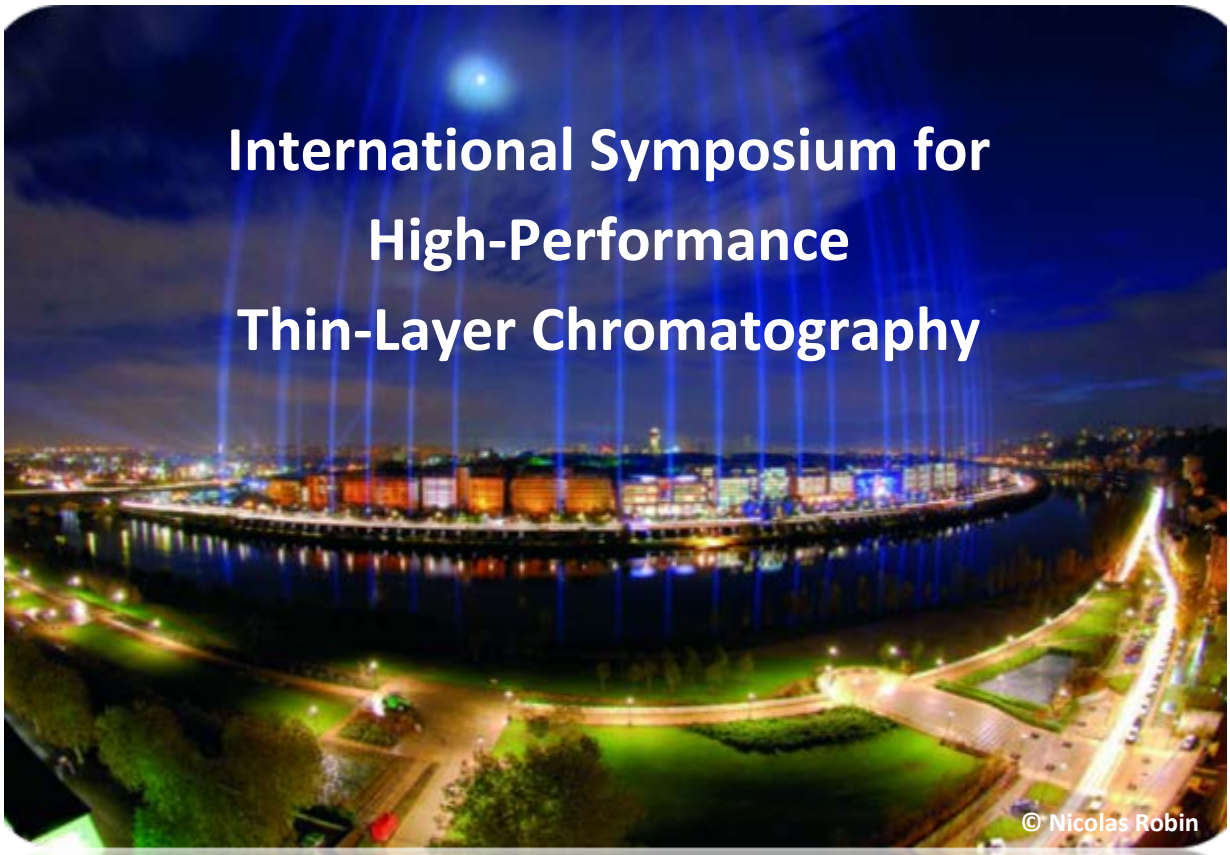
HPTLC 2014

Book of abstracts



Lyon, 2 – 4 July 2014

Edited and revised by Prof. Dr. Gertrud Morlock

Justus Liebig University Giessen, Germany, 4th July 2014

Welcome to Lyon!

The Scientific Committee of the HPTLC 2014 welcomes you to the beautiful city of Lyon with the well-known French spirit of hospitality, good food and friendship. The symposium will present an exciting and diverse scientific program with panel discussion, workshops, oral and poster presentations and an active social program. The Organizing Committee arranged a perfect location for this event and we all hope that you will enjoy this unique hospitality.

Enter into fruitful discussions with a number of world renowned experts in HPTLC. The exchange of experiences and ideas is essential for the promotion of HPTLC. The attractive field of HPTLC hyphenations, for example, with mass spectrometry or bioassays, increased the general interest in HPTLC in recent times. Analysts learned that HPTLC is a suitable tool to solve their analytical questions.

However, we all still have to face challenges. How to improve the teaching and training in HPTLC at educational sites? This efficient method deserves its space in the training of the next generation of scientists. It is necessary to advocate for the best analytical solution, in which decision finding process, HPTLC is often not seen as an option. Most analysts do not even know that we are liquid chromatography, and thus, have been contributing with LC-MS methods for over a decade. Hence, the situation changed and this knowledge needs to be spread more widely.

We will enjoy the views on HPTLC from the many different nations that will be present in Lyon. The many facets will contribute to the further development of the method. The flexibility of the method is unlimited, however, our brain seems to be the barrier. Get inspired by each other and new ideas will be born.

On behave of the Scientific Committee of the HPTLC 2014,
Colin Poole and Gertrud Morlock

The series of international HPTLC Symposia

2-4 July 2014, **Lyon**
(22nd) International Symposium for High-Performance Thin-Layer Chromatography, HPTLC 2014

6-8 July 2011, **Basel**
(21st) International Symposium for High-Performance Thin-Layer Chromatography, HPTLC 2011

11-13 June 2008, **Helsinki**
(20th) International Symposium for Thin-Layer Chromatography, HPTLC 2008

9-11 October 2006, **Berlin**
(19th) International Symposium for Thin-Layer Chromatography, HPTLC 2006

29-31 May 2005, **Siofok**
(18th) Planar Chromatography 2005

23-25 May 2004, **Visegrad**
(17th) Planar Chromatography 2004

15-18 October 2003, **Lyon**
(16th) International Symposium for TLC

21-23 June 2003, **Budapest**
(15th) Planar Chromatography 2003 (in honor of Professor Thihak)

4-6 October 2002, **Novo mesto**
(14th) Planar Chromatography Today 2002

11-13 May 2002, **Keszthely**
(13th) Planar Chromatography 2002 (in honor of Doctor Geiss)

23-25 June 2001, **Lillafüred**
(12th) Planar Chromatography 2001

11-13 May 2000, **Lillafüred**
(11th) Planar Chromatography 2000 (in honor of Professor Kaiser)

16-19 May 1998, **Visegrad**
10th International Symposium on Instrumental Planar Chromatography (60 years TLC + 10 years JPC)

9-11 April 1997, **Interlaken**
9th International Symposium on Instrumental Planar Chromatography

5-7 April 1995, **Interlaken**
8th International Symposium on Instrumental Planar Chromatography

23-26 March 1993, **Brighton**
7th International Symposium on Instrumental Planar Chromatography

23-26 April 1991, **Interlaken**
Sixth International Symposium on Instrumental Planar Chromatography

21-24 February 1989, **Brighton**
(Fifth) International Symposium on Instrumental High Performance Thin-Layer Chromatography

22–25 September 1987, **Selvino**
Fourth International Symposium on Instrumental High Performance Thin-Layer Chromatography

17–19 April 1985, **Würzburg**
Third International Symposium on Instrumental High-Performance Thin-Layer Chromatography

2-6 May 1982, **Interlaken**
Second International Symposium on Instrumental High-Performance Thin-Layer Chromatography

18-21 May 1980, **Bad Dürkheim**
First International Symposium on Instrumentalized High-Performance Thin-Layer Chromatography

International Scientific Committee

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The Scientific Community deeply recognizes the

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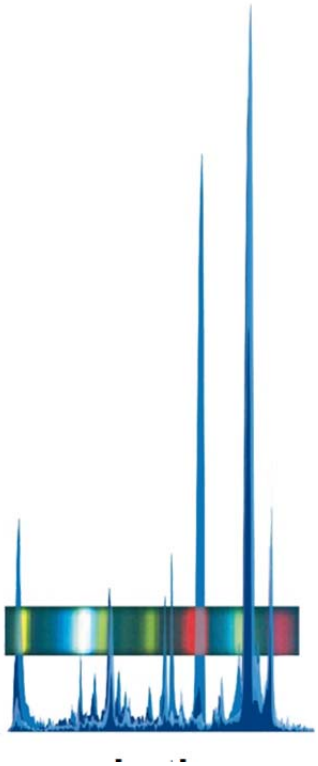

*See attachment (pages 204 ff)

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Prof. Dr. Colin Poole, USA

Prof. Dr. Gertrud Morlock, Germany

Thursday 3rd July 2014

20:00 Poster awards during the symposium dinner

Overall Schedule

University of Lyon[1]

WED 2nd	9:00-12:00	Practical Workshops on State-of-the-art of HPTLC applications

Congress Center[2]

WED 2nd	12:00-14:00	Mounting of the poster presentations
	14:00	Opening
	14:15-18:30	Tutorials and Panel Discussion
	18:30	Welcome lecture on Lyon
	19:00-21:00	Poster Session 1 and Evening Welcome Diner-Cocktail
THU 3rd	9:00-18:00	Oral Presentations and Poster Sessions 2 and 3
	18:00-19:00	Poster Session 4
	19:30-23:30	Symposium dinner with poster awards at Chateau de Janzè[3]
FRI 4th	9:00-17:30	Oral Presentations and Poster Session 5
	17:30	Demounting of the poster presentations

Social events (by bus)

SAT 5th	09:00-17:00	Visit of Grande Chartreuse Monastery and Chartreuse Cellars[4]
	10:00-17:00	Visit of vineyard: Tasting of wines at lunch in Domaine La Madone[5]
	14:30-16:30	Visit of Lyon historic center: from Fourvière to the Old Lyon[6]

[1]http://master-analyse-controle.univ-lyon1.fr/webapp/website/website.html?id=1498349&pageId=1499, Université Claude Bernard, Lyon 1, Bâtiment Claude Berthollet, 22 Avenue (Gaston Berger RDC), 69622 Villeurbanne cedex
[2]www.ccc-lyon.com, 50 Quai Charles de Gaulle, 69463 Lyon cedex 06
[3]www.chateaudejanze.com, Chateau de Janzè, 15, Chemin de Janzè, 69380 Marcilly d'Azergues
[4]www.musee-grande-chartreuse.fr/en, www.chartreuse.fr/index.php and www.chartreuse.fr/the-chartreuse-cellars;article;53;uk.html
[5]www.domaine-de-la-madone.com
[6]www.en.lyon-france.com/Guided-Tours-Excursions

Oral Presentations
(Congress Center)

WED 2nd Tutorials and panel discussion

14:00		Welcome by a representative of Lyon's city and opening
14:15	T-1	POOLE, USA: An interphase model to explain retention in (thin-layer) chromatography
15:15	T-2	MORLOCK, Germany: HPTLC-bioassays for effect-directed analysis
16:15	T-3	SCHWACK, Germany: HPTLC hyphenations - potential for structure elucidation
17:15		**Panel discussion** on HPTLC research & development with sponsors
18:30		**Welcome lecture** on Lyon BERTHOD, France: A thick layer of history for thin-layer chromatography
19:00-21:00		**Poster Session 1 and Evening Welcome Diner-Cocktail**

THU 3rd Oral and Poster Sessions

Session 1: Development (Chair: Berthod)

09:00		BERTHOD	France	Opening and announcements
09:10	O-1	COKSOYLER	Turkey	A new TLC technique and chamber
09:25	O-2	HALKA-GRYSINSKA	Poland	A new semi-automatic device with horizontal developing chamber for gradient TLC
09:40	O-3	CEBOLLA	Spain	A hyphenated technique based on AMD-FDIC-MS for separating and determining biomarkers of lysosomal storage diseases in human fluids

Session 2: Miniaturization (Chair: Poole)

10:00	O-4	OLESIK	USA	UTLC: Possibly the Future of Analytical Separation Science
10:20	O-5	MORLOCK	Germany	Electrospun polyacrylonitrile nanofibers as miniaturized layer materials for UTLC
10:35	O-6	CUSHMAN	USA	Microfabricated TLC plates based on carbon nanotube scaffolds infiltrated with SiO_2 via atomic layer deposition
10:50	O-7	LINFORD	USA	Fast, microfabricated, normal phase TLC plates based on carbon nanotube forest scaffolds

11:15 Coffee break (30 min)

Session 3: Hyphenation with MS (Chair: Schwack)

11:45	O-8	SHIEA	Taiwan	Detection of polar and nonpolar compounds on TLC plates using laser desorption/electrospray and atmospheric pressure chemical ionization/mass spectrometry (LD/ESI+APCI/MS)
12:05	O-9	CRAWFORD	USA	Planar chromatography meets direct ambient mass spectrometry: current trends
12:20	O-10	GRIESINGER	Germany	TLC-MS: elution- and desorption-based approaches to spatially resolved structure elucidation
12:35	O-11	DA SILVA	France	Analysis of flavonoids in plant extracts using HPTLC-MALDI-TOFMS: influence of MALDI parameters

13:00 Lunch and Poster session 2 (60 min)

Session 3 continued: Hyphenation with MS (Chair: Shiea)

14:00	O-12	SCHWACK	Germany	Planar SPE coupled to flow injection TOF/MS analysis - a rapid pesticide screening tool
14:15	O-13	SCHULZ	Germany	Influence of TLC plate parameters on MS results by coupling TLC to MS
14:30	O-14	SPINA	France	A rapid identification of anthocyanins in various plants from Burkina Faso by HPTLC/MS
14:45	O-15	HAEBE	Germany	Quantitative HPTLC surface analysis by DART-MS scanning

Session 4: Effect-directed detections (Chair: Choma)

15:00	O-16	MORICZ	Hungary	HPTLC-bioassay-MS, a rapid tool to search and analyze bioactive plant products
15:15	O-17	DE-EKNAMKUL	Thailand	A combination of TLC and a novel androgenic alopecia cell-based assay for screening reductase inhibitors in plants
15:30	O-18	SUN	China	Directly bioprospecting of amicoumacin-producing strains and efficient discovery of new amicoumacins by HPTLC profile
15:45	O-19	WEISS	Germany	Enhanced tests and evaluation procedures for the effect-directed analysis using HPTLC applied for aqueous environmental samples

16:00 Poster session 3 and coffee break (60 min)

Session 4 continued: Effect-directed detections (Chair: Moricz)

17:00	O-20	CHEN	Germany	HPTLC-bioluminescence screening for residues of antibiotics in food of animal origin
17:15	O-21	CHOMA	Poland	The novel TLC-direct bioautography tests for analysis of antimicrobials

17:30	O-22	SCHOENBORN	Switzer-land	Screening untreated drinking water on estrogenic activity using the planar-YES, a TLC-EDA tool
17:45	O-23	WEINS	Germany	The relevancy of effect directed analysis implementing the European Water Framework Directive 2000/60/EC in the field of water policy

18:00-19:00 **Poster session 4**

19:00 Bus departure to Chateau de Janzè in Marcilly d'Azergues
(in front of congress center entrance)

19:30-23:30 **Symposium dinner and poster award ceremony** at Chateau de Janzè

FRI 4th Oral and Poster Sessions

Session 5: Separation characteristics (Chair: Olesik)

09:00	O-24	DZIDO	Poland	Orthogonal pressurized planar electrochromatography
09:15	O-25	MALINOWSKA	Poland	TLC in investigation of complex properties in solutes
09:30	O-26	STUDZINSKI	Poland	Retention changes of 1,2,4-triazole derivatives in RP-TLC and micellar TLC systems under the influence of external static magnetic field
09:45	O-27	ZARZYCKI	Poland	Practical approach for temperature-controlled TLC

Session 6: Analysis of food and feed (Chair: Linford)

10:00	O-28	BESSIN	France	Streamlined analysis of sugars in chicory root juice: comparison of 2 chromatographic methods
10:15	O-29	VOVK	Slovenia	Planar chromatography and mass spectrometry in analysis of phytonutrients in the extracts of edible plants
10:30	O-30	SRIVASTAVA	India	Development and validation of HPTLC-MS determination of melamine in milk
10:45	O-31	AGATONOVIC-KUSTRIN	Australia	HPTLC quantification of phenolics in wine using high resolution plate imaging
11:00	O-32	CLENCH	UK	Fast Automated Food Safety Screening - FAFOSS

11:15 Coffee break and poster session 5 (60 min)

Session 7: Analysis of traditional medicines (Chair: De-Eknamkul)

12:15	O-33	GENTA-JOUVE	France	A toolbox for HPTLC data processing
12:30	O-34	FROMMEN-WILER	Switzer-land	HPTLC method for the identification of seven different resin species
12:45	O-35	BHARDWAJ	India	Characterization of medicinal mushrooms by hyphenated HPTLC

13:00 Lunch (60 min)

Session 7 continued: Analysis of traditional medicines (Chair: Spangenberg)

14:00	O-36	~~SHAKILA~~	~~India~~	Not presented ~~HPTLC fingerprint development of Siddha drugs for quality assessment~~
14:15	O-37	REICH	Switzer-land	Standardized and internationally harmonized HPTLC methods for describing the quality of reference material for *Angelica*, *Ligusticum* and related species

Session 8: Pharmaceutical analysis (Chair: Dzido)

14:30	O-38	PARMAR	India	Experimental design approach for robustness testing of HPTLC methods
14:45	O-39	DOMINGO	Spain	Monitorization, separation and quantification of antifungals used for invasive aspergillosis treatment by HPTLC
15:00	O-40	~~CHARDE~~	~~India~~	Not presented ~~Development of validated method for simultaneous estimation of benzhexol HCl and trifluperazine HCl in pharmaceutical dosage form~~

15:15 Coffee break (30 min)

Session 9: Environmental analysis (Chair: Di Marcello)

15:45	O-41	SPANGENBERG	Germany	Using chemiluminescence in TLC for the quantification of polyaromatic hydrocarbons
16:00	O-42	TYRPIEN-GOLDER	Poland	What does TLC mean?

Session 10: Toxicological analysis (Chair: Bernard-Savary)

16:15	O-43	LADROUE	France	Interest of (HP)TLC for forensic laboratories
16:30	O-44	JAISWAL	India	Extraction/isolation and detection of monocrotophos from blood using HPTLC
16:45	O-45	~~KUMAR~~	~~India~~	Not presented ~~A new method of extraction and detection of dicyclomine HCl from urine using HPTLC~~
17:00		Poole	USA	Closing remarks, Announcements for the social events

List of Poster Presentations

Poster group 1: Analysis of food, feed, food supplements and cosmetics

P-1 Analysis of pesticide residues in animal feed - method comparison

P-2 Accelerated screening of sulfonamides in animal-derived foods by HPTLC-FLD-ESI/MS

~~P-3 Analysis of sulfonamide residues in chicken and shrimp using TLC~~

P-4 Rapid HPTLC screening to study the reactivity of UV filter substances towards skin proteins

P-5 Streamlined analysis of lactose-free dairy products

P-6 Quantitation of coumarin in food, confirmed by mass spectrometry

P-7 Simultaneous determination of citrinin and lovastatin in lactone- und hydroxy acid form with validated HPTLC-UV/FLD method

P-8 HPTLC-FLD-ESI-MS and HPTLC-MALDI-TOF/TOF MS analysis of lecithins used in the production of chocolate

P-9 Fingerprinting of Serbian, Slovenian and Croatian propolis using HPTLC and pattern recognition methods

P-10 Solid-phase extraction for clean-up of sugar-rich plant material for improving identification by normal phase HPTLC

P-11 Quantitative HPTLC method for quercetin derivatives in onion extract

P-12 Analysis of multi-ingredient food supplements by fingerprint HPTLC approach

P-13 Rapid determination of oleuropein in food supplements and in *Olea europaea* L. leaves extracts by a densitometric-HPTLC approach

P-14 Screening of active biomolecules in broccoli extracts using HPTLC- coupled to a UV densitometer and MALDI-TOFMS

P-15 Application of HPTLC for the analysis of PAHs in food samples

P-16 Analysis of anthocyanins of coloured wheat varieties

P-17 Biorefinery analytics by means of HPTLC - separation and quantification of wood sugars by HPTLC

P-18 Quality assessment of commercial tea products sold as German chamomile (*Matricaria recutita* L.) by a validated HPTLC method in Turkish market

~~P-19 Validated HPTLC method for quantitation of hexetidine in bulk drug and in mouth-wash formulation~~

P-20 Japanese knotweed a good source of proanthocyanidins

P-21 Interactions between phenolic compounds and food proteins can alter antigenic properties

~~P-22 Determination of caffeine content of a commercial coffee samples with HPTLC~~

~~P-23 HPTLC Analysis of carbohydrates from the mesocarps of *Pithcellobium dulce*~~

~~P-24 Fingerprint profiling of polysaccharide kefiran extracted from kefir grains by HPTLC~~

P-25 Simultaneous determination of Sudan dyes (I-IV) in spices by TLC

P-26 The use of off-line 2DTLC-HPLC-ESI-MS in the qualitative analysis of lutein-containing dietary supplements

P-27 Comparison of an HPTLC method with the Reflectoquant assay for fast HMF determination in honey

Poster group 2: Hyphenations with MS

P-28 TLC/laser-induced acoustic desorption/atmospheric pressure chemical ionization MS

P-29 Separation, quantitative determination, and fatty-acid profiling of monoacylglycerides in fatty acid methyl esters (FAME) using an online, hyphenated technique based on AMD-FDIC-ESI-MS

P-30 HPTLC-MS using an elution-based TLC-MS interface

P-31 HPTLC-nanospray-MS using the advion nanomate® system

P-32 Characterization of proteins by HPTLC-MS - proteomics revisited?

P-33 Hyphenation of HPTLC with ESI-MS for the characterization of saponins in different plant matrices

P-34 Mass spectrometric characterization of enzyme-lactose derivatives

P-35 A further look at protein analysis - useful applications of nano-ESI-MS for the identification of proteins and possible modifications

P-36 LC-MS determination of the composition and biological activity of the drug Ukrain based on *Chelidonium majus* L. alkaloids

P-37 Planar solid phase extraction and flow injection TOF/MS analysis - (non-)target screening for pesticide residue analysis

P-38 Coupling HPTLC with MALDI-TOF MS for detection of flavonoids

P-39 Influence of the silica gel layer thickness on the quality of TLC-MALDI mass spectra of lipids

P-40 Rapid identification of antioxidant compounds of G*enista saharae* Coss. & Dur. by combination of DPPH scavenging assay and HPTLC-MS

P-41 Hyphenation of MALDI-TOF-MS methodology: A real improvement for plants extract characterization

P-42 TLC-MALDI investigation of a multi-compound flu medication

P-43 Development of an off-line HPTLC-MS technique for plant extracts

Poster group 3: Miniaturization

P-44 Surface analysis of microfabricated TLC plates by FTIR and TOF-SIMS to determine surface hydration

P-45 Inkjet application, chromatography and MS of sugars on nanostructured thin films

P-46 ULTC on SiO_2, $Al2O_3$, TiO_2 and ZrO_2 nanostructured thin films

P-47 Office Chromatography: Precise printing of sample solutions on miniaturized thin-layer phases

P-48 Fast, microfabricated, normal phase TLC plates based on carbon nanotube forest scaffolds

Poster group 4: Effect-directed analysis

P-49 Detection of antibacterial bell pepper ingredients by TLC/HPTLC-direct bioautography

P-50 Is HPTLC method a valuable solution for free radical scavenging activity evaluation? A comparison of two quantitative approaches on silica plates with the reference method in solution.

P-51 Determination of biological active compounds in *Asteraceae* tinctures by TLC-DB and LC-MS methods

P-52 Improved bioautographic xanthine oxidase assay: combining HPTLC separation and activity assessment for phytopharmaceutical research

P-53 p-YES - building a bridge between chemical analysis and biological effects

P-54 Development of a bioautographic HPTLC method for identification and quantitation of estrogen-effective compounds as a novel non-target method

P-55 Fast HPTLC-direct bioautography using *Bacillus subtilis* for screening of antibiotics in plant extracts

P-56 Effect-directed analysis of *Salvia officinalis*

P-57 Antimicrobial activity of *Fragaria x ananassa* Duch. and *Solanum lycopersicum* L. by TLC

P-58 Screening of mediterranean and alpine plant extracts for tyrosinase inhibition by an HPTLC autobiographic assay

P-59 Identification of antibacterial *Tanacetum vulgare* L. essential oil component by the use of HPTLC-bioautography-MS

P-60 Detecting quorum sensing compounds using TLC bioautography

P-61 Troubleshooting *Vibrio fischeri* bioassay for crude marine extracts

~~P-62 Identification of antimicrobial and anti-quorum sensing components from South African propolis using HPTLC-bioautography~~

~~P-63 Chemical composition and antibacterial activity of medicinally useful essential oil from the rhizomes of Alpinia Allughas Rosc.~~

P-64 HPTLC hyphenated with bioassays for the screening of bioactive constituents of Serbian *Salicaceae* bud extracts

Poster group 5: Plant and herbal analysis

P-65 CAMAG Method Library powered by visionCats - download & run compendial methods for identification of medicinal plants

P-66 HPTLC analysis of crude extracts of *Schoepfia schereberi* J.F. Gmel

P-67 HPTLC-UV densitometric detection method for quantification of xanthones in mangosteen (G*arcinia mangostana*) fruit hulls extracts

P-68 HPTLC analysis of secoiridoids and phenylpropanoids in different extracts of *Ligustrum vulgare* L. leaves

P-69 Optimization of the separation of caffeoyl derivatives from aqueous and ethanolic extracts of *Galinsoga parviflora* and *Galinsoga ciliata*

P-70 HPTLC and HPLC for the qualitative and quantitative analysis of *Calendula Officinalis* - advantages and limitations

~~P-71 Application HPTLC method for identification and stability study of compounding preparation with herbals~~

~~P-72 Comparative study of the pharmacological activity and HPTLC profiles of *Strobilanthes crispus* from various cultivation sources~~

~~P-73 Simultaneous separation and quantification of five phytohormones using HPTLC~~

P-74 Enhanced extraction of isoflavones from *Medicago sativa* L. by complexation with (2-hydroxypropyl)-β-cyclodextrin and l-lysine monohydrochloride

~~P-75 HPTLC for the diagnosis of stability of traditional polyherbal medicines~~

P-76 Anti-tubercular glycolipids from the leaves of *Sterculia setigera* Del. (Sterculiaceae)

P-77 Standardization of *Zanthoxylum zanthoxyloides* bark roots harvesting for the production of phytomedecine against sickle cell desease

P-78 HPTLC finger print profile of different extracts of *Cocculus hirsutus* (Linn.)

P-79 Method development and validation of rutin in flavanoidal fraction of *Hemidesmus indicus* Linn. by HPTLC

P-80 A simple and sensitive HPTLC method for simultaneous estimation of anti-malarial compound artemisinin and its precursor artemisinic acid in leaves of *A. annua* L. plants

P-81 Validated densitometric HPTLC method for the quantification of luteolin in *Portulaca oleracea* leaves

P-82 Quantification of phenylpropanoids in commercial *Echinacea* products with ANN modelling of HPTLC fingerprints

Poster group 6: Pharmaceutical analysis

P-83 Determination of adulteration of anti-obesity drug in Indian herbal medicinal products: development and validation of analytical method

P-84 Quantitative determination of trazodone in human serum by HPTLC

P-85 Simultaneous estimation of drotaverine HCl and nimesulide in pharmaceuticals by HPTLC method

P-86 Development of stability indicating HPTLC method for simultaneous estimation of irbesartan and amlodipine in combination

P-87 Stability indicating HPTLC method for simultaneous estimation of cilnidipine and telmisartan in their combined dosage form

P-88 Stability indicating HPTLC method for determination of flupirtine maleate in pharmaceutical preparations

P-89 Validated analytical HPTLC method & content uniformity for the determination of letrozole in tablet dosage form

P-90 Validated HPTLC analytical method & content uniformity test for the determination of cilnidipine and telmisartan in tablet dosage form

P-91 Development and validation of stability-indicating HPTLC method for determination of cilnidipine and its related substances in bulk drug and pharmaceutical preparations

P-92 Development and validation of HPTLC method for determination of edaravone in bulk and in injectable dosage form

P-93 HPTLC method for quantitation of diosgenin from extract of *Balanite aegyptiaca*

P-94 Simultaneous estimation of citicoline and methylcobalamin in pharmaceutical

formulation by HPTLC-densitometry

P-95 Simultaneous estimation of amiloride hydrochloride and torsemide in their combined dosage form by HPTLC

P-96 Simultaneous estimation of epalrestat and methylcobalamin in pharmaceutical formulation by HPTLC-densitometry

~~P-97 Simultaneous estimation of dosulepin and methylcobalamin in pharmaceutical formulation by HPTLC-densitometry~~

P-98 HPTLC method development and validation for determination of alfuzosin hydrochloride in bulk and tablet dosage form

P-99 Development of validated HPTLC method for estimation of balofloxacin in bulk and tablet dosage form

P-100 A novel method of sample preparation for quantitative analysis with HPLC and HPLC/MS techniques

~~P-101 A validated HPTLC method for simultaneous quantification of ramipril and hydrochlorothiazide in bulk and tablet formulation~~

~~P-102 Simultaneous estimation of cyclobenzaprine HCl and aceclofenac in pharmaceutical formulation by HPTLC-densitometry method~~

P-103 Quantification of metolazone and ramipril in their combined dosage form by HPTLC method

~~P-104 Designing a nutraceutical formulation with antioxidant property as a preventive remedy against diabetic retinopathy~~

~~P-105 Development and validation of the HPTLC quantitative method of the degradation products in bromchloramide~~

P-106 Evaluation of glycosylceramides in wheat germ by automated multiple development (AMD)-HPTLC

~~P-107 TLC is complementary technique to HPLC~~

~~P-108 Development of validated stability indicating HPTLC assay method for simultaneous quantification valacyclovir and acyclovir~~

~~P-109 Sarcosine as a stage dependant metabolomic marker to detect prostate cancer by using HPTLC~~

Poster group 7: Toxicological, clinical and environmental analysis

P-110 TLC method for simultaneous determination of seven chlorophenoxy and benzoic acid herbicides in ground water

~~P-111 HPTLC analysis of carbamate insecticides of forensic importance: separation evaluation and stability study~~

~~P-112 Pesticide screening in forensic toxicology by HPTLC~~

~~P-113 HPTLC analysis of three organophosphorus fungicides of forensic importance in whole blood samples~~

P-114 Extraction/isolation and detection of dicyclomine hydrochloride from blood using HPTLC plate

~~P-115 Determination of aflatoxins in livestock mixed feed and feed ingredients~~

~~P-116 Validated HPTLC method for identification and quantification of cyanogenic glycosides~~

~~in apricot kernels and bitter almonds~~

P-117 Validation of a HPTLC method for quantitative determination of hypoglycin A in methanol extracts of maple (Acer) samples

P-118 Tracing the diacylglycerol metabolism by click chemistry and TLC-fluorescence imaging

P-119 Assessment of cardiolipin content by HPTLC and cardiolipin fatty acid composition by GC in rat mammary tumors

P-120 Occupational exposure to polycyclic aromatic hydrocarbons (PHA) among asphalt and road paving workers

P-121 Quantitative analysis of base oils by HPTLC-FDIC

P-122 Chromatographic profiling of heavy petroleum products by AMD-densitometry - Repeatability, factors influencing separation and some examples of application

P-123 Application of AMD to petrochemical analysis: improved separation and expanded hydrocarbon group type analysis of heavy petroleum products

Poster group 8: Fundamentals

P-124 Influence of relative humidity on PH-EUR performance test

P-125 Development of a work-flow for HPTLC data processing for untargeted metabolomics

P-126 Simple and selective two-dimensional separation of complex mixtures of peptides with planar chromatography

P-127 Influence of some variables on migration distance and solute band shape of peptides in pressurized planar electrochromatography system

P-128 Berberrubine: a FDIC fluorophore for determining saturated hydrocarbons with a simple calibration via non-covalent interactions, and signal amplification from a keto-enol tautomerism

P-129 TLC and magneto-TLC as a method for investigation on selected d-electron ion element complexes with organic ligands

P-130 Application of 1 mL sample for analytic TLC

P-131 Thermal-wave investigation of physical non-uniformities within TLC stationary phases

P-132 New active thermography method for sensitive detection of fullerenes separated on micro-TLC plates

Deleted posters were not presented!

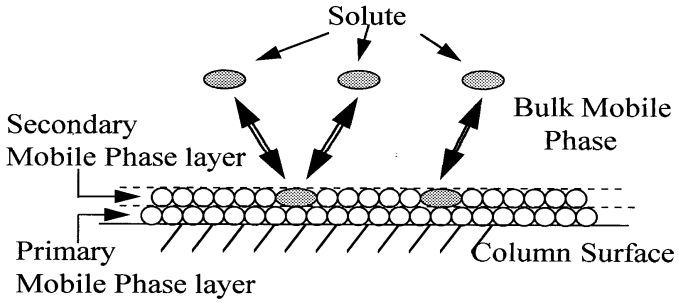
Tutorial 1

An Interphase Model for Retention in (Thin-Layer) Chromatography

Colin F. POOLE
Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA,
cfp@chem.wayne.edu

It is necessary to understand the difference between interfaces and interphases to understand retention in chromatography. An interface is simply a dividing plane between two phases that can affect retention by surface interactions. An interphase is a region immediately adjacent to a solvated stationary phase surface between the impenetrable region of the stationary phase and the bulk mobile phase. For all forms of liquid chromatography the interphase region acts as the stationary phase and retention occurs because of the distribution of a solute between the interphase region and the bulk mobile phase.

The composition of the interphase region is dynamic and is in equilibrium with the bulk mobile phase. The contribution of the different components of the interphase region to retention will be delineated for separations in normal- and reversed-phase chromatography. For chemically bonded phases, selective solvation by the mobile phase has a greater effect on relative retention than the type of siloxane-bonded ligands. The main difficulty in method translation results from the relatively large differences in phase ratio for the interphase region formed by ligands of different chain length. Site-specific and steric interactions on inorganic oxide adsorbents are responsible for the difficulty of modeling normal-phase separations for varied compounds. The interphase model, however, successfully reproduces the solvent strength parameter scale.



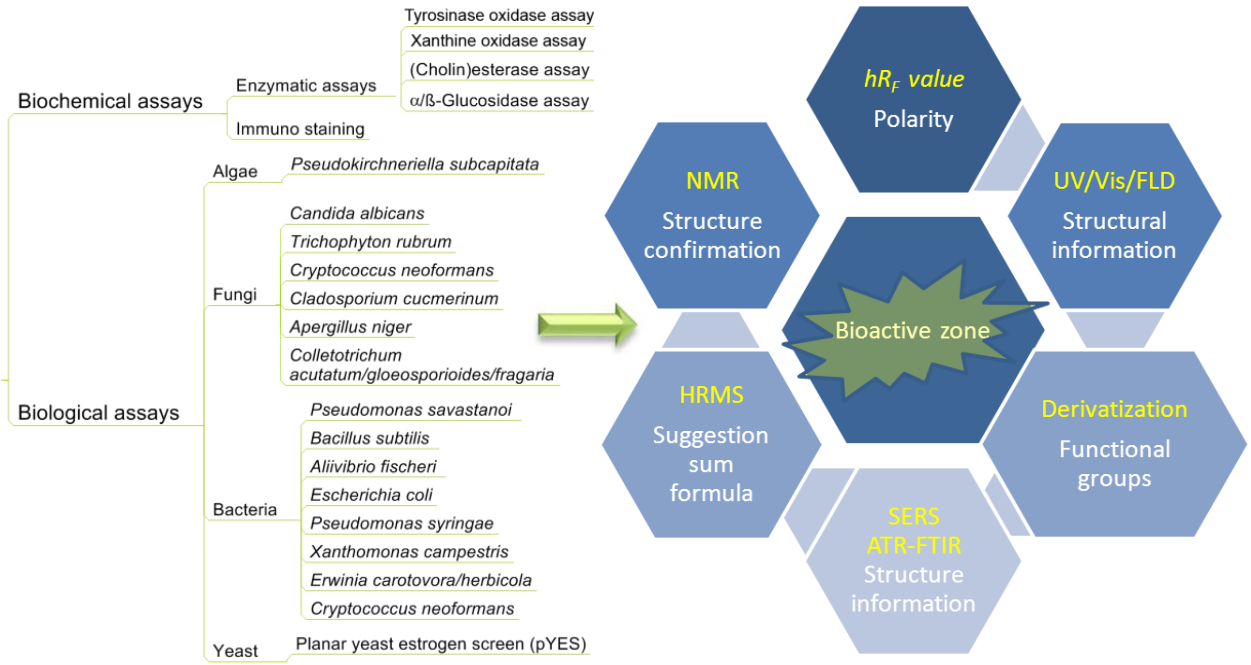
General schematic of solute interactions in the interphase region
for normal-phase chromatography

Tutorial 2

HPTLC-Bioassays for Effect-Directed Analysis

Gertrud E. MORLOCK
Justus Liebig University Giessen, Germany, Gertrud.Morlock@ernaehrung.uni-giessen.de

Recent progress in the application of bioassays in direct combination with chromatography (direct bioautography) led to a rapid and reliable characterization of unknown samples with regard to their activity profile. Planar chromatograms were directly immersed into bioassays, instantly evaluated or incubated for up to several hours followed by visualization of the activity profile via an enzyme-substrate reaction. Despite long aqueous incubation times, sharp-bounded HPTLC zones were obtained, allowing quantitation. Bacteria, yeast cells or enzymes specifically and sensitively detect active compounds in complex samples according to their distinct effect. HPTLC in combination with bioassays, derivatization reagents, spectroscopic and high-resolution mass spectrometric detections etc. led to a fast activity profiling and the direct link to the bioactive compounds of interest in complex raw samples. All these tools, inclusive of structure elucidating methods were performed at the analytical level directly from the bioactive zone of interest. [1, 2]



Bioassays employed for TLC/HPTLC and characterization tools to obtain the structure of bioactive zones (reproduced with permission from [1])

[1] Morlock, G., Q&more 2014, 1, 42-47; copyright G. Morlock
[2] Morlock, G., Chromatography combined with bioassays and other hyphenations – the direct link to the compound indicating the effect, in B.S. Patil, G.K. Jayaprakasha, F. Pellati, Instrumental Methods for the Analysis of Bioactive Molecules, ACS Books Publishing, in print.

Tutorial 3

HPTLC Hyphenations - Potential for Structure Elucidation

Wolfgang SCHWACK
University of Hohenheim, Institute of Food Chemistry, 70599 Stuttgart, Germany,
wolfgang.schwack@uni-hohenheim.de

Structure elucidation and characterization of an unknown compound separated by HPTLC requires differently sophisticated approaches, depending on the pre-information about the sample (degradation study *versus* a plant extract). Hyphenations can be selected as demanded to reach the relevant information and to sketch the compound's structure.

Applying the versatile TLC-MS interface to record low and high resolution mass spectra directly from the HPTLC zone, meanwhile is most easy and well established. Alternative elution based TLC-MS techniques are available, called Liquid Extraction Surface Analysis (LESA, Advion) or In situ microextraction (flowprobe, Prosolia), however only working on reversed phase plates. Additionally to a zone-targeted recording, a whole track or hR_F substance window can be scanned by matrix assisted laser desorption and ionization (TLC-MALDI, Bruker) or by ambient desorption techniques like Desorption Electrospray Ionization (2D-DESI, Prosolia) and Direct Analysis in Real Time (DART SVP 45A – 3+D, Ion Sense), all under the precondition of a high spatial resolution and a satisfying detectability in the low ng per band range.

Regarding the isotope pattern of the identified molecular ion (as the protonated or deprotonated molecule), the exact mass leads to the molecular formula, which can be searched in data bases like ChemSpider. To reduce the great number of hits (constitutional isomers), additional information about the presence of functional groups is essential. Therefore, attenuated total reflection (ATR)-FTIR spectra can be easily recorded from zones of interest, eluted by TLC-MS Interface, to identify hydroxyl or carboxyl groups and aromatic ring systems, for example. Diffuse reflectance infrared fourier transform (DRIFT) spectroscopy was shown by reflectance measurements directly from the analytical plate, which, however, is limited to the transparent IR windows of the layer material. After the elution of the zone of interest by the TLC-MS Interface and deuterated solvent exchange, recording of ^{1}H-NMR spectra is possible at the analytical scale, to obtain the final information concerning the proton spin systems.

Panel discussion

HPTLC research & development

Moderator: Pierre BERNARD-SAVARY, President CCCM and Chromacim, France
Sue KENNERLEY, Director, KR Analytical, UK
Markus WYSS, Director, Head Sales & Marketing, CAMAG, Switzerland
Petra LEWITS, Product Management Analytical Chromatography, Merck Millipore, Germany
Frank PORBECK, Senior European Support Specialist, Advion, UK
Colin F. POOLE, Wayne State University, USA

Create actively the future of HPTLC! Although HPTLC is a small emerging field, the impact is great due to its unique advantages. Discuss with manufacturers and opinion leaders the progress in the field – pros and cons are welcome, together we are strong!

- Novel layers: Electrospun? Monolithic? Nanostructured? Hardcore particles? HILIC layers? SEC layers?
- Layer improvements: Separation power? Reproducibility?
- Instrumental developments: Novel Hardware? Software improvements?
- Further hyphenations: Elution head-based TLC/HPTLC-MS with fully automated positioning on zones of interest? FTIR? FT-SERS? Coupling with column chromatography or supercritical fluid chromatography? Imaging MS?
- Novel detection tools: Image quality? Quantitative evaluation based on the image?
- Quantitative use of HPTLC: Validation? Significant numbers for precision values? Improved software tools, e.g. for integration of peaks (tangent peaks) or drawing the baseline (impacted by negative peaks)?
- Miniaturization: Layers (thickness and size)? Separation time? Online one-click system?
- Future bioassays: Transfer of cell tests? *Fungi*? Genetically modified organisms? Bioluminescent organisms?
- New derivatization reactions for compound classes difficult to detect: Selectivity? Detectability?
- Support: Need for books and training courses? Online CCBS database search? How to improve the research paper quality? How to train the journal reviewer? Are we LC? Are we LC-MS?

Welcome lecture

A thick layer of history for thin-layer chromatography

Alain BERTHOD
University of Lyon, Villeurbanne, France, berthod@univ-lyon1.fr

Paris is the shining capital of France and when I am asked: "where are you from?" and answer: "I am French", the person speaking to me starts to tell me how Paris is the nicest city in the world. When I go on saying that most French are NOT from Paris and I am from Lyon, I see surprise and distress: "I am sorry for you". What is that: Lyon? Never heard about it.

On April 24, 2014, CNN broadcasted a short show titled "8 ways Lyon outshines Paris" (http://edition.cnn.com/2014/04/24/travel/lyon-outshines-paris/) that went little noticed in the US but outraged the Parisian people. Lyon, Lugdunum at the time, was the capital of Gaul for six centuries and the birth place of two Roman emperors: Claude, born in 10 BC, emperor from 41 to 54 AD and Caracalla, born in 188 AD, and emperor from 198 to 217 AD. The talk will briefly present the rich history of Lyon pointing places that can still be seen today. For example, the house where André-Marie Ampère was born in 1775 is a museum worth the visit. Ampère (1775-1836) established electricity fundamental laws so that his name became the internationally accepted unit for electrical intensity.

Lyon is hosting HPTLC 2014. High quality talks will be given and novel HPTLC points will be presented in posters during the three days of the event. It is important to know that an extended stay can be programmed in this time of the year when the weather is favorable and there is so many things to see and to do.

O-1

A New TLC Technique and Chamber

Fikret Nafi COKSOYLER
University of Yuzuncu Yil, Foog Engineering Department, Van, Turkey, coksoyler@hotmail.com

A new TLC system (Turkish Patent No. 2007/08721) was tested for separation of aflatoxins (AFB1, AFB2, AFG1 and AFG2). The method and equipment is promising for developing countries for aflatoxin determination in food by TLC. This new system is compared with the classical system (unsaturated development in 20 x 20 cm trough chamber). In both systems, chloroform - acetone 9:1 and t-butylmethylether - methanol - water 96:3:1 were used as developing solvents on TLC silica gel 60 aluminum sheets (at 25 °C).

As a result, at the same migration distance, the new system has given much better resolution at both solvents, but separation time was longer. With the new system, equal or better resolutions were obtained at shorter migration distances and also separation time was shorter. The new technique needed only a few milliliter of solvent despite of 50-100 mL for the classical technique. Study showed that the new technique was more simple, more reproduceable and cheaper than the classical technique for separation of aflatoxins. In this study, quantification was performed by visual analysis of the chromatogram photographs at 365 nm. Determination coefficients were better than 0.99 for all calibration curves.

O-2

A New Semi-Automatic Device with Horizontal Developing Chamber for Gradient TLC

Aneta HAŁKA-GRYSIŃSKA
Medical University of Lublin, Lublin, Poland, aneta.halka@umlub.pl

Ewelina SITARCZYK, Medical University of Lublin
Wojciech MARKOWSKI, Medical University of Lublin
Tadeusz DZIDO, Medical University of Lublin
Anna KLIMEK-TUREK, Medical University of Lublin
Adam CHOMICKI, Medical University of Lublin

Gradient TLC offers many advantageous features, which enable to solve lots of analytical problems encountered with the separation of multicomponent mixtures. Unfortunately, practitioners of TLC can solve general elution problem using only one commercially available device so far, i.e. the AMD 2 system [1]. Therefore our research group have been looking for alternative solutions for gradient mode in TLC. In 2012 we reported a new horizontal developing chamber with practical advantages in comparison to other chambers especially in respect of its application to stepwise gradient elution [2]. Unquestionable disadvantage of this device was the necessity for applying special sealant margins on the periphery of each chromatographic plate that complicates the whole process. Consequently, we have designed the next semi-automatic device, which does not require this special procedure of chromatographic plate preparation.

In this presentation we are going to show and discuss the results obtained with the device. The new device was used for separation of multicomponent mixture with general elution problem. Additionally a general equation for determination of relative position, Rpg, of solutes chromatographed under conditions of stepwise gradient elution with one void volume of the mobile phase in TLC process has been applied. A satisfactory agreement between calculated (by computer program) and experimental values of Rpg has been obtained. Based on the results obtained we discuss the advantages and disadvantages of different devices for gradient mode of thin-layer chromatogram development.

[1] Burger K., Fresenius zur Anal. Chem. 318 (1984) 228-233
[2] Markowski W., Wróblewski K., Dzido T.H., J. Planar Chromatogr. 25 (2012) 3, 200-207

O-3

A Hyphenated Technique Based on AMD-FDIC-MS for Separating and Determining Biomarkers of Lysosomal Storage Diseases in Human Fluids

Vicente L. CEBOLLA
CSIC-ICB, Zaragoza, Spain, vcebolla@icb.csic.es

Andres DOMINGUEZ, CSIC-ICB
Carmen JARNE, CSIC-ICB
Luis MEMBRADO, CSIC-ICB
Maria SAVIRON, CSIC-CEQMA
Jesus ORDUNA, CSIC-ICMA
Javier GALBAN, Zaragoza University
Maria P. LAPIEZA, CSIC-ICB

Analytical techniques for determining several neutrals sphingolipids which are biomarkers of lysosomal storage diseases are required for their diagnostics and treatment monitoring. For this purpose, a hyphenated technique has been developed, based on sequential coupling of Automated Multiple Development (AMD), Fluorescence Detection by Intensity Changes (FDIC) and MS.

Different gradient separation strategies using AMD have been proposed depending on which biomarkers are considered. Primuline-postimpregnated silica gel plates have been used for FDIC and for further analyte quantification. Then, an on-line, direct transfer of the corresponding biomarker peak has been performed from the primuline-impregnated plate to a mass spectrometer through an elution-based interface. Peak identification has been done by ESI and APCI which provide useful complementary information. Primuline has been demonstrated to be compatible with MS, and does not interfere in the MS identification of studied metabolites.

Globotriaosylceramide (Gb3) has been identified in Fabry's patient plasma. Sphingomyelin (SM), a biomarker of Niemann-Pick disease, has been determined in human plasma from two healthy volunteers, under the following conditions. SM was separated from the other metabolites in 19 min using 2-step AMD gradient (from 80:20 to 50:50, v:v) over 60 mm total developing distance. FDIC was carried out with primuline (200 ppm; λexc= 365 nm). SM quantification from FDIC signals was performed using nonlinear calibration by the standard addition method ([SM]= 280, 289 ppm; *RSD* <6%).

