# Simultaneous Estimation of Zolmitriptan and Sumatriptan Succinate in Pure and Synthetic Mixture Using UV Spectrophotometer

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## ABSTRACT

There is no analytically validated method for the simultaneous estimation of zolmitriptan and sumatriptan using a UV-Visible spectrophotometer of has been reported or published to date. Hence an attempt was made to develop and validate a novel analytical method for the simultaneous estimation of zolmitriptan and sumatriptan succinate by Vierordt's method using a UV-visible spectrophotometer and forced degradation study. For the wavelength selection of drugs, 0.1 N HCl was optimized and spectra were monitored at 226 nm and 282 nm, respectively, using Shimadzu UV-1800. The linearity was found in the concentration range of 2-14 µg/mL for both zolmitriptan ($R^2 = 0.999$) and sumatriptan succinate ($R^2 = 0.998$) with an accepted regression value. The percentage recovery (80%, 100%, and 120% levels) of reference standards of zolmitriptan and sumatriptan succinate using the proposed method was found to be 99.1-99.8% w/w and for the synthetic mixture was found to be in the range of 99-99.8% w/w. Percentage RSD in intermediate precession of zolmitriptan and sumatriptan succinate was found to be 0.07 and 0.47 (intra-day), 0.05 and 0.39 (inter-day). The percentage drug content of zolmitriptan and sumatriptan succinate in a synthetic mixture was found to be $100.42 \pm 0.02\%$ and $103.90 \pm 1.02\%$. Furthermore, the degradation study for the mixture of zolmitriptan and sumatriptan succinate and synthetic mixture under acidic, basic, thermal, and photolytic conditions except peroxide degradation were within the acceptance limits. The proposed method was successfully validated by the ICH Q2 R1 guideline and was found to be specific, accurate, precise, robust, and rugged for the simultaneous estimation of zolmitriptan and sumatriptan succinate in bulk and Synthetic mixture.

## INTRODUCTION

Analytical techniques are routinely used in the determination of the composition, structure, properties, and purity of pharmaceutical and non-pharmaceutical substances [1]. Day by day, there is a surge in the number of new drugs introduced into the market for the treatment of different disease conditions. Normally, there will be a greater period between the date of introduction of drugs into the market and the date of their incorporation in the pharmacopoeia. Therefore, in most cases, there is a need for an appropriately validated analytical protocol for routine quality control analysis of drugs in pharmaceutical formulations. A forced degradation study is an essential step in the design of a regulatory-compliant stability program for both drug substances and products and was

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formalized as a regulatory requirement in ICH guideline $Q_1A$.

Globally, migraine is the 6th most prevalent disease and a major cause of disability [2]. Zolmitriptan is a member of the triptan class of agents with antimigraine properties [2, 3]. Zolmitriptan selectively binds to and activates serotonin (5-HT) 1B/1D receptors expressed in intracranial arteries. In addition, zolmitriptan may also relieve migraine headaches by inhibiting pro-inflammatory neuropeptide release [4, 5]. Sumatriptan is a sulphonamide that consists of N, N-dimethyltryptamine bearing an additional (N-methylsulfamoyl) methyl substituent at position 5. Selective agonist for a vascular 5-HT1 receptor subtype (probably a member of the 5-HT1D family), used (in the form of its succinate salt) for the acute treatment of migraine with or without aura in adults. It has a role as a serotonergic agonist and a vasoconstrictor agent. It is functionally related to a N, N-dimethyltryptamine and is a conjugate acid of a sumatriptan [2, 6, 7].

The combination of zolmitriptan, sumatriptan, and other drugs was estimated in pharmaceutical formulation using RP-HPLC and HPTLC [8, 9]. However, there is no analytically validated method for the simultaneous estimation of zolmitriptan and sumatriptan using a UV-Visible spectrophotometer of has been reported or published to date. Hence an attempt was made to develop and validate a novel analytical method for the simultaneous estimation of zolmitriptan and sumatriptan succinate by Vierordt's method using a UV-visible spectrophotometer and forced degradation study.

## MATERIALS AND METHODS

### Materials
Zolmitriptan and sumatriptan succinate working standards were purchased from Yarrow Chem Pharmaceuticals, Bangalore. In-house preparation of synthetic mixture containing 150 mg where 140 mg of excipients and 5 mg of each zolmitriptan and sumatriptan succinate was prepared in the laboratory. 0.1 N HCl of analytical grade solution was prepared using distilled water. Excipients are magnesium stearate, starch, Talc, PVP, SSG, mannitol, and MCC are of LR grade.

