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RP HPLC Method Development and Validation for the Estimation of Fenoverine In Bulk Drug and Dosage Form

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ABSTRACT

A new simple and sensitive RP-HPLC method was developed and validated as per the ICH guidelines for the estimation of fenoverine in bulk and pharmaceutical dosage form. The chromatographic separation was achieved on Enable 18H C_{18} column (250 x 4.6mm, 5µm) with a mobile phase containing acetonitrile and phosphate buffer pH 7 (55:45) at flow rate of 0.8ml/min using in isocratic elution mode. Detection was carried out at 262nm with the retention time of 4.7mins. Linearity in the calibration plot was achieved over the concentration range of 5-25ng/ml with an r^2 value of 0.997. The method was validated for accuracy, precision, specificity and selectivity, robustness, detection and quantification limits and system suitability parameters according to ICH guidelines Q2 R1. The detection limit and quantitation limit were found to be 1.3ng/ml and 4ng/ml respectively. Further the validated method was successfully applied for the analysis of fenoverine in bulk and pharmaceutical dosage forms.

Keywords: Fenoverine, RP HPLC, Validation.

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INTRODUCTION

Fenoverine is chemically 10-[(4-Piperonyl-1-piperazinyl) acetyl] phenothiazine and is indicated in the treatment of smooth muscle spasm of stomach, kidney, gallbladder or any other part of gastro intestinal tract. It is also reported to use in symptomatic treatment of gastrointestinal spasm, gastric and duodenal ulcer, monorrhagia, biliary duct and urinary tract spasm[1, 2, 3]. A limited number of analytical methods were reported for the determination of fenoverine in tissue samples by HPLC[4]; in pharmaceutical dosage forms by RP HPLC[5]; spectrofluorimetric method for estimation of fenoverine in pure and pharmaceutical preparation[6]. It was observed that only one RP HPLC method was reported for the estimation of Fenoverine in rat plasma and fenoverine HCl in the pharmaceutical dosage forms[7]. However, no RP-HPLC method was reported for the estimation of fenoverine in bulk drug and dosage forms. In view of this, efforts were made to develop new simple RP HPLC method for the estimation of fenoverine in the bulk drug and pharmaceutical dosage forms and to validate the method as per the ICH guidelines Q2 (R1)[8].

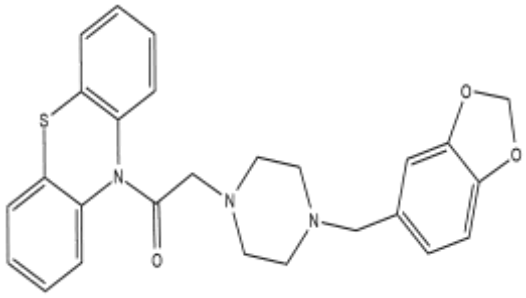

Figure 1: Structure of Fenoverine

MATERIALS AND METHOD

Materials

Fenoverine working standard was obtained as gift sample from Telangana state Drug control administration; Spasmopriv tablets (Fenoverine -Micro labs) purchased from local retail pharmacy. Methanol, acetonitrile, orthophosphoric acid, Potassium dihydrogen phosphate, disodium hydrogen phosphate Sigma Aldrich and all solvents were HPLC grade..

Instruments and Chromatographic conditions

The HPLC analysis was performed on Shimadzu LC-20AT prominence liquid chromatograph comprising a LC-20AT pump with LC Solution software, SPD-20A UV-visible detector and Enable-18H C_{18} column was used (250 x 4.6mm, 5µm). Mobile phases consisted of acetonitrile and phosphate buffer pH7 in the ratio of 55: 45 was filtered through Millipore filter and degassed and delivered with a flow rate of 8ml/min. Retention time was 10mins, injection volume was 20µl.

The Detection wavelength for fenoverine was set to 262nm. Chromatography was carried out by maintaining the column at room temperature.

Preparation of fenoverine standard preparation

10mg of fenoverine working standard was accurately weighed and transferred into a 10ml volumetric flask and 2ml of diluent i.e mobile phase was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent, this is standard stock-I solution with concentration of 1000µg/ml. From the standard stock – I final dilution 100ng/ml was prepared. Standard dilution were prepared by further diluting this solution to appropriate concentrations, degassed and filtered through filter before injecting into the HPLC system.