O-4

UTLC: Possibly the Future of Analytical Separation Science

Susan OLESIK
The Ohio State University, Columbus, USA, olesik.1@osu.edu

Michael BEILKE, The Ohio State University
Toni NEWSOME, The Ohio State University
Martin BERES, The Ohio State University

Chromatographic separation science often requires the use of a large volume of organic solvents. Moving toward separations that use a minimal amount of organic or hazardous solvents is a worthwhile goal. This capability requires the separation devices to be highly selective for the analytes of interest and also highly efficient. Also, the stationary phases should be made of materials that can be used for an extended period of time and/or be biodegradability after the separations are finished. UTLC has the potential of meeting many of these desired capabilities.

Chromatographic efficiency improves with the inverse square of the particle size of the support. As the dimension of the particle decreases the pressure drop across the chromatographic system also increases. However, nanostructured materials that are not particle-based but nanofiber-based materials and are also self-organized at the macroscopic and nanoscopic level seem to be violating Darcy's law for flow properties. This talk will illustrate substantial improvements in chromatographic efficiencies using organized nanostructures without the expected gain in enhanced pressure drop and the unique range of chromatographic selectivity, and applications. These nanofibrous materials also function quite well as matrices for laser desorption ionization MS.

O-5

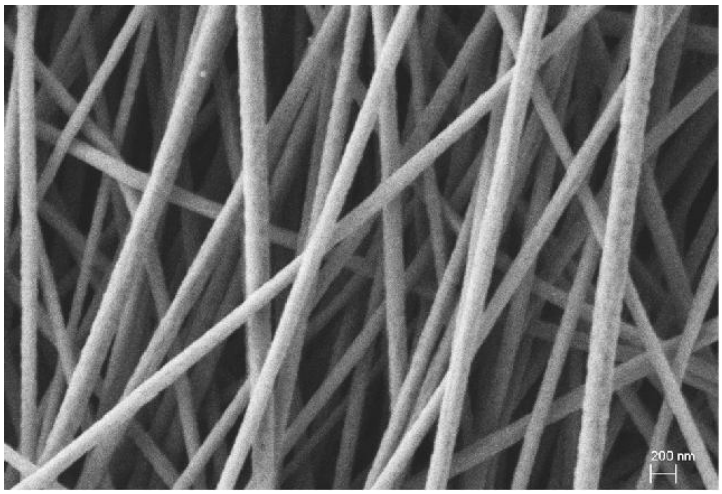
Electrospun Polyacrylonitrile Nanofibers as Miniaturized Layer Materials for UTLC

Gertrud E. MORLOCK
Justus Liebig University Giessen, Germany, Gertrud.Morlock@ernaehrung.uni-giessen.de

Pimolpun NIAMLANG, Department of Materials Engineering, Rajamangala University of Technology Rattanakosin, Nakorn Pathom, Thailand
Pitt SUPAPHOL, The Petroleum and Petrochemical College, Chulalongkorn University

The electrospun polycrylonitrile (PAN) nanofibers were used as UTLC stationary phases. Manganese-activated zinc silicate, a photoluminescent compound (UV 254), was directly mixed into a PAN solution, which was further fabricated into a fibrous substrate by the electrospinning process. The electrospun PAN nanofibrous phase, incorporating the UV 254 indicator, was employed to study the separation of 7 preservatives and an actual beverage sample. The thickness of layers and average diameters of individual fibers were calculated for additions of different UV 254 indicator concentrations. The separation efficiency on the photoluminescent layers was compared with HPTLC layers. [1]

Some benefits were the reduction in migration distance (3 cm), migration time (12 min), analyte (10 nL volumes) and mobile phase volumes (1 mL). As ultrathin stationary phase, such layers are suited for their integration into the Office Chromatography concept. For the first time, electrospun nanofiber layers were hyphenated with electrospray ionization MS (ESI-MS), and analytes were successfully confirmed by the ESI-MS spectra obtained.



Selected SEM image of an electrospun PAN nanofiber phase (12% PAN solution containing 10% UV 254 indicator, electric field of 18 kV, collection distance of 10 cm)

[1] P. Kampalanonwat*, P. Supaphol, G.E. Morlock, J. Chromatogr. A 1299 (2013) 110-117
*formerly name of Niamlang

O-6

Microfabricated TLC Plates Based on Carbon Nanotube Scaffolds Infiltrated with SiO_2 via Atomic Layer Deposition

Cody CUSHMAN
Brigham Young University, Provo, United States, mrlinford@chem.byu.edu

Supriya S. KANYAL, Brigham Young University
David S. JENSEN, US Synthetic Corp.
Andrew E. DADSON, US Synthetic Corp.
Mathew R. LINFORD, Brigham Young University

We have recently reported the preparation of microfabricated, carbon nanotube (CNT)-templated SiO2 nanowire TLC plates. One of the principle challenges in this work has been to find a suitable method to conformally coat carbon nanotubes with the desired stationary phase material. Here we present an overview of our fourth microfabrication process. In this development, true atomic layer deposition (ALD) in commercially viable equipment is used to coat the CNTs with SiO_2 for the production of robust plates capable of performing rapid, high-efficiency, normal phase separations.

O-7

Fast, Microfabricated, Normal Phase TLC Plates based on Carbon Nanotube Forest Scaffolds

Mathew R. LINFORD
Brigham Young University, Provo, United States, mrlinford@chem.byu.edu

Andrew E. DADSON, U.S. Synthetic Corp.
Gertrud MORLOCK, Justus Liebig University Giessen
Supriya S. KANYAL, Brigham Young University
Cody CUSHMAN, Brigham Young University
Tim HÄBE, Justus Liebig University Giessen

We have recently demonstrated that patterned carbon nanotube (CNT) forests can act as a template for the preparation of TLC plates. The resulting plates show higher efficiencies than commercial ones with significantly shorter run times. We have explored five methods for preparing these plates.

The first is the low-pressure chemical vapor deposition (LPCVD) of silicon onto the CNTs, followed by oxidation to make SiO_2 and remove the CNTs. The resulting SiO_2 was ideal for chromatography and white for easy analyte visualization. However, this process resulted in volume distortion which led to irreproducibility in the microfabrication process. To overcome this, we deposited a thin (< 5 nm) film of carbon on the CNTs, followed by atomic layer deposition of alumina (ALD), followed by a fast, pseudo (ψ) ALD of SiO_2. This approach led to undistorted features, and the ψ ALD process deposited ca. 10 nm of SiO_2 per cycle instead of the <1 nm/cycle deposited by traditional ALD. However, we discovered the aluminum catalyst used in the ψ ALD process was accessible to the analytes and compromised the resulting chromatography. An amino silane monolayer on these plates allowed good separations to be performed. In a third advance, patterned CNTs were treated with ozone, and SiO_2 was deposited directly on them by ψ ALD. While this third process required fewer steps than the second one, the Al contamination remained. Our fourth approach was to deposit SiO_2 by true ALD. The resulting plates showed no Al contamination, no bonded phase was necessary, and a test mixture of dyes could be separated on them. However, true ALD is slow.

We believe that our fifth, and latest, approach satisfies all the requirements of a manufacturable process. Here, silicon nitride is conformally deposited on CNTs by LPCVD. Upon oxidation, the CNTs are removed and the silicon nitride is converted to SiO_2. The resulting plates show outstanding performance.

O-8

Detection of Polar and Nonpolar Compounds on TLC Plates Using Laser Desorption/Electrospray+Atmospheric Pressure Chemical Ionization/Mass Spectrometry (LD/ESI+APCI/MS)

Jentaie SHIEA
National Sun Yat-Sen University, Kaohsiung, Taiwan (ROC), jetea@fac.nsysu.edu.tw

Sy-chyi CHENG, National Sun Yat-Sen University
Siou-sian JHANG, National Sun Yat-Sen University
Ruei-hung HUNG, National Sun Yat-Sen University

TLC coupled to ambient mass spectrometry (AMS) is able to rapidly characterize sample solutions with minimal pretreatment. However, TLC-AMS is limited by its ionization source, which uses either electrospray ionization (ESI) or ambient pressure chemical ionization (APCI) for the generation of polar and nonpolar analytes, respectively. Current AMS techniques are unable to detect both polar and nonpolar analytes in one analysis. The development of AMS that can simultaneously detect both compounds over a comprehensive mass range is therefore more applicable. In this study, polar and nonpolar compounds on TLC plates were desorbed via laser desorption and post-ionized by an ambient ESI+APCI source. Analyte ions were then detected by an ion trap MS.

The LD/ESI+APCI system consisted of a pulsed laser and a dual ESI+APCI ionization source. Analytes separated on the TLC plates were desorbed by laser irradiation and post-ionized via reactions with charged species generated by the dual ESI+APCI ion source. The dual ionization source was operated in positive ESI-only, APCI-only, or ESI+APCI modes, which determined the type of analytes detected. Polar and nonpolar compounds (e.g., rhodamine 6G and n-dodecane) in a mixture were separated and characterized based on their polarities. Rhodamine 6G ions at *m/z* 443 were characterized in ESI-only mode; on the other hand, nitrogen and oxygen adducts of n-dodecane were detected in APCI-mode at *m/z* 184 and m/z 186, respectively. Both rhodamine 6G and n-dodecane ions were simultaneously detected in ESI+APCI mode. Complex samples such as crude and essential oils were also analyzed by TLC-LD/ESI+APCI/MS. The experimental results indicated that the proposed LD/ESI+APCI/MS technique is useful for rapidly and simultaneously characterizing polar and nonpolar chemical compounds on TLC plates.

O-9

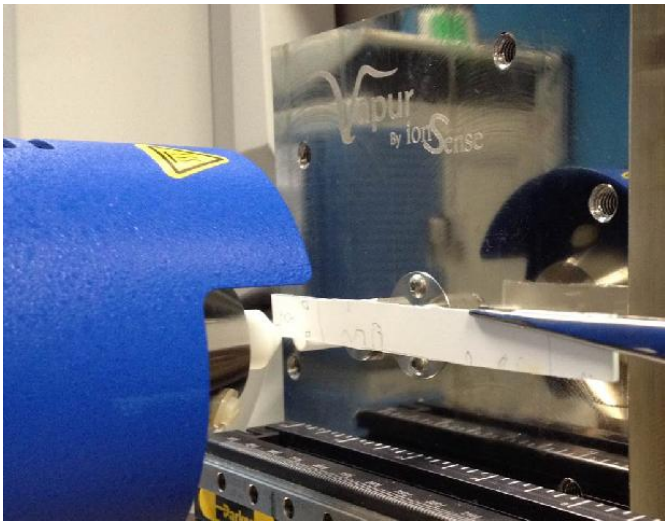
Planar Chromatography Meets Direct Ambient Mass Spectrometry: Current Trends

Elizabeth CRAWFORD
IonSense, Inc., Saugus, MA, United States, crawford@ionsense.com

Brian MUSSELMAN, IonSense, Inc.
Jason SHEPARD, University at Albany, SUNY, New York, United States

Regulation of dietary supplements and rapid screening for their quality and potential adulteration, in particular regards to regulated pharmaceuticals, including stimulants, has become an area of interest for regulatory bodies, as well as supplement manufacturers who need to protect their brand from counterfeiters. Ambient mass spectrometry offers a high level of sensitivity and specificity combined with high resolution MS with the advantage of rapid sample screening. An overview of coupling approaches and publications on the topic of combining HPTLC with ambient mass spectrometry will be given.

A Direct Analysis in Real Time (DART) ambient ionization source was coupled to a high resolution accurate mass (HRAM) Orbitrap MS. HPTLC glass backed plates were prepared from polar (methanol) extracts of several marketed herbal supplements. The HPTLC plates were cut using a smartCUT plate cutter to bisect the developed spots and were directly mounted onto the DART ionization source sampling stage via a customized holder for direct MS analysis.



Direct HPTLC MS analysis via DART-MS

O-10

TLC-MS: Elution- and Desorption-Based Approaches to Spatially Resolved Structure Elucidation

Hans GRIESINGER
Merck KGaA, Department of Bioanalytical Chemistry, Darmstadt, Germany,
hans.griesinger@merckgroup.com

Katerina MATHEIS, Merck KGaA
Beate FUCHS, University of Leipzig
Jürgen SCHILLER, University of Leipzig
Michael SCHULZ, Merck KGaA

The coupling of TLC separation with mass spectrometric detection and its analytical benefits are presented. The individual methods are well established and appreciated for their stability, reproducibility and sensitivity regarding separation and identification of analytes present in a given mixture. Nevertheless there are limitations to overcome. TLC, strong in detecting impurities and separating complex sample mixtures, is restricted by reference substances and staining methods/UV activity. On the other hand MS delivers excellent classification of substances by their mass, but leads to an overflow of information in analytical mixtures that may be hardly manageable.

We will introduce different setup supported combinations of these techniques, newest developments in sample preparation as well as corresponding applications. Using desorption-based as well as elution-based approaches and considering chromatographical parameters, the sample preparation and mass separation techniques lead to an outstanding versatility of TLC-MS.

The assignment of mass spectra to TLC bands produces a detailed and spatially resolved input of compound information for TLC technology. The focus will be the tremendous impact of silica gel layer thickness on the quality of mass spectra. Whereas electrospray ionization based MS shows a reduce in detection limits and increasing sensitivity and S/N ratios with "thinner" TLC layers, for matrix assisted laser desorption/ionization (MALDI)-MS, in particular, the intensity of the matrix background signals can be significantly reduced and a sensitivity gain is observed if silica gel layer thickness decreases. A clear correlation of matrix, sample peak intensities and TLC layer will be illustrated by using selected lipid classes such as phosphatidylcholines and phosphatidylethanolamines that are abundant in biological samples.

O-11

Analysis of Flavonoids in Plant Extracts using HPTLC-MALDI-TOFMS: Influence of MALDI Parameters

David DA SILVA
Institut de Chimie Organique et Analytique, Orléans, France, david.da-silva@univ-orleans.fr

Laetitia FOUGERE, Institut de Chimie Organique et Analytique
Emilie DESTANDAU, Institut de Chimie Organique et Analytique
Benoit MAUNIT, Institut de Chimie Organique et Analytique
Claire ELFAKIR, Institut de Chimie Organique et Analytique

The potential of MALDI-MS for the direct analysis of TLC plates has been demonstrated by several research groups. This technique has been used for analysis of variety of polymers, proteins, nucleotides and styrene oligomers. The main goal of this work is to develop a method involving a direct coupling of matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to HPTLC for characterization of flavonoids present in plant extracts. The key to successful HPTLC-MALDI-MS studies is the method used to prepare the HPTLC plates.

In this work, we shall be interested in the need of matrix, its nature (organic or nonorganic), the method used to deposit it and its amount. First, we have evaluated the influence of these HPTLC-MALDI preparation methods on flavonoid standard compounds (gallic acid, *p*-coumaric acid, rutin, naringenin, isorhamnetin, catechin, quercetin, cyanindin) before to analyze plant extracts. The HPTLC flavonoid separation has been realized according to the conditions routinely used in our group for the flavonoids screening from sea buckthorn, rose and licorice. HPTLC plates are analyzed thanks to TLC-adapted target for MALDI-TOF-MS. Positive and negative ionization modes were carried out on an Autoflex MS (Bruker Daltonik) in reflectron mode.

Although the MALDI ionization and plate preparation remain empirical, the automated analysis of the entire TLC plate, the non-destruction of spots, the high sensitivity and ability to ionize compounds avoiding significant fragmentation (e.g. detection of rutin $[M-H]^-$ ion) are major advantages of this TLC-MALDI coupling. In our approach, the use of innovative nonorganic (nanoparticles) matrix provides the best results: sensitivity, ionization of all standards and identification with a high degree of confidence. This optimized TLC-MALDI coupling proves its interest through fast characterization of flavonoids in complex plant extracts.

O-12

Planar SPE Coupled to Flow Injection TOF/MS Analysis - a Rapid Pesticide Screening Tool

Wolfgang SCHWACK
University of Hohenheim, Institute of Food Chemistry, Stuttgart, Germany,
wolfgang.schwack@uni-hohenheim.de

Claudia OELLIG, University of Hohenheim

The well-known problems in pesticide residue analysis concern the so-called "matrix effects" during liquid chromatography or gas chromatography coupled to mass spectrometry (LC-MS or GC-MS), which only can be avoided by an efficient clean-up of extracts. Therefore, a new clean-up concept was successfully developed and implemented in recent years, called high-throughput planar solid phase extraction (HTpSPE). For QuEChERS extracts of fruits and vegetables, pSPE was performed on TLC amino foils to separate the matrix from pesticides that were focused into a sharp zone. Applying the TLC-MS interface allowed the elution of the focused sample zones into autosampler vials [1]. Method performance was proven with selected pesticides spiked to fruits and vegetables [1] and to green and black tea [2] by LC-MS(/MS).

The aim of the present study was to combine our highly efficient pSPE clean-up with time-of-flight mass spectrometry (TOF/MS) to perform a rapid screening for pesticide residues. Therefore, a direct µL-flow injection analysis (µL-FIA)-TOF/MS strategy with-out chromatographic separation was developed, resulting in a single sample peak 1.5 minutes after injection. From the extracted mass spectrum under the peak, all pesticides present could be clearly identified at once, based on their exact masses.

The new approach was successfully checked with a pesticide mixture of different substance classes spiked to QuEChERS extracts of apples, grapes, cucumbers and tomatoes. All spiked pesticides were correctly identified by a target oriented database search and recovered close to 100% with relative standard deviations of 1.310% (n=5). As compared to the common dispersive SPE clean-up, matrix effects were almost completely eliminated by HTpSPE.

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O-13

Influence of TLC Plate Parameters on MS Results by Coupling TLC to MS

Michael SCHULZ
Merck KGaA - Merck Millipore, Darmstadt, Germany, michael.schulz@merckgroup.com

Susanne MINARIK, Merck KGaA - Merck Millipore
Michaela OBERLE, Merck KGaA - Merck Millipore
Katerina MATHEIS, Merck KGaA - Central Analytics
Hans GRIESINGER, Merck KGaA - Central Analytics

Thin layer chromatography (TLC) is a well-known effective separation technique. Very low cost and time per sample can be achieved by analyzing many samples in parallel on one plate. None, or very simple sample preparation can be applied due to the high matrix tolerance of TLC. By coupling TLC with MS (mass spectrometry) additional substance identification can be performed making TLC - MS a powerful analytical approach. Commercially available systems for direct measurements of TLC spots with MS are on the market making these techniques accessible for routine use.

Here we would like to present how the MS results can be influenced by different plate parameters, e.g. binder system, layer thickness or packaging. Results will be shown by coupling TLC to MS using an elution based interface and by coupling TLC to MALDI-MS (matrix assisted laser desorption ionisation - mass spectrometry) using an adapter device.

O-14

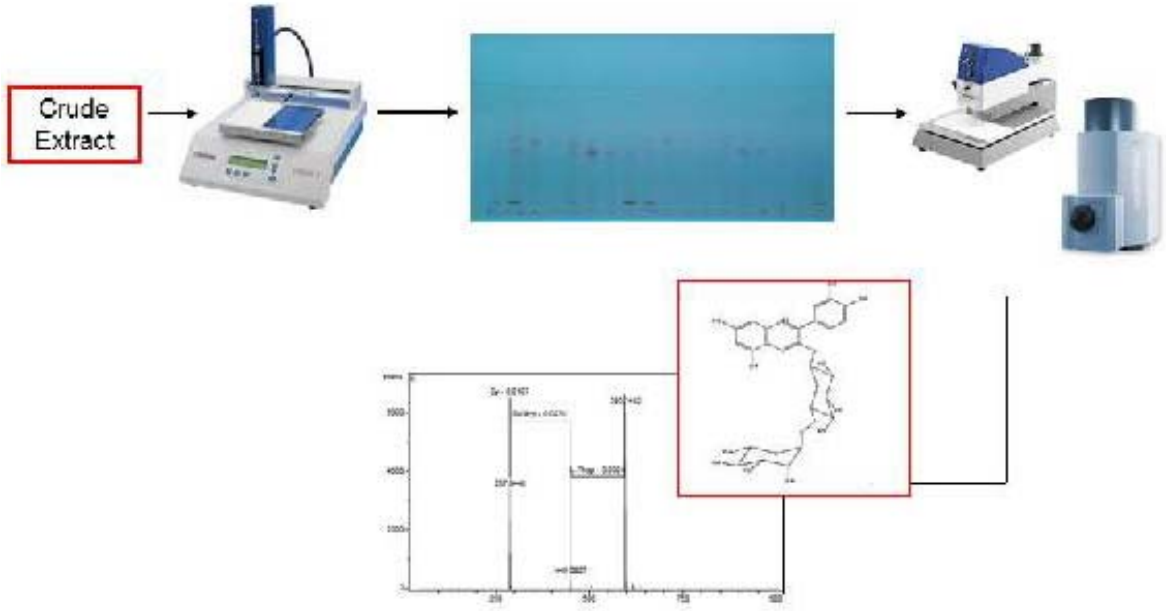
A Rapid Identification of Anthocyanins in Various Plants from Burkina Faso by HPTLC/MS

Rosella SPINA
UMR 7565 CNRS, Faculté des Sciences et techniques, BP 70239, Vandoeuvre-lès-Nancy 54506, France, rosella.spina@univ-lorraine.fr

Rosella SPINA, Université de Lorraine, France
Adama HÉMA, Université de Ouagadougou, Burkina Faso
Eloi PALÉ, Université de Ouagadougou, Burkina Faso
François DUPIRE, Service Commun de Spectrométrie de Masse, Université de Lorraine, France
Fabien LACHAUD, Service Commun de Spectrométrie de Masse, Université de Lorraine, France
Mouhoussine NACRO, Université de Ouagadougou, Burkina Faso
Dominique LAURAIN-MATTAR, Université de Lorraine, France

Anthocyanins are polyphenolic compounds responsible for cyanic colour ranging from salmon pink through red and violet to dark blue of most flowers, fruits, leaves and stems. They have significant antioxidant activity and several works showed an important role in the prevention of neuronal and cardiovascular illnesses, cancers and diabetes. Also anthocyanins have different industrial applications because they have been regarded as potential food colorants used to replace synthetic colorants.

This work focuses on the phytochemical analysis of some edible plants such as *Sarcocephalus latifolius* or medicinal plants such as *Tapinanthus ssp*. from Burkina Faso which are commonly used in traditional medicine. In order to identify isolated compounds in a mixture from plant extracts, HPTLC/MS analytical method was tested. This analytical system led rapidly to the identification of some anthocyanins.



Identification of anthocyanins by HPTLC/MS

O-15

Quantitative HPTLC Surface Analysis by DART-MS Scanning

Tim HÄBE
Justus Liebig University Giessen, Giessen, Germany,
Tim.T.Haebe@ernaehrung.uni-giessen.de

Gertrud E. MORLOCK, Justus Liebig University Giessen

Quantitative online surface analysis was demonstrated by coupling HPTLC with Direct Analysis in Real Time MS (DART-MS). DART is an upcoming desorption and ionization technique already hyphenated with GC and HPLC [1, 2]. Various adaptations showed the potential of this technique on a wide range of analytes and first attempts for coupling with HPTLC were made [3, 4]. The original DART SVPA-3DS interface was modified for exact positioning and movement of the TLC substrate as for the exact guidance of the DART gas stream.

Desorption, ionization and capturing of analytes out of planar substrates were improved. The proper alignment and consistent substrate movement were optimized to minimize mass signal variations. An angled substrate table reduced collisions of the deflected gas stream with the inner sampling tube wall and thus increased detectability. A proper gas stream guidance and low gas scattering after collision with the surface was supported by a short sampling tube with an angled ending towards the substrate. This optimized geometry with open access to the substrate surface and reduced ambient air gap increased efficiency and reproducibility of ion transportation to the MS orifice.

Scanning of butyl-4-hydroxybenzoat applied on the planar substrate showed high precisions. This confirmed the improved desorption efficacy and detectability. After chromatography of methyl-4-hydroxybenzoat and butyl-4-hydroxybenzoat, mean determination coefficients showed suitable quantitative capabilities. Spatial resolution was determined to be smaller then 1 mm and the scan lane width to be 3 mm.

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O-16

HPTLC-Bioassay-MS, a Rapid Tool to Search and Analyse Bioactive Plant Products

Ágnes M. MÓRICZ
Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, moricz.agnes@agrar.mta.hu

Peter G. OTT, Hungarian Academy of Sciences, Budapest
Gertrud E. MORLOCK, Justus Liebig University Giessen

There is an increasing demand for new bioactive substances because of the emergence and spread of bacterial resistance to commonly used antibiotics. This issue became a worldwide problem in human and animal health as well as in agriculture. The plant kingdom is still an underestimated pool of secondary metabolites possessing enormous therapeutic potential, so it can be the source of new, effective and easy-to-obtain agents. In the last decade the strategy to discover active substances has generally been changed leaving the time-consuming and expensive methods behind, in which bioactivity was studied only after isolation of the components.

The basics of the new strategy are that only the effective components are isolated. To search compounds in plant extracts with a desired activity, HPTLC hyphenated with an appropriate bioassay is a very useful tool that enables the in vitro biological study of the components previously separated in the adsorbent layer. Further combination of this system with spectroscopic and spectrometric techniques makes the characterization of the bioactive substances possible. In this presentation, we demonstrate through examples a rapid and relatively simple procedure to detect antibacterial and antioxidant plant products by the use of HPTLC combined with direct bioautography (bioassay) and the DPPH* reagent. Additionally, UV densitometry, UV spectra, DART SVPA-MS(/MS), ESI-MS via TLC-MS Interface, TLC-MS Interface off line TOF MS and LC-DAD-MS were applied to characterize the active compounds.

Á. M. Móricz is grateful to I. Klingelhöfer, S. Krüger, M. Jamshidi-Aidji and T. Häbe for support at JLU Giessen, Germany, where she spent 2 months funded by DAAD. This work was also supported by OTKA (PD83487) and Bolyai grants.

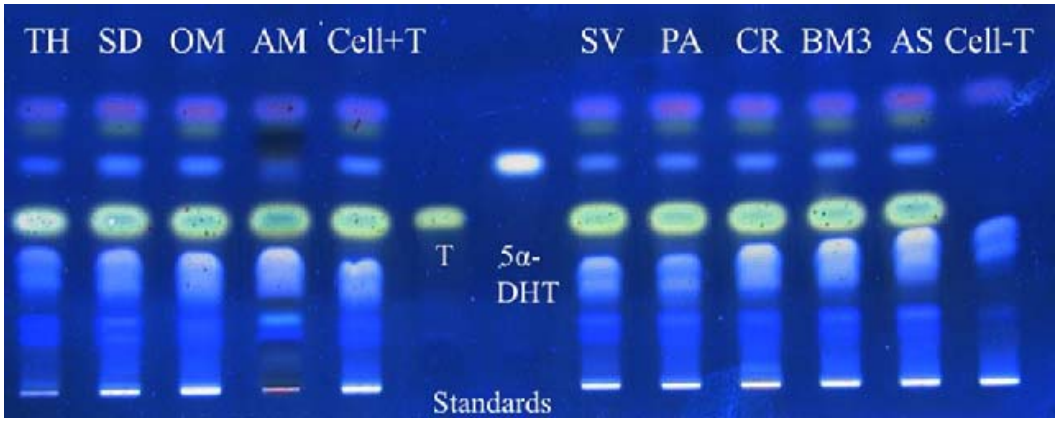
O-17

A Combination of TLC and a Novel Androgenic Alopecia Cell-Based Assay for Screening 5α-Reductase Inhibitors in Plants

Wanchai DE-EKNAMKUL
Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, dwanchai@chula.ac.th

Ruchy JAIN, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.
Orawan MONTHAKANTIRAT, Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.
Parkpoom TENGAMNUAY, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

5α-Reductase (5α-R) is an enzyme catalyzing the conversion of testosterone (T) to 5α-dihydrotestosterone (5α-DHT) that causes androgenic alopecia, a major type of scalp hair loss. The aim of this study is to develop a combined non-radioactive cell-based assay and TLC detection for direct screening of plant 5α-R inhibitors. The assay system was conducted on a 96-well plate, containing each well 10,000 human hair dermal papilla cells, 0.1 mM T and 5.0 µg/ml test compound in a total volume of 200 µl. After 48 hours, T and 5α-DHT were extracted by ethyl acetate, separated by TLC and visualized by *o*-phosphoric acid staining. The plate was scanned under 366 nm to obtain the amount of 5α-DHT produced. This optimized system was validated and tested with a specific 5α-R type 1 inhibitor, dutasteride, and further screened for potential plant extracts. The validated TLC system showed positive results with the plant extract of *Avicennia marina*.



TLC plate visualised under 366 nm showing 5α-R1 inhibitory activity of some methanolic plant extracts. *Micromelum minutum* - MM, *Tarenna hoaensis* - TH, *Scoparia dulcis* - SD, *Olendra musifolia* - OM, *Avicennia marina* - AM, *Salacia verrucosa* - SV, Pterygo – PA

O-18

Directly Bioprospecting of Amicoumacin-Producing Strains and Efficient Discovery of New Amicoumacins by HPTLC Profile

Cheng-Hang SUN
Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, chenghangsun@hotmail.com

Shao-wei LIU, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College

Pharmaceutical microorganisms have been major sources of antibacterial agents and remain very promising. Frequent rediscoveries of known compounds hampers progress of new antibiotic discoveries unless utilization of new strategies for rapid structural dereplication in the earlier discovering period. In our study, an automatic analytic HPTLC system was used to directly bioprospect microorganisms of producing amicoumacin group of compounds, which showed various important bioactivities, especially excellent inhibitory activity against MRSA. When HPTLC profiling is combined with MS or UPLC-UV-MS, known amicoumacin compounds can be rapidly dereplicated, meanwhile, new amicoumacin compounds and its producing strains can be found. Integration of miniaturized liquid culture of microorganisms, high-throughput activity screening and multiple samples parallel evaporating with the automatic analytic system will improve the efficiency of discovery.

Six isolates of amicoumacin-producing strains were found and two of them could produce new amicoumacin compounds. Four new amicoumacin antibiotics, named Hetiamacin A-D were separated and their chemical structures were identified. Antibacterial test showed that they exhibited remarkable growth inhibitory bioactivities against oxacillin-resistant *Staphylococcus aureus* and oxacillin-resistant *Staphylococcus epidermidis*.

The core technology based on HPTLC greatly accelerated discovery efficiency of amicoumacin-producing strains and new amicoumacins group antibiotics. When the system is combined with microbial resources in China, more new amicoumacin antibiotics will be found and lead to possible discovery of new leading compounds against MRSA *etc*.

O-19

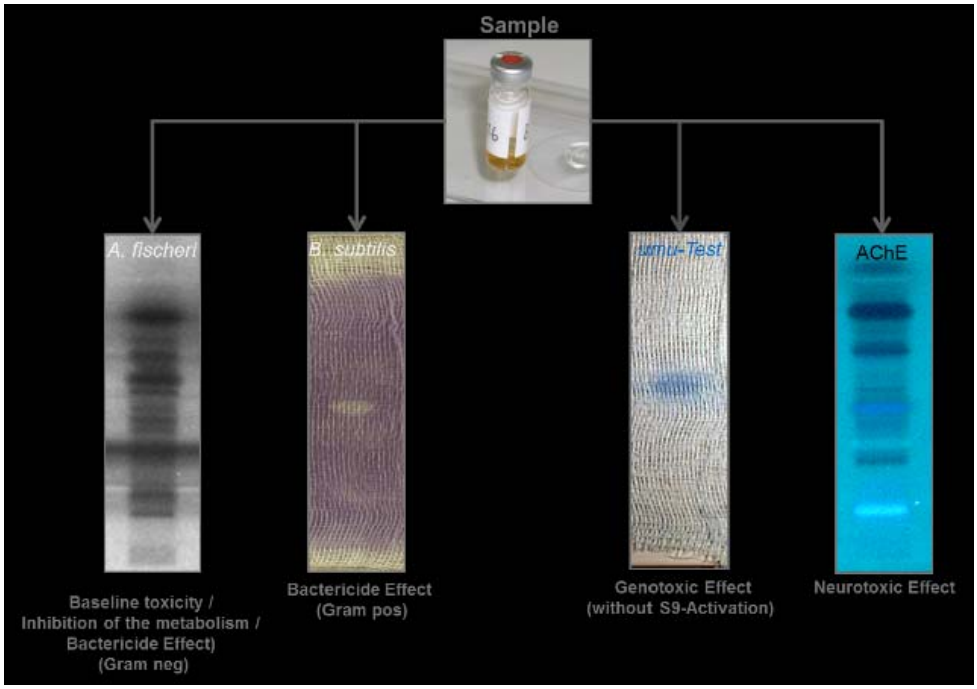
Enhanced Tests and Evaluation Procedures for the Effect-Directed Analysis using HPTLC Applied for Aqueous Environmental Samples

Stefan C. WEISS
Zweckverband Landeswasserversorgung, Langenau, Germany, weiss.s@lw-online.de

Nicole JUNG, Zweckverband Landeswasserversorgung
Wolfgang SCHULZ, Zweckverband Landeswasserversorgung
Walter H. WEBER, Zweckverband Landeswasserversorgung

The infiltration of anthropogenic organic trace substances is a potential risk for the water resources of drinking water production. For this reason, it is necessary for a drinking water supplier to monitor the water resources regularly and comprehensively. Therefore, Landeswasserversorgung (LW) water supply uses effect-directed analysis (EDA) after HPTLC separation in addition to conventional target analysis with physico-chemical detection. EDA enables a first impression of toxicological effects of an individual substance or a group of substances.

Up to now, only a few toxicological tests are available for routine analysis with HPTLC. Therefore, further tests and evaluation procedures for EDA with HPTLC have been developed or optimized and include: baseline toxicity (*Aliivibrio fischeri*), bactericide effects (*Aliivibrio fischeri, Bacillus subtilis*), neurotoxicity (acetylcholinesterase, AChE) and genotoxicity (*S. typhimurium* TA1535/psk 1002, umu test). In particular, their suitability for practical application will be shown for landfill leachates. Zones from the HPTLC plate which have shown effects were extracted and transferred to an QTOF/MS for further investigations.



Several HPTLC-EDA methods to detect different types of toxicity in the environment

O-20

HPTLC-Bioluminescence Screening for Residues of Antibiotics in Food of Animal Origin

Yisheng CHEN
Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany, Stuttgart, Germany, Yisheng.Chen@uni-hohenheim.de

Wolfgang SCHWACK, University of Hohenheim

Residue analysis of antibiotics is performed by tedious agar plate tests for screening or by LC-MS analyses, meeting the dilemma of a huge number of samples as compared to analytical capacity. Therefore, an HPTLC-bioluminescence assay as the ideal platform for a multi-sample rapid screening was developed. For the first time, the bio-compatibility of different plate layers were evaluated and optimized for the application of the photobacterium *Aliivibrio fischeri* (DSM No. 7151).

Exclusively on amino plates, adequate sensitivity meeting the EU MRLs was achieved for 16 first-line veterinary antibiotics belonging to tetracyclines, fluoroquinolones, amphenicols, gentamicins, and macrolides. Following QuEChERS extraction and sample application, plates were developed with MeOH - MeCN 2:3 prior to bioassay, allowing the clear separation of the target antibiotics from natural inhibitors arising from co-extractive matrices. The method was validated with milk and kidney samples, showing proof that the antibiotics under study unequivocally could be detected at their EU MRL levels.

Workflow scheme of HPTLC-bioluminescence screening

O-21

The Novel TLC-Direct Bioautography Tests for Analysis of Antimicrobials

Irena M. CHOMA
University of Maria Curie-Skłodowska, Lublin, Poland, irena.choma@poczta.umcs.lublin.pl

Edyta M. GRZELAK, University of Maria Curie-Skłodowska, Lublin, Poland
Wioleta JESIONEK, University of Maria Curie-Skłodowska, Lublin, Poland
Barbara MAJER-DZIEDZIC, University of Life Sciences, Lublin, Poland

TLC-direct bioautography (TLC-DB) can be applied to the analysis of various antimicrobial agents in complex matrices e.g. body fluids, pharmaceutical preparations, environmental and food samples. The principle or the method is that both separation and microbiological detection are performed directly on the same TLC plate. In details: a developed (HP)TLC plate is dipped in a suspension of microorganisms growing in a proper broth, incubated and visualized with tetrazolium salts. The bacteria cover the whole surface of the plate and during incubation grow directly on it excluding the spots where the substances possessing antibacterial properties are located. They form pale growth inhibition zones against the purple background of the (HP)TLC plate [1-4].

Because of the lack of commercially available tests, two bioautographic assays to be used after TLC separation were developed, optimized and fully validated. One of them is based on Gram negative bacteria, *Escherichia coli* [2] another one on Gram positive bacteria, *Bacillus subtilis* [3]. These tests were used successfully to determine antibiotics at their maximum residue level in milk. Other applications cover screening antibacterial properties of herbal drugs, plant extracts and their constituents as well as estimating quality of sample preparation procedures [4].

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O-22

Screening Untreated Drinking Water on Estrogenic Activity using the Planar-YES, a TLC-EDA Tool

Andreas SCHOENBORN
Zurich University of Applied Sciences, Waedenswil, Switzerland, andreas.schoenborn@zhaw.ch

Andrea A. GRIMMER, Zurich University of Applied Sciences

Drinking water suppliers are challenged by increasing amounts of microcontaminants in raw waters used for drinking water production. It is foreseeable that "emerging" contaminants need to be monitored in the future. A concept coupling sample preparation (using HPTLC) with effect-directed analysis (TLC-EDA) is an option to do this in a cost-efficient and informative way.

Four raw waters used for drinking water production by the drinking water supplier of Zurich (WVZ), Switzerland, were extracted and measured on estrogenic activity (EA), using the planar-YES as an example for a TLC-EDA method. Samples originated from river bank filtration, from the rivers Limmat and Rhine, and from the outlet of a wastewater treatment plant. Samples were divided, and half were spiked with estrogenic substances (positive controls). Evian water served as negative control. The extracts were produced by WVZ and processed according to Schoenborn & Grimmer (2013).

Calibration used 17-β-estradiol (E2) as standard, at masses of 1, 5, 10 and 25 pg per band. Calibration curves based on height and area units of the bioautograms had an R^2 between 0.92 and 0.98 (n= 28-32 per data point). Covariance analysis (ANCOVA) of the calibration data showed a consistent line slope and a variable y-axis intersection. One standard E2-spot per HPTLC plate may be sufficient for quantification in the future.

Weak EA was detected in Limmat water, in the range of 0.2-0.3 ng/l E2-equivalents. It can be attributed to 2 different estrogenic substances, one of them probably E2. Results for Rhine- and river bank filtration water were inconsistent. Treated wastewater contained 5 specifiable substances with EA (none of them E2), but couldn't be quantified, due to antagonistic effects in the assay. Evian water did not show EA, while in all spiked samples, EA was consistently detected. Detection of EA was repeatable with the planar-YES, but further development is needed for exact quantification.

O-23

The Relevancy of Effect Directed Analysis Implementing the European Water Framework Directive 2000/60/EC in the Field of Water Policy

Christel WEINS
WBA Büro für Wirkungsbezogene Analytik in Umwelt, Lebensmitteln und Medizin, Saarbrücken, Germany, c.weins@weins.de

In this talk the principle of Effect Directed Analyis (EDA) will be explainend and the need of EDA in HPTLC concerning the European Water Framework Directive 2000/60/EC in the field of water policy will be demonstrated.

The European Directive 2000/60/EC established a framework in the field of water policy to improve ecological quality in Community surface waters. This Directive recognised the need for action to avoid long-term deterioration of freshwater quality aiming at sustainable management and protection of freshwater resources. Especially the diversity had been taken into account in the planning and execution of measures to ensure protection and sustainable use of water in the framework of the river basin, regarding the vulnerability of aquatic ecosystems.

Public authorities and private companies spend billions of Euros in sewage treatment plants, renaturation programs of water bodies to improve the water quality and struture of surface water bodies aiming at protection the natural biodiversity. Nevertheless most of the measures misses its major the objectives. Elimination of priority hazardous substances, as described in this directive, seems not efficient enough to restore the natural biodiversity. In order to demonstrate the presence of one or more contaminants, which are responsible for the toxic effect in the ecosystem, it is necessary to detect and to quantify the smallest traces of these substances (e.g. in the ng to pg range). Substances and preparations, or the breakdown products of such, which have been proved to possess biologically hazardous effectiv properties or properties which may affect reproduction or other endocrine-related functions in the aquatic environment have to be identified to eliminate these contaminants successfully.

"Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy"

O-24

Orthogonal Pressurized Planar Electrochromatography

Tadeusz DZIDO
Medical University of Lublin, Lublin, Poland, tadeusz.dzido@umlub.edu.pl

Eryk ŁOPACIUK, Medical University of Lublin
RafałGAJOS, Medical University of Lublin
Beata POLAK, Medical University of Lublin
Radosław GWARDA, Medical University of Lublin

Planar chromatography is a very useful and convenient technique for two dimensional (2D) separations. This mode of separation with TLC is usually performed in two consecutive and orthogonal processes, i.e. chromatogram development in the first direction is followed by that in the second one. High peak capacity of such separation is based on different separation selectivity in each of two separation directions involved. An orthogonal combination of both separation directions into single simultaneous process stands for the real challenge. It is rather not possible to perform such separation with conventional TLC only. However, TLC in combination with planar electrochromatography/electrophoresis can be performed under conditions of ambient pressure. Such separations were reported previously [1, 2].

In this presentation we demonstrate the next development stage of 2D planar separation in which overpressured layer chromatography (OPLC) and pressurized planar electrochromatography (PPEC) are combined orthogonally into single simultaneous process named as orthogonal pressurized planar electrochromatography (OPPEC) [3]. We will show principle of action and important constructional details of the device for this new technique. Based on the results obtained for analytical and micropreparative separations of test dyes, enantiomers and peptides we will discuss advantages and challenges of OPPEC.

It seems that OPPEC, at its contemporary development stage, is especially suitable for continuous micropreparative separations.

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O-25

TLC in Investigation of Complex Properties in Solutes

Irena MALINOWSKA
Faculty of Chemistry, Department of Planar Chromatography, Maria Curie-Skłodowska Univ., Lublin, Poland, irena.malinowska@poczta.umcs.lublin.pl

Agnieszka WRONKA, Maria Curie-Skłodowska University
Wieslawa FERENC, Maria Curie-Skłodowska University

At present times, coordination compounds play a great role in many areas of live and science. In many laboratories a lot of different kinds of the complex compounds are synthesized. Some properties of the compounds, may be determined using a different methods as spectroscopy methods, magnetic, X-ray analysis. Those methods give some information about structure, bonds, magnetic properties - but in solid state of the compounds. Large group of the complexes are used in solutes - for example in medicine. There are practically no information about affinity of the compounds to surface, form of the compounds in solutes etc.

TLC can be a good method for that type investigations. Analyzing retention of the compounds in different chromatographic systems (with different stationary and mobile phases) the affinity of the compounds to the different surface can be determined. Analysing retention of the compounds and comparing it with the ligand retention give some information about interactions of the complexes with surface. Comparison of the retention of the complexes with the same ligand and different central ions can give some information about influence of the central ion of the complex to interaction with surface. Some information about form of the complexes in give solution can be obtained by use of electrocgromatography systems and magnetochromatography - about magnetic properties in solute. On the basis of the chromatography analysis in RP systems lipophilicity parameter can be determined - the parameter of the great importance especially in the case of the compounds with biological activity.

O-26

Retention Changes of 1,2,4-Triazole Derivatives in RP-TLC and Micellar TLC Systems under the Influence of External Static Magnetic Field

Marek STUDZINSKI
Department of Planar Chromatography, Faculty of Chemistry, M. Curie-Skłodowska University, Lublin, Poland, Lublin, Poland, marek.studzinski@poczta.umcs.lublin.pl

Irena MALINOWSKA, Curie-Skłodowska University
Henryk MALINOWSKI, Vexler and Baldin Laboratory of High Energy Physics, JINR, Dubna, Russia

Targeting biologically active substances at specific parts of living organism may be the way to improve applications of chemical substances in many areas of human activities. One of the most important parameters describing possible impact of given compound on living cell is its lipophilicity. According to Lipinski's rule of five, logarithm of partition coefficient (log P) in octanol/water system must be between 2 and 5 for given substance to assume that it is able to permeate cell membrane.

Magnetic field presence changes biological activity of some substances. The changes may be caused by changes of lipophilicity of the substances in magnetic field Investigated substances belong to the group of compounds widely used as pesticides and are also considered as potential drug candidates. Because of that lipophilicity of some 1,2,4-triazoles in external magnetic field was determined using TLC magnetochromatography and compared with those obtained without external magnetic field presence.

Chromatographic investigation, and data analysis basing on Soczewiński-Wachtmeister equation for RP-LC systems and Foley equation for MLC systems is a convenient way of log RM_0 experimental determination. According to above, retention changes of chosen 1,2,4-triazole derivatives in given chromatographic systems under the influence of external magnetic field may be considered as a result of their lipophilicity changes caused by presence of the field. Thus, it may be assumed that applying relatively weak external static magnetic field may be considered as method to control 1,2,4-triazoles cell membrane permeation and in consequence their concentration in intracellular fluid in different parts of living organism independently. Obtained results proved that lipophilicty changes of investigated compounds in magneto TLC appeared regardless of chosen method of its determination (RP-LC or MLC).

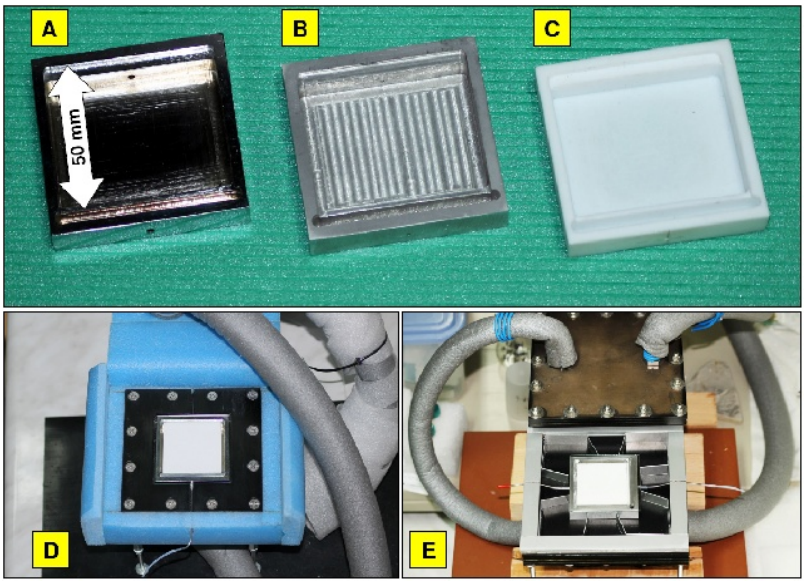
O-27

Practical Approach for Temperature-Controlled TLC

Pawel K. ZARZYCKI
Koszalin University of Technology, Koszalin, Poland, pkzarz@wp.pl

Planar chromatography is still rapidly evolving due to plates and equipment miniaturization, the discovery of new materials for stationary phases and the availability of sensitive and selective detectors. Non-forced flow separation that is performed in classical vertical or horizontal chambers, can be affected by many factors especially that mobile phase vapour is directly contacting with the whole stationary phase surface during chromatographic run. Under such conditions temperature plays critical role for stability of analytes retention, selectivity and can significantly influence robustness of separation protocol.

In this communication the temperature effects on analytes retention and typical problems associated with temperature controlling of TLC run will be discussed from practical point of view. This will be demonstrated, predominantly focusing on separation and fingerprinting of bioactive substances, including steroids, fullerenes or chlorophyll dyes in complex samples (e.g. fish bile, spirulina extracts, soot dust materials, untreated and treated sewage water), performed within home-made removable micro-TLC units designed for development of 5 x 5cm plates.



Perspective view of micro-TLC chambers, manufactured from chromium coated brass (A), aluminium (B) and PTFE (C), working within different types of temperature controlled ovens (fast heat exchanger D, developing and visualization module E)

O-28

Streamlined Analysis of Sugars in Chicory Root Juice: Comparison of Two Chromatographic Methods

Antoine BESSIN
Purifunction, Lille, France, abessin@purifunction.com

Gertrud MORLOCK, Justus Liebig University Giessen

Crops varietal selection requires quantifying quality markers in a high number of samples in a short time, following harvest. Therefore, conventional liquid chromatography methods could be limited in terms of analyses throughput, especially when interferences with the matrix occur in the detection of markers or when other analytes require long gradients to be eluted. Chicory root (*Cichorium intybus*) represent the main source of inulin in Europe. This group of fructopolysaccharides is mostly used for its prebiotic effect and its low nutritional value as a texturing agent and food additive.

