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Validation of high-performance liquid chromatographic assay methods for the analysis of carboplatin in plasma ultrafiltrate

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Abstract

Validation of two HPLC assays for the quantitation of carboplatin in human plasma ultrafiltrate is described. Both assay methods employed a YMC ODS-AQ 3.9×150 mm (3 μm) column for the chromatographic separation. The first method utilized direct UV detection, the second method utilized UV detection following post-column derivatization with sodium bisulfite. Structural analogues of carboplatin were synthesized and used as internal standards for the assays. With direct UV detection, sample clean-up using solid-phase extraction on amino cartridges was required prior to injection, with extraction recoveries ranging from 80 to 90%. This extraction procedure was not necessary with the post-column reaction method, which employed a more selective analytical wavelength. Unfortunately, instability of the post-column reagent was a problem and led to greater variability in predicted concentration values. For standard curves, a weighted ($1/y^2$) regression approach was used for plots of peak area or peak height ratio (carboplatin/internal standard) vs. carboplatin concentration. The limit of detection of both assays was 0.025 μg/ml and both were validated for carboplatin concentrations from 0.05 to 40 μg/ml. Accuracy and precision data were generated using three batches of validation samples, each batch consisting of a standard curve and five sets of quality control samples. Stability of carboplatin in blood, plasma, plasma ultrafiltrate, and reconstituted extracts was evaluated. The assay methods were employed for the pharmacokinetic analysis of blood samples drawn from a pediatric patient that received a 400 mg/m^2 dose of carboplatin. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Carboplatin

1. Introduction

Carboplatin (*cis*-diammine-1,1-cyclobutanedicarboxylatoplatinum(II)) is a second-generation platinum antineoplastic agent with demonstrated clinical efficacy, especially in bladder, lung, ovarian, and testicular carcinomas [1,2]. Investigations of the clinical pharmacokinetic and pharmacodynamic behavior of carboplatin are limited by the availability of analytical methods with suitable sensitivity and specificity. Liquid chromatographic separations are needed in order to isolate carboplatin in the presence of its in vitro and in vivo degradation products. Due to its polar nature, the first HPLC assays for determination of carboplatin in biological samples employed normal-phase separations on silica [3], diol [4], or amino [5] columns. Such methods are somewhat insensitive, providing detection limits around 0.5 μg/ml, and also result in the consumption of significant amounts of organic solvents required as mobile phase constituents. In contrast,

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HPLC assay methods based on RP separation of carboplatin from plasma components have the advantage of reduced organic solvent consumption. Furthermore, the greater efficiency of RP vs. normal-phase separations results in increased assay sensitivities. However, the physicochemical properties of carboplatin make development of RP methods particularly difficult. Carboplatin has minimal *k* values on most ODS columns, while the absence of a specific chromophore necessitates UV detection in the nonspecific absorbance region (below 230 nm). Unfortunately, plasma-based samples contain a myriad of polar constituents which are also detected at these wavelengths. A viable RP method for carboplatin requires either extensive sample pretreatment or more selective detection techniques. To further complicate matters, methods commonly used to concentrate samples and increase assay sensitivity are not applicable to carboplatin, since it is not sufficiently hydrophobic to be retained on RP-based extraction cartridges and will not partition extensively into water-immiscible solvents. Thus, investigators have relied mainly on advancements in HPLC technology or alternative detection strategies to improve the sensitivity and specificity of HPLC methods for carboplatin quantitation.

Allsopp et al. [6] utilized a column-switching approach to minimize interferences and allow detection at a more sensitive wavelength (210 nm). Unfortunately, column-switching technology is not present in most laboratories. On-line differential pulse polarography with a hanging mercury drop electrode [7] has been used for carboplatin, but the technique is difficult to reproduce and again requires equipment not found in most laboratories. Specific monitoring of the carboplatin MH^+ ion using electrospray-MS [8,9] and of the Pt^+ ion using inductively coupled plasma-MS [9] have been described; however, these techniques are costly, require a high degree of technical expertise, and have not yet been fully developed for carboplatin determination in biological fluids.

The utility of post-column (PC) derivatization of platinum compounds with sodium bisulfite has been demonstrated [10,11]. An HPLC–PC method employing potassium dichromate activation followed by sodium bisulfite derivatization has already been developed and validated for the determination of cisplatin in plasma ultrafiltrate [12]. Previously, we reported preliminary development and comparison of HPLC assay methods based on direct UV detection and on UV detection after PC derivatization for the quantitation of carboplatin in plasma ultrafiltrate samples [13]. The HPLC–PC method had a similar limit of quantitation (0.05 μg/ml) as the HPLC–UV method, yet required no sample extraction procedure and possessed a much shorter chromatographic run time.

