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Validated Spectrophotometric Determination of Ritodrine, Isoxsuprine and Raloxifene Using Nitrosation Reaction and Derivative of the Differential Absorbance

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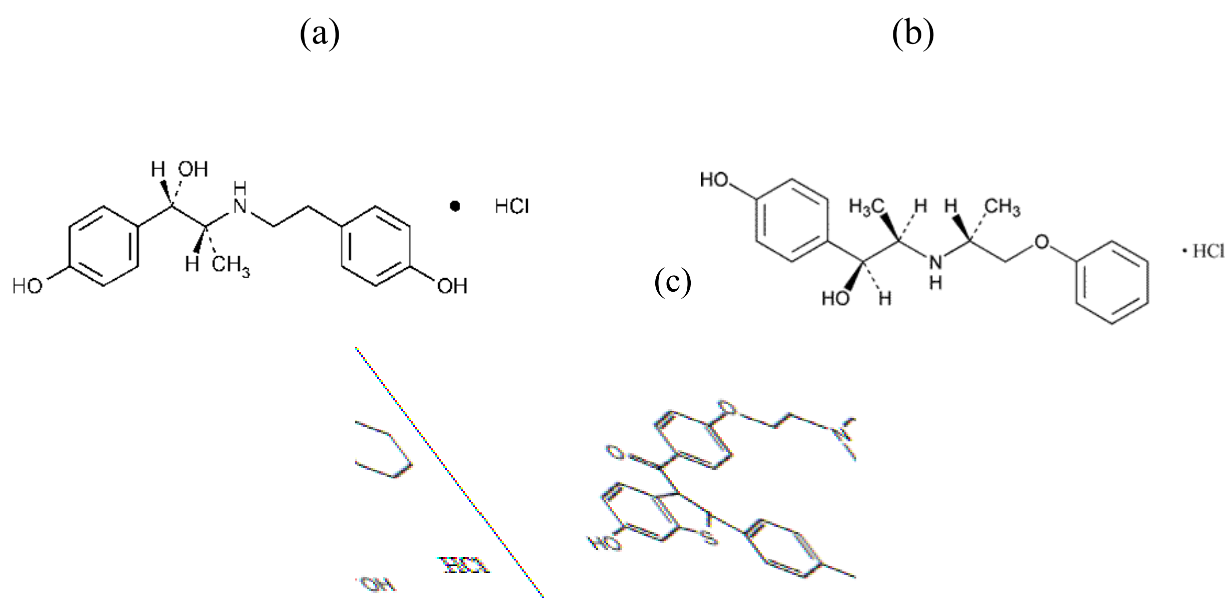
Summary: Two simple, accurate, sensitive and economic methods have been developed for the quantitative determination of ritodrine hydrochloride (RTH) isoxsuprine hydrochloride (ISP) and raloxifene hydrochloride (RLX). The first method is based upon a simple nitrosation reaction where the three drugs have been reacted with sodium nitrite in acidic medium to form the corresponding nitroso derivatives. They have λ max at 386, 387 and 389 nm, respectively (Method A). The second method is based upon the differential derivative spectrophotometry where the three investigated drugs were determined through measuring the amplitude of the first derivative of the difference absorption (ΔD^1) in alkaline solution against acidic solution as blank at 239- 251, 303 and 239- 271 nm, respectively (Method B). All the variables affecting the two methods were carefully studied and optimized. The two methods were successfully applied for determination of the drugs in bulk and in different pharmaceutical formulations where a thorough research was done to detect any interference of each formulation additive. Moreover a study of the formed nitroso derivatives was done and interpretation of the resulted data was presented.

Introduction

Ritodrine (RTH) is β_2 adrenergic receptor agonist that was developed as uterine relaxant used to delay premature labor [1], isoxsuprine (ISP) is a beta-adrenergic agonist that causes direct relaxation of uterine and vascular smooth muscles.

Therefore, it is used in women for treatment of premature labour [2] and raloxifene (RLX) is a selective estrogen receptor modulator (SERM); it is an estrogen receptor antagonist in both breast and endometrial tissue but has agonist action on bone and in lowering total cholesterol and low density lipoprotein (LDL). It is used for the prevention and treatment of osteoporosis in post menopausal women [1] [Fig.1]. All are used to treat female hormonal disorder. RTH and ISP are official in the British pharmacopoeia where RTH is determined by HPLC procedure and ISP is determined spectrophotometrically [3]. RLX is official in United State pharmacopoeia where an HPLC is reported for its determination [4]. Various analytical methods have been applied for the determination of RTH, ISP and RLX among which the spectrophotometric methods for RTH [5-8], ISP [9-11] and for RLX [12-14], and Fluorimetric methods for RTH and ISP [15]. Other methods were reported including gas chromatography for RTH [16, 17] and ISP [18, 19], thin layer chromatography for RTH and ISP [20], HPLC methods for RTH [21-23], ISP [22, 24] and for RLX [25] and electrochemical methods for ISP [26] and for RLX [27, 28].

