

## Strategies for Targeted and Untargeted Screening of Printing Ink Photoinitiators and Acrylates in Migration Studies Using UPLC and Quadrupole Time-of-Flight Mass Spectrometry

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## Abstract

In this study, UPLC, HRMS, and a custom library were used to detect photoinitiators and acrylates and perform quantitation of target analytes in a single-sided migration study using a food simulant as the testing medium.

### Benefits

- A comprehensive approach to identify photoinitiators and acrylates in printing ink formulations (e.g., in migration studies of printed food contact materials) by UltraPerformance Liquid Chromatography (UPLC) and high-resolution mass spectrometry (HRMS) in conjunction with the UNIFI Scientific Information System
- Enhanced confidence in elemental composition and component structural assignments via simultaneous collection of accurate mass precursor and product ion data combined with compound libraries and confirmatory targeted MS/MS
- Increased analytical flexibility, with the ability to detect compounds over wide molecular weight range, elucidation of unknown and untargeted analytes, while improving sample throughput resulting from rapid chromatographic separations

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## Introduction

Ultraviolet light (UV)-curing inks and varnishes are used to print and coat food contact materials, including cardboard cartons and both rigid and flexible plastic packaging.<sup>1</sup> Migration of printing ink components into food is a concern to ink formulators. Indirect contamination by a process known as set-off, which is the unintentional transfer of the printed side of packaging to the non-printed side of another adjacent package, is also a concern. The non-printed side of the packaging is usually in contact with the food, thus the presence of set-off can cause contamination.<sup>2</sup> The amount of ink components transferred during packaging manufacture can be heavily dependent on the type of ink used, the curing and drying method, or the method of stacking/storage. Permeation migration can occur when there is transfer of the external ink components through the package and to the unprinted internal side of the food container, which can cause contamination. Food safety is at the center of these concerns. In 2005, the Italian Food Control Authority alerted consumers to baby milk contaminated with isopropylthioxanthone (ITX), a photoinitiator.<sup>3</sup> The

contaminated milk was withdrawn from the market in Italy, France, Spain, and Portugal, with over 30 million liters of milk destroyed.<sup>4</sup>

Photoinitiators absorb light and generate a reactive species, which then initiates a chemical transformation. They accelerate drying times by acting as catalysts in the polymerization reactions that occur in the conversion of liquid coatings to cured films.<sup>5</sup> The photoinitiators range in molecular weight from small molecules to larger polymeric structures. Since the ITX incident, there has been increased impetus to prevent similar contamination occurrences. The polymeric photoinitiators (PPI) were developed to increase the molecular weight (MW) and make them less likely to migrate than smaller MW compounds. The structure of polymeric compounds contains the functional part of the molecule, which is bound to an oligomeric backbone resulting in increased molecular weight and decreased migration potential.<sup>6</sup>

Research interests into analytical methods that can be used to detect a wide variety of the photoinitiators, amine synergists, acrylates, and other printer ink components that could either migrate into foods or otherwise cause food contamination also increased. Many analytical methods have been used, including direct analysis in real time (DART) coupled to a time-of-flight mass spectrometer (MS), GC-MS/MS, HPLC-DAD, HPLC-FLR, or LC-MS/MS.<sup>2,6,7</sup> Quantitative analytical methods must be capable of detecting the specific migration limit (SML), which is the maximum amount of a given substance that can be released into food or a simulant for each photoinitiator.<sup>4</sup> According to EU Regulation (EC) No. 1935/2004, surfaces and materials that are in contact with food should not transfer their constituents into the food at levels that would endanger human health,<sup>8</sup> while other regions, such as the United States of America and China, adopt a similar framework to their food contact legislation (US FDA CFR 21 and China's Food Safety Law, respectively).

Switzerland has an extensive body of national legislation in place on food contact materials, including printing inks.<sup>9</sup> Within the European Union, guidance on industry practices exist,<sup>10</sup> however, extensive SMLs have not yet been set for printing inks migrating into food. Ultimately, the responsibility is placed on suppliers and manufacturers to ensure the materials and articles, when in contact with food, do not endanger human health. When toxicological data does not exist, a level of 10 µg/kg must be detectable by the analytical technique and migration of the compounds into the food, or simulant, must not exceed this limit.<sup>5,8</sup> Detecting false positives can lead to serious consequences, therefore unambiguous identification of photoinitiators and other contaminants is critical.

In this study, UPLC, HRMS, and a custom library were used to detect photoinitiators and acrylates and perform quantitation of target analytes in a single-sided migration study using a food simulant as the testing medium.

