Application Note

Clinical Research

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Quantitative determination of cisplatin in plasma and urine in clinical research by triple quadrupole LC/MS/MS

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Abstract

Cisplatin is a platinum-containing compound used for the treatment of different types of cancer. The quantitative determination of cisplatin has been carried out using several analytical methods including atomic absorption spectroscopy, high performance liquid chromatography with phosphorescence, ultraviolet detection, or with inductively coupled plasma mass spectrometry. Few liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) analytical methods have been reported for the analysis of cisplatin either for direct quantification or indirect by derivatizing with organic compounds. This Application Note presents an assay developed and verified using a highly sensitive LC/MS/MS analytical method for the quantitative determination of cisplatin following derivatization with diethyldithiocarbamate (DDTC) to detect the platinum (Pt) of cisplatin. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 column (3.0 × 50 mm, 2.7 µm) with a binary gradient mobile phase. Quantification was performed on an Agilent 6400 triple quadrupole LC/MS system with electrospray ionization, and detection was performed using multiple reaction monitoring (MRM). The analytical method has a limit of detection (LOD) of 1 ng/mL, and the quantifiable range was 3 to 3,000 ng/mL in rat plasma and urine. The analytical method was accurate and precise, with an intra-day and inter-day accuracy and precision of ±20 % for lower limit of quantitation (LLOQ), and of ±15 % for low, mid, and high-quality control samples. This analytical method was successfully applied to study the pharmacokinetic profile of cisplatin in rat plasma and urine given a range of doses from 0.5 to 3.5 mg/kg.

Introduction

Cisplatin (*cis*-diamminedicholroplatinum II, Platinol, Figure 1) is a chemotherapeutic agent used in the treatment of ovarian cancer as well as cervical cancer, testicular cancer, and germ cell tumors. Cisplatin is administered intravenously, and is rapidly distributed into the liver, prostate, and kidney. Approximately half of the dose of cisplatin is eliminated through urine. Cumulative renal toxicity of cisplatin is severe, and the drug is contraindicated in patients with pre-existing renal impairment. After initiating therapy, serum creatinine and blood urea nitrogen elevations are important biomarkers of kidney damage, and robust analytical methods compatible with biological matrices are needed. As cisplatin is a metal-based molecule, historical analyses include phosphorescence, high-performance liquid chromatography with ultraviolet visible detection (HPLC-UV), atomic absorption spectrometry, or inductively coupled plasma mass spectrometry (ICP-MS) due to its increased sensitivity. A few LC/MS/MS analytical methods have been developed, but not analytically validated, to analyze cisplatin either directly by measuring its concentration, or indirectly through derivatization of cisplatin with diethyldithiocarbamate (DDTC), which forms a platinum (Pt) complex with DDTC in different matrices, particularly cell suspensions. This Application Note shows an analytically validated method for the quantitative determination of cisplatin through derivatization with DDTC in rat plasma and urine samples.

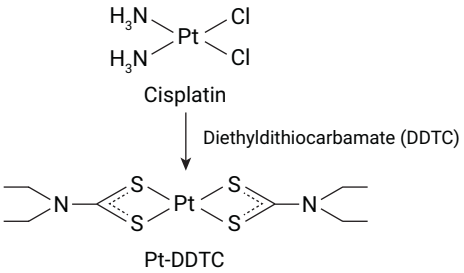

Figure 1. Structure of cisplatin complexed with diethyldithiocarbamate (DDTC) to form a Pt-DDTC complex.

Experimental

LC configuration and parameters

Configuration	
Components	Agilent 1260 Infinity II binary pump (G1312B)
	Agilent 1260 Infinity high performance autosampler (G1367E)
	Agilent 1260 Infinity thermostatted column compartment (G1316A)
Needle wash	MeOH:ACN:H_2O = 1:1:1
Autosampler temperature	4 °C
Injection volume	10 µL
Analytical column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm LC column(p/n 699975)
Column temperature	20-25 °C
Mobile phase A	0.1 % v/v formic acid in water
Mobile phase B	Acetonitrile
Flow rate	0.35 mL/min
Gradient	Time (min) %B 0.0 5 0.5 5 1.0 75 1.5 90 2.5 95 4.0 95 5.0 5
Stop time	7 minutes
Post time	1 minute

