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Short communication

Rapid determination of sumatriptan in human plasma by ultra performance liquid chromatography–tandem mass spectrometry and its application to clinical pharmacokinetic study

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ABSTRACT

A sensitive and simple detection method coupling ultra-performance liquid chromatography with tandem mass spectrometry was developed and validated to analyze sumatriptan levels in human plasma. The plasma sample preparations for the analysis were based on liquid–liquid extraction with ethyl acetate, evaporation, and reconstitution. MS/MS detection was performed on a triple-quadrupole tandem mass spectrometer by monitoring the protonated parent → daughter ion pairs at m/z 296 → 58 and m/z 388 → 71 for sumatriptan and terazosin (internal standard), respectively. The method was validated with respect to its specificity, linearity, sensitivity, accuracy, precision, recovery, and stability. The calibration curve was linear from 0.5 to 50 ng/mL ($r > 0.999$). The mean extraction recovery for sumatriptan was higher than 62.3%. The method accuracy was within 97.4%, and the relative standard deviation of the intra- and inter-day precision values was within 11.7% at all quality control levels. Plasma samples that contained sumatriptan were stable under three freeze–thaw cycles, short- and long-term storage, and autosampler conditions. This method was successfully applied to a pharmacokinetic study conducted with 10 healthy volunteers. After oral administration of 50-mg sumatriptan and serial blood sampling over 12 h, the mean area under the plasma concentration–time curve from time 0 to 12 h and the maximum plasma concentration were 116.2 ng h/mL and 33.2 ng/mL, respectively.

1. Introduction

Migraines are a common disease characterized by a unilateral pulsating headache, nausea, vomiting, and photophobia [1]. Sumatriptan, a selective 5-hydroxytryptamine (serotonin) type 1B receptor agonist, is the first-line therapy for migraines that have not responded to an acceptable dose of analgesics [2]. The low bioavailability (~14%) of sumatriptan is due to pre-systemic metabolism and incomplete absorption of the drug after its oral administration [3].

Several previously reported sumatriptan quantitation methods include gas chromatography (GC) with mass spectrometry (MS) [4], high-performance liquid chromatography (HPLC) with fluorescence [5], and UV [3,6,7]. These methods, however, suffer from relatively low sensitivity [1,3,5,6,8], high plasma volumes required for sample preparation [1,5], and longer runtimes [5,7]. Although several LC–MS/MS methods [1,8–10] for determining sumatriptan levels in plasma have been reported, they too suffered from low sensitivity [1,8,11] and high sample volumes and required complicated sample preparation and expensive instrumentation [1,8–11]. Thus, simpler and more sensitive assay methods are required to measure sumatriptan in human plasma samples. The current study describes a sensitive and simple U-HPLC–MS/MS method and its application to a clinical pharmacokinetic study of sumatriptan succinate in healthy male volunteers.

2. Experimental methodology

2.1. Reagents and chemicals

Sumatriptan (lot no. STS1106025) was purchased from SMS Pharmaceuticals, and the internal standard (IS), terazosin (lot no. 076K4116), and ACS reagent-grade formic acid were purchased

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from Sigma–Aldrich (St. Louis, MO, USA). Sumatriptan succinate for oral administration was supplied by Myung In Pharm. CO. Ltd (Seoul, Korea). HPLC-grade acetonitrile and ethyl acetate were obtained from Merck (Darmstadt, Germany). Ultrapure water for chromatography was prepared using a Milli-Q water purification system (Millipore). Heparinized, blank human plasma samples were obtained from healthy Korean male volunteers.

