Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 806 (2004) 299–303
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

High performance liquid chromatographic method for the determination of sumatriptan with fluorescence detection in human plasma

Z. Ge, E. Tessier, L. Neirinck, Z. Zhu*

Pharmascience, 6111 Royalmoount Avenue, Suite 100, Montreal, Que., Canada H4P 2T4

Received 1 October 2003; received in revised form 19 March 2004; accepted 29 March 2004

Available online 27 April 2004

Abstract

A rapid and sensitive high performance liquid chromatography (HPLC) method with fluorescence detection has been developed for the determination of sumatriptan in human plasma. The procedure involved a liquid–liquid extraction of sumatriptan and terazosin (internal standard) from human plasma with ethyl acetate. Chromatography was performed by isocratic reverse phase separation on a C18 column. Fluorescence detection was achieved with an excitation wavelength of 225 nm and an emission wavelength of 350 nm. The standard curve was linear over a working range of 1–100 ng/ml and gave an average correlation coefficient of 0.9997 during validation. The limit of quantitation (LOQ) of this method was 1 ng/ml. The absolute recovery was 92.6% for sumatriptan and 95.6% for the internal standard. The inter-day and intra-day precision and accuracy were between 0.8–3.3 and 1.1–6.3%, respectively. This method is simple, sensitive and suitable for pharmacokinetics or bioequivalence studies.
© 2004 Elsevier B.V. All rights reserved.

Keyword: Sumatriptan

1. Introduction

Sumatriptan succinate, 3-[2-(dimethylamino)ethyl]-*n*-methyl-1H-indole-5-methane-sulphonamide succinate (Fig. 1) is a 5-hydroxytryptamine (5-HT1B/1D) receptor agonist, efficacious in the treatment of migraine. The mechanism of action of the (5-HT1B/1D) receptor agonist has been thoroughly studied [1–4] and leads to two main theories. Sumatriptan acts as a vasoconstrictor of dilated intracranial blood vessels and, also as an inhibitor of the pro-inflammatory neuropeptide release which leads to headache relief.

Several analytical methods have been developed and published for the determination of sumatriptan in biological fluids. Among the methods described in the literature, there is high performance liquid chromatography (HPLC) with coulometric detection [5–8] and HPLC with mass spectrometric detection [9–11]. In these methods, the sumatriptan was extracted from plasma samples using liquid–liquid extraction or solid phase extraction. Among the published methods, all have used 1 ml plasma samples to achieve 1 ng/ml of limit of quantitation (LOQ) with the exception of the article by Biddlecombe et al. [12]. In this article, 0.1 ml of plasma was used to achieve 0.1 ng/ml LOQ. At the moment, there is no method available in the literature for the determination of sumatriptan in human plasma using a fluorescence detection technique.

In this paper, we present a method for the determination of sumatriptan in human plasma using a different detection technique. The method uses an HPLC with fluorescence detection and results in the same quantitation limit as previously published LC/MS/MS methods, 1 ng/ml. Fluorescence detection is simple, robust and available to most analytical laboratories. In addition, this method involves a simple liquid–liquid extraction with excellent reproducibility, which makes it suitable for pharmacokinetics or bioequivalence studies.

2. Experimental

2.1. Materials

Sumatriptan succinate (99.2%) and terazosin (98.4%) were obtained from Pharmascience (Montreal, Canada).

* Corresponding author. Tel.: +1-514-340-5040; fax: +1-514-340-9920.
E-mail address: zzhu@pharmascience.com (Z. Zhu).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.03.057

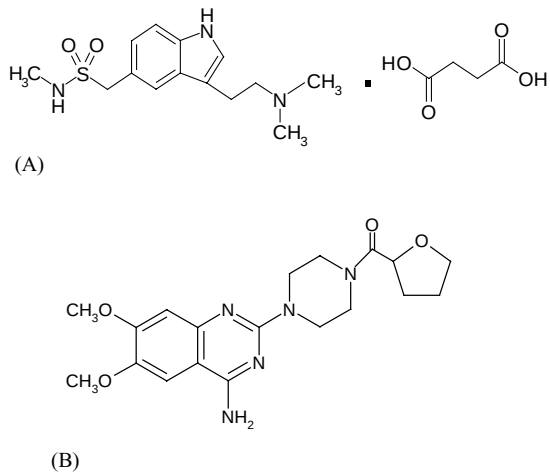

Fig. 1. Chemical structure of sumatriptan succinate (A) and terazosin (B).

