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A Simple, Fast, and Reliable LC-MS/MS Method for Determination and Quantification of Phthalates in Distilled Beverages

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APPLICATION BENEFITS

- Separation and detection of 7 phthalates in 11 minutes.
- Simple, quick "dilute and shoot" sample preparation method for routine applications where high sample throughput is required.
- Elimination of major phthalate contamination using the ACQUITY UPLC® Isolator Column.
- Selective mass spectra obtained for all seven phthalates with dominant precursor ion.
- Low limits of quantification achieved using Waters® ACQUITY UPLC H-Class System and Xevo® TQD.
- Single and robust method for a variety of distilled beverages.
- Maintain consumer confidence and ensure compliance in market.

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

Xevo TQD

ACQUITY UPLC BEH Column

ACQUITY UPLC Isolator Column

KEY WORDS

Phthalates, whisky, whiskey, brandy, gin, tequila, distilled beverages, spirits, DEHP, DBP, DINP, BBP, DMP, DNOP, DEP

INTRODUCTION

Phthalates, esters of phthalic acid, are often used as plasticizers for polymers such as polyvinylchloride. They are widely applicable in various products including personal care goods, cosmetics, paints, printing inks, detergents, coatings, and food packaging. These phthalates have been found to leach readily into the environment and food as they are not chemically bound to plastics. As such they are known to be ubiquitously present in our environment.

Phthalates have been reported to show a variety of toxic effects related to reproduction in animal studies, which has resulted in these compounds being considered as endocrine disruptors. Screening food and beverages for phthalates contamination is required by many legislative bodies, although regulations vary from country to country in regards to acceptable daily tolerances and specific migration limits.

Traditionally, phthalates have been analyzed by gas chromatography-mass spectrometry (GC-MS), where derivatization and/or extraction and sample preparation is often required to improve chromatographic separation.[1] The resulting GC-EI-MS spectra can lack selectivity, where the base ion, used for identification and quantification of many common phthalates is the non-selective *m/z* 149 ($C_8H_5O_3$) ion. Furthermore, background contamination of phthalates remains a significant challenge due to their ubiquitous presence.

In this application note phthalates were separated on a reversed-phase column within 11 minutes using UltraPerformance Liquid Chromatography (UPLC®) coupled to a tandem quadrupole mass spectrometer. In order to assess the method's applicability, various brands of distilled spirits were tested. Repeated injections of the samples were made to evaluate method robustness over a number of days. Limits of detection (LOD) and quantification (LOQ) of seven key phthalates (DEHP, BBP, DBP, DNOP, DEP, DMP, and DINP) in the sample will be presented.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC H-Class
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Total run time:	11.0 min
Column:	ACQUITY UPLC Isolator, 2.1 x 50 mm (p/n 186004476)
Needle wash:	90% Methanol + 0.1 % formic acid
Purge solvent:	10% Methanol
Seal wash:	10% Methanol

Time (min)	%A	%B	Curve
Initial	60	40	6
0.5	60	40	6
5.0	1	99	6
8.0	1	99	6
8.5	60	40	6
11.0	60	40	6

MS conditions

MS System:	Xevo TQD
Ionization mode:	ESI +
Capillary voltage:	0.5 kV
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas:	1000 L/hr
Acquisition:	Multiple Reaction Monitoring (MRM)

Standards

Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), and diiso-nonyl phthalate (DINP) were purchased from Sigma-Aldrich. The stock solutions for each phthalate were prepared at 1 mg/mL in methanol.

Samples

The samples analyzed in this application note consisted of an assortment of various distilled beverages purchased in the U.S.A. The sample description for each of the six samples is listed in Table 1.

	Sample name	Type
1.	Sample A	Brandy (Brand A)
2.	Sample B	Gin
3.	Sample C	Whisky (Brand A)
4.	Sample D	Brandy (Brand B)
5.	Sample E	Tequila
6.	Sample F	Whisky (Brand B)

Table 1. List of distilled beverages.

Sample preparation

The distilled beverage samples were diluted 1:1 with water and placed in LCGC Certified Glass 12 x 32 mm Screw Neck Total Recovery Vials (p/n 186000384C) for LC-MS/MS analysis.

