ELSEVIER

Journal of Chromatography B, 681 (1996) 416–420

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Determination of sumatriptan succinate in human plasma by high-performance liquid chromatography with coulometric detection and utilization of solid-phase extraction

M. Franklin*, J. Odontiadis, E.M. Clement

University of Oxford, Department of Psychiatry, Psychopharmacology Research Unit, Littlemore Hospital, Oxford OX4 4XN, UK

Received 6 November 1995; revised 12 January 1996; accepted 12 January 1996

Abstract

Sumatriptan succinate (the analyte) and naloxone (the internal standard) were extracted from plasma with a solid-phase extraction technique. Chromatography and detection were performed by isocratic reversed-phase high-performance liquid chromatography with coulometric end-point detection. The standard curve was linear over the range 0–100 ng/ml of sumatriptan succinate in plasma. The reproducibility (as defined by the coefficient of variation, C.V.) over the range of the standard curve was 4.9–7.3%. The recovery averaged 83%. The sensitivity was 0.25 ng of sumatriptan on column (allowing a concentration of 0.5 ng/ml to be determined from a 1-ml plasma sample volume). Plasma profiles of the analyte following subcutaneous (s.c.) administration in eight normal male volunteers, are presented.

Keywords: Sumatriptan succinate; Serotonin agonist

1. Introduction

The drug 1,3-[2-(dimethylamino)ethyl]-*n*-methyl-1*H*-indole-5-methanesulphonamide succinate (sumatriptan succinate) is a new and highly selective serotonin (5-HT_{1d}) agonist, which is used in the treatment of migraine [1].

The drug has been measured in both plasma and urine [2,3]. Both methods previously described utilized high-performance liquid chromatography (HPLC) procedures, the first [1] with mass spectrometry (MS) and the second with electrochemical end-point detection [2]. The former procedure has advantages over the latter, however it is both expensive to run and the equipment is costly. The latter technique uses a liquid–liquid extraction procedure, which is both slow and cumbersome. Both procedures quote limits of detection in the region of 1 ng/ml of plasma.

Our aim was to set up an assay procedure that was quick, simple, cheap to run, robust, gave a high degree of selectivity and could be used in an ordinary clinical laboratory. We describe here a method which we feel satisfies all the criteria. The method uses HPLC with coulometric end-point detection, solid-phase extraction (SPE) and an internal standard, the opiate antagonist, naloxone. The

*Corresponding author.

0378-4347/96/$15.00
PII S0378-4347(96)00040-0

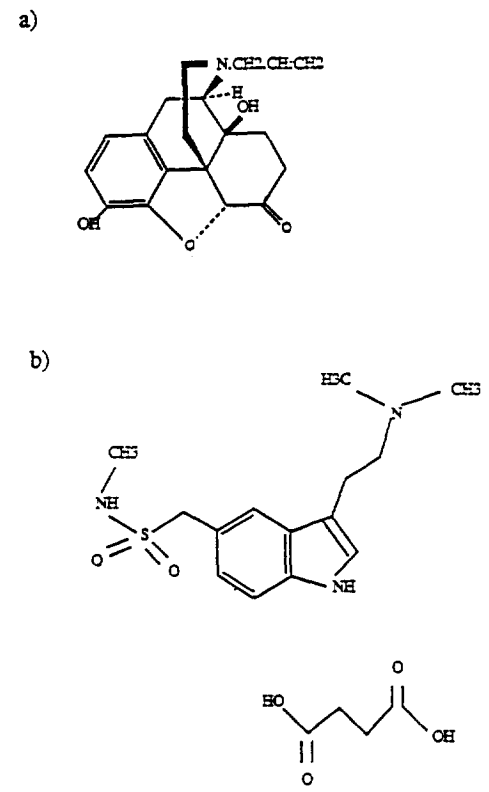

Fig. 1. Structures of (a) naloxone, the internal standard and (b) sumatriptan succinate.

structures for both the analyte and the internal standard are shown in Fig. 1.

2. Experimental

2.1. Materials

Sumatriptan succinate, the reference standard was donated by Glaxo (Ware, UK), whilst the internal standard, naloxone, was obtained from DuPont (Letchworth, UK). All other reagents used for the assay were of the highest grade available. Plasma for the preparation of standards for extraction was obtained from normal healthy volunteers. All water was deionised and glass-distilled prior to use.

Stock standard solutions of both the analyte and the internal standard were prepared at concentrations of 1.00 μg/ml in methanol. The assay standards were prepared freshly for each assay from these stock solutions.

2.2. Chromatographic apparatus and conditions

The HPLC system consisted of a Jasco PU-960 HPLC pump, a manual Rheodyne 7125 injection valve with a fitted 50-μl loop, a 25 cm×4.6 mm I.D., 5 μm mixed-mode (CN–C_{18}) analytical column (Capitol HPLC, Edinburgh, UK) and protected by a CN Brownlee New Guard column (Anachem, Luton, UK). A Coulochem II detector (ESA, Bedford, MA, USA) fitted with a Model 5020 guard cell and a Model 5011 analytical cell was used. A Model 4400 integrator (Varian, Harbor City, CA, USA) was used for quantification purposes.

