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Validation of Rapid and Sensitive Spectrofluorimetric Assay for Determination of Four Triptans in Pure and Dosage Forms; Application to Human Plasma and Content Uniformity Testing

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Abstract

A sensitive, simple, rapid and reliable spectrofluorimetric assay was developed for the assay of definite anti-migraine drugs namely; Almotriptan malate, Rizatriptan benzoate, Sumatriptan succinate and Zolmitriptan in their pharmaceutical preparations and biological fluid. The suggested procedure was established on determination of the quenching process resulting from the action of the studied drugs on the native fluorescence of Eosin Y via developing of a binary complex reaction between the cited antimigraine preparations and Eosin Y in 0.2 M acetate buffer (pH.3.5). Under the optimized experimental conditions, the relative fluorescence capacity was determined at λ_{ex}=301.3 nm and λ_{em}=542.8 nm. The calibration graphs were linear through extent from 0.07-1.0, 0.20-1.0, 0.2-1.0 and 0.1-1.0 µg/mL, for Almotriptan malate, Rizatriptan benzoate, Sumatriptan succinate and Zolmitriptan, respectively. The detection limits were 0.019, 0.041, 0.055 and 0.032 µg/mL while quantitation limits were 0.059, 0.125, 0.168 and 0.096 µg/mL for Almotriptan malate, Rizatriptan benzoate, Sumatriptan succinate and Zolmitriptan, respectively. The suggested assay has been validated according to ICH and USP guidelines and favorably has been applied to assay of cited drugs in their dosage forms and content uniformity testing. The high sensitivity of the developed assay allowed quantification of the studied antimigraine drugs in human plasma.

Keywords: Almotriptan; Rizatriptan; Sumatriptan; Zolmitriptan; Dosage forms; Human plasma; Eosin Y; Spectrofluorimetry

Introduction

A migraine headache has attacks that may persist from 4 to 72 h. A headache continuing for more than 72 h is called a status migrainous. Migraine trouble is a one-sided pulsating ache that is elevated from motion and has the ability to change the individual performance. Serotonin (5-HT_1) agonists as triptans were found to be useful in relieving ache and symptoms of the migraine attack. According to the FDA clinical reviews, triptans are effective in prevention of moderate to severe migraine. However, they are not used in peoples who suffering from cardiovascular complications [1].

Almotriptan malate (ALT), chemically known as 1- [({3-[2(dimethyl amino) ethyl] indol-5-yl} methyl) sulfonyl] pyrrolidine malate, Rizatriptan benzoate (RZT), is known N, N-dimethyl -5- (1H-1, 2, 4-triazol-1-yl methyl)-1H- indole-3- ethanamine mono benzoate, Sumatriptan succinate (SMT), chemically designated as 3-[2-(dimethylamino) ethyl]-N-methyl-indole-5-methane sulfonamide succinate and Zolmitriptan (ZLT), chemically (4S)-4-[[3-[2-(dimethylamino) ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone, (Figure 1) are selective serotonin agonists (5-HT_1) which are usually effective for the prevention and treatment of migraine complications [1,2]. Different analytical techniques were reported for their determination in different matrices including spectrophotometry [3-8], spectrofluorimetry [9-11], conductometry [12] and HPLC [13-20].

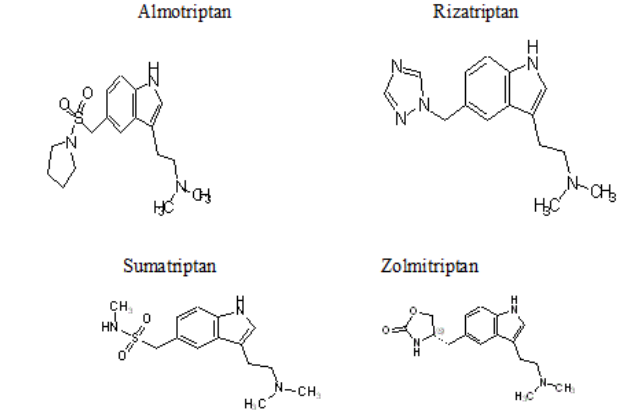

Figure 1: Chemical structures of the studied anti-migraine drugs.

The complex formation that occurs between a lot of drugs and Eosin Y as an ion pairing agent has been considerably investigated using spectrophotometric and spectrofluorimetric assay of such drugs with or without using of metal [21-27].

