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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF THIOGUANINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Thioguanine in pharmaceutical dosage form. Chromatographic separation of Thioguanine was achieved on Waters Alliance-e2695, by using Waters X- Bridge RP18, 150mm x 4.6mm, 3.5µm, column and the mobile phase containing 2.0gm Hexane-1-Sulphonic acid is dissolved in 1lt water adjust pH-2.5 with OPA **&** ACN in the ratio of 40:60% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 286nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Thioguanine was NLT 2000 and should not more than 2 respectively. %Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Thioguanine and study of its stability.

Key words: HPLC, Thioguanine

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INTRODUCTION

A drug may be defined as a substance meant for diagnosis, cure mitigation, prevention or treatment of diseases in human beings or animals or for alteringany structure or any function of the body. Drugs play a vital role in the progress of human civilization by curing diseases. Today majority of the drug used are of synthetic origin. These are produced in the bulk and used for their therapeutic effects in pharmaceutical formulations. There are biologically active chemical substances generally formulated in to convenient dosage forms such as tablets, capsules, ointments and injectableets, these formulations deliver the drug substance in stable, on-toxic and acceptable form, ensuring its bioavailability and therapeutic activity. Safety and efficacy are two fundamental issues of importance in drug therapy. The safety of a drug is determined by its pharmacological- toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The impurities in drugs often possess unwanted pharmacological or

toxicological effect by which any benefit from their administration may be outweighed. Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipients) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions. Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time." A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it. A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species.

Thioguanine is 2-amino-3,7-dihydropurine-6-thione, thioguanine competes with hypoxanthine and guanine for the enzyme hypoxanthine- guanine phosphor ribosyl transferase (HGPRTase) and is itself converted to 6- thioguanilyic acid (TGMP), which reaches high intracellular concentrations at therapeutic doses. TGMP interferes with the synthesis of guanine nucleotides by its inhibition of purine biosynthesis by pseudo feedback inhibition of glutamine-5- phospho ribosyl pyrophosphate amidotransferase, the first enzyme unique to the de novo pathway of purine ribonucleotide synthesis. TGMP also inhibits the conversion of inosinic acid (IMP) to xanthylic acid (XMP) by competition for the enzyme IMP dehydrogenase. Thioguanine nucleotides are incorporated into both the DNA and the RNA by phosphodiester linkages, and some studies have shown that incorporation of such false bases contributes to the cytotoxicity of thioguanine. Its tumor inhibitory properties may be due to one or more of its effects on feedback inhibition of de novo purine synthesis; inhibition of purine nucleotide inter conversions; or incorporation into the DNA and RNA. The overall result of its action is a sequential blockade of the utilization and synthesis of the purine nucleotides (1-5).

From the literature survey conducted, it was found that there are few analytical methods reported for estimation of Thioguanine by using HPLC. A comprehensive, validated and simple analytical method for assay of Thioguanine tablets and degradation products is therefore crucial. HPLC with PDA detector is a good selection as PDA detector is available in most laboratories. Therefore, in proposed project a successful attempt has been made to develop simple, accurate and economic methods for analysis of Thioguanine tablets and validated.

MATERIALS AND METHOD

Preparation of Standard Solution (6-7)

25 µg/ml of Thioguanine is prepared by diluting with mobile phase. This solution is used for recording chromatogram.

Preparation of sample Solution

2 tablets (each tablet contains Thioguanine-40 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solution of Thioguanine (µg/ml) was prepared by dissolving weight equivalent to 25 mg of Thioguanine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. further dilutions are prepared in 5 replicates of 25 µg/ml of Thioguanine was made upto mobile phase.

Forced degradation studies

Forced degradation studies on drug product, placebo formulation containing one drug substance and blank were performed by applying the following types of stress to obtained degradation of about 1% to 20%.Peak purity degradation table for forced degradation samples

Acid degradation

36.25mg of sample transferred into 100ml volumetric flask to this add 0.1N HCL and kept on bench top for 10min then neutralized it with 0.1N NaOH then the remaining procedure is same as the test preparation**.**

Base degradation

36.25 mg of sample transferred into 100ml volumetric flask to this add 0.1N NaOH and kept on bench top for 10min then neutralized it with 0.1N HCL then the remaining procedure is same as the test preparation**.**

Peroxide degradation

36.25mg of sample transferred into 100ml volumetric flask to this add 10%H_2O_2 and kept on bench top for 10min then the remaining procedure is same as the test preparation.

