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Development and validation of single analytical HPLC method for determination of flavoxate HCl in bulk, tablets and biological fluids

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Abstract: A simple, sensitive and precise high performance liquid chromatographic (HPLC) method was developed and validated for determination of flavoxate HCI in raw material, tablets and biological fluids. The method followed by using the Zorbax XDB-C18 column containing Di-isobutyl n-octadeceylsilane (4.6mm×150mm, 5µm). The mobile phase consisted of acetonitrile: methanol: 0.15M sodium perchlorate (17:35:48 v/v) having pH 3. UV detection was carried out at 229nm at 40°C. Results indicated that the method has successfully established and validated in accordance with ICH guidelines acceptance criteria for linearity (0.03-7.5µg), accuracy (101.18-101.28%), robustness of column age and column lot (peak area %CV≤0.04, purity %CV≤ 0.006) and robustness of HPLC condition (%CV≤ 0.02), precision (intra and inter day precision assay, %CV values for peak area and percent purity of flavoxate HCl≤2%) and system suitability parameters. The average noise, theoretical LOD and LOQ were found to be 0.01 mAU, 0.03 mAU and 0.6ng, respectively. The Coefficient of determination (r^2) ranging from 0.03µg to 7.5µg, 0.99 which was within acceptable criteria of r^2 & gt 0.99. The spiked recoveries of samples were 101.28, 101.18 and 101.18% respectively. All data revealed that this method can be used for *in-vitro* & *in-vivo* determination of flavoxate HCI in various pharmaceutical preparations.

Keywords: Flavoxate HCI, validation, HPLC, precision, accuracy.

INTRODUCTION

Simple, accurate and speedy analytical testing methods are always the preference of pharmaceutical analysts in order to meet the time constrain during the manufacturing (Deidda *et al*., 2018). The developed analytical method should be reproducible, sensitive and applicable to the variety of dosage forms/samples and if possible could be applicable to bulk and biological samples with minor modifications.

Flavoxate HCI is a flavone derivative that belongs to the class of drugs used as smooth muscle relaxants particularlyon pelvic muscles (Sheu *et al*., 2001). It is recommended drug for the treatment of urinary tract infections related to pain, urinary frequency and incontinence. It is given by oral route when administration it is metabolized into 3-methyl-flavone-8-carboxylic acid (Pedersen, 1977). Bioavailability of drug depends upon various factors i.e. drug physicochemical properties and excipients in formulation, its lumen decomposition, pH and perfusion of the digestive system, surface and time accessible for retention, food and hepatic first pass impact (Malangu, 2018)

According to FDA, that method can be used to estimate flavoxate if confidence interval is 90%. Such a method can be utilized for performing the bioequivalence study of flavoxate (Sheu *et al*., 2001).Till now different methods including ultra violet (UV) (B.P, 2005), high performance liquid chromatography (HPLC), liquid chromatography-mass spectroscopy (LC-MS/MS) (Loh *et al*., 2021), potentiometric (Ismail, 2016), electrophoresis (Zhang *et al*., 1993) etc have been developed to estimate flavoxate HCI in pharmaceutical preparations but still not a method has been developed which can be used to estimate flavoxate HCI simultaneously in raw material, tablets and biological fluids. Since in routine analysis a single method capable of identification and quantification of active ingredient is required therefore the current research work is based upon the development and validation of such type of RP-HPCL method for flavoxate HCl.

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MATERIALS AND METHODS

Instrumentation

HPLC Agilent 1260 infinity II (Germany), autosampler, equipped with quaternary pump, degasser and-UV-VIS detector. A reverse phase C18 column with dimensions 150 x 4.6mm and 5µm in diameter was used.

Reagents and chemicals

The authentic working standard of flavoxate was gifted by Pacific Pharmaceuticals Pvt. Lahore, Pakistan. Methanol (HPLC grade), perchloric acid, HCI, acetonitrile and sodium perchlorate were purchased from Merck, Germany. Flavoxate HCI raw material and tablets (600mg) and plasma were used for analysis. Tablets were prepared by Pacific Pharmaceuticals Pvt. Lahore, Pakistan.

