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Determination of the platinum drug *cis*-amminedichloro(2-methylpyridine)platinum(II) in human urine by liquid chromatography–tandem mass spectrometry

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Abstract

A validated stable isotope dilution liquid chromatography–tandem mass spectrometry assay for the novel platinum drug *cis*-amminedichloro(2-methylpyridine)platinum(II) (ZD0473) in human urine has been developed. This method uses selected reaction monitoring on the transition of *m/z* 393 $[M+NH_4]^+$ to *m/z* 304 $[M+NH_4-NH_3-2\times H^{35}Cl]^+$ for ZD0473, and *m/z* 400 $[M+NH_4]^+$ to *m/z* 310 $[M+NH_4-NH_3-H^{35}Cl-{}^2H^{35}Cl]^+$ for the internal standard $[{}^2H_7]$ZD0473. Standard curves were prepared over the range from 0.15 to 50 μg/ml. The lower limit of quantitation was 0.2 μg/ml for 100 μl of urine. This simple, rapid, reliable, and sensitive method of quantitation displayed acceptable accuracy and precision over the 3 days of assay validation. A novel platinum adduct was formed during the storage of ZD0473 in human urine. The adduct did not correspond to any of the typical sulfhydryl adducts that have been identified previously for platinum drugs. Formation of the adduct was prevented by the addition of 50% (w/v) sodium chloride to the urine. The assay can be used to quantify intact ZD0473 in the urine of subjects dosed with this new platinum drug.

Keywords: *cis*-Amminedichloro(2-methylpyridine)platinum(II); Platinum adduct

1. Introduction

Renal elimination is the major pathway by which platinum anticancer agents are excreted [1–4]. Thus, more than 50% of unchanged oxaliplatin was found in urine [5]. Renal clearance determined from the urinary concentration of unchanged drugs is an important pharmacokinetic parameter used in the design of dosage strategies. Urine samples are normally collected over a 12- or 24-h time period, therefore they may be kept at room temperature for extended periods in the clinic before freezing. Consequently, irreversible loss of the intact platinum drug may result from adduct formation through reaction with nucleophiles that are present in the urine (Scheme 1) [4,6,7].

The platinum agent *cis*-amminedichloro(2-methylpyridine)platinum(II) (ZD0473) (Scheme 1)

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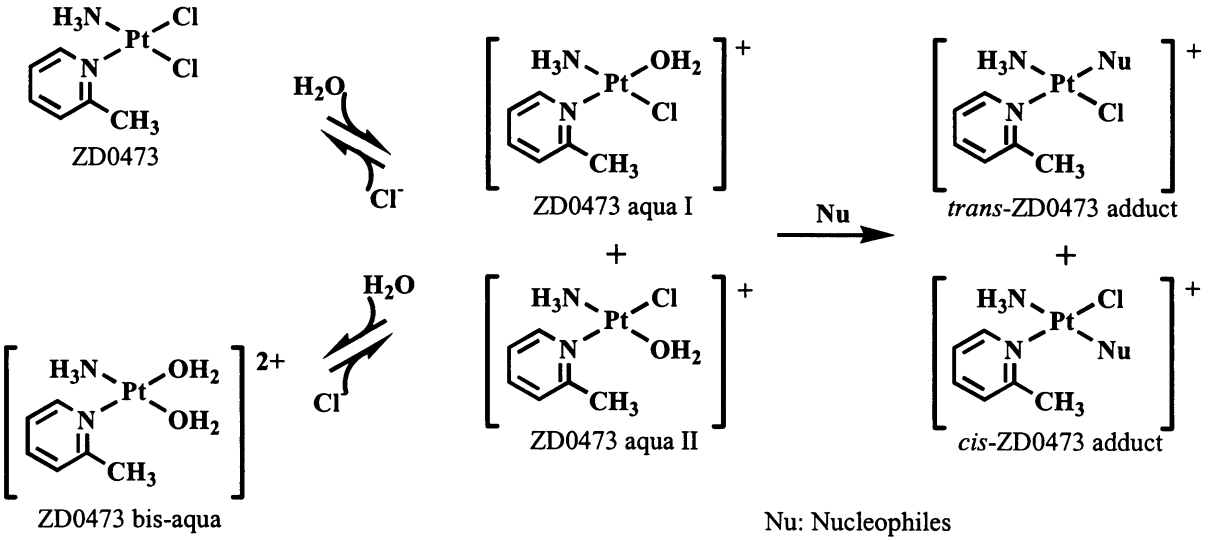

Scheme 1. Equilibration of ZD0473 with aquated forms and irreversible adduct formation. Mono-aquated forms are present as *cis* and *trans* species [41].

is a novel sterically hindered anti-tumor agent that is undergoing clinical trials [8]. ZD0473 was chosen from a panel of sterically hindered pyridine platinum complexes, due to its reduced reactivity with sulfur ligands and unique DNA binding properties [9]. LC–MS studies demonstrated that JM216 (a related platinum drug) underwent extensive biotransformations in vivo by reduction to the platinum(II) compound, JM118, and by hydrolysis to JM518 (Fig. 1) [10–12]. Furthermore, a substantial portion of the drug was excreted as cyclohexylamine [13], suggesting that the parent drug or one of the primary metabolites was undergoing ligand exchange. LC–MS studies have demonstrated that other platinum drugs with carbocyclic rings such as oxaliplatin and carboplatin (Fig. 1) can also undergo biotransformations in vivo [4,6,14]. These studies suggested that ZD0473 could undergo biotransformations through loss of the 2-methylpyridine ligand or loss of the chloride moieties. Thus, total Pt analyses would not reflect concentrations of the parent drug, which in turn could lead to erroneous pharmacokinetic profiles. Quantitative studies of Pt drugs in urine have generally relied upon the use of atomic absorption spectrophotometry [2,4], or more sensitive ICP-MS methodology [4,15].

