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Determination of carboplatin in plasma and tumor by high-performance liquid chromatography–mass spectrometry

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Abstract

Carboplatin is a platinum analogue that is used in a number of chemotherapeutic regimens for solid tumors, such as lung and ovarian carcinomas. Most often characterization of carboplatin's pharmacokinetic properties is based on measurement of platinum, rather than intact carboplatin. We have developed a sensitive LC–MS method for the determination of intact carboplatin in plasma ultrafiltrate and in tumor tissue. Carboplatin was extracted from rat plasma ultrafiltrate and tumor samples using solid-phase extraction cartridges and analyzed using reversed-phase chromatography with positive electrospray ionization followed by mass spectrometric detection. Using 50 μl of plasma ultrafiltrate or 140 μl of tumor homogenate supernatant, the extraction afforded a recovery of 58.7 and 45.8% for plasma and tumor, respectively. The mobile phase was 5% acetonitrile in 0.5% acetic acid at 0.2 ml/min that yielded a retention time of carboplatin of 2.2 min. The method has been validated at carboplatin plasma ultrafiltrate concentrations from 0.07 to 2.5 μg/ml, and from 0.03 to 1.3 μg/ml in tumor homogenates. The main advantages of this method compared with earlier methods are the ability to measure intact carboplatin in a sensitive and specific manner.

Keywords: Carboplatin

1. Introduction

Carboplatin (cis-diammine (1,1-cyclobutanedicarboxylato)-platinum (II)) is an antineoplastic drug with an activity profile similar to cisplatin, yet possessing an improved toxicity profile that has been attributed to greater chemical stability due to slower rates of conversion to active aquated species [1]. It is recommended for chemotherapy of ovarian cancer, head and neck cancer, and lung cancer [2].

Currently, the most widely used assays for carboplatin in biological samples are based on the quantitation of elemental platinum by atomic absorption spectroscopy [3–6] because of their high sensitivity. The water solubility and poor UV absorption properties (Fig. 1) of carboplatin have also contributed to the use of assays based on measurement of platinum. Platinum is the DNA alkylating species of carboplatin, forming DNA–platinum adducts. In this regard, measurement of platinum tumor concentrations or platinum–DNA adducts can provide useful intracellular pharmacodynamic information.

In order to characterize the pharmacokinetics of intact carboplatin, several HPLC methods have been reported [7–13]. Since carboplatin is irreversibly

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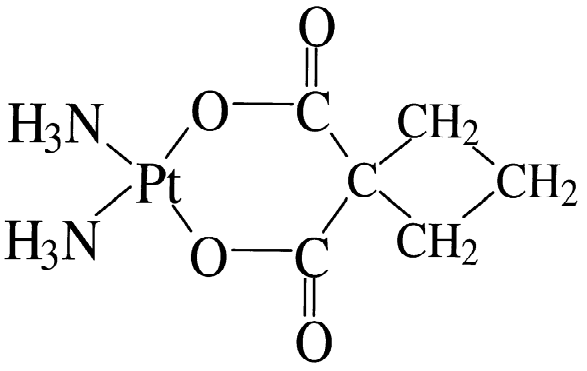


Fig. 1. Chemical structure of carboplatin.

bound to proteins, analyses of biological samples are normally based on the measurement of unbound drug contained in plasma ultrafiltrates (PUFs). HPLC methods that utilize UV detection have been relatively insensitive to carboplatin although one recent method has reported limits of quantitation (LOQ) of 0.05 μg/ml from PUF [13]. The inherent lack of favorable UV absorption properties of carboplatin has led to a post-column derivatization technique with sodium bisulfite [12,13] that yielded an LOQ of 0.05 μg/ml in PUF without a specific sample clean-up step [13]. Another factor that has contributed to the limited number of intact carboplatin assays is its very polar nature, which makes clean-up of plasma samples by either liquid–liquid extraction or solid-phase extraction difficult. Recently, an HPLC–UV method utilizing solid-phase extraction reported an LOQ of 0.05 μg/ml [13], yet a relatively large volume of PUF (200 μl) was required. Both derivatization and direct UV methods also led to relatively longer chromatographic run times, being 26 min for post-column derivatization and 52 min for direct UV, respectively [13]. Other alternative methods to improve assay sensitivity for carboplatin have used column switching techniques and electrochemical detection [11,14]. The column switching method reported a limit of detection of carboplatin of less than 0.2 μg/ml, whereas electrochemical detection led to an improved detection limit of 0.037 μg/ml from PUF.

