



Gas Chromatography/ Mass Spectrometry

Author:

Kathryn Lawson-Wood

PerkinElmer, Inc.

Seer Green, UK

Quantification of Phthalate Leaching from Food Contact Materials by GC/MS

Introduction

Migration of compounds from Food Contact Materials (FCMs) into food, also known as leaching, can result in food spoilage, and in some cases

may be detrimental to human health. This has resulted in regulations and guidelines on compound migration from FCMs into packaged food. Beside the chemical nature of the food and the plastic in contact with the food, the overall storage conditions, such as temperature and time, affect the migration from FCMs into food.

Among the FCMs, phthalates in plastics are known to leach into food. Phthalates are a diverse range of compounds predominately used as plasticisers to increase flexibility of plastic (Figure 1). Since phthalates are weakly bound, they have the potential to leach out into their surrounding environment. Although toxicological effects of all phthalates have not been evaluated, many which are widely used, such as bis(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate (BBP), and dibutyl phthalate (DBP), display endocrine disrupting capabilities. Therefore, use of phthalates in food packaging is limited¹.

This application demonstrates the use of the PerkinElmer Clarus® SQ 8 GC/MS to quantify three regulated phthalates (DBP, BBP, and DEHP), along with other common phthalates (DMP, DEP, and DnOP) with high sensitivity and repeatability based on Regulation EU No. 10/2011. The Clarus SQ 8 system was operated in Selected Ion Full Ion (SIFI™) MS mode. In SIFI mode, the analyst can set up the instrument to detect target analytes in Selected Ion Monitoring (SIM) mode while simultaneously probing for unknown leachable compounds in scan mode. Further attributes of the application demonstrate the effect of different temperatures and food types on leaching from FCMs.

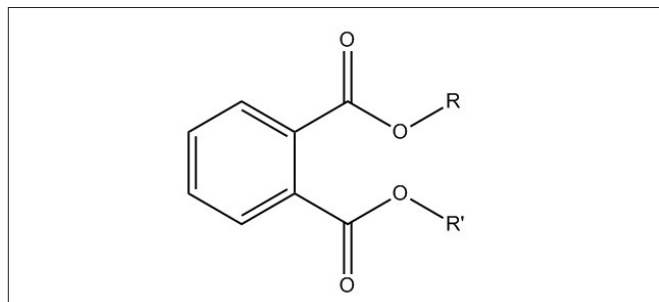


Figure 1. General phthalate structure, where R and R' represent various combinations of alkyl side chains.

Food Contact Materials Migration Regulations

Specific Migration Limit (SML) is the maximum permitted quantity of a specific substance released from a FCM into food or food simulant (fs) and is expressed in mg/kg fs². The European Union SMLs for phthalates used in FCMs, according to Directive 2007/19/EC, are 0.3 mg/kg fs for DBP, 1.5 mg/kg fs for DEHP, and 30.0 mg/kg fs for BBP³.

Simulants are often used, to simplify the migration analysis in different food types, as substitutes for food to mimic the interactions between the food and packaging, reduce matrix interferences, and decrease the amount of sample preparation⁴. The EU Regulation No. 10/2011 on 'Plastic materials and articles intended to come into contact with food' specifies certain test conditions for FCMs, with food simulants listed in Table 1.

Table 1. Food simulants in Regulation EU No. 10/2011.

| Type of Food | EU No. 10/2011 Food Simulants |
|-------------------------------|-------------------------------|
| Aqueous (pH > 4.5) | 10 % Ethanol (v/v) |
| Acidic (pH < 4.5) | 3 % Acetic acid (m/v) |
| Alcoholic/slightly lipophilic | 20 % Ethanol (v/v) |

A variety of migration testing conditions, representing accelerated storage conditions, are listed in Regulation EU No. 10/2011 dependant on the packaging. The temperature and time conditions considered to be the most severe are 10 days at 50 °C to simulate up to six months storage at room temperature and 10 days at 20 °C to simulate refrigerated conditions. For packaging intended for high temperature use, including microwavable packaging, the specified migration test condition is one hour at 100 °C. A surface to volume ratio of 6 dm² to 1 kg of fs should be used in specific migration testing and the final sample results reported in mg/kg fs⁴.

Experimental

HPLC grade n-hexane solvent and EPA 606 phthalate mix containing 2000 mg/L diethyl phthalate (DEP), dimethyl phthalate (DMP), bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), benzyl butyl phthalate (BBP), and dibutyl phthalate (DBP) in n-hexane were obtained from Sigma-Aldrich.

