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Note

Determination of carboplatin in canine plasma by liquid chromatography with ultraviolet–visible detection and confirmation by atomic absorption spectroscopy

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Carboplatin [cis-diamine(1,1-cyclobutane dicarboxylate)platinum, CBDCA, NSC No. 241240] (Fig. 1) is a second-generation cisplatin (CDDP) analogue with similar preclinical antitumor activity [1,2]. Clinical phase I and II trials with CBDCA have demonstrated near-equal efficacy as well as reduced nephrotoxicity and nausea making CBDCA one of the most promising CDDP analogues developed to date [3,4].

High-performance liquid chromatographic (HPLC) analysis of CBDCA has been recently described [5,6]. Both techniques employed ultrafiltration procedures to separate protein-bound and free components of CBDCA. However, these reports did not address the release of drug from the protein-bound fraction prior to HPLC analysis. The protein-bound fraction of CBDCA in human plasma is small (<10% at 37°C) at 2 h post-injection. The protein-bound

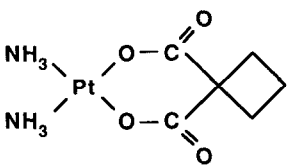

Fig. 1. Structure of carboplatin (CBDCA).

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fraction becomes significant at 24 and 48 h post-injection (>70% at 37°C). This paper describes the use of HPLC with an appropriate releasing reagent to determine CBDCA concentrations in canine plasma. In addition, the use of a photodiode array detector to obtain multi-wavelength chromatograms is employed to determine the chromatographic peak homogeneity. The HPLC method was confirmed by atomic absorption spectroscopic (AAS) determination of platinum [7].

EXPERIMENTAL

Materials and reagents

All reagents were of analytical grade. For the preparation of mobile phase acetonitrile, methanol and 2-propanol (Fisher Scientific, Pittsburg, PA, U.S.A.) were of HPLC grade. Carboplatin was supplied by the Drug Synthesis Branch of the National Cancer Institute (Bethesda, MD, U.S.A.).

A 1 mg/ml stock solution of carboplatin was prepared in HPLC-grade water. The working solutions of 20, 5 and 1 μg/ml were prepared daily from the stock solution.

The microseparation system, Centricon-10, molecular cut-off filter of 10 000 daltons was supplied by Amicon (Danvers, MA, U.S.A.), HPLC-grade water was obtained from a Model 1000-Hydro ultrapure water system (Hydro Services and Suppliers, Research Triangle Park, NC, U.S.A.).

Chromatographic conditions

The liquid chromatograph (Waters Chromatography Division, Milford, MA U.S.A.) was equipped with a W600 multi-solvent delivery system with U6K injector and temperature control accessory set at 50°C. This was coupled to a Model 990 photodiode array detector operated at 210–320 nm and 0.1–0.03 a.u.f.s. Chromatographic separation was achieved on a 3-μm Spherisorb phenyl column (10 cm×4.6 mm I.D.) obtained from Phenonenex (Rancho Palos Verdes, CA, U.S.A.).

CBDCA was eluted by an initial mobile phase consisting of 5% methanol running for 5 min followed by acetonitrile–methanol–2-propanol–water (45:45:5:5, v/v) with a flow-rate of 2.5 ml/min. This was used to purge the column of the remainder of the endogeneous components from the canine plasma ultrafiltrate. The column was then reequilibrated for 10 min with the initial mobile phase. With a mobile phase flow-rate of 0.5 ml/min the retention time of CBDCA was 3.6 min.

Plasma sample preparation procedure

Canine plasma (0.5 ml) was diluted in the microseparation system (Centricon-10, 10 000 molecular mass cut-off filter, Amicon) with 0.5 ml of a mixture of acetonitrile–methanol–water (10:40:50, v/v) and vortex-mixed. The mix-

ture was centrifuged for 30 min at 2677 *g* in a 45° fixed-angle rotor (International Equipment, Needham Heights, MA, U.S.A.). A 10–30 μl aliquot of a colorless filtrate was used for HPLC analysis.

Calibration

Linearity of the UV–VIS photodiode array detector response was studied by injecting 10-μl quantities of increasing concentrations of CBDCA standard solution containing 0.5–20 μg/ml analyte. Each measurement was repeated twice. The relationship between peak areas and concentration of CBDCA was linear over the entire range (correlation coeficient = 0.9985, $n = 10$).

Determination of platinum in canine plasma by electrothermal AAS

Canine plasma samples were analysed by electrothermal AAS using a Model 4000 AAS system and HGA-400 graphite furnace (Perkin-Elmer, Norwalk, CT, U.S.A.). Samples (10 μl) of undiluted plasma were pipeted onto L'vov platforms in pyrolytically coated graphic tubes. The wavelength was set at 265.9 nm using a Perkin-Elmer hollow cathode platinum lamp and deuterium arc background correction [7].

