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ORIGINAL ARTICLE

Complexation and oxidation of Flutamide with Fe^{3+} and 1,10-phenanthroline: Few analytical applications

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Abstract A simple and sensitive spectrophotometric method has been proposed for the determination of flutamide (FLD). The method is based on its complexation and oxidation of FLD by Fe^{3+} and 1,10-phenanthroline. Two sets of condition were established. In the first set only FLD reacted with Fe^{3+} ion, while in the second one FLD was oxidized by Fe^{3+} in the presence of 1,10-phenanthroline. The complexation and oxidation methods were monitored spectrophotometrically. All variables affecting the development of the color were investigated and the conditions obeying Beer's law were optimized. Plots of absorbance against concentration in both methods were rectilinear over the ranges 0.5–10 and 0.5–1.5 μg mL^{-1}, with mean recoveries of 99.51 and 99.83 for methods A and B respectively. The limits of detection for procedures 1 and 2 are 0.332 and 0.726 μg mL^{-1} 1, respectively. Both the methods were successfully applied for the determination of the FLD in its dosage form.

1. Introduction

Flutamide (FLD), chemically known as 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]-propanamide (Fig. 1) is widely used as a antineoplastic (hormonal) and antiandrogen drug (Budavari et al., 1989; Brogden and Chrisp, 1991; McLeod, 1993; APFH, 2004). It is a powerful nonsteroidal androgen antagonist, commonly used to treat prostate cancer, is believed to block androgen receptor sites. Flutamide and its primary hydroxyl metabolites decrease metabolism of C-19 steroids by the cytochrome P-450 system at the target cells in the

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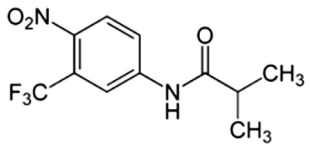

Fig. 1 Chemical structure of FLD.

secondary sex organ (Osol and Hoover, 1996). It has been recently included in the European Pharmacopoeia which involves a liquid chromatographic method for the determination of the drug (EP, 2005).

Several methods have been reported for the assay and the determination of FLD in pure form, in biological and pharmaceutical preparation methods, including spectrophotometric (Nagaraja et al., 2002; Reddy et al., 2003; Rangappa et al., 2000; Hemavathi et al., 2012), spectrofluorometric (Smith et al., 2008), Voltammetric (Reddy et al., 2011; Hammam et al., 2004) and HPLC (Filip et al., 2007; Niopas and Daftsios, 2001; Smith et al., 2009; Salgado et al., 2005).

The spectrophotometric method includes the formation of yellow color with hydrochloric acid with λ_{max} of 380 nm which suffers from various drawbacks Zarapkar et al., 1996). The recently reported three spectrophotometric methods for the determination of FLD using promethazine hydrochloride or resorcinol or (NEDA) N-(1-naphthyl) ethylenediamine dihydrochloride (Rangappa et al., 2000) have been reported. Two newly spectrophotometric methods published make use of p-dimethylamino cinnamaldehyde, NEDA, chromotropic acid and resorcinol (Reddy et al., 2001).

We have recently reported a detailed study of the interactions of copper(II) with flutamide in the presence of anionic surfactants by resonance rayleigh scattering, second-order scattering and frequency doubling scattering (Mohd et al., 2011).

Although highly expensive, sophisticated and time consuming techniques are available, we are reporting in this paper, a simple, precise and accurate spectrophotometric method for rapid determination of FLD. The method is based on the complexation and oxidation of the drug by Fe^{3+} ion in the presence and absence of phenanthroline. Under both the conditions the reactions were monitored spectrophotometrically by measuring the increase in absorbance at 510 nm.

2. Experimental

2.1. Apparatus

The absorption spectra were obtained with UVD-2960 Double beam PC connected UV–vis spectrophotometer EL20 Education Line pH Meter from Mettler-Toledo Inc.

