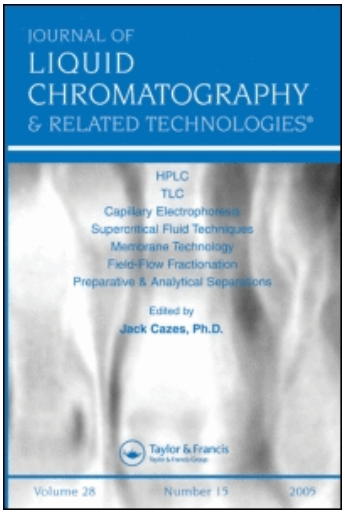

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HPLC-UV Detection for Analysis of p-Benzoquinone Dioxime and p-Nitrosophenol, and Chromatographic Fingerprint Applied in Quality Control of Industrial p-Benzoquinone Dioxime

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HPLC-UV Detection for Analysis of p-Benzoquinone Dioxime and p-Nitrosophenol, and Chromatographic Fingerprint Applied in Quality Control of Industrial p-Benzoquinone Dioxime

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Abstract: A reliable reversed-phase high performance liquid chromatographic (HPLC) method has been developed for simultaneous determination of p-benzoquinone dioxime (BQD) and its related impurity p-nitrosophenol (NSP). Separation was achieved on a Kromasil C_{18} column by using methanol-water-NH_4Ac-NH_3 solution (pH = 7.0, 50 mM) (30/50/20, v/v/v) as the mobile phase, and detection was operated by UV absorption at a wavelength of 305 nm. The method was seen to have good linearity, accuracy, and precision for the concentration range and to be an attractive choice for the quality control of BQD for industrial use. Moreover, the HPLC-UV-vis fingerprint of BQD has been established, and successfully applied to quality control of industrial BQD in laboratories of some rubber factories in China. Chromatographic fingerprints of intermediates would become an effective strategy for accelerating the progress of fine chemical industry.

Keywords: p-Benzoquinone dioxime, p-Nitrosophenol, HPLC, Quality control, Chromatographic fingerprint

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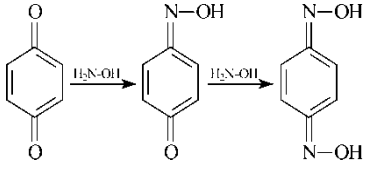
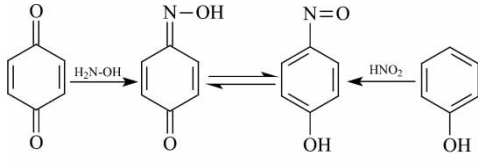
INTRODUCTION

p-Benzoquinone dioxime (BQD), a kind of crosslinker, can easily disperse in natural rubber, vulcanize quickly, and be able to endure heat and ozone. It can also be used as polymerization inhibitor of crylic acid, adhesive of metallic glass when it was hot-melted, regulator of olefin copolymers when they were crosslinked, and stabilizer of organic monomers. BQD can activate oxidant such as Pb_3O_4 and PbO_2. BQD was propitious to butyl rubber, natural rubber, butyl-benzene rubber, thiokol rubber, ethylene-propylene rubber, and some other rubbers inhibiting nuclear radiation.[1–3]

BQD was produced when p-benzoquinone reacted with hydroxylamine in factory. But, in the course of this reaction, a byproduct, p-benzoquinone monoxime (BQM), was easily found.



It was proven that BQM is the same compound as p-nitrosophenol (NSP), which is produced by nitrosification of phenol. The two structures are tautomeric compounds and can transform from each other.



Direct and accurate assays for the two compounds is critical for the quality control of BQD. However, it has not been reported how to determine BQD content and its impurity NSP. Japanese Industrial Standard (JIS)[4] prescribed the method for quality control of BQD, which controlled indirectly the quality just by the decomposition point; there was no determination of main components or impurities. In this paper, we established a high performance liquid chromatographic (HPLC) method to simultaneously determine BQD and NSP in BQD for industrial use.

