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A NOVEL SPECTROFLUORIMETRIC METHOD FOR THE DETERMINATION OF GEMIFLOXACIN IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Novel selective, rapid, simple, sensitive, economic and reproducible spectrofluorimetric method was developed for the determination of Gemifloxacin in bulk as well as pharmaceutical formulations. Gemifloxacin, as a primary aromatic amine, reacts fluorescamine, which is highly sensitive fluorogenic reagent used in many investigations. This method was based on the reaction between Gemifloxacin and fluorescamine in borate buffer solution of pH 8.5 to give a highly fluorescent derivative. This reaction product was measured spectrofluorimetrically at 483 nm after excitation at 353 nm under optimum conditions, linear relationship with best correlation coefficient 0.9999 and the linearity was detected in between the range of 100-1000 ng mL^{-1}. The average % recovery obtained was quantitatively as 100.37%. The intraday and interday precision was found to be 0.074 % and 0.096%, respectively. The limit of detection was found to be 0.123 µg/mL and the limit of quantification was 0.369 µg/mL. Therefore, this newly recommended spectrofluorimetric method is most suitable for estimation of Gemifloxacin and observed to be validated for calculation of accuracy, precision, robustness, LOD and LOQ. So this method becomes highly congenial for analysis of Gemifloxacin in tablet form. Therefore, spectrofluorimetric method is considered as most convenient analytical technical method for the drug determination in pharmaceutical preparations, quality control and clinical laboratories.

Key words: Gemifloxacin, Spectrofluorimetric method, Fluorescamine.

INTRODUCTION

Systematic (IUPAC) name of Gemifloxacin (GMF) is 7-[(4Z)-3-(Aminomethyl)-4-

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methoxyimino-pyrrolidin-1yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-corboxylic acid and it is a potent novel broad spectrum antibiotic belonging to fourth generation fluoroquinolones. GMF demonstrated improved activity against acute bacterial exacerbation of chronic bronchitis caused by *Streptococcus pneumonia* and respiratory pathogens like *Haemophilus influenzae* and *Moraxella catarrhalis*. GMF was proved to be cost effective treatment for oral bronchitis compared to Clarithromycin. The structural formula for GMF is shown in Fig. 1.

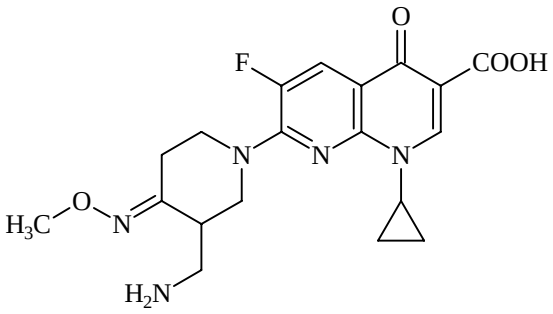


Fig. 1: Chemical structure of Gemifloxacin

A deep and thorough literature survey revealed that a few analytical methods have been reported for the estimation of GMF in pharmaceutical preparations i.e., human plasma by visible spectrophotometry[1,2], capillary zone electrophoresis[3], HPLC-tandem mass spectrometry[4], fluorescence method[5-9], HPTLC[10], microchip electrophoresis[11], RP-HPLC[12-14], etc. These methods have some drawbacks such as pertaining inadequate sensitivity, being time consuming, tedious and dedicated to sophisticated and requires expensive instruments. The spectrofluorimetric analysis is one of the major techniques of analytical chemistry. The primary aim of this research analysis study is to develop rapid, simple, accurate, sensitive and low cost alternative techniques for the determination of GMF in bulk and pharmaceutical dosage forms. So the present analysis was particularly centralized to examine fluorescamine as derivatizing reagent in the determination of sensitive spectrofluorimetric method to have routine determinations of GMF in drug substances. The above explained some techniques are sensitive, require high cost instruments, occasionally tedious and time taking. But the proposed spectrofluorimetric new method requires simple solvent and no need of complicated sample preparation.

