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Validation of a liquid chromatographic tandem mass spectrometric method for the determination of sumatriptan in human biological fluids

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Abstract

A liquid chromatographic tandem mass spectrometric method for the quantitative determination of sumatriptan base in human plasma and urine has been developed and validated over the concentration range 0.2–20 ng base ml^{-1}. Sumatriptan is a 5-HT_1 receptor agonist which has found widespread use in the treatment of migraine. Sumatriptan and its internal standard (D_3-sumatriptan) were extracted from human matrices using C_2 solid phase cartridges. The extracts were chromatographed on a C_{18} column, ionised using a heated nebuliser assisted atmospheric pressure ionisation (API) interface and detected by MS/MS in the multiple reaction monitoring mode. The completed validation demonstrated the method to be robust, accurate, precise and specific for the direct quantification of sumatriptan in human fluids. The method was used on a routine basis to determine the levels of sumatriptan in human volunteers following the oral administration of a 25 mg dose of sumatriptan succinate. © 1998 Elsevier Science B.V.

Keywords: Sumatriptan; LC-MS/MS; Validation; Human; Plasma; Urine; Bioanalysis

1. Introduction

Sumatriptan succinate (Imigran, Imitrex; GW compound GR43175C) is a 5HT_1 receptor agonist which is widely used in the treatment of migraine [1,2]. The clinical routes of administration are oral, subcutaneous and intranasal. The compound has a low oral bioavailability and is metabolised to an indole acetic acid, GR49336 (Fig. 1) as well as other minor metabolites [3,4]. Methods previously used for the determination of sumatriptan in human biological fluids centered largely on the use of HPLC with electrochemical detection [5,6]. The methods were linear over the range from 1 to 30 ng base ml^{-1}. An initial attempt to develop a thermospray LC–MS method with automatic

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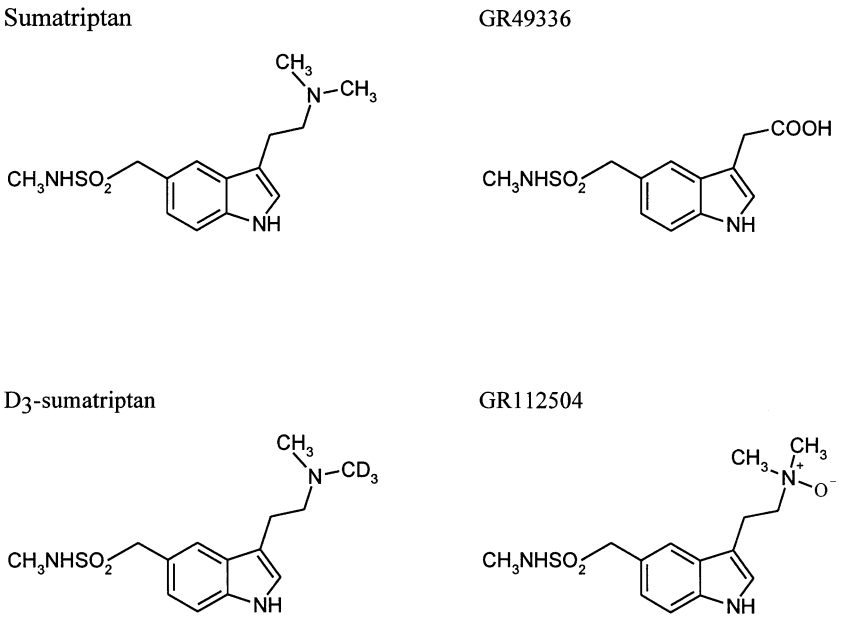

Fig. 1. The structure of sumatriptan, its deuterated analogue (analytical internal standard), and the circulating metabolites GR49336 and GR112504.

sample processing was able to detect down to 500 pg ml^{-1} [7]. However, the assay only had acceptable precision and accuracy over a calibration range of 2–50 ng ml^{-1} and so was considered to be limited in terms of sensitivity, cost and general applicability relative to the HPLC methodologies. However, the improvements in mass spectrometric methodology in recent years, such as atmospheric pressure chemical ionisation techniques, has meant that assay methods with enhanced sensitivity, improved robustness and high sample throughput, have become possible. These LC–MS/MS techniques have now been applied to the assay of several $5HT_1$ receptor agonists including sumatriptan[8,9]

This paper describes the development and validation of a LC–MS/MS method for the determination of sumatriptan in human plasma and urine, incorporating a deuterated internal standard; and the application of the method to the analysis of samples from a clinical study in healthy human volunteers. A preliminary report on the development of this method has been published earlier[10].

