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## Note

# High-performance liquid chromatographic determination of a new $H_2$ blocker (ORF 17 910) in plasma

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ORF 17 910, N-(4-amino-1-oxo-1,2,5-thiadiazol-3-yl)-3-[3-[(4-methylpiperidinyl)methyl]phenoxy]-*n*-propylamine (I, Fig. 1), is a new $H_2$ blocker, which has been under pre-clinical investigation [1]. This report describes a high-performance liquid chromatographic (HPLC) procedure for determination of I in plasma. This method has been used to analyse plasma samples obtained from dogs after administration of I and is suitable for pharmacokinetic studies of I in the dog.

## EXPERIMENTAL

### *Chemicals and reagents*

I and II (internal standard, Fig. 1) were supplied by the Chemical Development Division at Ortho. Glass-distilled acetonitrile, diethyl ether (preserved with ethanol) and methanol (HPLC grade, Burdick & Jackson Labs., Muskegon, MI, U.S.A.), phosphoric acid (Fisher Scientific, Fair Lawn, NJ, U.S.A.), sodium hydroxide (Anachemia Chemicals Champlain, NY,U.S.A.) and dibasic sodium phosphate (Baker, Phillipsburg, NJ, U.S.A.) were used as received.

### *Apparatus and chromatographic conditions*

The chromatographic system consisted of a Waters M-6000 pump, a Waters WISP 710B autosampler (Waters Assoc., Milford, MA, U.S.A.), an LDC Model 1203 UV detector (Milton Roy, Riviera Beach, FL, U.S.A.) operated at 229 nm and a linear recorder (Linear Instruments, Irvine, LA, U.S.A.) set at 30 cm/h and 10 MV. The WISP initiated chromatographic peak-height measurement was carried out by an HP 3357 Laboratory Automation System (Hewlett-Packard,

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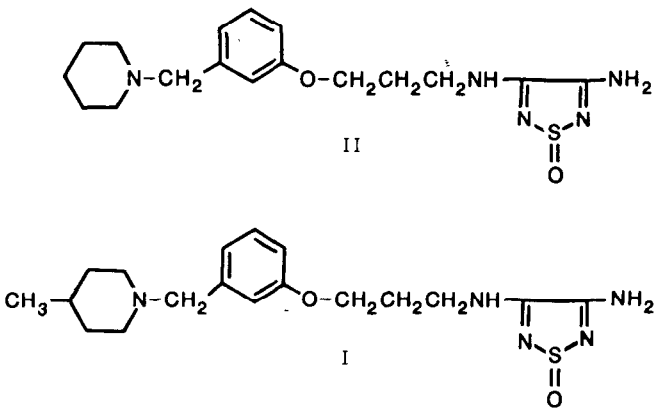


Fig. 1. Chemical structures of I and II.

Paramus, NJ, U.S.A.) through an HP 18652 A/D converter. A Whatman Partisil 10, ODS-3 column (10 cm × 4.6 mm I.D.) was used for separation and was operated at room temperature. The mobile phase consisted of 0.02 *M* dibasic sodium phosphate (pH ca. 3.5)–methanol–acetonitrile (5:1:1, v/v/v). The mobile phase was prepared daily and filtered through a 0.45-μm HA filter (Millipore, Bedford, MA, U.S.A.) before use. At a flow-rate of 2 ml/min, II and I eluted at ca. 3.2 and 4.8 min, respectively.

*Standards*

Stock solutions of I and II were prepared in methanol. A methanolic solution containing 0.5 mg/ml II was used as an internal standard.

*Extraction procedure*

A 0.5-ml sample of plasma was pipetted into a 125 × 16 mm disposable culture tube. To each sample, 5 μl of the internal standard solution (0.5 mg/ml) were added, followed by the addition of 0.1 ml of 0.1 *M* sodium hydroxide and 5 ml of diethyl ether. A piece of aluminium foil was placed on top of the tube before capping. The mixture was shaken for 10 min at 20 rpm and centrifuged for 10 min at ca. 900 *g*. The organic layer was transferred to another 125 × 16 mm disposable culture tube and evaporated to dryness in a water-bath (25–30°C) under a mild stream of nitrogen. The tube was rinsed with ca. 1 ml of diethyl ether. The contents were vortex-mixed for 1 min and evaporated to dryness. The residue was reconstituted with 100 μl of methanol, and 20 μl of the solution were injected onto the column.

*Quantification*

Blank plasma fortified with I was simultaneously analysed with study samples. Peak-height ratios were used for quantification.

