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Fully automated assay for the determination of sumatriptan in human serum using solid-phase extraction and high-performance liquid chromatography with electrochemical detection

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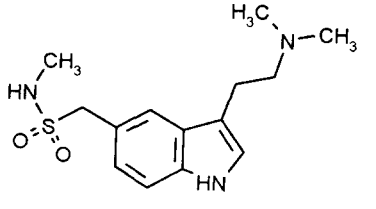
Abstract

A method is described for a fully automated, sensitive, accurate and precise assay for the determination of sumatriptan in human serum. The assay consists of solid-phase extraction followed by reversed-phase HPLC with electrochemical detection. The extraction procedure has been fully automated on a Zymate XP robot linked on-line to the HPLC system. The assay is linear over the analytical range 1–30 ng ml^{-1} and selective for sumatriptan with respect to endogenous plasma components and GR49336, the major circulating metabolite. The intra-assay data demonstrate a maximum bias and precision across the calibration range of 10% and 6.6%, respectively. The inter-assay data demonstrate a maximum bias and precision across the calibration range of 6.7% and 8.8%, respectively. The extraction efficiency of the assay is approximately 90% and is constant across the calibration range. The assay was used for the determination of sumatriptan in serum clinical samples and was shown to be robust in sustained use over several months. The use of a Zymate XP robot allowed complete automation of the assay, which resulted in high-quality, high-throughput analyses.

Keywords: Electrochemical direction; Reversed-phase chromatography; Robotics; Solid-phase extraction;

1. Introduction

Sumatriptan succinate {3-[2-(dimethylamino) ethyl]-*N*-methyl-1*H*-indole-5-methanesulphonamide succinate (**1**), Glaxo compound GR43175C} is a novel, highly selective 5-hydroxy-tryptamine-1 receptor agonist used for the treatment of migraine [1,2]. As part of the development of the drug, assays were required to determine plasma and serum concentrations to characterize the



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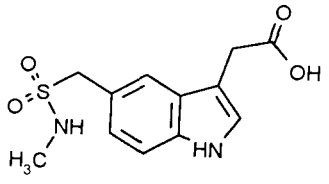

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pharmacokinetics of sumatriptan in humans. Previously, sumatriptan has been determined in plasma and serum using a liquid–liquid extraction procedure followed by reversed-phase high-performance liquid chromatography (HPLC) [3]. The assay was suitably sensitive, accurate and precise, but the extraction procedure was manually intensive. This paper describes a fully aotomated HPLC method which uses solid-phase extraction with electrochemical detection for the determination of sumatriptan in serum and validation according to published procedures [4].

2. Experimental

2.1. Chemicals, standards and materials

Sumatriptan was supplied by the Pharmaceutical Development Department of Glaxo Research and Development. Methanol (HPLC grade) was purchased from Rathburn Chemicals (Walkerburn, UK). Concentrated hydrochloric acid (AnalaR grade) and tris(hydroxymethyl)methylamine, potassium dihydrogenorthophosphate and potassium dihydrogenorthophosphate (all reagent grade) purchased from BDH Chemicals (Poole, UK). Water was doubly distilled from glass and stored in glass. Bond Elut C18 sample extraction cartridges were supplied by Jones Chromatography (Hengoed, UK). The disposable glass tubes and the disposable pipette tipes used on the robotic system for sample preparation were purchased from Zymark (Warrington, UK).

2.2. Robotic instrumentation

A Zymark System V XP robot was used for sample preparation. The robotic system consisted of the following modules: a Zymate XP core system (controller, robot arm and disc drive); a general-purpose hand, a 2.5 ml syringe hand, a liquid–solid extraction pysection with six solvent lines, an HPLC injection pysection (200 μl loop), balance pysection with Sartorius 1712 balance and a dilute and dissolve pysection.

