A HIGHLY SENSITIVE METHOD APPLICABLE FOR SOME SELECTED ANTIVIRAL DRUGS WITH USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: A rapid and stability-indicating ultra-performance liquid chromatography method was developed for quantification of perphenazine, trifluoperazine, mesoridazine, thiothixene to get some more advantages over other methods already developed in different methods we can single method. The method was developed according to ICH and to develop the calibration curve for all drugs using this method. In this method a simple isocratic conditions of mobile phase comprising water and Acetonitrile in a ratio of 20:80, v/v at a flow rate of 0.5 mL/minute over waters X bridge C18, 50 × 4.6 mm, 3.5μm column at room temperature was maintained. The method showed excellent linear response with correlation coefficient (R^2) values of 0.999 for perphenazine, trifluoperazine, mesoridazine, thiothixene which was within the limit of correlation coefficient

Keywords: UPLC, Perphenazine, Trifluoperazine, Mesoridazine, Thiothixene

PERPHENAZINE: It is roughly ten times as potent as chlorpromazine [1] at the dopamine-2 (d2) receptor [2] thus perphanizinen [3] is considered a medium potency antipsychotic [4] [5].
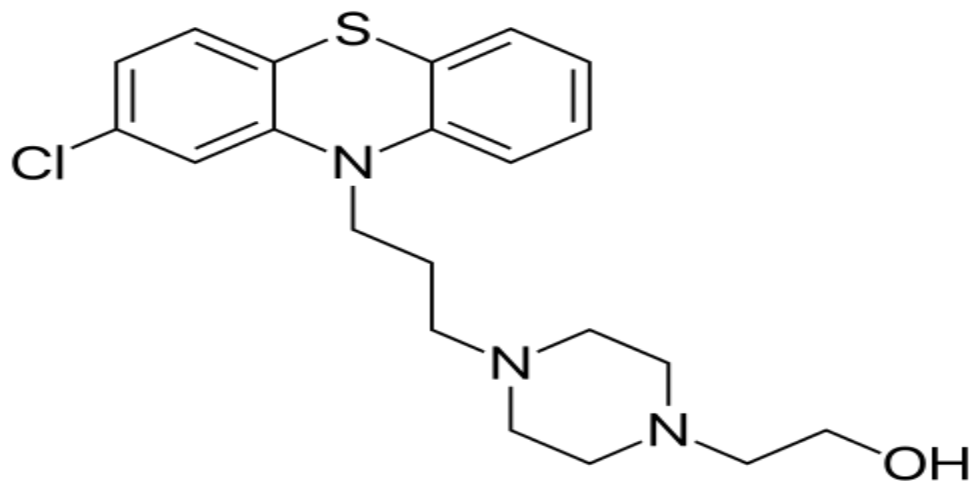
Fig 1: Chemical structure of Perphenazine

TRIFLUOPERAZINE: It is marketed under the brand name stelazine [6] among others, is a typical antipsychotic primarily used to treat schizophrenia.
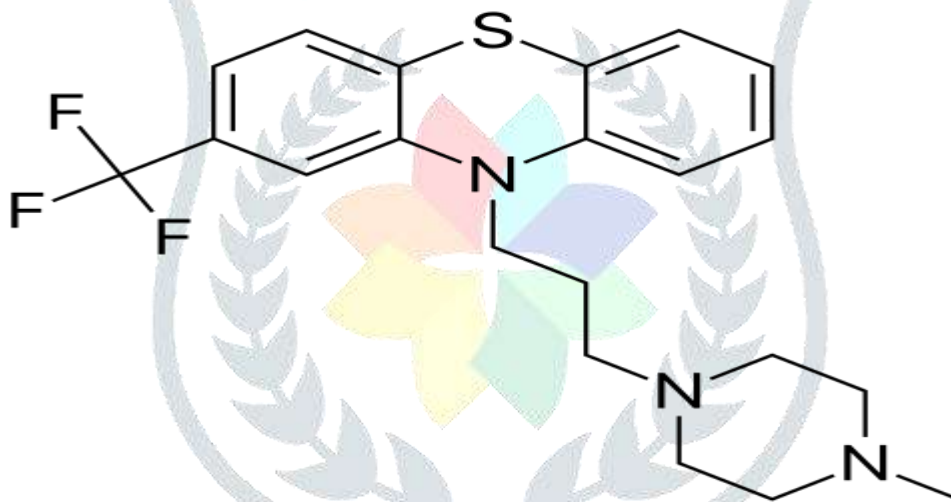

