

# Determination of 19 Phthalic Acid Esters (PAEs) in Vegetables Using Modified QuEChERS and Gas Chromatography-Triple Quadrupole Mass Spectrometry (GC/MS/MS)

## Application Note

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### Abstract

A practical method using an Agilent 7000B Triple Quadrupole GC/MS/MS system for the detection of 19 phthalic acid esters (PAEs) residues in vegetables was established. The PAEs were extracted from vegetable samples with acetonitrile using ultrasonic equipment. The extracts were salted with anhydrous magnesium sulfate and sodium chloride, centrifuged, and then purified by C18. The PAEs were separated using an HP-5 MS UI column and detected using a triple quadrupole multiple reaction monitoring mode. Vegetable samples were spiked with a 0.010 mg/kg and 0.10 mg/kg ( $n = 3$ ) mixed standard. The majority of recoveries were within 60–120 %, and the RSDs were below 15%. Good linearity of the 19 types of PAEs was obtained from 0.010 to 0.50 mg/L, with  $R^2$  of all compounds larger than 0.995. The method shows high sensitivity and good accuracy, and meets the general requirement for multiresidue analysis and, therefore, can be applied to the determination of PAEs in vegetables.



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## Introduction

Phthalic acid esters (PAEs) have the possibilities of increasing product transparency, flexibility, and durability. They are key additives of plastics, and are primarily found in polyvinylchloride (PVC) products. Due to the widespread application of plastic packaging materials and plastic greenhouses, large amounts of these compounds are released into the environment, passing on serious PAE pollution to vegetables and other foodstuffs. Due to their suspected carcinogenic and estrogenic properties, there has been considerable monitoring of the exposure of phthalates to humans.

The common pretreatment methods for PAE analysis are liquid-liquid extraction (LLE) and solid phase extraction (SPE) [1]. However, the LLE method is tedious and not environmental friendly due to the large consumption of organic solvent, while the SPE method requires multistage treatment, and is time-consuming. This study used the modified QuEChERS method to extract and purify the samples as an alternative. China has published a GB (National Standard) methodology with an extraction approach for 16 phthalates in foodstuffs [2]. This study analyzed a total of 19 phthalates including 15 compounds in the GB method and an extra four interesting PAEs (DPRP, DIDP, DINP, and DAP). The GC parameter used in this study contained a 28-minute separation with backflush to minimize analysis time and avoid elution of unexpected compounds with high molecular weight which could contaminate the detector [3]. The Agilent 7000B Triple Quadrupole GC/MS/MS System provided excellent sensitivity and selectivity in this research, and the established method was proven to be simple, efficient, and reliable.

## Experimental

### Reagents and chemicals

Acetonitrile (ACN) and hexane were of HPLC grade. The 15 phthalate standards were: dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), bis(2-methoxyethyl phthalate) (DMEP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis(2-ethoxyethyl) phthalate (DEEP), dipentyl phthalate (DPP), dihexyl phthalate (DHXP), benzyl phthalate (BBP), bis(2-n-butoxyethyl) phthalate (DBEP), dicyclohexyl phthalate (DCHP), bis(2-ethylhexyl) phthalate (DEHP), di-*n*-octyl phthalate (DNOP), and dinonyl phthalate (DNP), and were purchased from o2si smart solutions (America); bis-propyl ester phthalate (DPRP), di-iso-decyl phthalate (DIDP), and diisononyl phthalate (DINP) were purchased from Dr. Ehrenstorfer (German), and diallyl phthalate (DAP) was purchased from Sino-pharm Chemical Reagent (China).

## Instruments and conditions

### Chromatographic parameters

GC system	Agilent 7890A GC (The configuration is shown in Figure 2)
Column 1 and Column 2	Agilent HP-5 MS UI capillary column (15 m × 0.25 mm, 0.25 μm)
Oven temperature program	60 °C hold 1.5 minutes at 20 °C /min to 220 °C, hold 1 minute at 5 °C /min to 280 °C, hold 4 minutes (GB/T 21911-2008)
MMI inlet Injection mode	Splitless, purge on after 1 minute
Carrier gas	Helium
Flow rate	Column 1) 1.0 mL/min Column 2) 1.20 mL/min
Injection port temperature	280 °C
Injection volume	1 μL
Backflushing conditions	
Timing	5 minute duration during post-run
Oven temperature	280 °C
Aux EPC pressure	50 psi
Inlet pressure	2 psi

