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pyrilamine (as maleate) spectrophotometric analysis

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Abstract

Two Simple, accurate and reproducible UV spectrophotometric methods were established for the assay of Pyrilamine maleate (PYRA) based on the formation of diazotization-coupling reaction products between PYRA and p-sulphanilic acid (SAC), ethyl aceto acetate (EAA). The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (M_1 - M_2) are given. The absorbance was measured at 430 (SAC- M_1) and 400 nm (EAA- M_2). Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and standard error of estimation (Se) for each system. Determination of Pyrilamine in bulk form and in pharmaceutical formulations was also incorporated.

Keywords: Pyrilamine, Spectrophotometric determination, Statistical analysis, Recovery studies

Introduction

1,2-Ethanediamine pyrrolidine N-(4-methoxy phenyl)-N-methyl-(Z)-N, N-dimethyl-N, N-dimethyl-2-pyridinyl-1 part butyl-2-ene dioate to 1 part 2-[(2-dimethylamino)ethyl] (p-methoxy benzyl] amino acid As seen in Figure 1, (Figure 2) is a relatively safe antihistamine. Useful for mild, uncomplicated cases of urticaria and angioedema, angioedema, demographia, and ecoratum of reactions of blood or plasma, as well as perennial and seasonal allergic rhinitis, vasomotor rhinitis, allergic conjunctivitis due to inherent allergens and foods. Natural histamine is a biogenic amine found in most cells and tissues of the body, and this antagonizing drug competes with it for receptor sites. For the determination of PYRA in body fluids and pharmaceuticals, only a few number of

physico-chemical techniques have been published. The majority of them use HPLC [1-2], UV-VIS [3-5], HPLC-MS/TS [6], TSP/MS [7], GLC [8], or the Partition chromatographic technique [9]. There is still room to build a few more visible spectrophotometric techniques with improved sensitivity, selectivity, precision, and accuracy since the analytically valuable functional groups in PYRA have not been thoroughly utilized for constructing acceptable spectrophotometric methodologies. For the purpose of comparing the findings obtained with the suggested techniques, the author has devised two simple and sensitive UV spectrophotometric methods in isopropyl alcohol/ $CHCl_3$ for the quantification of PYRA in pure or pharmaceutical formulations.

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Material and methods

An Elico UV-Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements, an Elico LI-120 digital pH meter was used for pH measurements. All the chemicals and reagents used were of analytical grade and the aqueous solutions were freshly prepared with triple distilled water.

Preparation of the reagents

All reagents were prepared using by Double distilled water and all chemicals are AR grade

Preparation of standard drug solutions in free base form (M_1)

Pyrimilamine as maleate (mg/ml) was prepared by dissolving an appropriate amount of its salt (Pyrimilamine Maleate) equivalent to 100 mg of free base in 20 ml of water, adding 10ml of 0.1 M NaOH solution and extracting the separated base with chloroform (4 x 20 ml). The combined chloroform extract was washed with water, dried over anhydrous sodium sulphate and made upto 100ml with chloroform and this stock solution was diluted step wise with chloroform to get the working standard solution.

Preparation of standard solution of Hydrolysed PYRA (M_2)

25 mgs of Pyrimilamine maleate was boiled with 10 ml of 5N HCl in a round – bottomed flask under reflux for 1 hour. Cooled and the excess HCl was removed under vacuum. The residue was dissolved in distilled water and made up to 50ml with distilled water (500 g/ml) to get a stock solution of hydrolysed drug (H PYRA). Working solutions were prepared from stock solution.

Method- M_1 : SAc solution (Sd-fine; 1%, 5.774×10^{-2} M): Prepared by dissolving 1g of p-sulphanilic acid in 100ml of μ 0.2M HCl

NaNO₂ solution (E.Merck; 0.4%, 5.796×10^{-2} M): Prepared by dissolving 400mg of sodium nitrite in 100 ml of distilled water

NaOH solution (Loba; 4%, 1M): Prepared by dissolving 4g of sodium hydroxide in 100 ml of distilled water

Method- M_2 : EAA solution (Qualigens; 2%, v/v 1.569×10^{-1} M): Prepared by dissolving 1.2 g of citric acid in 5ml of methanol and made upto 100ml with acetic anhydride.