Chromatographic conditions

The HPLC analysis (Agilent 1260 infinity II) was carried out by using the Zorbax XDB -C18 column containing Di-isobutyl n- octadeceylsilane as stationary phase supported on porous silica. The flow rate of the mobile phase was maintained at1.5mL/min. The detector used was UV-Visible detector set at the wavelength of 229nm. The temperature of the column was maintained at 40ºC throughout the analysis. Injection volume was 5µL for both samples as well as the standard. Testing of biological fluids was carried out by using the same chromatographic conditions. However, the flow rate was adjusted to avoid any interference with plasma peaks. Firstly, the calibration curve was constructed for flavoxate HCl in human plasma after spiking collected plasma with different solutions of 0.01, 0.02, 0.03, 0.04 and 0.05mg/mL by diluting the stock solution and 1.2 and 1.4mg/mL.

Preparation of standard solution

Accurately weighed working standard of flavoxate HCl (50mg) was transferred into 100 mL volumetric flask and 50mL of 0.1N HCl was added into it. The flask was sonicated for 2min then was heated at 70°C for five min. After heating the flask was cooled to room temperature and the volume of the solution was made up to 100mL with methanol.

Preparation of sample solution

Preparation of bulk sample

The raw material powder was weighed 68mg approximately, which was equivalent to 50mg of flavoxate HCl and was transferred into a 100mL volumetric flask. 50mL 0.1N HCI was added to it and was sonicated for 2 min. The solution was heated at 70ºC for 5min then was cooled at room temperature and the final volume was made up to 100mL with methanol. Finally, the solution was filtered with Whatman filter paper.

Preparation of tablet sample

Simply compressed tablets (#10) were selected randomly and were powdered. Weighed the powder equivalent to 50mg of flavoxate HCI and was transferred into a100mL volumetric flask. 50mL of 0.1N HCl was added to this flask and was sonicated for 2min. Then the flask was heated at 70ºC for 5 min, cooled at room temperature and the final volume was made up to 100mL with methanol. Finally, the solution was filtered with Whatman filter paper (Fayyaz*et al*., 2015).

Determination in human plasma

Development of calibration cure

The calibration curve was constructed for flavoxate HCl in human plasma after spiking collected plasma with different solutions of 0.01, 0.02, 0.03, 0.04 and 0.05mg/mL by diluting the stock solution, and 1.2 and 1.4mg/mL.

Withdrawal and processing of blood samples

All the work on humans was carried out after getting permission and according to the guidelines established by the Punjab University College of Pharmacy, the University of the Punjab, under the Human ethical form number HEC/PUCP/1967-A.3mL of blood samples was collected from each volunteer (n=6) before oral drug administration (0h) and at 0.5, 0.67,1.0, 2.0, 3.0,3.5, 4.0,4.5, 5.0,5.5, 6.0,6.5, 7.0, 8.0, 10.0, 12.0, 18.0h administration in heparinized tubes and was mixed by gentle inversion. The heparinized blood samples were subjected to centrifugation at 3000 rpm for 15 min using frigerated centrifugation machine (Hettich Zentrifugen, Japan) at 5±3ºC. After centrifugation, the plasma layer was extracted with help of micropipette from blood layer and transferred to eppendorf tubes. These plasma samples were stored in specialized freezer capable of maintaining the temperature at -20ºC until they were analyzed for the estimation of flavoxate HCl.

Preparation for biological sample

The flavoxate HCl was extracted by using acetonitrile, 1mLof plasma was mixed with an equal amount of acetonitrile. The prepared sample was shaken for about 3 min and centrifuged for 10min at 4000rpm. The top layer of the sample was collected and evaporated under a steam of nitrogen until dried. The resulting residue was mixed with a200 µL mobile phase. 5 µL of the prepared sample was injected into HPLC for detection of drug in plasma samples.

