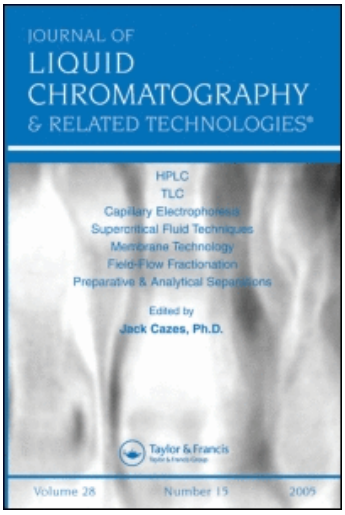

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SIMULTANEOUS DETERMINATION OF RITODRINE AND ISOXSUPRINE IN PHARMACEUTICAL PREPARATIONS USING LIQUID CHROMATOGRAPHY

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SIMULTANEOUS DETERMINATION OF RITODRINE AND ISOXSUPRINE IN PHARMACEUTICAL PREPARATIONS USING LIQUID CHROMATOGRAPHY

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□ *Simultaneous determination of two structurally related β_2 adrenergic receptor agonists namely, Ritodrine HCl (RTH) and Isoxsuprine HCl (ISP) was performed using reversed phase HPLC. The separation was performed on a C18 column adopting UV detection at 221 nm using a flow rate of 1 mL/min. The mobile phase consisted of acetonitrile: methanol: acid phosphate buffer (25:25:50) pH 4.5. The developed method was validated in terms of specificity, linearity, lower limit of quantification, lower limit of detection, precision, and accuracy. With the proposed method, satisfactory resolution between the two drugs was obtained (resolution factor= 2.76). Linear determination range of both drugs was 1–100 μg/mL. The proposed method was applied to the determination of RTH and ISP in synthetic mixtures and pharmaceutical samples. The method requires a minimum of sample handling and is rapid (within 3 min) and reproducible (RSD< 2.0%). The mean recoveries of the analytes in pharmaceutical preparations were in agreement with those obtained from reference methods, as revealed by statistical analysis of the obtained results using the Student's t-test and the variance ratio F-test.*

Keywords HPLC, isoxsuprine HCI, pharmaceutical preparations, ritodrine HCl

INTRODUCTION

Ritodrine Hydrochloride (RTH) and Isoxsuprine Hydrochloride (ISP) are Beta-adrenergic agonists, chemically they are named 1-(*p*-hydroxyphenyl)-2-(4-hydroxyphenethylamino) propan-1-ol hydrochloride and1-(4-hydroxyphenyl)-2-(1-methyl-2-phenoxyethylamino) propan-1-ol hydrochloride, respectively (Figure 1). RTH is a β_2 adrenergic agonist solely used as uterine relaxant.[1] It decreases uterine contractility and is used to arrest premature labor and as an emergency means of alleviating fetal asphyxia during labor.[2] ISP is a vasodilator that also stimulates ß-adrenergic receptors.[1] It causes direct

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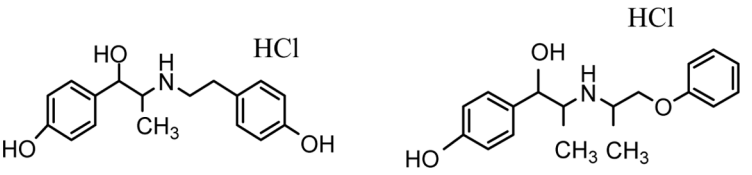

FIGURE 1 Structural formula of Ritodrine hydrochloride (a) and Isoxsuprine hydrochloride (b).

relaxation of vascular and uterine smooth muscle, and its vasodilating action is greater on the arteries supplying skeletal muscles than on those supplying skin.[2]

Both, B.P.[1] and U.S.P.[3] described HPLC methods for determination of RTH. The literature is enriched with several methods for the determination of RTH in pharmaceutical dosage forms including; UV spectrophotometry,[4] sequential injection spectrophotometry,[5] colorimetry,[6–13] spectrofluorimetry,[13] and HPLC.[14]

On the other hand, determination of ISP was described by B.P.[1] using a potentiometric acid base titration method and U.S.P.[3] using a UV spectrophotometric method.

