LC/MS/MS Analysis of Coproporphyrin I and Coproporphyrin III in Human Plasma

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The authors declare no financial competing interest.

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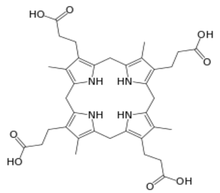
Introduction

Coproporphyrin I (CP-I) and Coproporphyrin III (CP-III) are potential endogenous biomarkers for hepatic organic anion transporting polypeptide (OATP)1B1/1B3 function. We developed and validated a bioanalytical assay for monitoring these biomarkers to assess OATP1B1/1B3 inhibition in place of a standalone prospective clinical drug-drug interaction (DDI) study. Currently, investigational drugs that alter the pharmacokinetics of other medications are subject to additional testing to understand how to manage a DDI risk safely. By monitoring the effect of an investigation drug on the levels of these endogenous substrates of OATP1B1/1B3 in early clinical development, the potential need for a dedicated clinical DDI study could be avoided.

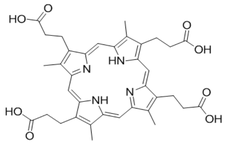
Method

Coproporphyrin I and Coproporphyrin III are extracted from human K2EDTA plasma using a supported liquid extraction (SLE) methodology. After recovering the analytes with ethyl acetate, the samples were evaporated, and reconstitution was done with formic acid and EDTA solution. The resulting extracts were analyzed by LC-MS/MS detection. The analytes were separated from potential interferences using a Water Acquity Premier BEH C18 (2.1 x 100mm) column and gradient conditions. A Sciex 6500+ mass spectrometer operating in positive electrospray (ESI) mode was used to detect CP-I, CP-III, and the labeled internal standards. Because CP-I and CP-III are endogenous compounds, calibrators and quality control samples were prepared in charcoal-stripped human K2EDTA plasma. The analytical range of the assay was 50.0 to 5000 pg/mL for both biomarkers. A 200-microliter aliquot of sample volume was required to achieve appropriate sensitivity.

Compounds



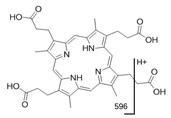
Coproporphyrin I



Coproporphyrin III

Method Development

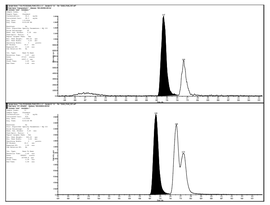
CP-I and CP-III were detected utilizing electrospray ionization. For quantitation, a precursor of 655.3 m/z and product ion of 596.3 m/z were monitored. Since CP-I and CP-III are isomers, the same MRM transition was collected for both analytes.



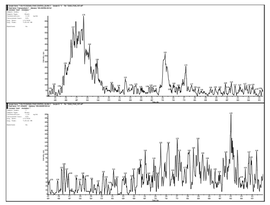
Isotopically labeled forms (15N4) of CP-I and CP-III were used as internal standards. For CP-III there was an additional chromatographic peak observed in the MRM transition. This second peak was likely due to a labeled (15N4) CP-IV impurity that was produced during synthesis. Due to the close retention time of the interference to the CP-III IS, both peaks were integrated for data processing.

For preparation of analyte free calibrators, charcoal-stripped human plasma was utilized. To prepare the surrogate matrix, ~ 1.5 g of activated charcoal was mixed with 30 mL of human K2EDTA plasma and incubated at 56 °C for one hour and then filtered and screened to ensure CP-I/CP-III background leaves were no greater than 20% of the LLOQ peak area.

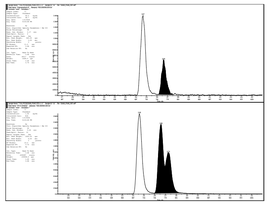
Chromatography



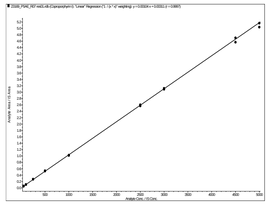
CP-I LLOQ (50 pg/mL)



CP-I Double Blank (0 pg/mL)



CP-III LLOQ (50 pg/mL)



CP-I Calibration curve
(50 to 5000 pg/mL)

Validation Design

The validation principles were modeled after the current FDA M10 bioanalytical validation guidance. To predict meaningful inhibition of OATB1B1/1B3 function, the method validation was designed to assess the following method attributes:

- Accuracy and Precision in both surrogate and authentic matrix
  - Surrogate matrix QC levels included an LLOQ, Low, Medium, and High
  - Authentic QC levels included an unfortified QC, Low and High fortification levels
- Parallelism
  - Comparison of analyte response in authentic matrix vs. surrogate at 2X, 5X, and 20X dilution
- Stability assessments
  - Long-term, short-term, and freeze-thaw matrix stability in both surrogate and authentic matrix
- Specificity
  - In authentic matrix for IS evaluation
  - Low endogenous QC for concomitant interference test
- Selectivity
  - Surrogate matrix for interference between analytes and internal standards.
- Matrix Effect and Recovery
  - Performed in authentic and surrogate matrix

Results of the Validation

	Parallelism CP-I			Parallelism CP-III		
Dilution	20X	5X	2X	20X	5X	2X
Mean (n=6)	3890	3990	4080	3680	3700	3790
Stdev.	46.5	41.9	50.0	86.5	70.6	71.3
%CV	1.2	1.0	1.2	2.4	1.9	1.9

CP-I Surrogate QC Inter-day accuracy and Precision				
Run	LLOQ (50.0 pg/mL)	LOW (150 pg/mL)	MEDIUM (2000 pg/mL)	HIGH (3750 pg/mL)
1	48.7	150	1990	3610
2	50.1	148	1970	3600
3	51.3	152	2040	3730
Mean (n=8)	50.0	150	2000	3650
Stdev.	1.85	4.14	50.3	87.9
%CV	3.7	2.8	2.5	2.4

Results of the Validation (continued)

CP-III Surrogate QC Inter-day accuracy and Precision				
Run	LLOQ (50.0 pg/mL	LOW (150 pg/mL)	MEDIUM (2000 pg/mL)	HIGH (3750 pg/mL)
1	55.5	154	1930	3620
2	50.4	140	1930	3490
3	51.1	153	2070	3850
Mean (n-18)	52.3	149	1980	3650
Stdev.	4.70	8.93	91.2	218
%CV	9.0	6.0	4.6	6.0

CP-I Authentic QC Inter-day Accuracy and Precision			
Run	EQC (207 pg/mL)	LEQC (717 pg/mL)	HEQC (3690 pg/mL)
1	202	732	3750
2	216	710	3610
3	202	709	3700
Mean	207	717	3690
Stdev.	7.40	17.8	88.5
%CV	3.63	2.5	2.4

CP-III Authentic QC Inter-day Accuracy and Precision			
Run	EQC (0.0 pg/mL)	LEQC (586 pg/mL)	HEQC (4040 pg/mL)
1	BLOQ	547	3930
2	BLOQ	579	3890
3	BLOQ	631	4310
Mean	NA	586	4040
Stdev.	NA	38.3	218
%CV	NA	6.5	5.4

Conclusion

A method of analysis for coproporphyrin I and coproporphyrin III in human plasma was developed and validated to support regulated clinical studies. Understanding the dose-dependent changes in these endogenous substrates of OATP1B1/1B3 makes it possible to identify investigation drugs with a potential DDI risk before conducting clinical DDI studies in patients.