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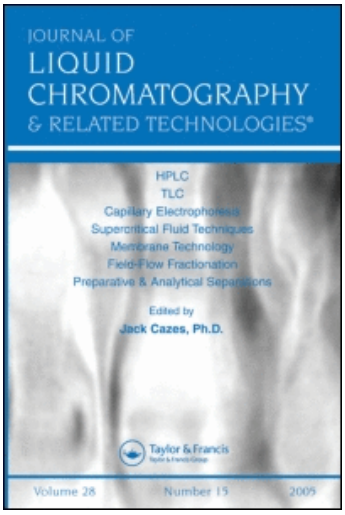

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SIMULTANEOUS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF CARBOPLATIN AND CISPLATIN IN INFUSION FLUIDS

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ABSTRACT

A simple high performance liquid chromatographic (HPLC) procedure for the simultaneous determination of cisplatin and paraplatin in infusion fluids is described. The method uses: a 5 μm C 18 column, 0.01 M phosphate buffer adjusted at pH 7.0 and 5.5 10^{-4} M hexadecyl trimethyl ammonium bromide as mobile phase, and 216 nm spectrophotometric detection to provide sensitive and specific assay for carboplatin and its major degradation products.

Stability indicating ability was demonstrated using degradation products and forcibly degraded samples with hydrochloric acid, sodium hydroxide and hydrogen peroxide.

This assay appears to be suitable for stability studies of carboplatin in infusion fluids or lyophilized powder.

INTRODUCTION

The platinum co-ordination complex cisplatin (cis-diammine-dichloroplatinum II) was introduced to the clinic as a cytotoxic agent in 1972. Clinical experience has shown that

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cisplatin is highly nephrotoxic. Carboplatin, cis-diammine 1,1-cyclobutane dicarboxylate platinum II, is a second generation platinum containing antitumor agent analogue of cisplatin. Carboplatin has a broad spectrum of antitumor activity similar to that of cisplatin and is less nephrotoxic and emetogenic than cisplatin (1,2).

For carboplatin to become biologically active it is envisaged that one or both of the cyclobutane dicarboxylate ligands are replaced by water molecules to produce highly reactive diaquated diammine platinum (II). This substitution is similar than loss of chlorine atoms from cisplatin in aqueous solutions (3). Paraplatin may also undergo nucleophilic substitution reactions with chloride ions resulting in conversion to cisplatin (4). Recently Allsopp et al. (5) have observed that the time for 5% degradation of paraplatin in 0,9 % sodium chloride was 29,2 h and have proposed a mechanism for the degradation of cisplatin (figure 1). Cisplatin formation have not been evaluated in this study.

Several stability indicating liquid chromatographic methods have been developped to quantify paraplatin in infusion fluids. All these methods consisted in reversed phase liquid chromatography. With water (4), phosphate buffer (5,6,7) or hexadecyltrimethylammonium (HTAB) solution pH 3.0 (8) as mobile phase, degradation products haven't interfere with paraplatin while cisplatin was not resolved from solvent front or was not detectable.

We report the development of an original HPLC method using solvent generated anion exchangers to quantify simultaneously paraplatin and cisplatin in infusion fluids. This method is rapid, selective and reproducible and has been used for the stability study of these drugs in infusion fluids.

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MATERIALS AND METHOD

Chemicals

Cisplatin and carboplatin were supplied by Eli Lilly laboratories (St Cloud, France) and Bristol laboratories (Paris la Défense, France). 1,1 cyclobutane dicarboxylic acid was obtained from Merck (Darmstadt, Germany), cyclobutane monocarboxylic acid,

