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Development and Validation of a Simple UV Method for In-Vitro Estimation of Zolmitriptan in an Intraoral Dosage form

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ABSTRACT

A simple, sensitive, rapid, specific, cost effective and reproducible UV spectrophotometric method was developed for the estimation of Zolmitriptan in a pharmaceutical formulation. Zolmitriptan exhibited maximum absorbance at 283 nm. Beer's law was obeyed in the concentration range of 5 to 60 µg/ml. The lower limit of detection and the limit of quantification were found to be 0.14 µg /ml and 0.43µg/ml respectively. Results of analysis were validated by statistical analysis and by recovery studies. The method was validated with respect to linearity, precision, LOD, LOQ and sensitivity. The determination of the zolmitriptan was not interfered by the excipients of the product. For Intraday and interday precision % RSD was less than 1.56%.The proposed method was found to be suitable for the analysis of Zolmitriptan in oral films and pharmaceutical formulation at pH6.8.

Keywords: zolmitriptan, migraine, validation, UV Spectrophotometer,

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INTRODUCTION

Zolmitriptan, 4S-4-({3-[2-(dimethylamino) ethyl]-1H-indol-5-yl} methyl)-1,3-oxazolidin-2-one, is a second-generation triptan prescribed for migraine patients, with or without an aura, and cluster headaches. It has a selective action on serotonin (5-HT1B/1D) receptors. Zolmitriptan mimics the action of serotonin by directly stimulating the serotonin receptors in the brain, causing the vasoconstriction of perivascular nerve terminals thus treating migraine pain. [1-3]

Analysis is an important aspect of formulation development of any drug molecule. A suitable and validated method should be available for the analysis of drug in the bulk, in drug delivery systems, for release dissolution studies.

For development of an intraoral drug delivery system for zolmitriptan and comparing of different formulations, it is desirable to quantify its release from the dosage form during *in vitro* studies.

The assay of ZMT in pure and dosage forms, as far as we know, is not official in any pharmacopoeia, and therefore, requires much more investigation. Literature survey reveals that only few analytical methods have been reported for the estimation of Zolmitriptan and are UV-Extractive Spectrophotometry, HPLC [4], LC[5] and LC-MS-MS [6, 7]. The reported methods are relatively expensive and complex. Although Raza A et al and NGR Rao *et al*, have developed and reported two methods but estimation of drug in phosphate buffer at pH 6.8 was not reported.

The objective of the study was to develop a simple, sensitive, rapid, precise, cost effective and reproducible UV method for estimation of Zolmitriptan in phosphate buffer pH 6.8.

MATERIALS AND METHODS

A UV/Vis double beam spectrophotometer (Schimadzu 1700) with 1 cm matched quartz cells was used for spectral measurement. The reference standard of Zolmitriptan was procured as a gift sample from Cipla Pharmaceuticals (India). HPMC E5 and Polyplasdone XL-10 were purchased from HIMEDIA Laboratories Pvt. Ltd. Mumbai and SIGMA Life science St. Louis USA respectively.

Method

Preparation of Standard Stock Solution

Standard solution of Zolmitriptan (100µg/ml) was prepared by dissolving 100 mg of pure Zolmitriptan in 1000 ml volumetric flask containing 100 ml phosphate buffer pH 6.8 and adjusted the volume up to 1000 ml.

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Preparation of Calibration Curve

Aliquots of 0.5 to 6 ml portions of the standard solution were transferred to a series of calibrated 10 ml volumetric flasks, and volume was adjusted with phosphate buffer pH 6.8. Solutions were scanned in the range of 200-400 nm against blank (phosphate buffer pH 6.8) and the absorbance of solutions was measured and calibration curve was constructed.

Method Development

UV method selection: A simple, sensitive, rapid, precise, cost effective and reproducible UV spectrophotometric method has been developed for *in-vitro* estimation of zolmitriptan.

Diluent selection: Selection of media was based on solubility and stability of zolmitriptan. Dissolution studies of drug loaded oral films required use of phosphate buffer pH 6.8. Hence phosphate buffer pH 6.8 was selected as analytical media for present work.

Lambda max selection: In the spectrum of zolmitriptan two peaks (Fig 1) 225nm and283nm were observed but **283 nm** was selected because if any small change in the λmax cannot change absorption significantly.

