Application Data Set from Shimadzu

● LCMSMS Analysis LCMSMS-004

Determination of Phthalates in Beverage by UFLC-Triple Quadrupole Mass Spectrometry

Abstract: A method was proposed for the determination of phthalates using Shimadzu ultra fast liquid chromatograph and triple quadrupole mass spectrometer. Samples were, after having been processed, separated by LC-30A ultra fast liquid chromatograph, and then quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. The calibration curves of 16 phthalates were plotted in the concentration range of 10-500 μg/L using internal standard method. The plotted calibration curves were of satisfactory linearity with correlation coefficients higher than 0.999. Standard solutions at concentrations of 20 μg/L, 50 μg/L, and 100 μg/L were used for precision test. The %RSDs of retention time and peak area data of 6 successive injections were below 1.04 % and 4.15 %, respectively, showing that the system had satisfactory precision.

Key words: phthalates, triple quadrupole mass spectrometry, beverage

Phthalates are a group of artificially synthesized chemicals which, when added into plastics, can improve the plastics' elasticity. They are a category of common elasticizers extensively present in agricultural film, plastic bags, toys, and rubber tubes. They can be carcinogenic and teratogenic if ingested. On May 24, 2011, Taiwanese media reported that some clouding agent products in Taiwan were elasticizer-tainted. The news sent food safety supervision authority into a turbulent state and brought the detection of phthalates under spotlight. The origin of this food safety scandal was that some clouding agents manufacturers had, in defiance of law, used phthalate elasticizers instead of palm oil in the production of food additives in order to reduce production costs.

A method was developed for the determination of phthalates using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer.

1. Experiments

1.1 Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment.

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The detailed configuration included two LC-30AD pumps, DGU-20A_5 online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions Ver. 5.41 chromatography workstation.

1.2 Conditions of Analysis

LC conditions
Column: Shim-pack XR-ODS III 2.0 mm I.D.×150 mm L., 2.2 μm
Mobile phase A: 5mM ammonium acetate aqueous solution
Mobile phase B: methanol
Flow rate: 0.4 mL/min
Column temperature: 45 °C
Injection volume: 10 μL
Mode: multiple reaction monitoring (MRM)
Dwell time: 10 ms
Pause time: 3 ms
MRM parameters: see Table 2

1.3 Preparation of standard solutions

Standard substances: A total of 16 standard substances were used, i.e. dimethylphthalate (DMP), dibutylphthalate (DBP), dimethoxyethyl phthalate (DMEP), dioctyl phthalate (DPP), butyl benzyl phthalate (BBP), bis(2-n-butoxyethyl) phthalate (DBEP), dicyclohexyl phthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), Di-n-octyl phthalate (DNOP), dinonyl phthalate (DNP), dihexylphthalate (DHXP), diethyl phthalate (DIBP), bis(2-ethoxyethyl) phthalate (DEEP), di-iso-decyl phthalate (DIDP), diethyl phthalate (DEP), and diphenyl phthalate (DIPP).

Internal standard substance: deuterated-di-2-ethylhexyl phthalate (D4-DEHP).

Elution mode: gradient elution, see Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
0.01	Pumps	Pump B Conc.	75
6.50	Pumps	Pump B Conc.	90
7.00	Pumps	Pump B Conc.	100
8.50	Pumps	Pump B Conc.	100
8.60	Pumps	Pump B Conc.	75
10.00	Controller	Stop	

MS condition
Ionization mode: ESI (+)
Ionization voltage: +4.5 kV
Nebulizing gas: Nitrogen 3.0 L/min
Drying gas: Nitrogen 15 L/min
Collision gas: Argon
DL temperature: 250 °C
Heater block temperature: 450 °C

Preparation of standard working solutions: Multi-standard intermediate solution was prepared using methanol as solvent, and then diluted with 50% methanol aqueous solution to get multi-standard working solutions at concentrations of 10, 20, 50, 100, and 500 μg/L.

Preparation of internal standard working solution: 10 mg/L standard intermediate solution was prepared using methanol as solvent, and then diluted with 50% methanol to get 100 μg/L standard working solution.

