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Original Research Paper

VALIDATED STABILITY INDICATING HPTLC METHOD FOR DIAZEPAM AND IMIPRAMINE IN BULK AND COMBINED DOSAGE FORM

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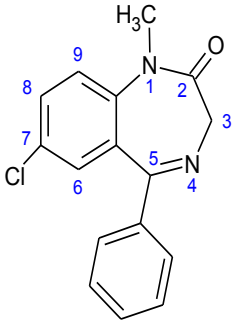
ABSTRACT

A Rapid, simple, specific, accurate and precise HPTLC method was developed for the simultaneous determination of Diazepam and Imipramine hydrochloride by using Silica gel 60 F254 precoated on aluminum sheet (10 cm × 10 cm) of 0.20 mm layer thickness (E. Merck KGaA) as stationary phase & Chloroform: Methanol: Hexane: Glacial Acetic Acid (3:3.5:3.5:0.2 v/v/v/v) as mobile phase. Camag Linomat 5 automatic application applicator, twin trough glass chamber, Camag TLC scanner and wincats software were used throughout the experiment. The Rf value were found to be 0.25 ± 0.01 for Imipramine hydrochloride & 0.47 ± 0.05 for diazepam (DIA) Respectively. Certification was done at 251 nm where Imipramine hydrochloride (IMI) & diazepam (DIA) have basic absorbance. The proposed method can be successfully employed for simultaneous quantitative analysis of Diazepam and Imipramine hydrochloride in bulk drugs and formulations.

Keywords: HPTLC, Validation, Imipramine hydrochloride (IMI), Diazepam (DIA).

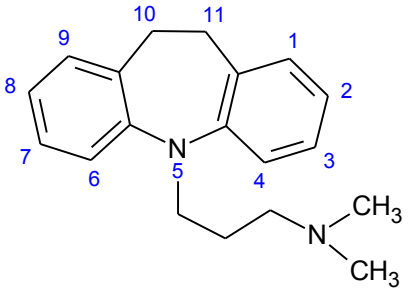
INTRODUCTION

Diazepam [7-chloro-1-methyl-5-phenyl-2, 3-dihydro-1H-1, 4-benzodiazepin-2-one] (figure 1), is a colorless to light yellow crystalline powder, almost odorless, freely soluble in water, methanol and solvent ether. Diazepam is anxiolytic, sedative & hypnotic, antiepeleptic and muscle relaxant. It is official in Indian Pharmacopoeia[1,2,3,4], which recommends a titrimetric method for its analysis. Imipramine hydrochloride [3-(5, 6-dihydrobenzo [b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine HCl] (figure 2) is a white to off-white powder, odorless, crystalline powder, sparingly soluble in water and freely soluble in methanol. It is commonly used as an antidepressant and urinary incontinancy agent. Imipramine is official in British Pharmacopoeia[5,6], which recommends HPLC and HPTLC methods for its analysis. Diazepam and Imipramine combination suspension is combination in Indian market.



7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one

Figure 1: Structure of Diazepam



3-(10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine

Figure 2: Structure of Imipramine hydrochloride

This paper reports validated HPTLC method for simultaneous determination of Diazepam and Imipramine HCl in pharmaceutical formulation. The proposed method is simple, accurate, reproducible and suitable for routine determination of Diazepam and Imipramine in combined dosage form. The method was validated in compliance with ICH guidelines[7]. Literature survey reveals that many analytical methods are reported for determination of Diazepam and Imipramine.[8-35]

MATERIALS AND METHODS

Cipla Pharmaceuticals (Maharashtra, India) supplied pure drug sample of Diazepam and Impiramine hydrochloride procured from Umedica Laboratories Ltd. (Gujarat, India) and were certified to contain 99.32% (w/w) and 99.16% (w/w) respectively, on dried basis. Methanol and water used were of HPLC grade and were purchased from Merck and CDH respectively. Potassium dihydrogen phosphate was purchased from Rankem. The suspension formulation (Parfil, Perron Pharmaceuticals, Karampura, New Delhi, India) containing 125 mg of Diazepam and 5 mg of Imipramine per 5 ml was procured from local market and used for analysis of marketed formulation. Camag Linomat 5 automatic application applicator, twin trough glass chamber, Camag TLC scanner and wincats software were used throughout the experiment. In addition, an electronic balance (Ohaus N-13123), a pH meter (Labtronics LT-11).

Selection of Solvent for Imipramine Hydrochloride (IMI) & Diazepam (DIA)

Initially water was used to check out solubility of both drugs, where Imipramine Hydrochloride (IMI) & Diazepam (DIA) was sparingly soluble in non-polar solvent, solubility is more in ethanol & methanol. Therefore methanol has been selected as common solvent for analysis.

Table 1: Solubility Data of Imipramine Hydrochloride & Diazepam

Solvent	Imipramine Hydrochloride (IMI)	Diazepam (DIA)
Water	Freely soluble	Soluble
Methanol, Ethanol	Freely soluble	Slightly soluble
Ether	Insoluble	Slightly soluble
Acetone	Freely soluble	Freely soluble

Preparation of Mobile Phase

The mobile phase was consisting of Chloroform: Methanol: Hexane: Glacial Acetic Acid (3:3.5:3.5:0.2 v/v/v/v). Mobile phase is prepared by mixing of 3ml of Chloroform, 3.5 ml of Methanol, 3.5 ml of Hexane & 0.2ml of Glacial acetic acid in HPTLC chamber & same was employed for elution & chromatographic separation.

