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Assay and single dose pharmacokinetics of a novel systemic acyl coenzyme A cholesterol O-acyltransferase inhibitor, RP 73163, in rat plasma using automated solid-phase extraction with high-performance liquid chromatography

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Abstract

RP 73163, (*S*)-2-[5-(3,5-dimethylpyrazol-1-yl)pent-1-yl]sulphinyl-4,5 diphenylimidazole (I), is a highly potent in vitro and in vivo inhibitor of acyl coenzyme A cholesterol O-acyltransferase (ACAT) (E.C. 2.3.1.26), and as such it has potential therapeutic use as a cholesterol lowering agent in man. A method has been developed for the extraction and assay of I from rat plasma, using a fully automated solid-phase extraction column (ASPEC) technique, coupled to a reversed-phase HPLC system with detection by native fluorescence. The method has been validated over the concentration range 10–500 ng/ml, with demonstrated linearity, precision and accuracy, the mean limit of detection being 6.6 ± 1.3 ng/ml. Application of the method to the assay of samples following administration of the compound to male and female rats is reported, together with determined pharmacokinetic parameters.

1. Introduction

The compound (*S*)-2-[5-(3,5-dimethylpyrazol-1-yl)pent-1-yl]sulphinyl-4,5-diphenylimidazole (I, Fig. 1a) [1,2] has been demonstrated to potently inhibit the acyl coenzyme A cholesterol O-acyltransferase (ACAT) enzyme (E.C. 2.3.1.26) in various animal species, both with in vitro preparations from various tissue sources and in vivo. This enzyme is fundamental in the intracellular formation of cholesteryl esters and has been implicated in the development of atherosclerosis by (a) its moderation of dietary cholesterol uptake from the gut and its ensuing transport of esterified cholesterol across the intestinal membranes [3–5], (b) the hepatic biosynthesis of cholesteryl esters, with subsequent formation and secretion of very low density lipoproteins (VLDLs) [6,7] and (c) the intracellular storage of cholesteryl esters and the resulting formation of arterial lesions [8,9].

Initial exploratory studies in the rabbit indicated that I was readily absorbed following oral administration and therefore that the product

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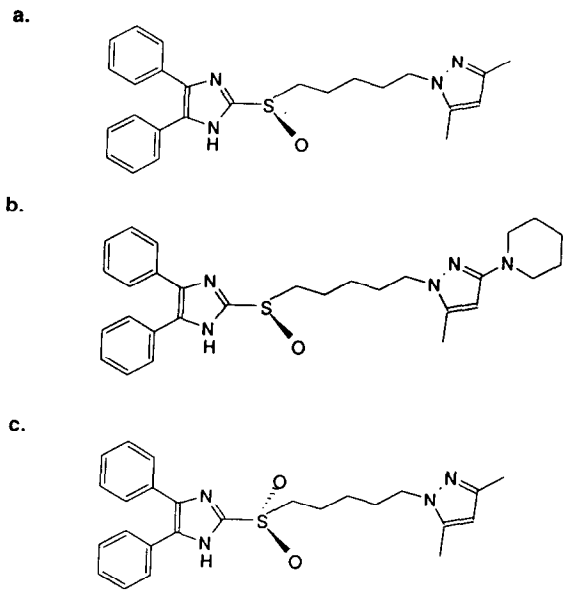

Fig. 1. Structures of (a) compound I, (b) compound II (internal standard), and (c) the sulphone metabolite of I, compound III.

has a potential therapeutic use as a systemic cholesterol lowering agent in man. Systemic availability at therapeutically useful concentrations suggests that this compound could act upon both circulating concentrations of low density lipoproteins (LDLs), a proven atherogenic factor derived from VLDLs and directly upon arterial cholesteryl ester formation, resulting in reduced plaque formation.

The methodology reported for the determination of I in rat plasma has been fully validated and applied to the assay of samples following single-dose oral (10, 30, 100 and 300 mg kg^{-1} body weight) and intravenous (2.5 mg kg^{-1} body weight) administration to male and female animals, thus allowing pharmacokinetic parameters, including the absolute bioavailability, of the compound to be determined.

2. Experimental

2.1. Materials

Acetonitrile (Chromanorm HPLC grade) was obtained from RP Prolabo (Rhône-Poulenc Laboratory Products, Manchester, UK), whilst high-purity water was prepared as required using an Elgastat UHQ PS purification system (Elga, High Wycombe, UK). Tetrabutyl ammonium bromide (TBAB, HPLC reagent grade) was purchased from FSA Laboratory Supplies (Loughborough, UK), laboratory grade (99.0% minimum purity) dipotassium hydrogen phosphate was purchased from BDH (Poole, UK) and orthophosphoric acid (90% w/v), from RP Prolabo. All of the above materials were used as supplied and without further purification or filtration.

Compound I, the chromatographic internal standard II (Fig. 1b) and the sulphone derivative and putative metabolite of I (III; Fig. 1c), were prepared within the Discovery Chemistry Department, Dagenham Research Centre, Rhône-Poulenc Rorer (UK).

2.2. Standard solutions

Stock solutions of each of the reference compounds were initially prepared in acetonitrile–water (1:1, v/v) at a concentration of 100 μg/ml. From these solutions, working standards were prepared by sequential dilution using the same solvent mixture to give 25, 5 and 1 μg/ml solutions of I, 50 and 25 μg/ml of II and 10 μg/ml of III.

