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Fluorimetric and colorimetric methods for the determination of some antimigraine drugs

Ramzia.I. El-Bagary [a]*, Nashwah G. Mohammed [b], Heba A. Nasr [b]

[a] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, EL-Kasr El Aini St. Egypt.*
[b] *National Organization for drug Control and research, Giza, Egypt.*

ABSTRACT

Two spectroscopic methods were proposed for the determination of almotriptan malate (AM), eletriptan hydrobromide (EH), and rizatriptan benzoate (RB), in pure form or in their pharmaceutical dosage forms. The first method is a quantitative fluorimetric method. For rizatriptan benzoate, the native fluorescence was measured for its solutions in acetonitrile upon excitation at λ 235 nm and emission at λ 357nm. Linear relationship was obtained over concentration range (1-12µg/ml). For almotriptan malate and eletriptan hydrobromide, induced fluorescence was measured by fluorogenic labeling with 4-chloro-7-nitrobenzofurazan (NBD-cl) upon excitation at λ 460nm, and emission at λ 550nm. Linear relationships were obtained over concentration ranges; (10-100µg/ml) & (10-80µg/ml) for almotriptan malate and eletriptan hydrobromide respectively. The second method was based on formation of charge transfer complex between the base of the studied drugs and 7,7,8,8 tetracyanoquinodimethane (TCNQ). The colored complexes have a maximum at λ 744nm. Linear relationships were obtained over concentration ranges (10-75µg/ml), (10-70µg/ml), and (10-100µg/ml), for almotriptan and eletriptan, and rizatriptan respectively. The proposed methods were reproducible, precise and accurate and were successfully applied for the determination of each of the studied drugs in pure form or in pharmaceutical dosage form in Quality Control Determinations.

Keywords: Almotriptan malate and Eletriptan hydrobromide, Rizatriptan benzoate, fluorimetry, 4-chloro-7-nitrobenzofurazan, 7,7,8,8 tetracyanoquinodimethane.

INTRODUCTION

Migraine is described as neurovascular headache, and it is characterized by recurrent attacks of headache which typically last from 4 to 72 hours. Simple analgesics and non steroidal anti-inflammatory drugs are effective if taken at the earliest signs of the attack. Attacks not responding to simple analgesics or non steroidal anti-inflammatory drugs may be treated with some selective serotonin (5HT1) agonists, e.g. almotriptan, eletriptan and rizatriptan [1, 2]. (Fig.1)

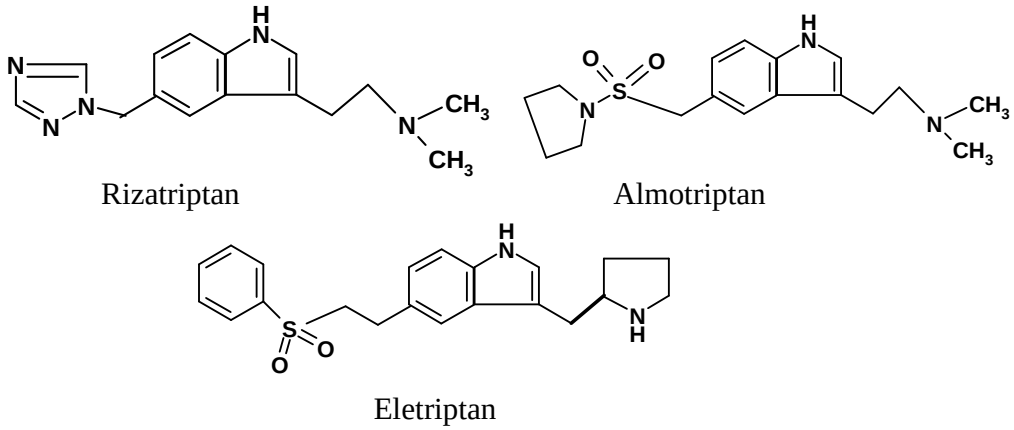

Fig. 1. Chemical structure of Rizatriptan, Almotriptan and Eletriptan

Few methods have been reported for the determination of almotriptan, eletriptan and rizatriptan in pure, pharmaceutical dosage forms and in biological fluids. The methods include RP-HPLC using UV detector [3-8]. HPLC methods using fluorimetric detector were used for the determination of rizatriptan in presence of its impurities or in plasma [9-12]. HPLC coupled with MS method was described for the determination of rizatriptan either in plasma or in serum [13-15]. Stability indicating methods for the determination of mixture of rizatriptan and naproxen were established using microemulsion electrokinetic chromatography (MEEC) [16].

