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Development and Validation of Spectroscopy Methods for the Estimation of Zolmitriptan in Pure and Pharmaceutical Dosage Form

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ABSTRACT
In the present work, two simple, sensitive and specific methods (Zero order Spectroscopy, Area under curve spectroscopy) have been developed for the quantitative estimation of Zolmitriptan in pure and pharmaceutical dosage forms.

Method A: Zero Order Spectroscopy
A simple, specific, accurate and precise Zero order Spectroscopy method was developed and validated for the estimation of Zolmitriptan in pharmaceutical dosage forms. The stock solution was prepared by weighing 100 mg of Standard Zolmitriptan in 100 ml volumetric flask containing methanol and distilled water (1:1). The final stock solution was made to produce 100 µg/ml with Diluent. Further dilutions were prepared as per procedure. The linearity was found in the concentration range of 1-6 µg / ml. The Correlation coefficient was 0.9996. The regression equation was found to be Y = 0.137 x + 0.0065. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, and ruggedness. The limit of detection and limit of quantitation for estimation of Zolmitriptan was found to be 0.018 (µg/ml) and 0.0.053 (µg/ml), respectively. Recovery of Zolmitriptan was found to be in the range of 99.44-100.24 %.
Proposed method was successfully applied for the quantitative determination of Zolmitriptan in pharmaceutical dosage forms.

Method B: Area Under Curve Spectroscopy
A simple, specific, accurate and precise Area under curve Spectroscopy method was developed and validated for the estimation of Zolmitriptan in pharmaceutical dosage forms. The stock solution was prepared by weighing 100 mg of Standard Zolmitriptan in 100 ml volumetric flask containing methanol and distilled water (1:1). The final stock solution was made to produce 100 µg / ml with distilled water. Further dilutions were prepared as per procedure. The linearity was found in the concentration range of 1-6 µg / ml. The Correlation coefficient was 0.9994. The regression equation was found to be Y = 0.148 x - 0.0017. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness. The limit of detection and limit of quantitation for estimation of Zolmitriptan was found to be 0.104 (µg / ml) and 0.318 (µg / ml), respectively. Recovery of Zolmitriptan was found to be in the range of 99.80-100.31 %. Proposed method was found to be simple, reproducible, and accurate and was successfully applied for the quantitative determination of Zolmitriptan in pharmaceutical dosage forms.

Keywords: Zolmitriptan, zero order spectroscopy, area under curve spectroscopy.

I. INTRODUCTION

Zolmitriptan is used for acute treatment of migraines. It is a serotonin receptor agonist having the following molecular structure.

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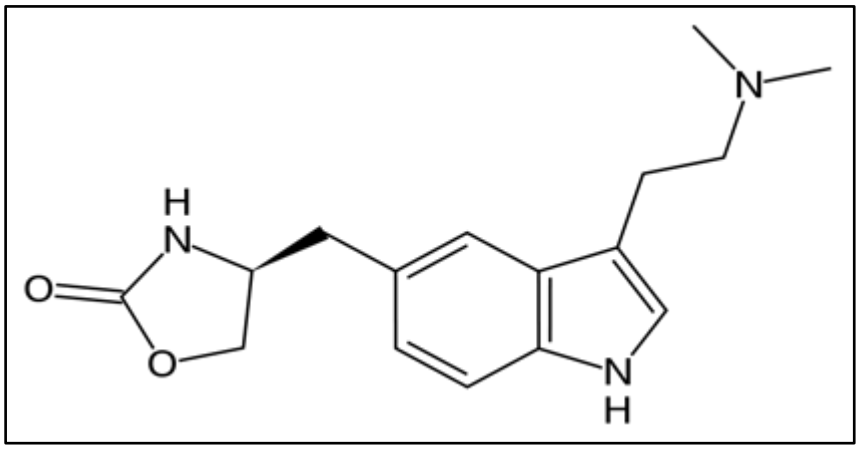

Nomenclature:(S)-4-({3-[2-(dimethylamino) ethyl]-1H-indol-5-yl} methyl)-1, 3-oxazolidin-2-one
Molecular formula: $C_{16}H_{21}N_3O_2$
Molecular weight : 287.357 g/mol
Characteristics: White or almost white crystalline powder.
Category: a serotonin receptor agonist used to treat migraine headaches.
Solubility: soluble in methanol, acetonitrile

II. MATERIALS AND METHODS

Reagents and Pharmaceutical Preparations

The pure sample of Zolmitriptan, its formulation and placebo was procured as gift sample from Aptex Pharma ltd. (Bangalore, India).

