Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008, Pages 26-35 藥物食品分析 第十六卷 第二期

Spectrophotometric Methods for Determination of Ritodrine in Bulk and in Pharmaceutical Dosage Forms

MOHAMED ABD EL-GHAFFAR, DINA EL-SHERBINY*, DALIA EL-WASSEEF AND SAADIA EL-ASHRY

Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

(Received: May 7, 2007; Accepted: September 4, 2007)

ABSTRACT

Three simple and sensitive spectrophotometric methods are described for determination of ritodrine hydrochloride (RTH) either in pure form or dosage forms. The first method was based on the reaction with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) in borate buffer of pH 8.0 producing a yellow colored product measured at 392 nm. The second and third methods were based on RTH oxidation with either 1,10-phenanthroline or Folin-Ciocalteu reagents producing red and blue chromogens which were measured at 510 and 760 nm, respectively. Beer's law was obeyed in the ranges of, 2-16, 0.2-2, 0.8-12 μg/mL with the minimum detectable values of 0.1, 0.05, 0.14 μg/mL for the three methods, respectively. Different experimental parameters affecting the development and stability of colored products were carefully studied and optimized. The effect of different foreign matters and sensitizers on the color development in the three proposed methods was also studied. The proposed methods were successfully applied to the determination of RTH in its dosage forms. The obtained percentage recoveries were 99.73 ± 0.72, 99.88 ± 1.2, 99.97 ± 0.42 for the three proposed methods, respectively. The obtained results were statistically validated and compared with those obtained with a reference method. Proposals of the reactions mechanisms were presented.

Key words: ritdorine HCl, spectrophotometry, NBD-Cl, 1,10-phenanthroline, Folin-Ciocalteu, dosage forms

INTRODUCTION

Ritodrine hydrochloride (RTH), *erythro*-1-(*p*-hydroxyphenyl)-2-(4-hydroxyphenethylamino) propan-1-ol hydrochloride is a β_2 adrenergic agonist solely used as uterine relaxant[1](Figure 1). RTH 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 labor and to alleviate fetal asphyxia during labor in case of emergency[2]. The British pharmacopoeia[1] recommended a HPLC method for its assay either in raw materials or pharmaceutical preparations.

The literature is enriched with several methods for determination of RTH in pharmaceutical dosage forms including UV spectrophotometry[3], sequential injection spectrophotometry[4], colorimetry[5-12], and spectrofluorometry[12]. Meanwhile, the assay of RTH in biological samples has been reported using HPLC[13-15] and GC-MS[16,17] methods. The therapeutic importance of RTH required the development of sensitive, simple and reliable methods for industrial quality control of its pharmaceutical preparations. Some of the reported methods for RTH determination are either laborious[13-17] or insufficiently sensitive[3,5,8-10]. In the present work three simple and sensitive colorimetric methods have been developed and validated for this purpose. The three methods are based on the reaction with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) (method 1) or application of the oxidation of ritodrine with either Fe(III)-1,10-phenanthroline (FPL) (method 2) and Folin-Ciocalteu (F-C) (method 3).

NBD-Cl is a labeling reagent specific for primary and secondary amines. Several pharmaceutical compounds have been determined through this approach, such as skeletal muscle relaxant and antihistaminic drugs[18], oxicams[19], trimetazidine[20], lisinopril[21], fenoterol[22], β-blockers[23] and tramadol[24].

1,10-Phenanthroline (FPL) is an oxidizing agent for many drugs with reducing properties particularly

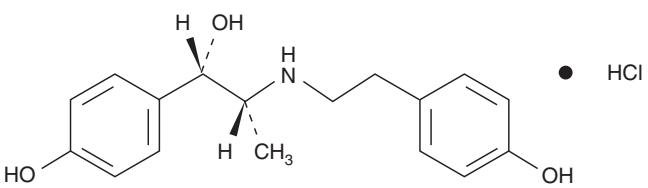


Figure 1. Structural formula of ritodrine hydrochloride.

* Author for correspondence. Tel: +20502247496;
Fax: +20502247496; E-mail: dina_elsherbiny@mans.edu.eg

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

phenolic compounds, in which dark red orange chelate is formed. This reaction was used in the determination of many drug substances such as ascorbic acid[25], amlodipine[26], amoxycillin, ciprofloxacin and piroxicam[27], and acetaminophen[28].

Folin-Ciocalteu reagent (F-C) is specially used for the determination of many phenolic compounds utilizing its liability to be reduced into blue colored product. Many drug substances such as salbutamol[29], minocycline[30], diclofenac[31], trimetazidine[32], acyclovir[33], methotrexate[34], omeprazole[35], sulphinpyrazone[36], and gliclazide[37], have been determined on this basis. The structural features of RTH allowed the use of the three reagents for its assay. The three proposed methods have been successfully applied to the assay of RTH in pharmaceutical dosage forms.

MATERIALS AND METHODS

I. *Materials and Reagents*

All materials and reagents used were of analytical reagent grade. Ritodrine hydrochloride (RTH) reference standard was kindly provided by Solvay Duphar (The Netherlands). Pharmaceutical preparations containing RTH, Yutopar tablets labeled to contain 10 mg RTH per tablet and Yutopar ampoules labeled to contain 10 mg RTH per 1 mL of ampoule, were purchased from a local pharmacy.

4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole reagent (NBD-Cl) (Sigma, St. Louis, USA) was prepared as 0.05% (w/v) ethanolic solution.

Ferric phenanthroline reagent (FPL) was prepared by mixing 0.193 gm of the 1,10-phenanthroline (Sigma-Aldrich, St. Louis, USA) with 2 mL of 1.0 M HCl and 0.16 gm of ferric ammonium sulfate dodecahydrate (Sigma, St. Louis, USA) and diluted with distilled water to 100 mL[27].

