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Determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum by liquid chromatography/electrospray tandem mass spectrometry

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Development of a rapid, sensitive and selective method for the determination of antimigraine drugs from human serum is essential for understanding the pharmacokinetics of these drugs when administered concurrently. Solid phase extraction (SPE) using Oasis HLB was used to extract the drugs (sumatriptan, naratriptan, zolmitriptan and rizatriptan) and the internal standard bufotenine from serum. A method based on liquid chromatography/tandem mass spectrometry (LC/MS/MS) was developed and validated to simultaneously quantitate these antimigraine drugs from human serum. The precursor and major product ions of the analytes were monitored on a triple quadrupole mass spectrometer with positive ion electrospray ionization (ESI) in the multiple reaction monitoring (MRM) mode. The base peak in all the analytes is formed by alpha cleavage associated with protonation of the secondary amine. Mechanisms for the formation of the collision-induced dissociation products of these antimigraine compounds are proposed. Linear calibration curves were generated from 1–100 ng/mL with all coefficients of determination greater than 0.99. The inter- and intraday precision (%RSD) were less than 9.3% and accuracy (%error) was less than 9.8% for all components. The limits of detection (LOD) for the method were 250 pg/mL for sumatriptan and 100 pg/mL for the remaining analytes based on a signal-to-noise ratio of 3.

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Migraine is a common disorder characterized by unilateral headache often accompanied by nausea and/or vomiting. Migraine usually begins in childhood, adolescence or early adult life. One of the first theories to explain migraine was described by Graham and Wolff[1] who suggested that the pathogenesis of the disease was primarily of vascular origin with the headache being associated with a marked and prolonged phase of cranial vasodilation. The role of neurotransmitters, particularly 5-hydroxytryptamine (5-HT), as a central mediator of a migraine attack has received the most attention to date.[2–5] The characteristics of the 5-HT receptors that mediate contraction of blood vessels have been studied for several years.[6,7]

Sumatriptan, naratriptan, zolmitriptan and rizatriptan have high affinity for 5-HT (1D/1B) receptors and their therapeutic activity is attributed to their agonistic activity at this receptor site. There are two theories that explain the efficacy of 5-$HT_{1D/1B}$ receptor agonists in migraine. One theory suggests that activation of 5-$HT_{1D/1B}$ receptors located on the intracranial blood vessels, including those on the arteriovenous anastomoses, leads to vasoconstriction, which is correlated with the relief of headache.[8] The other theory suggests that activation of 5-$HT_{1D/1B}$ receptors on sensory nerve endings in the trigeminal system results in the inhibition of pro-inflammatory neuropeptide release.[8]

Liquid chromatography is widely used for the quantitative determination of pharmaceutical compounds with UV, fluorescence or electrochemical detection. More recently, mass spectrometry (MS) has been introduced as a highly sensitive and selective detector for HPLC analyses. During the past 12 years, several approaches have been tried to interface LC to MS including thermospray ionization,[9,10] particle beam,[11,12] and atmospheric pressure ionization (API).[13,14] Thermospray ionization has limitations in that it requires critical control of the vaporizer temperature during analysis, as well as the possibility of thermal degradation of labile molecules. Particle beam ionization sources lack the necessary sensitivity for bioanalytical applications[15] and there have been reports of nonlinear responses for quantitative analysis.[16] For the determination of pharmaceutical compounds for clinical and pre-clinical studies, LC/MS with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) is rapidly becoming a powerful analytical tool.[17,18]

Sumatriptan has been quantitated by HPLC,[19–24] capillary electrophoresis[25] and LC/MS[26–29] from various biological matrices. An LC/MS/MS assay has been reported for the determination of naratriptan from rabbit plasma.[27] At this time, no method is available in the literature for the determination of rizatriptan and zolmitriptan from human serum. In this study an LC/ESI-MS/MS method was

