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High Throughput LC-MS/MS Assay of Phthalates Using PerkinElmer QSight® 220 LC-MS/MS

Introduction

Phthalates are a family of chemicals commonly used as plasticizers to increase the flexibility, transparency, durability, and longevity of plastics.

These compounds are easily leached out into the environment and migrate into the body. Studies have implicated phthalates in a number of health problems, including asthma, endocrine disruption, reproductive abnormalities, and cancer. The quantification of phthalate exposure is therefore of importance in environmental, occupational medicine, food, and toys. Sensitive and reliable methods to rapidly detect the presence of phthalates are clearly needed. An LC-MS/MS method for the analysis of phthalates comprising of 10 analytes has been developed on a PerkinElmer QSight® 220 triple quadrupole mass spectrometer. This LC-MS/MS method provides a fast, sensitive, and accurate solution for the analysis of phthalates.

2. Method

Samples were extracted with methanol. The aliquot was then centrifuged and supernatant was taken and diluted for LC-MS/MS analysis. In order to minimize the contamination, only glassware was used for all the sample preparation. Liquid-liquid extraction method was also developed for sample processing.

Phthalate standards were purchased from Sigma-Aldrich (MO, USA). Each phthalate standard solution containing 5 ug/mL was prepared by diluting with methanol for construction of the calibration curves.

2.1. Mass Spectrometry Conditions

The LC-MS/MS analysis was performed using the QSight® 220 triple quadrupole mass spectrometer. Table 1 outlines the instrumental parameter settings used during this method. Multiple MRM experiments were used to accommodate the fast UHPLC system. The optimized MRM parameters for all the 10 phthalates are shown in Table 2.

Table 1: MS conditions used on the QSight® 220 instrument during the method.

ESI Voltage (V)	5850
HSID Temp (°C)	300
Nebulizer Gas Setting	450
Drying Gas Setting	200
Source Temp. (oC)	300
Dwell Time (ms)	20
Pause time (ms)	5

Quick Facts:

- High sensitivity method for quantitation of Phthalates using the QSight® 220 triple quadrupole mass spec
- High Throughput analysis of 10 phthalates in 10 minutes LC run time
- Good linearity with $R^2 > 0.99$ for all 10 phthalate samples.
- LLODS are between 0.125 to 5 pg/ul with 1 uL injection volume.

Table 2: Optimized MRM Parameters

Compound	Precursor (m/z)	Fragment (m/z)	CCL 2	CE
DMP	195	163	-60	11
DEP	223	177	-76	11
DBP	279	205	-80	11
DPP	307	149	-80	21
BBP	313	205	-90	11
DHXP	335	149	-10	23
DCHP	331	149	-12	21
DEHP	391	167	-10	19
DNOP	391	261	-10	11
DIDP	447	141	-15	15

2.2. LC Conditions

Shimadzu® Prominence UFLC® system was used with a Phenomenex Kinetex® C18 (2.1 X 100) 2.6 µm particle size column. A Phenomenex Kinetex® C18 (4.6 X100) 2µm particle size trap column between the autosampler and the pump was used to remove any phthalate from the HPLC system. The LC conditions are listed below and in Table 3.

Mobile Phase:	A (0.1% Formic Acid in H ₂ O) B (100% MeOH))
Flow rate:	0.5 mL/min
Injection volume:	1 µL
Column temperature:	40 °C

Table 3: LC gradient

Time (min)	0.06	4	8	8.1	10
Solvent B%	50	98	98	50	50

3. Results

3.1. Extracted Ion Chromatograms (EICs)

LC system carryover issue was first examined. Figure 1&2 show the chromatograms of two phthalate samples. The top panel in each figure shows the injection of the highest concentration (2000 pg/μL) of DMP and DEP, respectively; bottom panel in each figure shows the overlapped three methanol blank injections immediately following the injection of the highest concentration of DMP and DEP, respectively. No carryover is observed. The injection volume is 1 μL.

Figure 1: Chromatograms of DMP injection (2000 pg/μL) and blank injection with methanol.

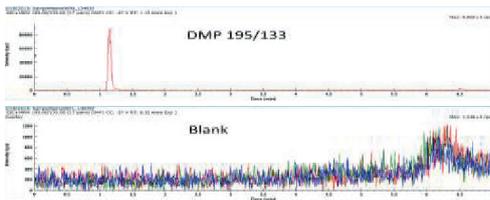


Figure 2: Chromatograms of DEP injection (2000 pg/μL) and blank injection with methanol.

