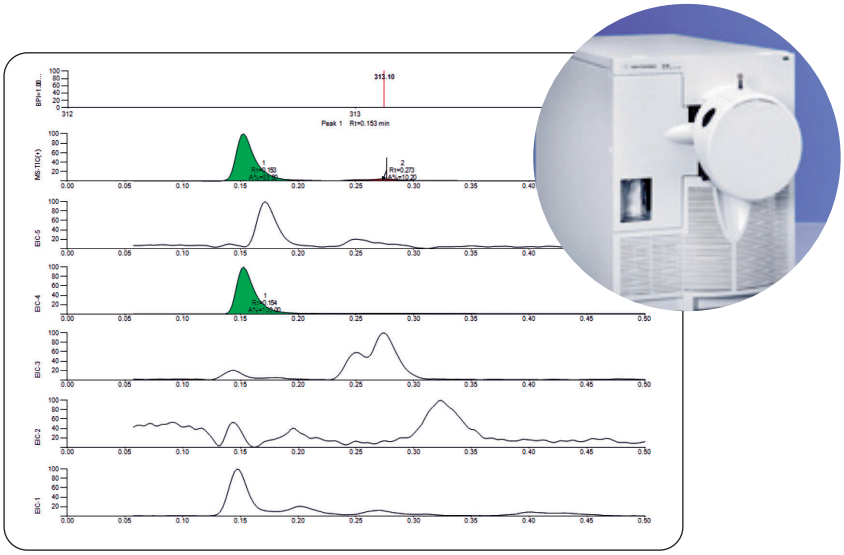
High throughput screening for phthalates in toys and childcare articles using the Agilent 6140 single quadrupole LC/MS system and Analytical Studio Browser software

Increased productivity by quick identification of samples that fail regulatory requirements

Application Note

Environmental



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Abstract

The European Commission has prohibited commercialization of toys or child care articles containing more than 0.1% by weight of any of the following six phthalates: di-isononyl phthalate (DINP), di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), di-iso-decyl phthalate (DIDP), butyl benzyl phthalate (BBP) and dibutyl phthalate (DBP). In addition, various other regulatory agencies throughout the world have banned the excessive use of these phthalates in toys.[1]

In this Application Note, we show high-throughput analyses with less than 1-minute run times for separating phthalates in toys or child care articles on sub-2 µm columns. The use of the Agilent 6140 single quadrupole LC/MS system and Agilent Analytical Studio Browser software allows quick visual identification of samples that exceed regulatory requirements. Fast analysis methods reduce analysis time for consumer product testing agencies or QC departments of toy manufacturers. The method presented here saves time and money, while quickly identifying toys that fail legislation requirements.

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Introduction

Phthalate esters (plasticizers) are used in polymeric materials, such as polyvinyl chloride (PVC) plastics to increase their flexibility. These noncovalently-bound phthalates can leach out of the product, and potentially cause environmental and health hazards. Six phthalates (DINP, DEHP, DNOP, DIDP, BBP and DBP) are classified as posing a health risk. Children can ingest these leachable phthalates from toys by way of mouth.

The Agilent 1200 Series Rapid Resolution LC system coupled to an Agilent 6140 single quadrupole LC/MS system referred to as mass selective detector (MSD) along with Agilent Analytical Studio Browser (ASB) provide versatile tools for high-throughput screening of phthalates such as these. High-throughput analysis is demonstrated with a simple extraction procedure, a run time of less than 1 minute, a short overall sequence time, and quick identification of samples containing excessive phthalates. The autotune feature of the MSD allows instrument tuning without significant user intervention. Agilent ZORBAX Eclipse Plus RRHT 1.8 µm columns facilitate fast chromatographic separations. A short overall analysis time is the result of the overlapped injection option of the Agilent 1200 Series SL Plus Autosampler.

Analytical Studio Browser (ASB) is a visual tool that allows rapid identification of samples exceeding threshold levels set by the analyst. The ASB allows the user to:

- Browse very large amounts of LC/MS data very quickly
- Make an assessment of the quality of data taken from a variety of detectors
- Edit data and override data processing decisions made by automated systems
- Report the data in a format that fits the particular needs of their work environment.

