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ORIGINAL PAPER

# Spectrofluorimetric Determination of Fluvoxamine in Dosage Forms and Plasma Via Derivatization with 4-Chloro-7-Nitrobenzo-2-Oxa-1,3-Diazole

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**Abstract** A highly sensitive and simple spectrofluorimetric method has been developed and validated for the determination of the antidepressant fluvoxamine (FXM) in its dosage forms and plasma. The method was based on nucleophilic substitution reaction of FXM with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in an alkaline medium (pH 8) to form a highly fluorescent derivative that was measured at 535 nm after excitation at 470 nm. The factors affecting the reaction was carefully studied and optimized. The kinetics of the reaction was investigated, and the reaction mechanism was presented. Under the optimized conditions, linear relationship with good correlation coefficient (0.9995) was found between the fluorescence intensity and FXM concentration in the range of 65–800 ng $ml^{-1}$. The limits of detection and quantitation for the method were 21 and 64 ng $ml^{-1}$, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2.17%. The proposed method was successfully applied to the determination of FXM in its pharmaceutical tablets with good accuracy; the recovery values were 97.8–101.4±1.08–2.75%. The results obtained by the proposed method were comparable with those obtained by the official method. The high sensitivity of the method allowed its successful application to the analysis of FXM in spiked human plasma. The proposed method is superior to the previously reported spectrofluorimetric method for determination of FXM in terms of its simplicity. The proposed method is practical and valuable for its routine application in quality control and clinical laboratories for analysis of FXM.

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## Introduction

Fluvoxamine (FXM); (E)-5-methoxy-4′-trifluromethyl-valerophenone O-2-aminoethyl-oxime is a new generation antidepressant drug. It exerts its antidepressant effect through a selective inhibition for the reuptake of the neurotransmitter serotonin by the presynaptic receptors. FXM is absorbed well following oral administration to healthy volunteers, and peak plasma concentrations are achieved within approximately 3–8 h, with half-life time of about 15 h. Because of the long half-life, FXM is given once-a-day [1]. These combined qualities made FXM one of the most widely prescribed antidepressant drugs [2].

FXM has been determined in its pharmaceutical dosage forms and/or biological fluids by titrimetry [3], voltammetry [4], nuclear magnetic resonance [5], high performance liquid chromatography [6–8], gas chromatography [9–11], capillary electrophoresis [12], spectrophotometry [13–18], and spectrofluorimetry [19]. These methods were associated with some major drawbacks such as inadequate sensitivity, time-consuming, tedious, and dedicated to sophisticated and expensive instruments. Although, spectrofluorimetry is a sensitive and simple technique, however the only reported spectrofluorimetric method for determination of FXM was time-consuming because of the long time required for carrying out the fluorogenic reaction [19]. For these reasons, the development of new of sensitive and simple spectrofluorimetric method that overcomes the drawbacks of the existing methodologies was very essential.

4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) has been used as a derivatizing reagent in the spectrofluorimetric

determination of many pharmaceutical amines [20–23]. The reaction of NBD-Cl with FXM has not been investigated yet. Therefore, the present study was devoted to investigate the reaction between FXM (being amine) and NBD-Cl, and employment the reaction in the development of sensitive and simple spectrofluorimetric method for determination of FXM in dosage forms and biological fluids.

## Experimental

### Apparatus

FP-6200 fluorometer (JASCO Co. Ltd., Kyoto, Japan), with 1-cm quartz cells was used for all measurements. The slit width of both the excitation and emission monochromators was set at 1.5 nm. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate (0.01 μg $ml^{-1}$). Wavelength calibration was performed by measuring $\lambda_{excitation}$ 275 nm and $\lambda_{emission}$ 430 nm; no variation in the wavelength was observed. pH meter, Model 350 (Bibby Scientific Ltd., T/As Jenway, Essex, England). MLW type thermostatically controlled water bath (Memmert GmbH, Co. Schwa bach, Germany).

