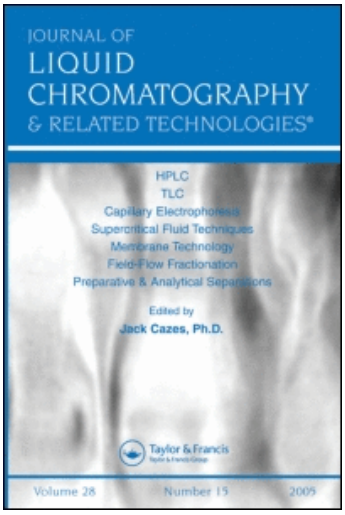

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DETERMINATION OF RITODRINE IN PLASMA USING HPLC

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ABSTRACT

An HPLC instrument coupled with an electrochemical detector was used to determine ritodrine (erythro-p-hydroxy-α-[1-[(p-hydroxyphenethyl)-amino]ethyl] benzyl alcohol hydrochloride) at nanogram levels in serum. Extraction of ritodrine was accomplished using a multistep ethyl acetate procedure, and the mobile phase consisted of acetonitrile, ammonium acetate, glacial acetic acid, and a counterion. The stationary phase was a Biophase ODS 5 μm column at ambient temperature. Nalbuphine hydrochloride (Nubain®) was used as an internal standard to quantitate the ritodrine levels of pregnant patients receiving ritodrine. The procedure's linearity for both ritodrine standards and spiked plasma samples was demonstrated. The precision of the assay was found to be 3.4% at 20 ng/ml ritodrine. The minimum detectable concentration, with a signal-to-noise ratio of 6, was determined to be 0.31 ng per 50 μl injected, corresponding to a concentration of 0.6 ng/ml plasma. The sensitivity, precision, and reproducibility of the assay were all found to be acceptable for determining ritodrine in patient serum.

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INTRODUCTION

Premature birth is the leading cause of neonatal mortality and morbidity(1). Ritodrine hydrochloride (Yutopar®) is the first product in the United States approved as tocolytic agent for the management of premature labor. However, little information is available describing its effective serum levels, pharmacokinetics, and side effects in pregnant women and neonates.

The only analytical technique available for determining ritodrine in serum is radioimmunoassay, a technique that requires the development of a specific antiserum(2). Therefore, the purpose of this study was to develop a high performance liquid chromatography (HPLC) procedure that would obviate the need for an antiserum and still provide a high level of sensitivity. Coupling HPLC with electrochemical detection (EC) provided a high level of sensitivity and precision, as well selectivity, for the analysis of ritodrine in serum. HPLC/EC methods similar in nature have been used to analyze tricyclic antidepressants(3).

MATERIALS AND METHODS

Reference Compounds

Reference ritodrine hydrochloride (Yutopar ®) was obtained from the Merrell Dow Research Center (Cincinnati, Ohio). The internal standard, nalbuphine hydrochloride (Nubain®), was donated by Endo Laboratories, Inc. (Garden City, New York).

Apparatus

An HPLC instrument (Perkin-Elmer Model 3B) coupled with an electrochemical detector (Bioanalytical Systems Model 4B) and a data terminal (Perkin-Elmer Model Sigma 10B) was used for determining ritodrine. The electrochemical detector was equipped with a glassy-carbon electrode; the chromatography column was a Biophase ODS 5 µm column. A Rheodyne injector (model 7125) was used with a 50 µl loop. The samples were centrifuged with an International centrifuge (model HN) and the samples were mixed in a Vortex shaker (model Vortex-Genie).

Chromatography Conditions

The mobile phase consisted of acetonitrile, ammonium acetate, and water (20/10/70) with 3mM ion-pair reagent (1-heptanesulfonicacid sodium salt) at pH 3.7. The ammonium acetate buffer (10x) contained 400mM ammonium acetate, 2.1M glacial acetic acid and water. Each liter of mobile phase contained 12ml of stock ion-pair solution (5.52gm ion-pair salt, 10ml glacial acetic acid, diluted to 100ml with water). The mobile phase was recirculated at a flow rate of 1ml/min.. The Biophase ODS 5 µm column was used at ambient temperature; the carbon electrode at 0.95 volts (vs. Ag/AgCl).

Treatment of Glassware

All test tubes and centrifuge tubes were surface treated with PROSIL-28 (PCR Research Chemicals, Inc.) an organosilane surface

treating agent. The procedure used was that recommended by the product's manufacturer.

