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High-performance liquid chromatographic determination of a potent AII receptor antagonist (DMP 811) in plasma

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ABSTRACT

An HPLC assay for DMP 811, 4-ethyl-2-propyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole-5-carboxylic acid (I) in rat and dog plasma has been developed. Compound I was isolated from plasma using a liquid–liquid back extraction procedure. The extraction recovery was greater than 81%. Separation of I from endogenous components in plasma was achieved on an E. Merck C_8 column using a mobile phase of 0.05 *M* ammonium acetate, brought to pH 3.75 with acetic acid, and acetonitrile (78:22, v/v). The eluent was monitored by fluorescence with excitation and emission set at 235 and 370 nm, respectively. The assay was linear from 2 to 2000 ng/ml. Inter- and intra-day coefficients of variation for the rat-plasma assay ranged from 0.9 to 5.2% (5–2000 ng/ml) and 2.7 to 16.5% (2–2000 ng/ml), respectively. The respective coefficients of variation for the dog-plasma assay were 1.9 to 5.6% and 1.2 to 14.0%. The percent differences from the accuracy results were 12% or less. Using 0.5 ml of plasma for extraction, the minimum quantifiable limit was 2 ng/ml. This method has been used to quantify plasma levels of I in rats or dogs following 3–10 mg/kg i.v. or p.o. doses.

INTRODUCTION

DMP 811, 4-ethyl-2-propyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole-5-carboxylic acid (I, Fig. 1), an AII receptor antagonist, has been shown to lower blood pressure in conscious renal hypertensive rats and furosemide-treated dogs [1]. Previously reported methods for other AII antagonists, DuP 753 and DuP 532 used UV detection at 254 nm [2–4]. The detection sensitivity was reported to be 5–6 ng/ml using 1 ml of human plasma or 25 ng/ml using 0.5 ml of dog plasma [2–4]. This paper describes an HPLC-fluorescence detection method for the analysis of DMP 811 in rat and dog plasma. Using 0.5 ml of plasma, the quantifiable limit was 2 ng/ml.

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EXPERIMENTAL

Chemicals and reagents

I and II (internal standard, Fig. 1) were synthesized at the DuPont Merck Pharmaceutical Company, Wilmington, DE, USA. Acetonitrile, methanol and glacial acetic acid, HPLC grade, were purchased from J. T. Baker (Phillipsburg, NJ, USA). HPLC grade ammonium acetate, approximately 98%, was purchased from Fisher Scientific (Pittsburg, PA, USA). All other chemicals were of reagent grade.

Chromatography and data system

The HPLC system consisted of a pump (Series 4, Perkin-Elmer, Norwalk, CT, USA), an autosampler (Perkin-Elmer ISS-100), a reversed-phase column (LiChroCART Lichrospher 100 RP-8, 25 cm × 4.6 mm I.D., 5 μm, E. Merck, Gibbstown, NJ, USA), with temperature main-

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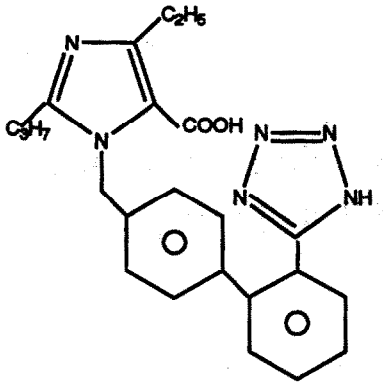
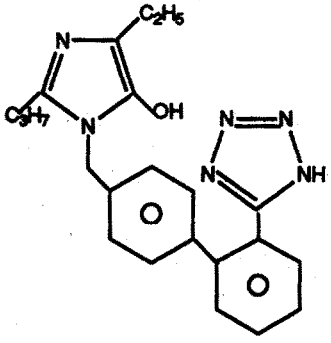


I



II

Fig. 1. Chemical structure of I and II.

tained at 35°C by a Jones Chromatography (Lakewood, CO, USA) column block heater and an ABI 980 fluorescence detector (Foster City, CA, USA) set at 235 nm excitation and 370 nm emission. The mobile phase was composed of 0.05 *M* ammonium acetate (pH lowered to 3.75 with glacial acetic acid and filtered)–acetonitrile (78:22, v/v for rats and 79:21, v/v for dogs). The flow-rate was set at 1.0 ml/min. The data system consisted of an A/D converter (Nelson Analytical, 900 series), a Compaq Deskpro 386/20E computer, an Epson EX-800 printer, and Turbochrom 3 software (version 3.1, Nelson Analytical, Cupertino, CA, USA).

