Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (9):286-294
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of stability indicating RP-LC method for estimation of related substances of flavoxate HCl in bulk and its pharmaceutical formulations

Pavani Peddi[1]*, T. Raja Rajeswari[2] and Ramana Reddy Ganji[3]

[1]Dept. of Chemistry, PVP Siddhartha Institute of Technology, Vijayawada-520010, India
[2]Reader in Chemistry, Dept. Of Chemistry , Govt. College for Women, Guntur
[3]Dept. of Chemistry, Acharya Nagarjuna Univ, Guntur, India

ABSTRACT

Estimation of related substances by using high-performance liquid chromatographic method was developed and validated for the determination of Flavoxate Hydrochloride. The method is simple, highly sensitive, and selective and is capable of quantitative determination of Flavoxate Hydrochloride. The chromatographic separation is achieved by injecting 20µL standard solution of Flavoxate Hydrochloride into HPLC system with PDA detector using a Hypersil ODS, 5µm (150 x4.6) mm column. The mobile phase consists of pH 2.75 buffer and Acetonitrile. The flow rate was set at 1.0 ml/min with column and sampler temperatures at 25˚C and ambient respectively and runtime was optimized to 25 min. The developed LC method was validated with respect to specificity, precision, linearity, ruggedness, stability of analytical solution and robustness.

Keywords: Flavoxate Hydrochloride, Liquid chromatography, Related Substances.

INTRODUCTION

Flavoxate HCl [1] is a urinary anti spasmodic (smooth muscle relaxant) acts as a direct antagonist at muscarinic acetylcholine receptors in cholinergically innervated organs. Chemically Flavoxate Hcl is 2-(1-piperidyl) ethyl 3-methyl-4-oxo-2- phenylchromene-8-carboxylate, having molecular formula as C24H25NO4•HCl and molecular weight as 427.92. Flavoxate Hcl is slightly soluble in Water and Ethanol and sparingly soluble in Methylene Chloride. The pKa Value of Flavoxate Hcl is 7.3. The structural formula of Flavoxate Hcl was shown in Figure: 1.1.

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and formulations is one of the most challenging tasks. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products. For these reasons, all major international pharmacopoeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product. [2-3] Flavoxate HCl is official in the United States pharmacopeia. [4] In the literature survey, there were several HPLC [5-11] and UV [12] methods that have been reported for the determination of Flavoxate Hcl in pharmaceutical preparation either individually or in combination with other drugs. Up to know there is no methods were available

for the determination of estimation of related substances for Flavoxate Hcl individually. Hence, the objective of this study is to develop simple, sensitive and accurate RP-HPLC method for estimation of Flavoxate Hcl in pharmaceutical dosage forms. The developed method was validated according ICH [13-14] guidelines including various Stability parameters proved for its accuracy.

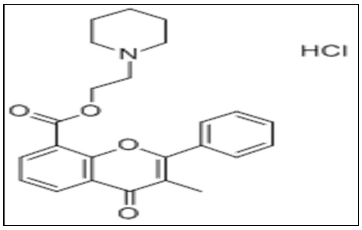

Figure: 1.1 Structure of Flavoxate Hydrochloride

1.1 Related Substance Structures:

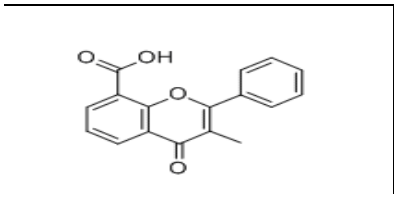

Figure: 1.2 Chemical Structure of 3-Methylflavone-8-carboxylic acid
Molecular Formula: $C_{17}H_{12}O_4$, Molecular Weight: 280.27

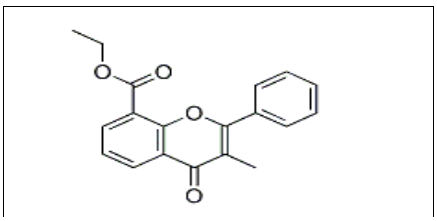

Figure: 1.3 Chemical Structure of 3-methylflavone-8-carboxylic acid ethyl ester
Molecular Formula: $C_{19}H_{16}O_4$, Molecular Weight: 308.32794

MATERIALS AND METHODS

2.1 Reagents and chemicals

Hexane-1-sulphonic acid sodium salt, Triethylamine, ortho phosphoric acid, Acetonitrile, were procured from Merck. Water (MIlli-Q). All chemicals were of an analytical grade and used as received.

