Research Article

Development and Validation of Stability-Indicating UV-Spectrophotometric Methods for the Determination of Zolmitriptan in Pharmaceuticals

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ABSTRACT

Two simple, selective and stability indicating UV-spectrophotometric methods have been developed and validated for the determination of zolmitriptan (ZMT) in bulk drug and in its dosage form. The proposed methods are based on the measurement of the absorbance of ZMT either in 0.1 M H_2SO_4 at 222 nm (method A) or in acetonitrile at 224 nm (method B). The calibration graphs are linear over the range 0.4-10.0 µg mL^{-1} in method A and 0.2-5.0 µg mL^{-1} in method B with correlation coefficients (r) of 0.9997 (method A) and 0.9999 (method B). The apparent molar absorptivity values are 4.00×10^4 and 4.74×10^4 L mol^{-1} cm^{-1}, for method A and method B, respectively. The other optical characteristics such as Sandell's sensitivity, limits of detection (LOD) and quantification (LOQ) values are also reported. The accuracy and precision of the methods were evaluated based on intra-day and inter-day variations. The methods were applied to the determination of ZMT in tablets with mean values of ≤ 102.0% and standard deviations falling under 1.25 %. No interference was observed from common additives formed in tablets formation. The accuracy of the methods was further confirmed also by standard addition procedure. The stability of the drug was studied by subjecting ZMT to acid and alkaline hydrolysis, oxidative, thermal and UV degradation. This study indicated that ZMT was degraded in alkaline and oxidative conditions.

Keywords: Zolmitriptan, UV-spectrophotometric determination, pharmaceuticals, degradation study.

INTRODUCTION

The antimigraine drug zolmitriptan (figure 1) 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. Zolmitriptan (ZMT) binds with high affinity to human $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors leading to cranial blood vessel constriction. The therapeutic activity of ZMT for the treatment of migraine headache can most likely be attributed to the agonist effects at the $5HT_{1B/1D}$ receptors on intracranial blood vessels (including the arterio-venous anastomoses) and sensory nerves of the trigeminal system which result in cranial vessel constriction and inhibition of pro-inflammatory neuropeptide release [1].

ZMT is not included in any pharmacopeias. Literature survey reveals that few analytical methods have been published for analysis of ZMT in human plasma and include high-performance liquid chromatography (HPLC) with fluorescence[2], coulometric[3], mass detection[4-6] and liquid chromatography-mass spectrometry[1,7,8].

High-performance liquid chromatography (HPLC) with UV-detection has been widely used for the quantitative determination of ZMT in pharmaceuticals [9-16]. Ultra- performance liquid chromatography (UPLC)[17], liquid chromatography-mass spectrometry[18], voltammetry[19], visible spectrophotometry[20,21] and UV-spectrophotometry methods[22-24] have also been reported.

Several techniques applied for the assay of ZMT in pharmaceuticals. Besides, a few UV-spectrophotometric methods have also been reported for ZMT. Acharjya et al[22] reported two UV-spectrophotometric methods for the determination of ZMT in bulk and pharmaceutical formulations. First method was the first derivative method in which the response of drug in 0.1 M H_2SO_4 measured at 298 nm and the second was based on calculation of area under curve (AUC) for drug in 0.1 M H_2SO_4 at 283 nm. In a UV-spectrophotometric method described by Murthy and Veditha[23], drug is

dissolved in different solvents such as water, water:acetonitrile (1:1) and water: Methanol (1:1) and the response measured at 283 nm in the case of water, 284 nm in the case of both acetonitrile:water, and MeOH:water. A UV-spectrophotometric method developed by Sankar et al[24] for the quantification of ZMT in which drug in methanol showed absorption maximum at 226 nm.

However, the reported chromatographic techniques such as HPLC and LC-MS are expensive, time-consuming, need skilled operators. Visible spectrophotometric methods involve strict pH control, use of large quantity of organic solvents and sometimes require pre-concentration and extraction steps that increase the risk of sample loss. In contrast, UV-spectrophotometry is characterized by its speed and simplicity, accuracy and inexpensive instrument needed, and hence it is an important alternative to other analytical techniques with clear advantages in terms of cost of analysis.

