Research Article

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Development and validation of stability indicating RP-HPLC method for Simultaneous Estimation of Imipramine Hydrochloride and Diazepam in pharmaceutical dosage form.

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Abstract:

A simple, precise, and stability indicating high performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of Imipramine hydrochloride and Diazepam in pharmaceutical dosage form. **Materials and Methods:** The method involves the use of easily available inexpensive laboratory reagents. The separation was achieved on Phenomenex Prodigy C-18 column (150*4.6 mm, i.d., 5 µm particle size) with isocratic flow with UV detector. The mobile phase at a flow rate of 1.0 mL/min consisted of methanol and 0.1%v/v ortho phosphoric acid (pH 3) in the ratio of 70:30 v/v. **Results:** A linear response was observed over the concentration range 5.00-50.00 µg/mL of diazepam and the concentration range 5.00-50.00 µg/mL of Imipramine HCl. Limit of detection and limit of quantitation for Imipramine were 2.73 µg/mL and 1.27 µg/mL, and for Diazepam were 1.80 µg/mL and 1.98 µg/mL, respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria for linearity, accuracy, precision, specificity, robustness. **Conclusion:** The analysis concluded that the method was selective for simultaneous estimation of Imipramine HCl and Diazepam can be potentially used for the estimation of these drugs in combined dosage form

Keywords: Diazepam Imipramine HCl, RP HPLC, anxiolytic, Antidepressant.

Introduction

Diazepam (**fig-1a**) chemically 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one. Diazepam is a benzodiazepine that binds to a specific subunit on the GABA receptor at a site that is distinct from the binding site of the endogenous GABA molecule. Diazepam appears to act on areas of the limbic system, thalamus and hypothalamus, inducing anxiolytic effects. Its actions are due to the enhancement of GABA activity. Benzodiazepine drugs including diazepam increase the inhibitory processes in the cerebral cortex [1]. Imipramine HCl (**fig-1b)** chemically 5-3-(DiMethylamino) propyl-10,11-dihydro-5*H*-dibenz[*b,f*]-azepine monohydrochloride [2]. Imipramine is prototypical dibenzazepine-derivative tricyclic antidepressant (TCA). Its primary use in multiple sclerosis is to treat bladder symptoms, including urinary frequency and incontinence. Several high-performance liquid chromatographic (HPLC) methods have also been reported for the

determination of IMI and DIA individually [3-8] and in combination with other drugs [9-12]. However, there is no simultaneous method reported for their simultaneous estimation. Hence we have planned to develop a validated reversed-phase HPLC method for the estimation of these drugs in combined dosage form as per ICH guidelines [13-18]. The aim of the present study was to develop accurate, precise and selective reverse phase HPLC methods for the simulated analysis of Imipramine HCl and Diazepam.

2. EXPERIMENTAL WORK

2.1. Reagents and chemicals

Orthophosphoric acid (AR Grade, Merck ltd), Methanol (HPLC grade, Merck ltd), Milli-Q water, Diazepam (99.8 % w/w is a gift sample from Corpus research Laboratories) and Imipramine HCl (100% w/w purchased from Corpus Research Laboratories), glacial acetic Acid (GR Grade, SD Fine Chem Ltd). All other chemicals are of the highest grade commercially available unless otherwise specified. DEPSONIL-DZ tablets for evaluation of the assay content were purchased for a local pharmacy.

2.2. Apparatus and chromatographic conditions

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 70:30 % (v/v) of Methanol and 20mM Orthophosphoric acid (pH adjusted to 3.0 with acetic acid) operated on isocratic mode. The flow rate is 1.0 ml/min. Chromatographic determination of Imipramine HCl and Diazepam was performed on Phenomenex Prodigy C_{18} column (150 X 4.6 mm id, ODS 2, 5µm). The wavelength of detection is 222 nm. The injection volume is 20µL.