Instrument Specifications

Spectrophotometer: Shimadzu-1800 UV/Vis double beam
Sample cell: Quartz (1cm)
Lamp: D2
Software: UV Probe

Table 1: Chemical Used

Chemicals/reagents	Maker	Grade
Methanol	Spectrochem	A.R
Water	Milli Q	Distilled

Method A: Zero Order Spectroscopy

Zero order spectroscopy is UV Spectrophotometric method which involves the determination of Zolmitriptan in bulk drug and pharmaceutical formulations and has an absorption maximum at 224 nm in methanol and distilled water (1:1) which is presented as Fig 1. Beer's law was obeyed in the concentration range of 1-6 µg/ml.

Method Development

Selection of diluent

As the sample was insoluble in water methanol and distilled water in the ratio of (1: 1) was used as diluent. Sample was readily soluble in the above mentioned diluent.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 100 mg of Zolmitriptan in methanol and distilled water (1: 1) and the volume was made up to 100 ml with methanol and distilled water(1: 1) in 100 ml volumetric flask (Stock solution-l, 1000 µg/ml). 10 ml of stock solution-l was diluted to 100 ml with methanol and distilled water (1:1) (Stock solution-l, 100 µg/ml). 1 ml of stock solution-II was taken in 10 ml standard flask diluted to 10 ml with methanol and distilled water (1: 1) to get the concentration 10 µg/ml. The absorbance of resulting solution was measured against respective blank solution in the UV region of 200-400 nm, maximum absorbance was shown at 224nm and is given in Fig 1.

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Preparation of standard curve

Appropriate volume of aliquots from standard Zolmitriptan stock solutions were transferred to a series of 10 ml volumetric flasks capacity. The volume was adjusted to the mark with methanol and distilled water ((1: 1) to obtain concentrations of 1 to 6 µg/ml. Absorbance spectra of each solution against methanol and distilled water (1:1) as a blank were measured at 224 nm and the absorbance values are shown inTable 2. The obtained absorbance values are plotted against the concentration of Zolmitriptan to get the calibration graph and are represented as Fig 2. Theregression equation and correlation coefficient was determined and are presented inTable 3.

Sample preparation of Zolmitriptan:

To determine the content of Zolmitriptan in tablet formulation (Label claim: 2.5 mg), twenty tablets were weighed, their average weight was determined and finelypowdered. Tablet powder equivalent to 100 mg of Zolmitriptan was weighed and transferred into 100 ml volumetric flask. The drug was dissolved in 70 ml of methanoland distilled water (1: 1) by sonication for 30 minutes and further diluted withmethanol and distilled water (1: 1) up to the mark. Clear solution was obtained bycentrifugation at 8000 rpm for 10 minutes, supernatant was collected then finaldilution was made with methanol and distilled water (1: 1) to get the final stocksolution of 100 µg/ml. From this stock solution, various dilutions of the samplesolution were prepared and analyzed.

Validation of Spectrophotometric method

Accuracy

Accuracy is the closeness of the test results obtained by the method to the truevalue. To study the accuracy, thecommercially available tablet formulation of Zolmitriptan (Label claim 2.5 mg) was taken and Analysis of the same wascarried out. Recovery studies were carried out at three different levels i.e.50%, 100% and 150% by adding standard drug solution to the sample solution. The % recovery was calculated and reported in Table 4.

Precision

The precision of an analytical method is the degree of agreement amongindividual test results when the method is applied repeatedly to multiple samplingsof homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).

