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Determination of 24 Phthalate Esters in Food Packaging Materials by Gas Chromatography-Mass Spectrometry*

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ABSTRACT Objective: To develop a gas chromatography-mass spectrometry (GC-MS) method for the determination of 24 phthalate esters in food packaging materials. **Methods:** The samples were extracted with hexane. The identification and quantification were performed by GC-MS in the selected ion monitoring (SIM) mode. **Results:** The calibration curves of phthalate esters showed good linearity in the range of 0.05 mg/L~10 mg/L (0.5 mg/L~10 mg/L for diisodecyl phthalate(DIDP) and diisononyl phthalate(DINP)) with the correlation coefficients (r^2) between 0.9848 and 0.9998. The limits of detection of phthalate esters in food packaging materials were from 0.002 mg/kg to 0.05 mg/kg (S/N=3). The average recoveries of 24 compositions spiked in packaging materials were 85.2%~108% and the relative standard deviations (RSDs, n=6) ranged from 5.9% to 10.2%. **Conclusion:** This method is fast, sensitive and accurate for the qualitative and quantitative determination of 24 phthalate esters in food packaging materials.

Key words: Gas chromatography-mass spectrometry (GC-MS); Phthalate esters (PAEs); Packaging materials

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Introduction

Phthalate esters (PAEs) are commonly used as plasticizers, because they can increase durability and longevity of plastic. Recently, they are widely used in commercial products, such as materials, toys, electronics, medical devices, packaging materials, detergents and so on^[1]. Phthalates have been categorized as “chemicals of concern” by the United States Environmental Protection Agency (EPA)^[2] due to their potential reproductive toxicities in laboratory animal studies^[3-6]. With the increasing of plastic industry scale and plastic products usage, study showed that the phthalate esters had already contaminated the water, atmosphere and soil^[7]. What's more, they were also found in organisms and human's body. Experimental data indicated that phthalate esters may cause hormonal and reproductive system disorders, such as the abnormalities of sperm, fetal sex and endocrine, even the testicular cancer^[8-10].

Recently, there are many determinations of phthalate esters, such as high performance liquid chromatography (HPLC)^[11], gas chromatography (GC)^[12-13], gas chromatography-mass spectrometry (GC-MS)^[15-17] and so on. But only sixteen kinds of plasticizers were tested by national standards for food packaging materials. This study was to develop a new method with mass spectrometric detection (GC-MS) which can determine the 24 kinds of phthalate esters simultaneously in food packaging materials with easy operation

and high accuracy.

1 Materials and methods

1.1 Materials

1.1.1 Reagents and experimental instruments 24 standards of phthalate esters were bought from Dr. Ehrenstorfer (Augsburg, Germany). All standards were pure substances (>98%). N-hexane and acetone were supplied by Merck (Darm. stad, Germany). Gas chromatograph-mass spectrometer was bought from Agilent (7890 A-5975C, America). Electronic balance was purchased from Mettler (XS205, Germany). Ultrasonic apparatus was bought from Kunshan ultrasonic instrument Co., LTD. Rotary evaporator was bought from BUCHI (R215, Switzerland). Colorimetric tubes were bought from Beijing Glass Instrument Factory. All the glass wares must be washed carefully with acetone and hexane, and then were heated at 500℃ for 4h. They must be washed with acetone and hexane before using. A reagent blank must be analyzed before sample analysis in each batch.

1.1.2 Preparation of standard solutions Each of the standard solution was prepared in n-hexane at a concentration of 1000 mg/L. From these solutions, a working mixture was prepared in n-hexane with the valid period of one month. It contained all the standards at a concentration of 100 mg/L each. Standard working solutions of different concentrations ranged from 0.5 mg/L to 10 mg/L with

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n-hexane. All solutions were protected from light and stored at 4℃.