This report describes the validation of HPLC–UV and HPLC–PC assay methods for carboplatin concentrations between 0.05 and 40 μg/ml. Specificity of the techniques is demonstrated in the presence of co-administered drugs and endogenous components of plasma ultrafiltrate. As well, both assay methods are employed for the pharmacokinetic analysis of clinical samples obtained from a pediatric patient receiving carboplatin.

2. Experimental

2.1. Chemicals

Carboplatin powder was purchased from Strem Chemicals (Newburyport, MA, USA). Etoposide injection (20 mg/ml) and ifosfamide for injection were from Bristol-Myers Squibb (Montreal, Canada), ondansetron injection (2 mg/ml) was from Glaxo Laboratories (Toronto, Canada), and trimethoprim powder was from Burroughs Wellcome (Kirkland, Canada). Dexamethasone, dimenhydrinate, nystatin, and sulfisoxazole powders were purchased from Sigma (Oakville, Canada). Water was HPLC grade (Milli-Q, Millipore Corp., Bedford, MA, USA) and prepared on site. Acetonitrile (HPLC grade) was obtained from Fisher Scientific (Fairlawn, NJ, USA) as were ACS grade monobasic sodium phosphate, sodium hydroxide, and sodium bisulfite.

2.2. Synthesis of internal standards

The compounds bis(methylamine)-1,1-cyclobutanedicarboxylatoplatinum(II) (MethCBDCA) and bis(methylamine)malonatoplatinum(II) (MethMAL) (Fig. 1) were synthesized as internal standards for

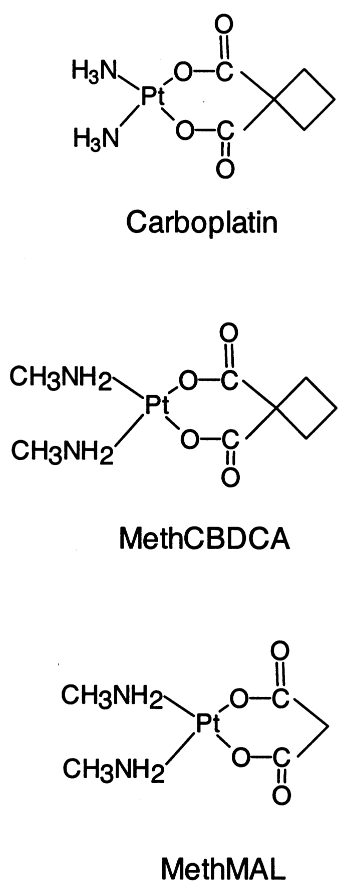

Fig. 1. Structures of carboplatin and analogues synthesized for use as internal standards.

the HPLC–UV and HPLC–PC assay methods, respectively. The compounds were produced in a two-step sequence according to the method of Cleare and coworkers [14]. Briefly, the first step involved reaction of potassium tetrachloroplatinate with sodium iodide followed by methylamine to produce bis(methylamine)diiodoplatinum(II), which rapidly precipitated as a yellow-colored product. In the second step, this product was reacted with silver nitrate followed by excess cyclobutanedicarboxylic or malonic acid to produce the MethCBDCA or MethMAL compounds, which precipitated as white crystals following extended cooling of the product mixture. The identity of the products was verified by HPLC–MS, the chromatographic peaks consisting of the expected molecular ions formed under positive electrospray conditions [8].

2.3. Chromatographic equipment and data analysis

The HPLC system consisted of two model 510 pumps, a model 712 WISP autoinjector, and a model 484 variable wavelength UV detector (Waters, Milford, MA, USA). Chromatographic separations were performed using a YMC ODS-AQ 4.6×150 mm column (Waters) with a 4×23 mm guard column containing the same 3 μm packing material. The PC reactor consisted of a 15.2 m×0.5 mm I.D. knitted PTFE coil (Supelco, Bellefonte, PA, USA). To improve flow precision, a model LP-21 LO-pulse dampening unit (Mandel Scientific, Guelph, Canada) was placed between the PC reagent pump and the PC reactor. For data analysis, peak integrations were performed using the Waters Maxima 820 computer software and peak area or peak height ratios (carboplatin/internal standard) subsequently fitted using weighted ($1/y^2$) least squares linear regression.

