

Determination of Phthalates in Drinking Water by UHPLC with UV Detection

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Key Words

Phthalate Esters, Acclaim C30 Column, EPA Method 606

Goal

To develop an efficient high-performance liquid chromatography (HPLC) method for the simultaneous determination of 19 phthalate compounds in drinking water. The 19 target analytes cover those specified in European Union (EU) Directive 2005/84/EC;¹ U.S. Environmental Protection Agency (EPA) Methods 606 and 8061A;^{2,3} the Chinese HJ/T 72-2001;⁴ and the Standardization Administration of China (SAC) GB/T 20388-2006⁵ and GB/T 21911-2008.⁶

Introduction

Phthalates are a class of chemical compounds widely used as plasticizers for polyvinyl chloride resins, adhesives, and cellulose film coating. To date nearly 20 kinds of phthalates (structures shown in Figure 1) have been used for these purposes. Phthalates are potentially hazardous to human health—especially to children's health—due to their classification as endocrine disruptors. This has resulted in regulations regarding the types and levels of phthalates allowable in plastic toys,¹ water containers,²⁻⁴ textiles,⁵ and foods.⁶ For example, Directive 2005/84/EC¹ lists six phthalates (Table 1) that need to be monitored when used as plasticizers in toys and childcare articles and, if present, must be at concentrations $\leq 0.1\%$ of the mass of the product.



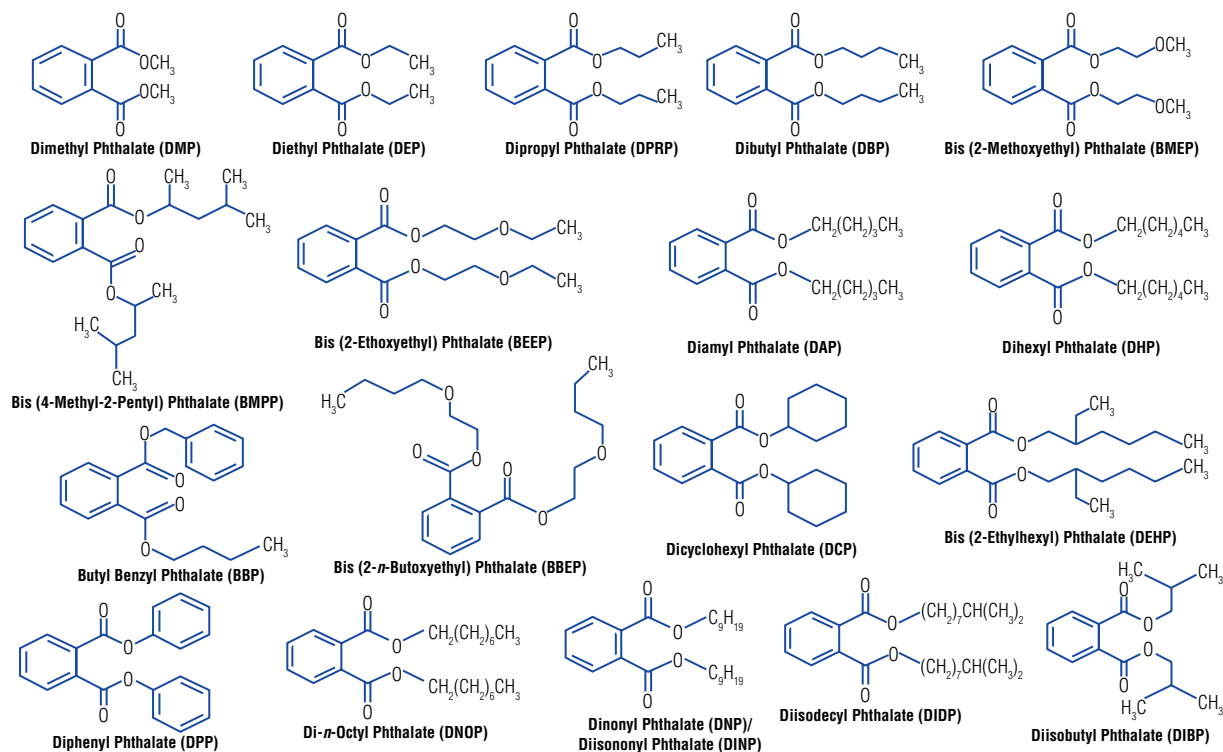


Figure 1. Structures of 19 phthalates (isomers DNP and DINP have the same structure).