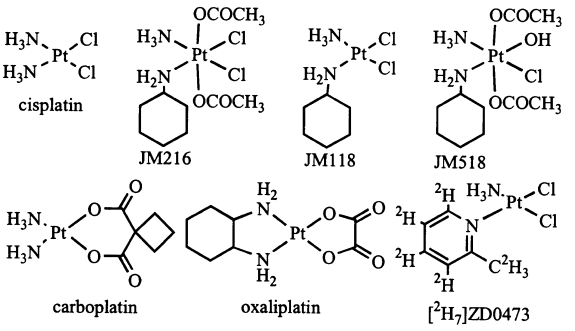

Fig. 1. Structures of platinum anticancer drugs and deuterated ZD0473.

We have recently developed a method for the quantitative analysis of ZD0473 in human plasma ultrafiltrate [16], which was the first validated stable isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS–MS) assay reported for a platinum drug. LC–MS–MS methodology is extremely robust and amenable to quantitation of biological samples. The high sensitivity and specificity of selected reaction monitoring (SRM) after collision-induced dissociation (CID) is attributable to the use of a specific precursor-to-product ion transi-

tion as we have demonstrated previously for DNA adducts, drugs, and their metabolites [17–21]. In the case of the platinum drugs, there are three major isotopes of platinum and two of chlorine. Molecular species are complex and sensitivity is limited by segregation of the MS signal into a number of different isotopes. Furthermore, in biological fluids, the dichloro form of the drug exists in equilibrium with various aquated forms such as *cis*, *trans*, and *bis*-aquated (Scheme 1) [22]. Further equilibration processes could result in various de-protonated forms [23]. We have already overcome these difficulties by careful choice of the precursor ion and LC–MS–MS conditions, and by using a stable-isotope-labeled internal standard (I.S.) to compensate for the equilibration between the aquated forms and the intact form. The method was sensitive enough to study ZD0473 disposition after administration of an intravenous dose of the drug to humans and has been employed for pharmacokinetic studies of ZD0473 [24]. This method has now been adapted to the analysis of ZD0473 in human urine incorporating a technique to avoid the formation of adducts during storage. We report the application of this new assay to monitor urinary concentrations of ZD0473 in cancer patients who received 60 mg/m^2 doses of ZD0473 as 1-h infusions.

2. Experimental

2.1. Apparatus

Liquid chromatography was performed on a Waters 2690 Separation Module equipped with an autosampler, vacuum degasser, and column heater (Waters, Milford, MA, USA). This was coupled to a Finnigan TSQ 7000, a triple-quadrupole mass spectrometer fitted with an electrospray ionization (ESI) source and an API 1 interface (Thermo Finnigan, San Jose, CA, USA). All standards and quality control (QC) solutions were prepared using certified volumetric flasks and calibrated pipettes. All tubes used were siliconized polypropylene centrifuge tubes. Autoinjector vials were used with siliconized insert tubes (250 μl). Vials and tubes were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Materials and reagents

HPLC-grade water, methanol, and hydrochloric acid were obtained from Fisher Scientific. Ammonium acetate, acetic acid, and sodium chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). ZD0473 and [$^2H_7$]ZD0473 were provided by AstraZeneca (Cheshire, UK). Liquid nitrogen and argon were obtained from BOC Gases (Bellmawr, NJ, USA).

2.3. Preparation of drug-free urine

Urine samples were collected from three healthy volunteers. A sample from each urine collection (approximately 67 ml of each) was mixed together in a 250 ml sterile square media bottle (total volume approximately 200 ml, pH 6.5–7.0). Sodium chloride (4 g) was added to the urine. The pH was adjusted to approximately 5.0 by adding 0.2 *M* HCl in 0.15 *M* sodium chloride. The urine was stored at −80 °C until used.

2.4. Preparation of standard solutions

ZD0473 is a light-sensitive compound, in common with other platinum drugs [25,26], so precautions were taken to exclude natural light wherever possible. *cis*-Amminedichloro(2-methylpyridine) platinum(II) (ZD0473) (10.0 mg) was transferred to a 25 ml volumetric flask. Aqueous sodium chloride (0.15 *M*, 20 ml) was added and the solution was shaken for 30 min. The solution was then made up to the mark with aqueous 0.15 *M* sodium chloride and stirred with a magnetic stirrer for 30 min. Sonication was avoided for preparing stock solutions as this could cause decomposition [26]. This solution (400 μg/ml) was used to prepare the QC samples. A similar method was used to prepare a solution of 100 μg/ml, which was then diluted with 0.15 *M* aqueous sodium chloride for the standard curve samples. All solutions were stored at 4 °C in the dark. QC urine samples were prepared by adding the appropriate amount of ZD0473 solution to drug-free human urine for the lower limit of quantitation QC (LLQ, 0.2 μg/ml), lower QC (LQC, 0.4 μg/ml), middle QC (MQC, 4 μg/ml), and higher QC (HQC, 40 μg/ml). Upper QC dilution standard (UQC, 400 μg/ml) was

prepared by dissolving ZD0473 (10.0 mg) directly in drug-free human urine (25 ml). Samples were stored in the dark at −80 °C in siliconized polypropylene centrifuge tubes (0.6 ml) until used. A stock solution of I.S. (100 μg/ml) was prepared by dissolving *cis*-amminedichloro([$^2H_7$]-2-methylpyridine)platinum (II) ([$^2H_7$]ZD0473) (10.0 mg) in 0.15 *M* aqueous sodium chloride (100 ml) as described above for the non-deuterated standard. This solution was diluted with 0.15 *M* aqueous sodium chloride to give a final concentration of 10 μg/ml. Stock solutions used for the assay were monitored by UV on a regular basis to make sure that no decomposition had taken place. Samples were diluted to 100 ng/μl with 0.15 *M* sodium chloride. The absorbance ratio between λ_{max} (268 nm) and λ_{min} (250 nm) was determined and compared with a freshly prepared standard ($A_{250}/A_{268} = 0.62$).

