應用手冊

Rapid Phthalate Screening in Consumer Products, Increasing Profits With Greater Sample Throughput

James Morphet

Waters Corporation

Abstract

This application note describes a rapid solution for identifying levels of phthalates that exceed levels defined in worldwide legislation in the analysis of children's toys.

Introduction

Phthalates are a class of chemicals that are used in polyvinyl chloride (PVC) plastic to improve flexibility and durability. In PVC plastic, the vinyl molecules form an attraction to one another to produce a very brittle plastic. To make this softer and more flexible, a plasticizer (often a phthalate) is added to allow the molecules to slide against each other. This can be as much as 50% by weight in some materials.

These phthalates are not chemically bonded to plastics, and in certain circumstances, they can be released by PVC. In children's toys, this process is augmented with bending, chewing, contact with oils, saliva, and temperatures over 30 °C. This class of chemicals has been widely investigated over several years as they have been considered a risk to human health regarding cancer, obesity and reproduction.

Legislation has been introduced to limit the amount of phthalates in PVC. In the United States, California became the first state to enact a prohibition on the use of Diethylhexyl phthalate (DEHP), Dibutyl phthalate (DBP), and Benzylbutyl phthalate (BBP) in all toys and child care articles, as well as a prohibition on the use of Di-isononyl phthalate (DINP), Di-isodecyl phthalate (DIDP), and Di-n-octyl phthalate (DnOP) in toys and child care articles intended for use by children under three years of age that can be put in the mouth[1].

Since then, the U.S. Congress has implemented a ban on their use. The bill imposes a permanent ban on three phthalates in objects used by children under 12: DEHP, DBP, and BBP. Three other compounds DINP, DIDP, and DnOP have interim bans pending additional safety studies.

The European Union has imposed legislation which states that DEHP, DBP, and BBP should not be used in toys or child care articles. In addition, the legislation states that DINP, DIDP and DnOP should not be used in toys or child care articles that are intended for a child's mouth[2]. Strict limits have been placed on these compounds at a level of 0.1%. The latest Japanese toy safety standard (ST-2002 – eighth edition, clause 1.9) will also enforce these levels for the six compounds.

The ever increasing amounts of children's toys that are covered by these legislative acts emphasize the need for a more cost-effective solution and faster types of analysis. This application note describes the screening of the six legislated and other non-legislated phthalates in a rapid one minute run.