The applicability of this methodology can be extended to other analyses that require high-throughput screening of many samples to meet pass or fail criteria.

Experimental

Twelve phthalates standards were purchased from Sigma Aldrich. Soft toys designed for children under the age of three were purchased from local stores in India and tested for the six restricted phthalates. Using the appropriate fragmentor voltage, each sample was screened in SIM mode for all six phthalates in one run.

Experimental Parameters	Details	
Column	Agilent ZORBAX Eclipse Plus C18 30 mm × 2.1 mm, 1.8 µm p/n 959731-902; operated at 27.5 °C	
LC	Agilent 1200 Series Rapid Resolution LC system	
Mobile phase	Methanol with 0.01% formic acid	
Isocratic run	Run Time (minutes): 0.5 minutes	
Flow	0.6 mL/minutes	
Sample preparation	Stock sample: All phthalates standards were dissolved in 100% methanol to a concentration of 40 µg/mL. 12-Phthalates working samples: Twelve phthalates (Table 1) standard were mixed to 1 µg/mL in methanol. Restricted phthalates samples: Six restricted phthalates were dissolved in methanol to different concentrations – 0.01 µg/mL, 0.05 µg/mL, 1.0 µg/mL and 1.5 µg/mL. Toy samples: Ten toys were cut into small pieces; 0.1 g were weighed and sonicated in 5 mL of methanol (20,000 µg/mL) for 30 minutes and the supernatant was syringe filtered. A phthalates content limit of 0.1% w/w would theoretically correspond to 20 µg/mL in thissolution. The toy sample was further diluted from 20 µg/mL to 1 µg/mL as a working sample concentration. Blank sample: Methanol without any sample was treated as described for a toy sample.	
Injection volume	2 µL	
1200 SL DAD	Bypassed	
Needle wash	50% mobile phase and 50% D-I water for 5 seconds.	
6140 MSD parameters	Drying gas Nebulizer pressure Dry gas temperature Capillary voltage	13 L/minutes 40 psig 250 °C 4000 V
	Source: ESI ionization: Positive mode SIM mode, peak width 0.01 minutes	

Results and Discussion

Rapid determination of MS source parameters using flow injection analysis

Flow injection analysis (FIA) is operated without a column and is used to quickly determine the optimal MS source parameters for a specific compound. All twelve phthalates were sequentially run using the "Run Multiple FIA Method" option to rapidly determine the best fragmentor voltage. Table 1 shows the list of twelve phthalates and their fragmentor voltages, determined using multiple FIA methods. The column "Molecular ion" represents the positively charged species of phthalates, although sodium adducts were also observed.

Less than 1-minute run time for analysis of a mixture of 12 phthalates

A mixture of 12 phthalates (each 1 µg/mL) was passed through an RRHT column. As shown in Figure 1, the elution of all phthalates occurred within 0.5 minutes with moderate separation. DEHP and DNOP are isomers that elute at the same time. Their quantification results can be coupled since both are restricted phthalates. DBP and Di-isobutyl phthalate are also isomers with the same retention time (Table 2), but only DBP is a restricted phthalate. Further analysis may be necessary to distinguish di-isobutyl phthalate from DBP if the total phthalates content for that sample exceeds specification limit.

Number	Compound name	Abbreviated compound name	Molecular ion $(M+H)^+$	Fragmentor voltage (V)	Dwell time (msec)
1	Diethyl phthalate	DEP	223.1	105	10
2	Dipropyl phthalate	DIPP	251.1	89	10
3	Di-n-butyl phthalate	DBP*	279.1	83	10
4	Diisobutyl phthalate	DIBP	279.2	84	10
5	Bis methyl glycol phthalate	DMGP	283.1	106	10
6	Dipentyl phthalate	DNPP	307.2	112	10
7	Dicyclohexyl phthalate	DCP	331.1	94	10
8	Benzyl butyl phthalate	BBP*	313.1	88	10
9	Di(2-ethyl hexyl) phthalate	DEHP*	391.1	106	10
10	Di(n-octyl) phthalate	DNOP*	391.2	93	10
11	Diisononyl phthalate	DINP*	419.1	97	10
12	Diisodecyl phthalate	DIDP*	447.2	90	10