Thermal degradation

Respective weights of sample, API subjected to heat 70^0c for 30min then the samples were analyzed as that of test preparation

RESULTS AND DISCUSSION

Determenation of Working Wavelength (λ_{max})

The spectra of drug shows at 286 nm (Fig-1), Thus 286 nm was selected as detector wavelength for the HPLC chromatographic method.

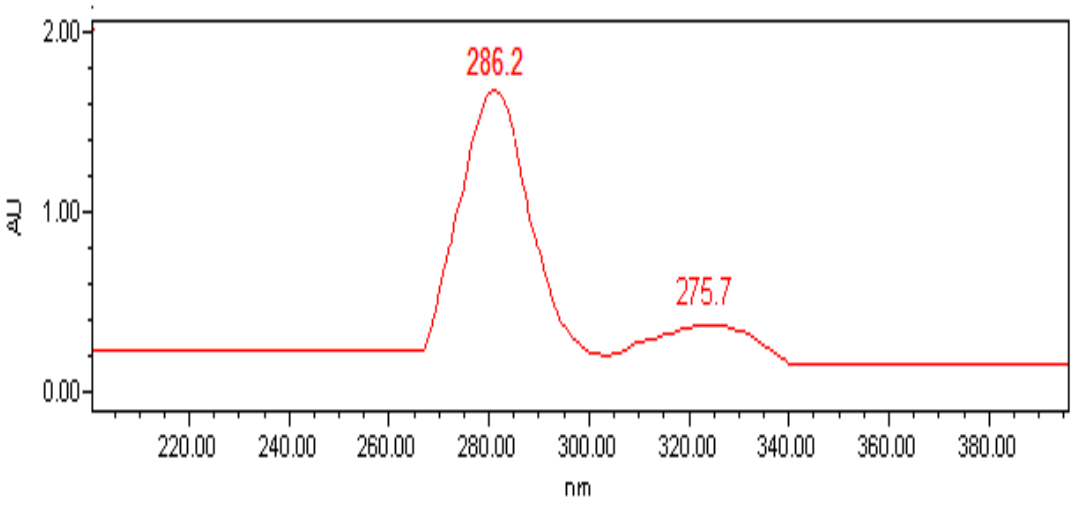

Fig-1 PDA spectrum of Thioguanine

Linearity

Linearity of detector response was established by plotting a graph of concentration vs response of Thioguanine peak. The detector response was found to be linear from about 4-60 µg/ml. The correlation coefficient, squared co-relation coefficient, slope, intercept and residual sum of squares were calculated and squared correlation coefficient was found to be within the acceptable limits (Table-1).

Table-1 Linearity of detector response of Thioguanine

S.no.	Conc.(µg/ml) of Thioguanine	Area	Acceptance criteria
		Thioguanine	
1	4.0 µg/ml	443054	Squared co relation coefficient should be not less than0.999.
2	6.25 µg/ml	783514	
3	12.5 µg/ml	1606209	
4	25.0 µg/ml	3134505	
5	31.25 µg/ml	3978517	
6	37.5 µg/ml	4785617	

Accuracy

A series of sample solution were prepared in triplicate (3 preparations for Thioguanine levels 50% to 150%) by spiking the Thioguanine API on placebo in the range of about 50% to 150% of test concentrations of 40 mg tablets, injected into HPLC system and analyzed as per the test method. Individual % recovery, mean % recovery, %RSD and squares correlation coefficient for linearity of the test method were calculated and the results were found to be within the acceptable limits (Table-2).

