ELSEVIER

Journal of Chromatography B, 766 (2002) 339–343

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Simultaneous measurement of zolmitriptan and its major metabolites *N*-desmethylzolmitriptan and zolmitriptan N-oxide in human plasma by high-performance liquid chromatography with coulometric detection

E.M. Clement*, M. Franklin

University of Oxford, Department of Psychiatry, Psychopharmacology Research Unit, Warneford Hospital, Oxford OX3 7JX, UK

Received 2 April 2001; received in revised form 14 September 2001; accepted 3 October 2001

Abstract

Zolmitriptan, *N*-desmethylzolmitriptan, zolmitriptan N-oxide and an internal standard (an analogue of zolmitriptan) were extracted from plasma by a solid-phase extraction (SPE). Chromatography was performed using isocratic reversed-phase high-performance liquid chromatography (HPLC) with coulometric end-point detection. The standard curves were linear over the range 2–20 ng/ml for zolmitriptan and its metabolites in plasma. The mean inter- and intra-assay coefficients of variation over the range of the standard curves were less than 11%. The absolute recovery averaged 87, 58 and 77% for zolmitriptan, *N*-desmethylzolmitriptan and zolmitriptan N-oxide, respectively. The assay sensitivity was 0.5 ng for each analyte.

Keywords: Zolmitriptan; *N*-desmethylzolmitriptan; Zolmitriptan N-oxide

1. Introduction

Zolmitriptan, an antimigraine drug is a novel 5-$HT_{1B/1D}$ receptor agonist and has recently been used as an effective neuroendocrine probe of 5-HT_{1D} receptor function in humans [1,2]. In humans, zolmitriptan is well absorbed and undergoes extensive metabolism by the liver. Its major metabolites are *N*-desmethylzolmitriptan and zolmitriptan N-oxide [3]. Two methods for the measurement of zolmitriptan and its metabolites in plasma have been reported [3]. These utilised HPLC with fluorescence and mass spectrometry, respectively. We describe here a simple highly sensitive, selective and reliable method which uses HPLC with coulometric end-point detection, SPE and an internal standard. The structures for zolmitriptan and its metabolites are shown in Fig. 1.

2. Experimental

2.1. Materials

Zolmitriptan (311C90), *N*-desmethylzolmitriptan (183C91), zolmitriptan N-oxide (1652W92) and the internal standard (890W92) were kindly donated by Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK. All reagents used for the assay

*Corresponding author.

1570-0232/02/$ – see front matter
PII: S0378-4347(01)00470-4

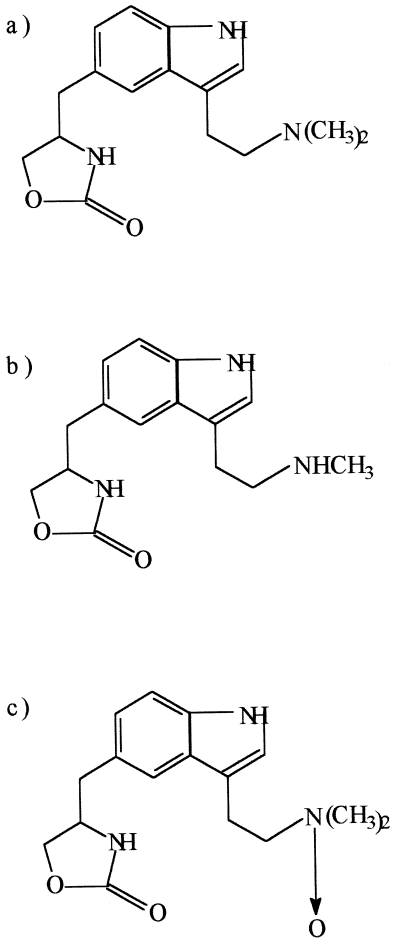

Fig. 1. Structures of (a) zolmitriptan, (b) *N*-desmethylzolmitriptan and (c) zolmitriptan N-oxide.

were of the highest grade available. Water was purified to HPLC standard prior to use. Drug-free plasma for the preparation of calibration standards was obtained from normal healthy volunteers.

