Determination of Phthalate Esters in Soft Drinks By GC-MS

Anila I. Khan, Thermo Fisher Scientific, Runcorn, UK

Key Words
TraceGOLD TG-5MS, phthalate esters, soft drinks, liquid-liquid extraction

Abstract
This application note demonstrates the quantitative analysis of phthalate esters in soft drinks. The combination of Thermo Scientific™ ultra low bleed TraceGOLD™ TG-5MS columns with a Thermo Scientific™ TRACE™ GC coupled with a Thermo Scientific™ ISQ™ mass spectrometer provides high sensitivity for the detection of phthalate esters in selected ion monitoring (SIM) acquisition mode.

Introduction

Phthalate esters are the main plasticizers used as softening agents in the production of PVC. These compounds are reported to act as endocrine disruptors, and exposure to high levels can cause harmful effects in the human reproductive system. There have been reports from the U. S. Food and Drug Administration that certain foods and beverages, particularly fruit juices, contain high levels of phthalates. In some cases, deliberate adulteration of soft drinks with phthalate esters has been reported.

The EU recently published a methodology without an extraction method for bis-(2-ethylhexyl)phthalate (DEHP) in sports drink at concentrations between 3000 and 100000 ng/mL [1]. In this application note, an analytical procedure for the quantitative analysis of 15 phthalate esters between the concentrations of 100 and 5000 ng/mL is reported. The extraction of the phthalate esters is based on liquid-liquid extraction. Extraction efficiencies are reported at low (300 ng/mL) and high (1000 ng/mL) concentrations in spiked drink sample.

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Experimental Details

Consumables		Part Number
Column:	TraceGOLD TG-5MS, 30 m × 0.25 mm × 0.25 µm	26098-1420
Septum:	Thermo Scientific BTO, 17 mm	31303211
Liner:	Thermo Scientific™ Splitless Straight Liner, 5 × 8 × 105 mm	45350033
Column ferrules:	100% graphite ferrules for Thermo Scientific™ TRACE™ injector, 0.1–0.25 mm i.d.	29053488
Column ferrules:	Graphite/Vespel® for transfer line 0.1–0.25 mm i.d.	29033496
Injection syringe:	10 µL fixed needle syringe for a Thermo Scientific™ TriPlus™ Autosampler	36500525

Sample Handling Equipment		Part Number
System:	Thermo Scientific™ eVol™ Sample Dispensing System (containing Sample Dispensing System, eVol XCHANGE™ Syringes in 5, 50, and 500 µL volumes and eVol stand)	66002-024
Vials and closures:	Thermo Scientific 9 mm Wide Opening Screw Thread Vial Convenience Kit, 2 mL Clear Vial with Patch, Blue Polypropylene Closure with Clear PTFE/Blue Silicone Septa	60180-599

Chemicals and Reagents	Part Number
Fisher Scientific™ HPLC grade acetone	A/0600/15
Fisher Scientific HPLC grade dichloromethane	D/1856/17
Fisher Scientific HPLC grade hexane	H/0406/15
Fisher Scientific HPLC grade water	W/0106/17

Preparation of Calibration Standard

A stock standard solution of 1000 µg/mL of phthalate esters listed in Table 1 was prepared in hexane / acetone (8:2 v/v) (Standard A). This was then used to prepare standard solutions in hexane of 5000, 3000, 1000, 500, 300, and 100 ng/mL. These were Standards B–G. For construction of the calibration curve, the standards B–G were fortified with benzyl benzoate as an internal standard to a level of 1000 ng/mL.

Sample Preparation

The standards and extracts were prepared in scrupulously clean glassware. Avoiding any contact with plastic is vital as phthalates can contaminate glassware and blank samples very easily. Glass analytical syringes, glass pipettes, and pesticide grade solvents were used for preparing samples.

Glassware was scrupulously cleaned by rinsing first with water and then with acetone and hexane. For accuracy, the calibration samples were prepared using an eVol dispensing system. This avoided the use of plastic pipettes as the eVol system has a glass syringe barrel and stainless steel dispensing needle. Any plastic containers were avoided to reduce potential phthalate contamination in the sample preparation.