Figure (1): Chemical structure of RTH (a), ISP (b) and RLX (c).



In this work, the reaction between the three drugs bearing phenolic groups and nitrous acid evolved as a reaction between sodium nitrite and hydrochloric acid was used to produce nitroso derivatives which were studied in an attempt to develop a simple spectrophotometric procedure for the drugs determination and the formed

nitroso derivatives were separated and investigations were done for its identification (method A). A second method was presented based upon the first derivative of the differential absorbance of the three drugs which was used for their quantitative determination (method B). The presented methods results were applied for the determination of RTH, ISP and RLX in pharmaceutical formulations and produced satisfactory results. Results were compared with pharmacopeial or reported methods where as no significant differences were observed.

Experimental

Apparatus

Schimadzu ultraviolet/ visible recording spectrophotometer (UV-160A PC, Schimadzu, Kyoto, Japan)

Ultrasonic processor, sonicator (type USR3/2 907, Julabo Labortechnik, D-7633 Seelbach, West Germany)

Magnetic stirrer (Wheaton, type rc-2, Rikakikai corp, Tokyo, Japan)

Schimadzu electronic balance (type AGE-220, Schimadzu, Kyoto, Japan)

Melting point were run on the Differential scanning calorimeter 50, Schimadzu, Kyoto, Japan

Mass spectra were run on Hewlett Packard 5988 spectrometer, Microanalytical Center, Cairo University.

Materials and Reagents

Ritodrine hydrochloride was kindly supplied by Pharco Pharmaceuticals Company Alexandria, Egypt, the dosage form "Yutopar®" tablets and ampoules manufactured by Pharco Pharmaceuticals Company Alexandria, Egypt, "Yutopar®" tablets labeled to contain 10 mg of RTH; "Yutopar®" ampoules labeled to contain 50 mg RTH/ 5 mL were purchased from local market.

Isoxsuprine hydrochloride was kindly supplied by Sedico Pharmaceuticals Company, October 6th City, Egypt, the dosage forms used were, "Vascular®" tablets manufactured by Sedico Pharmaceuticals Company, October 6th City, Egypt and labeled to contain 20 mg of ISP and "Duvadilan®" tablets manufactured by Pharco Pharmaceuticals Company Alexandria, Egypt and labeled to contain 20 mg ISP; were purchased from local market.

Raloxifene hydrochloride was kindly supplied by Amoun Pharmaceuticals Company, El-Obour City, Egypt, the dosage forms used were, "Evista®" film coated tablets manufactured by Eli Lilly and company, USA and labeled to contain 60 mg RLX and "Ralox®" film coated tablets manufactured by Amoun Pharmaceuticals Company, El-Obour City, Egypt and labeled to contain 60 mg RLX; were purchased from local market.

Hydrochloric acid, (Merck KGaA, Darmstadt, Germany)

Sodium nitrite powder (PRS, Barcelona, Spain)

Sodium hydroxide pellets (Merck KGaA, Darmstadt, Germany)

Methanol analar (SIGMA Pharmaceuticals Industries Company, October 6th City, Egypt)

The following reagents were also prepared:

1 mol L^{-1} and 2 mol L^{-1} sodium nitrite

0.05, 0.5, 0.8 and 1 mol L^{-1} hydrochloric acid

0.02 and 0.2 mol L^{-1} sodium hydroxide

Standard Stock Solutions

Ritodrine

RTH (25 mg) was accurately weighed, transferred into 100 mL volumetric flask, dissolved in water and then completed to volume. (RTH-A solution)

RTH (5 mg) was accurately weighed, transferred into 100 mL volumetric flask dissolved in water and then completed to volume. (RTH- B solution)

RTH-B solution (10 mL) was pipetted accurately into 100 mL volumetric flask and completed to volume with 0.2 mol L^{-1} sodium hydroxide solution (RTH-C solution)

RTH-B solution (10 mL) was pipetted accurately into 100 mL volumetric flask and completed to volume with 0.05 mol L^{-1} hydrochloric acid solution (RTH-D solution)

Isoxsuprine

ISP (25 mg) was accurately weighed, transferred into 100 mL volumetric flask, dissolved in warm water and then completed to volume. (ISP-A solution)

ISP (10 mg) was accurately weighed, transferred into 100 mL volumetric flask, dissolved in warm water and then completed to volume. (ISP-B solution)

ISP-B solution (40 mL) was pipetted accurately into 100 mL volumetric flask and completed to volume with 0.2 mol L^{-1} sodium hydroxide solution (ISP-C solution)

ISP-B solution (40 mL) was pipetted accurately into 100 mL volumetric flask and completed to volume with 0.05 mol L^{-1} hydrochloric acid solution (ISP-D solution).

