Development and validation of LC-MS method with electro spray ionization for quantitation of sumatriptan in human plasma: Application to bioequivalence study

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Sumatriptan is a potent and selective vascular 5-hydroxytryptamine1 (5-HT_1) receptor agonist effective for the treatment of migraine. We developed liquid chromatographic-mass spectrometric method for determination of sumatriptan in human plasma. Sumatriptan was extracted from samples using solid phase extraction technique and separated on analytical column, Waters ODS2, thermostated at 20°C.

The mobile phase was a mixture of 5mM ammonium-acetate and acetonitrile (80:20), with a flow rate of 0.3 mL/min. Total run time was 10 min. Mass spectral detector was optimized and adjusted in electrospray single ion monitoring (ESI-SIR) mode. Molecular ion masses (m/z) were 296.00 and 267.00 for sumatriptan and internal standard atenolol, respectively. Under these conditions retention times for internal standard atenolol and sumatriptan, were 3.4 ± 0.3 min and 7.3 ± 0.5 min, respectively.

The standard curve of plasma spiked with sumatriptan was linear in the range of 1.0-100.0 ng/mL, with correlation coefficient r = 0.9931. The limit of quantification (LOQ) was 1.0 ng/mL. Presented method was successfully applied in the pharmacokinetic study of generic tablets with the new formulation, containing sumatriptan, after single oral dose (100 mg) was given to 24 volunteers.

This method is simple, sensitive and suitable for pharmacokinetic or bioequivalence studies.

ДРАГАНА РАНЧИЧ, СЛАВИЦА ВУЧИНИЧ, СНЕЖАНА ДЖОРДЖЕВИЧ, ДРАГАНА ДЖОРДЖЕВИЧ. *РАЗРАБОТКА И ПРОВЕРКА ЭЛЕКТРОСПРЭЙ ИОНИЗАЦИОННОГО ЖХ-МС МЕТОДА КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ СУМАТРИПТАНА В ПЛАЗМЕ КРОВИ ЧЕЛОВЕКА: ПРИМЕНЕНИЕ ДЛЯ ИССЛЕДОВАНИЙ БИОЭКВИВАЛЕНТНОСТИ.*

- Суматриптан является мощным и селективным агонистом 5-hydroxytryptamine1 (5-HT_1) - васкулярного рецептора эффективного при лечении мигрени. Разработана методика для определения суматриптана в плазме крови человека с использованием метода жидкостной хроматографиии с масс- детектированием. Суматриптан экстрагировали из образцов с использованием метода твердофазной экстракции и разделяли на аналитической колонке (Waters ODS2), термостатированной при 20°С. Подвижная фаза представляет собой смесь 5 мМ водного раствора ацетата аммония и ацетонитрила (80 : 20), с расходом 0.3 мл/мин. Общее время развертки 10 мин. Масс-спектральный детектор оптимизированы и настроен для работы в режиме (ESI -SIR). Массы детектируемых молекулярных ионов составляли 296.00 и 267.00 для суматриптана и внутреннего стандарта - атенолола, соответственно. В этих условиях время удерживания для внутреннего стандарта атенолол и суматриптана были, соответственно, 3.4 ± 0.3 мин и 7.3 ± 0.5 мин. Стандартная кривая плазмы крови с пиками суматриптана показала линейную зависимость от концентрации в диапазоне 1.0-100.0 нг/мл, с коэффициентом корреляции R = 0.9931.

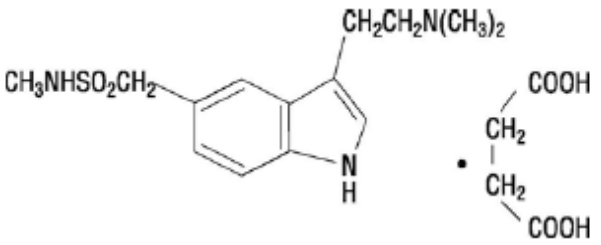
Предел количественного определения составляет 1.0 нг/мл. Разработанный метод определения был успешно применен в фармакокинетическом исследовании таблеток генерика с новым составом, содержавших суматриптан, в качестве действующего компонента, которые однократно и перорально в дозе (100 мг вещества) давали 24 добровольцам. Этот метод прост, чувствителен и подходит для фармакокинетических исследований и изучения биоэквивалентности.

