STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF CLOMIPRAMINE HYDROCHLORIDE CAPSULES BY RP-HPLC

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Abstract: A simple, precise, and accurate RP-HPLC method has been developed and validated for the quantitative analysis of clomipramine hydrochloride in capsule dosage form. An isocratic separation was achieved using by Inertsil ODS 3V (250X4.6 mm); 5µm particle size column with a flow rate of 1.2ml/min and PDA detector at 254nm. The mobile phase consisted of pH 3.2 buffer solution and Acetonitrile (50:50% v/v). The Diluent consisted of methanol 100%v/v. It is validated for specificity, linearity, precision, accuracy and robustness. The specificity of the method was determined by comparing interference from the placebo and by stress testing the drug product (forced degradation). The method was linear over the concentration range 4–200 µg/ml (r^2 = 0.999). The accuracy of the method was between 98.9–99.5%. The method was found to be Robust and suitable for the quantitative analysis of clomipramine hydrochloride in capsule dosage form. Degradation products resulting from the stress studies did not interfere with the detection of clomipramine hydrochloride peak in chromatogram, demonstrating the stability-indicating power of method.

IndexTerms **- Clomipramine hydrochloride, Stability-indicating, RP-HPLC.**

INTRODUCTION:

Clomipramine hydrochloride capsules are antiobessional drug which belongs to the class of pharmacologic agents known as tricyclic antidepressant (dibenzazepine). Clomipramine is a strong, but it is not completely selective serotonin reuptake inhibitor, its main active metabolite is a desmethyclomipramine which acts as an inhibitor of noradrenaline reuptake. It also shows α-receptor blockage and β-receptor down-regulation. The IUPAC name of clomipramine hydrochloride is 3-(3-ckloro-10, 11-dihydro-5H-dibenzo [b, f] azepin-5-yl)-N,N-dimethylpropan-1-amine hydochloride with molecular formula of $C_{19}H_{24}ClN_2$. Hcl and its molecular weight is 351.315 g/mol. Anafranil, clomicalm are the brand names available in the market.

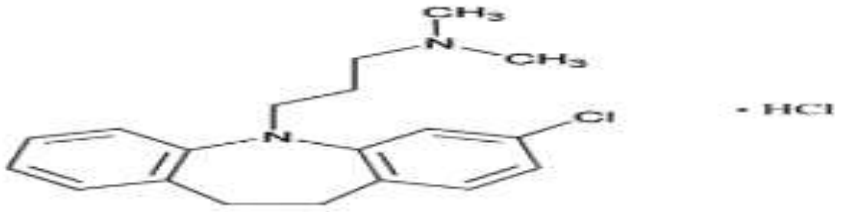


fig .no 1molecular structure of clomipramine hydrochloride

EXPERIMENTAL:

S.No	CHEMICALS	GRADE
1	Water	Purified water
2	Sodium 1-heptanesulfonate	IC grade
3	Phosphoric acid	AR grade
4	Acetonitrile	HPLC grade
5	Methanol	HPLC grade
6	Triethylamine	AR grade

Chemicals:

Equipment:

HPLC: Agilent system with VWD model: 1200 series/2690 , PDA detector and chromatographic software Empower-2.0 was used.

Chromatographic conditions:

Chromatographic separation was carried out in isocratic mode at room temperature using a Inertsil ODS 3V (250X4.6 mm); 5µm particle size column. The mobile phase consisted of pH 3.2 buffer solution and Acetonitrile (50:50% v/v).at a flow rate of 1.2 ml/min was used as a mobile phase. The injection volume was10µl and eluent was monitored at 254nm using PDA detector. The run time was 10min and each of the studied component was quantified by using total peak area.

Buffer preparation:

Weigh and transfer 5.5g sodium 1-heotanesulfonate in 100ml of volumetric flask, add 50ml of water, sonicate to dissolve and make up to the volume with GAA, mix well.

Mobile phase A:

Transfer 20ml of buffer solution and 2ml triethylamine to a 500ml volumetric flask, make up with water and adjust PH to 3.2 with orthophosphoric acid and mix well. Filter the solution through 0.45µ nylon membranes filter.

Mobile phase B: Acetonitrile

Mobile phase:

Mix 50:50 Mobile phase A and Mobile phase B and sonicate to degas for 10 minutes (**Note:** Mobile phase is stable for 3days on bench top).

Selection of wavelength

The absorption spectra of clomipramine hydrochloride solution was scanned over the range 200-400nm by using photodiode spectrophotometer and the spectra was recorded. The maximum absorbance was at wavelength 254nm. So an Optimized wavelength selected was 254nm.