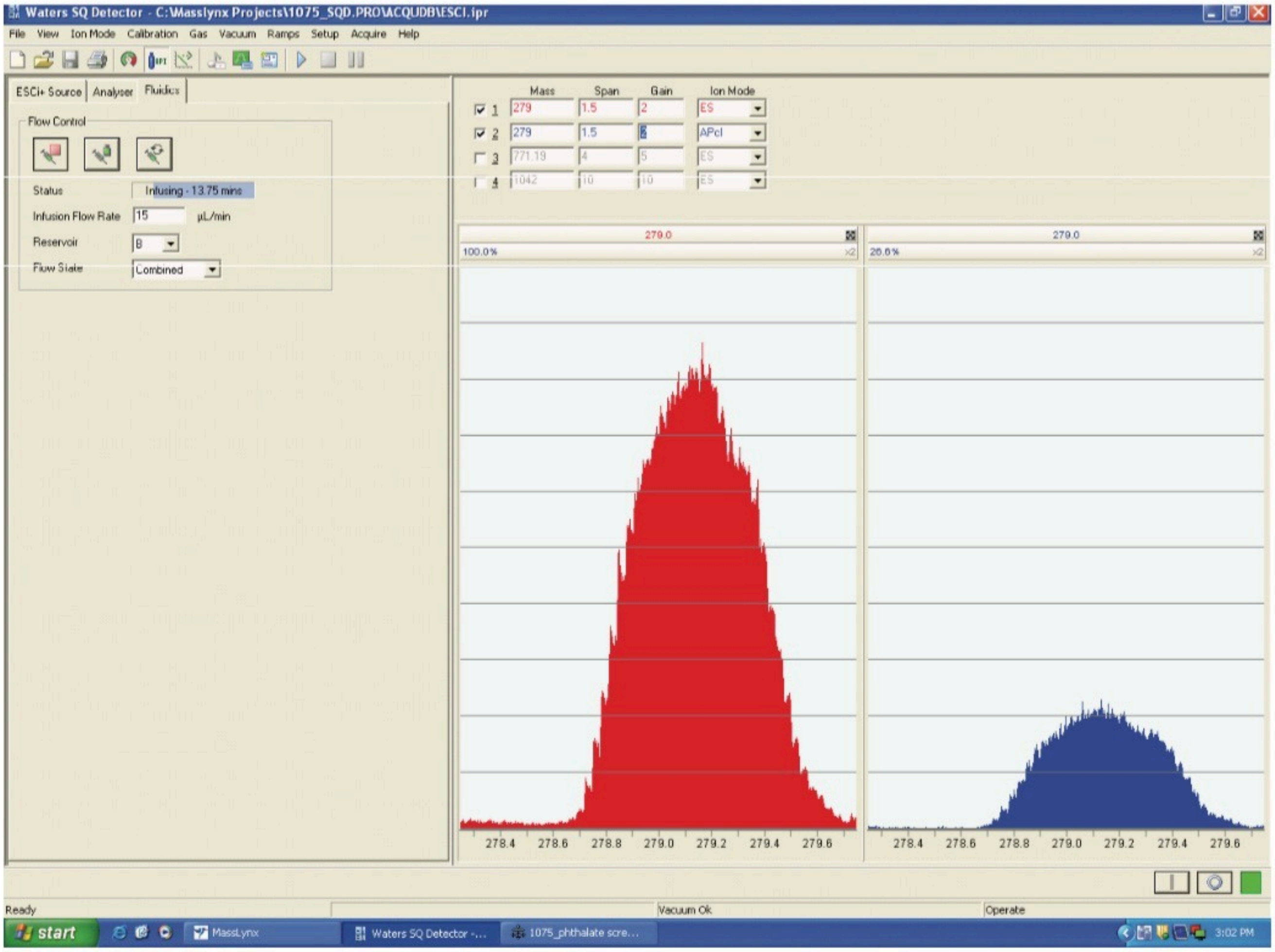
Experimental

Sample Preparation

A basic sample extraction was employed, the sample was homogenized; 2 g was taken and sonicated with 200 mLs of methanol for 10 min. The supernatant was filtered, diluted 10x using methanol, and placed into a Waters Certified vial, capped and racked in the ACQUITY Sample Manager for analysis.

The analysis of phthalates can be performed in both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) modes as this class of compounds is susceptible to both modes when using a mass spectrometer. The Waters ESCi multi-mode ionization source[3] combines high-speed switching between ESI and APCI within the ion source, enabling the user to run MS methods with differing combinations and durations of both ESI and APCI within the same analysis.

Figure 1 shows the infusion of DBP with ESCi ionization enabled with a corona current of 1 µA. The analyst is quickly able to see which ionization mode is more suited to the compound of interest, without having to waste time changing between source setups in different ionization modes. This quick experiment shows the relative intensity of positive mode ionization in ESI (red), versus APCI (blue). Although APCI gives a good response and could be used for the analysis[4], it is ESI that gives greater sensitivity for this compound and should therefore be the mode of ionization used for further experiments.



Status: Infusing - 13.75 mins

Infusion Flow Rate: 15 µL/min

Reservoir: B

Flow State: Combined

		Mass	Span	Gain	Ion Mode
☑	1	279	1.5	2	ES
☑	2	279	1.5	2	APcI
☐	3	771.19	4	5	ES
☐	4	1042	10	10	ES

Figure 1. Sample tune page using the ESCi probe.

LC conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Column temp:	27.5 ˚C
Sample temp:	4 ˚C
Isocratic flow rate:	0.60 mL/min.
Mobile phase:	Methanol + 0.1% formic acid

Weak needle wash:	98:2 Water: methanol + 0.1% formic acid
Strong needle wash:	Methanol + 0.1% formic acid
Total runtime:	1 min
Injection volume:	2 µL, partial loop injection with needle overfill and Load Ahead enabled