1.2 Methods

1.2.1 Conditions for gas chromatography (GC) and mass spectrometry (MS) The GC conditions for the experiment were as follows: HP-5ms capillary column (cross-linked 5% methyl silicone) of 30 m× 0.25 mm and with a phase thickness of 0.25 μm was selected to separate the analytes. Helium was used as carrier gas (flow rate, 1mL/min). The temperature of injection port was maintained at 250 ℃ in the splitless mode, and the injection volume was 1.0 μL. The initial oven temperature was 70 ℃, after

holding at the initial temperature for 1 min, elevated to 230 ℃ at a speed of 20 ℃ /min, then increased to 290 ℃ at a speed of 5 ℃ /min, and then to 300 ℃ at a rate of 10 ℃ /min for 2 min.

The MS conditions for the experiment were as follows: Electron ionization was used as the ionization mode. The ion source and quadrupole temperature were 230 ℃ and 150 ℃, the electron impact ionization mode (70eV) was operated in scan mode (50-550 amu). NIST mass spectral database was used. The external standard method is employed for quantitative analysis, and the ions used for selected ion monitoring (SIM) are summarized in Table 1.

Table 1 Retention times and characteristic ions of 24 phthalate esters

No	PAEs	Retention time/min	Characteristic ions (m/z)
1	DMP dimethyl phthalate	7.63	163* 135 194
2	DEP diethyl phthalate	8.49	149* 177 121
3	DIPrP diisopropyl phthalate	8.85	149* 150 191 209
4	DAP diallyl phthalate	9.361	149* 189 132 104
5	DPrP dipropyl phthalate	9.52	149* 150 191 209
6	DIBP diisobutyl phthalate	10.11	149* 223 205
7	DBP dibutyl phthalate	10.76	149* 223 205
8	DMEP bis(2-methoxy-ethyl) phthalate	11.04	59* 149 104 207
9	DIPP diisoamyl phthalate	11.58	149* 237 167 69
10	BMPP bis(4-methyl-2-pentyl) phthalate	11.69	149* 167 251
11	DEEP bis(2-ethoxyethyl)phthalate	11.96	72* 149 104 193
12	DPP dipentyl phthalate	12.28	149* 237 219
13	DHXP dihexyl phthalate	14.16	149* 251 233
14	BBP benzyl butyl phthalate	14.30	149* 91 206 104
15	DBEP bis(2-n-butoxyethyl) phthalate	15.60	149* 101 85 193
16	DCHP dicyclohexyl phthalate	16.21	149* 167 249
17	DHP di-n-heptyl phthalate	16.30	149* 265 104 247
18	DEHP bis(2-ethylhexyl)phthalate	16.42	149* 167 279
19	DPhP diphenyl phthalate	16.55	225* 77 153 104
20	DNOP di-n-octyl phthalate	18.65	149* 279 167
21	DINP diisononyl phthalate	19.23	293* 127 167 149
22	DPHP bis(2-propylheptyl)phthalate	20.07	149* 167 307
23	DIDP diisodecyl phthalate	21.56	307* 149 167 141
24	DNP dinonyl phthalate	23.25	293* 149 167 85

Note:* quantitative ion.

1.2.2 Sample preparation Food packaging materials were crushed into small pieces until they became the specified granularities of 0.02 g. 0.5000g sample was accurately weighed and put it into 25mL colorimetric tube. 20mL n-hexane was used as the solvent to extract all the samples for three times. The samples were filtered by filter paper, and all the liquid was concentrated to 1mL through distillation flask, and be stored in a sealed container. Afterwards,

the liquid was injected into GC-MS.

2 Results

2.1 The method detection limit, linear range and the correlation coefficient

The external standard method is employed for quantitative analysis. Typical SIM chromatograms of a mixture of 24 standard

phthalate esters is shown in Fig. 1. What is more, the mass concentration of every compound as the abscissa and response value of peak area as the ordinate establish standard curve. Linear range, correlation coefficient and the regression equation are showed in table 2. The calibration curves of phthalate esters showed good linearity in the range of 0.05 mg/L~10 mg/L(0.5 mg/L~10 mg/L for disononyl phthalate (DINP) and diisodecyl phthalate (DIDP)) with the correlation coefficients (r^2) between 0.9848 and 0.9998. The limits of detection of phthalate esters in food packaging materials ranged from 0.002 mg/kg to 0.05 mg/kg ($S/N=3$).