## Experimental

### Sample preparation

The authentic standard compounds of 12 photoinitiators and acrylates, listed in Table 1, which consisted of a mixture of liquids and solids, were dissolved in acetonitrile at a concentration of 1 mg/mL. Speedcure 7010 required sonication for 30 minutes to fully dissolve. For quantitative analyses the compounds were combined, and a stock solution of 10 µg/mL was prepared. The calibration curve was prepared by sequential dilution in the solvent used as a food simulant (95:5 ethanol/water).

| Name                                                                                                                                      | CAS No.      | Synonym                            |
|-------------------------------------------------------------------------------------------------------------------------------------------|--------------|------------------------------------|
| 1,1,1-trimethylolpropane, ethoxylated, ester with 2-benzoylbenzoic acid                                                                   | N/A          | Polymeric benzophenone derivative  |
| 1-(4-[(4-benzoylphenyl)thio]phenyl)-2-methyl-2-[(4-methylphenyl)sulfonyl]-1-propan-1-one                                                  | 0272460-97-6 | Esacure 1001                       |
| 1-butanone, 2-(dimethylamino)-1-[4-(4-morpholinyl)phenyl]-2-(phenylmethyl)-                                                               | 0119313-12-1 | Irgacure 369                       |
| Poly(oxy-1,2-ethanediyl), $\alpha$ -(2-benzoylbenzoyl)- $\omega$ -[(2-benzoylbenzoyl)oxy]-                                                | 1246194-73-9 | Omnipol 2702                       |
| 1,3-di({a-[1-chloro-9-oxo-9H-thioxanthen-4-yl]oxy}acetyl)poly[oxy(1-methylethylene)]oxy)-2,2-bis({a-[1-methylethylene]}oxymethyl) propane | 1003567-83-6 | Speedcure 7010                     |
| (Dimethylamino)benzoate, esters with branched polyols                                                                                     | N/A          | Polymeric aminobenzoate derivative |
| 1-butanone, 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-[4-(4-morpholinyl)phenyl]-                                                     | 0119344-86-4 | Irgacure 379                       |
| Phenyl bis(2,4,6-trimethylbenzoyl) phosphine oxide                                                                                        | 0162881-26-7 | Irgacure 819                       |
| Pentaerythritol ethoxylate tetraacrylate                                                                                                  | 51728-26-8   | PPTTA                              |
| Glycerol propoxylated, esters with acrylic acid                                                                                           | 52408-84-1   | GPTA                               |
| Trimethylolpropane triacrylate                                                                                                            | 15625-89-5   | TMPTA                              |
| Ethoxylated trimethylolpropane triacrylate                                                                                                | 28961-43-5   | TMP(EO)TA                          |

Table 1. Names, CAS numbers, and synonyms of the photoinitiators and acrylates analyzed in the study.

### Migration study

The migration study was performed using biaxially oriented polypropylene (BOPP), 55-µm-thick printed with three different UV opaque whites. A DN80 stainless steel migration cell system (Siegwerk/Sieg-Mi-Flex) from LABC-Labortechnik GmbH ([www.LABC.de](http://www.LABC.de)) was used for the study. One-sided approximate contact area: 0.5 dm<sup>2</sup>, fill volume: ca. 100 mL. The cell was filled with the simulant, 95% ethanol and 5% water, and maintained at 40 °C for 10 days in an oven (MaxQ 4450, Thermo Scientific).

### Instrumentation and software

LC separations and MS experiments were performed on the ACQUITY UPLC I-Class System and the Xevo G2-XS QToF Mass Spectrometer. UNIFI Scientific Information System Software was used for data acquisition and processing.

## Method conditions

### UPLC method

|                   |                                                                         |
|-------------------|-------------------------------------------------------------------------|
| Separation mode:  | Gradient                                                                |
| Column:           | ACQUITY UPLC BEH C <sub>18</sub> , 1.7 µm, 2.1 × 100 mm                 |
| Solvent A:        | 95:5 2 mM ammonium formate with 0.1% formic acid in water:acetonitrile  |
| Solvent B:        | 95:5 acetonitrile: 2 mM ammonium formate with 0.1% formic acid in water |
| Flow rate:        | 0.400 mL/min                                                            |
| Column temp.:     | 40 °C                                                                   |
| Injection volume: | 3 µL                                                                    |

### Gradient conditions:

| Time(min) | %A  | %B   | Curve |
|-----------|-----|------|-------|
| 0.00      | 95% | 5%   | –     |
| 10.00     | 0%  | 100% | 6     |
| 15.00     | 0%  | 100% | 6     |
| 15.20     | 95% | 5%   | 6     |