2.2 Chromatography

The analytical separations were carried out on Waters-2695 series HPLC system with PDA detector. The analytical column was Hypersil ODS, 5µm (150 x4.6) mm. The mobile phase consists of pH 2.75 buffer and Acetonitrile. The flow rate was 1.0 ml/min. and runtime was 25minutes. Column temperature was maintained at 25˚C and sample temperature was maintained at ambient. Detection was measured 293nm with PDA detector and 20 µL sample was injected. The control of HPLC system and data collection was Empower[2] software.

2.3 Preparation of Flavoxate Hydrochloride Standard Solution:

Weigh and transfer about 25 mg of Flavoxate Hydrochloride working standard or reference standard in to a 50 mL volumetric flask.Add about 35 mL of diluent, sonicate to dissolve and dilute to volume with diluent mix well. Pipette out 5 mL of above solution into 50 mL of volumetric flask and dilute to volume with diluent (50µg/ml).

2.4 Preparation of Flavoxate Hydrochloride Sample solution:
Weigh 10 tablets and calculate average weight. Crush the tablets into fine powder with the help of mortar and pestle. Weigh and accurately transfer tablet powder of 365 mg (equivalent to200 mg of Flavoxate Hydrochloride) into 200 mL volumetric flask. Add about 150 mL of diluent, sonicate for 30 minutes with intermediate shaking and dilute to volume with diluent and mix well. Pipette out 5 mL of above solution into 100 mL of volumetric flask and dilute to volume with diluents (50µg/ml). Filter the solution through 0.45µm Nylon filter.

RESULTS AND DISCUSSION

Method optimization parameters
An understanding of the nature of API (functionality, acidity, or basicity), the synthetic process, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting. Different mobile phases and Stationary phases were employed to develop a suitable LC method for quantitative determination of Flavoxate Hydrochloride in its drug. A number of columns containing various packing materials of ODS supplied by different manufacturers and different mobile phase composition were tried to obtain good peak shape and selectivity for impurities present in Flavoxate Hydrochloride. Peak tailing was observed when buffer and acetonitrile. In the next approach buffer and acetonitrile in a ratio of 65:35 v/v was employed using YMC Pro C-18 (150 X 4.6) mm, 5µm column. Under these conditions USP peak tailing was found to be 1.9 for Flavoxate Hydrochloride peak. In another attempt using the YMC- Pack ODS-AQ (150 X4.6) mm, 5µm column and mobile phase consisting of buffer and acetonitrile in the gradient program, Flavoxate Hydrochloride peak eluting at 6.3 min with better peak shape of Flavoxate Hydrochloride compared with other trials. In another trial using Hypersil ODS, 5µm (150 x4.6) mm column with a mobile phase consisting of pH 2.75 buffer and Acetonitrile was tried. In this eluent Flavoxate Hydrochloride gave a very good and well separated peak from all impurities. In another trial, solvent system and column are same as above and optimized the column temperature, flow and injection volume. After number of trials for flow and column temperature combination, in order to obtain best column temperature and flow, was set at column temperature 25˚C and flow rate was 1.0 ml/min. runtime 25 minutes with sample temperature maintained at ambient was finally selected. Detection was measured by 293nm with PDA detector and the sample injected was 20 µL. These chromatographic conditions were selected for validation studies. The system suitability results obtained using these chromatographic conditions are shown in Table: 1.1 and Flavoxate Hydrochloride peak eluting at 6.3min. The Flavoxate Hydrochloride standard chromatogram is shown in Figure: 1.4.

