IJBPAS, January, 2025, 14(1): 289-302

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STABILITY INDICATING UHPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IMIPRAMINE HYDROCHLORIDE AND DIAZEPAM IN A PHARMACEUTICAL DOSAGE FORM

SHINDE KB*[1], BHANGALE CJ[2] AND VADITAKE KT[3]

1: Department of Pharmaceutical Quality Assurance, PRES's College of Pharmacy (For Women), Chincholi, Nashik

2: Department of Pharmaceutical Chemistry, PRES's College of Pharmacy (For Women), Chincholi, Nashik

3: Department of Pharmaceutical Chemistry, PRES's College of Pharmacy (For Women), Chincholi, Nashik

***Corresponding Author: Dr. Kanchan Shinde: E Mail: charushila.bhangale@pravara.in**

Received 27th Oct. 2023; Revised 28th Nov. 2023; Accepted 8th April 2024; Available online 1st Jan. 2025

https://doi.org/10.31032/IJBPAS/2025/14.1.8614

ABSTRACT

The UHPLC method for simultaneous measurement of imipramine HCL and diazepam was developed and validated. The mobile phase was 70:30 Methanol:water.Imipramine HCL and Diazepam had retention times of 1.263 min and 2.027 min, respectively. Quantification was performed using ultraviolet detection at 252 nm based on the overlay UV spectrum. The linearity range of imipramine HCL was determined to be 5-25 g/mL and that of diazepam to be 1-5 g/mL. The calibration curve was plotted, and the regression equation for Imipramine HCL was y = 1,80,033.80x + 44,004.00 with a correlation coefficient (r2) of 0.9993, and the regression equation for Diazepam was y = 16,40,748.70x + 1,64,942.30 with a correlation coefficient (r2) of 0.9995. The percent recovery of Imipramine HCL was found to be in the range of 98.25-100.40% according to the ICH standards, while Diazepam was found to be in the range of 98.61-

100.40%. Imipramine HCL has a detection limit of 0.02g/mL - 0.05g/mL and a quantitation limit of 0.02g/mL - 0.06g/mL. It was determined that the procedure was simple, linear, rapid, accurate, precise, repeatable, and resilient. The % RSD was found to be within ICH guidelines. The % RSD was found to be within ICH guidelines. Stress degradation tests confirmed the drug's exposure to acid, basic, neutral hydrolysis, oxidative, thermal, and photolytic stress conditions. Under alkali and peroxide stress conditions, the medication degraded most significantly. The results showed that the proposed chromatographic technique was suitable for the accurate, precise, and rapid simultaneous detection of Imipramine HCL and Diazepam in bulk and medicinal dosage form.

Keywords: Imipramine HCL, Diazepam, UPLC method, ICH Guidelines

1. INTRODUCTION:

A tricyclic antidepressant called imipramine (IPM) is prescribed to treat depression and lessen infantile enuresis. The standard tricyclic antidepressant (TCA), imipramine, is a dibenzazepine derivative. TCAs and phenothiazines share a similar structural makeup. They have an alkyl amine substituent on the middle ring of a tricyclic ring structure. Chemically, IPM is 10,11-dihydro-5Hdibenz[b,f] azepine-5-(dimethylaminopropy1) hydrochloride. IPM may produce drowsiness but has no effect on mood or arousal in those who are not depressed. Imipramine elevates mood in those who are depressed. Children with nocturnal enuresis and depression may both benefit from imipramine treatment. Chronic and neuropathic pain, including diabetic neuropathy, panic disorder, ADHD, and post-traumatic stress disorder (PTSD) are examples of unlabelled indications **[1-2]**. Chemical structure of IPM is shown in **Figure 1**. Diazepam (DZP) is a long-lasting, rapidly acting benzodiazepine that is frequently used to treat seizures, severe anxiety, alcohol withdrawal, and panic disorders. a long-acting benzodiazepine having anticonvulsant, anxiolytic, sedative, muscle relaxant, and amnesic qualities. Its effects are brought about by increased gamma-aminobutyric acid activity. It is used to treat severe anxiety disorders, as a hypnotic to treat sleeplessness temporarily, as a sedative and premedicant to treat seizures, as an anticonvulsant, and to treat alcohol withdrawal syndrome **[3-4]**. Chemically DZP is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one. Chemical structure of DZP shown in **Figure 2**.