Preparation of Standard Stock Solution (Standard Stock Solution (1000 µg/ml)

The 50 mg of Imipramine Hydrochloride (IMI) were effectively weighed & exchanged to 50 ml volumetric glass containing few ml (10 ml) of methanol. Glasses were sonicated for 2 minutes to particular solids &

volume was made up to imprinting with diluent to pick up standard strategy containing 1000 µg/ml Imipramine Hydrochloride (IMI).

The 50 mg of Diazepam (DIA) were accurately weighed & exchanged to isolated 50 ml volumetric container containing few ml (10 ml) of methanol. Containers were sonicated for 2 minutes to independent solids & volume was made up to etching with diluent to get standard strategy containing 1000 µg/ml Diazepam (DIA).

Selection of Analytical Wavelength

The λmax exhibit estimation of wavelength maxima for solution that show most amazing maxima. From overlain UV scope of Imipramine Hydrochloride (IMI) & Diazepam (DIA) it was found that at 251 nm both prescription has huge absorbance showed up in figure. 3. Thusly 251 nm was picked as regular exploratory wavelength for examination of both drugs.

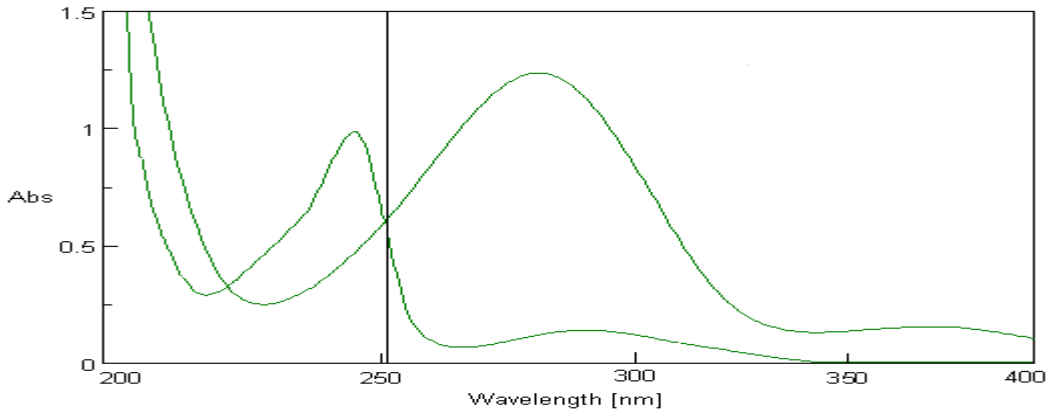


Figure 3: Overlain UV spectrum of Imipramine Hydrochloride (IMI) & Diazepam (DIA) in methanol in range of 200 nm-400 nm

Selection of Densitometry Condition

HPTLC was performed on precoated silica gel HPTLC plate 60 F254 (10 cm × 10 cm) of 0.20 mm layer thickness (E. Merck KGaA, Germany) of aluminum sheet. Camag HPTLC framework furnished with altered Linomat V self-loader sample complete (Camag, Switzerland), Hamilton syringe (100µl), CAMAG TLC Scanner-3 & combined programming WinCATs translation 1.3.4 was utilized for examination. Plates were prewashed with methanol & actuated at 110°C for 5 min before chromatography. Tests & norms were joined with plate as 8mm band under surge of N2 gas, 11.6 mm disengaged, 15 mm from base edge, beginning 15 mm from edge of HPTLC plate with Linomat V instrument. faithful illustration application rate was 100nL/s. straight climbing progress was done in CAMAG twin-trough glass chamber (10 cm × 10 cm) which was pre-immersed with advantageous stage Chloroform: Methanol: Hexane: Glacial Acetic Acid (3:3.5:3.5:0.2 v/v/v/v) for 30 min at room temperature (25°± 2°C). Straight rising progress of chromatogram run was 8 cm & change time around 25min. make plates were dried by framework for hot air with assistance of hair dryer. Densitometric checking was performed in absorbance mode at 251 nm by use of CAMAG TLC scanner III. Radiation source was deuterium light transmitting steady UV radiation some spot around 190 & 400nm. Checking rate is 20mm/sec was utilized. Slit estimation was 6 × 0.45 millimeters. Spotting parameter is a) Band width: 8 millimeter b) Syringe size: 100µl. Using to spot of tests was done by using Hamilton microliter syringe.

Validation of HPTLC Method

Exactly when framework has been made it is basic to acknowledge it to confirm that it is suitable for its normal reason. Acknowledgment tells how extraordinary procedures are, especially whether it is sufficient for arranged application. Strategy Validation is today key stress in activity of informative science labs. It is starting now all that much executed in pharmaceutical industry. US Food & Drug Administration (FDA) have changed draft rules with bare essential recommendations for technique acknowledgment of bio-

methodical systems (Shah, VP; 2001) in pharmaceutical business. International Conference on Harmonization (ICH) has given implications of acknowledgment issues joined into "demonstrative systems" for fields of bio-investigative procedure, pharmaceutical & biotechnological methods (ICH, Q2A, Q2B, Q6B, 2002). In like way US Pharmacopeia (USP) has disseminated rules for framework endorsement for explanatory schedules for pharmaceutical things (USP, 1995). However standards from ICH & USP are not as point by point as those from FDA, & in analytic biotechnology range there exists no bare essential acknowledgment rules. Most surely understood acknowledgment parameters will be immediately depicted underneath. On the basis of International Conference on Harmonization (ICH) the different validation parameters are:

- Accuracy
- Precision
- Specificity
- Limits of Detection
- Limits of Quantification
- Ruggedness
- Robustness
- System suitability

RESULTS AND DISCUSSION

Analysis of Marketed Formulations

The made HPTLC methodology was viably joined for estimation of diazepam & imipramine hydrochloride in advanced estimation structure. Publicized arrangement, Depranil plus Tablet (Marketed by La Pharma) & Depsol Forte Tablet (Marketed by Intas Pharma Ltd.) was inspected using made HPTLC system. Densitogram of tablet test exhibited only two peaks at hindrance part estimation of 0.25 & 0.47 minute for diazepam & imipramine HCl, independently, demonstrating that there is no obstacle of excipients present in tablet definition. Substance of diazepam & imipramine HCl was processed by differentiating peak zones of test & that of standard. Advanced arrangements were explored using proposed system which gave rate recovery of more than 97.0 for diazepam & imipramine hydrochloride. No impedance from excipients present in advanced tablet arrangement was watched which is showed up in figure 4.

