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Research Article

UV-Spectrophotometric Methods For The Determination Of Zolmitriptan in Bulk and Pharmaceutical Dosage Forms

Sasmita Kumari Acharjya*, M.E.Bhanoji Rao, B.V.V. Ravi Kumar, M.Mathrusri Annapurna

Department of Pharmaceutical Analysis and Quality Assurance
Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India, 760010.
**Corresponding Author: acharjya2009@gmail.com*

ABSTRACT

UV, first derivative, and AUC-spectrophotometric methods for the determination of zolmitriptan in bulk and pharmaceutical formulations have been developed. For the UV-spectrophotometry, standard solutions were measured at 283.0 nm. The linearity ranges were found to be 0.5–100 µg/mL in 0.1M HCl and the regression equation was $A=2.02\times10^{-2}C+4.6\times10^{-4}$ ($r^2=0.9999$). For first derivative spectrophotometry, the response ($dA/d\lambda$) of standard solutions was measured at 298.0 nm. Calibration curve was constructed by plotting $dA/d\lambda$ values against concentrations, 1–100 µg/mL of zolmitriptan. Regression equation of linear calibration graph was calculated as $D_1= -1.14\times10^{-3}C-2.00\times10^{-5}$ ($r^2=0.9999$). The AUC-spectrophotometric method was based on calculation of area under curve (AUC) for analysis of zolmitriptan in the wavelength range of 278.0–288.0 nm. Calibration curve was constructed by plotting AUC values against concentrations, 0.5–100.0 µg/mL of zolmitriptan. Regression equation of linear calibration graph was calculated as $AUC=1.963\times10^{-1}C+1.34\times10^{-3}$ ($r^2=0.9999$). The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of zolmitriptan in pharmaceutical formulations.

Keywords: Zolmitriptan, UV-spectrophotometry, Derivative-spectrophotometry, AUC- spectrophotometry

1. INTRODUCTION

Zolmitriptan, (4*S*)-4-[[3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl] methyl]-2-oxazolidinone, is a novel serotonin 5-hydroxytryptamine receptor agonist that has shown, in an extensive clinical trial program, to be highly effective in the acute oral treatment of migraine with or without aura [1-2]. It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. Zolmitriptan mimics this action of serotonin by directly stimulating the serotonin receptors in the brain and it relieves the pain of migraines.

Zolmitriptan is not official in IP, BP and USP. A detailed literature survey for zolmitriptan revealed that several analytical methods such as spectrophotometric [3-4] and HPLC [5- 8] were reported for the quantification of zolmitriptan. Some HPLC methods were reported for Zolmitriptan quantification in plasma samples: coulometric detection [9], liquid chromatography/tandem mass spectroscopy [10-14] and liquid chromatography with fluorescence detection [15-16]. A few validated chiral LC methods [17-19] and a capillary electrophoresis method [20] was also reported for zolmitriptan quantification.

In the previous studies, Aydogmus et al.[3] developed two extractive spectrophotometric methods which were based on the formation of yellow ion-pair complexes between zolmitriptan and tropaeolin (TPOO) and bromothymol blue (BTB) in citrate-phosphate buffer of pH 4.0 and 6.0, respectively. Both the methods were time consuming because it involves extractions steps. Raza et al. [4] developed an extraction-free spectrophotometric method and Beer's law is obeyed in the concentration range 10-250 µg/mL. This method is not sensitive to estimate low concentrations of zolmitriptan in biological fluids. The other reported methods [5-20] require special instruments, which are not commonly available in routine laboratories. In addition, some procedures can be laborious and time consuming. The UV spectrophotometric (UV) method is very simple, rapid and economical and allows the determination of drugs with sufficient reliability.

The aim of this study was to develop simple UV-spectrophotometric procedures for routine analysis of zolmitriptan in pharmaceutical dosage forms. The analytical method for zolmitriptan analysis is not officially available in any pharmacopoeia and previous methods were mainly focused on the analysis of the drug in biological fluids. Thus, it is important to develop methods, which is applicable for routine quality control of the drug. The developed methods were validated for its linearity, accuracy, precision, specificity, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

2. MATERIAL AND METHODS

2.1 Chemicals and reagents

Zolmitriptan working standard was obtained as gift from Dr. Reddy's Laboratories Limited, Hyderabad, India. The purity of zolmitriptan was evaluated by obtaining its melting point and

ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. A tablet formulation containing 2.5 mg of zolmitriptan was purchased from local market. 0.1M hydrochloric acid (HCl) of analytical grade solution was prepared in double distilled water.