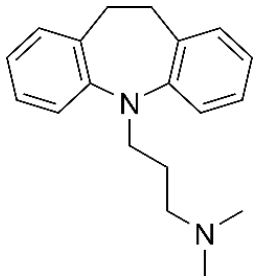

Figure 1: Structure of Imipramine HCL

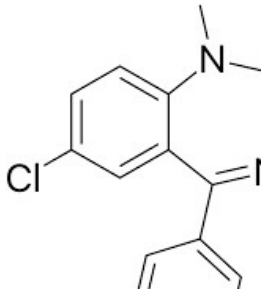

Figure 2: Structure of Diazepam Maleate

IPM and DZP combination is used to treat depression. It decreases the number of episodes of depression and also reduces the duration of each depressive episode. It improves mood, behavior, sleep and decreases anxiety. Literature survey reveals that few UV **[5-8]**, HPTLC **[9-12]** and HPLC **[13-25]** methods are reported for the determination of IPM,DZP in pharmaceutical formulation. The study's goal is to explore the full degradation behaviour of IPM, DZP in bulk and dose forms. The proposed method is quick, simple, accurate, and reproducible, and it can be used to routinely analyses IPM, DZP in bulk samples and pharmaceutical dosage forms. The approach has been validated in accordance with ICH guidelines **[26-28]**. The sample recoveries in the formulation were in good accord with their label claims, and no formulation excipients interfered with the estimate. As a result, this method is simple and convenient for regular examination of bulk medicines in pure form and mixtures.

2.MATERIALS AND METHOD:

2.1Instruments:

The chromatographic process was carried out using a Shimadzu PVT Ltd UPLC system, a variable wavelength programmable UV identifier, and a Rheodyne injector with a 5l fixed circle. A Phenomenex C18 opposing stage (100mm x 2.1ID, particle size: 1.7 micron) was used. Individually, Wenser High Precision Balance Model: PGB 100 electronic equilibrium were used for Spectrophotometric judgements and gauging purposes.

2.2 Reagents and chemicals

Imipramine HCL and Diazepam was procured from PharmaTech Solutions. UHPLC grade Acetonitrile and water were acquired from Merck specialities private restricted, Mumbai.

2.3 Chromatographic conditions

Phenomenex C18 (100mm x 2.1ID, Particle size: 1.7 micron) was utilized for the chromatographic method at wavelength of 252 nm. Methanol: Water (70:30) was used

as the mobile phase for elution, and the same solvent was used to prepare the standard and sample solutions. The elution was tested by infusing the 5µl and changing the flow rate to 0.3 mL/min.

2.4 Preparation of Standard Stock solutions

Accurately weighed and transferred 25 mg IPM and 5 mg DZP working standards into a 100mL clean dry volumetric flask, added 3/4th volume of diluent, sonicated for 5 minutes, and made up to final volume using diluents. IPM has a final concentration of 250 g/mL, while DZP has a concentration of 50 g/mL. These medications' working standard solutions were created by diluting the relevant stock solution with mobile phase.

2.5 Selection of mobile phase

IPM (250g/mL) and DZP (50g/mL) standard solutions were loaded into the RP-UHPLC apparatus and ran in different solvent systems. Initially, different mobile phase systems such as methanol and water were explored in the isocratic mode to determine the optimal conditions.

2.6 UHPLC Method Development

Optimisation of RP-UHPLC method

For the simultaneous measurement of IPM and DZP, the UHPLC method was devised. Different mobile phases were examined for technique optimisation, however Methanol provided excellent retention times, hypothetical plates, and good resolution: Water (70:30v/v) gradient method with Phenomenex C18 (100mm x 2.1mm ID, particle size: 1.7 m) **(Table 1)**.

Table 1: Optimized Chromatographic Conditions

Mobile phase	**Methanol: Water (70:30v/v)**
Selection of column	**Phenomenex C18 (100mm x 2.1mm ID, Particle size: 1.7 µm)**
Injection volume	**5 µL**
Flow rate	**0.3 mL/min**
Column temperature	**Room Temperature**
Detection wavelength	**252 nm**
Run Time	**4.0 minutes**
Retention time	**Imipramine HCl (1.2 min) and Diazepam (2.0 min)**

2.7 Method validation [26-28]

The optimised RP-UHPLC method was validated in compliance with the ICH Q2 (R) requirements.

