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DETERMINATION OF THE ENANTIOMERS OF SUPROFEN AND [$^2H_3$]SUPROFEN IN PLASMA BY CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

YOSHIHIKO SHINOHARA*, NORIHARU KIRII, HIROYUKI TAMAOKI, HIROSHI MAGARA and SHIGEO BABA

Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03 (Japan)

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SUMMARY

A method for the stereoselective assay of the (+)- and (−)-enantiomers of suprofen and [$^2H_3$]suprofen in human plasma was developed using gas chromatography–mass spectrometry-selected-ion monitoring. (±)-[$^2H_7$]Suprofen was used as an internal standard. The method involved diethyl ether extraction and chiral derivatization with *S*-(−)-1-(naphthyl)ethylamine to form diastereomeric amide. The diastereoisomers were separated on a capillary gas chromatograph–mass spectrometer. Quantitation was achieved by selected-ion monitoring of the quasi-molecular ions of the diastereoisomers. The sensitivity, specificity, accuracy and reproducibility of the method were demonstrated to be satisfactory for application to pharmacokinetic studies of suprofen enantiomers.

INTRODUCTION

Many drugs contain chiral centres and the stereoselective pharmacokinetics has recently been given much consideration [1–4]. Interest in enantioselective pharmacokinetics has been generated from the realization that constituent enantiomers of a chiral compound may exhibit different pharmacodynamic and toxicologic properties. The 2-arylpropionic acids (2APs) are an important group of chiral non-steroidal anti-inflammatory drugs. Several 2APs have stereoselective pharmacokinetics and also undergo a unidirectional metabolic chiral inversion of the less active *R*-enantiomer to the active *S*-enantiomer [5–7]. Therefore, it is important to know the pharmacokinetics of the individual enantiomers after administration of drugs as racemate.

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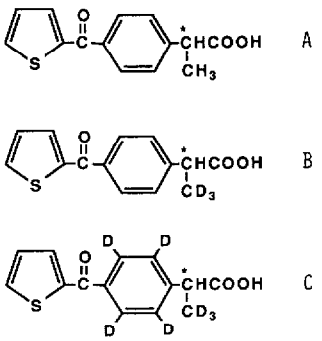

Fig. 1. Structures of suprofen (A), [$^2H_3$]suprofen (B) and [$^2H_7$]suprofen (C). Asterisks denote asymmetric carbon atoms.

Suprofen, α-methyl-4-(2-thienylcarbonyl)benzeneacetic acid (Fig. 1), is a relatively new, orally effective 2AP [8–11]. It is administered as the racemic mixture. The (+)-isomer has been shown to be more potent than the (−)-isomer for the anti-inflammatory activity [12]. Several pharmacokinetic studies have been reported [13–17]. However, there is no information on the pharmacokinetics of individual suprofen isomers in humans or in animals after administration of the racemate.

We have initiated studies to assess the pharmacokinetics of each enantiomer after administration of the racemic mixture. Stable isotope methodology can be uniquely applied to the pharmacokinetic study of each suprofen enantiomer. Information concerning chiral inversion after administration of the racemic mixture can also be obtained. This present paper describes a procedure for the simultaneous determination of suprofen enantiomers in human plasma after administration of the stable isotopically labelled pseudo-racemate of suprofen. The assay involved derivatization with an optically active reagent and gas chromatography–mass spectrometry (GC–MS) with selected-ion monitoring (SIM).

EXPERIMENTAL

Chemicals

Unlabelled racemic suprofen [(±)-SP-d_0] was obtained from Taiyo Pharmaceutical Industry (Gifu, Japan). The following compounds were purchased: 2-thiophenecarbonyl chloride and *S*-(−)- and *R*-(+)-1-phenylethylamine from Tokyo Kasei Kogyo (Tokyo, Japan); *S*-(−)-1-(naphthyl)ethylamine [(−)-NEA] from Norse (Newbury Park, CA, U.S.A.); [$^2H_5$]fluorobenzene (98 atom% ^2H) from Aldrich (Milwaukee, WI, U.S.A.); [$^2H_3$]methyl iodide (99.5 atom% ^2H) from Merck (Montreal, Canada); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD) and 1-hydroxybenzotriazole (HOBT)

from Peptide Institute (Osaka, Japan); anhydrous aluminum chloride and sodium hydride (60% dispersion in mineral oil) from Wako (Osaka, Japan).