Folin-Ciocalteu reagent (F-C) (2.0 N solution) (Sigma Aldrich, Buchs, Switzerland)

Hydrochloric acid (Prolabo, France)

Borate buffer, 0.2 M (pH 8.0)

10% Na_2CO_3 (w/v) solution was prepared in distilled water.

Sodium dodecyl sulfate (SDS), 99% purity (Park Scientific Ltd, Northampton, UK)

Sodium dioctyl sulfosuccinate (SDOSS), (UNIKEM, Copenhagen, Denmark). Cetyltrimethyl ammonium bromide (Cetrimide), (Merck, Darmstadt, Germany)

Polyoxyethylene 23-lauryl ether (Brij 35). (Sigma, St. Louis, MO, USA)

3-N,N-dimethylmyristyl ammonium propane sulfonate (MAPS), (Fluka AG, Buchs, Switzerland).

II. *Apparatus*

A Shimadzu UV-Visible 1601 PC spectrophotometer was used for the spectrophotometric measurements.

III. *Sample Preparation and Procedure*

Standard solution of RTH containing 100 µg/mL was prepared in distilled water and was further diluted with the same solvent as appropriate. The standard solution was kept in the refrigerator and was found to be stable for at least 7 days.

IV. *Construction of Calibration Graph for Method 1*

Increasing volumes from the stock solution of the drug were quantitatively transferred to a set of 10-mL volumetric flasks, so as to contain the drug within the concentration range 2.0-16 µg/mL. Borate buffer (3.5 mL) of pH 8 followed by 1.0 ± 0.2 mL of NBD-Cl solution (0.05%) were added to each flask. The solutions were heated for 15 min at 70°C. The reaction was quenched by cooling under tap water, and then 0.2 mL of concentrated HCl was added and each flask was made up to volume with distilled water. The absorbance was measured at 392 nm against a reagent blank.

V. *Construction of Calibration Graph for Method 2*

To a set of 10-mL volumetric flasks, increasing volumes from the stock solution of the drug were quantitatively transferred so as to contain the drug within the concentration range 0.2-2 µg/mL. To each flask 2.5 ± 0.2 mL of FPL was added. The solutions were heated in a boiling water bath for 20 min. The reaction was quenched by cooling under tap water and each flask was made up to volume with distilled water. The absorbance was measured at 510 nm against a reagent blank.

VI. *Construction of Calibration Graph for Method 3*

Increasing volumes from the stock solution of the drug were quantitatively transferred to a set of 10-mL volumetric flasks, so as to contain the drug within the concentration range 0.8-12 µg/mL. Five milliliter of Na_2CO_3 solution (10% w/v) followed by 0.75 ± 0.2 mL of F-C solution (2N) were added to each flask. The solutions were stored for 20 min at ambient temperature then each flask was made up to volume with distilled water. The absorbance was measured at 760 nm against a reagent blank.

The calibration curves for the three proposed methods were constructed by plotting the absorbance against the final concentration of the drug. Alternatively, the corresponding regression equations were derived.

VII. *Analysis of the Pharmaceutical Formulations*

For Yutopar tablets, 10 tablets were finely powdered after weighing. A portion of the tablet powder equivalent to 10 mg of the drug was extracted with 3 × 30 mL portions of distilled water. After sonication of each portion for 10

min, the extracts were transferred quantitatively into 100 mL measuring flasks and the flask was made up to volume with distilled water. The final solution was centrifuged (4000 ×g) for 15 min, and filtered. For Yutopar ampoules, the contents of five ampoules were mixed, and 1 mL volume of the mixture which equivalent to 10 mg of the drug was transferred into 100-mL volumetric flask. Afterwards, the flask was made up to volume with distilled water after mixing well. The final solution was used without filtration. For both prepared solutions of tablets and ampoules, further dilutions were made as appropriate with distilled water and processed as described under *Construction of calibration graphs*, adopting the three different proposed methods. The nominal content of the tablets and ampoules were calculated using either the calibration graph or the corresponding regression equation.

RESULTS AND DISCUSSION

I. *Method 1*

The reaction between NBD- Cl and the secondary amino group of RTH in borate buffer of pH 8.0 was found to produce a yellow colored product with a maximum absorbance at 392 nm (Figure 2A). The different experimental parameters affecting the intensity of the produced color were studied and optimized to obtain the maximum color intensity.

The pH was varied over the pH range of 7-10 using borate buffer where the maximum absorbance was obtained at pH 8.0 as shown in Figure 3. The effect of using different buffer solutions such as carbonate or phosphate buffers as alternatives to borate buffer at this optimum pH value was also investigated. It was found that borate buffer is superior in obtaining the maximum absorbance. This may be attributed to hydrolysis of the reagent with other buffers, as revealed in previous reports[22]. NBD-Cl is also hydrolyzed in alkaline medium to give NBD-OH which has a maximum absorbance at 462 nm. Therefore, it was necessary to acidify the reaction mixture to pH 2 (by adding 0.2 mL of concentrated HCl) before measurement. At this acidic pH, the reagent blank did not show any significant absorption peak.

The effect of temperature on the color intensity was studied by heating the reaction mixture over the range from 50 to 100°C for different periods of time. It was found that heating at relatively lower temperature (70°C) for 15 min gave more reproducible results as illustrated in Table 1.

The effect of different volumes of 0.05% NBD-Cl on the color intensity was studied over the range 0.2 to 1.6 mL. It was found that 1.2 ± 0.2 mL was the most suitable volume of the reagent (Table 1). These optimum conditions have been used for the proposed spectrophotometric method. As for stability of the produced derivative, it was found to be stable for at least 2 hr.

