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Validation of Spectrophotometric Methods for the Assay of Zolmitriptan in Dosage Forms

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ABSTRACT

The spectrophotometric methods M_6, M_7, M_8 and M_{10} for the determination of Zolmitriptan in pure and dosage forms have been described in this paper. The present methods involve the determination of Zolmitriptan in pharmaceutical dosage at the given optimum conditions. The stock solution (1.0 mg/mL) of Zolmitriptan was prepared by dissolving 100 mg of the drug in 10.0 mL of methanol and made up to 100 mL with distilled water to get a clear solution. Appropriate volumes of this stock solution were diluted step wise to get the working standard solutions of concentrations 200 µg/mL for Methods-M_8, M_{10},; 240 µg/mL for Method-M_6; and 300 µg/mL for Method-M_7 respectively. The proposed methods have the good sensitivity and higher λ_{max}. Statistical analysis of the results showed that the proposed procedures have good precision and accuracy. The spectrophotometric methods based on reactivity of the functional groups of Zolmitriptan with various organic reagents to produce colored species of reasonable stability, paving the possibility for spectrophotometric determination of Zolmitriptan in pure and pharmaceutical formulations.

Keywords: Zolmitriptan, Spectrophotometric Methods, Spectral Characteristics, Precision and Validation.

INTRODUCTION

The antimigraine drug Zolmitriptan is a selective agonist of serotonin (5-hydroxytryptamine; 5-HT) type 1B and 1D receptors and chemically known as (4S)-4-[[3-[2-(dimethylamino) ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone. The therapeutic activity of Zolmitriptan for the treatment of migraine headache can most likely be attributed to the agonist effects at the $5\text{-HT}_{1B/1D}$ receptors on intracranial blood vessels (including the arteriovenous anastomoses) and sensory nerves of the trigeminal system, which result in cranial vessel constriction and inhibition of proinflammatory neuropeptide release [1]. Zolmitriptan (Figure 1) (S)-4-{[3-(2-dimethylaminoethyl)-1H-indol-5-yl] methyl}-1, 3-oxazolidin-2-one is an oral, selective serotonin receptor. A detailed literature survey for Zolmitriptan revealed that several analytical methods such as spectrophotometric and HPLC were reported for the quantification of Zolmitriptan that are laborious and time consuming. This made the author an attempt to assay the above said drug using simple analytical tools.

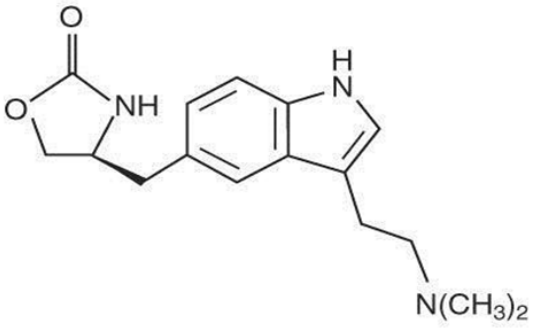


Figure 1: Molecular structure of Zolmitriptan

This paper describes the development and validation of some new UV-Visible spectrophotometric methods and RP-HPLC for the assay of Zolmitriptan in pure and dosage forms. This paper briefs the author experimental work in developing and validating some economical analytical methods in assaying Zolmitriptan in pure and dosage forms. Zolmitriptan is not included in any pharmacopeia. Literature survey reveals that few analytical methods have been published for analysis of Zolmitriptan in human plasma and include high-performance liquid chromatography with coulometric [2], mass spectrometric detection [3–5], and liquid chromatography-mass spectrometry [1, 6, 7].

Most of the reported methods require expensive instrumental setup, expertise personnel, and complicated procedure. Two of the reported visible spectrophotometric methods require liquid-liquid extraction and strict pH control. The aim of the present work is to develop simple, sensitive and cost-effective spectrophotometric method for the determination of Zolmitriptan in pharmaceutical formulation. The method makes use of vanillin as the reagent in presence of concentrated H_2SO_4 and has been demonstrated to be superior to the existing spectrophotometric methods in terms of simplicity, speed, working conditions, and accuracy and precision. However, it is evident that the above said reported methods are not simple and require expensive equipment and moreover, to our knowledge no simple UV-Visible spectrometric methods have been not yet reported in the literature for the determination of Zolmitriptan in pharmaceutical dosage forms and this fact prompted the author to develop accurate and inexpensive UV-Visible spectrophotometric methods for routine determination of Zolmitriptan in pure and tablet dosage forms. Rao et al. have published their results on different oxide materials, luminescent materials and polymers in their earlier studies [8-24]. The present paper describes UV-Visible spectrophotometric methods**,** which are based on reactivity of the functional groups of Zolmitriptan with various organic reagents to produce colored species of reasonable stability, paving the possibility for spectrophotometric determination of Zolmitriptan in pure and pharmaceutical formulations.