2. Experimental

2.1. Materials and reagents

Reference standards of sumatriptan succinate, of the deuterated internal standard of sumatriptan, of the known metabolite GR49336, and of the putative metabolite GR112504 (sumatriptan *N*-oxide) were obtained from Glaxo Group Research. HPLC grade acetonitrile and methanol were purchased from Fisons (Loughborough, UK), ammonium acetate and triethylamine from Aldrich (Gillingham, UK) and Tris base from Sigma (Poole, UK). Control human plasma was obtained from the Charterhouse Clinical Research Unit (London, UK) and control human urine from healthy volunteers at Huntingdon Life Sciences.

2.2. Instrumentation

The LC–MS/MS system consisted of a Sciex API III^{plus} mass spectrometer (PE Sciex, Toronto, Canada) equipped with an atmospheric pressure

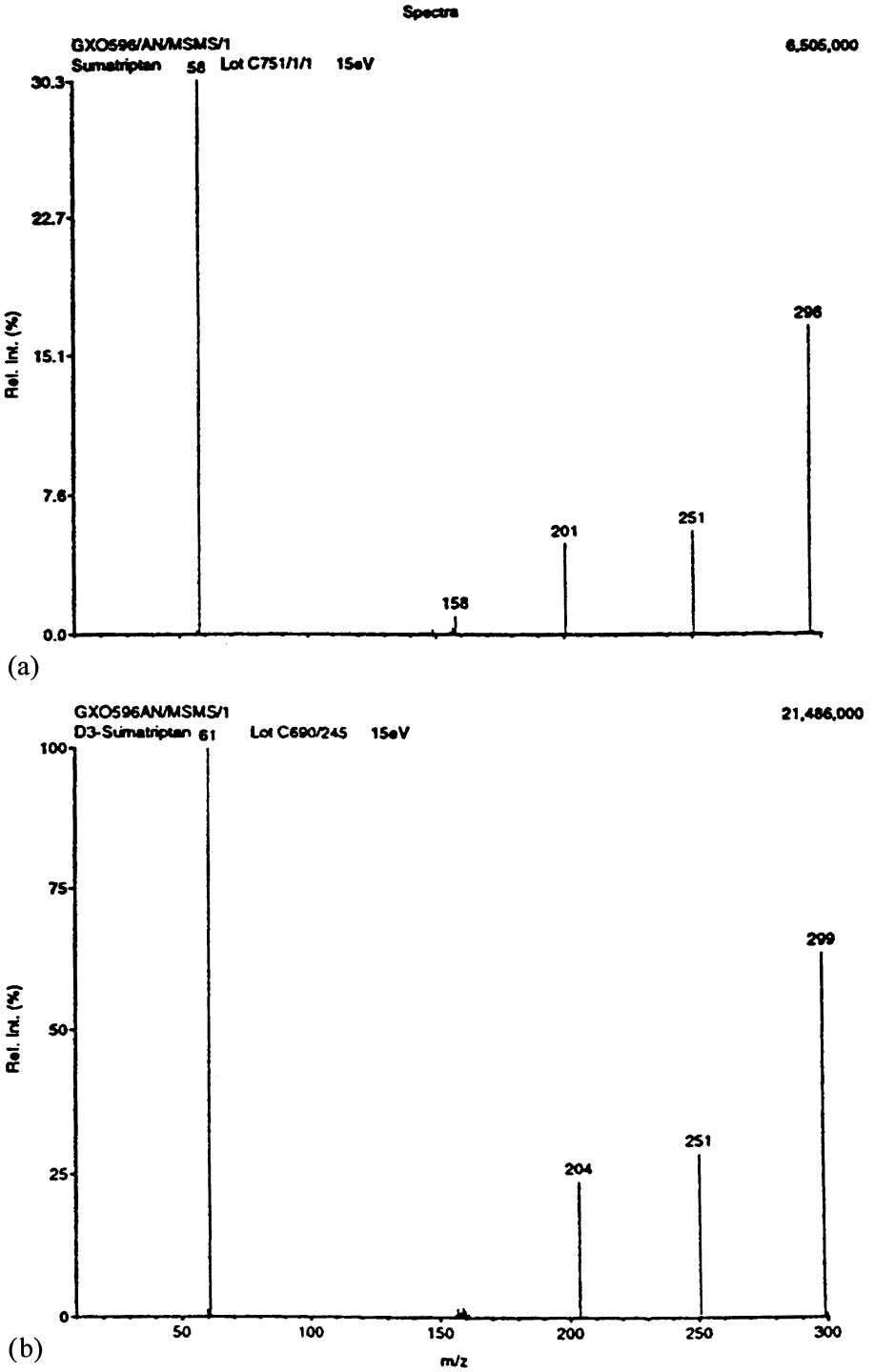

Fig. 2. The product ion mass spectra of (a) sumatriptan and (b) its deuterated analogue.

