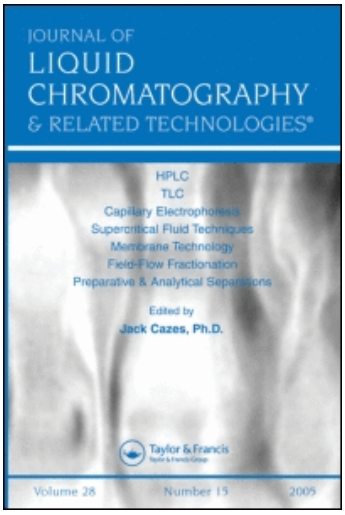


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Method for Quantitation of a New Antidiabetic Agent in Plasma

Yusuke Sato[a]; Masahiko Nishikawa[a]; Hisashi Shinkai[a]
[a] Central Research Laboratories Ajinomoto Co., Inc., Kawasaki, Japan

To cite this Article Sato, Yusuke , Nishikawa, Masahiko and Shinkai, Hisashi(1988) 'High Performance Liquid Chromatographic Method for Quantitation of a New Antidiabetic Agent in Plasma', Journal of Liquid Chromatography & Related Technologies, 11: 16, 3425 — 3433
To link to this Article: DOI: 10.1080/01483918808082265
URL: http://dx.doi.org/10.1080/01483918808082265

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(16), 3425-3433 (1988)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR QUANTITATION OF A NEW ANTIDIABETIC AGENT IN PLASMA

YUSUKE SATO, MASAHIKO NISHIKAWA AND HISASHI SHINKAI*
Central Research Laboratories
Ajinomoto Co., Inc.
Suzuki-cho, Kawasaki-ku
Kawasaki 210, Japan

ABSTRACT

An HPLC procedure for the detection and quantitation of a new antidiabetic agent, N-(*trans*-4-isopropylcyclohexylcarbonyl)-D-phenylalanine (A4166), in dog plasma was developed. The drug and internal standard were extracted from plasma using a reversed phase C_{18} extraction column (Sep-pak). Separation was accomplished on a ERC-ODS-1161 reversed-phase column with a mobile phase of acetonitrile / 0.1M phosphate buffer, pH 6.6 (30 / 70). Quantitation was achieved by monitoring the ultraviolet absorbance at 210 nm. A linear relationship between concentration and peak height ratio (A4166 / internal standard) was obtained. The method has been successfully used for analysis of plasma samples from beagle dogs following oral administration of A4166.

INTRODUCTION

N-(*trans*-4-Isopropylcyclohexylcarbonyl)-D-phenylalanine, A4166

*Correspondence

(Figure 1), is a new oral antidiabetic agent, which was developed in our laboratory (1, 2, 3). In connection with pharmacokinetic investigations of A4166, a quantitative method for the determination of plasma levels of the drugs is required. This paper presents a high-performance liquid chromatographic (HPLC) assay for A4166 on a reversed-phase column with UV detection at 210 nm. Sample preparation involves the use of solid-phase extraction on a Sep-pak C_{18} cartridge column with N-(*trans*-4-ethylcyclohexylcarbonyl)-D-phenylalanine (Figure 1) added as the internal standard. The assay was applied to the determination of plasma concentration of A4166 in dogs receiving various doses of A4166.