2.2 Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; derivative mode: ^{1}D (first order derivative, dA/dλ); band width ($\Delta\lambda$):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Denver, Germany).

2.3 Preparation of standard stock and working standard solution

The standard stock solution of zolmitriptan was prepared by dissolving accurately weighed 10 mg of the drug in 0.1M HCl and diluted to 10 mL with same solvent to obtain a final concentration of 1000 µg/mL. This stock solution was further diluted to get a working standard solution 100 µg/mL.

2.4 Method I: UV- spectrophotometry

Series dilutions of the standard solution were made by pipetting out 0.05, 0.1, 1.0, 2.0, 4.0, 6.0, 8.0 mL from working standard solution (1mL=100µg of zolmitriptan) and 1.0 mL from stock solution (1mL=1000µg of zolmitriptan) into separate 10 mL volumetric flasks and diluting to volume with 0.1M HCl to produce the concentrations ranging from 0.5-100.0 µg/mL. The above solutions were scanned over the range of 400 nm to 200 nm against blank. The λ_{max} was found to be at 283.0 nm. The calibration curve was constructed by plotting concentration (0.5-100.0 µg/mL) versus absorbance at 283.0 nm.

Method II: First- derivative spectrophotometry

The spectrum obtained in Method I was derivatised to get first order derivative spectra and the response (dA /dλ) of the spectra were measured at 298.0 nm and then calibration curve was constructed by plotting concentration (1.0-100.0 µg/mL) versus response (dA/dλ) at 298.0 nm.

Method III: Area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrum obtained in Method I was used to calculate AUC. The calibration curve was constructed by plotting concentration (0.5-100 µg/mL) versus AUC.

2.5 Preparation of Sample Solution

Twenty zolmitriptan tablets (2.5 mg each) were weighed, transferred to a clean dry mortar and ground into a fine powder using a pestle. Tablet powder equivalent to 10 mg of drug was transferred to a 100 mL volumetric flask and 50 mL 0.1M HCl was added. After ultrasonic vibration for 30 min, the mixture was diluted to volume with 0.1M HCl and filtered through Whatman filter paper (No. 41). From the filtrate an appropriate aliquot was taken in such a way that the final concentration in 10 mL lies within the linearity range tested. The responses were measured and concentration in the sample was determined by comparing the response of sample with that of the standard. The assay results for zolmitriptan in its pharmaceutical dosage forms obtained by using the different spectrophotometric methods were compared by using one-way ANOVA.

3. VALIDATION OF METHODS

The proposed methods were validated as per ICH guidelines [21]

3.1 Linearity

For all the methods, calibration curve was prepared on 3 different days. The calibration curve was constructed by plotting the response (*y*) versus the theoretical concentrations of standards (*x*), by using linear regression analysis. Linearity was expressed as a correlation coefficient; the value must be >0.9990.

3.2 Precision

The intraday and interday precisions of the proposed spectrophotometric methods were determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of zolmitriptan (10.0, 20.0, and 40.0 µg/mL) and the results are reported in terms of percent relative standard deviation.

3.3 Accuracy

The accuracy of the method was determined by calculating recoveries of zolmitriptan by the method of standard additions. The study was performed by spiking three known amounts of zolmitriptan (8.0, 10.0, and 12.0 µg/mL; ranging from 80% to 120%) into a prequantified sample solution (10 µg/mL). Three samples were prepared at each of these concentrations. The recovery of added drug was estimated by measuring the response and by fitting these values to the straight-line equation of calibration curve.

3.4 Specificity

Results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the methods were said to be specific.

3.5 Ruggedness

Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot (10 µg/mL) in different laboratories by different analyst using similar operational and environmental conditions. The results are reported in terms of percent relative standard deviation.

