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Research

Analytical Method Development and Validation of Thioridazine by RP-HPLC method

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Abstract:
Method development and validation are extremely important in the development of drug material. For many drug products, Scientists working on Investigational New Drug (IND), New Drug Application (NDA), and Abbrevated New Drug Application (ANDA) used to characterized API'S and excipients have not been sufficiently developed or validated [1,2]. The objective of the present study is to establish and generate inheriting Validation data for Thioridazine RP-HPLC method.
Keywords: Thioridazine, RP-HPLC, Validation.

Introduction:
Thioridazine blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain; blocks alpha-adrenergic effect, depresses the release of hypothalamic and hypophyseal hormones and is believed to depress the reticular activating system thus affecting basal metabolism, body temperature, wakefulness, vasomotor tone, and emesis. This work was planned on the conventional lines of procedure in the development of analytical method for Thioidazine in single component formulation. Also, validating accuracy of developed method as per ICH guidelines.

Materials and Method:

Selection of Analytical Technique[3]

HPLC was selected as analytical technique for estimation of Thioridazine

Instruments: The analysis of the drug was carried out on Younglin (S.K.) Gradient System UV Detector. Equiped with Reverse Phase (Cosmosil) C18 column (4.6 id x 250mm; 5µm), a SP930D pump, a 20µ l injection loop and UV630D Absorbance detector and running autochro-3000 software.

a) Selection of stationary phase: The column used in this method C18 Cosmosil. The configuration of the column is 4.6 x 250 mm, particle size 5 □m. C18 column gives high non polar retentivity, symmetric peak shape, highly reproducible and stable ideal for HPLC method

b) Solubility Studies: This study was carried out to find an ideal solvent in which drugs are completely soluble. Various solvents were tried for checking solubility of Thioridazine. From solubility studies it was concluded

that of Thioridazine is soluble in Methanol and water however it is soluble in Water PH adjusted 0.1% Orthophosphoric Acid with TEA, Buffer pH 3.0.

c) Chromatographic conditions: The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

List of Mobile Phase :

1. ACN+Methanol+0.05% (OPA with TEA)Water (10+30+60% v/v) 239 nm 0.7ml/min
2. ACN+Methanol+0.05% (OPA with TEA)Water (25+25+50% v/v) 239 nm 0.7ml/min
3. ACN+ 0.1% (OPA with TEA)Water (70+30 % v/v) 239 nm 0.7ml/min
4. ACN+ 0.05% (OPA with TEA)Water (70+30 % v/v) 239 nm 0.8ml/min
5. MEOH+ 0.05% (OPA with TEA)Water (60+40 % v/v) 239 nm 0.7ml/min
6. MEOH+ 0.05% (OPA with TEA)Water (70+30 % v/v) 239 nm 0.7ml/min

Preparation of Stock Standard Solution [4]:

Standard Solution Stock I : (Thioridazine)

Accurately weight and transfer 10mg Thioridazine working standard into 10 ml volumetric flask as about diluent Methanol completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol : Water (0.1%OPA with TEA)Water, prepared in (70ml MEOH : 30ml WATER v/v) solvent.

Validation of method for analysis of Thioridazine:

The developed method was validated as per ICH guidelines.

Analytical Method validation Analytical method validation was carried out as per ICH method validation guidelines Q2 (R1).

Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Determination:

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated**.**

Preparation of standard stock solution for linearity:

Average weight of tablet sample (equivalent to 10 mg of Thioridazine were weighed and transfered to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted upto 10 ml volumetric flask with diluents was added to make up the volume.

Table1 : Table for linearity

Sr No.	Concentration (µg/mL)
1	10
2	20
3	30
4	40
5	50

Accuracy (recovery)[5]:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Preparation of standard stock solution:

10 mg of Thioridazine working standards were weighed and transfered to 10 mL volumetric flask & diluent was added to make up the volume 0.2ml of this solution diluted upto 10 ml with diluent.

Application of proposed method for analysis of tablet formulation:

Accuracy

The accuracy was determined by Thioridazine (equivalent to10 mg of Thioridazine) (80%, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder mixture containing 10 mg of Thioridazine were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy**.**

Table2: Table for accuracy

Sample	Amount added (mg)
Accuracy 80%	8
Accuracy 100%	10
Accuracy 120%	12

Results and discussion:

Chromatogram of Trial 1:

