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ORIGINAL PAPER

Study on the Fluorescence Quenching Reaction of Amitriptyline and Clomipramine Hydrochlorides with Eosin Y and its Analytical Application

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Abstract Amitriptyline.HCl (AMI) and clomipramine.HCl (CMI) react with eosin Y (EY) in pH 3.8 NaAc-AcH buffer solution to form ion association complex which results in quenching of fluorescence of EY and appearance of a new resonance Rayleigh scattering (RSS) spectrum at 620 nm. The spectral characteristics of absorption, fluorescence and RSS spectra have been investigated. The factors influencing the reaction were studied and optimum conditions for the reaction have been determined. Based on fluorescence quenching, a simple and sensitive spectrofluorimetric method for determination of AMI and CMI has been developed. The fluorescence quenching intensity was measured at 550 nm using an excitation wavelength of 310 nm. The calibration graph was found to be rectilinear in the range 0.08–2.0 $\mu g mL^{-1}$ with detection limit of 0.017 $\mu g mL^{-1}$ for AMI and 0.06–2.0 $\mu g mL^{-1}$ with detection limit of 0.015 $\mu g mL^{-1}$ for CMI. The method can be satisfactorily applied to the determination of AMI and CMI in tablets without interference from commonly occurring exicipients. The recovery and RSD values obtained indicate good accuracy and precision of the method. The mechanism of the reaction and fluorescence quenching has also been discussed.

Keywords Eosin Y · Ternary complex · Amitriptyline.HCl · Clomipramine.HCl · Fluorescence quenching

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Introduction

Amitriptyline.HCl (AMI) and clomipramine.HCl (CMI) belong to the category of tricyclic antidepressant drugs (TCAs) and are widely used to treat various kinds of depression. Amitriptyline-HCl, (Fig. 1a) 3-(10,11-dihydro-5H-dibenzo [a,d] cycloheptene-5-ylidene)-N,N-dimethyl propan-1-amine hydrochloride, is useful in the treatment of involutional melancholia, anxiety and manic depression [1]. Clomipramine-HCl, (Fig. 1b) 3-(3-chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine hydrochloride, is mainly used for the treatment and relief of obsessive and compulsive disorders as well as in depression and other emotional disturbances. Side effects of AMI and CMI are essentially those of dibenzazepines which include drowsiness, hypothermia, arrhythmic abnormalities, congestive heart failure and death in cases of an overdose. [2]. The majority of the TCAs act primarily by inhibiting serotonin-norepinephrine reuptake in central nervous system which results in an elevation of the synaptic concentrations of these neurotransmitters, and therefore an enhancement of neurotransmission [3]. In spite of new atypical drugs, such as those of the SSRI group (fluoxetine, fluvoxamine, etc.), AMI and CMI are still the reference compounds in the treatment of many psychiatric disorders [4]. However, these are considered to have many undesirable side effects and overdosage may cause adverse reactions.

Due to their therapeutical relevance, several methods have been reported for determination of both the drugs in pharmaceutical preparations and in biological fluids. Various analytical techniques reported for AMI include spectrophotometry [4–9], chemiluminescence [10], fluorescence polarization immunoassay [11], high pressure liquid chromatography (HPLC) [12, 13], gas chromatography-mass

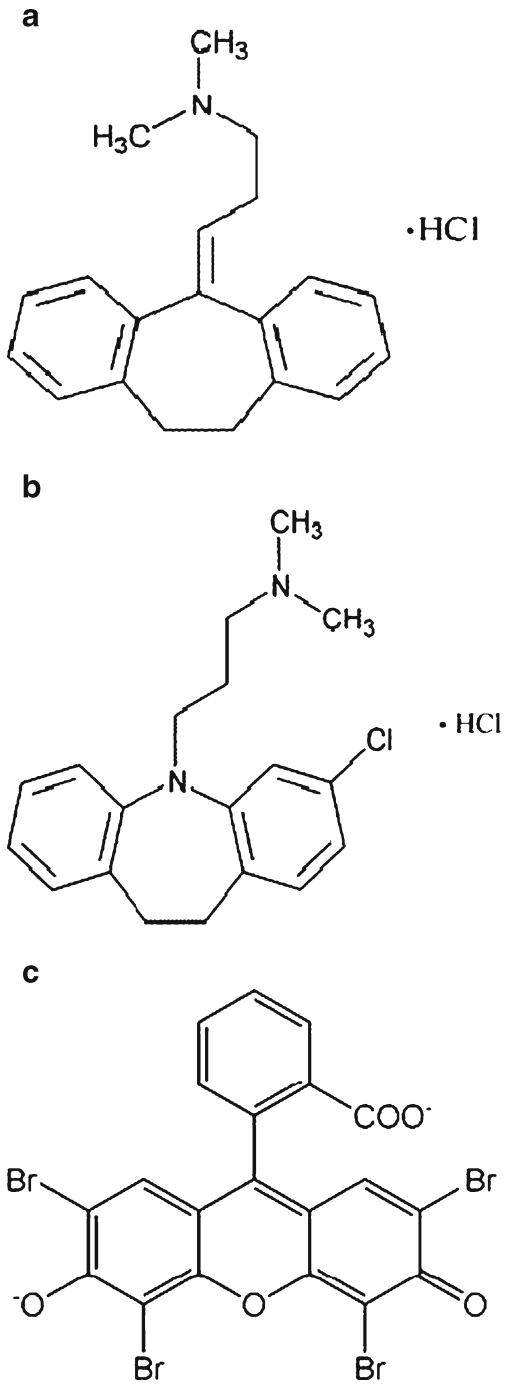


Fig. 1 **a** Structure of Amitriptyline.HCl. **b** Structure of Clomipramine.HCl. **c** Structure of Eosin Y

spectrometry (GC-MS) [14]. For determination of CMI, the reported methods include spectrophotometric [15–20] chemiluminescence [21], HPLC [22–24], GC [25], LC–MS [26], ion selective electrode [27], capillary zone electrophoresis (CZE) [28]. However, the existing methods are associated with some drawbacks. The spectrophotometric methods, though simple and cheap, are not very sensitive. Other methods (such as HPLC and GC-MS methods) involve elaborate sample pretreatment procedures, are complicated in operations and require expensive equipment. Stringent control of conditions is required in chemiluminescence, fluorescence polarization immunoassay methods. Therefore, it is still significant to develop a sensitive, rapid and economical method for determination of AMI and CMI.