Table 1. Regulated phthalates in standard methods.

Standard Methods and Directive	Directive 2005/84/EC	EPA Method 606	EPA Method 8061A	HJ/T 72-2001	GB/T 20388-2006	GB/T 21911-2008
Matrix	Toys/ Childcare Articles	Municipal/ Industrial Wastewater	Aqueous/ Solid Matrices	Water	Textile	Foods
Detection	—	GC-ECD	GC-ECD	HPLC-UV	GC-MS	GC-MS
DMP		•	•	•	•	•
DEP		•	•		•	•
DIBP					•	•
DBP	•	•	•	•	•	•
DPRP					•	
BMEP						•
BMPP						•
BEEP						•
DAP					•	•
DHP					•	•
BBP	•	•	•		•	•
BBEP						•
DCP						•
DEHP	•	•	•		•	•
DPP						•
DNOP	•	•	•	•	•	•
DNP	•				•	•
DINP	•				•	
DIDP	•				•	

Table 2. Preparation of calibration curve standards.

Stock Std of Phthalate Calibration Mixture	Vol of Stock Std of Phthalate Calibration Mixture (μL)	Vol of CH ₃ OH-H ₂ O Solution (3:1, v/v)	Final Vol of Calibration Std (μL)	Final Conc of Calibration Std (μg/mL)
Mixture 1: 50 (μg/mL)	20	980	1000	1.0
	100	900		5.0
	200	800		10
Mixture 2: 0.5 (μg/mL)	100	900		0.05
	200	800		0.1
	1000	0		0.5

Gas chromatography (GC) and HPLC are frequently used techniques for the determination of phthalate compounds,⁷⁻¹⁴ and use of Fourier transform infrared spectroscopy (FT-IR) has been reported as well.¹⁵ Some GC and HPLC standardized methods have been created.²⁻⁶ For example, EPA Methods 606² and 8106A³ contain a GC-electron capture detector (ECD) method to determine six phthalates (Table 1) in municipal/industrial wastewater and aqueous/solid matrices (including ground water, leachate, soil, sludge, and sediment), respectively. HJ/T 72-2001¹⁴ uses an HPLC-UV method for the determination of three phthalates (Table 1) in industrial wastewater and ground water. Because all phthalates are prohibited as a food additive, GB/T 21911-2008⁶ provides a GC-mass spectrometry (MS) method for the determination of 16 phthalates in food samples (Table 1).

Some of the existing phthalate determination methods have reported weaknesses. For example, a capillary GC method does not provide sufficient resolution for some phthalate isomers, such as DINP and DIDP, even with MS detection.¹⁶ The same report suggests a reversed-phase HPLC method might improve the resolution between DINP and DIDP using either a C8 or C18 stationary phase with gradient elution. However, a simultaneous determination of phthalates by HPLC that can separate all the phthalates listed in the enacted standard methods¹⁻⁶ (Table 1) has not been published. In this application note we describe a method that can separate all the phthalates listed in the standard methods.

Equipment

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system, including:
 - LPG-3400RS Quaternary Pump with SRD-3400 Integrated Solvent and Degasser Rack
 - WPS-3000TRS Wellplate Sampler, Thermostatted with 100 μL sample loop
 - TCC-3000RS Thermostatted Column Compartment
 - DAD-3000RS Diode Array Detector with 13 μL flow cell
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software version 6.80, SR9 or higher

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Methanol (CH₃OH), HPLC Grade (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH₃CN), HPLC Grade (Fisher Scientific P/N AC610010040)
- A mixture of 16 phthalates standard solution for GB/T 21911-2008, including DMP, DEP, DIBP, DBP, BMEP, BMPP, BEEP, DAP, DHP, BBP, BBEP, DCP, DEHP, DPP, DNOP, and DNP; 1000 μg/mL in hexane for each component (ANPEL Scientific Instrument Co., Ltd., Shanghai, China)
- DPRP, DINP, and DIDP; 1000 μg/mL in hexane (ANPEL Scientific Instrument Co., Ltd., Shanghai, China)