EXPERIMENTAL SECTION

2.1. Apparatus

1)Shimadzu UV spectrophotometer UV1601, A 10753580384 Japan.
2) Shimadzu spectroflourimeter. A 4013, Japan.
3) Digital pH meter, Jenway, England.
4) Heater ultrasonic shaker, Retscn UR 1, 306082174, Germany.

2.2. Materials and Reagents

1) Almotriptan malate, kindly provided by; European Egyptian Pharmaceutical Industries Company, Cairo, Egypt. (Purity 100.16%±0.163) [4]
2) Eletriptan hydrobromide, kindly provided by; Pfizer, Cairo, Egypt (Purity99.95%±0.166) [7]
3) Rizatriptan benzoate, kindly provided by; Epico, Cairo, Egypt. (Purity100.19%±0.315)[8]

4) Almotrip forte tablets, labeled to contain 17.5 mg almotriptan malate per tablet B.N. 8005, manufactured by European Egyptian Pharmaceutical Industries Company, Cairo, Egypt.
5) Relpax tablets, labeled to contain 40 mg eletriptan HBr per tablet, B.N. 6102007, manufactured by Pfizer, Cairo, Egypt.
6) Migratec tablet, labeled to contain 10 mg rizatriptan benzoate per tablet, B.N. MIG2, manufactured by Epico , Cairo, Egypt,
-Chloroform (ADWIC, Egypt), Diethyl ether (ADWIC, Egypt), Orthophosphoric acid (ADWIC, Egypt), Acetonitrile (HONIL Limited, England), Ethanol (HONIL Limited, England), Potassium dihydrogen orthophosphate (EL Nasr Co., Egypt), Sodium bicarbonate (EL Nasr Co., Egypt), Sodium carbonate anhydrous (EL Nasr Co., Egypt).
-Tetracyanoquinodimethane (SIGMA, Germany) (3-5mg/ml) in acetonitril.
- 4-Chloro-7-nitrobenzofurazan (Aldrich, Germany) (0.1M) in ethanol.
- Bicarbonate buffer pH 8: sodium bicarbonate (4.2g) and sodium carbonate (5.3g) was dissolved in 250ml distilled water and the pH was adjusted to pH8 with orthophosphoric acid.] [17].
- Phosphate buffer pH 3: potassium dihydrogen orthophosphate (34g) was dissolved in 250ml distilled water and the pH was adjust to pH3 with orthophosphoric acid. [17].

2.3. Procedure

2.3.1. Preparation of working standard solutions,

2.3.1.1. For Fluorimetric method

A solution of 20µg/ml of RB in acetonitrile was prepared. Two solutions of 1mg/ml of each of AM, and EH were prepared in acetonitrile.

2.3.1.2. For Colorimetric method

An accurate weight (50mg) of each of AM, EH, and RB was transferred into 125ml separating funnel and dissolved in distilled water. The solutions were rendered alkaline with 5ml 33% ammonia solution for AM and EH or with 5ml of 0.1N sodium carbonate solution for RB. The formed precipitate (free base) was extracted three times each with 15ml (chloroform for AM, and RB or ether for EH). The chloroformic or ethereal extracts were collected into a 100ml beaker after passing through anhydrous sodium sulphate and evaporate to dryness. The residue left after evaporation was dissolved in acetonitrile and transferred quantitatively into 50ml volumetric flask and the volume was completed with the same solvent to produce standard base solution equivalent to 1mg/ml of each drug.

2.3.2. Preparation of pharmaceutical dosage form solutions

2.3.2.1. For Fluorimetric methods

An accurate weight of the powdered tablets equivalent to100mg of RB or AM or EH was transferred into 100ml volumetric flask. Twenty ml aliquot of acetonitrile was added to the flask and the solution was shaken for 10 minutes using ultrasonic shaker. The volume was completed to the mark with acetonitrile to prepare solution of (1mg /ml) of pharmaceutical dosage form of each drug.

The solution was filtered through dry funnel and dry filter paper, the first 10 ml of the filtrate was rejected.

