



Novel Assay with Phospholipids Column Trapping and Switching Valve System for the Determination Of Carboplatin in Human Plasma by LC/MS/MS

Authors

Yifei Liu, Roger Demers, Daria Wentzel, Erika Hess, Laura Cojocar

Novel Aspect

Uses a pre-extraction column and a switching valve system to trap the phospholipids.

Introduction

The purpose was to develop a selective and reproducible LC/MS/MS method for the analysis of Carboplatin, a platinum based compound and chemotherapeutic agent for treating many types of cancer. Due to the extreme polarity of Carboplatin and its lack of retention on conventional reverse phase columns, a polar LC column (Alltima™ HP HILIC, 2.1x150 mm, 3 μ , Alltech®) was used to retain the compound. However, it requires more than 10 minutes to wash out the phospholipids so that the next injection is not affected.

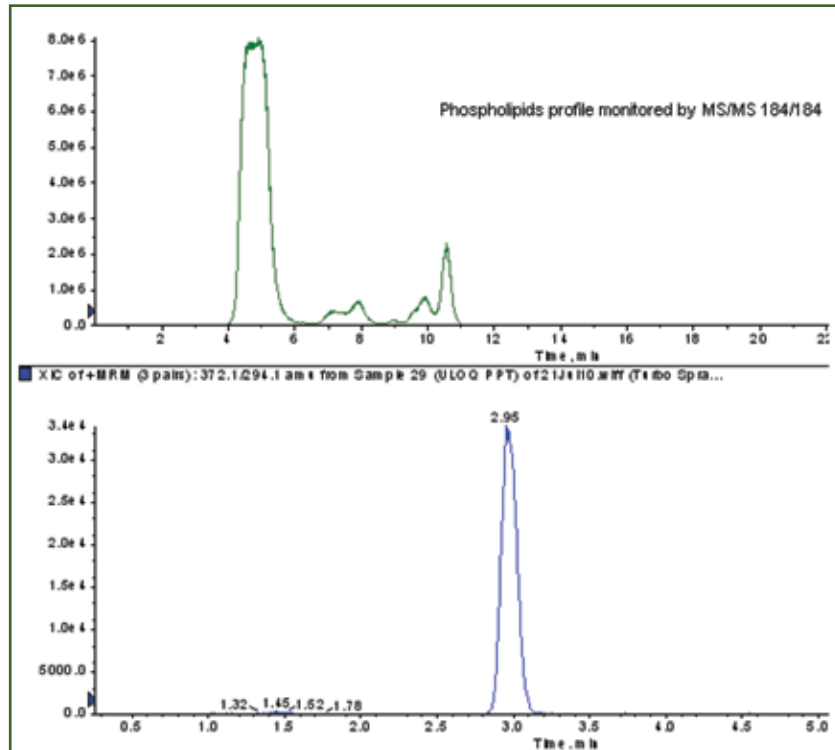
After tuning and optimizing the mobile phase, the phospholipids profile could be separated from the analyte peak as indicated in figure 1. However, it requires more than 10 minutes to wash out the phospholipids so that the next injection is not affected.

To develop a rapid and robust bio-analytical LC/MS/MS method using the HILIC column, it is necessary to shorten the re-equilibration time after analyte peak elution. Therefore, a pre-extraction column (Luna C18(2), 30x2 mm, 5 μ , Phenomenex®) was added to trap the phospholipids and a switching valve system was used to inject phospholipids-free sample into the analytical column. It is easy to implement the plot on cohesive HTLC with valve control and multiple pumps, but it is not required.



Introduction (continued)

Figure 1. Extracted protein precipitate plasma sample (200 ng/mL carboplatin) injected directly into a HILIC column with a mobile phase consisting of acetonitrile: 10 mM ammonium acetate (85:15, v:v) and 0.3 mL/min. flow rate



Methods

Human plasma, 50 μ L, fortified with Carboplatin, was mixed with 50 μ L of stable label internal standard solution, and then precipitated with acetonitrile. The supernatant was injected directly into the LC/MS/MS system. A dual switching valve system was used: first, a pre-extraction Luna C18(2) column retained the phospholipids and other endogenous interferences, while the compound was eluted into a second column (figure 3). An HP Alltima™ HILIC column was used to retain the compound and achieve the chromatographic separation (figure 4 and 5). The mass spectrometer was an AB Sciex API-4000™, operated in the positive ionization mode, using Turbo ion spray as the ion source. The MRM transitions 372/294 and 377/299 were monitored for Carboplatin and internal standard, respective.