Table 1
List of twelve phthalates analyzed using FIA method to determine the optimal fragmentor voltage.
***Restricted phthalates - for analysis of six phthalates only, dwell volume corresponds to 14 milliseconds.**

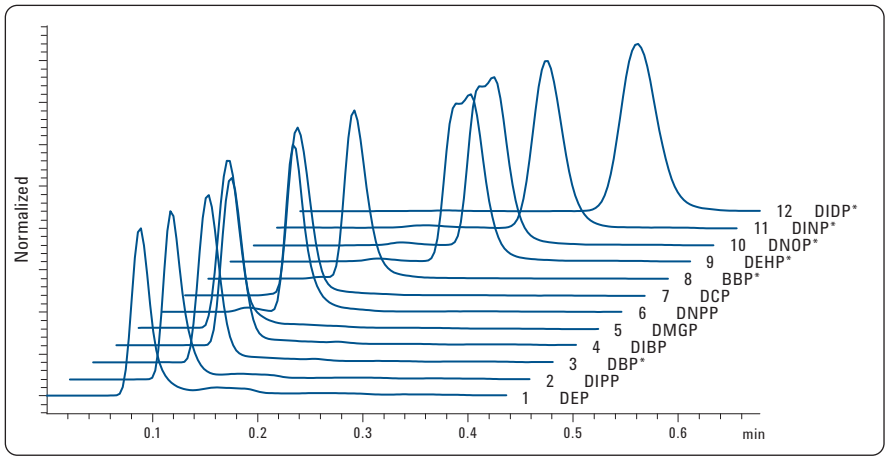

Figure 1
Extracted ion chromatogram from a single sample containing mixture of 12 phthalates.
***Phthalates numbers's 3, 8, 9, 10, 11 and 12 are restricted phthalates.**

High-throughput analysis of toy samples for six restricted phthalates using overlapped injections

Toy samples and restricted phthalates samples were analyzed using less than 1 minute isocratic runs and overlapped injections. In overlapped injections, the injector needle collects the sample and performs a needle wash when the previous run is still in progress. The time lag between injections is eliminated, which shortens overall sample analysis time. As shown in Figure 2, overlap injection was enabled after 0.3 minutes, which allowed subsequent samples to be injected soon after the end of the previous run.

Number	Compound name	Retention time (minutes)	Peak width at half height (sec)
1	Diethyl phthalate	0.153	1.6
2	Dipropyl phthalate	0.162	1.4
3	Di-n-butyl phthalate (DBP)*	0.173	1.4
4	Diisobutyl phthalate	0.173	1.4
5	Bis methyl glycol phthalate	0.145	1.4
6	Dipentyl phthalate	0.189	1.5
7	Dicyclohexyl phthalate	0.202	1.5
8	Benzyl butyl phthalate (BBP)*	0.171	1.6
9	Di(2-ethyl hexyl) phthalate (DEHP)*	0.282	2.5
10	Di(n-octyl) phthalate (DNOP)*	0.282	2.5
11	Diisononyl phthalate (DINP)*	0.317	1.9
12	Diisodecyl phthalate (DIDP)*	0.381	2.3

Table 2
The retention time of twelve phthalates and their peak width in seconds. The retention time of all compounds is within half a minute so the overall run time can be decreased further.

