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 20388-2006[5] 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 ≤0.1% of the mass of the product.

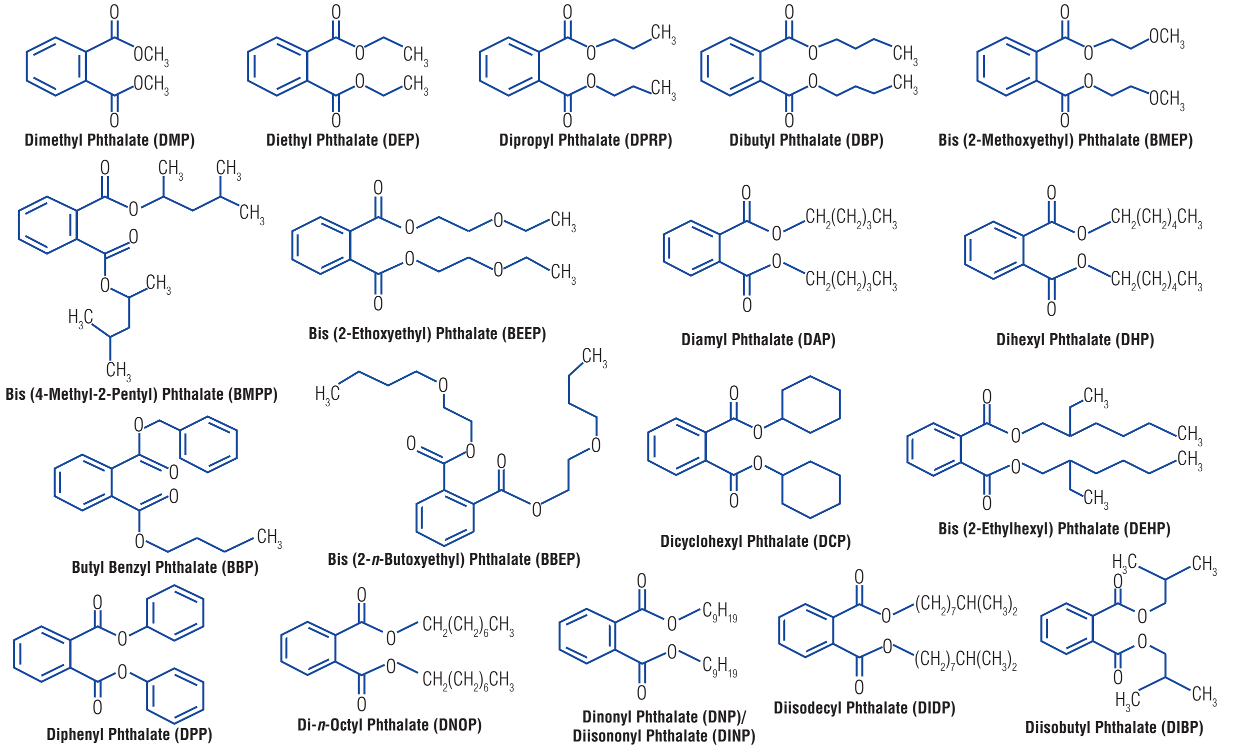

Figure 1. Structures of 19 phthalates (isomers DNP and DINP have the same structure).

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_3OH-H_2O 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 606[2] and 8106A[3] 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 72-200[14] 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 21911-2008[6] 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 methods[1-6] (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 (CH_3OH), HPLC Grade (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH_3CN), 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

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.

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: CH_3OH
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

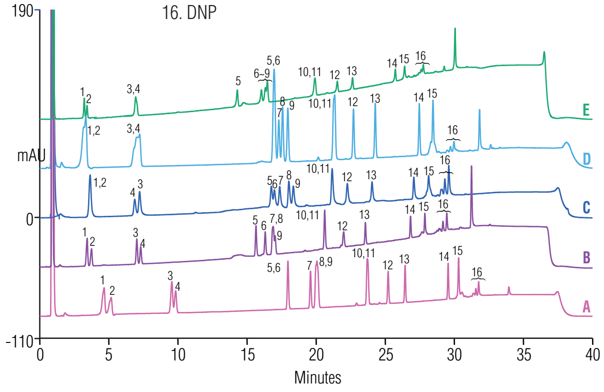

Figure 2. Chromatograms of phthalates listed in GB/T 21911-2008 using five different columns.

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—CH_3CN/water, CH_3OH/water, and CH_3CN/CH_3OH/water—were investigated for the separation of phthalates. Experiments showed that neither the CH_3CN/water nor CH_3OH/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 CH_3CN/CH_3OH/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 CH_3CN/CH_3OH/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.

Column: Acclaim C30, 3 µm, 3.0 × 150 mm
Mobile Phase: A: Water
B: CH_3CN
C: CH_3OH
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

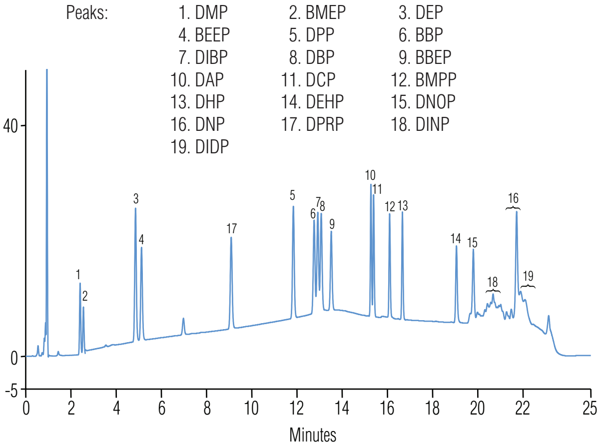

Figure 3. Chromatogram of DPRP, DINP, DIDP, and the 16 phthalates listed in GB/T 21911-2008 using an Acclaim C30 column.

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: CH_3CN
C: CH_3OH
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

Peaks (5 µg/mL each):
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 17. DPRP

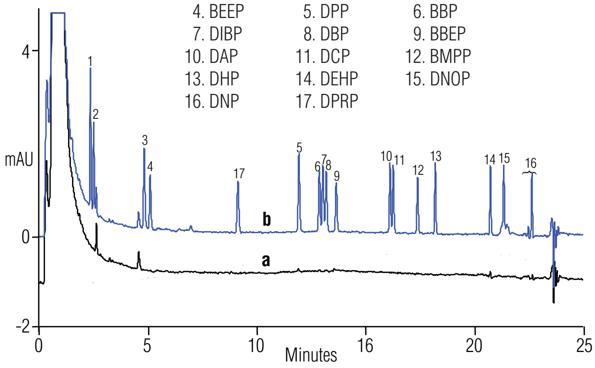

Figure 4. Blank-subtracted chromatograms of a drinking water sample and the same sample spiked with a phthalate mixed standard using an Acclaim C30 column.

Table 5. Sample analysis results.

	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

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