Sensitive and precise quantitation of 29 phthalate esters in food simulants and beverages

Using the QTRAP 4500 system from SCIEX

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Introduction

This technical note describes a sensitive, accurate and precise LC-MS/MS method for analyzing 29 phthalate esters (PEs) in food simulants and beverages. Contamination controls, optimized chromatographic separation of isomers and the sensitivity of the QTRAP 4500 system enabled the method to achieve limits of quantitation (LOQs) in the range of sub-to-low µg/kg for 29 PEs analyzed in food simulants.

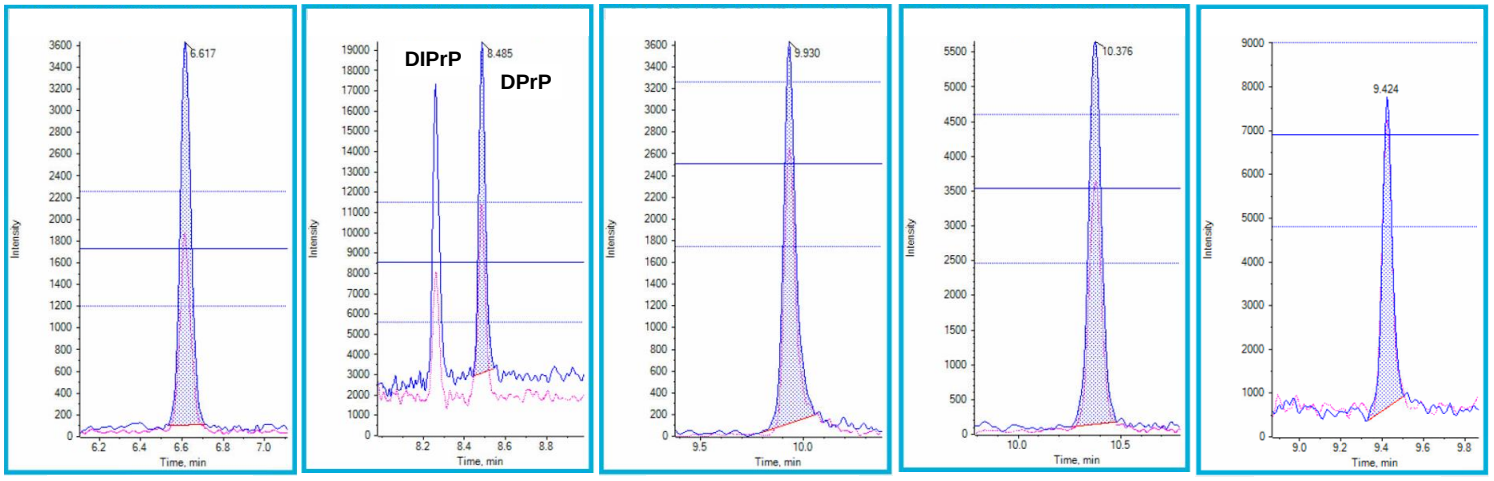
PEs are synthetic chemicals that are added to plastic food and beverage packaging to increase its flexibility and prolong its durability.[2] However, PEs can migrate into food and beverages through direct contact with the packaging materials, resulting in potential dietary exposure. Due to the toxicological risks of these chemicals to human health, the European Commission has established specific migration limits (SMLs) for several PEs as leachable components from plastic food contact materials.[1]

Analysis of PEs is challenged by the complexity of food and beverage matrices. This is further complicated by their ubiquity in plastic labware and consumables, which negatively impacts LOQs. Here, chromatographic conditions were optimized to achieve baseline resolution of isomers that typically coelute.

Coupled with rigorous controls to minimize background contamination and the sensitivity of the QTRAP 4500 system, 29 PEs were accurately quantified with excellent precision in 4 food simulants and a juice sample, even at the LOQ level (Figure 1).

