

DEVELOPMENT AND VALIDATION OF A SENSITIVE BIOANALYTICAL METHOD FOR THE DETERMINATION OF SUMATRIPTAN IN RAT PLASMA BY UPLC-MS

AMRUTA B. KUMBHAR², UPENDRA C.GALGATTE², SHRIKANT WARKAD¹, B. SANTHAKUMARI^{1*}

¹Department of Center for Materials Characterization, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008, ²Department of Quality Assurance Techniques, PES's Modern College of Pharmacy, Nigdi, Pune 411044, Maharashtra, India.
Email: b.santhakumari@ncl.res.in

Received: 04 July 2013, Revised and Accepted: 26 July 2013

ABSTRACT

Objective: To develop a new, sensitive and specific isocratic ultra performance liquid chromatography mass spectrometry (UPLC-MS) method for the determination of sumatriptan in rat plasma using paracetamol as an internal standard (IS).

Method: Chromatographic separation of the analyte and internal standard was achieved on a Hypersil Gold C18 (8 μ , 4.6×150mm) analytical column. The mobile phase was composed of methanol and MQ water (90:10, v/v), pumped isocratically at a flow rate of 500 μ L/min.

Result: The calibration curve was linear over a concentration range of 0.1 to 1 ng/mL ($r^2 = 0.9982$) with a limit of quantification 0.1 ng/mL of sumatriptan. The intra-day and inter-day precision and accuracy were between 1.003 and 2.401% and 0.317 and 2.317%, respectively. Sumatriptan containing plasma samples were stable under three freeze-thaw cycles, autosampler conditions, bench top stability and at -22°C for 30 days.

Conclusion: The proposed method uses less biological material and is MS compatible. This high sensitive and simple validated method can be used in pharmacokinetic application studies using HPLC or LC-MS.

Keywords: Sumatriptan, Paracetamol, UPLC-MS.

INTRODUCTION

Bioanalytical techniques employed for the quantitative determination of drugs and their metabolites in biological fluids, plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK) and toxicokinetic studies. The quality of these studies, which are often used to support regulatory filings, is directly related to the quality of the underlying bioanalytical data. It is therefore important that guiding principles for the validation of these analytical methods be more established and disseminated to the pharmaceutical community. There are various bioanalytical techniques which are used for qualitative & quantitative analysis of pharmaceutical products [1].

Migraine is a chronic neurological disorder characterized by recurrent moderate to severe headaches often in association with a number of autonomic nervous system symptoms. Typically the headache is unilateral (affecting one half of the head) and pulsating in nature, lasting from 2 to 72 hours. Associated symptoms may include nausea, vomiting, photophobia, phonophobia and the pain is generally aggravated by physical activity. In other words, Migraine is a familial disorder characterized by recurrent attacks of headache widely variable in intensity, frequency and duration [2, 3].

Sumatriptan is a synthetic drug belonging to the triptan class used in the acute treatment of migraine headaches. It is structurally similar to serotonin (5HT), and is a 5-HT (types 5-HT_{1D} and 5-HT_{1B}) agonist [4]. The specific receptor subtypes it activates are present on the cranial arteries and veins. Acting as an agonist at these receptors, sumatriptan reduces the vascular inflammation associated with migraines. Activation of these receptors causes vasoconstriction of those dilated arteries. Sumatriptan is also shown to decrease the activity of the trigeminal nerve, which, it is presumed, accounts for sumatriptan's efficacy in treating cluster headaches. The injectable form of the drug has been shown to abort a cluster headache within fifteen minutes in 96% of cases [5].

Sumatriptan succinate (SS) is a selective 5-hydroxytryptamine (5-HT) receptor subtype agonist. Chemically it is known as 3-[2-(dimethylamino) ethyl]-N-methyl-indole-5-methanesulfonamide succinate (1:1) Fig. 1. The empirical formula is C₁₄H₂₁N₃O₂S•C₄H₆O₄, representing a molecular weight of 413.5. It is indicated for the acute treatment of migraine attacks with or without aura in adults.

Sumatriptan was the first antimigraine agent approved by US FDA in 1991 for the treatment and management of acute migraine cases [6, 7].

Paracetamol is chemically N-(4-hydroxyphenyl) acetamide. It is effective as the non-steroidal anti-inflammatory drug and analgesic. In this study it was used as internal standard (IS) Fig.2.