Raloxifene

RLX (5 mg) was accurately weighed, transferred into 100 mL volumetric flask, dissolved in methanol and then completed to volume. (RLX-A solution)

RLX-A solution (10 mL) was pipetted accurately into 100 mL volumetric flask and completed to volume with 0.02 mol L^{-1} sodium hydroxide solution (RLX-B solution)

RLX-B solution (10 mL) was pipetted accurately into 100 mL volumetric flask and completed to volume with 0.5 mol L^{-1} hydrochloric acid solution (RLX-C solution)

Procedure

Recommended Procedure (method A)

Aliquots of the RTH-A, ISP-A and RLX-A solutions were transferred into three separate sets of 25 mL volumetric flasks; amounts of sodium nitrite solutions and hydrochloric acid solutions were added. The reactions were kept under the optimal temperature (15 c^{o} for RTH and room temperature for ISP and RLX); for specific time (10 min, 25 min and 40 min for RTH, ISP and RLX respectively). Each flask was completed to volume with the solvent. Table (1) shows the experimental condition for each drug. The absorbance of each of the yellow colored chromogen was measured at the maximum wavelength and the absorbance *vs.* the final concentration was plotted to get the calibration graph. Thus, the regression equations were driven.

Recommended Procedure (method B)

Different aliquots of both alkaline and acidic (RTH- C, D solutions), (ISP- C, D solutions) and (RLX- B, C solutions) were introduced into six different sets of 10 mL measuring flasks, the volume in each set was completed with the same solvent. Table (1) summarizes the experimental condition and the spectral characteristics. The first derivative of the difference absorption spectra (ΔD^{1}) of the alkaline sets of the drugs against those of the acidic sets were recorded using the following experimental parameters; Δ λ = 2 nm, scaling factor = 50, speed = medium, slit = 2 nm. The calibration curves were plotted using amplitudes of the first derivative of the difference absorbance *vs.* concentrations.

Procedure for Tablet Preparation

Twenty tablets of RTH, ISP and RLX were accurately weighed and pulverized, then a specified quantity of the active ingredient was transferred into 100 mL measuring flask; made up to volume with the solvent, stirred on a magnetic stirrer for

5 minutes using gentle speed, then filtered. The procedure was completed as mentioned under *2.4.1.a.* and *2.4.1.b.* using different aliquots of the tablet solutions. The specified quantity of the active ingredient of each drug, solvent and aliquots range used, all were mentioned in table (2). The nominal content of the drugs were computed from the corresponding regression equations.

Table (1) Experimental Condition of the Proposed Methods

items	RTH	ISP	RLX
Solvent used	distilled water	Warm distilled water	methanol
Method A (Nitrosation)			
Concentration range (µg/ml)	20-90	15-65	4-20
Amount, concentration of $NaNO_2$ solution	4mL of 2 mol L^{-1}	3mL of 2 mol L^{-1}	1mL of 1 mol L^{-1}
Amount, concentration of HCl	1mL of 0.8 mol L^{-1}	1mL of 1 mol L^{-1}	1mL of 0.5 mol L^{-1}
Temperature C°	15	room temp.	room temp.
Time of reaction (min.)	40	25	10
Wavelength of measurment (nm)	387	386	389
Method B (ΔD^1)			
Concentration range (µg/ml)	1-6	10-40	0.5-4.5
Concentration of NaOH	0.2 mol L^{-1}	0.2 mol L^{-1}	0.02 mol L^{-1}
Concentration of HCl	0.05 mol L^{-1}	0.05 mol L^{-1}	0.05 mol L^{-1}
Temperature C°	room temp.	room temp.	room temp.
Amplitude measured (nm)	239-251	303	239-271
Wavelength range (nm)	200-450	220-400	220 - 350
Scale	-0.60, +0.60	-0.99, +0.60	-0.60, +0.60

Table (2) Experimental Condition and Recovery Study for the Pharmaceutical Preparation

	RTH		ISP		RLX	
	Yutopar® tablets	Yutopar® ampoules	Vascular® tablets	Duvadilan® tablets	Evista® tablets	Ralox® tablets
Solvent Used	water		warm water		methanol	
Method A						
Concentration range (µg/ml)	20-60	25-60	20-40	20-40	4-12	4-12
% Recovery Tablets	101.2±1.42	101.6±0.52	99.75±0.72	100±0.752	100.23±0.63	99.96±0.819
% Recovery Added	98.6±1.41	100.7±1.79	100.26±1.65	99.4±1.029	99.03±1.524	99.66±1.456
Method B						
Weighed amount (µg)	50	50	$10x10^3$	$10x10^3$	5	5
Concentration range (µg/ml)	1-4	1.5- 2.5	15-25	15-25	1.5-2.5	1.5-2.5
% Recovery Tablets	100.12±1.26	100.91 ±1.24	100.86 ±1.12	100.21± 1.24	100.64 ± 0.51	100.43 ± 0.963
% Recovery Added	99.26± 1.66	101.13 ± 1.86	100.46 ± 1.42	99.7 ± 1.012	99.13 ±1.75	99.22 ±0.926