2.3. High-performance liquid chromatography

A Merck Lichrospher RP 18 analytical cartridge column (LiChroCART, 12.5 cm × 4 mm I.D., 5 μm particle size) was obtained from BDH, being used in combination with a disposable pre-column containing 100-μm particle-size end-capped RP18 material. Connection was via a Manu-CART zero dead-volume fitting, also supplied by BDH.

The chromatographic system consisted of an LDL ConstaMetric III high pressure pump (Stone, UK), a Gilson automated sample preparation with extraction columns (ASPEC) system (supplied by Anachem, Luton, UK) and its associated sample dilutor, Model 401. A Jasco Model 821-FP fluorescence detector (supplied by Ciba Corning Diagnostics, Halstead, UK) was

utilised for the detection of eluted compounds, excitation and emission wavelengths being set to 270 nm and 400 nm, respectively.

The temperature of both the pre-column and analytical column was controlled with a Jones Chromatography block heater Model 7960 (Hengoed, UK). Data acquisition was automatically performed using PE Nelson Model 2600 Chromatography Software, version 5.1.5, via a 900 Series Intelligent Interface (PE Nelson Systems) and an IBM PC AT computer.

The mobile phase consisted of a mixture of buffer (10 m*M* TBAB and 10 m*M* aqueous dipotassium hydrogen phosphate, adjusted to pH 6.5 with dropwise addition of orthophosphoric acid) and acetonitrile (1:1, v/v). This was helium degassed for approximately 5 min prior to use. Separation of eluting compounds was achieved at a flow-rate of 1.0 ml/min and a temperature of 40°C; back-pressure was of the order of 1500 psi.

2.4. Diode-array spectroscopy

To obtain specimen UV-Vis spectra (210–500 nm wavelength) of the eluting compounds of interest a Waters 990 photodiode-array detector was used in conjunction with the previously described HPLC system, instead of the Jasco fluorescence instrument.

2.5. Preparation and extraction of calibration standards

Drug-free Sprague–Dawley rat plasma, from either male or female animals, was obtained by centrifugation of freshly acquired heparinised blood (4°C, 3000 rpm, 15 min). Aliquots of this plasma (0.5 ml) were spiked with 5–12.5 μl of compound I working standards (1.0–25 μg/ml) to provide eight individual concentrations in the range of 10–500 ng/ml. This was followed by the addition of 10 μl of the working internal standard solution (25 mg/ml), to provide a final concentration of 500 ng/ml plasma. Vortex-mixing for 30 s per sample was used to ensure sample homogeneity prior to loading on to the ASPEC system. Technicol (Higher Hillgate, UK) end-capped C_2 disposable extraction cartridges of 1.0-ml capacity and containing 100 mg of solid-phase material, were previously found to be suitable for this assay. Immediately before use, each cartridge was conditioned by sequential washing with 2.0 ml of acetonitrile and then 2.0 ml of high-purity water; this was performed using a resident programme supplied with the ASPEC. For each plasma sample, a sub-aliquot of 0.45 ml was automatically transferred to a conditioned cartridge and then forced through under positive air pressure for 20 s. The same cartridge was washed successively with 2.0 ml of water and then with 1.0 ml of water–acetonitrile (9:1, v/v). Elution of the compounds of interest was achieved using 600 μl of the chromatographic mobile phase, of which 510 μl was actually injected onto the HPLC system.

Peak-area ratios (PHRs) of compound I to the internal standard II were automatically calculated by the chromatographic computer software and a calibration graph of PHR versus the concentration of I added to the plasma was plotted. The $1/x$ weighted least-squares regression line of best fit to the data was calculated, to allow quantitation of drug concentrations in the test samples.

2.6. Accuracy and precision

Drug-free rat plasma (3×4.0 ml) was spiked with I at a concentration of either 10, 100 or 500 ng/ml, and with II (500 ng/ml), using appropriate standard solutions. Six replicate aliquots (0.5 ml) were then removed from each of the three bulk preparations and extracted and assayed according to the described procedure. Concentrations of I in plasma were determined against a concurrently prepared but independent standard curve, in order to determine the intra-assay (within-day) precision and accuracy. The procedure was repeated on two more occasions, to calculate further intra-assay data in addition to the inter-assay (between-day) precision and accuracy.

2.7. Limit of detection

For each calibration line, the limit of detection was calculated as the lowest concentration for which the peak-area ratio was distinguishable

from the calibration intercept (as calculated by $1/x$ weighted least-squares regression) with a 95% confidence interval. In practice, this was achieved using our "in house" RS1 LIMDET programme, in which the straight-line equation ($y = mx + c$) is used and y is set to the 95% confidence limit at the intercept. By transposition, $x = (y - c)/m$, and x is defined as the limit of detection.

2.8. Plasma storage stability

Drug-free rat plasma (12.0 ml) was spiked with a standard solution of I to give final concentrations of 25 and 250 ng/ml. Following vortex-mixing, aliquots (3×0.5 ml) were assayed immediately (day 0), whilst the remainder were sub-aliquoted and frozen at −21 to −24°C. Subsequently, over a period of up to 64 days, individual aliquots were thawed and a further three 0.5-ml replicates were assayed against a concurrently prepared standard curve.

