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Journal of Chromatography B, 681 (1996) 339–345

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Automated quantitative determination of a new polyamine biosynthesis inhibitor (CGP 48 664) and a potential metabolite in human and animal plasma by high-performance liquid chromatography

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Received 26 July 1995; revised 9 January 1996; accepted 9 January 1996

Abstract

A specific and sensitive liquid chromatographic assay for the determination of 4-amidino-1-indanone-2'-amidinohydrazone (CGP 48 664, I) and a potential metabolite, 2-(4-carbamoyl-2,3-dihydro-1H-inden-1-yliden) hydrazine carboximidamide (CGP 53 391, II), in human and animal plasma was developed. CGP 51 467, a structural analog, was added to the plasma samples (up to 1 ml) as an internal standard. After mixing, the samples were processed automatically using an ASPEC solid-phase extraction system. The final extracts were chromatographed on a 5 μm Purospher RP-18 HPLC column. Chromatography was performed using a gradient system and UV detection. The described HPLC method is suitable for specific and quantitative measurement of concentrations of I, as well as its potential metabolite II down to 5–10 ng/ml in human and animal (dog, rat) plasma with acceptable reproducibility and accuracy.

Keywords: 4-Amidino-1-indanone-2'-amidinohydrazone; 2-(4-Carbamoyl-2,3-dihydro-1H-inden-1-yliden) hydrazine carboximidamide

1. Introduction

4 - Amidino - 1 - indanone - 2' - amidinohydrazone (CGP 48 664, I, Fig. 1) is a new sterically rigid and achiral compound and a potent inhibitor of the polyamine biosynthesis enzyme S-adenosylmethionine decarboxylase (SAMDC) [1]. This paper describes a sensitive analytical procedure for the parent compound I and a potential metabolite, 2 - (4 - carbamoyl - 2,3 - dihydro - 1H - inden - 1 - yliden) hydrazine carboximidamide (CGP 53 391, II; Fig. 1), in plasma samples using solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) with UV detection. The structure analog CGP 51 467 (I.S.; Fig. 1) was used as an internal standard.

2. Experimental

2.1. Chemicals and reagents

CGP 48 664 (I), CGP 53 391 (II) and CGP 51 467 (I.S.), (laboratory batches) were supplied by Dr. J.

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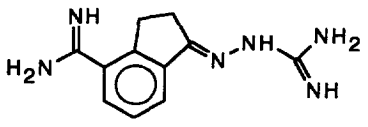
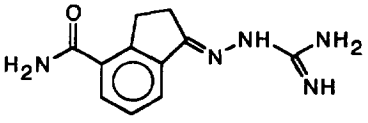
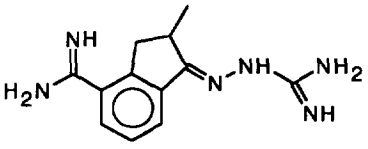
0378-4347/96/$15.00
PII S0378-4347(96)00026-6



CGP 48 664 (I)



CGP 53 391 (Potential metabolite), (II)



CGP 51 467 (Internal standard; I.S.)

Fig. 1. Structures of I (CGP 48 664), II (CGP 53 391) and I.S. (CGP 51 467).

Stanek (Research Basle, Drug Discovery, Ciba-Geigy, Basel, Switzerland). Phosphate buffer pH 8 (0.063 mol disodium hydrogenphosphate and 0.004 mol potassium dihydrogenphosphate per liter), 4 mol/l sodium hydroxide solution and Millipore-filtered water (SQC, HPLC-quality) were obtained from Elemental and Micro analytical Services, Ciba-Geigy. LiChrosolv-grade acetonitrile (ACN), LiChrosolv-grade methanol and sodium dodecylsulfate were obtained from Merck (Darmstadt, Germany). 85% Orthophosphoric acid, acetic acid and methyl acetate were obtained from Fluka (Buchs, Switzerland).