## Xevo G2-XS QToF conditions

|                        |                                  |
|------------------------|----------------------------------|
| Acquisition mode:      | MS <sup>E</sup> sensitivity mode |
| Start and end mass:    | 100–2000                         |
| Ionization mode:       | ESI+                             |
| Capillary voltage:     | 3.5 kV                           |
| Cone voltage:          | 30 V                             |
| Collision energy ramp: | 20–50 V                          |
| Desolvation temp.:     | 450 °C                           |
| Source temp.:          | 150 °C                           |
| Desolvation gas flow:  | 1000 (L/hr)                      |
| Cone gas:              | 50 (L/hr)                        |

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## Results and Discussion

### Library Generation

A custom library of the target analytes was created for the study. The theoretical formulae for the oligomeric series of the polymeric photoinitiators were generated and included in a Microsoft Excel spreadsheet along with other information pertaining to each compound, including the structure in the form of a .mol file, a brief description, adducts fragments, and retention times.

The information contained in the Microsoft Excel file was used to create a database within the UNIFI Scientific Library, which can then be used in the targeted screening of the photoinitiators and acrylates. Using multiple identification parameters greatly increases the confidence in the results and decreases the

incidence of false positives. An example of the library entry for Omnipol 2702 (n=4) is shown in Figure 1.

**Results**  
Search results (94 items found):

- 61 Omnipol 2702-00
- 62 Omnipol 2702-01
- 63 Omnipol 2702-02
- 64 Omnipol 2702-03
- 65 **Omnipol 2702-04**
- 66 Omnipol 2702-05
- 67 Omnipol 2702-06
- 68 Omnipol 2702-07
- 69 Omnipol 2702-08
- 70 Omnipol 2702-09
- 71 Omnipol 2702-10
- 72 Speedcure 7010-04
- 73 Speedcure 7010-05
- 74 Speedcure 7010-06
- 75 Speedcure 7010-07
- 76 Speedcure 7010-08
- 77 Speedcure 7010-09
- 78 Speedcure 7010-10
- 79 Speedcure 7010-11
- 80 Speedcure 7010-12
- 81 TMP(EQ)TA
- 82 TMP(EQ)TA-01
- 83 TMP(EQ)TA-02
- 84 TMP(EQ)TA-03
- 85 TMP(EQ)TA-04
- 86 TMP(EQ)TA-05
- 87 TMP(EQ)TA-06
- 88 TMP(EQ)TA-07

**Omnipol 2702-04 [Argus Analysen Service GmbH]**

| Property           | Value                                                                                                                   |
|--------------------|-------------------------------------------------------------------------------------------------------------------------|
| Item type          | Compound                                                                                                                |
| Item description   | Di ester of carboxymethoxybenzophenone and polyethyleneglycol 200                                                       |
| IUPAC name         |                                                                                                                         |
| Formula            | C <sub>36</sub> H <sub>34</sub> O <sub>9</sub>                                                                          |
| Hill formula       | C <sub>36</sub> H <sub>34</sub> O <sub>9</sub>                                                                          |
| Average molar mass | 610.6498                                                                                                                |
| Monoisotopic mass  | 610.2203                                                                                                                |
| Item tag           | 15/C <sub>36</sub> H <sub>34</sub> O <sub>9</sub> /c37-33<br>(27-7-3-1-4-8-27)<br>29-11-15-31(16-12-29)35<br>(39)       |
| InChI              | 44-25-23-42-21-19-41-20-<br>22-43-24-26-45-36(40)<br>32-17-13-30(14-18-32)34<br>(38)28-9-5-2-6-10-28/<br>h1-18H,19-26H2 |

**Detection results**

| Priority                                                                                                                                                                                                                    | Intensity | Formula                                        | Neutral Mass (Da) | Adduct           | Charge | Fragmentation type | Expected m/z | Expected RT (min) | Ionization technique |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------------------------------------|-------------------|------------------|--------|--------------------|--------------|-------------------|----------------------|
| Detection result: Instrument model: Unknown, Instrument serial no.: Manually created, Created by administrator on Apr 24, 2019<br>Omnipol 2702, Di ester of carboxymethoxybenzophenone and polyethyleneglycol 200 (3 items) |           |                                                |                   |                  |        |                    |              |                   |                      |
| 1                                                                                                                                                                                                                           |           | C <sub>36</sub> H <sub>34</sub> O <sub>9</sub> | 610.2203          | +NH <sub>4</sub> | +1     | None               | 628.2541     | 7.450             | ESI+                 |
| 2                                                                                                                                                                                                                           |           |                                                |                   |                  |        | CID                | 209.0597     |                   | ESI+                 |
| 3                                                                                                                                                                                                                           |           |                                                |                   |                  |        | CID                | 253.0859     |                   | ESI+                 |

Figure 1. An example of the library entry for Omnipol 2702 (n=4).

