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Fluorimetric and spectrophotometric determination of ritodrine hydrochloride in bulk and pharmaceutical formulations

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Abstract

Two simple sensitive and accurate methods have been developed for the determination of ritodrine hydrochloride in bulk and pharmaceutical preparations. The first method involves the direct measurement of the native fluorescence of the drug in the concentration range 4–9 μg ml^{-1}, the second method is based on the oxidation of ritodrine HCl with cerium(IV) followed either by spectrophotometric or fluorimetric measurement in the concentration ranges 0.5–1.0 and 0.05–0.1 μg ml^{-1}, respectively. The interference of various formulation excipients was examined. The reliability of the proposed methods was checked at three different concentrations; the standard deviation varied from 2.7×10^{-3}–0.109. The described methods have been applied to the determination of ritodrine HCl in tablets and ampoules. The assay results showed insignificant difference with those of the official USP 23 HPLC method.

Keywords: Cerium; Pharmaceuticals; Ritodrine; Spectrofluorimetry; Spectrophotometry

1. Introduction

Ritodrine hydrochloride, erythro-*p*-hydroxy-α-[1-[[*p*-hydroxyphenyl)ethyl]amino]ethyl]hydrochloride, is a direct-acting sympathomimetic agent with predominantly β-adrenergic activity and selective action on β_2 receptors. It decreases uterine contractility and is used to arrest premature labour and as an emergency means of alleviating foetal asphyxia during labour [1].

The drug, its tablets and injection are official in the USP 23 that describes an HPLC method for their determination [2].

The literature reveals only two papers concerning the determination of ritodrine in pharmaceutical dosage forms, through nitrosation or nitration with subsequent complexes formation [3,4]. In biological fluids, it has been determined by HPLC [5] and GLC methods [6].

Cerium(IV) as an oxidising agent has been used successfully for the determination of many drugs such as paracetamol, phenothiazines and penicillamine [7–10].

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In this paper ritodrine HCl was analysed through the measurement of its native fluorescence, and via its oxidation with Ce(IV) with subsequent either spectrophotometric or fluorimetric measurement.

2. Experimental

2.1. Apparatus

Absorbance was measured on a Perkin-Elmer double beam UV-VIS spectrophotometer model 550S.

The fluorimetric measurements were performed on a Perkin-Elmer 650-10S fluorescence spectrometer.

2.2. Material and reagent

Ritodrin HCl authentic was supplied by Solvay Duphar (The Netherlands), the content was determined by the USP 23 method and was found to be 99.5%.

Yutopar® tablets and ampoules (Pharco Pharmaceuticals, Alexandria, Egypt) under licence from Solvay Duphar (The Netherlands) labeled to contain 10 mg ritodrine HCl per tablet or per 1 ml of ampoule.

Cerium(IV) solutions were prepared by dissolving the appropriate quantities of ceric ammonium sulphate (Prolabo, France) in 2 M H_2SO_4 (E. Merck, Darmstadt, Germany), so as to obtain 10^{-3} M (solution A) and 10^{-4} M (solution B) solutions.

Silver oxide was prepared by mixing equimolar quantities of $AgNO_3$ and NaOH solutions. The brown precipitate was filtered, washed with water and dried for 5 h in a 110°C oven.

2.3. Ritodrine HCl standard solutions

An accurately weighed quantity of ritodrine HCl (50 mg) was dissolved in distilled water in a 100 ml calibrated flask. Aliquots of the above prepared stock solution were further diluted to obtain 50 μg ml^{-1} (standard solution A), 5 μg ml^{-1}, (standard solution B) and 0.5 μg ml^{-1}, (standard solution C) standard solutions.

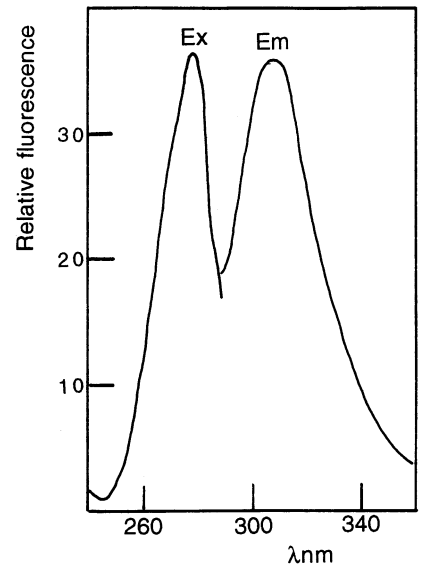

Fig. 1. Excitation (Ex) and emission (Em) spectra of ritodrine HCl.