Triple quadrupole mass spectrometer configuration and parameters

Configuration	
Instrument	Agilent 6460 triple quadrupole LC/MS system with Jet Stream technology (G6460A)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	300 °C
Drying gas flow	8 L/min
Nebulizer pressure	45 psi
Sheath gas temperature	250 °C
Sheath gas flow	11 L/min
Nozzle voltage	600 V
Capillary voltage, positive	3,500 V
Delta EMV, positive	400 V
Q1/Q2 resolution	0.7/0.7 Unit

MS/MS compound information for analytes and internal standards

Compound	ISTD?	Precursor ion	Product ion 1	Product ion 2	RT (min)	Fragmentor (V)	Collision energy (V)	CAV	Polarity
Platinum-DDTC		492	116	114.1	3.1	200	25	4	+
Palladium-DDTC		403	254	339.1	4.1	140	22	4	+
8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)	✓	305	178	254	3.3	170	33	4	+

Chemicals and reagents

Rat plasma and urine were purchased from Bioreclamation IVT, (Baltimore, MD, USA). Standards and internal standards were bought from Toronto Research Chemicals, Inc., (Ontario, Canada) and Sigma-Aldrich (MO, USA). Sample preparation and LC solvents were from Sigma-Aldrich (MO, USA) and Fisher Scientific (Waltham, MA, USA).

Cisplatin, palladium acetate (assay internal standard (IS)), and DPCPX (analytical internal standard) were separately weighed, dissolved in dimethyl sulfoxide (DMSO), and made up with methanol to prepare primary stock solutions of 1 mg/mL to a final concentration of DMSO ≤10 % v/v. All stock solutions were stored at −20 °C. Working stock solutions were prepared from the primary stock solutions, and the freshly prepared working stock solutions were used for the preparation of quality control (QC) samples and calibration standards (CS). For cisplatin, further dilutions were made with methanol to produce working stock solutions of 0.05, 0.1, 0.2, 0.5,1, 2, 5, 10, 15, 20, 25, and 30 mg/mL on the day of analysis to achieve final concentrations of 5, 10, 20, 50, 100, 200, 500, 750, 1000, 1,500, 2,000, 2,500, and 3,000 ng/mL. These samples were used for the calibration curve. A 1 ng/mL working internal standard solution was prepared in acetonitrile, and a working solution of 5 mg/mL palladium acetate was prepared in acetonitrile to be used as the incubation internal standard.

Sample preparation

CS and QS samples were prepared in rat plasma and urine by spiking 45 µL of plasma or urine with 5 µL of appropriate concentrations of cisplatin, and 5 µL of palladium acetate followed by addition of 15 µL of 1 % v/v DTCC in 0.1 N NaOH solution. This mixture was vortexed, followed by incubation using a water bath set at 40 °C for 30 minutes to form the Pt-DDTC complex. At the end of the incubation, 1.5 mL of acetonitrile containing IS (DPCPX, 1 ng/mL) was added for protein precipitation. The samples were vortexed for two minutes followed by centrifugation at 4,000 rpm in a tabletop centrifuge. The supernatant was separated in to a clean culture tube, and the contents were dried under nitrogen gas at 45 °C until dry. The dried samples were reconstituted with 80:20:0.1 % water:acetonitrile:formic acid, and loaded onto the LC/MS/MS system.

Similarly, samples were prepared for the determination of recovery, accuracy, and precision, at 1, 15, 1,200, and 2,400 ng/mL as low limit of quantitation (LLOQ), low quality control (LQC), mid quality control (MQC), and high quality control (HQC). To calculate the accuracy and precision, six replicates of either the LLOQ or the QCs were injected each time.

Data analysis

System control and data acquisition were performed by Agilent MassHunter acquisition software (B.06.00). MS/MS transitions were obtained using MassHunter optimizer software to determine optimal parent and fragment ions, fragmentor voltages, and collision energies upon injection of a neat solution of each compound or internal standard. Data were analyzed using MassHunter quantitative analysis software (B.06.00) and qualitative analysis software (B.07.00).