1.4 Sample pretreatment method: 5.0 mL beverage was taken, added with 2.0 mL n-hexane (residue analysis grade), shaken for 2 min, allowed to settle; 1.0 mL supernatant was taken and dried under nitrogen flush, added with 50 % methanol aqueous solution and brought to marked volume of 1.0 mL, and then injected for analysis.

Table 2 MRM Parameters

Compound	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
DMEP	283.15	59.10	-29	-15.2	-24
		207.1*	-29	-10.0	-26
DMP	195.10	163.1	-22	-11.3	-20
		77.10*	-22	-34.5	-29
DEEP	311.15	73.15	-32	-13.9	-29
		221.15*	-32	-10.0	-28
DEP	223.15	177.15	-40	-10.0	-21
		149.05*	-40	-16.5	-32
DIPP	319.15	225.20	-33	-13.9	-30
		77.10*	-33	-38.4	-29
DIBP	279.20	149.05	-32	-17.8	-32
		205.15*	-32	-10.0	-25
DBP	279.20	149.05	-36	-15.2	-31
		205.15*	-36	-10.0	-25
BBP	313.20	91.15	-33	-31.9	-20
		149.10*	-33	-13.9	-32
DBEP	367.25	101.30	-23	-13.9	-22
		249.25*	-23	-10.0	-20
DPP	307.20	149.10	-35	-13.9	-33
		219.20*	-35	-10.0	-29
DCHP	331.20	149.05	-40	-31.9	-32
		167.05*	-40	-13.9	-20
DHXP	335.25	149.20	-34	-13.9	-33
		233.30*	-34	-10.0	-32
DIDP	447.40	141.25	-21	-12.6	-31
		85.20*	-21	-21.6	-20
D4-DEHP	395.35	153.20	-20	-26.8	-33
		113.30*	-20	-10.0	-25
DEHP	391.35	149.15	-40	-31.9	-32
		113.30*	-40	-10.0	-25
DNOP	391.35	149.15	-40	-19	-33
		261.25*	-40	-10	-20
DNP	419.35	71.20	-20	-22.9	-29
		127.25*	-20	-12.6	-29

Note: *refers to qualitative ion

2. Results and Discussion

2.1 MRM Chromatograms of Standard Samples

The chromatograms of 10 ng/mL standard solutions were as shown in Fig.1-Fig. 17.

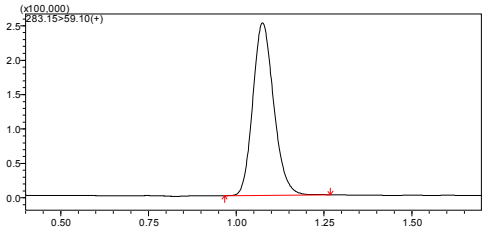

Fig.1 MRM chromatogram of DMEP (283.15>59.10)

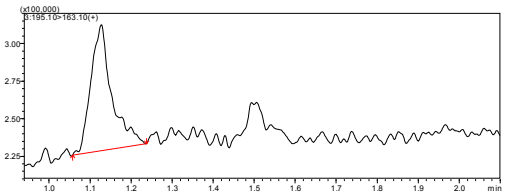

Fig. 2 MRM chromatogram of DMP (195.10>163.10)

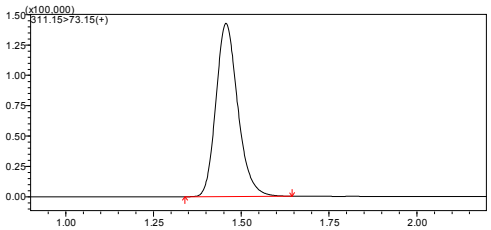

Fig. 3 MRM chromatogram of DEEP (311.15>73.15)