2.4. Sample solutions

2.4.1. Tablets

Twenty tablets were weighed and powered. An accurately weighed quantity equivalent to 2.5 mg ritodrine HCl was transferred into a 50 ml volumetric flask. The flask was half filled with distilled

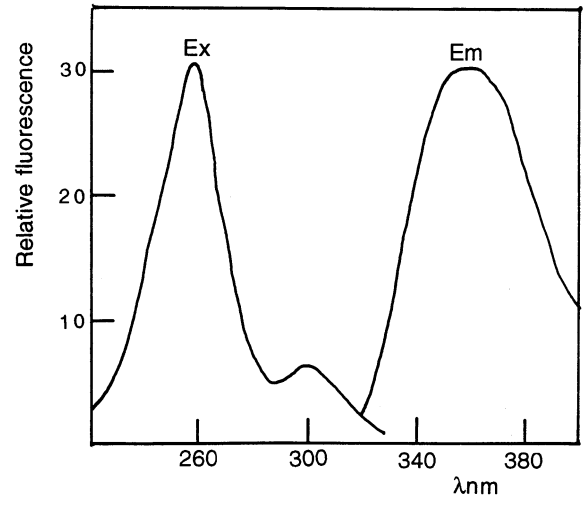

Fig. 2. Excitation (Ex) and emission (Em) spectra of cerium(III).

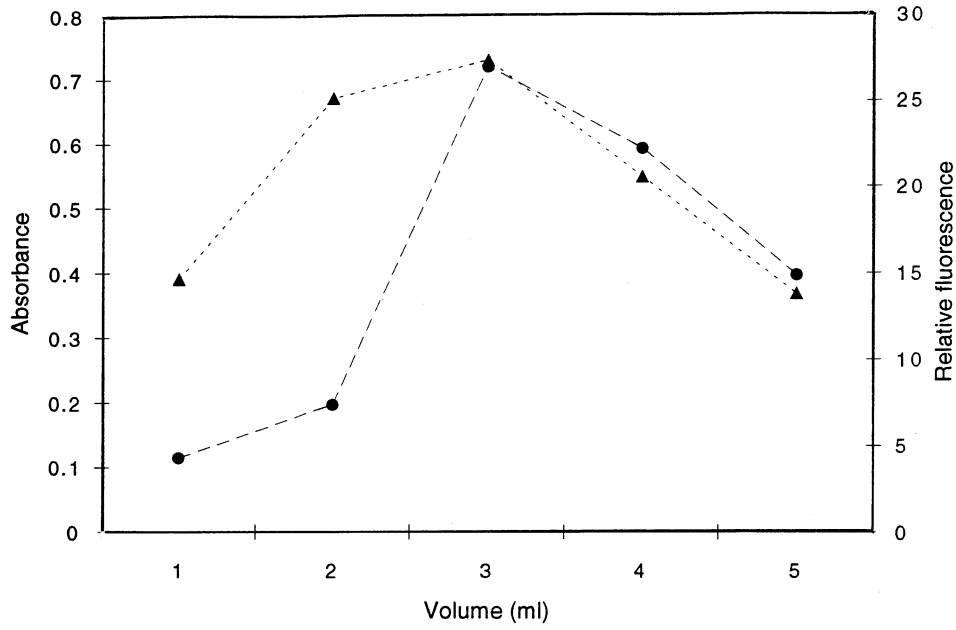

Fig. 3. Effect of volume of Ce(IV) on the oxidation reaction; (using 10^{-3} and 10^{-4} M Ce(IV) solution for the spectrophotometric and fluorimetric measurement, respectively) (▲ absorbance, ● relative fluorescence).

water, shaken for 15 min. The tablets extract was filtered into a separatory funnel and the residue was washed twice with distilled water. About 5 mg Ag_2O was added to the combined wash and filtrate and the liberated ritodrine base was extracted with chloroform. The chloroformic extract was filtered and evaporated using a rotary evaporator. The oily residue was dissolved in about 20 ml 2 M HCl and the solution was diluted to 100 ml with distilled water. The final solution was diluted to suit the application of the procedures.