2.5. Chromatographic operating conditions

HPLC was conducted using a YMC ODS-AQ column (150×2.0 mm I.D., 3 μm, 120 Å; Waters) with the column oven set at 30 °C. Solvent A was 5 m*M* aqueous ammonium acetate containing 0.1% (v/v) acetic acid and solvent B was 5 m*M* methanolic ammonium acetate containing 0.1% (v/v) acetic acid. The flow-rate was 180 μl/min. A linear gradient was run as follows: 0 min 1% B, 5 min 76% B, 6 min 1% B, 16 min 1% B. The samples (200 μl) were maintained at 4 °C in the autosampler tray and injections of 50 μl were made. The gradient was started 2 min before the sample injection to compensate for the dead volume of the gradient delivery system. The column effluent was diverted to waste for the first 5 min of the analysis to prevent excess sodium chloride and endogenous materials from entering the mass spectrometer.

2.6. Mass spectrometer operating conditions

ESI was conducted with a needle voltage of 4.5 kV. Nitrogen was used as the sheath gas (70 p.s.i.) and the auxiliary gas (10 units) to assist with nebulization. The capillary was heated to 210 °C to provide optimal desolvation, and the ESI interface and mass spectrometer parameters were optimized to obtain maximum sensitivity without sacrificing unit resolution. An SRM experiment was conducted by using the m/z 393→m/z 304 transition of ZD0473. For the I.S., the m/z 400→m/z 310 transition was monitored. During the SRM studies, argon was used as the collision gas. A collision offset energy of −18 eV and a collision cell pressure of 2.75 mT were used. The tube lens, the capillary voltage, parent offset voltage, daughter offset voltage, lens 1-1 voltage, lens 1-2 voltage, lens 2-1 voltage, lens 2-2 voltage, lens 2-3 voltage, lens 3-1 voltage, lens 3-2 voltage, lens 3-3 voltage, parent resolution, daughter resolutions, and octapole offset voltage were optimized for sensitivity without sacrificing resolution. The electron multiplier was set at 1650 V, the scan time was 0.4 s, and the daughter scan width was 1.0 amu.

2.7. LC–MS conditions for analyzing urinary adducts of ZD0473

Chromatography was performed using a Hypercarb column (porous graphite carbon, 150×2.1 mm I.D., 5 μm; Thermo Finnigan) at a flow-rate of 0.15 ml/min. Solvent A was 5 m*M* aqueous ammonium acetate containing 0.1% (v/v) acetic acid and solvent B was 5 m*M* ammonium acetate in methanol containing 0.1% (v/v) acetic acid. A linear gradient was run as follows: 0 min 1% B, 9 min 91% B, 12 min 91% B, 13 min 1% B, 30 min 1% B. MS was operated using the same conditions as described above for the quantitation except for using Q1 full scan mode from m/z 280 to m/z 980.

2.8. Stability of ZD0473 during storage

ZD0473 solution (40 μl of 100 ng/μl) was spiked into three different fresh urine samples (1.96 ml) or three different fresh human urine samples (1.96 ml) containing sodium chloride (1 g) and kept in the dark at room temperature. Immediately after (0 h) or 24 h later, 50 μl of each reaction mixture was transferred into the HPLC vial containing 50 μl of [$^2H_7$]ZD0473 solution (10 ng/μl), mixed, and placed on the autosampler maintained at 4 °C. Injections of 40 μl were made on the LC–MS system. The peak area ratio of ZD0473/[$^2H_7$]ZD0473 of the corresponding 0-h samples (n =

3, average value) was taken as 100% for the calculation.

2.9. Suppression of ESI signal from endogenous constituents in the urine

The response of the MQC samples (4 μg/ml) was compared with the response from standard samples containing 4 μg of ZD0473 and 10 μg of [$^2H_7$]ZD0473 in 1 ml of 0.15 *M* sodium chloride.

2.10. Sample preparation for analysis by liquid chromatography–mass spectrometry

Eight calibration standards were prepared in the range from 0.15 to 50 μg/ml using drug-free human urine (100 μl). A blank urine (drug-free urine), and a control zero (drug-free urine that was spiked with only I.S.) were included with each calibration curve. QC urine samples (100 μl) were transferred to labeled siliconized polypropylene centrifuge tubes (2 ml). UQC samples (100 μl) were diluted 10-fold with drug-free urine (900 μl). The I.S. solution (100 μl, 10 ng/μl) was added to each tube except the blank urine sample. Each tube was vortex-mixed for 20 s and an appropriate amount of 0.15 *M* sodium chloride and 10 μl of 0.1 *M* hydrochloric acid in 0.15 *M* sodium chloride were added to a total volume of 1000 μl. Each tube was again vortex-mixed for 20 s. The solutions were centrifuged at room temperature at 14 000 rpm for 5 min. The supernatant (200 μl) was transferred to HP auto-injector vials, which were kept at room temperature for 2 h to ensure complete equilibration of the aquated forms back to the dichloride form.

2.11. Data analysis

Data analysis was performed using LCQuan, version 1.2 (Thermo Finnigan) from raw mass spectral data. Calibration curves were plotted using a quadratic regression with weighting index of $1/x$ as this gave the best fit of the data over the concentration range. This was because of the minor contribution of ^{37}Cl and ^{198}Pt in the [$^2H_7$]ZD0473 channel (m/z 400→m/z 310) at high concentrations of ZD0473.

