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ARTICLES

HPLC Determination of Ritodrine Hydrochloride in Pharmaceutical Formulations[1]

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Abstract—A simple, rapid, and accurate HPLC method is described for the determination of ritodrine hydrochloride (RTH) in both pure form and pharmaceutical formulations. A Hypersil Shendon ODS column with a mobile phase of dibasic phosphate buffer and acetonitrile (75 : 25) and isoxsuprine hydrochloride were used as an internal standard. The flow rate was 1 mL min^{-1} and the effluent was monitored at 270 nm pH 4.0. The calibration graph is linear in the range 2–30 μg mL^{-1}. The proposed HPLC method has been successfully employed for the determination of RTH in Yutopar tablets and injection solutions.

INTRODUCTION

Ritodrine hydrochloride (RTH), chemically 1-(4-hydroxy phenyl)-2-[2-(4-hydroxy phenyl) ethyl amino] propanol, is a selective β_2-receptor agonist that is used to control premature labor and reverse fetal distress caused by excessive uterine activity [1]. Ritodrine is used widely in obstetrics [2]. In view of the increased pharmaceutical applications of ritodrine, its assay and quality control are very important.

The literature reveals that only a few methods concern the determination of RTH in pharmaceutical-dosage forms. Spectrophotometric methods based on the formation of colored dye by the coupling reaction between the oxidation product of MBTH [3] and 4-aminoantipyrine [4] and diazotized *p*-nitroaniline and sulphanilic acid [5]. Other reactions are based on nitration with subsequent complex formation [6]. Besides, fluorimetric and spectrophotometric [7] methods have been reported for the assay of RTH. The current United States Pharmacopoeia [8] describes an HPLC method for the analysis of RTH. In biological fluids, RTH has been determined by the HPLC [9] and GLC [10] methods. For routine quality control, the development of a simple, rapid, and reliable method is highly desirable.

The aim of the present work is to develop an HPLC method for the analysis of RTH in both pure and dosage forms. This new method is simple, rapid, accurate, and reliable.

EXPERIMENTAL

Apparatus. We used the HPLC equipment of a Shimadzu model class VP 10 series with a Shimadzu tunable absorbance detector and column of 250 × 4.6 mm i.d. containing Hypersil Shendon ODS packing material at ambient temperature with a chart speed 0.5 cm min^{-1}. The mobile phase consisted of 0.02 M KH_2PO_4 + acetonitrile (75 : 25), and the pH was adjusted to 4.0 with dilute phosphoric acid at flow rate of 1 ml min^{-1}. A UV detector at 270 nm was used.

Reagents. Potassium hydrogen orthophosphate (0.02 M) was prepared by dissolving 1.3609 g of KH_2PO_4 (Loba Chemie Pvt. Ltd., India) in 500 mL of distilled water. HPLC-grade acetonitrile and orthophosphoric acid A.R. were used.

Standard solution. Pure compounds of ritodrine (RTH) and isoxsuprine hydrochlorides (ISH) were received from Duphar-Interfran Ltd., India, and were used as such. The stock solutions of RTH and ISH were prepared in the mobile phase in a 100-mL standard flask so as to get a concentration of 1000 μg mL^{-1}. Solutions with lower concentrations were prepared by diluting the standard solutions. ISH was used as the internal standard.

Assay procedure. In a 100-mL calibrated flask, an accurately weighed amount (from the mixed and powdered contents of twenty tablets) of 50 mg of RTH was dissolved in 75 mL of the mobile phase and the contents were thoroughly shaken for about 30 min. Then, the volume was diluted up to the mark with the mobile phase, mixed well, and filtered using quantitative filter paper. The requisite amount of the drug solution was mixed with 10 μg mL^{-1} of ISH (IS) in a 10-mL calibrated flask and diluted to the mark with the mobile phase. A 50 μL of this solution was injected into the column. The peak area ratio of RTH was calculated. The amount of the drug was assayed from the calibration graph, which was constructed using a standard solution of RTH.