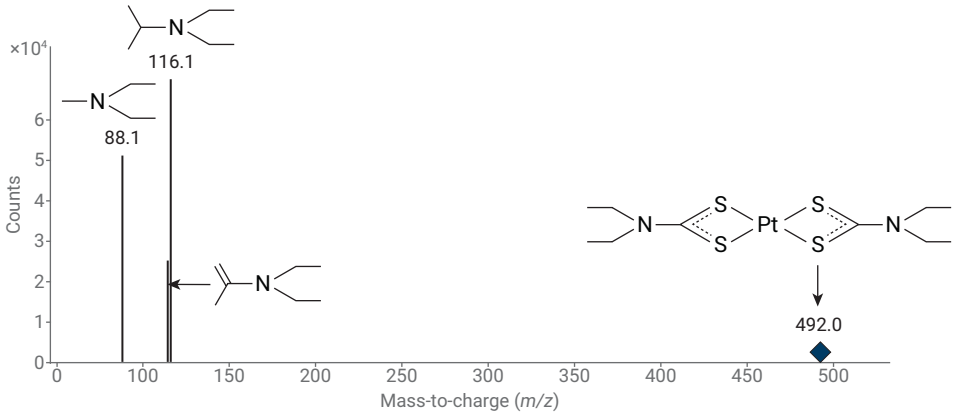

Figure 2. XIC chromatogram of cisplatin, overlaid with possible fragments. Pt-DDTC (492 *m/z*) was fragmented into three different possible product ions: 116.1, 114.1, and 88.1 *m/z*.

Results and Discussion

Chromatography, linearity, and specificity

The calibration concentration for cisplatin ranged from 5 to 3,000 ng/mL. R^2 values were greater than 0.999 using a $1/x^2$ weighting factor.

Chromatograms for blank plasma samples and plasma samples spiked with cisplatin (Figure 4), palladium (Figure 5), and DPXPX (Figure 6) did not show any interfering peaks. The lack of interfering peaks from endogenous plasma components at the retention times of cisplatin, palladium, and DPCPX confirmed the specificity and selectivity of the analytical method.

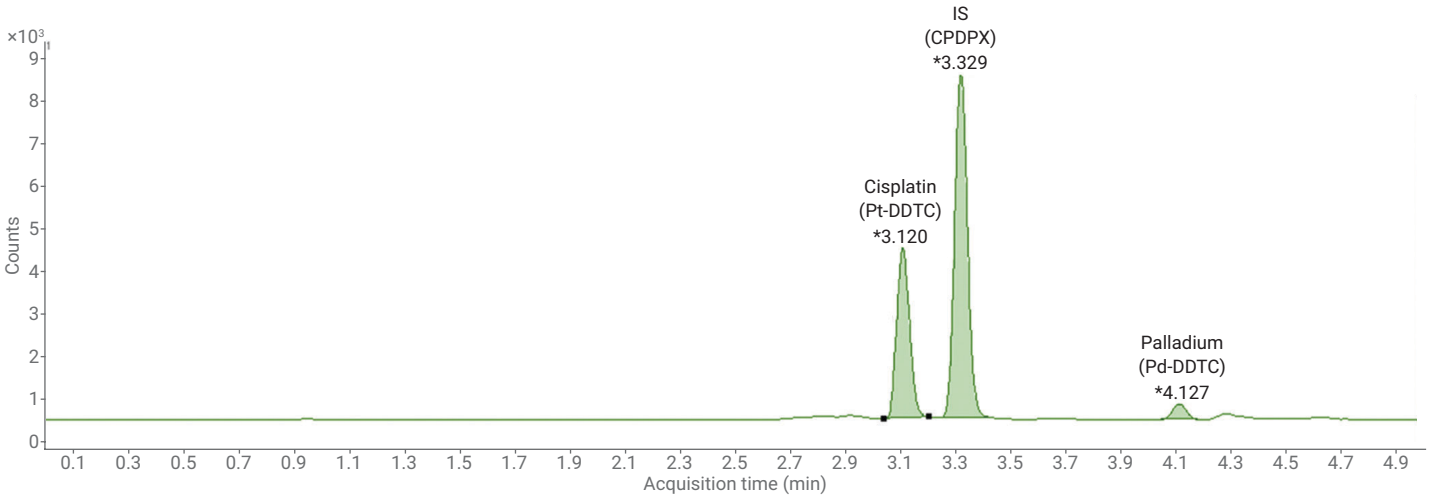

Figure 3. Extracted ion chromatogram of cisplatin (RT = 3.1 minutes), palladium (4.2 minutes), and DPCPX (3.3 minutes).

