Development of a Novel HILIC HPLC/MS/MS Bioanalytical Method for the Quantitative Analysis of Carboplatin from the Plasma of Mouse

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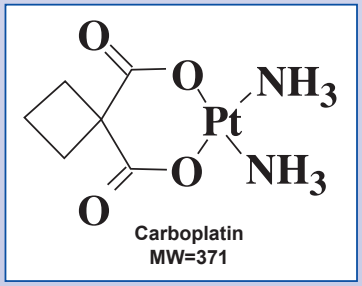
Overview

- **Purpose** - Develop an HPLC/MS/MS method to determine concentrations of Carboplatin, an organo-metallic drug, in plasma, when typical HPLC/MS/MS methods are unsuccessful
- **Methods** – 96 well-plate extraction and HPLC/ESI/MS/MS
- **Results** – Range from 25-10,000 nM with accuracies and precision better than 25% using Hydrophilic Interaction Liquid Chromatography (HILIC)

Introduction

Carboplatin is a drug that is used as a chemotherapy treatment for cancer. Measurement of carboplatin by HPLC/UV methods is insensitive due to the lack of significant UV absorption. Although derivitization of carboplatin has been used, these methods only produced limits of quantitation near 0.13 µM and required run times of >25 minutes per sample. Other HPLC/MS methods required extensive clean-up of the biological samples. In addition, the HPLC/MS methods suffered from assay interferences due to lack of retention of the polar carboplatin on the reversed phase column. Here we report on the development of a precipitation method with a polar HPLC column (HILIC) for the sensitive HPLC/MS/MS quantitative analysis of carboplatin from plasma.



Carboplatin
MW=371

Methods

Extraction

- Simple 3:1 acetonitrile precipitation of Carboplatin from mouse plasma in 96 well plates

HPLC USING HILIC

- Gradient from 18% to 32% aqueous in 4 minutes. Hold for 1.0 minute
- Flow rate = 0.4 mL/minute
- 1% formic acid Acetonitrile and Water
- Polyhydroxy Ethyl A 2.1x100 mm (PolyLC, Columbia,MD)
- Direct 30 µL injection of supernatant

Mass Spectrometry

- Sciex API3000 operating in MRM mode
- Turboionspray (400 °C)
- Positive ion mode
- MRM transitions for Carboplatin–
  - m/z 372 ➔ 294

Other HPLC Results

- Conventional C8 or C18 columns were not successful in retaining Carboplatin
- Polar embedded reversed-phase columns even with ion-pair reagents were not successful in retaining Carboplatin
- Bare silica was not successful in retaining Carboplatin – Thus not an ion-exchange mechanism
- pH of the mobile phase had an insignificant influence on the retention of Carboplatin on Polyhydroxy Ethyl A – Thus not an ion-exchange mechanism
- The percent organic had the most significant affect on the retention of Carboplatin on Polyhydroxy Ethyl A

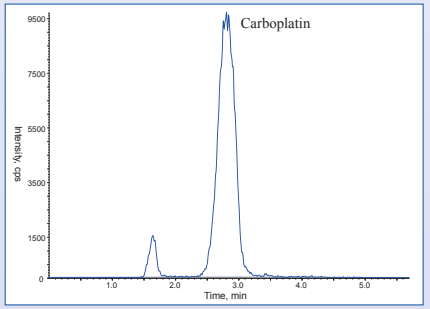
All the above results lead to the conclusion that the dominant chromatographic interaction is HILIC

Table 1. Standard Curve and QC Results for the HPLC/MS/MS Analysis of Carboplatin from Mouse Plasma

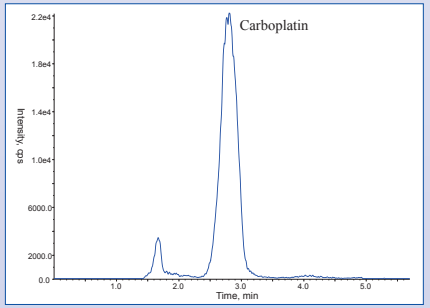
r^2=0.997

Standard Curve Level (nM)	Calculated Concentration (nM)	% Accuracy
25	27.4	110
50	61.8	124
100	112	112
500	493	98.6
1000	943	94.3
2500	2330	93.2
5000	4350	87.0
10000	7850	78.5
QC-2500	1950	78.0
QC-2500	1980	79.2

HPLC/MS/MS Chromatogram from the Analysis of a Standard Fortified with Carboplatin at 5000 ng/mL and Extracted from Mouse Plasma



HPLC/MS/MS Chromatogram from the Analysis for Carboplatin from a Mouse Plasma Sample 75 Minutes Post-Dose



Conclusions

- Developed HPLC/MS/MS method to quantify the organo-metallic drug Carboplatin from mouse plasma
- Found that the HPLC chromatographic interaction was predominantly HILIC
- Method supports PK studies for Carboplatin
- Future work will investigate other types of HILIC stationary phases to obtain more narrow peaks, and thus improve detection limits
- Investigate use of labeled internal standard to improve accuracy