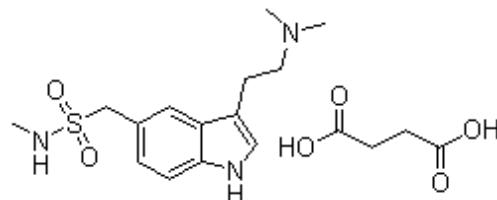


Fig. 1: Sumatriptan Succinate

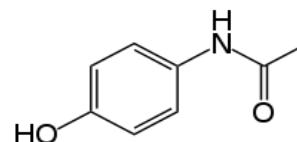


Fig. 2: Paracetamol

Several methods were reported for the determination of sumatriptan in biological matrices by HPLC with fluorescence, electro-chemical, ultraviolet detection and LC-MS/MS. In majority of these published methods, the sensitivity was ≥1 ng/mL with high volume of biological fluid (≥0.5 mL) for sample processing. In this study to achieve LOQ of 0.1 ng/mL 0.2 mL of plasma was used.

A critical comparison of different analytical methods for identification and determination of several triptans including sumatriptan has been reviewed. These methods, however, suffer from relatively low sensitivity, high plasma volumes required for sample preparation and longer runtimes. In this study a simpler and more sensitive UPLC-MS method was used for the determination of sumatriptan in healthy male rat plasma samples. Thus, in the present work a highly sensitive, selective and rapid UPLC-MS method has been developed for measurement of sumatriptan in plasma samples and fully validated as per the regulatory guidelines [3, 6, 8, 9].

MATERIAL & METHODS

Chemicals and reagents

Sumatriptan succinate was gifted by Cipla Ltd. and Paracetamol (IS) was obtained from Emcure pharmaceuticals Pvt. Ltd. UPLC Grade solvents (Methanol) was obtained from Merck and milli-Q water was from SG Series Compact Pretreatment Module.

Instrumental conditions

Analysis of sumatriptan was carried out on Q-Exactive Orbitrap UPLC-MS system. Chromatographic separation were performed using Hypersil Gold C18 (8 μ , 4.6 \times 150mm) column.

The UPLC pump was operated in isocratic mode at a flow rate of 500 μ L /min at ambient temperature. The mobile phase consisted of solvent A (100% methanol) and solvent B (100% MilliQ water). The autosampler was set to inject 5 μ L of extract with a chromatographic run time of 10 min. Mass detection was carried out in positive electrospray ionization (ESI) mode, using full mass scan. The MS parameters were capillary temperature 320°C, spray voltage 3.60kV, heater temperature 350°C.

Preparation of stock solutions, standards and quality control samples

A stock solution of Sumatriptan at a concentration of 1 mg/mL was prepared in methanol. The stock solution of paracetamol (IS) was prepared at a concentration of 1 mg/mL in methanol.

Spiking solutions of Sumatriptan for the preparation of calibration standards and quality control samples were prepared in methanol and spiked in to the plasma. The calibration curve from 20.0 ng/mL to 200.0 ng/mL was generated using six calibration standards at the concentrations of 20.0 ng/mL (STD 1), 40.0 ng/mL (STD 2), 80.0 ng/mL (STD 3), 120.0 ng/mL (STD 4), 160.0 ng/mL (STD 5) and 200.0 ng/mL (STD 6). Quality control (QC) samples were prepared at three concentration levels of 20 ng/mL (low), 80 ng/mL (medium) and 200 ng/mL (high).

For each solution, IS was added at a constant level of 40 μ L of 750ng/mL stock solution. All solutions were stored under refrigeration at 4°C prior to usage.

Sample preparation and extraction

Sumatriptan from the plasma was extracted using protein precipitation extraction technique [10, 11]. The plasma samples were stored at -22°C and allowed to thaw gradually to room temperature before processing. Aliquot of 220 μ L plasma was taken into eppendorff tubes and added 40 μ L of internal standard dilution and vortexed to mix the contents. Sumatriptan is extracted by using methanol as a precipitating solvent. Vortexed for 30sec then the solution was centrifuged at 4°C, 13,500 rpm for 10 min. The supernatant is taken and transferred to vials.

