

Rapid, Sensitive, and Robust Detection of Phthalates in Food Using GC/MS or LC/MS

Application Note

Food Safety

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Abstract

A recent series of international food safety crises has highlighted the need for the rapid development of sensitive and reliable methods that can be run on a wide range of mass spectrometry platforms for the detection of food contaminants. The most recent example is the illegal adulteration of juice drinks and other foods with phthalates in Taiwan. Agilent has developed highly sensitive and quantitative methods for the detection of phthalates in food that can be run on both GC/MS and LC/MS systems.



Agilent Technologies

Introduction

Phthalates are esters of phthalic acid and are used as plasticizers to increase the flexibility, transparency, durability, and longevity of plastics. Phthalates are used in a large variety of applications, from enteric coatings of pharmaceutical pills to emulsifying and suspending agents. They can be found in a wide range of products, including adhesives and glues, electronics, personal-care products, medical devices, tubing, packaging, and children's toys. These compounds are easily released into the environment as they are not covalently bound to the plastics in which they are mixed. Studies have implicated phthalates in a number of health problems, including asthma, endocrine disruption, reproductive abnormalities, cancer, low birth weight, autism, and ADHD. As a result, the European Union (EU), United States (USA), and several other countries have begun to regulate phthalate exposure. The use of six phthalates in children's toys has been restricted in the European Union (2005/84/EC) since 2007, and in the USA since 2009 (Table 1).

In May of 2011, it was discovered that Bis-(2-ethylhexyl) phthalate (DEHP) had been illegally added to sports drinks, fruit juice, tea beverages, fruit jam, and food powders, as a substitute clouding agent. Clouding agents (emulsifiers) improve the appearance of fruit juices and other products. Phthalates were used in this case as a substitute for palm oil. These products were immediately recalled and a global advisory was issued due to the possible export of these products. The maximum allowable limit for any phthalate is now 1 part per million (ppm) in China and Taiwan.

Sensitive and reliable methods to rapidly detect the presence of phthalates in food are clearly needed, in order to protect consumers from unscrupulous production processes. This application note describes methods that can be run on both GC/MS and LC/MS platforms to enable sensitive detection of phthalate compounds, including the six that are regulated in the US and EU. The GC/MS and LC/MS methods can be run on both single and triple quadrupole mass spectrometers. The GC/MS methods can detect as little as 50 parts per billion (ppb) of the phthalates, while the LC/MS methods are sensitive to as low as 1 ppb, using the triple quadrupole MS. Both detection levels are more than adequate to ensure that foods do not contain more than the levels of phthalates allowed by USA, EU, China and Taiwan regulations.

Table 1. The Most Commonly Used Phthalates

Compound name	Acronym
<i>Dibutyl phthalate</i>	DBP
<i>Benzylbutyl phthalate</i>	BBP
<i>Bis-(2-ethylhexyl) phthalate</i>	DEHP
<i>Di-n-octyl phthalate</i>	DNOP
<i>Diisononyl phthalate</i>	DINP
<i>Diisodecyl phthalate</i>	DIDP
Dimethyl phthalate	DMP
Diethyl phthalate	DEP
Dipropyl phthalate	DPRP
Dibutyl phthalate	DBP
Diisobutyl phthalate	DIBP
Dimethoxyethyl phthalate	DMEP
Dipropyl phthalate	DPP
Pentylisopentyl phthalate	PIPP
Diisopentyl phthalate	DIPP
Bis(2-ethoxyethyl) phthalate	DEEP
Diethylhexyl terephthalate	DEHT
Diallyl phthalate	DASP
Butylbenzyl phthalate	BBP
Dipropylheptyl phthalate	DPHP
Dicyclohexyl phthalate	DCHP
Dihexyl phthalate	DHP
Bis-methylphenyl phthalate	BMPP
Butyloctyl phthalate	BOP
Dibenzyl phthalate	DBZP
Diheptyl phthalate	DHEPP
Diisoheptyl phthalate	DIHEPP
Hexylethylhexyl phthalate	HEHP
Benzylbutyl phthalate	BBEP
Diisooctyl phthalate	DIOP
Diethylhexyl phthalate	DEHP
Dioctyl phthalate	DOTP
Dinonyl phthalate	DNP
Diundecyl phthalate	DUP
(Bis(2-ethylhexyl) adipate	DEHA
Di-n-hexyl phthalate	DNHP

The six phthalates in bold italic are regulated in the USA and EU.

