Optimizing Analysis of Phthalates in Plastic Toys Using the Agilent 1290 Infinity Method Development Solution

Application Note

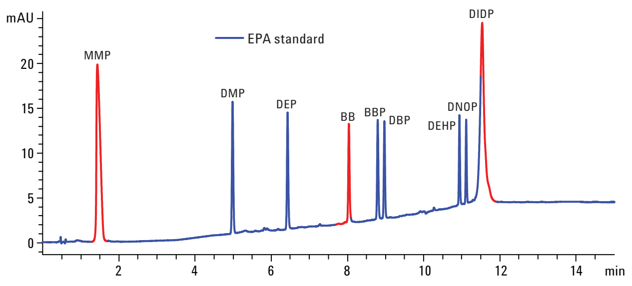
Food Testing & Agriculture

Author

A.G.Huesgen
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

Since the late nineteen eighties, phthalates have been under suspicion as a health risk for humans. From that time, official authorities in Europe, the US, China, and other countries have passed regulations for phthalates, especially in plastic toys. Many official methods are based on GC or GC/MS methods, but during the last decade LC and LC/MS methods were developed. This Application Note shows the development of an HPLC/UV method for nine phthalates using a phenyl-hexyl column and a ternary gradient. An Agilent 1290 Infinity Method Development Solution, in combination with an Agilent Method Scouting Wizard, was used for method development. For the final method precision of retention times and areas, limits of detection (LOD) and limits of quantitation (LOQ), as well as the linearity, was evaluated. Plastic material from a toy was analyzed, and the content of phthalates was determined. In addition, a fast UHPLC method was developed and compared to the high resolution method.



Agilent Technologies

Introduction

Worldwide regulations have been set for allowable levels of phthalates in plastic toys[1], municipal and waste water[2-4], textiles[5], and foods[6]. The 2005/84/EC1 directive lists six phthalates (Table 1) that need to be monitored when used as plasticizers in toys and childcare articles and must be ≤ 0.1 % of the mass of the product. Even more strict regulations are under consideration[7]. DEHP, DBP, and BBP were classified as toxic to reproduction and the EU banned them especially from baby products. A replacement could be DIDP and DINP for example, which are until now not regarded as toxic, however, these softeners are also forbidden in baby products. DINP and DIDP seem to quickly spread in the environment and to enrich in organisms. For this reason, their entry into the environment should be prohibited. The German "Umwelt Bundesamt" proposed to replace all phthalate-containing materials, such as flexible PVC, little by little with phthalate-free materials, such as polyethylene and polypropylene, where it is possible[8].

In the following, a HPLC/UV method was developed for nine phthalates, see Table 1. A phenyl-hexyl column and a C18 column were used for optimum separation. In addition, binary and ternary gradients using acetonitrile and methanol as organic phases were applied. The performance of the method was evaluated and a real-life sample was measured. In addition, the developed method was transferred to a fast UHPLC method.

Experimental

Instrumentation

	Agilent 1290 Infinity Method Development Solution
Quaternary Pump	G4204A
Autosampler	G4226A
ALS cooler	G1330B
Column 1 equipped with high pressure column switching valve	G1316C
Column 2 equipped with low pressure column switching valve	G1316C
Diode array detector	G4212A
Valve drives	G1353B
Method development kit	G4230B
Low dispersion capillary kit	G4212A

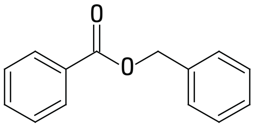
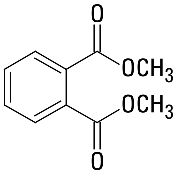
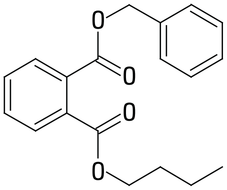
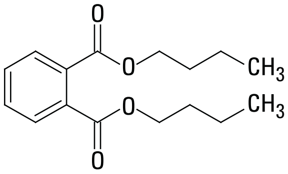
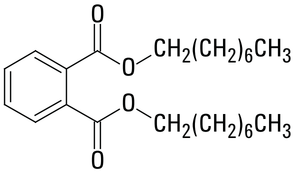
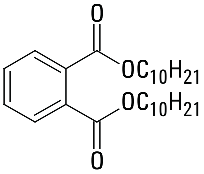
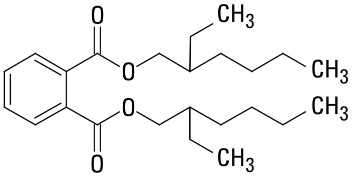
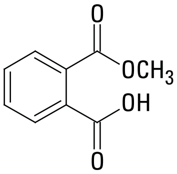
Sample preparation

