Analysis of Phthalates in Body Wash using Solid-Supported Liquid-Liquid Extraction

Application Note

Consumer Products

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Abstract

Phthalates are additives often used in flexible vinyl products and cosmetics. There is concern about phthalates because of their toxicity and the high levels of exposure humans have to them on a daily basis. This application note details the extraction of four phthalates (diethyl phthalate, dipropyl phthalate, benzylbutyl phthalate, and dicyclohexyl phthalate) from an infant shampoo/body wash using Agilent Chem Elut 5 mL unbuffered solid-supported liquid-liquid extraction (SLE) cartridges. Separation and quantitation of the phthalates was achieved using an Agilent 1200 Infinity Series with diode array detection. The recoveries of the phthalates ranged from 91% to 108% when extracted using the reported SLE method.

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Introduction

Phthalates are a family of compounds that are often used as additives in flexible vinyl products, or as solvents or fixatives in cosmetics [1,2]. They are mainly used as plasticizers but are also used to promote absorption of body washes and lotions [2,3]. Consumption of phthalates has been associated with reproductive issues attributed to the disruption of internal hormonal balances [4]. They have also been known to cause extremely low sperm count in males [5]. The U.S. Environmental Protection Agency (EPA) is concerned about phthalates because of their toxicity, and because it is common for humans to have a high daily level of exposure to phthalates through a multitude of cosmetic products and plastic materials. Phthalate exposure has also been linked to reproductive developmental issues in children. A 2008 study showed that phthalate exposure to infants was widespread. The study showed that increased concentration of phthalates measured in infant urine samples were associated with reported use of infant powder, infant lotion, and infant shampoo [6].

In these experiments, phthalates were extracted by solid-supported liquid-liquid extraction (SLE) and liquid-liquid extraction (LLE) from a spiked sample of shampoo/body wash. The percent recovery of the phthalates after extraction was determined by high performance liquid chromatography (HPLC). The calculated recoveries were then used to compare the two methods, SLE and LLE.

In SLE, a high purity, finely divided, inert, diatomaceous earth sorbent is used to aid the extraction of the analyte from an aqueous solution into an organic solvent. The aqueous solution containing the analyte is passed through the cartridge and the aqueous phase is adsorbed onto the diatomaceous earth. Once the solution has been adsorbed onto the sorbent, an immiscible organic solvent is used to extract and elute the analyte off the cartridge [7]. Because the aqueous solution is spread over the sorbent in a very thin layer, the two solvents are in intimate contact and the analyte can be extracted into the organic solvent without the shaking necessary in LLE. This helps to avoid the problem of emulsion formation that is common in LLE. SLE cartridges typically incorporate a phase separation filter at the outlet to prevent mixing which results in elution of the aqueous phase along with the organic solvent. Chem Elut SLE is available in several formats, and can be purchased in prepacked cartridges or by bulk.

Experimental

All organic solvents were HPLC grade. Acetonitrile was purchased from Burdick and Jackson, Muskegan, MI. Methanol was purchased from Fisher Scientific, Fairlawn, NJ. Acetone and ethyl acetate were purchased from Pharmco, Brookfield, CT. Ultrapure water was delivered using a Millipore Synergy UV purification system. Diethyl phthalate, dipropyl phthalate, benzylbutyl phthalate, and dicyclohexyl phthalate were purchased from Sigma-Aldrich Corp. A stock solution containing each of the four phthalates was prepared in methanol. The concentration of each phthalate in the stock solution was approximately 10 mg/mL. Standard solutions used to create the calibration curve were prepared by dilution of the stock solutions with methanol. Standards were prepared at nominal concentrations of 10, 25, 50, 100, 500, and 1,000 µg/mL.