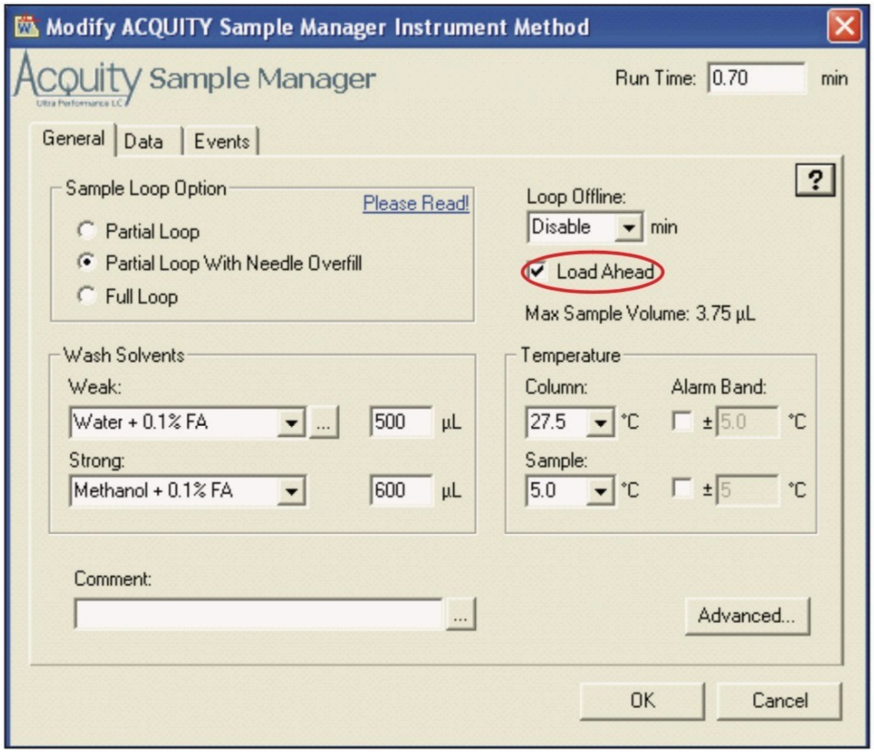
MS conditions

MS system:	ACQUITY SQ Detector
Ionization mode:	ESI +
Capillary voltage:	3 kV
Desolvation gas:	Nitrogen, 800 L/Hr, 450 ˚C
Cone gas:	Nitrogen, 5 L/Hr
Source temp:	130 ˚C
Acquisition:	Selected Ion Recording (SIR)

The Waters ACQUITY SQD has IntelliStart Software as a standard feature. This easy-to-use technology allows users to feel confident when using this single quadrupole mass spectrometer.

IntelliStart enables users to easily perform calibration, sample tuning, and automate daily checks. This ensures that the system is running at user-defined levels and will maximize the ability of the instrument to analyze samples, as the instrument will be self certified and ready-to-use when the analyst arrives for work.

Getting the best performance out of instrumentation is important in any analysis. Load ahead, as shown in Figure 2, allows users to further increase throughput when necessary by decreasing injection to injection runtimes.



Modify ACQUITY Sample Manager Instrument Method

Run Time: 0.70 min

Sample Loop Option
○ Partial Loop
● Partial Loop With Needle Overfill
○ Full Loop

Loop Offline: Disable min
☑ Load Ahead
Max Sample Volume: 3.75 µL

Wash Solvents
Weak: Water + 0.1% FA 500 µL
Strong: Methanol + 0.1% FA 600 µL

Temperature
Column: 27.5 °C Alarm Band: ☐ ± 5.0 °C
Sample: 5.0 °C ☐ ± 5 °C

Comment:

Figure 2. Screenshot showing Load Ahead functionality enabled.

This option is supported by ACQUITY UPLC and can be found when editing the Sample Manager instrument method. Checking this tick box will tell the ACQUITY Sample Manager to prepare the next injection in the sequence while the current one is being analyzed. For short runtimes like this, one minute injection can make a valuable difference and allows many more samples to be analyzed in the working day. In this instance, Load Ahead reduced the injection to injection run time by a third.

Results and Discussion

A one minute, injection-to-injection screen was achieved for 14 phthalates in a children's toy extract using ACQUITY SQD, as shown in Figure 3.

