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Short communication

Determination of SK&F 108566 (Teveten®) in human plasma by reversed-phase high-performance liquid chromatography

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Abstract

A sensitive, selective and rugged analytical method was developed for the determination of SK&F 108566 (eprosartan, Teveten) in human plasma. The new method employs a simple solid-phase extraction procedure to isolate the drug and its internal standard (SB-200062) from plasma samples. The assay is based on analysis by reversed-phase high-performance liquid chromatography with ultraviolet absorbance detection. The dynamic range of the assay is from 10.0 to 5000 ng/ml, based on 0.5-ml aliquots of plasma. No interference from the endogenous components of plasma, the anticoagulant, or sample collection devices have been noted. The assay has been fully validated. The mean within-run precision (6.5%), between-run precision (4.0%), accuracy (106%) and recovery (71.8%) of the method were considered acceptable. Additionally, SK&F 108566 was found to be stable in plasma under the storage and sample preparation conditions used. This assay has been successfully employed to provide pharmacokinetic data from clinical trials. © 1998 Published by Elsevier Science B.V.

Keywords: Teveten®; Eprosartan

1. Introduction

SK&F 108566 (eprosartan, Teveten®) (**I**) is a highly selective, non-peptide angiotensin-II antagonist. The compound has been shown to inhibit angiotensin II induced vasoconstriction in preclinical species and cause reductions in systolic and diastolic blood pressure at peak effect after dosing in clinical patients [1,2]. It is currently being developed for the treatment of hypertension.

A sensitive, specific and robust assay procedure was required for the determination of **I** in human plasma to obtain pharmacokinetic data in support of ongoing clinical studies. The new assay is based on high-performance liquid chromatography (HPLC) with ultraviolet (UV) absorbance detection. The development, validation and application of the assay procedure are described herein.

2. Experimental

2.1. Chemicals and reagents

SK&F 108566 {(E)-3-[2-butyl-1-[(4-carboxyphenyl)methyl]-1H-imidazol-5-yl]-2-[(2-thienyl)-

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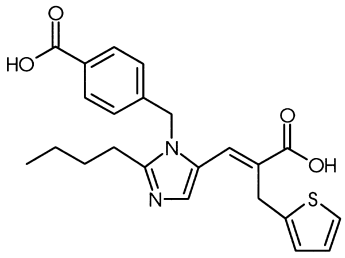
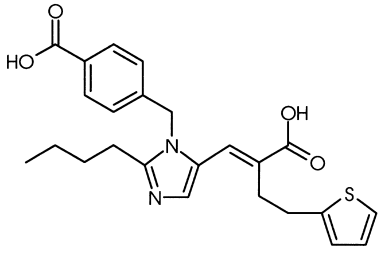
methyl]propenoic acid} (**I**, Fig. 1) and the internal standard SB-200062 {(E)-3-[2-butyl-1-[(4-carboxyphenyl)methyl]-1H-imidazol-5-yl]-2-[(2-thienyl)-ethyl]propenoic acid} (**II**, Fig. 1) were supplied by SmithKline Beecham (Swedeland, PA, USA). Reagent grade citric acid and sodium citrate, and HPLC grade tetrahydrofuran, methanol and ethyl acetate were obtained from J.T. Baker (Phillipsburg, NJ, USA). Reagent grade glacial acetic acid was obtained from Mallinckrodt (Paris, KY, USA). Sequanal grade triethylamine was obtained from Pierce (Rockford, IL, USA). Purified water from a Milli-Q Plus water system (Millipore, Bedford, MA, USA) was used in the preparation of buffer and reagent solutions. Phenyl solid-phase extraction (SPE) cartridges (100 mg bed packing, 1-ml volume capacity) were purchased from Analytichem International (Harbor City, CA, USA). Drug-free control human plasma (anticoagulant: heparin) was purchased from Biological Specialties (Lansdale, PA, USA).



SK&F-108566
I



SB-200062
II

Fig. 1. Chemical structures for SK&F 108566 (**I**) and the internal standard SB-200062 (**II**).