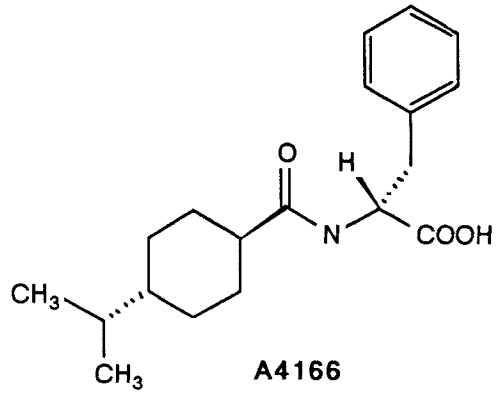
EXPERIMENTAL

Apparatus

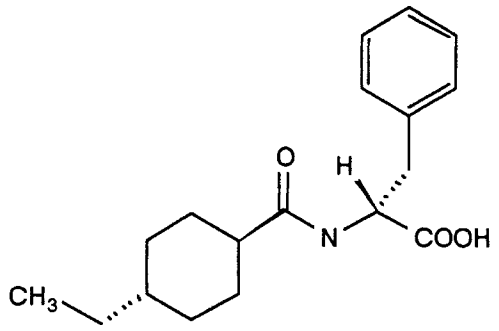
The chromatography was performed with a Model 655A-12 liquid chromatograph (Hitachi, Tokyo, Japan) equipped with a Model 7125 syringe-loading injector (Rheodyne, California, USA), a Model 655A-21 variable wavelength UV monitor (Hitachi, Tokyo, Japan) set at 210 nm and a Model 655-71 data processor (Hitachi, Tokyo, Japan). The column was a ERC-ODS-1161 reversed-phase column (Erma Optical Works, Tokyo, Japan). Acetonitrile / 0.1M phosphate buffer, pH 6.6, (30 / 70) was used as a mobile phase. A flow-rate of 1.5 mL / min was typically used at room temperature.

Chemicals and Reagents

A4166 and the internal standard were synthesized in our laboratory (1,2,3). Acetonitrile from Wako Chemicals (Osaka, Japan) was of HPLC



A4166



Internal Standard

Figure 1. Chemical structures of A4166 and the internal standard.

grade. The 0.1M phosphate buffer solution (pH 6.6) was made from commercially available materials and redistilled water of high quality.

Preparation of Standards

The stock solution of A4166 (1 mg / mL) and the internal standard (1 mg / mL) were prepared by accurately dissolving weighed samples in

acetonitrile. Working solution of appropriate concentrations were made every week by dilution of the stock solutions with acetonitrile. The calibration curve standards were prepared by adding known amounts of A4166 and the internal standard to blank plasma, and contained 2, 4, 10, 20 and 30 μg / mL of A4166 and 10 μg / mL of the internal standard.

Sample Work-up

A 200 μl volume of internal standard (25 μg / mL) and 2 mL of 0.1M phosphate buffer (pH 6.6) were added to 0.5 mL of plasma. Plasma samples were adsorbed on a disposable reversed phase extraction column, Sep-pak C_{18} (Waters) . The column was conditioned twice with 3 mL of methanol, twice with 3 mL of water and once with 2 mL of 0.1M phosphate buffer (pH 6.6). The sample solution was forced by a syringe through the column, followed by 15 mL volumes of water. The A4166-containing fraction was eluted with 2 mL of methanol. This solution was evaporated under vacuum. The residue was dissolved in 0.5 mL of the mobile phase and 20 μl was injected onto the column.

RESULTS AND DISCUSSION

Various compositions of acetonitrile and phosphate buffer were tried as the mobile phase, and different ionic strengths of the buffer component were evaluated. A 30 : 70 mixture of acetonitrile-phosphate buffer (0.1 M, pH 6.6) was found to be optimal. The separation was performed at room temperature on the reversed-phase column at a flow-rate of 1.5 mL / min. The detection wavelength was set to 210 nm. Under these conditions, the capacity factors (k') for A4166 and internal standard

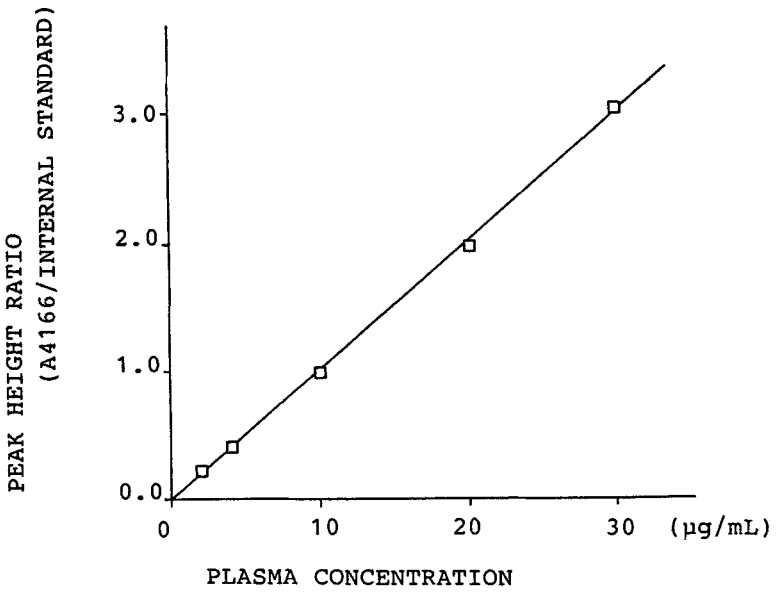


Figure 2. Calibration curve for the extraction of A4166 from blank dog plasma.

were determined as 6.97 and 3.69, respectively. The corresponding retention times were 12.44 and 7.32 min, respectively.

