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Research Article

Development and validation of Stability Indicating RP - HPLC Method for Analysis of Eletriptan

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ABSTRACT

A simple, rapid and precise Reversed phase high performance liquid chromatography method was developed for the analysis of Eletriptan. Chromatographic separation of Eletriptan is performed by using a Phenomenex Chromosil C_{18} (250 x 4.6mm, 5 µm) column as stationary phase. A mobile phase comprising of Acetonitrile, Triethylamine (TEA) and Tetrahydrofuran (THF) in the ratio of 50:25:25(v/v/v) (pH is adjusted to 6.3 with 1% ortho phosphoric acid) at a flow rate of 1.0ml/min and UV detection at 228 nm. The method is validated as per ICH and FDA guidelines. Linearity was observed in the range of 30-100 µg/ml. Recovery values are found to be 99.04 to 101.99%. The % RSD values for method precision and system precision are found to be less than 2%. The limit of detection and limit of quantification of method was found to be 0.8µg/ml and 2.5µg/ml respectively. The method was able to determine the stability of drug from pharmaceutical dosage form exposed to various stress conditions such as acidic, basic, photolytic oxidative, hydrolytic, and thermal degradation. Well-separated degrading peaks of Eletriptan are observed from the chromatograms of the samples subjected to stability studies.

Keywords: Eletriptan, HPLC, Method development, Validation, Degradation kinetics.

INTRODUCTION

IUPAC name of Eletriptan is (R)-3-[(-1-methylpyrrolidin-2-yl) methyl]-5-(2-phenylsulfonylethyl)-1H-indole and has a molecular mass 382.519 g/mol. Molecular formula of Eletriptan is $C_{22}H_{26}N_2O_2S$ and available with trade name of Relpax. Eletriptan is a second-generation triptan drug intended for treatment of migraine headache. It is used as an abortive Medication, blocking a migraine headache attack that is already in progress. Eletriptan is marketed and manufactured by Pfizer Inc. Eletriptan was approved by the U.S Food and Drug Administration (FDA) on December 26, 2002[1, 2] for the acute treatment of migraine with or without aura in adults[3]. It is not intended for the prophylactic therapy of migraine or for use in the management of hemiplegic or basilar migraine[3].

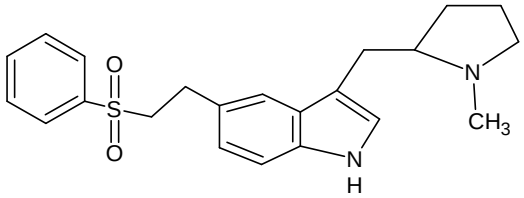


Fig. 1: Structure of Eletriptan

Few HPLC methods were reported for estimation of Eletriptan from biological fluids[4, 5]. However, stability indicating HPLC method employing UV-Visible detector has not been reported. Hence, an RP-HPLC method was developed and validated for linearity, precision, accuracy, and robustness, limit of detection and limit of quantification.

MATERIALS AND METHODS

Reagents used

Acetonitrile, TEA and THF (HPLC grade) were procured from Merck specialities private Ltd.,

Mumbai. Reference standard Eletriptan is procured from Divi's Labs Ltd., Hyderabad.

Preparation of Mobile phase

The required volume of Acetonitrile (50ml), TEA (25ml) and THF (25ml) were transferred separately into volumetric flask and pHwas adjusted to 6.3 and filtered through nylon membrane filter (0.45mc). The filtered solvents are mixed and degassed by subjected to sonication for 10min and the resulting solution is used as mobile phase[6].

Instrumentation

Chromatographic separation performed with Peak high performance liquid chromatography having LC-P7000 isocratic pump, equipped with Peak LC-UV7000 variable wavelength detector. Chromatograms and data recorded by means of Peak Chromatographic Software version 1.06.

Preparation of standard stock solution

Accurately weighed 100mg of Eletriptan was taken in a 100ml volumetric flask and 100ml of methanol was added to obtain 1000 µg/ml of Eletriptan standard solution (Stock solution). From the stock solution aliquots of different concentrations were prepared by taking 3ml-10ml of stock solutions into series of 100ml volumetric flasks and volume was made up to mark and concentration of 30µg/ml -100µg/ml was obtained .