Extractions

The body wash was spiked with the phthalate stock solution, then treated by SLE and LLE, and the percent recovery for each phthalate was determined. The SLE, LLE, and HPLC methods were adapted from published methods developed for measuring pesticide residues in honey [8,9] and for measuring parabens in body wash [10]. Prior to extraction by either SLE or LLE, the samples were prepared as described in Figure 1. The entire prepared sample was then poured into the SLE cartridge or into the separating funnel for extraction by SLE or LLE, respectively. Twenty-four individual samples were prepared as described in Figure 1. These samples were divided into two sets of 12. Twelve samples were spiked with 200 µg/mL of phthalates and 12 were spiked with 20 µg/mL of phthalates, so that recoveries could be calculated at both high and low levels. Six of the high-level spikes and six of the low-level spikes were extracted using the SLE method described in Figure 2. The remaining 12 samples were extracted using the LLE method described in Figure 3. This allowed for a direct comparison of the two methods. The SLE cartridges used were Chem Elut, 5 mL unbuffered.
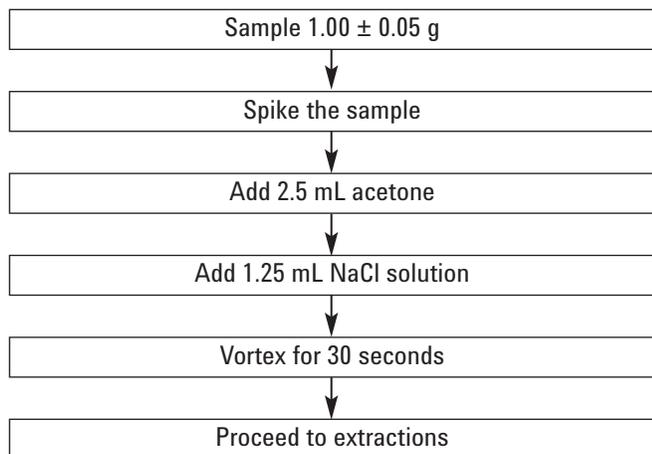
Sample 1.00 ± 0.05 g
↓
Spike the sample
↓
Add 2.5 mL acetone
↓
Add 1.25 mL NaCl solution
↓
Vortex for 30 seconds
↓
Proceed to extractions

Figure 1. Preparation of sample prior to extraction by SLE or LLE.

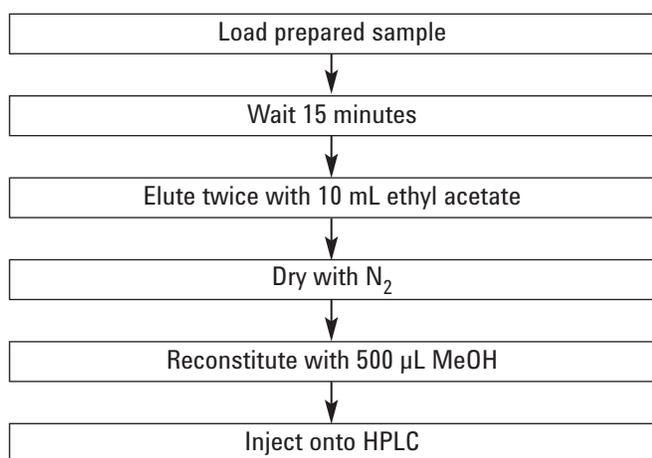| Load prepared sample |
| :---: |
| ↓ |
| Wait 15 minutes |
| ↓ |
| Elute twice with 10 mL ethyl acetate |
| ↓ |
| Dry with N_2 |
| ↓ |
| Reconstitute with 500 µL MeOH |
| ↓ |
| Inject onto HPLC |

Figure 2. Procedure used to extract phthalates from the shampoo/body wash using SLE.

HPLC

The analysis was performed on an Agilent 1200 Infinity Series with a binary pump, autosampler, inline degasser, and an 80 Hz Diode Array Detector. The detector flow cell chosen for this study was a micro flow cell with a 2 µL volume. ChemStation for LC 3D Systems, Rev. B.03.01, was used for data collection and analysis.

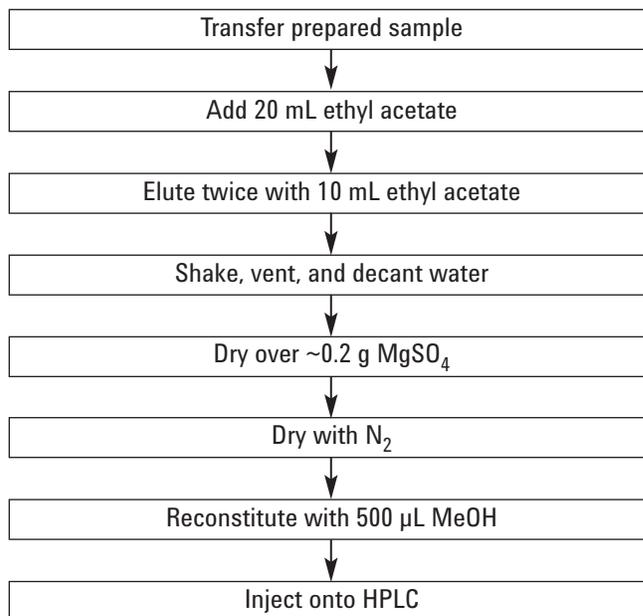| Transfer prepared sample |
| :---: |
| ↓ |
| Add 20 mL ethyl acetate |
| ↓ |
| Elute twice with 10 mL ethyl acetate |
| ↓ |
| Shake, vent, and decant water |
| ↓ |
| Dry over ~0.2 g $MgSO_4$ |
| ↓ |
| Dry with N_2 |
| ↓ |
| Reconstitute with 500 µL MeOH |
| ↓ |
| Inject onto HPLC |

Figure 3. Procedure used to extract phthalates from the shampoo/body wash using LLE in a separating funnel.