RESULTS AND DISCUSSION

Previous reports concerning the determination of drugs in serum of various species have demonstrated the usefulness of microseparation techniques to remove macromolecules and eliminate time-consuming extraction procedures [8–13]. Carboplatin exhibits minimal binding to plasma proteins during initial time periods (0–2 h) at normal body temperature (37–38°C) in the dog. By 24–48 h the protein binding increases to 100% (data not shown). Release of CBDCA from protein binding is thus necessary to insure analytical precision as to provide accurate pharmacokinetic data of total drug concentrations for clinical application.

Several stationary phases were evaluated for their ability to separate CBDCA from endogenous plasma compounds and from the solvent front. Based on

TABLE I

STATISTICAL SUMMARY OF CBDCA RECOVERIES FROM CANINE PLASMA

Amount added (ppm)	n	Amount recovered (ppm)		Coefficient of variation (%)	Mean recovery (%)
		Range	Mean ± S.D.		
20	10	17.9–21.9	19.5 ± 1.56	7.99	97.5
5	10	4.40–5.26	4.78 ± 0.31	6.39	95.6
1	10	0.77–1.07	0.90 ± 0.04	4.80	90.0

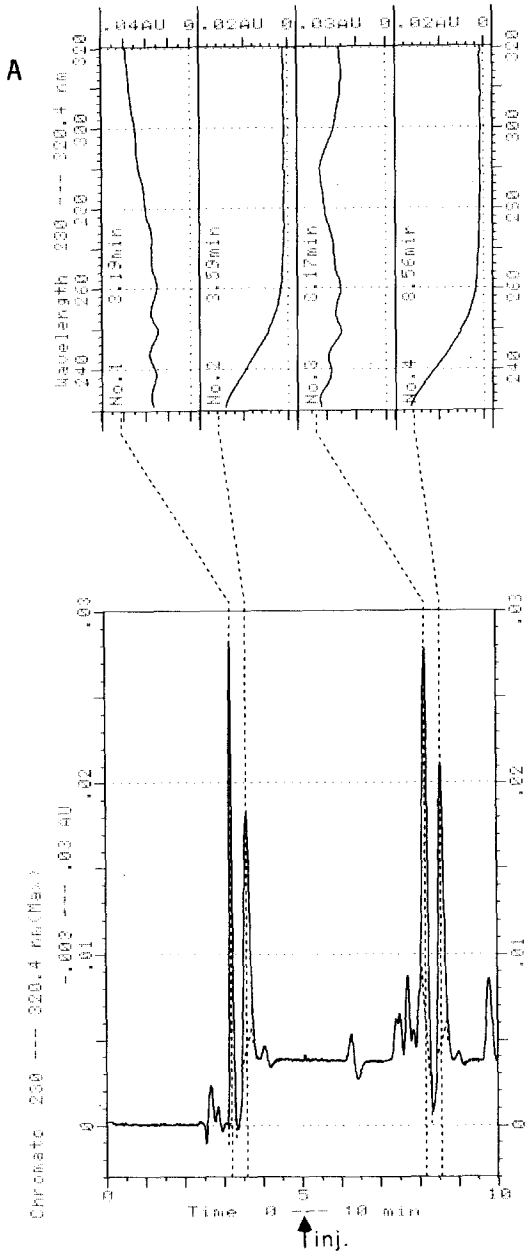
A
.04AU
.02AU
.03AU
.02AU
Wavelength 230 --- 320.4 nm
No.1
3.13min
No.2
3.69min
No.3
3.17min
No.4
3.56min
Chromato 230 --- 320.4 nm(Max)
-.002 --- .03 AU
Time 0 --- 10 min
inj.

Fig. 2.