2.9. Freeze–thaw stability

Drug-free rat plasma (20.0 ml) was spiked with a standard solution of I to give a final drug concentration of 100 ng/ml. From this bulk preparation, replicate aliquots (3×0.5 ml) were immediately taken and the concentration of I was determined; the remainder of the preparation was then frozen. On a further three occasions during the day, the bulk sample was again thawed, replicate aliquots were taken for assay and the remainder re-frozen.

2.10. Drug formulations

The solution of I used for intravenous administration was prepared at a concentration of 0.25% (w/v) and consisted of an aqueous solution of ethanol (20%, v/v), polyethylene glycol 400 (20%, v/v) and benzyl alcohol (0.5%, v/v). Suspensions of I for oral administration were prepared at concentrations of 0.2, 0.6, 2.0 and 6.0% (w/v), the vehicle consisting of aqueous sodium carboxymethylcellulose (1%, w/v) and Tween 80 (0.2%, w/v).

2.11. Animal study

Healthy male and female Sprague–Dawley CD rats (120–160 g on arrival) were obtained from Charles River UK (Margate, UK) approximately 1 week prior to experimentation. Animals were allowed water and food (ERD diet; Labsure, UK) ad libitum during this period of acclimatisation and experimentation.

Intravenous administration: For both sexes, a total of forty rats received the formulated product at 2.5 mg kg^{-1} body weight as a bolus injected into the jugular vein, previously exposed by surgery, whilst the animal was under isoflurane anaesthesia. Sacrifice of animals, four per time point, was by carbon dioxide asphyxiation at 5, 15, 30 min and 1, 2, 4, 6, 9, 12 and 24 h post dose.

Oral administration: For both sexes, a total of 144 rats were randomly assigned into four groups of thirty six animals. Group 1 of each sex received the formulated product by gavage at 10 mg kg^{-1} body weight, whilst groups 2, 3 and 4 received the drug at 30, 100 and 300 mg kg^{-1}, respectively. Groups of animals ($n = 4$ per time point) were sacrificed at 0.5, 1, 2, 3, 4, 6, 12, 24 and 96 h post dose.

For both animal phases, blood was removed into heparinised tubes by cardiac puncture; subsequent centrifugation (4°C, 3000 rpm, 15 min) was performed to obtain the corresponding plasma samples. These were then immediately frozen at −22 to −24°C until required for assay. All experimental work was performed in accordance with current UK Home Office guidelines.

2.12. Assay of test samples

Frozen plasma samples were allowed to thaw to room temperature immediately prior to use. To 0.5-ml aliquots, internal standard solution was added (10 μl of 25 μg/ml), followed by vortex-mixing (30 s). Samples were loaded on to the ASPEC system and assayed according to the

described method against a concurrently prepared calibration curve and process controls.

It should be appreciated that a number of samples, particularly at the earlier sampling times and for the higher dose groups, required dilution with drug-free plasma prior to processing, in order to remain within the upper limit of the validated calibration curve.

2.13. Preparation of process controls

Drug-free rat plasma (3 × 2.0 ml) was spiked with standard solutions of I at either 25, 100 or 375 ng/ml plasma, followed by internal standard solution (10 μl of 25 μg/ml). From each of these, 3 aliquots of 0.5 ml were removed and assayed.

2.14. Pharmacokinetics and statistics

Pharmacokinetics was undertaken using SIPHAR software package version 4.0 (Simed, Centre d'Etudes et de Recherches en Statistiques et Informatique Médicales, Creteil, France) on an IBM PS2 Model 60 personal computer. For the purposes of this study, the following pharmacokinetic parameters were determined: the time of maximal plasma concentration of I (T_{max}) and the maximum concentration of I (C_{max}), both after oral administration only. The terminal elimination rate constant β was calculated using non-weighted least-squares regression following natural log transformed elimination data points. β was subsequently used in the calculation of the elimination half-life ($T_{1/2}$) from the equation $T_{1/2} = \text{Ln } 2/\beta$ and the area under the plasma concentration–time curve ($AUC_{0-\infty}$) using the trapezoidal rule; that is,

$$AUC_{0-\infty} = \sum_{i=1}^{n-1} \frac{(c_{i+1} + c_i)(t_{i+1} - t_i)}{2} + \frac{c_n}{\beta}$$

where c_i denotes the plasma concentration of I at time t_i, c_n denotes the plasma concentration of I at the final sampling time n. Plasma clearance (Cl) was determined from intravenous data only using $Cl = \text{dose}/AUC_{0-\infty}$. Similarly, the apparent volume of distribution (Vd) was determined for intravenous data only from $Vd = Cl/\beta$. Bioavailability (F) was derived from the ratio of $AUC_{0-\infty}$ values (oral/i.v.) after dose normalisation:

$$\%F = \frac{AUC_{oral}}{AUC_{i.v.}} \cdot \frac{dose_{i.v.}}{dose_{oral}} \cdot 100$$

In order to statistically test for differences in systemic exposure to I following oral dosing, two-way analysis of variance was performed on $AUC_{0-\infty}$ data at all dose levels and with respect to sex, using the RS1 statistical package (B.B.N. Software).

3. Results

The described method was fully validated prior to its use; both method validation and sample assay were performed to the standards of Good Laboratory Practice (GLP).

