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Liquid chromatographic determination of *cis*-platin as platinum(II) in pharmaceutical preparation, serum and urine samples of cancer patients

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Abstract

Spectrophotometric and high performance liquid chromatographic (HPLC) methods have been developed for the determination of *cis*-platin and carboplatin based on the pre-column derivatization of platinum(II) with 2-acetylpyridine-4-phenyl-3-thiosemicarbazone. The complex was extracted in chloroform with molar absorptivity of 2.2×10^4 L mol^{-1} cm^{-1} at 380 nm. The complex eluted from a Phenomenex C-18 (150 mm × 4.6 mm i.d.) column with methanol:water:acetonitrile:tetrabutyl ammonium bromide (1 mM) (44:30:25:1, v/v/v/v) with a flow rate of 1 ml/min and UV detection at 260 nm. Ruthenium(IV) and selenium(IV) also separated completely. The linear calibration curve was with 0.5–12.5 μg/ml and detection limit of 10 ng/ml platinum(II).The analysis of *cis*-platin and carboplatin injections by spectrophotometric and HPLC methods indicated relative standard deviation (R.S.D.) of 0.66–2.1%. The method was used for the determinations of *cis*-platin in serum and urine of cancer patients after chemotherapy and platinum contents were found 148–444 and 50–90 ng/ml with R.S.D. of 0.3–3.0 and 0.6–2.4% for the serum and urine, respectively. The recovery of platinum(II) from serum was 97% with R.S.D. 2.2%.

Keywords: *cis*-Platin platinum(II); Cancer; LC

1. Introduction

cis-Platin [*cis*-dichlorodiamino-platinum(II)] and Carboplatin [*cis*-diammine (1,1-cyclobutanedicarboxylato) platinum(II)] are platinum containing drugs used for chemotherapy of cancer patients. A number of analytical and pharmacokinetic studies of *cis*-platin in biological fluids have been made [1–2]. The analytical methods are reported for the determination of intact *cis*-platin [3–5] and elemental platinum from biological samples. The methods include atomic absorption (flame and nonflame) [2,6–9], inductively coupled plasma atomic emission (ICP-AE) [10–11], electroanalytical techniques [12–13], neutron activation analysis [14] and gas [15] and liquid chromatography [16–24]. HPLC methods are based on spectrophotometric [16–18], ICP-AE [2], electrochemical [19–21] and quenched phosphorescence [22] detection. Spectrophotometric detection is convenient, but *cis*-platin is spectrophotometrically insensitive and post-column derivatizations with sodium bisulphite in the presence of potassium dichromate [17,18] and diethyldithiocarbamate [24] have been reported. The methods are reported to indicate comparable sensitivity to atomic absorption [16]. For column derivatization of platinum, HPLC elution and separation with 1-hydroxy-2-pyridine-thione [25], 4-(2′-pyridylazo) resorcinol [26], *n*-butyl-2-naphthyldithiocarbamate [27], β-quinolinol [28], 1-(2′-pyridylazo) naphthol [29] and bis(salicylaldehyde) teramethylethylenediimine [H_2SA_2Ten] [23] have been reported, but some have long retention time [26] or high relative standard deviation [25] for platinum. H_2SA_2Ten has been applied for HPLC determination of *cis*-platin with detection limit of 1 μg/ml [23].

Thiosemicarbazones and phenyl thiosemicarbazones are interesting complexing reagents, because they form highly stable and intensely colored complexes immediately, by

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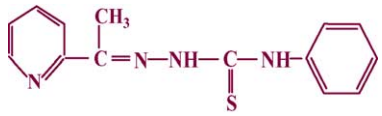

Fig. 1. Structural diagram of the reagent APPT.

bonding through the sulphur and hydrazine nitrogen atoms, which are ideally suitable for spectrophotometric detection [30–31]. Some of the useful HPLC separations and determinations of metal ions are also reported using the ligands: 2-piconaldehyde-4-phenyl-3-thiosemicarbazone, 2-thiophenaldehyde-4-phenyl-3-thiosemicarbazone, 2-pyrrolaldehyde-4-phenyl-3-thiosemicarbazone, 2-acetylpyridine-4-phenyl-3-thiosemicarbazone (APPT), glyoxal dithiosemicarbazone, glyoxal bis(4-phenyl-3-thiosemicarbazone), dimethylglyoxal bis(4-phenyl-3-thiosemicarbazone) and diacetyl bis(4-dimethylamino-phenyl)-3-thiosemicarbazone [30–36]. However, the reagents have not been examined for platinum or platinum based drugs.