2.4. Direct UV detection (HPLC–UV assay)

The mobile phase consisted of 1.3% acetonitrile in 20 m*M* monobasic sodium phosphate, pumped isocratically at 0.7 ml/min. The analytical wavelength was 230 nm. Retention times of carboplatin and MethCBDCA (internal standard) were 6.5 and 45 min, respectively. The total run time per sample was 52 min.

With the HPLC–UV method, plasma ultrafiltrate samples required solid-phase extraction prior to injection. An aliquot (200 μl) of ultrafiltrate was spiked with 30 μg/ml of MethCBDCA, diluted with 2 ml of acetonitrile, then pulled via vacuum suction onto the sorbent bed of a 3 ml Supelclean amino extraction cartridge (Supelco) pre-conditioned with 2 ml of acetonitrile–water (95:5). Following a wash step with 2 ml of acetonitrile–water (90:10), 2 ml of acetonitrile–methanol–water (50:25:25) was used to elute carboplatin and the internal standard, which were collected and evaporated under nitrogen gas at 40°C. Once dry, the sample was reconstituted with 150 μl of water and 60 μl injected onto the HPLC column.

2.5. PC reaction/UV detection (HPLC–PC method)

Using a detection wavelength of 290 nm, initial investigations had demonstrated that a mobile phase consisting of 20 m*M* buffer (pH 4.5) provided separation of carboplatin from endogenous components of plasma ultrafiltrate. Optimization studies were conducted on-line with changes made to the PC reagent only, the exception being to ensure that identical buffer salts (citrate, phosphate, or acetate) were used in both mobile phase and PC reagents. Our initial PC reagent consisted of 20 m*M* buffer and 40 m*M* sodium bisulfite, with the final pH adjusted to 5.7 via dropwise addition of a 1 *M* sodium hydroxide solution. Composition of the PC reagent was then altered to examine the effect of the following variables on detector response (λ_{290}): buffer type, buffer pH, acetonitrile concentration, and bisulfite concentration. Following equilibration of the mobile phase and PC reagents, mean peak heights were recorded after triplicate injections (30 μl) of a 5 μg/ml aqueous carboplatin standard.

Optimized mobile phase and PC reagents consisted of 20 m*M* monobasic sodium phosphate and 40 m*M* sodium bisulfite in 20 m*M* monobasic sodium phosphate (pH 5.4), respectively. Both pumps were operated isocratically at 0.7 ml/min. The detection wavelength was 290 nm. The PC reactor was kept at ambient temperature; however, the PC reagent was protected from light and kept in an ice-cooled water bath to minimize degradation. Plasma ultrafiltrate samples were spiked with 10 μg/ml internal standard (MethMAL) and an aliquot (60 μl) injected directly onto the HPLC column. Carboplatin and MethMAL eluted at 9 min and 11.5 min, respectively. The total run time was 26 min.

2.6. Preparation of plasma ultrafiltrate

Both HPLC–UV and HPLC–PC methods were used for the determination of unbound carboplatin. Plasma ultrafiltrate was prepared from clinical or drug-free plasma samples by centrifugation in a Centrifree micropartition unit (Amicon, Beverly, MA, USA) at 2000 *g* and 15°C. Typical yields were 350–500 μl following 60 min ultrafiltration of 0.75 ml of plasma.

2.7. Assay validation

Specificity in the presence of endogenous components of human plasma ultrafiltrate was evaluated using plasma obtained from fourteen pediatric patients receiving chemotherapeutic agents (other than carboplatin) at Calgary Children's Hospital, as well as plasma samples obtained from five healthy adult volunteers. Interference from drugs potentially co-administered with carboplatin was also investigated using aliquots of plasma ultrafiltrate to which 50 μg/ml of the appropriate compounds had been added.

The limits of detection of the procedures were established by injection of plasma ultrafiltrate samples to which carboplatin was added in decreasing concentration, until a signal-to-noise ratio of less than 3:1 was reached.

Precision and accuracy were evaluated for carboplatin concentrations ranging from 0.05 to 40 μg/ml. Three batches of validation samples were run. Each batch consisted of a standard curve (blank, 0.05, 0.1, 0.2, 0.5, 2, 8, 15, 25, and 40 μg/ml concentrations) followed by five sets of quality control (QC) samples (0.05, 0.2, 8, and 40 μg/ml concentrations). For each standard curve or QC set a separate weighing of powder was made followed by serial dilutions to the appropriate concentrations. Reproducibility of slope values was established by analysis of six standard curves injected in a single batch. Linearity was examined both in terms of the goodness-of-fit of the data to the linear equation used and its ability to accurately predict concentration values. Accuracy and precision figures were determined from these predicted concentrations across all three validation batches ($n=15$ at each concentration) and therefore reflect a combination of both intra- and inter-assay variability.