Key features of the QTRAP 4500 system for the analysis of PEs

- The sensitivity of the QTRAP 4500 system enabled the use of a rapid extraction protocol and an LC-MS/MS method that achieved in-vial LOQs of 0.025–15 ng/mL based on the lowest calibration standard used for each PE
- Effective chromatographic separation of PE isomers permitted the individual and summed concentrations of isomers to be reported
- A single solvent-based calibration curve (0.025–75 ng/mL, $r \geq 0.99$) was applied to the analysis of PEs in 4 food simulants and a juice sample with minimal matrix effects observed for most of the PEs tested
- Acceptable accuracies (70–130%) and precision values (%CV <30%) were achieved for all 29 PEs spiked at their corresponding LOQs and for the quality control spikes prepared in 4 food simulants and a juice sample



DMEP 0.1 ng/mL | **DPrP 0.05 ng/mL** | **DBzP 0.25 ng/mL** | **DCHP 0.05 ng/mL** | **BBP 0.1 ng/mL**

Figure 1. Detection of dimethoxyethyl phthalate (DMEP), dipropyl phthalate (DPrP), dibenzyl phthalate (DBzP), dicyclohexyl phthalate (DCHP) and butylbenzyl phthalate (BPP) at their corresponding limits of quantitation (LOQ) in food simulant D1. The blue trace represents the quantifier transition and the pink trace represents the qualifier transition of each analyte. The horizontal lines represent the ion ratio tolerance range. Chromatographic separation was achieved for the isomers of dipropyl phthalate (DPrP) and diisopropyl phthalate (DIPrP).

Experimental methods

Chemicals and samples: The target analyte list included 29 PEs. Individual neat standards were combined to prepare intermediate stock solutions from which calibration standards (0.025–75 ng/mL) were prepared in 50% (v/v), ethanol in water.

Sample preparation of food simulants: Quality control (QC) spikes were prepared by spiking the 4 food simulants (Table 1) at 5 ng/mL. QC spikes prepared in food simulants A, B and C were spiked at 2x the spiking concentration, then diluted 1:1 with ethanol to improve PE solubility before LC-MS/MS analysis. The QC spike prepared in food simulant D1 was analyzed without dilution.

Table 1. Food simulants listed in EU regulation 10/2011 for migration testing.

Food simulant	Composition	Dilution Ratio
Simulant A	*10% (v/v), ethanol in water*	*1:1 with ethanol*
Simulant B	*3% (v/v), acetic acid in water*	*1:1 with ethanol*
Simulant C	*20% (v/v), ethanol in water*	*1:1 with ethanol*
Simulant D1	*50% (v/v), ethanol in water*	*No dilution*

Sample preparation of juice: A 1 g sample of juice was combined with 10 mL of acetonitrile in a glass centrifuge tube and vortexed for 1 minute. After centrifuging at 2000 rpm for 10 minutes, the supernatant was collected, spiked at 5 ng/mL and an aliquot was transferred to an autosampler vial for LC-MS/MS analysis.

Contamination control: Due to the ubiquity of PEs in the laboratory environment, additional cleaning of all glassware was implemented and the use of plastic materials was minimized to reduce the background contamination of PEs and other interfering compounds. Procedural blanks were evaluated to assess the baseline background of known problematic compounds, such as dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), bis-(2-ethylhexyl) phthalate (DEHP), di-*n*-octyl phthalate (DnOP) and diisooctyl phthalate (DIOP).

Chromatography: LC separation was performed on an ExionLC AD system using a Phenomenex Kinetex Biphenyl column (100 x 3 mm, 2.6 µm) fitted with an Agela Ghost Hunter pre-column (50 x 4.6 mm). A flow rate of 0.425 mL/min, an injection volume of 3 µL and a column temperature of 25°C were used. The 20-minute gradient used is presented in Table 2.

Table 2. Chromatographic gradient.

Time (min)	%A	%B
0.01	*60*	*40*
2.67	*30*	*70*
4.80	*30*	*70*
5.33	*10*	*90*
13.00	*10*	*90*
13.20	*60*	*40*
20.00	*60*	*40*

Mobile phase A: *Water with 0.5% (v/v) acetic acid*
Mobile phase B: *Methanol*

Mass spectrometry: Analysis was performed using the QTRAP 4500 system with a Turbo V ion source in positive electrospray ionization mode. All 29 PEs were individually infused into the mass spectrometer to optimize the declustering potential (DP), collision energy (CE) and collision exit potential (CXP) for each analyte. Data were acquired in scheduled multiple reaction monitoring (sMRM) mode with a 1-minute detection window around the retention time (RT) of each analyte and a target cycle time of 1 second. Table 3 shows the source parameters for the mass spectrometer and Table 4 shows the list of 29 PEs with their corresponding MRM transitions and compound-dependent parameters. Figure 2 shows the overlaid extracted ion chromatograms (XICs) of all 29 PEs based on their quantifier transitions in a 5 ng/mL calibration standard.

