IJPSR (2018), Volume 9, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 May, 2017; received in revised form, 05 September, 2017; accepted, 17 September, 2017; published 01 February, 2018

ALKALINE DEGRADATION KINETICS AND STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF FLAVOXATE HYDROCLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

K. Srinivasa Rao

Department of Pharmacy, Indira Gandhi National Tribal University, Amarkantak - 484887, Madhya Pradesh, India.

Keywords:

Flavoxatehydrocloride, Stability studies, Assay, Validation, Degradation kinetics, Statistical analysis

Correspondence to Author:
Dr. K. Srinivasa Rao

Department of Pharmacy, Indira Gandhi National Tribal University, Amarkantak - 484 887, Madhya Pradesh, India.

E-mail: ksrao108@gmail.com

ABSTRACT: A simple stability indicating reversed-phase HPLC method was developed, validated and subsequently alkaline degradation kinetics are also determined for the estimation of Flavoxate Hydrocloride (FVH) present in pharmaceutical dosage forms. The proposed RP-HPLC method utilizes a LiChroCART - Lichrosphere 100, C18 RP column Hibar® (250 × 4 mm, 5 µm) in an isocratic separation mode with mobile phase consisting of methanol and water in the proportion of 50:50% (v/v), at a flow rate of 0.8 ml / min and the effluent was monitored at 315 nm. The retention time of FVH was found to be 2.92 min. Stability of FVH was investigated as per ICH - prescribed stress conditions including acidic, alkaline, thermal, oxidative and photolytic conditions. Significant degradation of FVH was observed under all studied stress conditions. A kinetic study was conducted to investigate the alkaline degradation of FVH at different temperatures; reaction rate constants, half-life times and activation energy were calculated. The described method was linear over a range of 1 - 300 µg/ml. The percentage recovery was 99.46. F-test and t-test at 95% confidence level was used to check the intermediate precision data obtained under different experimental setups; the calculated value was found to less than the critical value.

INTRODUCTION: Flavoxate hydrochloride (FVH) is a 3- methylflavone- 8- carboxylic acid β-piperidinoethyl ester hydrochloride, $C_{24}H_{26}ClNO_4$, MW: 427.93. It is a flavone derivative, exhibits smooth muscle relaxant activity, with selective action on the pelvic region [1]. It is used for the symptomatic relief of pain, urinary frequency, and incontinence associated with inflammatory disorders of the urinary tract. It is also used for relief of vesico-urethral spasms resulting from instrumentation or surgery [2]. Forced degradation study or stress testing is undertaken to demonstrate specificity while developing a stability indicating method, particularly when little information is available about potential degradation products.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(2).625-32

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(2).625-32

The ICH guideline entitled "Stability Testing of New Drug Substances and Products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substances [3]. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and

enables recommendation of storage conditions, retest periods, and shelf lives to be established. Regulatory agencies recommend the use of stability-indicating methods for the analysis of stability samples. Forced degradation of FVH was performed under stress conditions (acid, alkaline, photolytic, thermal and oxidative), to establish the stability indicating nature of the method, and the stressed samples were analyzed by the proposed method. The proposed RP-HPLC method was validated as per ICH guidelines [4, 5].

The literature survey reveals that FVH was estimated from its pharmaceutical preparations by using Ultraviolet spectroscopy [6, 7], high performance liquid chromatography [8 - 11], potentiometric [12], Voltammetry [13], and capillary electrophoresis [14] techniques. Also, few HPLC methods have focused on the analysis of FVH in biological fluids [15 - 17].

The retention time of the reported HPLC methods are more than five minutes and the flow rate of the mobile phase is 1.5 ml / min, which requires a greater mobile phase for the determination of FVH. Hence, in the present the authors developed a rapid, economical, simple, and precise alkaline degradation kinetic study and stability indicating RP-HPLC method for the estimation of flavoxate hydrocloride in bulk and pharmaceutical dosage formulations.

MATERIALS AND METHODS:

Chemicals and Reagents: Gift sample of FVH was received from Alkem Labalories, Mumbai, India. HPLC grade methanol was purchased from Rankem Ltd, India. Hydrogen peroxide was purchased from Qualigens Fine chemicals Ltd., India and sodium hydroxide was purchased from Merck Ltd., India. High pure water was prepared by using Millipore Milli Q plus purification system. Commercial formulations containing FVH were purchased from the local market.

