

Simultaneous Determination of Ritodrine and Isoxsuprine Using Coupling Technique of Synchronous Fluorimetry and H-Point Standard Addition Method

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Abstract Simultaneous determination of two structurally related β_2 adrenergic receptor agonists namely, Ritodrine HCl (RTH) and Isoxsuprine HCl (ISP) was performed using coupling technique of synchronous fluorimetry and H-point standard addition method. Under optimum conditions, linear determination ranges were $1.48 - 14.80 \times 10^{-6} \text{ mol L}^{-1}$ and $1.54 - 15.44 \times 10^{-6} \text{ mol L}^{-1}$ for ISP and RTH respectively. RTH and ISP could be determined simultaneously without interference from each other when their concentration ratio varies from 5:1 to 1:5 in the mixed sample. The proposed method was applied to the determination of RTH and ISP in synthetic mixture of pharmaceutical samples, the accuracy and precision of the results were satisfactory.

Keywords Coupling technique · Synchronous fluorimetry · H-point standard addition method (HPSAM) · Isoxsuprine HCl · Ritodrine HCl

Introduction

Ritodrine Hydrochloride (RTH), 1-(*p*-hydroxyphenyl)-2-(4-hydroxyphenethylamino) propan-1-ol hydrochloride (Fig. 1) is a β_2 adrenergic agonist solely used as uterine relaxant [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 labour and as an emergency means of alleviating foetal asphyxia during labour [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 determination of RTH in pharmaceutical dosage forms including; UV spectrophotometry [4], sequential injection spectrophotometry [5], colorimetry [6–13], spectrofluorimetry [13], and HPLC [14].

Isoxsuprine Hydrochloride (ISP), 1-(4-hydroxyphenyl)-2-(1-methyl-2-phenoxyethylamino) propan-1-ol hydrochloride (Fig. 1) is a vasodilator that also stimulates β -adrenergic receptors [1]. It causes direct 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]. B.P. [1] described an acid base titration assay using potentiometric detection. Meanwhile, the U.S.P. [3] described a UV spectrophotometric method for its determination.

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

The similar pharmacological action along with the closely related chemical structure of RTH and ISP required the development of sensitive, simple and reliable method for their simultaneous determination in quality control laboratory. To the best of our knowledge, no previous methods were reported for their simultaneous determination.

The simultaneous determination of multicomponent mixtures has always been an interesting question for analysts; many selective analytical techniques have been put forward. Synchronous fluorimetry and H-point standard

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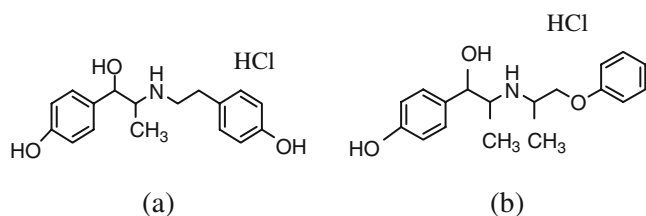


Fig. 1 Structural formula of Ritodrine hydrochloride **a** and Isoxsuprine hydrochloride **b**

addition method (HPSAM) are two selective analytical techniques.

Since synchronous fluorimetry was presented by Lloyd [22] in 1971, it has been developed fast and has been perfected [23, 24]. It was further developed from constant-wavelength difference synchronous fluorimetry [25] to variable-angle synchronous fluorimetry [26], constant-energy difference synchronous fluorimetry [27] and derivative synchronous fluorimetry [28–30]. Synchronous fluorimetry offers several advantages, including narrowing of the spectral band, enhancement in selectivity by spectral simplification and the elimination of scattered light. Therefore, it is a selective fluorimetry.

Although the standard addition method could remove the error resulting from the sample matrix, it can not remove the constant error resulting from other components in the system. In 1988, Reig et al [31] presented a technique called “H-point standard addition method (HPSAM)” that was based on the principle of dual wavelength spectrophotometry and the standard addition method. They further studied its principle and application [32]. The greatest advantage of HPSAM is that it can remove the errors resulting from the presence of an interferent and blank reagent. So, HPSAM could be used for simultaneous determination of components [33, 34].

Synchronous fluorimetry and HPSAM have the above advantages, but they have several shortcomings. Synchronous fluorimetry has difficulty in resolving multicomponent mixture with seriously overlapping spectra. The HPSAM has also difficulty in determining two components with symmetric and seriously overlapping spectra simultaneously. Synchronous fluorimetry combined with HPSAM is first proposed by Falco et al. [35], and further applied by Yang et al [36] for simultaneous determination of cephalexin and cefadroxil. Also applied by Guo et al for the simultaneous determination of epinephrine and norepinephrine after derivatization into fluorescent products [37].

In this work, synchronous fluorimetry combined with HPSAM was applied for the simultaneous determination of ISP and RTH in synthetic mixture with symmetric and seriously overlapping spectra and also in their single pharmaceutical preparations

Experimental

Apparatus

The fluorescence intensities were measured using a Perkin-Elmer Model LS 45 spectrofluorimeter equipped with 20 KW Xenon discharge lamp, excitation, emission grating monochromators and a 1×1 cm quartz cell. The apparatus is driven by *hp* computer.

Materials and reagents

All experiments were performed with analytical reagent grade chemicals. Distilled water was used throughout the experiments.