Spectrofluorimetric methods have received growing interest due to their simplicity, low cost equipment, high sensitivity and wide working concentration range. Due to these factors, they have been widely used to estimate pharmaceuticals [29, 30]. To the best of our knowledge, no spectrofluorimetric method for the determination of AMI has been reported. For the determination of CMI, so far only three spectrofluorimetric methods have been reported [18, 20, 31]. Since the reported spectrofluorimetric methods for determination of AMI were found to have certain limitations such as use of sulphuric acid, use of organic solvents, time consuming extractive procedures and narrow linear range so there is need for development of new methods. The aim of the present work was to develop a new spectrofluorimetric method for determination of the studied drugs that is more sensitive, rapid and less expensive than the reported methods for the determination of the studied drugs.

In the present work, the interaction between Eosin Y (EY) and selected drugs was studied by fluorescence measurements. It was observed that addition of AMI or CMI to EY leads to fluorescence quenching of the dye. Based on this, a fluorescence quenching method has been developed for the determination of AMI and CMI in pharmaceutical preparations. The reaction conditions were optimized and the method has been validated.

Experimental

Apparatus

A RF-5301 PC spectrofluorophotometer (Shimadzu Corporation, Japan) was used for recording fluorescence spectra and measuring the fluorescence intensity. A UV-1800 Pharmaspec UV-Visible spectrophotometer (Shimadzu Corporation, Japan) was used for recording the absorption spectra. An ATC pH meter model 132-E (Electronics, India) was used for adjusting pH values.

Reagents and Solutions

Amitriptyline-HCl (Sigma Aldrich, Steinheim Germany) standard solution was prepared by dissolving the requisite amount of drug in water. The concentration of stock solution was 100 $\mu g mL^{-1}$. Eosin Y disodium salt (sd fine-chem Ltd, India) standard solution was prepared by dissolving corresponding amount of EY in water. The concentration

of stock solution was 1.44×10^{-3}molL^{-1}. Sodium acetate-acetic acid buffer solutions of desired pH were prepared by mixing 0.1 molL^{-1} acetic acid and 0.1 molL^{-1} sodium acetate solutions according to certain proportion and adjusting the pH values by pH meter. NaCl solution of concentration 1 molL^{-1} was prepared by dissolving the corresponding amount of NaCl in water. The working solutions of desired concentration were prepared by dilution of stock solutions with triply distilled water.

All reagents used were of analytical reagent grade and purchased from Merck India unless stated otherwise. Triply distilled water was used throughout.

Measurement Procedure

In a 10 mL volumetric flask, the following solutions were added in given sequence: a certain aliquot of drug standard solution, 1 mL of NaAc-AcH buffer solution of pH 3.4 and 0.4 mL of 1.44×10^{-3}molL^{-1} EY solution. The resulting solution was diluted to mark with water and mixed thoroughly. The fluorescence measurements were then made at 550 nm using an excitation wavelength of 310 nm. The slit widths for excitation and emission monochromators were fixed at 10 nm. All measurements were performed in 1 cm quartz cell at room temperature. The fluorescence intensity for binding product (F) and reagent blank (F_0) at λex/ λem= 310 nm/ 550 nm was measured and the quenched fluorescence intensity was represented by $\Delta F=F-F_0$.

Pharmaceutical Sample Preparation

AMI and CMI tablets from different manufacturers were bought from the local market. An accurately weighed amount of powder obtained from ten tablets or capsules equivalent to 25 mg of the drug was transferred into 100 mL volumetric flask. About 50 mL distilled water was added to the flask. The mixture was sonicated in an ultrasonic bath for 10 min and then filtered. The filtrate was diluted further with water to a total volume of 100 mL. These solutions were then appropriately diluted with water so that final concentration was in the working range for both the drugs.

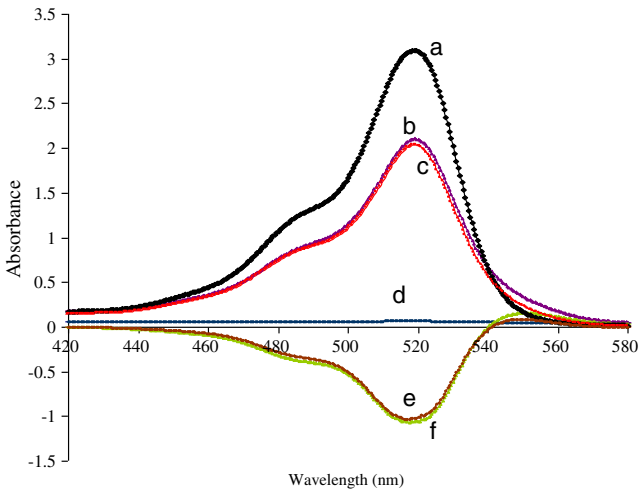


Fig. 2 Absorption spectra of (**a**) EY (**b**) AMI-EY (**c**) CMI-EY (**d**) AMI and CMI (**e**) AMI-EY (against EY blank) (**f**) CMI-EY (against EY blank) system. Conditions: AMI: 2 μgmL^{-1}; CMI: 2 μgmL^{-1}; EY: 5.76×10^{-5} molL^{-1}; acetate buffer: 1×10^{-2}molL^{-1}; pH 3.8; λexc 308 nm

