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Short Communication

Measurement of plasma concentrations of a combined histamine H_1- and H_2-receptor antagonist (SK&F 93319)

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Introduction

Effective H_1- and H_2-receptor antagonist activities have been combined in a single molecule, SK&F 93319 (2-[4-(3-methoxypyrid-2-yl)butylamino]-5-[(6-methyl-pyrid-3-yl)methyl]-pyrimidin-4-one]), which has approximately equipotent antagonist activities at both types of histamine receptor [1].

The possible utility of such combined H_1- and H_2-antagonist activity was first discussed by Black *et al.* [2] and the presence of both actions in a single molecule was described by Harvey and Owen [3]. It is desirable to be able to follow the plasma kinetics of such a compound, particularly during the period of pharmacological activity.

The present paper describes a normal-phase HPLC assay for SK&F 93319 in plasma, which has been developed for use in pharmacokinetic studies. The method is a modification of the original assay for cimetidine and cimetidine sulphoxide [4] and is similar to that used for oxmetidine, and another H_2-receptor antagonist [5].

Experimental

Materials

SK&F 93319 was used as the trihydrochloride salt and was >99% pure. 2-[4-3-chloropyrid-2-yl)butylamino]-5-(6-methyl-pyrid-3-yl methyl)pyrimid-4-one (SK&F 93333) was used as the internal standard and was 98.9% pure. Both substances were

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synthesized by the Chemical Support Department of SK&F Research Ltd (Welwyn, UK).

All other chemicals were analytical grade and were obtained from May & Baker Ltd (Dagenham, UK) except octan-1-ol (Puriss), which was obtained from Fluorochem Ltd (Glossup, Derbyshire, UK).

Organic solvents and water for HPLC were HPLC grade (Rathburn Chemical Ltd, Peebleshire, UK) and were filtered through Millipore type FH filters (organic solvents) or type HA filters (water) prior to use.

High-performance liquid chromatograpy

The chromatograph was equipped with a 710B automatic sampler (WISP), a M6000A pump and a 441 fixed wavelength (229 nm) detector (Waters Associates, Cheshire, UK) and an LDC 301 recording integrator (Laboratory Data Control, Staffordshire, UK). The 250 × 4.6 mm i.d. columns were packed with 5-μm Lichrosorb Si 60 (HPLC Technology Ltd, Cheshire, UK).

The mobile phase was a degassed mixture of acetonitrile–methanol–water (260:80:10, v/v/v) to which strong ammonia solution (0.88sg) (1.5, v) was added.

The flow rate was 2.0 ml min^{-1} and the column was maintained at ambient temperature.

Preparation and storage of stock solutions

Stock solutions of SK&F 93319 (trihydrochloride) and SK&F 93333 (base) were both prepared by dissolving the required weight of the compound in a minimal volume of methanol, then diluting with ethanol to give the required concentration. Stock solutions prepared in this manner may be stored at −20°C for 4 weeks.

Collection and storage of plasma samples

Blood was drawn into tubes containing lithium heparin as anticoagulant. The plasma was separated by centrifugation for 10 min at 1500 ***g*** and at 4°C, transferred to non-heparinized tubes and stored at −20°C. Under these conditions the plasma samples may be stored for at least 12 weeks.

Assay of SK&F 93319 in plasma

To 1 ml of plasma in a polypropylene centrifuge tube (12 ml) was added a solution of the internal standard in ethanol (50 μl, equivalent to 5.0 μg of SK&F 93333). Octan-1-ol (5 ml) was then added, the tube was stoppered, and the contents mixed gently for 15 min with a blood cell suspension mixer. After centrifugation for 5 min at 2000 ***g***, the octanol layer (4.5 ml) was transferred to a second polypropylene tube containing 0.02 M hydrochloric acid (3.0 ml). The mixing and centrifugation processes were repeated; the octanol was then removed as completely as possible by aspiration and discarded.

The acid layer (2.5 ml) was transferred to a third polypropylene tube; care was taken to avoid contamination from the residual octanol. Acetonitrile (300 μl) was added followed by sufficient solid potassium carbonate (approximately 5 ***g***) to saturate the aqueous extract. The contents of the tube were mixed with a vortex mixer. After centrifugation for 5 min at 2000 ***g***, the acetonitrile formed a discrete layer above the aqueous solution. This layer (200 μl) was transferred to a low-volume autosampler vial for analysis by HPLC.

