EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 2
452-467
Year: 2015

METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF FLAVOXATE HYDROCHLORIDE IN TABLET DOSAGE FORM

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Article Received on 01/03/2015 **Article Revised on 23/03/2015** **Article Accepted on 14/04/2015**

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ABSTARCT

A stability-indicating gradient RP-HPLC method has been developed for the estimation of impurities of Flavoxate Hcl in pharmaceutical formulations. Efficient chromatographic separation was achieved on a Inertsil ODS-3V (150×4.6 mm, 5µ) column with mobile phase containing a gradient mixture of solvents A and B. The flow rate of the mobile phase was 1 mL/ min with column temperature of 25°C and detection wavelength at 293nm. Regression analysis showed an r value (correlation coefficient) greater than 0.999 for Flavoxate Hcl. Flavoxate Hcl formulation sample was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal, and photolytic degradation. Flavoxate Hcl was found stable degrade significantly in Acid stress condition. The degradation products were well resolved Flavoxate Hcl, and their impurities. The peak purity test results confirmed that the Flavoxate Hcl peak was homogenous and pure in all stress samples thus proving the stability-indicating power of the method. The developed method was validated according to ICH guidelines with respect to specificity, linearity, limits of detection and quantification, accuracy, precision, and robustness.

KEYWORDS: Stability indicating RP-HPLC method; Flavoxate Hcl, Method validation; Gradient technique.

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 $C_{24}H_{25}NO_4$•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 Fig. 1.

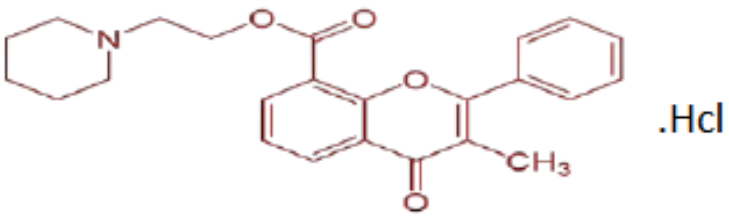

Fig. 1: Molecular structure of Flavoxate Hcl

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 Stability parameter for Flavoxate Hcl individually.

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

EXPERIMENTAL

Chemicals and Reagents

Flavoxate Hcl Pure drug was procured from Sai Ram Organic Pvt.Ltd., Hyderabad, URISPAS-200mg tablets (Each equivalent to 200 mg of Flavoxate Hcl) of Cipla were purchaged from market. Ortho phosphoric acid (Merck), Acetonitrile (HPLC grade) (Merck), Methanol (HPLC grade) (Merck), Hexane-1-Sulphonic acid (Merck), Hydrochloric acid (Merck), triethylamine, potassium nitrite (Merck) and Hydrogen peroxide (Merck) were procured from Suvarna Scintific Euipments, Vijayavada,. Triple distilled water (in-house preparation) was used. The Agilent 1200 LC system used for method development and validation with diode array detector (model: 2998 detector and e2695 separation module) operating Empower software. Weighing was performed with a Shimadzu AUX 220. Photo stability studies were carried out in a photo stability chamber (SUN TEST XLS+, Atlas, USA). Thermal stability studies were performed in a dry air oven.

Method Development

Selection of Wavelength

By using UV-Visible spectrophotometer, A standard solution of Flavoxate Hydrochloride was scanned in UV region and the absorbance maximum was found as 293nm. So wavelength maxima (λ max) selected as 293 nm for analysis.

Chromatographic Conditions

HPLC measurements were carried out using a reversed phase Inertsil ODS-3V (150×4.6 mm), 5µ particle size column operated at 25°C with gradient elution at 1 mL /min using a Mixture of buffer (Solvent A consists of Hexane-1 sulphonic acid sodium salt buffer) and Acetonitrile (Solvent B) as mobile phase. The HPLC gradient program was set as follows with respect to time (min)/% mobile phase B: 0.01/35, 10/35, 12/80, 18/80, 20/35 and 25/35.the analytes are detected at 293 nm and injection volume used is 20 µL. Mixture of Buffer Acetonitrile (65: 35 v/v) was used as diluent for sample preparation.

Mobile phase preparation

Mobile phase –A

Weigh and transfer 1 g of Hexane-1 sulphonic acid sodium salt in to 650 ml of water mix well and add 1 ml of Trimethyl amine and 1 mL of ortho phosphoric acid to this solution mix well. Filter through 0.45µm membrane filter.