The reagent APPT has been reported for the HPLC determination of copper(II), cobalt(II) and iron(II) in pharmaceutical preparations [33] (Fig. 1), but the present work reports HPLC separation of platinum(II), selenium(IV), ruthenium(IV) and sensitive and selective determination of platinum(II)- and platinum-based drugs in pharmaceutical preparations, serum and urine of cancer patients after chemotherapy.

2. Experimental

The reagent APPT was prepared as reported [33,37] by heating together equimolar (0.01 M) solution of 2-acetylpyridine and 4-phenyl-3-thiosemicarbazide in methanol. A Hitachi 655A liquid chromatograph connected with variable wavelength UV monitor and Rheodyne 7125 injector was used. The responses of the UV detector were connected with computer with CSW-32 (Data Apex Ltd© 2001 www.dataapex.com) software. A Phenomenex C-18 (150 mm × 4.6 mm i.d.) was used through out the study. The spectrophotometric studies were carried out on Hitachi 220 spectrophotometer. IR spectrum of the reagent APPT was recorded in KBr on Perkin–Elmer 1360 IR spectrophotometer.

The blood and urine samples of cancer patients on *cis*-platin chemotherapy were collected from the cancer ward of Atomic Energy Medical Center, Liaquat University of Medicine and Health Sciences (LUMHS) Jamshoro, after 1–2 h of infusion of *cis*-platin for blood samples and 4–6 h for urine samples. The blood samples were collected with a 5 ml hypodermic syringe by vein puncture. The urine samples were collected in clean plastic bottle (1 L). GR grade chemicals: sodium acetate, acetic acid, sodium bicarbonate, sodium carbonate, boric acid, borax, ammonium chloride, ammonia, chloroform, acetonitrile, hydrochloric acid (37%) (E. Merck) and tetrabutyl ammonium bromide (Fluka) were used. Freshly prepared doubly distilled water from all glass was used for HPLC studies. The buffer solutions in the pH range 1–10 at unit interval were prepared from the following; potassium chloride (1 M)–hydrochloric acid (0.1 M) pH 1–2, sodium acetate (1 M)–acetic acid (1 M) pH 3–6, ammonium acetate (1 M)–acetic acid (1 M) pH 7, boric acid (1 M)–borax (1 M) pH 8–9 and ammonium chloride (1 M)–ammonia pH 10.

2.1. Spectrophotometric procedure

An aliquot of solution (4–5 ml) containing platinum(II) (5–30 μg), selenium(IV) (50–400 μg) or ruthenium(IV) (20–70 μg) separately were transferred to separating funnel and added sodium acetate–acetic acid buffer pH 3 (2 ml), to platinum(II) and selenium(IV) solutions and borate buffer pH 9 (2 ml), to ruthenium(IV) solution. The reagent APPT solution (2 ml, 0.02%, w/v, in methanol) and chloroform (4 ml) was then added. The contents were mixed well and layers were allowed to separate. The organic layer was collected in 10 ml volumetric flask. The extraction was repeated with 3 ml of chloroform. Ethanol (1 ml) was added to each of the flask before adjusting the final volume with chloroform. The absorption spectra were recorded against reagent blank within 600–250 nm.