None of the three UV-spectrophotometric methods [22-24] reported for ZMT is stability-indicating. The reported two stability indicating methods using UPLC [13] and LC-MS[14] require expensive experimental set up and expertise personnel. Thus, much effort has been devoted to the development of simple, sensitive and accurate methods to determine ZMT in pure drug and pharmaceuticals as well as to study its degradation behavior.

According to International Conference on Harmonization (ICH) guidelines entitled 'stability testing of new drug substances and products', stress testing of the drug substance should be carried out to elucidate the inherent stability of the active substance. Susceptibility to oxidation is one of the required tests. Also the acid or base hydrolysis, thermal and photolytic stability studies are required. In the present study, two simple, sensitive and efficient UV-spectrophotometric methods were elaborated for the determination of ZMT in pure form and in tablet dosage form. The methods are based on the measurement of absorbance of ZMT solution in either 0.1 M H_2SO_4 (method A) or acetonitrile (method B). The proposed methods were validated as per ICH guidelines[25]. The developed methods were successfully applied to the quantification of ZMT in its tablets without any interference by the inactive ingredients. The stability-indicating ability of the methods was also investigated by subjecting the drug to forced degradation under various stress-conditions.

EXPERIMENTAL

Apparatus

The Spectrophotometric measurements were carried out using Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer.

Reagents and chemicals

All reagents and chemicals used were of analytical reagent grade whereas acetonitrile was of HPLC grade. Doubly-distilled water was used to prepare solutions wherever required.

0.1 M H_2SO_4: Aqueous solution of 1.0 M H_2SO_4 was prepared by diluting the concentrated H_2SO_4 (Merck, Mumbai, India; Sp. gr. 1.84).

1.0 M HCl: Aqueous solution of 1.0 M HCl was prepared by diluting the concentrated HCl (Merck, Mumbai, India; Sp. gr. 1.18).

1.0 M NaOH: Solution of 1.0 M sodium hydroxide was prepared by dissolving 4 g of NaOH (Merck, Mumbai, India) in 100 mL of water.

5% H_2O_2: A 5% (v/v) aqueous solution of hydrogen peroxide (LOBA Chemie Pvt. Ltd., Mumbai, India, 30% w/v) was prepared in the usual manner and used for degradation study.

Standard ZMT solution

Stock standard solutions equivalent to 20 µg mL^{-1} and 10 µg mL^{-1} ZMT were prepared separately in 0.1 M H_2SO_4 and acetonitrile, and used for the assay in method A and method B, respectively.

One brand of tablet namely Zomig-2.5 (Intas Pharmaceuticals, Dehradun, India) was purchased from local commercial sources.

Assay procedures

Method A (using 0.1 N H_2SO_4)

Aliquots of standard ZMT solution (0.2-5.0 mL of 20 µg mL^{-1}) were accurately transferred into a series of 10 mL calibrated flasks and the volume was made up to the mark with 0.1 M H_2SO_4. The absorbance of each solution was measured at 222 nm against the same solvent.

Method B (using acetonitrile)

Different volumes (0.2-5.0 mL) of standard 10 µg mL^{-1} ZMT solution were accurately transferred into a series of 10 mL calibrated flasks and the volume was made up to the mark with acetonitrile. The absorbance of each solution was measured at 224 nm against acetonitrile.

Procedure for tablets

Twenty tablets each containing 2.5 mg ZMT were weighed and finely powdered. An amount of the powder equivalent to 10 mg of ZMT was

accurately weighed and transferred to a 100 mL calibrated flask, 60 mL of the respective solvent (0.1 M H_2SO_4 in method A and acetonitrile in method B) was added and the content was shaken thoroughly for about 20 min. The volume in each flask was diluted to the mark with the same solvent, mixed well and filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was rejected and a suitable aliquot of the filtrate (100 µg mL^{-1} ZMT) was diluted appropriately to get a working concentration of 20 and 10 µg mL^{-1} ZMT and then subjected to analysis by applying the assay procedures described above.