### MS parameters

Mass system	Agilent 7000B Triple Quadrupole GC/MS System
Solvent delay	5.0 minutes
Ion source	EI
Ionization voltage	70 eV
Ion source temperature	280 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
Interface temperature	280 °C
Collision gas	Nitrogen, 1.50 mL/min
Quenching gas	Helium, 2.25 mL/min

MRM parameters are shown in Table 1. Most of the compounds contain three transitions to confirm the presence of the targets.

## Modified QuEChERS Approach

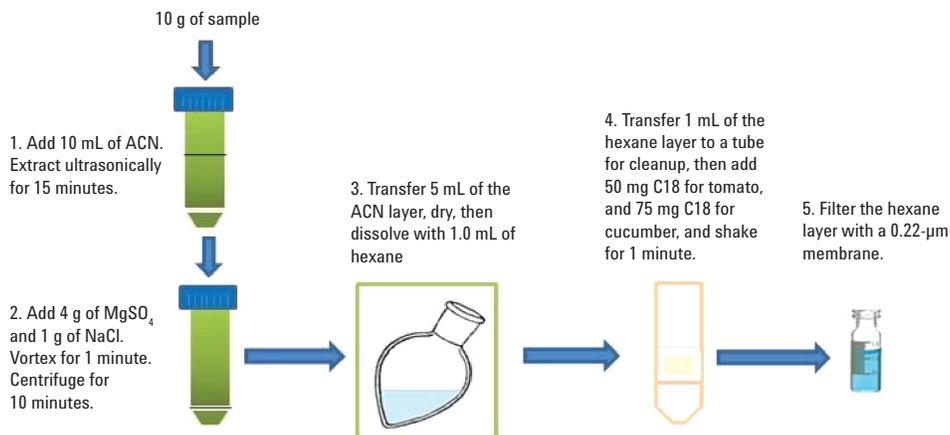


Figure 1. Flow chart of sample preparation.

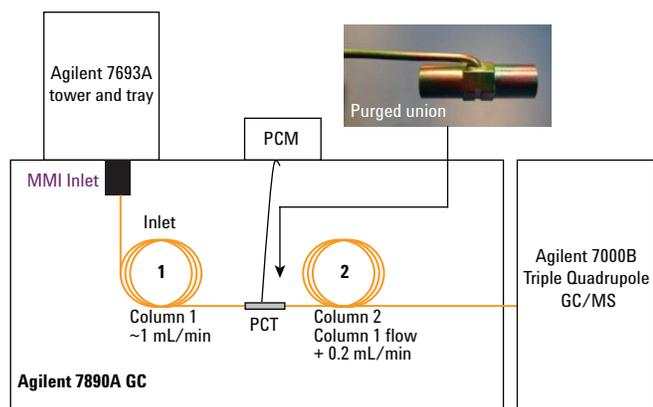


Figure 2. GC/MS/MS configuration with backflush.

## Results and Discussion

### Chromatographic separation results

GC analysis was finished in 28 minutes, with baseline separation for 17 of the 19 compounds. Although two types of PAE (DIDP and DINP) could not be separated ideally, the MRM function of GC/MS/MS allowed the two coeluting compounds to be separated by the transition ions. The results are shown in Figure 3. Table 1 shows the order and retention times.

### Advantage of the instrument configuration

The method is based on the multiresidue GC/MS/MS Analyzer, which is configured with Agilent Capillary Flow Technology (CFT), enabling rugged, reliable GC column backflush, using two 15 m HP-5MS UI columns. The backflush of the GC column shortens runtime, extends column life, reduces

chemical background, provides consistent retention times and spectra, and keeps the MS ion source clean. A Multi-Mode Inlet (MMI) provides the flexibility to inject samples in cold, hot, or solvent-vent modes. This configuration is based on a constant flow mode method with mid-column backflush. Overall, the method provides ultimate performance and shorter cycle-time with reduced carrier gas consumption.