Intra and inter-day precision

A variation of results within the same day (intra-day), variation of results betweendays (inter-day) was analyzed and was shown in Table 5. Intra-day precision wasdetermined by analyzing Zolmitriptan for six times in the same day at 224 nm.Inter-day precision was determined by analyzing the drug daily once for six days at224 nm.

Linearity

The linearity of analytical method is its ability to elicit test results that are directlyproportional to the concentration of analyte in sample within a given range and was given in Fig 2.

Range

The range of analytical method is the interval between the upper and lower levels ofanalyte that have been demonstrated to be determined within a suitable level ofprecision, accuracy and linearity.

Ruggedness

The solutions were prepared and analyzed with change in the analytical conditionslike different laboratory conditions and different analyst and are presented in Table 6.

Method B: Area Under Curve Spectroscopy

Area under curve Sspectrophotometric method which involves the determination of Zolmitriptan in bulk drug and pharmaceutical dosage forms andwas measured between 219 to 229nm. Which is presented as Fig3, Beer's law wasobeyed in the concentration range of 1-6 µg/ml.

Method Development

Preparation of standard stock solution

The Standard stock solution of Zolmitriptan was prepared same as described in method A. The absorbance of resulting solution was measured against methanol and distilled water (1: 1) as a blank solution in the UV region of 200-400 nm, which shows area at the wavelength range 219-229 nm.

Preparation of standard curve

Aliquots of standard solution of Zolmitriptan were prepared same as described in method A. The absorbances were measured between 219 to 229nm against blank andthe absorbance values were shown in Table 7. The obtained absorbance valueswere plotted against the concentration of Zolmitriptan to get the calibration curveand is represented in Fig4. The regression equation and correlation coefficientwere determined and are given in Table 8.

Sample preparation of Zolmitriptan

The Sample preparation of Zolmitriptan was prepared same as described in MethodA. From

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the finial stock solution, various dilutions of sample solution were preparedand analyzed.

Validation of Spectrophotometric method

All the validation parameters such as accuracy, precision, linearity, range andruggedness are same as described in Method A.

III. RESULTS

METHOD A: ZERO ORDER SPECTROSCOPY

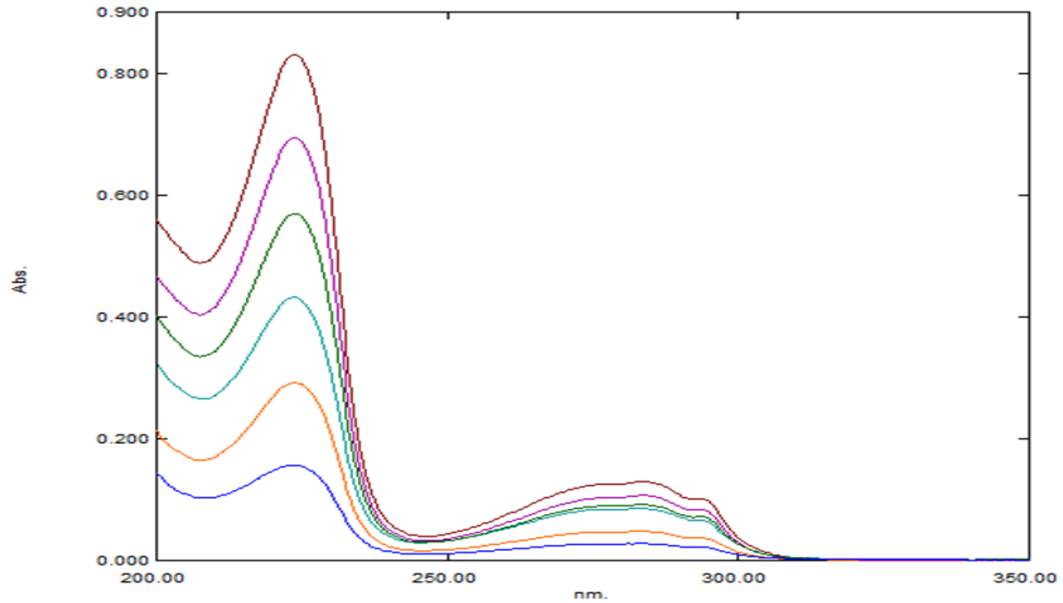

Fig 1- Zero order spectra of zolmitriptan at 224 nm

Table 2 - Results of Calibration Curve at 224 nm for Zolmitriptan by Zero Order Spectroscopy