A 0.05 g amount of the crushed polymer sample was dissolved in 5 mL of THF. Polymers were precipitated with 10 mL of methanol and cooled for 1 hour. When the polymers had settled, the solution was filtered through 0.45-µm Agilent Captiva Premium Syringe Filters (regenerated cellulose, p/n 5190-5111), evaporated, and then diluted with 500 µL acetonitrile.

Chromatographic Conditions for Method Development	
Compounds	9 Phthalates
Column 1	Agilent ZORBAX Eclipse Plus Phenyl Hexyl, 3 × 100 mm, 3.5 µm, p/n 959961-312
Column 2	Agilent ZORBAX Eclipse Plus C-18, 3 × 100 mm, 3.5 µm, p/n 959961-302
Mobile phases	Water (A), Acetonitrile (B), Methanol (C)
Gradient 1	at 0 minutes 10 % B, at 10 minutes 90 % B
Gradient 2	at 0 minutes 10 % C, at 10 minutes 90 % C
Gradient 3	at 0 minutes 5 % B, 5 % C, at 10 minutes 45 % B, 45 % C
Flow rate	1 ml/min
Injection volume	1 µL
Column temperature	40 °C
Detection	228/6 nm, Reference 380/80 nm, 20 Hz, 10-mm cell
Chromatographic Conditions for High-Resolution UHPLC	
Column 1	Agilent ZORBAX RRHT Eclipse Plus Phenyl Hexyl, 3 × 100 mm, 1.8 µm, p/n959964-312
Mobile phases	Water (A), Acetonitrile (B), Methanol (C)
Gradient	at 0 minutes 5 % B and 5 % C, at 10 minutes 45 % B and 45 % C, at 12.45 minutes 45 % B and 45 % C, at 12.5 minutes 90 % B, at 14 minutes 5 % B and 5 % C
Flow rate	1 mL/min
Run time	15 minutes
Post time	3 minutes
Injection volume	1 µL
Column temperature	40 °C
Detection	228/6 nm, Reference 380/80 nm, 20 Hz, 10-mm cell
Chromatographic Conditions for Fast UHPLC	
Column 1	Agilent ZORBAX RRHT Eclipse Plus Phenyl Hexyl, 2.1 × 50 mm, 1.8 µm, p/n 959757-912
Mobile phases	Water (A), Acetonitrile (B), Methanol (C)
Gradient	at 0 minutes 5 % B and 5 % C, at 2.5 minutes 45 % B and 45 % C
Flow rate	2 mL/min
Run time:	3.5 minutes
Post time:	1 minute
Injection volume:	1 µL
Column temperature:	50 °C
Detection:	228/6 nm, Reference 380/80 nm, 20 Hz, 10-mm cell