Procedures for selectivity study

A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was prepared by combining all components to form a homogeneous mixture. A 10 mg of the placebo blank was accurately weighed and its solution was prepared as described under 'tablets', and then subjected to analysis by following the general procedure.

A synthetic mixture was prepared by adding 10 mg of pure ZMT to 10 mg of the above mentioned placebo blank and the mixture was homogenized. Following the procedure described under procedure for tablets, the synthetic mixture solution (100 µg mL^{-1} ZMT) was prepared, and then diluted with the respective solvent to get working concentrations of 20 and 10 µg mL^{-1} in ZMT before subjecting to analysis following the procedures described above.

Procedure for stress degradation by hydrolysis under acidic, alkaline and neutral conditions

A 2.0 mL of 20 µg mL^{-1} standard ZMT solution in 0.1 M H_2SO_4 (method A) or 4.0 mL of 10 µg mL^{-1} standard solution of ZMT in acetonitrile (method B) was taken separately in three 25 mL calibrated flasks, 5.0 mL each of 1.0 M HCl (acid hydrolysis), 1.0 M NaOH (alkaline hydrolysis) and water (neutral hydrolysis) were added separately to each flask. The flasks were kept on a water bath for 2.0 h at 80 °C, and then cooled to room temperature. The first and second flasks were neutralized with 5.0 mL of 1.0 M NaOH (for acid hydrolysis) and 5.0 mL of 1.0 M HCl (for alkaline hydrolysis) followed by making all the flasks to the mark with the respective solvent (0.1 M H_2SO_4 in method A or acetonitrile in method B). The absorption spectrum of solution in each flask was recorded from 400-200 nm versus the corresponding blank.

Procedure for oxidative degradation

To 2.0 mL of standard ZMT solution (20 µg mL^{-1}) in 0.1 M H_2SO_4 (method A) or to 4.0 mL of 10 µg mL^{-1} standard ZMT solution in acetonitrile (method B) taken in a 25 mL calibrated flask, 5 mL of 5% hydrogen peroxide was added. The flasks were kept on a water bath at 80 °C for 2.0 h. The flasks were cooled to room temperature, made up to the mark with the respective solvent and the absorption spectrum was run from 400-200 nm against the corresponding blank.

Procedure for dry heat and photo-degradation

The powdered sample (0.1 g) of ZMT was taken on a Petri dish and kept in the oven at 105 °C for 24 h, the sample cooled to room temperature and used to prepare 20 µg mL^{-1} ZMT in 0.1 M H_2SO_4 (method A) and 10 µg mL^{-1} ZMT in acetonitrile (method B). To study the photostability powdered sample (0.1 g) of ZMT was taken on a Petri dish, exposed to UV radiation in a UV chamber of 1200 flux-hr for 48 h and then used to prepare 20 µg mL^{-1} ZMT in 0.1 M H_2SO_4 (method A) and 10 µg mL^{-1} ZMT in acetonitrile (method B). The absorption spectrum of each solution was run from 400-200 nm against the corresponding solvent.

RESULTS AND DISCUSSION

Absorption spectra

ZMT solution in 0.1 M H_2SO_4 (method A) and acetonitrile (method B) exhibited absorption peaks at 222 and 224 nm for method A and methods B, respectively (Figure 2) and the absorbance at these wavelengths was found to be linearly dependent upon the concentration of drug. In both the cases, the corresponding blank solutions showed negligible absorbance. Therefore these wavelengths were used as analytical wavelength throughout the investigation.

Forced degradation study

The absorption spectra of the ZMT solutions in 0.1 M H_2SO_4 and acetonitrile treated with acid, base and water hydrolysis, hydrogen peroxide, dry heat and UV radiation were run in the range of (200-400 nm). The degradation was evaluated based on the comparison of the UV

spectra of "stressed ZMT samples" with those of the "standard ZMT solution".

