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A STABILITY- INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTIFICATION OF ANTITHYROID DRUG PROPYLTHIOURACIL

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ABSTRACT:

A Simple, economic, selective, precise, and stability-indicating high performance liquid chromatography (HPLC) method has been established and validated for the determination of Propylthiouracil in bulk drug. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products using an Inertsil ODS 3V C18 (250 x 4.6) mm, 5µ column and the mobile phase containing phosphate buffer, orthophosphoric acid and acetonitrile 87:0.1:13 (%, v/v/v), as mobile phase at flow rate 1.0 ml min^{-1}. Detection was performed at 276 nm and a sharp peak was obtained for Propylthiouracil at a retention time of 15.0±1.5 min. The method is validated for its specificity, precision, accuracy, linearity and ruggedness accordance with ICH guidelines. Regression analysis data for the calibration plots were indicative of good linear relationships between response and concentration over the range 100.0µg mL^{-1} – 300.0µg mL^{-1}. The correlation coefficient was 0.9999. The value of slop and intercept of the calibration plot was 60776 and 153148. Statistical analysis proved the method is repeatable, selective and accurate for estimation of Propylthiouracil in bulk drug. Because the method could effectively separate the drug from their possible impurities like ethylbutyrylacetat and thiouea, it can be used as a stability indicating method. The wide linearity range, sensitivity, accuracy, short retention time and simple mobile phase imply the method is suitable for routine quantitation of Propylthiouracil with high precision and accuracy.

KEY WORDS: Propylthiouracil, Reverse phase, HPLC, UV-Vis detection and C18 column

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INTRODUCTION:

Propylthiouracil (6-propyl-2-thiouracil) is one of the thioamide derivative widely used in the treatment of hyperthyroid conditions. It forms complexes with metals and reacts with sulfhydryl-oxidizing agents. Despite its relatively safe profile, hepatotoxicity remains a serious, albeit rare, complication. Having been reported in nearly 30 cases in the

English-language literature since its introduction in 1947[1], Propylthiouracil induced toxic liver injury must be considered in any patient receiving this drug in which symptoms of hepatic dysfunction appear. Patients may present with jaundice, right upper quadrant abdominal pain, malaise, nausea, vomiting, and anorexia. Reported sequelae of propylhiouracil hepatotoxicity range from rapid recovery following discontinuation of the drug to fulminant hepatic failure and death[2]. A recent review of drug hepatotoxicity lists Propylthiouracil induced hepatitis as an "idiosyncratic" reaction with immunologic mechanisms, hypersensitivity, and direct toxicity among the postulated pathogeneses[3]. Propylthiouracil has been used since the 1940s as an antithyroid agent for the treatment of hyperthyroidism[4]. It may also be given to patients with alcoholic liver disease and has been shown to decrease mortality by half in these patients in a two-year double-blind study[5]. Propylthiouracil is also used to test taste perception for bitterness[6]. The ability to taste Propylthiouracil is genetically determined and affects an individual's food choices in daily life. Propylthiouracil was formerly used as a metabolic depressant to promote fattening of cattle.

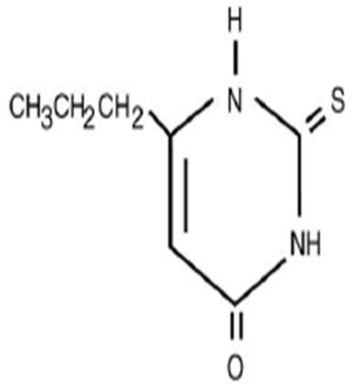

Figure: 1 Structure of Propylthiouracil

MATERIALS & METHODS:

Chemicals and reagents

All chemicals were of the highest purity available. Acetonitrile (HPLC Grade), Orthophosphoric acid (AR grade), Phosphate buffer and Purified water (HPLC grade) were used as mobile phase chemicals and purchased from Rankem.

Chromatography

Chromatography was performed on a Shimadzu LC-2010$_{HT}$ with UV/ PDA detector and LC solution software. Inertsil ODS-3V, (250 x 4.6) mm, 5µm was used for separation and quantification of Propylthiouracil. Mettler Toledo XS 205 analytical balance were used for weighing of sample and standard preparation. The mobile phase was a mixture of buffer -acetonitrile in the ratio of 87:13 (%, v/v). Chromatographic parameters were used as the detection was performed at 276 nm; the flow of mobile phase was 1.0 mL min^{-1}. The injection volume was 10 µL. The column oven temperature was 30°C. The total time required for chromatographic separation was 25 min.