Sample vials (borosilicate glass, 9 ml, 13×100 mm), collection tubes (polyethylene, 3.5 ml, 12×55 mm) and sealing caps for SPE cartridges (500 mg, polyethylene) were obtained from Gilson Medical Electronics (Villiers le Bel, France). Isolute SPE C_2 cartridges (500 mg end-capped, 3 ml) were obtained from International Sorbent Technology (Glamorgan, UK).

Micro-volume sample flask (cylindrical, polypropylene, 0.3 ml) and locking cap with star polyethylene were obtained from Weidemann Plastic (Romanshorn, Switzerland).

2.2. Automatic sample preparation

An ASPEC sample preparation system (Gilson Medical Electronics) was used for the SPE part of the sample preparation.

2.3. Chromatographic conditions

A Hewlett-Packard HP 1090 instrument equipped with a Kratos Spectroflow UV detector set at 230 nm was used. Data capture was achieved with a Merck-Hitachi Model D-2000 computing integrator. The column used was a LiChroCart 125×4 mm I.D. HPLC cartridge, Puroshper RP-18 (5 μm), protected by a 4×4 mm I.D. LiChroCart guard column.

A Si-saturation column, filled with S 10 unbonded 10 μm silica in a 60×4.6 mm I.D. column as protection against the low pH, was mounted between the pulse dampener and injector. The mobile phases A and B for the gradient elution were prepared from a stock mobile phase with 10% acetonitrile containing 10 mmol sodium dodecylsulfate, 20 mmol sodium hydroxide and 130 mmol phosphoric acid per liter; 2.5 l solution contained 7.21 g dodecylsulfate sodium salt, 14.4 g sodium hydroxide (4 mmol/l), 37.48 g of 85% phosphoric acid and 250 ml acetonitrile. All ingredients, except the acetonitrile, were filtered using pore size 0.45 μm cellulose nitrate membrane filters. This stock mobile phase was stable for at least two weeks. The preparation of A and B was as follows: (A) 700 ml stock mobile phase+1400 ml acetonitrile (70%, v/v); (B) 1750 ml stock mobile phase+500 ml acetonitrile (30%, v/v). The gradient elution (flow-rate 0.75 ml/min) program is listed in Table 1.

Table 1
Gradient elution program

Time (min)	B (%)	Acetonitrile (%)
0–0.5	97.5	31
5	65	44
16	50	50
17–22	0	70
23	97.5	31

Stop time=36 min

2.4. Preparation of solutions

Compound I, II and I.S. stock solutions were prepared in Millipore water (100 μg/ml, 5 min treatment with ultrasound). The elution solution (50% (v/v) acetic acid in methyl acetate) was found to be stable for at least one month. The dilution to 1% (v/v) in methanol was made daily. The "rinse mixture" contained 50% (v/v) 0.1 mol/l sodium hydroxide–methanol (30 ml of 4 mol/l sodium hydroxide, 1170 ml of Millipore water and 1200 ml of methanol).

2.5 Procedure

Up to 1.0 ml plasma, the solutions of I and II were added by weight; 150 to 500 ng I.S., in 100 μl were added volumetrically. The volume was completed with water to a total volume of 2.0 ml and 2.0 ml phosphate buffer (pH 8.0) was added to make up to a total volume of 4.0 ml. The samples were prepared in borosilicate glass disposable culture tubes, mixed and placed in the ASPEC. The ASPEC performs the following steps: (A) SPE conditioning with 3.0 ml methanol, followed by 1.0 ml water; (B) application of the samples to the SPE cartridges; (C) Washing of the SPE cartridges with 6.0 ml water and 0.55 ml methanol; and (D) elution with 3.0 ml eluting mixture, containing 1.0% acetic acid and 1.0% methyl acetate in methanol.

Between each of these steps, the sample transfer coil was rinsed with 1.5 ml of the "rinse mixture" to prevent cross-contamination and accumulation of proteins. To improve the stability of the analytes, the elution phase was partially neutralized. A 40-μl volume of 4 mol/l sodium hydroxide was added to the collection tubes and mixed by an ASPEC ram exit program. The eluates were removed from the ASPEC, transferred to 10-ml glass tubes by decanting and rinsing the collection vial with 300 μl of methanol. The transferred samples were evaporated to dryness under a stream of nitrogen in a water bath at 40°C for about 10 min. The residues were redissolved in 500 μl of water. After mixing and brief centrifugation, the final extracts are filled into plastic sample vials for HPLC. Aliquots of 250 μl were injected onto the HPLC column.

