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Simultaneous Determination of Coproporphyrin-I and Coproporphyrin-III in Human Plasms and Human Urine Using LC-MS/MS

Wei Zhang, Yong Yan, Jim Xiao, XiaoNan Tang, Luca C. Matassa, and ZhongPing (John) Lin.
Frontage Laboratories, Inc., 700 Pennsylvania Dr, Exton, PA 19341

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PURPOSE

Porphyrins and their metabolites, such as, Coproporphyrin-I(CP-I) and Coproporphyrin-III(CP-III), are involved in diverse cellular processes and proposed to be endogenous biomarkers for predicting OATP-mediated DDIS. Qualitative and quantitative analyses of these Porphyrins in human body fluids and tissues have become critically important for our understanding of human physiology and potential biomarkers for hepatic OATP1 B functional activity. Few studies on the determination of porphyrins by LC/MS/MS were reported due to some difficult technical issues.

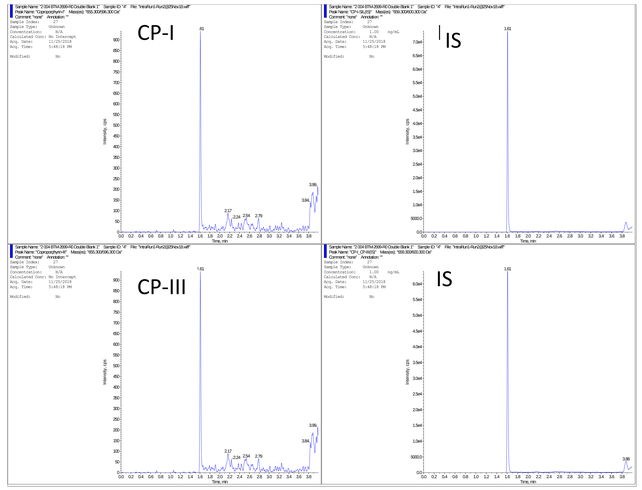
OBJECTIVE(S)

We developed and validated an LC-MS/MS method for simultaneous determination of CP-I and CP-III in human plasma with a lowest LLOQ limit (10 pictogram per milliliter for both CP-I and CP-III

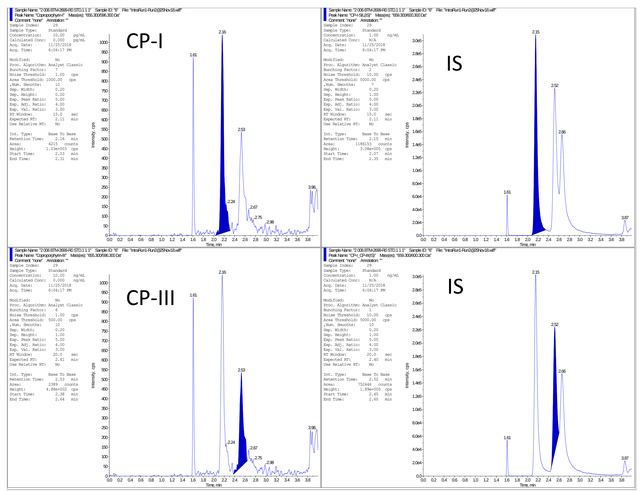
METHOD

CP-I, CP-III, and the internal standards were extracted by solid phase extraction from human plasma. Reversed-phase HPLC separation was achieved with a Waters, Acquity, UPLU BEH C18, 1.7µm, 2.1 x 100 mm column. MS/MS detection was set at mass transitions of *m/z* 655.3→596.3 for CP-I and CP-III and *m/z* 659.3→600.3 for CP-I-$^{15}N_4$ and CP-III-$^{15}N_4$ in MRM positive mode.

TYPICAL CHROMATOGRAM OF BLANK MATRIX



TYPICAL CHROMATOGRAM OF LOWEST STANDARD



VALIDATION SUMMARY

Analytes		CP-I and CP-III			
Internal standards		CP-I-$^{15}N_4$ and CP-III-$^{15}N_4$			
Linearity		0.9914 for CP-I 0.9905 for CP-III			
Lower limit of quantitation (LLOQ)		10.0 pg/mL for both analytes			
Average recovery of the Analyte (%)		47.8 for CP-I 41.0 for CP-III			
		LLOQ	Low	Mid	High
CP-I QC Intra-run precision range (%CV)	Run 1	9.5	6.4	1.8	1.6
	Run 2	14.9	3.3	2.1	1.8
	Run 3	7.1	6.1	2.2	2.5
CP-I QC Intra-run accuracy range (%Bias)	Run 1	6.9	10.0	10.0	9.2
	Run 2	7.0	2.7	2.7	-10.5
	Run 3	7.0	12.0	8.0	6.1
CP-I QC Inter-run precision range (%CV)		10.4	6.4	3.5	8.9
CP-I QC Inter-run accuracy range (%Bias)		6.0	8.3	6.7	1.6
		LLOQ	Low	Mid	High
CP-III QC Intra-run precision range (%CV)	Run 1	16.1	12.2	2.6	2.6
	Run 2	16.5	8.2	1.0	1.6
	Run 3	4.8	13.7	2.4	2.9
CP-III QC Intra-run accuracy range (%Bias)	Run 1	9.0	6.7	8.7	4.2
	Run 2	11.0	-3.0	2.7	-12.4
	Run 3	6.0	5.7	12.7	6.1
CP-III QC Inter-run precision range (%CV)		13.1	11.9	4.2	8.9
CP-III QC Inter-run accuracy range (%Bias)		8.0	3.0	8.0	-0.8

CONCLUSION(S)

The validation study successfully evaluated intra-run and inter-run accuracy and precision, selectivity, sensitivity, hemolysis, linearity, recovery, dilution integrity, processed sample stability, QC bench-top stability, matrix effect, batch-size, inter-conversion, analyte interference to IS, re-injection stability, QC freeze/thaw stability, carryover, and whole blood stability. Based on a 200 µL sample volume of human plasma, the LLOQ is 10.0 pg/mL. The dynamic range of the method is 10.0 – 5000 pg/mL. This method was determined to be suitable for the determination of CP-I and CP-III in K_2EDTA human plasma samples

NOVEL ASPECT

Lowest LLOQ limit(10 pg/mL for both CP-I and CP-III) was achieved by Optimizing the sample extraction condition and LC/MS/MS conditions. The method was used for the sample assay in determination of CP-I and CP-III in human plasms. This method was also used for urine sample analysis and the results successfully support the clinic researches.

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