In this research, a suggested method was developed through the formation of binary complexes between Eosin Y and antimigraine drugs in an attempt to create a rapid, reliable, sensitive, simple, applicable and extraction- free spectrofluorimetric process for assay of

the antimigraine drugs in their dosage forms and human plasma. Eosin Y is cheap and its solution remains unchanged for at least two weeks. The suggested assay depends on measuring the change in fluorescence intensity of Eosin Y when adding studied drugs at 542.8 nm after excitation at 301.3 nm.

Experimental

Apparatus

The fluorescence spectra and measurements of cited drugs have been determined using a fluorescence spectrometer FS-2 (Scinco, Korea), connected to Dell PC, equipped with 1 cm quartz cell, Xenon arc lamp, grating excitation and emission monochromators with slit widths set at 5 nm, PMT voltage 400 V. A Shimadzu UV-1601 PC UV–visible spectrophotometer (Tokyo, Japan) with 1 cm quartz cell, Laboratory centrifuge speed of (18,659 g-forces (Bremsen ECCO, Germany) and Jenway pH meter model 350 (E.U).

Materials and reagents

All reagent used have been of analytical grade. Double distilled water (obtained from all glass devices) has been used.

Pharmaceutical compounds: Almotriptan malate (ALT, 99.5 %), Rizatriptan benzoate (RZT, 99.9%), Sumatriptan succinate (SMT, 99.1 %) and Zolmitriptan (ZLT, 99.4%) were kindly supplied by European Egyptian for Pharmaceutical & Chemical Industries company, Alexandria, Delta Pharm for pharmaceutical & chemical industries company, Cairo, Sigma for Pharmaceutical & Chemical Industries company, Cairo and Global Nabi for Pharmaceutical & Chemical Industries company, Cairo, Egypt, respectively.

Pharmaceutical formulations: The following dosage forms available in Egyptian market were analysed:

Almotrip forte® tablets labeled to contain 17.5 mg Almotriptan malate/tablet that equal to 12.5 mg almotriptan base (batch # 3083001A), the product of European Egyptian Pharmaceutical company, Alex, Egypt. Migriza® tablet labeled to contain 10 mg Rizatriptan benzoate/tablet (batch # B.N. 21197) product of delta pharm for pharmaceutical industries company, Cairo, Egypt. IMIGRAN® tablet labeled to contain 50 mg Sumatriptan succinate/ tablet Product of GlaxoSmithKline NZ Ltd. for pharmaceutical industries company, Cairo, Egypt. Amigrawest® tablet that contains 2.5 mg zolmitriptan/tablet (batch #15295), from Western Pharmaceutical company, El Obour city, Cairo, Egypt.

Chemicals and reagents: Eosin Y (Riedel-De-Haen AG-D-3016 Seeize 1) used as 0.72×10^{-5} M prepared in distilled water. The solution was freshly prepared in double distilled water then diluted to final concentration with distilled water as appropriate.

Acetic acid, acetone, acetonitrile, disodium hydrogen phosphate, ethanol, citric acid, hydrochloric acid, methanol, 85% orthophosphoric acid, sodium acetate, and sodium hydroxide were purchased from El Nasr chemical co., Abu Zaabal, Cairo, Egypt.

Teorell and Stenhagen buffer solutions of pH range 2.5-6.0 were prepared using double distilled water. The buffer is composed of citric acid, orthophosphoric acid, sodium hydroxide and adjusted to the required pH with 0.1 M hydrochloric acid [28].

Acetate buffer 0.2 M were prepared by mixing appropriate volumes of 0.2 M Acetic acid with 0.2 M Sodium acetate solutions to attain the suitable pH (2.5-6).

Mcliavain buffers (pH ranging from 2.5-6) were prepared by good mixing of certain volumes of 0.2 M Disodium hydrogen phosphate with 0.2 M citric acid [28].

Pooled blank plasma was obtained from Assiut University Hospital, Assiut, Egypt.

Standard drug solution: An accurate weight equal to 10.0 mg of each studied drug was carefully and separately transferred into 100 mL volumetric flask. The powder was dissolved in double distilled water and diluted to the mark with the distilled water to obtain a stock solution of 100.0 µg/mL for each drug. Further dilutions were made with distilled water to obtain working standard solutions in the range of calibration curves.

General analytical procedure: An accurately measured volume of cited drugs working standard solutions were added into a series of 10 mL volumetric flasks so that the final concentrations were in the range of (0.07-1.0 µg/ mL) for ALT, (0.2-1.0 µg/mL) for RZT, (0.2-1.0 µg/ mL) for SMT and (0.1-1.0 µg/ mL) for ZLT. To each flask, 2.0 mL 0.72×10^{-5} M Eosin Y was added and the solutions have been mixed very well before adding 1.5 mL 0.2 M acetate buffer (pH 3.5) to each flask. Finally, each flask was completed to the required volume with double distilled water and the relative fluorescence intensity of the solutions was determined at 542.8 nm after excitation at 301.3 nm against a solution blank. The relative fluorescence intensity (ΔF) has been plotted against the final concentration of cited drugs (µg/mL), calibration curves were constructed and the regression equations were derived using GraphPad InStat version 3.05.

