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RP- HPLC Method for the Quantitation of Chlorpromazine HCL and Trihexiphenidyl HCL Simultaneously

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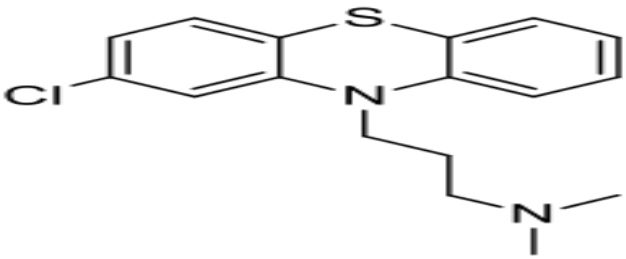
ABSTRACT
In the present it is aim to develop a RP- HPLC method for the quantitation of Chlorpromazine HCl and Trihexiphenidyl HCl simultaneously. The aim was achieved by using chromatographic conditions ODS C_{18} (250×4.6mm, 5µ) Column, mixture of Ammonium acetate Buffer and Methanol in the ratio of 15:85 with the flow rate of 1ml/min and column temperature was 20^0C. The effluents were detected at the wavelength of 211nm. Retention time was 2.5 min and 4.4 min for Chlorpromazine HCl and Trihexiphenidyl respectively. The proposed new method was validated according to ICH guidelines and successfully applied to the determination of both drugs in their combined pharmaceutical formulation with mean recoveries of 99.58-100.45 ± 0.386-0.931. Linearity obeys in the range of 60-140µg/ml and 2.4-5.6µg/ml for both drugs correspondingly with correlation coefficient values as 0.998 and 0.996 respectively. The results obtained were found to be satisfactory, when statistically compared to those obtained from a reference method.

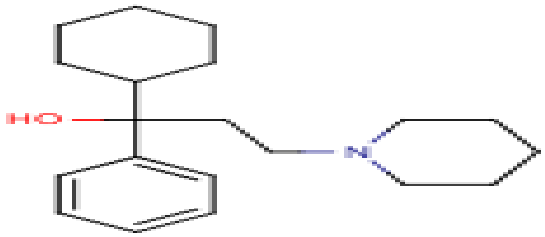
KEY WORDS: Chlorpromazine HCl, Trihexiphenidyl HCl, Ammonium acetate, RP-HPLC

I. INTRODUCTION:

Chlorpromazine is a dopamine inhibitor. It inhibits prolactin-release-inhibitory factor, considered to be dopamine, thereby stimulating the release of prolactin. The aggression of middle dopaminergic purpose may be related to the therapeutic effect in psychotic conditions. Chlorpromazine can produce alpha-adrenergic blockade which may produce hypotension. Chlorpromazine also has a tendency to produce elevated serum glucose and cholesterol levels.



CHLORPROMAZINE HCl



TRIHEXIPHENIDYL

II. Materials and Methods

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicater	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	HYPERSIL, BDS (C_{18} 250x4.6 ID) 5µm

Table 1: Optimized chromatographic conditions

Mobile phase	Buffer (Ammonium acetate): Methanol
PH	6.0
Column	ODS
Flow rate	1ml/min
Column temperature	20°c
Temperature	Ambient
Wavelength	211nm
Injection volume	20ul
Run time	6min
Retention time	4.403

Standard Preparation: Weigh accurately 50 mg of Chlorpromazine HCl and 2 mg of Trihexiphenidyl HCl in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of Chlorpromazine HCl and 4µg/ml of Trihexiphenidyl HCl is prepared by diluting 2ml to 10ml with mobile phase.

Sample preparation: 20 tablets (each tablet contains 50 mg of Chlorpromazine HCl and 2 mg of Trihexiphenidyl HCl) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Chlorpromazine HCl and Trihexiphenidyl HCl (µg/ml) were prepared by dissolving weight equivalent to 50 mg of Chlorpromazine HCl and 2 mg of Trihexiphenidyl HCl and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicate for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Chlorpromazine HCl and 4µg/ml of Trihexiphenidyl HCl was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Procedure: Separately inject 20µl of the standard preparation in 6 replicate and calculate RSD of standard area (RSD NMT 2.0%) , Tailing factor (NMT 2.0), Theoretical plate NLT 1000 and inject test solution into the chromatogram and record the chromatograph

METHOD DEVELOPMENT

Table 2: Assay Results

	CHLORPROMAZINE HCl		TRIHEXIPHENIDYL HCl	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	2344.463	2334.362	212.684	207.967
Injection-2	2351.614	2323.199	209.655	209.698
Injection-3	2337.863	2337.863	207.039	207.039
Injection-4	2341.801	2331.502	210.80	207.632
Injection-5	2241.801	2328.483	210.080	208.197
Average Area	2323.508	2331.082	210.052	210.1066
Tablet average weight	125mg		125mg	
Standard weight	100mg		4mg	
Sample weight	250mg		250mg	
Label claim	50mg		2mg	
std. Purity	99.2%		99.3%	
Amount found in mg	49.76mg		1.99mg	
Assay(%purity)	99.52%		99.39%	

The amount of Chlorpromazine HCL and Trihexiphenidyl HCL present in the taken dosage form was found to be 99.52 % and 99.39% respectively.