Procedure for Ampoules Preparation

The content of ten ampoules was mixed thoroughly; specified volume containing amount of the active ingredient was pipetted into 100 mL volumetric flask and the flask was made up to volume with distilled water and mixed well. The final solution was used to apply procedures *2.4.1.a* and *2.4.1.b* using aliquots equivalent to 25- 60 µg / mL and 1.5-2.5 µg/mL respectively. The nominal content of the drugs were computed from the corresponding regression equation.

Preparation of the Nitroso Derivative

A quantity of 0.3 g of RTH and ISP and 0.5 g of RLX was accurately weighed, transferred into three separated conical flask, dissolved in 15 mL water for RTH, ISP and in methanol for RLX then 10 mL of 1 mol L^{-1} hydrochloric acid was added to each flask, each solution was then, titrated using 15 mL of 2 mol L^{-1} sodium nitrite solution while stirring in ice bath using magnetic stirrer. The resulting solutions were kept refrigerated for two days. The resulting yellow precipitates were filtered and triturated with methylene chloride and then, left to dry.

Results and Discussion

Method A

The nitrosation of phenol group bearing molecules in the presence of nitrous acid resulted from a reaction between sodium nitrite and hydrochloric acid (29), ended with the introduction of nitroso group; this fact was used to produce the nitroso derivative of RTH and ISP followed by chelation (30) or formation of meisenheimer complex (31). A simple nitrosation reaction was used for the determination of RTH, ISP and RLX where maxima used for their determination were at 387, 386 and 389 nm, respectively, (Fig. 2).

Method B

The difference maxima intensities of the basic and acidic spectra of the cited drugs [Fig 3] encourage the authors to use another simple method for determination of RTH, ISP and RLX which is the first derivative of the difference absorption (ΔD^1) of the basic medium relative to that of an equivalent solution of the same drug concentration in the acidic medium. The wave lengths used were 239 to 251, 303 and 239 to 271 nm, respectively, [Fig. 4].

Figure (2): The UV spectra of the nitrosation derivatives of RTH at λ=387nm (90 μg mL^{-1}) (a), ISP λ=386nm (65 μg mL^{-1}) (b) and RLX at λ=389 nm (16 μg mL^{-1}) (c).