Experimental

Reagents and Standards

GC Standards: Dibutyl phthalate (DBP,CAS 84-74-2), benzyl butyl phthalate (BBP,CAS 85-68-7), bis[2-ethylhexyl] phthalate (DEHP CAS 117-81-7), di-n-octyl phthalate (DNOP,CAS 117-84-0), diisodecyl phthalate (DIDP,26761-40-0), di-isononyl phthalate(DINP,28553-12-0) were purchased from ChemService (West Chester, PA, USA). The standards were dissolved in dichloromethane.

LC Standards: Thirty phthalate standards were purchased from Dr.Ehrentorfer GmbH. Individual phthalate stock solutions containing 100–1,000 µg/mL were prepared in methanol. Working standard solutions containing 10 µg/mL of each phthalate were prepared by dilution with methanol for construction of the calibration curves.

Instruments

The GC/MS and GC/MS/MS experiments were performed on an Agilent 7890A GC System coupled to either an Agilent 5975C GC/MSD or a 7000B Triple Quadrupole GC/MS. The LC/MS and LC/MS/MS experiments were performed on an Agilent 1260 RRLC HPLC system coupled to either an Agilent 6150 Single Quadrupole LC/MS or an Agilent 6400 Series triple quadrupole LC/MS. The instrument conditions are listed in Tables 2–4.

Table 2. GC/MS and GC/MS/MS Conditions

GC run conditions

Columns	Agilent J&W DB-5MS Ultra Inert Capillary GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI)
Injection volume	1 µL
Inlet temperature	Isothermal at 290 °C
Injection mode	Splitless
Oven program	120 °C for one minute 120 °C to 300 °C at 20 °C/min Hold at 300 °C for 5 min
Post run	300 °C for 5 min
Carrier gas	Helium at 1.2 mL/min
Transfer line temp	300 °C

Agilent 5975C Series GC/MSD (single quadrupole) conditions

Acquisition parameters	EI, SIM/Scan
Scan mode	50–500 amu mass range

Agilent 7000B Triple Quadrupole GC/MS conditions

Mode	EI, MRM
Source temperature	230 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
Tune file	atunes.eiextune.xml
Collision gas flows	Nitrogen at 1.5 mL/min, Helium at 2.25 mL/min
Detector gain	15

Table 3. LC/MS Conditions for Separation of 30 Phthalates

LC run conditions						
Column	Analysis: Agilent ZORBAX Eclipse Plus, 2.1 × 50 mm, 1.8 μm (p/n 959741-912)					
Column temperature	25 °C					
Injection volume	10 μL					
Mobile phase	A = ddH ₂ O B = Methanol					
Run time	6.0 min					
Flow rate	0.5 mL/min					
Gradient table	Time(min)	0	2.0	5.0	5.5	5.51
	B%	60.0	80.0	100.0	100.0	60.0

Agilent 6150 MSD (single quadrupole) LC/MS system conditions

Source	ESI, Positive
Drying gas temperature	300 °C
Drying gas flow	4 L/min
Nebulizer pressure	50 psig
Capillary voltage (positive)	4000 V
Scan mode	SIM

Table 4. LC/MS/MS Conditions for Separation of 13 Phthalates

LC run conditions						
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 μm (p/n 959758-902)					
Column temperature	50 °C					
Injection volume	10 μL					
Autosampler temperature	6 °C					
Mobile phase	A = ddH ₂ O B = Methanol + 5.0 mM ammonium formate					
Run time	10 min					
Flow rate	0.55 mL/min					
Gradient tables	1260 RRLC					
	Time(min)	0	5.0	6.0	8	8.1
	Flow (mL/min)	0.2	0.55	0.55	0.55	0.55
	B%	90.0	90.0	95.0	95.0	100.0
	Agilent 1290 Infinity GC System					
Time (min)	0	5.0	8	8.1		
Flow (mL/min)	0.2	0.55	0.55	0.55		
B%	90.0	90.0	90.0	100.0		

Agilent 6400 Series Triple Quadrupole LC/MS/MS conditions

Ion mode	ESI, positive
Drying gas temperature	350 °C
Drying gas flow	10 L/min
Nebulizer pressure	40 psi
Capillary voltage	4000 V
MRM acquisition	Segment MRM (0–3.5 min for segment 1; 3.5–8.0 min for segment 2)
Delta EMV	50–100 V

Sample Preparation

GC/MS

Three drinking water samples were provided by the Food Industry Research and Development Institute of Taiwan. Four beverage samples, black tea, sports drinks, orange juice, and coffee latte, were obtained from retail stores to test the sample preparation method. A solid phase extraction (SPE) method was used in lieu of the traditional hexane liquid-liquid extraction, to reduce background and speed up the extraction process. A glass cartridge was loaded with 5g Agilent Chem Elut material (p/n 198003) and 5 mL of aqueous sample, which was absorbed onto the Chem Elut sorbent. The sorbent was washed three times with 5 mL of dichloromethane, to elute the analytes, leaving contaminants behind. The sample was then nitrogen evaporated to 2.5 mL before analysis.