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

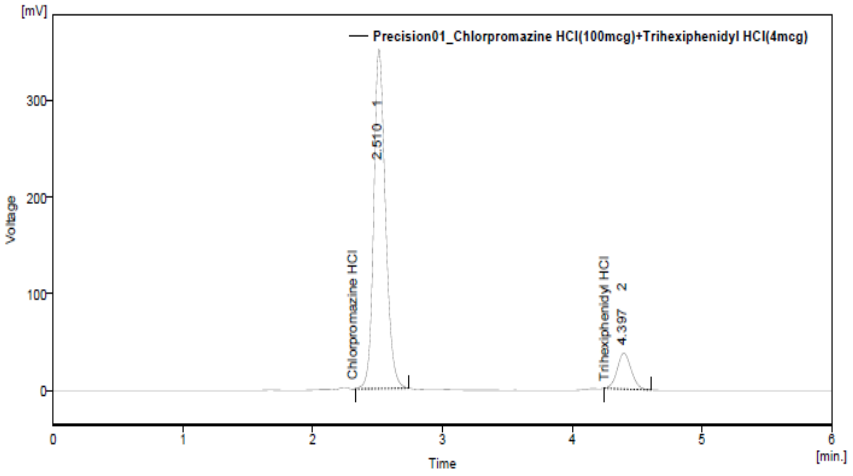


Fig.1: Chromatogram of system suitability injection-1

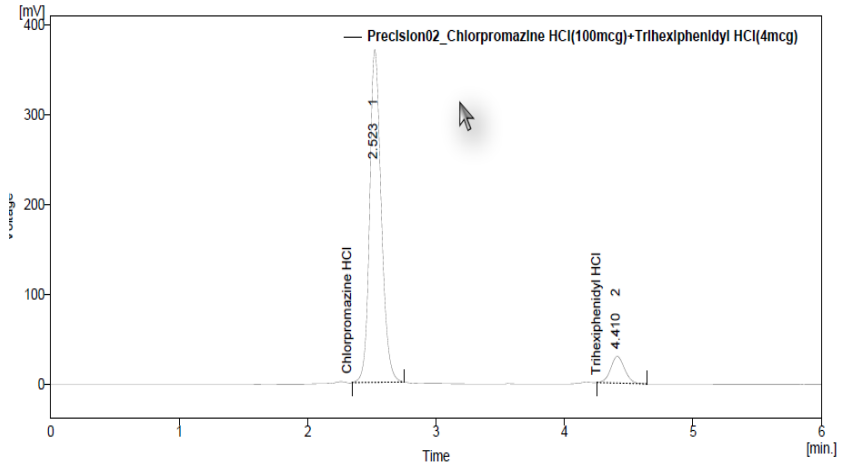


Fig.2: Chromatogram of system suitabulity injection-2

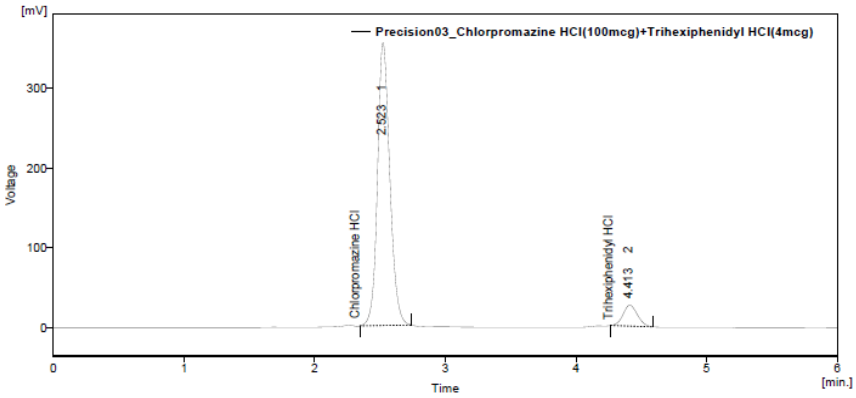


Fig.3: Chromatogram of system suitabulity injection- 3

III. RESULTS AND DISCUSSION:

Table 4: Results for system suitability of Chlorpromazine HCl

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.510	2311.866	3735	1.375
2	2.523	2325.117	3775	1.375
3	2.523	2321.138	3304	1.269
4	2.523	2333.196	3304	1.308
5	2.507	2350,119	3260	1.308
6	2.497	2341.355	3234	1.269
Mean	2.5138	2330.465	-	-
SD	0.0109	13.956	-	-
%RSD	0.43	0.60	-	-

Table 5: Results for system suitability of Trihexiphenidyl HCl

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.397	208.286	7868	1.194	10.408
2	4.410	204.128	7842	1.161	10.248

3	4.413	202.102	7094	1.156	9.813
4	4.413	200.853	7094	1.194	9.813
5	4.397	202.888	7437	1.233	9.959
6	4.390	208.551	7019	1.200	9.830
Mean	4.403	204.468			
SD	0.010	3.241			
%RSD	0.22	1.59			