Values for precision and accuracy were estimated at 2, 4, 10, 20 and 30 μg / mL A4166 in plasma and are presented in Table 1. The coefficient of variation ranged from 0.6 to 2.8 % with an average of 1.38 %.

The relationship between the concentration of A4166 added to dog plasma and the peak height ratio of A4166 relative to the internal standard is shown in Figure 2. Linear regression of the peak height ratio versus concentration gives a typical coefficient of determination (r) of 0.999.

TABLE 1

The Precision and Accuracy of the HPLC Method

Concentration (μg / mL)	Detected Concentration[1]	Coefficient of variation (%)
2	1.84 ± 0.04	2.1
4	3.92 ± 0.11	2.8
10	10.0 ± 0.08	0.8
20	19.7 ± 0.12	0.6
30	30.4 ± 0.18	0.6

[1]Mean ± SD, n = 5

TABLE 2

Recovery from Solid Phase Extraction (Sep-pak C_{18})

Sample	% Recovery[1]
A4166 (6 μg / mL)	96.5 ± 2.7
A4166 (30 μg / mL)	95.4 ± 0.6
Internal standard (10 μg / mL)	96.2 ± 0.6

[1]Mean ± SD, n = 3

To determine recovery, the peak height of the extract of plasma samples spiked with A4166 and the internal standard were compared with the same amount of drugs as the standard solution injected directly onto the column without extraction. The percent recoveries of A4166 and the internal standard from the C_{18} extraction column are shown in Table 2.

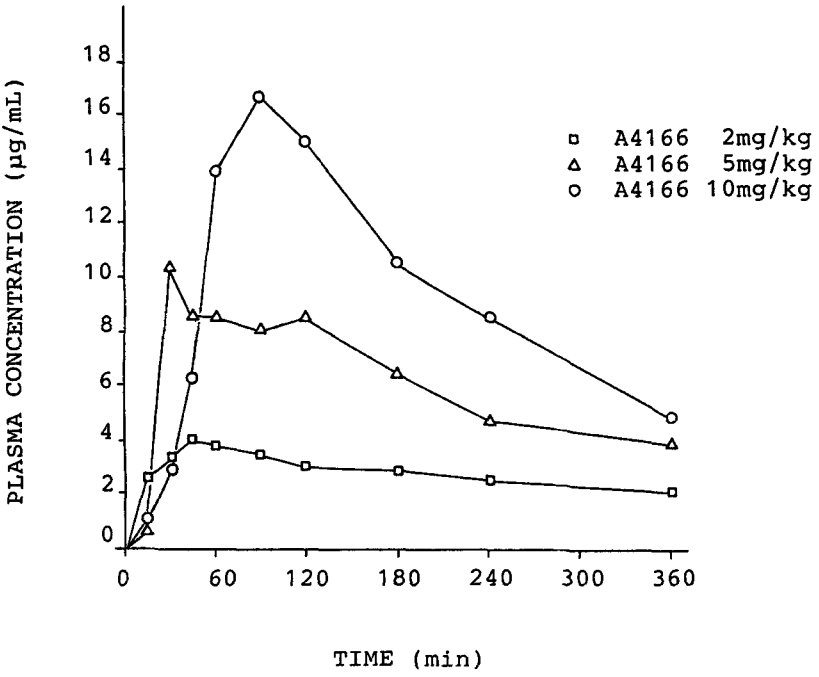


Figure 3. Mean concentration of A4166 in the blood of dogs following an oral dose of 2, 5 or 10 mg / kg (n = 3).

The utility of the assay was demonstrated by analyzing A4166 in plasma samples from beagle dogs having an oral dose of 2, 5 or 10 mg / kg. Figure 3 shows plasma concentration-time profiles. Typical chromatograms are shown in Figure 4.