### Reagents and materials

Fluvoxamine maleate (FXM; Solvay Pharma, Suresnes, France) was obtained and used as received; its purity was 100.2±1.25%. A solution of 0.2% (w/v) of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl; Sigma Chemical Co., St. Louis, USA) was freshly prepared by dissolving 100 mg in 50 ml acetone. Clark and Lubs buffer solution of pH 8 was prepared by mixing 50 ml of 0.2 M aqueous solution of boric acid and KCl (1 L contains 12.368 g of boric acid and 14.90 g of KCl) with 0.2 M NaOH in 200 ml standard flask [24], and adjusted by pH meter. Faverine film-coated tablets (Solvay Pharma) were labeled to contain 50 mg FXM per tablet. Human plasma samples were collected from normal healthy volunteer at King Khaled University Hospital (Riyadh, Saudi Arabia), and they were stored in deep-freezer at −20 °C until analysis. Double distilled water was obtained through WSC-85 water purification system (Hamilton Laboratory Glass Ltd., Kent, USA), and used throughout the work. All solvents and materials used throughout this study were of analytical grade.

### Preparation of standard and sample solutions

#### *Fluvoxamine maleate (FXM) standard solution*

An accurately weighed amount (50 mg) of FXM was quantitatively transferred into a 25-ml calibrated flask, dissolved in 20 ml distilled water, completed to volume with the same solvent to obtain a stock solution of 2 mg $ml^{-1}$. This stock solution was further diluted with water to obtain working solutions in the ranges of 0.6–8 μg $ml^{-1}$.

#### *Tablets sample solution*

Twenty tablets were weighed, and finely powdered. An accurately weighed quantity of the powdered tablets equivalent to 100 mg of FXM was transferred into a 100-ml calibrated flask, and dissolved in about 40 ml of distilled water. The contents of the flask were swirled, sonicated for 5 min, and then completed to volume with water. The contents were mixed well and filtered rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with distilled water to obtain a suitable concentration for the analysis.

#### *Plasma samples*

Aliquots of 1 ml of FXM solution (0.6–8 μg $ml^{-1}$) were dispensed into test tubes. A 1 ml of drug-free plasma and 2 ml of acetonitrile was added. After vortexing for 3 min, the tube was centrifuged at 4,500 rpm for 20 min. The mixture was extracted three times with 3×1.5 ml dichloromethane:n-butanol (4:1 v/v). The extract was transferred to another 5 ml tube and evaporated to dryness under stream of nitrogen gas at 40 °C. The residue was dissolved in 1 ml acetonitrile and used for analysis.

### General recommended procedure

Accurately measured aliquots of FXM solution containing 0.6–8 μg $ml^{-1}$ were transferred into separate 10-ml calibrated flasks. One milliliter of Clark and Lubs buffer solution (pH 8±0.2) was added followed by 1 ml of NBD-Cl solution (0.2%, w/v). The reaction solution was allowed to proceed at 50±5 °C for 20 min. After cooling, the reaction mixture was acidified by adding 1 ml of 0.1 M HCl, and completed to volume with acetonitrile. The fluorescence intensity of the resulting solution was measured at 470 nm after excitation at 535 nm against reagent blanks prepared in the same manner with 1 ml water instead of 1 ml sample solution.

### Determination of the stoichiometric ratio of the reaction

In the limiting logarithmic method [25], two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments were carried using increasing NBD-Cl concentrations ($6.26\times10^{-5}$–$5\times10^{-4}$ M) at fixed FXM concentration ($9.21\times10^{-7}$ M). The second set of experiments were carried using increasing FXM concentrations ($2.76\times10^{-7}$–$1.84\times10^{-6}$ M)

at fixed NBD-Cl concentration ($1\times10^{-3}$ M). The logarithms of the obtained fluorescence intensities were plotted as a function of the logarithms of the NBD-Cl and FXM concentration in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

## Results and discussion

### Excitation and emission spectra

Because of the absence of native fluorescence of FXM, its derivatization with fluorogenic reagent was necessary for its spectrofluorimetric determination. NBD-Cl was chosen as a derivatizing reagent because it forms highly fluorescent derivatives with amines using relatively mild reaction conditions [21–23]. Owing to the presence of labile chloride in the chemical structure of NBD-Cl, a daily fresh solution was prepared and tested in the present study. It was found that FXM reacts with NBD-Cl and forms a yellow fluorescent derivative. This derivative exhibited maximum fluorescence intensity ($\lambda_{em}$) at 535 m after its excitation at maximum wavelength ($\lambda_{ex}$) at 470 m. The excitation and emission spectra for the reaction product of FXM with NBD-Cl are given in Fig. 1.