HPLC–MS has been widely used for the determination of drugs and their metabolites in biological samples because of its high sensitivity and specificity. Two HPLC–MS methods have been reported to determine carboplatin in aqueous solution [15,16], with one method based on the use of inductively coupled plasma (ICP)-MS [16]. Both provided a high sensitivity with a detection limit of 130 pg [16] and a quantitation limit of 700 pg [15] in aqueous solution. No HPLC–MS method has been applied to the measurement of carboplatin in biological samples. This may in part be attributed to difficulties in sample clean-up that can cause interferences and inadequate ionization of the water-soluble carboplatin. This paper describes a new HPLC–MS method using positive electrospray ionization for the sensitive and specific determination of carboplatin in PUF and tumor.

2. Experimental

2.1. Chemicals and supplies

Carboplatin (cis-diammine (1,1-cyclobutanedicarboxylato)-platinum) was purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile and acetic acid were purchased from Fisher Chemicals. Purified water (Nanopure deionization system, Barnstead/Thermolyne, Dubuque, IA, USA) was used for all aqueous solutions. Drug-free rat plasma was purchased from Lampire Biological Laboratories (Pipersville, PA, USA). Bond Elut SCX cartridges (100 mg/1 ml) were purchased from Varian (Harbor City, CA, USA). Centrifree centrifugal filter devices were purchased from Millipore (Bedford, MA, USA).

2.2. Preparation of stock solutions, calibration standards and quality control samples

A standard stock solution containing carboplatin was prepared in water at a concentration of ~180 μg/ml and stored at 4 °C. This stock solution was diluted with purified water to obtain working solutions of 21.5, 6.5, 1.9, 0.6, and 0.2 μg/ml.

Calibration standards in PUF were prepared at concentrations of 2.6, 0.8, 0.2, 0.070, and 0.02 μg/ml by adding appropriate aliquots of each working solution to PUF. Quality control samples were

prepared in a similar way and assayed in triplicate on each day that samples were analyzed.

Tumor homogenates were prepared by adding purified water to tumor tissue at a ratio of 4:1 (ml:g) and then homogenized for 2 min. Calibration standards and quality control samples in tumor homogenate were prepared at carboplatin concentrations that ranged from ~0.03 to 1.3 μg/ml homogenate. Quality control samples were prepared in triplicate on each day that samples were analyzed.

2.3. Solid-phase extraction

Approximately 150 μl of rat plasma was placed in the centrifugal filter device and centrifuged at 2000 *g* for 10 min to obtain PUF. A volume of 50 μl of PUF was mixed with 56 μl of water by vortex. Under mild vacuum, 100 μl of the mixture was applied to an SCX cartridge preconditioned with 1 ml methanol and 2 ml water. The cartridge was then washed with 100 μl of 0.1% acetic acid, and then eluted with 200 μl of mobile phase. Aliquots of 10 μl were injected onto the HPLC–MS.

For tumor samples, 200 μl of tumor homogenate was centrifuged at 15 000 rpm for 3 min followed by the addition of 140 μl supernatant to an SCX cartridge, again preconditioned with 1 ml methanol and 2 ml water. An analogous extraction procedure was then followed as described for PUF.

2.4. Method validation

The precision and accuracy of the assay was based on analyses of PUF and tumor samples. PUF and tumor homogenate quality control samples were included in all calibration curves and processed in triplicate. The intra-day and inter-day means, standard deviations, and coefficients of variation (C.V.) were calculated by standard methods. The limit of quantitation (LOQ) in PUF and tumor homogenates was determined using quality control samples and defined as the lowest concentration at which the signal-to-noise ratio was greater than 6:1, and both intra-day and inter-day C.V.'s and % biases were less than 15%.

The specificity of the assay for carboplatin in the presence of endogenous components of rat PUF was evaluated using plasma obtained from different batches of commercial rat plasma as well as from plasma collected from Fischer 344 rats. Interference from drugs that might be co-administered with carboplatin was investigated in rat PUF to which topotecan (2.8 μg/ml) and paclitaxel (10 μg/ml) had been added.

2.5. LC–MS

The HPLC system consisted of an HP 1100 series chromatographic system coupled to a Finnigan Navigator Quadrupole MS. Positive ion electrospray mode was used. The nebulizer temperature was maintained at 150 °C and nitrogen was used as both the nebulizer gas and drying gas with a flow-rate of 310 l/h. A cone voltage of 11 V and capillary voltage of 3.22 V were used. The high mass and low mass resolution were 1.9 and 11.5, respectively. The analytes were detected at m/z 370.3 with a dwell time of 0.2 s in selected-ion recording (SIR) mode with the multiplier voltage set at 600 V.

The analytical column was an ODS Hypersil (Hewlett-Packard; 5 μm particle size, 100×2.1 mm) maintained at ambient temperature. The mobile phase consisted of 5:95 acetonitrile:0.5% (v/v) acetic acid pumped at a flow-rate of 0.2 ml/min.

