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ORIGINAL PAPER

Spectrofluorimetric Determination of Etodolac, Moxepril HCl and Fexofenadine HCl Using Europium Sensitized Fluorescence in Bulk and Pharmaceutical Preparations

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Abstract A simple, selective and sensitive luminescence method has been developed for the assay of etodolac (I), moxepril HCl (II) and fexofenadine HCl (III) in bulk drug and pharmaceutical formulations. The method is based on the luminescence sensitization of europium (Eu^{3+}) by complexation with the studied drugs. The fluorescence intensities of the products were measured at 667 nm for (I) and at 615 for (II) and (III) while exciting at 276 for all the studied drugs. The fluorescence intensity was directly proportional to the concentration over the range (20–280), (40–240) and (30–80) ng/ml with limits of detection (LOD)= 0.93, 0.92 and 0.95 μg/ml for drugs I, II and III respectively. Optimum conditions for the formation of the complex in methanol were carefully studied. The proposed method was successfully applied for the assay of the studied drugs in pharmaceutical formulations with excellent recovery.

Keywords Sensitized fluorescence · Europium · Etodolac · Moxepril HCl · Fexofenadine HCl

Introduction

Etodolac (I) is an important non-steroidal anti-inflammatory drug which is used for rheumatoid arthritis, osteoarthritis and the treatment of acute pain [1], very few fluorimetric methods have been used for its determination [2]; other methods used include high performance liquid chromatography (HPLC) [3] and spectrophotometric methods [2, 4].

Moexipril HCl (II) is a potent new orally active, non-sulfhydryl angiotensin-converting enzyme inhibitor, which is used for the treatment of hypertension and congestive heart failure [1]. Few analytical methods have been developed for determination of moexipril including: derivative spectrophotometric [5], spectrophotometric methods [6], and reversed phase-HPLC methods [7] have been developed for the determination of moexipril

Fexofenadine HCl (III) is a non-sedating antihistamine. It does not possess significant sedative or antimuscarinic actions. It is used as the hydrochloride in the symptomatic relief of allergic conditions including seasonal allergic rhinitis and urticaria [1]. Few analytical methods have been reported for its determination including spectrophotometric [8, 9] and HPLC [10] methods.

Europium (III) has been used for the determination of some drugs such as diclofenac sodium [11], piroxicam [12, 13], humic acid and its model compounds [14]. Lanthanide ions are known to have a very weak fluorescence because the absorption of these ions is very low. However, when these ions are chelated to organic ligands their fluorescence can be dramatically enhanced [15–17].

Since there are no or very few fluorimetric methods reported for the drugs of interest, we set out to capitalize on the opportunity to achieve increased sensitivity offered by europium chelation for the determination of trace quantities of drugs in bulk and pharmaceutical preparations. In this work, a simple and sensitive method for determination of etodolac, moxepril HCl and fexofenadine HCl either in pure form and its pharmaceutical formulations is shown using europium chloride. The method uses the increase in sensitivity brought about by enhancement and sensitization of the fluorescence of europium by the studied drugs.

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Experimental

Apparatus

Fluorescence spectra were obtained using LS45 fluorescence spectrophotometer (Perkin-Elmer, London, UK) equipped with a pulsed xenon lamp and 10 mm matched quartz cells.

Materials and Reagents

Etodolac was kindly supplied by Pharco pharmaceutical company (Alexandria, Egypt). Its pharmaceutical preparation Etodolac® tablets (labelled to contain 300 mg etodolac per tablet) were obtained from a local drugstore.

Moexipril hydrochloride was kindly supplied by Minapharm pharmaceutical company (10th of ramadan, Egypt). Its pharmaceutical preparation Primox® tablets (labelled to contain 15 mg moexipril hydrochloride per tablet) were obtained from a local drugstore.

Fexofenadine HCl was kindly supplied by SEDICO pharmaceutical company (6th of October, Egypt). Its pharmaceutical preparation Rapido® capsules (labelled to contain 120 mg fexofenadine HCl per capsule) and Telfast® tablets (labelled to contain 120 mg fexofenadine HCl per tablet) were obtained from a local drugstore.

Europium (III) chloride and Tris(hydroxymethyl)aminomethane HCl (TRIS) were obtained from Sigma-Aldrich (Munich, Germany). All other chemicals and reagents used were of analytical grade

Standard Solutions

Solutions of the drug analytes were prepared to a concentration 0.2 mg/ml by dissolving the appropriate quantities of the drugs in 10 ml methanol followed by dilution to a final total volume of 50 ml with methanol. Working solutions of lower concentrations were prepared by appropriate dilutions of the standard solutions with methanol. A solution of $EuCl_3$ was prepared to a concentration of 8×10^{-4} (w/v) by dissolving the appropriate quantity of $EuCl_3$ in 10 ml methanol followed by dilution with methanol to a final volume of 100 ml. Double distilled water was used in preparation of all solutions.