4. RESULTS AND DISCUSSION

The molecular structure of the zolmitriptan is presented in Figure No. 1. Zolmitriptan was completely soluble in methanol and 0.1M HCl, whereas the solution was turbid in the presence of water and 0.1M NaOH (sodium hydroxide). 0.1M HCl was selected as the solvent for zolmitriptan because it's cost is less as compared to methanol and also provides the highest solubility and UV absorbance without interference from sample matrix for direct UV, first derivative and AUC measurements. The first derivative was performed to prove whether sample matrices of the investigated marketed formulation would interfere with the zolmitriptan spectrum. Area under curve method was developed because zolmitriptan produces broad spectrum in fundamental spectrum. Results showed that, direct UV, first derivative and area under curve method measurements are feasible for the analysis of zolmitriptan without interference from sample matrices. Figure No. 2 and 3 shows overlaid UV-spectrophotometric (0.5–100 µg/mL) and first-derivative (1–100 µg/mL) absorption spectra of zolmitriptan respectively, and the spectra were found to be similar in nature and overlapping. Figure No. 4 shows the absorption spectrum of zolmitriptan (10 µg/mL) in 0.1M HCl for the method III. Optical characteristics of zolmitriptan were calculated by the proposed methods and presented in Table No. 1.

From the calibration curve (Graph No. 1), it was observed that with the increase in zolmitriptan concentration, the responses are increased. In Method I, the λ_{max} was found to be at 222.0 and 283.0 nm (Figure No. 2). But the study was carried out at 283.0 nm, because at this wavelength the Beer- Lambert's law was following properly with good linearity range. For Method II (Figure No. 3), 298.0 nm was selected because at 236.0 nm, maximum wavelength of the peaks as well as zero crossing point are not remaining constant and at 263.0 nm Beer- Lambert's law was not following properly. For Method III (Figure No. 4), study was carried out at two wavelength ranges i.e 273.0-293.0 nm and 278.0-288.0 nm, but good linearity range was obtained at the wavelength range of 278.0-288.0 nm.

A correlation coefficient of 0.9999 was observed for all the methods, suggests that the developed methods had an excellent linearity over the selected concentration range. Commercially available tablet formulation for zolmitriptan was obtained and assayed as described in Preparation of Sample Solution. The assay results obtained by all the methods for tablet dosage form were comparable with the label value claimed. The results indicate the recovery of zolmitriptan from the pharmaceutical preparation was quantitative (Table No. 2); there was no interference from the excipients present in the dosage form when compared to the control. It was also observed that there was no significant difference in the content of zolmitriptan obtained by using the different proposed spectrophotometric methods. Moreover one-way ANOVA was used to compare the data. *p*-Value obtained was 0.3093 ($p > 0.05$).

The intra-day and inter-day precision values (%RSD) were calculated (Table No. 3**)** and lying in the acceptable limits (≤2%) for zolmitriptan. The accuracy of zolmitriptan which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% were found to be in the acceptable limits (≤2%) (Table No. 4). This indicates that there was no interference from the excipients present in the dosage form. Ruggedness of proposed methods was determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range (Table No. 5).

Comparison of UV Spectrophotometric method with first derivative and Area under curve method

The assay results for zolmitriptan in its pharmaceutical dosage forms obtained by using the different spectrophotometric methods were compared by using one-way ANOVA. *p*-Value obtained was 0.3093($p > 0.05$), which indicates that there was no significant difference in the content of zolmitriptan determined by the different spectrophotometric methods.

Table No. 1: Optical characteristics of zolmitriptan

Parameters	Method I	Method II	Method III
Beer-Lambert's range(µg/mL)	0.5–100	1–100	0.5–100
λ_{max}(nm)/ wave length range (nm)	283.0	298.0	278.0-288.0
Molar absorptivity (L/mol.cm)	5632.19328	-316.09248	54885.1488
Sandell's sensitivity (µg cm^{-2}/0.001 A)	0.05102	-	-
Slope	0.020127	-0.00114	0.19677
Standard deviation of slope	0.0002	0.00001	0.0007
%RSD of slope	0.9379	-0.8772	0.3566
Intercept	0.000457	0.00002	0.001314
Standard deviation of intercept	0.00001	2.6 $X10^{-07}$	0.000025
%RSD of intercept	1.264	1.336	1.876
Correlation coefficient	0.9999	0.9999	0.9999
%RSD of Correlation coefficient	0.0026	0.003	0.0015
Limit of detection(µg/mL)	0.000947	0.000766	0.000413
Limit of quantitation (µg/mL)	0.002869	0.002321	0.001252

Table No. 2: Assay results of commercial zolmitriptan tablet

Zolmitriptan marketed formulation	Label claim/Tablet	% Recovery* ±SD			%RSD		
		Method I	Method II	Method III	Method I	Method II	Method III
Tablet	2.5mg	99.927±0.267	100.37±0.577	99.82±0.359	0.267	0.575	0.3599