Fig 2: Chemical structure of Trifluoperazine

MESORIDAZINE: It is a piperidine [7] neuroleptic drug belonging to the class of the drugs called phenothiazines [8] used in the treatment of schizophrenia.
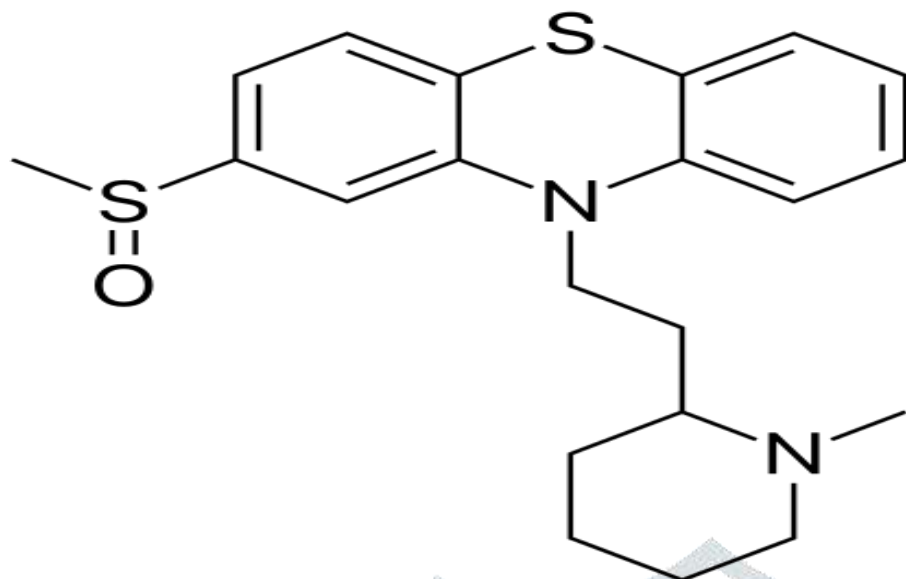
Fig 3: Chemical structure of Mesoridazine

THIOTHIXENE: Thiothixene or tiotixene sold under the brand name navane among others, is a typical antipsychotic of the thioxanthene class which is related to chlorprothixene [9] and is used in the treatment of psychosis [10] like schizoophrenia and bipolar mania.
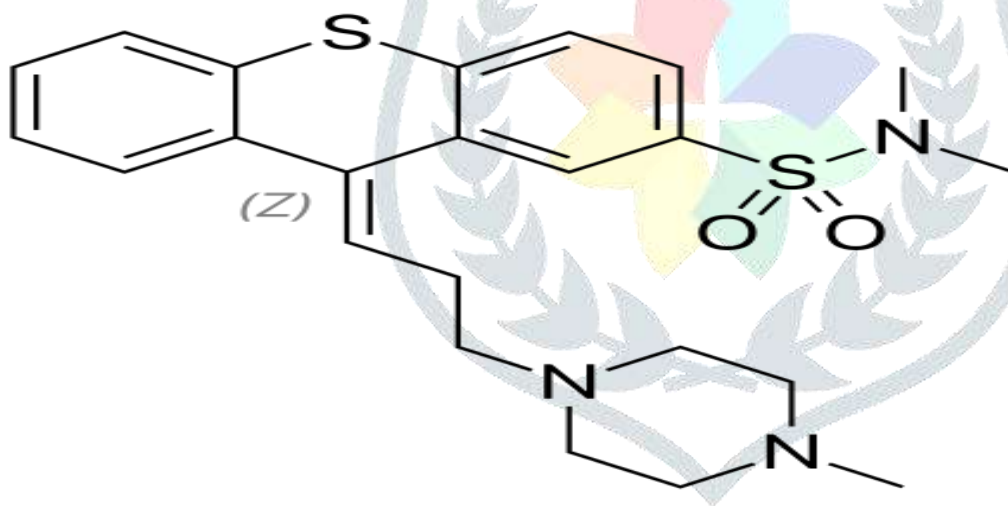

