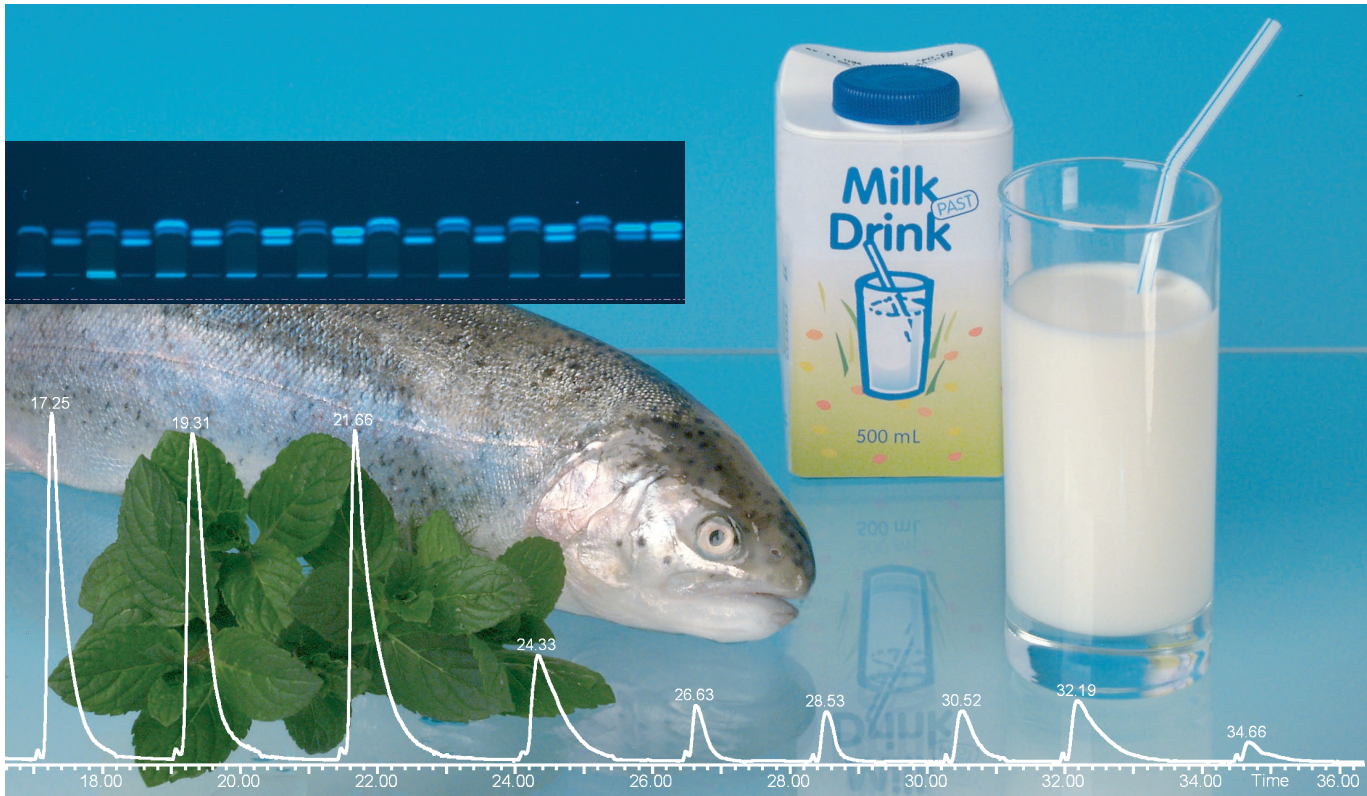



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Efficient methods for food control



INTERNATIONAL SYMPOSIUM
ON HIGH-PERFORMANCE
THIN-LAYER CHROMATOGRAPHY
Berlin (Germany), 9–11 October 2006

CAMAG

96

Quantification of ITX in food by HPTLC/FLD coupled with ESI-MS and DART-MS



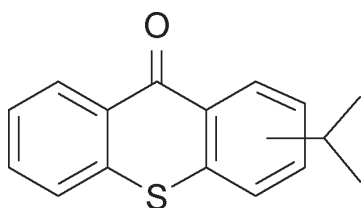
◀ Prof. Dr. Wolfgang Schwack and Dr. Gerda Morlock*

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The working group of Prof. Dr. Schwack, University of Hohenheim, Stuttgart, Germany, is actively engaged in planar chromatography (see CBS 94). In November 2005 isopropylthioxanthone (ITX) directed consumers' attention as new food contaminant. As no analytical method was published so far for quantification of ITX in concerned food, a new sensitive and selective planar chromatographic method with fluorescence detection (FLD) and confirmation of positive findings by ESI-MS (electrospray ionisation mass spectrometry) and DART-MS (Direct Analysis in Real Time mass spectrometry) was developed [1].