Method development

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent (s) in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimised so the peak of Propylthiouracil did not interfere with those from the solvent and possible impurities. Other criteria, viz. time required for analysis, appropriate k range ($1<k<10$) for elute peaks, sensitivity, solvent noise, were also considered. After each change of the mobile phase the column was re-equilibrated by passage of at least ten column volumes of the

new mobile phase [10]. To investigate the appropriate wavelength for simultaneous determination of Propylthiouracil solution of the compound in the mobile phase were scanned by UV-visible spectrophotometry (Shimazdu, Japan model UV-1800) in the range 200-400nm. From the UV spectra, suitable wavelength choices considered for monitoring the Propylthiouracil was 276 nm. Solutions of Propylthiouracil in the mobile phase were also injected directly for HPLC analysis and the responses (peak area) were recorded at 276 nm. It was observed there was no interference from the mobile phase or base line disturbance at 276 nm. It was, therefore, concluded that 276 nm is the most appropriate wavelength for analysis of the Propylthiouracil with suitable sensitivity. A variety of mobile phases were investigated to establish a suitable HPLC method for analysis of Propylthiouracil in bulk drug. This included mixture of acetonitrile-water, 25:75 (%, v/v), phosphate buffer-acetonitrile, 75:25 (%, v/v), phosphate buffer-orthophosphoric acid-acetonitrile, 75:0.1:25 (%, v/v/v). The final mobile phase was a mixture of phosphate buffer-orthophosphoric acid-acetonitrile, 87:0.1:13 (%, v/v/v). The suitability of the mobile phase and method was decided by study of the accuracy, precision, linearity, specificity and stability in analytical solution in accordance with USP and ICH guidelines[8-9].

Method validation

The method was validated for accuracy, intraday and inter-day precision, linearity, specificity and stability in analytical solution, in accordance with ICH guidelines[8-9]. System suitability tests are an integral part of liquid chromatographic method. It is used to verify that the resolution of the chromatographic system is adequate for the analysis to be done[8-9]. The tests are based on the concept that the equipment, electronics, analytical operations and sample to be analyzed constitute an integral system that can be evaluated as such. System suitability was evaluated by replicate (n= 6) injections of the standard solution containing Propylthiouracil at 200.0 µg mL^{-1}. The *RSD* of retention time, peak area, number of theoretical plates, and USP tailing factor were within 1%, indicating the suitability of the system (Table 1). The number of theoretical plates and the USP tailing factor were within the acceptance criteria of >2000 and ≤1.5, respectively, indicating good column efficiency and optimum mobile phase composition[8].

Table: 1 Results of system suitability

Property[a]	**Propylthiouracil**	
	Mean[b]	***RSD* (%)**
t_R	14.89	0.07
A	12286367	0.65
T	1.04	0.53
N	5259	0.61

[a] t_R, retention time; A, peak area; T, tailing factor; N, number of theoretical plates;

[b] Mean from of six replicate injections (n = 6)

Specificity and selectivity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, and matrix, etc.[8-9]. The specificity and selectivity of the proposed method was evaluated by estimating the amount of Propylthiouracil in the presence of possible impurities and Blank. The HPLC chromatograms were recorded for the spiked solution revealed no peaks within a retention

time of Propylthiouracil. The study of the absence of possible impurities and blank showed that none of the peaks appears at the retention time of Propylthiouracil and it was concluded that the developed method is selective in relation to the Propylthiouracil of the final preparation. The chromatograms of Propylthiouracil Blank, spiked sample, sample and standard using the proposed method is shown in [Figure 4 to Figure 7].

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision should be investigated using homogeneous, authentic samples[8-9]. In accordance with ICH recommendations precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability of sample application was determined as intraday variation whereas intermediate precision was determined by measuring inter-day variation from six preparation of Propylthiouracil sample at target concentration level. Results from determination of repeatability and intermediate precision as % *RSD* are show in Table 2. The low value of % *RSD* are indicative of the high repeatability of the method. Ruggedness of the method was established by comparing the results obtained on different days by different analyst on different instrument and HPLC column as % *RSD*.