2.2. Instrumentation and chromatographic conditions

The chromatographic system consisted of a Hitachi Model L6000A pump, a Waters WISP Model 712 autosampler, and an Applied Biosystems Model 785A programmable UV absorbance detector, operated at a wavelength of 300 nm. The HPLC analytical column was a BDS-Hypersil C_{18} (5 μ, 150×2 mm) from Keystone Scientific. The guard column was a BDS-Hypersil C_{18} (5 μ, 20×2 mm) also from Keystone Scientific. The isocratic mobile phase consisted of 0.05 *M* citrate buffer (pH 3.5)–tetrahydrofuran (34:16, v/v). The flow-rate was set to 0.25 ml/min. The data acquisition system was Access*Chrom from Perkin-Elmer/Nelson Systems.

A Spe-ed Wiz Model 6030 semi-automated SPE system from Applied Separations (Bethlehem, PA, USA) was used to perform the SPE steps.

2.3. Preparation of calibration standards and quality control samples

A 1.0 mg/ml stock solution of **I** was prepared in 1% glacial acetic acid in methanol. Dilutions of the stock solution to produce working solutions (100, 10, 1 and 0.1 μg/ml) were prepared with methanol–0.05 *M* acetic acid (50:50, v/v). A 1.0 mg/ml stock solution of the internal standard **II** was also prepared in 1% glacial acetic acid in methanol and diluted to produce a 16 μg/ml working solution with methanol–0.05 *M* acetic acid (50:50, v/v). All solutions were stored at room temperature for periods of up to two months.

Daily calibration curves were prepared in 0.5-ml aliquots of human plasma by spiking drug-free control plasma using the working solutions of **I**. Nine calibration standards were used, nominally ranging from 10 to 5000 ng/ml.

For validation of the assay, four pools of quality control (QC) samples were prepared containing 10, 20, 500 and 5000 ng/ml of **I**. Stock and working solutions of the analyte were prepared as above (from a separate weighing) and appropriate volumes of the working solutions of **I** were pipetted into drug-free, control plasma. These plasma samples were stored at −20°C until the analyses were performed. Eight replicates from each concentration

were extracted and analyzed on each of three separate days. The concentrations of the QC samples were determined by comparison with calibration standards run singly for each day of analysis.

2.4. Extraction procedure

Calibration standards, and 0.5-ml aliquots of the control blanks and validation QC samples were spiked with the working solution of **II** to produce an internal standard concentration of 400 ng/ml. An aliquot (500 μl) of 0.1 *M* citrate buffer, pH 3.5, was pipetted into all samples, which was followed by brief vortex mixing and centrifugation for 5 min in a high-speed centrifuge (8800 *g*). The SPE cartridges were conditioned with 2 ml of methanol, followed by 2 ml of Millipore water. The plasma samples were applied to the cartridges manually and washed with 2 ml of 0.05 *M* acetic acid, followed by a 45 s drying period using air. The cartridges were then washed with 1 ml of ethyl acetate containing 0.1% triethylamine to remove interfering endogenous compounds and again dried for 45 s. The cartridges were eluted with 2 ml of methanol–0.05 *M* acetic acid (90:10, v/v). The eluent was evaporated to dryness under nitrogen at 45°C. The residue was reconstituted with 125 μl of mobile phase, vortex mixed and transferred to autosampler vials. The vials were centrifuged for 10 min (1875 *g*) and 50-μl aliquots were injected onto the HPLC system for analysis.

2.5. Sample analysis and calculations

The quantitation of **I** from human plasma was based on the chromatographic peak height ratio of the analyte **I** to that of the internal standard **II**. Calibration curves were constructed from linear regression (weighted by the reciprocal of analyte concentration) of the peak height ratio versus the analyte concentration in the calibration samples. The concentration of **I** in QC and experimental samples was calculated by inverse prediction using the parameters established from regression of the daily calibration curves.

Precision and accuracy of the assay are given in Table 1. Acceptable precision was defined as ≤20% at the lower limit of quantitation (10 ng/ml) and ≤15% at all other concentrations. Acceptable average accuracy was defined as ≤15% error at all concentrations.

2.6. Recovery

Four concentration levels of QC samples (10, 20, 500 and 5000 ng/ml) were used to calculate recovery. Following extraction and analysis, the mean chromatographic peak height ($n=8$) for each concentration was compared to the mean peak height from unextracted reference injections of the equivalent concentration of **I** ($n=3$). Internal standard was not added to the extracted samples.

2.7. Evaluation of stability

The long-term freezer stability of **I** in human plasma from volunteers dosed with **I** was determined by analyzing samples stored at −20°C for a minimum of seven months following the original analysis.