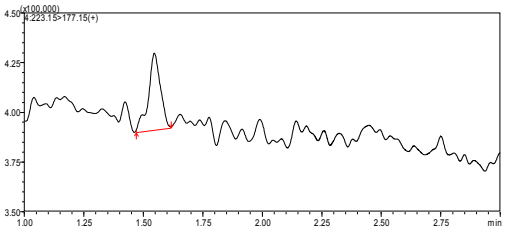

Fig. 4 MRM chromatogram of DEP (223.15>177.15)

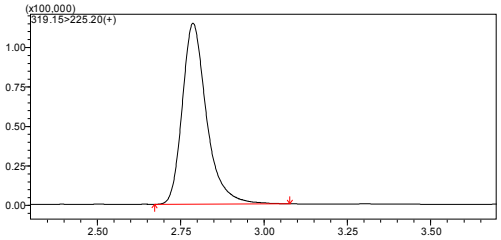

Fig. 5 MRM chromatogram of DIPP (319.15>225.20)

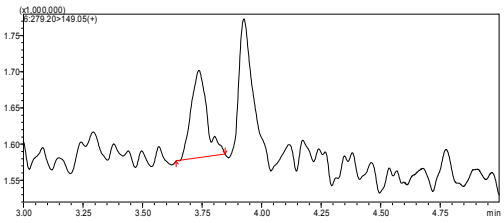

Fig. 6 MRM chromatogram of DIBP (279.20>149.05)

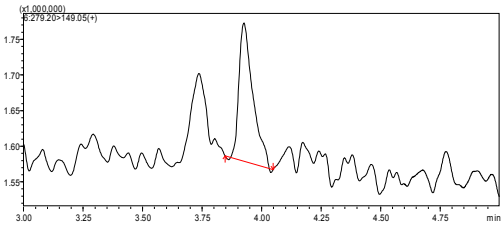

Fig. 7 MRM chromatogram of DBP (279.20>149.05)

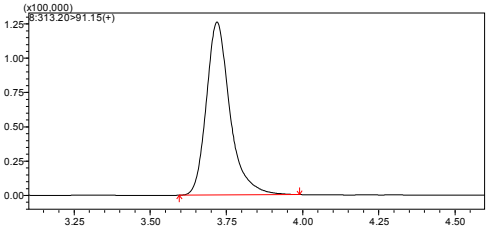

Fig. 8 MRM chromatogram of BBP (313.20>91.15)

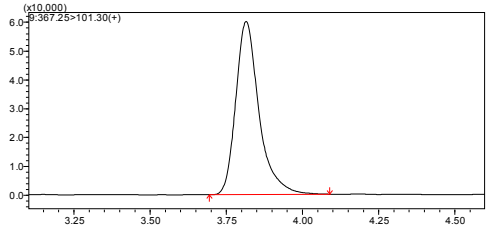

Fig. 9 MRM chromatogram of DBEP (367.25>101.30)

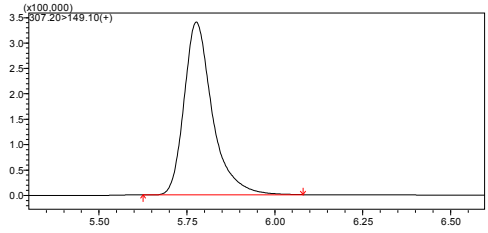

Fig. 10 MRM chromatogram of DPP (307.20>149.10)

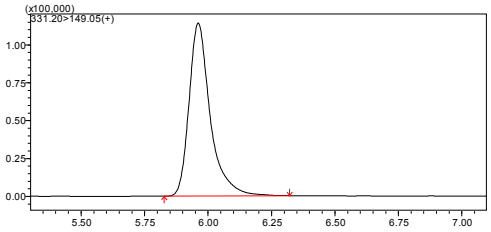

Fig. 11 MRM chromatogram of DCHP (331.20>149.05)

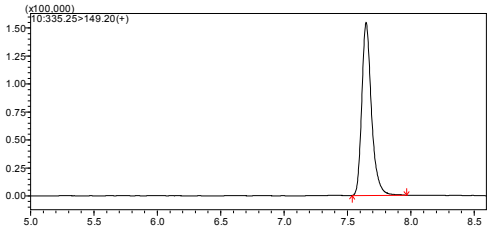

Fig. 12 MRM chromatogram of DHXP (335.25>149.20)