To prepare the spiked soft drink samples, 300 ng/mL and 1000 ng/mL of phthalate esters (listed in Table 2) prepared in acetone were spiked into 5 mL of soft drink followed by the addition of 5 mL of dichloromethane containing 1000 ng/mL internal standard. The solution mixture was shaken vigorously. An aliquot of organic layer was transferred to the GC vial.

A high injector temperature (320 °C) was used to release the high MW phthalates or any adsorbing phthalates in the injector head. The high temperature resistant BTO septa were used.

Separation Conditions	
Instrumentation:	Thermo Scientific™ TRACE™ GC Ultra gas chromatograph
Carrier gas:	Helium
Column flow:	1.0 mL/min, constant flow
Oven temperature:	80 °C (1 min), 10 °C/min, 320 °C (8 min)
Injector type:	Split/Splitless
Injector mode:	Splitless (1 min), 20 mL/min flow rate, constant septum purge
Injector temperature:	320 °C

MS Conditions	
Instrumentation:	Thermo Scientific™ ISQ™ GC single quadrupole mass spectrometer
Transfer line temperature:	300 °C
Source temperature:	260 °C
Ionization conditions:	EI
Electron energy:	70 eV
SIM scan parameters:	Table 1

Compound	Abbrev	Scan start time (min)	Quan ion (Qual Ion)	Dwell time (s)
Dimethylphthalate	DMP	7.0	163 (194, 77)	0.08
Diethylphthalate	DEP	10.50	149 (177, 121)	0.08
Benzyl benzoate (Internal standard)	ISTD	12.50	105 (212, 91)	0.08
Diisobutyl phthalate	DIBP	14.00	149 (223, 205)	0.08
Di-n-butyl phthalate	DBP	15.00	149 (223, 205)	0.08
Bis(2-methoxyethyl) phthalate	DMEP	15.75	59 (149,193)	0.08
Bis(4-methyl-2-pentyl) phthalate	BMPP	16.30	149 (251,167)	0.08
Bis(2-ethoxyethyl) phthalate	DEEP	16.80	45 72,149	0.08
Dipentylphthalate	DPP	17.20	149 (237, 219)	0.08
Di-n-hexyl phthalate	DHXP	18.20	104 (149, 76)	0.04
Benzyl butyl phthalate	BBP	18.20	149 (91, 206)	0.04
Bis(2-n-butoxyethyl) phthalate	DBEP	19.50	149 (223, 205)	0.08
Dicyclohexyl phthalate	DCHP	20.30	149 (167, 83)	0.08
Bis(2-ethylhexyl) phthalate	DEHP	20.30	149 (167, 279)	0.08
Di-n-octyl phthalate	DNOP	21.40	149 (279, 167)	0.08
Di-nonyl Phthalate	DNP	22.80	149 (293, 71)	0.08

Table 1: SIM Scan parameters

Injection Conditions	
Instrumentation:	TriPlus Autosampler
Injection volume:	1 µL
Pre- and post-injection dwell time:	3 s

Data Processing	
Software:	Thermo Scientific™ Xcalibur™ software

Results

The analysis was carried out using a TraceGOLD TG-5MS column. The chromatogram of 1000 ng/mL of spiked phthalate ester in soft drink, in a full scan mode, is shown in Figure 1.

Phthalate esters are environmentally ubiquitous and this may therefore affect measured recoveries. To minimize this problem, care was taken when preparing solutions for calibration and extractions. Longer chain phthalate esters such as DNP, DNOP, and DHXP can absorb to the glassware and the lower extraction recoveries were expected.

The results for the calibration data and for the phthalate esters extracted from a soft drink are displayed in Table 2. The coefficients of determination (R^2) between the area ratio of sample and internal standard for all phthalate esters were > 0.99 (Table 2), demonstrating excellent method linearity using the TraceGOLD TG-5MS GC column.

The extraction of phthalate esters at 300 ng/mL and 1000 ng/mL gave recoveries between 66–111% and 71–118%, respectively.