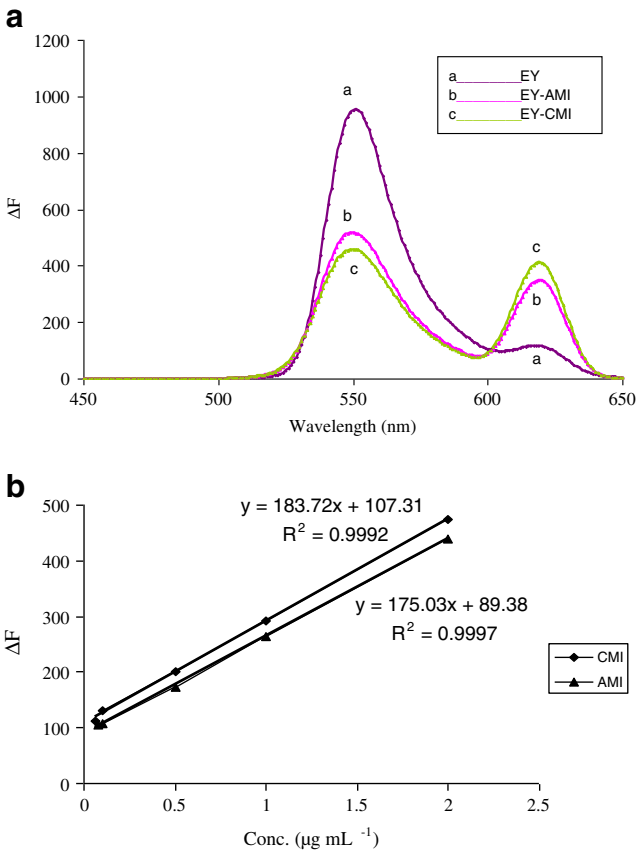


Fig. 3 **a** Fluorescence emission spectra of (**a**) EY (**b**) AMI-EY (**c**) CMI-EY system Conditions: AMI: 2 μgmL^{-1}; CMI: 2 μgmL^{-1}; EY: 5.76×10^{-5}molL^{-1}; acetate buffer: 1×10^{-2}molL^{-1}; pH 3.8; λexc 308 nm. **b** Plots of concentration versus fluorescence quenching

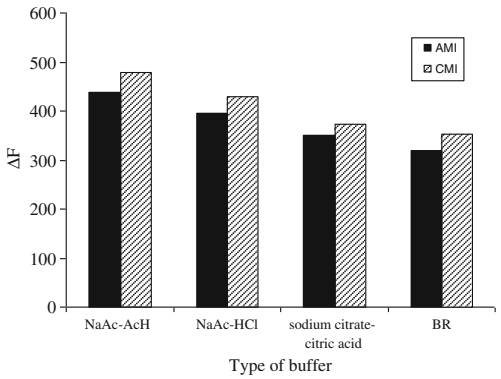


Fig. 4 Effect of different types of buffers on fluorescence quenching value for the studied drugs

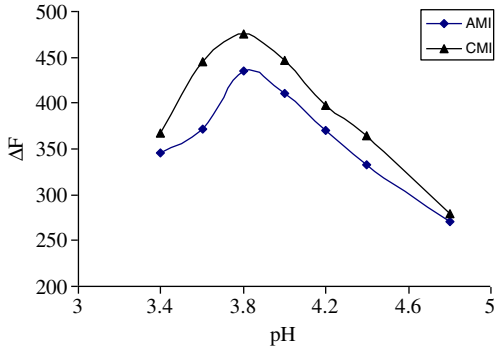


Fig. 5 Effect of pH on fluorescence quenching value for the studied drugs. Conditions: AMI: 2 μgmL^{-1}; CMI: 2 μgmL^{-1}; EY: 5.76×10^{-5} molL^{-1}; acetate buffer: 1×10^{-2}molL^{-1}

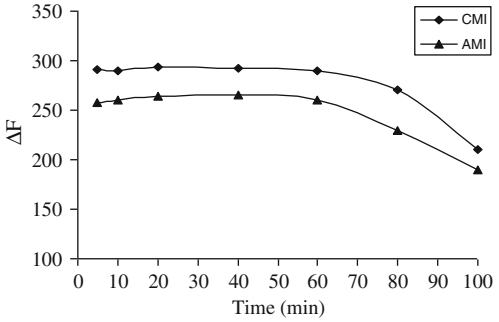


Fig. 7 Effect of time on fluorescence quenching value for the studied drugs. Conditions: AMI: 1 μgmL^{-1}; CMI: 1 μgmL^{-1}; acetate buffer: 1×10^{-2}molL^{-1}; pH 3.8

Results and Discussion

Spectral Characteristics

Absorption Spectra

The absorption spectra of amitriptyline hydrochloride, clomipramine hydrochloride, eosin Y, AMI-EY complex and CMI-EY complex are shown in Fig. 2. As shown in Fig. the maximum absorption wavelength of EY is located at 517 nm while the two chosen drugs have little absorption in 300–700 nm range. Upon reaction with AMI or CMI to form an ion-association complex, the absorption spectrum of eosin Y shows change. The absorbance intensity at about 517 nm drops significantly.

