I; however, it was not present in the antacid formulations studied (Fig. 2). It was found that salicyclic acid could be better resolved, if necessary, by utilizing a 5% OV-1 column and temperature programming (150°C for 10 min, then 32°C/min to 175°C) (Fig. 3). However, chromatography time increased from ~5 min (SE-30 column) to >16 min (OV-1 column) per injection.

Assay of the forced degradation antacid sample revealed that ~10% of I had been converted to V. Compound III had not appreciably hydrolyzed during this short interval. No additional degradation peaks were observed.

The linearity data for I–V, determined by plotting peak height ratios (sample/internal standard) *versus* weight ratios injected, are presented in Table I. The correlation coefficients and percent variations (9) all indicate good linearity over the range studied: 0.75–6 μg for I, 0.5–3.8 μg for II, 0.1–0.9 μg for III, 0.06–0.5 μg for IV, and 0.014–0.7 μg for V. The percent intercepts indicate no serious problem with single-point standard calculations, except for IV. The significant negative intercept for IV demands that standard and sample concentrations be matched within 10%. Alternatively, a standard curve analysis could be run.

Assays of eight synthetic liquid antacid samples made by spiking placebo with solutions containing known amounts of standard I–V at levels of 60–125% of the theoretical yielded average recoveries and relative standard deviations of 99.0 ± 2.0% for I, 99.3 ± 1.9% for II, 98.7 ± 1.8% for III, 100.3 ± 1.6% for IV, and 100.0 ± 1.4% for V.

Twelve replicate assays of an actual liquid antacid sample containing all four preservatives and their common hydrolysis product V yielded good precision, as shown in Table II. The assay results of two other commercial liquid antacids are also shown in Table II. Since these samples had been stored for ~2 years prior to assay, relatively high concentrations of V were encountered. Although only antacids were examined in the present work, the technique should be readily applicable to other pharmaceutical preparations, such as liquids, elixirs, parenterals, creams, and gels.

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Analysis of Ritodrine in Serum by High-Performance Liquid Chromatography with Electrochemical Detection

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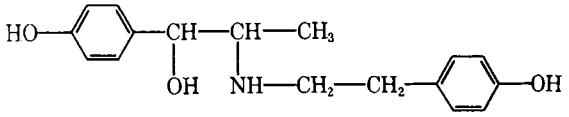
Abstract □ A sensitive and specific assay for ritodrine in serum was developed using high-performance liquid chromatography (HPLC) with electrochemical detection. Serum samples were alkalinized to pH 9.4 by the addition of a sodium carbonate buffer and extracted with ethyl acetate. The extracts were evaporated to dryness and the residues were reconstituted in the HPLC mobile phase and chromatographed on a octadecylsilane reverse-phase column. The detection of ritodrine was achieved by an electrochemical detector with a glassy carbon electrode. The sensitivity was 0.2 ng for on-column injection. The extraction efficiency was 80%.

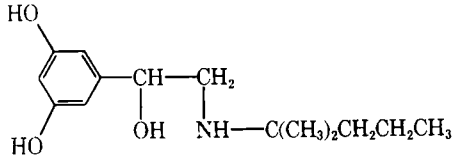
Keyphrases □ Ritodrine—analysis in serum, high-performance liquid chromatography with electrochemical detection □ High-performance liquid chromatography—analysis of ritodrine in serum, electrochemical detection

Ritodrine hydrochloride, *erythro*-*p*-hydroxy-α-[1-[(*p*-hydroxyphenethyl)amino]ethyl]benzyl alcohol hydrochloride (I), is a β-adrenergic receptor stimulant developed specifically for obstetrical use in the management of premature labor. Pharmacological studies have shown that ritodrine is a potent inhibitor of myometrial contractility with only minor cardiovascular effects (1–4). Studies on experimental animals have demonstrated the uterine relaxant properties of ritodrine. Depending on the animal species and the route of administration, the effective dose ranges from 5 μg/kg to 15 mg/kg (5). In humans, the therapeutic dose varies from 50 to 200 μg/min. The infusion rate is maintained 24–48 h according to the response of the patient and side effects (6). Treatment is usually followed with an oral 10-mg dose four to eight times daily for several days or until term. Human disposition studies have been carried out on healthy nonpregnant volunteers using tritiated ritodrine. It was found that the majority of the drug was excreted in the urine with a maximal excretion rate attained within 1 h after drug administration. The data suggested a two-compartment open model with half-lives of 2 and 13 h (5).