Synthesis of deuterated compounds

(±)-[$^2H_3$]Suprofen. A dispersion of sodium hydride (2.76 g, 0.069 mol), previously washed with dry benzene (three 50-ml portions), was suspended in dry N,N-dimethylformamide (100 ml), and diethyl-2-[4-(2-thienylcarbonyl)phenyl]propanedioate [8] (24.0 g, 0.069 mol) in dry N,N-dimethylformamide (10 ml) was added dropwise. The mixture was stirred at room temperature for 10 min, then [$^2H_3$]methyl iodide (10.0 g, 0.069 mol) was added dropwise. The reaction mixture was stirred for 1 h, benzene and water were added, the mixture was separated, and the aqueous layer was re-extracted. The combined extracts were washed with water and dried over sodium sulphate. Removal of the solvent by evaporation resulted in a pale yellow residue. A solution of the residue dissolved in 5% sodium hydroxide (100 ml) was refluxed under a nitrogen atmosphere for 7 h. After cooling, the reaction mixture was washed with benzene (two 100-ml portions) and acidified to pH 1 with 5 *M* hydrochloric acid. The solution was extracted with chloroform, washed three times with water and dried over sodium sulphate. The solvent was evaporated to dryness under reduced pressure and the resulting crystals were recrystallized from acetonitrile to give (±)-[$^2H_3$]suprofen [(±)-SP-d_3] as white crystals (8.8 g, 49%). m.p. 122.0–122.5°C. NMR [$\delta_H(C^2HCl_3)$]: 3.80 (1H, s, -CH-), 7.08–7.88 (7H, m, aromatic). MS [electron impact (EI)]: *m/z* 263 (M^+), *m/z* 218 ($M^+ - COOH$). Calculated for $C_{14}H_9{}^2H_3O_3S$: C, 63.86; H(^{2}H), 4.59. Found: C, 63.79; H(^{2}H), 4.72. The isotopic composition was 99.0% deuterium atoms ($^2H_3$, 98%; $^2H_2$, 2.0%; ^2H, 0.0%).

(±)-[$^2H_7$]Suprofen. To a stirred solution of 2-thiophenecarbonyl chloride (4.4 g, 0.03 ml) and anhydrous aluminum chloride (3.2 g, 0.02 mol) in 10 ml of dichloromethane was added [$^2H_5$]fluorobenzene (3.00 g, 0.03 mol). After the initial slightly exothermic reaction, the mixture was refluxed for 105 min. After cooling, benzene and water were added, the mixture was separated, and the aqueous layer was re-extracted. The combined extracts were washed with 5% sodium carbonate and water and dried over sodium sulphate. After removal of the solvent by evaporation, the solid residue was recrystallized from isopropyl alcohol to yield 1.88 g (30%) of 4-(2-thienylcarbonyl)-[$^2H_4$]fluorobenzene (I). m.p. 95–96°C. NMR [$\delta_H(C^2HCl_3)$]: 7.10–7.72 (3H, m, thienyl); MS (EI): *m/z* 210 (M^+).

Diethyl-2-[4-(2-thienylcarbonyl)-[$^2H_4$]phenyl propanedioate (II) was synthesized from I according to the method described by Van Daele et al. [8]. According to the procedure for (±)-SP-d_3 described above, II was treated with [$^2H_3$]methyl iodide to give (±)-[$^2H_7$]suprofen [(±)-SP-d_7] (0.28 g, 12%). m.p. 121.5–122.0°C. MS: *m/z* 267 (M^+), *m/z* 222 ($M^+ - COOH$). The iso-

topic composition was 98.3% deuterium atoms ($^2H_7$, 88.8%; $^2H_6$, 10.7%; $^2H_5$, 0.5%; $^2H_4$, 0.0%).

