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DEVELOPEMENT AND VALIDATION OF A SELECTIVE AND RAPID LC-MS/MS METHOD FOR QUANTIFICATION OF ZOLMITRIPTAN

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ABSTRACT

A sensitive and specific liquid chromatography electrospray ionization mass spectrometry (LC-MS/MS) method has been developed and validated for identification and quantification of zolmitriptan. The liquid chromatographic separation was performed with Symmetry C18 packed column (3.5 µm, 2.1 × 50 mm) using a mixture of Acetonitrile-Water-Formic acid (70 : 30 : 0.1) at a flow rate of 0.2 mL/min. Detection was performed on a single quadrupole mass spectrometer by selecting ion monitoring (SIM) mode via electrospray ionization (ESI) source. In positive mode, zolmitriptan produced a protonated precursor ion at m/z 289.41 and a corresponding product ion at m/z 244.35. Linearity was established for the range of concentrations 30-1000 ng/mL, with a coefficient of determination(r) of 0.994 and good back calculated accuracy and precision. The inter and intraday precision (R.S.D %) were lower than 5% and accuracy ranged from 95-110%. The lower limit of quantification was identifiable and reproducible at 1 ng/mL. The proposed method enables the unambiguous identification and quantification of zolmitriptan.

Key words: Zolmitriptan, Triptan, LC-MS/MS, LC-ESI-MS.

INTRODUCTION

Migraine is a recurrent incapacitating neurovascular disorder characterised by attacks of debilitating pain associated with photophobia, phonophobia, nausea and vomiting. Neurogenic theory considers migraine to be a spreading depression of cortical, electrical activity followed by vascular phenomenon[1,2].

Attacks of migraine typically last from several hours to 2 to 3 days, and many

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patients suffer one or more attacks a month[3]. Against this background the triptans, selective serotonin 5-HT (1B/1D) agonist are very effective acute migraine drugs with a well developed scientific rationale[4].

Zolmitriptan is a second generation triptan developed to provide improved pharmacokinetic and optimised trigeminovascular targeting of both the peripheral and central trigeminal terminals[3].

Zolmitriptan (s)-4-[3-[2-(dimethylamino) ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone. Clinical research indicates that it has a better efficacy and tolerability profile at low doses of 2.5-10 mg. Zolmitriptan is rapidly adsorbed when given as oral tablets both in fasting state and when given with food.

In the previous studies, Seaber et al.[5] developed a HPLC method to assay zolmitriptan and its three major metabolites with fluorescence detection and Clement and Franklin[6] established a HPLC method for quantification of zolmitriptan and its two major metabolites with coulometric detection.

A more sensitive liquid chromatography mass spectrometry (LC-MS-MS) method[7] was developed to assay zolmitriptan and its active metabolite 183C91. But the necessity of tandem mass spectrometry system was a restriction in terms of cost and general applicability.

Chen et al.[8] developed a method to determine zolmitriptan in human plasma by LC-MS-MS method. The mobile phase consisted of Acetonitrile-Water-Formic cid (70 : 30 : 0.5) at a flow rate of 0.5 mL/min, as the concentration of formic acid increase the MS signal was depressed.

All the above methods were based on plasma and biological data. In this paper, we describe a more simple, selective and highly sensitive method by using HPLC coupled with electro spray ionisation (ESI) single quadrupole mass spectrometry (MS) for the determination of Zolmitriptan.

EXPERIMENTAL

Chemicals and reagents

Zolmitriptan (purity > 99%) was a gift sample from a local manufacturing unit in Hyderabad, India. LC-MS acetonitrile was purchased from Sigma Aldrich, all other chemicals were commercially available analytical grade materials used as received. Milli-Q water was prepared from demineralised water was used throughout the study.

Apparatus

Liquid chromatographic-mass spectrometric analysis was undertaken with an alliance 2695 separation module (HPLC) and a Micromass Quattro micro mass spectrometer from WATERS (Waters Corporation, Milford, MA, USA) equipped with the electro spray ionization (ESI) interface. Data collection, integration and calibration were accomplished using LC solutions chromatography data systems.

