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ELECTROCHEMICAL AND DENSITOMETRIC DETERMINATION OF ZOLMITRIPTAN IN PHARMACEUTICAL DOSAGE FORMS

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Abstract

Two methods for the determination of antimigrain drug zolmitriptan in bulk powder and in pharmaceutical dosage forms by voltammetric and densitometric methods are developed. Voltammetric method depends on the oxidation of zolmitriptan on an activated carbon paste electrode. The compound is oxidized irreversibly at high positive potential. The response was evaluated with respect to pH; scan rate, nature of the buffer and other variables. The peak current at about 0.76V, with the maximum current at pH 5 \pm 0.2 acetate buffer. The mechanism of the reaction is controlled by oxidation of the N-H group in the indole ring. By differential pulse voltammetry, the calibration plot was linear in the range $3.99 \times 10^{-7} - 2.90 \times 10^{-6}$ M.

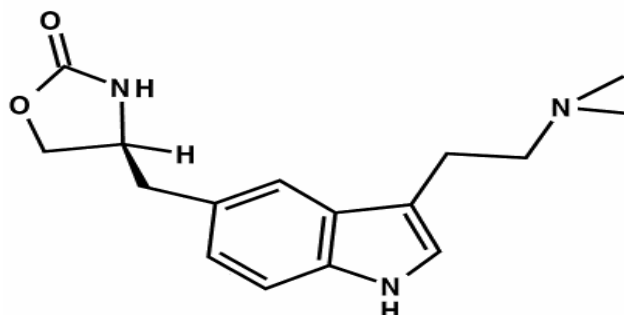
The second method is based on the application of thin layer chromatographic separation of the studied drug in the presence of its degradation product followed by the densitometric measurements of the spot areas of the drug. After separation on silica gel GF₂₅₄ plates using methanol: ammonia 25% (100:1.5 v/v) as mobile phase, the chromatographic zones corresponding to the spots of zolmitriptan were scanned at 285nm. The calibration function was established in the range of 1.00- 9.00 μ g/ spot. The suggested methods were used to determine the cited drug in bulk powder, and pharmaceutical dosage forms. Results were compared statistically with the reported method.

Key words: Voltammetry, Densitometry, Zolmitriptan

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Introduction

Zolmitriptan (zol), (S)-4-[[3-(2-(dimethylamine) ethyl)-1H-indol-5-yl]methyl]-2-oxazolidinone, is a novel and highly selective 5-HT_{1B/1D} receptor agonist used in the acute oral treatment of migraine and has recently been used as an effective neuroendocrine probe of 5-HT_{1D} receptor function in humans¹. Its structure is shown in scheme 1.

**Scheme 1**

It is worth-noting that there are no reported methods for the analysis of this drug in pharmaceutical dosage forms, whereas in biological fluids, only few attempts have been made. These were mainly chromatographic technique by HPLC method using coulometric detection² and with electro spray tandem mass spectrometry³ and solid phase extraction using liquid chromatography coupled to tandem mass spectrometry. No electro analytical or stability indicating methods for the determination of the drug have been reported in the literature.

This work is concerned with a study of the voltammetric behavior of zol using the particularly rapid and selective technique cyclic voltammetry at carbon paste electrode (CPE). Zolmitriptan is adsorbed on a CPE and this phenomenon was used in the design of an adsorptive stripping method for the determination of zol at nanomolar concentration levels. Alternatively, densitometric method was proposed for zol analysis. This method has been used as stability-indicating method for the determination of the cited drug in the presence of its degradation product. The proposed methods have been applied for the determination of the studied drug in bulk powder and in pharmaceutical dosage forms.

Experimental

Voltammetric measurements were carried out using a computer driven AEW₂ Analytical electrochemical workstation with ECProg 3. Electrochemistry software (Sycopel, England) in combination with C-2 stand with a three-electrode configuration, a carbon paste electrode (BAS model MF-2010, 3 mm diameter) working electrode, an Ag/AgCl/3M KCl (BAS model MF-2063) reference electrode and platinum wire (BAS model MW-1032) counter electrode Microcal Origin (V.5.10). Software was used for the transformation of the initial signal. A cyberscan

500 digital pH-meter with glass combination electrode served to carry out the pH measurements.