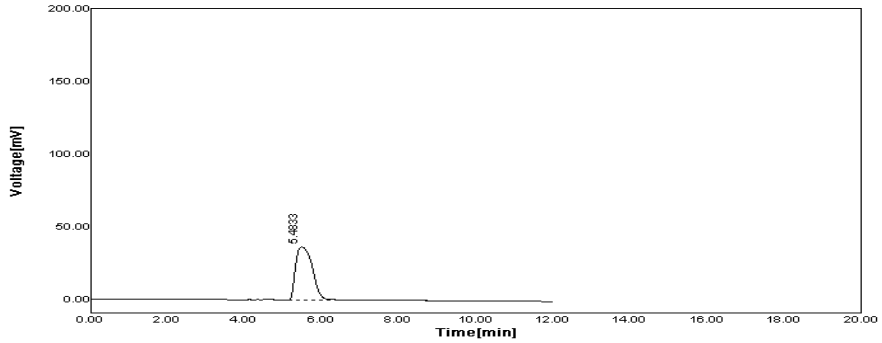

Fig No 1: Chromatogram of Trial 1

Table No 3: Result for Chromatogram of Trial 1

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
Sum	5.4933	1093.0880	100.00	900.4	1.45	0.000

Chromatogram of Trial 2:

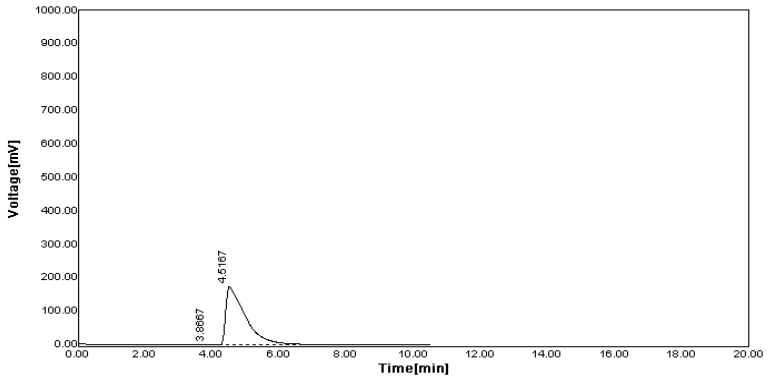

Fig No 2 : Chromatogram of Trial 2

Table No .4: Result for Chromatogram of Trial 2

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	3.8667	3604.02	0.51	649.5	1.3146	0.0000
2	4.5167	6975.5645	100.00	297.3	3.7266	0.7856
Sum		7011.6045				

Chromatogram of Trial 3:

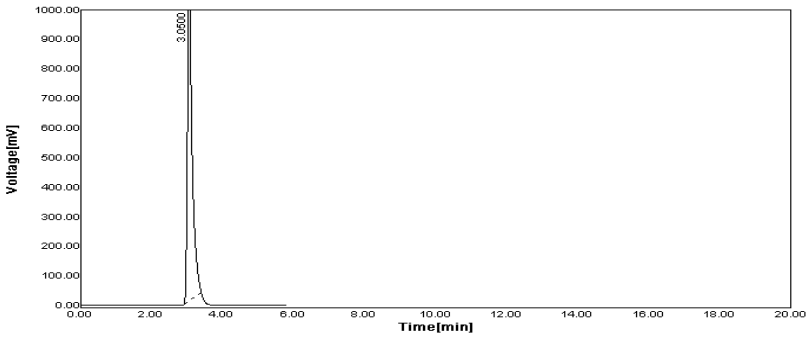

Fig No 3 : Chromatogram of Trial 3

Table No .5: Result for Chromatogram of Trial 3

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	3.0500	9977.7949	100.00	3718.4	1.9508	0.0000
Sum		9977.7949				

Chromatogram of Trial 4:

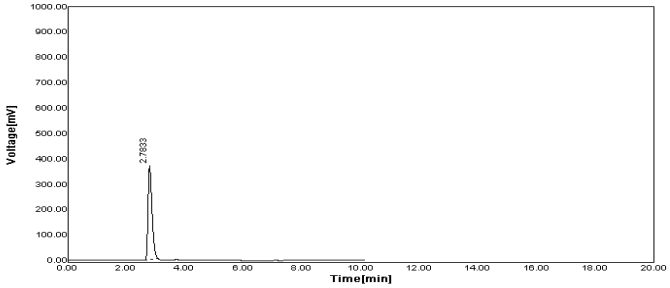

Fig No 4: Chromatogram of Trial 4

Table No .6: Result for Chromatogram of Trial 4

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	2.7833	3468.4341	100.0	2892.01	1.5317	0.0000
Sum		3171.7716				

Chromatogram of Trial 5:

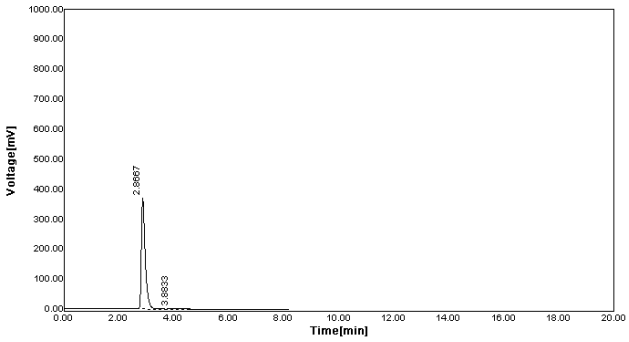

Fig No 5: Chromatogram of Trial 5

Table No .7: Result for Chromatogram of Trial 5

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	2.8667	3609.9768	99.13	3060.6	1.5317	0.0000
2	3.8833	31.6388	0.87	228.5	4.0387	1.6466
Sum		3641.6157				

Chromatogram of Trial 6:

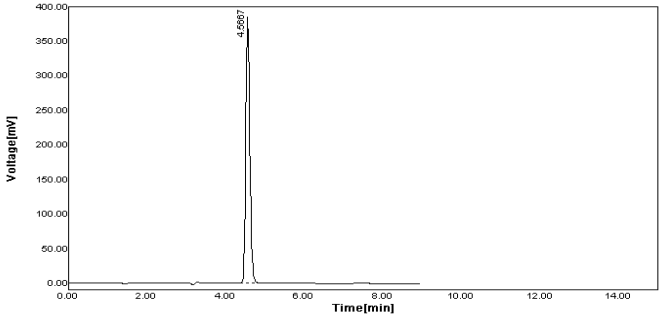

Fig No 6: Chromatogram of Trial 6

Table No .8: Result for Chromatogram of Trial 6

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	4.5867	2291.8047	100.0	11609.4	1.1004	0.0000
Sum		2291.8047				

The final chromatographic conditions selected were as follow:

Analytical column : Cosmosil C18 Column (250mm x 4.6mm, 5µ m partical size).

Injection volume : 20µl

Flow rate : 0.7ml/min
Mobile phase : Methanol : water (70: 30% V/V)
Detection : 239 nm
Run Time : 15 min

Preparation of Standard chromatogram of Thioridazine

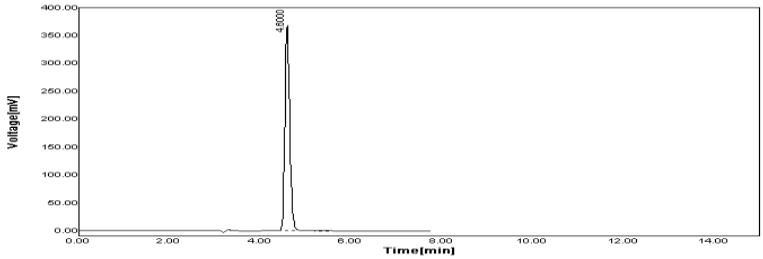

Fig No.7: Chromatogram of standard Thioridazine

Table No 9: Result for standard Chromatogram of Thioridazine

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	4.600	2169.0204	100.00	13719.6	1.1438	0.0000
Sum		2169.0204				

Analytical of Method Validation:

1. **Linearity:** From Thioridazine standard stock solution, different working standard solution (10-50µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using mixed volume loop injector Chromatograms were recorded.