Table: 1.1 System suitability results of Flavoxate Hydrochloride

Parameter	Retention time (min)	Tailing factor	Theoretical plates	%RSD
System suitability	6.3	1.2	7574	0.1

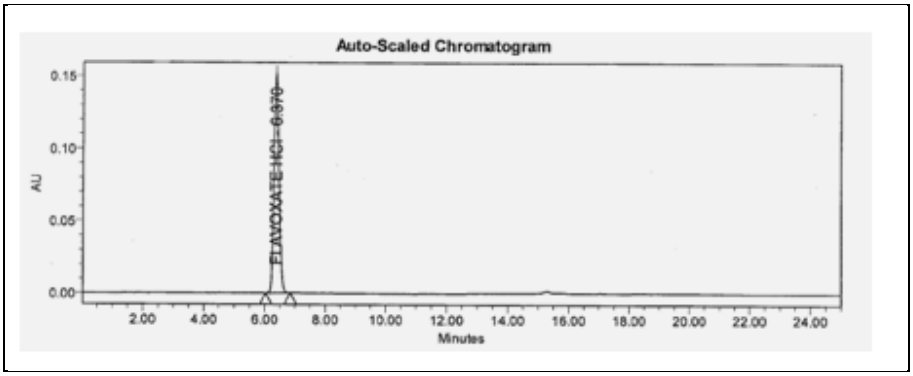

Figure: 1.4 HPLC Chromatogram of Flavoxate Hydrochloride

4.0 Method validation
4.1 Specificity
Blank and Placebo interference
A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were

recorded. Chromatograms of Blank & placebo solutions **Figure: 1.5 & Figure: 1.6** showed no peak at the retention time of Flavoxate Hydrochloride peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Flavoxate Hydrochloride in Flavoxate Hydrochloride tablets.

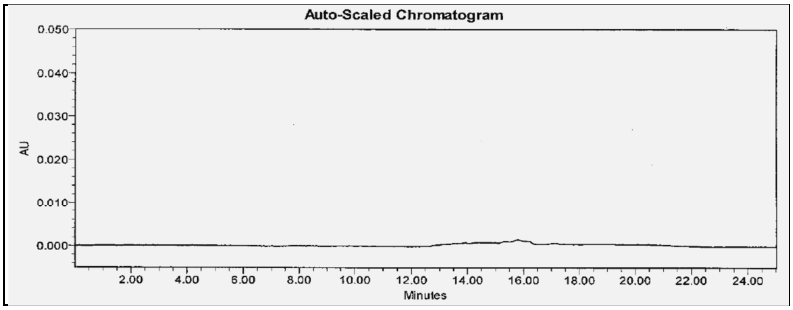

Figure: 1.5 typical chromatogram of Blank

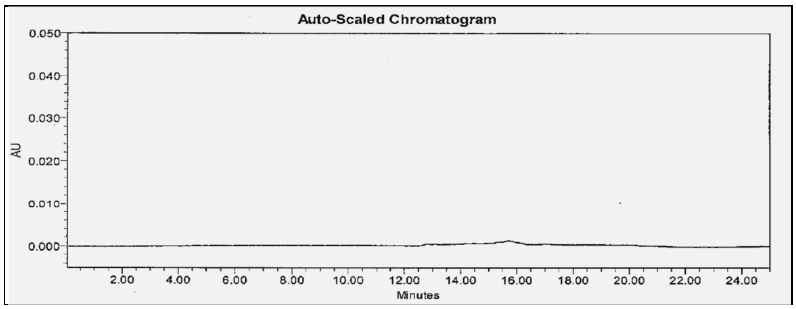

Figure: 1.6 typical chromatogram of Placebo

4.2 Impurity stock solution preparation for spiked test solution:

4.2.1 Spiking solution of impurity –A

Weighed and transferred 6.2541 .mg of impurity-A into 25 ml volumetric flask ,added 3 ml of Acetonitrile and sonicated for 5min.Added 15 ml of diluent and sonicated for 25 min with intermittent shaking .Made up the volume with diluent and mix well.

