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ANALYTICAL METHOD OF DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IMIPRAMINE AND CHLORDIAZEPOXIDE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 251 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Phenomenex Gemini C18 (4.6×250mm) 5µm particle size because it was giving good peak. 35º C temperatures was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.Mobile phase is Methanol and Phosphate buffer (pH-3.8) (40:60% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 6 min because analyze gave peak around 2.121, 3.643 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range 10-30mg/ml of Imipramine and 30-90mg/ml of Chlordiazepoxide of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

KEYWORDS: Imipramine, Chlordiazepoxide, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Imipramine, the prototypical tricyclic antidepressant (TCA), is a dibenzazepine-derivative TCA. TCAs are structurally similar to phenothiazines. They contain a tricyclic ring system with an alkyl amine substituent on the central ring. In non-depressed individuals, imipramine does not affect mood or arousal, but may cause sedation. In depressed individuals, imipramine exerts a positive effect on mood. TCAs are potent inhibitors of serotonin and norepinephrine reuptake. Tertiary amine TCAs, such as imipramine and amitriptyline, are more potent inhibitors of serotonin reuptake than secondary amine TCAs, such as nortriptyline and desipramine. TCAs also block histamine H1 receptors, α1-adrenergic receptors and muscarinic receptors, which accounts for their sedative, hypotensive and anticholinergic effects (e.g. blurred vision, dry mouth, constipation, urinary retention), respectively 5. Imipramine has less sedative and anticholinergic effects than the tertiary amine TCAs, amitriptyline and clomipramine. Imipramine may be used to treat depression and nocturnal enuresis in children Label. Unlabeled indications include chronic and neuropathic pain (including diabetic neuropathy), panic disorder, attention-deficit/hyperactivity disorder (ADHD), and post-traumatic stress disorder (PTSD).[1-5] IUPAC name is pentadeca-1(15),3,5,7,11,13-hexaen-2-yl} propyl) dimethylamine. Molecular Formula is $C_{19}H_{24}N_2$. Molecular weight is 280.4.

Chlordiazepoxide is a benzodiazepine used to treat the withdrawal symptoms of acute alcoholism, to treat preoperative anxiety, and to treat anxiety over a short-term period. Chlordiazepoxide binds to stereospecific benzodiazepine (BZD) binding sites on GABA (A) receptor complexes at several sites within the central nervous system, including the limbic system and reticular formation. This results in an increased binding of the inhibitory neurotransmitter GABA to the GABA(A) receptor. BZDs, therefore, enhance GABA-mediated chloride influx through GABA receptor channels, causing membrane hyperpolarization. The net neuro-inhibitory effects result in the observed sedative, hypnotic, anxiolytic, and muscle relaxant properties.[6-8] IUPAC name is 7-chloro-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepin-4-ium-4-olate. Molecular Formula is $C_{16}H_{14}ClN_3O$. Molecular weight is 299.7.

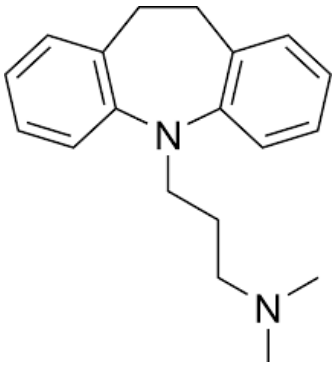

Figure 1: Structure of imipramine.

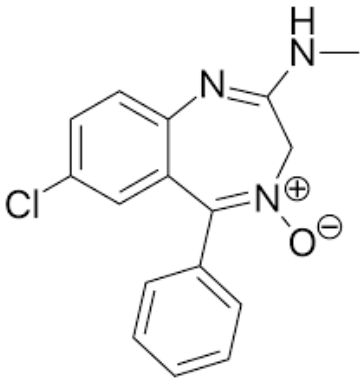

Figure 2: Structure of chlordiazepoxide.