Table 3. Source, gas and temperature conditions.

Parameter	Value
Curtain gas (CUR)	*40 psi*
Collision gas (CAD)	*Medium*
IonSpray voltage (ISV)	*5500 V*
Temperature (TEM)	*450°C*
Nebulizer gas (GS1)	*40 psi*
Heater gas (GS2)	*50 psi*

Data processing: All data were processed using SCIEX OS software, version 2.1.6. Linear regression of calibration curves was performed based on a linear regression with a weighting of 1/x. Data from the isomer groups that were not chromatographically separated were processed in the Analytics module of SCIEX OS software using a single transition (bolded in Table 4) to collectively represent all the unseparated isomers. The resulting concentration was corrected with a multiplier based on the number of isomers that coeluted.

Table 4. List of target analytes, their abbreviations, MRM transitions and compound-dependent parameters.

#	Compound	Abbreviation	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)	EP (V)
1	***Bis-(2-ethylhexyl) phthalate***	***DEHP***	***391.3***	***149.0***	***50***	***29***	***10***	***10***
2	*Di-n-octyl phthalate*	*DnOP*	*391.3*	*149.0*	*50*	*20*	*10*	*10*
3	*Disooctyl phthalate*	*DIOP*	*391.2*	*149.3*	*54*	*31*	*12*	*10*
4	***Dinonyl phthalate***	***DNP***	***419.3***	***149.1***	***50***	***22***	***17***	***10***
5	*Diisononyl phthalate*	*DINP*	*419.3*	*148.9*	*45*	*29*	*16*	*10*
6	*Dibutyl phthalate*	*DBP*	*279.1*	*149.2*	*35*	*20*	*11*	*10*
7	*Diisobutyl phthalate*	*DIBP*	*279.1*	*205.1*	*33*	*10*	*15*	*10*
8	***Diisodecyl phthalate***	***DIDP***	***447.4***	***149.1***	***56***	***36***	***14***	***10***
9	*Dipropylheptyl phthalate*	*DPrHP*	*447.4*	*149.1*	*46*	*36*	*10*	*10*
10	*Bis(4-methyl-2-pentyl) phthalate**	*BMPP*	*335.2*	*149.0*	*37*	*35*	*18*	*10*
11	*Bis-methylpentyl phthalate**	*DMPP*	*335.2*	*233.2*	*39*	*11*	*15*	*10*
12	***Dihexyl phthalate****	***DHxP***	***335.2***	***149.2***	***40***	***28***	***12***	***10***
13	*Butyloctyl phthalate**	*BOP*	*335.2*	*149.0*	*39*	*21*	*11*	*10*
14	*Dipropyl phthalate*	*DPrP*	*251.1*	*149.0*	*30*	*19*	*11*	*10*
15	*Diisopropyl phthalate*	*DIPrP*	*251.1*	*149.0*	*45*	*29*	*18*	*10*
16	*Dipentyl phthalate*	*DPP*	*307.2*	*149.2*	*29*	*18*	*14*	*10*
17	*Diisopentyl phthalate*	*DIPtP*	*307.1*	*219.2*	*34*	*11*	*15*	*10*
18	***Diheptyl phthalate***	***DHtP***	***363.2***	***149.1***	***43***	***21***	***16***	***10***
19	*Diisoheptyl phthalate*	*DIHP*	*363.3*	*149.3*	*52*	*26*	*18*	*10*
20	*Diphenyl phthalate*	*DPhP*	*319.0*	*225.1*	*79*	*16*	*15*	*10*
21	*Bis(2-n-butoxyethyl) phthalate*	*DBEP*	*367.2*	*249.2*	*63*	*11*	*14*	*10*
22	*n-butyl phthalyl n-butyl glycolate*	*BPBG*	*337.2*	*149.1*	*50*	*21*	*14*	*10*
23	*Dimethyl phthalate*	*DMP*	*195.1*	*163.2*	*22*	*12*	*11*	*10*
24	*Diethyl phthalate*	*DEP*	*223.1*	*177.1*	*24*	*12*	*15*	*10*
25	*Dimethoxyethyl phthalate*	*DMEP*	*283.2*	*207.1*	*34*	*10*	*16*	*10*
26	*Diallyl phthalate*	*DAP*	*247.2*	*189.0*	*24*	*11*	*16*	*10*
27	*Butylbenzyl phthalate*	*BBP*	*313.2*	*205.2*	*42*	*11*	*14*	*10*
28	*Dicyclohexyl phthalate*	*DCHP*	*331.1*	*149.0*	*45*	*32*	*17*	*10*
29	*Dibenzyl phthalate*	*DBzP*	*347.1*	*181.3*	*44*	*13*	*16*	*10*