For the matrix matched spiked calibration, Sample F was spiked with seven phthalates at 5, 10, 20, 50, and 100 ppb (µg/L) and diluted 1:1 with water. It is important to note that the water used for sample preparation should be screened for phthalates. Any background contamination from the water will add to any response in the sample and must be accounted for.

Data acquisition and processing

The data was acquired using MassLynx® Software, and processed using TargetLynx™ Application Manager.

For all compounds, two MRM transitions were acquired for quantification and confirmation purposes. The MRM transitions, cone voltages, and collision energies for all compounds, along with expected retention times are shown in Table 2.

Name	Retention time (min.)	Parent mass *m/z*	Cone voltage (V)	Daughter 1 m/z	Collision energy 1 (V)	Daughter 2 *m/z*	Collision energy 2 (v)
DMP	1.37	195	15	133.0	20	163.0	10
DEP	3.14	223.1	20	149.1	20	177.1	10
DBP	5.01	279.1	15	149.0	15	208.0	5
BBP	4.97	313.2	10	149.0	10	205.1	5
DEHP	6.50	391.3	25	113.1	10	167.0	15
DNOP	6.62	391.3	30	149.0	15	261.2	10
DINP	6.73	419.4	35	127.1	35	149.0	20

Table 2. List of phthalates with MRM transitions and retention times.

RESULTS AND DISCUSSION

The chemical formula, mass (M+H+), and structure of all phthalates are shown in Table 3. The separation of the seven phthalates was achieved using the ACQUITY UPLC H-Class System in 11 minutes. The retention times of all phthalates are shown in Table 2.

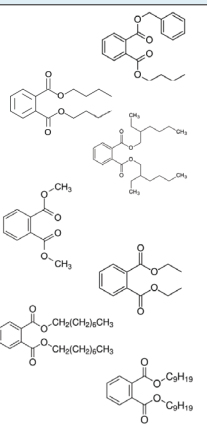
Name	CAS No.	Chemical Formula	*m/z* ($M+H^+$)	Structure
Benzylbutyl phthalate (BBP)	85-68-7	$C_{19}H_{20}O_4$	313.2	
Dibutyl phthalate (DBP)	84-74-2	$C_{16}H_{22}O_4$	279.1	
Bis (2-ethylhexyl) phthalate (DEHP)	117-81-7	$C_{24}H_{38}O_4$	391.3	
Dibutyl phthalate (DMP)	131-11-3	$C_{10}H_{10}O_4$	195.0	
Diethyl phthalate (DEP)	84-66-2	$C_{12}H_{14}O_4$	223.0	
DI-n-octyl phthalate (DNOP)	117-84-0	$C_{24}H_{38}O_4$	391.3	
Di iso-nonyl phthalate (DINP)	28553-12-0	$C_{26}H_{42}O_4$	419.4	

Table 3. Chemical formula, mass, and structure of all seven phthalates that were studied.

Due to the ubiquitous contamination of phthalates in the environment, the background response from phthalates can interfere during sample analysis. In order to remove this background interference, an ACQUITY UPLC Isolator Column (p/n 186004476) with an extension tube that was placed in the flow path between the mobile phase mixer and the sample manager injector. Background phthalates are retained on the Isolator Column until the gradient elutes them through the analytical column. In this way, the phthalates from the sample elute earlier than the background phthalates. Hence the Isolator Column separates any background phthalate response from the phthalates in the sample to be analyzed. In addition, the peak shape of the phthalates from the background is much broader than those coming from the sample. Figure 1 shows an example of the separation of background phthalate contamination from the analyte of interest (BBP) in a solvent standard.