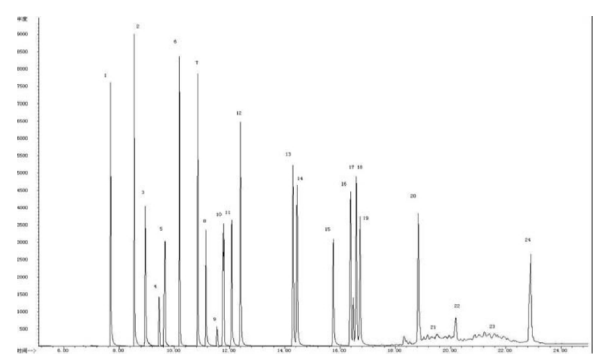


Fig.1 SIM chromatogram of 24 phthalate esters

Table 2 Linear ranges, linear equations, correlation coefficients (r^2), limits of detection (LODs) of 24 PAEs

No.	PAEs	Linear range (mg/L)	Linear equation	r^2	LOD (mg/kg)
1	DMP	0.05~10	$Y=1.24 \times 10^5 X - 1.12 \times 10^4$	0.9998	0.002
2	DEP	0.05~10	$Y=9.09 \times 10^4 X + 1.65 \times 10^4$	0.9993	0.004
3	DIPrP	0.05~10	$Y=1.08 \times 10^5 X - 1.41 \times 10^4$	0.9990	0.02
4	DAP	0.05~10	$Y=1.50 \times 10^4 X - 3.76 \times 10^3$	0.9974	0.03
5	DPRP	0.05~10	$Y=1.20 \times 10^5 X - 1.35 \times 10^4$	0.9996	0.005
6	DIBP	0.05~10	$Y=1.49 \times 10^5 X - 2.45 \times 10^3$	0.9978	0.002
7	DBP	0.05~10	$Y=1.68 \times 10^5 X - 9.09 \times 10^3$	0.9993	0.006
8	DMEP	0.05~10	$Y=3.79 \times 10^4 X - 1.45 \times 10^4$	0.9963	0.015
9	DIPP	0.05~10	$Y=6.78 \times 10^3 X - 6.78 \times 10^3$	0.9991	0.025
10	BMPP	0.05~10	$Y=3.88 \times 10^4 X - 2.46 \times 10^3$	0.9985	0.004
11	DEEP	0.05~10	$Y=1.82 \times 10^4 X - 7.59 \times 10^4$	0.9964	0.029
12	DPP	0.05~10	$Y=1.69 \times 10^5 X - 1.09 \times 10^4$	0.9971	0.003
13	DHXP	0.05~10	$Y=1.26 \times 10^5 X - 4.11 \times 10^4$	0.9975	0.002
14	BBP	0.05~10	$Y=6.04 \times 10^4 X - 1.81 \times 10^4$	0.9959	0.041
15	DBEP	0.05~10	$Y=2.07 \times 10^4 X - 1.06 \times 10^4$	0.9937	0.006
16	DCHP	0.05~10	$Y=9.29 \times 10^4 X - 1.06 \times 10^4$	0.9975	0.002
17	DHP	0.05~10	$Y=3.99 \times 10^4 X - 6.86 \times 10^3$	0.9989	0.023
18	DEHP	0.05~10	$Y=8.38 \times 10^4 X - 1.39 \times 10^4$	0.9984	0.003
19	DPhP	0.05~10	$Y=8.61 \times 10^4 X - 3.56 \times 10^4$	0.9962	0.017
20	DNOP	0.05~10	$Y=1.06 \times 10^5 X - 3.65 \times 10^4$	0.9975	0.012
21	DINP	0.5~10	$Y=1.06 \times 10^4 X - 7.76 \times 10^3$	0.9954	0.029
22	DPHP	0.05~10	$Y=1.95 \times 10^4 X - 3.68 \times 10^3$	0.9985	0.032
23	DIDP	0.5~10	$Y=9.62 \times 10^3 X - 8.77 \times 10^3$	0.9836	0.05
24	DNP	0.05~10	$Y=1.04 \times 10^5 X - 4.36 \times 10^4$	0.9925	0.026

Note: x = the concentrations of target compounds. y = the peak area of target compounds. LOD was defined at $S/N=3$.