Mobile phase –B: Acetonitrile -100%

Preparation of 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.

Test preparation

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. Filter the solution through 0.45µm Nylon filter.

Note: Maintain the sonication temperature below 25 ^{o}C throughout the sonication.

Method validation

The method was validated according to ICH analytical method validation guidelines[13]-[14] which should be considered are listed below.

- ✓ System suitability
- ✓ Linearity
- ✓ Range
- ✓ Accuracy
- ✓ Precision
- ✓ Specificity
- ✓ Robustness
- ✓ Detection Limit (LOD)
- ✓ Quantitation Limit (LOQ)

System suitability

The standard and check standard solutions were prepared as per test method and injected into HPLC system.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. A series of Flavoxate Hydrochloride standard solutions were prepared in the range of about50%-150% of test concentration and injected into HPLC system as per test method.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Precision

- ✓ The precision 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. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Department of Pharmaceutical Analysis,
- ✓ Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.
- ✓ The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

  Precision can be divided into three types, they are

i. Repeatability (Method precision)

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

- ✓ To evaluate the precision for assay (n=6) replicate samples were prepared and analyzed as per test method.
- ✓ The % assay of each individual preparation, mean % assay and % RSD (n=6) of assay results were calculated.

ii. Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

- ✓ To evaluate intermediate precision for assay (n=6), replicate samples were prepared and analyzed as per test method by using HPLC system (waters and Agilent), different column and by different analyst on different day.
- ✓ The % assay of each individual preparation, mean % assay and %RSD (n=6) of assay results were calculated.
- ✓ The overall %RSD for assay results (n=12) of method precision and intermediate precision were calculated.

iii. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

i. Blank interference

Blank was prepared and injected as per test method. It was observed that no blank peaks were eluting at the retention time of analyte peak.

ii. Placebo interference

Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering at the retention time of analyte peak.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

i. Flow rate variation

- ✓ A study was conducted to determine the effect of variation in flow rate.
- ✓ Standard, check standard and simple solutions (in triplicate) were prepared as per the test method and injected into HPLC system with flow rates of 0.8 ml/minute and 1.2 ml/minute.

ii. Column oven temperature variation

- ✓ A study was conducted to determine the effect of variation in column oven temperature.
- ✓ Standard, check standard solutions and sample solutions (in triplicate) were prepared as per test method and injected into HPLC system with column oven temperature of 30oC.

Detection Limit (LOD)

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.

Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Forced degradation

- ✓ Forced degradation studies performed on drug product, drug substance and placebo for Acid degradation, Alkali degradation, Peroxide degradation, Water degradation, Thermal degradation, Humidity degradation and UV light degradations and samples were injected as per test method.
- ✓ % net stress conditions using degradation was calculated and Flavoxate Hydrochloride peak purity was evaluated in all water Empower software.

i. Acid degradation

Weighed and transferred placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Then added 10 mL of 5N HCL for acid degradation, kept on water bath at 60oC for 6 hrs. After that solution kept a side to attain room temperature, then neutralized with 10 mL of 5N NaoH. Sonicated for 30 minutes, volume made up to mark with diluent. Pipette out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC system.

ii. Base degradation

Weighed and transferred placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Then added 10 mL of 0.1N NaoH for base degradation, kept on water bath at 60oC for 6 hrs. After that solution kept a side to attain room temperature, then neutralized with 10 mL of 0.1N HCL. Sonicated for 30 minutes, volume made up to mark with diluent. Pipette out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC.

iii. Peroxide degradation

Weighed and transferred placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Then added 10 mL of 10 %peroxide for peroxide degradation, kept on water bath at 60oC for 6 hrs. Sonicated for 30 minutes, volume made up to mark with diluent. Pipette out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC.

iv. Water degradation

Weighed and transferred placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Then add 10 mL water, kept on water bath at 60^{o}C for 6 hrs. Sonicated for 30 minutes, volume made up to mark with diluent. Pipette out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC.

v. Thermal degradation: (Condition: 36 hrs at 105 OC)

Weighed and transferred thermally degraded placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Sonicated for 30 minutes, volume made up to mark with diluent.Pipetted out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC.

vi. Humidity degradation: (Condition: 6 days at 90% RH)

Weighed and transferred humidity degraded placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Sonicated for 30 minutes, volume made up to mark with diluent. Pipetted out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC.

vii. UV degradation: (Condition: 4 days in UV cabinet)

Weighed and transferred UV degraded placebo, API and sample in to 200 mL volumetric flask, added 130 mL of diluent and shaken for few minutes to dissolve contents. Sonicated for 30 minutes, volume made up to mark with diluent. Pipette out 5 mL of above solution in to 100 mL volumetric flask, volume made up to mark with diluent and mixed well. Filtered through 0.45µm Nylon filter and injected into HPLC.

