Development and validation of a new analytical RP-HPLC method for the determination of flavoxate hydrochloride in bulk form and pharmaceutical dosage form

Vegesna Kalyan Varma [1*], Chaganti Mukesh [2], Neenavath Kalyan [3], Walliqul Hoque [4]
[1]Associate Professor, Department of Pharmaceutical Analysis, Sree Dattha Institute of Pharmacy Sheriguda(V), Ibrahimpatnam (M), Telangana, India
[2-5] Department of Pharmaceutical Analysis, Sree Dattha Institute of Pharmacy, Sagar Road, Sheriguda, Ibrahimpatnam, R.R, Telangana, India

* Corresponding Author: **Vegesna Kalyan Varma**

Article Info

ISSN (Online): 2582-8940
Volume: 05
Issue: 01
January-March 2024
Received: 02-01-2024;
Accepted: 05-02-2024
Page No: 42-48

Abstract
A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Flavoxate HCL, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and water (45:55% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 260nm. The retention time of the Flavoxate HCL was 2.379 ±0.02min respectively. The method produce linear responses in the concentration range of 24-120mg/ml of Flavoxate HCL. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations. The method was validated for accuracy, precision, linearity, robustness, ruggedness and LOD & LOQ of standard solution. The developed RP-HPLC method was found to be accurate, precise, linear, and robust and was successful applied to a pharmaceutical tablet formulation for qualitative estimation of Flavoxate HCL in Bulk form and Marketed Pharmaceutical Dosage forms.

Keywords: Flavoxate HCL, RP-HPLC, Method Development, Validation, Accuracy

Introduction

Flavoxate is a muscarinic antagonist and spasmolytic used for the symptomatic relief of conditions associated with lack of muscle control in the bladder, such as dysuria, urgency, and nocturia. A drug that has been used in various urinary syndromes and as an antispasmodic. Its therapeutic usefulness and its mechanism of action are not clear. It may have local anesthetic activity and direct relaxing effects on smooth muscle as well as some activity as a muscarinic antagonist. Flavoxate Hydrochloride [1] is the hydrochloride salt form of Flavoxate, a parasympatholytic agent with antispasmodic activity. Flavoxate hydrochloride competitively binds to muscarinic receptors, thereby preventing the actions of acetylcholine. This relaxes vascular smooth muscle, mainly of the urinary tract, and prevents smooth muscle contractions. Flavoxate is a spasmolytic flavone derivative that acts by relaxing the smooth muscle in the urinary tract. Flavoxate [2] is a competitive muscarinic receptor antagonist indicated for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and urinary frequency.Flavoxate [3] (flay vox' ate) is a synthetic quaternary ammonium anticholinergic which inhibits the muscarinic actions of acetylcholine on autonomic nerve endings, decreasing the smooth muscle tone of bladder and gastrointestinal tract. Flavoxate hydrochloride [4] is contraindicated in patients who have any of the following obstructive conditions: pyloric or duodenal obstruction, obstructive intestinal lesions or ileus, achalasia, gastrointestinal hemorrhage and obstructive uropathies of the lower urinary tract. The IUPAC Name of Flavoxate HCL [5] is 2-piperidin-1-ylethyl 3-methyl-4-oxo-2-phenylchromene-8-carboxylate; hydrochloride. The Chemical Structure of Flavoxate HCL is as follows

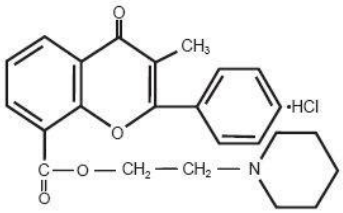

Fig 1: Chemical Structure of Flavoxate HCL

Several methods [27-30] have been reported for the determination of Flavoxate HCL by UV-visible Spectrophotometry and by HPLC, UPLC in bulk and also in human serum. In this context, a simple, sensitive and economic reversed phase HPLC method has been developed and validated for the quantification of Flavoxate HCL in bulk and pharmaceutical dosage forms.

Materials and Methods

Instruments Used

Table 1: Instruments used

S. No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, PDA 996 Detector.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals Used

Table 2: Chemicals Used

S.No.	Chemical	Brand names
1	Flavoxate HCL (Pure)	Local Market
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Method Development

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Flavoxate HCL working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.72ml of the above Flavoxate HCL stock solutions[6] into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [7-10].

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase [11] was optimized to Methanol and Water in proportion 45:55 v/v respectively.

Optimization of Column

The method was performed with various C18columns like ODS column, Xterra, and X Bridge C18 column. Symmetry C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution [12] at 1ml/min flow.

Preparation of Mobile Phase

Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of HPLC Water (55%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [13].