The effect of diluting solvents other than water such as acetone, acetonitrile, methanol, ethanol and dimethyl formamide (DMF) was also investigated to obtain

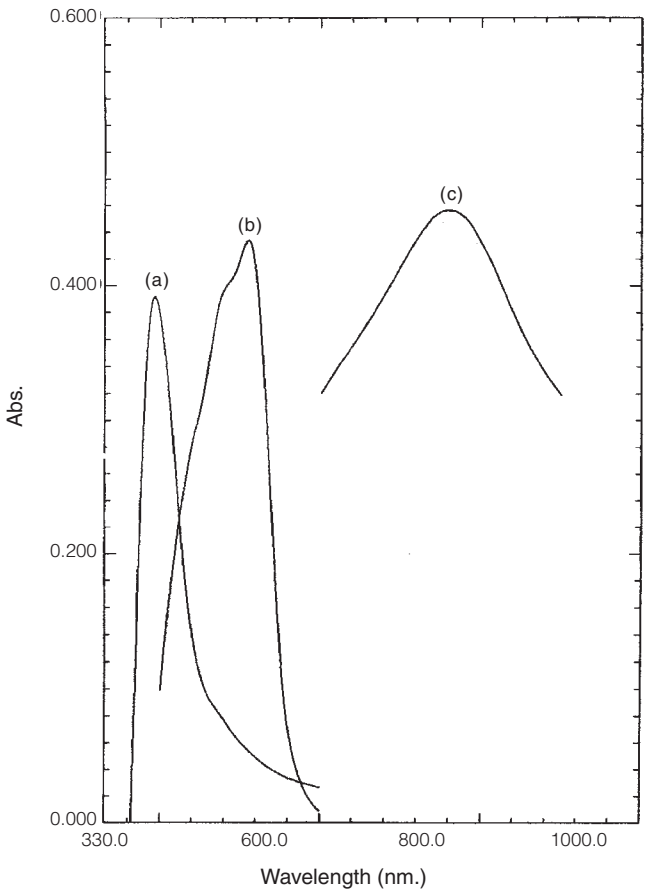


Figure 2. Absorption spectra of reaction products of RTH with (A) 0.05% NBD-Cl in borate buffer of pH 8.0 (16 μg/mL), (B) 0.01 M FPL in acidic medium (2 μg/mL), and (C) 2.0 N Folin-Ciocalteu in Na_2CO_3 solution (6 μg/mL).

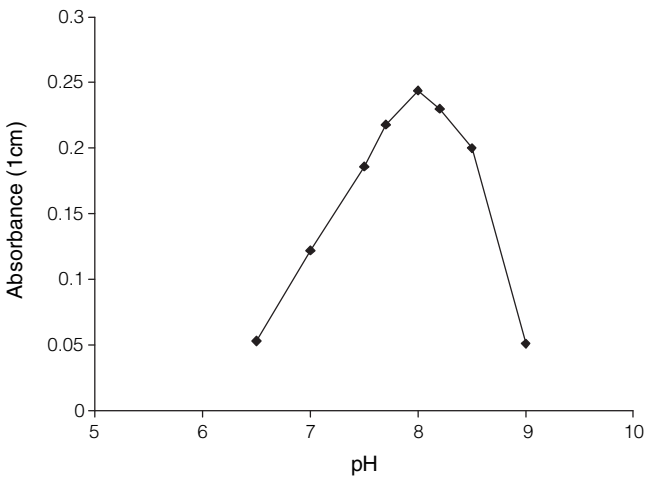


Figure 3. Effect of pH of borate buffer (0.2 M) on development of the reaction product of RTH (10 μg/mL) with NBD-Cl.

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

the maximum color intensity. It was found that water, acetone and acetonitrile are of similar effect. Meanwhile, ethanol, methanol and DMF slightly decreased the color intensity.

Stoichiometry of the Reaction

The stoichiometry of the reaction was studied using equimolar concentrations of the drug and NBD-Cl adopting Job's method of continuous variation[38], a molar ratio of 1:1 (drug: NBD-Cl) respectively, was obtained by the applied method as shown in Figure 4. Based on the observed molar reactivity of the reaction, the mechanism of the reaction between RTH and NBD-Cl was postulated in Scheme 1.

The formation constant of the reaction product K_f was calculated using the following formula[39]:

$$K_f = \frac{A/A_m}{\left(\frac{1-A}{A_m}\right)^{n+1} C^n n^n}$$

where A is the maximum absorbance of the continuous variation curve (Figure 4), A_m is the absorbance corresponding to intersection of the two tangents of the continuous variation curve, C is the molar concentration of RTH corresponding to maximum absorbance, n is the number of the molecules of the reagent in the reaction product, and K_f was found to be equal to 3695. This high value of K_f indicates a very stable reaction product.

The Gibbs free energy of the reaction ΔG was also calculated with the following equation:

$\Delta G = -2.303RT \log K_f$

where R is the universal gas constant (8.314 J), T is the absolute temperature (273 + 25°C) and K_f is formation constant of the reaction.

The value of ΔG was found to be (-20.36) K.J/mole. The negative sign of ΔG points out to the spontaneous nature of the reaction.

Table 1. Factors affecting formation of the reaction product of ritodrine (10 μg/mL) with NBD-Cl

Absorbance	Heating Temperature (°C)
0.033	25
0.163	50
0.216	60
0.244	70
0.245	80
Precipitation	100
Absorbance	**Heating time (min)**
0.140	5
0.195	10
0.244	15
0.240	20
0.246	30
0.206	40
0.200	50
0.174	60
Absorbance	**0.05% NBD-Cl (mL)**
0.073	0.2
0.151	0.4
0.205	0.6
0.245	0.8
0.246	1
0.248	1.2
0.245	1.4
0.222	1.6