The differential pulse voltametry conditions were: pulse amplitude 25 mv, pulse width 20 ms, pulse interval 400 ms, step potential 4 mv and scan rate 10 mv/ sec.

- Densitometer, Shimadzu dual wavelength CS-9301.
- UV lamp short wavelength, 254nm.
- TLC plate's 20x20 cm with 0.25 mm thickness, silica gel GF₂₅₄ (E. Merck, Darmstadt, Germany). The samples were applied to TLC plates using a 25 µl Hamilton micro syringe.

Materials

- Zolmitriptan, working standard, kindly supplied by Zenica (England). The purity of the sample was found to be $99.96 \pm 0.52\%$ according to the reported method.

- Zomic tablets-Zenica batch No. GA 8721. Each tablet was claimed to contain 2.5 mg zolmitriptan.

Reagents and Standard Stock Solutions.

All chemicals used were of analytical grade and solvents were of spectroscopic grade.

- 2M sodium hydroxide, aqueous solution.
- 2M hydrochloric acid, aqueous solution.
- Methanol (E. Merck).
- Acetate buffer pH 5⁴.

Preparation of stock standard solution

Zolmitriptan stock solution 1 mg/ml in methanol for densitometric method.

The working solution for voltametric investigations was prepared by dilution of the stock solution with selected supporting electrolyte pH 5 ± 0.2 to obtain a solution of Zol. 1×10^{-3} M.

Preparation of degradation products.

A 50 mg zol. bulk powder dissolved in 2ml methanol and then 25 ml 2M sodium hydroxide was added. The solution was heated in the oven at 105°C for 4

hrs. and then cooled. The solution was neutralized with 2M hydrochloric acid, evaporated in a boiling water bath for about 5ml and filtered if necessary. The solution was transferred into 50-ml volumetric flask and the volume was completed with methanol (1mg/ml) for densitometric method.

Laboratory prepared mixtures.

Mixtures of zol and its degradation product were prepared containing 0.50-4.50 mg of the first and 10-90% of the latter (1.00mg/ml) into a series of 5-ml volumetric flasks and the volume was completed with methanol. TLC using methanol-tested solution for complete degradation: ammonia 25% (100:1.5v/v) as mobile phase.

General Procedures for calibration curves

-By voltammetric method

The activation of carbon paste electrode using a high anodic potential is highly effective⁵. The appropriate initial potential was then selected and electrode allowed to stabilize for 30 s, a scan was then initiated from 0.2- 1.2V using a scan rate of 100 mv/s. The peak current was evaluated as the difference between each voltammogram and the background electrolyte to voltammogram.

All data were obtained at ambient temperature

-By densitometric method

An accurately volume of stock standard solution (1.00 mg/ml) equivalent to 0.50-4.50 mg zol were transferred into 5-ml volumetric flasks and completed to the decided volume with methanol. A 10.0 μ l of each solution was applied on the TLC plate (20x20cm) and placed in a chromatographic tank previously saturated for 30 min. with the developing mobile phase methanol: ammonia (100:1.5v/v). The plate was developed by ascending chromatography for a distance of 16 cm then removed and air-dried. The spots were detected under UV lamp at 254nm and scanned at 285nm. The calibration curve was constructed by plotting the area under the peaks versus the corresponding concentration and the regression equation was computed.

Assay of prepared mixtures

10.0 μ l of different prepared mixtures was applied on TLC plates and the procedure was followed as mentioned above. The concentration of zol was calculated either by substituting in the regression equation or by comparing to standard spotted, developed and scanned under the same condition.

Procedures for pharmaceutical dosage forms

Ten tablets were accurately weighed and finally crushed. A portion of the powder equivalent to 10 mg of zol was weighed, dissolved in 3x10 ml methanol and filtered. The filtrate was evaporated under nitrogen till about 5ml, transferred quantitatively into 10 ml volumetric flask and completed to volume with the same solvent (1.00mg/ml) for densitometric method. An aliquot of this solution was diluted with acetate buffer pH 5 \pm 0.2 to obtain a solution of 1×10^{-3} M for voltammetric method. The procedure was completed as under calibration curve of each method. The concentration of the drug was calculated from the corresponding regression equation.