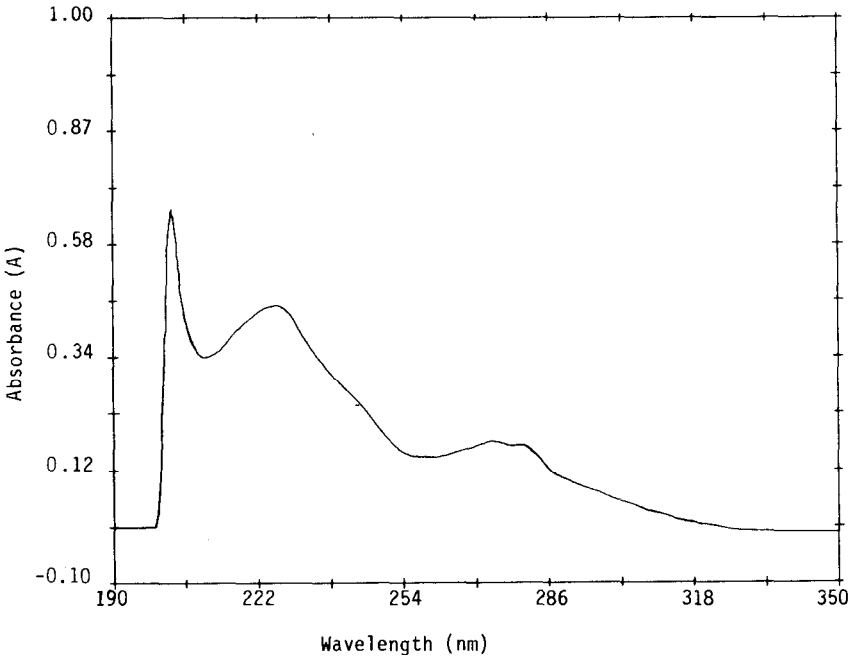


Fig. 2. UV spectrum of I in methanol (10 μg/ml). The absorption spectrum of methanol has been subtracted.

*Extraction recovery*

Plasma samples fortified with 0, 0.04, 0.2, 0.5, 2 and 10 μg/ml I were extracted according to the extraction procedure described above. The extraction recovery was calculated by comparing the peak height of extracted I or II with that of a methanolic standard injected directly onto the column.

## RESULTS AND DISCUSSION

Fig. 2 shows the UV absorption spectra of I in methanol, with a maximum at 226 nm. Therefore, detection at 229 nm (fixed-wavelength detector) or 226 nm (variable-wavelength detector) was chosen for quantification of I.

Typical chromatograms obtained from injections of a mixture of I and II in methanol (10 μg/ml) and a calibration standard (dog plasma spiked with 2 μg/ml I) are shown in Fig. 3.

*Intra- and inter-day precision*

For dog plasma containing 0.04, 0.2, 0.5, 2 and 10 μg/ml I the intra-day coefficients of variation (C.V.) were 4.3–15.3, 0.8–2.6, 1.4–6.5, 0.8–3.6 and 2.9%, respectively. The inter-day variations were 2.7, 1.6, 1.5, 0.5 and 2.2%, respectively (Table I).