Key words: Sumatriptan, LC-MS, plasma, bioequivalence study, vascular 5-hydroxytryptamine1 (5-HT_1) receptor agonist

Ключевые слова: суматриптан, ЖХ-МС метод определения, плазма крови, исследование биоэквивалентности, лечение мигрени, агонист 5-НТ1-рецепторов, 5-гидрокситрифтамин1 (5- HT_1), серотонин

INTRODUCTION

Sumatriptan (as the succinate), is a selective 5-hydroxytryptamine1 (5-HT1) receptor subtype agonist. Sumatriptan succinate is chemically designated as 3-[2-(dimethylamino) ethyl]-N-methylindole-5-methanesulfonamide succinate (1:1), and it has the following structure:



Sumatriptan is an agonist for a vascular 5-HT_1 receptor subtype. 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 migraine. This action in humans correlates with the relief of migraine headache. [1-4]

There are many methods described in the literature for the measurement of the sumatriptan in biological fluids. Methods for determination of this substance in biological materials include liquid chromatography with electrochemical (EC)[5], ultra violet (HPLC-UV)[6], fluorescence[7] and mass spectrometric (MS)[2,8-12] detection.

The aim of this study was to develop and validate a method for determination of sumatriptan in human plasma. We described LC-MS method development and validation parameters for human plasma with sufficient sensitivity and application of this method to determine pharmacokinetic parameters for sumatriptan in humans.

EXPERIMENTAL

Chemical and reagents. Sumatriptan succinate reference standard and the internal standard (IS), atenolol, were obdained from Sigma-Aldich Chemical Company (St Louis, MO, USA). Acetonitril, HPLC-grade, was purchased from Merck Company (Darmstad, Germany); deionized water was purified using the Milli-Q system (Millipore, Bedford, MA, USA); all other reagents were of analytical grade. Extraction cartridge Oasis® HLB 3cc (60 mg) was obtained from Waters Corporation, Manchester, Ireland. Blank human plasma was collected from healthy, drug free volunteers and stored at approximately -20°C until needed.

Instrumentation and chromatographic conditions. The LC-MS system consisted of a Waters 2695 separation module, with quaternary pump, degaser and autosampler, interfaced to a Micromass ZQ2000 mass spectrometer equipped with an electrospray ionisation source. The apparatus was managed with a Masslynx 4.0 software.

Chromatographic separation was performed on a Waters ODS2 column (150 mm x 4.6 mm i.d.,5 µm, Waters, UK). The colum temperature was maintained at 20°C. The mobile phase consisted of acetonitril and water (80:20, v/v), delivered at flow rate of 0.3 mL/min. The injection volume was 50 µL. An ESI source in positive ion mode was used for determination. The optimized ionization condisions were: capilary voltage 3 kV; cone voltage 30 V; extractor 3 V. The source and desolvation temperatures were 150 and 430°C, respectively. Nitrogen was used as a cone and desolvation gas at flow rates of 145 and 360 L/Hr, respectively. SIR mode (Single Ion Recording) was used for the quantification at *m/z* 296 and *m/z* 267 for sumatriptan and IS, respectively.

Preparation of stock and standard solutions. The stock solution of sumatriptan was prepared by disolving 10.0 mg accuretely weighed analytical standard in 10.0 mL methanol to produce a concentration of 1 mg/mL and stored at 4°C. Working solutions of sumatriptan at 1, 5, 10, 25, 50, 100 ng/mL were prepared fresh daily in methanol by appropirate dillutions of the stock solution. A stock solution of IS (1 mg/mL) was prepared by disolving of 10 mg analytical standard in methanol, from wich a 1 mg/L internal standard (IS) working solution was prepared in methanol as well.