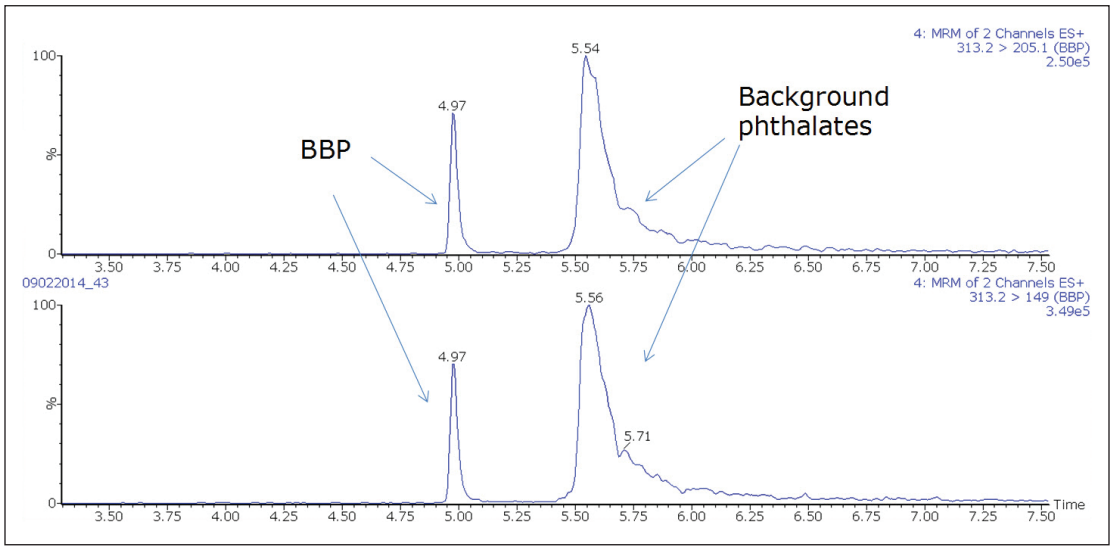


Figure 1. The ACQUITY UPLC Isolator Column separates BBP and background phthalates in the solvent standard.

Figure 2 shows a chromatogram of each of the phthalates spiked in whisky sample F at 100 ppb (µg/L). All of the phthalates including the pair of isomers (DEHP and DNOP) were easily separated in less than 10 minutes. As discussed previously, the smaller background peaks in the chromatograms of DEP, DEHP/DNOP, and BBP are background contamination separated from the analytes of interest using the ACQUITY UPLC Isolator Column.

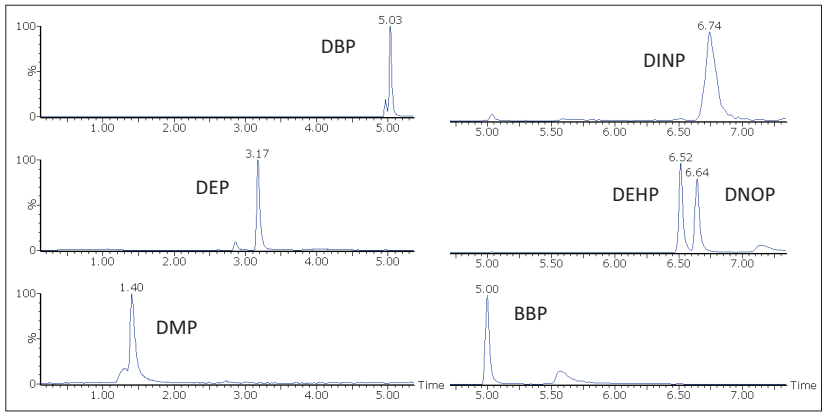


Figure 2. Separation of all the seven phthalates tested at 100 ppb (µg/L) in sample F.

Linearity

Sample F (whisky brand B) was chosen for the matrix spiked calibration curve. Sample F was pre-spiked with the seven phthalates at concentrations of 5, 10, 20, 50, and 100 ppb, then diluted 1:1 prior to analysis. Figure 3 shows the matrix spiked calibration curve for all of the phthalates in sample F. The co-efficient of determination (r^2) of each calibration curve was >0.99 for all phthalates in sample F (Figure 3), and also in a solvent calibration curve (data not shown).

Limit of detection (LOD) and quantification (LOQ):

All phthalates were spiked into sample F at various levels (1 to 10 ppb) to determine LOD and LOQ. With the exception of DINP, all phthalates were easily detected at 1 ppb with a signal-to-noise (S/N) ratio >10. For DINP, the LOD and LOQ limits were based on the matrix spiked calibration standard that provided a signal-to-noise ratio >3 and >10, respectively. These are shown in Table 4 for sample F.

Name	LOD	LOQ
DMP	1 ppb	1 ppb
DEP	1 ppb	1 ppb
DBP	1 ppb	1 ppb
BBP	1 ppb	1 ppb
DEHP	1 ppb	1 ppb
DNOP	1 ppb	1 ppb
DINP	5 ppb	10 ppb

Table 4. LOD and LOQ of all phthalates in sample F.

