Determination of Cisplatin in Human Plasma by LC-MS/MS using Hemoglobin to Overcome Matrix Effects

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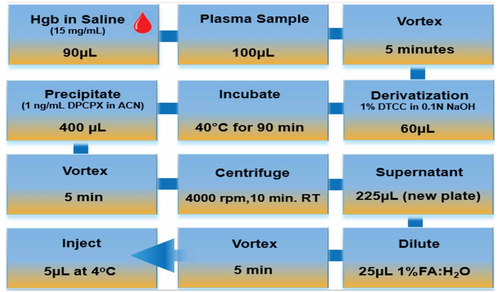
PURPOSE

Matrix effects occurring from endogenous components in plasma are very common and can have significant impacts on the accuracy and precision of a LC/MS/MS bioanalytical assay. Typically, these effects can be overcome by making simple adjustments to the analytical method, diluting the biological sample, or upgrading a simple technique, such as protein precipitation extraction (PPE) to solid-phase extraction (SPE) or liquid–liquid extraction (LLE). In some cases, practical method revisions to physically separate or minimize the interferent are not enough and the true interaction between the drug of interest and the interfering endogenous component can be overlooked.

METHOD

Presented here is a novel approach to overcoming matrix effects on the quantitation of cisplatin resulting from hemolysis within the plasma sample by utilizing a strategic sample treatment with hemoglobin (Hgb) itself prior to extraction. The magnitude of this effect was determined through data comparison from multiple unique lots of human plasma with varying degrees of visually gradable and non-gradable hemolysis. After observing a promising correction to the plasma assay when treating all samples with human whole blood, a reagent solution approach was devised to provide a dependable and controllable treatment. Lyophilized human hemoglobin, sourced through a vendor, was prepared and optimized in saline to mimic the expected level found in human whole blood. In this method, human plasma samples (100 µL) were pre-treated with 15 mg/mL of hemoglobin in saline to counterbalance the interaction between cisplatin and free hemoglobin within plasma. All samples were then derivatized with 1% Sodium diethyldithiocarbamate trihydrate (DDTC) prior to release of cisplatin by PPE with 100% acetonitrile (ACN) and 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) as internal standard (IS). The supernatant was finally acidified with a 1% formic acid (FA) solution before being chromatographed using reversed phase HPLC with an Agilent InfinityLab Poroshell 120 EC-C18 analytical column and 0.1% FA: Water / 0.1% FA: ACN as mobile phases.

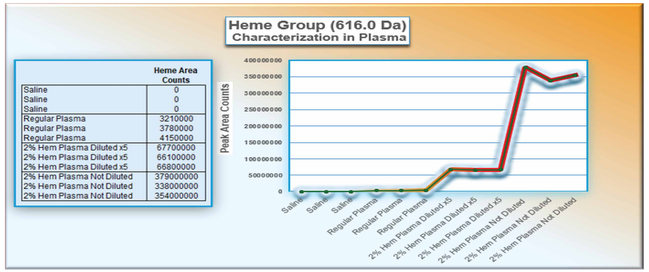
Final Extraction Procedure:



Effect of hemolysis issue suspected and further tested by serially diluting with regular plasma.

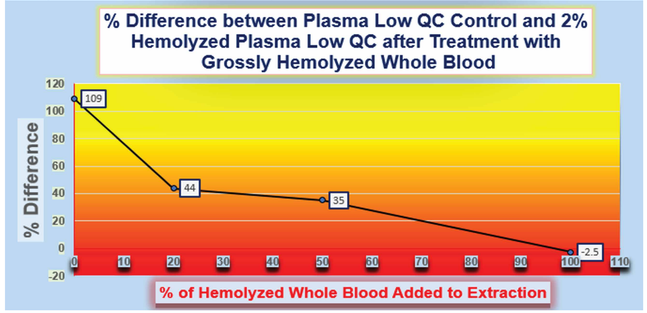
	2% Hemolyzed Plasma QC (6.00 ng/mL) diluted with Regular Plasma						
	Control	200x	100x	50x	20x	10x	5x
Mean (ng/mL)	6.57	7.38	8.47	8.30	8.25	8.78	10.2
%Bias	9.6	22.9	41.1	38.3	37.5	46.4	69.9

Heme group response monitored in regular versus hemolyzed plasma based on previously documented increase of heme observed as cisplatin concentration increases in association with the formation of a cisplatin-Hgb complex 1 .



Percentage of hemoglobin treatment required to overcome various levels of cisplatin-Hgb complexes in regular and hemolyzed plasma was optimized.

Optimization - Using % Additions of Hemolyzed Whole Blood to Treat Plasma Samples (Area Ratio)								
	0%		20%		50%		100%	
	Control	2% Hem	Control	2% Hem	Control	2% Hem	Control	2% Hem
	0.001510	0.002800	0.002950	0.005410	0.006000	0.006480	0.008880	0.011000
	0.001210	0.002150	0.002990	0.004520	0.005740	0.008740	0.009230	0.008400
	0.000932	0.002110	0.003270	0.003660	0.005570	0.006320	0.008170	0.009040
	0.000971	0.002080	0.003000	0.003970	0.005350	0.010000	0.008830	0.007960
	0.000882	0.002480	0.002670	0.004260	0.005280	0.007990	0.009370	0.008450
	0.000989	0.001940	0.003050	0.003990	0.006040	0.006380	0.010600	0.008860
Mean	0.001082	0.002260	0.002988	0.004302	0.005663	0.007652	0.009180	0.008952
% Diff		108.8		43.9		35.1		-2.5



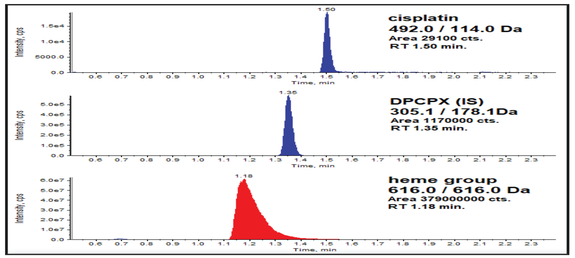
A hemoglobin solution (15 mg/mL) extraction treatment was finally selected and it corrected the recovery of cisplatin in the presence of various degrees of hemolysis.

