IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 15, Issue 01, 2026

NOVEL RP-HPLC METHOD FOR THE DETERMINATION OF VILOXAZINE: DEVELOPMENT, VALIDATION, AND APPLICATION TO STABILITY STUDIES

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Abstract:

Background: Viloxazine hydrochloride is a non-stimulant norepinephrine reuptake inhibitor used for the treatment of attention deficit hyperactivity disorder (ADHD).

Materials and Methods: From the literature review, we have noticed the use of Methanol, Acetonitrile, Potassium phosphate, Isopropyl alcohol and Ortho phosphoric acid (OPA), HPLC water, as the mobile phases for the analysis work of Viloxazine Hydrochloride and the reported retention time was in between 2.2 to 3.8 min at the flow rates of greater than 1ml/min. **Results and Discussion:** In the present work, we have used Methanol and 0.1% OPA (75:25v/v) as the mobile phase and the retention time was 3.1 minutes at the flow rates of 1ml/min. The Column was C_{18}(4.6mm x 250mm;5µm) and detector was UV-PDA (λ_{max} 230nm). Linearity concentrations were 10µg/ml to 50µg/ml(R^2-0.9985). All the validation parameters and degradation studies complied with the limits of ICH Q2R1 guidelines. **Conclusion:** This developed method consumes mobile phase with methanol and water containing OPA (0.1%) which are cheaper and environment friendly. Thus, the method can be considered as green method as compared to the already reported methods.

Key words: Methanol, Ortho phosphoric acid, RP-HPLC, Viloxazine Hydrochloride.

INTRODUCTION:

2-[(2-ethoxyphenoxy) methyl] morpholine hydrochloride, also known as viloxazine hydrochloride. It is freely soluble in organic solvents such as Water, 0.1N HCl, DMSO. It is Very slightly soluble in acetonitrile, acetic acid, isopropyl alcohol, and sparingly soluble in methanol. The FDA has approved QELBREE, an extended-release viloxazine formulation, for the treatment of attention deficit hyperactivity disorder (ADHD). The molecular weight is 237.299 g/mol^{-1}, and the empirical formula is $C_{13}H_{20}ClNO_3$. Its pKa value is 8.19.

Viloxazine extended-release has a roughly 88% relative bioavailability when compared to an immediate-release formulation. Viloxazine dosage ranges from 100 mg to 600 mg once daily, with a proportionate increase in Cmax and AUC. The range of the Cmax is 540–1600 ng/mL. The median T_{max} was almost five hours after a single 200 mg dose was administered, with a range of three to nine hours. Two days of once-daily administration resulted in a steady condition with no buildup. A high-fat meal causes a two-hour delay in T_{max} and a 9% and 8% drop in Cmax and AUC, respectively. IR elimination half-life: 2–5 hours, ER elimination half-life: 7.02 ± 4.74 hours, and excretion urine (~90%), faeces (<1%)[1-5].