2.7.1 Linearity

Different concentrations of test solutions were injected individually, and the chromatograms were recorded. A series of IPM and DZP test preparations were made by taking 0.2 mL - 1.0 mL from the stock solution containing IPM (250g/mL) and DZP (50g/mL) in five 10 mL volumetric flasks and making up the final volume with mobile phase. Under optimised chromatographic

conditions, a 20l volume of each concentration was injected into UHPLC three times.

2.7.2 Accuracy

Recovery studies by standard addition were used to test the accuracy of the proposed method. Samples were prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples were analyzed and the recoveries of each are calculated.

2.7.3 Precision

The intraday precision research was carried out by creating a test solution of the same concentration and analysing it three times during the day. To determine interday precision, the identical process was used on two different days. The outcome was reported as %RSD.

2.7.4 Limit of Quantitation (LOQ) & Limit of Detection (LOD)

The detection and quantification limits were determined using concentrations in the calibration curve's lower linear range.The LOD and LOQ were calculated using the formulas LOD = 3.3 s/s and LOQ = 10 s/s from the slope(s) of the calibration curve and the standard deviation (SD) of the peak regions.

2.7.5 Robustness

Robustness was determined by varying the chromatographic conditions such as mobile phase composition, detection wavelength, flow rate, and so on, and the percentage RSD should be provided. Small changes were permitted in the optimal settings, and the robustness of the approach was determined. Individual variances in detecting wavelength of 2 nm and flow rate of 0.1 mL/min were attempted. In the system were injected in duplicate solutions of 100% test concentration with the necessary changes under the optimum circumstances.

2.7.6 Ruggedness:

Ruggedness is the investigation of the influence of external factors on the approach. To assess the robustness of the suggested approach, factors were purposefully altered. These factors included system variation, various analysts, and atmospheric fluctuations. Two distinct analysts prepared the test solution according to the test procedure and injected three concentrations of test solution into the UHPLC system at a flow rate of 0.3 mL/min.

2.7.7 Assay of marketed formulation

20 tablets of Intas Pharmaceutical's marketed formulation (Depsol forte) were taken, weighed separately, and crushed into fine powder. The average weight of the tablet sample was weighed and transferred to a 100 mL volumetric flask, where diluent was added to fill the volume. Sonicate for 10

minutes, spinning occasionally. The produced stock solution contains 250 g/mL of IPM and 50 g/mL of DZP and was filtered via a 0.45m membrane filter. For the analysis, 0.6 mL of the solution was taken in volumetric flask, diluted to 10 mL, and injected into the system

2.7.8 System suitability

System suitability characteristics were tested to validate the system, procedure, and column performance. A standard solution of IPM and DZP was injected into the system six times, and system suitability features were evaluated.

2.8 Forced degradation of IPM and DZP

A stock solution of IPM and DZP was prepared. This solution was used for forced degradation to provide an indication of the stability-indicating property of the method **(Table 2)**.

Table 2: Forced Degradation Conditions according to ICH guidelines

Test Condition	Acidic degradation	Alkaline degradation	Oxidative degradation	Thermal degradation	Photolytic degradation
Imipramine HCl (25 mg) and Diazepam (5 mg)	1N HCl, 1 hr at 60^0c	1N NaOH, 1 hr at 60^0c	3%H_2O_2, 24hrs	Thermal stress for 24 hrs	Photolytic stress for 24 hrs

2. RESULTS

3. 3.1 Optimization of method

Several mobile phases were created by varying the amounts of various aqueous phases and organic modifiers. Methanol: Water (70:30) was eventually chosen as the mobile phase. At a flow rate of 0.3mL/min, the chromatogram obtained from the analysis of the standard solution was IPM (Rt=1.263 min) and DZP (Rt=2.027 min). The UV detection at 252nm was used for quantification. Chromatogram are shown in **Figure 3**.

3.1 Linearity*:*

The analytical method linearity was defined as the technique's capacity to produce test findings that are directly proportionate to the analyte concentration within a given range. The calibration graph was determined by plotting the peak area from the UHPLC chromatograph against the matching concentrations.IPM was shown to be linear in the concentration range of 5-25g/mL, whereas DZP was linear in the concentration range of 1-5g/mL. The calibration curve are shown in **Figure 4 and Figure 5**.

3.2 Accuracy

The method's accuracy defines how close the method's results are to the true value. The accuracy testing results showed that the procedure is accurate within acceptable limits. The % RSD for Imipramine HCL and Diazepam is determined, and all findings are within limits. Acceptable accuracy was

within the range with a maximum RSD of 2.0%. Results are shown in **Table 3**.

