# Determination of Sumatriptan Succinate in Plasma and Urine by High-Performance Liquid Chromatography with Electrochemical Detection

PETER D. ANDREW[x], HELEN L. BIRCH, AND DEBORAH A. PHILLPOT

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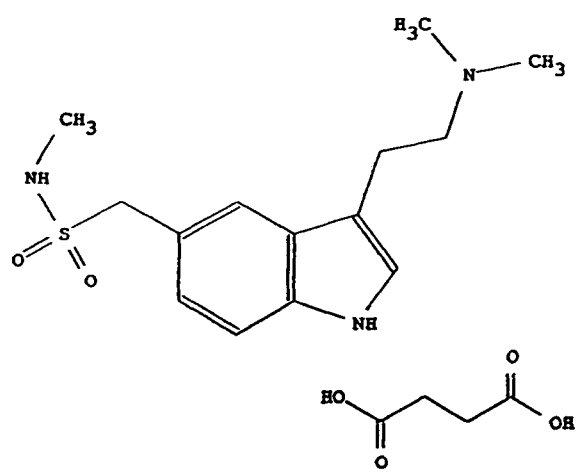
**Abstract** □ Methods are described for the determination of sumatriptan succinate (**1**) in plasma and urine. Prior to chromatography, plasma is subjected to liquid/liquid extraction and urine is diluted in pH 7 buffer without further pretreatment. Both procedures use reversed-phase high-performance liquid chromatography with electrochemical detection. The analytical range for the plasma assay is 1–30 ng/mL and that for the urine assay is 0.2–12 μg/mL. The assays are linear over the analytical ranges and specific with respect to endogenous interference and the major metabolite (**2**) of sumatriptan. For the plasma assay, intra-assay data (n = 6) indicate a maximum coefficient of variation (CV) and bias across the calibration range of 6.0 and 3.0%, respectively. The interassay CV (n = 4) is ~15% at the bottom of the calibration range, falling to 4% or less at 8 ng/mL and above. Bias is ~12% at the bottom, reducing to <2% at 8 ng/mL and above. The urine intra-assay data indicate a maximum CV and bias of 8.9 and 8.3%, respectively. The interassay is CV 15% for the lowest calibrant, reducing steadily across the calibration range to <2% for the top calibration value, and bias is <7% across the range.

Sumatriptan succinate (3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methanesulfonamide succinate, **1**, Glaxo compound GR43175C), is a novel, highly selective 5-hydroxytryptamine-1 agonist used for the treatment of migraine.[1,2] As part of the development of the drug, assays were required to determine plasma and urine concentrations to characterize the pharmacokinetics of **1** in humans. The method was applied to plasma from rat, dog, primate, and humans and to urine from humans. Previously, a liquid chromatography–mass spectrometric (LC–MS) method[3] had been used but was limited in terms of cost, sample throughput, and general applicability.




**1**

For the purpose of measuring concentrations of **1** in plasma and urine, the UV spectral properties of **1** were not sufficiently sensitive. In contrast, electrochemical detection provided a means of more sensitive detection, probably as a consequence of oxidation of the indole ring. This paper describes a high-performance liquid chromatographic (HPLC) method with electrochemical detection for the determination of **1** in plasma and urine and its validation according to published procedures.[4]

## Experimental Section

**Chemicals and Reagents**—Compound **1** (analytical grade 98.5% minimum) was supplied by the Analytical Research Department, Glaxo Group Research, Ware, U.K. Sodium hydroxide, disodium hydrogen orthophosphate dihydrate, potassium dihydrogen orthophosphate, methylene chloride, hexane, and ethyl acetate were all analytical reagent quality. Methanol was HPLC grade (Rathburn Chemicals, Walkerburn, Scotland). Water was double distilled from glass and stored in glass. The solvent extraction mixture comprised 20% methylene chloride in ethyl acetate that was then washed twice with water and filtered through a Whatman PS1 phase separation filter paper.