Analytical assay of ritodrine had been limited to the use of radioactively labeled drug (5) until recently when a sensitive RIA (7) was reported. The RIA has a sensitivity of <1 ng and has been used to measure serum levels of ritodrine in humans after oral or parenteral administration. Whereas good sensitivity was achieved by this procedure, some unexplained variability was observed in the data, possibly due to cross-reactivity with some endogenous interferences in the samples. The RIA antiserum is cur-



I



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rently unavailable precluding the routine clinical monitoring of ritodrine. Thus, it was deemed desirable to develop an alternative method for the assay of this drug in biological fluids. This report describes a simple, sensitive, and specific high-performance liquid chromatographic (HPLC) assay using electrochemical detection for the measurement of nanogram quantities of ritodrine in serum or plasma.

EXPERIMENTAL

Reagents and Materials—Ritodrine hydrochloride[1] and the internal standard[2], 1-(3,5-dihydroxyphenyl)-2-(1,1-dimethylbutylamino)ethanol (II), were used as received. Distilled-in-glass ethyl acetate[3] and methanol[4] were used in the extraction and chromatographic procedures, respectively. All other chemicals were reagent grade.

Apparatus—Chromatographic analyses were performed at ambient temperature on a liquid chromatograph[5] equipped with an electrochemical detector[6] fitted with a thin-layer cell containing a glassy carbon electrode. The electrochemical potential of the glassy carbon working electrode was set at +0.90 V relative to a silver–silver chloride reference electrode. A 4.6-mm × 26-cm prepacked octadecylsilane reverse-phase column[7] with a precolumn[8] (1.2 mm × 7.1 cm) was used. Samples were introduced onto the column through a septumless injector[9] with a 100-μL syringe. Chromatograms were recorded on a strip-chart recorder[10].

Chromatographic Condition—The chromatography was performed at an isocratic mode. The mobile phase was a methanol–phosphate buffer (25:75, v/v) with a flow rate of 1.5 mL/min. The phosphate buffer was composed of 0.01 M KH_2PO_4, 0.3 mM sodium octanesulfonate, and 0.1 mM EDTA (disodium ethylenediaminetetraacetate) and had a final pH of 4.5. The solvent mixture was deaerated by stirring under vacuum before use.

Extraction—A serum sample (1 mL) was adjusted to pH 9.4 by the use of 2 M sodium carbonate buffer. The internal standard (200 ng) was added to each sample, and the mixture was then vortexed and extracted with 6 mL of ethyl acetate by reciprocal shaking for 15 min. After centrifugation, the organic layer was transferred to another tube and evaporated to dryness under a nitrogen stream at 35°C. The dry residue was then redissolved in 400 μL of the HPLC mobile phase by vigorous vortexing. A 40-μL aliquot of the mixture was injected into the chromatograph for quantitation.

A set of standard samples prepared from blank serum spiked with known amounts of ritodrine and 200 ng of the internal standard were processed as above. The peak height ratios of ritodrine to the internal standard in these samples were determined, and a calibration curve was constructed from the resulting data. The concentrations of unknown samples were derived from this calibration curve. The recovery of ritodrine by the extraction procedure was 80%.

Mass Spectrometry—The identity of the ritodrine peak on the HPLC chromatogram was verified by MS. The eluate corresponding to the ritodrine fraction was collected from the HPLC, and the pH was adjusted to 9.4 with sodium carbonate. Ritodrine was extracted with ethyl acetate and evaporated to dryness under a nitrogen stream. The residue was applied to a sample vial of the spectrometer[11]. MS analysis was performed *via* a direct probe inlet in the electron-impact ionization mode at 70 eV. The mass spectrum of authentic ritodrine[1] showed the following characteristic fragment ions: *m/z* 121 (100%), 164 (65%), 107 (23%), 77 (21%), and 91 (10%). No molecular ion was observed for ritodrine. The HPLC peak of interest exhibited ions at *m/z* 121 (100%), 164 (65%), 107 (23%), 77 (21%), and 91 (10%). MS data were identical to those of the authentic sample.