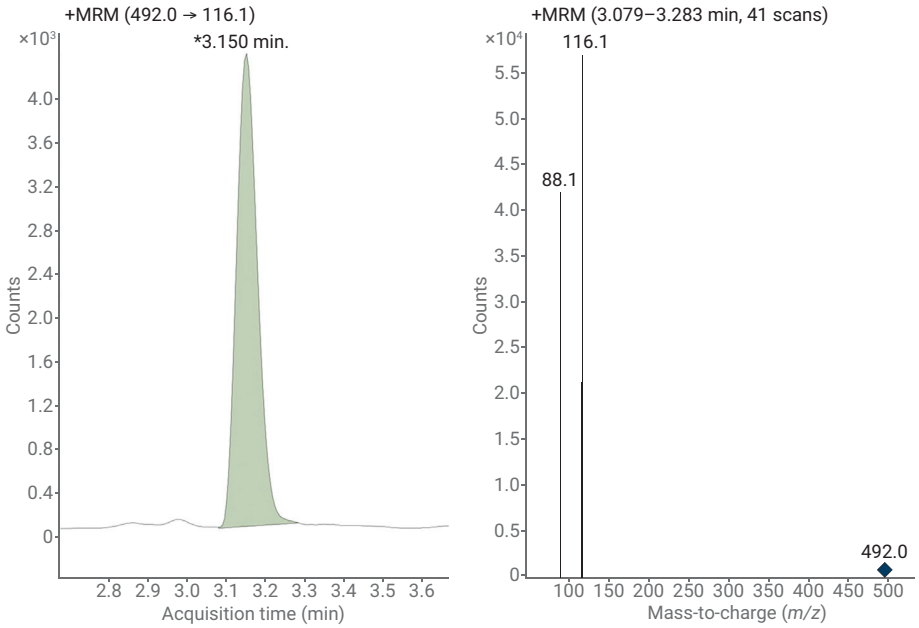

Figure 4. A) XIC chromatogram of Pt-DDTC, with a retention time of 3.1 minutes. B) Pt-DDTC (492 *m/z*) was fragmented into three different possible product ions: 116.1, 114.1, and 88.1 *m/z*.

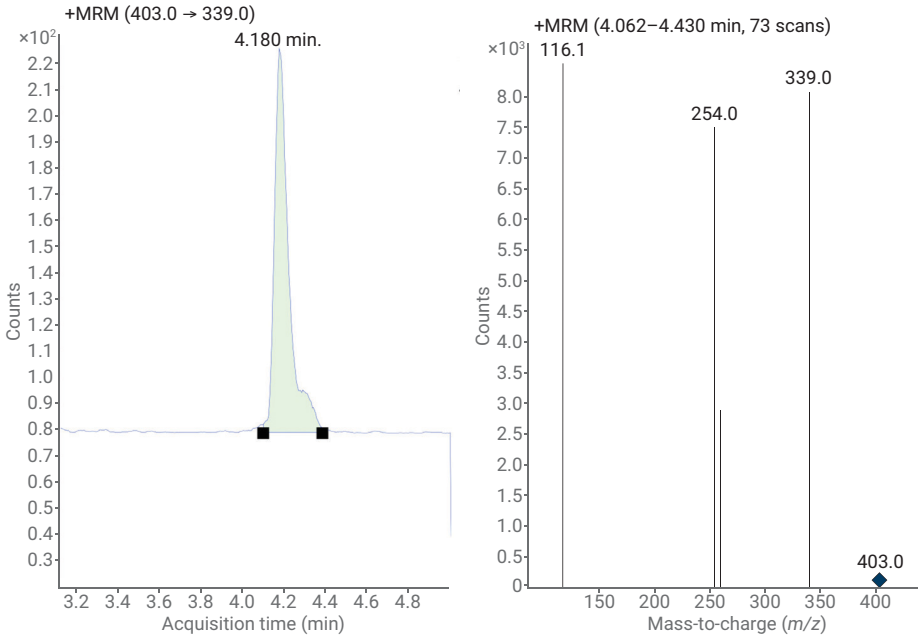

Figure 5. A) XIC chromatogram of Pd-DDTC with a retention time of 4.1 minutes. B) Pd-DDTC (403 *m/z*) was fragmented into three different possible product ions:, 339.0, 254.0, and 116.1 *m/z*.

