Research Article

# Development of Validated Spectrofluorimetric Method for the Estimation of Fenoverine

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**ABSTRACT**

For the estimation of fenoverine in pure and pharmaceutical preparation a sensitive spectrofluorimetric method was developed. This wass based on the oxidation of fenoverine with cerric ammonium sulphate to produce cerrous ion, whose fluorescence was observed at 376 nm while excited at 261 nm. The calibration graph was linear over the range of 1-10 µg/ml. This is applied successfully for the assay of fenoverine in marketed formulations with a mean value of 98.70 ± 0.5219. Recoveries were found to be 99.14% and 99.42 % at 50 and 100% level with the % RSD values of 0.432% and 0.673%, respectively.

**Keywords:** Fenoverine, , fluorescence, excitation, emission, ICH guidelines.

## INTRODUCTION

Fenoverine[1] (piperonyl-4-piperazinyl)-2-(phenothiazinyl-10)-1-ethanone, is an antispasmodic drug which is known to inhibit contraction of smooth muscles elicited either by electrical stimulation or by potassium depolarization. Since the activation of voltage dependent $Ca^{2}$+ channels is associated with electrical and mechanical activity in smooth muscle, the inhibition of these channels by drugs which abolish excessive myoelectric activity might be useful to reduce gastro-intestinal disorders. Literature survey reveals that fenoverine can be assayed by UV spectrophotometric[2], HPLC[3] and HPTLC[4] methods individually or in combination with other drugs[5]. Among the various methods developed spectrofluorimetry could serve as a better method for the determination of fenoverine, due to its simplicity, specificity, speed of analysis and low cost.

## MATERIALS AND METHOD

Pharmaceutical grade of fenoverine was used without further purification. A commercial formulation (*Spasmopriv*) was purchased from the local market. All other reagents used in this study were of AR grade. Jasco FP-750 spectrofluorimeter connected to computer loaded with spectra manager 1.54.03 version was employed with a 10 mm matched glass cells. All weights were taken on Shimadzu Electronic balance BL-220 H.

### Preparation of stock and working standards

The standard solution of fenoverine was prepared by weighing 10 mg of drug, dissolved in 10 ml of methanol (1000 µg/ml) and further diluted to get a concentration 100 µg/ml. This solution was protected from light, and stored at 4°C for a week. A concentration 5 µg/ml was used for optimization of reagents and instrumental parameters.

### Selection of wavelength

Selecting a proper detection wavelength is vital to ensure the active compounds are detected precisely. Here the very dilute solution of fenoverine was scanned between 270 – 650 nm for emission, by keeping the excitation wavelength as 261 nm.

### Optimization of experimental variable

### Effect of acid strength

Various strength of sulphuric acid ranging from 0.05 M - 0.5 M were tried by keeping the strength of cerric ammonium sulphate (CAS) as 0.001 M and volume of reagent as 5 ml and the fluorescence intensities were noted at selected wavelength.

### Effect of strength of oxidant

Different strength of CAS ranging from 0.001 M - 0.004 M was tried by fixing the strength of sulphuric acid as 0.2 M and volume of reagent as 5 ml. From shape of the spectrum and fluorescence intensity the strength was selected for the study.

**Fixing the volume of oxidizing mixture**
To 0.5 ml of fenoverine solution different volumes of oxidating mixture (0.001 M CAS in 0.2 M Sulphuric acid) such as 1ml, 2ml, 3ml, 4ml and 5ml were added, these solutions were diluted up to 10ml with methanol and the intensity of fluorescence were noted.

**Optimization of spectrofluorimetric variables**
The response time, excitation and emission bandwidth and sensitivity are the important factors that affect the fluorescence intensity of any compound. Increase in the bandwidth usually increases the fluorescence intensity. It is due to the reason, as the bandwidth increased, the light falls on the sample is also increases and the fluorescence intensity is based on the amount of light, it tends to increase the intensity.

**Response time**
The excitation and emission bandwidth were fixed as 20nm and 5nm at different responses the fluorescence intensities were measured. The solution of 5 µg/ml of fenoverine solution was used for the study.

**Excitation and emission bandwidth**
Response was fixed as 0.05 sec and emission bandwidth as 5nm, fluorescence intensity of 5 µg/ml solution of fenoverine were measured with different excitation bandwidth such as 5,10,20 nm. Similarly it was also done for fixing emission bandwidth.