### Optimization of reaction variables

#### *Effect of NBD-Cl concentration*

The studying the effect of NBD-Cl concentration on its reaction with FXM revealed that the reaction was dependent on NBD-Cl concentration as the fluorescence intensity (FI) of the reaction solution increased as the NBD-Cl concentration increased (Fig. 2). The highest readings were

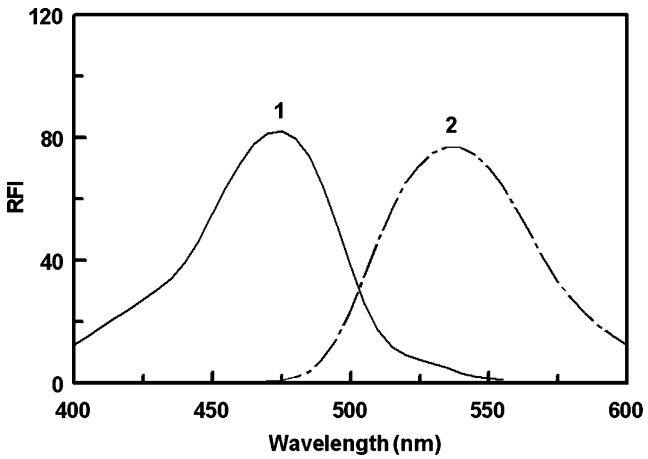


**Fig. 1** Excitation (1) and emission (2) spectra of the reaction product of FXM (400 ng $ml^{-1}$) with NBD-Cl

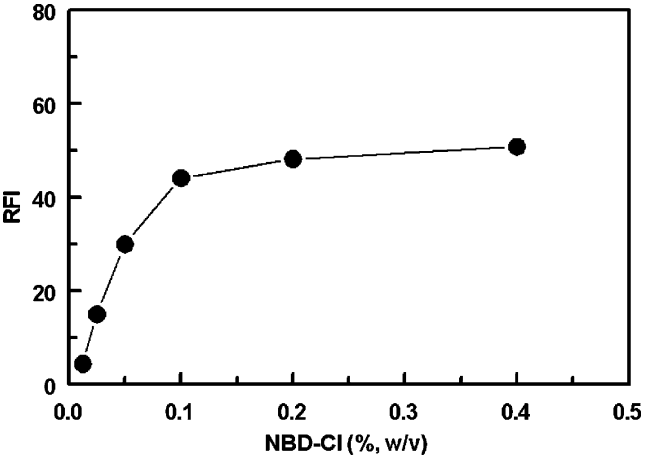


**Fig. 2** Effect of NBD-Cl concentration on its reaction with FXM (400 ng $ml^{-1}$)

attained at NBD-Cl concentration of 0.1% (w/v), and higher concentrations of NBD-Cl up to 0.4% had no effect on the fluorescence intensity. For more precise readings, further experiments were carried out using 0.2%.

#### *Effect of alkalinity and pH*

In order to generate the nucleophile from FXM, the reaction should be carried out in alkaline medium. Different alkaline buffer systems (borate, phosphate, and carbonate) having the same pH value (8) were tested. The highest FI was obtained when the reaction was carried out in borate-NaOH-HCl buffer system (Clark and Lubs buffer). With other buffers, either precipitation of white colloid occurred upon addition of NBD-Cl reagent solution, non reproducible results, and/or weak sensitivities were observed.

The effect pH of the reactions was studied by carrying out the reaction in Clark and Lubs buffer solution of pH 5–9.5. The results indicated that the FI increased as the pH increased and maximum readings were obtained at pH 8± 0.2 (Fig. 3). This increase in the FI with the pH was possibly due to the conversion of amino group of FXM from the maleic acid salt form (in acidic pH values) to the free amino group (as the pH turns alkaline). This facilitates the nucleophilic substitution reaction. At pH above 8.2, sharp decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the condensation reaction between FXM and NBD-Cl. In order to keep the high sensibility for determination of FXM, the subsequent experiments were carried out at pH 8±0.2.