Method validation

The method performance was evaluated for selectivity, accuracy, precision, linearity, and stability during various stress conditions including bench top stability, freeze thaw stability, autosampler stability etc [12,13].

Selectivity

The selectivity of method was evaluated by analyzing 6 replicates of plasma samples spiked at LLOQ (Lower Limit of Quantification = 0.1 ng/mL)

Linearity

Calibration curves were constructed using linear regression within the range of 20 -200ng/mL of sumatriptan.

Recovery

Recovery of analyte was evaluated by comparing response of sumatriptan in three quality control samples (LQC, MQC and HQC) with the response of sumatriptan in equivalent aqueous solutions.

Precision and Accuracy

For precision and accuracy studies, samples of three concentration levels were prepared as low (LQC), medium (MQC) and high (HQC) quality controls, corresponding to 20, 40 and 200ng/mL respectively with six replicates each. Precision was evaluated with inter and intra batches.

Stability studies

The stability of sumatriptan in plasma samples were evaluated during method validation. Sumatriptan stability was evaluated using two concentration levels (low and high quality control, corresponding to 20 and 200ng/mL respectively). The stability of Sumatriptan was also evaluated in post extracted samples kept in the autosampler at 4°C for 60 hours, as well as plasma samples kept at -22°C and after being stressed to 3 freeze-thawing cycles (24 hours each cycle). All samples described above were compared to freshly prepared Sumatriptan samples at the same concentration levels.

RESULTS AND DISCUSSION

Chromatographic Optimization

A UPLC-MS method was developed for Sumatriptan, which can be conveniently employed for routine analysis in biological fluids. The chromatographic conditions were optimized in order to provide a good performance of the assay. The mobile phase for drug was selected based on its polarity. Different trials were taken and the finally the optimized mobile phases are methanol: water (90:10) with flow rate of 500 μ L/min. The Fig. 3 and 4 represent the mass spectra of sumatriptan and paracetamol (IS). The retention times of sumatriptan and paracetamol were 2.36 and 3.75 min, respectively. The chromatogram of sumatriptan and sumatriptan with IS have been shown in Fig. 6. The method is validated as per regulatory guidelines.

Selectivity

The described method of UPLC-MS was used for analysis and identification of sumatriptan and was shown to be selective for the analyte and its IS (retention times for sumatriptan and paracetamol were 2.36 and 3.75 minutes respectively). No interfering peaks were observed with the same retention time of the analyte when different plasma samples were analysed. Fig. 5 and Fig.6 represent the chromatograms of blank plasma sample and plasma sample spiked with sumatriptan and IS respectively.

Linearity

The LOQ of Sumatriptan in human plasma was 1ng/mL. The LOQ is defined as the concentration level of Sumatriptan at five times its half-life and providing an S/N ratio of >10/1. A linear response was observed for the Intensity of peak ratio versus concentration over a working range of 20–200ng/mL for Sumatriptan with an average correlation coefficient of 0.9982.

Recovery

The recovery was evaluated by comparing response of extracted and unextracted samples. Extracted samples include six replicates of extracted LQC, MQC and HQC samples. Unextracted samples included the aqueous solutions equivalent to extracted samples. The average recovery for sumatriptan in plasma was ranged from 85.58 to 90.92 % for the low, medium and high quality control samples with an average of 87.42 % represented in table 1.

Accuracy and Precision

Accuracy and precision was evaluated by analysing 3 batches. Each batch consists of six replicates of LQC, MQC and HQC. Precision was evaluated for both interday and intraday batches. The interday and intraday precision and accuracy of the method for each sumatriptan concentration levels (20.0, 40.0 and 200.0ng/mL) are represented in Table 2. The mean accuracy for each concentration level ranged from 94.06 to 101.25% and the mean precision for each concentration level ranged from 1.003 to 2.401%.

Stability Studies

Stability studies were performed to evaluate the stability of sumatriptan in plasma after exposing to various stress conditions. The stability studies performed include bench top stability, freeze thaw stability, auto sampler stability and long term stability of

processed samples. All Stability evaluations were performed as per international regulatory guidelines.

Sumatriptan was found to be stable in the blank rat plasma for 6 h at bench top, and 24 h in an autosampler at room temperature, at repeated freeze-thaw cycles (three cycles). Sumatriptan was stable in plasma sample for 30 days when stored at -22°C.