The analytical column was an Agilent ZORBAX Eclipse Plus C18. The run time was 9 minutes with a re-equilibration time of 2 minutes. The run time was 7 minutes with a re-equilibration time of 2 minutes.

Conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 x 150 mm, 5 µm (p/n 959993-902)	
Sample prep:	Agilent Chem Elut, 5 mL (p/n 12198006)	
Eluent:	A, 90% Water:10% acetonitrile; B, acetonitrile	
Injection volume:	1.7 µL	
Flow rate:	2.00 mL/min	
Gradient:	Time (min)	% B
	0.00	50
	3.00	65
	5.00	70
Response time:	0.02 s	
Detection:	230 nm	

Results and Discussion

Figure 4 shows the calibration curves for the four phthalates. The linear regression results for the calibration curves of four phthalates are given in Table 1.
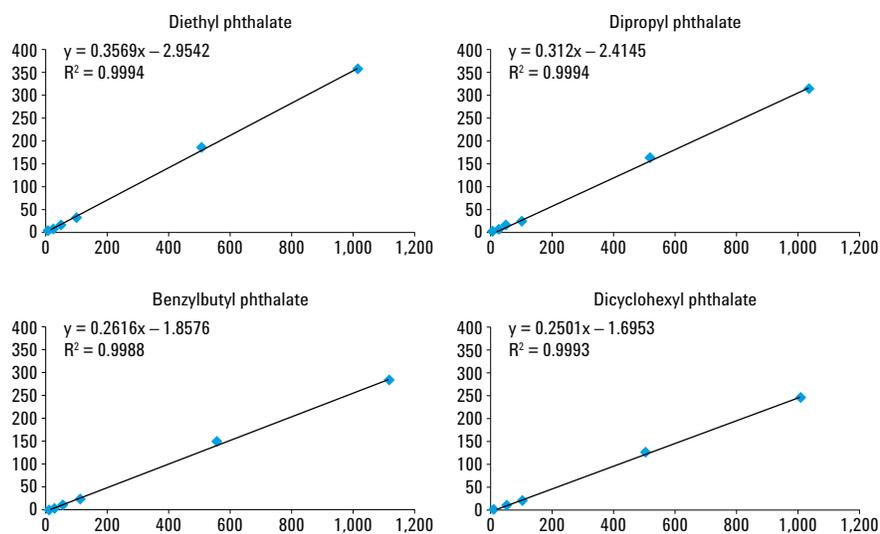

Figure 4. Calibration curves for the four phthalates.

Table 1. Linear regression results for calibration curves of four phthalates.

Compound	Least squares line of best fit	R^2
Diethylphthalate	y = 0.3569x - 29542	0.9994
Dipropylphthalate	y = 0.312x − 2.4145	0.9994
Benzylbutylphthalate	y = 0.2616x − 1.8576	0.9988
Dicyclohexylphthalate	y = 0.2501x − 1.6953	0.9993

The shampoo/body wash was chosen because the label stated it did not contain any phthalates. Chromatograms of the extracts from the unspiked body wash after SLE and LLE were performed are shown in Figure 5. Chromatograms of the extracts from the spiked samples after extraction by SLE and LLE are shown in Figure 6. The samples extracted by LLE (shown in Figures 5A and 6A) have large interference peaks for compounds extracted from the matrix along with the analytes of interest. These interference peaks made it impossible to accurately quantify the peaks of interest at the low level spikes and even for some of the analytes at the high level spikes. The chromatograms show that in the area of the chromatogram that the phthalates elute, the sample extracted by SLE (shown in Figures 5B and 6B) was much cleaner and the peaks of interest did not coelute with the interference peaks.

