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Note

Identification of degradation products from aqueous carboplatin injection samples by electrospray mass spectrometry

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Abstract

Four different carboplatin injection samples in water (~10 mg/ml) stored at room temperature were investigated for degradation products by electrospray liquid chromatography-mass spectrometry (ESI/LC–MS). A mass spectrometer compatible mobile phase system with 0.02% formic acid and methanol was used to resolve the impurities. Possible chemical structures of the unknown impurities and its degradation mechanism were proposed based on its experimental results and literature findings.

Keywords: Carboplatin; Electrospray; LC–MS

1. Introduction

Platinum complexes (Fig. 1) are widely used as drugs against cancer (Boulikas and Vougiouka, 2003). Cisplatin (*cis*-diamminedichloroplatinum II), carboplatin (*cis*-diammine 1,1-cyclobutane dicarboxylato platinum II), oxaliplatin (1R, 2R-diaminocyclohexane)oxalatoplatinum(II) and nedaplatin *cis*-diamine (glycolato) platinum(II) are used for the treatment of cancers such as melanoma, sarcoma and leukemia tumor models and have shown clinical efficacy for head, neck, bladder, lung, ovarian, colorectal and testicular cancers (Oliver and Mead, 1993; Stathopoulos et al., 1999). Platinum complexes target DNA (Galea and Murray, 2002); form DNA adducts and trigger apoptosis (programmed cell death). Cisplatin acts on tumor by forming positively charged species in aqueous solutions. These activated species binds with DNA forming mono adducts as well as intra and interstrand cross links. Although cisplatin is widely used, its clinical use is hampered due to its high renal, nephro, gastrointestinal, ototoxicity (Rosenberg, 1977; Hill and Speer, 1982; Cheung et al., 1987).

Carboplatin has shown lesser toxicity for kidneys and nervous system than cisplatin due to the formation of six-membered carboplatin chelate ring structure that shields platinum atom from nucleophilic attack. Thus under aqueous conditions the positively charged activated species of carboplatin are formed at much slower rate than in cisplatin (Harland et al., 1984). Recent studies however, have shown that the presence of other nucleophiles such as chloride ions increases the toxicity of infusion solutions due to the formation of cisplatin (Allsopp et al., 1991). Carboplatin undergoes hydrolysis into diammineaqua [O1-carboxylato-1-carboxy-cyclobutane] platinum(II) followed by release of cyclobutane dicarboxylic acid (CBDCA). There are number of methods such as high performance liquid chromatography (Pujol et al., 1997), atomic absorption spectrometry (Schnurr et al., 2002), electrochemical methods (Elferink et al., 1987), inductively coupled plasma-mass spectrometry (Hann et al., 2005), X-ray (Curis et al., 2000; Kuroda et al., 1984), nuclear magnetic resonance (Kuroda et al., 1984) and liquid chromatography mass spectrometry (LC–MS) (Burns et al., 1996; Henderson and Mcindoe, 2005) were used to for the identification of degradation products. Earlier study on carboplatin using LC–MS was essentially focused on low level quantification, and has shown that the method could be used for detecting up to 0.03–1.3 $\mu g\,ml^{-1}$ in plasma homogenates (Guo et al., 2003). Since there are no reports published on identification

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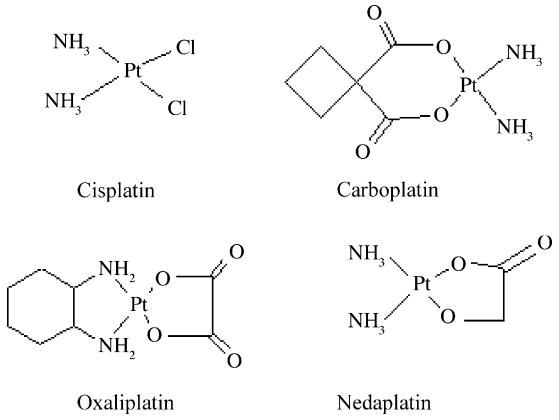


Fig. 1. Chemical structure of representative platinum anticancer drugs.

of unknown degradation products of aqueous carboplatin solutions by LC–MS, an attempt has been made to identify chemical structures of unknown impurities.

2. Materials and methods

2.1. Materials

Carboplatin injection samples were purchased from Bristol Myers Ltd., UK for experimental use. Dabur carboplatin were obtained from Dabur Pharma, Gordon, UK. HPLC grade methanol was supplied by Qualigens. Formic acid (grade) was purchased from Spectrochem, India. HPLC-grade water (Milli-Q, Millipore, and Bedford, MA, USA) was prepared on-site.