2.3. Preparation of standard solutions, Calibration Standards & Quality Control Samples

Stock solutions of Imipramine HCl (2.5mg/mL),&Diazepam(0.5mg/mL) were prepared separately in a volumetric flask using methanol and labeled accordingly. Suitable dilutions were then prepared using 50:50 %v/v Methanol & Milli-Q water as Diluent Solution. A Linear Calibration curve containing 8 non-zero standards were prepared using Diluent solution in the concentration range of 5.00-50.00 µg/mL for Imipramine & 5.00-50.00 µg/mL for Diazepam. The calibration standard sample is then transferred into the auto sampler for analysis. Samples for Specificity (Sample with Imipramine alone, sample with Diazepam alone, Blank Sample and sample containing both the drugs) were also prepared accordingly.

For the preparation of quality control samples, a separate stock containing approximately the same concentration of the Imipramine and Diazepam were prepared and labeled as quality control stocks. From these stocks, quality control samples containing Imipramine and Diazepam were prepared at three concentration levels namely LQC, MQC, HQC so as to obtain low, median and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples.

2.4. Assay

The assay of tablets containing Imipramine and Diazepam (Brand name: DEPSONIL-DZ) is done using the procedure given in Indian Pharmacopoeia under tablets. The active ingredients in each of 10 dosage units is taken by random sampling and analyzed by the developed method. The tablets are said to be compliant if the each individual content is 85 – 105 % of the average content or labeled claim. For the current assay ten tablets were randomly taken and transferred separately into 100ml volumetric flasks and dissolved in 20 ml methanol. The solution was then ultrasonicated for 10min and then made up to volume. Required amount of solution is then taken and filtered through 0.45µ nylon membrane and diluted with diluent solution so that the resultant concentrations are within the calibration range of the developed method. The samples are then analyzed by using the validated method. The sample is then injected in triplicate.

2.5 Method Validation

2.5.1 System Suitability

A sample containing mixture of Imipramine (at concentration of 25.00µg/ml) and Diazepam (at concentration of 25.00µg/ml) was used as system suitability sample. System suitability was assessed by six replicate analysis. A percent coefficient of variation (% CV) less than 1 % for retention times for the drugs is taken as the acceptance criterion.

2.5.2 Detection and Quantitation Limits (Sensitivity)

Limits of detection (LOD) and quantification (LOQ) (**Fig-2**) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, with precision (%CV) and accuracy with (±) 20%.

2.5.3 Linearity (Calibration Curve)

The calibration curve was constructed with eight non-zero standards ranging from 5.00 to 50.00µg/mL for Imipramine and 5.00–50.00 for Diazepam. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (**Fig-3**).

2.5.4 Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (2 days).

2.5.5 Specificity

For demonstration of specificity, 4 samples namely blank sample, sample containing Imipramine alone, sample containing Diazepam alone and sample containing the mixture of Imipramine and Diazepam were prepared separately. Specificity of the method was determined by comparing results of all the samples (**Fig-4**). The developed method is said to be specific if the % interference calculated as peak area (if any) at the retention time of each of the analytes in the blank sample is less than 20% of peak area at the corresponding retention times of each of the drugs in the lowest calibration standard. Sample Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

2.5.6 Stability

The stability of the drug is determined by placing the MQC samples for the short term stability at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hrs was studied and established.

2.5.7 Stress Degradation Studies

For Stress Degradation Analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100.00µL of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 5% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for Photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples. The analysis is performed in triplicate.

Fig-1a: Structure of Imipramine HCl

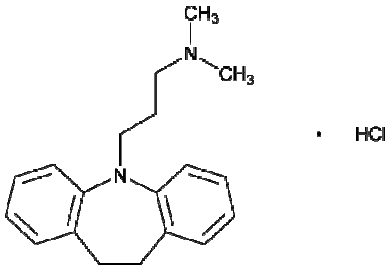
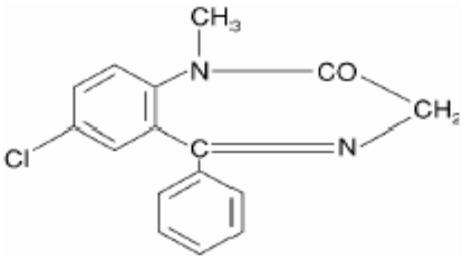