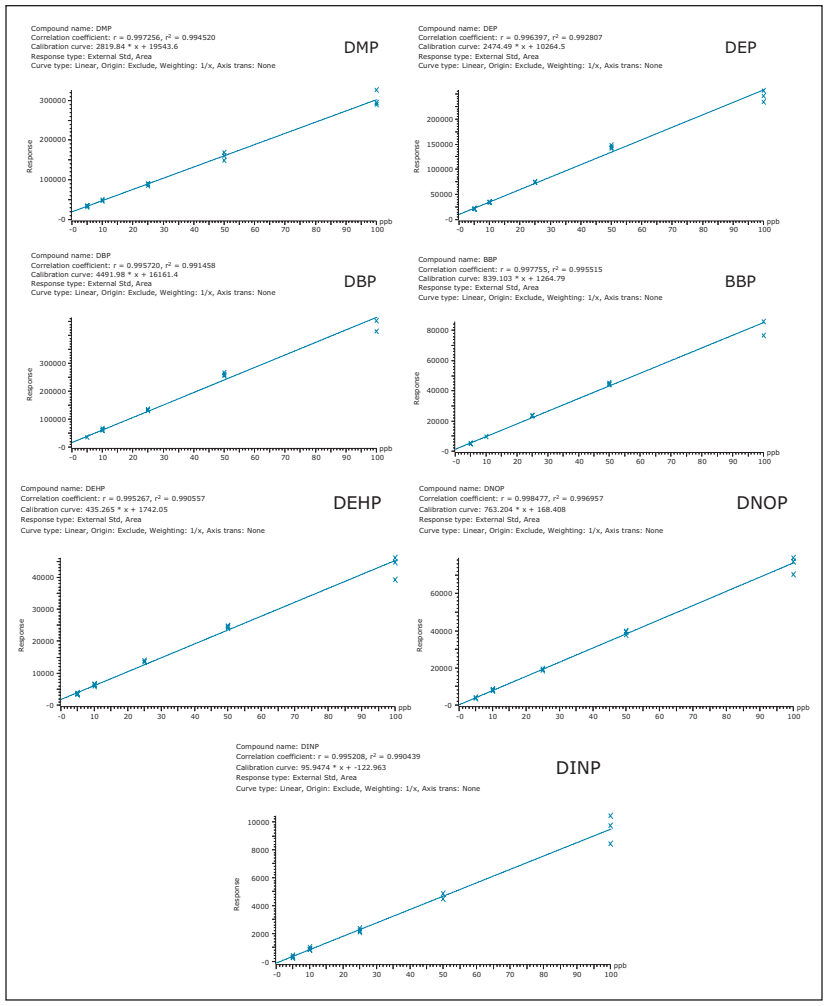


Figure 3. Matrix spiked calibration curve of all phthalates from 5 ppb (µg/L) to 100 ppb (µg/L) in sample F.

To study the applicability of the method to other distilled beverage types, all distilled beverage samples listed in Table 1 were spiked with the seven phthalates at 100 ppb (µg/L) and analyzed. All phthalates were successfully detected in all of the samples. Figure 4 shows the MRM chromatograms of BBP for each of the samples. BBP was successfully detected in all of the distilled beverage samples at 100 ppb.