Ritodrine hydrochloride and Isoxsuprine hydrochloride were kindly provided by Pharco Pharmaceutical Company, Alexandria, Egypt. Stock solutions have concentrations of 617.67×10^{-6} mol L⁻¹ and 592.07×10^{-6} mol L⁻¹ for RTH and ISP respectively were prepared by dissolving 10.0 mg of each drug in 50 mL measuring flask separately. The working solutions were then prepared by dilution with water as appropriate to obtain (77.21 and 74.0×10^{-6} mol L⁻¹ for RTH and ISP respectively) final concentration. All the solutions were kept at 4°C in a refrigerator and they were stable for at least 1 week.

0.2 M Britton-Robinson buffer was prepared by addition of 12.37 gm boric acid to solution containing 12 mL glacial acetic acid and 23.1 mL 85% phosphoric acid (M.wt.=98)

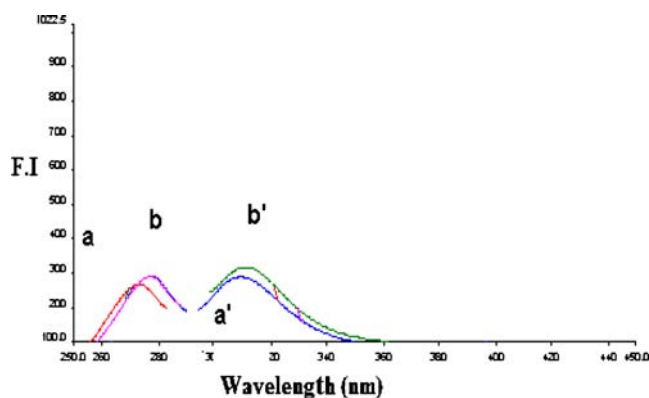
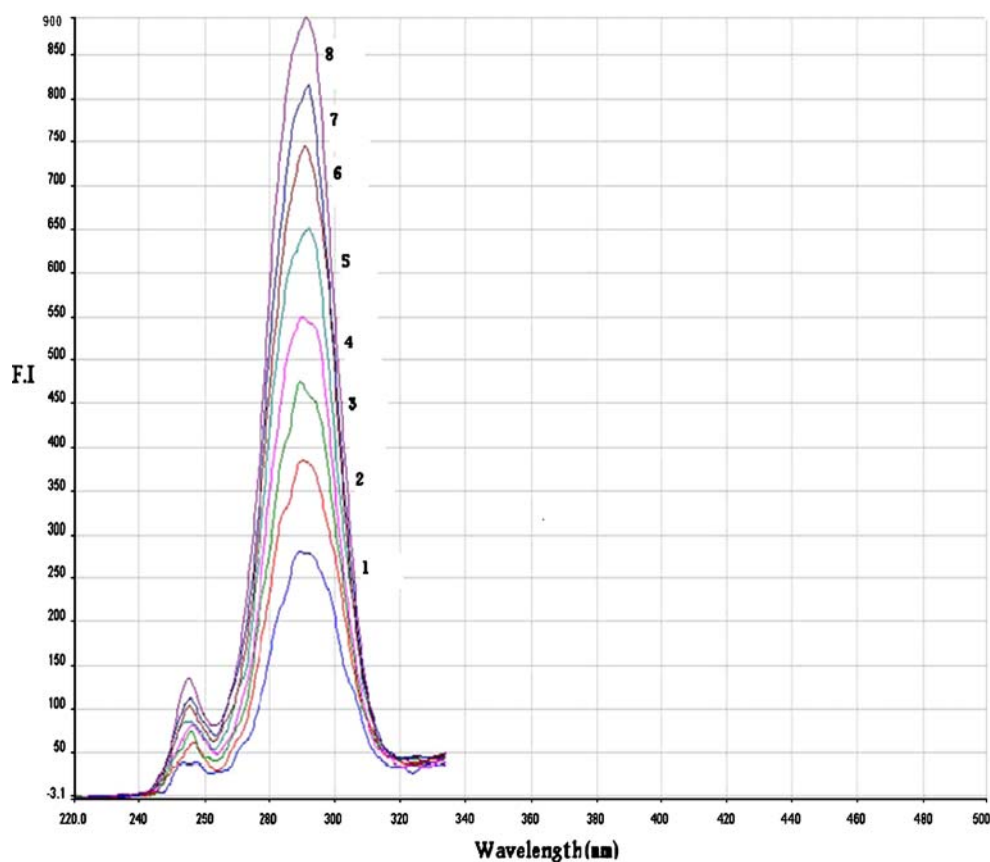


Fig. 2 Excitation and emission spectra of both ISP (a,a') and RTH (b,b') respectively in aqueous media

Fig. 3 (1-8) Synchronous fluorescence emission spectra upon using 3.09×10^{-6} mol L⁻¹ RTH, 2.37×10^{-6} mol L⁻¹ ISP and standard addition was performed using RTH (1.54, 3.09, 4.63, 6.18, 7.72, 9.26, 10.81 and 12.35×10^{-6} mol L⁻¹ respectively)



into 1 L volumetric flask. The solution was made up to volume with distilled water. Different amounts of 1 M NaOH were added to obtain full pH range (2–12) [1].