RESULTS AND DISCUSSION

Method Development

After completion of several trials with same mobile phase and different columns and different mobile phase compositions, we have obtained chromatograms with good peak shape and retention time.

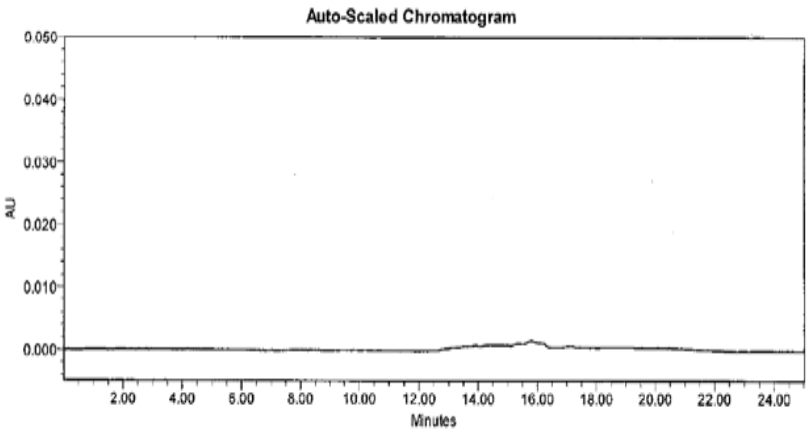

Fig.2: Typical chromatogram of Blank

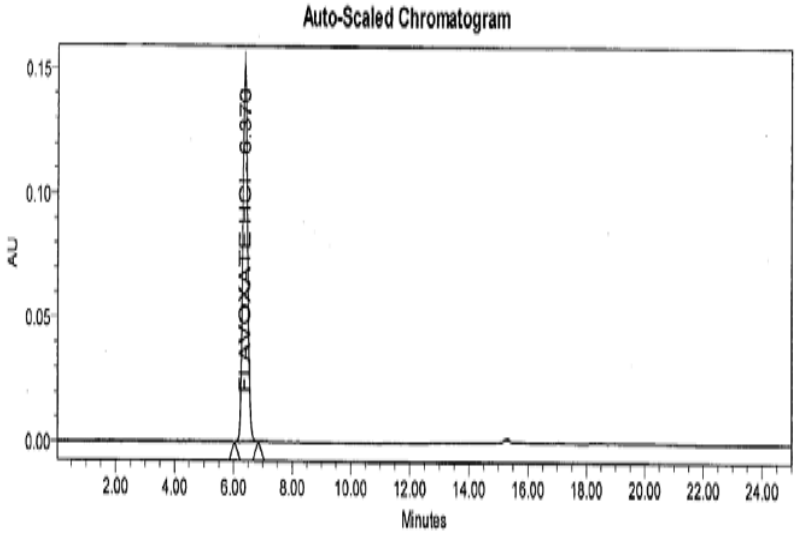

Fig.3: Typical chromatogram of standard solution

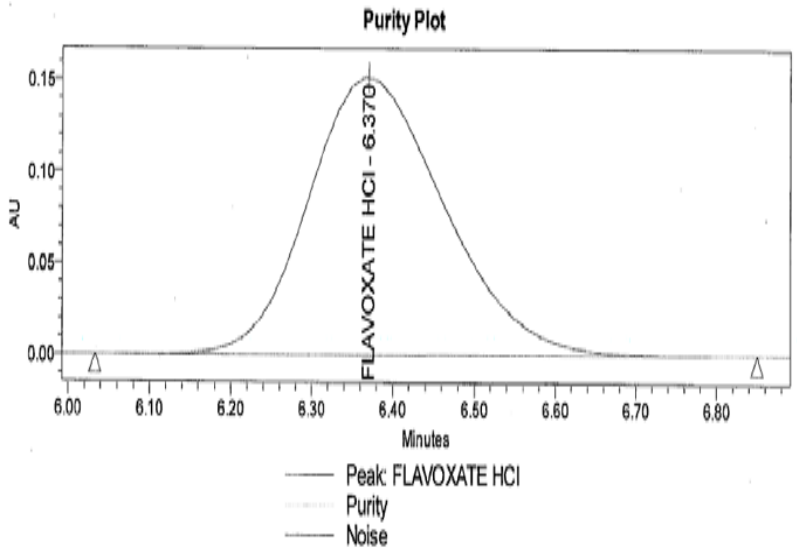

Fig.4: Purity plot of Flavoxate Hydrochloride in sample

Table: 1. Peak Purity of Flavoxate Hcl

Sample	Purity Angle	Purity Threshold
Flavoxate Hcl	0.256	0.276

Acceptance criteria: The software peak angle should be less than peak threshold.

Conclusion: Flavoxate Hydrochloride peak was observed at RT 6.37. Peak angle found to be less than peak threshold.

Method Validation

System suitability

Table: 2. System suitability Parameters of Flavoxate Hcl