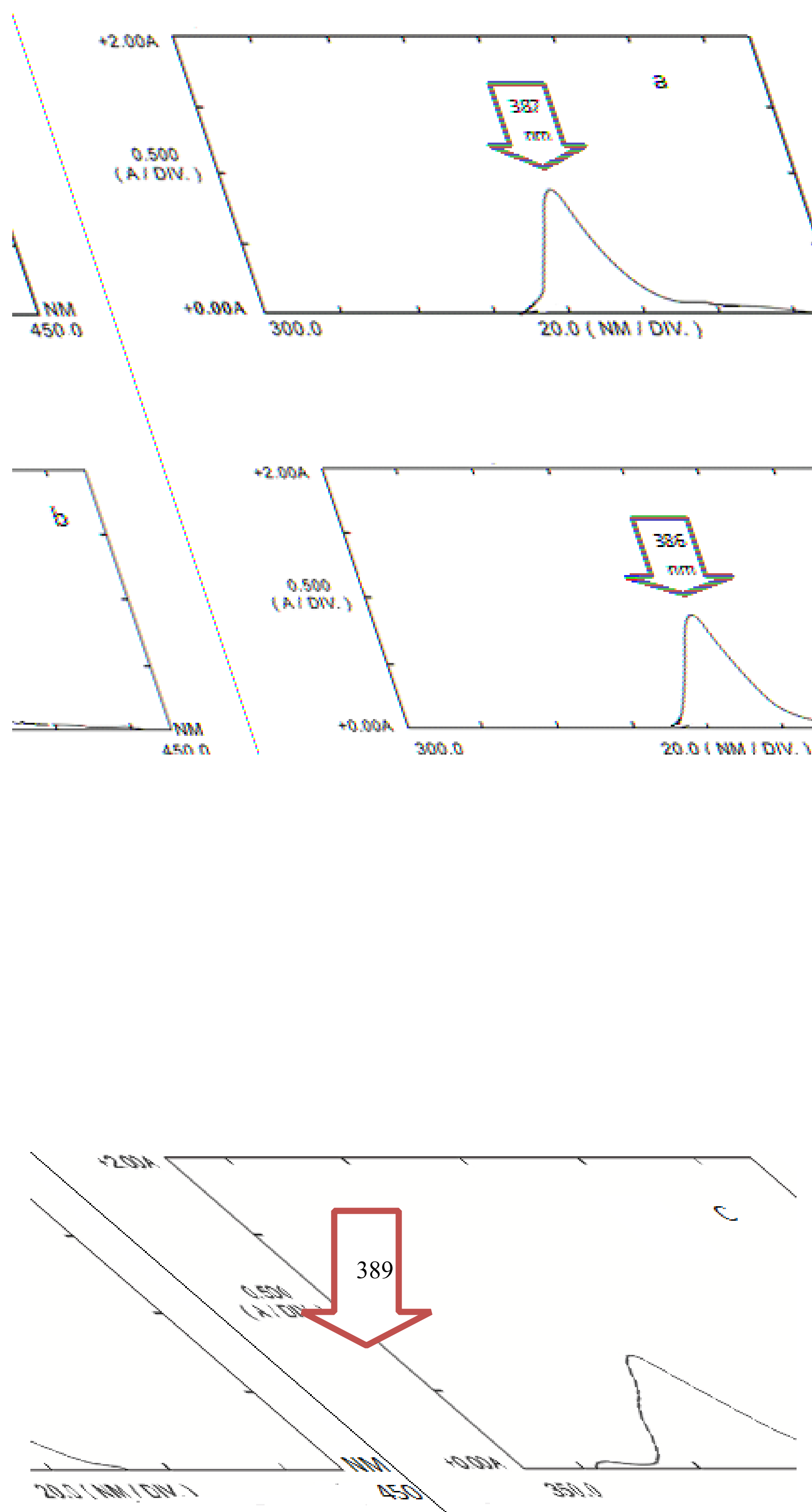
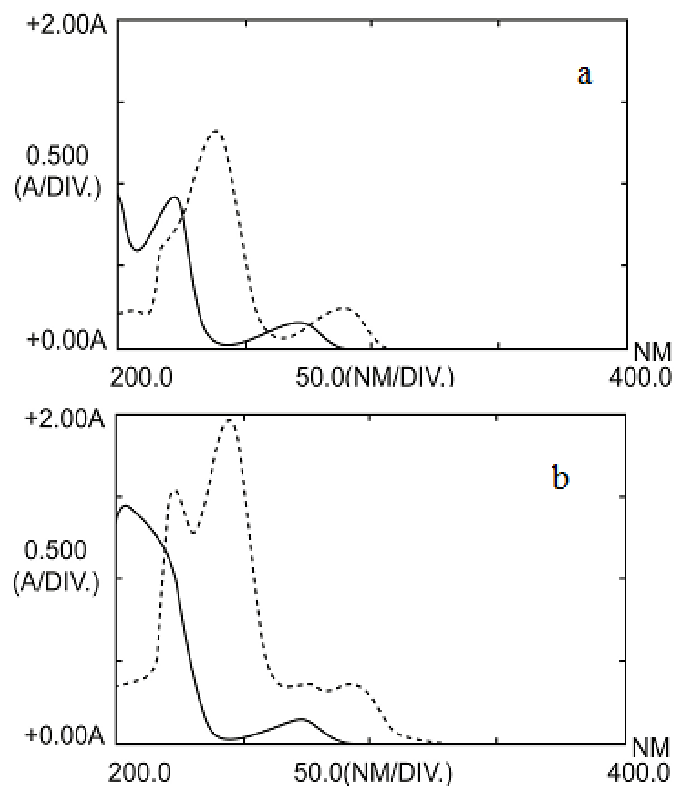


Figure (3): The zero order UV spectra of RTH (12 µg mL^{-1}) (a), ISP (45 µg mL^{-1}) (b) and RLX (8.5 µg mL^{-1}) (c) (-) acidic spectra of (a), (b) and (c) in 0.05 mol L^{-1} HCl, (---) alkaline spectra of (a) and (b) in 0.2 mol L^{-1} NaOH and (c) in 0.02 mol L^{-1} NaOH.



