

# Determination of 20 Phthalic Acid Esters in Alcoholic Drinks by Ultra High Performance Liquid Chromatography/Tandem Mass Spectrometry

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## 1. Introduction

Phthalic acid esters (PAEs) are a group of commercial chemicals widely used to make plastics more malleable and help lotions penetrate skin. A number of phthalate esters are known to cause birth defects or reproductive harm. PAEs can migrate from plastic materials to the environment. They are often found in water, soil, air, food products and the human body. As well known, alcoholic drinks have always been popular around the world. In alcoholic drinks production, plastic containers are typically

used in the storage and transportation process, which could make some phthalate esters leak easily from PVC tubes or vessels as well as plastic caps. The aim of this study is to determine the level of phthalate esters migration in alcoholic drinks by fast liquid chromatography-electrospray tandem mass spectrometry. This method is simple and rapid with acceptable sensitivity to meet the requirements for the analysis of PAEs in alcoholic drinks.

## 2. Method and Materials

### Sample Preparation

Accurately weigh 5.0 g of alcoholic drinks into a glass tube. After centrifugation for 20 min at 6000 rpm, the supernatant was analyzed by LC/MS/MS.

### UHPLC/MS/MS Analysis

#### UHPLC

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30As degasser, and an SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-ODSIII column (150 mmL. × 2.0 mm i.d., 2.2 μm) with the column temperature at 45°C. The delay column (Inertsil ODS-4 50 mmL. × 3.0 mm i.d., 2.0 μm) was used to reduce the PAEs interference which comes from the mobile phases. The mobile phase consisted of (A) 5 mmol/L ammonium acetate-water and (B) methanol using gradient elution of 45%-90%B at 0-6.5 min, 90%-100%B at 6.5-7.0 min, 100%B at 7.0-9.9 min, and 100%-45%B at 9.9-10 min. The flow rate was 0.4 mL/min. The injection volume was 10 μL.

#### Mass spectrometry

A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was connected to the Shimadzu UHPLC instrument via an ESI interface. The mass spectra were acquired in positive ion mode. The DL temperature was set at 250°C, with heat block temperature at 450°C, nebulizing gas at 3 L/min and drying gas at 15 L/min. The dwell time was 15 ms and the pause time was 3 ms. The MRM parameters were shown in Table 1.

#### Standard PAEs

Dimethyl phthalate (DMP), diethyl phthalate (DEP), bis(2-ethoxyethyl) phthalate (DEEP), bis(2-methoxyethyl) phthalate (DMEP), diallyl (o-)phthalate (DAP), dipropyl phthalate (DPRP), diisopropyl phthalate (DiPRP), diphenyl phthalate (DIPP), benzyl butyl phthalate (BBP), diisobutyl phthalate (DIBP), bis(2-butoxyethyl) phthalate (DBEP), dibutyl phthalate (DBP), dipentyl phthalate (DPP), diisoamyl phthalate (DiAP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), dicyclohexyl phthalate (DCHP), di-n-hexyl phthalate (DHXP), and diheptyl phthalate (DHP). All of the standard PAEs were purchased from ANPEL Scientific Instrument Co., Ltd.

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## 3. Results and Discussion

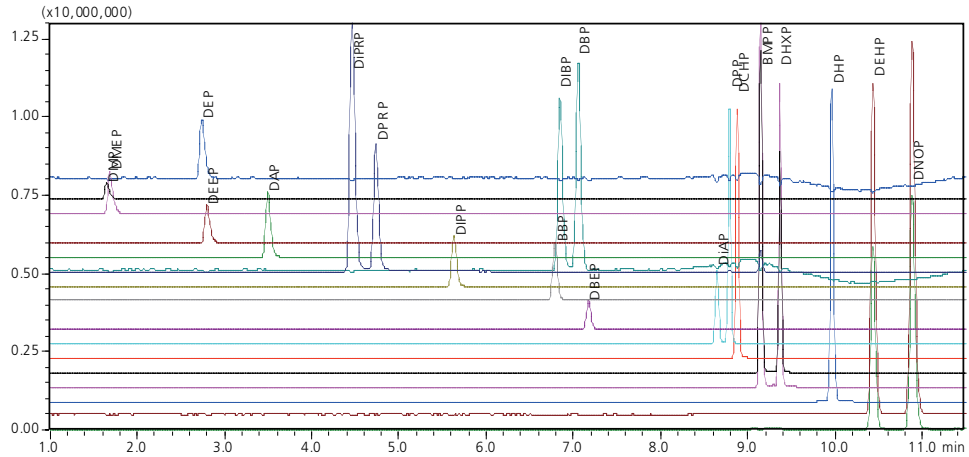


Fig. 1 MRM chromatograms of 20 phthalic acid esters (200 µg/L)

