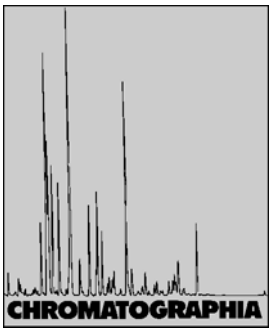
Quantitative High-Performance Liquid Chromatographic Analysis of Nitroxoline and Structurally Related Compounds



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Key Words

Column liquid chromatography
Nitroxoline
8-Hydroxyquine
8-Hydroxy-5-nitrosoquinoline and 8-hydroxy-5,7-dinitroquinoline
Plasma and urine

Summary

Nitroxoline (8-hydroxy-5-nitroquinoline) and the structural related compounds 8-hydroxyquine, 8-hydroxy-5,7-dinitroquinoline, and 8-hydroxy-5-nitrosoquinoline have been investigated by reversed-phase high-performance liquid chromatography on C_{18}/ODS. Complete separation and symmetric peaks were obtained by use of THF-methanol-water, 3:3:4, containing 10 mmol L^{-1} disodium ethylenediamine tetraacetic acid (EDTA) and 10 mmol L^{-1} citric acid in the water, as mobile phase. The pH and the concentration of EDTA in the mobile phase were found to be critical for eliminating tailing and for full separation. The calibration plot was linear for concentrations between 3.0 and 300 μg mL^{-1}; the regression coefficient was 0.99996. Assay of the nitroxoline standard showed that recovery was from 99.3 to 102%, with a mean standard deviation for nitroxoline of 0.9%. The method is suitable for quality control of nitroxoline.

Introduction

Nitroxoline (8-hydroxy-5-nitroquinoline) has been widely used for treatment of urinary tract infections caused by Gram-negative and Gram-positive microorganisms. 8-Hydroxy-5,7-dinitroquinoline, 8-hydroxyquinoline, and 8-hydroxy-5-nitrosoquinoline have been found to be produced as impurities during the synthesis of nitroxoline. A variety of chromatographic methods has been used for quality control of nitroxoline. The study by thin-layer chromatography has been reported [1], but interfering traces of metal ions must be removed from the silica gel by a complicated procedure. Sorel and coworkers used high-performance liquid chromatographic (HPLC) methods [2] for analysis of nitroxoline in plasma and urine, but it was shown that nitroxoline and its above mentioned structurally related impurities co-eluted.

The aim of the work discussed in this report was to develop a simple but accurate HPLC procedure for the simultaneous analysis of nitroxoline and structurally related compounds. HPLC separation conditions were optimized and all the target compounds were fully separated. The method is highly suitable for the quality control of nitroxoline.

Experimental

Reagents and Materials

Nitroxoline (I) was supplied by Jiangsu import and export company, China. 8-Hydroxy-5,7-dinitroquinoline (III) and 8-hydroxy-5-nitrosoquinoline (IV) were purchased from Danyang No. 6 chemical factory, China. 8-Hydroxyquine (II) (pa), perchloric acid (pa), acetic anhydride (pa), citric acid (pa), sodium hydroxide (pa), and potassium biphthalate (ga) were obtained from Shanghai Chemical Reagent Corporation, China. Tetrahydrofuran (THF) and methanol were HPLC-grade, from Jiangsu Huaiyin Fine Chemical Research Institute, China. Perchloric acid solution in acetic anhydride (0.1 mol L^{-1}) was standardized with potassium biphthalate.

Equipment

HPLC analysis was performed with a Series 200 IC pump coupled to a 235C diode-array detector (Perkin-Elmer, Norwalk, USA) and equipped with a Kromasil C_{18} column (200 mm × 4.0 mm, 5-μm particles; Jiangsu Huaiyin Fine Chemical Research Institute). UV absorbance was

