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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NARATRIPTAN AND ZOLMITRIPTAN BY SPECTROPHOTOMETRY

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## ABSTRACT

**Background**: Naratriptan and Zolmitriptan are drugs used for the treatment of migraine headaches. Zolmitriptan and Naratriptan is a group of medicines known as serotonin 5-HT$_{1B/1D}$ receptor agonist. **Objective:** To develop analytical methods suitable for validation of Naratriptan and Zolmitriptan by UV spectrophotometric methods as per the ICH guidelines. **Methods:** For UV-Method water: glycerol (1% v/v) is used as solvent as well as Methanol water used as solvent. Solution of drugs were scanned in spectrophotometer in the range 200-400nm. **Results:** In UV Method the wavelength was chosen for analysis of Zolmitriptan 238nm, Naratriptan 328nm. The Linearity of proposed methods found to be Zolmitriptan 1-10μg/mL and Naratriptan 1-12μg/mL LOC and LOQ values shows that the method was sensitive and specific. %RSD values found to be <2. The $R^2$ values were found to be >0.99.

Keywords: Naratriptan, Zolmitriptan, Methanol, UV, Accuracy

## INTRODUCTION

The current Global Burden of Diseases, Injuries, and Risk Factors Study continues to spot migraine as prominent reason for disability worldwide [1, 2] mostly in the persons younger than 50 years of age [3]. Migraine is the fifth principal cause of disability worldwide and it was found that migraine is three times more

common in females than males [4]. Nearly 3 billion individuals were likely to have a migraine or headache in 2016 [5]. Naratriptan HCl and Zolmitrptan both are 5 hydroxytryptamine$_{1B/1D}$ (5-HT$_{1B/1D}$) agonist used for the treatment of migraine headaches [6, 7]. The chemical structure of Naratriptan and Zolmitritan are shown in **Figure 1 and Figure 2** respectively. The International Union of Pure and Applied Chemistry name of Naratriptan and Zolmitriptan is (N-methyl-3-(1-methyl-4-piperidinyl)-1$H$-indol-5-ethane sulfonamide monohydrochloride [8] and (4S)-4-[[3-[2-(dimethyl amino) ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone respectively [9]. After doing the literature review it was found that very few analytical methods have been investigated till now for the determination of Zolmitriptan and Naratriptan in pharmaceutical dosage form and biological fluid. Few UV spectroscopic [10-13], HPLC [14-17] and UPLC [18, 19] methods had been developed for the estimation of Zolmitriptan and a few UV [20, 21], HPLC [17, 22-24] and UPLC [25] methods had been developed for the estimation of Naratriptan. In this study, a simple, sensitive, precise, accurate, and cost-effective UV-Spectrophotometric technique has been developed for the assessment of Zolmitriptan and Naratriptan in bulk drug.
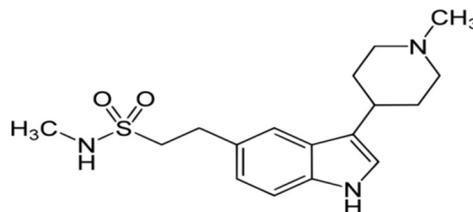


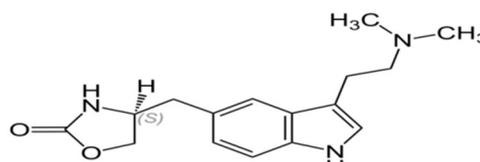**Figure 1: Naratriptan**



**Figure 2: Zolmitriptan**

## MATERIALS AND METHODS

### Instrument

UV – visible spectrophotometer (UV 1800) shimadzu, Weighing/electronic balance (1 mg sensitivity AUY-220) Shimadzu, Ultrasonics (1.5L50) sonicator was used for sonication.

### Chemicals and reagents

Naratriptan and Zolmitriptan were provided by K.P Laboratories, Hyderabad, India, and Methanol was purchased from SD fine chemical limited NBD-Cl Reagent (4-Chloro-7-nitrobenzo furzan) purchased from Sigma Aldrich Sodium hydroxide pellets purchased from Fine chemicals Limited.

### Preparation of Stock Solution (Standard) Zolmitriptan and Naratriptan:

Weigh accurately 10 mg of Zolmitriptan and Naratriptan separately into a 100ml volumetric flask. Add 10 ml of solvent water: glycerol (1% v/v) and shake well to dissolve the drug completely. Make up the volume to

100ml with solvent to get 100 mg/mL of both Zolmitriptan and Naratriptan.

**Procedure:**

Aliquots standard solution containing (5-15µg/mL) Zolmitriptan and (4-16µg/mL) Naratriptan were transferred to series of 10ml of volumetric of flasks. The volume was made up to the mark with solvent. The absorbance of the solutions measured at $\lambda_{max}$ 200-400 nm against solvent blank.

**Preparation of NBD-Cl reagent:**

This solution was prepared by dissolving 0.1996 gm of NBD-Cl in 100 ml of methanol in 100 ml volume flask.
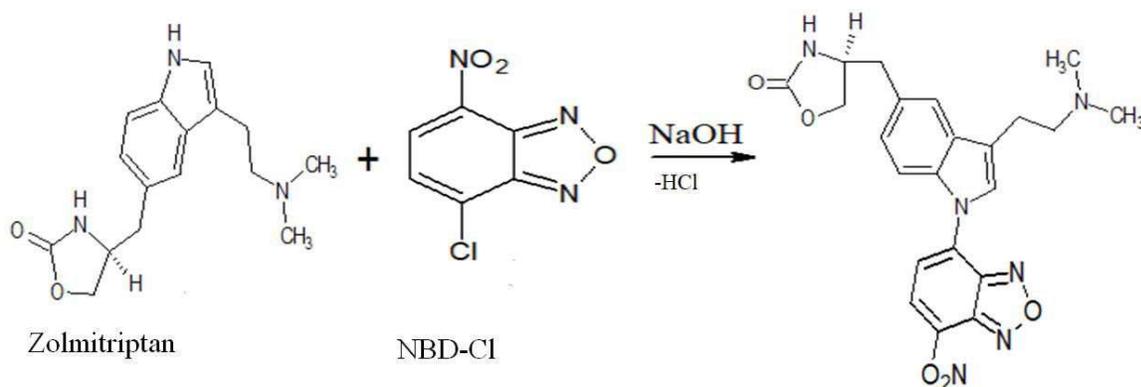
**Preparation of 1 M sodium hydroxide solution:**

This solution was prepared by dissolving 4 gm of NaOH in 100 ml of distilled water in 100 ml volume flask.
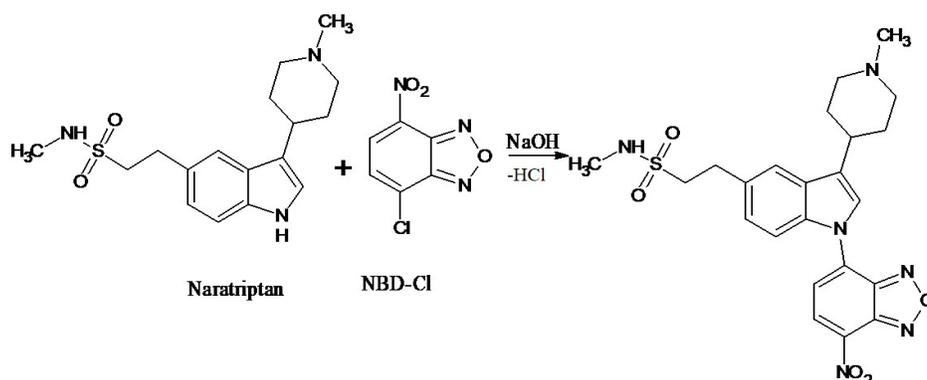