Table 1 MRM parameters of 20 phthalic acid esters

Compound	Precursor ion (m/z)	Production (m/z)	Q1 pre bias (V)	CE (V)	Q3 pre bias (V)
DMP	194.7	163.0*	-23	-11	-17
		77.1	-23	-31	-14
DEMP	283.1	207.0*	-21	-6	-22
		149	-21	-28	-29
DEP	223.1	149.0*	-30	-20	-30
		177.1	-30	-10	-18
DEEP	311.1	73.1*	-23	-13	-30
		221.1	-23	-6	-24
DAP	247.1	189.1*	-28	-11	-20
		149.1	-28	-16	-27
DPRP	251.1	149.0*	-30	-20	-30
		191.1	-30	-9	-20
DiPRP	251.1	149.0*	-30	-20	-30
		191.1	-30	-9	-20
DIPP	319.1	225.0*	-23	-9	-24
		77.1	-23	-36	-30
BBP	313.1	91.1*	-30	-20	-17
		149	-30	-12	-16
DIBP	279.1	149.0*	-30	-20	-30
		205.1	-30	-8	-22
DBP	279.1	149.0*	-30	-20	-30
		205.1	-30	-8	-22
DBEP	367.1	101.1*	-27	-12	-19
		249.1	-27	-7	-27
DPP	307.1	149.1*	-30	-20	-30
		219.1	-30	-7	-24
DiAP	307.1	149.1*	-30	-20	-30
		219.1	-30	-7	-24
DCHP	331.1	149.1*	-30	-25	-28
		167.1	-30	-13	-18
BMPP	335.1	149.1*	-30	-25	-27
		167	-30	-11	-18
DHXP	335.2	149.1*	-30	-20	-30
		233.2	-30	-8	-25
DHP	363.2	149.1*	-30	-20	-30
		247.2	-30	-8	-27
DEHP	391.3	149.1*	-30	-25	-30
		167.1	-30	-13	-17
DNOP	391.4	149.0*	-30	-20	-30
		261.1	-30	-8	-29

\*for quantitation

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20 phthalic acid esters were separated in 11 min. The MRM chromatograms in positive mode were shown in Fig. 1. A linear relationship was found between peak area and different concentrations of 20 phthalate esters within 5,

10, 20, 50 and 200 µg/L. Correlation coefficients (r) more than 0.999, the limits of detection (LODs) and the limits of quantitation (LOQs) were obtained as shown in Table 2.

Table 2 The calibration curve, LOD and LOQ of 20 PAEs

Compound	Calibration curve	r	LOD (µg/L)	LOQ (µg/L)
DMP	Y = 12673.3X + 2300.05	0.9998	0.62	1.85
DMEP	Y = 26744.3X - 12057.1	0.9999	0.23	0.70
DEP	Y = 41199.3X + 60434.8	0.9998	1.67	5.00
DEEP	Y = 24761.6X - 25617.3	0.9999	0.18	0.56
DAP	Y = 43997.2X - 53600.0	0.9999	0.12	0.36
DiPRP	Y = 166476X - 70183.0	0.9999	0.05	0.15
DPRP	Y = 82151.0X - 14189.4	0.9999	0.09	0.26
DIPP	Y = 28688.5X - 89879.0	0.9990	0.16	0.47
BBP	Y = 36060.8X + 13664.7	0.9999	0.10	0.29
DIBP	Y = 105063X + 1597740	0.9999	1.63	4.94
DBP	Y = 131073X + 373016	0.9999	0.83	2.50
DBEP	Y = 16453.9X - 2429.68	0.9999	0.13	0.38
DiAP	Y = 39687.3X + 274520	0.9989	0.03	0.10
DPP	Y = 115069X + 108679	0.9999	0.02	0.06
DCHP	Y = 114309X + 233683	0.9999	0.02	0.05
BMPP	Y = 143127X + 451840	0.9996	0.02	0.05
DHXP	Y = 129006X + 132748	0.9999	0.02	0.06
DHP	Y = 128802X - 125656	0.9999	0.03	0.10
DEHP	Y = 164622X - 14942.3	0.9998	0.47	1.42
DNOP	Y = 120832X - 232093	0.9998	0.46	1.40