Sample preparation

A composite of 20 tablets (Relpax-20mg) was prepared by grinding it into a finely divided powder with uniform size. 10 mg of Eletriptan was accurately weighed and transferred into a 10ml volumetric flask and volume was made up to the mark using mobile phase to obtain 1000 µg/ml of Eletriptan solution (Stock solution). The solution was sonicated for 15 min and it was filtered, 6ml of filtrate was transferred into 100 ml volumetric flask the volume made up to the mark with mobile phase Acetonitrile, TEA and THF in the ratio of 50:25:25(v/v/v) and mixed thoroughly to obtain a concentration of 60 µg/ml.

Chromatographic conditions

Column	: Phenomenex Chromosil C_{18} (250 mm x4.6mm, 5 µm)
Flow rate	: 1.0 mL/minute
Wavelength	: UV-228 nm
Column temperature	: Ambient
Injection Volume	: 20 µL
Runtime	: 10minutes

Validation parameters

The method developed for the determination of Eletriptan is validated as per ICH and FDA guidelines. Validation studies are performed considering 20µg/ml of Eletriptan as the specification level[6].

Linearity

Linearity is studied by preparing standard, solutions in the range of 30 µg/ml -100 µg/ml with mobile phase. A calibration graph is plotted between concentration of drug (20µg/ml) and chromatographic peak area (mv).

System suitability

System performance parameters for the developed HPLC method were determined by injecting five replicates of standard solution (20µg/ml). Parameters such as number of theoretical plates (N)*, tailing factor**[7] and relative standard deviation of peak area and retention time are calculated by employing empower Chromatographic software.

Calculation of the number of Theoretical Plates*

$N=5.54(V_e/W_{1/2})^2$, Where, N = Number of theoretical plates, Ve = elution volume or retention time (mL, sec, or cm), $W_{1/2}$ = width of the peak at half peak height (mL, sec, or cm)

Calculation of Tailing Factor**

T= (a + b)/2a, Where, T = tailing factor (measured at 5% of peak height), a = distance from the leading edge of the peak to the midpoint, b = distance from the point at peak midpoint to the trailing edge.

Precision

60 µg/ml standard solutions are prepared to calculate the precision for the developed method. The prepared solution is injected into injector at same concentrations and same chromatographic conditions. The chromatograms are recorded. The % R.S.D values are calculated.

a. System precision

The system precision is established by six replicate injections of the standard drug solution (20µg/ml). The corresponding peak area is measured and percentage RSD was calculated.

b. Method precision[8,9]

The sample solution (20µg/ml) was prepared separately five times with the solvent. The % R.S.D values calculated are 0.32 for intraday precision and 0.05 for inter-day precision. Hence, the developed method shows high precision.

Accuracy

Accuracy is the measure of closeness between the actual value and the analytical value

(calculated by applying the test procedure for a number of times). Recovery is done at three different levels viz. 20%, 50% and 100% within the beer's limit for drug. The accuracy is studied by recovery experiment. The solutions are injected in triplicates for each spike and the assay was performed as per the test method of the method. From this % recovery and the quantity present (mg) or recovered were calculated.[8]

Robustness[10]

Three sample solutions are prepared and analysed using the established conditions and by variation of the following analytical parameters; wavelength (223nm to 233 nm), the flow rate of mobile phase (0.8 to 1.2 ml/min) and buffer pH (6.1 to 6.5). Eletriptan contents and RSD were determined for each condition.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and the Limit of Quantification (LOQ**)** are calculated as defined by ICH (2003)[8]. Using the mean values of three independent analytical curves, determined by a linear-regression model, where the factors 3.3 and 10 for the detection and quantitation limits, respectively, are multiplied by the ratio from the standard deviation of the intercept and the slope.

Forced Degradation studies

Stress studies are performed under conditions of hydrolysis (acidic, alkaline and neutral), photolysis, oxidation, hydrolytic and dry heat (Thermal studies), as mentioned in ICH QIA (R2)[12-13]. It is performed by incubating the standard of Eletriptan solution for 8 to 48 hours in different conditions. The standard solution of 20µg/ml is prepared in methanol and injected after incubation.

a. Acid hydrolysis (0.1 N HCl)

In acid degradation studies, 20 µg/ml solution of Eletriptan injection is prepared in methanol. From that, 5ml solution is pipette out into a 25ml volumetric flask and added 20 ml of 0.1 N HCl to it and stored in dark room at 40 ^{0}c for 24 hours.

b. Base hydrolysis (0.1 N NaOH)

In base degradation studies, 20 µg/ml solution of Eletriptan injection is prepared in methanol. From that, 5ml solution is pipette out into a 25ml volumetric flask and added 20 ml of 0.1 N NaOHto it and stored in dark room at 40 ^{0} c for 24 hours.

c. Photolytic degradation

Photolytic degradation studies of the dry drug and the drug in methanol solution at a concentration of 20µg/ml are performed after exposing them to sunlight during daytime (60,000-70,000 lux) for 24 hours.