Chromatography

Chromatography was performed on a Symmetry C18 packed column (3.5 µm, 2.1 × 50 mm particle size, Waters, Ireland) maintained at 60^0C. The HPLC system consists of Shimadzu LC-20 AT liquid chromatographic pump, Rheodyne injection port (Rheodyne, Cotati, CA, USA) with a 20 µL sample loop and SPD-M20A photo diode array (PDA) detector (Shimadzu, Kyoto, Japan). The mobile phase consisted of LC-MS Acetonitrile: milli-Q water: formic acid (70 : 30 : 0.1 v/v) delivered at 0.2 mL/min.

Mass spectrometry

The MS detector was equipped with electro spray ionization (ESI) interface and operated in positive polarity. Operational optimised ESI parameters were as follows; capillary temperature 200^0C; ion spray voltage 3000 V; capillary voltage 60 V; extractor lens 3 V.

Calibration standards

Stock solutions of zolmitriptan with a concentration of 1000 µg/mL was prepared by dissolving 25 mg of zolmitriptan into 25 mL volumetric flask and adds about 25 mL of diluents (80 : 20 of acetonitrile and water) and sonicate to dissolve completely, make volume up to the mark with the same diluent. Seven standard solutions of 30, 50, 70, 100, 500, 700 and 1000 ng/mL of zolmitriptan were prepared by further dilution of the stock solution with appropriate volumes of diluents.

RESULTS AND DISCUSSION

Chromatography and mass spectrometry

The positive ionisation mode was selected for the determination of Zolmitriptan because of the presence of amino group, which was easily protonated. Electron spray ionisation source under positive ion detection mode was found to better than the other

sources evaluated during the early stage of method development. By positive ESI mode the analyte formed predominantly protonated molecules at m/Z 288.87 and 244.87 in Q1 full scan mass spectra as shown in Fig. 1.

Table 1: Precision and accuracy for the determination of Zolmitriptan[a]

Nominal concentration (ng/mL)	Calculated concentration (ng/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Accuracy (%) recovery
50	50 ± 2.46	2.25	2.56	104.93
100	100 ± 5.72	1.4	2.06	95.72
600	600 ± 8.91	3.27	4.52	101.48

[a]Data are based on assay of 6 replicates on 3 different days

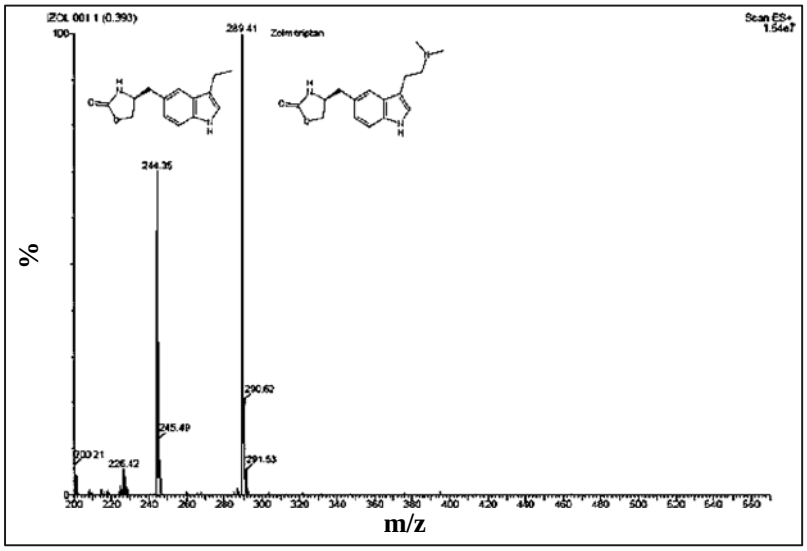

Fig. 1: Mass spectra of zolmitriptan

The chromatographic conditions were optimized by flow injection analyses with mobile phases containing varying percentage of organic phase to achieve maximum peak responses and good reproducibility.

During method development different mobile phases were used initially ammonium acetate buffer: methanol (45 : 55), where the peak shape was not good and background noise was higher (1×10^3) (Fig. 2). Then ammonium acetate buffer: acetonitrile (30 : 70) was

studied when the peak shape was not good and the MS signal was suppressed due to high concentration of buffer (Fig. 3).