2.2. Reagents

Distilled water and analytical-reagent grade chemicals were used. Standard solution of FLD (Sigma Aldrich) was prepared by dissolving 0.0276 g of the drug in (40:60) ethanol–water mixture. Working solution containing 25 ml was prepared from the stock solution. Phenanthroline and ferric chloride $FeCl_3.6H_2O$ (Merck) solution were prepared in distilled water in a 100 ml flask. Acetate buffer of pH 3.5 and 5 was prepared and calibrated with pH meter.

2.3. Method A

An aliquot solution containing 0.5–10 μg mL^{-1} of FLD was transferred to a 10 ml volumetric flask containing 1 ml of 2.50 mol L^{-1} Fe^{3+} solution and 1 ml acetate buffer of pH 3.5. This mixture made up to the mark with distilled water. Its absorbance was measured at 510 nm against a reagent blank.

2.4. Method B

An aliquot solution containing 0.5–1.5 μg mL^{-1} of FLD was transferred to a 10 ml volumetric flask containing 1.5 ml of 1.2×10^{-2} mol L^{-1} 1,10-phenanthroline and 1 ml acetate buffer of pH5. The solution was warmed on a water bath at 50 °C for 10 min followed by the addition of 1.0 mL of 6.5×10^{-93} mol L^{-1} Fe^{3+} solution made up with distilled water. The increase in absorbance was measured after 25 min at 510 nm against a blank.

3. Results and discussion

The methanolic solutions of FLD and FLD–Fe^{3+} complex were found to absorbance at maximum wavelength at 210, 230, 270 and 510 nm, respectively (Figs. 2 and 3). FLD–Fe^{3+} was allowed to react with 1.10-phenanthroline resulting in the formation of red colored product and obtain the maximum absorbance at 510 nm (Fig. 3).

The FLD reacted with Fe^{3+} at pH 3.5 at room temperature, forming a colored complex according to the equation given below. However, in acetate buffer of pH 5 at 50 °C, Fe^{3+} is reduced by FLD in Eq. (2) and subsequently forms a colored complex with 1,10-phenanthroline which is called ferroin in Eq. (3) (Marczenko, 1986). The mechanism of ferroin is shown in Scheme 1.

$$FLD + Fe^{3+}(1) \rightarrow \text{Colored} \quad \text{Complex} \tag{1}$$

$$FLD + Fe^{3+}(2) \rightarrow \text{Oxidized} \quad \text{Products} \tag{2}$$

$$Fe^{2+} + 3\text{Phen} \rightarrow Fe(\text{Phen})_3^{2+}(\text{Ferroin}) \tag{3}$$

The effect of reaction condition was studied for FLD and its optimum values for procedures were selected. Two sets of conditions must be fulfilled. Under Method A only FLD reacted

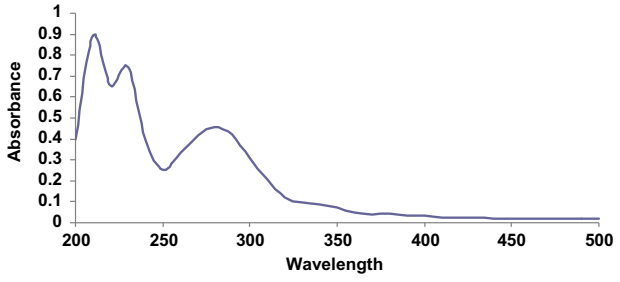

Fig. 2 Absorbtion spectra of flutamide.

