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Preparation and application of 3D solid structure performance liquid chromatography fillers in the detection of phthalates

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Abstract: *Materials of 3D structure for HPLC columns have been prepared. Silica spheres with high purity were first funetionalized with Substance A and a range of other substances, and finally followed by quaternization with iodomethane to form the final product. A standard solution of four certain phthalates was used to verify the chromatographic performance of the resulting packing materia1. Quantification analysis was performed by the external standard method. Linear relationships were found between the peak areas and the mass concentrations of the four certain phthalates in definite ranges, with detection limits (3S/N) of 5 mg·kg-1 and the low limits of determination (10S/N) of 10 mg·kg-1. Recovery rates obtained by standard addition method were in the range of 100.8~106.8% and RSDs (n=6) were less than 4%, which meeting the requirement of routine experiment.*

Keywords: *Electronic and electrical product; High performance liquid chromatography; Phthalates; RoSH*

1. Introduction

Plasticizer can increase the plasticity of polymer resin, enhance the softness of products, is the output and consumption of the largest type of additives, among which phthalate esters are the most widely used, the most common plasticizer, widely used in electrical and electronic products production and manufacturing links. However, phthalates are highly migratory and easily released from products, and enter the human body through the respiratory tract, digestive tract, skin, etc., causing toxicity to multiple systems of the human body. At the same time, phthalates, a kind of environmental hormones, can interfere with endocrine, may lead to premature puberty in children. In addition, with the abandonment and recycling of electrical and electronic products, the internal phthalates will flood into the ecological environment and have an impact on the ecological environment and human health [1][2]. In view of the above situation, it is very necessary to reduce or avoid the use of harmful substances in electrical and electronic products from the source.

In June 2015, EU Directive (EU) 2015/863[1] is an amendment to Annex II of EU Directive 2011/65/EU. Officially listed four phthalates (di-(2-ethylhexyl) phthalate (DEHP), butylbenzyl phthalate (BBP), dibutyl phthalate (DBP) and diisobutyl phthalate (DIBP) in Appendix II of RoHS 2.0, Meanwhile, the content limit of the four newly added PAEs is 0.1%(w/w). At present, the main detection method used at home and abroad is gas chromatography-mass spectrometry (GC-MS), which is expensive to purchase, use and maintain. Based on this, considering the high boiling point of phthalates and the properties suitable for liquid chromatography detection, the research of liquid chromatography with relatively low cost and relatively high accuracy is imminent.

Because DBP and DIBP are isomers, the peaks overlap and have the same mass spectrometry under normal chromatographic conditions, and the detection by liquid chromatography is limited[2]. In this paper, the packing of 3D three-dimensional structure for high performance liquid chromatography was synthesized and the column was packed.

In this study, a HPLC method for the determination of four kinds of phthalates (PAEs) in electronic and electrical products was established by using the above synthetic liquid chromatography column and optimizing the chromatographic conditions.

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2. Experimental part

2.1 Instruments and reagents

Agilent 1260 high performance liquid chromatograph with UV detector (VWD), Milli-Q ultra pure water machine.

Four standard substances (purity ≥99.5%): methanol (chromatographic pure, Merck, Germany), n-hexane (chromatographic pure, Merck, Germany), DBP, DIBP, BBP and DEHP (purity ≥99.5%), high purity silica gel (5μm, YMC, Japan), iodomethane (analytical pure, West Asia reagent), Toluene (analytical pure, national medicine reagent), acetone (analytical pure, national medicinereagent). Triethylamine (analytical pure, Chinese medicine reagent)

2.2 Preparation method

2.2.1 Synthesis of silica gel matrix mixed stationary phase

(1) Weigh and activate 10 g spherical porous silica gel with acid, wash with ultra-pure water until the filtrate becomes neutral, and vacuum dry at 100-150 ℃ for at least 12 h;

(2) Weigh 5 g of activated silica gel and add it into a three-mouth bottle, add 100 mL of dried toluene, and heat reflux under mechanical stirring; 0.5h later, appropriate amount of substance A and 1.0mL triethylamine were added and heated for reflux for 4 h under stirring. Then cool to room temperature, add 1.0 mL of methane iodide and stir at room temperature for 1 h. After the reaction, the chromatographic fillers were washed with toluene, acetone and methanol for several times, and dried by vacuum at 60-90 ℃ for at least 12 h. That is, the stationary phase of silica gel matrix is obtained (the surface of silica gel is a 3D three-dimensional structure functional group).