Results and Discussion*-Voltammetric method.*

Cyclic voltammograms of zol obtained in acetate buffer pH5.0 at scan rate of 100 mV/S are shown in Figure 1. In cyclic voltammograms one well-defined anodic peak was observed. The fact that no peak was observed in the reverse scan suggests that the oxidation process is an irreversible one. The peak currents decrease with succeeding potential scans suggesting formation of adsorbed species on electrode surface.

The number of electrons involved in the oxidation process indicating that one electron was transferred in the oxidation process⁶. A comparison with one oxidation obtained for indole-3-acetic acid, which occurred at a similar potential to that of zol, indicated that the oxidation occurred at nitrogen atom in the indole ring of the molecule.

The effect of potential scan rate *ip*, on the peak current and peak potential of zol was evaluated as shown in Figure 2.

The variation *iP* vs V is linear over the scan range 25-300 mv/s giving straight line which fitted the equation:

$iP (\mu A) = (1.1288 \pm 0.14125) + (0.01973 \pm 8.2630) X$, $r = 0.9947$. The peak potential was shifted to less positive values on increasing the scan rate, which conforms the irreversible nature of the oxidation process.

The effect of pH and composition of the supporting electrolyte was evaluated for 2.5×10^{-5} M zol solution using cyclic voltammetry in various electrolytes such as

acetate, phosphate and Britton Robinson buffers of different ionic strength in the range (0.04-0.2 M). Maximum size peaks were obtained with 0.05 M acetate buffer (pH 5 ± 0.2). Figure 3 illustrates the differential pulse voltammetric response to different concentrations of zol (acetate buffer pH5). The peak current was linearly related to zol concentration within the range 0.394- 2.99 μg zol (3.99×10^{-7} - 2.99×10^{-6} M) according to the regression equation.

The proposed method was then applied to pharmaceutical preparation samples. Nine samples from different dissolved tablets were analyzed using the proposed voltammetric method. Appropriate dilution to produce solution within the linear range of the calibration curve was used in the analysis. The values found ranged from 3.99×10^{-7} to 2.99×10^{-6} M according to the regression equation.

-Densitometric method

TLC densitometric method was used for stability indicating method for determining zol in the presence of its alkaline induced degradation products. Experimental conditions such as mobile phase composition scan mode, speed, and wavelength of detection were optimized to provide accurate, precise and repeatable results for zol. The chosen scan mode was the zigzag mode, and the wavelength of scanning was chosen to be 285nm. The greatest differences between the R_f values of the cited drug and its degradation product (0.65 and 0.32, respectively,) were obtained by the system-containing methanol: ammonia 25%(100:1.5v/v). With this technique a linear correlation was obtained between the concentrations 1.00-9.00 $\mu\text{g}/\text{spot}$ of zolmitriptan and the spot area. The linear regression equation was computed and found to be:

$$A = 5.59 \times 10^2 + 9.68 \times 10^2 C \qquad r = 0.9991$$

Where A is the peak area, C is the concentration in $\mu\text{g}/\text{spot}$ and r is the correlation coefficient.

To assess the stability indicating efficiency of the TLC densitometric method the degradation product of zol were mixed with the intact sample in different ratios (10-90%) and the mixtures were analyzed by this method. The results obtained are shown in Table 1. It is clear that the suggested method is valid for determining zol in laboratory prepared mixtures containing up to 90% of its degradation products.