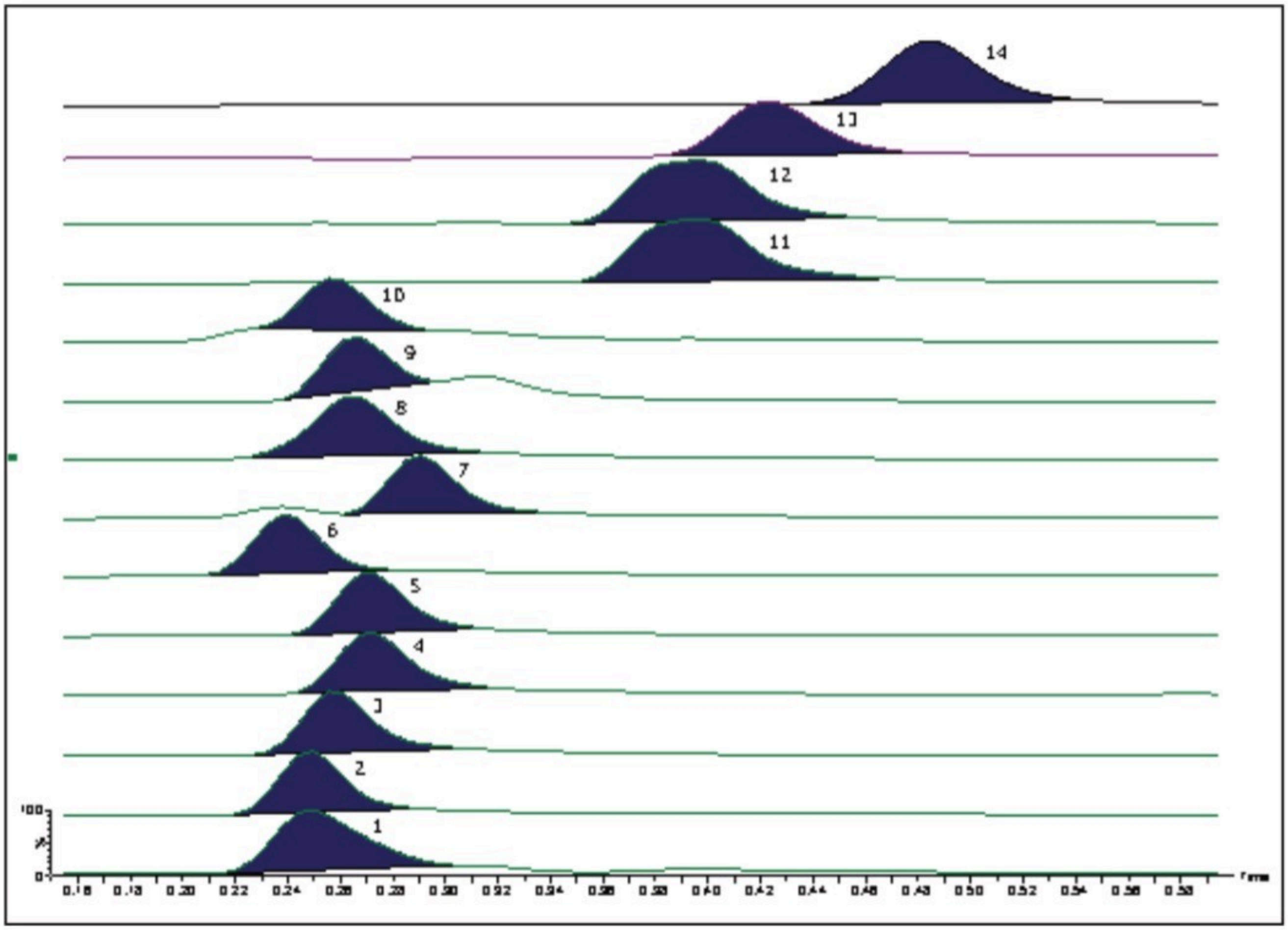


Figure 3. Chromatogram showing separation of 14 phthalates, which are listed in Table 1.