Experimental conditions are listed in Table 2. The analysis was carried out on a PerkinElmer Clarus SQ 8 GC/MS system with a 5 µL syringe liquid autosampler and programmable split/splitless injector. A silicone rubber injection port septum BTO® rated to 400 °C with low bleed was fitted (Part No. N9302972). A glass injector-port liner (2 mm internal diameter) with deactivated quartz wool (Part No. N9306232) was used to improve vaporisation and prevent non-volatile material entering the column.

SIFI MS mode was used to obtain library searchable spectral information and data with the sensitivity of Selected Ion Monitoring (SIM). This MS function is achieved through combining SIM with a full scan over a desired mass range. In SIM mode, the parameters are set to monitor a single or a few selected ions. Table 3 shows the SIM quantitation and qualifier ions for each phthalate. The most abundant ion for most phthalates is m/z 149 except DMP, whose most abundant ion is m/z 163. Referring to Figure 2, R and R' represent various alkyl side chains. However, in the case of DMP, both R and R' represent CH₃ and the hydrogen on the oxygen is replaced by CH₃. The most abundant peak then becomes m/z 163¹.

Table 2. GC/MS Conditions.

| Gas Chromatograph: | PerkinElmer Clarus 680 GC |
|---------------------------|---|
| Column | PerkinElmer Elite™ - 5 MS capillary column (30 m x 0.25 mm x 0.25 µm) |
| Injection Port Type | Programmable Split/Splitless |
| Injector Temperature | 280 °C |
| Injection Type | Split, 20:1 |
| Injection Volume | 1 µL (5 µL syringe) |
| Carrier Gas | Helium, constant pressure (22.7 psi) |
| Oven Programme | 100 °C for 0.5 min, ramp to 320 °C at 15 °C/min and hold for 2.5 min |
| Mass Spectrometer: | PerkinElmer Clarus SQ 8C MS |
| Ionisation Mode | EI |
| GC Inlet Line Temperature | 280 °C |
| Ion Source Temperature | 280 °C |
| Function Type | SIFI |
| Scan Range | m/z 45-400 |
| Scan Time | 0.060 s |
| InterScan Delay Time | 0.050 s |
| Dwell Time | 0.070 s |
| InterChannel Delay Time | 0.005 s |
| Solvent Delay Time | 3 min |

Table 3. SIM ions used for phthalates.

| Phthalate | Quantitation Ion (m/z) | Qualifier Ion (m/z) | Retention Time Window (min) |
|-----------|------------------------|---------------------|-----------------------------|
| DMP | 163 | 77 | 4.80-5.60 |
| DEP | 149 | 177 | 6.00-6.80 |
| DBP | 149 | 205 | 8.50-9.70 |
| BBP | 149 | 91 | 11.20-12.00 |
| DEHP | 149 | 167 | 12.20-13.10 |
| DnOP | 149 | 279 | 13.30-14.20 |

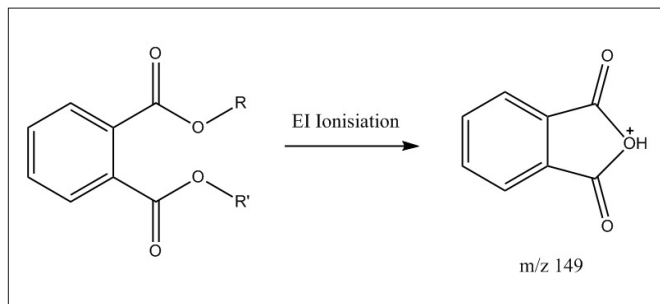


Figure 2. General structure of phthalate esters and their major fragmentation in EI ionisation where R and R' represent alkyl side chains¹.

Sample Preparation and Spike Recovery

Sample preparation was based on Regulation EU No 10/2011. Four plastic food packaging types (polytetrafluoroethylene bottle, polyvinyl chloride cling film, polyethylene terephthalate microwavable tray, and polypropylene bottle) were obtained from local supermarkets. 10% ethanol, 3% acetic acid, and 20% ethanol were used as food simulants to represent aqueous, acidic, and alcoholic or slightly lipophilic food mediums respectively.

Each plastic was cut into 0.06 dm² pieces and placed in separate sealed glass vials containing 10 mL of different food simulant. Each vial was stored as stated in Table 4. Finally, to test the effect of heating on microwavable packaging, the migration testing was conducted at room temperature and at 100 °C for one hour.