Note: Compounds in the non-separated isomer groups were quantified using the bolded transition in each group.
Note: Compounds denoted with an asterisk (Compound 10–13) belong to the same isomer group. Chromatographic separation was achieved for BMPP and DMPP from DHxP and BOP, but not between DHxP and BOP.

Separated isomer group

Non-separated isomer group

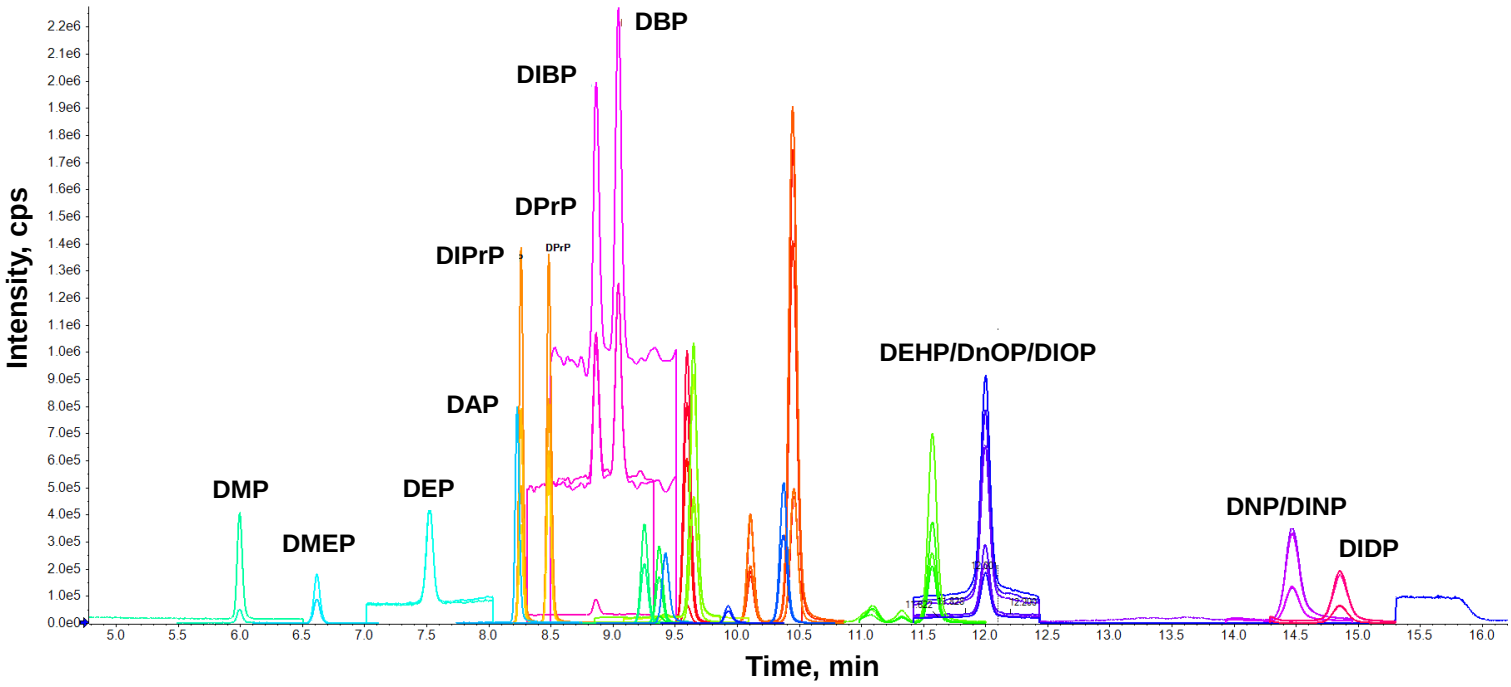


Figure 2. Overlaid extracted ion chromatograms (XICs) of the quantifier transitions of all 29 PEs in a 5 ng/mL standard.