2.2. Liquid-chromatography–tandem mass spectrometry

The ACQUITY™ U-HPLC system was coupled with a Quattro Premier XE™ Micromass® triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The chromatographic separation was performed at 30 ± 5 °C on an ACQUITY™ U-HPLC BEH C_{18} Column (50 mm × 2.1 mm, 1.7 μm, Waters). Sumatriptan was eluted with an isocratic mobile-phase water–acetonitrile–formic acid (83:17:0.1, v/v/v) at a flow rate of 0.2 mL/min and with a total run time of 5 min for each sample. The mobile phase was filtered through a 0.22-μm membrane filter (Millipore, Dublin, Ireland). The autosampler was maintained at 10 ± 5 °C, and the samples (5 μL, a partial loop in the needle overfill mode) were injected through the column into the mass spectrometer. Mass detection was used in the positive electrospray ionization (ESI) ion mode, which was performed using multiple reaction monitoring (MRM). The MS parameters were as follows: source temperature, 80 °C, desolvation temperature, 250 °C, capillary voltage, 4.0 kV, cone gas flow, 47 L/h of nitrogen gas, and desolvation gas flow 599 L/h of nitrogen gas. The cone voltage and collision energy were 30 V and 19 eV for sumatriptan, and 52 V and 37 eV for terazosin (IS), respectively. Argon was used as the collision gas at a flow rate of 0.25 mL/min. All the data were collected, processed, and calculated using MassLynx™ software with QuanLynx™ (Waters Corp.).

2.3. Preparation of standards and quality controls

Stock solutions of sumatriptan and IS were prepared separately in ultrapure water at concentrations of 1 mg/mL and stored at −20 °C. Standard sumatriptan solutions at concentrations of 5, 10, 50, 100, 200, 400, and 500 ng/mL and an IS solution at 1 μg/mL were prepared via serial dilution of the stock solutions of sumatriptan and IS with ultrapure water. Heparinized drug-free blank plasma was spiked with standard solutions to prepare calibration standards of 0.5, 1, 5, 10, 20, 40, and 50 ng/mL of sumatriptan with 0.1 μg/mL of IS. Quality control (QC) samples were prepared at four concentrations: 0.5 ng/mL (lower limit of quantitation, LLOQ), 1 ng/mL (low, LQC), 10 ng/mL (middle, MQC), and 50 ng/mL (high, HQC). All standard stock solutions were kept at −20 °C until analysis. The standards and QC samples were extracted daily before analysis using the following procedure for the plasma samples.

2.4. Plasma sample preparation

The plasma samples were stored at −80 °C and allowed to thaw gradually to room temperature before processing. After 200-μL aliquots of plasma were transferred into microcentriguge tubes, 10 μL of IS solution (1 μg/mL of terazosin) were added to each tube, and the tubes were briefly vortexed. Ethyl acetate (1.7 mL) was added to each tube, and the mixture was vortexed for 5 min and centrifuged at 16,100 × *g* for 5 min at 4 °C. Next, 1.5 mL of the upper organic layer were transferred to another glass tube and evaporated until dry for 40 min at 45 °C using a SpeedVac vacuum evaporator (Savant Instruments, Holbrook, NY, USA).

The dry residue was reconstituted in 100 μL of diluent (water–acetonitrile–formic acid, 83:17:0.1, v/v/v) and vortexed for 1 min. The resulting solution was filtered through a nylon filter (0.22 μm, 4 mm; Millipore) into glass vials, and 5 μL of this solution were injected into the U-HPLC–MS/MS system.

2.5. Method validation

The full validation was performed according to the KFDA guidelines for human plasma [12,13].

2.5.1. Specificity

To determine whether endogenous compounds interfered with the analysis, drug-free plasma samples from six different individuals were analyzed to detect potential interferents at the LC retention times for sumatriptan and IS.

2.5.2. Linearity and sensitivity

The calibration curve was determined to be in the range of 0.5–50 ng/mL. The linearity was evaluated by constructing a linear regression equation that fit the peak-area ratio of sumatriptan to IS versus the sumatriptan concentration using a weighted ($1/x$) least-squares regression.

The analyte signal at the LLOQ should be at least 10 times the signal of the blank plasma when carry-over effects are considered.

2.5.3. Accuracy and precision

The intra-day accuracy and precision were assessed by analyzing five replicates of each QC sample (0.5, 1, 10, and 50 ng/mL) on the same day. The inter-day accuracy and precision were determined by analyzing the QC samples on five different days. The accuracy was calculated as (measured concentration/nominal concentration) × 100%, and the precision was expressed as the relative standard deviation (RSD) defined as standard deviation/mean × 100%. The accuracy should deviate not more than ±15% from the nominal concentration and the precision should be ≤15%, except at the LLOQ where an accuracy of ±20% and a precision of ≤20% were considered acceptable.