## Migration Study

The migration study was performed using cells designed to allow single sided migration experiments. An example of the stainless-steel migration cell system used in the study is shown in Figure 2. The cell is assembled once the packaging material is put in place with the non-printed side in contact with the simulant. The simulant is added to the cell, and the migration experiment is conducted over 10 days in an oven maintained at a constant temperature (40 °C).





*Figure 2. Photograph of the Siegwerk (Sieg-Mi-Flex) migration cell system (LABC-Labortechnik GmbH) used to perform single-sided migration study.*

The results are determined after the duration of the study by quantifying the residual target compounds in the simulant. Calibration curves were used to quantify the target compounds. The curves ranged in concentration from 1 ng/mL to 10 µg/mL (equivalent to 1.2 µg/kg–12,000 µg/kg) though not all compounds were linear over this range. In the case of the polymeric photoinitiators, the most intense oligomers were selected as the primary and secondary ions for quantitation and confirmation of the detected analytes (Table 2). Migration was observed for TMPTA (detected as its sodium adduct) in all tested samples printed with different opaque whites. TMPTA exceeded the SML of 50 µg/kg in two of the three tested samples with results ranging from 15.5–360.4 µg/kg in three samples tested (Figure 3).



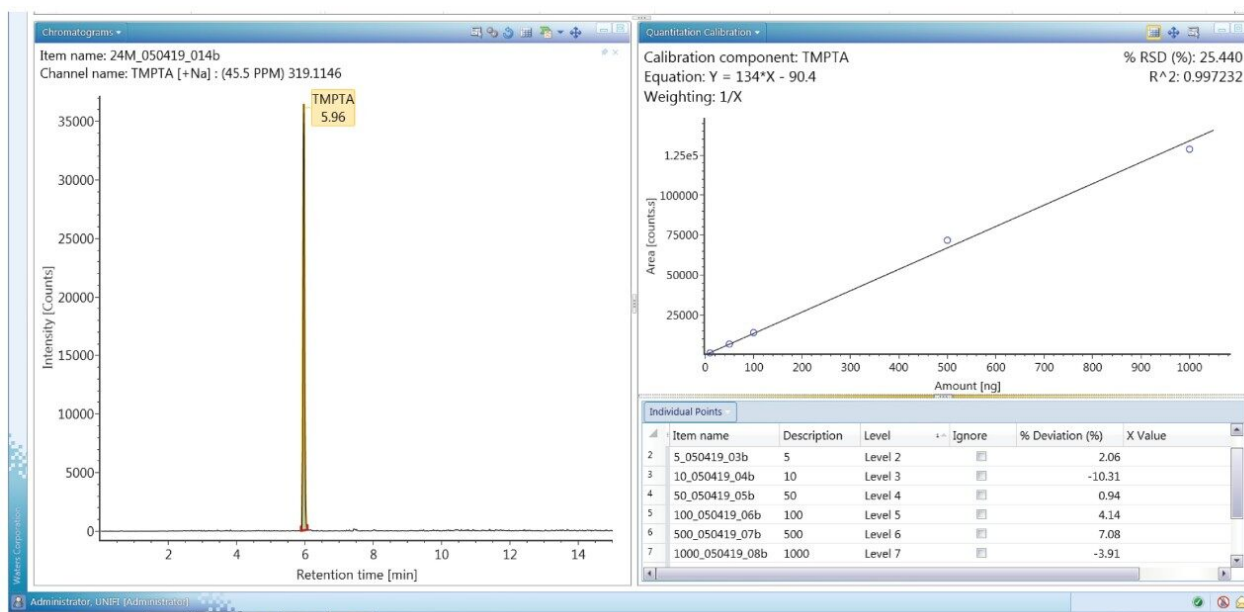


Figure 3. Chromatogram of the TMPTA migration measured in the simulant, 360.4  $\mu\text{g/kg}$  (calculations based on contact area: 0.5  $\text{dm}^2$ ). The linear calibration curve over the range 5–1000 ng/mL (6–1200  $\mu\text{g/kg}$ ) is also shown as well as the % deviations.