The literature survey revealed that There are really few approaches reported in the literary works for evaluation of Imipramine and Chlordiazepoxide alone or in combination with various other drugs in the pure form as well as drugs formulations by RP-HPLC. [9-15] In view of the demand for an appropriate, cost-effective RP-HPLC method for routine analysis of Imipramine and Chlordiazepoxide synchronized evaluation of in pharmaceutical dose type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Imipramine and Chlordiazepoxide. The recommended approach will be validated according to ICH guidelines. The objective of the recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Imipramine and Chlordiazepoxide in pharmaceutical dose kind by utilizing RP-HPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS

Chemicals and Reagents: Imipramine and Chlordiazepoxide were Purchased from Sura Lab. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 251 nm with column Phenomenex Gemini C18 (4.6×250mm) 5µm particle size, dimensions at 25^0C temperature. The optimized mobile phase consists of Methanol and Phosphate buffer (pH-3.8) (40:60% v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions

Preparation of mobile phase:

Accurately measured 400ml of Methanol (40%) of and 600ml of HPLC Water (60%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent preparation:

The Mobile phase was used as the diluent.

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Imipramine and Chlordiazepoxide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Imipramine and 0.6ml of Chlordiazepoxide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of sample solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Imipramine and Chlordiazepoxide sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.2ml of Imipramine and 0.6ml of Chlordiazepoxide Sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions

RESULTS AND DISCUSSION

Method: The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient

temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Phenomenex Gemini C18 (4.6×250mm) 5µm particle size, the mobile phase of composition Methanol and Phosphate buffer (pH-3.8) (40:60% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1 and 2.

Table 1: Results of system suitability for imipramine.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Imipramine	2.152	513652	78542	4698	1.2
2	Imipramine	2.157	513524	78654	4785	1.2
3	Imipramine	2.141	513425	78541	4682	1.2
4	Imipramine	2.133	513647	78454	4854	1.2
5	Imipramine	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std. Dev.			572.2004			
% RSD			0.111363			

Table 2: Results of system suitability for chlordiazepoxide.

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Chlordiazepoxide	3.674	1635285	265421	7985	1.1	10.1
2	Chlordiazepoxide	3.631	1635241	265484	7898	1.1	10.1
3	Chlordiazepoxide	3.625	1652547	253498	7954	1.1	10.1
4	Chlordiazepoxide	3.692	1658458	265241	7965	1.1	10.1
5	Chlordiazepoxide	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std. Dev.			10865.58				
% RSD			0.659766				

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Imipramine and Chlordiazepoxide in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-3.

Table 3: Assay results for Imipramine and Chlordiazepoxide.

	Label Claim (mg)	% Assay
Imipramine	10	99.7
Chlordiazepoxide	10	99.7

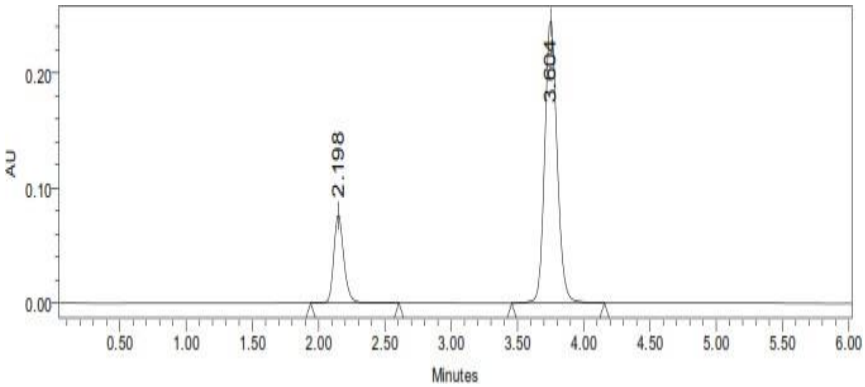

Figure 3: Standard chromatogram.

