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Short Communication

Quantitation of a new cholecystokinin and gastrin receptor antagonist (L-365,260) in dog and rat plasma by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic (HPLC) procedure has been developed for the quantification of L-365,260 (I), a cholecystokinin and gastrin receptor antagonist, in dog and rat plasma. The method involves liquid–liquid extraction and HPLC with ultraviolet detection. Standard curves were linear over the range 7.5–2000 ng/ml for rat and dog plasma. The method is reproducible and reliable with a detection limit of 7.5 ng/ml in biological fluids. The mean coefficients of variation for concentrations within the range of the standard curve range were 3.84 and 2.56%, respectively, for intra-day analysis and 4.48 and 4.26%, respectively, for inter-day analysis. Application of the development was successfully demonstrated by quantifying the concentration of I in both dog and rat plasma samples following an intravenous or oral dose of 5 mg/kg I.

INTRODUCTION

L-365,260, [3*R*-(+)-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl]-N-(3-methylphenyl)urea (I), whose chemical structure is shown in Fig. 1, is a potent and selective non-peptide brain cholecystokinin (CCK-B) and gastrin receptor antagonist. Compound I promises to be a potentially powerful new tool for investigating gastrin and brain CCK-B receptors, and

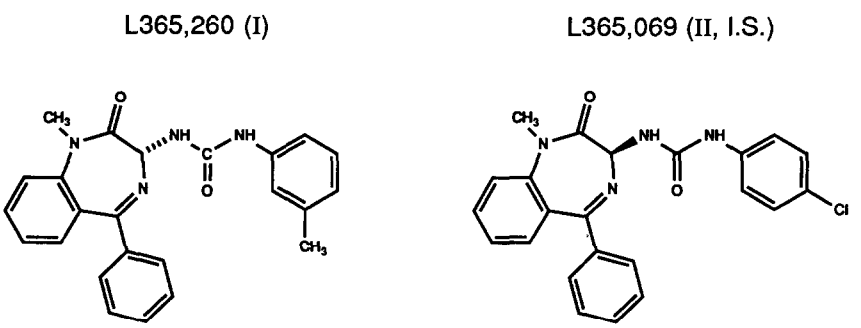

Fig. 1. Structures of I and II.

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possibly their role in physiology and disease [1–3]. It is appropriate, therefore, that the pharmacokinetics of I be determined in laboratory animals and man. The analytical procedure described in this report involves liquid–liquid extraction and reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet detection. L-365,069 (II) (Fig. 1) served as an internal standard (I.S.).

EXPERIMENTAL

Chemicals

I and II (internal standard, I.S.) were synthesized at Merck, Sharp & Dohme Research Labs. (Rahway, NJ, USA). Acetonitrile, methanol, phosphoric acid and triethylamine (TEA) were obtained form Fisher Scientific (Fair Lawn, NJ, USA). Diethyl ether was purchased from Burdick & Jackson Baxter (Muskegon, MI, USA).

Standard preparation

Working standards for I were prepared by diluting a stock solution of 1.0 mg/ml in acetonitrile to 200, 20, 10 and 2.5 μg/ml. Compound II at a concentration of 12.5 μg/ml in acetonitrile served as internal standard stock solution.

Apparatus

The high-performance liquid chromatograph consisted of three components: a WISP Model 710B automatic sample injector with a 96-sample card reader (Waters Assoc., Milford, MA, USA), a 8700XR gradient pump with an SP8500 dynamic mixer (Spectra-Physics, San Jose, CA, USA) and an SP873XR UV variable-wavelength detector operating at 236 nm. A 100 mm × 4.6 mm I.D. column, packed with 5-μm Partisil ODS-3 RAC 2 (Whatman, Clifton, NJ, USA), was used with an in-line RP-18 guard column (30 mm × 4.6 mm I.D.) (Brownlee Labs., Santa Clara, CA, USA). The mobile phase consisted of acetonitrile–0.015 *M* phosphoric acid (40:60, v/v), adjusted to pH 3.2 with TEA. The flow-rate was 2 ml/min.

Sample preparation

Plasma samples were prepared by mixing 0.2 or 0.5 ml of rat or dog plasma with 10 μl of internal standard stock solution (12.5 μg/ml) in a 100 mm × 13 mm screw-cap (PTFE Liner, Kimble, Vineland, NJ, USA) test tube. The samples were adjusted to 1.0 ml with distilled water. After vortex-mixing, the diluted plasma was extracted with 5.0 ml of diethyl ether by shaking for 10 min on an Ederbach shaker. After centrifugation for 5 min at 4500 *g*, 4.5 ml of the organic phase were transferred to a 100 mm × 13 mm culture test tube and evaporated to dryness under nitrogen. The residue was reconstituted in 250 μl of acetonitrile–phosphoric acid buffer solution (30:70) and vortex-mixed for 1 min. An aliquot (200 μl) of the final solution was injected into the HPLC system.