General Procedure

Specific volumes of TRIS buffer were added into a 5 ml volumetric flask followed by specified volumes of europium chloride (8×10^{-4})% w/v (Table 1). Then different aliquots of drug standard solutions equivalent to (0.1–1.4), (0.2–1.2), (0.15–0.4) μg of (I), (II) and (III) were added prior to dilution with methanol. The fluorescence intensities of the products were measured at 667 nm for (I) and at 615 for (II) and (III) while exciting at 276 for all the studied drugs.

Table 1 Experimental parameters for the spectrofluorimetric study of etodolac, moxepril HCl and fexofenadine HCl with europium chloride

Parameter	Etodolac	Moxepril HCl	Fexofenadine HCl
λ excitation (nm)	276	276	276
λ emission (nm)	667	615	615
Linear range (ng/ml)	20–280	40–240	30–80
Tris buffer concentration (M)	0.005	0.01	0.03
Tris buffer pH	5.5–6	8	7.5
Tris buffer volume (ml)	0.2	0.5	0.2
Vol of $EuCl_3$ (8×10^{-4})% (ml)	0.2	1	0.7
Regression equation[a]			
Slope, b	2.53	1.01	3.39
Intercept, a	70.99	57.73	−72.02
Correlation coefficient (r^2)	0.9999	0.9995	0.9994
LOD (μg/ml)	0.93	0.92	0.95
LOQ (μg/ml)	3.11	3.06	3.18

Average of three experiments

[a] (Fluoresence emission) = a + b (drug molarity)

Procedure for Tablets and Capsules

Accurately weighted quantities of well mixed drug powders were dissolved in known volumes of methanol. Drug sample solutions were filtered prior to completing the fluorimetric assay as above.

Results and Discussion

Etodolac (I), moxepril HCl (II) and fexofenadine HCl (III) were determined spectrofluorimetrically by complex formation with europium chloride. The fluorescence intensities of the products were measured at 667 nm for (I) and at 615 for (II) and (III) while exciting at 276 for all the studied drug. It is important to mention that the first peak of the emission spectra at around 550 nm should be assigned to the first harmonic of the excitation beam at 276 nm (276 nm×2=552 nm), due to second order defect of the emission. The enhancement of Eu^{3+} fluorescence was through complex formation with the studied drugs in the presence of TRIS buffer. The widely accepted explanation for this enhancement is that the excitation light is absorbed and collected by the organic ligand (or drug molecule in this case), which serves as an "antennachromophore". This is followed by an internal transfer of the collected energy to

the encapsulated lanthanide ion, which is then emitted as the line spectrum of the lanthanide ion. The result of this process is a profound fluorescence enhancement of the lanthanide emission due to the energy transfer [17].

It is clear that the intensity of the emission line is greatly enhanced in the presence of the studied drugs in comparison with Eu^{3+} alone. A very weak signal is observed from the spectral characteristics of Eu^{3+}. However, the introduction of the studied drugs to this mixture resulted in an intense well known structured emission spectrum of Eu^{3+} (Figs. 1 and 2).

The emission spectrum of Eu^{3+} with the studied drugs reveals the well known bands of Eu^{3+} luminescence based on the $^5D_0 \rightarrow {}^7F_J$ (J=0, 1, 2, 3, 4) and $^5D_1 \rightarrow {}^7F_J$ (J=1, 2, 3, 5, 6) transitions. The most intense transitions are $^5D_0 \rightarrow {}^7F_2$, and $^5D_0 \rightarrow {}^7F_1$, with emissions around 610–660 nm and 585–600 nm.

The effect of TRIS buffer concentration, Eu^{3+} concentration and volume were investigated in order to optimize the luminescence intensity.