Stock standard solutions of zolmitriptan, *N*-desmethylzolmitriptan, zolmitriptan N-oxide and internal standard were prepared at concentrations of 100 μg/ml in methanol and stored at 4°C. These were stable for at least 3 months, however as a precaution stock solutions were prepared monthly. The assay standards were prepared freshly for each assay from the stock solutions in distilled water. Experiments to determine the inter-assay coefficient of variation were performed over a month. The stock solution standards of zolmitriptan and its metabolites used for these experiments gave consistent values (see Table 1).

2.2. Chromatography

The HPLC system consisted of a Jasco PU-980 HPLC pump (Jasco Corporation, Tokyo, Japan), a GBC Model LC 1610 autosampler (GBC Scientific Equipment, Victoria, Australia), a 250×4.6 mm (I.D.) column (Phenomenex, Macclesfield, UK) packed with 5 μ spherisorb ODS/CN (a mixed mode combination of C_{18} (octadecyl) and cyanonitrile material) and a Model 5100A coulometric detector with a Model 5010 analytical cell and a Model 5020 guard cell (ESA, Bedford, MA, USA). A Varian Model 4400 integrator (Varian Associates, Harbour

Table 1
Inter- and intra-assay coefficients of variation (C.V.s) and precision data for the determination of zolmitriptan (Z) and its major metabolites, *N*-desmethylzolmitriptan (NDZ) and zolmitriptan N-oxide (NOZ) in plasma (n=5 replicates per assay). Results are presented as mean±S.E.M. Results for the inter-assay data are the means taken from three individual assay runs. The C.V.s were calculated from results obtained from drug-free plasma spiked with known amounts of the analytes

Actual value (ng/ml)	Observed value (ng/ml)			Coefficient of variation (%)		
	Z	NDZ	NOZ	Z	NDZ	NOZ
(a) Inter-assay						
5	4.5±0.53	3.3±0.37	5.2±0.79	11.6	11.2	15.1
10	8.7±1.03	7.5±1.11	9.3±0.89	11.9	14.9	9.6
20	18.4±0.98	17.4±1.10	17.3±1.22	5.35	6.3	7.1
(b) Intra-assay						
5	4.1±0.47	4.1±0.45	4.7±0.37	11.4	11.1	7.9
10	8.5±0.94	7.9±0.38	9.1±0.40	11.1	4.8	4.4
20	18.2±1.07	18.1±1.64	17.9±0.78	5.9	9.1	4.3

City, CA, USA) was used for quantification purposes.

The selected operating potentials for the detectors 1, 2 and guard cell were 0.35 V, 0.75 V and 0.85 V, respectively, as indicated by the voltammogram (see Fig. 2). The response time was 10 s. The mobile phase consisted of 0.05 *M* potassium phosphate buffer (pH 3.5) in acetonitrile (87:13, v/v). The mobile phase was filtered through a 0.2 μ filter and degassed prior to use. The flow-rate was 1 ml/min with an operational back pressure of 110 kg/cm^2. The chromatography was carried out at room temperature. Peak heights rather than peak areas, were calculated by integrator. Unknown plasma concentrations of zolmitriptan and its metabolites were quantified using linear regression response of the standard curves (drug/internal standard peak height ratio) [4].

2.3. Procedure

Blood samples were collected into tubes containing lithium heparin as anticoagulant and spun at 1500 *g* in a refrigerated centrifuge at 4°C for 15 min. The plasma was separated and stored at −20°C until required for assay.

Zolmitriptan, *N*-desmethylzolmitriptan and zolmitriptan N-oxide standards for each assay were prepared freshly in drug-free 0.5 ml plasma and consisted of five concentration points over the range 2–20 ng/ml. To each 0.5 ml of standard and unknown samples were added 20 ng in 20 μl of the internal standard. Blank plasma samples were also run in parallel. The analytes were extracted using SPE with an internal standard to monitor extraction recovery and detection fluctuation.

