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Article

Simultaneous Determination of Nine Related Substances in *p*-Phthalic Acid Residue by RP-HPLC

Na Yuan, Jun-Qin Qiao and Hong-Zhen Lian*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry & Chemical Engineering and Center of Materials Analysis, Nanjing University, 22 Hankou Road, Nanjing 210093, China

*Author to whom correspondence should be addressed. Email: hzlian@nju.edu.cn

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A high-performance liquid chromatography with ultraviolet detection (HPLC-UV) is established for the simultaneous determination of *p*-phthalic acid, benzoic acid, 4-carboxybenzaldehyde, *m*-phthalic acid, *o*-phthalic acid, phthalide, *o*-toluic acid, *m*-toluic acid and *p*-toluic acid in the residue generated during the production of *p*-phthalic acid (PPA). On a narrow-diameter reversed-phase C18 column, gradient elution is applied with a methanol–water–ammonium acetate-acetic acid buffer (100 mmol/L, pH 4.70) as mobile phase at a flow rate of 0.2 mL/min, and detection is operated by UV absorption at a wavelength of 254 nm. Under the conditions, these nine relative compounds, including organic acids, are well separated. The detection limits (S/N = 3) are 0.05 μg/mL–0.20 μg/mL, and the correlation coefficients of standard curves are between 0.99995 and 0.99999. Relative standard deviations for analyses of real samples are below 5.3%, and recoveries of these determined compounds ranges from 90.0 to 104.9%. The method, which provides accurate and reliable results, can effectively guide the recycling and reutilization of organic acids and related substances in PPA residue.

Introduction

As an important chemical raw material and fundamental organic intermediate, *p*-phthalic acid (PPA) is widely used in chemical industries (1). It is primarily applied in producing polyester fibers, plasticizers, dyes and pesticides (2, 3). The traditional method of PPA production in industry is liquid phase air oxidation technology, which uses *p*-xylene as a starting material and metal halide as catalyst. With the growing global demand of PPA, people are increasingly concerned with the study and application of industrial PPA.

Owing to the existence of toluene and ethylbenzene in *p*-xylene and decarboxylation generated from excessive oxidation, benzoic acid (BA) is inevitably present in the final product of PPA. In addition, 4-carboxybenzaldehyde (4-CBA) can always be detected due to partial oxidation of *p*-xylene. Moreover, the tiny isomeric impurities coexisting in *p*-xylene, such as *o*-xylene and *m*-xylene, can form corresponding oxidation products in the reaction. As a result, BA, 4-CBA, *m*-phthalic acid (MPA), *o*-phthalic acid (OPA), phthalide (PA), *o*-toluic acid (OTA), *m*-toluic acid (MTA) and *p*-toluic acid (PTA) are all the possible related substances in industrial PPA (4). The byproducts originating from *p*-xylene oxidation not only affect the quality of target product PPA, but also lead to blockage of pipes in the manufacturing devices.

To control the quality of PPA and to prevent certain hazardous substances from accumulating in the production system, the impurities, often referred to as oxide residues, have to be excluded from the oxidation unit. A plant for large-scale production of PPA can generate considerable amounts of oxide residue (5). Taking into account the important application of oxidation products of *p*-xylene and their correlated compounds in industry, it is important to reuse organic acids found in the residue.