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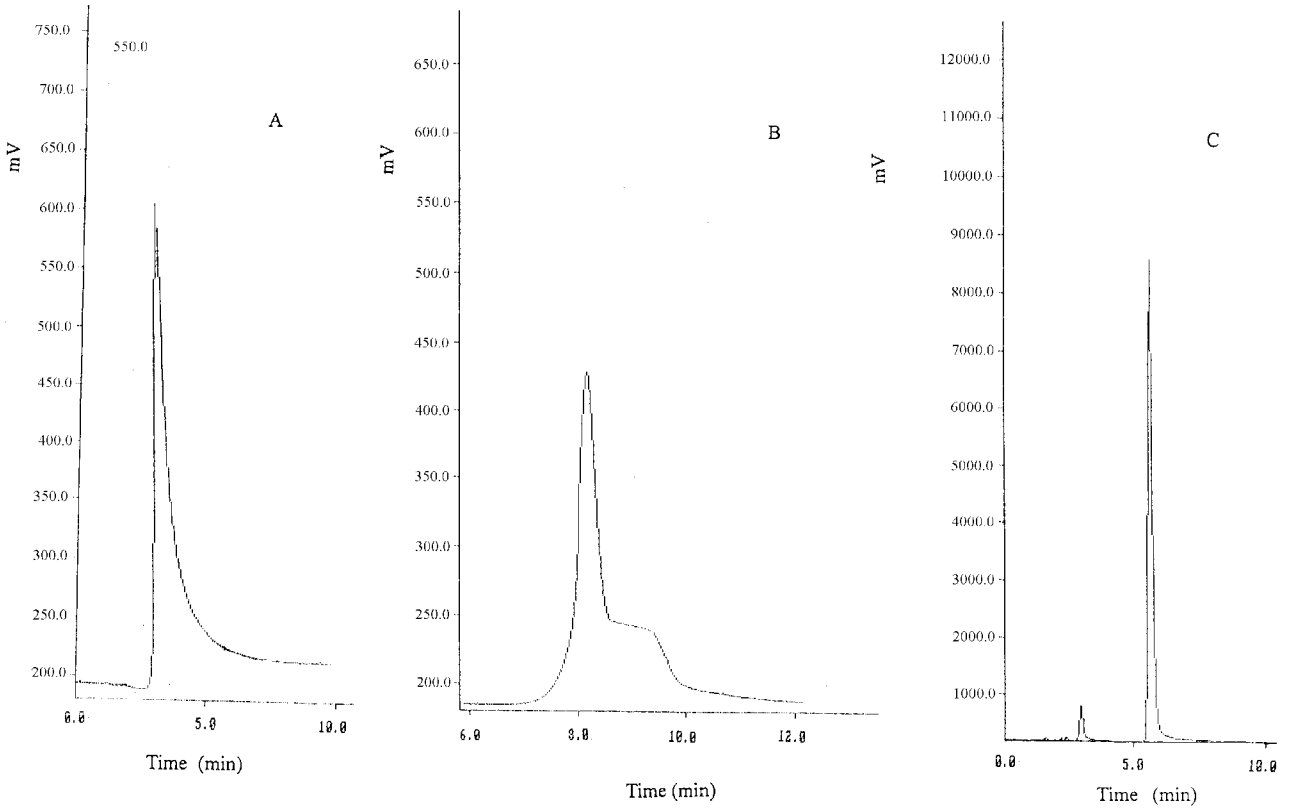

Figure 1. UV-absorbance chromatograms obtained from chromatography of nitroxoline with different mobile phases: **A**. Methanol-water, 1:1, containing 50 mmol L^{-1} $NiSO_4$ and 10 mmol L^{-1} ammonium acetate in the water; **B**. THF-methanol-water, 3:3:4, containing 10 mmol L^{-1} citric acid in the water; **C**. THF-methanol-water, 3:3:4, containing 10 mmol L^{-1} disodium ethylenediamine tetraacetic acid (EDTA) and 10 mmol L^{-1} citric acid in the water. The mobile phase pH was 6.0.

monitored at 245 nm. THF-methanol-water, 3:3:4, containing 10 mmol L^{-1} disodium ethylenediamine tetraacetic acid (EDTA) and 10 mmol L^{-1} citric acid in the water, was used as mobile phase. Mobile phase pH, monitored by means of an Orion (USA) model SA 720 pH meter, was adjusted to 6.0 by addition of sodium hydroxide (2 mol L^{-1}). All separations were performed at room temperature at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL.

Preparation of Standards and Samples

Separate stock solutions of nitroxoline and related compounds were prepared by dissolving each compound in THF at 1 mg mL^{-1}. Calibration standards were prepared by diluting stock solution with mobile phase. Linear calibration plots for the concentration range from 3.0 to 300 μg mL^{-1} were constructed by regression of nominal concentration against peak area.

Six batches of nitroxoline were collected from DanYang 6th Chemical Factory at different times, and samples from each batch (10 mg) were dissolved in mobile phase to furnish 0.05 mg mL^{-1} solutions.

Quantification of nitroxoline was achieved by measurement of peak area and comparison with the calibration plot. Quantification of the structurally related compounds was achieved by the method of normalization.

