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Spectrophotometric Methods for The Determination of Raloxifene Hydrochloride and Isopreterenol Hydrochloride

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Summary- Spectrophotometric methods for the determination of raloxifene hydrochloride (I) and isoproterenol hydrochloride (II) in pure form and in pharmaceutical formulations are developed. Method A is based on the reaction of the studied drugs with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and cerium (IV) ammonium sulfate in an acidic medium. The colored compounds are measured at 565 and 519 nm. Method B is, mainly, based on the nitration of the drug molecule followed by the subsequent formation of Meisenheimer complex with acetone in alkaline medium. The experimental conditions leading to optimum chromogen intensity and stability were carefully studied. Method C for the determination of raloxifene hydrochloride is based on the selective oxidation of the drug with N-bromosuccinimide to give an intense yellow product measured at 450 nm.

The results of analysis were found to agree statistically with those obtained with either the official or reported methods. The proposed procedures are characterized by their simplicity with accuracy and precision.

Introduction

Raloxifene, [6-Hydroxy-2-(4-hydroxyphenyl)-benzo[b] thien-3-yl][4-[2-(1-piperidinyl)ethoxy] phenyl] methanone, is used as antiosteoporotic[1]. Only one HPLC method [2] is reported for its determination.

Isoproterenol, 4-[1-Hydroxy-2-[(1-methylethyl)-amino]ethyl]- 1,2-benzenediol, is used as bronchodilator[1]. Several methods have been reported for its determination including HPLC[3-7] either in pharmaceutical formulations or in biological fluids, G.C[8] and capillary electrophoresis[9]. El-Obeid determined isoproterenol colorimetrically using 4-aminobenzohydrazide[10]. Doty[11] investigated the reaction between isoproterenol and iron citrate in the presence of buffer pH 8. Wahbi et al[12] showed that the drug reacted with p-benzoquinone at pH 5.4. Other indicators such as 0.2% potassium persulfate, 0.1% sodium chloride in the presence of phosphate buffer pH 5.5[13] and

sodium cobaltinitrite in the presence of glacial acetic acid[14] were also applied for the spectrophotometric determination of isoproterenol. The structures of the studied drugs are shown in scheme I.

The purpose of this work is to determine the cited drugs in bulk powder and in pharmaceutical formulations using simple, rapid, precise, accurate and low cost methods.

Experimental

Apparatus

All spectral and absorbance measurements were made on SHEMADZUE UV/VIS.- 1601 PC spectrophotometer.

All chemicals used were of analytical grade and all solutions were freshly prepared.

1. Raloxifene hydrochloride bulk powder (Eli Lilly).
2. Isopreterenol hydrochloride (Neo pharma).
3. MBTH (Aldrich), 0.2% m/v aqueous solution.
4. Cerium (IV) ammonium sulfate solution 0.1% m/v in 5% sulfuric acid.
5. N-bromosuccinimide solution, 0.005 M in methanol.
6. Acetone (BDH Chemicals, laboratory-reagent grade).
7. Sodium hydroxide aqueous solution, 20% m/v.
8. The nitrating mixture was prepared by mixing concentrated nitric acid and sulfuric acid in a 1:1 ratio.
9. Raloxifene working standard, 200 $\mu g.ml^{-1}$, 2.0 mg ml^{-1} and 50 $\mu g.ml^{-1}$ in methanol for methods A, B and C, respectively.
10. Isopreterenol hydrochloride working standard, 200 $\mu g.ml^{-1}$, 2.0 mg. ml^{-1} in methanol for methods A and B, respectively.

Pharmaceutical formulations

* Evesta tablets (Eli Lilly) containing raloxifene hydrochloride equivalent to 60 mg/tablet.

*Isuprel injections (Abbott Laboratories) containing isoproterenol hydrochloride equivalent to 0.2 mg / ml.

*Sabex injections (Neo pharma) containing isoproterenol hydrochloride equivalent to 0.2 mg/ml.

General procedures for bulk powder

Method A

Aliquots of standard drug solution containing 50-400, 50-250 µg for (I) and (II), respectively were transferred into a series of 10-ml volumetric flasks then 2.0 ml 0f MBTH were added. After 5 min, 2.0 ml of cerium (IV) ammonium sulfate solution were added for both drugs and the contents were mixed thoroughly. After 20 and 30 min. for (I and II), respectively, the volumes were made up to 10 ml with water. The absorbance of the solutions were measured at 565 and 519 nm. for I and II, respectively against a blank reagent. The calibration curves were constructed and the regression equations were calculated.

Method B

Aliquots from standard drug solutions (2 $mg.ml^{-1}$ of each) 0.5-5 ml and 0.5-3.5 ml for (I) and (II), respectively, were pipette into a wide mouth thick wall test tube and subjected to evaporation using heated water bath. To the residue lefted in each test tubes, carefully, 2 ml from the nitrating mixture were then added (care must be taken during handling the nitrating mixture). The tubes were heated on boiling water bath for (I) and at 80 ^0C for (II) for 20 minutes for both. The cooled mixtures were transferred to separate 50-ml volumetric flasks and the volume was completed with water. From the nitrated solutions 1 ml were transferred into a set of 10-ml volumetric flasks, treated with 2 ml of acetone and 2 ml of 20% sodium hydroxide solution and diluted to volume with water. The absorbance of the resulting solutions was measured at 390 and 350 nm. for (I) and (II), respectively, against a reagent blank. The

calibration curves were constructed and the regression equations were calculated.