3.3 Precision

The repeatability of test results is ensured by intraday and interday precision. Both Imipramine HCL and Diazepam had % RSD values that were less than 2. Results are shown in **Table 4**.

3.4 Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions i.e. Change in flow rate and wavelength. From robustness study % RSD was found to be within limit of 2 % for the Imipramine HCL and Diazepam. Hence it is robust and complies per ICH guidelines. Results are shown in **Table 5**.

3.5 Ruggedness

The proposed method was evaluated by two different analysts. The IPM and DZP results were 99.78%, 99.65 %, and 99.88%, 100.05 %t, respectively. From ruggedness study % RSD was found to be within limit of 2 % for the Imipramine HCL and Diazepam. Hence it complies with the ICH criteria.

3.6 Specificity

The method's specificity was determined by completely separating standard drugs in the presence of additional excipients often seen in dosage forms. Excipients and contaminants had no effect on the conventional medications. As a result, the procedure is distinct.

3.7 Assay of Marketed formulation

The proposed approach was used for pharmaceutical formulation, and the% label claim for IPM and DZP was determined to be 98.28% and 99.41%, respectively. The amount of drugs determined by the proposed approach agreed well with the label claim.

3.8 System Suitability Parameters:

System appropriateness characteristics were measured in order to validate the system, method, and column performance. Six times a standard solution of imipramine HCL and diazepam was injected into the system, and system suitability characteristics were examined. Results are shown in **Table 6**.

3.9 Force degradation studies

Stress testing of the drug material can aid in determining the anticipated degradation products, as well as the stability and specificity of the analytical technique. Degradation experiments were carried out on solutions containing IPM (15µg/mL) and DZP (3µg/mL). In UHPLC, the chromatograms of samples degraded with acid **(Figure 6)**, base **(Figure 7)**, hydrogen peroxide **(Figure 8)**, dry heat **(Figure 9)** and light **(Figure 10)** revealed clearly separated spots of pure IPM and DZP, as well as several additional peaks at varied retention durations. Results of degradation are shown in **Table 7**.