2.3. Chromatographic instrumentation and conditions

The HPLC system consisted of a Severn Analytical pump fitted with a high-efficiency pulse damper (Severn Analytical, Macclesfield, UK), a Spherisorb ODS-1 HPLC column (5 μm, 100 × 4.6 mm i.d.; Anachem, Luton, UK) preceded by an in-line guard column of the same packing and an electrochemical detector (Coulochem 5100A detector, Severn Analytical, UK). The chromatographic separation was performed at 40°C using a mobile phase of 35% phosphate buffer (0.075 M, pH 7.0)–methanol (35:65, v/v). The flow rate was 1 ml min^{-1} and detection was performed using electrochemistry at the following settings: guard cell, +0.9 V; analytical cell detector 1, +0.55 V; analytical cell detector 2, +0.80 V; gain, 99 on range 1; response, 0.1 s. Under these conditions, sumatriptan had a capacity ratio (k') of approximately 5.5, giving a retention time of typically 6 min. Data handling was carried out using a Multichrom Chromatography Data Handling System (VG Data Systems, Altringham, UK).

2.4. Preparation of standards

Calibration standards (1, 3, 5, 10, 20 and 30 ng ml^{-1}) and quality control (QC) samples (4, 10 and 25 ng ml^{-1}) were prepared by mixing control serum with aqueous solutions of sumatriptan. The calibration standards were prepared either prior to each assay run or in batch to be stored frozen (−20°C) until required. The QC samples were prepared in batch and stored frozen (−20°C) until required. Sumatriptan is stable in control serum for 2 years when stored at −20°C [3].

2.5. Sample preparation

Prior to analysis by HPLC, study samples, calibration standards and QC samples were prepared using solid-phase extraction. The entire procedure was automated on a Zymate XP robot. A 1 ml aliquot of serum sample was added to 1 ml of 0.05 M Tris buffer (pH 9.0) and vortex mixed using the dilute and dissolve module. A solid phase-extraction Bond Elut C18 column was conditioned with 1 ml of methanol followed by 1 ml of 0.05 M Tris buffer (pH 9.0), each of which was pushed through the column with air for 30 s. The diluted sample was transferred on to the conditioned extraction column and pushed through the column bed with air for 150 s. The extraction column was washed with 2 ml of 0.05 M Tris buffer (pH 9.0) and pushed through with air for 150 s; then 1 ml each of 0.1 M phosphate buffer (pH 7.0) and 10% methanol in distilled water were each pushed through the column with air for 40 s. The analyte was eluted with 0.4 ml of elution reagent, methanol–0.1 M phosphate buffer (pH 7.0) (65:35), pushed through with air for 40 s. Each air push was performed at 5 psi. An aliquot of the extract (200 μl) was injected on to the HPLC system via the Rheodyne loop of the LC injection station. After each injection, the LC injector was washed with 5 ml of wash solution [phosphate buffer (0.075 M; pH 7.0)–methanol (35:65)] and thoroughly dried with air. Data acquisition was initiated automatically from the robotic system via an instrument interface module.

2.6. Quantification

Calibration standards and QC samples were analysed in duplicate, interspersed with the study samples throughout the assay run. Peak areas were recorded and calibration lines were constructed from the calibration standard data using weighted linear regression ($1/x^2$). Concentrations of sumatriptan in QC and study samples were quantified by comparison with these calibration lines. The calibration range 1–30 ng ml^{-1} was generally suitable for sumatriptan pharmacokinetic studies. Samples containing sumatriptan in excess of 30 ng ml^{-1} were analysed after dilution with control serum to within the calibration range of the assay.

2.7. Validation

Validation of the assay procedure was carried out to establish intra- and inter-assay variability, selectivity and extraction efficiency over the calibration range. The intra- and inter-assay variabil-

Table 1
Intra-assay variation and bias for the determination of sumatriptan in serum over the range 1–30 ng ml^{-1}