**Chemical Reaction:**

**Orange Coloured derivative ($\lambda_{max}$= 352nm)**

**Figure 3: Possible Chemical Reaction of Naratriptan**



**Light yellow orange coloured derivative ($\lambda_{max}$= 329nm)**

**Figure 4: Possible Chemical Reaction of Zolmitriptan**

**Procedure:**

A 1.5 ml from 500 µg/mL of Zolmitriptan & Naratriptan was carried into 25 ml of volume flask followed by adding 2.0 ml of NBD-Cl and 1.0 ml of NaOH solution. After 15 minutes, the volume was made up with methanol for (Zolmitriptan), distilled water for (Naratriptan), in representing 0.7-1.8 ml Zolmitriptan and 1-2.2 ml Naratriptan in a series are prepared 10 ml of volume flask. The absorbances of the solution were measured in 1cm cells, against blank at the specific wavelength of maximum wavelength.

**Determination of $\lambda_{max}$:**

Standard solution (10 µg/mL) of Zolmitriptan derivative was scanned in UV Spectrophotometer from 300-800nm. $\lambda_{max}$ Zolmitriptan derivative was found to be 329nm. Standard solution (6µg/mL) of Naratriptan derivative was scanned in UV-Visible Spectrophotometer from 300-800nm and $\lambda_{max}$ Naratriptan derivative was found to be 352nm.

**Determination of $\lambda_{max}$:**

Standard solutions of Zolmitriptan and Naratriptan were scanned in UV spectrophotometer in the range 200-400nm to determine the $\lambda_{max}$ of each drug.

**Validation as per ICH guidelines [26]**

The developed method for Naratriptan and Zolmitriptan was validated for parameters such as system suitability, precision, linearity, accuracy, robustness, and solution stability as per ICH guidelines

**Linearity:**

Aliquots standard solution containing (0.1-1ml) Zolmitriptan and (0.1-1.2ml) Naratriptan were transferred to series of 10ml of volumetric of flask. The volume was made up to the mark with solvent. The absorbance of the solutions measured at $\lambda_{max}$ 238 nm (Zolmitriptan) 328 nm (Naratriptan) against solvent blank. The amount of sample was computed from calibration graph.

**Precision:**

Repeatability of the method was performed by measuring the absorbance of same sample in replicates of five.

%RSD =SD x 100/Mean

Where, SD $\longrightarrow$ standard Deviation

**Robustness:**

Robustness of the developed method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters like variations in $p^H$, solvent, $\lambda_{max}$ etc. In the present study, robustness was measured by varying the wavelengths.

**Accuracy:**

The Recovery of Standard solutions of 10µg/mL Zolmitriptanin Water: glycerol (1%

v/v Glycerol) were prepared separately. Sample solutions of 5, 10, 15µg/mL were prepared as 50%,100%,150% samples respectively. All the solutions were scanned

under UV-range (200-400nm) and the %recovery was calculated from the absorbance values.

$$\% \, Recovery \; = \; \frac{Amount \; found}{Amount \; taken} \; \times 100$$

## LOD & LOQ:

LOD is the smallest amount of sample that can be detected but not essentially quantified. LOQ is the smallest amount of sample that

can be quantified.

LOD & LOQ are calculated from the formula:

$$LOD = 3.3 \times \sigma/s \quad LOQ = 10 \times \sigma/s$$

Where, $\sigma \longrightarrow$ standard deviation and
S $\longrightarrow$ slope

## RESULTS AND DISCUSSION

**UV Methods:** In the proposed method the $\lambda_{max}$ of Zolmitriptan was found to be 238nm
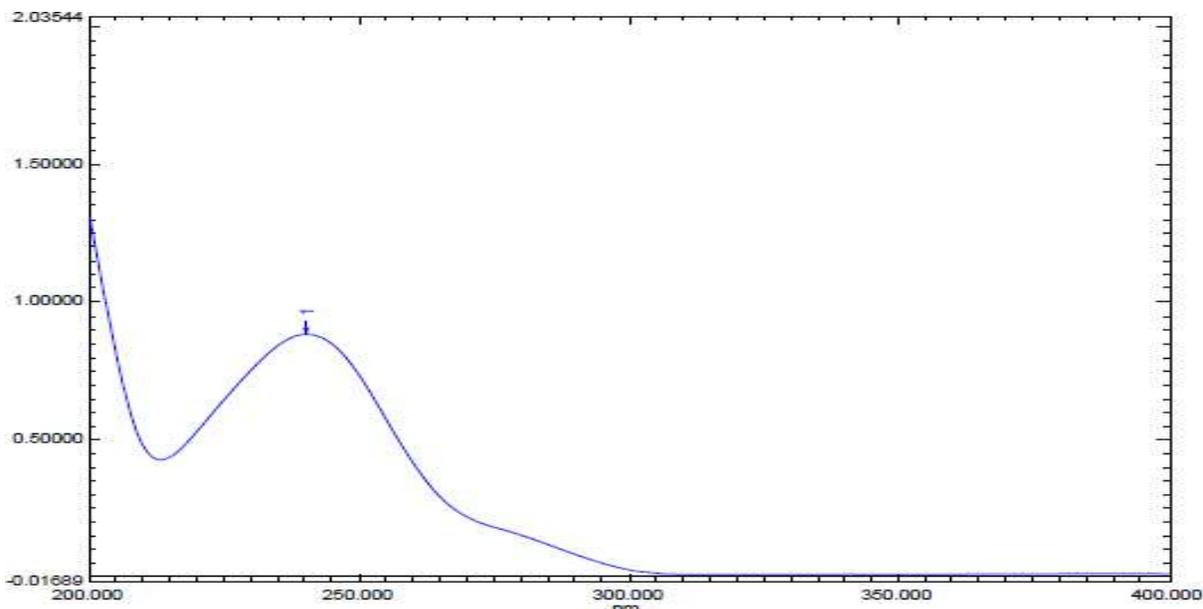
when used with 1% v/v glycerol in water as solvent.



