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Aplication of some anionic dyes for the spectrophotometric determination of phenazopyridine hydrochloride

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ABSTRACT

In the present study the authors were developed three simple, economic and sensitive spectrophotometric methods for the assay of phenazopyridine hydrochloride either in pure form or pharmaceutical formulations .The described methods are based on the formation of colored chloroform extractable ion-pair complex of the drug with bromophenol blue (Method A), Amidoblack (Method B) and Bromothymol blue (Method C) in acidic medium. The extracted chromagen complexes showed absorbance maxima at 370nm, 620nm and 400nm respectively. These methods permitted determination of the studied drug over a concentration range 1-5 µg\ml with molar absorptivity of 3.73 X 10^4, 3.241 X 10^4 and 5.437 X 10^4 $Lmole^{-1}cm^{-1}$ respectively. These methods have been successfully applied for the assay of the drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvents. Results of analysis were validated statistically and through recovery studies and were in good agreement with those obtained by the official and reference methods.

Key words: Phenazopyridine, absorbance maxima, ion-pair complex.

INTRODUCTION

The aim of present work is to develop reliable and accurate spectrophotometric methods for the quality control of the pharmaceutical formulations containing the cited drug. Phenazopyridine (2, 6-diamino-3-phenylazo-pyridine), 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] and High performance liquid chromatography[10]. By exploiting the various functional groups in the Phenazopyridine the authors had developed three simple and sensitive spectrophotometric methods for the determination of Phenazopyridine in pharmaceutical formulations and bulk.

METERIAL AND METHODS

Apparatus

Elico UV – Visible Double beam spectrophotometer model SL-159.

Reagents

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

1. 0.1% Bromophenolblue (method A)
2. 0.2 %Amidoblack (method B)
3. 0.2 %Bromothymol blue (method C)
4. 0.1N HCl

Standard drug 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, B and C.

Analytical procedure

Into a series of 125 mL separating funnels, aliquots of drug sample (0.2-1.0mL) was added. 2mL of bromophenol blue (Method A), Amidoblack (Method B) and Bromothymol blue (Method C) and 0.1N HCl. The funnels were shaken vigorously with 5 mL chloroform for 2 min, and then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a 50 mL beaker, dried over anhydrous sodium chloride, and transferred to a 10 mL volumetric flask. Then the combined extract was made up to the mark with chloroform and mixed well. The absorbance of the organic phase was measured at 370nm (Method A), 620nm (Method B) and 400nm (Method C) respectively against reagent blank .The standard calibration curve was prepared to calculate the amount of the drug.

Assay of pharmaceutical formulations

Powdered drug equivalent to 100 mg was accurately weighed and dissolved in water and filtered. The filtrate was made up to 100 mL and appropriate aliquots of the drug solution were treated as described above and the results were tabulated in Table-2

RESULTS AND DISCUSSION

The proposed methods are based on the formation of ion-pair complex between the positively charged drug and anionic dyes bromophenol blue (Method A), Amidoblack (Method B) and Bromothymol blue (Method C). Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction.

$$X^{+}_{(aq)} + d^{-}_{(aq)} \leftrightarrow X^{+}D^{-}_{(aq)} \leftrightarrow X^{+}D^{-}_{(org)}$$

Where x^{+} and d^{+} represent the protonated drug and anion form of the dye respectively and subscript (aq) and (org) refer the aqueous and organic phases respectively. The different optical parameters were calculated for all the methods and the results were 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

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

Parameters	Method A	Method B	Method C
λ_{max} (nm)	370	620	400
Beer's law limit (µg/ mL)	1-5	1-5	1-5
Sandell's Sensitivity (ìg/cm^2/0.001 abs. unit)	0.0057	0.0065	0.0039
Molar absorptivity(Litre.mole^{-1}.cm^{-1})	3.73×10^4	3.241×10^4	5.437×10^4
Stability of Color (hours)	24	24	24
Regression equation (Y)*			
Intercept (a)	0.022	0.031	0.057
Slope(b)	0.017	0.014	0.027
% RSD$	1.15	1.06	0.472
% Range of errors (95% confidence limits):			
0.05 significance level	0.961	0.886	0.394
0.01 significance levelCorrelation coefficient	1.422	1.311	0.583

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

Table 2: Results of analysis of tablet formulations containing Phenazopyridine

Formulations	Labeled amount(mg)	Recovery by reference method*(%)	Recovery by proposed methods (%)**		
			Method A	Method B	Method C
Pyridium®	100	99.90	99.89	99.75	99.88
Re-Azo®	95	94.86	94.40	94.65	94.58

* Reference method was UV method developed in the laboratory.

** Recovery amount was the average of six determinants

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.

CONCLUSION

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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REFERENCES

1. Pyridium (Parke-Davis): Physician's desk reference 46th edn ., 1763-4(1992)
2. Rockville, MD .The United States Pharmacopoeia Convention, Inc (2000) United States Pharmacopeia, USP 24, NF 19. The United States Pharmacopoeia Convention, Inc, Electronic Version
3. Pyridium (Parke-Davis), Krogh CME, (ed): Compendium of pharmaceuticals and specialties, 26th edn. Ottawa,1006 (1991).
4. Product Information: Pyridium, phenazopyridine. Warner Chilcott Laboratories, Rockaway, NJ, USA, Rev. 06(1998)
5. Li, Kai-jun; Chen, Qin-hua; Zhang, Zhuo; Zhou, Peng Li, Peng Liu, Jia; Zhu, Jun., *J. Chroma. Sc.,* **46**(8): 686-4 (2008).
6. Tarek Saied Belal., *J, Fluore.,* **18**(5): 771-780 (2008)
7. Osman Çakir, Ender Biçer., *J.Elec.anal.,* **9**(1): 87-90 (2005).
8. Palabiyik, M, Onur, F., *Ana., Let.*, **37**(10): 2125 - 50 (2004).
9. Nevin Erk, U. Gulay Gonullu., *Ana., Let*., **35**(1): 83-98 (2002).
10. Farin, D. Piva, G. Kitzes-Cohen., *J.Chromat*., **52**(3): 179-180 (2002).
11. Wu Yan-Wei; Xiang Bing-Ren; Shang Er-Xin; Zhang Wei Yao xue bao., *Ac. Pharma. Sin.,* **40**(7): 668-72 (2005).