Effect of pH, Concentration and Volume of TRIS Buffer

The influence of TRIS buffer concentration on the luminescence intensity of Eu^{3+} was studied using various concentrations, volumes, pHs of the buffer in the range 0.005–0.08 M, 0.1–1.5 ml, and pH 5.56–8.5, respectively. The effect of buffer concentration can be seen in Fig. 3. In the case of etodolac, an isolated and extreme maximum in fluorescence emission was observed at a buffer concentration 0.005 M. At buffer concentrations higher than this, however, the etodolac emission decreased to about 50% of its maximum value and remained fairly constant up to buffer concentrations as high as 0.06 M. In the case of moxepril HCl, a very subtle increase in fluorescence intensity was observed upon increasing the buffer concentration from 0.005 to 0.01 M, but thereafter, the fluorescence intensity decreased gradually (by 20% at a buffer concentration of 0.06 M relative to the highest emission at 0.01 M). In the case of fexofenadine HCl, the fluorescence emission doubled upon increasing the buffer concentration from an initial value of 0.02 M to 0.03 M, but decreased thereafter in a fashion similar to that observed for moxepril HCl.

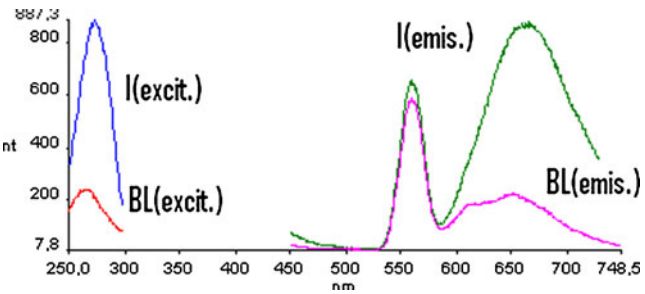

Fig. 1 Excitation and emission spectra of the reaction product of 240 ng/ml etodolac (I) with europium chloride relative to the blank (BL)

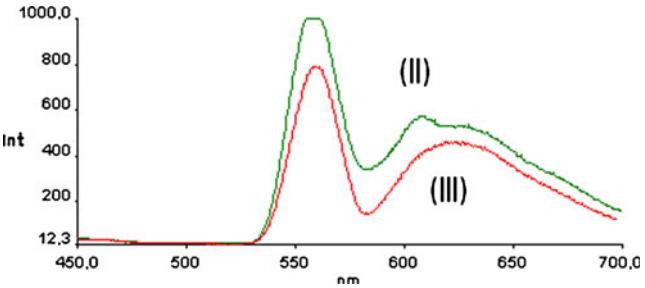

Fig. 2 Emission spectra of the reaction product of 240 ng/ml moxepril HCL (II) and 80 ng/ml Fexofenadine (III) with $8\times10^{4-}$% w/v europium chloride, excitation was at 276 nm for both

On exceeding the optimum buffer concentration, it is possible that TRIS competes with the studied drugs for Eu^{3+} binding sites. This may result in less Eu-chelates being formed with the studied drugs and hence, a decrease in luminescence intensity. Similar behaviour was observed previously by Rieutord et al. [18], where it was reported that TRIS buffer may have chelating properities towards lanthanide ions. Thus, the optimum TRIS concentration for the determination of drug I, II, and III was found to be 0.005 M, 0.01 M, and 0.03 M, respectively.

Increasing the proportion of buffer volume present in the reaction mixtures has the practical effect of increasing buffer ion concentration, and so we would expect that this change would result in similar trends in fluorescence intensity as those observed previously for increasing buffer concentration (in Fig. 3).

The buffer pH was increased from 5.5 to 8 to determine the effect on fluorescence emission. An emission maximum was observed at a different pH for each drug studied, although each of the drugs displayed similar trends in emission as a function of pH; namely, passing through a slight maximum at a given pH, followed by a decrease in

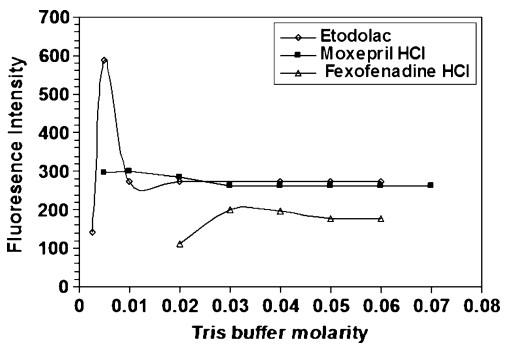

Fig. 3 Effect of TRIS buffer molarity on fluorescence intensity of 200 ng/ml etodolac, 240 ng/ml moxepril HCl and 80 ng/ml fexofenadine HCl

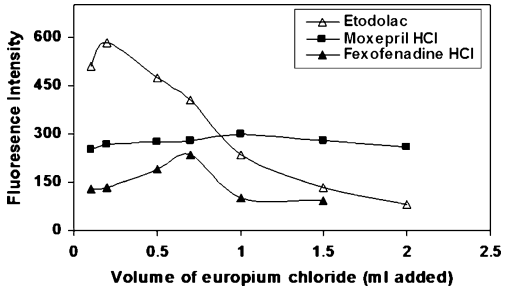

Fig. 4 Effect of europium volume on absorbance of 200 ng/ml etodolac, 240 ng/ml moxepril HCl and 80 ng/ml fexofenadine HCl

the emission at increasingly higher pH values. For etodolac, moxepril HCl and fexofenadine HCl, pH values of 5.5–6.0. 8.0, and 7.5, respectively, were found to be optimal (as illustrated in Table 1).