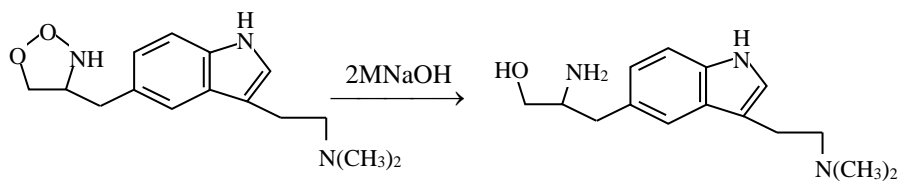
The proposed methods were successfully applied for the analysis of the studied drug in its dosage forms as shown in Table 2. Applying the standard addition

technique further assessed the validity of the proposed methods. Results obtained are presented in Table 2, proving no interferences from additives or excipients, which are normally, present in dosage forms.

Table 3, shows the analytical data and statistical comparison of analytical result for pure sample by the proposed methods and reported one (HPLC method). Calculated t- value and F- ratio are less than the theoretical one indicating with 95 % confidence that there is no significant difference between the proposed methods and the reported HPLC method with respect To accuracy and precision.

Identification of degradation product

When zolmitriptan was heated with 2M sodium hydroxide for 4 hrs, complete degradation was obtained. With the above mentioned TLC system, the R_f values were (0.65 and 0.32 for the cited drug and degradation product. , respectively,). By spraying the plate with Marshal' reagent a violet colour was produced for free amino group. This was confirmed by IR spectrum that showed the presence of an absorption band at 3350 cm^{-1} and 3300 cm^{-1} corresponding to the amino function and an absorption band at 3200 cm^{-1} corresponding to OH group. In addition to the C=O function at 1700 cm^{-1} totally disappears in the product confirming the hydrolytic step followed by decarboxylation Figure 4. The mass spectrum of the hydrolytic product showed a molecular ion peak at 262, which is in accordance with the suggested formula as shown in Figure 5. Therefore, the suggested pathway for the degradation of zolmitriptan in 2M sodium hydroxide is presented in the following equation:



Zolmitriptan

2-Amino-3-(3-(2-(dimethylamino)ethyl)-1H-indol-5-yl)-1-propanol

Validation of analytical proposed methods.

Linearity, in quantitative analysis of zolmitriptan, calibration curves was plotted representing the relationship between i_p current and potential E_p in cyclic voltammetry and between the peak area versus concentration in densitometry. The resulting data was studied by linear regression analysis. In all cases, linearity plots had correlation coefficient 0.9947 and 0.9991, respectively.

Accuracy and precision were studied using solutions containing different concentrations and analyzed in five replicates by the two proposed methods. The results were accurate and reproducible with low standard deviations. This indicates that the developed methods have good precision.

Specificity / selectivity. Specificity is the ability of the methods to measure the anyolute response in the presence of additives, excipients and degradation products. It was found that assay results were not changed in pure forms, in drug formulations in both methods and in laboratory prepared mixtures in densitometric method. In the proposed methods, there was no need of pre separation and only filtration was applied to make the solution clear.

Ruggedness, according to USP is a measure of the degree of reproducibility expressed, as relative standard deviation under variety of conditions among which is the experimental periods. The intraday (n=5) and interday (n=3) variations were evaluated by repetitive analysis of samples containing 3.99×10^{-7} , 2.99×10^{-6} M in voltammetric method and 2.00, 8.00 $\mu\text{g/spot}$ in densitometric method. The relative standard deviations were then calculated.

The results of assay validation are represented in Table 4, from the data obtained it is proved that the proposed methods are accurate, precise and rugged over the specified range.

Conclusion

The high sensitivity of the voltammetric method allowed the determination of zolmitriptan in biological fluids. The proposed TLC densitometric method is very simple, rapid and uses a minimal volume of solvents, compared to the other separation techniques. Furthermore, an extremely large number of samples can be analyzed at the same time without compromising accuracy and also, can be used as stability-indicating method. The proposed methods are suitable for quality control laboratories.

