Screening and confirmation of 35 photoinitiators in food products by LC/MS/MS using triggered MRM acquisition and library comparison

Thomas Glauner[2]; Teelke Jung[1]; Diane Fügel[1]; Bernhard Wüst[2]

[1] CVUA Stuttgart, Schaflandstrasse 3/2, 70736 Fellbach, Germany
[2] Agilent Technologies Sales & Services GmbH & Co. KG, Hewlett-Packard-Str. 8, 76337 Waldbronn, Germany

Agilent Technologies

Introduction

- Increasing concern about contaminants in food including plasticizers or printing ink components introduced via packaging.
- Although printing inks are only applied to the external surface, migration, gas phase transfer or accidental contamination by set-off has been reported.

- In this presentation we show:
  - Development of a sensitive UHPLC/MS/MS method for the analysis of 35 photoinitiators.
  - Use of triggered multiple reaction monitoring (tMRM) acquisition for data dependent acquisition of confirmatory ions
  - Additional qualification of positive detects by spectral library comparison.
  - Results of a validation study for dried pasta and fruit juice beverages.

Conclusions

- Broad screening of packaging contaminants in food is becoming increasingly important.
- A sensitive UHPLC-MS/MS method for the simultaneous quantitation of 35 photoinitiators using triggered MRM acquisition has been developed.
- In addition to accurate quantitation, tMRM allows for enhanced identification by comparing acquired spectra with reference library spectra.
- The method was successfully validated for wheat pasta and orange juice beverages.
- Linear calibration curves and excellent precision for replicate injections were observed.
- Triggered MRM produces high quality spectra even at low concentrations by using optimized collision energies for each MRM transition.
- Two photoinitiators were identified in fruit juice beverage and noodle samples at low levels but with high confidence as high library match scores were obtained.

Results and Discussion

Method performance characterization

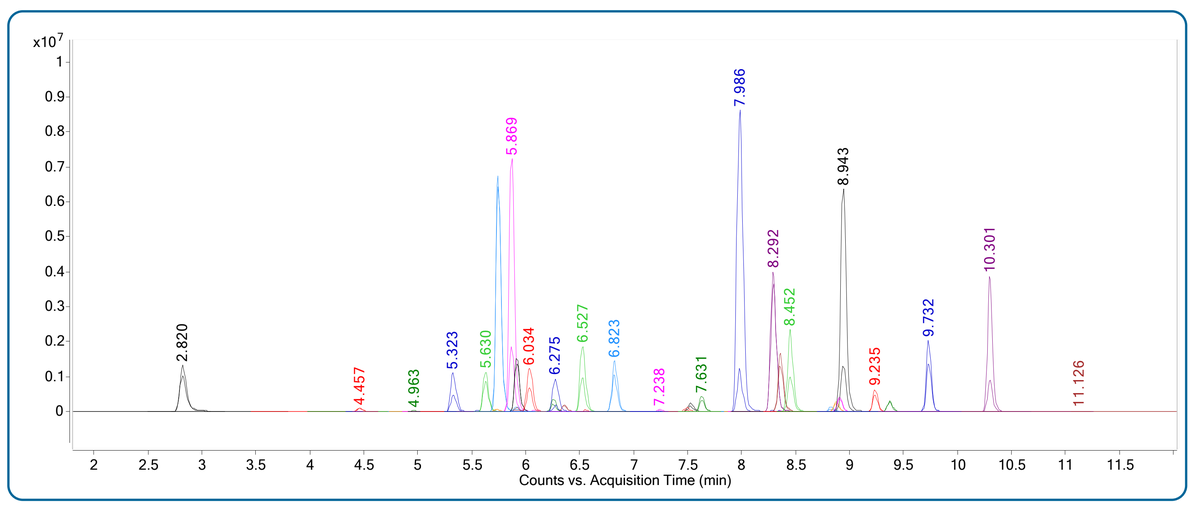

Figure 1: UHPLC-MS/MS chromatogram of 35 photoinitiators spiked in a pasta extract corresponding to a concentration of 40 µg/kg and acquired with tMRM with 2 primary transitions per compound.

