



## Spectrophotometric determination of pyrilamine (As maleate)

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### Abstract

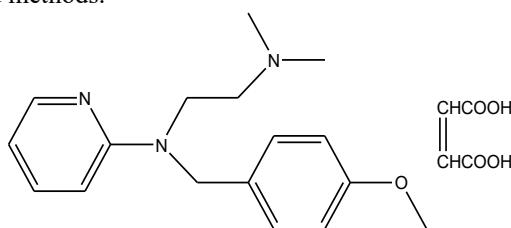
Two Simple, accurate and reproducible UV spectrophotometric methods were established for the assay of Pyrilamine maleate (PYRA) based on the formation of diazotization-coupling reaction products between PYRA and p-sulphanilic acid (SAc), ethyl aceto acetate (EAA). The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (M<sub>1</sub>-M<sub>2</sub>) are given. The absorbance was measured at 430 (SAc-M<sub>1</sub>) and 400 nm (EAA-M<sub>2</sub>). 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

Pyrilamine {1, 2-Ethane diamine N-[(4-methoxy phenyl) methyl]-N<sup>1</sup>, N<sup>1</sup>-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 urticaria 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.

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<sub>3</sub> 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.



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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<sub>1</sub>)

Pyrilamine as maleate (mg/ml) was prepared by dissolving an appropriate amount of its salt (Pyrilamine 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<sub>2</sub>)

25 mgs of Pyrilamine 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  $\mu$ g/ml) to get a stock solution of hydrolysed drug (H PYRA). Working solutions were prepared from stock solution.

**Method-M<sub>1</sub>:** SAc solution (Sd-fine; 1%,  $5.774 \times 10^{-2}$  M): Prepared by dissolving 1g of p-sulphanilic acid in 100ml of 0.2M HCl

NaNO<sub>2</sub> solution (E.Merck; 0.4%,  $5.796 \times 10^{-2}$  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<sub>2</sub>:** EAA solution (Qualigens; 2%, v/v  $1.569 \times 10^{-1}$  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<sub>1</sub>:** To a series of 10ml graduated test tube 0.8 ml of sulphanilic acid and 1 ml of NaNO<sub>2</sub> solutions were added and allowed to stand for 2 min. Aliquots of hydrolysed drug (0.5-2.5ml, 50  $\mu$ g/ml<sup>-1</sup>) 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<sub>2</sub>:** Aliquots of hydrolysed PYRA (0.5ml – 3.0ml, 25  $\mu$ g ml<sup>-1</sup>) ethanolic solution of EAA were delivered into series of 25 ml calibrated tubes. One ml of 2 % ( $1.569 \times 10^{-1}$  M) was added and then 2.5ml of conc H<sub>2</sub>SO<sub>4</sub> 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  $\mu$ g/ml. It was further diluted step wise with distilled water to get the concentration of 50  $\mu$ g/ml. Aliquots of PYRA solution 1.0-5.0 ml, 50  $\mu$ 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 ( $\lambda_{max}$ ) 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  $\mu\text{g}/\text{ml}$  for M<sub>1</sub> and 2.5  $\mu\text{g}/\text{ml}$  for M<sub>2</sub>. 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

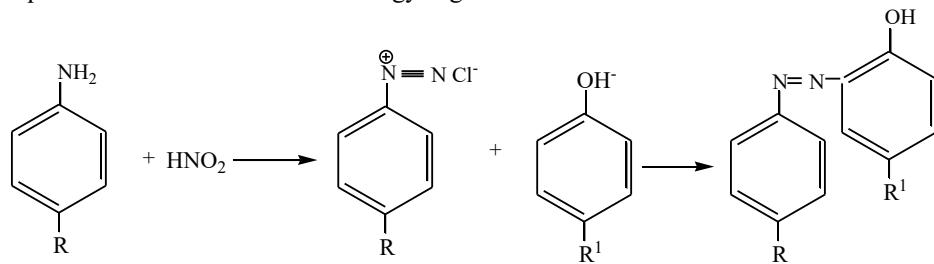
**Accuracy:** To determine the accuracy of each proposed method, different amounts of bulk samples of PYRA within the Beer's law limits were taken and analyzed by the proposed method. The results (percent error) are recorded in Table 1.

**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.

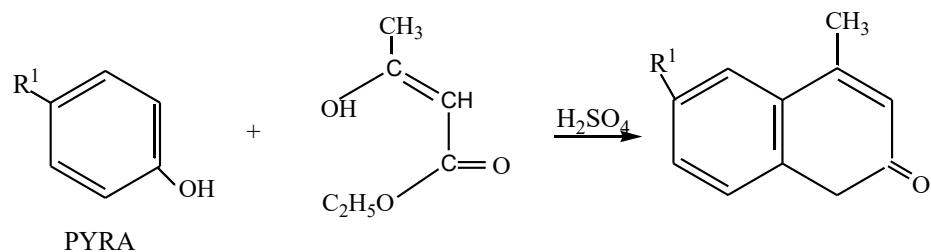
#### Chemistry of the colored species

**Method M<sub>1</sub>:** 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 phenolic compound. The probable sequence of reactions based on analogy is given in scheme 1.



Scheme 1

**Method M<sub>2</sub>:** Condensation of phenolic compound with ethyl acetoacetate in sulphuric acid medium, to give a coumarin is the basis for this method scheme 2.



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	M <sub>1</sub>	M <sub>2</sub>
$\lambda_{\text{max}}$ (nm)	430	400
Beer's law limits ( $\mu\text{g/ml}$ )	2.5-15.0	0.5-3.0
Detection limit ( $\mu\text{g/ml}$ )	0.1579	0.02683
Molar absorptivity (l.mol/cm)	$1.928 \times 10^4$	$5.683 \times 10^4$
Sandell's sensitivity ( $\mu\text{g/cm}^2/0.001$ absorbance unit)	$7.570 \times 10^{-2}$	$3.682 \times 10^{-2}$
Optimum photometric range ( $\mu\text{g/ml}$ )	4-12.5	2.5-6.25
Regression equation (Y=a+bc)		
slope (b)	0.05225	0.1413
Standard deviation on slope (S <sub>b</sub> )	$3.049 \times 10^{-4}$	$7.580 \times 10^{-4}$
Intercept (a)	$4.249 \times 10^{-3}$	$7.5 \times 10^{-4}$
Standard deviation on intercept (S <sub>a</sub> )	$2.528 \times 10^{-3}$	$1.257 \times 10^{-3}$
Standard error on estimation (S <sub>e</sub> )	$2.411 \times 10^{-3}$	$1.198 \times 10^{-3}$
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 <sup>a</sup>	Amount taken (mg)	Amount found by proposed methods <sup>b</sup>		Reference method	Percentage recovery by proposed methods <sup>c</sup>	
		M <sub>1</sub>	M <sub>2</sub>		M <sub>1</sub>	M <sub>2</sub>
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 IV	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

<sup>a</sup>Tablets from four different pharmaceutical companies. <sup>b</sup>Average  $\pm$  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; <sup>c</sup>Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations)

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