Table 1. Comparison of results obtained by the proposed densitometric method and reported method for the determination of zolmitriptan in laboratory prepared mixtures.

| Sample No. | Degradation product, % | Densitometric method, Recovery,% | Reported method ^b Recovery, % |
|------------|---------------------------|-------------------------------------|---|
| 1 | 10 | 100.21 | 98.94 |
| 2 | 20 | 99.26 | 99.15 |
| 3 | 30 | 101.00 | 100.12 |
| 4 | 40 | 99.96 | 99.14 |
| 5 | 50 | 98.97 | 101.24 |
| 6 | 60 | 100.67 | 99.29 |
| 7 | 70 | 99.73 | 99.15 |
| 8 | 80 | 99.21 | 100.64 |
| 9 | 90 | 100.51 | 99.56 |
| Mean | | 99.95 | 99.69 |
| RSD% | | ± 0.71 | ± 0.80 |

^a Recoveries were calculated with reference to weight (drug- degradation in mixtures).

^b HPLC manufacturer procedure method.

Table 2. Determination of zolmitriptan in pharmaceutical formulations by the proposed and reported method.

| Preparation | Voltammetric method Recovery ^a , % | Densitometric method Recovery ^a , % | Reported method ^b Recovery ^a , % |
|---------------------------------|---|--|---|
| Zomic Tablets 2.5 mg/ tablet | 99.25 ± 0.66 | 99.74 ± 0.44 | 99.31 ± 0.81 |
| B.N. | | | |
| F ^c | 1.49 | 3.39 | |
| T ^c | 0.13 | 1.04 | |

^a Values are mean ± RSD% (n=5). ^b HPLC manufacturer procedure.

^c Theoretical values of F- ratio and t- values at the 95% confidence level are 6.39 and 2.31, respectively.

Table 3. Analytical data and statistical comparison of results for the determination of zolmitriptan bulk powder by both the proposed methods and reported method

| Parameters | Voltammetric method | Densitometric method | Reported method ^b |
|------------------------------|---|------------------------|------------------------------|
| Concentration range | 3.99×10^{-7} - 2.99×10^{-6} M | 1.00-9.00 μ g/spot | |
| Mean \pm RSD% ^a | 99.22 \pm 0.75 | 99.71 \pm 0.82 | 99.62 \pm 0.63 |
| Variance | 0.56 | 0.67 | 0.40 |
| F ^c | 1.41 | 1.68 | |
| t ^c | 0.820 | 0.196 | |

^a n= 5^b HPLC manufacturer method using Spherisorb 5 ODS2 as a column, water: acetonitrile: trifluoroacetic acid: triethylamine (1730: 270: 2: 0.5) as mobile phase, flow rate 1.0 ml/ minute and UV detection at 225 nm.^c Theoretical values of F- ratio and t- values at 95% confidence level are 6.39 and 2.31, respectively.**Table 4. Results of assay validation⁷ obtained by applying the proposed voltammetric and densitometric methods**

| Parameters | Voltammetric method | Densitometric method |
|-------------------------|---|------------------------|
| Range | 3.99×10^{-7} - 2.99×10^{-6} M | 1.00-9.00 μ g/spot |
| LOD | 3.35×10^{-7} M | 0.25 |
| LOQ | 1.11×10^{-6} M | 0.90 |
| Slope | 0.1973 | 9.68 |
| Intercept | 1.1288 | 5.59×10^2 |
| Mean \pm RSD % | 99.22 \pm 0.75 | 99.71 \pm 0.82 |
| Correlation Coefficient | 0.9947 | 0.9991 |
| RSD % ^a | 5.55×10^{-8} - 2.36×10^{-7} M | 0.666- 0.794 |
| RSD % ^b | 8.41×10^{-8} - 4.65×10^{-7} M | 0.763- 1.023 |

^a The inter day (n=3) and ^b the intra day (n=5) relative standard deviations concentrations 3.99×10^{-7} - 2.99×10^{-6} M and 3.00-8.00 μ g/spot for cyclic voltammetry and densitometry, respectively.

.LOD and LOQ is the limit of detection and limit of quantitation.

Fig. 1. Cyclic voltammogram for 5.0×10^{-3} M Zlmitriptan in acetate buffer pH 5.0 at carbon paste electrode, scan rate = 100 mV s^{-1} .

