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Determination of Phenazopyridine in Bulk and Pharmaceutical Dosage Forms with Spectrophotometry

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The present work describes two new spectrophotometric methods for the determination of phenazopyridine hydrochloride (PPH) in bulk and formulations. The methods are based on the reduction of Fe3+ into Fe2+ by the drug followed by the complex formation of Fe2+ with 1,10 phenanathroline (method A) and 2,2' bipyridyl (method B) to form red colored chromogen having maximum absorbance at 530nm and 520nm for methods A and B respectively. Beer's law is obeyed in the range of 2-10 ?g/mL with molar absorptivity of 2.185 × 104 for method A and 2.305 X 104 for method B respectively. The results were favorably compared with those obtained by reference UV spectrophotometric method. The accuracy, precision, and the linearity ranges of the methods have been determined, and they have been validated by analyzing the title drug. No interference was observed from common pharmaceutical adjuvants.These two methods were successfully applied to the pharmaceutical formulations, capsules, tablets.

Key words: Sandell's sensitivity, Phenazopyridine, Beer's law.

Phenazopyridine (2, 6-diamino-3-phenylazopyridine), is frequently used as an adjunct to sulfonamides, antibiotics and other urinary tract antiseptics to treat bacterial mucosal infections of the lower urinary tract[1-4], because of its putative analgesic effect on the mucosa of the urinary tract, high selectivity and no anti-choline activity. The drug has been determined by a variety of analytical techniques such as GC-MS[5], Spectrofluorimetric[6], Electro analytical methods[7], UV spectrophotometric[8], Ratio spectra derivative spectrophotometry[9] ,High performance liquid chromatography[10] and chromatography[11]. By exploiting the various functions groups in the Phenazopyridine the authors had developed two simple and sensitive spectrophotometric methods for the determination of Phenazopyridine in pharmaceutical formulations and bulk.

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Methodology

Apparatus

Elico UV – Visible Double beam spectrophotometer model SL-156.

Materials and Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared in distilled water.

Method A

1M 1,10 - phenanathroline
1M Ferric chloride
0.2M Ortho phosphoric acid

Method B

1M 2,2‘ Bipyridyl
1M Ferric chloride
0.2M Ortho phosphoric acid

Preparation of standard and sample solution

Accurately weighed 100mg of Phenazopyridine was dissolved in 100mL distilled water to give a concentration of 1mg/mL. The final concentration was brought to 100 µg/mL for both Methods A and B.

Assay procedure for the determination of Phenazopyridine

Method A

Aliquots of the standard solution containing (20 -100µg) of PPH, 1ml of ferric chloride solution and 1ml 1, 10 phenanathroline were added to heating tubes. The mixture was homogenized by shaking, immersed in a water bath at 100°C for 20 minutes, then cooled to room temperature then 2 ml of 0.2M Ortho phosphoric acid was added. The contents in all the tubes were transferred into a series of 10 ml volumetric flask then diluted up to the mark with distilled water and the absorbance was measured at ëmax 530 nm against reagent blank. The calibration curve was prepared to calculate the amount of the drug

Method B

Aliquots of the standard solution containing (20 -100µg) of PPH, 1ml of ferric chloride solution and 1ml 2, 2‘Bipyridyl were added in heating tubes. The mixture was homogenized by shaking, immersed in a water bath at 100°C for 15 minutes, then cooled to room temperature then 2 ml of 0.2M Ortho phosphoric acid was added. The mixture was transferred into a series of 10 ml volumetric flask then diluted up to the mark with distilled water and the absorbance was measured at λmax 520 nm against reagent blank. The calibration curve was prepared to calculate the amount of the drug.

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

Parameters	Method A	Method B
λ_{max} (nm)	530	520
Beer’s law limit (µg/ mL)	2 – 10	2 - 10
Sandell’s Sensitivity (ig/cm^2/0.001 abs. unit)	0.0097	0.0082
Molar absorptivity (Litre.mole^{-1}.cm^{-1})	2.185×10^4	2.305×10^4
Stability of Color (hours)	1	1
Regression equation (Y)*		
Intercept (a)	0.002	0.022
Slope(b)	0.01	0.0098
% RSD$^{\$}$	0.727	1.15
% Range of errors (95% confidence limits):		
0.05 significance level	0.607	0.961
0.01 significance level	0.899	1.42
Correlation coefficient®	0.9986	0.9973

*Y= a + bx, where Y is the absorbance and x is the concentration of PPH in µg/ mL
$ = for six replicates

RESULTS AND DISCUSSION

The method A and B are based on the reduction of Fe^{3+} into Fe^{2+} by the drug followed by chelation of Fe^{2+} with 1,10 phenanathroline (method A) and with 2,2‘ bipyridyl (method B) to form a red colored chromogen. The colored chromogens have λ_{max} at 530nm and 520nm for methods A and B respectively. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity for these methods are presented in Table-1.The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1

The accuracy of these methods was ascertained by comparing the results obtained with the proposed and reference methods in the case of formulation are presented in Table-2. As an additional check on the accuracy of these methods, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed formulation and percent recovery values obtained are listed in Table-2. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

Table 2. Results of analysis of tablet formulations containing PPH

Formulations	Labeled amount(mg)	Recovery by reference method*(%)	Recovery by proposed methods (%)**	
			Method A	Method B
Pyridium®	100	99.90	99.89	99.75
Re-Azo®	95	94.86	94.40	93.65

* Reference method was UV method developed in the laboratory.
** Recovery amount was the average of six determinants.

CONCLUSIONS

The proposed methods were found to be simple, economical and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing Phenazopyridine showed no interference from the common excipients. Hence, these methods could be considered for the determination of Phenazopyridine in the quality control laboratories.

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