Extraction Procedure

One ml of plasma was added to 1.0 ml of distilled water (Type 1, one megaohm) and 1.0 ml of 0.6 M K_2CO_3 in a 15 ml conical centrifuge tube with screw cap(3); this resulted in a pH of 9.4.. Then 8.0 ml of ethyl acetate was added. The tube was then mechanically shaken for 5 minutes, and then centrifuged at 2000 rpm for 15 minutes. The organic layer was transferred to a 15 ml conical centrifuge tube with a screw cap containing 1.2 ml of 0.1 M HCl. The tube was again shaken for 5 min. and centrifuged at 2000 rpm for 10 minutes. The top layer was aspirated to waste. Five-tenths ml of 0.6 M K_2CO_3 and 1.0 ml ethyl acetate was added (pH 9.4) and the tube was again shaken and centrifuged as mentioned above. The ethyl acetate layer was transferred to a 12 ml conical test tube and evaporated under a stream of purified nitrogen to dryness at room temperature. The residue was reconstituted in 300 µl mobile phase and 50 µl of the sample was injected into the liquid chromatograph.

RESULTS

LC Chromatograms

A chromatogram of ritodrine in the mobile phase (10 ng/ml) is shown in Figure 1. The retention time of the ritodrine was

approximately 9.33 min.; the contaminant came from the reagents but it was not further identified since it did not interfere with the procedure. Also shown in Figure 1 is a chromatogram from a plasma extracted sample; the concentration of ritodrine in this sample was also 10 ng/ml. The retention time of the internal standard, nalbuphine, was 13.58 min..

Standard Curves

The linearity for both rithodrine standards and plasma extracted samples over a range of 0-50ng/ml is demonstrated in Figure 2. The minimum detectable concentration was 0.6ng/ml in plasma, which yielded a signal-to-noise ratio of 6; this corresponds to 0.31 ng/ml per 50 µl injected.

Oxidation Potential

The oxidation potential for ritodrine (+0.95 volts) was optimized by plotting peak height versus applied potential. The appropriate range for the applied potential was first determined by Bioanalytical Systems of West Lafayette, Indiana.

Mobile Phase

Methanol was first used in the mobile phase; however, satisfactory results were not obtained. This led us to experiment with an acetonitrile solvent system. The mobile phase described in the methods section was found to be satisfactory after experimenting with the various solvent variables.