Extraction Treatment Comparison (Mean % Bias)		
Sample Type	No Hgb Treatment	15 mg/mL Hgb Treatment
Control Plasma	-2.0	9.0
0.5 % Hem Plasma	48.0	2.3
1.0 % Hem Plasma	58.0	7.7
2.0 % Hem Plasma	133.7	11.0

RESULTS

Detection was conducted in positive ion mode using a Sciex 6500 mass spectrometer monitoring molecular (parent) ion (Q1)/molecular (product) ion (Q3) of m/z 492.0/114.0 and 305.1/178.1 for cisplatin and DPCPX (IS) respectively. To characterize and track the relationship between cisplatin and hemoglobin content in plasma, the heme group was simultaneously measured during development by monitoring a pseudo molecular (parent) ion (Q1)/molecular (parent) ion (Q1) of m/z 616.0 /616.0. The assay was linear over the concentration range 2.00 – 2000 ng/mL using a 1/x2 weighting factor. Intra-day precision (as percent coefficient of variation, %CV) was ≤8.0% and accuracy (as %bias) ranged from -10.4% to -3.0%. Inter-day precision (as percent coefficient of variation, %CV) was ≤6.3% and accuracy (as %bias) ranged from -7.8% to -5.0%.

Cisplatin, DPCPX (IS) and heme group chromatograms and LC/MS/MS parameters:



Analyte	Precursor (m/z)	Product (m/z)	Dwell Time (msec)	Declustering Potential (V)	Collision Energy (V)
cisplatin	492.0	114.0	100	200	48
DPCPX (IS)	305.1	178.1	20	120	47
heme group	616.0	616.0	15	200	5

Mass Spectrometer:	Sciex 6500
Source Type:	Electrospray
Scan Type:	MRM
Polarity:	Positive
Resolution Q1:	Unit
Resolution Q3:	Unit

Gradient Table

Time (minutes)	MPA %	MPB %
0.50	50	50
2.25	0	100
3.25	0	100
3.30	50	50
4.00	50	50

Column:	Agilent InfinityLab Poroshell 120 EC-C18, 2.7 µm 3.0 x 50 mm
Mobile Phase A (MPA):	0.1% Formic Acid in Water
Mobile Phase B (MPB):	0.1% Formic Acid in ACN
Flow Rate:	0.600 mL/min

Validation accuracy and precision results for cisplatin in human plasma.

Accuracy and Precision - Plasma QC Samples				
Curve	2.00 ng/mL	6.00 ng/mL	800 ng/mL	1600 ng/mL
1	1.81	5.60	748	1452
	1.71	5.48	781	1599
	1.94	5.38	731	1490
	1.93	5.96	719	1404
	1.69	5.37	728	1477
	1.95	5.30	740	1455
Intrarun Mean	1.84	5.52	741	1479
Intrarun SD	0.120	0.240	22.1	65.3
Intrarun %CV	6.5	4.3	3.0	4.4
Intrarun %Bias	-8.0	-8.0	-7.3	-7.5
n	6	6	6	6
2	1.94	5.81	777	1399
	1.86	5.48	732	1428
	1.85	5.27	719	1525
	1.87	5.57	800	1735
	2.05	5.55	739	1554
	2.07	5.67	765	1464
Intrarun Mean	1.94	5.56	755	1518
Intrarun SD	0.100	0.180	30.6	121
Intrarun %CV	5.2	3.2	4.1	8.0
Intrarun %Bias	-3.0	-7.3	-5.6	-5.1
n	6	6	6	6
3	2.11	5.41	740	1435
	2.01	5.59	713	1394
	1.96	5.67	702	1461
	1.86	5.67	711	1463
	1.83	5.33	703	1463
	1.77	5.55	732	1423
Intrarun Mean	1.92	5.54	717	1440
Intrarun SD	0.130	0.140	15.4	28.1
Intrarun %CV	6.8	2.5	2.1	2.0
Intrarun %Bias	-4.0	-7.7	-10.4	-10.0
n	6	6	6	6
Inter-run Mean	1.90	5.54	738	1479
Inter-run SD	0.120	0.180	27.5	83.0
Inter-run %CV	6.3	3.2	3.7	5.6
Inter-run %Bias	-5.0	-7.7	-7.8	-7.6
n	18	18	18	18

CONCLUSION

This high throughput assay was successfully validated and selectivity, matrix effect, hemolytic/lipemic interference, stability and dilution integrity all met current FDA and EMA requirements. The method will be applied for the determination of cisplatin in human clinical samples for patients undergoing solid tumor treatment.

REFERENCE

[1] Rupasri Mandal, Robyn Kalke and Xing-Fang Li. Mass spectrometric studies of cisplatin-induced changes of hemoglobin. Rapid Commun. Mass Spectrom. 2003; 17: 2748–2754 [accessed 2023 Nov 09]. Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1259