2.6. Calibration

To construct calibration curves, plasma samples with known concentrations were prepared ($n=3$) by adding I and II to 1 ml of drug-free human plasma. After addition of the I.S., the samples were processed as described. After chromatography, the peak-height ratios of the compounds to the I.S. were plotted against the given I and II concentrations. The calibration curve was calculated by quadratic ($y=a+bx+cx^2$) rather than linear regression. The following terms for a calibration curve of I and II in the range of 5–1600 ng/ml were obtained. Intercept (a), −0.0042 (I) and −0.0046 (II). Slope (b), 0.0028 (I) and 0. 0039 (II). Slope (c), −0.00000014 (I) and −0. 00000006 (II). Correlation coefficient (r), 0.99966 (I) and 0.99997 (II).

3. Results and discussion

3.1. Stability

Stock solutions of I, II and I.S. were stable for at least three months if stored in the refrigerator (4–8°C). Recoveries of solutions re-analyzed after three months of storage were all within ±10%. In plasma, I and II were stable for at least three freeze–thaw cycles. Recoveries of spiked plasma samples re-analyzed after three months of storage were all within ±10%. Spiked plasma samples were also stable at ambient temperature (+26°C) for up to 3.5 h.

3.2. Selectivity

The chromatograms of extracts of drug-free plasma of human, dog and rat plasma showed no interfering substances and the peaks of I, II and I.S. are well separated from plasma constituents (Fig. 2, Fig. 3 and Fig. 4). The retention times under these conditions were as follows: II, ca. 8.2 min; I, ca. 13.0 min; I.S., ca. 13.7 min.

3.3. Within-day precision and accuracy

Human plasma samples spiked with eight different concentrations of I and II in the range 10–1500

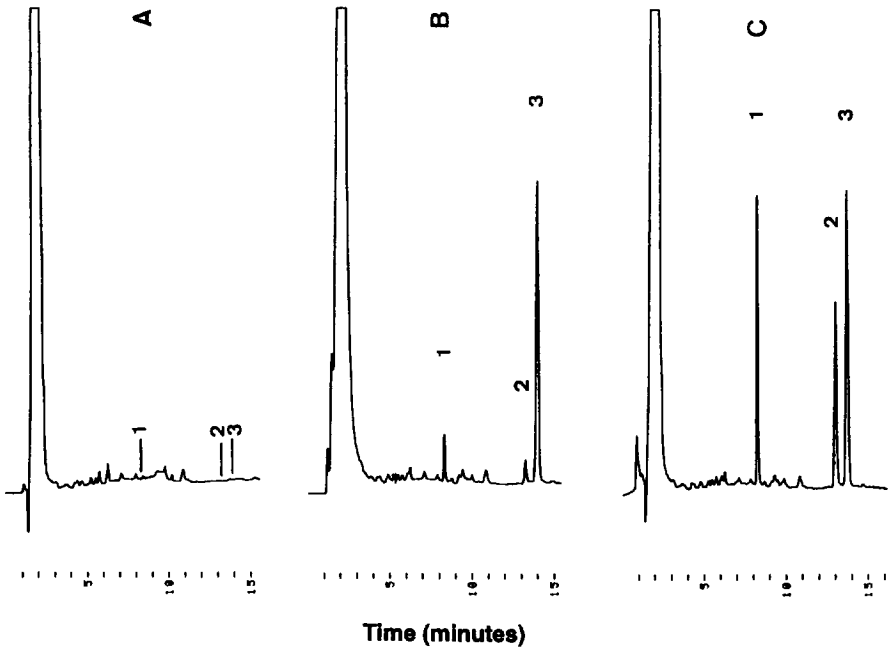


Fig. 2. Chromatograms of extracts of 1 ml human plasma samples. (A) Blank plasma; (B) blank plasma spiked with 37.6 ng II (1), I (2) and 500 ng I.S. (3); (C) blank plasma spiked with 242.4 ng II (1), I (2) and 500 ng I.S. (3).