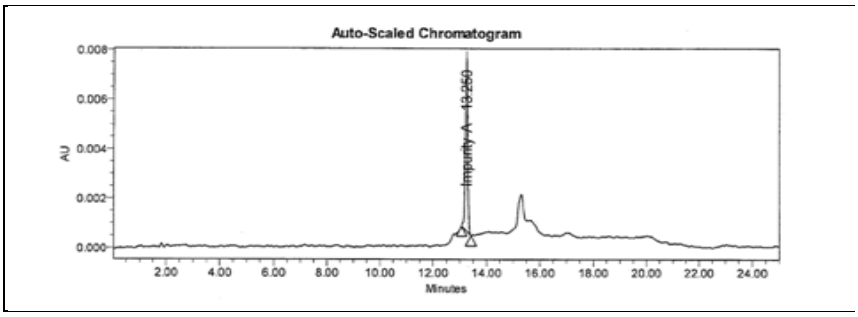

Figure: 1.7 typical chromatogram impurity-A

4.2.2 Spiking solution of impurity –B

Weighed and transferred 5.0402 mg of impurity-B into 25 ml volumetric flask ,added 3 ml of Acetonitrile ,sonicated for 5min.Added 15 ml of diluent and sonicated for 25 min with intermittent shaking .Made up the volume with diluent and mixed well.

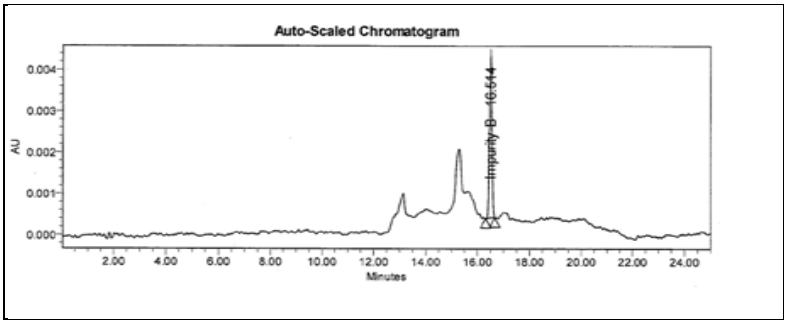

Figure: 1.8 typical chromatogram impurity-B

4.2.3 Preparation of spiked sample preparation at 1 % of test concentration:
Pipetted out 5 mL of above solution into 100 mL volumetric flask, added 0.3 mL of impurity stock solution –A and impurity stock solution-B each into same volumetric flask. Volume made with diluent and filtered through 0.45µm nylon filter and injected into HPLC system.

4.2.4 Preparation of individual impurity at 1 % of test concentration:
Pipette out 0.23 mL of impurity-A stock solution into 100 mL volumetric flask individually and 0.2 mL of impurity stock solution into 100 mL volumetric flask individually.Volume made up to mark with diluent and mixed well and injected into HPLC system