2.2. Preparation of standards and reagents

Aqueous stock solutions 1 mg ml^{-1} carboplatin, diamminediaquaplatinate II (DIAQUA) and CBDCA were prepared prior to LC–MS experiments and stored at 4 °C. Stock solutions were diluted with Milli-Q water to obtain working solutions of 10 μg ml^{-1}.

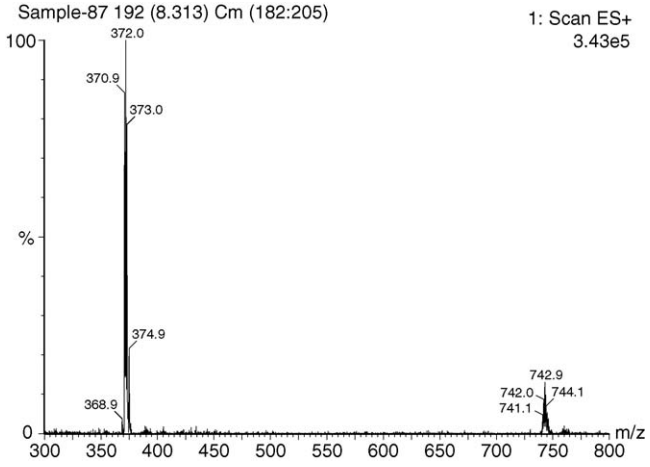


Fig. 2. Full scan positive ion electrospray mass spectrum of carboplatin.

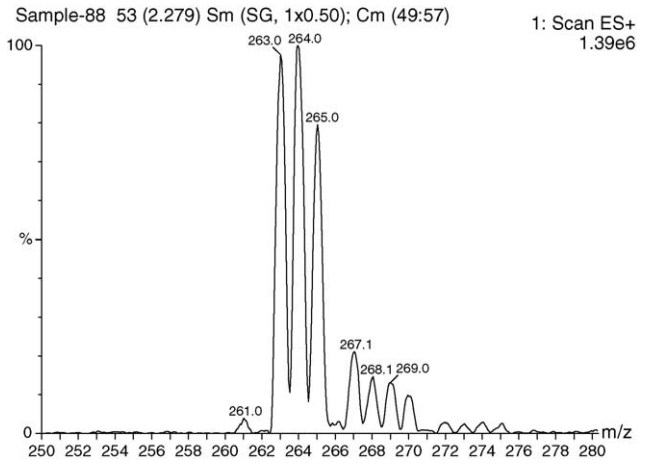


Fig. 3. Full scan positive electrospray mass spectrum of diamminediaquaplatinate II.

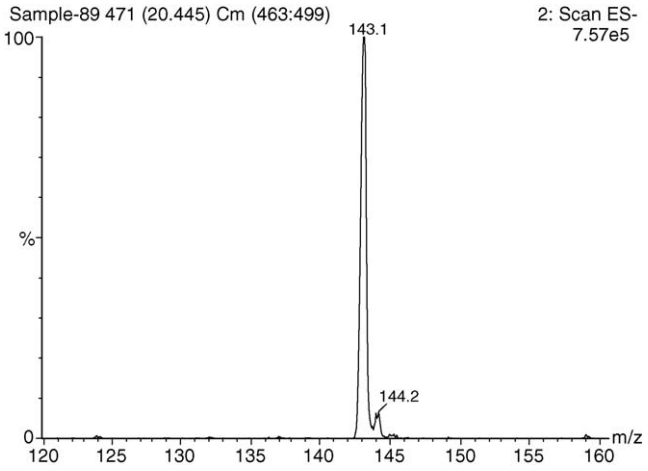


Fig. 4. Full scan negative electrospray mass spectrum of cyclobutane dicarboxylic acid.

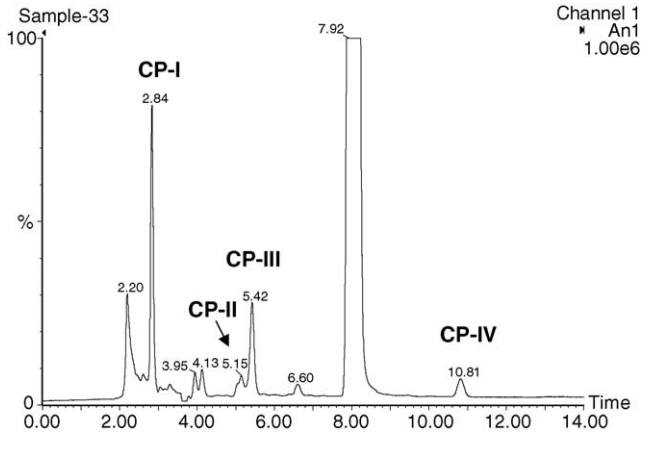


Fig. 5. High performance liquid chromatography profile of carboplatin injection sample collected at 210 nm.