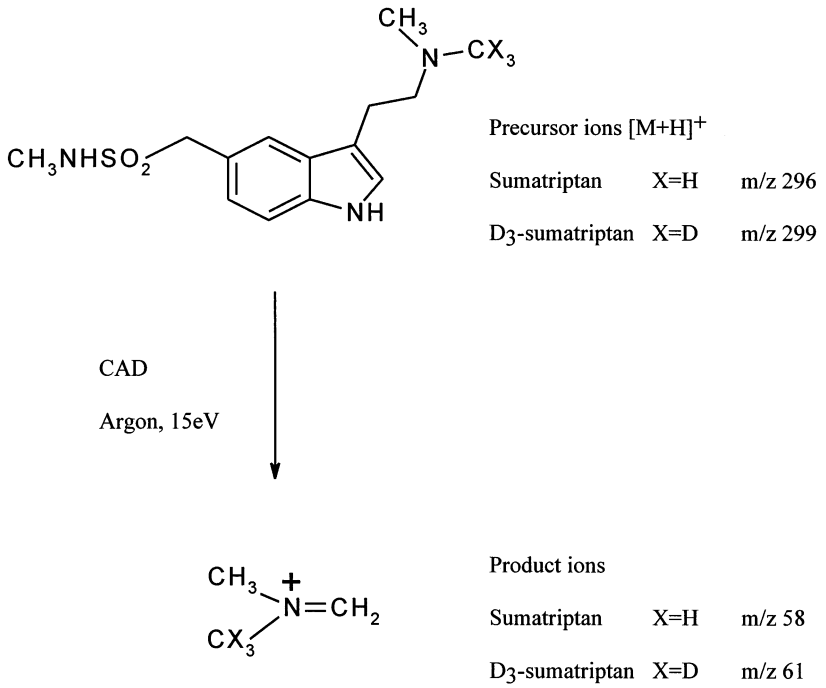

Fig. 3. Fragmentation pathway for sumatriptan and D_3-sumatriptan.

chemical ionisation (APCI) interface and linked to a Shimadzu LC-10AD liquid chromatograph and SIL-10A autoinjector (Dyson Instruments, Houghton-le-Spring, UK). The HPLC column was a C_{18} Prodigy, 5 μm, 30 × 4.6 mm i.d. column (Phenomenex, Macclesfield, UK).

2.3. Instrument conditions

The HPLC pump was operated in isocratic mode at a flow rate of 1 ml min^{-1} at ambient temperature. The mobile phase consisted of 85% ammonium acetate solution (0.05 M, pH7):15% methanol. The autosampler was set to inject 50 μl of extract with a chromatographic run time of ~2.5 min. The mass spectrometer was set to use APCI with a heated nebuliser interface at about 500°C. The nebuliser pressure was set at 80 psi. The corona discharge current was typically 4 μA and the orifice voltage was set at 40 V. Argon was used as the collision gas at a gas thickness of 3×10^{15} atoms cm^{-2} and the collision energy was 15 eV. The ions monitored were m/z 296 → 58 for sumatriptan, and m/z 299 → 61 for D_3-sumatriptan.

2.4. Preparation of standard solutions

Primary stock solutions of sumatriptan (100 μg base ml^{-1}) and D_3-sumatriptan at 20 ng base ml^{-1} were prepared by dissolving the compound(s) in HPLC grade water. Working standard solutions of sumatriptan at 2.5, 100 and 10 ng base ml^{-1} and of D_3-sumatriptan at 20 ng base ml^{-1} were prepared from the primary stock solutions. Solutions of GR112504 and GR49336 in water at ~100 μg ml^{-1} were prepared and diluted to provide working solutions at ~100 ng ml^{-1}.