3. Results and discussion

3.1. Mass spectrometry and chromatography

The molecular weight of carboplatin is 371.3. The full scan spectrum is dominated by masses at m/z 370.3, 371.3 and 372.3 with similar relative intensity. Blank biological samples showed there were no interfering peaks at m/z 370.3 at the retention time of carboplatin. Trace peaks were observed at m/z of 371.3 and 372.3 with a signal intensity of about two- to three-fold higher than baseline noise. Therefore, the m/z at 370.3 was chosen for detecting intact carboplatin in biological matrices. The MS responses to carboplatin with either acetic acid or formic acid in the mobile phase were compared. Mobile phases containing acetic acid showed a better response than those that contained formic acid, with response increasing in proportion to acid concentrations up until 0.5% after which no further response increase

was observed. The polar nature of carboplatin limited its retention on a reverse phase column; however, we found the use of 5% acetonitrile provided adequate retention and peak shape for carboplatin. This mobile phase composition unmasked a baseline shift in biological samples prior to the retention time of carboplatin, yet acetonitrile concentrations of less than 5% that eliminated this shift yielded broad carboplatin peaks and reduced sensitivity. Since the validation process demonstrated that the LOQ was not affected by the baseline shift, the mobile phase of 5% acetonitrile was used for analysis.

3.2. Extraction of carboplatin from biological matrices

The biological matrices had a pronounced effect on the MS response of carboplatin. Use of methanol precipitation of protein in plasma and tumor tissue did not provide adequate sample clean-up as the carboplatin peak was masked. Direct injection of PUF, which is protein-free, also yielded a poor MS response even at very high concentrations that could be attributed to inhibition of ionization. It appeared solid-phase extraction was necessary to obtain an adequate MS response for carboplatin. Numerous types of solid-phase materials (i.e. C_{18}, C_2, CN, NH_2 and SAX) were tried without success due to the inability to retain carboplatin and resulted in very low recoveries. The SCX cartridge was able to retain carboplatin more than the other types of cartridges. Carboplatin is a strong hydrophilic compound and cannot be retained on most solid-phase materials. However, due to the two amine groups on carboplatin that could bind with benzenesulfonic acid anions, the strong cation-exchange groups on SCX columns, adequate retention of carboplatin was attained. The relatively longer retention on the SCX cartridges resulted in suitable separation and higher recoveries. The recoveries of carboplatin were sensitive to the washing and elution volumes. The final volume of 100 μl for the washing solution (0.1% acetic acid) and 200 μl of mobile phase for elution provided adequate recovery without an evaporation step. The mean recoveries ($n=3$) from PUF were 59.3, 52.4, 60.6, and 62.4% at carboplatin concentrations of 2.58, 0.77, 0.23, and 0.07 μg/ml, respectively, whereas from tumor homogenate supernatant the recoveries were 50.3, 44.1, and 43.1% at concentrations of 1.08, 0.32, and 0.097 μg/ml, respectively. Following extraction, sample solutions were stable for 1.5 h at room temperature and for 24 h at 4 °C, whereas stock solutions were stable for at least 1 month when stored at 4 °C.

3.3. Method validation

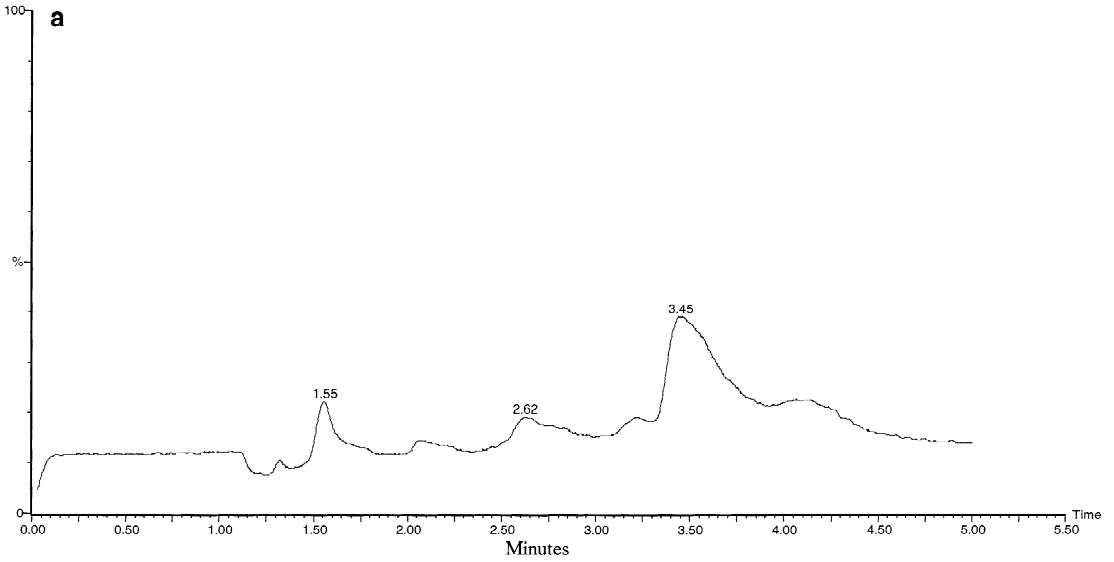
The method was validated for carboplatin in PUF and in tumor homogenate over concentration ranges of 0.07–2.58 μg/ml in PUF and 0.034–1.27 μg/ml in tumor homogenate. Calibration curves were linear with average correlation coefficients greater than 0.999 in the range of 0.02–2.6 μg/ml for PUF, and 0.034–1.3 μg/ml in tumor homogenates. At carboplatin concentrations greater than 1 μg/ml in the injection solution both peak area and peak height MS responses were saturable. Therefore sample concentrations greater than 1 μg/ml were diluted with mobile phase before injection. The method yielded mean intra-day and inter-day precision and accuracy values of less than ±15% (Tables 1 and 2).The LOQs were 0.07 μg/ml for PUF and 0.034 μg/ml for tumor homogenate. There were no interfering peaks in plasma ultrafiltrate samples containing topotecan and paclitaxel at concentrations of 2.8 and 10 μg/ml, respectively.