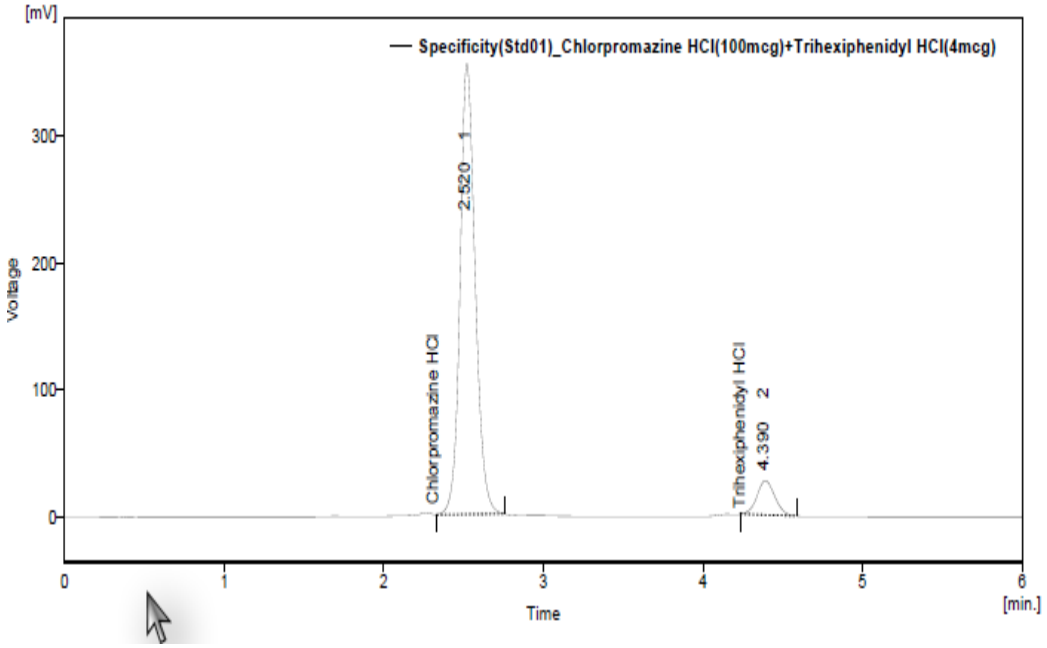


Fig. 4: Chromatogram for Specificity of Chlorpromazine HCl and Trihexiphenidyl HCl standard

Table 6: Results for Specificity of Chlorpromazine HCl and Trihexiphenidyl HCl

Name	Rt	Area	Th.Plates	Asymmetry	Resolution
Chlorpromazine HCl	2.520	2335.666	3295	1.008	9.709
Trihexiphenidyl HCl	4.390	210.340	7019	1.088	

Linearity and range

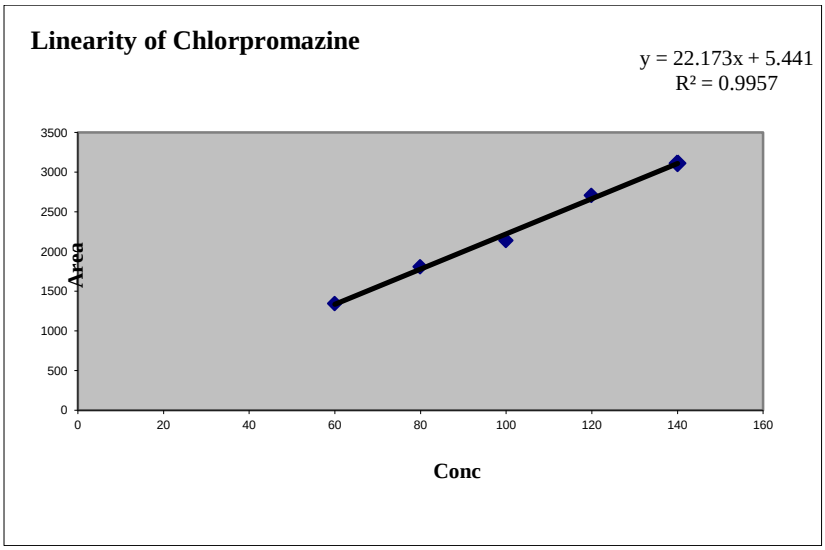


Fig. 5: Linearity graph of Chlorpromazine HCl

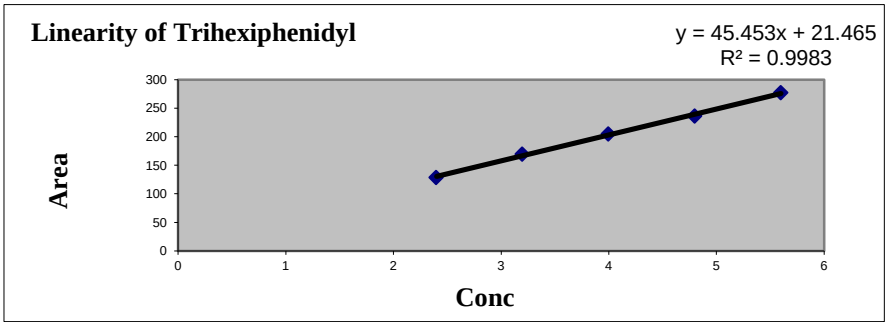


Fig. 6: Linearity graph of Trihexiphenidyl HCl

Accuracy:

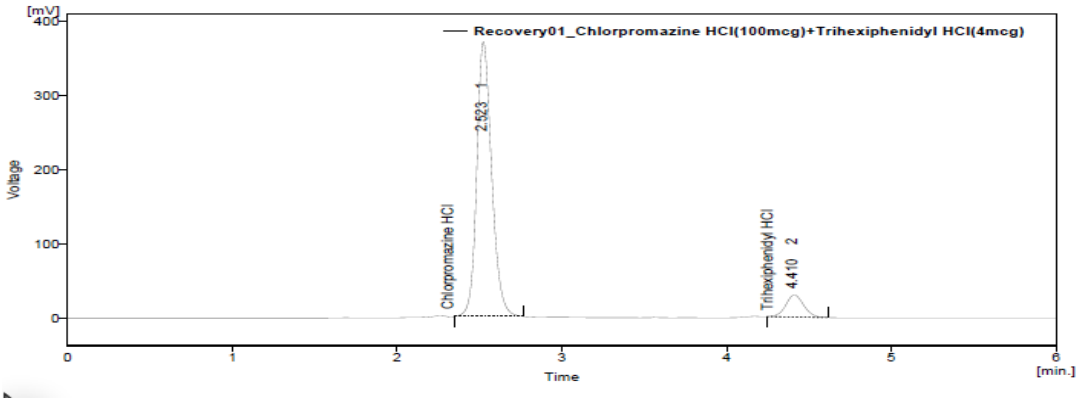


Fig. 7: Chromatogram of 80% recovery (injection 1)

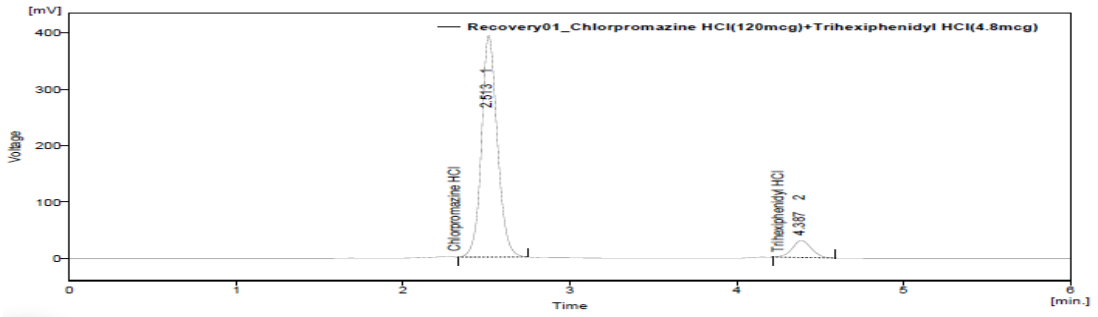


Fig. 8: Chromatogram of 100% recovery (injection 1):

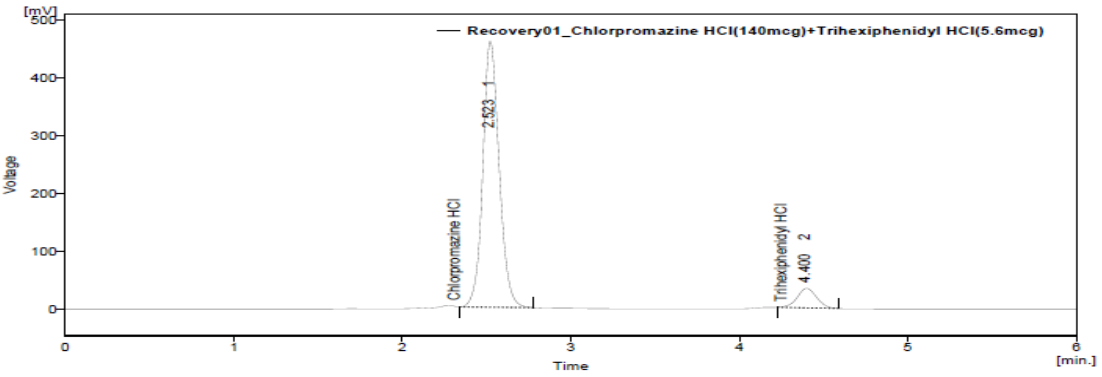


Fig. 9: Chromatogram of 120% recovery (injection 1)

Table 7: Results for 120% recovery (injection 1)

Name	Rt	Area	Th.Plates	Asymmetry	Resolution
Chlorpromazine HCl	2.510	2194.643	3735	1.375	10.408
Trihexiphenidyl HCl	4.397	210.051	7868	1.125	

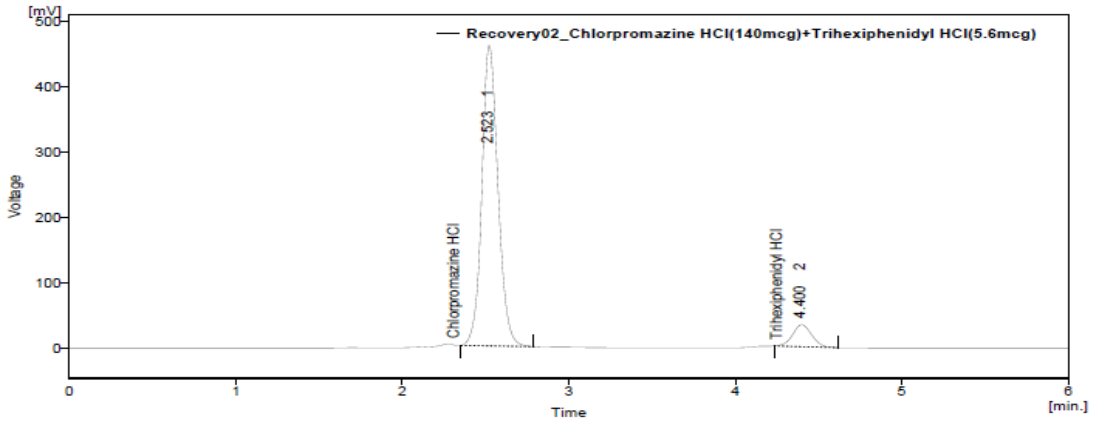


Fig. 10: Chromatogram of 120% recovery (injection 2)