HPLC Instrumentation and Conditions: Quantitative HPLC was performed on Shimadzu HPLC with LC 10 ATVP series pumps besides SPD10A VP UV-Visible detector. The chromatographic separations were performed using LiChroCART - Lichrosphere 100, C18, RP column Hibar® (250 × 4 mm, 5 µm) maintained at ambient temperature, eluted with mobile phase at a flow rate of 0.8 ml/min for 10 min. The mobile phase consisted of methanol - water (50:50% v/v). Measurements were made with injection volume 20µl and ultraviolet detection at 315 nm.

Preparation of Mobile Phase: Methanol and water was properly mixed in the ratio of 50:50.

Standard and Sample Preparation: The standard stock solution of FVH (1 mg / ml) was prepared by dissolving 25 mg each of FVH in 25 ml volumetric flask containing 10 ml of methanol and 5 ml of water. The solutions were sonicated for about 10 minutes and later diluted to desired volume with mobile phase. Standard calibration solutions of FVH having concentration in the range of 1 - 300 µg/ml were prepared by diluting stock solution with mobile phase.

Twenty tablets of FVH each containing 200 mg were weighed and powdered. A quantity equivalent to 25 mg of FVH was weighed and transferred in to a 25 ml volumetric flask containing 15 ml of the mobile phase and sonicated for 10 minutes. Subsequently, the volume was made up to the mark with the mobile phase. The solution was filtered using a nylon 0.45 µm membrane filter. This solution was diluted with the mobile phase to yield the concentrations in the Beers range of 1 - 300 µg/ml.

Forced Degradation Study: The stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation, and UV degradation, as defined by ICH.

Acidic and Thermal Degradation: Aliquots of FVH standard solution (containing 100.0 mg) were transferred into two small conical flasks; 5 mL aliquots of 1.0 M HCl and distilled water were added. The solutions were heated in a thermostatically controlled water bath at 80 °C for 30 min. At the specified time, the contents of the flasks were cooled, acid solutions are neutralized to pH 7.0 using 1.0 M NaOH and 1.0 mL of each solution was then transferred into a series of 10 mL volumetric flasks. The volumes were completed with the mobile phase and the solutions were mixed well. Triplicate 20 mL injections were made for each sample.

Alkaline Degradation: Aliquots of FVH standard solution (containing 100.0 mg) were transferred into a series of small conical flasks and 5 mL of 0.1M NaOH were added to each flask. The solutions were heated in a thermostatically controlled water bath at different temperature settings (60, 70, 80 °C) for different time intervals (10 - 40 min). At the specified time, the contents of each flask were cooled and the solutions are neutralized to pH 7.0 using 1.0 M HCl. The solutions were transferred into a series of 10 mL volumetric flasks, the volumes were completed with the mobile phase and the solutions were mixed well. Triplicate 20 mL injections were made for each sample.

Oxidative Degradation: Aliquots of FVH standard solution (containing 100.0 mg) were transferred into a series of small conical flasks and 2 mL of H_2O_2 solution (30% w/v) were added to each flask. The solutions were heated in a thermostatically controlled water bath at 80 °C for 30 min. At the specified time, the contents of each flask were cooled. 1.0 mL of each solution was transferred into a series of 10 mL volumetric flasks, the volumes were completed with the mobile phase and the solutions were mixed well. Triplicate 20 mL injections were made for each sample.

Photolytic Degradation: Aliquots of FVH solution (100.0 mg/mL) were transferred into a series of small conical flasks containing solvent (methanol - water (1:1 v/v)) and exposed to UV-light at a wavelength of 254 nm at a distance of 15 cm placed in a UV cabinet for 3 h. At the specified time, 1.0 mL of each solution was transferred into a 10 mL volumetric flask, completed to the mark with the mobile phase and mixed well. Triplicate 20 mL injections were made for each sample.

RESULT AND DISCUSSION:

Optimization of the Method: The proposed RP-HPLC method utilizes a LiChro CART-Lichrosphere 100, C18 RP column Hibar® (250 × 4 mm, 5 µm) in an isocratic mode with mobile phase methanol and water in the proportion of 50:50% (v/v), at a flow rate 1 ml / min and the effluent was monitored at 315 nm. The retention time of FVH was 3.058 min. Degradation products resulting from the stress studies did not interfere with the detection of FVH and the assay is thus stability indicating.