Preparation of fenoverine sample preparation

100mg of tablet powder was accurately weighed and transferred into 100ml clean dry volumetric flask, 20ml of diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent. From this stock solution final dilution 100ng/ml was prepared. Final sample concentration prepared by diluting 100ng/ml solution to appropriate concentrations.

Preparation of phosphate buffer

0.7907 grams of potassium dihydrogen phosphate and 3.25 grams of disodium hydrogen phosphate were weighed and taken into 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 7 with ortho-phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

450ml (45%) of the above buffer mixture was mixed with 550ml of acetonitrile (55%) – HPLC grade and degassed in ultrasonic water bath for 5 minutes. It was filtered through 0.22µ filter under vacuum filtration.

System Suitability

System suitability of the developed method was evaluated from the number of theoretical plates(N) and tailing factors of six replicates of standard solution.

METHOD VALIDATION

The RP-HPLC method was validated according to ICH Q2 R1 guidelines. Validated parameters were specificity, linearity, accuracy, precision, detection limit, quantitation limit and robustness.

Specificity

Specificity of the developed method was ascertained by representative chromatograms of standard drug and sample to assess unequivocally the analyte in the presence of the other components

which may be expected to be present blank, standard and sample solutions were prepared and injected into HPLC system and were analyzed as per the test method.

Linearity

Linearity was determined within the specified range of fenoverine 5ng/ml to 25ng/ml as per guidelines. Three independent calibration curves were constructed with the concentration and peak area of five standards. The experimental results were graphically plotted and linearity of each calibration curve was statistically analyzed by adopting linear least square methodology and calibration equation and correlation coefficient were determined.

Accuracy

The accuracy of the method was evaluated by determining the percentage recovery of the standard samples after adding known amount of fenoverine. The sample solutions prepared in the range of 80% -120% of test concentration, three replicates of each sample were prepared and injected into HPLC system and were analyzed as per method and accuracy was expressed in percentage recovery and %RSD .

Precision

Precision of the method was assessed in terms of repeatability, intraday and inter day precision. Repeatability of the method for the analysis of fenoverine was determined by injecting standard fenoverine solution six times into the chromatographic system and effect on peak area was examined. The intra-day precision was determined by analysis of the standard fenoverine solutions three times on the same day in triplicates. Inter-day precision was assessed by analysis of the standard solutions on three consecutive days. The results of the precision were statistically expressed in terms of mean+SD and %RSD.

LOD and LOQ

LOD and LOQ were calculated based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines. The LOD and LOQ were calculated using LOD=3.3σ/S; LOQ = 10σ/S; Where σ = Standard deviation; S= Slope.

Robustness

The robustness of the method capacity was determined by deliberately varying the method parameters such as mobile phase flow rate (0.6 ml/min and 1 ml/min) and mobile phase volume ratio in six replicates of fenoverine sample solution. Robustness was studied by influence of

change in flow rate in six replicates. The variations in the results of system suitability parameters such as theoretical plates, tailing factor, percent recovery RSD were analyzed.

ASSAY PROCEDURE

Spasmopriv 10 tablets were taken and powdered. 100mg equivalent amount of drug was taken from powder sample in 100ml volumetric flask and 20ml of mobile phase was added and sonicated to dissolve drug completely and make volume up to the mark with the mobile phase(1000 µg/ml stock solution). From the stock solution 15ng/ml was prepared and injected into the chromatographic system in six replicates and the obtained peak area was used for calculating the % assay of the fenoverine.

RESULTS AND DISCUSSION

Chromatography

Optimization of chromatographic conditions was done by performing various trials by changing the mobile phases, flow rate and run time. Several trails were performed by changing the ratios of acetonitrile-water in the mobile phase, but none of them resulted in a favorable peak. In the next attempts acetonitrile- phosphate buffer pH7 was taken as mobile phase and tests were carried out by keeping constant injecting volume (20µl) and detection wavelength (262 nm). Finally mobile phase consisting of acetonitrile-phosphate buffer pH 7(55:45) with flow rate 0.8ml/min gave symmetrical peak at approximately 4.702 min when detected at 262 nm (Figure 2). The system suitability was determined as per the described method and the tailing factor(t) was 0.891±0.08 theoretical plate was 3097±54.2.