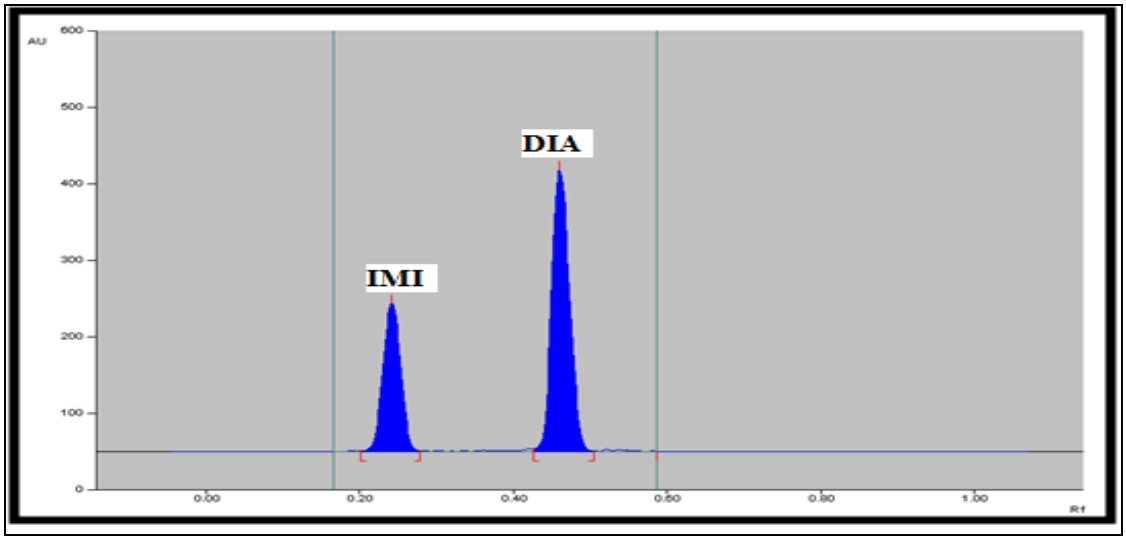


Figure 4: Densitogram of marketed formulation of IMI (150ng/spot) & DIA (300ng/spot)

Linearity & Range

Peak degrees were found to have better direct relationship with obsession than top statures. Linearity of exploratory framework is its ability to move test works out of course that are particularly relating to centralization of analytes in tests within given scope. Change turns were gotten by plotting peak district

versus obsession with level of 50 - 300 ng/band for Imipramine & 100 - 600 ng/band for Diazepam, self-rulingly in mix standard. Change turn data of Imipramine & Diazepam was showed. Data of plummet into sin examination of arrangement turns are showed. Made TLC plate of Imipramine & Diazepam is showed. The fall far from confidence association was seen to be y= 84403x + 29304 & connection coefficient was seen to be 0.999 for Imipramine. Apostatize trial declaration was seen to be y = 43512x + 60109 & alliance coefficient was seen to be 0.996 for Diazepam. Each response was ordinary of three determinations. Statistical examination data of likeness turn get, incline, & slide into sin relationship are showed. Three dimensional overlain chromatogram of Diazepam in obsession level of 100-600 ng/band & Imipramine in center level of 50 - 300 ng/band in mix standard.

Table 2: Result of calibration curve for Imipramine

Concentration (ng/spot)	Area Mean ± S.D.	% RSD
50	1557.22 ± 37.40	2.40
100	2514.75 ± 60.03	2.38
150	3428.50 ± 40.13	1.17
200	4273.27 ± 45.52	1.06
250	5094.68 ± 52.92	1.03
300	5836.48 ± 42.56	0.72

a = Average of three determinant
S.D. =Standard Deviation
RSD= Relative Standard deviation

Table 3: Result of calibration curve for Diazepam

Concentration (ng/spot)	Area Mean ± S.D.	% RSD
100	3910.52 ± 084.74	2.16
200	5516.55 ± 100.84	1.82
300	7086.62 ± 074.24	1.47
400	8339.52 ± 083.88	1.00
500	9612.28 ± 096.69	1.00
600	10911.70 ± 060.62	0.55

[a] = Average of three determinant
S.D. =Standard Deviation
RSD= Relative Standard deviation

Table 4: Statistical analysis data of calibration curve

Parameters	IMI	DIA
Linear Range (ng/spot)	100 – 600	50 – 300
Slope	13.87	17.13
Intercept	2708	786
Regression Co-efficient (r^2)	0.9960	0.9980
Standard deviation of slope	0.17	0.32
Standard deviation of intercept	83.78	66.74
Limit of Detection (ng/spot)	30.30	15.16
Limit of Quantitation (ng/spot)	100	50