Several analytical technologies have been used for the identification and quantification of organic acids. Acid-base titration can only assay total acid number, and non-acid components cannot be determined. 4-CBA can be detected by polarography, in which toxic mercury is used. Capillary electrophoresis (CE) can also be applied to determine the organic acids, but the reproducibility is usually poor (6, 7). Gas chromatography (GC), a technique for quantification of volatile compounds, needs chemical derivatization when analyzing aromatic acids, which is tedious and time-consuming. Although high-field asymmetric waveform ion mobility spectrometry (FAIMS) can separate OPA, MPA and PPA (8), the complexity of asymmetric waveform confines the application of FAIMS (9). Ion-exchange chromatography (IEC) can be used in the determination of residue generated during PPA refining, but the peak shape is not good enough, and the re-equilibrium time is relatively long (10). Reversed-phase high-performance liquid chromatography (RP-HPLC) is a suitable technology for the analysis of organic acids, but most of the reported methods in the literature (11, 12) have only determined a few carboxylic acid related compounds. An RP-HPLC method had been established by us to simultaneously separate and determine eight relative compounds, including organic acids in industrial toluic acids (4), which can be used for analysis of PPA product. However, the method is primarily suited for quality control, and a relatively long period is needed when applying isocratic elution, even at the flow rate of 1.5 mL/min. The most significant omission is that the crucial impurity 4-CBA was not included. 4-CBA is crucial for several reasons. First, as an important intermediate in organic synthesis, 4-CBA can be widely applied in the production of medicine, pesticide and liquid crystal. Thus, it is important to reuse 4-CBA in PPA residue. Second, 4-CBA is inevitably present in the final product of PPA. As a chain terminating agent in condensation polymerization, 4-CBA directly affects the quality of polyethylene terephthalate (PET); e.g., intrinsic viscosity and molecular weight (7). Therefore, for both positive and negative reasons, it is important to detect the content of 4-CBA in PPA products and residue. Based on this

work, we adopted gradient elution and a small diameter column to simultaneously separate and determine nine related substances in PPA residue, which can provide a satisfactory result for a sample with complex matrix.

Experimental

Reagents and materials

Reference substance (RS) of MPA (99.5%) was provided by Nanjing Huahong Chemical Industry Co. (Nanjing, China); RSs of PPA, OPA, BA and PA (all 99.0%) were purchased from Shanghai First Reagent Factory (Shanghai, China); RS of OTA (99.0%) was supplied by Shanghai Institute of Materia Medica (Shanghai, China); MTA and PTA (both 99.5%) were obtained from Taixing Seventh Chemical Factory (Taizhou, China); RS of 4-CBA (99.0%) was purchased from Shanghai Bangcheng Chemical Co. (Shanghai, China); PPA residue was provided by GPRO Jiangsu Zhongshan Chemical Co. (Nanjing, China). Methanol was HPLC-grade (Merck, Darmstadt, Germany). Acetic acid ($\geq$ 99.5%, analytical reagent) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China); perchloric acid (70–72%, guaranteed reagent) was from Tianjin Third Reagent Factory (Tianjin, China); ammonium acetate ($\geq$ 98.0%, analytical reagent) was from Guangdong Guanghua Chemical Factory Co. (Shantou, China); sodium hydroxide ($\geq$ 96.0%, analytical reagent) was from Sinopharm Chemical Reagent Co. (Shanghai, China). Wahaha purified water (Wahaha Group, Hangzhou, China) was used throughout the experiment. The mobile phase was filtered through a 0.45-μm membrane filter before use.

Apparatus and chromatographic conditions

Instrumentation for analysis was a Waters Alliance 2695 Separations Module equipped with a vacuum degasser, a quaternary pump, an auto-sampler and a 996 UV–Vis photodiode-array detector (PDA) (Waters, Milford, MA). The separation was controlled and the chromatograms were recorded with a Waters Empower chromatography manager system. HPLC separation was carried out on a Diamonsil C18 column (250 × 2.1 mm i.d., 3 μm) (Dikma Technologies, Tianjin, China). The column temperature was constantly maintained at 30°C and the separation was carried out by gradient elution with a flow rate of 0.2 mL/min. The mobile phase was methanol–water–buffer. The buffer was NH_4Ac-HAc (100 mmol/L, pH = 4.70). The gradient used for elution was methanol–water–buffer (5/40/55, *v*/*v*/*v*) for the initial 22 minutes, then a linear gradient from methanol–water–buffer (5/40/55, *v*/*v*/*v*) to methanol–water–buffer (35/10/55, *v*/*v*/*v*) for 3 min, and a hold at methanol–water–buffer (35/10/55, *v*/*v*/*v*) for 35 min. Then, the mobile phase was back to methanol–water–buffer (5/40/55, *v*/*v*/*v*) in 3 min, and the column was re-equilibrated for at least 10 min before the next injection. The injection volume was 2 μL each. The effluent was monitored by the PDA detector set at 254 nm.