Application of the suggested assay

Application to tablets dosage forms: Twenty tablets of each pharmaceutical formulation containing ALT, RZT, SMT or ZLT were weighed, grounded in a mortar and mixed very well. An accurate weight of the grounded tablets equal to 10.0 mg of each studied drug was added into a 100 mL volumetric flask, about 80 mL of double distilled water was added and the flasks were sonicated for 20 min, and completed to the mark with double distilled water and then filtered. The first portion of filtrate was discarded. Aliquots of these solutions were added into a series of 10 mL volumetric flasks to get sample solutions (0.07-1.0 µg/ mL) for AMT, (0.2-1.0 µg/mL) for RZT, (0.2-1.0 µg/ mL) for SMT and (0.1-1.0 µg/ mL) for ZLT and then general analytical procedure was followed.

Application to spiked human plasma: A sample of 5.0 mL of the drug-free human blood sample was obtained from healthy volunteers and transferred into a tube containing heparin and then centrifuged at 18,659 g-forces for 20 min. Into 10 mL stoppered, well-sealed and calibrated tube, 1.0 mL of the drug- free plasma was spiked by 1.0 mL of either studied anti-migraine drug and then the flasks were diluted to 10.0 mL with methanol and the obtained solutions were centrifuged at 18,659 g-forces for 20 min. A determined amount of the obtained supernatant was appropriately diluted with double distilled water to be at concentration within the concentration range of all antimigraine studied drugs, and then the general analytical procedure was performed as described previously. Blank experiment was conducted by subjecting the antimigraine-free blood sample to the same procedure. The relative fluorescence intensity was measured and the

percentage recoveries were calculated by utilizing the respective regression equations.

Results and Discussion

Eosin Y (a tetrabromofluorescein derivative) is a yellowish red dye with green fluorescence. It possesses a single carboxyl group and it is classified as an acidic dye. It is reported that Eosin Y fluorescence is quenched by the development of stable complex with cationic drugs under acidic conditions [29]. Eosin Y has been widely used to develop accurate and precise analytical methods for determination of several compounds of pharmaceutical importance through spectrofluorimetric and/or spectrophotometric measurement [23,27,30-35].

The aim of the present work was to study the resultant quenching effect caused by ALT, RZT, SMT and ZLT on the native fluorescence of Eosin through the formation of binary complexes in an attempt to develop and validate rapid, reliable, sensitive and simple method for their determination in pharmaceutical preparations and biological fluids.

Fluorescence spectrum: The fluorescence of Eosin was found to be quenched through the formation of a stable non-fluorescent ion pair complex with ALT, RZT, SMT and ZLT at pH 3.5, Figure 2. The formation of the complex may be caused by the electrostatic attraction between the anionic functional group of Eosin Y and cationic quaternary amine group of the cited antimigraine drugs in acidic pH [25].

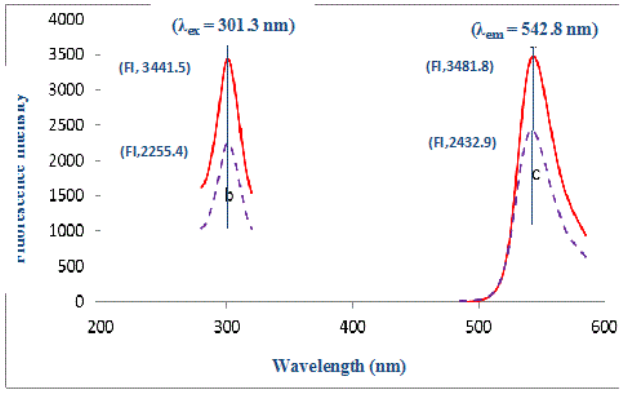

Figure 2: (b, c) Fluorescence spectra of the reaction product of ALM (1.0 µg/mL) as an example of studied drugs with 0.72 × 10^{-5} M) Eosin at pH 3.5, (a, a`), Fluorescence spectra of Blank Eosin (0.72 × 10^{-5} M) at pH 3.5, where: (a, b) are the excitation spectra and (a, c′) are the emission spectra.