Recovery of carboplatin following ultrafiltration was evaluated at carboplatin concentrations of 0.5, 8, and 25 μg/ml via direct comparison of peak area values before and after ultrafiltration of aqueous carboplatin standards. Recovery of carboplatin following solid-phase extraction was also evaluated at

similar concentration values. Peak area ratios of five extracted ultrafiltrate samples were compared to those from five samples prepared by adding carboplatin to an extract of blank plasma ultrafiltrate. In both cases, the internal standard was added after the extraction.

2.8. Carboplatin stability

Stability of carboplatin in blood, plasma, plasma ultrafiltrate, and solid-phase extracts of plasma ultrafiltrate was examined using the HPLC–UV assay method. Stability of blood at 4°C was evaluated over a 24 h period at concentrations of 0.5, 8, and 25 μg/ml. At times 0, 12, and 24 h, three aliquots were removed from a large-volume standard at each concentration, centrifuged to obtain plasma, and assayed. Stability of plasma, plasma ultrafiltrate, and solid-phase extracts was evaluated for carboplatin concentrations of 0.5 and 25 μg/ml. Plasma standards were subjected to freeze thaw cycles, with multiple assaying ($n=5$) of each standard being performed prior to freezing at −70°C and again after 1 and 2 months. For plasma ultrafiltrate, carboplatin was added to multiple samples ($n=10$), then five samples were extracted immediately and five were cooled at 15°C for 1 h prior to extraction. For solid-phase extracts of plasma ultrafiltrate, injections were made immediately following extraction and repeated after the samples ($n=5$) had remained in the autosampler tray for 60 h.

2.9. Pharmacokinetic samples

Blood samples were obtained from a five-year-old female given a carboplatin dose of 400 mg/m^2 via a 1 h i.v. infusion. Samples were collected in heparinized tubes pre-infusion and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 13, and 21.5 h post-infusion. Once drawn, the blood samples were refrigerated at 4°C until all samples had been collected. Following collection of the last sample, the blood was centrifuged at 1500 *g* for 10 min to obtain plasma, which was then ultrafiltered and assayed using both HPLC–UV and HPLC–PC methods. WinNonlin (Version 1.5, Scientific Consulting Inc.) was used to fit the concentration-time data to open one- and two-compartment models and to derive the appropriate pharmacokinetic parameter estimates.

3. Results

3.1. Optimization of PC reagent conditions

Results of our optimization experiments are shown in Fig. 2. Peak heights obtained for phosphate and acetate were significantly better than those observed for citrate. The optimal PC reagent pH was between 5.3 and 5.5. For acetonitrile concentrations of 2–10% added to the PC reagent, no obvious relationship between acetonitrile concentration and peak height response was observed. Further comparison of PC reagents with and without 4% acetonitrile showed no significant differences between the two. While peak height responses increased for bisulfite concentrations up to 70 m*M*, increases in the magnitude of the baseline noise increased substantially at higher bisulfite concentrations. Thus, with respect to signal-to-noise ratios, the maximal response was achieved for a PC reagent containing 40 m*M* bisulfite.

3.2. Chromatography

Figs. 3 and 4 show representative chromatograms obtained using the HPLC–UV and HPLC–PC assay methods, respectively. The increased selectivity of the latter method is evident from the chromatograms and results due to the higher analytical wavelength employed (290 nm). Despite the solid-phase extraction procedure used with the HPLC–UV method, significant endogenous material is still observable in the chromatograms, especially in the baseline region prior to carboplatin elution. The HPLC–PC chromatograms, on the other hand, appear relatively free from endogenous material, with only a single major peak observed in addition to carboplatin and the internal standard.

3.3. Assay validation

For both HPLC–UV and HPLC–PC methods, no interferences were noted for plasma ultrafiltrate