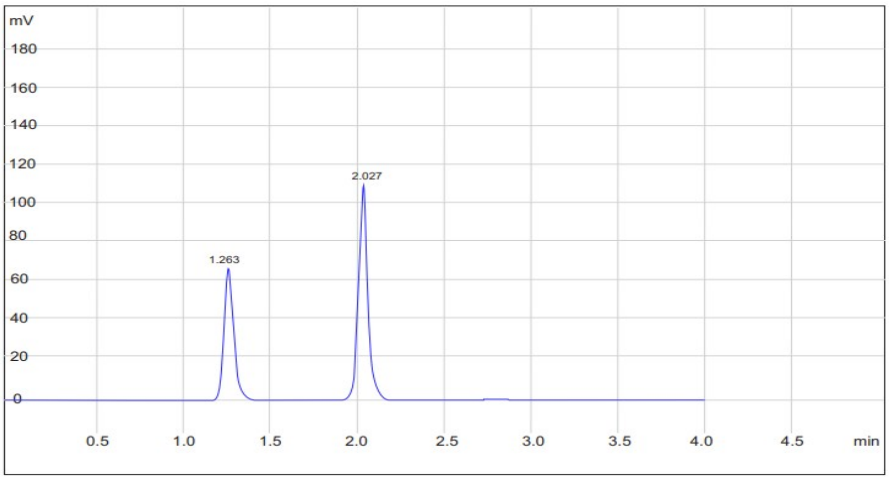

Figure 3: Chromatograph of IPM and DZP

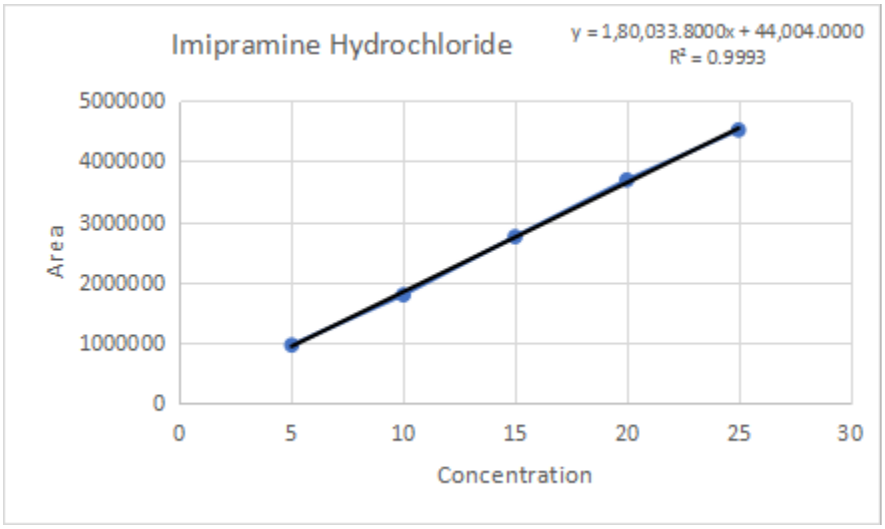

Figure 4: Calibration curve for Imipramine HCL

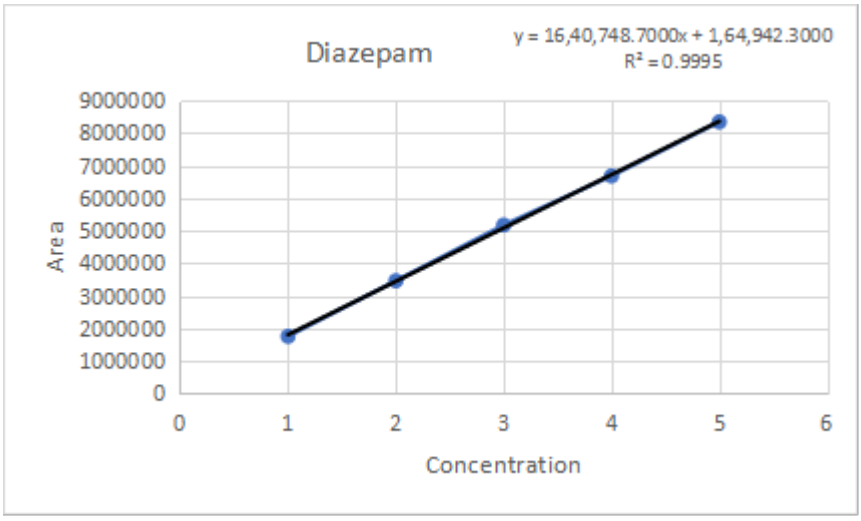

Figure 5: Calibration curve for Diazepam

Table 3: Recovery studies

Level of addition	% Mean recovery*		SD		% RSD	
	IPM	DZP	IPM	DZP	IPM	DZP
50%	99.27	99.29	1.01	0.59	1.01	0.60
100%	99.27	100.08	1.03	0.20	1.03	0.20
150%	99.56	99.90	0.82	0.47	0.83	0.47

Table 4: Precision studies Intraday and Interday

Drug	Conc. [µg/mL]	Intra- day Amount found [µg/mL]		Inter- day Amount found [µg/mL]	
		SD [n= 3]	% RSD	SD [n= 3]	% RSD
IPM	5	2263.72	0.23	955.96	0.10
	15	18776.47	0.68	7871.12	0.29
	25	9179.17	0.20	13742.77	0.30
DZP	1	9117.68	0.52	15033.57	0.84
	3	16873.71	0.33	10049.30	0.19
	5	10774.85	0.13	13881.67	0.17

Table 5: Data for Robustness study of IPM and DZP

Sr. No	Parameter	Condition	Imipramine HCL				Diazepam			
			Area	Mean	SD	% RSD	Area	Mean	SD	%RSD
1	Change in Flow rate (mL/min)	0.2	2754813	2756253	8955.26	0.32	5174869	5172130	8090.45	0.16
2		0.3	2748105				5163026			
3		0.4	2765841				5178496			
1	Change in Wavelength (nm)	250	2751402	2757567.7	10611.77	0.38	5198456	5179124	17459.86	0.34
2		252	2769821				5174413			
3		254	2751480				5164503			

Table 6: System suitability parameter

System Suitability Parameters	Observations	
	IPM	DZP
RetentionTime (min)	1.255	2.097
Capacity Factor(K')	1.12	1.11
Asymmetry factor	1.07	1.09
Tailing factor(T)	1.05	1.09
Therotical plates	9846	9626