**Figure 5: Absorption spectrum of Zolmitriptan against blank (1% v/v Glycerol in water)**
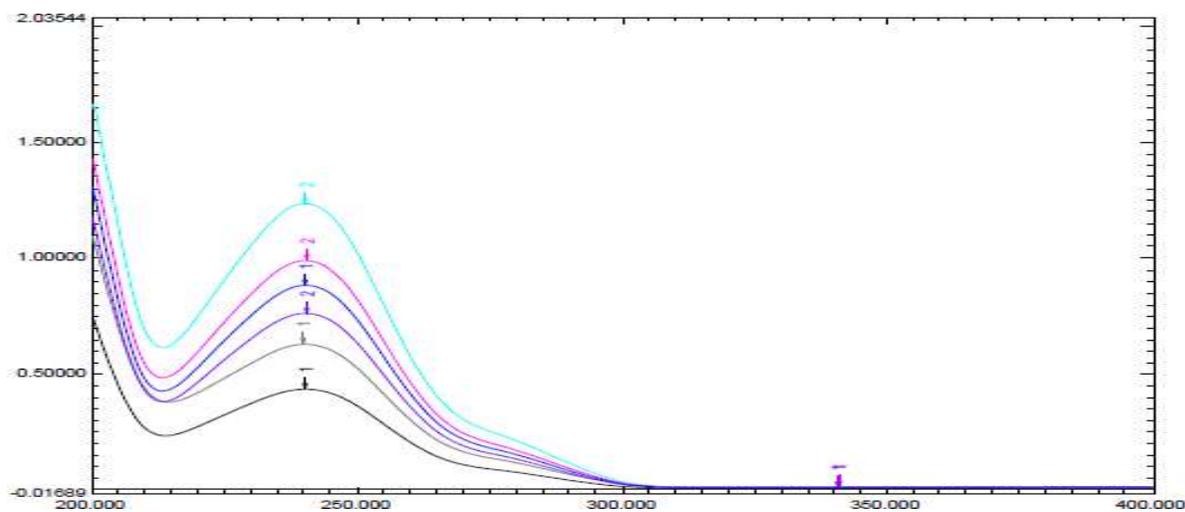
**Figure 6: Overlain spectra of Zolmitriptan against blank**

The overlain spectra the concentration range of Zolmitriptan was found to be 5-15µg/mL
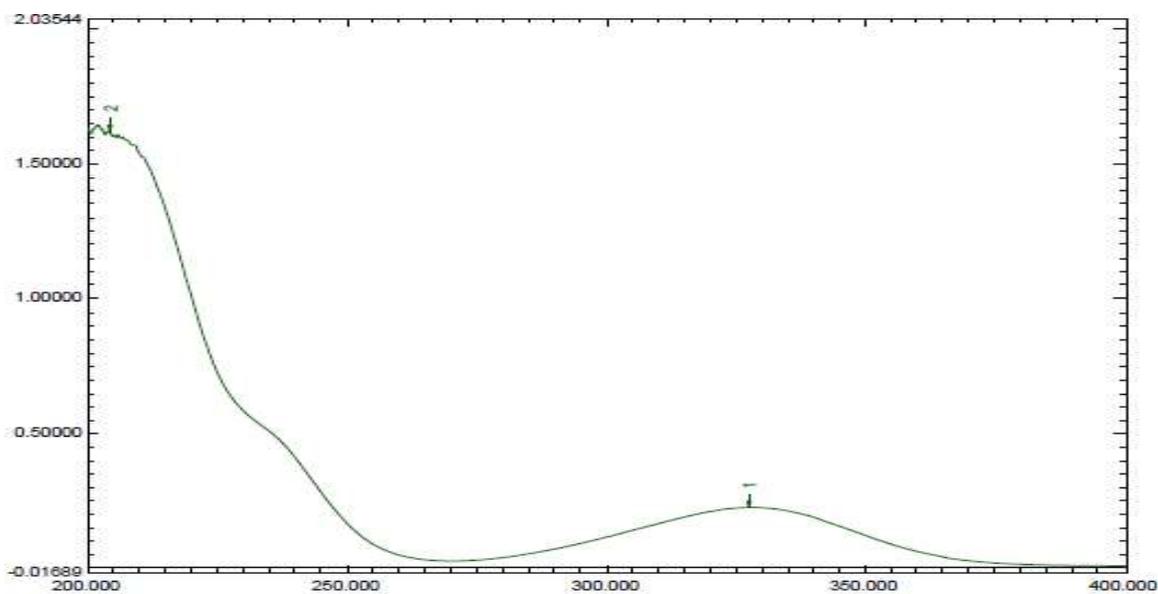


**Figure 7: Absorption spectrum of Naratriptan against blank (1% v/v glycerol in water)**

In the proposed method the $\lambda_{max}$ of Naratriptan was found to be 328nm when used with 1% v/v glycerol in water as solvent

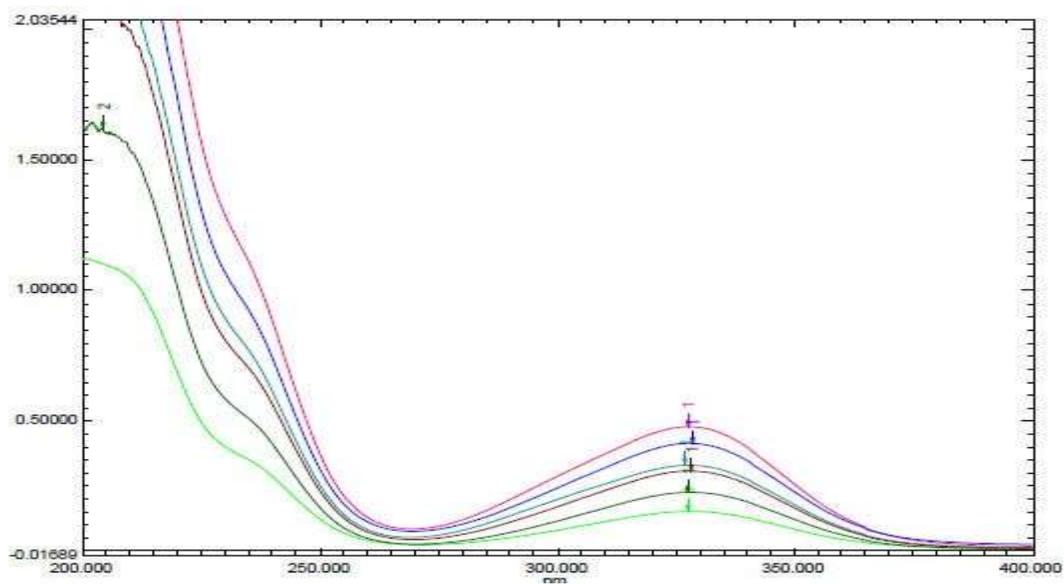**Figure 8: Overlain spectra of Naratriptan against blank**

The overlain spectra of Naratriptan was found to be in the concentration range 4-16 µg/mL

**Colorimetric Methods:**

In the proposed method the $\lambda_{max}$ of Zolmitriptan derivative was found to be 329nm when used with methanol as solvent.
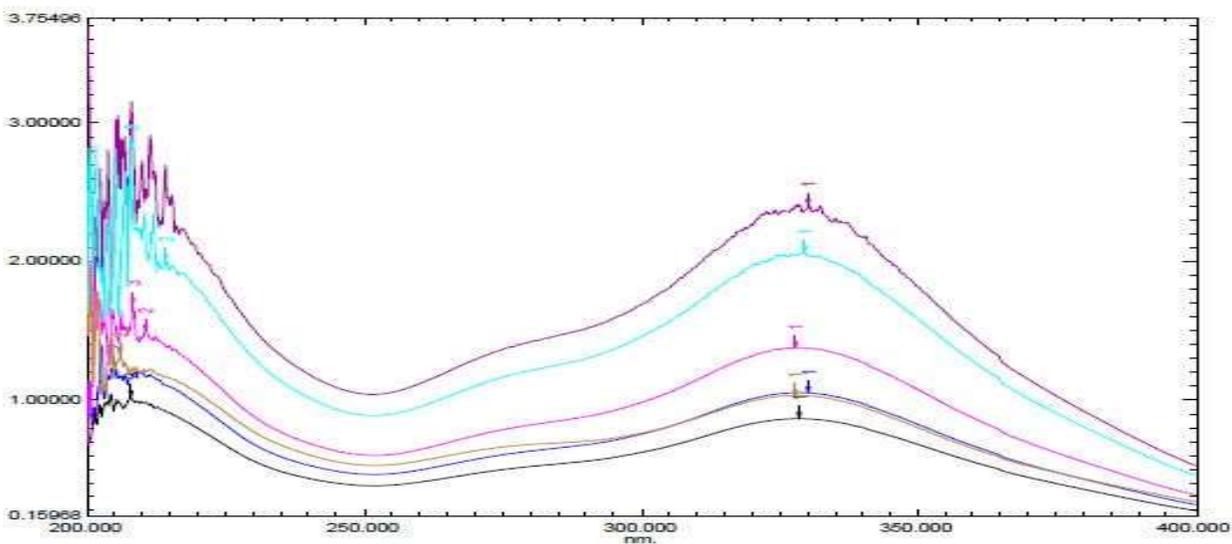