LOD= Limit of Detection
LOQ=Limit of Quantitation

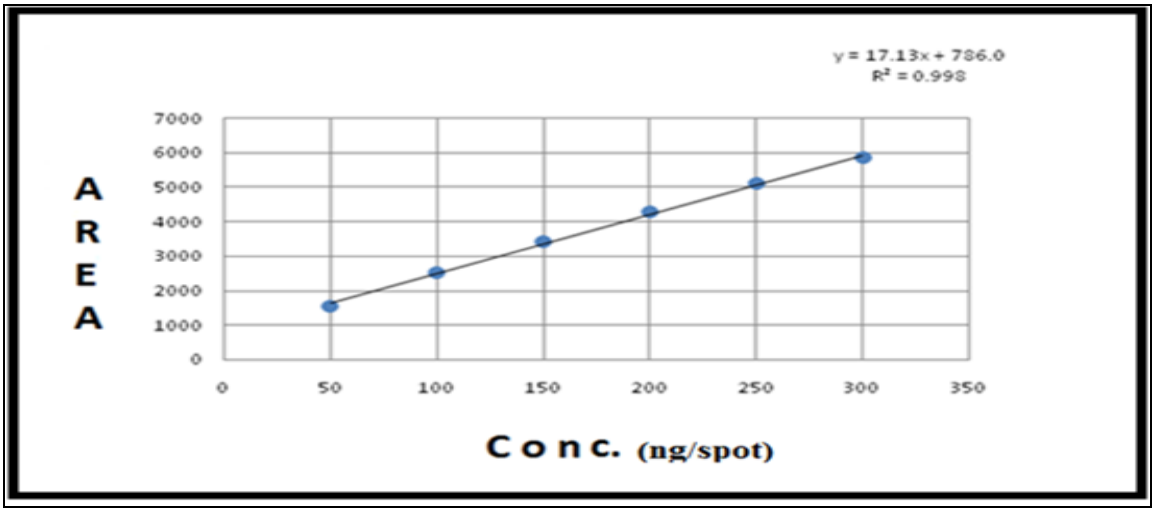


Figure 1: Calibration curve of IMI standard

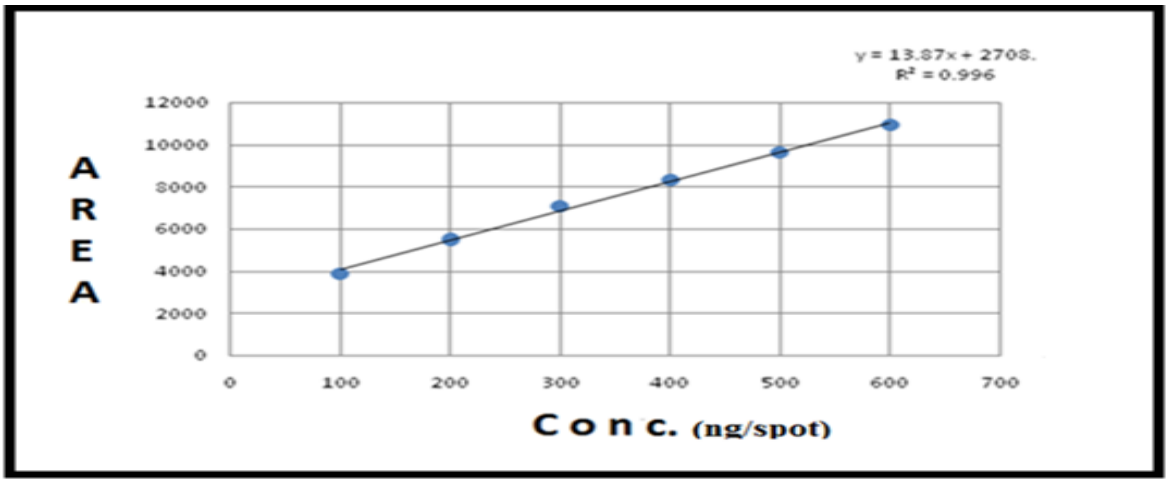


Figure 2: Calibration curve of DIA standard

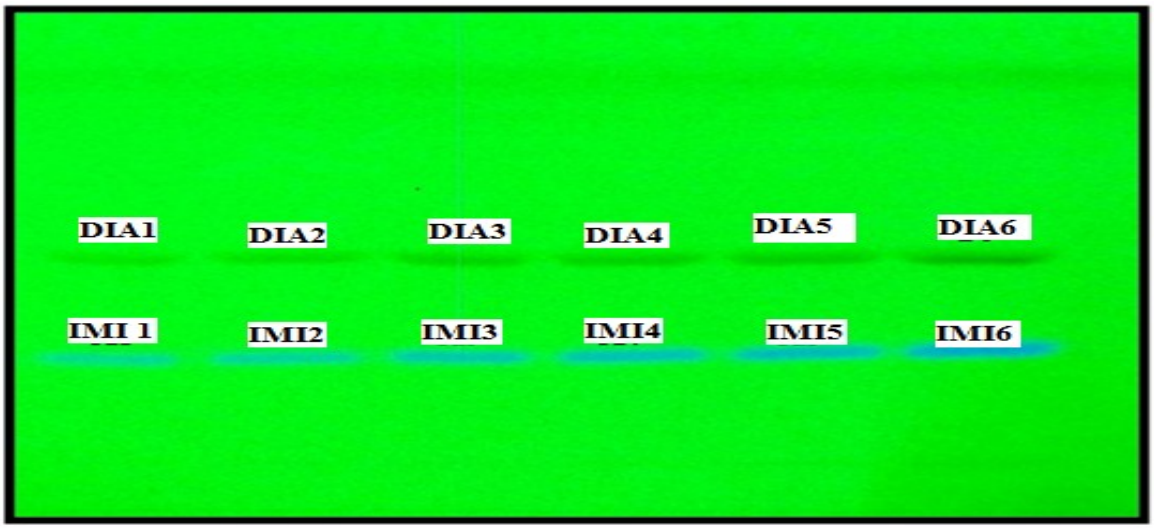


Figure 3: Photograph of developed TLC plate

(DIA1-DIA6 = 100 – 600 ng/band (DIA), IMI1-IMI6 = 50 – 300 ng/band (IMI))