Phthalate	Peak number	Parent ion	Dwell time (s)	Cone voltage (V)
Dimethyl phthalate	1	195.0	0.02	16
Diethyl phthalate	2	223.1	0.02	16
Dipropyl phthalate	3	251.1	0.02	16
Di-n Butyl Phthalate (DBP)	4	279.0	0.02	20
Diisobutyl phthalate	5	279.1	0.02	18
Bis methylglycol phthalate	6	283.1	0.02	15
Dipentyl phthalate	7	307.1	0.02	20
Benzyl Butyl Phthalate (BBP)	8	313.1	0.02	20
Dihexyl phthalate	9	335.2	0.02	18
Butyl phthayl butylglycolate	10	337.2	0.02	18
Bis 2 Ethylhexyl Phthalate (DEHP)	11	391.1	0.02	25
Di-n-Octyl Phthalate (DNOP)	12	391.2	0.02	25
Di-isononyl Phthalate (DINP)	13	419.2	0.02	24
Di-isodecyl phthalate (DIDP)	14	447.3	0.02	30

Table 1. MS parameters for phthalate analysis with acronyms for those legislated against.

A separation of the six legislated phthalates is shown in Figure 4. Co-elution of Bis-2-Ethylhexyl Phthalate

(DEHP) and Di-n-Octyl Phthalate (DNOP) in the middle trace is due to the compounds being isobaric, because they share a parent ion of 391 *m/z*. This does not matter for the purposes of fast screening, as the aim is to find any positive sample close or above the legislated limit and make it a candidate for quantifiable reanalysis.

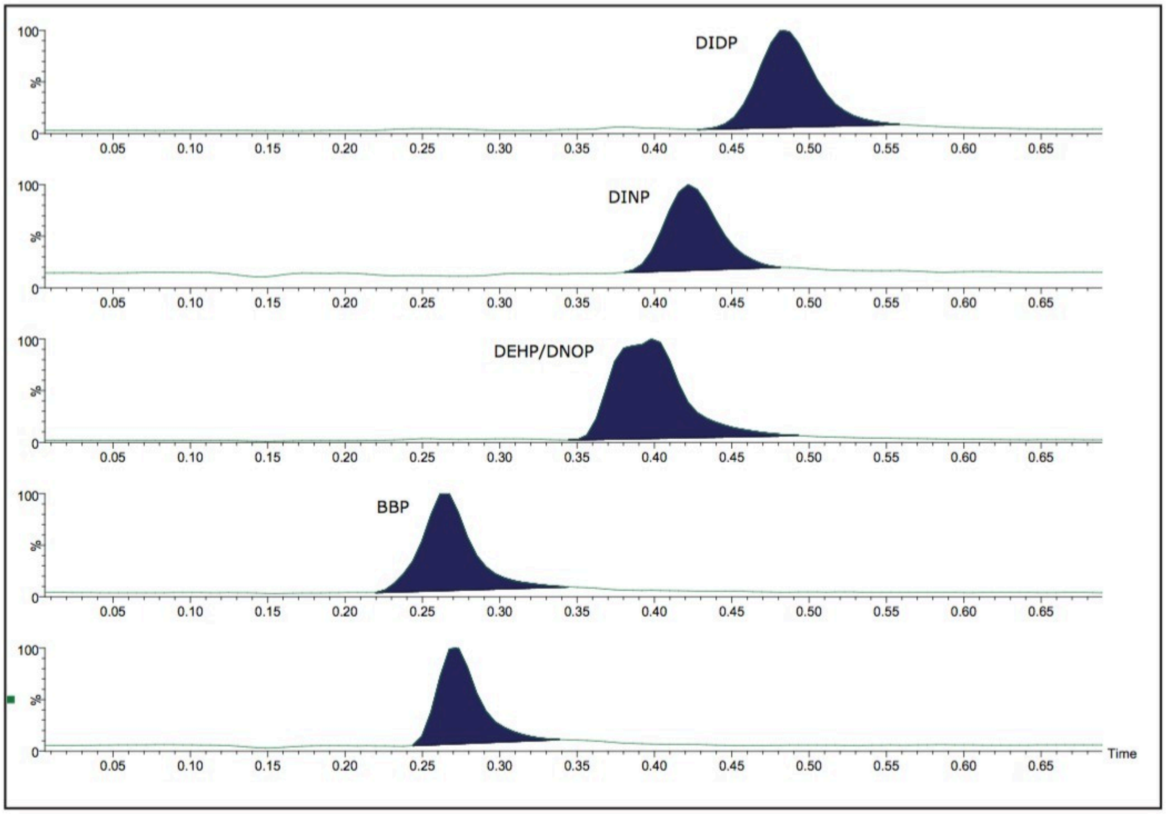


Figure 4. Chromatogram showing legislated phthalates.