Quality markers of chicory include mono- and disaccharides contents of their roots. Indeed, their presence lowers the average degree of polymerization of saccharides and lowers the sticky point of the sugar isolate at drying steps. Some of the many advantages of HPTLC are the high throughput of analyses, the low cost of consumables and the fact that it does not require complex sample preparation. A simple and fast HPTLC method was successfully developed for the quantification of glucose, fructose and sucrose in chicory juice. Sample preparation consisted in simple dilutions and centrifugations in well plates. Separation of these compounds was achieved with a mixture of water, acetonitrile and aminoethyldiphenylborinate in a horizontal chamber, allowing application of eleven samples in duplicates and six calibration points on each side of the HPTLC plate silica gel 60. p-Aminobenzoic acid was used for derivatization to selectively enhance glucose detection, which is present in a 10 times lower concentration than fructose and sucrose. Quantification was performed by fluorescence measurement at 366/> 400 nm.

Comparison with liquid column chromatographic methods was performed regarding consumables, labor costs and analysis time. HPTLC proved to be 2.5 times cheaper and 3.5 times faster than HPLC, and allowed to quantify 5000 samples in duplicates in 7 weeks, that is to say 1.5 minute per sample.

O-29

Planar Chromatography and Mass Spectrometry in Analysis of Phytonutrients in the Extracts of Edible Plants

Irena VOVK
National Institute of Chemistry, Laboratory for Food Chemistry, and
EN-FIST Centre of Excellence, Ljubljana, Slovenia, irena.vovk@ki.si

V. GLAVNIK, National Institute of Chemistry and EN-FIST Centre of Excellence
A. ALBREHT, National Institute of Chemistry and EN-FIST Centre of Excellence
K. NAUMOSKA, National Institute of Chemistry
B. SIMONOVSKA, National Institute of Chemistry
S. SMRKE, National Institute of Chemistry

Edible plants (e.g. vegetables, fruits, grains, legumes, nuts, spices, different teas) are rich sources of many phytonutrients, some known and some still unknown. The major groups of phytonutrients represent carotenoids, some triterpenoids, phytosterols, polyphenols (e.g. flavonoids, phenolic acids), isothiocyanates etc. Phytonutrients are obviously a big group of compounds with different bioactivities. Many of them are antioxidants, some are capable of enhancing immune response or cell-to-cell communication, some can lower blood pressure and/or cholesterol level, some are even causing death of cancer cells etc. Through many epidemiological, human nutrition and cell culture studies came cognition that fruit and vegetables protect human health. However, much research is still needed to connect the mode of action with specific compounds in foods. There are also other important aspects of investigation of phytonutrients (e.g. their interaction with medicines). Firstly, methods for screening, qualitative and quantitative analysis in different matrices need to be developed to study the composition of different foods especially regarding the minor constituents. Chromatographic techniques are an indispensable tool in the research of phytonutrients, especially their combined use and hyphenation to mass spectrometry and UV/vis spectrophotometry.

The presentation will show the potential of planar chromatography and mass spectrometry in screening, qualitative and quantitative analysis of triterpenoids, carotenoids and proanthocyanidins and flavanols in foods derived from plants. Our new analytical methods (HPTLC, HPTLC-MS, HPTLCxMS, HPTLC-LC-MS) with improved stability of the analytes, selectivity of detection, established database for MS and MS^2 spectra lead to improvements in chemical analysis of these compounds and to discovery of some interesting phytonutrients not known before to be present in some common vegetables.

O-30

HPTLC Qantification of Phenolics in Wine using High Resolution Plate Imaging

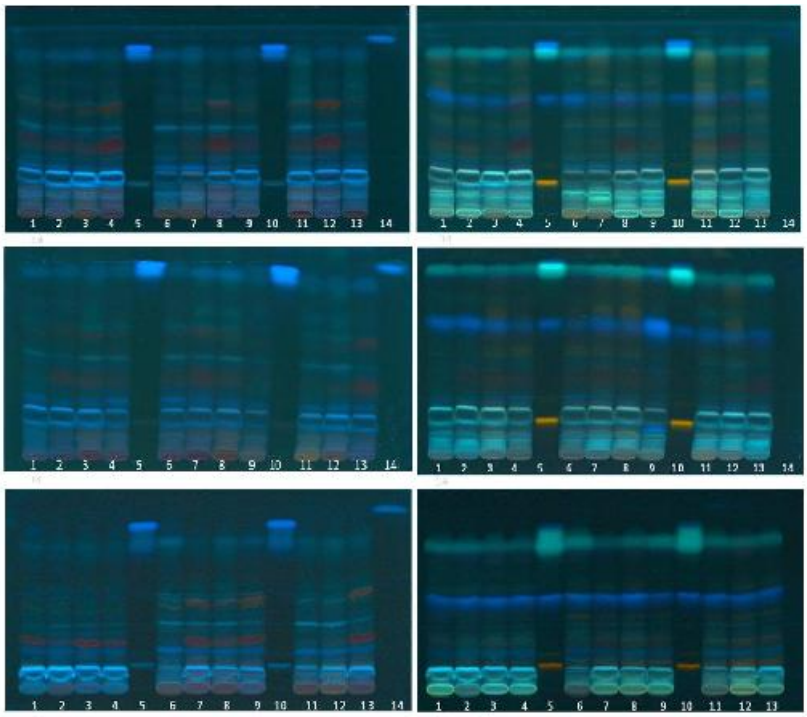
Snezana AGATONOVIC-KUSTRIN
La Trobe University, School of Pharmacy and Applied Science, Bendigo, Australia,
s.kustrin@latrobe.edu.au

Chandima HETTIARACHCHI, La Trobe University
David MORTON, La Trobe University
Slavica RAZIC, University of Belgrade

The protective role of red wine has been attributed to the presence of a wide range of phenolic antioxidants. However, there is limited information on the phenolic composition of wines. The aim of this study was to develop and optimise a simple HPTLC method to quantify levels of gallic acid, caffeic acid, resveratrol and rutin in wine samples and to correlation their amounts with total antioxidant capacity (TAC) and total polyphenolic content (TPC).

A total of 45 wine samples were collected from different regions of Australia and oversees. A HPTLC method for the analysis of caffeic acid, gallic acid, resveratrol and rutin from wine samples was developed and validated.

A HPTLC chromatography may not have the same efficiency as HPLC separation but is simple to run and multiple wine samples could be analysed and compared on the same plate. Furthermore, TLC is the only chromatographic method offering the choice of presenting the results as an image. The different colours of the individual bands for the different phenolics act as an extra dimension. Thus, in combination with visual evaluation HPTLC could provide extremely rapid screening of wine samples.



HPTLC chromatograms of phenolics in wine

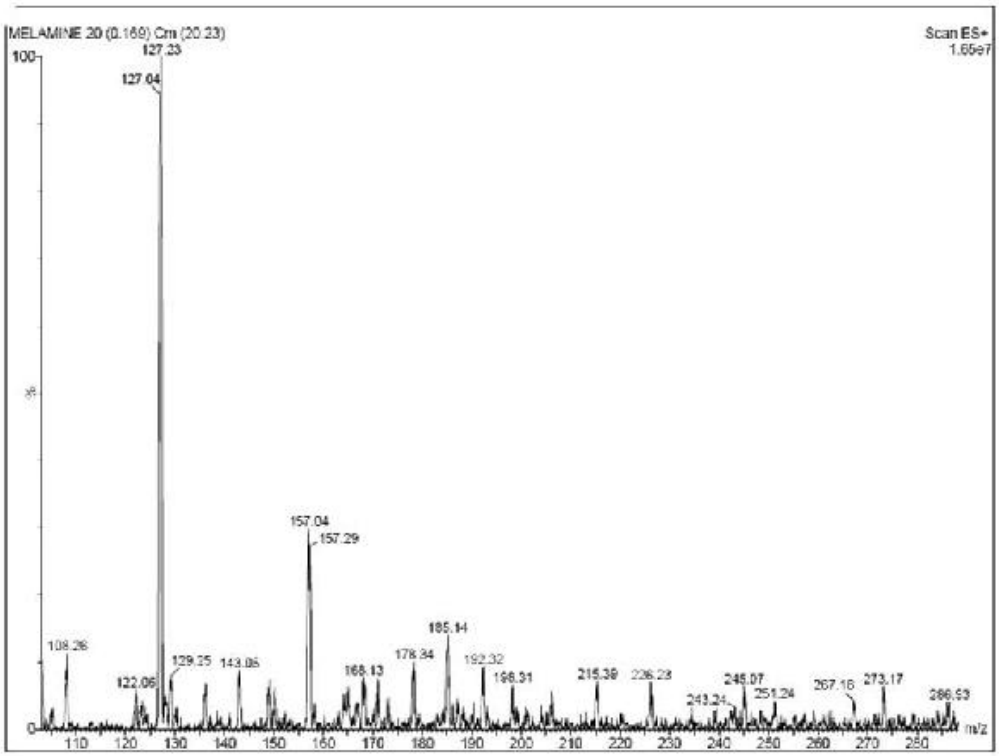
O-31

Development and Validation of HPTLC-MS Determination of Melamine in Milk

Manmohan SRIVASTAVA
Dayalbagh Educational Institute, Agra, India, dei.smohanm@gmail.com

Roopa RANI, Dayalbagh Educational Institute, Agra
Sharad MEDHE, Dayalbagh Educational Institute, Agra

Melamine containing large amount of nitrogen is deliberately added to milk to increase the protein content which is absorbed in the gastrointestinal tract of animal or human beings and later precipitates in the kidney to form crystals. HPTLC-MS based quantification of melamine was carried out on Silica gel 60 F254s HPTLC plates using optimized solvent system of iso-propanol: dichloro methane: water; 5:2.5:3; v/v/v in a twin trough chamber saturated (5 min) at pH 6.8, having absorbance measurements at 200nm. Validation has been carried out by testing its linearity (1-10 µg), accuracy (95.90-98.712%), precision (1.578- 1.670%), and limits of detection (2.688 ng) and quantification (8.146 ng). Confirmation of the presence of melamine content was obtained using Gas Chromatography. Out of the twenty four markets purchased samples of liquid and powder milk, five of (liquid milk) and three (powder milk) were found contaminated with melamine in the range 65-354 µg mL^{-1} and 98-314 µg mL^{-1}, respectively. HPTLC in combination with exactness of MS has been found suitable and rapid approach for routine analysis of melamine as adulterant to market purchased milk samples.



HPTLC-MS spectrum

O-32

Fast Automated Food Safety Screening - FAFOSS

Malcolm R. CLENCH
Sheffield Hallam University, UK, m.r.clench@shu.ac.uk

David PARKINSON, SI Biologics
Jiliian NEWTON, Sheffield Hallam University
Jamie YOUNG, Sheffield Hallam University
Martin HEMMINGWAY, Alcontrol Laboratories
K. Clive THOMPSON, Alcontrol Laboratories
Andrew BAKER, Advion

Current methods for measuring contaminants in foodstuffs tend to be slow and costly and often involve an initial screen by immunoassay followed up, where necessary, by analysis using conventional GC-MS and/or LC-MS. The initial testing is expensive and the follow up testing is often characterised by high cost and delay. In this project, methods for fast and robust parallel measurements of food samples for important contaminants have been developed . This has been achieved by using high throughput laboratory robots to carry out unattended sample preparation for high performance thin layer chromatography.

Example data from the analysis of patulin in apple juice and rhodamine B in childrens's sweets are presented. The use of the TLC-MS Interface coupled to an Expression CMS mass spectrometer has been investigated for the quantitative analysis of samples that were indicated as "positive" in the initial HPTLC screen. The use of TLC-MS removes the need for the additional sampling/sample processing and analysis by LC-MS as is currently required. The proposed technique should prove invaluable in emergency situations when large numbers of samples need to be screened to determine which samples are contaminated and which product batches should be withdrawn. The proposed new methodologies combine ideas in simple sample preparation, parallel sample processing and quantitative analysis directly from TLC plates.

O-33

A Toolbox for HPTLC Data Processing

Grégory GENTA-JOUVE

Laboratoire de Pharmacognosie, UMR CNRS 8638 COMETE, Faculté des Sciences Pharmaceutiques et Biologique, Université Paris Descartes, Paris Cedex 06, France, gregory.genta-jouve@parisdescartes.fr

In the last few years, an increasing scientific interest for the use of HPTLC as a tool for chemotaxonomy or plant extract certification has emerged, but despite some undeniable assets, HPTLC remains relatively unused in metabolomics.

Here we present a toolbox working in the Matlab environment called ChromCorr v 0.1. In this toolbox are implemented all common features used in metabolomics such as, data selection, peak detection, data normalisation, data alignment and a new feature developed exclusively to correct the diffusion drift correction that can be observed during the HPTLC development step. Within ChromCorr v 0.1, users can import HPTLC pictures and process their data and compare samples using uni- and multivariate statistical analyses.

O-34

High Performance Thin Layer Chromatography (HPTLC) Method for the Identification of Seven Different Resin Species

Débora ARRUDA FROMMENWILER
CAMAG, Basel, Switzerland, debora.frommenwiler@camag.com

Eike REICH, CAMAG
Eliezer CENIVIVA, CAMAG
Grigorios THEOCHARIS, CAMAG

For decades, planar chromatography has been closely connected with plant analysis. Traditionally classical TLC is described in all monographs for herbal drugs of the European and other pharmacopoeias as a method of identification. Today HPTLC has become one of the primary tools for identification of medicinal plants. Many publications by standard setting organizations (e.g. the USP Dietary Supplement Compendium, the TLC Atlas of Crude Chinese Medicines, the American Herbal Pharmacopoeia, Quality Standards of Indian Medicinal Plants, etc.) utilize HPTLC fingerprints to describe the quality of plant drugs.

The use of plant resins was already documented in ancient Greece by Theophrastus and in ancient Rome by Pliny the Elder. Resins known as frankincense and myrrh (were highly prized in ancient Egypt and used in religious rites as incense). Resins are hydrocarbon secretions of many plants, particularly coniferous trees. They are values as an important source of starting materials for organic synthesis, and as constituents of incense and perfume.

In the European Pharmacopoeia eight different TLC and HPTLC monographs are described to identify Myrrh (*Commiphora myrrha*), Myrrh tincture, Siam benzoin (*Styrax tonkinensis*), Siam-benzoe tincture, Sumatra benzoin (*Styrax benzoin*), Sumatra-benzoe tincture, Dammar gum (species from the genera Shorea, Balanocarpus or Hopea), Tolu balm (*Balsamum tolutanum*) and Indian Frankincense (*Boswellia* sp). No method is included for identification of Guggul (*Commiphora mukul*).

This work proposes a single harmonized HPTLC method with simple sample preparation for the discrimination of all seven types of resins and distinguishes individual species and their adulterants: *Commiphora myrrha, Styrax tonkinensis, Styrax benzoin, Dammar gum, Boswellia serrate, Commiphora wightii* and *Balsamum tolutanum*.

O-35

Characterization of Medicinal Mushrooms by Hyphenated HPTLC

Anuja BHARDWAJ
Defence Institute of Physiology and allied Sciences, Lucknow Road, Timarpur, Delhi 110054, India, kmisra99@yahoo.com

Mamta, Defence Institute of Physiology and allied Sciences
Rajkumar Tulsawani, Defence Institute of Physiology and allied Sciences
Kshipra Misra, Defence Institute of Physiology and allied Sciences

Medicinal mushrooms such as Cordyceps sinensis (CS) and Ganoderma lucidum (GL) are known to possess large contingent of bioactive compounds, prominently flavonoids with antioxidant activities and are used as traditional medicine for the promotion of health in many Asian countries for centuries. HPTLC hyphened with FTIR and MS was used for the characterization of Indian tissue cultured variety of CS and GL which are being developed to be used as performance enhancers under hypoxic conditions.

Accelerated solvent extraction was employed to prepare the extracts of CS (CSAq, CS25%Alc, CS50%Alc, CS75%Alc , CS100%Alc) using different solvent compositions whereas a sequential fractionation of fruiting body (GLF), mycelium (GLM) and their mixture (GLMF) was carried out using water, methanol and ethyl acetate for GL at room temperature. A new, simple, precise, rapid and sensitive HPTLC method was developed for the quantification of antioxidant compounds (ascorbic acid, gallic acid, hesperidin, quercetin, kaempferol, rutin). Ascorbic acid, rutin and hesperidine were identified and quantified in all the CS samples. Rutin and gallic acid were identified and quantified in GLMaq and GLMFaq, whereas hesperidine and quercetine were identified and quantified in all the aqueous extracts of GL. Besides, several unknown bands were also observed during HPTLC profiling of all the CS and GL extracts.

The confirmation of respective compound was carried out by FTIR and MS using the TLC-MS Interface. Further, the results of HPTLC analysis of antioxidant compounds and the phytochemical evaluation of all the extracts demonstrated a very good correlation. It was concluded that since all the aqueous extracts possessed higher content of antioxidant compounds these can be used as potential candidates to ameliorate hypoxia induced pathological conditions.

O-36

HPTLC Fingerprint Development of Siddha Drugs for Quality Assessment

Shakila R
Siddha Central Research Institute (Central Council for Research in Siddha),
Arignar Anna Hospital Campus, Arumbakkam, Chennai, India, shakilasiva@gmail.com

Gopakumar K, Siddha Central Research Institute

In India different traditional systems viz., Siddha, Ayurveda, Unani, Yoga & Naturopathy and Homoeopathy are practiced since many years. The prime moto of AYUSH, a department under the Ministry of Health & Family Welfare, Government of India is to develop these systems through the Councils functioning individually for each system. In recent years there is a vast demand for traditional medicines due to their safety and efficacy. But still the traditional drugs are yet to be standardized and validated. Distinguishing characteristic features are to be developed.

In Siddha and Ayurveda systems of medicine, there are certain drugs which have same name but different composition and therapeutic uses. For example, Thalisathy chooranam, Sowbhagyasundi legiyam, Eladi chooranam are mentioned in both Siddha and Ayurveda systems of medicine. Their irgredients are not similar and their medicinal properties are not similar. The aim of this investigation is to develop HPTLC finger print profiles of different extracts of these drugs and to document their TLC chrmatograms at UV 254 nm, 366 nm and after derivatization which would be helpful to distinguish and to assess the quality of these drugs commercially available in the market.

O-37

Standardized and Internationally Harmonized HPTLC Methods for Describing the Quality of Reference Material for *Angelica*, *Ligusticum* and Related Species

Eike REICH
CAMAG Laboratory, Muttenz, Switzerland, eike.reich@camag.com

Jonghwan KIM, Ministry of Food and Drug Safety, Osong, Korea
Shuangsheng MA, National Institute for Food and Drug Control, Beijing, China
Valeria MAIRE- WIDMER, CAMAG Laboratory
Eliezer CENIVIVA, CAMAG Laboratory
Nino GIACOMELLI, Univsersity of Zurich, Department of Ethno Botany
Anita ANKLI, CAMAG Laboratory
Candy TANG, Nikyang, Shanghai, China
Débora ARRUDA FROMMENWILER, CAMAG Laboratory

Under current Good Manufacturing Practice medicinal plants as raw material for use as herbal drugs, dietary supplement, or cosmetic ingredient require proper identification whenever they change custody. Global supply chains and international trade therefore are in need of methods for identification that can give reliable and reproducible results, regardless of the laboratory applying them. HPTLC has great potential to become the method of choice because of its principal fitness for this purpose. Multiple samples can be compared and evaluated for similarity. The technique can very well deal with the natural variability of plants and is able to detect and distinguish adulterant species. Two fundamental requirements must be fulfilled to establish identity of a material with certainty: the specifications of the material must be established and the employed method of analysis must prove the agreement of a sample with the specifications.

Regulatory agencies of several Asian countries are working together in establishing Reference Materials for Medicinal Plants that can be used internationally to define species identity based on chemical profiles, distinguish materials that originate from different species, and also allow assessing the proficiency of testing laboratories.

This paper describes the development of a harmonized HPTLC method for the identification of Angelica, Ligusticum and other related species that are used as traditional medicines or dietary supplements in Asia, Europe and North America. More than 20 species have been included in the study and all of them can be clearly discriminated. The method was validated in laboratories of three collaborating countries and then used to generate data for eventually setting specifications for reference materials with respect to fingerprint and minimum content of marker compounds. Based on this collaboration the first reference material has been established and is currently under investigation by national authorities.

O-38

Experimental Design approach for Robustness Testing of HPTLC Methods

Vijaykumar PARMAR
Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, CHARUSAT Campus, Changa - 388 421. Ta. Petlad, Dist. Anand, Gujarat, INDIA., Anand, India, vijayparmar.ph@charusat.ac.in

Bhavin PATEL, Ramanbhai Patel College of Pharmacy
Hetvi PATEL, Ramanbhai Patel College of Pharmacy
Sanketkumar PATEL, Ramanbhai Patel College of Pharmacy
Ketan VARIYA, Ramanbhai Patel College of Pharmacy

Robustness tests are performed at the end of method development to test the susceptibility of an assay procedure to small changes in the experimental conditions [1]. The test stimulates the changes that can be expected when transferring the method between laboratories, instruments and/ or operators. The use of experimental design helps to predict the possible interactions between the factors with limited number of experiments. Design of Experiment (DoE) is found to be an alternative tool for robustness testing of chromatographic methods [2].

In present paper, Plackett-Burman (PB) designs have been employed for robustness testing of two developed HPTLC methods. The first method is a sensitive HPTLC method for simultaneous determination of Beclomethasone dipropionate (BDP) and Formoterol fumarate dihydrate (FFD) in Rotacaps. The second HPTLC method is developed for estimation of diosgenin from Balanites aegyptiaca extract using spraying reagent.

PB designs for the testing of seven factors using eight experiments were used for robustness testing. The percent recoveries and Rf values were observed as responses at each experiment designed. In order to evaluate the robustness, the significance of the factor effects was determined statistically using error estimates in the calculation of critical effects and graphically by means of Pareto charts. The statistical analysis of the data showed that all absolute factor effects on the responses were found to be smaller than the corresponding critical effects. Thus, in all situations, the percent recoveries and Rf values for both the drugs were not significantly affected by factor changes. The robustness of the proposed methods was studied using DoEs and found to be robust at deliberate changes made in experimental conditions.

[1] ICH, Q2 (R1); Validation of analytical procedure: Text and Methodology, 2005, 1-17
[2] Parmar, V. K., Patel, H. N., & Patel, B. K. (2014). Journal of chromatographic science, bmt208

O-39

Monitorization, Separation and Quantification of Antifungals Used for Invasive Aspergillosis Treatment by HPTLC

M.P. DOMINGO
Instituto de Carboquímica (ICB-CSIC), Zaragoza, Spain, eva@icb.csic.es

Eva GALVEZ, Instituto de Carboquímica (ICB-CSIC)
Matxalen VIDAL, Departamento Microbiologia, Hospital Universitario Miguel Servet, IIS Aragón, Zaragoza, Spain
Beatriz DE LA RUEDA, Departamento Hematologia, Hospital Universitario Miguel Servet, IIS Aragón, Zaragoza, Spain
Lourdes ROC, IIS Aragón, Zaragoza, Spain
M.J. REVILLO, IIS Aragón, Zaragoza, Spain
A. REZUSTA, IIS Aragón, Zaragoza, Spain
J. PARDO, Grupo Inmunidad Celular Efectora en Infeccion, Inflamacion y Cancer, Dpto. Bioquimica y Biologia Molecular y Celular, Biomedical Research Centre of Aragón (CIBA), Universidad de Zaragoza, Zaragoza, Spain. Fundación Aragón I+D (ARAID), Gobier

Treatment with antifungal drugs is often used in patients at risk of Invasive Aspergillosis (IA). The mortality rate of invasive infections due to Aspergillus spp. is very high mainly due to the difficulty in diagnosing of these infections and the limited efficacy of antifungal agents. Therapeutic drug monitoring can help to minimize the risk of toxicity and maximize the efficacy. The most commonly prescribed antifungals are Voriconazole, Itraconazole, Fluconazol, Posaconazol and Anfotericin B. There is no optimal therapy for IA, and therefore sometimes a combination of antifungals is used to improved outcomes. The aim of this study is to monitor the most commonly antifungal used, Voriconazole, and to separate all of them in the same analysis.

Voriconazole is an extended-spectrum triazole antifungal with activity against a wide variety of pathogens, including Aspergillus and Candida. Optimum Voriconazole trough levels are between 1 and 5 mg/L but it is unclear whether these levels are reached with currently dosing schedules used. We have developed a fast and easy HPTLC method to monitor the blood levels of Voriconazole after sera extraction. Besides in this work we report a multi separation and detection HPTLC method of the antifungals Voriconazole, Itraconazole, Fluconazole, Posaconazole and Anfotericin B commonly used as treatment of IA. Using the same HPTLC method we are able to detect the diagnostic biomarker of IA, bismethylthiogliotoxin and the antifungal treatment of this invasive infection.

O-40

Development of Validated Method for Simultaneous Estimation of Benzhexol HCl and Trifluperazine HCl in Pharmaceutical Dosage Form by HPTLC

Manoj CHARDE
Government College of Pharmacy, Kathora Naka, VMV Road, Amravati, India, manojudps@rediffmail.com

Ritu CHAKOLE, Department of Pharmacy, Government Polytechnic, Gadge Nagar, Amravati-Maharashtra, India

A simple, rapid, precise and accurate HPTLC method has been developed and validated for simultaneous estimation of Benzhexol hydrochloride (BENZ) and Trifluperazine hydrochloride (TRIFLU) in pharmaceutical preparations. Separation was achieved on HPTLC aluminum sheets silica gel 60 F254 (0.2 mm thickness) using methanol – acetone – toluene - ammonia, 1:2:7:0.1 (v/v) as mobile phase. Densitometric quantification was performed at ≥ 210 nm by reflectance measurement. The hR_F values of BENZ and TRIFLU were 82 and 37, respectively.

The linearity of proposed method was investigated in the range of 0.4 to 0.8 ≤ µg spot-1 and 1.0 to 2.0 µg spot-1 for BENZ and TRIFLU respectively. The result obtained for laboratory mixture of BENZ and TRIFLU was found to be 99.9% ± 0.4% and 99.8% ±0.2% by height and 99.9% ± 0.5% and 99.8 ± 0.2% by area, respectively. The result obtained for marketed formulation of BENZ and TRIFLU was found to be 99.7% ± 0.6% and 99.9 % ± 0.2% by height and 100.1% ± 0.5% and 99.9% ± 0.3% by area, respectively. The percentage recoveries for BENZ and TRIFLU were 100.1% ± 0.7% and 100.1% ± 0.2% by height and 99.7% ± 0.8% and 99.9% ± 0.3% by area, respectively.

O-41

Using Chemiluminescence in TLC for the Quantification of Polyaromatic Hydrocarbons

Bernd SPANGENBERG
University of Applied Sciences Offenburg, Offenburg, Germany, Spangenberg@HS-Offenburg.de

Barbara MILZ, University Offenburg
Regina BRAEMER, University Offenburg
Andrea SEIGEL, University Offenburg

TLC is widely used in the field of environmental analysis. In the last few years it was shown that simple CCD-cameras (charged coupled device-cameras) can replace slit-scanners, showing advantages in comparison to commonly used scanners. A CCD-device measures the whole TLC-plate simultaneously and stores the result in a single image which can be evaluated using special software. It is possible to measure in absorption and in fluorescence and it is possible to evaluate 2D separations. The drawback of absorption and fluorescence measurements by CCD-cameras is the illumination problem. It is nearly impossible to evenly illuminate the whole HPTLC-plate (10 x 10 cm). That is the real reason why TLC fluorescence analysis by a CCD-device is so restricted because the excitation intensity directly influences the measurement signal.

Chemiluminescence is a widely used method for ultra-trace analysis. Surprisingly, in TLC quantification is rarely used in chemiluminescence [1]. This may be due to questions of quantification limits and synchronisation problems. We will show that chemiluminescence is a suitable method for ultra-trace analysis to quantify polyaromatic hydrocarbons.

[1] B. Spangenberg, J. Planar Chromatogr 24 (2011) 357-359

O-42

What Does TLC Mean?

Krystyna TYRPIEŃ-GOLDER
Medical University of Silesia, Katowice, Poland, ktyrpien@sum.edu.pl

Cezary DOBOSZ, Medical University of Silesia

Among various meanings of TLC like *The Last Cigarette - a smoking cessation program that offers numerous resources and tools to assist smokers in their quit efforts*, there is also TLC, the analytical technique, which can be useful to check the process of quitting smoking and allows to observe differences in nicotine metabolism.

The task of this survey was checking sufficiency of TLC followed by densitometry for monitoring process of cutting down on smoking. Assembled data suggested that during one week after stopping smoking small amounts of main nicotine metabolites were still removed with urine from the organism.

Moreover, TLC with densitometry has also been applied in similar studies carried out to assess of exposure to tobacco smoke among pregnancies, children, students of medicine as well as young people suffering from disorders of the central nervous system in Upper Silesia, Poland.

However, the analysis of nicotine and its main metabolites is a multi-stage process which is characterized by not so high recoveries (about 80%) but a satisfactory reproducibility (2.8 – 9.2%). These results also depend on stationary phases applied for TLC/HPTLC. If the TLC plates used for these research were properly marked by HPTLC, confusion caused by various meaning of TLC abbreviation would be eliminated.

O-43

Interest of (HP)TLC for Forensic Laboratories

Virginie LADROUE
Police Forensic Laboratory of Lyon, Ecully, France, virginie.ladroue@interieur.gouv.fr

Laurence DUJOURDY, French Police Forensic Institute
Pierre BERNARD-SAVARY, Chromacim

In the past few years the French police forensic institute has been exploring applications of HPTLC to illicit drugs in various situations. On the one hand, the Institute was involved in international collaborations with countries where the forensic activity is just emerging. Our expertise was required to train forensic partners in countries where supplies in electricity, compressed gases and chemicals can not be guaranteed (Afghanistan, Cape Verde). In such contexts TLC has proven to be a reliable technique, bringing an adequate solution to local needs and resources.

On the other hand, like other cutting edge technology forensic laboratories throughout Europe, the Institute has been facing the emerging phenomenon of New Psychoactive Substances (NPS). Nowadays, many synthesis laboratories worldwide offer new psychoactive drugs derived, yet slightly different, from regulated drugs. In this particular area, creativity of clandestine chemists flourishes and several new molecules are encountered in our laboratories every year.

When analysing a molecule for the very first time, our routine GC-MS and HPLC-UV characterising methods are helpless. In the past, we resorted to NMR in order to achieve formal structural elucidation of seized samples. In cases of mixtures of NPS though, a separation of the components is required prior to NMR analysis. In the example presented here, HPTLC at a semi-preparative scale was investigated in order to resolve a mixture of cathinones.

O-44

Extraction/Isolation and Detection of Monocrotophos from Blood using HPTLC

Ashok Kumar JAISWAL
All India Institute of Medical Sciences, New Delhi, India, ashokjaiswal72@gmail.com

Susheel KUMAR, All India Institute of Medical Sciences
Supriya KRISHNA, All India Institute of Medical Sciences
Tabin MILLO, All India Institute of Medical Sciences

Monocrotophos is a broad spectrum organophosphorus insecticide. It is extensively used in the field crops of different states of India such as Uttar Pradesh, Haryana, Bihar etc. This pesticide is easily available in the market and encountered in accidental/suicidal cases majority of time. Its analysis is usually done from biological samples such as blood and urine which is the sample of choice in management and medicolegal cases. Routinely, HPLC, Gas Liquid Chromatography (GLC), GC-MS, and LC-MS are used for the analysis of monocrotophos. These techniques are not only costly but also time consuming and require more sophisticated instruments.

An attempt has been made to develop a new method for analysis of monocrotophos in biological sample, blood, using HPTLC plate. Monocrotophos was extracted from blood using liquid-liquid extraction and analyzed by HPTLC. For chromatographic separation, various binary and tertiary solvent systems were used as mobile phase. Developed plates were viewed under UV light followed by spray of chromogenic reagents which successfully increased the sensitivity without dispensing with the simplicity of the method. The method developed is a simple, rapid, inexpensive, non-destructive and reproducible which can be performed in any laboratory easily.

O-45

A New Method of Extraction and Detection of Dicyclomine Hydrochloride from Urine using HPTLC

Rajesh KUMAR
All India Institute of Medical Sciences, India, drrajesh_mmc@yahoo.com

Dicyclomine hydrochloride is an antispasmodic and anticholinergic drug which is easily available in the market. Nowdays dicyclomine hydrochloride abuse is increasing in India. Many deaths were reported due to its poisoning in the department of Forensic Medicine and Toxicology, AIIMS, New Delhi. In such cases urine is the sample of choice for qualitative and quantitative analysis of drug. Routinely, highly sophisticated instruments like HPLC, Gas Liquid Chromatography(GLC) are used for the analysis of drug. An attempt has been made to develop a new method for analysis of Dicyclomine in biological sample, urine, using HPTLC plate.

Dicyclomine was extracted from urine using liquid-liquid extraction (chloroform– ether 1:3) and analyzed using HPTLC. For chromatographic separation, various binary and tertiary solvent systems (toluene – acetone – ammonia, 7:3:0.5; ethyl acetate – chloroform – methanol 7.5:1.5:0.5) were used as mobile phases. Developed plates were viewed under UV light followed by spraying of chromogenic reagents which successfully increased the sensitivity without interfering with the simplicity of the method. The method developed is an easy, simple, rapid, cost effective, and can be performed in any laboratory as a preliminary examination before any instrumentation.

P-1

Analysis of pesticide residues in animal feed - method comparison

Wolfgang SCHWACK
University of Hohenheim, Institute of Food Chemistry, Stuttgart, Germany,
wolfgang.schwack@uni-hohenheim.de

Andreas THIELEN, University of Hohenheim
Claudia OELLIG, University of Hohenheim

For pesticide residue analysis in feedingstuff, the DFG S19 multi-method, also known as EN 12393 [1] is widely applied by laboratories responsible for the control of animal feed. However, the multi-step method is rather solvent- and time-consuming, although very sensitive due to high sample concentration of the final extract.

Therefore, other methods of extraction were tested and compared with the S19, especially in terms of matrix load and sensitivity, including the QuEChERS method [2], the Swedish ethyl acetate extraction [3], and the BfR ChemElut method [4]. As test samples of different fat content, soy beans, linseeds, and a mixed feed pellet material were studied. The matrix load of extracts was made visible by TLC on amino plates, which also were used for a planar solid phase extraction clean-up (pSPE) [5].

As to be expected, both the QuEChERS extraction by acetonitrile and the extraction by ethyl acetate resulted in highest matric loads, last of which completely extracted lipids as the S19 method also did. Lipids were nearly no problem for the BfR method, since the sample extraction is performed by methanol/water, followed by partition into dichloromethane with the help of a ChemElut cartridge. The BfR extracts were both lowest in matrix components, clearly shown by TLC, and also highest concentrated resulting in high sensitivity. However, strong matrix effects still occurred during GC/MS analyses. An additional dispersive SPE clean-up (PSA) only partly reduced matrix effects, but they were completely eliminated by our new pSPE cleanup.

[1] European Committee for Standardization (2009), EN 12393-2, Brussels, Belgium
[2] http://www.crl-pesticides.eu/library/docs/fv/CRLFV_Multiresidue_methods.pdf
[3] http://www.crl-pesticides.eu/library/docs/fv/ethyl_acetate_extraction.pdf
[4] Klein, J., Alder, L., J. AOAC Internat. (2003) 86, 1015-1037
[5] Oellig, C., Schwack, W., J. Chromatogr. A (2011) 1218, 6540-6547

P-2

Accelerated screening of sulfonamides in animal-derived foods by HPTLC-FLD-ESI/MS

Yisheng CHEN
Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany,
Yisheng.Chen@uni-hohenheim.de

Wolfgang SCHWACK, University of Hohenheim

Sulfonamides (SAs) are referred to a group of synthetic compounds characterized by a common p-amino-benzene sulfonamide moiety. In EU, SAs are among the most widely administrated veterinary antibiotics. Keeping in mind of the rather low non-compliant rates (< 0.08 %) disclosed by EU veterinary antimicrobial surveillance programs, crudely screening out target samples from the huge sample batches played an important role in the framework ensuring food security. However, many factors make this task difficult to be accomplished.

Here, an HPTLC method coupled to fluorescence densitometry (FLD) and elution-head based electrospray ionization mass spectrometry (ESI/MS) was established for the screening of twelve representative SAs at their EU MRLs [1]. Following separation on HPTLC plates silica gel 60 F254 with methanol - ethyl acetate - ammonium hydroxide solution (28%), (2+8+0.1 mL) and fluram derivatization, sensitive and selective quantitation of the analytes can readily be accomplished with fluorescent densitometry. Limits of detection and quantitation were 15-40 and 35-70 µg/kg, respectively. Additionally, a confirmative detection by HPTLC-electrospray ionization mass spectrometry (HPTLC-ESI/MS) was optimized, allowing straightforward identification of target zones. Therefore, the risk of potential false positive findings can efficiently be reduced.

Including a quick extraction procedure, the method was validated to meet the enforced EU regulation No 37/2010, regarding different food matrices (bovine milk, porcine liver and kidney). Satisfactory precision (*RSD* < 14.3 %) and recoveries (75-115 %) were achieved. Therefore, the established method displays the potential being a relatively more cost-efficient alternative to conventional LC-MS approaches, showing exciting sample throughput and simplicity.

[1] Chen, Y., Schwack, W., J. Chromatogr. A, 2014, 1331:108-116

P-3

Analysis of sulfonamide residues in chicken and shrimp using TLC

J. M. K. J. K. PREMARATHNE
Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka, krissjayaruk@yahoo.com

D. A. SATHARASINGHE, University of Peradeniya
A. R. C. GUNASENA, University of Peradeniya
A. WANIGASEKARA, University of Peradeniya
D. M. S. MUNASINGHE, University of Peradeniya
P. ABEYNAYAKE, University of Peradeniya

The broad spectrum of activity, availability and low cost in sulfonamides has led to become a widely used group of antimicrobials in veterinary practice especially in developing countries. The objective of this study was to establish a TLC method to detect residues of sulfonamides commonly used Sri Lanka in chicken and shrimp. A reliable, inexpensive, simple, liquid-liquid extraction for the rapid analysis of the five sulfonamides sulfadiazine (SDZ), sulfadoxin (SD) sulfamethazine (SMZ), sulfathiazole (STZ) and sulfaquinoxaline (SQ) was developed.

A 3 g of homogenized chicken and shrimp samples were treated with 0.3 ml of 0.1 M HCl, 3 ml of distilled water, and 4.5 ml of ethyl acetate were added and vortexed for 1 min. The tube was then centrifuged at 524 *g* for 10 min. The supernatant was collected and repeated the same procedure. The supernatant was evaporated under a mild nitrogen flow at 55 °C. The dry residue was dissolved in 1 ml of methanol - water mixture, 3:1 (v/v). The prepared solution was extracted twice with 1 ml petroleum spirit. The resulting solution was evaporated under a mild stream of nitrogen at 90 °C, reconstituted with 100 µl of methanol and used for TLC analysis.

Silica gel plates were spotted with 25 µl of standard solutions and extracted samples. The mobile phase was chloroform – n-butanol, 9:1, saturated with distilled water. Sulfonamides were visualized using fluroscamine solution at 366 nm by TLC scanner. The quantity of the sulfonamide in samples were calculated via calibration curves (peak area; 200, 150, 100, 50, 30 and 25 ng/g). The SQ had the highest LOD and LOQ values, 60 and 100 ng/g, respectively. All the other analytes' LOD values were 40ng/g and STZ had the lowest LOQ values of 50 ng/g.

P-4

Rapid HPTLC screening to study the reactivity of UV filter substances towards skin proteins

Wolfgang SCHWACK
University of Hohenheim, Institute of Food Chemistry, Stuttgart, Germany,
wolfgang.schwack@uni-hohenheim.de

Constanze STIEFEL, University of Hohenheim

Different organic UV filter substances (UVFS) contain reactive carbonyl groups, which are possible reaction partners for proteins or free amino acids of the skin. As the formation of protein adducts is seen as key step in the development of contact allergies, the determination of the reactivity towards nucleophilic reaction partners can be used to estimate the (photo) sensitizing potential of a substance.

This is of major importance for cosmetic ingredients, as these substances are intentionally applied on the skin and must pass the legally binding safety assessment prior usage. As the new EU cosmetics regulation prohibits animal testing, the development of alternative methods is of great importance.

Therefore, we developed a fast and simple HPTLC screening method, using an amino phase as simple protein model to estimate the reactivity of common UVFS towards skin proteins [1]. Solutions of the UVFS were applied onto an HPTLC plate, treated by slight heating or UV irradiation, followed by plate development. The plate-bound species remained at the start zones, while the migrating UVFS were quantified by a TLC scanner.

Among the studied UVFS, the ketones BP-3, HMBS, and BM-DBM showed the highest reactivity. UV irradiation of the plate additionally induced the reactions, especially of BM-DBM. The triazones DEBT and EHT, and the camphor derivatives 3 BC and 4 MBC only slightly reacted with the amino phase to form adducts.

Comparing the results of the HPTLC screening with human (photo) patch test data, it became obvious that especially those UVFS, which are common triggers for allergic and photoallergic reactions, also showed a high tendency to bind to the amino phase. Concludingly, the rapid and simple HPTLC screening seems to be well suited to estimate the potential of UVFS to form protein adducts and may contribute to identify skin sensitizers.

[1] Stiefel, C, Schwack, W., Int. J. Cosmetic Sci. (2013), 35, 588-599

P-5

Streamlined analysis of lactose-free dairy products

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Lauritz MORLOCK, Justus Liebig University Giessen
Carot LEMO, Justus Liebig University Giessen

The global prevalence of lactose intolerance has already created a large assortment of commercially available lactose-free food products. Nevertheless, the market for functional food for lactose-intolerant consumers is increasing. A broad range of dairy products, even with high fat and protein contents, were investigated for their lactose content. For their analyses, the most streamlined approach for detection and quantitation of lactose in lactose-free dairy products was developed [1].

After a simple sample preparation, the resulting 10 % food sample solution was directly subjected to the chromatographic system. For application volumes up to 250 µL on a rectangular start zone, LODs for lactose in dairy products were obtained down to the 0.04 mg/L range, which is the lowest LOD reported in matrix so far. For 11 types of dairy products spiked at a lactose content of 0.01 %, the mean recovery rate was 90.5 ± 10.5 % with a mean repeatability of 1.3 ± 1.0 % (n = 11). The running costs were low (0.3 Euro or 0.4 USD/analysis) and analysis time was fast (3 min/analysis).

Thus, the developed, streamlined approach enabled a fast product screening, and at the same time, the reliable quantitation of lactose in relevant samples. Such an analysis is highly attractive to the field of food safety and quality control of lactose-free products, as a limit value for lactose is in discussion in the EU and expected soon. The efficacy of a more advanced production technology for lactose-free dairy products can be investigated by this method due to its very low detectability of lactose. This methodological concept can be transferred to other challenging fields.

[1] G. Morlock, L. Morlock, C. Lemo, J. Chromatogr. A 1324 (2014) 215–223.

P-6

Quantitation of coumarin in food, confirmed by mass spectrometry

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Luisa WINHEIM, Justus Liebig University Giessen
Stephanie KRÜGER, Justus Liebig University Giessen

Coumarin is a phytochemical which is naturally occurring, *e.g.* in woodruff, tonka beans and cinnamon. Since its potential hepatotoxic effect was figured out, the interest in fast and matrix-robust analytical methods increased. An efficient and sensitive HPTLC method was newly developed for analysis of coumarin in cinnamon and cinnamon products with a minimal sample preparation and a short analysis time.

10 different commercially available cinnamon samples and 16 different cinnamon containing foods were dissolved in methanol and analyzed. For some foods, a short sample preparation (grinding) was necessary. Coumarin in solution remained stable for at least 9 days. The development was performed on HPTLC plates silica gel 60 with a mixture of *n*-hexane – ethyl acetate – ammonia. Most of the samples were treated this way, however, for milk rice, a rectangular application and an additional front elution with tetrahydrofuran was necessary due to the strong matrix influence. For detection of coumarin zones, the plates were immersed into an ethanolic KOH solution. The fluorescence was measured at 366/>400 nm. LOD was 200 pg/band and LOQ 400 pg/band. The repeatabilities and the intermediate precisions (both *%RSD*) were mostly ≤ 6 %, with some exceptions.

For verification, zones of different samples and standards were eluted via the TLC-MS Interface into the ESI-MS. Bioactivity of coumarin was shown with the bacteria *Aliivibrio fischeri*. To conclude, the newly developed HPTLC method can be used for fast and reliable food control of cinnamon samples and cinnamon containing foods.

P-7

Simultaneous determination of citrinin and lovastatin in lactone- und hydroxy acid form with validated HPTLC-UV/FLD method

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Ines KLINGELHÖFER, Justus Liebig University Giessen

Lovastatin (Mevinolin, Monacolin K) acts as a lipid-lowering agent and is synthesized, among others, in fermenting rice with *Monascus* fungus strains. In this fermentation, the nephrotoxic mycotoxin citrinin is also formed, which acts carcinogenic and mutagenic, but also antibiotic.

For simultaneous detection of the three components of interest a normal phase HPTLC method was developed. Separation was performed on HPTLC plates silica gel 60 using an eluent mixture of n-hexane, acetone, and acetic acid. The densitometric analysis was carried out in the multi-wavelength scan: Lovastatin was detected in lactone form (LL, hR_F 35) and hydroxy acid form (LH, hR_F 23) by measuring the absorption at 238 nm, whereas citrinin (hR_F 7) was detected by measuring the fluorescence at 313/>400 nm.

The validation of the method showed very good performance characteristics: The average LOD and LOQ (n = 3) were 10 and 50 ng/band for LH and LL as well as 1 and 4 ng/band for citrinin, respectively. The calibration range was linear in the range of 25 and 500 ng/band for LH, 25 and 350 ng/band for LL and 2.5 and 50 ng/band for citrinin. The average determination coefficient (R^2, n = 5) was 0.9998 for LH, 0.9999 for LL and 0.9989 for citrinin. The mean recovery rate for the three substances was 109.7 ± 5.4 % for three replicate measurements per sample preparation, and 3 concentration levels in the lower, middle and upper working range over 5 repetitions of the entire process (l = 3, i = 3, j = 5). The mean repeatability over 3 concentration levels (*%RSD*, l = 3, i= 3) was 2.0 % for LH, 2.6 % for LL and 2.6 % for citrinin. The mean laboratory precision over three concentration levels (*% RSD*, l = 3, j = 5) was 3.9 % and 5.3 % for LH and LL, and 6.1 % for citrinin.

The application of the newly developed and validated HPTLC method was shown for 17 commercial samples. Red rice samples, including Zhibituo and Xuezhikang, were extracted with a solvent mixture of acetone and water and analyzed. Both, LH and LL, were detected in all samples. The reproducibility (*%RSD,* k = 3) was in the range of 0.7 % and 10.3 % and the average reproducibility was 4.4 %. Citrinin could not be detected in the samples at the detection limit of 1.5 mg/kg for a given application volume of 50 µL.

P-8

HPTLC-FLD-ESI-MS and HPTLC-MALDI-TOF/TOF MS analysis of lecithins used in the production of chocolate

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Stephanie KRÜGER, Justus Liebig University Giessen
Laura BÜRMANN, Justus Liebig University Giessen
Günter LOCHNIT, Justus Liebig University Giessen

Lecithin is used as additive E322 in many food products and has excellent emulsifying properties [1]. In the chocolate industry glycerophospholipids' emulsifying properties are important i.a. for the ingredients' miscibility and the chocolate mass' stability. Soybeans were the most common source for lecithin but problems regarding genetic modification and intrinsic allergens made it necessary to consider alternative sources. The focus of this study was primarily on phosphatidylcholine (PC) and phosphatidylethanolamine (PE) since both effect the rheology of molten chocolate differently. Soybean and sunflower lecithins were analyzed by HPTLC-FLD/MS regarding quantitative phospholipid content and fatty acid pattern as well as rheological behavior. [2]

Separation for quantitative analysis was done according to [3]. However, for visualization, derivatization with the primuline reagent was employed. For densitometric evaluation, fluorescence measurement was performed at 366 nm. The results showed sunflowers and soybeans to have significantly different PC and PE contents. Dark and milk chocolate masses showed slightly deviating rheological behavior, when using sunflower lecithin instead of soybean. Comparison of the quantitative results with the rheological data revealed no causal relation between PE content and yield value in any of the chocolates. For neither dark nor milk chocolates any relation was found for PC content and viscosity. For white chocolate a negative correlation was indicated. HPTLC-ESI^+-MS spectra were recorded with a single quadrupol MS. Additionally HPTLC-MALDI-TOF spectra were recorded for comparison. Two derivates for each PC and PE could be identified in accordance with literature. [2]

[1] Zusatzstoff-Zulassungsverordnung (ZZulV), 2012
[2] S. Krüger, L. Bürmann, G. Morlock, in submission
[3] E. Reich, D. Handloser, V. Eidmer, J. Liq. Chromatogr. Relat. Technol. 31 (2008) 1857-1870

P-9

Fingerprinting of serbian, slovenian and croatian propolis using HPTLC and pattern recognition methods

Petar RISTIVOJEVIC
Innovation center of the Faculty of Chemistry, Belgrade, Serbia, ristivojevic@chem.bg.ac.rs

Jelena TRIFKOVIC, Faculty of Chemstry, University of Belgrade
Irena VOVK, Laboratory for Food Chemistry, National Institute of Chemistry,
Filip ANDRIC, Faculty of Chemstry, University of Belgrade
Dušanka MILOJKOVIC-OPSENICA, Faculty of Chemistry, University of Belgrade

Propolis is a resinous natural substance collected by honeybees (*Apis mellifera* L.) from different plant. It has been recognized as antibacterial, antiviral, antifungal, antioxidant, antiinflammatory agents. The main constituents of propolis are plant resin (50%) and beeswax (30%), while minor constituents are aromatic oils (10%), pollen (5%) and other organic compounds (5%).

