Spectrophotometric Methods for the Assay of Pylamine Maleate Using Chromogenic Reagents

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Abstract: Simple, accurate and reproducible UV spectrophotometric methods were established for the assay of pylamine maleate (PYRA) based on the formation of oxidative coupling and precipitation, charge transfer complexation products. Method A includes the oxidative coupling reaction of PYRA with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in presence of Ce(IV). The formation of oxidative coupling product with 4-amino phenazone (4-AP) in presence of $K_3Fe(CN)_6$ is incorporated in method B. Precipitation/charge transfer complex formation of the PYRA with tannic acid (TA)/Metol-Cr(VI) in method C were proposed. The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (A-C) are given. 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 pylamine in bulk form and in pharmaceutical formulations were also incorporated.

Keywords: Estimation, Pylamine, Precipitating agent, Charge transfer complex.

Introduction

Pylamine (as maleate PYRA) 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 angiodema, angioedema, demographism and anecoratum 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 physicochemical methods appeared in the literature for the assay of PYRA¹ in biological fluids and pharmaceutical formulations. Most of them are based on visible spectrophotometric methods²⁻³, HPLC⁴⁻⁸, GC⁹⁻¹⁰, fluorimetry¹¹⁻¹³, LC-MS¹⁴, GC-MS¹⁵⁻¹⁷, & TLC¹⁸, Mass¹⁹. 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 made some attempts in this direction and succeeded in developing visible spectrophotometric methods by exploiting various functional groups of PYRA. All these methods have been extended to pharmaceutical formulations as well.

Experimental

An Elico, UV - Visible digital spectrophotometer with 1 cm 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 analytical grade and the aqueous solutions were freshly prepared with triple distilled water. A 1 mg/mL solution was prepared by dissolving 100 mg of pure PYRA in 100 mL distilled water and the stock solution was diluted step wise with distilled water to get the working standard solutions of required concentrations. MBTH Solution (Aldrich; 0.2%, 8.56×10^{-3} M), Ce(IV) Solution (Wilson labs; 1%, 1.58×10^{-2} M) for method A 4-AP solution (Ferak; 1%, 4.92×10^{-2} M), $K_3[Fe(CN)_6]$ (BDH; 4.0%, 1.22×10^{-1} M), Pyridine (Qualigens, 12.4 M) for method B and TA (Loba 0.2%, 1.17×10^{-3} M); PMAP (Loba, 0.3%, 8.71×10^{-3} M), Cr(VI) (BDH, 0.3% 1.01×10^{-2} M), Buffer pH 3 were prepared.

Recommended procedures

Method A

Aliquots of standard PYRA solution (0.5-3.0 mL, $25 \mu\text{g} \cdot \text{mL}^{-1}$) were transferred into a series of 25 mL calibrated tubes. Then 0.5 mL (8.56×10^{-3} M) of MBTH solution was added and kept aside for 5 min. After that 1 mL (1.58×10^{-2} M) of ceric ammonium sulphate was added and kept aside for 10 min. The volume was made up to the mark with distilled water. The absorbance was measured at 460 nm against a similar reagent blank. The amount of PYRA was computed from its calibration graph (Figure 1).

Figure 1. Beer's law plot of PYRA - MBTH-Ce(IV)

Method B

Aliquots of standard PYRA (0.5-3.0 mL, $25 \mu\text{g} \cdot \text{mL}^{-1}$) solution, 0.5 mL of pyridine, 1 mL of 4AP (4.92×10^{-2} M) and 0.5 mL of $K_3[Fe(CN)_6]$ (1.22×10^{-1} M) were added successively into a series of 10 graduated tubes and the total volume in each flask was brought to 10 mL with distilled water and kept aside for 5 min. The absorbances were measured at 500 nm against a reagent blank. The coloured species was stable for 30 min. The drug concentration was deduced from a calibration graph (Figure 2).

Figure 2. Beer's law plot of PYRA - 4-AP - $K_3Fe(CN)_6$

Method C

Aliquots of standard drug solution (0.5-3.0 mL 400 $\mu\text{g/mL}$) were delivered in to a series of centrifuge tubes and the volume in each test tube was adjusted to 3.0 mL with 0.01 N HCl. Then 1.0 mL of Tannic acid was added and centrifuged for 5 min. The precipitate was collected through filtration and subsequently washed with 2.0 mL of distilled water. The filtrate and washings were collected in a 25 mL graduated test tube. Then 15 mL of p^H 3.0 buffer and 1.5 mL of PMAP solution were successively added. After 2 min, 2.0 mL of Cr (VI) solution was added and the volume was made up to the mark with distilled water. The absorbance was measured after 5 min at 560 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and intern drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of drug was calculated from Beer's law plot (Figure 3)

Figure 3. Beer's law plot of PYRA - (TA/PMAP-Cr(VI))

CN(C)CCN(c1ccccc1OC)Cc2ccccc2C(=O)O

Method A

Step I

Step II

Results and Discussion

The optimum conditions for the color development of methods A, B and C were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

The list of proposed and reported methods was given in Table 1. The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (A-C) are given Table 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.

