Increasing Selectivity and Confidence in Detection when Analyzing Phthalates by LC-MS/MS

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Overview

Recent issues with the determination of phthalates in food and beverages like yogurt, sport drinks and fruit juices have highlighted the need for both food manufacturers and regulatory agencies to utilize fast and accurate analytical techniques to proactively ensure product safety.

A fast and sensitive LC-MS/MS method was developed for the analysis of 22 phthalates utilizing a simple extraction, fast LC separation using a Phenomenex Kinetex™ C18 column with a run time of 10 minutes, and selective MS/MS detection using a SCIEX QTRAP® 5500 System operated in Multiple Reaction Monitoring (MRM) mode. Major challenges of method development were the presence of chemical background and matrix interferences. To address these challenges we successfully applied the unique MRM[3] mode to enhance detection selectivity by detecting second generation product ions and Enhanced Product Ion (EPI) scanning to increase confidence in identification using the molecular fingerprint of each target analyte saved into the MS/MS spectrum. In addition, the SCIEX SelexION® technology was used to separate critical isomers using Differential Mobility Spectrometry (DMS).

Introduction

Phthalates are widely used industrial chemicals with an estimated annual production of over 8,000,000 tons. Phthalates are added to plastics to increases flexibility, transparency, and longevity. By weight, they contribute 10-60% of plastic products. Phthalates are used in a variety of products, including building materials (caulk, paint, adhesives), household products (vinyl upholstery, shower curtains, food containers and wrappers), and cosmetics.[1]

The use of various phthalates is restricted in many countries because of health concerns.[2-3]

In 2011, the illegal use of bis(2-ethylhexyl) phthalate (DEHP) and Diisononyl phthalate (DINP) in clouding agents for use in food and beverages has been reported in Taiwan.[4]

As a result fast and reliable methods for the detection of different phthalates in food and beverages are needed. Chromatographic techniques coupled to mass spectrometry are methods of choice because of their sensitivity and selectivity.[5]

Here we present a new and unique LC-MS/MS method using the SCIEX QTRAP 5500 System operated in MRM, MRM[3], and EPI mode to detect 22 phthalates. In comparison to GC-MS the developed LC-MS/MS method has several advantages:

- Reduced sample preparation and no need for derivatization
- Superior quantitative results with shorter run times
- Higher degree of confidence due to the presence of the quasi-molecular ion and characteristic fragment ions

In addition, DMS was used to separate isomeric phthalates using the SCIEX SelexION technology.

Experimental

Sample Preparation

One gram sample was homogenized and extracted with 45 mL methanol using ultra sound for 30 min. An aliquot of 5 mL was transferred into a vial and centrifuged for 10 min (3500 rpm). The supernatant was further diluted for LC-MS/MS analysis.

LC Separation

LC separation was achieved using an Agilent 1200 system with a Phenomenex Kinetex C18 (100 x 4.6 mm; 2.6 µm) column and a fast gradient of water + 10 mM ammonium acetate and methanol at a flow rate of 500 µL/min.

MS/MS Detection

The SCIEX QTRAP® 5500 system was used with Turbo V™ source and Electrospray Ionization (ESI) source. Two selective MRM transitions were monitored for each targeted analyte (Table 1). MRM[3] was used to differentiate between isomers and to increase selectivity to reduce interferences.

DMS Separation

The SCIEX SelexION® technology was used to selectively detect isomeric phthalates. A Separation voltage (SV) of 3800 V was used with acetonitrile as chemical modifier. The Compensation Voltage (CoV) was optimized for each target analyte specifically.

Results

Phthalates are esters of 1,2-benzenedicarboxylic acid.
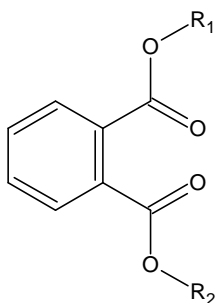
Targeted analytes of this project are listed in Table 1.

All plastic material (i.e. pipette tips) was avoided when handling samples and making dilutions. All glassware was cleaned carefully to avoid contamination. Different organic solvents (LC and LC-MS grade) were evaluated and distilled water was used to minimize background interferences.

Solid Phase Extraction (SPE) is known to be a major source of phthalate contamination resulting in over-estimation and false positive results.[5] Thus, a simple and fast procedure using liquid extraction was developed and successfully applied to the analysis of food and beverage samples.