Due to its simplicity and low cost, HPTLC was recognized as a tool for fingerprint analysis of food samples. A set of characteristic chromatographic signals is treated as unique multivariate fingerprint, i.e. multidimensional vector, and combined with multivariate image analysis and pattern recognition methods could be used for sample classification. Propolis samples from Serbia and Germany were classified in two major types, orange and blue, supporting the idea of existence of two types of European propolis [1, 2]. As a continuation of this research, the aim of the current study is comprehensive phenolic profiling of Serbian, Croatian and Slovenian propolis using HPTLC, fully optimized image analysis and unsupervised technique, Principal Component Analysis (PCA), in order to determine biological and geographical origin of propolis from three neighboring countries.

Development of the chromatographic profile of analyzed propolis samples was based on application of HPTLC conditions optimized regarding the resolution of the phenolic acids and flavonoids. PCA indicate the existence of two clearly separated clusters which correspond to two varieties of propolis. Studied samples were, also, classified into three clusters according to their geographical origin. Serbian propolis samples are mainly orange and formed one cluster, while Slovenian propolis are mainly blue and formed second cluster. Croatian samples are positioned between these two groups. Signals at specific RF values responsible for classification of studied extracts have also been isolated and underlying.

[1] Morlock, G.E., Ristivojevic, P., Chernetsova, E.S, J. Chromatogr. A 1324 (2014) 104–112
[2] Morlock, G.E., Scholl, I., Kunz, N., Schroeder, A. CBS 111 (2013) 13-15

P-10

Solid-phase extraction for clean-up of sugar-rich plant material for improving identification by normal phase HPTLC

Grigorios THEOCHARIS
CAMAG Laboratory, Muttenz, Switzerland, grigorios.theocharis@camag.com

Debora FROMMENWILER, CAMAG Laboratory

Berries constitute a significant dietary source of a multitude of phytochemicals such as flavonoids and coumarins, which exert beneficial effects on human health. However, they also have a high sugar content, hence disturbing identification by HPTLC fingerprints obtained on normal phases. Therefore, the preparation of such samples requires an efficient clean-up step to avoid matrix-derived interferences that may adversely influence the analysis sensitivity, specificity, and reproducibility.

In two individual studies, existing HPTLC methods for the identification of Cranberry (*Vaccinium macrocarpon*) and Goji berry (*Lycium barbarum*) fruits were evaluated. The thereby encountered poor reproducibility has been greatly improved by the removal of matrix compounds employing a C18 SPE clean-up. Moreover, the yield of substances of interest has been increased whereas the overall solvent consumption decreased.

From the experiments, a generalized approach has been derived, which is applicable for future projects targeting other sugar-rich sample matrices.

P-11

Quantitative HPTLC method for quercetin derivatives in onion extract

Céline BERGER
BioEurope, Anet, France, celine.berger@solabia.fr

Aina QUEIROZ, BioEurope

Onion (*Allium cepa*) is a widely food in the world and its preparation generates a lot of waste. Solabia wants to promote these by-products to make a rich antioxidant active. Skins, inedible parts, are very rich in flavonoids including quercetin derivatives : quercetin, quercetin-4'-glucoside and quercetin-3, 4'-diglucoside.

A HPTLC method has been developed in order to quantify these three molecules in onion extracts on a single plate. These three compounds are well separated and applied by overspotting. The quantification is performed by densitometer at 366 nm.

The simultaneous determination of 3 molecules by HPTLC therefore saves time and use less solvent. It also helped to choose the extraction parameters to obtain optimized yields in these three derivatives.

P-12

Analysis of multi-ingredient food supplements by fingerprint HPTLC approach

Marcello NICOLETTI
Department of Environmental Biology, University Sapienza of Rome, Rome, Italy,
marcello.nicoletti@uniroma1.it

Chiara TONIOLO, Department of Environmental Biology, University of Rome

Analysis of herbal food supplement (botanicals) is a difficult task, in particular in the determination of the composition a multi-ingredient botanical. We propose an analytical approach based on HPTLC fingerprints.

For a long time, the utilization of a single chemical substance was the basis of any development of medicinal drug and modern pharmacology. In products derived from plant raw materials, the analytical approach for their validation consisted into the selection of one or few compounds, as either active constituents or "markers", for purposes of identification and quality assessment. However, the last decay evidenced a clear tendency for the use of pharmaceutical multi-ingredients in modern medicine. Even more, the presence of multi-ingredients became clear in the new entries of the food sector, from nutraceuticals to functional foods.

If in a pharmaceutical drug the composition is based on several identified and known substances including the active ones, in botanicals the total composition is the sum of several extracts from different species. If the extract of a single species contains at least 20-30 principal constituents, this number must be multiplied by the number of species utilized. However usually the chemical composition of each herb is very different from the others.

We report several examples of multi-ingredient botanicals where the fingerprint of the product is compared with fingerprints of the extracts of the species reported in the label, obtaining a rapid and low cost identification of the composition. Furthermore an example of fine identification of very similar raw materials is reported as evidence of the sensibility and reliability of the proposed method.

P-13

Rapid determination of oleuropein in food supplements and in *Olea europaea* L. leaves extracts by a densitometric-HPTLC approach

Stefano MULAS

Phytolab - Department of Statistics, Informatics and Applications "G.Parenti" (DiSIA), University of Florence - via Ugo Schiff , 6 -50019 Sesto Fiorentino (FI), Firenze, Italy,
stefano.mulas@unifi.it

Annalisa ROMANI, University of Florence
Silvia A. CORAN, University of Florence

A research aiming to a rapid HPTLC method for the screening and the determination of the oleuropein in aqueous and hydro-alcoholic olive leaves extracts and in commercial products claiming oleuropein as their major component is described.

Oleuropein is a natural secoiridoid present especially in *Olea europaea* L. Oleuropein and its derivatives exhibit several biological activities, e.g. anti-hypertensive, anti-inflammatory, free radical scavenging, antioxidant, prevention of neoplastic diseases and anti-platelet. The *Olea europaea* L. leaves are the main source of these compounds; therefore their extracts have been recently used to prepare affordable dietary supplements.

A HPTLC-densitometric method, based on external standard approach combined with $FeCl_3$ derivatization, was developed for routine analysis of oleuropein in olive leaves extracts. The separation has been carried out by reversed-phase wettable C 18 plates. The method was optimized and validated; the obtained results were comparable with those from a HPLC/DAD/MS method.

Moreover the method was set up with the aim to obtain a simultaneous cross comparative evaluation of different *Olea* leaves extracts and extra virgin olive oils by the HPTLC fingerprint approach. It highlights the presence of four classes of compound: secoiridoids, polyphenols, lignans and hydroxycinnamic acids.

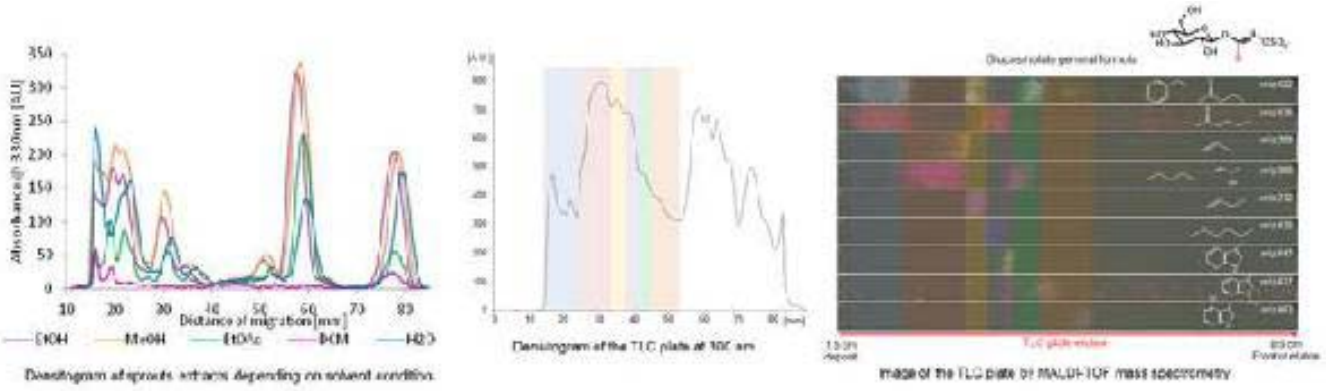
P-14

Screening of active biomolecules in broccoli extracts using HPTLC- coupled to a UV densitometer and MALDI-TOF MS

Muriel MATT
Laboratoire de Chimie et Physique, Approche Multiéchelle des Milieux Complexes LCPA2MC
Université de Lorraine, METZ, France, muriel.matt@univ-lorraine.fr

Estelle SIBILLE, Université de Lorraine
Raffaella DI MATTIA, Université de Lorraine
Manuela DI MARCELLO, Université de Lorraine
Patrick CHAIMBAULT, Université de Lorraine

The aim of the work consists in the optimization of the extraction of compounds from Broccoli (vegetable of *Brassicaceae* family) and their identification based on HPTLC analysis. Broccoli is a good source of compounds promoting health since it contains a large variety of phytochemicals including glucosinolates (and breakdown products), phenolics and other antioxidants like vitamins. This vegetable is subject to large medical and chemical studies in the field of prevention and therapy relating cancer. In order to determine the molecular composition of the broccoli in floret, stalk, sprout and seed extracts, HPTLC coupled with UV densitometry proved to be an effective tool to optimize the sample treatment (broccoli pretreatment, extraction mode, solvent efficiency, extracts stability). Direct UV detection with or without derivatization enables to monitor principally flavonoid derivatives, whereas the coupling of HPTLC with MALDI–TOF MS in positive or negative mode highlighted the potentiality of TLC separation for a larger screening of biomolecules, and especially for the glucosinolates detection.



HPTLC analysis of broccoli extracts, UV and MALDI-TOF MS detection

P-15

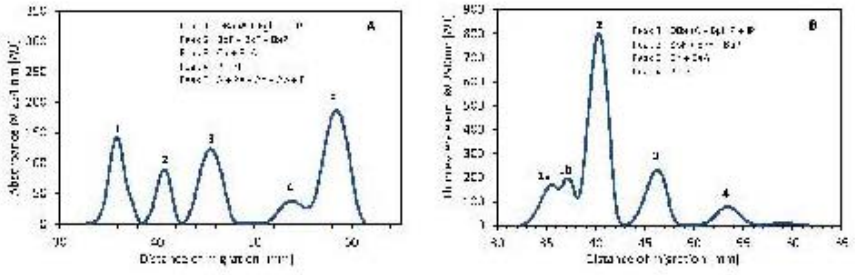
Application of HPTLC for the analysis of PAHs in food samples

Manuela DI MARCELLO
Delft University of Technology, Delft, Netherlands, M.DiMarcello@tudelft.nl

Maria PARIS, University of L'Aquila
Muriel MATT, University of Lorraine

Polycyclic aromatic hydrocarbons (PAHs) are considered to be key pollutants involved also in food contamination. The aim of the work is the analysis of EPA16 (16 PAHs priority pollutants listed by US EPA) in food, qualitatively and quantitatively. PAHs are separated on HPTLC RP-18 plates with concentrating zone, eluting with hexane - dimethylformamide 98:2 at -23 °C. Detection is performed by UV and fluorescence.

Quantification was achieved on broccoli, semi-skim and skim milk. Samples were fortified with a known amount of EPA16 and a mix of 4 selected PAHs and extracted with hexane (dried broccoli) or ethanol and hexane (milk). The quantification was realised by external calibration, using the quadratic regression ($0.97<R^2<0.99$) for EPA 16 and the linear model for the mixture ($R^2 \geq 0.99$). Best results in terms of recovery were between 80% and 120%. This range was respected in the 80% of the measurements made. A certain variability should be addressed to the operative wavelength, which strongly influences the results. No significant interference of food matrix should be reported. These promising results evidence the opportunity to use HPTLC for monitoring PAHs in food.



Separation of EPA16 (30 ng/zone) on HPTLC plate with concentration zone

P-16

Analysis of anthocyanins of coloured wheat varieties

Stefan BÖHMDORFER
University of Natural Resources and Life Sciences, Department of Chemistry, Vienna, Austria, stefan.boehmdorfer@boku.ac.at

Klaus ATZGERSDORFER, Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna
Stephanie KRÜGER, Justus Liebig University Giessen
Gertrud MORLOCK, Justus Liebig University Giessen
Thomas ROSENAU, Department of Chemistry, University of Natural Resources and Life Sciences, Vienna
Heinrich GRAUSGRUBER, Department of Crop Sciences, University of Natural Resources and Life Sciences, Vienna

Anthocyanins, the blue and red dye in many plant organs, are considered to be valuable antioxidants. They can be found in certain wheat varieties, either in the aleurone (blue-grained wheat) or in the pericarp (purple-grained wheat). To increase the total anthocyanin content, crosses between several coloured wheat varieties were made and progenies selected according to grain colour. To evaluate changes in the expressed anthocyanin composition, an HPTLC method was developed and the results compared to an HPLC method that had been used in a previous project [1].

Sample preparation consisted of a simple acidic extraction with methanol and water of the wholemeal flour followed by filtration. This resulted in a sample solution that was well suited for determination of total anthocyanin content and oxygen radical absorbance capacity. A previous project had shown that reversed phase HPLC columns deteriorate quite quickly if these extracts are analyzed directly due to the high load of sample matrix. This rendered the development of an applicable planar chromatographic system challenging. Nonetheless, a normal phase HPTLC method was developed that was able to tolerate the high matrix content. Compared to the previously used HPLC method, normal phase HPTLC offered the known benefits of parallel analysis on single-use plates, higher selectivity of the normal phase, and smaller solvent and sample consumption. The HPLC method was advantageous in terms of labor time and method development.

The anthocyanin patterns of blue and purple wheat varieties differed according to the number of bands and the total peak area. The chromatograms were statistically evaluated and correlated to the total anthocyanin content. The chromatograms reflected not only the total anthocyanin content but also the genetic backgrounds.

[1] Syed Jaafar, *et al.*, Plant Breed. 2013, 132, 546-552

P-17

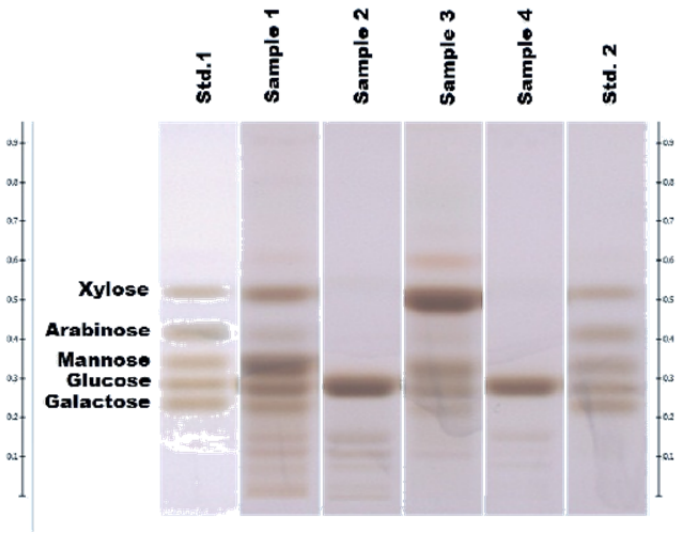
Biorefinery analytics by means of HPTLC - separation and quantification of wood sugars by HPTLC

Antje POTTHAST
University of Natural Resources and Life Sciences, Department of Chemistry, Christian Doppler Laboratory *Advanced Cellulose Chemistry and Analytics*, Vienna, Austria,
antje.potthast@boku.ac.at

Josua Timotheus OBERLERCHNER, BOKU, Vienna, Austria
Stefan BÖHMDORFER, BOKU, Vienna, Austria
Thomas ZWECKMAIR, BOKU, Vienna, Austria
Stefan KOCH, BASF Ludwigshafen, Germany
Alois KINDLER, BASF Ludwigshafen, Germany
Thomas ROSENAU, BOKU, Vienna, Austria

Crude oil as a starting material for the chemical industry will be more and more replaced by renewable materials in the future. In comparison to a petrol refinery the concept of a biorefinery uses lignocellulosics as starting materials. Either the polymeric constituents are used directly or in a more degraded form after different pretreatments. As the composition of natural feedstock varies fast and robust screening methods are required in order to define quality or reaction conditions. Plant hydrolysates are often difficult to analyze by GC or HPLC due to their complex matrix. The application of HPTLC allows to overcome such matrix associated problems.

In this study, a HPTLC method for the quantification of the most common sugars existing in plant hydrolysates is presented. To evaluate this method it was compared with GC-MS and HPLC-RI. Liquid and gas chromatography, with all their inherent benefits and drawbacks are by far the most common techniques currently applied for quantification of plant-derived sugars. However, HPTLC is able to outperform these classical methods with regard to analysis time and matrix effects, while delivering reliable results.



HPTLC chromatogram of wood-hydrolyzate sugars

P-18

Quality assessment of commercial tea products sold as German chamomile (*Matricaria recutita* L.) by a validated HPTLC method in Turkish market

Etil GUZELMERIC
Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy and Phytotherapy, Istanbul, Turkey, etil.ariburnu@yeditepe.edu.tr

Erdem Yesilada, Yeditepe University, Istanbul

German chamomile tea composed of dried flower heads of *Matricaria recutita* L. is one of the most popular single ingredient herbal teas. It has been safely consumed for centuries either its delicious flavor or medicinal purposes such as anti-spasmodic, anti-inflammatory and mild sedative which are mainly related with its phenolic constituents (e.g., apigenin-7-O-glucoside). The annual consumption of chamomile flowers in the world is recorded as several thousands of tons [1]. In parallel with global trend, there is also strong and increasing demand to chamomile flowers, either crude or processed, in Turkey.

On the other hand, misidentification of chamomile like flowers by lay people due to some morphological similarities with *M. recutita* L. is led to unintentional adulteration. It is the most important drawback for the promotion of chamomile products, besides adulteration may result in not only loss of product efficacy but also cause tendency to toxicity. Eventually it is obvious that detection of adulteration plays a vital role for the public health.

Apigenin-7-O-glucoside is one of the major active components in chamomile flowers and also stated as a standard marker in the European Pharmacopoeia [2], therefore comparative assessment of Apigenin-7-O-glucoside was performed by a validated HPTLC method in *M. recutita* L. as reference and commercial chamomile tea products sold in different food stores or spice shops (akhtars) in Turkey. The analyses were performed on HPTLC plates silica gel 60 NH_2 F254s using ethyl acetate - formic acid - acetic acid - water (30:1.5:1.5:3, v/v/v/v) [3]. Results of the present study have revealed that most of the chamomile tea products sold in spice shops were found to be adulterated with the flowers of other *Asteraceae* members.

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P-19

Validated HPTLC method for quantitation of hexetidine in bulk drug and in mouth wash formulation

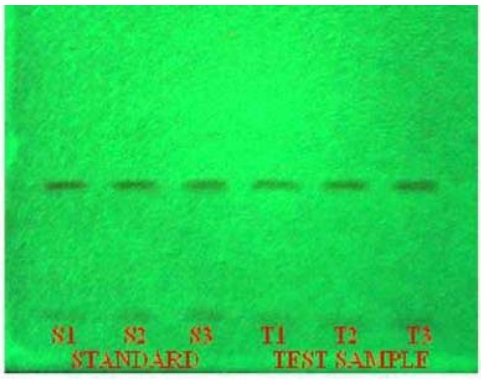
Safeena SHEIKH
Unijules life sciences Ltd 34-35 MIDC Area kalmeshwar, Nagpur, India, ard@unijules.com

Suhail ASGHAR, Nagpur, India
Showkat AHMAD, Nagpur, India

A new, simple, precise, and accurate HPTLC method for quantitation of hexetidine as the bulk drug and in mouth wash formulation have been developed. Chromatographic separation of the drug was performed on aluminium backed plates coated with silica gel 60F254 as the stationary phase and the solvent system consisted of toluene - methanol (8:2, v/v). Densitometric evaluation of the separated zones was performed at 525 nm. The drug was satisfactorily resolved with the formulation excipients with hR_F values of 33, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity which found in the range of 1 to 6 µg/spot with the correlation coefficient of 0.999, intra-day precision 0.9 to 1.0% and interday precision 0.3% and accuracy afford 98-102%, and specificity in accordance with ICH guidelines.

Weigh accurately hexetidine 10 mg in 50 ml volumetric flask and dissolve the content in 30 ml methanol mix the content and make up the volume to 50 ml with methanol. The drug response was linear, regression correlation coefficient was found to be 0.999 over the concentration range 1-6 µg/spot. The LOD and LOQ were found to be 150 ng/spot and 250 ng/spot, respectively. Experimental results of the amount of hexetidine in mouth wash formulations, t was found to be 100.9%.

Good recoveries of the hexetidine in the range from 99.8 to 100.7. Mobile phases having different composition like toluene - methanol 8.1:1.9 (v/v), 8.3:1.7 (v/v), 7.9:2.1 (v/v) and 7.8:2.2 (v/v) were investigated. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 min. Also detection wavelength was altered, duration of saturation (± 5 min), and development distance (± 1 cm). Robustness of the method was found satisfactory.



Chromatogram with separated spots of hexetidine in standard and sample

P-20

Japanese knotweed a good source of proanthocyanidins

Vesna Glavnik
Laboratory for Food Chemisty, National Institute of Chemisty, Ljubljana, Slovenia,
vesna.glavnik@ki.si

Anže ROGELJ, University of Ljubljana, Biotechnical faculty, Department of Food Science and Technology, Ljubljana, Slovenia
Irena VOVK, National Institute of Chemisty, Ljubljana

Japanese knotweed (*Fallopia japonica*) was brought to Europe from Japan as an ornamental plant in the 19th century, but now knotweed presents one of the 100 most world invasive alien species in Europe. Besides Japanese knotweed is also known sakhalin knotweed (*Fallopia sachalinensis*) and their interspecific hybrid *Fallopia* x *bohemica*, which are less invasive than Japanese knotweed. Japanese knotweed is used as a traditional medicinal plant in Japan and China for centuries, because of its health benefits to humans. It is well-known as a good source of resveratrol, but not as a source of catechins and proanthocyanidins.

The aim of this study was to make a comparison of the content of catechins in cocoa which is a good source of proanthocyanidins, green tea a good source of catechins and Japanese knotweed not known for proanthocyanidins by TLC. Extraction of the proanthocyanidins was made by 70% aqueous acetone. TLC analysis was performed on HPTLC silica gel 60 [1] and cellulose plates [2] using different developing solvents [1,2], while detection was performed after derivatization by 4-dimethylaminocinnaldehyde reagent [3]. The estimated content of selected catechins and proanthocyanidins in different tissues (leaves, stems, flowers and roots) in all three known species *Fallopia japonica*, *Fallopia sachalinensis*, *Fallopia* x *bohemica* showed that the richest tissues of all three species with proanthocyanidins are roots and flowers.

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P-21

Interactions between phenolic compounds and food proteins can alter antigenic properties

Kathrin Tscherch
Department of Chemistry, University of Tennessee, Knoxville, United States, ktscherc@utk.edu

Lena MORSCHEUSER, Institute of Food Chemistry, University of Hamburg
Mareen LEHMANN, Hamburg
Julia BILLER, Hamburg
Marina DECKWART, Hamburg
Markus FISCHER, Hamburg
Markus FISCHER, Hamburg
Angelika PASCHKE-KRATZIN, Hamburg
Sascha ROHN, Hamburg

Allergic reactions to food are caused by certain food ingredients and can result in a variety of symptoms. In recent years, food allergies have evolved from being a problem for food-allergic individuals to a major public health concern. Research studies have gained increased knowledge about the influence of food processing on the stability and analytical detectability of food allergens. However, there is still a lack of knowledge on this topic making it difficult to predict the impact of processing on the allergenicity of foods. There are no general rules as to how different allergenic foods respond to processing methods. For instance, structural alterations as well as chemical modifications of the protein may occur. These may involve formation of covalent bonds between for instance lysine residues of a protein and other constituents of the food matrix, such as phenolic compounds, leading to various adducts. The resulting phenol-protein complexes are barely understood, not least because of the lack of suitable analytical methods. The combination of HPTLC and in situ immunostaining (HPTLC-IS) represents a promising new approach enabling the simultaneous chromatographic separation and characterization regarding immunological properties.

The aim of this study was to develop a procedure for separating a model protein via NP-HPTLC and detecting its epitop by IS. Then, the developed method was used to analyze the influence of various phenolic compounds on myoglobin's antigenicity. Results showed that the attachment of different phenolic compounds to myoglobin caused a hR_F-value shift and an affected detectability by immunostaining suggesting an alteration of antigenic properties.

P-22

Determination of caffeine content of a commercial coffee samples with HPTLC

Fatih GÖGER
Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, ESKIŞEHIR, Turkey,
fatihgoger@gmail.com

TLC is one of the best and fastest chromatographic technique. It is not as easy as someone has think about it. Because it's an open chromatographic technique and separation can be effect from too many factors for example: humidity, tank saturation time, migration time, edge effect, spraying time, dipping time, drying time.

HPTLC is a upper level technique. If this technique is used with some equipment, which controls (limits) the environmental and personal effects, it is very effective and reliable. HPLC and GC were the major quantification technique for European Pharmacopoeia, but in EP 7.0 qualitative HPTLC determination is receiving acceptance, which shows HPTLC to be a reliable method.

Coffee is the most well-known and important commodities for world trade and cultivated Arabica, Brazil and the other tropical countries. Major pharmacologically active compound of coffee is caffeine which is a purine alkaloid.

In this study a commercial water soluble coffee and its gold form were investigated for their caffeine content. 1 g water soluble coffee solved in 25 ml hot water and diluted 5 times. Caffeine standard and sample solution were analyzed. Calibration curve of caffeine at 275 nm ($y = 460.559 + 12.170\ x$, $r^2 = 0.99845$) was used for determination. Classic form water soluble type's caffeine content is found as 2.9% ± 0.1% and gold form was found as 2.7% ± 0.2%.

P-23

HPTLC analysis of carbohydrates from the mesocarps of *Pithcellobium dulce*

Mary SARAL A
Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore – 632014 India, amarysaral@vit.ac.in
Preethi SARAVANAN, VIT Vellore, India

The main aim of this work is to develop a rapid, simple, precise HPTLC method for the quantification of carbohydrate contents from the hydroalcholic extract of *Pithcellobium dulce* pods. The extracts were tested to determine the total polysaccharide compound by Beronic *et al.* The presence of carbohydrates was confirmed by phenol-sulphuric acid method. TLC and HPTLC methods were carried out according to Harborne and Wagner *et al*. Different composition of mobile phase was employed for the optimization of solvent system by TLC. Fingerprinting analysis was developed by HPTLC for good resolution and separation.

TLC profiling of the hydroalcholic extract confirms the presence of carbohydrate. Glucose, fructose, galactose, xylose, lactose, rhamnose, sucrose are the standards were spotted using n-butanol - acetic acid - water 6.5:1.5:2 on silica gel 60 plates. HPTLC fingerprinting of extracts confirm the presence of glucose, galactose and fructose with different hR_F values.

HPTLC fingerprinting of *Pithecellobium dulce* is useful in the characterisation of different phytoconstituent present in this species. Further, separation and characterisation of bioactive compound from the fruit is to be evaluated and reported in future.

P-24

Fingerprint profiling of polysaccharide kefiran extracted from kefir grains by HPTLC

Carmen POP
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, CLUJ-NAPOCA, Romania, lazar_rody@yahoo.com

Sorin APOSTU
Ancuţa M. ROTAR
Cristina A. SEMENIUC
Liana SALANĂ
Marianne SINDIC, University of Liege, Agro-Bio Tech Gembloux
Carmen SOCACIU

Kefiran is a water-soluble polysaccharide, which contains D-glucose and D-galactose units, which may be extracted from kefir grains a complex population of lactic acid bacteria and yeasts. The quantification of kefiran monosaccharide composition was carried out by complete acid hydrolysis of the polysaccharide with 0.2 M TFA at 80°C for 24h. A rapid and sensitive extraction method was developed using a mixture of n-propanol:acetic acid:water (70:20:10, v/v), and for the derivatization was used: p-amino benzoic acid 7g/L and o-phosphoric acid in methanol 30 g/L. The separation of monosaccharides was performed by thin layer chromatography on HPTLC plates silica gel 60 F254, 20 x 10 cm. The polynomial regression data for the calibration plots exhibited good correlation (r = 0.99087 for galactose and r = 0.99703 for glucose standards, respectively).

Quantitative analysis was carried by comparing samples to the calibration curve performed on the same plate. The identified HPTLC fractions of the hydrolysate were glucose (hR_F 71) and galactose (hR_F 66), which indicates the high purity of kefiran. The relative concentrations of each monosaccharide identified in samples are dependent on the initial molecular weight of the polymer chain. The results also demonstrated that the kefiran isolated from kefir grains grown in milk is a heteropolysaccharide which contains D-glucose and D-galactose units in a ratio of 0.94:1.1.

This simple and fast HPTLC procedure can be performed for the quantitative determination of monosaccharide composition of the kefiran. The physical properties of the polysaccharide kefiran depend on extraction parameters (temperature and time). The release of glucose and galactose by polysaccharide hydrolysis occurred at the same time, the maximum rate of hydrolysis has been identified after 24 h.

P-25

Simultaneous determination of sudan dyes (I-IV) in spices by TLC

Jana SIMONOVSKA
Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia, Skopje, Macedonia,
j_klopcevska@yahoo.com

Olga POPOVSKA, Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia
Zoran KAVRAKOVSKI, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Vodnjanska 17, 1000 Skopje, Republic of Macedonia
Vesna RAFAJLOVSKA, 1Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia

Sudan dyes (I-IV) are synthetic, lipophilic azo dyes. During recent years, they have been widely used as coloring agents in the industry. The use of these dyes in the food and spices does not comply with the EU food safety requirements, as well as with Food and Drug Administration (FDA), due to their carcinogenic and toxic effects. Nowadays, determination of Sudan dyes have achieved great attention taking in matter their unlawfully use as food colorants in red pepper spices and other related products.

A TLC method was developed for simultaneous determination of Sudan dyes (I-IV) in spice samples supplied from local market in the Republic of Macedonia. A mobile phase consisted of water – acetone – methanol - acetic acid 3:1:7:0.1 v/v/v/v and TLC aluminium sheets silica gel 60 F254 (20 × 20 cm, 0.2 mm thickness layer) were used in the developed method. The chromatograms of Sudan dye standards, spice samples and spiked samples were evaluated in term of retention factor (hR_F) values. The limit of detection based on visual evaluation of the coloring spots was found 0.006 mg/mL, 0.0048 mg/mL, 0.0050 mg/mL and 0.0099 mg/mL for Sudan I Sudan II, Sudan III and Sudan IV, respectively. In 6 from 17 analyzed spice samples Sudan dyes were detected. The method can be used for routine quality analysis of Sudan dyes in spices.

P-26

The use of off-line 2DTLC-HPLC-ESI-MS in the qualitative analysis of lutein-containing dietary supplements

Piotr MIGAS
Department of Pharmacognosy with Medicinal Plants Garden, Medical University of Gdańsk, Poland, Gdańsk, Poland, pmig@gumed.edu.pl

Natalia STEMPKA, Medical University of Gdańsk, Poland
Mirosława KRAUZE-BARANOWSKA, Medical University of Gdańsk, Poland

Lutein is the main carotenoid of retina but not synthesized by human organism. Although lutein is present in fruits and vegetables its amount in diet is often too low. Therefore lutein occurring in organism is enriched with the use of dietary supplements, mainly in the prophylaxis of macular degradation. However, both lutein and its esters with fatty acids present in the supplements are unstable. As a result, the amount of lutein in dietary supplements differs from that specified in the leaflets.

The aim of the presented work was to establish off-line 2DTLC-HPLC-ESI-MS analysis conditions for determination of lutein in dietary supplements available in Poland. The extraction of carotenoid compounds from the analyzed matrices was carried out with a mixture of methanol - chloroform 1:1, v/v. The developed conditions of 2DTLC separation on TLC Si60 glass plates coupled initial purifying of the sample and the proper separation of lutein. The more lipophilic compounds were eluted in the first direction with the use of n-heptane - ethyl acetate (9:1, v/v) as mobile phase and cut off with the part of the plate. The plate was dried and the separation was continued in the opposite direction with the use of n-heptane - acetone - ethyl acetate 55:25:20, v/v/v. The lutein was identified basing on co-chromatography with standard, UV and ESI-MS spectra. Its concentration was analyzed densitometrically.

The developed method of separation shortens the time of sample preparation what is crucial in lutein determination due to its fast degradation. The higher concentration of lutein was assigned in soft capsules especially in the preparations contained other antioxidants like vitamin E, unsaturated fatty acids or polyphenols.

P-27

Comparison of an HPTLC method with the Reflectoquant assay for fast HMF determination in honey

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Anna HOŠŤÁLKOVÁ, Justus Liebig University Giessen
Ines KLINGELHÖFER, Justus Liebig University Giessen

A recently developed HPTLC method [1] for 5-hydroxymethylfurfural (HMF), a widespread process contaminant in carbohydrate-rich food and beverages, was improved to obtain reliable results for a wider range of honey matrices. After alcoholic extraction and rectangular application, a two-step development on HPTLC plates silica gel 60 was introduced. The first short development up to 2 cm with ethanol - methanol 9:1 (v/v) was essential for HMF release from the start area, followed by the separation with ethyl acetate up to 5 cm. Absorbance measurement was performed at 290 nm. Using this modified method the reliability and robustness of the former HPTLC method was improved and the application was extended to a broader range of honey matrices.

The analytical response of HMF (hR_F 74 ± 1) in the working range (4 - 60 ng/band) showed correlation coefficients of r ≥ 0.9994 (n = 8) for polynomial calibrations. The intra-day precision (repeatability, *%RSD,* n = 6*)* ranged 3.4 - 4.7 %. The inter-day precision (reproducibility, *%RSD,* n = 3) was 0.4 - 6.6 %. The reproducibility over the whole procedure inclusive sample preparation (*%RSD*, n = 2) was 0.4 - 7.2 %. Recovery rates for a range of different application volumes, and thus honey matrix applied, differed only by 4.2 %. HMF findings calculated by external calibration *versus* standard addition method (honey was spiked or oversprayed with different HMF solutions) differed on average by 2.4 % and showed that the matrix influence was minor.

Finally, the modified method was compared with the Reflectoquant® assay. The reflectometric assay was performed as specified by Merck. Quantitation limits corresponded to 4 mg/kg for both methods and were suited for quantitation of HMF in honey at the strictest regulated level of 15 mg/kg. 17 honey samples of different botanical and geographical origin were analyzed. Having in mind that spectrometric methods (sum parameter generated by staining) can vary up to 20 % if compared to chromatographic methods (additional separation from matrix), comparable results were obtained with both methods: The mean deviation between both methods was 15 %, which underlines that both methods are well-suited for fast HMF determinations.

The project was partially co-financed by the European Social Fund and the state budget of the Czech Republic, TEAB, project no. CZ.1.07/2.3.00/20.0235 and SVV 267 002.

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P-28

Thin layer chromatography/laser-induced acoustic desorption/atmospheric pressure chemical ionization mass spectrometry

Jentaie SHIEA
National Sun Yat-Sen University, Kaohsiung, Taiwan (ROC), jetea@fac.nsysu.edu.tw
Sy-chyi CHENG, National Sun Yat-Sen University

Laser-induced acoustic desorption (LIAD) is an ambient technique developed for desorbing and ionizing organic and biological materials from solid substrates. LIAD involves the laser ablation of a substrate, which generates acoustic waves that propagate through the substrate to desorb analytes on the other side. This technique is able to desorb analytes without a matrix as well as generating fewer fragments than traditional laser desorption techniques, and has been combined with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) for direct characterization of biological and petroleum samples without tedious pretreatment. TLC has been combined with LIAD/ESI to separate and characterize sample mixtures such as dyes, drugs, and essential oils. Since APCI can provide information on nonpolar components that is not obtained using ESI, the combination of LIAD with APCI is useful for characterizing less-polar and nonpolar compounds on separated TLC plates.

The LIAD/APCI source consisted of a pulsed laser beam, a sample plate, and an APCI-plasma source. The separated TLC plates were placed on glass slides, where the gap between the glass slides and the TLC plates was filled with a viscous solution. A pulsed laser beam was used to irradiate the underneath of an aluminum TLC plate to desorb analyte spots placed on the other side. Desorbed analytes were post-ionized via reactions with charged species (i.e. electrons, metastable molecules, and hydronium ions) generated in an APCI-plasma source and then detected by an ion trap mass analyzer. This TLC-LIAD/APCI/MS approach has been used to separate and characterize mixtures of saturated hydrocarbons and aromatic compounds as well as perfumes and essential oils. The results indicated that the hyphenation of LIAD with APCI-plasma/MS is useful for characterizing less-polar and nonpolar chemical compounds on TLC plates.

P-29

Separation, quantitative determination, and fatty acid profiling of monoacylglycerides in Fatty-Acid Methyl Esters (FAME) using an on-line, hyphenated technique based on AMD-FDIC-ESI-MS

Vicente L. CEBOLLA
CSIC-ICB, Zaragoza, Spain, vcebolla@icb.csic.es

Carmen JARNE, CSIC-ICB
Luis MEMBRADO, CSIC-ICB
Maria SAVIRON, CSIC-CEQMA
Jesus ORDUNA, CSIC-ICMA

FAME is a biodiesel obtained from vegetal or animal oils through esterification and transesterification processes, which is used as a total or partial substitute for petroleum derived-diesel. Monoacylglycerides are one class of the impurities coming from an incomplete esterification reaction. Due to their high fusion point and low solubility at low temperatures, monoacylglycerides can produce deposits in motor engines. The maximum concentration of monoacylglycerides tolerated in FAME by UNE-EN14214:2013 standard is 0.8 wt%.

We evaluate here the determination by HPTLC of monoacylglycerides in 9 FAMEs from vegetal and animal origins. Monoglycerides have been separated from other FAME components (fatty acids, diglycerides, triglycerides and fatty acid-methyl esters) using a 3-step AMD separation based on a t-butyl methyl ether – dichloromethane - *n*-heptane gradient, over a total migration step of 90 mm, in one hour. Densitometric Fluorescence Detection by Intensity Changes and quantitative determination of monoacylglycerides have been carried out by plate post-impregnation using primuline (200 ppm) by excitation at 365 nm. Identification of monoacylglycerides was done in FDIC by using glyceryl stearate as standard, and also by ESI-MS, after an on-line extraction of the peak using a head-elution based TLC-MS interface. Moreover ESI-MS provides the fatty acid profile of the corresponding FAME-derived monoglyceride.

One plate per analyzed FAME was used. In each plate, the corresponding FAME was applied on triplicate (three 12 mm-bands, 2500 g each), together with 10 sample bands corresponding to different sample loads of the standard. Intra-plate and inter-plate calibrations show adequate repeatability, and a good sensitivity to determine monoacylglycerides far below the limit required by the UNE standard.

P-30

HPTLC-MS using an elution-based TLC-MS interface

Hans GRIESINGER
Merck KGaA - Department of Bioanalytical Chemistry, Darmstadt, Germany,
hans.griesinger@merckgroup.com

Michaela OBERLE, Merck KGaA
Katerina MATHEIS, Merck KGaA
Michael SCHULZ, Merck KGaA

A straightforward way to couple TLC with MS is via the TLC-MS Interface. It is an elution-based, semi-automatic system to extract zones from the TLC plate and transfer them online into the MS. It is suitable for all thin layer materials and every eluent that can be sprayed in the ion source. The interface can be connected to any kind of LC-coupled mass spectrometer.

We show how the TLC-MS Interface can be used for the development of TLC-MS applications in the areas of food & beverage, pharmaceutical ingredients, cosmetic actives and peptide & protein analysis.

- separation and identification of insulin species
- investigation of UV-filters in suncream
- analysis of steroids
- determination of caffeine in energy drinks

All experiments were performed on newly developed HPTLC plates with a reduced separation layer thickness. After chromatographic separation the analytes were extracted with acetonitrile/water (95:5, v/v) and transferred online into the MS with a flow rate of 0.2 ml/min. The ionization mode was electrospray ionization (ESI) in the positive mode.

TLC allows sample preparation and chromatographic separation in one step. This is possible because of the high sample matrix tolerance of TLC. Through the coupling of TLC with MS substance identification is possible. It is shown that the use of thinner TLC plates leads to improved detection limits, increased sensitivity and improved S/N ratios. The sample matrix is clearly separated from the target analytes. This leads to clean mass spectra due to a very low level of ion suppression. Further instrument and method developments might be useful to overcome technical challenges, e.g. the replacement of silica gel particles from the plate.

P-31

HPTLC-nanospray-MS using the Advion NanoMate® system

Michaela OBERLE
Merck KGaA, Merck Millipore - Lab Essentials R&D Analytical Chromatography, 64293 Darmstadt, Germany, michaela.oberle@merckgroup.com

Hans GRIESINGER, Merck KGaA
Susanne MINARIK, Merck KGaA
Katerina MATHEIS, Merck KGaA
Michael SCHULZ, Merck KGaA

A relatively new field of great interest is coupling TLC to MS. An attractive approach is the TriVersa NanoMate® system from Advion. It is a chip-based nano-electrospray ionization source combined with a flexible pipetting system.

Direct surface analysis can be performed automatically in the Liquid Extraction Surface Analysis (LESA) mode. The extraction solvent is brought in contact with the TLC plate surface to extract sample material. Optimally suited TLC surfaces are hydrophobic modified silica gel layers in combination with extraction solvents containing at least 30% water.

We show how the NanoMate® system can be used for the development of TLC-MS applications in the areas of food and beverage, cosmetic actives, pharmaceutical ingredients and protein analysis, for example:

- separation and identification of food dyes
- determination of UV-filters
- analytical investigation of Hydrocortisone, Cortodoxone, Metyltestosterone
- analysis of Myoglobin

All experiments were performed on HPTLC RP-18 F254s plates. After chromatographic separation the analytes were extracted with methanol - water (50:50, v/v + 0.1% formic acid) by a flexible pipetting system and transferred via the combined chip-based nano-electrospray ionization source into the MS. The ionization mode was electrospray ionization (ESI) in the positive ion mode.

P-32

Characterization of proteins by HPTLC-MS - proteomics revisited?

Julia BILLER
University of Hamburg, Institute of Food Chemistry, HAMBURG SCHOOL OF FOOD SCIENCE, Hamburg, Germany, biller@chemie.uni-hamburg.de

Maria TRUSCH, University of Hamburg
Sascha ROHN, University of Hamburg

Due to the wide range of functionality and reactivity, proteins are in significant focus of research in food and nutritional science. In addition to the protein identification, also changes in the protein (post-translational modifications - PTMs) are of particular interest. So far, the analysis has been based on traditional chromatographic and electrophoretic separation methods. However, studies of such protein derivatives with these conventional methods often led only to unsatisfactory results.

In this work, a new methodology is introduced, combining traditional mass spectrometry based protein analysis with thin-layer chromatography. The first step is an optimized separation of proteins and protein species by means of the multidimensional high-performance thin-layer chromatography (HPTLC). The identification and characterization of proteins and protein species will be carried out by mass spectrometry, which requires the establishment of innovative coupling techniques such as HPTLC-MALDI, HPTLC-ESI, or HPTLC-LESA-ESI-MS.

The implementation and development of these methods are initially based on purified proteins as well as complex protein-mixtures relevant in foods. In the further course the evolved procedures will be extended to food- and feed-related questions.

P-33

Hyphenation of HPTLC with ESI-MS for the characterization of saponins in different plant matrices

VALERIA REIM
University of Hamburg, Institute of Food Chemistry, HAMBURG SCHOOL OF FOOD SCIENCE, Hamburg, Germany, reimv@chemie.uni-hamburg.de

Sascha ROHN, Univerisity of Hamburg

Saponins are naturally occurring glycosides, widely distributed in the plant kingdom. Based on a lipophilic aglycone (sapogenin), several hydrophilic oligosaccharide moieties are attached. The lack of distinctive chromophores hampers the analysis of saponins by HPLC-DAD. None of the currently applied chromatographic techniques allows an all-embracing characterization and quantitative analysis. However, HPTLC is a notable technique permitting a variety of detection possibilities. Especially offline coupling of HPTLC with mass spectrometry via a TLC-MS interface is increasingly used in recent years.

The aim of this study was to characterize saponins in different plant materials by HPTLC-ESI-MS. Chromatography was performed on HPTLC plates silica gel 60 F254 using (1) CHCl3/MeOH/ddH2O (6.0/4.0/0.9; v/v/v) and (2) CHCl3/AcOH/MeOH/dd H2O (6.4/3.2/1.2/0.8; v/v/v/v) as mobile phase. Detection was carried out at UV 366 nm/white light after derivatization with p-anisaldehyde sulphuric acid and Ehrlich's reagent. Spots were eluted semi-automatically with (A) 0.1 % FA in dd H2O and (B) ACN (40/60; v/v) and a flow rate of 0.25 mL/min into an ESI-MS. Mass spectra were obtained at positive ion mode within a range of *m/z* 200-3000.

Meanwhile, hyphenation of HPTLC with mass spectrometry via commercially available TLC-MS interface(s) is applicable for saponin analyses as well. Triterpenoid saponins were identified in peas and the soap bark tree *Quillaja saponaria*. Asparagus, *Tribulus terrestris* and fenugreek seeds mainly contain furostanols. In contrast, steroid alkaloids were detected in potato sprouts. The elution-based interface technique is target-orientated. Spatial resolution and highly sensitive mass spectrometric signals which are nearly free of contamination are obtained within a few minutes.

P-34

Mass spectrometric characterization of enzyme-lactose derivatives

Julia BILLER
University of Hamburg, Institute of Food Chemistry, HAMBURG SCHOOL OF FOOD SCIENCE, Hamburg, Germany, biller@chemie.uni-hamburg.de

Marten KLUKKERT, University of Hamburg
Lena MORSCHHEUSER, University of Hamburg
Claudia S. LEOPOLD, University of Hamburg
Maria TRUSCH, University of Hamburg
Sascha ROHN, University of Hamburg

Enzymatic drugs are often used in context of pharmacotherapy for substitution of endogenous proteins. For example, in pancreatic insufficiency a lack of endogenous digestive enzymes is medicated by giving pancreatic enzymes (e.g. α-amylase and lipase). Pharmaceutical excipients such as lactose, a commonly used filler in tableting, can contribute to a transformation and denaturation of the protein structure of the drug. Thus may lead to declining biological activity and immunological reactions. The characterization and identification of the reaction products form the basis for understanding the influence on the properties of the preparation and its risk-benefit evaluation. In this regard HPTLC coupled to MALDI-TOF-MS offers a suitable method.

For a more specific detection and an improved allocation of possible modifications preceding thin layer chromatographic separation was necessary. The simultaneous separation of numerous samples enabled a direct comparison of treated and non-treated α-amylase. Also effects of different parameters in tableting and storage were examined. Further supplemental information about the resulting products was obtained by staining with various derivatization reagents. Spots were detected in the enzyme-lactose derivatives by UV detection as well as by derivatization with the sugar-specific diphenylamine-p-anisidine being absent in the pure α-amylase control sample. For these spots divergent *m/z* values were assigned by direct HPTLC-MALDI-MS.

