



A Validated HPTLC Method for Determination of Propyl Thiouracil in the Presence of its Impurities

Arti V. Gajbhar*, B. S. Kuchekar, V. P. Choudhari

Dept. of pharmaceutical Chemistry, MAEER's Maharashtra Inst of Pharmacy, Ex- Serviceman Colony, Paud Road, Kothrud, Pune, India.

*Corresponding author's E-mail: arti.gajbhar@mippune.edu.in

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ABSTRACT

In the current trend various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the identification and quantitation of impurities in Active Pharmaceutical Ingredient's (API's) and it is now emerging as a mandatory requirement for the new drug application. Hence their level should be controlled and monitored. Thus a simple sensitive High performance thin layer chromatographic (HPTLC) method for estimation of Propyl thiouracil and its process related impurities has been developed. Propyl thiouracil and its impurities were separated and identified on silica Gel 60 F₂₅₄ TLC plates with Hexane: Acetone: Ethyl Acetate: Formic acid 6:4:1:0.5 (v/v) as mobile phase. The plates were developed to a distance of 8 cm and quantification was performed at $\lambda = 254$ nm. The method was validated for linearity, accuracy, precision and robustness according to ICH guidelines. The calibration plots were linear in the range 2500-5625 and 50-112.5 ng/band for API and its three impurities respectively. Here recovery value for API and impurities were found to be in the range of 99.99 to 100.75 % with % RSD value always less than 2. Hence the developed method was found to be simple, sensitive, accurate and precise which can be used for propyl thiouracil impurity profile study and for routine analysis of propyl thiouracil from its dosage forms and for IPQC study during manufacturing of its formulations.

Keywords: Propyl thiouracil (PTU), High performance thin layer chromatography, Validation, Impurities.

INTRODUCTION

There is an ever increasing interest in impurities present in API and Formulation. Recently, not only purity profile but also impurity profile has become essential as per various regulatory requirements. With ever increasing regulatory and compendial stringency on the control of impurities and degradation products in API and Formulations, a profound emphasis is being paid on their characterization and analysis at trace levels. Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research.¹ Propyl Thiouracil (PTU) (Propylthiouracilum) [2, 3-dihydro-6-propyl-2-thioxypyrimidin-4(1H)-one.] **Figure 1**, has been used since the 1940s as an antithyroid agent for the treatment of hyperthyroidism.²⁻⁵ Its notable side effects include a risk of agranulocytosis and aplastic anemia. Propyl thiouracil inhibits the enzyme thyroperoxidase which normally acts in thyroid hormone synthesis by oxidizing the anion iodide (I⁻) to iodine (I⁰), facilitating iodine addition to tyrosine residues on the hormone precursor thyroglobulin. This is one of the essential steps in the formation of thyroxine.⁶

Literature survey revealed that different analytical methods for the determination of propyl thiouracil have been reported, which include high performance liquid chromatography (HPLC) method for determination of API from formulation⁷, from Plasma⁸ and a stability indicating LC method for quantification.⁹ The impurity named Thiourea (Impurity 1) is reported in British

Pharmacopoeia.¹⁰ However no HPTLC method has been reported for determination of propyl thiouracil and its three impurities. As HPTLC is the most simple separation technique today available to the analyst. It is referred as time machine that can speed up the work and allows performing many things at a time usually not possible with other analytical technique¹¹. Hence this article describes a simple, precise, accurate validated HPTLC method for quantitative estimation of API and its impurities.

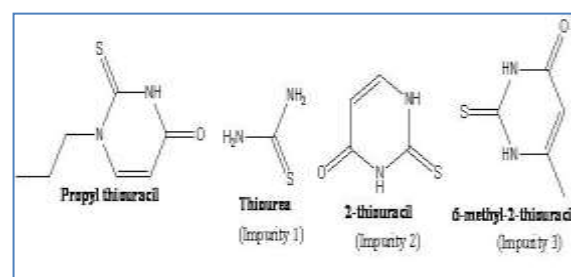


Figure 1: Structure of propyl thiouracil and its three impurities

MATERIALS AND METHODS

Reagents and Pharmaceutical Preparations

Propyl thiouracil pure analytical sample was obtained as a gift sample from Macleods Pharmaceutical Ltd., Daman and its impurities, impurity 1 (Thiourea) [9], impurity 2 (2-Thiouracil) and impurity 3 (6-methyl-2-thiouracil) were procured from Sigma-Aldrich Ltd. Propyl thiouracil commercial tablets-PTU containing propyl thiouracil 50 mg per tablet, Batch No. HPB402A, manufactured by