The guard cell is used to pre-oxidise any impurities that reside in the mobile phase prior to oxidation at the analytical electrodes, detectors 1 and 2. Detector 1 is used to pre-oxidise unwanted materials (that have lower oxidation potentials than the analytes of interest) that might interfere at the analytical electrode, detector 2.

The potentials for the guard cell and detectors 1 and 2 were 0.9, 0.5 and 0.85 V, respectively. The response time was 10 s.

The mobile phase consisted of 0.04 *M* potassium phosphate buffer (pH 6.6)–methanol (55:45, v/v). The mobile phase was filtered through a 0.2-μm filter and degassed prior to use. The flow-rate was 1 ml/min. The chromatography was carried out at ambient room temperature.

Peak heights rather than peak areas in the chromatograms were normally measured. Plasma concentrations of sumatriptan were assessed by using the slope of the standard curve for peak-height ratios for the analyte and the internal standard.

3. Procedures

Blood samples were generally collected into tubes containing lithium heparin as anticoagulant, centrifuged and the plasma separated and stored at −20°C until required for assay.

Standards for assay were prepared freshly each time and consisted of five concentration points over

the range 5–50 ng/ml plus blanks, in duplicate, in drug-free plasma. To each 1 ml of standard or sample were added 30 ng of the internal standard, prior to column addition.

Carboxymethyl (CBA) sorbent columns (50 mg Isolute, Jones Chromatography, Hengoed, UK) were conditioned with full column volumes (1 ml) of methanol followed by distilled water. The vacuum was diverted to prevent the columns from drying out and the standards and samples were transferred to the columns. The vacuum was again applied allowing the complete passage of the materials through the column. Each column was washed with two column volumes of water. Each column was then taken to dryness under vacuum. The vacuum was again diverted, the manifold needles were wiped dry and a collection tray containing 75×10 mm glass tubes was inserted in the manifold system. Compounds were eluted with a single column volume of 1% NH_3–methanol. The eluates were evaporated to dryness, under vacuum at 40°C. Residues were reconstituted in mobile phase (200 μl), vortex-mixed and made ready for injection onto the HPLC system.

4. Results

Resolution and sensitivity were determined by injection of an extracted plasma (see Fig. 2). The retention times of sumatriptan and the internal standard were 8.8 and 7.8 min, respectively. The linearity of both the extraction procedure and the detector were verified over the standard range for sumatriptan (0–100 ng/ml). The linearity was determined by assaying pooled drug-free plasma spiked with known amounts of sumatriptan. A calibration curve was calculated for sumatriptan concentration and the peak-height ratio over the concentration range studied. The mean equation for the curve was $y=16.7x-0.04$ ($r=0.997$, $n=6$). Precision data for the assay procedure is given in Table 1. The absolute recoveries of sumatriptan for spiked pools containing 4.5 and 25.9 ng/ml of sumatriptan were 81.6±6.4% and 85.2±9.4% respectively ($n=6$ for each concentration).

Sample extracts were stable for at least one week when stored at 4°C and out of light. A number of common psychotropic drugs were tested for assay interference. However, none of those tested were found to interfere.

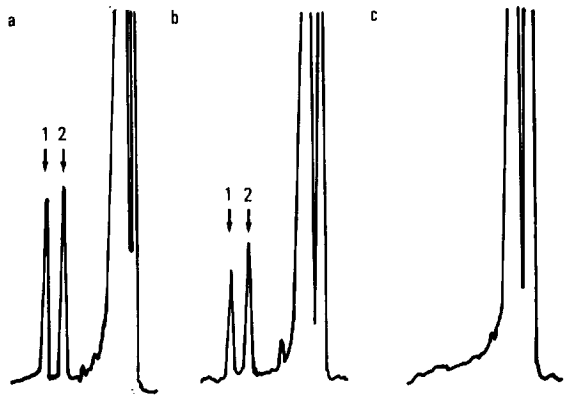

Fig. 2. Chromatograms of (a) sample from a normal volunteer following s.c. administration (6 mg), estimated to be 10.4 ng/ml, (b) drug-free plasma spiked with 8 ng/ml of sumatriptan, (c) plasma blank (i.e. drug-free plasma only). Peaks 1 and 2 represent sumatriptan and the internal standard, respectively. The retention time for peaks 1 and 2 were 8.8 and 7.8 min, respectively. NB. The amount of internal standard added to each sample extract was 30 ng.

Mean plasma profiles of sumatriptan following s.c. administration of sumatriptan in eight normal healthy male volunteers are shown in Fig. 3.