Table 1
Intra-day precision and accuracy of carboplatin in plasma ultrafiltrate (PUF) and tumor homogenate ($n=3$)

	PUF (μg/ml)				Tumor (μg/ml)			
Concentration added	0.070	0.232	0.775	2.58	0.0342	0.114	0.380	1.266
Concentration measured	0.075	0.243	0.740	2.44	0.039	0.122	0.367	1.142
SD	0.01	0.024	0.083	0.232	0.002	0.012	0.009	0.110
C.V. (%)	13.1	9.7	11.2	9.5	4.5	10.0	2.5	9.6
Bias (%)	7.1	4.6	−4.5	−5.5	12.7	7.1	−3.4	9.7

Table 2
Inter-day precision and accuracy of carboplatin in plasma ultrafiltrate (PUF) and tumor homogenate ($n=5$)

	PUF (μg/ml)				Tumor (μg/ml)			
Concentration added	0.070	0.232	0.775	2.58	0.0342	0.114	0.380	1.266
Concentration measured	0.071	0.241	0.708	2.469	0.0338	0.118	0.371	1.181
SD	0.008	0.021	0.071	0.121	0.004	0.006	0.0105	0.076
C.V. (%)	10.9	10.0	8.8	4.9	11.1	5.1	2.8	6.5
Bias (%)	10.7	−8.6	3.9	−4.4	−1.1	3.8	−2.3	−6.7