Table 8: Recovery results for Trihexiphenidyl HCl

Recovery level	Accuracy Trixehiphenidyl HCl					Average %Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	%Recovery	
4.0%	1	214.953	211.685	4.00	100.02	99.09%
	1	210.051				
	1	210.051				
4.8%	2	240.294	241.728	4.72	98.34	

	2	242.445				
	2	242.445				
5.6%	3	279.916	272.801	5.54	98.92	
	3	269.243				
	3	269.243				

Precision

Method precision

Prepared sample preparations of Trihexiphenidyl HCl and Chlorpromazine HCl as per test method and injected 6 times in to the column.

Table 9: Result for precision injection 1

Name	Rt	Area	Th.Plates	Asymmetry	Resolution
Chlorpromazine HCl	2.510	2211.866	3735	1.375	10.408
Trihexiphenidyl HCl	4.397	208.286	7868	1.161	

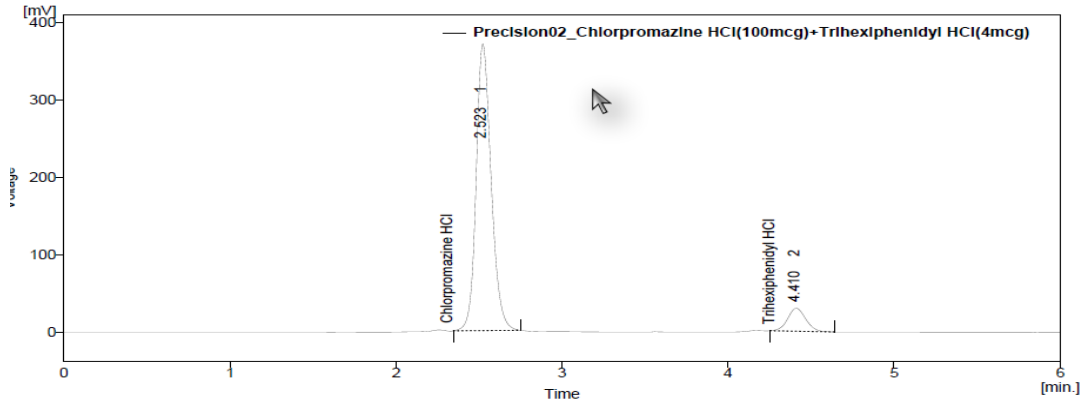


Fig. 11: Chromatogram of precision injection 2

Table 10: Result for precision injection 2

Name	Rt	Area	Th.Plates	Asymmetry	Resolution
Chlorpromazine HCl	2.523	2325.117	3775	1.375	
Trihexiphenidyl HCl	4.410	204.128	7482	1.161	10.248

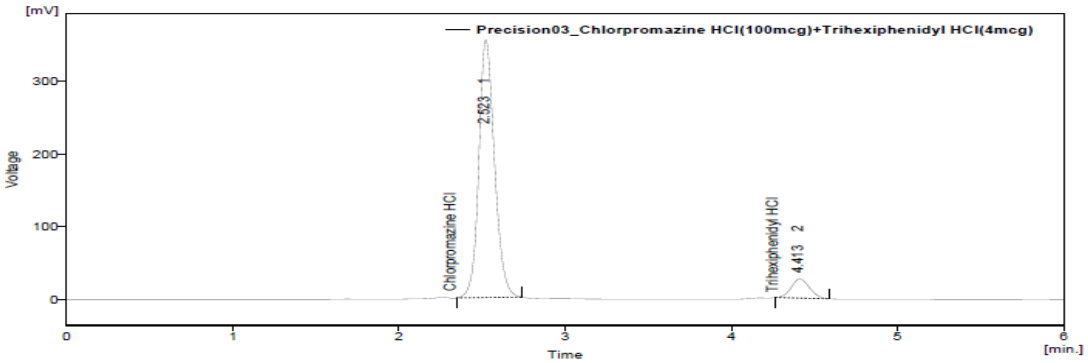


Fig. 47: Fig. 12: Chromatogram of precision injection 3

Table 11: Result for precision injection

Name	Rt	Area	Th.Plates	Asymmetry	Resolution
Chlorpromazine HCl	2.523	2321.138	3304	1.269	
Trihexiphenidyl HCl	4.413	202.102	7094	1.156	9.813