3.1. Chromatographic separation

Fig. 2 provides representative chromatograms of extracted (a) drug-free rat plasma, (b) drug-free rat plasma, spiked with 100 ng/ml of I, 500 ng/ml of the internal standard II and 20 ng/ml of the putative sulphone metabolite III, and (c) sample plasma, taken 2 h post oral administration and spiked with internal standard only. As shown, all three compounds of interest were separated from co-extracted species endogenous to the plasma, eluting from the column with retention times of the order of 5.5 min (I), 7.0 min (III) and 11.3 min (II). It should be noted that the putative sulphone metabolite was indeed detected in a number of the study plasma samples.

No differences were detected in the chromatographic profiles of extracted drug-free plasma taken from male and female animals.

3.2. Peak assignment

Peak assignment was based upon both retention time data and diode-array spectra. Fig. 3 shows photodiode-array spectra obtained from

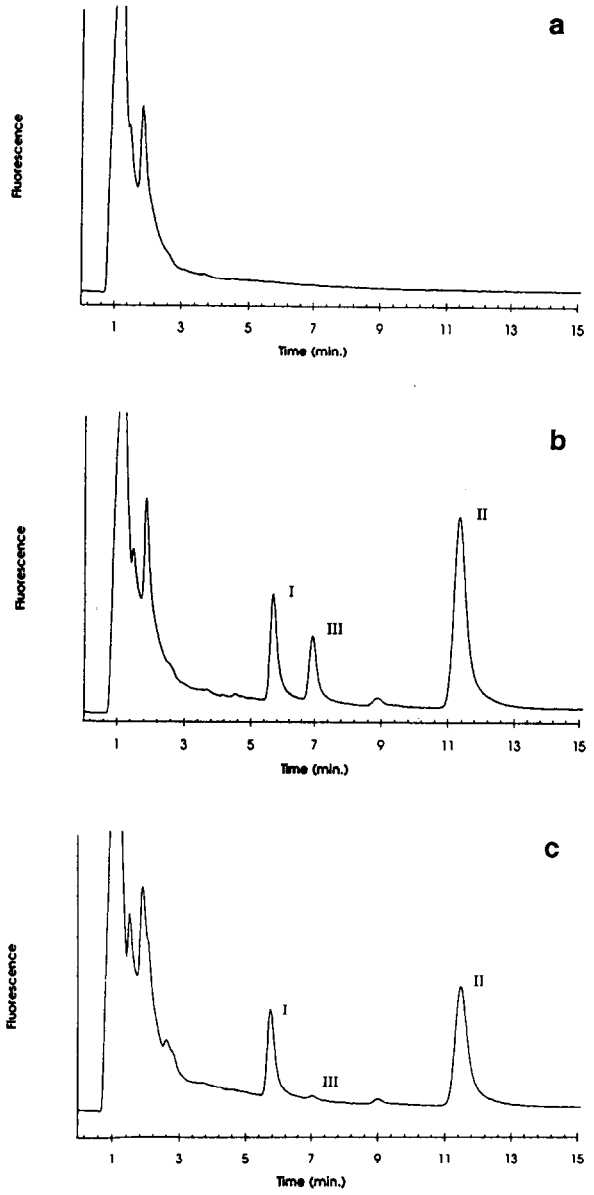

Fig. 2. Chromatograms of extracted (a) drug-free rat plasma, (b) drug-free rat plasma spiked with 100 ng/ml of I, 500 ng/ml of II and 20 ng/ml of III, and (c) sample plasma, taken 2 h post oral administration at 30 mg/kg (1:20 dilution), spiked with internal standard only.

the start, middle and trailing edges of the peak corresponding to the retention time of the suspected sulphone metabolite III, for both an extracted test sample and the authentic standard solution. As shown, these spectra were indistinguishable, thus confirming the peak assignment and the lack of any major interference in extracted samples. Comparable diode-array spectra were additionally obtained for compound I itself and the internal standard, again from extracted samples and standard solutions.

3.3. Extraction efficiency

The mean ± S.D. efficiency of plasma extraction for I (77.2 ± 1.2%) and II (51.1 ± 6.6%) was determined at a concentration of 100 ng/ml and 500 ng/ml, respectively. This was achieved by comparison of peak areas for extracted and non-extracted standards on 5 separate occasions.

3.4. Plasma storage stability

Sub-ambient storage (−21 to −24°C) of I in rat plasma was investigated at a concentrations of 25 and 250 ng/ml. These spiked plasma standards were assayed at the time of preparation (day 0) and subsequently on days 18, 36 and 64 of storage (25 ng/ml) or 6, 15, 27 and 41 days of storage (250 ng/ml). These data are summarised in Table 1. As shown, there is no apparent time-dependent decrease in the determined concentration at 25 ng/ml, although there was a possible non-significant downward trend at the higher concentration.

3.5. Freeze–thaw stability

Considering that plasma samples would require freezing and thawing on at least one occasion, and possibly more if re-assay was required, the freeze–thaw stability of I was investigated. Mean concentrations and associated standard deviation values determined following up to three freeze–thaw cycles are given in Table 2. The data suggest that the freeze–thaw process has no significant effect upon the determined plasma concentration. Consequently, there is no need to sub-divide the study plasma samples prior to freezer storage.

