

Determination of phthalate migration from toys

Using the Agilent 1260 Infinity Analytical SFC System with an Agilent 6130 Single Quadrupole LC/MS

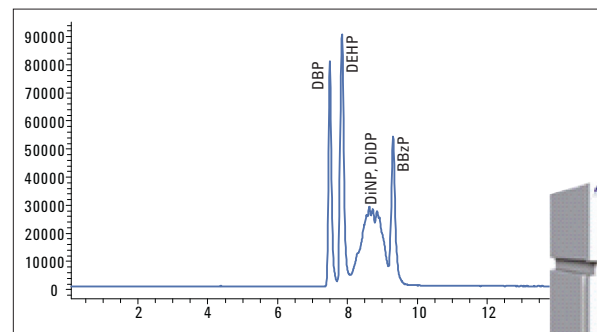
Application Note

Consumer Products

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Abstract

Supercritical fluid chromatography (SFC) can be used for the determination of polymer additives such as phthalates that can migrate or leach out of plastics. In this Application Note, the analysis of phthalates in a migration test from PVC toys is performed using the Agilent 1260 Infinity Analytical SFC System in combination with an Agilent 6130 Single Quadrupole LC/MS. The SFC/MS approach facilitated identification and quantification of mixed isomer phthalates in less than 15 minutes analysis time with good repeatability.



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Introduction

Phthalates form a group of well known plasticizers that are often applied to soften polymers, such as PVC, in toys. High molecular weight phthalates are used as polymer additives. Phthalates are not chemically bound to PVC and can leach out or evaporate into the environment. It has been shown that, in rodents, phthalates can act as hormone-like compounds. They therefore have to be considered as a potential health risk to humans. Some well known phthalates, such as di(ethylhexyl) phthalate (DEHP), consist of a single isomer; others, such as diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP), consist of a mixture of isomers with different branching of the alkyl groups. It poses a significant analytical challenge to perform the analysis of these mixed isomer phthalates. Using capillary gas chromatography (GC), a partial separation of the individual isomers (within a sample of diisononyl or diisodecyl phthalate) is obtained, but this separation is not required since quantification is done on the sum of the isomers. Conversely, no GC separation is available, allowing the complete chromatographic separation of all DiNP isomers from all DiDP isomers. Moreover, in GC/MS using electron ionization (EI), all phthalates give very similar mass spectra with a most abundant ion at m/z 149. Differentiation of both mixed isomer phthalates is only possible by using the $[M\text{-alkyl}]^+$ ion (m/z 297 for DiNP, m/z 307 for DiDP)¹. In contrast, different HPLC techniques can cover only a portion of leachable polymers, therefore reversed-phase separation and normal phase chromatography have to be used in order to cover the complete polarity range. In addition, leachables are often obtained in apolar solvents, which require solvent exchange prior to analysis if reverse phase separation is the method of choice.

SFC has an advantage because it is useful for a very broad range of analytes from polar to apolar. In addition, the analysis time is significantly lower

than that of HPLC methods due to the superior diffusion characteristics of the mobile phase, and due to the fact that solvent exchange is usually not required.

In this Application Note, the analysis of phthalates by SFC-MS is demonstrated. By using atmospheric pressure chemical ionization (APCI), $[M+H]^+$ ions are obtained that allow good differentiation of DiNP and DiDP from each other and from other phthalates. The method was applied to the determination of DiNP from a toy, according to a procedure described by the European Commission².

Experimental

Samples and extraction procedure

Stock solutions of dibutyl phthalate (DBP), butyl benzyl phthalate (BBzP), DEHP, DiNP and DiDP were prepared at 1,000 ppm in dichloromethane and stored at 4 °C. From these

stock solutions, a 10 ppm mixture was prepared; additionally, a dilution series for DiNP was made from 50 ng/mL–1,000 ng/mL in MeOH.