| Primary                                  | Adduct                      | Primary target > fragments observed $m/z$ | Secondary                                | Secondary target > fragments observed $m/z$ |
|------------------------------------------|-----------------------------|-------------------------------------------|------------------------------------------|---------------------------------------------|
| Polymeric benzophenone derivative (n=6)  | $[\text{M}+\text{NH}_4]^+$  | 832.3917 > 209.0594, 253.0869             | Polymeric benzophenone derivative (n=5)  | 788.3692 > 209.0594, 253.0869               |
| Esacure 1001                             | $[\text{M}+\text{H}]^+$     | 515.1339 > 331.1143                       | Esacure 1001                             | 515.1339 > 360.1184, 139.0233               |
| Irgacure 369                             | $[\text{M}+\text{H}]^+$     | 367.2381 > 190.0871, 176.1442, 294.1855   | Irgacure 369                             | 367.2381 > 176.1442, 294.1855               |
| Omnipol 2702 (n=4)                       | $[\text{M}+\text{NH}_4]^+$  | 628.2543 > 209.0594, 253.0869             | Omnipol 2702 (n=5)                       | 672.2908 > 209.0594, 253.0869               |
| Speedcure 7010 (n=8)                     | $[\text{M}+2\text{H}]^{2+}$ | 905.1697 > 361.0290, 320.9980, 379.0394   | Speedcure 7010 (n=9)                     | 935.1909 > 320.9980, 361.0290, 379.0394     |
| Polymeric aminobenzoate derivative (n=6) | $[\text{M}+\text{H}]^+$     | 840.4656 > 148.0773, 192.1029             | Polymeric aminobenzoate derivative (n=7) | 884.4947 > 148.0773, 192.1029               |
| Irgacure 379                             | $[\text{M}+\text{H}]^+$     | 381.2532 > 190.0869                       | Irgacure 379                             | 381.2532 > 308.2006, 176.1076               |
| Irgacure 819                             | $[\text{M}+\text{Na}]^+$    | 441.1594 > 147.0812                       | Irgacure 819                             | 441.1594 > 227.0982                         |
| PPT(T)A (n=5)                            | $[\text{M}+\text{NH}_4]^+$  | 590.2823 > 501.2334, 413.1802, 369.1526   | PPT(T)A (n=6)                            | 634.3069 > 457.2067, 413.1802, 501.2334,    |
| GPTA (n=4)                               | $[\text{M}+\text{NH}_4]^+$  | 504.2819 > 241.1070, 113.0582, 183.0636   | GPTA (n=5)                               | 562.3233 > 113.0589, 241.1065, 183.0642,    |
| TMPTA                                    | $[\text{M}+\text{Na}]^+$    | 319.1151 $[\text{M}+\text{Na}]^+$         | TMPTA $[\text{M}+\text{NH}_4]^+$         | 314.1593                                    |
| TMP(EO)TA (n=4)                          | $[\text{M}+\text{NH}_4]^+$  | 490.2654 > 269.1370, 313.1633, 357.1890   | TMP(EO)TA (n=5)                          | 534.2867 > 313.1633, 357.1890, 269.1370     |

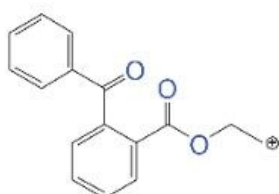
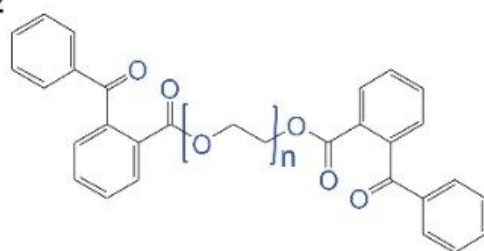
Table 2. Primary and secondary target ions monitored as well as the most intense fragments, adducts, and oligomers observed for the photoinitiators and acrylates analyzed in the study.

## Untargeted Screening

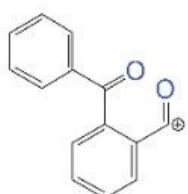
The chemical constituents of ink formulations make them complex substances to analyze, particularly if there are polymeric substances present. It is important that the correct analytical tools are used to accurately identify ink formulation components in migration testing experiments. This can be done with targeted methods of analysis, such as a multiple reaction monitoring using a tandem quadrupole.<sup>3,7</sup> The ability to also perform untargeted screening is beneficial as the information about the chemical composition of the ink formulation can sometimes be incomplete. The use of high-resolution chromatography and HRMS can generate results which can greatly aid in untargeted screening studies.

The structures of the polymeric photoinitiators often share structural features which can lead to common fragment ions for each of the oligomers following collision induced dissociation (CID). This information can be used in untargeted analysis where an unexpected analyte might be suspected. When the oligomers of the polymeric benzophenone derivative-1A series undergo CID, the most prominent fragments are  $m/z$  253.0858 and  $m/z$  209.0597 (Figures 4 and 5). Polymeric photoinitiators that have the same substructure, such as Omnipol 2702, can also give rise to these fragments.