In the past two decades, China has been become a main exporter of fine chemical intermediates in the world. Because quality control in the fine chemical industry is of primary importance, some countermeasures and suggestions have been put forward by authors, based on the current situation of analysis and testing of fine chemicals and intermediates in China. The most important one is the concept of fingerprinting. We had suggested that chromatographic

fingerprinting of fine chemical intermediates should be established by using the experience of fingerprinting of Traditional Chinese Medicine and the methodology of impuritomics, and regarded it as a key technique for quality control. The significance of the chromatographic fingerprint for research, development, manufacture, and trade of fine chemical intermediates was presented. It was indicated that the fingerprinting would become the powerful support for the progress of fine chemicals in China. The feasibility of establishment of chromatographic fingerprints of intermediates was discussed, as well as introducing the general procedures and the essential techniques for the strategy.[5,6] Without doubt, HPLC is one of the dominating techniques for this purpose. The HPLC fingerprint of synthetic arbutin (p-hydroxyphenyl-β-D-glucopyranoside) has been developed by HPLC combined with UV-vis and mass spectrometric detection, and successfully applied to quality control in some chemical plants manufacturing arbutin.[7] The fingerprint of BQD established in this paper is another application example of fine chemical intermediate fingerprints.

EXPERIMENTAL

Apparatus

Instrumentation for quantitative analysis included a Varian 5060 liquid chromatograph (Varian, Walnut Creek, USA), a Rheodyne 7725i injector value equipped with a 10 μL loop (Rheodyne, Cotati, USA), a Waters 486 tunable UV-vis absorbance detector (Waters, Milford, USA) operating at 305 nm. Data acquisition and processing was performed on a JS-3050 chromatographic working station (Dalian Johnsson Separation Science and Technology Corporation, Dalian, PRC) and a Yokogawa Hokushin Electric Type 3066 pen recorder (Sino-Japanese Sichuan Fourth Meter Factory, Chongqing, PRC). A Waters Alliance 2695 Separations Module equipped with a vacuum degasser, a quaternary pump, and an auto-sampler, and a 996 UV-vis photodiode-array detector (PDA) (Waters, Milford, MA, USA) was used to get a series of UV-vis spectra needed. The separation was controlled and the chromatograms and spectra were recorded by a Waters Millinium^{32} chromatography manager system.

Reagents and Chemicals

Reference substances (RS) of BQD (95%) and NSP (99%) were provided by Taixing Shengming Fine Chemical Company (Taizhou, PRC). Methanol was HPLC grade (Jiangsu Hanbang Science & Technology Co. Ltd., Huai'an, PRC). Water used for all solutions; dilution and mobile phase was Wahaha purified water (Wahaha Co. Ltd., Hangzhou, PRC).

Chromatographic Conditions

The analytical column was a Kromasil C_{18}, 15 μm, 50 mm × 4.6 mm I.D. (Jiangsu Hanbang). The column temperature was constantly maintained at 30°C. The separation was carried out by isocratic elution of methanol-water-NH_4Ac-NH_3 solution (pH = 7.0, 50 mM) (30/50/20, v/v/v) mobile phase, with a flow rate of 1.0 mL/min. The injection volume was 10 μL. The optimum UV wavelength was 305 nm.

RESULTS AND DISCUSSION

HPLC Analytical Method

Linear Range and Detection Limit

Under the conditions described above, BQD and NSP can be separated completely (shown in Fig. 1). The BQD standard solution used for calibration purposes was prepared by weighting accurately about 25.00 mg of BQD RS into a 25 mL volumetric flask and adding methanol up to the mark. The final concentration of BQD standard stock was 1.0 mg/mL. Standard solutions at concentration of 0.00001 ~ 0.2 mg/mL of BQD were prepared by serial dilution of the stock solution with methanol. In the same way, NSP standard solution used for calibration purposes was prepared by weighting accurately about 10.00 mg of NSP RS into