2.4.2. Ampoules

The content of ten ampoules was mixed. A volume equivalent to 10 mg ritodrine HCl was transferred to a 100 ml volumetric flask. The flask was completed to mark with distilled water. Suitable dilution was made to fit the applicable concentration range.

2.5. General procedure

2.5.1. For the direct fluorimetric method

The relative fluorescence intensity of ritodrine HCl aqueous solution was measured at $\lambda_{em} = 308$ nm with $\lambda_{ex} = 278$ nm.

2.5.2. For the oxidation with cerium(IV)

An aliquot of ritodrine HCl aqueous solution was transferred into a 10 ml volumetric flask, to which 3 ml cerium(IV) solution was added. The flask was heated in a thermostated water-bath at 100°C for 20 min, cooled and diluted to the mark with distilled water.

2.5.2.1. For the spectrophotometric measurement. The absorbance of the blank solution was measured at 317 nm against the experimental solution.

2.5.2.2. For the fluorimetric measurement. The relative fluorescence was measured at $\lambda_{em} = 357$ nm with $\lambda_{ex} = 257$ nm.

2.6. Construction of calibration graphs

2.6.1. For the direct fluorimetric method

To a set of 10 ml volumetric flasks, volumes of standard solution A ranging from 0.8–1.8 ml (in

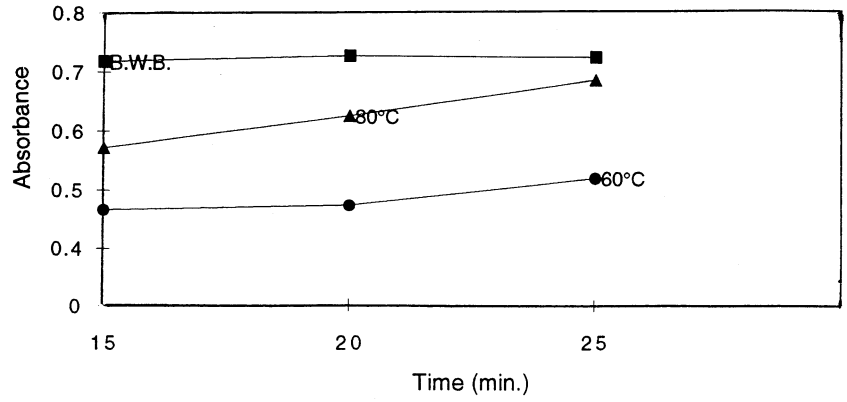

Fig. 4. Effect of temperature and heating time on the oxidation reaction.

0.2 ml increment) were transferred. The volume was completed with distilled water and the relative fluorescence intensity was measured at the specified excitation and emission wavelengths.

2.6.2. For the oxidation with cerium(IV)

The general procedure was followed, using aliquots of 1–2 ml (in 0.2 ml increment) from standard solution B and 3 ml of Ce(IV) solution A for the spectrophotometric procedure. Whereas for the fluorimetric procedure, 1–2 ml (in 0.2 ml increment) from standard solution C and 3 ml of Ce(IV) solution B were used.

3. Results and discussion

Aqueous solution of ritodrine HCl fluoresce at 308 nm with an excitation wavelength at 278 nm (Fig. 1). The fluorescence is destroyed by alkalinization, remained unchanged by acidification.

Cerium(IV) is a powerful oxidising agent. It's acidic solution is yellow in colour while in its reduced form it is colourless and possesses a native fluorescence (Fig. 2). Ritodrine is oxidised with cerium(IV) and the spectrophotometric analysis was based on monitoring Ce(IV) equivalent to ritodrine through the absorbance measurement of the reagent blank against the reaction product solution containing excess unconsumed Ce(IV). Due to the fluorescent nature of the formed cerous ion, the quantitative method has been extended to be measured fluorimetrically.

3.1. Effect of experimental variables

Investigations were carried out to establish the most favourable conditions for the oxidation. The reaction was optimized using 3 ml of Ce(IV) (10^{-3} and 10^{-4} M for the spectrophotometric and fluorimetric measurements, respectively) solution in 2 M H_2SO_4 and by heating in a boiling water-bath for 20 min (Figs. 3 and 4).