Oxidative degradation

In Oxidative degradation studies, 20 µg/ml solution of Eletriptan injection is prepared in methanol. From that, 5ml solution is pipette out into a 25ml volumetric flask and added 20 ml of 3% H_2O_2 prepared in water. The resultant solution is allowed standing for eight hours in a dark room to facilitate oxidation of the drug.

d. Hydrolytic degradation

20 µg/ml solution of Eletriptan injection is prepared in methanol. From that, 5ml solution is pipette out into a 25ml volumetric flask and added 20 ml of water at 40^{0}C. The resultant solution is allowed standing for 48 hours.

e. Thermal degradation

In thermal degradation studies, 20 µg/ml solution of Eletriptan injection was prepared in methanol. The resultant solution is heated at 60^{0}C and stored in dark room for 24 hour then brought to room temperature. After that, their corresponding absorbance values are recorded.

RESULTS AND DISCUSSIONS

The chromatographic parameters were initially evaluated using Phenomenex Chromosil C_{18} column by adopting various mobile phase acetonitrile, TEA and THF at pH 6.3 in the ratio of 50:20:30, 50:35:15, 50:30:20 and 50:25:25. The system suitability parameters such as theoretical plates count and tailing factor observed from the chromatograms obtained with the mobile phase containing acetonitrile, TEA and THF at pH 6.3 in the ratio of 50:20:30, 50:35:15, 50:30:20 were not within the acceptable limits. The desired system suitability parameters are obtained with the mobile phase containing acetonitrile, TEA and THF in the ratio of 50:25:25 at pH 6.3 and maintained at a flow rate of 1.0ml/min. The suitable chromatographic conditions for elution of Eletriptan are presented in Table 1. The retention time of the drug is 4.85min. The typical chromatogram of standard drug is shown in fig: 2 , and the typical chromatogram of Eletripan formulation is shown in fig: 3. Calibration curve of Eletriptan showed good linearity in the range of 30-100 µg/ml (fig: 4) Table 2. A linear correlation coefficient (r) was found to be 0.9996. The corresponding regression equation was found to be Y= 2742*X+1375. The system suitability parameters such as number of theoretical plates and tailing factor were found to be 7299 and 1.06. Low values of %RSD indicated that the method is precise and reproducible. The LOD

and LOQ were calculated from slope of calibration curve and standard deviation. These values were found to be 0.8 and 2.5 respectively Table 3.The obtained lower values of LOD and LOQ showed better sensitivity of the proposed method. The peak areas of intra and inter day analysis was shown in Table 4. The % recovery values of Eletriptan observed by analysing the tablets prepared in the laboratory with the proposed analytical method were within acceptable limits Table 5. The suitability of the proposed method for the estimation of the Eletriptan is tested with the formulation and the result obtained was 99.13% Table 6. Further, the suitability of the proposed method for elution and quantification of decomposed components (if any) is investigated by injecting the samples that are subjected to forced degradation studies Table 7. The chromatograms observed from the samples previously subjected to acid hydrolysis (0.1N HCl), alkaline Hydrolysis (0.1N NaOH), photolytic degradation, oxidative degradation(3%hydrogen peroxide), hydrolysis degradation, and thermal degradation(40^{0}c) (1× ICH, NA and 3× ICH, NA) are shown in fig. 5. The presence of new peaks in the chromatogram indicated the presence of decomposed products. Thus, the developed experimental method was found to be suitable for quantification of the drug in presence of decomposed products as these peaks clearly resolved from the drug peak. The drug is found to more decompose in acidic, light (1× ICH, NA and 3× ICH, NA) and high temperature conditions while it was found to be stable under aqueous conditions. It proves the sensitivity of method.