Working Standard Solutions for Calibration

Mixture 1 Stock Standard of Phthalate Calibration (50 μg/mL)

Dilute 50 μL of the mixture of 16 phthalates standard solution (1000 μg/mL for each component) and 50 μL of DPRP (1000 μg/mL) to 1 mL with 900 μL of a methanol/water solution (3:1, v/v).

Mixture 2 Stock Standard of Phthalate Calibration (0.5 μg/mL)

Dilute 10 μL of Mixture 1 (50 μg/mL) to 1 mL with 990 μL of a methanol/water solution (3:1, v/v).

Prepare six working standard solutions for the calibration with 0.05, 0.1, 0.5, 1.0, 5.0, and 10 μg/mL concentrations by adding the proper amounts of stock standard of phthalate calibration Mixtures 1 and 2 and a methanol/water solution (3:1, v/v). The volumes of each solution needed to make the calibration standards are shown in Table 2.

Sample Preparation

Bottled drinking water samples were purchased from a local market.

Pipet 4 mL of each drinking water sample into a 5 mL volumetric flask, bring to volume with methanol, and mix for 2 min. Filter the solutions through a 0.45 μm filter prior to injection.

Table 3. Separation status of 16 phthalates on different columns.

Peak No.	Phthalates	Columns				
		Acclaim 120, C18	Acclaim C30	Acclaim PA	Acclaim PA2	Hypersil GOLD
1	DMP	√	√	✕	✕	√
2	BMEP	√	√			√
3	DEP	√	√	√	✕	✕
4	BEEP	√	√	√		
5	DPP	✕	√	√	✕	√
6	BBP		√	√		√
7	DIBP	√	✕	√	√	✕
8	DBP	✕		√	√	
9	BBEP			√	√	
10	DAP	✕	✕	✕	✕	✕
11	DCP					
12	BMPP	√	√	√	√	√
13	DHP	√	√	√	√	√
14	DEHP	√	√	√	√	√
15	DNOP	√	√	√	√	√
16	DNP	√	√	√	√	√

Note: ✓ represents separated and ✗ represents not separated.

Conditions

Column:	Thermo Scientific™ Acclaim™ C30, 3 µm, Analytical, 3.0 × 150 mm (P/N 075724)
Mobile Phase:	A: Water B: Acetonitrile C: Methanol
Gradient:	0 min, B: 35%, C: 0%; 12–22 min, B: 25–100%, C: 45–0%, curve 5–3; 22.5–25 min, B: 35%, C: 0%
Flow Rate:	1.0 mL/min
Injection Volume:	5 µL
Temperature:	45 °C
Detection:	UV absorbance at 228 nm

Columns: A. Acclaim 120, C18, 3 µm, Analytical, 2.1 × 150 mm
B. Acclaim C30, 3 µm, Analytical, 3.0 × 150 mm
C. Acclaim PA, 3 µm, Analytical, 2.1 × 150 mm
D. Acclaim PA2, 2.2 µm, Analytical, 2.1 × 150 mm
E. Hypersil GOLD, 3 µm, 100 L × 4.6 mm i.d.

Mobile Phase: A: Water
B: CH₃OH

Gradient: 0–30 min, 40–100% B; 35 min, 100% B; 35.5–40 min, 40% B

Flow Rate: A,C: 0.5 mL/min; D: 0.35 mL/min; B: 1.0 mL/min; E: 1.5 mL/min

Inj. Volume: 5 µL

Temperature: 30 °C

Detection: UV absorbance at 228 nm

Peaks: 1. DMP 2. BMEP 3. DEP
4. BEEP 5. DPP 6. BBP
7. DIBP 8. DBP 9. BBEP
10. DAP 11. DCP 12. BMPP
13. DHP 14. DEHP 15. DNOP
16. DNP