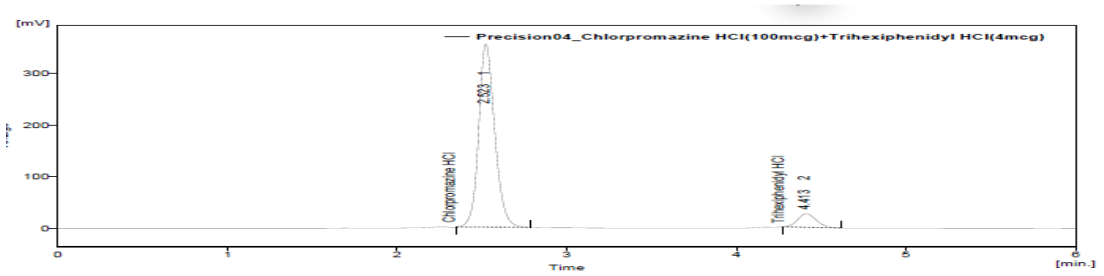


Fig. 13: Chromatogram of precision injection 4

Table 13: Result for precision injection 4

Name	Rt	Area	Th.Plates	Asymmetry	Resolution
Chlorpromazine HCl	2.523	2333.196	3304	1.308	
Trihexiphenidyl HCl	4.413	200.853	7094	1.194	9.813

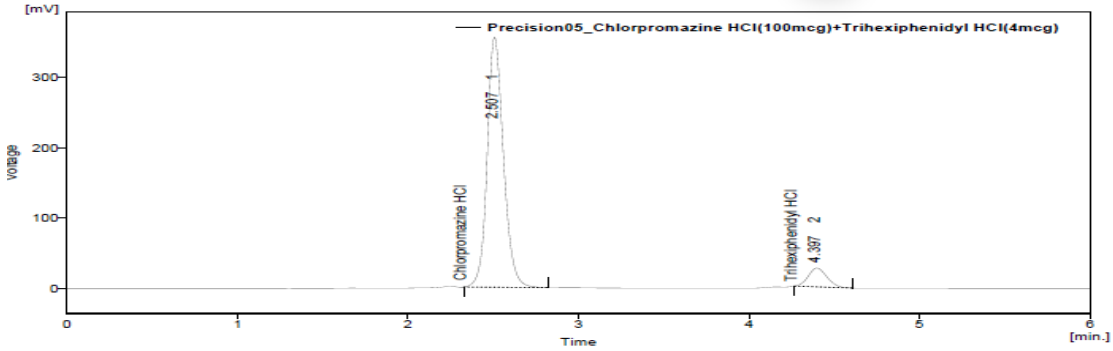


Fig. 14: Chromatogram of precision injection 5

Table 14: Results for Method precision of Chlorpromazine HCl and Trihexiphenidyl HCl

Chlorpromazine HCl		
S. No.	**Rt**	**Area**
1	2.150	2311.866
2	2.523	2325.117
3	2.523	2321.138
4	2.523	2333.196
5	2.507	2350.119
6	2.497	2341. 355
Avg	2.5138	2330.465
SD	0.0109	13.956
%RSD	0.43	0.60

Trihexiphenidyl HCl		
S. No.	**Rt**	**Area**
1	4.397	208.286
2	4.410	204.128
3	4.413	202.102
4	4.413	200.853
5	4.397	202.888
6	4.390	208.551
Avg	4.403	204.468
SD	0.010	3.241
%RSD	0.22	1.59

Robustness

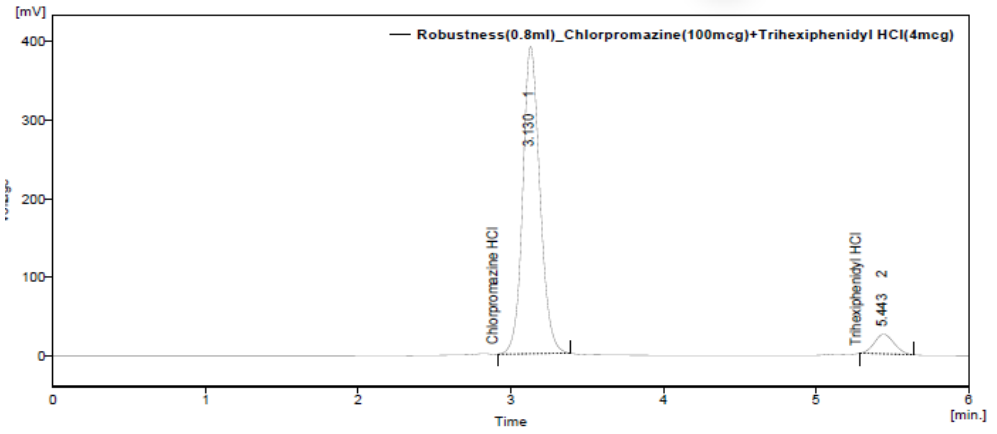


Fig. 15: Chromatogram of Chlorpromazine HCl and Trihexiphenidyl HCl Robustness (0.8 ml)