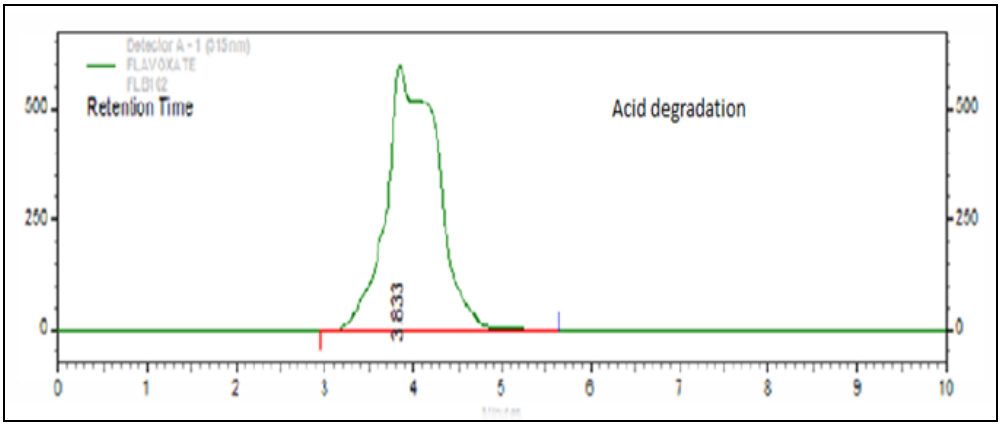
Results of Forced Degradation Studies: Much degradation was observed in FVH samples under all stress conditions like acid, alkaline hydrolysis, oxidative, photolysis and thermal degradation **Fig.1**. **Table 1** indicates the extent of degradation of FVH under various stress conditions. Under acidic conditions (1.0 M HCl, 80 °C, 30 min), about 21.94% of the drug was degraded with a broad chromatogram at Retention time 3.8 min. Meanwhile, in the case of neutral hydrolysis (water, 80 °C, 30 min), only 12.07% of the parent drug was degraded, resulting in the appearance of broad chromatogram at Retention time 3.4 min.

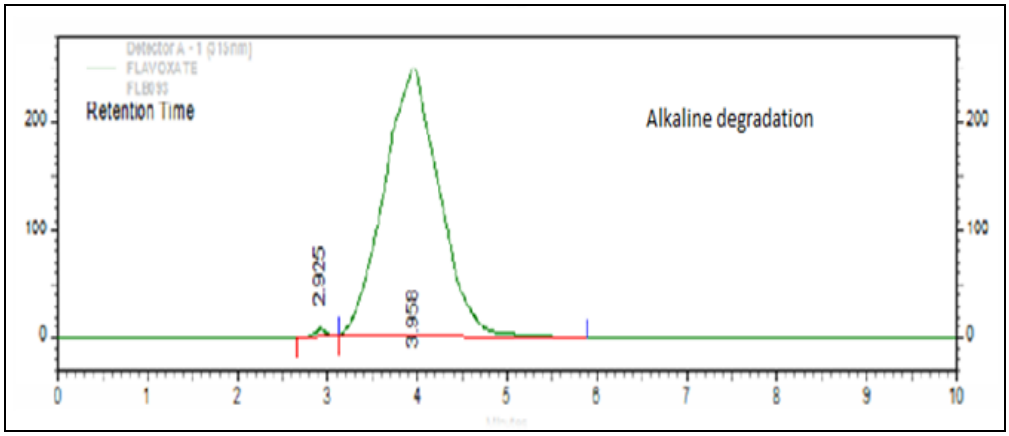
TABLE 1: RESULTS OF ANALYSIS OF FORCED DEGRADATION STUDY