Chromatographic separation of PE isomers

The column selection and gradient conditions were optimized to chromatographically separate as many PEs as possible within the 8 isomer groups, as shown in Table 4. Separation was achieved for 8 compounds in the 4 isomer groups highlighted in green. The remaining non-separated isomers, highlighted in orange, were quantified using the summed concentrations of the PEs within each isomer group. Isomer separation is not required by the EU regulation for individual reporting, however, the ability to separate PE isomers enabled lower LOQs to be achieved.

Calibration performance and LOQ

A calibration curve ranging from 0.025 to 75 ng/mL was prepared in 50% (v/v) ethanol in water, also used as food simulant D1. Excellent regression ($r \geq 0.99$) was achieved for all analytes tested (Table 5), however, the linear dynamic range was constrained for some compounds due to high background levels, as will be discussed below. This calibration curve was applied to the analysis of PEs in the 4 food simulants and a juice sample. Select compounds required a solvent-matched composition in the calibration standards to correct for matrix effects.

The in-vial LOQs ranged from 0.025 to 15 ng/mL based on the lowest calibration standard meeting acceptance criteria for accuracy (±30%), precision (%CV <30%) and ion ratio (±30%) (Table 5, blue highlighted data). Expressed on a per mass basis using the food simulant density of 0.91 g/mL and a 1 mL sample size, the LOQs ranged from 0.027 to 16 µg/kg. Several analytes, such as DEHP+DnOP+DIOP, DEP, DBP and DIBP, were impacted by blank contamination and high baselines were observed in their XICs (Figure 2). These interferences yielded higher LOQs for these PEs. Extensive measures, such as frequent cleaning of glassware and elimination of plastic materials, were employed to control laboratory contamination.

Method performance in food simulant spikes

As specified in the EU regulation, the 4 food simulants listed in Table 1 reflect different properties of food, such as hydrophilicity, acidity and lipophilicity, and are used to test the migration of substances that leach from food contact materials. The food simulant QC samples were spiked at 5 ng/mL of each PE, which corresponded to approximately 5 µg/kg by assuming a density of 1 g/mL across all 4 food simulants. Most of the PEs exhibited acceptable average accuracies (80–120%) and precision values in all 4 food simulants at this spiking concentration (Table 5, green highlighted data). Some PEs, including DBP, DPhP, BPBG and DEP, were impacted by matrix effects, resulting in average accuracies that exceeded 130%, as denoted by an asterisk (Table 5). The use of matrix-matched calibration standards resulted in acceptable accuracies for the impacted PEs. Only the results for *n*-butyl phthalyl *n*-butyl glycolate (BPBG) will be described in detail.

Table 5. Linear range, correlation coefficient (*r*), average accuracy and precision (%CV) at the calibration LOQ prepared in food simulant D1 and QC spikes in 4 food simulants and a juice sample. The number of replicates corresponds to the number of injections of each spike.