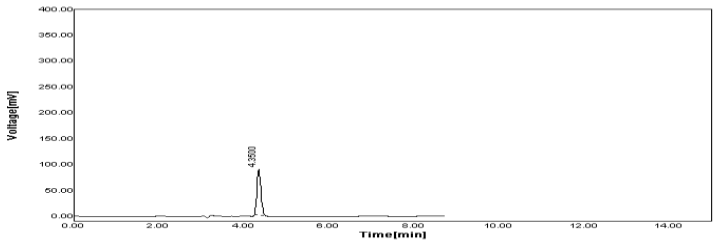

Fig.No.8 .Chromatogram of linearity 10 MCG

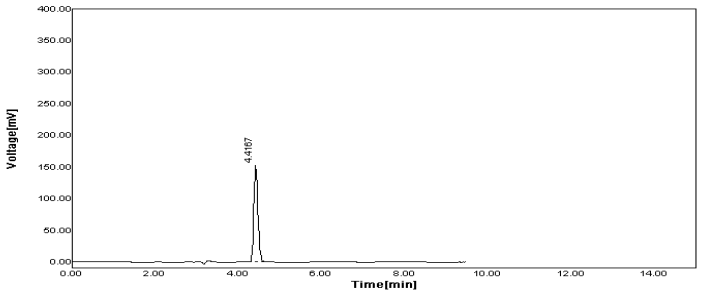

Fig.No.9 .Chromatogram of linearity 20 MCG

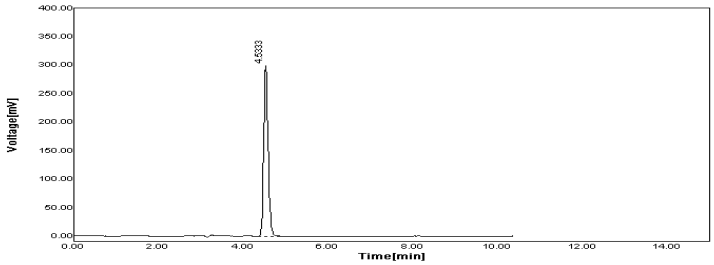

Fig.No.10 .Chromatogram of linearity 30 MCG

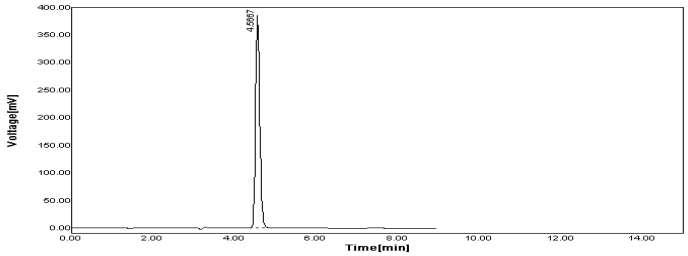

Fig.No11 .Chromatogram of linearity 40 MCG

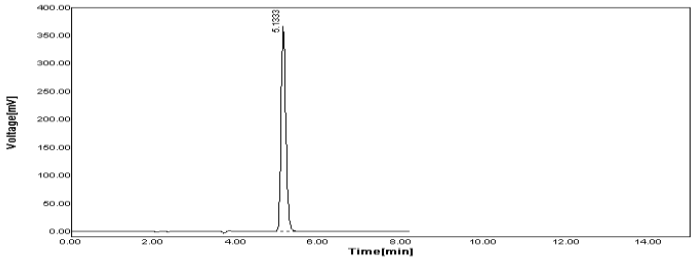

Fig.No.12 .Chromatogram of linearity 50 MCG

Table No 10. Linearity of Thioridazine

S. No.	Conc. (Mcg/ml)	Area Thioridazine
1	**10**	**567.94**
2	**20**	**1034.08**
3	**30**	**1583.81**
4	**40**	**2164.20**
5	**50**	**2771.86**

Regression equation data for Thioridazine

Regression Equation Data Y=mx+c

Slope(m) 55.38x , Intercept(c) 37.01 , Correlation Coefficient 0.997

Accuracy:- Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed

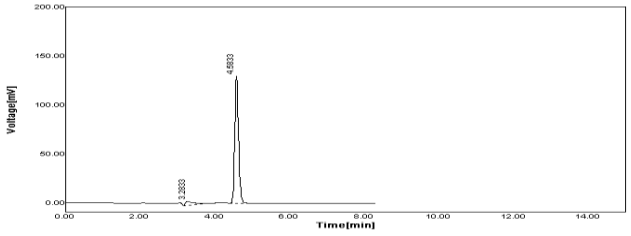

Fig.13. Chromatogram of Accuracy 80%

Accuracy 100%

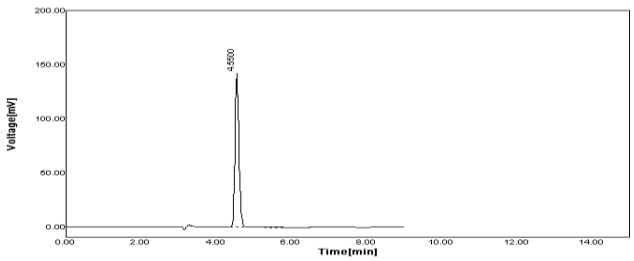

Fig.14. Chromatogram of Accuracy 100%

Accuracy 120%

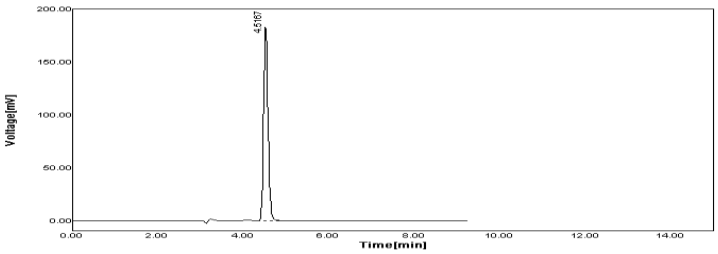

Fig.15. Chromatogram of Accuracy 120%

Conclusion:

Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Thioridazine in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Thioridazine in Single-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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