SPECTROPHOTOMETRIC AND ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION AND VALIDATION OF AZATHIOPRINE IN API AND PHARMACEUTICAL DOSAGE FORM

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This paper describes two simple, sensitive and selective spectrophotometric and atomic absorption spectrometric (AAS) procedures for the determination of compounds of Azathioprine. These procedures are based on the formation of ion-pair complexes between the drugs and ammonium reineckate. The formed precipitates are quantitatively determined either spectrophotometrically or by AAS procedures. The methods consist of reacting drugs with Reinecke's salt in an acidic medium at a temperature of 25 ± 2°C. The spectrophotometric procedure (procedure I) is based on dissolving the formed precipitate with acetone. The volume was completed quantitatively and absorbance of the solution was measured at 391.4 nm against blank. Also, the AAS procedures (procedure II) are quantitatively determined directly or indirectly through the chromium precipitate formed or the residual unreacted chromium in the filtrate at 307.3nm. The optimum conditions for precipitation have been carefully studied. Beer's law is observed for the studied drugs in the ranges 0.1-1.5 mg mL-1 or 5-70 µg mL-1 using spectrophotometric or AAS methods, with correlation coefficients ≥ 0.9965, respectively. Both procedures I and II were accurate and precise when applied to the analysis of the cited Azathioprine in different dosage forms with good recovery percent ranged from 98.90 ± 0.94 to 100.15 ± 0.97 without interference from additives.

(Received November 2, 2010; accepted November 24, 2010)

Keywords: Azathioprine, reinecke's salt, spectrophotometry, atomic absorption

1. 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).

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Table: 1 Quantitative parameters for determination of Azathioprine Spectrophotometric procedure

Bulk drug	Linearity range	slope	Intercept	Coefficient Corr.
Azathioprine	2.0-16.0	0.019	0.81	0.294

Table: 2 Determination of the investigated drugs in tablets by Atomic Absorption spectrometry

Sample	Label Claimed	Amount Found	%age of Label Clamed Found	Percentage Recovery	Coefficient Corr.
Azathioprine	10	10.08	99.986	99.98	0.41

*Mean of six estimations.

2 Experimental

2.1 Materials

All chemicals used are of AR grade from S.D.Fine chemicals, Mumbai Commercial tablets of Azoran marketed by RPG Life Sciences, Mumbai were procured from local market.

Apparatus

UV/Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). A Shimadzu atomic absorption flame spectrophotometer model with a chromium hollow cathode lamp under the following observations: height above burner 1 cm,single slot type burner, air flow-rate 21.51/min; acetylene flow-rate 3.41/min operation conditions: lamp current 10 mA; slit width 5.2 nm; wavelength current 54 mA; slit width 1.5nm; wavelength 307.5 nm.

2.2 Pharmaceutical Preparations

Stock solutions 15.0 mg mL^{-1} were prepared by accurately weighing (1.5 g) of the examined pure drug into a 100-mL calibrated flask, dissolved in double distilled water and kept in the dark to avoid degradation of the drugs. Stock solution, 15×10^{-5} M ammonium reineckate (Aldrich product) solution was also prepared by dissolving the appropriate weight in 100 mL of triple distilled water.

2.3 Spectrophotometric procedure

Aliquot containing 1.00–15 mg of the investigated drugs was transferred into a 10-mL calibrated flask; 3.0 mL of 15×10^{-5} M of ammonium reineckate and 5.0 mL of 0.01 M H_2SO_4 were added successively. The mixture was left to stand for 10 min and completed the volume with water. The precipitate was filtrated through a sintered glass funnel after 1 hr, and washed three

times with 50 mL of ice water. Afterwards, the precipitate was dried in a vacuum desiccator the volume was completed quantitatively

with acetone to the appropriate volume. Absorbance of the solutions was measured at 391.2 nm, against a reagent blank solution prepared in the same way without the drug.

The calibration graph was obtained by applying the procedure and using standard drug solutions.

2.4 Atomic absorption spectrometry

Reagents precipitates were collected on a glass funnel and washed with 10mL portions of distilled water. Dissolved in 50 mL of ether. The solution was then nebulizer in an flame of AAS measurement of chromium ion concentration at 307.3 nm. Concentration of the tested drug was calculated from the relevant calibration graph. Indirect Procedure

Of the filtrate and washings from the direct procedure were Collected in a 100-mL volumetric flask; and Completed to volume with ether. The resulting solution (5 mL) was diluted to 25 M with ether. Concentration of the tested drug was calculated from the relevant calibration graph.

2.5 Preparation of samples

Twenty tablets of the drug were thoroughly ground.A quantity equivalent to 5 mg of drug was accurately weighed into a 100-mL volumetric flask, completed to volume with water and filtered per method I or II.

3. Results and discussion

For the spectrophotometric procedure the absorption spectrum of the reaction products was measured at 391.2 nm. For the atomic absorption spectrometric procedure (procedure II), acidic solutions of the drugs yielded purple coagulated precipitates with ammonium reineckate.

3.1 Calibration graph for spectrophotometric procedure

A calibration graph was Solutions having concentrations of 2.0, 4.0, 6.0, 8.0, 10.0 _g/mL ammonium reineckate were measured. Each measurement was performed at least four times to check the reproducibility.

3.2 Calibration graph for AAS

A calibration graph was Solutions having concentrations of 2.0, 4.0, 6.0, 8.0, 10.0 _g/mL ammonium reineckate were measured. Each measurement was performed at least four times to check the reproducibility. Spectrophotometric procedure, Atomic Absorption spectrometry provided a new and alternate route for analysis of like Azathioprine. In the present work investigated drugs are found to react with ammonium reineckate to form suitable molecular complexes. These complexes are insoluble in aqueous phase. Therefore they were extracted with dilute Sulphuric acid and measured their atomic absorptions at 307.3nm. Azathioprine can be determined in the concentration ranges 2.0-16.0 and 2.5-21.0 $\mu g \cdot ml^{-1}$ with mean percentage recovery 100.01 ± 0.02. (Table 1) These methods were also applied for the analysis of pharmaceutical formulations of both the drugs as shown in Table (2) along with recovery studies. These methods can be employed for routine analysis of Azathioprine in quality control laboratories.

4. Conclusions

We developed a new spectrophotometric method: Atomic Absorption spectrometry with ammonium reineckate in the pharmaceutical preparation.This paper reports a new example of associate complex application in drug analysis. Spectrophotometric procedure or Atomic Absorption spectrometry has the advantages of being fast and simple compared to other analytical techniques. The AAS method showed wide dynamic range, high sensitivity, low quantification limit and no interference Azathioprine formulation. The proposed methods proved to be sensitive, accurate and precise, as well as simple to handle with higher tolerance limits relative to previously published methods.

Acknowledgement

The author is thankful to Prof.D.V. Kohli for given valuable suggestion to project.

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