Fig-1b: Structure of Diazepam



3.0 RESULTS AND DISCUSSION

3.1 Method Development and Validation

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Functional group analysis revealed the presence of acidic character to the molecules. Therefore we evaluated the chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 have been tried with different buffer salts such ammonium Formate, ortho phosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran. However less tailing and high theoretical plates are obtained with Phenomenex column C18 150 X 4.6 cm 5µm column.. The peak response of Imipramine decreased with decreased composition of Methanol in the mobile phase. Mobile phase composition consisted of (70:30 v/v) of Methanol and 20mM Orthophosphoric acid (pH adjusted to 3.0 ± 0.1 with glacial acetic acid) on isocratic mode. The flow rate of the method is 1.0 ml/min. Calibration standards were prepared in diluents solution containing 50:50 % v/v of Methanol and Milli-Q water. The wavelength of detection is 222nm. The column temperature is maintained at 25 ^{O}C. At the reported flow rate, peak

shape was excellent; however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape. Hence 1.0 ml/min was optimized flow rate decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. To evaluate the feasibility of the experiment under regular lab conditions, we assessed the stability of Imipramine and Diazepam under room temperature and under normal light conditions.

3.2 Method Validation

3.2.1 System Suitability

The % RSD of the peak area for both drugs is within the acceptable criteria (**Table-1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 10436 ± 50 for Imipramine and 19595 ± 75 for Diazepam.The USP tailing factor for Diazepam and Propranolol HCl is not more than 2.0 Imipramine is 1.45 ±0.02 while that of Diazepam is 1.08 ±0.02.

3.2.2 Determination and Quantification Limits (Sensitivity)

Fig-2 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table-2**).

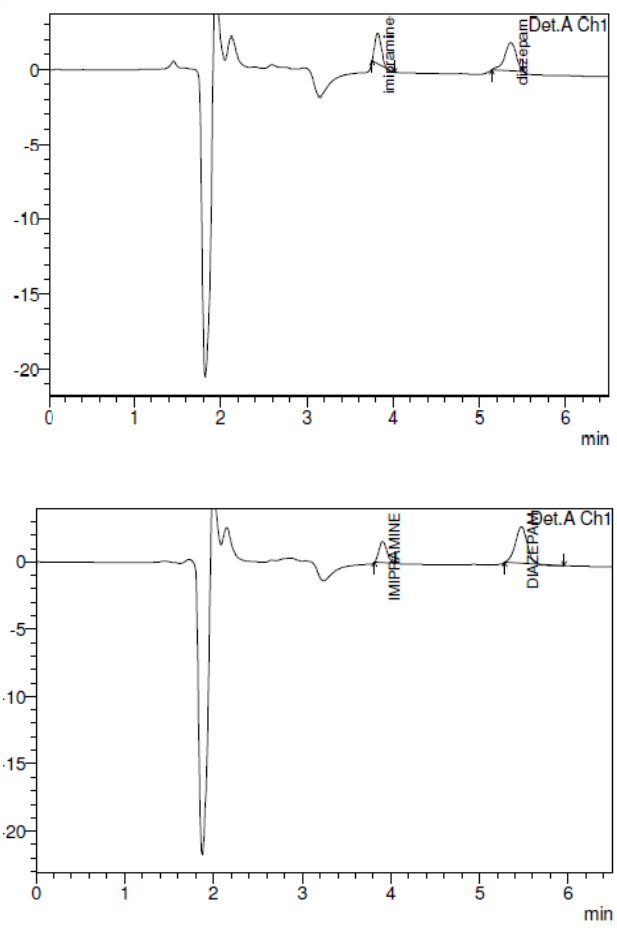
3.2.3 Linearity

The linearity was demonstrated in triplicate. The results of the best fit line (y = mx + c) for the triplicate analysis is given in **Table 3**. The accuracy of the calibration standards was evaluated from the back calculated concentrations **(Table 4)**. The mean accuracies of calibration curve standards for Imipramine were found to be within the range is 96.18-109.35% and for Diazepam in the range of 94.12-107.86%.

3.2.4 Accuracy and Precision

Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given the (**Table-5**). The intra-day (day-1) accuracy for Imipramine ranged from 93.37- 118.86% while that of Diazepam ranged from 96.86 – 119.0 %. The intra-day (day-1) precision for Imipramine ranged from 1.50-2.09 % while that of Diazepam ranged from 1.56-2.58 %. The inter-day (day-2) accuracy for Imipramine ranged from 91.62-123.51 % while that of Diazepam ranged from 96.38-119.80 %. The inter-day (day-2) precision for Imipramine ranged from 0.56-2.32% while that of Diazepam ranged from 1.57-2.96%. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

Fig-2: Chromatograms shown below indicate limit of Detection (LOD) above and Limit of Quantitation (LOQ) below.