Fluorescence Spectra

The fluorescence spectra of AMI-EY and CMI-EY systems are shown in Fig. 3a. Under the experimental conditions, the selected drugs did not show any fluorescence but EY had a strong fluorescence. The excitation and emission wavelengths selected for EY were 310 nm and 550 nm, respectively. It was observed that addition of drug solution to EY system causes decrease in fluorescence intensity of EY system though fluorescence spectral characteristics of EY were not changed. This fluorescence quenching of EY by drug was found to be directly proportional to drug concentration in a certain range for both the drugs (Fig. 3b). Therefore, it can be applied to the determination of AMI and CMI.

Optimum Reaction Conditions

Effect of pH and Buffers

The effect of pH and various buffers on the fluorescence quenching system of EY-AMI and EY-CMI was investigated in the pH range 2–5. Four buffer solutions including NaAc-AcH, HCl-NaAc, sodium citrate-citric acid and Britton Robinson (BR) buffer were tested for their effect on fluorescence intensity of the system. As shown in Fig. 4, NaAc-AcH buffer solution was found to give best results. The optimum pH value for both the systems was determined to be 3.8 as can be seen from Fig. 5. ΔF value was highest at this pH and as pH value was raised, ΔF decreased sharply.

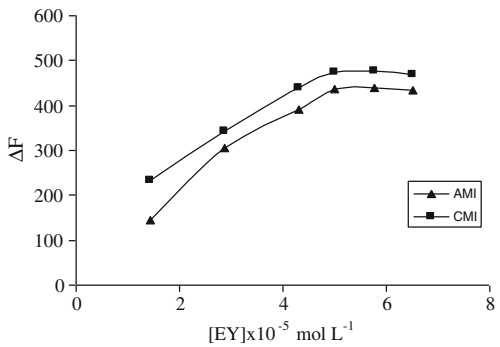


Fig. 6 Effect of EY concentration on fluorescence quenching value for the studied drugs. Conditions: AMI: 2 μgmL^{-1}; CMI: 2 μgmL^{-1}; acetate buffer: 1×10^{-2}molL^{-1}; pH 3.8

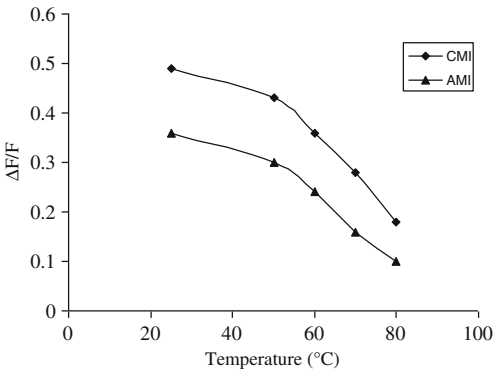
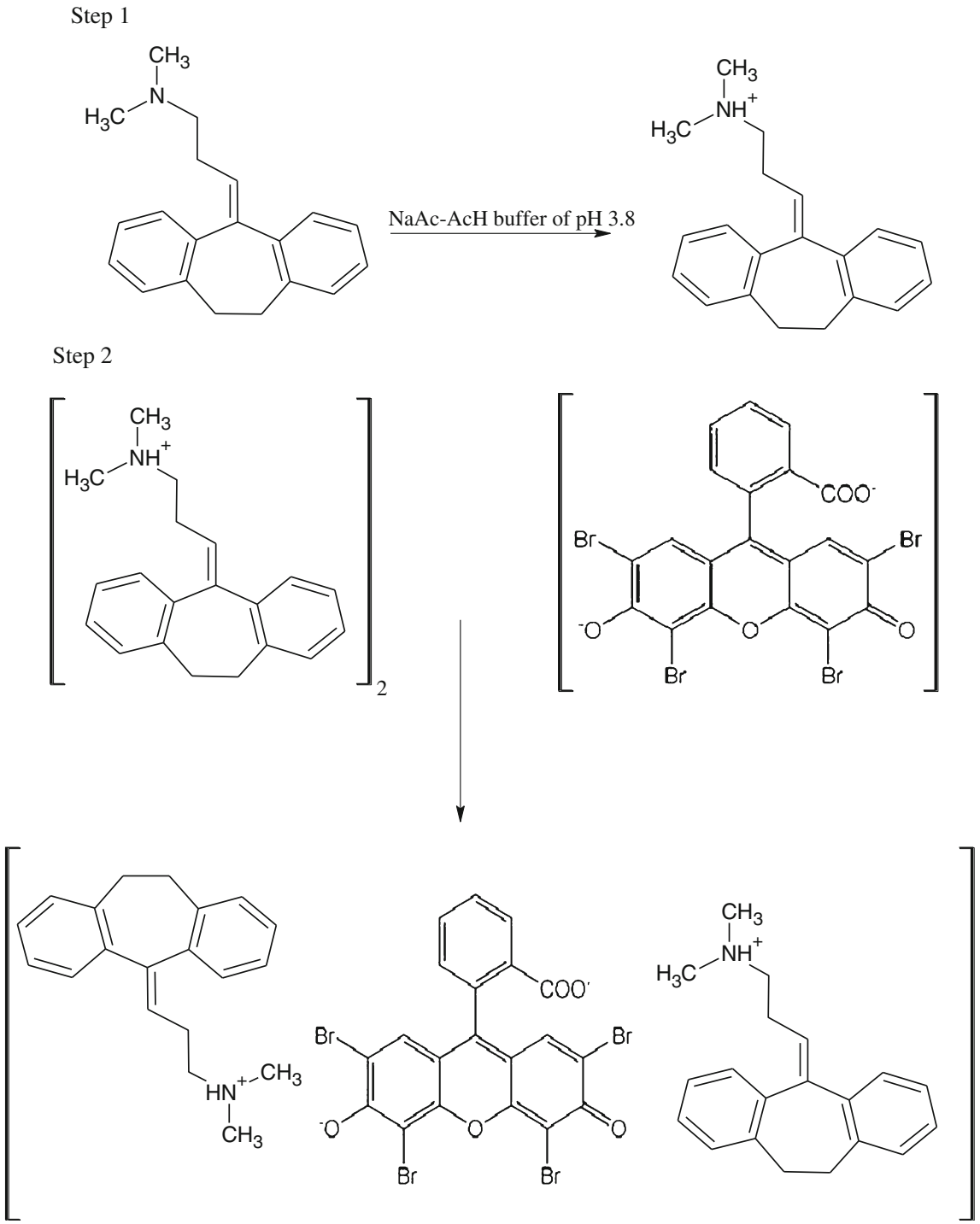