2.12. Accuracy and precision

Precision and accuracy studies were performed ($n=5$) on the LLQ, LQC, MQC, HQC, and 10-fold-diluted UQC samples on three separate days and after 2 weeks, 1 months, 3 months, and 6 months. The LQC, MQC, and HQC samples were examined after three freeze–thaw cycles which consisted of thawing for 1 h at room temperature followed by freezing for 23 h or longer at −80 °C. Stability on the autosampler was assessed by re-analyzing samples kept at 4 °C for 4 days. Assay accuracy was assessed by comparing the mean of the study samples with the theoretical concentration of the QC samples and expressed as a percentage. Within-day precision was calculated from the ratio of the relative standard deviation (% RSD) to the appropriate mean value expressed as a percentage for five replicates ($n=5$). Inter-day precision, defined as % RSD of three different day validation runs (day 1–day 3), was also assessed. For validation studies, the mean of all the lower limit of quantitation QC samples had to demonstrate between 80 and 120% accuracy with a precision of ±20% or better [27]. The other QC samples (LQC, MQC, HQC, and UQC) had to demonstrate between 85 and 115% accuracy with a precision of ±15% or better [27].

2.13. Intravenous dosing with ZD0473

ZD0473 (60 mg/m^2) was administered by 1-h intravenous infusion. Appropriate Institutional Review Board approval was obtained for these studies.

3. Results and discussion

3.1. Stability of ZD0473 in fresh human urine

A number of studies have examined the stability of dichloro-Pt drugs in aqueous solutions [23,25,26,28–32]. Substitution of chlorine by water to form various charged aquated species occurs reversibly as shown for ZD0473 in Scheme 1. The equilibrium is affected by several parameters, such as temperature, pH and chloride-ion concentration. Therefore, we have employed isotonic saline (0.15 *M* sodium chloride) as a solvent for stock solutions and

kept the chloride-ion concentration constant at 0.15 *M* during all analytical procedures. A stable isotope-labeled analog used as I.S. has made it possible to compensate for the equilibration between aqua forms and the intact drug. Low-molecular-mass adducts of platinum drugs may be derived from reaction with urinary glutathione or methionine [4,6]. Although ZD0473 is known to be less reactive against sulfhydryl groups than other platinum drugs [8,33], it could potentially react with other nucleophiles that may be present in urine.

ZD0473 (40 μg) in fresh human urine (1 ml) was kept at room temperature for 1 day and the sample was then analyzed by LC–MS using the full scan mode. ZD0473 was found to generate two isomeric low-molecular-mass adducts. The major adduct (t_R = 15.0 min) was well separated from both ZD0473 (t_R = 17.1 min) and aquated ZD0473 (t_R = 8.3 and 12.3 min) using a porous graphite carbon column (Hypercarb) instead of a YMC ODS AQ column (Fig. 2). The mass spectrum showed an ion cluster of *m*/*z* 452, 453, 454, 455, and 456 (Fig. 3). The ratio was much closer to the compound which has one platinum and one chlorine (calculated ratio as PtCl: 0.93:0.94:1.00:0.30:0.43) rather than the compound that has one platinum and two chlorines (calculated ratio as $PtCl_2$: 0.72:0.73:1.00:0.47:0.58). A minor adduct eluted at 12.2 min (*m*/*z* 453; Fig. 2) with a retention time very similar to one of the aqua isomers (t_R = 12.3 min, *m*/*z* 358; Fig. 2). This resulted in a relatively large signal in the TIC at 12.3 min (Fig. 2). The mass spectrum of the minor adduct was identical to the major adduct. These data suggested that a nucleophile in the urine had replaced one of the chlorine atoms attached to ZD0473 to generate a mixture of *cis*- and *trans*-adducts as shown in Scheme 1. However, the increase in molecular mass compared with ZD0473 did not correspond to adduct formation by any nucleophilic sulfhydryl compound known to react with platinum drugs such as glutathione, cysteine, *N*-acetyl cysteine, or methionine [34–39].

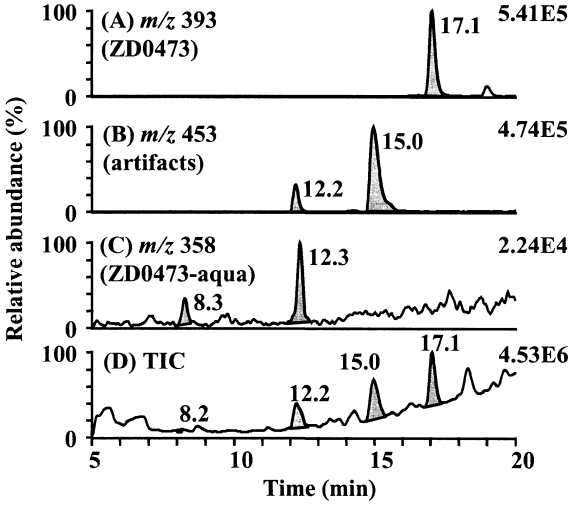

Fig. 2. LC–ESI-MS analysis of the UQC (40 μg/ml) sample which was prepared using fresh urine and kept at room temperature for 1 day. Chromatography was performed using a Hypercarb column (5 μm, 150×2.1 mm I.D.) at a flow-rate of 0.15 ml/min. (A) Ion chromatogram of *m*/*z* 393, (B) ion chromatogram of *m*/*z* 453, (C) ion chromatogram of *m*/*z* 358, and (D) total ion chromatogram (*m*/*z* 280–980).

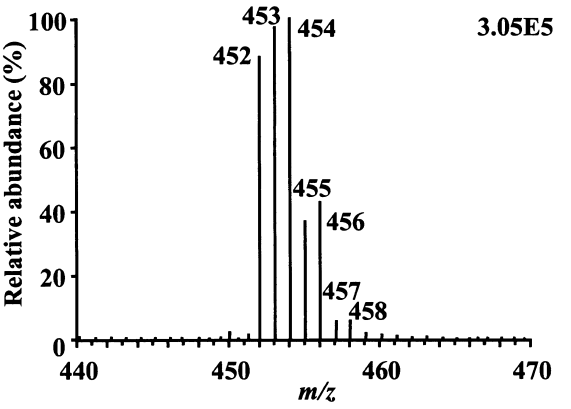

Fig. 3. Mass spectrum (*m*/*z* 440 to *m*/*z* 470) of the unknown adduct peak (t_R = 15.0 min) from Fig. 2. No significant ions were observed below *m*/*z* 440.