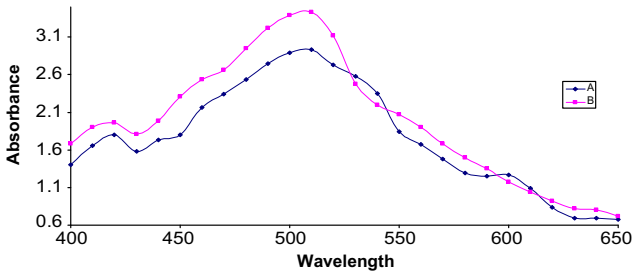

Fig. 3 Absorption spectra of (A) mixture condition for FLD = 0.8 μg mL^{-1}, Fe^{3+} = 2.2×10^{-3} mol L^{-1},pH = 3.5 at T = 25 °C (B) FLD = 1.0 μg mL^{-1}, 1,10-phenanthroline = 1.30×10^{-3} mol L^{-1}, Fe^{3+} = 2.2×10^{-3} mol L^{-1} and pH = 5.0 at 50 °C.

$$FLD + Fe^{3+}\ (1) \rightarrow Colored\ Complex \quad (1)$$

$$FLD + Fe^{3+}\ (2) \rightarrow Oxidized\ Products \quad (2)$$

$$Fe^{2+} + 3Phen \rightarrow Fe(Phen)_3^{2+}\ (Ferroin)\ (3)$$

Scheme 1 A detailed mechanistic scheme for the complexation and oxidation of FLD with Fe3^{+} and 1,10-phenanthroline.

while in method B the FLD was oxidized by Fe^{3+} ion in the presence of 1,10-phenanthroline.

4. Effect of pH

The effect of pH on the reaction of FLD with Fe^{3+} was studied in the range of 2–7. In the first procedure only FLD forms complex with Fe^{3+}. The absorbance of FLD with pH increases and attains a maximum at pH 3.5. Since at higher pH absorption decreases an optimum pH of 3.5 was chosen for the first set. Fig. 4 shows the effect of pH on the oxidation of FLD by Fe^{3+} in the presence of 1,10-phenanthroline under the second set of conditions. Since in this case the absorbance attains a maximum at pH 5 the subsequent experiments were done at this pH.

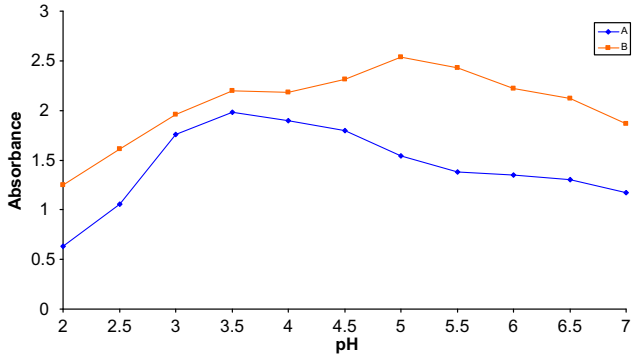

Fig. 4 Effect of pH on the complex formation reaction and oxidation of FLD (A) FLD = 0.8 μg mL^{-1}, Fe^{3+} = 2.2×10^{-3} mol L^{-1}, at 25 °C (B) FLD = 1.0 μg mL^{-1}, 1,10-phenanthroline = 1.30×10^{-3} mol L^{-1} and Fe^{3+} = 2.2×10^{-3} mol L^{-1} at 50 °C.

5. Effect of the concentration of Fe^{3+}

The effect of Fe^{3+} concentration on the complex formation in Fig. 5 of FLD was studied in the range of 6.0×10^{-5} to 5.8×10^{-3} mol L^{-1}. The absorbance of FLD increased with increasing Fe^{3+} concentration up to 2.2×10^{-3} mol L^{-1} and remained constant at higher concentration. A concentration of 2.2×10^{-3} mol L^{-1} Fe^{3+} ion was therefore chosen for the first set of conditions. Fig. 3 also shows the effect of concentration of Fe^{3+} on the oxidation of FLD under the second set of condition in the range of 6.4×10^{-5} to 5.8×10^{-3} mol L^{-1}. The absorbance of FLD increases with increasing Fe^{3+} concentration and attains a maximum at 3.8×10^{-4} mol L^{-1}. The concentration 3.8×10^{-4} mol L^{-1} of Fe^{3+} was therefore, selected for the second set of conditions.