2.2 Recovery and precision of the method

Recovery tests were performed by packaging materials without phthalates in order to verify the viability of this method. Overall recoveries and relative standard deviations (RSDs, $n=6$) were satisfactory; The results were 85.2 % to108 % and 5.9 %~ 10.2 %, respectively (Table 3).

2.3 Determination of the samples

60 kinds of packaging materials were bought to determinate PAEs from the market. Results showed that there was one or two PAEs in the plastic packaging materials generally. The most common type of PAEs was DEHP with range of 0.14 mg/kg~ 7.5 mg/kg, followed by DBP with the range of 0.13 mg/kg~ 0.29 mg/kg.

Table 3 Recoveries and RSDs of 24 PAEs in the samples (n=6)

No	Compound	Spiked/(mg/kg)	Found/(mg/kg)	Recovery/%	RSD/%
1	DMP	0.05	0.0436	87.2	6.8
		1.0	0.965	96.5	8.5
		10	9.88	98.8	7.6
2	DEP	0.05	0.0452	90.4	8.5
		1.0	0.974	97.4	9.1
		10	10.06	100.6	9.2
3	DIPrP	0.05	0.0474	94.8	7.1
		1.0	0.986	98.6	8.2
		10	9.32	93.2	7.2
4	DAP	0.05	0.0426	85.2	8.7
		1.0	0.973	97.3	8.4
		10	9.55	95.5	9.6
5	DPRP	0.05	0.0448	89.6	8.2
		1.0	0.984	98.4	6.8
		10	9.75	97.5	6.4
6	DIBP	0.05	0.0472	94.4	9.3
		1.0	0.939	93.9	9.2
		10	9.59	95.9	8.7
7	DBP	0.05	0.0471	94.2	8.7
		1.0	0.957	95.7	8.5
		10	10.05	100.5	10.1
8	DMEP	0.05	0.0449	89.9	8.6
		1.0	0.945	94.5	10.2
		10	9.58	95.8	8.4
9	DIPP	0.05	0.0476	95.2	8.3
		1.0	0.985	98.5	7.9
		10	9.63	96.3	8.7
10	BMPP	0.05	0.0428	85.6	7.1
		1.0	0.877	87.7	8.6
		10	9.22	92.2	9.2
11	DEEP	0.05	0.0473	94.6	6.5
		1.0	0.893	89.3	6.8
		10	10.4	104	8.4
12	DPP	0.05	0.0534	106.8	7.8
		1.0	0.929	92.9	7.6
		10	9.88	98.8	9.4
13	DHXP	0.05	0.0458	91.6	7.8
		1.0	0.987	98.7	7.6
		10	9.51	95.1	9.5
14	BBP	0.05	0.0537	107.4	9.4
		1.0	0.967	96.7	8.6
		10	9.11	91.1	7.8

15	DBEP	0.05	0.0486	97.2	8.4
		1.0	0.982	98.2	9.1
		10	9.68	96.8	6.8
16	DCHP	0.05	0.0536	107.2	8.3
		1.0	0.986	98.6	8.9
		10	10.06	100.6	9.5
17	DHP	0.05	0.0458	91.6	6.9
		1.0	1.025	102.5	7.3
		10	10.13	101.3	8.5
18	DEHP	0.05	0.0467	93.4	6.1
		1.0	0.923	92.3	6.8
		10	9.56	95.6	9.6
19	DPhP	0.05	0.0432	86.4	7.4
		1.0	0.917	91.7	9.1
		10	9.45	94.5	7.8
20	DNOP	0.05	0.0502	100.4	7.6
		1.0	0.968	96.8	8.2
		10	9.59	95.9	9.5
21	DINP	0.5	0.521	104.2	7.3
		1.0	0.987	98.7	8.6
		10	9.76	97.6	7.6
22	DPHP	0.05	0.0459	91.8	8.1
		1.0	0.968	96.8	9.3
		10	9.77	97.7	8.5
23	DIDP	0.5	0.426	85.2	8.6
		1.0	0.895	89.5	7.5
		10	9.77	97.7	9.3
24	DNP	0.05	0.0437	87.4	7.7
		1.0	0.976	97.6	7.5
		10	9.26	92.6	5.9

The detection rate of DIBP was the lowest with range of 1.14 mg/kg~5.09 mg/kg.