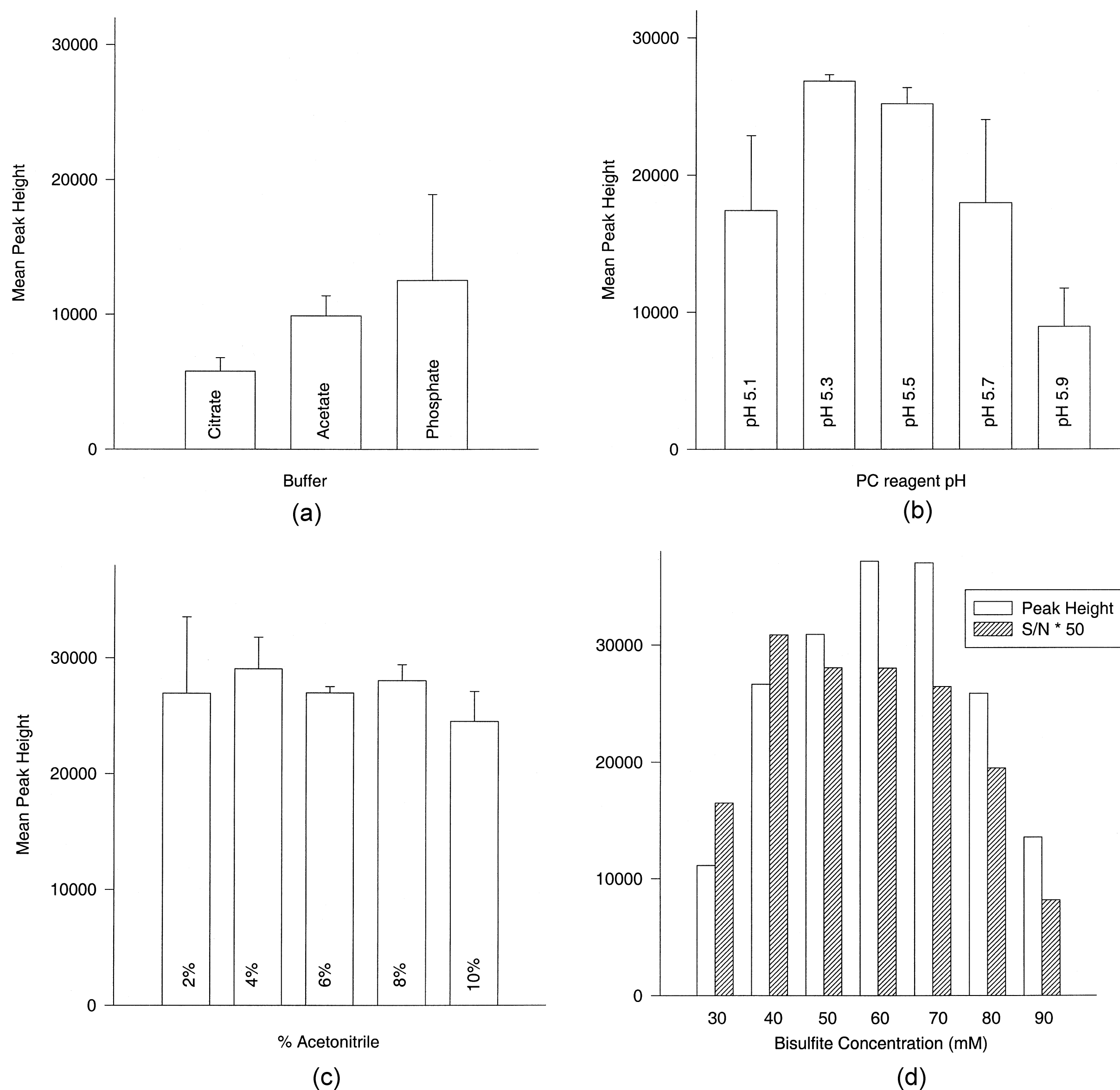

Fig. 2. Optimization of PC reagent composition with respect to (A) buffer type, (B) pH, (C) acetonitrile concentration, and (D) bisulfite concentration.

samples containing the following co-administered drugs: dexamethasone, dimenhydrinate, etoposide, ifosfamide, nystatin, ondansetron, sulfisoxazole, and trimethoprim. No significant peaks were found at the retention time of carboplatin in control plasma obtained from healthy adult volunteers and from pediatric patients receiving chemotherapeutic agents other than carboplatin.

Limits of detection, corresponding to signal-to-noise ratios of 3:1, were similar (0.025 μg/ml) for both assays. In all cases, calibration curves from 0.05 to 40 μg/ml had r^2 values greater than 0.999. Slope