Results from analysis by potentiometric titration were compared with those from HPLC-UV. Each sample (0.1320 g) was weighed, transferred to a 40-mL beaker, and dissolved in 20 mL acetic anhydride. This solution of nitroxoline was titrated against a solution of perchloric acid, also in acetic anhydride [3].

Results and Discussion

Optimization of the HPLC Separation

It has been reported that severely tailing peaks are obtained for nitroxoline with frequently used chromatographic systems – reversed-phase, ion-exchange, and dynamic ion-exchange [2]. It has been suggested that strong interaction of nitroxoline with the free silanol groups of the column-packing material and the presence of trace metal ions were responsible for the tailing but addition of neither 8-hydroxyquinoline [2] nor Ni ion [3] to the mobile phase improved the separation of nitroxoline from structurally related compounds. For compounds with a chelating molecular structure it has been found that addition of chelating agents to the mobile phase could eliminate the effect of metal ions on chromatographic behavior, e.g. tailing peaks [5–7].

In our study three different mobile phases were investigated. The optimum separation was achieved by use of THF-methanol-water, 3:3:4, containing 10 mmol L^{-1} disodium ethylenediamine tetraacetic acid (EDTA) and 10 mmol L^{-1} citric acid in the water, at pH 6.0. We found that mobile phase pH and EDTA concentration were dominant critical factors affecting the separation of nitroxoline and peak asymmetry – in general, the higher the concentrations of EDTA and citric acid the greater the reduction of the tailing of the principal peaks. Although

better separation was always achieved at lower pH, it was necessary to maintain the pH at values above the point at which EDTA precipitated. Figure 1 illustrates the chromatography of nitroxoline with three different mobile phases; a chromatogram obtained from the separation of nitroxoline, 8-hydroxyquine, 8-hydroxy-5,7-dinitroquinoline, and 8-hydroxy-5-nitrosoquinoline is shown in Figure. 2.

Analysis of Nitroxoline

A calibration plot was constructed after chromatography of a series of nitroxoline standards. The linear regression equation for the calibration plot was $A = -2.306 \times 10^2 + 1.081 \times 10^4 C$ (n = 8, correlation coefficient 0.9999), where A is the peak area and C the concentration of nitroxoline (μg mL^{-1}). Quantitative analysis of nitroxoline was achieved by measurement of peak area and comparison with the calibration plot. The reproducibility of the method was examined for three concentrations of nitroxoline – 15.1, 45.3, and 75.5 μg mL^{-1} (n = 5). The coefficients of variation were 1.1, 0.8, and 0.2%, respectively.

Assay of nitroxoline in the pure standard at three different concentrations, 15.1, 45.3, and 75.5 μg mL^{-1}, each repeated five times, showed that recovery was 99.3 – 102%, with a mean standard deviation of 0.9%. Quantification of the structurally related compounds was achieved by the method of normalization. The nitroxoline content and the relative percentage composition of the structurally related compounds II, III, and IV, determined by HPLC analysis, are shown in Table I.

Potentiometric titration was not suitable for precise analysis of the mixture, because when the purity of a sample is high a distinct point of inflection is observed in the shape of the titration curve whereas when the purity is low the titration curve obtained contains two barely distinguishable points of inflection (Figure 3). Despite this, the first potentiometric break was adopted in our work for comparison with the result from HPLC-UV. The results listed in Table I indicate that there was little difference between results obtained by use of the two methods.