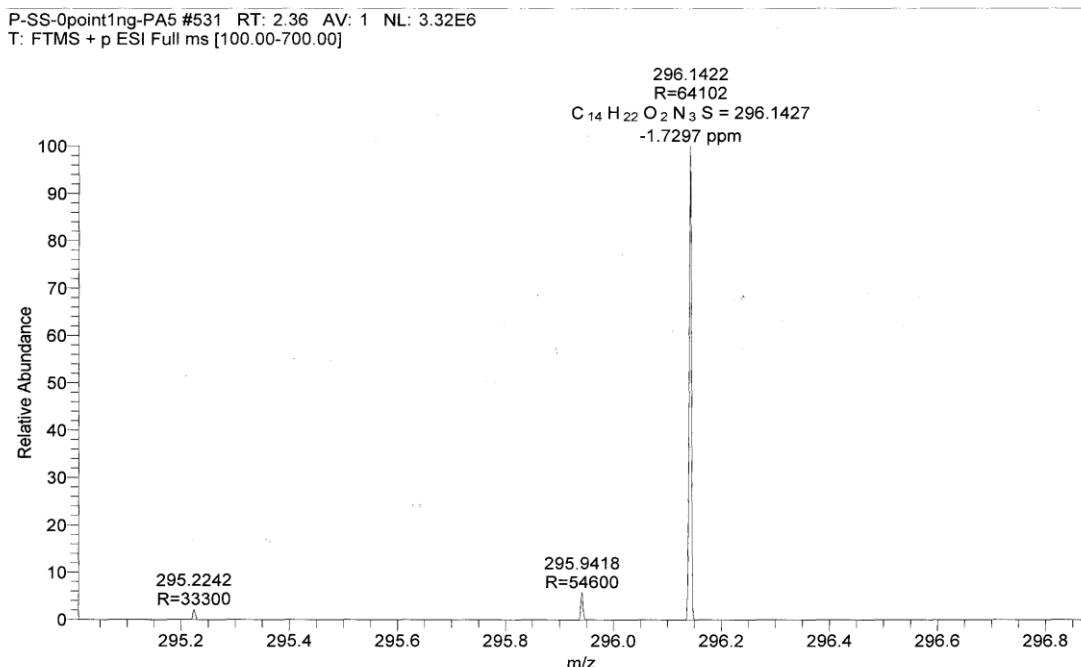


Fig. 3: Mass spectrum of Sumatriptan

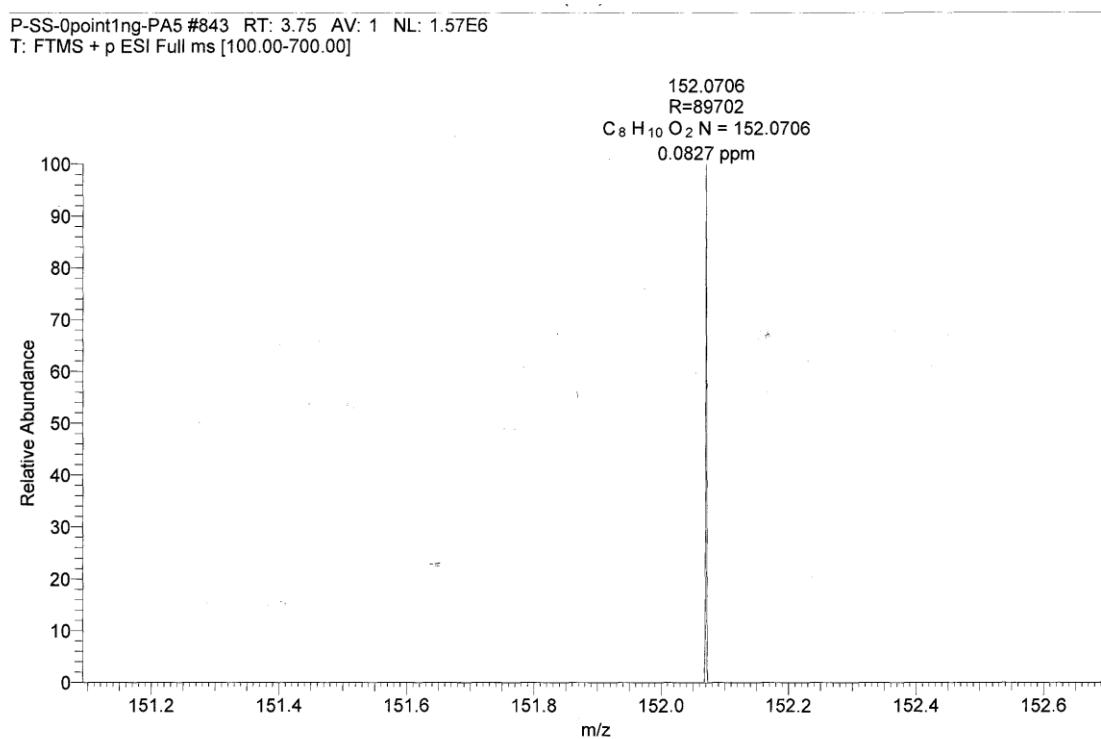


Fig. 4: Mass spectrum of Paracetamol

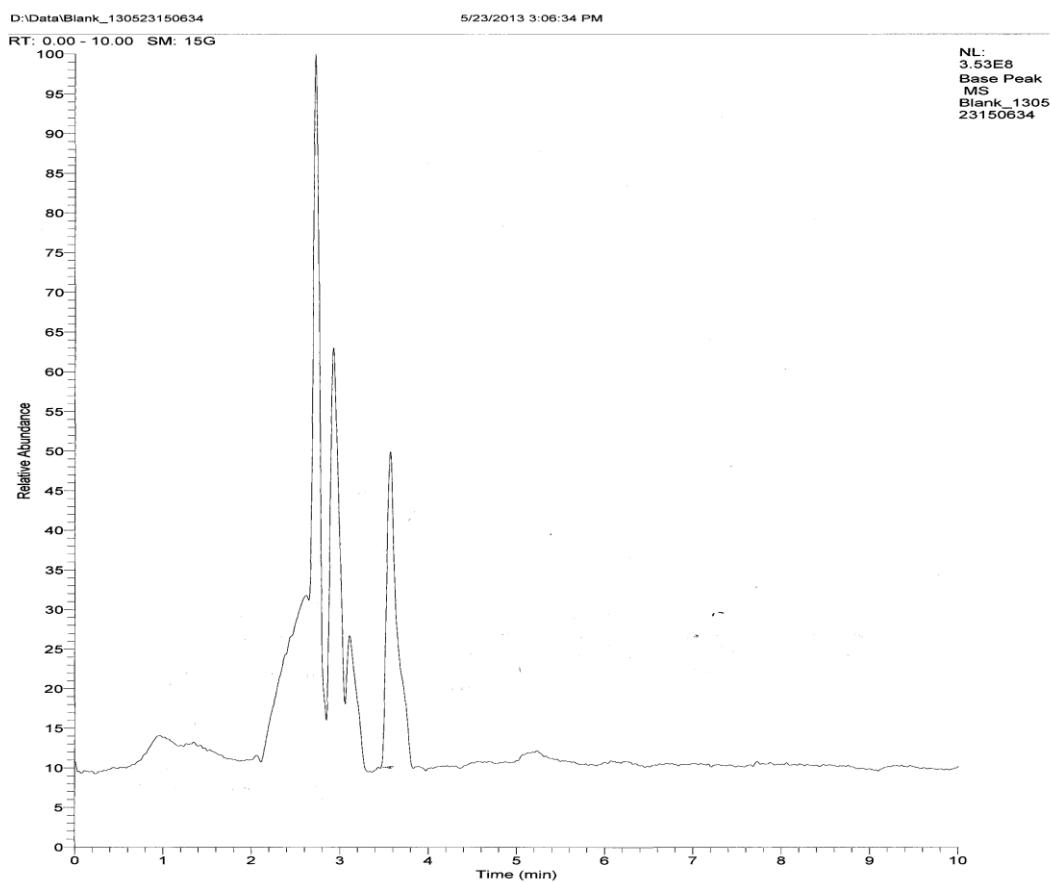