For RB, Two ml aliquot was further diluted to 100ml with the same solvent to form a solution of 20µg/ml of RB pharmaceutical dosage form.

2.3.2.2. For Colorimetric methods

An accurate weight of the powdered tablets of AM,EH, and RB, equivalent to (50mg) was transferred to 125ml separating funnel, dissolved in distilled water and the procedure was repeated as mentioned under " **Preparation of working standard solutions for colorimetric method**"; starting from; the solution was rendered alkaline……"

The formed solutions were 1mg/ml of pharmaceutical dosage form of each drug.

2.3.3. General procedure and linearity

2.3.3.1. Fluoremetric methods:

2.3.3.1.1. For rizatriptan benzoate:

Aliquots (0.5-6ml) of the prepared standard stock solution of RB equivalent to (10-120µg/ml) were transferred into series of 10ml volumetric flasks. The volume was completed to the mark with acetonitrile, and the fluorescence intensity of each solution was recorded at 357nm against a solvent blank using the following parameters:
Spectrum type: emission.
Sensitivity: low
Excitation wavelength: 235nm
Emission scanning range: 220 – 900
Ordinate upper: 1000 lower: 0.00

The fluorescence intensities were plotted versus the concentrations (µg/ml) to construct the calibration curve and the following regression equation (1) was computed.

F_{357}= 649.07 C+ 41.628……...r^2 =0.9999 for RB…….1

Where, F_{357} is the fluorescence intensities at 357nm, C is the concentration of the drug in (µg/ml), and r^2 is the regression coefficient.

2.3.3.1.2. For almotriptan malate and eletriptan hydrobromide:

Different aliquots (0.5-5ml), or (0.5-4ml) equivalent to (0.5-5mg), or (0.5-4mg) of AM, and EH standard stock solutions, respectively, were transferred into two series of volumetric flasks (50ml). One milliliter of bicarbonate buffer pH 8 was added to each flask followed by 1ml distilled water to avoid precipitation of buffer. Two milliliters of NBD-Cl solution in ethanol (0.1M) were added followed by 2ml ethanol to avoid precipitation of the reagent. The solutions were completed to 25ml using distilled water and left in ambient temperature for 30 minutes. The reaction was allowed to complete in water bath at 90^{o}C for fifteen minutes. After cooling the volumes were completed with ethanol to 50ml and the fluorescence intensity of each solution was recorded at 550nm against a solvent blank using the following parameters:
Spectrum type: emission.
Sensitivity: low
Excitation wavelength: 460nm
Emission scanning range: 220 – 900
Ordinate upper: 1000 lower: 0.00

The fluorescence intensities were plotted versus the concentrations (µg/ml) to construct the calibration curve for AM and EH and the following regression equations (2&3) were computed.

F_{550}= 8.4539 C+ 0.1014……...r^2 =0.9992 for AM…….2

F_{550}= 12.433 C+ 8.00 ……...r^2 =0.9992 for EM…….3

Where, F_{550} is the fluorescence intensities at 550nm, C is the concentration of the drug in (µg/ml), and r^2 is the regression coefficient.

2.3.3.2. Colorimetric method

Different aliquots of the prepared standard base solutions equivalent to (0.1-0.75mg), (0.1-0.7mg) or (0.1-1mg); for AM, EH, or RB, respectively, were transferred into three series of volumetric flasks (10ml). Four milliliters of 0.4%TCNQ solution in acetonitrile were added to each flask. The reactions were allowed to proceed for 15 minutes on a water bath at 90^{o}C. After cooling the volumes were completed with acetonitrile and the absorbance was recorded at 744nm against reagent blank to plot calibration curves.

2.3.4.Determination of Almotriptan malate, Eletriptan hydrobromide, and Rizatriptan benzoate in tablet form using the proposed methods:

2.3.4.1. Fluoremetric methods:

2.3.4.1.1.For rizatriptan benzoate:

Different aliquots (0.5- 6ml) of the pharmaceutical dosage form stock solution were transferred into series of 10 ml volumetric flasks and the procedures were completed as mentioned under "**General procedure and linearity**". The same experiment was repeated applying the standard addition technique. The concentrations of the labeled and added standard of RB were calculated using the regression equation (1).