P-35

A further look at protein analysis - Useful applications of nano-ESI-MS for the identification of proteins and possible modifications

Lena MORSCHHEUSER
University of Hamburg, Hamburg, Germany, lena.morschheuser@chemie.uni-hamburg.de

Julia BILLER, University of Hamburg
Valeria REIM, University of Hamburg
Maria TRUSCH, University of Hamburg
Sascha ROHN, University of Hamburg

Within the last decade, proteomics devoted to map the entirety of proteins. By now focus is on detailed analytics of individual proteins. Of special interest is the characterization of posttranslational modified (PTM) proteins. To explore the diversity of the different modifications possible, there are only a few methods available. Using an off-line nanoESI-MS (e.g. TriVersa Nanomate® Advion, USA) provides the possibility to identify purified proteins and/or modifications, respectively.

Based on preliminary investigations with myoglobin, potential facilities were shown. Especially the ETD/PTR-experiment gains a benefit of the long term measurement by off-line nanoESI-MS. Furthermore, the coupling of MS with the mentioned interface leads to an innovative extraction technique: via a liquid extraction surface analysis (LESA) the substances of interest are leached out of a matrix e.g. a high-performance thin-layer chromatography (HPTLC) plate. Besides the analysis of intact proteins also a characterization of tryptic peptides might lead to the protein identification purposed or rather the clarification of modifications. Due to a higher resolution of single peptides by MS-analysis, a previous separation is beneficial. For this purpose the application of liquid chromatography (LC) or the above-mentioned HPTLC is successfully exercised. An advantage of using a HPTLC separation is a selective excitation of interesting spots (peptides) which where reckoned to bear a modification. High resolution and the opportunity of hyphenation with MS, the pre-separation of peptides by HPTLC exhibits an excellent alternative besides the commonly applied LC-separation.

P-36

LC-MS determination of the composition and biological activity of the drug Ukrain® based on *Chelidonium majus* L. alkaloids

Wioleta JESIONEK
Maria Curie-Skłodowska University, Lublin, Poland, wioleta.jesionek@o2.pl

Emilia FORNAL, Laboratory of Separation and Spectroscopic Method Appliacations, Center for Interdisciplinary Research, The John Paul II Catholic University of Lublin, Poland
Irena M. CHOMA, Department of Chromatographic Methods, M. Curie-Skłodowska University, Lublin, Poland
Barbara MAJER-DZIEDZIC, Department of Veterinary Microbiology, University of Life Sciences, Lublin, Poland
Agnes M. MORICZ, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

The greater celandine (*Chelidonium majus* L.) has very long history of use as a medicinal plant, especially in traditional medicine. The latex isolated from plant is a source of benzo-phenandrine, protoberberine and protopine alkaloids. The fresh extract from the plant inhibits the growth of fungi, bacteria, viruses and protozoa. Celandine related drugs have calming, analgesic, anticonvulsant and hypnotic effects. One of the therapeutic agents based on *Chelidonium majus* extract is *Ukrain*® semi-synthetic *Chelidonium majus* alkaloid derivative which has strong cytostatic and cytolytic properties.

In the presented work antimicrobial activity both of *Ukrain*®and *Chelidonium majus* L. alkaloid root extracts were investigated. Determination of bioactivity was carried out by TLC combined with microbiological detection, called direct bioautography (TLC-DB). The antimicrobial activities of the plant extracts against *Bacillus subtilis* were investigated.

Main fractions of *Ukrain*®visible under 254 nm and 366 nm in the sample, were isolated with methanol from TLC plate and analyzed by liquid chromatography coupled with a tandem quadrupole - time of flight mass spectrometer equipped with the HPLC-chip-cube (Agilent LC-Q/ToF 6538).

The presence of chelidonine, sanguinarine, chelerythrine, berberine, coptisine, allocryptopine were confirmed by the standards. Nano-LC-chip-Q/ToF-MS/MS analysis allowed to tentatively determine five compounds previously undetected in Ukrain stylopine, norchelidonine, hydroberberine, α-homochelidonine and protopine with high sensitivity and high mass accuracy. Biological detection provided information that compounds like sanguinarine, α-homochelidonine, cheletythtine are active against *Bacillus subtilis* strain.

P-37

Planar solid phase extraction and flow injection TOF/MS analysis - (non-)target screening for pesticide residue analysis

Wolfgang SCHWACK
University of Hohenheim, Institute of Food Chemistry, Stuttgart, Germany,
wolfgang.schwack@uni-hohenheim.de

Claudia OELLIG, University of Hohenheim, Institute of Food Chemistry

The recently introduced clean-up of QuEChERS extracts of fruit and vegetables by high-throughput planar solid phase extraction (HTpSPE) resulted in LC-MS analyses free of matrix effects [1]. The HTpSPE clean-up was now combined with time-of-flight mass spectrometry (TOF/MS) to perform a rapid screening for pesticides.

Therefore, a direct µL-flow injection analysis (µL-FIA)-TOF/MS strategy without chromatographic separation was developed, resulting in a single sample peak. The mass spectrum extracted from the entire peak represents the sum of all spectra collected during an alternative LC-MS run. The obtained mass spectra were rather free of co-extracted matrix compounds and easily allowed the identification of pesticides present in a sample by a target-oriented database search, based on their exact masses.

As compared to the common dispersive SPE (dSPE), the high efficiency of pSPE could clearly be shown by visual comparison of the respective mass spectra. The sum of all detected mass signals strongly supported the visual inspection.

Applying the µL-FIA-TOF/MS to blank pSPE extracts of apples, grapes, tomatoes and cucumbers, resulted in only one false-positive pesticide hit for tomatoes; but after dSPE clean-up, the number of false-positive hits ranged between 6 (grapes) and 20 (apples). From extracts spiked with seven representative pesticides, all were correctly identified after pSPE clean-up. After dSPE, however, 4, 3, 2, and 1 of the spiked pesticides were not recovered from tomato, cucumber, apple, and grape extracts, respectively.

The mass data obtained by our HTpSPE-µL-FIA-TOF/MS approach also can simply be searched for unknowns, just by listing all masses that are extracted from the sample peak mass spectrum, but not listed in the target database, allowing a real non-target screening from only one mass spectrum per sample.

[1] Oellig, C., Schwack, W., J. Chromatogr. A 1218 (2011) 6540-6547

P-38

Coupling HPTLC with MALDI-TOF MS for detection of flavonoids

Evelyn WOLFRAM
Zurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy, Waedenswil, Switzerland, wola@zhaw.ch

Ivana KROSLAKOVA, Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, CH-8820 Wädenswil, Switzerland

Direct coupling of HPTLC and MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry) has been so far successfully applied for the analysis of oligosaccharides (Dreisewerd 2006), lipids and their derivatives (Torretta *et al.* 2013, Stübiger 2009, Fuchs *et al.* 2008) as well as tetracycline antibiotics (Meisen *et al.* 2010).

Analysis of flavonoids, namely flavonol aglycones and glycosides, is very important for the examination of plant extracts for quality control purposes. The aim of this study was to establish an optimal MALDI-TOF MS method including choice of matrix, matrix concentration and deposition of matrix on the chromatographed silica coated aluminium backed TLC plate for analysis of flavonol aglycones and glycosides which are commonly found in plant extracts. Results of successful direct HPTLC coupling with MALDI-TOF MS for analysis of rutin, luteolin-7-O-glucoside, apigenin-7-O-glucoside and their aglycones including determination of limits of detection are presented. Further, the method will be applied for molecular mass determination of unknown flavonoid bands detected in HPTLC separated plant extracts.

P-39

Influence of the silica gel layer thickness on the quality of TLC-MALDI mass spectra of lipids

Hans GRIESINGER
Merck KGaA - Department of Bioanalytical Chemistry, Darmstadt, Germany,
hans.griesinger@merckgroup.com

Sabrina PRZYSAMBOR, Merck KGaA
Beate FUCHS, University of Leipzig
Jürgen SCHILLER, University of Leipzig
Katerina MATHEIS, Merck KGaA
Michael SCHULZ, Merck KGaA

Coupling TLC with mass spectrometry (MS) is a relatively new field of great interest [1]. MALDI mass spectra can be recorded directly from a developed TLC plate. The matrix is applied on the TLC plate after separation and then the TLC plate is mounted onto a TLC -MALDI adapter target followed by direct measurement. Limitations of TLC like spot capacity and sensitivity of UV detection or staining methods can be overcome by the spatial resolution and high sensitivity using MALDI-MS.

Here we present how the quality of TLC-MALDI mass spectra of lipids is influenced by the thickness of the separation layer. TLC-MALDI MS analysis of lipids benefits significantly from a reduced silica gel layer thickness.

It will be shown that the intensity of the matrix background signals can be significantly reduced and a sensitivity gain can be achieved if the silica gel layer thickness is decreased from 200 µm to 60 µm. This will be illustrated by using selected lipid mixtures with the focus on phosphatidylcholines (PC) and phosphatidylethanolamines (PE) which are abundant in biological samples.

The positive effect of reduced layer thickness can be explained by the reduced amount of DHB on thinner layers (2.1, 2.9 and 5.1 mg/cm² of DHB on the 60, 100 and 200 µm plates determined by weighing the plate before and after matrix application) and improved detectability because the analyte is closer to the surface.

[1] G. Morlock, W. Schwack TrAc 29 (2010) 1157-1171

P-40

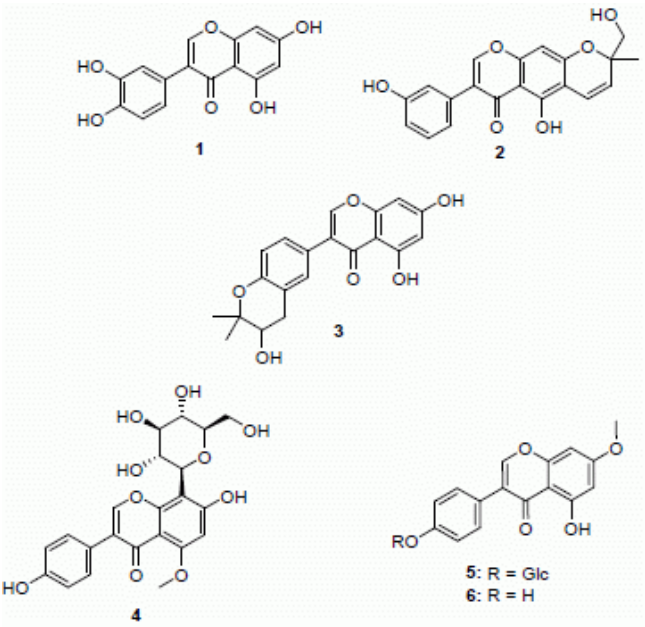
Rapid Identification of Antioxidant Compounds of *Genista saharae* Coss. & Dur. by Combination of DPPH Scavenging Assay and HPTLC-MS

Sabrina BOUTEFNOUCHET
Laboratoire de Pharmacognosie, U.M.R./C.N.R.S. 8638, Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Sorbonne Paris Cité, 4 Avenue de l'Observatoire, F-75006 Paris, France, Paris, France, sabrina.boutefnouchet@parisdescartes.fr

Djamila MERIANE, Laboratoire de Phytothérapie Appliquée aux Maladies Chroniques, Université Ferhat Abbas, Sétif 19000, Algeria
Grégory GENTA-JOUVE, Laboratoire de Pharmacognosie, Université Paris Descartes, France
Mohamed KAABECHE, Laboratoire de Phytothérapie Appliquée aux Maladies Chroniques, Université Ferhat Abbas, Algeria
Sylvie MICHEL, Laboratoire de Pharmacognosie, Université Paris Descartes, France

Genista species are sources of antioxidant phenolic compounds such as O- and C-glycosylflavonoids and isoflavonoids. A combination of a DPPH scavenging assay with HPTLC-MS, a fast and efficient method for identification of bioactive compounds, has been applied for evaluation of the radical scavenging activity of metabolites from *Genista saharae* Coss. & Dur. Different organs collected at various periods have been compared. Identification of antioxidant compounds was obtained by elution of the major DPPH-inhibition zones.

The resulting HPTLC-MS analysis under moderately polar conditions, coupled to the DPPH results led to the putative identification of two antioxidant isoflavone aglycones: 3',4',5,7-tetrahydroxyisoflavone (1) and ficuisoflavone (3), whereas polar migration conditions led to the identification of the glycosides 5-methoxy-4',7-trihydroxy-8-glucopyranosylisoflavone (4) and 4',5-dihydroxy-7-methoxyisoflavone-4'-O-β-D-gluco-pyranoside (5). Evaluation of percentage of inhibition of DPPH radical by the purified isoflavone 4 from the root extract showed that it affords a moderate contribution to the total radical scavenging activity of the extract.



P-41

Hyphenation of MALDI-Tof-MS methodology: A real improvement for plants extract characterization

Laëtitia FOUGERE
Univ.Orleans, CNRS, ICOA, UMR 7311, Orleans, France, laetitia.fougere@univ-orleans.fr

David DA SILVA, ICOA
Emilie DESTANDAU, ICOA
Benoit MAUNIT, ICOA
Claire ELFAKIR, ICOA

It was yet well established that TLC method is a method of choice for a rapid screening of the molecular families present in different plant extracts. However, even if fundamental information about the nature of these families could be obtained from hR_F, spot color and the UV absorption spectra, the unambiguous identification of molecules remains difficult and fastidious. Therefore, the coupling of TLC to MS seems to be a good alternative to obtain a more detailed characterization bringing as new information, the molecular mass of analytes.

Flavonoids were selected as relevant molecules to the development of a TLC/MALDI-TOF coupling, because these molecular families are in plants in quantity. This method developed on standards is promising. Therefore plants were chosen, beforehand characterized by LC/MS² or HRMS, and recovering flavonoid family (e.g. sea buckthorn - flavonol; apple - dihydrochalcone; cherry - anthocyanidin; licorice - flavone; mangostana - xanthone; rose and robinia - flavonol).

During this study, we were able to observe and confirm three main advantages for specific characterization of flavonoid compounds with this coupling (TLC/MALDI-MS). In apple, at the same hR_F, the detection method (MALDI-TOF) allows distinguishing phloridzin (*m/z* 435) and quercitrin (*m/z* 447). In the same way, rose ethanolic extract is rich in kaempferol derivatives, giving several spots green, the MS allows us to distinguish with more accuracy the nature of the different compounds. However, MS is limited for detection of isobar compounds as ellagic acid, quercetin and robinetin (*m/z* 301). Therefore, separative dimension obtained by chromatography remains essential in this situation. Moreover, the results obtained in the characterization of plant samples correlate well with studies already performed in our laboratory. In conclusion, this study confirms the feasibility TLC/MALDI-TOF coupling for the fast screening and characterization of different plants.

P-42

TLC-MALDI investigation of a multi-compound flu medication

Hans GRIESINGER
Merck KGaA - Department of Bioanalytical Chemistry, Darmstadt, Germany,
hans.griesinger@merckgroup.com

Sabrina PRZYSAMBOR, Merck KGaA
Katerina MATHEIS, Merck KGaA
Knut BEHREND, University of Hamburg
Michaela OBERLE, Merck KGaA
Michael SCHULZ, Merck KGaA

Thin layer chromatography (TLC) and mass spectrometry (MS) are well established and appreciated for their reproducibility, sensitivity and stability regarding separation and identification of analytes present in a given mixture. With TLC complex sample mixtures can be separated and impurities can be detected, but no information about mass is obtained. Therefore reference substances, staining methods or UV activity are required. Mass spectrometry in addition to TLC allows an excellent classification of substances by their mass. The combination of those two powerful techniques leads to a new level of information based on chromatographically separated and mass selected analytes as an orthogonal technique to LC-MS but with the advantage of visualization.

We show an application for TLC-MS on a 100 µm HPTLC silica gel MS-grade plate for MALDI (matrix assisted laser desorption/ionization). Characterization of small molecules in a multi-compound flu medication is shown. After chromatographical separation of all compounds, a mix of matrix with 2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid was applied via spraying the solution homogeneously onto the TLC-plate. By using a spray method in comparison to a standard dip process, wash-out of spots is reduced. The single compounds of the drug like paracetamol, caffeine and chlorphenamine could be identified with MS and UV detection. Those molecules are detected at *m/z* 150. This is a rather unusual mass range for MALDI-MS as most matrices ionize at *m/z* < 700 and the matrix signals may overlap the compound masses and can cause complex spectra. With the new plate type a good reproducibility and sensitivity could be shown and even analyses in this small mass range lead to high quality mass spectra.

P-43

Development of an off-line HPTLC-MS technique for plant extracts

Laure PASQUIER
LVMH Recherche, Parfums et Cosmétiques, Saint-Jean-de-Braye, France,
lpasquier@research.lvmh-pc.com

Lucille BLANCHOT, LVMH Recherche, Parfums & Cosmétiques
Jean François TRANCHANT, LVMH Recherche, Parfums & Cosmétiques

Our department of Ethnobotanical Innovation is dedicated to the development of plant extracts. Plants of interest are selected in a first time with local partners around the world. Then, their phytochemical composition is studied in order to find molecules with potential cosmetic properties (biological, coloring or structural).

HPTLC is an efficient analytical method to separate non volatile mixtures like plant extracts in order to identify different components. Sometimes, when mixtures are really complex or when two compounds are too similar in terms of polarity, accurate identification becomes really fastidious. In those cases, HPTLC can be connected to MS to allow structural characterization.

A very original approach has been developed by our department and consists of an off-line coupling. Now, the TLC-MS Interface has been used to elute spots from HPTLC plates and introduce them directly into the mass spectrometer in order to identify compounds of interest. This poster reports our results and a gives a global view of the different steps to follow during the analysis of molecules from plant extracts by HPTLC-MS. Indeed, once the molecule has been extracted from the HPTLC plate, a drop of its solution is laid down on a Direct Exposure Probe (DEP)'s filament and introduced directly into a Polaris Q mass spectrometer. Then, thanks to a Direct Probe Controller (DPC), the sample is desorbed from the filament, ionized and fragmented by Electronic Impact (EI).

Families of the vegetable kingdom have been studied. Up to now, standards have been tested on the entire coupling to optimize the detection of each family. Then, the method will be assessed on plant extracts. Best candidates for this analytical method seem to belong to the sterol family and give high signal at a very low concentration.

P-44

Surface analysis of microfabricated TLC plates by FTIR and ToF-SIMS to determine surface hydration

Mathew R. LINFORD
Brigham Young University, Provo, United States, mrlinford@chem.byu.edu

Cody CUSHMAN, Brigham Young University
Supriya S. KANYAL, Brigham Young University
David S. JENSEN, US Synthetic Corp.
Andrew E. DADSON, US Synthetic Corp.

The surface chemistry of a stationary phase strongly affects the efficiency of a separation on it. In the case of SiO_2, silicon may be present in siloxane (Si-O-Si) linkages, and it may also be hydrated, showing isolated, germinal, or vicinal silanol (SiOH) groups. This hydration of SiO_2 has been studied in HPLC. This work showed that (i) isolated silanol groups in a silica stationary phase result in poor chromatography, and (ii) good hydration of the silica with hydrofluoric acid (HF) or ammonium hydroxide can reduce the number of isolated silanols so that good chromatography can be performed. The traditional figure of merit for the quality of silica for chromatography has been the peak position of the silanol signal in FTIR at ca. 3740 cm^{-1}. If the silanol peak is above this value, isolated silanols are present in excess and good chromatography is not possible. Conversely, a peak position below this value indicates that a well-hydrated material has been produced. However, in our work in this space, we have found that HF treatment of our TLC plates is so effective that the resulting silanol peak position cannot be well measured. Accordingly, we have turned to another analytical technique to allow us to probe our surfaces: time-of-flight secondary ion mass spectrometry (ToF-SIMS). The resulting $SiOH^+/Si^+$ peak ratio from ToF-SIMS appears to correlate well with the 3740 cm^{-1} peak from FTIR and allow us to successfully assay the quality of our HF treatments.

P-45

Inkjet application, chromatography and mass spectrometry of sugars on nanostructured thin films

Gertrud E. MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Simone KIRCHERT, Justus Liebig University Giessen
Zhen WANG, University of Alberta, Edmonton, Canada
Michael T. TASCHUK, University of Alberta, Edmonton, Canada
Steven R. JIM, University of Alberta, Edmonton, Canada
Michael J. BRETT, University of Alberta, Edmonton, Canada and NRC National Institute for Nanotechnology, Edmonton, Alberta T6G 2M9, Canada

Potential improvements in planar chromatography performance motivate recent trends towards miniaturization. Ultrathin-layer chromatography (UTLC) can reduce analysis time and expense by enabling separations over short migration distances that require only small solvent and sample volumes. One recent technique for producing ultrathin layers is glancing angle deposition (GLAD). Aligned macropores in these media produce interesting separation capabilities. So far the use of GLAD UTLC plates has been limited to model dye systems, rather than realistic analytes. The goal of the presented research was to transfer a HPTLC sugar analysis method to GLAD UTLC plates using Office Chromatography.

A commercial inkjet printer applied very small and sharp bands of water-soluble sugar solutions with volumes between 7 nL and 15 nL to the GLAD UTLC films. Effective transfer of the HPTLC method to GLAD UTLC required stationary phase nanostructure optimization, mobile phase adjustment, and adaptation of the sugar visualization technique. Appropriate derivatization enabled observation of otherwise clear zones with ~ 100 ng applied sugar mass. Based on the signal-to-noise measurements of the respective peaks, LODs were estimated to be ~ 10 ng per zone. We also successfully separated sugars extracted from a commercial chocolate sample, representing the first reported separation of 'real-world' samples analyzed by GLAD UTLC. We further performed electrospray ionization mass spectroscopy (ESI-MS) of underivatized sugars spotted onto GLAD UTLC plates, demonstrating the possible advantages of coupling GLAD UTLC with MS.

P-46

Ultrathin-layer Chromatography on SiO_2, Al_2O_3, TiO_2 and ZrO_2 nanostructured thin films

Gertrud E. MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Julia WANNENMACHER, University of Hohenheim, Stuttgart, Germany
Steven R. JIM, University of Alberta, Edmonton, Canada
Michael T. TASCHUK, University of Alberta, Edmonton, Canada
Michael J. BRETT, University of Alberta, Edmonton, Canada and NRC National Institute for Nanotechnology, Edmonton, Alberta T6G 2M9, Canada

Although planar stationary phases continue to evolve, few exploit the unique properties of non-traditional inorganic oxides. This work explored the chromatographic behaviours of nanostructured thin films made of SiO_2, Al_2O_3, TiO_2, and ZrO_2. Glancing angle deposition (GLAD) was used to engineer nanostructured thin films in these oxides for ultrathin-layer chromatography (UTLC). All of the thin films had similar high surface area anisotropic channel-like structures but exhibited different chromatographic properties in separations of water soluble food dyes and carotenoids. TiO_2 and ZrO_2 proved to be especially interesting since food dye separations on these materials could be modified by simple post-deposition UV exposure and oxidation heat treatments.

In general, analyte retention was increased by UV irradiation but was decreased by oxidation. Separation performance was evaluated by calculating figures of merit using advanced video instrumentation and time-resolved UTLC methods designed for GLAD UTLC media. As per the Office Chromatography concept, consumer inkjet printers were used to apply food dye mixtures in sharp zones. Some of the best GLAD UTLC performance was achieved by the combining Office Chromatography, alternative oxides, and time-resolved UTLC. Theoretical plate heights below 4 µm and detection limits below 2 ng per zone for the food dye tartrazine were measured. For the first time, GLAD UTLC separations were successfully coupled with ESI-MS, allowing separation of a food dye mixture and subsequent mass spectrometric analysis within a few minutes. This research demonstrates the chromatographic merits of alternative oxides in GLAD UTLC.

P-47

Office Chromatography: Precise printing of sample solutions on miniaturized thin-layer phases

Tim HÄBE
Justus Liebig University Giessen, Giessen, Germany,
Tim.T.Haebe@ernaehrung.uni-giessen.de

Gertrud MORLOCK, Justus Liebig University Giessen

Office chromatography merges new achievements in office technologies with future trends in planar chromatography. Its full analytical power is based on reproducible analytical tools and automated devices. Application via ink jet printing of solutions on planar stationary phases was shown for food dye samples [1], derivatisation reagents [2] and whole cells [3]. Using high resolution scanner and picture evaluation software quantitative determinations are possible, latest shown for hydrazine [4] and ochratoxin A [5]. A new challenge is the precise application of samples on miniaturized or nanostructured layers. These ultra-thin layer phases are more sensitive and tolerate only a minimum of solvent. Therefore a thermal ejecting Bubble Jet printer was modified for exact application.

Modifications include the removal of unnecessary parts of the printing unit and a plate guide system for save and precise plate handling. The original cartridge system was replaced by filter vials for small sample volumes and for handling different viscous solutions. The application range of the print head was determined in different modes and its purge unit was improved to avoid sample diversion. The worthwhile application range of the print head was determined as 5-50 nL per printjob depending on application area and resolution. This application method was used for sample deposition on different miniaturized thin layer substrates. Office chromatography involves several advantages such as very low reagent consumption by a clean, versatile and cheap working station.

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P-48

Fast, microfabricated, normal phase TLC plates based on carbon nanotube forest scaffolds

Mathew R. LINFORD
Brigham Young University, Provo, United States, mrlinford@chem.byu.edu

Andrew E. DADSON, U.S. Synthetic Corp.
Gertrud MORLOCK, Justus Liebig University Giessen
Supriya S. KANYAL, Brigham Young University
Cody CUSHMAN, Brigham Young University
Tim HÄBE, Justus Liebig University Giessen

We have recently demonstrated that patterned carbon nanotube (CNT) forests can act as a template for the preparation of TLC plates. The resulting plates show higher efficiencies than commercial ones with significantly shorter run times. We have explored five methods for preparing these plates. The first is the low-pressure chemical vapor deposition (LPCVD) of silicon onto the CNTs, followed by oxidation to make SiO_2 and remove the CNTs. The resulting SiO_2 was ideal for chromatography and white for easy analyte visualization. However, this process resulted in volume distortion which led to irreproducibility in the microfabrication process.

To overcome this, we deposited a thin (< 5 nm) film of carbon on the CNTs, followed by atomic layer deposition of alumina (ALD), followed by a fast, pseudo (ψ) ALD of SiO_2. This approach led to undistorted features, and the ψ ALD process deposited ca. 10 nm of SiO_2 per cycle instead of the <1 nm/cycle deposited by traditional ALD. However, we discovered the aluminum catalyst used in the ψ ALD process was accessible to the analytes and compromised the resulting chromatography. An amino silane mololayer on these plates allowed good separations to be performed. In a third advance, patterned CNTs were treated with ozone, and SiO_2 was deposited directly on them by ψ ALD. While this third process required fewer steps than the second one, the Al contamination remained. Our fourth approach was to deposit SiO_2 by true ALD. The resulting plates showed no Al contamination, no bonded phase was necessary, and a test mixture of dyes could be separated on them. However, true ALD is slow.

We believe that our fifth, and latest, approach satisfies all the requirements of a manufacturable process. Here, silicon nitride is conformally deposited on CNTs by LPCVD. Upon oxidation, the CNTs are removed and the silicon nitride is converted to SiO_2. The resulting plates show outstanding performance.

P-49

Detection of antibacterial bell pepper ingredients by TLC/HPTLC-direct bioautography

Ágnes M. MÓRICZ
Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, moricz.agnes@agrar.mta.hu

F. Gábor BOGNÁR, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences
Péter G. OTT, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences

Bell pepper (*Capsicum annuum*) can be severely affected by the pathogenic bacterium *Xanthomonas euvesicatoria* causing leaf spot disease. Relying on a bioassay-guided methodology, we examined plant metabolites as potential mediators of a novel antibacterial resistance type in pepper lines having or lacking this resistance trait. Ethyl acetate extracts were found to be optimal to obtain non-macromolecular metabolites from samples taken at three time points from different parts of resistant plants and their susceptible counterparts that were infected with *X. euvesicatoria* by leaf injection. The components of the samples were separated by TLC/HPTLC.

The antibacterial activity of separated compounds has been tested by direct bioautography (layers were submerged in bacterium suspension) against the phytopathogens *X. euvesicatoria* and *Pseudomonas syringae* pv. *maculicola*, the rhizosphere inhabitant *Bacillus subtilis* and the marine *Aliivibrio fischeri*. The visualization of the bioautogram was performed with methylthiazolyldiphenyl-tetrazolium (MTT) vital stain or by the detection of luminescent light emitted by naturally or bioluminescence-tagged transgenic bacteria. We found at least four different compounds active against *X. euvesicatoria*, and one of them visibly increased at 6 h post-infection, both in resistant and susceptible plants. These components also inhibited *B. subtilis*, and two of them had a negative effect on *P. s.* pv. *maculicola* and *A. fischeri*. The components that showed antibacterial effect were transferred for further analysis by spectroscopic and spectrometric techniques.

P-50

Is HPTLC method a valuable solution for free radical scavenging activity evaluation? A comparison of two quantitative approaches on silica plates with the reference method in solution

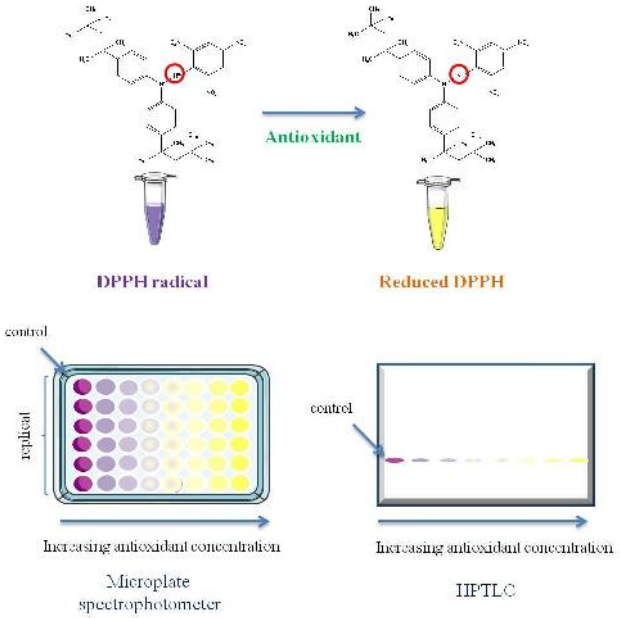
Anne-Sylvie FABIANO-TIXIER
GREEN Team extraction, Avignon, France, anne-sylvie.fabiano@univ-avignon.fr

Ruiz KARINE, GREEN Team extraction
Bernard-savary PIERRE, Chromacim sas
Chemat FARID, GREEN Team extraction

Scavenging of DPPH free radical is the basis of a common antioxidant assay. This method mesuring the antioxidant activity with the free radical scavenging of DPPH. The samples properties are reported in terms of EC50 which is the concentration to get 50% of the total reaction with DPPH, and their reduction kinetics. The DPPH radical has a deep violet color in solution corresponding to a high absorption spectrum in visible light with a maximum at 520 nm. After the neutralization reaction, the DPPH solution becomes colorless or pale yellow.

The aim of this work is to report the development of a new method for evaluation of DPPH reduction by HPTLC. In this work, chemistry-based assays, as reduction or scavenging of DPPH with different antioxidant like ascorbic acid, romarinic acid etc are realized and discussed.

The HPTLC method validation shown on this poster is based on the comparative study of the antioxidant potential profile results given by two different HPTLC methods and the results of spectrometry in 96-well micro-plate reference method for a selection of molecules known for their radical scavenging properties.



P-51

Determination of biological active compounds in *Asteraceae* tinctures by TLC-DB and LC-MS methods

Wioleta JESIONEK
Maria Curie-Skłodowska University, Department of Chromatographic Methods, Lublin, Poland, wioleta.jesionek@o2.pl

Agnes M. MORICZ, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
Peter G. OTT, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
Emilia FORNAL, Laboratory of Separation and Spectroscopic Method Appliacations, Center for Interdisciplinary Research, The John Paul II Catholic University of Lublin, Poland
Irena M. CHOMA, M. Curie-Skłodowska University
Bela KOCSIS, Institute of Medical Microbiology and Immunology, Medical School, University of Pecs, Hungary
Györgyi HORVATH, Department of Pharmacognosy, Medical School, University of Pecs, Hungary

Matricaria recutita L. (chamomile) and *Achillea millefolium* L. (yarrow) belong to *Asteraceae* family and they are very common in meadows, pathways and home gardens. Their preparations e.g. infusions or alcohol extracts are widely used as remedies to treat many health problems. Both chamomile and yarrow have anti-inflammatory, antimicrobial and antioxidant properties. Besides, chamomile has anti-spasmodic and sedative properties while yarrow is used for digestive problems, diabetes, hepato-biliary diseases and amenorrhea symptoms.

Most of microbiological assays used nowadays give information only on an activity of a whole extract under investigation and do not provide information on its composition and components activities. The problem can be solved by combination of two techniques: thin-layer chromatography with microbiological detection i.e. direct bioautography (TLC-DB). TLC is an ideal separation method for analyzing samples in complicated matrices, like plant extracts. TLC-DB allows for separation of tested compounds and verification of their biological activity directly on a TLC plate. However, to obtain complete information on investigated compounds like structures, it is necessary to use for other techniques e.g. LC-MS.

Our research work was divided into few steps. First of them concerned optimization of TLC separation and visualization of the main compounds with the following reagents: ethanolic KOH for coumarins, NP/PEG reagent for polyphenols and anisaldehyde/sulphuric acid for steroids and terpenes. Then, TLC-DB was used for biological detection against several bacteria strains: two soil bacteria *B. subtilis* and *M. luteus*, four human patogenic strains: *S. epidermidis*, *S. aureus*, methicillin-resistant *S.aureus* and *E. coli*. Additionally, two plant pathogenic strains: *P. syringae* pv. *maculicola* and *X. vesicatoria* as well as *A. fischeri* were tested. Isolated biologicaly active fraction were investigated by LC-MS/MS technique.

P-52

Improved bioautographic xanthine oxidase assay: combining HPTLC separation and activity assessment for phytopharmaceutical research

Sarah BRÄM
Zurich University of Applied Sciences (ZHAW), Institute of Biotechnology, Phytopharmacy, Wädenswil, Switzerland, Waedenswil, Switzerland, wola@zhaw.ch

Beat MEIER, Zurich University of Applied Sciences (ZHAW)
Kalina DANOVA, Bulgarian Academy of Science, Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria
Evelyn WOLFRAM, Zurich University of Applied Sciences (ZHAW)

Bioautography offers a rapid and simple tool for screening of secondary metabolite profiles of medicinal plants by HPTLC combined with screening of potential health beneficial activities. The aim of this work has been to optimize and validate a bioautographic Xanthine oxidase (XO) Inhibition assay first described by Ramallo *et al.* (2006) to obtain reliable and reproducible results. Xanthine oxidase (XO) catalyses the oxidation of hypoxanthine and xanthine to uric acid under the formation of superoxide radicals and hydrogen peroxide. XO inhibition is a valuable assay in bioactivity screening for active ingredient discovery.

The assay procedure has been improved by optimizing concentrations of redox dye, substrate, enzyme activity as well as the buffer conditions in combination with low gelling agarose and adjustment of incubation time and temperatures according to the XO thermal activity characteristics. XO inhibitory effects were visualised as white zones on a purple coloured thin layer chromatogram based on the reaction of superoxide radicals with nitroblue tetrazolium chloride. The visual detection limit of the competitive XO inhibitor allopurinol was 45.4 ng. Extracts of *Camellia sinensis* and *Artemisia alba* showed also to contain constituents with XO inhibitory activity, that could be visually detected down to an applied amount of 10 µg dry weight (dw) for *C. sinensis* extract and 100 µg dw for *A. alba*.

From the results it can be concluded, that the improved bioautographic XO inhibition assay is a rapid and valid research tool for assessment of active secondary metabolites from medicinal plants.

[1] Ramallo I.A., Zacchino S.A., Furlan R.L.E. (2006). Phytochemical Analysis 17: 15-19.

Acknowledgements: We thank SNF for the financial support of this work within the Bulgarian-Swiss Joint Research Project Project No. IZEBZ0_142989; DO2-1153

P-53

p-YES - building a bridge between chemical analysis and biological effects

Sebastian BUCHINGER
Federal Institute of Hydrology, Koblenz, Germany, buchinger@bafg.de

Denise SPIRA, Federal Institute of Hydrology, Germany
Kathrin BROEDER, Federal Institute of Hydrology, Germany
Michael SCHLUESENER, Federal Institute of Hydrology, Germany
Thomas TERNES, Federal Institute of Hydrology, Germany
Georg REIFFERSCHEID, Federal Institute of Hydrology, Germany

The identification of compounds with advers biological effects in complex environmental samples is one of the main challenges in ecotoxicology and environmental chemistry. Chemical analysis alone does not provide information about effects per se, on the other hand bioassays detect the overall effect of a mixture and it is not possible to assign an observed effect to single compounds. Therefore, chemical separation techniques are often combined with bioassays in effect directed analysis.

The planar Yeast Estrogen Screen (p-YES) which combines compound separation by HPTLC with a specific bioassay for the detection of estrogenic effects was recently developed. This combination resulted in a rapid and robust method for the detection of estrogenic effects in mixtures with demanding matrices and even in toxic samples. Furthermore, the method supports compound identification by the analysis of the thin layer chromatogram and the direct accessability of the analytes for a subsequent analysis by mass spectrometry. The presented work shows various applications of the p-YES for the analysis of surface waters, river sediments and waste water for estrogenic effects.

P-54

Development of a bioautographic HPTLC method for identification and quantitation of estrogen-effective compounds as a novel non-target method

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Ines KLINGELHÖFER, Justus Liebig University Giessen

Food, food supplements, cosmetics or commodities can be unknowingly pharmaceutically active. Traditional medicines are supposed to be active. Environmental samples can be hazardous. For the first time, microorganisms detect quantitatively and effect-directed single bioactive compounds in complex samples separated on a planar chromatogram in parallel. The bioassay workflow was substantially improved. Sharp-bounded, narrow compound zones linking to the biological effect were obtained. This advancement was demonstrated for quantitation of food for endocrine disrupting compounds (EDCs) reacting with the human estrogen receptor and detectable down to the femtogram-per-zone range in the chromatogram (ng/kg range in samples).

Exemplarily the discovery of up to 6 endocrine disrupting compounds (EDCs) was shown in seven propolis samples. One of these compounds, preliminarily assigned as CAPE, was verified through HPTLC-ESI-MS via the elution-head based TLC-MS Interface. To demonstrate the general applicability of this substantial improvement, it was successfully transferred to another bioassay linked with chromatography. This new era of quantitative direct bioautography will accelerate the scientific understanding in a wide application field via the access to fast and reliable information on effect-causing components directly in complex samples.

P-55

Fast HPTLC-direct bioautography using *Bacillus subtilis* for screening of antimicrobial components in plant extracts

Maryam JAMSHIDI-AIDJI
Justus Liebig University Giessen, Giessen, Germany,
Maryam.Jamshidi-Aidji@ernaehrung.uni-giessen.de

Gertrud MORLOCK, Justus Liebig University Giessen

Hyphenation of HPTLC with direct bioautography (HPTLC-DB) using *Bacillus subtilis* can play a key role for target identification of antibacterial substances in botanicals. Two limits are encountered: first, the method could be strongly affected by all factors influencing the growth rate of the bacteria since *B. subtilis* is incubated on the developed plates for a long time; secondly, the steps of this method are very time consuming, *i.e.* incubation of bacteria in broth and on plate as well as visualization. Until now, there are only few studies to tackle these issues.

The aim of the current study is to present an optimized, rapid and straightforward technique for HPTLC-DB using *B. subtilis*. To achieve this goal, growth rate of the bacteria, as dependent variable, was compared in two different broths (M1 and M2) with various broth culturing times (6 and 8 h) and plate incubation times (1, 2 and 3 h). The extract of *Ocimum basilicum* L. was used as test sample. HPTLC plates silica gel 60 were developed with toluene – ethyl acetate - formic acid and immersed into the different mediums. After incubation, plates were revealed with MTT solution, and the obtained fingerprints of antibacterial zones were compared.

The results showed that the medium can influence the whole process. The growth rate measured was higher in the broth M1, rich in nutrients, if compared to M2. Consequently, the incubation time for M1 (to obtain OD ≥0.4) was shorter than for M2. Additionally, the higher viscosity of M1 reduced running off the culture medium from the plate surface during immersion. After the visualization step, the background color on the plates, and thus the contrast to the antibacterial zones, was much stronger with M1 than with M2. It was concluded that the procedure using M1 with 6 h broth incubation time combined with 2 h plate incubation time took the shortest time and generated the sharpest inhibition zones.

P-56

Effect-directed analysis of *Salvia officinalis*

Maryam JAMSHIDI-AIDJI
Justus Liebig University Giessen, Giessen, Germany,
Maryam.Jamshidi-Aidji@ernaehrung.uni-giessen.de

Christina STENFERT-KROESE, Justus Liebig University Giessen
Gertrud MORLOCK, Justus Liebig University Giessen

Pharmacological studies of *Salvia officinalis* showed that it contains a variety of chemical constituents with biological activity. Hyphenation of HPTLC with bioassays, microchemical detection and MS enabled targeted identification of substances from plant extracts by HPTLC-UV/FLD/Vis-EDA-MS. Analysis was performed on HPTLC plates silica gel 60 with a mixture of toluene, ethyl acetate, methanol and formic acid. For universal microchemical derivatization, anisaldehyde sulfuric acid was used. For selective microchemical derivatization, the diphenylpicrylhydrazyl (DPPH*), fast blue salt B, Neu's and 2, 4-dinitrophenylhydrazine reagents were used to detect specific functional groups of phytochemical interest. Effect-directed detection with the Aliivibrio fischeri and *Bacillus subtilis* bioassays were carried out directly on the developed plates to detect A. fischeri bioactive and antibiotic compounds, respectively.

The presence of rosmarinic acid was confirmed by overlapped application, microchemical derivatization and HPTLC-ESI-MS. With the DPPH* reagent, the ethanolic *Salvia* extract showed many strong antioxidative compounds. One of major response was rosmarinic acid. Based on the chromatographic fingerprint, strong antioxidants in *Salvia* were polar to middle-polar. With regard to the *A. fischeri* bioassay, strong bioactive substances were mostly non-polar, except for rosmarinic acid. Two polar compounds, which enhanced the luminescence, were also evident. The *Bacillus subtilis* bioassay enabled the detection of nonpolar antibacterial substances. These nonpolar antibiotics were separated with an optimized apolar mobile phase system based on toluene, ethyl acetate and formic acid. The unknown antibiotics were characterized by ESI-MS via the TLC-MS Interface.

P-57

Antimicrobial activity of *Fragaria* x *ananassa* Duch. and *Solanum lycopersicum* L. by TLC

Yurina KWACK
Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea, Seoul, South Korea, greenrina.k@gmail.com

Dong Sub KIM , Department of Plant Science, Seoul National University, Seoul 151-921, Korea

Strawberry (*Fragaria* x *ananassa* Duch.) and tomato (*Solanum lycopersicum* L.) are known as vegetables containing many secondary metabolites. Previous studies also showed that their non-edible parts include higher contents of secondary metabolites than edible parts. For instance, Ellagic acid content of strawberry leaves was 12.96 ± 2.30 mg/g DW while that of petioles and fruits was below 6 mg/g FW [1]. The contents of α- and dehydro-tomatine in tomato leaves were 0.89 and 1.42 mg/g FW, respectively, and was the greatest content among all the tested parts [2].

We tested antimicrobial activity of parts of the two vegetables extracted by methanol, acetone, dichloromethane, and hexane against *Phytophthora capsici* as secondary metabolites in non-edible parts of strawberry and tomato plant were higher than those in edible parts. The selected method was TLC combined with microbiological detection, called direct bioautogrphy. The extracts were developed with polar eluent system (ethyl acetate:methanol:water = 8:1:1). After staining with 2 mg/ml solution *p*-iodonitrotetrazolium violet, antimicrobial activity was observed as white inhibition zones on a red background.

[1] D.S. Kim, H. Na, Y. Kwack, S.K. Kim, J.W. Heo, C. Chun, Kor. J. Hort. Sci. Technol. 31, 2013, 224-230.
[2] D.S. Kim, H. Na, Y. Kwack, C. Chun, Kor. J. Hort. Sci. Technol. (in press)

P-58

Screening of Mediterranean and Alpine plant extracts for tyrosinase inhibition by an HPTLC autobiographic assay

Anita ANKLI
CAMAG Laboratory, Muttenz, Switzerland, anita.ankli@camag.com

Camille MAGNENAT, School of pharmaceutical sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland
Claudia SIMOES-PIRES, University of Geneva, University of Lausanne
Judith TAIBON, University of Innsbruck, Institute of Pharmacy/Pharmacognosy, CCB-Center of Chemistry and Biomedicine Innrain 80/82, 6020 Innsbruck, Austria
Vasiliki-Ioanna BOKA , Department of Pharmacognosy and Natural Product Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece
Ekaterini ARGIROPOULOU, University of Athens, Greece
Hermann STUPPNER, University of Innsbruck, Austria
Nektarios ALIGIANNIS , University of Athens, Greece
Leandros SKALTSOUNIS , University of Athens, Greece
Muriel CUENDET, University of Geneva, University of Lausanne
Eike REICH, CAMAG Laboratry, Muttenz, Switzerland

The main aim of the EU funded project with the acronym NATPROTEC is the discovery and development of innovative products for the cosmetic industry by using emerging and environmentally friendly technologies. The scientific concept involves the discovery of bioactive natural products originating from the Mediterranean and Alpine biodiversity. The objectives are implemented through an extended exchange of researchers from Academia to Industry and *vice versa*. In the first half of the project a broad spectrum of bioassays was incorporated for the evaluation of skin-protecting, anti-aging and anti-hyperpigmenting activity.

The evaluation of the anti-hyperpigmenting action of the extracts was performed through the measurement of tyrosinase inhibition. For this, an HPTLC autobiographic assay was developed and optimized. Around 300 plant species were collected and extracted by various partners with a polar and an apolar solvent to produce about 600 extracts. Depending on the kind of extraction (consecutive/non-consecutive), 15 or 25 µg of extract were applied on the plate, which was then developed using an appropriate solvent mixture.

Based on the HPTLC bioautographic assay, 1.8 % of the tested extracts contained substances exhibiting strong tyrosinase inhibition (and 3.3 % had a moderate activity). Mainly plant species from the *Cistaceae*, *Ericaceae*, *Fabaceae*, *Moraceae*, *Rosaceae*, *Saxifragaceae*, and *Melianthiaceae* families showed promising results. Most of the active compounds were found in the polar extracts.

P-59

Identification of antibacterial *Tanacetum vulgare* L. essential oil component by the use of HPTLC-bioautography-MS

Agnes M. MORICZ
Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, moricz.agnes@agrar.mta.hu

Andrea BOSZORMENYI, Semmelweis University, Faculty of Pharmacy, Department of Pharmacognosy, Budapest, Hungary
Peter G. OTT, Hungarian Academy of Sciences, Budapest, Hungary
Gertrud E. MORLOCK, Justus Liebig University Giessen, Giessen, Germany

Common tansy (*Tanacetum vulgare* L.) from the family *Asteraceae* is a native plant in Europe. Tansy was formerly used to treat intestinal worms, rheumatism, digestive problems, fevers, sores, as well as flavouring for foods. It is an aromatic plant; its leaves and tubular yellow flowers are rich in volatile compounds. Tansy essential oil is an antibacterial, antifungal, anti-inflammatory, antihistaminic, antiviral, febrifuge, insecticide, sedative and a vermifuge extract. However, because it can contain toxic thujone, its use is regulated and is not suggested without medical supervision.

Tansy plants were grown in greenhouse in Budapest using seeds collected in Hungary at Lake Balaton. The oil was steam distilled from the fresh flower of the 2-years old plants. According to GC-MS analysis, the oil does not contain thujone. The antioxidant and antibacterial oil components were searched by the use of HPTLC combined with the DPPH* assay and direct bioautography using soil bacterium *Bacillus subtilis*, marine bacterium *Aliivibrio fischeri*, as well as plant pathogen bacteria *Xanthomonas vesicatoria* and *Pseudomonas syringae* pv. *maculicola*. The main active compound was further characterized and identified as chrysanthenol by means of UV densitometry (UV spectrum), and optionally, DART SVPA-MS, LC-DAD-MS and SPME-GC-MS.

A. M. Moricz spent 2 months of a DAAD scholarship at JLU Giessen, Germany. Thanks to I. Klingelhöfer, S. Krüger, M. Jamshidi-Aidji and T. Häbe for their assistance. This work was also supported by OTKA (PD83487) and Bolyai grants.