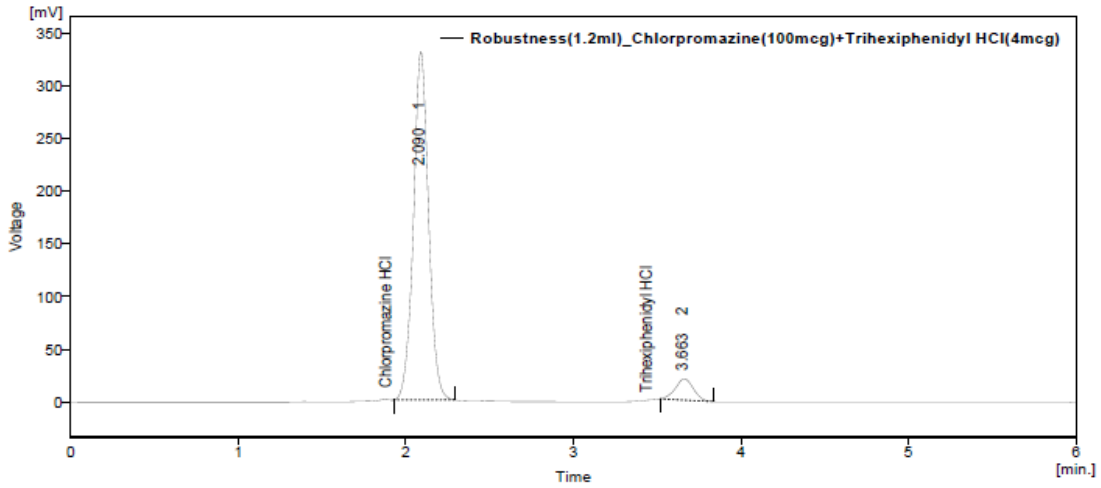


Fig. 16: Chromatogram of Chlorpromazine HCl and Trihexiphenidyl HCl for Robustness (1.2 ml)

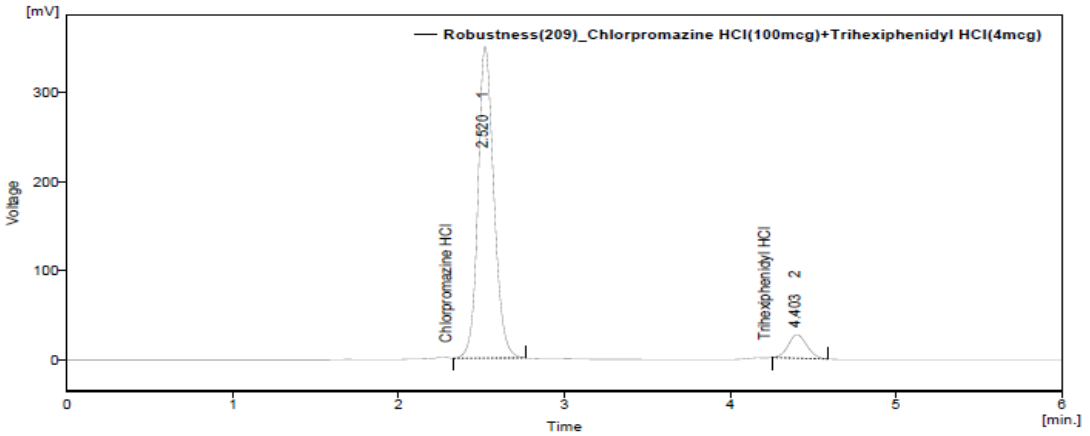


Fig. 17: Chromatogram of Chlorpromazine HCl and Trihexiphenidyl HCl for Robustness (209nm)

Table 15: Results of Robustness study

Parameter	Chlorpromazine HCl		Trihexiphenidyl HCl	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor

Flow Rate				
0.8 ml/min	3.130	1.058	5.443	1.067
1.0 ml/min	3.130	1.058	5.443	1.067
1.2 ml/min	2.090	1.036	3.663	1.043
Wavelength				
209nm	2.520	1.400	4.403	1.150
211nm	2.520	1.308	4.403	1.150
213nm	2.517	1.308	4.387	1.121