Optimization of reaction variables: The various reaction variables that affect the development and stability of binary complexes formed between Eosin Y and studied drugs have been faithfully studied and optimized. One reaction parameter has been changed individually while the other parameters kept constant. These factors include the influence of pH, different kinds of buffer solutions, the volume of buffer, the different volumes of Eosin Y solutions, diluting solvent and the reaction time.

The influence of pH: The influence of pH on the formation of Eosin Y- drug complex has been studied as it has important effect on the ionization of Eosin Y. The effect of pH of acetate buffer [28] on the quenching of the fluorescence capacity of Eosin has been monitored through pH range 2.5-6.0. It was found that the pH values that resulted in highest ΔF were from pH 3.3 to 3.7, and then a decrease in the relative fluorescence intensity has occurred. Other buffers having the same pH values such as Torell and Stenhagen buffer and Mcliavain buffer [28] were studied. Acetate buffer gave maximum fluorescence intensity difference than Torell and Stenhagen buffer and Mcllvaine buffer, so acetate buffer was used at pH (3.5) as optimum pH through the reaction (Figure 3).

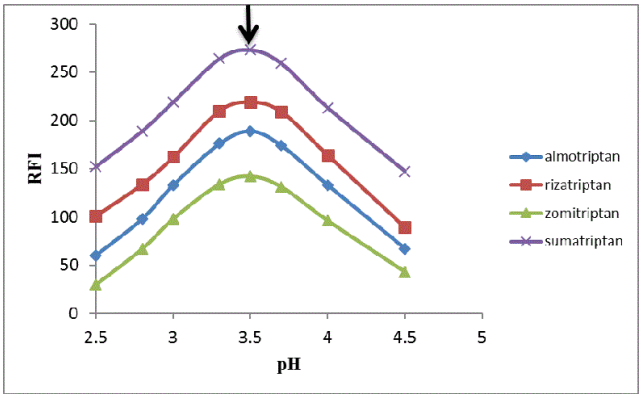

Figure 3: pH effect of 0.2 M acetate buffer on the reaction of the studied drugs (1µg/mL) with Eosin (0.72 × 10^{-5} M).

Effect of buffer volume: Different volumes (ranging from 0.5:3.0 mL) of 0.2 M Acetate buffer system (pH 3.5) were used for the general assay procedure; it was found that the maximum RFI were obtained when the buffer volumes were 1.0-2.0 mL for all studied drugs, (Figure 4). Lower or higher volumes showed a marked decrease in the RFI.

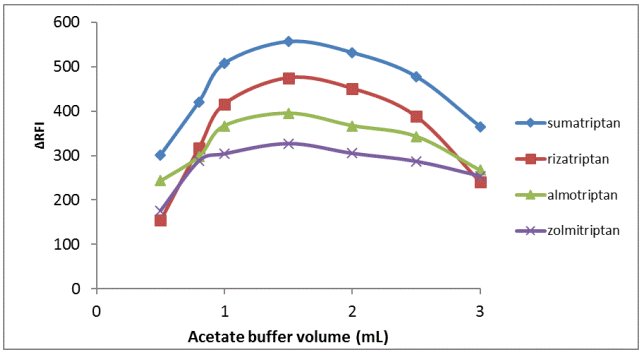

Figure 4: Acetate buffer volume effect (pH 3.5) on the reaction between the studied drugs (1 µg/ mL) and Eosin (0.72 × 10^{-5} M).

Effect of Eosin Y concentration: The effect of the volume of Eosin Y reagent was studied using various quantities of (0.72×10^{-5} M) of the reagent. It was found that increasing volume of the reagent up to 1.5 mL produced a proportional increase in ΔF. However, no further increase in ΔF was observed upon increasing the volume of the reagent

up to 2.5 mL. Therefore, 2 mL of 0.72×10^{-5} M Eosin solution was chosen as the optimal volume of the reagent, (Figure 5).

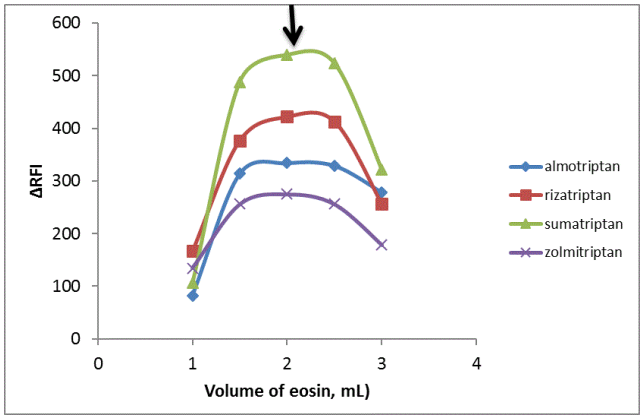

Figure 5: Effect of Eosin Y volume (0.72×10^{-5} M) on the reaction between the studied drugs (1 µg/ mL) and Eosin Y.