3.2. Effect of adding sodium chloride to stabilize ZD0473 in fresh urine during collection

The amount of ZD0473 in three different spiked fresh urine samples from healthy volunteers was decreased by 27 to 40% when stored at room temperature for 24 h (Fig. 4). This was prevented by the addition of excess sodium chloride. Ligand exchange on ZD0473 is known to occur through aquated forms (Scheme 1) [23]. Therefore, the excess chloride prevents formation of aquated forms

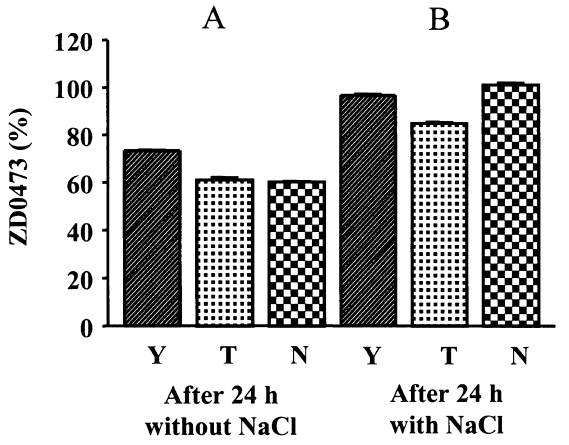

Fig. 4. Stability of ZD0473 in fresh urine ($n=3$). (A) Without the addition of sodium chloride. (B) With the addition of sodium chloride. Y, T, or N is the code letter for each of the volunteers.

of the drug so that nucleophilic displacement reactions cannot occur.

3.3. Mass spectrometry

We have recently developed an LC–MS–MS method for the quantitative analysis of ZD0473 in human plasma ultrafiltrate [16]. The mobile phase system of 5 m*M* ammonium acetate containing 0.1% acetic acid made it possible to generate only ammonium adduct ions $[M+NH_4]^+$ rather than mixtures of $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[2M+H]^+$, and $[2M+Na]^+$ as had been observed previously for platinum drugs [10,40]. The most intense product ion from $[M+NH_4]^+$ was $[M+NH_4-NH_3-2\times HCl]^+$. The most intense ion $[M+NH_4]^+$ at m/z 394 contained two platinum species (^{194}Pt and ^{196}Pt) with combinations of $^{194}Pt/^{35}Cl/^{37}Cl$ and $^{196}Pt/^{35}Cl_2$. The next most intense ions at m/z 393 contained ^{195}Pt and $^{35}Cl_2$. Therefore, the ion at m/z 393 gave the most intense product ion ($[M+NH_4-NH_3-2\times H^{35}Cl]^+$ at m/z 304) when subjected to CID. Similarly, the most intense product ion from $[^2H_7]$ZD0473 (I.S.), obtained by CID of m/z 400 ($^{195}Pt^{35}Cl_2,^2H_7$), appeared at m/z 310 ($[M+NH_4-NH_3-H^{35}Cl-^2H^{35}Cl]^+$). It is noteworthy that one of the deuterium atoms attached to the 2-methylpyridine substituent on the I.S. was also lost during CID. Therefore, the product ion used for SRM was only six amu higher than the analyte, although the I.S. contained seven deuterium atoms.

3.4. Liquid chromatography–selected reaction monitoring mass spectrometry

The SRM transitions were m/z 393→m/z 304 for ZD0473 and m/z 400→m/z 310 for the I.S. The seven deuterium atoms present in the internal standard prevented any interference from the analyte in the SRM channels used for the internal standard. As expected, the deuterated I.S. eluted slightly ahead (0.06 min) of the protium form during LC–MS analysis.

Chromatography was performed using a reversed-phase 2-mm internal diameter YMC ODS-AQ column. No interference was observed at the retention time of ZD0473 (Fig. 5A, upper) or its I.S. (Fig. 5A, lower) in drug-free urine. Cross-talk between $[^2H_7]$ZD0473 and the protium form of ZD0473 was less than 0.03% (Fig. 5B, upper). The signal-to-noise (S/N) ratio for the lower limit of quantitation QC (LLQ) was >40 (Fig. 5C, upper). No interfering peaks were observed in LC–SRM-MS chromatograms for the LQC, MQC, or HQC and the intensity of the I.S. channel remained relatively constant. The mean retention time for the analyte was 7.06 min (precision <0.3%) and I.S. was 7.00 min (precision <0.3%) during 3 days of validation.

3.5. Calibration curves

The calibration curves were prepared by plotting the peak area ratio between ZD0473 and $[^2H_7]$ZD0473 against the concentration of ZD0473 in the urine. All calibration curves were fitted ($R^2=$ 0.9992–0.9998 with a quadratic regression) in the range of 0.15 to 50 μg/ml (Fig. 6). The accuracy of the back-calculated values from theoretical values ranged from 97.2 to 104.0%.

3.6. Precision and accuracy

The lower limit of quantitation of the ZD0473 assay was 0.2 μg/ml (990 pg/on column). The accuracy over the three validation days for the LLQ was 108.8% and the precision was 4.4% (Table 1).

