Development and Validation of a UHPLC/MS/MS Method for a Panel of Drugs and Metabolites to Support Drug-Drug Interaction Studies

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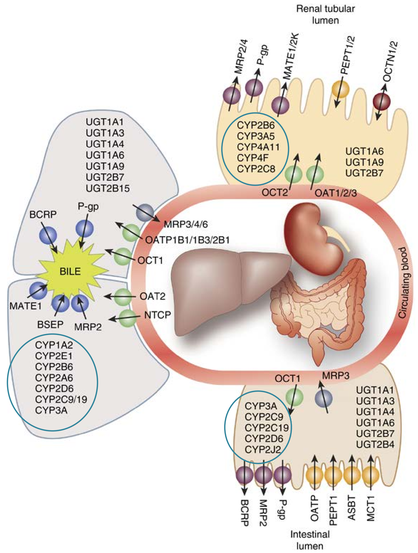
Introduction

The impacts of drug-drug interactions on the pharmacokinetics and pharmacodynamics of drugs in development must be evaluated relative to guidances from all major regulatory agencies. In a world of aging populations and increasing polypharmacy, this data is critical to avoid unintended adverse reactions and even fatalities. To support and simplify the clinical aspects of these studies, combination cocktails, such as the "Inje" or "Cooperstown 5+1" cocktails have been developed. This poster outlines a method for simplifying the DDI bioanalytical analysis utilizing a validated 6-in-1 UHPLC/MS/MS DDI marker/metabolite multiplex method for omeprazole/5-hydroxy omeprazole, tolbutamide/4-hydroxytolbutamide, and dextromethorphan/dextrorphan. Separate methods already existed at AIT Bioscience for the other 2 components of the Coopertown W/T cocktail, caffeine/paraxanthine and midazolam/1'-hydroxymidazolam.

The October 2017 FDA Draft Guidance Clinical Drug Interaction Studies: Study Design, Data Analysis, and Clinical Implications prescribes the following as sensitive index substrates of CYP enzymes, and these are the focus of the method developed.



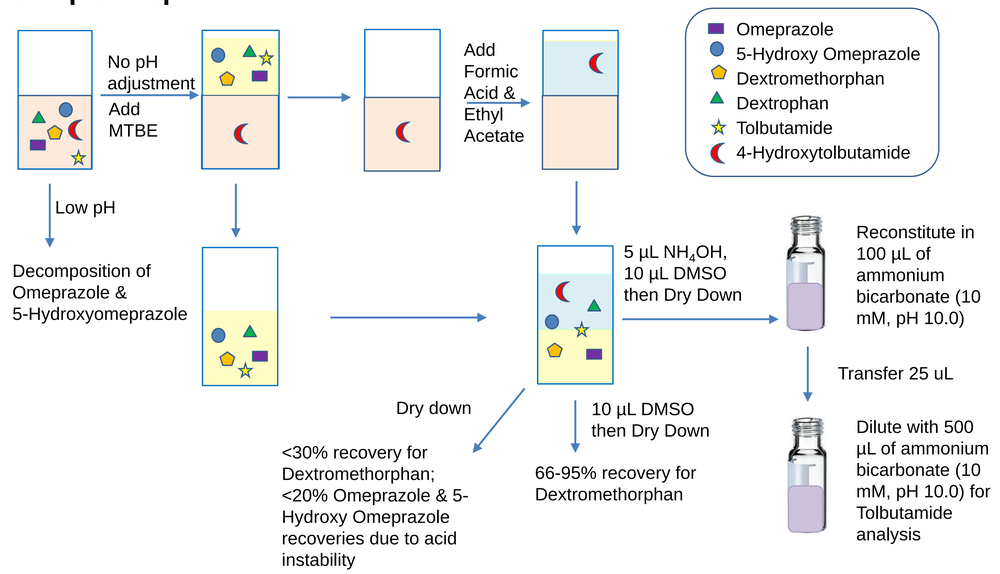
CYP1A2: caffeine, tizanidine
CYP2C8: repaglinide (also a substrate for OATP1B1)
CYP2C9: warfarin, tolbutamide (both are moderate sensitive substrates)
CYP2C19: S-mephenytoin, omeprazole
CYP2D6: atomoxetine, desipramine, dextromethorphan
CYP3A: midazolam, triazolam