Introduction

Isopropylthioxanthone (ITX) used as photoinitiator in UV inks applied to packaging materials has recently been found in milk and ready-to-feed infant formula. Over 30 millions liter of milk were called back by producers in Italy, France, Spain and Portugal in November 2005.



▲ Structure formula of isopropyl-9H-thioxanthen-9-one (ITX)

Migration of ITX into the food from prefabricated packaging material or copying by rolling of the packaging material are sources of food contamination discussed. Obviously fat-containing foods are more affected than water-based products. Besides infant formula also milk and milk-based products, soy bean beverages, fruit juices, fruit nectars and other drinks and foods, like cacao powder or olive oils, packed in the same manner, are of high concern to be investigated for absence of ITX.

Based on the levels reported up to some hundred $\mu\text{g}/\text{kg}$, ITX is judged as not to give cause for health concern. However, there are no data available at present on aspects other than genotoxicity making a final conclusion difficult. So far not only the monitoring of ITX is of concern, also further photoinitiators used, like 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) as well as 4,4'-bis(diethylamino)-benzophenone and 4,4'-bis(dimethylamino)-benzophenone, are in discussion.

The method fully serves the interests of food industry and food control to monitor absence of ITX in concerned food considering a high sample throughput to be managed. The results prove modern planar chromatography as a rapid and cost-efficient alternative method to quantify ITX in milk-based or fatty matrices in the low $\mu\text{g}/\text{kg}$ range. Only positive results are confirmed by online ESI-MS in the SIM mode or by DART-MS involving a minimal employment of the MS device as further decisive advantage of HPTLC.

Sample preparation

4 mL milk or 4 g yoghurt were extracted with cyclohexane – ethyl acetate 1:1 (v/v) by employment of accelerated solvent extraction (ASE). Prior to extraction 25 μL of the 2,4-diethyl-9H-thioxanthen-9-one (DTX) solution (8 $\mu\text{g}/\text{mL}$) were added as internal standard. The extract was dried over 4 g sodium sulphate (water free), filtered and evaporated to dryness by a centrifuge evaporator. The residue was taken up in 1.5 mL acetonitrile, concentrated again and finally taken up in 250 μL acetonitrile.

For soy bean oil and margarine, a simple partitioning step of ITX into acetonitrile (at 50 °C for 30 min at 600/min) was used. 1 mL acetonitrile and 25 µL DTX solution were added to 1 g fat (plus 200 mg magnesium sulphate for margarine samples). The clear upper acetonitrile layer was subjected to HPTLC analysis.

Standard solutions

ITX (3.2 µg/mL) and DTX (8 µg/mL) were dissolved in acetonitrile each.

Layer

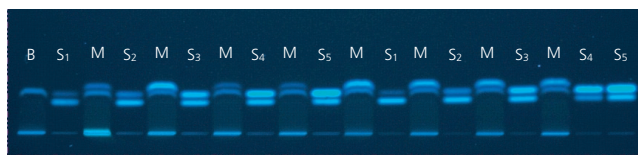
HPTLC plates silica gel 60 (Merck) 20 x 10 cm

Sample application

Bandwise with Automatic TLC Sampler 4, 18 tracks, application volume 30 µL of sample and 3–30 µL of ITX standard solution (9.6–96 ng), band length 7 mm (track distance 9 mm), distance from lower edge 8 mm, distance from both sides 20 mm. For internal standard evaluation all ITX standard zones were oversprayed with 3 µL DTX standard solution (24 ng/zone).