The advantage of using a single quadrupole mass spectrometer as the detection system over a point detector, such as photo diode array, is that it gives increased selectivity from background interferences. This will reduce false positive results and make the screening more effective. It will show blank samples to be blank and not appear to contain a phthalate which in reality is could be a matrix interferent at a similar retention time.

Cost-effective technology

A typical quantitative phthalate analysis on a standard GC-MS instrument usually takes approximately 20 minutes per sample. This low throughput option limits users to fewer than 100 samples per day, and thus puts a restriction on the profitability of the laboratory. The advantage achieved by using an ACQUITY SQD is that a 60 second injection to injection runtime is used, producing 60 samples per hour. This screening process quickly shows samples that meet or fail legislative levels. The majority of samples will not come close to phthalate legislative levels, as the toys were not made using any of the six previously mentioned phthalates. An increase in reported results each day will lead to greater laboratory profitability.

Those samples that show positive results close to, or above the level of legislation can then become nominees for quantitation on a slower GC instrument.

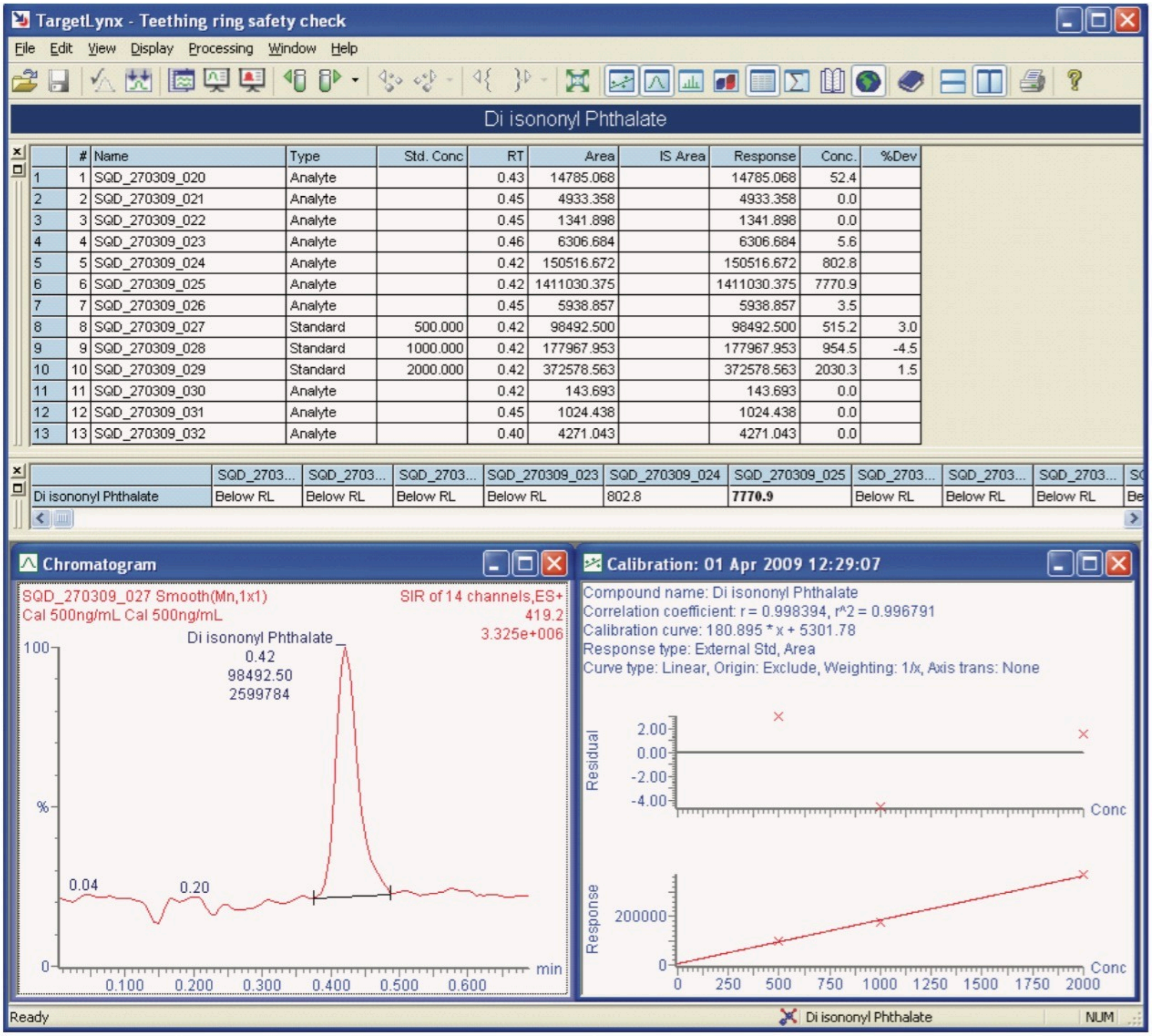
Data acquisition and processing methods

The data were acquired using Waters MassLynx Software, v.4.1. Incorporated into MassLynx, IntelliStartTM Technology automates optimization of MS parameters for the sample, and monitors the health of the MS system, reducing the time for operator-intensive troubleshooting and upkeep. With its fast acquisition rates,the ACQUITY SQD was employed to give 14 data points across each peak. These points help define the peak shape and give more confidence in the semiquantitative results produced in these analytical runs.

This data was processed using TargetLynx Application Manager. This quantification software enables processing and reporting for quantitative data, incorporating a range of confirmatory checks that identify samples that fall outside user-specified or regulatory thresholds.