Several methods were used for determination of ISP in pharmaceutical dosage forms including, sequential injection spectrophotometry,[15] colorimetry,[16,17] simple kinetic spectrophotometry,[18] spectrofluorimetry,[19] voltammetry,[20] and HPLC.[21]

The similar pharmacological action, along with the closely related structure of RTH and ISP, required the development of a sensitive, simple, and reliable method for their simultaneous determination in quality control laboratory of the producing company (Solvay). To the best of our knowledge, no previous methods were reported for their simultaneous determination. HPLC has been the most widely used separation technique to obtain specificity. In this work, reversed phase HPLC was applied for the simultaneous determination of RTH and ISP using UV detection. Adequate separation of two adjacent peaks of RTH and ISP within very short time (3 min), was obtained as revealed by the value of resolution factor ($R = 2.76$). The proposed method was successfully applied to their simultaneous determination in pharmaceutical preparations in a single chromatographic run.

EXPERIMENTAL

Chemicals

Ritodrine hydrochloride and isoxsuprine hydrochloride reference standards were kindly provided by Solvay Duphar (The Netherlands).

Pharmaceutical preparations containing RTH and ISP are Yutopar tablets (labeled to contain10 mg RTH per tablet) and Duvadilan tablets (labeled to contain 20 mg ISP per tablet), respectively, were purchased from the local pharmacy. Acetonitrile and methanol (of HPLC grade) were obtained from Merck (Darmstadt, Germany). Sodium dihydrogen phosphate (0.02 M solution) was obtained from Sigma (St. Louis, MO) Orthophosphoric acid for analysis (0.2 M solution) was obtained from Prolabo (Paris, France).

Apparatus

Separation was performed with a Perkin Elmer™ Series 200 Chromatograph equipped with a Rheodyne injector valve with a 20.0 μL loop and a UV/VIS detector operated at 221 nm. Total Chrom Workstation (Massachusetts, USA) was applied for data collecting and processing. Mobile phase was degassed using Merck solvent L-7612 degasser. A Consort P-901 pH-meter was used for pH measurements.

Column and Mobile Phase

Separation was achieved on a Hibar®, Lichrosorb® RP-18 (5 μm particle size) (150 mm × 4.6 mm i.d.) from Merck. The column was operated at ambient temperature. The mobile phase components were mixture of acetonitrile-methanol-20 mM sodium dihydrogen phosphate (25:25:50 v/v/v) the final pH of the mobile phase was adjusted to 4.5 using 0.2 M orthophosphoric acid, at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45 μm membrane filter (Millipore, Ireland).

Sample Preparation and Procedures

Stock solutions of ritodrine and isoxuprine of 1.0 mg/mL were prepared in methanol and were further diluted with the same solvent to obtain another working solution (0.1 mg/mL). All the solutions were kept at 4°C in a refrigerator and they were stable for at least 1 wk.

Construction of Calibration Graphs

To a series of 10 mL, volumetric flasks with increasing volumes of the working standard solutions of ISP and RTH were quantitatively transferred, in order to contain the drug within the concentration range of 1.0–100.0 μg/mL, after being diluted to 10.0 mL with the mobile phase. Injection into the HPLC was performed at ambient temperature (25°C).

Twenty μL aliquots were injected (in triplicate) and the calibration curves were constructed by plotting the peak area against the final concentration of both drugs. Alternatively, the corresponding regression equations were derived.

Analysis of the Pharmaceutical Preparations

For both Yutopar tablets and Duvadilan tablets, 10 tablets were finely powdered after weighing, and a portion of the tablet powder equivalent to 100 mg active substance was transferred quantitatively into a small beaker and suspended in 20 mL methanol. The drug was extracted with 3×15 mL portions of methanol. After sonication of each portion for 10 minutes, the extracts were transferred quantitatively into 100 mL measuring flask and the flask was made up to volume with methanol to give a stock solution of 1.0 mg/mL. The final solution was centrifuged ($4000 \times g$) for 15 min, and filtered. For both prepared solutions of tablets, further dilutions were made as appropriate with methanol then proceed as described under "Construction of Calibration graphs." All samples were filtered through 0.45 μm sample filters (RC 25, Sartorius AG, Goettingen, Germany) prior to injection into the HPLC system. "The nominal content of the pharmaceutical preparation were calculated using the corresponding regression equation."

RESULTS AND DISCUSSION

Optimization of the Chromatographic Performance and System Suitability

The chromatographic experimental variables affecting the peak shape and retention of both drugs RTH and ISP were carefully studied in order to obtain the most suitable chromatographic conditions that provide the least peak broadening and maximum symmetry as well as satisfactory resolution of both drugs (Figure 2). Optimization of separation was achieved by measuring the highest number of theoretical plates and the best resolution factor.