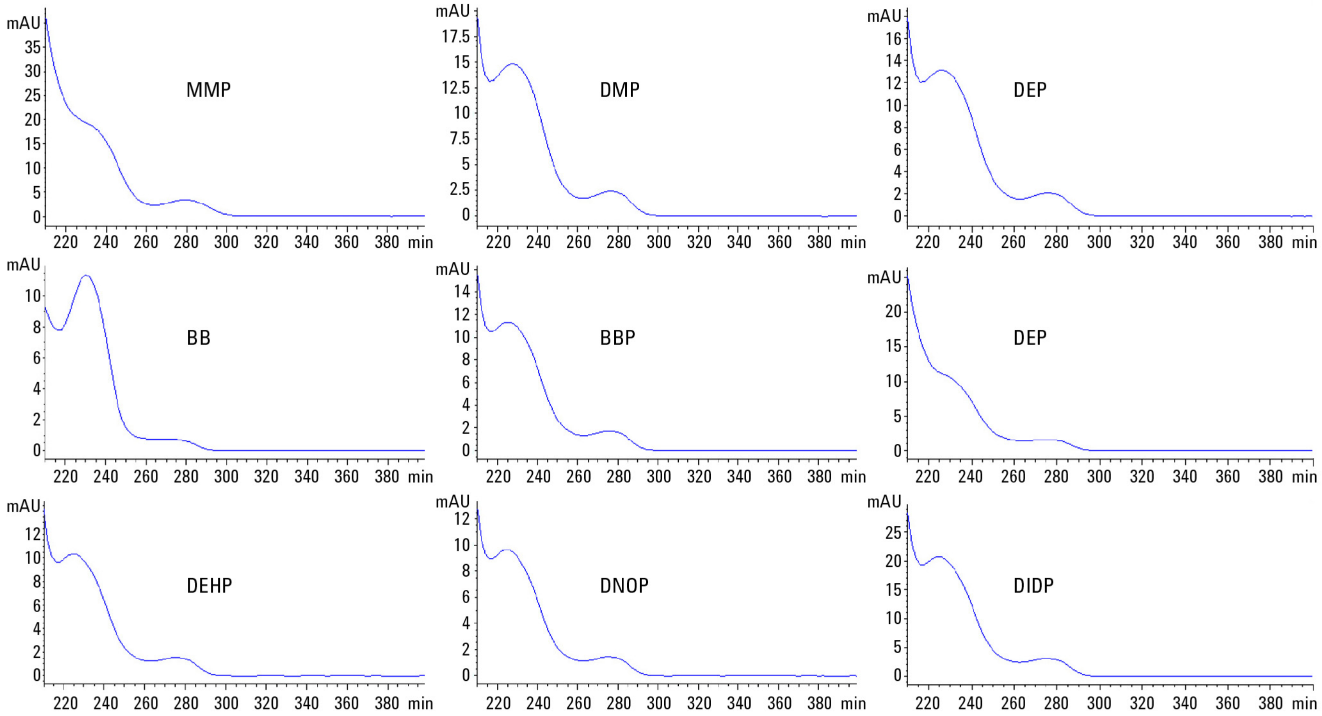
Analyzed compounds

Table 1. Analyzed samples.

Name classification by EU	Abbreviation	Structure
Benzylbenzoate suspected allergen	BB	
Dimethyylphthalate (EPA standard)	DMP	
Butyl benzyl phthalate reprotoxic (EPA standard)	BBP	
Dibutyl phthalate reprotoxic (EPA standard)	DBP	
Di-n-octyl phthalate potential risk (EPA standard)	DNOP	
Di-isodecyl phthalate potential risk	DIDP	
Di-(2-ethylhexyl) phthalate reprotoxic	DEHP	
Mono-methylphthalate (degradation product in urine)	MMP	

The EPA Phthalate ester mix (48805-U), diisodecylphthalate (80135-10 mL) and monomethylphthalate (36926-250 mg) and Bezylbenzoate (N11182-1g) were purchased from Sigma-Aldrich, Germany.

Spectra of analyzed compounds



Acquisition and evaluation software

Agilent OpenLAB CDS ChemStation version C.01.05

Agilent Method Scouting Wizard version A.02.02

Results and Discussion

The following workflow was used:

- Method development using different columns and different mobile phases
- Method validation of the final high resolution UHPLC method
- Analysis of real life sample
- Development of a fast UHPLC method
- Comparison of high resolution UHPLC versus fast UHPLC

Method development

Two columns of different selectivity, three gradients with either acetonitrile, methanol, or a combination of acetonitrile and methanol as organic phase were applied. The EPA standard with six compounds was analyzed in one vial. Two more vials containing the other compounds were tested the same way.

