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Innovative RP-HPLC Technique for Method Development and Validation of Propylthiouracil Tablets

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This work focuses on the use of high-performance liquid chromatography (HPLC) for the quantitative analysis and validation of propylthiouracil tablets through reverse phase chromatography (HPLC- RP). The analysis was optimised using a C18 column with a mobile phase that includes acetonitrile and buffer in a ratio of 20:80 (v/v). The buffer is made from monobasic potassium phosphate with a pH of 4.6 ± 0.05. The detection of propylthiouracil was performed at ? = 272 nm. Samples were extracted using methanol and water. The newly developed method exhibited good linearity between 24.916 and 74.748 μg/mL, with an R2 > 0.999. Precision expressed in terms of % relative standard deviation (RSD) was within the acceptable range, whereas accuracy in terms of % recovery varied between 98-102%. Therefore, the proposed and validated HPLC-RP method is reliable for the quantitative analysis of propylthiouracil in pharmaceutical formulations, ensuring accurate dosage determination and quality control. The technique shows promising potential for pharmacokinetic studies and routine quality assurance in the pharmaceutical industry.

Keywords: High- performance liquid chromatography; Pharmaceutical formulation; Propylthiouracil tablets; Quantitative analysis; validation RP-HPLC.

The pharmaceutical industry represents companies that manufacture morale and medication over the counter. Pharmaceutical research focuses on the discovery and development of pharmaceutical agents for administration¯# whether self-administered by patients or provided by healthcare professionals¯# for treatment, protection, or prevention. As the field advances, innovative and skilled formulations are being introduced to the market. Although some dosage forms may be susceptible to contamination, many are highly effective. Such developments require precise, easy-to-use, and flexible chemical analysis techniques due to the significance of quality control in pharmaceuticals. Unlike other consumer goods, medicines must adhere to strict standards because they directly affect human health. Ensuring quality requires a dedicated department for quality control and assurance [1]. The medication does not reduce the efficiency of oral or injectable thyroid Propylthiouracil is useful in treating hyperthyroidism because it prevents the production of thyroid hormone treatments, nor does it neutralize the thyroxine or triiodothyronine present in the bloodstream. Propylthiouracil works by inhibiting the conversion of thyroxine

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to triiodothyronine in peripheral tissues, making it a possible option for managing thyroid storm. The drug is rapidly absorbed and extensively metabolized. Within 24 hours, approximately 35% of the medication is excreted in the urine, both in its intact and conjugated forms [2]. The primary goal of analytical chromatography is to obtain target analytes with a sufficient resolution in the shortest possible time. The solutes diffuse slowly in and out of the tiny pores in the nonporous particles, which eliminates the stagnant mobile phase in the intra-particle void volume [3]. Validation refers to assessing validity and efficiency. The validation group consists of individuals from various regulations. Validation is the process of providing written proof that ensures the product meets the standards for analysis applications [4].

This article presents the systematic approach taken in the quantitative analysis for the validation of propylthiouracil tablets by using reverse phase HPLC. The study elaborates on the criteria applied for selecting methods, optimization performed for chromatographic conditions, parameters checked during the validation process, and analysis of real-world samples. This research will furnish detailed information at each step involved in development and validation to act as a comprehensive guide for analysts and researchers working in the pharmaceutical field.

MATERIALS AND METHODS

Chemicals and reagents used

All chemicals used in the analysis should be either AR grade or an equivalent grade; solvents should be HPLC grade or equivalent. Monobasic potassium phosphate, sodium hydroxide, orthophosphoric acid, acetonitrile, methanol, and HPLC-grade milliQ water.

Working standard and sample used

Propylthiouracil standard, Propylthiouracil 50 mg tablets, and Placebo for propylthiouracil 50 mg tablets were given by Bio Plus Life Science Pvt Ltd., Hosur, Tamil Nadu, India.

Instruments used

Analytical and precision balance from Denver Instrument, HPLC with openlab software from Agilent 1200 and Agilent 1260 Infinity II, ultrasonicator from PCI Analytics, and pH meter from Weibfr.

Preparation of solution[5,6]

Preparation of buffer: In a 1000 mL beaker, 3.4 g of monobasic potassium phosphate was added with 500 mL of water and then sonicated to dissolve. After adjusting to a pH of 4.6± 0.05. A 0.1 N of sodium hydroxide or diluted phosphoric acid was added with 500 mL of water. A pH of 4.6± 0.05 was achieved by adding 0.1 N of sodium hydroxide or diluted phosphoric acid to the resultant solution. A 0.45µ membrane filter was used to filter this mixture, then degassed.

Mobile phase preparation: In a ratio of 20:80 (v/v), a suitable amount of degassed acetonitrile and buffer were prepared.

Preparation of Diluent: Use 1% methanol in water as diluent

Standard preparation: About 50 mg of the working standard for propylthiouracil was accurately weighed and transferred into a 50 mL dry volumetric flask. Then, 10 mL of methanol was added, sonicated to dissolve it, and water was added to fill the flask to the recommended volume. A 5 mL of this solution was diluted to 100 mL with water and mixed.

Sample preparation: A minimum of 20 tablets was weighed to determine the average weight. The tablets should be crushed into a fine powder. The powder weight was taken and added to a 100 mL clean, dry flask. Next, 20 mL of methanol was added and sonicated for 5 minutes. After that, 50 mL of water was added and sonicated for 15 minutes with intermittent shaking. The mixture was cooled and made up the volume with water. Using a 4.5µ membrane filter (PVDF), the mixture was filtered. The first 4 mL of the filtrate was discarded. Further, 5 mL of the solution was diluted to 1000 mL with water and mixed.