Fig. 8 Effect of temperature on fluorescence quenching value for the studied drugs. Conditions: AMI: 1 μgmL^{-1}; CMI: 1 μgmL^{-1}; acetate buffer: 1×10^{-2}molL^{-1}; pH 3.8



Scheme 1 Reaction pathway of the proposed method

Therefore, NaAc-AcH buffer solution of pH 3.8 was selected for further experiments. In addition, the effect of volume of buffer solution on the fluorescence intensities was also examined and the results showed that too much buffer solution would result in decrease of ΔF value. Thereby, the suitable volume of buffer solution was 1 mL.

Effect of Concentration of Eosin Y

The effect of concentration of EY on ΔF value was examined in the range 1.44–6.5 × 10^{-5} mol L^{-1} of EY. As shown in Fig. 6, it was observed that when the concentration of EY was too low, ΔF was low because reaction was incomplete.

Table 1 Analytical parameters for the studied drugs by proposed method

Drug	Linearity range (μg mL^{-1})	LOD	LOQ	Slope	Intercept	Correlation coefficient
Amitriptyline.HCl	0.08–2.0	0.017	0.056	175.03	89.38	0.9997
Clomipramine.HCl	0.06–2.0	0.015	0.049	183.72	107.31	0.9992

ΔF was found to increase with increase in concentration of EY and reached a maximum value when concentration of EY was 5.0×10^{-5} molL^{-1} and remained constant upto 6.5×10^{-5} molL^{-1}. So for further experiments, 5.76×10^{-5} molL^{-1} concentration of EY was selected.

Reaction Time and Stability of Fluorescence Quenching Value

The effect of reaction time on EY-AMI and EY-CMI systems was studied and stability of both the systems was investigated. It was observed that the complexes got stabilized immediately and ΔF value remained stable for at least 1 h for both the systems (Fig.7). So the fluorescence measurements could be taken immediately after the addition of reagents.

Effect of Addition Order of Reagents

The effect of addition order of the reagents on ΔF value of the system was also investigated. For this, the reagents were added in different sequences and ΔF value was measured. It was observed that there is negligible effect of the addition order of reagents on the fluorescence intensity. The chosen sequence was: drug, buffer, eosin Y.

Effect of Temperature

The effect of temperature on ΔF value was also investigated. For this, the above mentioned procedure was carried out at five different temperatures of 25 (room temperature), 50, 60, 70 and 80 °C. It was observed that high temperature decreases the ΔF value (Fig. 8). Maximum and constant ΔF value was obtained at 25±5 °C. Therefore further experiments were carried out 25 °C.

Effect of Ionic Strength

The effect of ionic strength on ΔF value for both the systems was investigated using NaCl solution. It was observed that when the concentration of NaCl is lower than 0.05 mol•L^{-1}, the effect of ionic strength on ΔF is weak. But, when the concentration of NaCl is higher than 0.05 mol•L^{-1}, ΔF decreases gradually. This shows that a lower concentration (≤0.050 mol•L−1) of NaCl was allowed but the ionic strength in higher concentration affects the interaction of AMI and CMI with EY.

Mechanism of Reaction

The composition ratio of the complex was determined using Job's method of continuous variation and mole ratio methods [32]. The combining molar ratio was determined to be 2:1 for AMI: EY as well as CMI: EY system. Both AMI and CMI are tricyclic antidepressant drugs which are derivatives of dibenzazepine group possessing tertiary amine group in the aliphatic chain (Fig. 1a, b). Literature survey reveals that these drugs are capable of interacting with negatively charged dyes and reagents through this nitrogen. The tertiary nitrogen in these drugs gets protonated easily at acidic pH creating a positive centre. which interacts with negative groups in dyes and reagents through electrostatic interactions. EY (Fig. 1c) is a tetrabromofluorescein disodium salt. It is a weak acid and its pKa_1 and pKa_2 values are 2.6 and 3.6, respectively [33]. In acidic medium of pH 3.8, it loses its two sodium ions in distilled water creating two negative centres in the molecule. Therefore, in a certain acidic condition, nitrogen of the aliphatic chain is easily protonated providing one positive centre. Also 1 mol of eosin Y

Table 2 Effect of foreign species on the determination of 0.5 μgmL^{-1} AMI and CMI

Foreign species	AMI		CMI	
	Concentration (μg mL^{-1})	ΔF(%)	Concentration (μg mL^{-1})	ΔF(%)
1.Sucrose	1200	+2.1	1250	+2.5
1.Glucose	1000	+2.4	1000	+3.8
2. Lactose	2600	−4.8	2700	−4.2
3. Starch	2000	+3.2	2000	−3.5
4. Citric acid	120	−4.5	130	−4.6
5. Sodium benzoate	400	+3.2	430	+3.2
6. Magnesium stearate	250	+4.8	270	+4.0