#### *Effect of temperature and time*

In order to determine the optimum temperature and time required for completion the reaction, the derivatization

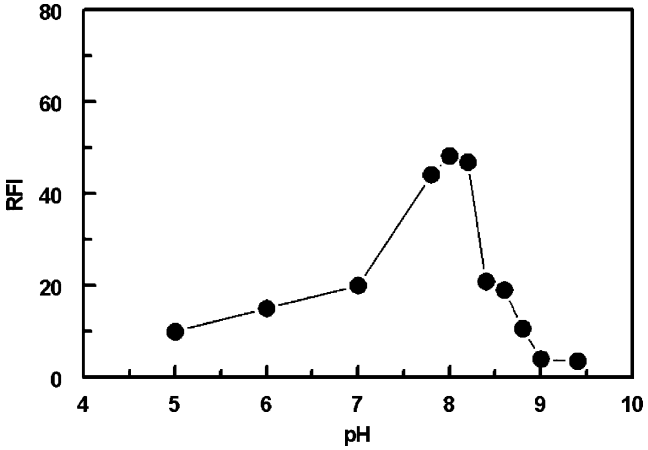


**Fig. 3** Effect of pH on the reaction of NBD-Cl (0.2%, w/v) with FXM (400 ng $ml^{-1}$). RFI is the relative fluorescence intensity

reaction was carried out at room temperature (25±5 °C) and the induced FI values were monitored at different time intervals. It was found that the reaction was very slow, and did not go to completion in reasonable time; it required more than 1 h (Fig. 4). Therefore, investigations were carried out at varying elevated temperatures (40–70 °C), and the intensities of the induced fluorescence were monitored for 60 min. The optimum conditions were considered as the conditions at which the high FI values, high reproducible results, and comfortable measurements (wide plateau region on the FI-time curve) could be obtained. The results indicated that the reaction was dependent on temperature, and the optimum condition was achieved by heating at 50 °C for 20 min. At higher temperatures, the maximum FI was obtained in shorter times (~10 min), however rapid progressive decrease in the readings was observed as the reaction time increased. This was probably attributed to the degradation of the reagent at high temperature. This observation was coincident with the results that have been previously reported by Aktas E.S. et al. [26].

*Effect of HCl concentration*

Under the above mentioned conditions, significantly high fluorescence backgrounds were also observed. This was attributed to the hydrolysis of NBD-Cl to the corresponding hydroxy derivative namely, 4-hydroxy-7-nitrobenzo-2-oxa-1,3-diazole (NBD-OH) [27]. The fluorescence of NBD-OH was found to be quenched by decreasing the pH of the reaction medium to less than one [28]. Therefore acidification of the reaction mixture prior to measurement of the FI was necessary to remarkably decrease the background fluorescence. Meanwhile, the reaction product was not affected, thus the sensitivity was ultimately increased. The concentration of HCl required for acidification was found to be 0.01 M in the final assay solutions (i.e. 1 ml of 0.1 M).

*Effect of diluting solvent*

Upon diluting the reaction with water, colloids were obtained indicating the incomplete solubility of FXM-NBD in water. Therefore, water could not be used for dilution. In order to select the most appropriate organic solvent for diluting the reaction solution, different solvents were tested: methanol, ethanol, isopropanol, acetone, and acetonitrile. The highest readings were obtained when acetonitrile was used. Therefore, acetonitrile was used for diluting the reaction mixture in the subsequent experiments.

*Stability of the fluorescent derivative*

The effect of time on the stability of the FXM-NBD fluorescent derivative was studied by following the FI of the reaction solution (after dilution) at different time intervals. It was found that the FI values remain constant for at least 1 h. This allowed the processing of large batches of samples, and their comfortable measurements with convenience. This increased the convenience of the method as well as made it applicable for large number of samples.

A summary for the optimization of the variables affecting the reaction of FXM with NBD-Cl is given in Table 1.

Stoichiometry and kinetics of the reaction

The stoichiometry of the reaction between FXM and NBD-Cl was investigated by limiting logarithmic method [25]. As shown in Fig. 5, two straight lines with comparable slopes indicating the 1:1 ratio for the reactions. Based on this ratio, the reaction pathway between FXM and NBD-Cl was postulated to proceed as shown in Fig. 6.