Preparation of placebo: A 100 mg equivalent of propylthiouracil placebo powder was accurately weighted and transferred into a 100 mL clean, dry volumetric flask, methanol (20 mL) was added and sonicated for 5 minutes, and then 50 mL of water was added, sonicated for 15 minutes, cooled, and made up to the flask water and mixed. Using a 0.45µ membrane filter (PVDF) for filtration. Further, 5 mL of the solution was diluted to 100 mL with water and mixed.

Calculation for % Assay:

Calculation of the percentage assay for propylthiouracil by using the following formula.

$$\% \text{ Assay} = \frac{A_T}{As} \times \frac{Ws}{50} \times \frac{5}{100} \times \frac{100}{W_T} \times \frac{100}{5} \times \frac{P}{100} \times \frac{A_w}{L} \times 100$$

Where,

A_T = Average area of propylthiouracil peak in the chromatogram of sample solution

As = Average area of propylthiouracil peak in the chromatogram of standard solution, as obtain under system suitability.

Ws = Weight of working standard taken in mg

P = Percent potency of working standard used (as is basis)

W_T = Weight of sample in mg

A_W = Average weight in mg

L = Label claim in mg

RESULTS AND DISCUSSION

Method validation[7-13]:

Specificity

Blank, placebo, standard, control test solution, spiked test solution, and individual impurity solution related to propylthiouracil were prepared and analysed as per the method. The chromatograms show there is no interference from the blank, placebo and the known impurity with a retention time of peak due to propylthiouracil. Results are shown in Tables 1-4.

Table 1. Results obtained from standard, control and spiked test solution

Sample ID	Retention Time of Propylthiouracil peak (in minutes)
Standard solution	2.760
Test solution (Control)	2.753
Spiked test solution	2.753

Table 2. Results obtained from individual impurities solution

Impurity Name	Retention time (minutes)
Thiourea Impurity	Not Detected in 272 nm
Thiourea Impurity	238 nm RT-1.18

Forced degradation study

Forced degradation study in propylthiouracil 50 mg tablets and its placebo. No peak was detected at the retention time of propylthiouracil in the chromatogram of diluent and placebo. Forced degradation studies are shown in Table 5.

Table 3. Results obtained from control and spiked test solution

Sample	(% Assay)	Absolute difference in % Assay values
Test solution (Control)	98.2	0.2
Spiked test solution	98.4	

Table 4. Results obtained for peak purity of Propylthiouracil

Sample ID	Purity factor	Result
Blank solution	within the threshold limit	Pass
Placebo solution	within the threshold limit	Pass
Standard solution	within the threshold limit	Pass
Test solution (Control)	within the threshold limit	Pass
Spiked test solution	within the threshold limit	Pass

Filter study

Using the analysis method as an outline, standard and sample solutions are prepared. 0.45µm Nylon and 0.45µm PVDF filters were used to filter the standard solution as well as the sample solution. By discarding 2 mL, 4 mL, and 6 mL of the solution after it has passed through the previous filter, three sub-fractions will be gathered. Unfiltered standard solution is used as the standard. Results obtained by using different syringe filters and unfiltered standard solutions are summarised in Tables 6 and 7.