The UV absorption spectrum of RTH and ISP in methanol showed different maxima (Figure 3). The two drugs were found to have seriously overlapped spectra. The simultaneous determination of the two drugs by conventional, derivative and derivative ratio spectrophotometry is hindered by strong spectral overlap throughout the wavelength range. Their separation was achieved by application of reversed phase HPLC adopting UV

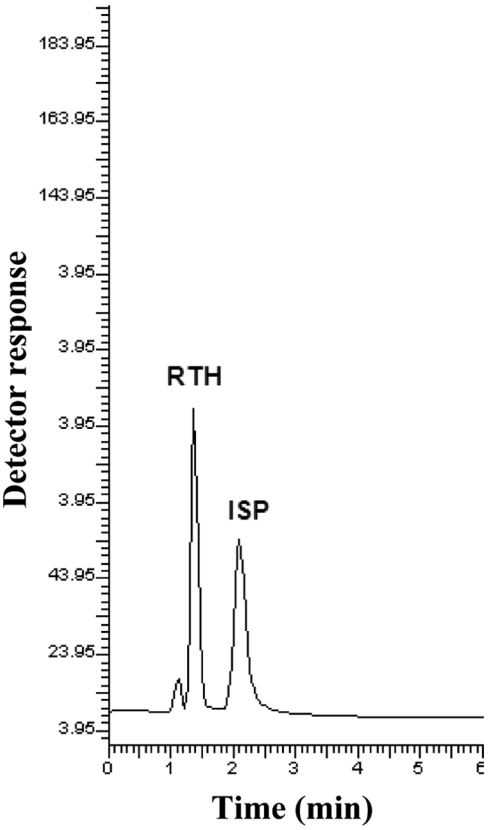

FIGURE 2 Typical chromatogram of RTH (15 μg/mL) and ISP (15 μg/mL) under the optimum chromatographic conditions [C18 Column, 5 μm (150 mm × 4.6 mm i.d.)] operated at ambient temperature, the mobile phase components were acetonitrile-methanol-sodium dihydrogen phosphate buffer (pH = 4.5) (25:25:50), flow rate of 1.0 mL/min and UV detection at 221 nm.

detection at 221 nm to assure reasonable sensitivity for both RTH and ISP, using a mobile phase composed of acetonitrile-methanol-20 mM sodium dihydrogen phosphate (25:25:50) with pH 4.5 adjusted with 0.2 M orthophosphoric acid at flow rate 1.0 mL/min.

Several modifications in the mobile phase composition were performed in order to study the possibilities of improving the efficacy of the chromatographic system and resolution of the eluted peaks. These modifications included changing the proportions of the mobile phase components, the pH and the flow rate. Mass distribution ratio (D_m), relative retention time (R_r), number of theoretical plates (N), and resolution factor (R) were measured *versus* each variable.

Different ratios were investigated and the optimum ratio was found to be 25:25:50, acetonitrile: methanol: sodium dihydrogen phosphate buffer

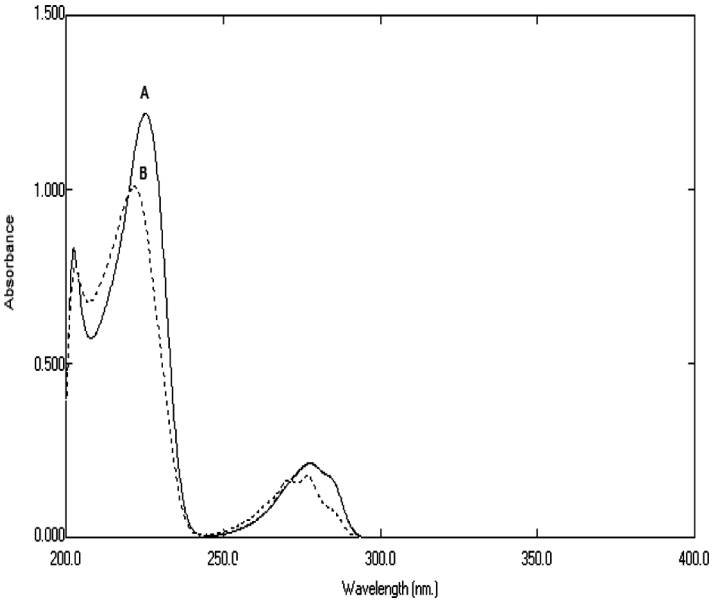

FIGURE 3 Absorption spectra of RTH (10 μg/mL) (A) and ISP (10 μg/mL) (B) in methanol.

as revealed by the values of resolution factor (R) along with the number of theoretical plates (N) for both solutes as shown in Table 1.