Table 1: Chromatographic Conditions

S. No.	HPLC conditions	Result
1	Elution	Isocratic
2	A.P.I Concentration	60 µg/ml
3	Mobile Phase	Acetonitrile, TEA and THF in the ratio of 50:25:25(v/v/v)
4	Wavelength	228 nm
5	Column	C_{18} Column
6	PH	6.3
7	Pump Pressure	10.9 M Pa
8	Temperature	Ambient
9	Retention Time	4.85 min
10	Run Time	10min
11	Area	167533
12	Th. Plates	7299
13	Tailing Factor	1.06
14	Flow	1ml/min

Table 2: Linearity result

S. No.	Concentration (µg/ml)	Area(mille volts/sec)
1	30	82018
2	40	112176
3	50	140915
4	60	167533
5	70	191445
6	80	224903
7	90	244444
8	100	274568

Table 3: System suitability and validation

Parameters	Values
Accuracy	99.74
Retention time(min)	4.85 min
Tailing factor	1.06
LOD(µg/ml)	2.5
LOQ(µg/ml)	0.8

Table 4: Intra and inter day Precision of the proposed method

S. No.	Concentration	Intraday	Interday
1	60 µg/ml	167533	167240
s2	60 µg/ml	167699	167340
3	60 µg/ml	167016	167331
4	60µg/ml	167006	167417
5	60 µg/ml	167034	167412
6	60 µg/ml	166165	167292
Result	% RSD	**0.32**	**0.05**

Table 5: Recovery studies

S. No.	Recovery	Labeled claim	Practically detected	% of Recovery	Mean % Recovery
1	20 %	50µg/ml	49.52	99.04	99.45
		50µg/ml	49.17	98.34	99.45
		50µg/ml	50.50	100.99	99.45
2	50%	60µg/ml	60.12	100.20	99.90
		60µg/ml	59.13	98.55	99.90
		60µg/ml	60.57	100.96	99.90
3	100 %	80µg/ml	80.71	100.89	99.87
		80µg/ml	79.70	99.24	99.87
		80µg/ml	79.58	99.48	99.87

Table 6: Recovery of Eletriptan from formulation

Formulation	Dosage	Concentration	Amount found	% Assay
Relpax	20 mg	60µg/ml	59.48	99.13

Table 7: ForcedDegradation studies of Eletriptan

Type of Degradation	Observation
Acid hydrolysis (0.1 N HCl)	Standard peak was split in to four peaks.
Base hydrolysis (0.1 N NaOH)	Standard peak was split in to four peaks.
Photolytic degradation	Standard peak was split in to four peaks.
Oxidative degradation	Standard peak was split in to four peaks.
Hydrolytic degradation	Standard peak was split in to two peaks
Thermal degradation	Standard peak was split into four peaks.