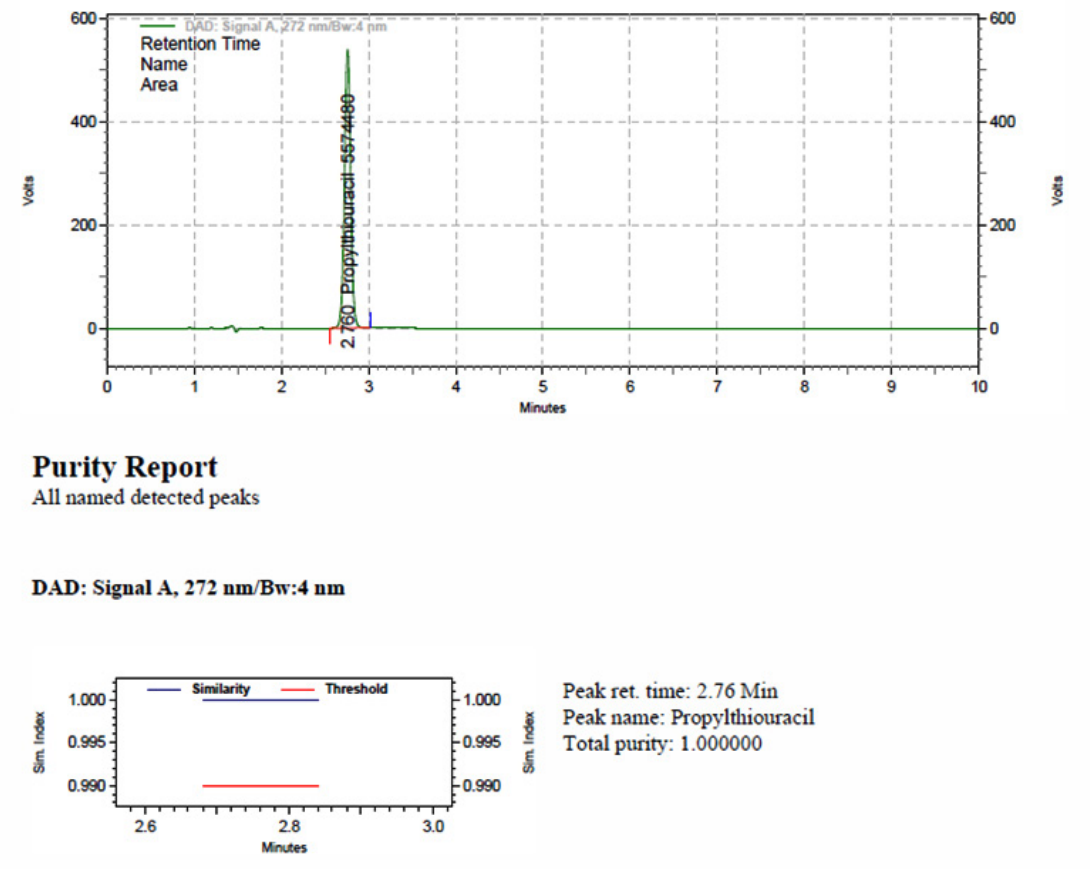

Fig. 1. Chromatogram of specificity for standard solution with peak purity

Table 5. Forced degradation of Propylthiouracil 50mg tablets results below

Mode of degradation	Condition	% Assay	% Degradation
Control sample	Not applicable	100.6	NA
Acid degradation	5mL of 5N HCl, at 80°C for 60 minutes	97.7	2.9
Base degradation	5mL of 5N NaOH, at 80°C for 60 minutes	91.6	9.0
Photolytic degradation	1.2million lux hours/200watt hours square meter	98.4	2.2
Thermal degradation	3 days at 105 °C	100.9	No degradation
Control sample	Not applicable	100.2	NA
Oxidation degradation	5 mL of 5% v/v H_2O_2, bench top for 30 minutes	93.1	7.1

Solution stability

Standard solution stability

According to the test protocol, a standard solution was prepared and stored at room temperature (23°C- 27°C). The study of the stability of the solution on various days when compared to freshly made standard solution is necessary. The results are shown in Table 8.

Sample solution stability

According to the test method, the sample solution was prepared and stored at room temperature (23°C- 27°C). The study of

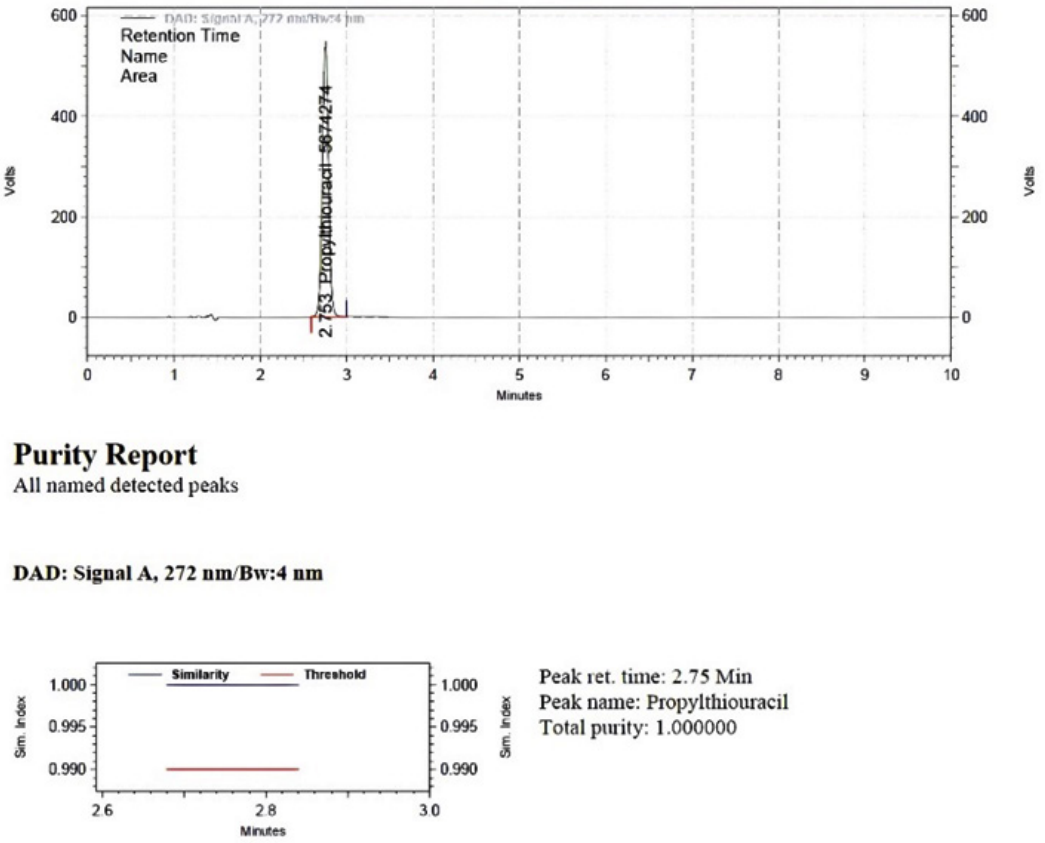

Fig. 2. Chromatogram of specificity for sample solution with peak purity

Table 6. Results obtained from filter saturation study for standard solution

No	Filter size and type	Quantity Discarded in ml	Average Area	Absolute % difference
1	Unfiltered standard solution	NA	5733363	NA
2	0.45 µm Nylon	2 ml	5723530	0.2
		4 ml	5719118	0.2
		6 ml	5720648	0.2
3	0.45 µm PVDF	2 ml	5729777	0.1
		4 ml	5734088	0.0
		6 ml	5731705	0.0

the stability of the solution at various days when compared to a freshly made standard solution is necessary. The results are shown in Table 9.