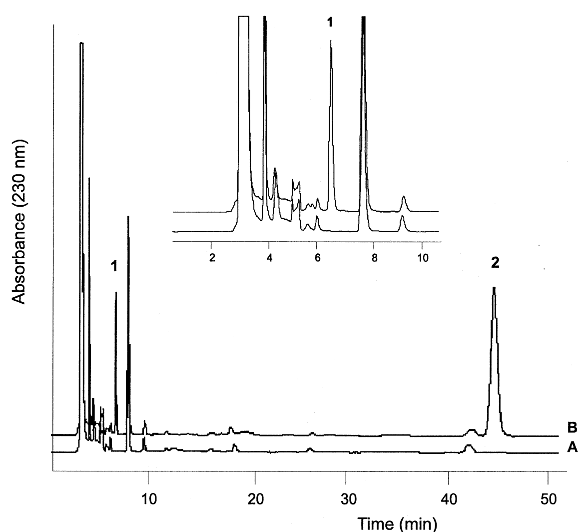

Fig. 3. Representative chromatograms from HPLC–UV assay of (A) blank plasma and (B) plasma containing 8 μg/ml carboplatin. Peaks are (1) carboplatin and (2) MethCBDCA.

precision values ($n=6$) were 2.4% and 4.9% for the HPLC–UV and HPLC–PC methods, respectively. To examine the ability of the linear calibration curve to

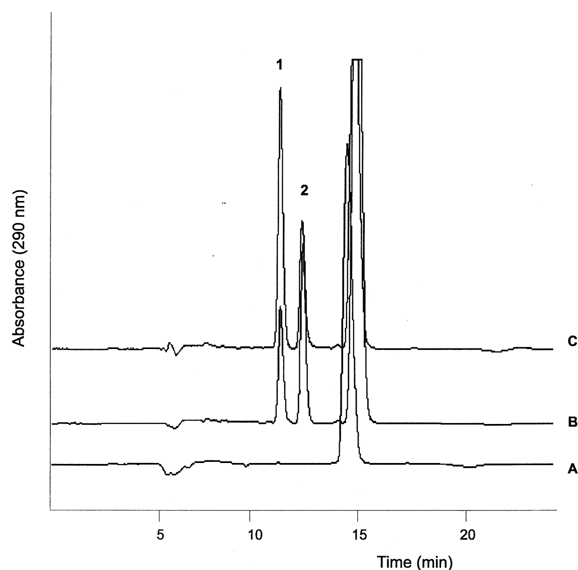

Fig. 4. Representative chromatograms from HPLC–PC assay of (A) blank plasma, (B) plasma containing 8 μg/ml carboplatin, and (C) patient plasma obtained 1 h post-infusion following a 400 mg/m^2 dose of carboplatin. Peaks are (1) carboplatin and (2) MethMAL.

predict concentration, values were calculated for each QC sample using the calibration curve run during the same validation batch and compared to the actual concentrations. A statistical summary of the accuracy and precision of these predicted values is given in Tables 1 and 2 for the HPLC–UV and HPLC–PC methods, respectively. As evident from comparison of the %RSD values, the variability observed for the HPLC–PC assay was about twice that observed for the HPLC–UV assay. Indeed, this variability was even larger when we evaluated the precision of the untransformed peak height or area ratio values themselves. The %RSD values for this untransformed data ranged from 12 to 34% for the HPLC–PC method vs. 6–20% for the HPLC–UV method, with the greatest variability observed at the lowest QC concentration evaluated. One-way analysis of variance on these mean peak height or area ratio values showed that they were significantly different across the three batches of validation samples, demonstrating the need to run a calibration curve prior to evaluation of each new batch.

Recovery of carboplatin following ultrafiltration was greater than 98% at all concentrations evaluated. For the solid-phase extraction procedure, mean recoveries ($n=5$) were 83.4%, 86.2%, and 88.5% for carboplatin concentrations of 0.5, 8, and 25 μg/ml, respectively. These results were in agreement with those anticipated following method development, in which observed recoveries ranged from 80 to 90%.

Carboplatin was stable in blood stored at 4°C for 24 h. Although decreases were observed after 12 and 24 h for all three concentrations studied, the observed decreases were small (less than 5%) and one-way analysis of variance indicated that there was no significant difference between the mean peak area ratios obtained at the different time points. For the plasma samples stored at −70°C for 2 months, peak

Table 1
Statistics for predicted carboplatin in plasma ultrafiltrate (HPLC–UV method)

QC concentration (μg/ml)	Mean ($n=15$)	%Bias	%RSD
0.050	0.055	9.1	15
0.200	0.190	−5.2	5.8
8.00	7.55	−5.6	4.8
40.0	39.0	−2.6	4.4

Table 2
Statistics for predicted carboplatin in plasma ultrafiltrate (HPLC–PC method)

QC concentration (μg/ml)	Mean (n=15)	%Bias	%RSD
0.050	0.050	−0.3	11
0.200	0.169	−15	15
8.00	7.96	−0.4	11
40.0	43.3	8.3	8.5

area ratios declined by 3.5% and 4.9% for carboplatin concentrations of 0.5 and 25 μg/ml, while the solid-phase extracts left in the autosampler at ambient temperature for 60 h had mean area ratio decreases of 6.5% and 1.8%, respectively. No significant differences in area ratio values were observed for plasma ultrafiltrate samples cooled for 1 h at 15°C.

