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Determination of Ciramadol in Plasma by Gas–Liquid Chromatography

SAMUEL F. SISENWINE ˣ, HAZEL B. KIMMEL, CESARIO O. TIO, ANN L. LIU, and HANS W. RUELIUS

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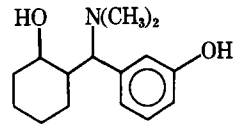
Abstract □ An analytical method for determining ciramadol concentrations in plasma was developed and evaluated for its specificity, precision, linearity, and sensitivity. GLC–electron capture detection of a dipentafluorobenzoyl derivative of the drug was used for quantitation. An isomer of the drug served as an internal standard. Resulting mean ratios of the peak height of derivatized drug to that of derivatized internal standard varied with a coefficient of variation that ranged from 3.8 to 11.1%. The mean ratio was linearly related to ciramadol content (8.75–175 ng) with a correlation coefficient >0.999. The minimum quantifiable concentration was 4 ng/ml with a 2-ml specimen. An application of this method is presented.

Keyphrases □ Ciramadol—analgesics, GLC determination in plasma □ GLC—determination of ciramadol in plasma, analgesics □ Analgesics—GLC determination of ciramadol in plasma

Ciramadol [(−)*cis*-2-(α-dimethyl (I) amino-*m*-hydroxybenzyl)cyclohexanol hydrochloride] is a potent analgesic when administered to animals, and indications of its effectiveness in relieving mild to moderate pain in malignant disease and moderate to severe postoperative pain in humans have been reported (1–3). During previous metabolic disposition studies (4), it was recognized that a very sensitive assay for the drug in biological fluids would be required during preclinical studies and especially when clinical bioavailability studies were performed. Maximum concentrations in the plasma of rats given single 1-mg/kg intragastric doses of the drug were highest (105 ng/ml) at the earliest sampling time (15 min), and when studies were conducted in rhesus monkeys, maximum concentrations in plasma never exceeded 4 ng/ml after a similar dose. In the current report, an assay for ciramadol, which meets the desired requirements, is presented. It is based on the electron-capturing capability of a dipentafluorobenzoylated derivative following a GLC separation.

EXPERIMENTAL

Standards and Reagents—Ciramadol (I) and the internal standard [*trans*-2-(α-dimethylamino-*p*-hydroxybenzyl)cyclohexanol hydrochloride hemiethanolate, II] were synthesized in these laboratories[1]. Deionized water was prepared by passing distilled water through an ion-exchange system[2]. Pentafluorobenzoyl chloride and all other reagents and solvents were purchased from commercial sources.



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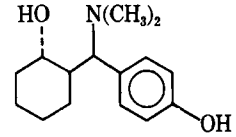

II

Pentafluorobenzoyl chloride was purified by distillation prior to use. The fraction boiling at 158–159° was collected and stored in a sealed container at 5°.

Calibration standards were prepared from a stock solution containing 1 mg of drug/ml of deionized water. Subsequently, solutions containing 0.01, 0.02, 0.05, 0.1, and 0.2 μg of drug/100 μl, or, respectively, 8.75, 17.5, 43.75, 87.5, and 175 ng of free base/100 μl were prepared. All concentrations of ciramadol in biological fluids are expressed as free base.

The concentration of the internal standard solution prepared in water was 100 ng/100 μl.

Preparation of Pentafluorobenzoyl Derivatives—One gram of ciramadol (as free base) was dissolved in 80 ml of benzene and 30 ml of pyridine. To the solution, 2.5 g of pentafluorobenzoyl chloride was added dropwise with stirring at 25° for 2 hr. The reaction mixture was washed with 100 ml each of water, 10% Na_2CO_3, water, 0.5 *N* H_2SO_4, 10% Na_2CO_3, and water. The organic layer was separated, dried over anhydrous sodium sulfate, treated with 2 g of charcoal[3], filtered, and evaporated. A pale yellow syrup was obtained. After treating again with charcoal the syrup was analyzed.

Anal.—Calc. for $C_{29}H_{21}NO_4F_{10}$: C, 54.64; H, 3.32; N, 2.20. Found: C, 54.64; H, 3.44; N, 2.27.

Compound II (200 mg) was dissolved in 16 ml of benzene and 6 ml of pyridine. To the solution, 0.5 g of pentafluorobenzoyl chloride was added dropwise with stirring at 25° for 2 hr. The reaction mixture then was washed sequentially (20-ml portions) and treated with charcoal as described above. After the solvent was removed under reduced pressure, a syrup was obtained. A pale yellow crystalline product precipitated after the syrup was dissolved in warm ethanol and cooled at 4°. The precipitate was collected, recrystallized from ethanol, and dried in air. Yield: 75 mg.