2.3.4.1.2. For Almotriptan malate and Eletriptan hydrobromide

Different aliquots equivalent to (0.5-5mg), or (0.5-4mg) of AM, and EH of the pharmaceutical dosage form stock solution, respectively; were transferred into two series of volumetric flasks (50ml) and the procedures were completed as mentioned under "**General procedure and linearity**". The same experiment was repeated applying the standard addition technique. The concentrations of the labeled and added standard of AM and EH were calculated using the regression equations(2&3).

2.3.4.2. Colorimetric method

Different aliquots of the prepared pharmaceutical dosage form stock base solutions equivalent to (0.1-0.75mg), (0.1-0.7mg) or (0.1-1mg) of AM, EH, or RB, respectively, were transferred into three series of volumetric flasks (10ml) and the procedures were completed as mentioned under "**General procedure and linearity**". The same experiment was repeated applying the standard addition technique. The concentrations of the labeled and added standard of AM, EH and RB were calculated using the regression equations (4-6).

RESULTS AND DISCUSSION

3.1.Fluorimetric methods

For RB: a native fluorescence was displayed for RB upon using acetonitrile as a solvent with maximum emission at 357 nm when excited at 235nm (fig.2). The fluorescence of RB in different solvents and in presence of different pH buffers was studied. No fluorescence has been obtained upon using isopropyl alcohol or butanol as a solvent. While the drug exhibits fluorescence on using methanol, ethanol, and acetonitrile as a solvent. It was noticed that the fluorescence is pH dependent. At basic pH, the fluorescence was enhanced whereas at acidic pH the fluorescence was inhibited as the basic medium enhance the effect of auxochrome group (amino group) in RB molecule [18]. The better sensitivity and linearity were achieved upon using acetonitrile due to its chromophoric moiety thus; acetoniltrile was the solvent of choice for the fluorimetric determination of RB. Linear relationship was obtained over the concentration range (1-12µg/ml) RB and the regression equation (1) was computed as follows:

$F= 649.07C+ 41.628$……...$r^2 =0.9999$ for RB…….(1)

For AM & EH: there is no native fluorescence for either AM, or EH in different media. Therefore, induced fluorescence has been proposed by derivatisation with NBD chloride (4-chloro-7-nitrobenzofurazan) where a proton from the drug molecule was replaced with the fluorogenic moiety of NBD-Cl. The derivatisation products have a maximum emission at 550 ±2 nm when excited at 460 nm (fig. 3, 4).

Factors affecting the derivatisation product (e.g. amount of the reagent, buffer, time and temperature of reaction) have been studied.

Maximum fluorescence intensities were given on using 2ml of 0.1M solution of NBD-Cl in ethanol, and on using 1ml of bicarbonate buffer pH 8. The fluorescence intensity and the stability of the solutions were decreased by increasing the volume of reagent or buffer or increasing of their concentrations. .

Maximum fluorescence intensities were obtained upon leaving the solutions at ambient temperature for 30 minutes, then heating for 15 minutes in water bath (90^oC) to complete the addition reaction.

Linear relationships were obtained over the concentration ranges (10-100µg/ml) & (10-80µg/ml) for AM and EH, respectively; the regression equations (2&3) were computed as follows:

$F=8.4529C+0.1014$……..$r^2=0.9992$ for AM…….2
$F=12.433C+8.00$……$r^2=0.9996$ for EH…….3

The mean percentage accuracies are 100.36±0.474, 99.99±0.793 and 99.94±0.349 for RB, AM and EH respectively.

Limit of detection and limit of quantification were calculated based on standard deviation of the response and the slope of the calibration curve and found to be 0.021µg/ml & 0.068µg/ml for RB, 2.07µg/ml, & 6.88µg/ml for AM, and 1.31µg/ml& 4.38µg/ml for EH; respectively.

The methods were successfully applied for the determination of the studied drugs in tablets without interference from the additives and the standard addition technique was applied. The mean percentage recoveries of the added RB, AM and EH are 100.43± 0.84, 99.98±0.94 and 99.44±0.87, respectively (table1).