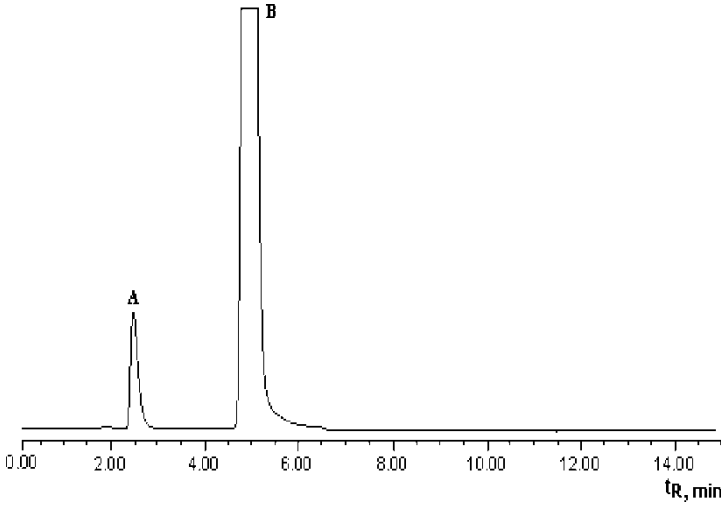


Figure 1. Chromatogram of NSP and BQD standards. Peaks: A. 0.006 mg/mL NSP; B. 0.06 mg/mL BQD.

a 10 mL volumetric flask and adding methanol up to the mark. The final concentration of NSP standard stock was 1.0 mg/mL. Standard solutions at concentration of $0.00001 \sim 0.04$ mg/mL of NSP were prepared by serial dilution of the stock solution with methanol.

Linear regression analyses of the relationship between peak areas versus amounts of standards were carried out within the range $0.00002 \sim 0.2$ mg/mL for BQD and $0.00004 \sim 0.04$ mg/mL for NSP, respectively. The equations were A_{BQD} (area) $= -60055.92 + 9.65E7 \times C_{BQD}$ and A_{NSP} (area) $= 1743.87 + 1.51E7 \times C_{NSP}$, respectively, with correlation coefficients of 0.9993 and 0.9998. The limits of detection ($S/N = 3$) were 0.00001 mg/mL for both BQD and NSP.

Sample Analysis

Analysis of Main Content in Real Samples

The sample solution was prepared by weighting accurately about 6.00 mg of BQD sample into a 10 mL volumetric flask and adding methanol up to the mark. The obtained solution (1.00 mL) was transferred into a 10 mL volumetric flask, then completed to volume and mixed. Five of the same BQD samples (1#) were analyzed, and each solution was injected two times under the optimum HPLC conditions (shown in Fig. 2). In the five analyses, the average content of BQD was 91.34%, and the relative standard deviation (RSD) was 1.0%. Four other different batch BQD samples

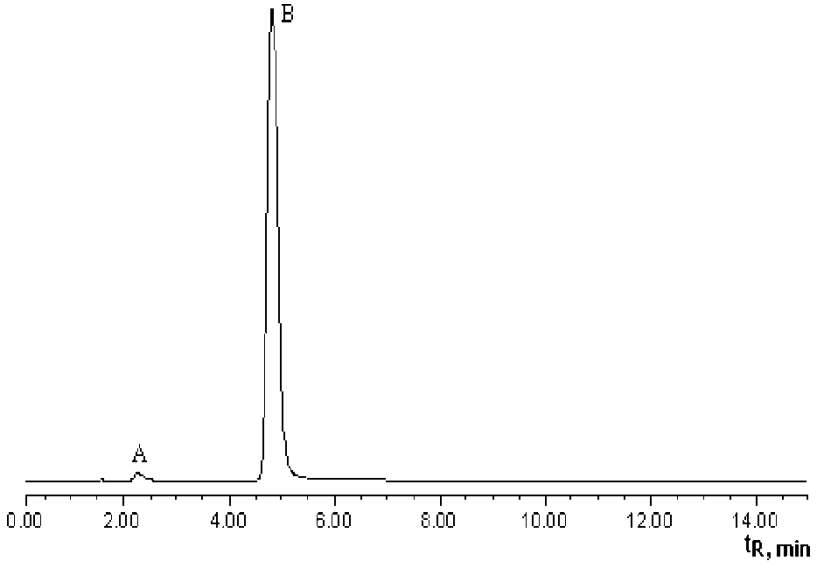


Figure 2. Chromatogram for main content in real samples. Peaks: A. NSP; B. BQD.