2.3. LC–MS

Degradation products of aqueous carboplatin samples were analyzed on a Waters, Micromass quattro micro triple quadrupole LC–MS. Except for CBDCA all samples were recorded by electrospray positive ion mass spectrometry. For positive ions the capillary and cone voltages were set at 3.34 kV and 12.0 V, respectively, while for CBDCA polarity of the voltages were reversed to negative. Ion source was maintained at a temperature of 100 °C and the desolvation gas temperature was kept at 120 °C. Nitrogen gas was used for desolvation and nebulisation gas with a flow rate of 500 and 50 l H^{-1}, respectively. The mass resolution was at 800 U full width half maximum (FWHM) over a mass range 100–1000 amu and the scan speed was 700 amu s^{-1}. MS–MS analyses were carried out by (i) selecting the parent ion in the first quadrupole, (ii) inducing fragmentation of the selected ion in the second quadrupole used as collision cell with argon (3.7×10^{-3} mbar), and (iii) analyzing the product ions in the third quadrupole. Chromatographic separations were performed using an YMC-pack ODS-A column (4.6 mm × 250 mm, 5 μm) at room temperature with an injection volume of 10 μl. The UV detector was set at 210 nm.

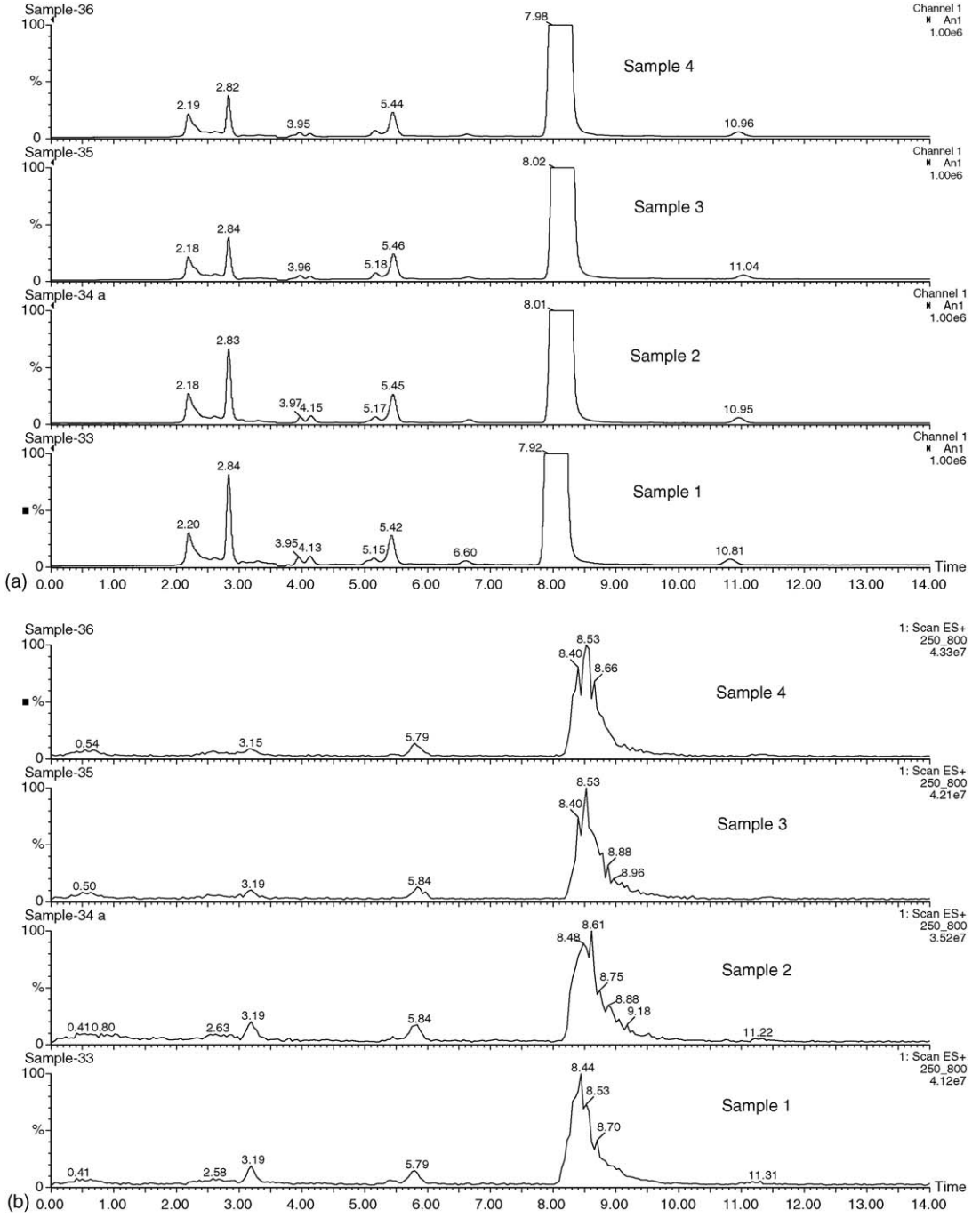


Fig. 6. Comparison of HPLC (a) and respective mass chromatograms (b) of four carboplatin injection samples.

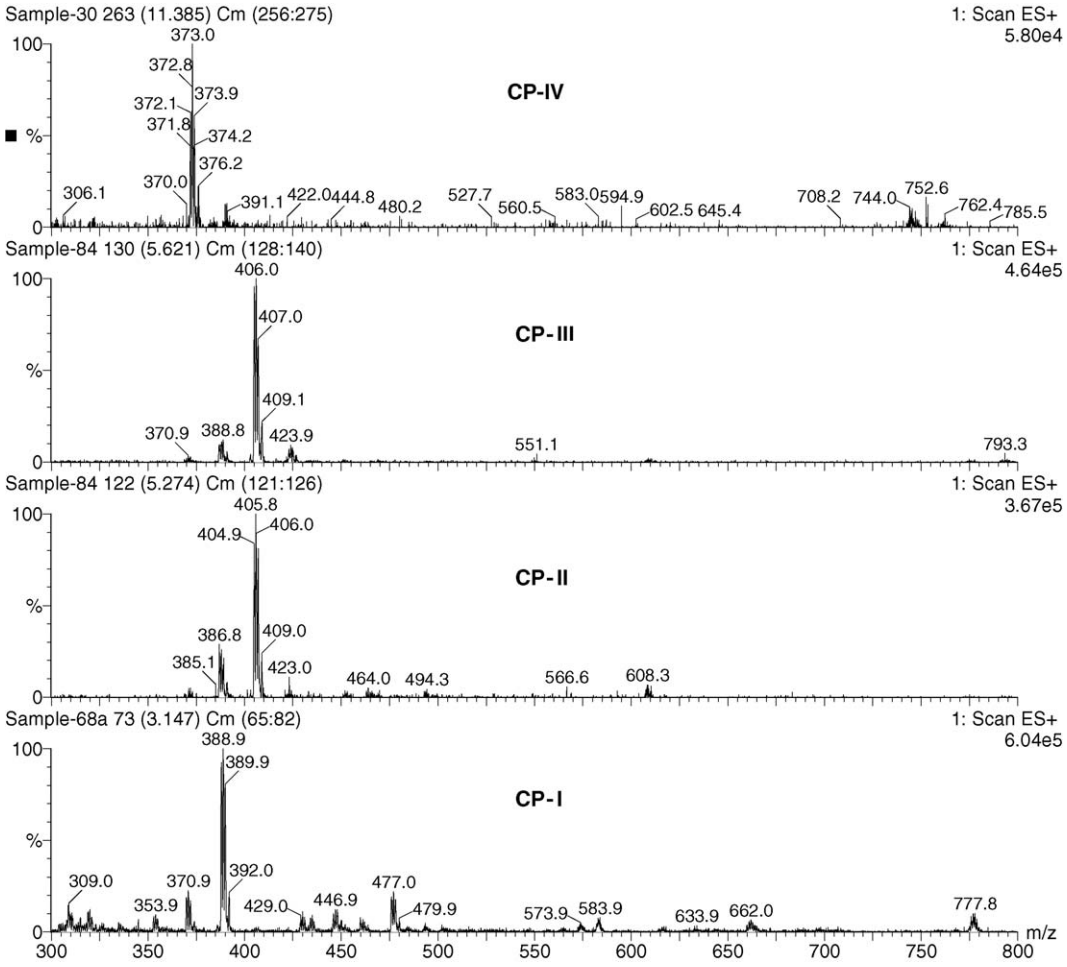


Fig. 7. Positive electrospray mass spectra of major impurities collected at different retention times.