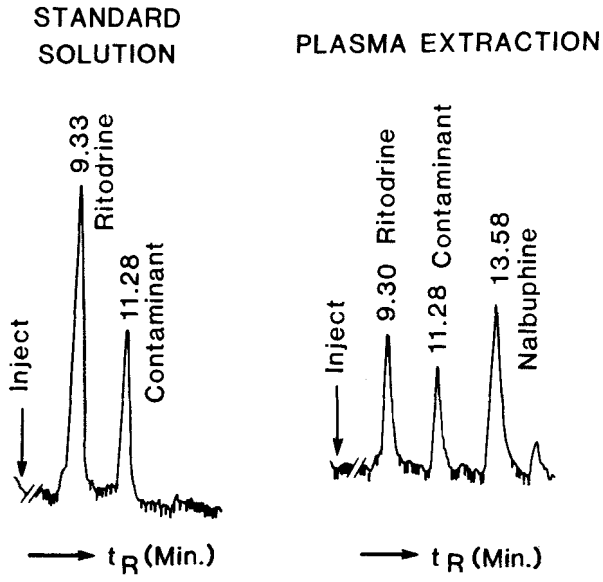

FIGURE 1 LC of Ritodrine Hudrochloride

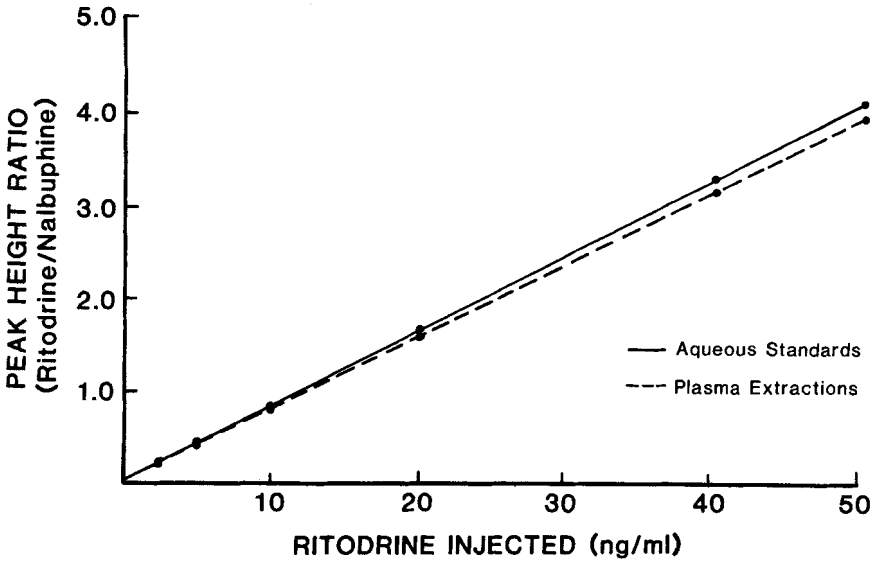

FIGURE 2 Linearity of Ritodrine Standards Using LCEC

TABLE I

Recovery of Ritodrine Added to Plasma

Tissue	Number	Recovery %	SD	CV%
Plasma (1 ml)	10	80.1	1.2	2.5%

TABLE II

Assay Precision for Plasma Ritodrine

Conditions	Concentration (ng/ml)	Mean + SD	CV%
Separate Extractions (n=12)	20	19.6 + 0.7	3.4

Recovery

The recovery of ritodrine was determined using spiked plasma samples (25ng/ml). The results are shown in Table 1. The mean recovery was 80.1% with a coefficient of variation of 2.5%. The extraction solvent used (ethyl acetate) was arrived at by experimenting with the solvents used in our laboratory for drug extraction (see Discussion).

Precision

The analytical precision at a plasma concentration of 20ng/ml was found to be excellent. The coefficient of variation was 3.4% (Table 2).

DISCUSSION

A radioimmunoassay technique for analyzing ritodrine was not used by us because of the difficulty in producing a suitable antiserum. Instead, an HPLC/EC technique was developed that gave a level of sensitivity (0.6ng/ml plasma) comparable to that reported by Gandar et al. (0.3ng/ml plasma)(2) using radioimmunoassay. We found the HPLC/EC technique to be sufficiently sensitive for analyzing ritodrine in pregnant women receiving the drug to prevent premature labor.

Since our lab does not have cyclic voltammetry, the oxidation potention for ritodrine was determined by Bioanalytical Systems of West Lafayette, Indiana. They found the optimum potential to be approximately +900 mv with a glassy carbon electrode. The potential used in our method was +950 mv; this value was arrived at experimentally and was within the appropriate range suggested by the voltammogram produced by Bioanalytical Systems.

A considerable amount of experimentation was done to determine which solvent system would provide the best results. The first system tried was that used by Merrell Laboratories, the previous manufacturer of ritodrine. Their mobile phase contained methanol, water, sodium-n-heptal sulphinate, and ammonium acetate. The solvent system we report here, using acetonitrile, etc., gave us good sensitivity and precision.

Several organic extraction solvents were tried before one was found that was satisfactory. Among those tried were: acetonitrile, methyl-t-butyl ether, diethyl ether, and ethyl acetate. Ethyl acetate gave an extraction efficiency of 80%; however, adjustment of

the pH as described in the procedure section is vital to obtain this efficiency (personal communication with Dr. Lan K. Wong of the University of Pittsburgh). The extraction procedure followed was that described by Suckow and Cooper with the exception of using ethyl acetate and adjustment of the pH.

Overall, the sensitivity, precision, and reproducibility of the assay were all found to be acceptable for determining ritodrine in the serum of pregnant mothers.

REFERENCES

1. Caritis, S.N., Edelstone, D.I., and Mueller-Heubach, E.: Pharmacologic inhibition of preterm labor. Am. J. Obstet. Gynecol. 133 :557-580, 1979.

2. Gandar, R., de Zoeten, L.W., and van der Schoot, J.B.: Serum level of ritodrine in man. Eur. J. Clin. Pharmacol. 17 :117-122, 1980.

3. Suckow, R.F., and Cooper, T.B.: Simultaneous determination of imipramine, desipramine, and their 2-hydroxy metabolites in plasma by ion-pair reverse-phase high-performance liquid chromatography with amperometric detection. J. Pharm. Sci. 70 :257-261, 1981.