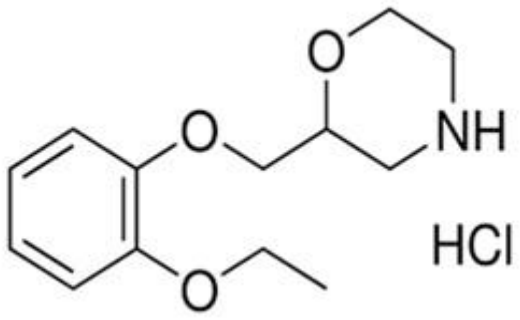

Figure1: Chemical structure of Viloxazine Hydrochloride

Y. venkatarao, D. Ramachandran, P. Bhrath, K. Rameseshaiah and Sampath Upparapalli create a new stability indicating technique utilising RP-HPLC to identify related compounds of viloxazine hydrochloride in an API form. The techniques make use of an Agilent Eclipse XDB C8 column (250x4.6x5µm) with mobility phase such as potassium phosphate and acetonitrile, mobile phase (A) 20 mM monobasic potassium phosphate, and mobile phase (B) acetonitrile and water (90:10%, v/v), with phosphoric acid to bring the pH down to 2.35, at a flow rate of 0.8 mL/min[6]. Shaik Mastanamma, ChunduriAkhila, M.S. Dhandapani reported the development and validation of the Chiral HPLC Method for S-Viloxazine Estimation and FD Characterisation by MS. The techniques use an achiral pack IM C 18 column (250 x 10 mm, 5 µm) with a flow rate of 1 mL/min and a mobile phase such as methanol, n- hexane, or IPA (30:50: 20 v/v)[7]. Nakka Madhuri, G. Surekha, M. Venkatesh, Dr.A. Yasodha outlined the novel stability suggesting high performance liquid chromatography for pharmaceutical dose form and bulk viloxazine determination. The techniques employ a C 18 column (150 x 4.6 mm, 5 µm) with a flow rate of 1 mL/min and a mobile phase such as 0.01 N Phosphate buffer: methanol (80: 20) and 0.01 N Disodium Phosphate buffer: methanol (80: 20)[8]. Dr. K. Swathi Priya, S. Sravani, and Dr. K. Atchuta Kumar explain how to estimate viloxazine using the rp-hplc approach. The majority of techniques employ a C 18 column (250 x 4.6 mm, 5 µm) with a mobile phase such as 0.01N KH2po4: acetonitrile (70:30). phase (A) 0.1% orthophosphoric acid (35:65) and mobile phase (B) acetonitrile at a flow rate of 1 mL/min[9].

MATERIALS AND METHODS

Materials

The instruments were Shimadzu LC-20AD HPLC, Shimadzu UV-1800ENG240V UV Visible Spectroscopy, Shimadzu ATY224 electronic balance, digital ultrasonic cleaner

(SONICA2200MH), hot air oven (VISION LAB EQUIPMENT), and UV Cabinet (MONOQUARTZ).

Chemicals

The chemicals used in this work included Viloxazine Hydrochloride (JSL Laboratories Pvt Ltd), Methanol-HPLC grade (Merck), Water (HPLC grade, Merck), ortho phosphoric acid (s d fine-chem limited).

RP-HPLC Chromatographic conditions:

HPLC with PDA was made use of to carry out the project. Methanol and 0.1% orthophosphoric acid (75:25, v/v) was the ratio that was selected after experimenting with several mobile phase solvent combinations to achieve the required system suitability parameters. In order to achieve a working standard of 10 µg/ml, 10 mg of Viloxazine Hydrochloride was dissolved in 10 ml of diluent to create the standard solution. Subsequent dilutions were made using the mobile phase. A range of Viloxazine Hydrochloride dilutions with different concentrations (10 to 50 µg/ml) were made. After injecting the solutions into the chromatographic apparatus using a 20 µl fixed loop (manual injector) at a flow rate of 1 ml/min, chromatograms were produced and the effluents were seen at 230 nm. At 3.1 minutes, the peak was eluted with a run duration of 4.5 minutes. The procedure was expanded to determine the pharmaceutical dose form of Viloxazine hydrochloride. Viloxazine hydrochloride capsules were prepared by the direct blending method. Viloxazine hydrochloride pills preparation quantities are provided in the assay formulation table 1.

Accurately weighed quantities of Viloxazine Hydrochloride, microcrystalline cellulose, lactose monohydrate, and croscarmellose sodium were individually passed through sieve No. 40 to remove aggregates and to ensure uniform particle size. The sifted materials were transferred into a clean and dry mortar and blended thoroughly for 10–15 minutes to obtain a homogeneous mixture. To increase the flow qualities of the powder blend, colloidal silicon dioxide was added to the above blend and gently stirred for 3-5 minutes after passing it through sieve No. 60.

Finally, magnesium stearate (passed through sieve No. 60) was added as a lubricant and mixed for 2-3 minutes, taking care not to over-lubricate. Using a manual capsule filling machine, the finished lubricated blend was packed into appropriate-sized hard gelatin capsules. The filled capsules were properly sealed, polished to remove any sticking powder, and stored in airtight containers for further analysis.

The 30 mg strength medicinal dosage form was taken, and 20 Viloxazine Hydrochloride pills (label claim 30 mg) were weighed, then ground into a powder using a glass mortar and pestle.