EXPERIMENTAL

Materials and methods

Chemicals and reagents: GMF drug was kindly supplied by Hetero Labs, Ltd.,

Hyderabad, India. Fluorescamine was procured from Sigma-Aldrich. All chemicals and reagents were of analytical grade used throughout the estimation of the drug. Vignan Pharmacy College, Vadlamudi, freely supplied triple distilled water from their own plant. G-Cin 320 mg tablets were procured from local market.

Apparatus and instruments

Model SL-174 Elico Spectrofluorimeter with 1 cm quartz cells was utilized to obtain spectral and fluorescence measurements. ELICO LI120 pH meter was used for adjusting pH. ESSAE VIBRA AJ (0.001 g), ESSAE-Teraoka Ltd weighing balance and ultrasonicator of Ultrasonic bath sonicator, PCI Ltd., Mumbai were used. 10 mL and 100 mL volumetric flasks, 0.5-10 µL adjustable-volume micropipet, 1 mL, 5 mL & 10 mL pipettes, beakers, measuring cylinders etc., were used in this research work.

Preparation of reagents and standard solutions

Fluorescamine as derivatization reagent

15 mg of fluorescamine was accurately weighed and transferred to 10 mL volumetric flask and dissolved in some acetone and the content of the flask was diluted with the acetone to 10 mL.

Aqueous borate buffer pH 8.5

65.25 mL of $Na_2B_4O_7$ (0. 05 mol/L) was taken into 100-mL volumetric flask. The content of the flask was diluted with the hydrochloric acid (0.1 mol/L) to 100 mL.

Preparation of standard drug solution

A quantity of 100 mg of GMF was correctly weighed, poured into 100 mL volumetric flask and kept till it was dissolved in 30 mL distilled water. It was sonicated for 5 min and the resultant solution was diluted to volume with triple distilled water to get a stock solution of 1 mg/mL (1000 µg/mL) concentration. This solution was utilized as a working standard solution. For spectrofluorimetry, the above prepared stock solution was diluted further with triple distilled water to get working standard solutions of 100 µg/mL and 10 µg/mL.

General procedure

An aliquot of 0.10 to 1.00 mL of standard solution of 10 µg/mL were poured into a series of 10 mL of separate volumetric flasks. To each flask, 0.2 mL of borate buffer

solution of pH 8.5 and 0.05 mL of fluorescamine solution were added, mixed well and then made up to the mark with triple distilled water. After five min., the fluorescence intensity of the resulting solutions was measured at 483 nm with excitation at 353 nm. A calibration curve was plotted by taking fluorescence intensity on Y-axis and concentration of GMF on X-axis.

Assay procedure for GMF tablets

20 GMF tablets were exactly weighed and ground into smooth powder. A quantity of the tablet powder equivalent to 50 mg of GMF was accurately weighted, put into 50 mL of volumetric flask containing 30 mL triple distilled water and extraction was performed mechanically for 25 min. Then it was sonicated continuously for 20 min till it was entirely dissolved and the volume was brought to 50 mL with triple distilled water and eventual solution was filtered. The said filtrate was diluted further with triple distilled water to get a working sample solution. Then it was assayed as mentioned under general procedure. The amount of GMF in tablets was calculated using its calibration graph.

RESULTS AND DISCUSSION

For the wavelength detection, the working standard solution of GMF was scanned at wavelengths ranging from 200-600 nm in spectrofluorimeter and got 353 nm as excitation wavelength and 483 nm as the emission wavelength. The results so obtained are graphically shown in Fig. 2 and 3.