Standards

Stock solutions of I and II were prepared in methanol. A 0.05 *M* sodium hydroxide solution containing 0.2 μg/ml of II was used as an internal standard.

Extraction procedure

A 0.5-ml aliquot of 0.1 *M* phosphoric acid was added to 0.5 ml of rat or dog plasma in a 20 mm × 125 mm test tube. For plasma standards and samples with 1000 ng/ml or higher concentration, only 0.25 ml of plasma was used. An additional 0.25 ml of blank plasma was added prior to extraction. The mixture was extracted with 13 ml of ether–isopropanol (95:5, v/v) and shaken for 20 min. After centrifugation at 1900 *g* for 5 min, 10 ml of the upper organic layer was transferred to a clean centrifuge tube containing 200 μl of 0.2 μg/ml II in 0.05 *M* sodium hydroxide and shaken for 15 min. The ether–isopropanol mixture was discarded after centrifugation at 1900 *g* for 5 min. Residual organic solvents in the aqueous layer were evaporated under nitrogen. Fifty μl of the sodium hydroxide layer was analyzed by HPLC.

Calibration

The peak-height ratios (PHR) of I to the internal standard of the extracted plasma samples were evaluated against those of spiked concentrations of the compound. Two power curves for each compound were used to determine slopes, exponents, and correlation coefficients of the plasma standards. One power curve was used to evaluate standard concentrations ranging from 2 to 20 ng/ml (low-standard curve) and the second power curve was used to evaluate standard concentrations ranging from 20 to 2000 ng/ml (high-standard curve). The formula for the calculation of an unknown sample concentration is:

$$\text{concentration} = \left(\frac{\text{PHR}}{a}\right)^{1/b}$$

where a and b are the slope and the exponent of the respective power curve.

The low-standard curve was used for the calculation of concentration of samples whose PHR was less than or equal to the PHR of the 20 ng/ml standard. For samples whose PHR was higher than that of 20 ng/ml, the high-standard curve was used to calculate the sample concentrations.

RESULTS AND DISCUSSION

Specificity

Figs. 2A and 2B show typical chromatograms obtained from rat blank plasma spiked with II (internal standard) and blank plasma spiked with I and the internal standard, II. Fig. 2C shows a rat plasma sample 16 h after receiving a 3 mg/kg oral dose of I. Figs. 3A and 3B show typical chromatograms of dog blank plasma spiked with II only and spiked with both I and II, respectively. Fig. 3C shows a dog plasma sample 12 h after receiving a 3 mg/kg oral dose of I. No interfering peaks were observed in the blank plasma. The retention times were approximately 10.5 min for I and 14.9 min for II.