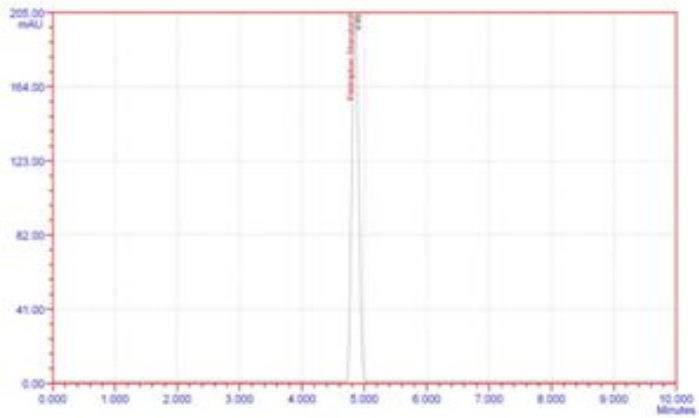


Fig. 2: Typical chromatogram of Eletripan standard

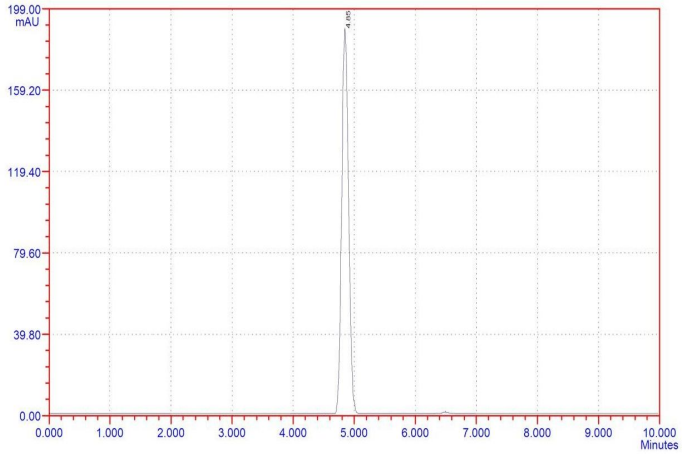


Fig. 3: Typical chromatogram of Eletripanformulation

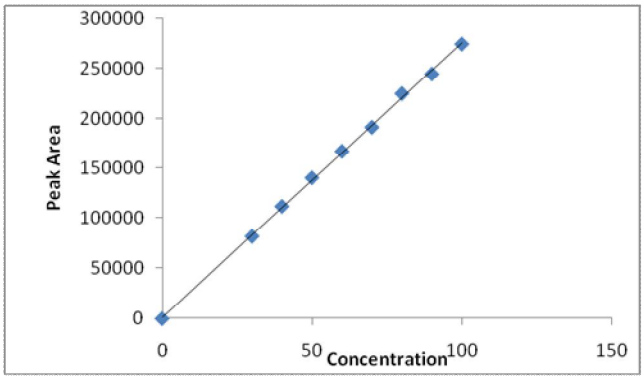
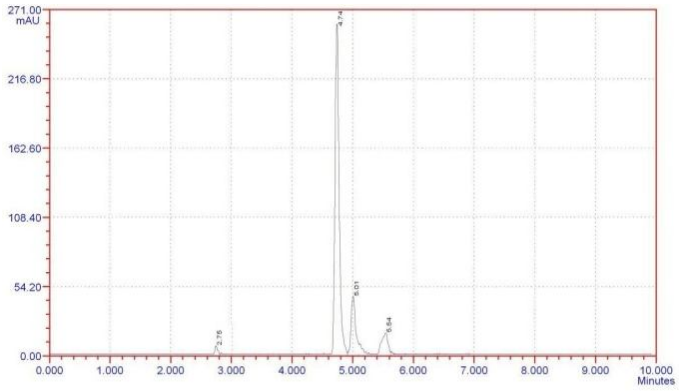
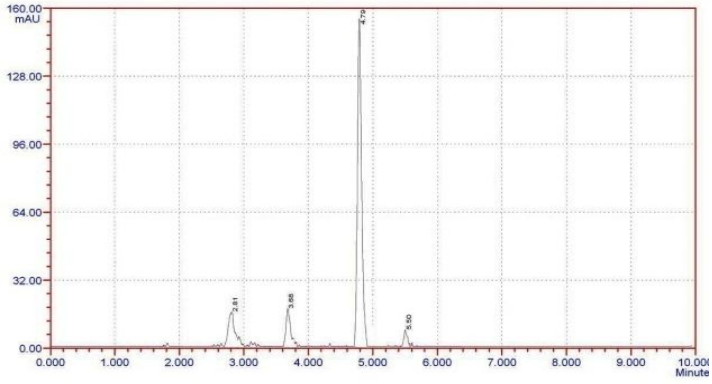


Fig. 4: Calibration curve



(a)



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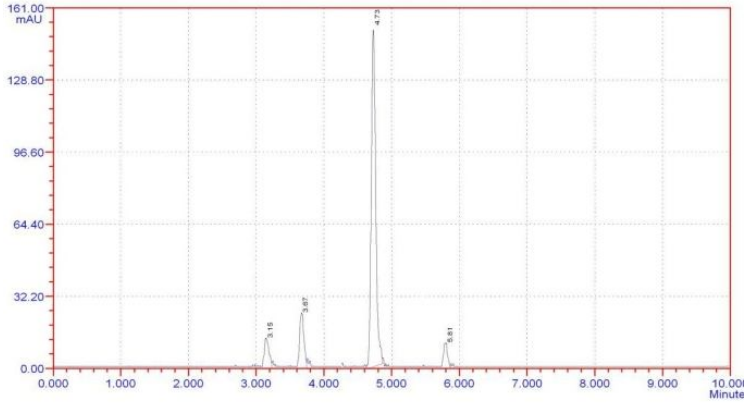

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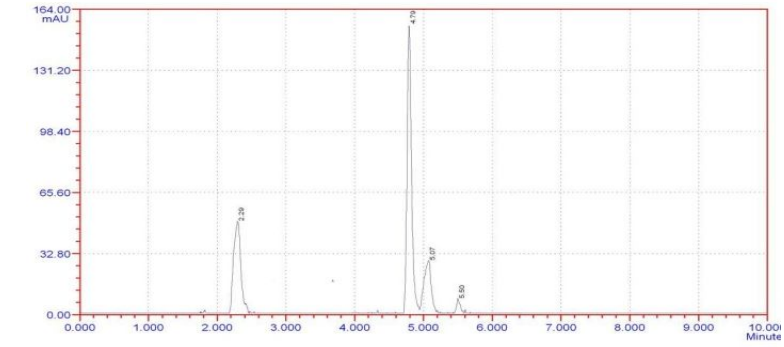

