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High-performance liquid chromatographic determination of cisplatin as platinum(II) in a pharmaceutical preparation and blood samples of cancer patients

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Abstract

An high-performance liquid chromatographic (HPLC) method has been developed for the determination of cisplatin, based on precolumn derivatization of platinum(II) with bis(salicylaldehyde)tetramethylethylenediimine, extraction in chloroform and elution from a 3 μm Hypersil ODS column with methanol–acetonitrile–water as mobile phase and detection at 254 nm. Copper(II), iron(II), nickel(II), palladium(II), dioxouranium(IV) separated completely and did not affect the determination of platinum(II). The method was applied for the determination of cisplatin as platinum(II) in a pharmaceutical preparation and in blood samples of cancer patients after infusion of cisplatin.

Keywords: Cisplatin; Platinum(II)

1. Introduction

Cisplatin [*cis*-dichlorodiaminoplatinum(II)] is a platinum containing drug used for chemotherapy of cancer patients. A number of analytical and pharmacokinetic studies of cisplatin in biological fluids have been made [1,2]. The analytical methods reported for the determination of platinum from biological samples include atomic absorption (flame and nonflame) [2–6], inductively coupled plasma atomic emission (ICP–AE) [7,8], electroanalytical techniques [9,10] and liquid chromatography [11–20]. HPLC methods are based on spectrophotometric, [11–14] ICP-AE, [2] electrochemical [15–19] and quenched phosphorescence [20] detection. Spectrophotometric detection is convenient, but cisplatin is not sensitive spectrophotometrically and post column derivatization with sodium bisulphite in the presence of potassium dichromate [11,12] and diethyldithiocarbamate [13] have been reported. The methods are reported to indicate comparable sensitivities to atomic absorption [11]. For precolumn derivatization of platinum, HPLC elution and separation with 1-hydroxy-2-pyridinethione [21], 4-(2-pyridylazo) resorcinol [22], *n*-butyl-2-naphthyldithiocarbamate [23], β-quinolinol [24] and 1-(2-pyridylazo)-2-naphthol [25] have been reported, but some have long reaction times [22] or high relative standard deviation (R.S.D.) [21] for platinum. Moreover, the precolumn derivatization procedures have not been applied for HPLC determination of cisplatin or platinum based anticancer drugs in formulation or

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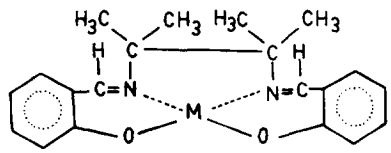

M = Cu(II), Ni(II), Fe(II), Pd(II), Pt(II), UO_2(II).

Fig. 1. Structural diagram of metal chelates.

biological fluids. In the present work bis(salicylaldehyde)tetramethylethylenediimine (H_2SA_2Ten) has been examined as a reagent for precolumn derivatization and HPLC determination of platinum(II) in a cisplatin injection and blood samples (Fig. 1).

The reagent H_2SA_2Ten has been reported for the gas chromatographic (GC) and HPLC determination of copper and nickel [26] and HPLC determination of vanadium [27], cobalt, iron [28] and uranium [29].

2. Experimental

2.1. Solvent extraction of platinum(II)

An aliquot of solution (1–10 ml) containing platinum(II) (0–100 μg) was transferred to a well stoppered test tube and sodium bicarbonate buffer pH 8 (2 ml) and reagent H_2SA_2Ten solution (2 ml, 1% w/v in ethanol) were added. The mixture was warmed on a warming bath for 15 min and cooled, to the contents was added chloroform (4 ml). The layers were mixed well and 2 ml of extract was transferred to a sample vial. The solvent was removed and the residue was dissolved in 1 ml of ethanol. The solution (5 μl) was injected onto a Hypersil ODS, 3 μm column (150×4.6 mm I.D.) and the complex was eluted with a ternary mixture of methanol–acetonitrile–water (50:30:20, v/v/v) with a flow-rate of 0.4 ml/min. Detection UV was at 254 nm.

2.2. Solvent extraction procedure for metals

A solution (5–10 ml) containing cobalt(II), copper(II), nickel(II), iron(II), palladium(II), platinum(II) and dioxouranium(VI) (25–150 μg each) was transferred to a stoppered test tube and to it was added sodium acetate buffer pH 6 (2 ml, 1 *M*) and reagent H_2SA_2Ten solution (2 ml, 1% in ethanol). The procedure was followed as in Section 2.1 and the complexes were eluted with methanol–acetonitrile–water (10:70:20, v/v/v) with a flow-rate of 0.4 ml/min and detection was at 254 nm.