The drug free human plasma was obtained from Biological Specialty (Colmar, PA, USA). HPLC grade water was supplied from an in house nano-pure water purification system. Acetonitrile, methanol, ethyl acetate, sodium phosphate monobasic, *o*-phosphoric acid 85% and 1 M sodium hydroxide were purchased from Fisher Scientific (Nepean, Ont., Canada).

2.2. Stock solutions and standards

Stock solutions of sumatriptan and terazosin (internal standard) were prepared by mixing an appropriate amount of sumatriptan or terazosin with methanol to a final concentration of 1.0 mg/ml. The final concentration calculation of sumatriptan is expressed on the amount of base form. The stock solutions were stored at $-20 \pm 5\,^{\circ}C$ and they are stable for at least 1 month at this temperature. The stability of stock solution was determined by comparing a freshly prepared stock solution with the solution that was stored at $-20 \pm 5\,^{\circ}C$ for 1 month.

A seven point non-zero calibration standard, ranging from 1 to 100 ng/ml was prepared by spiking the drug free human plasma containing EDTA with an appropriate amount of sumatriptan. The quality control (QC) samples at three concentration levels (3, 30 and 60 ng/ml) were prepared in a similar way as the calibration standard. Six different lots of drug free human plasma were tested before spiking to ensure that there was no endogenous interference at retention times of sumatriptan and terazosin (internal standard). The quality control samples were extracted with the calibration standards to verify the integrity of the method.

2.3. Extraction procedure for plasma samples

A 0.5 ml aliquot of human plasma sample was placed in a screw cap glass tube. A 0.1 ml of internal standard working solution (100 ng/ml terazosin) and 0.5 ml of 1 M sodium hydroxide solution were added and the mixture was vortexed for 3 s. The human plasma samples containing sumatriptan were then extracted with 7 ml of ethyl acetate. The mixture was shaken for 10 min and centrifuged for 10 min at room temperature. The extract was transferred to a culture tube, and then evaporated to dryness under a nitrogen stream. The extraction residue was reconstituted in 0.25 ml of the mobile phase, then, injected into the HPLC system.

2.4. Chromatography and quantitation

The chromatographic system consisted of an Agilent 1100 series HPLC system with a fluorescence detector (Agilent Technologies, Palo Alto, CA, USA). The separation was achieved by using a 150 mm × 4.6 mm, Zorbax C18 column (Agilent Technologies, Palo Alto, CA, USA) with the mobile phase consisting of acetonitrile 25 mM, pH 7.5 sodium phosphate monobasic, (60:40, v/v). The mobile phase was delivered at a flow rate of 1.0 ml/min. The fluorescence detector excitation wavelength was set at 225 nm and the emission wavelength was set at 350 nm. Both excitation and emission are at their maximum absorption wavelength. A peak height ratio method (sumatriptan/terazosin) was used for quantitation. When using fluorescence detection, the peak height ratio method is more consistent and reliable than the peak area method. The sumatriptan concentration in human plasma samples was determined by a standard curve that was analyzed with weighted least squares linear regression (weighting factor $1/x^2$).

3. Results

3.1. Limit of quantitation, linearity and precision

The LOQ of sumatriptan in human plasma was 1 ng/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 for the peak height ratio versus concentration over a working range of 1–100 ng/ml was observed for sumatriptan with an average correlation coefficient of 0.9997 ($n = 5$). The results of the sumatriptan calibration samples are presented in Table 1.