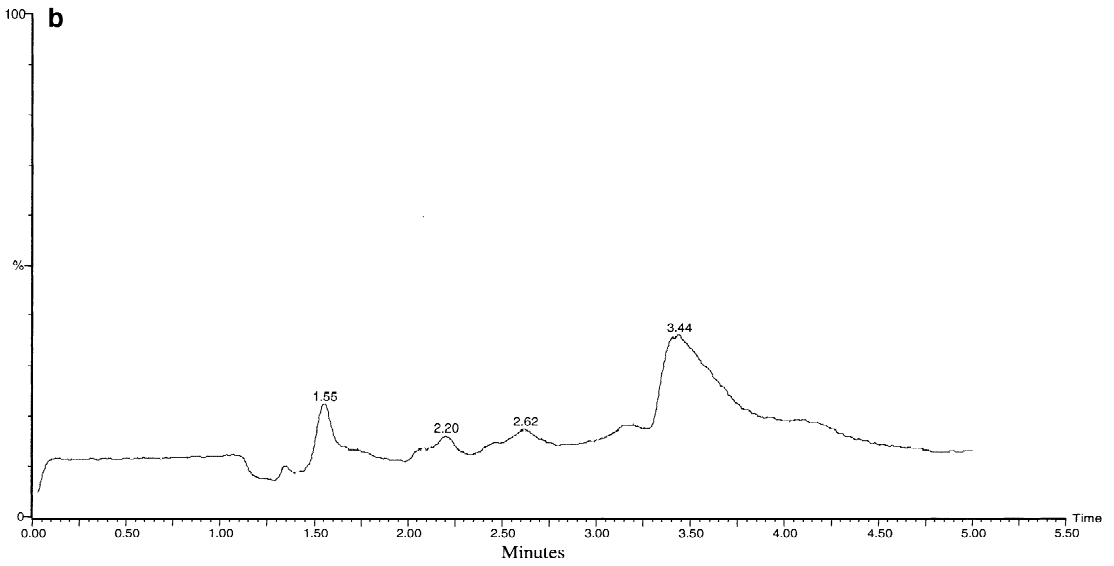
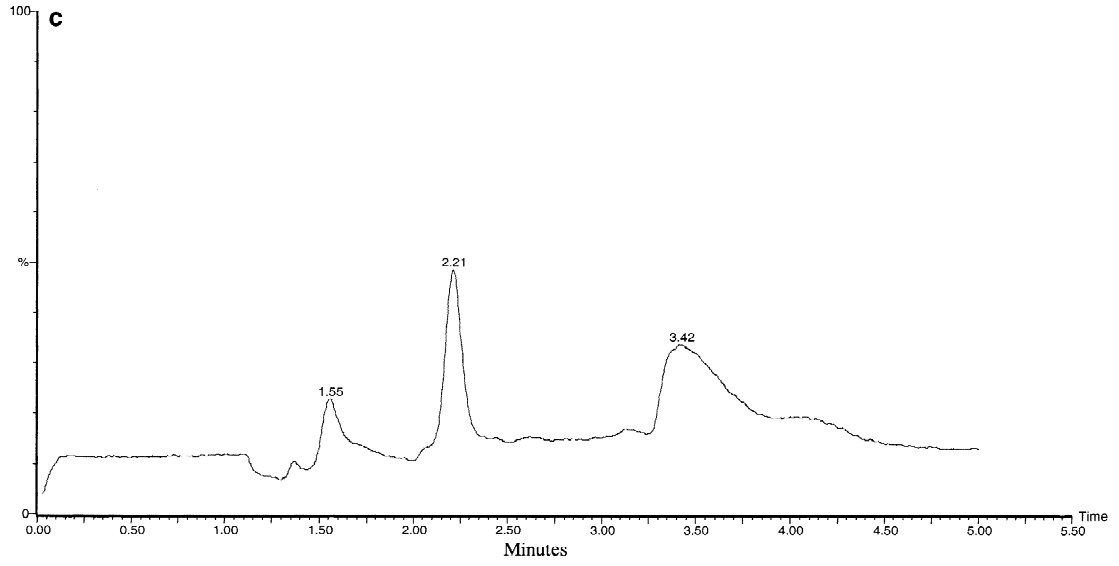


Fig. 2. Chromatograms of carboplatin in plasma ultrafiltrate (PUF): (a) blank; (b) 0.070 μg/ml; (c) 0.770 μg/ml; (d) rat PUF obtained at 4 h after administration of 30 mg/kg carboplatin. The retention time of carboplatin is 2.2 min.

3.4. Method application

The analytical method was subsequently used to analyze samples from pharmacokinetic studies in a syngeneic rat model of ovarian cancer. Briefly, Fischer 344 