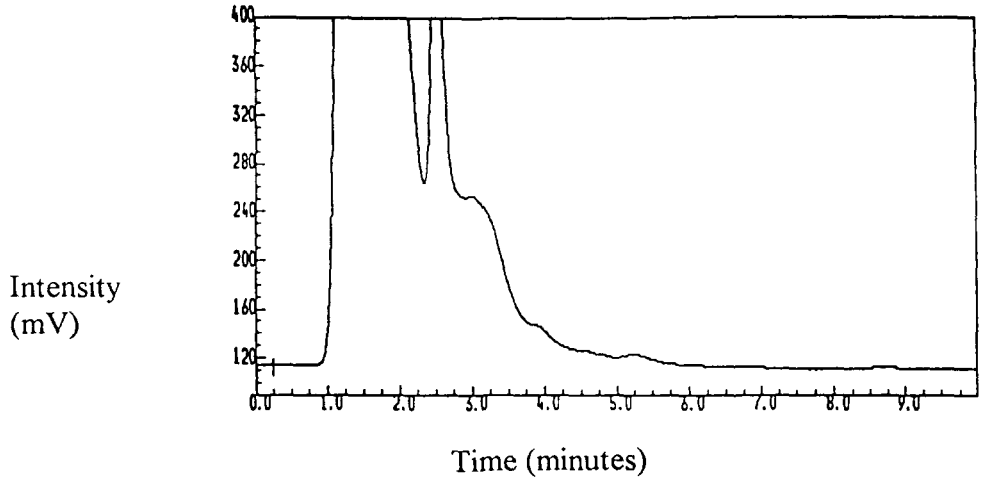
Nominal concentration of sumatriptan (ng ml^{-1})	Mean observed concentration (ng ml^{-1})	Number of replicates	RSD (%)[a]	Bias (% error)[b]
1	1.1	6	3.8	10
3	2.8	6	4.3	−6.7
5	4.9	6	4.4	−2.0
10	10.0	5[c]	3.7	0
20	19.8	6	6.6	−1.0
30	30.0	6	4.8	0

[a] RSD (standard deviation/mean concentration) × 100.
[b] Bias (% error = [(mean concentration − nominal concentration)/nominal concentration × 100.
[c] Sample lost during analysis.

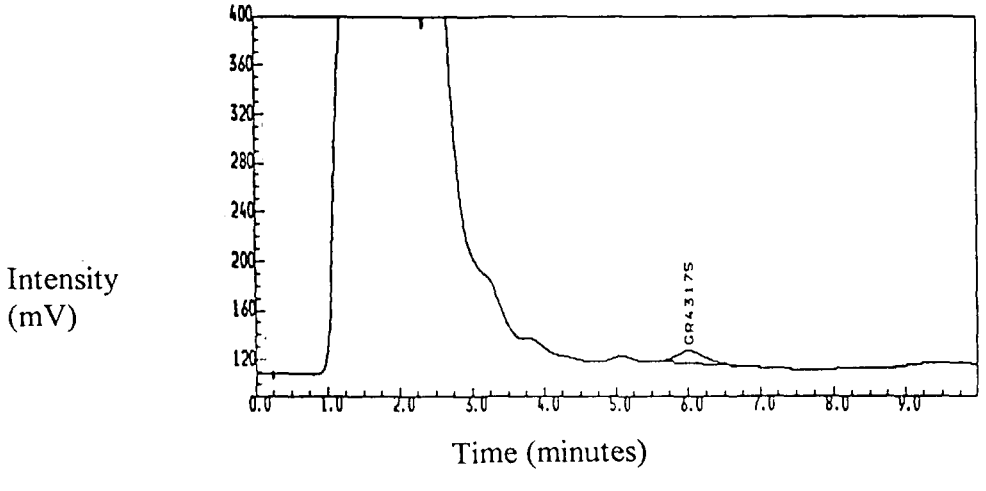
Table 2
Inter-assay variation and bias for the determination of sumatriptan in serum over the range 1–30 ng ml^{-1}

Nominal concentration of sumatriptan (ng ml^{-1})	Mean observed concentration (ng ml^{-1})	Number of replicates	RSD (%)[a]	Bias (% error)[b]
1	0.99	6	8.8	−1
3	2.8	6	6.0	−6.7
5	4.9	6	3.8	−2.0
10	9.5	6	4.0	−5.0
20	19.8	6	2.6	−1.0
30	29.3	6	2.0	−2.3

[a, b] See Table 1.



