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Short communication

Measurement of a new anticonvulsant, (*S*)-3-(aminomethyl)-5-methylhexanoic acid, in plasma and milk by high-performance liquid chromatography

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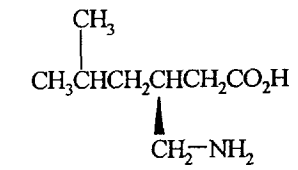
Abstract

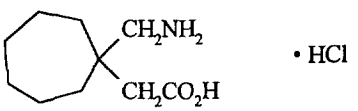
A specific and sensitive isocratic method for the measurement of a new anticonvulsant, (*S*)-3-(aminomethyl)-5-methylhexanoic acid, in rat plasma and milk is described. Following deproteinization, the compound and internal standard [1-(aminomethyl)cycloheptaneacetic acid] were derivatized utilizing 2,4,6-trinitrobenzene sulfonic acid and extracted with cyclohexane. Analytes were resolved on a 5 μm Spherisorb ODSII column (250 mm × 4.6 mm) using a mobile phase of 57% acetonitrile in 0.1 *M* ammonium acetate, pH 4.0. Absorbance was monitored at 350 nm. Limit of quantitation was 1.00 μg/ml for a 100-μl aliquot of plasma or milk.

1. Introduction

(*S*)-3-(Aminomethyl)-5-methylhexanoic acid (CI-1008, I; Fig. 1) (Parke-Davis Pharmaceutical Research, Ann Arbor, MI, USA) is a γ-aminobutyric acid (GABA) analogue being developed as an anticonvulsant [1,2]. I is structurally related to the novel anticonvulsant gabapentin; however, its mechanism of action, as well as that of gabapentin, is unknown. To date, I (IC_{50} 0.037 μM) is the most potent compound capable of displacing [^{3}H]gabapentin from its high affinity binding site in rat brain. The anticonvulsant activity of I and gabapentin are presumed to be mediated via interaction with this receptor.



I



II

Fig. 1. Structures of I (CI-1008) and II (PD 403609, I.S.).

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A study was conducted in lactating rats to determine the toxicokinetics of I and drug-exposure potential during lactation. We present here a versatile HPLC–UV procedure which allows determination of plasma and milk concentrations of I.

2. Experimental

2.1. Chemicals

CI-1008 (I) (100% parent) was synthesized by the Department of Chemistry, Parke-Davis Pharmaceutical Research. Internal standard (II) PD 403609 (84.4% active compound), 1-(aminomethyl)cycloheptaneacetic acid (Fig. 1), was synthesized by Gödecke Research and Development (Freiburg, Germany). All reagents and solvents were of HPLC grade purity, where available. Acetonitrile and cyclohexane were obtained from Burdick and Jackson (Muskegon, MI, USA). HPLC water was obtained from EM Science (Gibbstown, NJ, USA). Derivatizing agent, 2,4,6-trinitrobenzene sulfonic acid [3,4] (TNBSA), was obtained as a 5% w/v aqueous solution from Pierce Chemical (Rockford, IL, USA). Standard solutions of I and II were prepared in HPLC water and stored at 4°C. No special handling procedures were required for the stock solutions or actual study samples. Working standard solutions (1.00 to 500 μg/ml of I and 20.3 μg/ml of II) were freshly prepared before daily analysis by dilution in HPLC water.

2.2. Blood and milk collections

Pregnant Wistar rats received single daily oral doses of 250, 1250, or 2500 mg/kg beginning on gestation day 6 and continuing through lactation. On lactation days 12, 13, or 14, blood and milk samples were obtained from surviving dams. Serial blood samples were collected in heparinized tubes at 0, 1, 4, 7, and 12 h postdose. Plasma was isolated by centrifugation. Milk was collected at 4 h postdose from anesthetized (Ketamine, 40 mg/kg) dams by massage 3 to 5 min after administration of 1 to 2 IU Oxytocin [5]. Plasma and milk samples were stored frozen until analyzed.

2.3. HPLC conditions

The HPLC system consisted of a multi-solvent delivery system (Waters Model 600, Waters Associates, Milford, MA, USA), an autosampler (Waters, WISP Model 712) and a variable-wavelength UV detector (Waters, Lambda Max, Model 481).

