ejpmr, 2017,4(5), 455-458

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

TO STUDY THE EFFECT OF BASIC SOLVENT ON MERCAPTOPURINE BY USING UV-SPECTROPHOTOMETER

Rajashri Patil*, Mahananda Ghodke, Vishal Jadhav and Mukesh Mohite

Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044, Maharashtra, India.

***Corresponding Author: Dr. Rajashri Patil**

Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044, Maharashtra, India.

Article Received on 14/03/2017 **Article Revised on 04/04/2017** **Article Accepted on 25/04/2017**

ABSTRACT

Three precise and economical UV methods have been studied for estimation of Mercaptopurine in bulk formulation. Method A involves measurement of UV absorbance in Zero order derivative & Method B involves first order derivative are at 219 & 299 nm respectively. Method C deals with Area Under Curve measurement (AUC method), which involves the calculation of integrated value of absorbance with respect to wavelength between 306-315 nm. The drug follows Beer-Lambert's law in the concentration range of 1-10 µg/ml in all three methods. Results of analysis were validated statistically and were found to be satisfactory. Thus proposed anhydrous solvent i.e. Sodium hydroxide and methods can be successfully applied for estimation of Mercaptopurine in routine analytical work.

KEYWORDS: Mercaptopurine, Sodium hydroxide, Zero Order derivative, First order derivative, Area Under Curve method (AUC), UV Spectrophotometer.

INTRODUCTION

Mercaptopurine is 3,7-dihydropurine-6-thione. It is an oral anti-cancer drug. Mercaptopurine was originally developed and continues to be used for chemotherapy, either alone or in combination with other agents. It is effective for the treatment of a number of cancers, including: Acute lymphoblastic leukemia (ALL), Acute promyelocytic leukemia (APL), Ulcerative colitis, Lymphoblastic lymphoma, and Crohn's disease. Mercaptopurine is thought to affect cancer by following pathway. For cancer, Mercaptopurine converted to thioinosinic acid, by hypoxanthine-guanine phosphoribosyl-transferase and then metabolised to thioguanine ribonucleotide and deoxyribonucleotide; incorporation of these compounds into RNA and DNA results in the antitumor effect of the drug. The drug is official in Indian pharmacopoeia, USP and BP.

The method was validated according to the ICH guidelines.[10,11]

MATERIALS AND METHODS

Materials

Mercaptopurine was obtained as gift sample from Aribindo pharmaceuticals and distilled water and Sodium Carbonate (Anhydrous) were used as a solvent in the study.

Instrument

A Shimadzu UV-1700 UV/VIS Spectrophotometer was used with 1cm matched quartz cells were used for spectral measurements.

Stock Solution

Accurately about 5 mg of Mercaptopurine was weighed and transferred to 50 ml volumetric flask; 20 ml of Sodium hydroxide solution (0.1 N) was added to dissolve the drug completely with vigorous shaking. Then the volume was make up with the distilled water up to the mark.

Method a

The Zero order derivative spectra at n=0 showed a sharp peak at 219 nm (Fig. 1). The absorbance difference at n=1 (dA/dλ) was calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. A calibration curve was plotted taking the absorbance difference (dA/dλ) against the concentration of Mercaptopurine. The coefficient of correlation (r^2), slope and intercept values of this method are given in table 1.

Method b

The First order derivative spectra at n=1 showed a sharp peak at 299 nm (Fig. 2). The absorbance difference at n=1 (dA/dλ) was calculated by the inbuilt software of the instrument which were directly proportional to the concentration of the standard solution. In the first order derivative spectra the standard drug solutions were

scanned. A calibration curve was plotted taking the absorbance difference (dA/dλ) against the concentration of Mercaptopurine. The coefficient of correlation (r^2), slope and intercept values of this method are given in table 1.

Method c

The AUC (Area Under Curve) method involves the calculation of absorbance with respect to the wavelength between range of wavelengths λ1 and λ2. To get the linearity between area under curve and concentration this range is selected on the basis of repeated observations. Suitable dilutions of standard stock solution of Mercaptopurine were prepared and scanned in the spectrum mode from the wavelength range 400 nm to 200 nm (Fig. 3) and the calibration curve was plotted as AUC against concentration of Mercaptopurine. The method was checked by analyzing the samples with known concentration.