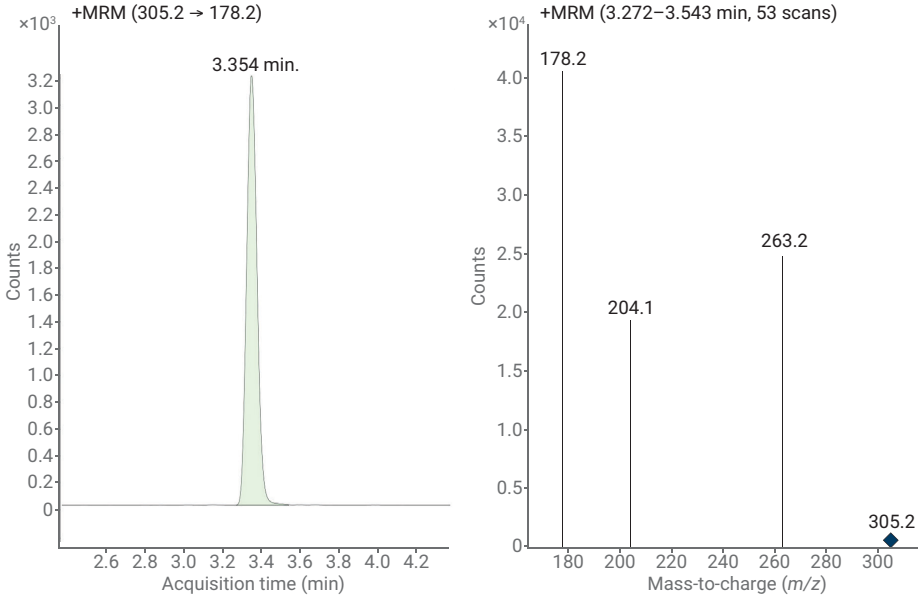

Figure 6. A) XIC chromatogram of IS (DPCPX) with a retention time of 3.3 minutes. B) DPCPX (305.2 *m/z*) was fragmented into three different possible product ions: 263.2, 204.1, and 178.2 *m/z*.

Accuracy and reproducibility

The accuracy, and intra-day and inter-day assay precision, were determined by analyzing six replicates of QC samples at four concentrations of cisplatin in plasma and urine on different days. All QCs were within the limit of ±15 % deviation for accuracy (SD) and precision (RSD), and the LLOQ samples were within ±20 % of SD and RSD[16]. Tables 1 and 2 show the values.

Conclusion

The developed and analytically validated LC/MS/MS method is highly sensitive for the determination of derivatized cisplatin using small amounts of biological sample material. The analytical method uses only 45 µL of plasma with an improved LOD of 1 ng/mL and a final injection volume of 10 µL dissolved in mobile phase. The effective concentration on-column was 2.25 pg. All the QCs were within the limits of ±15 % for accuracy and precision, and the LLOQ was within the range of ±20 % for accuracy and precision. This Application Note shows the development of an LC/MS/MS analytical method in clinical research demonstrating improvement in analytical sensitivity with this technology.

Table 1. Accuracy and precision values of cisplatin in plasma; six replicates were analyzed at each concentration for intra-day and inter-day variability studies.

	Intra-day variability								Inter-day variability			
Concentration (ng/mL)	3		15		1,200		2,400		3	15	1,200	2,400
Run	1	2	1	2	1	2	1	2	1	1	1	1
Mean (ng/mL)	2.6	2.8	14.7	14.9	1,170.6	1,312.4	2,106.5	2,291.8	2.8	15.2	1,243.3	2,324.2
SD	0.3	0.4	2	2.1	47.7	62.9	106.5	85.3	0.5	0.8	103.1	201.9
RSD	11.9	15.2	13.8	14.3	4.1	4.8	5.1	3.7	17.1	5.1	8.3	8.7
Accuracy (%)	87.1	93.9	98.1	99.6	97.6	109.4	87.8	95.5	93.2	101.3	103.6	96.8

Table 2. Accuracy and precision values of cisplatin in urine; six replicates were analyzed at each concentration for intra-day and inter-day variability studies.

	Intra-day variability								Inter-day variability			
Concentration (ng/mL)	3		15		1,200		2,400		3	15	1,200	2,400
Run	1	2	1	2	1	2	1	2	1	1	1	1
Mean (ng/mL)	3.1	2.6	16.1	15.9	1,265.8	1,204.2	2,093.8	2,163.4	2.7	16.0	1,235.0	2,089.1
SD	0.4	0.4	2.6	1.6	52.3	54.1	100.5	94.2	0.4	2.1	60.0	107.7
RSD	12.4	13.6	16.3	10.4	41	4.5	4.8	4.4	14.0	13.0	4.9	5.2
Accuracy (%)	104.9	88.3	107.0	106.0	105.5	100.4	87.2	90.1	91.1	106.5	102.9	87.0

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Printed in the USA, March 28, 2018
5991-9189EN

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