Preparation of standard and sample solutions

Individual PPA, MPA, OPA, 4-CBA, PA, BA, OTA, MTA and PTA standard stock solutions (1.0 mg/mL) used for calibration purposes were prepared by separately weighing 10.00 mg of RS into nine 10-mL volumetric flasks, then adding 4 mL diluent methanol–water–buffer (10/30/60, *v*/*v*/*v*) and 400 μL 48% sodium hydroxide solution. After 5 min sonication, pH was adjusted to 4.70 by adding approximately 250 μL perchloric acid. The diluent was added to make up to the mark after another 10 min sonication. To get the mixed standard solution, 100 μL each of PPA and 4-CBA, and 1.00 mL each of the remaining standard stock solutions were transferred into a 10-mL volumetric flask, mixed and diluent was added to the volume. Other mixed standard solutions were prepared by serial dilution of the previously described mixed standard solution with diluent. Considering the relatively strong UV absorption of PPA and 4-CBA, the concentrations of these two components were 1/10 of the other organic acids in all mixed standard solutions.

The sample solutions for analysis were prepared in quintuplicate by accurately weighing approximately 10.00 mg PPA residue sample into 10-mL volumetric flasks and dissolving as described previously, then transferring 1.00 mL of each of the five solutions into five 10-mL volumetric flasks, mixing and adding diluent to the volume.

Results and Discussion

Optimization of gradient elution program

Separation of nine relative compounds, including organic acids, was carefully tested under different elution programs. The initial mobile phase containing 5% methanol was the most suitable for clearly separating the peaks before 22 min; i.e., PPA,

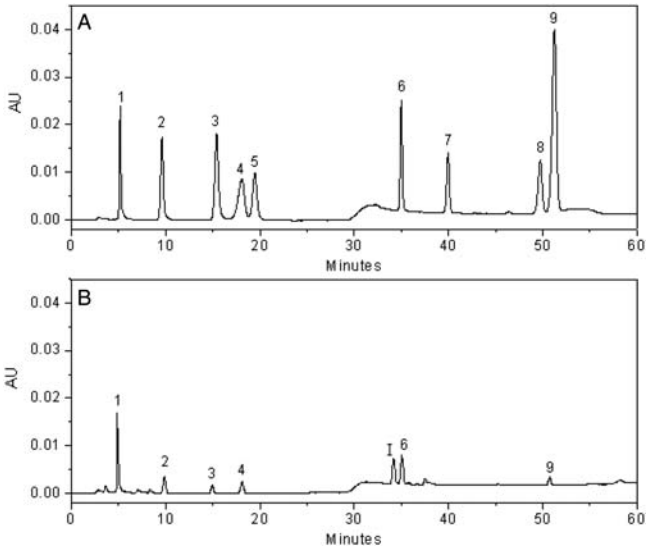

Figure 1. Chromatograms of (A) mixed standard solution; (B) PPA residue sample; column: Diamonsil C18 column (250 × 2.1 mm i.d., 3 μm), column temperature: 30°C, mobile phase: gradient elution of mixture of methanol, water and NH_4Ac-HAc buffer (100 mmol/L, pH = 4.70): methanol–water–buffer (5/40/55, *v*/*v*/*v*) for the initial 22 minutes, then a linear gradient from methanol–water–buffer (5/40/55, *v*/*v*/*v*) to methanol–water–buffer (35/10/55, *v*/*v*/*v*) for 3 minutes, and a hold at methanol–water–buffer (35/10/55, *v*/*v*/*v*) for 35 min, flow rate: 0.2 mL/min, injection volume: 2 μL, wavelength used for UV detection: 254 nm, peaks: 1, PPA; 2, MPA; 3, OPA; 4, 4-CBA; 5, PA; 6, BA; 7, OTA; 8, MTA; 9, PTA; I, unidentified impurity.