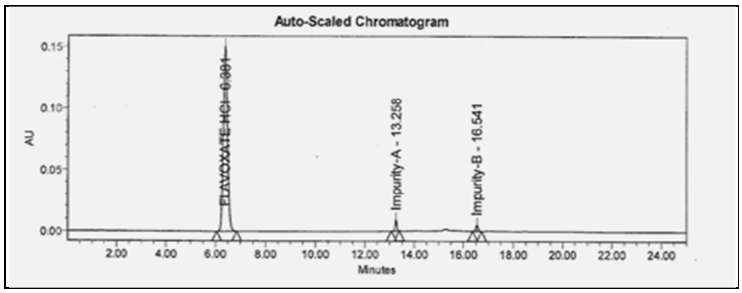

Figure: 1.9 typical chromatogram of spiked solution

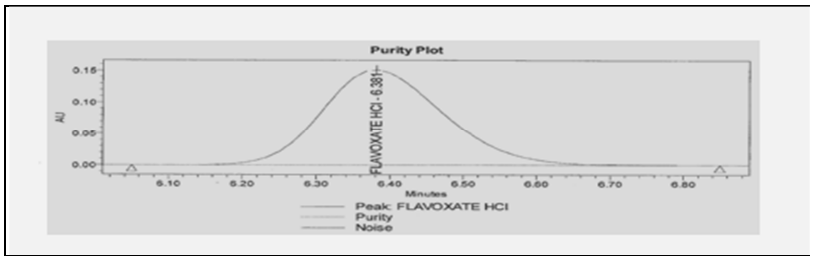

Figure: 2.0 Purity plot of Flavoxate Hydrochloride in spiked sample

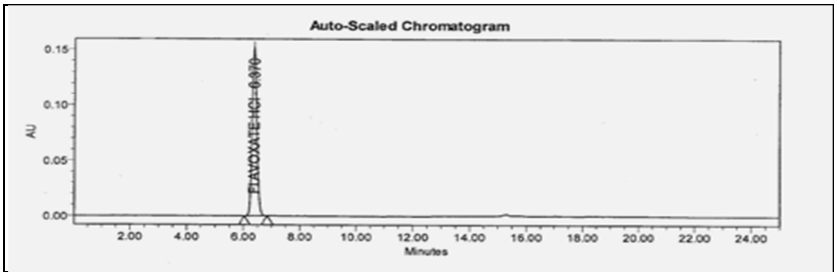

Figure: 2.1 typical chromatogram of unspiked sample

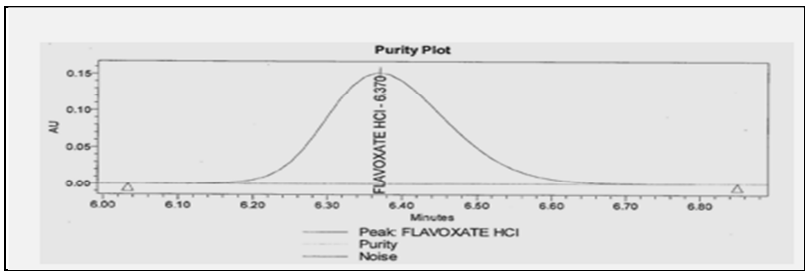

Figure: 2.2 Purity plot of Flavoxate Hydrochloride in unspiked sample

Table: 1.2 Impurity interference data

Peak Name	RT (minutes)	
	Standard & individual impurities	Spiked sample
Flavoxate Hydrochloride	6.41	6.38
Impurity-A(3-methylflavone-8-carboxylic acid)	13.25	13.26
Impurity-B(3-methylflavone-8-carboxylic acid ethyl ester)	16.51	16.54

It was observed that known impurities are not co eluting with each other and main analyte peak.

Table: 1.3 Peak purity table for unspiked and spiked samples

Peak name	Total peak purity			
	Unspiked sample		Spiked sample	
Flavoxate Hydrochloride	Purity angle	Purity threshold	Purity angle	Purity threshold
	0.256	0.276	0.047	0.276

Peak purity of the Flavoxate Hydrochloride in the as such sample preparation and in spiked test preparation was calculated and found to be within the acceptable limit.

4.3 System Precision

Six replicate injections of standard solution were given into the HPLC system. Data along with the % RSD of area of Flavoxate Hydrochloride peak is shown in **Table:1.4** The peak areas indicate an acceptable level of precision for the analytical system.

Table: 1.4 System precision for the Flavoxate Hydrochloride

Injection No	Flavoxate Hydrochloride
1	113685
2	113564
3	113758
4	113024
5	113348
6	112928
Mean area	113385
%RSD	**0.30**

4.4 Method Precision

Precision of the impurities and degradants method was determined by injecting six sample solutions spiked with impurity-A, impurity-B at specification level. The samples were prepared as per the method and the result for precision study is tabulated in **Table: 1.5**

Table: 1.5 Results of method precision

Sample No.	% Impurity (w/w)	
	Impurity-A	Impurity-B
1	0.200	0.208
2	0.197	0.205
3	0.201	0.209
4	0.198	0.206
5	0.203	0.209
6	0.204	0.207
Mean	**0.200**	**0.207**
% RSD	**1.36**	**0.78**