Procedure

Construction of calibration graphs

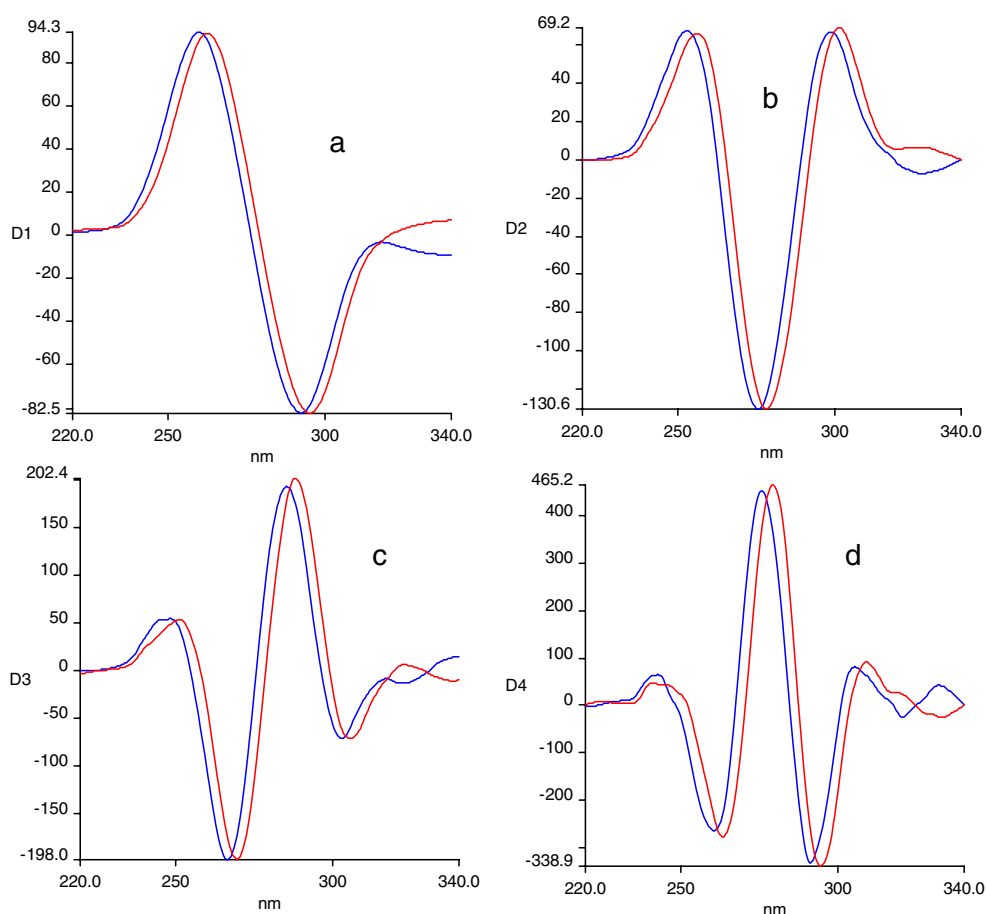
To a set of 25 mL volumetric flasks, different volumes of the stock solution of both drugs were quantitatively transferred and were made up to the volume with distilled water. Synchronous scanning was carried out at $\Delta\lambda = 30$ nm, the excitation and emission slit widths were 10 nm, wavelength scanning speed was 600 nm/min. at appropriate wavelengths. Synthetic samples containing different concentration ratios of ISP and RTH were prepared and standard addition of ISP or RTH (up to 15.44 and 14.80×10^{-6} mol L⁻¹ for RTH and ISP respectively) was made. Simultaneous determination of RTH and ISP with HPSAM was performed by measuring the fluorescence at $\lambda_1 = 268.76$ nm and $\lambda_2 = 289$ nm (when standards of ISP were added), or $\lambda_1 = 264.72$ nm and $\lambda_2 = 287$ nm (when standards of RTH were added), for each

sample solution. The concentration ranges for construction of HPSAM calibration graph were $1.54 - 15.44 \times 10^{-6}$ mol L⁻¹ and $1.48 - 14.80 \times 10^{-6}$ mol L⁻¹ for RTH and ISP respectively.

Assay of dosage forms

For both Yutopar tablets (labeled to contain 10 mg RTH/tablet) and Duvadilan tablets (labeled to contain 20 mg/tablet). Both are products of Pharco Pharmaceutical Company, Alexandria, Egypt. Ten tablets were finely powdered after weighing, and a portion of each tablet powder equivalent to 10 mg of the active substance was extracted separately with 3×15 mL portions of distilled water. After sonication of each portion for 10 min, the extracts were transferred quantitatively into 50 mL measuring flask and the flask was made up to volume with distilled water. The final solution was centrifuged ($4000 \times g$) for 15 min, and filtered. For both prepared solution of tablets, further dilutions were made as appropriate with distilled water then proceed as described under "Construction of calibration graphs". The nominal content of the tablets were calculated using either the calibration graph or the corresponding regression equation.