Table 1
Intra-assay accuracy and precision for the sumatriptan procedure in human plasma and inter-assay precision for the sumatriptan procedure in human plasma, as estimated on three separate occasions

Nominal value (ng/ml)	Observed value (ng/ml)	Coefficient of variation (%)
Intra-assay (n=5)		
5	4.5±0.2	4.9
10	9.9±0.4	3.8
25	25.9±1.0	4.0
Inter-assay (n=5)		
5		4.9
10		7.3
25		5.5

The precision (coefficient of variation (C.V.) was calculated from results for pooled drug-free plasma spiked with known amounts of sumatriptan.

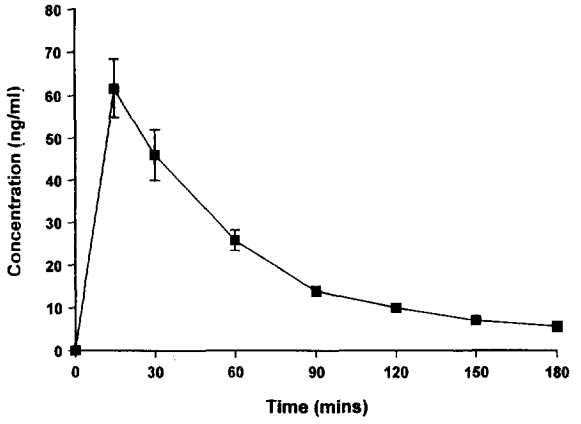

Fig. 3. Plasma profiles of sumatriptan in eight male volunteers (mean ± standard error) following s.c. administration of sumatriptan (6 mg).

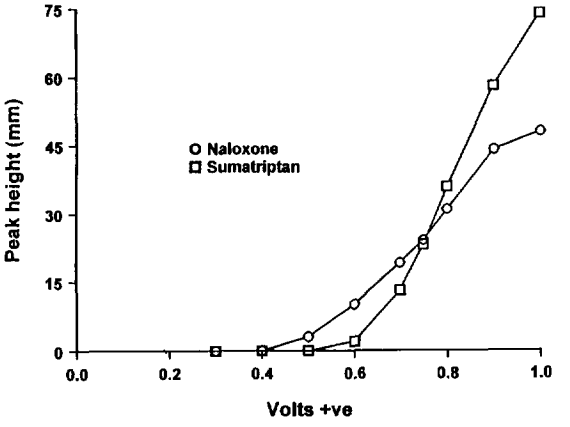

Fig. 4. Voltammogram of sumatriptan and the internal standard, naloxone, for detector 2 (the analytical electrode) at different potentials. These were determined when the guard cell and detector 1 were at zero potential. The full scale deflection was 0.3 nA.

5. Discussion

We describe a simple and highly selective procedure for the measurement of sumatriptan in plasma. The HPLC results were consistent and reliable. The detection limit (the peak height equal to three times the baseline) was 0.25 ng; this allowed for measurements of 0.5 ng/ml in a 1-ml plasma sample.

Similarly to that reported [2], we too used a sub-maximal operating potential at the analytical electrode (i.e., 0.85 V rather than above 0.9 V, see Fig. 4), as this gave adequate sensitivity and offered the advantage of greater selectivity at the reduced potential.

The detector response and the extraction were shown to demonstrate good linear relationships for both the analyte and the internal standard over the range. Although we found the analytical recovery of the analyte to be reasonably consistent, occasionally we did get spurious recoveries. We have therefore concluded that the use of an internal standard to monitor the recovery is an absolute necessity (unlike the conclusion of a previously reported method [2], which concluded that, in their view, it was unnecessary with a consistent recovery). This allows for the detection of any variation that may occur, which, if not monitored, would give rise to unreliable and spurious data.

The "mixed mode" analytical column was produced to give greater selectivity for the chromatography of these typically basic psychotropic drugs. The use of a "mixed mode" analytical column allows for the use of the greater retentive properties of the C_{18} phase, coupled together with the use of the more selective properties of the CN phase.

Adequate reliability and precision were demonstrated, whilst the sample extracts were shown to be stable for at least one week under the stated conditions. The use of an SPE procedure to extract the analyte and internal standard has advantages over the multi-stage liquid–liquid extraction process described previously [2], the most obvious of these being the time factor. When it is possible, and this is not the case in all instances, it is very much quicker to extract using the SPE technique than by the more traditional liquid–liquid extraction method. In general, SPE also gives higher percentage recoveries when the extraction process is fully optimised.

The usefulness of the method is demonstrated by the fact that we have successfully determined sumatriptan plasma profiles in normal male volunteers following s.c. administration of that drug.

6. Conclusions

We have described a simple, robust, highly selective and reproducible method for the determination

of sumatriptan in plasma. This is cheap to run and may easily be set up in any clinical laboratory. We have demonstrated clear advantages over previously described methods.

References

[1] K.L. Denchant and S.P. Clisshold, Drugs, 43 (1992) 776–798.
[2] P.D. Andrew, H.L. Birch and D.A. Phillpot, J. Pharm. Sci., 82 (1993) 73–76.
[3] J.M. Oxford and M.S.J. Lant, J. Chromatogr., 496 (1989) 137–146.