Method validation. Method validation was carried out according to the currently accepted European Medicines Agency (EMEA) guideline on validation of bioanalytical methods[13].

Calibration standards and quality control samples. Calibration curves were prepared by spiking 900 µL samples of blank plasma with working standard solutions, to achieved concentrations of 1, 5, 10, 25, 50, 100 ng/mL of sumatriptan. Standard curves were constructed using unweighted least-squares regression of the peak areas ratios of the analyte to IS (*y*) against the corresponding plasma concentration of the analite (*x,* ng/mL).

Quality control samples (QCs) were prepared by adding appropriate volumes of QC working solutions to blank human plasma at concentrations of 1, 25 and 100 ng/mL. The concentration of the IS was 10 ng/mL in all samples.

Matrix effect. The matrix effect (co-eluting, undetected endogenous matrix compounds that may influence the analyte ionization) was examined by extracting blank human plasma and reconstituting with methanol containing a known amount of the analytes, analyzing the reconstituted extracts and then comparing the peak areas of the analyte with

that of analytes in methanol. The experiment was performed in six samples for each QC concentration and the blank plasma used in this study was obtained as six different batches.

Application the pharmacokinetic study. A single oral dose of 100 mg sumatriptan was given to 24 healthy, fasted volunteers with a washout period of 1-week between the successive treatments. Thirteen men and 11 women (mean age, 32.2 years; mean weight, 72.9 kg) completed the study. Plasma samples were obtained before dosing and up to 12 hours afterwards. Around 5 mL bloods were collected in tubes with EDTA before and after dosing. The samples have been collected in the following pharmacokinetic times measured in hours: 0, 0.17, 0.33, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 9.0 and 12.0 h. Aliquot of 1-2 mL plasma was immediately separated by centrifugation for 10 min at 3000 rpm and kept frozen at -20^{o}C until analysis.

Pharmacokinetic parameters (PK) of sumatriptan (maximal concentration - C_{Max}, time to reach maximal concentration - T_{Max}, partial and total areas under curve – AUC (0-12) and (0- ∞), lag time- Tlag, elimination-rate constant – Kel, elimination half-time – T1/2el, and mean residence time – MRT) were estimated using noncompartmental calculations performed with EquivTest™ (Statistical Solutions Ltd., Cork, Ireland).

RESULTS AND DISCUSSION

Method development. The aim of our work was to develop and validate a simple, rapid and sensitive assay method for the quantification of sumatriptan in human plasma. During method development different options were evaluated to optimize sample extraction, detection parameters and chromatography.

We used atenolol as an internal standard to obtain good accuracy and precision.

Sample preparation has an important role in determination of drugs in biological samples. Sumatriptan could be isolated from plasma both by liquid-liquid[5,8] and solid-phase extraction[2,10].

An evaporation-free solid-phase extraction (SPE) method was developed and validated for sumatriptan. High organic washing (50% methanol) and low organic elution (20% methanol) were used.

At first we applied alkaline liquid-liquid extraction with chloroform, but recovery was lower than 60%. Therefore, we decided to perform solid-phase extraction that gave better recovery. Furthermore, solid-phase extraction gave cleaner extract and saved time for preparation of samples, which is very important in analysis of high number of samples. All samples, QCs, and standards with a sample volume of 1 mL spiked with IS working solution were extracted on Oasis HLB Extraction Cartridge. Before extraction, plasma samples were alkalinized by adding 50 μL of 25% ammonia and 50 μL of IS. Cartridges were conditioned with 1 mL of methanol and 1mL of water, and then alkalinized samples with internal standard were loaded. After washing the cartridges with 1 mL of 5 % methanol, sumatriptan and IS were eluted with 1 mL of mobile phase. The samples were transferred into auto sampler vials and 50 μL was injected onto the LC-MS column.

MS detecting conditions were operated according to the MS signal response of the target compound and the results indicated that the positive SIR mode head best sensitivity. Full mass spectrum of sumatriptan and internal standard (atenolol) are shown in Fig. 1. In Fig. 2. the chromatographic conditions, were optimized through several trails to achieve good resolution and symmetric peak shapes for sumatriptan and IS.