6. Effect of the concentration of 1,10-phenanthroline

The effect of 1,10-phenanthroline concentration on the absorbance of the solution under the second set of condition was studied in the range of 6.0×10^{-5}–2.40×10^{-3} mol L^{-1} shown in Fig. 6. The absorbance of the solution increases with increasing 1,10-phenanthroline concentration with a maximum at 1.30×10^{-3} mol L^{-1}. It was therefore, selected as optimum concentration.

7. Effect of temperature

In order to examine the effect of temperature, the reaction of FLD with Fe^{3+} was studied between 25 and 50 °C. The variation in temperature had no effect on the absorbance and hence experiment was done at 25 °C for the first set. Since in the second set of conditions the absorbance increases rapidly up to 50 °C and becomes slow at higher temperature, a temperature of 50 °C was chosen for the reaction.

7.1. Kinetic studies

It is clear from the plots in Figs. 7 and 8 that the rate is proportional to the concentration of FLD and obeys the equation:

$$\text{Rate} = K'[\text{FLD}]^n \quad (4)$$

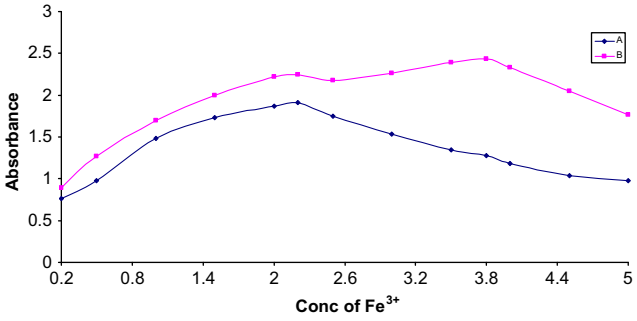

Fig. 5 Effect of Fe^{3+} ion concentration on the complex formation and oxidation of FLD (A) FLD = 0.8 μg mL^{-1}, pH = 3.5 M, at 25 °C (B) FLD = 1.0 μg mL^{-1}, 1,10-phenanthroline = 1.30×10^{-3} mol L^{-1} and pH = 5.0 at 50 °C.

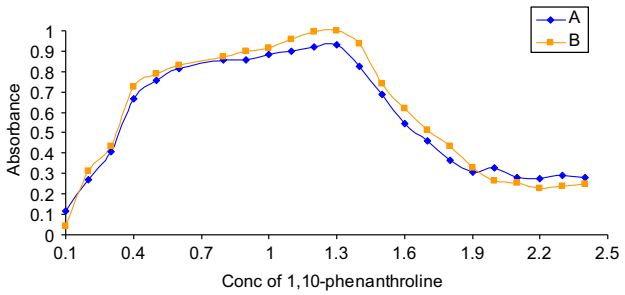

Fig. 6 Effect of 1,10-phenanthroline concentration on the absorbance of the ferroin produced from the oxidation of (A) FLD (B) FLD Fe^{3+} ion. Condition FLD = 1.0 μg mL^{-1}, Fe^{3+} = 2.2×10^{-3} mol L^{-1} and pH = 5.0 at 50 °C.

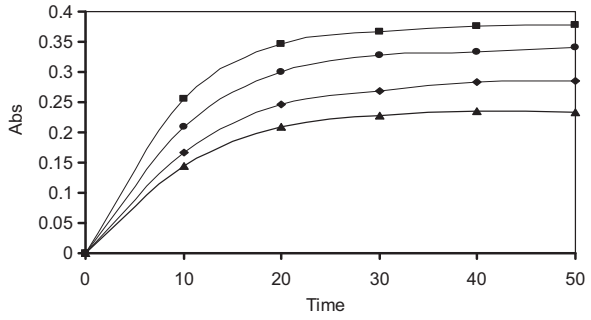

Fig. 7 Absorbance vs. time graphs for the reaction of FLD Oxidation with Fe^{3+} ion in the presence of 1,10-phenanthroline by method B.

