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A Simple, Direct Quantification of Carboplatin in Human Plasma Using Liquid Chromatography-Tandem Mass Spectrometry

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Purpose

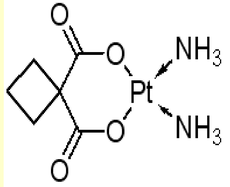
Carboplatin is a platinum complex chemotherapy drug used for the treatment of various forms of cancer, such as lung and ovarian carcinoma, etc. The purpose of this study was to develop and validate a simple LC-MS/MS method for direct quantitation of carboplatin in human to support clinical studies.

Introduction

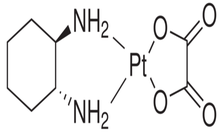
Carboplatin is commonly measured by ICP or ICP-MS after digestion to platinum. This is actually a measurement of total platinum not carboplatin drug form in plasma. Due to its unique chemical structure and its hydrophilicity, it posted a challenge for the development of an LC-MS/MS method for the quantitation of carboplatin in human plasma. The derivatization and hybridSPE-precipitation methods have been reported. However, the reproducibility for the quantitation may be in question through such lengthy sample preparation procedures. In order to support clinical studies, we have developed a simple and specific LC-MS/MS method for the determination of carboplatin in human plasma. The calibration curve ranged from 50 to 10,000 ng/mL. Carboplatin and the added internal standard were extracted from human plasma and injected to an HPLC system and detected in positive ESI-MRM mode on an API-4000 LC-MS/MS system.

The assay has been used for analysis of carboplatin in human plasma to support clinical studies.

Structure



Carboplatin



Oxaliplatin (IS)

Methods

Sample Preparation:

Carboplatin and the added internal standard, oxaliplatin, were extracted from 100 µL of plasma by protein precipitation using acetonitrile. After vortexing and centrifugation, the supernatant was transferred to a 96-well plate, dried under nitrogen and reconstituted for LC-MS/MS analysis. LC separation was performed on a C18 HPLC column (Luna 100 x 2.0 mm, 5 µ, 100 Å). Analytes were detected by multiple reaction monitoring (MRM) in positive ion electrospray mode on an AB Sciex API-4000 LC-MS/MS analysis.

Liquid Chromatography:

Pump:	Shimadzu UFLC LC-20AD
Autosampler:	Shimadzu UFLC SIL-20A
System Controller:	Shimadzu CBM-20A
Analytical Column:	Luna C18, 100 × 2.0 mm, 5 µ
Gradient:	Analytes were eluted using a gradient with formic acid in both MPA (water) and MPB (methanol).
Flow Rate:	0.3 mL/min.
Injection Volume:	2 µL

Mass Spectrometry:

MS System:	AB Sciex API-4000
Scan Mode:	(+)ESI-MRM
MRM transitions:	Carboplatin: 371.9 → 294.2
	Oxaliplatin (IS): 398.0 → 306.3

Results and Discussion

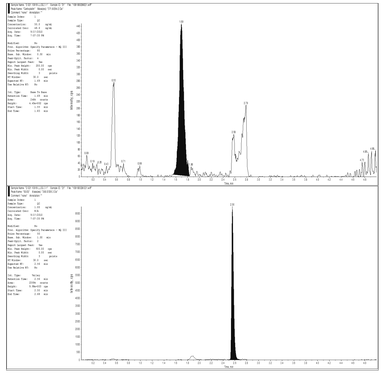


Figure 1. Chromatograms for Carboplatin (LLOQ, 50 ng/mL, Top) and IS (bottom)

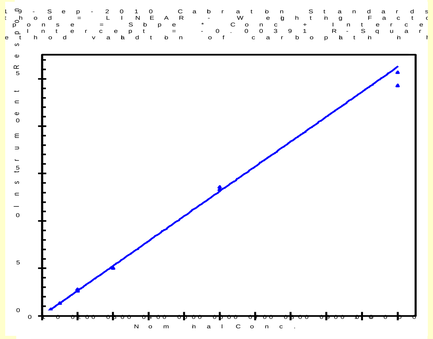


Figure 2. Typical Calibration Curve of Carboplatin in Human Plasma (50- 10,000 ng/mL)

Table 1. Carboplatin Inter-day and Intra-day Precision and Bias

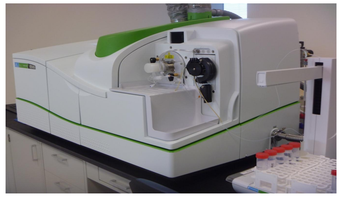
Run Number	Carboplatin Nominal Concentrations (ng/mL) LLOQ (50)	Low (150)	Mid (1000)	High (9000)
	Carboplatin Measured Concentrations (ng/mL)			
1	50.4	155	847	8700
	49.2	144	1000	8830
	44.4	150	1000	8240
	48.9	151	1020	8760
	49.1	148	1030	9010
	49.0	142	1060	8200
Mean	48.5	148	993	8620
S.D.	2.08	4.76	74.8	330
%CV	4.3	3.2	7.5	3.8
%Bias	-3.0	-1.3	-0.7	-4.2
n	6	6	6	6
2	50.7	148	1050	9290
	48.8	146	993	8850
	48.6	146	996	9250
	45.3	145	1010	8220
	49.4	155	1020	8860
	47.6	154	1040	9200
Mean	48.4	149	1020	8950
S.D.	1.83	4.38	23.2	405
%CV	3.8	2.9	2.3	4.5
%Bias	-3.2	-0.7	2.0	-0.6
n	6	6	6	6
3	47.9	151	1080	9120
	51.6	158	1120	9290
	56.1	163	1070	8670
	50.7	160	1040	8530
	51.3	161	1010	8960
	49.8	161	1010	9250
Mean	51.2	159	1060	8970
S.D.	2.73	4.24	43.2	312
%CV	5.3	2.7	4.1	3.5
%Bias	2.4	6.0	6.0	-0.3
n	6	6	6	6
Overall Mean	49.4	152	1020	8850
Overall S.D.	2.51	6.54	55.2	368

Figure 3 Incurred Sample Reanalysis (ISR) of Carboplatin in Human Plasma

- This assay was validated within a nominal range of 50 to 10,000 ng/mL for human plasma with the correlation coefficients (r^2) ≥0.9979. The intra-day precision and accuracy ranged from 2.3% to 7.5%, and -4.2% to 6.0%, respectively. The inter-day precision and accuracy ranged from 4.2% to 5.4% and –1.7% to 2.0%, respectively. No interference peak from matrix was observed at the retention time of carboplatin. The recovery was from 70.5% to 74.1% from the human plasma. The method was successfully apply for the analysis of metformin in clinical studies.
- The method's selectivity was evaluated by analyzing six different lots of blank plasma samples. Internal standard was not added to those blank plasma samples. The mean peak area of the control plasma samples was compared to the mean peak area of the LLOQ QC samples prepared from the same six different lots of plasma as blank samples. No substantial interferences from endogenous plasma components or from IS were observed.
- The stability of carboplatin in human plasma has been evaluated after storage at approximately -70 °C for 46 days. It was found that carboplatin was stable in human plasma stored at ~-70 °C for at least 46 days.
- Hemolyzed plasma test was conducted in the presence of 2% of whole blood. No obvious effect on carboplatin in hemolyzed plasma samples was observed.
- Incurred Sample Reanalysis (ISR) of clinical study samples proved this method is reproducible (Figure 3).

Conclusions

A simple, specific and reproducible LC-MS/MS assay for direct quantification of carboplatin in human plasma has been developed and validated. The assay has been used for the analysis of carboplatin in human plasma in support of clinical studies.



ICP-MS for total Pt