LC/MS

One gram samples of beverage, food powder, health-care products, or baked goods were extracted with 45 mL methanol and sonicated for 20 minutes. After extraction, the extracted solvent was brought to room temperature (about 25–30 °C) and methanol was added to fill a 50 mL flask. One milliliter of the extraction solution was centrifuged (3,500 rpm) for 10 minutes before analysis.

Analysis Parameters

The MS and MS/MS parameters used are shown in Tables 5–8.

Table 5. Agilent 5975 GC/MS Single Quadrupole SIM Analysis Parameters

No.	Compounds	CAS no.	Target ion	Q1	Q2	Q3
1	DBP	84-74-2	149	150	223	205
2	BBP	85-68-7	149	91	206	238
3	DEHP	117-81-7	149	167	279	104
4	DNOP	117-84-0	279	149	167	261
5	DINP	28553-12-0	293	149	127	167
6	DIDP	26761-40-0	307	149	141	167

Table 6. Agilent 7000B Triple Quadrupole GC/MS Analysis Parameters

No.	Compounds	Quantitative transitions	Qualitative transitions
1	DBP	149→65@ 30 eV	149→93@ 20 eV 223→149@ 10 eV
2	BBP	149→65@ 30 eV	149→93@ 20 eV 206→149@ 10 eV
3	DEHP	149→65@ 30 eV	149→93@ 20 eV 279→149@ 15 eV
4	DNOP	279→71@ 10 eV	279→149@ 15 eV
5	DINP	293→71@ 10 eV	293→149@ 10 eV
6	DIDP	307→71@ 10 eV	307→149@ 10 eV

Table 7. Agilent 6150 Single Quadrupole LC/MS System Analysis Parameters for 30 Phthalates

Compound	m/z	Frag V
DMP	195.00	90
DEP	223.00	90
DPRP	251.00	90
DBP/DIBP	279.00	130
DMEP	283.00	130
DPP/PIPP/DIPP	307.00	130
DEEP	311.00	130
BBP	313.00	130
DPHP	319.00	130
DCHP	331.00	130
DHP/BMPP/BOP	335.00	130
DBZP	347.00	130
DHEPP/DIHEPP/HEHP	363.00	130
BBEP	367.00	130
DNOP/DIOP/DEHP/DEHT/DOTP	391.00	130
DNP/DINP	419.00	130
DIDP	447.00	130
DUP	475.00	130

Frag V = Fragmentation voltage

Table 8. Agilent 6400 Triple Quadrupole LC/MS System Analysis Parameters for 13 Phthalates

Compound	RT (min)	MRM	Dwell time (ms)	Fragmentor voltage (V)	Collision energy (eV)
DMP	0.98	195.1→163	30	57	5
		195.1→91.9			40
		195.1→77			40
DEP	1.05	223.1→177	30	72	1
		223.1→148.9			13
		223.1→65			45
DAP	1.07	247.1→189	50	72	1
		247.1→149			15
DPP	1.18	251.1→191	30	72	1
		251.1→149			9
		251.1→65			53
DBP	1.39	279.2→205.1	30	72	1
		279.2→149			9
		279.2→120.9			37
BBP	1.35	313.2→205	30	72	1
		313.2→148.9			5
		313.2→91			25
DCP	1.84	331.2→167	30	72	9
		331.2→148.9			21
		331.2→120.9			49
DNHP	2.28	335.2→233.1	30	72	1
		335.2→149			9
		335.2→120.9			45
DEHP	3.96	391→279	100	140	5
		391→167			15
		391→149			15
DEHA	4.07	371.3→129	100	104	9
		371.3→111			25
		371.3→55			53
DNOP	4.45	391.3→261.1	50	104	1
		391.3→149			9
		391.3→120.9			53
DINP	5.41	419.3→149	50	104	13
		419.3→71			21
		419.3→57			25
DIDP	6.93	447.3→149	50	104	25
		447.3→85			25
		447.3→71			25

Results and Discussion

Sample Preparation

One of the keys to success for both the GC and LC methods is a sample preparation technique that is rapid and reduces background noise. There are many sources of phthalate contamination in the laboratory, including plastic tubing and containers. Careful sample preparation is critical to obtaining a result free of contamination and background noise from chemical interferences. One of the traditional methods used for GC analysis of phthalates in food in China, per regulation GB21911-2008, is a liquid-liquid extraction method using

hexane. An alternative method using the Agilent Chem Elut solid phase extraction cartridge is much easier, faster, less than 10 minutes, and costs less, with less exposure to phthalate contamination sources. Figure 1 illustrates the low background and absence of contamination using Chem Elut, versus the traditional liquid-liquid extraction.