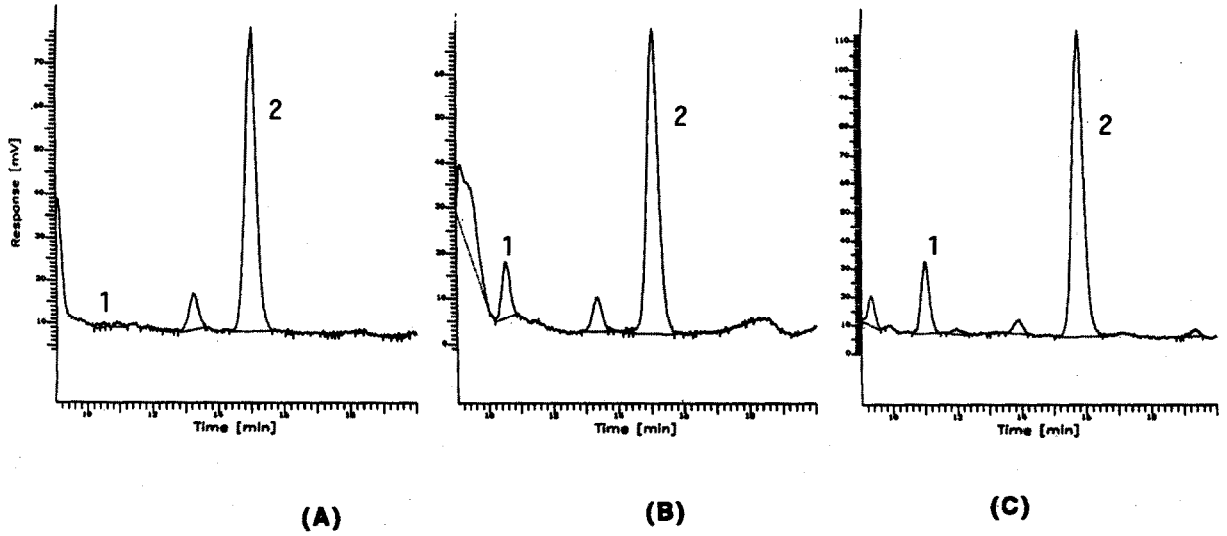


Fig. 2. HPLC from blank rat plasma spiked with internal standard (A), blank rat plasma spiked with internal standard and 10 ng/ml of DMP 811 (B), and a rat plasma sample taken 16 h after administration of a 3 mg/kg oral dose of DMP 811, showing a concentration of 17.9 ng/ml for DMP 811 (C). Peaks: 1 = DMP 811; 2 = XC978 (internal standard).

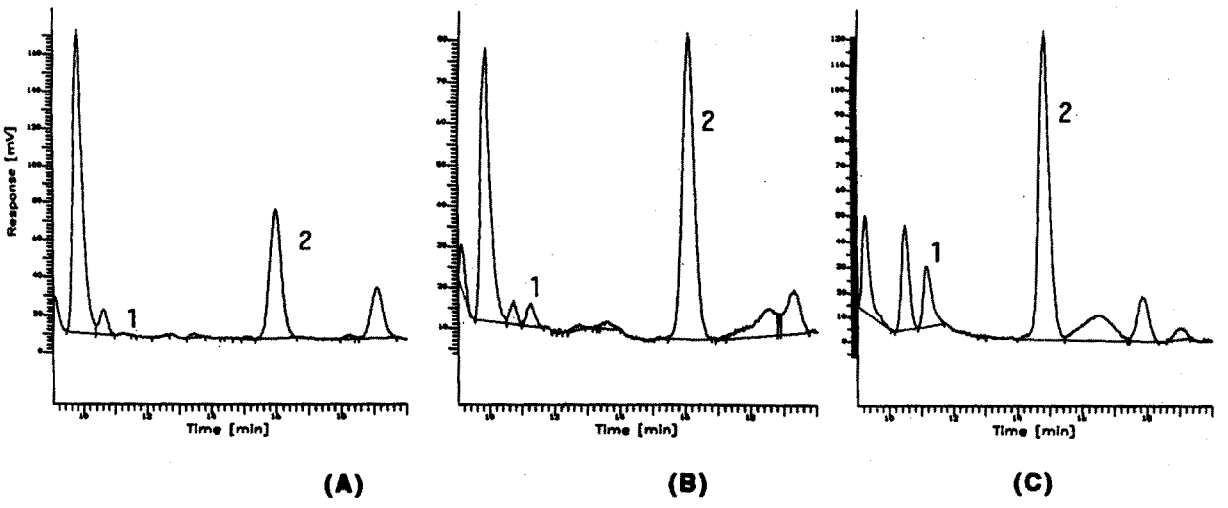


Fig. 3. HPLC from blank dog plasma spiked with internal standard (A), blank dog plasma spiked with internal standard and 5 ng/ml of DMP 811 (B), and a dog plasma sample taken 12 h after administration of a 3 mg/kg oral dose of DMP 811, showing a concentration of 24.6 ng/ml for DMP 811 (C). Peaks: 1 = DMP 811; 2 = XC978 (internal standard).