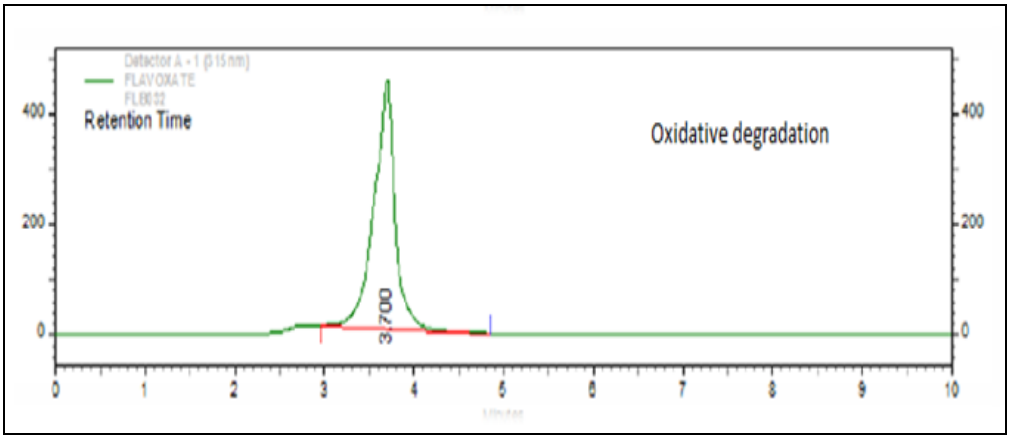
Stress condition / duration	FVH	
	% Recovery	Retention Time
Acid degradation 1.0M HCl, 80 °C, 30 min	77.66	3.833
Alkaline degradation 0.1M NaOH, RT, 10 min,	78.1	2.925, 3.958
Photolysis UV light (254 nm), MeOH-H_2O (1:1 v/v), 3 hrs	61.72	3.083
Thermal degradation Water, 80 °C, 30 min	87.93	3.450
Oxidative degradation 30% w/v H_2O_2, 80 °C, 30 min	53.28	3.700

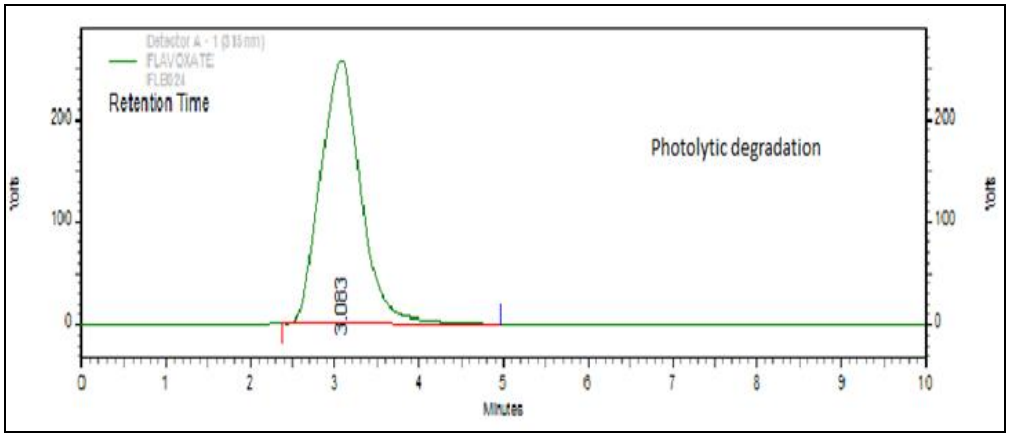
RT: Room Temperature

The alkaline degradation of FVH was investigated by heating with 0.1 M NaOH. Preliminary studies revealed that FVH is susceptible to alkaline degradation, where considerable degradation was observed at Retention time 2.9 and 3.9 min. Consequently, the kinetics of alkaline degradation was explored at different temperature settings (60 - 80 °C) for increasing time intervals (10 - 40 min). The alkaline degradation of FVH was found to follow first order degradation kinetics as shown in **Fig. 2**. The apparent first order reaction rate constants and the half-lives were calculated and the results are presented in **Table 2**. By plotting log k_{obs} values versus 1/T, an Arrhenius plot was obtained. From the Arrhenius equation, activation energy (Ea) of the alkaline degradation of FVH was calculated and was found to be 5.36 kcal /mol.