Table 7: Results of Forced Degradation Studies

Sr. No.	Condition	Drugs	Area of sample	Area of Standard	% Drug recovered	% Degradation
1	Acid stress	IPM	2501565	2754816	90.81	9.19
		DZP	4806421	5178534	92.81	7.19
2	Alkali Stress	IPM	2554567	2754816	92.73	7.27
		DZP	4835643	5178534	93.38	6.62
3	Oxidative Stress	IPM	2265440	2754816	82.24	17.76
		DZP	4082418	5178534	78.83	21.17
4	Thermal Stress	IPM	2617515	2754816	95.02	4.98
		DZP	4998562	5178534	96.52	3.48
5	Photolytic Stress	IPM	2715420	2754816	98.57	1.43
		DZP	5035357	5178534	97.24	2.76

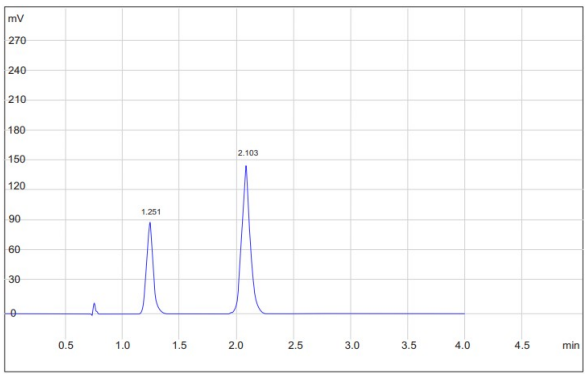

Figure 6: Acid Stressed Chromatogram

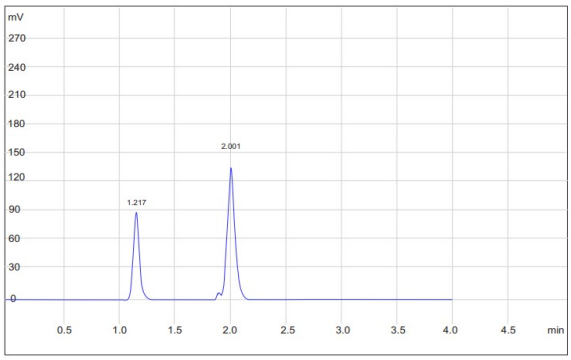

Figure 7: Alkali Stressed Chromatogram

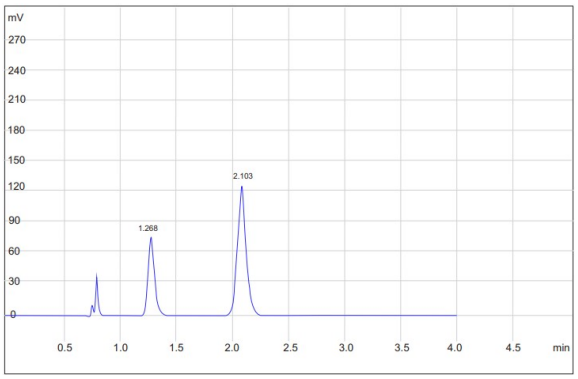

Figure 8: Peroxide Stressed Chromatogram

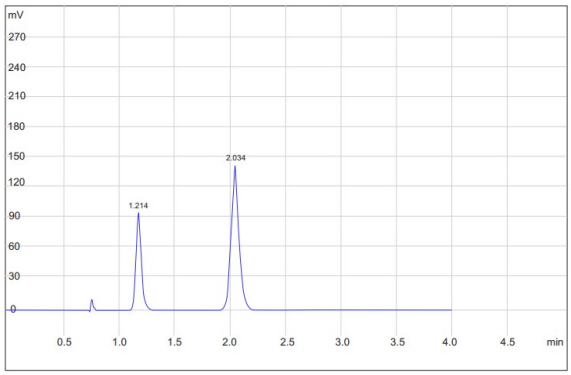

Figure 9: Thermal Stressed Chromatogram

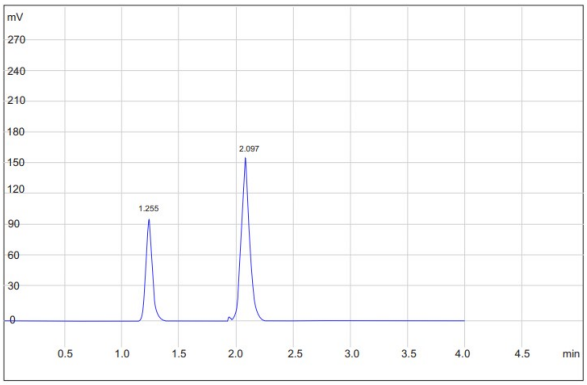

Figure 10: Photolytic Stressed Chromatogram

4. DISCUSSION

The IPM was found to be linear in the concentration range of 5-25 g/mL, while DZP is 1-5 g/mL. According to the accuracy study, the percentage recovery of IPM was found to be in the range of 98.25-100.40% and DZP was found to be in the range of 98.61-100.40%, which is within the ICH requirements. Intraday and interday precision ensure that % RSD was within ICH requirements, i.e., NMT 2 for both IPM and DZP. IPM has a detection limit of 0.02g/mL - 0.05g/mL and a quantification limit of 0.02g/mL - 0.06g/mL. Robustness was

investigated using purposeful variation, i.e., a change in flow rate and a change in wavelength that was within 2% of RSD according to ICH criteria. The ruggedness analysis revealed results within 2% of the range in Analyst examined. The % assay of Depsol forte was discovered to be IPM (98.28%) - DZP (99.41%). There were no chromatographic interferences from the tablet excipients observed. The forced degradation study was used to determine the stability of IPM and DZP **[32-33]**. The chromatograms of materials degraded with acid, base, hydrogen peroxide, and heat exhibited clearly separated spots of pure IPM and DZP, as well as some additional peaks at varied Rt values.

5. CONCLUSION:

The proposed chromatographic method for determining IPM and DZP from pure and dose forms was found to be simple, precise, accurate, quick, and specific. The mobile phase employed for method development is simple to prepare and inexpensive. The sample recoveries in the formulation were excellent. Among all the established methods, this approach is the most cost-effective and has the shortest run time, allowing for rapid analysis. As a result, this method may be simply and conveniently used for in-vitro dissolution and routine analysis of IPM and DZP in Pharmaceutical dosage form.