Table 1
Precision and accuracy data of sumatriptan calibration standards

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E.[a] (%)	C.V.[b] (%)	*n*
1	0.99	−1.0	0.6	5
2	2.0	1.6	1.4	5
10	10.1	1.1	0.7	5
25	25.2	0.6	0.3	5
50	50.3	0.6	0.9	5
80	78.7	−1.6	0.7	5
100	95.6	−1.4	0.9	5

[a] R.E.: Relative error.
[b] C.V.: Coefficient of variation.

Table 2
Precision and accuracy data of sumatriptan quality control samples

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E.[a] (%)	C.V.[b] (%)	*n*
Inter-assay				
3	3.2	6.2	1.0	18
30	30.8	2.6	1.4	18
60	60.7	1.1	3.3	18
Intra-assay				
1	1.0	1.3	2.8	6
3	3.2	6.3	0.8	6
30	30.6	1.9	0.9	6
60	61.8	3.0	3.2	6

[a] R.E.: Relative error.
[b] C.V.: Coefficient of variation.

The inter-assay precision and accuracy were determined by analyzing five calibration curves with quality control samples at three-concentration levels on five different days. The intra-assay precision and accuracy was determined by analyzing six replicates of LOQ samples and quality control samples at three-concentration levels extracted on the same day. Detailed results of the precision and accuracy for intra-assay and inter-assay precision are listed in Table 2.

3.2. Recovery

The absolute recovery of sumatriptan was assessed by comparing the peak height of extracted QC samples in six replicates (at low, mid and high range) to reference QC's prepared in solutions at the same concentration levels. The concentrations of the six replicates were 3, 30 and 60 ng/ml. The overall recovery of sumatriptan was 92.6%, while the recovery of the internal standard was 95.6%. Results are shown in Table 3.

3.3. Specificity

Screening of six different sources of drug free human plasma showed no endogenous interference at the retention times of sumatriptan and the internal standard. A chromatogram of an extracted blank human plasma sample as well as representative chromatograms of extracted calibration samples at the lower limit of quantitation and extracted high QC samples are provided in Fig. 2.

Table 3
Extraction yield of sumatriptan and terazosin (internal standard)

Analyte	Concentration (ng/ml)	Recovery[a] (%)	C.V.[a] (%)	*n*
Sumatriptan	3	95.5	1.7	6
Sumatriptan	30	93.6	1.7	6
Sumatriptan	60	88.6	4.7	6
Terazosin	100	95.6	5.8	18

[a] C.V.: Coefficient of variation.