Table 2 shows the calculated recoveries for the four phthalates after extraction from the spiked shampoo/body wash by SLE and LLE. Note that the injected concentrations are twice as high as the concentration spiked because the extracted samples were dried and reconstituted in half the original volume. Poor results were obtained for the four phthalates when the low-level spikes were extracted using the LLE method. When those results were compared to the results obtained by the SLE method, it was obvious that SLE was a better choice for this determination. For the low concentration spikes, the SLE method outperformed the LLE method in both accuracy and precision. For the high spikes, the results were comparable except for dipropylphthalate. For this compound, only the SLE method gave reasonable results due to a large interference peak that was seen when the LLE method was used.
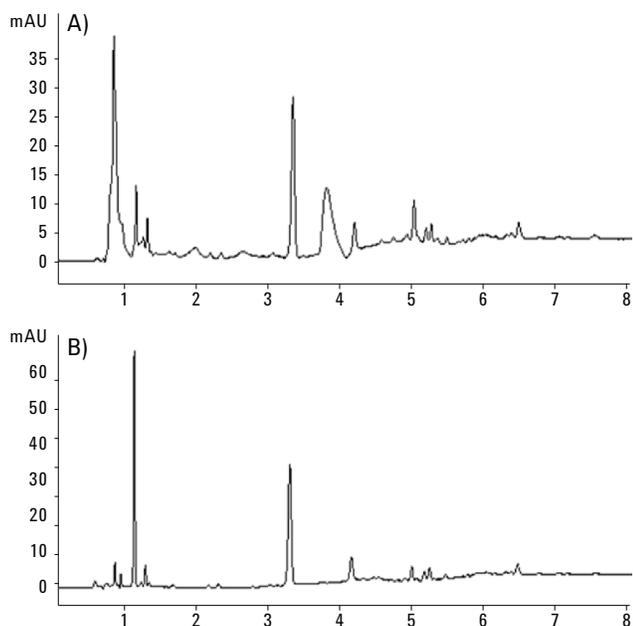
Figure 5. Chromatograms of infant shampoo/body wash (not spiked) after A) LLE, B) SLE.
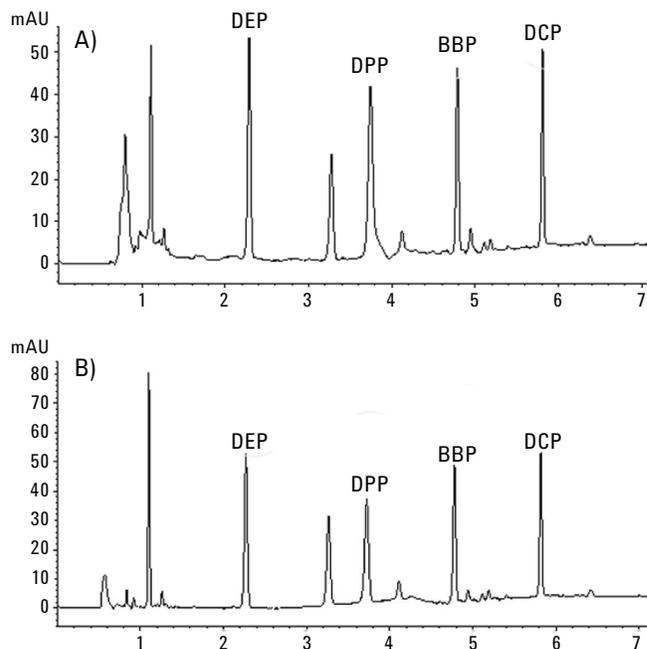

Figure 6. Chromatograms of extract from infant shampoo/body wash spiked with phthalates after A) LLE, and B) SLE.

Table 2. Calculated percent recoveries for the extraction of four phthalates from infant shampoo/body wash using SLE and LLE.

	% Recovery (LLE)				% Recovery (SLE)			
	Spiked at 20 µg/mL		Spiked at 175 µg/mL		Spiked at 20 µg/mL		Spiked at 175 µg/mL	
	avg	std dev	avg	std dev	avg	std dev	avg	std dev
Diethylphthalate	124.13	14.53	92.50	3.37	91.72	4.04	108.18	2.12
Dipropylphthalate	898.39	99.58	147.71	14.05	100.55	4.00	104.88	4.87
Benzylbutylphthalate	113.94	11.43	92.66	2.49	96.02	3.74	91.11	3.69
Dicyclohexylphthalate	378.82	16.33	93.50	2.94	95.53	3.72	95.37	5.79

Conclusions

This application note illustrates that Agilent Chem Elut SLE cartridges offer an effective method for the extraction of phthalates from a shampoo/body wash matrix. When SLE was used, the interferences that were extracted from the matrix along with the phthalates were minimal. In contrast, when LLE was used the interferences that were extracted did not allow for good quantitation of the analytes. The chromatograms of the extracts obtained by SLE were cleaner than those obtained by LLE and the overall results for SLE were superior to those obtained by LLE.

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