**Sensitivity**
By keeping the response, excitation and emission bandwidth as constant, fluorescence intensity of 5 µg/ml solution was recorded by varying sensitivity as low, medium and high. Finally it was selected on the basis of maximum fluorescence intensity.

**Validation methods**
The developed method was validated as per ICH guidelines[6] in terms of linearity and range, precision and accuracy.

**Linearity**
10 mg of fenoverine was weighed accurately and was dissolved in methanol to get 100µg/ml. A solution of 0.001 M CAS in 0.2 M sulphuric acid was prepared. Aliquots of standard stock solution of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0ml were added into a series of 10ml standard flask and 5ml of reagent was added to all standard flasks and the volume was made upto the mark with methanol. The fluorescence intensity of the solutions was recorded with the fixed instrumental conditions and calibration graph was prepared by plotting fluorescence intensity against concentration of the drug.

**Precision**
Precision of the method was determined by the repeatability studies. Intra-day and inter-day studies were carried out for the method by repeating the procedure six times and the % RSD was calculated.

**Accuracy**
Accuracy of the developed method was determined by conducting the recovery studies. To the powdered formulation of fenoverine, standard drug was added at 50% and 100% level. The concentration of the drug present in the resulting solutions was determined by the proposed method. The recovery procedure was repeated for six times and % recovery was calculated.

**RESULTS AND DISCUSSION**
Luminescent spectroscopy is the most sensitive method for the quantitative estimation of trace level analyte[7]. CAS is a powerful oxidizing agent and is non-fluorescent under acidic conditions, while its reduced form exhibits native fluorescence[8,9]. The latest property has been used for the indirect determination of several drugs. The oxidation of fenoverine with CAS is the basis of the present analytical procedure developed for determination of fenoverine. The increase in fluorescence intensity due to Cerrous ions formed after the addition of the drug to an acidic CAS solution was measured at 261 nm as excitation wavelength and 376 nm as emission wavelength (Fig. 1).
Several investigations were carried out to establish the optimum experimental conditions for oxidation of fenoverine. The optimized parameters included the concentration and volume of CAS, type of acid, strength of sulphuric acid and reaction time. Instrumental variables like excitation and emission band width as well as response time were also optimized.The nature and concentrationof the acid used in the reaction have a very significant influence on the fenoverine emission intensity. Therefore, various kinds of acids, such as HCl, $H_2SO_4$ and $HNO_3$ were added to the CAS solutions to test the effect of acid on the fluorescence intensity. The highest emission was observed from sulphuric acid treated CAS solutions, and the signal was stable. Hence, sulphuric acid was chosen for further study. The concentration of sulphuric acid in CAS solutions was subsequently

optimized. These responses were plotted against different strength of the sulphuric acid in Fig.2. Fluorescence intensity of cerrous ion obtained by fenoverine was increased till 0.2M and remained constant at higher concentration. Hence, the concentration of acid was fixed as 0.2M for fenoverine in order to carry out the following work.

The effect of cerric ion concentration on the fluorescence intensity was assessed in the range 0.001-0.004M. In Fig.3, it was shown that cerric ion at concentrations of 0.001M leads to the saturation signals in the case of fenoverine. At concentrations lower than this range, the fluorescence intensity dropped due to insufficient CAS for oxidation. On the other hand, higher concentrations of CAS were reported to probably quench the fluorescence thus decreasing the detected intensity. Optimized volume of CAS was 5 ml. The effect of dilution of cerrous ion might be one of the factors causing the quenching. Hence, various diluting solvents were tried, such as water, acetonitrile, methanol, and ethanol. It was found that maximum fluorescence intensity was obtained when methanol was used as solvent for dilution.

When the excitation and emission band width were kept as 20 and 5 nm, a smooth spectrum was obtained. After selecting this, the response time was fixed as 0.05sec which would be sufficient to give higher fluorescence intensity. A good linearity was found in the concentration range of 1-10µg/ml (Fig. 4). Precision was confirmed by replicate analysis of two working standards of fenoverine for repeatability and intermediate precision, the %RSD was found to be below two which indicated the method was highly precise.

The accuracy was carried out at 50 % and 100 % level from the amount determined. % recovery ranged from 99.14–99.42 % for the method. The results are shown in table 1. Stability studies indicated that the samples were stable when kept at room temperature for thirty minutes and under refrigeration temperature for twenty hours. It was evident from the assay of formulation that the percentage content was in good agreement with the labeled claim. Results are shown in table 2.