The method scouting was finished after 10.5 hours as each sample run took approximately 18 minutes. For more information about the 1290 Infinity Method Development Solution, see References[9,10]. The best separation was obtained using the phenyl-hexyl column in combination with acetonitrile and methanol as organic phase (Figure 1).

Using the same chromatographic conditions, the C18 column provided less resolution for DEHP, DNOP, and DIDP. To further increase resolution and signal-to-noise, the phenyl-hexyl column with 3.5-µm particles was replaced by a phenyl-hexyl column with 1.8-µm particles (Figure 2). All other column dimensions were kept the same.

Resolution, peak width, and peak height were improved.

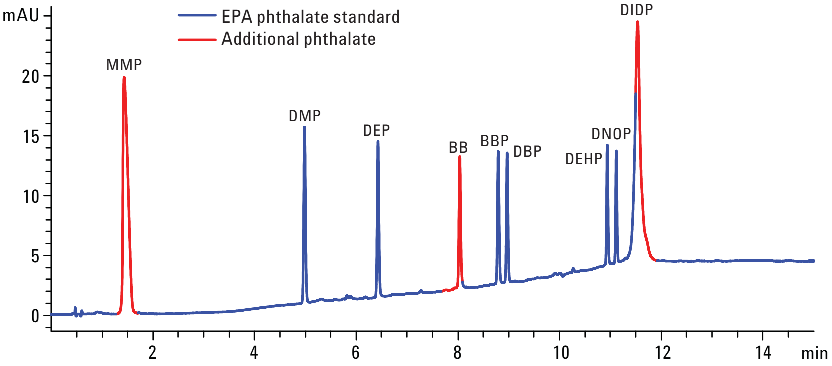


Figure 1. Analysis of phthalates using the phenyl-hexyl column and acetonitrile and methanol in combination.

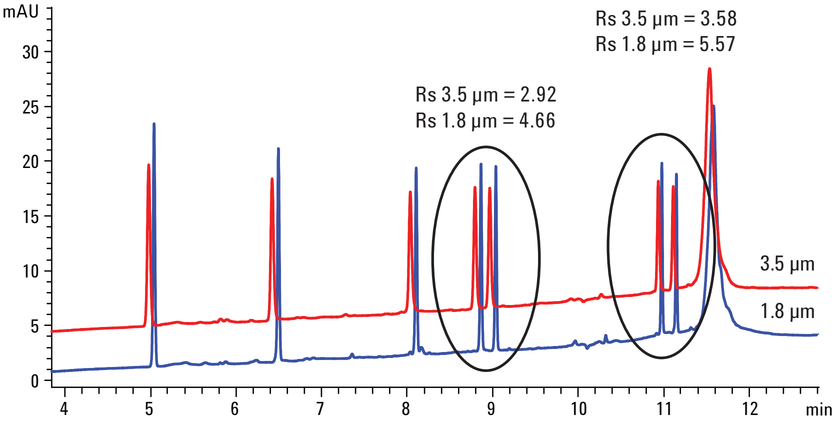


Figure 2. Comparison of different particle sizes.

Method Performance

Based on experiments obtained on the phenyl-hexyl column with 1.8-µm particles, the following method performance parameters were evaluated (Tables 2 and 3):

- Precision of retention times
- Precision of areas
- Linearity
- LOD and LOQ

Table 2 shows the combined precision data for retention times and areas. The precision for retention times was typically < 0.01 % RSD. The precision for the areas was typically < 0.73 % RSD.

The LOD and LOQ was determined by injecting low-level amounts of phthalates (Figure 3).

Table 3 shows the combined results for LOD, LOQ, and resolution.

Table 2. Precision of retention times and areas.