Boulton et al. used the mobile phase which consisted of methanol:water:formic acid (90:10:0.1, v/v/v) [8].

We reached a good chromatographic separation with a mixture of acetonitrile with 0.1% glacial acetic acid and 5 mM ammonium-acetate, pH 3.6 (80:20, v/v).

Specificity and selectivity. The specificity of the method was evaluated by analyzing human plasma samples from at last six different sources to investigate the potential interferences at the LC peak region for analyte and IS. Figure 3. showed a typical chromatogram for the control human plasma (free of analyt and IS). No interfering peaks from endogenous compounds were observed at the retention times of analyte and IS. Figure 4. showed a chromatogram human plasma spiked with sumatriptan at LLOQ and IS.

The low limit of quantification of 1 ng/mL was higher in comparison with LLOQ described by McLoughlin (0.5 ng/mL)[11] and Boulton (0.7 ng/mL) [8], but it was very good due to the fact that we used single mass not tandem mass spectrometric detector.

The retention times were 7.3 min for sumatriptan, and 3.4 min for IS. The total chromatographic run time was 10.0 min. It is a short run time when it comes to analyzed more than 150 plasma samples per day, with possible interfering organic impurities from matrix, which could be retain on chromatographic column.

Linearity. The calibration standards were prepared and assayed in three replicates on three different days to demonstrate the linearity of this method. The lower limit of quantification (LLOQ) was defined as the lowest concentration at which the signal to noise (S/N) ratio was larger than 10 and both the precision and accuracy were less than or equal to 20% by analyzing the three replicates of samples spiced with each analyte.

