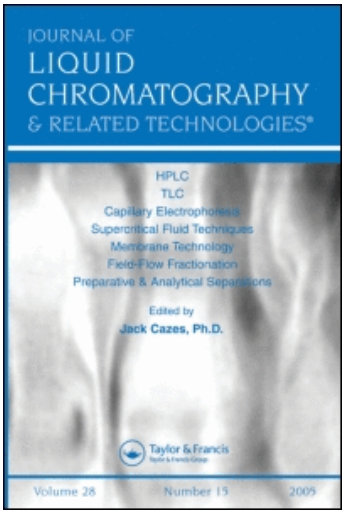

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HPLC Determination of Norepinephrine Bitartrate in 5% Dextrose Injection on Underivatized Silica with an Aqueous-Organic Mobile Phase

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HPLC DETERMINATION OF NOREPINEPHRINE BITARTRATE IN 5% DEXTROSE INJECTION ON UNDERIVATIZED SILICA WITH AN AQUEOUS-ORGANIC MOBILE PHASE

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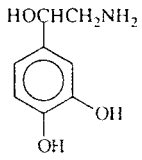
ABSTRACT

A high performance liquid chromatographic procedure has been developed for the assay of norepinephrine bitartrate in 5% dextrose injection containing up to a 500 fold excess of ranitidine. The separation and quantitation are achieved on a 22-cm underivatized silica column at ambient temperature (22 ± 1°C) using a mobile phase of 50:50 v/v 5 mM phosphate buffer, pH 3.0 - acetonitrile at a flow rate of 1.0 mL/min with detection at 280nm. It was shown that the predominant mechanism of retention for the drug on silica was cation exchange. The method showed linearity for norepinephrine over the 1 - 128 μg/mL range (r^2 = 0.9999, n=8). Accuracy and precision were in the 0.9 - 2.3 % and 0.63 - 3.3% ranges, respectively. The limit of detection was 26 ng/mL based on a signal-to-noise ratio of 3.



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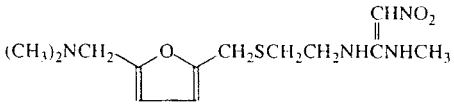

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Figure 1 - Chemical structures of Norepinephrine (A) and Ranitidine (B).

INTRODUCTION

Norepinephrine (Fig. 1) is chemically classified as a catecholamine and is used as a sympathomimetric in pharmaceutical preparations. It is easily oxidizable under the influence of air and light, expecially in neutral or alkaline solutions (1). Therefore, it is important to develop a rapid and direct analytical method for the drug.

Spectrophotometric (2-7), titrimetric (8,9), and liquid chromatographic (10,11) methods have been widely used for the determination of catecholamines such as norepinephrine in pharmaceuticals. A reverse-phase high performance liquid

chromatographic (HPLC) procedure is reported for the assay of norepinephrine in USP norepinephrine bitartrate injection. (12).

The purpose of this investigation was to develop a fast and direct injection assay for the quantitation of norepinephrine in 5% dextrose injection containing up to a 500 fold excess of ranitidine. In this paper, we report an HPLC procedure for the determination of norepinephrine in a ranitidine-dextrose mixture which uses underivatized silica with an aqueous buffer-acetonitrile mobile phase and direct sample injection.

EXPERIMENTAL

Reagents and Chemicals

Norepinephrine bitartrate was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD 20852). Ranitidine hydrochloride was obtained from Glaxo, Inc. (Research Triangle Park, NC 27709). Acetonitrile (J.T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076). Monobasic sodium phosphate and concentrated phosphoric acid were Baker analyzed reagents. 5% Dextrose Injection was obtained from Baxter Healthcare (Morton Grove, IL 60053).

Instrumentation

The chromatographic system consisted of a Beckman Model 110B Solvent Delivery Module (Beckman, San Ramon, CA 94583), an Alcott Model 738 HPLC Autosampler (Alcott Chromatography, Norcross, GA

30093) and an ABI Model 759A Absorbance Detector (Applied Biosystems, Foster City, CA 94404). Data acquisition and reduction were performed on an HP Model 3394A Integrator (Hewlett-Packard Company, Avondale, PA 19311). Separation was accomplished on a silica column (220 mm x 4.6 mm i.d., 5 μm Brownlee Labs, Santa Clara, CA 95050). The mobile phase consisted of 50:50 v/v 5 mM aqueous monobasic sodium phosphate, pH 3.0 (adjusted with concentrated phosphoric acid) - acetonitrile. The mobile phase was filtered through a 0.45 μm Nylon-66 filter (MSI, Westborough, MA) and degassed by sonication prior to use. The flow rate was set at 1 mL/min and the UV detector was set at 280 nm.