For the analysis of injection solution, the requisite volume was transferred to a 100-mL calibrated flask

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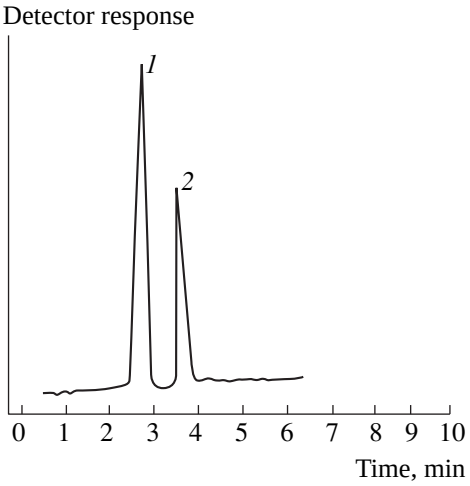

The HPLC chromatogram. (*1)* RTH; (*2)* ISH. See text for explanation.

and diluted to the mark with the mobile phase. The drug content in the diluted solution was assayed as described above. The results are presented in Table 1.

RESULTS AND DISCUSSION

A Hypersil Shendon reverse phase column is used for the separation of a large number of organic compounds, including some chemotherapeutic agents. It was observed that this column gives a satisfactory separation of RTH and ISH in the solvent system comprised of a dibasic phosphate buffer (pH 4) and acetonitrile.

Mobile phase. The composition of the mobile phase that gave the optimum chromatographic conditions was investigated. A mobile phase consisting of acetonitrile and water (50 + 50) adjusted to pH 3 did not give well-defined sharp peaks for RTH and ISH. The use of a mobile phase consisting of an acetonitrile–phosphate buffer (25 + 75) adjusted to pH 6 did not yield the required separation within a short time (the retention time of RTH was >8 min). A mobile phase consisting of a phosphate buffer (0.02 M) and acetonitrile (75 + 25) adjusted to pH 4.0 with dilute orthophosphoric acid gave the optimum resolution of RTH and ISH (see figure). The presence of organic solvent in the mobile phase resulted in the reduction of the column back-pressure, shorter retention times, and sharper peaks. No interference from the other components in the pharmaceutical formulations was encountered using this mobile phase.

The pH of the mobile phase was a critical factor in obtaining good resolution and sharp peaks. It also influenced the retention times of the two components. The concentration of the phosphate buffer only influenced the retention times of the two components: increasing the concentration to 75 mL reduced the retention time of RTH to 2.7 min. The use of the mobile phase to dilute the mixture of the RTH and ISH aliquots to the final volume before injection into the column was also necessary to achieve the optimum conditions. The retention times for RTH and ISH were 2.71 and 3.36 min, respectively. The method was found to be linear in the concentration range from 2 to 30 μg mL^{-1} of RTH.

Internal standard. Isoxsuprine hydrochloride was selected as the internal standard because of its structural similarity to RTH. It was observed that a good resolution was obtained under the operating conditions.

Precision and accuracy. The precision of the proposed method was evaluated by replicate analyses of samples containing RTH at three different concentrations (low, medium, and high).

The within-day precision showed a RSD of 0.60 or 0.14% at the low concentration (5 μg mL^{-1}). The between-day precision evaluated over a period of five days showed a RSD of 0.61 or 0.10% at the low concentration. The low values of both the within- and between-day RSDs at the low concentration reflect the

Table 1. Analysis of RTH in pharmaceutical preparations (n = 5)

Preparation	Analyte taken (μg mL^{-1})	Analyte found (μg mL^{-1})	% Recovery ± SD		t-value*	F-value**
			Officinal method	Proposed method		
Yutopar***,	5	5.06	99.9 ± 0.4	101.2 ± 0.7	2.6	3.06
tablet	15	14.63	98.0 ± 0.9	99.5 ± 1.2	2.4	1.77
	25	24.98	101.2 ± 0.8	99.9 ± 1.0	1.1	1.52
Yutopar***, for	5	4.99	99.5 ± 1.1	99.8 ± 1.3	0.6	1.39
injection	15	15.03	99.9 ± 0.4	100.2 ± 0.5	1.7	1.56
50 mg/10 mL	25	24.93	100.5 ± 0.5	99.7 ± 0.6	2.4	1.44

*Tabulated value, 2.78.