Parametrs	Result*	Acceptance
% RSD for Area	0.1	NMT 2%
Tailing Factor	1.2	NMT 2
Theoritical Plates	7374	NLT 3000
% Assay	100.4	98-102%

* Average of six readings

Observation: The system suitability parameters were evaluated as per the test method and found to be within the limits.

Specificity

i. Blank interference

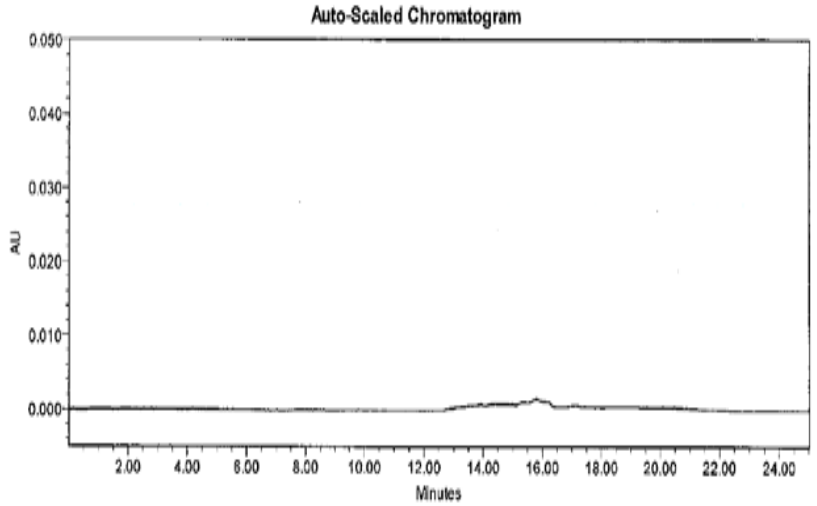

Fig. 5: Typical chromatogram of Blank

Placebo interference

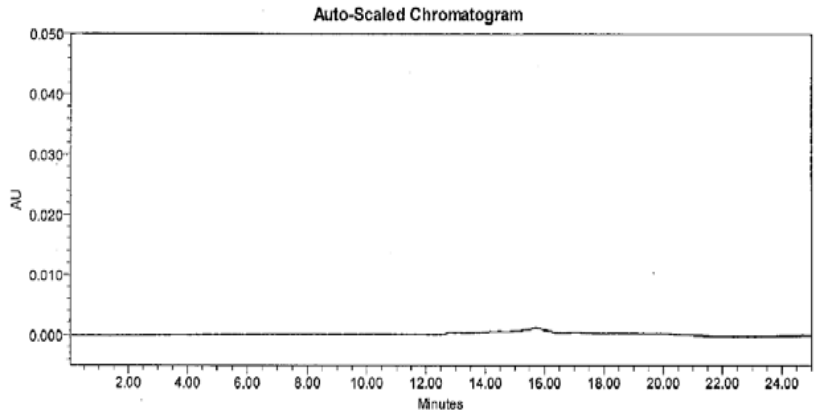

Fig. 6: Typical chromatogram of Placebo

Table 3: Blank and placebo interference

Sample No.	Peak found at RT of Analyte peak (YES/NO)	
Blank	Placebo	
1	No	No
2	N/A	No

Observation: It was observed that no blank and placebo peaks were interfering at the retention time of analyte peak.

Linearity

Linearity of detector response was established by plotting a graph of concentration Vs response of Flavoxate Hydrochloride peak.