The powder, equivalent to 10 mg of Viloxazine Hydrochloride, was then transferred to a 10-ml volumetric flask and adjusted to 10 ml using diluents (mobile phase). To reach the working standard of 10 µg/ml, further dilutions were done using the mobile phase, and a Micropore filter of 0.45 µm pore size was used to filter the solution. The drug's concentration in the capsule sample solution was determined by comparing it to that of the standard's peak area. The API handles characteristics including system appropriateness, linearity, LOD, and LOQ, while the

capsule powder handles precision, accuracy, robustness, and degradation studies. The suggested approach was verified in accordance with ICH principles.

Table 1: Assay formulation table

S.no	Excipients	Official Formula (For 1 Capsule)	Working Formula (For 20 Capsules)
1	Viloxazne Hydrochloride (API)	50 mg	1000 mg
2	Microcrystalline Cellulose	30 mg	600 mg
3	Croscarmellose Sodium	5 mg	100 mg
4	Magnesium Stearate	1 mg	20 mg
5	Collidal Silicon Dioxide	0.5 mg	10 mg
6	Lactose Monohydrate	q.s. to 150mg	q.s. to 3000mg

RESULTS AND DISCUSSION

System suitability

Six replicates of the prepared concentration were taken in order to assess system suitability. After injecting six duplicates at 30 µg/ml under optimum HPLC conditions, the chromatograms were evaluated to determine the peak heights, retention lengths, tailing factors, theoretical plates, and peak areas. Figure 2 displays the chromatogram that was produced with optimal circumstances at a concentration of 30 µg/ml whereas Table 2 contains the values of the system suitability parameters.

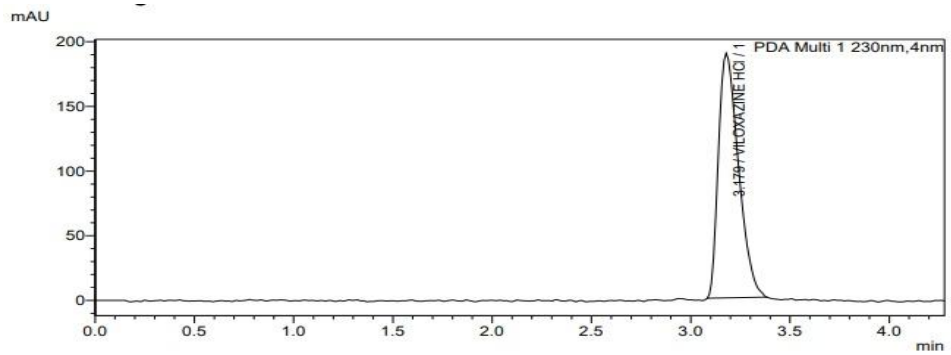

Figure 2: Chromatogram of Viloxazine Hydrochloride under optimized conditions.

Table 2: System suitability parameters for the suggested HPLC technique.

S.NO	Peak Area	Retention duration(min)	Plate Count	Peak Height	Tailing
1	1333164	3.179	3861	189150	1.473
2	1362576	3.178	3807	189875	1.511
3	1324598	3.18	3869	186576	1.46
4	1327429	3.178	3875	188155	1.465
5	1325818	3.178	3843	187124	1.481
6	1347637	3.18	3834	189325	1.491
Average	1336870.33	3.18	3848.17	188367.50	1.26
STDEV	15181.19	0.00	25.46	1311.69	0.02
% RSD	1.14	0.03	0.66	0.70	1.49

Limits	_	_	>2000	_	<2.0
% RSD	<2.0	<2.0	<2.0	<2.0	<2.0

Linearity

Viloxazine hydrochloride was found to have passed the linearity range of 10–50 µg/ml. An R^2 of 0.9985 was discovered. A Plot the peak area against medication solution concentration resulted in a calibration curve. The values of peak area for the corresponding concentrations are displayed in Table 3, and Figure 3 displays the corresponding graph.