Table 4. Storage conditions for each plastic.

| Food Simulant | Time (days) | Temperature(°C) | |
|----------------------|-------------|-----------------|----|
| 10% Ethanol (v/v) | 10 | 20 | 50 |
| 3% Acetic acid (m/v) | 10 | 20 | 50 |
| 20% Ethanol (v/v) | 10 | 20 | 50 |

After the migration testing times, the plastics were removed and the samples extracted with hexane (2 mL) to concentrate the analytes by a factor of 5. Sodium chloride (0.5 g) was added to prevent emulsion between the two layers. Each vial was shaken vigorously for 2 min, and the contents allowed to separate for 10 min. The hexane layer was placed into autosampler vials for analysis. Extraction blanks were prepared and analysed to monitor for any external sources of contamination.

Spike recovery was used to determine the efficiency of the extraction process of phthalates in each food simulant. This was achieved by spiking each food simulant (10 mL) to give expected concentrations of 3.0 mg/L and 140.0 mg/L phthalate in hexane. The spike samples were treated according to the extraction procedure previously described.

Phthalate Calibration

Ten calibration standards spanning the range 0.2 – 200.0 mg/L mixed phthalate in hexane were prepared in 10 mL volumetric flasks using the EPA 606 phthalate mix and run using the GC/MS method. These standards were equivalent to the concentration range of 0.04 – 40.00 mg/kg phthalate in fs accounting for the concentration factor of five used in sample preparation. Calibration curves were constructed using the specified SIM quantitation ions using TurboMass™ software and external standardization. A low calibration range (0.2 – 12.0 mg/L phthalate in hexane) was used for DBP and DEHP whilst a high calibration range (12.0 - 200.0 mg/L phthalate in hexane) was used for BBP to ensure coverage of regulatory parameters. The high range was also used for DnOP, DEP, and DMP to illustrate linearity and repeatability for other common phthalates.

To evaluate the accuracy of both calibration ranges, 75.0 mg/L and 0.8 mg/L mixed phthalate validation standards in hexane were independently made and the calculated concentrations obtained from the calibrations compared with the known concentrations. The stability of the instrument and repeatability of results was evaluated by performing six repeat injections on the validation standards and the RSD % calculated.

Results and Discussion

Phthalate Calibration

The SIM chromatogram of the 100.0 mg/L phthalate standard is shown in Figure 3. External standard calibration curves for all six phthalates showed linear regression coefficients (R²) greater than 0.994 (Table 5), indicating excellent linearity. Good levels of accuracy and repeatability were obtained for the validation standards for 6 continuous injections, with RSDs below 4% (Table 6).