European Union phthalate legislation states that the phthalate concentration should not be greater than 0.1%. The TargetLynx Application Manager can be used to show which samples require further analysis. In this example, the screening batch shows 10 samples and a 3-point calibration curve which is set with a midpoint to coincide with legislated limits. Due to the dilution factors in the sample extraction, the 0.1% legislated level is equivalent to 1000 ng/mL.

The results are seen in the central overview bar of the TargetLynx browser (Figure 5). In this example, the analyst has set the reporting concentration to be 800 ng/mL (slightly lower than legislative limit) and the maximum concentration to be 1000 ng/mL (legislative limit) in the TargetLynx method editor. Samples with concentration levels lower than the reporting concentration will be displayed as "below RL" and will not need further analysis. The reporting level flag in method editor was intentionally set lower than the legislation to highlight samples that may require further analysis, even though the result shows they are below legislative limits. Any sample over the legislative limit (maximum concentration flag in method editor) is highlighted with bold text and should be quantitatively analyzed.



	#	Name	Type	Std. Conc	RT	Area	IS Area	Response	Conc.	%Dev
1	1	SQD_270309_020	Analyte		0.43	14785.068		14785.068	52.4	
2	2	SQD_270309_021	Analyte		0.45	4933.358		4933.358	0.0	
3	3	SQD_270309_022	Analyte		0.45	1341.898		1341.898	0.0	
4	4	SQD_270309_023	Analyte		0.46	6306.684		6306.684	5.6	
5	5	SQD_270309_024	Analyte		0.42	150516.672		150516.672	802.8	
6	6	SQD_270309_025	Analyte		0.42	1411030.375		1411030.375	7770.9	
7	7	SQD_270309_026	Analyte		0.45	5938.857		5938.857	3.5	
8	8	SQD_270309_027	Standard	500.000	0.42	98492.500		98492.500	515.2	3.0
9	9	SQD_270309_028	Standard	1000.000	0.42	177967.953		177967.953	954.5	-4.5
10	10	SQD_270309_029	Standard	2000.000	0.42	372578.563		372578.563	2030.3	1.5
11	11	SQD_270309_030	Analyte		0.42	143.693		143.693	0.0	
12	12	SQD_270309_031	Analyte		0.45	1024.438		1024.438	0.0	
13	13	SQD_270309_032	Analyte		0.40	4271.043		4271.043	0.0	

	SQD_2703...	SQD_2703...	SQD_2703...	SQD_270309_023	SQD_270309_024	SQD_270309_025	SQD_2703...	SQD_2703...	SQD_2703...	S
Di isononyl Phthalate	Below RL	Below RL	Below RL	Below RL	802.8	**7770.9**	Below RL	Below RL	Below RL	Be