### Omnipol 2702



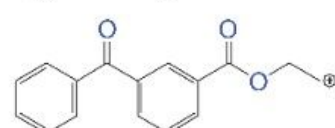
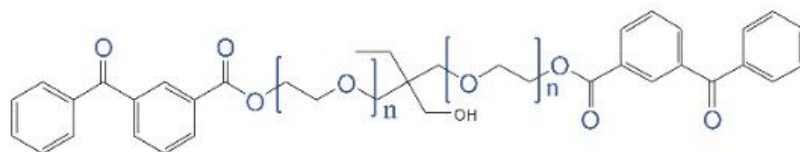
Proposed structure for  
fragment with  
 $m/z$  253.0858,  
 $C_{16}H_{13}O_3$



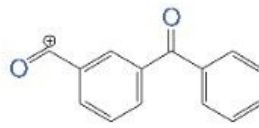
Proposed structure  
for fragment with  
 $m/z$  209.0597,  
 $C_{14}H_9O_2$

### Polymeric benzophenone derivative-1A

(proposed structure)



Proposed structure for  
fragment with  $m/z$  253.0858,  
 $C_{16}H_{13}O_3$



Proposed structure for  
fragment with  $m/z$  209.0597,  
 $C_{14}H_9O_2$

### Polymeric benzophenone derivative-1B

(proposed structure)

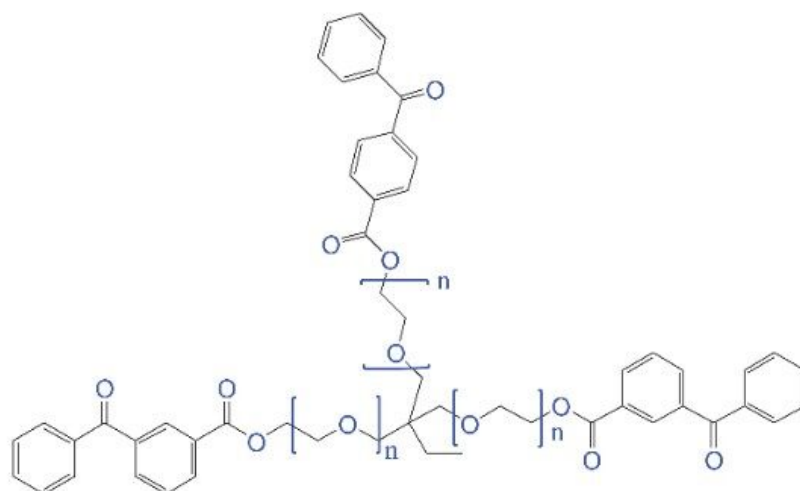


Figure 4. Proposed structure of polymeric benzophenone derivative-1A,

In the high collision energy spectrum, the most prominent fragment observed had  $m/z$  190.0863. A common fragment search of  $m/z$  190.0863 revealed a second compound with this fragment at a retention time ( $t_R$ ) of 4.18 min (Figure 6).

