Application Note

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Multiresidual Determination

Validating Specific Migrations from Packaging into Food Simulant Using LC-MS/MS

Authors

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Abstract

The objective of this work was to develop and validate an analytical method for measuring five substances present in plastic materials, and one internal standard by UHPLC-MS/MS. The substances studied were terephthalic acid (PTA), isophthalic acid (*i*-PTA), bisphenol S (BPS), bisphenol A (BPA), and benzophenone (BP)

PTA and *i*-PTA are monomers of polyethylene terephthalate (PET), while BPS and BPA are monomers of polyethersulphone (PES) and polycarbonate (PC), respectively. BPA is also used in the composition of epoxy coating for metal packaging. BP is a photoinitiator used in UV-cured printing ink. Orthophthalic acid (*o*-PTA) was chosen as the internal standard.

All of the substances have a specific migration limit, described in Brazil/Mercosur and European Union legislation, and European Standards have established individual methods for determining each substance studied.

The simulant selected for the analysis was 50 % aqueous ethanol solution (v/v), representative of dairy products and food with a concentration of ethanol up to 50 %.

For selected reaction monitoring, we used an Agilent 6460 Triple Quadrupole LC/MS system, which included an Agilent 1290 Infinity LC with Agilent Jet Stream technology. Linearity, limits of detection (LODs), and limits of quantification (LOQs) were determined, as well as the precision on different days and at three different concentrations. Recovery was carried out after 24 hours of contact at 40 °C.

The results obtained for LOD/LOQ were:

- **PTA:** 3.9/12.5 µg/kg
- ***i*-PTA:** 3.7/11.9 µg/kg
- **BPS:** 1.9/5.9 µg/kg
- **BPA:** 12.6/40.2 µg/kg
- **BP:** 7.6/24.3 µg/kg

The precision relative standard deviations (RSDs) obtained were between 1 and 9 % for repeatability. The recovery was 89 to 112 %, within the appropriate range for each concentration.

The multiresidues method that we developed can detect these substances in 8 minutes, while traditional methods are restricted to one-by-one determinations.

Introduction

Various substances used in plastic packaging materials, such as additives and monomers, and nonintentionally added substances (NIAS) formed during the process are of safety concern because they can migrate from packaging into food. Interactions between food and packaging occur, and small quantities are transferred to food. This migration should be evaluated to ensure that it is minimal and does not present consumer health concerns[1].

Terephthalic acid (PTA) and isophthalic acid (*i*-PTA) are examples of migrant compounds from polyethylene terephthalate (PET) packaging, and their harmful effects on laboratory animals have been demonstrated. The European Food Safety Authority (EFSA) has established maximum levels of *i*-PTA and PTA migration from plastics of 5 mg/kg and 7.5 mg/kg of food, respectively, and the same value is valid for Brazil/Mercosur. PET is made by polymerizing ethylene glycol with PTA or by transesterification with dimethyl terephthalate (DMT), commonly using antimony trioxide as a catalyst. Many processes incorporate the *i*-PTA as a comonomer.

PET is commonly used in the production of bottles, sheets, films, and oven-compatible food trays[1].

Bisphenol A (BPA) is widely used in the production of phenolic-epoxy resins and polycarbonate plastics, in a variety of applications such as epoxy food-can coatings and plastic food containers. During the sterilization process in cans or polycarbonate plastics, heat and contact with acidic or basic foods increase the hydrolysis of the ester bonds that link BPA molecules in the polycarbonate and epoxy resins. As a result, compounds are released to food[2]. BPA is permitted for use in food contact materials (FCMs) in the EU under Regulation 10/2011/EU, relating to plastic materials and articles intended for contact with foodstuffs having a specific migration limit (SML) of 0.6 mg/kg, or 600 µg/kg, Brazil/Mercosur adopted the same value. BPA is recognized as an endocrine disrupting chemical (EDC)[3]. In January 2011, the EU adopted Commission Directive 2011/8/EU, prohibiting the use of BPA for the manufacture of polycarbonate infant-feeding bottles[4,5]. There is an EU draft regulation to amend the specific migration limit of BPA to 0.05 mg/kg, or 50 µg/kg[6].

