

# Quantitative Analysis and Identification of Migrants in Food Packaging Using LC-MS/MS

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## Introduction

Packaging improves the quality and safety assurance of food, especially from micro-organisms, biological and chemical contaminants. Packaging is therefore an essential component for the food industry and the manufacturing processes.

However, over the last couple of years there has been a growth in the number of materials and substances used in food packaging so in order to improve food safety a migration study for compounds is becoming more important to prevent the use of compounds that can migrate into food.

Currently, an upper limit for the overall migration of 60 mg/kg or 10 mg/dm<sup>2</sup> has been set by the European Union (EU).<sup>1</sup>

In the USA, the regulations for food packaging material are more complex, because the types of raw and processed foods, and conditions of use are separated.<sup>2</sup>

In this study three compounds: ITX, Irgacure, and TRP are investigated (Figure 1). ITX is a mixture of 2-Isopropylthioxanthone and 4-Isopropylthioxanthone. Irgacure contains Irgacure 819 (Phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide). Both are used as photo-initiators in UV cured inks. TRP (Tri(propylene glycol) diacrylate is an ingredient of cured inks.

The data presented discusses linearity of response, robustness and the use of the Multiple Reaction Monitoring combined with Enhanced Product Ion scanning (MRM-EPI) using an AB SCIEX 3200 QTRAP<sup>®</sup> LC/MS/MS System as a way of gaining additional information for the presence of these migrants.



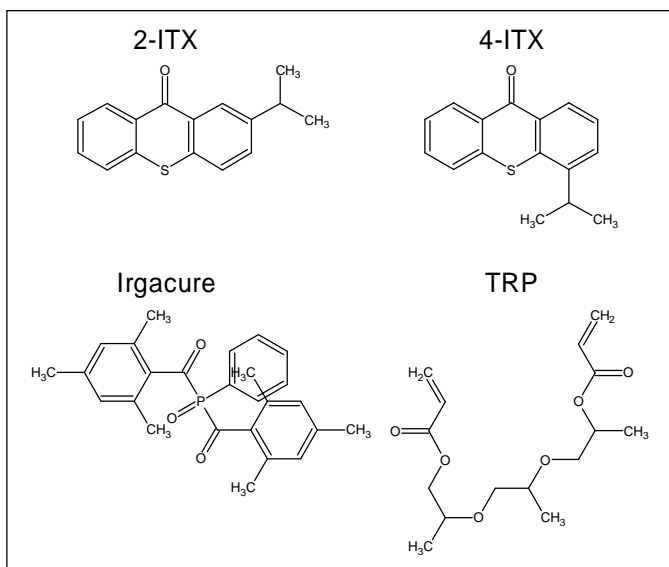
## Experimental

### Sample Extraction

Standards were prepared in the solvent composition at the start of the LC run (water/acetonitrile + 0.1% formic acid 70/30). Three sorts of real samples were analyzed: a packaging cap with only decoration (inks), a packaging cap with only varnish and a packaging cap with decoration and varnish. 1 dm<sup>2</sup> of each sample was extracted with acetonitrile. The extracted sample was evaporated and reconstituted in initial mobile phase before analysis.

### LC

An Agilent 1200 system containing a binary pump flowing at 250 µL/min, autosampler, and a column oven set at 20°C were used with a Hypersil BDS C18 column (5 µm, 100 x 2 mm). 10 µL injections of standards and extracts were separated using a gradient (Table 1) of mobile phase A (0.1 % formic acid in water) and B (0.1 % formic acid in acetonitrile). 5 minutes column equilibration time was used between runs.



**Figure 1.** Investigated migrants from food packaging

**Table 1.** LC gradient

Step	Time (min)	% A	% B
0	5.0	70	30
1	2.0	5	95
2	7.0	5	95
3	7.1	70	30
4	12.0	70	30

## MS/MS

All experiments were performed on an AB SCIEX 3200 QTRAP<sup>®</sup> LC/MS/MS System with Turbo V<sup>™</sup> source at 450°C using Electrospray Ionization (ESI) in positive polarity. The following source conditions were used:

Curtain Gas (CUR)	25 psi
IonSpray Voltage (IS)	5000 V
Gas1	40 psi
Gas2	50 psi
CAD Gas	Medium
Temperature	450 °C

Analyses were based on two different Information Dependent Acquisition (IDA) experiments using Multiple Reaction Monitoring (MRM) in the survey scan and dependent Enhanced Product Ion (EPI) scanning. MRM transitions were previously optimized (see Table 2). A dwell time of 100 ms was used for each transition and the pause time was set to 5 ms.