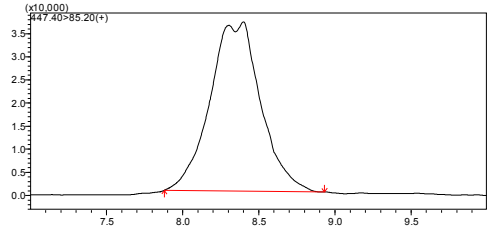

Fig. 13 MRM chromatogram of DIDP (447.40>141.25)

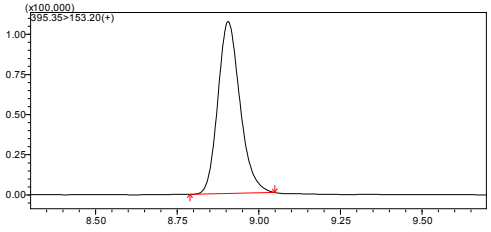

Fig. 14 MRM chromatogram of D4-DEHP (395.35>153.20)

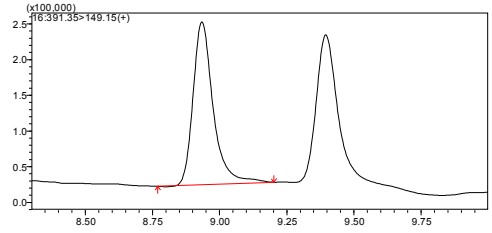

Fig. 15 MRM chromatogram of DEHP (391.35>149.15)

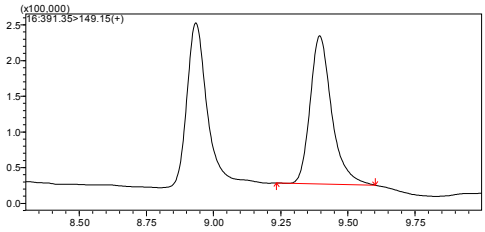

Fig. 16 MRM chromatogram of DNOP (391.35>149.15)

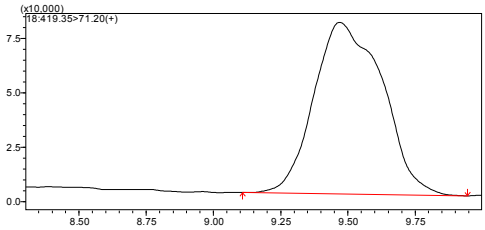

Fig. 17 MRM chromatogram of DNP (419.35>127.25)

2.2 Linearity

Multi-standard working solutions at concentrations of 10, 20, 50, 100 and 500 μg/L were assayed with internal standard method under the analysis conditions as specified in 1.2 and calibration curves were plotted. The plotted calibration curves were of satisfactory linear relation and relevant information is shown in Table 2.

