APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Rapid LC/MS/MS Analysis of Phthalates

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 QSight™ 220 Triple Quadrupole Mass Spectrometer. This LC/MS/MS method provides a fast, sensitive, accurate and reproducible solution for the analysis of phthalates.

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Experimental

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.

The LC/MS/MS analysis was performed using a 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. QSight MS conditions.

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

Table 2. Optimized MRM parameters.

Compound Name	Precursor	Fragment	CCL2	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

An Altus™ UPLC® system was used with a Brownlee™ C18 (100 X 2.1 mm, 2.7 µm) column. A Brownlee C18 (100 X 4.6 mm) 2.7 µm particle size trap column between the autosampler and the pump was used to trap any phthalate from the UHPLC system. The LC conditions are listed below and in Table 3.

Mobile Phase: A (0.1% formic acid in 100% H_2O)
B (100% MeOH)

Flow Rate: 0.5 mL/min.

Injection Volume: 1 µL

Column Temperature: 40 °C

Table 3. LC gradient.

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

Results

LC system carryover issue was first examined. Figures 1 and 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.

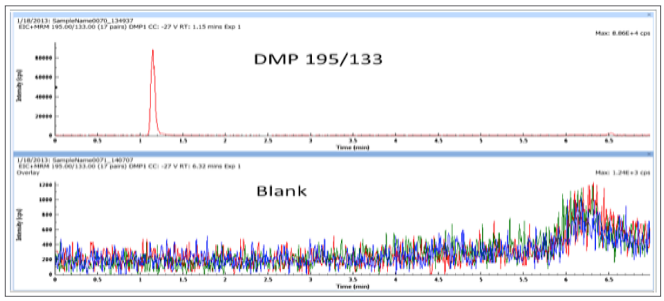

Figure 1. Chromatograms of DMP injection (2000 pg/µL) and blank injection with methanol.

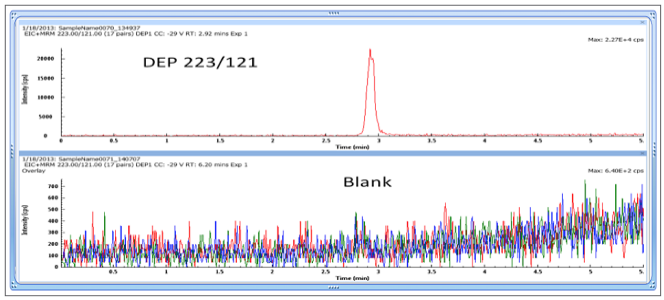

Figure 2. Chromatograms of DEP injection (2000 pg/µL) and blank injection with methanol.

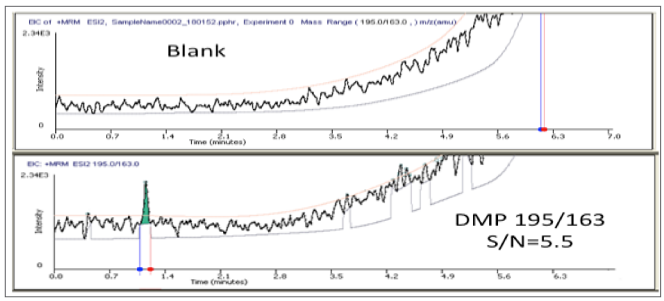

Figure 3. The EIC chromatograms of DMP (195/163) at LLOD 1.25 pg/µL. Injection volume is 1 µL. Signal to noise ratio is 5.5.

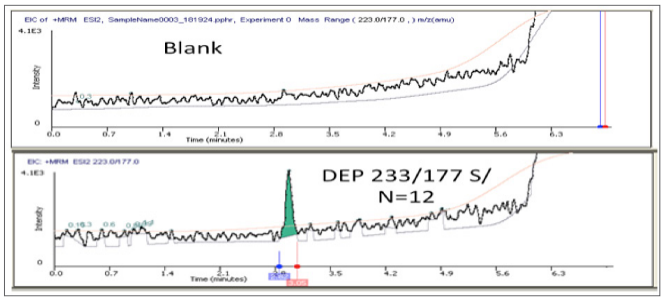

Figure 4. The EIC chromatograms of DEP (233/177) at LLOD 0.625 pg/µL. Injection volume is 1 µL. Signal to noise ratio is about 12.

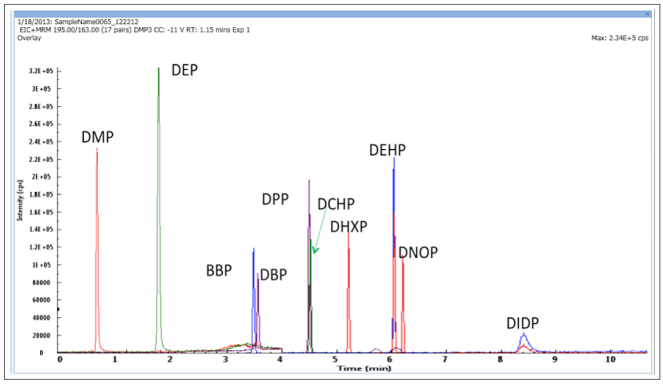

Figure 5. The overlap of LC/MS/MS chromatograms for the separation and detection of 10 phthalates at a concentration of 20 pg/µL with a 10-min. LC run time.

The calibration curves for all the 10 phthalates were constructed; only DMP and DEP were shown as representatives in Figure 6. The calibration curves are 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 CV values < 15%. The linear dynamic range and linearity for all the phthalates are listed in Table 4.

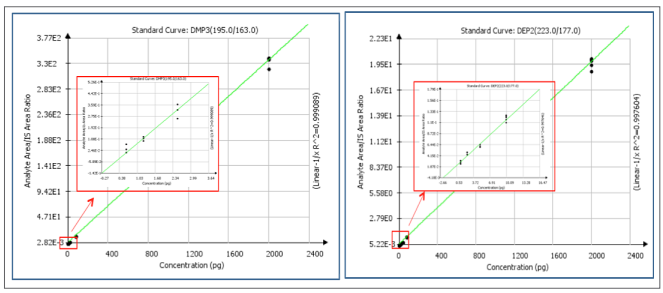

Figure 6. Calibration curves for DMP with 195/163 (left) and DEP with 223/177 (right).

Conclusion

A fast, accurate, and reproducible LC/MS/MS method has been developed for 10 phthalate samples on a QSight 220 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/µL with 1 µL injection volume. Good linearity was obtained with $R^2 > 0.99$ for all analytes. The accuracy was typically between 85 and 115% with CV values < 15%. The LC/MS/MS method described in this study provides a rapid and accurate method for analysis of phthalates.

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

Phthalates	MRM	Linear dynamic range (pg/uL)	Accuracy (%)	R2
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	0.998
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

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