Macleods Pharmaceutical Ltd., Daman, India were purchased locally. All chemicals used were of Analytical reagent grade including methanol, hexane, acetone, ethyl acetate, formic acid and purchased from Research Lab Fine Chem Industries, Mumbai.

Standard and Sample solution preparation

Standard Solution: Standard stock solution containing 1000 µg/mL of propyl thiouracil was prepared in methanol by weighing 10 mg of API dissolving it in sufficient quantity of methanol and finally volume was made up to 10 mL with methanol.

Similarly working standard for the three impurities, i.e impurity 1 (Thiourea), impurity 2 (2-Thiourasil) and impurity 3 (6-methyl-2-thiouracil) was prepared having concentration of 100 µg/mL in methanol.

Mixed standard solution: From the above standard solution propyl thiouracil (1000 µg/mL) 10 ml was taken and to it 2 ml of stock solution (100 µg/mL) of each impurities was added to get the mixed standard solution of API and impurities. From this solution equivalent volume was spotted on plate to give the desired concentration of API and impurities.

Sample Solution: Twenty PTU tablets were weighed and powdered and quantity of powder equivalent to 100 mg was weighed and transferred to 100 mL volumetric flask. The powder was dissolved in methanol by sonication for 5 min, the solution was filtered through a whatmann filter paper no. 41 and finally volume was made with the methanol. Further from the above stock solution 5 mL was diluted to 50 mL with methanol to get the solution containing 100 µg/mL of propyl thiouracil.

Chromatography

Chromatography was performed on 20 x10 cm aluminium backed silica gel 60 F₂₅₄ TLC plates (Merck, Darmstadt, Germany). Before use, the plates were washed with methanol and activated at 110 °C for 5 min. Hexane: Acetone: Ethyl acetate: Formic acid, 6:4:1:0.5 (v/v) were used as the mobile phase. Samples (5 µL) were applied to the plates as 6 mm bands, keeping 17.7 mm distance between bands and 8 mm distance from plate side edge and 8 mm distance from bottom of plate. CAMAG (Muttens Switzerland) Linomat V was used as sample applicator equipped with 100 µL syringe (Hamilton, Reno, Nevada, USA) with the spraying rate as 150 nL/sec. The plates were developed in CAMAG 20 x 10 cm twin trough chamber lined with filter paper and saturated with mobile phase vapors for 10 min at room temperature. The solvent front position was set as 80 mm. After development of the plate in the mobile phase, it was dried in hot air oven at 50°C for 5 min. Densitometric scanning of the developed plate was performed at wavelength λ= 254 nm using CAMAG TLC scanner III with win CATS Software and deuterium lamp as light source with the slit dimension 4.00 x 0.30 mm and scanning speed of 20 mm/s.

Optimization of Method

HPTLC procedure was optimized to develop a method for identification and quantification of drug and its three impurities simultaneously. Firstly from the literature survey, Chloroform: 2-propanol: Glacial acetic acid (50:6:0.1 v/v)¹¹, was tried but in this mobile phase the bands were observed near to the baseline. So again another mobile phase containing Hexane: Acetone: Methanol (6:2:0.5 v/v) was tried, here only API got separated but three impurities were not moving at all. The next trial was done with Hexane: Acetone (5:5 v/v) as the mobile phase, but in this case drug was moving sufficiently but impurities were not at all resolved. Hence it was concluded that, one has to select the mobile phase containing compulsorily Hexane and acetone, but along with that another one composition of solvent need to be included which is less polar as compared to methanol. Hence mobile phase containing Hexane: Acetone: Ethyl acetate (6:4:0.5 v/v) was tried but in this, impurities got moved but peaks were not well resolved. After this the next mobile phase selected was Hexane: Acetone: Ethyl acetate (6:4:1 v/v), but here all peaks were well resolved with tailing effect. Hence finally mobile phase containing Hexane: Acetone: Ethyl acetate: Formic acid (6:4:1:0.5 v/v) was tried to reduce the tailing effect. This mobile phase showed well resolved peaks of drug and its three impurities with the reproducible results. So the method was said to be optimized.