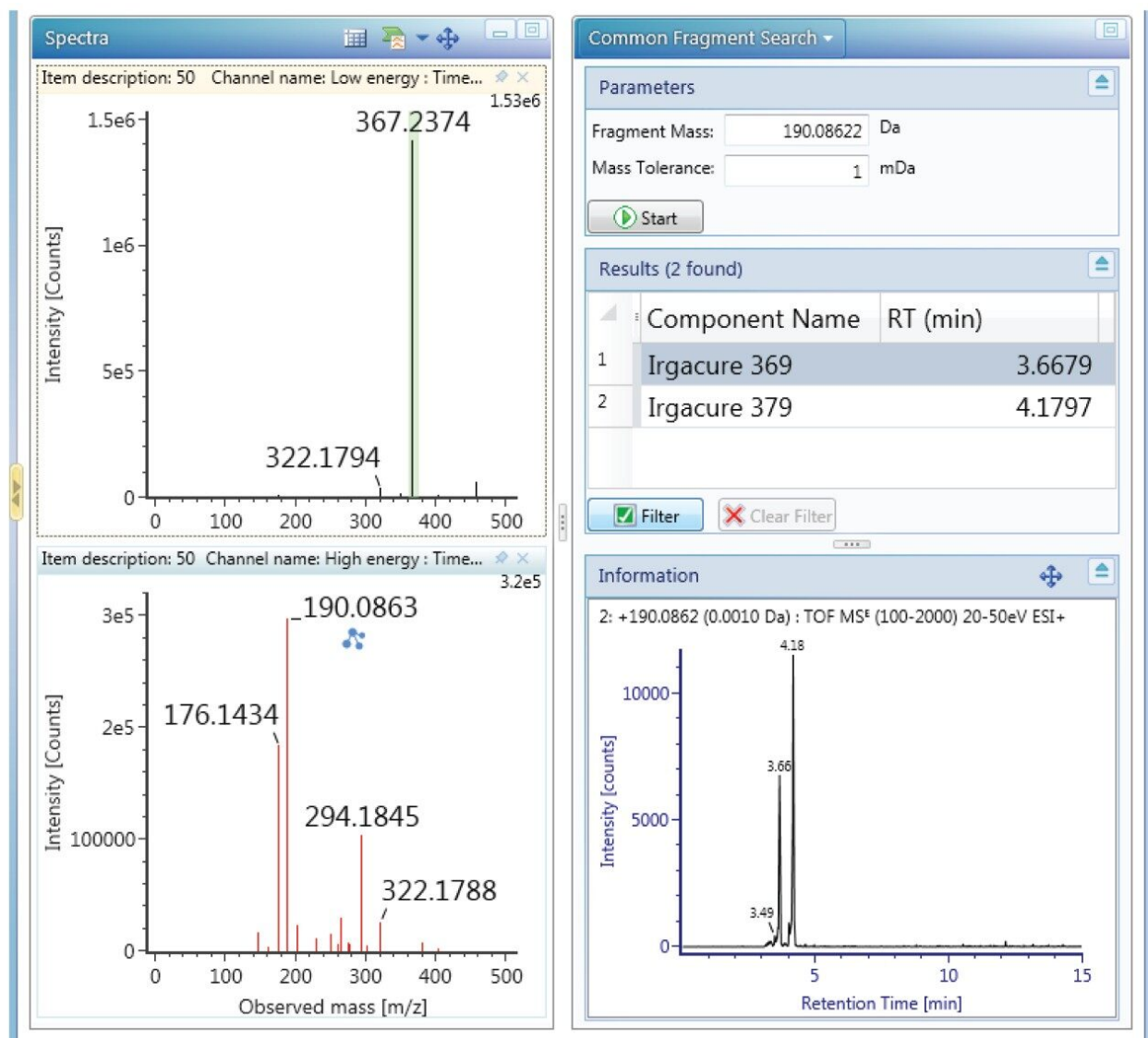


Figure 6. High and low CE spectra of Irgacure 369 ( $t_R$  3.66 min) at 50 ng/mL (60  $\mu$ g/kg). The structural elucidation tool for Search Common Fragment shows the fragment ion  $m/z$  190.0863 is common to Irgacure 369 and Irgacure 379.

The compounds Irgacure 369 and Irgacure 379 each produce a fragment with a  $m/z$  190.0863 as they possess very similar chemical structures. The Fragment Match structural elucidation tool can be used to assign the most likely structures to fragments based on a scoring system and other parameters. As is shown

in Figure 7, the most likely structure for the common fragment is based on the 4-morpholinophenyl(oxo)methylium ion (Figure 8).

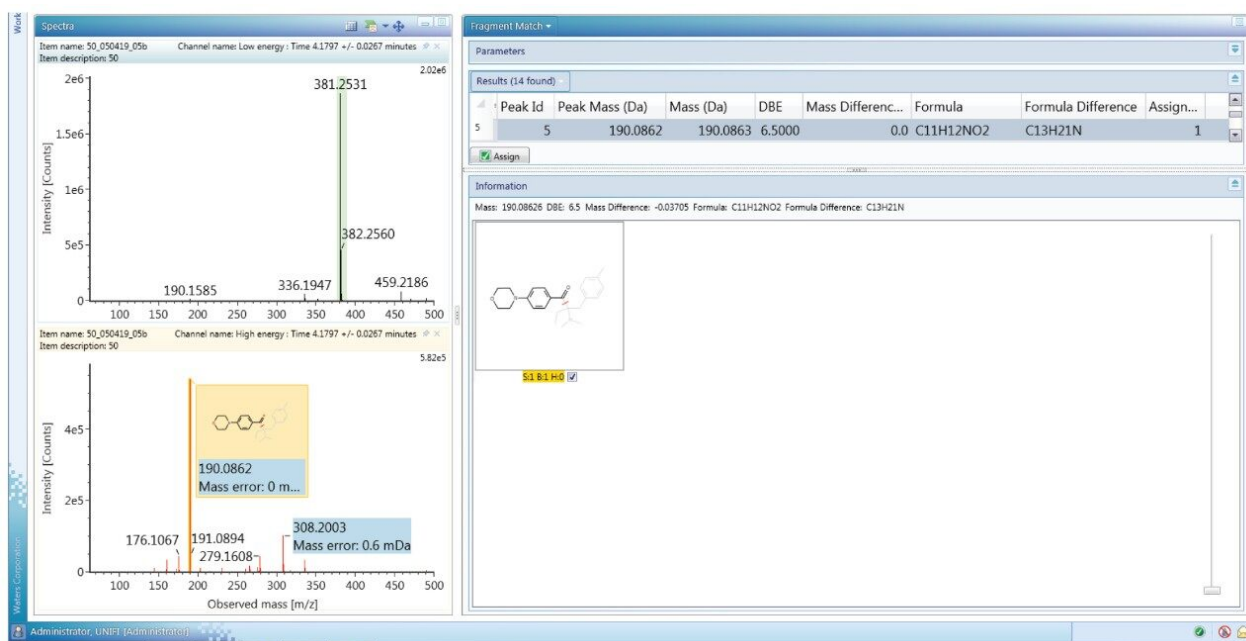
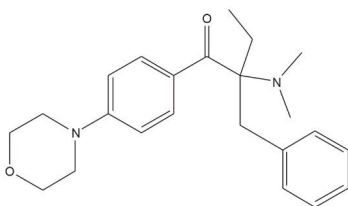
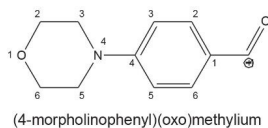
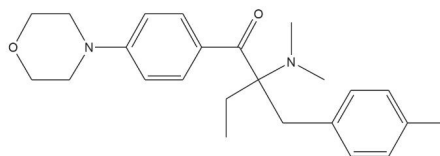