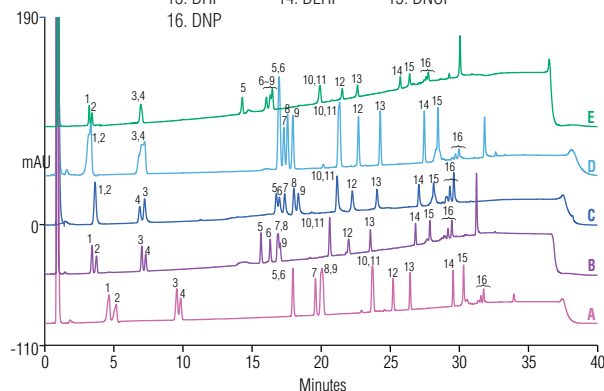


Figure 2. Chromatograms of phthalates listed in GB/T 21911-2008 using five different columns.

Results and Discussion

Phthalate Separation

The HJ/T 72-2001 HPLC method for the determination of DMP, DBP, and DNOP in industrial wastewater uses a cyano column.⁴ This method uses a C18 column for both the determinations of DMP, DEP, and DBP from materials that come in contact with food,¹⁰ as well as for DBP and DEHP in environmental water samples.¹²

In the work shown here, five columns—the Acclaim PolarAdvantage (PA); Acclaim PA2; Acclaim 120, C18; Acclaim C30; and the Thermo Scientific™ Hypersil GOLD™ columns—were evaluated for the separation of the 16 phthalates listed in GB/T 21911-2008. Figure 2 shows the chromatograms and Table 3 summarizes the separation results on the five columns under the same chromatographic conditions. Two phthalates—DAP (Peak 10) and DCP (Peak 11)—were not separated on any of the columns. Two other phthalates—DMP (Peak 1) and BMEP (Peak 2)—were not separated on the Acclaim PA and PA2 column. Three phthalates—DIBP (Peak 7), DBP (Peak 8), and BBEP (Peak 9)—were not separated on

the Acclaim C30 column. Four phthalates—DPP (Peak 5), BBP (Peak 6), DBP (Peak 8), and BBEP (Peak 9)—were not separated on the Acclaim 120, C18 column. Five phthalates—DEP (Peak 3), BEEP (Peak 4), DIBP (Peak 7), DBP (Peak 8), and BBEP (Peak 9)—were not separated on the Hypersil GOLD column. Six phthalates—DMP (Peak 1), BMEP (Peak 2), DEP (Peak 3), BEEP (Peak 4), DPP (Peak 5), and BBP (Peak 6)—were not separated on the Acclaim PA2 column.

Although the number of unresolved phthalates was smallest using the Acclaim PA column, it can be deduced that the separation of DMP (Peak 1) and BMEP (Peak 2) will be more difficult to achieve due to their stronger polarity (earliest elution), as compared to unresolved compounds on the other columns. Therefore, the Acclaim C30 column was chosen for further evaluation.

Optimization of Chromatographic Conditions

The effect of mobile phase composition was also explored to optimize chromatographic conditions for this analysis. Three mobile phase systems—CH₃CN/water, CH₃OH/water, and CH₃CN/CH₃OH/water—were investigated for the separation of phthalates. Experiments showed that neither the CH₃CN/water nor CH₃OH/water mobile phase system provided satisfactory resolution for the separation of DPP (Peak 5), BBP (Peak 6), DIBP (Peak 7), DBP (Peak 8), and BBEP (Peak 9). Therefore, the CH₃CN/CH₃OH/water mobile phase system was tried and the effect of column temperature on resolution was investigated.

Figure 3 shows the chromatogram of DPRP, DNP, and DIDP—together with the 16 phthalates listed in GB/T 21911-2008—under optimized chromatographic conditions. Good separation of the 19 phthalates was achieved with the exception of two compounds—DNP (Peak 16) and DIDP (Peak 19). The incomplete resolution of DNP and DIDP may affect their quantification by UV detection. However, the incomplete resolution between DNP and DIDP will not be a hindrance to MS detection because the CH₃CN/CH₃OH/water mobile phase is MS compatible.