Table 3 Determination of studied drugs in some commercial tablets using the proposed procedures

Drug	Product	Labelled content (mg)	% Recovery ± R.S.D.
Amitriptyline.HCl	Amline[a](tablets)	10 mg	98.3±1.02
	Tryptomer[b](tablets)	25 mg	99.0±1.25
	Amitor[a] (tablets)	50 mg	101.1±1.40
Clomipramine.HCl	Anafranil[c] (tablets)	10 mg	101.5±1.10
	Clomipramine[d](capsule) 10 mg		99.6±1.45
	Clofranil[e] (tablets)	25 mg	98.7±1.52

[a]Torrent Pharmaceuticals Ltd, India
[b]Wockhardt Ltd. Mumbai, India
[c]Novartis India Ltd
[d]Unicare (India) Pvt. Ltd
[e]Sun Pharma India

provides two negative sites under the selected experimental conditions. So EY^{2-} :AMI^{+} or CMI^{+} will form a 1:2 neutral ion association complex by the electrostatic attraction and the hydrophobic force. EY showed obvious fading at the maximum wavelength of 517 nm. The scheme of the reaction between AMI and EY is shown in Scheme 1.

This complex formation resulted in fluorescence quenching of EY. A variety of processes can result in quenching, such as excited state reactions, energy transfer, complex-formation and collisional quenching. It is associated with the property of the fluorophore, conjugation mode, and local environment [34]. The fluorescence quenching is considered to be static quenching reaction due to change of absorption spectra on formation of the complex and the decrease of ΔF value on increase of temperature.

Resonance Rayleigh Spectrum (RRS Spectrum)

Rayleigh scattering (RS) is an elastic scattering by particles much smaller than the incident wavelength (λ) in which the scattered wavelength is equal to the incident wavelength. The signal level of scattered wavelength is generally low because the intensity of scattered Rayleigh light is proportional to 1/λ4. Resonance Rayleigh scattering (RRS) occurs when the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. This happens when the wavelength of Rayleigh scattering is located at or close to its molecular absorption band. The scattering intensity in this case is very high. It provides new information concerning molecular structure, size, form, charge distribution, state of combination and so on.

The formation of ion association complex between drug and EY results in appearance of a new second order Resonance Rayleigh scattering spectrum at 620 nm. It can be seen from Fig. 3 that when Eosin Y reacts with the drug to form ion association complex, the fluorescence intensity of the emission spectrum at 550 nm is quenched but RRS intensity enhances. This can be explained as follows: The light energy absorbed by the molecule is released mainly in the form of fluorescence emission. However energy loss also occurs through resonance scattering and nonradiation. So quenching of fluorescence is not only due to loss of energy through nonradiative process instead of radiative but also due to transfer of fluorescence energy to scattering. The resonance scattering is mostly ignored in case of small molecules but becomes important in case of large molecules like ion association complexes and could not be ignored. So a part of radiative energy is transferred to scattering through resonance effect to produce a resonance light scattering. Due to this, as the fluorescence emission intensity decreases, RSS intensity increases. This synchronous change can be seen from Fig. 3. So it can be considered that enhancement of RSS is the result of energy transfer from light emission to the scattering.

Table 4 Recoveries of studied drugs added to their commercial dosage forms

Sample	Amount added (μg mL^{-1})	Amount found[a] (μg mL^{-1})	Recovery[a] (%) ± RSD
Amline (tablet)	0.10	0.102	97.5±1.10
	0.50	0.485	102.0±0.90
	1.00	1.03	103.0±1.00
Anafranil (tablet)	0.10	0.101	101.0±1.12
	0.50	0.048	99.0±1.20
	1.00	0.97	97.0±1.42

[a] average value of five determinations ± RSD

Calibration Graph

Under the optimum experimental conditions, the relationship between decrease in fluorescence intensity (ΔF) and concentration for both the drugs was studied. The intercepts, slopes, correlation coefficients, limits of detection (LOD) and limits of quantitation (LOQ) for both the studied drugs are summarized in Table 1. LOD and LOQ values were calculated as: LOD=3S/N; LOQ=10S/N. Results in Table 1 show that the studied drugs, AMI and CMI, can be detected at concentrations as low as 17 ngmL^{-1} and 15 ngmL^{-1}, respectively with the present method. The values of slopes of the calibration curves for both the drugs indicate high sensitivity of the proposed procedures.

Selectivity of the Method

The effects of some common excipients used in pharmaceutical preparations were studied by analyzing sample solutions containing a fixed amount of AMI (0.5 $\mu g mL^{-1}$) or CMI (0.5 $\mu g mL^{-1}$) with various amounts of each excipient. The studied excipients were sucrose, glucose, lactose, starch, citric acid, sodium benzoate, and magnesium stearate. The maximum permissible concentrations of foreign species causing a ±5 % relative error in ΔF value are shown in Table 2. It was found that none of the studied excipients were fluorescent in nature and did not interfere in the determination of drugs at concentrations commonly found in pharmaceutical formulations. So, this method can be used for the determination of AMI and CMI in pharmaceuticals.

Application to Pharmaceutical Preparations

Some commercial dosage forms of the studied drugs were successfully analyzed by the proposed method. The results are shown in Table 3. The results indicated that there were no significant differences between labelled contents and those obtained by the proposed method.