Different LC conditions were evaluated during method development. In general C18 material with a neutral buffer of ammonium acetate was found to give good separation. Methanol is organic modified was more efficient in separating isomers. The Phenomenex Kinetex C18 column was finally chosen because of its UHPLC like efficiency and resolution at significantly lower column pressure resulting in high robustness and long instrument up time.

The final gradient started at 50% methanol and included a cleanup step at 98% methanol at a flow rate of 1000 µL/min to reduce background levels.

In addition, a trap column was used between pump and autosampler to retain any phthalates originating from the HPLC system.

MRM transitions were fully optimized with $M+H^+$ as precursor ion and two compound dependent fragment ions. The dominating fragment ions were protonated phthalic acid (167), phthalic anhydride (149), and different esters of phthalic acid and phthalic anhydride (Figure 1).
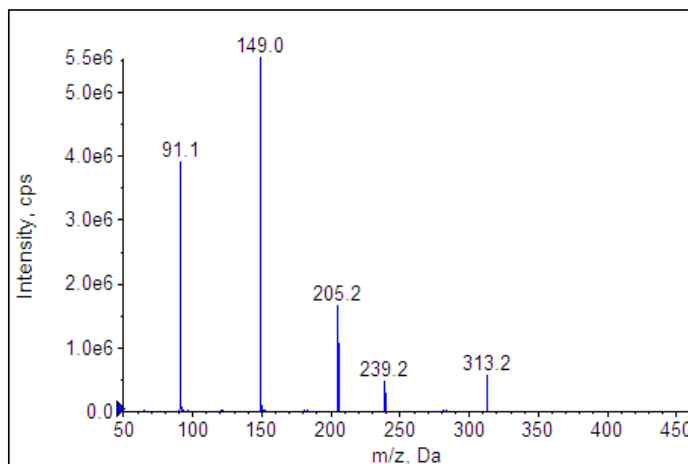

Figure 1. EPI spectrum of BBP, the molecular fingerprint saved into the MS/MS spectrum was used for compound identification with highest confidence

Table 1. Targeted phthalates, compound information, and optimized MRM transitions (Q1 and Q3 ions)

Phthalate		CAS	Formula	M.W.	Q1	Q3
Dimethyl phthalate	DMP	131-11-3	$C_{10}H_{10}O_4$	194.18	195	163 / 133
Diethyl phthalate	DEP	84-66-2	$C_{12}H_{14}O_4$	222.24	223	149 / 177
Diallyl phthalate	DAP	131-17-9	$C_{14}H_{14}O_4$	246.26	247	189 / 149
Dipropyl phthalate	DPrP	131-16-8	$C_{14}H_{18}O_4$	250.29	251	149 / 191
Diisopropyl phthalate	DIPrP	605-45-8	$C_{14}H_{18}O_4$	250.29	251	149 / 191
Dibutyl phthalate [EU, EPA]	DBP	84-74-2	$C_{16}H_{22}O_4$	278.34	279	149 / 205
Diisobutyl phthalate [EPA]	DIBP	84-69-5	$C_{16}H_{22}O_4$	278.34	279	149 / 205
Bis(2-methoxyethyl) phthalate	DMEP	117-82-8	$C_{14}H_{18}O_6$	282.29	283	207 / 59
Dipentyl phthalate [EPA]	DPP	131-18-0	$C_{18}H_{26}O_4$	306.40	307	219 / 149
Diisopentyl phthalate	DIPP	605-50-5	$C_{18}H_{26}O_4$	306.40	307	219 / 149
Bis(2-ethoxyethyl) phthalate	DEEP	605-54-9	$C_{16}H_{22}O_6$	310.34	311	221 / 149
Benzyl butyl phthalate [EU, EPA]	BBP	85-68-7	$C_{19}H_{20}O_4$	312.37	313	149 / 205
Diphenyl phthalate	DPhP	84-62-8	$C_{20}H_{14}O_4$	318.32	319	225 / 77
Dicyclohexyl phthalate	DCHP	84-61-7	$C_{20}H_{26}O_4$	330.42	331	167 / 249
Bis(4-methyl-2-pentyl) phthalate	BMPP	146-50-9	$C_{20}H_{30}O_4$	334.46	335	167 / 251
Dihexyl phthalate	DHXP	84-75-3	$C_{20}H_{30}O_4$	334.46	335	149 / 233
Di-n-heptyl phthalate	DHP	3648-21-3	$C_{22}H_{34}O_4$	362.51	363	149 / 233
Bis(2-n-butoxyethyl) phthalate	DBEP	117-83-9	$C_{20}H_{30}O_6$	366.45	367	101 / 249
Bis(2-ethylhexyl) phthalate [EU, EPA]	**DEHP**	**117-81-7**	**$C_{24}H_{38}O4$**	**390.56**	**391**	**167 / 279**
Di-n-octyl phthalate [EU, EPA]	DNOP	117-84-0	$C_{24}H_{38}O_4$	390.56	391	261 / 149
Diisononyl ortho-phthalate [EU, EPA]	**DINP**	**28553-12-0**	**$C_{26}H_{42}O_4$**	**418.61**	**419**	**275 / 149**
Diisodecyl ortho-phthalate [EU, EPA]	DIDP	26761-40-0	$C_{28}H_{46}O_4$	446.66	447	149 / 289