Table: 2 Results of measurement of intraday and inter-day precision (*n* = 6)

Concn ($\mu g\ mL^{-1}$)	Repeatability (Intraday precision)		Intermediate precision (Inter–day)		Ruggedness (%) *RSD*
	Mean Area	Mean (%) *RSD*	Mean Area	Mean (%) *RSD*	
	12369808	0.96	12399866	0.70	1.16
	12360530		12549557		
	12396478		12551449		
	12234953		12299149		
	12265254		12473156		
	12390796		12550685		

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample[8-9]. The calibration graph was linear, i.e. the system adhered to Beer's law, over the range 25% to 150% of test concentration. The graph of area versus concentration is plotted. The correlation co-efficient (r), Y-intercept, slope

of regression line, residual sum of squares are calculated and recorded in Table 4.

Table: 3 Calibration plot data for Propylthiouracil.

Concn (µg mL^{-1})	Mean Area
100.100	6236313
150.150	9228897
200.200	12359018
250.250	15436777
300.300	18341585

Table: 4 Linearity regression data for calibration plot for Propylthiouracil

Data	Propylthiouracil
Linearity Range (µg mL^{-1})	100.100–300.300
Regression equation	y =60776x + 153148
Correlation coefficient (r)	0.9999
Slope	60776
Intercept	153148
Residual sum of squares	22577243418

Table: 5 Results from recovery studies (n = 3)

Amount added (%)	Theoretical conc. (µg mL^{-1})	Conc. Found (µg mL^{-1})	Mean (%) recovery	Mean (%) Recovery RSD
50	100	100.100	99.86	0.82
75	150	150.150	99.38	0.38
100	200	200.200	99.83	0.43
125	250	250.250	99.82	0.03
150	300	300.300	99.58	0.20

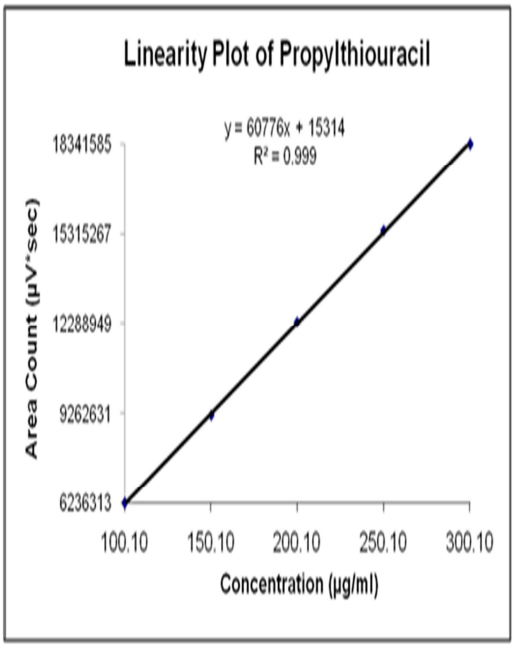

Figure: 3 Linearity plot of Propylthiouracil

Accuracy

A study of recovery of Propylthiouracil sample was established. Samples were prepared in triplicate of Propylthiouracil in test preparation at 50%, 75%, 100%, 125% and 150% of the target concentration level. The average % recovery of Propylthiouracil was calculated and given in Table 5. The recovery was found in the range of 99.38% to 99.86%, which indicated the accuracy of the method was adequate.

Stability in Solution

The stability of Propylthiouracil spiked in sample in aqueous solution at a concentration of 200.0µg ml^{-1} was studied by storing the solution in a tightly capped volumetric flask at room temperature on a laboratory bench for 24 hours and found it was stable. The amount of the Propylthiouracil was checked at 0 hrs, 6hrs, 12hrs, 18hrs and 24hrs intervals.

Table: 6 Summary of validation data

Parameters	**Concentration**	**Result**
Linearity		R^2 = 0.9999
Slope	100.100–300.300 µg mL^{-1}	60776
Intercept		153148
Precision		
(i) System Precision	200.00 µg mL–1 (standard)	0.65
% RSD (n=6)		
(ii) Repeatability (Inter-day)		0.96
by recovery % RSD (n=6)	0.02 % w/w	
(iii) Intermediate precision (Intra-day) % RSD (n=6)	0.02 % w/w	0.70
(iv)Ruggedness % *RSD* (n=6)	0.02 % w/w	1.16
Accuracy		
(Mean % Recovery, ± % *RSD*)		
(*n* =3) at 50 % level	0.01 % w/w	99.86 ± 0.82
(*n* =6) at 100 % level	0.02 % w/w	99.83 ± 0.43
(*n* =3) at 150 % level	0.03 % w/w	99.58 ± 0.20
(*n* = 12) Over all recovery	-	99.69 ± 0.37
Specificity	Specific	Specific

n = number of determinations

RESULTS & DISCUSSION:

During method development different approaches were tried. Presented method was found to be simple and sensitive with linearity in the concentration range of 100.10–300.30 µg mL^{-1}. Method is specific and indicated no interference in the Propylthiouracil peak, accuracy of method was established by recovery, the recovery values are within acceptable limits at different concentration levels and the data in table-5 indicated that the method is precise and rugged. Representative chromatogram is presented in Figure-4 to Figure-7 and the different values of validation data; linearity, precision, ruggedness, accuracy, limit of quantification and limit of detection are given in table-6.

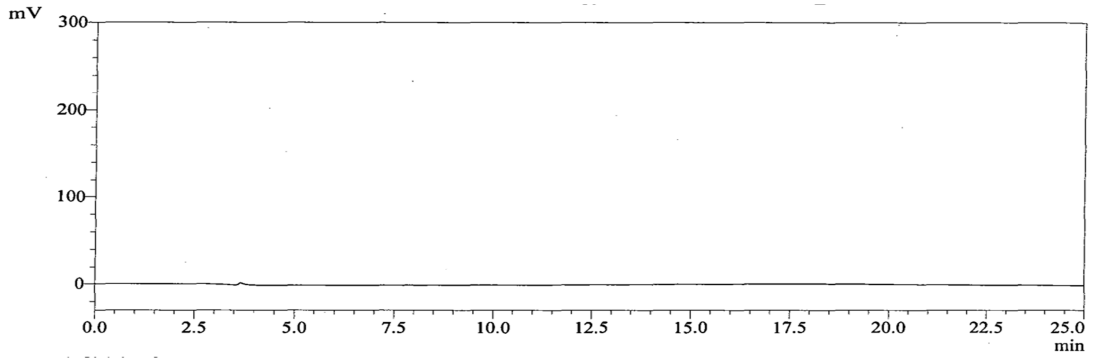

Figure: 4 HPLC Chromatogram of Propylthiouracil Blank

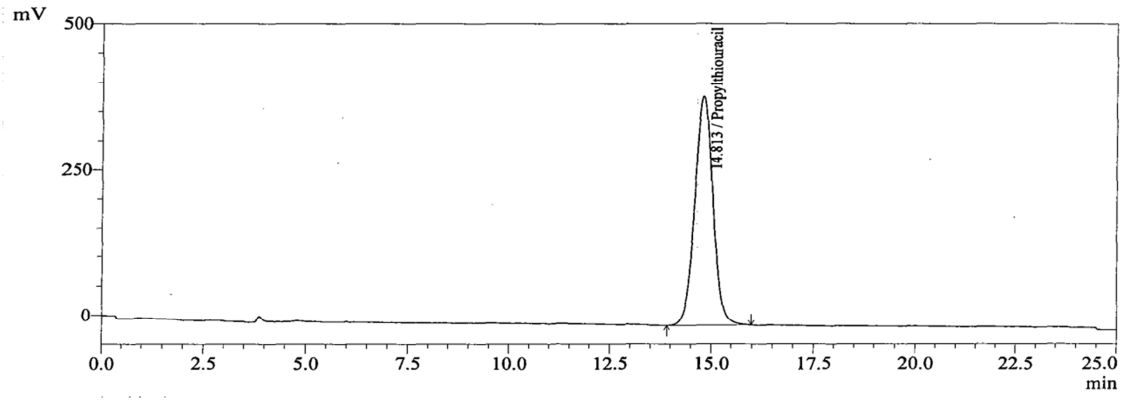

Figure: 5 HPLC Chromatogram of Propylthiouracil standard

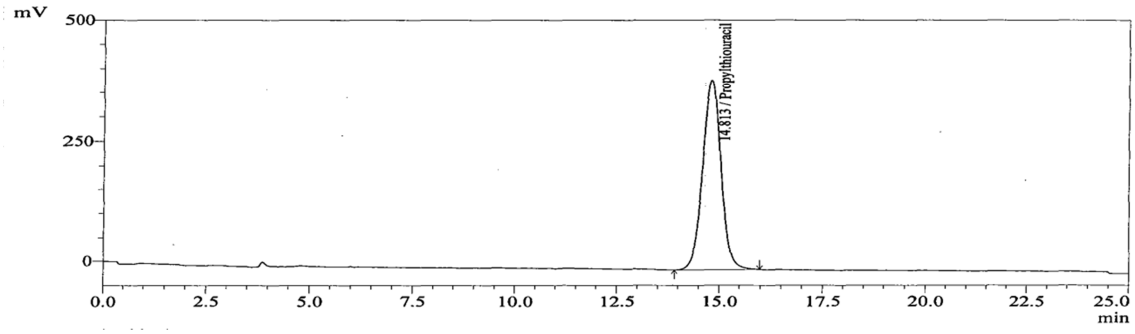

Figure: 6 HPLC Chromatogram of Propylthiouracil sample

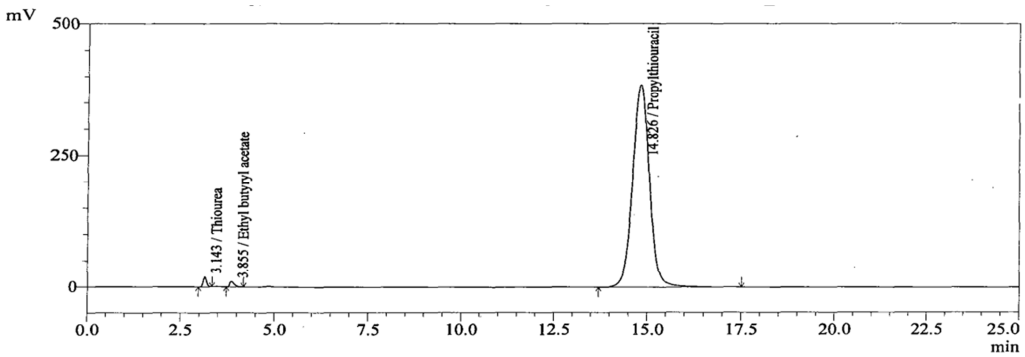

Figure: 7 HPLC Chromatogram of Propylthiouracil spiked sample

CONCLUSION:

This HPLC method is precise, specific, accurate, and stability indicating. Statistical analysis proved the method is repeatable and selective for the analysis of Propylthiouracil. The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities. It may be extended to determination of the possible impurities. Because the method separated the drug from its possible impurities, it can be used as a stability indicating method.