Figure 7. High and low CE spectra of Irgacure 379 (tR 4.18 min) at 50 ng/mL (60  $\mu$ g/kg). The Fragment Match structural elucidation tool shows the structure that has been assigned to the fragment ion with  $m/z$  190.0862.

Irgacure 369



Irgacure 379



Proposed structure for fragment with  $m/z$  190.0863,  $C_{11}H_{12}NO_2$

Figure 8. Structure of Irgacure 369 and Irgacure 379 shown with proposed structures for the common fragment with  $m/z$  190.0863.

Once common fragments have been identified they can be entered in the search criteria of the processing

method and used in subsequent analyses (Figure 9). This enables automatic searching of the data for shared structural features.

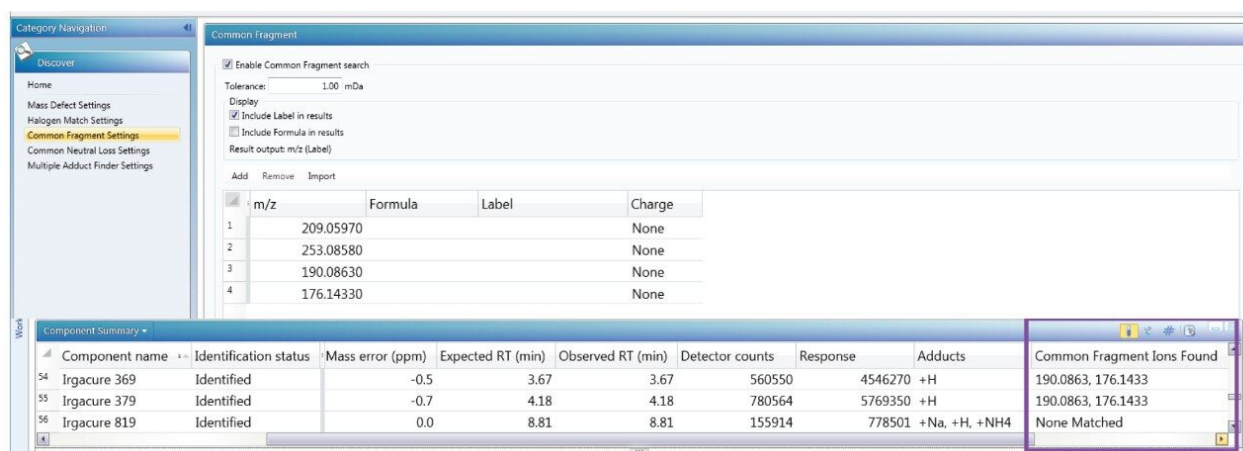


Figure 9. Discovery tools and common fragment settings shown as well as automatic common fragment search results (highlighted in purple).

## Conclusion

A custom library greatly aided in the targeted analysis of the migration samples under test. The library consisted of acrylates and photoinitiators, some polymeric in nature with entries and identification information specific for each oligomer. TMPTA was identified and quantified in the single-sided migration study. The level of the TMPTA quantified in the simulants exceeded the SML of 50 µg/kg in two of the three samples printed with different opaque whites. The structural elucidation tools available in the UNIFI Software helped to identify photoinitiators with common fragments. The workflow illustrated here can be used to identify targeted and untargeted photoinitiators in migration studies.

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