**Equipment and Chromatographic Conditions**—The HPLC equipment comprised a Spectroflow 400 pump fitted with a high-efficiency pulse dampener (part no. 2500-0567, ABI, Warrington, U.K.), a WISP autosampler (Waters, Milford, MA), a column thermostat (Jones Chromatography, Hengoed, Mid Glam., U.K.), and a Coulochem model 5100A electrochemical detector fitted with a guard cell and 5011 analytical cell (ESA, Bedford, MA). Data capture and integration was with Trilab 3000 (Trivector, Sandy, Beds., U.K.).

The chromatography column was stainless steel, with i.d. dimensions of 125 × 4.6 mm, fitted with an integral 10-mm guard cartridge, packed with 5-μm Spherisorb ODS-1 (MH Scientific, Aylesbury, Bucks., U.K.), and maintained at 40 °C. The mobile phase was 40% aqueous pH 7.0 phosphate buffer (5.25 g of disodium hydrogen orthophosphate dihydrate and 2.79 g of potassium dihydrogen orthophosphate dissolved in 1.0 L of water) in methanol and was used at a typical flow rate of ~1 mL/min to give a retention time for **1** of ~400 s.

The electrochemical analytical cells were set at 0.55 and 0.80 V. The first cell acted as a conditioning cell, with the output from the second cell collected at a set gain of 99 with minimum filtering. The guard cell was set at 0.9 V. The detector was used to investigate the electrochemical behavior of **1** in the mobile phase, commencing at an applied potential of 0.4 V and incrementing in stages to 1.0 V. The resultant hydrodynamic voltamogram is illustrated in Figure 1.

**Analytical Procedure**—Plasma (1.0 mL) was basified with 4 M sodium hydroxide solution (100 μL) and then extracted with the methylene chloride:ethyl acetate mixture (2.60 mL). The mixture was agitated on a rotational shaker for 15 min and then centrifuged at 1000 × *g* for 4 min. An aliquot of the upper layer (2.0 mL) was quantitatively transferred to a second tube, and hexane (2 mL) and pH 7 phosphate buffer (300 μL) were added. The tube contents were agitated and centrifuged as before. The tube was then immersed in a freezing mixture of acetone and solid carbon dioxide to freeze the lower aqueous layer. The upper layer was aspirated off under reduced pressure. After thawing, an aliquot (200 μL) of the remaining aqueous solution was examined by HPLC. Typical chromatograms of

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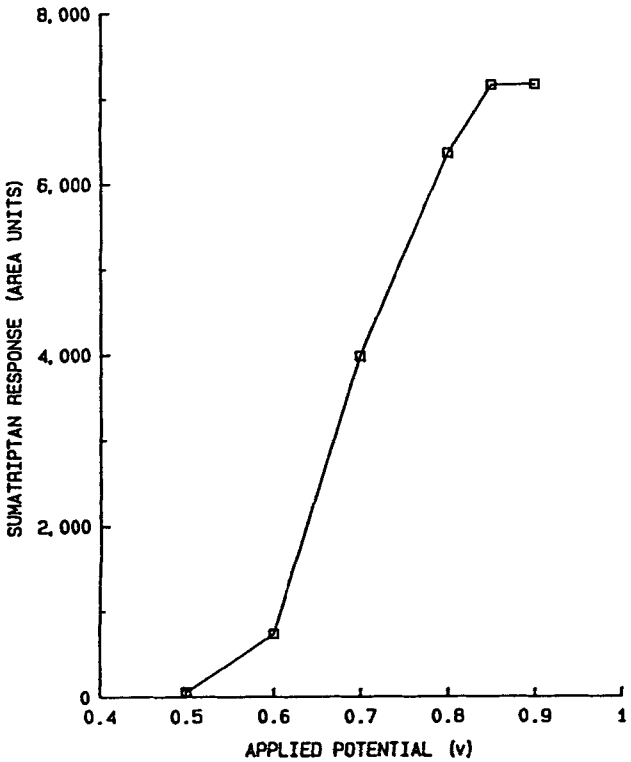



**Figure 1**—Hydrodynamic voltamogram of 1 in mobile phase obtained with an ESA Coulochem 5011 cell.

plasma extracts are shown in Figure 2.