P-60

Detecting quorum sensing compounds using TLC bioautography

Margaret RAE
National University of Ireland Galway, Galway, Ireland, margaret.rae@nuigalway.ie

Andrew MCKIERNAN, University of Ireland, Galway

In this work, we used the luminescent gram-negative bacterium *Allivibrio fischeri* in an attempt to detect eleven different acyl homoserine lactone (AHL) quorum sensing compounds using the TLC bioautography technique. We demonstrate that some AHLs could be detected by this method in our laboratory and show that selection of the stationary phase/mobile phase gave different results in terms of AHL detection.

P-61

Troubleshooting *Aliivibrio fischeri* bioassay for crude marine extracts

Margaret RAE
Ryan Institute, National University of Ireland, Galway and Marine Biodiscovery Laboratory, Marine Institute, Rinville, Oranmore, Co. Galway., Galway, Ireland, margaret.rae@nuigalway.ie

Oisìn MCMANAMON, National University of Ireland, Galway
Deniz TASDEMIR, School of Chemistry, National University of Ireland, Galway
Oisìn MCMANAMON, Marine Biodiscovery Laboratory, Marine Institute, Rinville, Oranmore, Co. Galway

The luminescent gram-negative bacterium *Aliivibrio fischeri* has been used a predictor of bioactivity in TLC bioautography assays. However, the assay when applied to crude marine extracts throws up many positive results. This work is an attempt to critically look at the applicability of the bioassay to crude extracts. We examine the crude extracts and fractions made from liquid-liquid partitions. In addition, we compare the results of the TLC bioautography technique with that of cell viability assays.

P-62

Identification of antimicrobial and anti-quorum sensing components from South African propolis using HPTLC-bioautography

Deepak KASOTE
Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa, KasoteDM@tut.ac.za

Aijaz AHMAD, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa
Weiyang CHEN, Tshwane University of Technology, Pretoria, South Africa
Alvaro VILJOEN, Tshwane University of Technology, Pretoria, South Africa
Sandy VAN VUUREN, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, Parktown, Johannesburg 2193, South Africa

HPTLC-bioautography is becoming powerful tool for rapid identification of bioactive components from crude natural products. Propolis is a resinous natural product composed of plant exudates produced by honeybees. The anti-infective property of propolis is well known and the composition of propolis is variable with the floristic composition in the area of the beehive. Some studies have reported the antimicrobial properties of South African propolis, however, this study is the first to correlate the bioactivity to a specific compound in South African propolis.

Hence, the aim of the study was to identify antimicrobial and anti-quorum sensing components from South African propolis using HPTLC-bioautography in tandem with UPLC-QTOF-MS/MS. Silica gel pre-coated aluminum plate was used for HPTLC bioautography analysis and the separation was carried out by using methanol: water (60:40, v/v). HPTLC bioautographic assay was performed against two Gram-positive bacteria, Gram-negative bacteria, and one fungal isolates. Similarly, HPTLC bioautographic anti-quorum sensing potential of propolis sample was further assessed using *Chromobacterium violaceum*. Cinnamic acid and pinobankin-3-*O*-pentanoate or 2-methylbutyrate was found to be active against all the tested Gram-positive and Gram-negative bacteria, and pinocembrin was identified as an only active antifungal compound. Caffeic acid was identified as the anti-quorum sensing component in South African propolis.

P-63

Chemical composition and antibacterial activity of medicinally useful essential oil from the rhizomes of *Alpinia allughas* Rosc.

Nikhil KUMAR
Monad University Hapur-245101, (U.P.), India, Hapur, India, nikhilkaushik01@gmail.com

Charu AGARWAL, Galgotias University, G.B.Nagar (U.P.), India
Vijayata, Meerut College Meerut, (U.P.), India

To obtain much better information about the medicinally useful essential oil of the rhizome of *Alpinia allughas* Rosc., the oil from the rhizome collected from Guwahati, Assam, has been isolated by hydrodistillation and analysed by GC and GC/MS. Twenty-eight compounds representing 95.14% of the oil have been identified. Norisoprenoid related antioxidant β-ionol is the major component (15.53%). In a rare observation, the sesquiterpenes & derivatives (39.47%) and monoterpenes & derivatives (39.42%) are almost in equal amounts. Among the monoterpenes & derivatives, the terpinen-4-ol (11.51%), 1,8-cineole (8.91%) and fenchyl acetate (7.55%) are main. Among sesquiterpenes & derivatives, the major components are found to be β-selinene (13.77%) and γ-elemene (6.30%). The oil shows good antibacterial activity against *Staphylococcus aureus*.

P-64

HPTLC hyphenated with bioassays for the screening of bioactive constituents of Serbian *Salicaceae* bud extracts

Gertrud MORLOCK
Justus Liebig University Giessen, Chair of Food Science, Giessen, Germany,
Gertrud.Morlock@ernaehrung.uni-giessen.de

Salim HAGE, Justus Liebig University Giessen

Salicaceae tree buds are increasingly used in gemmotherapy, and moreover importantly contribute to the production of propolis by European bee *Apis mellifera* L. An HPTLC method hyphenated to several bioassays was developed, with the purpose to detect chemical constituents with pharmacological activity.

21 bud extracts from *Populus* and *Salix* species were obtained from Serbia. Analysis was performed on HPTLC plates silica gel 60 with a mixture of hexane - ethyl acetate - acetic acid. For microchemical derivatization of phenolic compounds, Neu's reagent with polyethylenglycol was used. The extracts were also subjected, directly on the developed plates, to the following bioassays: 1° effect-directed detection with *Bacillus subtilis* and *Aliivibrio fischeri*, to detect antimicrobial and bioactive compounds, respectively; 2° planar yeast estrogen screen (pYES) for substances with phytoestrogenic activity; 3° Marston's colorimetric method for cholinesterase inhibitors.

At least two (unpolar) compounds found in *Populus* bud samples have antimicrobial activity against *B. subtilis*, whereas at least 3 compounds present in all *Populus* samples were detected as active in the *A. fischeri*. One phytoestrogenic compound was found in all *Populus* samples. In these trials, no activity was found in *Salix* buds, except in cholinesterase assay (at least two inhibitors); however, *Populus* buds contained far more inhibitors, several of them being selective for acetylcholinesterase *vs.* butyrylcholinesterase.

HPTLC hyphenation was thus useful for the detection, in *Salicaceae* buds, of substances, with potential pharmacological activities, e. g. antibacterial and phytoestrogenic. Selective acetylcholinesterase inhibitors are interesting for the symptomatic treatment of Alzheimer's disease and some other dementias. In the future, this hyphenated HPTLC method would be easily adapted for quantitative control analysis, and for structural identification of bioactive molecules, by hyphenation to targeted mass spectrometry.

The research was supported by a DAAD mobility fellowship (A/13/95848). Prof. Dušanka MILOJKOVIC- OPSENICA, University of Belgrade, Serbia, and her group are acknowledged for the preparation of the bud extracts.

P-65

CAMAG Method Library powered by visionCats - download & run compendial methods for identification of medicinal plants

Eliezer CENIVIVA
CAMAG, Muttenz, Switzerland, eliezer.ceniviva@camag.com

Nicolas RICHERDT, CAMAG
Valeria MAIRE-WIDMER, CAMAG
Daniel HANDLOSER, CAMAG
Anita ANKLI, CAMAG
Debora FROMMENWILER, CAMAG
Reich EIKE, CAMAG

HPTLC is an established analytical technique for the identification of medicinal plants. Based on standardized procedures in general chapters and numerous individual plant monographs in official compendia HPTLC is recognized by regulation authorities. For the evaluation and documentation of the comprehensive data provided by HPTLC chromatograms (fingerprints) electronic images are invaluable provided that they were generated in a standardized HPTLC process without "photoshopping". Digital images allow for visual comparison of fingerprints from multiple plant samples, botanical reference material and chemical reference standards.

With state-of-the-art HPTLC software such as visionCATS documentation and comparison of digital HPTLC fingerprints has become easy and intuitive while the process remains controllable, well documented, and therefore GMP compliant. The software features an electronic method library which provides the user with an online database of compendial and fully validated HPTLC methods for identification of medicinal plants, ready for download. Using the detailed method description the users run their samples by HPTLC and compare the results (the digital HPTLC fingerprints) with the library's digital HPTLC fingerprints of botanical reference material, typical and atypical samples and known adulterants.

Based on the monograph of the European Pharmacopoeia, the HPTLC method helps the user identifying samples of European elder flower (*Sambucus nigra*) and at the same time discriminating two other adulterant species of Sambucus.

P-66

HPTLC analysis of crude extracts of *Schoepfia schereberi* J.F. Gmel

Marcos SOTO-HERNANDEZ
Colegio de Postgraduados, Texcoco, Mexico, msoto@colpos.mx

Maurilio LOPEZ-ORTEGA, Universidad Veracruzana

Crude extracts of wood, bark, leaves and fruits of *Schoepfia schreberi* were obtained and separated by HPTLC. Extracts were obtained with hexane, dichloromethane and methanol. Fractionation was performed of the leaf and fruit extracts. The fractions were analyzed by HPTLC.

The results showed great quantity and quality of flavonoids in leaves and terpenoids in fruits. The data recorded by the HPTLC analysis can explain the anti-herbivory activity of the main fractions towards insects and that the leaves are better protected than the fruits against the attact by insects.

P-67

HPTLC-UV densitometric detection method for quantification of xanthones in mangosteen (*Garcinia mangostana*) fruit hulls extracts

Aline GILBERT
Centre de Recherche Yves Rocher, 101 Quai Roosevelt, 92444 Issy les Moulineaux Cedex France, Issy-les-Moulineaux, France, aline.gilbert@yrnet.com

Benedicte PORTET, Centre de Recherche Yves Rocher, France
Jocelyn GIBOULOT Centre de Recherche Yves Rocher, France
Christian LUBRANO, Centre de Recherche Yves Rocher, France
Jean-Renaud ROBIN, Centre de Recherche Yves Rocher, France

Mangosteen (*Garcinia mangostana* L., *Clusiaceae*) is a tropical tree native to Southeast Asia and largely distributed in India, Indonesia, Malaysia and Thailand. The fruit, considered as the "queen of tropical fruits", has a size as a little orange with a thick and red-purple pericarp that contains a famous and delicious pulp inside. The hull has been used for hundreds of years in Southeast Asia for a great variety of medicinal properties. More recently, mangosteen fruit products (juice or extract) are also used as raw materials for nutritional supplements and cosmetic applications. Previous phytochemical investigations reported that the major bioactive secondary metabolites are prenylated xanthones derivatives which occur in high concentration in the hull. Due to the increasing interest of this plant, the development of a convenient and reliable analysis method is needed to assess the quality control of fruit hulls extracts.

The aim of our study was to develop a quantitative analysis of the main xanthones (α-mangostins and 9-hydroxycalabaxanthone) in fruit hull extracts by HPTLC-UV. The method was performed on a TLC scanner apparatus on analytical RP-18 HPTLC plates. The mobile phase was acetonitrile/water/formic acid in the ratio (70/27/3, v/v/v) and the plates were scanned at 280 nm and 320 nm before derivatization with Neu reagent. We applied our method for quantification of xanthones on our fruit hull samples native to India and the results obtained were compared to those of HPLC-UV ones at 280 nm. The total average amount of xanthones is about 55 % (w/w) of dry matter. The concentration of the two main compounds α- and y-mangostins could reach respectively 44 % and 6 % (w/w) of dry matter. This HPTLC-UV method could be considered as a powerful tool to confirm the quality control of mangosteen fruit hulls.

P-68

HPTLC analysis of secoiridoids and phenylpropanoids in different extracts of *Ligustrum vulgare* L. leaves

Monika CZERWINSKA
Medical University of Warsaw, Warsawa, Poland, monika.czerwinska@wum.edu.pl

Marlena ZIAREK, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
Monika E. CZERWINSKA, Medical University of Warsaw, Poland
Agnieszka BAZYLKO, Medical University of Warsaw, Poland
Ewa OSINSKA, Department of Vegetable and Medicinal Plants, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland
Anna K. KISS, Medical University of Warsaw, Poland

The genus *Ligustrum* (*Oleaceae*) is distributed in Europe and Asia (south China and Korea), where it is used to prevent hypertension, sore throats, inflammation and diabetes [1]. Among the species of the genus *Ligustrum*, common privet (*Ligustrum vulgare* L.) is one of the most popular decorative shrub in Poland. According to previous study the main groups of compounds in extracts of *Ligustrum vulgare* are secoiridoids and phenylpropanoids [2].

The aim of the study was to determine the changes of composition of aqueous and ethanolic extracts, as well as decoctions and infusions prepared from leaves of *Ligustrum vulgare* collected in different seasons of plant growth using HPTLC method. The second part of our study focused on the quantitative determination of oleuropein, oleacein and echinacoside in these preparations.

Aqueous and ethanolic extracts, as well as decoctions and infusions of leaves were applied on HPTLC plates silica gel 60 F254 using Linomat 5. The mixture of ethyl acetate, methanol, water and acetic acid was used as a mobile phase. The plates was developed in the pre-saturated ADC2 chamber. Densitometric quantification was performed at 240 and 350 nm using TLC Scanner 3 with winCATS software. The procedure was validated according to ICH guidelines.

The presence of oleacein was confirmed in aqueous extracts, whereas oleuropein was present in particular in ethanolic extracts, decoctions and infusions. Echinacoside was detected in all prepared extracts.

[1] Gao B-B., She G-M. and She D-M.: Chem. Biodivers. 2013, 10, 96-128.
[2] Czerwinska M.E., Granica S. and Kiss A.K.: Plant. Med. 2013, 79, 924-932.

P-69

Optimization of the separation of caffeoyl derivatives from aqueous and ethanolic extracts of *Galinsoga parviflora* and *Galinsoga ciliata*

Agnieszka BAZYLKO
Department of Pharmacognosy and Molecular Basis of Phytotherapy, Warsaw Medical University, WARSAW, Poland, agnieszka.bazylko@wum.edu.pl

Karolina BORUC, Students' Scientific Association at the Department of Pharmacognosy and Molecular Basis of Phytotherapy, Warsaw Medical University, ul. Banacha 1, 02-097 Warsaw, Poland
Anna K. KISS, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Warsaw Medical University, ul. Banacha 1, 02-097 Warsaw, Poland

Galinsoga parviflora Cav. and *Galinsoga ciliata* Raf. Blake. (*Galinsoga quadriradiata* Ruiz et Pav.) belong to the *Asteraceae* family. They were imported to Europe from Central and South America, from the region of the Andes. *G. ciliata* in Poland occurs more seldom than *G. parviflora*. In the traditional medicine extracts from the herb are used in skin problems, dermatological diseases, eczemas, lichens, sores and hard healing wounds, to cure flu and colds, and as treatment for snakebites.

On the basis of phytochemical analysis, flavonoids like patulitrin (6-methoxyquercetin--7-O-glucoside), quercimeritrin (quercetin-7-o-glucoside), and quercitagetrin (quercitagetin--7-O-glucoside), and phenolic acids - caffeoyl conjugates (e.g. chlorogenic acid), were found in extracts of *Galinsoga* herb [1].

The aim of our present study was optimization of HPTLC separation of caffeic acid derivatives. Different stationary phases, including unmodified silica gel, silica gels modified with CN, NH_2, Diol and RP18 groups were tried. The best separation of tested compounds was achieved on HPTLC plates silica gel 60 F254 with mixtures of ethyl acetate - acetic acid - formic acid - water and ethyl acetate - methyl ethyl ketone - formic acid - water. The proposed systems can be used for *Galinsoga* extracts identification by chemical fingerprinting.

[1] Bazylko A., Stolarczyk M., Derwinska M., Kiss A.K. Natural Product Research, 26 (17), 1584-1593, 2012

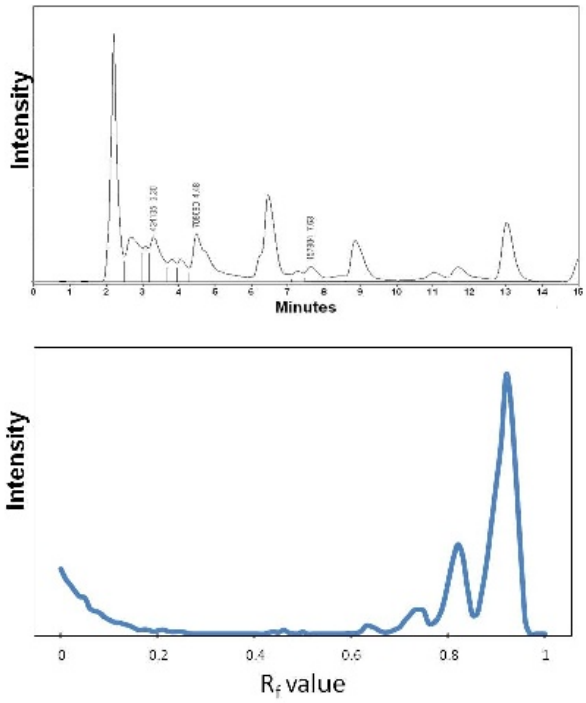
P-70

HPTLC and HPLC for the qualitative and quantitative analysis of *Calendula Officinalis* - advantages and limitations

Snezana AGATONOVIC-KUSTRIN
La Trobe University, Bendigo, Australia, s.kustrin@latrobe.edu.au

David MORTON
Christine LOESCHER

Chromatography techniques such as HPTLC and HPLC are commonly used to produce a chemical fingerprint of a plant to allow identification and quantify the main constituents within the plant. The aims of this study were to compare HPTLC and HPLC, for qualitative and quantitative analysis of the major constituents of *Calendula officinalis* and to investigate the effect of different extraction techniques on the *C. officinalis* extract composition from different parts of the plant. The results found HPTLC to be effective for qualitative analysis, however, HPLC was found to be more accurate for quantitative analysis. A combination of the two methods may be useful in a quality control setting as it would allow rapid qualitative analysis of herbal material while maintaining accurate quantification of extract composition.



Comparison of HPLC and HPTLC chromatograms

P-71

Application HPTLC method for Identification and stability study of compounding preparation with herbals

Oeksandr ZDORYK
National University of Pharmacy, Kharkiv, Ukraine, oleksandr_zdoryk@ukr.net

Kateryna KHOKHLOVA, National University of Pharmacy

There are some traditional formulations in Ukraine, which contain herbal substances as active ingredients. Among them there is an ointment for treatment of hemorrhoids prepared as stock preparation. The active ingredients of the ointment are Marigold tincture and Arnica tincture. As the procedure of quality assurance of extemporaneous drugs should include development of quality control methods and stability study, the scope of this work was development and validation of identification methods of the ointment and its stability study.

Take into account the nature of active substances of the ointment, for its identification and stability study of identification tests the (HP)TLC method was chosen. Application of HPTLC gives the possibility for specific determination of the drug and is a particularly suitable tool for its stability study. For identification and stability study of the ointment two groups of constituents were chosen - flavonoids and calendulosides. The optimal conditions of chromatography which provide specific, robust and precision identifications were determined. The mobile phase: Ethyl acetate, anhydrous formic acid, glacial acetic acid, water (100:11:11:27), derivatization reagent: NP/PEG spraying solution, heating at 100-105°C for 3-5 min, examination under UV 365 nm were selected for flavonoids. The mobile phase: chloroform, glacial acetic acid, methanol, water (35:16:6:4), derivatization reagent: anisealdehyde - sulfuric acid spraying solution, heating at 100-105°C for 2-5 min, examination in white light were selected for calendulosides. Stability tests of the ointment were carried out at refrigerated temperature, ambient and accelerated conditions over 2 months. Based on the obtained results, the ointment is stable for at least 2 months under the storage condition of t = 5 ± 3°C when protected from light, and approximately 1 month at the storage condition of t = 25 ± 2°C/RH 60% when protected from light.

P-72

Canceled, not presented

Comparative study of the pharmacological activity and HPTLC profiles of *Strobilanthes crispus* from various cultivation sources

Sutha DEVARAJ
Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia, sutha133@yahoo.com

Choon Sheen LAI, USM
Nik Soriani YAACOB, USM

Strobilanthes crispus (*S. crispus*) is a plant consumed traditionally for its anticancer and various other properties. The plant extracts exhibited cytotoxic effect against various cancer cell lines and showed hepatoprotective effect in vivo. Hence *S. crispus* may be further explored for its potential as a functional food. It is known that the phytochemicals in plants are largely affected by a number of environmental factors. The present study is intended to compare the antioxidant and cytotoxic activities as well as the chemical profiles of *S. crispus* collected from five different cultivation sites in the Northern Peninsular of Malaysia. The environmental factors differ in these locations in terms of the amount of sunlight, surrounding conditions as well as crop management practices. Antioxidant activities were determined using total phenols, DPPH radical scavenging and FRAP assays. Cytotoxicity on MCF-7 human breast cancer cell lines was evaluated using LDH assay.

The chemical composition of the plant extracts of *S. crispus* obtained from various cultivation sites were evaluated by HPTLC-densitometry. The reductive potential and free radical scavenging capacity of *S. crispus* was mainly concentrated in the methanol extract and were largely varied among difference sources. This variation may be attributed mainly to the content of flavonoid glycosides justified by the HPTLC profiles which changes as the plant mature. The cytotoxic effect of *S. crispus* was mainly exerted from the dichloromethane extracts which are rich in porphyrin pigments. Unlike the antioxidant activities, the cytotoxicity of *S. crispus* was rather consistent with the variation in plant sources. Marked differences were observed among *S. crispus* obtained from different cultivation sites in terms of antioxidant activities but the differences in cytotoxicity were marginal.

P-73

Simultaneous separation and quantification of five phytohormones using HPTLC

Vasudev THAKKAR
Sardar Patel University, Vallabh Vidyanagar, India, vasuthakkar@gmail.com

Ravi PATEL, Sardar Patel University
Bagavathi SUBRAMANIAN, Sardar Patel University

Estimation of phytohormones is routinely required in plant biochemistry, plant physiology, tissue culture and PGPR studies for understanding of plant growth and development. A protocol for separation and quantification of five structurally related phytohormones ABA, Kinetin, JA GA3 and IAA was designed using HPTLC method. Culture filtrate of Pseudomonas bacteria was acidified with 7M HCl and extracted with an equal volume of ethyl acetate to separate ABA, JA, GA3 and IAA. Kinetin was extracted from the remaining water fraction of the same extract.

Along with different concentration of standards, various extracted sample were applied on TLC foils silica gel 60 F254 and developed with isopropanol – ammonia - water 10:1:1 (v/v) as mobile phase. Quantification of ABA, Kinetin, JA, GA3 and IAA were done by absorbance measurement at 260, 275, 295, 265 and 280 nm, respectively. HPTLC method was found to be rapid, cost-effective and sensitive, which allowed quantification of phytohormones at nM levels. Moreover, this sensitivity can be improved further by adding internal standard without any specific sample preparation.

P-74

Enhanced extraction of isoflavones from *Medicago sativa* L. by complexation with (2-hydroxypropyl)-β-cyclodextrin and L-lysine monohydrochloride

Barbara FUMIĆ
Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia,
bfumic@pharma.hr

Sara ŠIMIĆ, Faculty of Pharmacy and Biochemistry, University of Zagreb
Marijana ZOVKO KONČIĆ, Faculty of Pharmacy and Biochemistry, University of Zagreb
Mario JUG, Faculty of Pharmacy and Biochemistry, University of Zagreb

The importance of isoflavones as natural phytoestrogens is currently the subject of intense research and discussions. Phytoestrogens have structural similarity with the human female hormone 17-β-estradiol, which can bind to both α- and β-estrogen receptors, and mimic the action of estrogens on targeted organs, thereby exerting many health benefits when used in some hormone-dependent diseases such as prostate cancer, cardiovascular disease and osteoporosis as well as in relieving postmenopausal symptoms. One of the major difficulties in developing isoflavone-based healthcare products is their low water solubility.

Isoflavone aglycones from *Medicago sativa* L. were isolated in form of ternary complexes with (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) and L-lysine monohydrochloride, using an ultrasound assisted extraction method. Upon hydrolysis using hydrochloric acid total content of isoflavone aglycones was determined by HPLC. Analysis was run on Agilent 1220 HPLC equipped with variable wavelength detector. Separation was performed on a Agilent Zorbax SB-C18 column, using mobile phases: water/methanol/formic acid (93:5:2) (A) and (3:95:2) (B). Elution was performed at a flow rate of 1 ml/min at the temperature of 40 °C using gradient method.

Isoflavone aglycones were identified by the comparison of their retention times with standards acquired commercially. Content of isoflavone aglycones was determined using calibration curves of standards ($r^2 > 0.999$). Results of the performed HPLC analysis showed that complex formation gained in more efficient extraction of isoflavone aglycones, especially daidzein compared to water extract at HP-β-CD and L-lysine monohydrochloride concentration of 10 mM. Further studies will examine the influence of higher concentration HP-β-CD and L-lysine monohydrochloride on isoflavone aglycones extraction.

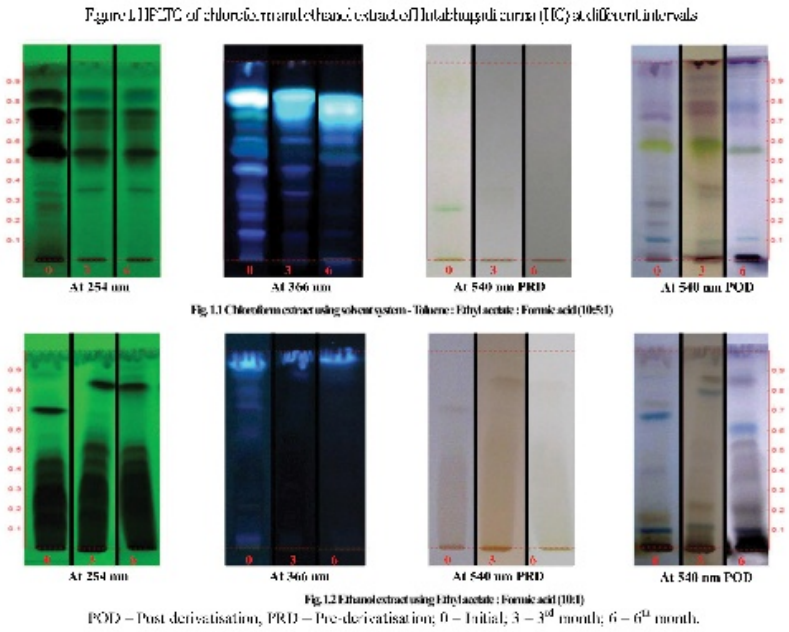
P-75

HPTLC for the diagnosis of stability of traditional polyherbal medicines

K.N. Sunil Kumar
SDM Centre for Research in Ayurveda and Allied Sciences, Laxminarayana Nagar, Kuthpadi- 574 118 INDIA, Udupi, India, sunilkumarnarayanan@gmail.com

PRIYADARSHINI
PUSHPENDRA
B.S. HOLLA
B. RAVISHANKAR
B. YASHOVARMA
B. YASHOVARMA

Measuring chemical stability of multi-herb formulation is a complex job. As there are no comprehensive guidelines for stability testing of herbal products, there is an urgent need to develop a most sensitive tool for the purpose. Keeping this problem in view a thought was given validate the employability of HPTLC as an effective tool for stability testing. HPTLC fingerprint analysis has been used as a tool to evaluate real time stability of *Hutabhugadi Curna* (HC). The chromatograms were developed using aluminium plates pre-coated with silica gel 60 F 254 as a stationary phase. Samples were analysed at the time of preparation of HC and after 3rd and 6th months of storage. Alteration of fingerprint profile from the initial pattern in terms of number spots was employed as diagnostic tools. Percentage variation in composition at given period of time can be calculated easily using formula; Months when 10 % degradation occurs = ([0 Month Assay value-{(0 Month Assay value x 10)/100}]-Intercept)/ Slope. The HPLTC methodology developed was found to be highly sensitive and reliable in proposing shelf for the formulation.



HPTLC of chloroform and ethanol extract of *Hutabhugadi Curna* (HC) at different intervals

P-76

Anti-tubercular glycolipids from the leaves of *Sterculia setigera* Del. (*Sterculiaceae*)

Esther Adebola ADELAKUN
University of Jos, Jos, Jos, Nigeria, debola2000@yahoo.com

Ibrahim Tijjani BABALOLA, Yobe State University, Damaturu, Nigeria
Scott G. FRANZBLAU, Institute for Tuberculosis Research, ITR, University of Illinois at Chicago, 833South Wood Street Chicago, IL 60612, United States

Natural products form a rich source in the search for new anti-tubercular agents with many groups undertaking screening of natural products as a preliminary step to finding new lead compounds. The anti-TB activity of the leaves of *Sterculia setigera*, a medicinal plant in Nigeria, was investigated by bioactivity-guided fractionation against virulent strains of *Mycobacterium tuberculosis* (H37Rv (ATCC27294)) in vitro, using the Alamar Blue Assay.

Repeated purifications of the active fractions using combination of normal and reverse phase chromatography led to the isolation of a pure fraction with an interesting anti-TB activity (minimum inhibitory concentration of 15.13 µg/ml). The cytotoxicity of this compound was evaluated against African green monkey kidney cells (Vero cells) and human hepatocellular carcinoma (HepG2) cell lines and were found non-toxic to both cell lines (IC50 102.4 and 81.08 µg/ml respectively).

Preliminary spectroscopic studies showed that the most active fraction is a mixture of two closely related glycolipids (1H, 13C NMR). Further purification of the mixture is ongoing to separate them for complete individual structure elucidation. This preliminary report provides a scientific evidence for the presence of glycolipids as the antimycobacterial agent in *S. setigera* leaves. This is the first report on the occurrence of biologically active glycolipids from S*. setigera* leaves.

P-77

Standardization of *Zanthoxylum zanthoxyloides* bark roots harvesting for the production of phytomedecine against sickle cell desease

Jean Claude W. OUÉDRAOGO
Département Médecine-Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé, Ouagadougou, Burkina Faso, ouedraclaude@yahoo.fr

Félix B. KINI, Département Médecine-Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé (Burkina Faso)
Kamlesh K. BHUTANI, Department of Natural Product, National Institute of Pharmaceutical Education and Research, S A S Nagar, Mohali (India)

Sickle cell disease is one of the most common severe monogenic disorders in the world, affecting millions of people worldwide, but most common among people whose ancestors come from Africa.

Zanthoxylum zanthoxyloides (syn. *Fagara zanthoxyloides* Lam.) (*Rutaceae*) is used for the treatment and the prevention of sickle cell disease crisis. A phytomedecine has been developed (FACA) with combination of some plants but is mainly composed by *Zanthoxylum zanthoxyloides* which contained the actives principles (Ouattara *et al.*, 2004; Ouattara *et al.*, 2009). It is well known that the secondary metabolites production by plants of the same species is influenced by an enormous quantity of environmental factors, hence the need to standardize the harvest in order to standardize the phytomedecine production.

Raw materials, consisting of root barks collected in six (06) localities of Burkina Faso, were examined by HPTLC fingerprint. The results show that the composition of the samples varies with the localities. Quantitative evaluation of vanillic acid in the samples by densitometry found it to be present in the range of 0.46 and 0.97%. So, the raw material will be collected in the locality with high percentage of vanillic acid because the isolated actives principles have each, two vanillic acid moieties.

P-78

HPTLC fingerprint profile of different extracts of *Cocculus hirsutus* (Linn.)

Samuel THAVAMANI
PSG College of Pharmacy, Peelamedu, Coimbatore, India, Coimbatore, India,
samtmani78@rediffmail.com

HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant during the manufacture of herbal medicine. The aim of the present study is to develop the HPTLC fingerprint profile of different extracts of *Cocculus hirsutus* (CH) namely petroleum ether, hexane, chloroform, methanol, ethyl acetate and aqueous extract. The results of preliminary phytochemical studies confirmed the presence of alkaloid, glycoside, steroid, carbohydrate, protein, flavonoid and phenolic compounds. Chromatogram was developed on silica gel 60 aluminum sheets using the mobile phase toluene – ethylacetate, 9:1.

No significant spot was observed in petroleum ether extract. The hexane extract of CH showed 3 spots (hR_F values 43, 52, 95), chloroform extract of CH showed 9 spots (hR_F values 18, 23, 37, 44, 52, 59, 70, 79, 92), ethyl acetate extract of CH showed 8 spots (hR_F values 19, 24, 37, 44, 52, 70, 79, 91), methanol extract of CH showed 7 spots (hR_F values (19, 24, 38, 45, 52, 70, 79).

In the present study, HPTLC fingerprints of various extracts of *Cocculus hirsutus* will provide referential information for the standardization of CH. HPTLC method for routine quality control of the present species can be carried out using this method. HPTLC fingerprint is suitable for rapid and simple authentication and comparison of subtle differences among samples of identical plant resources.

P-79

Method Development and validation of Rutin in flavanoidal fraction of *Hemidesmus indicus* Linn. by HPTLC

Dinesh Kumar CHELLAPAN
International Medical University (IMU), Kualalumpur, Malaysia,
kumargubbiyappa@imu.edu.my

Kumar Shiva GUBBIYAPPA, International Medical University (IMU)

The present study was undertaken to determine the rutin content in flavanoidal fraction of methanolic extract of roots of *Hemidesmus indicus* roots by HPTLC. A simple, precise and accurate high-performance thin-layer chromatographic method has been established for the determination of rutin in the root extract of *Hemidesmus indicus*. *Hemidesmus indicus* (L.) (*Asclepiadaceae*) is a twining shrub which has been used as folk medicine and as ingredient in Ayurvedic and Unani preparations against disease of biliousness, blood diseases, diarrhea, skin diseases, respiratory diseases, fever, syphilis, bronchitis, eye diseases, loss of appetite, burning sensation rheumatism and gastric disorders.

Flavanoidal fraction of methanolic extract of the root powder was used for the experimental work. Separation was performed on HPTLC plates silica gel 60 F254 with n-butanol - acetic acid – water - ammonia 3:1:1:1 (v/v), as mobile phase. The determination was carried out using the densitometric absorbance mode at 286 nm. Method development and validation was carried out for linearity, precision, accuracy, sensitivity, limit of detection, limit of quantification, assay was carried out. Rutin response was found to be linear over the range 50-400 ng/µl. The concentration of rutin in the flavanoidal fraction was found to be 9.6%. The HPTLC method was evaluated in terms of sensitivity, accuracy, precision and reproducible. In the present study HPTLC method developed can be used for routine quality control analysis of marketed preparation.

P-80

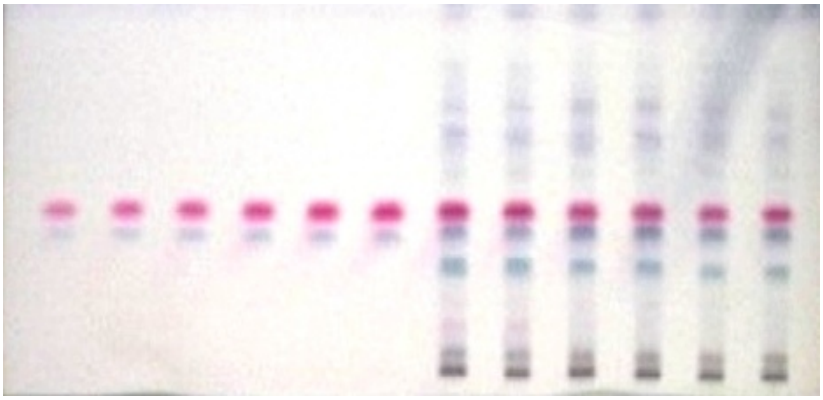
A simple and sensitive HPTLC method for simultaneous estimation of anti-malarial compound artemisinin and its precursor artemisinic acid in leaves of *A. annua* L. plants

Shahzad AHMAD
Center for Transgenic Plant Development, Faculty of Science, Jamia Hamdard, New Delhi, India, sahmad.sch@jamiahamdard.ac.in

Shazia KHAN
Athar ALI
Malik Zainul ABDIN

A simple, selective, reliable and rapid HPTLC method has been developed for simultaneous analysis of a potent antimalarial molecule, artemisinin (AM) and its precursor molecule, artemisinic acid (AA) in the leaf extract of *Artemisia annua* L.. The chromatographic separation was performed on silica gel plates 60 F254. The separation was achieved with hR_F 40 ± 3 for artemisinin and hR_F 30 ± 3 for artemisinic acid. After derivatization with anisaldehyde solution (spraying reagent), absorbance measurement at 536 nm followed.

The method was validated as per ICH guidelines in terms of linearity, specificity, sensitivity, accuracy and precision. The method was linear in the range of 700 ng to 4200 ng for artemisinin and 300 ng to1800 ng for artemisinic acid. The specificity was checked by comparing hR_F values and overlay spectra of standards with samples. The sensitivity of the method was studied in term of LOD (30 ng for both AM and AA) and LOQ (80 ng for both AM and AA). The accuracy of the method was checked by the recovery study and the average percentage of the recovery was found to be 98% for artemisinin and 99% for artemisinic acid. Therefore, this method was found to be rapid, precise, simple, economic and accurate.



Chromatogram at 536 nm, tracks 1-6: standard mix of artemisinin (hR_F 40) and artemisinic acid (hR_F 30) , tracks 6-12 : leaves extract of *Artemisia annua* L.

P-81

Validated densitometric HPTLC method for the quantification of luteolin in *Portulaca oleracea* leaves

Pradeep KAMBOJ
JCDM College of Pharmacy, Sirsa, India, dr.pradeepkamboj@gmail.com
Ajudhia Nath KALIA, ISF College of Pharmacy, Moga

A rapid and reliable quantitative densitometric HPTLC method for the determination of luteolin in *Portulaca oleracea* leaves was developed. The crude plant material was refluxed with 70% (v/v) aqueous methanol for 30 min for the preparation test sample. The sample and standard luteolin were applied on TLC aluminium sheets silica gel G 60 F254. Luteolin was separated in the sample mixture using a saturated mixture of toluene - ethyl acetate - formic acid 5:3.5:0.1, v/v/v. Spectrodensitometric scanning was carried out at 350 nm in the absorption mode.

The developed solvent system showed compact spot for luteolin at hR_F-value 34 ± 2. The linear regression analysis data for the calibration curve showed linear relationship (r^2=0.9946) in the concentration range 200-800 ng with respect to peak area. The HPTLC method was further validated successfully for precision, recovery and robustness with respect to International Conference on Harmonization (ICH) guidelines. The statistical analysis of the data showed that the proposed method is precise, reproducible and accurate, and can consequently be employed for the standardization of the plant and its marketed formulations on the basis of luteolin content.

P-82

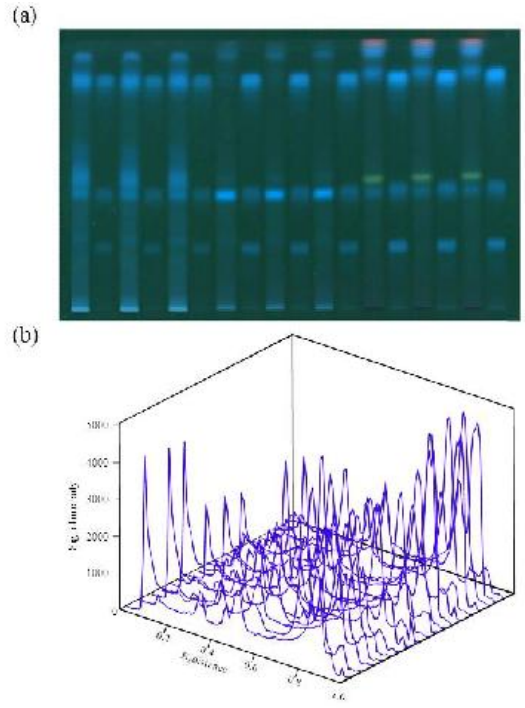
Quantification of phenylpropanoids in commercial *Echinacea* products with ANN modelling of HPTLC fingerprints

Snezana AGATONOVIC-KUSTRIN
La Trobe University, School of Pharmacy and Applied Science, Bendigo, Australia,
s.kustrin@latrobe.edu.au

Christine LOESCHER, La Trobe University
David MORTON, La Trobe University

While it is generally assigned immune enhancement activities, the effectiveness of *Echinacea* formulation is highly dependent on the *Echinacea* species, on the part of the plant used, on the plant age and on the method of extraction. The aim of this study was to investigate the capacity of an artificial neural network (ANN) to analyse TLC chromatograms as fingerprint patterns and quantify three phenylpropanoid markers, chicoric acid, chlorogenic acid and echinacoside in commercial *Echinacea* products.

A simple TLC method for routine evaluation of the phytochemical variability in *Echinacea* formulations has been developed. By applying samples with different weight ratios of marker compounds to the system, a database of chromatograms was constructed. A hundred and one signal intensities in each of the TLC chromatograms were correlated to the amounts of applied echinacoside, chlorogenic acid and chicoric acid using an ANN. The developed ANN model was used to quantify 3 markers in *Echinacea* commercial formulations. The minimum LOQ of 63, 154 and 98 ng and the LOD of 19, 46 and 29 ng were established for echinacoside, chlorogenic acid and chicoric acid respectively.



Captured TLC plate image (a) with corresponding densitograms (b)

P-83

Determination of adulteration of anti-obesity drug in Indian herbal medicinal products: development and validation of analytical method

Kashyap THUMAR
Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, India,
kasu_patel5@yahoo.co.in

Lav BHATIA, Saurashtra University
Navin SHETH, Saurashtra University

In this era of fast growth, the problem arises very rapidly and that makes all the governments of various countries of the world to take preventive steps very immediately. In the list of various problems health problems are the prime, and obesity is the one of the health problems in various countries of the world. Obesity is a rapidly growing problem in the whole world, and is the most highlighted by every type of media of communications.

These days slimming Herbal Medicinal Products (HMPs) are more in demand in Indian market and it encourages manufacture of slimming HMPs to be adulterate the HMPs with active synthetics to increase the efficiency of the products. Sibutramine is anorexic drug by acting on serotonin neurotransmitter. But sibutramine is the drug which is recently banned due to few life threatening cardiac side effects. Sibutramine is one of the possible adulterant of the slimming HMPs that can be added by the manufacturer and has to be detected by the analyst to determine the adulteration in the HMPs.

The purpose of this study was to identify and quantify sibutramine in slimming HMPs in less time with accurate result. This method will be useful for the routine analysis of illegally added sibutramine in the HMPs.

HPTLC separation was performed on aluminum sheets silica gel G60 F254 (10 x 10 cm, 20 x 10 cm) using chloroform – acetone - methanol (8.5:1.0:0.55, v/v/v) and detection at 223 nm. Linearity was given in the range of 300-2500 ng/band and the method was validated for accuracy, precision, specificity, robustness and successfully applied to local marketed products. As a conclusion this method was found to be useful for the routine analysis of illegally added sibutramine in HMPs.

P-84

Quantitative determination of trazodone in human serum by HPTLC

Sigrid MENNICKENT
University of Concepcion, Concepcion, Chile, smennick@udec.cl

Ana GONZALEZ, University of Concepcion, Chile
Mario VEGA, University of Concepcion, Chile
Gisela RIOS, University of Concepcion, Chile
Marta DE DIEGO, University of Concepcion, Chile

A simple, rapid, specific, precise and accurate HPTLC method for quantification of trazodone in human serum was developed and validated. The method includes a liquid-liquid extraction of the analyte from the matrix using a mix of *n*-hexane - isoamyl alcohol (99:1) as extraction solvent.

In the developed method, chromatography was performed on HPTLC plates silica gel F254 (10 cm x 10 cm and 10 cm x 20 cm, layer tickness 0.2 mm) prewashed with methanol, and with toluene – acetone – ethanol - ammonium (9:7:2:0.5, v/v/v/v), as mobile phase. The developing solvent was run up to 80 mm in a trough chamber previously saturated with solvent mixture for 20 min. Densitometric detection was done at 258 nm. The regression data for the calibration plots showed good linear relationship (r=0.999) in the range of 20 and 200 ng/band, corresponding to 0.20 and 2.00 ng/µL of trazodone in human serum after extraction process and applying 10 µL to the chromatographic plates. The %RSD of intra-assay and inter-assay precision, were in the range of 1.0% to 3.0% (n=3) and 1.1% to 3.5% (n=9), respectively. The limit of detection and limit of quantitation were found to be 0.016 ng/µL, and 0.048 ng/µL. The recovery values were between 94.7% and 99.0% (RSD ± 4.3%), and the hR_F for trazodone and m-chlorophenilpiperazine (its major metabolite) were 82 and 39, respectively. Patient serum samples were analyzed by this HPTLC method successfully. Therefore, this HPTLC method is suitable for quantitative determination of trazodone in human serum.

P-85

Simultaneous estimation of drotaverine HCl and nimesulide in pharmaceuticals by HPTLC

Rita CHAKOLE
Department of Pharmacy, Government Polytechnic, Gadge Nagar, Amravati, India,
kdc_ritu@rediffmail.com

Manoj CHARDE, Government College of Pharmacy, Kathora Naka, VMV Road, Amravati, Maharashtra, India

A simple, rapid, precise and accurate HPTLC method was developed and validated for simultaneous determination of drotaverine HCl (DRO) and nimesulide (NIM) in pharmaceutical preparations. Separation was achieved on a HPTLC aluminum sheets silica gel 60 F254 (0.2 mm thickness) as the stationary phase using cyclohexane – methanol - ethyl acetate (5:2:3, v/v/v) as mobile phase. Densitometric quantification was performed at 295 nm by reflectance scanning. The hR_F values of DRO and NIM were 41 and 62, respectively.

The linearity was investigated in the range of 0.1 to 0.6 µg/spot and 0.2 to 0.7 µg/spot for DRO and NIM, respectively. The percentage drugs estimated in the marketed formulation for DRO is 99.8% ± 0.5%, 99.7% ± 0.01% and for NIM is 99.6% ± 0.6%, 99.7 ± 0.07%. The percentage recoveries for DRO and NIM were 99.9% ± 0.6% and 100.2% ± 1.1% by area and 99.6 %± 0.3% and 100.0% ± 0.2% by height, respectively. The developed method was suitably validated for precision, accuracy, specificity and ruggedness.

P-86

Development of stability indicating HPTLC method for simultaneous estimation of irbesartan and amlodipine in combination

Dipal PATEL
Indukaka Ipcowala College Of Pharmacy, New V.V.Nagar, India, pateldipal10@gmail.com

Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Falgun MEHTA, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy
Shahin VAHORA, Indukaka Ipcowala College of Pharmacy

A simple, sensitive, and precise HPTLC method has been developed for the estimation of irbesartan and amlodipine besylate in combination. TLC aluminum sheets silica gel 60 F254 were employed as the stationary phase, while the solvent system was chloroform – toluene – methanol - acetic acid (6:2.5:1.5:0.5, v/v/v/v). The hR_F values were observed to be 57 ± 2, and 30 ± 2 for irbesartan, and amlodipine besylate, respectively. The separated spots were densitometrically analyzed in absorbance mode at 244 nm. The method was linear in the range of 50-500 ng/band for irbesartan and 400-900 ng/band for amlodipine besylate. The limits of detection for irbesartan and amlodipine besylate were found to be 15.6 and 13.8 ng/band, respectively. The respective limits of quantification were 47.4 and 42.0 ng/band, respectively.

Irbesartan and amlodipine stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photo degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their hR_F values. Stressed samples were assayed using developed HPTLC method. The proposed method was validated with respect to linearity, accuracy, precision and robustness. The method was successfully applied to the estimation of irbesartan and amlodipine besylate in synthetic mixture.

P-87

Stability indicating HPTLC method for simultaneous estimation of cilnidipine and telmisartan in their combined dosage form

Nimmi DALWADI
Indukaka Ipcowala College of Pharmacy, New V.V. Nagar, India, nimmidalwadi_18@yahoo.in

Shahin VAHORA, Indukaka Ipcowala College of Pharmacy
Falgun MEHTA, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy
Arpita PATEL, Indukaka Ipcowala College of Pharmacy

A sensitive, selective and precise HPTLC method has been developed and validated for the simultaneous estimation of cilnidipine and telmisartan both as a bulk drug and in formulation. TLC aluminium sheets silica gel 60 F254 were employed as stationary phase, while the solvent system was chloroform - methanol - toluene (6:0.7:2, v/v/v). The hR_F values of cilnidipine and telmisartan were observed to be 59 and 30, respectively. The densitometric analysis was carried out in absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for cilnidipine and telmisartan over a concentration range of 100-600 ng/spot and 400-2400 ng/spot, respectively. The method was validated for precision, robustness and recovery. The limit of detection and limit of quantification for cilnidipine and telmisartan were found to be 15.0 and 10.3 ng/spot, 45.3 and 31.4 ng/spot, respectively. Statistical analysis showed that the method is repeatable, selective, and precise. Cilnidipine and telmisartan were subjected to acid, base, peroxide, and UV-induced degradation. In stability tests the both drugs were susceptible to acid and basic hydrolysis, oxidation and photodegradation. Statistical analysis proved the method is repeatable, selective, and accurate for estimation of cilnidipine and telmisartan. Because the method could effectively separate the drugs from their degradation products, it can be used as a stability-indicating method.