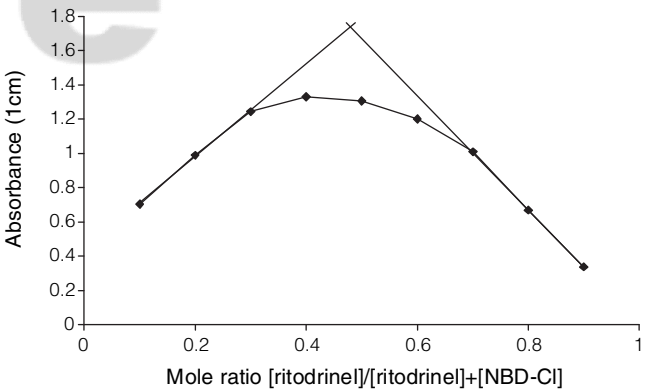
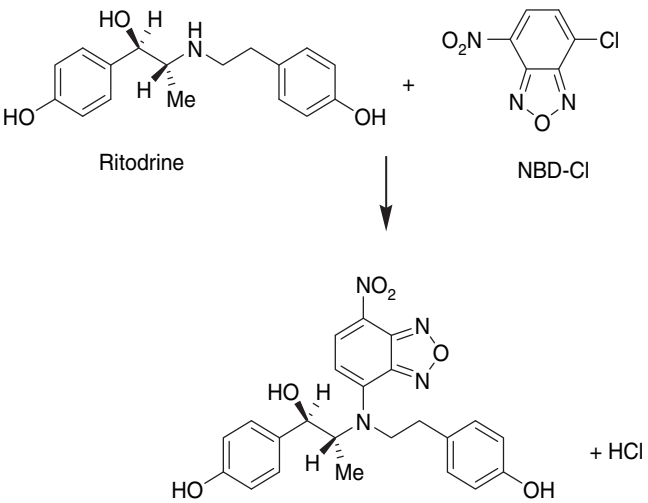


Figure 4. Determination of molar reactivity of NBD-Cl - RTH using Job's method of continuous variation (0.005 M).



Scheme 1. Proposal of the reaction mechanism between ritodrine and NBD-Cl.

II. *Method 2*

Ferric salts play a prominent role in the spectrophotometric determination of many pharmaceutical

drug substances acting as an oxidant. The Fe^{3+}/1,10-phenanthroline (Fe^{3+}/phen) system is a valuable reagent for analytes with reducing properties, because the final product is the intensely colored and extractable chelate $[Fe(phen)_3]^{2+}$ [27]. RTH as a phenolic compound can undergo oxidation by FPL reagent in a weakly acidic medium, forming an orange red colored complex with absorption maximum at 510 nm. Absorption spectrum of this colored complex is shown in Figure 2B. The optimum reaction parameters were established via number of preliminary experiments.

The color intensity of the formed complex $[Fe(phen)_3]^{2+}$ was measured against different concentrations of the reagent ranged from 0.25 to 3.0 mL. As shown in Figure 5, reagent ranging from 1.5 to 3.0 mL gave the maximum absorbance values. Hence 2.5 mL of the reagent was used through out this study.

The formation of colored complex was slow at room temperature and required longer time for maximization because of kinetic hindrance. The reaction was accelerated by heating for 20 min, at higher temperature of 40°C to 100°C using thermostatically controlled water bath. It was observed that the maximum absorbance was obtained upon boiling. Different boiling times were then further investigated over time intervals ranged from 5 to 30 min. The maximum color intensity was obtained after boiling the reaction mixture for 15 min, thus boiling for 20 min was chosen as an optimum boiling time to assure complete reaction. The absorbance of the resulting colored product was stable at the room temperature for more than 24 hours.

The stoichiometry of the reaction was studied adopting the limiting logarithmic method[40]. The two straight lines obtained upon using increasing concentrations of the drug while keeping the concentration of the reagent constant (Figure 6A) and upon using increasing concentrations of the reagent while keeping the concentration of the drug constant (Figure 6B). The two lines gave two slopes with the values of 0.224 and 1.001 for RTH and FPL, respectively, therefore the molar ratio for the reaction between RTH and FPL, is approximately 1:4. Based on the observed molar ratio, the reaction pathway shown in scheme 2 is proposed.

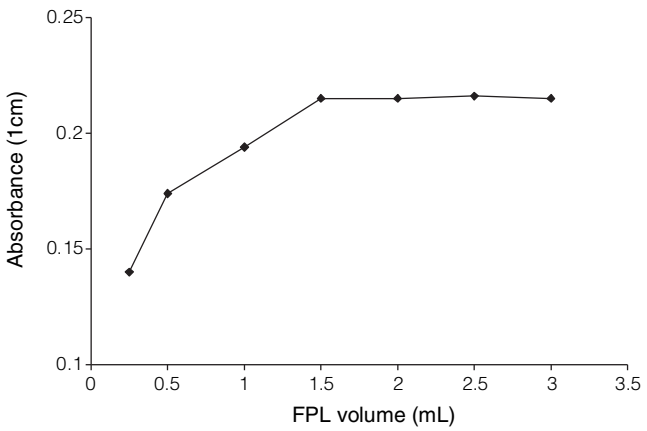
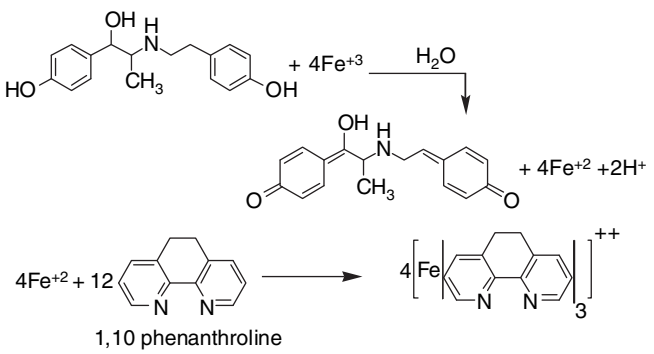


Figure 5. Effect of different volumes of 0.01 M FPL on the development reaction product with RTH (1 μg/mL).



Scheme 2. Proposal of the reaction mechanism between ritodrine and 1,10-phenanthroline.