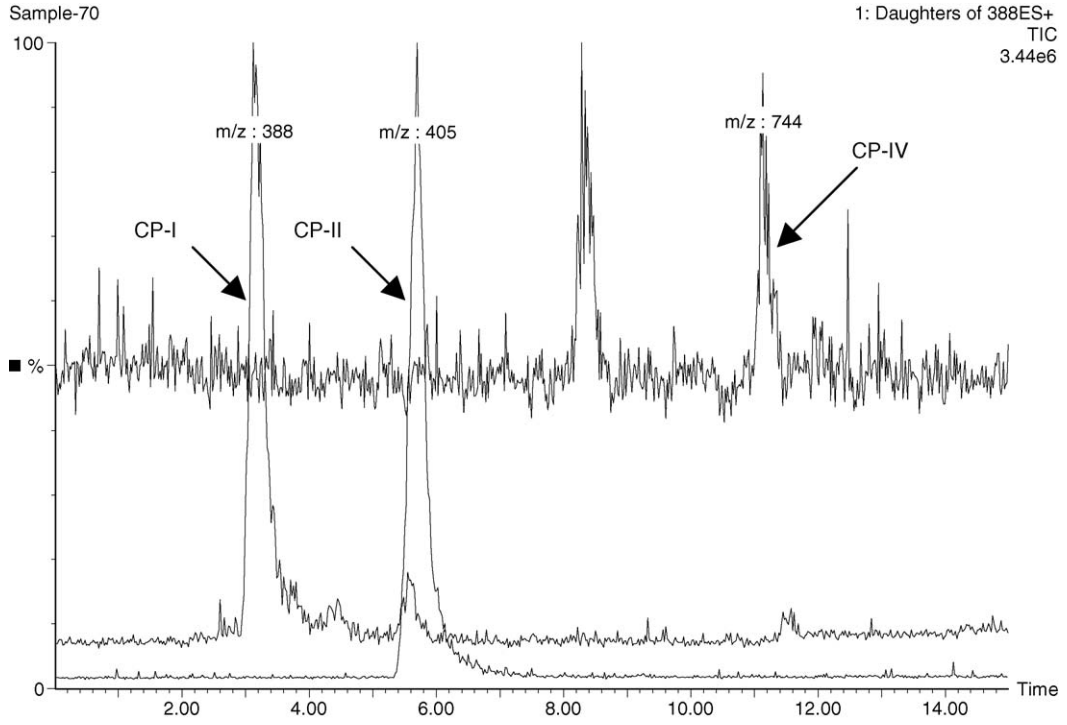


Fig. 8. Extracted mass chromatogram for the aqueous degradation products at various retention times.

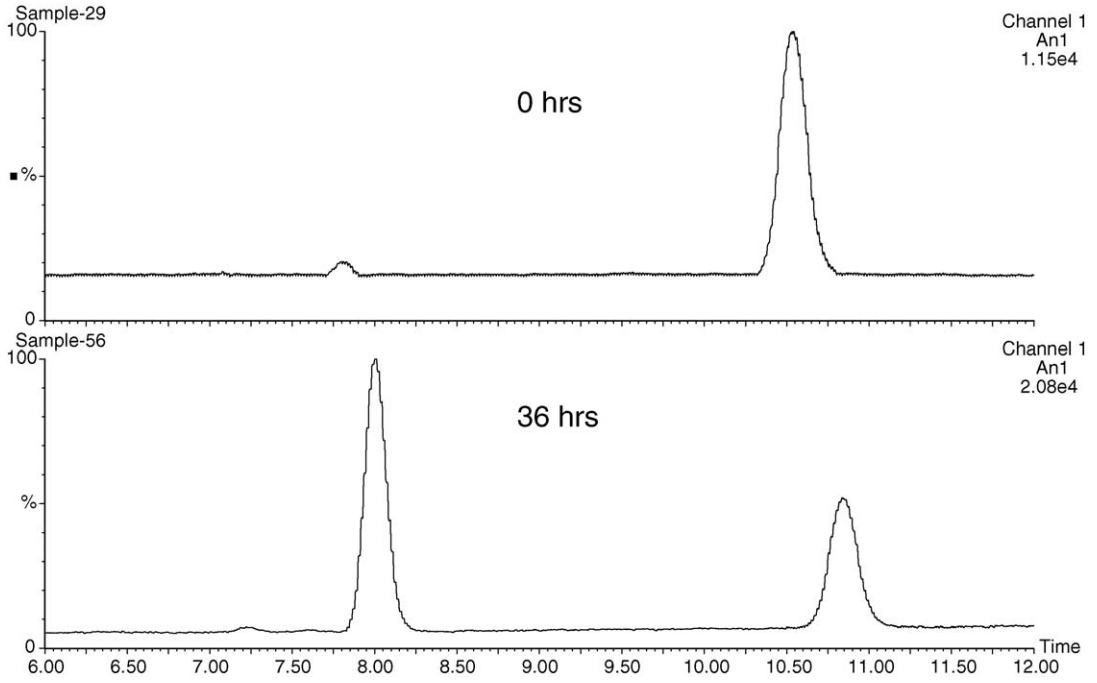


Fig. 9. HPLC Profile of CP-IV recorded at 0 and 36 h.