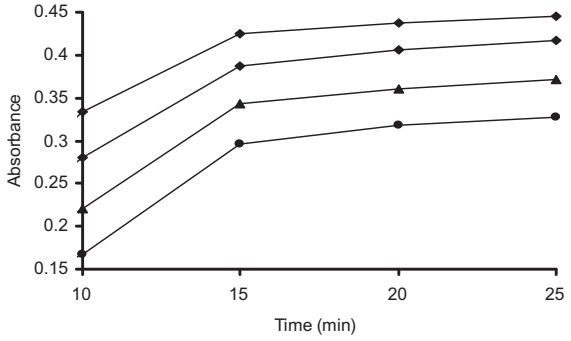

Fig. 8 Absorbance vs. time graphs for the reaction of FLD with Fe^{3+} ion by the method A.

where K' is the pseudo first-order rate constant of the reaction and n is the order of the reaction. The rate may be estimated by the variable-time method measured as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds (Weisberger et al., 1953). Taking logarithms of rates and concentration as shown in Table 1, Eq. (4) is transformed into:

$$\log(\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log[\text{FLD}] \quad (5)$$

Regression of log (rate) versus log (FLD) gave the regression equations:

$$\log(\text{rate}) = -0.9874 + 1.9920 \log C \ (r = 0.9996) \text{ Method A}$$

Table 1 Logarithms of the rates for different concentrations of FLD applying methods A and B.

Log $\Delta A/\Delta t$	Log [FLD], (mol L^{-1})
Reaction with Fe^{3+} (Method A)	
−3.942	−4.854
−3.690	−4.553
−3.491	−4.376
−3.360	−4.244
Oxidation with Fe^{3+} ion in the presence of 1,10-phenanthroline (Method B)	
−4.066	−3.824
−3.750	−3.523
−3.552	−3.251
−3.500	−3.222

$$\log(\text{rate}) = 9.48 \times 10 - 3 + 0.848 \log C \ (r = 0.9998) \text{ Method B}$$

Hence $K' = 1.023S^{-1}$ or $1.054S^{-1}$, applying Methods A or B, respectively, and the reactions can be approximated to first order ($n \approx 1$) with respect to FLD concentration under the optimized experimental conditions. The concentration of FLD was determined using an excess of Fe^{3+} and acetate buffer of pH 3.5, applying Method A.

Under second procedure excess of 1,10-phenanthroline, Fe^{3+} and acetate buffer of pH 5 with respect to the initial concentration of FLD was taken.

However, the rate will be directly proportional to drug concentration in a pseudo-first rate equation as follows:

$$\text{Rate} = K'[\text{drug}] \quad (6)$$

where K' is the pseudo-order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to Eq. (6). The rate constant, fixed-concentration and fixed time methods (Yatsimirskii, (1966), Laitinen and Harris, (1975)) were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r) and the intercept.

7.2. Fixed-time method

The absorbance was measured at a preselected fixed time. Absorbance versus FLD concentration was plotted at 5 min interval applying method A and 10 min interval under the method B with the regression equation shown in Table 2.

The slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time interval for measurements in Table 2.

7.3. Rate-constant method

Graphs of log (absorbance) versus time over the concentration range of FLD 1.2×10^{-5}–4.8×10^{-5} mol L^{-1}, and 1.5×10^{-4}–7.5×10^{-4} mol L^{-1} were plotted by methods A and B respectively. The pseudo-first order rate constants corresponding to different FLD concentrations were then calculated from the slopes multiplied by −2.303 and listed in Table 3.

Table 2 Calibration equations at different fixed times for FLD in the ranges 0.5–10 μg mL^{-1} and 0.5–1.5 μg mL^{-1} applying methods A and B., respectively.