**Average of three determinations*

Table No. 3: Precision

Conc. µg/mL	Intraday						Interday					
	Response[a]± SD[a]			%RSD[a]			Response[a]± SD[a]			%RSD[a]		
	Method I	Method II	Method III	Method I	Method II	Method III	Method I	Method II	Method III	Method I	Method II	Method III
10	0.197± 0.00153	-0.0112± 0.000153	1.93± 0.02	0.774	-1.368	1.0363	0.197± 0.001	-0.011± 0.00015	1.933± 0.021	0.508	-1.372	1.077
20	0.413± 0.00153	-0.023± 0.0002	4.037± 0.0025	0.3696	-0.8772	0.0623	0.414± 0.0021	-0.023± 0.00021	4.047± 0.0111	0.502	-0.914	0.275
40	0.807± 0.00153	-0.0457± 0.00031	7.813± 0.0404	0.189	-0.669	0.517	0.808± 0.001	-0.046± 0.0004	7.847± 0.0751	0.124	-0.831	0.957

[a] *Mean, SD and %RSD (n = 3).*

Table No. 4: Accuracy

Nominal value,%	C_{sample} µg/mL	$C_{standard\ added}$ µg/mL	Recovery, %			Average recovery,(n=3)± SD			RSD, %		
			Method I	Method II	Method III	Method I	Method II	Method III	Method I	Method II	Method III
80	10	8	98.93 99.78 99.21	99.23 97.34 100.34	100.23 100.34 98.23	99.307± 0.4332	98.97± 1.517	99.6± 1.188	0.4362	1.5326	1.1925
100	10	10	101.23 100.46 100.67	102.34 101.43 101.5	100.23 100.98 100.34	100.787± 0.398	101.75± 0.5064	100.517± 0.405	0.395	0.498	0.403
120	10	12	100.56 100.48 100.87	100.27 100.31 100.36	102.32 102.23 103.32	100.637± 0.206	100.31± 0.0451	102.623± 0.605	0.205	0.045	0.5895
Mean (n=9)						100.243± 0.346	100.34± 0.689	100.913± 0.732	0.345	0.692	0.728

Table No. 5: Ruggedness

Analyst I, %RSD			Analyst II, %RSD		
Method I	Method II	Method III	Method I	Method II	Method III
0.44	0.54	0.38	0.46	0.52	0.41

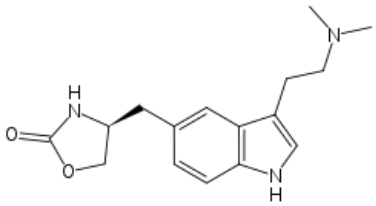


Figure No. 1: Chemical structure of Zolmitriptan

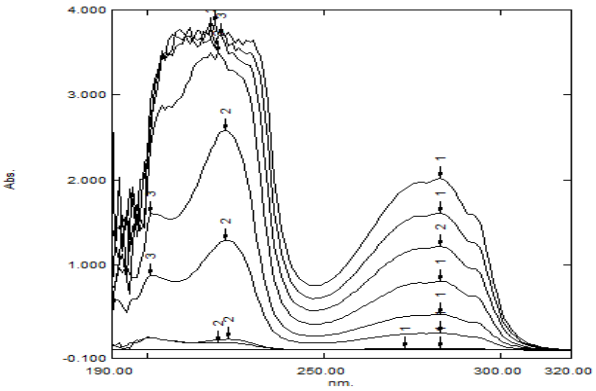
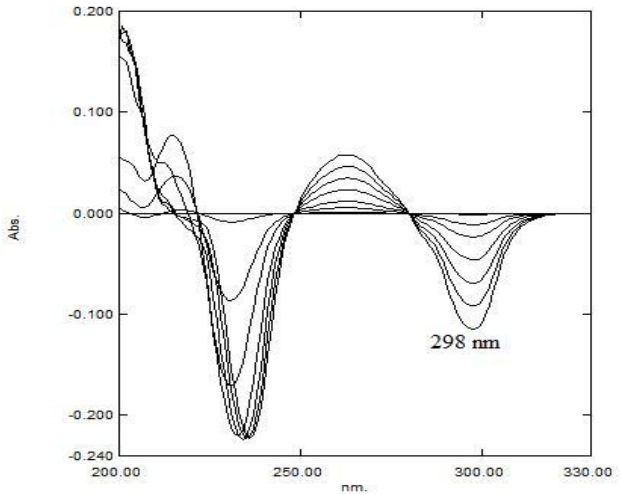
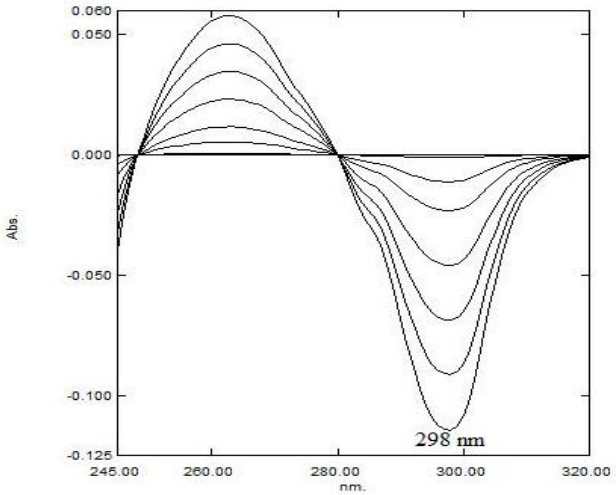