Methods (continued)

FIGURE 2. The diagram of switch valve system using a cohesive HTLC.

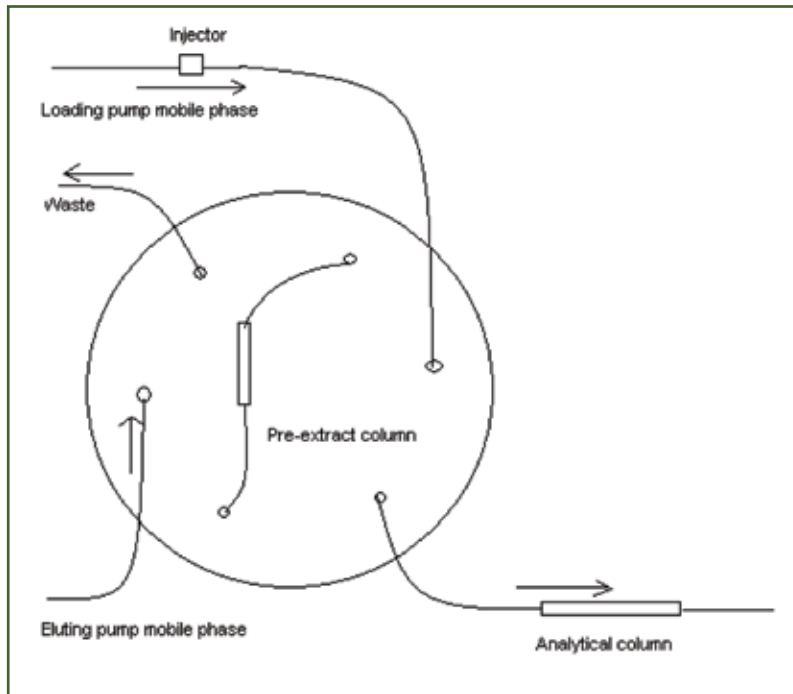
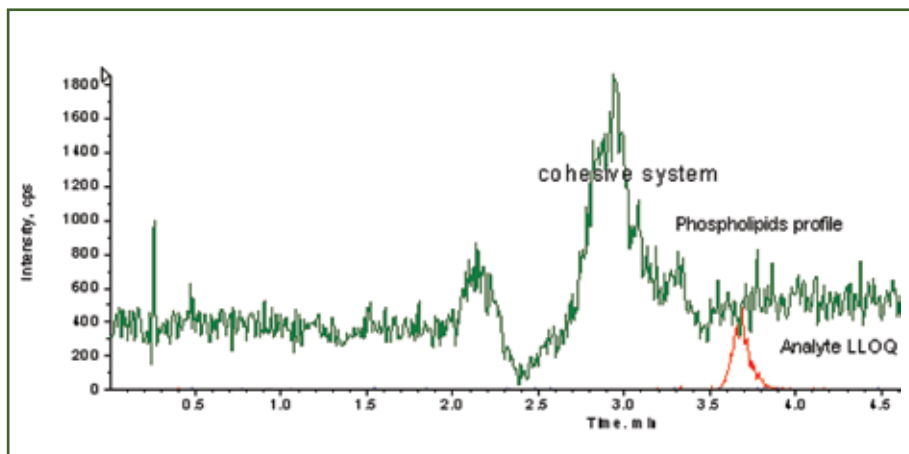


FIGURE 3. Phospholipids profile after 96 sample injections with a pre-extraction column. The phospholipids do not build up on analytical column.





Methods (continued)

FIGURE 4. Optimized LC gradient on cohesive HTLC.