Table 1. Results of determinations

	BQD		NSP	
Batch no.	Content (%)	RSD (%, n = 3)	Content (%)	RSD (%, n = 3)
1	91.34	1.0[a]	6.24	1.1[a]
2	94.81	0.4	5.20	0.3
3	91.85	0.3	5.27	0.3
4	76.05	0.6	4.04	0.7
5	76.48	0.3	0.54	0.6

[a]n = 5.

(2#∼5#) were weighted and analyzed three times following the aforementioned method. The results were summarized in Table 1.

Analysis of Impurity Content in Real Samples

The sample solution was prepared by weighting accurately about 6.00 mg of BQD sample into a 10 mL volumetric flask and adding methanol up to the mark. Five BQD samples were analyzed and each solution was analyzed two times (shown in Fig. 3). In the five analyses the average content of NSP was 6.24%, and RSD was 1.1%. Four other different batch BQD samples (2# ∼ 5#) were weighted and analyzed three times following the aforementioned method. The results were summarized in Table 1.

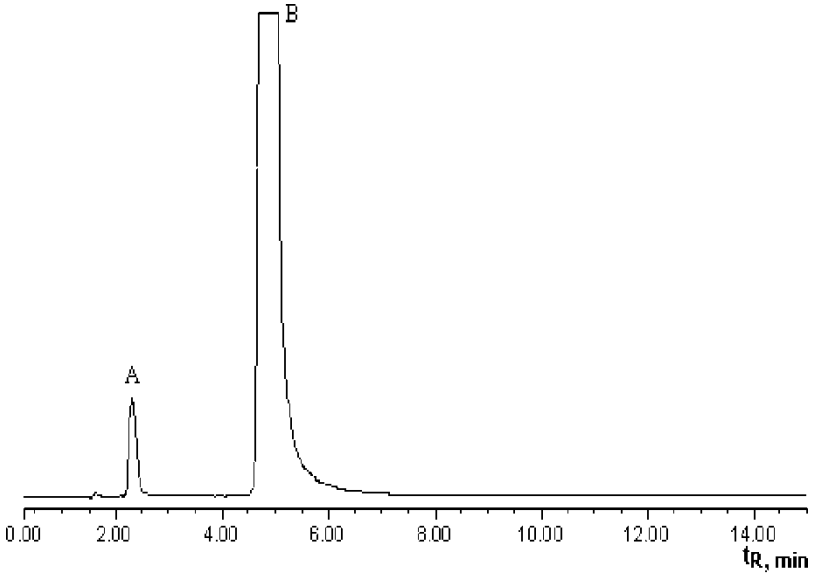


Figure 3. Chromatogram for impurities in real samples. Peaks: A: NSP; B: BQD.

Choice of Experimental Parameters

Effect of Mobile Phase pH

A key factor for the separation of BQD and NSP was the pH of the mobile phase. The composition of the mobile phase was fixed at methanol-water-NH_4Ac solution (50 mM) (30/50/20 v/v/v). The NH_4Ac solution pH value was adjusted by acetic acid or ammonium hydroxide. The result of the experiment (pH from 5.0∼7.5) was shown in Fig. 4. As can be seen, BQD and NSP could be separated completely when pH was over 7.0. On the other hand, it was convenient that NSP transformed into its tautomeric compound. The quinoid structure had stronger UV-vis absorption in alkalescent medium than its phenolic structure in acidic medium. The reason that pH 7.0 was selected as mobile phase pH was to protect chromatographic column packing materials.

From the Sadtler Standard IR spectrum, we found that NSP had some characteristics of tautomerism. The peaks at 1446 cm^{-1}, 1558 cm^{-1}, and 1610 cm^{-1} were assigned to phenyl stretching vibrations. The heights of the three peaks were very similar because of the participation of the two double bonds in the quinoid structure. The peak at 1626 cm^{-1} was assigned to its stretching vibrations that carbonyl and double bond conjugated. There was a peak at 1732 cm^{-1}, which was a typical stretching vibration of N═O. The wide absorption band at 2200∼3200 cm^{-1} was the superposed result of