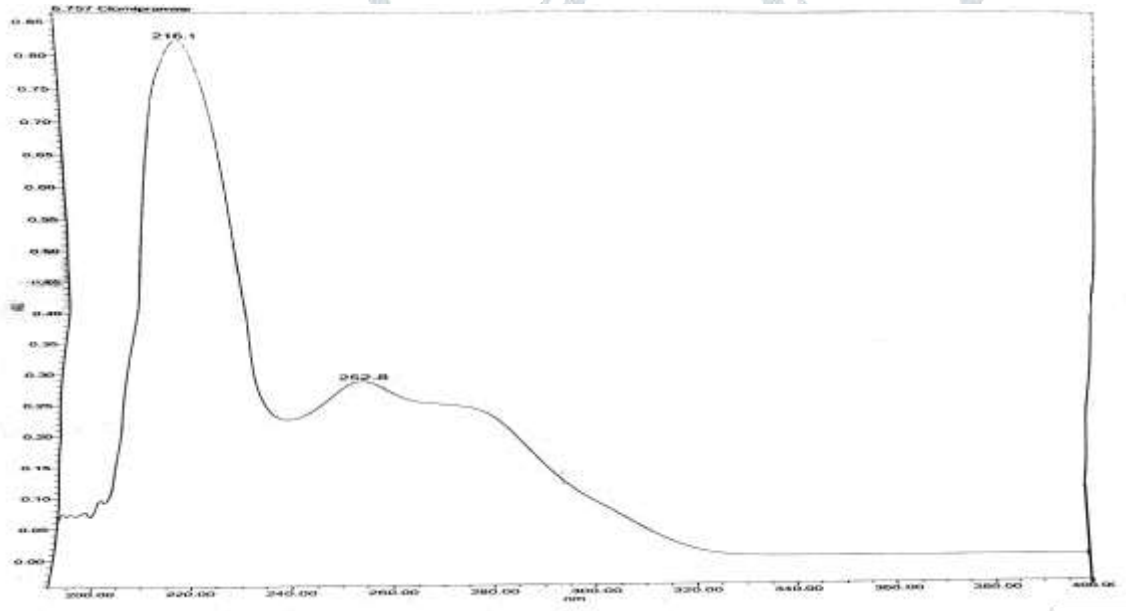


fig.no.2 λ_{max} spectrum

Diluent: Methanol

Standard preparation:

Weigh and transfer about 32 mg of Clomipramine hydrochloride working or reference standard in to a 100 mL volumetric flask, add about 70mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix well.

Note: Standard solution is stable for 1day on bench top and 3days in refrigerator.

Check standard preparation:

Prepare check standard similar to the standard preparation.

Preparation of sample solution:

Weigh and drop 8 capsules into 250ml volumetric flask. Add about 25ml of water, sonicate with intermediate shaking for 10min and assure that capsule shell was dispersed into water completely then add 50ml of diluent, sonicate with intermediate shaking for 10min. Then again add 50ml of diluent, sonicate with intermediate shaking for another 10min. keep the flask on bench top to

attain room temperature then dilute up to mark with diluent and mix well. Filter the solution through 0.45µ nylon membranes filter.
Dilute 10ml of the above solution into 25ml of diluent and mix well.

OPTIMIZATION METHOD:

Objective: To develop intact sample preparation method for Clomipramine HCL capsule.

PARAMETERS	CONDITIONS
Column	Inertsil ODS 3V 250X4.6 mm, 5 µm
Flow rate	1.2ml/min
Wavelength	254nm
Column temperature	Ambient
Injection volume	10µl
Run time	10min
Elution mode	Isocratic

table.no. 1 chromatographic parameters of optimized method

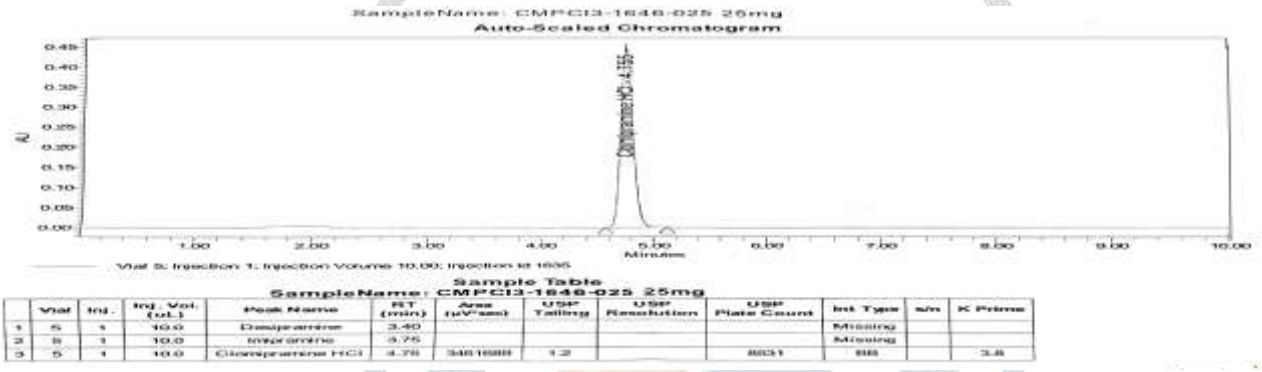


fig.no.3 optimized method chromatogram

Validation Procedure

The analytical method was validated as per ICH Q2(R1) [24] guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), forced degradation and stability.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

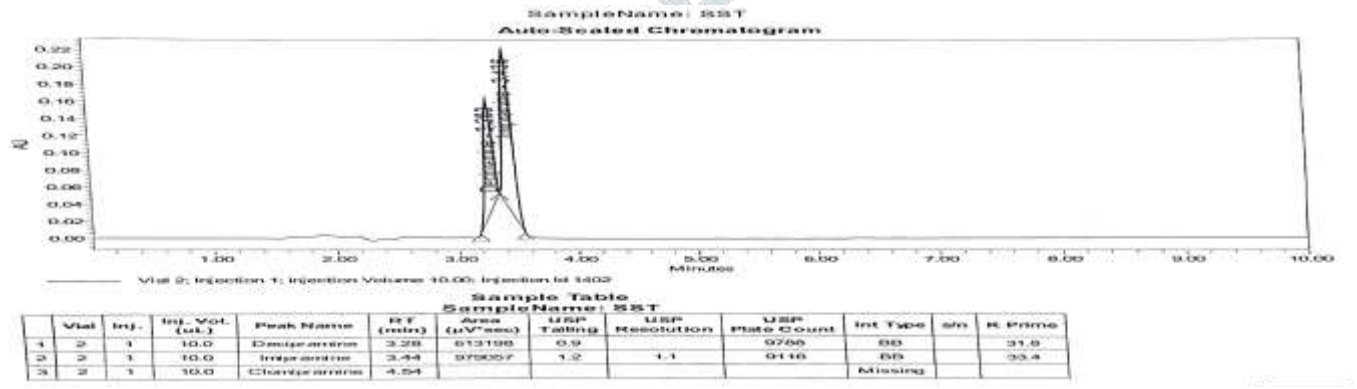


fig.no.4 system suitability sst chromatogram

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excepients), which may be expected to be present in the sample and standard solution. Retention time of clomipramine hydrochloride was 4.565 min. There are no interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. For sample refer fig.no.3