Urine sample preparation comprised a 20-fold dilution with pH 7 phosphate buffer prior to examination by HPLC (20 μL), with the same chromatographic and detector conditions as for plasma extracts. Typical chromatograms of diluted urine are shown in Figure 3.

**Calibration**—Calibration curves were prepared in both matrices over the ranges 1–30 ng/mL for plasma and 0.2–12 μg/mL for urine. These ranges are generally suitable for pharmacokinetic studies after a 3-mg subcutaneous dose or a 100-mg oral dose of 1 base provided, in the case of plasma, that samples taken at the early time points are diluted (usually one-to-one) with control plasma. Curves comprised

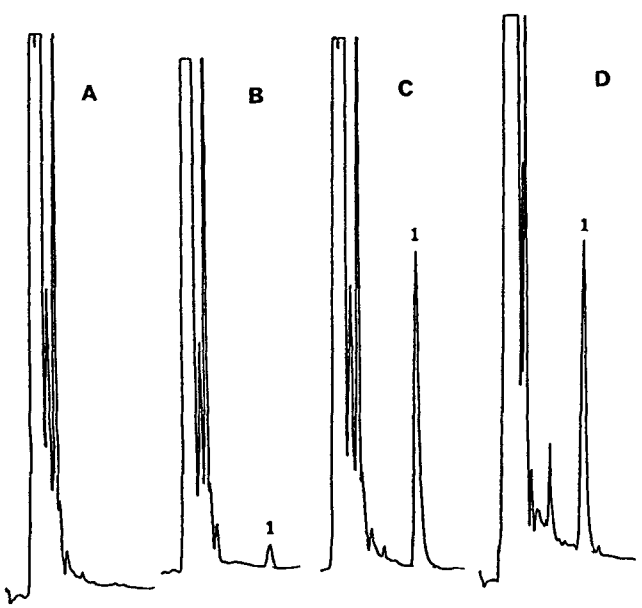



**Figure 2**—Chromatograms of plasma extracts: (**A**) blank; (**B**) and (**C**) standards containing 1 at 1 and 10 ng/mL, respectively; (**D**) 7-h postdose sample from a volunteer administered 100 mg of 1 orally.

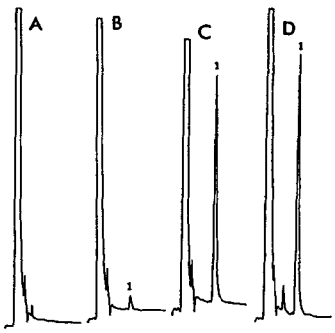



**Figure 3**—Chromatograms of dilute urine: (**A**) blank; (**B**) and (**C**) standards containing 1 at 0.2 and 3 μg/mL, respectively; (**D**) 0–3-h sample from a volunteer administered 100 mg of 1 orally.

six duplicate concentrations dispersed evenly across the range, plus a duplicate blank. All calibration samples were extracted and analyzed as described. Equations of curves of detector response versus concentration of 1 were calculated by the least squares method. These curves were consistently linear, with minimum coefficients of correlation of 0.999 and intercepts corresponding to <20% of the lowest calibrant value.

**Validation**—Validation of the assay procedures was carried out to establish intra- and interassay variability over the calibration range. The specificity and linearity of the assay, the recovery of the plasma extraction procedure, and stability of both diluted urine and extract solutions were also examined. The inter- and intra-assay variability were determined to assess the performance of the assay by calculation of accuracy and precision. The accuracy was expressed by the bias (difference from the theoretical) and the precision was expressed by the coefficient of variation (CV). Both bias and CV are presented as percentages.