The resulting UV spectra of stress ZMT solutions (4 µg mL^{-1} in 0.1 M H_2SO_4 and acetonitrile) subjected to acid hydrolysis showed the same spectra (Figure 3) of the standard solution which indicated that ZMT does not undergo degradation under this condition.

Under alkaline conditions ZMT solutions in 0.1 M H_2SO_4 and in acetonitrile undergoes degradation in both methods (Figure 4). Also, the absorption spectra of ZMT solutions in 0.1 M H_2SO_4 and in acetonitrile treated with hydrogen peroxide showed that ZMT undergoes significant degradation in both methods (Figure 5).

The UV spectra of stress ZMT samples subjected to dry heat treatment and UV-degradation were similar to that of the standard ZMT sample in both methods and it showed that ZMT did not undergo degradation under these conditions (Figure 6). The results of degradation study are summed up in Table 1.

Method Validation Procedures

The proposed methods were validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and Sensitivity

Under optimum conditions a linear relation was obtained between absorbance and concentration of ZMT in the range of 0.4 - 10.0 and 0.2 - 5.0 µg mL^{-1} for method A and method B, respectively (Figure 3). The calibration graph is described by the equation:

$$Y = a + b\,X$$

(Where Y = absorbance, a = intercept, b = slope and X = concentration in µg mL^{-1}) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines [26] are compiled in Table 2 speak of the excellent sensitivity of the proposed methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s$$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

Precision and accuracy

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of the ZMT were prepared and analyzed in seven replicates. The analytical results obtained from this investigation are summarized in Table 3. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% R.E) indicate the high precision and the good accuracy of the proposed methods. The percentage relative error was calculated using the following equation:

$$\%R.E = \left[\frac{found - taken}{taken}\right] \times 100$$

The assay procedure was repeated seven times, and percentage relative standard deviation (% R.S.D) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. The placebo blank solution was subjected to analysis according to the recommended procedures. The resulting absorbance readings for both the methods were same as the reagent blank, inferring no interference from placebo.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution at three concentration levels yielded percent recoveries which ranged from 97.58 to 101.1 with standard deviation of 0.96 – 1.73 in both the cases.

Robustness and ruggedness

The robustness of the methods was evaluated by measuring the absorbance at three different wavelengths whereas the method ruggedness was performed by four different analysts and also using three different cuvettes by a single analyst. Intermediate precision values (%RSD) were in the range 0.95 – 1.81% indicating acceptable ruggedness. These results are presented in Table 4.

Application to tablets

In order to evaluate the analytical applicability of the proposed methods to the quantification of ZMT in commercial tablets, the results obtained by the proposed methods were compared to

those of the reference method [11] by applying Student's t-test for accuracy and F-test for precision. The reference method describes chromatographic separation of ZMT with UV-detection at 225 nm. The results (Table 5) show that the calculated Student's t- and F-values at 95 % confidence level are less than the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ZMT at three concentration levels (50, 100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In both cases, the added ZMT recovery percentage values ranged of 98.20 – 102.0 % with standard deviation of 0.57 – 1.25 (Table 6) indicating that the recovery was good, and that the co formulated substance did not interfere in the determination.

CONCLUSIONS

In the proposed study, stability-indicating UV-spectrophotometric methods were developed for the determination of ZMT and validated as per ICH guidelines. From statistical analysis of the results, it is inferred that methods are accurate, precise, and repeatable. The developed methods were found to be simple, sensitive and selective for analysis of ZMT in dosage form without any interference from the excipients. Assay results for dosage form using proposed methods showed 99.37±1.34% (method A) and 101.6±1.46% (method B) of ZMT. The results indicated the suitability of the method to study stability of ZMT under various forced degradation conditions viz. acid, base, dry heat, neutral, photolytic and UV degradation. It can be concluded from degradation study that ZMT undergoes extensive degradation under alkaline and oxidative conditions and stable to acidic, photolytic and thermal stress conditions. The chromatographic methods require complex and expensive equipment, use of large quantities solvents, labour-intensive sample preparation procedure and require skilled personnel. Thus, proposed methods can be used in quality control and routine analysis of ZMT in pharmaceuticals since the methods are both precise and accurate, and free from interference from common additives and excipients that might be found in commercial tablets.