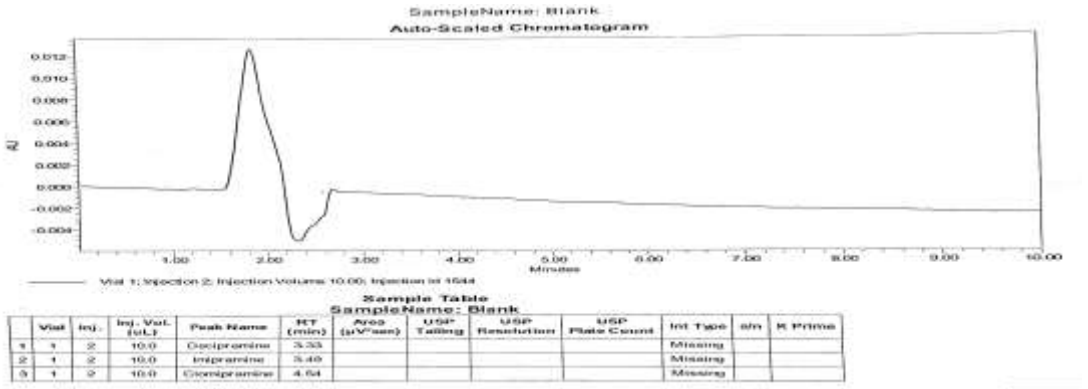


fig.no.5 specificity blank chromatogram

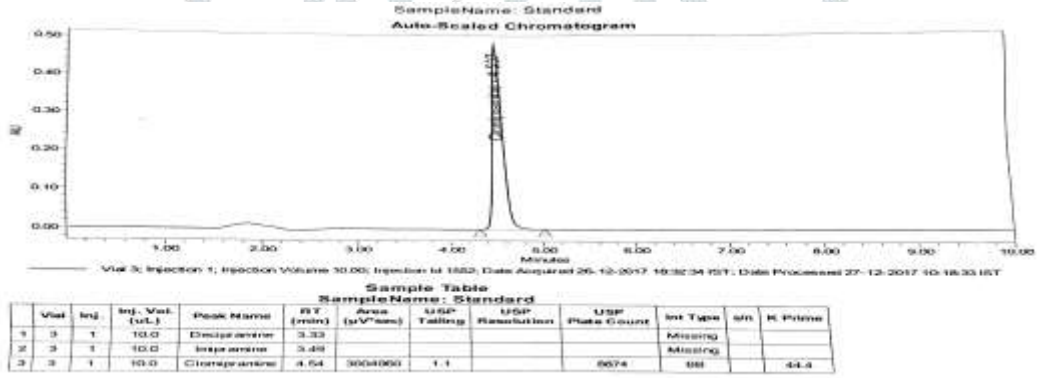


fig.no.6 specificity standard chromatogram

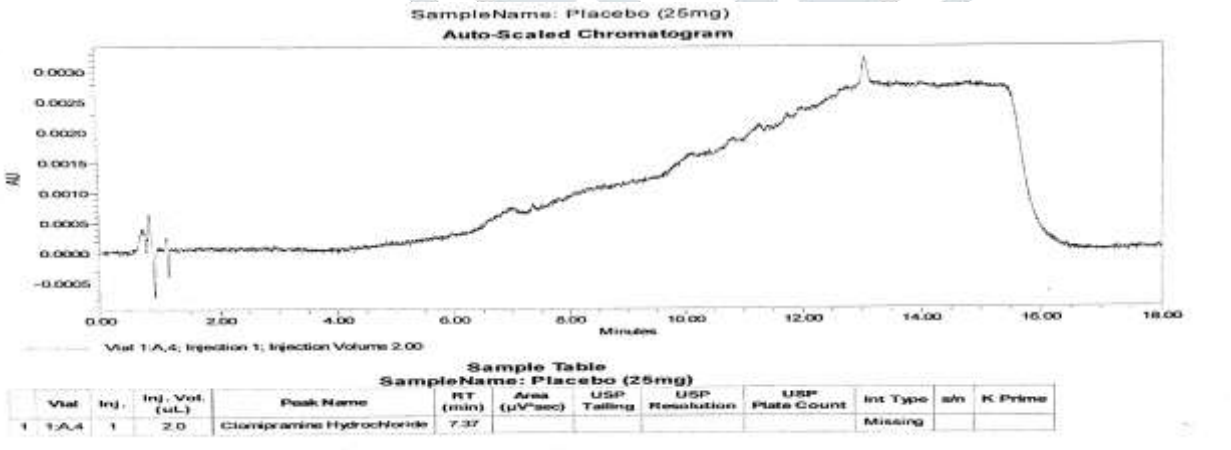
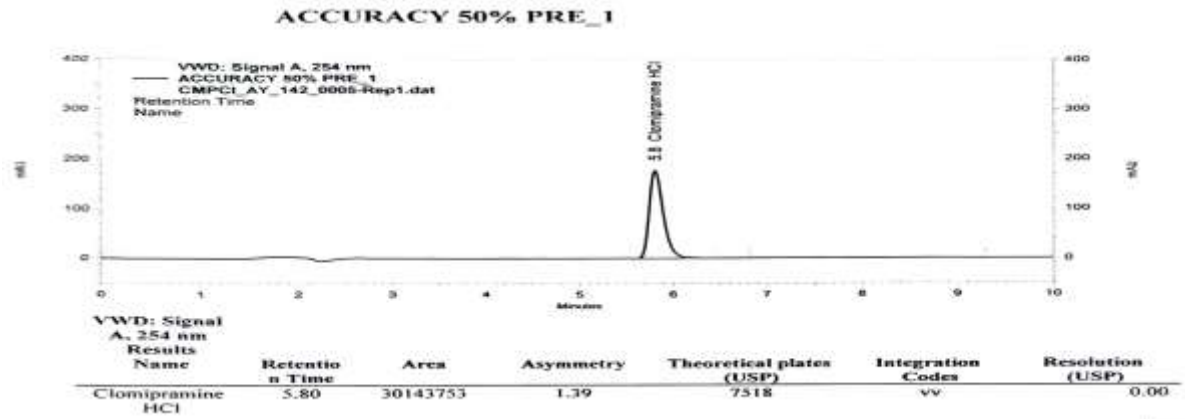