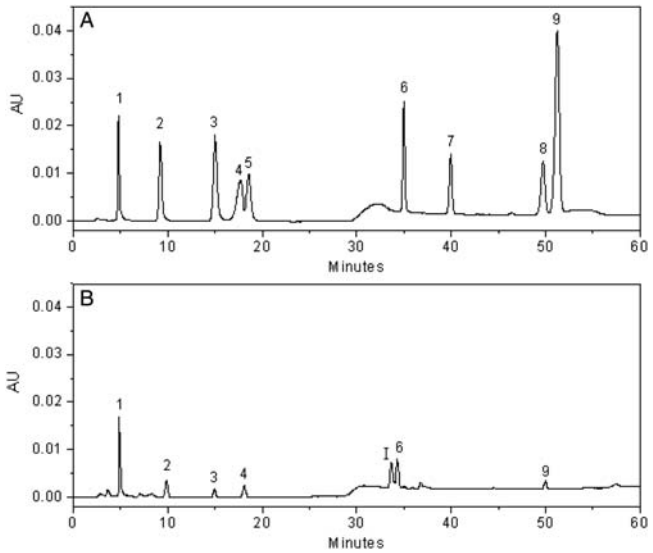

Figure 2. Chromatograms of mixed standard solution when applying higher initial proportions of: (A) methanol; (B) PPA residue sample when applying steeper gradient; mobile phase: gradient elution of mixture of methanol, water and NH_4Ac-HAc buffer (100 mmol/L, pH = 4.70); (A) methanol–water–buffer (8/40/52, *v/v/v*) for the initial 22 min, then a linear gradient from methanol–water–buffer (8/40/52, *v/v/v*) to methanol–water–buffer (35/13/52, *v/v/v*) for 3 min, and a hold at methanol–water–buffer (35/13/52, *v/v/v*) for 35 min; (B) methanol–water–buffer (5/40/55, *v/v/v*) for the initial 22 min, then a linear gradient from methanol–water–buffer (5/40/55, *v/v/v*) to methanol–water–buffer (40/5/55, *v/v/v*) for 3 min, and a hold at methanol–water–buffer (40/5/55, *v/v/v*) for 35 min; peak numbers and other chromatographic conditions were the same as in Figure 1

Table I
Standard Curves and Detection Limits

Number	Component	Linear range (μg/mL)	Linear equation	*r*	LOD (μg/mL)
1	PPA	0.20 ~ 10.0	$A = 45874.186 + 2.388 \times 10^7 C$	0.99995	0.05
2	MPA	0.60 ~ 100	$A = 3433.990 + 3.274 \times 10^6 C$	0.99998	0.16
3	OPA	0.30 ~ 100	$A = 5401.653 + 5.107 \times 10^6 C$	0.99998	0.10
4	4-CBA	0.30 ~ 10.0	$A = 34876.637 + 3.145 \times 10^7 C$	0.99996	0.08
5	PA	0.60 ~ 100	$A = 4473.690 + 3.106 \times 10^6 C$	0.99999	0.20
6	BA	0.40 ~ 100	$A = 3010.112 + 3.054 \times 10^6 C$	0.99997	0.10
7	OTA	0.40 ~ 100	$A = 3380.415 + 2.681 \times 10^6 C$	0.99998	0.10
8	MTA	0.60 ~ 100	$A = 3278.494 + 3.010 \times 10^6 C$	0.99999	0.20
9	PTA	0.40 ~ 100	$A = 10832.447 + 9.979 \times 10^6 C$	0.99998	0.10

MPA, OPA, 4-CBA and PA (Figure 1A), and methanol increment at 10% per min after 22 min was the most suitable for rapid elution of BA, OTA, MTA and PTA. If the initial proportion of methanol was lower than 5%, analysis time was even longer than isocratic elution (4). In this case, to shorten analysis time, a steeper gradient has to be applied, despite the protuberant baseline near 32 min.