Recovery experiments were carried out for the studied drugs in their respective pharmaceutical formulations. For this, three different concentrations of standard solutions of studied drugs were added and analysed in five replicates. The recovery experiments were performed through standard addition technique to reduce the interference from pharmaceutical additives and excipients. The results of the analysis are presented in Table 4. The obtained recovery and RSD values indicate good accuracy and precision of the proposed method.

The results in Table 4 indicate that there is no interference from frequently encountered excipients or additives. So the proposed method is suitable for analysis of the studied drugs in their dosage forms and application in quality control laboratories.

The performance of the present method was compared with other existing methods (Table 5). It can be seen from the table that the linear range of the proposed method is appreciable and detection limit is quite low making the method more sensitive compared to the other reported methods. RSD values, though slightly higher than some of the reported methods are still acceptable and comparable to other spectrofluorimetric methods. It can be seen from the table, that majority of the reported methods involve elaborate extractive procedures, costly and polluting organic solvents, toxic metals etc thus limiting their use as analytical methods of choice. Moreover for AMI, this is the first

Table 5 Comparison of the proposed method with other existing methods for determination of AMI and CMI

Drug	Reagent	Linear range ($\mu g\ mL^{-1}$)	LOD ($\mu g\ mL^{-1}$)	RSD	Ref
AMI	SPECTROPHOTOMETRY				
	Mercuric thiocyanate and Fe (III)	0–60	_____	_____	5
	Methyl orange	1–25	_____	0.127–0.865	6
	Bromocresol green	1–25		0.103–0.588	
	7,7,8,8-tetracyanoquinodimethane	10–300	_____	0.212–0.915	7
	Mo and thiocyanate	1–30	_____	0.095–0.485	8
	Ammonium molybdate	1–140	1	0.35 %	9
	SPECTROFLUORIMETRY				
	Eosin Y	0.08–2	0.017	1.02–1.40	Present method
	CHEMILUMINESCENCE				
	Ru bipyridine	_____	0.094	_____	10
CMI	SPECTROPHOTOMETRY				
	Thymol blue	4–26	_____	1.20–2.80	15
	Picryl chloride	0.4–2.4	_____	_____	16
	p-phenylenediamine dihydrochloride in sulfuric acid	0.1–4.0	_____	1.92	17
	SPECTROFLUORIMETRY				
	Ce(IV) and sulfuric acid	0.05–1.1	0.04	1.16–1.48	18
	Potassium dichromate	0.1–3.0	_____	_____	19
	Alizarin red S	1.0–20.0	0.32	0.24	20
	Eosin Y	0.06–2	0.015	1.12–1.42	Present method
	FLOW INJECTION ANALYSIS				
	9,10 dimethoxyanthracene-2-sulfonate (DMAS)	0.25–3.0	<0.30	_____	21

spectrofluorimetric method developed and no other method using this technique has been reported to the best of our knowledge.

Conclusions

Eosin Y can react with AMI and CMI in NaAc-AcH buffer solution of pH 3.8 to form ion association complex which results in quenching of fluorescence of EY. They interact by an electrostatic mechanism resulting in the formation of a 1:2 complex between EY and the selected drugs. The interaction also leads to appearance of a new RSS spectrum. Based on fluorescence quenching, a new spectrofluorimetric method for the determination of AMI and CMI has been proposed. The decrease in fluorescence intensity was found to be proportional to the concentration of AMI in the range of 0.08–2.0 $\mu g mL^{-1}$ and concentration of CMI in the range of 0.06–2.0 $\mu g mL^{-1}$. The proposed method is simple, rapid and inexpensive and can be considered as a good alternative to high cost HPLC methods. The method can be considered non-polluting and does not involve complex extractive procedures. Besides being the first reported spectrofluorimetric method for determination of AMI, the method is simple and sensitive as compared to existing spectrofluorimetric methods for determination of CMI. The method may be satisfactorily applied to the determination of AMI and CMI in tablets without interference from common excipients which make the method suitable for routine quality control particularly in laboratories where expensive equipments like HPLC and LC-MS are not available.