2.5. Preparation of calibration standards, validation samples and quality control samples

Samples of control human plasma (or urine) were fortified with sumatriptan working standard solutions to give bulk calibration standards over

Table 1
Reproducibility of plasma validation samples (intra-assay precision and accuracy)

Concentration added (ng base ml^{-1})	Mean measured concentration (ng base ml^{-1})	*n*	SD	CV (%)	RE (%)
0.2	0.19	6	0.01	3	−5
0.5	0.50	6	0.02	3	−1
1.0	0.98	6	0.02	3	−2
2.0	1.98	6	0.07	3	−1
5.0	5.18	6	0.12	2	4
10.0	10.16	6	0.26	3	2
20.0	20.22	6	0.90	4	1

SD, standard deviation; CV, coefficient of variation; RE, relative error of mean.
All values calculated using non-rounded data.

Table 2
Reproducibility of plasma validation samples (inter-assay precision and accuracy)

Concentration added (ng base ml^{-1})	Mean measured concentration (ng base ml^{-1})	*n*	SD	CV (%)	RE (%)
0.2	0.20	5	0.01	6	−2
0.5	0.51	5	0.04	7	2
1.0	1.01	6	0.06	6	1
2.0	2.03	6	0.08	4	1
5.0	5.08	5	0.24	5	2
10.0	10.28	6	0.38	4	3
20.0	20.23	6	0.76	4	1

See footnotes to Table 1.

the range 0.2–20 ng (0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 ng) sumatriptan base ml^{-1}. Validation samples were prepared to cover the same concentration range and QC samples were prepared at three concentrations of 0.4, 6 and 15 ng base ml^{-1}. Separate QC samples also fortified with GR112504 or GR49336 were prepared at a concentration of $\sim$10 ng ml^{-1} for the assessment of assay specificity. Samples fortified with sumatriptan at 100 ng base ml^{-1} were also prepared for dilution with control human fluid.

2.6. Extraction procedure

All samples were thawed and vortex mixed. A portion of the internal standard working solution (20 ng base ml^{-1}, 100 μl) was added to 1 ml of plasma/urine, except the blank, and mixed thoroughly. Tris buffer (pH 7.2, 25 mM, 0.5 ml) was added to each tube which was mixed again. The urine samples, but not the plasma samples, were centrifuged at 3500 rpm for 10 min. The solid phase cartridges were conditioned by the addition of methanol (1 ml) followed by Tris buffer solution (1 ml). The buffered samples were loaded onto the activated solid phase cartridges and washed with a solution of methanol:Tris buffer [1:9 v/v] under vacuum to dryness. The experimental samples were eluted with 2 × 1 ml of acetonitrile containing 1% triethylamine. The eluates were dried at about 35°C under nitrogen and reconstituted in ammonium acetate solution (0.05 M, 150 μl). These solutions were centrifuged at 13000 rpm (Micro Centaur) for $\sim$10 min and a portion (50 μl) of the sample was analysed by LC–MS/MS.

2.7. Assay validation

The method was validated over the concentration range 0.2–20 ng base ml^{-1} in human plasma/urine. Calibration lines for each batch analysed were defined by seven calibration standards plus blank matrix (zero standard and blank were not used in the calculation). Calibration