ANALYSIS OF TABLET FORMULATION

For the estimation of Mercaptopurine in tablet formulation, tablets were weighed and ground into a fine powder. Tablet powder equivalent to 50 mg of Mercaptopurine weighed and transferred to 50 ml volumetric flask and dissolve in 20 ml of Sodium hydroxide Solution. It was kept for ultra sonification for 15 min, finally the volume was made up to the mark with distilled water, and this was then filtered through Whatman filter paper to get tablet stock solution. Various dilutions of the tablet solution were prepared and analyzed for six times and concentration was calculated by using calibration curve for the three methods. All the methods were validated according to ICH guidelines. Recovery studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug (4, 6 and 8 mg respectively) to previously analyzed tablet powdered sample (0.5 mg) as per ICH guidelines and percentage recovery was calculated as shown in table 3. All the methods were validated for linearity, accuracy and specificity.

METHOD OF VALIDATION

Precision

Precision of the method was determined by repeating the assay 3 times for six replicate dilutions of the same concentrations after every two hours on the same day for intraday precision. Performing the assay of the same sample solution after 24 hours and 48 hours carried out interday & intraday precision. The results are shown in the table 4.

Linearity

A series of volumetric flasks of 10 ml capacity were arranged. To each of these flasks 1, 2, 3, 4, 5 ml of the drug stock solution ware added. The volume was made up with distilled water. The absorbance was measured at 219 nm in method A, 299 nm in method B and 306-315 nm in method C against the reagent blank. A linear graph of absorbance v/s concentration was obtained. The concentration range over which the drugs to obeyed Beers-Lamberts law was found to be 1-10 µg/ml for Mercaptopurine.

RESULT AND DISCUSSION

All the methods A, B & C for the estimation of Mercaptopurine in tablet form were found to be simple, precise, accurate, rapid and reproducible. Beer-Lambert's law was obeyed in the concentration range of 1-10 µg/ml in all the methods (Graph.1). The values of standard deviation were satisfactory low and the recovery studies were close to 100% (Graph.2). The spectroscopic method applied has the advantage that it provides the hidden peaks in the normal spectrum when the spectrum is not sharp and it also discard the interference caused by the excipients present in the formulation. The AUC method has advantage that it is applicable to be drug which shows the broad spectra without a sharp peak. Hence the three methods can be employed for routine analysis of the drugs in Quality Control, R&D laboratories by using Anhydrous solvent like Sodium carbonate.

CONCLUSION

It has been proved that the stability of the drug in proposed solvent is up to the mark, it can be evaluated by interday and intraday study and all three methods were developed and validated as per ICH guidelines for estimation of Mercaptopurine. These methods and solvent were applied for estimation of marketed formulation. The method has been evaluated for the linearity, precision, LOD and *L*OQ in order to ascertain the suitability of the method. The results of the study can be used in quality control departments with respect to routine analysis for the assay of the tablet containing Mercaptopurine.

Table 1: Optical characteristics & parameters

Sr. No.	Parameters	Method A	Method B	Method C
1	Wavelength(nm) (λ Max)	219	299	306-315
2	Beer's – Lambert's range (µg/ml)	1-10	1-10	1-10
3	Coefficient of correlation (r^2)	0.9990	0.9980	0.9980
4	Regression equation	Y =0.057x + 0.015	Y=0.003x+0.0003	Y=0.802x+ 0.072
5	a – Slope (m)	0.057	0.003	0.802
6	b – Intercept (c)	0.015	0.0003	0.072
7	LOD	10.84	10.92	10.85
8	LOQ	32.54	32.76	32.55

Table 2: Assay of the tablet

Sr. No.	Method	Conc.(µg/ml)	Amount found (mg)*	% Mean	S.D.	R.S.D. %	S.E.
1	A	10	10.00	99.92	0.945	1.06	0.479
2	B		10.02	100.97	1.775	1.97	0.887
3	C		10.11	112.87	1.317	1.36	0.687