Two methods can be used to condense the time interval from 20 to 35 min. One is to increase the initial proportion of methanol, and the other is to adopt a steeper gradient. As shown in Figure 2A, an initial concentration of methanol higher than 5% led to poorer resolution of 4-CBA and PA. As shown in Figure 2B, when applying a sharper gradient than 10% per min, the baseline became worse, and BA was partially overlapped by an unidentified impurity (peak I) in the real PPA residue sample.

Calibration curve and limit of detection

Linearity of calibration curve was obtained from the regression of peak area versus concentration of standard. Each mixed standard solution was injected three times consecutively (Figure 1A), and the limit of detection (LOD) was defined as the concentration at which the signal to noise ratio equaled 3 (Table I). The linear range of the calibration curves for all analytes were over two (PPA and 4-CBA) or three orders (the others) of magnitude with excellent correlation coefficients of 0.99995 ~ 0.99999. The LODs of all analytes were 0.05 ~ 0.20 μg/mL, which are suitable for the direct analysis of these nine compounds in residue samples. The LODs of PPA, MPA, OPA and PA were close to the previous results (4), and those of the rest were significantly improved because gradient elution was used and their peaks with longer retention became sharper and higher. Taking into account that the injection volume was only 2 μL instead of 10 μL in the previous work, the absolute detectable amounts of all analytes were significantly lower. Similarly, compared to Yang *et al.*'s work (12), our LODs were slightly higher. However, considering that the injection volume was 20 μL in their experiment, the absolute detectable amounts of the present method were actually lower.

Recovery test

Recoveries were obtained by spiking a suitable volume of mixed standard solution to sample solution of PPA residue and

Table II
Result of Recovery Test

Component	Background	1			2			3		
	(μg/mL)	Added (μg/mL)	Found (μg/mL)	Recovery (%)	Added (μg/mL)	Found (μg/mL)	Recovery (%)	Added (μg/mL)	Found (μg/mL)	Recovery (%)
PPA	1.56	0.10	1.65	92.6	0.49	2.07	104.9	0.97	2.57	103.9
MPA	4.24	1.02	5.26	100.3	5.11	9.24	97.9	10.22	14.26	98.1
OPA	1.43	0.96	2.38	98.5	4.82	6.25	100.0	9.64	11.09	100.2
4-CBA	0.52	0.10	0.61	90.0	0.50	1.04	104.6	1.00	1.49	97.0
PA	nd	1.00	1.04	103.4	5.01	5.19	103.6	10.01	10.03	100.2
BA	3.69	0.98	4.66	98.8	4.92	8.79	103.7	9.84	13.96	104.4
OTA	nd	0.97	0.97	99.9	4.83	4.86	100.6	9.66	9.98	103.3
MTA	nd	1.01	0.98	96.7	5.06	4.91	98.9	10.12	9.88	97.6
PTA	0.34	0.96	1.32	102.3	4.80	5.32	103.8	9.60	10.40	104.8

Table III
Determination of the Organic Acids and Related Substances in PPA Residue (%)

Number	Component	Intra-day ($n = 5$)		Inter-day ($n = 4$)	
		Content	RSD	Content	RSD
1	PPA	3.14	3.2	3.12	4.0
2	MPA	8.45	3.4	8.47	4.5
3	OPA	2.89	3.9	2.86	4.3
4	4-CBA	1.02	3.0	1.04	3.4
5	PA	nd	–	nd	–
6	BA	7.44	5.1	7.38	5.3
7	OTA	nd	–	nd	–
8	MTA	nd	–	nd	–
9	PTA	0.66	4.2	0.68	4.8

analyzing under the HPLC conditions described previously. Three replicates were performed for the test, with results shown in Table II. Recoveries of nine related compounds in PPA residue ranged from 90.0 to 104.9%. In Yang *et al.*'s work (11), PA, OPA, BA, OTA, PTA and MTA were determined, with recoveries ranging from 96.5 ~ 103.2%. Recoveries of these six compounds in our experiments ranged from 96.7 ~ 104.8% under three spike levels, which looked nearly the same. In addition, recovery tests of PPA, MPA and 4-CBA also gained reasonable results. The peak shape of aldehyde is generally regarded to be a little asymmetrical; this is why recovery of 4-CBA (90.0 ~ 104.6%) was not as good as those of other compounds, especially at lower spike levels.