2.2. Spectrophotometeric determination of platinum in *cis*-platin and carboplatin injections

Solution (4 ml) from *cis*-platin injection (Platosin, PCH Pharma Cheme, Karachi) containing 0.5 mg/ml *cis*-platin and carboplatin (1 ml) [David Bull Laboratories (DBL), Australia] containing 10 mg/ml carboplatin were added hydrochloric acid (4 ml, 37%), and the mixture was heated on hot plate gently. Most of the acid was evaporated and the residue was added hydrochloric acid (2 ml). Most of the acid was again evaporated and residue was dissolved in water and volume was adjusted to 25 ml. The solution (1–2 ml) was taken and spectrophotometric procedure was followed. The absorbance was measured at 380 nm against reagent blank and the amount of platinum from *cis*-platin and carboplatin injections were calculated from calibration curve.

2.3. HPLC analytical procedure

An aliquot of solution (2–5 ml) containing platinum(II) (0–12.5 μg), selenium(IV) (0–12.5 μg) and ruthenium(IV) (0–10 μg) was added sodium acetate–acetic acid buffer (2 ml) pH 3. Reagent APPT solution (2 ml, 0.02% in methanol) and chloroform (2 ml) were then added. The contents were mixed well and layers were allowed to separate. The organic layer was collected and aqueous layer was added borate buffer pH 9 (3 ml). Chloroform (2 ml) was added and extraction was repeated. The organic layers were mixed together. The solvent was evaporated under nitrogen

atmosphere and the residue was dissolved in methanol (0.5 ml). The solution (20 μl) was injected onto a Phenomenex C-18 (150 mm × 4.6 mm i.d.) column and eluted with methanol:water:acetonitrile:tetrabutyl ammonium bromide (1 mM) (44:30:25:1, v/v/v/v) with a flow rate of 1 ml/min. The detection UV was at 260 nm.

2.4. HPLC determination of platinum in cis-platin and carboplatin injections

Solution (2 ml) from *cis*-platin injection (*cis*-plasol, Delta West Ltd, Upjohn Company, USA) containing 1 mg/ml *cis*-platin and carboplatin (1 ml) [David Bull laboratories (DBL), Australia] containing 10 mg carboplatin/ml were taken and treated as 2.2. The final volume was adjusted to 25 ml. Solution (1 ml) was taken and were added sodium acetate–acetic acid buffer pH 3 (2 ml), reagent APPT solution (2 ml, 0.02% in methanol) and chloroform (4 ml). The contents were mixed well and layers were allowed to separate. Exactly 2 ml from organic layer was pipetted out and further processed as 2.3.The amount of platinum in *cis*-platin and carboplatin injections was evaluated from the calibration curve.

2.5. HPLC determination of platinum from serum samples

The blood sample (5 ml) was centrifuged at 12,000 rpm for 15 min, and supernatant layer was collected. Methanol (6 ml) was added and the mixture was again centrifuged for 15 min. The above layer was collected in the beaker and hydrochloric acid (2 ml, 37%) was added. The contents were heated to near dryness and the residue was dissolved in water (3 ml). The extraction procedure 2.4 was carried out and 20 μl of the solution was injected onto the column as in section 2.3. The amount of platinum in blood was evaluated from calibration curve.

2.6. % Recovery of platinum from blood

Blood sample (5 ml) from two healthy volunteers, who have not taken any drug during last one week was collected. The blood samples were processed as 2.5 and after removing of protein was added platinum(II) (10 μg). The remaining procedure was followed as 2.5. The amount of platinum(II) was calculated from calibration curve.

2.7. Determination of platinum from urine samples

The urine sample (50–250 ml) was added hydrochloric acid (20 ml, 37%) and was heated on hot plate gently to about 5 ml. Hydrochloric acid (10 ml) was added and again heated to 2–3 ml. Methanol (6–8 ml) was added and centrifuged at 12000 rpm for 20 min. The supernatant liquid was collected and pH adjusted to 3. The complete solution was consumed and remaining procedure used was as 2.5.

2.8. Determination of platinum from serum and urine samples by standard addition

A blood sample (S. no. 2) after removal of the proteins was divided exactly in two equal parts and part A was treated as 2.5. Part B was added 10 μg platinum(II) and again was treated as 2.5. The amount of platinum in fraction A was evaluated from calibration curve and platinum contents in fraction B was calculated from average increase in the response from the standard.