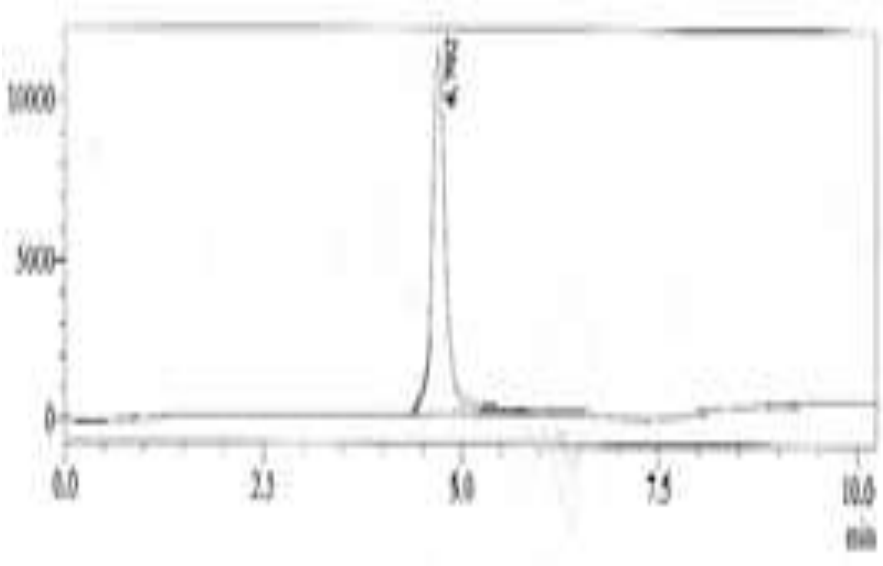

Figure 2: Chromatogram of fenoverine

Specificity

Specificity of the method was checked by analyzing the representative chromatograms of blank, standard and sample. Chromatograms were checked for interference in the blank chromatogram at the retention time of drug, no peak was observed at the retention times of fenoverine. The chromatogram of standard and sample drug was compared, retention time was same and sample did not show any interference peak of diluents, excipients.

Linearity

The linearity of the method was established by performing linear regression analysis for the calibration curves constructed between concentration and peak area. The response of the drug was found to be linear in the concentration range of 5–25ng/ml for fenoverine. The calibration curve was found to be linear with an r^2 value of 0.997 for fenoverine with regression equation y = 1136x + 22857 respectively. For these studies obtained r^2 value was considered to be appropriate to demonstrate the linearity of the method in proposed range. The standard calibration graph is presented in figure 3.

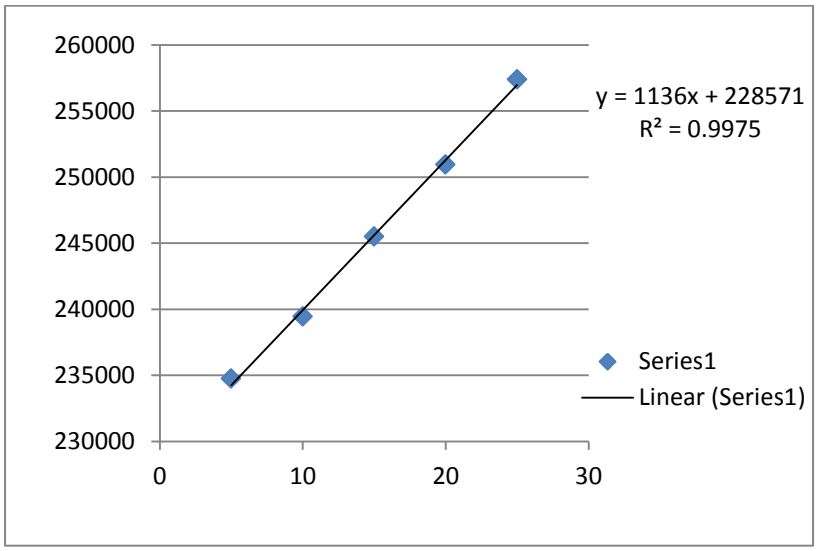

Figure 3: Standard calibration graph for fenoverine

Accuracy

Accuracy of the method was determined at three different concentration levels in six replicates (n=6). The %RSD value was less than 2 and the mean % recovery was between 98.1 to 99.3%, indicating the accuracy of the method. Accuracy data and recovery data was shown in the table 1.