## Results and Discussion

The HPLC results were consistent and reliable. Good peak shape was achieved and column performance did not deteriorate over many hundreds of injections (the guard cartridge was changed after two analytical runs, typically 150–200 injections). The electrochemical detector stabilized quickly and provided noise-free measurements even at the lowest calibration standard. The use of the first cell as a conditioning cell, set at 0.55 V, greatly improved the stability of the baseline, without compromising peak area, and marginally reduced endogenous material response in the early part of the chromatogram. The choice of 0.8 V as the setting for monitoring 1 gave an adequate signal while offering some selec-

**Table I—Plasma Intra-assay Variability**

| Calibration Concn, ng/mL | Observed Concn, ng/mL | | | Mean Concn, ng/mL | SD[a] | CV, % | Bias, % |
|---|---|---|---|---|---|---|---|
| 1 | NR[b] | 0.86 | 1.01 | 0.97 | 0.07 | 6.9 | −3.0 |
| | 1.00 | 1.03 | 0.97 | | | | |
| 2 | 2.82[c] | 2.01 | 2.11 | 1.98 | 0.10 | 5.3 | −1.0 |
| | 1.86 | 2.02 | 1.88 | | | | |
| 4 | 4.17 | 4.32 | 4.01 | 4.05 | 0.17 | 4.2 | 1.3 |
| | 3.95 | 3.84 | 4.00 | | | | |
| 8 | 8.48 | 8.45 | 8.18 | 8.11 | 0.30 | 3.7 | 1.4 |
| | 7.91 | 7.80 | 7.85 | | | | |
| 12 | 12.23 | 12.34 | 11.89 | 11.97 | 0.48 | 4.0 | −0.25 |
| | 12.05 | 12.26 | 11.04 | | | | |
| 20 | 20.53 | 20.15 | 18.89 | 19.76 | 0.62 | 3.2 | 1.2 |
| | 19.68 | 19.54 | —[d] | | | | |
| 30 | 29.26 | 29.60 | 29.13 | 29.19 | 0.29 | 1.0 | −2.7 |
| | 28.78 | 29.17 | — | | | | |

[a] Standard deviation. [b] No result. [c] Outlier, excluded from data. [d] —, Not applicable.

**Table II—Urine Intra-assay Variability**

| Calibration Concn, μg/mL | Observed Concn, μg/mL | | | Mean Concn, μg/mL | SD[a] | CV, % | Bias, % |
|---|---|---|---|---|---|---|---|
| 0.2 | 0.21 | 0.17 | 0.18 | 0.19 | 0.02 | 8.9 | −7.5 |
| | 0.17 | 0.20 | 0.18 | | | | |
| 0.4 | 0.50 | 0.42 | 0.42 | 0.43 | 0.04 | 8.5 | 8.3 |
| | 0.40 | 0.41 | 0.45 | | | | |
| 1.0 | 1.04 | 1.09 | 1.00 | 1.07 | 0.05 | 4.7 | 6.7 |
| | 1.04 | 1.09 | 1.14 | | | | |
| 3 | 3.01 | 3.07 | 3.06 | 3.07 | 0.04 | 1.4 | 2.3 |
| | 3.08 | 3.06 | 3.14 | | | | |
| 7 | 7.11 | 6.92 | 7.04 | 7.10 | 0.13 | 1.9 | 1.5 |
| | 7.04 | 7.22 | 7.29 | | | | |
| 12 | 12.07 | 12.99 | 12.06 | 12.29 | 0.26 | 2.1 | 2.4 |
| | 12.32 | 12.49 | 12.68 | | | | |

[a] Standard deviation.