Bisphenol S (BPS) is also used in many industrial applications, including polycarbonate plastics and resins. It is the monomer of polyethersulfone (PES), and is also used in curing fast-drying epoxy glues[2]. Recent studies demonstrated the likelihood that BPS may have similar endocrine-disrupting characteristics as BPA[7]. Because of this, an SML of 0.05 mg/kg, or 50 µg/kg, was established for BPS in Europe and Brazil/Mercosur[4,8,9].

Photo-initiators have widely been used in packaging materials as a main component of UV inks. These compounds contain photo-sensitive groups that start the polymerization process to cure the ink by UV radiation. Benzophenone (BP) is the most used photo-initiator in UV-cured printing inks, with a final content in the ink of 5–10 %. UV inks are used to print packaging materials such as rigid plastics, multilayer laminates, cardboard, and paper. The unintentional transfer of printing-ink components from the external printed surface onto the food-contact surface can occur when the printed material is rolled on reels or stacked during storage. In Europe, specific legislation established an SML for BP of 0.6 mg/kg, or 600 µg/kg, for food-contact plastics[4].

Generally, specific migration is determined separately as established by the EN standards series 13130. However, the analysis is time-consuming, expensive, and requires a lot of solvent. Some authors have developed multiresidue methods to determine monomers, additives, and amines in different packaging materials.

Lago; *et al.*[10] determined 14 photo-initiators and amine synergists in a single run using UHPLC-DAD. The limits of detection (LODs) were ≤1.56 µg/dm^2. Different types of packaging materials were analyzed, and positive samples were confirmed by LC/MS/MS in positive electrospray ionization (ESI) mode.

Lin; *et al.*[11] developed a method using an UHPLC tandem mass spectrometer to identify and quantify two photo-initiators, nine plasticizers, three primary aromatic amines, and six types of bisphenol. The separation was performed in 12 minutes, and the validation parameters indicated that the method was effective for those substance classes.

Surma; *et al.*[12] determined 10 perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFASs) using LC/MS/MS, while Li; *et al.*[13] analyzed 16 phthalic acids in food simulants using LC/triple quadrupole mass spectrometry.

Paseiro-Cerrato; *et al.*[14] proposed a simultaneous determination of polyfunctional amines used as monomers by high-performance liquid chromatography with diode-array detection (HPLC-DAD) after derivatization with dansyl chloride. The analyses were carried out by HPLC with different detectors, although a mass spectrometer was the most widely used detector.

The objective of this study was to develop and validate a method for determining *i*-PTA (CAS 121-91-5), PTA (CAS 100-21-0), BPA (CAS 80-05-7), BPS (CAS 80-9-1), and BP (CAS 119-61-9) simultaneously and rapidly, using an ultra-high performance LC/MS/MS method. Orthophthalic acid (*o*-PTA, CAS 88-99-3) was used as the internal standard. Figure 1 shows the molecular structure of compounds analyzed in this work.

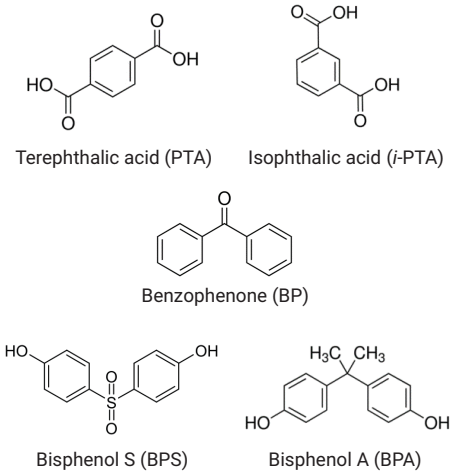

Figure 1. Molecular structure of substances present in the plastic materials analyzed in this work.