Anal.—Calc. for $C_{29}H_{21}NO_4F_{10}$: C, 54.64; H, 3.32; N, 2.20. Found: C, 54.43; H, 3.49; N, 2.65.

Mass Spectrometry—Mass spectrometric analysis of the two derivatives was performed on a mass spectrometer[4] (electron beam, 70 eV; source temperature, 200°) equipped with a data system[5]. Samples were examined by direct introduction in the electron-impact mode.

Analysis of Ciramadol in Plasma—*Extraction and Derivatization*—Standards (0.01, 0.02, 0.05, 0.1, and 0.2 μg of ciramadol) were added to a series of 16 × 125-mm culture tubes (polytef lined[6] screw cap) containing 1 ml of control (drug free) dog plasma. To each sample was added

[1] Dr. J. P. Yardley, Medicine Chemistry Section II, Wyeth Labs., Inc., Radnor, Pa.
[2] Bion Exchanger System, Pierce Chemical Co., Rockford, Ill.
[3] Norit, Fisher Scientific Co., Fair Lawn, NJ 07416.
[4] AEI-MS 902, Associated Electronic Ind., Ltd., U.K.
[5] Nova 3 Computer system with DS-50S software, Data General Corp., Southboro, Mass.
[6] Teflon, DuPont Co., Wilmington, Del.

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Table I—Precision of the Ciramadol Assay

Concentration[a], Ciramadol ng/ml			Intra-assay Replications of Standards			
Hydrochloride Salt	Free Base	*N*	Mean Ratio ± *SD* I/II	*CV*, %	Slope[b]	Correlation[b] Coefficient
Experiment A						
10	8.75	5	0.160 ± 0.006	3.8		
20	17.5	5	0.307 ± 0.025	8.1		
50	43.75	5	0.805 ± 0.051	6.3		
100	87.5	5	1.544 ± 0.144	9.3		
200	175	5	3.273 ± 0.215	6.6		
					0.0187	0.9996
Experiment B						
10	8.75	5	0.126 ± 0.014	11.1		
20	17.5	4	0.232 ± 0.021	9.1		
50	43.75	5	0.610 ± 0.037	6.1		
100	87.5	5	1.151 ± 0.096	8.3		
200	175	5	2.433 ± 0.179	7.4		
					0.0138	0.9995
Experiment C						
10	8.75	5	0.153 ± 0.015	9.8		
20	17.5	5	0.294 ± 0.022	7.5		
50	43.75	5	0.757 ± 0.067	8.9		
100	87.5	5	1.522 ± 0.109	7.2		
200	175	5	2.988 ± 0.180	6.0		
					0.0172	1.0000

[a] Internal standard (100 ng) was added to a 1-ml sample of control dog plasma. [b] Values were obtained by linear regression analysis of known concentrations of ciramadol (as free base) *versus* mean ratios of peak heights.

100 ng of the internal standard in 0.10 ml of water, 1 ml of 5 *N* NH_4OH, and 5 ml of ether. Each sample was mixed thoroughly and centrifuged, and 4 ml of the ether extract was transferred to another tube. The aqueous phase was extracted once more with 5 ml of ether, and the ether phase was combined with the original extract. One ml of 5 *N* acetic acid was added to the combined ether extracts, and the contents were mixed thoroughly and centrifuged. The ether phase was aspirated, and the aqueous extract was washed with 5 ml of ethyl acetate. After aspirating the ethyl acetate wash, any remaining ethyl acetate was removed by heating the aqueous extract in a water bath at 60° for 5 min under flowing nitrogen. Sodium chloride (800 mg), 1 ml of 5 *N* NH_4OH, and 1 ml of toluene were added to each sample. The contents were mixed and centrifuged, and then 0.7 ml of the toluene extract was transferred to a 12 × 75-mm test tube and reacted with 0.10 ml of pyridine and 0.05 ml of pentafluorobenzoyl chloride at 25° for 30 min with shaking after 15 min. The reaction tube containing the mixture was washed with 1 ml of 10% Na_2CO_3, 1 ml of 0.2 *N* H_2SO_4, 1 ml of 10% Na_2CO_3, and 1 ml of water, successively, and refrigerated until analysis by GLC.

GLC Conditions—GLC analyses were performed with a gas–liquid chromatograph[7] equipped with a 1.8 m × 2-mm glass column containing 2% OV-101 Chromosorb W-HP (80/100 mesh)[8]. The column was maintained at 230°, the injection port at 260°, and the electron detector (nickel 63) at 340°. The carrier gas was ultra-high pure nitrogen. The column was pretreated by injecting 4 μl of a toluene–lecithin solution (1 mg/ml).