3 Discussion

Phthalate esters are fat-soluble substances and they may dissolve in most organic solvents. Phthalates can contaminate food through food-contact packaging materials and during processing, storage, and transport^[18]. The extracting effects of some organic solvents were compared which were commonly used in the experiments, such as methanol, acetonitrile, acetone, n-hexane, ethyl acetate and dichloromethane. The results showed methanol was easy in the extraction, but not easy to concentrate the extract because of its miscibility with water. It is easy to extract pigment which could pollute the detector of GC-MS when acetone, ethyl acetate and dichloromethane were used to extract phthalate esters from some food packing material which contained ink using; There was no significant difference between the extracting effects of n-hexane and acetonitrile, which could both meet the challenge of analysis. Acetonitrile is more poisonous than n-hexane, therefore n-hexane was finally chosen as the extractive agent. As plasticizers, phthalates can be released easily from the products into the environment

since they are not chemically bound to products. Experiments showed that 24 kinds of PAEs with the HP-5ms type capillary column were well separated under the appropriate conditions. Fig 1 showed the chromatogram for the separation of a mixture of these compounds under optimized conditions. DINP and DIDP each has a variety of isomers with a wide range of boiling points and the chromatogram was composed of a series of chromatographic peak appeared in a certain range, presenting a five-finger peak shape. DINP, DIDP and DNOP overlap each other, but respectively extract fragment ions m/z 279, 293 and 307 as DNOP, DINP and DIDP quantitative ion can differentiate three components quantitative well.

Recent researches showed that DEHP exposure levels were significantly decreased when people were restricted to contact with packaging materials^[19]. Because plastic products are commonly be used in recent years, and its contamination to food can not be overlooked. Phthalate acid esters are regarded as endocrine disrupters^[1,20]. Therefore, the quality of food plastic products should be regulated in the future. This research showed that the qualified rate of 24 plasticizer esters content was 98.3% in 60 food packing materials, and the main detection components were DEHP,

DBP and DIBP. The PVC packing materials have higher detection rate and are easy to migrate out into food in high temperature or in the oil, so we suggest that consumers endeavor to avoid eating food wrapped in PVC material of plastic products.

In summary, a sensitive method for the simultaneous determination of 24 phthalate acid esters in food packaging material by gas chromatography-mass spectrometry (GC-MS) was firstly proposed. The method is suitable for the determination of 24 phthalate esters simultaneously in packaging material with easy operation, high accuracy. It is strongly recommended that food regulatory departments strengthen the detection of phthalate esters, because it is significant for us to safeguard human health, reduce the pollution of the environment. According to the relevant EC framework regulation 1935/2004, the regulation of food contact materials was established^[22]. Based on the results, hazardous substances of food contact materials should not be released into food in quantities which could endanger human health.

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气相色谱质谱法测定食品包装材料中 24 种邻苯二甲酸酯 *

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摘要 目的:建立同时检测食品包装材料中 24 种邻苯二甲酸酯类化合物的气相色谱质谱法(GC-MS)分析方法。**方法:**用正己烷提取包装材料,GC-MS 选择离子监测模式(SIM)测定,运用气质联用仪测定 24 种邻苯二甲酸酯类物质。**结果:**24 种邻苯二甲酸酯类物质的线性范围为 0.05 mg/L~10 mg/L,除了邻苯二甲酸二异壬酯(DINP)和邻苯二甲酸二异癸酯(DIDP)为 0.5 mg/L~10 mg/L,相关系数(r^2)除 DINP、DIDP 外均大于 0.99,方法的检出限(信噪比为 3)为 0.002 mg/kg~0.05 mg/kg,在食品包装材料基质中 3 个加标水平的平均回收率为 85.2%~108%,相对标准偏差(RSD,n=6)为 5.9%~10.2%。**结论:**该方法快速、灵敏、准确可靠,适用于食品包装材料中邻苯二甲酸酯类化合物的分析检测。

关键词:气相色谱质谱;邻苯二甲酸酯;塑化剂;包装材料

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