MATERIALS AND METHODS

Instruments used: Genesys 10 UV-Spectrophotometer 10 mm matched quartz cells procured from Thermo Scientific Company with were used for all spectral measurements. A Systronics digital pH meter [Model-362] was used for pH measurements.

Preparation of Reagents: All the chemicals and reagents used were of analytical grade and solutions were prepared with doubled distilled water.

Method - M_6 [0.1 %, 2, 4-Dinitro Phenyl Hydrazine Reagent]: Prepared freshly by dissolving 100 mg of 2, 4-Dinitro Phenyl Hydrazine in a mixture of 10 mL of methanol and 0.5 mL of Conc. HCl was added and finally diluted to 100 mL with methanol.

Method - M_7 [2-Chlorophenyl Hydrazine (0.25 % W/V)]: Prepared by dissolving 0.25 g of 2-chlorophenyl hydrazine in 100 mL methanol.

Method - M_8: Solution of FC reagent (0.2 % W/V) was prepared freshly with distilled water.

Method-M_{10}: Various solutions of 2, 2$^{\alpha}$-Bipyridyl reagent (0.01 M), Ferric chloride (0.003 M) and ortho phosphoric acid (0.2 M) were prepared with double distilled water.

Preparation of stock and working standard solutions: The stock solution (1.0 mg/mL) of Zolmitriptan was prepared by dissolving 100 mg of the drug in 10.0 mL of methanol and made up to 100 mL with distilled water to get a clear solution. Appropriate volumes of this stock solution were diluted step wise to get the working standard solutions of concentrations 200 µg/mL for Methods-M_8, M_{10},; 240 µg/mL for Method-M_6; and 300 µg/mL for Method-M_7 respectively.

Procedure for Tablets: Twenty Zolmitriptan tablets (ZOMIG; 5.0 mg) were weighed, transferred to a clean dry mortar and ground into a fine powder using a pestle. Tablet powder equivalent to 100mg of drug was transferred to a 100 mL volumetric flask transferred into a 100 mL calibrated flask, 60mL of methanol was added and the content shaken thoroughly for 15-20 min and the volume was finally diluted to the mark with distilled water, mixed well and filtered through Whatman filter paper No 41. A suitable volume of the filtrate was accurately diluted with distilled water and this solution was used for the determination of Zolmitriptan as per the recommended procedures described below.

RESULTS AND DISCUSSION

Method Development: It involves the Optimization studies for the proposed procedures involve the study of the influence of various factors on the color development [optimal conditions] such as reagent concentration, order of addition of reagents, time, temperature and choice of solvent for maximum color development.

Optimization Studies for the Proposed Methods: The optimization studies for the color development for the proposed methods M_8 and M_{10}, for the assay of Zolmitriptan were found to be same.

Method – M_6 [2, 4-DNPH]: The optimum conditions in this method were established basing on the study of the effects of various parameters such as volume of 2, 4-DNPH solution, volume of solvents solution used initially and subsequently for final dilution and the stability of colored species after final dilution.

Method–M_7 [2- CPH]: The optimum conditions for the proposed methods were found basing on the study of the effects of various parameters such as volume of CPH solution, volume of solvents solution used initially and subsequently for final dilution.

Recommended Procedures: After a systematic and detailed study of the various parameters, as described in optimum condition the following procedures {M_6 [2, 4-DNPH], M_7 [CPH], M_8 [FC] and M_{10} [2, 2-BPL], were proposed for the assay of Zolmitriptan in pure and formulations.