2.5.4. Recovery

The extraction recoveries of sumatriptan from the three QC samples (1, 10, and 50 ng/mL) were determined by comparing the peak-area ratio of the spiked sumatriptan in the blank plasma sample before and after extraction at the corresponding concentrations. IS recovery was determined at a single concentration (1 μg/mL) in a similar manner.

2.5.5. Stability experiments

Stability was measured at the low (1 ng/mL) and high (50 ng/mL) QC concentrations in triplicate. Four stability conditions were tested: post-preparation stability at 10 °C for 24 h, freeze–thaw stability for three cycles, short-term stability at room temperature for 6 h, and long-term stability at −70 °C for 37 days. The stability of stock solutions was also analyzed for 25 day at −20 °C. All samples were analyzed with the same calibration curve, as discussed above.

2.6. Pharmacokinetic application

The validation method was applied to a pharmacokinetic study of sumatriptan among healthy volunteers. The pharmacokinetic study protocol was approved by the institutional review board of Kyungpook National University Hospital (Daegu, Korea), and all the volunteers provided written informed consent. After an overnight fast of 12 h, the volunteers received a single, 50-mg oral dose of sumatriptan succinate with 240 mL of water. An additional water intake was permitted 2 h after dosing. No volunteers were allowed to take any concomitant medication during the study period. Blood samples (8 mL) were collected in tubes containing sodium heparin before (0 h) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and

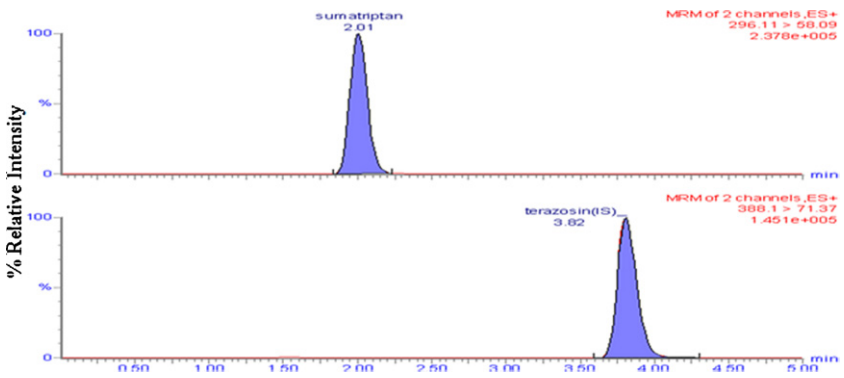

Fig. 1. Typical MRM chromatogram of sumatriptan (upper panel) and terazosin (lower panel) from a plasma sample from a volunteer 1 h after oral administration of a 50-mg sumatriptan tablet spiked with terazosin (experimental conditions were as described in the text).

12 h after sumatriptan administration. The plasma samples were immediately separated from the blood via centrifugation at 800 × *g* for 10 min at 4 °C and were stored at −70 °C until their subsequent analysis. The pharmacokinetic parameters were calculated using WinNonlin 5.2 software (Pharsight Corporation, CA, USA), including the area under the curve (AUC), maximum plasma concentration (C_{max}), the time to reach C_{max} (T_{max}) and the half-life ($t_{1/2}$) of sumatriptan.

3. Results and discussion

3.1. Liquid-chromatography–tandem mass spectrometry

To optimize ionization, 1 μg/mL of sumatriptan and 1 μg/mL of IS solutions were infused along with the mobile phase (0.2 mL/min), directly into the mass spectrometer in positive- and negative-ion mode. The observed ion abundances of sumatriptan and IS were significantly greater in positive-ion mode. In the fully scanned mass spectra, the positive ESI of sumatriptan and IS produced molecular ions ($[M+H]^+$) at *m*/*z* 296 and 388, respectively. The most abundant and stable product ions were observed at *m*/*z* 58 and 71 for sumatriptan and IS, respectively.

Analyses were initiated under isocratic conditions. Mobile phases were evaluated for symmetric peak shape, sensitivity, and low background noise. Acetonitrile and methanol, buffered with formic acid and ammonium formate, resulted in the highest resolution. A mobile phase of water–acetonitrile–formic acid (83:17:0.1, v/v/v) was chosen for this study. Fig. 1 shows a typical, representative chromatogram of extract from volunteer plasma that had been spiked with IS. The retention times were 2.0 and 3.8 min for sumatriptan and IS, respectively.