fig.no.7 specificity placebo chromatogram

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. The method was performed by calculating the recovery experiments at three levels (50%, 100% and 150%). The test solution was injected six times for each spike level and assay was performed as per the test method. The recovery results were close to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and

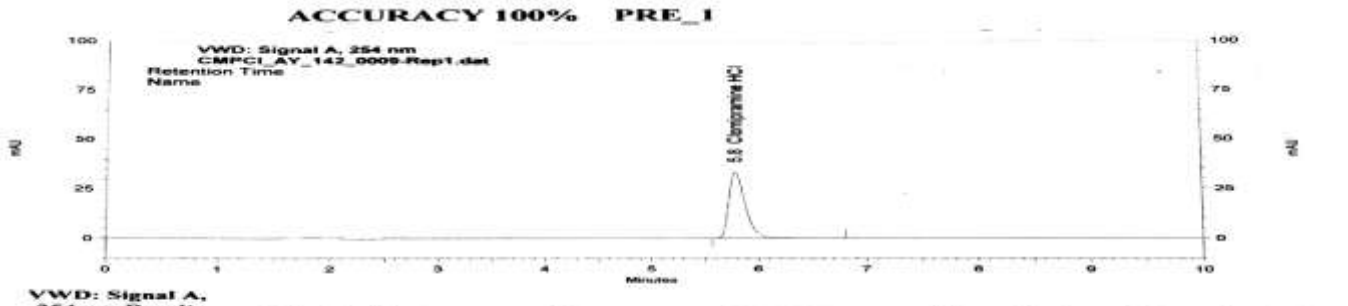
relative standard accuratewithin the desired range. The results are summarized below.



VWD: Signal A, 254 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Integration Codes	Resolution (USP)
Clomipramine HCl	5.80	30143753	1.39	7518	vv	0.00

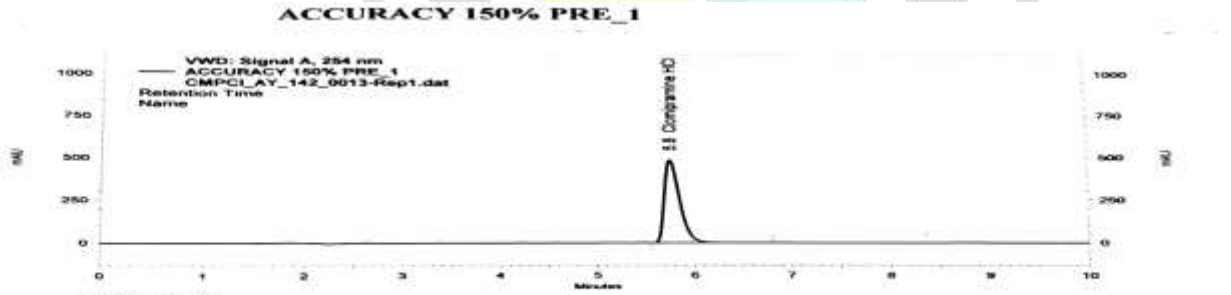
fig.no. 8accuracy 50% chromatogram



VWD: Signal A, 254 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Integration Codes
Clomipramine HCl	5.78	60244538	1.50	6952	vv

fig.no. 9accuracy 100% chromatogram



VWD: Signal A, 254 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Integration Codes	Resolution (USP)
Clomipramine HCl	5.75	91005678	1.56	6386	vv	0.00