3.2. Identification of the reaction product

It was reported that *p*-hydroxy benzaldhyde is the main decomposition product of ritodrine infusion due to photooxidation sensitized by vitamin B_2 [11]. Identification tests were carried out for the reaction product of ritodrine with Ce(IV) under the previously mentioned conditions (TLC, HPLC and IR). The chromatographic studies were based on the comparison of the TLC plates and HPLC chromatograms of the reaction product with standard *p*-hydroxy benzaldhyde and *p*-hydroxy benzoic acid.

For the TLC, plates covered with silica and eluting solvents composed of chloroform, methanol in different ratios were used. The

Table 1
Optical characteristics, statistical analysis for calibration graphs and validation data for the determination of ritodrine

Item	Direct fluorimetric method	Oxidation with Ce(IV)	
		Spectrophotometric measurement	Fluorimetric measurement
Concentration range (μg ml^{-1})	4–9	0.5–1.0	5×10^{-2}
λ_{max} (nm)	—	317	
$\lambda_{excitation}$ (nm)	278	—	257
$\lambda_{emission}$ (nm)	308	—	357
Regression equation[a]			
Intercept (a)	0.819	−0.148	−2.124
Variance of intercept (S_a^2)	0.453	8.4×10^{-5}	0.143
Slope (b)	6.671	0.873	0.293
Variance around slope (S_b^2)	1×10^{-2}	1.4×10^{-4}	2.4×10^{-5}
Correlation coefficient (r)	0.9996	0.9996	0.9994
Variance (S_{yx}^2)	0.176	2.5×10^{-5}	4.2×10^{-2}
Limit of detection (μg ml^{-1})	0.156	1.4×10^{-2}	1.7×10^{-3}
Relative sensitivity[b]	89.655	8.046	1.000
Precision[c]			
Standard deviation	0.109	6.5×10^{-3}	2.7×10^{-3}
Standard analytical error	4.9×10^{-2}	2.9×10^{-3}	1.2×10^{-3}
Limit of error (95% confidence limit)	0.136	8.1×10^{-3}	3.3×10^{-3}

[a] Ritodrine HCI concentration expressed in terms of ng ml^{-1} for the fluorimetric measurement after oxidation, in term of μg ml^{-1} for the direct fluorimetric and spectrophotometric methods.
[b] Calculated with respect to the fluorimetric measurement after oxidation.
[c] Calculated using the average recoveries obtained from three different concentration levels in five replicates.

HPLC procedure used the USP XXII mobile phase [12] and diode-array system for detection.

The results obtained confirm that there is no cleavage in the ritodrine molecule after oxidation with Ce(IV) in acidic medium and that the oxidation product is neither *p*-hydroxy benzaldhyde nor *p*-hydroxy benzoic acid.

The IR spectrum (KBr) of a purified specimen separated from the reaction product revealed a stong absorption band at 1646 cm^{-1}, which can be attributed to a C=O group (due to oxidation of ritodrine alcoholic OH). The appearance of the keto group at that relatively low frequency was attributed to the hydrogen bonding with the amino group on the adjacent carbon.

From the above results, most probably the reaction product of ritodrine with Ce(IV) in acidic medium is the amino ketone, 1-(4-hydroxyphenyl)-2-[[2-(4-hydroxyphenyl)ethyl]amino]-1-propanone which is the related substance mentioned in the USP XXII monograph [12].

Further study will be done.

3.3. Validation of the methods

By using the above mentioned fluorimetric and spectrophotometric procedures linear regression equations were obtained over the concentration ranges stated in Table 1. The good linearity of the calibration graphs is clearly evident from the values of the variances around the slopes (Table 1).

The detection limits varies from 1.7×10^{-3} to 0.156 μg ml^{-1} (Table 1).

In order to evaluate the precision of the proposed methods, standard solutions containing three different concentration of ritodrine HCl were reacted, five absorbance or fluorescence measurements were made on each reaction product formed according to the recommended general procedure (or ritodrine HCl aqueous solution for the direct fluorimetric method). The

Table 2
Assay results of ritodrine HCl in pharmaceutical preparations using the proposed methods

Item	Direct fluorimetric method	Oxidation with Ce(IV)		USP 23 (HPLC)
		Spectrophotometric measurement	Fluorimetric measurement	
Yutopar tablets				
Recovery (%)[a]	95.75	95.45	95.84	95.91
± SD	0.85	0.75	0.84	0.39
t	0.38	1.22	0.17	
F	4.74	3.68	4.69	
Standard addition[a]				
Recovery (%)	99.90	100.14	99.60	
± SD	0.88	0.30	1.07	
Yutopar ampoules				
Recovery (%)[a]	99.46	100.06	99.90	99.63
± SD	0.63	0.90	0.89	0.39
t	0.51	0.99	0.63	
F	2.70	5.42	5.30	
Standard addition[a]				
Recovery (%)	99.57	99.24	100.23	
± SD	0.39	0.44	0.66	

[a] Each value is the mean of five measurements.
Theoretical values for t and F at $p = 0.05$ are 2.31 and 6.39, respectively.

overall standard deviation, standard analytical error and limit of error (95% confidence limit) are listed in Table 1.