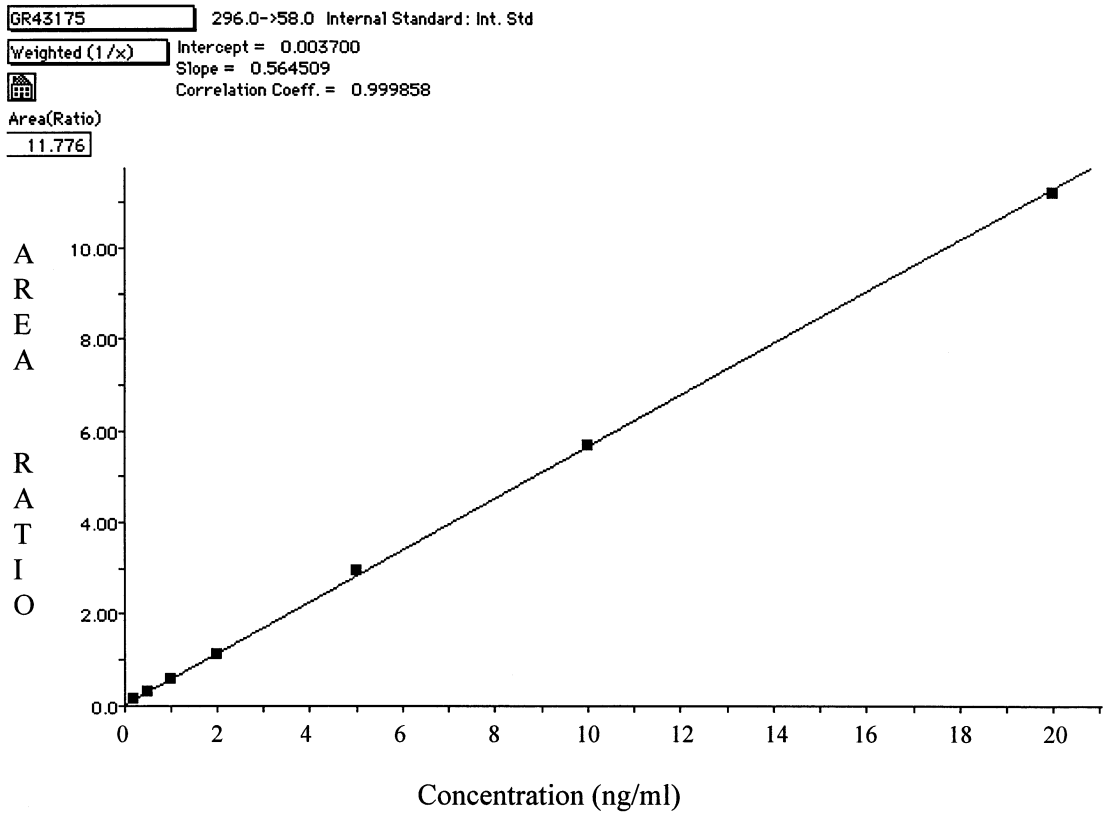

Fig. 4. A typical calibration plot for plasma analyses illustrating the linearity of the assay over the calibration range.

measurements were subjected to least squares regression analysis using $1/x$ weighting by a computer program (Macquan® version 1.3, PE Sciex) to provide data on the slope, y-intercept, correlation coefficient and the back calculated calibration standard concentrations.

The precision of the assay was evaluated by determining the coefficients of variation occurring within (intra-) and between (inter-) day analyses. Plasma validation samples prepared at the concentrations of each point on the calibration line were analysed in replicate ($n = 6$) on day-1 and singly on 6 separate days, along with duplicate quality control samples and a set of calibration standards. For the urine assay the intra-day precision was determined by the analysis of replicate ($n = 6$) quality control samples on day-1. The inter-day precision was evaluated by the coefficient of variation of quality control samples on 6 days ($n = 6$).

The accuracy of the assay was determined by measuring the concentrations of sumatriptan in the validation samples and comparing them to their nominal concentrations, and expressing the results as relative error (RE%) of measurement. The lower limit of quantification (LLOQ) of the assay was taken as the lowest concentration of sumatriptan on the calibration range with acceptable precision (20%) and accuracy ($100 \pm 20\%$). The absolute recovery of sumatriptan through the extraction procedure was determined for each concentration on the calibration line, in replicate ($n = 6$).

The specificity of the assay was assessed qualitatively by comparison of chromatograms of blank human plasma/urine and human plasma/urine fortified with sumatriptan and deuterated sumatriptan. Blank human plasma/urine from ten volunteers was also assessed for endogenous inter-