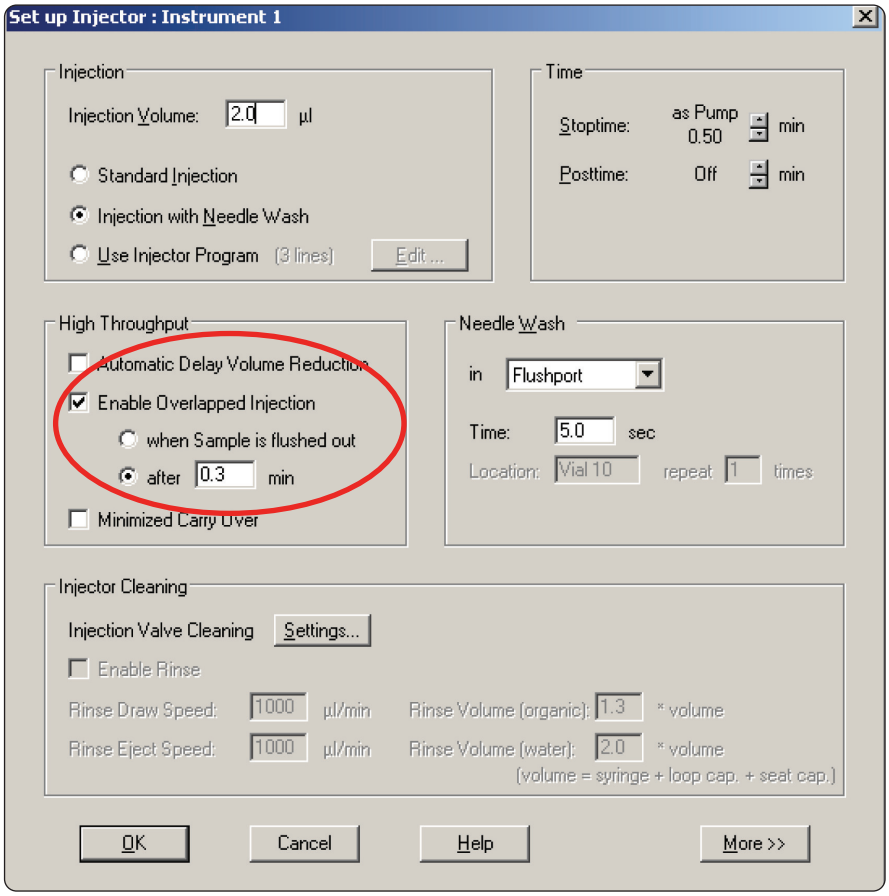

Figure 2
The use of overlapped injections for high throughput sample injections in the autosampler setting of the ChemStation (B03.02).

Easy assessment of results using Analytical Studio Browser:

ASB is an add-on software to the Agilent ChemStation that allows quick identification of samples containing specific compounds. After data acquisition and ChemStation data analysis, ASB displays the results of the analysis and correlates them to the sample location in the autosampler. The parameters of integration events define which peaks are integrated. Only the integrated peaks are used in ASB calculations to show the presence or absence of the compound in a particular sample.

Initially a mixture of standard phthalates of low concentration (0.1 µg/mL) is injected in the MS to determine the area of the peaks. These areas are used in "integration events" to integrate peaks above this threshold. Any phthalate peak whose area exceed the value set in "integration parameter" is integrated and shows up as a green spot in ASB. A screenshot of Analytical Studio Browser is shown in Figure 3. In this example DINP (di-isononyl phthalate) is identified as a 419.1 peak in sample 4E. Although other phthalates are also present in 4E, they do not exceed the limit as defined in the integration settings.

The samples marked in red do not have detectable phthalates within the limit of 0.01% w/w. ASB helps the analyst visually determine which sample needs careful analysis. The analyst can proceed to look at the area of each phthalate to determine if it crossed regulatory limits, or if the sum of the areas of other phthalates exceed the regulatory limits.

As show in Figure 3, green spots indicate those samples that contain one or more phthalates that have crossed the limit set by 0.1 µg/mL standard (0.01% phthalate content for each phthalate).