Similarly urine sample (S. no. 6) was treated as 2.7 and after removal of protein and adjustment of pH to 3 was again divided into two equal parts. The remaining procedure was followed as above for blood.

3. Results and discussion

The reagent APPT reacts quickly with platinum(II), selenium(IV) and ruthenium(IV) to form water-soluble colored complexes. The complexes are also extractable in chloroform. The effect of pH on the formation of the complexes within pH 1–10 was investigated. Maximum color development for platinum(II) and selenium(IV) was observed in acidic medium at pH 3, but ruthenium(IV) indicated in basic conditions at pH 9 (Table 1). The compositions of the metal chelates at optimized pH were examined by variation of metal:ligand mole ratio. It was observed that platinum(II) formed metal chelate with 1:2, but selenium(IV) and ruthenium(IV) with 1:3 metal:ligand ratio (Table 1). The complexes formed were highly stable and did not show any change in absorbance up to 24 h. The complexes indicated a reasonable sensitivity with molar absorptivities within 2.3×10^3 to 2.2×10^4 L mol^{-1} cm^{-1}, within 370–380 nm (Table 1). Platinum(II) gave highest spectrophotometric sensitivity and was examined for the determination of platinum from *cis*-platin and carboplatin injections. *cis*-Platin

Table 1
Quantitative spectrophotometric data for metal chelates of APPT

Metal chelates metal:ligand	pH of max: derivation	λ-max (nm)	$\sum$ (L mol^{-1} cm^{-1})	Composition of chelate	Calibration range (μg/ml)	r^2
Ru(IV)	9	370	1.8×10^4	1:3	2–7	0.994
Se(IV)	3	370	2.3×10^3	1:3	5–40	0.999
Pt(II)	3	380	2.2×10^4	1:2	0.5–3	0.995

Table 2
Analysis of *cis*-platin and carboplatin injections by spectrophotometry and HPLC

Sample	Metal ion	Amount reported (μg/ml)	Amount found (μg/ml)	R.S.D.% ($n=3$)	R.D.%
By spectrophotometry					
cis-Platin	Pt(II)	2.62	2.55	0.7	2.0
Carboplatin	Pt(II)	2.10	2.14	0.9	1.8
By HPLC					
cis-Platin	Pt(II)	5.23	5.1	2.7	2.1
Carboplatin	Pt(II)	10.51	10.44	1.6	0.66

contains labile two chlorides and reacts with APPT to develop yellow color, which absorbed maximally at 385 nm, but some decrease in the absorbance was observed due to two amino groups attached to platinum(II) [5]. In case of carboplatin, color development with APPT was poor because of the substitution of 1,1-cyclobutanedicarboxylato with more binding strength in carboplatin then chloro substituents in *cis*-platin [5]. However, after acid digestion with hydrochloric acid, followed by complexation with APPT and extraction in chloroform, resulted in the quantitative recovery of platinum(II) with relative standard deviation (R.S.D.) 0.7 and 0.9% ($n=3$) for *cis*-platin and carboplatin, respectively (Table 2).

Spectrophotometric method gave reasonable sensitivity but to increase the selectivity, HPLC was investigated. Platinum(II)–APPT eluted from reversed phase Phenomenex C-18 column and separated from the excess of the reagent APPT. Ruthenium(IV) and selenium(IV) chelates also separated completely, when eluted with methanol:water:acetonitrile:tetrabutyl ammonium bromide (1 mM) (44:30:25:1, v/v/v/v) with a flow rate of 1 ml/min and UV detection at 260 nm (Fig. 2). Reproducibility of the elution of platinum(II) chelate in terms of average peak height and retention time ($n=6$) with 10 μg/ml was examined and R.S.D. obtained were 2.5 and 1.5%, respectively. Copper(II), iron(II), cobalt(II), palladium(II) and lead(II) also react with APPT to form colored complexes. Their effect on determination of platinum(II) was investigated. The metals separated and did not affect the determination of platinum(II).