2.3. Determination of platinum in cisplatin injection

From the cisplatin injection (Nippon Kayakli Co. Ltd. Tokyo, Japan) 1 g was taken and to it was added hydrochloric acid (37%) (30 ml) and the mixture was heated on a hot plate. Most of the acid was evaporated, the residue was dissolved in water and the volume was adjusted to 10 ml with water. The solution (5 ml) was taken and the procedure was followed as in Section 2.1.

2.4. Spectrophotometric determination of platinum in cisplatin injection

Dithizone (0.01% w/v) (5 ml) was transferred to a separating funnel and to it was added sulphuric acid (2 *M*) (5 ml) and benzene (5 ml). The contents were mixed well and the layers were allowed to separate. The organic layer was discarded and to the aqueous layer was added platinum(II) solutions (0.2–1.2 ml) containing (20–120 μg). Benzene (5 ml) was then added and the contents were well mixed. The organic layer was collected and absorption spectra were recorded from 800–350 nm, against blank reagent.

From the cisplatin injection 1 g was taken and processed as in Section 2.3, and solutions of 1 and 2 ml were taken and the procedure was followed as above. The absorbance was measured at 550 and 490 nm. The amount of platinum was evaluated from the calibration curve.

2.5. Determination of platinum in blood samples

To the blood sample (5 ml) placed in a centrifuge tube was added trichloroacetic acid (2 ml, 10% w/v in water). The contents were mixed thoroughly and

allowed to stand for 15 min. The contents were centrifuged for 20 min and the supernatant layer was separated and to it was added hydrochloric acid (37%) (2 ml) and trichloroacetic acid (8 ml, 10% w/v in water). The mixture was again centrifuged for 15 min. The above liquid layer was transferred to a beaker and to it was added hydrochloric acid (37%) (2 ml). The solution was heated to near dryness and the residue was dissolved in 3 ml of water. The pH of the solution was adjusted to 6 and the extraction procedure was followed as in Section 2.1. The total organic layer (chloroform 4 ml) was transferred to a sample vial and the solvent was evaporated. The residue was dissolved in methanol (0.1 ml) and 5 μl of the solution was injected onto the column as in Section 2.1. The amount of platinum in blood was evaluated from standard calibration and standard addition techniques. For standard addition a blood sample was spiked with platinum (20 μg) and the procedure was followed as stated earlier in this section.

The reagent H_2SA_2Ten was prepared as reported [30] by heating together salicylaldehyde and tetramethylethylenediamine in a 2:1 molar ratio in ethanol. The results of elemental microanalysis agreed to the expected value. The elemental microanalysis was carried out by Elemental Micro-analysis, Devon, UK. The blood samples of cancer patients were collected from the Cancer Ward of Atomic Energy Centre, Liquat Medical College Hospital, Jamshoro, Pakistan, after 1 to 2 h of infusion of cisplatin (20 ml), containing 10 mg of *cis*-dichlorodiaminoplatinum(II). The blood sample was collected with a 5 ml hypodermic syringe by venipuncture.

A Hitachi 220 was used for spectrophotometric studies. A Hitachi 655A liquid chromatograph connected with variable wavelength UV monitor, Rheodyne 7125 injector and Hitachi D-2500 chromato integrator was used.

Hypersil ODS, 3 μm (150×4.6 mm I.D.) (Shandon, USA) was used throughout the study.

G.R. grade chemicals (sodium carbonate, sodium bicarbonate, sodium acetate, acetic acid, dithiozone, chloroform, acetonitrile, benzene, ethanol, hydrochloric acid (37%) (E. Merck) were used. Freshly prepared doubly distilled water was used for HPLC studies. Well stoppered test tubes were from Quickfit, UK.

3. Results and discussion

The reagent H_2SA_2Ten reacts with platinum(II) to give a yellow solution, which is extractable in chloroform. The complex absorbs maximally at 483 nm (ϵ=1225 l/mol.cm). The effect of pH on the extraction of platinum in chloroform was examined spectrophotometrically. It was observed that transfer of platinum from aqueous to organic phase occurred within the pH range 3–10, with a maximum at pH 8.

The platinum(II) complex of H_2SA_2Ten easily eluted from the Hypersil ODS 3 μm column, and complete separation from the complexing reagent occurred when eluted with a mixture of methanol–acetonitrile–water using a flow-rate of 0.4 ml/min (Fig. 2).