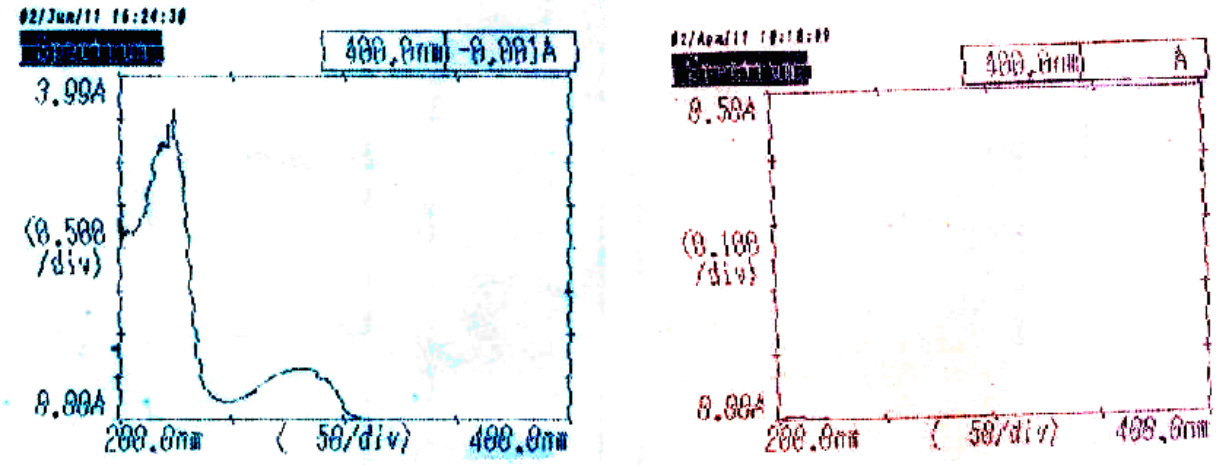

Figure 1 UV spectra of a)standard , b) placebo

Validation of Analytical Procedure [11-14]

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

Linearity was assessed by three methods;

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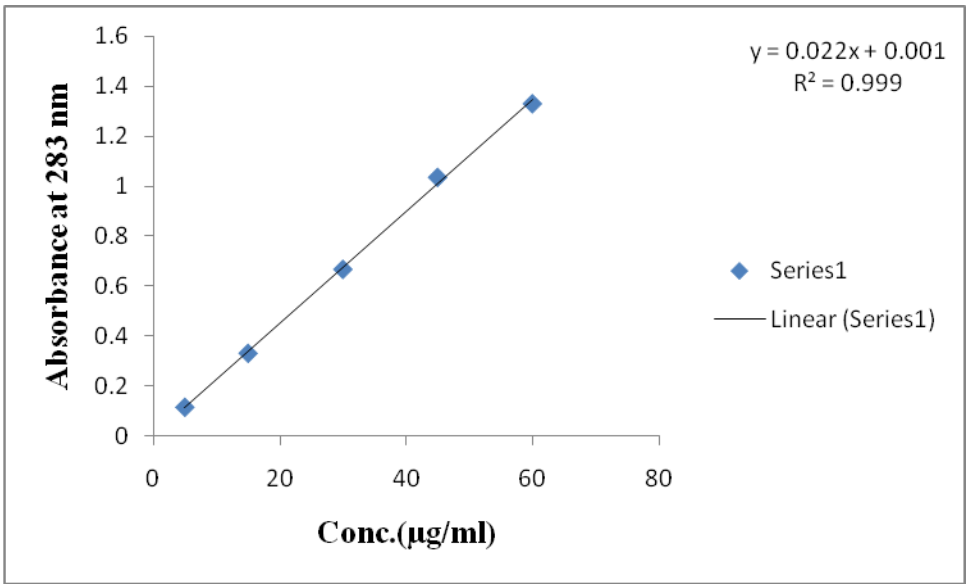
Figure 2 shows calibration curve of Zolmitriptan at 283 nm



A) Visual Examination

Linearity was calculated with suitable aliquots of standard stock solution of zolmitriptan (0.5, 1.5, 3.0, 4.5, 6.0 ml) taken, diluted up to 10 ml with phosphate buffer pH 6.8 and corresponding conc. of solutions were approximately 5,15,30,45 and 60 µg/ml. Linear regression of absorbance Vs concentration yielded the equation y = 0.022x + 0.001, where the correlation coefficient was 0.999 given in table 1.