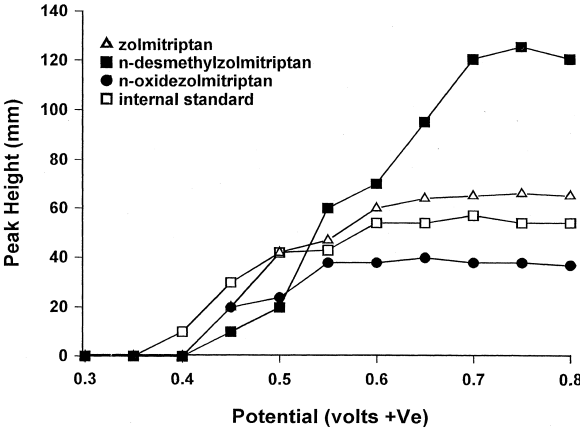

Fig. 2. Voltammogram of zolmitriptan, *N*-desmethylzolmitriptan, zolmitriptan N-oxide and internal standard for detector 2 (the analytical electrode) at different potentials. The voltammogram was determined when the guard cell and detector 1 were at zero potential.

Isolute mixed-mode SPE columns (50 mg HCX type, Jones Chromatography Ltd, Hengoed, UK) were conditioned sequentially with full column volumes (1 ml) of methanol and 0.025 *M* phosphate buffer (pH 6.8). The vacuum on the vacuum manifold system (VacMaster, IST, Mid Glamorgan, UK) was diverted to prevent the columns from drying out and the standards/plasma samples were loaded. The vacuum was again applied to allow the complete passage of the materials through the column. Each column was washed with two column volumes of 0.025 *M* phosphate buffer (pH 6.8) and 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 into the vacuum manifold. Compounds were eluted with a single column volume of 1% ammonia in methanol. Eluates were evaporated to dryness under vacuum at 40°C. The residue was re-constituted in methanol (100 μl), vortex-mixed and made ready for injection onto the HPLC system. A 25-μl sample of the reconstituted extract was injected into the HPLC system in the auto-sampler. Reconstituted extracts were stable for up to 7 days when stored at 4°C and out of light. We ran a maximum of 30 samples per run with the auto-sampler at room temperature. Under these conditions and even after a full re-run of the whole batch we have not noticed any significant change in sample values.

3. Results

Assay resolution and sensitivity were determined by injection of extracted drug-free plasma spiked with known amounts of zolmitriptan and its metabolites (see Fig. 3). The retention times of zolmitriptan (Z), *N*-desmethylzolmitriptan (NDZ), zolmitriptan N-oxide (NOZ) and the internal standard were 7.7,

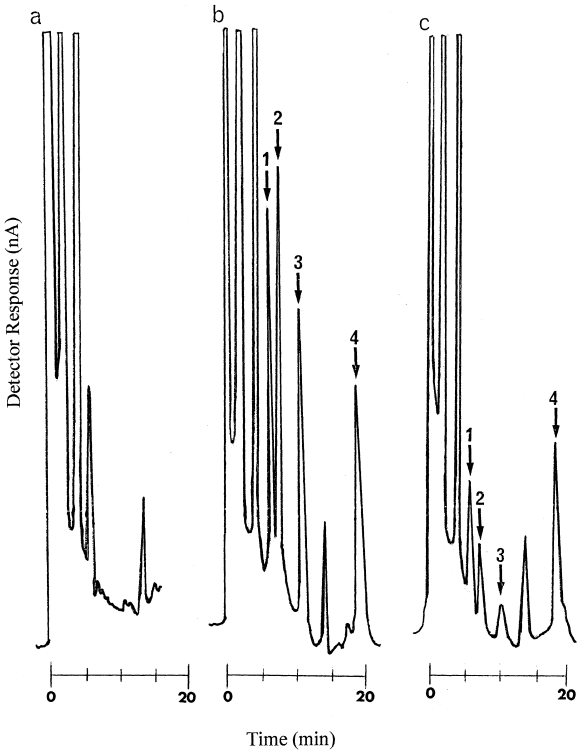

Fig. 3. Chromatograms of (a) blank drug-free plasma, (b) drug-free plasma spiked with 10 ng/ml of each of the analytes, (c) sample from a normal volunteer following oral administration of zolmitriptan (the dose was 5 mg) the peak for zolmitriptan was estimated to be 3 ng/ml. Peaks 1, 2, 3 and 4 represent *N*-desmethylzolmitriptan, zolmitriptan, zolmitriptan N-oxide and internal standard, respectively. The retention time for peaks 1, 2, 3 and 4 were 7.7, 6.7, 10.5 and 17.2 min, respectively.