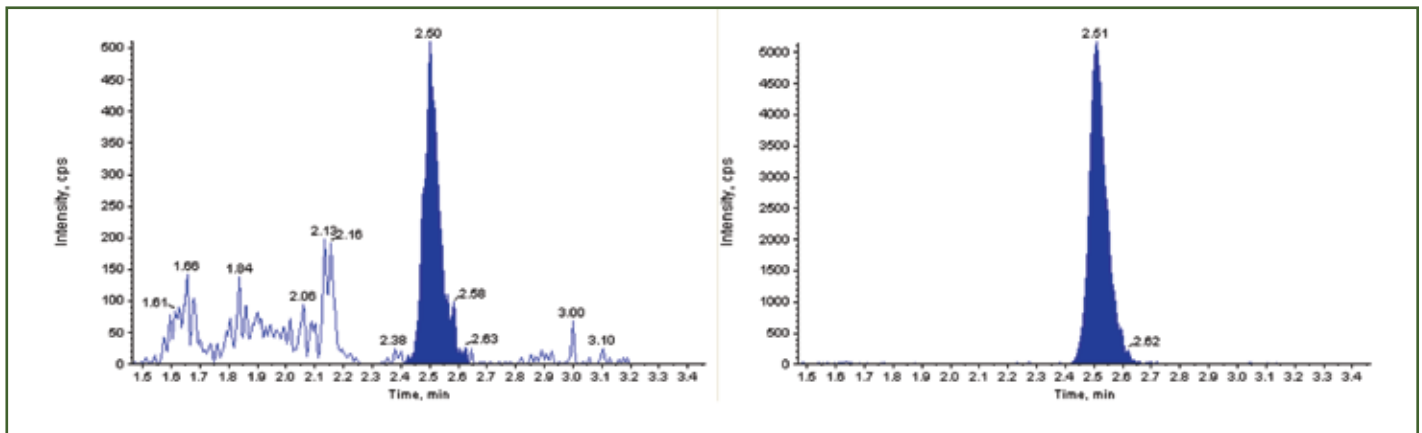
Loading/eluting pump mobile Phase A: 10 mM ammonium acetate (a.q.)

Loading pump mobile Phase B: 0.1% formic acid in acetonitrile

Eluting pump mobile phase B: Methanol: acetonitrile (50:50, v:v)

Step	Start	Sec	Flow	Grad	%A	%B	%C	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	0.30	Step	20.0	80.0	-	====	out	0.20	Step	-	100.0
2	0.50	210	0.30	Step	20.0	80.0	-	====	in	1.00	Step	-	100.0
3	4.00	60	0.30	Step	20.0	80.0	-	====	out	0.20	Step	-	100.0

Figure 5. Extracted LLOQ (10.0 ng/mL) with internal standard using optimized method.



Results

An LLOQ of 10.0 ng/mL was achieved and the method reproducibility was excellent. The assay linearity was proved over a 10.0 - 5000 ng/mL calibration range with accuracy and precision within 10% at low, mid and high level. The compound stability in the matrix was evaluated after 4 FT cycles and 24h in the refrigerator at 1-8 °C. Selectivity, recovery and matrix effect were evaluated (for details see table 1).



Results (continued)

TABLE 1: Assay summary	
Method	LC/MS/MS
Matrix	Human Plasma
Anti-coagulant	K2EDTA
Internal Standard:	Carboplatin-D4
Aliquot Volume	50.0 µL
LLOQ / ULQ	10.0 ng/mL / 5000 ng/mL
LLOQ QC	
Inter-Assay Accuracy (%Bias):	4.0 %
Inter-Assay Precision (%CV):	9.8 %
Low, Medium Low, Medium, and High QC	
Inter-Assay Accuracy (%Bias):	-0.00 to 4.0 %
Inter-Assay Precision (%CV):	1.6 to 7.3 %
Ability to Dilute:	25000 ng/mL (DF = 10)
Analyte Stability in Solution:	24.5 Hours at room temperature
Freeze-Thaw Matrix Stability:	4 Cycles from -70 °C nominal to 1 – 8 °C nominal
Bench-Top Matrix Stability:	24 Hours at 1 – 8 °C nominal
Long-Term Matrix Stability:	85 Days at -70 °
Reinjection Reproducibility:	80 Hours 15 minutes at room temperature
Processed Extract Stability	148 Hours 15 minutes at room temperature

Conclusions

A sensitive and robust LC/MS/MS assay was developed and validated for the determination of Carboplatin in human plasma. The use of an HILIC column and switching valve system was essential in improving the assay selectivity and reproducibility. The method is simple, fast and has excellent reproducibility.