Table 3: Viloxazine Hydrochloride Linearity

S. No	Concentration(µg/ml)	Peak area
1	10	857078
2	20	1074091
3	30	1341813
4	40	1551875
5	50	1820183

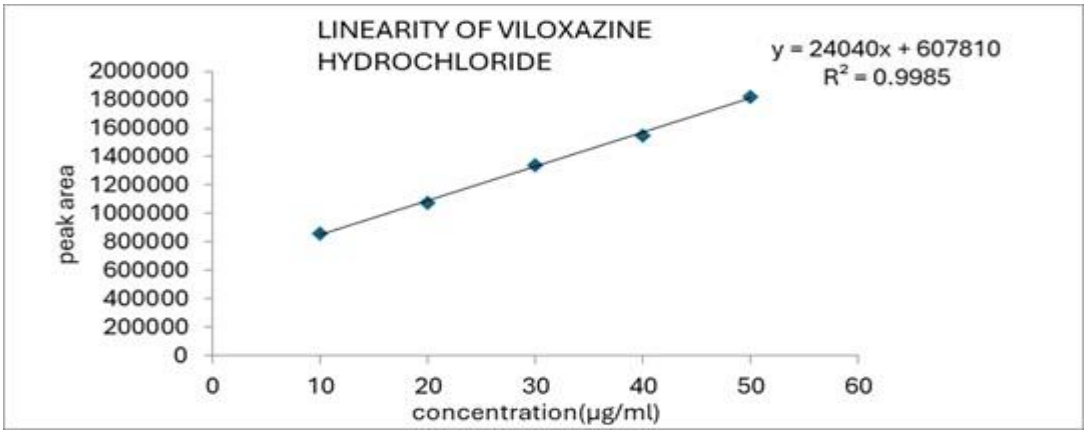

Figure 3: Viloxazine Hydrochloride Linearity graph

Precision

The precision test was passed when a 100% concentration (30µg/ml) was dispensed into the chromatograghic apparatus and the percentage RSD seem less than 2%. The percision values are shown in table 4.

Table 4: The suggested HPLC method's precision

	Peak Area		
S.No	**Day 1**	**Day 2**	**Day 3**
1	1349575	1303691	1382132
2	1344980	1339921	1370939
3	1342639	1327733	1397440
4	1338366	1330125	1333438
5	1351333	1322394	1363470
6	1358130	1322041	1361919
Average	1347503.83	1324317.50	1368223.00

STDEV	7005.77	12026.61	21572.19
% RSD	0.52	0.91	1.58
Limits	% RSD: <2.0	% RSD: <2.0	% RSD: <2.0

Accuracy

A series of solutions containing 50%, 100%, and 150% were made using the powdered capsule equal to the medication Viloxazine. Analysis of the chromatograms that resulted from the aforementioned concentrations revealed that the percentage recovery was within the acceptable range of 98–102%. The values being displayed in table 5.

Table 5: Accuracy displaying recovery percentage

Accuracy level	Conc Taken (µg/ml)	Conc Added (µg/ml)	Peak area	Conc Recovered (µg/ml)	% Recovery	lean % ecovery
50%	30	15	1655099	13.56	90.42	8.78
50%	30	15	1688963	14.97	98.32	
50%	30	15	1711606	15.91	106.09	
100%	30	30	2087837	31.56	105.21	9.53
100%	30	30	1969631	26.64	88.82	
100%	30	30	2082944	31.36	104.53	
150%	30	45	2434421	45.98	102.18	01.71
150%	30	45	2439345	46.18	102.63	
150%	30	45	2414233	45.14	100.31	

LOD & LOQ

The results show that the limit of quantification (LOQ) was 5.744µg/ml and the limit of detection (LOD) was 1.895µg/ml. Table 6 summarises these parameters.

Table 6: HPLC Method LOD and LOQ

Parameters	Slope from Linearity	SD of peak from system suitability
	24040	13809.22
LOD =3.3xSD/Slope	1.895µg/ml	
LOQ = 10xSD/Slope	5.744µg/ml	

Robustness

A sample solution of 100% concentration was prepared and injected into the chromatographic

apparatus by following the below chromatographic conditions. The observed values fall within the acceptable ranges.