TABLE 1: Analytical validation parameters:

Parameter	Result
Absorption maxima (nm)	283
Linearity range	5 to 60 µg/ml
Regression equation Slope Intercept	y= 0.022x+0.001 0.022 0.001
Correlation coefficient r^2	0.999
Accuracy	98.67 ± 0.39%
LOD	0.14 µg /ml
LOQ	0.43µg/ml.
System suitabilty	0.5492±0.004

B) Residual Analysis

Residual curve and response curve were calculated for statistical specification of calibration curve. For the residual curve there were five points same as calibration curve and residual point were calculated by the difference of predicted absorbance and observed

absorbance. The residual curve limit showed sum of residual was zero or with in limit respective to method as in table 2.

Residual = Observed value - Predicted value

Table 2 Response factor analysis

S. No.	Conc. (µg/ml)	Observed Value	Predicted Value	Residual	Response factor
1	5	0.115	0.111	0.004	0.0230
2	15	0.331	0.331	0.000	0.0221
3	30	0.667	0.661	0.006	0.0222
4	45	1.035	0.991	0.044	0.0230
5	60	1.33	1.321	0.009	0.0222

C) Response curve

Response curve were calculated with suitable aliquots of standard stock solution (5, 15, 30, 45 and 60µg/ml). Response curve were calculated by response factor (response/concentration). Response curve were showed good result and showed equally positive point from the mean of response point.

Range

Linearity range: 5-60µg/ml
Working range: 1-60µg/ml
Target concentration: 32.5µg/ml.

Accuracy (Recovery Study)

In order to ascertain the suitability and reproducibility of the proposed method, recovery studies were carried out by adding known quantities of standard Zolmitriptan (80,100,120%) to the placebo sample solution and the mixtures were analyzed by the proposed method. Three samples were prepared for each recovery level. The percentage recovery of was found to be 98.67 ± 0.39% indicating that there is no interference by the excipients in the method.

Precision

Intra-day method precision(method precision) was evaluated by analyzing by six samples of fast dissolving oral film of Zolmitriptan on same day .The intermediate precision (inter-day precision) of the method was determined by evaluating the samples of Zolmitriptan film on different days and by two different analysts on two different U.V. spectrophotometer in the same laboratory.

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Its calculation was based on based on the Standard Deviation of the Response and the Slope. Standard deviation of response was calculated by calibration curve and slope from calibration curve.

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Several approaches for determining the detection and quantitation limit are based on visual evaluation, Signal-to-Noise ratio and standard deviation of the response and the slope. Its calculation was based on based on the Standard Deviation of the Response and the Slope. Standard deviation of response was calculated by calibration curve and slope from calibration curve.

The LOD and LOQ of Zolmitriptan were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ were found to be 0.14 µg /ml and 0.43µg/ml.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. It was carried out by varying ±2nm in working λ_{max} i.e.281nm and 285nm and observed the absorbance given in table 3.

Table 3 Results of Robustness at λmax± 2

	λmax 283nm	λmax 281nm	λmax 285nm
Mean	0.116	0.119	0.118
SD	0.001	0.001	0.002
RSD	0.86	0.84	0.85

System Suitability Testing

System suitability testing was done by injecting six samples of 25µg/ml zolmitriptan solution and absorbance measured by UV spectrophotometer at 283 nm. Mean o absorbance and relative standard deviations were calculated.

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DISCUSSION

In the proposed method, the λ max of zolmitriptan was found to be 283nm and there was no interference of placebo (blank oral film) in determination of zolmitriptan form dosage form.

The quantification was linear in the concentration range of 5 to 60 µg/ml. The regression equation of the linearity plot of concentration of zolmitriptan over its absorbance was found to be y = 0.022x + 0.001 (r^2 = 0.999) **.** The accuracy of the method at 80%,100% and 120% level was performed and shows the mean range of 98.67±0.39 and % RSD =0.41. The results of intraday and inter day evaluation of zolmitriptan film shows the mean of 2.28 mg and 2.33mg, RSD of 1.05 and 0.76 respectively indicating repeatability of method. . Also reproducibility is confirmed as %RSD = 0.6 was obtained on employing U.V.-Pharmaspec 1700 Shimadzu and Double beam spectrophotometer 2203 ^{smart} respectively. The Robustness of developed method was checked by changing λmax ±2 nm and result was found to be satisfactory with %RSD = 0.85.The limit of detection and limit of quantification were found to be 0.14 µg /ml and 0.43 µg /ml respectively which indicate sensitivity of the method.

CONCLUSION

The proposed method is simple, rapid accurate, precise, reproducible, and economical with good precision. It is specific while estimating dosage form without interference of excipients and other additives. Thus this method can be used for routine quantitative analysis of Zolmitriptan in bulk and oral film dosage form.

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