**Table 2.** MS/MS conditions

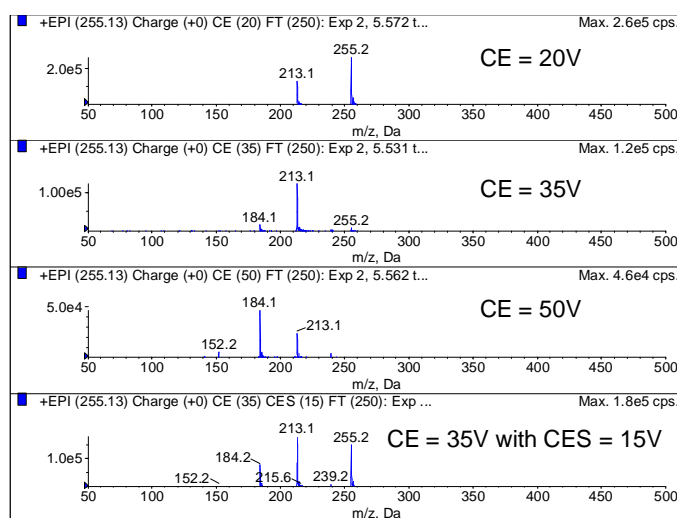
Compound	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	CE (V)
ITX	255.1	213.1	66	35
	255.1	184.1	66	61
Irgacure	419.2	147.2	21	23
	419.2	119.2	21	57
TRP	301.2	113.2	21	13

Experiment 1 triggered three EPI scans at collision energies (CE) of 20; 35 and 50 V. Experiment 2 used a single dependent scan with a CE of 35 V and Collision Energy Spread (CES) of 15 V. CES was found to give more reproducible and richer MS/MS spectra, in comparison to dedicated and fixed Collision Energies, and thus greatly enhancing the quality of library searching. The scan speed of the EPI scans were 4000 amu/s and Dynamic Fill Time (DFT) was used for all EPI scans. In both experiments peaks were identified in the MRM survey using Dynamic Background Subtraction (DBS).

Identification of analytes in the real samples was based on searching against the mass spectral library created from MRM-EPI analyses of standards.

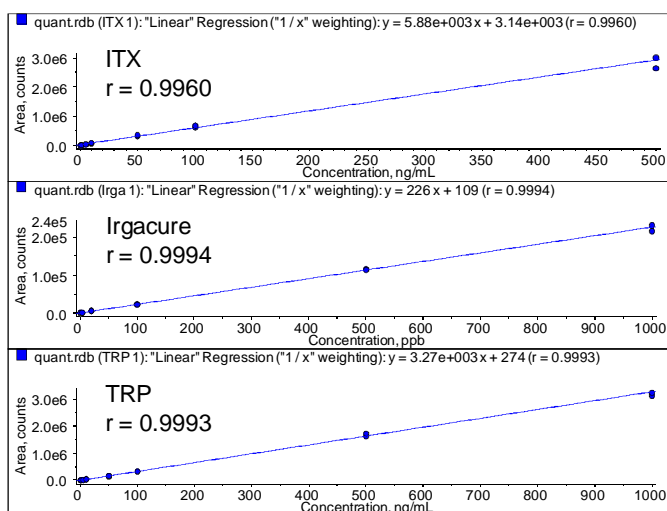
## Results and Discussion

Standards at 10 ng/mL were used to build a mass spectral library. An example of reference spectra is shown in Figure 2. Standards were used over a range 0.1 to 1000 ng/mL to produce calibration lines.



**Figure 2.** An example of the effect of collision energy on the EPI spectra of a migrant standard used for generating library data (10 ng/mL ITX standard)

Figure 3 shows calibration lines that were obtained from standards analyzed in MRM-EPI mode with each standard analyzed in duplicate. The 'r' values obtained from these calibration lines (0.5 – 500 ng/mL for ITX, 2 – 1000 ng/mL for Irgacure and 0.5 – 1000 ng/mL for TRP) were greater than 0.996 when a linear fit with 1/x weighting was applied.