When*n=6 at each level of recovery

Table 3: Recovery studies

Sr. No.	Tablet Sample	Level of recovery %	Mean*			S.D.*			C.O.V			S.E.*		
			A	B	C	A	B	C	A	B	C	A	B	C
01	T1	80	108.9	103.3	101.8	0.127	0.981	0.988	0.016	0.018	0.021	0.052	0.048	0.031
02		100	107.0	105.89	101.37	0.191	0.762	0.793	0.036	0.039	0.04	0.078	0.071	0.081
03		120	99.11	99.06	97.64	0.117	0.696	0.897	0.013	0.026	0.028	0.048	0.043	0.032

When *n=3 at each level of recovery, T1: Folitrex (5mg)

Table: 4 Statistical validation for precision.

Sr. No.	Component	Mean			S.D.			R.S.D.			S.E.		
		A	B	C	A	B	C	A	B	C	A	B	C
1	Intra-day	99.07	98.89	98.79	0.49	0.44	0.56	0.49	0.49	0.57	0.20	0.23	0.31
2	Inter-day	99.03	98.58	98.80	0.25	0.31	0.55	0.25	0.29	0.55	0.10	0.22	0.23

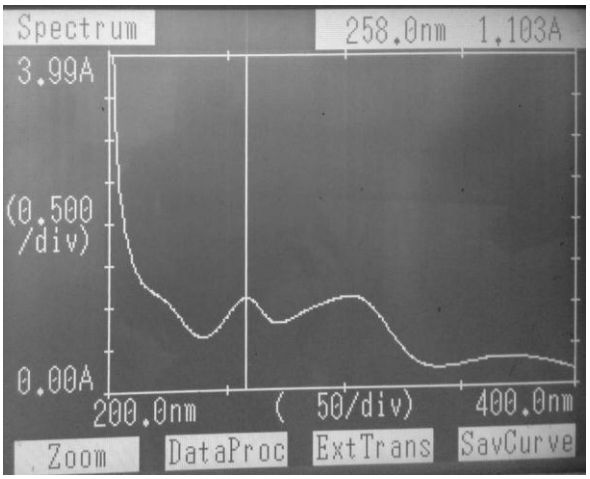


Fig: 1 Spectrum by zero order derivative method.

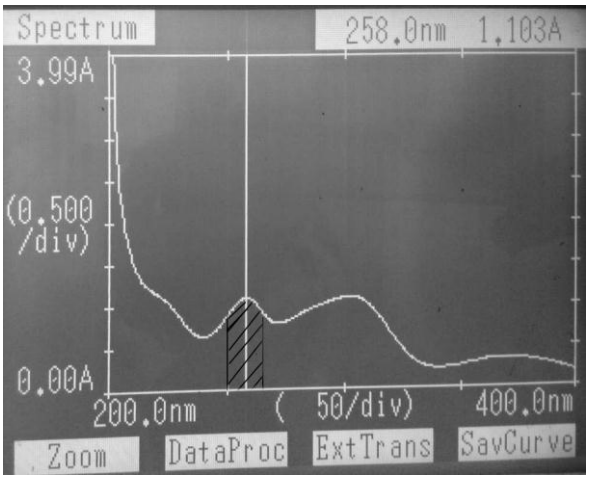


Fig. 3: Spectrum by AUC method.