Method Validation

The method was validated for accuracy, precision, linearity, specificity and robustness in analytical solution, in accordance with ICH guidelines.¹²⁻¹⁴

Linearity

The equivalent volume of mixed standard solution (4-9 µL) was spotted on plate to cover the range of and 2500-5625 ng per band and 50 to 112.5 ng per band of standard drug and its three impurities. Chromatographic plates were developed using optimized procedure and scanned as described above. The peak areas were recorded for propyl thiouracil and its impurities. A good linear relationship between response (peak area) and concentration was obtained. As the calibration can be accepted as linear, only if it is proven statistically hence to prove the linearity of the data; non numerical test method (testing the residuals) was performed.^{15,16}

Method sensitivity (LOD and LOQ)

Calibration plot for API and its three impurities was performed three times and its standard deviation and mean slope was calculated. All this data was processed as per the formula for limit of detection LOD and limit of quantitation LOQ given as $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$, where σ is standard deviation and S is slope of calibration plot.



Assay

For the assay from the sample solution the equivalent volume was spotted to obtain the concentration 3750 ng/band. The same procedure was repeated 3 times and plate was developed and scanned using the optimized procedure. The area under curve for the sample solution was compared with the data of calibration curve and the % assay was calculated.

Accuracy (Recovery)

As the drug was qualifying the assay, the validation was performed with the first parameter as accuracy. The accuracy of the method was determined by calculating recoveries of propyl thiouracil and its impurities by the standard addition method. Recovery was performed at three levels i.e. 80, 100 and 120 %. Known amount of standards were spiked to the sample to get the concentration as 3125, 3750 and 4370 ng per band. Similarly known amount standard impurities (62.5, 75, 87.5 ng per band) were spiked to the sample solution of impurities. The amount of Propyl thiouracil and its impurities were determined by measuring the peak areas.

Precision

For the precision both repeatability and reproducibility of the method for drug and its impurities was checked. For repeatability the peak area of sample band was measured repeatedly (n=6) and the relative standard deviation was calculated. For intermediate precision, the peak area of sample at three different concentration were measured for three times (n=3) on the same day for intraday precision. For inter day precision the peak area was measured for three times (n=3) on different day and the relative standard deviation was calculated.

Robustness

To study the method robustness the parameters which were changed deliberately include volume of mobile phase, saturation time, composition of mobile phase, TLC plate form different lot and time from spotting to development and development to scanning. By changing the above parameters the peak area of the API and its impurities were recorded. Robustness of method for each parameter change was assessed by using % assay of analyte and its R_f value.

Specificity

The specificity of the method was determined by analyzing standard drug, test sample and its three impurities. The spot for Standard, sample drug and impurities was confirmed by comparing the R_f and spectrum of the spot to that of a standard. The peak purity was determined by comparing R value of the spectrum at different regions of the spot, i.e. peak start (S) to peak apex (M) and peak apex (M) to peak end (E).

RESULTS AND DISCUSSION

Linearity

Linearity between concentrations and response was checked as described in earlier section. Calibration curves were obtained for propyl thiouracil and its 3 impurities from which linear regression equation was computed and a correlation coefficient was obtained. Linear regression data for the calibration plots (n = 3) are listed in Table 1. The correlation coefficient was found to be in the range of 0.997-0.999 for all the components, which shows excellent linearity between the concentration and response. From non numerical test; the plot of residuals was obtained which doesn't show any trend in it that implies results are satisfactory. This proves the linearity of calibration.

Method sensitivity (LOD and LOQ)

Method sensitivity was performed as explained in section 2.4.2. The LOD and LOQ were found to be 191.1 ng and 580.09 ng for API and 3.26, 9.89 for Impurity 1, 2.08, 6.31 ng for impurity 2 and 1.08, and 3.28 ng for impurity 3 respectively (Table 1). The low values of LOD and LOQ reveals that the method is sensitive (Figure 2).