REFERENCES:

1. James T. Hardee, Angela L. Barnett, Abdul Thannoun, Bijan Eghtesad, Donald Wheeler, M. Mazen Jamal, Albuquerque "Propylthiouracil-Alerts, Notices, and Case," September -1996- vol 165. Livingston HJ, Livingston SF "Agranulocytosis and hepatocellular jaundice "Toxic reaction following propylthiouracil therapy," JAMA. 135: 422-425, (1947).
2. Colwell AR, Sando DE, Lang SJ "Propylthiouracil-induced agranulocytosis, toxic hepatitis, and death," JAMA. 148:639-641, (1952)
3. Lee WM "Drug-induced hepatotoxicity," N Engl J Med. 333: 1118-1127, (1995).
4. Farwell AP, Braverman LE "Thyroid and antithyroid drugs". In The Pharmacological Basis of Therapeutics10th ed., Goodman LS, Gilman A, eds. New York, McGraw-Hill. pp. 1563-1596, (2001).
5. Orrego H, Blake JE, Blendis LM, Compton KV, Israel Y. "Long-term treatment of alcoholic liver disease with Propylthiouracil," N Engl J Med. 317(23): 1421-1427, (1987).
6. Ly A, Drewnowski A. "PROP (6-n-propylthiouracil) tasting and sensory responses to caffeine, sucrose, neohesperidin dihydrochalcone and chocolate," Chem Senses. 26(1): 41-47, (2001).
7. United States Pharmacopeia, USP32. The United States Pharmacopeial Convention, Maryland, USA, General Chapters, 621, 1225, (2009).
8. International Conference on Harmonization tripartite guideline, ICH Secretariat, Geneva, Switzerlan, Q2R1, (2005).
9. Lloyd R. Snyder, Joseph J.Kirkland, Joseph I.Glajch, "Practical HPLC Method Development Second Edition", (1997).