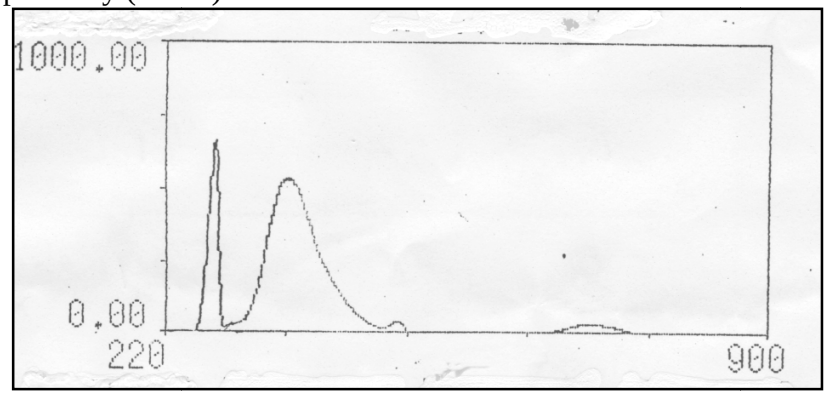

Fig (2): Excitation and emission spectra of rizatriptan benzoate (8µg/ml)

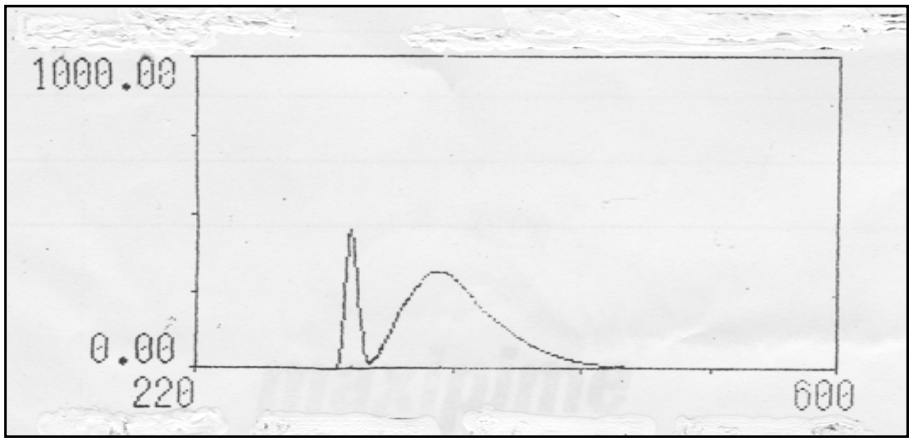

Fig (3) Excitation and emission spectra of almotriptan malate (60µg/ml)

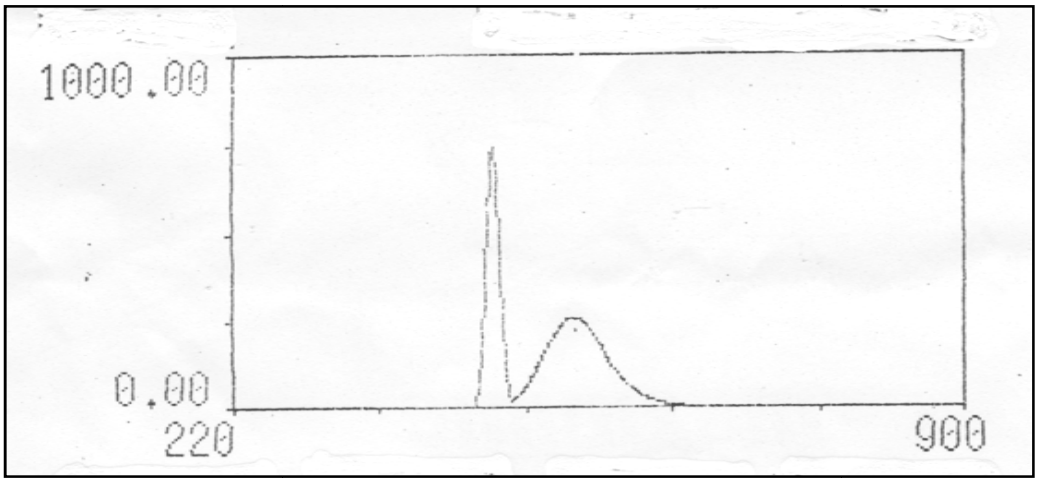

Fig (4) Excitation and emission spectra of Eletriptan hydrobromide (40µg/ml)

3.2. Colorimetric methods:

In the present study, spectrophotometric method has been utilized for the determination of RB, AM and EH (as n-electron donor) using 7,7,8,8 tetracyanoquinodimethane (TCNQ) as π-electrons acceptor. The reaction of the drugs with TCNQ results in the formation of an intense bluish green color exhibiting maximum absorbance at 744nm (fig 5, 6,7).