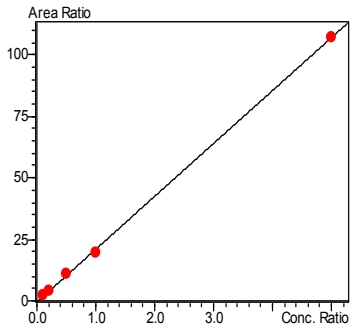

Fig. 18 Calibration curve of DMEP

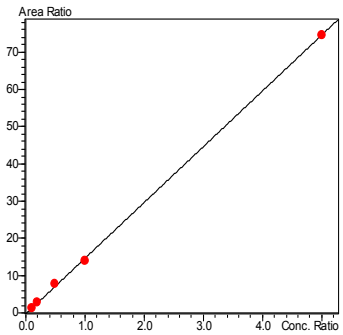

Fig. 19 Calibration curve of DMP

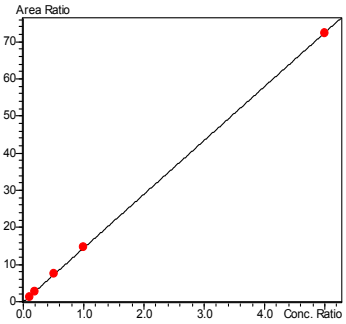

Fig. 20 Calibration curve of DEEP

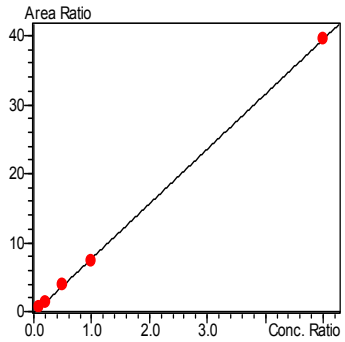

Fig. 21 Calibration curve of DEP

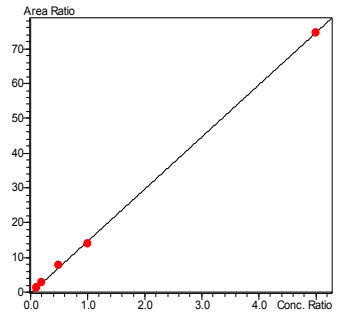

Fig. 22 Calibration curve of DIPP

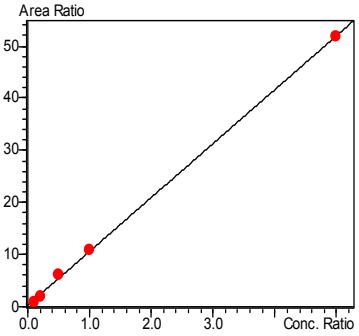

Fig. 23 Calibration curve of DIBP

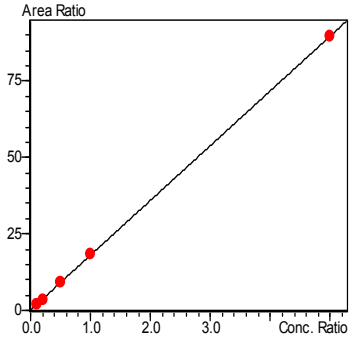

Fig. 24 Calibration curve of DBP

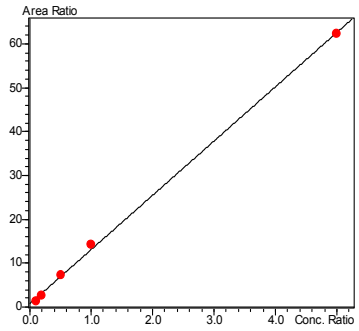

Fig. 25 Calibration curve of BBP

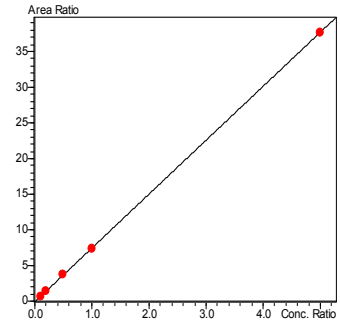

Fig. 26 Calibration curve of DBEP

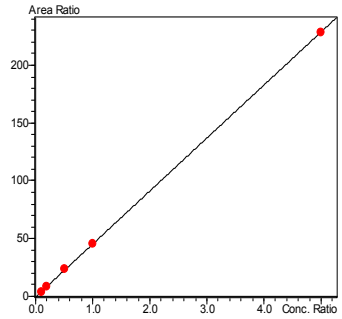

Fig. 27 Calibration curve of DPP

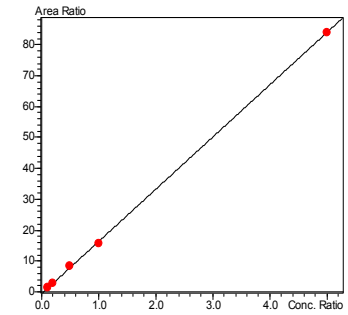

Fig. 28 Calibration curve of DCHP

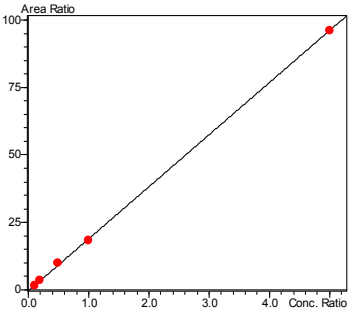

Fig. 29 Calibration curve of DHXP

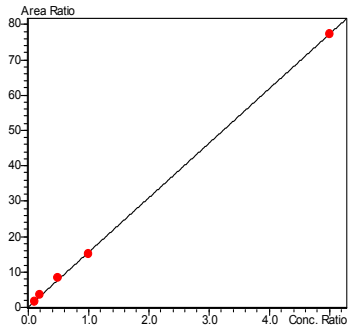

Fig. 30 Calibration curve of DIDP

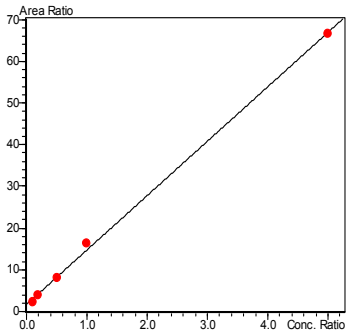

Fig. 31 Calibration curve of DEHP

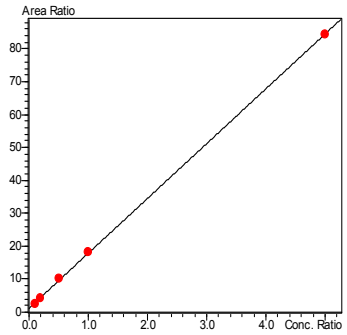

Fig. 32 Calibration curve of DNOP

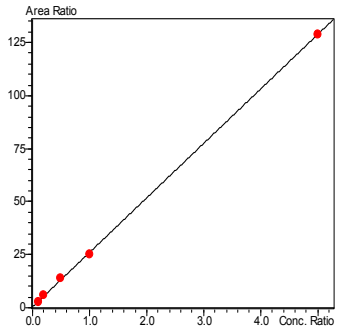

Fig. 33 Calibration curve of DNP

Table 2 Calibration curves and LOQ information of the 16 phthalates

