Fast GCMS Method for Analysis of Phthalate Esters in Beverages

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1: Introduction

Phthalate esters are well known toxic plasticizers. They are primarily used in polyvinyl chloride (PVC) polymers to enhance properties such as flexibility, transparency, longevity and durability. However, human consumption of certain plasticizers such as di-2-ethylhexyl phthalate, could lead to health problems such as cancer, endocrine disruption and fertility problem. In May 2011, the Food and Drug Administration (FDA) uncovered a case on the substitution of palm oil based emulsifier with phthalate esters (di-2-ethylhexyl phthalate and diisononyl phthalate) by 2 Taiwan companies. This led to a huge public outcry as this massive scale food contamination has been on-going for more than 20 years. Therefore, it is of great importance to develop analytical methods to ensure the rapid and reliable detection of phthalate esters in food and drink products.

In this study , we aim to make use of GCMS-QP2010 *U*ltra to develop a Fast GCMS method with narrow-bore capillary column for the simultaneous analysis of 7 phthalate esters in beverages. This state of the art technology is utilized as it promises to achieve high resolution separation within a short elution time. Such a feat is feasible as GCMS-QP2010 *U*ltra contains the ASSP™ (Advanced Scanning Speed Protocol) function. This ASSP™ function enables the scan rate to reach 20,000 u/sec and the data acquisition speed to attain 100Hz, thereby increasing the data points collection in Scan/SIM (FASST) data acquisition mode. In addition, the ASSP™ function causes a shorter SIM dwell times in contrast to the conventional GCMS system (up to 5 times difference) and thus enabling more SIM channels to be monitored. Finally, GCMS-QP2010 *U*ltra posses the capabilities of heating and cooling the oven rapidly for the reduction of analysis cycle. Therefore, with the aid of these features, GCMS-QP2010 *U*ltra promises to achieve the aim set out in this study.

2: Instrument & Analytical Conditions

Instrument

GC-MS : GCMS-QP2010 *U*ltra
Auto injector : AOC5000

GC		MS	
Injection Temp.	: 290 ° C	Ion Source Temp.	: 230 ° C
Column	: DB-5	Interface Temp.	: 280 ° C
	(10 m L. * 0.1 mm I.D. , δf =0.1 μm)	Acquisition Mode	: Scan/SIM (FASST)
Column Temp.	: 80° C – (60° C/min) – 220° C –	Tuning Mode	: Normal
	(50° C/min) – 310° C (1 min)	Emission Current	: 60 mA
Injection Mode	: Splitless	Scan	
Carrier Gas	: He (constant linear velocity mode)	Scan Range	: m/z 50 – 450
Linear Velocity	: 60 cm/sec	Event Time	: 0.05 sec
Purge Flow	: 3 mL/min	Scan Speed	: 10000 u/sec
Injection Volume	: 1 mL	SIM	
		Event Time	: 0.05 sec

Compound	SIM Monitoring Ions
BB (ISTD)	m/z 105.1, 194.1, **212.1***
DBP	m/z 149.1, 167.1, 205.1, **223.1**
BBP	m/z 91.1, 149.1, **206.1**
DEHP	m/z 149.1, 167.1, **279.2**
DnOP	m/z 149.1, 167.1, 261.2, **279.2**
DINP	m/z 149.1, 167.1, **293.3**
DIDP	m/z 149.1, 167.1, **307.3**
DnDP	m/z 149.1, 289.2, **307.3**