### Preparation of standard solution
Both zolmitriptan and sumatriptan succinate were accurately weighed (10 mg) and transferred into a 10 mL clean dry volumetric flask separately, about 3 mL of 0.1 N HCl was added to dissolve it completely and the volume was made up to the mark with the same solvent to a concentration of 1000 µg/mL. Further, each sub-stock was prepared by pipetting out 1 mL from the stock solution into 10 mL volumetric flasks separately and diluted up to the

mark with 0.1 N HCl to a concentration of 100 µg/mL. From the sub-stocks, 0.8 mL of both zolmitriptan and sumatriptan succinate was pipetted out into the separate 10 mL clean dry volumetric flask, mixed well, and made up to the mark by using the 0.1 N HCl (8 µg/mL).

### Preparation of synthetic mixture solution
The synthetic mixture consists of 5 mg of zolmitriptan and sumatriptan succinate each and 140 mg of excipients equivalent to one tablet. Similarly, 1500 mg of the synthetic mixture was prepared in ten portions ($10 \times 150$ mg). An equivalent quantity of a powdered mixture (150 mg) was weighed accurately and transferred to a clean, dried 50 mL volumetric flask diluted with 0.1 N HCl solvent and made up to the mark. Then, mix well the content by shaking for 10 min, and the solution was filtered by using Whatman filter paper. Then 0.8 mL was pipetted out in a clean, dried 10 mL volumetric flask diluted it with the same solvent, and made up to the mark.

### Method validation
The objective of validating an analytical method is to demonstrate the appropriate intended use. The validation parameters such as specificity, linearity, accuracy, precision, LOD, LOQ, ruggedness, and robustness were carried out according to ICH $Q_2 R_1$ guidelines [10, 11].

### Specificity
According to the specificity test, aliquots of reference standard and synthetic mixture were prepared (8 µg/mL). There was no interference due to the blank. All the solutions were subjected to spectral scanning from 200-400 nm to find out the λmax and analytical wavelengths.

### Linearity study
#### Preparation of reference standard solution
From the prepared sub-stock solution, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL were pipetted out to a separate 10 mL clean, dry volumetric flask and made up to the mark with the 0.1 N HCl to get 2, 4, 6, 8, 10, 12, 14 µg/mL.

### Accuracy
Zolmitriptan (5 mg) and sumatriptan succinate (5 mg) concentrations corresponding to 80%, 100%, and 120% of the reference standard amount were added and the absorbance was measured using a UV spectrophotometer in triplets. The percentage recovery was calculated at all three concentration levels.

### Precision
#### Repeatability
From the sub-stock aliquots of the desired strength of 8 + 8 µg/mL were prepared by pipetting out 0.8 mL of

zolmitriptan and sumatriptan succinate 10 mL clean, dry volumetric flask and made up to the mark with the solvent.

*Intermediate precision*
Precision was conducted on multiple days to assess the method's Intermediate precision.

*Intra-Day and Inter-day*
The absorbance of prepared aliquot solutions was measured six times on the same day with a time difference of 0, 2, and 4 hours by UV-spectrophotometer.

*Robustness*
A deliberate modification in wavelength was performed as a part of the robustness. The wavelength was adjusted by ± 2 nm. The prepared aliquot solution was introduced to measure the absorbance by UV-spectrophotometer with variable wavelengths in conjunction with the technique wavelength.

*Limit of Detection (LOD)*
It was computed as:

$$LOD = 3.3 \times \sigma/S \qquad (1)$$

*Limit of quantification (LOQ)*
LOQ was calculated using:

$$LOQ = 10 \times \sigma/S \qquad (2)$$

*Degradation studies*

Forced degradation tests for zolmitriptan and sumatriptan succinate pharmaceuticals were performed as per ICH $Q_1A$ $(R_2)$ criteria. A degradation study was conducted in a standard zolmitriptan and sumatriptan succinate mixture and a synthetic mixture under acidic, basic, thermal, oxidative, and photolytic conditions [12].