Sr. no.	Conc. (µg/ml)	Absorbance at 224 nm
1	0	0
2	1	0.148
3	2	0.291
4	3	0.415
5	4	0.569
6	5	0.694
7	6	0.831

Table 3 - Optimum Conditions, Optical Characteristics and Statistical Data of the Regression Equation in Zero Order Spectroscopy

Parameters	UV method
λ_{max} (nm)	224
Beer's law limits (µg/ml)	1-6
Molar extinction coefficient (L mol^{-1} cm^{-1})	0.151

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Sandell's sensitivity(µg/cm)	0.006
Regression equation(Y*)	Y=0.138x0.0097
Slope(b)	0.135
Intercept(a)	0.0065
Correlation coefficient(r^2)	0.9996
Intraday Precision(% RSD**)	0.669
Interday Precision(% RSD**)	0.794
Limit of detection(µg/ml)	0.0017
Limit of quantitation(µg/ml)	0.105

*Y= bx+a where x is the concentration of Zolmitriptan in µg/ml and Y is the absorbance at the respective λ_{max}
**average of six determinations

Table 4 - Determination of Accuracy Results for Zolmitriptan at 224 nm by Zero Order Spectroscopy

Brand used	**Amount of sample (µg/ml)**	**Amount of drug added (µg/ml)**	**Amount recovered (µg/ml)**	**% recovery ± SD****
Zolmitriptan	2	1	0.999	99.95±0.32
	2	2	2.001	100.36±0.47
	2	3	2.999	99.98±0.19

**average of six determinations

Table 5 - Determination of Precision Results for Zolmitriptan at 224 nm by Zero Order Spectroscopy

Concentration (µg/ml)	**Inter-day absorbance Mean ± SD****	**% CV**	**Intra-day absorbance Mean ± SD****	**% CV**
1	0.149± 0.00163	1.09	0.148±0.00216	1.64
2	0.285±0.00348	1.22	0.285±0.00147	0.99
3	0.414±0.00176	0.42	0.434±0.00605	0.57
4	0.556±0.00606	1.09	0.548±0.00565	1.03
5	0.687±0.00662	0.96	0.685±0.00433	0.63
6	0.823±0.00876	1.06	0.817±0.00365	0.45

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**average of six determinations