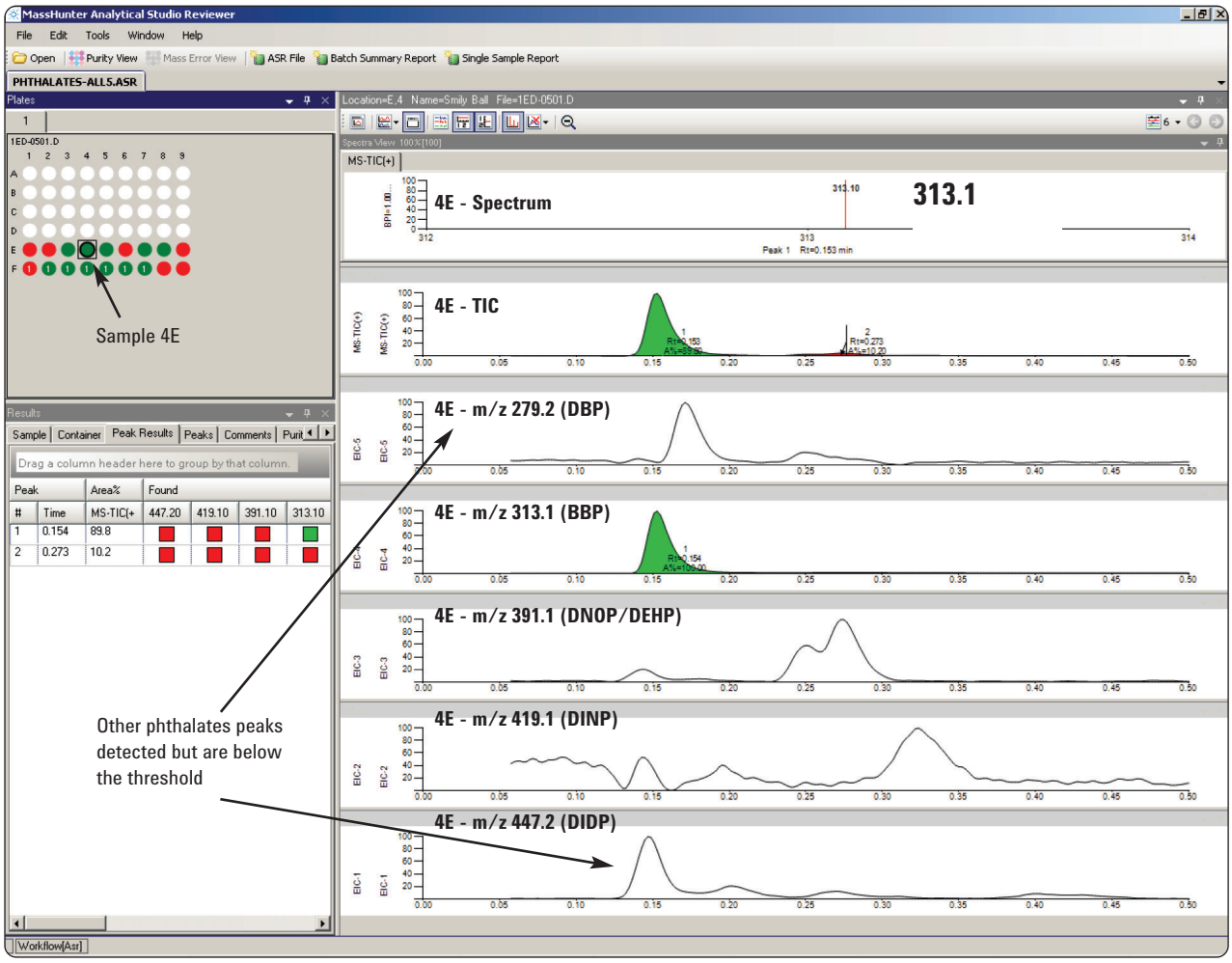

Figure 3
Screen shot of Analytical Studio Browser.

Conclusion

The combination of the Agilent 6140 single quadrupole LC/MS system along with Analytical Studio Browser software provides a versatile tool for high-throughput screening of phthalates in toys. High-throughput is demonstrated in analysis of phthalates in toys by simple extraction procedures, short run times, reduced sequence analysis with overlapped injections, and quick identification of samples containing phthalates using Analytical Studio browser. Sample analysis time is significantly reduced, providing a cost-effective solution.

References

1. http://europa.eu/legislation_summaries/consumers/consumer_safety/l32033_en.htm

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