The effect of the presence of copper(II), iron(II), nickel(II), palladium(II) and dioxouranium(VI) on the extraction and determination of platinum(II) was examined. The extraction was carried out at pH 6, because at pH 8 iron precipitated out as iron oxide. It was observed that they did not interfere with platinum(II) when eluted with a ternary mixture of methanol–acetonitrile–water (10:70:20, v/v/v) with

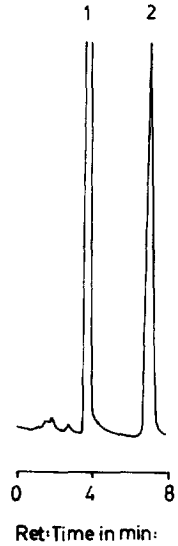

Fig. 2. HPLC separation of (1) H_2SA_2Ten, (2) platinum(II) 40 μg/ml as a complex. Column, Hypersil ODS 3 μm (150×4.6 mm I.D.). Eluent, methanol–acetonitrile–water (50:20:30, v/v/v). Flow-rate, 0.4 ml/min. Detection, UV 254 nm.

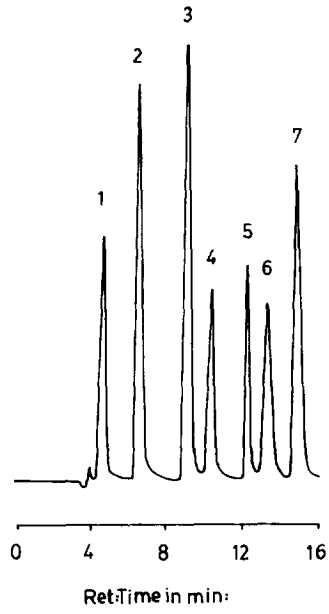

Fig. 3. HPLC separation of (1) H_2SA_2Ten, (2) dioxouranium(VI), (3) platinum(II), (4) iron(II), (5) pallaidum(II), (6) nickel(II) and (7) copper(II). Column, Hypersil ODS 3 μm (150×4.6 mm I.D.). Eluent, methanol–acetonitrile–water (10:70:20, v/v/v). Flow-rate, 0.4 μl/ml. Detection at 254 nm.

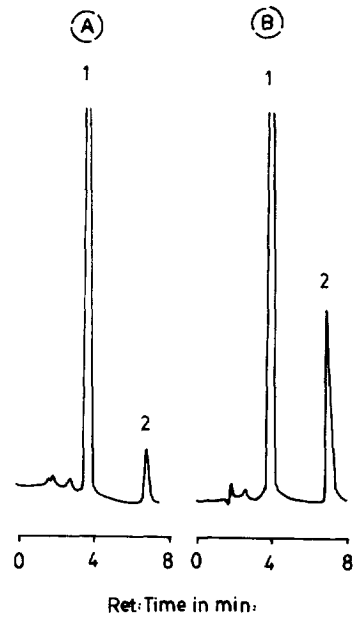

Fig. 4. HPLC determination of platinum from blood sample. (1) H_2SA_2Ten, (2) platinum(II). (A) Platinum in blood serum, (B) blood serum spiked with 20 μg platinum. Conditions as in Fig. 2.

a flow-rate of 0.4 ml/min (Fig. 3). Cobalt(II) and cobalt(III) also eluted close to the reagent peak and did not affect the determination of platinum.

The linear calibration curve for platinum as a complex of H_2SA_2Ten was found by plotting average peak height ($n=3$) of known standard concentration in the range 0–50 μg/ml, with the coefficient of correlation $r=0.9986$. The relative standard deviation (R.S.D.) for the analysis of 25 μg/ml was 2.5% ($n=5$). The detection limits measured were at least three times the background noise at 1 μg/ml, corresponding to 5 ng/injection of 5 μl. The platinum in the cisplatin injection was 6.28 mg/injection corresponding to 9.7 mg cisplatin with R.S.D.=4.61% ($n=3$). The amount of platinum found using dithiozone spectrophotometric method [31] was 6.25 μg/injection, corresponding to 9.62 mg/injection cisplatin. The cisplatin contents reported was 10 mg/injection. The blood samples of cancer patients after infusion of cisplatin were analysed for the platinum content (Fig. 4). The amount of platinum found in blood serum were 212 and 214 ng/ml with R.S.D. values of 4.6 and 4.8%, respectively. The blood of a cancer patient was also analysed for the contents of platinum using standard addition. The amount of platinum found was 300 ng/ml with R.S.D.=3.9%.

4. Conclusion

A method has been proposed for the determination of platinum based on precolumn derivatization with H_2SA_2Ten and HPLC elution from Hypersil ODS, 3 μm column. Copper, cobalt, nickel, iron, palladium and uranium completely separated from platinum. The detection limit was 5 ng/injection platinum. The method was applied for the determination of platinum in a cisplatin injection and blood samples after infusion of cisplatin to cancer patients.

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