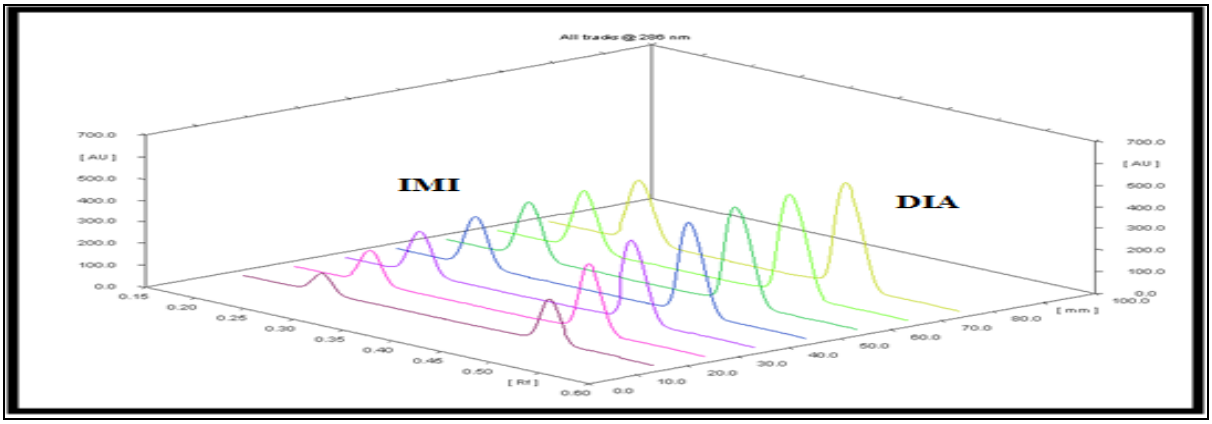


Figure 4: Three dimensional overlain spectra of & IMI (50-300 ng/band) & DIA (100-600 ng/band)

Precision

The repeatability of developed method was evaluated by applying 10µl solution of 150ng/band for IMI solution on TLC plate six times on same day. CV was found to be 1.17 for IMI. Repeatability of developed method was evaluated by applying 10µl solution of 300ng/band for DIA solution on TLC plate six times on same day. CV was found to be 1.04 for DIA. The deferred results of halfway accuracy (Intraday precision & Interday exactness) trials are appeared in Tab.44 for IMI. For intraday accuracy recreate examinations of three specific focuses 50, 150 & 300 ng/spot of IMI arrangements were related on TLC plate in triplicate around same time displayed wonderful reproducibility. For Interday accuracy repeat examinations of three momentous focuses 50, 150 & 300 ng/spot of IMI approaches were joined on TLC plate in triplicate on three unmistakable day's demonstrated magnificent reproducibility. CV of intraday & Interday studies was seen to be 0.93 - 1.54 & 1.06 - 3.42 freely for IMI. The results of generally engaging accuracy (Intraday precision & Interday precision) trials are appeared in Tab.45 for DIA. For intraday accuracy go over examinations of three novel focuses 100, 300 & 600 ng/spot of DIA arrangements were related on TLC plate in triplicate around same time exhibited great reproducibility. For Interday precision repeat examinations of three exceptional focuses 100, 300 & 600 ng/spot of DIA courses of action were joined on TLC plate in triplicate on three unmistakable days demonstrated unfathomable reproducibility. CV of intraday & Interday studies was seen to be 0.65 - 2.57 & 0.70 - 2.83 freely for DIA. The made system was seen to be right & repeatable on reason of mean CV values for repeatability & broadly engaging precision concentrates on which were <2.9 for DIA & <3.5 % for IMI freely. Portions of pharmaceutical & particular corruption things in blend of focused on tests were seen to be relative when examinations were performed with LC structure on diverse days.

Reproducibility of made philosophy was overseen by two stand-out authorities under same densitometry condition & on same HPTLC instrument for DIA & IMI at 300 ng/spot & 150 ng/spot fixation level only. Impact on top degree was assessed by applying F-test. There was no imperative capability was discovered exhibiting that made system was reproducible. Reproducible results are appeared in Tab. 46 & 47 for IMI & DIA autonomously.

Table 5: Repeatability study

Conc.	IMI (150 ng/spot)	DIA (300 ng/spot)
Area	3463.8	7195.2
	3407.3	7020.6
	3421.8	7023.7
	3397.1	7150.2
	3490.7	7029.8
	3390.3	7100.2
Mean	3428.50	7086.62
SD	40.13	74.24
% RSD	1.17	1.04

Table 6: Intraday & Interday precision study for IMI

Intraday Precision		
Conc. (ng/spot)	Area ± S.D. (n=3)	% RSD
50	1578.43 ± 24.32	1.54
150	3427.40 ± 44.94	1.28
300	5833.70 ± 54.35	0.93
Interday Precision		
50	1561.53 ± 53.47	3.42
150	3412.90 ± 48.34	1.41
300	5825.57 ± 62.12	1.06

a = Average of three determinant
S.D. =Standard Deviation
RSD= Relative Standard deviation