Linearity

A serious number of solutions containing propylthiouracil at concentrations listed below were analysed to determine the linearity of the method. The response of linearity for propylthiouracil is determined from the range of 24.916 µg/ml to 74.748 µg/ml. Results are illustrated in Table 10 and the corresponding Figure 3- 8.

Table 7. Results obtained from filter saturation study for sample solution

No	Filter size and type	Quantity Discarded in ml	Average Area	Assay %	Absolute % difference
1	Unfiltered sample solution	NA	5651723	99.0	NA
2	0.45 µmNylon	2 ml	5658626	99.1	0.1
		4 ml	5652407	99.0	0.0
		6 ml	5682430	99.6	0.6
3	0.45 µmPVDF	2 ml	5660532	99.2	0.2
		4 ml	5667759	99.3	0.3
		6 ml	5661367	99.2	0.2

Table 8. Results of standard solution stability at 23°C-27°C

Interval	Standard solution stability at 23°C-27°C	
	Average Area	Similarity factor
Initial	5728333	0.99
24 hours	5719319	0.98
48 hours	5714535	0.99

Table 9. Results of sample solution stability at (23- 27) °C

Interval	Sample solution stability at 23°C-27°C		
	Average Area	% Assay	Absolute % difference
Initial	5703944	99.9	Not Applicable
24 hours	5648200	98.4	1.5
48 hours	5648391	98.2	1.7

Table 10. Results obtained for linearity

Linearity Solution No	Level	Concentration (µg/mL)	Average Area of Propylthiouracil
1	50%	24.916	2765964
2	75%	37.374	4110765
3	100%	49.832	5441493
4	125%	62.290	6828044
5	150%	74.748	8171130
Slope		108585.7361	
Intercept		52434.80000	
Correlation coefficient (r)		0.99998	
% Deviation of Y-Intercept		0.96	
Regression co-efficient (r^2)		0.99996	

Accuracy

The accuracy is determined by examining the solutions of placebo spiked with propylthiouracil active pharmaceutical ingredient (API) at three different levels of concentrations in triplicate. The solutions were prepared and analysed as per the method. % recovery results are shown on Table 11 and corresponding Figure 9-11.

Precision

Repeatability of standard solution- System precision

Repeatability solution -1 was injected into the chromatographic system five times in replicates. Results are summarised in Table 12.