Fig 4: Chemical structure of Thiothixene

MATERIALS AND METHOD

Perphenazine, Trifluoperazine, Mesoridazine, Thiothixene standards were provided by Glenmark pharmaceuticals, Mumbai. Water, acetonitrile HPLC grade from Merck company, Mumbai.

Selection of wavelength of detection

Perphenazine, Trifluoperazine, Mesoridazine, Thiothixene standard solution of 100 ppm was scanned at 200-400 nm and UV Spectrum was recorded. By observing the spectrum of standard solution, λ_{max} of 225 nm was taken for trails to develop the proposed method.

Instrumentation and Chromatographic Conditions

Ultra performance liquid chromatography Agilent 1200 series equipped with PDA detector and waters X bridge C18 (50 mm × 4.6 mm, 3.5µm) containing 3.5 µm particle size column was used. Mobile phase comprising of water and acetonitrile in a ratio 20:80 % v/v at a flow rate of 0.5 ml/min and the effluent was detected at 225nm. The Column temperature was maintained at ambient and the volume of injection is 5µL.

Mobile phase- A

HPLC grade water.

Mobile phase - B

Acetonitrile

Preparation of Mobile phase A+B:

Take mobile phase A and B in the ratio of 20:80 v/v.

Diluent : Water and acetonitrile in the ratio of 20:80 v/v

Preparation of solutions

Standard stock solution: Weigh each 5 mg of perphenazine, trifluoperazine mesoridazine, thiothixene accurately weighed and transferred into 10 ml volumetric flask add 7 ml diluent sonicate for 30 minutes to dissolve the contents completely, then make up to the mark with diluent.

Working Standard solution: 1 mL of standard stock solution was pipetted into 10 mL volumetric flask and diluted up to the mark with diluents and filtered through 0.45µ Millipore Nylon filter to obtained concentration of 50µg/ml.

Preparation of Linearity stock solution:

Perphenazine, trifluoperazine, mesoridazine, thiothixene is weighed 5mg of each and taken in 10ml volumetric flask and make up to the mark with the suitable solvents (water, acetonitrile).

Linearity 25%:

0.25 ml of stock solution is taken in to the 10ml volumetric flask and make up to the mark with HPLC grade water.

Linearity 50%:

0.5 ml of stock solution is taken in to the 10ml volumetric flask and make up to the mark with HPLC grade water.

Linearity 75%:

0.75 ml of stock solution is taken in to the 10ml volumetric flask and make up to the mark with HPLC grade water.

Linearity 100%:

1 ml of stock solution is taken in to the 10ml volumetric flask and make up to the mark with HPLC grade water.

Linearity 125%:

1.25 ml of stock solution is taken in to the 10ml volumetric flask and make up to the mark with HPLC grade water.

Linearity 150%:

1.5 ml of stock solution is taken in to the 10ml volumetric flask and make up to the mark with HPLC grade water.

Results and Discussion

System suitability

The UPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of the standard solution   assessed to check the system suitability. The number of theoretical plate count and Tailing factor all the parameters were found to be within limit.

