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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF THIORIDAZINE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Thioridazine in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol.[1] The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics.[2] It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity[3,4,5,6] The UV spectroscopic method was developed for estimation of Thioridazine in tablet dosage form and also validated as per ICH guidelines.[7] **Results:** The drug is freely soluble in analytical grade methanol, soluble in water and practically insoluble in ether. So, the analytical grade methanol is used as a diluent in method. The melting point of Thioridazine was found to be 158-159˚C (uncorrected). It showed absorption maxima 260 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 50µg/ml (PPM). The linearity was observed between 30-70 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 100.92, 101 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.64% and for Ruggedness is 0.40%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Thioridazine in tablet dosage form. The method could be considered for the determination of Thioridazine in quality control laboratories.

KEYWORDS: Thioridazine, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Thioridazine is a phenothiazine antipsychotic used in the management of psychoses, including schizophrenia, and in the control of severely disturbed or agitated behaviour. It has little antiemetic activity.[1] Phenothiazines also possess antiemetic, sedative, antipruritic, antidyskinetic, analgesic, and antihistaminic properties.[2] Thioridazine has been known as one of the most potent phenothiazines which inhibit trypanothione reductase irreversibly and is commonly used as an antipsychotic and antimuscarinic drug to treat behavioral symptoms.[3,4] Additionally, thioridazine was effective in the treatment of sweating as an antidepressant and sedative drug.[5]

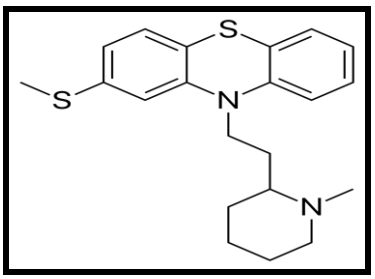

Fig. 1: Chemical Structure of Thioridazine

Due to the clinical importance of thioridazine hydrochloride, it is significant to establish a simple and sensitive method for its determination in biological fluids. Different pharmacopoeias recommend the determination of thioridazine base or the hydrochloride by titrating the drug in glacial acetic acid and acetic anhydride against perchloric acid. The BP recommends the titration of the drug in acetone solution containing about 7% mercury (II) acetate solution and using methyl

orange as indicator.[6] Several analytical methods have been described for determination of thioridazine hydrochloride. Among the methods conventional high performance liquid chromatography are most often used.[7-10] Voltammetry[11-13], chemiluminescence methods have been reported for the determination of thioridazine hydrochloride.[14] Supercritical fluid chromatography[15] and LC combined with other techniques were also reported for determination of thioridazine hydrochloride[16,17] and their main metabolites or derivatives. Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Thioridazine in tablet dosage form.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials**

Thioridazine was obtained as a gift sample. Thioridazine tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method development

A. Determination of λ max (15 PPM)[18, 19]

100 mg weighed amount of Thioridazine was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 5 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

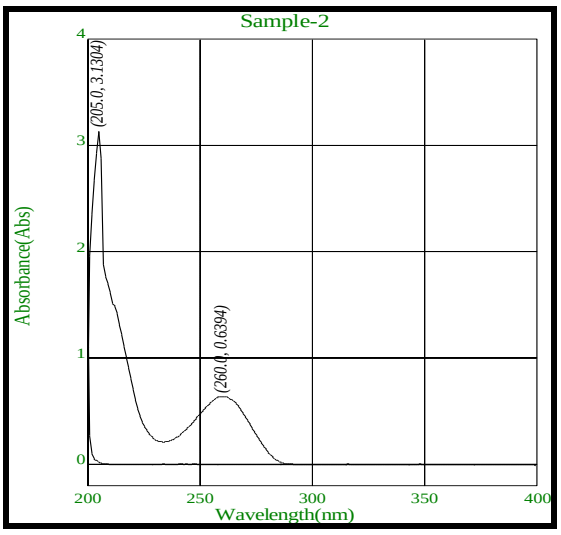

Fig. 2: Calibration Curve.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 100 mg of Thioridazine in 100 ml of analytical grade methanol to get concentration of 1000 µg/ml (PPM).