Table 1: Forced degradation summary

Degradation condition	% Assay* (method A)	% Assay* (method B)	Observation
Control sample	99.8	99.8	Not applicable
Acid hydrolysis (1M HCl , 80°C, 2 hours)	99.8	98.7	No degradation observed
Base hydrolysis (1M NaOH , 80°C, 2 hours)	-	-	Extensively degraded
Oxidation (5% H_2O_2 , 80°C, 2 hours)	-	-	Extensively degraded
Thermal (105°C, 3 hours)	98.9	98.4	No degradation observed
Photolytic (1.2 million lux hours)	100.0	98.7	No degradation observed
* Percentage against standard ZMT			

Table 2: Regression and analytical parameters

Parameter	Method A	Method B
λ_{max}, nm	222	224
Beer's law limits (µg mL^{-1})	0.4-10.0	0.2-5.0
Molar absorptivity (L mol^{-1} cm^{-1})	4.00×10^4	4.74×10^4
Sandell sensitivity* (µg cm^{-2})	0.0072	0.0061
Limit of detection (µg mL^{-1})	0.06	0.06
Limit of quantification (µg mL^{-1})	0.19	0.19
Regression equation, Y** Intercept,(a)	0.0034	-0.0021
Slope,(B)	0.1371	0.1702
Correlation coefficient (r)	0.9997	0.9999

Table 3: Evaluation of intra-day and inter-day precision and accuracy

Method	ZMT taken (µg mL^{-1})	Intra-day (n = 7)			Inter-day (n = 5)		
		ZMT found[a] (µg mL^{-1})	%RSD[b]	%RE[c]	ZMT found[a] (µg mL^{-1})	%RSD[b]	%RE[c]
Method A	2.00	2.03	0.93	1.48	2.03	1.09	1.61
	4.00	3.95	1.25	1.20	3.95	1.31	1.31
	6.00	5.96	0.83	0.67	6.08	1.12	1.25
Method B	1.00	1.01	1.08	0.92	1.01	1.33	1.08
	3.00	3.04	1.04	1.19	3.04	1.38	1.36
	5.00	4.96	1.35	0.84	4.95	1.62	0.97

[a.] Mean value of five determinations; [b.] Relative standard deviation (%); [c.] Relative error (%).

Table 4: Robustness and ruggedness

Method	ZMT taken, µg mL^{-1}	Method robustness	Method ruggedness	
		Wavelengths, nm[a] RSD, % (n = 3)	Inter-analysts RSD, % (n = 3)	Inter- cuvettes RSD, % (n = 3)
Method A	2.00	1.17	1.81	1.00
	4.00	1.24	1.06	1.35
	6.00	1.09	1.33	1.79
Method B	1.00	0.95	1.52	1.14
	3.00	1.53	0.98	1.60
	5.00	0.83	1.10	1.48

[a]Wavelengths used were 221, 222 and 223 in method A and 223, 224 and 225 in method B.

Table 5: Results of analysis of tablets by the proposed methods

Tablet Brand name	Label claim mg/tablet	Found (Percent of label claim ±SD)[a]		
		Reference method	Proposed methods	
			Method A	Method B
Zomig-2.5	2.5	100.59± 0.90	99.37 ± 1.34 *t* =1.69 *F*=2.22	101.6 ± 1.46 *t* =1.32 *F* =2.63

[a]Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.78.
Tabulated F-value at the 95% confidence level is 6.39.