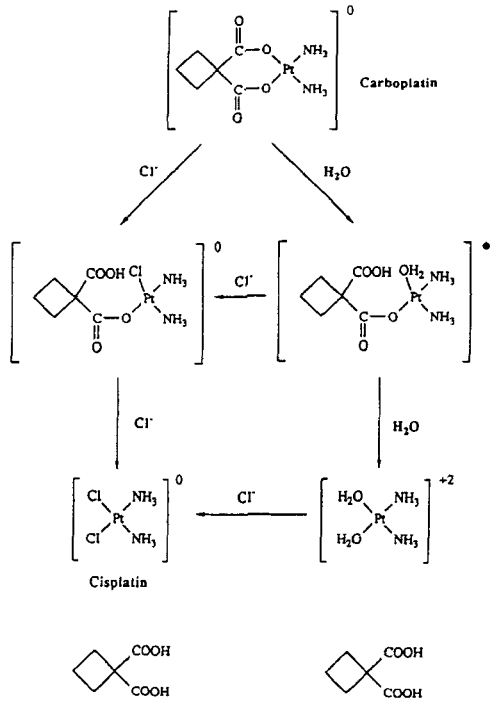

figure 1: Overall reaction of cisplatin with chloride ion in aqueous solution (Allsopp et al.).

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transplatin, hexadecyltrimethylammonium bromide from Sigma (St Louis Mo 63178 USA). Distilled water was provided from Biosedra (Malakoff, France). Analytical grade sodium dihydrogen phosphate, sodium monohydrogen diphosphate and sodium citrate were obtained from Merck (Darmstadt, Germany).

HPLC equipment

The liquid chromatographic system consisted of a L6000 pump (Merck), a model Wisp 710B sample injector (Waters associates Inc. St Quentin Yvelines, France), a Waters 990 UV-Visible photodiode array dectector Waters, a model APC IV NEC personnal computer with Waters 990 software and plotter.

Chromatographic conditions

Isocratic reversed phase chromatography was performed at ambient temperature with a Nucleosil C18 column (150 x 4.2 mm i.d., 5 μm particle size, Merck)

Solvent generated anion exchangers were prepared by pumping 0.5 % (w/v) hexadecyltrimethylammonium bromide aqueous solution through the column until equilibration of the system.

Mobile phase consisted of 0.01 M phosphate buffer M pH 7.0 containing hexadecyl-trimethylammonium bromide (5.5 10^{-4} M). It was filtered through 0.45 μm Millipore filter , degassed in vacuo. All separations were carried out at room temperature with flow rate set at 1.0 ml/min.

Absorbance column effluent was monitored from wavelength 200 to 400 nm and peak integration performed at 216 nm. Chromatograms were recorded using 5 mm/min chart speed.

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Standard solutions:

Cisplatin and carboplatin were accurately weighed. Cisplatin was diluted with sodium chloride 0.9% carboplatin with water for injection to give a final concentration of 100 μg/ml.

Validation of HPLC assay

Linearity: A six point calibration plot of carboplatin and cisplatin concentrations against peak area was constructed over the concentration range 5-50 μg/ml. The best fit straight line was determined by least squares regression.

Precision of the method: Within-day accuracy and precision of the assay were determined by preparing and analysing ten replicate samples of three concentrations of carboplatin (5, 20, 50 μg/ml) and two concentrations of cisplatin (5, 20 μg/ml). Mean observed

concentration, percentage standard deviation and percentage of the theoretical concentration were calculated.

Control samples (n=9) of paraplatin (5, 20, 50 μg/ml) and cisplatin (5, 20 μg/ml) were assayed over a one month period to calculate between-day precision and accuracy.

Stability indicating ability : The stability indicating ability of HPLC assay system was determined using degradation products and forced degraded samples. To four glass tubes (*a-d*) was added 1 ml of carboplatin solution (1 mg/ml). To glass tube *a* was added 1 M HCl (1ml), glass tube *b* 1 M NaOH (1ml), glass tube *c* 6 volumes hydrogen peroxide solution and glass tube *d* water for injection (1 ml). Tubes *a-c* were heated in a water bath at 70°C for 2 hours. Control tube was protected from light and stored at + 4°C.

Each tube was then equilibrated to room temperature, adjusted to pH 7.0 and the resulting solution was subjected to the HPLC assay after appropriate dilution.

The diode array spectrophotometric detector has also helped to establish the specificity of the method. We have recorded UV spectra of paraplatin and cisplatin peaks as eluted from the column . We have established spectral purity of the HPLC peaks and specificity of each compound by superimposing upslope, downslope and apex spectra.

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RESULTS

Optimization of chromatographic conditions.