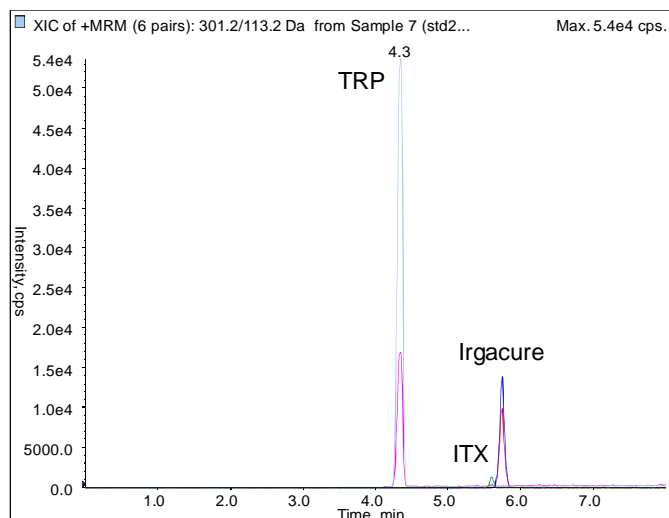
**Figure 3.** Calibration lines obtained from ITX, Irgacure and TRP with r values > 0.996 (no internal standard used)

Repeatability and %CV were assayed by 5 repeat injections of a standard close to the limits of quantitation of each analyte and results are summarized in Table 3 with all coefficients of variation <10% (no internal standard was used).

**Table 3.** Reproducibility data from 5 replicate injections

Compound	Transition	Concentration (ng/mL)	% CV (n=5)
ITX	255.1/213.1	0.5	8.2
Irgacure	419.2/147.2	0.844	5.2
TRP	301.2/113.2	0.515	9.5

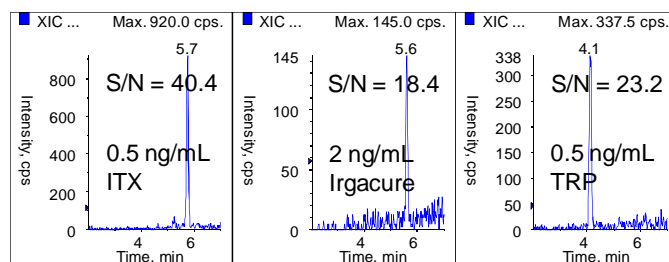
Figure 4 shows a typical trace obtained from the analysis of migrant standard prepared in the initial mobile phase, all migrants were detected below 1 ng/mL as shown in Table 4 with Figure 5 giving the sensitivity of migrants at a concentration of 0.5 ng/mL (ITX and TRP) and 2 ng/mL (Irgacure).



**Figure 4.** 10  $\mu$ L injection of migrants standards in initial mobile phase

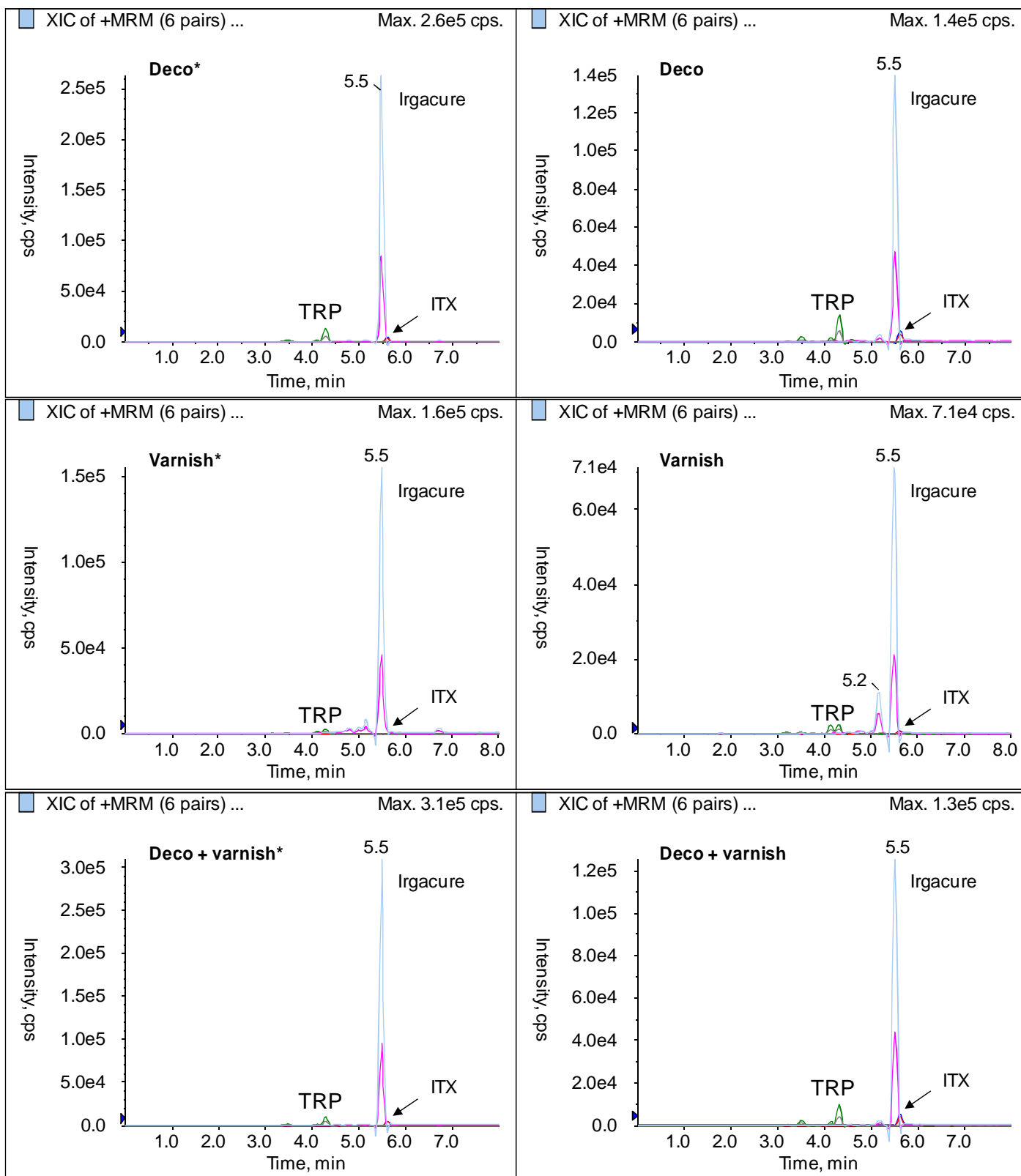
**Table 4.** Estimates for limits of detection (LOD), limits of quantitation LOQ), and linearity for food migrants