Validation procedure

Validation is the analytical procedure through which the performance characteristics of a procedure meet the requirements for intended analytical applications. The main purpose of the validation is to reveal that the developed method is appropriate for its indented use. Various analytical methods have been used for drug estimation however, HPLC is still popular and being utilized successfully for the quantification of many chemicals by researchers (Kapupara *et al*., 2018; Koppala *et al*., 2017). Furthermore the method is precise, accurate and specific for the analysis of analyte according to the official range. It is performed when a new method is developed or in case of any change in the existing method (Hanif *et al*., 2021).

Linearity

Linearity was conducted to determine the range over which flavoxate hydrochloride exhibited the linear responses. The stock solution was prepared by dissolving 1mg/mL of flavoxate HCI. Solutions of strength 0.01, 0.02, 0.03, 0.04 and 0.05mg/mL were prepared by using stock solution and 1.2 and 1.4mg/mL. 5µL of each concentration was injected to find out the proportional relation (Shah *et al*., 2019; Mehmood *et al*., 2019).

Precision
It is the measure of how close data values are to each other for a series of measurements under the same analytical conditions by different analysts (Alquadeib, 2019). It was performed on a lot of tablets on a single day. All samples were prepared as described in sample preparation and inject into the column 6 times to determine repeatability. The sample solution was injected 6times.Intermediate Precision comprised of Intra assay/ Inter day assay was performed. It was evaluated by two analysts. Each analyst tested the samples on three separate days (USP, 2017).

Accuracy
The known amount of flavoxate HCl spiked could be determined with the matrices of samples by using this assay. The reference standard stock solution was prepared with diluting solution having concentration of 0.01 mg/mL. The stock solution of reference material diluted gravimetrically i.e. 80, 100 and 120%and spiked it to flavoxate HCl. The spiked and unspiked samples of flavoxate HCl were tested in triplicate (USP, 2017).

Limit of detection (LOD)
LOD was measured by injecting the four replicates of blanks (Dayyih *et al*., 2021). The noise was measured from 0-6 minutes (determined as+1 min from the retention time of flavoxate hydrochloride main peak (approximately 4.0 min) in 0.5 min intervals. LOD was calculated theoretically as 3X base noise while LOQ was calculated as 10X base noise. The values were expressed as µg load of flavoxate HCl based on peak height of standard injection (5 µg)(USP, 2017).

Robustness
It is the measure of the capacity of a method to remain unaffected by small variations in method parameters, and is an indication of reliability during normal use. Comparison of the performance of the different columns of different HPLC instruments and variation of the column temperature and flow rate assessed the reliability of C18 RP-HPLC assay in this study (USP, 2017).

STATISTICAL ANALYSIS

Statistical analysis was performed using the Microsoft Office Excel version 2018.

RESULTS

Development of analytical method
The first step in developing an HPLC method development is the selection of optimum absorption wavelength when using UV-Visible detector. As per the literature, flavoxate HCl showed maximum absorption at the wavelength (λmax) of 229 and is used in this study. Chromatographic results were investigated under various chromatographic conditions. Mobile phases of different compositions comprising methanol, acetonitrile and sodium perchlorate buffers were evaluated. After various attempts, the mobile phase consisted of acetonitrile: methanol: 0.15M sodium perchlorate (17:35:48 v/v) having pH 3 adjusted with perchloric acid and with flow rate 1.5mL/min give better results. The representative chromatogram depicting sharp gaussian peak with retention time at ~3.80 minutes is shown in fig. 1.Calibration curve using flavoxate HCl standard solution in the range of 0.039µg to 7.5 µg showed a liner curve with R2 value of 0.999. The calibration curve was used to calculate the % amount of raw material and tablets.

Assay of raw material
Fig. 1 shows chromatograms obtained by the samples prepared with (right) and without (left) flavoxate HCl raw material. As evident there was no peak observed in the sample without any drug, the baseline is very smooth showing no interference due to solvent. The raw material shows sharp single peak at the retention time of drug.

Assay of tablets
Fig. 2 shows representative chromatogram obtained from the samples obtained from prepared tablets of flavoxate HCl. A smooth baseline was observed indicating a valid extraction procedure with 100% drug recovery. The sample shows similar results as shown for the assay of raw material with retention time at 3.80 minutes.