Resolution of suprofen enantiomers

(±)-SP-d_0 (25.0 g, 0.096 mol) was dissolved in 250 ml of ethanol. *S*-(−)-1-Phenylethylamine (11.6 g, 0.096 mol) was added slowly to the solution. The mixture was allowed to stand at room temperature for 1 h. The resulting precipitate was collected by filtration. The precipitate was then recrystallized from ethanol–water (5:1). The recrystallization procedure was repeated four times to give the colourless needle crystals. The crystals were dissolved in 150 ml of 25% hydrochloride acid. The solution was stirred for 1 h at room temperature, diethyl ether was added, the mixture was separated, and the aqueous layer was re-extracted. The combined extracts were dried over sodium sulphate, and the solvent was evaporated to dryness under reduced pressure. The residue was recrystallized from acetonitrile to give 1.12 g (8.96%) of white crystals. A 1% solution of the SP enantiomer in methanol had an optical rotation ($[\alpha]_D^{20}$) of +41.7°.

A similar procedure was followed using *R*-(+)-1-phenylethylamine as chiral base. (−)-SP-d_0 ($[\alpha]_D^{20}$ −43.5°) was obtained in 5.40% yield.

(±)-SP-d_3 was resolved into its enantiomers in the same manner as (±)-SP-d_0. (+)-SP-d_3 ($[\alpha]_D^{20}$ +41.4°) and (−)-SP-d_3 ($[\alpha]_D^{20}$ −41.5°) were obtained in 9.45 and 10.8% yield, respectively.

The enantiomeric purities were determined by GC–MS–SIM using (±)-SP-d_7 as an internal standard.

Stock solutions

Stock solutions of (+)-SP-d_0 (10.145 mg per 50 ml), (−)-SP-d_0 (9.915 mg per 50 ml), (+)-SP-d_3 (10.102 mg per 50 ml), (−)-SP-d_3 (9.904 mg per 50 ml) and (±)-SP-d_7 (10.016 mg per 50 ml) were prepared in methanol. Storage of these solutions at 4°C did not result in any detectable decomposition for more than six months. All analyses were performed by diluting the stock solutions with methanol (20 ng/μl, 2 ng/μl).

GC–MS–SIM

GC–MS–SIM measurements were made with a Shimadzu QP-1000 gas chromatograph–mass spectrometer equipped with a data processing system. A methylsilicone bonded-phase fused-silica capillary column SPB-1 (10 m × 0.25 mm I.D.) with a 0.25-μm thin film (Supelco, Bellefonte, PA, U.S.A.) was connected directly to the ion source. Helium was used as the carrier gas at 0.8 kg/cm^2. A splitless injection system SPL-G9 (Shimadzu, Kyoto, Japan) was used with a septum purge flow-rate of 1.0 ml/min and a split vent flow-rate of 30

ml/min. The purge activation time was 1 min after injection. The column temperature was set at 220°C and increased at 40°C/min to 280°C. The mass spectrometer was operated in chemical ionization mode with isobutane as the reactant gas at a pressure of $2 \cdot 10^{-5}$–$5 \cdot 10^{-5}$ Torr. The ionization voltage and ionization current were 200 eV and 150 μA, respectively. The ion source temperature was 280°C. The multiple-ion detector was focused on the quasi-molecular ions of the (−)-NEA derivatives of suprofen ($^2H_0$, *m/z* 414; $^2H_3$, *m/z* 417; $^2H_7$, *m/z* 421).

Sample preparation for GC–MS–SIM

Frozen plasma samples were thawed at room temperature. To a PTFE-lined screw-cap culture tube (100 mm×16 mm I.D.) were added 1.0 ml of plasma and 2 μg of (±)-SP-d_7 dissolved in 100 μl of methanol. The plasma sample was allowed to stand for 30 min at room temperature. Following addition of 1 ml of 1 *M* hydrochloric acid, the plasma sample was extracted with diethyl ether (two 3-ml portions). The extract was back-extracted with 1 ml of 0.1 *M* sodium hydroxide. The aqueous layer was acidified with 1 ml of 1 *M* hydrochloric acid and extracted with diethyl ether (two 3-ml portions). The organic layer was transferred to a PTFE-lined screw-cap conical centrifuge tube (100 mm×10 mm) and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 1 ml of dichloromethane, and 100 μg of HOBT in 100 μl of dichloromethane containing 1% pyridine, 100 μg of WSCD in 100 μl of dichloromethane and 100 μg of (−)-NEA in 100 μl of dichloromethane were added. The mixture was shaken for 30 s on a vortex mixer and left at room temperature for 1.5 h. The solution was washed with water (two 1-ml portions) and then evaporated under a stream of nitrogen. The residue was dissolved in dichloromethane and subjected to thin-layer chromatography (TLC) on Kieselgel $60F_{254}$ plates (0.25 mm thickness; Merck, Darmstadt, F.R.G.). The TLC plate was developed with ethyl acetate and the UV-positive zone corresponding the standard (±)-SP-d_0-(−)-NEA with an R_F value of 0.58–0.65 was scraped off. Suprofen-(−)-NEAs were eluted with 5 ml of dichloromethane and the solvent was evaporated. The residue was dissolved in 50 μl of dichloromethane and a 0.5-μl of the solution was subjected to GC–MS.