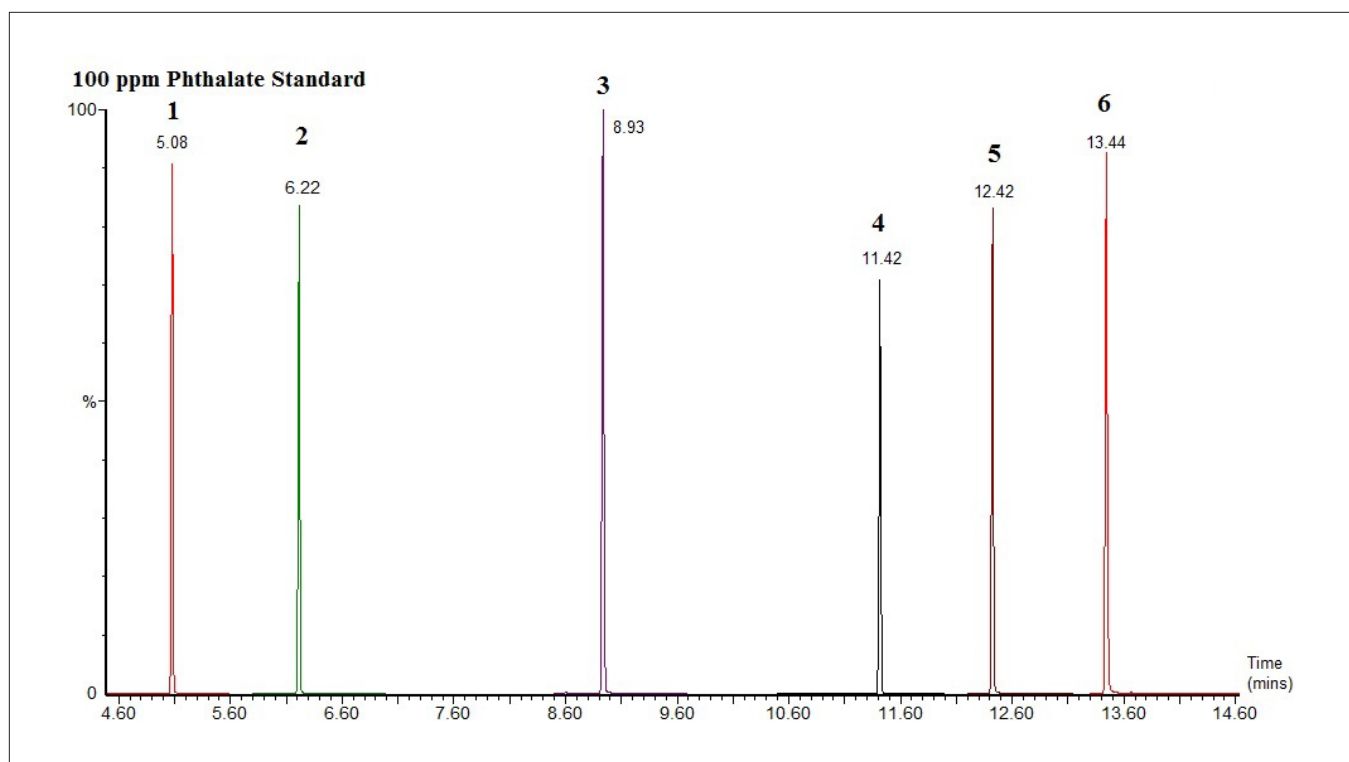


Figure 3. SIM chromatogram of 100.0 mg/L phthalate standard (1- DMP, 2- DEP, 3 – DBP, 4- BBP, 5- DEHP, and 6- DnOP).

Table 5. Retention times and calibration of phthalates with concentrations .

| Phthalate | Retention Time (mins) | Calibration Range in Hexane (mg/L) | Calibration Range in FS (mg/kg) | R ² |
|-----------|-----------------------|------------------------------------|---------------------------------|----------------|
| DMP | 5.08 | 12.0 – 200.0 | 2.40 – 40.00 | 0.9981 |
| DEP | 6.22 | 12.0 – 200.0 | 2.40 – 40.00 | 0.9997 |
| DBP | 8.93 | 0.2 – 12.0 | 0.04 – 2.40 | 0.9974 |
| BBP | 11.42 | 12.0 – 200.0 | 2.40 – 40.00 | 0.9948 |
| DEHP | 12.42 | 0.2 – 12.0 | 0.04 – 2.40 | 0.9960 |
| DnOP | 13.44 | 12.0 – 200.0 | 2.40 – 40.00 | 0.9971 |

Table 6. Phthalate accuracy and repeatability with concentrations reported to one decimal place.

| Phthalate | True Concentration in Hexane (mg/L) | Average Measured Concentration in Hexane (mg/L) | RSD (%) |
|-----------|-------------------------------------|---|---------|
| DMP | 75.0 | 79.9 | 3.66 |
| DEP | 75.0 | 77.8 | 3.95 |
| DBP | 0.8 | 0.8 | 2.21 |
| BBP | 75.0 | 69.2 | 2.24 |
| DEHP | 0.8 | 0.8 | 2.64 |
| DnOP | 75.0 | 76.2 | 1.91 |

Samples and Spike Recovery

Results of the spiked food simulants are shown in Table 7. Good recoveries were obtained for most phthalates, ranging from 83 – 107 %. DMP, a lighter and slightly polar phthalate, was the only compound with lower recoveries, ranging from 46 – 57 % recovery due to fewer interactions with the hexane layer. In general, the highest recovery was observed in 20 % ethanol for most phthalates except DMP and DEP.