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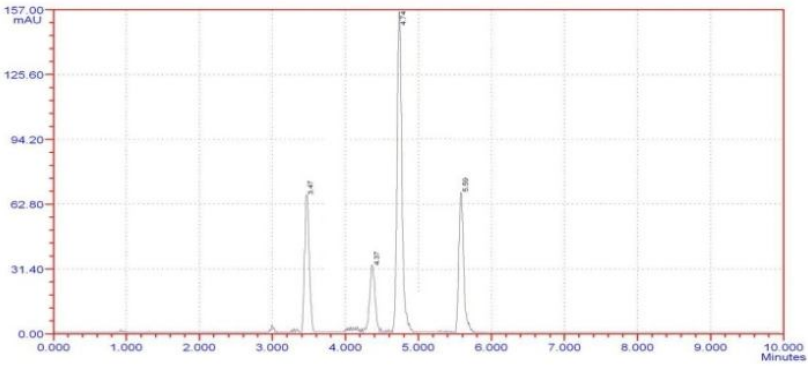

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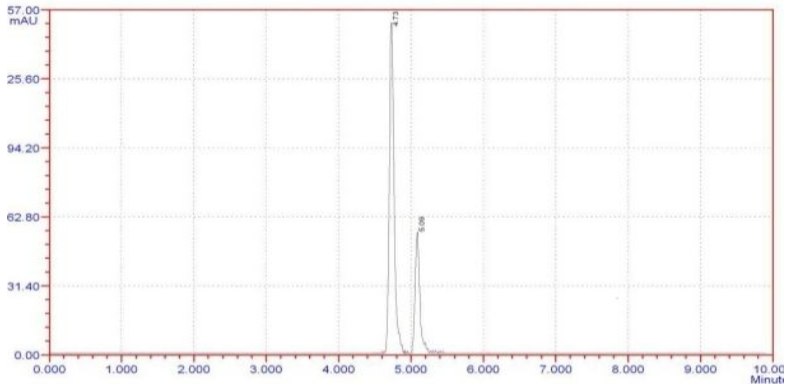
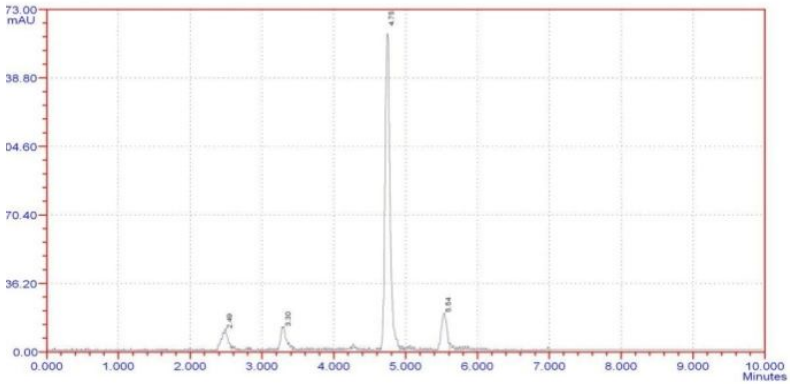


(f)



(g)

Fig. 5: chromatogram of Eletriptan enclosed to (a) acid hydrolysis (0.1N HCl), (b) base hydrolysis (0.1N NaOH), (c) & (d) photolytic degradation (normal light& UV), (e) peroxide degradation, (f) hydrolytic degradation, (g) thermal degradation

CONCLUSION

The proposed analytical method can be used for the determination of Eletriptan in bulk drug and in pharmaceutical dosage forms as a part of quality control analyzing. The RP HPLC method showed linear response in the stated range was accurate, precise, robust, reliable and specific. Based on the data obtained from forced degradation studies, of all the samples previously subjected to acid hydrolysis (0.1N HCl), base hydrolysis(0.1N NaOH), oxidative degradation (3%hydrogen peroxide), thermal degradation(40ºc), Eletriptan was degraded into four peaks in case of photolytic degradation , (1× ICH, NA and , 3× ICH, NA) Eletriptan was degraded into two peaks. The chromatograms are shown in fig.5. The presence of new peaks in the chromatogram indicated the presence of decomposed products. Thus, the developed experimental method was found to be suitable for quantification of the drug in presence of decomposed products as these peaks clearly resolved from the drug peak. It can be concluded that there was no other co-eluting peak with the main peak. Hence, this method can also used as a stability indicating assay method of Eletriptan in its tablet dosage form.

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