Method

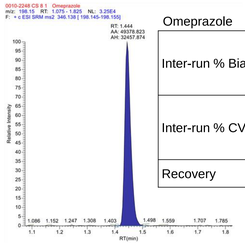
Method development focused on the challenge of combining six analytes with varying chemical properties into a single method. The concentration ranges needed for the analytes also varied significantly, with LLOQs ranging from 0.1 ng/mL to 200 ng/mL. Omeprazole and metabolite 5-Hydroxy omeprazole were found to be very unstable at acidic pH, requiring a pH of 8-9 or higher throughout sample processing to prevent analyte loss. Ultimately, a two-step LLE scheme was devised. MTBE without pH adjustment is used to extract the majority of the five analytes other than 4-Hydroxy tolbutamide. Residual MTBE is evaporated prior to adding dilute formic acid and ethyl acetate to the plasma samples to extract 4-Hydroxy tolbutamide. Additional challenges including range linearity for tolbutamide, dextromethorphan carry-over and low recovery, as well as stability of omeprazole and its metabolite were all addressed.

Chromatography was performed on a Waters BEH C18 column in 2 injections, one for all analytes except tolbutamide and a second with 20X dilution for tolbutamide. Injection 1 was quantitated by ESI+ MS/MS and injection 2 by ESI- MS/MS.

Sample Preparation

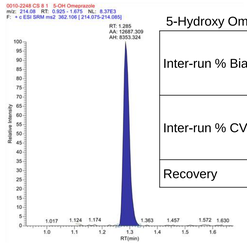


Results



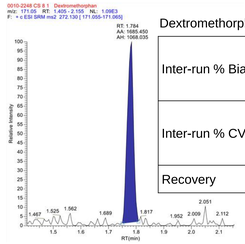
Omeprazole

Inter-run % Bias	LLOQ VS (2.00 ng/mL): -1% Low VS (6.00 ng/mL): 0.2% Middle VS (75.0 ng/mL): 1.6% High VS (750 ng/mL): 1.7%
Inter-run % CV	LLOQ VS (2.00 ng/mL): 6.2% Low VS (6.00 ng/mL): 3.3% Middle VS (75.0 ng/mL): 3.6% High VS (750 ng/mL): 2.8%
Recovery	92.4-96.4%



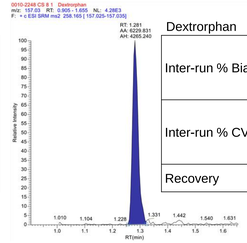
5-Hydroxy Omeprazole

Inter-run % Bias	LLOQ VS (1.00 ng/mL): -2.2% Low VS (3.00 ng/mL): -2% Middle VS (37.5 ng/mL): -2.9% High VS (375 ng/mL): -5.3%
Inter-run % CV	LLOQ VS (1.00 ng/mL): 7.2% Low VS (3.00 ng/mL): 3% Middle VS (37.5 ng/mL): 3.5% High VS (375 ng/mL): 3.5%
Recovery	84.6-88.6%



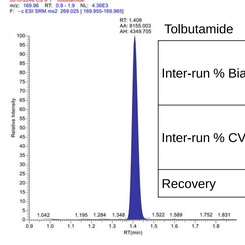
Dextromethorphan

Inter-run % Bias	LLOQ VS (0.100 ng/mL): 1% Low VS (0.300 ng/mL): -3% Middle VS (3.75 ng/mL): 0.5% High VS (37.5 ng/mL): 0.8%
Inter-run % CV	LLOQ VS (0.100 ng/mL): 11.3% Low VS (0.300 ng/mL): 8.5% Middle VS (3.75 ng/mL): 4.5% High VS (37.5 ng/mL): 3.8%
Recovery	66.2-95.1%



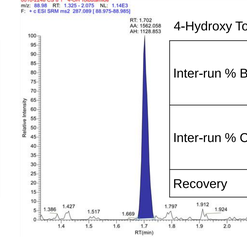
Dextrorphan

Inter-run % Bias	LLOQ VS (0.100 ng/mL): 3% Low VS (0.300 ng/mL): -1.7% Middle VS (3.75 ng/mL): -1.9% High VS (37.5 ng/mL): -0.3%
Inter-run % CV	LLOQ VS (0.100 ng/mL): 13.1% Low VS (0.300 ng/mL): 5.3% Middle VS (3.75 ng/mL): 2.8% High VS (37.5 ng/mL): 2.9%
Recovery	61.4-65.7%