3.2.5 Specificity

Specificity was determined by comparison of the Blank chromatogram with that of the Standard chromatogram (**Fig-4**)

3.2.6 Room Temperature Stability

Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 92.89 % for Imipramine and 90.13 % for Diazepam. The results are tabulated in **Table-6**.

Fig-3a: Linear calibration curve of Imipramine HCl

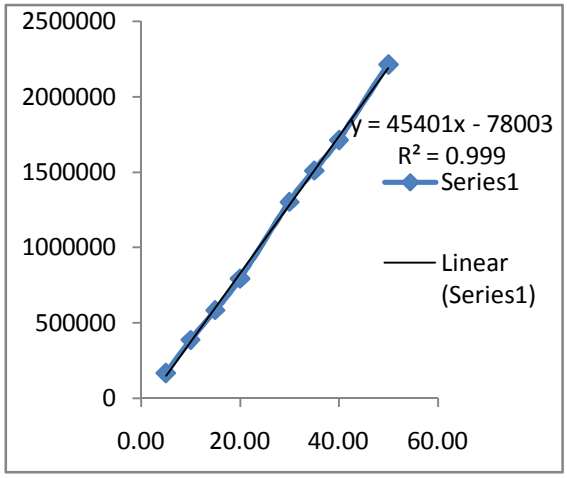
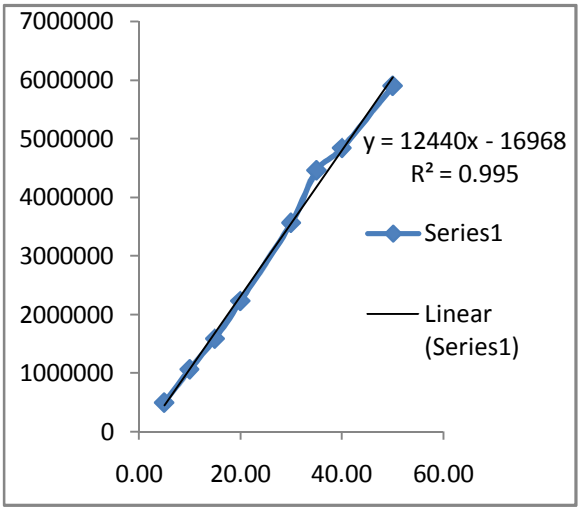


Fig-3b: Linear calibration curve of Diazepam



3.2.7 Stress Degradation

Stress studies revealed that Imipramine is not susceptible to degradation under acid, light (UV) and oxidative stress conditions (**Fig 5**). However, in alkaline conditions (0.1N NaOH), the drug was instable and the degradation peak eluted earlier accompanied with a drastic peak distortion and increased tailing. Except for alkaline conditions, the drug content was within 95 –105 % for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Stress studies on Diazepam indicated instability under alkaline and photolytic conditions. This has been clearly demonstrated by the help of overlap spectra of all the stress samples as compared with that of freshly prepared sample of similar concentration **(Fig 5)**.

3.2.8 Robustness study

Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (1.0 ± 0.1 ml/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time, theoretical plates, and tailing factor, were studied**.** At lower flow rate, the retention time of Imipramine was 2.18 ± 0.02 minutes (n=6) while that of Diazepam was 5.49 ± 0.02 minutes. At lower flow rate, the tailing factor for Imipramine increased to 2.44 ± 0.03 and Diazepam increased to 6.01 ± 0.03. At higher flow rate, tailing factor for both Imipramine and Diazepam remained unchanged as compared to normal flow. The elution was earlier at higher flow rate; Imipramine and Diazepam eluted at 2.00± 0.01 and 4.93 ± 0.02 minutes respectively. The retention time of Imipramine and Diazepam were and 2.00 ± 0.03 minutes and 5.83 ± 0.03 minutes respectively (n=6) when the mobile phase composed of 75 methanol and 25 parts of 20m orthophosporic acid (pH 3.0).