Reproducibility, Linearity, and Detection Limits

Method precision was estimated by making eight consecutive injections of a calibration standard composed of 17 phthalates (DINP and DIDP not included) with a concentration of 5 µg/mL for each. The RSD of each of the 17 analytes was ≤0.1% for retention time and ≤1.5% for peak area, showing good precision.

Calibration linearity for UV detection of the 17 phthalates was investigated by making five consecutive injections of a mixed standard solution prepared at six different concentrations (30 total injections). The external standard method was used to establish the calibration curve and quantify these phthalates in drinking water samples. Excellent linearity was observed from 0.05 to 10 µg/mL when plotting the concentration versus the peak area, and the coefficients of determination were ≥0.99 for all analytes (Table 4).

Method detection limits (MDLs) of 17 phthalates using UV detection were calculated using the single-sided Student's *t* test method (at the 99% confidence limit). Eight consecutive injections of a drinking water sample mixed with a mixed standard solution (1 µg/mL) were used to determine the standard deviation value for calculating MDLs; the results were MDLs ≤0.02 µg/mL for each analyte, showing good method sensitivity.

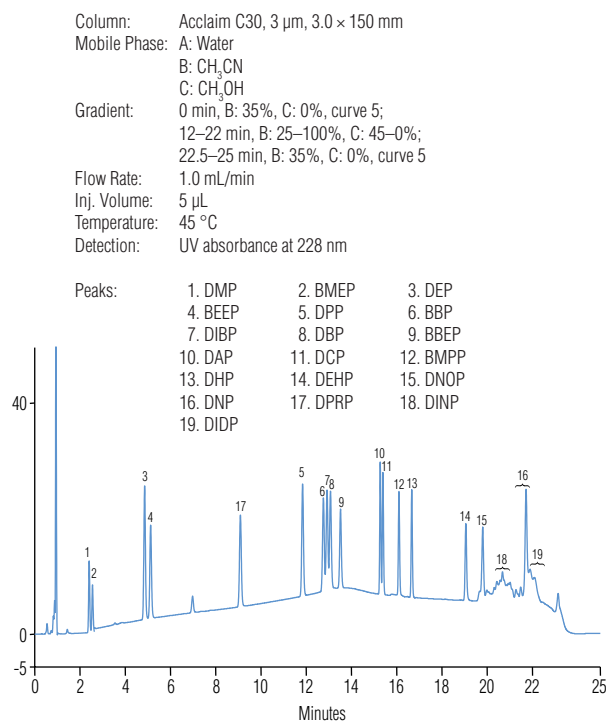


Figure 3. Chromatogram of DPRP, DINP, DIDP, and the 16 phthalates listed in GB/T 21911-2008 using an Acclaim C30 column.

Table 4. Method linearity data.

Analyte	Regression Equation	r ²	Range (µg/mL)
DMP	A = 2.0813c + 0.3169	0.9987	0.05–10
BMEP	A = 4.7338c + 0.3031	0.9990	
DIBP	A = 3.4175c + 0.3236	0.9991	
DBP	A = 3.4175c + 0.3236	0.9962	
DPP	A = 3.5302c + 0.2286	0.9935	
BBP	A = 2.8745c + 0.2430	0.9946	
DIBP	A = 2.6872c + 0.1741	0.9947	
DBP	A = 3.0726c + 0.2305	0.9982	
BBEP	A = 2.8730c + 0.2143	0.9997	
DAP	A = 1.9798c + 0.2256	0.9987	
DCP	A = 2.4258c + 0.1394	0.9963	
BMPP	A = 1.7087c + 0.1628	0.9994	
DHP	A = 2.1026c + 0.1985	0.9996	
DEHP	A = 1.9963c + 0.3536	0.9994	
DNOP	A = 2.3658c + 0.1879	0.9902	
DNP	A = 2.6389c + 0.1836	0.9936	
DPRP	A = 2.3759c + 0.1565	0.9993	

Sample Analysis

Figure 4 compares the chromatograms of an unadulterated drinking water sample with the same sample spiked with a 5 µg/mL mixed phthalate standard. No detectable levels of phthalates were found in the unspiked sample. The analysis results and related data are summarized in Table 5. These data show excellent spiked recovery (77–110%) for each phthalate, thereby demonstrating method accuracy.