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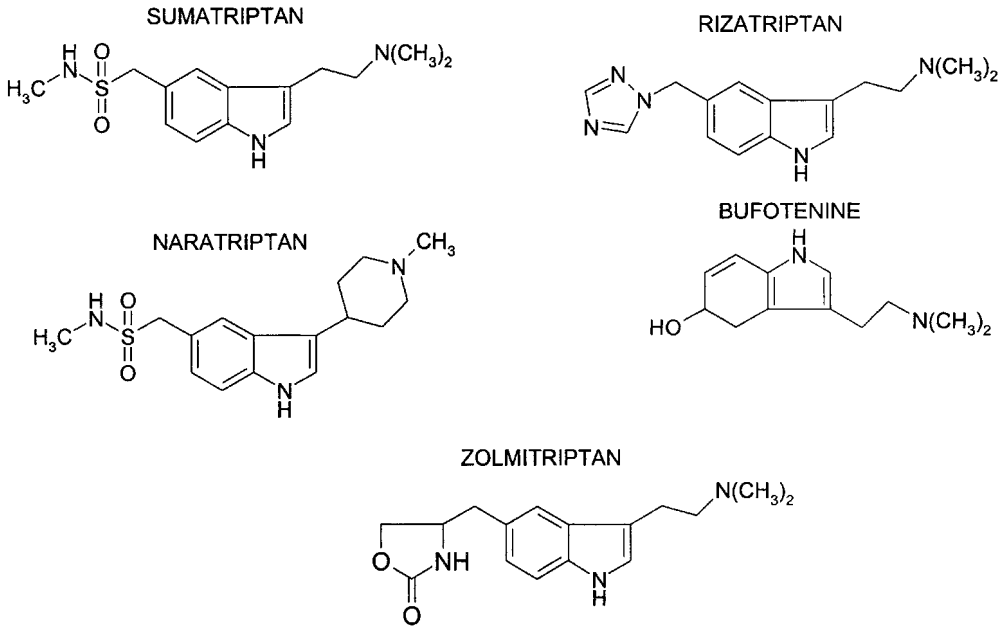

Figure 1. Structural formulae of the antimigraine compounds studied.

developed to analyze individual or a mixture of triptans concurrently from human serum.

Electrospray tandem mass spectrometry (MS/MS) provides the analyst with a rugged, sensitive and widely used technique to mass select a precursor and a characteristic product ion of an analyte, making it a highly specific method for the analysis of drug mixtures from human serum. In the present study, solid phase extraction (SPE) and online LC/ESI-MS/MS using bufotenine as an internal standard was utilized to quantitate sumatriptan, naratriptan, zolmitriptan and rizatriptan from human serum. Collision-induced dissociation (CID) data on the compounds are presented and mechanisms for the formation of the observed product ions are proposed.

EXPERIMENTAL

Materials

Sumatriptan succinate and naratriptan hydrochloride were gifts from Glaxo Wellcome (RTP, NC 27709). Zolmitriptan was a gift from Zeneca Pharmaceuticals (Wilmington, DE 19850) and rizatriptan benzoate was a gift from Merck Research Labs (Rahway, NJ 07065). Bufotenine (internal standard) was obtained from Radian International (Austin, TX 78720). Acetonitrile and methanol (HPLC grade) were obtained from J. T. Baker (Phillipsburg, NJ 08865). Deionized water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076). Oasis HLB solid phase cartridges were obtained from Waters Corporation (Milford, MA 01757).

Preparation of stock and sample solutions

Stock solutions of sumatriptan, naratriptan, rizatriptan and zolmitriptan were prepared at a concentration of 100 μg/mL in water. Bufotenine was obtained as a solution of 100 μg/mL in methanol. A working internal standard solution was prepared by diluting the bufotenine stock solution with water to provide a concentration of 250 ng/mL. Structural formulae of the triptans and the internal standard are shown in Fig. 1. An intermediate stock solution of 1000 ng/mL containing each analyte was prepared by diluting the original stock solution with water. For preparation of standard curves, seven dilutions containing a mixture of sumatriptan, naratriptan, rizatriptan and zolmitriptan were prepared at 1, 2.5, 5, 10, 25, 50 and 100 ng/mL in blank serum. Quality control (QC) samples were prepared at concentrations of 3, 15 and 75 ng/mL in blank serum and were stored at −20 °C until assayed or used for validating the analytical method.