Reproducibility of sample solution- Method precision

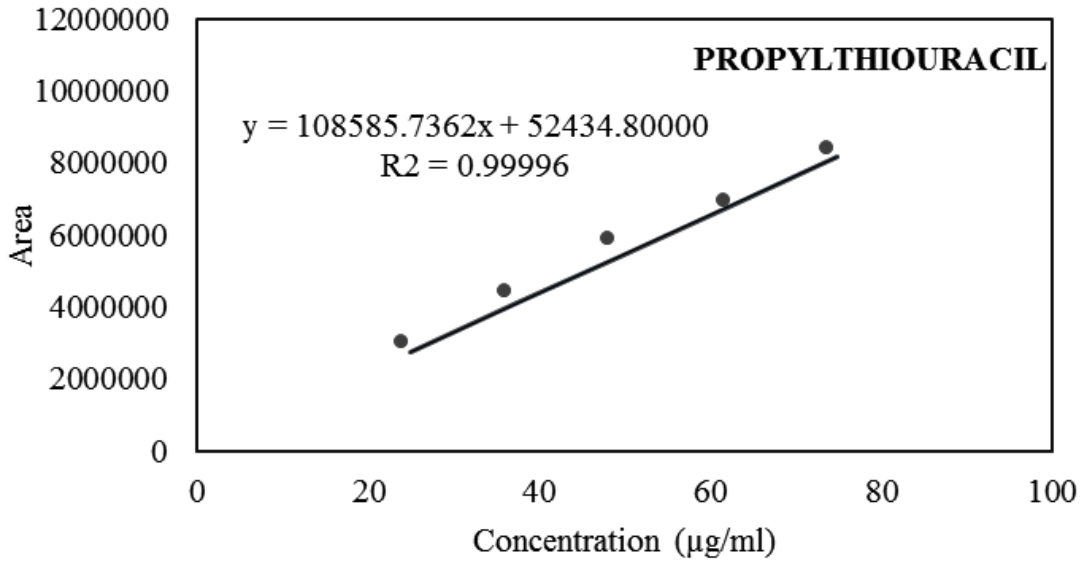

Fig. 3. Linearity plot of Propylthiouracil

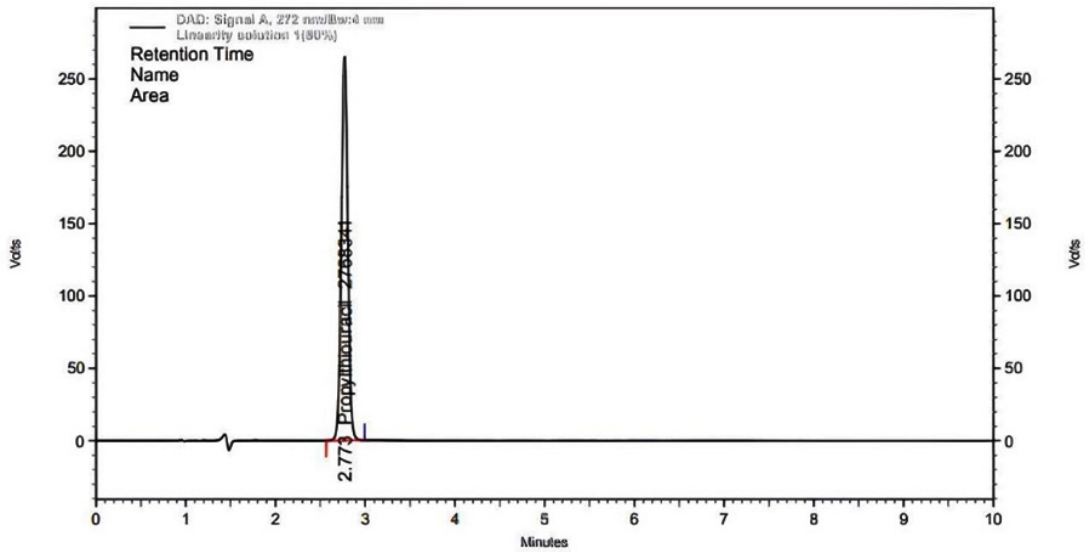

Fig. 4. linearity 50%

Six test solutions of a single batch were analysed as per the proposed methodology and the results as given in Table 13.

Intermediate precision – Ruggedness

By comparing the analysis of the propylthiouracil 50 mg tablet samples conducted on different days by different analysts using different instruments and columns, the robustness of the method was verified. The outcomes of Analyst 2's examination of the sample solution are displayed in Table 14. Table 15 shows the mean, standard deviation, and percentage RSD for the two sets of data.

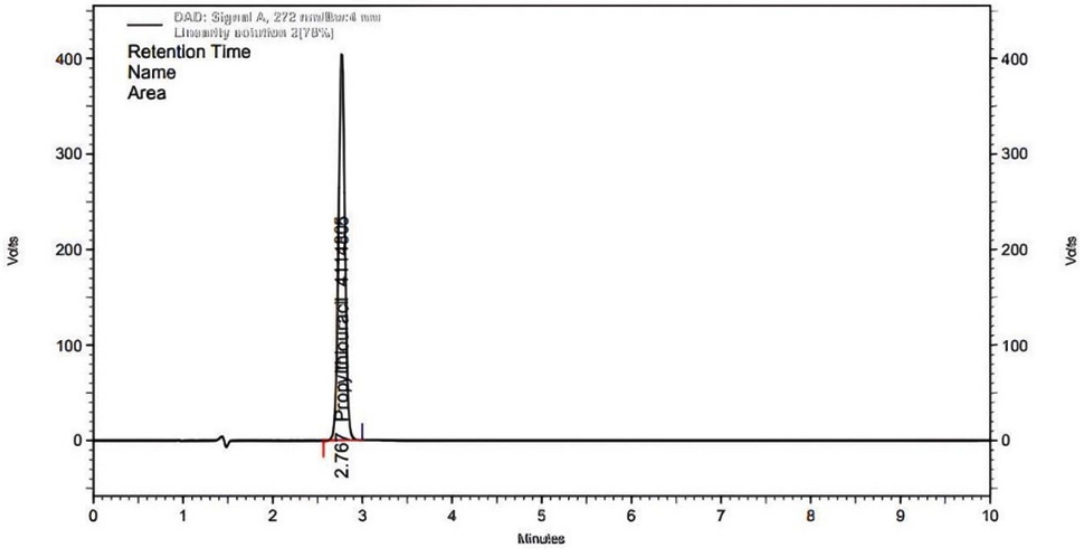

Fig. 5. Linearity 75%

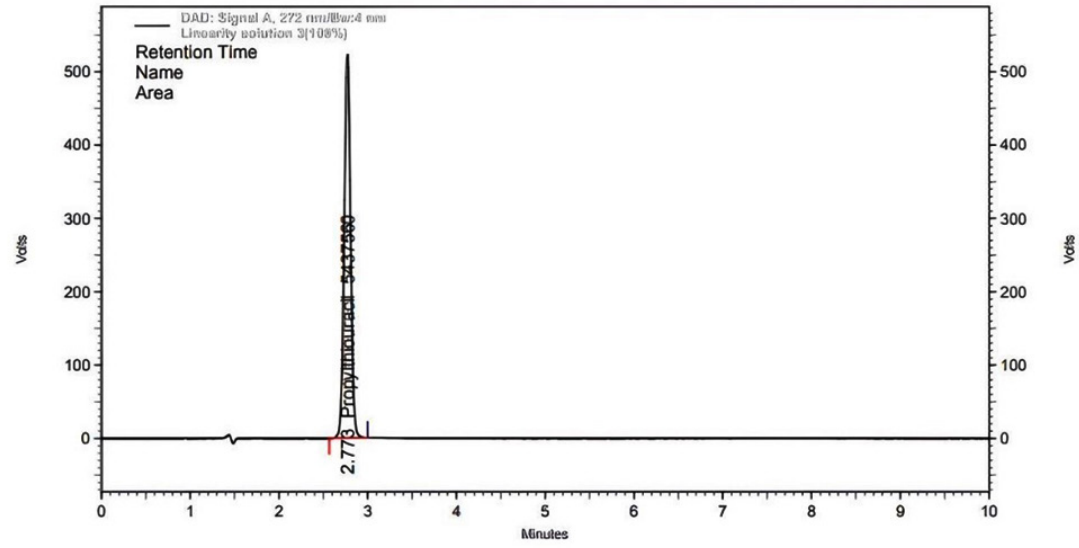

Fig. 6. Linearity 100%

Robustness

By verifying that the system suitability parameters were met and deliberately changing variables such as flow rate, organic content of the mobile phase composition, pH variation and detection wavelength, the procedure's robustness was tested. Every condition was examined for a sample solution. Propylthiouracil assay percentage and system suitability were determined. Altered parameters from an optimised method for the robustness of the assay and results are tabulated from Tables 16 and 18.