Compound	RSD RT (%)	RSD amount (%)
MMP (600 ng)	0.099	0.594
DMP (20 ng)	0.007	0.701
DEP (20 ng)	0.005	0.163
BB (90 ng)	0.007	0.717
BBP (20 ng)	0.01	0.574
DBP (20 ng)	0.009	0.506
DEHP (20 ng)	0.007	0.521
DNOP (20 ng)	0.007	0.568
DIDP (1,800 ng)	0.009	0.728

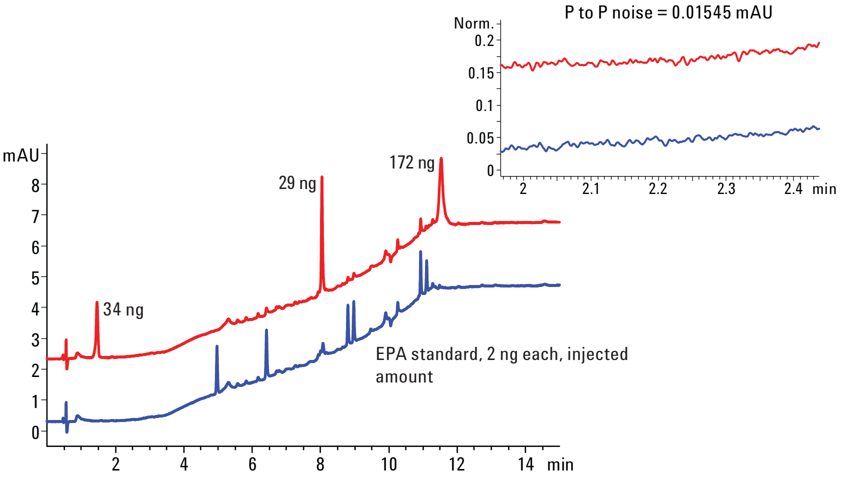


Figure 3. Chromatograms used for determination LOD and LOQ.

Table 3. LOD, LOQ, and resolution.

	Amount used for LOD and LOQ (ng/µL)	Signal-to-noise (S/N)	LOD with S/N=3 ng/µL	LOQ with S/N =20 ng/µL	Resolution based on EPA 20 ng each MMP 340 ng BB 290 ng DIDP 1720 ng
MMP	34	96.6	1.1	7.3	–
DMP	2	74.6	0.08	0.54	65.15
DEP	2	74.6	0.08	0.54	34.86
BB	29	200.3	0.44	2.9	39.03
BBP	2	63	0.1	0.63	19.41
DBP	2	62.3	0.1	0.64	4.66
DEHP	2	68.9	0.09	0.58	57.19
DNOP	2	48.2	0.12	0.83	5.57
DIDP	172	113.6	4.5	30	4.33

The linearity was evaluated by injecting 2,000, 500, 125, 31.25, 7.812, and 1.953 ng/µL of the EPA standard. Linearity was given from 7.8 up to 2,000 ng injected amount related to response factors, see the example in Figure 4. For all standards, the coefficient of correlation was > 0.99998.

Analysis of a real-life sample

The recovery rate was measured by spiking the plastic material of a toy with the EPA standard. The resulting theoretical concentration after sample preparation was 100 ng each for all six compounds (Figure 5). The recovery rate was between 66 and 76 %.

The analysis of plastic material from a baby toy showed that the phthalate concentration was far below the allowed limit of 0.1 % = 100 ng total, related to the extracted material of 0.05 g, (Figure 6). The standard contained the six EPA phthalates and the three additional compounds MMP, BB, and DIDP.

The presence of DIDP was affirmed. The DIDP spectrum of the UV library did not comply with the peak spectrum of the sample at the same time.

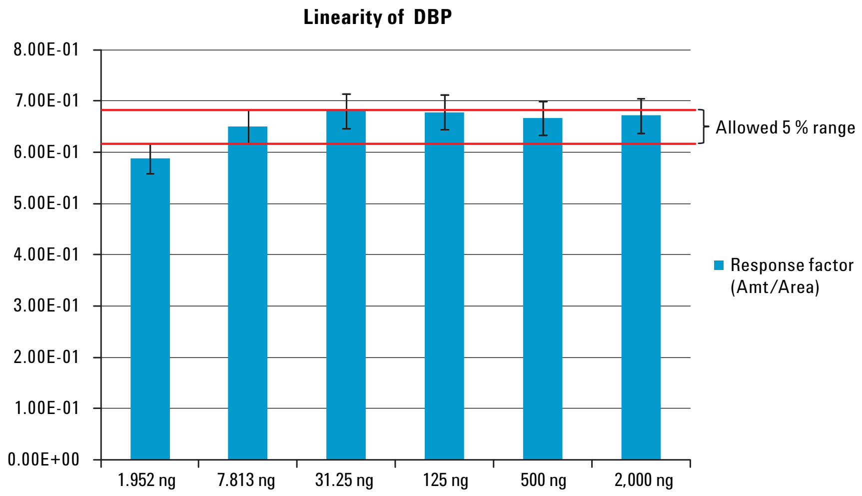


Figure 4. Linearity of DBP as example.