Table-2 Accuracy data of Thioguanine

Recovery level	Accuracy of Thioguanine					%RSD
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	
50%	18.13	1625742	1625437	100.45	100.1	0.74
	18.13	1637252				
	18.13	1613317				
100%	36.25	3459312	3462423	100.36	100.3	0.30
	36.25	3453831				
	36.25	3474127				
150%	54.38	3881148	3874889	100.28	100.3	0.21
	54.38	3878082				
	55.36	3896121				

Degradation Studies

Forced degradation studies on drug product, placebo formulation containing one drug substance and blank were performed by applying the following types of stress to obtained degradation (Fig-2-5) of about 1% to 20%. Peak purity degradation table-3 is given for forced degradation samples.

Table-3 Degradation Studies Data

S.no	Degradation Parameters	Time	Peak Area	%Recovery	%Degradation
1.	Acid	30min	2539466	72.4	15.3
2.	Alkali	30 min	2557829	72.8	15.5
3.	Humidity	30min	2593330	71.6	14.6
4.	Photolytic	30 min	2577028	74.5	16.3
5.	Reduction	30 min	2586907	76.1	17.2
6.	Thermal	30 min	2576285	79.2	18.8

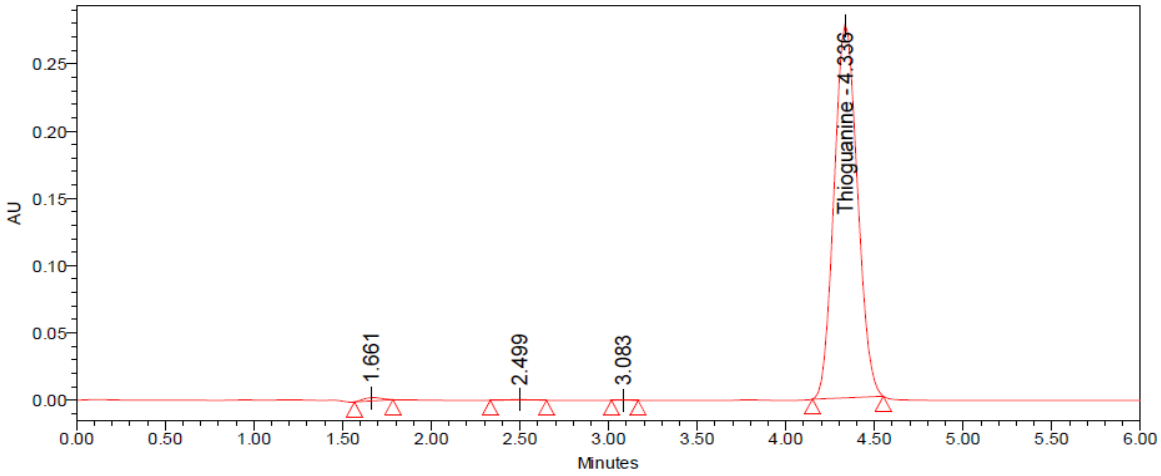

Fig-2 Acidic degradation study

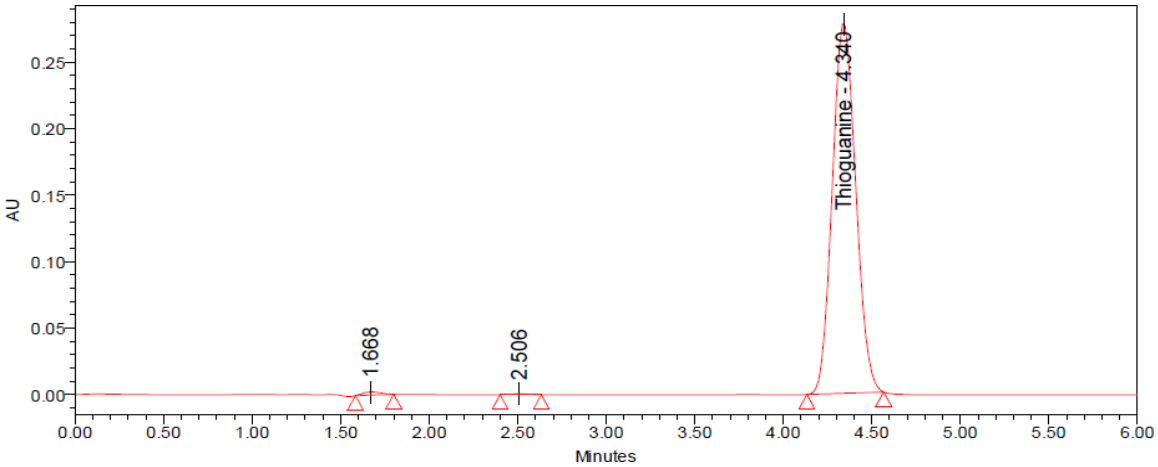

Fig-3 Base degradation study