** Tabulated value, 6.39.

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Table 2. Within- (I) and between-day (II) precision of the RTH assay

Preparation	I			II		
	Analyte taken (μg mL^{-1})	Analyte found* (μg mL^{-1}) ± SD	RSD, %	Analyte taken (μg mL^{-1})	Analyte found* (μg mL^{-1}) ± SD	RSD, %
RTH	5	4.98 ± 0.03	0.60	5	4.94 ± 0.03	0.61
Pure form	15	14.98 ± 0.02	0.17	15	14.91 ± 0.02	0.13
	25	25.19 ± 0.04	0.14	25	25.92 ± 0.03	0.10
Yutopar tablet	5	4.97 ± 0.02	0.40	5	4.92 ± 0.02	0.45
	15	14.93 ± 0.03	0.20	15	14.98 ± 0.01	0.06
	25	25.91 ± 0.02	0.08	25	25.98 ± 0.02	0.08
Yutopar for injection	5	4.96 ± 0.03	0.50	5	4.92 ± 0.02	0.45
	15	14.99 ± 0.02	0.40	15	14.98 ± 0.01	0.06
	25	24.89 ± 0.06	0.25	25	24.98 ± 0.02	0.08

* Average recovery from five determinations (n = 5).

high precision of the method. At the high concentration (25 μg mL^{-1}) both the within- and between-day RSDs were very low, which further indicates that the method is highly precise. The results are presented in Table 2.

APPLICATIONS

The developed HPLC method was applied to the determination of RTH in pharmaceutical formulations. The experimental results of the assay of RTH in Yutopar tablets and injections using the proposed HPLC method compare favorably with the officinal method of United States Pharmacopoeia [8] and are shown in Table 1. A statistical analysis of the results by Student's *t*- and *F*-tests showed no significant difference in the accuracy and precision between the proposed and officinal methods (Table 1).

CONCLUSIONS

In conclusion, a specific, rapid, and accurate HPLC method has been developed to estimate RTH in both pure and pharmaceutical-dosage forms.

No extra peaks were obtained even when sample chromatograms were run for longer periods. These observations suggest that the inactive ingredients or degradation products are absent. Also, there is no interference from common excipients like starch, talc, dextrose, etc., which are generally present in the formulation. The developed HPLC method can serve as an alternative to other methods for the determination of RTH in pharmaceutical formulations.

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REFERENCES

1. Harden, T.P., Peter, J.B., and Merkatz, I.R., *Obstet. Gynecol.*, 1980, vol. 56, p. 1.
2. Bari, V.R., Dhorda, U.J., and Sundaresan, M., *Indian Drugs*, 1999, vol. 36, p. 679.
3. Revanasiddappa, H.D., Manju, B., and Ramappa, P.G., *Anal. Sci.*, 1999, vol. 15, p. 661.
4. Revanasiddappa, H.D. and Manju, B., *J. AOAC Int.*, 2000, vol. 82, p. 1440.
5. Revanasiddappa, H.D. and Manju, B., *Farmaco*, 2001, vol. 56, p. 615.
6. Bakry, R., Sei-Walily, A.F.M., and Belal, S.F., *Anal. Lett.*, 1995, vol. 28, p. 2503.
7. Razak, O.A., *J. Pharm. Biomed. Anal.*, 1998, vol. 18, p. 359.
8. *United States Pharmacopoeia 24*, Rockville, 2000, p. 1493.
9. Gross, A.S., Brown, K.F., Baird-Lambert, J.A., and Nation, R.L., *J. Chromatogr.*, 1987, vol. 416, p. 400.
10. Wright, M.R., Axelson, J.E., Abbolt, F.S., Riggs, K.W., Van der Weyde, M.P., Taylor, S.M., McMorland, G.H., and Rurak, D.W., *J. Chromatogr.*, 1991, vol. 565, p. 225.