For LC/MS analysis, an innovative extraction method was created by the Taiwan FDA for beverages, food powder, health-care products, and baked goods using methanol and sonication for 20 minutes. An internal standard was added before sample preparation, to assure that the method was quantitative.

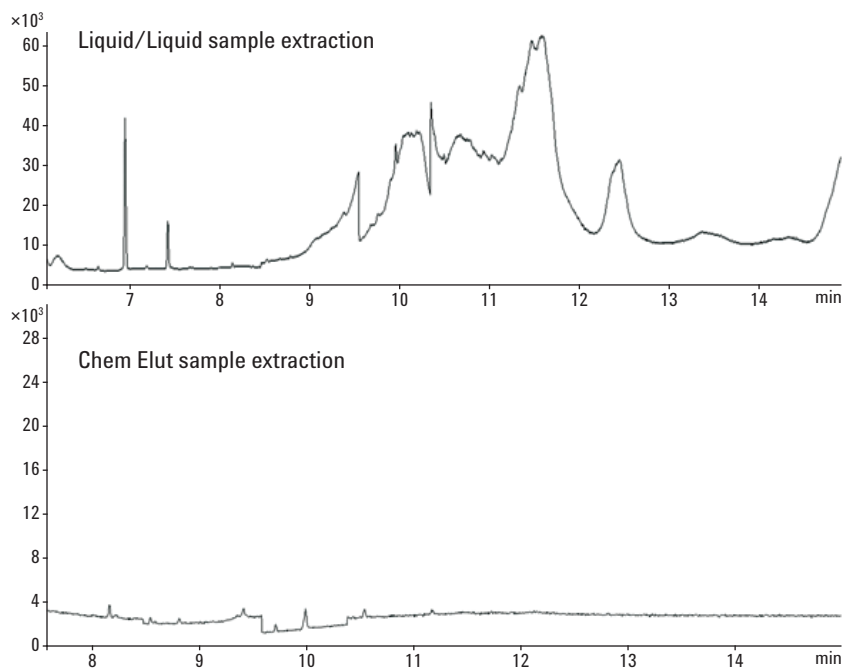
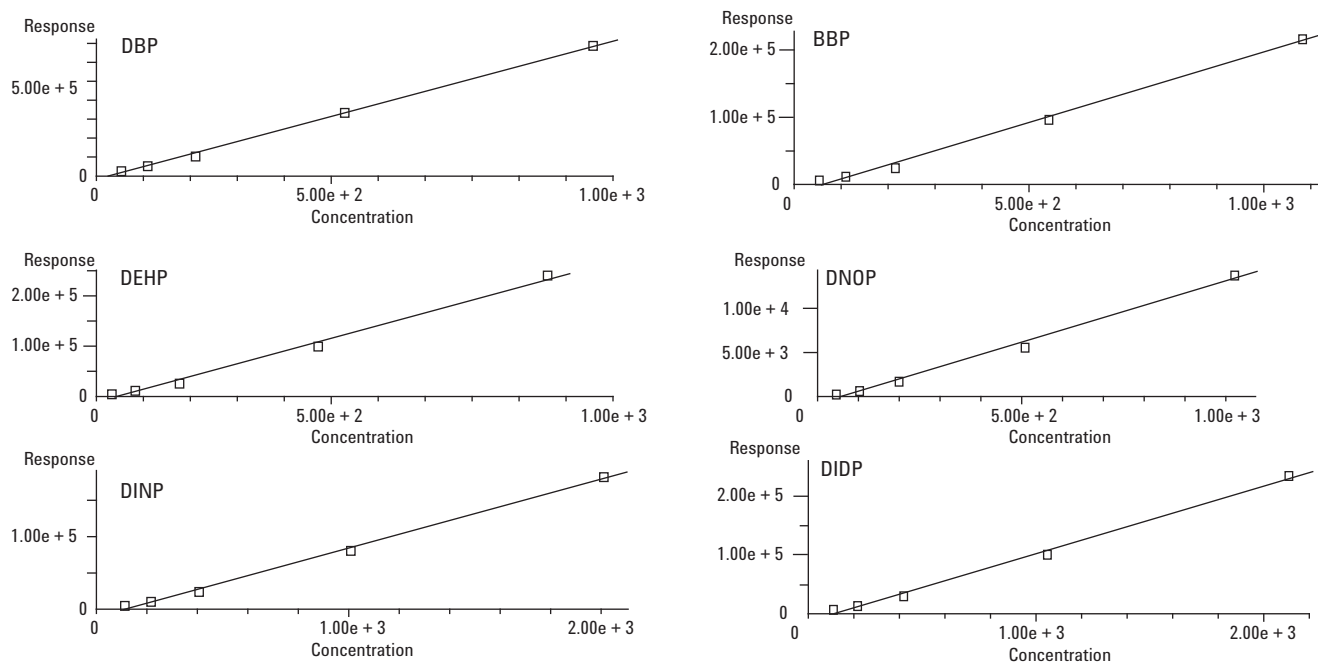