Chromatography

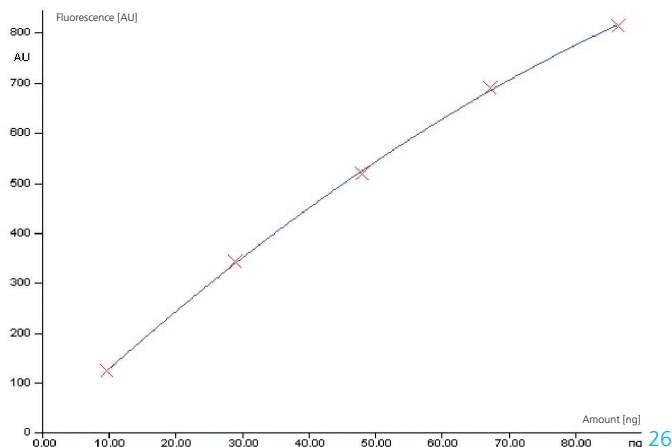
In a horizontal developing chamber with toluene – n-hexane 4:1 (v/v); migration distance 50 mm from the lower edge. After chromatography the plate was dried in a stream of warm air for 1 min. Development was performed anti-parallel from both plate sides leading to a throughput of 36 separations in 7 min migration time. Per separation chromatography needs less than 0.2 min and 0.3 mL solvent.



▲ Plate showing the determination of ITX in milk (hR_f 26, internal standard DTX at hR_f 20); illumination at UV 254/>400 nm; B: blank sample (milk plus DTX), S_1 – S_5 : standard level 1–5, M: spiked milk samples

Densitometry

TLC scanner 3 with winCATS software; fluorescence measurement at UV 254/>400 nm; polynomial calibration via peak height



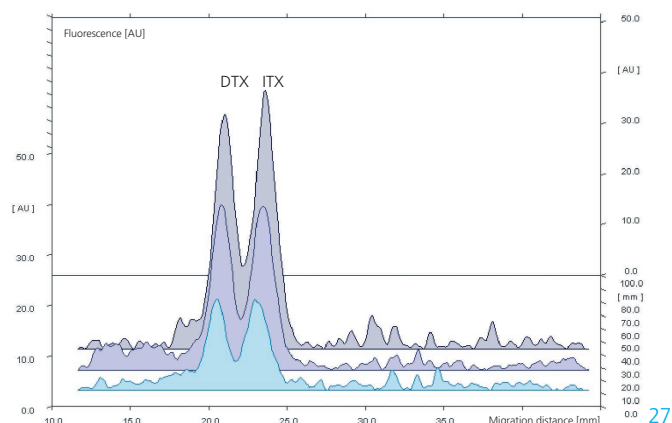
▲ Polynomial regression of ITX ($y = -0,037 x^2 + 12.610 x + 5.994$, $sdv = \pm 1.51\%$, $r = 0.99981$) in the working range of 20–200 µg/kg (9.6–86.4 ng/zone)

Documentation

With DigiStore 2 documentation system by illumination at UV 254/>400 nm

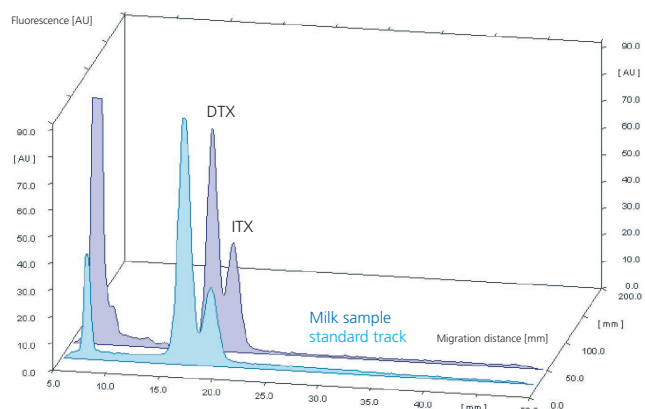
Results and discussion

Limits of detection (S/N of 3) have been established to be 64 pg both for ITX and DTX. In fatty matrix (spiked butter) LOD of ITX was determined to be 1 µg/kg.