Fig. 5: Typical chromatogram of blank plasma sample

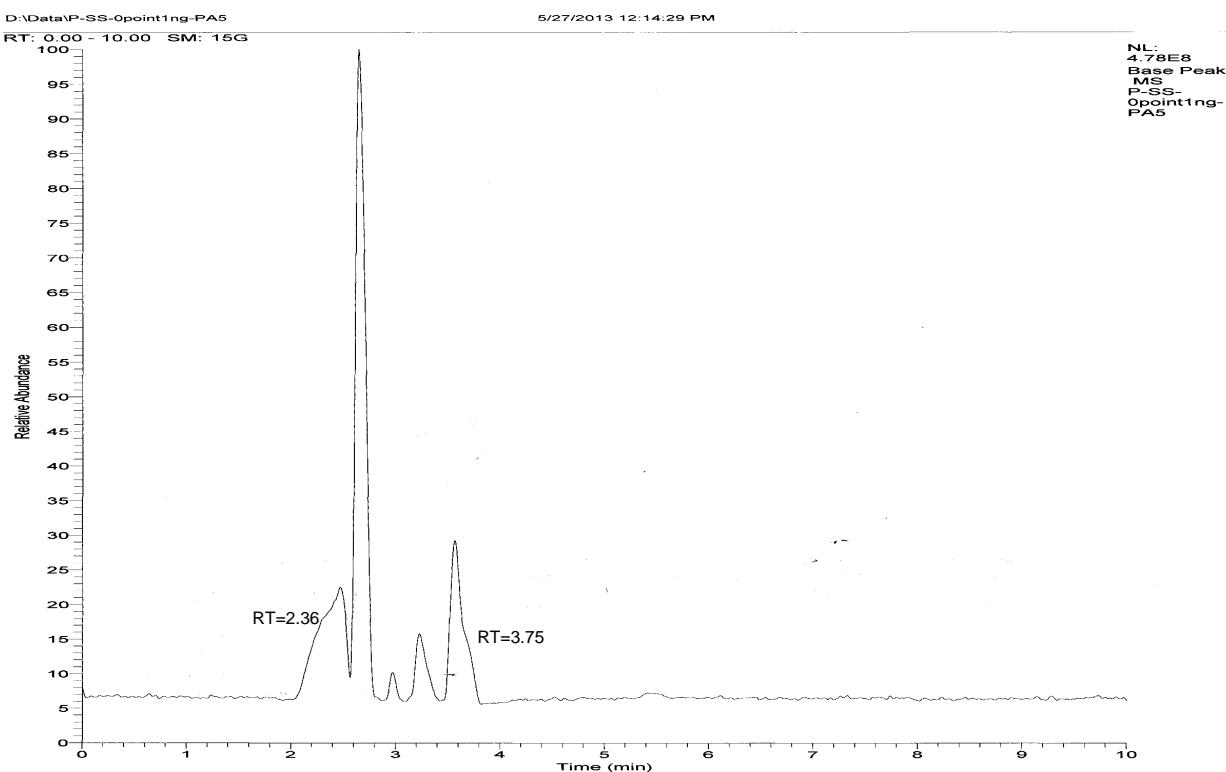


Fig.6: Chromatogram of blank plasma spiked with Sumatriptan (2.36RT) and Paracetamol (3.75RT)

