Analysis of Roxatidine Acetate Hydrochloride in Bulk and from Sustained Release Formulations

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Two spectrophotometric methods for the estimation of roxatidine acetate hydrochloride (RAH) in bulk drug and in its sustained release tablets have been developed. The method I is based on the formation of a yellow coloured ion pair with bromophenol blue whereas the method II is based on the reaction of RAH with hydroxylamine hydrochloride to get a hydroxamic acid which on further reaction with ferric chloride gives a reddish brown complex. Beer's law was followed in the concentration range of 2 to 15 µg/ml in method I and 50 to 300 µg/ml in method II.

Roxatidine acetate hydrochloride (RAH) is a hostamine H_2 receptor antagonist used in patients with peptic ulcers. Chemically, it is 2-(acetyloxy)-N-(3-(1-piperidinyl methyl) phenoxy) propyl) acetamide[1]. A survey of literature revealed that a couple of HPLC methods[2,3] are available but no colorimetric method was reported till date. Two simple, rapid and precise methods are developed for the estimation of RAH in bulk drug and in its sustained release formulations. Method I is based on the formation of an ion pair complex with an acidic dye bromophenol blue at pH 3 acid phthalate buffer. The ion pair complex was then extracted into chloroform and was measured at 422 nm against reagent blank whereas the method II is based on the reaction of RAH with hydroxylamine hydrochloride to get hydroxamic acid which forms a reddish brown complex with ferric chloride and it was measured at 511 nm against a reagent blank.

A Hitachi UV 2000 UV/Vis spectrophotometer with 1 cm matched quartz cells was used for all the spectral measurements. All the reagents used were of AnalaR grade. Aqueous solutions of bromophenol blue (0.1%), acid phthalate buffer of pH 3, hydroxylamine hydrochloride (2 N), sodium hydroxide (3.5 N) and hydrochloric acid (3.5 N) were prepared as per IP. Ferric chloride (0.74 M) was prepared in 0.1 N hydrochloric acid. Standard drug solution (1 mg/ml) was also prepared in double distilled water. Sustained release tablets (75 mg and 150 mg) of RAH, Rotane (Hoechst Marion Roussel) were obtained from local market.

Aliquots ranging from 0.2 to 3 ml of standard solution (100 µg/ml were transferred to separating funnels and 5 ml of acid phthalate buffer of pH 3 and 1 ml 0.1% BPB solution were added to each solution. The yellow ion pair formed was then extracted with 5 ml of chloroform and diluted to 10 ml with chloroform. Absorbance of these solutions were measured against reagent blank at 422 nm. The curve was linear in the concentration range of 2 to 15 µg/ml in method 1.

Aliquots (0.5 to 3 ml) of standard drug solution (100 µg/ml) were taken into borosil test tubes. To this, 0.5 ml of 2 N hydroxylamine hydrochloride solution and 1 ml of 3.5 N NaOH were added. The tubes were kept in boiling water bath for five minutes. The reaction was then quenched by cooling the tubes in an ice bath for five minute. Now 1.5 ml of 3.5 N HCl and 2 ml of 0.74 N ferric chloride were added. The solutions were transferred to 10 ml volumetric flasks and volume was made up to the mark. Absorbance was measured at 511 nm against the reagent blank prepared in the same manner without the drug. The curve was linear in the concentration range of 50 to 300 µg/ml.

Twenty tablets of each formulation were weighed accurately and ground to a fine powder. The powder

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TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION

Data	Method I	Method II
λ max (nm)	422	511
Beer's law limits (μg/ml)	2 - 15	50 - 300
Molar extinction coefficient (L mole^{-1}cm^{-1})	1.257×10^4	0.875×10^3
Sandell's sensitivity. (μg cm^{-2}/0.001 absorbance units)	3.06×10^3	4.40×10^2
Regression equation	Y = 0.034x + 0.048	Y = 0.002x + 0.022
Slope	0.034	0.002
Intercept	0.048	0.022
Correlation coefficient	0.997	1.0
Precision (% RSD)	0.032	0.010

equivalent to 25 mg of RAH was weighed and transferred to a beaker. To this 15 ml of distilled water was added and the suspension was stirred for 20 min to dissolve the drug. The solution was filtered through a Whatman's filter paper into a 25 ml volumetric flask. The filter paper was rinsed twice with 3 ml of distilled water and the volume was then made upto the mark. The above stock solution was further diluted with distilled water to get a working solution of 100 μg/ml.