Linear calibration curves for platinum(II), selenium(IV) and ruthenium(IV) were obtained with 0.5–12.5, 2.5–12.5 and 2–10 μg/ml with coefficient of determination r^2 0.9965, 0.9962 and 0.9970, respectively. The detection limits measured as three times the background noise were 10, 120 and 50 ng/ml of platinum(II), selenium(IV) and ruthenium(IV) corresponding to 0.2, 2.4 and 1 ng/injection (20 μl), respectively. The analysis of test mixtures for platinum(II), selenium(IV) and ruthenium(IV) indicated relative error within ±3.5%.

cis-Platin and carboplatin injections were analyzed for the contents of platinum in injections and the results obtained

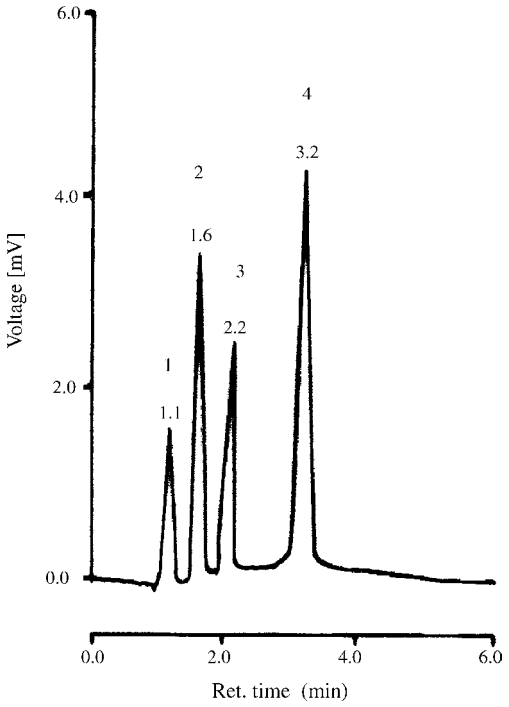

Fig. 2. HPLC separation of (1) Ru(IV), (2) APPT, (3) Se(IV) and (4) Pt(II) complexes of APPT. Column Phenomenex C-18 (150 mm × 4.6 mm i.d.). Elution with methanol:water:acetonitrile:tetrabutyl ammonium bromide (1 mM) (44:30:25:1, v/v/v/v), with flow rate 1 ml/min and UV detection at 260 nm.

(Table 2) are with R.D. of 0.66–2.1% from reported value by manufacture with R.S.D. 1.6–2.7%. *cis*-Platin contents in the serum and urine samples of cancer patients on *cis*-platin chemotherapy were examined. The blood and urine

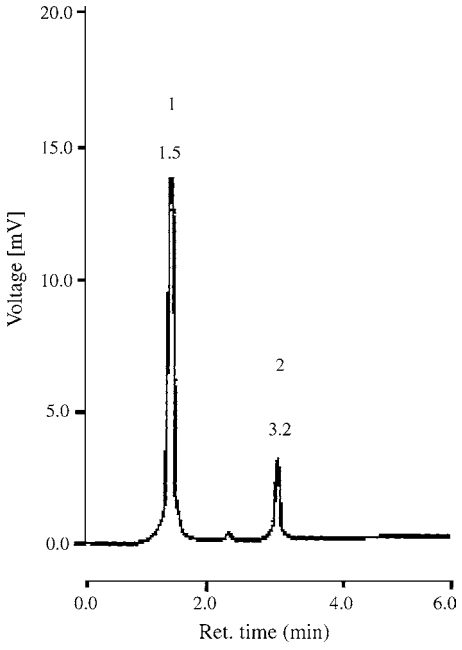

Fig. 3. HPLC response of (1) APPT, (2) Pt(II) from serum sample of cancer patient. Conditions as Fig. 2.