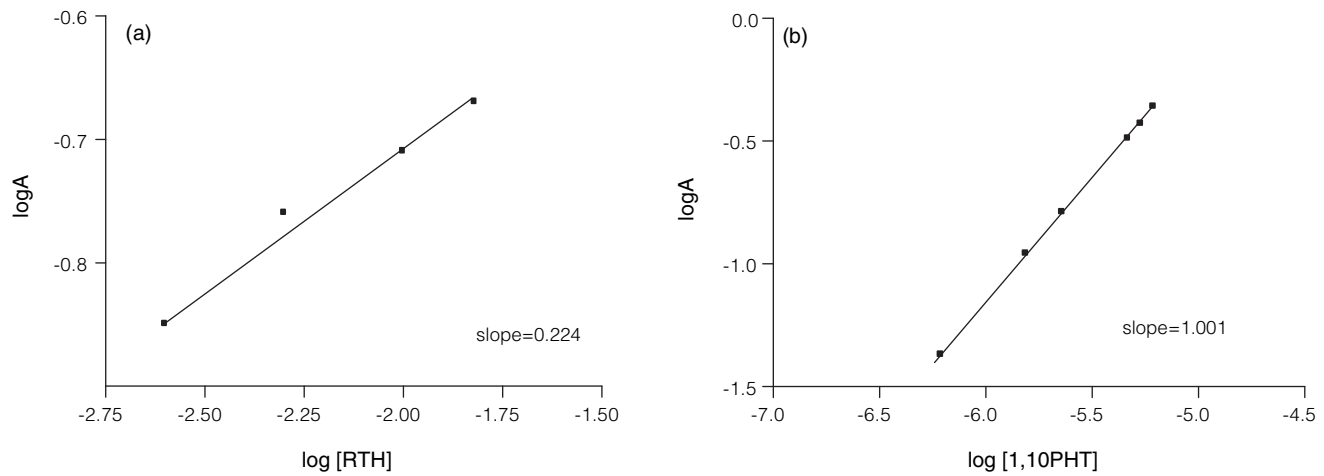


Figure 6. Limiting logarithmic plots for the molar reactivity of RTH with FPL: (A) log A vs. log [RTH] with [1,10PHT] kept at 1.5×10^{-3} M; (B) log A vs. log [1,10PHT] with [RTH] kept constant at 3.1×10^{-6} M.

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

III. *Method 3*

This method is based on formation of blue colored chromogen, following the reduction of phosphor-molybdo tungestic mixed acid of the Folin-Ciocalteu reagent (F-C) by RTH in the presence of sodium carbonate, which could be measured at 760 nm as shown in Figure 2C. The mixed acids in the F-C reagent are the final chromogen and involve the following chemical species: $3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$ and $3H_2O{\cdot}P_2O_5{\cdot}14WO_3{\cdot}4MoO_3{\cdot}10H_2O$.

RTH probably affects the reduction of 1, 2 or 3 oxygen atoms from tungestate and/or molybdate in F-C reagent, thereby producing one or more possible reduced species with characteristic intense blue color. The effect of different variables such as volume of F-C reagent, selection of the reaction medium, optimum volume of Na_2CO_3, and reaction time were studied and optimized for attainment of maximum color intensity and stability.

The influence of F–C reagent concentration on the color development was investigated and the obtained results are shown in Figure 7. It is apparent that 0.5 to 1.0 mL of reagent gave the maximum color intensity, thus 0.75 mL of reagent was used throughout the work.

To find a suitable medium for the reaction, different aqueous bases were investigated, such as sodium hydroxide, sodium carbonate, sodium acetate and sodium hydrogen phosphate. The maximum color intensity was obtained upon using sodium carbonate. The optimum concentration of sodium carbonate solution was further investigated. After different volumes of 10% sodium carbonate solution were attempted at a constant concentration of RTH (8 μg/mL), it was found that different volumes ranged from 4.0 to 6.0 mL were optimum thus 5.0 mL was used throughout the work.

Development of the colored reaction product was not instantaneous. Maximum color development was obtained within 15 min of mixing the reactants, and was stable for at least 30 min thereafter.

IV. *Effect of Foreign Matters and Different Surfactants*

The effect of different surfactants and foreign

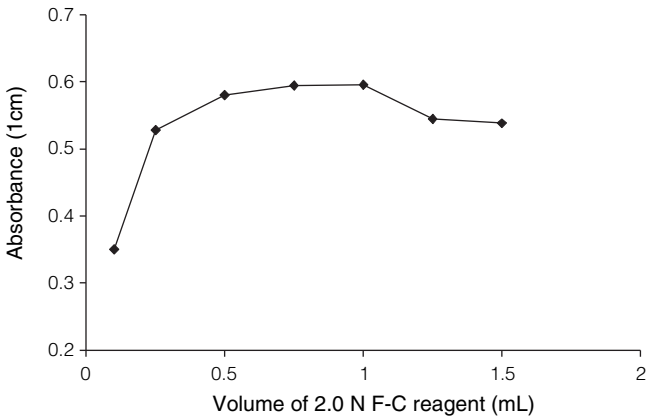


Figure 7. Effect of different volumes of (2.0 N) F-C reagent on the development of the reaction product with RTH (8 μg/mL) in Na2CO3 solution.