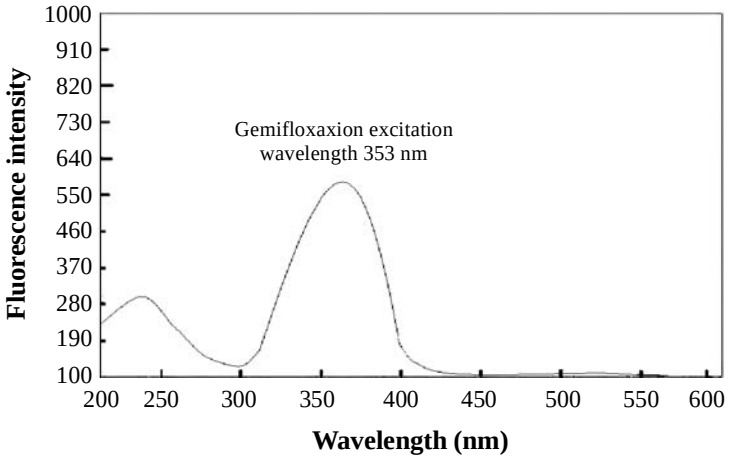


Fig. 2: Excitation spectrum of GMF with fluorescamine

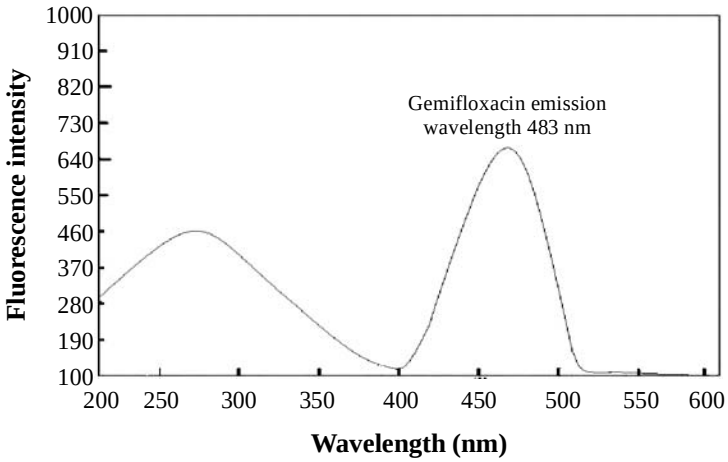
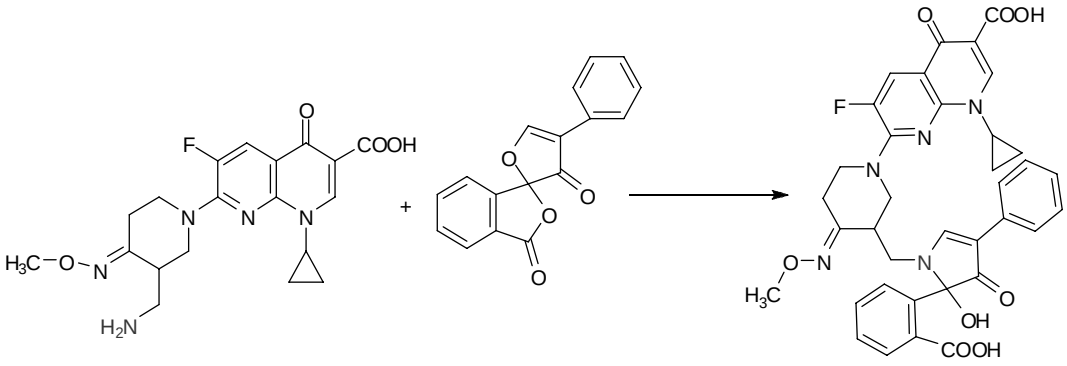


Fig. 3: Emission spectrum of GMF with fluorescamine

Because the fluorogenic technique is among the most sensitive method, it has been preferred for developing a method of analysis of GMF. GMF contains amino group, which is an appropriate candidate for derivatization by fluorescamine. Fluorescamine is a fluorogenic reagent, which has been widely used in the field of pharmaceutical analysis. Fluorescamine reacts instantaneously with primary amines in aqueous solutions to give highly fluorescent pyrrolinone derivatives. This method is conducted in borate buffer of pH 8.5 to yield a highly fluorescent derivative that is measured at 483 nm with excitation at 353 nm. **Scheme 1** shows the proposed reaction pathway between GMF with fluorescamine at pH 8.5 utilizing borate buffer.



