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Letter to the Editor

High-performance liquid chromatographic determination of *cis*-diamminedichloroplatinum(II) (cisplatin) as the *o*-phenylenediamine complex

Sir,

cis-Diamminedichloroplatinum(II) (cisplatin) is an important anticancer drug. Cisplatin is highly reactive in the body, and its biodegradation products may have activity and toxicity behaviour different from that of the parent drug. In order to evaluate the drug in clinical situations and to optimize therapeutic regimens, analytical methods capable of separating the drug and its individual biotransformation products and detecting these species at therapeutically relevant levels (ng/ml) are required. However, a major obstacle remaining in the clinical analysis of cisplatin is detection.

Atomic absorption (AA) [1–3] and X-ray fluorescence [4] are non-resolution methods and give only total platinum levels without identifying the drug. HPLC separation followed by AA is an off-line method and, therefore, time-consuming and highly dependent on the sample matrix. UV absorption [1,2,5] with a refractive index detector connected in series [6], electrochemical detection [7–9], neutron activation and radioactive detection from ^{193} Pt, which also gives total platinum [1], have also been used. Recently, determination of cisplatin in urine and plasma in the range from $5 \cdot 10^{-7}$ to $5 \cdot 10^{-5}$ M by quenched phosphorescence detection was reported [10].

The following derivatization agents have been used for cisplatin.

(1) Diethyldithiocarbamate [11–13]: this is a convenient method because the adduct can be extracted from water into chloroform and it strongly absorbs at 254 nm (ε = 43 000 M^{-1} cm^{-1}).

(2) Thiourea (Kurknakow test): as a result of the *trans* directing effect of thiourea (Tu), cisplatin is converted into $Pt(Tu)_4Cl_2$ whereas the *trans* isomer yields *trans*-$Pt(NH_3)_2(Tu)_2Cl_2$; these complexes can be separated by high-performance liquid chromatography (HPLC) [14].

(3) 3,4-Diaminobenzoic acid: this yields a blue complex (λ_{max} = 715 nm) but no HPLC separation was mentioned [15].

(4) $Pt(NO_3)_2$–1,2-diaminodicyclohexane with sodium D-glucuronate: this reaction is very slow, requiring three weeks at room temperature [16].

(5) Sodium bisulphite as a derivatizing agent and sodium dichromate as an

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activating agent react with cisplatin to give a strongly absorbing complex (λ = 290 nm) [9,17].

The aim of this work was, therefore, to develop a rapid, reliable and sensitive precolumn derivatization technique [18] with cisplatin to allow drug detection in the ppm range.

EXPERIMENTAL

Chemicals

HPLC-grade solvents were filtered through 0.5-μm Millipore FHUP-04500 filter paper. Distilled water was passed through a Waters Milli-Q system. *o*-Phenylenediamine (Fluka, Buchs, Switzerland), was recrystallized from toluene. Cisplatin was obtained from Abic (Ramat Gan, Israel).

A μBondapak reversed-phase C_{18} column (300 mm × 4.6 mm I.D., particle size 10 μm) was obtained from Waters (Milford, MA, U.S.A.).

Apparatus

A Waters HPLC system with Model 6000 A solvent-delivery pump, Model U6K injector and Model 480 UV–VIS detector was used. The output was recorded on an HP Model 3390 A integrator. The sample injection volume was 100 μl.

Cisplatin–o-phenylenediamine complex

Cisplatin (*cis*-DDP, 1.5 mg) was dissolved in water (2 ml) and added to an aqueous solution (1 ml) of *o*-phenylenediamine (OPDA, 1.8 mg). After 20 min at 100°C, a blue precipitate was formed, which was filtered through Whatman No. 41 filter paper, washed with water and then dissolved in dimethylformamide (DMF) (100 ml). The reaction mixture was chromatographed on the C_{18} column with a mobile phase of chloroform[a] (at a flow-rate of 1 ml/min), and detection at 703 nm. At this wavelength, only one peak, at a retention item of 3 min, is seen. The reaction was carried out with several concentrations of cisplatin and the same amount (1.8 mg) of OPDA, and a linear calibration curve was obtained.

RESULTS

In order to establish reaction conditions for binding *cis*-DDP with new fluorescent diamines [19], the reaction of cisplatin with OPDA, a model compound, was explored. Platinum(II) or platinum(IV) reacted (as Na_2PtCl_4 or Na_2PtCl_6) with OPDA at pH 6.5 to form a light blue solution with an absorption maximum at 703 nm [20]. The *cis*-DDP–OPDA complex is formed conveniently after heating for 20 min. The complex is soluble in DMF, is time-stable and obeys Beer's

[a]Some instability in chloroform was noted, but this is insignificant in the duration of the analysis.

law, and calibration curves of 2–20 μg/ml were readily prepared. The sensitivity measured (in a 1-cm cell) was 0.4 μg/ml Pt.

The relative ease of formation of the *cis*-DDP–OPDA complex and its fast elution form the reversed-phase C_{18} column show the value of this method as a rapid analytical tool for determining *cis*-DDP by precolumn derivatization, followed by HPLC analysis.

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