Solid phase extraction procedure

1 mL samples of each calibration standard and QC samples were thawed and mixed well with a vortex mixer. To each sample were added 50 μL of 250 ng/mL internal standard solution (bufotenine) in a glass tube and the mixture was mixed well. Oasis HLB SPE cartridges (30 mg, 1cc) were conditioned with 1mL of methanol and 1mL of deionized water. The samples were added to the cartridges and vacuum was applied. The cartridges were washed with 1 mL of water and then 2 × 1mL of 95:5 v/v water/methanol. The sample was eluted using 2 × 0.75mL of absolute methanol followed by sample concentration in a vacuum centrifuge (Savant Instruments Inc., Farmingdale, NY, USA). Extracts were then reconstituted in 200 μL of mobile phase, mixed, and 50 μL were injected into the LC/ESI-MS/MS system.

LC/ESI-MS/MS conditions

The LC separation was performed using a Hewlett-Packard 1100 HPLC system equipped with a quaternary pump and an autosampler (Hewlett-Packard, Palo Alto, CA, USA). The column utilized was an Alltech Solvent Miser Silica (150 × 2.1mm i.d., 5 μm particle size, Deerfield, IL, USA). An Optiguard 1-mm guard column containing C-8 sta-

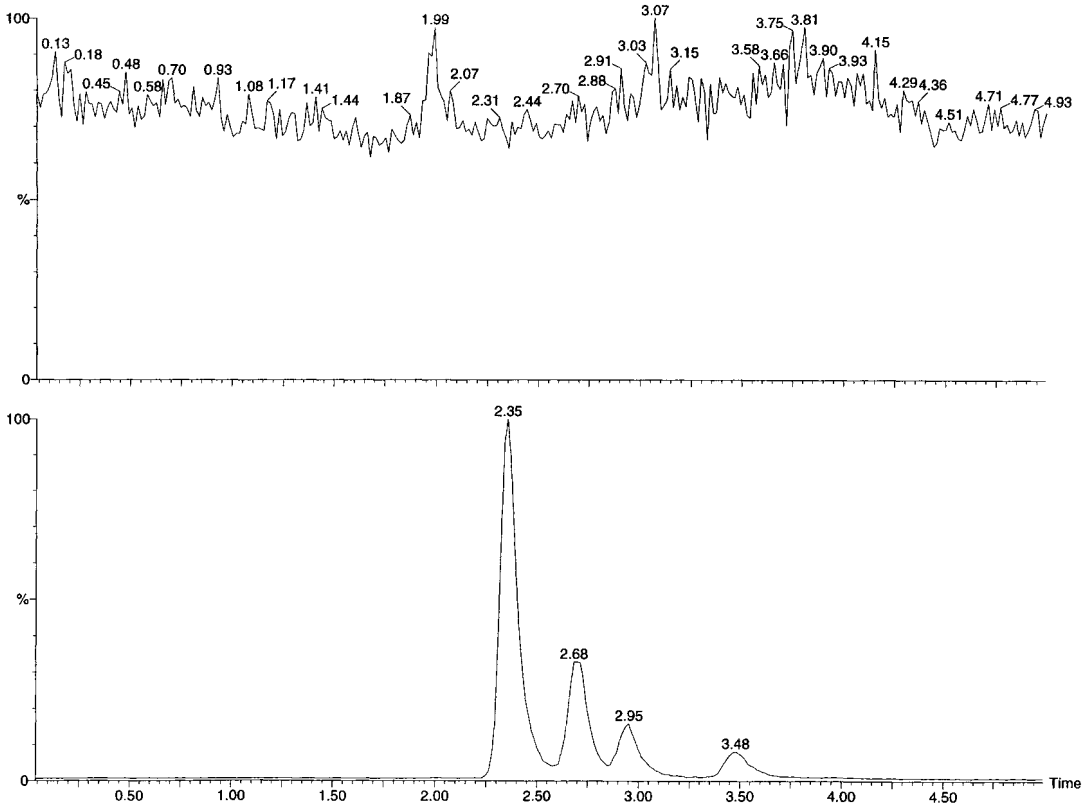

Figure 2. Typical chromatogram of (a) blank human serum and (b) human serum spiked with sumatriptan (2.68 min), naratriptan (2.71 min), zolmitriptan (2.95 min), rizatriptan (3.48 min) and the internal standard, bufotenine (2.35 min).