Under the optimum conditions (Table 1), the fluorescence intensity-time curves for the reaction at varying FXM

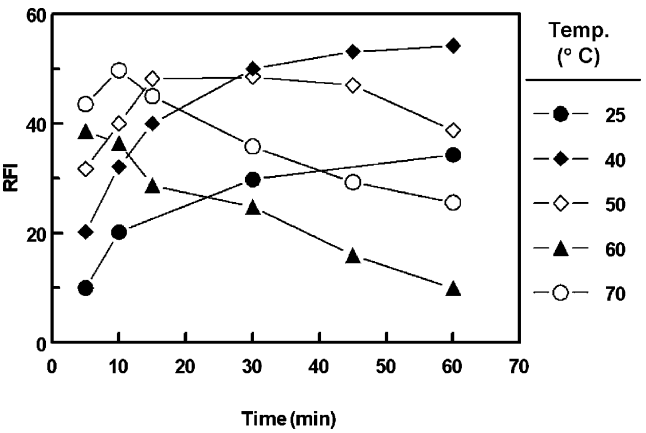


**Fig. 4** Effect of time on the reaction of NBD-Cl (0.2%, w/v) with FXM (400 ng $ml^{-1}$) at different temperatures. RFI is the relative fluorescence intensity

**Table 1** Optimization of variables affecting the reaction of FXM with NBD-Cl

| Variable | Studied range | Optimum condition |
|---|---|---|
| NBD-Cl (%, w/v) | 0.01–0.5 | 0.2 |
| pH | 5–9.5 | 8±0.2 |
| Temperature (°C) | 25–70 | 50 |
| Time (min) | 5–60 | 20 |
| HCl (M) | 0.02–0.5 | 0.1 |
| Solvent | Different [a] | Acetonitrile |
| Stability of FXM-NBD (min) | 10–60 | 60 |

[a] Solvents tested: methanol, ethanol, isopropanol, acetone, and acetonitrile.
[b] The stability of the FXM-NBD was studied after dilution of the reaction solution.

concentrations ($2.88\times10^{-7}$–$9.21\times10^{-7}$ M) with a fixed concentration of NBD-Cl ($1\times10^{-3}$ M) were generated (Fig. 7). The initial reaction rates (K) were determined from the slopes of these curves. The logarithms of the reaction rates (Log K) were plotted as a function of logarithms of FXM concentrations (log C). The regression analysis for the values was performed by fitting the data to the following equation:

$$\text{Log K} = \log \text{K}' + n \log C$$

where K is reaction rate, $K'$ is the rate constant, $C$ is the molar concentration of FXM, and $n$ (slope of the regression line) is the order of the reaction. As seen in Fig. 8, a straight line with slope values of 0.8106 (≈1) confirming that the reaction was first order. However under the optimized

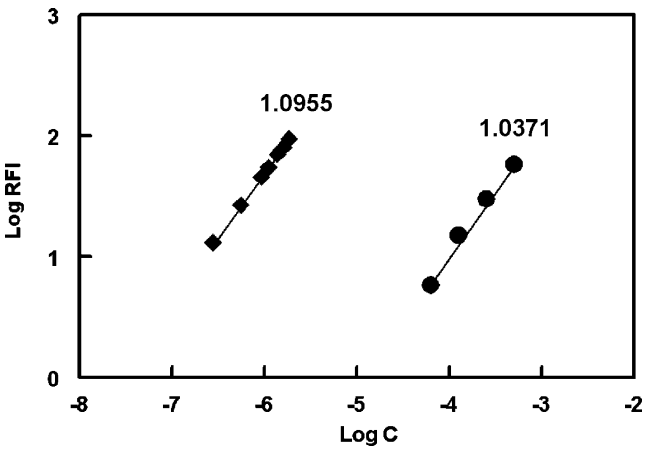


**Fig. 5** Limiting logarithmic plot for molar reactivity of FXM with NBD-Cl. C and RFI are the concentration and relative fluorescence intensity, respectively. The first line (♦) was generated using fixed FXM concentration ($9.21\times10^{-7}$ M) and varying NBD-Cl concentrations ($6.26\times10^{-5}$–$5\times10^{-4}$ M). The second line (●) was generated using varying FXM concentrations ($2.76\times10^{-7}$–$1.84\times10^{-6}$ M) and fixed NBD-Cl concentration ($1\times10^{-3}$ M). Figures on the lines are the slopes