Bold   Illegally used in food and beverages in Taiwan in 2011[4]
[EU]   Restricted use in toys and childcare articles in Europe[2]
[EPA]   Addressed in the phthalates action plan of the U.S. Environmental Protection Agency[3]

An example chromatogram of LC-MS/MS detection of 22 phthalates is shown in Figure 2.

Limits of detection (LOD), linearity and accuracy of quantitation were determined. Example chromatograms of six high priority phthalates (from 1 to 100 ng/mL) are shown in Figure 3a and 3b.

For all targeted phthalates an LOD of at least 1 ng/mL was achieved. Please note that the final LOD greatly depends on background interferences which can greatly vary from laboratory to laboratory.

Table 2. Accuracy and linearity of six high priority phthalates

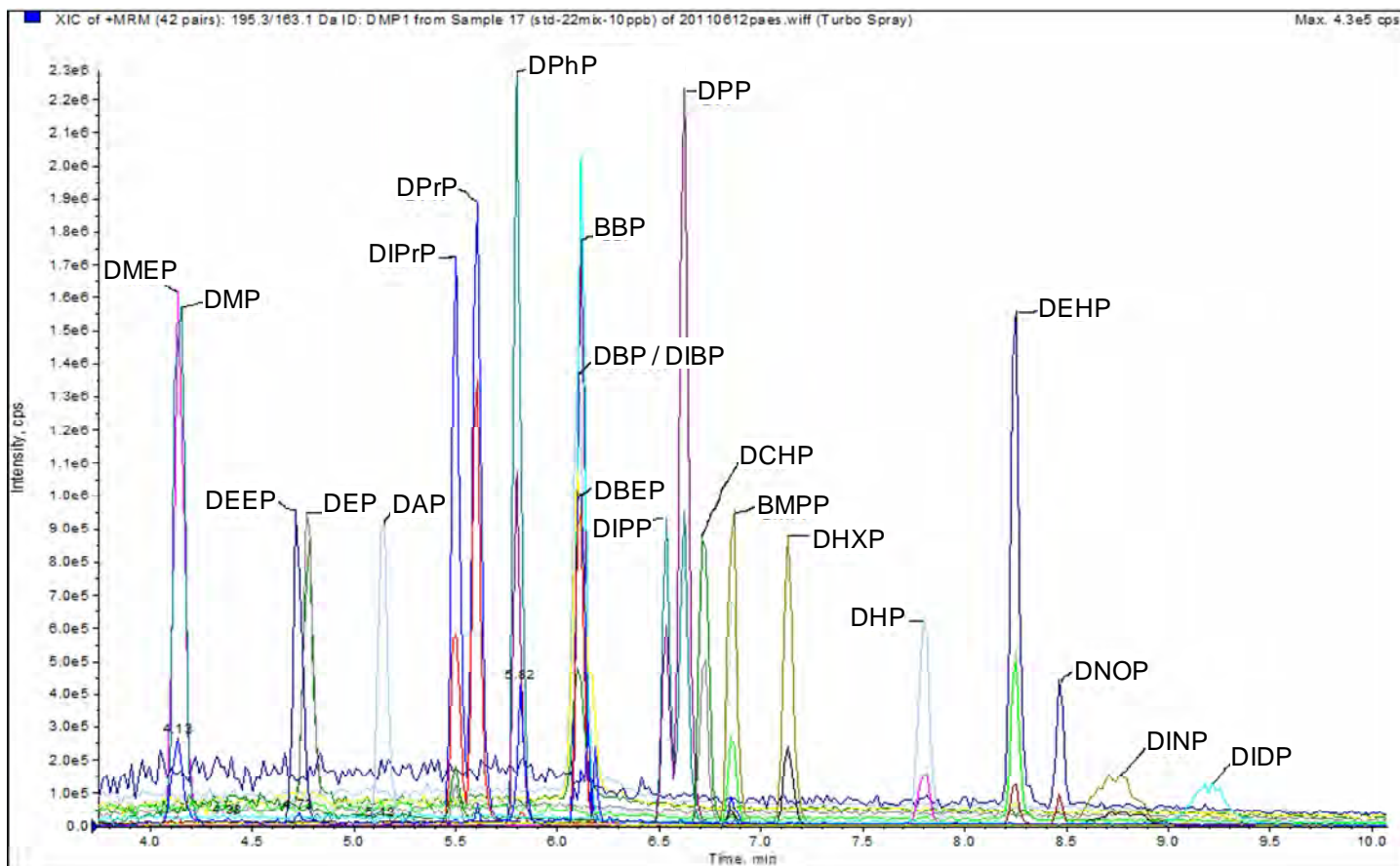| Phthalate	Accuracy (%)	Regression
DBP	97-103	0.9998
BBP	91-108	0.9999
DEHP	88-108	0.9989
DNOP	85-113	0.9982
DINP	92-111	0.9998
DIDP	94-109	0.9931