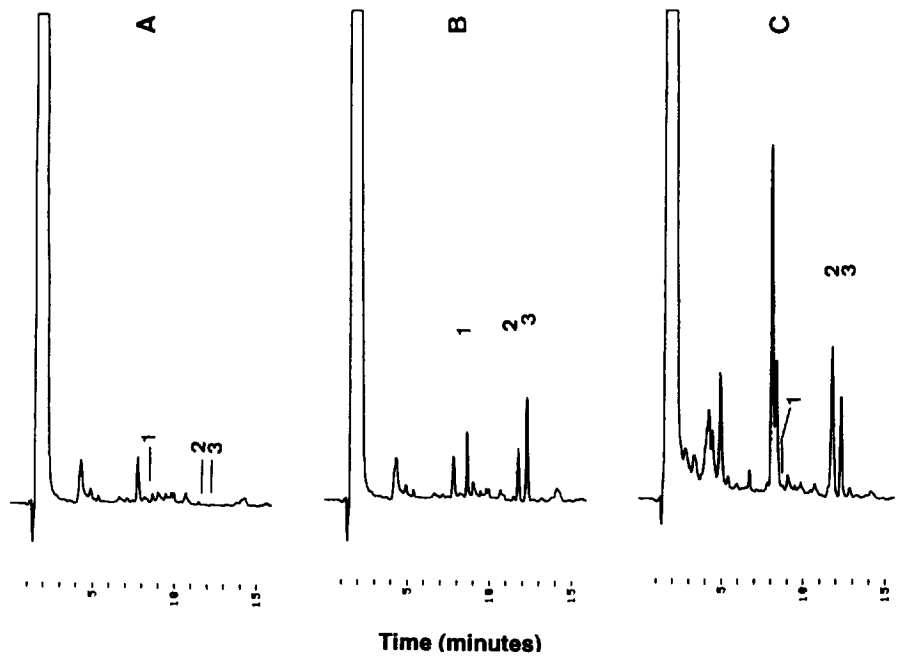


Fig. 3. Chromatograms of extracts of 1 ml dog plasma samples. (A) Blank plasma; (B) blank plasma spiked with 50 ng II (1), I (2) and 150 ng I.S. (3); (C) 2 h after 1 mg/kg I, intravenously.