TABLE I

INTRA-DAY REPRODUCIBILITY RESULTS OF DMP 811 IN RAT AND DOG PLASMA HPLC ASSAY (n = 3)

Spiked concentration (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)
Rat		
2.0	2.1 ± 0.3	16.5
5.0	5.1 ± 0.6	11.7
10.0	9.6 ± 0.6	5.8
20.0	20.2 ± 0.7	3.3
500.0	456.9 ± 12.4	2.7
2000.0	2141.5 ± 76.2	3.6
Dog		
2.0	2.2 ± 0.1	5.5
5.0	5.1 ± 0.4	6.9
10.0	9.9 ± 1.2	11.7
20.0	20.0 ± 0.4	1.9
500.0	552.3 ± 77.3	14.0
2000.0	2232.5 ± 35.2	1.6

TABLE ITABLE I

INTER-DAY REPRODUCIBILITY RESULTS OF DMP 811 IN RAT AND DOG PLASMA HPLC ASSAY (n = 3)

Spiked concentration (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)
Rat		
5.0	5.4 ± 0.1	2.4
10.0	8.8 ± 0.5	5.2
20.0	19.9 ± 0.2	0.9
500.0	486.6 ± 17.8	3.7
2000.0	2029.9 ± 35.2	1.7
Dog		
5.0	5.7[a]	–[a]
10.0	9.4 ± 0.5	5.6
20.0	20.9 ± 1.0	4.6
500.0	514.3 ± 9.5	1.9
2000.0	1997.7 ± 70.3	3.5

[a] n = 2.

Intra-day reproducibility

Three sets of I in rat and dog plasma samples were prepared and analyzed on the same day. The results are shown in Table I. The coefficients of variation ranged from 2.7 to 16.5% and from 1.6 to 14.0% for rats and dogs, respectively.

Inter-day reproducibility

Plasma standards of I were prepared and analyzed on three different days. The results are shown in Table II. The coefficients of variation ranged from 0.9 to 5.2% and from 1.9 to 5.6%, respectively, for rat and dog plasma samples.

Accuracy

The accuracy was calculated using the results of the intra-day reproducibility studies. The results are shown in Table III. For rats and dogs, the absolute differences ranged from 1.2 to 8.6% and from 0.1 to 11.6%, respectively.

TABLE III

ACCURACY OF DMP 811 RAT AND DOG PLASMA ASSAYS

Spiked concentration (ng/ml)	Rat		Dog	
	Found concentration (ng/ml)	Difference (%)	Found concentration (ng/ml)	Difference (%)
2	2.1	4.1	2.2	8.3
5	5.1	2.8	5.1	1.6
10	9.6	−3.7	9.9	−1.3
20	20.2	1.2	20.0	−0.1
500	456.9	−8.6	552.3	10.5
2000	2141.5	7.1	2232.5	11.6

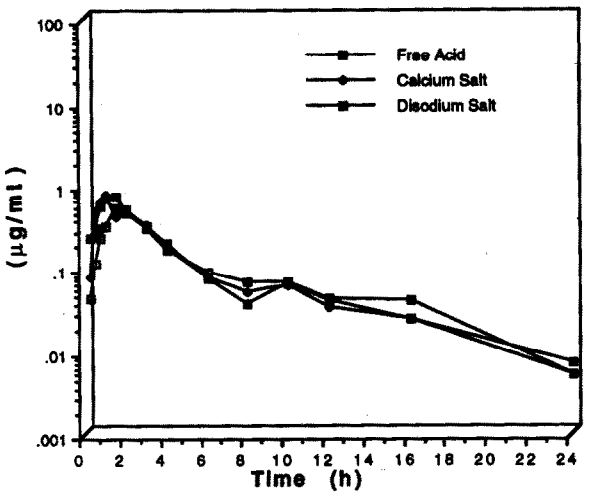


Fig. 4. Plasma levels of DMP 811 in six dogs after oral administration of 3 mg/kg of DMP 811 free acid, calcium salt, or disodium salt (capsules) in a three-way cross-over study.

Recovery

The recovery was determined by comparing the peak height of I after extraction with an unextracted sample. The mean ± C.V. (n = 9) extraction recovery for I at concentrations of 100–2000 ng/ml was 111.0 ± 6.4%.

Application

The method has been used successfully to quantify plasma levels in rats or dogs following 3–10 mg/kg i.v. or p.o. doses. Fig. 4 shows plasma levels of I in six dogs after oral administration of 3 mg/kg of I.

CONCLUSION

In summary, an HPLC method for the analysis of I in plasma has been developed and used for analysis of plasma samples obtained from dogs and rats after administration of I. It was found to be suitable for pharmacokinetic studies of I in dog and rat.

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