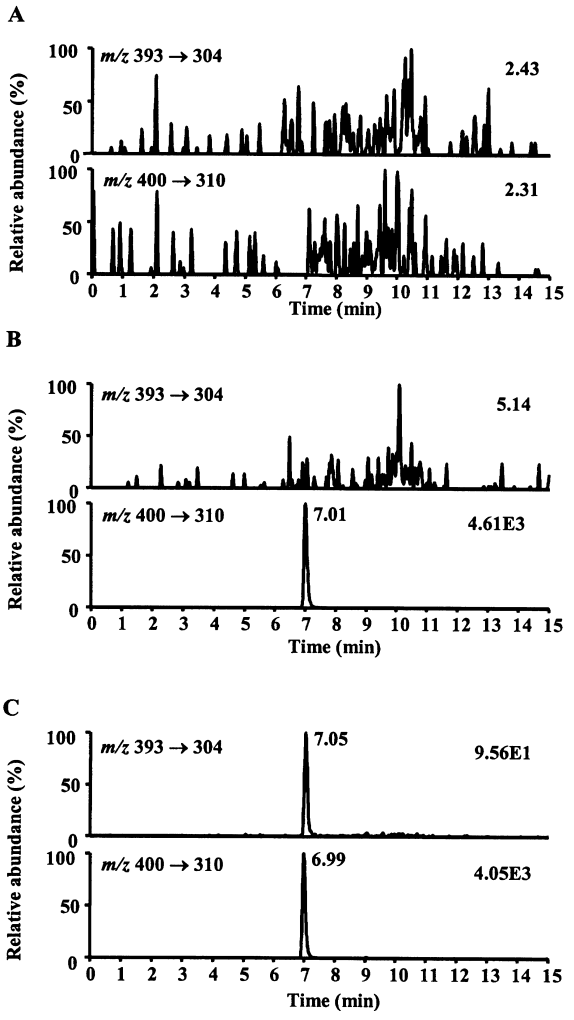

Fig. 5. LC–ESI-SRM-MS analysis of 100 μl of urine: (A) blank urine, (B) control zero (blank urine that was spiked with I.S.), and (C) LLQ samples (0.2 μg/ml). The maximum signal intensity (DAC units) is shown at the top right of each chromatogram.

Accuracy was in the range 93.7 to 104.7% and precision was 5.0% or better for the other QC samples (Table 1). Therefore, all of the QC samples readily met the criteria established by Shah et al. for assay validation [27].

The LLQ, LQC, MQC, HQC, and UQC validation samples from day 1 were re-analyzed after 4 days' storage on the autosampler at 4 °C. The accuracy for the LLQ, LQC, MQC, HQC, and UQC was 117.4, 107.2, 101.5, 99.6, and 94.0%, respectively. The precision for the LLQ, LQC, MQC, HQC, and UQC

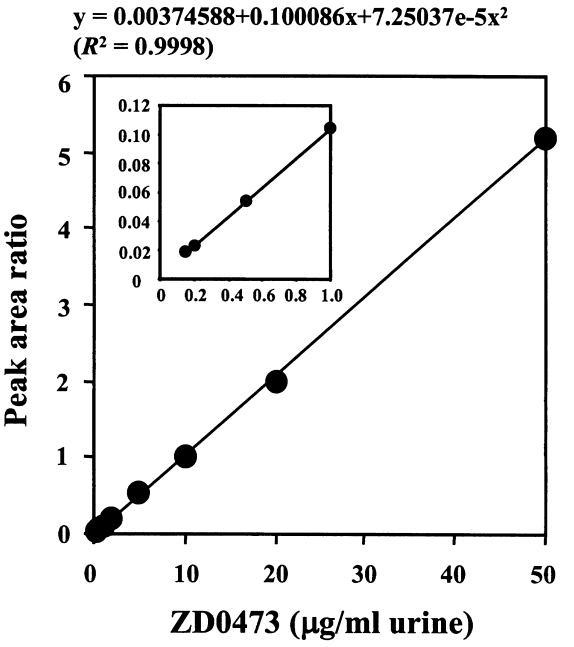

Fig. 6. Typical calibration curve for ZD0473 using [$^2H_7$]ZD0473 as the I.S. (day 1). The equation of the regression line was $y = 0.00374588 + 0.100086x + 7.25037e - 5x^2$ ($R^2 = 0.9998$).

Table 1
Accuracy and precision of assay for QC samples ($n = 5$)

	Day 1	Day 2	Day 3	Overall
LLQ (0.2 μg/ml)				
Mean (μg/ml)	0.216	0.218	0.219	0.218
Accuracy (%)	108.0	109.0	109.4	108.8
Precision (%)	5.1	6.0	2.3	4.4
LQC (0.4 μg/ml)				
Mean (μg/ml)	0.413	0.407	0.418	0.413
Accuracy (%)	103.4	101.8	104.4	103.2
Precision (%)	4.3	4.3	4.4	4.1
MQC (4 μg/ml)				
Mean (μg/ml)	4.083	4.189	4.052	4.108
Accuracy (%)	102.1	104.7	101.3	102.7
Precision (%)	1.6	1.8	1.1	2.2
HQC (40 μg/ml)				
Mean (μg/ml)	39.41	40.66	40.05	40.04
Accuracy (%)	98.5	101.7	100.1	100.1
Precision (%)	1.6	0.8	2.1	2.0
UQC (400 μg/ml)				
Mean (μg/ml)	377.7	374.7	377.9	376.8
Accuracy (%)	94.4	93.7	94.5	94.2
Precision (%)	2.1	2.6	1.7	2.1

was 3.1, 4.8, 1.1, 2.3, and 0.7%, respectively. These data confirmed the samples were stable enough to be stored on the autosampler for up to 4 days. After 6 months storage at −80 °C, the accuracy for the LLQ, LQC, MQC, HQC, and UQC was 113.1, 108.4, 104.2, 107.8, and 99.2%, respectively. The precision for the LLQ, LQC, MQC, HQC, and UQC was 2.3, 2.7, 1.2, 2.3, and 1.7%, respectively. These data confirmed that ZD0473 was stable in urine for at least 6 months, if the samples were stored at −80 °C.

LQC, MQC and HQC samples were analyzed after three freeze–thaw cycles (Table 2). The freeze–thaw cycle consisted of thawing for 1 h at room temperature, followed by freezing for 23 h or longer at −80 °C. The accuracy for the LQC, MQC, and HQC was 103.4, 98.5, and 98.9%, respectively. Similarly, the precision for the LQC, MQC, and HQC was 3.8, 2.4, and 1.3%, respectively. Therefore, samples could be re-analyzed on two further occasions if repeat analyses were required.