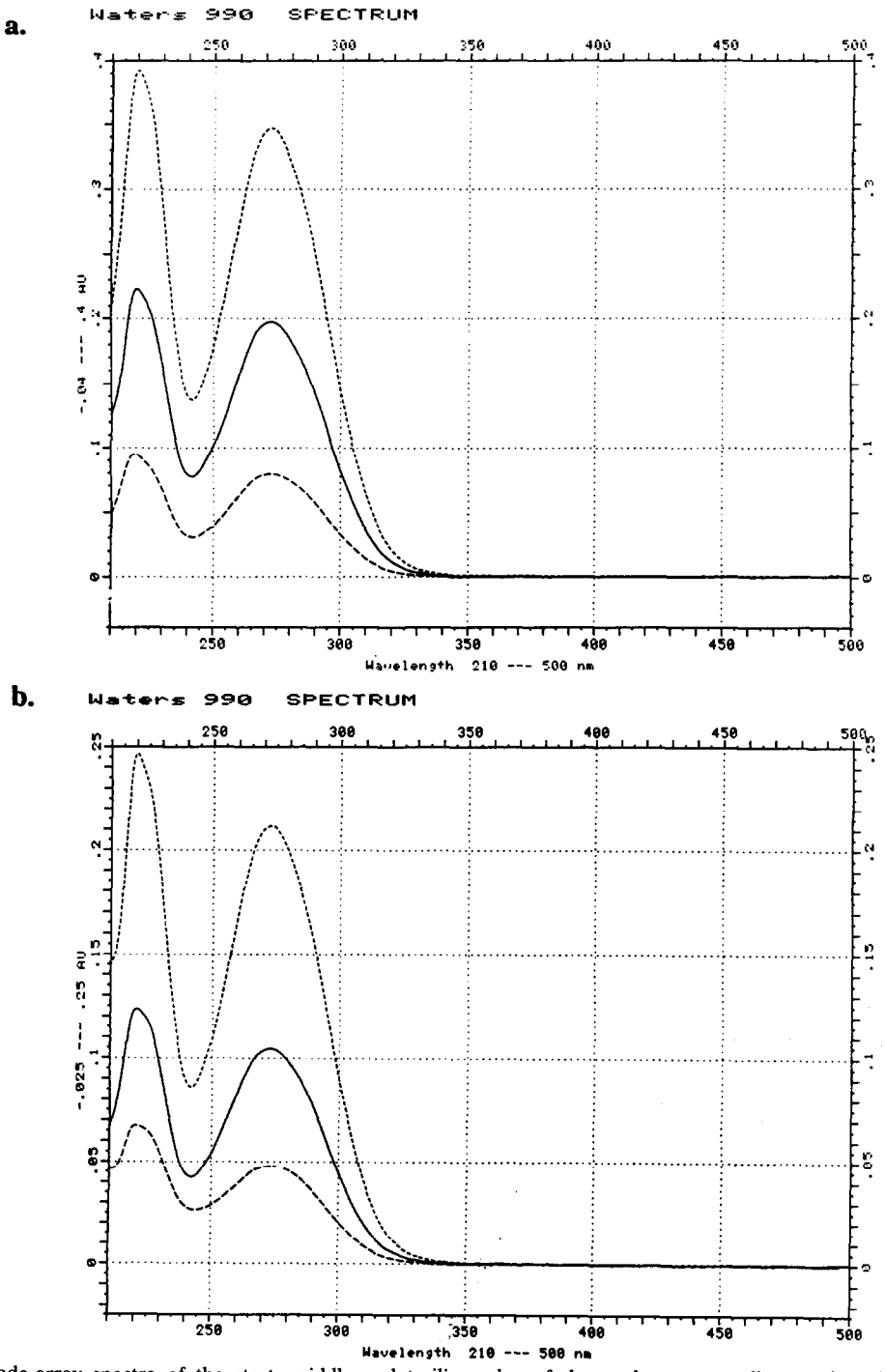

Fig. 3. Photodiode-array spectra of the start, middle and trailing edge of the peak corresponding to the retention time of compound III, the sulphone metabolite of I, from (a) an extracted test sample and (b) a standard solution of compound III.

Table 1
Plasma storage stability at −21 to −24°C

Nominal concentration (ng/ml)	Day of assay	Plasma concentration (mean ± S.D.) (ng/ml)	Deviation from day 0 (%)
25	0	24.2 ± 0.92	–
25	18	23.5 ± 2.60	−2.9
25	36	25.5 ± 0.96	+5.4
25	64	24.3 ± 1.55	+0.4
250	0	270.2 ± 29.6	–
250	6	290.8 ± 21.2	7.6
250	15	288.5 ± 8.9	6.8
250	17	237.2 ± 10.7	−12.2
250	41	243.1 ± 6.9	−10.0

Table 2
Plasma freeze–thaw stability at 100 ng/ml

Freeze–thaw cycle	Plasma concentration (mean ± S.D.) (ng/ml)	Deviation from preparation (%)
–	101.7 ± 14.5	–
1	100.7 ± 2.4	−0.98
2	100.3 ± 14.4	−1.4
3	103.2 ± 13.6	1.5

3.6. Linearity of method response and limit of detection

Linearity of response over the concentration range 10–500 ng/ml was demonstrated on the basis of peak-area ratios (I/II) versus concentration plots, using $1/x$ weighted least-squares linear regression analysis; this was performed both during method validation and sample assay, with correlation values (r^2) being greater than 0.99 in all cases. Data generated as part of the method validation are presented in Table 3, together with the limits of detection, calculated on the basis of 95% confidence limits. The mean (± S.D.) limit of detection was 6.6 ± 1.3 ng/ml. An eight-point calibration graph, prepared in drug-free rat plasma, with a single replicate at each concentration, representative of those used for method validation and sample analysis, is presented in Fig. 4.