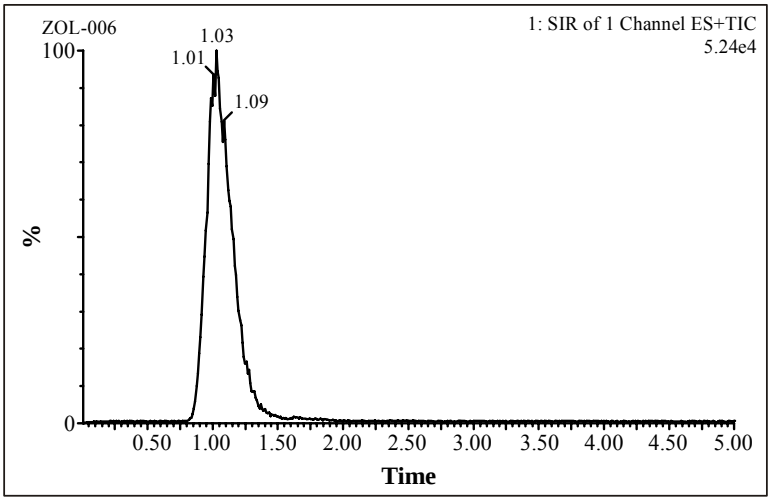

Fig. 2: Chromatogram of zolmitriptan with mobile phase - ammonium acetate buffer : methanol (45:55)

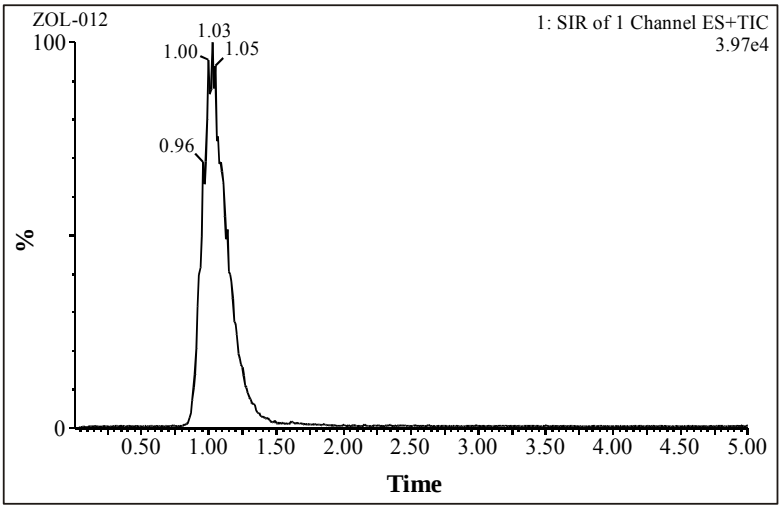

Fig. 3: Chromatogram of zolmitriptan with mobile phase - ammonium acetate buffer : acetonitrile (30:70)

Acetonitrile: Water: Formic acid (70 : 30 : 0.1% v/v) was used as a mobile phase where the peak shape was good, sample was completely vaporised and auto sampler stability was also found to be good, and the results were reproducible (Fig 4).

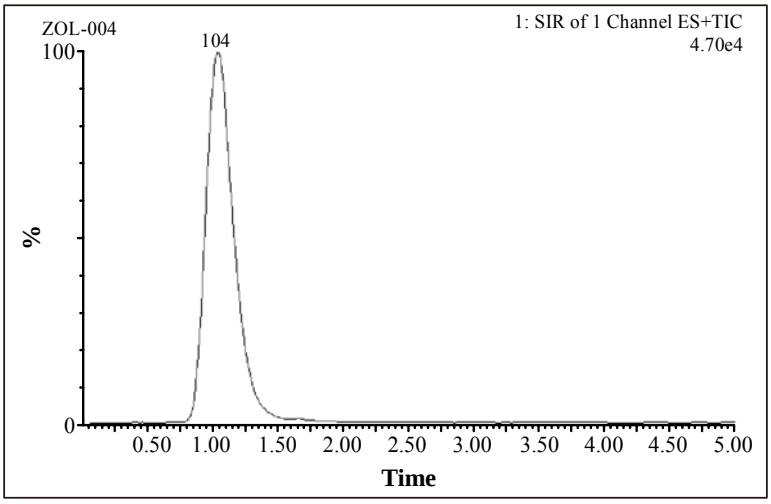

Fig. 4: Chromatogram of zolmitriptan with mobile phase - acetonitrile : water : formic acid (70:30:0.1% v/v)