Figure 2. Example LC-MS/MS chromatogram showing the separation and detection of 22 phthalates at a concentration of 10 ng/mL
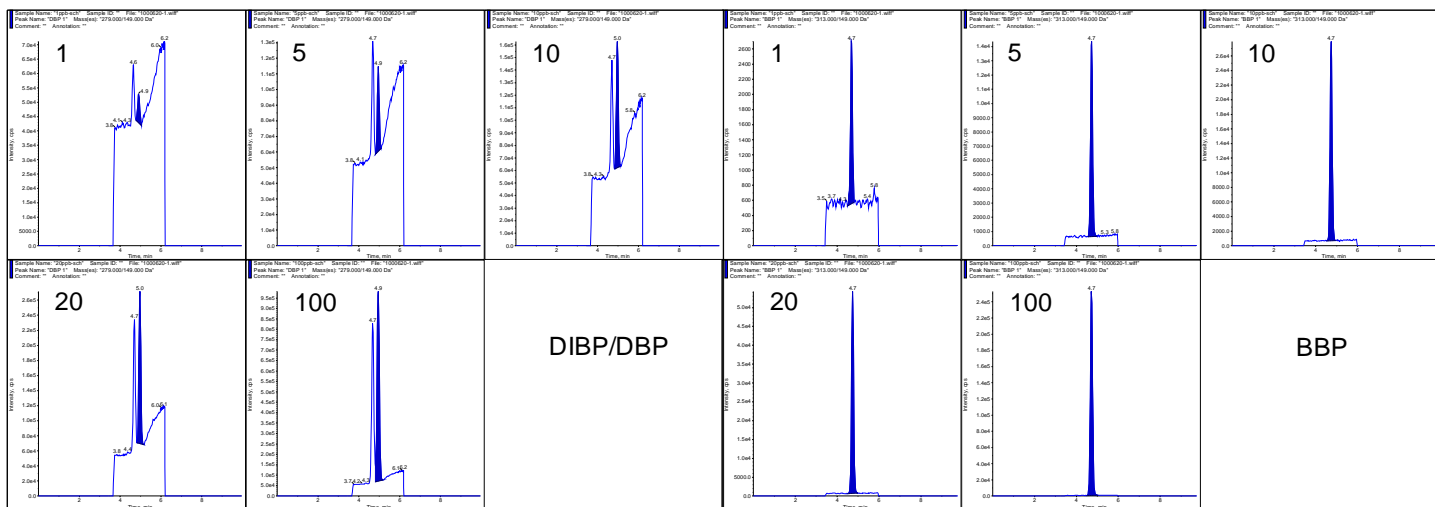

