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Quantitative determination of Pyrilamine (as maleate) by UV-Visible spectrometry

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ABSTRACT

Three Simple, accurate and reproducible UV spectrophotometric methods were established for the assay of pyrilamine maleate (PYRA) based on the formation of ion-association products between PYRA and cobaltous thiocyanate (CTC), citric acid (CA) and Alizarin red S (ARS). The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (M_1-M_3) are given. The absorbance was measured at 620 (CTC-M_1), 580 (CA-M_2) and 420 nm (ARS M_3). 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.

Key words: Pyrilamine, Spectrophotometric determination, statistical analysis, recovery studies

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INTRODUCTION

Pyrilamine {1, 2-Ethane diamine N-[(4-methoxy phenyl) methyl]-N^1, N^1-dimethyl-N-2piridinyl-(Z)-2 butene dioate (1:1) (or) 2-[(2-Dimethyl amino) ethyl) (p-methoxy benzyl) amino] pyridine maleate (1:1)} (Figure 1) is an antihistamine with a low incidence of side effects. It is effective for use in perennial and seasonal allergic rhinitis, vasomotor rhinitis, allergic conjunctivitis due to inherent allergens and foods, mild uncomplicated allergic skin manifestations of urticarea and angiodesma, angioedema, demo graphism and aneceoratum of reactions of blood or plasma. It is an antagonizing agent that competes for receptor sites with natural histamine, a biogenic amine present in most body cells and tissues.

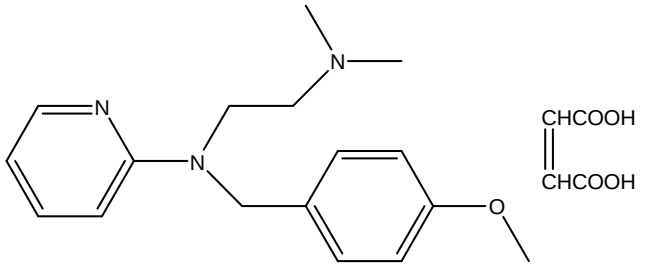


Figure 1

A very few Physico-chemical methods appeared in the literature for the assay of PYRA in biological fluids, and pharmaceutical formulations. Most of them are based on HPLC [1-2], UV-VIS [3-5], HPLC-MS/TS [6], TSP/MS [7], GLC [8] and Partition chromatographic method [9]. The analytically useful functional groups in PYRA have not been fully exploited for designing suitable visible spectrophotometric methods and so still offer a scope to develop few more visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy.

The author has developed two simple and sensitive UV spectrophotometric methods in isopropyl alcohol/$CHCl_3$ for the estimation of PYRA in pure or pharmaceutical formulations and adopted it as a reference method to compare the results obtained with the proposed methods.

MATERIALS AND METHODS

Experimental

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 solutions

A 1 mg/ml solution was prepared by dissolving 100 mg of pure PYRA in 10 ml of distilled water followed by dilution to 100 ml with distilled water and the stock solution was diluted step wise with distilled water to get the working standard solutions of concentrations of 25µg/ml.

Method-M_1: *CTC solution* ($2.5x10^{-1}$M): Prepared by dissolving 7.25 g of cobaltous nitrate (BDH) and 3.8g of ammonium thiocyanate (BDH) in 100 ml of distilled water.
Buffer solution (pH 2.0): Prepared by mixing 306 ml of trisodium citrate (0.1M) with 694 ml of HCl (0.1M) and the pH of the solution were adjusted to 2.0.
Nitrobenzene (Qualigens): AR grade nitrobenzene was used.

Method-M_2: *Citric acid – acetic anhydride reagent* (E.Merck; 1.2%,$6.245x10^{-2}$M): Prepared by dissolving 1.2 g of citric acid in 5ml of methanol and made upto 100ml with acetic anhydride.
Methanol (qualigens): AR grade methanol was used.
Acetic anhydride (qualigens): AR of acetic anhydride was used.

Method-M_3: *ARS reagent* (0.2%, $5.84x10^{-3}$M): 200 mg of Alizarin red S dissolved in 100ml distilled water.

HCl solution (0.1M): 8.6 ml of conc HCl in 100ml with distilled water

Procedure - *M_1*: Into a series of 125 ml separating funnels, aliquots of standard PYRA solution (0.5-3.0 ml, 100 µg/ml) were taken. Then 2.0 ml of buffer (pH 2.0) and 5.0 ml ($2.5x10^{-1}$M) of CTC solutions were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 ml with distilled water. To each separating funnel, 10.0 ml of nitrobenzene was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured at 620 nm against a similar reagent blank. The amount of PYRA was computed from its calibration graph.

