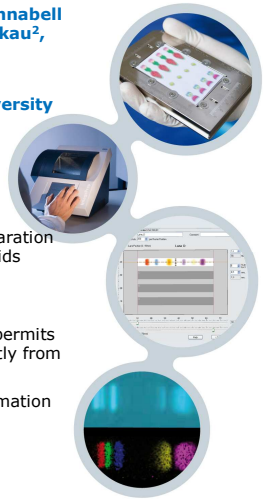
High Performance TLC-MALDI

Martin Schürenberg[1], Beate Fuchs[2], Annabell Bischoff[2], Rosemarie Süß[2], Detlev Suckau[2], Jürgen Schiller[2] and Gerda Morlock[3]

[1]Bruker Daltonik GmbH, Germany; [2]University of Leipzig, Germany; [3]University of Hohenheim, Stuttgart, Germany



Introduction

- HPTLC is routinely used as efficient separation method for difficult analytes such as lipids
- Automated devices provide robust high performance TLC separations
- Hyphenation with MALDI-TOF analysis permits high resolution molecular readout directly from the TLC/HPTLC-plates [1,2]
- MALDI-MS/MS provides structural information where required

Methods

- Lipid standards (Avanti Polar Lipids)
- HPTLC silica gel 60 F_{254} aluminum backed sheets, 50×75 mm, 200 µm layer thickness (Merck, # 1.05556.0001)
- ATS4 (CAMAG) for automated sample application
- DHB applied using the ImagePrep matrix coating device (Bruker)
- TLC-adapted target for MALDI (Bruker)
- TLC-MALDI software (Bruker) settings: 100 µm raster width
- UltrafleXtreme MALDI-TOF (Bruker) acquisition time ~ 5 min/lane

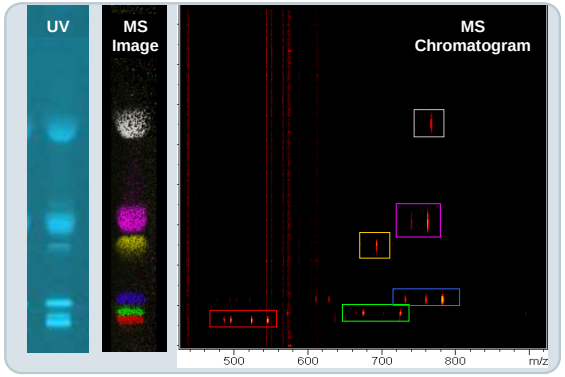


Fig. 1: HPTLC analysis of 532 ng of a lipid standard mixture: (**UV**) primuline, fluorescence at 366 nm; (**MS Image**) MALDI-TOF image analysis, false color representation of ion distribution; and (**MS Chromatogram**) heat map mass chromatogram with lipid signals (Rf vs. m/z) boxed corresponding to the MS image. The chromatographic representation provides a 2D high resolution access to TLC-MALDI data. DHB was used as MALDI matrix.

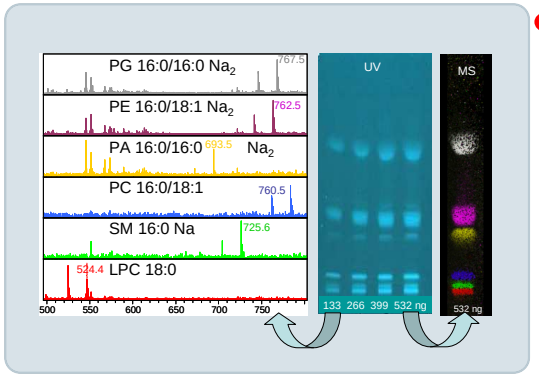


Fig. 3: **Detectability** of HPTLC-MALDI: A dilution series of lipid standard containing 133-532 ng of each lipid was analyzed. Even at the 133 ng level intense MALDI spectra were obtained that can be well interpreted. Parent ion masses refer to MH^+ or sodiated peaks.

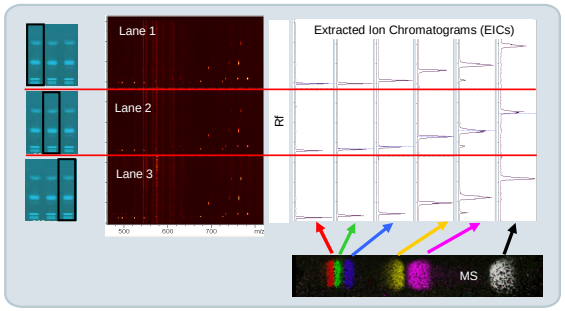


Fig. 2: **Repeatability** of HPTLC-MALDI. 3 repetitive analyses with 330 ng lipid standard on 2 TLC plates were developed by primuline (left) and MALDI (center). Repeatability of the intensities of extracted parent ion chromatograms (right) is **better than 10 %.**

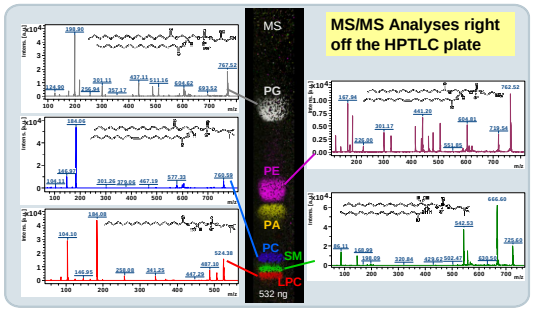


Fig. 4: **Structural elucidation** Software supported MALDI-TOF/TOF MS/MS spectra acquisition right from the HPTLC plate after MS acquisition. Fragment ions correlate with the respective lipid structure and permit its unequivocal identification and head group assignment.

IMSC 2009, Poster PMM: 386

References

- B Fuchs et al. *A direct and simple method of coupling matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) to thin-layer chromatography (TLC) for the analysis of phospholipids from egg yolk*, ABC 2007, **389**: 827-834
- B Fuchs et al. *Analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS*, ABC 2008, **392**: 849-860

Conclusions

HPTLC-MALDI is a largely automated, software-supported new method:

- Chromatographic resolution of HPTLC is maintained throughout the MALDI analysis **Fig. 1**
- Reproducibility of HPTLC-MALDI is in the 10 % range **Fig. 2**
- Sensitivity of HPTLC-MALDI for lipids is in the 100 ng range **Fig. 3**
- Structural analysis can directly follow up using HPTLC-MALDI-MS/MS **Fig. 4**
- **suitable for lipid analysis for clinical, nutritional, pharma-ceutical and cosmetic analysis**