**Figure 9: Absorption spectrum of Zolmitriptan against blank (methanol)**

**Figure 10: Overlain spectra of Zolmitriptan derivative (methanol)**
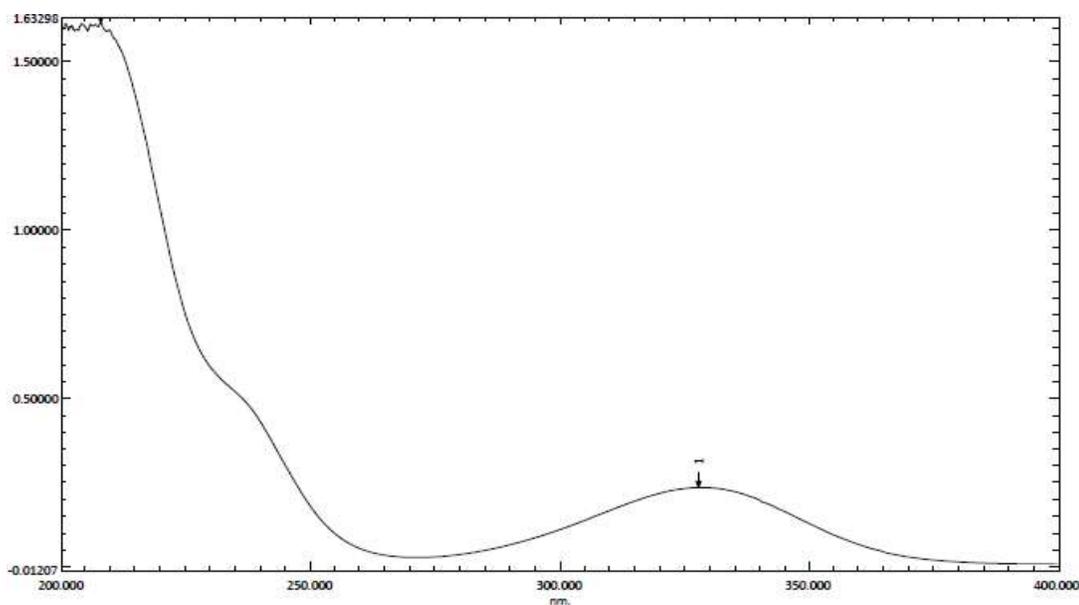


**Figure 11: Absorption spectrum of Naratriptan against blank (water)**

The overlain spectra of Zolmitriptan was found to be in the concentration range 7-18 g/mL In

Zolmitriptan visible spetrum, obsevred that, $\lambda_{max}$ may not shift towards longer wavelength because NBD-Cl reagent not interact enough to provide intense colour.

**Figure 12: Overlain spectra of Naratriptan derivative (water)**

In the proposed method the $\lambda_{max}$ of Naratriptan derivative was found to be 352nm when used with water as solvent.The overlain spectra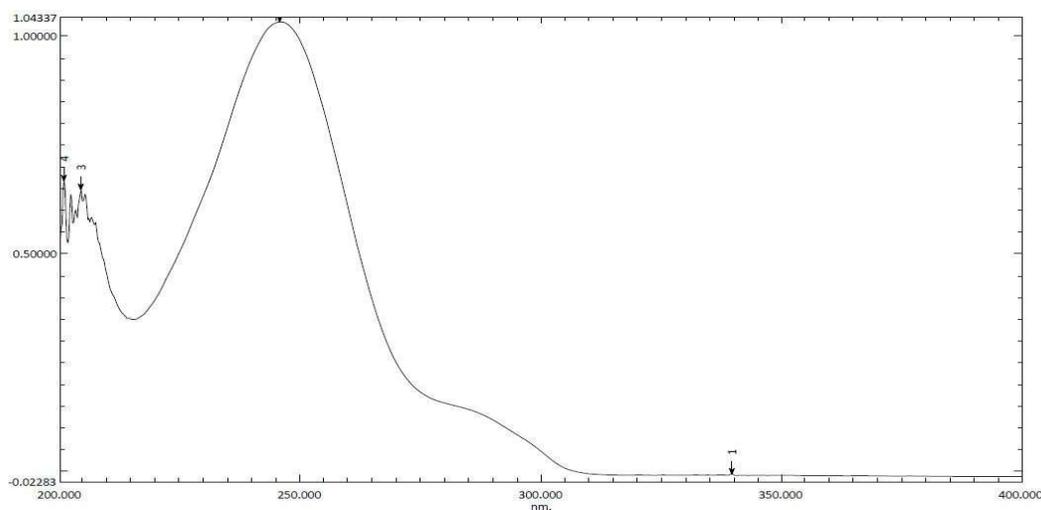 of Naratriptan was found to be in the concentration range 10-20 µg/mL. In Naratriptan visible spetrum, obsevred that, $\lambda_{max}$ may not shift towards longer wavelength because NBD-Cl reagent not interact enough to provide intense colour.
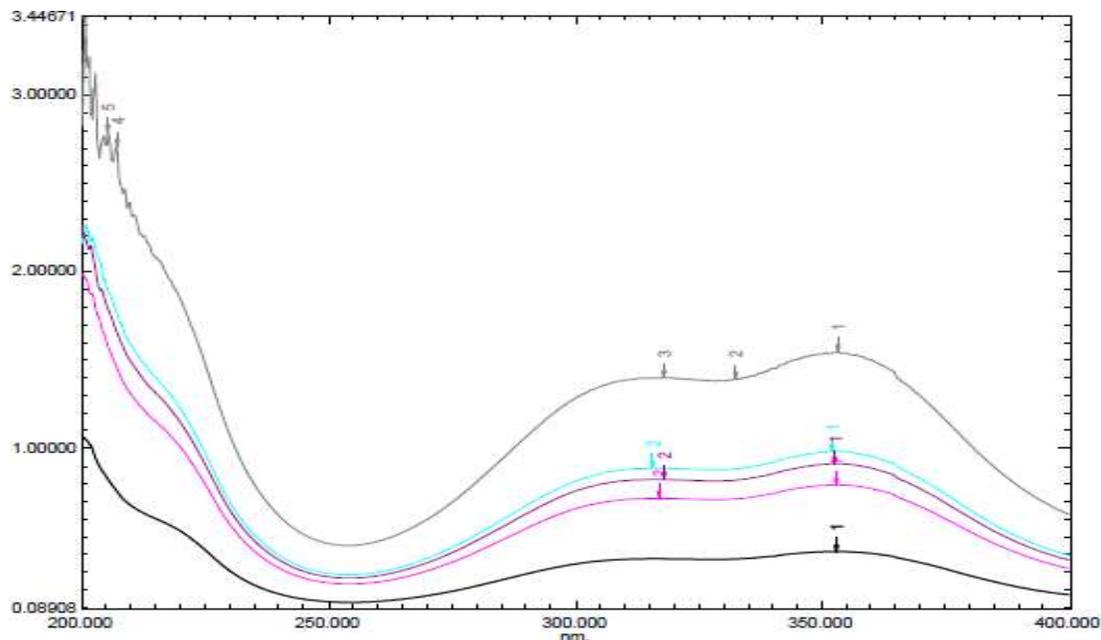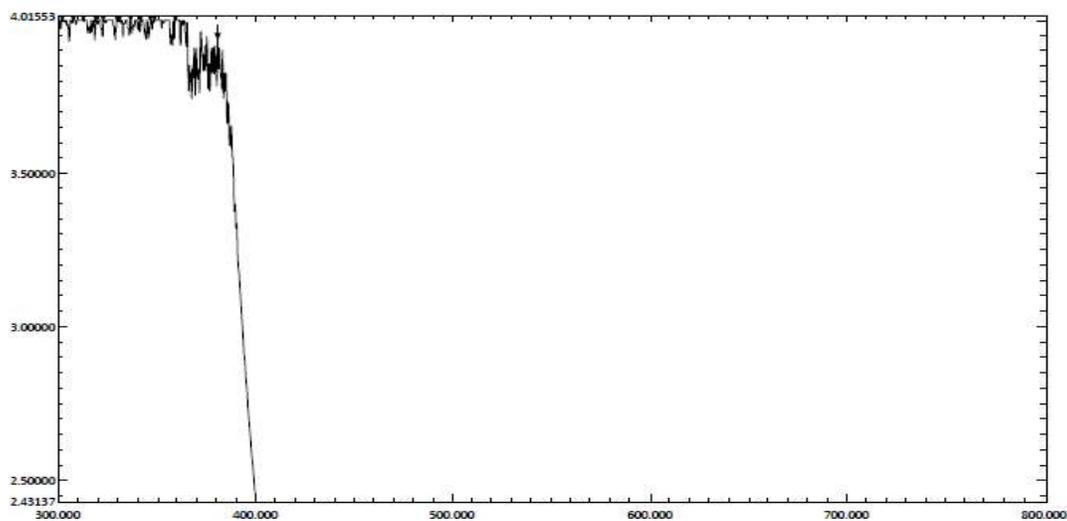