Riley et al (9) have described a high performance liquid chromatography of inorganic platinum (II) complexes, using solvent generated anion exchangers and have studied the effect of electrolytes on solute retention. Presence of bromide or nitrate in the mobile phase decreases retention, while low concentration of citrate ($0.5\ 10^{-3}$ mol/l) and acetate cause increased retention of cisplatin; this effect increases with pH over the range 3.0 to 7.0. At higher concentration of citrate, retention of cisplatin decreases

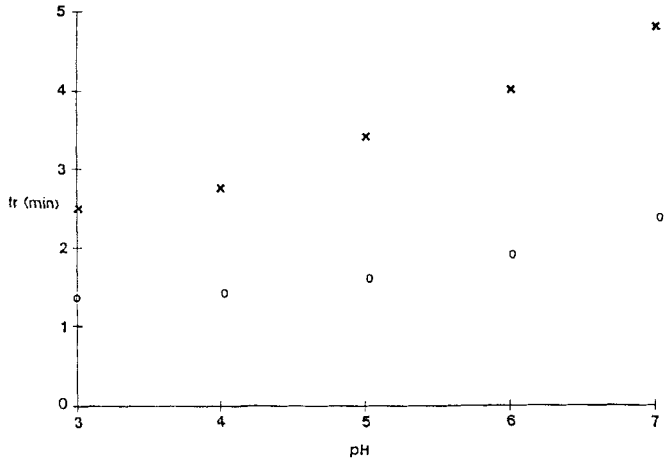

figure 2: effect of pH on retention of carboplatin (o) and cisplatin (x), column C18 Nucléosil 5 µm, mobile phase; phosphate buffer 0.05 M, HTAB 5.5 10^{-4} M.

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gradually with increasing concentration of citrate over the 5 10^{-3} to 1 10^{-1} mol/l range owning to the secondary contribution of electrostatic effects.

According to Riley data, we applied this method to paraplatin. The mobile phase was a mixture of citrate buffer (1 mg/ml) and HTAB (10^{-4} mol/l). Figure 2 shows the relationship between the reciprocal of the retention time of paraplatin and cisplatin and the effect of pH.

Increase in pH has resulted in increase in the retention of both compounds. This effect was more pronounced with cisplatin than carboplatin.

To choose a detector wavelength suitable to quantify cisplatin and paraplatin, we have injected a standard solution containing the two analytes. Figure 3 presents the data as *isoabsorbance* contours for each HPLC peak, and demonstrate wavelength absorption maxima appeared for cisplatin at 216 and 300 nm and for paraplatin at 216 nm. Accurate determination of both compounds requires a common wavelength with an high extinction and figure 3 shows that 216 nm seems to be an appropriate wavelenght

At 216 nm presence of chloride ion in injected sample induces negative peaks interfering

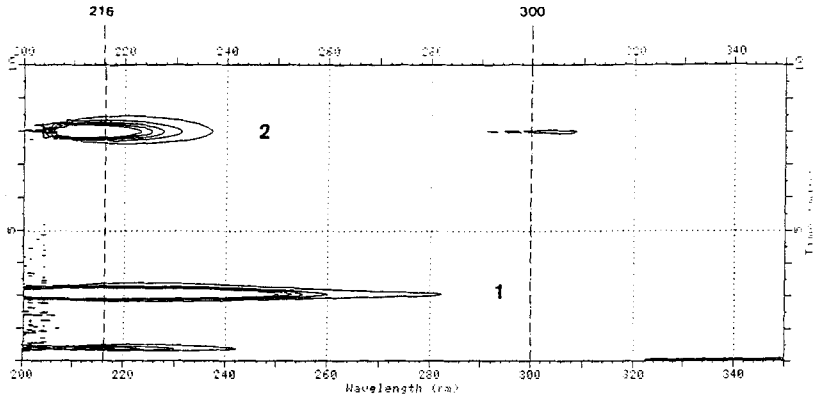

figure 3: Isoabsorbance contour plot of cisplatin (1) and carboplatin (2) standard solution.

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with cisplatin peak. Low detection limit for cisplatin is necessary for indicating stability method of paraplatin and sensitivity at 300 nm was not sufficient.

In order to avoid this problem, we have replaced citrate buffer with 0.01 M phosphate buffer pH 7.0. Resolution and retention times were similar to that observed with citrate buffer, baseline was better and negative peaks haven't occured on chromatogram with chloride ion injection.