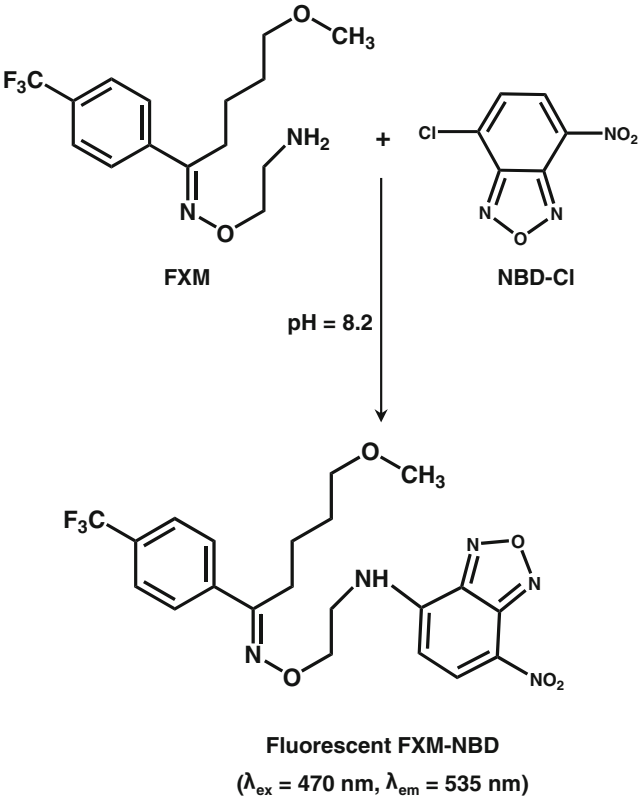


**Fig. 6** Scheme for the reaction pathway of FXM with NBD-Cl

reaction conditions, the concentration of NBD-Cl was in much more excess than that of FXM in the reaction solution. Therefore, the reaction was regarded as a pseudo-first order reaction.

## The apparent rate constant and activation energy

The fluorescence intensity-time curves at different temperatures (25, 40, 50, 60, and 70 °C) were generated using fixed concentration of FXM ($4.60\times10^{-7}$ M) and NBD-Cl ($1\times10^{-3}$ M). The reaction time was set at maximum

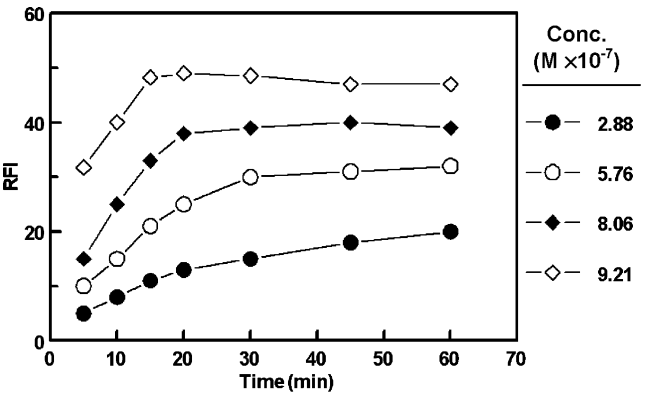


**Fig. 7** Relative fluorescence intensity (RFI)-time curves for the reaction of NBD-Cl ($1\times10^{-3}$ M) with FXM at different concentrations

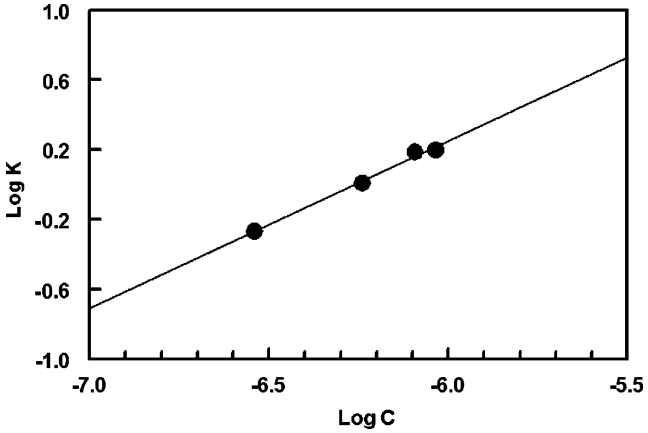


**Fig. 8** Linear plot for Log C versus Log K for the kinetic reaction of FXM with NBD-Cl. C is the FXM concentration ($2.88\times10^{-7}$–$9.21\times10^{-7}$ M), and K is the reaction rate (second$^{-1}$)

10 min, before the decrease of the FI occurs at high temperatures (Fig. 4). From these curves the apparent rate constants were calculated. The activation energy, defined as the minimum kinetic energy that a molecule possess in order to undergo a reaction, was determined using Arrhenius equation [29]:

$$\text{Log k} = \log A - \text{Ea}/2.303\ \text{RT}$$

where k is the apparent rate constant, $A$ is the frequency factor, Ea is the activation energy, T is the absolute temperature (°C+273), and R is the gas constant (1.987 cal degree$^{-1}$ mole$^{-1}$). The values of log k were plotted as a function of 1/T. Straight line with slope value of −0.7971 (=−Ea/2.303 R) was obtained (Fig. 9). From these data, the activation energy was calculated and found to be 3.65 kcal mol$^{-1}$. This low activation energy explained that the nucleophilic substitution reactions between FXM and NBD-Cl could be easily takes place under mild conditions, and NBD-Cl could be used as a useful reagent in the spectrofluorimetric determination of FXM.