**Table IV—Urine Interassay Variability**

| Calibration Concn, μg/mL | Observed Concn, μg/mL | | | | Mean Concn, μg/mL | SD[a] | CV, % | Bias, % |
|---|---|---|---|---|---|---|---|---|
| 0.2 | 0.18 | 0.23 | 0.21 | 0.24 | 0.21 | 0.03 | 15 | 6.3 |
| | 0.25 | 0.24 | 0.18 | 0.17 | | | | |
| 0.4 | 0.34 | 0.39 | 0.41 | 0.41 | 0.41 | 0.06 | 14 | 1.9 |
| | 0.54 | 0.41 | 0.38 | 0.38 | | | | |
| 1 | 0.92 | 1.24 | 0.86 | 0.98 | 1.01 | 0.11 | 11 | 1.1 |
| | 1.00 | 0.98 | 1.07 | 1.04 | | | | |
| 3 | 2.70 | 2.83 | 2.96 | 3.22 | 3.11 | 0.27 | 8.6 | 3.5 |
| | 3.35 | 3.32 | 3.16 | 3.12 | | | | |
| 7 | 6.81 | 6.98 | 6.80 | 6.91 | 6.97 | 0.12 | 1.7 | −0.43 |
| | 7.01 | 7.04 | 7.10 | 7.11 | | | | |
| 12 | 12.35 | 12.31 | 12.07 | 12.25 | 12.07 | 0.22 | 1.8 | 0.58 |
| | 11.90 | 12.05 | 11.76 | 11.87 | | | | |

[a] Standard deviation.

tivity over that available at 0.9 V, the applied potential offering maximum output (Figure 1).

Intra-assay variability was measured from sixfold replicates of sample matrix spiked at each calibrant concentration (fivefold for the two top calibrant values), when assayed against a duplicate calibration line prepared in the same way. For the plasma assay, the data indicated maximum CV and bias across the calibration range of 6.0 and 3.0%, respectively (Table I). The urine assay indicated maximum CV and bias of 8.9 and 8.3%, respectively (Table II).

Interassay variability was measured by analysis of duplicate spiked plasma and urine samples, corresponding to each calibration point, on four separate occasions. An independent calibration line was prepared in duplicate each time. For the plasma assay (Table III), the CV was ~15% at the bottom of the calibration range, falling to 4% or less at 8 ng/mL and above. Bias was ~12% at the bottom, reducing to <2% at 8 ng/mL and above. For the urine assay (Table IV), the CV was 15% for the lowest calibrant, reducing steadily across the range to <2% at the top calibration value. Bias was <7% across the calibration range.

Recovery of the plasma extraction procedure was checked over the range 2–30 ng/mL by comparison with duplicate injections of accurately prepared aqueous solutions of concentration similar to each calibrant point. The mean peak area for each calibration value (*A*) was compared with that of the aqueous standard (*B*). The values obtained for *A* and *B* and the calculated recoveries are shown in Table V. The mean recovery was 68.1% over the range 2–30 ng/mL. This estimate does not include adjustment for the incomplete transfer (2.0 mL from an added 2.6 mL) of the upper layer to the second extraction stage. Precision of the recovery across the range was good (CV, 4.9%), so the inclusion of an internal standard was not thought likely to make a significant improvement to the quality of data obtained.

Specificity was demonstrated in several ways. Numerous samples of plasma and urine from humans, dog, and rat were examined by the described procedures, and the visible absence of interfering endogenous compounds in the chromatograms was noted. The single major metabolite **2** eluted on the solvent front and did not interfere with measurements of **1**. As a further indication of specificity, comparison of the results for plasma samples analyzed by this method and a thermospray LC–MS method, which used selective ion recording,[3] gave a coefficient of correlation of 0.999, a slope of 0.94, and an intercept of 0.7 ng/mL. The LC–MS and HPLC plasma assay data are shown in Table VI. It was confirmed that ergotamine, dihydroergotamine, aspirin, paracetamol, and dihydrocodeine did not interfere with the assay.