Diluent Preparation

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

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

Further pipette 0.72ml of the above Flavoxate HCL stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [14].

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [15].

Specificity

Preparation of Standard Solution

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

Further pipette 0.72ml of the above Flavoxate HCL stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Flavoxate HCL sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent [16] and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.72ml of Flavoxate HCL above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay [17] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity

Accurately weigh and transfer 10 mg of Flavoxate HCL working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[18]. (Stock solution)

Preparation of Level – I (24ppm of Flavoxate HCL)

Pipette out 0.24ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – II (48ppm of Flavoxate HCL):

Pipette out 0.48ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – III (72ppm of Flavoxate HCL):

Pipette out 0.72ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – IV (96ppm of Flavoxate HCL):

Pipette out 0.96ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – V (120ppm of Flavoxate HCL):

Pipette out 1.2ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Procedure

Inject each level into the chromatographic system[19] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [20].

Precision

Repeatability

Preparation of Flavoxate HCL Product Solution for Precision

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

Further pipette 0.72 ml of the above Flavoxate HCL stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For Preparation of 50% Standard Stock Solution

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

Further pipette 0.36ml of the above Flavoxate HCL stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution

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

Further pipette 0.72ml of the above Flavoxate HCL stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 150% Standard Stock Solution

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

Further pipette 1.08ml of the above Flavoxate HCL stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Flavoxate HCL and calculate the individual recovery and mean recovery values.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution

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

Further pipette 0.72ml of the above Flavoxate HCL stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.7 ml/min and 0.9 ml/min instead of 0.8ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of Mobile Phase Organic Composition
The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio and 40:60, 50:50 instead of 45:55, remaining conditions are same.10µl of the above sample was injected and chromatograms were recorded.

Results and Discussion

Development of a Method

Different chromatographic conditions were tried for better separation and resolution. Symmetry C_{18} (4.6×150mm) 5µmcolumn was found satisfactory. Peak purity of Flavoxate HCL was checked using UV detector and 260 nm was considered satisfactory for detecting the drug with adequate sensitivity. A number of solvents in the different ratio over a wide range of pH were tried, but either peak shape was broad or resolution was not good. Repeated trials to obtain good, sharp peak with an efficient resolution between two peak of Flavoxate HCL done on a C_{18} column in isocratic HPLC gave satisfactory results. The run time was good in isocratic trial with mobile phase consisting of Methanol and Water (45:55% v/v) and Symmetry C_{18} (4.6×150mm) 5µm Column, flow rate 0.8 ml/min and detection wavelength 260 nm gave the satisfactory results in terms of retention time, resolution, symmetry and sensitivity. A typical RP-HPLC chromatogram for determination of Symmetry C_{18} (4.6×150mm) 5µmfrom bulk and standard preparation and from pharmaceutical formulation was shown in (fig-2).

Optimized Chromatographic Conditions:

Mobile phase ratio : Methanol: water (45:55 v/v)
Column : Symmetry C18 (4.6×150mm) 5µm
Column temperature : 40ºC
Wavelength : 260nm
Flow rate : 0.8ml/min
Injection volume : 10µl
Run time : 6minutes

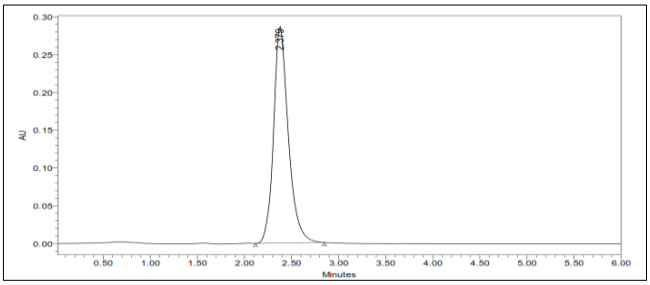

Fig 2: Optimized Chromatographic Condition

Validation of an Analytical Method

The developed RP-HPLC method was validated for parameters like system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness according to ICH guidelines [21].

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The system suitability parameters [22] were tabulated in table 3. All the parameters were found to be within the limits.

Table 3: Results of system suitability for Flavoxate HCL

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Flavoxate HCL	2.317	2274631	239458	5728	1.2
2	Flavoxate HCL	2.302	2284721	239582	5093	1.2
3	Flavoxate HCL	2.323	2238127	236493	5391	1.2
4	Flavoxate HCL	2.343	2259349	249482	6139	1.2
5	Flavoxate HCL	2.321	2204850	239452	5281	1.2
Mean			2252336			
Std. Dev.			31827.08			
%RSD			1.41307			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.
Analytical method was tested for specificity [22] to measure accurately quantitates Flavoxate HCL in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Flavoxate HCL in pharmaceutical dosage form was found to be 99.7%.