Table 7. Phthalate extraction recoveries in different food simulants (n=3).

| Spiked Food Simulant | Average Phthalate Recovery (%) | | | | | |
|------------------------------|--------------------------------|------|------|-------|------|-------|
| | DMP | DEP | DBP | BBP | DEHP | DnOP |
| 20 % EtOH (3.0 mg/L) | - | - | 99.8 | - | 99.0 | - |
| 20 % EtOH (140.0 mg/L) | 46.3 | 93.1 | - | 104.0 | - | 106.6 |
| 10 % EtOH (3.0 mg/L) | - | - | 88.8 | - | 96.4 | - |
| 10 % EtOH (140.0 mg/L) | 56.7 | 97.6 | - | 102.9 | - | 101.8 |
| 3 % Acetic acid (3.0 mg/L) | - | - | 83.6 | - | 82.8 | - |
| 3 % Acetic acid (140.0 mg/L) | 48.3 | 85.4 | - | 90.5 | - | 92.2 |

Under the different storage conditions, no phthalates were detected from any of the FCM samples. Cling film was the only FCM found to have detectable leaching. Figure 4 shows the Total Ion Chromatogram (TIC) of the cling film sample at 50 °C in 20 % ethanol with several unknown low-intensity chromatographic peaks. These peaks were not found to be any identifiable common plastic additives using the National Institute of Standards and Technology (NIST) Mass Spectral Database.

It was observed that increasing the temperature at which samples were exposed, increased the amount of leaching from the FCM to the food simulant. This is evident in Figure 5 for cling film in 20 % ethanol at 20 °C and 50 °C. Leaching was further affected by the type of food simulant. The most leaching was observed in 20 % ethanol, which simulated an alcoholic or slightly lipophilic food medium, followed by 10 % ethanol, and 3 % acetic acid (Figure 6). Therefore, leaching is likely to be higher when cling film is in contact with lipophilic foods compared with acidic foods.

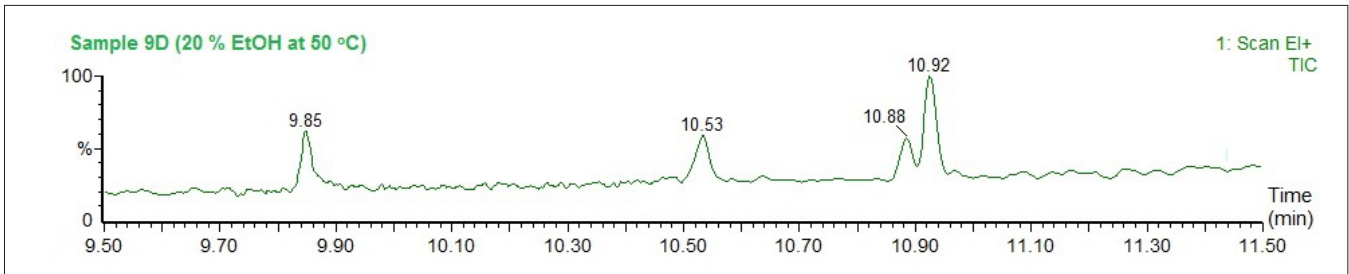


Figure 4. TIC of cling film in 20% EtOH at 50 °C.

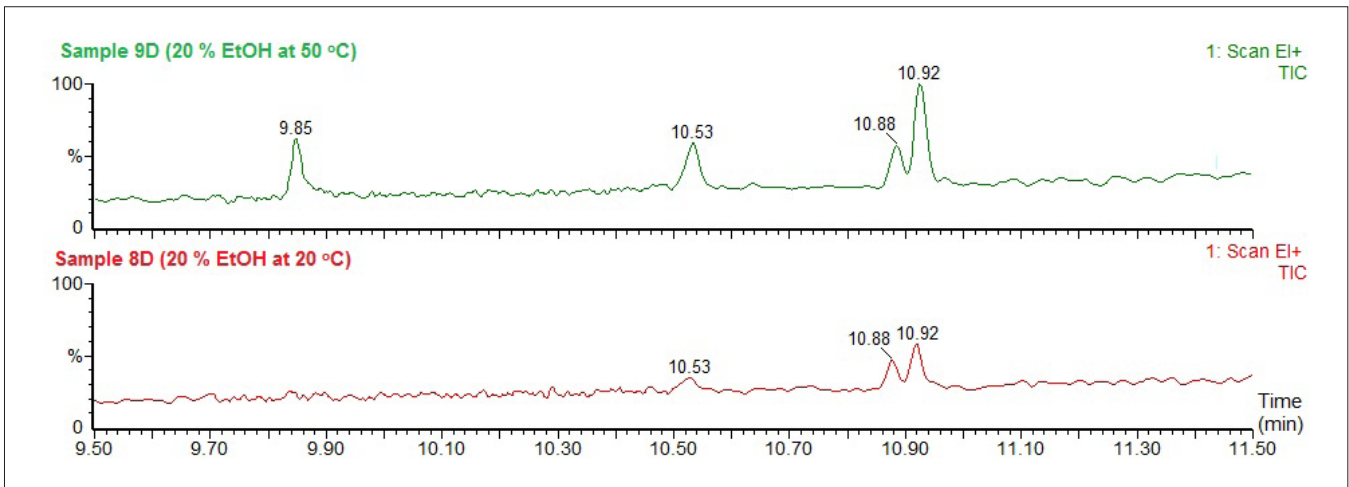


Figure 5. Effect of temperature on leaching in cling film in 20% EtOH.