RESULTS AND DISCUSSION

The sensitivity of detection for ritodrine during on-column injection was found to be 0.2 ng. This was established by observing that the peak height of 0.2 ng of ritodrine *versus* the internal standard was significantly different from zero when it was compared with the 95% confidence interval of the *y*-intercept. Under the described chromatographic conditions, the retention times of ritodrine and the internal standard were 7.8 and 12.0 min, respectively. Comparing the chromatogram (Fig. 1A) of an extracted blank serum sample pooled from pregnant women with that of a similar serum sample spiked with 40 ng of ritodrine hydrochloride and 200 ng of the internal standard (Fig. 1B), it can be concluded that little or no interferences from endogenous substances are present. A typical chromatogram from a patient receiving ritodrine for premature

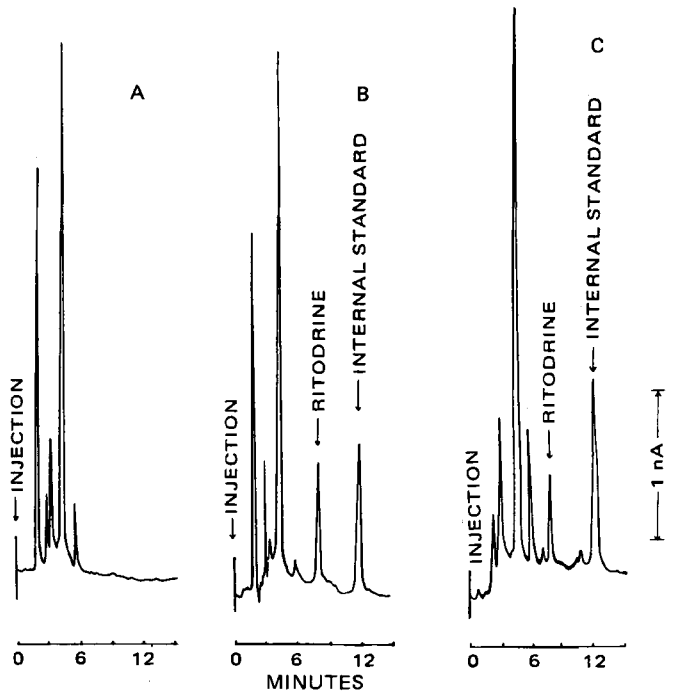

Figure 1—*Chromatograms of samples prepared from blank serum pooled from pregnant women (A), blank serum spiked with 40 ng of ritodrine hydrochloride and 200 ng of the internal standard (B), and serum of a patient receiving ritodrine therapy (C).*

Table 1—Analysis of Ritodrine Level in Serum

Ritodrine Spiked, ng/mL	Peak Height Ratio[a]	Mean Value Assayed, ng/mL	Ratio of Assayed to Spiked
2	0.095 0.091 0.119 0.098	2.08	1.04
5	0.131 0.165 0.144 0.130	5.31	1.06
10	0.207 0.210 0.237 0.186	9.20	0.920
20	0.400 0.345 0.366 0.460	19.6	0.978
40	0.731 0.760 0.708 0.752	39.9	0.998
80	1.35 1.57 1.50 1.43	82.3	1.03

[a] Ritodrine/internal standard.

[1] Philip-Duphar Research Laboratories, Weesp, The Netherlands.
[2] Research Laboratories of A B Draco, Lund, Sweden.
[3] Mallinckrodt, Paris, Ky.
[4] Burdick & Jackson Laboratories, Muskegon, Mich.
[5] Model M-45; Waters Associates, Milford, Mass.
[6] Model LC-4B; Bioanalytical Systems, West Lafayette, Ind.
[7] μBondapak C18; Waters Associates, Milford, Mass.
[8] HC Pellosil; Whatman, Clifton, N.J.
[9] Model U6K; Waters Associates, Milford, Mass.
[10] Linear Instruments, Irvine, Calif.
[11] Model 3200; Finnigan Corp., Sunnyvale, Calif.

labor is shown in Fig. 1C. The specificity of this method was also confirmed by mass spectral studies. The HPLC eluate corresponding to ritodrine was found to exhibit MS characteristics identical to those of pure ritodrine. The spectrum shows a base peak at m/z 121 and a strong fragment ion at m/z 164. No molecular ion is detectable for ritodrine in the electron-ionization spectrum.