P-88

Stability indicating HPTLC method for determination of flupirtine maleate in pharmaceutical preparations

Kamalesh GADAPAYALE
Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur (M.S.) India 440 033, Nagpur, India, kamaleshkg@gmail.com

Rahul DAHALE, Department of Pharmaceutical Sciences
Dinesh SATONE, Department of Pharmaceutical Sciences
Rajendra KAKDE, Department of Pharmaceutical Sciences

A sensitive, precise and stability indicating HPTLC method for determination of flupirtine maleate in bulk drug and in formulations was developed and validated. The method employed TLC aluminium sheets silica gel 60 F254 as stationary phase. The solvent system consist of toluene – methanol - glacial acetic acid in the ratio of 16:3:1, v/v/v. This system was found to give compact spots for flupirtine maleate (hR_F value 52 ± 1). Densitometric analysis was carried out in the absorbance mode at 351 nm.

Calibration plots obtained by plotting peak area were linear over the concentration range 200-800 ng per band with r^2 of 0.994 ± 0.001 for peak height and 0.999 ± 0.001 for peak area. The calibration equations obtained were y = 1.886 x + 194.366, RSD 2.1%, for peak height, and y = 78.897 x + 3690.291, RSD 1.3% for peak area. The method was validated as per the ICH guidelines. The LOQ that produced the requisite precision and accuracy was 11.3 ng per band for peak height and 161.0 ng per band for peak area. The LOD were 3.7 ng and 53.1 ng per band for peak height and peak area, respectively.

Mentioned chromatographic parameter were strictly monitor during method validation such as band width of 4 mm, 5 µL sample volume, application rate 5 s/µL, ascending separation technique, migration distant of 80 mm, scanning speed of 20 mm/s and chamber saturation time of 20 min. The drug undergoes degradation under acidic, basic, neutral, photo, oxidation and dry heat treatment. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and dry heat degradation. Statistical analysis proves that the method is reproducible and selective for the determination as the method could effectively separate the drug from its degradation products. It can be employed as a stability indicating method for determination of flupirtine in bulk drug and pharmaceutical preparations.

P-89

Validated analytical HPTLC method & content uniformity for the determination of letrozole in tablet dosage form

Falgun MEHTA
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
mehta.falgun@gmail.com

Shahin VAHORA, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy

A simple HPTLC Content Uniformity Test was developed and validated for the analysis of letrozole (LET) in its commercial single components tablet formulations (2.5 mg/tablet). The method employed TLC aluminium sheets silica gel 60 F254 as stationary phase. The solvent system consisted of toluene - ethyl acetate 5: 5, v/v.

This system was found to give compact spots for LET (hR_F value 30). Densitometric analysis of letrozole was carried out in the absorbance mode at 238 nm. The linear regression data for calibration plots showed good linear relationship with r^2 0.998 in the concentration range of 50-250 ng/ml. The mean value of correlation coefficient, slope and intercept were 0.998 ± 0.002, 18.98 ± 0.07 and 478.9 ± 12.6, repectively. The method was validated in terms of linearity (50-250 ng/spot), precision, accuracy and specificity.

For content uniformity test, letrozole content of 10 individual tablet units of market formulation was determined after extracting with methanol. The formulation complied with the USP specifications. The proposed Content Uniformity Test can analyze ten tablets simultaneously on a single plate and provides a faster and cost- effective quality control tool.

P-90

Validated HPTLC analytical method and content uniformity test for the determination of cilnidipine and telmisartan in tablet dosage form.

Shahin VAHORA
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
shahin121190@gmail.com

Falgun MEHTA, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Dipal PATEL, Indukaka Ipcowala College of Pharmacy

A HPTLC method and Content Uniformity Test was developed and validated for the analysis of cilnidipine(CIL) and telmisartan (TEL) in its combined tablet formulations (10 mg CIL and 40 mg TEL/tablet). The method employed TLC aluminium plates silica gel 60 F254 as stationary phase. The solvent system consisted of chloroform - toluene - methanol 6:2:0.7, v/v/v. This system was found to give separate spots for CIL and TEL (hR_F value of 59 ± 1 and 30 ± 3). Densitometric analysis of CIL and TEL was carried out in the absorbance mode at 254 nm.

The linear regression data for calibration plots showed good linear relationship with r^2 of 0.9992 and 0.9993 for CIL and TEL, respectively in the concentration range of 100-600 ng/spot and 400-2400 ng/ml for CIL and TEL, respectively. For CIL and TEL, the mean value of slope and intercept were 7.66 and 1.943 as well as 395.6 and 1026.2, respectively. The method was validated in terms of linearity (100-600 ng/spot and 400-2400 ng/spot for CIL and TEL, respectively.), precision, accuracy (98-102%) and specificity.

For content uniformity test, 10 individual tablet was determined after extracting with methanol. Both the drug complied with the USP specifications. The proposed content uniformity test can analyze ten tablets simultaneously on a single plate and provides a fast and cost-effective quality control tool.

P-91

Development and validation of stability-indicating HPTLC method for determination of cilnidipine and its related substances in bulk drug and pharmaceutical preparations

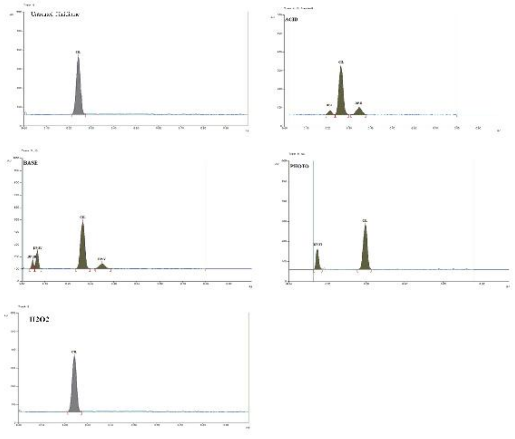
Rajendra KAKDE
Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur (M.S.) India 440 033, Nagpur, India, drkakde@yahoo.com

Gajanan KALYANKAR
Kamalesh GADAPAYALE
Dinesh SATONE

A simple, precise and stability-indicating HPTLC method has been developed for determination of cilnidipine in bulk drug and formulations. The method employed HPTLC aluminium sheets silica gel 60 F254 as stationary phase. The solvent system consist of acetonitrile - toulene 1:8, v/v. Densitometric analysis of cilnidipine was carried out in the absorbance mode at 240 nm. A single spot of hR_F value of 26 ± 3 was observed in chromatogram of cilnidipine. Extracted cilnidipine from tablet shows no interference of excipients commonly present in the tablet.

The cilnidipine content was found to be 99.20% ± 0.63%. The low *%RSD* value indicated the suitability of this method for routine analysis of cilnidipine in pharmaceutical dosage forms. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 0.9980 ± 0.0013 by peak height and r^2 0.9980 ± 0.0012 with respect to peak area in the concentration range 50-800 ng per spot. The mean *%RSD* of slope and intercept were 11.027 ± 0.72 and 279.81 ± 8.26 with respect to peak area. The method was validated as per ICH guidlines. The LOD and LOQ were found to be 3 ng per spot and 11 ng per spot, respectively. Mentioned chromatographic parameter were strictly monitor during method validation such as band width of 5 mm, 5 µL sample volume, application rate 5 s/µL, ascending separation technique, migration distant of 80 mm and scanning speed of 20 mm/s.

The drug was susceptible to acid, base hydrolysis, oxidation and photolysis. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of cilnidipine. Optimised method is specific for cilnidipine along with the related substances as well as sensitive at low concentration. The proposed HPTLC method can be applied for identifcation and quantitative determination of cilnidipine in bulk drug and dosage forms.



Degradation behavior of cilnidipine at various stress conditions

P-92

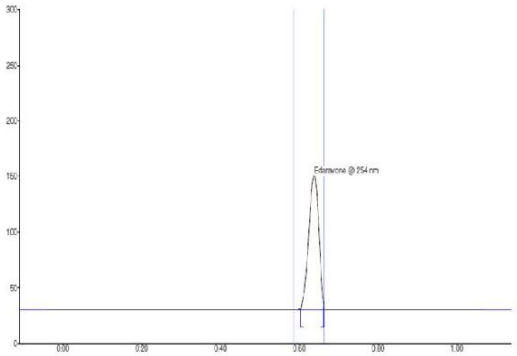
Development and validation of HPTLC method for determination of edaravone in bulk and in injectable dosage form

Dhananjay MESHRAM
Pioneer Pharmacy Degree College, Vadodara, India, dbmeshram@yahoo.com

Krunali THAKKAR, Pioneer Pharmacy Degree College
Dinesh SATONE , Sun Pharmaceutical Advanced Research Center

A simple, rapid, reliable and accurate HPTLC method has been developed for the estimation of Edaravone in bulk and pharmaceutical dosage form. The chromatographic development was carried out on aluminum sheets silica gel 60 F254 using a mixture of toluene - methanol (3:2, v/v) as mobile phase. Detection was carried out densitometrically at 254 nm. The hR_F value of the analyte was found to be 66 ± 2.

The method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantification and specificity. The linear regression analysis data for the calibration plots showed a good linear relationship with r^2 of 0.9995 in the concentration range 200-600 ng/spot. The % assay (mean *%RSD*) was found to be 100.3% ± 0. 7%. Accuracy of the method was accessed by percentage recovery and found to be 99.8% ± 0.7%. The method is new, simple and economical for routine estimation of edaravone in bulk, pre-formulation studies and pharmaceutical formulation rapidly at low cost in routine analysis.



Chromatogram of standard edaravone at hR_F 63

P-93

HPTLC method for quantitation of diosgenin from extract of *Balanites aegyptiaca*

Vijaykumar PARMAR

Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, CHARUSAT Campus, Changa - 388 421. Ta. Petlad, Dist. Anand, Gujarat, INDIA., Anand, India, vijayparmar.ph@charusat.ac.in

Sanketkumar PATEL, Ramanbhai Patel College of Pharmacy
Ketan VARIYA, Ramanbhai Patel College of Pharmacy

Balanites aegyptiaca Del. (*Zygophyllaceae*), known as 'desert date,' is traditionally used in treatment of various ailments i.e. jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever [1]. It is widely distributed in dry land areas of Africa and South Asia. In India, it is particularly found in Rajasthan, Gujarat, Madhya Pradesh and Deccan. It is reported that whole and extracted pulp of B. aegyptiaca fruits reported a hypocholesterolemic and hepatoprotective activity [2].

A HPTLC method was developed for quantitation of diosgenin as marker from fruit-pulp extract of *Balanites aegyptiaca*. Separation was achieved on HPTLC plates silica gel 60 F254 using toluene – ethyl acetate - formic acid (7:2.8:0.2, v/v/v) as mobile phase. The quantitation of diosgenin was carried out using the densitometric reflection/absorption mode at 426 nm after post chromatographic derivatization using anisaldehyde sulphuric acid reagent. This system was found to give a spot of diosgenin at hR_F 49 ± 2 and linearity was found in the ranges 10-50 ng/spot (r^2 = 0.9998) with second order polynomial curve. Limit of detection (2 ng/spot), limit of quantification (10 ng/spot) and recovery (94.3% to 97.3%) were found satisfactory.

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[2] Abdel-Rahim EA, El-Saadany SS, Wasif MM, Food Chem. 1986, 20, 69-78

P-94

Simultaneous estimation of citicoline and methylcobalamin in pharmaceutical formulation by HPTLC densitometry

Falgun MEHTA
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
mehta.falgun@gmail.com

Shweta SINGH, Indukaka Ipcowala College of Pharmacy
Dhara PATEL, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy

A simple, precise, rapid, selective, and economic reversed phase HPTLC method has been established for simultaneous analysis of citicoline and methylcobalamin. HPTLC method was developed on TLC plates silica gel F254 G60 as stationary phase using methanol - acetonitrile - water - triethylamine (8.5:1.5:1:0.5, v/v/v) as mobile phase. The plates were scanned at 254 nm for both, citicoline and methylcobalamin. Both drugs were resolved and hR_F value was found to be 39 for citicoline and 61 for methylcobalamin. The method was linear in the range 1-6 µg/band for both, citicoline and methylcobalamin. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is important tool for analysis of combined dosage form.

P-95

Simultaneous estimation of amiloride hydrochloride and torsemide in their combined dosage form by HPTLC method

Falgun MEHTA
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
mehta.falgun@gmail.com

Smit BHADJA, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOITYA, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Shahin VAHORA, Indukaka Ipcowala College of Pharmacy

A simple, sensitive and precise high performance thin layer chromatographic method has been developed for the estimation of torsemide (TOR) and amiloride HCl (AML) in the pharmaceutical dosage form. TLC aluminum sheets silica gel 60 F254 used as stationary phase, while chloroform - methanol - ammonia (7.5:3.5:1, v/v/v) was used as mobile phase. The hR_F value was observed 46 ± 1 and 24 ± 1 for TOR and AML, respectively. The densitometric analysis was carried out in absorbance mode at 286 nm. The method was linear in the range of 100-600 ng/spot for TOR and 50-300 ng/spot for AML. The method was validated as per ICH guidelines. The limit of detection and limit of quantitation were found to be 20 ng/spot and 100 ng/spot, respectively for TOR. The limit of detection and limit of quantitation were found to be 13 ng/spot and 50 ng/spot, respectively for AML. The proposed method was successfully applied to the estimation of TOR and AML in the pharmaceutical dosage form.

P-96

Simultaneous estimation of epalrestat and methylcobalamin in pharmaceutical formulation by HPTLC densitometry

Falgun MEHTA
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
mehta.falgun@gmail.com

Krima PATEL, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Usman CHHALOTIYA, Indukaka Ipcowala College of Pharmacy

A simple, precise, rapid, selective, and economic reversed phase HPTLC method has been established for simultaneous analysis of epalrestat and methylcobalamin. The HPTLC method was developed on TLC plates silica gel G60 F254 as stationary phase, using methanol - ethyl acetate - triethylamine (5:5:0.5, v/v/v) as mobile phase. The plates were scanned at approximately 254 nm for both epalrestat and methylcobalamine, respectively. In HPTLC method both the drugs were resolved using proposed mobile phase and hR_F value was found to be 69 for epalrestat and hR_F 25 for methylcobalamin. The method was found to be linear in the range 200-800 and 800-2600 ng/band for epalrestat and methylcobalamin, respectively. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is important tool for analysis of combined dosage form.

P-97

Simultaneous estimation of dosulepin and methylcobalamin in pharmaceutical formulation by HPTLC-densitometry method

Falgun MEHTA
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
mehta.falgun@gmail.com

Dhara DESAI, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy

A simple, precise, rapid, selective, and economic reversed phase HPTLC method has been established for simultaneous analysis of dosulepin and methylcobalamin. HPTLC method was developed using on TLC plates silica gel G60 F254 as stationary phase and methanol - ethyl acetate -triethylamine (8:2:0.5, v/v/v) as mobile phase. The plates were scanned at 272 nm for both dosulepine and methylcobalamine, respectively. In HPTLC method both the drugs were resolved and hR_F value was 52 for dosulepin and hR_F 25 for methylcobalamin. The method was found to be linear in the range 200-700 and 800-2300 ng/band for dosulepin and methylcobalamin, respectively. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is important tool for analysis of combined dosage form.

P-98

HPTLC method development and validation for determination of alfuzosin hydrochloride in bulk and tablet dosage form

Pradip DHORE
University Department of Pharmaceutical Sciences, Nagpur, Nagpur, India,
nishikantraut29@gmail.com

A simple, rapid and reproducible HPTLC method has been developed for the estimation of alfuzosin HCl in bulk and tablet dosage form using TLC aluminum sheets silica gel 60 F254. The mobile phase consists of toluene - methanol (7:3 v/v). Detection at 248 nm resulted in sharp peak at 35. The validation of the method was accomplished as per International Conference on Harmonization (ICH) guidelines for specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness.

The method linearity was between 100-500 ng/ml. The percentage recovery was 100.6% ± 0.9% and 100.9% ± 0.7% by area and height, respectively. LOD and LOQ were 30 ng and 50 ng per spot. The peaks in acid, base and oxide forced degradation study are well resolved from the analyte peak. The developed method can be suitably applied for the estimation of drug during routine analysis and quality control studies. As the drug can be estimated unequivocally in presence of its degradants, hence the data required for shelf life, storage and handling conditions can also be generated from the said method.

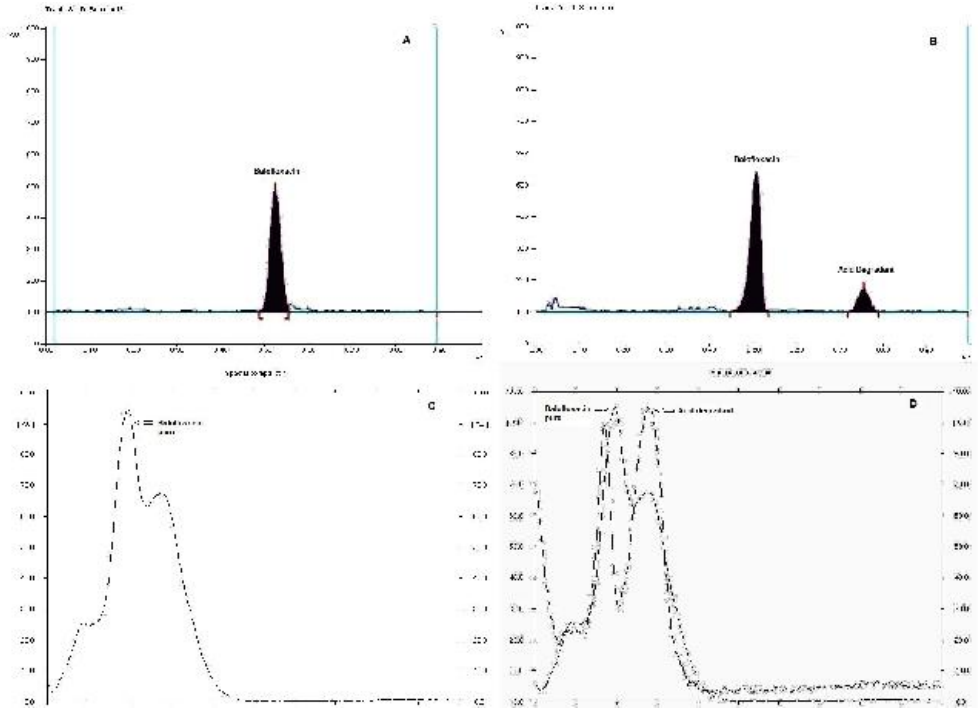
P-99

Development of validated HPTLC method for estimation of balofloxacin in bulk and tablet dosage form

Nishikant RAUT
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India, nishikantraut29@gmail.com

Shrenik BEDE, Department of Pharmaceutical Sciences
Pradip DHORE, Department of Pharmaceutical Sciences

A simple, specific and sensitive HPTLC assay method has been developed for balofloxacin in bulk and pharmaceutical dosage form. TLC aluminum sheets silica gel 60 F254 and *n*-butanol – ethanol - ammonia (5:3:2 v/v/v) were employed for the study. The assay performed on tablet showed 99.6% ± 0.8% content with sharp peak at hR_F 47. The linearity was studied in the concentration range of 100-1000 ng/ml with correlation coefficient of 0.9991 by area and 0.9990 by height. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to precision, accuracy, linearity, specificity, limit of detection, limit of quantitation and robustness. The method was found to be accurate with % drug recoveries 99.1% ± 0.7% and 99.5% ± 0.9% by height and area, respectively. Degradation peaks were observed under acidic condition and peak of drug was well resolved with ability to estimate it quantitatively. The method can be suitably applied for assay of balofloxacin during routine analysis, quality control, for setting storage conditions and shelf life.



Densitogram and spectrum of balofloxacin

P-100

A novel method of sample preparation for quantitative analysis with HPLC and HPLC/MS techniques

Anna KLIMEK-TUREK
Medical University of Lublin, Poland, Lublin, Poland, anna.klimek@umlub.pl

Mateusz SIKORA, Medical University of Lublin, Poland
Aneta HAŁKA-GRYSIŃSKA, Medical University of Lublin, Poland
Tadeusz DZIDO, Medical University of Lublin, Poland

When the analytes of interest are presented in complex matrices, for example biological or pharmaceutical origin, then the sample is usually not suitable for direct introduction into analytical instruments as liquid chromatograph or mass spectrometer. Commonly used sample preparation method include filtration, centrifugation, liquid-liquid extraction (LLE) or solid phase extraction (SPE). These procedures are time and cost consuming. For this reason, in recent years Dried Blood Spot (DBS) method, where blood samples are blotted and dried on filter paper, is becoming popular. The dried samples can be easily shipped to an analytical laboratory and analysed using various methods such as HPLC or MS.

The success of DBS in clinical analysis inspired us to seek sample preparation method for quantitative analysis based on TLC advantages (low cost and short time of analysis, rapid and cost-efficient optimization of the separation, minimal effect of matrix).

Our procedure allows quantitative TLC/MS or TLC/LC/MS sample analysis without its sophisticated preparation. The method is characterized by higher reproducibility and accuracy than classical TLC/MS quantitative analysis and shows greater linearity compared to TLC-densitometry methods. The use of commercial TLC/HPTLC plates for sample preparation to quantitative analysis with HPLC or LC/MS makes the analytical procedure reproducible, convenient and economical. In our opinion, the proposed mode can be especially applied to drug monitoring in clinical analysis.

P-101

A validated HPTLC method for simultaneous quantification of ramipril and hydrochlorothiazide in bulk and tablet formulation

Patel NIRALI
Indukaka Ipcowala College of Pharmacy, Vallbh Vidyanagar, India, nirali.pharma90@gmail.com

Mehta FALGUN, Indukaka Ipcowala College of Pharmacy
Shah DIMAL, Indukaka Ipcowala College of Pharmacy
Chhalotia USMANGANI, Indukaka Ipcowala College of Pharmacy

A HPTLC method for the estimation of ramipril and hydrochlorothiazide has been developed. It employs aluminum backed silica gel 60 F254 TLC plates, (10 cm × 10 cm, layer thickness 0.2 mm) pre-washed with methanol and mobile phase comprising ethyl acetate - methanol - ammonia (9:1:1, v/v/v). The developing solvent run up to 70 mm in a trough chamber, previously saturated with 10 ml of solvent mixture for 20 min. Densitometric scanning was performed at 210 nm. The hR_F values were 21 and 43 for ramipril and hydrochlorothiazide, respectively. The limit of detection and limit of quantitation were found to be 1000 ng/band and 6000 ng/band for ramipril, and 625 ng/band and 3750 ng/band for hydrochlorothiazide, respectively. The proposed method can also be used for routine quality control to accurately determine ramipril and hydrochlorothiazide in bulk and tablet dosage form.

P-102

Simultaneous estimation of cyclobenzaprine HCl and aceclofenac in pharmaceutical formulation by HPTLC densitometry

Roshni PATEL
Indukaka Ipcowala college of Pharmacy, New Vallabh Vidhyanagar, India,
roshni_ramdev@yahoo.com

Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy
Falgun MEHTA, Indukaka Ipcowala college of Pharmacy
Dimal SHAH, Indukaka Ipcowala college of Pharmacy

A simple, precise, rapid, selective, and economic reversed phase HPTLC method has been established for simultaneous analysis of cyclobenzaprine HCl and aceclofenac. HPTLC method was developed on TLC plates silica gel G60 F254 as stationary phase using toluene - ethyl acetate – methanol - acetic acid (5:3:2:0.4) as mobile phase. The plates were scanned at approximately 280 nm for both cyclobenzaprine HCl and aceclofenac, respectively. In HPTLC method both the drugs were resolved and hR_F value was 30 for cyclobenzaprine HCl and hR_F 65 for aceclofenac. The method was found to be linear in the range 60-420 ng/band and 800-5600 ng/band for cyclobenzaprine HCl and aceclofenac, respectively. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is important tool for analysis of combined dosage form.

P-103

Quantification of metolazone and ramipril in their combined dosage form by HPTLC

Pratik MODI
Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, India,
pratikmodi97@gmail.com

Usmangani CHHALOTIYA, Indukaka Ipcowala College of Pharmacy
Dimal SHAH, Indukaka Ipcowala College of Pharmacy
Falgun MEHTA, Indukaka Ipcowala College of Pharmacy

A simple, sensitive and precise high performance thin layer chromatographic method has been developed for the estimation of metolazone (MET) and ramipril (RAM) in the combined dosage form. TLC aluminum plates pre-coated with silica gel F254 G60 used as the stationary phase, while ethyl acetate – methanol – toluene - ammonia (5:3:2:1 v/v/v/v) used as mobile phase. The hR_F value was observed 44 ± 2 and 62 ± 1 for RAM and MET, respectively. The densitometric analysis was carried out in absorbance mode at 210 nm. The method was linear in the range of 100-700 ng/band for MET and 500-3500 ng/band for RAM. The method was validated as per ICH guideline. The limit of detection and limit of quantification were found to be 14.6 ng/band and 48.6 ng/band, respectively for MET. The limit of detection and limit of quantification were found to be 46.4 ng/band and 154.6 ng/band for RAM. The proposed method was successfully applied to the estimation of MET and RAM in the combined dosage form by HPTLC method.

P-104

Designing a nutraceutical formulation with antioxidant property as a preventive remedy against diabetic retinopathy

Rajesh SHARMA
VidyaPratishthan's Arts Science and Commerce College., Baramati, Pune, India,
rajesh_vpascc@yahoo.com

Sushama CHAPHALKAR, VidyaPratisthan's School of Biotechnology
Madurima DIKSHIT, Chemistry Department,University of Pune

Diabetes is the leading cause of blindness in working age group accounting approximately 12% of all cases of registrable blindness amongst those < 65 years old. Retinopathy, damage caused to retina by complications of diabetes is third major cause of vision loss followed by cataract and glaucoma. The etiology of retinopathy can be tracked by four phases: Oxidative stress, hyperglycemia, improper angiogenesis and weakening of pericytes the smooth muscle cells lining the vascular endothelium in retina. So overcome these phases, the objectives of the current study was to design an herbal formulation that will act as preventive remedy against Diabetic retinopathy.

To combat phase 1, 2 and 3, the formulation was subjected to extractions of polyphenols and flavonoids using HPTLC, micro preparatory HPTLC was used for purification of the secondary metabolites. A bank of molecules was generated by scraping the stationary phase and efficacy of each was established by FRAP assay. Radical scavenging ability was determined by DPPH and ABTS assay, Lipid peroxidation by TBARS assay.

Since the study is recommending edible formulation, the cytotoxicity MTT assay was performed. The formulation was evaluated for its absorption via intestine (using goat intestine model). Pericyte culture and CAM assay assessed the ability to imparts strength to vascular pericytes. Safety of the formulation can be guaranteed for it is reducing lipid peroxidation (TBARS assay). No DNA damage was observed with the Comet assay. The results of the entire assays are promising and the drug can be safely consumed.

P-105

Development and validation of the HPTLC quantitative method of the degradation products in bromo chloro amide

Veronika CICCONE
DSM Nutritional Products Ltd, CH-4334 Sisseln, Switzerland, veronika.ciccone@dsm.com
Evelyn RECK, DSM Nutritional Products Ltd

TLC is a very important tool in the in-process control in the production of APIs. Facile sample preparation and application as well as the instant receipt of results are the major benefits of this technique. The use of HPTLC with quantitative data interpretation offers even additional advantage.

In this study we describe the development of a new HPTLC method for the determination of five specific impurities in bromo chloro amide. The method was adapted for the release analysis in a QC laboratory and validated. Several method parameters had to be standardized, among them exposure conditions and incubation time. In this context the robustness of the method was studied.

The following method parameters were studied during the method development:
- The influence of relative humidity on the separation capacity
- Saturation of the developing chamber
- Stability of the test substances on a TLC plate
- Stability during the chromatography (studied by 2D-chromatography)

The validation included specificity, determination of the LOQ, linearity of each test substance, precision (system precision, repeatability and intermediate precision), accuracy and robustness. The method is suitable for the quantitative determination of the degradation products of bromo chloro amide and is also ready for the implementation for the release analysis.

P-106

Evaluation of glycosylceramides in wheat germ by automated multiple development (AMD)-HPTLC

Mathias REISBERG
Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany, mathias.reisberg@pharmazie.uni-halle.de

Peter CHLEBOWSKI
Reinhard NEUBERT
Birgit DRÄGER

Ceramides play a crucial role in the formation of the skin barrier. A quantitative ceramide dysbalance seems to be a factor for skin disease manifestation like psoriasis and neurodermatitis. Ceramides are commercially available, however, semisynthetic and very expensive; thus there is a need for cheap alternatives. Nature itself provides an alternative source in plants: glycosylceramides occur ubiquitously and may serve as a resource for human skin ceramides.

Glycosylceramides comprise a wide range of structures. d18:2 h16:0-Glu is a major component of wheat sphingolipids (Sullards *et al.*, 2000. J Mass Spectrom. 35: 347-353). It was detected in many other plants and therefore was used as a reference substance in these studies. Especially seeds contain high amounts of glycosylceramides, they may present a naturally source for growth. Wheat germ, a by-product of milling, is an attractive glycosylceramide resource.

Most HPTLC methods focus on the separation of ceramides from skin samples. For glycosylceramides from plants, a reliable and effective method for the identification and evaluation was necessary.

A high performance chromatography technique AMD-HPTLC method was adapted for the determination of lipid classes from plant samples with special regard to glycosylceramides. Therefore, an established 18-step AMD-HPTLC method for the separation of skin ceramides was used as a start for method development (modified after Opitz *et al.* 2011, Chromatographia. 73: 559-565). Modifications in solvent and gradient composition led to band sharpening and a better separation of glycosylceramides from glycosylsteroids, which is essential for glycosylceramide determination. The total lipid extract and a glycosylceramide fraction after column chromatography were investigated. Densitometric analysis revealed two sharp and separated peaks of glycosylceramides and glycosylsteroids and proved the feasibility of the method for future screening procedures.

P-107

TLC is complementary technique to HPTLC

Charu AGARWAL
Galgotias university, Greater Noida, India, tina.icfai@gmail.com

TLC using ligand exchange and impregnation methods provided a direct, simple, sensitive, rapid and economical approach to enantiomeric resolution of certain analytes including those from pharmaceutical preparations along with a method to recover the pure enantiomer for further use or application in small amounts and can be practiced both in analytical laboratories and industry for routine analysis and R&D activities in comparison to high-performance liquid chromatography and capillary electrophoresis that have high equipment and running costs. The CDRs provided a high sensitivity with limits of detection at ng level that could be applied for the trace analysis in pharmaceutical and biological samples. Thus, the methods have potential applications in quality control in pharmaceutical formulations of many pharmaceutically and biologically important compounds.

P-108

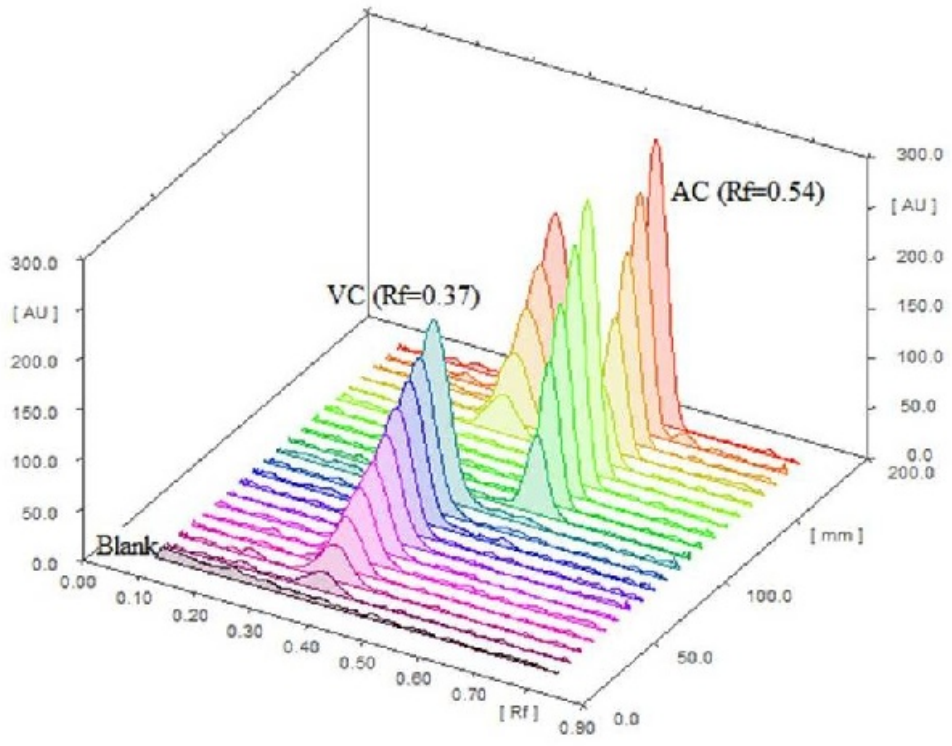
Development of validated stability indicating HPTLC assay method for simultaneous quantification of valacyclovir and of acyclovir

Achintya SAHA
University of Calcutta, Kolkata, India, achintya_saha@yahoo.com

Plaban BHATTACHARYA, University of Calcutta

Valacyclovir (VC) is the valine ester of acyclovir (AC). Plasma AC concentration was found to be enhanced after valine esterification and frequency of solitary administration of AC was reduced. The bioavailability of VC is sometimes observed to be poor due to undesired hydrolysis in the intestinal lumen. Hence, it is essential to analyze VC and AC simultaneously during various formulation development studies.

A specific, precise, robust and accurate HPTLC method has been developed for simultaneous quantification of VC and AC. The drugs, VC and AC were separated isocratically on silica gel 60 F254 with n-butanol - glacial acetic acid - 1M triethyl ammonium acetate, 4:1.5:1.5 (v/v/v) as mobile phase. Densitometric evaluation of the separated zones was carried out at 254 nm. There was no chromatographic interference from the other components and compact spots were observed for VC (hR_F 35 ± 2) and AC (hR_F 0.5 ± 2). Regressional analysis of the calibration plot revealed good linearity over the concentration range of 0.1-1 µg and 0.1-0.5 µg for VC and AC, respectively. Stability of VC was checked under acidic, alkaline and aquatic environmental stress conditions.



Densitograms of valacyclovir and acyclovir

P-109

Sarcosine as a stage-dependant metabolomic marker to detect prostate cancer by using HPTLC

Rajesh SHARMA
Vidyapatisthans School of Biotechnology, Baramati, India, rajesh_vpascc@yahoo.com

Sushma CHAPHALKAR , Vidyapatisthans School of Biotechnology
Chakraborty CHAKRABORTY, Vidyapatisthans School of Biotechnology
Kaustubh DESHPANDE, Vidyapatisthans School of Biotechnology

Prostate cancer (CaP) is the most common type of tumors disease in men. Early diagnosis of cancer of the prostate is very important, because the sooner the cancer is detected, the better it is treated. According to the fact, there is great interest in the finding of new markers including amino acids, proteins or nucleic acids. Prostate specific antigen (PSA) is commonly used and is the most important biomarker of CaP. This marker can only be detected in blood and its sensitivity is approximately 80%. Moreover, early stages cannot be diagnosed using this protein. Currently, there does not exist a test for diagnosis of early stages of prostate cancer. This fact motivates us to find markers sensitive to the early stages of CaP, which are easily detected in bio-fluid urine.

P-110

TLC method for simultaneous determination of seven chlorophenoxy and benzoic acid herbicides in ground water

Olga POPOVSKA
Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia, Skopje, Macedonia, olga_popovska@yahoo.com

Jana SIMONOVSKA, Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia
Zoran KAVRAKOVSKI, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Vodnjanska 17, 1000 Skopje, Republic of Macedonia
Vesna RAFAJLOVSKA, Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia

Thin layer chromatography (TLC) densitometric method for simultaneous determination of seven chlorophenoxy and benzoic acid herbicides (2,4-D; 2,4,5-T; MCPA; 2,4-DP; MCPP; Dicamba and MCPB) in water samples was developed and validated. The method was developed using a mobile phase consisted of benzene-dioxane-glacial acetic acid in ratio of 45 : 10 : 2, TLC silica gel 60 F254 aluminium plate and densitometry scanning at 280 nm wavelength. The linearity was obtained in the range 20-200 ng per band with the correlation coefficient between the peak area and concentration for each analyte higher than 0.9976.The limit of detection and quantification were 18 and 60 ng per band, respectively. The extracted recoveries of analyzed herbicides varied from 84.5 to 92.7%. The method can be used for routine quality analysis of chlorophenoxy and benzoic acid herbicides in natural surface and ground water samples.

P-111

HPTLC analysis of carbamate insecticides of forensic importance: separation evaluation and stability study

Praveen U. SANGANALMATH
Toxicology Division, Forensic Science Laboratory, Karnataka, Bangalore, India,
praveen_usp@yahoo.com

Purigali M. NAGARAJU, Forensic Science Laboratory, Karnataka, India

Methomyl (MM), carbofuran (CF), fenobucarb (FC), and carbosulfan (CS) are the widely used carbamate insecticides due to their broad spectrum of activity, short environmental persistence, and low mammalian toxicity. Lethal intoxication cases due to intentional self-poisoning are reported, so assays are required for the evaluation of separation parameters for these insecticides. Hence, a study was undertaken to evaluate the efficacies of separation of four carbamate insecticides by NP-TLC on silica gel 60 F254 and RP-HPTLC on silica gel 60 RP-18 W F254.

The study revealed that the system of NP-TLC with mobile phase n-hexane-acetone (7.5:2.5) and system of RP-HPTLC with mobile phase methanol-water (8.0:2.0) provided the optimum conditions for the separation of MM, CF, FC and CS. Densitometric detection was performed at 200 nm for CF, FC and CS and at 240 nm for MM. These wavelengths resulted in higher values for peak area and peak height. Retention (RF and RM) and separation (Δ;RF, RS, α, RFα) data were calculated. Under the chromatographic conditions used, methomyl and carbosulfan were found to have higher RM values in normal phase and reverse phase, respectively. Methomyl was therefore adsorbed strongly in NP-TLC and carbosulfan in RP-HPTLC. RF values of all four carbamate insecticides increased with increasing mobile phase acetone content in case of NP-TLC and decrease of the mobile phase water content in RP-HPTLC. Peak resolution (RS) was greater than 1.5 for all compound pairs (CF-MM, FC-MM, CS-MM, FC-CF, CS-FC, and CS-CF) in NP-TLC. RS values in RP-HPTLC were also higher for all the pair compounds. Four carbamates were stable for at least 6 h in solution and 6 h on the plate before chromatography. No decomposition of carbamates was observed by two-dimensional chromatography.

P-112

Pesticide screening in forensic toxicology by HPTLC

Praveen U. SANGANALMATH
Toxicology Division, Forensic Science Laboratory, Karnataka, Bangalore, India,
praveen_usp@yahoo.com

P. M. NAGARAJU, Forensic Science Laboratory, Karnataka, India

The identification of unknown or unsuspected poisons in lethal intoxication cases is one of the most challenging aspects of forensic toxicology. Identification is primarily based on the comparison of the analytical data gathered on the chemical properties (e.g. retention behavior in a given chromatographic system, and/or mass, UV or IR spectrum) with those of reference compounds contained in a database.

Pesticides, because of their potential toxicity are commonly used as poisons in homicides, suicides and also in cases of crime against animals. Incidents of poisoning due to pesticides could not be ascertained by clinical signs alone and in most instances, the identity of the pesticide involved was not known with certainty, resulting in deficiencies or delays in the care afforded to patients. Thus, a rapid identification of causal pesticide would provide very useful information to clinicians for making treatment decisions in emergencies, which is also important in forensic cases.

A simple, rapid and inexpensive HPTLC method is adapted for the screening of organophosphates, carbamates, synthetic pyrethroids, and other group of pesticides. The pesticides have been selected due to their market presence and reported cases of lethal intoxication.

Separation of pesticides was achieved on precoated silica gel 60 F254 HPTLC plates. In this study, preliminary data such as mobile phase optimization, detection wavelength and in situ UV spectrum was recorded for each of the pesticide and the analytical data gathered was stored in the HPTLC library for detection and identification of pesticides reported in cases of fatal intoxication.

The availability of preliminary analytical databases is of paramount importance in a forensic toxicology laboratory for solving poisoning cases such as suicide and homicide attempts in which the poisoning source is uncertain.

P-113

HPTLC analysis of three organophosphorus fungicides of forensic importance in whole blood samples

Praveen U. SANGANALMATH
Toxicology Division, Forensic Science Laboratory, Karnataka, Bangalore, India,
praveen_usp@yahoo.com

P.M. NAGARAJU, Forensic Science Laboratory, Karnataka, India
K. KEMPARAJU, Department of Biochemistry, Mysore University, Karnataka, India

Poisoning by organophosphorus fungicides (OPFs) is although uncommon; recently, a few cases of deaths owing to the ingestion of OPFs were reported. Ditalimfos (D), edifenfos (E), and tolclofos-methyl (TM) are the commonly used OPFs by the farming community. The availability of a large number of OP compounds has added complexities in toxicological analysis, resulting in more confusion. Therefore, it is a challenging task for forensic scientists to find out the exact fungicide used for suicide and homicide purposes.

Efficacies of separation of three OPFs was evaluated by NP-TLC on silica gel 60F254 and RP-HPTLC on silica gel 60RP-18 WF254. A HPTLC method is developed for the determination of three OPFs in whole blood samples. Different extraction procedures were compared for optimum recovery of OPFs from spiked blood samples over a pH range of 4 to 6.5.

OPFs were best separated by NP-TLC by use of n-hexane-acetone 9:1 (v/v) as mobile phase. RP-HPTLC with methanol-water as mobile phase has not provided optimum conditions for the separation, because complete separation of E from TM was not achieved. An average analytical recovery of 93.12, 92.5, and 91.33 % was achieved from a dichloromethane extract at pH 5.5 for D, E, and TM, respectively. Calibration curve for the OPFs in blood were linear from 0.5 to 100 g/ml^{-1}. The lower limit of quantification was 1.22, 1.27, and 1.5 g/ml^{-1} for D, E and TM. The method showed excellent intra and inter-assay precision for spiked blood samples at concentrations of 1, 10, and 50 g/ml^{-1}.

The proposed HPTLC method has proven to be simple, rapid, inexpensive, and does not require extensive cleanup procedures even though whole blood is a complex matrix. The method is highly suited to toxicological analysis, because the calibration graph obtained by use of linear regression led to excellent analytical results during validation.

P-114

Extraction/isolation and detection of dicyclomine hydrochloride from blood using HPTLC

Supriya KRISHNA
All India Institute of Medical Sciences, New Delhi, India, cheenu_krishna@yahoo.co.in

Tabin MILLO, All India Institute of Medical Sciences
Ashok KUMAR JAISWAL, All India Institute of Medical Sciences

Dicyclomine hydrochloride belongs to the class of synthetic anticholinergics. It is regularly used for the treatment of spasmodic pain, spasmodic dysmenorrhea, and renal, ureteric and biliary colic. This drug is easily available in the market and nowadays abused for suicidal purposes. Biological sample of choice for quantitative and qualitative analysis of drug is Blood. Routinely, highly sophisticated instruments like HPLC, Gas Liquid Chromatography (GLC) are used for the analysis of drug. An attempt has been made to develop a new HPTLC method for analysis of dicyclomine in biological sample, *i.e.* blood.

Dicyclomine was extracted from blood using liquid-liquid extraction method. For chromatographic separation, various binary and tertiary solvent systems (toluene – acetone - ammonia 7:3:0.5); ethyl acetate – chloroform - methanol 7.5:1.5:0.5) were used as mobile phases. Developed plates were viewed under UV light followed by spray of chromogenic reagents which successfully increased the sensitivity without interfering with the simplicity of the method. The method developed is simple, rapid, inexpensive, and can be performed in any laboratory as a preliminary examination before any instrumentation.

P-115

Determination of aflatoxins in livestock mixed feed and feed ingredients

Halis OGUZ
Selcuk University Faculty of Veterinary Medicine, Konya, Turkey, hlsgz@hotmail.com

Hasan AYDIN, Konya Veterinary Control Institute, Turkey
Riza UCMAN, Konya Veterinary Control Institute, Turkey

During 2009 - 2012 period, mixed feeds (n=56) and feed ingredients (n=59), from different eight provinces (Afyonkarahisar, Aksaray, Antalya, Burdur, Isparta, Karaman, Konya and Niğde), submitted to Toxicology Laboratory, Konya Veterinary Control Institute with suspected aflatoxins(B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2)) were analyzed in this study.

Samples were analyzed for aflatoxin using thin layer chromatographic and semi-quantitative method for detection and quantification. A floriosil column was used during preparation of samples. AFB1 was found in 16 samples of mixed feed (28.6 %) and 9 feed ingredient (15.3 %) whereas AFB2 in 2 mixed feed (3.6 %) and 3 feed ingredients (5.1 %). AFG1 and AFG2 were detected in only one sample of feed ingredients (1.7 %). It was found that AFB1 levels in mixed feed samples ranged from 1 to 20 g/kg and were 0.5 to 60 g/kg in feed ingredient samples. However AFB2 levels in mixed feed samples ranged from 0.2 to 2 g/kg and were 0.5 to 15 g/kg in feed ingredients. Levels of AFG1 and AFG2 in feed ingredients samples were detected 0.5 g/kg and 1 g/kg, respectively. Only two samples of feed ingredients (60 ppb AFB1 and 35 ppb AFB1) exceeded the maximum permissible levels of aflatoxins established by the Ministry of Food, Agriculture and Livestock.

P-116

Validated HPTLC method for identification and quantification of cyanogenic glycosides in apricot kernels and bitter almonds

Younes ZEBBICHE
National Center of Toxicology, Algiers, Algeria, dr.y.zebbiche@gmail.com

Imene REBAI, Laboratory of Toxicology, Hospital Bab El Oued, Algiers, Algeria
Mohamed AZZOUZ, Laboratory of Biology and Toxicology, Hospital Ait Idir, Algiers, Algeria
Rania ABTROUN, Laboratory of Toxicology, Hospital Bab El Oued, Algiers, Algeria
Mohamed REGGABI, Laboratory of Biology and Toxicology, Hospital Ait Idir, Algiers, Algeria
Barkahoum ALAMIR, National Center of Toxicology, Algiers, Algeria

The toxicity of apricot kernels and bitter almonds is related to the release of cyanide, highly toxic, from cyanogenic compounds. Identification and dosage of these cyanogenic glycosides are an important source of information in evaluating the toxicity of these plants. This paper presents an HPTLC method for identification and quantification of cyanogenic glycosides from apricot kernels and bitter almonds. Following this, we correlate results with those of cyanide concentrations measured by GC-FID HS.

After grinding bitter almonds and apricot kernels, 500 mg of ground material is extracted with 10 mL of methanol in a water bath at 60 °C for 1 h, then centrifuged and filtered using Whatman filter paper. The HPTLC method utilizes separation on HPTLC plates silica gel 60 F254 with methanol - ammonia (100:1.5, v/v) as mobile phase, derivatization with combined action of hydroxylamine hydrochloride and ferrous iron chloride reagents. Scanning densitometry is performed at 200 nm. A precise and accurate assay can be performed in the linear working range of 4 ng/spot absolute (0.1 % in the dried plant material) up to an amount of 80 ng/spot (2 %). The method has been validated, addressing specificity, stability, reproducibility, and robustness

The results show good correlation between cyanogenic glycosides measured by HPTLC and data obtained from analysis of cyanide released by GC-FID, the correlation coefficient R = 0.953 (R^2 = 0.909, n = 16). The validated HPTLC quantification method can easily be automated and is applicable to the analysis of multiple samples in parallel. As an indication, the number that can be responsible for a fatal poisoning by released cyanide varies from 11 to 21 bitter almonds (mean = 16) and from 25 to 211 apricot kernels (mean = 99).