The degradation of IPM (15µg/mL) and DZP(3µg/mL) solutions was studied. The force degradation investigation was carried out under five different conditions: acidic, alkaline, oxidative, thermal, and photolytic degradation. The degradation products generated during the stability investigation were well isolated from the pure medication, demonstrating the uniqueness of the developed technique. The predominant degradation of the drugs was discovered to occur under acid and peroxide stress conditions.

6.ACKNOWLEDGEMENT

The authors are very much thankful to PRES's College of Pharmacy, For Women, Nashik for his support and also thank to the management for providing the necessary facilities to carry out this research work.

7.CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERNCES

[1] https://go.drugbank.com/salts/DBSALT000099

[2] https://go.drugbank.com/drugs/DB00829

[3] https://pubchem.ncbi.nlm.nih.gov/compound/Imipramine-hydrochloride

[4] https://pubchem.ncbi.nlm.nih.gov/compound/Diazepam

[5] Umapathi P, Parimoo P, Second-order derivative spectrophotometric assay for imipramine hydrochloride and diazepam in pure admixtures and in dosage forms. J Pharm Biomed Anal; 13:1003–1009.

[6] Ullah Khan I, Aman T. Kazi AA, Azizuddin R. Spectrophotometric Determination of Imipramine-HCl in Pure and Pharmaceutical Preparations. Anal Lett. 1999;32:2061–2069.

[7] Sadeghi S,Takjoo R, Haghgoo S. Quantitative determination of diazepam in pharmaceutical preparation by using a new extractive-spectrophotometric method. Anal Lett. 2002;35:2119–2131.

[8] Daharwal S J. Development and validation of UV spectrophotometric method for simultaneous estimation of diazepam and propranolol in bulk drug and its formulations. Asian J. Pharm. Ana. 2013; 3(1):20-23.

[9] Srivastava V, Kumar P, Jat RK. Validated stability indicating HPTLC method for diazepam and imipramine in bulk and combined dosage form.Int. J. Drug Res. Tech. 2016;6 (1): 7-21

[10] Patel S. Development and Validation of HPTLC method for simultaneous determination of diazepam and propranolol hydrochloride in tablet dosage form. Int J Pharma Front Res. 2011; 1(3): 29-37

[11] Patel SK, Patel NJ .Simultaneous Determination of Imipramine Hydrochloride and Chlordiazepoxide in Pharmaceutical Preparations by Spectrophotometric, RP-HPLC, and HPTLC Methods Journal of AOAC International.2010; 93(3) :904–910, https:// doi. org/10.1093/jaoac/93.3.904

[12] Prakash SG Neeraj U, Gopal G. Development and validation of spectrophotometric, HPTLC and HPLC methods for the determination of Imipramine and Chlordiazepoxide in pharmaceutical dosage forms. Der Pharmacia Sinica. 2012; 3(2): 185-192.