Table 1: System Suitability Results

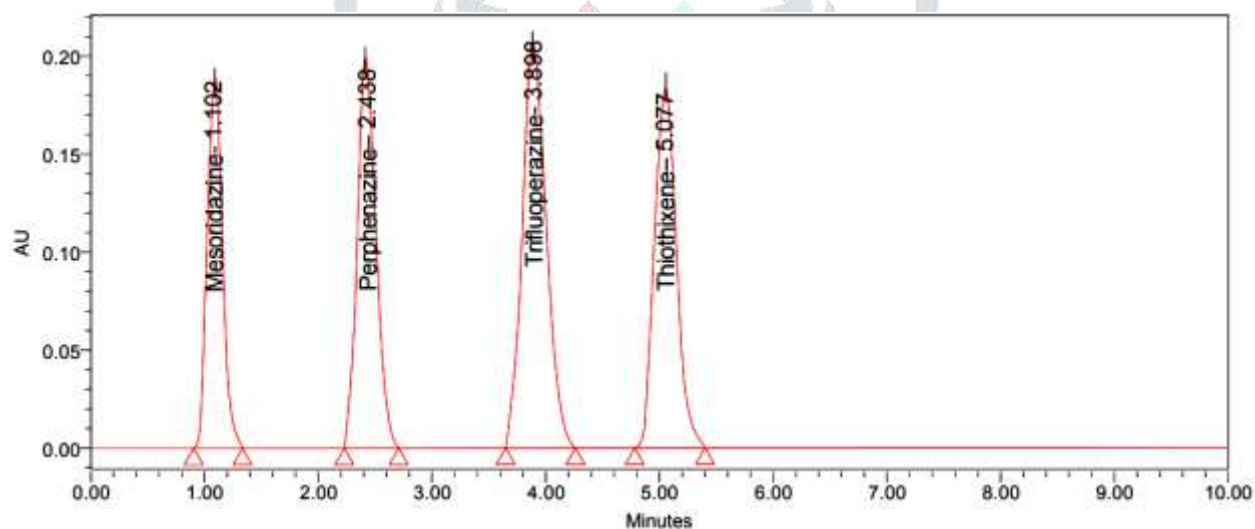| Drug Name	USP Plate count	USP Resolution	USP Tailing
Mesoridazine	5842		1.08
Perphenazine	6974	5.28	1.17
Trifluoperazine	3210	5.32	1.01
Thiothixene	4988	3.41	1.06

Fig 5: Chromatogram for Standard

Linearity

Linearity of the method was evaluated by preparing a standard solution. Sequential dilutions were performed to the given solutions at 25, 50, 75, 100, 125 and 150% of the target concentrations. These were injected and the peak areas are used to plot calibration curves against the concentration. The correlation coefficient values of these analytes were 0.999. The results were shown in table.

Table 2: Linearity results for Mesoridazine

S.No.	Linearity	Conc. of Mesoridazine	Area Counts of Mesoridazine
1	25%	12.5	361562
2	50%	25	782659
3	75%	37.5	1113407
4	100%	50	1568356
5	125%	62.5	1856494
6	150%	75	2256725

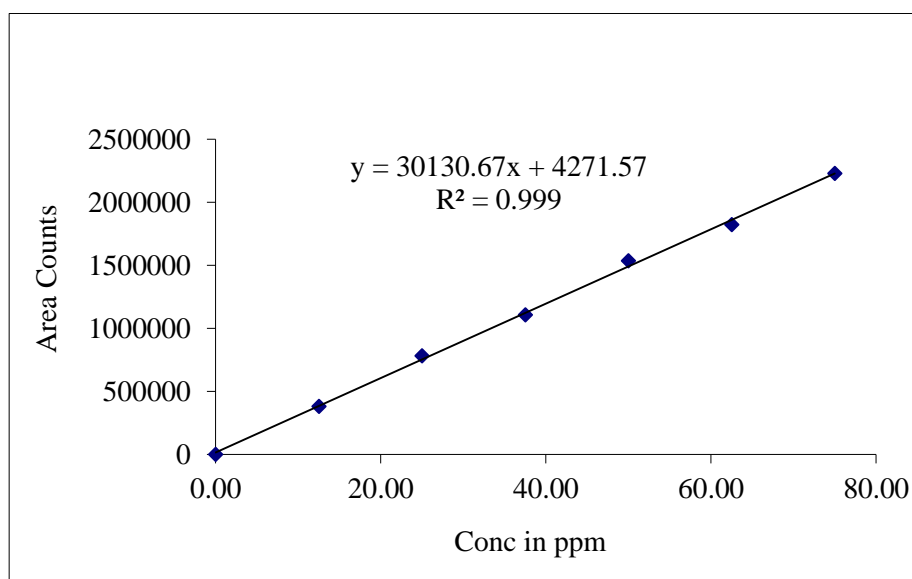$$y = 30130.67x + 4271.57$$
$$R^2 = 0.999$$

