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ORIGINAL RESEARCH

Validation and application of a high-performance liquid chromatography/tandem mass spectrometry assay for sumatriptan in human plasma

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ABSTRACT: A sensitive and convenient high-performance liquid chromatography–tandem mass spectrometry(HPLC-MS/MS) assay is described for the (5-$HT_{1B/1D}$) receptor agonist sumatriptan in human plasma. Sumatriptan was recovered from plasma ($81.8 \pm 6.8\%$) by liquid–liquid extraction. The mobile phase flow rate was 0.3 mL/min and consisted of methanol:water:formic acid (90:10:0.1, v/v/v). The analytical column (4.6×100 mm) was packed with Partisil C_8 (5 μm). The standard curve was linear from 0.7 to 70.4 ng/mL ($r^2 > 0.99$). The lower limit of quantitation was 0.7 ng/mL. The assay was specific, accurate (percentage deviation from nominal concentrations were <15%), precise and reproducible (within- and between-day coefficients of variation <10.3%). Sumatriptan in plasma was stable over three freeze/thaw cycles and at room temperature for one day. The utility of the assay was demonstrated by following sumatriptan plasma concentrations in two healthy subjects for 8–12 h following a single 20 mg intranasal dose. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: 3-[2-(dimethylamine)ethyl]-*N*-methyl-1H-indole-5-methanesulfonaminde; 5-hydroxytryptamine type 1B and 1D receptors; serotonin; nasal spray formulation; intranasal does; pharmacokinetic study

INTRODUCTION

Sumatriptan 3-[2-(dimethylamino) ethyl]-*N*-methyl-1H-indole-5-methanesulfonamide, see Fig. 1 is an agonist at vascular 5-hydroxytryptamine (serotonin) type 1B and 1D (5-$HT_{1B/1D}$) receptors which play a role in the mediation of vasoconstriction. This property makes sumatriptan a useful drug in managing some types of migraine headaches (Buzzi and Moskowitz, 1991). Previously reported methods for quantitating sumatriptan in human plasma have included high-performance liquid chromatography (HPLC) with electrochemical or coulometric detection (Andrew *et al.*, 1993; Dunne and Andrew, 1996; Franklin *et al.*, 1996), gas chromatography with mass-spectrometry (MS) detection (Rochholz *et al.* 1995), HPLC-MS (Dulery *et al.*, 1997; Oxford and. Lant, 1989), and, more recently, HPLC with tandem mass-spectrometry (MS/MS) detection (McLoughlin *et al.*, 1996; Cheng *et al.*, 1998; Vishwanathan *et al.*, 2000; Biddlecombe *et al.* 2001). Of the HPLC-MS/MS assays that have been applied to clinical samples, only plasma concentrations of sumatriptan following oral administration have been reported to date (McLoughlin *et al.*, 1996; Cheng *et al.*, 1998).

Sumatriptan is now available in a nasal spray formulation and is administered at lower doses than those normally given by the oral route (5 and 20 mg vs 25 or 50 mg for intranasal and oral, respectively). This report describes the validation of an HPLC-MS/MS assay for sumatriptan in human plasma and demonstrates its applicability to clinical samples following a single intranasal dose of sumatriptan to two healthy volunteers.

EXPERIMENTAL

Drug and reagents. Sumatriptan succinate reference standard (98.6% pure, concentrations hereafter are reported as free base

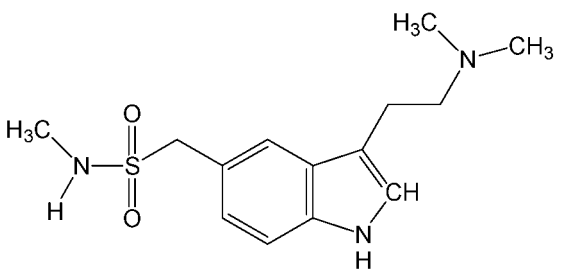

Figure 1. Structure of sumatriptan.

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corrected for purity unless otherwise stated) was obtained from Glaxo Wellcome Inc. (Research Triangle Park, NC, USA). BC-002605 was employed as an internal standard and was synthesized by Bristol-Myers Squibb Co. (Syracuse, NY, USA). Drug-free human plasma, containing EDTA as an anticoagulant, was obtained from the Metropolitan-Washington Blood Bank (Washington, DC, USA). HPLC-grade methanol was obtained from Burdick and Jackson (Muskegon, MI, USA). Formic acid, methyl *t*-butyl ether, and ammonium hydroxide, all ACS-grade, were obtained from EM Science (Cincinnati, OH, USA). ACS-grade ammonium phosphate was obtained from Mallinkrodt (Paris, KY, USA). Water was distilled and deionized by reverse osmosis and subsequently filtered through a 0.45 μm filter.