Assay of plasma samples
Fig. 3 shows results obtained from assay of plasma samples. Blank plasma peaks of observed below 2 minutes showing no interference with the drug peak. A sharp gaussian drug peak was observed at 5.8 minutes. The increase in the retention time of drug was due to the adjustment of flow rate to avoid interaction of drug peak with plasma. The mean % recovery values of spiked were found to be 92.38-103.87% with RSD <5%in plasma with flavoxate HCl over the concentration range of 0.039µg to 7.5µg claiming the accuracy of method.

Validation of developed method
Table 1 summarizes the validation parameter of developed method along with their acceptable criteria. All the parameters were in acceptable range.

System suitability testing
The theoretical plate at half height on various days for different instruments ranged from 4844 - 6469. The USP tailing on various days for different instruments ranged from 1.168-1.358. The results have been given in table 2 and 3.

Linearity
The peak area and % peak purity for ten solutions of flavoxate HCl tablets $DCT_{HM\text{-}WG}$-IV are given in table 4. The linearity of flavoxate HCl ranging from 0.039-7.5µg.

Table 1: Summary of parameters and acceptance criteria

Validation Parameters	Acceptance Criteria	Results	Pass/ Fail
Accuracy	95-105%	101.18 – 101.28%	Pass
Precision-Repeatability	%CV for peak purity ≤ 2	% CV≤ 0.12	Pass
Precision, Intra-Assay	%CV for peak purity ≤ 2 %CV for peak area ≤ 2	% CV≤ 0.01 to 0.23 % CV≤ 0.09 to 0.45	Pass
Precision, InterAssay	%CV for peak purity ≤ 2 %CV for peak area ≤ 2	% CV≤ 0.08 to 0.18 % CV≤ 0.20 to 0.40	Pass
Robustness, Column age, Column lot	%CV for peak Area ≤ 2 %CV for % Purity ≤ 2	% CV≤ 0.04 % CV≤ 0.006	Pass
Robustness, HPLC Conditions	%CV for % Purity ≤ 2	% CV≤ 0.02	Pass
Linearity	$r^2 \geq 0.9900$	$r^2 \geq 0.9999$	Pass
Limit of Detection	Report Value	0.2 ng	Pass
Limit of Quantitation	Report Value	0.6 ng	Pass

Table 2: System Suitability Intra-Assay

System suitability	Plate count	Pass/Fail	USP tailing	Pass/ Fail
Reference Standard instrument 1,Inj. 1	6190	Pass	1.168	Pass
Reference Standard instrument 1, Inj.2	6177	Pass	1.178	Pass
Reference Standard instrument 1, Inj.3	6229	Pass	1.168	Pass
Reference Standard instrument 2, Inj.1	5299	Pass	1.355	Pass
Reference Standard instrument 2, Inj.2	5297	Pass	1.348	Pass
Reference Standard instrument 1, Inj.3	5423	Pass	1.345	Pass
MEAN	5769		1.260	
STDEVA	473	NA	0.098	NA
% CV	8.2		7.745	

Table 3: System Suitability Inter-Assay (3 days, two different HPLCs)

System Suitability	Mean	STDEVA	% CV
Plate count	5784	474.9	8.2
USP tailing	1.264	0.096	7.55

Table 4: Linearity-Variation of concentration on % purity

Target Concentration (mg/ mL)	Load (µg)	Peak Area	% Peak Purity
0.0078	0.039	72.50	100
0.0156	0.078	125.97	100
0.0313	0.156	248.53	100
0.0625	0.312	489.23	100
0.1250	0.625	983.70	100
0.2500	1.250	1915.20	100
0.5000	2.500	3865.07	99.8
0.7500	3.750	5821.17	99.8
1.0000	5.000	7818.23	99.9
1.5000	7.500	11768.80	99.93
% CV for Peak Purity = 0.10			

Table 5: Repeatability of the C18 RP-HPLC assay for quantitative determination of flavoxate HCI

Injection	Retention Time (min)	% Purity	Peak Area (mAU)
1	4.068	100	3990.80
2	4.068	100	4003.90
3	4.068	99.76	4034.06
4	4.071	99.77	3985.80
5	4.069	99.79	3999.57
6	4.074	99.78	4023.19
Average	4.0696	99.85	4006.22
%CV	0.06	0.12	0.47