The flow rate was maintained at 1.0 ml min^{-1}. Gradient elution was used with aqueous formic acid (0.02%, v/v) as component A and methanol as component B. The gradient consisted of 100%A for the first 10 min, followed by 100 A to 75% A–25% B in 1 min with additional 9 min at 75% A–25% B. The gradient was reverted back to 100% A after 20 min. Mass spectra for all samples were collected for a mass range from 100 to 1000 amu.

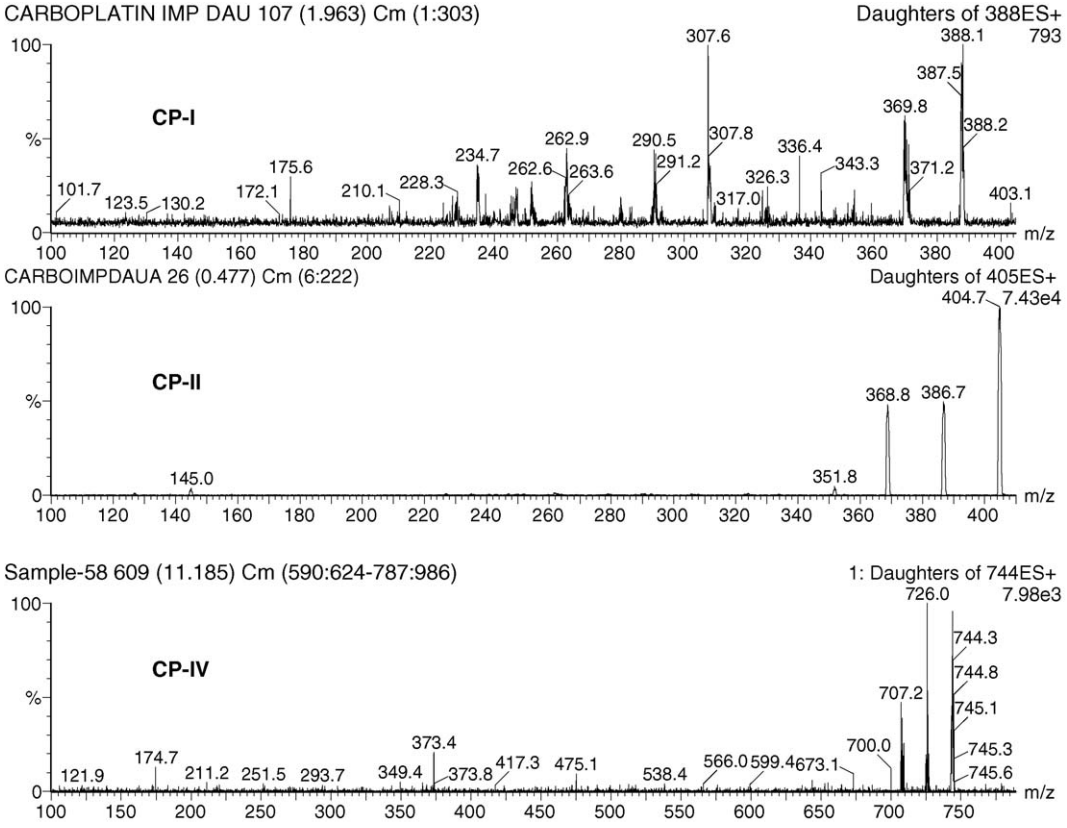


Fig. 10. Product ions observed for the aqueous degradation products of carboplatin.

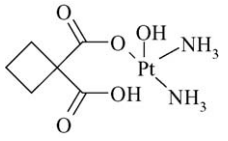
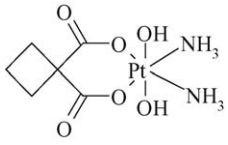
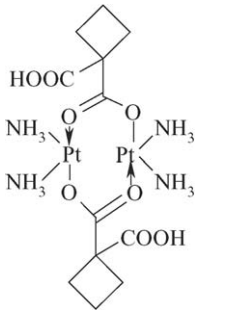
3. Results and discussion

Full scan positive electrospray mass spectrum of standard carboplatin ($C_6H_{12}N_2O_4Pt$) show intense ion peaks at *m/z*: 372 and 743 amu which could be accounted for the molecular ion $[MH]^+$ and its dimer $[M2H]^+$ (Fig. 2). Molecular ions that contain platinum exhibited clusters of isotopic peaks corresponding to the major platinum isotopes ^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt with relative abundance of platinum isotopes as 32.9, 33.8, 25.3 and 7.2%.