#	Compound	Range (*r*)	Accuracy (%CV)								
			Calibration LOQ in food simulant D1 (*n* = 2)		QC spike in 4 food simulants (*n* = 6)					QC spike in juice (*n* = 6)	
			Conc (ng/mL)	Calibration LOQ	Conc (ng/mL)	Sim A	Sim B	Sim C	Sim D1	Conc (mg/kg)	Juice
1	DEHP DnOP DIOP	15–75 (0.992)	15	101 (2.6)	15	103 (8.4)	100 (6.7)	90.5 (8.2)	109 (6.1)	0.05	100 (3.4)
2	DNP DINP	1–50 (0.998)	1	104 (0.5)	10	130 (1.3)	126 (0.7)	117 (1.3)	108 (0.5)	0.05	93.4 (1.6)
3	DBP	5–25 (0.996)	5	97.6 (7.9)	5	155* (17.2)	115 (5.4)	104 (3.7)	102 (4.3)	0.05	93.5 (5.1)
4	DIBP	5–25 (0.996)	5	95.4 (6.3)	5	130 (11.2)	113 (8.1)	103 (2.8)	98.9 (3.4)	0.05	91.4 (2.5)
5	DIDP DPrHP	1–50 (0.998)	1	103 (0.3)	10	124 (1.5)	118 (1.8)	115 (1.9)	107 (0.8)	0.05	90.6 (1.4)
6	BMPP	0.025–25 (0.999)	0.025	84.8 (3.7)	5	124 (1.5)	118 (2.2)	113 (1.7)	108 (0.9)	0.05	92.2 (1.2)
7	DMPP	0.25–25 (0.999)	0.25	104 (2.9)	5	124 (1.3)	121 (0.9)	112 (0.6)	106 (1.0)	0.05	92.8 (0.9)
8	DHxP BOP	0.05–50 (0.999)	0.05	98.6 (1.9)	10	128 (0.7)	123 (1.1)	115 (0.5)	108 (1.2)	0.05	95.1 (0.7)
9	DPrP	0.05–25 (0.999)	0.05	90.6 (1.9)	5	130 (0.5)	120 (1.2)	116 (1.1)	109 (0.9)	0.05	98.9 (0.7)
10	DIPrP	0.05–25 (0.990)	0.05	98.9 (3.2)	5	124 (0.7)	115 (1.0)	113 (1.2)	106 (0.8)	0.05	101 (1.5)
11	DPP	0.25–25 (0.999)	0.5	95.1 (4.0)	5	124 (1.3)	121 (0.9)	113 (0.8)	106 (1.4)	0.05	90.8 (1.1)
12	DIPtP	5–25 (0.997)	5	101 (3.7)	5	124 (2.0)	117 (2.3)	94.3 (3.9)	100 (4.5)	0.05	84.3 (3.3)
13	DHtP DIHP	0.05–50 (0.999)	0.1	98.2 (2.2)	10	127 (1.0)	123 (0.7)	116 (0.7)	108 (1.0)	0.05	90.0 (1.4)
14	DPhP	2.5–25 (0.996)	2.5	105 (6.0)	5	141* (6.4)	128 (3.2)	112 (8.3)	109 (5.8)	0.05	91.2 (3.4)
15	DBEP	0.025–25 (0.999)	0.05	102 (5.5)	5	121 (1.5)	117 (1.4)	108 (0.8)	103 (1.4)	0.05	99.7 (1.0)
16	BPBG	0.25–25 (0.993)	0.25	76.0 (0.4)	5	158* (1.4)	157* (0.9)	131* (1.3)	115 (1.0)	0.05	130 (2.7)
17	DMP	0.25–25 (0.999)	0.5	86.0 (1.7)	5	123 (2.8)	117 (2.7)	111 (1.1)	111 (0.5)	0.05	100 (1.5)
18	DEP	2.5–25 (0.997)	2.5	106 (1.2)	5	133* (9.4)	120 (6.4)	106 (1.2)	109 (1.5)	0.05	107 (1.8)
19	DMEP	0.1–25 (0.999)	0.1	103 (0.04)	5	118 (0.9)	116 (1.7)	111 (0.9)	103 (0.9)	0.05	100 (1.8)
20	DAP	0.05–25 (0.999)	0.05	94.2 (3.6)	5	127 (1.4)	121 (1.1)	112 (1.1)	104 (1.4)	0.05	103 (1.3)
21	BBP	0.1–25 (0.999)	0.1	120 (0.9)	5	122 (1.1)	115 (1.4)	103 (0.7)	105 (1.4)	0.05	84.3 (2.0)
22	DCHP	0.05–25 (0.999)	0.05	110 (3.6)	5	124 (0.5)	116 (1.4)	112 (1.1)	105 (0.9)	0.05	90.3 (0.6)
23	DBzP	0.25–25 (0.998)	0.25	122 (2.0	5	122 (3.6)	121 (2.4)	111 (1.9)	103 (2.1)	0.05	95.9 (0.7)

Note: All results met the acceptance criteria for precision (%CV <30%). Accuracies above 130% are denoted with an asterisk.
Note: All samples were quantified using the calibration standards prepared in food simulant D1, unless otherwise noted. The compounds that yielded accuracies >130% were re-analyzed using a solvent-matched calibration curve.
Note: The LOQ was determined based on S/N > 10, calibration curve linearity r > 0.99, accuracy ±30%, %CV <30% and ion ratio confidence ±30%. An exception was made for the ion ratio of DAP, due to the weaker ionization of the qualifier ion of this compound.