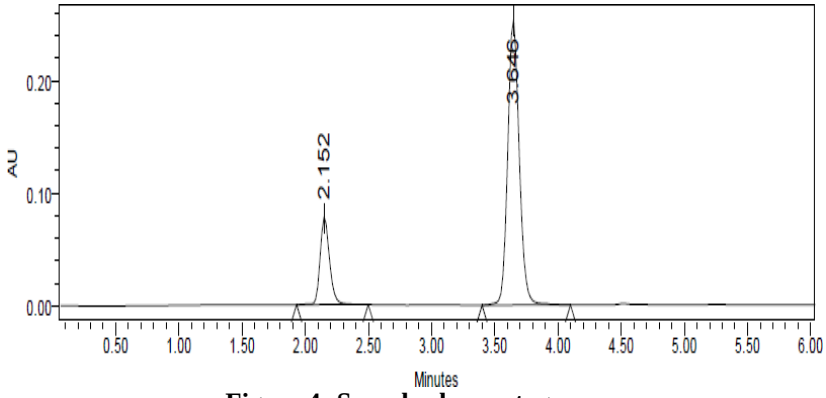

Figure 4: Sample chromatogram.

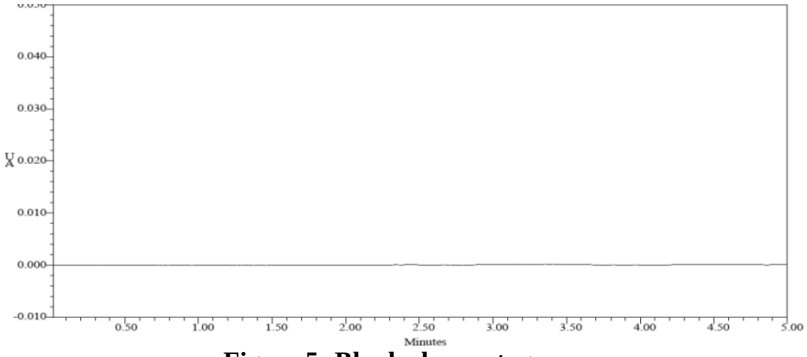

Figure 5: Blank chromatogram.

Validation of analytical method:

Linearity: The linearity study was performed for the concentration of 10 ppm to 30 ppm and 30 ppm to 90 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 4,5.

Table 4: Linearity results of Imipramine.

Concentration	Average peak area
10	245899
15	365687
20	481526
25	589854
30	705882

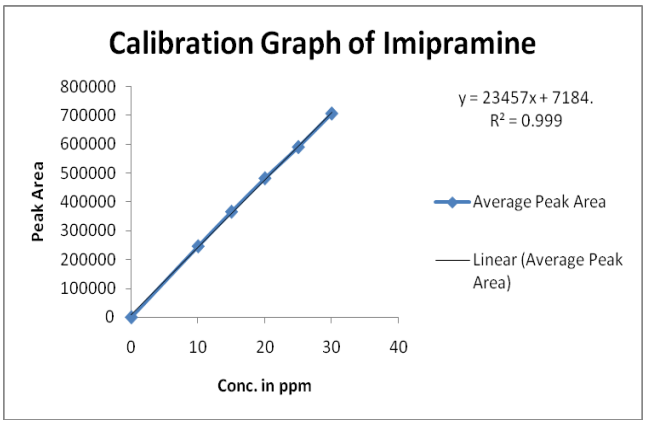

Figure 6: Linearity graph for imipramine.

Table 5: Linearity results of chlordiazepoxide.