Table 6: Intermediate precision – Peak area

		Intra-Assay	Inter-Assay				
		Prep-to-Prep, n=9	Day-to-Day, n= 27			Analyst-to-Analyst/ instrument-to-instrument n= 54	
Peak Area	Days	Mean of 3 Injections	%CV	Mean	% CV	Mean	%CV
Analyst 1 Instrument 1 Day 1 (batch 1)	Prep 1,2,3	3936.96	0.17	3939.75	0.20	3947.99	0.38
Analyst 1 Instrument 1 Day 2 (batch 1)	Prep 1,2,3	3948.35	0.09				
Analyst 1 Instrument 1 Day 3 (batch 1)	Prep 1,2,3	3933.95	0.12				
Analyst 2 Instrument 2 Day 1 (batch 1)	Prep 1,2,3	3951.52	0.39	3956.22	0.40		
Analyst 2 Instrument 2 Day 2 (batch 1)	Prep 1,2,3	3963.08	0.45				
Analyst 2 Instrument 2 Day 3 (batch 1)	Prep 1,2,3	3954.07	0.35				

Table 7: Intermediate Precision – Peak Area

		Intra-Assay		Inter-Assay			
		Pre-to-Prep, n=9		Day-to-Day, n=27		Analyst-to-Analyst/ instrument-to-Instrument n= 54	
Peak Area	Days	Mean	%CV	Mean	%CV	Mean	%CV
Analyst 1 Instrument 1 Day 1 (batch 2)	Prep 1,2,3	3933.95	0.22	3935.43	0.24	3944.34	0.38
Analyst 1 Instrument 1 Day 2 (batch 2)	Prep 1,2,3	3939.72	0.26				
Analyst 1 Instrument 1 Day 3 (batch 2)	Prep 1,2,3	3932.61	0.23				
Analyst 2 Instrument 2 Day 1 (batch 2)	Prep 1,2,3	3946.28	0.30	3953.22	0.36		
Analyst 2 Instrument 2 Day 2 (batch 2)	Prep 1,2,3	3952.95	0.29				
Analyst 2 Instrument 2 Day 3 (batch 2)	Prep 1,2,3	3960.45	0.43				

Table 8: Intermediate precision - % Peak purity

		Intra-Assay	Inter-Assay				
		Pre-to-Prep, n=9	Day-to-Day, n= 27			Analyst-to-Analyst/ instrument-to-instrument n= 54	
Peak Area	Days	Mean	%CV	Mean	%CV	Mean	%CV
Analyst 1 Instrument 1 Day 1 (batch 2)	Prep 1,2,3	99.81	0.10	99.79	0.18	99.85	0.15
Analyst 1 Instrument 1 Day 2 (batch 2)	Prep 1,2,3	99.68	0.23				
Analyst 1 Instrument 1 Day 3 (batch 2)	Prep 1,2,3	99.89	0.14				
Analyst 2 Instrument 2 Day 1 (batch 2)	Prep 1,2,3	99.96	0.06	99.90	0.08		
Analyst 2 Instrument 2 Day 2 (batch 2)	Prep 1,2,3	99.88	0.09				
Analyst 2 Instrument 2 Day 3 (batch 2)	Prep 1,2,3	99.87	0.07				

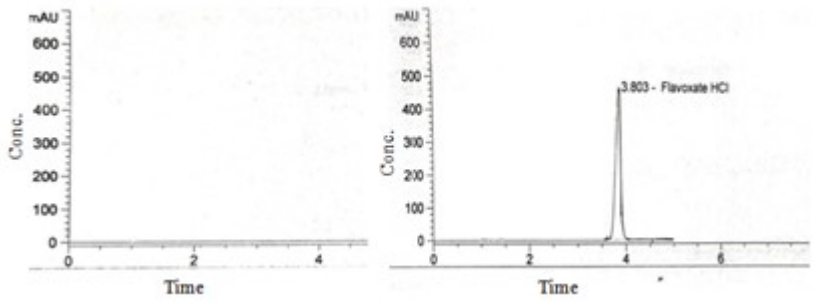

Fig. 1: Chromatogram of samples with (right) and without (left) flavoxate HCl raw material.