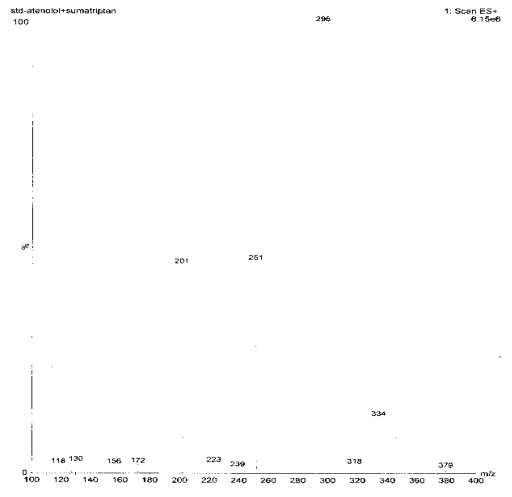


Fig 1. Full-scan mass spectrum of sumatriptan

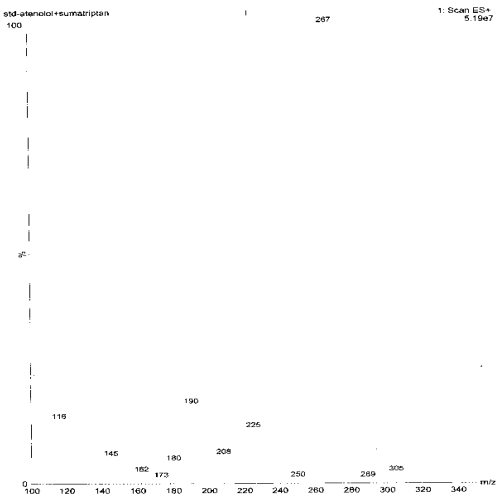


Fig 2. Full-scan mass spectrum of IS

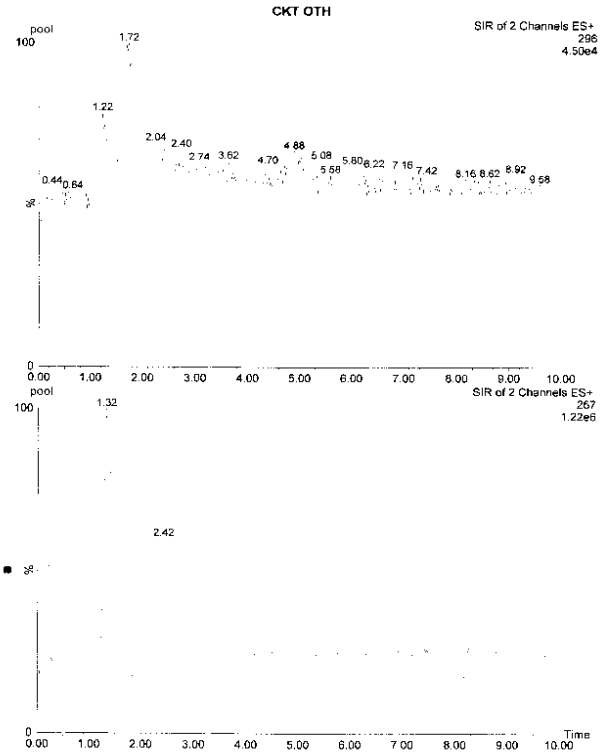


Fig 3. Chromatogram of blank sample in absence of endogenous material at the RT of sumatriptan and IS

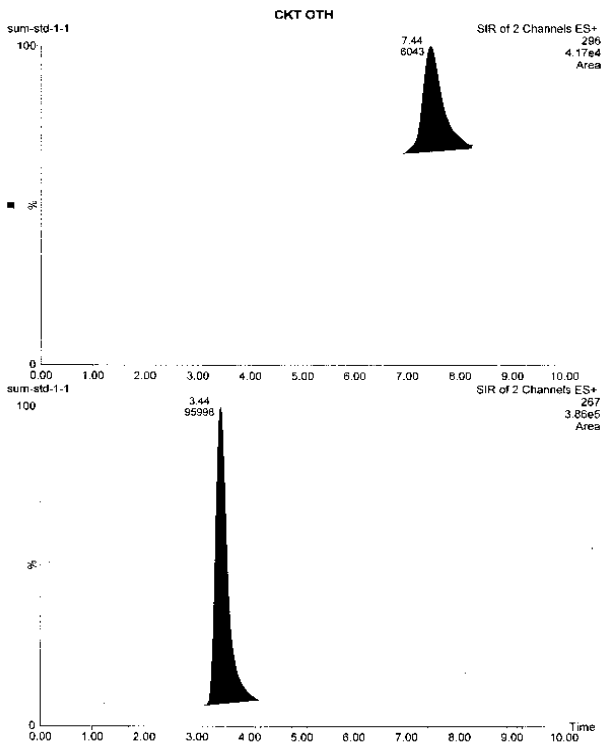


Fig 4. Chromatogram of LLOQ Level and IS

Standard curves prepared for sumatriptan in plasma were linear over range of 1-100 ng/mL. The mean (n=3) calibration curve for sumatriptan was $y = 0.0071x + 0.0047$, $r^2=0.9931$, where y and x are the peak area ratio of analyte to internal standard and concentration (ng/mL) of anayte, respectively.

A good linearity was observed over the concentration ranges of 1-100 ng/mL for sumatriptan like Ge et al. after applying of HPLC with fluorescence detection[7].

Tan et al described LC-MS-MS method with good linearity in the same concentration range of 1 to 100 ng/mL[10].

The LOQ under the optimized conditions was 1 ng/mL, which was estimated from the fact that the precision and accuracy were less than 5%, and the S/N ratios were much higher than 10.