Chromatography and mass spectroscopy. The assay was performed on a Waters 2690 separations module (combined pump and autosampler, Waters Corp., Milford, MA, USA). The mobile phase flow rate was 0.3 mL/min and consisted of methanol: water:formic acid (90:10:0.1, v/v/v). The mobile phase was degassed under vacuum and filtered (0.45 μm) prior to use. The injection volume was 20 μL. The analytical column was a 10 cm × 4.6 mm i.d. Partisil C_8, 5 μm particle size (Whatman, Clifton, NJ, USA). The assay was performed at ambient temperature. The mass spectrometer was an API Sciex Model 300 (Foster City, CA, USA). The ion polarity was set in positive mode, and the source was electrospray ionization (TurboIon Spray). The nebulizer gas was air (zero grade) and the auxiliary, curtain, and collision gas was nitrogen (99.999%). The heated capillary temperature was 400°C. The ion spray, orifice and ring voltages were 5100, 37 and 200 V, respectively. The Q0, Q1, Q2 and Q3 rod offset voltages were −6.0, −7.0, −32.0 and −34.0 V, respectively. The MS/MS transition monitored for sumatriptan was at 296.0–251.0 amu and was at 314.2–296.2 amu for BC-002605. Mass spectrometry data were acquired by the system software supplied by PE Sciex (Sample Control version 1.2). Chromatographic data were integrated using MacQuan software version 1.4 (Applied Biosystems, Foster City, CA, USA).

Sample preparation. In a 15 mL glass centrifuge tube, 25 μL of the internal standard spiking solution (containing 0.5 ng BC-002605) were added to 1 mL of the plasma sample to be analyzed. After vortexing for 1 min, 0.5 mL of 1 M sodium carbonate solution was added to the mixture. Subsequently, 5 mL of methyl *t*-butyl ether were added and the mixture vortexed for 3 min, followed by an additional 10 min on a mechanical shaker. The tube was then centrifuged for 10 min at 1200***g***. The aqueous layer was frozen by placing the tube in a dry ice–acetone bath, and the organic layer was transferred to a clean glass centrifuge tube. The organic solvent was evaporated to dryness by a stream of nitrogen and mild heat in a multivap concentrator (Organomation, Berlin, MA, USA). The residue was reconstituted in 200 μL of methanol and the mixture transferred into autosampler vials for analysis.

Quantification and validation. Sumatriptan stock solutions (70 μg/mL) were prepared in methanol and serially diluted to produce a 704 ng/mL stock solution. The stock solution was used to prepare various concentrations of spiking solutions in water (14.1–1408 ng/mL). These spiking solutions were added to drug-free plasma (50 μL of spiking solution to 1 mL of plasma) to produce quality control and standard curve standards. A 20 ng/mL spiking solution of the internal standard, BC-002605, in water was similarly prepared.

The recovery of sumatriptan from the extraction procedure was determined by a comparison of the peak area of sumatriptan in spiked plasma samples (in triplicate at 3.5 and 35.2 ng/mL) to the peak area of sumatriptan in samples prepared by spiking extracted drug-free plasma samples with the same amounts of sumatriptan at the step immediately prior to chromatography. The specificity of the extraction procedure and the assay was assessed by extracting and analyzing drug-free plasma from six different individuals. The resultant ion chromatograms were examined for the presence of any endogenous constituents which may potentially interfere with the analysis of sumatriptan or the internal standard.

Triplicate seven point standard curves ranging from 0.7 to 70.4 ng/mL of sumatriptan were run on three separate days. The integrated peak areas of sumatriptan and BC-002605 were used to construct a standard curve from the peak area ratio vs added sumatriptan concentration by linear regression analysis with $1/x$ weighting. Four replicates of quality control samples at three concentrations of sumatriptan (2.8, 28.2 and 56.3 ng/mL) were included in each analytical run to determine the within- and between-run precisions of the assay. The lower limit of quantitation (LLQ) of the assay was assessed analyzing plasma samples from six different individuals spiked at 0.7 ng/mL, the lowest concentration in the standard curve.