Tolbutamide

Inter-run % Bias	LLOQ VS (200 ng/mL): -1.5% Low VS (600 ng/mL): -2.0% Middle VS (7500 ng/mL): -0.7% High VS (75,000 ng/mL): -0.4%
Inter-run % CV	LLOQ VS (200 ng/mL): 2.9% Low VS (600 ng/mL): 2.4% Middle VS (7500 ng/mL): 1.9% High VS (75,000 ng/mL): 2.4%
Recovery	65.4-68.7%



4-Hydroxy Tolbutamide

Inter-run % Bias	LLOQ VS (2.00 ng/mL): 6.5% Low VS (6.00 ng/mL): 1.3% Middle VS (75.0 ng/mL): 2.1% High VS (750 ng/mL): 2.3%
Inter-run % CV	LLOQ VS (2.00 ng/mL): 11.3% Low VS (6.00 ng/mL): 9.3% Middle VS (75.0 ng/mL): 7.1% High VS (750 ng/mL): 7.2%
Recovery	75.6-83.8%

Instrumental Analysis

UPLC: Waters Acquity
Column: Waters BEH C18 (2.1 x 50 mm; 1.7 µm)
Mobile Phase A: 100 Water : 0.1 Formic Acid
Mobile Phase B; 100 Acetonitrile: 0.1% Formic Acid
Column Temp: 30°C
Injection Volume: 20uL

5 analytes

Time (min)	Flow Rate (mL/min)	%MP A	%MP B
0.00	0.400	90.0	10.0
0.20	0.400	90.0	10.0
2.00	0.400	50.0	50.0
2.10	0.400	10.0	90.0
2.70	0.400	10.0	90.0
2.80	0.400	90.0	10.0
3.00	0.400	90.0	10.0

Thermo Vantage HESI+

Compound	Expected Retention Time (min)	Precursor Exact Mass/Charge (m/z)	Product Observed Mass/Charge (m/z)	Charge State of Precursor Ion
4-OH Tolbutamide	1.72	287.107	88.98	+1
4-OH Tolbutamide-D9	1.70	296.163	88.99	+1
Omeprazole	1.46	346.122	198.15	+1
Omeprazole-D3	1.46	349.141	198.12	+1
5-OH Omeprazole	1.30	362.117	214.08	+1
5-OH Omeprazole-D3	1.30	365.136	214.06	+1
Dextromethorphan	1.80	272.201	171.06	+1
Dextromethorphan-D3	1.80	275.220	171.04	+1
Dextrorphan	1.30	258.186	157.03	+1
Dextrorphan-D3	1.30	261.205	157.02	+1

Tolbutamide

Time (min)	Flow Rate (mL/min)	%MP A	%MP B
0.00	0.400	70.0	30.0
0.20	0.400	70.0	30.0
2.00	0.400	20.0	80.0
2.10	0.400	70.0	30.0
2.50	0.400	70.0	30.0

Thermo Vantage HESI-

Compound	Expected Retention Time (min)	Precursor Exact Mass/Charge (m/z)	Product Observed Mass/Charge (m/z)	Charge State of Precursor Ion
Tolbutamide	1.4	269.096	170.08	-1
Tolbutamide-D9	1.4	278.150	170.08	-1

Conclusion

Analyte	Range (ng/mL)	Internal Standard
Tolbutamide	200-100,000	Tolbutamide-D9
4-OH Tolbutamide	2.00-1000	4-OH Tolbutamide-D9
Omeprazole	2.00-1000	Omeprazole-D3
5-OH Omeprazole	1.00-500	5-OH Omeprazole-D3
Dextromethorphan	0.100-50.0	Dextromethorphan-D3
Dextrorphan	0.100-50.0	Dextrorphan-D3

A single, validated bioanalytical method was developed for 3 components of the Cooperstown W/T cocktail and their metabolites for use in DDI interaction analysis. Inter-run biases ranged from 3.0 to -5.3% across all compounds and inter-run precision from 1.9 to 13.1%. Carry-over was <20% for all analytes except dextromethorphan which varied from 8.9 – 81% and will be subjected to ordering restrictions and carry-over scrutiny in sample analysis runs. Recoveries ranged from the 60-70% for dextrorphan and tolbutamide to the >90% for omeprazole. This method was successfully used for the bioanalysis of a Phase-I DDI study.