4.5 Limit of detection (LOD) & Limit of Quantitation (LOQ)

Table: 1.6 LOD&LOQ for Flavoxate Hydrochloride and impurities

Solution No.	Flavoxate Hydrochloride		Impurity-A		Impurity-B	
	Concentration (µg/ mL)	Peak Area Response	Concentration (µg/ mL)	Peak Area Response	Concentration (µg/ mL)	Peak Area Response
1	0.001	251	0.003	550	0.003	467
2	0.007	1109	0.015	2449	0.016	2004
3	0.015	2004	0.030	4754	0.032	3917
4	0.030	3948	0.060	9282	0.064	8133
5	0.046	5528	0.090	13705	0.096	11825
6	0.061	7442	0.120	18307	0.128	15968
7	0.076	9456	0.151	23101	0.160	20202
8	0.092	11704	0.181	27743	0.192	24225
9	0.122	15133	0.241	36340	0.256	31769
10	0.153	18917	0.302	46063	0.320	39766
Slope	122866.421		151513.276		124398.621	
Residual SD	159.235		149.774		168.373	
LOD (µg/mL)	**0.004**		**0.003**		**0.004**	
LOQ (µg/mL)	**0.012**		**0.009**		**0.013**	

The limit of detection and limit of quantitation values obtained for each impurity and Flavoxate Hydrochloride are within the acceptance criteria.

4.6 Precision at LOQ

The precision at LOQ is performed by analyzing six replicate injections of the standard solution containing all known impurities and Flavoxate Hydrochloride at LOQ level.Determine the percentage relative standard deviation of peak areas of each impurity and Flavoxate Hydrochloride. Results of peak area of impurities and Flavoxate Hydrochloride are summarized in **Table: 1.7**

Table: 1.7 Summary of peak areas for precision at LOQ

Injection No.	Peak area of LOQ level		
	Flavoxate Hydrochloride	Impurity-A	Impurity-B
1	1684	1299	1460
2	1685	1253	1570
3	1646	1279	1461
4	1710	1237	1543
5	1714	1310	1458
6	1632	1253	1500
Avg.	**1679**	**1272**	**1499**
% RSD	**1.98**	**2.27**	**3.21**

4.7 Linearity and Range

The linearity is determined by injecting the solutions in duplicate containing known impurities and Flavoxate Hydrochloride ranging from LOQ to 200% (LOQ, 50%, 80%, 100%, 120%, 150% and 200%) of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Determine the response factor for each impurity with respect to Flavoxate Hydrochloride. Report the linearity range as the range

for determining the impurities.Results obtained are in the tables & figures show the line of best fit for peak area versus concentration for each impurity.

Table: 1.8 Linearity of Flavoxate Hydrochloride and impurities

Sample No.	% level	Peak area		
		Flavoxate Hydrochloride	Impurity-A	Impurity-B
1	LOQ	1679	1272	1499
2	50	9370	22708	19863
3	80	14999	36749	31927
4	100	18814	45810	39816
5	120	22693	55980	47988
6	150	28557	70238	60613
7	200	37832	93267	80586

Table: 1.9 Regression analyses for the linearity graph

Linear regression analysis	Flavoxate Hydrochloride	Impurity-A	Impurity-B
Correlation coefficient (r^2)	0.9998	0.9998	0.9999
Y- intercept	114.444	-505.373	-275.937
Slope	123052.580	155307.075	126209.397

4.8 Accuracy

Recovery of Flavoxate Hydrochloride impurities in Flavoxate Hydrochloride was performed. The sample was taken and varying amounts of Flavoxate Hydrochloride impurities representing LOQ to 200 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table: 2.0.**

Table: 2.0 Accuracy study of Flavoxate Hydrochloride

No. of Injection	Theoretical (%)	% Mean Recovery ± %RSD	
		Impurity-A	Impurity-B
3	50	92.27 ± 2.07	95.65 ± 3.02
6	100	94.16 ± 1.33	95.86 ± 0.66
3	200	93.60 ± 0.60	97.80 ± 0.38