## CONCLUSION

This report describes a validated spectrofluorimetric method for the estimation of fenoverine without interference of common excipients. This may be recommended as a method for fenoverine testing either in bulk or the corresponding dosage form in routine quality control. The precision of the method was statistically evaluated. From the economic point of view, the proposed method is simple, selective, specific, rapid and inexpensive, and thus seems a good alternative to previously reported methods.

## ACKNOWLEDGEMENT

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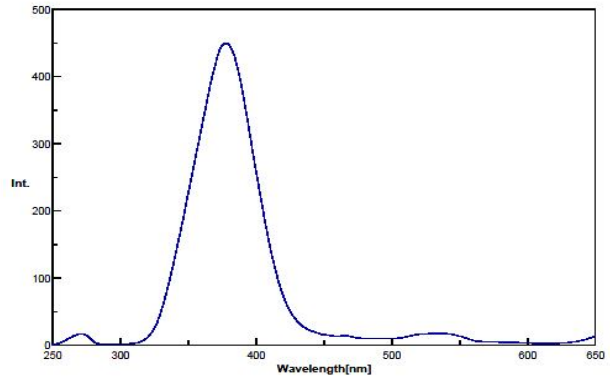


**Fig. 1: Emission spectrum of fenoverine in CAS**

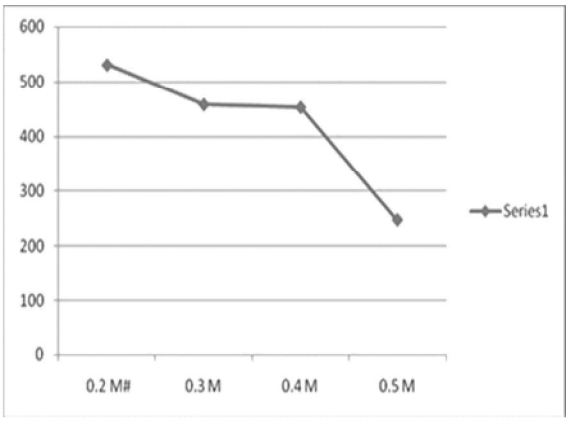


**Fig. 2: Effect of acid strength on fluorescence intensity**

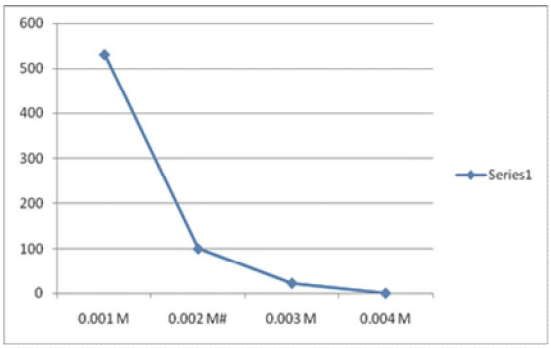


**Fig. 3: Effect of strength of oxidant on fluorescence intensity**

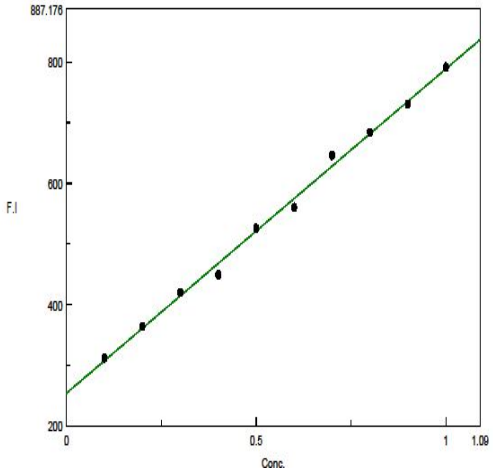


**Fig. 4: Calibration graph of fenoverine**

**Table 1: Recovery study**

| Drug | Amount added (mg) | | Amountof drug recovered (mg) | | % Recovered± RSD* | |
|---|---|---|---|---|---|---|
| | 50% | 100% | 50% | 100% | 50% | 100% |
| Fenoverine | 5 | 10 | 4.95 | 9.94 | 99.14 ± 0.432 | 99.42 ± 0.673 |

*Mean of six determinations

**Table 2: Analysis of formulation**

| Formulation | Amount (mg/cap) | | Amount estimated ±RSD* |
|---|---|---|---|
| | Labeled | Found | |
| Spasmopriv | 100 | 98.70 | 98.70±0.5219 |

*Mean of three determination

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