Preparation of Standard Solutions

A stock solution of norepinephrine base (128 μg/mL) was prepared by accurately weighing 12.11 mg of USP reference standard norepinephrine bitartrate powder, transferring to a 50-mL volumetric flask, 5% dextrose injection added to volume, and the solution shaken manually for 30 sec. Additional 1:10 and 1:100 dilutions were made in 5% dextrose injection to obtain standard solutions containing 12.8 and 1.28 μg/mL of norepinephrine base. Additional dilutions (1:1.25, 1:2.5, 1:5, 1:25, 1:50) of the norepinephrine stock solution were prepared in 5% dextrose injection to serve as spiked samples for the determination of accuracy and precision of the method. Quantitation was based on

linear regression analysis of norepinephrine peak height versus its concentration in μg/mL.

Assay Method

All samples containing norepinephrine including standard and spiked samples, were placed into autosampler vials, and 50-μL aliquots were directly injected into the HPLC system.

RESULTS AND DISCUSSION

The aim of this study was to develop an isocratic HPLC assay for the analysis of norepinephrine in the presence of up to a 500 fold excess of ranitidine contained in 5% dextrose injection. A stability study of norepinephrine in ranitidine admixtures would require that an assay procedure would separate, detect and quantitate the drug with reasonable accuracy and precision.

There are no reports in the scientific literature describing a separation of norepinephrine and ranitidine in 5% dextrose injection. Initial studies to develop a single isocratic HPLC method for norepinephrine involved reverse-phase systems using octadecylsilane (ODS) columns with various organic/aqueous mobile phases. Unfortunately, none of the ODS systems investigated would adequately separate norepinephrine from the large amount of ranitidine that would be present in the 5% dextrose injection admixture.

Our attention then turned to the use of an underivatized silica column with a buffered aqueous-organic mobile phase for the separation

and quantitation of norepinephrine in the dextrose-ranitidine mixture. This laboratory has previously reported HPLC methods to analyze basic, acidic and neutral compounds in pharmaceutical dosage forms and biological samples using underivatized silica (13-15). The separation mechanism for basic drugs with buffered aqueous mobile phases has been ascribed to the interaction of silanols with an amine group to produce a cation exchange mechanism. Since there were no reports in the scientific literature describing the separation of norepinephrine on silica, we investigated several mobile phases differing in pH, ionic strength, and type and concentration of organic modifier.

The pH of the mobile phase was varied from 2.5 to 7.0. Initial trials showed that pH did not affect the retention of norepinephrine. It has been our experience with silica columns that they show shorter equilibration times, less prominent solvent fronts and are much more stable when operated at low pH. Therefore, a mobile phase pH of 3 was selected for use in this study.

The ionic strength of the phosphate buffer component of the mobile phase was varied between 0 to 0.04 u. It was found that the ionic strength of the mobile phase was the predominant parameter affecting retention of norepinephrine. Increasing the ionic strength significantly decreased retention of norepinephrine. A 5 mM buffer strength was finally selected for the assay based on the best resolution of norepinephrine and ranitidine within a reasonable chromatographic run

time. The low concentration of buffer also aided in preventing excessive pump seal wear.

The concentration of acetonitrile in the mobile phase had little or no effect on the retention time. As the acetonitrile composition was increased from 40 to 60% of the mobile phase, the retention of norepinephrine essentially remained the same. Substituting methanol for acetonitrile in the mobile phase required about twice the concentration to achieve the same retention time, but caused a significant effect on retention. As the methanol composition in the mobile phase increased from 70 to 90%, norepinephrine showed decreased retention.