Time (min)	Regression equation	Correlation coefficient (r)
Reaction with Fe^{3+} (Method A)		
10	$A = -.05655 + .04192\ C$	0.9912
15	$A = -.04687 + .04412\ C$	0.9832
20	$A = -.02116 + .05038\ C$	0.9997
25	$A = -.00095 + .0538\ C$	0.9981
Oxidation with Fe^{3+} ion in the presence of 1,10-phenanthroline (Method B)		
10	$A = -.04045 + .03912\ C$	0.9928
20	$A = -.01945 + .03645\ C$	0.9969
30	$A = -.0016 + .00386\ C$	0.9972
40	$A = -.00045 + .0012\ C$	0.9991
50	$A = -2.15 \times 10^{-3} + .03745\ C$	0.9949
60	$A = 2.12 \times 10^{-3} + .03932\ C$	0.9994

Table 3 Values of K' calculated from slopes of log A versus t graphs multiplied by −2.303, for different concentrations of FLD, by applying methods A and B.

K' (s^{-1})	Log [FLD], (mol L^{-1})
Reaction with Fe^{3+} (Method A)	
-9.58×10^{-4}	1.2×10^{-5}
-8.40×10^{-4}	2.4×10^{-5}
-7.36×10^{-4}	3.6×10^{-5}
-6.26×10^{-4}	4.8×10^{-5}
Oxidation with Fe^{3+} ion in the presence of 1,10-phenanthroline(Method B)	
-2.44×10^{-4}	1.5×10^{-5}
-1.66×10^{-4}	3.0×10^{-5}
-1.57×10^{-4}	4.5×10^{-5}
-1.25×10^{-4}	6.0×10^{-5}
-1.10×10^{-4}	7.5×10^{-5}

Regression of (C) versus K' gave the equations:

$$K' = -1.050 \times 10^{-3} + 9.92\ C\ (r = 0.9912)\ \text{Method A}$$

$$K' = -2.12 \times 10^{-3} + 2.441\ (r = 0.9795)\ \text{Method B}$$

The value of r is indicative of poor linearity, probably because of inconsistency of K'.

7.4. Fixed-concentration method

Rate of reaction was recorded for different concentrations of FLD (3.35×10^{-5}–5.58×10^{-5} mol L^{-1}, and 5.36×10^{-5}–6.7×10^{-5} mol L^{-1}). A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time ($1/t$) versus the initial concentration of FLD was plotted in Table 4. The following equations for calibration graphs were obtained by linear regression:

$$1/t = -5.125 \times 10^{-3} + 173.92\ C\ (r = 0.9887)\quad \text{for Methods A}$$

$$1/t = -2.137 \times 10^{-3} + 63.1\ C\ (r = 0.9991)\quad \text{for Methods B}$$

Table 4 Values of reciprocal of time taken at fixed absorbance (0.4 and 0.3) for different rates of various concentrations of FLD applying methods A and B.

$1/t$ (s^{-1})	Log [FLD], (mol L^{-1})
Reaction with Fe^{3+} (Method A)	
8.40×10^{-4}	3.35×10^{-5}
2.75×10^{-3}	4.46×10^{-5}
4.70×10^{-4}	5.58×10^{-5}
Oxidation with Fe^{3+} ion in the presence of 1,10-phenanthroline (Method B)	
1.21×10^{-3}	4.5×10^{-3}
1.63×10^{-3}	6.02×10^{-4}
2.06×10^{-3}	6.70×10^{-4}

Table 5 Evaluation of precision of the proposed kinetic spectrophotometric method for determination of investigated FLD by applying methods A and B.

Amount taken (μg mL^{-1})	Amount found (μg mL^{-1})	% Recovery ± S.D.
Reaction with Fe^{3+} (Method A)		
10	10.05	100.50
20	20.16	99.55
30	29.91	98.50
		Mean ± S.D. = 99.51
Oxidation with Fe^{3+} ion in the presence of 1,10-phenanthroline (Method B)		
55	54.05	100.50
150	149.98	99.75
250	250.02	99.25
		Mean ± S.D. = 99.83

Table 6 Analytical parameters for the fixed time method of the kinetic spectrophotometric determination of investigated FLD in the pure form by applying methods A and B.