*The figures in bold are the quantitative ions

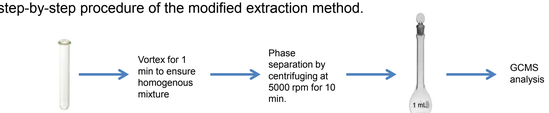
3: Samples and Sample Preparation

3-1: Standard Samples

Phthalate esters standard mixture III [contains 100 ppm of dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP), and di-isodecyl phthalate (DIDP)] was purchased from Kanto Chemical Co., Inc., while di-n-decyl phthalate (DnDP) was purchased from Sigma Aldrich Pte. Ltd. Benzyl benzoate (BB), the internal standard, was purchased from Wako Pure Chemical Industries, Ltd. All organic solvents used were of pesticide residue grade. The calibration was performed in accordance to [2].The Internal Standard (BB) was diluted in cyclohexane to create an internal standard stock solution (ISSS) of 100 ppm. Five calibration standard solutions at concentration of 0.25, 0.50, 1.00, 5.00 and 10.00 ppm were prepared in cyclohexane. All 5 calibration standards contain an internal standard (BB) of concentration at 1.00 ppm.

3-2: Sample Preparation

Grape juice purchased from a local convenience store was used as the sample for analysis. The sample extraction method was modified from [1]. Figure 1 illustrates the step-by-step procedure of the modified extraction method.



1. Take 5 mL of sample.
2. Add 2 mL of n-hexane (extractant).

1. Pipette 0.5 mL of the supernatant.
2. Add 10 μL of ISSS
3. Top up to 1 mL with cyclohexane.

Figure 1: Sample preparation flow chart

4: Result and Discussion

Figure 2 shows the overlay total ion chromatograms (TICs) of the 7 phthalate esters using conventional GCMS method which utilize a typically middle bore capillary column (30 m L. * 0.25 mm I.D., δf=0.25 μm) and Fast GCMS method. The use of Fast GCMS has effectively reduced the elution time from 13.6 to 4.5 min (3 times speed gain in comparison with the conventional GCMS method). In addition, the resulting peaks appeared sharper as more than 10 data points were collected for each peak during the high scan speed analysis.

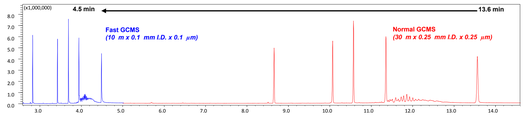


Figure 2: Overlay of TICs of Fast GCMS method (in blue) and Normal GCMS method (in red)

TIC of the internal standard (BB) and the 7 phthalate esters (DBP, BBP, DEHP, DnOP, DINP, DIDP and DnDP) analyzed by using Fast GCMS method is shown in Figure 3. Effective chromatographic separation of DBP, BBP, DEHP, DnOP and DnDP is achieved in the analysis. On the other hand, DINP and DIDP appeared as finger peaks due to the presence of numerous isomers. As such, area summation integration was used for the quantitation of DINP and DIDP.

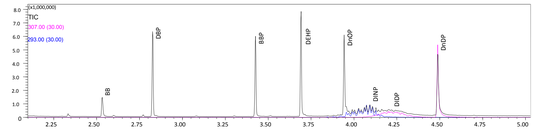


Figure 3: TIC of internal standard the 7 phthalate esters by using Fast GCMS method

The overlay mass chromatograms in Figure 4 illustrates an excellent injection-to-injection repeatability of DIDP at 0.50 ppm. The repeatability (%RSD) of the peak area for the phthalate esters at 0.50 ppm (n = 7) is within 3% (Table 1).

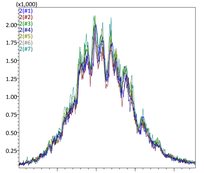


Figure 4: Mass chromatograms of DIDP at 0.50 ppm (n=7)

Table 1: Area repeatability, detection limit and quantitation limit of phthalate esters at 0.50 ppm in solution (n=7)