### Calibration curve and linear fit

Calibration curves were prepared at 0.010–0.50 mg/L (6 points: 0.010, 0.020, 0.050, 0.10, 0.20, and 0.50 mg/L), and the results are shown in Table 1. The linearity of the 19 PAEs was good, from 0.010 to 0.50 mg/L, with the  $R^2$  of all compounds higher than 0.995. Figure 4 shows the MRM chromatograms for each of 19 PAEs in the standard mixture.

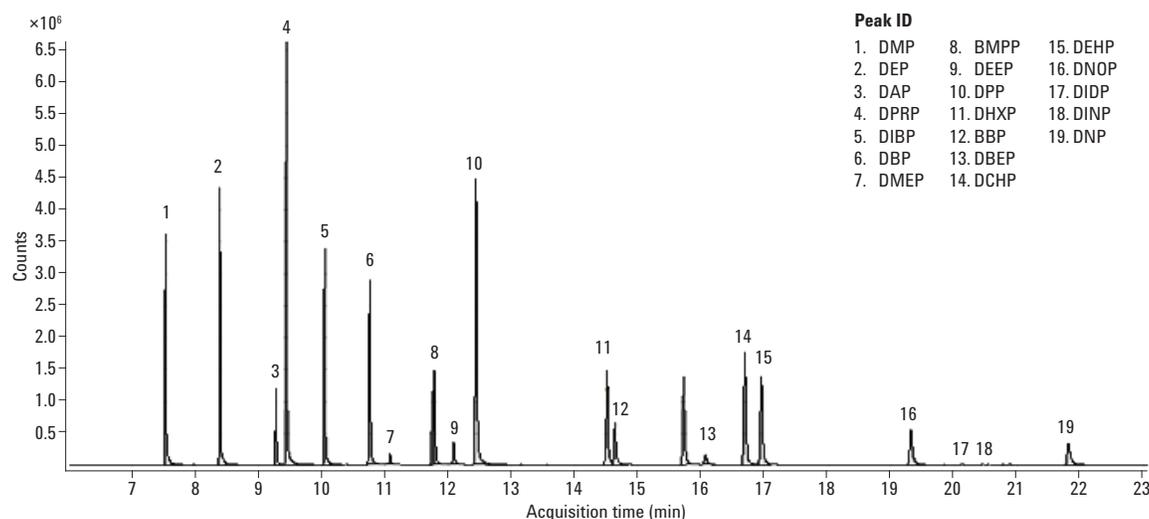


Figure 3. MRM total ion chromatogram for the analysis of standard mixture containing 19 PAEs.

Table 1. Retention Time, MRM Parameters and  $R^2$  of 19 PAE Compounds

No.	CAS	Compound	RT	Quant. MRM	Qual. MRM	Correlation coefficient ( $R^2$ )
				CE/ev	CE/ev	
1	131-11-3	DMP	7.5	163 → 77(20)	163 → 135 (10) 163 → 92 (30)	0.999
2	84-66-2	DEP	8.4	149 → 65(20)	149 → 93 (15) 177 → 149 (5)	0.999
3	131-17-9	DAP	9.3	149 → 65(25)	149 → 121 (10) 149 → 93 (15)	0.997
4	131-16-8	DPRP	9.6	149 → 65(25)	149 → 93 (20) 191 → 149 (5)	0.999
5	84-69-5	DIBP	10.1	149 → 65(25)	149 → 93 (15) 149 → 121 (15)	0.999
6	84-74-2	DBP	10.8	149 → 65(25)	149 → 93 (15) 149 → 121 (15)	0.996
7	117-82-8	DMEP	11.1	104 → 76(15)	104 → 50 (30) 207 → 59 (5)	0.995
8	146-50-9	BMPP	11.8	149 → 65(25)	149 → 93 (20) 167 → 149 (5)	0.995
9	605-54-9	DEEP	12.2	149 → 65(25)	149 → 93 (15) 193 → 149 (15)	0.998
10	131-18-0	DPP	12.5	149 → 65(25)	149 → 93 (15) 149 → 121 (15)	0.995
11	84-75-3	DHXP	14.6	149 → 65(25)	149 → 93 (20) 149 → 121 (15)	0.995
12	85-68-7	BBP	14.7	149 → 65(25)	149 → 93 (15) 91 → 65 (15)	0.995
13	117-83-9	DBEP	16.2	149 → 65(25)	149 → 93 (15) 191 → 149 (15)	0.996
14	84-61-7	DCHP	16.8	149 → 65(25)	149 → 93 (20) 167 → 149 (5)	0.995
15	117-81-7	DEHP	17.0	149 → 65(25)	149 → 93 (15) 167 → 149 (5)	0.997
16	117-84-0	DNOP	19.4	149 → 65(25)	149 → 93 (20) 149 → 121 (15)	0.995
17	84-76-4	DNP	21.9	149 → 65(25)	149 → 93 (20) 149 → 121 (15)	0.996
18	28553-12-0	DINP	19.0–22.0	293 → 149(10)	293 → 71 (10)	0.996
19	26761-40-0	DIDP	20.0–24.0	307 → 149(10)	307 → 71 (10)	0.995