The influence of different diluting solvents: For the selection of the most suitable solvent for the procedure, various diluting solvents have been examined; water, methanol, ethanol, acetonitrile, and acetone. Water was found to be an ideal diluting solvent as it afforded maximum sensitivity, and therefore, it was selected for further investigations.

Reaction time and stability of the reaction products: The influence of timing on the formation of stable complexes between the cited antimigraine drugs and Eosin Y reagent has been examined. It has been established that the quenching effect of cited drugs on the relative fluorescence capacity of Eosin Y has been instantaneous and the color of the complex has been developed within few seconds and remained stable for at least 30 min, (Figure 6).

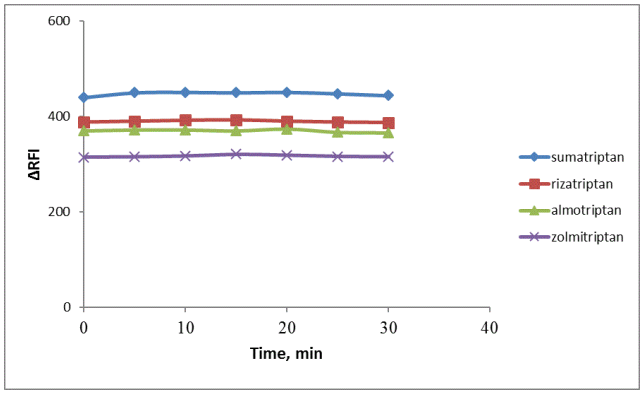

Figure 6: Time effect on the reaction between studied drugs (1 µg/mL) and Eosin (0.72×10^{-5} M).

Investigation of the molar ratio between Eosin Y reagent and cited antimigraine drugs: The molar ratio between the investigated antimigraine drugs and Eosin Y reagent was determined using Job's method of continuous variations [36] using (0.72×10^{-5} M) master equimolar solutions. The method revealed a 1:1 ratio between Eosin and all studied antimigraine drugs, Figure 7.

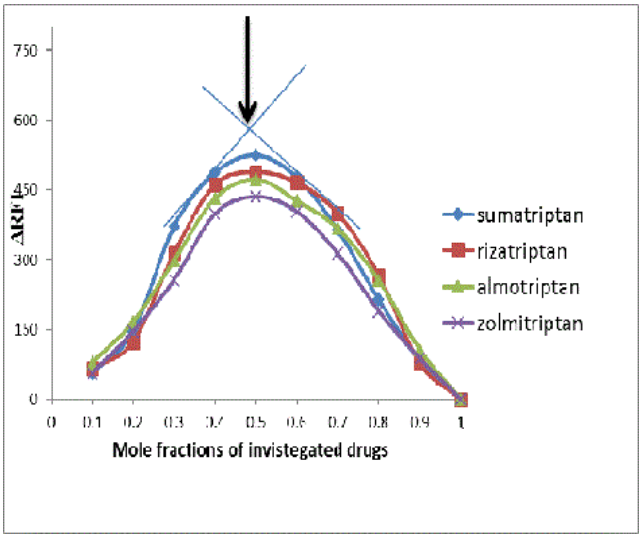

Figure 7: Job's method plot for determination of molar ratio of studied drugs and Eosin Y using 0.72×10^{-5} M equimolar solutions.

The results obtained from Job's method studies were in agreement with the suggested reaction mechanism [25,26]. This complex has been established from electrostatic attraction through the reaction between the acidic carboxylate group of Eosin Y reagent and the alkaline moiety (amino group) of the cited antimigraine drugs. Such reaction mostly takes place in an acidic pH [26]. The suggested reaction mechanism is outlined in Figure 8.