Compound	Calibration Curve	Correlation Coefficient (r)	LOQ (μg/L)	LOD (μg/L)

DMEP	Y = (21.5056)X + (0.507982)	0.9999	0.51	0.17
DMP	Y = (3.84968)X + (0.6499)	0.9993	8.65	2.85
DEEP	Y = (14.5116)X +(-0.0910578)	1.0000	0.13	0.04
DEP	Y = (7.9453)X + (-0.229857)	0.9999	67.80	22.40
DIPP	Y = (14.9911)X + (-0.349504)	0.9999	0.27	0.09
DIBP	Y = (10.3416)X + (0.280682)	0.9997	24.30	8.10
DBP	Y = (17.9339)X + (0.142112)	1.0000	12.90	4.26
BBP	Y = (12.3748)X + (0.731055)	0.9996	0.09	0.03
DBEP	Y = (7.56302)X + (-0.109707)	1.0000	0.30	0.10
DPP	Y = (45.8435)X + (-0.495981)	1.0000	0.12	0.04
DCHP	Y = (16.9033)X + (-0.553426)	0.9999	0.18	0.06
DHXP	Y = (19.2892)X + (-0.36547)	1.0000	0.09	0.03
DIDP	Y = (15.4162)X + (0.149031)	0.9999	0.63	0.21
DEHP	Y = (13.0563)X + (1.66949)	0.9994	0.76	0.25
DNOP	Y = (16.667)X + (1.19159)	1.0000	0.72	0.24
DNP	Y = (25.6942)X + (0.384239)	1.0000	0.69	0.23

2.3 Precision test

Multi-standard working solutions at concentrations of 20, 50, and 100 μg/L were assayed for 6 times in succession to assess the precision of the method. Repeatability of retention time and peak area is shown in Table 3. The results showed that the %RSDs of retention time and peak area data of standard solutions at 3 concentrations (high, medium, low) were 0.03%~1.04 % and 0.19 %~4.15 %, respectively, indicating that the method's precision was satisfactory.