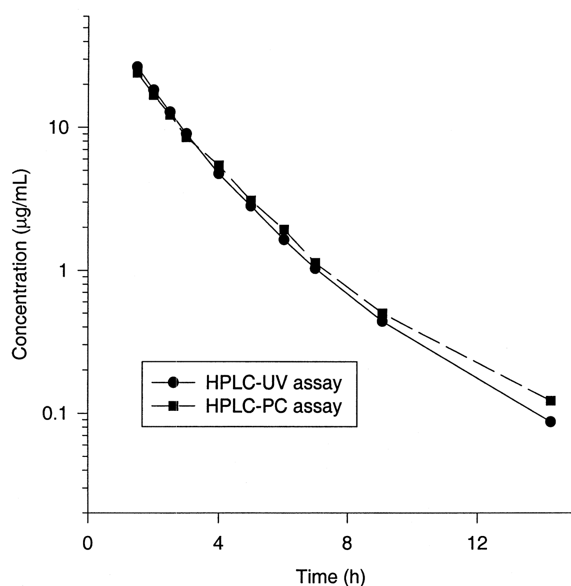

Fig. 5. Plasma concentration-vs.-time data derived from HPLC–UV and HPLC–PC assays following i.v. infusion of a 400 mg/m^2 dose of carboplatin to a pediatric patient.

3.4. Pharmacokinetic samples

Fig. 5 shows the elimination profiles determined by HPLC–UV and HPLC–PC assay methods following a 400 mg/m^2 dose of carboplatin given by 1 h i.v. infusion. Concentration values calculated by the two methods were similar. Both assays were able to determine the concentration of a sample obtained 13 h post-infusion; however, neither assay detected carboplatin in a sample obtained 21.5 h post-infusion. The observed elimination profile was best described by a two-compartment model, from which the pharmacokinetic parameter estimates shown in Table 3 were calculated. These estimates were in reasonable agreement with those reported previously by Riccardi and coworkers [15].

4. Discussion

During initial development, concerns regarding a fluctuating baseline due to poor mixing of the mobile phase and PC reagents were resolved by going to larger (0.5 mm vs. 0.3 mm I.D.) PTFE tubing and using equal mobile phase and PC reagent flow-rates. In addition, on-line optimization of the PC reaction

Table 3
Pharmacokinetic parameter estimates derived using a two-compartment model

Parameter	HPLC–UV assay	HPLC–PC assay	Riccardi et al. [15]
AUC (mg/ml*min)	4.70	4.51	2.46
C_{max} (μg/ml)	38.6	35.8	30.7
$t_{1/2\ (\alpha)}$ (h)	0.77	0.56	1.05
$t_{1/2\ (\beta)}$ (h)	1.86	1.54	6.44
Cl (ml/min)	56.6	59.9	not given
Cl/m^2 (ml/min)	83	88	87
V_d/m^2(L)	5.6	6.3	8.8

conditions was used since the products formed by the reaction show limited stability and the reaction is affected by various factors. These include time, temperature, pH, and the presence of buffer salts, metal ions, oxygen, and organic modifiers [10,11].

The HPLC–PC assay method provides several advantages over the HPLC–UV method. The improved selectivity afforded by UV monitoring of the bisulfite reaction product at 290 nm removed the need for sample clean-up of plasma ultrafiltrate prior to analysis, resulting in both time and cost savings. As well, the MethMAL internal standard used in the HPLC–PC method was more polar and thus eluted more quickly than MethCBDCA under the chromatographic conditions employed. Unfortunately, MethMAL could not be used as an internal standard for the HPLC–UV method since it showed poor extraction recovery and co-chromatographed with endogenous compounds of plasma ultrafiltrate samples. Additional platinum analogues evaluated (enloplatin, DWA2114R, and iproplatin) had significantly greater retention times than both MethMAL and MethCBDCA.