Scheme 1: The proposed reaction pathway of fluorescamine with GMF

Study of experimental parameters

The different experimental parameters affecting the development of the reaction product were carefully studied and optimized. Such factors were altered individually, while others were kept constant. The effect of pH, volume of the reagent, temperature and heating period have been extensively studied.

Effect of pH

The effect of pH on fluorescent intensity was studied using different pH values. Infact, the pH was varied over the pH range of 7-10 using borate buffers, where the maximum absorbance was obtained at pH 8.5 is shown in Fig. 4.

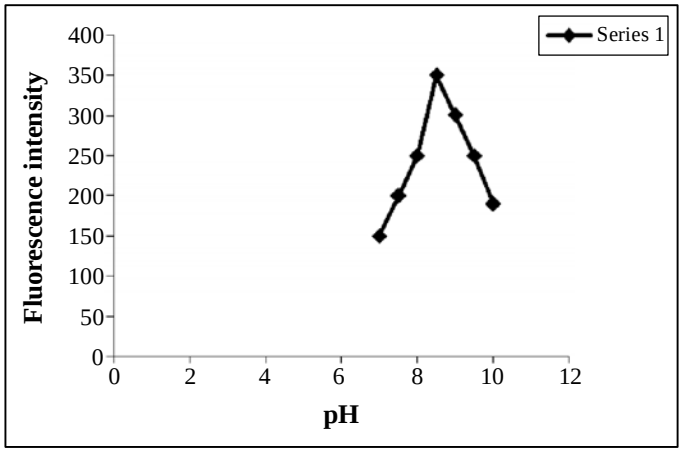


Fig. 4: Effect of pH on the development of the reaction products of GMF with fluorescamine

Effect of temperature and time

The effect of temperature on fluorescence intensity was studied. The derivatization reaction was carried out at 55°C to 75°C. Total color development was achieved instantly at 75°C and reached maximum intensity after five min.

Effect of volume of fluorescamine

The effect of the concentration of fluorescamine on the fluorescence development was studied. It was found out that 0.05 mL of 0.15% fluorescamine solution was enough for the maximum fluorescence. Fig. 5 shows the effect of volume of fluorescamine (0.5% w/v) on development of reaction products of GMF with fluorescamine.

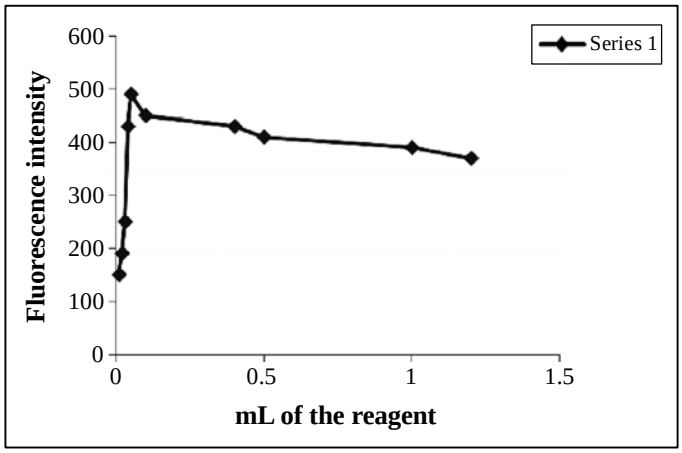


Fig. 5: Effect of volume of fluorescamine (0.5% w/v) on development of reaction products of GMF with fluorescamine

Effective of organic solvents and stability of the derivative

In order to select the most appropriate organic solvent, different solvents were tested. The organic solvents are methanol, chloroform, acetonitrile, dichloromethane, ethyl acetate, ethanol and water. No significant changes were observed with ethanol, methanol and water. So eventually water was preferred for the experimental work. Infact, the sample prepared under these conditions remained stable for at least seven hrs.