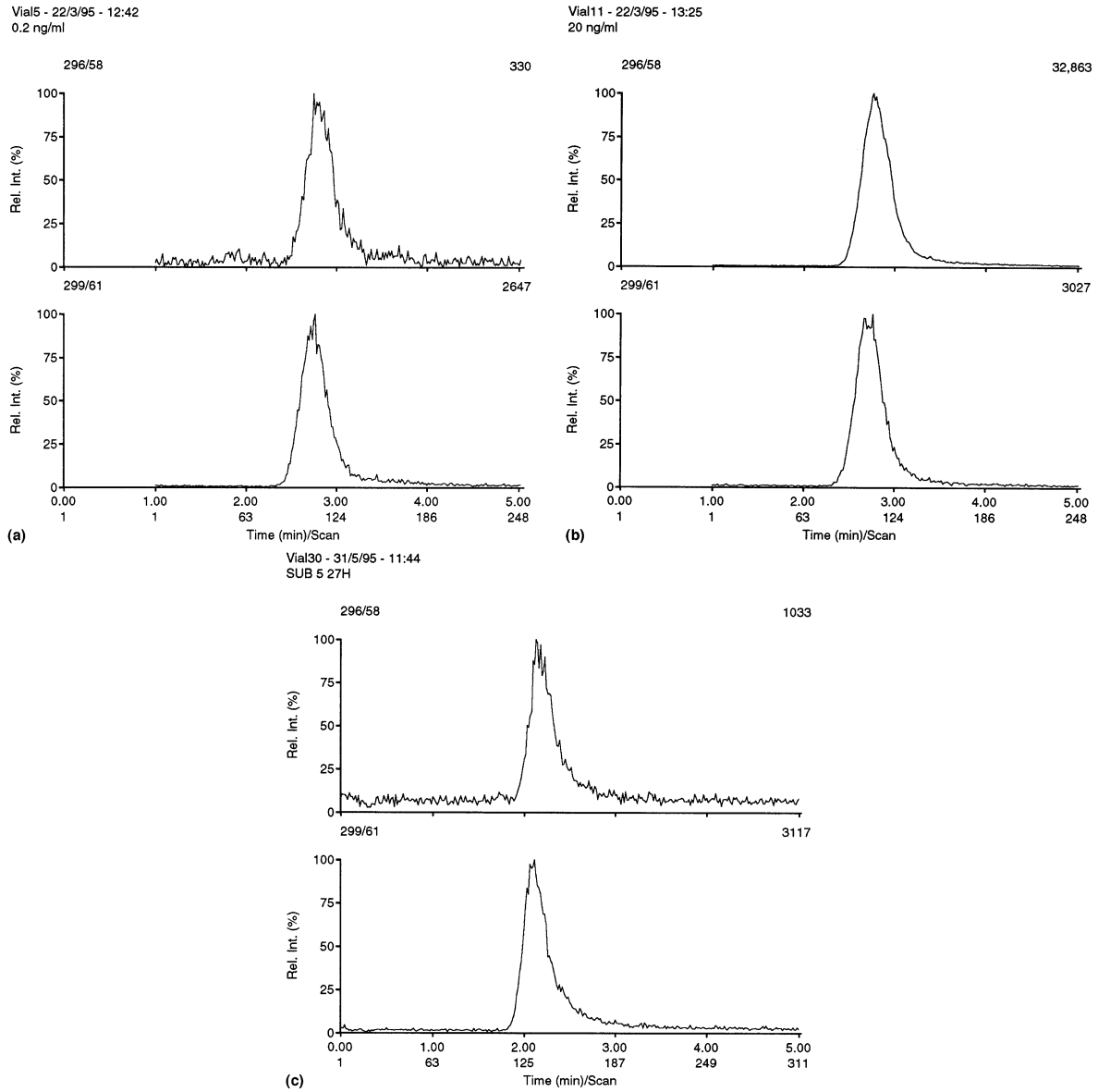

Fig. 5. Representative chromatograms showing (a) a calibration standard at 0.2 ng ml^{-1} (LLOQ); (b) a calibration standard at 20 ng ml^{-1}; and (c) a 27-h plasma sample from a subject who received an oral dose of sumatriptan. The concentration of sumatriptan was determined as 0.5 ng ml^{-1}. The top and bottom ion chromatograms correspond to sumatriptan and D_3-sumatriptan, respectively.

fering peaks. Samples of blank plasma containing the known metabolite GR49336 or the putative metabolite GR112504 were also checked for interference. In addition the specificity of the assay was assessed quantitatively by analysing plasma QC samples containing sumatriptan at 10 ng base ml^{-1}, which had been further fortified by the addition of either GR49336 or GR112504 at about 10 ng ml^{-1}, in replicate ($n = 3$).

Table 3
The reproducibility of plasma quality control samples

Concentration added (ng base ml^{-1})	Mean measured concentration (ng base ml^{-1})	n	SD	CV (%)	RE (%)
0.40	0.44	15	0.03	6	11
6.00	6.18	16	0.20	3	3
15.00	15.32	16	0.51	3	2

See footnotes to Table 1.

The effect of dilution was assessed by preparing a control sample fortified with sumatriptan at 100 ng base ml^{-1}, and diluting this 10-fold with blank matrix. The new concentration of sumatriptan was determined in replicate samples ($n = 6$) and compared to the nominal concentration. The LC–MS/MS system was routinely checked for injection carry-over by injecting re-constitution solvent blanks after the injection of a high concentration standard in the relevant matrix.