Experimental

Table 1 lists the compounds, retention times (RTs), precursor ions (*m/z*), product ions (*m/z*), dwell times, fragmentation energies, collision energies, and capillary polarites used in this study. The most intense transition was used for quantification, and the second most intense was used for confirmation of the analyte (qualification) for each compound.

Table 1. Retention time and MRM conditions of selected compounds.

Compound	RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (ms)	Fragmentor (V)	CE (V)	Polatity
o-PTA	2.9	165.0	121.0 77.1	50	70	4 12	Negative
PTA	3.3	165.0	121.0 77.1	50	80	8 16	Negative
i-PTA	3.9	165.0	121.0 77.1	50	90	8 8	Negative
BPS	4.4	249.0	108.0 92.0	50	45	24 32	Negative
BPA	6.2	227.1	212.0 133.0	200	45	12 20	Negative
BP	6.9	183.1	77.2 51.2	50	90	36 72	Positive

Ultra-high performance LC/MS/MS

LC Conditions	
Instrument	Agilent 1290 Infinity LC system
Column	Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm (p/n 959758-902)
Column temperature	40 °C
Injection volume	2 µL
Mobile phase	A) Methanol B) Water acidified with 0.1 % acetic acid
Gradient	Time (min) %A %B 0 85 15 4.2 50 50 4.5 30 80 8.01 85 15 Post time 9.0 minutes
Flow rate	0.3 mL/min
MS Conditions	
Instrument	Agilent 6460 Tandem Quadrupole LC/MS
Ion mode	AJS-ESI, negative and positive ionization
Capillary voltage	3,500 V (+) and 5,000 V (−)
Drying gas (N_2)	10 L/min
Drying gas temperature	280 °C
Nebulizer	45 psi
Sheath gas heater	300 °C
Sheath gas flow	10 L/min

For all validation parameters, the concentration of the internal standard *o*-PTA was 322.4 µg/kg, and the food simulant used was a 50 % aqueous ethanol solution (v/v). The limits of detection (LODs) and limits of quantification (LOQs) were obtained by performing seven injections from different solutions of the same concentration (56.1 µg/kg for PTA, 56.3 µg/kg for *i*-PTA, 5.1 µg/kg for BPS, 36.0 µg/kg for BPA, and 36.0 µg/kg for BP) as established by Inmetro DOQ-CGCRE-008[15].

The LOD was determined by Equation 1:

$$LOD = t_{(n-1,\ 1-\alpha)} \times s$$

Equation 1.

t = Student distribution. The value of t was 3.143 (99 % confidence interval, 6 degrees of freedom)
s = standard deviation

The LOQ was determined by Equation 2:

$$LOQ = 10 \times s$$

Equation 2.

s = standard deviation

According to established legislation[4], preparation was based on migration determination for recovery. Triplicate or duplicate samples of food simulant spiked with three different levels of analyte (92.1, 452.5, and 900.0 µg/kg for PTA, 92.6, 454.8 and 905.6 µg/kg for *i*-PTA, 40.5, 80.3 and 120.0 µg/kg for BPS, 150.0, 450.0, and 900.0 µg/kg for BPA and 149.8, 449.3, and 898.6 µg/kg for BP) were placed in an oven for 24 hours at 40 °C, simulating expected package/food contact at room temperature for 24 hours.Then we analyzed the samples.

Precision tests were performed at one-day intervals (repeatability) and three-day intervals (intermediate precision). Six or seven replicates of solution were spiked with the same quantity of analyte described for recovery, and injected into the equipment.

Results and Discussion

The LODs for PTA, *i*-PTA, BPS, BPA, and BP were 1,923, 1,351, 26, 47, and 79 times lower than the specific migration limit established by legislation, while the LOQs were 600, 420, 8, 15, and 25 times lower than the limit established by legislation (Table 2). The values obtained for BPA were in agreement with the draft of a new limit for BPA (50 µg/kg).