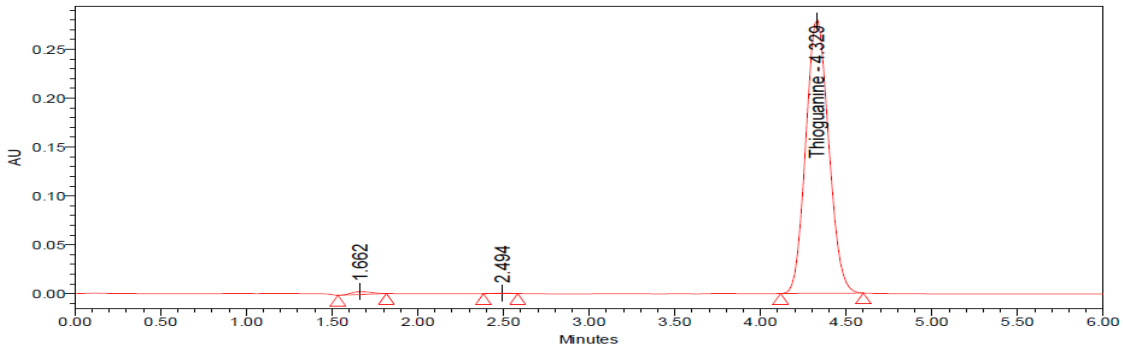

Fig-4 Humidity degradation

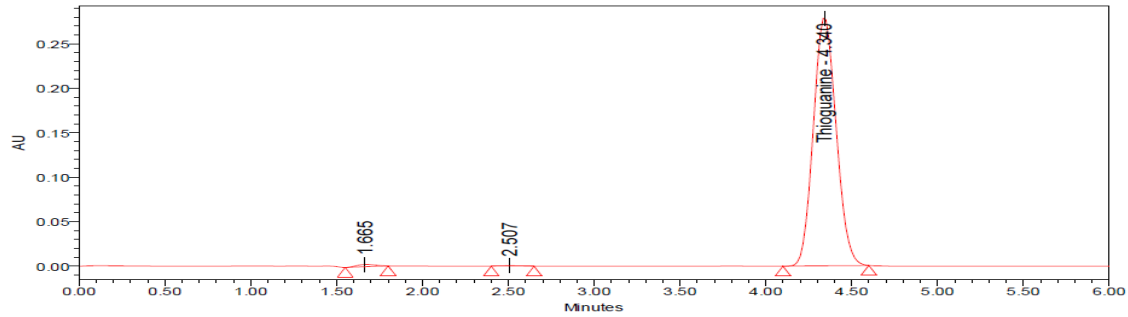

Fig-5 Thermal degradation study

CONCLUSION

Development and validation of RP-HPLC method for the estimation of Thioguanine in bulk and pharmaceutical dosage forms" with the facilities and the results are incorporated in this thesis. In conclusion a validated RP-HPLC method has been developed for determination of Thioguanine the bulk and tablet dosage forms. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of Thioguanine tablet dosage form. Several analytical procedures have been proposed for the quantitative estimation of Thioguanine separately and in combination with other drugs. So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Thioguanine in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. Thioguanine sample was injected into rat body collected samples at different time intervals like 0.5, 1.0, 2.0, 6.0, 12.0, 18.0, 24.0 Hr. After that sample are prepared as per test method injected into chromatographic system record their values. At 12 hr the sample reaches the maximum result, suddenly down to 18.0 hr.

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