3.7. Method accuracy and precision

Plasma concentrations of I were determined following spiking at 10, 100 and 500 ng/ml using six replicates per concentration per day. Intra- and inter-day parameters calculated from these data are summarised in Table 4. At concentrations of 100 and 500 ng/ml, good precision and accuracy is demonstrated both intra- and

Table 3
Linear regression parameters calculated on the basis of peak-area ratios

Assay validation	Slope of line	y-Intercept	Correlation coefficient (r^2)	Limit of detection (ng/ml)
1	$6.81 \cdot 10^{-3}$	$-7.67 \cdot 10^{-3}$	0.9948	6.1
2	$7.02 \cdot 10^{-3}$	$-2.26 \cdot 10^{-2}$	0.9960	5.6
3	$6.87 \cdot 10^{-3}$	$-9.85 \cdot 10^{-4}$	0.9909	8.1

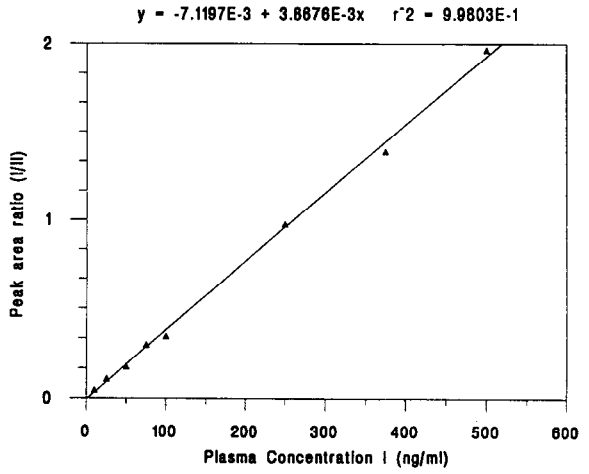

Fig. 4. A representative calibration curve for the assay of I, prepared in drug-free rat plasma.

inter-assay, mean concentrations being within the range 89–109% of the nominal value with a coefficient of variation (C.V.) of less than 10%. At 10 ng/ml, the assay proved to be accurate, but less precise than at the higher concentration, intra-assay accuracy being within the range of 81–109% of the nominal value. On the basis of these findings, the lower limit of quantitation for this method was defined as a concentration of 10 ng/ml, being within the previously published guidelines (within ± 20% precision and accuracy) for pharmacokinetic studies [10].

3.8. Assay of samples

During the assay of the study samples, triplicate process controls (PCs) at three concentrations were prepared and assayed as a check of the method's performance. On all occasions, accuracy and precision of these PC standards was within ± 15%; the criteria used for the rejection of an assay run.

3.9. Pharmacokinetics following oral administration

Mean compound I plasma concentration–time profiles (0–24 h) for male and female Sprague–Dawley rats following single oral administration at 10, 30 100 and 300 mg/kg are presented in Figs. 5 and 6, respectively. Quantifiable concentrations of I were not detected in any samples taken at 96 h post dose.

Following oral dosing, it appears that I becomes rapidly systemically available in high concentrations (Table 5). Mean T_{max} values tended to be in the range 0.5–4 h for male animals and 3–6 h for females, with a general trend to longer T_{max} values with increased dose. Also apparent is that mean C_{max} values in females were markedly higher than for males at corresponding dose levels. This was confirmed by comparison of the area under the mean

Table 4
Intra-assay ($n = 6$) and inter-assay ($n = 3$) precision and accuracy

Nominal concentration (ng/ml)	Precision (C.V., %)		Accuracy (%)	
	Intra-assay	Inter-assay	Intra-assay	Inter-assay
10	2.4		108	
10	5.3	15.9	109	99.3
10	8.9		81	
100	5.2		96.5	
100	2.2	5.5	89.3	95.1
100	3.8		99.6	
500	1.4		99.8	
500	4.2	7.8	93.4	100.8
500	2.6		109.1	

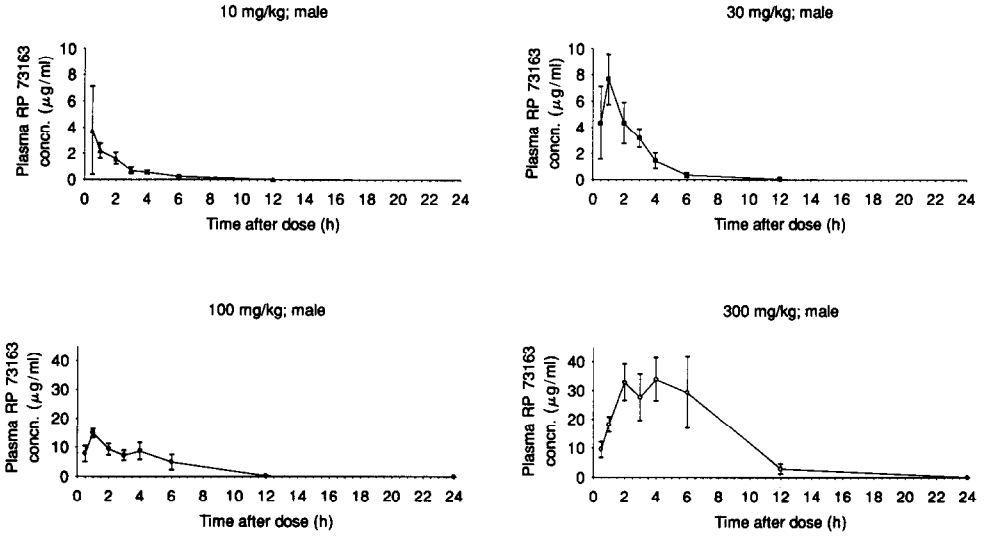

Fig. 5. Plasma concentration–time profiles for compound I following single oral administration to male rats ($n = 4$ per time point) at 10 (▲), 30 (■), 100 (●) and 300 (○) mg/kg.