The stability of sumatriptan in human plasma was assessed by analyzing triplicate quality control samples at 2.8, 28.2 and 56.3 ng/mL stored for one day at room temperature and also following one, two or three cycles of freezing at −70 °C and thawing. Concentrations following storage were compared to freshly prepared samples of the same concentrations. The samples were considered stable if the mean concentration of sumatriptan in the stability test samples was ≥90% of the mean concentration of sumatriptan observed in the freshly prepared samples.

Single intranasal dose pharmacokinetic study. Two healthy young male subjects participated in a pharmacokinetic study. Each subject received a 20 mg dose of sumatriptan (base) nasal spray (Imitrex®, Glaxo Wellcome Inc.). Serial blood samples were collected into EDTA vacuum tubes immediately before dosing and at 5, 10, 15, 20, 30, 35, 40 and 45 min, and at 1, 1.25, 1.5, 2, 2.5, 4, 6, 8, 12 and 24 h after dosing. Within 1 h of collection, plasma was harvested from the blood samples following centrifugation for 15 min at 2000 rpm. The plasma was immediately frozen at −20°C, and remained frozen until analyzed. The Western Institutional Review Board (Olympia, WA) approved the protocol and informed consent and the subjects gave written informed consent to participate.

RESULTS

Chromatography and mass spectroscopy

The mass spectrum and product ion mass spectrum of sumatriptan are shown in Fig. 2. Ion chromatograms for an extracted drug-free plasma sample and a plasma sample spiked with 0.7 ng/mL of sumatriptan (the LLQ) are shown in Fig. 3.

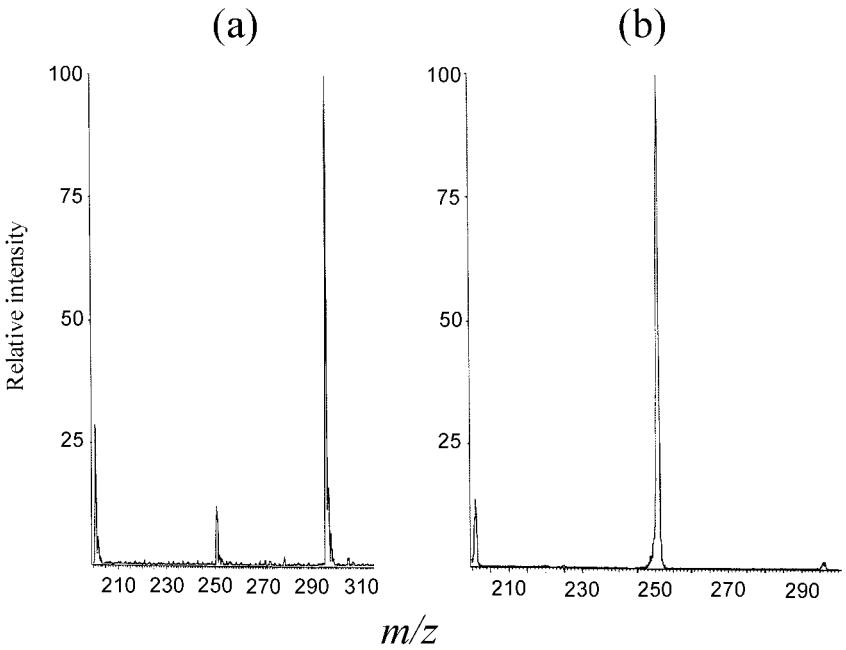

Figure 2. (a) The mass spectrum of sumatriptan and (b) the production mass spectrum of sumatriptan (*m/z* 296.0).

Quantification and validation

The mean recoveries of sumatriptan from human plasma following the extraction procedure were 80.7 ± 10.4 and 82.8 ± 1.5% at 3.5 and 35.2 ng/mL, respectively. The mean overall recovery of sumatriptan was 81.8 ± 6.8% ($n = 6$). No significant peaks interfering with sumatriptan or the internal standard were observed in drug-free plasma samples from six different individuals [see Fig. 3(a)].