Fig. 4 a, b, c and d First, second, third and fourth derivatives synchronous peaks of both ISP and RTH (5.92 and 6.18×10^{-6} mol L⁻¹ for ISP and RTH respectively) (from a - d respectively)



Results and discussion

Fluorescence spectra

In accordance with previous reports [13, 19], RTH and ISP show emission peaks at 308 and 305 nm after excitation at 278 and 270 nm, respectively as shown in Fig. 2. Their emission spectra overlapped seriously and the spectral band widths are rather wide. So, their determination using HPSAM only is very difficult. However, the resolution of the spectra was slightly enhanced with synchronous scanning (Fig. 3). Also further application of derivative synchronous fluorimetry up to fourth derivative was incapable of determination of these seriously overlapped peaks Fig. 4a, b, c and d. Thus, their successful resolution was obtained upon coupling of synchronous fluorimetry with HPSAM where synchronous scanning was carried out for the two components presenting spectra with a narrower band width. HPSAM requires the spectrum of the interferent to be known, where it is based on measurements of a standard addition line at two wavelengths λ_1 and λ_2 . The wavelengths selected at two points at which the interferent possesses the same fluorescence intensities whereas the analyte fluorescence intensities are different (Fig. 5a, b). The two standard addition lines intersect at the so

called H-point (Fig. 6) with co-ordinates $(-C_H, A_H)$, where C_H is the concentration of the analyte and A_H is the analytical signal due to the interferent [32]:

The addition lines obtained at the λ_1 and λ_2 are given by:

$$A(\lambda_1) = B + A_1 + M(\lambda_1) \cdot C_X \quad (1)$$

$$A(\lambda_2) = B + A_2 + M(\lambda_2) \cdot C_X \quad (2)$$

Where A_1 and A_2 denote the fluorescence of the analyte in the sample at λ_1 and λ_2 respectively, B is the fluorescence of the interferent which is the same at λ_1 and λ_2 , $M(\lambda_1)$ and $M(\lambda_2)$ are the slopes of the addition lines, and C_X is the concentration of the analyte added.

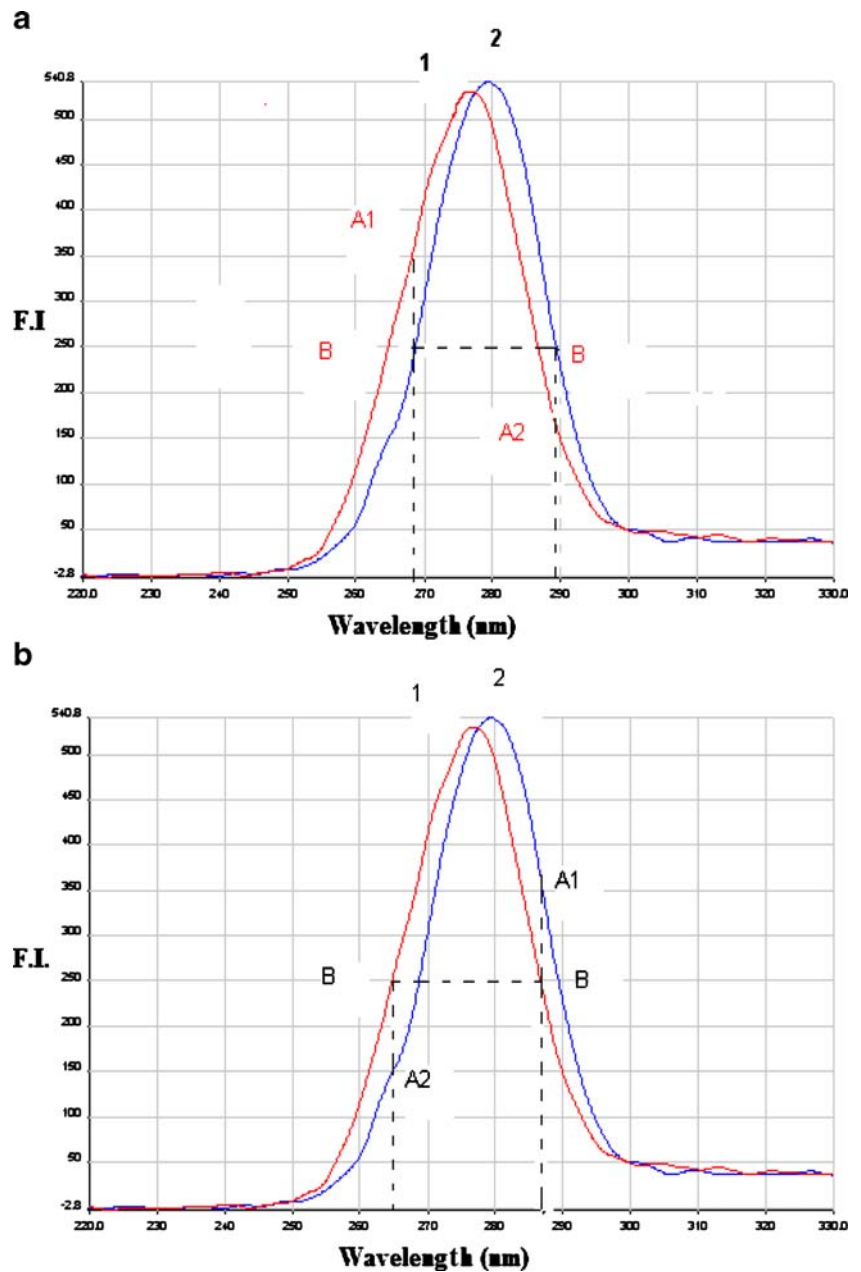
The addition lines intersect at the H-point, $(-C_H, A_H)$, so;

$$A_1 + M(\lambda_1) \cdot (-C_H) = A_2 + M(\lambda_2) \cdot (-C_H)$$

This can also be written as:

$$C_H = \frac{A_2 - A_1}{M(\lambda_1) - M(\lambda_2)} = \frac{C^0_X \cdot (M(\lambda_2) - M(\lambda_1))}{M(\lambda_1) - M(\lambda_2)} = -C^0_X \quad (3)$$