RESULTS AND DISCUSSION

A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Hypersil ODS, (150× 4.6mm) with 5µm particle size. Injection volume of 20µl is injected and eluted with the mobile phase consists of pH 2.75 buffer and Acetonitrile, which is pumped at a flow rate of 1.0 ml/min with column and sampler temperatures at 25˚C and ambient respectively and runtime was optimized to 25 min.. Detection, was carried out at 293 nm. The peaks obtained were sharp with retention time of 13.25 for Impurity-A, 16.51 for Impurity-B and 6.41 for Flavoxate Hydrochloride. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness, and stability of solution.

For Selectivity, the chromatograms were recorded for standard and sample solutions of Flavoxate Hydrochloride and its related substances. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of related substances in Flavoxate Hydrochloride.

The limit of detection (LOD) and limit of quantitation (LOQ) for impurity-A was found to be 0.003µg/ml,0.009µg/ml, for impurity-B 0.004µg/ml,0013µg/ml and Flavoxate Hydrochloride 0.004µg/ml, 0.012µg/ml, respectively. The linearity results for Flavoxate Hydrochloride and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for Flavoxate Hydrochloride and its impurities found to be 0.9998, 0.9998 and 0.9999 respectively.

The accuracy studies were shown as % recovery for Flavoxate Hydrochloride and its impurities at 50%, 100% and 200%.The limit of % recovered shown is in the range of 90 and 110% and the results obtained were found to be

within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Flavoxate Hydrochloride and its related substances in the range 92.27 -97.80 respectively.

For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of Flavoxate Hydrochloride and its impurities. The acceptance limit should be not more than 10, and the results were found to be within the acceptance limits.

Hence, the chromatographic method developed for Flavoxate Hydrochloride and its related substances are rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of the active pharmaceutical ingredients for assurance of its quality during its formulation.

REFERENCES

[1] Rx drug list, drug description; Available from http://www.rxlist.com/urispas-drug.htm.
[2] ICH, Impurities in new drug substances Q3A (R2). **2006** Oct 25; Available from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2__Guideline.pdf.
[3] ICH, Impurities in new drug products Q3B (R2), **2006** Jun 2; Available from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2__Guideline.pdf.
[4] USP, 32–NF27, 2378, Pharmacopeia Forum: 33(6): 172.
[5] Attimara M *J Young Pharm*., **2010**; 2(3): 280–283.
[6] Dr Mahesh V Attimarad, Sree Harsha N, and Ramachandra S. Setty. Simultaneous Determination of Ofloxacin And Flavoxate Hydrochloride In Human Plasma By RP HPLC., **2012**; 35(5-8): 768-777.
[7] Md Azheruddin1, Jomol Joseph1, Quddus Junaidyl. *Am. J. PharmTech Res*., **2014**; 4(2): 337-346.
[8] Sheu MT, Yeh GC, Ke WT, Ho HO. *J chromatogr, B, Biomed sci appl*., **2001**; 751(1): 79-86.
[9] Hardik Modi, Priyanka Patil, Priyanka Patel, Hardiksinh Baria, Sanjay Patel, Nehan Kabani. *Inventi: ppaqa*, **2013**; 626.
[10] Rania Nabih El-Shaheny, Nahed Mahmoud El-Enanya and Fathalla Fathalla Belala. *Analytical Methods*, **2014**; 6: 1001-1010.
[11] Mohammad Yunoos, M. Sowjanya, K Pavan Kumar and Ch Ashok Kumar. *Journal of Chemical and Pharmaceutical Research*, **2014**, 6(9): 381-388.
[12] Mahesh Attimarad, *Journal of Basic and Clinical Pharmacy*, **2011**; 2(1): 53-61.
[13] International conference on Harmonization guidance for Industry In: Q2A Text on Validation of Analytical methods. Switzerland, IFPMIA, **1994**; 1-4.
[14] International conference on Harmonization guidance for Industry In: Q2B Text on validation of Analytical methods. Switzerland, IFPMIA, **1996**; 1-8