Concentration	Average peak area
30	863094
45	1249397
60	1678592
75	2050412
90	2468444

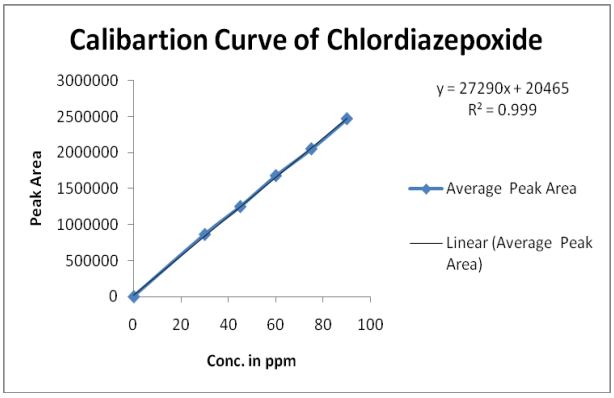

Figure 6: Linearity graph for chlordiazepoxide.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Imipramine and Chlordiazepoxide and calculate the individual recovery and mean recovery values. The results are shown in table 6,7.

Table 6: Showing accuracy results for Imipramine.

%Concentration (at specificationLevel)	Area	AmountAdded (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	
150%	715961	30	30.	100.72%	

Table 7: Showing accuracy results for chlordiazepoxide.

%Concentration (at specificationLevel)	Area	AmountAdded (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

Precision studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 8.

Table 7: Precision results for imipramine.

S. No	Peak name	tention time	Area (µV*sec)	Height(µV)	USP Plate Count	USP Tailing
1	Imipramine	2.157	513568	78546	1.2	4528
2	Imipramine	2.159	513685	78541	1.2	4572
3	Imipramine	2.186	513659	79852	1.2	4598
4	Imipramine	2.160	513254	78498	1.3	4529
5	Imipramine	2.170	513647	77898	1.2	4572

Table 9: Precision results for chlordiazepoxide.

S. No	Peak name	etention time	Area(µV*sec)	Height(µV)	SP PlateCount	USP Tailing
1	Chlordiazepoxide	3.603	1635625	265325	1.1	7985
2	Chlordiazepoxide	3.608	1658744	264588	1.1	7859
3	Chlordiazepoxide	3.600	1652985	265985	1.2	7845
4	Chlordiazepoxide	3.696	1645898	264898	1.1	7969
5	Chlordiazepoxide	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 10,11.

Table 10: Ruggedness results of imipramine.

S. No	Peak name	RT	Area (µV*sec)	Height (µV)	SP Plate count	USPTailing
1	Imipramine	2.198	514658	78698	4658	1.2
2	Imipramine	2.196	514354	78599	4598	1.2
3	Imipramine	2.160	513985	79854	4652	1.2
4	Imipramine	2.160	514875	79879	4561	1.2
5	Imipramine	2.160	514658	79865	4659	1.2
6	Imipramine	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std. Dev.			852.3705			
% RSD			0.165563			

Table 11: Ruggedness results of chlordiazepoxide.

S. No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	SP Plate count	USP Tailing	Resolution
1	Chlordiazepoxide	3.623	1645875	266589	7985	1.1	10.1
2	Chlordiazepoxide	3.611	1658554	265898	8001	1.1	10.1
3	Chlordiazepoxide	3.696	1649854	265415	7985	1.1	10.1
4	Chlordiazepoxide	3.696	1659842	265154	7956	1.1	10.1
5	Chlordiazepoxide	3.696	1645985	266598	7985	1.1	10.1
6	Chlordiazepoxide	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std. Dev.			6838.733				
% RSD			0.413635				

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1 ml/min. The results are shown in table12,13.

Table 12: Robustness results for imipramine.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.121	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

Table 13: Robustness results for Chlordiazepoxide.

Parameter used for sampleanalysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.643	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 14.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 14: LOD, LOQ of Imipramine and Chlordiazepoxide.

Drug	LOD	LOQ
Imipramine	11.0	35.2
Chlordiazepoxide	1.0	3.1

CONCLUSION

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Imipramine and Chlordiazepoxide in its bulk and tablet dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Chlordiazepoxide and Imipramine in its bulk and tablet dosage form.

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