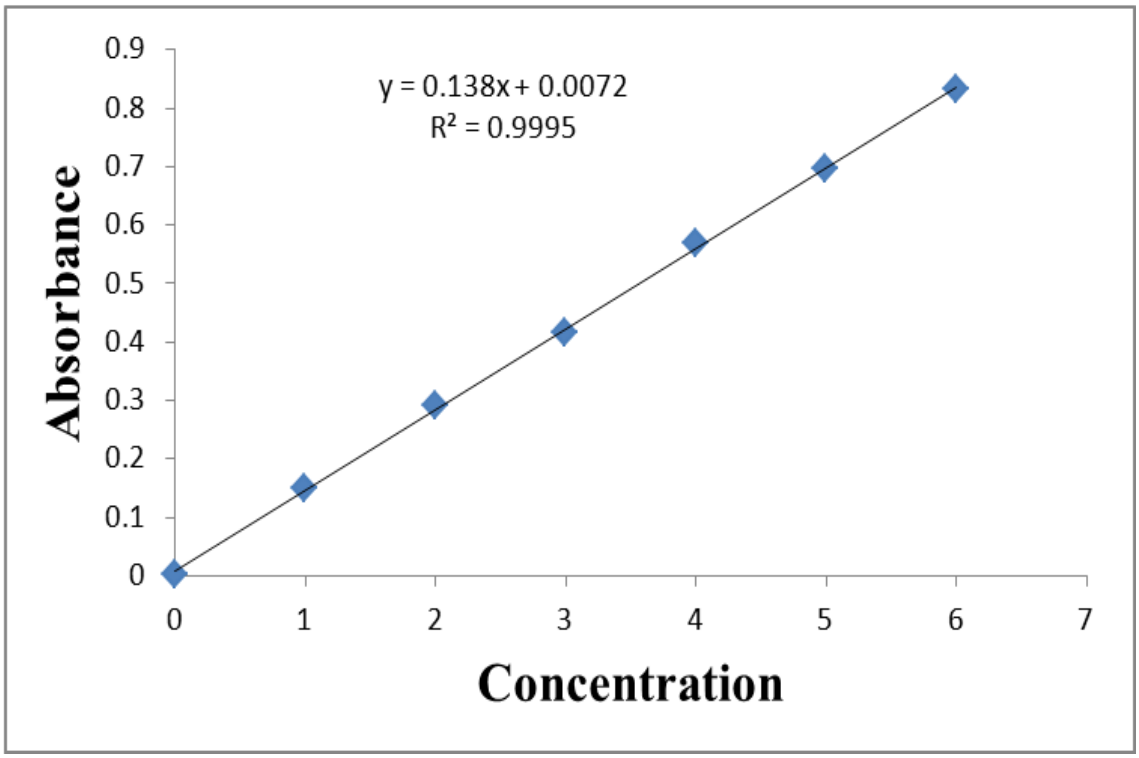

Fig 2 – Linearity Curve for Zolmitriptan at 224 nm by Zero Order Spectroscopy

Table 6 - Ruggedness Results for Zolmitriptan at 224 nm by Zero Order Spectroscopy

Brand used	Label claim (mg)	Analyst I		Analyst II	
		Amount found** (mg)	% Recovery ±SD**	Amount found** (mg)	% Recovery ±SD**
zolmitriptan	2.5	2.5	100.0±0.014	2.48	99.20±0.010

**average of six determinations

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METHOD B: AREA UNDER CURVE SPECTROSCOPY