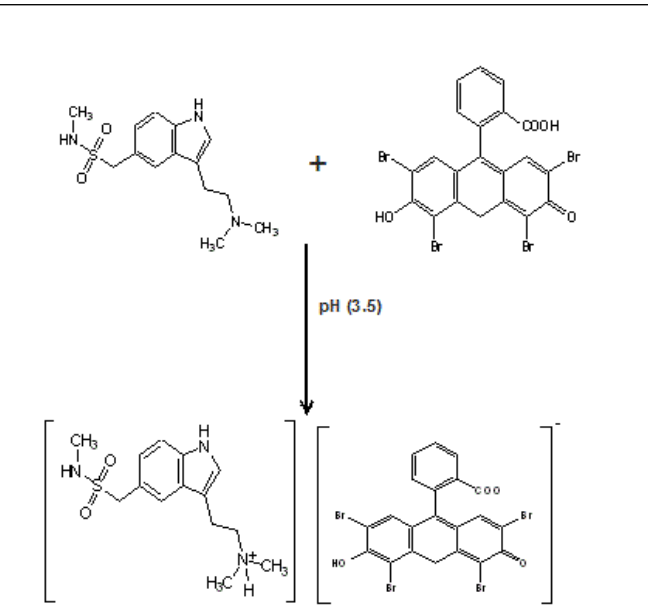

Figure 8: Suggested reaction mechanism between Sumatriptan as a representative example of studied drugs and Eosin Y.

Method validation: The suggested assay has been verified and validated according to ICH guidelines [37] in order to show that the developed procedures agree with the demands of the cited analytical performance. All validation operations have been checked through the defined calibration range scale of the developed assay to confirm the validation of the proposed method.

Linearity: The linearity of an analytical method is defined as the capacity (through a defined range) to elicit test results which proportional to the drug concentrations using at least nine concentrations [37]. Under the optimized reaction conditions, standard calibration curves for the studied antimigraine drugs have been investigated by analyzing a series of nine to eleven concentrations for each cited drug, taking the mean of three determinations for each concentration to minimize the relative error, and then plotting the corrected fluorescence intensity versus concentrations within the specified range, then test results were treated statistically by calculation of the regression equations by least squares method [38]. In this work, concentrations ranging from 0.07-1.0 µg/mL has been examined in all studied drugs and all validation procedures have been taken place through this range. Different analytical parameters for the calibration data together with the results from statistical evaluation of correlation co-efficient (r) of the regression equation such as intercept (Sa), slope (Sb), detection Limit (LOD) and quantification limit (LOQ) were presented in Table 1.

Parameter	Almotriptan (ALT)	Rizatriptan (RZT)	Sumatriptan (SMT)	Zolmitriptan (ZLT)
λex (nm)	301.3			
λem (nm)	542.8			
linear range (µg/mL)	(0.07-1.0)	(0.2-1.0)	(0.2-1.0)	(0.1-1.0)
LOD (µg/mL)	0.019	0.041	0.055	0.032
LOQ (µg/mL)	0.059	0.125	0.168	0.096
Correlation coefficient (r)	0.9999	0.999	0.9989	0.999
Determination coefficient (r2)	0.9998	0.998	0.9978	0.998
Slop(b)	0.982	1.036	0.847	0.984
Intercept(a)	73.43	-75.18	90.01	-41.67
SD of the intercept (Sa)	5.8	12.99	14.21	9.43
SD of slope (Sb)	0.01	0.989	0.022	0.015
SD of residual (Sy.x)	10.048	12.987	16.856	13.797

Table 1: Analytical parameters for the analysis of the studied drugs by Eosin Y method.

Accuracy and precision: The accuracy was checked by three times analysis for six different concentrations of pure drugs. The values presented in Table 2 showed the high acceptance between the correct estimations and the experimental estimations indicating good accuracy for the suggested assay. Intraday and Interday precision have been determined using three different concentrations for each drug and six determinations of each concentration. The calculated relative standard deviation (RSD) values were below 2 % indicating good repeatability and reliability for the suggested assay. The results and their statistical analysis were summarized in Table 3.

No	Almotriptan			Rizatriptan			Sumatriptan			Zolmitriptan		
	taken µg/mL	found* µg/mL	% recovery	taken µg/mL	found* µg/mL	% recovery	taken µg/mL	found* µg/mL	% recovery	taken µg/mL	found* µg/mL	% recovery
1	0.2	0.19	97.4	0.2	0.194	97	0.2	0.19	98	0.2	0.19	96.6
2	0.3	0.29	97.5	0.3	0.29	98.3	0.3	0.29	97.8	0.3	0.29	97.5
3	0.5	0.49	98	0.5	0.48	97.2	0.5	0.48	96.8	0.5	0.48	97
4	0.6	0.59	97.6	0.6	0.58	97	0.6	0.59	97.7	0.6	0.59	98.4
5	0.7	0.68	97.3	0.7	0.69	98.4	0.7	0.68	96.9	0.7	0.68	96.7
6	1	0.98	98.4	1	0.97	97.2	1	0.98	98.4	1	0.97	97.6
Mean			97.7			97.5			97.6			97.3
SD			0.42			0.65			0.63			0.68
RSD			0.43			0.67			0.65			0.7

Table 2: Accuracy evaluation of the proposed Eosin spectrofluorimetric method.*: Average of six replicate measurements, SD: standard deviation, RSD: relative standard deviation.