Resolving matrix effects for BPBG in food simulants

The accuracies of BPBG were consistently above 130% in QC spikes prepared in food simulants A, B and C (Table 5). Re-analysis of these QC spikes using calibration standards prepared in a matched solvent composition improved the average accuracies, as shown for food simulant B in Table 6.

Table 6. Comparison of average accuracies and %CV of QC spike in food simulant B analyzed using calibration standards prepared in 50% (v/v) ethanol in water (food simulant D1) and 3% (v/v) acetic acid in water (food simulant B).

Compound	Conc (ng/mL)	50% (v/v), ethanol in water (Food simulant D1)		3% (v/v), acetic acid in water (Food simulant B)	
		Average Accuracy	% CV	Average Accuracy	% CV
BPBG	5.00	157	0.86	114	2.31

Method applicability in a real juice sample

Application of the method to the analysis of a locally purchased juice sample revealed no detection of any PEs above their corresponding LOQs. Following the sample preparation, the juice extract was spiked at 5 µg/kg and injected 6 times. Excellent method performance was observed for the juice sample, as demonstrated by accuracies of 80–130% and %CV <5% (Table 5, orange highlighted data). Representative XICs of BMPP in a calibration standard and a spiked juice extract are shown in Figure 3.

Conclusions

- An accurate and precise LC-MS/MS method was developed using the QTRAP 4500 system for the quantitation of 29 PEs. Calibration LOQs of 0.025–15 ng/mL were achieved.
- Chromatographic separation was achieved for 7 groups of isomers, which enabled improved quantitation at lower LOQs
- The method achieved acceptable performance for linearity ($r \geq 0.99$), accuracy (80–130%) and precision (%CV <30%) in 4 food simulants and a juice sample
- Frequent cleaning of glassware and elimination of plastic material controlled the contamination of DEHP+DnOP+DIOP, DEP, DBP and DIBP in blank samples, enabling lower LOQs to be achieved, despite their high chromatographic baselines

References

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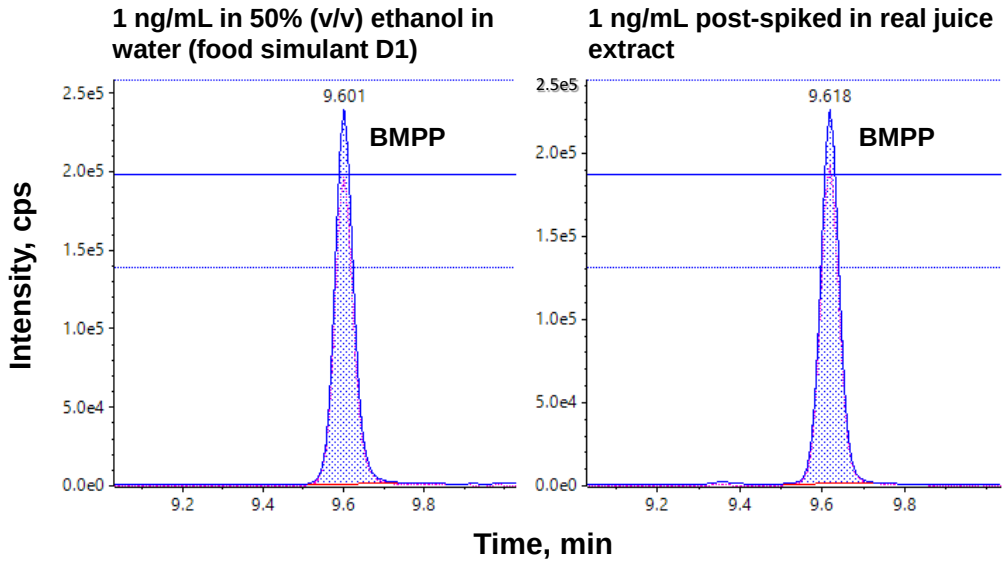

Figure 3. XICs of the quantifier transitions of BMPP in a 1 ng/mL calibration standard prepared in food simulant D1 and 1 ng/mL post-spiked juice extract.

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