Table7: Intraday & Interday precision study for DIA

Intraday Precision		
Conc. (ng/spot)	Area ± S.D.	%RSD
100	03931.10 ± 101.18	2.57
300	07073.33 ± 088.66	1.25
600	10930.60 ± 071.90	0.65
Interday Precision		
100	03928.30 ± 111.21	2.83
300	07133.07 ± 099.08	1.38
600	10903.10 ± 077.22	0.70

a = Average of three determinant
S.D. =Standard Deviation
RSD= Relative Standard deviation

Table 8: Reproducibility data for IMI (150 ng/spot)

Analyst 1 Area ± S.D (n =3)	Analyst 2 Area ± S.D (n = 3)	Result of F-test	Inference
3421.45 ± 69.32	3415.64 ± 82.51	1.41	No significant difference

* At 95% confidence interval, (F-Tabulated = 9.28)

Table 9: Reproducibility data for DIA (300 ng/spot)

Analyst 1 Area ± S.D (n = 3)	Analyst 2 Area ± S.D (n = 3)	Result of F-test	Inference
7082.74 ± 36.63	7067.21 ± 30.00	1.49	No significant difference

* At 95% confidence interval, (F-Tabulated = 9.28)

Accuracy

The recuperation of procedure was done by standard augmentation to preanalyzed test at three different fixation levels half, 100% & 150%. Triplicate determinations were made at every inside level. Known measure of gages of IMI (0, 50, 100 & 150 ng for each band) & DIA (0, 100, 200 & 300 ng for every band) were spiked to pre-evaluated test (100 ng for each band) of IMI & (200 ng for every band) of DIA from tablet dose structure & blends were poverty stricken around proposed HPTLC system. Rate recuperation of IMI & DIA was coordinated by measuring top ranges & fitting these qualities into lose faith logical

announcement of game plan plot. Recuperations were seen to be 97.67 ± 0.18 – 99.59 ± 0.51 % & 97.39 ± 0.23 – 99.52 ± 0.46% for IMI & DIA, autonomously. Qualities exhibit that method is accurate (table 48).

Limit of Detection & Limit of Quantitation

By, rationality in light of standard deviation of reaction & mean of slant was utilized for picking Limit of affirmation (LOD) & most remote reason for quantitation (LOQ). Past what numerous would consider workable for IMI & DIA were seen to be 15.16 ng/band & 30.30 ng/band, freely, while quantitation cutoff centers were seen to be 50 ng/band & 100ng/band, only. Above information shows that microgram measure of both drugs can be definitively & precisely picked. Estimations of LOD & LOQ of IMI & DIA independently exhibit affectability of proposed structure.

Table 10: Accuracy study

Level	Drug added (ng/band)	Drug Recovered (ng/band) [a]	% Drug Recovered ± SD
Imipramine Hyrdochloride			
50	100	97.86	98.93 ± 0.60
100	200	197.13	98.56 ± 0.51
150	300	394.79	97.39 ± 0.23
Diazepam			
50	50	48.60	98.60 ± 0.46
100	100	98.83	98.83 ± 0.28
150	150	149.59	99.59 ± 0.51

a=Average of Three determination

Specificity & Selectivity

Selectivity of framework proposes extent to which it can pick particular analytes under given conditions in mixes or arranges, crucial or complex, without hindrances from unmistakable parts. Specificity study was done to check impedance from excipients used as part of blueprints by planning conveyed mix containing both cures & excipients. Top goodness list & HPTLC chromatogram showed tops for both meds (IMI & DIA) with no interfering top & estimation of both arrangements were seen to be tasteful. Brilliant relationship was gotten amidst standard & test spectra of IMI & DIA. essentially indistinguishable UV degree of standard & test is seen to be same. In like way aftereffects of relationship between's tops start, most persuading, & end display closeness in these positions between estimation shapes & measures. Area of IMI & DIA spot at specific impediment variable not exactly same as it's undermines shows specificity of proposed framework.

The selectivity of test is measure of extent to which strategy can pick particular compound in disengaged cross segments without check from structure parts. Proposed system could be seen as specific & can be used to pick IMI & DIA in district of its corruption things or co-requested blends contained in pharmaceutical definition. Using perfect conditions of proposed structure IMI & DIA are pulled back at RF =0.25 & Rf = 0.47 openly in tablet estimation structure displaying high selectivity of system. All were awesome & common for enormous specificity of framework for examination of constancy of IMI & DIA. Test game-plan is prepared by mixing of IMI & DIA with tablet powder excipients. top convictions of both arrangements was assessed by taking gander at changed spectra of standard solutions & tests at top start, top summit & beat end positions of spot showed up in fig. 98. Specificity is showed up by taking gander at chromatogram of Diluent, standard system & test planning outline & by top goodness file to demonstrate that there was no any impedance of excipients with highest point of MET, PIO & GLI (figure 99, fig. 100 & fig. 101).

Robustness

Power is measure of limit of methodology to stay unaffected by little varieties in structure parameters. Nature of strategy was resolved in triplicate at fixation level of 10 µg/ml of Imipramine hydrochloride & 50 µg/ml of Diazepam. After little changes in this parameter influence top locales were resolved & mean & RSD of crest areas discovered. Intentional changes in running with parameters which affects % measure of 10 µg/ml of Imipramine hydrochloride & 50µg/ml of Diazepam & framework suitability parameters were studied.

a) Change in % organic phase of mobile phase by ± 5.0 %
b) Change in detection wavelength by ± 5.0 nm
c) Change in solvent migration distance
d) Change in chamber saturation time

The low estimation of RSD was found to exhibiting that proposed framework was capable, as meager however think changes in methodology parameters have no unfavorable effect on procedure execution as showed up in tabble 49. Low estimation of rate relative standard deviation shows that procedure is robust.