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References

1. Gilman AG (1985) The Phamacological basis of therapeutics, 7th edn. The McMillan Co, New York, p 450
2. Reynolds JEF (1982) Martindale: The extra pharmacopeia, 26th edn. The Pharmaceutical Press, London, p110
3. Tatsumi M, Groshan K, Blakely RD, Richelson E (1997) Pharmacological profile of antidepressants and elated compounds at human monoamine transporters. Eur J Pharmacol 340:249–258
4. Greist JH, Jefferson JW, Kobak KA, Katzeinick DJ, Serlin RC (1995) Efficacy and tolerability of serotonin transport inhibitors in obsessive compulsive disorder: a meta analysis. Arch Gen Psychiatry 52:53–60
5. Venkatesan P, Subrahmanyam PVRS, Pratap DR (2010) Spectrophotometric determination of pure amitriptyline hydrochloride through ligand exchange on mercuric ion. Int J ChemTech Research 2:54–56
6. Nour El-Dien FA, Mohamed GG, Mohamed NA (2006) Spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochlorides through ion-pair formation using methyl orange and bromocresol green reagents. Spectrochim Acta A Mol Biomol Spectrosc 65:20–26
7. Mohamed GG, El-Dien FA, Mohamed NA (2007) Utility of 7,7,8,8-tetracyanoquinodimethane charge transfer reagent for the spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochlorides. Spectrochim Acta A Mol Biomol Spectrosc 68:1244–1249
8. Mohamed GG, El-Dien FA, Mohamed NA (2006) Spectrophotometric determination of trazodone, amineptine, and amitriptyline hydrochlorides through ion pair formation with molybdenum and thiocyanate. Spectrochim Acta A Mol Biomol Spectrosc 65:1221–1226
9. Aman T, Kazi AA, Hussain MI, Firdous S, Khan IU (2000) Spectrophotometric dtermination of Amitriptyline HCl in pure and pharmaceutical preparations. Anal Lett 33:2477–2490
10. Dolman SJL, Greenway GM (1996) Determination of amitriptyline using electrogenerated chemiluminescence. Anal Commun 33:139–141
11. Rao ML, Staberock U, Baumann P, Hiemke C, Deister A, Cuendet C, Amey M, Hartter S, Kraemer M (1994) Monitoring tricyclic antidepressant concentrations in serum by fluorescence polarization immunoassay compared with gas chromatography and HPLC. Clin Chem 40:929–933
12. Linden R, Antunes MV, Ziulkoski AL, Wingert M, Tonello P, Tzvetkov M, Souto AA (2008) Determination of amitriptyline and its main metabolites in human plasma samples using HPLC-DAD: application to the determination of metabolic ratios after single oral dose of amitriptyline. J Braz Chem Soc 19:35–41
13. Ferguson GK (1998) Quantitative HPLC analysis of a psychotherapeutic medication: simultaneous determination of amitriptyline hydrochloride and perphenazine. J Chem Educ 75:1615–1617
14. Martinez Ruiz D, Menendez Gallego M (1988) Determination of amitriptyline and cocaine by GC and GC/MS in biological fluids. Vet Hum Toxicol 30:413–416
15. Nagaraja P, Silwadi MF, Syed AA (2002) Extractive spectrophotometric determination of certain dibenzazepine antidepressants in pharmaceutical preparations. Acta Pharm 52:289–297
16. Hussein SA, Mohamed AMI, Hoda HY (1989) Spectrophotometric determination of some dibenzazepine drugs with picryl chloride. Talanta 36:1147–1149
17. Nagaraja P, Silwadi MF, Syed AA (2000) Sensitive spectrophotometric determination of some dibenzazepine drugs with daizotized p-phenylenediamine dihydrochloride. Anal Lett 33:2913–2926
18. Mohamed FA, Mohamed HA, Hussein SA, Ahmed SA (2005) A validated spectrofluorimetric method for determination of some psychoactive drugs. J Pharm Biomed Anal 39:139–146
19. Mohamed A, Refaat IH (1999) Spectrofluorimetric determination of some dibenzazepine drugs. Bull Pharm Sci 22:91–196
20. Rahman N, Afaq N (2010) Optimization and validation of spectrofluorimetric method for the determination of clomipramine hydrochloride *via* ion-pair complexation with alizarin red S. Anal Methods 2:513–518
21. Valenzuela MA, Diaz TG, Diez NM, Rodriguez AS (2005) Response surface methodology for the optimisation of flow-injection analysis with insitu solvent extraction and fluorimetric assay of tricyclic antidepressants. Talanta 66:952–960
22. Berzas JJ, Guiberteau C, Contento AM, Rodriguez V (2002) Sensitive and rapid high-performance liquid chromatographic method for simultaneous determination of antidepressants in pharmaceutical formulations. Chromatographia 56:545–551
23. Yoshida H, Hidaka K, Ishida J, Yoshikuni K, Nohta H, Yamaguchi M (2000) Highly selective and sensitive determination of tricyclic antidepressants in human plasma using high-performance liquid chromatography with post-column (2,2′-bipyridyl) ruthenium (III) chemiluminescence detection. Anal Chim Acta 413:137–145

24. Xiong CM, Ruan JL, Cai YL, Tang Y (2009) Extraction and determination of some psychotropic drugs in urine samples using dispersive liquid-liquid microextraction followed by high-performance liquid chromatography. J Pharm Biomed Anal 49:572–578
25. Cholobowska Z, Chudzikiewicz E, Koscielniak P, Pickoszewski W, Stolarz A (2003) Application of gas chromatography with NPD detection to the analysis of tricyclic antidepressants in blood. Chem Anal 48:255–263
26. Kłys M, Scislowski M, Rojek S, Kolodziej J (2005) A fatal clomipramine intoxication case of a chronic alcoholic patient: application of postmortem hair analysis method of clomipramine and ethyl glucuronide using LC/APCI/MS. Legal Med 7:319–325
27. Ortuno JA, Hernandez J, Sanchez Pedreno C (2006) Ion selective electrode for the determination of some multidrug resistance reversers. Sens Actuators B 119:282–287
28. Berzas Nevado JJ, Villasenor Llerena MJ, Contento Salcedo AM, Aguas Nuevo E. Assay validation for three antidepressants in pharmaceutical formulations: practical approach using capillary gas chromatography. J Pharm Biomed Anal 38:52–59
29. Tabrizi AB (2007) A simple spectrofluorimetric method for determination of piroxicam and propranolol in pharmaceutical preparations. J Food and Drug Analysis 15:242–248
30. Berzas JJ, Alanon A, Lazaro JA (2002) Cyclodextrin enhanced spectrofluorimetric determination of fluoxetine in pharmaceuticals and biological fluids. Talanta 58:301–309
31. Tong C, Zhuo X, Liu W, Wu J (2010) Synchronous fluorescence measurement of enrofloxacin in the pharmaceutical formulation and its residue in milks based on the yttrium (III)-perturbed luminescence. Talanta 82:1858–1863
32. Job P (1928) Ann Chim 9:113–203
33. Zhou GD (2003) Dictionary of chemistry. Chemical Industry Press, Beijing, p 654
34. Zhu Z, Yang RH, You MX, Zhang XL, Wu YR, Hong W (2010) Single-walled carbon nanotube as an effective quencher. Anal Bioanal Chem 396:73–83