female rats (130–150 g) were implanted intraperitoneally with 15×10^6 Nutu 19 ovarian tumor cells [17] and allowed to grow for ~30 days. Carboplatin was dissolved in 5% D-glucose and administered intrarterially over 1 min at doses of 5 and 30 mg/kg. Serial blood samples were collected from 5 to 480 min following drug administration, from which plasma was harvested by centrifugation and then stored at −80 °C until analysis. To obtain



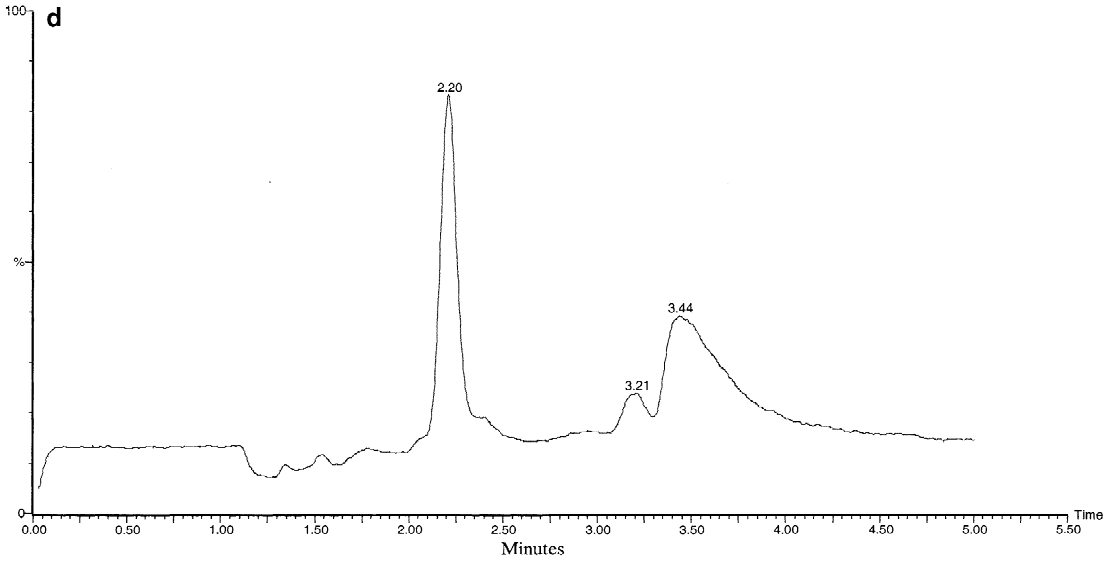


Fig. 2. (*continued*)