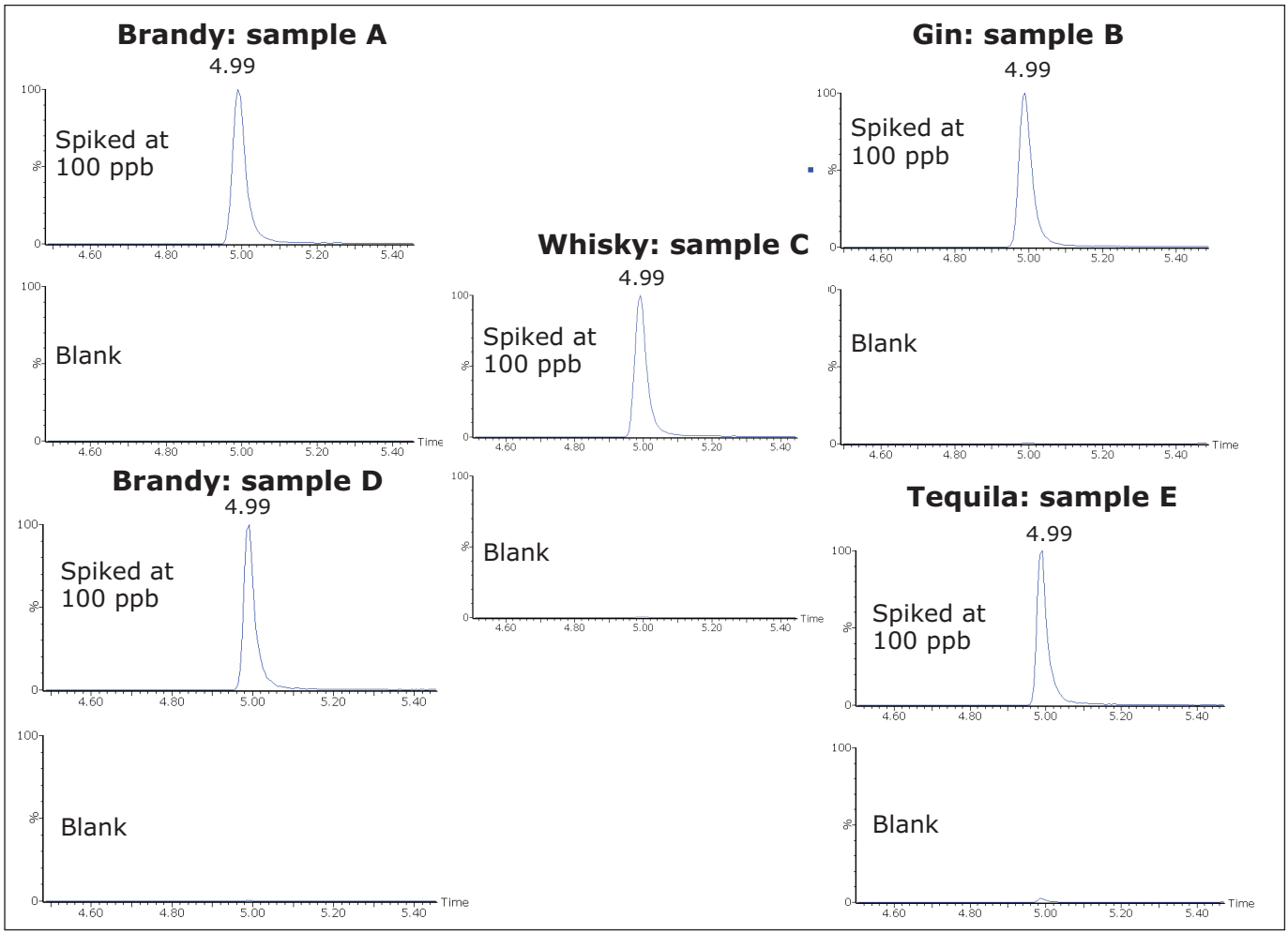


Figure 4. Chromatograms of BBP in brandy A, brandy B, gin, whisky, and tequila samples: spiked at 100 ppb (µg/L) and blank.

Sample analysis

All of the alcoholic beverages (samples A to F) were analyzed in triplicate. For the majority of samples, no phthalates were detected. There was a trace level of DEHP detected in brandy A (<5 ppb). Figure 5 shows a chromatogram of DEHP (2 MRM transitions) in a solvent standard and in brandy A.

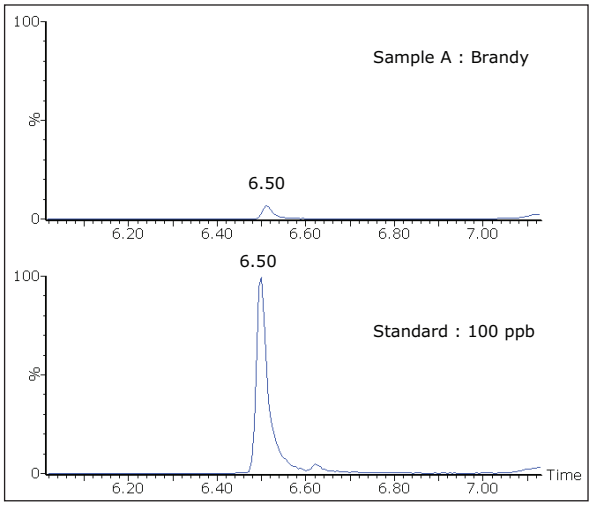


Figure 5. Chromatogram of DEHP in a solvent standard and brandy (sample A). Low level detection of DEHP in sample A at <5 ppb.