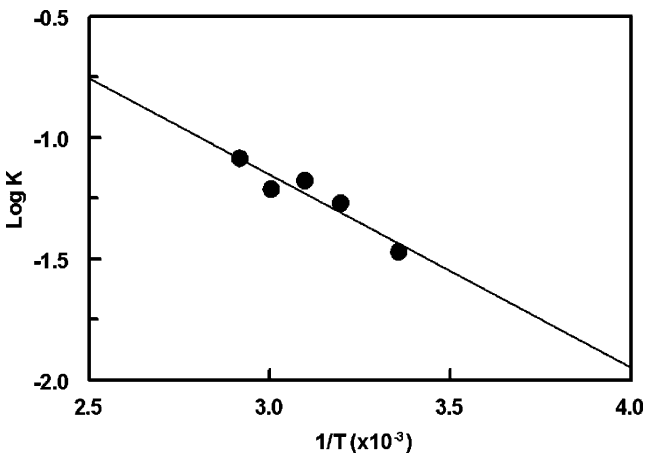


**Fig. 9** Arrhenius plot for the reaction of NBD-Cl and FXM. T and K are the absolute temperature and the apparent rate constant, respectively. [FXM] is $4.60\times10^{-7}$ M and [NBD-Cl] is $1\times10^{-3}$ M

## Validation of the method

### *Calibration and sensitivity*

Under the optimum conditions (Table 2), calibration curve for the determination of FXM by its reaction with NBD-Cl was constructed by plotting the FI as a function of the corresponding FXM concentration. The regression equation for the results was: FI=a+b C, where FI is the fluorescence intensity, C is the concentration of FXM in ng ml$^{-1}$. Linear relationship with small intercept and good correlation coefficient (0.9995) was obtained in the range of 65–800 ng ml$^{-1}$. The LOD and LOQ were determined according to ICH guidelines for validation of analytical procedures [30]. The LOD and LOQ values were found to be 21 and 64 ng ml$^{-1}$, respectively. The parameters for the analytical performance of the proposed spectrofluorimetric method are summarized in Table 2.

### *Reproducibility*

The reproducibility of the proposed method was determined by replicate analysis of five separate solution of the working standard. The method gave satisfactory results; RSD was 2.17% indicating its good reproducibility. This precision level is adequate for the precision and routine analysis of FXM.

### *Accuracy and specificity*

The accuracy of the proposed spectrofluorimetric method was evaluated by the recovery studies for added concentrations. The recovery values were 97.8–101.4±1.08–2.75% (Table 3), indicating the accuracy of the proposed

**Table 2** Statistical parameters for the determination of FXM by the proposed spectrofluorimetric method based on its reaction with NBD-Cl

| Parameter | Value |
|---|---|
| $\lambda_{ex}$ (nm) | 470 |
| $\lambda_{em}$ (nm) | 535 |
| Linear range (ng ml$^{-1}$) | 65–800 |
| Intercept | −3.3182 |
| SD of intercept | 1.5029 |
| Slope | 0.2366 |
| SD of slope | 0.0028 |
| Correlation coefficient ($r$) | 0.9995 |
| LOD (ng ml$^{-1}$) | 21 |
| LOQ (ng ml$^{-1}$) | 64 |

**Table 3** Recovery studies for determination of FXM by the proposed spectrofluorimetric method based on its reaction with NBD-Cl

| Added (ng ml$^{-1}$) | Recovery (% ±SD)[a] |
|---|---|
| 100 | 98.5±2.54 |
| 150 | 97.8±2.02 |
| 200 | 100.1±1.24 |
| 250 | 99.5±2.75 |
| 300 | 101.4±1.08 |

[a] Values are mean of three determinations.