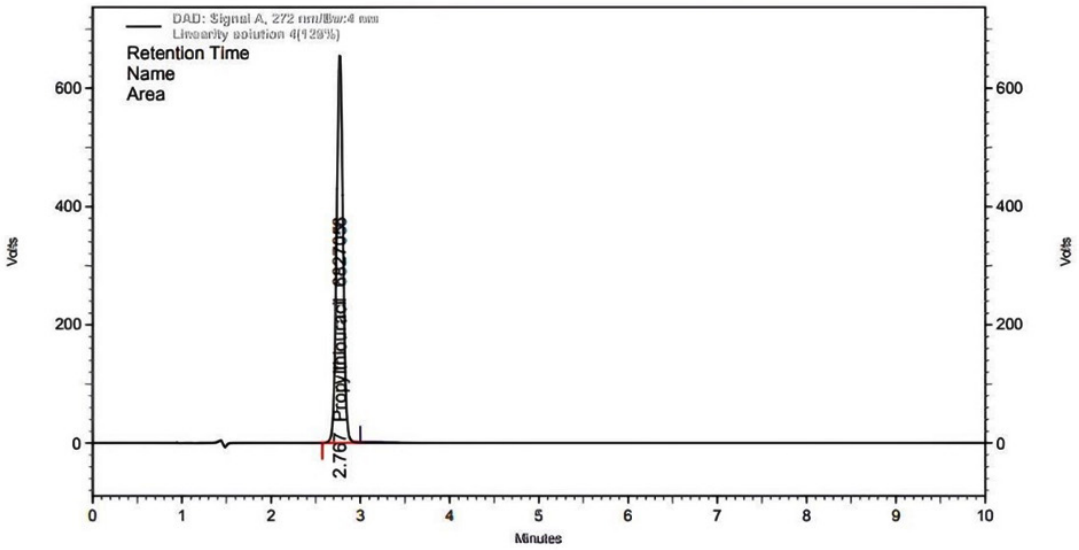

Fig. 7. Linearity 125%

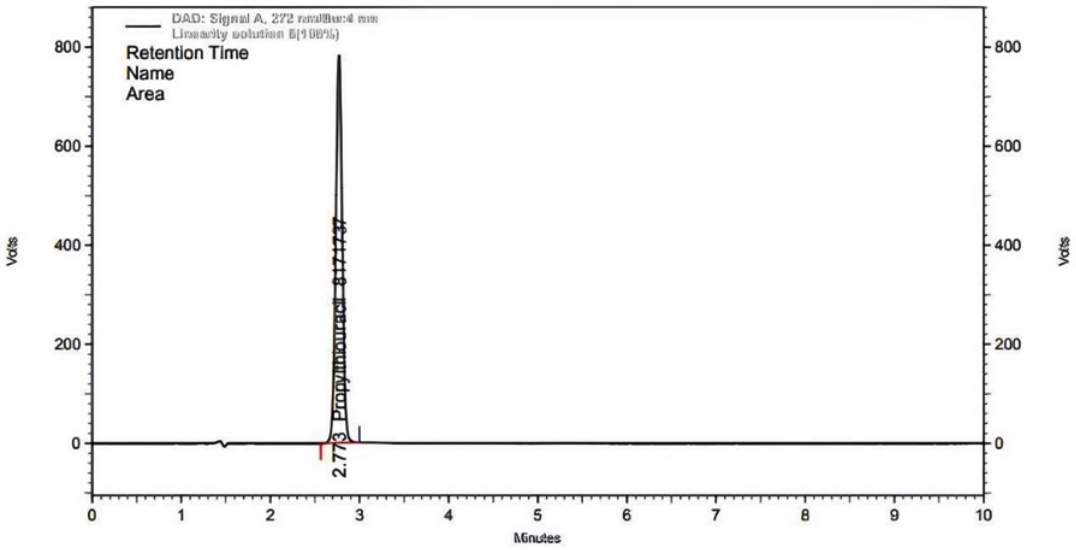

Fig. 8. Linearity 150%

System suitability

During the validation investigation, a standard solution was injected on various days. The propylthiouracil peak was calculated from a standard solution using system suitability software. Using standard solution-1, the percentage RSD of the area ratio of the five replicate injections for the propylthiouracil peak was determined. % similarity factor calculated from standard solution-2 against standard solution-1. Data is shown in Table 18. A typical set of system suitability chromatograms is shown in the appendix.