Method C for raloxifene hydrochloride

Aliquots of standard drug solution equivalent to 20-150 µg were pipetted into 10-ml volumetric flasks. To each flask 1.5 ml of 0.005 M N- bromosuccinimide solution was added and the volume was made up to 10 ml with methanol. The absorbance was measured at 450 nm against a blank reagent prepared similarly. The calibration curve was constructed and the regression equation was calculated.

Procedure for dosage forms

Tablets.

Ten tablets were weighed, powdered, mixed and an amount of the powder equivalent to 50 mg of raloxifene hydrochloride, was dissolved in methanol and transferred quantitatively to 25-ml volumetric flask. The volume was completed to the mark with methanol and filtered. The assay was completed as described above and the concentration was calculated from the regression equations.

Ampoules

a- An aliquot equivalent to 0.2 mg/ml was subjected to method A.

b- An aliquot from the mixed solution of fifteen ampoules equivalent to 2 mg was evaporated on a boiling water bath and the procedure for nitration and complex formation was completed as mentioned under method B.

Results and Discussion

Method A

The studied drugs form pink product (λmax. at 565, 519 nm for (I) and (II), respectively) with MBTH in the presence of cerium (IV) ammonium sulfate in acidic media. The molar absorptivety of the chromogen was found to be 1.30 and 1.07 x 10^4 (l mol^{-1} cm^{-1}) for (1) and (II), respectively.

Under the reaction conditions, MBTH on oxidation with Ce^{4+} ions loses two electrons and one proton forming an electrophilic intermediate which is the active coupling species[15].This intermediate undergoes electrophilic substitution with the phenolic moiety of the drug to form a colored product .

The optimum concentration of sulfuric acid solution in which cerium (IV) ammonium sulfate was dissolved was found to be 5%. Higher concentrations did not affect the color intensity. The optimum concentrations of MBTH and cerium (IV) ammonium sulfate leading to maximum color stability was found to be 2 ml of each. The maximum color intensity was obtained after 20 or 30 min. at room temperature for (I) and (II), respectively. The color was stable for 2 hours for both drugs. To obtain higher absorbance it is recommended that addition of water before the time required for development of the reaction should be avoided.

The linear correlation was found in the range 5-40 and 5-25 $\mu g.ml^{-1}$ with mean percentage accuracy 100.05±0.54 % and 98.99±0.74 % for (I) and (II), respectively..

Method B

Nitration of the cited drugs probably, results in the formation of the dinitro or polynitro derivatives in the para and ortho positions to the phenolic groups. By the introduction of these nitro groups either in the ortho or para, they will result in creating positive center which will favor nucleophilic attack by the acetone carbanion. The formed complex between the electron deficient polynitro derivative and the created acetone carbanion will result in the formation of colored complexes. These complexes are of Meisenheimer type [16] and differ from those obtained by adding only an alkali to the nitro derivatives.

A preliminary investigation was carried out to determine the optimum conditions for the nitration and subsequent Meisenheimer complex formation for the phenolic drugs.

Different procedures for nitration were tried, using sodium nitrate in sulfuric acid, fuming nitric acid or different proportions of concentrated nitric

acid to sulfuric acid. The best results were obtained using 2 ml of 1:1 mixture of concentrated nitric acid and sulfuric acid for both drugs.

A period ranging from 15-20 minutes on boiling water bath for (I) and 80 ^{0}C for (II) was found to be necessary for color development. The formed nitro precursors and the Meisenheimer complexes of the investigated drugs were stable for at least 2 hours.

Generally, maximum absorbance reading was obtained when using 2 ml of each of acetone and sodium hydroxide.

The absorption spectra of the reaction product of acetone-sodium hydroxide mixture with the nitrated compounds show maximum absorbance at 390 and 350 nm for I and II, respectively. Beer's law was validated from 2-18 and 2-14 μg.ml^{-1} with mean percentage accuracy of 99.31± 0.6 % and 99.54±0.31 % for I and II, respectively.

Method C for raloxifene hydrochloride

The drug was found to yield an intensely colored product with NBS in methanol medium probably due to oxidizing properties of this agent. Factors affecting the reaction were carefully studied to achieve quantitative results and it was established that 1 ml of 0.005 M NBS was sufficient to give maximum color in the range of the drugs concentration studied. The color developed immediately and remained stable for at least 1 hour. Water, methanol and ethanol were tested as diluting solvents and it was found that methanol was the best solvent for the procedure.

Under the specified reaction conditions, the absorbance at 450 nm. was found to be proportional to the concentration in the range 1-15 μg ml^{-1}. The most important spectral characteristics and quantitative parameters of the reaction investigated are shown in Table 1.