Table 1: Accuracy of the method to determine fenoverine

Sl. no	Amount of standard(ng/ml)	Amount of sample added (ng/ml)	Total conc (ng/ml)	Total conc found(ng/ml)	%Recovery	%RSD
1	15	12	27	26.5	98.1	1.7
2	15	15	30	29.8	99.3	0.3
3	15	18	33	32.5	98.4	1.6

Precision

Repeatability of the method was performed by six injections of fenoverine. The standard concentration of 20ng/ml was injected into chromatographic system and from the peak area % RSD was calculated, the % RSD for the area of six standard injections was found to be 1.6 which indicated that the developed method was precise.

Intra-day and inter day precision were performed for six replicates of three concentrations i.e 16ng/ml, 20ng/ml, 24ng/ml of fenoverine. The samples were analyzed at different sessions of the day(repeatability) and on different days(intermediate precision) and the samples did not show any variations in the measured peak areas and the calculated %RSD values are below 2 indicating that the method was precise. Intraday and inter day precision of the method is presented in the table2.

Table 2: Intraday and inter day precision of the method for the analysis of fenoverine

S. No	Conc. (ng/ml)	Intra day %RSD	Inter day %RSD
1	16	0.6%	0.4%
2	20	1.2%	0.1%
3	24	1.0%	0.2%

LOD and LOQ

The detection limit and quantification limits of the developed method was determined from the calibration curve with standard deviation (σ) and slope of the curve. The RP-HPLC method developed was sensitive in the analysis of fenoverine with the detection limit 1.3ng/ml and quantification limit 4ng/ml.

Robustness

The robustness of the RP-HPLC method was determined for the variations in flow rate and mobile phase ratio and changes in the system suitability, assay % recovery was studied. Six replicates of fenoverine sample concentration 20 ng/ml were taken and injected into the HPLC system with flow rates 0.6 ml/min and 1 ml/min and the effect on system suitability and assay was studied, similarly samples were analyzed by varying mobile phase ratio Acetonitrile and phosphate buffer

pH 7 60:40 and 40:60 and its effect also studied. The method was found to be robust considering the changes in mobile phase flow rate and changes in mobile phase volume ratio. The robustness data is presented in table 3.

Table 3: Robustness of the RP-HPLC method for the analysis of fenoverine

		Flow rate variation	
		0.6 ml/min	**1 ml/min**
System suitability parameters	**%RSD (Peak area)**	0.9	1.2
	Tailing factor (t)	0.9	1.1
	Theoretical plates(N)	3128	2888
Assay	**%Recovery± %RSD**	95.29±0.8	96.57±0.5

		Mobile phase variation	
		Acetonitrile and phosphate buffer pH 7(60:40)	**Acetonitrile and phosphate buffer pH 7(40:60)**
System suitability parameters	%RSD (Peak area)	1.1	1.6
	Tailing factor (t)	0.8	1.4
	Theoretical plates(N)	3226	2996
Assay	%Recovery± %RSD	97.12±0.9	96.72±1.1

Assay of fenoverine in the marketed formulation

The developed and validated method was applied for the determination of fenoverine in the marketed formulation SPASPOPRIV tablets. The table shows mean percentage recovery of fenoverine from the formulation and %RSD values indicating that the validated method could be applied for the determination of fenoverine in the formulation without the interference of other substances.

CONCLUSION

A new method was developed and validated for the estimation of fenoverine by RP – HPLC method and has various advantages like less retention time, good peak symmetry, phenominal linearity, highly sensitive. The analytical method was validated according to ICH guidelines Q2 R1. All the parameters validated were within the acceptable criteria. From the study, it was concluded that the described RP-HPLC method is a simple, sensitive, precise, accurate and stable method for the estimation of fenoverine and it could be applied successfully for the routine analysis fenoverin in of bulk drug and in marketed formulations.

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