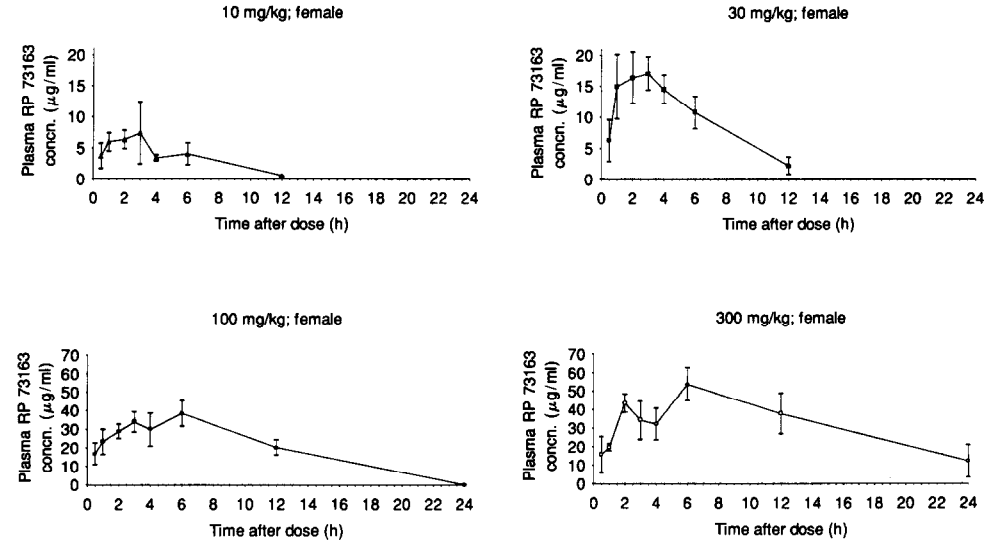

Fig. 6. Plasma concentration–time profiles for compound I following single oral administration to female rats ($n = 4$ per time point) at 10 (▲), 30 (■), 100 (●) and 300 (○) mg/kg.

plasma concentration–time profiles ($AUC_{0-\infty}$), a direct measure of systemic exposure of the animals to the drug. Application of two-way analysis of variance to the individual animal data rejected the null hypothesis that male and female results came from populations with the same mean ($p < 0.001$ at all dose levels). For both male and female animals, plots of $AUC_{0-\infty}$ versus dose (Fig 7: with the application of least squares linear regression analysis to obtain the

Table 5
Pharmacokinetic parameters determined for male and female Sprague–Dawley rats

Dose (mg/kg)	Sex	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	Cl (L/h/kg)	Vd (L/kg)	$AUC^{a}_{0-\infty}$ (μg/ml h)	F (%)
2.5; i.v.	M	na	na	2.87	0.520	2.15	4.81 (11.9)	na[b]
2.5; i.v.	F	na	na	3.28	0.157	0.741	15.97 (13.6)	na
10; p.o.	M	3.72	0.5	1.41	na	na	7.53 (12.7)	39.1
10; p.o.	F	7.38	3	2.67	na	na	45.25 (7.0)	70.8
30; p.o.	M	7.64	1	1.36	na	na	19.06 (6.0)	33.0
30; p.o.	F	17.05	3	2.61	na	na	127.01 (7.7)	66.3
100; p.o.	M	14.75	1	2.01	na	na	65.38 (3.0)	34.0
100; p.o.	F	38.60	6	2.39	na	na	470.3 (1.0)	73.6
300; p.o.	M	33.79	4	2.07	na	na	273.2 (0.9)	47.3
300; p.o.	F	53.69	6	8.22	na	na	918.4 (16.1)	47.9

[a] Values in brackets represent% of $AUC_{0-\infty}$ values extrapolated.
[b] na: not applicable.
The in-life phase of this experiment did not involve serial bleeding. Accordingly, for each dose and route of administration, the individual drug concentrations obtained for each of the 4 animals constituting a sacrifice group were meaned, at each of the designated time points, to obtain a mean drug concentration versus time profile. Pharmacokinetic parameters were calculated from these mean data. Consequently, it is not possible to express $t_{1/2}$, Cl, Vd, $AUC_{0-\infty}$ and F in terms of ± S.D.

line of best fit) indicated linear pharmacokinetics in both males ($r^2 = 0.9915$) and females ($r^2 = 0.9656$). There is however, a suggestion of plateauing towards the higher doses in the female animals.

3.10. Pharmacokinetics following intravenous administration

Mean plasma concentration–time profiles following intravenous dosing are presented in Fig. 8. As observed from the oral data, plasma concentrations in female animals were generally higher than for males for corresponding sampling times up to and including 12 h post administration. This is again reflected by comparison of calculated $AUC_{0-\infty}$ values; 15.97 and 4.81 μg/ml h for females and males, respectively. Sex differences are also apparent in the values determined for total plasma clearance, 0.16 and 0.52 l/h/kg, and the corresponding volumes of distribution, 0.74 and 2.15 L/kg, for female and male animals, respectively. Calculated plasma apparent terminal half-life values were 3.3 h (females) and 2.9 h (males) (Table 5).