Preparation of standard curve

Calibration standards were prepared by adding 2.5–10 μl of an appropriate working standard to 0.2 or 0.5 ml of control rat or dog plasma. Nine calibration concentrations of I were used for the standard curves (7.5, 12.5, 25, 50, 100, 200, 500, 1000 and 2000 ng/ml) in each matrix. The samples were extracted and processed as described above. Concentrations of I were calculated from the linear regression equation of the daily calibration curve constructed by plotting the ratio of the peak height of I to that of the internal standard against the concentration of I in plasma.

Biological sample

Rats. Two groups of adult male Sprague–Dawley rats, weighing 250–350 g with free access to food and water, were used in this study. Under light pentobarbital anesthesia (40 mg/kg intraperitoneally), a cannula for blood sampling was implanted in the right jugular vein one day before the experiment. The animals received I orally or intravenously at 5 mg/kg, and blood samples were drawn via the cannula at specific time intervals over a 3-h period. All samples were stored at −15°C until the day of analysis.

Dogs. Adult Beagle dogs, weighting 8–10 kg

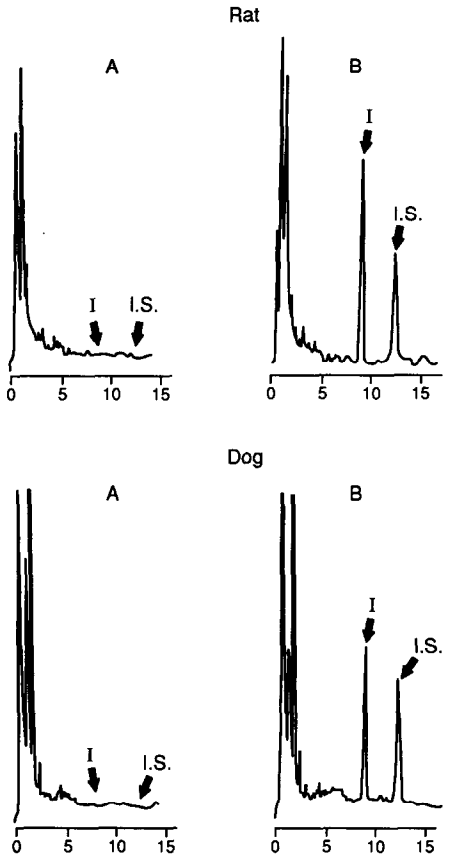

Fig. 2. Typical chromatograms of I and II in rat and dog plasma. (A) Sample containing 125 ng/ml II after intravenous administration of 5 mg/kg I; (B) control plasma.

with free access to food and water, were used in this study. The animals received the compound orally or intravenously at 5 mg/kg in a cross-over fashion and blood samples were drawn via the jugular vein at specific time intervals over a 6-h period. All samples were stored at −15°C until the day of analysis.

RESULTS AND DISCUSSION

An HPLC method with UV detection combined with liquid–liquid extraction has been developed for the determination of I in rat and dog plasma. Under the chromatographic conditions described in the Experimental section, I and the internal standard II were completely separated with retention times of 9.6 and 13.3 min, respectively. Fig. 2 shows representative chromatograms of control (A) and dosed (B) rat and dog plasma samples. No interfering peaks at the retention times of I and II were seen in control samples of any matrix. Optimal conditions for the analytical procedure were determined by alternately varying the molarity (7.5–30 m*M*) and pH (2.2–6.5) of phosphoric acid. Changes in molarity from 7.5 to 30 m*M* and in pH from 2.2 to 6.5 had negligible effects on the retention times of I and the internal standard, II.