3.7. Suppression of ESI signal from urine

The response of the MQC samples (4 μg/ml) was compared with the response from standard samples prepared using 0.15 *M* sodium chloride instead of drug-free urine. The responses of ZD0473 and [$^2H_7$]ZD0473 were reduced by only 19 and 16%, respectively, of the control values. Therefore, endogenous constituents in the urine that could have caused suppression of the ESI signal must have eluted at a different retention time from the analyte on the YMC ODS-AQ column. Chromatograms of pre-dose urine and post-dose urine obtained from a patient dosed with ZD0473 are shown in Fig. 7.

Table 2
Accuracy and precision of LQC, MQC, and HQC samples after three freeze–thaw cycles[a] ($n=5$)

LQC (0.4 $\mu g/ml$)	
Mean (μg/ml)	0.413
Accuracy (%)	103.4
Precision (%)	3.8
MQC (4 $\mu g/ml$)	
Mean (μg/ml)	3.940
Accuracy (%)	98.5
Precision (%)	2.4
HQC (40 $\mu g/ml$)	
Mean (μg/ml)	39.57
Accuracy (%)	98.9
Precision (%)	1.3

[a] The freeze–thaw cycle consisted of thawing for 1 h at room temperature followed by freezing for 23 h or longer at −80 °C.

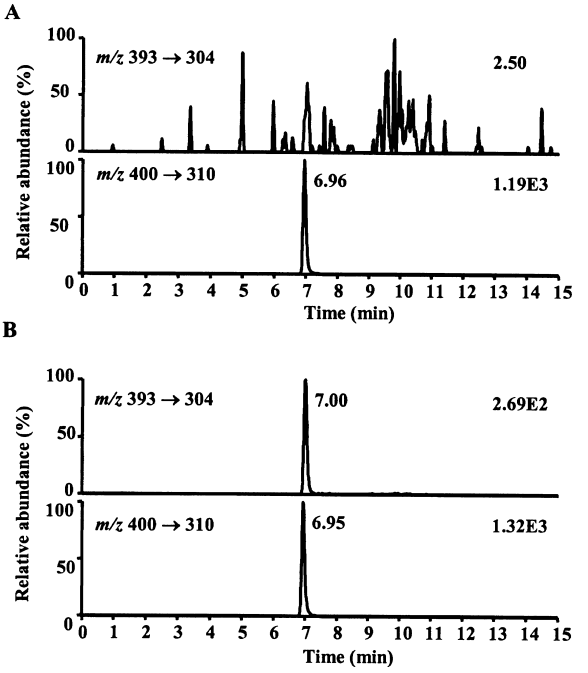

Fig. 7. LC–ESI-SRM-MS analysis of urine from a cancer patient infused with 60 mg/m^2 ZD0473: (A) pre-dose sample urine, (B) post-dose 0–12 h sample (17.6 μg/ml). The maximum signal intensity (DAC units) is shown at the top right of each chromatogram.

4. Summary

Platinum drugs have found wide utility in the treatment of cancer [41,42]. However, it can be difficult to develop rational dosing strategies for platinum drugs because of analytical problems associated with quantitation of the intact drug entities. The development of a validated LC–MS assay for the new platinum drug *cis*-amminedichloro(2-methylpyridine)platinum(II) (ZD0473) in urine will allow accurate and precise quantitation of urinary excretion to be determined. The method is based upon the use of stable isotope dilution LC–ESI-SRM-MS. Urine samples require no cleanup or

derivatization. However, a novel platinum adduct was formed during storage in the urine. The adduct did not correspond to any of the typical sulfhydryl adducts that have been identified previously for platinum drugs. Formation of the urinary adduct was prevented by the addition of excess sodium chloride. Standard curves were fitted to a quadratic regression over the range 0.15 to 50 μg/ml in human urine because of the minor contribution of ^{37}Cl and ^{198}Pt in the internal standard channel at high concentrations of ZD0473.

The LLQ for ZD0473 was 0.20 μg/ml. This simple, rapid, reliable, and sensitive method of quantitation had excellent accuracy and precision for ZD0473. Only 100 μl of sample was required. The 19 min total run time (allowing for column re-equilibration) made it possible to analyze 50 samples with a calibration curve in a single overnight run. The method is sensitive enough to permit the quantitation of the drug in urine after administration of an intravenous dose of 60 mg/m^2 ZD0473 to humans.