Figure 1. Comparison of injections of a blank after a GC/MS sample run, using either the liquid-liquid sample extraction (top), or the Chem Elut sample preparation procedure (bottom). Notice the heavy carryover in the liquid extracted blank, even in the blank prepared with Chem Elut, only one injection after a sample prepared the same way.

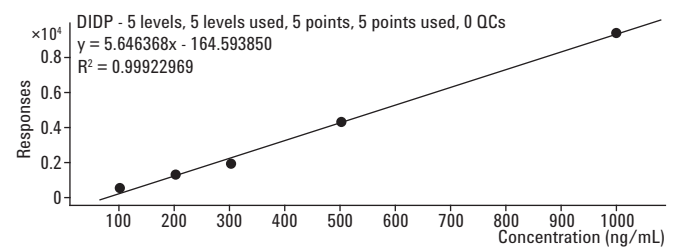
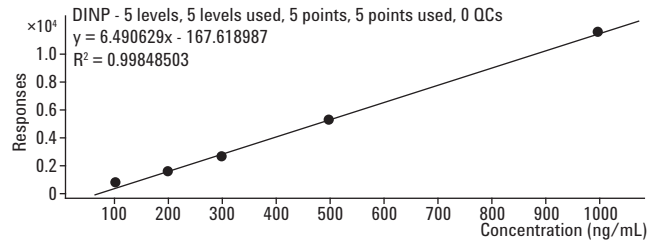
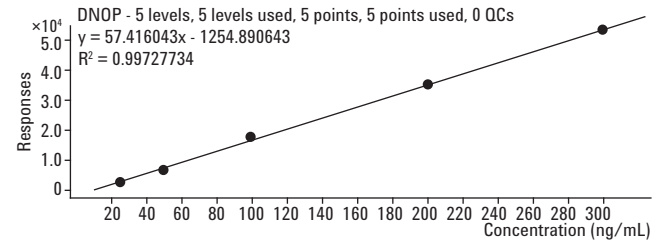
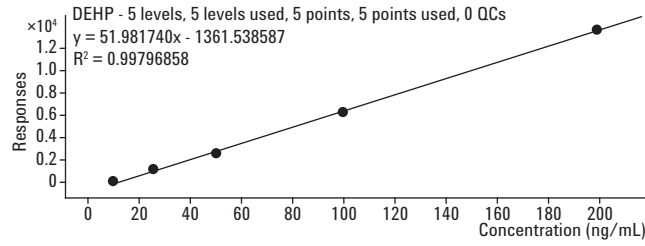
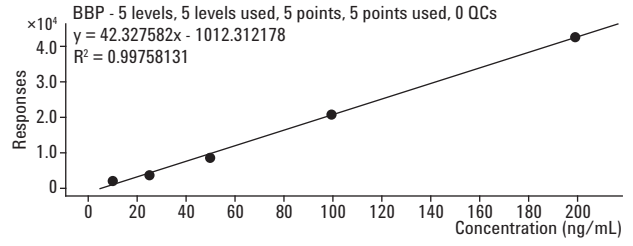
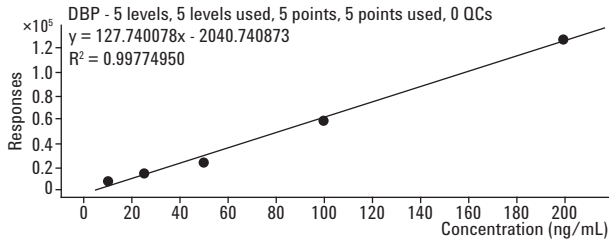
Sensitivity and Linearity of Quantification

The GC/MS and GC/MS/MS methods provide sensitivity for standards in solvent that easily exceeds the China (GB21911-2008) and Taiwan regulations for phthalates, which require that the level of any phthalate in food cannot exceed 1ppm. Using either single (MSD) or triple quadrupole MS platforms, the sensitivity ranges from 50 to 100 ppb, and the linearity of quantification (R^2) is greater than or equal to 0.992 (see Figures 2 and 3).