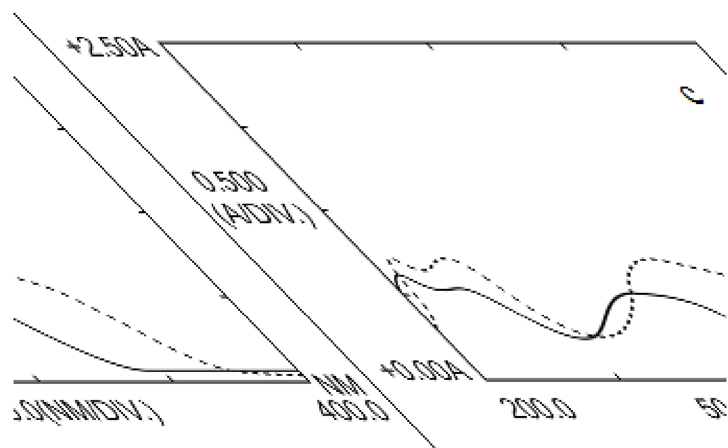
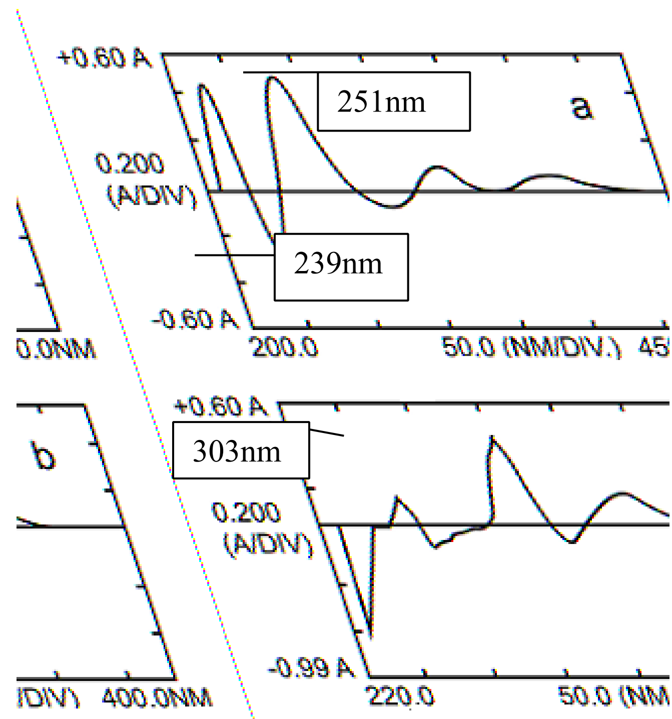
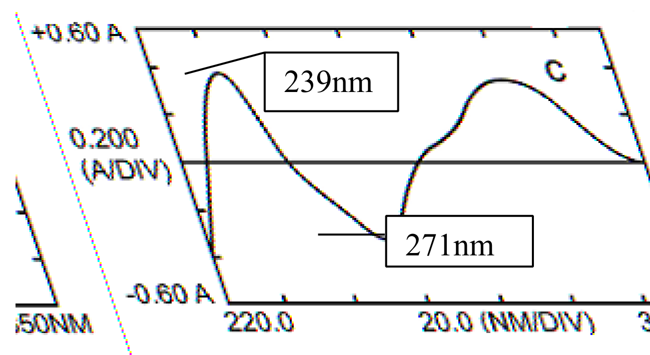


Figure (4): The first derivative spectra of the differential absorbance of RTH (4.5 µg mL^{-1}) (a), ISP (34 µg mL^{-1}) (b) and RLX (4 µg mL^{-1}) (c).





Study of the Experimental Conditions

Method A

Factors affecting the formation of the nitroso derivative were first studied. All factors were kept fixed where only one factor was changed in turn. These factors were; time of reaction, reaction temperature, amount and concentration of sodium nitrite, the same for hydrochloric acid. The formed nitroso derivatives were stable for at least for 1 hour for *RTH* and *ISP* and 30 minutes for *RLX*. All optimized factors were incorporated in the used procedures.

Method B

Different alkali and acid concentrations were tried and 0.2 mol L^{-1} sodium hydroxide was enough to establish both sensitivity and reading stability for RTH and ISP where 0.02 mol L^{-1} sodium hydroxide was acceptable for RLX, also 0.05 mol L^{-1} hydrochloric acid ensures the former requirements for the three drugs.

Linearity

The linearity of either absorption (method A) or amplitude (method B) responses vs. concentration was studied over the examined concentration ranges. Regression equations, analysis and average, other optical characteristics are presented in table (3).

Accuracy

Accuracy was studied using five different concentrations of the three drugs. Recovery data are shown in table (3).

Validity and Specificity

To ascertain validity of the method; known amounts of pure drug were added to the pharmaceutical dosage forms previously analyzed by the proposed methods. The average drug content of the labeled claim for all products tested and the added pure drugs amounts are tabulated in table (2). Also, the specificity was tested through check of the additives added to each pharmaceutical preparation used in the study and after excluding the non water soluble material for RTH or ISP dosage forms and the non- alcoholic soluble material for RLX dosage forms, a study have been made to

ensure a free interference results. Each additive was added separately to two concentrations of the respective drug in five different trials where a negative interference was obtained. Percent recovery achieved of the interferences studies were shown in table (4). Results revealed no interference from the excepiants used in the manufacture of the tested pharmaceutical products at their regularly added levels.