Table 9: Intermediate precision – % Peak purity

	Intra-Assay	Inter-Assay					
	Pre-to-Prep, n=9	Day-to-Day, n= 27				Analyst-to-Analyst/ instrument-to-instrument n= 54	
Peak Area	Days	Mean	% CV	Mean	% CV	Mean	%CV
Analyst 1 Instrument 1 Day 1 (batch 1)	Prep 1,2,3	99.88	0.01				
Analyst 1 Instrument 1 Day 2 (batch 1)	Prep 1,2,3	99.71	0.20	99.84	0.15		
Analyst 1 Instrument 1 Day 3 Lot (batch 1)	Prep 1,2,3	99.92	0.03				
Analyst 2 Instrument 2 Day 1 (batch 1)	Prep 1,2,3	99.98	0.05			99.90	0.13
Analyst 2 Instrument 2 Day 2 (batch 1)	Prep 1,2,3	99.92	0.11	99.96	0.08		
Analyst 2 Instrument 2 Day 3 Lot (batch 1)	Prep 1,2,3	99.97	0.05				

Table 10: Robustness-Effect of column age and column lot on peak area and percent purity

Injection	"Used" Column	"New" Column	Mean	% CV
PEAK AREA				
I	4072.28	3982.97	4008.80	0.04%
2	4009.04	3985.71		
3	4016.82	3986.00		
% PURITY				
1	99.25	99.79	99.79	0.006%
2	99.52	99.80		
3	99.19	99.79		

Table 11: Robustness-Variations of HPLC conditions on purity determination

Conditions	% Purity Injection 1	%Purity Injection 2	% Purity Injection 3	Mean	% CV
Flow rate, 1.25mL/min, Temp. 35°C	99.72	100	99.71	99.81	0.16
Flow rate, 1.5mL/min, Temp. 40°C	99.65	99.70	99.69	99.68	0.03
Flow rate, 1.75mL/min, Temp. 45°C	99.71	99.71	99.71	99.71	0.0
Average value of all conditions				99.73	0.02

Table 12: Accuracy results for analytical validation

Conditions	Peak Area	Actual Mean	Theoretical Value	Spiked Recovery
Reference Material	3830.20	3838.63	3838.63	NA
	3849.90			
	3835.80			
Flavoxate HCl tablets	3800.77	3800.68	3800.68	NA
	3797.57			
	3803.69			
80% spiked	6952.67	6959.27	6871.58	101.28%
	6959.65			
	6965.49			
100% spiked	7733.62	7729.38	7639.31	101.18%
	7730.07			
	7724.44			
120% spiked	8517.16	8506.02	8407.04	101.18%
	8491.13			
	8509.77			

Table 13: Determination of noise level for flavoxate HCl assay and theoretical limit of detection

Peak Height of Noise	Retention time 2.0-3.0 min, mAU	Retention time 5.0-6.0 min, Mau	Mean, mAU	Total Mean mAU
Height (mAU)Injection # 1	0.010	0.08	0.09	0.013
Height (mAU)Injection # 2	0.012	0.010	0.011	
Height (mAU)Injection # 3	0.025	0.010	0.018	
Height (mAU)Injection # 4	0.012	0.010	0.011	
Detection Limit = 3 X Mean Noise				0.039

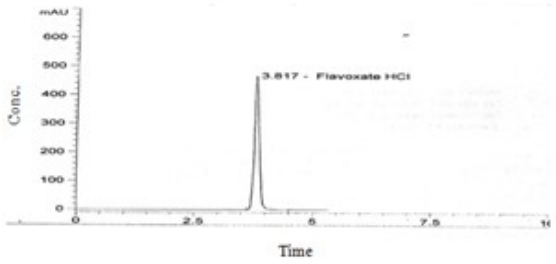

Fig. 2: Representative chromatogram of obtained flavoxate HCl tablets.