Table 7: HPLC method Robustness

Parameter	Condition	RT	Peak area	Theoritical plates	% Assay
Flow rate (ml/min), 1±0.2	0.8ml/min	3.921	1375621	4250	102.66
	1.0 ml/min	3.187	1322041	4272.00	98.66
	1.2ml/min	2.663	1361589	3536	101.62
Temp (°C), 30±5°C	25	3.196	1352732	4229	100.95
	30	3.185	1322041	4272.00	98.66
	35	3.182	1318979	4260	98.44
Wave length (nm), 230±2	228nm	3.189	1341120	4220	100.09
	230nm	3.185	1322041	4272.00	98.66
	232nm	3.19	1318891	4237	98.43

DEGRADATION STUDIES

1.Acid degradation

From the prepared stock, 0.3 ml of the sample solution was transferred into a 10 ml volumetric flask. solution, 1 ml of 0.1M HCl was added, and it was heated to 50°C for 15 minutes. chilled. After adding 1 ml of 0.1N NaOH to this cooled solution to neutralise it and make up the mark with mobile phase, it was injected into an HPLC, and the peak responses were noted.

2. Base degradation

Using the prepared stock, 0.3 ml of the sample solution was transferred into a 10 ml volumetric flask. solution, 1 ml of 0.1N NaOH was added, and it was heated to 50°C for 15 minutes before being cooled. To neutralise the solution and make up the mark with mobile phase, 1 ml of 0.1M HCl was added to the cooled solution. The mixture was then injected into an HPLC, the peak responses were recorded.

3.Peroxide degradation

A 10 ml volumetric flask was filled with 0.3 ml of the sample solution from the prepared stock solution. 1 ml of 3% H_2O_2 was added to this, and the flask was filled to the mark using mobile phase. The solution was then allowed to sit at room temperature for 12 hours before being injected into the HPLC.

4. Thermal degradation

From a 10 ml volumetric flask containing the stock solution, 0.3 ml of the sample solution was extracted, and using mobile phase, the volumetric flask was filled to the mark to reach 30µg/ml. This solution was maintained at 60°C above the heating mantle. This solution was immediately injected into the HPLC when the 30-minute period was over and cooled. The peak responses were noted.

5. Photolytic Degradation

In order to reach 30µg/ml, a volume of 0.3 ml of sample solution was extracted from a 10 ml volumetric flask that contained the stock solution and filled with mobile phase to the flask's

mark. This resolution was stored in a UV cabinet for one hour under UV light. After this, the solution was directly injected into the HPLC after the one-hour period was up and the peak responses were noted.

Table 8: Degradation studies

S. No	Condition	Peak Area	% Assay	% Degradation
1	Acid	1200483	89.592	10.408
2	Base	1208899	90.221	9.779
3	Temperature	1218741	90.955	9.045
4	Peroxide	1216797	90.810	9.190
5	UV	1229051	91.725	8.275

Summary table of the parameters for the method development, validation, and degradation studies

Table 9: Summary

S. No	Parameters	Results
1	Mobile phase	Methanol: OPA(75:25)
2	Wavelength detection	230nm
3	Calibration range(µg/ml)	10-50µg/ml
4	Regression equation	y = 24040x + 607810
5	Slope(m)	24040
7	Correlation coefficient(r^2)	0.9985
8	Retention time	3.1min
9	Systems Suitability	1.14(%RSD)
10	LOD(µg/ml)	1.895µg/ml
11	LOQ(µg/ml)	5.744µg/ml
12	Accuracy	98.42-101.71%
13	Inter-day Precision	0.52-1.58(%RSD)
14	Intraday Precision	0.34-1.05(%RSD)
15	Robustness Flowrate	98.66-102.66%
16	Robustness Temperature	98.44-100.95%
17	Robustness Wavelength	98.43-100.09%
18	Acid degradation	89.5%
19	Base degradation	90.2%
21	H_2O_2 degradation	90.8%
22	Thermal degradation	90.9%
23	Photolytic degradation	91.7%

Conclusion

RP-HPLC was used to create a method for estimating the amount of viloxazine hydrochloride in API and its capsule form. Methanol and OPA, which are relatively less expensive, environmentally friendly than the solvents used in the previously published methods. Thus, in the assay of viloxazine hydrochloride in API and dosage form, we may regard this approach as sensitive, cost-effective, repeatable, significantly quick and can be considered as green method. For the regular analysis of Viloxazine Hydrochloride, this approach can be utilised confidently and without hesitation.

Acknowledgement

This research was not funded by anyone government or private organization. It is self-financed.

Author contributions

All authors made contributions to design, acquisition of data, and interpretation of data, agreed to submit to the current journal, and gave final approval of the version to be published.

Ethical approvals

In this study, no experimental animals or human subjects were involved.

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