Method – M_6: Aliquots of working standard Zolmitriptan solution ranging from 0.5-2.5 mL (240 µg/mL) were transferred into a series of 10 mL calibrated test tubes. To this 2.0 mL of 2, 4-DNPH reagent was added followed by one drop of Conc. HCl. The mixtures were placed on a boiling water bath for 10 min and were cooled to room temperature. The contents of the tubes were mixed thoroughly and allowed to stand for 10 minutes with occasional shaking at room temperature and the final volume in each tube was made up to the mark with distilled water. The pink colored chromogen was measured spectrophotometrically at 490 nm against a reagent blank.

Method-M_7: Aliquots of (0.5-2.5 mL, 300 µg/mL) standard Zolmitriptan drug solution were transferred into a series of 10 mL volumetric flasks. To each of the above aliquots, 2.0 mL of 2-Chloro phenyl hydrazine was added followed by one drop of concentrated hydrochloric acid and heated to 50-55 ^oC for color development. The absorbance of the color derivatives were measured at 512 nm against reagent blank. The amount of Zolmitriptan was computed from its calibration graph.

Method-M_8: A series (0.5-2.5 mL, 200 µg/mL) of standard Zolmitriptan solutions were transferred into a series of 10.0 mL calibrated tubes and then solutions of NaOH (5.0 mL) and FC (1.5 mL) were added successively. The total volume in each test tube was brought up to 8.5 mL with distilled water. The absorbance of the greenish blue colored complex solution was measured after 5minutes at 750 nm against reagent blank prepared similarly. The amount of Zolmitriptan was computed from the Beer-Lambert plot.

Method-M_{10}: Aliquots (0.5-2.5 mL; 200 µg/mL) of standard Zolmitriptan drug solution were transferred into a series of 10 mL calibrated tubes and then solutions of $FeCl_3$ (1.0 mL) and 2, 2-Bipyridyl (1.0 mL) was added successively. The total volume in each test tube was brought up to 4.0 mLwith distilled water and heated for 10

minutes in a boiling water bath at 90 ^{o}C. After cooling to the room temperature, 2.0 mL of o-phosphoric acid was added in each test tube. The absorbance of the orange colored complex was measured after 5minutes at 494 nm against reagent blank prepared similarly. A calibration curve was prepared by plotting absorbance against concentration of the above said drug and the unknown was read from this plotted calibration curve.

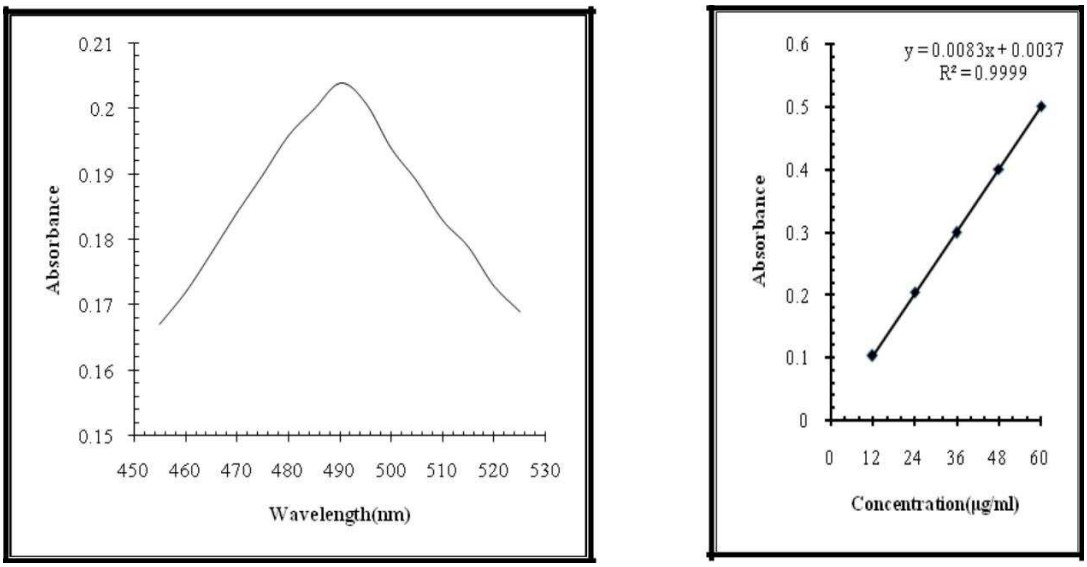


Fig. 2(a&b): Absorption spectra and Beer's law plot of Zolmitriptan for Method-M_6