Calculations—The ratio of the peak heights for derivatized ciramadol to derivatized internal standard at each concentration of the drug in plasma was determined. The line of best fit relating the ratio of peak heights to ciramadol concentration was calculated by a linear regression analysis.

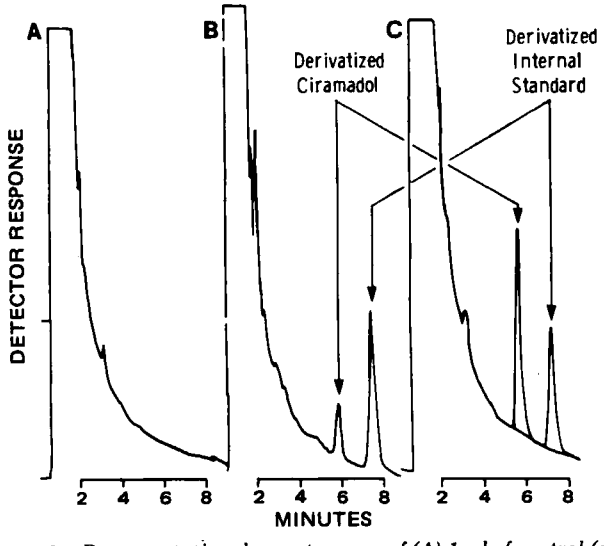

Figure 1—*Representative chromatograms of (A) 1 ml of control (drug free) plasma; (B) plasma sample (1 ml) from a dog given ciramadol; (C) 100 ng of ciramadol and 100 ng of internal standard added to 1 ml of control dog plasma.*

RESULTS

Mass Spectrometry—A mass ion at *m/z* 637 in the mass spectrum of derivatized ciramadol demonstrated that a dipentafluorobenzoyl derivative, which is consistent with the elemental analysis, occurred. The most predominant fragment was observed at *m/z* 344 and represented

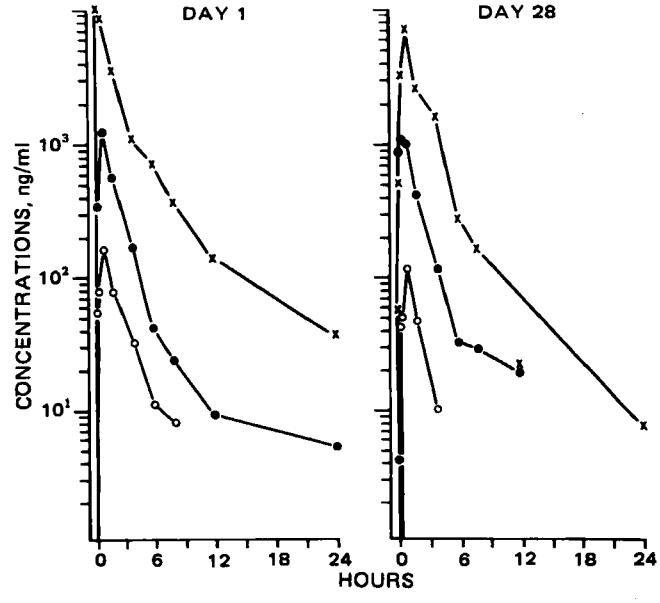

Figure 2—*Mean concentrations of ciramadol in plasma of dogs on day 1 and day 28 following the administration of daily 2- (○), 12- (●), and 72-mg/kg (×) oral doses of ciramadol.*

[7] Varian 3700, Varian Instrument Group, Palo Alto, Calif.

[8] Supelco, Inc., Bellefonte, Pa.

the facile cleavage of the derivative to the fragment $(CH_3)_2\dot{N}{=}CH{-}C_6H_4{-}OCOC_6F_5$. A fragment observed at m/z 195 indicated the loss of a pentafluorobenzoyl moiety. The mass spectrum of the derivatized internal standard was similar.

GLC—The retention time of the derivatized drug and internal standard was 5.8 and 7.6 min, respectively. A representative chromatogram is shown in Fig. 1. No interfering peaks were observed in control (drug free) plasma.

The mean ratio of the peak height for derivatized ciramadol to derivatized internal standard and its coefficient of variation at each concentration of drug added to control plasma (three experiments) is shown in Table I. The coefficient of variation for each mean ratio ranged from 3.8 to 11.1%. Standard curves relating ciramadol concentration to peak height ratio were linear with correlation coefficients ranging from 0.9995 to 1.0000. With a 2-ml plasma sample, the minimum quantifiable concentration was 4 ng/ml (as free base).