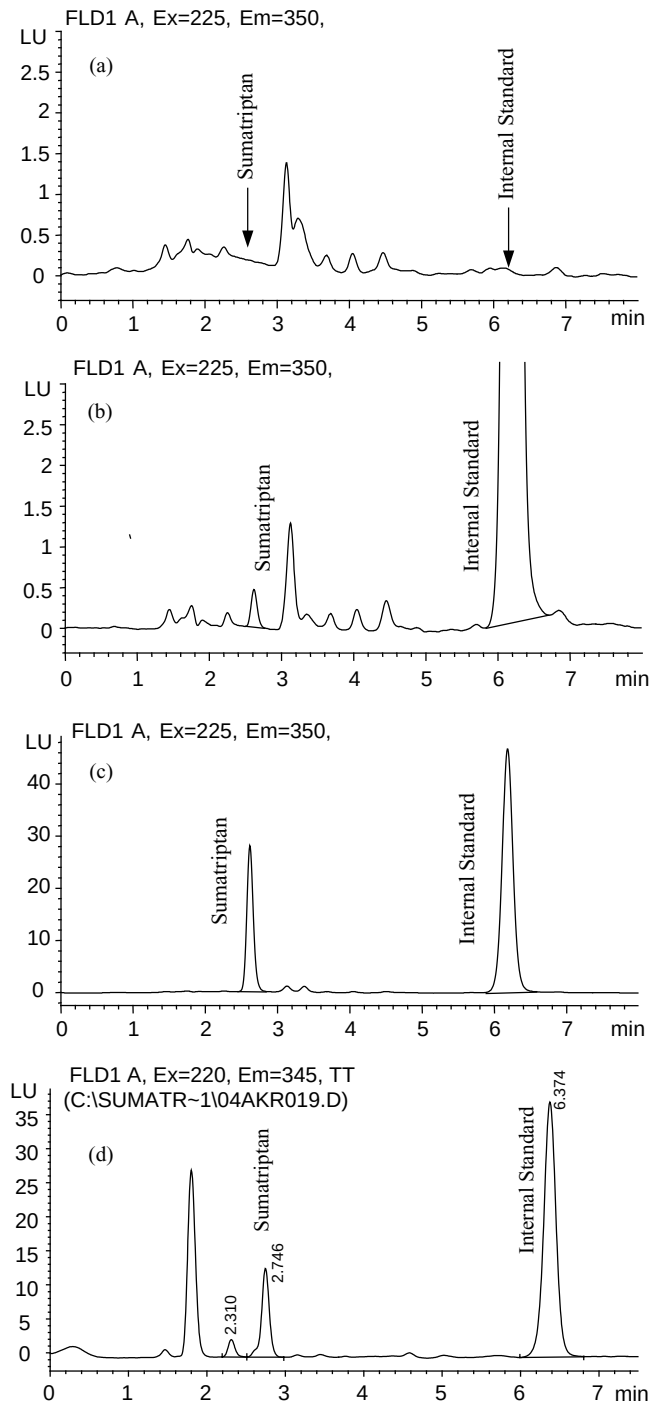

Fig. 2. Chromatograms of extracted plasma samples. (a) Plasma blank (no sumatriptan and no internal standard added), (b) plasma spiked with 1.00 ng/ml of sumatriptan (LOQ), (c) plasma spiked with 60 ng/ml of sumatriptan (QC high), (d) representative chromatogram of subject sample.

Commonly used over-the-counter drugs (OTCs) were also tested for possible interferences. The OTCs were spiked into the plasma to provide a concentration of 2 μg/ml, and were extracted using the same procedure as for sumatriptan. No interference was observed at the retention time of sumatriptan and the internal standard. The OTCs tested

were as follows: caffeine, acetylsalicylic acid, ibuprofen, acetaminophen and dextromethorphan.

3.4. Stability

The stability of sumatriptan under different conditions was evaluated. The acceptance criterias for all stability tests were at ±15% of the nominal value.

The stability of extracted sumatriptan and the internal standard in mobile phase (processed sample stability) was evaluated and the results showed that processed samples are stable at 4 ± 2 °C for at least 65 h. The processed sample stability was evaluated by comparing the extracted plasma samples that were injected immediately (time 0) with the samples that were re-injected 65 h after sitting in the autosampler at 4 ± 2 °C. Evaluation was based on back-calculated concentrations.

The human plasma samples containing sumatriptan were also evaluated for stability following freeze and thaw. The freeze-thaw stability evaluation was conducted by comparing the back-calculated concentrations of the stability samples which had been frozen and thawed three times, with the freshly plasma samples thawed only once. Sumatriptan was stable for at least three freeze-thaw cycles.

The stability of spiked human plasma samples after 118 days of storage at −20 ± 5 °C was evaluated as well. The stability evaluation involved an analysis of the low, mid and high quality control samples that were stored at −20 ± 5 °C for at least 118 days, together with a freshly spiked calibration standard, and quality control samples. The analysis was performed on the same day. The back calculated values for the stability were between −2.0 and 4.6%, which demonstrated the stability of sumatriptan in human plasma for at least 118 days at −20±5 °C.

3.5. Application

The method has been applied to the analysis of plasma samples from a study with human volunteers after oral administration of a 100 mg dose of sumatriptan tablets. Fig. 3 shows a characteristic concentration versus sampling time profile for sumatriptan in human plasma.