**Figure 13: NBD-Cl against methanol blank spectrum**
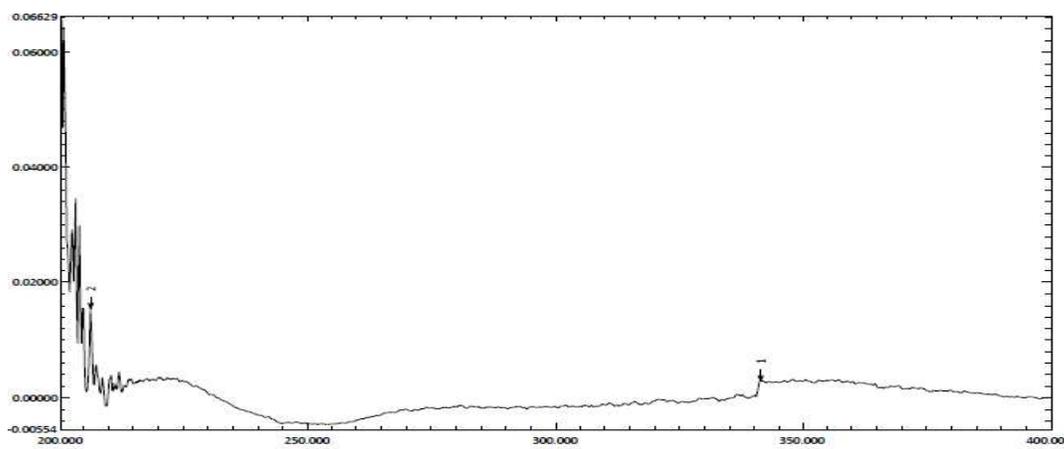
**Figure 14: Methanol solvent blank spectrum**

## Linearity: (UV method)

Linearity of Zolmitriptan and Naratriptan in Water: glycerol (1% v/v) were found to be (1- 10μg/mL) and (1-12 μg/mL) respectively. The results are present in **Tables 1 and 2**. The graphs pertaining to them are depicted in **Figure 15 and 18.**

**Tables 1: Linearity of Zolmitriptan:**

| Concentration(μg/mL) | Absorbance |
|---|---|
| 1 | 0.069 |
| 2 | 0.139 |
| 4 | 0.269 |
| 6 | 0.429 |
| 8 | 0.549 |
| 10 | 0.679 |



y = 0.0683x + 0.0027

R² = 0.9986
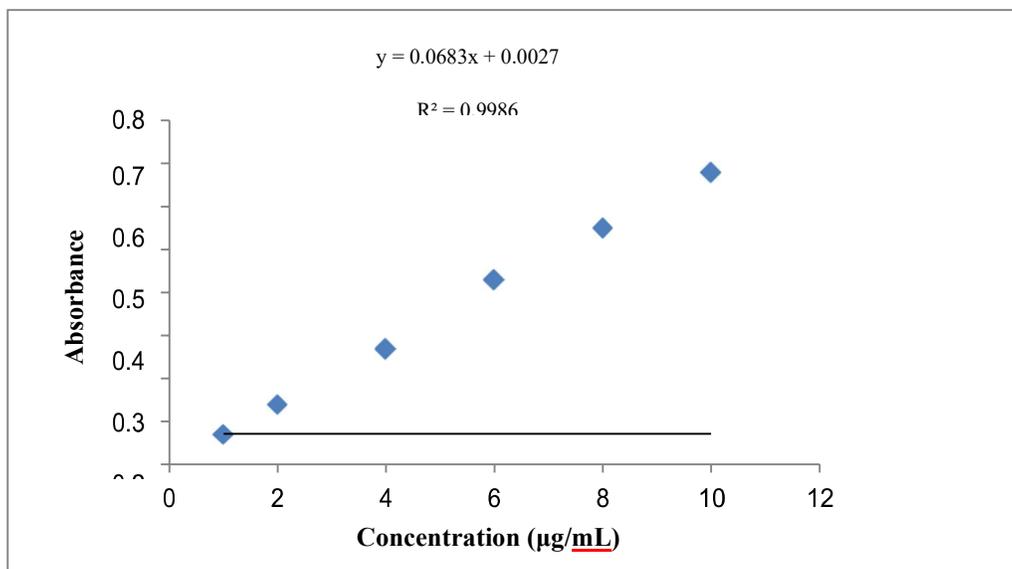
**Figure 15: Calibration curve for Zolmitriptan at 238nm (1% v/v Glycerol in water)**

**Tables 2: Linearity of Naratriptan**

| Concentration (μg/mL) | Absorbance |
|:---:|:---:|
| 1 | 0.036 |
| 2 | 0.053 |
| 4 | 0.102 |
| 6 | 0.145 |
| 8 | 0.190 |
| 10 | 0.203 |
| 12 | 0.275 |