However, RTH and ISP have octanol/water partition coefficient (Log P) values of 1.54 and 2.40,[22] respectively. This difference in lipophilicity was expressed as difference in their elution order.

TABLE 1 Effect of the Ratio of Mobile Phase Components on the Chromatographic Performance for the Simultaneous HPLC Determination of ISP and RTH

Ratio (Acetonitrile: methanol:acid phosphate buffer)	D_m		R_r	N		R
	RTH	*ISP*		*RTH*	*ISP*	
30:30:40	0.23	0.60	2.67	866	908	1.76
27.5:27.5:45	0.24	0.74	3.10	936	962	2.10
25:25:50	0.25	0.98	4.22	866	948	2.76
30:20:50	0.28	1.25	4.55	577	450	2.82
20:30:50	0.28	1.30	4.73	599	519	2.80
22.5:22.5:55*	0.29	1.39	4.75	973	831	4.10
20:20:60**	0.36	2.07	5.80	889	716	4.90

D_m is the mass distribution ratio [also known as the capacity factor (K') or the retention factor (K)].
R_r is the relative retention times [also known as the unadjusted relative retention (r_G)].
N is the number of theoretical plates for RTH and ISP.
R is the resolution factor.
*,**Band broadening (especially in case of ISP) and increased retention for both drugs.

TABLE 2 Effect of pH of the Mobile Phase on the Chromatographic Performance for the Simultaneous HPLC Determination of ISP and RTH

	D_m			N		
pH	*RTH*	*ISP*	R_r	*RTH*	*ISP*	*R*
3.5	0.25	0.88	3.80	856	866	2.37
4.5	0.25	0.98	4.22	866	948	2.76
5.5	0.24	0.95	4.00	849	643	2.70
6.0	Bad separation for the two drugs					

The pH of mobile phase was changed over the range of 3.5–6 using orthophosphoric acid. The study revealed that, changing the pH value did not greatly affect the separation of the two drugs. Efficient separation was achieved over the pH range 3.5–5.5, after which overlapping of the two peaks was obtained. Selected optimum pH value was 4.5, as it provides the best peak symmetry for both drugs as revealed by the values of number of theoretical plates (Table 2). However, the pK_a values of ISP are 8.0 and 9.8 and of RTH is 9.0;[22] thus, they still in the unionized form up to pH 8.0, and the differences in their elution is expressed only as a difference in their lipophilicity.

The flow rate was changed over the range of 0.6–1.4 mL/min. Flow rate of 1.0 mL/min was optimum for efficient separation in a reasonable time.

Method Validation

The proposed method was validated using the following criteria: sensitivity, linearity, intraday and interday precision, accuracy, specificity, and robustness. The sensitivity of the proposed method was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) according to ICH guidelines (ICH Topic QR).[23]

LOD was defined as:

$$3.3 \times \sigma/\mathrm{S}$$

and LOQ was:

$$10 \times \sigma/\mathrm{S}$$

where σ is the standard deviation of the y-intercept of the regression lines, (the standard deviation of the response) and S is the slope of the calibration curve. Linearity was evaluated by calculation of the regression equations over the ranges given in Table 3. The table also shows the detection limits, slopes, intercepts, and correlation coefficients obtained by linear least squares treatment of the results, standard deviation of slopes (S_b),

TABLE 3 Analytical Data for the Simultaneous HPLC Determination of RTH and ISP

Parameters	RTH	ISP
Concentration range (μg/mL)	1–100	1–100
Correlation coefficient	0.9999	0.9999
Slope	70.90	56.09
Intercept	90.53	85.07
LOD (μg/mL)	0.55	0.69
LOQ (μg/mL)	1.65	2.10
$S_{y/x}$	18.79	18.86
S_a	11.72	11.76
S_b	0.21	0.21
% RSD	0.63	0.57
% Er	0.20	0.18

[a]Limit of detection.
[b]Limit of quantitation.
[c]Percentage relative standard deviation for ten replicate samples.
[d]Percentage relative error for ten replicate samples.

and intercepts (S_a) on the ordinates, and standard deviation of the residuals ($S_{y/x}$).[24]

The intraday precision and accuracy were assessed by analyzing three samples of each concentration of RTH and ISP in their authentic samples using three different concentrations in one day. The interday precision and accuracy were assessed over three successive days by analyzing three samples of each concentration of RTH and ISP in their authentic samples using three different concentrations. The obtained results for both the intra- and inter-day precision and accuracy are abridged in Tables 4 and 5, respectively. As shown in these tables (4 and 5), the repeatability and reproducibility in the proposed method were fairly good, as indicated by the small values of standard deviation (SD), relative standard deviation (RSD), and error (% Er). The robustness of the method is demonstrated by the consistency of the separation efficiency with minor changes in experimental variables, such as the percentage of the organic solvent in the mobile phase over the range 45–55% and pH of the mobile phase from 3 to 6.5. These minor changes that might reasonably be expected to take place during the course of the operation of the method did not adversely affect the separation.