Effect of Europium (III) Concentration

Since luminescence sensitization is believed to occur via complex formation between Eu^{3+} and the studied drugs, it is necessary to optimize the ratio of the concentration of Eu^{3+} to ligands that will afford maximum complexation and hence maximum intensity of the emission line.

The optimum concentration of Eu^{3+} was determined by measuring the luminescence intensity with an increase in concentration of Eu^{3+} ranging from (8×10^{-4}) to (8×10^{-3})%.

In the case of etodolac, an increase in the fluoresence emission was observed at 0.2 ml of added europium chloride 8×10^{-4}% w/v. At europium concentration higher than this, the fluorescence intensity decreased gradually (by 15% at 2 ml of 8×10^{-4}% w/v europium chloride relative to the highest emission at 0.2 ml). In the case of moxepril HCl, an observable increase in fluorescence intensity was with increasing the volume from 0.1 to 1 ml of added europium 8×10^{-4}% w/v, but decreased thereafter by about 20% at 2 ml relative to the highest emission. For fexofenadine HCl, the fluorescence emission doubled by increasing the volume of europium chloride 8×10^{-4}% from 0.1 to 0.7 ml then decreased by about 40% at 1.5 ml of the same concentration of europium chloride as can be seen in Fig. 4.

It is expected as an excess of Eu^{3+} is required for complex formation. However, large excess above the optimum may result in quenching of the luminescence due to non-radiative collisions where the free ions may act as a quencher of the excited states of the studied drugs. Similar results were previously reported by Arnaud and George [19].

Method Validation

Under the described experimental conditions, relative fluorescence intensities were found to be linear over the concentration range cited in Tables 1 and 2. An increase in the luminescence intensity was observed with an increase in the concentration of drug as illustrated for etodolac in Fig. 5. The linear regression equations are listed in Table 1 with correlation coefficients from 0.9994 to 0.9999, indicating excellent linearity.

Analytical Applications

To examine the applicability of the method, the proposed method was used to determine the concentration of the studied drugs in their pharmaceutical dosage forms. The results of

Table 2 Fluorimetric determination of etodolac, moxepril HCl and fexofenadine HCl using europium chloride

Etodolac			Moxepril HCl			Fexofenadine HCl		
Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery%	Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery%	Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery%
20	20.16	100.81	40	40.03	100.08	30	30.06	100.20
80	79.06	98.82	60	60.91	101.51	40	39.49	98.72
120	118.98	99.15	140	138.44	98.89	50	50.68	101.37
160	159.29	99.56	160	160.31	100.20	60	60.11	100.19
200	201.98	100.99	200	199.08	99.54	70	69.54	99.34
240	240.68	100.28	240	241.82	100.76	80	80.15	100.18
280	279.06	99.66						
Mean		99.9			100.16			99.99
Variance		0.68			0.84			0.81
SD		0.82			0.92			0.90
SE		0.31			0.37			0.37