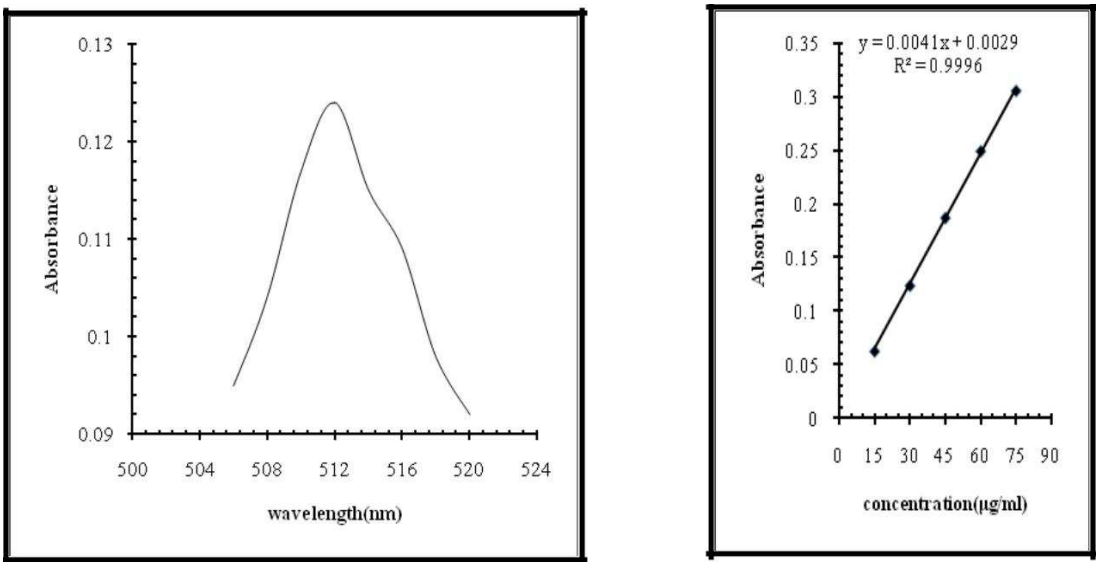


Fig. 3(a&b): Absorption spectra and Beer's law plot of Zolmitriptan for Method-M_7

Method Validation

Spectral Characteristics: The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900 nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in Figure 2(a) for M_6, Figure 3(a) for M_7, Figure 4(a) for M_8 and Figure 5(a) for M_{10}, respectively. The absorption curves of the colored species in each method show characteristic absorption maxima whereas the blank in each method has low or no absorption in this region.

Optical Characteristics: The Beers law plots and Ringbom plots (Figure 2(b) for M_6, Figure 3(b) for M_7, Figure 4(b) for M_8 and Figure 5(b) for M_{10}) of the developed methods were recorded graphically. Beers law limits, molar absorptivity, Sandells sensitivity and optimum photometric range for Zolmitriptan in each method were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values (Table 1).