	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Area 7	%RSD	Detection limit	Quantitation limit
BB	64,154	63,884	65,336	62,668	66,374	64,524	65,542	1.90	-	-
DBP	13,880	14,061	14,627	14,112	14,478	14,524	14,950	2.60	0.0371	0.1125
BBP	25,118	24,842	25,804	25,078	25,856	24,663	26,329	2.42	0.0276	0.0836
DEHP	13,351	12,928	13,787	13,327	13,851	13,365	13,921	2.67	0.0292	0.0886
DnOP	16,011	15,151	16,060	15,539	15,253	15,100	15,621	2.53	0.0391	0.1185
DINP	19,430	19,429	20,299	19,959	19,789	19,213	20,640	2.60	0.0254	0.0770
DIDP	17,817	16,756	18,000	17,274	17,848	17,197	18,040	2.78	0.0227	0.0689
DnDP	14,548	13,876	13,954	14,448	13,844	14,125	14,514	2.19	0.0209	0.0634

All 7 phthalate esters achieved excellent linearity with correlation coefficient (R^2) greater than 0.999 across the calibration range of 0.25 ppm -10.00 ppm (Figure 5)..

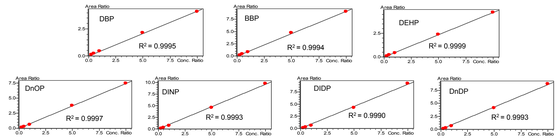


Figure 5: Calibration curves of 7 phthalate esters (0.25-10.00 ppm)

Figure 6a shows the TIC of grape juice sample. TIC of spiked sample (Figure 6b) and 0.50 ppm phthalate esters standard solution (Figure 6c) are utilized to serve as a comparison and confirmation for the presence of phthalate esters in the samples. Based on the TIC (Figure 6), it is found that minute amount of DEHP was present in the sample which is of no safety concern as the quantity is far below the EU specific migration limits (SML) of 1.5 ppm [3]. Additional substances such as palmitic acid butyl ester, octanoic acid triglyceride and decanoic acid vinyl ester are discovered from this sample.

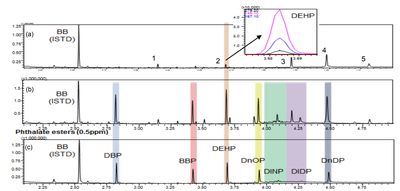


Figure 6: TIC of (a) grape juice together with the SIM chromatogram of detected DEHP; (b) grape juice spiked with 0.50 ppm phthalate esters; (c) 0.50 ppm phthalate esters standard solution .
Legend: Peak 1 - Palmitic acid butyl ester; Peak 2 - DEHP; Peak 3, 4 – Octanoic acid triglyceride ; Peak 5 – Decanoic acid vinyl ester.

Table 2: Concentration of phthalate esters in grape juice.

Target	Concentration (ppm)
DBP	N.A
BBP	N.A
DEHP	Detected
DnOP	N.A
DINP	N.A
DIDP	N.A
DnDP	N.A

Table 3: Concentration and recovery of phthalate esters in grape juice spiked at 0.50 ppm.

Target	Concentration (ppm)	Recovery %
DBP	0.59	118
BBP	0.57	114
DEHP	0.55	110
DnOP	0.48	96
DINP	0.47	94
DIDP	0.46	92
DnDP	0.47	94

5: Conclusion

The optimized Fast GCMS method in this study permits the elution of 7 phthalate esters within 4.5 min (3 times faster than the conventional GCMS method). Excellent linearity (R^2>0.999) was obtained for the calibration curves across the concentration range of 0.25 ~10.00 ppm. The repeatability is satisfactory with the %RSD < 3 for all the targeted analytes. Real sample analysis shows that a minute amount of DEHP is present in grape juice sample. However, the detected amount is below SML of 1.5 ppm.

6: References

[1] National Standards of the People's Republic of China, *GB/T 21911 -2008 : Determination of Phthalates in Food,* 2008.
[2] Consumer Product Safety Commission , *CPSC-CH-C1001-09.3:Standard Operating Procedure for the Determination of Phthalates*, http://www.cpsc.gov/about/cpsia/CPSC-CH-C1001-09.3.pdf
[3] European Union, *Commission Regulation (EU) No 10/2011 : Plastic Materials and Articles Intended to Come into Contact with Food.*