RAH has a secondary amino group in its molecular structure which makes it possible to form the ion pair complex with various acidic dyes. Bromophenol blue has been used to form the chromogen. Various immiscible solvents were tried to extract the yellow ion pair complex and it was found that it could be extracted with chloroform and methylene chloride. But as the colour intensity of the chromogen was maximum with chloroform, it was selected as the medium. The ion pair was stable for more than 20 h. The optical parameters and other constants are listed in Table 1. The stoichiometry of ion pair formed was studied by Job's method of continuous variation and it was found to be 1:1 reaction. The carbonyl-group present in RAH reacts with hydroxylamine hydrochloride to form hydroxamic acid which, in turn, forms a brown coloured complex with ferric ions. The colour of the complex was stable for 6 h. The completion of reaction is highly dependant upon temperature, so the method of Lossen[4] was adopted with some modifications.

The recovery studies were carried out to ascertain the accuracy and precision of the methods by adding known amounts of standard solution at three levels to previously analyzed sample solution and measuring the absorbance. The amount of the drug was computed from the calibration curve and was found to be in agreement with the labeled claim (Table 2).

RAH is quite resistant to oxidizing agents but it is easily hydrolyzed by acids as well as bases. The applicability of both the methods to analyze RAH in presence of it's acid and base hydrolyzed products was also studied. Hydrolysis was carried out by refluxing 10 mg of the drug with 10 ml of 2 M HCl or NaOH respectively for 30 min. The solution was cooled to room temperature then diluted to 50 ml. The solution was filtered through a Whatman filter paper after neutralization of excess acid or base and further diluted with distilled water to get a solution of 100 μg/ml. These solutions, alone and after mixing with standard RAH solution were analyzed by method I and II and the results are reported in Table 3, which indicates that the method II can be used to analyze RAH in presence of its acid or base hydrolyzed products.

The proposed methods I and II were validated by studying several parameters like Beer's law limits, slope, intercept and correlation coefficient and are recorded in Table 1. Linearity was checked for three consecutive days for the same concentration range from the same stock solution. Precision and accuracy of the method I and II were evaluated by assaying freshly prepared tablet solutions in triplicate at two different concentrations. To determine the robustness of the methods, experimental conditions were deliberately altered. Method I was found

TABLE 2: ANALYSIS OF MARKETED FORMULATIONS AND RECOVERY STUDY

Formulation	Amount taken (mg)	Amount found* (mg)	% recovery**
Method I	25	24.89	99.57
Method II	25	25.01	100.90

* Average of three determinations, **After spiking the sample with known amounts of standard solution at three levels.

TABLE 3: APPLICABILITY OF METHODS FOR THE ESTIMATION OF DRUG IN PRESENCE OF ITS HYDROLYZED PRODUCTS

Sample	Amount taken (μg/ml)	Amount found Method I (μg/ml)	Amount found Method II (μg/ml)
Base hydrolyzed	5.0	4.50	0.00
Base hydrolyzed + unhydrolyzed	5.0 + 5.0	6.75	4.90
Unhydrolyzed	5.0	4.95	5.10
Acid hydrolyzed	5-0	3.95	0.00
Acid hydrolyzed + Unhydrolyzed	5.0 + 5.0	9.50	5.10

to be dependant upon pH, whereas slight change in heating and cooling temperature affects the results in method II, so optimized conditions are essential for the best results. The proposed methods I and II are not very robust but are quite sensitive, precise, inexpensive and reproducible. In the absence of any available spectrophotometric method for the estimation of RAH, these methods can be used for the estimation of drug in bulk drug and in sustained release formulations.

REFERENCES

1. Budavan, S., Eds., In;The Merck Index, 12th Edn., Merck and Co. Inc., Whitehouse Station, N.J., 1996, 8431.
2. Burrows, J.L., Jolly, K.W. and Sullivan, D.J., J. Chromatogr. Biomed. Appl., 1988, 76, 163.
3. Argekar, A.P. and Kunjir, S.S., Indian Drugs, 1995, 32, 333.
4. Siggia, S. and Hanna, J.G., In; Qualitative Organic Analysis via Functional Groups, 4th Edn., John Wiley and Sons, New York, 1979, 172.