- UHPLC/MS/MS method was developed for 35 photoinitiators using triggered MRM with 2 primary transitions and up to 6 additional confirmatory ions per compound (Figure 1).
- Good separation was achieved using a 2-step gradient with ammonium formate and acetonitrile as the mobile phase.
- Thresholds for data dependent triggering were set on a compound by compound basis.
- Triggered MRM produces high quality spectra even at very low concentrations by using adequate dwell times and optimized collision energies for each transition (Figure 2).

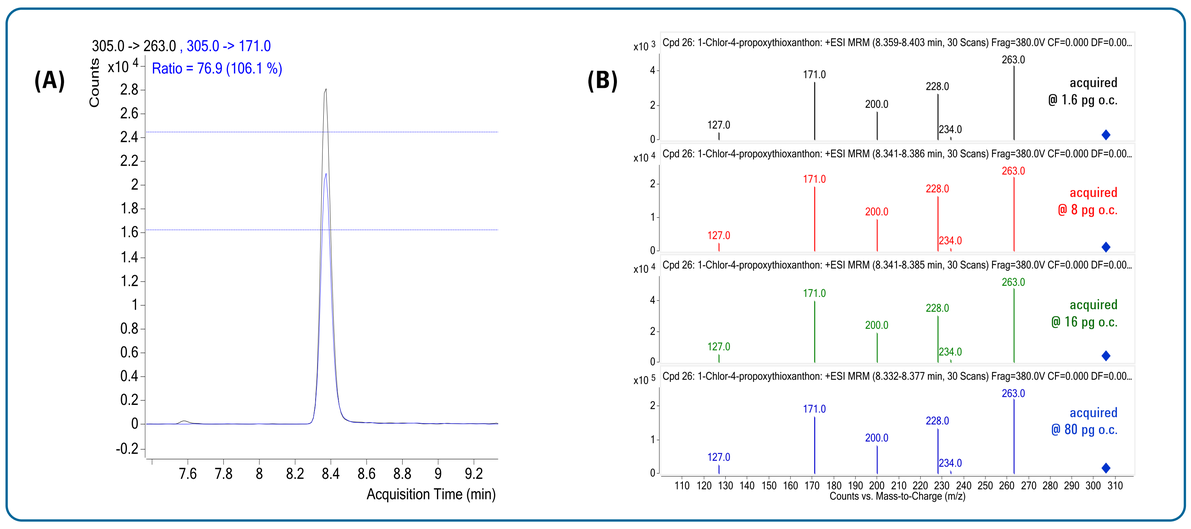

Figure 2: MRM chromatograms of the primary transitions for 1-Chloro-4-propoxy thioxanthone *spiked into pasta extract at a concentration of 0.8 µg/kg (A) and tMRM spectra of the same compound (B) acquired at concentrations of 0.8 (black), 4 (red), 8 (green) and 40 µg/kg (blue).*

- Spiking level of 0.8 µg/kg for 1-Chloro-4-propoxythioxanthone is 10 times below the specific migration level (SML) for not yet evaluated compounds [1].
- Area ratio of the primary transitions at this level is well within the expected range and in-spectrum fragment ratio is extremely reproducible also for different spiking levels.
- Area RSDs well below 5% for 5 replicates at 4 spiking levels and two matrices.

Experimental

- **Sample Preparation:**
  - Extraction of samples by citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits and 10 g fruit juice beverage or homogenized noodle sample (wetted with10 ml ultrapure water). Clean-up with dispersive SPE using PSA.
  - For method validation homogenized samples were spiked at 4 levels with a mixed stock solution containing all target compounds.
- **UHPLC MS/MS parameters:**
  - Agilent 1290 Infinity UHPLC system coupled via an Agilent Jet Stream electrospray ionization source to an Agilent G6490 QQQ system. Acquisition in positive electrospray ionization and with triggered multiple reaction monitoring (tMRM) mode. Confirmatory ions were measured over 5 acquisition cycles, thresholds were defined compound specific and were set to 50% of the signal height of the lowest calibration standard.
  - Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 x 150 mm, 1.8 µm @ 30°C; Injection volume 2 µl of the final extract.
  - Mobile phase A: 5 mM NH4 formate, mobile phase B: acetonitrile; flow rate 0.3 ml/min; 0.5 min isocratic at 10% B, linear gradient to 60% B in 3 min, linear gradient to 95% B in 5.5 min, 2.5 min isocratic at 95% B, linear gradient to 10% B in 0.1 min. Stop time 13 min.
- **References:**