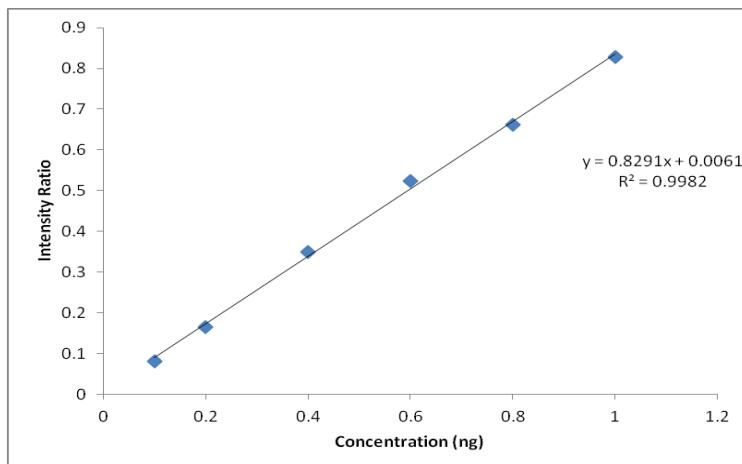


Fig. 7: Calibration Curve of Sumatriptan

Table 1: Recovery result of Sumatriptan from plasma

Concentration (ng/mL)	Mean relative recovery (%) (n=6)
20	85.58
40	85.77
200	90.92