tumor samples, a separate serial sacrifice study was conducted in which animals were administered 5 and 30 mg/kg of carboplatin in the same manner. Animals were sacrificed at 5, 120, 300 and 480 min following drug administration at which times tumors were removed and stored at −80 °C prior to analysis.

Representative chromatograms of carboplatin in PUF and in tumor homogenate are shown in Figs. 2 and 3. The retention time of carboplatin was 2.2 min with a total run time of 9 min required to avoid interfering peaks. There were no interfering peaks at the retention time of carboplatin in blank PUF and tumor samples.

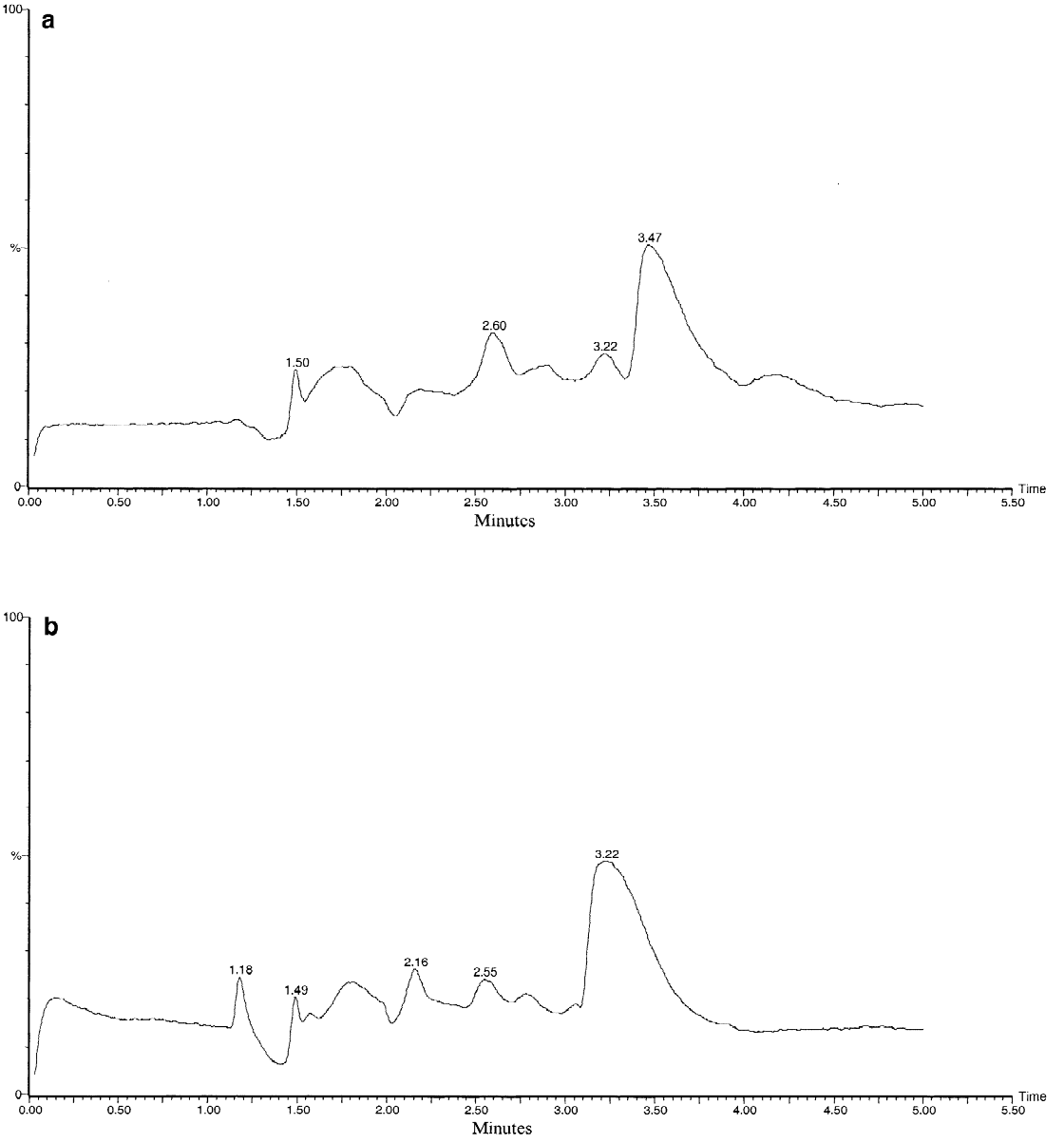
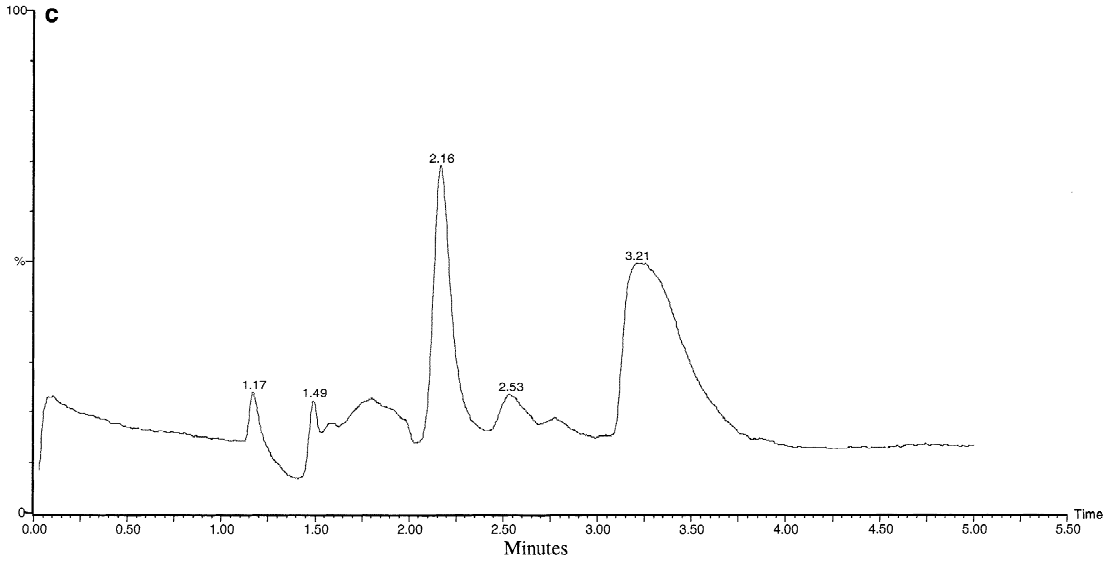