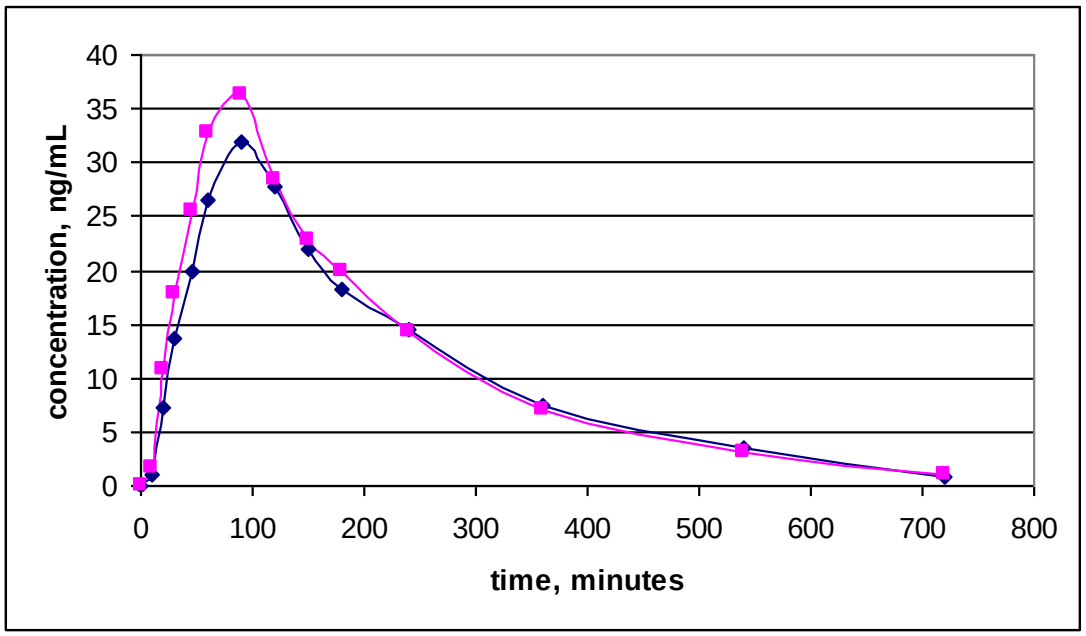


Fig 5. Mean concentration–time profile for sumatriptan in the plasma of healthy volunteers (n=24) after administration of a single 100-mg sumatriptan tablet

Table 1. Accuracy and precision for the determination of sumatriptan (3 days, five replicates per day)

Added concentration (ng/mL)	Found concentration (ng/mL)	Intra-day RSD (%)	Inter-day RSD (%)	Relative error, RE (%)
1	1.03	0.05	0.55	3.0
25	25.06	0.43	1.38	-0.2
100	99.96	1.86	1.72	-0.4

Relative error RE (%) = 100 x (found concentration – added concentration) / added concentration

Table 2. Stability data of sumatriptan quality controls in human plasma

QC (spiked) concentration (ng/mL)	Stability	Mean SD (ng/mL), n=6	Accuracy (%)	Precision (%CV)
1	3rd freeze-thaw	0.98	101.33	6.90
	6 h (bench-top)	1.03	94.44	11.07
	12 h (in injector)	0.91	95.56	10.65
	40 day at -20°C	0.98	90.37	9.42
100	3rd freeze-thaw	99.46	91.00	1.71
	6 h (bench-top)	100.53	90.37	2.69
	12 h (in injector)	101.33	96.83	2.40
	40 day at -20°C	99.96	95.97	1.95

Table 3. Relative bioavailability of sumatriptan

Parameter	90% Confidence interval (T/R)	P.E.	90% Confidence interval (T-R)
c_{Max} (ng mL^{-1})	0.808-1.072	0.945	0.819-1.091
$AUC_{(0–12)}$ (ng h mL^{-1})	0.823-1.130	0.979	0.832-1.151
$AUC_{(0–\infty)}$ (ng h mL^{-1})	0.823-1.130	0.979	0.832-1.151
t_{Max} (h)	0.964 -1.158	0.083	-0.050-0.220

Accuracy and precision. The accuracy and precision of the method were evaluated based on the data from the QC plasma samples at three concentrations (1, 25 and 100 ng/mL) each extracted and analyzed in five replicates on the same day (intra-day precision and accuracy) and on three consecutive days (inter day precision and accuracy).