Statistical Comparison

The proposed method was compared to the UV spectrophotometric methods (32, 3 and 13) to verify the results obtained from the proposed methods. A calibration graph was obtained for all the cited drugs. The concentration range, the reported maxima, the solvent used and the regression equations; all are listed in table (5). The results showed no significant difference between the reported and the proposed methods at the 95% probability level for *t* and *F* tests.

Table (3) Optical Characteristics, Accuracy and Precision of the Proposed Method

Items	RTH		ISP		RLX	
	Method A	Method B	Method A	Method B	Method A	Method B
λ max. of measurement (nm)	387	239 to 251	386	303	389	239 to 271
Beer's law limits **(**µg/ml**)**	20-90	1-6	15-65	12-40	4-20	0.5-4.5
*Sandell's sensitivity	0.1215	0.0048	0.1091	0.0475	0.0072	0.0325
Regression equation $A_{\lambda max}$=bC±a	0.0079 C±0.005	0.135c±0.026	0.0097C±0.006.	0.016c±0.015	0.043 C ±0.016.	0.222±0.028
Slope (b)	0.0079	0.135	0.0097	0.016	0.043	0.222
Intercept (a)	0.005	0.026	-0.006	0.015	0.016	-0.028
R^2	0.999	0.999	0.9997	0.999	0.9998	0.999
S_b	0.0108	0.233	0.0080	0.013	0.0147	-0.0219
S_a	0.0078	0.135	0.0097	0.016	0.0429	0.219
LOD (µg)	2.28	1.449	2.32	2.094	1.5	2.041
LOQ (µg)	7.6	4.83	7.73	6.98	5	6.804
CL of slope.	0.081 ± 0.00745	0.279±0.0187	0.0099 ± 0.0094	0.238±0.0013	0.0441 ± 0.0418	0.0151±0.0289
CL of intercept.	0.0317 ±0.0101	0.0702±0.0666	0.0051 ±0.021	0.0165±0.0155	0.028 ± 0.0018	0.222±0.0217
Standard error of estimation.	0.00315	0.0008	0.0031	0.0016	0.0028	0.00163
**CL of accuracy	99.44±1.604	99.2±1.093	99.21±1.5	99.34±1.487	99.53±1.095	100.05±1.469

- $\mu g ml^{-1}$ 0.001 absorbance unit
- ** Average of five concentrations of triplet replications.

Table (4) Selectivity Results

		Method A						Method B					
Amount added to pure drug		5mg	2.5mg	5mg	2.5mg	5mg	2.5mg	5mg	2.5mg	5mg	2.5mg	5mg	2.5mg
	n	Recovery%		SD		SE		Recovery%		SD		SE	
Yotupar® tablets													
Polyvinylpyrrolidone	5	100.00	100.10	0.561	0.640	0.251	0.286	99.9	99.4	0.510	0.540	0.287	0.286
Lactose	5	100.40	100.10	0.717	0.7396	0.321	0.331	99	99	0.514	0.526	0.211	0.277
Yotupar® Ampoules													
Sodium chloride + gl. acetic acid (pH 7)	5	99.70	99.30	0.804	0.458	0.360	0.205	99.39	99.2	0.348	0.39	0.149	0.196
Sodium lauryl sulfate	5	100.40	100.60	0.766	0.909	0.343	0.407	100	99.8	0.363	0.394	0.152	0.183
Vascular® tablets/ Duvadilan® tablets													
Mannitol	5	99.90	99.70	0.600	0.598	0.268	0.267	99.68	99.2	0.730	0.764	0.332	0.275
Lactose	5	99.50	99.90	0.452	0.736	0.202	0.329	99.3	99.3	0.719	0.7771	0.297	0.358
Evista® tablets/ Ralox® tablets													
Macrogol 400	5	99.98	99.80	0.867	0.663	0.388	0.296	100.3	100.1	0.630	0.643	0.280	0.291
Lactose	5	99.60	99.60	0.488	0.634	0.218	0.284	99.80	99.80	0.627	0.643	0.279	0.240
Polysorbate 80	5	99.80	100.10	0.643	0.817	0.288	0.365	100.5	100.2	0.653	0.603	0.288	0.295
Propylene glycol	5	99.30	99.90	0.769	0.506	0.344	0.226	100.2	100.3	0.670	0.610	0.264	0.276