Preparation of Standard solution

Pipette out 5 ml from standard stock solution and diluted up to 100 ml with analytical grade methanol to get concentration of 50 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[18,19,20]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 100 mg of Thioridazine was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through whatmann filter paper. Pipette out 5 ml of the above solution and diluted up to 100 ml with analytical grade methanol. The absorbance was measured at 260 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form.

Torrent Pharmaceutical Limited (50 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.6275
3	Sample	0.6241

Table 2: Dosage Form Specifications.

Type	Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	Torrent Pharma LTD (50mg)	05/2021	07/2023	8056872-9092	0.1041	99.5

E. Method of validation[18,20,21]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 30 - 70 PPM at 260 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	30 PPM	0.3954
2	40 PPM	0.5188
3	50 PPM	0.6304
4	60 PPM	0.7481
5	70 PPM	0.8845
Correlation coefficient		**0.999**

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade methanol and measuring the absorbance at 260nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.81	100.92
100	1	1.01	101.00
120	1.2	1.18	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.6215
2	Sample Solution 2	0.6305
3	Sample Solution 3	0.6214
4	Sample Solution 4	0.6212
5	Sample Solution 5	0.625
MEAN		0.62392
SD		0.00400
% RSD		0.6414

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.6219	0.6223	99.28	0.3977
		0.6226			
2	Analyst 2	0.6245	0.6265	99.84	
		0.6284			

RESULTS

1. Solubility of Thioridazine

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Analytical grade Methanol	Freely Soluble
2	Water	soluble
3	Ether	Practically insoluble

2. Melting point of Thioridazine

The melting point of Thioridazine was found to be 158-159˚C (uncorrected).

3. Results for linearity for assay method of Thioridazine

The linearity of method was determined at concentration level ranging from 30 to 70 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9989 ~ 0.999**

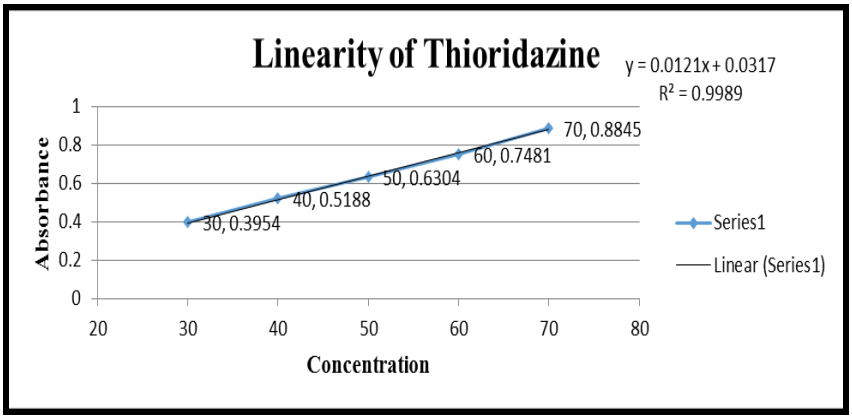

Fig. 3: Thioridazine Standard Curve.

4. Results for accuracy for assay method of Thioridazine

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Thioridazine

The % RSD for different sample of precision was found to be 0.6414 ~ 0.64 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Thioridazine

The %RSD for different sample of ruggedness was found to be 0.3977 ~ 0.40 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Thioridazine in tablet form has been developed. From the spectrum of Thioridazine, it was found that the maximum absorbance was 260 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 30-70 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Thioridazine in solid dosage forms. Hence, the method could be considered for the determination of Thioridazine in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. BP - British Pharmacopoeia
6. LC - Liquid Chromatography
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. C - Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date
14. µg/ml – Microgram per milliliter
15. Avg – Average
16. Wt – Weight
17. g - gm

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