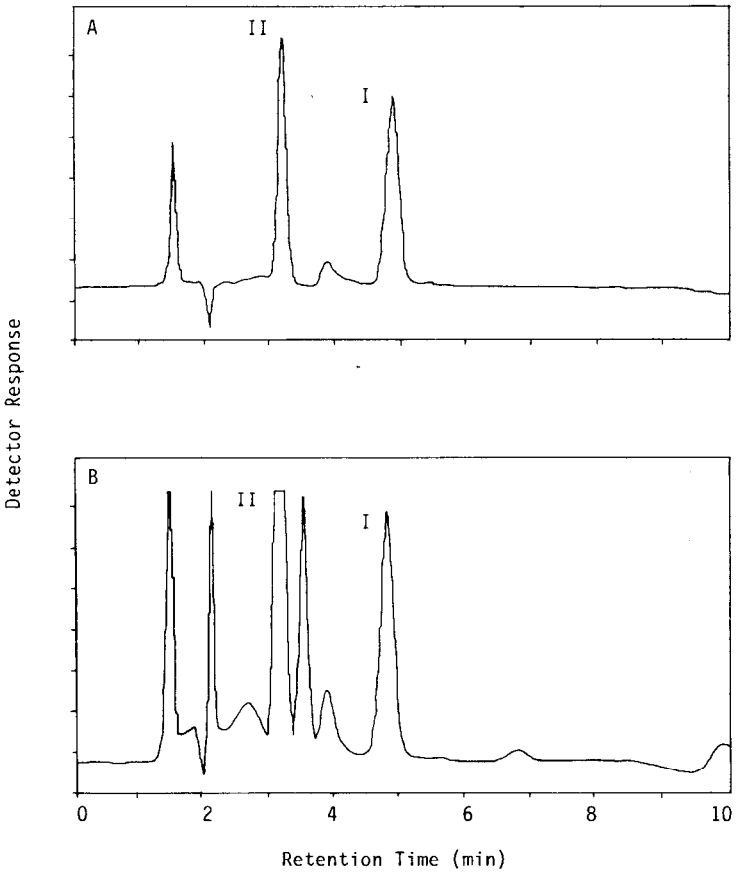


Fig. 3. Chromatograms obtained from (A) a 20-$\mu$l injection of a mixture of II (10 $\mu$g/ml) and I (10 $\mu$g/ml) in methanol and (B) of spiked dog plasma (2 $\mu$g/ml I) with II (2.5 $\mu$g per sample) added.

TABLE I

INTRA- AND INTER-DAY PRECISION STUDY

Dog plasma standards of I were prepared and stored frozen as 0.5-ml aliquots; three replicates at each concentration were analysed each day on three different days. A plasma standard containing 2 $\mu$g/ml I was used for calibration. Values in parentheses are coefficients of variation.

| Spiked concentration ($\mu$g/ml) | Measured concentration (mean, $n=3$) ($\mu$g/ml) | | | | Deviation** (%) |
|---|---|---|---|---|---|
| | Intra-day | | | Inter-day* | |
| | Day 1 | Day 2 | Day 3 | | |
| 0.04 | 0.038 (5.3%) | 0.037 (15.3%) ($n=2$) | 0.036 (4.3%) | 0.037 (2.7%) | −7.5 |
| 0.2 | 0.95 (2.6%) | 0.189 (0.8%) | 0.194 (1.1%) | 0.193 (1.6%) | −3.5 |
| 0.5 | 0.473 (1.4%) | 0.480 (2.7%) | 0.466 (6.5%) | 0.473 (1.5%) | −5.4 |
| 2 | 2.00 (1.9%) | 2.02 (0.8%) | 2.01 (3.6%) | 2.01 (0.5%) | 0.5 |
| 10 | 9.91 (2.9%) | 9.72 (2.9%) | 9.48 (−) ($n=1$) | 9.70 (2.2%) | −3.0 |

*Means of the three daily mean values.

**Percentage deviation = 100% × (measured concentration − spiked concentration)/spiked concentration; inter-day values were used for calculation.

TABLE II

RECOVERY OF I AND II FROM DOG PLASMA

| Spiked concentration of I ($\mu$g/ml) | Recovery (%) | |
|---|---|---|
| | I | II* |
| 0.04 | 69.2 | 54.8 |
| 0.2 | 77.8 | 62.4 |
| 0.5 | 72.9 | 61.9 |
| 2 | 80.9 | 65.2 |
| 10 | 74.1 | 60.3 |
| Mean ± S.D. | 75.0 ± 4.5 | 60.9 ± 3.9 |
| C.V. (%) | 6.0 | 6.4 |

*II (2.5 $\mu$g in 5 $\mu$l of methanol) was spiked in these plasma samples.