Table (5) Statistical results of the proposed methods

	RTH			ISP			RLX		
	Method A	Reference method *	Method B	Method A	Reference method **	Method B	Method A	Reference method ***	Methoc
ent used	water	1molL^{-1} hydrochloric acid	water	warm water	0.1molL^{-1} hydrochloric acid	**wa**rm water	methanol	methanol	methar
. range	20-90	3-8µg/ml	1-6	15-65	5-45µg/ml	12-40	4-20	5-25µg/ml	0.5-4.
x.	387 nm	274 nm	(251-239) nm	386 nm	275 nm	303 nm	389 nm	289 nm	(239-271)
ession equation	A = 0.0079C + 0.005	A = 0.052C + 0.007	A = 0.135C + 0.026	A = 0.0097C - 0.006	A= 0.008C + 0.007	A = 0.016C + 0.015	A = 0.043C + 0.016	A = 0.016C - 0.002	A = 0.22 0.028
n	99.44	100	99.24	99.21	99.24	99.40	99.53	99.78	100.0
	0.753	0.954	0.513	0.704	0.4827	0.715	0.514	0.4604	0.680
	0.337	0.427	0.229	0.315	0.2159	0.320	0.230	0.206	0.304
D	0.757	0.954	0.515	0.710	0.4845	0.717	0.516	04609	0.680
	5	5	5	5	5	5	5	5	5
	0.567	0.910	0.263	0.496	0.233	0.511	0.264	0.212	0.462
ue(2.13)****	1.03			0.0681			0.810		
		1.569			0.3627			0.7076	
ue(6.39)****	1.603			2.125			1.246		
		3.460			2.197			2.184	

*Spectrophotometric method [32] **BP 2009[3]

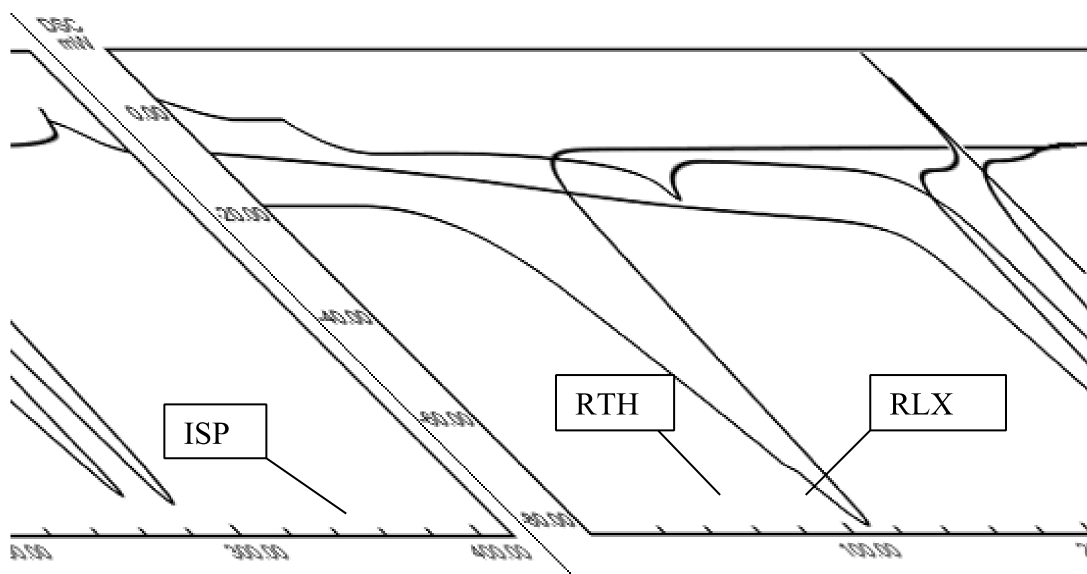
*** Spectrophotometric method [13].

**** Figures in parentheses are the tabulated *t* and *F* values at p= 95%.

Identification of the Nitroso Derivatives

The reported melting points of RTH, ISP and RLX were 196, 102 and 258 °C respectively. The practical ones were found to be 200,100 and 260 °C, where those of the formed nitroso derivatives were 267, 110 and 285 °C for RTH, ISP and RLX nitroso derivatives, respectively, (Fig. 5).

Figure (5): The melting point of RTH, ISP and RLX nitroso derivatives by DSC.



The mass spectrometric fragmentation of the formed nitroso derivatives of RTH, ISP and RLX, show the base peak at 383.5 369, and 569 respectively which was inaccordance with the expected mass of the nitroso derivative (381.5, 366.5 and 567.5 respectively). It is obvious and evidence from the comparison of M.wt of each drug 323.82, 337.84 and 510.05 respectively and the molecular ion peak of its nitroso derivative that two nitroso groups were introduced into RTH and RLX where only one

was introduced into ISP as shown by the matching of the calculated and found molecular weight.

Conclusion

The proposed methods have been evaluated over the linearity, accuracy and specificity and proved convenience and effectiveness for the quality control of RTH, ISP and RLX in their pharmaceutical dosage forms. The methodology requires simple reagents and apparatus which represent an economic procedure for routine laboratory analysis and quality control.

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