Fig.no.10 Accuracy 150% chromatogram

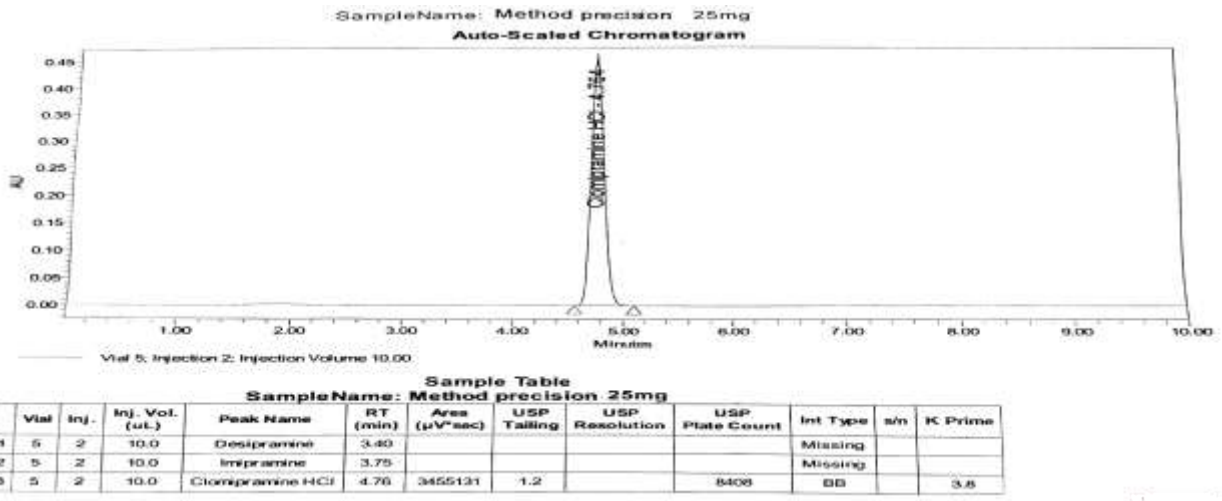
Method Precision:

Precision of an analytical method is the degree of agreement among individual test results.

Sample no	%Assay	
	25mg	**75mg**
1	101.6	97.7
2	101.4	95.9
3	103.5	98.6
4	100.6	95.6
5	100.3	96.4
6	103.9	96.3

Mean	101.9	96.8
%RSD	1.5	1.2

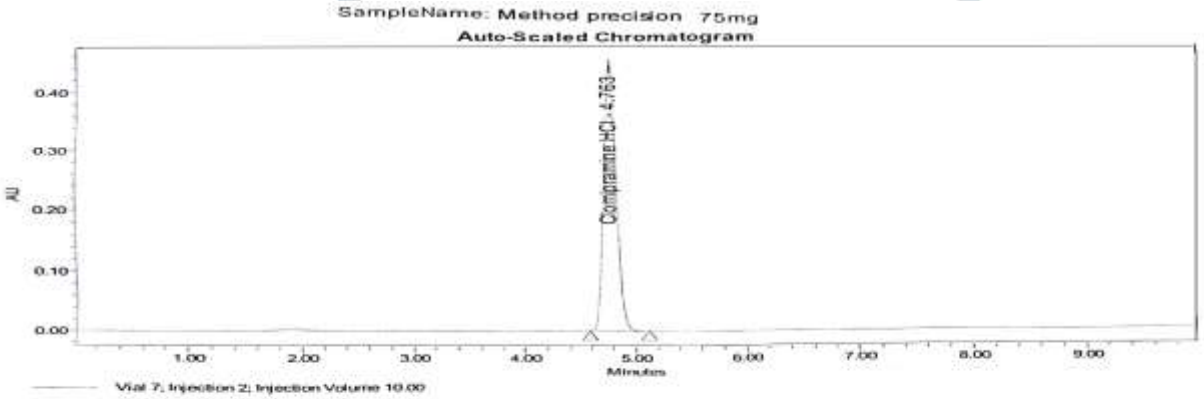
table.no. 2 method precision data



Sample Table
SampleName: Method precision 25mg

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	5	2	10.0	Desipramine	3.40					Missing		
2	5	2	10.0	Imipramine	3.75					Missing		
3	5	2	10.0	Clomipramine HCl	4.76	3455131	1.2		8408	BB		3.8

fig.no 11method precision 25mg chromatogram



Sample Table
SampleName: Method precision75mg

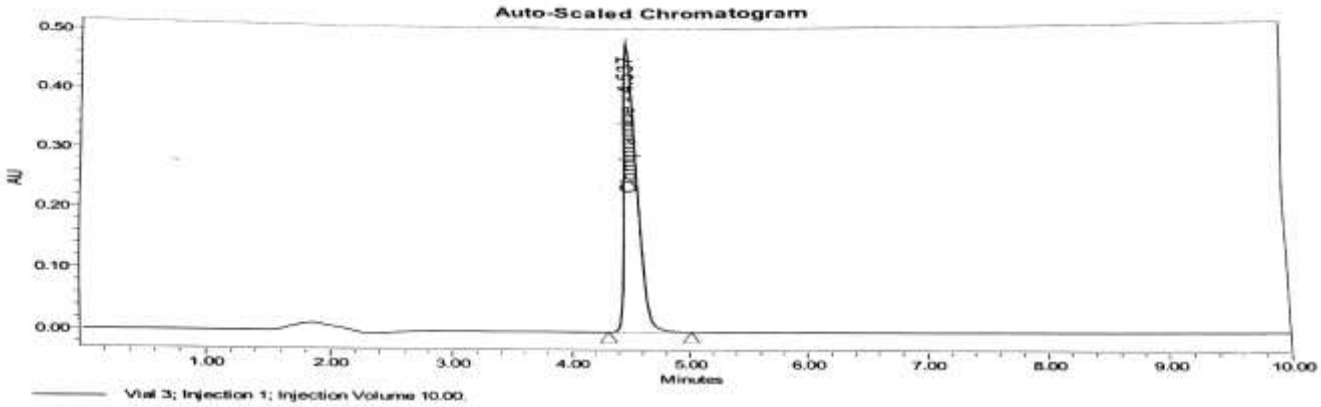
	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	7	2	10.0	Desipramine	3.40					Missing		
2	7	2	10.0	Imipramine	3.75					Missing		
3	7	2	10.0	Clomipramine HCl	4.76	3489855	1.2		8420	BB		3.8

fig.no 12method precision 25mg chromatogram

Intermediate Precision:

Sample no	**%Assay**	
	25mg	**75mg**
1	102.5	100.3
2	101.2	98.8
3	102.0	101.2
4	102.0	99.3
5	102.6	99.6
6	102.5	100.5
Mean	102.1	100.0
%RSD	0.5	0.9

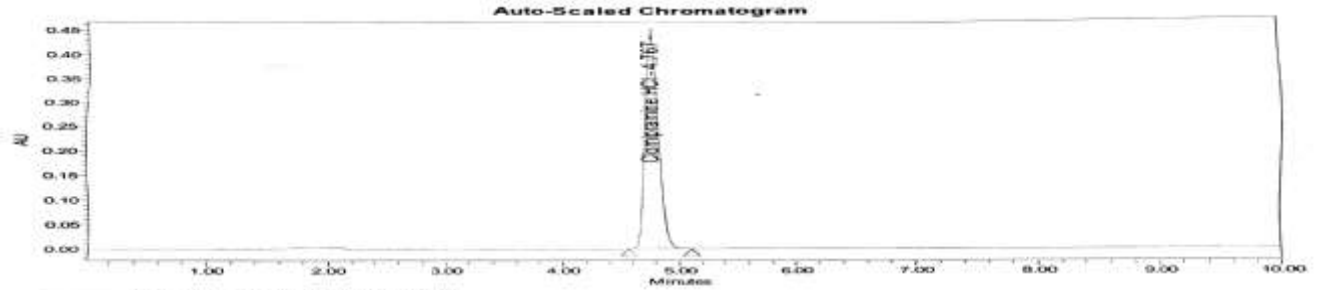
table.no. 3 intermediate precision data



Sample Table
SampleName: CMPCI5

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	3	1	10.0	Desipramine	3.33					Missing		
2	3	1	10.0	Imipramine	3.49					Missing		
3	3	1	10.0	Clomipramine	4.54	3604960	1.1		8874	BB		44.4

fig.no 13intermediate precision 25mg chromatogram



Sample Table
SampleName: CMPCI5

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	6	2	10.0	Desipramine	3.40					Missing		
2	6	2	10.0	Imipramine	3.75					Missing		
3	6	2	10.0	Clomipramine HCl	4.77	3407880	1.2		8438	BB		3.6

fig.no 14intermediate precision 75mg chromatogram

Linearity

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a different range. The six replicates of standard solutions were injected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. To calculate the slope, intercept and correlation coefficient the least squares method was used.

% Linearity level	concentration	Average area (Response)
4	12.0210	129813
50	160.2800	1727704
100	320.5600	3538587
150	480.8400	5301800
200	641.1200	7117339
Slope	11116.9095	
Y-intercept	-27315.1993	
% Y-intercept at 100% level	-0.8	
Correlation coefficient	1.000	
Square of Correlation coefficient	1.000	
Residual sum of squares	1.844275620.5	

table.no. 4 linearity data

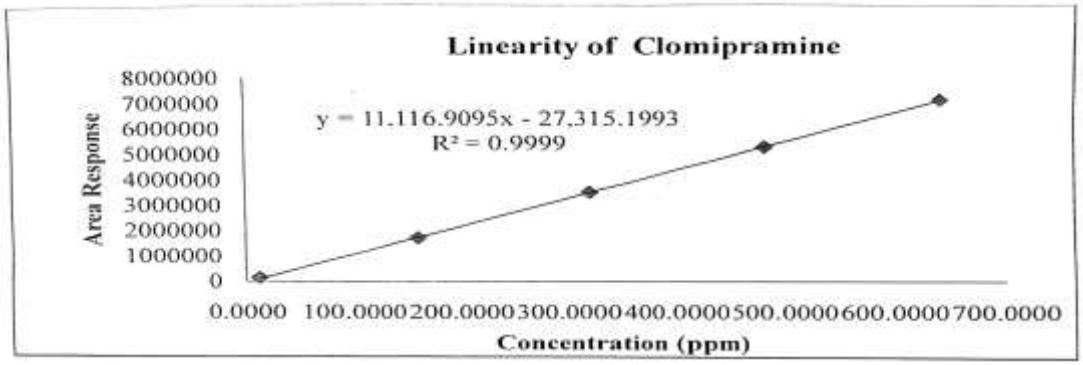


fig.no.15 linearity graph of clomipramine hydrochloride

Robustness

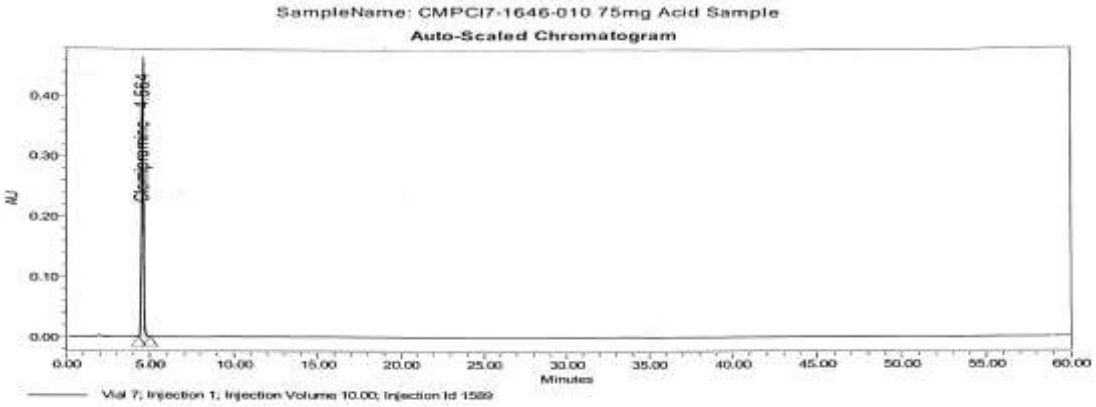
The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness conditions like Flow minus (1.0ml/min), Flow plus (1.4ml/min), temperature minus (20°C) and temperature plus (30°C), wavelength plus (256nm) and wavelength plus (252nm), filter variation like nylon and PVDF were maintained and samples injected in duplicate manner. System suitability parameters was not much affected and all the parameters were passed. %RSD were within the limit.