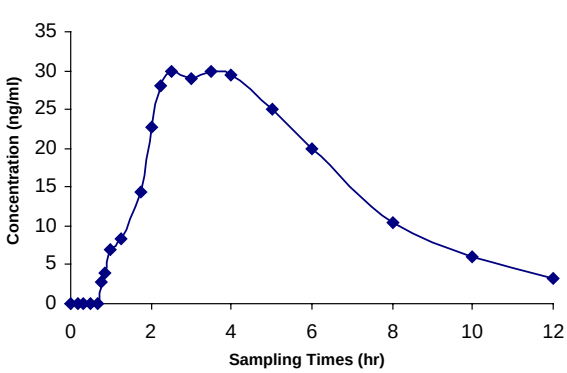

Fig. 3. Example of plasma concentration profile of sumatriptan (100 mg dose) in human subject.

4. Discussion

In this study, fluorescence detection was used instead of coulometric detection [5–8] or mass spectrometry detection [9,10]. This mode of detection is simple, stable, robust and gave a similar limit of quantitation (1 ng/ml when using 0.5 ml of plasma) to those previously published methods. The signal-to-noise ratio at 1 ng/ml was greater than 10/1. The specificity of the fluorescence detector was tested with six different plasma donors, and no endogenous compounds were found at the retention time of the drug and the internal standard.

The liquid–liquid extraction allowed an efficient cost effective extraction of sumatriptan and the internal standard. As demonstrated, the recovery was very good and variation in drug recovery is very small (C.V. = 3.3%).

The detector response was linear over the validated range. The results obtained from this method, in terms of precision and accuracy for the calibration samples and the quality control samples, demonstrate the reproducibility of the assay. Variation observed was less than 3.3% for the precision and less than 6.3% for the accuracy.

5. Conclusion

A high performance liquid chromatography method for the determination of sumatriptan in human plasma using fluorescence detection was developed. This method gives an alternative detection method for the analysis of sumatriptan. This method is inexpensive and easy to run with excellent reproducibility. It is robust and suitable for routine analysis that involves a large number of samples. This method has been successfully applied to a clinical study.

Acknowledgements

The authors would like to thanks M. Ghamlouche and L. Wang for providing the validation results.

References

[1] P.P.A. Humphrey, J. Neurol. 238 (1991) 538.
[2] W. Fenuik, P.P.A. Humphrey, M.J. Perren, A.D. Watts, J. Pharmacol. 86 (1985) 697.
[3] H.C. Dioner, H. Kaube, V. Limmouth, J. Neurol. 246 (1999) 515.
[4] S.J. Tepper, A.M. Rapoport, F.D. Sheftell, Arch. Neurol. 59 (7) (2002) 1084.

[5] M. Franklin, J. Odontiadis, E.M. Clement, J. Chromatogr. B Biomed. Appl. 681 (2) (1996) 416.
[6] M. Franklin, J. Odontiadis, J. Chromatogr. B Biomed. Appl. 679 (1–2) (1996) 199.
[7] M. Dunne, P. Andrew, J. Pharm. Biomed. Anal. 14 (6) (1996) 721.
[8] P.D. Andrew, H.L. Birch, D.A. Phillpot, J. Pharm. Sci. 82 (1) (1993) 73.
[9] K. Vishwanathan, M.G. Bartlett, J.T. Stewart, Rapid Commun. Mass Spectrom. 14 (3) (2000) 168.
[10] D.A. McLoughlin, T.V. Olah, J.D. Ellis, J.D. Gilbert, R.A. Halpin, J. Chromatogr. A. 726 (1–2) (1996) 115.
[11] D.W. Boulton, G.F. Duncan, N.N. Vachharajani, J. Chromatogr. B Biomed. Appl. 17 (1) (2003) 48.
[12] R.A. Biddlecombe, C. Benevides, S. Pleasance, Rapid Commun. Mass Spectrom. 15 (1) (2001) 33.