Application of Job's method of continuous variation using equimolar solutions of the studied drugs and NBS (1x 10^{-3} M) revealed a 1: 4 molar ratio (drug to NBS).

Quantitation, Accuracy and Precision

The reproducibility and accuracy of the proposed methods were assessed by using different concentrations of raloxifene and isoproterenol. The validity was checked occasionally during the assay. Standard calibration curves for the studied drugs were prepared by taking a series of different concentrations and applying the proposed methods. Beer's law is valid within the microgram concentration range of the studied drugs, Table 1. The regression equations of these calibration graphs were used to determine unknown concentrations of the drugs in pharmaceutical dosage forms. The results obtained were of good accuracy and precision.

Table 1: Spectral and quantitative parameters of the colored reaction product

Parameters	Raloxifene hydrochloride			Isoproternol hydrochloride	
	MBTH	Nitration	NBS	MBTH	Nitration
λ_{max} nm.	565	390	450	519	350
$\varepsilon \times 10^4$ (L/mol/cm)*	1.30	2.78	3.24	1.7	1.99
Linear range µg ml^{-1}	5-40	2-18	1-15	5-25	2-14
Intercept	0.0001	0.0065	0.0057	-0.0013	00076
Slope	0.0275	0.0578	0.0668	0.0393	0.0789
Correlation coefficient	1.00	1.00	0.9999	1.00	0.9998
Detection limit** µg ml^{-1}	0.52	0.19	0.1	0.47	0.20
Ringbom concentration µg ml^{-1}	5-35	2-15	1.5-14	5-22	2-12
Sandell sensitivity, µg. ml^{-1}	0.036	0.016	0.015	0.026	0.013

* Calculated on the basis of the molecular weight of the hydrochloride of the compound

** Calculated according to the formula stated in reference 18.

For more accurate results, Ringbom optimum concentration range was determined by plotting (c) in µg/ml against percent absorbance, and the linear portion of the S- shaped curve gave an accurate range of analysis.

The results of the proposed methods were statistically compared with those obtained by the reported [17] or official method[3]. Table 2 shows that the calculated F- values and t- test were less than the theoretical one, conforming accuracy and precision at the 95% confidence limit.

Table 2: Statistical comparison between results of analysis of bulk powder using the proposed methods and the reported or official methods.

	Raloxifene hydrochloride				Isoproterennol hydrochloride		
	MBTH	Nitration	NBS	Reported method[17]	MBTH	Nitration	Official method[3]
Mean%*	100.05	99.31	99.16	99.72	98.99	99.54	99.84
S.D.	0.54	0.61	0.80	0.37	0.74	0.31	0.40
Variance	0.29	0.37	0.64	0.14	0.55	0.10	0.16
t-	1.14	1.28	1.44		2.24	1.30	
F-	2.07	2.64	4.57		3.44	1.60	

Mean percentage recovery of five determinations.

Tabulated value for t- test =2.31 and for F- value = 6.39 at p = 0.05

The proposed methods were applied for the determination of the cited drugs in dosage forms and the validity was assessed by applying the standard addition technique. The results were reproducible with low standard deviations Tables 3 and 4.

Conclusion

The proposed methods are fairly sensitive, accurate and of good reliability owing to the high stability of the formed complexes. The results were reproducible and when compared with the reported or official methods using the student t- test and variance ratio F- test, no significant differences

were observed in respect of accuracy and precision. The methods could be considered as general methods for determining drug substances having phenolic OH groups. These merits, in addition to the use of simple reagents suggest its use in drug quality control laboratories.

Table 3: Analysis of raloxifene hydrochloride in its dosage forms by the proposed and reported method.

Preparations	Claimed mg/tablet	Found ± S.D*			
		MBTH	Nitration	NBS	Reported method (17)
Evesta Tablets	60	100.20 ± 0.78	99.52 ± 0.93	99.67± 0.84	99.80 ± 1.50
B.N.B9505B		t-= 0.53	t-= 0.35	t- =0.17	
		F-= 3.67	F-=2.26	F-= 3.17	

· Average of five determinations.

Tabulated value for t- test = 2.31 and for F- =values = 6.39 at p = 0.05.

(17) HPLC method.

Table 4: Analysis of isoproterenol hydrochloride in its dosage forms by the proposed and official method.

Preparations	Claimed mg/ml	Found ± S.D*		
		MBTH	Nitration	Official method(3)
Isuprel injections	0.20	99.49 ± 0.78	98.95 ± 0.97	98.79 ± 0.59
		t-= 1.59	t-= 2.27	
B.N.500803A		F-= 1.74	F-=2.69	
Sabex injections	0.20	100.23± 0.81	99.63 ± 0.72	99.86 ± 0.67
B.N.105597		t-= 0.79	t-= 0.52	
		F-= 1.47	F-=0.20	

· Average of five determinations.

Tabulated value for t- test= 2.31 and for F- ratio= 6.39 at p=0.05

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