Alturas Analytics, Inc.
The LC-MS Experts

Development of validated LC-MS methods using surrogate matrices for the quantification of biomarkers of clinically significant drug-drug interactions

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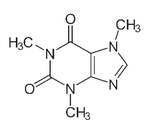
INTRODUCTION

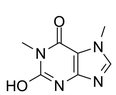
When developing a quantitative LC-MS/MS method, ideally the standard curve and quality controls should be prepared in a matrix as similar as possible to the study samples to limit the potential for dissimilar matrix components to interfere with accurate quantification. However, if blank matrix is unobtainable, or if the analyte of interest is endogenous, then use of a surrogate matrix may be necessary. The plasma ratio of caffeine/paraxanthine is used as a reliable indicator of the drug metabolizing enzyme CYP1A2. Similarly, coproporphyrin I/III are used as biomarkers of potential drug-drug interactions mediated by hepatic transport. The widespread use of caffeine makes obtaining drug-free plasma impractical, and coproporphyrin I/III are endogenous compounds. Therefore, we developed validated bioanalytical methods using surrogate matrices.

RESULTS

We confirmed the suitability of the surrogate matrix by assessing precision and accuracy of each analyte spiked into human plasma and quantified against calibration curves prepared in the surrogate matrix. In order to minimize matrix effects, an ethyl acetate extraction was chosen over a more simple protein precipitation method. This cleaner extraction method provided better parallelism between the surrogate matrix and the plasma samples. The assays were validated with concentration ranges appropriate for expected analyte concentrations based upon historical data from previous clinical trials. The accuracy and precision of each method met FDA guidance. The back-calculated accuracy of the calibrators and QCs was within 15% of the nominal concentration (20% at the LLOQ level), and the %CV was within 15% (20% at the LLOQ level).



Structure 1: Caffeine



Structure 2: Paraxanthine

METHODS

Caffeine and Paraxanthine

- Surrogate matrix-5x phosphate buffered saline (PBS) with 0.9% bovine serum albumin (BSA)

Extraction

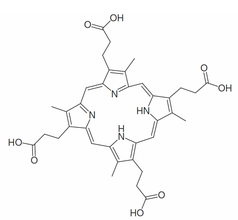
- Samples extracted via liquid-liquid extraction with ethyl acetate
- Upper phase dried under nitrogen at 40°C and reconstituted with 80:20 water:methanol with 0.1% formic acid
- Dynamic range 20.0-10,000 ng/mL

HPLC Parameters

- Mobile phase A: water with 0.1 % FA
- Mobile phase B: Acetonitrile with 0.1% FA
- Column: Discovery HS-C18, 2.1x50mm, 3µm
- Flow rate 0.7 mL/min
- Runtime: 4.5 min

Mass Spectrometry

- Sciex API-5500
- ESI-Positive mode
- 195.0 → 138.6 (caffeine)
- 181.1 → 124.2 (paraxanthine)
- 198.1 → 85.1 (caffeine-$^{13}C_3$)
- 188.1 → 129.1 (paraxanthine$^{13}C_4$,$^{15}N_3$)



Structure 3: Coproporphyrin I

Coproporphyrin I and Coproporphryin III

- Surrogate matrix-5x phosphate buffered saline (PBS) with 0.9% bovine serum albumin (BSA)

Extraction

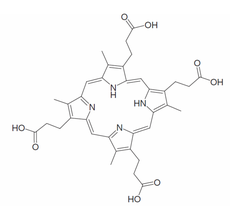
- Samples extracted via liquid-liquid extraction with ethyl acetate
- Upper phase dried under nitrogen at 40°C and reconstituted with 1:1 acetonitrile:water
- Dynamic range 0.02-10.0 ng/mL

HPLC Parameters

- Mobile phase A: 3:1 (v:v) 20 mM ammonium formate: 1 mM methylphosphonic acid
- Mobile phase B:Acetonitrile with 0.1% formic acid
- HPLC column: Luna® Omega C18, 2.1x50 mm
- Flow rate 0.5 mL/min
- Runtime: 6 min

Mass Spectrometry

- Sciex API-6500
- Copro-I Transition, m/z 655.3 → 596.3
- Copro-III Transition, m/z 655.4 → 596.3
- $^{15}N_4$-Copro I Transition, m/z 659.4 → 541.4
- $^{15}N_4$-Copro III Transition, m/z 659.3 → 541.4
- ESI-Positive Mode



Structure 4: Coproporphyrin III

Analyte	Caffeine		Paraxanthene		Coproporphyrin I		Coproporphyrin III	
Matrix	Human Plasma							
Surrogate Matrix	5X PBS with 0.9% BSA							
Extraction Method	Liquid-liquid extraction with ethyl acetate							
Calibration Model	Linear (1/X2)							
	Quantification in plasma using calibration curve prepared in surrogate matrix							
Accuracy in Plasma (%Bias)	MQC (700 ng/mL) -4.7 %	HQC (8000 ng/mL) +8.0%	MQC (700 ng/mL) +0.9%	HQC (8000 ng/mL) -4.9%	MQC* (0.75 ng/mL) -2.1%	HQC* (8.0 ng/mL) -0.4%	MQC (0.75 ng/mL) -12.7	HQC (8.0 ng/mL) -9.1%
Precision in Plasma (%CV)	MQC 3.5%	HQC 3.6%	MQC 4.7%	HQC 2.5%	MQC 1.4%	HQC 1.5%	MQC 2.2%	HQC 3.7%

*Concentrations corrected for presence of endogenous Coproporhyrin I in plasma.

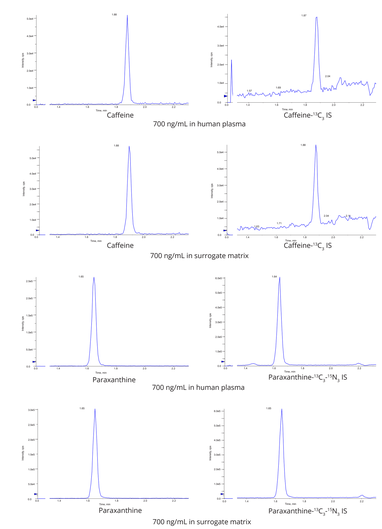


Figure 1: Chromatography of caffeine, paraxanthine, and internal standards (IS) extracted from human plasma or surrogate matrix.

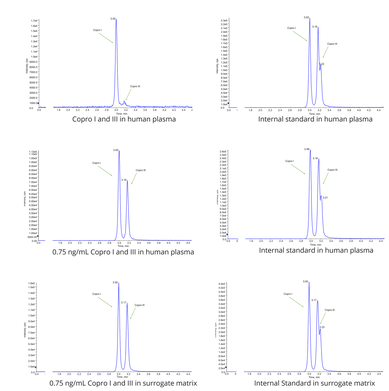


Figure 2: Chromatography of coproporphyrin I, coproporphyrin III and internal standards (IS) extracted from human plasma or surrogate matrix.

CONCLUSION

- Simple surrogate matrices containing PBS and BSA are suitable for differing compounds.
- When suitable, liquid-liquid extractions can help eliminate matrix effects.