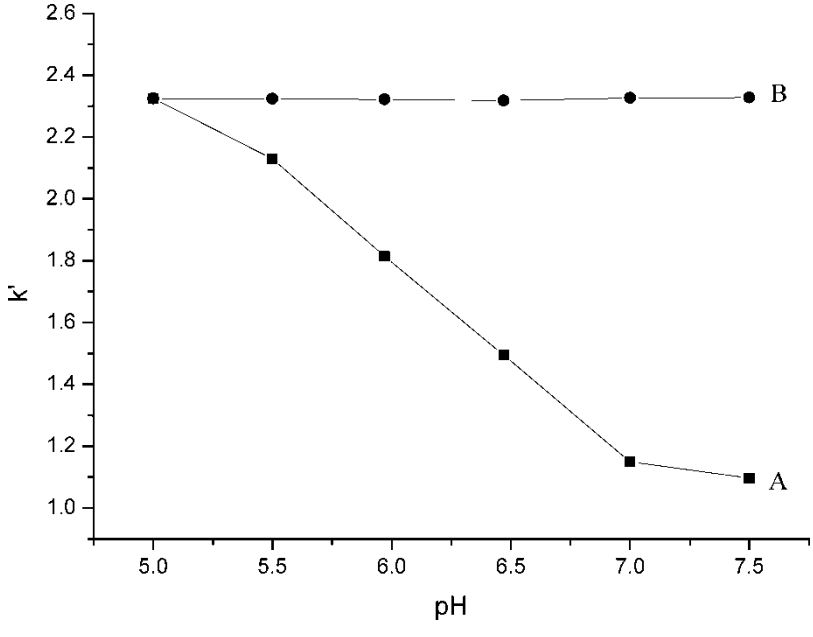


Figure 4. Effect of mobile phase pH. A: NSP; B: BQD.

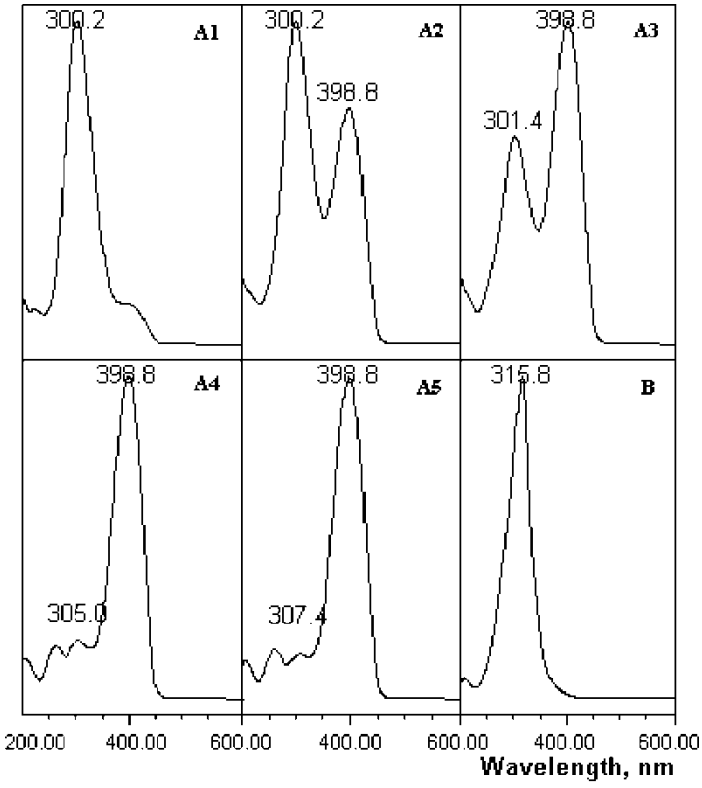


Figure 5. UV-vis absorption spectra for (A) NSP and (B) BQD. A1: pH 5.0; A2: pH 5.5; A3: pH 6.0; A4: pH 7.0; A5: pH 7.5; B: pH 7.0.

hydroxy in the phenolic structure and hydroxy in the quinoid structure. In this experiment, we found that NSP retention time would decrease when mobile phase pH increased, and UV-vis absorption spectrum for NSP changed accordingly (shown in Fig. 5, A1∼A5). It can be explained, that NSP transformed from phenolic to quinoid structure due to the reduction of shorter wavelength absorption (300 nm) and the augmentation of longer wavelength absorption (399 nm). In this case, the retention time of BQD did not change with pH (Fig. 4), and its UV-vis absorption spectrum did not change either (Fig. 5B). This was the first report about the tautomerism of NSP by HPLC separation and UV-vis detection.