$y = 0.0219x + 0.0129$
$R^2 = 0.9995$
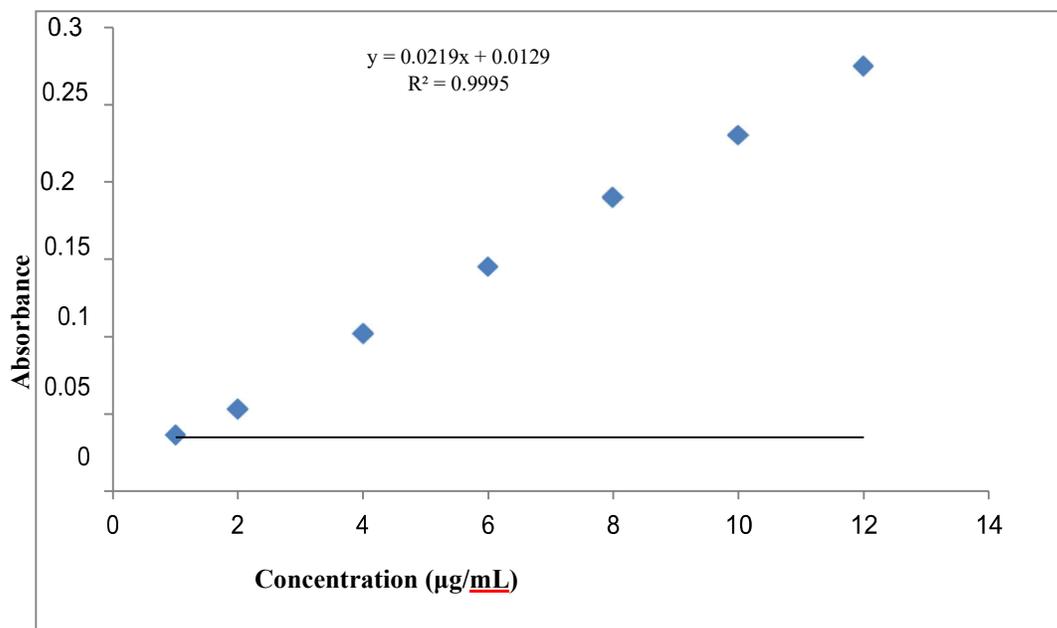
**Figure 16: Calibration curve for Naratriptan at 328nm (1% v/v Glycerol in water)**
**The $R^2$ values for both drugs was found to be > 0.99. which is acceptable**

**Linearity: (colorimetric method)**

Linearity values of (1-10μg/mL) Zolmitriptan derivative in Methanol and (1-12μg/mL) Naratriptan derivative in water used as solvent. The results are present in **Tables 3 and 4**. The graph pertaining to they are depicted.

**Tables 3: Linearity of Zolmitriptan derivative: (colorimetric method)**

| Concentration(μg/mL) | Absorbance |
|:---:|:---:|
| 1 | 0.092 |
| 2 | 0.136 |
| 4 | 0.320 |
| 6 | 0.478 |
| 8 | 0.623 |
| 10 | 0.800 |

y = 0.0796x - 0.0031
R² = 0.9978
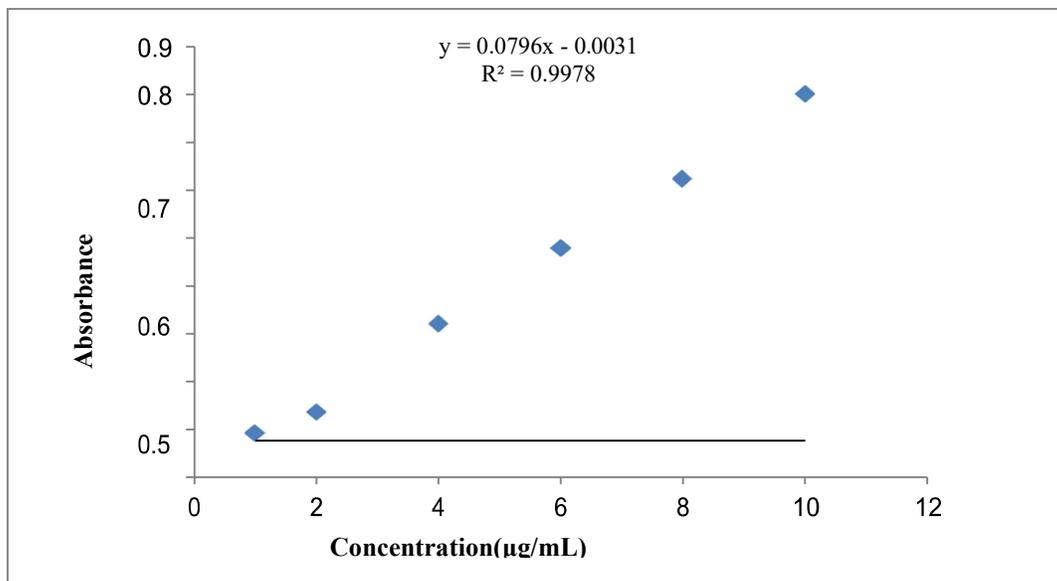
**Figure 17: Calibration curve for Zolmitriptan derivative at 329nm (methanol)**

**Tables 4: Linearity of Naratriptan derivative**

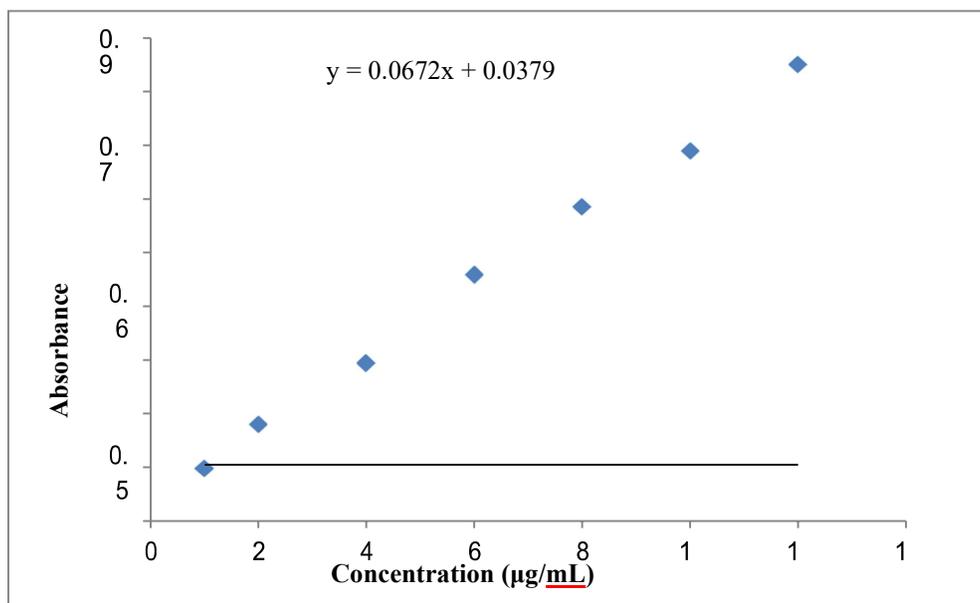| Concentration(μg/mL) | Absorbance |
|---|---|
| 1 | 0.098 |
| 2 | 0.180 |
| 4 | 0.278 |
| 6 | 0.459 |
| 8 | 0.585 |
| 10 | 0.689 |
| 12 | 0.850 |



y = 0.0672x + 0.0379

**Figure 18: Calibration curve for Naratriptan derivative at 352nm (Water)**

The $R^2$ values for both drugs was found to be > 0.99. which is acceptable.