Chromatographic separation was achieved using a 5 μm Spherisorb ODSII column (250 mm × 4.6 mm; Phase Separations, Deeside, UK) maintained at 40°C. The mobile phase consisted of 57% acetonitrile in 0.1 *M* ammonium acetate buffer, pH 4.0. Flow-rate was maintained at 1.0 ml/min. UV absorbance was monitored at 350 nm. The output signal generated by the detector was processed by a Hewlett Packard 3392A integrator (Hewlett Packard, Palo Alto, CA, USA).

2.4. Deproteinization

Aliquots (100 μl) of rat plasma or milk were pipetted into 1.5-ml screw-cap micro-centrifuge tubes containing 100 μl appropriate standard or water, 100 μl (2.03 μg) internal standard (II), and 200 μl of 2 *M* perchloric acid. Tubes were capped, inverted, and vortex-mixed vigorously for 30 s. Following centrifugation at 11 000 *g* in a Heraeus Biofuge (Model 15, Baxter Scientific Products, McGaw Park, IL, USA) for 10 min, the aqueous phase was transferred to 16 × 100 mm screw-cap glass tubes.

2.5. Derivatization

Following addition of TNBSA (75 μl) and 50% NaOH (25 μl) to the aqueous phase, tubes were vortex-mixed and let sit for 30 min. Reaction was terminated by the addition of 6 *M* HCl (100 μl).

2.6. Extraction

Saturated NaCl (100 μl) and cyclohexane (4.0 ml) were added. Tubes were shaken horizontally for 10 min, centrifuged at 1600 *g* for 10 min, and solvent phase transferred to 13 × 100 mm disposable tubes. Following evaporation at 50°C under vacuum, the residue was reconstituted in 200 μl mobile phase containing 10% EtOH. A 25-μl aliquot was injected into the HPLC system.

2.7. Calculations

A calibration curve was constructed by plotting the peak-height ratios (I/II) as a function of the amounts of I added to control rat plasma or milk. The best-fit line was determined using least-squares regression with a weighting factor of 1/(concentration^2) [6]. Concentrations of I in the unknown samples were calculated by interpolation from the standard curve.

3. Results

Validation was performed by assaying nine standards in triplicate. Precision, expressed as relative standard deviation (R.S.D.,%) and accuracy, expressed as relative error (R.E., %) between measured and theoretical amounts of drug, are given in Tables 1 (plasma) and 2 (milk). Irrespective of matrix, retention times for I and II were 5.8 and 9.7 min, respectively (Figs. 2 and 3).

Peak-height ratios (I/II) were linearly related to drug concentration over a range of 1.00 to 500

Table 1
Precision and accuracy of the measurement of I in rat plasma samples

	Theoretical calibration standard concentration (μg/ml)								
	1.00	2.00	5.00	10.0	20.0	50.0	100	200	500
Day 1	0.997	2.02	4.89	9.89	20.1	50.1	100	208	522
	1.02	1.97	4.92	9.30	19.7	49.2	102	204	508
	1.04	1.90	4.90	9.98	19.8	50.2	101	203	508
Mean	1.02	1.96	4.90	9.72	19.9	49.8	101	205	513
R.S.D.[a] (%)	2.1	2.6	0.3	3.8	1.0	1.1	1.0	1.3	1.6
R.E.[b] (%)	2.0	−2.0	−2.0	−2.8	−0.5	−0.4	1.0	2.5	2.6
Day 2	1.04	1.85	4.97	9.53	19.3	52.1	104	207	523
	1.03	1.84	4.75	9.61	20.3	50.6	102	195	487
	1.04	1.97	4.73	9.92	20.6	51.4	105	205	501
Mean	1.04	1.89	4.82	9.69	20.1	51.4	104	202	504
R.S.D.[a] (%)	0.6	3.8	2.8	2.1	3.4	1.5	1.5	3.2	3.6
R.E.[b] (%)	4.0	−5.5	−3.6	−3.1	0.5	2.8	4.0	1.0	0.8
Day 3	0.973	2.00	4.80	9.88	19.1	49.7	103	205	503
	1.00	1.95	5.02	10.1	19.3	50.3	101	196	510
	1.05	2.03	4.81	10.2	19.6	50.1	101	206	514
Mean	1.01	1.99	4.88	10.1	19.3	50.0	102	202	509
R.S.D.[a] (%)	3.9	2.0	2.5	1.6	1.3	0.6	1.1	2.7	1.1
R.E.[b] (%)	1.0	−0.5	−2.4	1.0	−3.5	0.0	2.0	1.0	1.8
Mean ($N = 9$)	1.02	1.95	4.87	9.82	19.8	50.4	102	203	508
R.S.D.[a] (%)	2.5	3.5	2.0	2.9	2.5	1.7	1.6	2.3	2.2
R.E.[b] (%)	2.0	−2.5	−2.6	−1.8	−1.0	0.8	2.0	1.5	1.6