CONCLUSIONS

A liquid chromatographic procedure has been described for the quantitative analysis of the new antidiabetic agent, A4166. This

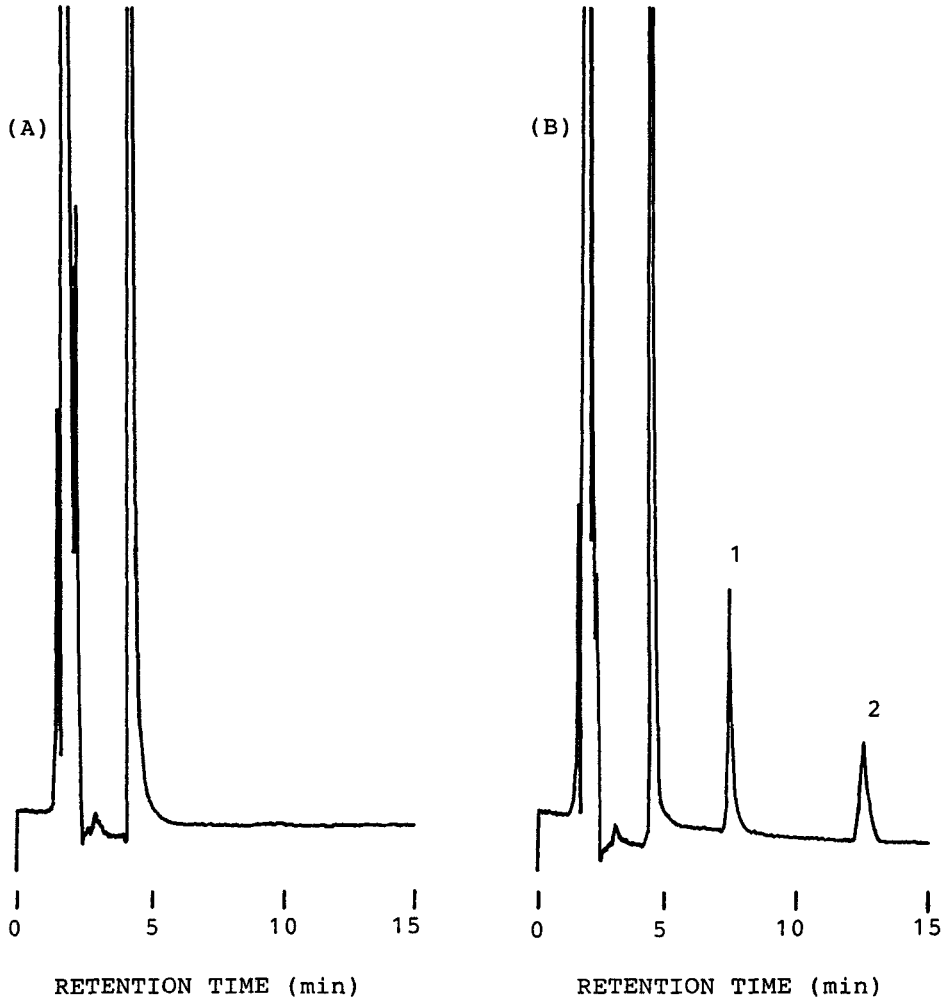


Figure 4. Chromatograms from the analysis of A4166 in plasma from a dog before (A) and 60 min after (B) oral administration of a 10 mg / kg.

1 = Internal standard; 2 = A4166.

compound and the internal standard can be isolated from plasma in high yield using a reversed phase (C_{18}) solid extraction procedure. The assay is sufficiently rapid and simple to allow accurate and precise measurements of plasma levels of A4166 during pharmacokinetic studies in dogs.

ACKNOWLEDGMENTS

We thank Keizo Matsuda, Kiyoe Karaki and Masanao Ozaki for guidance in the initial stages of the development of this assay.

REFERENCES

1. Shinkai, H., Toi, K., Kumashiro, I., Seto, Y., Fukuma, M., Dan, K., Fukushima, K. and Fujita, H., The Japanese - United States Congress of Pharmaceutical Sciences, Honolulu, Dec. 1987, p. s173.

2. Shinkai, H., Toi, K., Kumashiro, I., Seto, Y., Fukuma, M., Dan, K. and Toyoshima, S., J. Med. Chem., in press.

3. Shinkai, H., Nishikawa, M., Sato, Y., Toi, K., Kumashiro, I., Seto, Y., Fukuma, M., Dan, K. and Toyoshima, S., J. Med. Chem., submitted.