[13] Srikantha D, Raju RR. RP-HPLC estimation of Imipramine Hydrochloride and Diazepam in tablets. Indian J Pharm Sci. 2015 ; 77(3): 343–347.doi: 10.4103/0250-474x.159672

[14] Laloup M, Mdel FM, Wood M, Maes V, De Boeck G, Vanbeckvoort Y.

Detection of diazepam in urine, hair and preserved oral fluid samples with LC-MS-MS after single and repeated administration of Myolastan and Valium. Anal Bioanal Chem. 2007;388:1545–56.

[15] Reddy RK, Swathi D. RP-HPLC Method for Simultaneous Estimation of Alprazolam and Imipramine as Per ICH Guidelines, Indo Am. J.P. Sci, 2018; 05(04):2027-2039.http://doi.org/10.5281/zenodo.1214513

[16] Pydiratnam P, Kumar TS. Satyanarayana SSS. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Imipramine Hydrochloride and Diazepam in combined pharmaceutical dosage form. International Journal of Pharmaceutics and Drug Analysis. 2013:13-24, https://www.ijpda.com/index.php/ journal/article/view/4.

[17] Maheshwari M, Soni P. RP-HPLC Method development and validation for rapid estimation of Diazepam in bulk and pharmaceutical dosage form. Research Journal of Pharmacy and Technology. 2022, 15(5),1938-1942. doi: 10.52711/0974-360X.2022.00322

[18] Rezazadeh M, Emami J. A simple and sensitive HPLC method for analysis of imipramine in human plasma with UV detection and liquid-liquid extraction: Application in bioequivalence studies, Research in Pharmaceutical Sciences. 2016; 11(2): 168-176

[19] Abbara C, Bardot I, Cailleux A, LallementG, Le Boui A, Tureant A. High performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) method for simultaneous determination of diazepam, atropine and pralidoxime in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2008;874:42-50.

[20] Choudhury T, Ghosh J, Bagchi D. Reverse phase high performance liquid chromatographic method and method validation of imipramine by using single mobile phase. Int J Pharma Sci Tech. 2010; 4(1): 54-62.

[21] Yadav D, Dave M. Development of RP-HPLC method and stability studies for simultaneous estimation of Imipramine HCL and Diazepam in

bulk and pharmaceutical formulations., International Journal of Pharmaceutical Research and Applications. 2021;6(3): 558-592.DOI: 10.35629/7781-0603558592

[22] Mercolini L, Mandrioli R, Iannello C, Matrisciano F, Nicoletti F, Raggi MA. Simultaneous analysis of diazepam and its metabolites in rat plasma and brain tissue by HPLC-UV and SPE. Talanta. 2009;80:279–285. doi: 10.1016/j. talanta.2009.06.074.

[23] Rouini MR, Ardakani YH, Moghaddam KA, Solatani F. An improved HPLC method for rapid quantitation of diazepam and its major metabolites in human plasma. Talanta. 2008;75:671–676. doi: 10.1016/j. talanta. 2007. 11.060.

[24] Rani TG, shankar GD, Kadgapathi P, Satyanarayana B. Development of an RP-HPLC method for the simultaneous estimation of propranolol hydrochloride and diazepam in combined dosage form. Ind J Pharm Edu Res. 2011; 45(4): 296-300.

[25] Kim DH, Cho JY. Development of a simple and sensitive HPLCMS/MS method for determination of diazepam in human plasma and its application to a bioequivalence study. Transl Clin Pharmacol, 2017;25(4):173-178. doi: 10.12 793/tcp.2017.25.4.173

[26] ICH, Q2 (R1) Validation of Analytical Procedure: Test and Methodology, International Conference on Harmonization, Geneva, 2005.

[27] ICH, Q2A Validation of Analytical Procedures: Consensus Guidelines; ICH Harmonized Tripartite Guidelines, 1994.

[28] ICH, Q2B Validation of Analytical Procedures: Methodology, Consensus Guidelines ICH Harmonized Tripartite Guidelines, 1996.