Stability of **1** at ambient temperatures in both dilute urine and plasma extracts was demonstrated over 10- and 14-h periods, respectively, the times for a typical batch analysis. A concentration was chosen, approximating the midpoint of the calibration range, and six identical aliquots of the prepared solution for each matrix were compared over the prescribed time period. Accurately prepared aqueous solutions of an approximately equivalent concentration were included to demonstrate system stability. The urine solution gave a constant peak area of **1** (CV, 3.6%; n = 6) compared with the aqueous reference peak area (CV, 2.2%; n = 6), indicating

**Table III—Plasma Interassay Variability**

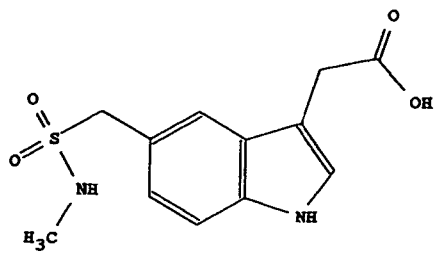
| Calibration Concn, ng/mL | Observed Concn, ng/mL | | | | Mean Concn, ng/mL | SD[a] | CV, % | Bias, % |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.86 | 1.01 | 1.02 | 1.21 | 0.98 | 0.13 | 13 | −2.0 |
| | 0.93 | 0.81 | 0.90 | 1.10 | | | | |
| 2 | 1.85 | 1.83 | 2.07 | 2.43 | 2.24 | 0.36 | 16 | 12 |
| | 2.91 | 2.07 | 2.36 | 2.43 | | | | |
| 4 | 3.84 | 4.31 | 3.94 | 4.92 | 4.31 | 0.41 | 9.6 | 7.8 |
| | 3.99 | 4.85 | 4.13 | 4.53 | | | | |
| 8 | 8.01 | 8.00 | 7.54 | 8.11 | 7.90 | 0.32 | 4.0 | −1.3 |
| | 8.25 | 7.34 | 7.81 | 8.14 | | | | |
| 12 | 12.27 | 11.35 | 11.93 | NR[b] | 11.95 | 0.31 | 2.6 | −0.42 |
| | 12.19 | 11.99 | 12.10 | 11.80 | | | | |
| 20 | 20.80 | 19.49 | 20.11 | 19.18 | 19.73 | 0.75 | 3.8 | −1.4 |
| | 20.53 | NR | 18.83 | 19.20 | | | | |
| 30 | 31.86 | 29.14 | 29.57 | 27.31 | 29.59 | 1.45 | 4.9 | −1.4 |
| | 30.53 | NR | 30.08 | 28.65 | | | | |

[a] Standard deviation. [b] NR, No result.

**Table V—Recovery of 1 from Plasma over the Range 2–30 ng/mL**

| Calibrator Concn, ng/mL | Peak Area Calibrator (*A*) | Peak Area Aqueous Standard (*B*) | Amount of 1 Injected, ng | Recovery, %[a] |
|---|---|---|---|---|
| 30 | 5567 | 6285 | 16.0 | 70.8 |
| 20 | 3431 | 3815 | 10.0 | 67.4 |
| 12 | 2048 | 2269 | 6.0 | 67.7 |
| 8 | 1274 | 1452 | 4.0 | 65.8 |
| 4 | 737 | 757 | 2.0 | 73.0 |
| 2 | 357 | 420 | 1.0 | 63.7 |

[a] Recovery = $A \times$ amount of **1** injected in aqueous standard $\times$ $100/B$ $\times$ calibrator concentration $\times$ 0.667; mean, 68.1%; standard deviation, 3.4%; CV, 4.9%.




**2**

**Table VI—HPLC and LC–MS Correlation**

| LC–MS, ng/mL | HPLC, ng/mL | | |
|---|---|---|---|
| | (i) | (ii) | Mean |
| 2.0 | 2.33 | 2.45 | 2.4 |
| 7.9 | 9.65 | 8.00 | 8.8 |
| 25.5 | 24.11 | 24.60 | 24.4 |
| 2.1 | 2.72 | 2.14 | 2.4 |
| 11.0 | 10.95 | 12.13 | 11.5 |
| 20.3 | 19.49 | 19.89 | 19.7 |
| 7.0 | 7.55 | 7.30 | 7.4 |
| 4.3 | 4.42 | 4.44 | 4.4 |

that dilute solutions of **1** are stable over this period. Similarly, chromatographic peak areas for **1** in plasma extracts remained constant (CV, 2.6%; n = 6) compared with the aqueous reference solution (CV, 4.0%; n = 6), demonstrating that plasma extracts are stable over the 14-h period. Subsequent practical experience in using the method has shown that the extracts remain satisfactory at ambient temperatures for at least 24 h.