Average of three experiments

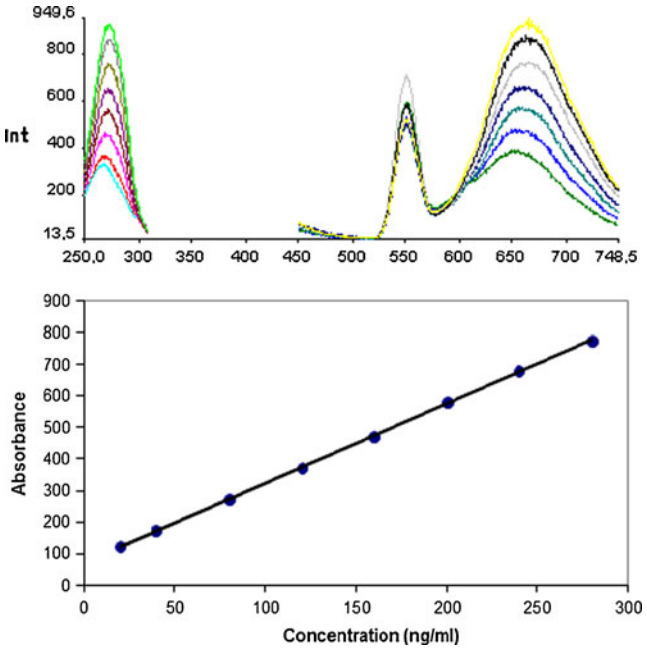

Fig. 5 The influence of increasing etodolac concentration from 20 (lower curve) to 240 ng/ml (upper curve) on the luminescence of etodolac $-Eu^{3+}$-system. $[Eu^{3+}] = (0.32\times10^{-4})\%$; [TRIS] = 0.005 M. λex=276 nm, λem=667 nm

analysis of the commercial dosage forms and the recovery study are shown in Tables 3 and 4. It is clear from these results that excellent recoveries with no interference from the excepients were obtained. The results obtained were compared with the previously presented methods. Statistical comparison of the results was performed with regard to accuracy and precision using student-*t*-test and f-ratio at 95% confidence level and the results of this analysis are shown in

Table 3 Fluorimetric determination of etodolac and moxepril HCl in their pharmaceutical dosage forms using europium chloride

Etodolac tablets			Primox tablets		
Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery %	Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery %
20	19.96	99.82	40	40.03	100.08
40	40.52	101.29	80	79.80	99.74
80	79.45	99.31	100	101.66	101.66
120	119.17	99.31	120	120.55	100.46
160	158.50	99.06	160	159.32	99.57
200	201.79	100.90	180	179.20	99.55
240	239.24	99.68	200	202.06	101.03
280	278.26	99.38	220	220.95	100.43
			240	241.82	100.76
Mean		99.85			100.37
Variance		0.66			0.51
SD		0.81			0.71
SE		0.29			0.24

Average of three experiments

Table 4 Fluorimetric determination of fexofenadine HCl in its pharmaceutical dosage forms using europium chloride

Fastel tablets			Rapido capsules		
Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery %	Actual conc. (ng/ml)	Found conc. (ng/ml)	Recovery %
30	29.76	99.21	30	30.06	100.20
40	40.37	100.93	40	40.37	100.93
50	50.68	101.37	50	49.80	99.60
60	61.00	101.66	60	60.41	100.68
70	70.72	101.03	70	69.83	99.76
80	80.74	100.92	80	79.26	99.08
Mean		100.85			100.04
Variance		0.73			0.48
SD		0.85			0.69
SE		0.35			0.28

Average of three experiments

Table 5. No significant differences were found between the novel fluorimetric method presented herein and previously presented methods [2, 5, 9].

Conclusion

The development of a sensitive and robust procedure has been described for the determination of etodolac, moxepril

Table 5 Spectrofluorimetric determination of etodolac, moxepril HCl and fexofenadine HCl using europium chloride compared with reported methods

Drug		Spectrofluorimetric method	Reported methods
Etodolac	Mean±R.S.D	99.90±0.825	100.48±0.846 [2]
	Variance	0.68	0.72
	Student-t-test	1.19 (1.81)[a]	–
	f-test	1.06 (4.53)[a]	–
	n	7	5
	LOD (μg/ml)	0.02–0.28	10–80
Moxepril HCl	Mean±R.S.D	100.16±0.915	99.86±0.66 [5]
	Variance	0.84	0.44
	Student-t-test	0.76 (1.76)[a]	–
	f-test	1.92 (3.48)[a]	–
	n	6	10
	LOD	0.04–0.24	1–11
Fexofenadine HCl	Mean±R.S.D	99.99±0.9	100±0.85 [9]
	Variance	0.81	0.72
	Student-t-test	0.02 (1.83)[a]	–
	f-test	1.12 (5.19)[a]	–
	n	6	5
	LOD	0.03–0.08	5–16

[a] The figures in parenthesis are the theoretical values for *t*- and *f*-tests ($p<0.05$).

HCl and fexofenadine HCl in pure form based on sensitized europium fluorescence. The enhancement of the europium fluorescence upon complexation with the studied drugs has enabled the assay of these drugs with high sensitivity and selectivity which can be observed from the decrease in limit of detection (LOD) brought about by the use of Eu^{3+} for complexation (0.93, 0.92 and 0.95 μg/ml) for etodolac, moxepril HCl and fexofenadine HCl respectively. The procedure was successfully applied for the determination of the target drugs in capsules and tablets with excellent reproducibility and no interference was observed from excepients commonly found in pharmaceutical preparations.

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