The correlation coefficients for the standard curves ranged from 0.991 to 0.997 ($n = 3$), indicating linearity of the assay over the range of 0.7–70.4 ng/mL of sumatriptan. The mean (SE) slope and intercept for the regression lines of best fit were 0.0269 (0.0028) response units/ng/mL and −0.0026 (0.009) response units, respectively ($n = 3$). The coefficient of variation (SD/mean) of the regression slopes was 10.4% and the *y*-intercept was not significantly different from zero (two-sided Student's *t*-test). The mean absolute deviations of the predicted

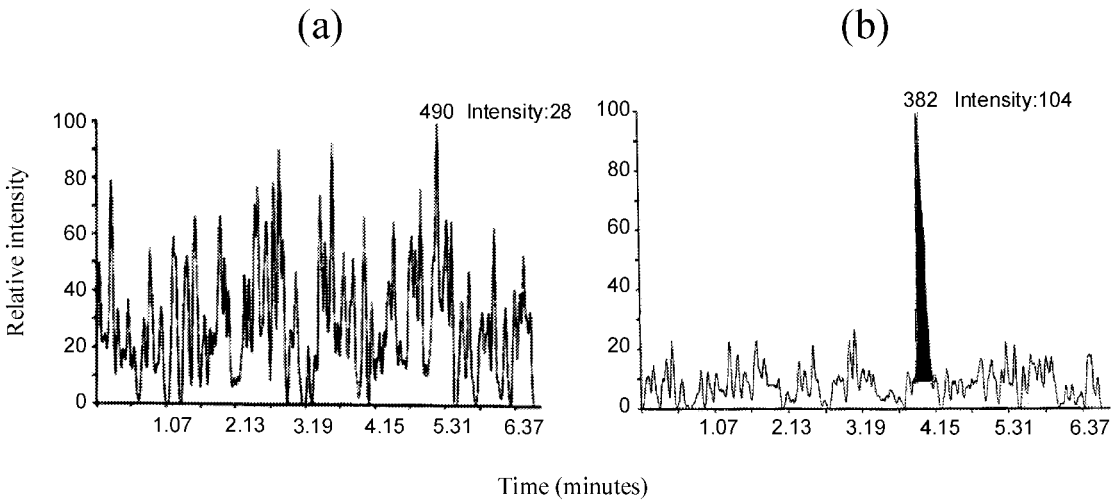

Figure 3. Ion chromatograms (*m/z* 296.0 to 251.0 amu) for (a) drug-free plasma and (b) plasma spiked with 0.7 ng/mL of sumatriptan, the concentration representing the lower limit of quantitation.

Table 1. Quality control summary for sumatriptan in human plasma. Data are collated from five replicates at each concentration run on three separate occasions

Nominal sumatriptan concentration (ng/mL)	2.82	28.2	56.3
Mean observed concentration (ng/mL)	2.78	29.8	59.6
Percentage deviation	−1.38	5.59	6.35
Between-run precision (%RSD)	3.04	1.30	0.06
Within-run precision (%RSD)	10.28	9.41	8.29

concentrations compared with nominal sumatriptan concentrations in LLQ samples were 12.1% with a coefficient of variation (precision) of 16.2%. A representative LLQ ion chromatogram for sumatriptan is shown in Fig. 3(b).

The number of quality control samples which exhibited deviations of <15% (predicted:nominal) were 13 of 15 at 2.8 ng/mL, 11 of 15 at 28.2 ng/mL, and 13 of 15 at 56.3 ng/mL. The within-run and between-run precision values showed <15% variability, and are summarized in Table 1. The range of concentration values obtained for all three quality control standards deviated from −17.6 to 23.2% of the nominal concentrations ($n = 45$). Sumatriptan was considered to be stable in human plasma (>90% of nominal concentration) at room temperature for 24 h (between 95.0 and 96.2% of the sumatriptan concentration in freshly prepared samples), and for three freeze/thaw cycles (between 102.1 and 116.5% of the sumatriptan concentration in freshly prepared samples). Previous studies have shown that sumatriptan is stable in frozen human plasma for at least 71 weeks (McLoughlin *et al.*, 1996).

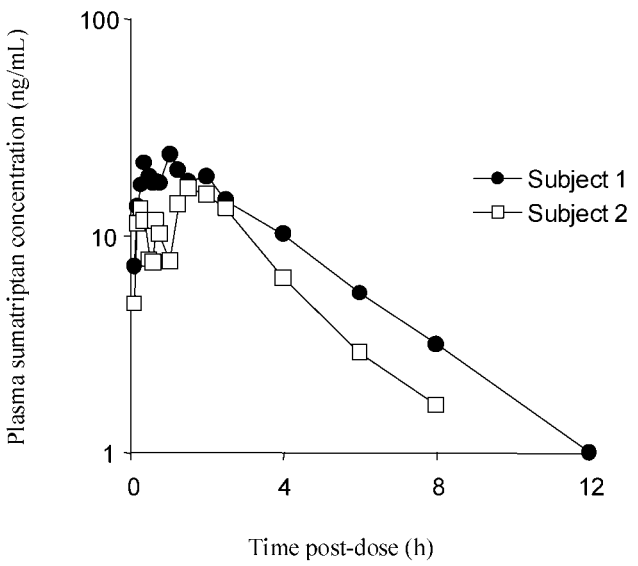

Figure 4. Plasma concentration–time profile for sumatriptan in two healthy subjects following a single 20 mg intranasal dose of sumatriptan succinate.