Figure 3a. MRM chromatograms of the high priority phthalates DBP and BBP at 1, 5, 10, 20, and 100 ng/mL
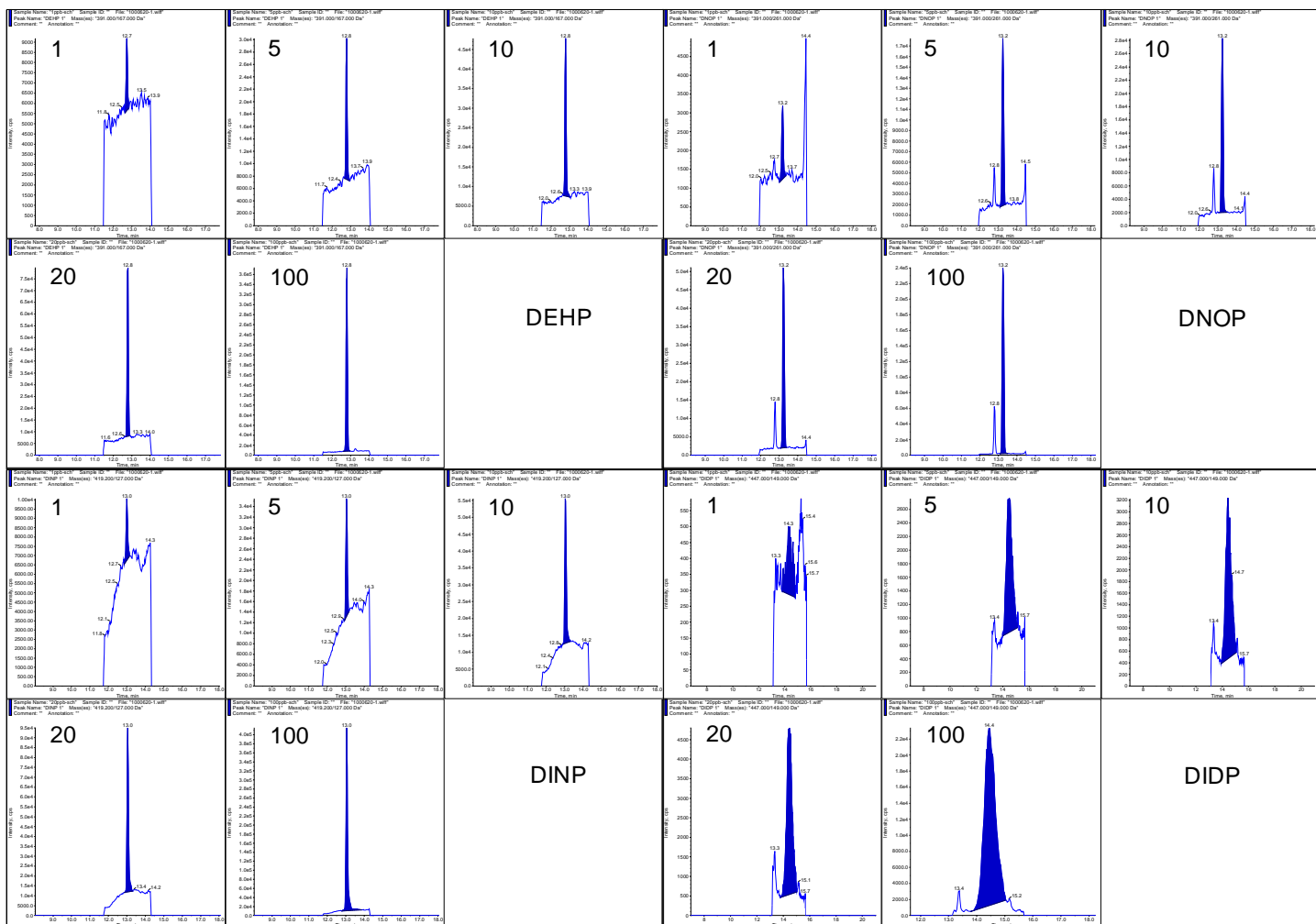
Figure 3b. MRM chromatograms of the high priority phthalates DEHP, DNOP, DINP, and DIDP at 1, 5, 10, 20, and 100 ng/mL

The accuracy was typically between 85 and 115% and quantitation was performed with linear regression and 1/x weighting. The coefficient of regression was above 0.999 for all analytes. Examples for accuracy and linearity are of six high priority phthalates are listed in Table 2.

The unique scan function of MRM[3] on the SCIEX QTRAP® 5500 System was investigated for its potential to differentiate isomeric species.