Table 6: Results of recovery study by standard addition method

Tablets studied	Method A				Method B			
	ZMT in tablets, µg mL^{-1}	Pure ZMT added, µg mL^{-1}	Total found, µg mL^{-1}	Pure ZMT recovered*, Percent±SD	ZMT in tablets, µg mL^{-1}	Pure ZMT added, µg mL^{-1}	Total found, µg mL^{-1}	Pure ZMT recovered*, Percent±SD
Zomig-2.5	1.99	1.00	2.98	98.70±1.25	1.52	0.75	2.28	101.1±1.25
	1.99	2.00	4.03	102.0±0.57	1.52	1.50	2.99	98.20±0.83
	1.99	3.00	5.02	101.0±0.80	1.52	2.25	3.81	101.8±0.76

Mean value of three determinations.

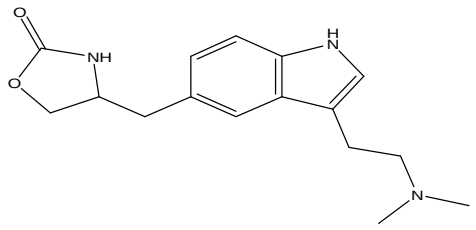
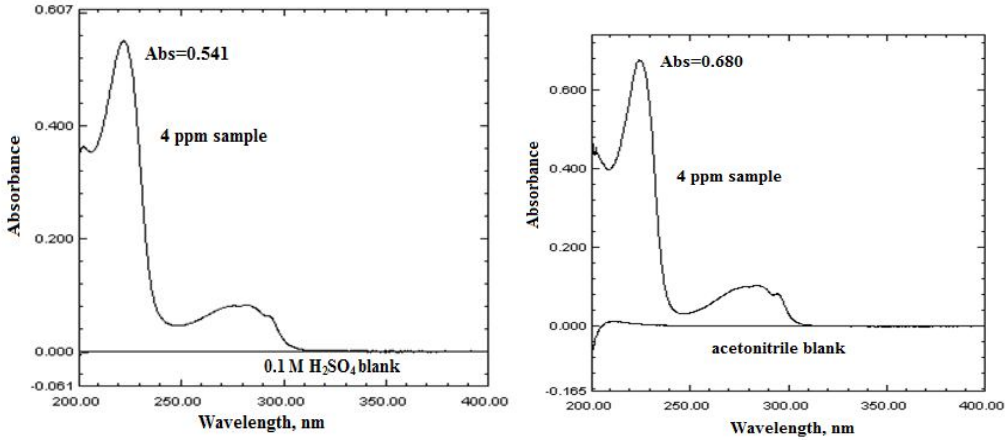
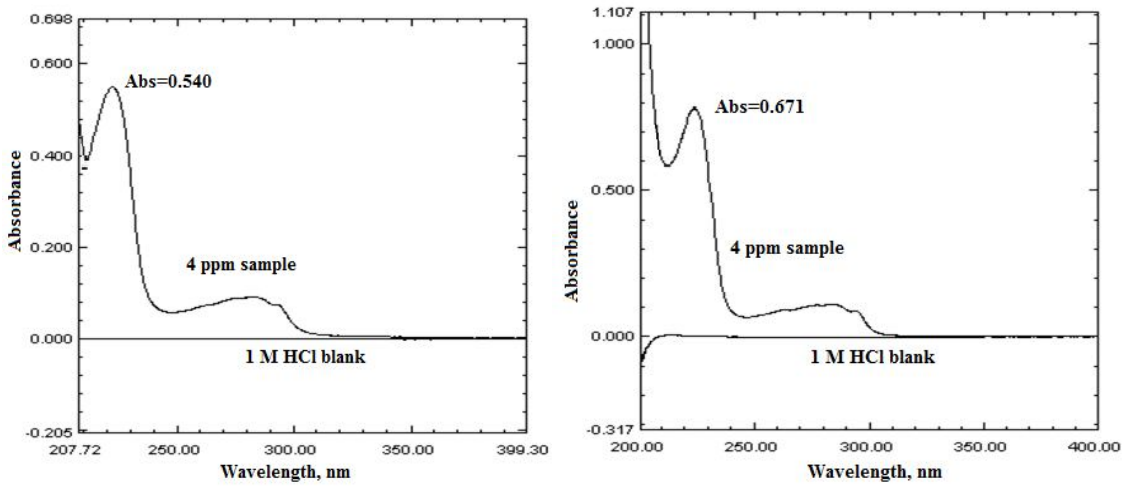