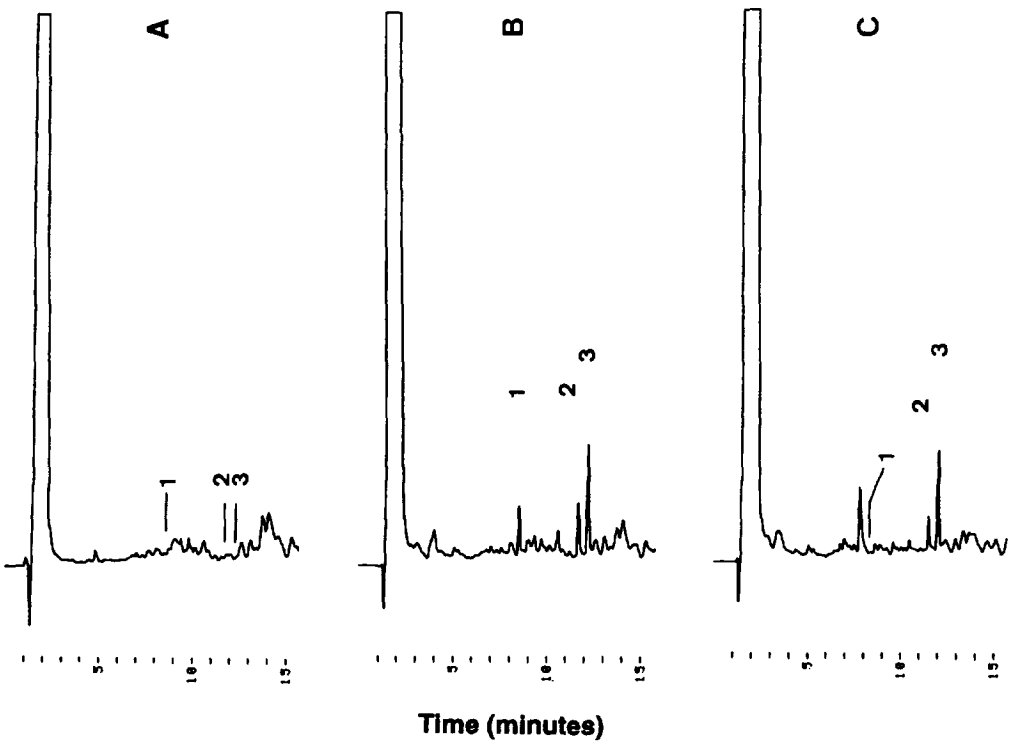


Fig. 4. Chromatograms of extracts of 0.4 ml rat plasma samples. (A) Blank plasma; (B) blank plasma spiked with 50 ng II (1), I (2) and 150 ng I.S. (3); (C) 2 h after 6 mg/kg I, intravenously.

ng/ml, were analyzed four times on the same day. The individual results are given in Table 2 (I) and Table 3 (II). The inter-assay coefficient of variation ranged from 1.0 to 6.8% for I and from 0.7 to 10.1% for II. Deviations of the mean values ranged from −3.6 to 11.9% for I and from −5.3 to 0.1% for II.

The correlation between given and found was subjected to a linear least-squares regression analysis ($y=a+bx$). For I, intercept (a)=2.7241, slope (b)=0.9833, correlation coefficient (r)=0.9998. For II, intercept (a)=0.6430, slope (b)=0.9660, correlation coefficient (r)=0.9998. No significant differences

Table 2
Within-day precision and accuracy of I

Given (ng/ml)	Found (mean±S.D.) (ng/ml)	Inter-assay precision (C.V., %)	Deviation from theory (%)
10.1	11.3±0.2	1.8	+11.9
18.1	17.9±0.2	1.1	−1.1
30.8	29.7±0.3	1.0	−3.6
74.2	76.8±5.2	6.8	+3.5
397.6	398.2±11.5	2.9	+0.1
709.0	705.5±17.3	2.5	−0.5
998.0	983.5±14.0	1.4	−1.4
1522.0	1495.9±15.4	1.0	−1.7

Table 3
Within-day precision and accuracy of II

Given (ng/ml)	Found (mean±S.D.) (ng/ml)	Inter-assay precision (C.V., %)	Deviation from theory (%)
10.1	9.9±1.0	10.1	−2.2
18.1	17.2±0.5	2.9	−5.0
30.8	29.7±0.7	2.4	−3.6
74.2	74.3±0.9	1.2	+0.1
397.6	392.4±7.4	1.9	−1.3
709.0	688.5±9.8	1.4	−2.9
998.0	944.6±6.8	0.7	−5.3
1522.0	1480.7±13.2	0.9	−2.7

Table 4
Between-day precision and accuracy of I

Given (ng/ml)	Found (day 1) (ng/ml)	Deviation from theory (%)	Found (day 2) (ng/ml)	Deviation from theory (%)
5.23	5.20	−0.6	4.95	−5.4
24.10	23.45	−2.7	23.22	−3.7
116.09	113.07	−2.6	111.14	−4.3
488.95	502.70	+2.8	503.48	+3.0
978.97	1001.49	+2.3	1012.20	+3.4
1444.73	1477.51	+2.3	1482.25	+2.6

were observed when rat or dog plasma was used for the validation.

3.4. Between-day precision and accuracy

Six human plasma pools with different concentrations in the range of 5–1500 ng/ml of unchanged I were analyzed on two different days (Table 4). On both days deviation from theory was below 10% in all cases. The metabolite II was not found in plasma samples of any of the species tested so far, therefore no plasma pools were prepared for this compound.