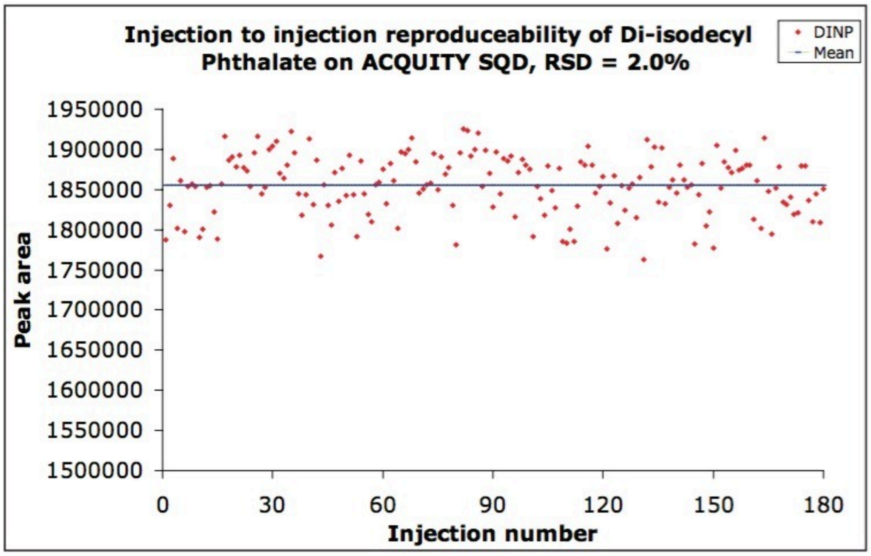
Compound name: Di isononyl Phthalate
Correlation coefficient: r = 0.998394, r^2 = 0.996791
Calibration curve: 180.895 * x + 5301.78
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 5. TargetLynx Application Manager shows samples above user-defined concentration limits.

This three-tiered approach of highlighting well below, near to, and over legislative limit samples empowers the analyst to make quick decisions with regard to candidates for quantitation.

Robust instrumentation is necessary for an efficient laboratory. ACQUITY SQD shows good injection to injection reproducibility throughout an analytical run.

A relative standard deviation of 2% over a 180 injection batch is shown in Figure 6. This minimal variation leads to accurate reporting of results and a reduced need to perform sample retests.



Conclusion

A one minute ACQUITY SQD screening technique was developed for the determination of phthalates which is capable of detecting levels below those set by worldwide legislation.

Analytical time and cost savings can be achieved when using ACQUITY SQD to rapidly identify the phthalate containing toy samples which need quantification, using lengthy time-consuming analytical techniques.

TargetLynx data processing software uses analyst specified limits to easily identify samples that are close to, or exceed legislated levels, giving the analyst a clear indication of which samples to further analyze.

ESCi ion sources can save valuable time in method development procedures, and reduce the number of injections required when dealing with mixtures of compounds that require different ionization techniques.

Improved efficiency and increased sample throughput was realized through the combination of powerful UPLC and fast MS acquisition technologies. The Waters ACQUITY SQD, as shown in Figure 7 offers a revenue conscious laboratory:

- n IntelliStart Technology
  Designed to lower costs by reducing the burden of complicated operation, training new users, time-intensive troubleshooting, and upkeep.
- n Robust performance
  Minimal variation over large sample batches gives confidence in system reproducibility, and reduces the need

for sample retests.

- n Automated system setup and quality control system checks

  Can be performed before analysts arrive to maximize availability during the working day.

Figure 7. ACQUITY SQD.

References

1. Commission of the European Communities EC 396/2005, OJ 2005; L70:1.
2. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040: 0043:EN:PDF
3. ESCi multi mode ionization demonstration http://www.waters.com/waters/promotionDetail.htm?id=10084787
4. Lee, P. Rapid analysis of 25 common polymer additives, Waters Application Note no. 720002488EN, March, 2008.

Featured Products

- [ACQUITY UPLC System <https://www.waters.com/514207>](https://www.waters.com/514207)
- [MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)
- [TargetLynx <https://www.waters.com/513791>](https://www.waters.com/513791)
- [SQ Detector 2 <https://www.waters.com/134631584>](https://www.waters.com/134631584)

720003021, January 2010