[a] Relative standard deviation
[b] Relative error

Table 2
Precision and accuracy of the measurement of I in rat milk samples

Theoretical concentration (μg/ml)	Measured concentration (μg/ml)	R.S.D.[a] (%)	R.E.[b] (%)
1.00	1.01	1.5	1.0
2.00	1.94	1.1	−3.0
5.00	4.95	0.8	−1.0
10.0	10.1	0.6	1.0
20.0	19.8	2.0	−1.0
50.0	49.7	1.4	−0.6
100	100	3.4	0.0
200	201	2.8	0.5
500	513	1.7	2.6

Interday variability not available since a 1-day validation was performed due to limited supply of control milk
[a] Relative standard deviation
[b] Relative error

μg/ml in plasma or milk. Extraction recovery of I or II from plasma was not determined at these concentrations; however, it is anticipated that recovery from plasma would be comparable to that from milk. Extraction recovery ($n = 6$/concentration) of I from rat milk relative to aqueous (H_2O) was 91.9 ± 1.5%, 89.4 ± 1.7% and 82.7 ± 1.9% for 2.00, 20.0, and 200 μg/ml, respectively. Extraction recovery ($n = 6$) of II from rat milk relative to aqueous was 92.3 ± 1.7% for 20.0 μg/ml.

Stability in plasma and milk was determined by analyzing triplicate quality control samples following exposure to three freeze–thaw cycles. Measured concentrations were within ± 4.3% R.E. of mean values obtained during validation, indicating that I is stable in plasma or milk following repeated freezing and thawing.

Limit of quantitation under the experimental conditions described was 1.00 μg/ml for a 100-μl aliquot of plasma or milk based on R.E. and R.S.D. values of ≤10% for calibration standards

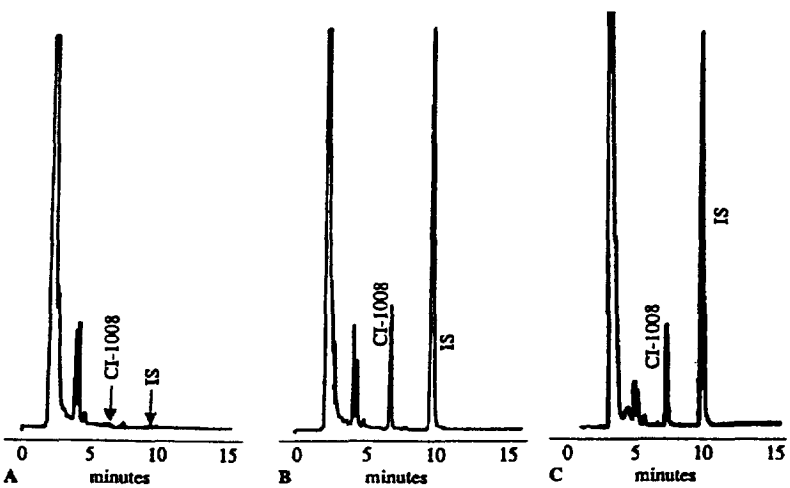

Fig. 2. Representative extracted plasma chromatograms of a blank (A); 100 μg/ml I (CI-1008) standard with I.S. (B); and 94.7 μg/ml observed 4-h following a 250 mg/kg oral dose (C).