3.5. Limit of quantitation

The limit of quantitation (LOQ) was evaluated for I by repeated analysis ($n=5$) of a plasma sample spiked with 5.25 ng/ml I. The mean (±S.D.) recovery was 126.2±29.5% (23.3% C.V.). Thus the LOQ was estimated to be between 5 and 10 ng/ml

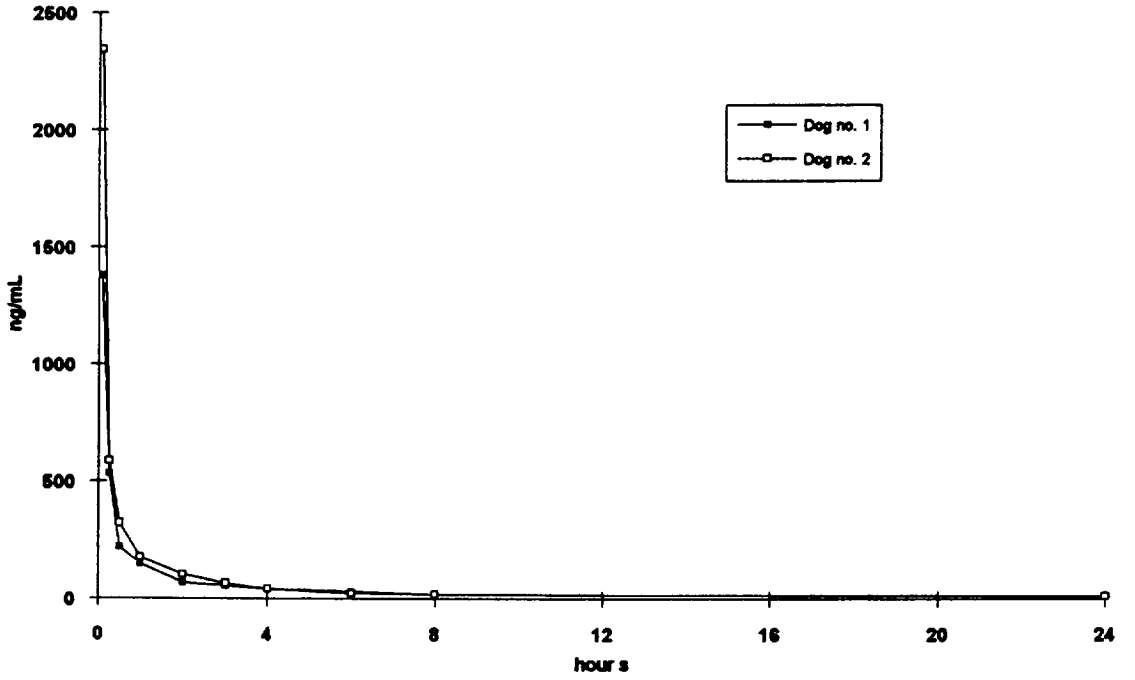


Fig. 5. Application. Plasma concentrations of I in two dogs treated with an intravenous dose of 1 mg/kg I. None of the samples contained measurable concentrations of the potential metabolite II.

depending on the chromatographic background due to the biological material.

3.6. Application

Two beagle dogs were treated with a single intravenous dose of 1 mg/kg I (hydrochloride salt). I and its metabolite II were determined in plasma. No measurable concentrations of II were found in any of the samples. The plasma concentration profiles of I are illustrated in Fig. 5. The kinetics of I was characterized by a rapid initial clearance from plasma followed by a very slow terminal phase ($t_{1/2\beta}$ = ca. 5 days). Total plasma clearance (Cl) was 8.9 and 10.5 l/h.

4. Conclusions

The described liquid chromatographic method is suitable to specifically and quantitatively measure concentrations of I, as well as its potential metabolite II down to 5–10 ng/ml in human and animal (dog, rat) plasma with acceptable reproducibility and accuracy.

References

[1] D. Fan, U. Regenass, H. Kaufmann, M. Gutman, P.J. Beltran, T.E. Campbell, Y.F. Wang, D. Bielenberg and I.J. Fidler, Ann. Oncol., 52 (1994) 81.