Compound	Range of linearity (mg/L)	Correlation coefficient (R^2)
1 DBP	0.05 - 1	0.998
2 BBP	0.05 - 1	0.996
3 DEHP	0.05 - 1	0.992
4 DNOP	0.05 - 1	0.992
5 DINP	0.1 - 2	0.997
6 DIDP	0.1 - 2	0.996

Figure 2. Examples of calibration curves as well as linearity range and correlation coefficient for the six phthalates of most interest, using the Agilent 5975 Series GC/MS in simultaneous SIM and Scan modes.



Compound	Range of linearity (mg/L)	Correlation coefficient (R ²)
1 DBP	0.05 - 1	0.998
2 BBP	0.05 - 1	0.998
3 DEHP	0.05 - 1	0.998
4 DNOP	0.05 - 1	0.997
5 DINP	0.1 - 2	0.999
6 DIDP	0.1 - 2	0.998

Figure 3. Examples of calibration curves as well as linearity range and correlation coefficient for the six phthalates of most interest, using the Agilent 7000B Triple Quadrupole GC/MS system in MRM mode.

Reproducibility is also excellent with the GC/MSD platform, as shown in Figure 4. An important feature of the Agilent 5975 GC/MS is its ability to provide SIM and Scan data simultaneously, as shown in Figure 5 for the separation of 15 phthalates. This feature reduces the probability of false positives.

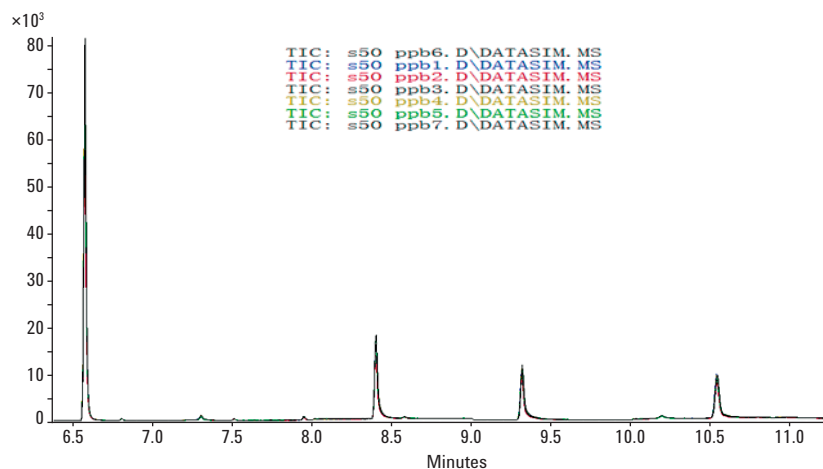


Figure 4. Reproducibility of total ion current (TIC) traces of six different injections of the six regulated phthalates at 0.05 mg/L (50 ppb). All six runs are virtually identical.

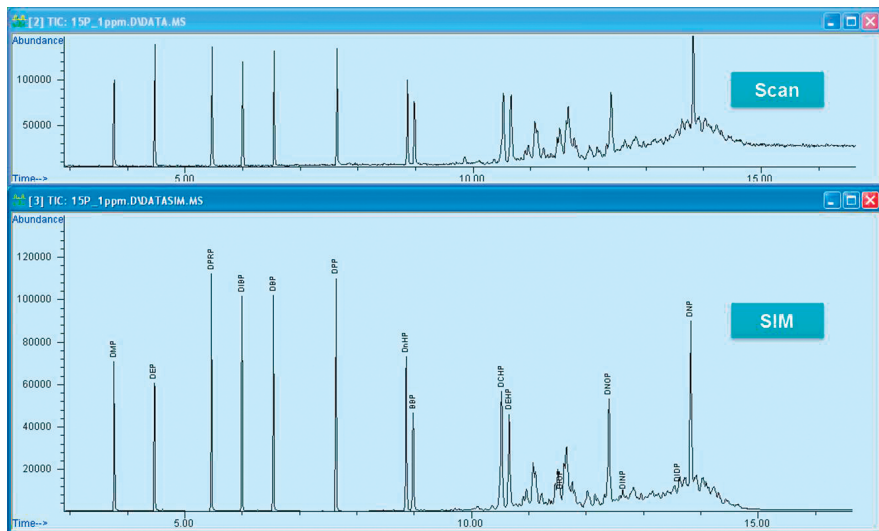


Figure 5. Synchronous GC/MS scan and SIM analysis of a mixture of 15 phthalates.

The LC/MS method that analyzes 30 phthalates simultaneously provides instrument detection limits from 50 to 500 ppb in methanol. The LC/MS/MS methods provide instrument detection limits (IDLs) well below the Taiwan FDA limit of 20 ppb, as shown in Table 9 ($\mu\text{g}/\text{mL}=\text{ppb}$), and the R^2 values for quantification are all greater than or equal to .9947, using the 13 phthalate method (extracted ion current chromatogram shown in Figure 6). An LC/MS/MS method has also been developed using a C18 column that can separate the isomers of 14 phthalate compounds especially DIBP and DBP (data not shown).