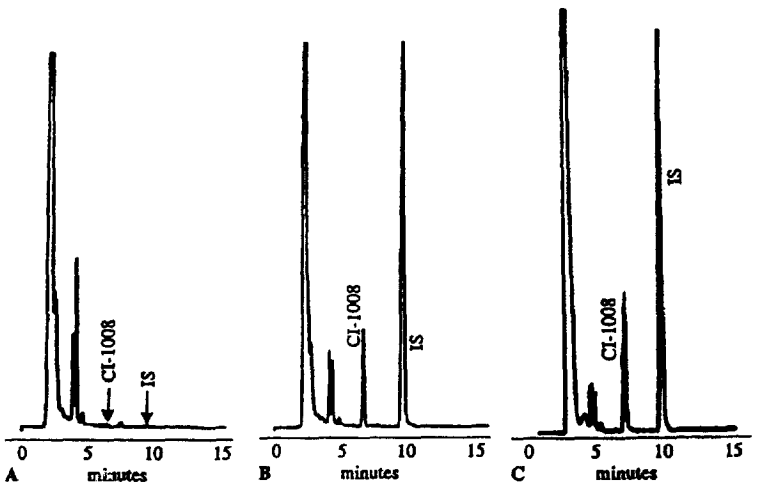

Fig. 3. Representative extracted milk chromatograms of a blank (A); 100 μg/ml I (CI-1008) standard with I.S. (B); and 127 μg/ml observed 4-h following a 250 mg/kg oral dose (C).

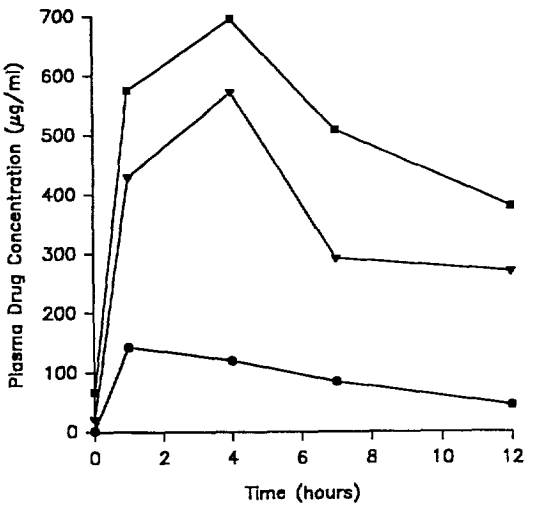

Fig. 4. Mean plasma concentration–time profiles in lactating rats following gavage administration of (●) 250 ($n = 6$), (▼) 1250 ($n = 2$), or (■) 2500 ($n = 1$) mg/kg per day.

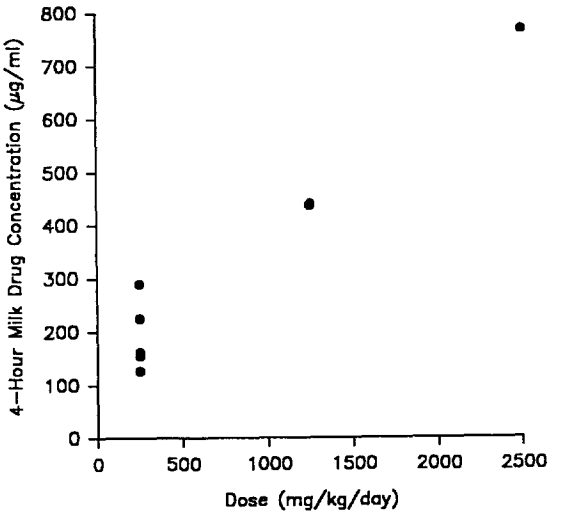

Fig. 5. Individual 4-h drug concentrations in milk from lactating rats receiving single daily oral doses of 250 ($n = 6$), 1250 ($n = 2$), or 2500 ($n = 1$) mg/kg per day.

and quality control samples. Representative chromatograms are provided in Figs. 2 and 3. Mean plasma drug concentration–time profiles in lactating rats are shown in Fig. 4. Individual 4-h drug concentrations in milk are plotted as a function of dose in Fig. 5.

4. Conclusion

This method is suitable for pharmacokinetic analysis of I in plasma and milk. Moreover, the HPLC–UV procedure can be used for quantita-

tion of drug levels in mouse, rabbit, and monkey plasma, demonstrating assay versatility.

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