Fig. 1: **Structure of zolmitriptan**



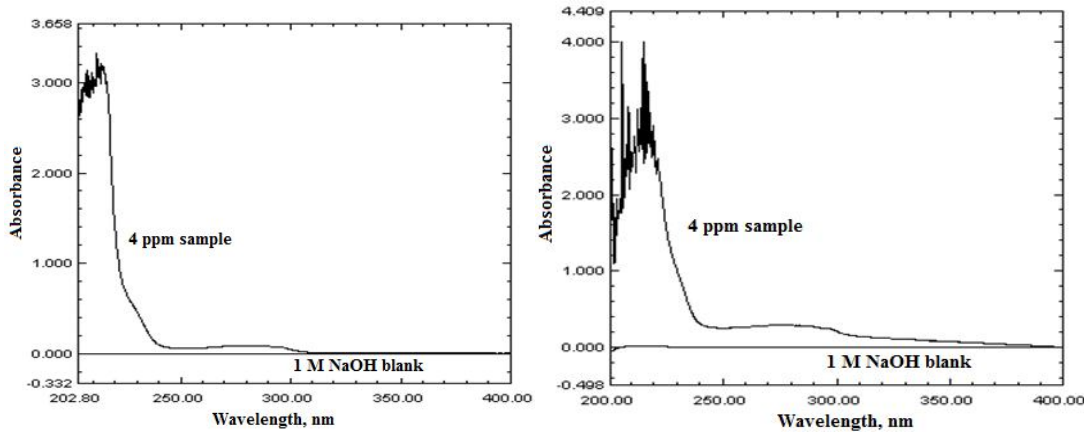
(a) (b)

Fig. 2: Absorption spectra of STS in 0.1 M H_2SO_4 (method A) and in acetonitrile (method B)



(a) (b)

Fig. 3: Acid degradation. a) (method A), b) (method B)



(a) (b)

Fig. 4: Base degradation. a) (method A), b) (method B)

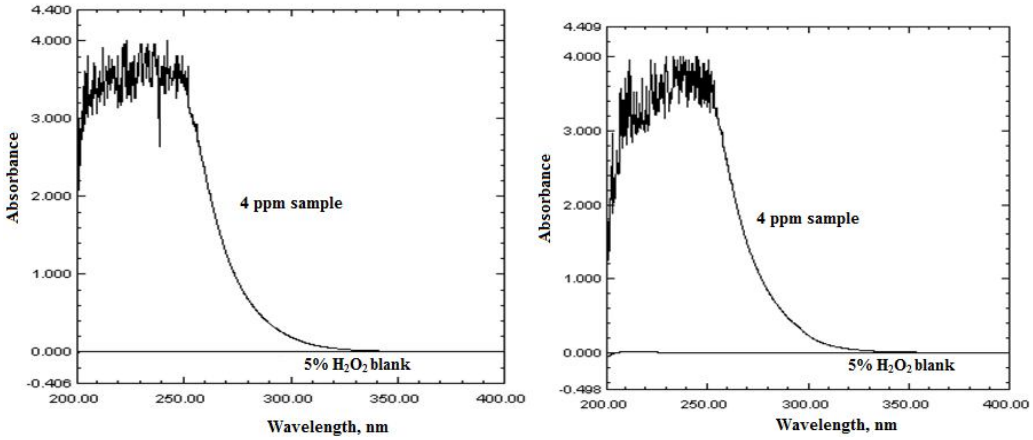


Fig. 5: Peroxide degradation a) (method A), b) (method B)