DISCUSSION

The absolute difference between the % assay of spiked and un-spiked samples meets the acceptance criteria, and the peak purity plot of propylthiouracil peak from standard, control and spiked test solutions shows that the peak is homogenous and no co-eluting peaks are seen in specificity. The peak purity plot of the propylthiouracil peak from degradation sample solutions indicates that the results are within the threshold limits. Based on the above data, a 0.45µm

Table 11. Results obtained for Propylthiouracil 50mg tablets (% Recovery)

Accuracy Level	Trails	Amount of Propylthiouracil added (mg)	Amount of Propylthiouracil found (mg)	% Recovery	% Mean Recovery	% RSD
50 %	1	50.86	50.813	99.9	100.2	0.3
	2	50.09	50.353	100.5		
	3	50.24	50.356	100.2		
100%	1	100.55	100.558	100.0	100.1	0.2
	2	100.75	100.617	99.9		
	3	100.49	100.811	100.3		
150%	1	150.30	149.693	99.6	99.6	0.2
	2	150.12	149.242	99.4		
	3	150.49	150.216	99.8		

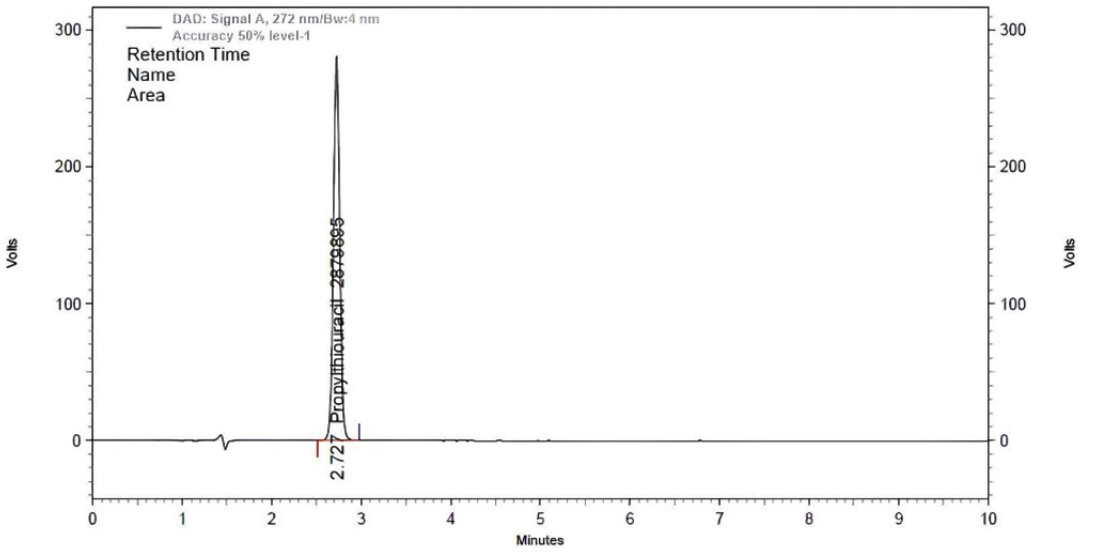

Fig. 9. Accuracy 50% level-1

PVDF filter is suitable for standard and sample preparation, and discard the 4 ml of filtrate. The standard and sample solutions are stable up to 48 hours at ambient temperature (23°C-27°C). The data response in linear throughout the working range. The method's acceptable recovery level is between 50% and 150% of the sample concentration. The method's precision is of acceptance level. Robustness indicates that the method is robust. All validation parameters were met in terms of system suitability, meeting the predetermined acceptance criteria.