Assay of Dosage Forms

The applicability of the proposed method was tested by determination of RTH and ISP in their dosage forms (Yutopar tablets for RTH) and (Duvadilan tablets for ISP). Excellent recoveries and SD values were

TABLE 4 Evaluation of the Accuracy and Precision Data of the Proposed HPLC Method for the Simultaneous Determination of RTH

Amount Added (μg/mL)	Found (%)		
	40	50	60
Intra-day			
	100.47	100.84	99.47
	100.10	100.36	99.72
	100.23	100.14	100.82
$\bar{X} \pm SD$	100.27 ± 0.19	100.44 ± 0.35	99.67 ± 0.18
% RSD	0.19	0.35	0.18
% Er	0.11	0.20	0.11
Inter-day			
	100.81	100.84	99.47
μg/mL	99.96	100.28	99.68
	100.17	100.06	99.95
$\bar{X} \pm SD$	100.20 ± 0.26	100.39 ± 0.40	99.70 ± 0.24
% RSD	0.26	0.40	0.24
% Er	0.15	0.23	0.14

(*)Each result is the average of three separate determinations.

obtained, as illustrated in Table 6. Common tablet excipients, such as sucrose, lactose, starch, and talc powder did not interfere with the assay. Statistical analysis of the results obtained by the proposed method and those given by the reference spectrofluorimetric[19] and spectrophotometric[7] methods for ISP and RTH, respectively, was performed using the student's

TABLE 5 Evaluation of the Accuracy and Precision Data of the Proposed Simultaneous HPLC Method for the Determination of ISP

Amount Added (μg/mL)	Found (%)*		
	40	50	60
Intra-day			
	99.61	100.02	99.39
	99.95	99.66	99.53
	100.04	99.39	99.82
$\bar{X} \pm SD$	99.87 ± 0.23	99.69 ± 0.31	99.58 ± 0.35
% RSD	0.23	0.31	0.35
% Er	0.13	0.16	0.20
Inter-day			
	99.61	100.02	99.39
	99.82	101.00	99.65
	100.13	99.86	99.97
$\bar{X} \pm SD$	99.85 ± 0.26	100.63 ± 0.35	99.67 ± 0.29
% RSD	0.26	0.35	0.29
% Er	0.15	0.20	0.17

(*)Each result is the average of three separate experiments.

TABLE 6 Assay Results for the Determination of RTH and ISP in Pharmaceutical Dosage Forms by the Proposed HPLC Method and Comparison Methods

	Found[a] of Drugs (%)		
Dosage Form	Proposed Method		Comparison Method[7]
Yutopar® tablets[b1]	99.40		100.5
	99.88		101.0
	100.40		100.5
Mean ($\bar{X}$) ± S.D.	99.89 ± 0.50		100.67 ± 0.29
t		1.76 (2.78)[c]	
F		3.59 (19.00)	
	Proposed Method		Comparison Method[19]
Duvadilan®tablets[b2]	99.12		99.40
	100.43		99.90
	99.75		100.54
Mean ($\bar{X}$) ± S.D.	99.77 ± 0.66		99.95 ± 0.57
t		0.42 (2.78)	
F		1.29 (19.00)	

[a]The average of three separate determinations.

[b1]Yutopar tablets labeled to contain 10 mg RTH per tablet manufactured by Pharco pharmaceutical, Alexandria, Egypt; batch number 198.

[b2]Duvadilan tablets labeled to contain 20 mg ISP per tablet manufactured by Pharco pharmaceutical, Alexandria, Egypt; batch number 269.

[c]The figures between parentheses are the tabulated values of *t* and *F* at $P = 0.05$.[24]

t test and the variance ratio *F* test. The calculated values did not exceed the theoretical ones, indicating that no significant difference between the performance of the compared methods regarding accuracy and precision.[24]

CONCLUSION

A rapid and simple method was developed for the simultaneous determination of RTH and ISP in a short turnover time (less than 3 minuets). The proposed method was applied for the determination of the two drugs either in pure forms or in their tablet dosage forms. The good validation criteria of the proposed method allow its use in quality control laboratories using a simple chromatographic system in a single chromatographic run.

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