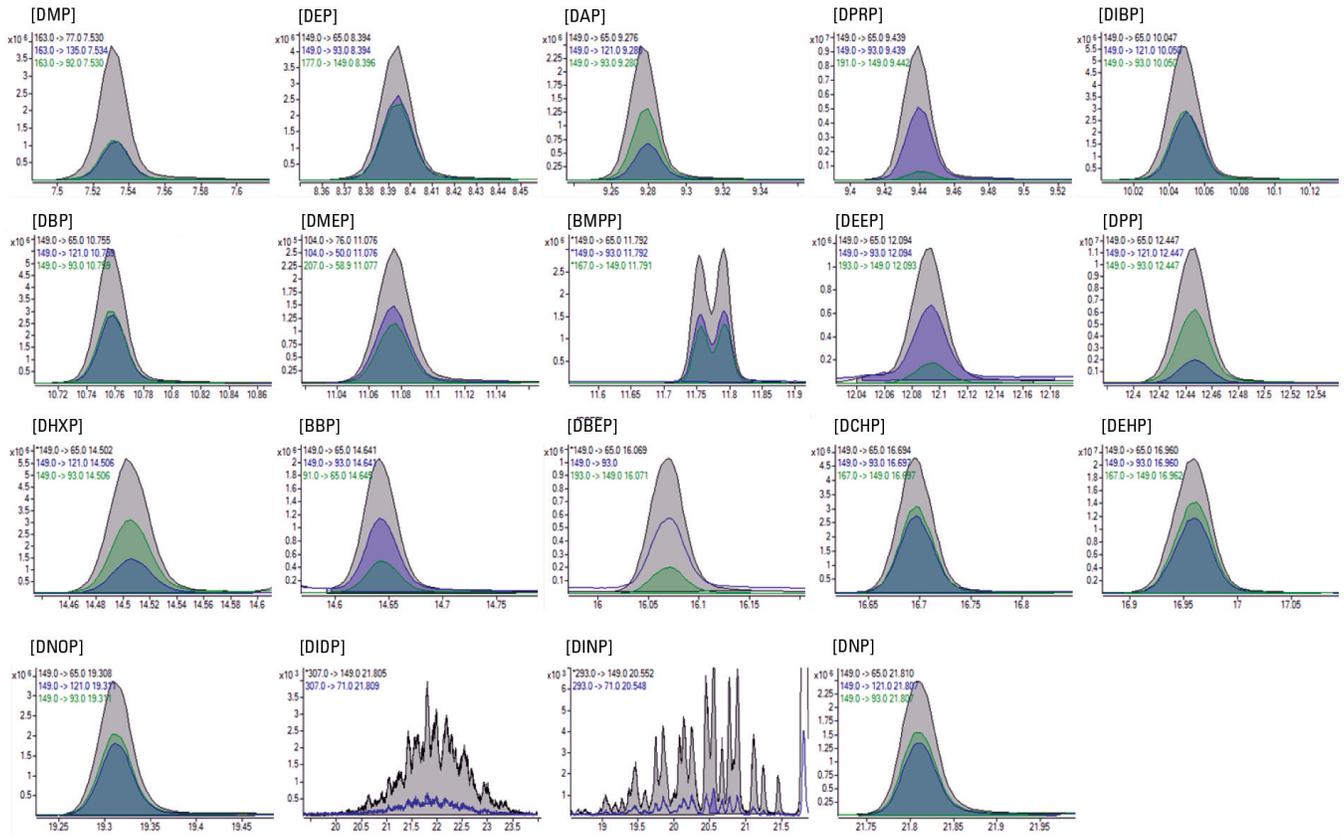


Figure 4. MRM chromatograms for each of the 19 PAEs in the standard mixture.