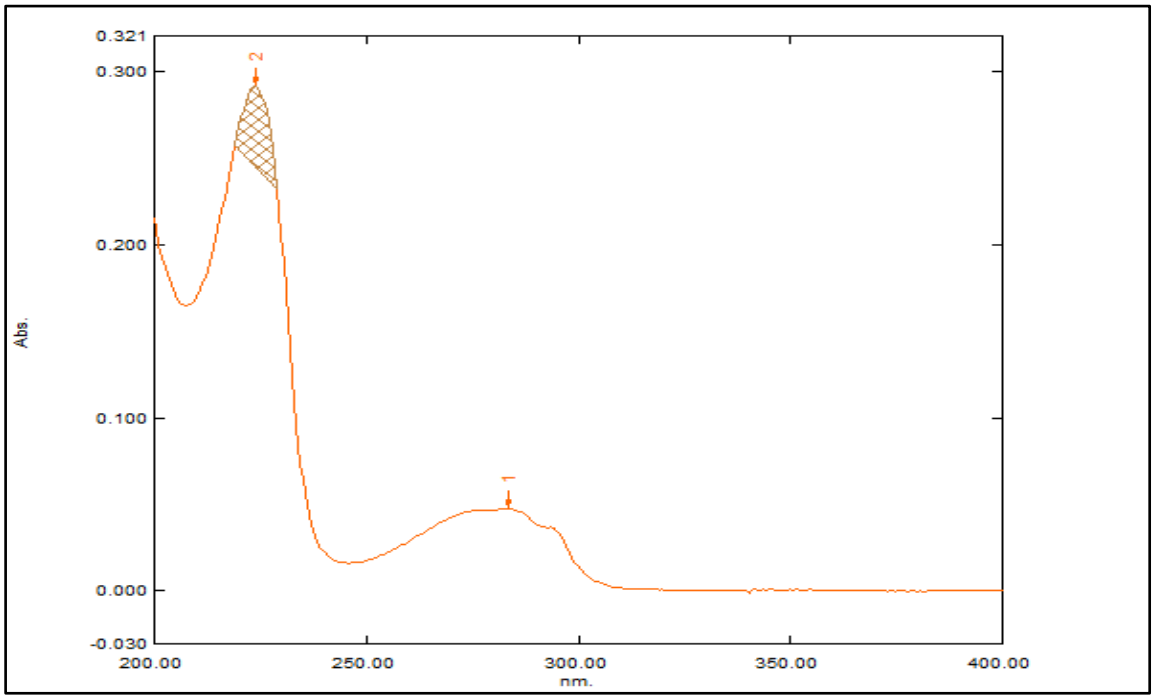

Fig 3 - Area Under Curve Spectra of Zolmitriptan at 219-229 nm

Table 7 - Results of Calibration Curve at 219-229 nm for Zolmitriptan by Area Under Curve Spectroscopy

Sr. no.	Conc. (µg/ml)	Absorbance at 224 nm
1	0	0
2	1	0.145
3	2	0.285
4	3	0.439
5	4	0.590
6	5	0.727
7	6	0.861

Table 8 - Optimum Conditions, Optical Characteristics and Statistical Data of the Regression Equation in Area Under Curve Spectroscopy

Parameters	UV method
λ_{max} (nm)	219-229
Beer's law limits (µg/ml)	1-6
Molar extinction coefficient (L mol^{-1} cm^{-1})	$0.0154x10^4$

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Sandell's sensitivity (µg/cm)	0.007
Regression equation (Y*)	Y=0.1447x0.0011
Slope (b)	0.1447
Intercept (a)	0.0011
Correlation coefficient (r^2)	0.9994
Intraday Precision (% RSD**)	0.441
Interday Precision (% RSD**)	0.975
Limit of detection (µg/ml)	0.105
Limit of quantitation (µg/ml)	0.318

*Y= bx+a where x is the concentration of Zolmitriptan in µg/ml and Y is the absorbance at the respective λ_{max}
**average of six determinations

Table 9 - Determination of Accuracy Results for Zolmitriptan by Area Under Curve Spectroscopy

Brand used	Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount recovered (µg/ml)	% recovery ± SD**
Zolmitriptan	2	1	0.998	99.8 ± 0.59
	2	2	2.006	100.31 ± 0.87
	2	3	2.996	99.73 ± 0.35

**average of six determinations

Table 10 - Determination of Precision Results for Zolmitriptan by Area Under Curve Spectroscopy

Concentration (µg/ml)	Inter-day absorbance Mean ± SD**	% CV	Intra-day absorbance Mean ± SD**	% CV
1	0.144 ± 0.00164	1.13	0.0423±0.001211	2.86
2	0.301 ± 0.00485	1.61	0.0888±0.001751	1.98
3	0.437 ± 0.0306	0.70	0.1363±0.00216	1.58

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4	0.594 ± 0.00509	0.86	0.1836±0.00216	1.17
5	0.731 ± 0.01088	1.49	0.2333±0.001966	0.84
6	0.874 ± 0.01051	1.20	0.2738±0.002408	0.87