3. Results and discussion

A LC–MS/MS method for the determination of sumatriptan in human plasma was developed and validated over the calibration range of 0.2–20 ng base ml^{-1} for a 1 ml sample and then cross-validated using human urine. Using a heated nebuliser APCI interface, sumatriptan and D_3-sumatriptan gave intense protonated molecular ions at m/z 296 and m/z 299, respectively. These ions (precursor ions) were fragmented by collision activated dissociation with argon at 15 eV in the Q2 region of the mass spectrometer to generate product ions (Fig. 2). The product ions of sumatriptan and of D_3-sumatriptan (at m/z 58 and 61 respectively, Fig. 3) were monitored by multiple reaction monitoring (MRM) from the corresponding precursor ions.

3.1. Recovery

The mean recovery (extraction efficiency) of sumatriptan at seven different concentrations ranged between $101 \pm 3\%$ at 0.2 ng base ml^{-1} and $90 \pm 3\%$ at 20 ng base ml^{-1} from control human plasma. Least squares regression analysis of a plot of recovery against concentration gave a slope of -0.31% per ng ml^{-1} and an intercept value of 97%. No statistical significance was detected. Comparable values were obtained for the recoveries from urine.

3.2. Performance characteristics

The relationship between the peak area ratio of sumatriptan to its deuterated internal standard and to the concentration of sumatriptan in matrix was linear over the calibration range (weighted $1/x$). The intra-day precision of the assay as indicated by the coefficients of variation of the measured concentrations of replicate validation samples were $< 5\%$ over the range 0.2–20 ng base ml^{-1}, and the accuracy of measurement was $\pm$ 5% or lower (Table 1). The inter-day precision was 6% at 0.2 ng base ml^{-1} and 4% at 20 ng base ml^{-1}; and the mean relative error was calculated as -2 and 1%, respectively (Table 2). The regression coefficient was typically 0.999 for both plasma and urine. A calibration plot demonstrating the linearity over the calibration range is shown in Fig. 4.

The lower limit of quantification (LLOQ) was 0.2 ng base ml^{-1} using a 1 ml sample of plasma or urine. Typical chromatograms of calibration standards at 0.2, 20 ng ml^{-1} and of a clinical sample are shown in Fig. 5. At this concentration the inter-day precision over five occasions using plasma as the matrix was 6% and the mean relative error was -2%. The reproducibility of the quality control samples in plasma (Table 3) and urine was acceptable, with the CV and RE of a standard at 0.4 ng base ml^{-1} being calculated as 6 and 11% in plasma ($n = 15$ occasions) and 10 and 0% in urine ($n = 12$ occasions).