## Recovery and repeatability

By spiking the blank matrix with levels of 0.01 mg/kg and 0.1 mg/kg for three replicates at each level, the recovery and precision of the method were validated. As shown in Table 2, the majority of recovery values were within 60–120 %, with RSD  $\leq$  15 %.

## Some tips for PAE detection

Phthalate is ubiquitous in the environment, and easily contaminated, thus, the preparation process must use all glass products (hot roasted) instead of any plastic containers. The purity of the solvent should be chromatographic grade or above. Initially, the purity of the solvent should be determined by GC/MS/MS. Use consumables free of plasticizers, for

example, a 30 m DB-5MS UI (122-5532UI) or 30 m HP-5MS UI (19091S-433UI) column, an individually wrapped green high temperature septum (p/n 5183-4759), and an Agilent Ultra Inert deactivated, single taper splitless inert liner (p/n 5190-2292). Using foil in the vial septum and avoiding multiple injections could prevent the dissolution of plasticizer.

## Vegetable sample analysis

Ten tomato samples obtained from local markets were analyzed. DIBP, DBP, and DEHP were detected in all the samples at levels up to hundreds of ppb. The concentration ranges of DIBP, DBP, and DEHP were 0.12–0.27 mg/kg, 0.13–0.22 mg/kg, and 0.10–0.76 mg/kg, respectively.

Table 2. The Recoveries and RSDs of 19 PAEs in Tomatoes and Cucumbers

Compound	Tomato(n = 3)				Cucumber(n = 3)			
	0.01 mg/kg		0.1 mg/kg		0.01 mg/kg		0.1 mg/kg	
	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %	Recovery %	RSD %
BBP	95.6	7.9	76.7	7.5	109.3	1.3	97.3	5.9
BMPP	98.3	8.5	89.7	5.4	104.4	4.5	96.3	6.3
DAP	92.2	4.1	68.2	12.5	103.4	6.4	97.7	3.7
DBEP	89.3	9.0	74.4	7.1	102.2	0.5	99.6	5.9
DBP	118.5	11.6	90.6	6.9	127.8	5.7	108.1	2.2
DCHP	91.0	12.4	81.3	6.7	110.2	0.7	98.4	6.5
DEEP	80.8	11.5	97.3	2.9	99.1	4.6	92.7	5.0
DEHP	105.0	10.3	63.9	3.8	63.3	5.7	91.9	6.3
DEP	62.3	8.3	92.0	13.6	106.6	6.3	91.3	8.2
DHXP	95.6	7.9	84.8	5.9	103.9	2.2	98.3	5.8
DIBP	128.3	10.5	89.5	8.2	126.5	13.3	112.9	6.0
DIDP	101.6	15.0	93.8	11.3	105.7	5.7	105.9	8.2
DINP	110.6	12.4	80.9	8.8	125.6	9.6	117.3	6.5
DMEP	68.4	9.1	101.7	13.0	79.4	6.8	76.2	8.1
DMP	89.2	6.9	66.7	8.4	89.3	7.9	76.2	14.9
DNOP	95.7	12.5	84.7	3.5	111.1	2.9	99.5	7.6
DNP	92.8	13.3	89.5	0.8	102.0	3.1	97.3	7.0
DPP	106.8	12.7	85.4	7.1	121.4	2.5	99.3	5.2
DPRP	94.9	6.8	72.3	12.2	101.2	6.1	96.7	3.5

## Conclusions

Using modified QuEChERS with GC/MS/MS MRM monitoring, a sensitive method for the rapid detection of 19 PAEs in vegetables has been developed and applied to real sample analysis. Three out of 19 PAEs were detected in vegetables randomly collected from local markets, showing the threat of food contamination and human exposure. The method is proven to be simple and reliable for the analysis 19 PAEs in vegetables, and can be extended to the analysis in other foodstuffs. Further survey of PAE levels in foodstuffs should be conducted.

## References

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