Table 1
Summary of within-run and between-run precision and accuracy, for SK&F 108566 QC samples in human plasma

Nominal concentration (ng/ml)	Mean found concentration (ng/ml)			Average within-run within-run (%)	Between-run precision precision	Average accuracy (%) (%)
	Day 1	Day 2	Day 3			
10	11.7	10.6	10.6	13.8	5.8	110
20	22.5	19.8	21.2	7.9	6.4	106
500	501	520	520	2.7	2.1	103
5000	5170	5320	5210	1.6	1.5	105

3. Results and discussion

3.1. Calibration curves

The dynamic range of the assay was set at 10.0 ng/ml to 5000.0 ng/ml. The precision for the calibration points over this range was acceptable with a coefficient of variation (C.V.) range of 0.2% to 10.2%. The accuracy was also acceptable with a range of 96.3% to 103%.

3.2. Linearity

A linear equation was determined to adequately represent the concentration/response relationship using the reciprocal of the analyte response as the weighting factor. The correlation coefficient (r) ranged from 0.9996 to 0.9999. The slope and intercept values were also reproducible.

3.3. Selectivity

The selectivity of this assay has proven to be sufficient for the routine determination of human plasma samples. A chromatogram of drug-free control plasma is shown in Fig. 2A. Evident in the chromatogram, there were no interfering peaks corresponding to the retention time of **I**. There were also no observable interferences at the retention time of the internal standard. A total of eight control plasma