A plasticized PVC toy was analyzed according to the official procedure². The extraction procedure consisted of cutting two disks from the toy (about 2.5-cm diameter, corresponding to a 10-cm² surface taking both sides into consideration). Both disks were placed into a sealable glass vessel with 30 mL of artificial saliva solution and three metal spheres. The container was then sealed and placed on a horizontal shaker for 30 minutes. The metal spheres were added to simulate a child chewing on the toy. The solution was then transferred to a separation funnel, to which 10 mL of cyclohexane were added. The cyclohexane layer was collected, evaporated down, and reconstituted in 1 mL of cyclohexane prior to injection.

Name	Abbreviation	Formula	MW (g/mol)	Ion APCI (+)	Ion mass
Dibutyl phthalate	DBP	C ₁₆ H ₂₂ O ₄	278.34	M+H	279
Benzybutyl phthalate	BBzP	C ₁₉ H ₂₀ O ₄	312.36	M+H	313
Di(2-ethylhexyl) phthalate	DEHP	C ₂₄ H ₃₈ O ₄	390.56	M+H	391
Diisononyl phthalate	DiNP	C ₂₆ H ₄₂ O ₄	418.61	M+H	419
Diisodecyl phthalate	DiDP	C ₂₈ H ₄₆ O ₄	446.66	M+H	447

Table 1
Names, abbreviations, molecular formulae, and MW information of selected phthalates.

Agilent 1260 Infinity SFC/MS solution	Part number
1260 Infinity Analytical SFC System consisting of: <ul style="list-style-type: none">• 1260 Infinity Degasser• Aurora Fusion A5 module• 1260 Infinity SFC Autosampler• 1260 Infinity SFC Binary Pump• 1290 Infinity Thermostatted Column Compartment• 1260 Infinity Diode Array Detector VL Plus	G4309A
External heating device	Caloratherm heater
1260 Infinity Micro Degasser	G1379A
1100 Series Binary Pump	G1312A (used as make-up flow pump)
6130 Quadrupole LC/MS	G6130B

Table 2
Instrument configuration used in the Agilent 1260 Infinity SFC/MS solution.

Instrumentation

The analyses were performed on an Agilent 1260 Infinity Analytical SFC System coupled to an Agilent 6130 Single Quadrupole LC/MS System. The system configuration is listed in Table 2. Details on how to couple the Agilent 1260 Infinity Analytical SFC to MS systems are described in another Agilent publication³.

Three Agilent ZORBAX SB CN columns (4.6 x 250 mm, 5 μ m) were coupled together to give a total column length of 75 cm. The modifier was acetonitrile isocratic at 4%, and detection was performed in positive ion mode using an APCI source. The experimental conditions are summarized in Table 3.

Results and discussion

Analysis of standard samples

A mixture containing DBP, BBzP, DEHP, DiNP, and DiDP was analyzed using SFC-APCI(+)-MS. The total ion chromatogram of the SIM trace is shown in Figure 1. The peak shapes for DiNP and DiDP are broader than the rest in the phthalate mixture, which is due to the isomers present. As in gas chromatography and liquid chromatography, these phthalates cannot be chromatographically resolved, and DiNP isomers overlap (at least partially) with DiDP isomers. However, DiNP and DiDP can easily be differentiated based on their MS spectra. Using the extracted ion chromatograms at the respective $[M+H]^+$ ions, the peaks for DiNP and DiDP can be deconvoluted (Figure 2). In comparison to GC/MS analysis, the SFC/MS analysis is faster and deconvolution is easier since the most abundant ion is the $[M+H]^+$ ion, while in electron ionization (EI) MS, all phthalates have a common m/e 149 as the most abundant ion, which cannot be used for deconvolution.