tionary phase was used (Optimize Technologies Inc., Oregon City, OR, USA). The flow rate was 300 μL/min. 50 μL injections of the reconstituted extracts were injected onto the HPLC system for analysis. An isocratic elution of 80:10:10 (v/v/v) 20mM ammonium acetate pH 2.70 (adjusted with glacial acetic acid to pH 4 and then with formic acid to pH 2.70)/methanol/acetonitrile was used and the assay was carried out in 4 min. Positive ion electrospray MS/MS was performed on a Micromass Quattro II triple quadruple mass spectrometer (Beverly, MA, USA) interfaced to the HP1100 HPLC system using a megaflow electrospray probe with nitrogen as the sheath gas. MS control and spectral processing was carried out using MassLynx software, version 2.22 (Micromass, Beverly, MA, USA). The positively charged molecule of each analyte was selected by mass and focused into the collision cell containing argon gas (99.999% purity) maintained at a pressure of approximately 1.3×10^{-3} Torr. The precursor and collision-induced fragment ions were monitored by the post-collision quadrupole analyzer. The measurements were made at a 120 °C source temperature, 4.0 kV needle voltage, 35 V cone voltage and 15–20 eV collision energy.

Table 1. *m/z* ratios and relative intensities from positive ion MS/MS spectra

Naratriptan	Sumatriptan	Zolmitriptan	Rizatriptan	Bufotenine
336 (15%)	296 (17%)	288 (12%)	270 (12%)	205 (14%)
241 (5%)	251 (21%)	243 (22%)	201 (100%)	160 (100%)
210 (5%)	201 (14%)	182 (26%)	158 (17%)	58 (68%)
98 (100%)	158 (6%)	58 (100%)	58 (4%)	
70 (10%)	58 (100%)			

RESULTS AND DISCUSSION

Solid phase extraction allowed an efficient extraction of all the analytes and the internal standard in a relatively short time with excellent recovery. Extraction recoveries (n = 5) were determined by external standard comparison and were approximately $101 \pm 4.6\%$ for naratriptan, $84 \pm 5.8\%$ for zolmitriptan, $76 \pm 4.9\%$ for sumatriptan, $82 \pm 6.4\%$ for rizatriptan and $81 \pm 4.1\%$ for the internal standard, bufotenine, based on a mixture with analyte concentrations at 5 ng/mL in serum. It was determined that the most efficient extraction was performed using Oasis HLB cartridges under the conditions described in the extraction procedure (see Experimental section). Other cartridges studied were C18, C8, C2 and silica, but recoveries were low (<60%). The Oasis HLB cartridge yielded good reproducible recoveries for all the analytes of interest. The extracts were also free of particulate matter and endogenous interferences. Hence, Oasis HLB cartridges were used for the extraction of the antimigraine drugs.

For quantitative LC/ESI-MS/MS, the positive ionization mode was selected because of improved sensitivity due to the presence of amine groups, which were easily protonated. Lower pH (pH < 3) was used to improve the formation of positive ions. Representative chromatograms of the blank serum and blank serum spiked with the internal standard and analytes are shown in Fig. 2.

Mass spectra and total ion chromatograms

In all of the analytes, the precursor ion $[M + H]^+$, where M is the molecular mass of the respective analyte, is formed as