Calibration curves and quantitation

Calibration curves for (+)-SP-d_0, (−)-SP-d_0, (+)-SP-d_3 and (−)-SP-d_3 were prepared from a series of sample solutions containing known amounts of (+)-SP-d_0, (−)-SP-d_0, (+)-SP-d_3 and (−)-SP-d_3 (10–0.1 μg) dissolved in methanol. Following the addition of 2 μg of (±)-SP-d_7 dissolved in methanol, the solvent was evaporated. The samples were purified and derivatized as described above. Each sample for GC–MS–SIM was prepared in triplicate. The peak-area ratios of *m/z* 414 versus *m/z* 421 ($^2H_0/^2H_7$) and *m/z* 417 versus *m/z* 421 ($^2H_3/^2H_7$) were determined by GC–MS–SIM in triplicate. The curves

were obtained by an unweighted least-squares linear fitting of the peak-area ratios versus the mixed molar ratios on each sample. Plasma concentrations were calculated by comparing the peak-area ratios obtained from the unknown samples with those obtained from the standard mixtures.

Drug administration

Two healthy adult male volunteers (subject A: 24 years, 62 kg; subject B: 23 years, 75 kg) participated in the study. After an overnight fast, they were administered orally a near-equimolar mixture of (±)-SP-d_0 and (±)-SP-d_3 as their sodium salts prepared by dissolving 100 mg of (±)-SP-d_0, 100 mg of (±)-SP-d_3 and 30.6 mg of sodium hydroxide in 200 ml of water. No food was permitted for 3 h after drug administration. Heparinized blood samples (8 ml) were drawn just before the oral dose and at 10, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after dosing. Plasma was separated and stored at −20°C until analysis.

RESULTS AND DISCUSSION

In the synthesis of deuterium-labelled suprofen for use as the pseudo-racemate, special attention was paid to the selective deuterium labelling of the methyl group. The methyl position was chosen not only because it offered the possibility of introducing three deuterium atoms but also because this position seemed not to suffer from any primary isotope effect. The main metabolic pathways of suprofen [18], i.e. reduction of the ketone group to an alcohol, hydroxylation of the thiophene ring and conjugation with glucuronic acid, do

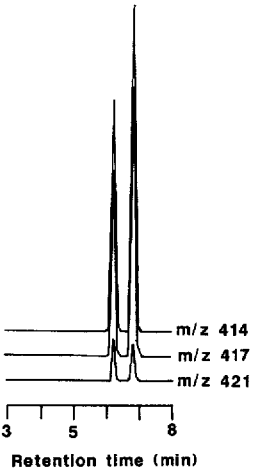

Fig. 2. SIM profiles of extracts from human plasma following the oral administration of a near-equimolar mixture of (±)-SP-d_0 and (±)-SP-d_3. (±)-SP-d_7 was added as an internal standard.