3.2. Sample preparation and recovery

Tan et al. [8] recently reported that the solid-phase extraction (SPE) method can reduce the matrix effect and is sensitive and easy to apply to the analysis of human plasma samples. However, this method requires a special set-up and greater overall time and cost. In this study, the liquid–liquid extraction (LLE) method was used to extract sumatriptan from the plasma samples.

The mean percent recovery values of sumatriptan were 69.8, 72.9, and 62.3% for the plasma samples ($n = 3$) at concentrations of 1, 10, and 50 ng/mL, respectively. The mean percent recovery for IS was 82.6% for the plasma samples at a concentration of 1000 ng/mL. The RSDs (%) of these values were within 11.8%. This extraction procedure for sumatriptan and IS was deemed consistent, precise, and reproducible.

3.3. Specificity

In this study, the specificity was investigated using independent drug-free plasma. No interfering endogenous peaks at the retention times (supplementary Figure) of sumatriptan (0.5 ng/mL) and IS (1 μg/mL) were observed in typical MRM chromatograms of blank human plasma and blank human plasma spiked with IS.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2013.01.004.

3.4. Lower limit of quantitation (LLOQ) and linearity

The linear regression equation was $y = 0.146x - 0.00607$, and the correlation coefficient (r) was 0.999 for all calibration curves, wherein y is the peak-area ratio (peak area of sumatriptan/peak area of IS) and x is the concentration of sumatriptan (ng/mL) in the plasma samples. This method had increased sensitivity (0.5 ng/mL) compared to previous studies [4,5] and was sufficiently sensitive for the determination and pharmacokinetic analysis of sumatriptan in humans. Patel et al. [9] reported an LLOQ of 0.05 ng/mL for sumatriptan. While this is lower than the LLOQ obtained in the current study, it required complicated sample preparation and expensive instrumentation.

3.5. Accuracy and precision

Accuracy and precision were determined through replicate analyses at four concentrations. The results are summarized in Table 1. The data indicate that accuracy and precision were within an acceptable range for analytical applications.

3.6. Stability

The QC plasma samples at two concentrations (1 and 50 ng/mL) were stable during sample preparation procedures, storage, and after sample extraction. The stability of sumatriptan is summarized in Table 2. Stored sumatriptan and IS stock solutions were stable for up to 24 days at −20 °C. Sumatriptan levels in human plasma varied only slightly, indicating adequate sample stability under the examined conditions.

3.7. Pharmacokinetic application

The established U-HPLC–MS/MS analysis method was successfully applied to a pharmacokinetic study of sumatriptan. The sumatriptan concentrations in plasma were quantifiable after oral administration of a 50-mg dose to 10 healthy Korean male volunteers. Mean concentration–time profiles of sumatriptan in plasma

Table 1
Intra-day ($n = 5$) accuracy and precision of quality control samples containing sumatriptan at four concentrations (0.5, 1, 10 and 50 ng/mL) in plasma.

Nominal con. (ng/mL)	Calculated con. (mean ± ng/mL)		Precision (RSD,%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.5	0.51 ± 0.04	0.50 ± 0.05	8.44	11.73	102.88	99.96
1	0.97 ± 0.09	1.00 ± 0.08	9.15	7.55	97.40	99.92
10	9.78 ± 0.88	10.08 ± 0.46	8.96	5.98	97.77	100.77
50	49.38 ± 2.92	50.66 ± 3.14	5.92	5.91	98.76	101.32

Table 2
Stability of sumatriptan standard in plasma at four different concentrations ($n = 3$).

Added QC concentrations (ng/mL)	Calculated concentrations (mean ± SD, ng/mL)			
	Post-preparation[a]	Freeze–thaw cycles ($n = 3$)	Short-term[b]	Long-term[c]
1				
Mean ± SD	0.92 ± 0.02	1.00 ± 0.04	1.07 ± 0.04	1.07 ± 0.03
% Relative concentration	96.6	90.1	112.2	99.8
50				
Mean ± SD	49.03 ± 1.92	50.55 ± 0.34	53.98 ± 2.08	49.79 ± 1.11
% Relative concentration	98.4	92.61	108.4	101.1
10 (sumatriptan stock)				
% Relative concentration[d]		100.1		
100 (IS stock)				
% Relative concentration[d]		97.8		

[a] After 24 h at 10 °C.
[b] After 6 h at room temperature.
[c] After 37 days at −20 °C.
[d] 24 days at −20 °C.