Retention Time
Acid degradation
3.833


Retention Time
Alkaline degradation
2.925
3.958


Retention Time
Oxidative degradation
3.700


Retention Time
Photolytic degradation
3.083
Minutes

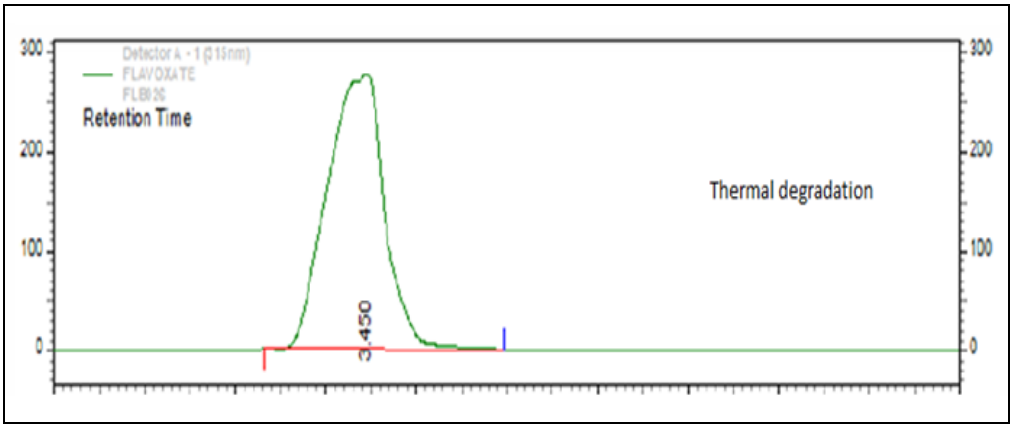


FIG. 1: REPRESENTATIVE CHROMATOGRAMS SHOWING FVH AFTER EXPOSURE TO DIFFERENT HYDROLYTIC CONDITIONS

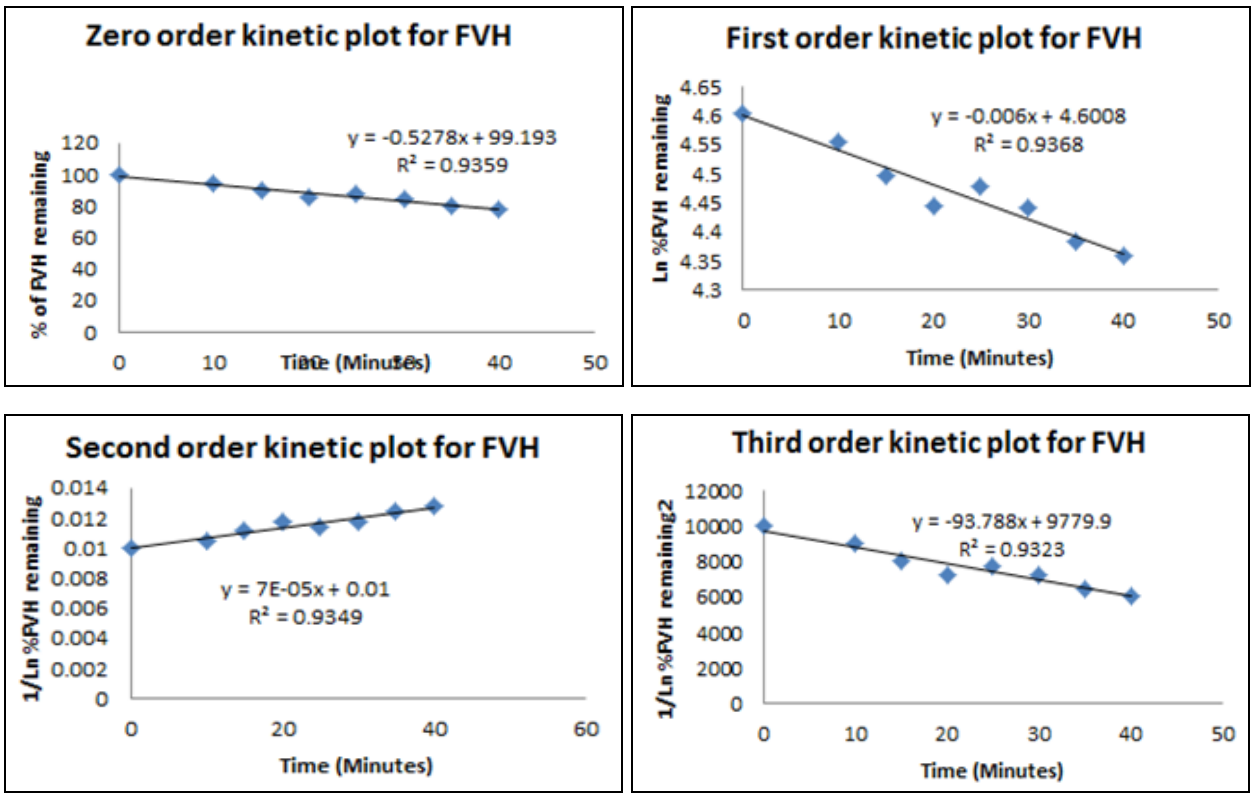


FIG. 2: REPRESENTATIVE ALKALINE DEGRADATION KINETIC PLOTS FOR FVH AT 60 °C

TABLE 2: REACTION RATE CONSTANTS AND HALF - LIVES OF FVH IN ALKALINE DEGRADATION AT DIFFERENT TEMPERATURE

Temperature (°C)	k (min^{-1})	$t_{1/2}$ (min)
60	6.0×10^{-3}	115.5
70	7.12×10^{-3}	97.33
80	8.43×10^{-3}	82.20