TABLE I

CONTRIBUTIONS TO ION INTENSITIES OF VARIOUS SPECIES IN CHANNELS MONITORED

Compound	*m/z* 414	*m/z* 417	*m/z* 421
(+)-SP-d_0	100	2.39	0.00
(+)-SP-d_3	0.42	100	0.58
(+)-SP-d_7	0.25	0.55	100
(−)-SP-d_0	100	2.35	0.00
(−)-SP-d_3	0 54	100	0 61
(−)-SP-d_7	0 35	0.66	100

TABLE II

ACCURACY OF SELECTED-ION MONITORING OF (+)-SP-d_0 AND (−)-SP-d_0 IN HUMAN PLASMA

Added (μg)	Found (μg)				C.V. (%)	Relative error (%)
	Individual values			Mean ± S.D.		
(+)-SP-d_0						
9.33	9.53	9.55	8.88	9.32 ± 0.31	3.33	−0.1
3.73	3.49	3.46	3.72	3.56 ± 0.12	3.37	−4.6
0.93	0.91	0.92	0.98	0.94 ± 0.03	3.19	1.1
0.37	0.38	0.39	0.37	0.38 ± 0.01	2.63	2 7
0.093	0.103	0.091	0.092	0.095 ± 0.005	5.26	2.2
(−)-SP-d_0						
10.73	11.00	10.75	10.51	10.75 ± 0.20	1.86	0 2
4.29	4.01	3.80	3.91	3.91 ± 0.09	2.30	−8.9
1.07	1.11	0.99	1.05	1.05 ± 0.05	4.76	−1.9
0.43	0.42	0.43	0.43	0.43 ± 0.005	1.16	0.0
0.107	0.114	0.102	0.100	0.105 ± 0.006	5.71	−1.9

not involve any reaction at the methyl position. Introduction of three deuterium atoms was achieved by treatment of the sodium malonate derivative with [$^2H_3$]methyl iodide followed by hydrolysis. EI–MS analysis of the final product demonstrated very high isotopic purity of (±)-SP-d_3 (99.0% deuterium atoms). (±)-SP-d_7 for use as an analytical internal standard was prepared from [$^2H_5$]fluorobenzene and [$^2H_3$]methyl iodide. The isotopic purity of the product was sufficiently high for use as an analytical internal standard, being 98.3% deuterium atoms ($^2H_7$, 88.8%; $^2H_6$, 10.7%; $^2H_5$, 0.5%; $^2H_4$, 0.0%).

In the separation of the 2AP enantiomers using GC or high-performance liquid chromatography, a diastereoisomeric amide derivative is formed by re-

TABLE III

ACCURACY OF SELECTED-ION MONITORING OF (+)-SP-d_3 AND (−)-SP-d_3 IN HUMAN PLASMA

Added (μg)	Found (μg)				C.V. (%)	Relative error (%)
	Individual values			Mean ± S.D.		
(+)-SP-d_3						
10.24	10.81	10.48	10.31	10.53 ± 0.21	1.99	2.8
4.10	3.92	4.15	4 16	4.08 ± 0.11	2.70	−0.5
1.02	0.95	0.99	1.03	0.99 ± 0.03	3.03	−2.9
0.41	0.40	0.42	0.41	0.41 ± 0.01	2.44	0.0
0.102	0.108	0.102	0.100	0.103 ± 0.003	2.91	1.0
(−)-SP-d_3						
9.77	10.47	10.28	10.28	10.34 ± 0.09	0.87	5.8
3.91	3.74	3.85	3.76	3.78 ± 0.05	1.32	−3.3
0.98	0.95	0.96	0.97	0.96 ± 0.01	1.04	−2.0
0.39	0.37	0.40	0.38	0.38 ± 0.01	2.63	−2.6
0.098	0.096	0.092	0.093	0.094 ± 0.002	2.13	−4.1

action of the carboxylic acid group of the 2APs with a chiral amine, such as amphetamine [19], leucinamide [20–22], phenylethylamine [23–25] or NEA [26,27]. The derivative most suitable for GC separation of (+)-suprofen from (−)-suprofen was found by the preparation of the amide of those compounds with (−)-NEA. The derivatization procedure was essentially the same as that proposed by Hutt et al. [26]. (±)-[^{14}C]Suprofen was used to determine the overall formation of the diastereomeric amides. The radioactivity in the reaction mixture was determined by capillary radio-GC [28]. The total GC peak yield [29] of (+)- and (−)-suprofen derivatives was 74 ± 1.5%. The ratio of the (+)- to (−)-suprofen diastereoisomer was 1.0, indicating that there was no stereoselective derivatization of suprofen under the conditions employed.