M_2: Aliquots of standard drug solution (0.5-3.0 ml, 100 µg/ml) were taken into a series of 25 ml graduated tubes and gently evaporated on a boiling water bath to dryness. To this 10 ml of citric acid – acetic anhydride reagent was added and the flasks were immersed in a boiling water bath for 30 min. The tubes were cooled to room temperature and made upto the mark with acetic anhydride. The absorbance of the colored solutions was measured after 15 min at 580 nm against a reagent blank. The amount of PYRA was calculated from the calibration graph.

M_3: Into a series of 125 ml separating funnels containing aliquots of standard PYRA solution (0.5-3.0 ml) (50 µg/ml) 6.0 ml of 0.1 M HCl solution and 2.0 ml of 0.2 % dye solution were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15 ml with distilled water. To each separating funnel 10 ml of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 420 nm against a similar reagent blank. The amount of PYRA was deduced from the concerned calibration curve.

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 µg/ml. It was further diluted step wise with distilled water to get the concentration of 50 µg/ml. Aliquots of PYRA solution 1.0-5.0 ml, 50 µg/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 DISCUSSION

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 10 µg/ml for M_1, 4µg/ml for M_2 and 5µg/ml for M_3. 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 blanks. 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 the 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.

Table 1.Optical and regression characteristics, precision and accuracy of the proposed methods for PYRA

Parameter	M_1	M_2	M_2
λ_{max} (nm)	620	580	420
Beer's law limits (µg/ml)	5-30	2-12	2.5-15.0
Detection limit (µg/m)	2.824	9.760	0.4098
Molar absorptivity (l.mol/cm)	4.096×10^3	1.416×10^4	1.269×10^4
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.2126	9.299×10^{-2}	9.921×10^{-2}
Optimum photometric range (µg/ml)	10-27.5	4 -10	4.5-12.59
Regression equation (Y=a+bc), slope (b)	0.013205	0.03852	0.0329
Standard deviation on slope (S_b)	5.651×10^{-3}	1.717×10^{-2}	5.256×10^{-4}
Intercept (a)	3.249×10^{-3}	3.5×10^{-3}	9.999×10^{-4}
Standard deviation on intercept (S_a)	9.372×10^{-2}	1.086×10^{-1}	4.358×10^{-3}
Standard error on estimation (S_e)	8.935×10^{-2}	1.1393×10^{-1}	4.158×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9996
Relative standard deviation (%)*	1.626	1.048	0.1261
% Range of error (confidence limits)			
0.05 level	1.870	1.205	0.1450
0.01 level	2.931	2.889	0.2273
% error in Bulk samples **	0.157	-0.300	-0.064

**average of three determinations ** Average of six determinations*

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 prenalysed formulations. The results of the recovery experiments by the proposed methods are also listed in the Table 2.

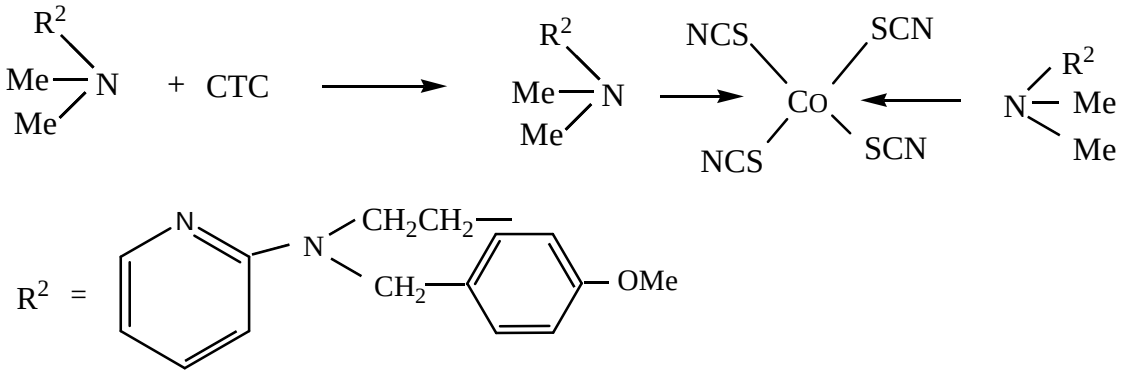
Table 2: Assay of PYRA in Pharmaceutical Formulations