3.3 Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of Imipramine and Diazepam. Also the method is validated for different parameters; hence it has been applied for the simultaneous estimation in pharmaceutical dosage forms. DEPSONIL-DZ was evaluated. The % Assay of Diazepam in the tablet is 95.139 ± 0.06mg and% Assay of Imipramine HCl is 98.313 ± 0.11 mg. None of the tablets ingredients interfered with the analyte peak. The spectrum of Imipramine HCl and Diazepam in the extracted tablet was matching with that of standard compounds indicating the purity of the compounds in the tablets.

Conclusions

The method gave accurate and precise results in the concentration range of 5 - 50 µg/mL for imipramine and 5 to 50µg/mL for Diazepam. The mobile phase composition consists of (70:30 v/v) of Methanol and ortho phosphoric acid (pH adjusted to 3.0 with glacial acetic acid), at the flow rate of 1.0 ml/min. The retention time of Imipramine is 2.01 ± 0.2 minutes and that of Diazepam is 5.98 ± 0.2 minutes. The column is Phenomenex 150 X 4.6mm, C18 column with the particle size of 5µm. A rapid sensitive and specific method for the simultaneous estimation of Imipramine and Diazepam in the pharmaceutical tablet formulations has been developed and validated.

Fig-4: Comparison of (a)Blank Chromatogram, (b) Imipramine alone (c) Diazepam alone and (d) sample containing both Imipramine and Diazepam