The chemical ionization mass spectra of (±)-SP-d_0-(−)-NEA, (±)-SP-d_3-(−)-NEA and (±)-SP-d_7-(−)-NEA demonstrated that the respective quasi-molecular ions at m/z 414, 417 and 421 were prominent. When the quasi-molecular ions were monitored, the sensitivity limit of the GC–MS–SIM assay was found to be 50 pg. A signal-to-noise ratio of at least 2.5 was used as a criterion for a significant response for the injected suprofen.

(±)-SP-d_0 and (±)-SP-d_3 were each resolved into their enantiomers by fractionated crystallization as their phenylethylamine salts. The enantiomeric purities were determined by GC–MS–SIM using (±)-SP-d_7 as an internal standard and were found to be 92.77% for (+)-SP-d_0, 93.46% for (−)-SP-d_0, 92.44% for (+)-SP-d_3 and 90.38% for (−)-SP-d_3. After resolution of the en-

TABLE IV

DAY-TO-DAY PRECISION

$n = 10$ for each concentration.

Added (μg)	Found (μg)	C.V. (%)	Relative error (%)
(+)-SP-d_0			
9.33	9.27 ±0.36	3.88	−0.6
3.73	3.62 ±0.16	4.42	−2.9
0.93	0.93 ±0.05	5.38	0.0
0.37	0.35 ±0.01	2.86	−5.4
0.093	0.096 ±0.005	5.21	3.2
(−)-SP-d_0			
10.73	10.77 ±0.40	3.71	0.4
4.29	4.15 ±0.15	3.61	−3.3
1.07	1.05 ±0.05	4.76	−1.9
0.43	0.41 ±0.01	2.44	−4.7
0.107	0.109 ±0.004	3.67	1.9
(+)-SP-d_3			
10.24	10.45 ±0.38	3.64	2.1
4.10	4.16 ±0.12	2.88	1.5
1.02	1.05 ±0.03	2.86	2.9
0.41	0.39 ±0.01	2.56	−4.9
0.102	0.110 ±0.003	2.73	7.8
(−)-SP-d_3			
9.77	10.09 ±0.41	4.06	3.3
3.91	4.04 ±0.12	2.97	3.3
0.98	0.98 ±0.03	3.06	0.0
0.39	0.37 ±0.01	2.70	−5.1
0.098	0.104 ±0.004	3.85	6.1

antiomers, the elution order was determined. The (+)-suprofen derivatives eluted at 6.2 min and the (−)-suprofen derivatives eluted at 6.7 min (Fig. 2).

Because of the natural abundance of ^{2}H, ^{13}C and ^{18}O, a small peak at m/z 417 may appear in the mass spectrum of (±)-SP-d_0-(−)-NEA. In addition, there is also the possibility that (±)-SP-d_3-(−)-NEA could contribute to the m/z 414 peak and (±)-SP-d_7-(−)-NEA to the m/z 414 and/or m/z 417 peaks. The mutual contributions to ion intensities of various species in channels monitored were shown in Table I. The labelled compounds possessed sufficiently high isotopic purity and the contributions to the other ions were minor. However, there was the possibility that the concentration of SP-d_3 in the pseudo-racemate study was 10- to 100-fold greater than that of SP-d_0, and the

TABLE V

PLASMA CONCENTRATIONS OF (+)-SP-d_0, (−)-SP-d_0, (+)-SP-d_3 AND (−)-SP-d_3 IN HUMANS FOLLOWING ORAL ADMINISTRATION OF A NEAR-EQUIMOLAR MIXTURE OF (±)-SP-d_0 AND (±)-SP-d_3