The accuracy was determined by calculating the percentage deviation observed in the analysis of QC samples and expressed in the relative error (RE). The intra- and inter-day precisions were expressed as relative standard deviations (RSD). As shown in Table 1, for each QC level, inter- and intra-day precisions (RSD) were ≤1.8 and ≤1.7.% and accuracy (RE) was ± 0.4%, indicating the acceptable accuracy and precision of the method developed.

Interassay precision was evaluated by analyzing ten spiked plasma extracts prepared at the same way as volunteer`s plasma samples for concentration value of 1 ng/mL. The mean calculated concentration was 0.95 ng/mL, with RSD of 3.88%.

Recovery. The extraction recoveries of sumatriptan from human plasma were determined by comparing peak areas from plasma samples spiked before extraction, with those from standard solution at the same levels.

Extraction recovery after liquid-liquid extraction described by Boulton et al. was 81.8 ± 6.8% [8] and 92.6%[7], while after solid phase extraction it was 92%[10]. Our results showed that the extraction recoveries of sumatriptan from human plasma were 94.9%, which was better than data described in literature. The absolute recovery of IS at concentration 10 ng/mL was 85.8%.

Stability. The stability of sumatriptan after solid-phase extraction was determined by analyzing QC samples under next conditions: over a period of 12 h injection time in the auto-sampler at ambient temperature and over the bench-top for a period of 6 h. After 40 days at -20^{o}C and after three freeze-thaw cycles. For each of the storage conditions, six replicates were analyzed at two concentrations levels. Table 2. summarized the short-term stability, long-term stability and the freeze and thaw stability data of sumatriptan. The results were found to be within the assay variability limits.

Matrix effect. No significant matrix effect was observed for analyte in the plasma samples. The possibility of matrix effect caused by ionization competition between the analytes and the endogenous co-eluents was evaluated by QC low and QC high samples in six replicates. The results of matrix effect were acquired from comparing the peak responses of the post-extraction spiked samples with those of the standard solution and suggested negligible matrix effect in this method. For all the tested concentrations, the ratios of the peak response from 93 to 103% were within an acceptable range.

Application to the pharmacokinetic study. The method was successfully applied for analysis of serum samples obtained after oral administration of a single sumatriptan tablet (dose 100 mg) to 24 healthy human volunteers. Figure 5 shows the mean serum concentration–time curve for sumatriptan. Pharmacokinetic parameters representing the early (c_{max}, t_{max}) and total exposure ($AUC_{0-\infty}$) to sumatriptan were obtained.

Standard statistical methods were used to analyze relative differences between the drugs. Bioequivalence was concluded if 90% CIs or the geometric mean ratios of c_{max} and $AUC_{0-\infty}$ were between 0.80 and 1.25 (Table 3.). The respective point estimates of ratios of the geometric means of long-transformed c_{max} and $AUC_{0-\infty}$ of sumatriptan (test-reference) were 0.945 and 0.979, with 90% CIs of 0.819-1091 and 0.832-1.151, respectively.

Mean maximal sumatriptan plasma concentrations, amounted to 35.0 ± 10.1 ng/mL (test) and 37.7 ± 12.8mg/mL (reference). The half-life was 2.01 ± 0.67 h. The area under the serum concentration–time curve (AUC_{0-12}) was 0.799 - 1.108 ng.h/mL. Differences in Tmax, analyzed by a non-parametric test, also did not reach statistical significance. These results are comparable with those reported elsewhere[12,14-15].

No adverse effects were reported by the subjects or revealed by clinical or laboratory tests. The study failed to demonstrate any statistically significant differences in the primary PK variables of sumatriptan between the 2 formulations of oral sumatriptan 100 mg in the select population of healthy volunteers. On the basic and according to both the early and total exposure to parent drug, the test and reference formulations were concluded bioequivalent.

CONCLUSIONS

The novel method has been developed for the determination of an antimigraine compound sumatriptan in human plasma using solid phase extraction procedure and liquid chromatography with mass spectrometric detection.

The method is rapid, sensitive, selective and reproducible. We show the suitability of the method for a pharmacokinetic study with human volunteers after oral administration of sumatriptan tablets.

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