## RESULTS AND DISCUSSION

*Optimized method*
After considering the solubility and spectral linearity of both drugs, 0.1 N HCl was used as a solvent. The spectral data were captured between 200-400 nm. The $\lambda_{max}$ of zolmitriptan was found to be 226 nm and sumatriptan succinate was found to be 282 nm, respectively.

*Method validation* [13]
*Specificity*
The UV spectra of the synthetic mixture containing zolmitriptan and sumatriptan succinate were similar to those of the standard mixture. There was no interference in the blank spectra.

*Linearity*
The overlain spectra zolmitriptan and sumatriptan succinate were represented as (**Figures 1a and 1b**). The absorbance of all linearity levels of both drugs was found at wavelengths of 226 nm and 282 nm. Linear regression $(R^2)$ of zolmitriptan and sumatriptan succinate was found to be 0.999 and 0.998, respectively (**Figures 1c and 1d**).

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a)



b)

c)                                          d)
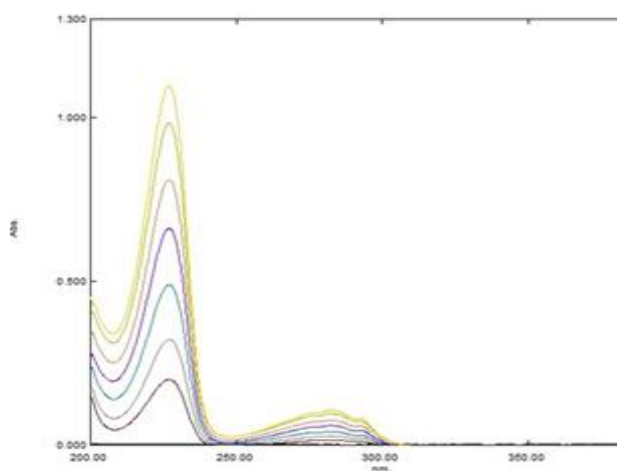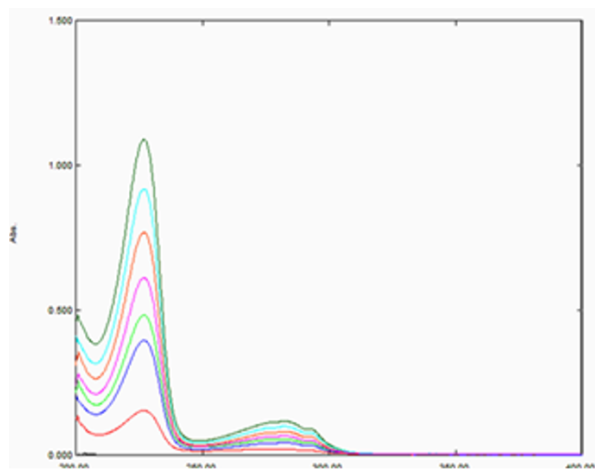
**Figure 1.** a) Overlay UV spectra of zolmitriptan and b) Overlay UV spectra of sumatriptan succinate mixture, c) Standard calibration curve of zolmitriptan, and d) Standard calibration curve of sumatriptan succinate.

*Accuracy*

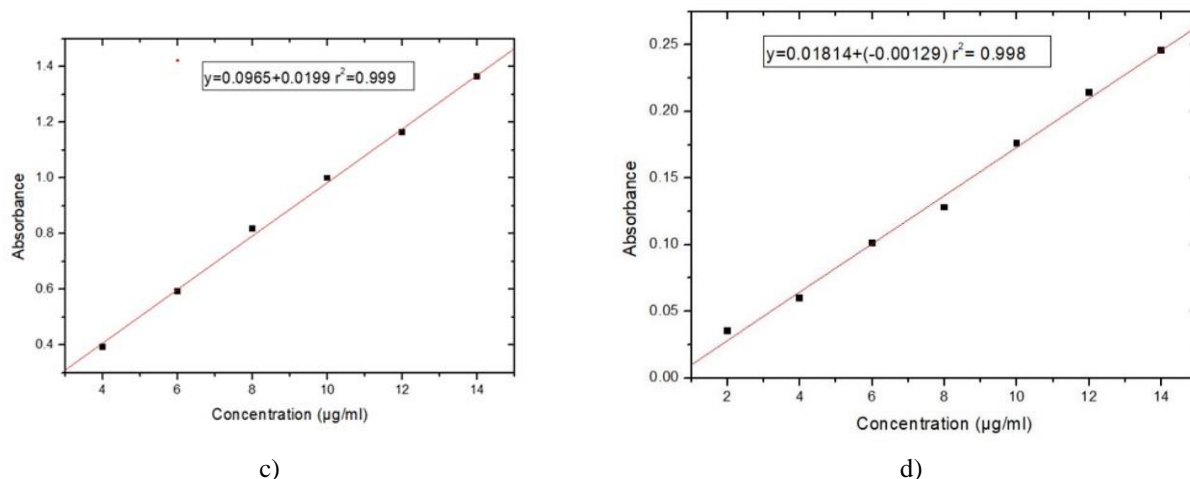The accuracy findings for zolmitriptan and sumatriptan succinate have been summarized in **Table 1**. A recovery study was performed from the formulated solution of zolmitriptan at 226 nm was 99.7%, 99.2%, and 99.1%, and sumatriptan succinate at 282 nm was 99.6%, 99.8%, and 99.6%, respectively.