Sulphuric acid (Qualigens 18M): AR grade was used as it is.

Procedure- M_1 : To a series of 10ml graduated test tube 0.8 ml of sulphanilic acid and 1 ml of NaNO₂ solutions were added and allowed to stand for 2 min.

Aliquots of hydrolysed drug (0.5-2.5ml, 50 g/ml⁻¹) were delivered into the test tubes. Then 5ml of NaOH solution was added and the volume in each tube was made upto 10ml with distilled water. The absorbance of the solutions was measured after 5 min at 430 nm against a reagent blank. The amount of drug in a sample was obtained from the calibration graph. **M_2 :** Aliquots of hydrolysed PYRA (0.5ml –

3.0ml, 25 g ml⁻¹) ethanolic solution of EAA were delivered into series of 25 ml calibrated tubes. One ml of 2 % (1.569×10^{-1} M) was added and then 2.5ml of conc H₂SO₄ was added slowly along the walls of each of tube and kept under running water for cooling. After 15 min, the total volume was brought to mark with ethanol. The absorbance was read against a reagent blank at 400 nm between 1-60 min the amount of drug was computed from the calibration graph.

For pharmaceutical formulations: An accurately weighed portion of tablet content equivalent to about 100 mg of PYRA was dissolved in a few ml of isopropyl alcohol and filtered to get 1mg/ml. The filtrate is evaporated to dryness and dissolved in distilled water. This stock solution (1 mg/ml) was further diluted step wise with distilled water use organic solvent isopropyl alcohol as under ZPD. These solutions were analyzed as under procedures described for bulk solutions.

Reference method [5]: An accurately weighed amount of formulation (Tablets powder) equivalent to 100 mg was

dissolved in a few ml of methyl alcohol and filtered. The filtrate was evaporated to dryness. The residue was dissolved in distilled water and further diluted to 100 ml with methyl alcohol to obtain concentration of 500 g/ml. It was further diluted step wise with distilled water to get the concentration of 50 g/ml. Aliquots of PYRA solution 1.0-5.0 ml, 50 g/ml were taken into a series of 5ml calibrated tubes and made upto the mark with methyl alcohol. The absorbance of each solution was measured at 323 nm against distilled water. The concentration of the drug was computed from its calibration graph.

Results and Conclusion

Spectral Characteristics: In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of PYRA were taken and colors were developed

separately by following the above procedures. The amounts of PYRA present in total volume of colored solutions were 5 g/ml for M_1 and 2.5 g/ml for M_2 . The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900 nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The absorption curves of the colored species in each method show characteristics absorption maximum.

Optical Characteristics: In order to test whether the colored species formed in above methods adhere to Beer's law, the absorbance at appropriate wavelength of a set of solutions containing varying amounts of PYRA and specified of amounts of reagents were recorded against the corresponding reagent b lanks. The Beer's law plots of these recorded graphically. Beer's law limits, molar absorptivity, sandell's sensitivity and optimum photometric range for PYRA in each method were calculated. Least square regression analysis was carried out for getting th e slope, intercept and the correlation coefficient values given Table 1.

Precision: The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of PYRA in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods

Accuracy: To determine the accuracy of each

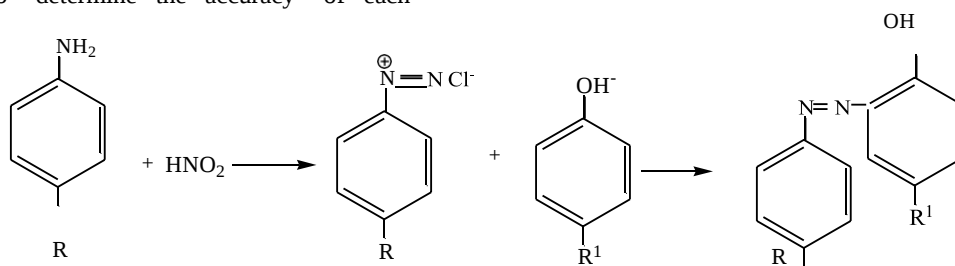
proposed method, different amounts of bulk samples of PYRA within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in Table 1.

Interference studies: The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of PYRA in methods under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amounts than they usually exist in formulations.