Table 2. Effect of foreign substances and different surfactants on performance of the three proposed methods for determination of ritodrine HCl

	Method 1			Method 2			Method 3		
Concentration of ritodrine HCl (μg/mL)	10			1.5			8		
	Absorbance								
With out foreign substances or surfactant	0.24			0.33			0.60		
Concentration of foreign substances (μg/mL)	10	5	1	10	5	1	10	5	1
Glucose	0.18	0.19	0.20	0.33	0.33	0.33	0.60	0.60	0.61
Lactose	0.22	0.23	0.24	0.34	0.34	0.35	0.60	0.60	0.60
Sucrose	0.17	0.18	0.23	0.32	0.32	0.33	0.60	0.60	0.60
Starch	0.16	0.18	0.24	0.33	0.33	0.33	0.60	0.60	0.61
Concentration of surfactant (μg/mL)	15	10	5	15	10	5	15	10	5
Sodium dodecyl sulfate	0.19	0.24	0.25	0.34	0.34	0.34	0.61	0.61	0.61
Cetrimide	0.14	0.22	0.22	0.33	0.33	0.33	0.61	0.61	0.6
Brij 35 (polyoxyethylene 23 lauryl ether)	0.15	0.18	0.25	0.33	0.33	0.33	0.58	0.58	0.60
Sodium dioctyl sulfosuccinate	0.23	0.26	0.25	0.32	0.32	0.33	0.58	0.58	0.60
3(N,N-Dimethylmyristylammonio) propanesulfonate	0.17	0.22	0.25	0.32	0.33	0.34	0.58	0.58	0.60

Table 3. Application of the three proposed spectrophotometric methods for determination of RTH in dosage forms

Sample	Recovery, % ± SD[a] Proposed methods			Reference method
	Method 1	Method 2	Method 3	
Yutopar tablet[b] (10 mg rito drine HCl/tab)	102.28 ± 0.13	100.21 ± 0.42	99.94 ± 0.46	101.00 ± 0.16
	t = 2.29 (2.776)	1.98 (2.776)	2.36 (2.571)	
	F = 1.49 (9.28)	6.89 (9.28)	8.27 (9.12)	
Yutopar ampoule[b] (10 mg rito drine HCl/amp)	99.99 ± 0.37	100.14 ± 0.35	100.18 ± 0.25	100.00 ± 0.19
	t = 0.05 (2.776)	1.05 (2.571)	1.36 (2.571)	
	F = 3.79 (9.28)	3.39 (9.12)	1.73 (9.12)	

[a]Mean recovery of 3 separate determinations.
The figure in parenthesis are the tabulated values of t and F at p =/0.05.
[b]Products of Pharco pharmaceutical, Alexandria, Egypt.

substances on the development of the colored products in the three proposed methods was investigated by adding three different concentrations of each substance to the reaction mixture. The investigated surfactants are either anionic such as sodium dodecyl sulfate (SDS) and sodium dioctyl sulfosuccinate (SDOSS), cationic such as cetrimide (CTR), or nonionic such as polyoxyethylene 23 lauryl ether (Brij 35) and 3(N,N-dimethylmyristylammonio propanesulfonate) (MAPS). The foreign substances included glucose, lactose, sucrose and starch. The obtained results are illustrated in Table 2. It was found that some of the tested substances had slight diminishing effect on absorbance of the developed colored reaction product with NBD-Cl (method 1). However, this effect increased upon increasing the concentration of the tested substances. Meanwhile, methods 2 & 3 were more resistant to this effect even at higher concentrations of the investigated substances.

V. *Assay of Dosage Forms*

Applicability of the three proposed methods was tested by determination of RTH in its dosage forms Yutopar tablets and Yutopar ampoules. Excellent recoveries and SD values were obtained (Table 3). Common tablet excipients, such as lactose, starch, talc, starch, avisil, gelatin, and magnesium stearate, did not interfere with the assay in the three applied methods. However, the slight diminishing effect of either lactose or starch on the absorbance of the reaction product of method 1 (Table 2) did not affect the percent recoveries of the tablets due to either its presence at low tolerable concentration (lactose) or its insolubility in water where the tablets were extracted (starch). A previously reported spectrophotometric method[5] was used for comparison using Student's *t*-test and the variance ratio *F*-test. As illustrated in Table 3, the calculated values did not exceed the reference figures indicating lack of significant difference in the performance of the compared methods regarding accuracy and precision[41].

Table 4. Analytical parameters for the assay of ritodrine adopting the three proposed spectrophotometric methods

Parameter	Method 1	Method 2	Method 3
λ_{max} (nm)	392	510	760
Linearity range (μg/mL)	2-16	0.2-2.0	0.8-12
Apparent molar absorptivity (L/mol/cm)	7.9×10^3	7×10^4	2.4×10^4
Linear regression Intercept (a)	8.08×10^{-4}	7.3×10^{-4}	-1.8×10^{-3}
Slope (b)	0.02	0.22	0.07
Correlation coefficient (r)	0.9999	0.9999	0.9999
$S_{y/x}$	9.30×10^{-4}	2.55×10^{-3}	2.00×10^{-3}
S_a	7.20×10^{-4}	3.51×10^{-3}	3.02×10^{-3}
S_b	7.17×10^{-5}	1.56×10^{-3}	3.10×10^{-4}
LOD (μg/mL)	0.10	0.05	0.14
LOQ (μg/mL)	0.30	0.16	0.41
RSD (%)	0.72	1.21	0.42
Er (%)	0.25	0.50	0.17

$S_{y/x}$: standard deviation of the residuals; S_a: standard deviation of the intercept of regression line; S_b: standard deviation of the slope of regression line; RSD % = (%)/$\sqrt{n}$; Er % = error (%).

VI. *Validation*

The three proposed methods were validated based on sensitivity, linearity, intraday and interday precision, accuracy, specificity, and robustness.

Sensitivity of the three methods was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) according to the ICH guidelines (ICH Topic Q2B; [42]).

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

LOD was defined as:

$$3.3 \times \sigma/S$$

and LOQ was:

$$10 \times \sigma/S$$

where σ is standard deviation of the y-intercept of the regression lines (the standard deviation of the response) and S is slope of the calibration curve.

Linearity was evaluated by calculation of the regression equations over the ranges given in Table 4. 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) and intercepts (S_a) on the ordinates, and standard deviation of the residuals ($S_{y/x}$)[41].