[1] Swiss Ordinance on Materials & Articles in Contact with Food (SR 817.023.21),

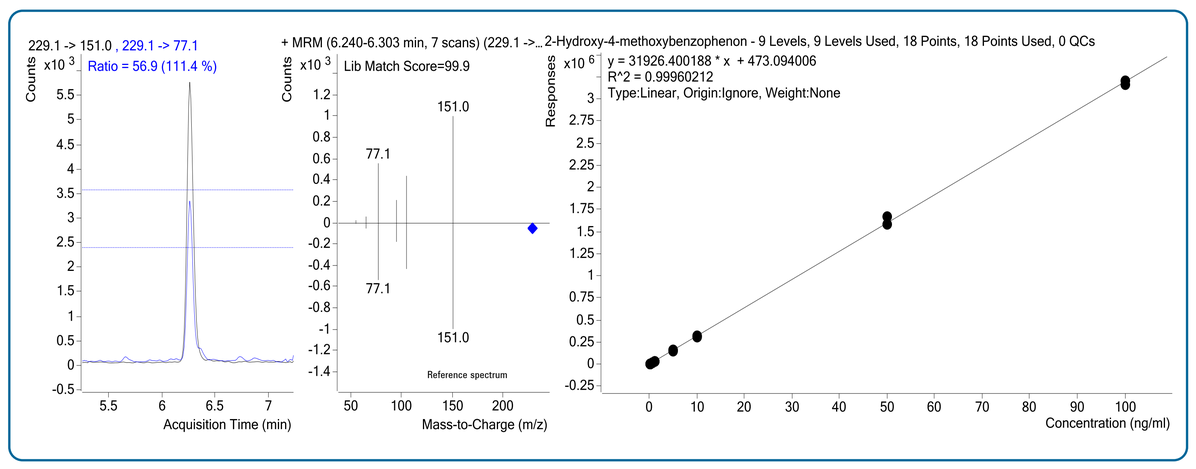

Figure 3: Chromatogram of 2-Hydroxy-4-methoxybenzophenone in pasta extract (1 µg/kg), tMRM spectrum in comparison to library spectrum and calibration curve.

- LOQs (S/N ratio ≥ 10) were below 0.01 µg/kg for 26 compounds, further 7 compounds had LOQs between 0.05 and 0.5 µg/kg, 4-Phenoxy-2,2-dichloracetophenone and 2,2-Diethoxy-acetophenone had LOQs of 1 and 5 µg/kg, respectively.
- Apparent recoveries for the QuEChERS extraction for majority of compounds were between 70 to 120 % based on solvent calibration.