Table 3. Repeatability of 16 phthalates (n=6)

Compound	20 μg/L		50 μg/L		100 μg/L	
	%RSD (RT)	%RSD (Area)	%RSD (RT)	%RSD (Area)	%RSD (RT)	%RSD (Area)
DMEP	0.05	2.68	0.45	0.94	0.12	0.52
DEEP	0.10	2.95	0.68	1.05	0.13	0.86
DMP	0.10	3.90	0.39	4.03	0.14	3.13
DEP	0.53	3.66	0.70	3.02	0.62	3.89
DIPP	0.09	3.28	1.04	0.50	0.10	0.19
DIBP	0.05	2.12	0.93	2.53	0.20	3.65
DBP	0.11	3.09	0.84	3.72	0.13	2.17
BBP	0.09	2.87	0.88	1.04	0.09	0.55
DBEP	0.10	1.91	0.87	1.24	0.08	0.78
DPP	0.07	1.63	0.49	1.24	0.05	1.26

DCHP	0.06	1.23	0.46	1.35	0.04	0.71
DHXP	0.05	1.63	0.31	1.52	0.03	0.76
DIDP	0.44	2.39	0.50	2.34	0.16	1.82
DEHP	0.05	3.54	0.20	3.72	0.05	2.22
DNOP	0.06	2.71	0.26	1.82	0.07	1.82
DIDP	0.06	4.15	0.26	4.11	0.07	2.37

2.4 Spike recovery test

An off-the-shelf green tea beverage was taken as matrix for determination of DEHP. DEHP was detected in the off-the-shelf green tea beverage and its content was determined to be 4.0 μg/L; and the MRM chromatogram is shown in Fig. 34. The above-mentioned green tea was spiked with DEHP at spike level of 50 μg/L and then subject to analysis; the concentration of DEHP was assayed to be 49.2 μg/L; after the DEHP content in matrix (4.0 μg/L) was deducted, the recovery of spiked samples was calculated to be 90.4 %. The chromatogram of off-the-shelf green tea samples spiked with standards is shown in Fig. 35.

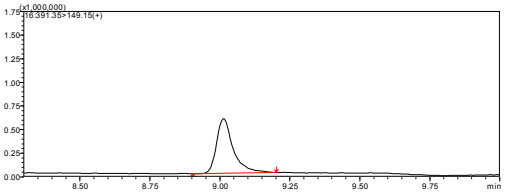

Fig. 34 MRM chromatogram of an off-the-shelf green tea (391.30>149.05)

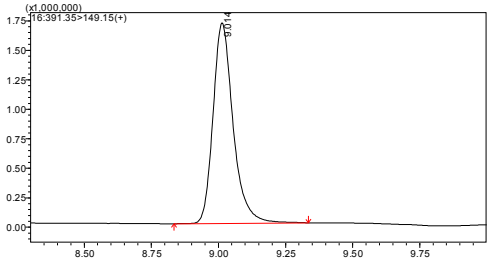

Fig. 35. MRM chromatogram of an off-the-shelf green tea spiked with standards (391.30>149.05)

2.5 Real sample assay results

4 types of off-the-shelf beverage were assayed. The quantitative analysis results of the tested samples were deducted solvent blank. Samples whose phthalates content is outside the range of the calibration curve were diluted first before injected for analysis. The quantification results were as shown in Table 4. DEHP was detected at different levels in all 4 types of beverage.

Table 4. Quantification results of the tested samples

Tested sample	Green tea	Sports drink	Guava juice	Milk tea
DEHP concentration (mg/L)	0.004	0.785	0.103	0.081

3. Conclusion

A method was developed for the determination of phthalates in beverage using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The method was of fast analysis speed and good precision. The correlation coefficients of all calibration curves were of good linearity and higher than 0.999. At the meantime, DEHP was detected at various levels in all 4 types of the off-the-shelf beverage.