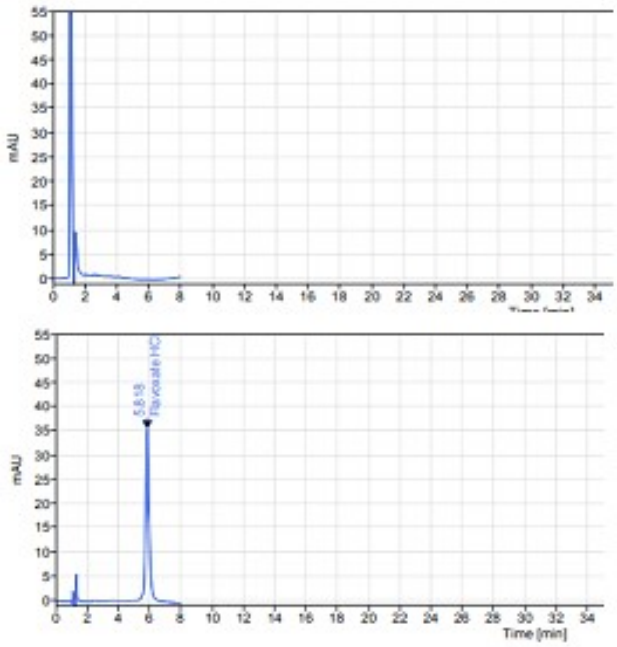

Fig. 3: Representative chromatogram of blank plasma (top) and spiked plasma with flavoxate HCl (bottom)

Repeatability

The results of flavoxate HCl tablets demonstrate the repeatability. The % CV for retention time, % purity and peak area were 0.0595, 0.116 and 0.4692 given in table 5.

Intermediate precision (Intra assay/ Inter assay)

The Intra-assay %CV values for peak area and percent purity for of flavoxate HCl finished products batch no DCTHM-WG-IV were 0.09% to 0.45% and 0.01% to 0.23% respectively. The values for peak area of % CV in inter -assay and % purity were 0.20% to 0.40% and 0.08% to 0.18% (table 6), respectively.

Robustness - column age and HPLC condition

The results of robustness are given in table 10. The range of peak area and percentage purity were 0.04% and 0.006% when premeditated the % CV between the "new" and "used" column lots.

Accuracy

The known concentration of reference material at 80, 100 and 120% could be determined through accuracy assay of the normal load (2.5µg). Results are given in table 12.The spiked recoveries were 101.28%, 101.18% and 101.18% respectively.

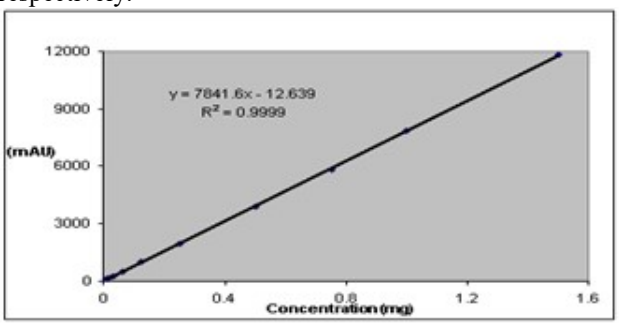

Fig. 4: Calibration curve of flavoxate HCl in blood ranging from 0.039µg -7.5µg

Limit of detection

The retention time of flavoxate HCl peak was ~ 4.0 min while range of noise fall between two ranges from 2.0–3.0 and 5.0-6.0 min. Noise was expressed as average of these two values. The theoretical LOD and average noise were found to be 0.039 mAU and 0.013 mAU (table 13).

The coefficient of determination (R^2) for sample solution from 0.039 µg to 7.5 µg was 0.9999 (fig. 4). This value was well within the acceptance criteria for the coefficient of determination as given as R^2>0.990 set. Furthermore linearity was calculated for comparison between percentage purity and % CV of ten solutions. The %CV for percentage purity for the range 0.039-7.5µg was calculated to be 0.10 %.