Conclusion

The work shown here describes an efficient HPLC method using UV detection for the determination of phthalates in drinking water samples. All 19 phthalates listed in key environmental regulatory documents—EU Directive 2005/84/EC; U.S. EPA Methods 606 and 8061A; the Chinese HJ/T 72-2001; and the Standardization Administration of China (SAC) GB/T 20388-2006 and GB/T 21911-2008—are well separated on the Acclaim C30 column (3 µm, 3.0 × 150 mm), and the separation time is <25 min.

Column: Acclaim C30, 3 µm, 3.0 × 150 mm
 Mobile Phase: A: Water
 B: CH₃CN
 C: CH₃OH
 Gradient: 0 min, B: 35%, C: 0%, curve 5;
 12–22 min, B: 25–100%, C: 45–0%;
 22.5–25 min, B: 35%, C: 0%, curve 5
 Flow Rate: 1.0 mL/min
 Inj. Volume: 5 µL
 Temperature: 45 °C
 Detection: UV absorbance at 228 nm
 Chromatograms: (a) Drinking Water Sample #1
 (b) A sample spiked with a phthalate mixed standard

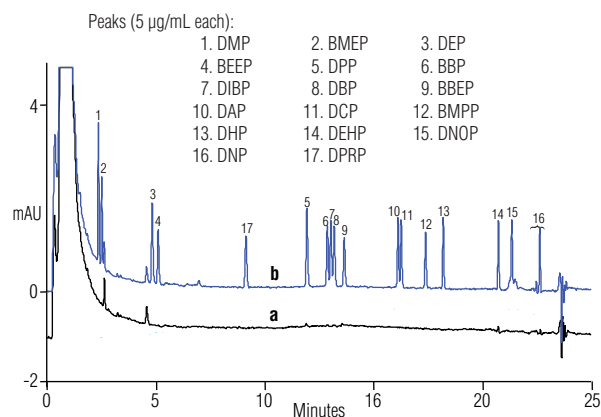


Figure 4. Blank-subtracted chromatograms of a drinking water sample and the same sample spiked with a phthalate mixed standard using an Acclaim C30 column.

Sample #1					Sample #2			
Analyte	Detected (µg/mL)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Detected (µg/mL)	Added (µg/mL)	Found (µg/mL)	Recovery (%)
DMP	Not Detected	1.0	0.95	95	Not Detected	1.0	0.87	87
		5.0	4.6	92		5.0	4.7	94
BMEP		1.0	1.1	110		1.0	1.04	104
		5.0	4.7	94		5.0	5.5	110
DEP		1.0	0.96	96		1.0	0.97	97
		5.0	4.9	98		5.0	4.4	88
BEEP		1.0	0.93	93		1.0	0.95	95
		5.0	5.1	102		5.0	5.1	102
DPP		1.0	0.97	97		1.0	0.94	94
		5.0	5.2	104		5.0	4.5	90
BBP		1.0	1.04	104		1.0	1.1	110
		5.0	5.2	104		5.0	5.3	106
DIBP		1.0	0.87	87		1.0	0.85	85
		5.0	4.9	98		5.0	4.9	99
DBP		1.0	0.89	89		1.0	0.96	96
		5.0	4.8	98		5.0	4.9	98
BBEP		1.0	0.96	96		1.0	0.85	85
		5.0	4.9	98		5.0	5.2	104
DAP		1.0	1.0	100		1.0	0.89	89
		5.0	5.0	100		5.0	4.6	92
DCP		1.0	0.85	85		1.0	0.96	96
		5.0	4.6	92		5.0	4.4	88
BMPP		1.0	0.94	94		1.0	1.0	100
		5.0	4.8	96		5.0	5.0	100
DHP		1.0	0.98	98		1.0	0.94	94
		5.0	5.1	102		5.0	4.7	94
DEHP		1.0	0.87	87		1.0	0.98	98
		5.0	4.4	88		5.0	4.7	94
DNOP		1.0	1.1	110		1.0	0.85	85
		5.0	5.3	106		5.0	4.5	90
DNP		1.0	0.85	85		1.0	0.88	88
		5.0	4.3	86		5.0	4.4	88
DPRP		1.0	0.78	78		1.0	0.77	77
		5.0	4.0	80		5.0	4.1	82