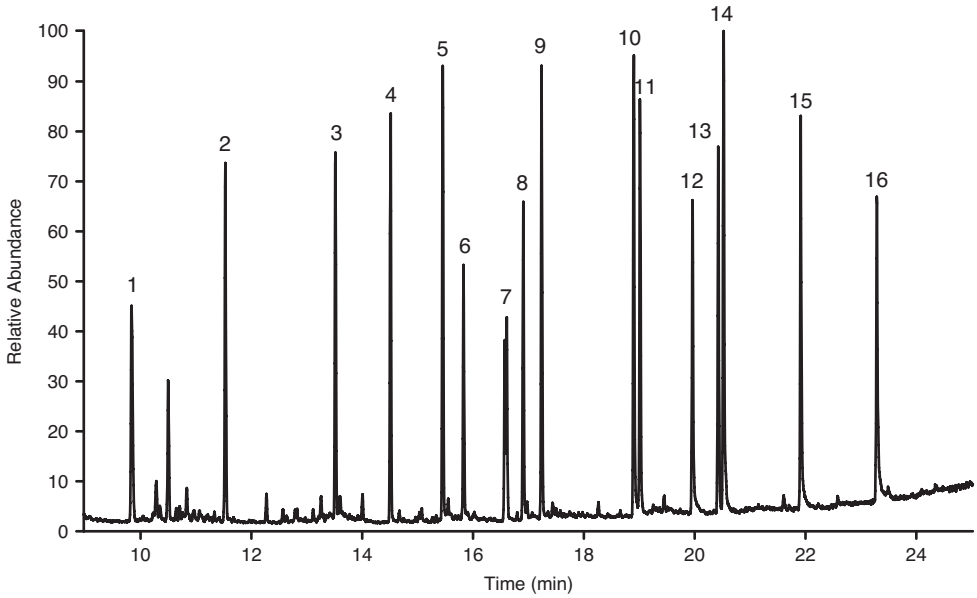

Figure 1: TIC of 1000 ng/mL phthalate esters standard in full scan 40–450 amu

Peak	Compound	Abbrev	t_R (min)	R^2	% Recovery at 300 ng/mL (n=3)	% Recovery at 1000 ng/mL (n=2)
1	**Dimethylphthalate**	DMP	9.91	0.9944	103	85
2	**Diethylphthalate**	DEP	11.60	0.9953	106	108
3	**Benzyl benzoate (Internal standard)**	ISTD	13.60			
4	**Diisobutyl phthalate**	DIBP	14.58	0.9970	92	118
5	**Di-n-butyl phthalate**	DBP	15.52	0.9964	91	92
6	**Bis(2-methoxyethyl) phthalate**	DMEP	15.90	0.9918	93	96
7	**Bis(4-methyl-2-pentyl) phthalate**	BMPP	16.6, 16.68	0.9975	95	82
8	**Bis(2-ethoxyethyl) phthalate**	DEEP	16.98	0.9913	100	93
9	**Dipentylphthalate**	DPP	17.31	0.9940	107	87
10	**Di-n-hexyl phthalate**	DHXP	18.98	0.9945	84	71
11	**Benzyl butyl phthalate**	BBP	19.09	0.9937	111	86
12	**Bis(2-n-butoxyethyl) phthalate**	DBEP	20.03	0.9930	77	77
13	**Dicyclohexyl phthalate**	DCHP	20.50	0.9925	107	84
14	**Bis(2-ethylhexyl) phthalate**	DEHP	20.60	0.9941	100	78
15	**Di-n-octyl phthalate**	DNOP	21.99	0.9962	71	80
16	**Di-nonyl Phthalate**	DNP	23.37	0.9947	66	79

Table 2: Recoveries and linearity of phthalate esters according to their retention times

Conclusion

A method for the quantification of phthalate esters in soft drinks has been developed on a TraceGOLD TG-5MS column. The ultra low bleed characteristics of the column enable the detection of low levels of phthalate esters using the ISQ mass spectrometer in SIM mode.

References

[1] EU commission JRC technical notes: Determination of bis-(2-ethylhexyl)phthalate (DEHP) in sports drinks by isotopic dilution headspace solid-phase micro extraction gas chromatography mass spectrometry (HS-SPME-GC-MS).

USA and Canada +1 800 332 3331
Australia 1300 735 292 (free call domestic)
China 800 810 5118 (free call domestic)
400 650 5118
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
India +91 22 6742 9494
+91 27 1766 2352
Japan 0120 753 670 (free call domestic)
0120 753 671 (fax)
Korea +82 2 3420 8600
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
Singapore +65 6289 1190
All Other Enquiries +44 (0) 1928 534 050

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