2.2.2 Column loading

A clean and dry empty column tube (150 mm×4.6 mm stainless steel column) was connected to the outlet of the homogenate tank, and methanol was used as the homogenate replacement liquid. Weigh 3.2g of the fixed phase in a conical flask and add 40 mL methanol as homogenate solvent. After ultrasonic dispersion, add it into the homogenate tank immediately and seal it. Open the intake valve of the high-pressure liquid pump, start the high-pressure pump, homogenate pressure rises to 60 MPa, and the homogenate enters the column tube at a flow rate of 30 mL/min. When the displacement fluid discharged exceeds 200 mL, the intake valve is closed. After the homogenizer is reduced to normal pressure, the column is stationary for 10 min. Then the column is removed and the column joint is installed for chromatographic detection.

2.3 Chromatographic conditions

3D three-dimensional liquid chromatography column (4.6*150 mm, 5 μm); Column temperature 35 ℃; Flow rate 1.0 mL •min-1; Injection volume 10 μL; Detection wavelength: 228 nm; The mobile phase A is water and B is methanol. Gradient elution: when 0~6 min, B is 80%; At 6-8 min, B increased from 80% to 95%, and remained for 4 min. At 12-13 min, B decreased from 95% to 80% and remained for 2 min.

3. Results and discussion

3.1 Optimization of chromatographic detection conditions

3.1.1 Mobile phase selection

IEC 62321-8:17 [2] uses acetonitrile-sodium formate system as the mobile phase. This study found that methanol-water system can be used as the mobile phase and play a good elution effect. At the same time, methanol is used to replace acetonitrile because methanol has less toxicity and lower cost.

3.1.2 Column comparison

Under optimized chromatographic conditions, three chromatographic columns (Zorbax SB C18, Eclipse XDB C18 and 3D three-dimensional structure column) (chromatographic column specifications are 4.6×150 mm, 5 μm) were investigated. The separation effect of the four phthalates (DIBP, DBP,

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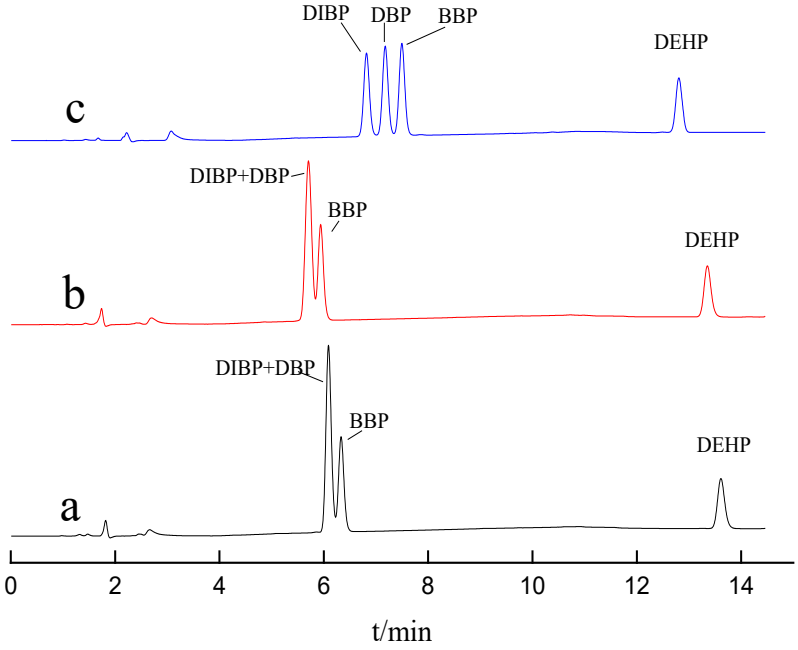
BBP and DEHP) was shown in Figure 1, and the separation degree was shown in Table 1.



a—Zorbax SB C18;b—Eclipse XDB C18;c—Capcell Pak ADME

Figure 1: Chromatograms of 4 PAEs on different 3 chromatographic columns under the same conditions

Table 1: Chromatographic resolution of 4 PAEs on different 3 chromatographic columns under the same conditions

PAEs	Separation efficiency		
	Zorbax SB C18	Eclipse XDB-C18	Capcell Pak ADME
DIBP	-	-	-
DBP	0	0	2
BBP	1.11	1.37	1.83
DEHP	32.25	32.95	24.53

It can be seen from Figure 1 and Table 1 that the four phthalates can be separated well by the 3D three-dimensional structure column, thus ensuring the accurate quantification of the determination of phthalates under the condition of liquid chromatography.