Time (min)	(+)-SP-d_0 (nmol/ml)	(−)-SP-d_0 (nmol/ml)	(+)-SP-d_3 (nmol/ml)	(−)-SP-d_3 (nmol/ml)	(+)-SP-d_0/ (+)-SP-d_3	(−)-SP-d_0/ (−)-SP-d_3
Subject 1						
10	12.14	14.44	12.34	14.66	0.98	0.99
20	30.77	39.22	30.99	38.39	0.99	1.02
30	31.89	45.79	32.51	46.26	0.98	0.99
45	20.28	31.62	20.58	31.37	0.99	1.01
60	13.91	24.24	14.05	23.51	0.99	1.03
90	6.45	13 45	6.53	12.91	0.99	1.04
120	4.19	9.72	4.14	9.30	1.01	1.04
180	1.92	5.46	1.90	5.20	1.01	1.05
240	0.77	2.23	0.76	2.16	1.01	1.03
360	0.28	0.58	0.26	0.57	1.07	1.01
$t_{1/2}$ (min)	61	58	60	59	1.02	0.98
AUC[a]	2050	3579	2065	3502	0.99	1.02
Subject 2						
10	25.20	31.58	25.48	31.67	0.99	1.00
20	24.43	31.89	24.65	31.22	0.99	1.02
30	16 60	23.86	16.75	22.79	0.99	1.05
45	11.52	18.17	11.77	18.53	0.98	0.98
60	9.18	15.94	9.30	15.49	0.99	1.03
90	4.73	9.03	4.86	9.34	0.97	0.97
120	3.07	6 84	3.08	6.94	1.00	0.98
180	1.42	3.57	1.37	3.34	1.04	1.07
240	0.77	2.11	0.76	2.01	1.01	1.05
360	0 32	0.54	0.31	0.53	1.04	1.01
$t_{1/2}$ (min)	75	66	74	66	1.01	1.00
AUC[a]	1568	2631	1580	2579	0.99	1.02

[a]Units are nmol min/ml.

converse. In such cases the contributions were significant, and corrections were made based on the values in Table I.

Calibration curves were prepared from a series of samples containing various amounts (0.4–10.0 μg) of (+)-SP-d_0, (−)-SP-d_0, (+)-SP-d_3 or (−)-SP-d_3, and a constant amount (2.0 μg) of (±)-SP-d_7. Each sample was then analysed in triplicate by monitoring the ions at m/z 414, 417 and 421. When the peak-area ratios were plotted against the mixed molar ratios, a good correlation was found between the observed peak-area ratios and the mixed molar ratios. The correlation coefficients were greater than 0.999.

The accuracy of the assay was determined for (+)-SP-d_0, (−)-SP-d_0, (+)-

SP-d_3 and (−)-SP-d_3 added to 1.0-ml aliquots of blank human plasma. The plasma samples were spiked with multiple standard solutions of (+)-SP-d_0, (−)-SP-d_0, (+)-SP-d_3 and (−)-SP-d_3 in the concentration range 0.09–10.7 μg/ml. The results are presented in Tables II and III. The estimated amounts were in good agreement with the actual amounts added. The inter-assay coefficients of variation (C.V.) for each enantiomer at all concentrations were less than 6%. Day-to-day precision of the assay was determined for a period of ten working days by performing triplicate analyses on plasma samples. The results listed in Table IV demonstrated an excellent reproducibility.

The present GC–MS–SIM method was applied for the quantitation of plasma concentrations of (+)-SP-d_0, (−)-SP-d_0, (+)-SP-d_3 and (−)-SP-d_3 after oral administration of a near-equimolar mixture of (±)-SP-d_0 and (±)-SP-d_3 (100 mg each) to two healthy subjects. There was no interference from endogenous compounds or metabolites of (±)-SP-d_0 and (±)-SP-d_3 in the vicinity of the peaks of analytes in the mass fragmentograms (Fig. 2). Plasma concentrations of (±)-SP-d_0 and (±)-SP-d_3 could be followed for up to 6 h (Table V). No appreciable differences between the plasma concentrations of the unlabelled and $^2H_3$-labelled analogues were observed. Also, the half-lives and areas under the curve of $^2H_3$-labelled compounds were practically the same as those of the unlabelled compounds. From these results, the pharmacokinetics of (±)-SP-d_3 could be considered to be equivalent to that of (±)-SP-d_0. A pharmacokinetic study of suprofen enantiomers after administration of stable isotopically labelled pseudo-racemate is now in progress and will be described in detail elsewhere.

The present method provides a sensitive and reliable technique to determine plasma levels of suprofen enantiomers with good accuracy and precision, and can be applied to pharmacokinetic studies of suprofen enantiomers.

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