Table 9. Instrument Detection Limits for the LC/MS/MS Analysis of 13 Phthalates

Compound	R^2	IDL ng/mL (S/N > 10)
DMP	0.9955	2
DEP	0.9968	3
DAP	0.9947	0.3
DPP	0.9983	0.5
DBP	0.9949	5
BBP	0.9962	0.5
DCP	0.9981	0.3
DNHP	0.9981	0.5
DEHP	0.9949	5
DEHA	0.9972	0.5
DNOP	0.9979	0.5
DINP	0.9993	5
DIDP	0.9998	5

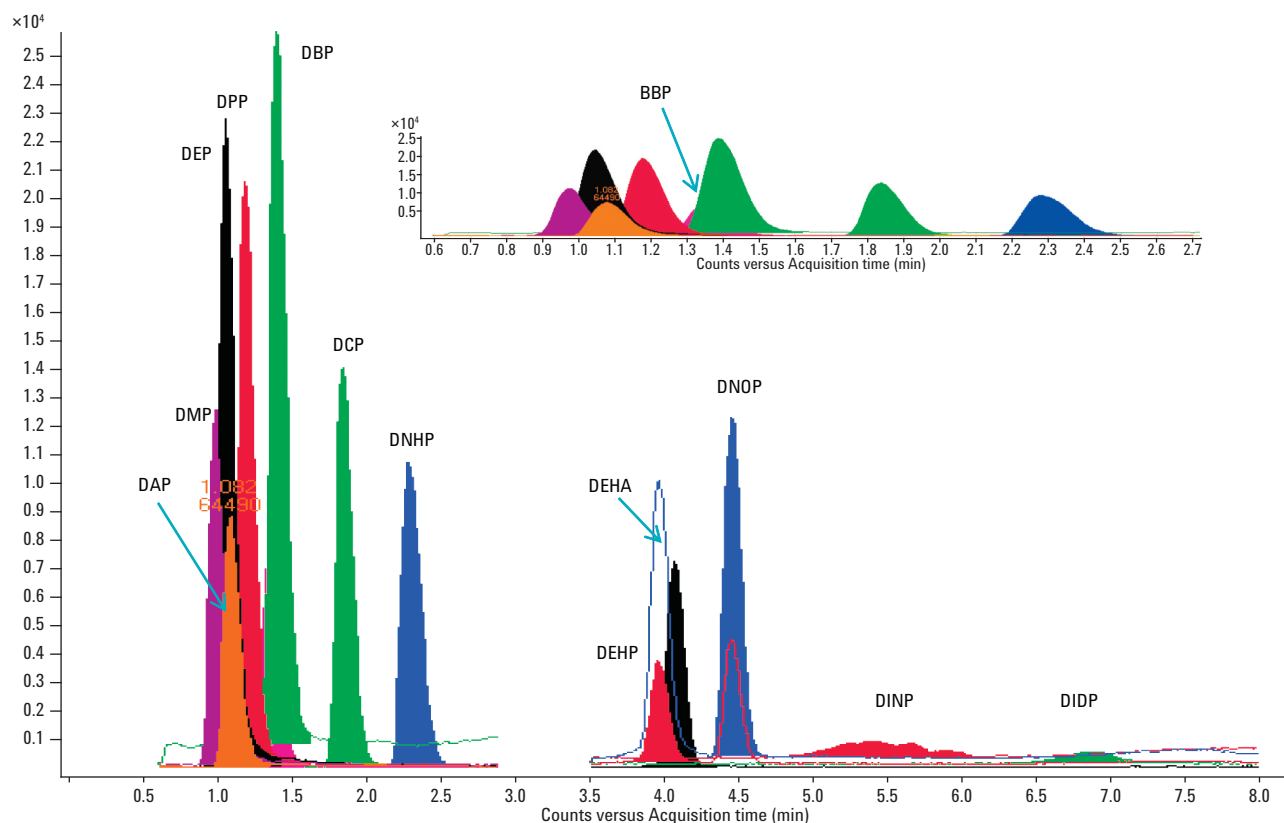


Figure 6. Extracted ion current (EIC) trace of the separation of 13 phthalates using LC/MS/MS.

Results of Sample Testing

Three beverage samples obtained from retail stores were tested using the GC methods developed here. The results of the GC/MS testing are shown in Table 10. The level of DINP in sample SN1874 and the levels of DEHP, DINP, and DNOP in sample SA2549 are above China and Taiwan mandated limits (1 ppm). In fact, the level of DINP in sample 2549 was more than 100 times the allowed limit.