An example of successfully differentiating between the isomers DIBP and DBP using the different fragmentation pattern in MRM[3] mode is shown in Figure 4. Using traditional MRM mode both compounds had the exact same transitions and needed to be separated on the LC time scale. Thus, MRM[3] allows speeding up the LC method if throughput requires.
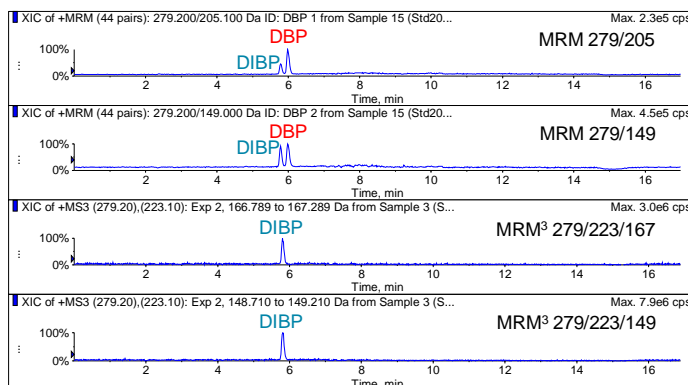

Figure 4. Differentiation of DIBP and DBP using the different fragmentation pattern in MRM[3] mode in comparison to MRM mode

Another possibility to enhance selectivity of detection is the use of Differential Mobility Spectrometry (DMS). The SCIEX SelexION® technology uses a planar DMS cell attached between the curtain plate and orifice plate of the mass spectrometer. Ions are separated based on difference in their high field and low field mobility.SV and CoV are optimized to correct the trajectory of a desired ion. In addition, a chemical modifier can be introduced to alter separation characteristics.
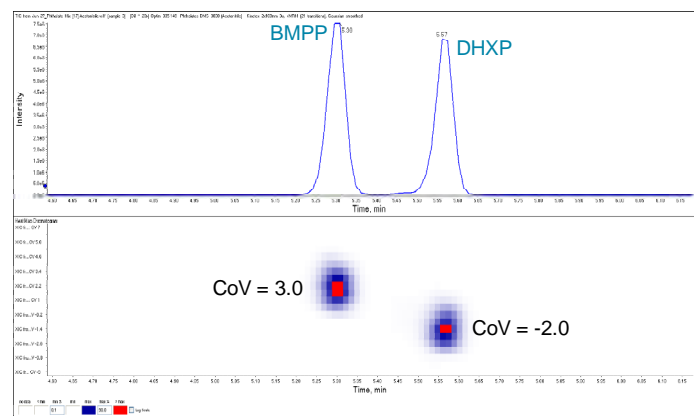

Figure 5a. Separation of the isomers BMPP and DHXP, both phthalates can be separated in the LC and DMS space resulting in increased selectivity
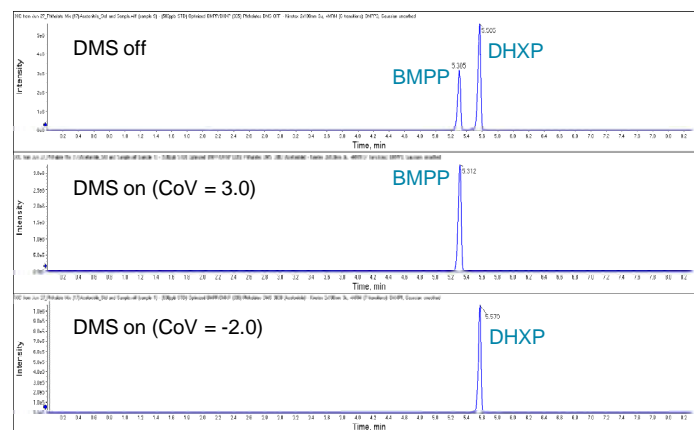

Figure 5b. Selective detection of BMPP and DHXP by compound specific CoV for each analyte, acetonitrile was introduced as chemical modifier

The example presented in Figure 5a and 5b highlights the unique selectivity achieved using DMS. The isomers BMPP and DHXP were separated using different CoV. Acetonitrile was introduced as chemical modifier to enhance separation.

Summary

A fast and sensitive LC-MS/MS method was developed for the detection of 22 phthalates in food and beverage samples. All possible precautions were taken to reduce chemical background. This included the avoidance of plastic material, careful handling of laboratory glassware, systematic evaluation of different LC solvents, a simple extraction procedure, and the use of a trap column inside the LC system.

All 22 phthalates were detected with an LOD of 1 ng/mL or lower, good accuracy, and linearity using two MRM transitions per analyte. Characteristic EPI spectra can be used to further increase confidence of compound identification based on characteristic MS/MS spectra and library searching.

In addition, the unique scan function MRM[3] of the QTRAP® 5500 system and SelexION technology were successfully used to separate isomeric species enhancing the selectivity of LC-MS/MS detection.

Acknowledgement

References

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