DISCUSSION

A precise, simple and selective method has been established and validated for the quantification of flavoxate HCI in bulk, tablets and human plasma simultaneously. Previously developed methods were employed only for estimation of flavoxate HCI in bulk and dosage form while for the estimation of flavoxate HCI in biological fluids a complicated method has to be adopted (Attimarad, 2010; Bertoli *et al*., 1976). Moreover, the currently developed method is more efficient and quick as the run time is less (4min) as compared to the other methods. The developed method is sensitive and can detect the minimum concentration of 0.2ng in plasma.

In this method validation, the theoretical plate at half height and USP tailing on various days for different instruments ranged, were well within the criteria for system suitability, i.e., theoretical plate at half height ≥1500 and USP tailing ≤1.5. For intra and inter day precision, different repeatability test methods such as % CV for retention time, % purity and peak area were used (table 2 and table 3), which met the required acceptance criteria of % of ≤2%. The value of intra and inter day assay % CV values for peak area and percent purity for

flavoxate HCl tablets was within the acceptance criteria for %CV of ≤2%. The % CV for the robustness of method for the column age was reported as the peak area, percent purity for the new and old column and for the robustness at different HPLC conditions, the % CV become double at variable column temperature and flow rates for triplicate injections of flavoxate HCl double (below, above and normal values) also appropriate for % CV of ≤5%. Therefore no significant effect was found on the % purity of flavoxate HCl upon any change in temperature and flow rate. The linearity of flavoxate HCl ranging from 0.039-7.5µg. The Coefficient of determination (r^2) for samples ranged from 0.039µg to 7.5 µg was 0.999. It was within acceptable criteria of r^2>0.990.

For further linearity, evaluation comparison was done on percent purity and % CV of ten solutions. The range of % purity was 0.039-7.5 µg to be found 0.1 for % CV. Nominal concentration (5µL of injection of 0.5 mg/mL) was indicated in data used for the quantitative determination of flavoxate HCl tablets at which flavoxate HCl purity was evaluated was within the linear range. The assays could determine the different reference concentrations at 80, 100 and 120% of normal load (2.5 µg) as in table 4. The spiked recoveries of the above samples were 101.28, 101.18 and 101.18% respectively. The average noise and theoretical LOD was found to be 0.013 mAU and 0.039 mAU, respectively (table 9). Based on the flavoxate HCl peak height of a nominal injection of linearity experiment (0.039 µg, flavoxate HCl, peak height =8.5 mAU), the theoretical LOD of 0.039 mAU was 0.2ng flavoxate HCl. The theoretical limit of quantitation was calculated as 10X the baseline noise (10X 0.013 =0.13mAU). Based on the flavoxate HCl peak height of a nominal injection of linearity experiment (0.039 µg,), the theoretical LOQ of flavoxate HCl was 0.6ng. This validation study successfully demonstrated the accuracy, precision, robustness, limit of detection, limit of quantification and linearity of the test method for quantitative determination of flavoxate HCl in tablets in all samples.

The assay method could be used for testing of biological samples as minimum limit of detection (LOD) was 0.2ng based on flavoxate HCl peak height of a nominal injection of linearity experiment (0.039µg, flavoxate HCl, peak height =8.5mAU), the theoretical LOD of 0.039 mAU. The value of the average noise and theoretical LOD were found to be 0.013 mAU and 0.039 mAU, respectively. Based on the flavoxate HCl peak height of a nominal injection of linearity experiment (0.039µg), the theoretical LOQ was 0.6ng flavoxate HCl. These values indicated that the current HPLC method was sensitive to determine the minimum quantity of flavoxate HCl in the plasma. It is a sensitive technique, more emphasis should be on finding the new methods to develop for determining the maximum quantity of any dosage form in plasma. This method is very sensitive so need to improve the increase the extent of conducting this study in plasma concentrations at different intervals.

CONCLUSION

The developed method is accurate, precise, robust, reliable and an alternate method for the simultaneous analysis of flavoxate HCl as raw material, pharmaceutical dosage forms and plasma samples. Furthermore, there is no need for any special pre-treatment of the sample for analysis. Compared to a previously reported HPLC method the present method is cost-effective, less time-consuming, and no special expertise is required for analysis of the drugs and handling of equipment.

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