Figure No. 2: Absorption spectrum of zolmitriptan in 0.1M HCl (0.5–100 µg/mL)



(A)



(B)

Figure No. 3: First- derivative absorption spectrum of zolmitriptan in 0.1M HCl (1–100 µg/mL): (A) Normal View (B) Large View

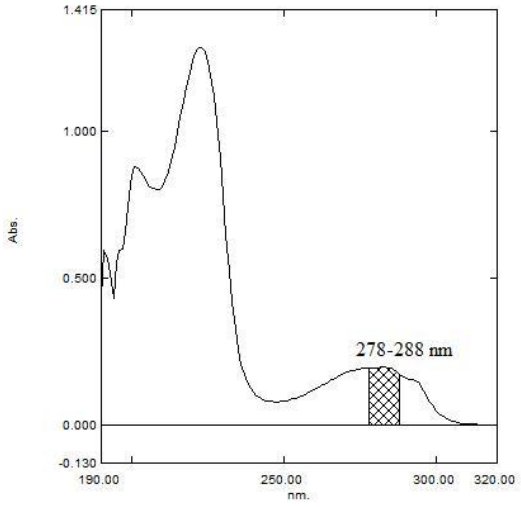
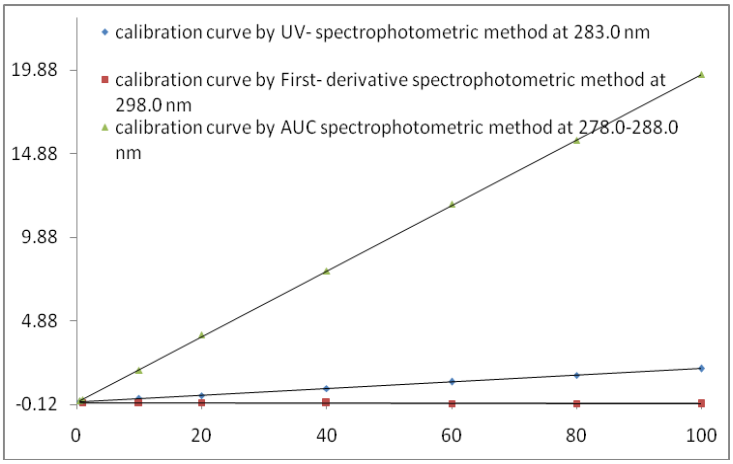


Figure No. 4: Absorption spectrum of zolmitriptan (10 µg/mL) in 0.1M HCl [278.0-288.0 nm range was selected for MethodIII]



Graph No. 1: Calibration curves of zolmitriptan in 0.1M HCl (Method I, II and III)

5. CONCLUSION

Three methods that were developed for the determination of zolmitriptan are based on different analytical techniques, UV-spectrophotometric, first-derivative and AUC method. All the methods were validated and found to be simple, sensitive, accurate, and precise. Hence, all the methods can be used successfully for routine analysis of pharmaceutical dosage forms of zolmitriptan. The proposed spectrophotometric methods will not replace the presently known methods available for the analysis of zolmitriptan. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

6. ACKNOWLEDGEMENTS

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