Sport drink samples were also analyzed for several phthalates using LC/MS/MS. A matrix calibration curve was constructed by spiking DEHP standard into a sport drink and then extracted using the sample preparation method (Figure 7). The curve was used to quantify DEHP in another sports drink sample. The qualifier ion ratios were 1.7 and 2.0 for two qualifier ions, matching the criteria for identification of DEHP standards. The concentration of DEHP in the sports drink was measured at 1.05 ppm, just above the Taiwan FDA limit of 1ppm.

Table 10. GC/MS Analysis Results for Three Beverage Samples

	DEHP (mg/L)	DINP (mg/L)	DNOP (mg/L)
SN610	ND	ND	ND
SN1874	ND	2.13	ND
SA2549	72.5	137	19.0

ND = not detected

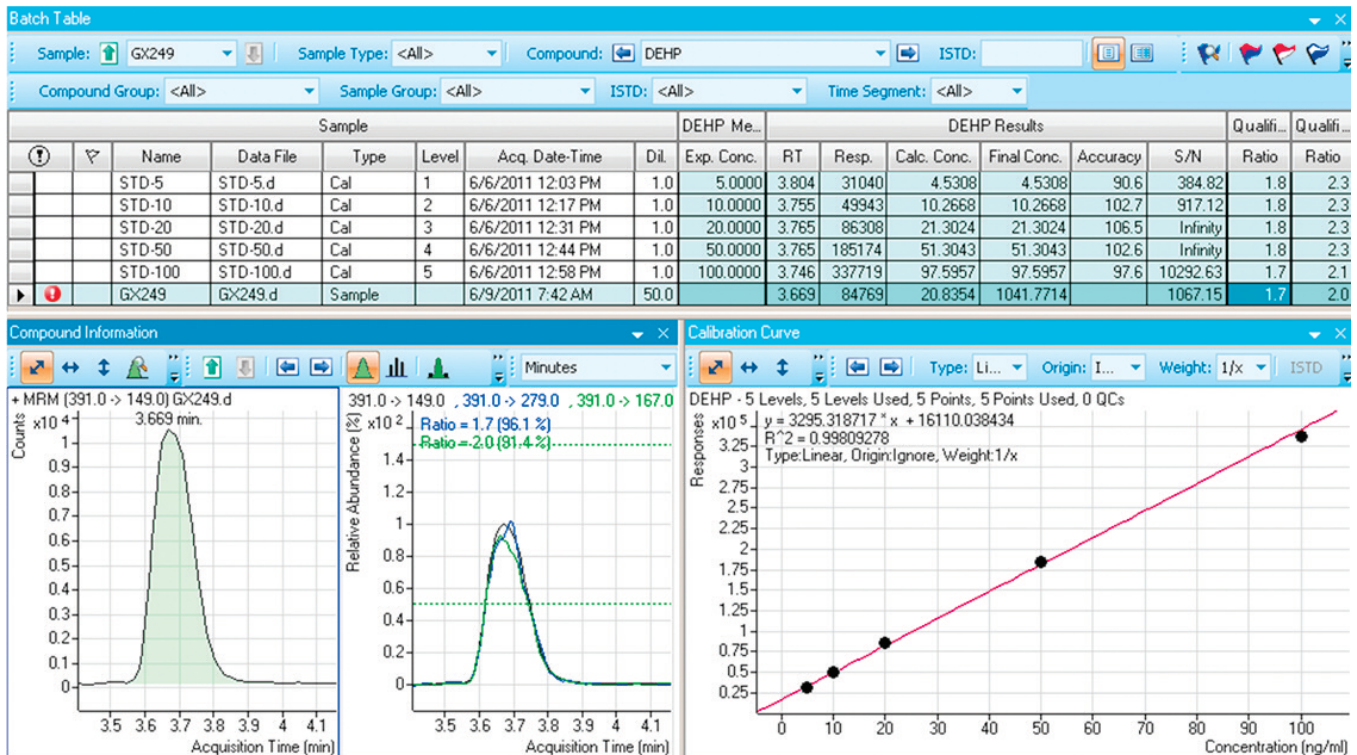


Figure 7. LC/MS/MS calibration curve for DEHP spiked into a sports drink sample.

Conclusions

Agilent has developed methods for the rapid detection and quantification of phthalates in food to meet the needs of most laboratories, regardless of the MS platform that they use or whether they need a qualitative survey of all 30 phthalates or careful quantification of very low levels of the 6 that are highly regulated in the EU and USA. The versatility and capability of Agilent GC/MS and LC/MS systems enable rapid response to emerging food safety crises.

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