Parameter	ALT (%found)*			RZT (%found)*			SMT (%found)*			ZLT (%found)*		
	0.3	0.6	1	0.2	0.7	1	0.3	0.6	1	0.3	0.6	1

Intraday assay	1	97.6	98.5	97.7	98.6	99.4	98.8	98.6	98.8	99.5	98.1	98.7	98.6
	2	97.3	98.6	97.8	98	99.4	98.8	98.3	98.8	99.4	98.5	98.5	98.4
	3	97.8	98.5	97.6	98.5	99.5	98.7	98.5	98.6	99.4	97.8	98.4	98
	mean	97.6	98.5	97.7	98.3	99.4	98.8	98.5	98.7	99.4	98.1	98.5	98.3
	SD	0.3	0.6	0.1	0.3	0.6	0.6	0.2	0.1	0.1	0.4	0.2	0.3
Interday assay	RSD	0.3	0.6	0.1	0.3	0.6	0.6	0.2	0.1	0.1	0.4	0.2	0.3
	1	98.5	97.7	97.3	98.9	99.4	98.8	98.3	98.5	99.4	98.1	98.5	98.5
	2	98.8	97.8	97.5	98.8	99.5	98.9	98.5	98.3	99.3	98.5	98.7	98.4
	3	98.6	97.6	97.3	98.7	99.2	98.8	98	98.6	98.5	98.3	98.6	98.2
	mean	98.6	97.7	97.4	98.8	99.4	98.8	98.3	98.5	99	98.3	98.6	98.4
	SD	0.2	0.1	0.1	0.1	0.2	0.1	0.3	0.2	0.5	0.2	0.1	0.2
	RSD	0.2	0.1	0.1	0.1	0.2	0.1	0.3	0.2	0.5	0.2	0.1	0.2

Table 3: Interday and Intraday precision of the proposed Eosin spectrofluorimetric method.

Detection limit (LOD) and Quantitation limit (LOQ): Detection limit and quantitation limit were calculated according to ICH Q2 (R1) recommendation [37] through the equations-LOD=3.3* standard deviation of the intercept/slope, While LOQ=10* standard deviation of the intercept /slope (Table 1).

Robustness of the Method: Robustness of suggested procedure was checked for little and fixed change in the variables of the developed assay as: the difference in pH value, (3.5 ± 0.2 mL), the difference in the amount of acetate buffer solution (0.2 M), (1.5 ± 0.5 mL) and the difference in the amount of Eosin Y dye (0.72×10^{-5} M), (2.0 ± 0.5 mL). During those assay, one parameter was varied whereas the other parameters remained constant and the recovery percentage was determined each time. The obtained recoveries and standard deviations indicated that little difference in each of studied parameter had no significant effect on the fluorescence intensity of the resulting complex of the suggested assay, (Table 4). It reveals the high accuracy of the suggested assay through its routine analysis for the studied antimigraine drugs.

Parameter	% Recovery* ± SD			
	ALT.(0.3 µg/mL)	RZT.(0.3 µg/mL)	SMT.(0.3 µg/mL)	ZLT (0.3 µg/mL)
Optimum condition	99.6 ± 0.4	99.3 ± 0.2	100.2 ± 0.6	99.8 ± 0.2
1-pH of 0.2 M acetate buffer solution (pH 3.5)				
pH 3.3	98.1 ± 0.3	98.5 ± 0.4	97.9 ± 0.5	98.4 ± 0.4
pH 3.7	98.1 ± 0.3	98.5 ± 0.4	97.8 ± 0.5	98.3 ± 0.3
2- volume of 0.2 M acetate buffer (pH 3.5)				
1 mL	98.1 ± 0.5	97.8 ± 0.2	98.6 ± 0.7	97.8 ± 0.3
2 mL	98 ± 0.6	97.7 ± 0.2	97.6 ± 1.0	97.9 ± 0.3
3- volume of Eosin (0.72×10^{-5} M)				
1.5 mL	97.4 ± 0.4	97.1 ± 0.3	97.6 ± 0.4	97.5 ± 0.5
2.5 mL	97.3 ± 0.4	97.9 ± 0.3	97 ± 0.6	97.4 ± 0.4

Table 4: Robustness of the proposed spectrofluorimetric method for analysis of the studied drugs. *Average of six determinations.