Linearity

The linearity of the test solutions for the assay method was prepared from Flavoxate HCL standard stock solution at five concentration levels from 24µg/ml to 120µg/ml of assay concentration. The peak area versus concentration data was treated by least-squares linear regression analysis (fig-3). The results have shown an excellent correlation between peak areas and concentration within the concentration range of 24–120 µg/ml for Flavoxate HCL (table-4). The correlation coefficient was found to be 0.9989 for the drug, which meet the method validation acceptance criteria and hence the method was said to be linear for both the drugs.

Chromatographic Data for Linearity Study

Table 4: Linearity Data of Flavoxate HCL

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	24	791554
66	48	1647073
100	72	2283804
133	96	3058339
166	120	3839630

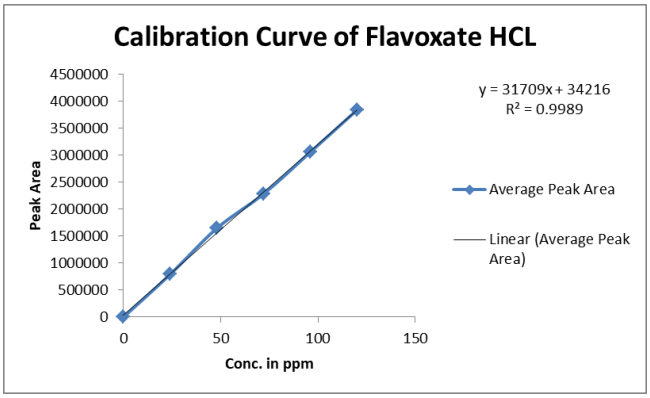

Fig 3: Calibration Curve of Flavoxate HCL

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Flavoxate HCL is a straight line.

Y = mx + c

Slope (m) = 31709

Intercept (c) = 34216

Correlation Coefficient (r) = 0.998

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 34216. These values meet the validation criteria.

Precision

The precision [23] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 5: Results of repeatability for Flavoxate HCL:

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Flavoxate HCL	2.356	2259464	245362	5938	1.2
2	Flavoxate HCL	2.356	2275915	248293	5827	1.2
3	Flavoxate HCL	2.357	2282117	240795	5032	1.2
4	Flavoxate HCL	2.358	2278675	230139	5978	1.2
5	Flavoxate HCL	2.359	2282448	249605	6183	1.2
Mean			2275724			
Std.Dev			9476.485			
%RSD			0.416416			

Intermediate Precision

Analyst 1

Table 6: Results of Intermediate Precision for Flavoxate HCL

S.No.	PeakName	RT	Area (µV*sec)	Height (µV)	USPP late count	USPT ailing
1	Flavoxate HCL	2.380	2236184	202188	5472	1.2
2	Flavoxate HCL	2.383	2238020	201837	6193	1.2
3	Flavoxate HCL	2.385	2239352	201273	5980	1.2
4	Flavoxate HCL	2.385	2242466	203923	7163	1.2
5	Flavoxate HCL	2.389	2244692	202938	6182	1.2
6	Flavoxate HCL	2.389	2247654	201982	7684	1.2
Mean			2241395			
Std.Dev.			4333.851			
%RSD			0.193355			

Analyst 2

Table 7: Results of Intermediate Precision Analyst 2 for Flavoxate HCL

S.No.	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Flavoxate HCL	2.380	2236184	217363	5928	1.2
2	Flavoxate HCL	2.383	2238020	218467	6183	1.2
3	Flavoxate HCL	2.385	2239352	218346	5927	1.2
4	Flavoxate HCL	2.385	2242466	221736	5163	1.2
5	Flavoxate HCL	2.389	2244692	228361	4827	1.2
6	Flavoxate HCL	2.346	2263431	217553	5019	1.2
Mean			2244024			
Std.Dev.			9988.458			
%RSD			0.445114			

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery[24] was calculated.

Table 8: The Accuracy Results for Flavoxate HCL

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1172485	36	35.8	99.4	99.5%
100%	2314753	72	71.6	99.4	
150%	3480210	108	107.9	99.9	

Limit of Detection for Flavoxate HCL

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

=5.5µg/ml

Quantitation Limit

The quantitation limit[25] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

=16.7µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Flavoxate HCL. The method is robust[26] only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Flavoxate HCL were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 9: Results for Robustness

Parameter Used For Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 0.8mL/min	3119086	2.379	5837	1.2
Less Flow rate of 0.7mL/min	2640811	2.763	5361	1.2
More Flow rate of 0.9mL/min	2640354	2.234	5231	1.2
Less organic phase	2640758	2.765	4503	1.5
More organic phase	2640125	2.236	4491	1.5

Summary and Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 260nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C_{18} because it was giving good peak.40 º C temperatures was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.8ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: water was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol: water was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around 2.3 and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 24-120ppm of the Flavoxate HCL target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Flavoxate HCL in bulk drug and in Pharmaceutical dosage forms.