Figure 4 shows three dimensional chromatograms of cisplatin and paraplatin standard solutions. Cisplatin and paraplatin eluted at approximatively 8 min and 3 min respectively. Chloride ion doesn't interfere with cisplatin peak and absorbance measurement of both compounds was possible at 216 nm.

Linearity:

Standard curves were linear over 5 to 60 μg/ml range of carboplatin in water for injection. Mean slope was 1956 for eleven standard curves (sd=37), and correlation coefficient was up 0.990.

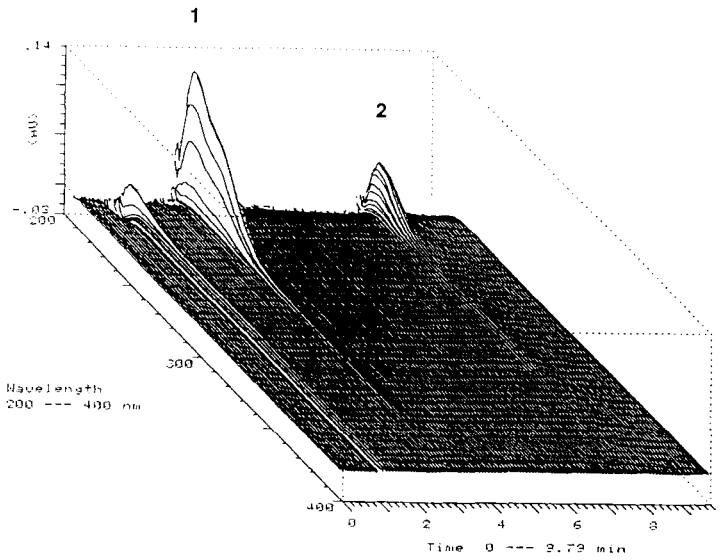

figure 4: Three dimensional chromatogram of carboplatin (1) 50 μg/ml and cisplatin (2) 50 μg/ml.

Relationship between peak area and cisplatin concentration was found to be linear over the 5-60 μg/ml concentration range. Equation of the least squares regression line was y= 2886 x -0.21 (r= 0.999, n=6).

Precision and accuracy of the method.:

Tables 1 and 2 show accuracy and precision of the method of determination of paraplatin and cisplatin for within-day and between-day analysis.

Within day and between day accuracy of control samples were greater than 98 % for carboplatin and greater than 95 % for cisplatin.

The precision of the method was illustrated by coefficients of variation. Within day variability of paraplatin was, 0.8 % for 5 , 20, and 50 μg/ml and between day variability was, 1.55, 2.06, 2.06 for 40, 50 ,60 μg/ml respectively. Coefficients of variation for cisplatin

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Table 1: Within and between day variability in measured carboplatin concentration.

Within day variability				Between day variability			
conc. spiked μg/ml	concentration found μg/ml mean (sd)	accuracy %	coefficient variation %	conc. spiked μg/ml	concentration found μg/ml mean (sd)	accuracy %	coefficient variation %
5	4.94 (0.04)	98.8	0.84	40	40.34 (0.63)	100.8	1.55
20	20.52 (0.18)	102.6	0.87	50	49.78 (1.02)	99.5	2.06
50	50.86 (0.39)	101.7	0.77	60	60.07 (1.24)	98.8	2.06

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Table 2: Within and between day variability in measured cisplatin concentration

Within day variability				Between day variability			
conc. spiked μg/ml	concentration found μg/ml mean (sd)	accuracy %	coefficient variation %	conc. spiked μg/ml	concentration found μg/ml mean (sd)	accuracy %	coefficient variation %
5	4.75 (0.24)	95.2	4.9	5	4.83 (0.41)	96.6	8.4
20	20.02 (0.60)	100	3.0	20	19.67 (0.51)	98.4	2.6

controls were: 4.9 % and 3.0 % for within day study and 8.4 and 2.6 % for between day assay for 5 and 20 μg/ml respectively.

The limit of detection for cisplatin was 1 μg/ml allowing a signal noise ratio of 3, and coefficient of variation for 1 μg/ml was 3% (n=10).