Pharmacopoeia methods (USP and EP) available for the resolution of carboplatin impurities utilize ion pair agents, strong acids and salts and suppress ion signals in a mass spectrum. To profile impurity masses by LC–MS a mass spectrometry compatible mobile phase system was used. Although ammonium acetate buffer is commonly employed mobile phase system for LC–MS due to adduct formation, formic acid method reported in literature (Burns et al., 1996) was adopted for identification of degradation products of aqueous carboplatin solutions. Solutions of standards of CBDCA and DIAQUA, known aqueous degradation products of carboplatin were analyzed by ESI-MS. Since protons has affinity for lone pair of electrons on nitrogen atoms, a positive electrospray ionization of DIAQUA (Fig. 3) resulted in protonated molecular ion (*m/z*: 264; MH^+) and since acids have the tendency to loose protons negative electrospray ionization of CBDCA (Fig. 4) showed deprotonated molecular ion (*m/z*: 143; $M - H^-$).

LC–MS analyses of four carboplatin injection samples recorded at 210 nm by formic acid method are given in Fig. 5. UV profiles show impurities at 2.20, 2.84, 3.95, 4.13, 5.15, 5.42, 6.60 and 10.8 min. Out of these impurities only impurities at retention time 2.8, 5.1, 5.4 and 10.8 were identified for molecular weights by LC–MS and marked as CP-I, CP-II, CP-III and CP-IV as shown in Fig. 5. The other impurities were not detectable by LC–MS due to low concentration or poor ionization. A comparison of HPLC and mass chromatogram of all four carboplatin injections samples show similar pattern as given in Fig. 6. Out of five impurities three major impurities were enriched and analyzed by LC–MS. Positive ion electrospray mass spectra of CP-I, CP-II, CP-III and CP-IV observed at respective retention times 2.8, 5.1, 5.4 and 10.8 min are given in Fig. 7. The mass spectra of CP-I, CP-II, CP-III and CP-IV showed protonated molecular ion peaks at *m/z*: 390, 406, 406 and 744, respectively. Appearance of ion peak *m/z*: 406 at two different retention times (5.1 and 5.4 min) may be due to isomerization during chelate ring opening step as described in Fig. 11. The positional isomers results in hydroxyl group occupying either axial or equatorial position in octahedral geometry (Kuroda et al., 1984). The molecular ion peak at retention time 2.8 min is *m/z*: 390 which could be explained by aqueous degradation as shown in Fig. 11. Conversion of coordinated water molecule into hydroxyl group has already been reported (Allsopp et al., 1991). At retention time 10.8 min, the molecular ion observed was 744. This molecular ion can be explained by the dimeric structure of the platinum complex. Similar chemical structure has been proposed by Schnurr et al. (2002). The high retention time of the complex also is explained by the dimeric structure. All three impurities (CP-I, CP-II and CP-IV) were difficult to isolate in solid state due to equilibrium conditions and lability of the ligands in solutions (Oliver and Mead, 1993). The extracted

Table 1
Retention time, molecular mass and proposed chemical structure of impurities

Impurity code	Retention time (min)	Molecular mass (*m/z*)		Molecular formula	Proposed structure
		Observed	Calculated		
CP-I	2.8	390.0	389.0	$C_6H_{14}N_2O_5Pt$	
CP-II	5.4	406.0	405.1	$C_6H_{14}N_2O_6Pt$	
CP-IV	10.8	744.0	744.1	$C_{12}H_{26}N_4O_8Pt_2$	