Recovery experiments were performed by comparing the analytical results for extracted samples with the quantities added to them. Table 3 shows the values obtained for the concentrations studied (low, medium, and high). According to Huber[16], the recovery range accepted for concentrations of 10–90 µg/kg is between 60–115 %, while recovery for concentrations of 100–1,000 µg/kg, is between 80–110 %. The recovery was 89–112 % for concentrations of 40.5–150.0 µg/kg, and 90–110 % for concentrations of 449.3–905.6 µg/kg.

Table 2. LOD and LOQ using LC/MS/MS.

Monomer	LOD (µg/kg)	LOQ (µg/kg)	Limit of specific migration (µg/kg)
PTA	3.9	12.5	7,500
i-PTA	3.7	11.9	5,000
BPS	1.9	5.9	50
BPA	12.6	40.2	600
BP	7.6	24.3	600

Table 3. Recovery using LC/MS/MS.

Monomer	Level (µg/kg)	Standard deviation	Recovery %[a]
PTA	92.1[b]	±13.1	112
	452.5[b]	±26.2	99
	900.0[b]	±49.8	106
i-PTA	92.6[b]	±9.1	105
	454.8[c]	±12.6	98
	905.6[c]	±10.6	106
BPS	40.5[b]	±2.5	108
	80.3[b]	±3.8	94
	120.0[b]	±2.9	90
BPA	150.0[b]	±18.2	109
	450.0[b]	±30.9	110
	900.0[c]	±34.7	106
BP	149.8[b]	±17.7	89
	449.3[b]	±15.5	105
	898.6[b]	±34.3	104

[a] After 40 °C for 24 hours
[b] Average of three determinations
[c] Average of two determinations

Table 4 shows that precision tests were performed at one-day intervals (repeatability), and three-day intervals (intermediate precision) with three different concentrations. According to Huber[16], the limit of relative standard deviation (RSD) expected for the concentration range of 10–90 µg/kg was 21 %, and for 100–900 µg/kg was 15 %. These values were obtained, and the results remained at 1–9 % (repeatability) and 3–15 % (intermediate precision), except for benzophenone, whose value was 23 %.

We studied the linearity of analytical curves (Table 5) using standard solutions in five concentrations for PTA ranging 60–1,000 µg/kg, six concentrations for *i*-PTA ranging 60–1,000 µg/kg, and six concentrations for BPS, BPA, and BP ranging 100–1,000 µg/kg. The coefficient of determination (R^2) calculated by linear regression showed values greater than 0.992.

Table 4. Precision (repeatability and intermediate precision) using LC/MS/MS.

Monomer	Level (µg/kg)	Repeatability RSD (%)	Intermediate precision RSD (%)
PTA	92.1	5[a]	7[a]
	452.5	2[a]	3[a]
	900.0	2[a]	3[a]
i-PTA	92.6	7[a]	9[a]
	454.8	3[a]	4[a]
	905.6	3[a]	4[b]
BPS	150.0	6[a]	13[a]
	450.0	2[a]	10[a]
	900.0	1[b]	14[b]
BPA	150.0	4[a]	15[a]
	450.0	3[a]	11[a]
	900.0	3[a]	6[a]
BP	149.8	4[a]	23[a]
	449.3	3[a]	10[a]
	898.6	9[a]	9[a]

[a] Average of seven determinations
[b] Average of six determinations

Table 5. Calibration curves and coefficients of determination.

Monomer	Calibration curve (y = ax + b)	Coefficient of determination (R^2)
PTA	0.020 + 1.229	0.998
i-PTA	0.026 + 0.869	0.997
BPS	0.012 + 0.449	0.998
BPA	0.002 + 0.025	0.995
BP	0.009 + 0.075	0.992

Figure 2 shows the multiple reaction monitoring (MRM) results obtained for *o*-PTA, PTA, *i*-PTA, BPS, BPA, and BP in spiked 50 % aqueous ethanol solution (v/v).