Fig 6: Linearity plot for Mesoridazine

Table 3: Linearity results for Perphenazine

S.No.	Linearity	Conc. of Perphenazine	Area Counts of Perphenazine
1	25%	12.5	403155
2	50%	25	799247
3	75%	37.5	1133462
4	100%	50	1594123

| 5 | 125% | 62.5 | 1921356 |
| 6 | 150% | 75 | 2291635 |

$$y = 30589.09x + 16191.54$$
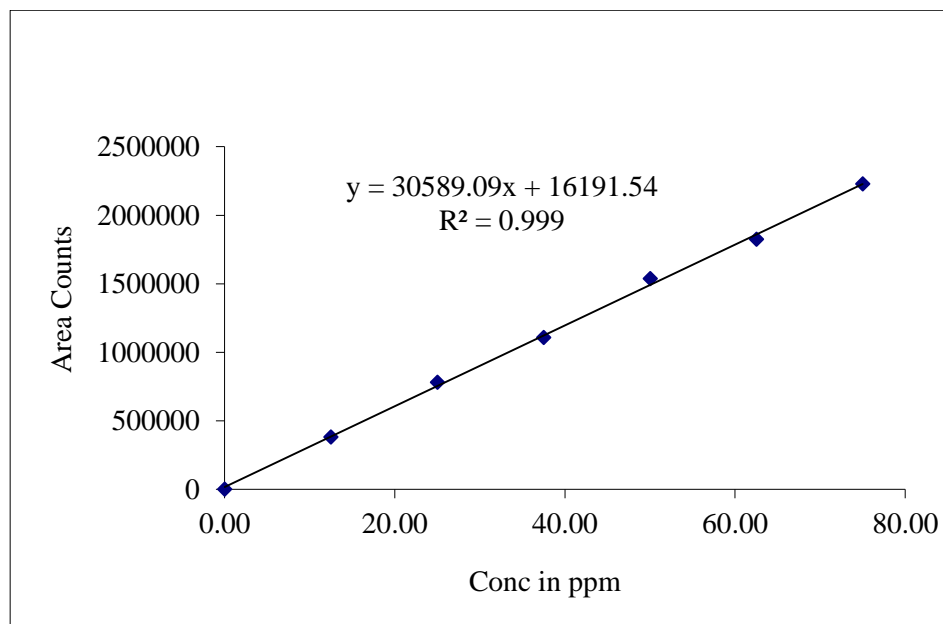$$R^2 = 0.999$$

Fig 7: Linearity plot for Perphenazine

Table 4: Linearity results for Trifluoperazine

S.No.	Linearity	Conc. of Trifluoperazine	Area Counts of Trifluoperazine
1	25%	12.5	409587
2	50%	25	829401
3	75%	37.5	1217546
4	100%	50	1642585
5	125%	62.5	2086591
6	150%	75	2431084