(a)



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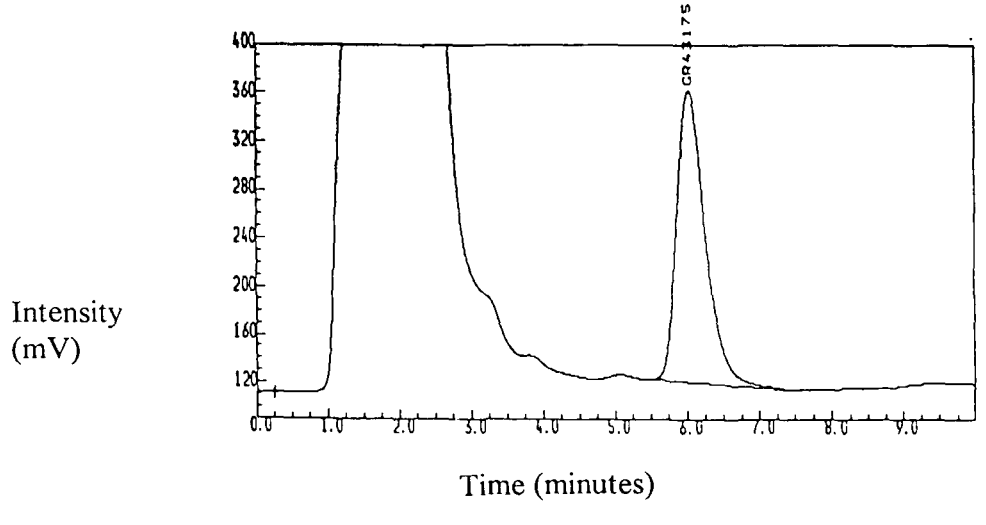

(c)

Fig. 1. Chromatograms of serum extracts: (A) and (B) standards containing sumatriptan at 0 and 1 ng ml^{-1}, respectively; (C) QC sample containing sumatriptan at 25 ng ml^{-1}.

ity were determined by calculation of the accuracy and precision of the assay. The accuracy is expressed by the bias (difference from the theoretical value) and the precision is expressed by the relative standard deviation (RSD). Both bias and RSD are quoted as percentages.

Intra-assay variability was determined from the analysis of sixfold replicates of control serum spiked at each calibrant concentration which were assayed against a calibration line.

Inter-assay variability was determined from the analysis of control serum spiked at each calibration concentration assayed against an independent calibration line on six separate occasions.

Selectivity was studied by assaying control human serum from six different subjects by the described procedures and the chromatograms examined for visible evidence of interfering endogeneous compounds. The major metabolite of sumatriptan, GR49336X (**2**) [5], was examined under the same conditions to ascertain its retention time.

The extraction efficiency of the method was measured by comparing the HPLC peak area of duplicate QC samples at three concentrations (4, 10 and 25 ng ml^{-1}), extracted as described, with direct HPLC analysis of aqueous solutions of sumatriptan at the corresponding concentrations.

3. Results and discussion

The validation data presented in Tables 1 and 2 demonstrate that the robotic assay for the determination of sumatriptan in serum is accurate, precise, selective and linear.

Examples of chromatograms of calibration samples and pre- and post-dose study samples are shown in Fig. 1.

The intra-assay precision, derived from mean data, was 8.8% at 1 ng ml^{-1}, decreasing to 2.0% at 30 ng ml^{-1}. The bias decreased from 10% at 1 ng ml^{-1} to 0% at 30 ng ml^{-1} (Table 1). The inter-assay precision, derived from mean data, varied from 3.7% to 6.6% over the calibration range 1–30 ng ml^{-1} and the bias was −1% at 1 ng ml^{-1}, −6.7% at 3 ng ml^{-1} and −2.3% at 30 ng ml^{-1} (Table 2). As these data demonstrate, a high degree of accuracy and precision was achieved with the automated assay using the external standard method, so the inclusion of an internal standard is not likely to make a significant improvement to the quality of the data obtained.