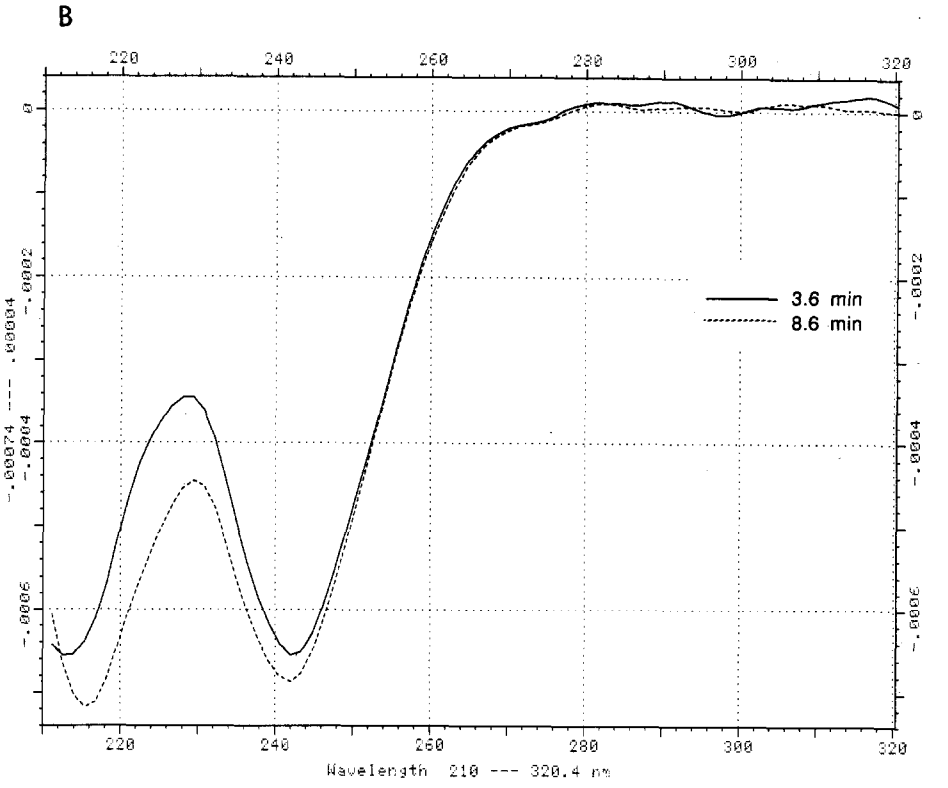

Fig. 2. (A) Fragment of HPLC-UV-VIS profile acquired at the maximum wavelength for all peaks in the range 230–320 nm (bottom) and UV spectra for each peak (top); No. 2. is 50 ng CBDCA standard solution acquired at 3.59 min running time (injected at 0 min) and No. 4 is 52 ng CBDCA from spiked canine plasma acquired at 8.56 min running time (injected at 5 min). Injection volume was 10 μl. (B) Comparison of the first derivative of spectra acquired at carboplatin standard peak at 3.6 min running time and carboplatin peak in canine plasma ultrafiltrate injected at 5 min running time and acquired 3.6 min after injection. (—) Standard; (---) sample.

resolution and recovery of CBDCA, Spherisorb 3-μm phenyl (100 mm×4.6 mm I.D.) yielded optimal results.

Several solutions used to release protein-bound CBDCA were evaluated (mixtures of differing amounts of acetonitrile, methanol, ethanol and water). Canine plasma diluted with an equal volume of 10% acetonitrile, 40% ethanol and 50% water yielded a recovery of CBDCA of 97.5% at 20 μg/ml, 95.6% at 5 μg/ml and 90.0% at 1 μg/ml (Table I).

HPLC-UV-VIS profiles of 50 ng CBDCA standard (0–5 min) and canine plasma spiked with 5 μg/ml CBDCA (5–10 min; injected at 5 min) are shown in Fig. 2A (bottom). This fragment of the chromatogram was computer-generated at the maximum wavelengths for all existing peaks in the 230–320 nm range with their respective UV spectra (top). The similarity of the UV-VIS

TABLE II

COMPARISON OF HPLC AND AAS MEASURED VALUES OF CARBOPLATIN INCUBATED WITH CANINE PLASMA AT 44°C

Incubation time (h)	*n*	Quantity of carboplatin (ppm)	
		HPLC	AAS
0	2	4.55	4.51 (2.37 Pt)
0.25	2	4.40	4.45 (2.34 Pt)
1.00	2	4.41	4.34 (2.28 Pt)
1.50	2	3.65	3.72 (1.95 Pt)
12.00	2	2.80	2.93 (1.56 Pt)

spectrum for the CBDCA in standard solution (curve 2, retention time 3.59 min) and plasma ultrafiltrate (curve 4, retention time 8.56 min, injected at 5 min) proves the homogeneity of the carboplatin peak in canine plasma. The blank canine plasma chromatograms showed an excellent analytical window for CBDCA (not shown).

Since absorption spectrum for CBDCA has no distinct absorption maximum (Fig. 2A, top) computer-generated first derivatives of UV–VIS spectra for CBDCA in standard solution and canine plasma ultrafiltrate were used to enhance resolution (Fig. 2B). These spectra are essentially identical.

Comparing the measured quantity of the drug in canine plasma by HPLC–UV–VIS photodiode array detection and by AAS determination of platinum in this matrix shows a good agreement between the two methods (Table II). The mean difference in measured values between HPLC and AAS was 2.6%.

The UV–VIS photodiode array detection limit was estimated to be 10 ppb using an injection volume of 100 μl and based on a 2:1 signal-to-noise ratio at 210 nm.

In summary, the method described herein is relatively simple, sensitive and selective for the HPLC–UV–VIS determination of CBDCA in canine plasma. This method was confirmed by AAS determination of platinum. Use of this procedure should facilitate clinical investigations of CBDCA by allowing the plasma concentration of total drug to be easily monitored.

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