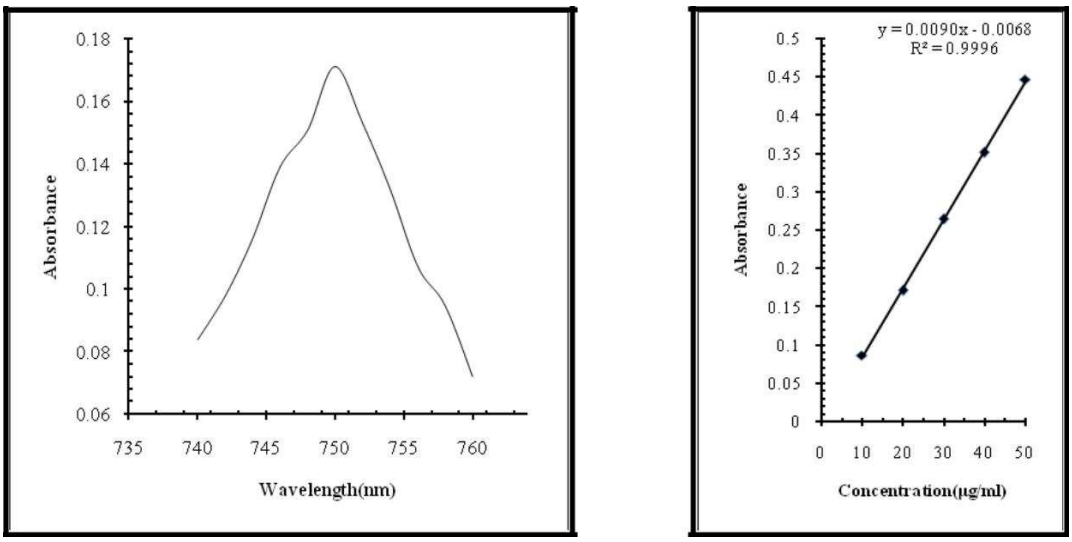


Fig. 4(a&b): Absorption spectra and Beer's law plot of Zolmitriptan for Method-M_8

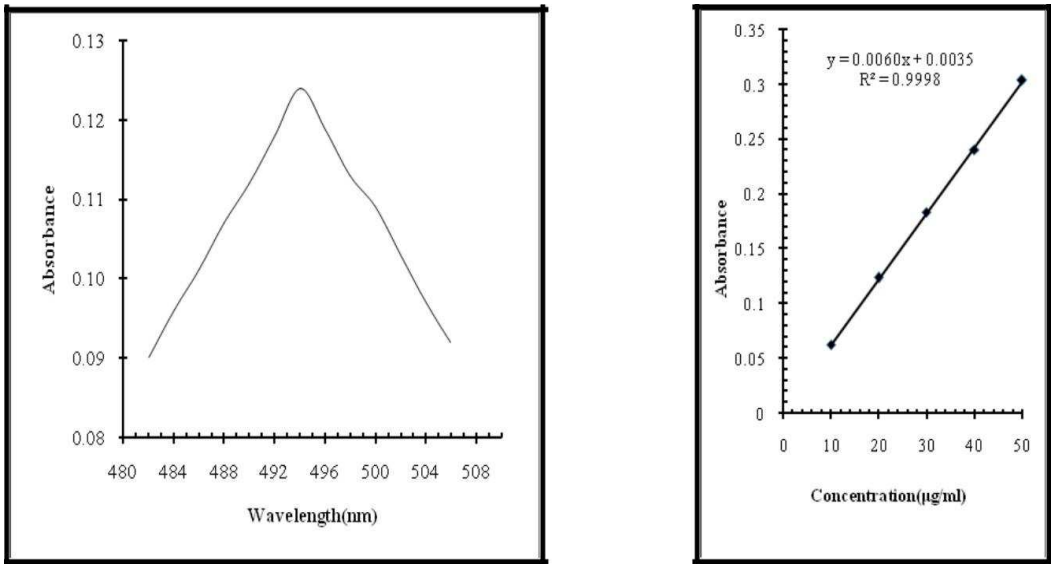


Fig. 5(a&b): Absorption spectra and Beer's law plot of Zolmitriptan for Method-M_{10}

Precision: The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of Zolmitriptan in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods.

Recovery Studies (Accuracy): Recovery studies were conducted by analyzing each pharmaceutical formulation in the instance for the active ingredient by the proposed methods. Known amount of pure drug was added to each previously analyzed formulation and the total amount of the drug was once again determined by all proposed methods after bringing the active ingredient concentration within the Beers law limits.

Analysis of Formulations: Commercial formulations (tablets) containing Zolmitriptan were successfully analyzed by the proposed methods. The values obtained by the proposed and reference method for formulations were compared statistically with F and t tests and found not to be different significantly. Percent recoveries were determined by adding standard drug to preanalyzed formulations.