In our preliminary comparisons of PC and direct UV detection methods [13], we observed better precision in peak height response values for PC detection than for direct UV detection. These initial results, however, were based on the analysis of a limited number of samples over a smaller concentration range (below 8 μg/ml). For the HPLC–UV method, precision values were similar for both preliminary and validation studies. In fact, prior to validation we made three changes to the HPLC–UV method in order to enhance its precision. Firstly, we decreased the total run time from 60 min to 52 min, ending the run shortly after elution of the internal standard (MethCBDCA). In addition, we made a slight adjustment to the wash and elution solvents used in our solid-phase extraction procedure to ensure complete recovery of MethCBDCA, which has a slightly weaker affinity for the extraction cartridge. Finally, we employed peak area in place of peak height integration. Due to the much longer elution time of MethCBDCA vs. carboplatin, changes in mobile phase composition or column performance seemed to have a more significant impact on MethCBDCA peak heights than on carboplatin peak heights, resulting in changes in peak height ratios. Use of area ratios helped to compensate for this problem. With the HPLC–PC method, however, some samples contained an endogenous component that eluted near carboplatin (R_s 1.0), making the use of peak height over peak area ratios preferable [16].

During our validation study, the variability observed for the HPLC–PC method was much higher than anticipated. The likely cause of this variability is instability of the PC reagent, specifically sodium bisulfite oxidation. Kizu et al. [11] reported that the sensitivity of their PC system remained unchanged for at least 15 h when the PC reagent was kept in an ice-cooled water bath. We found that the peak height values started to change significantly after as little as 10 h (Fig. 6). Addition of the internal standard helped account for this problem; however, peak height ratios still showed time-dependent changes, likely owing to different reaction rates of carboplatin and the internal standard with sodium bisulfite.

Gaver and Deeb [4] previously demonstrated the limited stability of carboplatin in water, plasma, plasma ultrafiltrate, and urine. In our study, we observed a small albeit statistically significant degradation of carboplatin in plasma and plasma ultrafiltrate extracts over time-periods extending past those

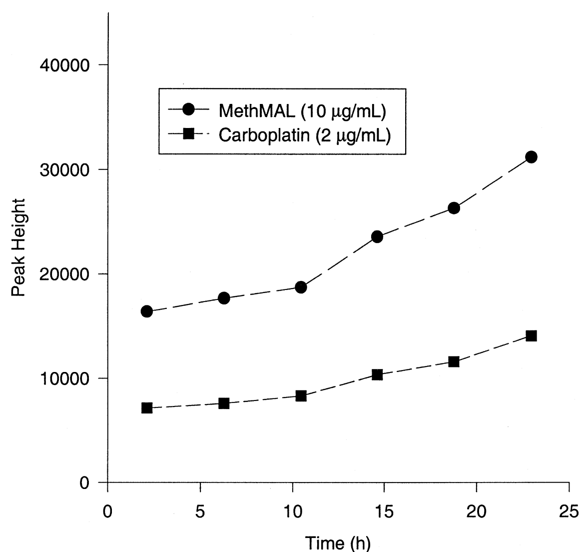

Fig. 6. HPLC–PC responses observed after injection of an aqueous standard containing carboplatin (2 μg/ml) and MethMAL (10 μg/ml), repeated at 4.3 h intervals.

typically employed during routine analyses. However, within the time-periods used for routine analyses, no degradation of carboplatin was observed.

Currently, we are studying the pharmacokinetics of carboplatin in pediatric patients receiving fixed body surface area-based doses of carboplatin. These studies employ plasma ultrafiltrate rather than plasma concentrations, since the majority of binding of carboplatin to plasma components is irreversible and results in inactivation of the drug. The rapid elimination profile of carboplatin from plasma necessitates the use of an assay method with both sensitivity and a broad linear range. The HPLC assay methods described in this report allow for carboplatin quantitation over nearly three orders of magnitude, without requiring sample dilutions or multiple calibration curves. This combination of sensitivity and range make these methods particularly suitable for the evaluation of carboplatin clinical pharmacokinetics. While results from our clinical patient suggest that both HPLC–UV and HPLC–PC assay methods can be used to generate similar pharmacokinetic profiles and parameter estimates, the variability of the HPLC–PC method is of concern. Indeed, for the HPLC–PC method the %RSD values in the predicted concentrations were greater than 10% at three of the four QC concentrations examined, with the precision at 0.2 μg/ml almost being outside the acceptable limits proposed by Shah and coworkers [17]. For this reason, we are presently using the HPLC–UV method for carboplatin determination in our ongoing pharmacokinetic study.

5. Conclusions

The validation of HPLC methods for the quantitation of carboplatin in plasma ultrafiltrate samples has been described. The increased selectivity of the HPLC–PC method results in shorter run times and no sample extraction requirements; however, validation experiments demonstrated that the HPLC–UV method provides superior accuracy and precision.

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