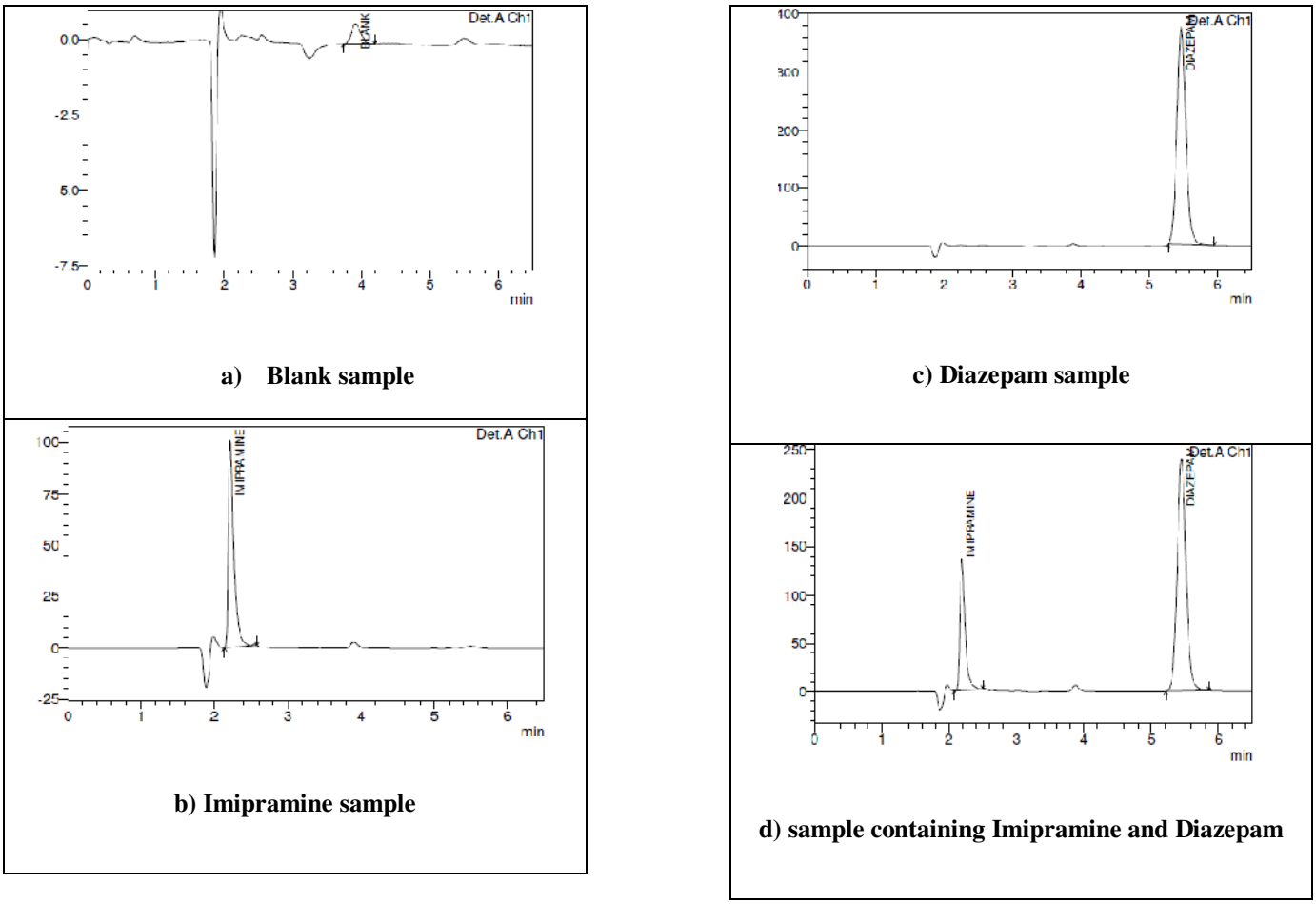


Fig-5a: Overlay Chromatogram showing the influence of various stress conditions on Diazepam; Data 1- fresh sample data2-Acid Stress, Data 3 – Oxidative Stress; Data 4 –Photolytic Stress; Data 5 – Alkaline Stress. Data 4 clearly indicates the spectral degradation of Diazepam due to alkaline instability.

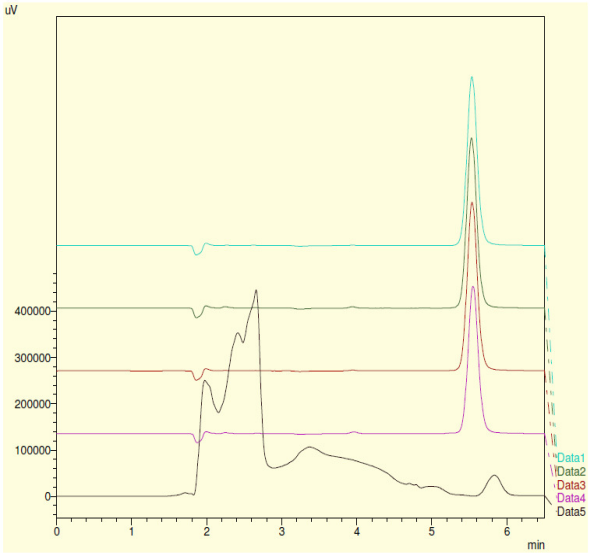


Fig-5b: Overlay Chromatogram showing the influence of various stress conditions on Imipramine HCl; Data 1- fresh sample data2-Acid Stress, Data 3 – Oxidative Stress; Data 4 –Photolytic Stress; Data 5 – Alkaline Stress. Data 4 clearly indicates the spectral degradation of Diazepam due to alkaline instability

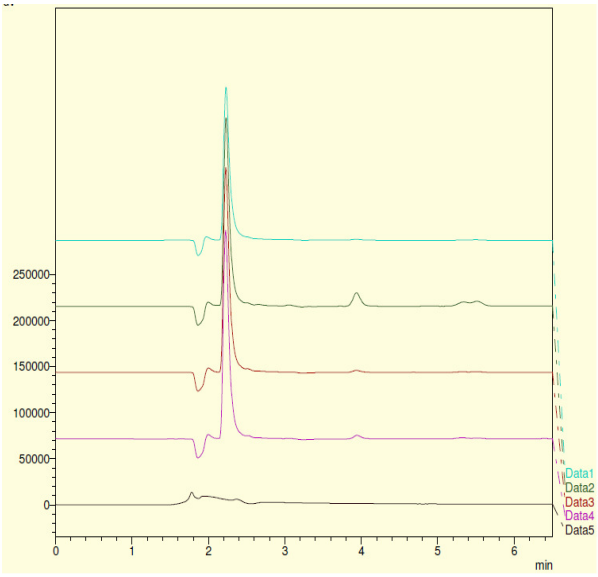


Table 1. System Suitability test for Imipramine (above) and Diazepam (below)