The acetonitrile phase may be stored for 3 days at −20°C prior to analysis.

Results

The HPLC method allowed base line separation of the internal standard (SK&F 93333) and the drug (SK&F 93319). The retention times for the internal standard and the drug were 4.5 and 5.5 min, respectively (Fig. 1). Chromatograms of extracted control (drug-free) plasma from humans, cynomolgus monkeys, dogs and rats contained no peaks that interfered with the method. The limit of detection was therefore set by instrument noise and was 7.5 ng on column for SK&F 93319. The limit of reliable measurement for SK&F 93319 was set at 100 μg l^{-1} for 1 ml of plasma because the precision (>20%) at lower concentrations was not considered acceptable.

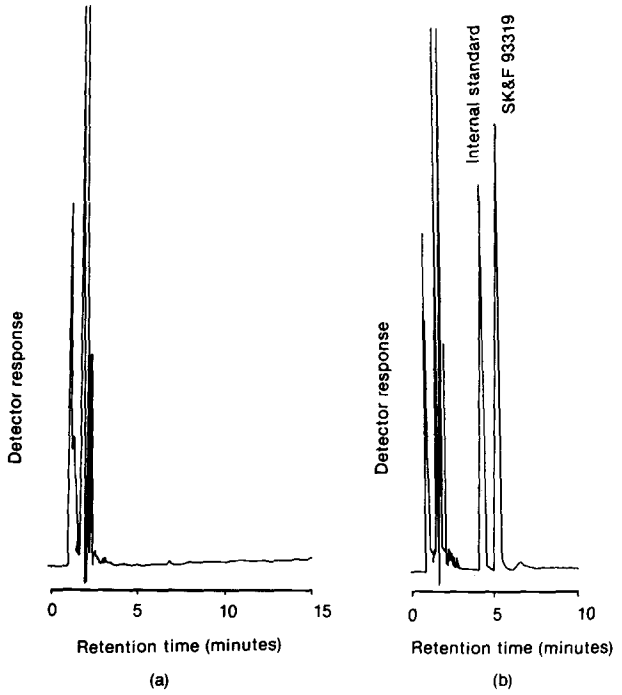

Figure 1
HPLC chromatograms of extracts from (a) control plasma and (b) plasma containing SK&F 93319. For chromatographic conditions, see text.

Preparation of calibration graphs

Calibration graphs for the determination of the drug in plasma were derived from results obtained in the assay of standards prepared by the addition of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg of SK&F 93319 to 1.0 ml of drug-free plasma; 2–6 replicate measurements were made at each concentration. The response was rectilinear within this range and under well-controlled conditions one calibration graph should hold good for several days. Equations of typical calibration graphs for a 5-day period are shown in Table 1. Not more than 100 μl of ethanolic solution should be added to 1.0 ml of plasma when adding the drug and internal standard. If this volume is exceeded, the recovery of SK&F 93319 relative to that of the internal standard becomes more variable so that the precision of the method is reduced.

Table 1
Equations of calibration graphs for the determination of SK&F 93319 in plasma

Date	Intercept	Slope	σ^{2}*
19/5/83	−0.00099	0.2422	0.00012
19/5/83	−0.0001	0.2402	0.00008
20/5/83	−0.0112	0.2425	0.00016
23/5/83	0.0021	0.2410	0.00013
23/5/83	−0.0001	0.2487	0.00020

* σ^{2} is the estimate of variance obtained from the pooled residual [6].

Precision

The precision of the method (Table 2) is reported as the 95% confidence limits for an estimate of the amount of drug using the calibration graph for plasma extracts. In the range 0.5–20.0 mg l^{-1} the precision of the method was better than 6%. The precision was 12% at 0.2 mg l^{-1} and 20% at 0.1 mg l^{-1}.

Recovery

The recoveries of SK&F 93319 and the internal standard were determined by comparing the HPLC responses of these compounds in extracted standards with responses of known amounts on column. It was assumed that no volume changes occurred during extraction. The mean recovery of SK&F 93319 was 85.9% and that of the internal standard 82.1% (Table 3), after adjustment for planned transfer losses.