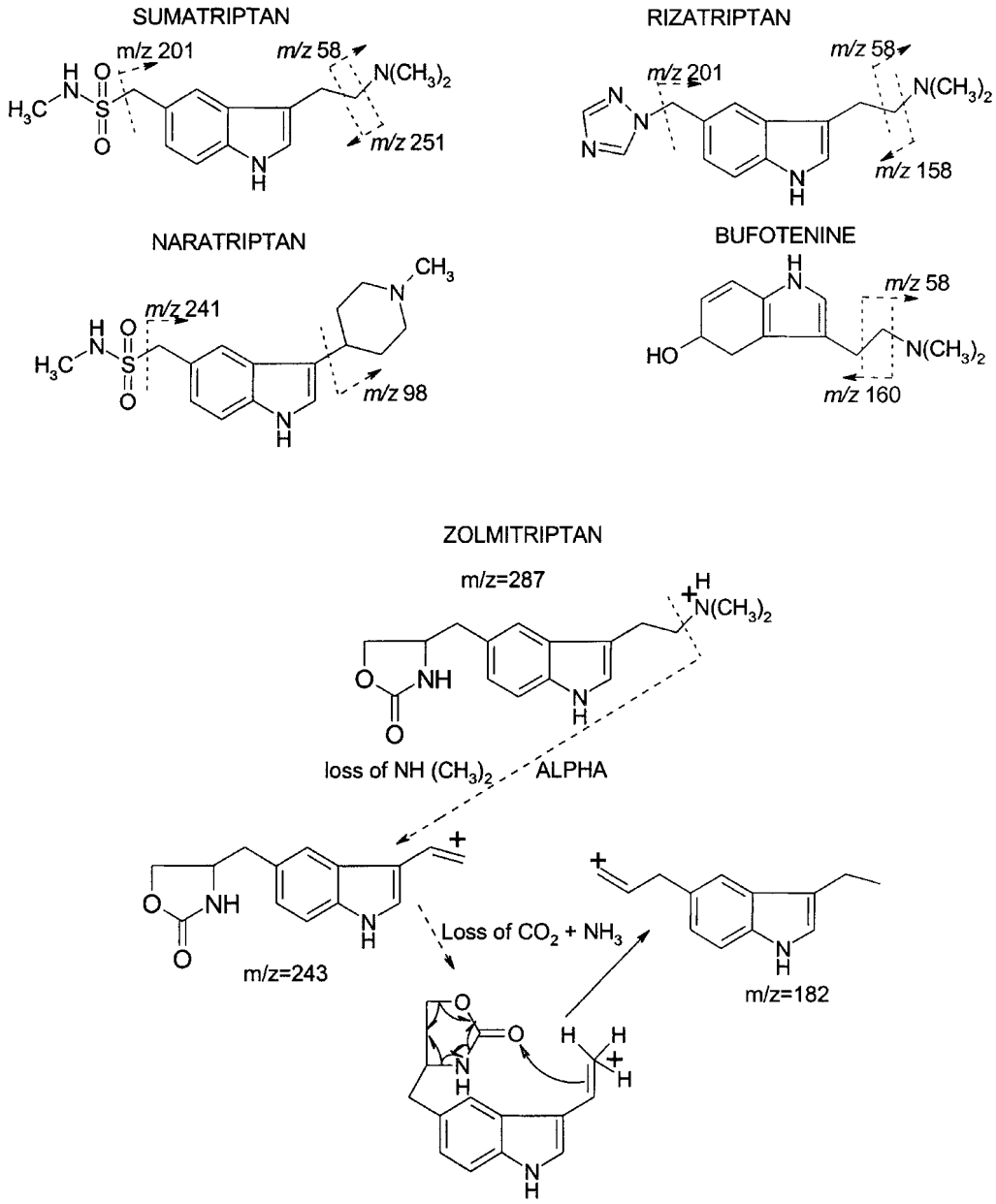

Figure 3. Fragmentation pathway for the antimigraine compounds studied.

a result of the addition of a proton to form the positively charged molecular ion. The positive ion tandem mass spectral data of the four triptans, as well as the internal standard bufotenine, are shown in Table 1. The base peak in the CID mass spectrum is *m/z* 58 for sumatriptan and zolmitriptan, *m/z* 98 for naratriptan and *m/z* 201 for rizatriptan. In the case of bufotenine, loss of neutral dimethylamine [$NH(CH_3)_2$] gives rise to the base peak at *m/z* 160. The precursor and major product ions of the analytes were monitored in the multiple reaction mode as follows: (1) naratriptan (*m/z* 336→98), (2) sumatriptan (*m/z* 296→58), (3) zolmitriptan (*m/z* 288→58), (4) rizatriptan (*m/z* 270→201) and (5) bufotenine (*m/z* 205→160). The base peak in all cases is formed by alpha cleavage associated with protonation of a secondary amine. Other fragment ions result from the loss of the remaining side chain containing the sulfonamide moiety.