The calibration curve for ritodrine using the internal standard bears a linear relationship ($r^2 = 0.9910$) over the range (2–80 ng/mL) studied. Table I illustrates the reproducibility of the method. To demonstrate the applicability of this method for the therapeutic monitoring of ritodrine, a serum sample from a pregnant woman receiving ritodrine for premature labor was analyzed. The patient received an intravenous infusion of ritodrine at 100 μg/min for 3.5 h. The serum level of ritodrine 20 min after the end of the infusion was 12.8 ng/mL. The concentration compares favorably with the results obtained by the RIA method (7). The degree of sensitivity achieved by the HPLC–EC method is suitable for drug monitoring and pharmacokinetic studies of ritodrine, since serum concentrations of the drug during clinical use usually range from several to ~100 ng/mL. The linearity of the calibration curve indicates that the assay methodology is appropriate for these measurements.

The usefulness of electrochemical detection has been demonstrated in the HPLC analysis of catecholamines (8–10), uric acid (11), ascorbic acid (11), and acetaminophen (12) in biological fluids and pharmaceutical preparations. Ritodrine, a compound structurally related to catecholamines, evokes a strong electrochemical response due to the two phenolic moieties in the molecule. The method described herein permits a rapid analysis of ritodrine at the nanogram level.

The sensitivity of the electrochemical detector is quite comparable with that of the RIA technique, and the assay speed of the two methods is also very comparable. The RIA method appears to be more convenient because no extraction is required. However, it takes ~40 min to incubate and equilibrate a sample with the antiserum plus additional time for scintillation counting, not to mention the time for preparing the antiserum. On the other hand, the analysis time by HPLC requires ~15 min according to the protocol. Most importantly the HPLC–EC method may be superior to the RIA in terms of specificity. Previous work in the assay of ritodrine by RIA showed negligible cross-reactivity with the drug conjugates (7). However, the possibility of cross-reactivity with endogenous catecholamines has not been evaluated. The fact that abnormal variability was observed in the data during the RIA quantitation of ritodrine (7) points to the potential problem of cross-reactivity. It is known that serum catecholamine levels are elevated during labor (13). This may cause erroneous determination of ritodrine by RIA under such conditions. On the other hand, the accuracy of the HPLC–EC method is not affected by these endogenous substances.

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Remarks on the Structure–Activity Relationship of Silver Sulfanilamides

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Received February 1, 1982, from the **Department of Pharmaceutical Analysis and Analytical Chemistry, State University of Leiden, 2300 RA Leiden, The Netherlands* and the ‡*Laboratory for Pharmaceutical and Analytical Chemistry* and the §*Laboratory for Pharmaceutical Technology and Dispensing, State University of Groningen, Groningen, The Netherlands.* Accepted for publication December 8, 1982.

Abstract □ The biological activity of a series of 10 silver sulfanilamides is studied in relation to the physical parameters pK_a, log K, and the aqueous solubility. None of the parameters demonstrate a simple relationship with the activity. A discussion of the significance of log K and the solubility in relation to the activity is given.

Keyphrases □ Silver sulfanilamides—structure-activity relationships, correlation between activity and pK_a, log K, and solubility □ Structure–activity relationships—silver sulfanilamides, correlation between activity and pK_a, log K, and solubility

An increasing interest in the silver sulfanilamide complexes was stimulated by the successful use of silver sulfadiazine (I) as an efficacious topical antibacterial agent in burn treatment (1). A number of efforts were made to account for the good *in vivo* activity of I as compared with other silver sulfanilamides (2–4). In this study the relevance of some physical parameters in relation to the *in vivo* antimicrobial activity are discussed.

BACKGROUND

The antimicrobial activity of I is thought to result from alteration of the mesosomal function of the microbial cell by the silver moiety of the molecule (5). The sulfadiazine moiety does not enter the cell and does not contribute appreciably to the antimicrobial action; therefore, I is not antagonized by *p*-aminobenzoic acid (3). A possible role of sulfadiazine is to localize the action of silver to the cell. The undissociated molecule I seems to interact with the microbial cell and is dissociated next at the cell surface into silver and sulfadiazine (6, 7).

The role of the sulfadiazine anion and other anions is rather unclear. Wysor assumed as a prerequisite for an active silver sulfanilamide that, like I, these compounds need to be stable in a chloride-containing solution

0022-3549/84/0100-0133$01.00/0

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