Analysis of formulations: Commercial formulations (tablets) containing PYRA were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found not to different significantly. The results were summarized in Table 2. Percent recoveries were determined by adding standard drug to preanalysed formulations. The results of the recovery experiments by the proposed methods are also listed in the Table 2.

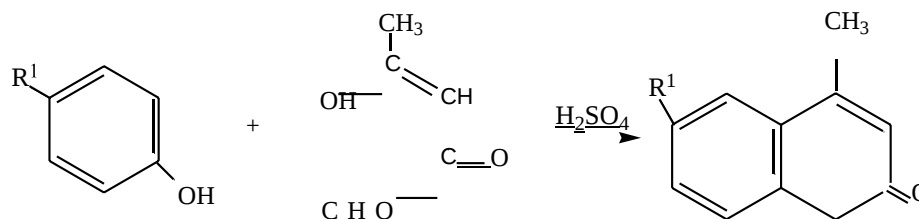
Chemistry of the colored species

Method M_1 : A study of the colored product formation of PYRA by the reaction with diazotised sulphanilic acid was carried out in the usual manner for diazotisation and coupling under alkaline conditions, with p henolic compound. The probable sequence of reactions based on analogy is given in scheme 1.



Scheme 1

Method M_2 : Condensation of phenolic compound with ethyl acetoacetate in sulphuric acid medium, to given a coumarin is the basis for this method scheme 2.



The developed UV-Vis Spectrophotometric methods for the estimation of PYRA were found to be simple and useful with high accuracy, precision, and reproducible. Sample recovery in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, this suggesting the validity of the methods and non interference of formulation excipients in the estimation. Table 1: Optical and regression characteristics, precision and accuracy of the proposed methods for PYRA

Parameter	μ	M_1	M_2
λ_{max} (nm)	μ	430	400
Beer's law limits (g/ml)		2.5-15.0	0.5-3.0
Detection limit (g/m)		0.1579	0.02683
Molar absorptivity (l.mol/cm)		1.928×10^4	5.683×10^4
Sandell's sensitivity (g/cm ² /0.001 absorbance unit)		7.570×10^{-2}	3.682×10^{-2}
Optimum photometric range (g/ml)		4-12.5	2.5-6.25
Regression equation (Y=a+bc) slope (b)		0.05225	0.1413
Standard deviation on slope (S _b)		3.049×10^{-4}	7.580×10^{-4}
Intercept (a)		4.249×10^{-3}	7.5×10^{-4}
Standard deviation on intercept (S _a)		2.528×10^{-3}	1.257×10^{-3}
Standard error on estimation (S _e)		2.411×10^{-3}	1.198×10^{-3}
Correlation coefficient (r)		0.9999	0.9999
Relative standard deviation (%)*		0.7211	0.9294
% Range of error (confidence limits)			
0.05 level		0.8291	1.06867
0.01 level		1.300	1.6758
% error in Bulk samples **		0.200	-3.60

*average of three determinations ** Average of six determination



Table 3: Assay of PYRA in Pharmaceutical Formulations

Formulations ^a	Amount taken (mg)	Amount found by proposed methods ^b		Reference method	Percentage recovery	
		M ₁	M ₂		by proposed M ₁	methods ^c M ₂
Tablet I	25	24.74±0.64 F = 1.373 t = 0.55	24.59±0.53 F = 2.002 t = 1.020	24.96±0.75	99.90±0.95	99.38±0.81
Tablet II	25	24.65±0.63 F = 1.9511 t = 0.73	24.63±0.72 F = 1.493 t = 0.736	24.97±0.88	99.46±0.82	99.72±0.46
Tablet III	25	24.72±0.52 F = 1.421 t = 0.60	24.56±0.49 F = 1.600 t = 0.81	24.92±0.62	99.94±0.73	99.90±0.97
Tablet V	25	24.52±0.56 F = 1.841 t = 1.18	24.64±0.63 F = 1.4553 t = 0.82	24.97±0.76	99.86±0.65	99.56±0.97

aPills manufactured by four separate drug firms. The t- and F-test scores for comparing the proposed approach with the reference method are expressed as the mean standard deviation of six separate determinations. cRecovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three measurements) Theoretical values at 95% confidence limit, F = 5.05, t = 2.57)

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