Table 11: Robustness study for IMI & DIA

Parameters		IMI (150ng/spot)		DIA (300ng/spot)	
		Area ± SD	% RSD	Area ± SD	% RSD
Changed Proportion of mobile phase	3.5:3:3.5:0.2	3229.87 ± 71.20	2.19	6705.22 ± 93.56	1.39
	3.5:3.5:3:0.2	3280.98 ± 40.38	1.23	6713.54 ± 99.82	1.48
	2.5:4:3.5:0.2	3226.42 ± 51.68	1.82	6718.26 ± 74.12	1.51
	2.5:3.5:4:0.2	3249.22 ± 42.53	1.82	6595.03 ± 81.51	1.21
Proportion of Mobile phase used	3:3.5:3.5:0.2	3315.53 ± 13.08	0.39	6823.90 ± 73.15	1.07
Changed Migration distance	70 mm	3266.42 ± 61.68	1.88	6681.66 ± 84.12	1.25
	90 mm	3241.59 ± 51.97	1.60	6696.90 ± 93.93	1.40
Migration distance used	80 mm	3321.41 ± 29.56	0.89	6810.56±47.83	0.70
Changed chamber saturation time	20 min	3234.22 ± 54.53	1.68	6665.03 ± 81.51	1.22
	40 min	3223.77 ± 50.53	1.56	6674.73 ± 99.79	1.49
Saturation time used	30 min	3345.99 ± 2.83	0.08	6798.08 ± 53.62	0.78
Changed in Detection wavelength	246nm	3272.72 ± 44.31	1.31	6731.41 ± 97.72	1.41
	256nm	3193.77 ± 50.53	1.65	6704.73 ± 56.09	1.69
Detection wavelength Used	251nm	3321.41 ± 29.56	0.89	6810.56±47.83	0.70

Solution Stability

The game-plan quality study uncovered that IMI & DIA courses of action were continuing for 48 h without detectable corruption. Rate measure of both medications was seen to be satisfying (Table 50).

Table12: Solution stability study

Time	Area of IMI (150ng/spot)	Area of DIA (300ng/spot)	% Amount Drug Found (n=3)	
			IMI (150ng/spot)	DIA (300ng/spot)
0 hr.	3367.83	6821.14	100.48	98.85
4.0 hrs.	3334.68	6800.75	99.19	98.36
8.0 hrs.	3333.91	6786.19	99.16	98.01
24.0 hrs.	3304.11	6779.95	98.00	97.86
48.0 hrs.	3298.54	6769.96	97.78	97.62

The acceptance criteria for various method validation parameters & their result are shown in Tab. 51 & compare with obtained result. Summary of method validation parameter & their result are shown in Tab.52 indicating that developed is validated as per ICH guidelines & result are within ICH guidelines values.

Table 13: Various validation parameter & their acceptance criteria

Validation Parameters	Acceptance Criteria
Correctness	Recovery 98- 102% (individual)
Reproducibity	Relative Standard Deviation < 2%
Repeatability	Rel. Std Dev. < 2%
Ruggedness	Rel. Std Dev. < 2%
Specificity/ Selectivity	No interference, P. P. I/ > 0.999
Regression range of linearity	Correlation coefficient r^2 > 0.999 or 0.995
Solution Stability	> 12 hour
Detection Limit	Signal /Noise > 2 or 3
Quantitation Limit	Signal /Noise > 10

Table14: Summary of validation parameters

Parameters	**IMI**	**DIA**
Linear Range (ng/band)	50 – 300	100 – 600
Regression Coefficient	0.998	0.996
Regression equation	y= 84403x + 29304	y = 43512x + 60109
Recovery (%)	97.67 – 99.59	97.39 – 99.52
Repeatability (% RSD, n=6)	1.17	1.04
Precision (% RSD) Intra - day (n=3) Inter - day (n=3)	 0.93 – 1.54 1.06 – 3.42	 0.65 – 2.57 0.70 – 2.83
Reproducibility	Reproducible	Reproducible
Limit of Detection (ng/ band)	15.16	30.30
Limit of Quantitation (ng/ band)	50	100
Robustness	Robust	Robust
Solvent stability	Stable for 48hrs	Stable for 48hrs
Specificity	Specific	Specific

System Suitability

The structure suitability tests are used to affirm that determination & reproducibility of chromatographic system are attractive for examination to be done. Tests rely on upon thought that equipment, devices, deliberate operations, & test to be dismembered constitute key structure that can be evaluated in light of present circumstances. System suitability parameters like RF qualities, top ideals peak area Imipramine Hydrochloride (IMI) & Diazepam (DIA) were figured. Structure suitability tests were done on recently organized mix standard stock plan of 100ng/band for Imipramine Hydrochloride (IMI) & 200 ng/band for Diazepam (DIA) & parameters got are laid out in table 15.

Table15: System suitability test parameters

System Suitability Parameter	IMI	DIA
Peak Purity	0.9968	0.9976
Rf value	0.25 ± 0.0051	0.47 ± 0.0083
Peak Area	3421.45 ± 69.32	7082.74 ± 36.63

Table 16: Assay results of marketed formulation

Formulation	Drug	Amount Taken (mg)	Amount Found (mg)	% IMI ±SD	% DIA ±SD
Depranil Plus Tablet	IMI	25	24.68	98.72 ± 0.93	100.40 ± 1.52
	DIA	5	5.02		
Depsol Forte Tablet	IMI	25	25.05	100.20 ± 1.04	99.40 ± 1.41
	DIA	5	4.97		

n= Average of Three determination
IMI=Imipramine, DIA=Diazepam

Stress Study

The heartiness measure techniques are getting vitality for evaluation of component pharmaceutical substance. USFDA underscore constancy demonstrating take gander at techniques for estimation of component fixings in pharmaceutical estimations structure. Recognized, quantitative, illustrative strategies is made that can perceive developments with time in blend, physical, or microbiological properties of arrangement substance & sedate thing. These are particular so that substance of component settling, degradation things & different pieces of recreation movement can be unequivocally measured without impedance. Term adequate decay is taken in broadest sense, which suggests 80-100% rot, if goal is allotment of contamination things or between 20-80% separating when goal is to add to corruption pathways (Bakshi, *et al.*; 2002). To review soundness demonstrating properties of made HPTLC structure, constrained contamination studies were done in perception to ICH rules. Strain studies were done under states of hydrolysis, photolysis, oxidation & dry warmth, as portrayed in ICH guideline Q1A (R2) (ICH, 2002).