The accuracy of the methods was ascertained by comparing the results by proposed and reference methods, statistically by the *t*- and *F*- tests. The comparison shows that there is no significant difference between the results of studied methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods, the excipients are usually present in pharmaceutical formulations do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were carried out. The recoveries of the added amounts of standard drug were studied at three different levels. Each level was repeated for 6 times. From the amount of drug found, the % recovery was calculated. The higher $\lambda_{\max}$ values of all the proposed methods have a decisive advantage since the interference from the associated ingredients should be generally less at higher wavelengths than at lower wavelengths. Thus the proposed visible spectrophotometric methods are simple and sensitive with reasonable precision, accuracy and constitute better alternatives to the existing ones to the routine determination of PYRA in bulk forms and pharmaceutical formulations.

Table 1. List of proposed and reported visible spectrophotometric methods

Type of reaction	Reagent	Method	Optical characteristics		
			$\lambda_{\max}$ nm	$\epsilon_{\max}$ l.mole ⁻¹ cm ⁻¹	Beer's limits $\mu\text{g mL}^{-1}$
Oxidative coupling	MBTH –Ce(IV)	Method A	460	7.288×10^4	0.5-3.0
Oxidative coupling	4AP-K ₃ [Fe(CN)] ₆	Method B	500	3.103×10^4	1.25-7.5
Precipitation/charge transfer complex formation	TA/Metol-Cr(VI)	Method C	560	5.045×10^3	8-48

Table 2. Optical and regression characteristics, precision and accuracy of the proposed methods for PYRA

Parameter	Method A	Method B	Method C
$\lambda_{\max}$, nm	460	500	560
Beer's law limits, $\mu\text{g/mL}$	1.0-6.0	1.25-7.5	8 - 48
Detection limit, $\mu\text{g/mL}$	0.07374	0.06312	1.316
Molar absorptivity, $\text{L mol}^{-1}\text{.cm}^{-1}$	2.998×10^4	2.971×10^4	2.899×10^3
Sandell's sensitivity ($\mu\text{g.cm}^{-2}/0.001$ absorbance unit)	6.486×10^{-2}	6.238×10^{-2}	0.2937
Optimum photometric range, $\mu\text{g/mL}$	2.5-4.5	3.6-7.5	20 – 48
Regression equation ($Y=a+bc$) slope (b)	0.0655	0.0645	0.0130
Standard deviation on slope (S_b)	8.705×10^{-4}	3.2485×10^{-4}	9.852×10^{-5}
Intercept (a)	6.75×10^{-3}	4.999×10^{-4}	6.999×10^{-3}
Standard deviation on intercept (S_a)	1.443×10^{-3}	1.347×10^{-3}	2.614×10^{-3}
Standard error on estimation (S_e)	1.376×10^{-3}	1.2841×10^{-3}	2.492×10^{-3}
Correlation coefficient (r)	0.9999	0.9996	0.9996
Relative standard deviation, %	0.2428	1.350	1.542
0.05 level	0.2791	0.15	1.773
0.01 level	0.4378	2.43	2.780
% Error in bulk samples	0.10	0.164	0.282

Conclusion

The proposed methods exploit the various functional groups in PYRA molecule. The decreasing order of sensitivity ($\epsilon_{\max}$) among the proposed methods are (Method A > Method B > Method C) respectively. The concomitants which do not contain the functional groups chosen in the present investigation do not interfere in the color development by proposed methods. Thus the proposed methods are simple, sensitive and selective with reasonable precision and accuracy and constitute better alternatives to the reported ones in the assay of PYRA in bulk form and pharmaceutical formulations (Table 3).

Table 3. Assay of PYRA in pharmaceutical formulations

Formulations	Amount taken, mg	Amount found by proposed Methods				Percentage recovery by proposed methods		
		Method A	Method B	Method C	Reference method	Method A	Method B	Method C
Tablet I	25	24.66 $\pm$ 0.55	24.75 $\pm$ 0.52	24.8 $\pm$ 0.45	25.1 $\pm$ 0.84	99.81 $\pm$ 0.48	99.72 $\pm$ 0.68	99.69 $\pm$ 0.98
		F=1.528	F=2.609	F=3.4844				
		t=0.9012	t=0.8914	t=0.8055				
Tablet II	25	24.69 $\pm$ 0.46	24.73 $\pm$ 0.28	24.59 $\pm$ 0.25	24.99 $\pm$ 0.42	99.77 $\pm$ 0.77	99.81 $\pm$ 0.83	99.74 $\pm$ 0.69
		F=1.9357	F=2.25	F=2.82				
		t=0.6613	t=0.80	t=1.27				
Tablet III	25	24.55 $\pm$ 0.45	24.71 $\pm$ 0.31	24.48 $\pm$ 0.42	24.92 $\pm$ 0.56	99.61 $\pm$ 0.95	99.69 $\pm$ 0.80	99.49 $\pm$ 0.86
		F=3.320	F=3.253	F=1.777				
		t=1.0910	t=0.50	t=0.98				
Tablet IV	25	25.10 $\pm$ 0.28	24.69 $\pm$ 0.19	24.63 $\pm$ 0.31	24.95 $\pm$ 0.37	99.69 $\pm$ 0.98	99.82 $\pm$ 0.44	99.88 $\pm$ 0.88
		F=3.719	F=3.792	F=1.753				
		t=0.8448	t=0.96	t=1.63				

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