**average of six determinations

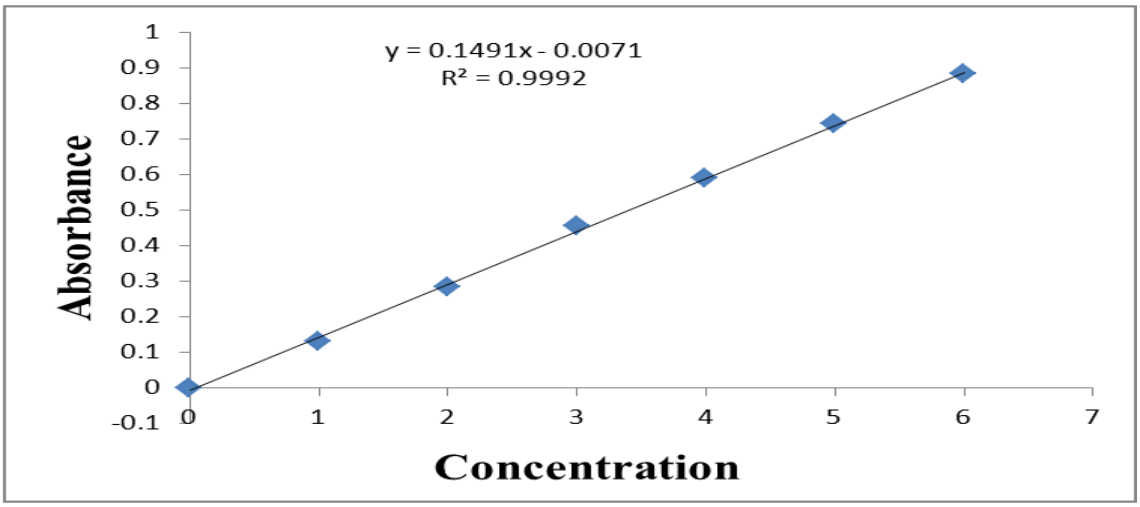

Fig 4 – Calibration Curve for Zolmitriptan between 219-229 nm by Area Under Curve Spectroscopy

Table 11 - Ruggedness Results for Zolmitriptan between 219-229 nm by Area Under Curve Spectroscopy

Brand used	Label claim (mg)	Analyst I		Analyst II	
		Amount found** (mg)	% Recovery ±SD**	Amount found** (mg)	% Recovery ±SD**
zolmitriptan	2.5	2.5	99.8±0.033	2.47	98.80±0.008

**average of six determinations

IV. CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision.

Few analytical methods were appeared in the literature survey for the determination of zolmitriptan, which includes HPLC and LCMS. In view of the above fact few simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical.

A UV Spectrophotometry method for the determination of Zolmitriptan was developed byusing Shimadzu-1800 UV/Vis double beam Spectrophotometer with 1 cm matched quartz cells at maximum wavelength of 224 nm which was determined by recording the spectra in the wavelengthregion of 200-400 nm using Methanol and Distilled water in the ratio of (1:1). And the spectra were presented as Fig: 5.1 and 5.3.

The optical characteristics such as Beer's law limits, Molar absorptivity, Sandell's sensitivity, Limitof detection and Limit of quantitation etc., in each method were calculated and the results werepresented in Table 3 andTable 8, respectively. Also the regression characteristics like slope (b),intercept (a), and correlation coefficient (r) using the method of least squares were calculated and were presented in Table 3 and Table8, respectively. The results showed that the methods is precise.

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Accuracy of the developed method was determined by recovery studies by adding known amount ofthe pure drug to the pharmaceutical formulation and the percentagerecovery studies were determined and data were presented in Table 4 and Table 9, respectively. Theresults were within the range of 99.73 ± 0.35 to 10.36 ± 0.47 and were found to be highly accurate.

The interference studies were carried out to the excipients present in the dosage forms of Zolmitriptan. Excipients did not interfere, when estimated by the proposed methods. The reported methods werefound to be simple, sensitive, accurate, precise, and economical and can be used in the determination of Zolmitriptan in pharmaceutical formulations. The precision of an analytical method was calculated by performing intra-day precision and inter-day precision studies. The valueswere found to be precise and were presented in Table 5 and Table 10, respectively. The linearity was foundin the concentration range of 1-64g/ ml tor Zero order and Area under curve-spectroscopy. Thecorrelation coefficients were found to be 0.9996 and 0.9994, respectively. The obtained (r^2) valuesshow that the selected concentration range gives good linearity.

The ruggedness studies were performed by the two analysts for the dosage form.The % recoveries were calculated and were given in Table 6 and Table 11 respectively. The values were between the ranges of 99.20 ± 0.010 - 100.0 ± 0.014.These values were found to be within the limit and the method is found to be rugged.

In the present investigation, two simple and sensitive UV spectrophotometric methods were developed for the quantitative estimation of zolmitriptan in bulk drug and pharmaceutical formulations. In addition to positive requirements of these analytical methods, the striking advantage of all the presently developed methods was that they were economical.

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