3.2 Standard curve and detection limit

Dilute the mixed standard stock solution of four phthalates with methanol to prepare 0.25, 0.5, 1, 2, 5 and 10 μg • mL-1 mixed standard solution. Test the standard solution of this sequence according to the chromatographic conditions in Section 1.2. Take the mass concentration of the detected target as the abscissa and the corresponding response peak area as the ordinate, carry out linear fitting and draw the standard working curve. See Table 2 for the linear range, linear regression equation and correlation coefficient of the four phthalates.

Prepare the mixed standard solution with low concentration. Inject the sample for analysis, calculate the detection limit of the method with 3 times the signal-to-noise ratio (3S/N), and calculate the lower limit of the method with 10 times the signal-to-noise ratio (10S/N). See Table 2 for the results.

Table 2: Linearity parameters, detection limits and lower limits of determination

PAEs	Linear range(μg•mL-1)	Linear equation	Related coefficient	Detection limit(mg•kg-1)	Determination limit(mg•kg-1)
DIBP	0.05~20	y=19.39015x-0.153911	0.9999	5	10
DBP	0.05~20	y=19.30035x-0.337004	0.9999	5	10
BBP	0.05~20	y=18.88449x-0.271419	0.9999	5	10
DEHP	0.05~20	y=13.97567x-0.121904	1.0000	5	10

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3.3 Precision and recovery test

Weigh 500 mg ± 10 mg of blank sample (accurate to 0.1 mg), respectively add 25, 100 and 500μg•kg-1 the mixed standard solution with three concentration levels, such as g • kg-1. According to the method in Section 1.3, the precision and recovery results are shown in Table 3.

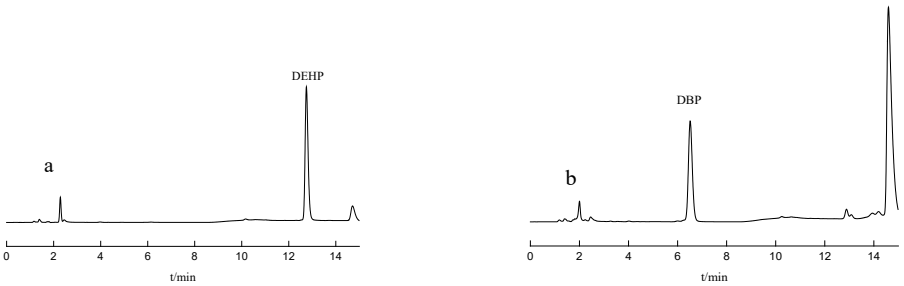
Table 3: Results of tests for precision and recovery (n=6)

PAEs	the adding standard matter amount		the adding standard matter amount		the adding standard matter amount	
	25 μg•kg-1		100 μg•kg-1		500 μg•kg-1	
	Spike-and-recovery experience/%	RSD/%	Spike-and-recovery experience /%	RSD/%	Spike-and-recovery experience /%	RSD/%
DIBP	102.3	3.58	101.9	1.63	101.5	1.35
DBP	106.8	3.46	102.6	1.77	100.8	1.85
BBP	103.8	3.82	102.3	1.40	100.9	1.63
DEHP	106.7	3.03	102.6	1.97	101.6	1.84

It can be seen from the table that the recoveries of the four phthalates in the sample are between 100.8% and 106.8%, and the relative standard deviation (n=6) of the measured value is within 4%, meeting the determination requirements.

3.4 Practical sample analysis

After sampling, the test was carried out according to this method. DEHP and DBP were more detected in typical positive samples. The test results are shown in Figure 2. The final phthalate content of typical samples 1 and 2 is 0.5% and 0.2%, respectively, which are 5 times and 2 times of the limit value (0.1%), and do not meet the standard requirements.



(a) representative sample1; (b) representative sample2

Figure 2: Chromatograms of typical samples

4. Conclusion

In this work, a high performance liquid chromatography (HPLC) filler with 3D three-dimensional structure was synthesized, which can be used for the chromatographic separation and qualitative and quantitative analysis of four PAEs in electronic and electrical products. The results showed that the four phthalates were well separated, and the mass concentration had a linear relationship with the peak area within a certain range. The detection limit (3S/N) was 5 mg•kg-1, and the determination limit (10S/N) was 10 mg•kg-1.The recoveries ranged from 100.8 to 106.8%, and the relative standard deviations of the measured values were within 4%, which met the requirements of conventional experiments.

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