The interference of the Yutopar® tablet's excepients, lactose, yellow iron oxide, povidone, maize starch and magnesium stearate, was studied. Lactose and povidone interfered in the oxidative method. This interference has been removed by liberating ritodrine base (using Ag_2O) [13] then extracting it from the tablets with $CHCl_3$.

Ritodrine ampoule contains sodium metabisulphite (Pharco Pharmaceuticals, Alexandria, Egypt, personal communication). This antioxidant showed no interference in the ampoule assay, however, positive interference was detected (for the oxidative method) when the concentration of sodium metabisulphite in the final reaction mixture is ≥ 1 μg ml^{-1}.

3.4. Commercial preparations analysis

The applicability of the proposed methods was tested by the determination of ritodrine HCl in its commercial tablets and ampoules, and through calculating the recovered percentage from the added drug (standard addition technique).

The results obtained were compared statistically with those obtained from the USP 23 HPLC method (Table 2), using Dunnett's procedure [14]. The test ensures that there is no significant difference between the percentage recoveries in the determination of ritodrine (the calculated significant difference (d') = 1.217 and 1.215 for the assay of tablets and ampoules, respectively).

4. Conclusion

The proposed methods are accurate, simple and can be used in the routine analysis for ritodrine HCl.

The direct fluorimetric procedure is the most selective one, while the oxidation method

followed by fluorimetric measurement is the most sensitive and could be applied for the assay of ritodrine in biological fluids.

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References

[1] E.F. Reynolds, Martindale The extra Pharmacopoeia, Royal Pharmaceutical Society, London, 31 edn., 1996, pp. 1589–1590.
[2] United States Pharmacopoeia, 23 Revision, United States Pharmacopoeial convention, Board of Trustees, 1995, 1389–1390.
[3] R.S. Bakry, A.F.M. ElWalily, S.F. Belal, Anal. Lett. 29 (3) (1996) 409–422.
[4] R.S. Bakry, A.F.M. El Walily, S.F. Belal, Anal. Lett. 28 (14) (1995) 2503–2519.
[5] A.S. Gross, K.F. Brown, J.A. Baird-Lambert, R.L. Nation, J. Chromatogr. Biomed. Appl. 60 (2(J. Chromatogr. (416)) (1987) 400–408.
[6] M.R. Wright, J.E. Axelson, F.S. Abbolt, K.W. Riggs, M.P. Vander Weyde, S.M. Taylor, G.H. McMorland, D.W. Rurak, J. Chromatogr. Biomed. Appl. 103 (1–2(J. Chromatogr. 565)) (1991) 225–236.
[7] S.M. Sultan, I.Z. Alzamil, A.M.A. Alrahman, S.A. Altamrah, Y. Asha, Analyst 111 (1986) 919–921.
[8] J.M. Calatayud, Anal. Chem. Acta 264 (1992) 283–289.
[9] T. Perez-Rinz, C. Martinez-Lozano, V. Tomas, C. Sidrach de Cardona, Talanta 40 (1993) 1361–1365.
[10] S.A. Abdel Fattah, Bull. Fac. Pharm., Cairo Univ. 34 (1996) 1–4.
[11] Y. Alkira, H. Taleshi, M. Yutaka, Byoin Yakugaku 13 (1987) 298–303.
[12] United States Pharmacopoeia, 22 Revision, United States Pharmacopoeial Convention, Mack, Easton, PA, 1990, p. 1230.
[13] M.M. Amer, A.M. Taha, S.R. El-Shabouri, P.Y. Khashaba, J. Assoc. Off. Anal. Chem. 65 (4) (1982) 894–900.
[14] R.G.D. Steel, J.H. Torrie, Principles and Procedures of Statistics, 2, McGraw-Hill, New York, 1980, pp. 111–112.