References

1. DrugBank Online. Flavoxate [Internet]. Available from: https://go.drugbank.com/drugs/DB01148
2. DrugBank Online. Flavoxate hydrochloride [Internet]. Available from: https://go.drugbank.com/salts/DBSALT000407
3. PubChem. Flavoxate hydrochloride [Internet]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Flavoxate-hydrochloride
4. PubChem. Flavoxate [Internet]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Flavoxate
5. Wikipedia. Flavoxate [Internet]. Available from: https://en.wikipedia.org/wiki/Flavoxate
6. Pavani Peddi, Raja Rajeswari T, Ramana Reddy Ganji. Development and validation of stability indicating RP-LC method for estimation of related substances of Flavoxate HCl in bulk and its pharmaceutical formulations. Der Pharmacia Lettre. 2016; 8(9):286-94.
7. Rashid AJ, Bashir S, Bukhari NI, Abbas N, Raza A, Ijaz QA, *et al*. Development and validation of single analytical HPLC method for determination of Flavoxate HCl in bulk, tablets and biological fluids. Pak J Pharm Sci. 2021 Jul; 34(4 Suppl):1629-37.
8. Attimarad M. Liquid Chromatographic Determination of Flavoxate HCl in Pharmaceutical Formulation. J Young Pharm. 2010 Jul-Sep; 2(3):280-83. doi: 10.4103/0975-1483.66787.
9. Attimarad M, Chohan MS, Balgoname AA. Simultaneous Determination of Moxifloxacin and Flavoxate by RP-HPLC and Ecofriendly Derivative

Spectrophotometry Methods in Formulations. Int J Environ Res Public Health. 2019 Apr; 16(7):1196. doi: 10.3390/ijerph16071196.
10. Scholars Research Library. Der Pharmacia Lettre. 2012; 4(1):76-86. ISSN 0974-248X.
11. FARMACIA. 2009; 57(5).
12. J Pharm Bioallied Sci. 2011 Apr-Jun; 3(2):310-4. doi: 10.4103/0975-7406.80766.
13. Journal of Pharmaceutical and Biomedical Analysis. 1999 Nov 1; 21(2):371-82.
14. Tropical Journal of Pharmaceutical Research. 2009 Oct; 8(5):449-54.
15. Sankar R. Instrumental Method of Analysis. 1st ed. Vallabh Prakashan; 2005. p. 18-6, 18-3.
16. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. John Wiley & Sons; 1997. p. 503.
17. U.S. Department of Health and Human Services FDA. Guidance for industry, Analytical Procedure and Method Validation [Internet]. 2000 Aug. Available from: http://www.fda.gov/guidance/index.htm
18. Cheng YF, Walter TH, Lu Z, Iraneta P, Gendreau C, Neue UD, *et al*. LCGC. 2000; 18(10):1162.
19. The United States Pharmacopeia 25/National Formulary 20. Rockville, MD: The United States Pharmacopeia Convention, Inc.; 2002. Ch. 1225. p. 2256-9.
20. ICH Q2B: Validation of Analytical Procedure; Methodology. International Conference on Harmonization of Technical Requirements for the Registration of Drugs for Human Use. Geneva, Switzerland; 1997 May.
21. ICH Q2B: Validation of Analytical Procedure; Methodology. International Conference on Harmonization of Technical Requirements for the Registration of Drugs for Human Use. Geneva, Switzerland; 2003 Nov.
22. Gorenstein MV, Li JB, Van Antwerp J, Chapman D. LCGC. 1994; 12(10):768-72.
23. Young PM, Gorenstein MV. LCGC. 1994; 12(11):832-8.
24. Warren WJ, Stanick WA, Gorenstein MV, Young PM. BioTechniques. 1995; 2:282-97.
25. Swartz ME. J Liq Chromatogr Relat Technol. 2005; 28(7-8):1253-63.
26. Swartz M, Murphy B. Am Lab. 2005; 37(3):22-7.
27. Swartz M. Pharm Formul Qual. 2004; 6(5):40-2.
28. Biomed Chromatogr. 2008 May; 22(5):469-77.
29. J Chromatogr B Analyt Technol Biomed Life Sci. 2008 Mar 1; 863(2):258-65.
30. Tagesschau.de. Kassen Mussen Nicht fur “Acomplia” Zahlen [Internet]. 2006 Oct 17 [cited 2007 Jun 13]. Available from: https://www.tagesschau.de
31. Matheson AJ, Noble S. Drugs. 2000 Apr; 59(4):829-35.