Experimental conditions

Column:	Three Agilent ZORBAX SB CN (4.6 x 250 mm, 5 μ m)
Supercritical fluid:	CO ₂
Modifier:	Acetonitrile (isocratic at 4%)
Outlet pressure:	120 bar
Flow rate:	2.0 mL/min
Temperature:	40 °C
Injection volume:	5 μ L
Caloritherm heater (*):	60 °C
Make-up flow (*):	Isopropanol at 0.5 mL/min
Detection:	MS in SIM mode, monitoring $[M+H]^+$ ions (Table 1)
APCI:	Capillary V \pm 3000 V, Corona I = 4.0 μ A
	Drying gas = 8.0 L/min at 325 °C
	Nebulizer = 50 psig
	Vaporizer = 350 °C

(*): Caloritherm heater and make-up flow prevent solute deposition/condensation before the back-pressure regulator³.

Table 3
Experimental conditions.

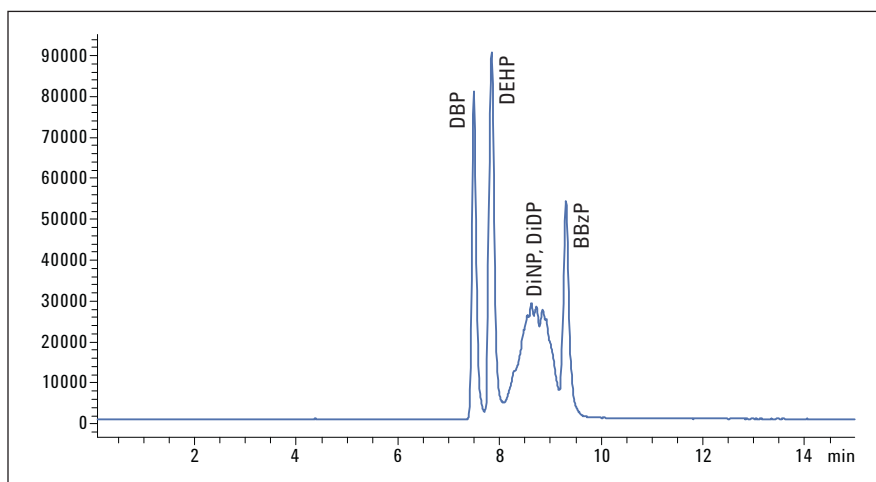


Figure 1
TIC of the SFC-APCI (SIM) analysis of the phthalate mixture (separation conditions are listed in Table 3).

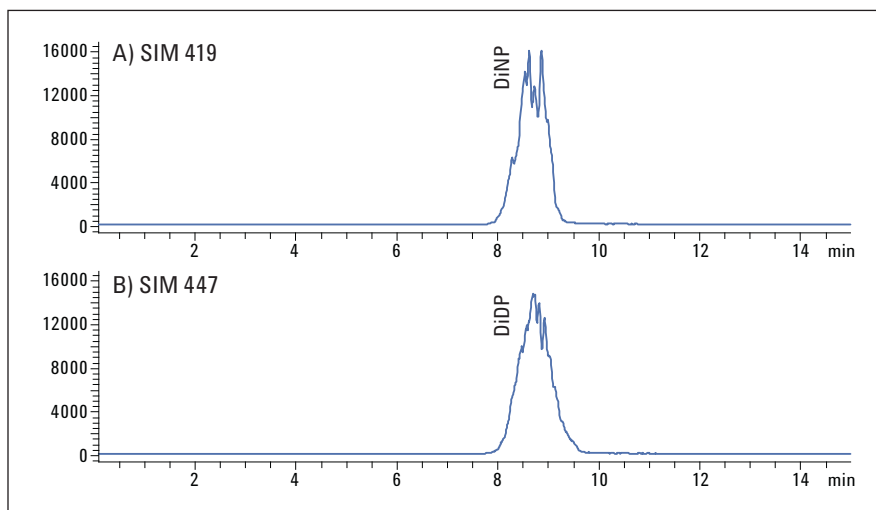


Figure 2
Extracted ion chromatograms showing the deconvolution of: A) DiNP at 419 amu B) DiDP at 447 amu.

The analytical method was applied to the analysis of a toy made from plasticized PVC. Sample preparation was done according to an official validated procedure, simulating migration of phthalates from the toy into artificial saliva. The extract was analyzed by SFC/APCI(+)MS operated in SIM mode. The chromatogram obtained (extracted ion chromatogram at m/z 419) is shown in Figure 3. In the sample, DiNP was clearly detected.