Stress degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q2(R1). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies was carried out by different types of stress conditions.

S.NO	DEGRADATION	CONDITION	DRUG PRODUCT			
			%Assay	%Net degradation	Peak angle	Peak threshold
1	Acid	10% 1N HCl, 2hrs heating at 60 °C	98.6	3.2	0.109	0.338
2	Base	10% 1N NaOH, 2hrs heating at 60 °C	101.8	3.2	0.086	0.319
3	Peroxide	10% of 30% H_2O_2, 2hrs bench top	98.3	0.3	0.094	0.314
4	Thermal	At 100°C for 5days	97.2	1.4	0.074	0.296
5	Humidity	90%RH 25°C for 5days	95.5	3.1	0.01	0.300

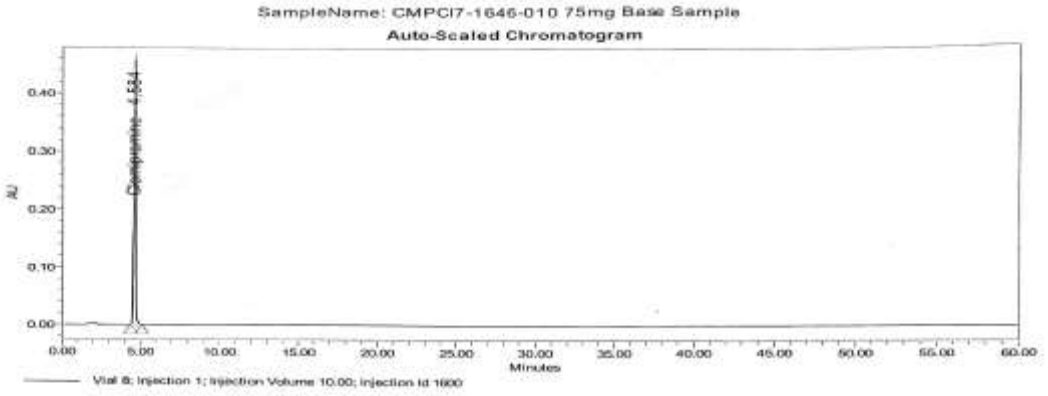
table.no. 5 forced degradation data



Sample Table
SampleName: CMPCI7-1646-010 75mg Acid Sample

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	7	1	10.0	Desipramine	3.33					Missing		
2	7	1	10.0	Imipramine	3.49					Missing		
3	7	1	10.0	Clomipramine	4.56	3388153	1.1		8801	BB		44.6

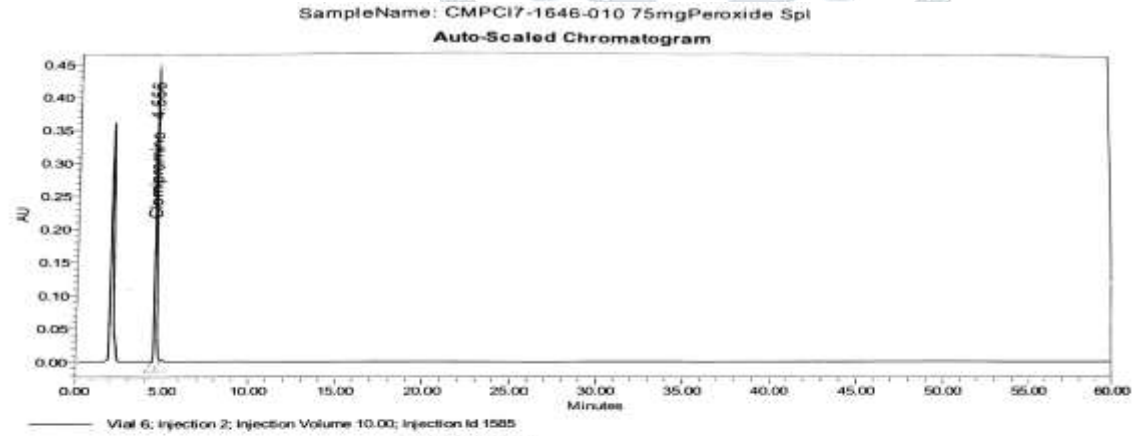
Fig.no.16 Acid sample chromatogram



Sample Table
SampleName: CMPCI7-1646-010 75mg Base Sample

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	8	1	10.0	Desipramine	3.33					Missing		
2	8	1	10.0	Imipramine	3.49					Missing		
3	8	1	10.0	Clomipramine	4.56	3380137	1.1		8861	BB		44.6