Effect of Ionic Strength of Mobile Phase

When methanol proportion in mobile phase was fixed at 30% we also observed the effect of NH_4Ac concentration in mobile phase on separation by adding different proportion of NH_4Ac-NH_3 solution (pH = 7.0, 50 mM) in mobile phase. The result of the experiment was shown in Fig. 6. From

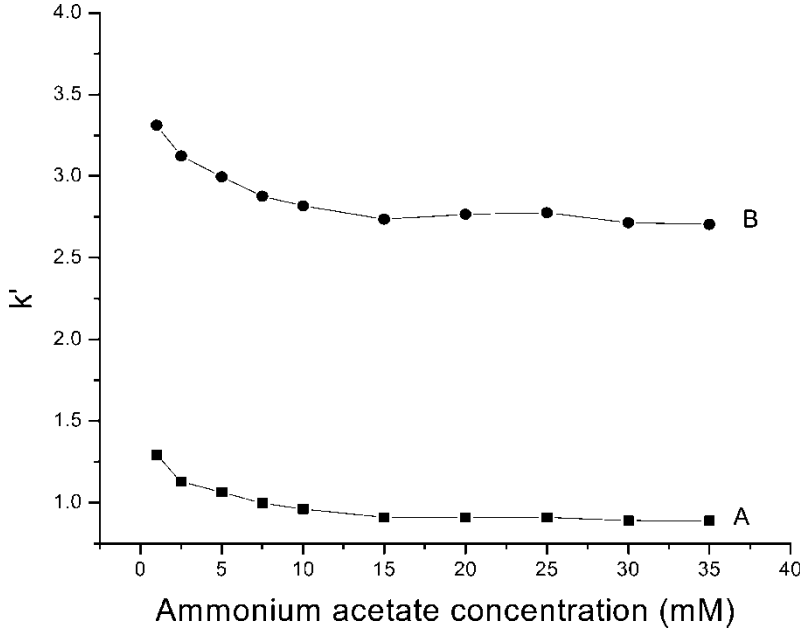


Figure 6. Effect of ion strength of mobile phase. A: NSP; B: BQD.

the figure, we can see that increasing NH_4Ac concentration would reduce the retention time of BQD and NSP, and then calm down simultaneously when the concentration was over 5.0 mM. Accordingly, we chose NH_4Ac concentration as 10 mM, namely, the proportion of NH_4Ac-NH_3 solution in the mobile phase being 20% (v/v).

Effect of Methanol Proportion in Mobile Phase

In this experiment, we fixed the proportion of NH_4Ac-NH_3 solution and altered the proportion of methanol (shown in Fig. 7). By comparing with separation phenomenon under various methanol proportions in mobile phase, it indicated that BQD and NSP can be separated perfectly on baseline when methanol-water-NH_4Ac-NH_3 solution (pH = 7.0, 50 mM) (30/50/20, v/v/v).

Detection Wavelength

The optimum UV wavelength of BQD was 315 nm from the PAD chromatogram (Fig. 5-B), but the NSP had strong absorption at 305 nm and 400 nm (shown in Fig. 5A4 and 5B). The wavelength of 305 nm was selected as the detection wavelength because the content of NSP was rather low.