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

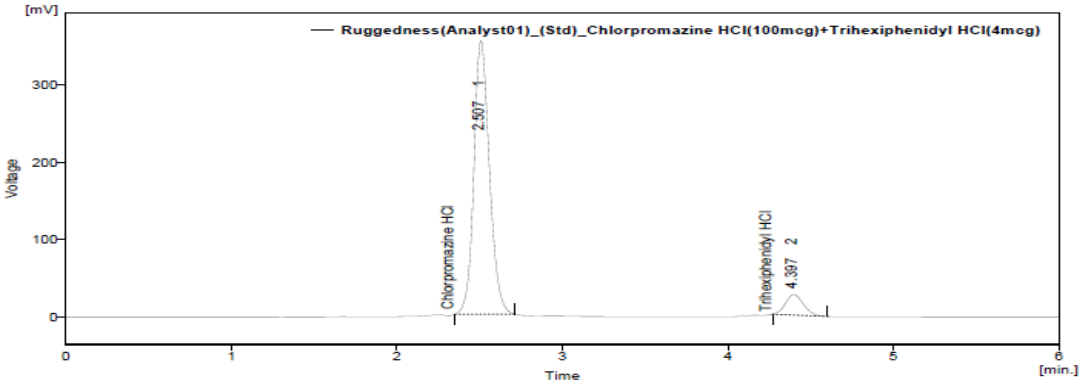


Fig. 18: Chromatogram of Analyst 01 standard preparation

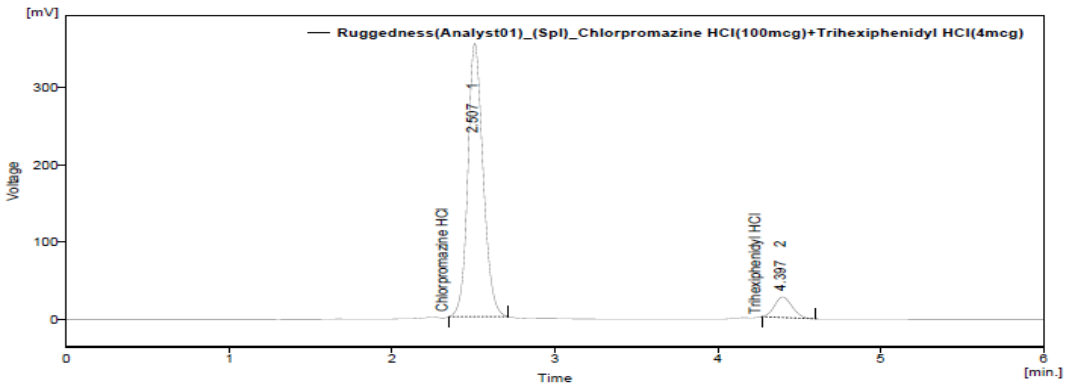


Fig. 19: Chromatogram of Analyst 01 sample preparation

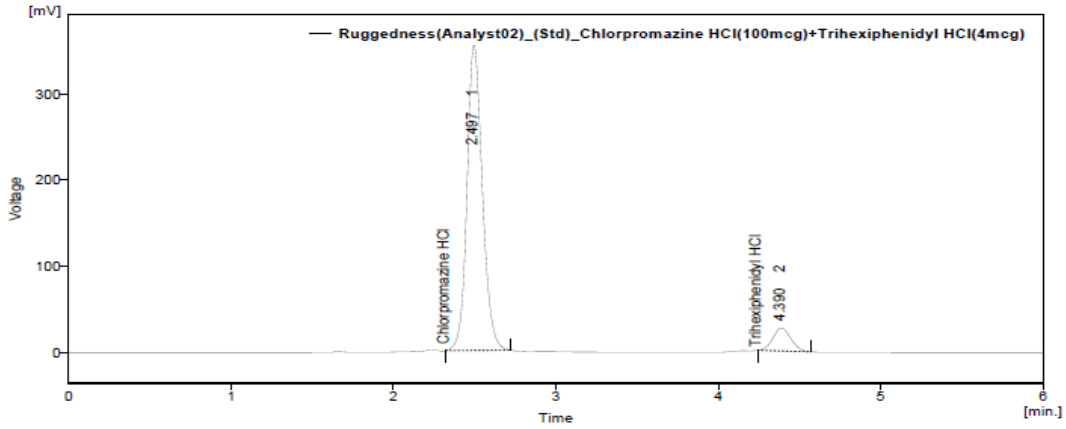


Fig. 20: Chromatogram of Analyst 02 standard preparation

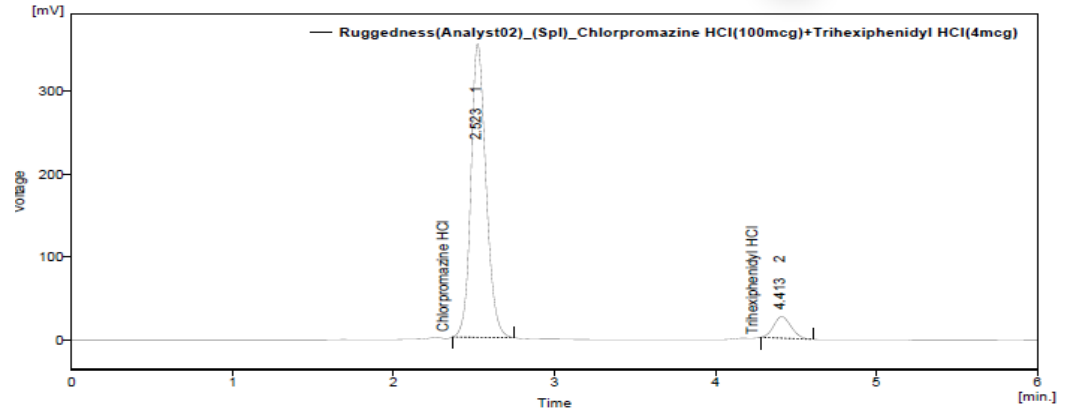


Fig. 21: Chromatogram of Analyst 02 sample preparation

Table 16: Results for Ruggedness

Chlorpromazine HCl	**%Assay**	**Trihexiphenidyl HCl**	**%Assay**
Analyst 01	99.30	**Analyst 01**	100.01
Analyst 02	100.01	**Analyst 02**	99.10
%RSD	0.54%	**%RSD**	0.65%

IV. CONCLUSION:

A simple, rapid, specific, precise and accurate RP-HPLC method was developed for simultaneous estimation of chlorpromazine HCl and Trihexiphenidyl HCl in a pharmaceutical dosage form. The developed method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability.

Parameters of validation prove the precision and selectivity of the method and it's applicability for the Assay of chlorpromazine HCl and Trihexiphenidyl HCl. The assay of chlorpromazine HCl and Trihexiphenidyl HCl was unaffected by the presence of its impurities.

The proposed method was found to be simple, rapid, specific, precise and accurate for simultaneous estimation of chlorpromazine HCl and Trihexiphenidyl HCl. Hence the developed method was suitable for quality control of raw materials and formulations.

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