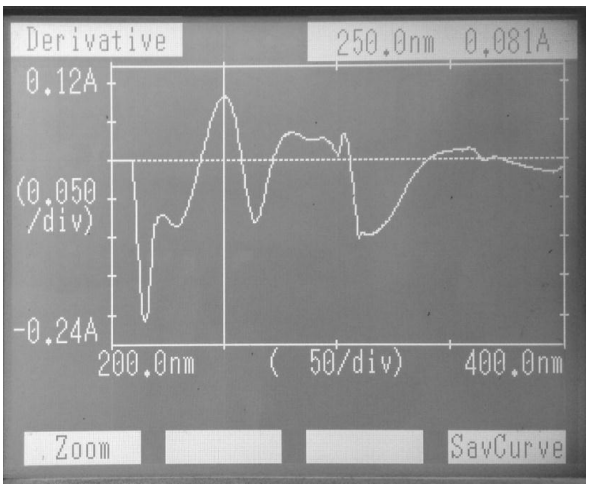
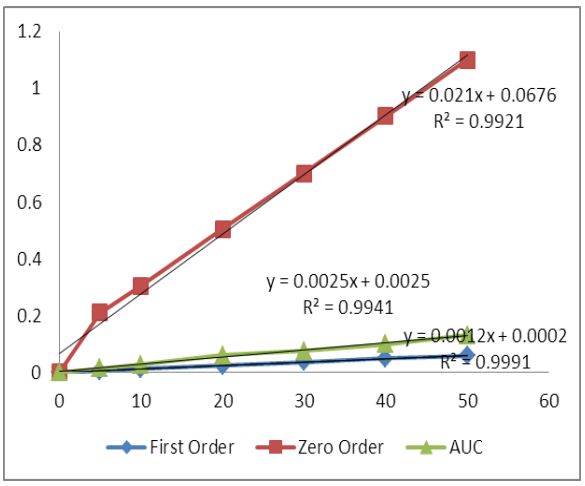
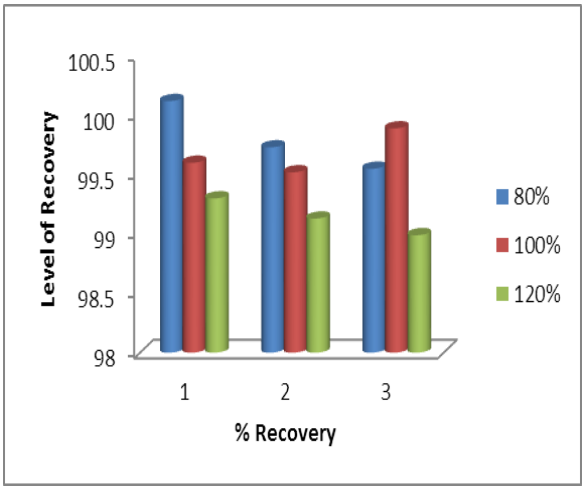


Fig. 2: Spectrum by first order derivative method.



Graph 1: optical parameters of method A, B and C.



Graph 2: recovery study by method A, B and C.

REFRENCES

1. Sean C.S Martindale the complete drug reference: Mercaptopurine. 6th edition Published by the pharmaceutical press an imprint of RPS publishing, 2009; 745-51.
2. The Merck index, An Encyclopedia of Chemicals: Mercaptopurine. Drugs & Biologicals, white house, 13th ed., 2001; 1071.
3. Remington, The Science and Practice of Pharmacy: Mercaptopurine. published by Wolters Kluwer (India) pvt. ltd, 21st ed., New Delhi, II : 1580.
4. The Indian pharmacopoeia, Mercaptopurine Tablets, The controller of publication, volume-II, 4th ed., New Delhi, 1996; 474.
5. British Pharmacopeia, Mercaptopurine, amended by supplements, 2004, II: 1278.
6. USP NF, The Official Compendia of Standards, volume-III Asian ed., 2008; 3: 2662-2663.
7. Subbarayan S and Karthikeyan V. Analytical method development and validation of layer by layer magnetic naoparticles of Mercaptopurine and Melphalan. World journal of pharmacy and pharmaceutical sciences, 2014; 3(3): 1221-1253.
8. Oliveira AR, Caland LB, Oliveira EG, Matheus FF, and Arnobio AS. HPLC-DAD and UV-Visible Spectrophotometric Methods for Mercaptopurine Assay in Different Biodegradable Microparticles. J Braz Chem Soc., 2015; 26(4): 649-659.
9. Bandi R, Naidu VS, Sugunb P and Kantipudi R. Validation of UV–Visible Spectrophotometric method for the analysis of Mercaptopurine in pharmaceutical formulations. International Journal of Pharmacy and Pharmaceutical Science Research, 2013; 3(3): 108-114.
10. ICH Q2A, Textbook on validation of analytical procedures, International Conference on Harmonization, Geneva Oct. 1994.
11. ICH Q2B, validation of analytical procedures, International Conference on Harmonization, Geneva Nov, 1994.