Application to assay of commercial samples: The proposed analytical procedure has been used for the analysis of pharmaceutical dosage forms of the investigated drugs and results obtained were compared by those of the described ones [3,5,8,11] by applying student's t-test and F-test at the 95% confidence level [38]. No significant difference was noticed between the values of the suggested and reported assays indicating high accuracy as well as good precision in the assay of the investigated compounds in their dosage forms (Table 5).

Dosage forms	Drug	% Recovery a ± SD		t-value b	F-value b
		Proposed method	Reported methods [3,5,8,11]		
Almotrip forte® 12.5 mg	ALT	98.1 ± 0.1	98.0 ± 0.1	1.1	2.4
Migriza® 10 mg	RZT	98.3 ± 0.4	98.4 ± 0.4	0.8	1.2
Imigran® 50mg	SMT	99.8 ± 0.1	99.7 ± 0.1	0.9	2.1

Amigrawest® 2.5 mg	ZLT	97.2 ± 0.1	97.2 ± 0.1	0.8	3

Table 5: Application of the proposed spectrofluorimetric and the reported methods for determination of the studied antimigraine drugs in their pharmaceutical dosage forms. A: Average of five determinations, b: Tabulated values at 95% confidence limit are t=2.306, F=6.338.

Content uniformity test: The method is ideally applied to content uniformity test that is a time-consuming procedure when applying normal experiment techniques, owing to the great accuracy of the suggested assay and its capacity to quickly determine the content of the drugs in only one tablet obtained with adequate precision. Ten different tablets have been assayed by applying the same experiment which used to analyze the cited drugs in tablets. The uniformity contents have been checked using the official United State Pharmacopeia guidelines (Chapter 905: Uniformity of Dosage Units). The acceptance value (AV) has been determined for all dosage forms and it has been established to be very small than the maximum allowed acceptance value (L1). The results of content uniformity of commercial preparation are shown in Table 6.

Dosage form no.	Almotrip forte® 12.5 mg	Migriza® 10 mg	Imigran® 50 mg	Amigrawest® 2.5 mg
	% labeled claim	% labeled claim	% labeled claim	% labeled claim
1	98.1	99.5	98.8	99.3
2	98.6	98.6	99	99.6
3	98.9	99	99.4	98.9
4	99.1	100	99.7	99.4
5	98.9	99.3	98.6	99.8
6	98.6	99.5	98.7	99
7	99.4	99.7	99.3	100
8	99.1	99.8	99.6	99.2
9	98.9	99	99.4	99.6
10	99.1	99.5	98.7	98.8
Mean	98.9	99.4	99.1	99.4
SD	0.4	0.4	0.4	0.4
RSD	0.4	0.4	0.4	0.4
Acceptance value (AV)	1	1	1	1
Max. allowed AV(L1)	15	15	15	15

Table 6: Content uniformity testing of studied drug tablets using the proposed method. Acceptance value [39]=2.4 × SD.

Application to biological fluid: The highest accuracy of the suggested assay permits the assay of antimigraine drugs in spiked human plasma. The concentrations of the drugs have been calculated from those analogous regression equations. The resulting mean recovery values have been obtained as presented in Table 7. It can be concluded that the suggested assay is convenient for the assay of investigated drugs in the biological fluid.

Add.conc (ng/mL)	Found conc.(ng/mL)	%Recovery* ± SD
Almotriptan		
70	68.9	98.4 ± 0.7
80	79.6	99.4 ± 0.5
200	197.1	98.5 ± 0.3
Rizatriptan		
200	196.4	98.2 ± 0.5
300	298.6	99.5 ± 0.4
400	392.5	98.1 ± 0.3
Sumatriptan		
200	196.9	98.4 ± 0.2
300	295.6	98.5 ± 0.2
600	595.6	99.2 ± 0.1
Zolmitriptan		
100	97.3	97.3 ± 0.0
200	196.5	98.3 ± 0.3
300	297.9	99.3 ± 0.3

Table 7: Application of the proposed method to spiked human plasma. *Mean of six determinations.

Conclusion

In this study, Eosin Y has been selected as an ion-pairing reagent to form binary complexes with Almotriptan malate, Rizatriptan benzoate, Sumatriptan succinate and Zolmitriptan. The suggested assay possesses the advantages of being sensitive, simple, rapid, reliable, and accurate for the assay of anti-migraine drugs in their commercial medications and spiked human plasma without interference from the general additives and complex matrices. In addition, it is a time-saving method and there is no need for pre-treatment or extraction of the samples. Moreover, the suggested assay is very suitable to be applied in tablet content uniformity test and for routine assay and quality control inspection of the studied antimigraine drugs.

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