IMIPRAMINE				
Sample ID	**Peak Retention Time**	**Peak Area**	**Theoretical Plates**	**Tailing Factor**
1	2.22	1030917	10563	1.47
2	2.24	985573	10436	1.48
3	2.24	924564	10701	1.49
4	2.23	939872	10416	1.51
5	2.21	998782	10668	1.5
6	2.21	1030393	10914	1.5
MEAN	**2.225**	**985016.8**	**10616.3**	**1.492**
STDEV	**0.0138**	**44829.04**	**186.52**	**0.0147**
%CV	**0.62**	**4.55**	**1.76**	**0.99**
DIAZEPAM				
Sample ID	**Peak Retention Time**	**Peak Area**	**Theoretical Plates**	**Tailing Factor**
1	5.53	3160019	19595	1.06
2	5.54	3020415	19643	1.07
3	5.55	2796834	20424	1.07
4	5.52	2879720	20365	1.08
5	5.51	2987526	20681	1.08
6	5.5	3171547	20887	1.08
MEAN	**5.525**	**3002676.8**	**20265.8**	**1.073**
STDEV	**0.0187**	**149202.91**	**534.98**	**0.01**
%CV	**0.34**	**4.97**	**2.64**	**0.76**

Table 2. Sensitivity

IMIPRAMINE

LOD

SR NO	DRUG	
	Retention Time	Peak Area
1	3.79	8603
2	3.82	8978
3	3.8	8545
MEAN	**3.8**	**8708.7**
ST DEV	**0.02**	**235.05**
% CV	**0.40**	**2.70**

DIAZEPAM

LOD

SR NO	DRUG	
	Retention Time	Peak Area
1	5.33	11231
2	5.36	11587
3	5.32	11236
MEAN	**5.3**	**11351.3**
ST DEV	**0.02**	**204.11**
% CV	**0.39**	**1.80**

IMIPRAMINE

LOQ

SR NO	DRUG	
	Retention Time	Peak Area
1	3.91	9056
2	3.9	9257
3	3.88	9245
MEAN	**3.9**	**9186.0**
ST DEV	**0.02**	**112.74**
% CV	**0.39**	**1.23**

DIAZEPAM

LOQ

SR NO	DRUG	
	Retention Time	Peak Area
1	5.49	24550
2	5.47	24644
3	5.43	25447
MEAN	**5.5**	**24880.3**
ST DEV	**0.03**	**492.99**
% CV	**0.56**	**1.98**

Table 3. Results of best-fit line for triplicate analysis for Imipramine (above) and Diazepam (below)

Imipramine			
Curve	**Slope**	**Intercept**	**r^2**
1	45401.07	78002.86	0.9990
2	44472.87	50341.24	0.9983
3	43484.51	-31109.01	0.9982
Mean	**44452.81**	**-53151.03**	**0.9984**

Diazepam			
Curve	**Slope**	**Intercept**	**r^2**
1	124405.62	-169680.65	0.9954
2	120419.60	-96467.34	0.9959
3	118255.57	-82869.87	0.9960
Mean	**121026.93**	**-115339.28**	**0.9957**

Table4. Linearity and Range for Imipramine (above) and Diazepam (below) demonstrating accuracy, carryover effect and specificity of the method (Curve 1).

IMIPRAMINE					
SAMPLE ID	**Concentration (Microgram/mL)**	**Retention Time**	**Peak Area**	**Back Calc Concentration**	**% Accuracy**
BLANK	0	**NA**	**0**	NA	
CC 1	5.00	2.19	170236	5.47	109.35
CC 2	10.00	2.18	388863	10.28	102.83
CC 3	15.00	2.18	586325	14.63	97.55
CC 4	20.00	2.16	795326	19.24	96.18
CC 5	30.00	2.15	1303594	30.43	101.44
CC 6	35.00	2.16	1511380	35.01	100.02
CC 7	40.00	2.17	1713908	39.47	98.67
CC 8	50.00	2.17	2213565	50.47	100.95
CO BLANK	**0**	**NA**	0	**NA**	**NA**