The assay was shown to be selective with respect to GR49336, the major human metabolite of sumatriptan. The two analytes were well separated, eluting at 1 and 7 min, respectively. Chromatograms from control serum were free from endogeneous interference at the retention time of sumatriptan. Thus the assay has been shown to be selective for sumatriptan with respect to endogenous components for serum. In earlier work [3], the chromatography was also shown to be selective with respect to ergotamine, dihydroergotamine, aspirin, paracetamol and dihydrocodeine. Using weighted ($1/x^2$) linear regression due to the 30-fold range of calibration, the coefficient of determination was typically 0.999. The intercept value was never greater than 0.33 ng

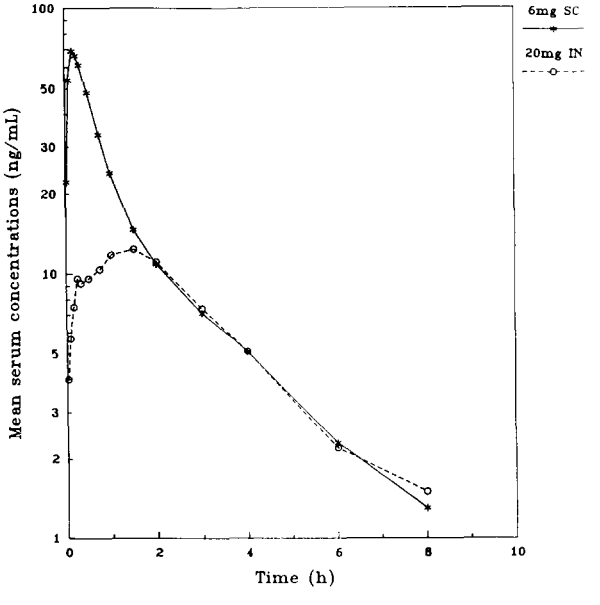

Fig. 2. Semilogarithmic plot of the median plasma concentration–time profile for six volunteers following a single 6 mg subcutaneous dose (x) and a 20 mg intranasal dose (○) of sumatriptan.

ml^{-1}. The mean extraction efficiency of sumatriptan from human serum on comparison with direct aqueous injections, was 89.8% (RSD 6.6%) and was independent of concentration over the range 1–30 ng ml^{-1}, an observation which contributes to the good precisions and accuracy of the method.

This method was of sufficient sensitivity to provide good pharmacokinetic data from the analysis of serum study samples. A typical profile of serum concentrations of sumatriptan versus time after administration of an intra-nasal dose (20 mg) or subcutaneous dose (6 mg) is shown in Fig. 2.

4. Conclusions

The robotic method for the determination of sumatriptan in serum is accurate, precise and selective over the calibration range 1–30 ng ml^{-1}. This assay was shown to be robust in sustained use over several months. The use of a Zymate XP robot has allowed complete automation of the assay, resulting in high-quality, high-throughput analyses in support of sumatriptan pharmacokinetic studies.

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References

[1] W. Feniuk, P.P.A. Humphrey, M.J. Perren, H.E. Connor and E.T. Whally, J. Neurol., 238 (1991) S57–S61.
[2] P.P.A. Humphrey, W. Feniuk, M.L. Perren, I.J.M. Beresford and M. Skingle, Ann. N, Y. Acad. Sci., 600 (1990) 587–600.
[3] P.D. Andrew, H.L. Birch and D.A. Phillpot, J. Pharm. Sci., 82 (1993) 73–76.
[4] R.J.N. Tanner, in E. Reid and I. Wilson (Eds), Methods Surveys in Biochemistry and Analysis, Vol. 20, Royal Society of Chemistry, Cambridge, 1990, pp. 57–63.
[5] C.M. Dixon, D.A. Saynor, P.D. Andrew, J. Oxford, A. Bradbury and M.A. Tarbit, Drug Metab. Dispose, 21 (1993) 761–769.