Determination of organic acids and related substances in PPA residue sample

The first sample solution was injected five times (Figure 1B). The peak area relative standard deviations (RSDs) were 1.4, 0.9, 0.1, 1.9, 0.3 and 3.1% for PPA, MPA, OPA, 4-CBA, BA and PTA, respectively, although PA, OTA and MTA were not detected. The analytical results of nine relative compounds, including organic acids, in PPA residue sample, with intra-day and inter-day precisions, are summarized in Table III.

Conclusions

The HPLC-UV procedure established for the simultaneous determination of nine relative compounds including organic acids in PPA residue is accurate, precise and reliable. Compared with our previous work (4), the crucial component 4-CBA is considered. Moreover, because of the application of a narrow-diameter column with small particle size, the proposed method greatly reduced solvent consumption and achieved better separation with higher response intensity, especially for BA, OTA, MTA and PTA with longer retention, which is of high practical value.

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References

1. Han, J.Y., Dong, Y., Fang, J., Wei, W.Y., Mao, W.; Progress in synthesis of terephthalic acid, *Chemical Industry and Engineering*, (2007); 24(81): 81–85.
2. Whinfield, J.R., Dickson, J.T.; British Patent 578079, (1941).
3. Whinfield, J.R., Dickson, J.T.; U.S. Patent, 2465319, (1949).
4. Lian, H.Z., Wei, Y.N.; Chromatographic fingerprints of industrial toluic acids established for their quality control; *Talanta*, (2007); 71(1): 264–269.
5. Ping, D.L.; Analysis of oxidation residue of p-xylene using high performance liquid chromatography; *Petrochemical Technology*, (1998); 27(4): 281–283.
6. Ma, Z.F., Huang, L.P., Xue, Y.X.; Determination of 4-carboxybenaldehyde and toluic acid in purified terephthalic acid by capillary electrophopresis; *Synthetic and Applied Technology*, (1998); 14(2): 54–55.
7. Li, M.G., Wu, S.Y., Ding, Y.S.; Determination of contents of benzoic acid, 4-carboxybenzaldehyde and *p*-toluic acid in purified terephthalic acid; *Petrochemical Technology*, (2008); 37(5): 518–521.
8. Barnett, D.A., Purves, R.W., Ells, B.; Separation of *o*-, *m*- and *p*-phthalic acids by high-field asymmetric waveform ion mobility spectrometry (FAIMS) using mixed carrier gases; *Journal of Mass Spectrometry*, (2000); 35(8): 976–980.
9. Guevremont, R.; High-field asymmetric waveform ion mobility spectrometry (FAIMS); *Canadian Journal of Analytical Sciences and Spectroscopy*, (2004); 49(3): 105–113.
10. Li, B., Han, F.; Simultaneous determination of eight compounds in residue from purification of terephthalic acid by high performance liquid chromatography; *Specialty Petrochemicals*, (2005); 22(5): 67–69.
11. Zhang, A.P., Hou, J., Wang, H., Wang, H., Wang, H.W.; Determination of main organic impurities in purified terephthalic acid by high performance liquid chromatography; *Journal of Analytical Sciences*, (2008); 24(5): 603–605.
12. Yang, H.J., Ding, M.Y.; Determination of *o*-toluic acid and its micro amounts of impurities in industrial products by HPLC; *Journal of Liquid Chromatography and Related Technologies*, (2002); 25(17): 2709–2715.