The recovery of I added to control rat or dog plasma at concentrations between 12.5 and 2000 ng/ml were at least 95 ± 6.5%; the recovery of II

TABLE I

ACCURACY AND PRECISION DATA FOR THE DETERMINATION OF I IN DOG PLASMA

Concentration (ng/ml)	Intra-day (n = 4)		Inter-day (n = 3)	
	Peak-height ratio of I/I.S.	C.V. (%)	Peak-height ratio of I/I.S.	C.V. (%)
7.5	0.070	1.08	0.066	7.09
50	0.382	4.34	0.356	0.7
200	1.17	5.69	1.39	10.1
2000	12.1	4.23	14.6	0.040
Mean		3.84		4.48
r^2		0.9998		0.9994

TABLE II

ACCURACY AND PRECISION DATA FOR THE DETERMINATION OF I IN RAT PLASMA

Concentration (ng/ml)	Intra-day (n = 4)		Inter-day (n = 3)	
	Peak-height ratio of I/I.S.	C.V. (%)	Peak-height ratio of I/I.S.	C.V. (%)
7.5	0.074	3.30	0.077	1.70
50	0.438	4.45	0.371	3.87
200	1.31	0.80	1.19	2.44
2000	13.9	1.70	13.0	9.02
Mean		2.56		4.26
r^2		0.9996		0.9968

(internal standard) was 92.4 ± 6.3%. Table I shows that for dog plasma, the intra-day and inter-day correlations were 0.9998 and 0.9994, respectively, over the range of the standard curve (7.5–2000 ng/ml, n = 3 or 4). The mean coefficients of variation (C.V.) were 3.84 and 4.48%, respectively.

Data for rat plasma are provided in Table II. The mean intra-day and inter-day correlations were 0.9996 and 0.9968, respectively, and the coefficients of variations were 2.56 and 4.26%, respectively.

Replicate standards (n = 5) were analyzed to assess the reproducibility of I in rat and dog plasma. The mean coefficients of variation were 2.87% for rat plasma and 4.03% for dog plasma, respectively, and the mean percentages difference between calculated and nominal concentration were 2.74% for rat plasma and 6.26% for dog plasma.

This analytical procedure has been successfully applied in pharmacokinetic studies of I and has provided reproducible and reliable results [4]. Typical results in rats and dogs receiving an oral or intravenous dose of 5 mg/kg are reported in Fig. 3. The mean bioavailability of I for dog and rat was 8.8 and 13.6%, respectively, and the terminal half-life of I was 35.7 min for rat plasma and 101.6 min for dog plasma [4].

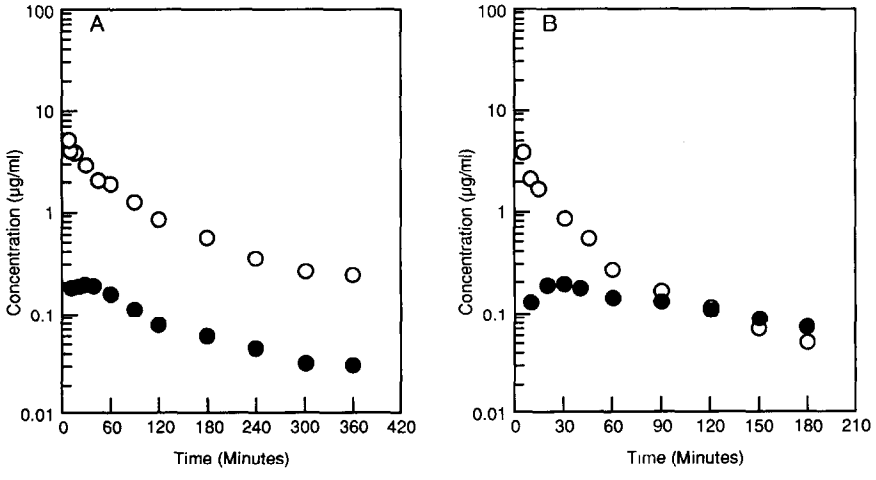

Fig. 3. Concentrations of I in rat (A) and dog (B) plasma following intravenous (○) or oral (●) administration of 5 mg/kg I.

REFERENCES

1 R. S. L. Chang, T. B. Chen, M. G. Bock, R. M. Freidinger, R. Chen, A. Rosegay and V. J. Lotti, *Mol. Pharmacol.*, 35 (1989) 803–808.
2 M. G. Bock, R. M. Dipardo, B. E. Evans, K. E. Rittle, W. L. Whitter, D. F. Veber, P. S. Anderson and R. M. Freidinger, *J. Med. Chem.*, 32 (1989) 13–16.
3 V. J. Lotti and R. S. L. Chang, *Eur. J. Pharmacol.*, 162 (1989) 273–280.
4 I.-Wu Chen, J. H. Lin, J. M. Dorley, H. G. Ramjit and S. M. Pitzenberger, *Drug Metab. Dispos.*, (1992) in press.