Compound	S/N (at ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)
ITX	40.4 (0.5)	0.04	0.12	0.12 - 500
Irgacure	18.4 (2.0)	0.33	0.5	0.5 - 1000
TRP	23.2 (0.5)	0.2	0.6	0.6 - 1000



**Figure 5.** Signal to noise (S/N) of low level migrant standards (S/N calculated using peak-to-peak algorithm)

This MRM data was then used to quantify migrants in cap extracts, examples of various extracts are given in Figures 6 and concentrations of migrants were summarized in Table 5.



**Figure 6.** A comparison of food packaging samples extracted with acetonitrile and where the acetonitrile extract of the same sample had been evaporated to dryness and reconstituted in mobile phase\* (cap with decoration (top), cap sealed with varnish (middle), and cap with decoration and sealed with varnish (bottom))

**Table 5.** Quantitation results from real samples (\* sample was evaporated to dryness and reconstituted in the same volume of mobile phase A to improve HPLC peak shape)

Extract	ITX (ng/dm <sup>2</sup> )	Irgacure (ng/dm <sup>2</sup> )	TRP (ng/dm <sup>2</sup> )
<i>Deco</i>	4.43	6320	5.39
<i>Deco*</i>	4.96	4347	5.77
<i>Varnish</i>	0.08	3940	0.67
<i>Varnish*</i>	0.54	2100	0.69
<i>Deco + varnish</i>	4.65	6750	3.97
<i>Deco + varnish*</i>	4.26	3687	3.99

To further identify the migrant the automatically acquired EPI spectra was searched against a mass spectral library previously created with spectra obtained from 10 ng/mL standards. DBS enabled the acquisition of high quality MS/MS spectra even for co-eluting compounds. The Purity Fit shown in Table 6 indicated if the spectrum, in the extract, was a good match for the library spectrum, generally a fit above 70% indicated a positive identification of the migrant in the extract.

**Table 6.** The Purity Fit (%) results taken from the spectra obtained from contaminants in real samples when compared with those in a library of spectra of standards (\* sample was evaporated to dryness and reconstituted in the same volume of mobile phase A to improve HPLC peak shape)

Extract	ITX (%)	Irgacure (%)	TRP (%)
<i>Deco</i>	78	88	81
<i>Deco*</i>	87	28	31
<i>Varnish</i>	63	60	98
<i>Varnish*</i>	34	81	44
<i>Deco + varnish</i>	97	44	65
<i>Deco + varnish*</i>	91	57	95

## Summary

The LC-MS/MS method developed can be used for quantitation of migrants in food packaging material. The sensitivity levels of the 3200 QTRAP<sup>®</sup> system were high enough to detect migrants at 0.01 mg/kg in extracts. A mass spectral library containing of EPI spectra at different standardized Collision Energy and Collision Energy Spread values can then be used to identify the compound at the required matrix detection levels, enabling direct injection analysis on extracts.

## Acknowledgements

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## References

- 1 European Commission – Health & Consumer Protection Directorate general - SANCO D3/AS D(2005)
- 2 FDA 21 CFR 170.100 - Submission of a premarket notification for a food contact substance (FCN) to the Food and Drug Administration (FDA). Code of Federal Regulations (December 2005)

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