k reaction rate constant (min^{-1}), $t_{1/2}$ half life (min)

The oxidative degradation of FVH was investigated by heating with H_2O_2 for 30 min, where considerable degradation of about 46.7% was observed with the formation of broad peak at retention time 3.4 min. When exposed to direct UV light for 3hrs, 38.28% of FVH was degraded and showing a broad peak at 3.08 min, which indicate that FVH is sensitive to light. When FVH was treated with heat at 80 °C for 30 min it was degraded only 12.07% with a broad peak at 3.4 min. Assay of FVH was unaffected by the presence of other degradants which confirms the stability-indicating power of the method.

Method Validation: The described method has been validated for linearity, precision, accuracy, specificity, LOD and LOQ, system suitability parameters, ruggedness and robustness.

Linearity: Least square regression analysis was carried out for the slope, intercept and correlation coefficient **Table 3**. The linear fit of the system was illustrated graphically. The linearity range was found to be 1 - 300 µg/ml. Regression equation for FVH was, y = 24341x + 4144.8 (R^2 = 0.9999).

TABLE 3: REGRESSION CHARACTERISTICS OF THE PROPOSED HPLC METHOD

Linearity experiment (n = 5)	FVH
Range (µg/ml)	1-300
Mean 'R' value	0.9999
Slope	24341
Intercept	4144.8

Accuracy: This experiment was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 80, 100 and 120% of labelled amount of the drug (400 mg FVH) in tablet. Three replicate samples of each concentration level were prepared and the % recovery at each level (n = 3), and mean % recovery (n = 9) were determined **Table 4**. The mean recovery was 99.46%.

TABLE 4: RESULTS OF ACCURACY EXPERIMENT USING PROPOSED METHOD

	FVH		
	Taken (µg)	Recovered (µg)	% Recovery
Level 1 (80)	8	7.96	99.53
Level 2 (100)	10	9.96	99.64
Level 3 (120)	12	11.90	99.23
Mean % recovery (n=9)		99.46 ± 0.25	
% RSD		0.253	

Precision: The precision of the proposed method was evaluated by carrying out eight independent assays of test sample. RSD (%) of eight assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument. F - test and t - test was applied to the two sets of data at 95% confidence level, and no statistically significant difference was observed. The resultant data was presented in **Table 5**.

Specificity: Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to present in the sample matrix. It was found that the proposed method was specific as there is no interference of other active ingredients and excipients ensuring that the peak response is due only to a single component. Based on the results, obtained from the analysis of forced degraded samples using the described method, it can be concluded that the method is specific for estimation of FVH in presence of degradants.

TABLE 5: RESULTS OF PRECISION STUDY

Precision	FVH
	Mean assay (%) / % R. S. D
Set 1 (n = 5)	99.7 / 0.939
Set 2 (n = 5)	99.4 / 0.771
	Calculated value/ critical value
F-test	0.921 / 3.328
t-test	0.434 / 2.103

LOD and LOQ: The detection and quantification limits were evaluated from calibration curves plotted in concentration range of 1 - 300 µg/ml. The acceptance criterion for these replicate injections was RSD not more than 30% for LOD concentration and not more than 10% for LOQ concentration. The formulae used were LOD = 3.3σ/S and LOQ = 10σ/S (where σ = standard deviation of response and S = slope of calibration curve). The standard drug solutions, for each value of LOD and LOQ concentration were injected 5 times. % RSD values for the area of replicate injections were calculated. LOD and LOQ for this method were found to be 0.120 and 0.378 respectively. These values indicated that the method was very sensitive to quantify the drug.

System Suitability Parameters: System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of FVH in pharmaceutical formulations was validated or not. System suitability is usually developed after method development and validation has been completed. The obtained value of Theoretical plates (N) in this method was 6869 and the tailing factor was found to be 1.324.