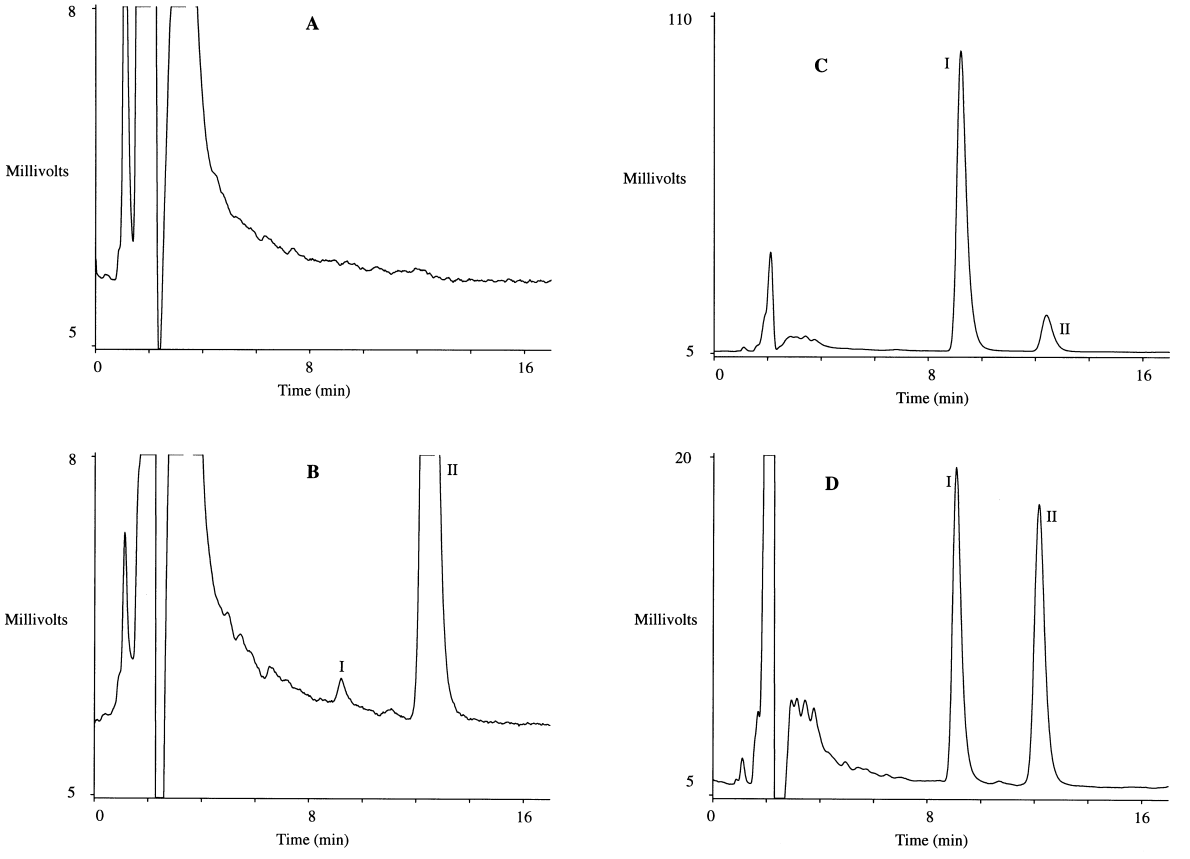


Fig. 2. Representative chromatograms of (A) drug-free control, (B) control plasma fortified with 10.0 ng/ml **I** and 400 ng/ml **II** (the lowest calibration standard); (C) control plasma fortified with 5000 ng/ml **I** and 400 ng/ml **II**; (D) extract of a plasma sample taken 3 h post-dose from a volunteer given 350 mg of **I** orally, concentration of **I**=683 ng/ml.

samples from various lots of plasma were screened during the validation of this assay procedure, and no significant interferences with either the analyte or the internal standard were observed.

A chromatogram from a plasma sample taken from a volunteer 3 h post a 350 mg oral dose of **I** is shown in Fig. 2D. This chromatographic peak represents a plasma concentration of 683 ng/ml of **I**.

3.4. Sensitivity

The limit of reliable quantitation was set at the concentration of the lowest non-zero standard, 10.0 ng/ml. A representative chromatogram is shown in Fig. 2B, and it can readily be seen that the signal-to-noise ratio for this concentration is acceptable, especially when compared to the response at this retention time for control blank plasma (Fig. 2A). The precision and accuracy of the back-calculated calibration standard at this concentration were acceptable at 10.2% and 98.6%, respectively ($n=3$). For QC samples at this concentration, the within-run precision averaged 13.8%, and the between-run precision was 5.8% (Table 1). The accuracy averaged 109.5%.

3.5. Precision and accuracy

The precision and accuracy of the assay procedure were determined by the analysis of QC samples. The results are summarized in Table 1. The average within-run precision ranged from 1.6% to 13.8%. The between-run precision ranged from 1.5% to 6.4%. The average accuracy ranged from 103% to 110%. These results demonstrated acceptable precision and accuracy for the assay procedure.

3.6. Recovery

The absolute recovery of **I** from human plasma ranged from 64.9% to 79.1% (Table 2). The recovery did not appear to be concentration dependent. Although the variability was somewhat high at the 10 ng/ml concentration (C.V. 35.7%), this did not appear to affect the performance of the assay. It is likely that the absence of the internal standard in the plasma during the recovery experiment contributed to the higher variability.

3.7. Stability

The stability of **I** in clinical samples during long-term storage at −20°C was demonstrated. Summarized in Table 3, the concentrations of **I** in clinical samples were found to be essentially unchanged after seven to eight months of storage. In addition, the stability of **I** in the methanolic stock solutions at room temperature was found to be acceptable for at least two months.

4. Conclusions

HPLC–UV with SPE proved to be a sensitive, accurate and reproducible technique for the quantitative determination of **I** in human plasma. The plasma extracts were found to be free from interferences and **I** was shown to be stable in human plasma for at least eight months when stored frozen at −20°C. The assay method was successfully validated over a concentration range of 10.0 to 5000 ng/ml according to generally accepted guidelines for assay validation [3–5] and has been used to quantitate **I** in over 10 000 clinical pharmacokinetic samples.

Table 2
Absolute recovery of SK&F 108566 from 0.5 ml of human plasma

	Nominal concentration (ng/ml)			
	10	20	500	5000
Extracted mean peak height ($n=8$)	207	385	9505	84 071
S.D.	74	29	1593	7874
Unextracted mean peak height ($n=3$)	296	526	12 017	129 573
% Recovery	69.8	73.3	79.1	64.9

Table 3
Plasma concentrations of SK&F 108566 in clinical samples reanalyzed after seven to eight months storage at −22°C

Subject No.	Time since dose (h)	Original value (ng/ml)	Reassay value (ng/ml)	% Difference	Time since original analysis (months)
564	1	69.9	68.0	−2.7	8
577	2	33.3	40.3	21.0	7
579	4	47.2	48.0	1.7	7
564	0.5	139	199	43.2	8
583	2	228	194	−14.9	7
565	1	268	292	9.0	8
567	1.5	1060	1210	14.2	8
583	2	1510	1620	7.3	7
573	2	664	669	0.8	7
578	12	29.8	31.1	4.4	7

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