- NA - Not applicable

DIAZEPAM					
SAMPLE ID	**Concentration (Microgram/mL)**	**Retention Time**	**Peak Area**	**Back Calc Concentration**	**% Accuracy**
BLANK	0.00	**NA**	**0**	NA	
CC 1	5.00	5.44	501256	5.39	107.86
CC 2	10.00	5.42	1068676	9.95	99.54
CC 3	15.00	5.42	1586752	14.12	94.12
CC 4	20.00	5.4	2231842	19.30	96.52
CC 5	30.00	5.39	3564432	30.02	100.05
CC 6	35.00	5.44	4458976	37.21	106.30
CC 7	40.00	5.45	4837226	40.25	100.62
CC 8	50.00	5.47	5896547	48.76	97.52
CO BLANK	0	**NA**	**0**	NA	**NA**

- NA - Not applicable

Table 5a. Results of inter and intra-day accuracy & precision for Imipramine by HPLC

	Nominal Concentration (µg/mL)		
	12.5	25.00	37.5
DAY 1			
MEAN (n=6)	14.56	23.34	35.81
SD	0.30	0.38	0.54
% CV	2.09	1.64	1.50
DAY 2			
MEAN (n=6)	14.85	23.19	35.25
SD	0.31	0.46	0.52
% CV	2.10	1.96	1.47
MEAN (n=6)	15.13	22.91	34.89
SD	0.35	0.42	0.20
% CV	2.10	1.83	0.56

Table 5b. Results of inter and intra-day accuracy & precision for Diazepam by HPLC

	Nominal Concentration (µg/mL)		
	12.5	25.0	37.5
DAY 1			
MEAN (n=6)	14.87	24.63	36.96
SD	0.24	0.64	0.58
% CV	1.60	2.58	1.56
DAY 2			
MEAN (n=6)	14.92	24.31	36.50
SD	0.31	0.70	0.57
% CV	2.07	2.88	1.57
MEAN (n=6)	14.97	24.02	36.14
SD	0.41	0.71	0.63
% CV	2.71	2.96	1.74

Table 6a. Room Temperature Stability of Imipramine (n = 6).

IMIPRAMINE

FRESH SAMPLE

SR NO	SAMPLE ID	CONC (µg/mL)	DRUG	
			RETENTION TIME	PEAK AREA
1	FRESH	25.00	2.15	988084
2	FRESH	25.00	2.14	995208
3	FRESH	25.00	2.14	972452
4	FRESH	25.00	2.13	902062
5	FRESH	25.00	2.13	957144
6	FRESH	25.00	2.13	962213
MEAN				**962860.50**
STDEV				**33178.72**
% CV				**3.45**

STABILITY SAMPLE

SR NO	SAMPLE ID	CONC (µg/mL)	DRUG	
			RETENTION TIME	PEAK AREA
1	STABILITY	25.00	5.39	895502
2	STABILITY	25.00	5.38	960507
3	STABILITY	25.00	5.38	859319
4	STABILITY	25.00	5.37	843544
5	STABILITY	25.00	5.35	861571
6	STABILITY	25.00	5.34	945770
MEAN				**894368.83**
STDEV				**48795.44**
% CV				**5.46**

% Stability 92.89

Table 6b. Room Temperature Stability of Diazepam (n = 6).

DIAZEPAM

FRESH SAMPLE

SR NO	SAMPLE ID	CONC (µg/m)	DRUG	
			RETENTIO N TIME	PEAK AREA
1	FRESH	25.00	2.12	3118390
2	FRESH	25.00	2.12	3154127
3	FRESH	25.00	2.12	3104326
4	FRESH	25.00	2.13	3080722
5	FRESH	25.00	2.14	3025992
6	FRESH	25.00	2.14	3206837
MEA				**3115065.67**
STD				**61947.98**
%				**1.99**

STABILITY SAMPLE

SR NO	SAMPLE ID	CONC (µg/m)	DRUG	
			RETENTIO N TIME	PEAK AREA
1	STABILIT	25.00	5.35	2892382
2	STABILIT	25.00	5.35	3121336
3	STABILIT	25.00	5.34	2697060
4	STABILIT	25.00	5.39	2847700
5	STABILIT	25.00	5.41	2280303
6	STABILIT	25.00	5.41	3006784
MEAN				**2807594.17**
STDE				**295689.62**
% CV				**10.53**

% Stability 90.13

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