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Spectrophotometric Determination of Azathioprine in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT: Two simple, accurate and precise Spectrophotometric methods have been developed for the determination of Azathioprine in bulk and pharmaceutical dosage forms.**Method A** is based on oxidation followed by complexation reaction of reduced Azathioprine with potassium ferricyanide in presence of ferric chloride to form a greenish blue coloured chromogen with an absorption maximum at 667.5nm and Beer's law is obeyed in the concentration range of 60-120 µg /ml. **Method B** is based on the diazotization of reduced Azathioprine in presence of nitric acid and followed by direct coupling with Bratton Marshall reagent to form dark pink colored chromogen with an absorption maximum at 519nm and obeyed Beer's law in the concentration range of 20-50 µg/ml.
Key words: Azathioprine, U.V / Visible Double Beam Spectrophotometer,Bratton Marshall Reagent, Potassium ferricyanide.

INTRODUCTION

Azathioprine[1-8] is chemically 6-[(1-methyl-4-nitro-1*H* imidazol-5yl) sulfanyl]-7*H*-purine. It is having immunosuppressive action which is given orally or by I.V route. It has marked effect on T-lymphocytes and suppresses cell mediated immunity. It is mainly used to prevent rejection in organ transplantation and also useful in a variety of auto-immune disorders. It is converted in the body to the anti-metabolite Mercaptopurine. It is official in U.S.P, European Pharmacopoeia and in British Pharmacopoeia. In literature no simple analytical methods were reported for its quantitative estimation in bulk drug as well as its formulations (Tablets).The present work deals with the development of two simple and sensitive colorimetric methods for the quantitative estimation in bulk drug as well as its formulations (Tablets).

MATERIALS AND METHODS

A Shimadzu U.V/ Visible double beam spectro photometer (Model 1700) with 1cm matched quartz cells are used for all spectral measurements. Azathioprine was obtained from RPG Life Sciences, Mumbai and all chemicals used are of AR grade from S.D.Fine chemicals, Mumbai

Standard drug solution:

Azathioprine equivalent to 100 mg of bulk drug was weighed accurately which was obtained from the RPG Life Sciences, Mumbai and dissolved in 20 ml of 1N NaOH and reduction is carried over using 0.75 gm of Zinc granules and 10 ml of 4N Hcl. After standing for 1hour at room temperature, filtered through whatmann paper grade1. The residue washed with 1N NaOH (10 ml×3 times each) and made upto 100 ml with 1N NaOH. Then final concentration made upto 1000 µg /ml. Further dilution was made to 100 µg/ml.

Sample Preparation:

20 Commercial tablets of Azoran each containing 50 mg of Azathioprine, marketed by RPG Life Sciences, Mumbai were analysed by the proposed methods. The uncoated tablets were finely grounded and weight equivalent to 100 mg was taken, then dissolved in 20 ml of 1N NaOH and to that 0.75 gm of Zinc granules and 10 ml of 4N Hydrochloric acid were added. After

standing for 1 hour at room temperature, filtered through whatmann paper grade1. The residue washed with 1N NaOH (10 ml × 3 times each) and made upto 100 ml with 1N NaOH. Then final concentration made upto 1000 µg /ml. Further dilution was made to 100 µg/ml.

Assay:

Method A: Aliquots of Azathioprine ranging from 0.6-1.2 ml (1ml=100 µg) were transferred into a series of 10 ml volumetric flasks. To each of these flasks 1 ml of potassium ferricyanide (0.5% w/v) and 1 ml of sulfuric acid (4N) were added. These are kept aside for 10 minutes for color development and made upto 10 ml with distilled water. The absorbance of the blue colored chromogen was measured at 667.5nm against reagent blank. The color was stable for more than 8 hours. The amounts of Azathioprine present in the sample and pure were computed from the calibration curve.

Method B: Aliquots of Azathioprine ranging from 0.2-0.5 ml (1ml=100 µg) were transferred into a series of 10 ml volumetric flasks. To each of these flasks 1 ml of Nitric acid (0.1% w/v),1 ml of Hcl (0.5 M), 2 ml of Bratton Marshall reagent (0.2% w/v) and 1 ml of Sodium hydroxide (2% w/v) were added and kept aside for 10 minutes and made upto 10 ml with distilled water. The absorbance of the dark pink colored chromogen was measured at 518 nm against reagent blank. The color was stable for more than 14 hours. The amount of Azathioprine present in the sample and pure was computed from the calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in **Table -1**. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations and the results are summarized in **Table-1**. The % RSD and % range of errors (0.05, 0.01 levels of confidence limits) are calculated from the eight measurements. The results showed that these methods have reasonable precision. Comparison of results obtained with the proposed methods and with that of U.V method for dosage forms (Table-2) confirm the suitability of the methods for pharmaceutical preparations (Tablets).

The optimum conditions for color development for Methods A, B have been established by varying the parameters one at a time and keeping the other parameters fixed and the effects of product on the absorbance of the colored species and incorporated in the procedures. To evaluate the validity and accuracy of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparations and then the results were compared with the proposed methods. The % recoveries are given in **Table-2**. Interference studies revealed that the additives like antioxidants, preservatives and solubilizers that were usually present in tablets did not interfere at their regularly added levels.

The proposed visible spectrophotometric methods are found to be simple, sensitive, accurate, precise and economical and can be used in the determination of Azathioprine in bulk drug and its pharmaceutical preparations in a routine manner.

TABLE 1: OPTICAL CHARACTERISTICS

Parameter	Method A	Method B
λ_{max} (nm)	667.5	518
Beer's Law Limits (µg/ml)	60-120	20-50
Molar absorptivity (l/mole/cm)*	2.1075×10^3	5.5914×10^3
Sandell's Sensitivity (µg/cm x 0.001 Absorbance unit)*	0.1304	0.0505
Regression equation * (y = mx + c)		
Slope (m)	0.0008	0.0020
Intercept (c)	0.0197	0.0147
Correlation Coefficient (r)	0.9996	0.9998
% RSD	0.1101	0.3524
Range of errors †		
Confidence limits with 0.05 level	0.0007	0.0017
Confidence limits with 0.01 level	0.0010	0.0026

† Average of eight determinations. * Y = bC + a, where C is the concentration of Azathioprine in µg / ml and Y is the absorbance of the respective λ_{max}.

TABLE-2 EVALUATION OF AZATHIOPRINE IN PHARMACEUTICAL PREPARATIONS

Sample*	Labelled Amount(mg)	Amount obtained (mg) Proposed Method			% Recovery†		
		I	II	U.V Method	I	II	U.V Method
S1	50	49.31 ±0.04	49.28 ±0.06	49.87 ±0.03	98.13 ±0.04	98.78 ±0.01	99.35 ±0.02
S2	50	49.40 ±0.02	49.36 ±0.03	49.91 ±0.01	98.56 ±0.03	98.66 ±0.03	99.61 ±0.04
S3	0	49.62 ±0.06	49.71 ±0.02	49.95 ±0.02	98.87 ±0.04	98.77 ±0.06	99.49 ±0.04

* Tablets from different manufacturers.
† Average of ± S.D of three measurements.

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