6.7, 10.5 and 17.2 min, respectively. The linearity of the extraction procedure and the detector response were verified over the standard range for both zolmitriptan and its metabolites (2–20 ng/ml). This was determined by measuring pooled drug-free plasma spiked with known amounts of the analytes. Calibration curves were calculated for each analyte utilizing the peak-height ratio versus analyte concentration. The mean slopes±S.E.M. were 0.041±0.0006 for Z, 0.046±0.0003 for NDZ and 0.0226±0.002 for NOZ, respectively (n=4). The mean assay coefficients of variation (C.V.s) for Z, NDZ and NOZ over the range of standard assay curve were 9.6, 10.8, and 10.5 (inter) and 9.5, 8.3, and 5.5 (intra), respectively. The absolute recoveries for these compounds were 87, 58 and 77%, respectively. The detection limit for all analytes was ~0.5 ng on column (with a signal-to-noise ratio of 3:1). Sample extracts were stable for at least 1 week when stored at 4°C and out of light.

4. Discussion

We describe here a simple, sensitive and highly selective HPLC assay procedure for the measurement of zolmitriptan and two of its major metabolites in plasma.

Following a screening process, HCX SPE columns were found to be the more selective and most efficient in terms of recovery for the analytes of interest. The use of 1% ammonia in methanol as the eluting reagent allowed for increased selectivity of the extraction and reduced unwanted interfering substances to a minimum. The detection limit for all analytes was ~0.5 ng which is similar to that reported previously using HPLC with mass spectrometry [3]. Precision profiles were all within acceptable limits.

It has been established that the ratio between the analytical recovery of the analytes and that of the internal standard submitted to the same operations was constant over a wide range of concentrations. Furthermore, the detector response was linear for both analytes and the internal standard. The requirement for an internal standard assay procedure was therefore satisfied. Although analytical recoveries of the analytes were generally consistent, occasionally we did get reduced recoveries from some plasma samples, which was possibly due to matrix effects. We therefore concluded that the use of an internal standard to monitor the recovery and the day-to-day variation of the detector response was an essential requirement.

Since *N*-desmethylzolmitriptan required a higher electrode potential for full and optimal oxidation (see Fig. 2), it was necessary to run the analytical electrode at a higher setting than would have been used for the other analytes. This may lead to a reduction in the working life of the analytical electrode.

High recoveries for zolmitriptan and its metabolites, lower sample volumes, improved analyte res-

olution and a reduced sample preparation time have all been attained by utilisation of SPE technology.

It can be argued that the metabolites of zolmitriptan, *N*-desmethylzolmitriptan and zolmitriptan N-oxide might get converted to the parent compound. However, by comparing the actual values of the analytes with that of the observed values, there is no evidence for such a case (see Table 1).

5. Conclusion

A simple, robust, highly selective and reproducible method for the measurement of zolmitriptan, *N*-desmethylzolmitriptan, zolmitriptan N-oxide has been described. The method has obvious advantages over those previously reported. It is reasonably cheap to run and can easily be set up in a clinical/analytical laboratory.

References

[1] K.W. Johnson, J.M. Schaus, M.M. Durkin, Neuroreport 8 (1997) 2237.
[2] R. Whale, Z.A. Bhagwagar, P.J. Cowen, Psychopharmacology 145 (1999) 223.
[3] R. Dixon, A. Warrander, Cephalalgia 17 (Suppl. 18) (1997) 15.
[4] J. Neter, W. Wasserman, M.H. Kutner, in: Applied Linear Regression Models, R.D. Irwin, Homewood, IL, 1983, p. 167.