Parameters	Method A	Method B
Optical characteristics		
λ_{max}, nm	510	510
Linearity range (μg mL^{-1})	0.5–10	0.5–1.5
Regression equation:	0.075210	0.089392
Intercept (a)		
Standard deviation of intercept	0.571274	0.471283
(Sa)	0.012716	0.002194
Slope (b)	0.00189	0.000325
Standard deviation of slope		
(Sb)	0.9865	0.9928
Correlation coefficient (r)	0.332	0.726
LOD (μg mL^{-1})	0.371	0.1615
LOQ (μg mL^{-1})		

7.5. Validation of the proposed method

7.5.1. Accuracy and precision of the proposed methods

Accuracy and precision were checked according to USP vali-

Table 7 Result for determination of FLD in urine samples by the RRS method [13].

Urine	Amount taken (μg mL^{-1})	Amount found (μg mL^{-1})	Mean (g)	% RSD	% Recovery
Sample 1	0.5	ND	0.499	0.44	99.9
Sample 2	1.0	ND	1.003	0.62	100.3
Sample 3	1.5	ND	1.504	0.89	100.2

dation guidelines (TUSP, 2002) at three concentration levels within the specified range, six replicate measurements were recorded at each concentration level presented in Table 5.

7.5.2. Limit of detection (LOD)

LOD was calculated based on standard deviation of response and the slope of the calibration curve. The limit of detection was expressed as:

$$LOD = 3\sigma/S$$

where σ is the standard deviation of intercept. S is the slope of the calibration curve. The results are summarized in Table 6 indicating good sensitivity of the proposed method. According to USP validation guidelines (TUSP, 2002), the calculated LOD values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOD equations was actually detected in these experiments.

7.5.3. Limit of quantitation (LOQ)

LOQ was calculated based on standard deviation of intercept and slope of the calibration curve. In this method, the limit of quantitation is expressed as:

$$LOQ = 10\sigma/S$$

The results indicate good sensitivity of the proposed method in Table 5. According to USP validation guidelines (TUSP, 2002), the calculated LOQ values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOQ equations was actually quantitated in these experiments.

8. TLC of FLD and its reaction products

The FLD drug, Fe^{3+} and its reaction products were analyzed by thin layer chromatography. The spots test of FLD, reaction products such as red color ternary complex of methods A and B were with *Rf* values of 0.65, 0.45 and 0.60, detected respectively.

9. Determination of FLD in urine

Authors have already carried out a detailed investigation on the FLD determination in urine by the resonance rayleigh scattering (RRS) method in their previous study (Mohd et al., 2011) as shown in Table 7. The results portrayed that the RRS method has good repeatability. The % RSD and % recovery are found to lie between 0.44 and 0.89 and 99.9 and 100.3 respectively. Therefore, the method can be applied for the determination of FLD in urine sample by future researchers.

10. Application to pharmaceutical dosage forms

The optimized methods have been applied for the determination of FLD in commercial pharmaceutical dosage forms. The concentration of FLD determination was computed from the regression method. The obtained results of proposed methods were statistically compared with those of reported literatures (Saleh et al., 2003; Khan et al., 2015). The mean recovery values were 99.51 and 99.83 respectively, which ensures that there is no interference in the FLD of other active compounds. The calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicates good precision and accuracy for the determination of FLD in pharmaceutical dosage forms.

11. Conclusion

The determination of FLD by the proposed method is based on its complexation and oxidation by Fe^{3+}. Two sets of conditions were established. In one set of conditions only FLD forms complex with Fe^{3+} while in the other set FLD is oxidized by Fe^{3+} in the presence of 1,10-phenanthroline. In both the sets, the reactions can be monitored spectrophotometrically by measuring the increase in absorbance at 510 nm. The proposed method is sensitive enough to enable determination of low amount of drug. These advantages encourage the application of the proposed method in routine analysis of FLD in industrial laboratories.

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