In the case of naratriptan, protonation of the nitrogen on the sulfonamide side chain results in loss of CH_3NHSO_2H via alpha cleavage to give *m/z* 241. Alternatively, the nitrogen in the piperidine ring may be protonated yielding the fragment ion at *m/z* 98 which can subsequently undergo a retro Diels-Alder reaction to form *m/z* 70.

Sumatriptan and rizatriptan undergo the loss of neutral CH_3NHSO_2H through a mechanism identical to that for naratriptan yielding *m/z* 201. There is also a loss of dimethylamine from the molecular ion by alpha cleavage to give rise to *m/z* 251. Loss of dimethylamine is supported by the presence of the fragment ion at *m/z* 58 [$H_2C = {}^+N(CH_3)_2$]. The loss of both side chains yields the fragment ion at *m/z* 158.

The ion at *m/z* 58, [$H_2C = {}^+N(CH_3)_2$], is the base peak in the fragmentation of zolmitriptan. The corresponding fragment ion at *m/z* 243 is formed by a neutral loss of dimethylamine. The neutral loss of $HN(CH_3)_2$ is also accompanied by the loss of CO_2 and NH_3 to yield the ion of *m/z* 182. The loss of dimethylamine results in the formation of a carbocation. The transfer of charge from the carboca-

Table 2. Intraday accuracy and precision data for spiked samples

Analyte	Conc added (ng/mL)	Conc found* (ng/mL)	Percent error	RSD (%)
Naratriptan	3	3.19 ± 0.13	6.57	4.11
	15	14.94 ± 1.05	0.38	7.07
	75	75.89 ± 3.48	0.40	4.63
Sumatriptan	3	2.70 ± 0.16	9.83	6.10
	15	13.94 ± 1.44	7.05	5.16
	75	74.14 ± 2.04	1.14	2.76
Zolmitriptan	3	2.89 ± 0.27	3.65	3.25
	15	15.25 ± 0.80	1.72	5.25
	75	72.12 ± 2.29	3.84	3.18
Rizatriptan	3	3.23 ± 0.02	7.83	0.67
	15	14.51 ± 0.32	3.20	2.18
	75	70.80 ± 1.61	5.58	2.28

* Mean ± Std dev.; Based on n = 3.

Table 3. Interday accuracy and precision data for spiked samples

Analyte	Conc added (ng/mL)	Conc found* (ng/mL)	Percent error	RSD (%)
Naratriptan	3	2.91 ± 0.27	3.13	9.25
	15	14.37 ± 0.83	4.20	5.76
	75	73.94 ± 3.62	1.42	4.90
Sumatriptan	3	2.77 ± 0.20	7.58	7.30
	15	14.32 ± 1.02	4.51	7.17
	75	74.30 ± 2.38	0.94	3.21
Zolmitriptan	3	3.15 ± 0.24	4.85	7.77
	15	14.82 ± 1.10	1.22	7.42
	75	71.88 ± 2.20	4.16	3.05
Rizatriptan	3	3.08 ± 0.25	2.80	7.95
	15	14.48 ± 0.67	3.47	4.67
	75	73.75 ± 3.20	1.66	4.35

* Mean ± std dev.; Based on n = 9.

tion to the ketone in the ring results in the loss of CO_2 and NH_3. A proposed mechanism is shown in Fig. 3.

Bufotenine was chosen as the internal standard because of its structural similarity to the analytes. Therefore, it is not surprising that its fragmentation is similar. Bufotenine fragments to form *m/z* 58, $[H_2C = {}^{+}N(CH_3)_2]$, by an alpha cleavage. The loss of neutral dimethylamine results in the formation of the other major fragment ion at *m/z* 160.

Linear regression and detection limits

Standard curves were produced on three different days in human serum over the range of 1–100 ng/mL, encompassing the therapeutic range of these antimigraine drugs. The response was linear for each analyte throughout this concentration range and the correlation coefficients (r^2) were greater than 0.99 for all standard curves. The limits of detection (LOD) for each analyte in human serum, based on a signal-to-noise ratio of 3, were 250 pg/mL for sumatriptan and bufotenine (IS) and 100 pg/mL for the remaining analytes.