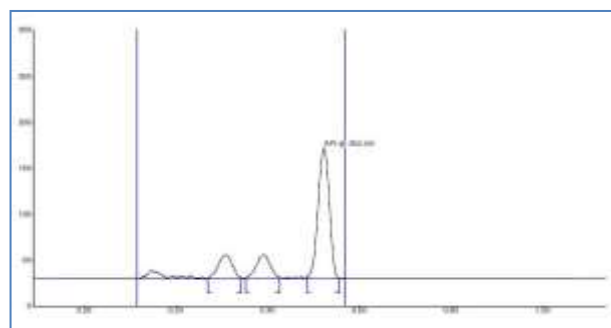


Figure 2: Densitogram of Propyl Thiouracil (PTU) with its impurities

Assay

The drug content obtained from standard and the sample was found to be comparable with no interference from excipients commonly present in tablet. The R_f value was found to be 0.48 (Figure 3) and the assay value was found to be 100.07 % and % RSD value as 0.95. The result indicates that the method is suitable for routine analysis of PTU and its impurities in pharmaceutical dosage form.

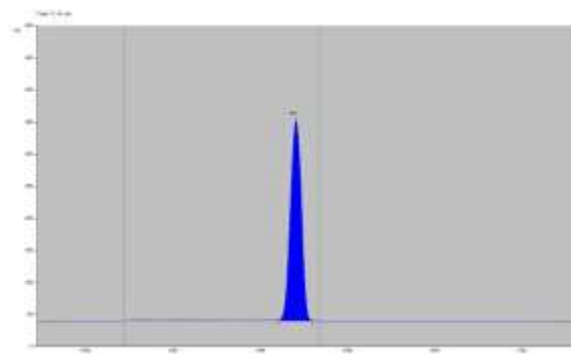


Figure 3: Densitogram of PTU tablet

Accuracy

The accuracy was performed as described in section 2.4.3 by standard addition method at three level i.e 80%, 100%, 120 %. The % recovery was found in the range of 99.99–100.75% and % RSD value was less than 2 for PTU and all the 3 impurities (Figure 4). The result shows that the method is accurate (Table 2).

Precision

The precision was performed as described in section 2.4.4. To check the repeatability, the response of sample solution was recorded six times for three different concentrations. The intraday and inter day precision was performed as per the procedure given in earlier section. The results are as shown in Table 3.

Table 1: Linear Regression data for the calibration plot (n=3) and Method Sensitivity

	PTU	Impurity 1	Impurity 2	Impurity 3
Linearity Range (ng)	2500-5625	50-112.5	50-112.5	50-112.5
Correlation Coefficient	0.999	0.997	0.999	0.998
Slope	1044	0.508	1.856	1.479
Intercept	1142	-1.83	1.52	20.26
LOD (ng)	191.1	3.26	2.08	1.08
LOQ (ng)	580.09	9.89	6.31	3.28

Table 2: Recovery Study (n=6)

Spike level	Mean % Recovery				% RSD			
	PTU	Impurity 1	Impurity 2	Impurity 3	PTU	Impurity 1	Impurity 2	Impurity 3
80 %	100.26	100.63	100.31	99.99	1.13	0.65	0.95	0.96
100 %	100.51	100.75	100.36	100.03	1.17	0.63	0.98	0.97
120 %	100.13	100.63	100.34	100.07	1.19	0.56	0.96	0.93

Table 3: Precision Study (n=3)

Concentration (ng/band)	PTU			Impurity 1			Impurity 2			Impurity 3		
	3125	3750	4370	62.5	75	87.5	62.5	75	87.5	62.5	75	87.5
Intra day (% RSD)	1.29	1.78	1.91	1.65	1.62	1.57	1.11	1.21	1.25	1.32	1.3	1.27
Inter day (% RSD)	1.75	1.64	1.62	1.89	1.91	1.94	0.98	0.95	0.96	1.03	0.99	1.1