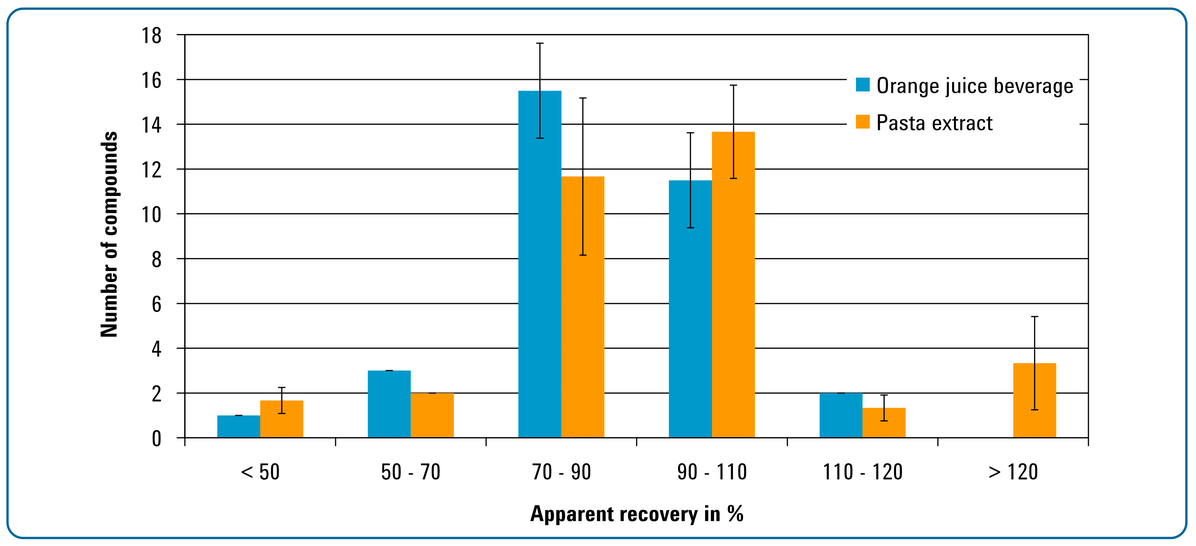

Figure 4: Histogram of apparent recoveries for photoinitiators in the QuEChERS extraction of orange juice beverage and pasta spiked at 3 µg/kg.

- Comparison of recoveries obtained for spiked blank extracts indicate that lower apparent recoveries were mainly caused by matrix effects in ESI and not by low extraction yield.
- Accurate quantitation in matrix is achieved when using matrix matched calibrations.

Analysis of real samples

- Lasagna noodles packed in cardboard boxes and orange juice beverages packaged in multi-layer pouches were analyzed for photoinitiators.
- For the lasagna noodles 2 samples were taken per package: one in direct contact with the cardboard box and one from the center of the package.
- Two photoinitiators were found at low levels with generally higher concentrations in the sample with direct contact to the packaging.

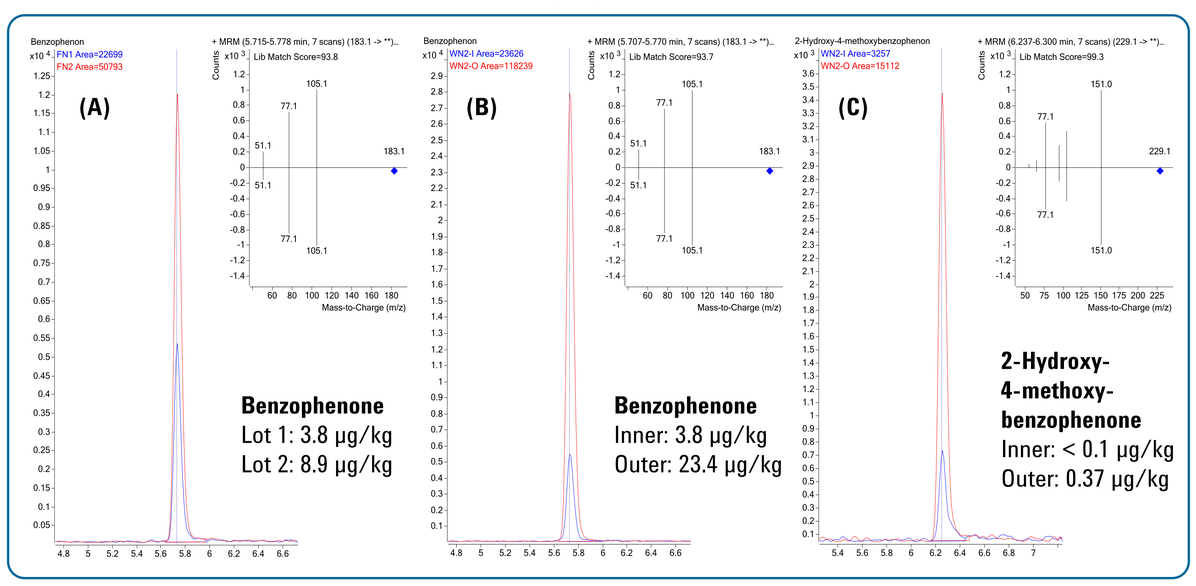

Figure 5: Chromatograms and tMRM spectra of benzophenone in the orange juice beverage (A) and noodle sample (B), and 2-Hydroxy-4-methoxy-benzophenone in a noodle sample (C).