Stock courses of action were readied by totally measuring 25 mg each of Imipramine Hydrochloride (IMI) & Diazepam (DIA) exchanging to two separate 25 ml volumetric containers containing few ml of methanol. Containers were spun to discrete solids & weakened up to etching with methanol. These stock approaches were utilized for constrained debasement considers.

The % degradation was calculated by following formula:

% degradation = [(Actual initial area of untreated stock solution – Reduced area of treated stock solution)/Actual initial area of untreated stock solution] * 100

Table17: Optimized stress degradation studies conducted on Imipramine hydrochloride & Diazepam

Stress degradation condition	**Stressor**
Base Induced Degradation	0.5 N NaOH, reflux at 75°C for 2hrs
Acid induced Degradation	0.5 N HCl, reflux at 75 °C for 4 hrs
Neutral hydrolysis	Double distil Water, reflux at 75 °C for 4 hrs
Oxidative degradation	3 % Hydrogen Peroxide (H_2O_2) reflux at 75 °C for 4 hrs
Thermal degradation	Dry Heat: Drug powder kept in hot air oven at 75 °C for 4 hrs, Wet Heat: Drug solution kept in boiling water bath for 4 hrs
Photolytic degradation	Drug powder was exposed to direct sunlight for 72 hrs Drug powder was exposed to UV light (254nm & 365nm) for 4 hrs

Table18: Forced degradation studies data of Imipramine & Diazepam by Proposed HPTLC Method

Stress condition	Time	Imipramine HCl (IMI)		Diazepam (DIA)	
		% Assay	% Degrade	% Assay	% Degrade
Alkaline hydrolysis (0.5N NaOH)	2hr	96.52	3.48	91.95	8.05
Acidic hydrolysis (0.5N HCl)	2hr	94.85	5.15	89.85	10.15
Neutral Hydrolysis (Double distil water)	2hr	98.28	1.72	98.16	1.84
Oxidative Degradation (6% H_2O_2)	4hr	98.85	1.15	96.52	3.48
Dry heat (75 °C)	4hr	98.09	1.91	95.23	4.77
Wet Heat (Boiling Water bath)	4hr	98.53	1.43	97.49	2.51
Sun light	72 hr.	97.49	2.51	97.52	2.48
UV radiation (254nm)	4hr	99.02	0.98	97.76	2.24
UV radiation (365nm)	4hr	98.16	1.84	97.06	2.94

CONCLUSION

Clear, questionable & quality demonstrating two particular chromatographic frameworks, for case, HPTLC & RP-HPLC were made for estimation of Imipramine hydrochloride (IMI) & diazepam (DIA) in their solidified pharmaceutical estimation structure.

Clear, questionable & Stability showing RP-HPLC structure was made using C18 range as stationary stage & Methanol & Water (Phosphate support) (75:25) v/v, pH 6.6 adjusted with Potassium Hydroxide as

versatile stage. Stream rate was kept up at 1 ml/min & ID was done at 251 nm where Imipramine hydrochloride (IMI) & diazepam (DIA) have focal absorbance. Upkeep times of Imipramine hydrochloride (IMI) & diazepam (DIA) were 2.85 min & 5.25 min. Compelled debasement studies were done & corruption thing tops were all around looked over prescription tops. Structure was perceived & saw to be sensitive, right & amend & unfaltering quality appearing. Quality indicating HPTLC structure was made using Silica gel GF254 pre-secured on aluminum sheet (10 cm × 10 cm) of 0.20 mm layer thickness (E. Merck KGaA) as stationary stage & Chloroform: Methanol: Hexane: Glacial Acetic Acid (3:3.5:3.5:0.2 v/v/v/v). Tangle section (RF) estimations of 0.25 ± 0.01 for Imipramine hydrochloride & 0.47 ± 0.05 for diazepam (DIA) was found. Certification was done at 251 nm where Imipramine hydrochloride (IMI) & diazepam (DIA) have basic absorbance. Compelled contamination studies were done & debasement thing tops were all around browsed cure tops. Structure was grasped & saw to be unstable, right & reexamine & stability indicating.

Table19: Comparison of developed methods for Imipramine Hydrochloride

Sr. No.	HPTLC method	RP-HPLC method
1	101.12	98.12
2	98.31	97.2
3	97.28	98.25
$\mathbf{F_{cal}}$	1.58	
$\mathbf{F_{tab}}$	4.06	

Table 20: Comparison of developed methods for Diazepam

Sr. No.	HPTLC method	RP-HPLC method
1	98.5	100.75
2	97.65	98.45
3	99	99.13
$\mathbf{F_{cal}}$	1.26	
$\mathbf{F_{tab}}$	4.06	

ANOVA test was applied for comparison of assay results of all proposed methods. F_{cal} was found to be less than F_{tab} & hence it was concluded that all four methods do not differ significantly.

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