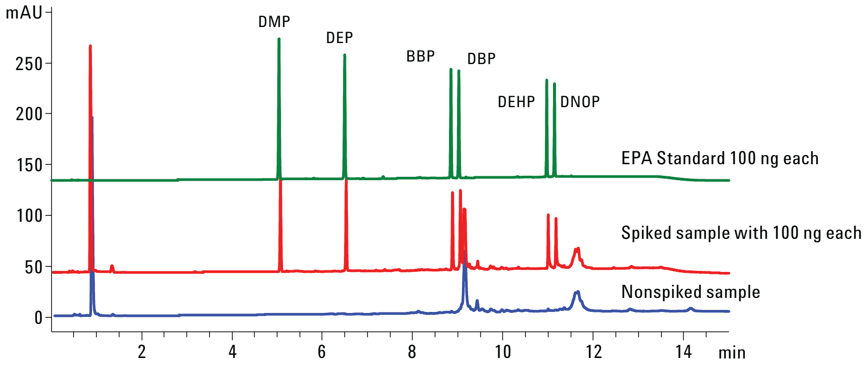


Figure 5. Analysis of EPA standard spiked into the plastic matrix.

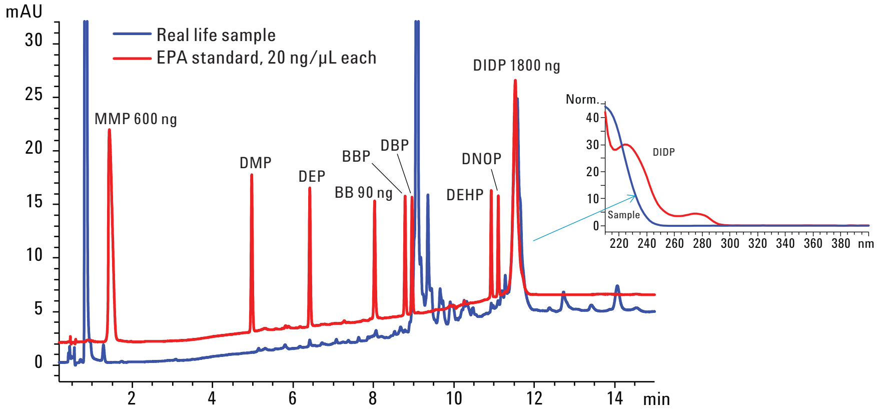


Figure 6. Overlay of standard chromatogram with chromatogram of extracted plastic material.

Transfer to a fast UHPLC method

The developed high resolution UHPLC method took approximately 18 minutes cycle time, which enabled the analysis of samples with high resolution and high precision. In some cases, it was advantageous to get results faster, for example, for fast screening of a bulk of samples. To reduce cycle time, the length of the column was halved and the flow rate was doubled. The internal diameter was reduced to 2.1 mm by using a ZORBAX RRHD Phenyl Hexyl column, which allows backpressures up to 1,200 bars. At 2 mL/min flow rate, the maximum pressure for the fast UHPLC analysis, was approximately 1,100 bar. The cycle time was reduced to 4.5 minutes (Figure 7).

Injecting the spiked matrix showed that identification and quantitation was possible also applying the fast UHPLC condition (Figure 8).