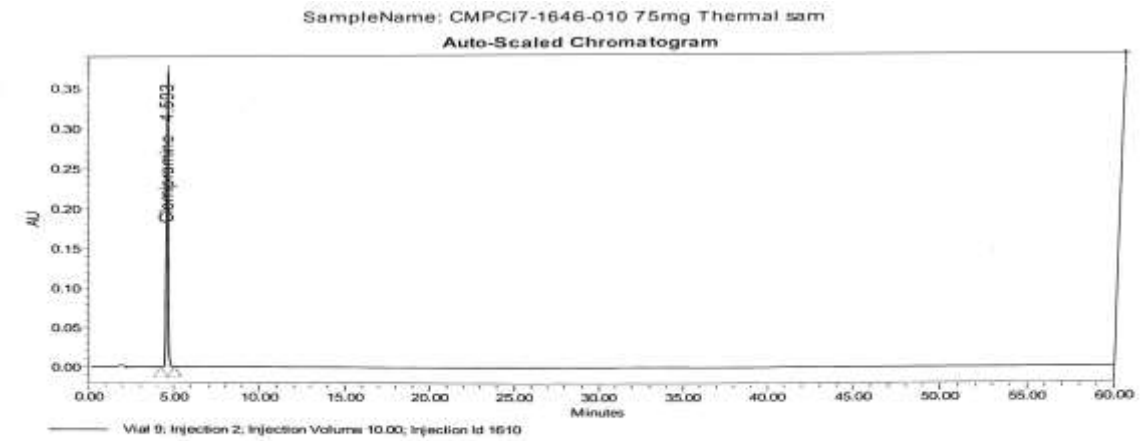
Fig.no.17 Base sample chromatogram



Sample Table
SampleName: CMPCI7-1646-010 75mgPeroxide Spl

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	6	2	10.0	Desipramine	3.33					Missing		
2	6	2	10.0	Imipramine	3.49					Missing		
3	6	2	10.0	Clomipramine	4.56	3276163	1.1		8892	BB		44.6

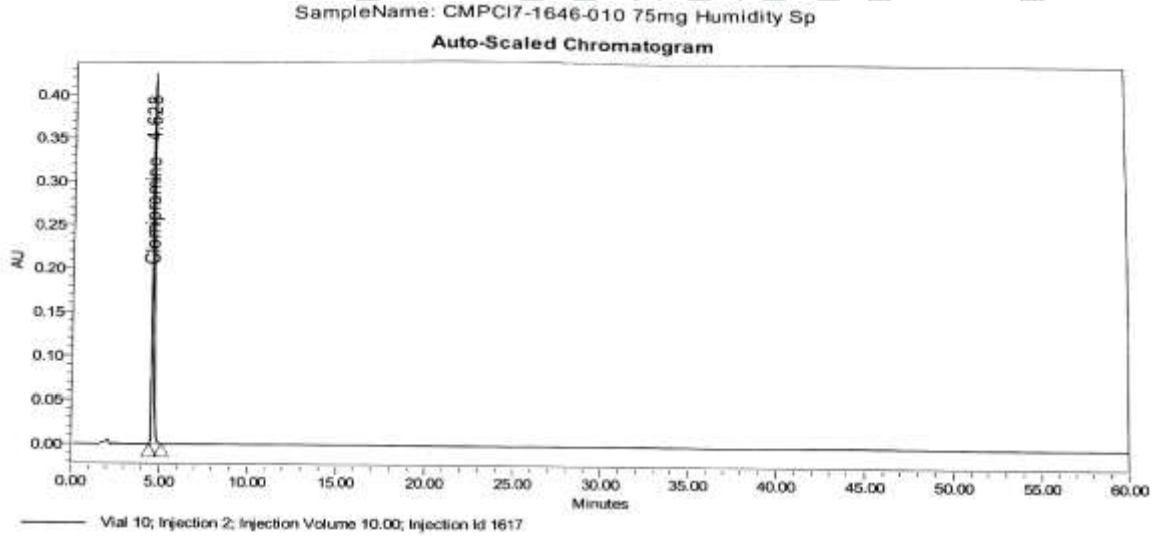
Fig.no.18 Peroxide sample chromatogram



Sample Table
SampleName: CMPCI7-1646-010 75mg Thermal sam

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	9	2	10.0	Desipramine	3.33					Missing		
2	9	2	10.0	Imipramine	3.49					Missing		
3	9	2	10.0	Clomipramine	4.59	2709026	1.1		8966	BB		44.9

Fig.no19 Thermal sample chromatogram



Sample Table
SampleName: CMPCI7-1646-010 75mg Humidity Sp

	Vial	Inj.	Inj. Vol. (uL)	Peak Name	RT (min)	Area (µV*sec)	USP Tailing	USP Resolution	USP Plate Count	Int Type	s/n	K Prime
1	10	2	10.0	Desipramine	3.33					Missing		
2	10	2	10.0	Imipramine	3.49					Missing		
3	10	2	10.0	Clomipramine	4.63	3133466	1.1		8965	BB		45.3

Fig.no20 Humidity sample chromatogram

SUMMARY:

Parameters	Results		Limits
Linearity Range (µg/ml)	4-200 (µg/ml)		
Regression coefficient	0.9999		
Slope(m)	11116		
Intercept(c)	-27315		
Regression equation(Y=mx+c)	11,116.905x-27,315.1993		R< 1
Specificity	Specific		No interference of any peak
Method precision	25mg	75mg	NMT 2.0%

%RSD		1.5	1.2		
Intermediate precision %RSD		25mg	75mg		NMT 2.0%
		0.5	0.9		
Accuracy % recovery		50%	100%	150%	98-102%
		98.9	98.5	99.5	
Robustness	FM	0.0			
	FP	0.1			
	TM	0.0			
	TP	0.1			
	WM	0.1			
	WP	0.1			%RSD NMT 2.0

Table.no. 6 Summary table

Conclusion:

The method was found to be specific, precise, robust, linear in the range of 50% of assay initial stock to 200% of assay sample concentration and accurate in the range of 50% of assay initial stock concentration to 150% of assay test concentration of clomipramine hydrochloride capsules 25mg, 50mg and 75mg by HPLC.

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