Robustness: The percentage recovery of FVH was good under most conditions and didn't show any

significant change when the critical parameters were modified. The tailing factor was always less than 2.0 and the components were well separated under all the changes carried out. Thus the method conditions were robust.

Assay: The validated method was applied to the determination of FVH in commercially available tablet formulations. **Fig. 3** and **4** illustrates a representative HPLC chromatograms obtained from FVH reference standard solution and from the assay of tablet formulations respectively.

The observed concentration of FVH was found to be 397.67 ± 0.27 mg (mean ± SD) for tablet formulation taken. The results of the assay (n = 9) under retention time taken yielded 99.06% (% RSD = 0.1) of label claim for FVH in formulation. The retention times of FVH were found to be 2.925 and 2.983 for standard drug and formulation respectively. The results of the assay indicate that the method is selective for the estimation of FVH without interference from the excipients used to formulate and produce these tablets.

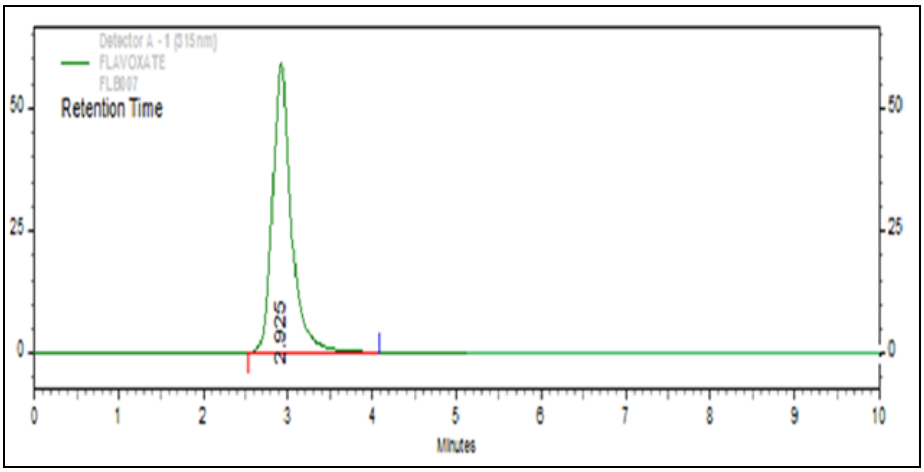


FIG. 3: REPRESENTATIVE CHROMATOGRAM OF FVH IN REFERENCE STANDARD SOLUTION (10µg/ml)

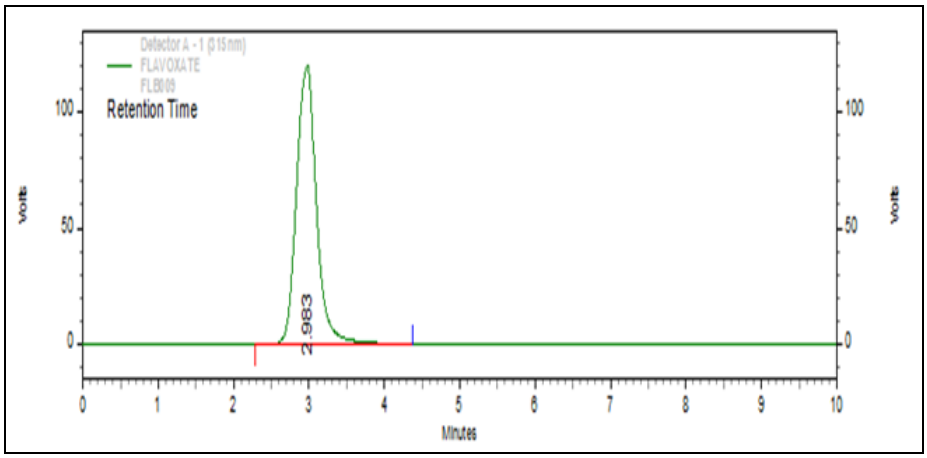


FIG. 4: REPRESENTATIVE CHROMATOGRAM OF FVH (20µg/ml) IN FORMULATION

CONCLUSION: The LC method described here is a very simple, sensitive, and accurate procedure for estimation of FVH. The developed and validated LC method is stability - indicating and enables specific, accurate, robust and precise analysis of FVH in formulations. The method is sensitive enough for quantitative detection of the analyte in pharmaceutical preparations. Nevertheless, this paper is the first report that investigates kinetics of alkaline degradation process of this drug.