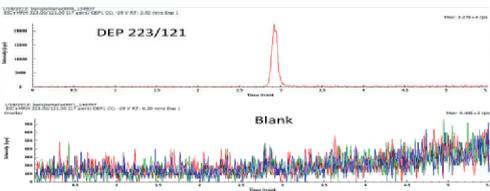


Figure 3: shows the EIC chromatograms of DMP (195/163) at LLOD 1.25 pg/μL. Injection volume is 1μL. Signal to noise ratio is about 5.5.

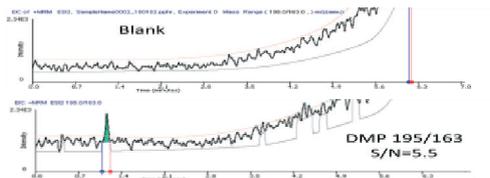


Figure 4: shows the EIC chromatograms of DEP (233/177) at LLOD 0.625 pg/μL. Injection volume is 1μL. Signal to noise ratio is about 12.

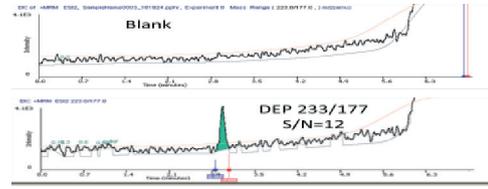
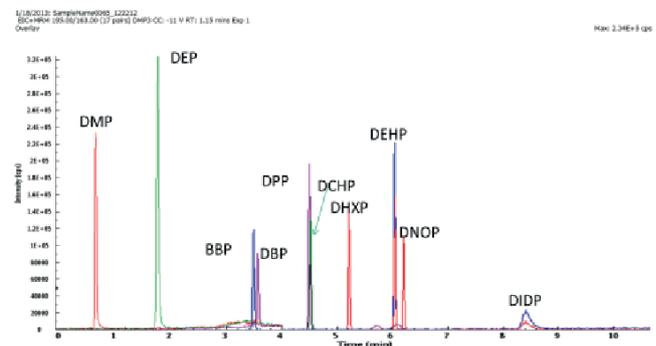


Figure 5: shows the overlap of LC-MS/MS chromatograms for the separation and detection of 10 phthalates at a concentration of 20 pg/μL with a 10-min LC run time.



3.2 Linearity

The calibration curves for all the 10 phthalates were constructed; only DMP and DEP were shown as representatives in Figure 6. The calibration curves were fit with a weighting factor of 1/x. Good linearity was obtained with $R^2 > 0.99$ for all analytes. The accuracy was typically between 85 and 115% with CVs < 15%. The linear dynamic range and linearity for all the phthalates are listed in Table 4.

Figure 6: Calibration curves for DMP with 195/163 (left) and DEP with 223/177 (right).

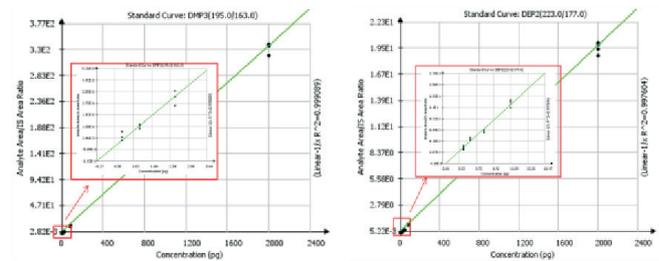


Table 4: List of linear dynamic range, accuracy, and linearity for 10 phthalates

Phthalates	MRM	Linear dynamic range (pg/uL)	Accuracy (%)	R ²
DMP	195/163	1.25-2000	90-108	0.999
DEP	223/177	0.625-2000	93-115	0.997
DBP	279/205	5-1000	87-112	0.999
DPP	307/149	0.125-200	94-113	0.997
BBP	313/205	1.25-1000	97-110	0.998
DHXP	335/149	0.125-200	86-109	0.998
DCHP	331/149	0.125-200	95-111	0.999
DEHP	391/167	2.5-2000	87-113	0.998
DNOP	391/261	0.5-200	91-105	0.999
DIDP	447/141	0.5-200	93-113	0.998

4. Conclusion

A fast and accurate LC-MS/MS method has been developed for 10 phthalate samples on the PerkinElmer QSight® mass spectrometry system. This method simplifies sample preparation and eliminates derivatization process. The capability of running multiple MRM experiments combined with the UHPLC system made the total run time much shorter (10 minutes). The quantitation results showed that the LLODs for all 10 analytes were between 0.125 to 5 pg/uL with 1 ul injection volume. Good linearity was obtained with R² > 0.99 for all analytes. The accuracy was typically between 85 and 115% with CVs < 15%. Therefore, this LC-MS/MS method is a good fit for fast and accurate measurements of phthalates.

5. Contact Information

To learn more about PerkinElmer Mass Spectrometry, our products or services please visit our website or contact us directly.

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