Fig. 5 a Separate synchronous fluorescence emission spectra of ISP $5.92 \times 10^{-6} \text{ mol L}^{-1}$ (1) and RTH $6.18 \times 10^{-6} \text{ mol L}^{-1}$ (2) in the aqueous medium. Conditions: $\Delta \lambda = 30 \text{ nm}$, the wavelength scanning speed = 600 nm/ min . the wavelengths selected for determination of ISP as analyte in the presence of RTH as interferent using HPSAM were 268.76 and 289 nm. **b** Separate synchronous fluorescence emission spectra of ISP $5.92 \times 10^{-6} \text{ mol L}^{-1}$ (1) and RTH $6.18 \times 10^{-6} \text{ mol L}^{-1}$ (2) in the aqueous medium. Conditions: $\Delta \lambda = 30 \text{ nm}$, the wavelength scanning speed = 600 nm/ min . the wavelengths selected for determination of RTH as analyte in the presence of ISP as interferent using HPSAM were 264.72 and 287 nm



The unknown analyte concentration C_X^0 therefore corresponds to C_H .

The fluorescence at the H-point, A_H , corresponds to the fluorescence of the interferent since from Eq. 1:

$$\begin{aligned}
 A_H &= A(\lambda_1) = B + A_1 + M(\lambda_1) \cdot (-C_H) \\
 &= B + M(\lambda_1) \cdot C_X^0 + M(\lambda_1) \cdot (-C_H) \\
 \text{So } A_H &= B
 \end{aligned}$$

Thus, A_H value is only related to the fluorescence of the interferent Y at the two selected wavelengths. To evaluate the interferent concentration from the ordinate value of the

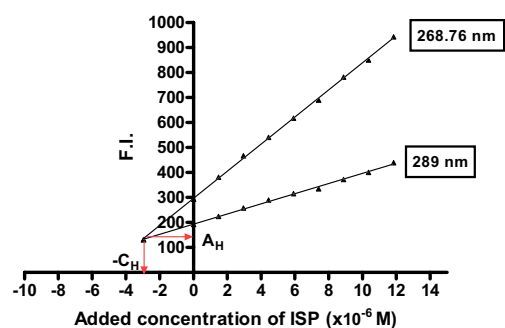


Fig. 6 Plots of H-point standard addition method for simultaneous determination of ISP and RTH when ISP is added, $\text{ISP} = 2.96 \times 10^{-6} \text{ mol L}^{-1}$ and $\text{RTH} = 2.47 \times 10^{-6} \text{ mol L}^{-1}$

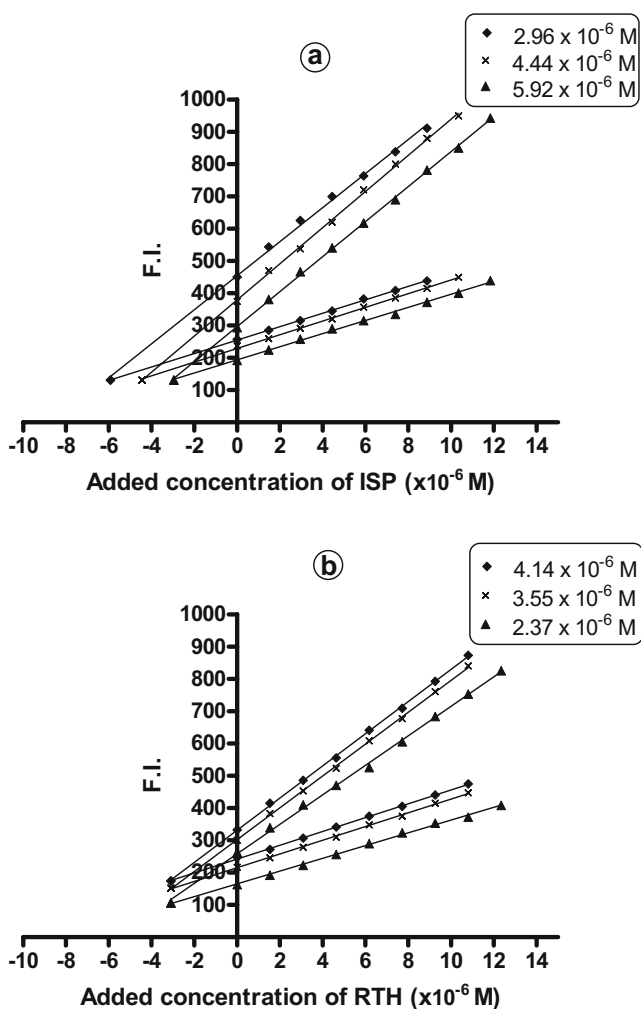


Fig. 7 **a** Plots of H-point standard addition method for; Fixed RTH concentration ($2.47 \times 10^{-6} \text{ mol L}^{-1}$) and different concentrations of ISP (2.96 , 4.44 and $5.92 \times 10^{-6} \text{ mol L}^{-1}$). [Standard additions were performed with ISP standards]. **b** Plots of H-point standard addition method for; Fixed RTH concentration ($3.09 \times 10^{-6} \text{ mol L}^{-1}$) and different concentrations of RTH (2.37 , 3.55 and $4.14 \times 10^{-6} \text{ mol L}^{-1}$). [Standard additions were performed with RTH standards]

H-point (A_H), a calibration graph of the fluorescence value of an interferent standard is needed.