Conclusions

The validated method can be applied to study the specific migration of terephthalic acid (PTA), isophthalic acid (*i*-PTA), bisphenol S (BPS), bisphenol A (BPA), and benzophenone (BP) from different plastic materials, internal coatings of cans, and photo-initiators used in UV printing inks. The method is rapid, taking eight minutes to separate PTA, *i*-PTA, BPS, BPA, and BP, including the internal standard *o*-PTA using the Agilent 1290 Infinity LC system combined with the Agilent 6460 Tandem Quadrupole LC/MS system. The LOD/LOQ results were compatible with the specific migration limits of 7,500 µg/kg (PTA), 5,000 µg/kg (*i*-PTA), 600 µg/kg (BPA and BP), 50 µg/kg (BPS) that have been established by legislation. Precision measured as %RSDs were between 1–9 % (repeatability). The recovery was between 89–112 %, within the expected range for each concentration. The method is simple, quick, and showed linear calibration curves.

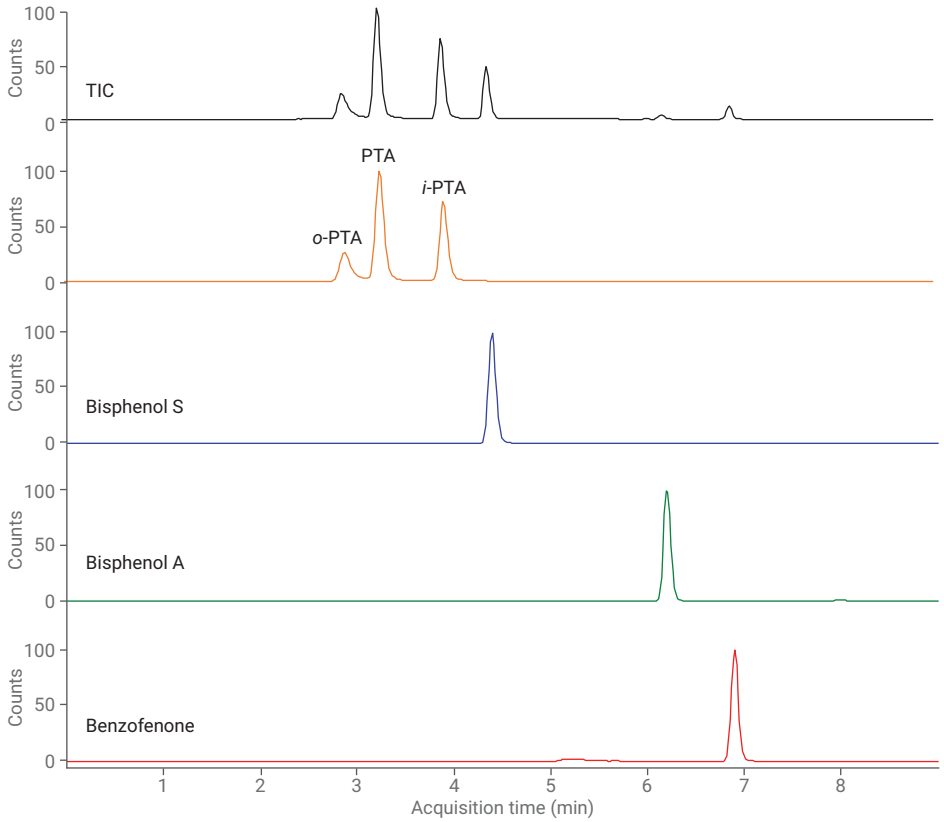

Figure 2. LC/MS/MS chromatogram of monomers in spiked 50 % aqueous ethanol solution (v/v). Concentration of analytes: *o*-PTA: 322.4 µg/kg; PTA: 1,001 µg/kg; *i*-PTA: 1,006 µg/kg; BPS: 1,000 µg/kg; BPA: 1,000 µg/kg; BP: 999 µg/kg.

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