**Table 1.** Recovery study of standard zolmitriptan and sumatriptan succinate mixture

| Level (%) | Sample conc. (mg) | Amount added (mg) | Total conc. (mg) | Amt. Recovered (µg/mL) | Mean Recovery (%) |
|---|---|---|---|---|---|
| **Zolmitriptan at 226 nm** | | | | | |
| 80% | 5 | 4 | 9 | $9.01 \pm 0.005$ | $99.7 \pm 0.003\%$ |
| 100% | 5 | 5 | 10 | $9.93 \pm 0.004$ | $99.2 \pm 0.004\%$ |
| 150% | 5 | 6 | 11 | $10.84 \pm 0.008$ | $99.1 \pm 0.57\%$ |
| **Sumatriptan succinate at 282 nm** | | | | | |
| 80% | 5 | 4 | 9 | $8.96 \pm 0.003$ | $99.6 \pm 0.002\%$ |
| 100% | 5 | 5 | 10 | $9.99 \pm 0.007$ | $99.8 \pm 0.001\%$ |
| 150% | 5 | 6 | 11 | $10.97 \pm 0.005$ | $99.6 \pm 0.003\%$ |

*Precision*

*Repeatability*

The repeatability was confirmed by measuring the absorbance of the mixture of standard zolmitriptan and sumatriptan succinate solution at 226 nm and 282 nm respectively and found to be within the acceptable values (RSD < 2%) **(Table 2)**. Similarly, the repeatability study of zolmitriptan and sumatriptan succinate present in the synthetic mixture was also within the acceptable values **(Table 3)**.

*Intermediate precision*

The results of an intermediate precision study (intra-day and inter-day) of standard zolmitriptan and sumatriptan succinate mixture and zolmitriptan and sumatriptan succinate present in the synthetic mixture were tabulated in **(Tables 2 and 3)**. Absorbance was measured six times for both the standard mixture and synthetic mixture were found to be within the acceptable range.

**Table 2.** Precision study of standard zolmitriptan and sumatriptan succinate in mixture

| No. Trials | Repeatability | | Intra-day precision of zolmitriptan ($A_{226}$) | | | | | | Inter-day precision of sumatriptan succinate ($A_{282}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **0 h** | | **2 h** | | **4 h** | | **0 h** | | **2 h** | | **4 h** | |
| | Zolmitriptan ($A_{226}$) | Sumatriptan succinate ($A_{282}$) | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ |

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| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.769 | 0.105 | 0.769 | 0.105 | 0.767 | 0.106 | 0.766 | 0.106 | 0.756 | 0.104 | 0.756 | 0.103 | 0.756 | 0.102 |
| 2 | 0.768 | 0.104 | 0.768 | 0.105 | 0.767 | 0.105 | 0.767 | 0.107 | 0.757 | 0.104 | 0.756 | 0.104 | 0.756 | 0.103 |
| 3 | 0.768 | 0.105 | 0.768 | 0.106 | 0.768 | 0.106 | 0.767 | 0.107 | 0.757 | 0.105 | 0.765 | 0.103 | 0.755 | 0.102 |
| 4 | 0.769 | 0.104 | 0.769 | 0.105 | 0.768 | 0.106 | 0.766 | 0.107 | 0.757 | 0.104 | 0.756 | 0.103 | 0.755 | 0.103 |
| 5 | 0.768 | 0.105 | 0.768 | 0.105 | 0.767 | 0.106 | 0.766 | 0.107 | 0.757 | 0.104 | 0.756 | 0.103 | 0.755 | 0.102 |
| 6 | 0.768 | 0.105 | 0.768 | 0.105 | 0.767 | 0.106 | 0.766 | 0.106 | 0.757 | 0.104 | 0.756 | 0.103 | 0.755 | 0.102 |
| Mean | 0.768 | 0.105 | 0.768 | 0.105 | 0.767 | 0.106 | 0.766 | 0.107 | 0.757 | 0.104 | 0.757 | 0.103 | 0.756 | 0.102 |
| SD | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0004 | 0.0004 | 0.0004 | 0.0004 | 0.0005 | 0.0004 |
| RSD (%) | 0.07 | 0.48 | 0.07 | 0.48 | 0.07 | 0.47 | 0.07 | 0.47 | 0.05 | 0.38 | 0.05 | 0.39 | 0.06 | 0.39 |

**Table 3.** Precision study of zolmitriptan and sumatriptan succinate in synthetic mixture

| No. Trials | Repeatability | | Intra-day precision of zolmitriptan ($A_{226}$) | | | | | | Inter-day precision of sumatriptan succinate ($A_{282}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 h | | 2 h | | 4 h | | 0 h | | 2 h | | 4 h | |
| | Zolmitriptan ($A_{226}$) | Sumatriptan succinate ($A_{282}$) | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ | $A_{226}$ | $A_{282}$ |
| 1 | 1.328 | 0.179 | 1.328 | 0.179 | 1.327 | 0.178 | 1.328 | 0.177 | 1.321 | 0.176 | 1.320 | 0.176 | 1.319 | 0.174 |
| 2 | 1.329 | 0.178 | 1.329 | 0.178 | 1.328 | 0.179 | 1.327 | 0.176 | 1.322 | 0.177 | 1.321 | 0.175 | 1.319 | 0.175 |
| 3 | 1.329 | 0.178 | 1.329 | 0.178 | 1.328 | 0.178 | 1.327 | 0.177 | 1.321 | 0.176 | 1.320 | 0.175 | 1.319 | 0.174 |
| 4 | 1.329 | 0.179 | 1.329 | 0.179 | 1.327 | 0.178 | 1.327 | 0.176 | 1.322 | 0.176 | 1.320 | 0.175 | 1.319 | 0.174 |
| 5 | 1.328 | 0.179 | 1.328 | 0.179 | 1.328 | 0.178 | 1.328 | 0.177 | 1.321 | 0.176 | 1.320 | 0.175 | 1.319 | 0.174 |
| 6 | 1.329 | 0.179 | 1.329 | 0.179 | 1.328 | 0.178 | 1.327 | 0.177 | 1.321 | 0.176 | 1.320 | 0.175 | 1.319 | 0.174 |
| Mean | 1.329 | 0.179 | 1.329 | 0.179 | 1.328 | 0.178 | 1.327 | 0.177 | 1.321 | 0.176 | 1.320 | 0.175 | 1.319 | 0.174 |
| SD | 0.0008 | 0.0005 | 0.0008 | 0.0005 | 0.0008 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0004 | 0.0004 | 0.0004 | 0.0005 | 0.0004 |
| RSD (%) | 0.055 | 0.28 | 0.055 | 0.28 | 0.040 | 0.27 | 0.038 | 0.27 | 0.038 | 0.50 | 0.030 | 0.23 | 0.038 | 0.23 |

*Limit of detection (LOD)*
The limit of detection was calculated from the linearity curve method using the slope, and standard deviation of the calibration curve. The LOD of zolmitriptan was found to be 5.37 and sumatriptan succinate was found to be 0.00280 respectively.

*Limit of quantification (LOQ)*
The limit of quantification was calculated from the linearity curve method using the slope, and standard deviation of the calibration curve. The LOD of zolmitriptan was found to be 163.12 and sumatriptan succinate was found to be 1.377, respectively.

*Robustness*
The Robustness was found by changing the wavelengths ± 2 nm to the actual wavelength. The percentage RSD was found to be within the range.

*Ruggedness*
In Ruggedness, there was a slight change in the absorbance values observed in analyst 1 to analyst 2, however, the values were within the acceptable range. The percentage RSD was within the range.