According to the above discussion at H-point, C_H is independent from the concentration of interferent and so, A_H is also independent from the analyte concentration. Figures (7a, b) clearly show the effect of changing in the concentration of analyte and interferent on the position of H-point.

Optimization of synchronous fluorimetry for HPSAM

Effect of pH

The effect of pH on the fluorescence intensity of both drugs was investigated using Britton-Robinson buffer

covering the whole pH range (2–12). It was found that the fluorescence intensity is destroyed by alkalization, and remained nearly unchanged in acid medium over pH range 2–7. Meanwhile it is completely quenched in alkaline pH (8–12) as demonstrated in Figure (8). This is in agreement with previous reports [13, 19]. However, no buffer was used in this study, where the aqueous medium offered the maximum fluorescence intensities for both drugs.

Effect of diluting solvent

The effect of different diluting solvents on the fluorescence intensity of both drugs was investigated as alternatives to water such as, ethanol, methanol, and acetone. It was found that the fluorescence intensity for both drugs was increased upon using solvents other than water but with less symmetric peaks. However, the use of water as solvent gives less fluorescence intensity but with good symmetric peak which is essential in application of HPSAM. Thus water was used as solvent throughout this work.

The selection of $\Delta \lambda$ in synchronous scanning

It is important for synchronous fluorimetry to select the proper $\Delta \lambda$, because its value can affect the shape, the bandwidth and the intensity of the synchronous fluorescence spectra. Smaller $\Delta \lambda$ values are beneficial for decreasing the bandwidths of the spectra and enhancing the resolution, but it also reduces the sensitivity of the determination. However larger $\Delta \lambda$ values increase the sensitivity but reduce the resolution and selectivity. Synchronous scanning was carried out with $\Delta \lambda$ varied from 10 to 120 nm. The best result was obtained with $\Delta \lambda = 30 \text{ nm}$ as demonstrated in Fig. 9a, b.

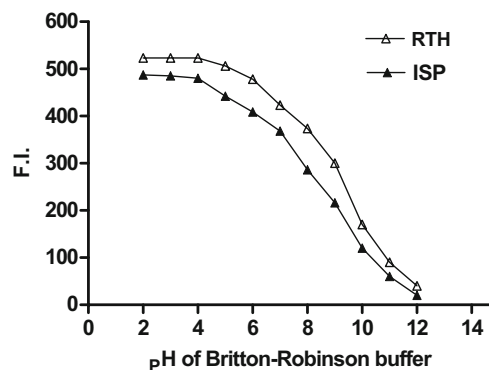


Fig. 8 Effect of pH of Britton-Robinson buffer on the fluorescence intensities of ISP ($5.92 \times 10^{-6} \text{ mol L}^{-1}$) and RTH ($6.18 \times 10^{-6} \text{ mol L}^{-1}$) in the aqueous medium