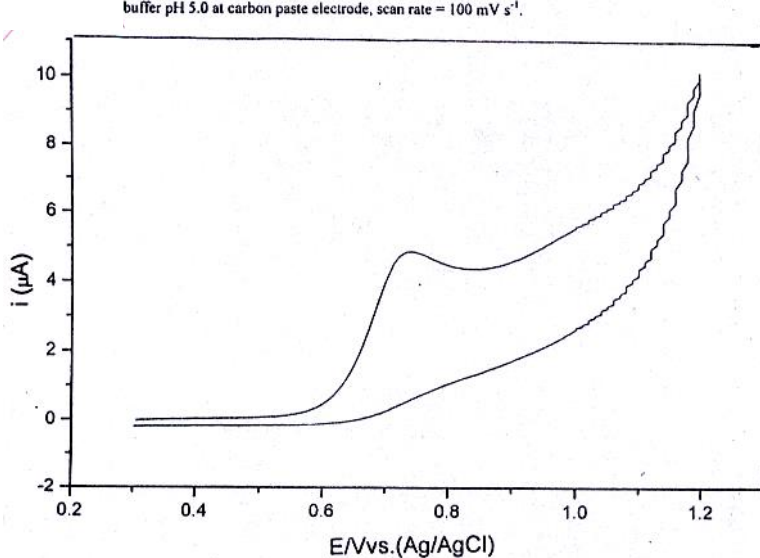


Fig. 2. Effect of scan rates (25-300 mV/s) on peak current for 5.0×10^{-3} M Zalmitriptan.

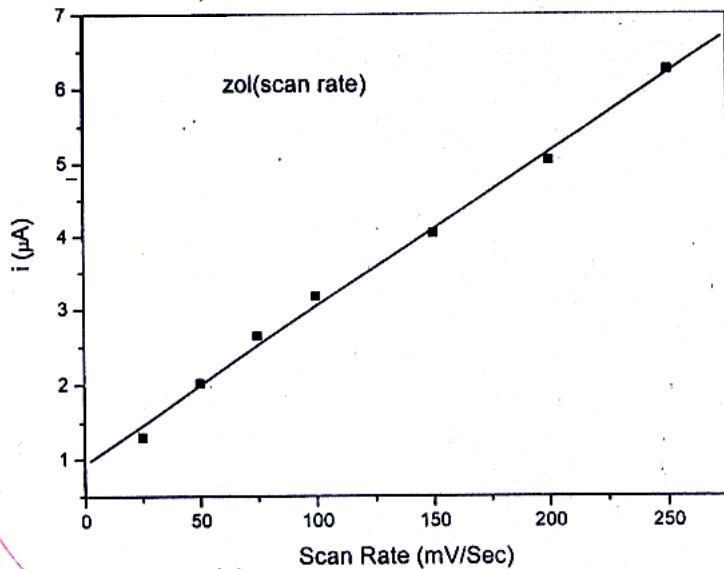


Fig.3. Anodic differential pulse voltammograms obtained for increasing concentration Zolmitriptan (b) 2.0 (c) 4.0 (d) 6.0 (e) 8.0 and (f) 10.0 $\mu\text{g ml}^{-1}$. Inset is the calibration plot. Dotted lines represent the blank.