*Determination of drug content of zolmitriptan and sumatriptan succinate in synthetic mixture using a validated method*
The percentage drug content of zolmitriptan and sumatriptan succinate in a synthetic mixture was within the limit as mentioned in Indian Pharmacopeia **(Table 4)**.

**Table 4.** Determination of drug content of zolmitriptan and sumatriptan succinate in synthetic mixture

| Trial | Zolmitriptan content (%) | Sumatriptan succinate content (%) |
|---|---|---|
| 1 | 102.5% | 105.0% |
| 2 | 100.5% | 103.7% |
| 3 | 98.25% | 103.0% |
| Mean ±SD | **100.42 ± 0.02** | **103.90 ± 1.02** |
| Acceptance criteria | 95-105% | 97.50-102% |

*Degradation studies of standard and synthetic mixture*
*Degradation study of standard zolmitriptan and sumatriptan succinate mixture*
When drugs were subjected to varied stress conditions, both the drugs zolmitriptan and sumatriptan succinate and synthetic mixture almost showed stable signs of deterioration [14]. This approach was able to identify the changes brought on by stressful conditions even though the deteriorated product under various stress conditions had not yet been seen (**Tables 5 and 6**).

**Table 5.** Degradation study of standard zolmitriptan and sumatriptan succinate mixture

| Degradation condition | Zolmitriptan (8 μg/mL) | Sumatriptan succinate (8 μg/mL) | Zolmitriptan (8 μg/mL) | Sumatriptan succinate (8 μg/mL) |
|---|---|---|---|---|
| | Absorbance | Degraded (%) | Absorbance | Degraded (%) |
| Acid degradation | 0.4 ± 0.0015 | 11.90 ± 0.22 | 0.03 ± 0.0015 | 10.1 ± 1.38 |
| Base degradation | 0.65 ± 0.0015 | 7.07 ± 0.22 | 0.08 ± 0.0015 | 7.4 ± 1.69 |
| Thermal degradation | 0.67 ± 0.002 | 4.2 ± 0.295 | 0.08 ± 0.0014 | 5.5 ± 1.11 |
| Photolytic degradation | 0.68 ± 0.001 | 3.13 ± 0.14 | 0.09 ± 0.001 | 4.44 ± 1.11 |
| Peroxide degradation | 0.59 ± 0.001 | 15.1 ± 0.15 | 0.07 ± 0.001 | 18.9 ± 1.12 |

**Table 6.** Degradation study of standard zolmitriptan and sumatriptan succinate mixture in synthetic mixture

| Degradation condition | Zolmitriptan (8 μg/mL) | Sumatriptan succinate (8 μg/mL) | Zolmitriptan (8 μg/mL) | Sumatriptan succinate (8 μg/mL) |
|---|---|---|---|---|
| | Absorbance | Degraded (%) | Absorbance | Degraded (%) |
| Acid degradation | 1.15 ± 0.002 | 10.6 ± 0.44 | 0.14 ± 0.0015 | 10.89 ± 0.911 |
| Base degradation | 1.16 ± 0.002 | 10.4 ± 0.16 | 0.15 ± 0.0015 | 8.72 ± 0.911 |
| Thermal degradation | 1.18 ± 0.001 | 8.77 ± 0.75 | 0.16 ± 0.001 | 7.13 ± 0.595 |
| Photolytic degradation | 1.19 ± 0.001 | 8.54 ± 0.075 | 0.16 ± 0.001 | 5.35 ± 0.60 |
| Peroxide degradation | 0.10 ± 0.001 | 23.2 ± 0.08 | 0.14 ± 0.001 | 17.9 ± 0.60 |

## CONCLUSION

The developed simultaneous UV-spectrophotometric method for standard zolmitriptan and sumatriptan succinate and Synthetic mixture was found to be simple, accurate, sensitive, precise, specific, economical, and rapid. The present analytical method was validated according to the ICH $Q_2(R_1)$ and the forced degradation study was performed according to the ICH $Q_1A(R_2)$ guidelines. The proposed UV-spectrophotometric method was validated over the specificity, precision, linearity, accuracy, ruggedness, and robustness. In addition, the forced degradation study was performed using a developed and validated UV method. The developed analytical method meets to specific acceptance criteria of both the guidelines. Furthermore, the percentage of drug contents in the synthetic mixture was found to be within the limit.

**Ethics statement:** None

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