Stoichiometry of the reaction

The molar ratio of fluorescamine to GMF in the reaction mixture was studied according to Job's method of continuous variation. By using equimolar solution of GMF and fluorescamine, the stoichiometry of the reaction was found as 1:1 ratio (drug/reagent, confirming that one molecule of GMF reacts with one molecule of fluorescamine.

The results of optical characteristics and regression data of proposed method is shown in Table 1.

Method validation

Linearity

Various aliquots were prepared from working solutions of GMF-fluorescamine (10 µg mL^{-1}) ranging from 0.10, 0.20, 0.30, 0.40 and 0.50 mL. These were transferred into a

series of 10 mL volumetric flasks. To each flask, 0.2 mL of borate buffer solution of pH 8.5 and 0.05 mL of fluorescamine solution was added, mixed well and the volume was brought up with triple distilled water to attain the 5 different concentrations of the drug ranging from 100-500 ng mL^{-1} of solution. The solution was allowed to stand for 5 min. The fluorescence intensity was measured at 483 nm and calibration curve was formed by plotting concentration of GMF on X-axis and resultant fluorescence intensity on Y-axis. Under optimum reaction conditions, RFI found to be linear in the range of 100-500 ng mL^{-1} having correlation coefficient $r^2 = 0.9999$. The calibration curve of GMF drug is shown in Fig. 6. The calibration data of standard GMF is represented in Table 2.

Table 1: Optical characteristics and regression data for proposed spectrofluorimetric method

Parameter	Results
λ_{ex} (nm)	353
λ_{em} (nm)	483
Linearity range (ng/mL^{-1})	100 – 1000
Regression equation (Y = a + bc)	y = 1.3456 x + 0.0501
Intercept (a)	0.0501
Slope (b)	1.3456
Standard deviation of intercept (S_a)	0.0500
Standard deviation of slope (s_b)	1.345
Standard error of estimation (S_e)	0.517
Correlation coefficient (r^2)	0.9999
% Relative standard deviation*	0.0561
Limit of detection (µg/mL)	0.123
Limit of quantitation (µg/mL)	0.369
% Range of error (Confidence limits)*	
0.05 level	0.182
0.01 level	0.240

** n = 6. Average of six determinations

Table 2: Calibration data of standard GMF

Concentration (ng mL^{-1})	Fluorescence intensity
0	0
100	134.45
200	268.904
300	404.35
400	538.768
500	672.25

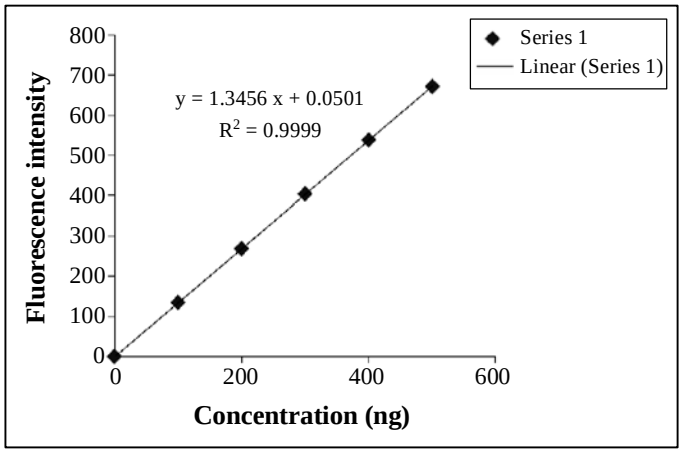


Fig. 6: Calibration curve of GMF

Specificity

The effect of wide range of excipients and other additives usually present in the formulations of GMF in the determinations under optimum conditions was investigated. The interference liabilities form the common tablet excipients such as lactose, talc, magnesium sterate and manitol were studied. The specificity of the method was investigated by considering the interference liabilities from the common tablets excipients. The method shows no or minimum interference with these possible interferences in sample.