Therefore, it appeared from our studies that the predominant mechanism of retention of the basic analytes on underivatized silica was cation exchange. The addition of methanol to the mobile phase probably replaced water held in the third and even second layer adsorbed to silica and caused differences in retention due to the increased lipophilicity of the stationary phase as compared to equal amounts of acetonitrile in the mobile phase. This effect of methanol versus acetonitrile has been previously reported by our laboratory in the separation of non-steroidal anti-inflammatory agents and anabolic steroids on underivatized silica (14,15). Thus a mobile phase consisting of 50:50 v/v 5 mM phosphate buffer pH 3.0 - acetonitrile was selected for the assay. It was decided to use 280 nm as the detection wavelength since the absorption maxima

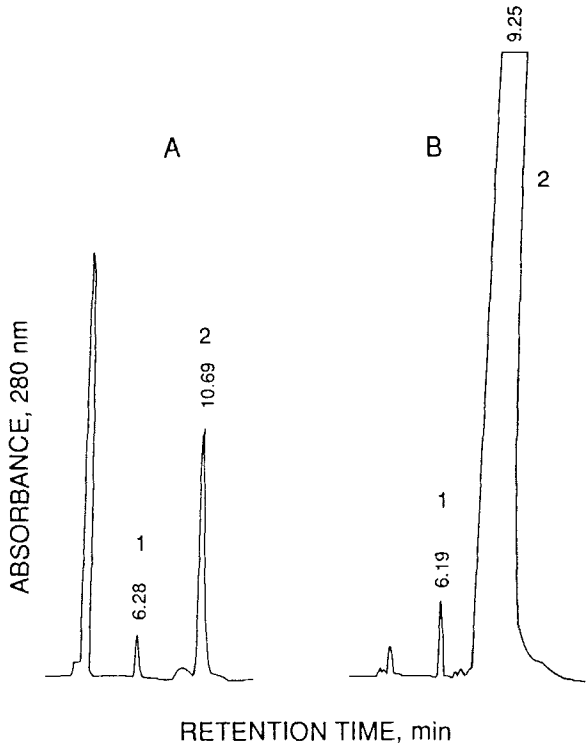

Figure 2 - HPLC chromatograms showing the separation of (A) a mixture of 4 μg/mL norepinephrine and 50 μg/mL ranitidine, and (B) a mixture of 8 μg/mL norepinephrine and 2 mg/mL ranitidine. See Experimental Section for chromatographic conditions.

for norepinephrine was 280 nm in the acetonitrile - phosphate buffer mobile phase.

Spiked samples of norepinephrine were prepared in 5% dextrose injection both at low concentration (4 μg/mL of norepinephrine and 50 μg/mL of ranitidine) and at high concentration (8 μg/mL of norepinephrine

Table 1 - Precision and Accuracy of Spiked Norepinephrine Solutions

Concentration added, μg/mL	Concentration found, μg/mL	n*	Relative Error,%	RSD %
Inter-day				
2.57	2.51 ± 0.08	4	2.2	3.3
25.68	25.94 ± 0.34	4	1.0	1.3
102.72	103.68 ± 1.33	4	0.93	1.3
Intra-day				
5.14	5.09 ± 0.07	3	0.9	1.4
51.36	52.55 ± 0.33	3	2.3	0.63

* n represents the number of days for the inter-day study and the number of samples analyzed on one day for the intra-day study.

and 2 mg/mL of ranitidine). Typical chromatograms shown in Fig. 2 demonstrate that the norepinephrine and ranitidine peaks at low and high concentrations are well separated on the silica column. The large ranitidine peak does not interfere with the determination of the small amounts of norepinephrine present in the admixtures.

Determination of norepinephrine levels in 5% dextrose injection was carried out using external calibration. A calibration curve was generated by least-square regression of the norepinephrine peak height against its concentration in 5% dextrose injection. The regression analysis showed linearity for norepinephrine over the 1 - 128 μg/mL range at 280 nm with a correlation coefficient of 0.9999 (n = 8). The detection limit was 26 ng/mL based on a signal-to-noise ratio of 3.

Percent error (accuracy) and precision of the method were evaluated using spiked samples. The results shown in Table 1 indicate that the procedure gives acceptable accuracy and precision.

In summary, an underivatized silica column with an aqueous pH 3.0 buffer -acetonitrile mobile phase has been shown to be amenable for the separation and quantitation of norepinephrine bitartrate in 5% dextrose injection containing up to a 500 fold excess of ranitidine. The HPLC method has advantages of using a simple and inexpensive mobile phase and direct sample injection onto an underivatized silica column.

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