References

1. Directive 2005/84/EC. Official Journal of the European Union, Amending for the 22nd Time Council Directive 76/769/EEC on the Phthalates in Toys and Childcare Articles, 2005.
2. *Method 606: Method for Organic Chemical Analysis of Municipal and Industrial Wastewater—Phthalate Esters*; U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984.
3. *Method 8061A: Phthalate Esters by Gas Chromatography with Electron Capture Detection (GC/ECD)*; Revision 1; U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1996.
4. HJ/T 72-2001: *Water Quality-Determination of Phthalates (Dimethyl, Dibutyl, and Dioctyl)-Liquid Chromatography*; Ministry of Environmental Protection of the People's Republic of China, Environmental Protection Industry Standards of the People's Republic of China: Beijing, 2001.
5. GB/T 20388-2006: *Textiles—Determination of the Content of Phthalates*; Standardization Administration of China (SAC), General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China: Beijing, 2006.
6. GB/T 21911-2008: *Determination of Phthalate Esters in Foods*; Standardization Administration of China (SAC), General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China: Beijing, 2008.
7. Thermo Scientific Application Note 52282: Determination of DEHP in Culture Media by GC-MS/MS Using PCI Ammonia. Austin, TX, 2012. [Online: www.thermoscientific.com/ecommm/servlet/techresource?resourceId=99636&storeId=11152&from=search# (accessed Dec 3, 2012).
8. Zhang, Y.H.; Zheng, L.X.; Chen, B.H. Phthalate Exposure and Human Semen Quality in Shanghai: A Cross-Sectional Study. *Biomed. and Environ. Sci.* **2006**, *19*, 205–209.
9. Amiridou, D.; Voutsas, V. Alkylphenols and Phthalates in Bottled Waters. *J. Hazard. Mater.* **2011**, *185*, 281–286.
10. Jen, J.F.; Liu, T.C. Determination of Phthalate Esters from Food-Contacted Materials by On-Line Microdialysis and Liquid Chromatography. *J. Chromatogr., A* **2006**, *1130*, 28–33.
11. Carrillo, J.D.; Salazar, C.; Moreta, C.; Tena M.T. Determination of Phthalates in Wine by Headspace Solid-Phase Microextraction Followed by Gas Chromatography–Mass Spectrometry: Fibre Comparison and Selection. *J. Chromatogr., A* **2007**, *1164*, 248–261.
12. Cháfer-Pericás, C.; Campíns-Falcó, P.; Prieto-Blanco M.C. Automatic In-Tube SPME and Fast Liquid Chromatography: A Cost-Effective Method for the Estimation of Dibutyl and Di-2-Ethylhexyl Phthalates in Environmental Water Samples. *Anal. Chim. Acta* **2008**, *610*, 268–273.
13. Casajuana, N.; Lacorte, S. Presence and Release of Phthalic Esters and Other Endocrine Disrupting Compounds in Drinking Water. *Chromatographia* **2003**, *57*, 649–655.
14. Determination of Seven Phthalates and Four Parabens in Cosmetic Products Using HPLC-DAD and GC-MS Methods. *J. Sep. Sci.* **2007**, *30*, 48–54.
15. Thermo Scientific Application Note 52157: Enhanced Sensitivity to Detect Phthalates by FT-IR. Madison, WI, 2011. [Online: www.thermoscientific.com/ecommm/servlet/techresource?resourceId=97830&storeId=11152&from=search (accessed Dec 3, 2012).
16. Fang, L.P.; Niu, Z.Y.; Cai, F.; Sun, J. Development of the Analysis Method of Phthalate Plasticizers. *Polymers & Additives* **2006**, *226* (4), 30–35.

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