Accuracy

The accuracy of the proposed method was checked by calculating the recovery of GMF by standard addition method. Different amount of pure sample solution was added to 3

different concentrations of standard drug solution and assayed. The % recovery of the added standard to the assay samples was calculated from the following equation. The percentage recovery = $[(C_t-C_u)/C_a]$ x 100, where C_t is the total concentration of the analyte determined. C_u is the concentration of the analyte present in the formulation and C_a is the concentration of the pure analyte added to the formulation. The mean accuracy was found to be 99.82% to 99.97%, which indicates good accuracy of the method. The results of analysis obtained of the commercial dosage forms and the recovery study are shown in Table 3.

Table 3: Accuracy data of Gemifloxacin

Amount taken (ng mL^{-1})$^{\$}$	Amount added (ng mL^{-1})	Total amount found* (ng mL^{-1}) (Mean ± S.D$^{@}$)	% Recovery	%RSD
200	100	299.92 ± 1.44	99.97	0.36
	300	499.10 ± 2.10	99.82	0.42
	500	699.30 ± 2.50	99.9	0.35

$^{\$}$ = G-C in 320 mg, *Average of five determinations, $^{@}$SD = Standard deviation

Precision

In order to know the repeatability of the assay, precision was done. It was carried out by the following sections.

Repeatability (Precision on replication)

From working solution (100 ng mL^{-1}), aliquot of 0.1 mL was transferred to the 10 mL volumetric flask and made up to mark with triple distilled water (1 µg/mL). The fluorescence intensity of this solution was measured at 483 nm. The fluorescence intensity of the same solution was measured 5 times and % RSD was calculated.

Intra-day and inter-day precision

Intra-day precision was decided by analyzing GMF (100, 300 and 500 ng mL^{-1}) 3 times in the same day and % RSD was calculated. Inter-day precision was determined by analyzing GMF (100, 300 and 500 ng mL^{-1}) daily for 7 days and % RSD was calculated. The RSD values for intra-day and inter-day precision were found 0.074 and 0.096, respectively indicating good precision. The results of intra- and inter- day precision of GMF by spectrofluorimetry is presented in Table 4.

Table 4: Intra-day and inter-day precision of GMF

Concentration (ng/mL^{-1})	Intra-day precision[$]		Inter-day precision[$]	
	Flurescence intensity Mean[a] ± SD[b] (n = 5)	% RSD[c]	Flurescence intensity Mean[a] ± SD[b] (n = 5)	% RSD[c]
100	134.47 ± 0.225		134.76 ± 0.242	
300	403.35 ± 0.222	0.074	404.76 ± 0.258	0.096
500	672.25 ± 0.421		672.54 ± 0.311	

[$] = Three times repetition were done, [a]Average of five determinations,
[b]SD = Standard deviation. [c]% RSD = Relative standard deviation

LOD: Based on the standard deviation of the response and the slope, the limit of detection may be expressed as LOD = 3.3*s/S. Where s = the standard deviation of the response and S = the slope of the calibration curve. The limit of detection was found to be 0.123 µg/mL

LOQ: Based on the standard deviation of the response and the slope, the quantification limits may be expressed as LOQ = 10s/S where s = the standard deviation of the response and S = the slope of the calibration curve. The limit of quantification was 0.369 µg/mL.

Robustness: The robustness was evaluated by the analysis of GMF under different experimental conditions such as making small changes in fluorescamine concentrations (%, w/v ± 0.5), time (optimum ± 0.25 minutes) and temperature (optimum ± 2^{o}C). Regarding robustness studies, variations in the fluorescamine concentrations did not significantly affect the procedures.