$$y = 32744.13x + 3065.86$$
$$R^2 = 0.999$$

Area Counts (y-axis): 0, 500000, 1000000, 1500000, 2000000, 2500000

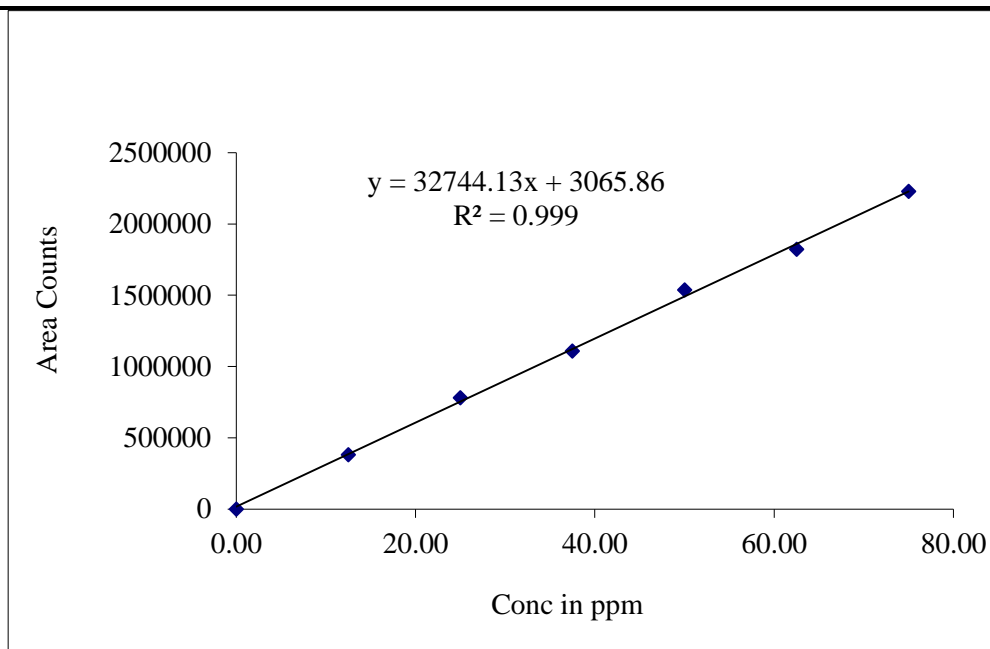Conc in ppm (x-axis): 0.00, 20.00, 40.00, 60.00, 80.00

Fig 8: Linearity plot for Trifluoperazine

Table 5: Linearity results for Thiothixene

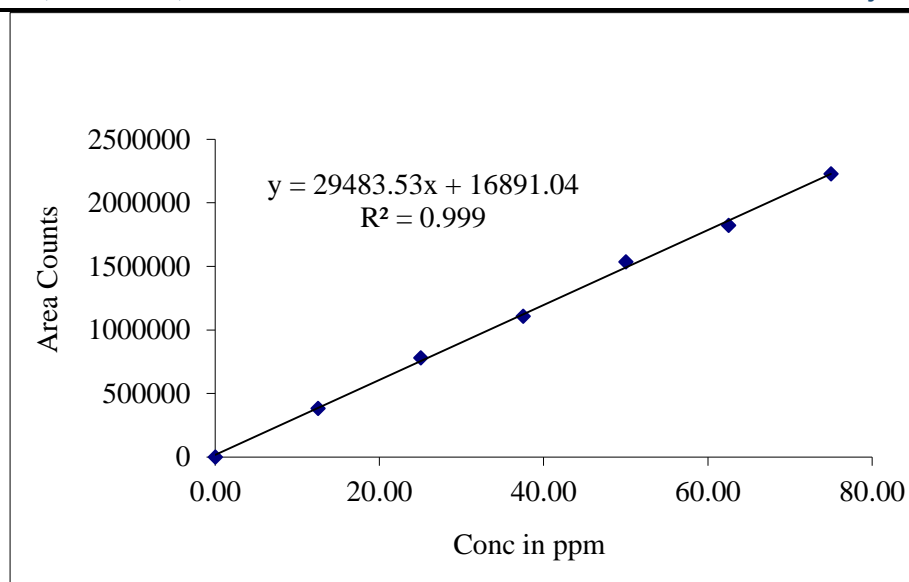| S.No.	Linearity	Conc. of Thiothixene	Area Counts of Thiothixene
1	25%	12.5	382122
2	50%	25	781248
3	75%	37.5	1107546
4	100%	50	1536247
5	125%	62.5	1823024
6	150%	75	2227478

$y = 29483.53x + 16891.04$
$R^2 = 0.999$

Fig 9: Linearity plot for Thiothixene

Conclusion:

This study showed that the proposed single UPLC method can be used for the assessment of drug purity, stability, solubility and lipid-formulation release profile with no interference of excipients or related substances of active pharmaceutical ingredient for Mesoridazine, Perphenazine, Trifluoperazine and Thiothixene drugs.

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