Fig. 3. Chromatograms of carboplatin in tumor homogenate: (a) blank; (b) 0.034 μg/ml; (c) 0.380 μg/ml; (d) rat tumor homogenate obtained at 2 h after administration of 30 mg/kg carboplatin. The retention time of carboplatin is 2.2 min.

Concentration–time profiles of carboplatin in PUF after administration of 5 and 30 mg/kg carboplatin are shown in Fig. 4.

The pharmacokinetic parameters were calculated by non-compartmental analyses [18] from the carboplatin PUF concentration–time profiles. The results are summarized in Table 3. There were no significant differences ($P > 0.05$) between any of the pharmacokinetic parameters (i.e. system clearance, half-life and volume of distribution) at the two dose levels which is consistent with dose-independent pharmacokinetics.

Carboplatin concentrations in tumors after administration of 5 and 30 mg/kg are shown in Fig. 5. Carboplatin concentrations could be measured for up to 8 h at both dose levels and were proportional to



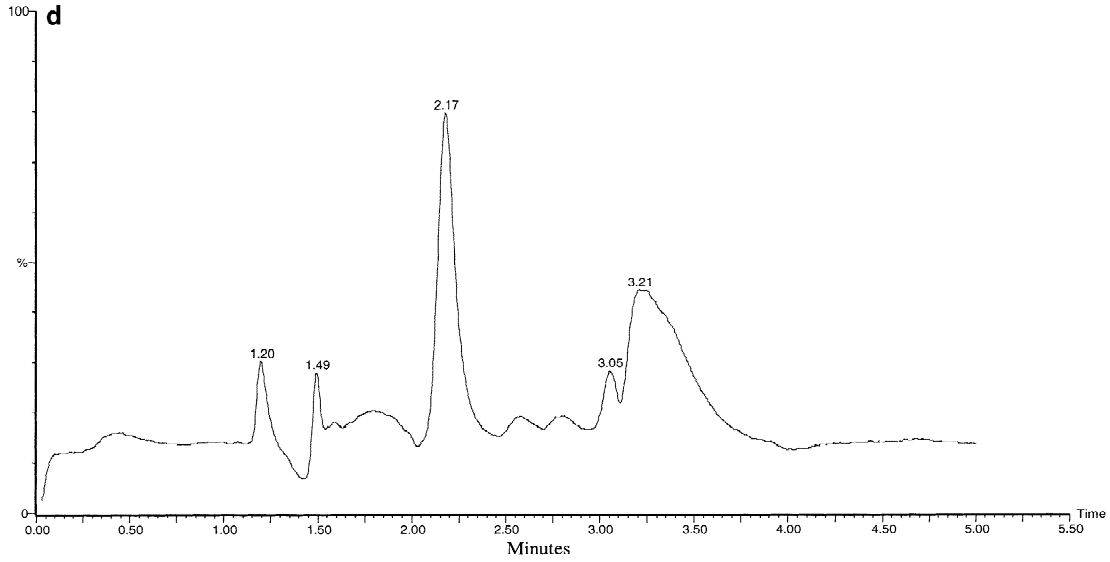


Fig. 3. (*continued*)

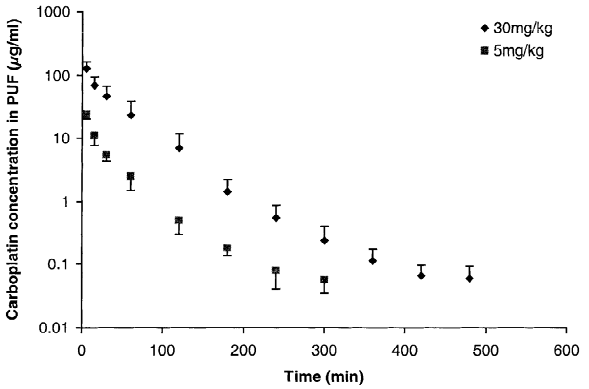


Fig. 4. Concentration–time profiles of carboplatin in rat plasma ultrafiltrate (PUF) after administration of 5 and 30 mg/kg ($n=4$, except for the first two points at the 30-mg/kg dose level in which $n=3$) of carboplatin intraarterially.

Table 3
Pharmacokinetic parameters of carboplatin after administration of 5 and 30 mg/kg of carboplatin intraarterially

Parameter	5 mg/kg	30 mg/kg
CL (ml/min per kg)	8.06±1.82	7.32±2.75
CLr (ml/min per kg)	2.70±0.72	3.72±0.57
V_{ss} (ml/kg)	285.9±57.7	284.6±63.9
$T_{1/2\ (terminal)}$ (min)	75.52±20.04	75.15±4.37
AUC (μg/ml per min)	638.9±142.0	4561.2±1682

AUC, area under curve in PUF; CL, total systemic clearance; CLr, renal clearance; $T_{1/2\ (terminal)}$, terminal elimination half-life; V_{ss}, volume of distribution at steady state. Values are means±SD; $n=4$.

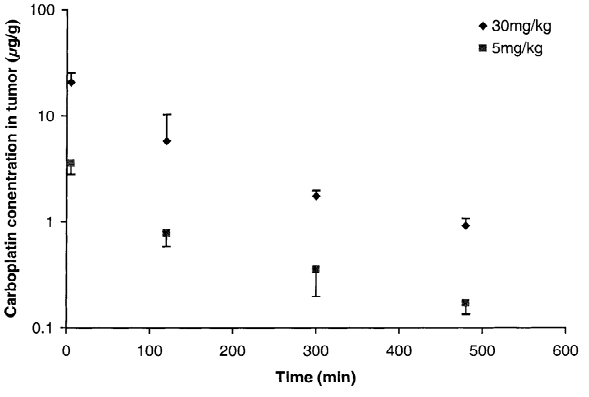


Fig. 5. Concentration–time profiles of carboplatin in rat tumor after administration of 5 and 30 mg/kg ($n=3$) of carboplatin intraarterially.

dose. Tumor to plasma AUC ratios were 0.41 and 0.32 at the 5- and 30-mg/kg dose levels, respectively.

Urine concentrations of carboplatin were very high and required dilution with mobile phase prior to injection onto the LC–MS. There was no ionization problem for the diluted urine samples and solid extraction was not required. The percentages of the administered carboplatin doses excreted in urine over 8 h were 41.9±20.9% ($n=4$) and 48.0±14.0% ($n=4$) at the 5- and 30-mg/kg doses, respectively.

4. Conclusion

A new validated LC–MS method is presented for the quantitation of intact carboplatin in rat plasma and tumor tissue. Carboplatin was extracted from PUF and from tumor homogenate by solid-phase extraction and then subjected to positive electrospray ionization.

The methods were validated and yielded % biases and C.V.'s of less than 15% and a quantitation limit of 175 pg of carboplatin. The method was successfully used in pharmacokinetic and tissue distribution studies in rats bearing Nutu 19 tumors, a preclinical model of ovarian cancer. The methods developed are simple and could likely be adapted to measure carboplatin in other biological samples.

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