DISCUSSION

The results of the present study demonstrate that the specificity, linearity, precision, and sensitivity of the GLC assay for ciramadol is satisfactory for measuring the drug in plasma in concentrations >4 ng/ml. This method has been applied to samples obtained in studies on ciramadol disposition in rhesus monkeys, dogs, and humans. Urine specimens have also been analyzed by this procedure. An example of the application of the assay, which demonstrates its utility in a multiple dose study in dogs, is presented in Fig. 2. The assay permitted the observation that multiple dosing did not affect the metabolic disposition of ciramadol in dogs. No peaks interfered with the detection of drug or internal standard in samples obtained from other investigated species.

Other methods of analysis have been attempted but were not pursued for various reasons. These included GLC and HPLC analysis of underivatized ciramadol as well as other nonfluorinated derivatives of ciramadol. None proved to be as sensitive as the described procedure.

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GLC–Mass Fragmentographic Determination of Mannitol and Sorbitol in Plasma

TERUYOSHI MARUNAKA ˣ, EIJI MATSUSHIMA, YUKIHIKO UMENO, and YOSHINORI MINAMI

Received September 18, 1981, from the *Research Laboratory, Taiho Pharmaceutical Co., Ltd., Kawauchi-cho, Tokushima, 771-01, Japan.* Accepted for publication February 25, 1982.

Abstract □ A GLC–mass fragmentographic method was developed for the simultaneous determination of mannitol and sorbitol as their *n*-butyldiboronate derivatives in plasma. The plasma sample was deproteinized, and the subsequent supernatant was concentrated to dryness; the resulting residue was then dissolved in pyridine containing *n*-butylboronic acid to allow derivation. An aliquot of this solution was injected into the gas chromatograph–mass spectrometer and analyzed by a selected-ion monitoring method using galactitol as the internal standard. Detection was limited to 20 ng/0.1 ml of plasma for both mannitol and sorbitol. A rapid, precise, and sensitive assay for the determination of mannitol and sorbitol in plasma was established.

Keyphrases □ Mannitol—GLC–mass fragmentographic analysis in plasma □ Sorbitol—GLC–mass fragmentographic analysis in plasma □ GLC–mass fragmentography—analysis, mannitol and sorbitol in plasma □ Hexiol—mannitol and sorbitol in plasma, GLC–mass fragmentographic analysis

Mannitol and sorbitol (glucitol) have been used as artificial sweeteners in pharmaceutical preparations.

Many methods employing GLC with a flame ionization detector have been reported for the determination of common hexitols. Authentic samples of mannitol and sorbitol have been analyzed by this GLC method in studying the acetyl (1–7), trifluoroacetyl (8, 9), trimethylsilyl (10), phenyldiboronate (11), and *n*-butyldiboronate derivatives (12). However, the mannitol and sorbitol contents were determined (13) in pharmaceuticals as their *n*-butyldiboronate derivatives. Mannitol, sorbitol, and other polyols were assayed (14) in human plasma or cerebrospinal fluid as their acetyl derivatives. Furthermore, mannitol was measured (15) in body fluids as an *n*-butyldiboronate derivative.

However, these GLC methods indicate several problems in determining mannitol and sorbitol in plasma samples, such as poor sensitivity or the influence of glucose pooled in plasma. Thus, a GLC–mass fragmentographic determination of mannitol and sorbitol in plasma was examined, and a rapid, precise, and sensitive analytical method for their assay was established. The present method was also found applicable to other human biological fluids.

EXPERIMENTAL

Materials—Mannitol[1], sorbitol[1], galactitol[1] (dulcitol), zinc sulfate[1], barium hydroxide[1], and other chemicals used were obtained commercially. *n*-Butylboronic acid[2] and pyridine[3] were derivating agents for *n*-butyldiboronate formation.

GLC–Mass Fragmentographic Conditions—A mass spectrometer[4] with an electron-impact ion source connected to a gas chromatograph[5] was used.

The coiled glass column (1 m × 2-mm i.d.) of the gas chromatograph was packed with 3% OV-17 on Chromosorb W-AW, 80–100 mesh[6] and conditioned at 280° for 24 hr. The temperatures of the injector, column,

[1] Wako Pure Chemical Co., Osaka, Japan.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Model JMS-D 300, JEOL, Tokyo, Japan.
[5] Model JGC-20kP, JEOL, Tokyo, Japan.
[6] Gaschro Kogyo Co., Tokyo, Japan.

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