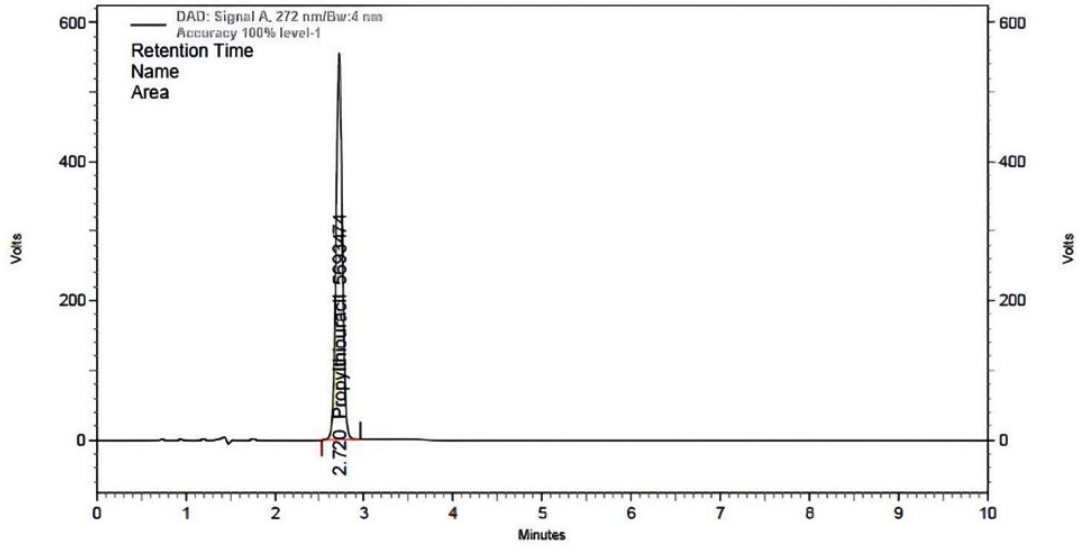

Fig. 10. Accuracy 100% level-1

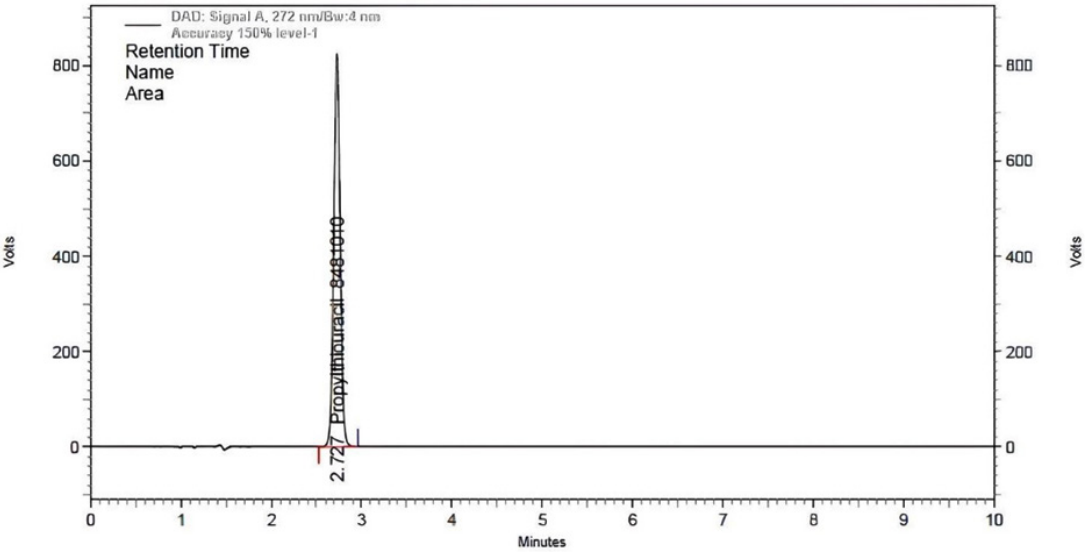

Fig. 11. Accuracy 150% level-1

Table 12. Results obtained for five replicate injections of standard solution

System Precision	Area of Propylthiouracil
1	5762988
2	5763640
3	5761356
4	5764861
5	5781881
Mean	5766945
%RSD	0.1

Table 13. Results obtained from six sample preparation from Method precision -Assay: Analyst 1

Method Precision	Propylthiouracil % assay
1	99.8
2	99.9
3	100.0
4	99.1
5	99.0
6	98.6
Mean	99.4
%RSD	0.6

Table 14. Results obtained from six sample preparation of intermediate precision -Assay: Analyst 2

Intermediate Precision	% Assay
1	98.8
2	99.1
3	98.3
4	98.7
5	99.0
6	100.2
Mean	99.0
%RSD	0.6

Table 15. Overall compilations of method precision and intermediate precision: Assay

Sample Number	% Assay	
	Method Precision	Intermediate precision
1	99.8	98.8
2	99.9	99.1
3	100.0	98.3
4	99.1	98.7
5	99.0	99.0
6	98.6	100.1
Mean	99.4	99.0
(%)RSD	0.6	0.6
Overall mean	99.2	
Overall RSD (%)	0.6	

Table 16. Altered parameters from optimized method for robustness of assay

No.	Parameter	Actual Method	Lower	Higher
1	Flow rate ± 0.1 mL/min	1.0 mL/min	0.9 mL/min	1.1 mL/min
2	Wavelength ± 2 nm	272 nm	270 nm	274 nm
3	Buffer pH ± 0.1	4.6	4.5	4.7
4	Mobile phase organic ± 2%	ACN: buffer (200:800)	ACN: buffer (196:804)	ACN: buffer (204:796)

Table 17. System suitability for flow rate variation, organic variation and wavelength variation

Flow Variations	%RSD	Column Efficiency	Tailing factor	% Recovery	% Assay	Absolute % Difference
Unchanged (1.0 mL/min)	0.2	73186	1.0	100.2	98.8	NA
High Flow rate 1.1 mL/min	0.2	63286	1.0	100.3	98.2	0.6
Low Flow rate 0.9 mL/min	0.1	74076	1.0	100.2	98.3	0.5
High M.P organic (ÿ2%)	0.0	71688	1.0	100.5	98.4	0.4
Low M.P organic (- 2%)	0.1	70992	1.0	100.4	98.2	0.6
High Wavelength (274nm)	0.1	73350	1.0	100.2	98.3	0.5
Low Wavelength (270nm)	0.2	73029	1.0	100.2	98.2	0.6

Table 18. System suitability from variation in buffer pH

Variations	%RSD	Column Efficiency	Tailing factor	% Recovery	% Assay	Absolute % Difference
Unchanged (pH 4.6)	0.1	67533	1.0	100.6	98.4	NA
High pH (pH 4.7)	0.1	73033	1.0	100.5	98.4	0.0
Low pH (pH 4.5)	0.1	66959	1.0	100.5	98.3	0.1

Table 19. System suitability observed on different days

Parameters	%RSD	Asymmetry factor	Plate count	% Recovery
Specificity	0.1	1.0	68693	100.3
Forced degradation	0.1	1.0	69139	101.1
Method precision and Filter study	0.1	1.1	65511	98.1
Linearity	0.2	1.0	67151	99.4
Accuracy	0.2	1.0	65918	101.2
Intermediate Precision	0.1	1.1	61400	100.5
Robustness	0.2	1.0	73186	100.2

CONCLUSION

A novel simple and sensitive reversed-phase HPLC isocratic method has been developed and validated for the assay of propylthiouracil tablets. The method was found to be linear (R2) is 0.999 within the analytical range of 24.916 µg/mL to 4.748 µg/mL. The results demonstrated that the method was both accurate and reproducible. Therefore, the developed chromatographic method can be used for estimation of propylthiouracil tablets.

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Conflict of Interest

The author(s) do not have any conflict of interest

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author's Contribution

The study was designed by Umamaheshwari D, who also headed the study's analyses and literature searches, wrote the protocol, carried out the statistical analysis, and wrote the first draft of the manuscript. The final manuscript was read and approved by all authors.

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