Single intranasal dose pharmacokinetic study

The assay was able to quantify sumatriptan in the plasma of the healthy subjects for at least 8 h, and up to 12 h following a single 20 mg intranasal dose. Subject sumatriptan plasma concentration vs time profiles are shown in Fig. 4. The concentration of sumatriptan in the 24 h plasma samples was below the LLQ in both cases. Two sumatriptan peaks were observed in the profiles of both subjects (see Fig. 4). The first peak was due to nasal absorption of sumatriptan [0.33 h (21.6 ng/mL) and 0.25 h (13.4 ng/mL), for subjects 1 and 2, respectively], while the later peak was presumably due to the oral absorption of swallowed sumatriptan [1.0 h (23.8 ng/mL) and 1.5 h (16.6 ng/mL), for subjects 1 and 2, respectively]. The area under the sumatriptan plasma concentration vs time curve (AUC) was 98.5 and 64.4 ng.h/mL for subjects 1 and 2, respectively. The apparent terminal-phase half-life of sumatriptan was 2.44 and 2.04 h for subjects 1 and 2, respectively.

DISCUSSION

The pharmacokinetic parameter values for sumatriptan in the two subjects in this study were comparable to previously reported values following single 20 mg intranasal doses in studies that were carried out during the development of the nasal spray formulation: mean C_{max} of 14.4 ng/mL; median T_{max} of 1.00 h; mean AUC of 49.9 ng.h/mL; and mean half-life of 2.0 h (Moore *et al.*, 1997). These studies employed an electrochemical detection method that had a lower limit of quantitation for sumatriptan of 1 ng/mL (Andrew *et al.*, 1993). However, electrochemical detection can require a high level of maintenance and the signal:noise ratio may reduced over time due to electrode contamination by assaying biological samples.

The lower limit of quantitation for sumatriptan in the assay described here was 0.7 ng/mL. While a small improvement in this value may be possible (see Fig. 3), a lower limit of quantitation of 0.7 ng/mL allowed the quantitation of sumatriptan in plasma for at least 8 h following a single 20 mg intranasal dose to two healthy subjects. This lower limit of quantitation was slightly higher than previously-published LC-MS/MS assay

methods for sumatriptan in human plasma [0.5 ng/mL (McLoughlin *et al.* 1996); 0.2 ng/mL (Cheng *et al.* 1998); and 0.1 ng/mL (Biddlecombe *et al.* 2001)]. However, in our pharmacokinetic study, a lower limit of quantitation of 0.7 ng/mL was sufficient for quantitation of sumatriptan concentrations over 4–5 half-lives, and allowed characterization of >95 % of the AUC extrapolated to infinity. This suggests that the lower limit of quantitation reported in the aforementioned LC-MS/MS assays would only marginally improve the pharmacokinetic characterization of sumatriptan following a 20 mg intranasal dose.

Automated sample preparation systems used in sumatriptan assays reported by Biddlecombe *et al.* (2001) and McLoughlin *et al.* (1996) are likely to be very useful in high-throughput situations. However, for pharmacokinetic studies of sumatriptan where numbers of samples are not large and where a sumatriptan assay is not routinely needed, the high capital cost of these robot systems means that the manual sample preparation described in our assay method may be more cost-effective since no specialized equipment is required. Additionally, when using an established manual extraction method in studies with relatively small numbers of subjects, such as is common in drug–drug interaction studies, there is also a potential to save on time spent developing automated extraction methods.

The assay presented here was specific, precise, and accurate according to commonly accepted criteria (Shah *et al.*, 1991). The assay is straightforward and does not require equipment that is not standard to most analytical laboratories. It will therefore be useful in pharmacokinetic studies using single or multiple doses of sumatriptan including those that are designed to examine concentration–response relationships, formulation bioequivalence, or potential drug–drug interactions.

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