Table 4: Results of Robustness study

Parameter	PTU		Impurity 1		Impurity 2		Impurity 3	
	% Assay, % RSD	% Assay, % RSD	% Assay, % RSD	% Assay, % RSD	% Assay, % RSD	% Assay, % RSD	% Assay, % RSD	
Volume of mobile phase (± 1 mL)	99.70, 1.02	99.56, 0.99	99.67, 1.12	100.45, 0.97				
Time of saturation (± 5 min)	99.85, 1.25	99.75, 1.06	100.5, 0.98	100.6, 1.09				
Composition of mobile phase Hexane (± 0.1 mL)	99.58, 1.5	99.69, 1.5	101.2, 1.6	100.85, 1.4				
Time from application to chromatography	99.45, 0.99	99.86, 0.89	101.02, 0.85	101.4, 1.05				
Time from chromatography to scanning	99.25, 1.2	99.63, 1.56	101.07, 1.7	99.67, 1.23				
Plate from different lot: Lot HX007716	101.2, 0.95	99.37, 0.68	100.16, 0.97	100.19, 0.99				
Plate from different lot: Lot HX007511	99.39, 1.03	101.42, 0.28	100.32, 1.2	100.01, 1.26				

Table 5: Peak purity values

	PTU	Impurity 1	Impurity 2	Impurity 3
r,s,m	0.999884	0.999825	0.999852	0.999979
r,m,e	0.999576	0.999936	0.999801	0.999983



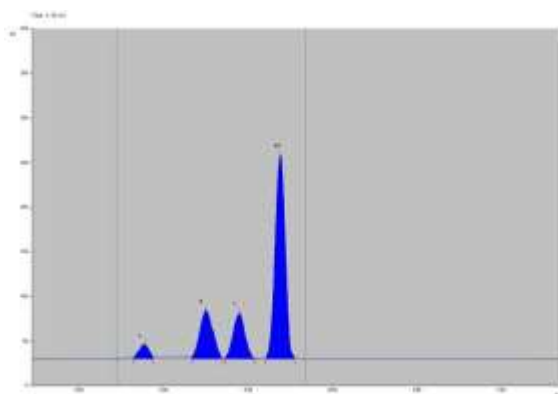


Figure 4: Densitogram of PTU and its 3 impurities

Robustness

Statistical analysis reveals that there is no significant difference between results obtained by applying some deliberate changes in the experimental conditions. Thus method was found to be robust. Table 4 shows the result of robustness study. (Table 4)

Specificity

Well resolved peaks were obtained for PTU and its impurities (Figure 5). The values of peak purity are found to be in the range of 0.999801 to 0.999979 (Table 5) which shows that the method is specific for the determination of impurities and drug simultaneously. Table 5 shows the values of peak purity.

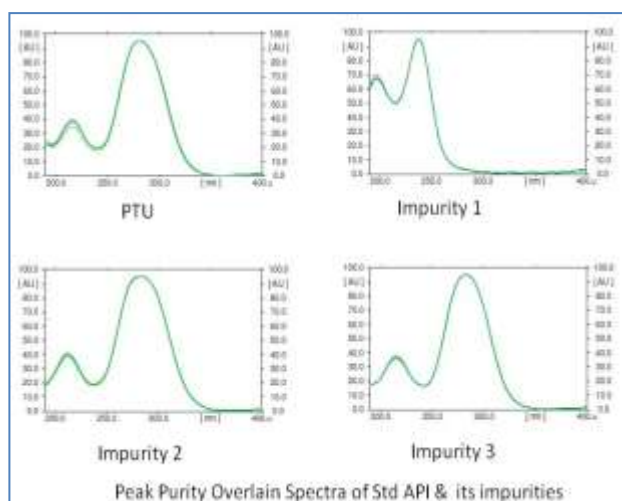


Figure 5: Peak purity overlay spectra of Standard PTU & its impurities

CONCLUSION

A simple, Specific, accurate, precise, robust and sensitive HPTLC method is developed. This method can be used in routine analysis of Propyl thiouracil in presence of its impurities which will serve as a quality control tool for the routine analysis of API and formulation. The method is reported first time & is novel. HPTLC is the fastest chromatographic technique and it gives more information

of sample. HPTLC technique is most suited for impurity profile of drug substances as per the compendial specifications.

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