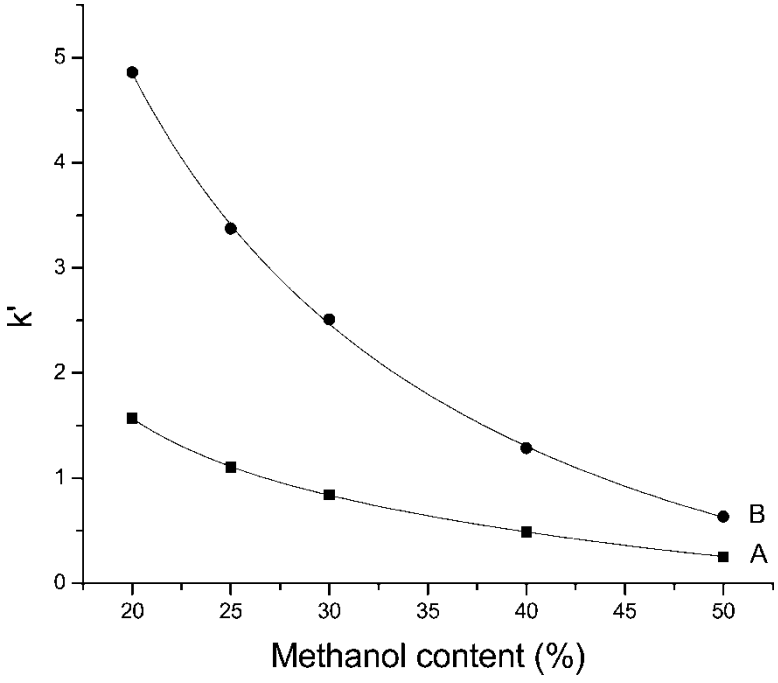


Figure 7. Effect of methanol proportion. A: NSP; B: BQD.

Samples Concentration

Considering determination of both substances in the sample, the low sample concentration was selected to determine the major component, whereas, high sample concentration was selected to determine impurities (0.6 mg/mL). If sample concentration was too dense for the main peak to fall into linear range while determining BQD content, the quantitation was incorrect. Experimental precision looked satisfying after 10 times of dilution (0.06 mg/mL).

Chromatographic Fingerprints of BQD for Industrial Use

The chromatographic fingerprint of BQD is shown in Fig. 8. The six industrial samples of BQD were different batches from the same manufacturer. As can be seen, the chromatograms of BQD samples were similar, all of which have two common peaks. The qualitative analysis of BQD and NSP can be achieved by matching UV-vis spectra to standards from PDA. From this sense, the chromatographic fingerprint of the established BQD was an HPLC-UV-vis fingerprint. The sole impurity in BQD was NSP. In the international trade, it was considered as an unqualified sample when NSP content in industrial BQD was over 5%, while the main content of BQD has not been stated. If a chromatogram of a BQD

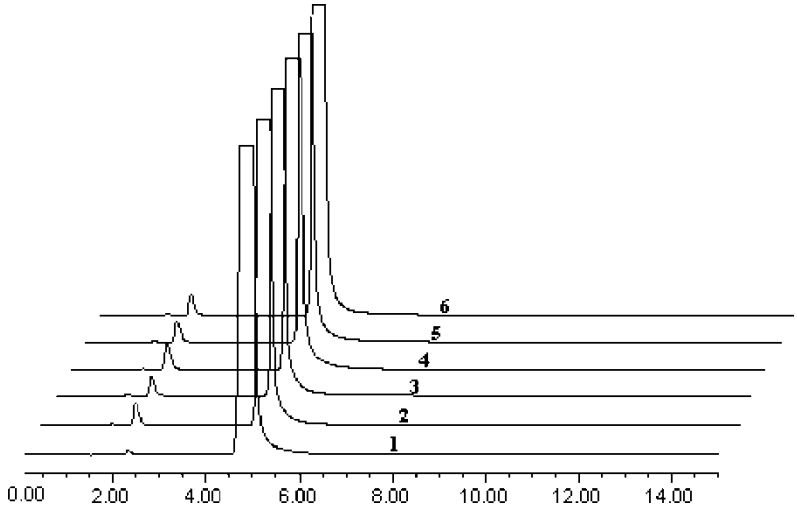


Figure 8. Chromatographic fingerprint of industrial BQD.

sample was similar with the chromatographic fingerprint, we can fleetly estimate that it was primarily eligible. After determination of NSP by the proposed HPLC procedure, it is found that the NSP contents were all less than 5% in Fig. 8. Otherwise, there was no use to carry through any other further analysis and testing.[5]

ACKNOWLEDGMENT

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