**Table 5** Analysis of FXM-containing-tablets by the proposed and the official methods

| Dosage form | Recovery (% ±RSD) [a] | | *t*-value [c] | *F*-value [c] |
|---|---|---|---|---|
| | Proposed | Official [b] | | |
| Faverin ds | 100.1±1.61 | 99.7±1.43 | 1.64 | 1.27 |

[a] Values are mean of 5 determinations.
[b] Reference 6.
[c] The tabulated values of *t*- and *F*- at 95% confidence limit are 2.78 and 6.39, respectively

method. The specificity of the method was evaluated by investigating the interference liabilities from the common excipients that might be added during pharmaceutical formulation. Samples were prepared by mixing known amount (50 mg) of FXM with various amounts of the common excipients: starch, glucose, lactose, acacia, talc, and magnesium stearate. These laboratory-prepared samples were analyzed by the proposed method applying he general recommended procedure. The recovery values were 98.47–102.84±1.58–2.64% (Table 4). These data confirmed the absence of interference from any of the common excipients with the determination of FXM by the proposed spectrofluorimetric method.

*Robustness and ruggedness*

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that variation in the NBD-Cl concentrations (0.15–0.25%, w/v), temperature (optimum±5 °C), and time (optimum± 5 min) did not significantly affect the procedures; recovery values were 96.8–104.2, and the RSD values did not exceed 4%. The most critical factor affecting the results was the pH that should be adjusted to be in the range of 8±0.2. Ruggedness was also tested by applying the method to the assay of FXM using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the RSD did not exceed 3.75%.

**Table 4** Analysis of FXM in presence of common excipients by the proposed fluorimetric method

| Excipient | Recovery (% ± SD) [a] |
|---|---|
| Starch (50)[b] | 102.84±1.58 |
| Glucose (10) | 98.47±2.10 |
| Lactose (10) | 100.87±1.85 |
| Acacia (10) | 102.21±2.64 |
| Talc (5) | 101.58±2.21 |
| MS [c] (10) | 100.43±1.98 |
| Average ± SD | 101.07±0.50 |

[a] Values are mean of three determinations.
[b] Figures in parenthesis are the amounts in mg added per 50 mg of FXM.
[c] MS = Magnesium stearate.

Applications of the method

It is evident from the above-mentioned results that the proposed method gave satisfactory results with FXM in its bulk. Thus its pharmaceutical dosage forms (tablets) were subjected to the analysis of their FXM contents by the proposed and the official [6] methods. The label claim percentage was 100.1±1.61% (Table 5). This result was compared with that obtained from the official method by statistical analysis with respect to the accuracy (by *t*-test) and precision (by *F*-test). No significant differences were found between the calculated and theoretical values of *t*- and *F*-tests at 95% confidence level proving similar accuracy and precision in the determination of FXM by both methods.

The high sensitivity of the proposed method promoted us to check its applicability to the determination of FXM in spiked human plasma. FXM is readily absorbed following oral ingestion, and a peak concentration in plasma occurs within about 3–8 h. Therapeutic level (normal steady state value that would occur after treatment with 100 mg/day) is 400 ng ml$^{-1}$ [31], which is much higher than the LOQ of the proposed method. The high sensitivity of the proposed

**Table 6** Analysis of FXM spiked in plasma samples by the proposed spectrofluorimetric method

| Nominal conc. (ng ml$^{-1}$) | Measured conc. (ng ml$^{-1}$) | Recovery (% ±RSD) |
|---|---|---|
| 80 | 77.9 | 97.4±2.41 |
| 120 | 117.0 | 97.5±1.85 |
| 240 | 245.2 | 102.2±2.97 |
| 500 | 490.5 | 98.1±1.56 |

method allowed the determination of FXM in spiked human plasma. The extraction procedure described by Ulu ST [32] was adopted here. The results were satisfactorily accurate and precise as the recovery was 97.4–102.2% with RSD less than 3% (Table 6).

## Conclusions

New simple and sensitive spectrofluorimetric method for the determination of FXM has been successfully developed and validated. The method involved simple derivatization of FXM with NBD-Cl reagent, and subsequent measuring the fluorescence intensity of the fluorescent reaction product. The proposed method is specific, accurate, reproducible, and highly sensitive to be applied on the analysis of tablets as well as the plasma samples. Furthermore, the analysis is relied on a simple apparatus, thus the proposed method is suitable for routine analysis of FXM in quality control and clinical laboratories.

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