Stability indicating ability:

Cisplatin and carboplatin peaks are separated from degradation products of cisplatin. Retention times of transplatin and diaquodiammine platinum are respectively 1.3

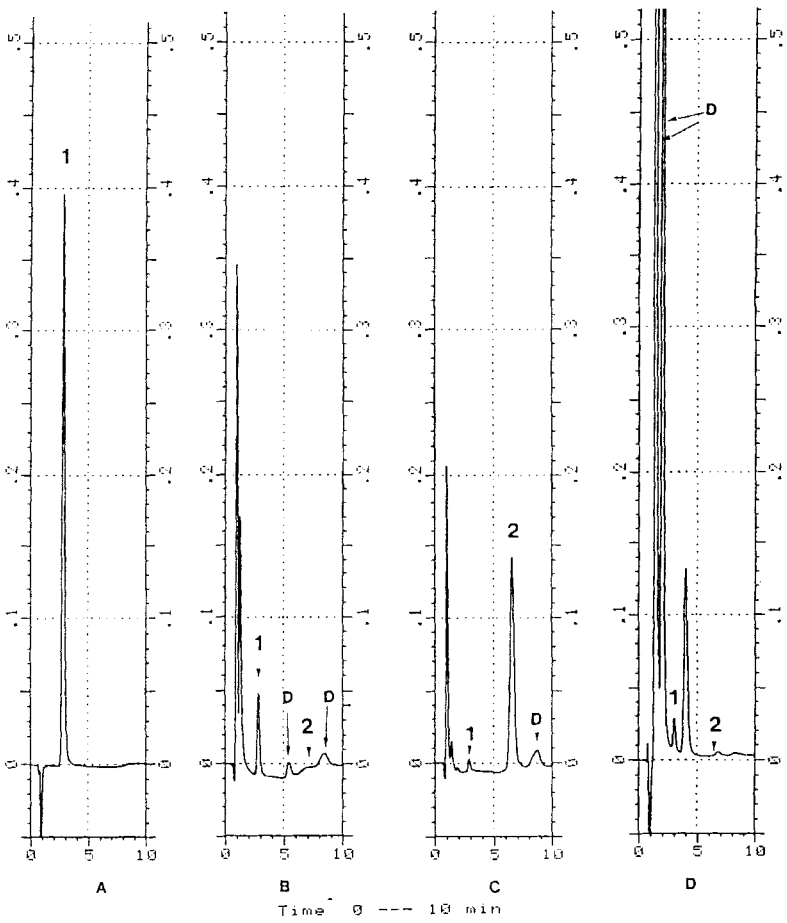

figure 5: HPLC chromatograms of control solution of carboplatin (**A**) and same solution after 2 hours at 70°C with 1 N NaOH (**B**), 1N HCl (**C**), and 6 vol hydrogen peroxide (**D**). (1): carboplatin, (2) cisplatin, (D) degradation products

and 1.5 min.. Mono and dicarboxilic cyclobutane acid are not eluted with this mobile phase.

HPLC chromatograms of carboplatin control solution, and samples of the same solution subjected to forcibly degradation (1 N HCl, 1 N NaOH and 6 v H_2O_2 heated solutions at + 70 ° C during 2 hours) are shown in figure 5.

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With hydrochloric acid, 98 % carboplatin was degraded after two hours at + 70°C . Peaks of carboplatin decreased 84 % in alkaline solution and 94 % with solution containing hydrogen peroxide. Cisplatin is detectable only in acidic solution containing chloride ion. Others degradation products peaks appears on all HPLC chromatogramms, but are eluted and detected without interference with paraplatin and cisplatin peaks.

DISCUSSION.

The development of this assay is required to determine carboplatin concentration in infusion fluid from stability assays. It was necessary for the assay to be specific for carboplatin and cisplatin, with a low limit of detection for cisplatin.

It was difficult to retain the highly polar carboplatin and cisplatin on typical reversed phase column. It was necessary to use solvent generated anion exchangers to separate inorganic platinum compounds from the solvent front.

Quantitation at 216 nm of both compounds was possible with only mobile phase containing phosphate buffer. Limit of detection of cisplatin with this method was 1 mg/l.

In conclusion we report a simple, specific and reproducible HPLC assay for carboplatin in infusion fluid that permit the simultaneous quantition of cisplatin and paraplatin.

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