Determination of GMF in tablets

The proposed method was successfully subjected to the analysis of marketed formulation G-Cin-320 mg. The obtained results are satisfactorily precise, accurate, with excellent recovery and SD is less than 2. Infact, experiments showed that there was no interference from the excipients and additives such as lactose, magnesium sterate, glucose and starch. The determination of GMF in tablet dosage form by spectrofluorimetry is shown in Table 5.

Table 5: Estimation of GMF in tablets

Tablet formulation	Labeled claim (mg/tablet)	Mean[a] ± S.D.[b]	% Assay	% RSD
G-Cin Lupin Laboratories Ltd., (Mumbai)	320 mg	319.89 ± 0.14	99.8	0.043

[a]Five independent determinations; [b] = Standard deviation

CONCLUSION

After going through the facts of relevant literature on this subject, the author, observed that no analyst has hitherto analyzed GMF drug in tablet form with fluorescamine as reagent with spectrofluorimetric method. Therefore, the proposed method is quite simple and sensitive spectrofluorimetric method and can be successfully carried out the analysis of GMF. The results pertaining to the statistical analysis confirmed that the present developed method possesses good precision and accuracy without interference of normal additives existing in the pharmaceutical preparations. This method doesn't require any tedious extraction procedure. The current study illustrated the utility of fluorescamine as reagent for the estimation of GMF drug with spectrofluorimetric method in bulk and pharmaceutical dosage form and found that this developed method is better than previously reported methods with regard to its selectivity and sensitivity features. The linearity range of the proposed spectrofluorimetric method is less than previously reported methods on GMF. The statistical results of the analysis of the tablets by this method were reproducible, reliable and were in good agreement with labeled claim of the drug and also without interference of the present excipients in the tablets. Finally, it is concluded that this proposed method can be utilized for the routine determination of GMF in research laboratories and pharmaceutical industries.

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REFERENCES

1. M. V. Krishna and D. G. Sankar, E-J. Chem., **5(3)**, 493-498 (2008).
2. M. V. Krishna and D. G. Sankar, E-J. Chem., **5(3)**, 515-520 (2008).

3. A. A. Elbashir, B. Saad, A. S. M. Ali, K. M. M. Al-Azzam and H. Y. Aboul- Enein, J. Liquid Chromatogr. Related Technol., **31(10)**, 1465-1477 (2008).

4. E. Doyle, S. E. Fowles, D. F. Mc Donnell, R. Mc Carthy and S. A. White, J. Chromatogr. B **746**, 191-198 (2000).

5. B. M. H. Al-Hadiya, A. A. Khady and G. A. E. Mostafa, Talanta, **83(1)**, 110-116 (2010).

6. N. F. Yousef and L. I. Bebawy, Bull. Faculty Pharm., **44(3)**, 215-227 (2006).

7. S. K. Tekkeli and A. Onal, J. Fluorescence, **21(3)**, 1001-1007 (2011).

8. J. A. Ocana, F. J. Barragan and M. Callejon, J. Pharmaceut. Biomed. Anal., **37(2)**, 327-332 (2005).

9. J. A. O. Gonzalez, M. Callejon Mochon and F. J. B. De La Rosa, Talanta, **52(6)**, 1149-1156 (2000).

10. A. R. Rote and S. P. Pingle, J. Chromatogr. B, **877(29)**, 3719-3723 (2009).

11. S. I. Cho, J. Shim, M. S. Kim and Y. K. Kim, J. Chromatogr. A, **1055**, 241-245 (2004).

12. W. Lee and C. Yong Hong, J. Chromatogr. A, **879(2)**, 113-118 (2000).

13. P. Ravisankar, G. Devala Rao, Ch. Devadasu, S. K. Afzal Basha, N. Sandhya Rani and P. Srinivasa Babu, J. Chem. Pharmaceut. Sci., **6(1)**, 46-54 (2013).

14. P. Ravisankar and G. Devala Rao, Int. J. Adv. Pharmaceut. Res., **4(5)**, 1751-1764 (2013).

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