Different parameters affecting the reactions were studied e.g. effect of the acceptors concentrations, reaction temperature, time, stability of the color and different solvents.

Different solvents as methanol, acetonitrile, benzene, acetone, chloroform, ethylene chloride and methylene chloride were examined. Acetonitrile afforded the maximum sensitivity when compared with all other solvent.

Studying of the effect of acceptors concentrations revealed that maximum color intensity was attained upon using 4ml of 0.4% w/v solution of TCNQ in acetonitrile. Studying the effect of the reaction time and temperature revealed that maximum color intensity was attained after heating for15 minutes at 900C water bath. The color was stable for at least 2 hrs.

The stoichiometry of the reactions was studied using Job's of continuous variation method and found to be (1mole drug: 2 mole TCNQ).

Beer's law is obeyed over the concentration ranges (10-75µg/ml), (10-70µg/ml) or (10-100µg/ml), for AM, EH, and RB, respectively. The regression equations (4-6) were computed, and they were as follows;

A=0.0135C+0.0053 r^2=0.998 for AM…….....4
A=0.0141C+0.0236 r^2=0.998 for EH ……....5
A=0.0103C+0.0015 r^2=0.999 for RB ……6

Where A is the absorbance at 744nm, C is the concentration in µg/ml, and r^2 is the regression coefficient.

The mean percentage accuracies were; 99.81±0.851, 100.26±0.912, and 99.96±0.437 for AM, EH and RB respectively.

The methods were successfully applied for the determination of the studied drugs in tablets without interference from the additives. The standard addition technique was applied to assess the validity of the proposed method and the mean percentage recoveries of the added authentic AM, EH, and RB were100.05±0.7, 100.01±0.38, and 99.96±0.53 respectively. (Table1).

Statistical comparisons between the results obtained by the suggested fluorimetric and colorimetric methods with the reported methods of analysis of the cited drugs [4, 7, 8], were carried out, and no significant difference between them (tables 1).

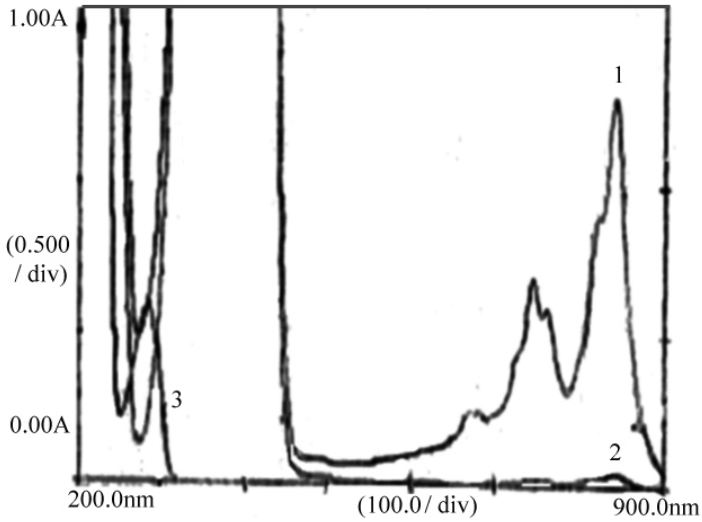

Fig (5): Absorption spectrum of 1- almotriptan (60µg/ml) complex with TCNQ solution (4%w/v), 2- blank reagent; 3- almotriptan (60µg/ml) in acetonitrile.