Formulations[a]	Amount taken (mg)	Amount found by proposed Methods[b]				Percentage recovery by proposed methods***		
		M_1	M_2	M_3	Reference method	M_1	M_2	M_3
Tablet I	25	24.79±0.7 F=1.338 t=0.75	24.62±0.47 F=2.546 t=0.962	24.74±0.6 F=1.373 t=0.55	24.96±0.75	99.83±0.9	99.62±0.76	99.90±0.9
Tablet II	25	24.63±0.6 F=3.808 t=0.7	24.54±0.59 F=2.224 t=1.01	24.65±0.6 F=1.9511 t=0.73	24.97±0.88	99.31±0.9	99.66±0.55	99.46±0.8
Tablet III	25	24.56±0.3 F=1.846 t=1.5	24.81±0.41 F=2.286 t=1.161	24.72±0.5 F=1.421 t=0.60	24.92±0.62	99.90±0.3	99.63±0.98	99.94±0.7
Tablet IV	25	24.65±0.4 F=2.215 t=0.88	24.73±0.48 F=3.008 t=0.67	24.52±0.5 F=1.841 t=1.18	24.97±0.76	99.54±0.6	99.68±0.98	99.86±0.6

[a]Tablets from four different pharmaceutical companies. [b]Average ± standard deviation of six determinations, the t- and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57; [c]Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations)

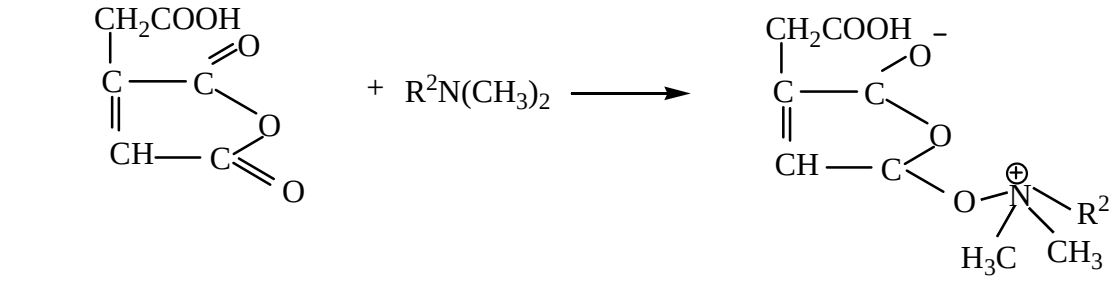
Chemistry of the colored species

M_1: Reaction between PYRA due to the presence of tertiary amine with cobalt thioyanate to give a soluble complex is the basis in this method scheme 1.



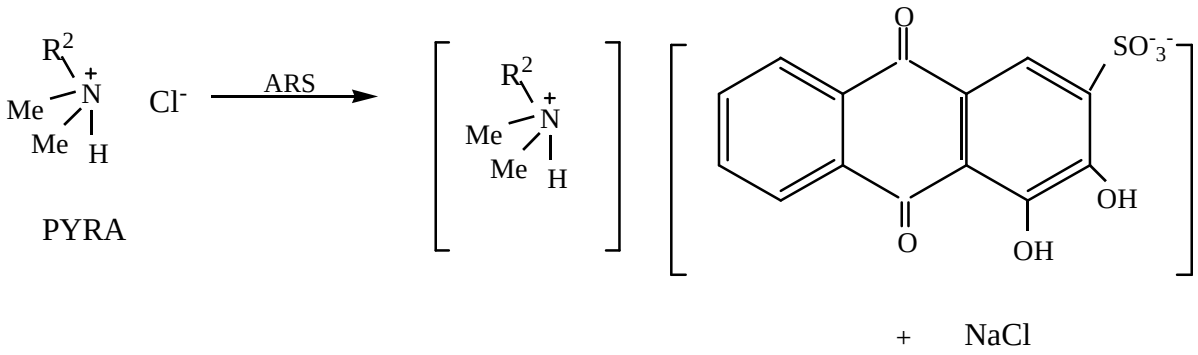
Scheme 1

Method M_2: Formation of red violet color internal salt with involvement of aliphatic tertiary amine in PYRA and aconitic anhydride (dehydration product of citric acid) is the basis in this method development scheme 2.



Scheme 2

Method M_3: PYRA possesses aliphatic tertiary amine group. It forms an ion association complex with an acid dye (ARS) which is extractable into chloroform from the aqueous phase. The protonated nitrogen (positive charge) of PYRA as hydrochloride is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction. It is supported by slope ratio method. Based on the analogy the structures of ion association complexes are shown in scheme 3.



Scheme 3

CONCLUSION

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.

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