3.11. Bioavailability

Bioavailability was shown to be greater in females, ranging from 47.9 to 73.6%, compared to 33.0–47.3% in males (Table 5). There was no apparent correlation between bioavailability and dose administered.

4. Discussion

The present paper is the first to describe an assay method for the determination of compound I in rat plasma. Using a coupled ASPEC solid-phase extraction and HPLC system with fluorescence detection, the method can be fully automated and is capable of the unattended analysis of up to 108 samples and standards, the maximum capacity of the ASPEC instrument. Using 0.5 ml of plasma, the linear range was determined to be from 10.0 ng/ml (the lower limit of accurate quantitation) to 500 ng/ml; the limit of detection was 6.6 ± 1.3 ng/ml. Potential difficulties, such as deterioration of sample during storage and the apparent loss of drug due to protein precipitation during the freeze–thaw

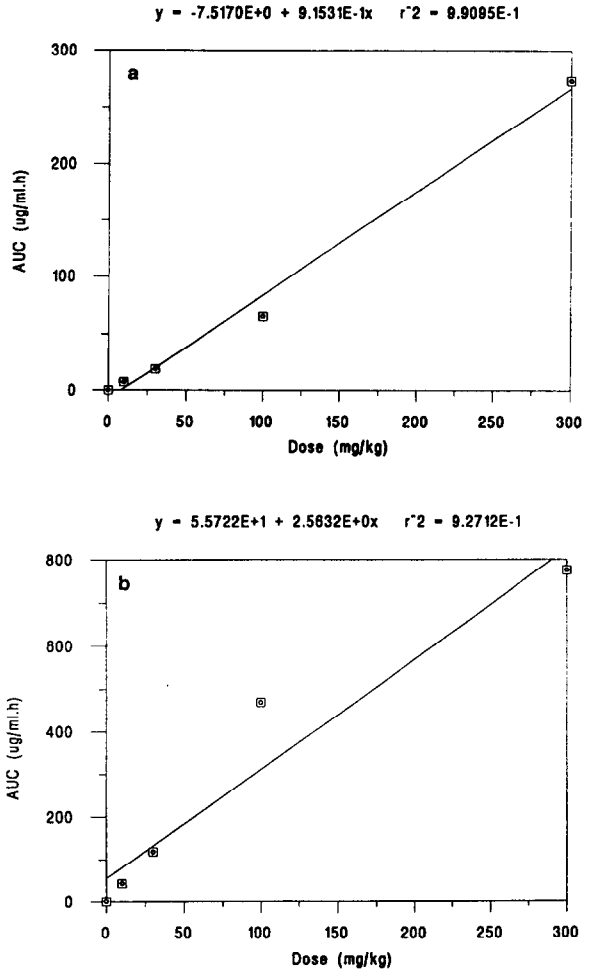

Fig. 7. Plot of plasma $AUC_{0-\infty}$ versus dose received for (a) male and (b) female Sprague–Dawley rats.

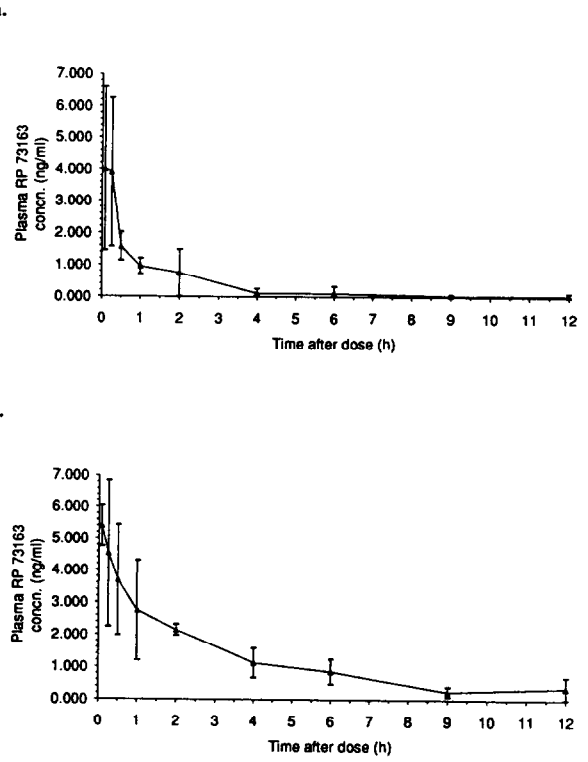

Fig. 8. Plasma concentration–time profiles for compound I following single intravenous administration to (a) male and (b) female rats ($n = 4$ per time point in both sexes).

process have been investigated and found not to occur.

The method has been successfully applied to the assay of both male and female plasmas, following i.v. and oral administration of the compound. As anticipated from preliminary investigations, compound I was demonstrated to be bioavailable in the rat, although this was dependent upon sex, as were other determined parameters. The greater systemic exposure in females is considered to be a reflection of the relatively higher metabolic potential of the male rat [10,11]. The sulphone metabolite of I was detected in post administration samples (male and female), the corresponding peak being well separated from that of the drug and internal standard.

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