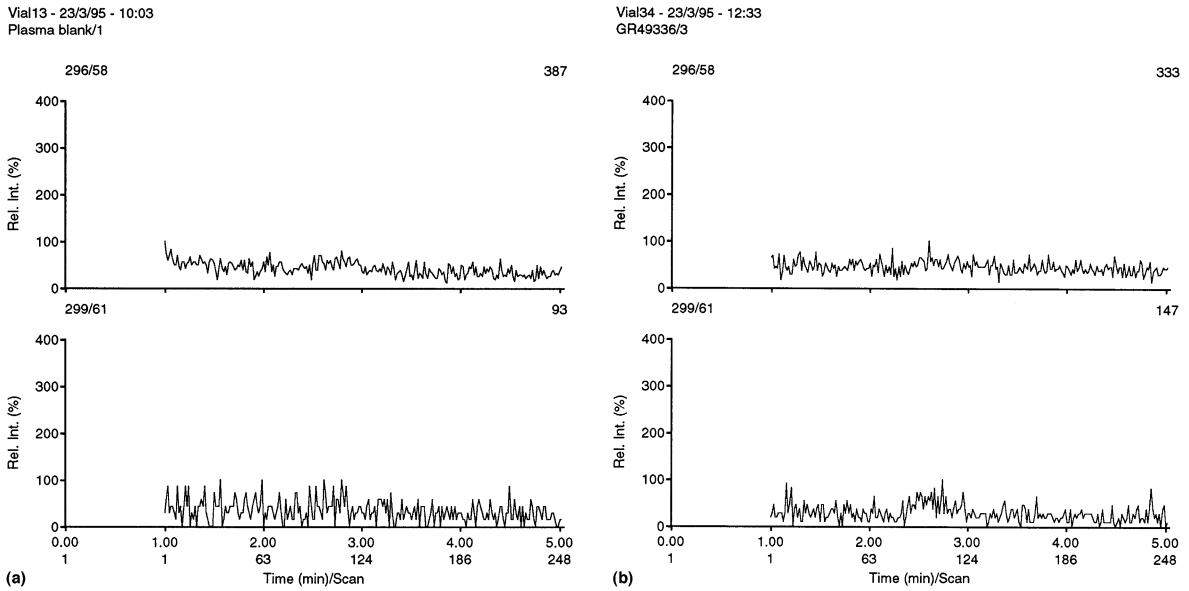

Fig. 6. Representative chromatograms of specificity samples. The top and bottom ion chromatograms correspond to sumatriptan and D_3-sumatriptan, respectively: (a) blank plasma; and (b) plasma + GR49336.

3.3. Specificity and storage stability

High assay specificity was achieved using liquid chromatographic separation in addition to multiple reaction monitoring (MRM) detection as only the dissociations of the precursor ions to their respective daughter ions were monitored. Control human plasma (or urine) was shown not to contain any endogenous compounds that could interfere with the assay (Fig. 6a). The putative metabolite GR112504 was found to elute at a different retention time (~ 2 min) compared to sumatriptan (~ 2.5 min) under the chromatographic conditions used and so did not interfere in the assay. The known metabolite, GR49336, was not observed under the extraction and chromatographic conditions used (Fig. 6b). Quality control samples containing sumatriptan which had been further fortified with GR49336 and GR112504 gave values comparable to the nominal sumatriptan concentration.

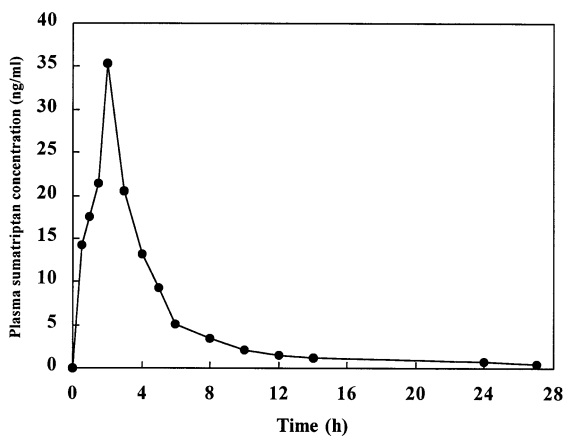

Fig. 7. A plasma concentration vs. time profile for one human volunteer following oral administration of 25 mg of sumatriptan succinate.

Slight injection carry over was noted but was not at a level likely to interfere with the assay. The concentration of sumatriptan in fortified control samples of plasma or urine following 10-fold dilution with blank plasma or urine was in good agreement with the theoretical values. As previous studies had indicated that sumatriptan was stable under various freeze-thaw conditions, upon storage at -15°C, and upon storage at ambient temperature for at least 14 h [5] no further checks on the stability of sumatriptan in plasma and urine were carried out.

3.4. Clinical application

This LC–MS/MS method was used to determine the concentrations of sumatriptan in human plasma and urine following oral administration of a 25 mg tablet of sumatriptan succinate. A total of 230 plasma and 104 urine samples were analysed. Sumatriptan was detectable in plasma (0.3–0.8 ng base ml^{-1}) for three of eight volunteers at 24 h after administration but not for the other five volunteers. A plot of the plasma concentration profile for one of the three volunteers is shown in Fig. 7. The median pharmacokinetic data from the study indicated that sumatriptan was absorbed reasonably rapidly (T_{max} 1 h) at this low dose giving a maximum concentration of 18.7 ng base ml^{-1}. Sumatriptan was shown to be cleared from the plasma with an initial half-life of 2.3 h.

The *N*-oxide of sumatriptan was also detected in some of the plasma samples, but the concentrations were not quantified. This establishes that the *N*-oxide is a metabolite of sumatriptan, but animal pharmacology studies have indicated that it is not active (H.E. Connor, Private communication).

4. Conclusion

An improved method for the determination of sumatriptan in human plasma and urine was developed and validated using tandem mass spectrometric methodology. The assay has an LLOQ of 200 pg base ml^{-1} and has been used for the analysis of samples from clinical studies. The method is reliable for measuring low concentrations of sumatriptan, and is suitable for generating reliable pharmacokinetic data.

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