Table 1: Results of method validation obtained by applying the proposed methods for the determination of Zolmitriptan

Parameter	M6	M7	M8
λ_{max} (nm)	490	512	750
Beer's law limits (µg/mL)	12.0-60.0	15.0-75.0	10.0-50.0
Molar absorptivity (1 mol^{-1}. cm^{-1})	$1.15x10^3$	$8.784x10^3$	$1.00x10^3$
Sandell's sensitivity (µg.cm^{-2}/0.001 A.U)	0.0598	0.0983	0.0569
Regression equation (Y=a+bc); Slope (b)	0.0083	0.0041	0.0090
Intercept (a)	0.0037	0.0029	0.0068
Correlation coefficient (r)	0.9999	0.9996	0.9996
Relative standard deviation (%)*	1.389	1.179	0.892
% Range of error (confidence limits)			
0.05 level	1.161	0.986	0.746
0.01 level	1.718	1.459	1.104
LOD	0.0240	0.0201	0.0193

** Average of six determinations considered*

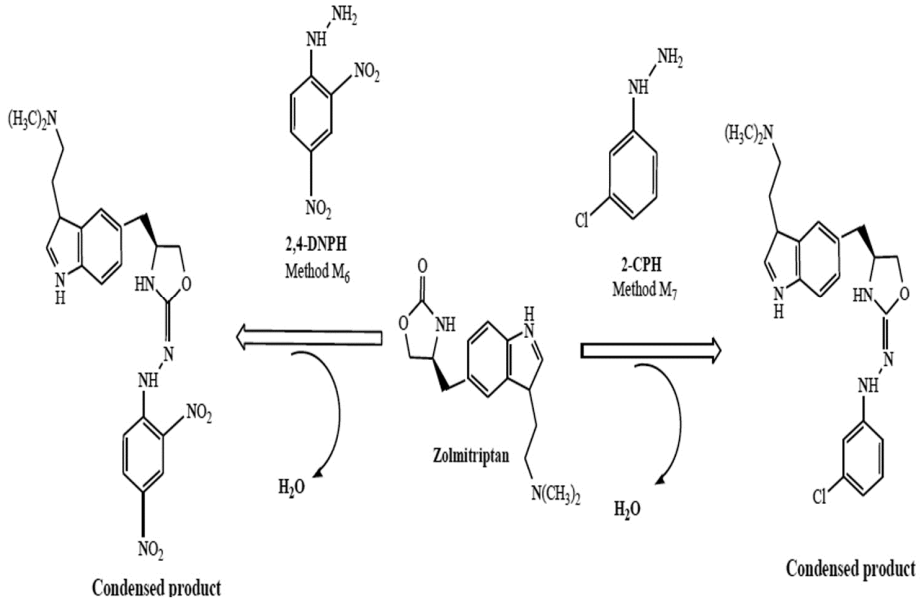
Interference Studies: The effect of wide range of excipients and other inactive ingredients usually present in the formulations for the assay of Zolmitriptan under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

Nature of the Colored Species: An attempt has been made by the author to indicate the nature of colored species in each of the proposed methods for Zolmitriptan is based on analogy of reactivity of the functional moiety (Keto and tertiary nitrogen group) in drug with appropriate reagents.

Method–M_6 & M_7: The proposed methods developed by the author were based on condensation reaction of the keto group of Zolmitriptan with 2, 4-DNPH (M_6) & 2-CPH (M_7) forming various colored chromogens. The predicted reaction mechanism is represented in Scheme 1 for method M_6 and M_7 respectively.

Method–M_8: This method is based on Lewis acid-base theory. The above mentioned drug (Zolmitriptan) contains nitrogen containing group having unshared pair of electrons [reducing agent] that reduce tungstate and/or molybdate, which are present in Folin Ciocalteus (FC) reagent in alkaline medium forming blue colored chromogen molybdenum blue.

Method – M_{10}: This method is based on oxidation followed by complex formation that involved the reaction of Zolmitriptan with 2, 2-bipyridyl, ferric chloride and ortho phosphoric acid to form orange colored chromogen that exhibited maximum absorption at 494 nm against the corresponding reagent blank.



Scheme-1

CONCLUSION

The spectrophotometric methods M_6, M_7, M_8 and M_{10} for the determination of Zolmitriptan in pure and dosage forms have been described in this paper. The present methods involve the determination of Zolmitriptan in pharmaceutical dosage at the given optimum conditions. It can be observed from the validation results of Zolmitriptan presented above, that the proposed methods have the good sensitivity and higher λ_{max}. Statistical analysis of the results showed that the proposed procedures have good precision and accuracy. Results of the analysis of pharmaceutical formulations of Zolmitriptan revealed that the proposed methods are suitable for its analysis with virtually no interference of the usual additives present in pharmaceutical formulations.

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