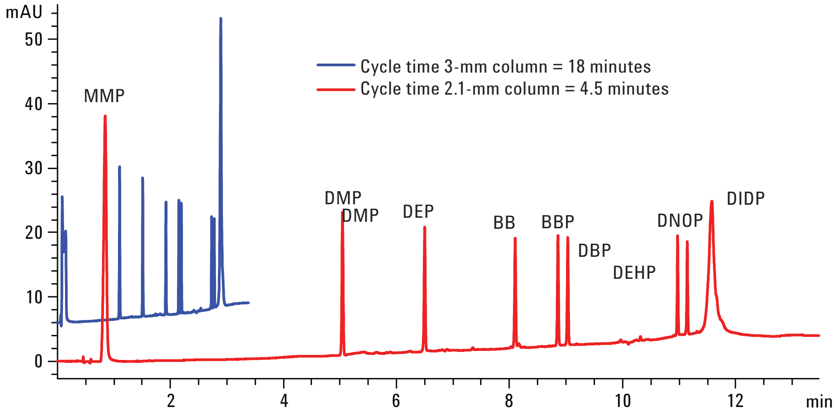


Figure 7. Overlay of chromatograms received from a high resolution and a fast UHPLC run.

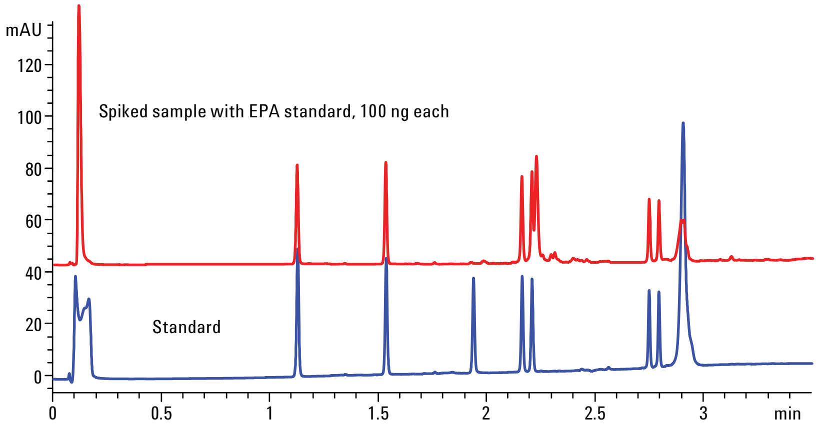


Figure 8. Overlay of standard and spiked matrix chromatogram.

Comparison of High-Resolution UHPLC and Fast UHPLC

The shortened cycle time of 4.5 minutes is advantageous if fast screening is the most important analysis requirement. As expected, the performance of the fast UHPLC method was not as good as the high resolution UHPLC method (Figures 9 and 10).

The resolution for the high resolution UHPLC method was, on average, 60 % better than the fast UHPLC method.

Precision of retention times was a factor 10 better, on average, for the high resolution method, but for the fast analysis, the maximum SD value was 0.00868 minutes. This means the retention time standard deviation was as small as 0.52 seconds, for example, for peak BB eluting at 1.938 minutes. The precision of areas was comparable for the well-resolved peaks except for DEHP and DNOP.

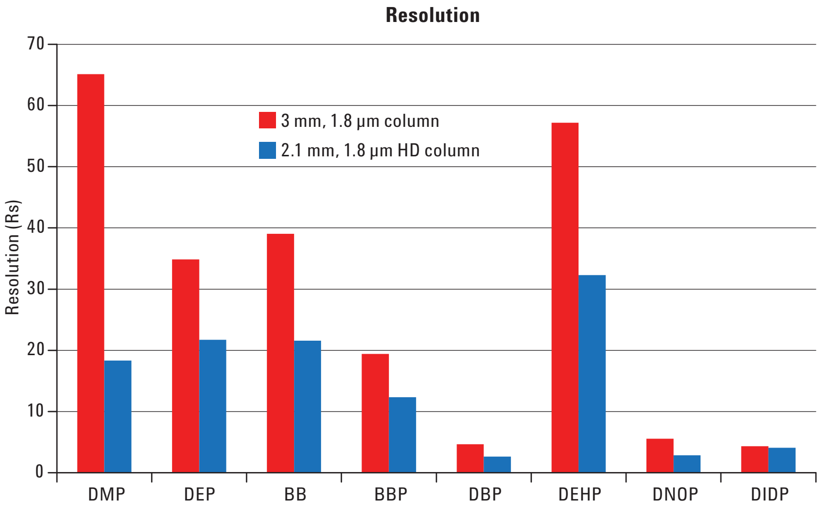


Figure 9. Comparison of resolution.