Reliability

Day-to-day variation of the calibration graph was small (<4%) so that the performance of the method was limited mainly by the deterioration of the column with time. The chromatographic conditions could be rapidly restored by a change of column; this change was shown to have little or no effect on the calibration graph.

Table 2
The precision of the assay for SK&F 93319 in plasma

Estimated amount of SK&F 93319 (μg)	95% confidence limits* for estimated amount of SK&F 93319	
	Lower limit (μg)	Upper limit (μg)
0.145	0.117	0.173
0.250	0.222	0.278
0.496	0.469	0.523
0.930	0.904	0.957
2.052	2.027	2.077
4.805	4.782	4.828
10.369	10.330	10.390
19.880	19.820	19.940

* Confidence limits were calculated by the method of Draper and Smith [7].

Table 3
Recovery of SK&F 93319 and SK&F 93333 from plasma by HPLC

Amount added to 1.0 ml plasma (μg)	Peak area* extracted sample (counts)	Peak area* non-extracted sample (counts)	Mean recovery (%)
SK&F 93319 0.5	748.2 ± 2.2% (n = 6)	899.8 ± 1.1% (n = 6)	83.2
2.0	3488 ± 3.0% (n = 5)	3897 ± 2.0% (n = 4)	89.5
10.0	17566 ± 3.2% (n = 5)	20692 ± 1.1% (n = 6)	84.9
SK&F 93333 5.0	6391 ± 5.8% (n = 18)	7785 ± 1.8% (n = 17)	82.1

*Results for peak area are expressed as mean ± relative standard deviation; n = number of replicate samples.

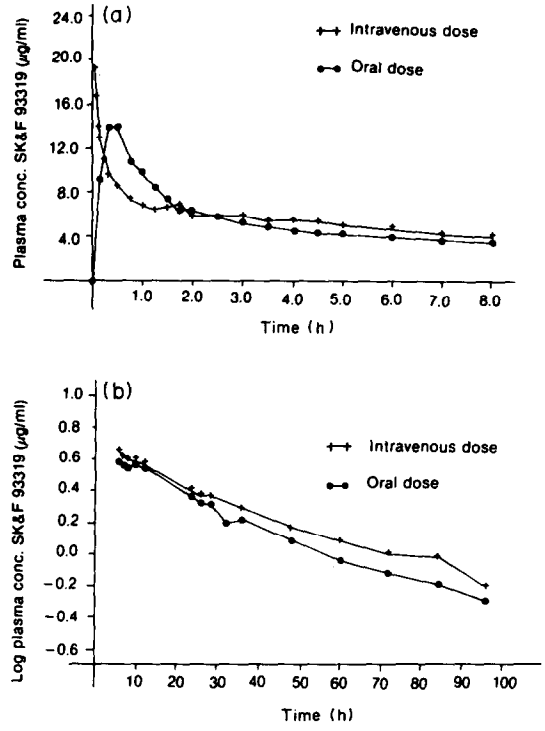

Figure 2
(a) Plot of mean plasma concentration of SK&F 93319 against time (0–8 h).
(b) Plot of the logarithm of mean plasma concentration of SK&F 93319 against time (6–96 h).

Plasma concentrations of SK&F 93319 after oral and intravenous administration to man

In a controlled study, five fully-informed male volunteers received an intravenous bolus injection of 2 mg kg^{-1} or an oral dose (as solution) of 5 mg kg^{-1} of SK&F 93319.

Blood samples were taken at various times after administration of these doses and the plasma was assayed for SK&F 93319. The results are shown in Fig. 2 and demonstrate that the technique was sufficiently sensitive to follow the distribution kinetics of the drug for at least 96 h after administration.

The protocol for this study demanded that the subjects should fast for 10 h before administration of SK&F 93319, and for 4 h after dosing. During that period the only refreshment allowed was 250 ml of fruit juice. Concomitant medication was specifically excluded as were xanthine-containing drinks, smoking and alcohol consumption from 12 h before dosing and for 8 h after dosing. Subjects remained supine or seated for the first 4 h of the study except when passing urine.

Discussion

This method, which has been used by different analysts within the authors' laboratories for assaying a combined histamine H_1- and H_2-receptor antagonist, presented no practical difficulties and is considered to be robust and reliable. The method is suitable for application to pharmacokinetic studies and has been used to define the pharmacokinetics of SK&F 93319 in man [7].

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