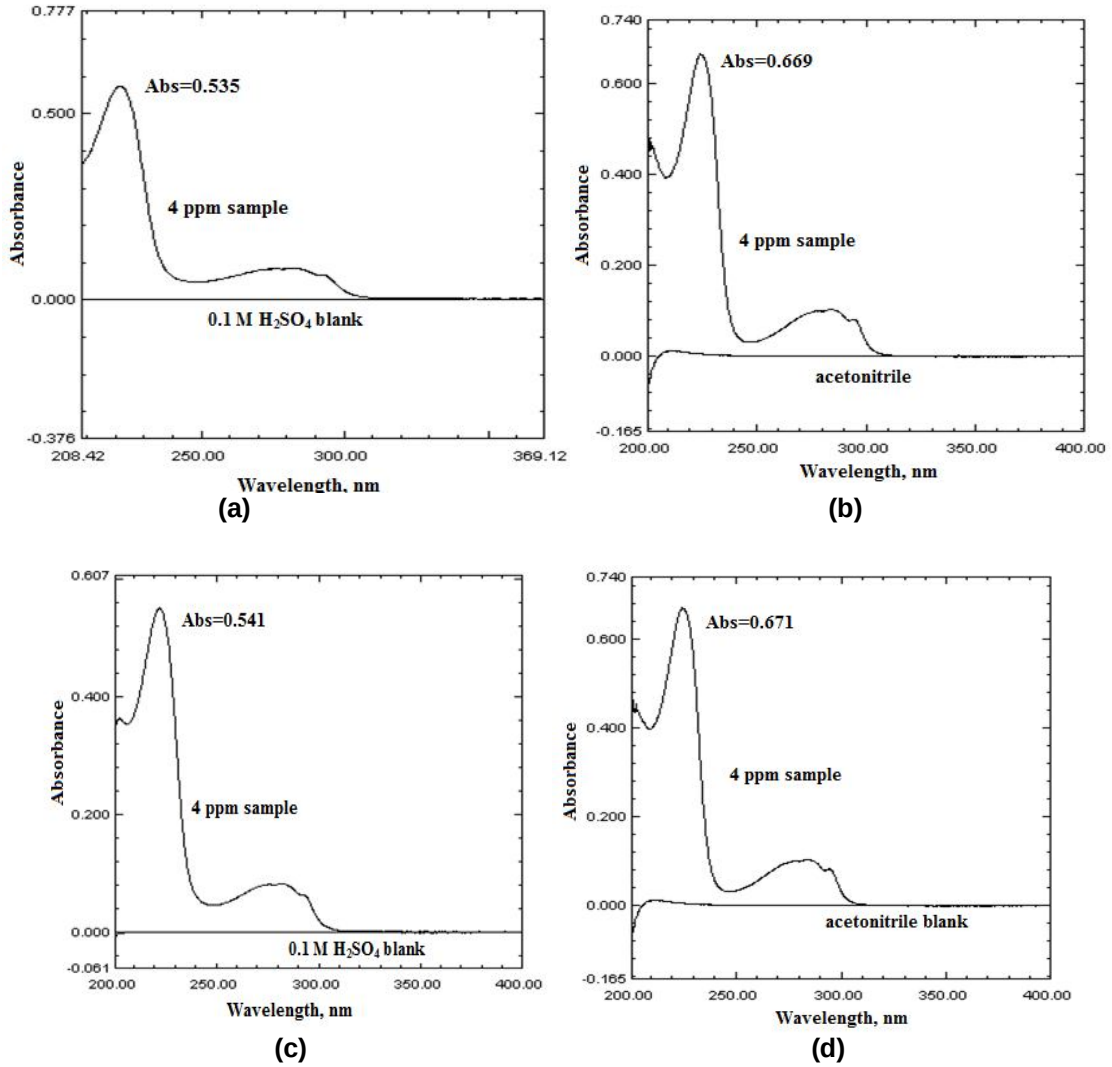


Fig. 6: Thermal degradation a) (method A), b) (method B) Photo degradation c) (method A), d) (method B)

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