Table 4: Linearity data

S. No	% Linearity level	Concentration in ppm	Response	Acceptance criteria
1	50	25.36	969864	r^2 should be NLT 0.999
2	60	30.43	1205080	
3	80	40.57	1550600	
4	100	50.71	1937176	
5	120	60.86	2389186	
6	150	76.07	2965780	

Table 5: Linearity Parameters

Parameter	Result
Slope	39204
Y-intercept	19352
Correlation coefficient	0.99948
Residual sum of squares	27146.78

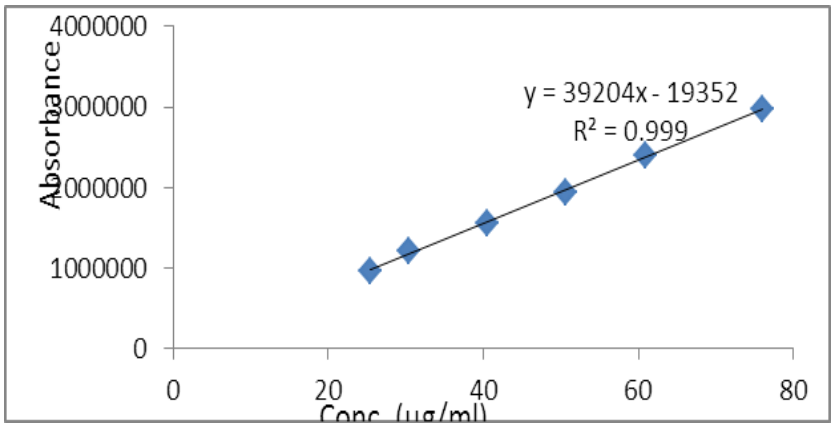

Fig. 7: Calibration Curve of Flavoxate Hcl

Observation

The detector response was found to be linear from about 50%-150% of test concentration. The correlation coefficient, squared correlation coefficient, slope, intercept and residual sum of squares were calculated and squared correlation coefficient was found to be within the acceptable limit.

Accuracy

Table 6: Accuracy data of Flavoxate Hydrochloride

S.No	% spike level	Amount added (mg)	Amount recovered(mg)*	% recovery	%RSD
1	50	100.03	101.59	101.53	0.4
2	75	149.89	150.51	100.40	0.5
3	100	199.80	201.04	100.6	0.1
4	125	249.78	251.65	100.76	0.2
5	150	299.66	301.60	100.65	0.2

*Average of three readings

Precision

i. System precision

Table 7: System Precision studies

Concentration (mg)	Peak Area*	% RSD
199.85	1826575	0.17

*Average of six readings

ii. Method Precision

Table 8: Method Precision studies

Concentration (mg)	Assay*	% RSD
199.85	99.13	0.58

*Average of six readings

Observation

The % assay of each individual preparation, mean % assay and % RSD (n=6) of assay results were calculated and found to be within the acceptance criteria.

iii. Intermediate Precision

Table 9: Intermediate Precision studies

Concentration (mg)	Assay*	% RSD
199.85	98.94	0.83

Observation

The % assay of each individual preparation, mean % assay and %RSD (n=6) of assay results were calculated and found to be within the acceptance criteria.

Robustness

Table 10: Robustness studies

Parameter		% RSD*
Robustness	Change in Flow rate (± 0.2ml)	0.413
	Changes Column oven Temperature (± 5 ^{o}C)	0.632

Observation

System suitability parameters were evaluated as per test method and the % assay and %RSD results were calculated and found to be within the specified limits.

Forced degradation

Table 11: Forced degradation studies

Method	% Assay*	%RSD	Purity Angle	Purity Threshold
Acid Degradtion	67.73	0.32	0.03	0.23
Alkali Degradation	99.72	0.62	0.03	0.25
Peroxide Degradation	97.45	0.26	0.02	0.23
Water	98.21	0.03	0.02	0.23
Humidity	97.75	0.17	0.03	0.24
UV light	101.93	0.39	0.03	0.25
Thermal	98.2	0.55	0.04	0.23

* Average of three readings

CONCLUSION

All the above results lead to the conclusion that the proposed method is accurate, precise, simple, robust and cost effective, stability indicating and can be applied successfully for the routine estimation of Flavoxate Hcl in pharmaceutical formulation by gradient RP-HPLC method.

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