Intra- and interday precision and accuracy

Intra- and interday precision and accuracy were calculated from QC samples analyzed on three days for each analyte at concentrations of 3, 15 and 75 ng/mL and are tabulated in Tables 2 and 3. All RSDs and percent errors were $<10.0\%$ over the period of 3 days (n = 9).

CONCLUSIONS

Solid phase extraction and LC/ESI-MS/MS provides the analyst with a novel method to determine the levels of antimigraine drugs in human serum. SPE can generate quantitative extractions without the need for excessive cleanup steps. LC/ESI-MS/MS, in addition to its high specificity, ruggedness and ease of use, provides sensitivity in the low ng/mL range.

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REFERENCES

1. Graham JR, Wolff HG. *Arch. Neurol.* 1938; **39**: 737.
2. Humphrey PPA, Fenuik W, Perron MJ. *Ann. NY Acad. Sci.* 1990; **600**: 587.
3. Moskowitz MA. *Neurol. Clin.* 1990; **8**: 801.
4. Olsen J. *Headache* 1990; **30**: 269.
5. Humphrey PPA. *J. Neurol.* 1991; **238**: 538.
6. Fenuik W, Humphrey PPA in *The Peripheral Action of 5-HT* Fozard (ed). Oxford University Press: Oxford, 1989; 100–122.
7. Fenuik W, Humphrey PPA, Perren MJ, Watts AD. *Br. J. Pharmacol.* 1985; **86**: 697.
8. Diener HC, Kaube H, Limmroth V. *J. Neurol.* 1999; **246**: 515.
9. Vestal ML, Ferguson GJ. *Anal. Chem.* 1985; **57**: 2373.
10. Voyksner RB, Haney CA. *Anal. Chem.* 1985; **57**: 991.
11. Willoughby RC, Browner RF. *Anal. Chem.* 1984; **56**: 2626.
12. Voyksner RB, Smith CS, Knox PC. *Biomed. Mass Spectrom.* 1990; **19**: 523.
13. Mutlib AE, Strupczewski JT. *J. Chromatogr. B* 1995; **669**: 237.
14. Gilbert JD, Greber TF, Ellis JD, Barrish A, Olah TV. et al. *J. Pharm. Biomed. Anal.* 1995; **13**: 937.
15. Poon G. in *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation and Applications*, Cole R (ed). John Wiley: New York, 1997; 323.
16. Watson J. in *Introduction to Mass Spectrometry*, Placito M, Bialer M (eds). Lippincott-Raven Publishers: Philadelphia, 1997; 402.
17. Whitehouse CM, Dreyer RN, Yamashita M, Fenn JB. *Anal. Chem.* 1985; **57**: 675.
18. Bruins AP. *Mass Spectrom. Rev.* 1991; **10**: 53.
19. Shirsat VA, Gabhe SY, Deshpande SG. *Indian Drugs* 1998; **35**: 404.
20. Singh S, Jain R. *Indian Drugs* 1997; **34**: 527.
21. Avandhanulu AB, Srinivas JS, Anjaneyulu Y. *Indian Drugs* 1996; **33**: 334.
22. Franklin F, Odontiadis J, Clement EM. *J. Chromatogr. B* 1996; **681**: 416.
23. Dunne M, Andrew P. *J. Pharm. Biomed. Anal.* 1996; **14**: 721.
24. Andrew PD, Birch HL, Phillpot DA. *J. Pharm. Sci.* 1993; **82**: 73.
25. Altria KD, Fibley SD. *Anal. Proc.* 1993; **30**: 363.
26. Cheng KN, Redrup MJ, Barrow A, Williams PN. *J. Pharm. Biomed. Anal.* 1998; **17**: 399.
27. Dulery BD, Petty MA, Schoun J, David M, Huebert ND. *J. Pharm. Biomed. Anal.* 1997; **15**: 1009.
28. McLoughlin DA, Olah TV, Ellis JD, Gilbert JD, Halpin RA. *J. Chromatogr. A* 1996; **726**: 115.
29. Oxford J and Lant MS. *J. Chromatogr. B* **88**: 137 (1989).