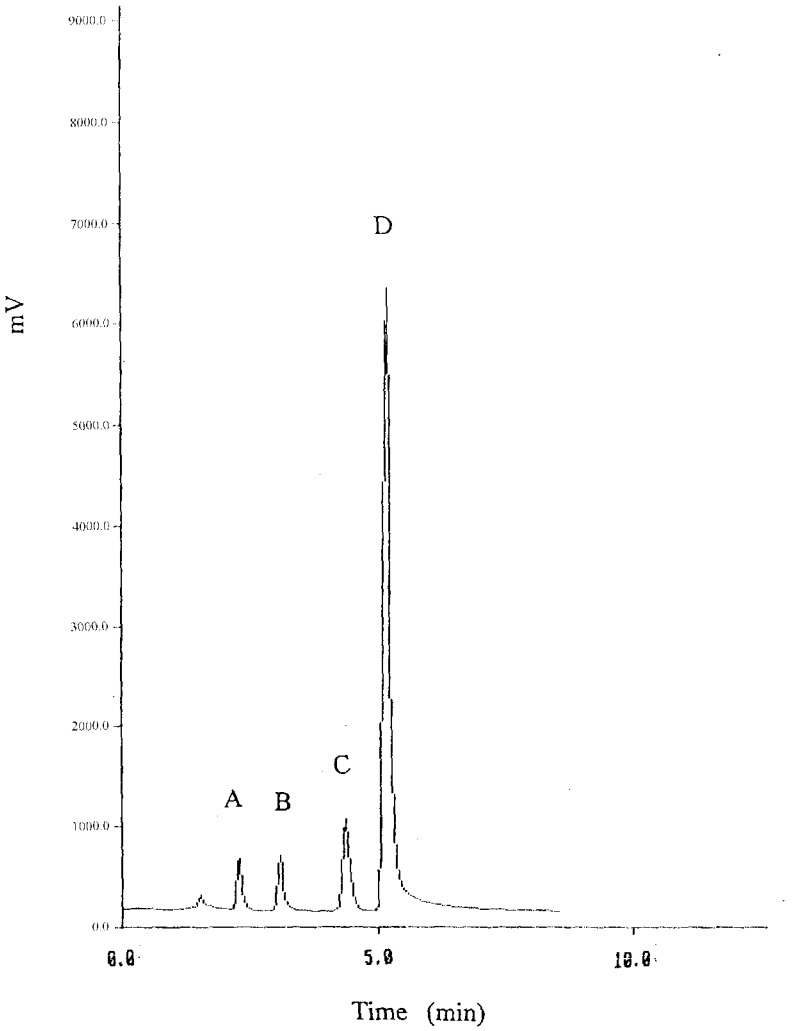

Figure 2. UV-absorbance chromatogram obtained from separation of nitroxoline and structurally related compounds: **A** = 8-hydroxy-5-nitrosoquinoline; **B** = 8-hydroxy-5,7-dinitroquinoline; **C** = 8-hydroxyquine; **D** = nitroxoline. Solid phase: Kromasil C18; mobile phase: THF-methanol-water, 3:3:4, containing 10 mmol L^{-1} disodium ethylenediamine tetraacedic acid (EDTA) and 10 mmol L^{-1} citric acid in the water (pH = 6.0).

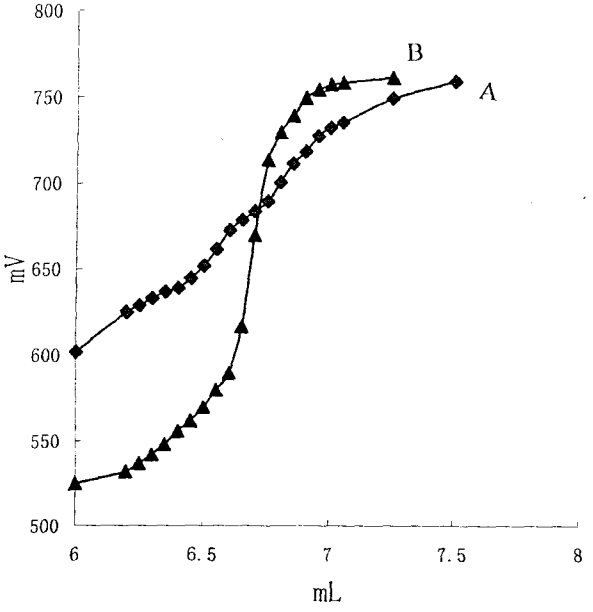

Figure 3. Results from potentiometric titration of nitroxoline against 0.1 mol L^{-1} perchloric acid: **A** = sample No. 1; **B** = sample No. 3.

Table I. Amounts of nitroxoline (I) and relative percentage composition of structurally related compounds II, III, and IV as determined by HPLC and potentiometric titration.

Batch number	HPLC external standard method (%) I	HPLC relative percentage composition			Potentiometric titration (%) I
		II	III	IV	
1	88.9	0.22	11.0	0	90.43
2	99.0	0.65	1.25	0.12	98.80
3	99.3	0.16	0.57	0.15	99.17
4	98.5	0.73	2.09	0.42	97.26
5	94.2	0.51	6.59	0	93.97
6	98.6	0.25	1.32	0.16	98.19
7	97.1	0.14	2.61	0.63	97.09
Standard	100	0.08	0.48	0	99.23

Conclusion

C_{18} reversed-phase high-performance liquid chromatography with controlled mobile phase pH and optimized EDTA concentration enables simple and accurate quantitative analysis of nitroxoline and the structural related compounds 8-hydroxy-5,7-dinitroquinoline, 8-hydroxy-5-nitrosoquinoline, and 8-hydroxyquine. Addition of EDTA and citric acid to the mobile phase results in significant reduction of peak tailing. Complete separation of the four compounds shows the method to be suitable for quality control of nitroxoline.

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