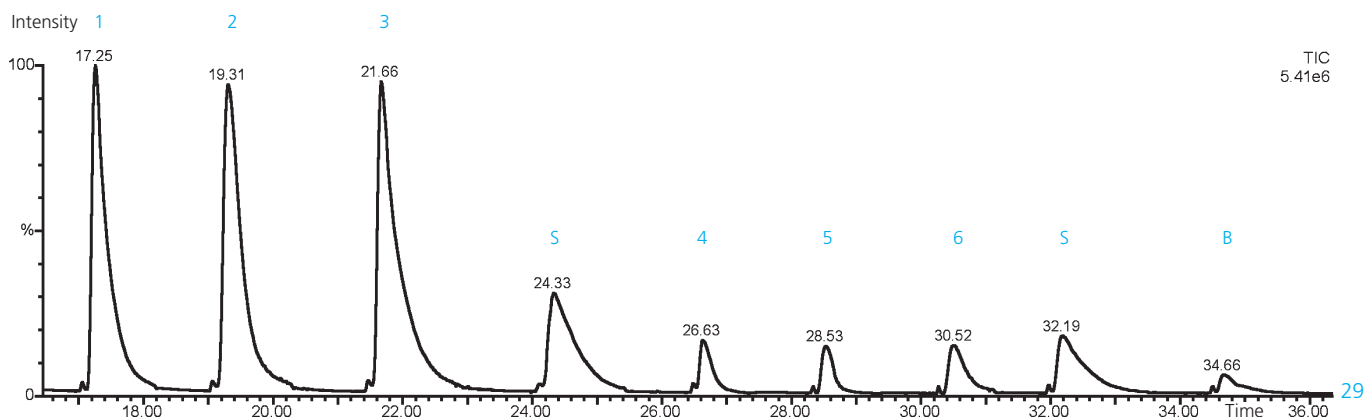
▲ Track overlay of 64 pg, 128 pg and 192 pg DTX and ITX; LOD (S/N of 3) for ITX and DTX were established to be 64 pg and LOQ 192 pg.

In the working range monitored (20–200 µg/kg) polynomial regression of ITX showed a relative standard deviation of $\pm 1.51\%$ ($r = 0.99981$). Regarding repeatability ($n = 9$) a coefficient of variation (CV) of 1.1% was obtained for ITX at 32 ng. Repeatabilities ($n = 4$) of ITX determination at 20, 50 and 100 µg/kg in milk, yoghurt, soybean oil and margarine showed CV between ± 1.0 and 6.4%. Via internal standard correction recoveries were about 130% for milk and yoghurt and 70 and 97% for margarine and soy bean oil, respectively.



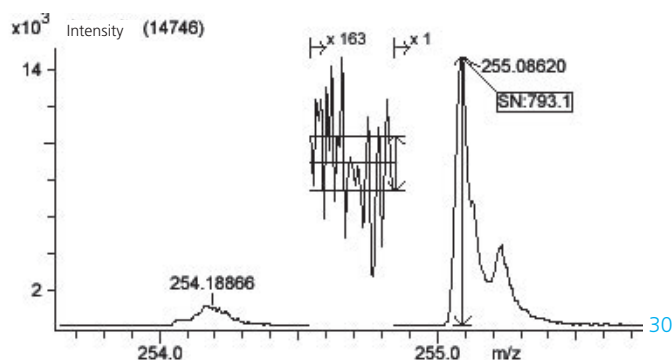
▲ Overlay of an ITX standard track and a milk sample track spiked with DTX at 50 µg/kg (internal standard) and ITX at 20 µg/kg

For confirmation by ESI-MS of positive findings in the µg/kg range, ITX zones were recorded in the selective ion monitoring (SIM) mode at m/z 255 and 277 using a plunger-based extraction device [2].



▲ SIM elution profiles recorded at m/z 255 and 277 of 3 ITX zones each in a yoghurt sample spiked at 100 ppb (peak 1–3) and 20 ppb (peak 4–6) besides extractions of standard zones S and blank extraction B

Further confirmation was performed by DART [3] which was directly coupled with a time-of-flight mass spectrometer (TOF-MS). This kind of versatile new ion source is working in open air under ambient conditions using an excited gas stream. The employment of DART in the field of planar chromatography was successfully demonstrated in first investigations.



▲ HPTLC/DART-TOF spectrum of a 48 ng ITX zone at m/z 255.0862

Further information is available from the authors on request.

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