Robustness study

To assess the repeatability and robustness of the method, 100 injections of sample F spiked at 100 ppb were performed. Figure 6 shows an example TrendPlot of DMP over these 100 injections. The %RSD (retention time and area count) and relative precision (CV) for all compounds are shown in Table 5. The %RSDs for area count and retention time were <6% and 0.1% respectively for all compounds over the 100 injections.

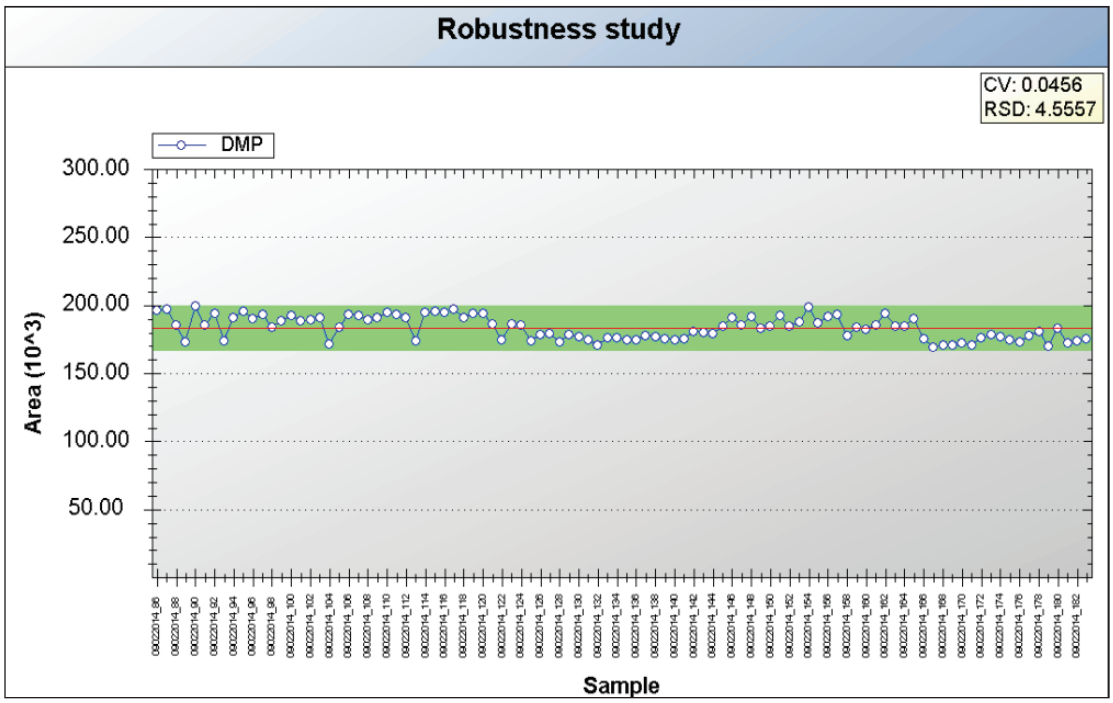


Figure 6. TrendPlot of DMP for 100 injections.

Name	RSD % (RT)	RSD % (area)	CV (area)
DMP	0.096	4.55	0.04
DEP	0.092	1.87	0.01
DBP	0.050	2.05	0.02
BBP	0.059	1.95	0.01
DEHP	0.035	3.82	0.03
DNOP	0.053	5.90	0.05
DINP	0.067	4.98	0.04

Table 5. Percentage relative standard deviation (retention time and area) and CV (area) for all phthalates (n= 100 injections).

CONCLUSIONS

- A simple and quick "dilute and shoot" method has been developed for accurate quantification of seven phthalates.
- The phthalates of interest were easily separated from background contamination using the ACQUITY UPLC Isolator Column.
- Excellent robustness, linearity, and levels of detection <10 ppb for all of the phthalates were achieved using the ACQUITY UPLC H-Class System coupled with Xevo TQD.

Acknowledgment

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Reference

1. H Y Shen. Simultaneous screening and determination eight phthalates in plastic products for food use by sonication-assisted extraction/GC-MS methods. *Elsevier*. 2005: 734–739.

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