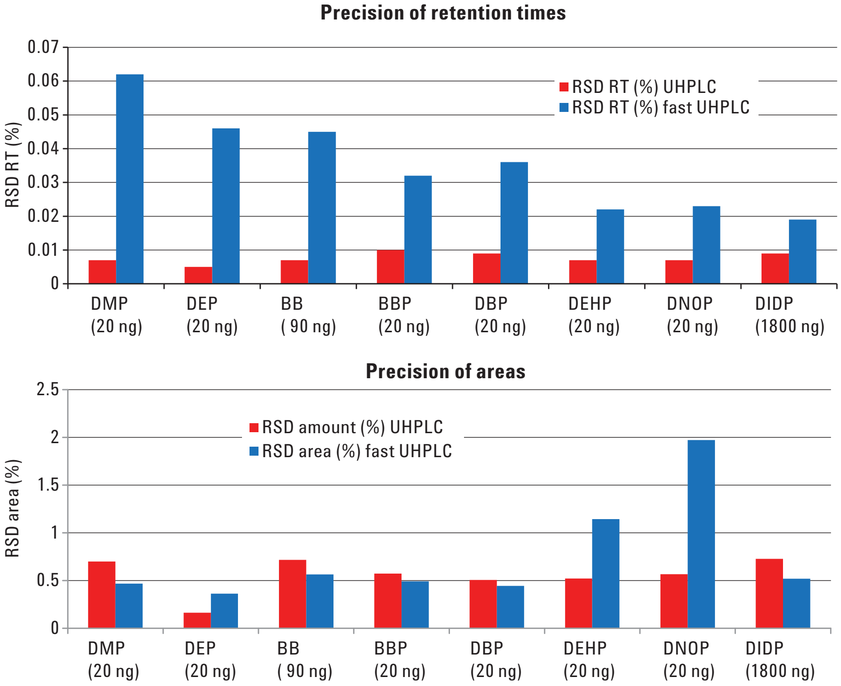


Figure 10. Comparison of precision.

Conclusion

Six restricted EPA phthalates, DMP, DEP, BBP, DBP, DEHP, and DNOP, plus MMP, BB, and DIDP were analyzed using an UHPLC/UV method, which provided a separation within an 18-minute cycle time. A phenyl-hexyl column and a ternary gradient using acetonitrile and methanol as organic phases had to be used for optimum separation. Method development was done using the 1290 Infinity Quaternary Method Development Solution in combination with the Method Scouting Wizard. The performance of the final high resolution UHPLC method of 18 minutes was evaluated, and a real-life sample was measured. The determination of 0.1 % of any restricted EPA phthalate of the mass of the product was feasible with high precision. For identification, UV spectra stored in a spectral library were used in addition to retention times. In addition, a fast screening method was developed with cycle times as low as 4.5 minutes.

References

1. 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. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_106.pdf

8. Umwelt Bundesamt. Phthalate – Die Nützlichen Weichmacher mit den unerwünschten Eigenschaften. www.umweltbundesamt.de, **2007**.

9. A.G.Huesgen, "Agilent 1260 Infinity Method Development Solution - Automatic Scaling of Gradient Times and Flow Rates for Different Column Lengths and Diameters Using the Agilent ChemStation Method Scouting Wizard", Agilent Application Note, Publication number 5990-6863EN, December **2010**.

10. A.G.Huesgen, "Agilent 1290 Infinity Multi-method Solution, Analysis of seven different food applications on one instrument – no column change, no mobile phase change", Agilent Application Note, Publication number 5990-5600EN, May **2010**.

www.agilent.com/chem/1200mds

Published in the USA, September 1, 2013
5991-2784EN

Agilent Technologies