The intraday and interday accuracy and precision for the three proposed methods were examined by analysis of samples of RTH in its dosage forms in two different concentrations. Intraday precision was assessed by five determinations/concentration in one day. Interday precision was assessed by determination of each concentration on three separate days. Repeatability and reproducibility in the three proposed methods were fairly good, as indicated by the small values of standard deviation (SD), relative standard deviation (RSD), and error (%Er) (Table 5).

Specificity of the three methods was proven by the negligible effect of the presence foreign matters and different surfactants on the absorbance intensity.

Robustness of the three methods is demonstrated by the consistency of the absorbance intensity with minor changes in experimental variables, such as changing pH (method 1), changing heating times (methods 1 and 2), changing the reaction times (method 3), molar concentration of the buffer (method 1) and reagent volume (methods 1, 2 and 3). The minor changes expected to take place during the course of the operation of the method did not adversely affect the absorbance intensity.

CONCLUSIONS

Three simple and sensitive spectrophotometric methods were developed for the determination of RTH.

Table 5. Accuracy and precision data of the three proposed spectrophotometric methods for determination of RTH in pharmaceutical dosage forms

Dosage form		Proposed methods		
		Method 1	Method 2	Method 3
Yutopar tablet (10 mg ritodrine HCl/tab)	Inter day			
	$\overline{X} \pm SD$	99.63 ± 0.23	100.22 ± 0.39	100.42 ± 0.07
	RSD%	0.23	0.39	0.07
	Er%	0.13	0.23	0.04
	Intra day			
	$\overline{X} \pm SD$	99.5 ± 0.4	100 ± 0.67	100.67 ± 0.14
	RSD%	0.4	0.67	0.14
	Er%	0.23	0.39	0.08
Yutopar ampoule (10 mg ritodrine HCl/amp)	Inter day			
	$\overline{X} \pm SD$	99.77 ± 0.23	100.45 ± 0.39	100.42 ± 0.07
	RSD%	0.23	0.39	0.07
	Er%	0.13	0.23	0.04
	Intra day			
	$\overline{X} \pm SD$	99.77 ± 0.23	100.22 ± 0.39	100.58 ± 0.14
	RSD%	0.23	0.39	0.14
	Er%	0.13	0.23	0.08

RSD % = (%)/$\sqrt{n}$; Er % = error (%).

Simplicity of the three methods is evident by the direct measurement of the produced reaction products within 20 min. Concentrations down to 2.0, 0.2 and 0.8 μg/mL could be measured using the three methods, proving high sensitivity especially for method 2. These three methods could be successfully applied to the determination of RTH in pharmaceutical dosage forms.

REFERENCES

1. The British Pharmacopoeia, Her Majesty's Stationary Office, London. 2007. p. 1444. (electronic copy)
2. Reynolds, J. G. F. and Martindale. 1999. The Extra Pharmacopoeia. 32 ed. pp. 1589-1590. Royal Pharmaceutical Society, London.
3. Huang, W., Wei, X. F. and Li, C. H. 1997. Ultra-violet spectrophotometric determination of ritodrine hydrochloride in tablets. Yaowu Fenxi Zazhi 17: 127-128.
4. Jacobus, F., Negussie, V. S., Beyene, W., Stefan, R. I. and Aboul-Enein, H. Y. 2005. Sequential injection spectrophotometric determination of ritodrine hydrochloride using 4-aminoantipyrine. Talanta 68: 401-405.
5. Sastry, C. S. P., Chintalapati, R., Prasad, A. V. S. S. and Sastry, B. S. 2001. Application of oxidative coupling reactions for the estimation of ritodrine hydrochloride in bulk sample and dosage forms. Talanta 53: 907-914.
6. Revanasiddappa, H. D. and Bochhegowda, M. 2001. Spectrophotometric methods for the determination of ritodrine hydrochloride and its application to pharmaceutical preparations. Il Farmaco. 56: 615-619.
7. Revanasiddappa, H. D. and Manju, B. G. 2000. Spectrophotometric determination of ritodrine and isoxsuprine hydrochlorides using 4-aminoantipyrine. J. AOAC Int. 83: 1440-1445.
8. Revanasiddappa, H. D., Manju, B. G. and Ramappa, P. G. 1999. Spectrophotometric method for the determination of ritodrine hydrochloride and amoxicillin. Anal. Sci. 15: 661-664.
9. Bakry, R. S., El Walily, A. F. M. and Belal, S. F. 1997. Spectrophotometric determination of some phenolic sympathomimetic drugs through reaction with 2,6-dihaloquinone chlorimides. Mikrochim. Acta. 127: 89-93.
10. Bakry, R. S., El Walily, A. F. M. and Belal, S. F. 1996. Spectrophotometric determination of etilefrine hydrochloride, prenalterol hydrochloride and ritodrine hydrochloride in pharmaceutical dosage form through nitrosation and subsequent copper chelation. Anal. Lett. 29: 409-422.
11. Bakry, R. S., El Walily, A. F. and Belal, S. F. 1995. Spectrophotometric determination of etilefrine, ritodrine, isoxsuprine and salbutamol by nitration and subsequent Meisenheimer complex formation. Anal. Lett. 28: 2503-2519.
12. Razak, O. A. 1998. Fluorimetric and spectrophotometric determination of ritodrine hydrochloride in bulk and pharmaceutical formulations. J. Pharm. Biomed. Anal. 18: 359-365.
13. Gross, A. S., Brown, K. F., Baird-Lambert, J. A. and Nation, R. L. 1987. Determination of ritodrine in blood and plasma by high-performance liquid chromatography with fluorescence detection. J. Chromatogr. Biomed. Appl. 416: 400-408.
14. Lin, L. S., Caritis, S. N. and Wong, L. K. 1984. Analysis of ritodrine in serum by high-performance liquid chromatography with electrochemical detection. J. Pharm. Sci. 73: 131-133.
15. Kuhnert, P., Erhard, P., Dixon, A., Kuhnert, B. and Gross, T. 1983. Determination of ritodrine in plasma using HPLC. J. Liquid Chromatogr. 6: 2775-2783.
16. Bocca, B., Fiori, M., Cartoni, C. and Brambilla, G. 2003. Simultaneous determination of zilpaterol and other beta agonists in calf eye by gas chromatography/tandem mass spectrometry. J. AOAC Int. 86: 8-14.
17. Hernandez-Carrasquilla, M. 2000. Gas chromatography-mass spectrometry analysis of β2-agonists in bovine retina. Anal. Chim. Acta 408: 285-290.
18. Saleh, H. M., El-Henawee, M. M., Ragab, G. H. and Abd El-Hay, S. S. Utility of NBD-Cl for the spectrophotometric determination of some skeletal muscle relaxant and antihistaminic drugs. Spectrochim. Acta A (In Press)
19. Taha, E. A., Salama, N. N. and Fattah, L. E. 2006. Spectrofluorimetric and spectrophotometric stability-indicating methods for determination of some oxicams using 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-Cl). Chem. Pharm. Bull. 54: 653-658.
20. Ibrahim, A. D. 2005. Kinetic spectrophotometric methods for determination of trimetazidine dihydrochloride. Anal. Chim. Acta. 551: 222-231.
21. El-Emam, A. A., Hansen, S. H., Moustafa, M. A., El-Ashry, S. M. and El-Sherbiny, D. T. 2004. Determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) followed by spectrophotometry or HPLC with fluorimetric detection. J. Pharm. Biomed. Anal. 34: 35-44.
22. El-Shabrawy, Y., Belal, F., Sharaf El-Din, M. and Shalan, Sh. 2003. Spectrophotometric determination of fenoterol hydrobromide in pure form and dosage forms. Il Farmaco. 58: 1033-1038.
23. Amin, A. S., Ragab, G. H. and Saleh, H. 2002. Colorimetric determination of β-blockers in pharmaceutical formulations. J. Pharm. Biomed. Anal. 30: 1347-1353.
24. Abdellatef, H. E. 2002. Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation. J. Pharm. Biomed. Anal. 29: 835-842.
25. Güçlü, K., Sözgen, K., Tütem, E., Özyürek, M. and Apak, R. 2005. Spectrophotometric determination of ascorbic acid using copper (II)–neocuproine reagent in beverages and pharmaceuticals. Talanta. 65: 1226-1232.
26. Rahman, N., Singh, M. and Hoda, M. N. 2004. Application of oxidants to the spectrophotometric determination of amlodipine besylate in pharmaceutical formula-