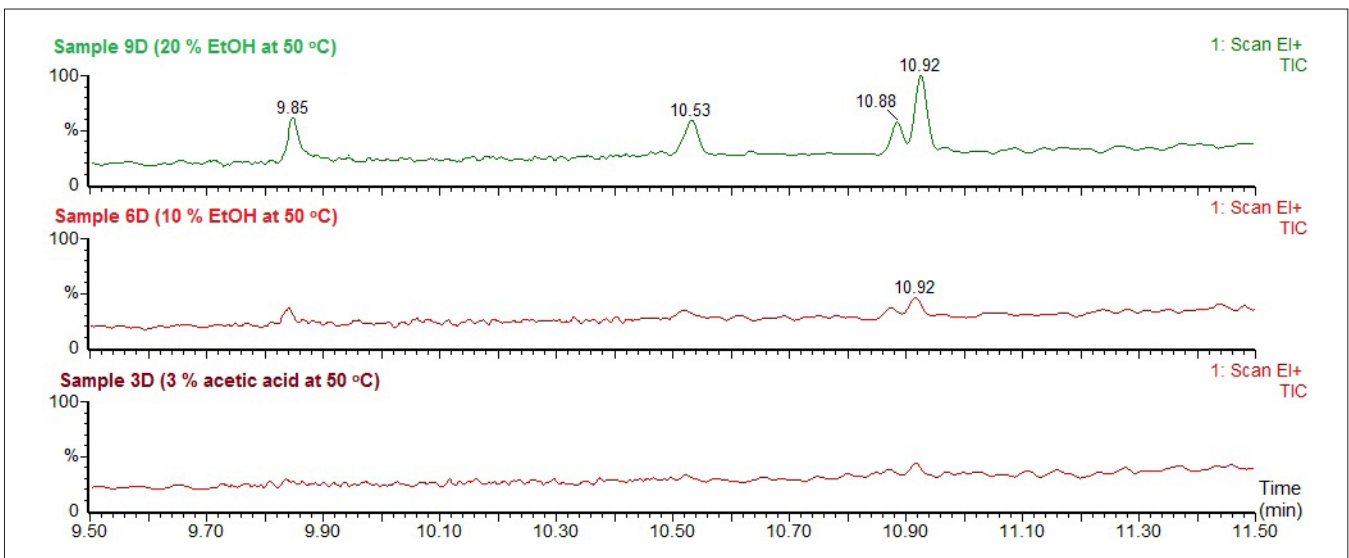


Figure 6. Effect of food simulant on leaching in cling film at 50 °C.

Conclusions

Regulations relating to safety and quality of packaged foods have placed major importance on migration testing. The use of some phthalates in FCMs, in particular ones which possess endocrine disrupting capabilities, are limited under EU regulation, thus a suitable and reliable technique is required to identify levels.

This application demonstrates the use of the PerkinElmer Clarus SQ 8 GC/MS to quantify three regulated phthalates (DBP, BBP, and DEHP), along with other common phthalates (DMP, DEP, and DnOP), from FCMs with high sensitivity and repeatability based on Regulation EU No. 10/2011. Analysing samples in SIFI MS mode, allowed for analysis of non-target compounds that may have leached into the food, whilst simultaneously obtaining high sensitivity for target compounds. The GC/MS analysis demonstrated increased leaching from FCMs at higher temperatures and more lipophilic food simulants.

References

1. C.A. Staples. (2003). "Phthalate Esters", The Handbook of Environmental Chemistry, Springer.
2. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Official Journal of the European Union.
3. <http://echa.europa.eu/documents/10162/c6781e1e-1128-45c2-bf48-8890876fa719> Date Accessed: 24th June 2016.
4. L. Nollet. (2004). "Handbook of Food Analysis: Residues and other food component analysis", Marcel Dekker, Volume 2.