The concentration of DiNP was determined using a calibration curve obtained for a serial dilution of a DiNP external standard (50 ng/mL – 1,000 ng/mL). The calibration curve showed good linearity ($R^2 > 0.999$). Good repeatability was obtained at low, mid and high levels. These figures of merit and the limit of detection (LOD) at $S/N > 3$ and limit of quantitation (LOQ) at $S/N > 10$ are listed in Table 4.

The measured concentration of DiNP in the extract from the toy was 122 ng/mL in the concentrated cyclohexane solution, corresponding to 4.1 ng/mL in the artificial saliva solution. This concentration corresponds to a relatively low DiNP release of 2.1 ng/10 cm²/min.

Conclusion

The Agilent 1260 Infinity Analytical SFC System coupled to an Agilent 6130 Single Quadrupole LC/MS was used for the determination of phthalates in a plasticized PVC toy. The method allowed fast separation and, due to the APCI/MS detection, differentiation between DiNP and DiDP was possible. The method showed good linearity and repeatability and could be applied to measure phthalate migration from the toy into an artificial saliva simulant. The SFC/MS method proved to be a good alternative to GC/MS or LC/MS methods.

Parameter	DiNP
LOD (ng/mL)	12.5
S/N (LOD)	3.2
LOQ (ng/mL)	50
S/N (LOQ)	10.3
Linearity (R^2) (50–1000 ng/mL)	0.9995
RSD (%) on peak area, 50 ng/mL level (n=5)	9.68
RSD (%) on peak area, 500 ng/mL level (n=5)	2.85
RSD (%) on peak area, 1000 ng/mL level (n=5)	1.68

Table 4
Figures of merit for DiNP analysed by SFC-APCI-MS.

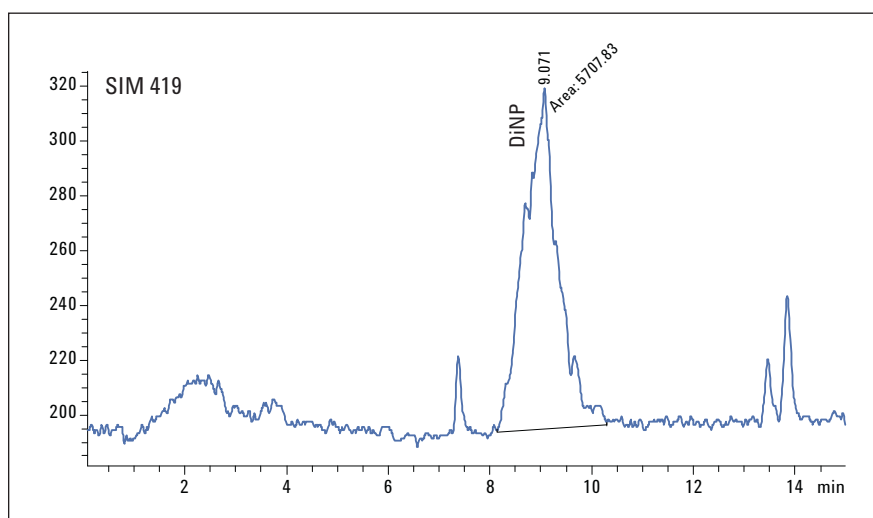


Figure 3
Extracted ion chromatogram (m/z 419) of the SFC-APCI-MS analysis of the concentrated extract of a plasticizer PVC toy. The separation conditions are the same as in Figure 1.

References

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3. M. Dunkle, G. Vanhoenacker, F. David, P. Sandra, M. Vollmer, "Agilent 1260 Infinity SFC/MS Solution - Superior sensitivity by seamlessly interfacing to the Agilent 6100 Series LC/MS system", Agilent Technologies Technical Overview, **2011**, Publication Number 5990-7972EN.

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