Table 3 Repeatability of peak area and RT at different concentrations (n=6)

Compound	%RSD (20 µg/L)		%RSD (50 µg/L)		%RSD (100 µg/L)	
	Area	R.T.	Area	R.T.	Area	R.T.
DMP	3.23	0.08	3.14	0.21	1.76	0.24
DMEP	0.73	0.08	0.71	0.19	0.74	0.20
DEP	4.79	0.17	3.90	0.18	2.76	0.24
DEEP	1.04	0.18	1.18	0.19	1.22	0.26
DAP	0.85	0.18	1.25	0.18	0.81	0.26
DiPRP	0.93	0.18	0.49	0.14	1.17	0.24
DPRP	0.61	0.17	0.87	0.13	0.91	0.22
DIPP	0.84	0.16	1.00	0.10	1.27	0.19
BBP	1.29	0.13	0.36	0.07	1.19	0.15
DIBP	3.55	0.12	3.34	0.07	1.82	0.15
DBP	4.79	0.11	1.50	0.07	1.40	0.14
DBEP	1.33	0.12	0.66	0.06	0.41	0.14
DiAP	1.56	0.05	1.44	0.02	1.10	0.05
DPP	1.30	0.09	0.26	0.09	1.74	0.07
DCHP	1.51	0.04	0.75	0.02	0.56	0.05
BMPP	1.35	0.04	0.83	0.02	1.23	0.03
DHXP	1.24	0.04	0.66	0.02	1.57	0.02
DHP	1.86	0.04	0.99	0.03	1.45	0.02
DEHP	0.59	0.06	0.42	0.04	1.65	0.04
DNOP	1.76	0.08	0.85	0.06	0.98	0.06

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In this study, the repeatability of 20 phthalic acid esters at different concentrations (20, 50 and 100 µg/L) was investigated. The %RSDs of retention time were better than 0.26% and %RSDs of peak area were less than 4.79%, as shown in Table 3.

The mixed standard sample was spiked into the blank alcoholic drink at the levels of 50 µg/kg and 100 µg/kg to evaluate the recovery of this method developed in this study. A good recovery of 78% to 127% was obtained for

each of the compound. The results are shown in Table 4. A 50-percent alcohol distilled liquor made by Hunan-based liquor producer in China contained a maximum of 1.04 mg of DBP per kg. Three samples, including Chinese liquor, wine and whisky from the local market were chosen for analysis. The results showed that 154.1 µg/kg DBP and 18.7 µg/kg DIBP were detected in one of Chinese liquor, and no detection in other samples.

Table 4 Recovery of 20 PAEs in an alcoholic drink

No.	Compound	50 µg/kg		100 µg/kg	
		Measured value (µg/kg)	Recovery (%)	Measured value (µg/kg)	Recovery (%)
1	DMP	42	91	92	100
2	DMEP	48	104	101	110
3	DEP	45	97	101	110
4	DEEP	49	107	102	111
5	DAP	49	107	102	111
6	DiPRP	48	104	101	110
7	DPRP	48	105	105	114
8	DIPP	54	118	117	127
9	BBP	47	102	98	106
10	DIBP	45	99	98	106
11	DBP	48	104	101	110
12	DBEP	48	104	102	111
13	DiAP	36	78	77	84
14	DPP	46	99	91	99
15	DCHP	43	92	85	93
16	BMPP	45	99	91	99
17	DHXP	46	101	91	99
18	DHP	46	99	89	97
19	DEHP	46	100	97	105
20	DNOP	45	99	96	105

## 4. Conclusions

A UHPLC/MS/MS method has been developed for determination of 20 PAEs in alcoholic drinks. All of them were separated in 11 minutes, and analyzed in positive mode. The calibration curves of 20 PAEs were constructed over a concentration range of 50-200 µg/L with correlation coefficients (r) more than 0.999. Good

repeatability on both retention time and peak area was obtained. The limits of detection (LODs) and the limits of quantitation (LOQs) for 20 PAEs were better than 2 µg/L, 5 µg/L, respectively. The method was established for fast, reliable quantitative determination of 20 PAEs in alcoholic drinks.