Similarly, under typical storage conditions of −15 °C, **1** is stable in plasma and urine. A batch of plasma with theoretical concentration of **1** of 20.6 ng/mL (found 20.0 ng/mL; CV, 3.6%; n = 6) was assayed at the end of a 2-year period of storage at −15 °C. The mean value was 19.4 ng/mL (CV, 1.4%; n = 6), indicating that **1** was stable in plasma under these conditions for a considerable period. Three cycles of freeze-thawing did not affect the measured concentration of **1** in plasma. Urine of theoretical concentration 2.8 μg/mL gave an initial measured concentration of 2.8 μg/mL (CV, 1.7%; n = 4) and, after 10 months storage, was found to be unchanged (CV, 3.4%; n = 4).

In humans, despite the high apparent volume of distribution and a short half-life of **1**, the method was of sufficient sensitivity to provide good pharmacokinetic data from analysis of plasma. A typical profile of plasma concentrations

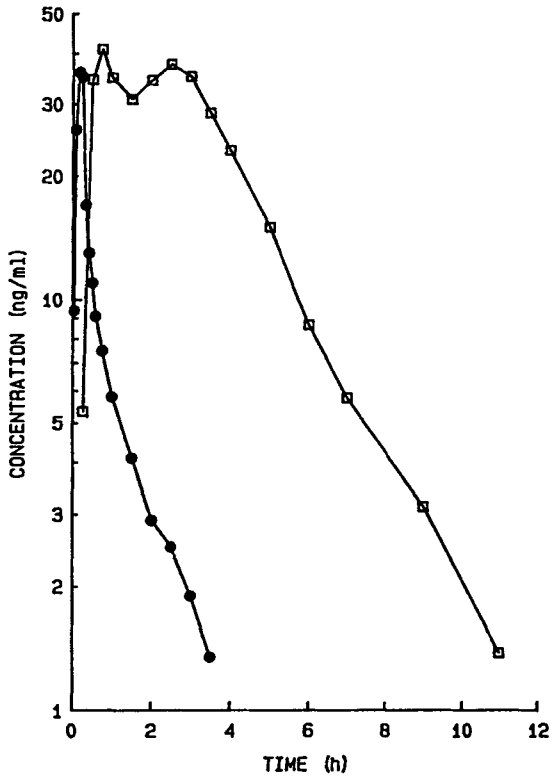



**Figure 4**—Semilogarithmic plot of the median plasma concentration–time profile for six volunteers following a single 100-mg oral (□) and a 2-mg intravenous (●) dose of **1**.

versus time after administration of an intravenous (2 mg) and oral (100 mg) dose of **1** is shown in Figure 4. The compound is eliminated from the body by a combination of renal and metabolic clearance. A single major metabolite, the indole acetic acid **2**, accounts for >50% of the administered dose. This metabolite, which is present in both circulating plasma and urine in much greater concentrations than **1**, does not interfere with the measurement of the parent compound.

The amount of unchanged sumatriptan cleared renally is typically 2–4% of an oral dose and 20% of an intravenous or subcutaneous dose. The range of the urine assay enabled accurate quantification of **1** in 24-h urine samples or in samples from timed intervals up to 12 h postdose, accounting for >90–95% of all unchanged **1** that was eliminated in the urine following a normal therapeutic dose.

## Conclusions

The method for determination of **1** in plasma and urine is accurate and precise. It is specific and sufficiently sensitive to elucidate pharmacokinetic parameters in humans after administration by a variety of dose routes.

## References and Notes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