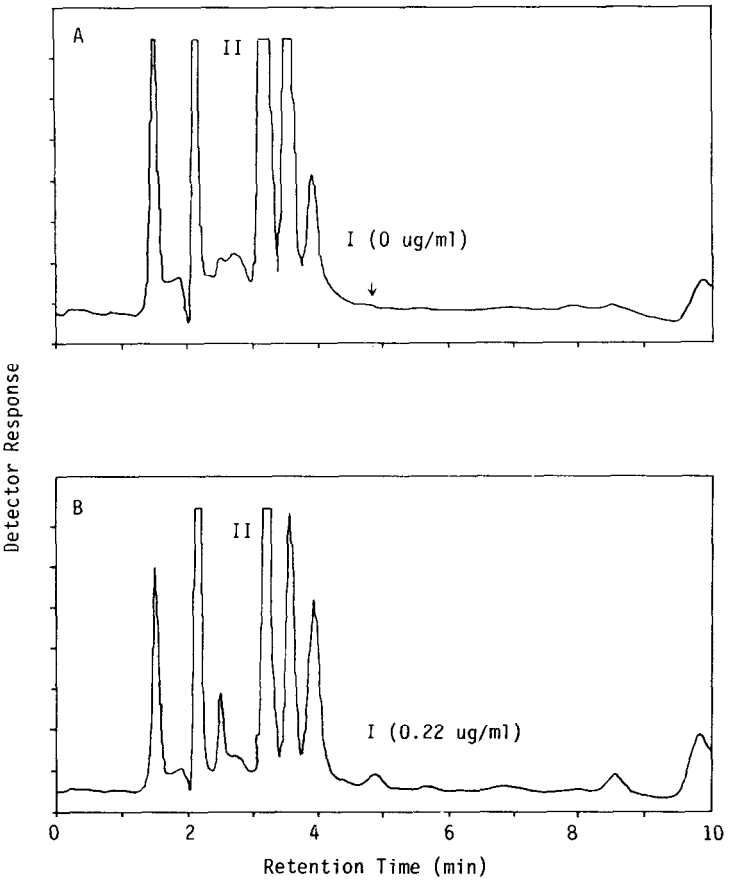


Fig. 4. Chromatograms obtained from a 20-$\mu$l injection of extracted plasma obtained (A) prior to and (B) at 8 h after an intravenous administration of 150 mg of I in one female beagle dog.

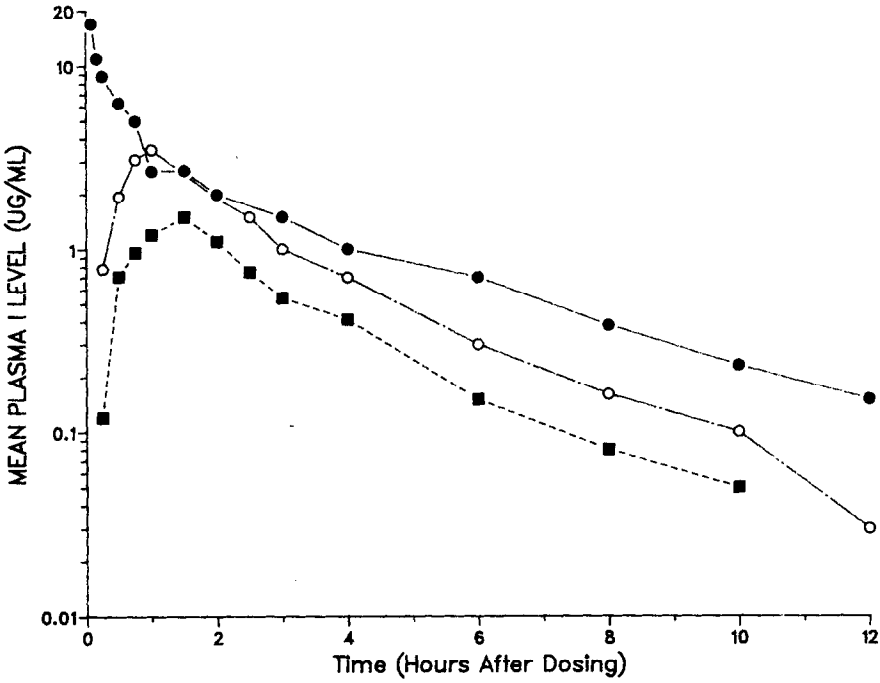


Fig. 5. Mean plasma concentration of I versus time data obtained following intravenous or oral administration of 150 mg of I to six female beagle dogs. Symbols: ● = intravenous; ■ = oral suspension; ○ = oral solution.

*Recovery*

As shown in Table II, the mean recovery of I was 75% at plasma concentrations of 0.04–10 μg/ml. The mean recovery of II (2.5 μg spiked in plasma) was 61%.

*Selectivity*

Different batches of blank dog plasma were extracted and chromatographed as described earlier. No endogenous components were found to elute at retention times close to that of I. As shown in Figs. 3 and 4, an endogenous substance found in some batches of plasma eluted at a similar retention time (3.54 min) to that of the internal standard (3.18 min). However, satisfactory separation was usually achieved by a minor variation ($\pm 5\%$) of the composition of methanol in the mobile phase.

*Linearity of the assay*

This assay is linear for plasma samples containing 0.04–10 μg/ml I. The linearity was demonstrated in the accuracy of the mean calculated concentrations for a series of plasma standards when a single-point calibration was used (Table I).

*Limit of detection*

The limit of detection of this assay is estimated to be 0.04 μg/ml.

*Application*

This assay has been used to analyse plasma samples obtained from dogs after administration of I. After intravenous injection of 15 mg/kg I to six female beagle dogs, the levels of I at 5 min and 8 h were 13.9–22.4 and 0.18–0.97 μg/ml, respectively (Fig. 5). After oral administration of 15 mg/kg I in solution to the same six female dogs, peak levels of 1.59–6.47 μg/ml were attained at 0.5–2 h and levels of 0.08–0.37 μg/ml at 8 h were observed (Fig. 5).

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 after administration of I. It was found to be suitable for pharmacokinetic studies of I in the dog.

REFERENCE

1 L.B. Katz, C.K. Scott and D.A. Shriver, J. Pharmacol. Exp. Ther., 238 (1986) 587.