Table 2: Intraday and Interday Precision and Accuracy of Sumatriptan

Concentration (ng /mL)	Accuracy (% nominal) (n=6)	Precision (%CV) (n=6)	
		Interday	Intraday
20	94.06	1.220	1.612
40	101.25	1.159	1.003
200	100.2	1.292	2.401

CONCLUSION

A new, sensitive and specific isocratic UPLC-MS method was developed and validated for the determination of sumatriptan in rat plasma. The validated method showed satisfactory evaluation results for all the validation parameters tested. The method was validated for sumatriptan concentration in the range of 0.1 to 1 ng/mL. This high sensitive and simple validated method can be used in pharmacokinetic application studies. Compared with previously published validation methods, our LLOQ (0.1ng/mL) is significantly sensitive to the 1 ng/mL reported method by Karthic et al and Tan et al. This validated method was successfully applied to a clinical pharmacokinetic study of sumatriptan.

ACKNOWLEDGEMENT

The authors express their gratitude to Cipla Ltd. (Mumbai, India) for the gift sample of pure sumatriptan succinate.

REFERENCES

- Atanu Kumar et al., Integrated Application of Bioprocess Engineering and Biotechniques for Quality & Bulk Drug Manufacturing. *J Bioequiv Availab* 2011; 3(11): 277-285.
- Nisha GS, Maithi P, Charyulu RN. Formulation and Development of Nasal in situ gels of triptans for Anti Migraine activity. *Int J Pharm and Biomedical Sci* 2012; 3 (2): 861-864.
- Sheshala R, Khan N, Darwis Y. Validated high performance liquid chromatography (HPLC) method for the determination of sumatriptan in rabbit plasma: Application to pharmacokinetic study. *African Journal of Pharmacy and Pharmacology* 2012; 6(2): 98-107.
- Razzaque Z, Heald M A, Pickard J D et al. Vasoconstriction in human isolated middle meningeal arteries: determining the contribution of 5-HT1B- and 5-HT1F-receptor activation .*Br J Clin Pharmacol* 1999; 47(1): 75-82.
- The Sumatriptan Study Group, Treatment of acute cluster headache with sumatriptan. *N Engl J Med* 1991; 324(6): 322-325.
- Shrivastava PS. Challenges in the simultaneous quantitation of Sumatriptan and naproxen in human plasma: Application to a bioequivalence study. *Journal of Chromatography B* 2012; 902:122– 131.
- Reddy YR, Kakuman K, Reddy MR, Reddy et al. Rapid Simultaneous Determination of Sumatriptan Succinate and Naproxen Sodium in Combined Tablets by Validated Ultra Performance Liquid Chromatographic Method. *J Anal Bioanal Techniques* 2011; 2(3): 1-6.
- Z Ge, E Tessier, L Neirinck, Z Zhu. High performance liquid chromatographic method for the determination of Sumatriptan with fluorescence detection in human plasma. *Journal of Chromatography B* 2004; 806: 299-303.
- Jeong Ju Seoa, Jeonghyeon Parka, Min Ho Baea, Sung Min Parka, Hae Won Lee et.al. Rapid determination of sumatriptan in human plasma by Ultra performance liquid chromatography-tandem mass spectrometry and its application to clinical pharmacokinetic study. *Journal of Chromatography B* 2013; 919– 920: 38- 42.
- Pharne A B, B Santhakumari, Ghemud A S, Jain H K, Kulkarni M J. Bioanalytical method development and validation of vildagliptin a novel dipeptidyl peptidase IV inhibitor by RP-HPLC method. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4(3): 119-123.
- Ghemud A S, B. Santhakumari, Pharne A B, Jadhav M M, K. S. Jain, Kulkarni M. J. Bioanalytical method development and validation of levalbuterol A β 2-adrenergic agonist by RP-HPLC method. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4(1): 249-253.
- International Conference on Harmonization (ICH), Validation of analytical methods definitions and terminology, ICH Q2 A, 1994.
- International Conference on Harmonization (ICH), Validation of analytical methods: Methodology, ICH Q2 B, 1996.