## Precesion: (UV method)

The results of precision of Zolmitriptan & Naratriptan are presented in **Tables 5 & 6**.

The %RSD values for both the derivatives were found to be $\leq 2$.

**Tables 5: Precision of Zolmitriptan**

| Concentration(µg/mL) | Intraday Precesion | Interday precesion |
|---|---|---|
| 2 | 0.135 | 0.181 |
| 2 | 0.136 | 0.184 |
| 2 | 0.137 | 0.185 |
| 2 | 0.138 | 0.186 |
| 2 | 0.136 | 0.183 |
| Mean | 0.1364 | 0.1838 |
| Standard deviation | 0.0011 | 0.0019 |
| % RSD | 0.83 | 0.95 |

**Tables 6: Precision of Naratriptan:**

| Concentration(µg/mL) | Intraday Precesion | Interday precesion |
|---|---|---|
| 10 | 0.215 | 0.258 |
| 10 | 0.218 | 0.262 |
| 10 | 0.216 | 0.264 |
| 10 | 0.215 | 0.263 |
| 10 | 0.215 | 0.262 |
| Mean | 0.2158 | 0.262 |
| Standard deviation | 0.0013 | 0.0024 |
| % RSD | 0.60 | 0.91 |

## Precesion: (Colorimetric method)

The results of precision of Zolmitriptan derivative & Naratriptan derivative are presented in **Tables 7 & 8**. The %RSD values for both the derivatives were found to be $\leq 2$.

**Tables 7: Precision of Zolmitriptan Derivative:**

| Concentration(µg/mL) | Intraday Precesion | Interday precesion |
|---|---|---|
| 6 | 0.472 | 0.494 |
| 6 | 0.473 | 0.496 |
| 6 | 0.473 | 0.491 |
| 6 | 0.474 | 0.493 |
| 6 | 0.475 | 0.495 |
| Mean | 0.473 | 0.493 |
| Standard deviation | 0.016 | 0.0019 |
| % RSD | 0.33 | 0.39 |

**Tables 8: Precision of Naratriptan Derivative:**

| Concentration(µg/mL) | Intraday Precesion | Interday precesion |
|---|---|---|
| 6 | 0.412 | 0.430 |
| 6 | 0.408 | 0.429 |
| 6 | 0.409 | 0.430 |
| 6 | 0.409 | 0.431 |
| 6 | 0.407 | 0.429 |
| Mean | 0.409 | 0.4298 |
| Standard deviation | 0.0018 | 0.008 |
| % RSD | 0.45 | 1.9 |

## Robustness: (UV method)

The robustness results of derivatives of Zolmitriptan and Naratriptan are presented in **Tables 9 & 10**. Noticeable changes were not found on small changes in wavelength and hence the method is found to be robust.

**Tables 9: Robustness study of Zolmitriptan (8μg/mL)**

| Changes in wavelengths(±2nm) | Absorbance |
|---|---|
| 236 | 0.533 |
| 237 | 0. 541 |
| 238 | 0. 546 |
| 239 | 0. 549 |

**Tables 10: Robustness study of Naratriptan (4μg/mL)**

| Changes in wavelengths(±2nm) | Absorbance |
|---|---|
| 325 | 0.092 |
| 326 | 0.097 |
| 327 | 0.102 |
| 328 | 0.106 |
| 329 | 0.109 |

## Robustness: (Colorimetric method)

The robustness results of derivatives of Zolmitriptan and Naratriptan are presented in **Tables 11 &12**. Noticeable changes were not found on small changes in wavelength and hence the method is found to be robust.

**Tables 11: Robustness study of Zolmitriptan derivative (2μg/mL)**

| Changes in wavelengths(±2nm) | Absorbance |
|---|---|
| 327 | 0.137 |
| 328 | 0. 139 |
| 329 | 0. 143 |
| 330 | 0. 145 |
| 331 | 0.149 |

**Tables 12: Robustness study of Naratriptan Derivative (10μg/mL)**

| Changes in wavelengths(±2nm) | Absorbance |
|---|---|
| 350 | 0.693 |
| 351 | 0.695 |
| 352 | 0.697 |
| 353 | 0.699 |
| 354 | 0.699 |

## LOD and LOQ: (UV method)

The limit of Detection and limit of Quantification results are presented in **Table 12**. The method was found to be sensitive for both Zolmitriptan and Naratriptan based on their LOD&LOQ values.

**Table 13: LOD and LOQ**

| Parameters | Zolmitriptan | Naratriptan |
|---|---|---|
| LOD (μg/ml) | 0.09 μg/ml | 0.19 μg/ml |
| LOQ (μg/ml) | 0.27 μg/ml | 0.59 μg/ml |

However the method was found to be relatively more sensitive for Zolmitriptan.

**LOD and LOQ (Colorimetric method):**

The limit of Detection and limit of Quantification results are presented in **Table 13 & 14**. The method was found to be sensitive for both Zolmitriptan derivative and Naratriptan derivative based on their LOD&LOQ values.

**Table 14: LOD and LOQ**

| Parameters | Zolmitriptan Derivative | Naratriptan Derivative |
|---|---|---|
| LOD (µg/ml) | 0.08µg/ml | 0.084 µg/ml |
| LOQ (µg/ml) | 0.24µg/ml | 0.26 µg/ml |

However the method was found to be relatively more sensitive for Zolmitriptan derivative.

The drug method found to be reproducible when subjected analyst and instrument variation.

**Table 15: Ruggedness: (Colorimetric method) 6**

| Variation | Naratriptan | Zolmitriptan |
|---|---|---|
| Actual | 0.583 | 0.604 |
| Analyst to analyst | 0.580 | 0.601 |
| Instrument to instrument | 0.545 | 0.578 |

**Accuracy of Zolmitriptan: (UV method)**

The accuracy results of Zolmitriptan are presented in **Table 16**.The %Recovery in all the three methods was within the limits

**Table 16: Accuracy results of Zolmitriptan**

| %Concentration (at specification Level) | %Recovery | % Mean Recovery |
|---|---|---|
| 50% | 98.12 | |
| 100% | 97.5 | 98 |
| 150% | 98.3 | |

**Effect of time:**

Under the optimum conditions, the effect of reaction time of Zolmitriptan and Naratriptan with reagent in alkaline was constructed, and the product remained stable for 50 minutes.