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References

[1] W.J.F. van der Vijgh, Clin. Pharmacokinet. 21 (1991) 242.
[2] S.B. Duffull, B.A. Robinson, Clin. Pharmacokinet. 33 (1997) 161.
[3] W. Kern, J. Braess, C.C. Kaufmann, S. Wilde, E. Schleyer, W. Hiddemann, Anticancer Res. 20 (2000) 3679.
[4] F. Levi, G. Metzger, C. Massari, G. Milano, Clin. Pharmacokinet. 38 (2000) 1.
[5] W. Kern, J. Braess, B. Bottger, C.C. Kaufmann, W. Hiddemann, E. Schleyer, Clin. Cancer Res. 5 (1999) 761.
[6] J.P. Allain, O. Heudi, A. Cailleux, A. Le Bouil, F. Larra, M. Boisdron-Celle, E. Gamelin, Drug Metab. Dispos. 28 (2000) 1379.
[7] R.R. Barefoot, J. Chromatogr. B 751 (2001) 205.
[8] J. Holford, F. Raynaud, B.A. Murrer, K. Grimaldi, J.A. Hartley, M. Abrams, L.R. Kelland, Anticancer Drug Des. 13 (1998) 1.
[9] F.I. Raynaud, F.E. Boxall, P.M. Goddard, M. Valenti, M. Jones, B.A. Murrer, M. Abrams, L.R. Kelland, Clin. Cancer Res. 3 (1997) 2063.
[10] G.K. Poon, P. Mistry, F.I. Raynaud, K.R. Harrap, B.A. Murrer, C.F.J. Barnard, J. Pharm. Biomed. Anal. 13 (1995) 1493.
[11] F.I. Raynaud, P. Mistry, A. Donaghue, G.K. Poon, L.R. Kelland, C.F.J. Barnard, B.A. Murrer, K.R. Harrap, Cancer Chemother. Pharmacol. 38 (1996) 155.
[12] V.K. Arora, F.J. Belas, A.K. Chaudhary, R.F. Mayol, R.C. Gaver, P.J. Gale, I.A. Blair, in: Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs, CA, June 1–5, 1997, p. 1076.
[13] V.K. Arora, G. Singh, R.F. Mayol, R.C. Gaver, P.J. Gale, I.A. Blair, in: Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, May 31–June 4, 1998, p. 689.
[14] D.H.J. van den Bongard, R.A.A. Mathot, J.H. Beijnen, J.H.M. Schellens, Clin. Pharmacokinet. 39 (2000) 345.
[15] J.G. Morrison, P. White, S. McDougall, J.W. Firth, S.G. Woolfrey, M.A. Graham, D. Greenslade, J. Pharm. Biomed. Anal. 24 (2000) 1.
[16] T. Oe, Y. Tian, P.J. O'Dwyer, D.W. Roberts, M.D. Malone, C.J. Bailey, I.A. Blair, Anal. Chem. 74 (2002) 591.
[17] T. Oe, S.J. Kambouris, V.E. Walker, Q. Meng, L. Recio, S. Wherli, A.K. Chaudhary, I.A. Blair, Chem. Res. Toxicol. 12 (1999) 247.
[18] G. Singh, V.K. Arora, P.T. Fenn, B. Mets, I.A. Blair, Anal. Chem. 71 (1999) 2021.
[19] K. Xu, V.K. Arora, A.K. Chaudhary, R.B. Cotton, I.A. Blair, Biomed. Chromatogr. 13 (1999) 455.
[20] K. Xu, M. Lanuti, E.S. Lambright, S.D. Force, S.M. Albelda, I.A. Blair, Biomed. Chromatogr. 14 (2000) 93.
[21] S. Pang, N. Zheng, C.A. Felix, J. Scavuzzo, R. Boston, I.A. Blair, J. Mass Spectrom. 36 (2001) 771.
[22] L.R. Kelland, S.Y. Sharp, C.F. O'Neill, F.I. Raynaud, P.J. Beale, I.R. Judson, J. Inorg. Biochem. 77 (1999) 111.
[23] M. El-Khateeb, T.G. Appleton, L.R. Gahan, B.G. Charles, S.J. Berners-Price, A.-M. Bolton, J. Inorg. Biochem. 77 (1999) 13.
[24] J.P. Stevenson, I.A. Blair, M. Redlinger, T. Sun, T. Oe, M. Koehler, D.W. Roberts, M.D. Malone, P.J. O'Dwyer, in: Proceedings of the 37th American Society of Clinical Oncology Annual Meeting, San Francisco, CA, May 12–15, 2001, p. 113a, Vol. 20.
[25] P.A. Zieske, M. Koberda, J.L. Hines, C.C. Knight, R. Sriram, N.V. Raghavan, B.E. Rabinow, Am. J. Hosp. Pharm. 48 (1991) 1500.
[26] M. Macka, J. Borak, L. Semenkova, F. Kiss, J. Pharm. Sci. 83 (1994) 815.
[27] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Pharm. Res. 9 (1992) 588.
[28] A. Andersson, H. Ehrsson, J. Pharm. Biomed. Anal. 13 (1995) 639.
[29] R.F. Greene, D.C. Chatterji, P.K. Hiranaka, J.F. Gallelli, Am. J. Hosp. Pharm. 36 (1979) 38.
[30] M. Jennerwine, P.A. Andrews, Drug Metab. Dispos. 23 (1995) 178.

[31] N. Nagai, R. Okuda, M. Kinoshita, H. Ogata, J. Pharm. Pharmacol. 48 (1996) 918.
[32] M.S. Davies, S.J. Berners-Price, T.W. Hambly, Inorg. Chem. 39 (2000) 5603.
[33] L.R. Kelland, in: B. Lippert (Ed.), Cisplatin, Helvetica Chimica Acta, Zürich, 1999, p. 497.
[34] P. Mistry, C. Lee, D.C.H. McBrien, Cancer Chemother. Pharmacol. 24 (1989) 73.
[35] D. Appenroth, K. Winnefeld, H. Schroter, M. Rost, J. Appl. Toxicol. 13 (1993) 189.
[36] O. Heudi, S. Mercier-Jobard, A. Cailleux, P. Allain, Biopharm. Drug Dispos. 20 (1999) 107.
[37] A. Kung, D.B. Strickmann, M. Galanski, B.K. Keppler, J. Inorg. Biochem. 86 (2001) 691.
[38] M.H. Hanigan, E.D. Lykissa, D.M. Townsend, C.N. Ou, R. Barrios, M.W. Lieberman, Am. J. Pathol. 159 (2001) 1889.
[39] E. Volckova, L.P. Dudones, R.N. Bose, Pharm. Res. 19 (2002) 124.
[40] R.B. Burns, R.W. Burton, S.P. Albon, L. Embree, J. Pharm. Biomed. Anal. 14 (1996) 367.
[41] I. Judson, L.R. Kelland, Drugs 59 (Suppl. 4) (2000) 29.
[42] S.W. Johnson, J.P. Stevenson, P.J. O'Dwyer, in: V.T. DeVita-Jr., S. Hellman, S.A. Rosenberg (Eds.), Principles and Practice of Oncology, 6th ed., Lippincott, Philadelphia, 2001, p. 376.