P-117

Validation of a HPTLC method for quantitative determination of hypoglycin A in methanol extracts of maple (*Acer*) samples

Jean HABYARIMANA
Faculty of Veterinary Medicine, Liège, Belgium, J.habyarimana@ulg.ac.be

Etienne BAISE, Département des Maladies Infectieuses et parasitaires, FARAH, Faculty of veterinary Medicine, University of Liège, Belgium
Mireille WEIBER, Département des Sciences Fonctionnelles, FARAH, Faculty of veterinary Medicine, University of Liège, Belgium
Thierry FRANCK, Centre de l'Oxygène, Recherche et Développement (CORD), FARAH, University of Liège, Belgium
Gilbert GAULT, Usc 1233 Ediss, VETAGROSUP, Ecole nationale vétérinaire de Lyon, France
Jérémy CARLIER, Latlumtox, EDISS University of Lyon France
Dominique VOTION, Equine Pole, FARAH, University of Liège, Belgium
Pascal GUSTIN, FARAH, Faculty of veterinary Medicine, University of Liège, Belgium

Hypoglycin A toxin variably contained in seeds of *Acer pseudoplatanus* and *A. negundo* is the highly suspected cause of atypical myopathy (AM) in Europe and seasonal pasture myopathy (SPM) in the US. For unknown reasons, incidence of AM increases over the last decade. In 2013, Atypical Myopathy Alert Group (AMAG) registered the largest outbreak with 415 European cases. As no curative treatment of these fatal myopathies exists, prevention is of major importance. The seasonality of AM and link with climatic conditions deserve further investigations to improve preventive measures based on the identification of environmental components that would favour the toxicity of these common trees.

The present study aims to validate a reliable HPTLC method for determination and quantification of the toxin in plants. Method-Standard stock of 200 µg/ml hypoglycin A from Toronto Research Chemical were prepared. The separation was performed on silica 60 Å F254, 20 X 10 or 10 X 10 cm HPTLC plates as stationary phase and achieved by using a mobile phase of the following composition: methanol, acetic acid, water 70/ 20/ 10 (v/v/v). Determination and quantification were performed at 490 nm in the absorbance mode. In respect of ICH guidelines, we observed a repeatable hR_F value of 59 with a valuable selectivity and specificity. Linearity in interval of interest was demonstrated. Precision is still to be improved but is less than 6%. Limit of detection reached 11.6 ± 0.5 ng, limit of quantification 65.2 ± 2.7 ng and accuracy of the method were tested by performing a complete extraction determination and quantification process.

These results confirm suitability of HPTLC for detection of hypoglycin A in plant extracts. This methodology will be used to determine the concentration of hypoglycin in autumnal samples of *A. pseudoplatanus* trees collected on pastures, where AM had occurred in 2013 and 2014.

P-118

Tracing the diacylglycerol metabolism by click chemistry and TLC-fluorescence imaging

Kathrin Tscherch
Department of Chemistry, University of Tennessee, Knoxville, United States, ktscherc@utk.edu

Sammy ENI ENI, University of Tennessee
Shahrina ALAM, University of Tennessee
Tanei RICKS, University of Tennessee
Michael Douglas BEST, University of Tennessee

Lipids are well-known for their fundamental role in forming biological membranes and as intracellular signaling molecules. In relationship to numerous diseases, lipids have been implicated in disease onset. Furthermore, the molecular signature of individual lipids is increasingly identified to encode for highly specific functions. While understanding the lipid metabolism and the specific role of individual lipid species is of crucial importance, such efforts are hindered by analytical difficulties such as low intracellular concentrations and complexity of the cellular environment. The development of chemical strategies and in vivo analytical methods to investigate lipids on a cellular level is therefore critical for enhancing the understanding of their biological role. The copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC), commonly known as click reaction, describes the reaction between an azide and alkyne to form a triazole. Click Chemistry enables the sensitive and specific detection of compounds containing azido groups or terminal alkynes, which can be integrated into the lipid molecule without major disturbance of the structure of hydrophobic hydrocarbon chains.

In this study, we present the design and synthesis of a diacylglycerol (DAG) analogue containing a functional azido moiety. DAG possesses crucial relevance in various biological processes and diseases, specifically cancer, and represents an important precursor for numerous species such as phospholipids. Studies were carried out in Saccharomyces cerevisae. Progress towards the selective labeling of biosynthetic products by introducing a fluorescent reporter molecule carrying an alkyne group will be presented, along with chromatographic separation and detection of fluorescently labeled lipids using TLC-fluorescence imaging.

P-119

Assessment of cardiolipin content by HPTLC and cardiolipin fatty acid composition by GC in rat mammary tumors

Michelle PINAULT
UFR Medecine de TOURS INSERM U1069, TOURS, France, michelle.pinault@univ-tours.fr

Julie DARTIER, UFR Medecine TOURS, France
Cyrille GUIMARAES, UFR Medecine TOURS, France
Caroline GOUPILLE, UFR Medecine TOURS, France
Stephane SERVAIS, UFR Medecine TOURS, France
Stephan CHEVALIER, UFR Medecine TOURS, France
Jean-françois DUMAS, UFR Medecine TOURS, France
Pierre BESSON, UFR Medecine TOURS France
Karine MAHEO, UFR Medecine TOURS, France

Studies suggest that cardiolipin (CL), a mitochondria-specific phospholipid (PL), could be implicated in the increase of drug efficacy by docosahexaenoic acid (DHA, 22:6n-3) in mammary tumors. Our hypothesis is that it could be due to a change in the CL content of mitochondria, and/or the fatty acid (FA) composition of CL. The aim of this study was to quantify CL as well as other membrane PL (phosphatidyl -choline PC, -ethanolamine PE, -serine PS, -inositol PI, and sphingomyelin, SM), and determine its FA composition in tumors of rats fed diets supplemented or not with fish oil.

Total tumor lipids were deposited on HPTLC plates with Camag ATS4 applicator and separated with an appropriate solvent. After revelation, PL bands were quantified by densitometry with Reprostar Camag TLC scanner 4. The amount of PL classes was determined by reference to a standard curve, and expressed as % of total membrane PL. In order to assess the FA composition of CL, samples were separated by conventional TLC. The CL spot was scraped and FA were methylated. FA methyl esters were quantified by gas chromatography.

The proportions of CL and other PL classes were not different between tumors from rats fed the control or DHA-rich diets. In contrast, the FA composition of CL was altered by fish oil: there was a 5-fold enrichment in total n-3 FA compensated by a 1.3-fold decrease in mono-unsaturated FA. N-3 FA were also enriched in PC, PS, PE and PI, but to a lower extent, and were not changed in SM.

This study shows that dietary supplementation with polyunsaturated n-3 FA increased DHA content of CL, which could affect mitochondrial functions and participate to chemosensitization.

P-120

Occupational exposure to polycyclic aromatic hydrocarbons (PHA) among asphalt and road paving workers

Elalia BOULARAS
Medicine Faculty of Sidi Bel Abbes, Oran, Algeria, a_boularas@hotmail.fr

Haciba REZK-KALLAH, Medicine Facutly of Oran

The assessment of the exposure to PHA has been performed on workers belonging to two companies, allocated into several activities. With whom a group of control was associated. In a first stage, we reconstituted the history, the economic, commercial and technical dimensions of both companies. The internal exposure to PHA was investigated by measurement of the urinary excretion of 2-naphtol, urine metabolite of naphtalene, one of the biomarkers of total PHA exposure. The aim was:

- To describe the characteristics of certain activities in the sector of asphalt and Roadworks (road paving, manufacturing of coated bituminous warm , manufacturing of asphalt cut-back, manufacturing of emulsion of asphalt),
- To assess the current exposure to the PHA among various workers in the sector of asphalt and road paving,
- To propose appropriate prevention measures.

Urine samples were collected from the exposed workers, at the beginning of the week, at the beginning of the work shift (BWBS) and at the end of the work shift, at the end of the week (ESEW). For control subjects, singles samples of urine were collected after the end of the work shift.Every subject was invited to answer a questionnaire for the collection of technical and medical data as well as the smoking habits, and food.

The concentration of 2-naphtol in the hydrolysate of urine was determined spectrophotometrically, after its reaction with the Fast Blue BB salt (diazotized 4-benzoylamino-2,5-diethoxyaniline). For all the workers included in the study (n=14), the 2-urinary naphtol concentrations are more important than those of the control subjects (n=18, median=9.55 mg/g creatinine) whether it is at (BWBS) (Md=16.2 mg/g creatinine) or at (ESEW) (n=18, median d=32.22 mg/g creatinine).

Considerable differences are observed according to the category of job. The concentrations are also higher among smokers. The results show a significant exposure, mainly during manual laying, reveals an important risk particularly for the respiratory system. Considering the current criteria, carcinogenic risk due to the HAP seems not insignificant.

P-121

Quantitative analysis of base oils by HPTLC-FDIC

Vicente L. CEBOLLA
CSIC-ICB, Zaragoza, Spain, vcebolla@icb.csic.es

Carmen JARNE, CSIC-ICB, Zaragoza, Spain
Luis MEMBRADO, CSIC-ICB, Zaragoza, Spain

AMD has been used in isocratic mode, with n-heptane as mobile phase over increasing migration distances (20-40 mm, in 10 mm steps) to obtain a rapid separation of base oils in Saturates and Aromatics. In addition, an extended AMD separation using a THF-DCM-n-heptane 20-step gradient with 2 mm step-1 over 90 mm total migration distance can also be applied to base oils. This allows an improved separation of the apolar families of these samples to be carried out.

Attribution of the nature of separated peaks using both AMD conditions has been done by comparison with migration distances of standards (applied individually and spiked with the base oils), selective UV detection at different wavelengths, and on-silica UV recording of peak's spectra. LOD and LOQ of aromatics in base oil have also been obtained from UV chromatograms.

Detection has also been done using fluorescence densitometry by FDIC. Quantification of saturates involved post-impregnation of silica gel plates with a solution of either berberine sulfate or berberrubine in MeOH (60 mg L-1).

As fluorescent responses of saturated hydrocarbons in berberine depend on the chain length, calibration procedures using this fluorophore must be done by normalization using a calibrant which is representative of the problem sample. Different calibrants have been tested: 1) a mixture of pure alkanes; 2) fractions of Saturates (obtained from the base oils either by flash chromatography, or using a TLC-MS interface).

Unlike in the case of berberine, fluorescence responses of saturated hydrocarbons on berberrubine plates are roughly constant with the number of carbon atoms in the hydrocarbon chain. Then, base oils may be determined using a pure alkane (e.g. C24, C28 or C32) as a single calibrant. Results are discussed and compared with those obtained from HPLC.

P-122

Chromatographic profiling of heavy petroleum products by AMD-densitometry - Repeatability, factors influencing separation and some examples of application

Vicente L. CEBOLLA
CSIC-ICB, Zaragoza, Spain, vcebolla@icb.csic.es

Carmen JARNE, CSIC-ICB, Zaragoza, Spain
Luis MEMBRADO, CSIC-ICB, Zaragoza, Spain

Automated Multiple Development (AMD) can be used for semi-detailed chromatographic characterization of heavy petroleum products. An optimized 20-step, THF-DCM-n-heptane gradient has been used for profiling a variety of samples that covers the whole range of petroleum heavy products.

Peak migration distances, chromatographic profiles, and fluorescence intensity signals obtained using this method have shown to be repeatable (intra- and inter-plate in the same laboratory) and reproducible (in two different laboratories).

Factors influencing separation have been studied. THF, coming from either mobile phase or plate cleaning solvent, plays a crucial role in the separation of the "Resins" zone of chromatogram. A mechanism previously proposed by Scott explains THF role on the behaviour of heavy hydrocarbon compounds.

Although this separation does not provide a complete molecular resolution of heavy petroleum products, the resulting profiles adequately represent the complexity of the analyte, and can provide a useful information for identification, comparison purposes, or for evaluating the effect of process variables on a crude oil. Some examples of application are presented. In the case of bitumes, different profiles have been obtained in function of their origin. A deasphalting process of an extra-heavy raw oil has also been monitored, showing different profiles for the crude oil and its derived deasphalted products under different conditions.

P-123

Application of AMD to petrochemical analysis: improved separation and expanded hydrocarbon group type analysis of heavy petroleum products

Vicente L. CEBOLLA
CSIC-ICB, Zaragoza, Spain, vcebolla@icb.csic.es

Carmen JARNE, CSIC-ICB
Luis MEMBRADO, CSIC-ICB

A complete molecular separation of heavy petroleum products is not feasible for their quality control. They are instead characterized in terms of chemical family composition, using classical Hydrocarbon Group Type Analysis such as SARA (Saturates, Aromatics, Resins, Asphaltenes). However, SARA does not provide information enough to correlate chemical differences of products with their conversion parameters in the case of heavy products.

AMD appears as an interesting alternative to obtain different separations with increasing level of complexity for these products. In general, and for a given sample, AMD can use the same mobile phase over increasing migration distances (refocusing effect) or use a combination of gradient development and refocusing in the search for an improved separation. Conditions can be fine tuned to obtain at will either an increased expansion of the asphaltenic-resinic chromatographic zone, or an increased expansion of the apolar zone (saturates-naphtheno aromatics) of products such as vacuum gas oil and residues, bitumes, asphaltenes or base oils.

In the simplest approach, SARA can also be obtained by AMD using a sequence of several elution solvents in order of growing or decreasing polarity, as when using a conventional developing chamber. Detection has been carried out by UV and fluorescence densitometry. Saturates can be detected by Fluorescence Detection by Intensity Changes (FDIC) as a positive peak using berberine-impregnated plates.

P-124

Influence of relative humidity on PH-EUR performance test

Peter SCHAUB
Merck KGaA, Darmstadt, Germany, peter.schaub@merckgroup.com

Michael SCHULZ, Merck KGaA
Susanne MINARIK, Merck KGaA

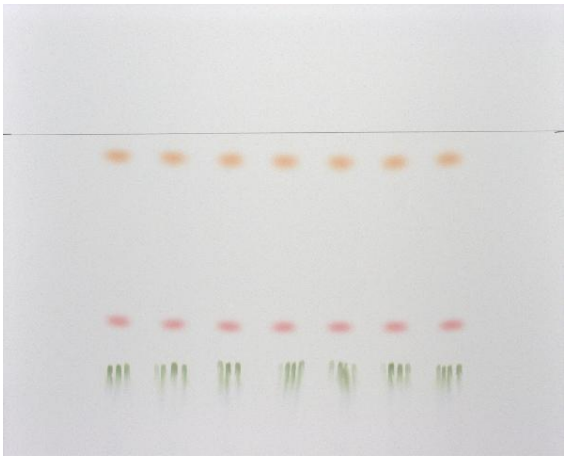
Water content is a well known source of influence during chromatographic separations. This especially plays a role when considering moisture on the stationary phase of a TLC plate. Therefore, for most analytical methods an activation step or storage at a certain humidity as part of the method preparation is prescribed. The relative humidity of the laboratory room still plays a big role during sample application directly before separation caused by the fast transfer of water onto the plate. This fact becomes more important when using non polar mobile phases.

This non-regulated variance of humidity around the globe leads to aberrations in results when using the PH-EUR performance test for silica gel plates [1]. The separation is performed with a mixture of bromcresol green, methyl orange, methyl red and sudan red separated in a normal flat bottom chamber without saturation with toluene/methanol 8:2 as mobile phase.

At a relative humidity of greater than 40 % at 21°C, the test does not work, even if the plate is activated directly before sample application. The two lower-hR_F spots begin to smear on the plate, which makes a clear separation impossible. The test criteria, to have four clearly separated spots is not fulfilled. At a low relative humidity of 20 % at 21°C, the separation can be easily conducted with good results. All four spots are clearly separated.

This result can also be achieved by "warming" the plate after application for a short time at 30°C in a laboratory oven. But this step causes a degradation of methyl red, which becomes more and more visible depending on the temperature and residue time.

[1] European Pharmacopoeia VIIIth edition, 2014 (8.1), monograph 1116700: TLC silica gel plate



Development of a PH-EUR test at humidity >40%

P-125

Development of a work-flow for HPTLC data processing for untargeted metabolomics

Grégory GENTA-JOUVE
Laboratoire de Pharmacognosie, UMR CNRS 8638 COMETE, Université Paris Descartes, 4 Avenue de l'Observatoire 75006 Paris, France, gregory.genta-jouve@parisdescartes.fr

Coralie AUDOIN, Chanel Parfumes Beaute, France
Vincent COCANDEAU, Chanel Parfumes Beaute, France
Lionel WEINBERG, Chanel Parfumes Beaute, France
Serge HOLDERITH, Chanel Parfumes Beaute, France

Nowadays, most of the metabolomics studies are performed using NMR or MS techniques [1]. One technique however seems to have been left apart in this field: the high performance thin layer chromatography (HPTLC). Although the different practical steps of a HPTLC experiment are now all automated, this technique is suffering the lack of computational treatment of the data. A large number of qualitative and quantitative protocols have been developed using HPTLC for the study of plants extracts [2-4]. While for quantitative analyses UV detection is mainly use, qualitative comparisons of different samples are often realized by direct visualization of the plates.

In this context we developed the first work-flow dedicated to the analysis of HPTLC data, including the steps commonly used in metabolomics analyses. This development resulted in the increase of features detected, and more importantly, in the reduction of the common diffusion drift observed during HPTLC plate development.

[1] R. Schuhmacher, R. Krska, W. Weckwerth, R. Goodacre, Anal. Bioanal. Chem. (2013) 1-2
[2] X.-H. Wang, P.-S. Xie, C. W. Lam, Y.-Z. Yan, Q.-X. Yu, J. Pharm. Biomed. Anal. 49 (2009) 1221-1225
[3] S. Bonny, L. Paquin, D. Carrié, J. Boustie, S. Tomasi, Analytica Chimica Acta 707 (2011) 69-75
[4] J. P. Piwowarski, A. K. Kiss, Phytochem. Anal. (2013)

P-126

Simple and selective two-dimensional separation of complex mixtures of peptides with planar chromatography

Radosław GWARDA
Department of Physical Chemistry, Chair of Chemistry, Medical University of Lublin, Lublin, Poland, Lublin, Poland, radoslaw.gwarda@umlub.pl

Monika ALETAŃSKA-KOZAK, Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Medical University of Lublin, Lublin, Poland
Dariusz MATOSIUK, Medical University of Lublin, Poland
Tadeusz DZIDO, Department of Physical Chemistry, Lublin, Poland

High efficient two-dimensional (2D) separation belongs to important advantageous features of planar separation techniques. This separation mode can be useful for analysis of many types of complex samples, such as protein digests. Different selectivity in each dimension is required to obtain good 2D separation and this is fulfilled if various mechanisms of separation selectivity or/and retention of analytes are involved in each of two chromatographic systems.

In one of our papers we have presented an example of simple and selective 2D separation of tryptic bovine serum albumin digest using high performance thin layer chromatography [1]. Here we show 2D separations of other complex mixtures of peptides (mixture of some synthetic oligopeptide standards as well as tryptic digests of proteins) using silica-based reversed-phase HPTLC plates and mobile phases composed of alcohols and water (with addition of proper buffer/ion-pairing reagent), with various modifications.

[1] Gwarda, R.L., Dzido, T.H. J Chromatogr A, 2013, 1312, 152-154

P-127

Influence of some variables on migration distance and solute band shape of peptides in pressurized planar electrochromatography system

Radosław GWARDA
Department of Physical Chemistry, Chair of Chemistry, Medical University of Lublin, Poland, radoslaw.gwarda@umlub.pl

Monika ALETAŃSKA-KOZAK, Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Medical University of Lublin, Poland
Dariusz MATOSIUK, Medical University of Lublin, Poland
Tadeusz DZIDO, Department of Physical Chemistry, Lublin, Poland

Pressurized planar electrochromatography (PPEC) is relatively new separation technique in which flow of the mobile phase is driven by electric field (electroosmotic effect). It is characterized by many advantages of thin layer chromatography and by some new, typical just for PPEC (such as short time of separation process, possibility of long-distance separation and different selectivity relative to chromatographic as well as electrophoretic systems). However, despite of development of this technique for last ten years, there are still quite few examples of its application to separation of particular groups of compounds. There is only one example of peptide separation using PPEC (with monolithic polymer layer) reported so far [1]. Anyway, influence of particular variables on peptide behavior in PPEC systems have not been presented yet.

Here we show influence of some variables (such as concentration of organic modifier of the mobile phase, type and concentration of ion-pairing reagent and/or surfactant, pH, etc.)on migration and zone shape of several oligopeptides in PPEC system using standard HPTLC RP-18 W chromatographic plates. We also indicate and try to explain some restrictions and difficulties related to use of such system for separation of these compounds.

[1] Woodward, S.D., *et al.*, Anal Chem, 2010, 82, 3445-3448

P-128

Berberrubine: a FDIC fluorophore for determining saturated hydrocarbons with a simple calibration via non-covalent interactions, and signal amplification from a keto-enol tautomerism

Vicente L. CEBOLLA
CSIC-ICB, Zaragoza, Spain, vcebolla@icb.csic.es

Arantzazu DELGADO-CAMÓN, CSIC-ICB
Carmen JARNE, CSIC-ICB
Andrés DOMINGUEZ, CSIC-ICB
Javier GALBÁN, Zaragoza University
Luis MEMBRADO, CSIC-ICB
Luis MEMBRADO, CSIC-ICB
Rosa GARRIGA, Zaragoza University
Fernando P. COSSIO, Euskal Herriko Unibertsitatea

Hydrocarbon-containing heavy products from petroleum consist of an important proportion of saturated hydrocarbons, having increasingly chain lengths with increasing boiling point. A rapid and accurate determination of these compounds as a single group (Saturates) is a challenge in petrochemistry.

Berberrubine is proposed here as a quantitative fluorescence sensor for saturated hydrocarbons that can be incorporated on HPTLC silica gel plates by adsorption, through impregnation. When n-alkanes are applied on a berberrubine-impregnated plate, and the system is excited at 365 nm, we found that fluorescent response (emission collected from 400 nm) is almost constant with the number of carbon atoms in the hydrocarbon chain. This behaviour is different to that of berberine and coralyne cations. In these cases, fluorescent responses depend on the alkane chain length, and any calibration procedure should take this into account.

Results show that berberrubine can be used as a "mass detector" for the analysis of Saturates in heavy petroleum products, simply using a pure alkane as a single calibrant. Saturates were determined in different heavy petroleum samples: one lubricating oil, 10 bitumes, 7 vacuum gas oil, 3 refining products and 2 synthetic mixtures, on berberrubine post-impregnated plates (60 ppm), and using alternatively three n-alkanes (C24, C28 and C32) as calibrants. Limits of application of this calibration are discussed

Berberrubine has two fluorescent forms in acidic and basic media, which correspond to a keto-enol tautomerism. Fluorescent signals can be rationalized from the predominance of enol form when berberrubine is adsorbed onto silicagel. OH bonds in enol form may difficult the freedom of orientation to interact with alkane molecules. Berberrubine was obtained from berberine chloride in a simple way, either by microwave-assisted or by vacuum pyrolysis synthesis.

P-129

TLC and magneto-TLC as a method for investigation on selected d-electron ion element complexes with organic ligands

Agnieszka WRONKA
Faculty of Chemistry, Maria Curie-Skłodowska University. Pl. M. Curie-Skłodowskiej 3, 20-031 Lublin, POLAND., Lublin, Poland, agnieszkawronka1@wp.pl

Irena MALINOWSKA, Maria Curie-Skłodowska University, Lublin, POLAND
Wiestawa FERENC, Maria Curie-Skłodowska University, Lublin, POLAND

The investigations of physicochemical properties of coordination compounds, not only as solids, but also in liquid state seem to be very important. The behaviours of these complexes being changed depending on the environments around the central ion may create various action.The retention analysis of investigated compounds may give us some information about their affinity to different stationary phase surfaces and about the influence of the central ion or organic ligand structure on the retention of these compounds.

The properties of 9 new complexes and their ligands have been characterized by chromatographic analyses. The parameter of relative lipophilicity (RM_0) of the tested compounds were determined experimentally by reversed-phase high-performance thin layer chromatography method with mixtures of aceton and water as a mobile phase. We also try to describe interactions between chromatographed substances and various surfaces (silica - SiO2 and modified by hydrocarbon chains - RP phases).

TLC combined with magnetic field has been proposed as complementary method for determination of physicochemical properties of investigated compounds. The chromatograms in the field and outside it were developed simultaneously in three identical chromatographic chambers. Two of them was placed in external magnetic field with two values of vector of inductivity (0.2 and 0.4 T). In magnetic field, retention of some complexes have changed, which means, that presence of mentioned field influences physicochemical properties of the compounds and their interactions with the stationary phase.

P-130

Application of 1 mL sample for analytic TLC

Fikret Nafi COKSOYLER
University of Yuzuncu Yil, Food Engineering Department, Van, Turkey, coksoyler@hotmail.com

Agan CANSU , University of Yuzuncu Yi I
Aydeniz RUKYETE, University of Yuzuncu Yi I

Immuno Affinite Column (IAC) clean up is an essential step for mycotoxin analysis by HPLC. 0.1-0.5 ml of IAC methanolic eluate is applied to sampling port of RP-HPLC. Increasing the sample volume decreases the quantification limits. In the case of TLC neither to increase sample volume more than 0.05 ml nor application of methanolic extract on the silica-gel plates is practical. In this study, a "high volume sample application" technique is developed for TLC. By means of this technique, up to 1.00 ml of methanolic eluate of the IAC could be applicable on plates. Five samples and a series of standards can be applied on the same 20X20 cm silica-gel coated aluminum plate. This simple and practical technique enables TLC separation of IAC cleaned -up extracts.

P-131

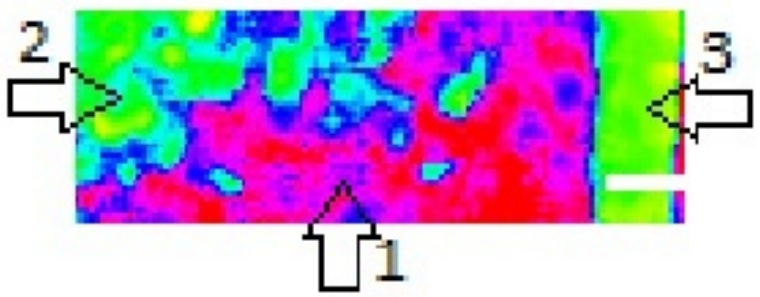
Thermal-wave investigation of physical non-uniformities within TLC stationary phases

Zbigniew SUSZYŃSKI
Koszalin University of Technology, Poland, Koszalin, Poland, zbigniew.suszynski@tu.koszalin.pl

Michał BEDNAREK, The Main School of Fire Service, Warszawa
Paweł ZARZYCKI, Koszalin University of Technology

Successful planar chromatographic separation requires high quality stationary phase layers in terms of their homogeneity. In this paper we report the results of investigation concerning detection of structural changing and thickness non-uniformities in planar chromatographic layers. Number of TLC and HPTLC stationary phases including silica, cellulose, aluminum oxide, polyamide and octadecylsilane with adsorbent layer ranging from 100 to 250 um were investigated.

Data acquisition and signal processing protocols are based on active pulse thermo-vision measurements. Active thermography methods for uniform spatial density of dissipated power is dedicated for detection and identification of thermal nonuniformities such as local geometrical (layer thickness), thermal effusivity and diffusivity changes. The last two parameters are affected by thermal conductivity, mass density and type of matter. Proposed measurement approach has revealed the changes in stationary phase thickness as well as detection of structural changes that appeared as result of the plate cutting.



Thermal Correlation image of nonuniformities in stationary phase layer:
1- proper quality, 2 - contrast of structural changes, 3 - contrast of the thickness increasing

P-132

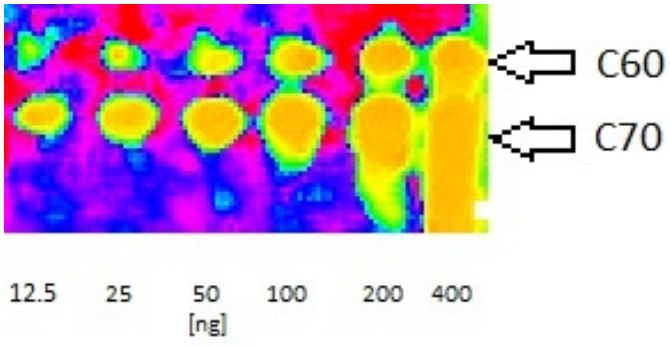
New active thermography method for sensitive detection of fullerenes separated on micro-TLC plates

Zbigniew SUSZYŃSKI
Koszalin University of Technology, Koszalin, Poland, zbigniew.suszynski@tu.koszalin.pl

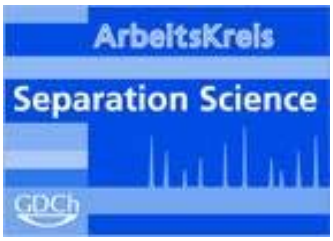
Paweł ZARZYCKI, Koszalin University of Technology
Robert ŚWITA, Koszalin University of Technology

In this research communication the new sensitive method for detection and identification of C60/70 fullerenes on RP18W HPTLC micro-plates (5x5 cm^2) is presented. Target analytes with mass ranging from 12.5 to 400 ng/spot were separated using mobile phase consisted of pure n-hexane and micro-plate covered with low-carbon loaded adsorbent RP18WF254S using unsaturated chamber.

Data acquisition and signal processing protocols are based on active pulse thermo-vision measurements. The source of energy excitation was composed of six flash lamps providing the total power density of 600 W/cm^2 for 10 ms pulse in visible spectra diapason. Temperature fields were registered by fast IR camera with resolution of 128x128 pixels captured with 500 fps.



Thermal Correlation image of C60/C70 fullerenes of mass ranging from 12.5 to 400 ng



Thanks to the working party *Separation Science*
of the German Chemical Society
that provided three travel grants
for PhD students and postdocs!

There were 24 applications for the 3 grants:

- Poland: 7
- Germany: 4
- India: 2
- Australia: 1
- Burkina Faso: 1
- Malaysia: 1
- Rumania: 1
- Serbia: 1
- South Africa: 1
- Turkey: 1
- Ukrain: 1
- 3 after deadline

Due to this high request, we would like to encourage further sponsors for support students with regard to travel grants for the next symposium! Be part of it.

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For attending HPTLC 2014
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Thank you!


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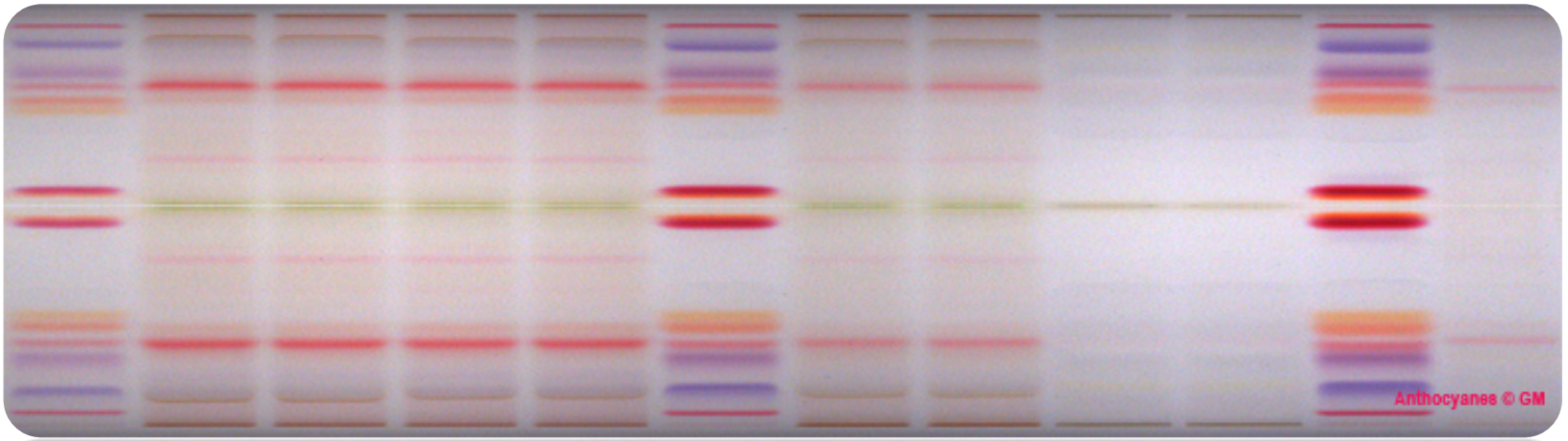

Attachment

The Scientific Community deeply recognizes the lifetime achievement of the Honorary Board Members for planar chromatography!

The inspiring researcher and thinker ahead!

12.02.1930
rudolf.kaiser@t-online.de

Prof. Dr. Rudolf Kaiser, Germany

- HPTLC has been developed at his *Institute for Chromatography* in 1976 together with Ute Hezel, J. Blome, H. Halpaap, D. Jaenchen and J. Ripphahn; documented in his book „HPTLC“, Elsevier, 1977, edited with A. Zlatkis, ISBN 0-444-41525-4
- Pioneer of planar chromatography → book on „Planar Chromatography“ (1986), ISBN 3-7785-0780-X
- www.planar-chromatography-by-kaiser.com, the micro PLC SITE
- started the first International Symposium HPTLC in Bad Duerkheim 1980 and founded the Journal of Planar Chromatography in 1988
- Inspiring research
  - Circular reversed phase planar chromatogram of synthetic fatty acid mixtures, in his dissertation, University Leipzig, 1954
  - “Direkte automatische Kopplung DC an GC”, Z. anal. Chem. 205 (1964) 205
  - „TLC in direct coupling with GC and MS“, Chemistry in Britain 5 (1969) 54
  - Book „Einführung in die Hochdruck-PLC“, 1987, ISBN 3-7785-1563-2

THE dedicated software programmer!

03.02.1934
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Prof. Dr. Siegfried Ebel, Germany

- Strong supporter of quantitative TLC/HPTLC
- Programming software for TLC/HPTLC automatization and contributing to the progress in instrumental TLC/HPTLC
- Creating calibration functions and providing fundamentals of quantitative TLC/HPTLC in many research paper
- Dedicated research in pharmaceutical analysis
- Member in many committees like the German and European Pharmacopoeia committee and the advisory board of the Federal Institute for Drugs and Medical Devices in Germany

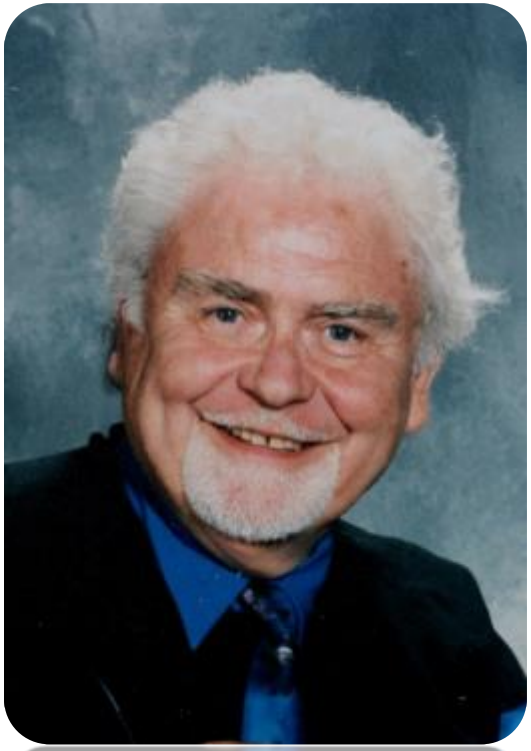
The father of modern instrumentation!

06.06.1927
dieter.jaenchen@camag.com

Dr. Dieter Jänchen, Switzerland

- Founder of CAMAG (1958) which rapidly developed into the world leader of instrumental planar chromatography
- Mentor and coordinator of the development of state-of-the-art instruments and software for planar chromatography
- Creator of CAMAG Bibliography Service with its literature database
- Missionary activities all over the world for the promotion of modern planar chromatography
- In the Scientific and Organizing Committee of the international HPTLC symposia until 1998

The pope of understanding!



25.02.1932
fgeiss@compuserve.com

Dr. Friedrich (Fritz) Geiss, Italy

- Defined terms, created understanding and turned the trial and error approach into a scientific and sound methodology
- Author of the books - our scientific bible
  - Die Parameter der Dünnschichtchromatographie, Vieweg 1972, Japanese Version 1980
  - Fundamentals of Thin Layer Chromatography (Planar Chromatography), Hüthig 1987, Russian Version 1989
- Invention of the Vario KS Chamber with his team, whose successor is frequently in use for optimizations
- Awardee of the Tswett Medal in 2002
- Author of numerous papers and books in the area of chemistry and of several books related to societal and political matters

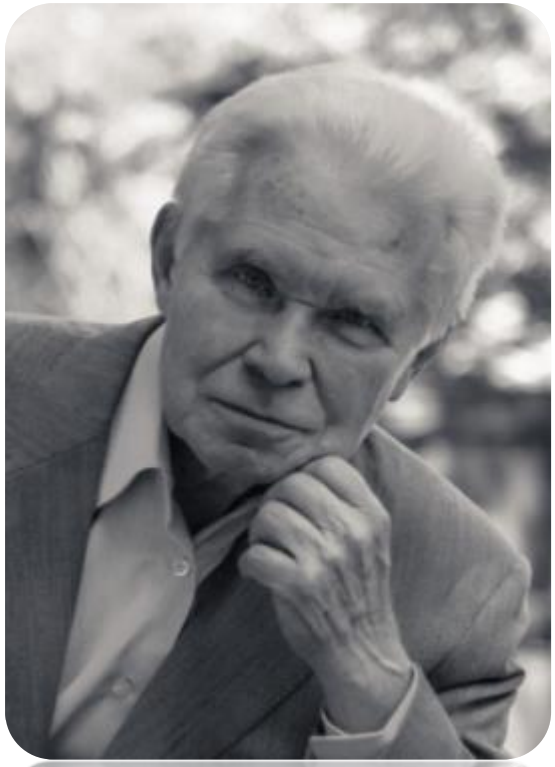
The creator of fundamentals in retention!

04.09.1928

Prof. Dr. Edward Soczewiński, Poland

- Author of 333 chapters of books and papers as well as of many patents (*e.g*. for production of cadmium oxide, chelidonine, protopine, and chambers)
- Research on molecular model of retention in normal-phase systems: Soczewiński equation (Anal. Chem., 1968)
- Coauthor of Soczewiński-Wachtmeister equation used in QSAR investigations
- Editorial Board Member of several chromatographic journals
- Awardee of many prizes and distinctions, *e.g.* Officers and Chevalier's of the Polonia Restituta Order, Golden Cross of Merit of Poland, the Tswett Medal, the doctor *honoris causa* of Medical Academy of Lublin and many others from the State and Ministry of Health and Social Welfare of Poland

The prominent voice for TLC in Russia!



18.04.1931
berezkin@ips.ac.ru

Prof. Dr. Victor Grigor'evich Berezkin, Russian Federation

- Chief Investigator of Institute for Petrochemical Synthesis of the Russian Academy of Sciences
- Laureate of the State Award of the Russian Federation, Honored Personality of Science of the Russian Federation, Honored Petrochemist of the USSR
- Books on TLC like "What is Chromatography?", the discovery of TLC and "Quantitative TLC"
- Papers on TLC on plates with closed sorption layer – the new variant of planar chromatography
- Member of Advisory Boards like
  - Journal of Analytical Chemistry (Russian journal)
  - Journal of Chromatography A
  - Journal of High Resolution Chromatography

The hands-on analyst and India's top pharmaceutical analyst!

18.11.1936
prabhu.sethi@gmail.com

Dr. P. D. Sethi, India

- India's first Government Scientist who realized the potential of HPTLC in 1985; Former Director of Central Indian Pharmacopeia Lab and Central Drug Testing Lab
- Set up QTLC method for analysis of birth control pills in 1987 and analyzed 10 000 samples per year.
- Member of several Government Committees for pharmaceutical analysis and herbal product standardization
- A primary force to adopt fingerprinting for herbal medicines as a first choice in India
- 13 books on pharmaceutical analysis including three volumes on multi drug formulation analysis by TLC/HPTLC methods and one on Content Uniformity Testing by HPTLC

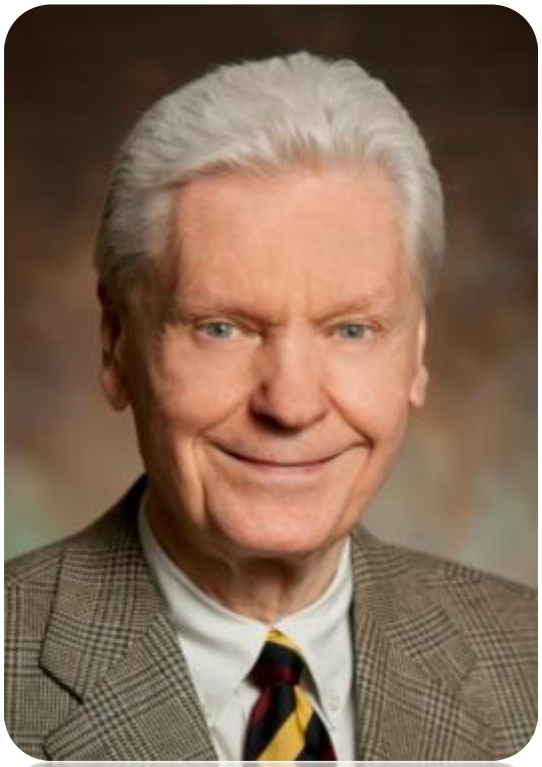
The originator of Pressurized Planar Electrochromatography!

01.11.1938
dnurok@iupui.edu

Prof. Dr. David Nurok, USA

- Published initial report on pressurized planar electrochromatography (PPEC). Also published the initial report on planar electrochromatography (PEC) in the reversed-phase mode.
- Founding member of InChromatics LLC, a company dedicated to promoting PPEC. The first instruments will be placed in a few selected laboratories by the end of 2014.
- Demonstrated, together with Frantisek Svec, the use of polymeric monoliths for the rapid separation of oligopeptides by PPEC.
- Developed, together with Robert Kleyle, statistical methods for predicting separation quality in TLC.
- Published several reports on the optimization of 2-D TLC.

The exceptionally distinguished author!



shermaj@lafayette.edu

Prof. Dr. Joseph Sherma, USA

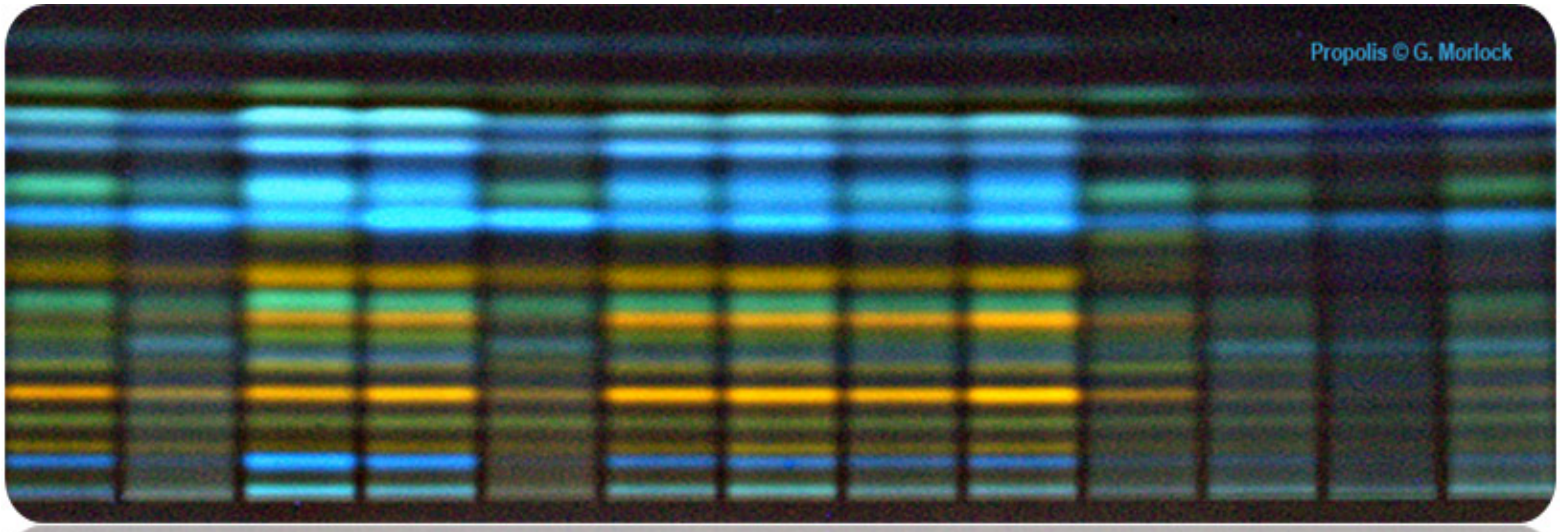
- Biennial reviews of the field of planar chromatography since 1970
- Co-guest edited with Professor Bernard Fried yearly special issues on TLC since 1999
- Co-editor of 10 books on TLC
- More than 750 published original research papers, review papers, manuals, books, and book chapters, the majority of which have been on planar chromatography topics
- Received the 1995 American Chemical Society Award for Research at an Undergraduate College, which resulted in 290 publications with 175 different students as coauthors

The outstanding American trainer!

29.05.1938
f.rabel@comcast.net

Fred Rabel, Ph.D., USA

- Trained over 5000 scientists on the use and optimization of TLC/HPTLC through training and short courses
- A dozen articles and chapters in textbooks on TLC/HPTLC applications, sorbents and layers
- Assisted in commercializing TLC/HPTLC sorbent and plate production at two American companies
- Continues to promote TLC/HPTLC in every HPLC talk/training and reminds people of its advantages and utility even in this age of laboratory automation
- The International community viewing this slide should remember that, unfortunately, the USA market has generally not bought into the advances in TLC/HPTLC plate and instrument technology that you are seeing at this meeting.



Feel free to submit photo and achievements of further

distinghuised, retired TLC/HPTLC researcher to

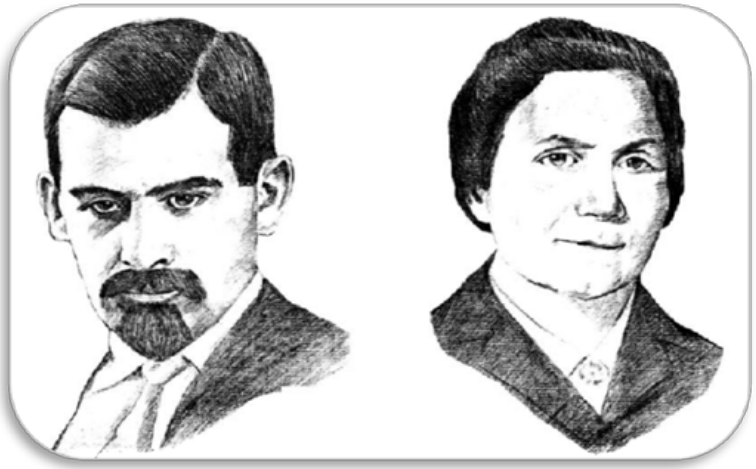
cfp@chem.wayne.edu and Gertrud.Morlock@ernaehrung.uni-giessen.de

Thank you in advance!

For sure, they enjoy a birthday card!

75+1 years of planar chromatography

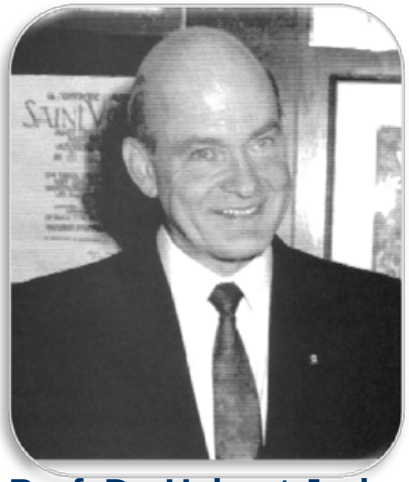
In memoriam



Prof. Dr. N.A. Izmailov
1907-1961

Dr. Maria S. Shreiber
1904–1992

Prof. Dr. Egon Stahl
1924-1986

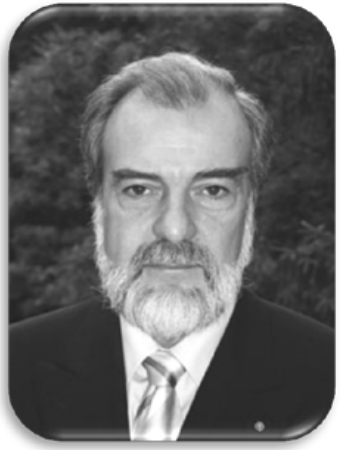


Prof. Dr. Helmut Jork
1934-1994

Prof. Dr. Ludimir Kraus
1923-1994

Prof. Dr. Werner Funk
1944-1996

Dr. Klaus Burger
1942-2005



Prof. Dr. Szabolcs Nyiredy
1950-2006