**Table 17: Naratriptan Derivative**

| Time in minutes | Absorption |
|---|---|
| 0 | 0.307 |
| 5 | 0.307 |
| 10 | 0.308 |
| 15 | 0.307 |
| 20 | 0.305 |
| 25 | 0.305 |
| 30 | 0.308 |
| 35 | 0.311 |
| 40 | 0.309 |
| 45 | 0.317 |
| 50 | 0.316 |

**Table 18: Zolmitriptan Derivative**

| Time in minutes | Absorption |
|---|---|
| 0 | 0.247 |
| 5 | 0.248 |
| 10 | 0.252 |
| 15 | 0.249 |
| 20 | 0.246 |
| 25 | 0.253 |
| 30 | 0.252 |
| 35 | 0.250 |
| 40 | 0.253 |
| 45 | 0.254 |
| 50 | 0.255 |

## CONCLUSION:

The developed UV-VIS spectrophotometric method was found to be simple, accurate, sensitive, precise, specific, economical and rapid. The method is very simple and involving no complicated sample preparations. The proposed UV-Spectrophotometric method was evaluated over the linearity, Ruggedness, Robustness, LOD and LOQ proved to be convenient and effective for the Naratriptan and Zolmitriptan. Colorimetric methods were developed using NBD-Cl reagent for Naratriptan and Zolmitriptan, but the results obtained gives an inference that NBD-Cl reagent had not reacted with both the drugs due to reason that the amino groups of the drugs were secondary. If drugs had primary amino group there may be a possibility for the reaction.

## ACKNOWLEDGEMENTS

## REFERENCES:

[1] GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries,1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet,388, 2016,1545–602.

[2] GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lance, 390, 2017,1211–59.

**[3]** Steiner TJ, Stovner LJ, Vos T. GBD 2015: migraine is the third cause of disability in under 50s. J Headache Pain,17, 2016,104.

**[4]** Li, Jie Jack; Johnson, Douglas S.; Sliskovic, Drago R.; Roth, Bruce D. Contemporary Drug Synthesis (Li/Drug Synthesis), Triptans for Migraine,2004.161-187. doi: 10. 1002 /04716 8674 3.ch12

**[5]** Stovner, Lars Jacob; Nichols, Emma; Steiner, Timothy J; Abd-Allah, Foad; Abdelalim, Ahmed; Al-Raddadi, Rajaa M; Ansha *et al*. Global, regional, and national burden of migraine and tension-type headache, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology,17(11), 2021, 954–976. doi:10.1016/S1474-4422(18)30322-3

**[6]** Tfelt-Hansen P. Naratriptan is as effective as sumatriptan for the treatment of migraine attacks when used properly. A mini-review. Cephalalgia,41(14), 2021,1449 1505. doi: 10 .1177 / 03331 024211028959

**[7]** Yang C, Liang C, Chang C, *et al*. Comparison of New Pharmacologic Agents With Triptans for Treatment of Migraine: A Systematic Review and Meta-analysis. JAMA Netw Open,4(10), 2021,1-15. doi:10.1001/jamanetworkopen.2021.28544

**[8]** Rizk, M.; Sultan, M.; Elshahed, M.; Ali, M. Eur. J. Chem., 9(3), 2018, 251-257. doi:10.5155/eurjchem.9.3.251-257.1731

**[9]** Ashour S, Khateeb M. Spectrophotometric determination of Zolmitriptan in pure form and in tablets through charge transfer complex reactions. J Biomed Allied Res., 1(1), 2019,1-11.

**[10]** Daswadkar SC, Gaikwad JD. Determination of Concentration of Zolmitriptan in Solid Dosage Form by UV Spectroscopic Method. Asian Journal of Pharmacy and Technology.,9(4), 2019,244-248, doi: 10.5958/2231-5713.2019.00040.0

**[11]** Raza A, Ansari TM, Niazi SB. A novel spectrophotometric method for the determination of zolmitriptan in pharmaceutical formulations. Journal of the Chinese Chemical Society.,54(6), 2007,1413-1417.

**[12]** Rao Raghavendra; Humaira Syeda; Munde Mangesh R. Validated UV

Spectroscopic Method For Estimation Of Zolmitriptan From Tablet Formulations. International Journal of Biomedical and Advance Research, 1(3). 2011. 82-87, doi:10.7439/ijbar.v1i3.

[13] Garima, G. & Nehal, S.M.D. & Vipin, G., Development and validation of a simple UV method for in-vitro estimation of zolmitriptan in an intraoral dosage form. Research Journal of Pharmaceutical, Biological and Chemical Sciences,4(2),2013,649-656.

[14] Mr. Rahul M. Sagde, Mr. Pawan N. Karwa, Mr. Vivek M. Thorat, Sanjay S. Jadhav. Analytical Method Development and Validation for the Estimation of Zolmitriptan by RP HPLC Method. International Journal of Trend in Scientific Research and Development, 3(5), 2019,804-810. https://doi.org/10.31142/ijtsrd26474

[15] Dighe N, Hodgar SK, Musmade DS. Development and Validation of RP-HPLC Method for the Identification of Process Related Impurities of Zolmitriptan. J Anal Pharm Res.,4(1), 2017,24-32

[16] Girotra P, Singh SK, Kumar G. Development of zolmitriptan loaded PLGA/poloxamer nanoparticles for migraine using quality by design approach. International journal of biological macromolecules,85, 2016,92-101.

[17] Jebali S, Belgacem C, Louhaichi MR, Bahri S, Latrous El Atarche L. Application of factorial and Doehlert designs for the optimization of the simultaneous separation and determination of antimigraine drugs in pharmaceutical formulations by RP-HPLC-UV. International journal of analytical chemistry. 2019, Aug 15;1-11

[18] Yusop Z, Jaafar J, Aris AB, Majid ZA, Umar K, Talib J. Development and validation of a selective, sensitive and stability indicating UPLC–MS/MS method for rapid, simultaneous determination of six process related impurities in darunavir drug substance. Journal of pharmaceutical and biomedical analysis,128,2016,141-148.

[19] Patel BM, Jangid AG, Suhagia BN, Desai N. Novel bio analytical method development, validation and

application for simultaneous determination of nebivolol and S-amlodipine in human plasma using ultra performance liquid chromatography-tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis,151,2018,244-51.

[20] Hernández CN, Martín-Yerga D, González-García MB, Hernández-Santos D, Fanjul-Bolado P. Evaluation of electrochemical, UV/VIS and Raman spectroelectrochemical detection of naratriptan with screen-printed electrodes. Talant,178,2018,85-88.

[21] Gupta A, Kumar J, Narang JK, Verma S, Singh H, Haque A. Development and validation of a stability indicating UV-spectrophotometric assay method for the determination of naratriptan hydrochloride. Pertanika J. Sci. Technol,27, 2019,933-941.

[22] Sai VM, Guptha DV, Krishna BS, Vasantharaju SG. Stability indicating assay method development and validation of naratriptan hydrochloride by RP-HPLC. Research Journal of Pharmacy and Technology,9(8), 2016,1177-1182.

[23] Balasekhara Reddy Challa, Bahlul Zayed Shtaiwy Awen and Babu Rao Chandu *et al*. Method development and validation for naratriptan determination in human plasma by HPLC with tandem mass spectrometry detection, and its application to bioequivalence study. Braz. J. Pharm. Sci.,47(1),2011,13-22. DOI: 10.1590/S1984-82502011000100003

[24] Shiva U. A Novel RP-HPLC method for the estimation of naratriptan HCl in bulk and dosage form. International Research Journal of Pharmaceutical and Applied Sciences,2(6), 2012,110-116.

[25] Shelke S, Shahi S, Kale S. Development and validation of UV spectrophotometric method of naratriptan hydrochloride in bulk and pharmaceutical formulation. Asian Journal of Biomedical and Pharmaceutical Sciences,5(47), 2015,36.

[26] Guideline, ICH Harmonized Tripartite. "Validation of analytical procedures: text and methodology." Q2 (R1) 1 (2005).