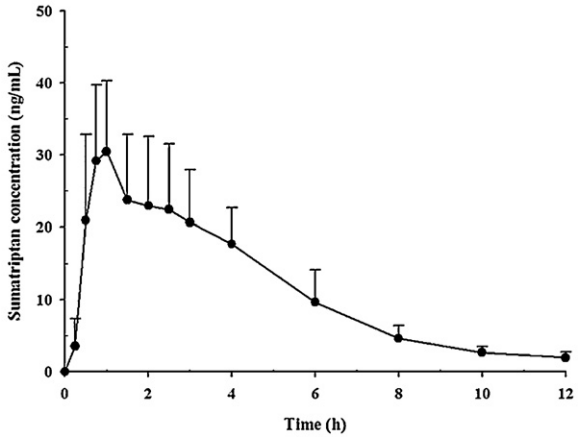

Fig. 2. Plasma concentration as a function of time after a single oral dose of 50-mg sumatriptan for 10 healthy male volunteers. Data show the mean and standard deviation.

are shown in Fig. 2, and calculated pharmacokinetic parameters are given in Table 3. At equivalent doses, the pharmacokinetic parameters in the current study were comparable to those obtained by Moore et al. [14] (Dose normalized pharmacokinetic parameters: AUC_{last} of 2.21 ng h/mL, C_{max} of 0.64 ng/mL, T_{max} of 1.0 h, and $t_{1/2}$ of 2.1 h). These results also agree well with the results of the authors' previous study.

Table 3
Calculated pharmacokinetic parameters of sumatriptan after oral administration of 50-mg to healthy male volunteers ($n = 10$).

Pharmacokinetic parameters	Mean ± SD
AUC_{last} (ng h/mL)	116.23 ± 24.42
C_{max} (ng/mL)	33.21 ± 9.08
Dose-normalized (to 1 mg)	
AUC_{last} (ng h/mL)	2.32 ± 0.49
C_{max} (ng/mL)	0.66 ± 0.18
T_{max} (h)	1.13 ± 0.63
$t_{1/2}$ (h)	2.96 ± 1.11

This study had some apparent limitations. The LC–MS-based ion suppression and enhancement profiles of the individual human serum samples, including those of the patients, were considerably different [15]. Accordingly, isotope-labeled internal standards with similar physicochemical properties as the target compound are usually considered state-of-the-art for LC–MS-based assay as well as a powerful approach for compensating for the suppression and enhancement effects in a matrix (as a deproteinized plasma) [16]. The matrix effect was represented as a practical disadvantage of instrumental techniques such as LC–MS analysis that has a major effect on quantitative analysis, reducing its accuracy and precision [17]. In this study, the retention times of sumatriptan and IS in a blank plasma sample were determined for consideration as a replacement for matrix effect examination. However, it was shown to be even more inaccurate method, compared to the other strategies for determining the matrix effect degree such as postextraction addition, and postcolumn infusion [15,18,19]. In a biological sample, the best way to adjust for the matrix effect is for the analysts to use an isotope-labeled internal standard. Accordingly, further study is required to confirm the matrix effect using an isotope-labeled internal standard in sumatriptan validation.

4. Conclusion

An U-HPLC–MS/MS analysis method for the quantification of sumatriptan was developed and validated using human plasma. This validated method had increased sensitivity and simplicity for application in human pharmacokinetic studies. Compared with previously published validation methods, our LLOQ (0.5 ng/mL) is significantly lower than the 1 ng/mL reported by Karthic et al. [1] and Tan et al. [8]. The LLE sample preparation method in our study was more simplistic than previous SPE sample preparation methods [1,8]. The sensitivity and speed of sumatriptan analysis were achieved with an LLOQ of 0.5 ng/mL and a complete LLE analysis time of 5 min per sample, which makes it suitable for high-throughput bioanalysis of sumatriptan. This method was

successfully applied to a clinical pharmacokinetic study of sumatriptan.

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