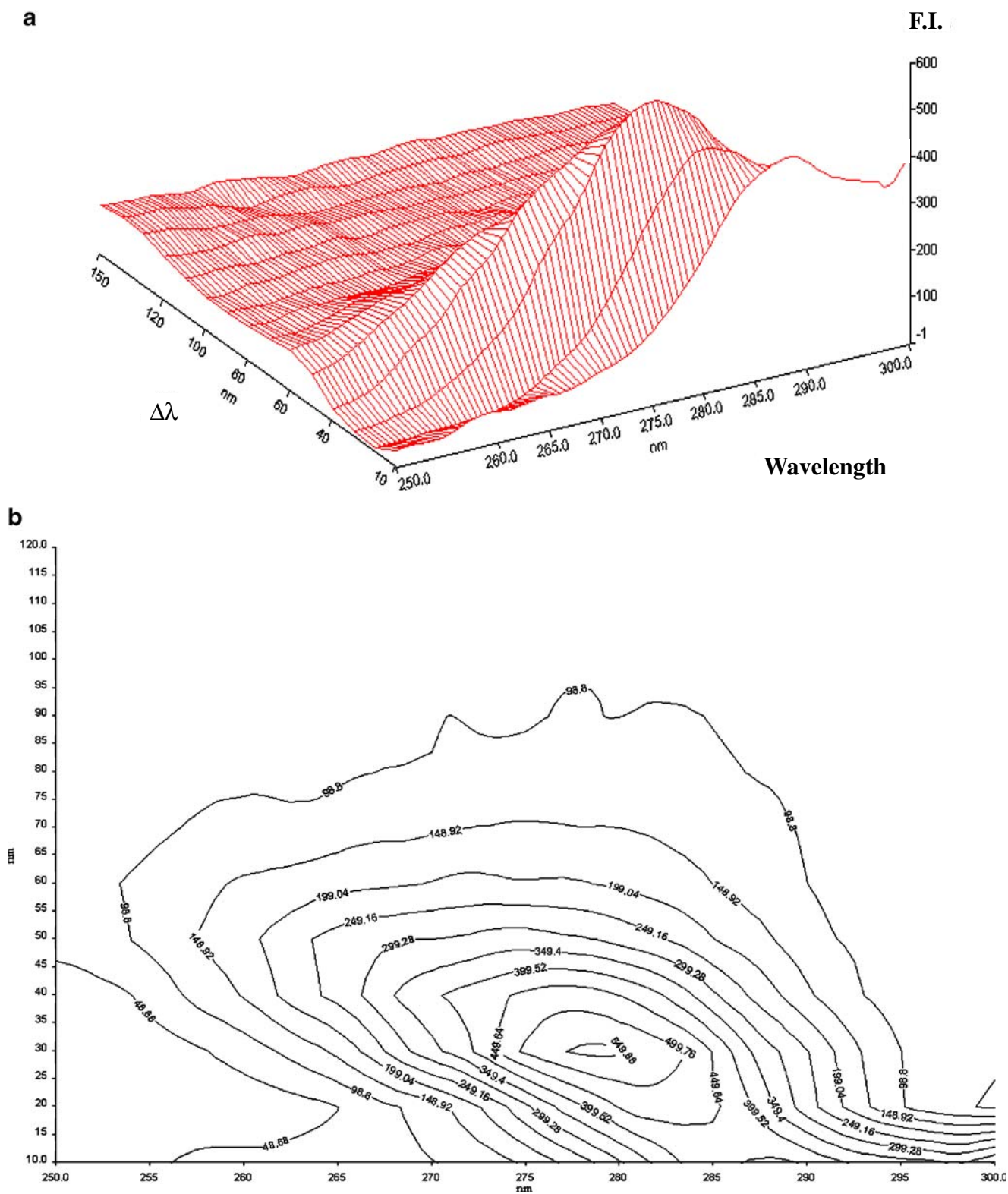


Fig. 9 **a** Surface projection of RTH 6.18×10^{-6} mol L⁻¹ in the aqueous medium. Conditions: $\Delta\lambda = 30$ nm and wavelength scanning speed = 600 nm/min. **b** Contour map of RTH 6.18×10^{-6} mol L⁻¹ in aqueous medium at $\Delta\lambda = 30$ nm

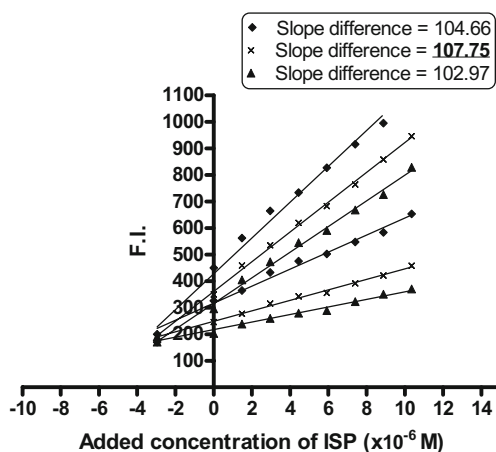


Fig. 10 Graph for selection of the best wavelengths pair according to the slope difference between the two line (at two different wavelengths) when ISP was added

The selection of λ_1 and λ_2

At optimum λ_1 and λ_2 the analyte signals must be linear with the concentrations; the interferent signals must remain constant, even if the analyte concentrations are changed. The analytical signals of the mixture composed from the analyte and the interferent signal should be equal to the sum of the individual signals of the two compounds. In addition, the slope difference of the two straight lines obtained at λ_1 and λ_2 must be as large as possible in order to get good accuracy. Figure 10 shows the H-point standard addition plots for synthetic test solutions of different pairs of wavelengths selected when ISP was added. Where the higher the value for the slope increment, the lower the error for the analyte concentration. Where the wavelength pair that gives the greatest slope increment was selected. It is clear that the synchronous fluorescence intensities of RTH are equal with higher slope difference of the two line obtained at $\lambda_1=268.76$ nm and $\lambda_2=289$ nm were selected in further experiments for determination of ISP in the presence

of RTH as interferent. Also, the synchronous fluorescence intensities of ISP are equal with higher slope difference of the two line obtained at $\lambda_1=264.72$ nm and $\lambda_2=287$ nm which were selected in further experiments for determination of RTH in the presence of ISP as interferent.

Data analysis using H-point standard addition method

Under optimum conditions, speciation of RTH and ISP was carried out by using HPSAM. As shown in Table 1, for four replicate experiments, RTH and ISP concentrations found are in good agreement with the added concentrations. The concentration of interferent was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H-point (A_H). Several synthetic mixed samples with different concentration ratios of RTH and ISP were analyzed using the proposed method. As shown in Table 2, the accuracy and precision of the results are all satisfactory, when the concentration ratio of RTH and ISP vary from 1:5 to 5:1. In addition, the method was applied for determination of both drugs in synthetic mixture of pharmaceutical preparations. The quantitative results of this analysis are summarized in Table 3. As it is shown, RTH and ISP can be determined with satisfactory accuracy and precision in pharmaceutical preparations.