tions. Il Farmaco. 59: 913-919.
27. Nagaralli, B. S., Seetharamappa, J. and Melwanki, M. B. 2002. Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations. J. Pharm. Biomed. Anal. 29: 859-864.
28. Carmona, M., Silva, M. and Pérez-Bendito, D. 1989. Stopped-flow kinetic determination of acetaminophen by oxidation with a 1,10-phenanthroline/iron (III) complex. Anal. Chim. Acta 218: 313-322.
29. Sadler, N. P. and Jacobs, H. 1995. Application of the Folin-Ciocalteau reagent to the determination of salbutamol in pharmaceutical preparations. Talanta 42: 1385-1388.
30. Prasad, A. V., Devi, P. A., Sastry, C. S. and Prasad, U. V. 2003. Assay of minocycline and tylosin using folin ciocalteu reagent. Eastern Pharmacist. 2: 67-68.
31. Basavaiah, K. and Prameela, H. C. 2002. Spectrophotometric determination of diclofenac sodium using folin-ciocalteu reagent in bulk drug and in dosage form. Eastern Pharmacist. 1: 61-63.
32. Murthy, T. K., Sankar, G. D. and Rao, Y. S. 2002. Visible spectrophotometric methods for the determination of trimetazidine dihydrochloride in pharmaceutical formulation. Indian Drugs. 39: 230-233.
33. Basavaiah, K. and Prameela, H. C. 2002. Simple spectrophotometric determination of acyclovir in bulk drug and formulations. *Il Farmaco*. 57: 443-449.
34. Sastry, C. S. P. and Lingeswara-Rao, J. S. V. M. 1996. Spectrophotometric methods for the determination of methotrexate in pharmaceutical formulations. Anal. Lett. 29: 1763-1768.
35. Sastry, C. S. P., Naidu, P. Y. and Murthy, S. S. N. 1997. Spectrophotometric methods for the determination of omeprazole in bulk form and pharmaceutical formulations. Talanta 44: 1211-1217.
36. Sastry, C. S. P., Sailaja, A., Rao, T. T. and Krishna, D. M. 1992. Three simple spectrophotometric methods for the determination of sulphinpyrazone. Talanta 39: 709-713.
37. Raghuveer, S., Avadhanulu, A. B. and Pantulu, A. R. 1992. Spectrophotometric determination of gliclazide in its pharmaceutical dosage forms using folin-ciocalteu reagent. Eastern Pharmacist. 35: 129-130.
38. Christian, G. D. and O'Rilly, J. E. 1980. Instrumental Analysis. 2nd ed. p. 186. Prentice Hall, New Jersey, U. S. A.
39. Inczedy, J. 1976. Analytical Application of Complex Equilibria. p. 101. Wiley, Budapest, Hungary.
40. Rose, J. 1964. Advanced Physico-Chemical Experiments. Pitman, London, U. K.
41. International Conference on Harmonization. 1996. Note for Guidance on Validation of Analytical Procedures: Methodology, Committee for Proprietary Medical Products, CPMP/ICH/281/95, Approval 18 December.
42. Miller, J. N. and Miller, J. C. 2005. Statistics and Chemometrics for Analytical Chemistry. 5th ed. Prentice Hall, U. K.