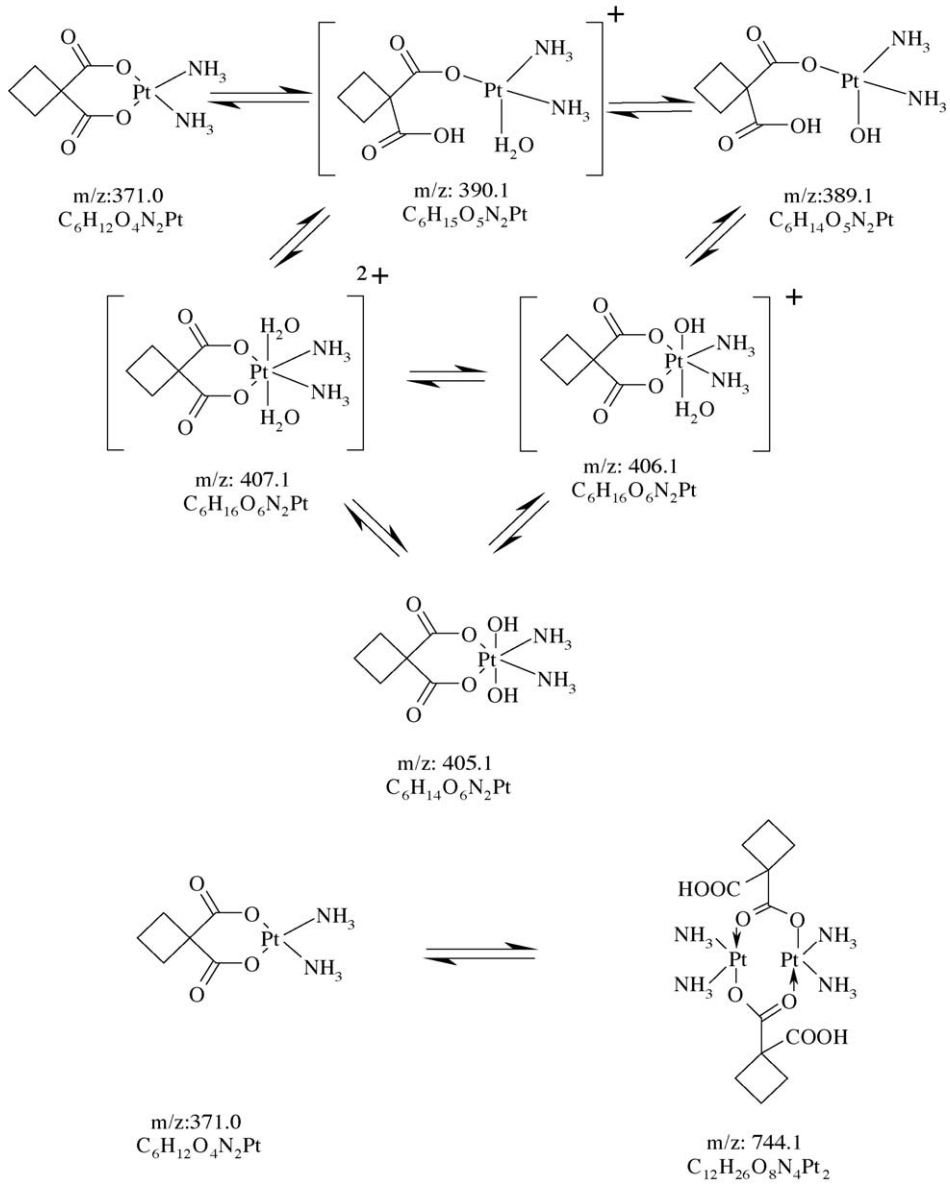


Fig. 11. Proposed degradation mechanism and inter conversion of dimer to monomer.

mass chromatogram of enriched impurities of CP-I, CP-II and CP-IV are given in Fig. 8. Enrichment of CP-IV also shows conversion of carboplatin dimer to carboplatin monomer when LC–MS was recorded at two different time intervals (Fig. 9), which further confirms the existence of equilibrium conditions in carboplatin solutions. Collision induced dissociation (CID) mass spectra of CP-I, CP-II and CP-IV are given in Fig. 10. The fragmentation of CP-I, CP-II and CP-IV showed ion peaks corresponding to loss of water (M-H_2O), ammonia (M-NH_3) and CBDCA (*m/z*: 145). Molecular masses of CP-I, CP-II and CP-IV and probable chemical structures with retention times are given in Table 1. Chemical structures of the molecular ions were proposed based on molecular ions, fragment pattern and literature findings (Schnurr et al., 2002). The formation degradation products of aqueous carboplatin solutions under equilibrium conditions are summarized in Fig. 11.

4. Conclusions

The degradation products of aqueous carboplatin solutions were studied by electrospray mass spectrometry using formic acid method. Four carboplatin injection samples showed comparable UV profiles and mass chromatogram indicating formation of similar impurities. The molecular masses of four impurities identified and three major impurities CP-I, CP-II and CP-IV were enriched and studied by LC–MS. Probable chemical structures have been proposed based on molecular masses, fragmentation and published literature. A degradation mech-

anism for the formation of impurities and dimer has also been proposed.

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