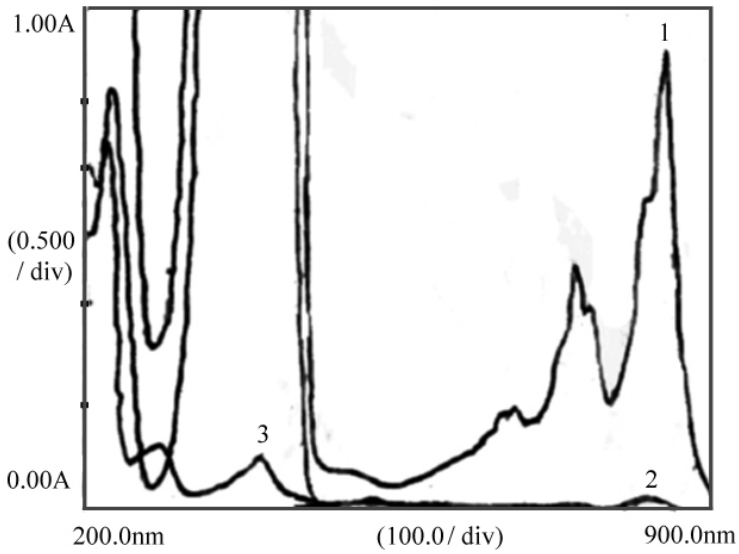

Fig (6): Absorption spectra of 1- eletriptan (20µg/ml) complex with TCNQ solution (4%w/v); 2- blank reagent; 3- eletriptan (20µg/ml) in acetonitrile.

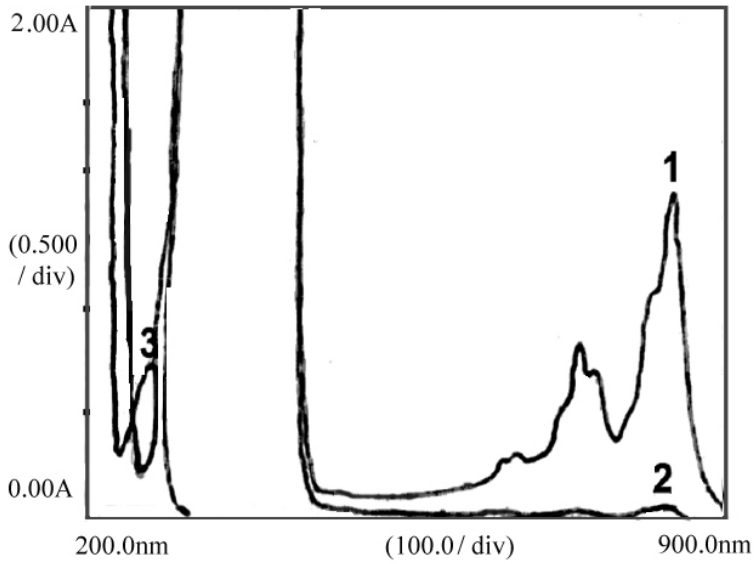

Fig (7): Absorption spectra of 1- rizatriptan (50µg/ml) complex with TCNQ solution (4%w/v); 2- blank reagent; 3- rizatriptan (50µg/ml) in acetonitrile.

(Table 1): Statistical comparison between results of analysis of pure samples of almotriptan malate, eletriptan hydrobromide and rizatriptan benzoate applying proposed methods and the reference methods

Item	Rizatriptan benzoate			Almotriptan malate			Eletriptan hydrobromide		
	Fluori-metric method	TCNQ method	Reference method [8]	Fluori-metric method	TCNQ method	Reference method [4]	Fluori-metric method	TCNQ method	Reference method [7]
Mean	100.37	99.96	100.66	99.99	99.81	100.31	99.94	100.26	99.68
n	6	6	5	6	6	5	6	6	5
SD	0.474	0.437	0.738	0.793	0.851	1.03	0.349	0.912	0.950
RSD	0.472	0.438	0.733	0.793	0.853	1.02	0.349	0.909	0.953
Variance	0.225	0.191	0.545	0.629	0.724	1.061	0.122	0.832	0.903
SE	0.193	0.179	0.33	0.324	0.3473	0.460	0.142	0.372	0.648
Tablets ±SD	100.68 ±0.556	99.79 ±0.317		101.22 ±0.181	99.93 ±0.698		99.42 ±0.722	100.17 ±0.112	
Added authentic± SD	100.43 ±0.84	99.96 ±0.53		99.98 ± 0.94	100.05 ±0.70		99.44 ±0.87	100.01 ±0.38	
t- test	0.483 (1.833)*	1.188 (1.833) *		0.329 (1.833)*	0.536 (1.833)*		0.0411 (1.833)*	0.419 (1.833)*	
F- ratio	2.404 (5.19)*	2.846 (5.19)*		1.688 (5.19)*	1.247 (5.19)*		4.048 (5.19)*	1.688 (5.19)*	

Figures in parenthesis are the theoretical F and t values at confidence limit 95%
n is the number of experiments.

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