The proposed method can thus be used for routine analysis, quality control and for studies of the stability of pharmaceutical tablets containing these drugs. The validation data indicative good precision, accuracy and reliability of the method. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation.

ACKNOWLEDGEMENT: The author is thankful to the Anwarul - Uloom College of Pharmacy, Hyderabad, for providing the laboratory facilities.

CONFLICT OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Pedersen E: Studies on the effect and mode of action of flavoxate in human urinary bladder and sphincter. Urol Int. 1977; 32: 202–8.
2. Sweetman SC: 33rd ed. London: Pharmaceutical Press; 2002. Maretention time indale. The Complete Drug Reference.
3. International Conference on Harmonization, IFPMA. Stability testing of new drug substances and products Geneva 2003.
4. International conference on Harmonization guidance for Industry In: Q2A Text on Validation of Analytical methods. Switzerland, IFPMIA 1994; 1-4.
5. International conference on Harmonization guidance for Industry In: Q2B Text on validation of Analytical methods. Switzerland, IFPMIA 1996; 1-8.
6. Zheng YJ: Ultra-violet spectrophotometric determination of flavoxate hydrochloride tablets. Yaowu - Fenxi-Zazhi 1993; 13: 339–40.
7. British Pharmacopoeia. London: Her Majesty's Stationary Office British Pharmacopoeia Commission 2005; 1.
8. Wang YT, Wang TL and Zhang J: RP-IP-HPLC determination of flavoxate hydrochloride tablets. Yaowu-Fenxi-Zazhi 2002; 22: 202–5.
9. Attimarad M: Liquid Chromatographic Determination of Flavoxate HCl in Pharmaceutical Formulation. Journal of Young Pharmacists 2010; 2(3): 280-283.
10. El-Gindy A, Abdel-Salam RA and Sallam S: High-performance liquid chromatographic determination of flavoxate hydrochloride and its hydrolysis product. Drug Development and Industrial Pharmacy 2008; 34: 1311–22.
11. Attimarad M, Fredy H and Armagon O: Liquid Chromatographic Determination of Flavoxate HCl in Pharmaceutical Formulation. E-Journal of Chemistry 2009; 5(3): 715-720.
12. Heba M, Ramadan N and El-Laithy M: Polymeric matrix membrane sensors for stability-indicating potentiometric determination of oxybutynin hydrochloride and flavoxate hydrochloride urogenital system drugs. Journal of AOAC International 2008; 91: 1318–30.
13. Ghoneim MM, El-Attar MA and Razeq SA: Voltammetric quantitation at the mercury electrode of the anticholinergic drug flavoxate hydrochloride in bulk and in a pharmaceutical formulation. Central European Journal of Chemistry 2007; 5: 496–507.
14. Zhang CX, Sun ZP, Ling DK, Zheng JS, Guo J and Li XY: Determination of 3-methylflavone-8-carboxylic acid, the main metabolite of flavoxate, in human urine by capillary electrophoresis with direct injection. Journal of Chromatography A 1993; 612: 287–94.
15. Gilliland MD, Carnes EJ, Saretention time well CJ, Dzerk AM and Lin PP: LC-MS/MS Determination of Flavoxate and 3-Methylflavone-8-Carboxylic acid (MFA) in Human NaF Plasma. MDS Pharma Services. Available from: http:// www.aapsj.org / abstracts / Am 2003/AAPS2003-000732.PDF.
16. El-Gindy A, Sallam S and Abdel-Salam RA: High performance liquid chromatographic determination of 3-methylflavone-8-carboxylic acid, the main active metabolite of flavoxate hydrochloride in human urine. Journal of Pharmaceutical and Biomedical Analysis 2007; 44: 274–8.
17. Sheu MT, Yeh GC, Ke WT and Ho HO: Development of a high performance liquid chromatographic method for bioequivalence study of flavoxate tablets. Journal of Chromatography B Biomedical Sciences and Applications 2001; 751: 79–86.

How to cite this article:
Rao KS: Alkaline degradation kinetics and stability indicating RP-HPLC method for the estimation of flavoxate hydrocloride in bulk and pharmaceutical dosage forms. Int J Pharm Sci & Res 2018; 9(2): 625-32. doi: 10.13040/IJPSR.0975-8232.9(2).625-32.