Table 3
HPLC determination of Pt(II) from serum and urine samples of cancer patients

S. no.	Sample (B/U)	Type of cancer	Age (years) and sex (M/F)	Dose of *cis*-platin infused (mg)	Amount found of Pt(II) (ng/ml) (R.S.D.%) ($n = 4$)
1	B	Esophagus	70 (M)	100	444 (0.4)
2	B	Vaginal	60 (F)	100	388 (3.0)
3	B	Testicular	18 (M)	25	148 (2.1)
4	B	Larynx	40 (M)	120	330 (2.5)
5	B	Larynx	40 (F)	100	260 (0.3)
6	U	Esophagus	70 (M)	100	90 (1.9)
7	U	Testicular	18 (M)	25	50 (2.4)
8	U	Larynx	40 (M)	120	83 (0.6)
By standard addition					
1	B	Vaginal	60 (F)	100	402 (2.6)
2	U	Esophagus	70 (M)	100	99 (3.2)

Blood and urine samples collected after 2 and 4–6 h of infusion of *cis*-platin injection; B, blood samples; U, urine samples.

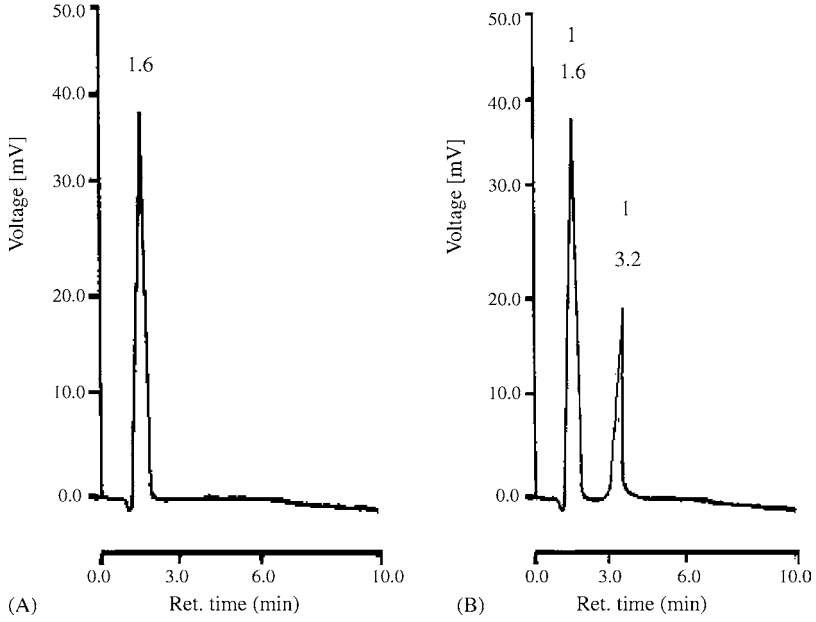

Fig. 4. HPLC response of the blood sample (A) from healthy volunteer and (B) after spiking with 10 μg of platinum(II). Conditions as Fig. 2.

samples were collected after 1–2 and 4–6 h after chemotherapy and analyzed following analytical procedure. The results of analysis are summarized in (Table 3). The amounts of platinum found in blood and urine were within the range 148–444 and 50–90 ng/ml with R.S.D. ($n = 4$) of 0.3–3.0 and 0.6–2.4%, respectively (Fig. 3). The higher amounts of platinum were found from patients suffering from esophagus cancers. Recovery percentage of platinum from blood was calculated by spiking the serum samples of healthy persons with 10 μg and response was compared with the same amount of platinum(II) 10 μg from aqueous solutions (Fig. 4). The recovery of platinum from serum was found 97% with R.S.D. 2.2%. A sample of blood and a sample of urine were also analyzed for platinum contents by standard addition and the results obtained correlated with observed values by calibration.

4. Conclusion

An analytical method has been developed for the sensitive and selective HPLC determination of platinum from pharmaceutical preparations, serum and urine samples of cancer patients after chemotherapy with platinum-based drugs. The detection limit for platinum is observed 200 pg/injection (20 μl). A number of elements tested did not interfere the determination of platinum. Pre-column chelating reagent used was 2-acetylpyridine 4-phenyl-3-thiosemicarbazone.

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