Conclusion

The proposed method was accurate, simple, not time consuming. Compared with synchronous fluorimetry or HPSAM, the coupling technique gave better resolution for binary mixtures of RTH and ISP with symmetric and seriously overlapped peaks. This coupling technique is simple in comparison with derivative synchronous fluorimetry where it does not require the apparatus with a derivative function.

Table 1 Accuracy and precision for the analysis of synthetic mixtures of RTH and ISP

Regression equation	r	Conc. taken ($\times 10^{-6}\text{molL}^{-1}$)		Conc. found ($\times 10^{-6}\text{molL}^{-1}$)	
		RTH	ISP	RTH	ISP
$I_{f\ 268.76}=172.7 C_i+373.51$	0.9989	4.32	2.96	4.35	2.93
$I_{f\ 289}=63.401 C_i+260.73$	0.9974				
$I_{f\ 268.76}=172.23 C_i+374.19$	0.9987	4.32	2.96	4.26	2.93
$I_{f\ 289}=63.514 C_i+260.45$	0.9973				
$I_{f\ 268.76}=172.32 C_i+372.95$	0.9988	4.32	2.96	4.29	2.90
$I_{f\ 289}=63.614 C_i+259.75$	0.9968				
$I_{f\ 268.76}=171.65 C_i+373.92$	0.9990	4.32	2.96	4.29	2.87
$I_{f\ 289}=64.104 C_i+260.93$	0.9964				

I_f is fluorescence intensity at the specified wavelength, C_i is concentration of analyte added, r is correlation coefficient

Table 2 Analysis of Ritodrine and Isoxsuprine in synthetic mixtures of different concentrations and proportions

Regression equation	r	Conc. taken (x 10 ⁻⁶ molL ⁻¹)		Conc. found (x 10 ⁻⁶ molL ⁻¹)	
		RTH	ISP	RTH	ISP
I _{f 268.76} =156.16 C _i +454.08	0.9982	2.47	5.92	2.53	6.04
I _{f 289} =61.513 C _i +254.77	0.9995				
I _{f 268.76} =165.53 C _i +379.15	0.9992	2.47	4.44	2.5	4.53
I _{f 289} =62.692 C _i +228.96	0.9987				
I _{f 268.76} =160.42 C _i +296.54	0.9995	3.08	2.44	2.96	2.47
I _{f 289} =60.066 C _i +193.43	0.9976				
I _{f 268.76} =172.7 C _i +373.51	0.9989	2.93	4.35	2.96	4.32
I _{f 289} =63.401 C _i +260.73	0.9974				
I _{f 268.76} =170.63 C _i +349.22	0.9993	3.08	3.71	2.96	3.71
I _{f 289} =61.252 C _i +240.1	0.9965				
I _{f 268.76} =160.42 C _i +296.54	0.9995	3.08	2.53	2.96	2.47
I _{f 289} =60.066 C _i +193.43	0.9976				
I _{f 287} =152.2 C _i +413.5	0.9971	2.34	6.39	2.37	6.17
I _{f 264.72} =67.108 C _i +248.35	0.9943				
I _{f 287} =159.03 C _i +341.3	0.9971	2.37	4.73	2.37	4.63
I _{f 264.72} =60.987 C _i +201.81	0.9952				
I _{f 287} =141.18 C _i +257.89	0.9986	2.46	3.15	2.37	3.09
I _{f 264.72} =61.117 C _i +164.82	0.9983				
I _{f 287} =153.73 C _i +331.04	0.9996	4.11	3.15	4.14	3.09
I _{f 264.72} =66.105 C _i +241.95	0.9993				
I _{f 287} =152.28 C _i +301.77	0.9996	3.55	3.0	3.55	3.09
I _{f 264.72} =65.586 C _i +215.13	0.9991				
I _{f 287} =141.18 C _i +257.89	0.9986	2.34	3.15	2.37	3.09
I _{f 264.72} =61.117 C _i +164.82	0.9983				
I _{f 268.76} =153.75 C _i +232.14	0.9991	1.54	3.0	1.48	3.09
I _{f 289} =61.649 C _i +178.06	0.9975				
I _{f 268.76} =184.24 C _i +431.65	0.9980	1.57	7.78	1.48	7.72
I _{f 289} =67.762 C _i +364.55	0.9981				
I _{f 287} =160.26 C _i +220.5	0.9990	3.02	1.61	2.96	1.54
I _{f 264.72} =72.402 C _i +167.28	0.9957				
I _{f 287} =132.34 C _i +510.51	0.9971	7.31	1.48	7.40	1.54
I _{f 264.72} =68.479 C _i +472.36	0.9952				

Table 3 Analysis of Ritodrine and Isoxsuprine in Yutopar and Duvadilan tablets

Sample	Conc. found (x 10 ⁻⁶ molL ⁻¹)		Conc. taken (x 10 ⁻⁶ molL ⁻¹)		Recovery (%)	
	RTH	ISP	RTH	ISP	RTH	ISP
1	2.47	2.96	2.56	3.08	103.75	104
2	3.09	3.55	3	3.46	97	97.5
3	6.18	2.37	6.39	2.34	103.5	98.75

Yutopar tablets labeled to contain 10.mg RTH per tablet

Batch number 198

Duvadilan tablets labeled to contain 20 mg ISP per tablet.

Batch number 269

Both are products of Pharco Pharmaceutical Company, Alexandria, Egypt

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