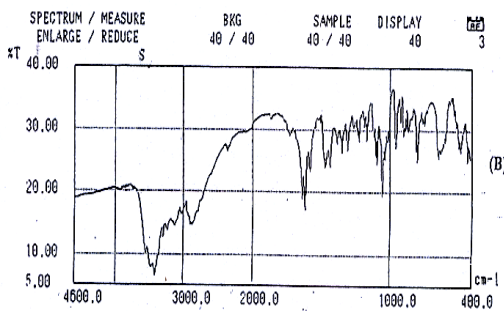
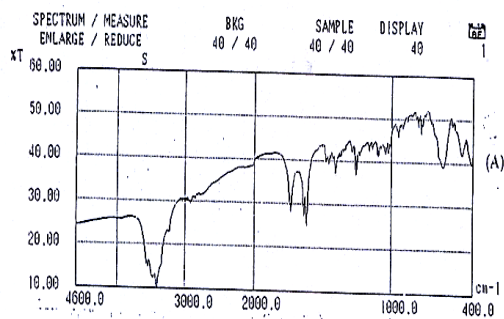
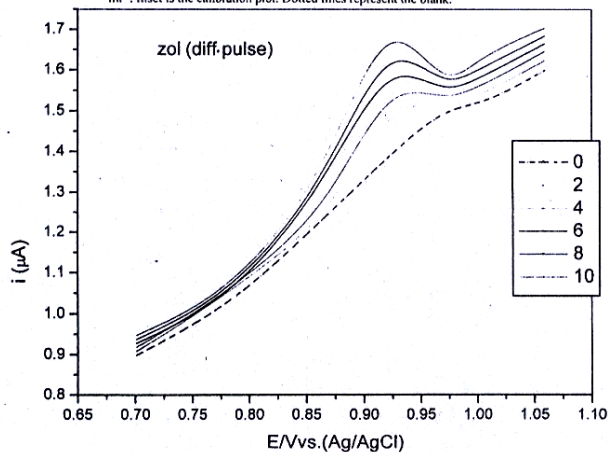


Figure 4: IR spectrum of zolmitriptan and its degradate (A & B)

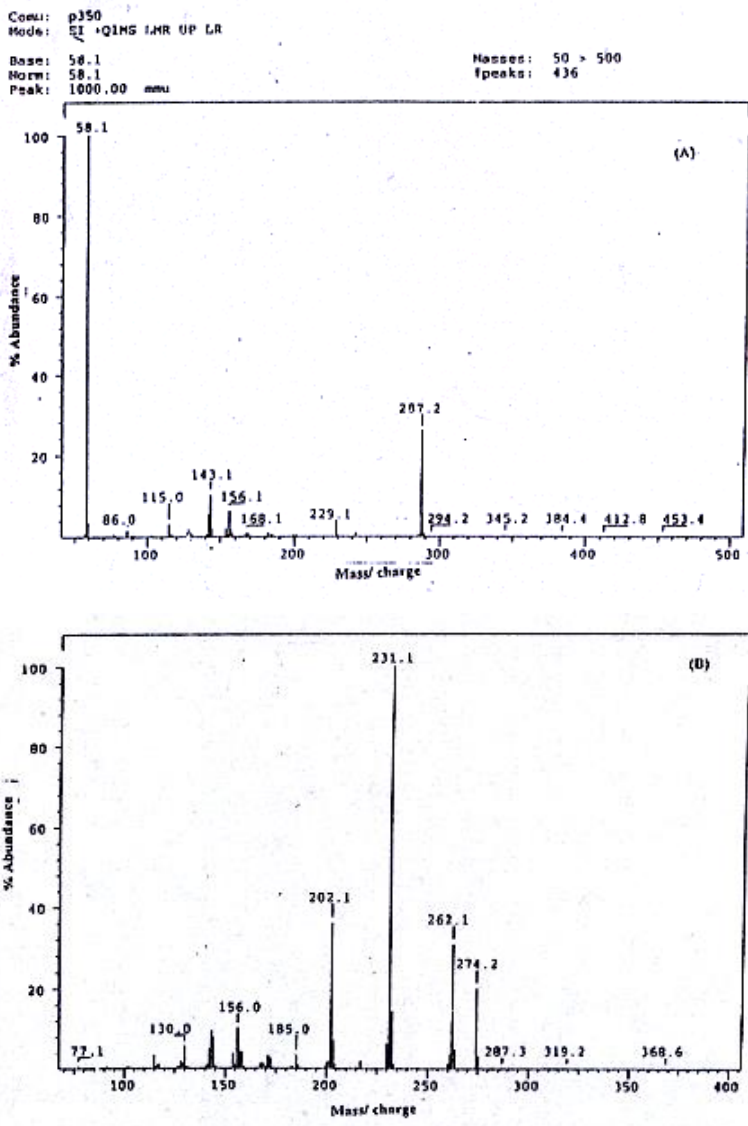


Figure 5 The mass spectra of (A) Intact zolmitriptan, (B) Degradation product.

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