The high organic content shortened the chromatographic cycle time and the acidic modifier (Formic acid) in the mobile phase improved sensitivity by promoting ionization of the analysis in ESI source.

With the selected chromatographic condition the chromatographic run time for each sample was completed within 2.0 min.

Column selection

The Symmetry C – 18 packed column (2.1 × 50 mm, 3.5 µm particle diameter) manufactured by WATERS (Ireland). Zhang et al.[9] used a Lichrospher CN Column where the retention time for zolmitriptan was found to be 5 to 4 min. in our hands Symmetry C – 18 packed column provided adequate retention and good peak shape.

Method validation

Linearity of calibration curve and LOQ

The assay was linear in the concentration range 30-1000 ng/mL ($r > 0.994$) with an LOD of 0.5 ng/mL.

The calibration curve was prepared and analysed linearity of the method. LOQ was the lowest concentration of analyte that could be determined with precision ≤ 20% and accuracy ± 20%. The limit of detection (LOD) was determined as the concentration with noise ratio of 3.11.

To evaluate linearity, plasma calibration curve were prepared over the concentration range of 30-100 ng/ml, encompassing the therapeutic range of this antimigraine drug. Calibration curve was calculated utilizing the peak area v/s analyte concentration. The response was linear for zolmitriptan throughout this concentration range and the correlation coefficient was 0.994. The typical equation was $Y= - 1.225 \times 10^2 + 130.8x$. The LLOQ was found to be 1 ng/mL.

Precision and accuracy

Accuracy and production were assessed and expressed by relative standard deviation. The lower limit of qualification was 1 ng/mL defined as the lowest concentration at which both the precision and accuracy were < 20%.

Intra and inter-day precision and accuracy (as relative standard deviation, RSD) were assessed by assay of samples (n : 3) on two separate days. Intra and inter day precisions were 2.06-4.52% and 1.4-2.25%, respectively with accuracy of 99-101.48%.

System suitability testing

Standard solution for the determination of system suitability contained 225 ng/mL which was prepared by diluting the corresponding standard stock solution.

System suitability was determined from 6 replicate injections of standard solution before sample analysis. The % RSD was found to be 4.42.

Robustness

ICH defines robustness as a measure of the methods capability to remain unaffected by small but deliberate variations in method parameters. As a part of determining robustness, deliberate change in the flow rate (± 0.023 mL/min) and temperature (± 5^0C) were carried out with a solution of 100 ng/mL of Zolmitriptan. Results are tabulated in Table 2 and Table 3.

Table 2: Robustness at different flow rates [100 ng/mL]

Flow rate (mL/min)	RT	Mean peak area	Mean concentration	SD concentration	RSD concentration
0.19	1.02	11486	96.35	1.84	1.91
0.20	1.04	11337	95.25	2.64	2.77
0.21	0.99	10798	92.36	1.58	1.71

Table 3: Robustness at different temperatures [100 ng/mL]

Temp. (^{0}C)	RT	Mean peak area	Mean concentration	SD concentration	RSD concentration
55	1.03	11351	95.30	1.89	1.93
60	1.01	11337	95.25	2.64	2.77
65	1.00	10329	95.23	2.01	2.36

CONCLUSION

The described LC-MS/MS method is a sensitive accurate and specific assay for the determination of Zolmitriptan with a chromatographic run time less than 2 min. More than 200 samples could be assayed daily, including sample preparation data acquisition and processing. The method has a LLOQ of 0.5 ng/mL and proved to be superior in sensitivity and speed of analysis in comparison to the reported methods.

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