Development and Validation of LC-MS/MS Methods for the Quantitation of Commonly Co-administered Drugs in Support of Several Clinical Studies

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PURPOSE

The 2018 FDA Guidance for the Validation of Bioanalytical Methods requires an assessment of the impact of the presence of co-administered drugs on the analytical measurement and stability of novel drug candidates. GLP-compliant methods were developed and validated in support of a clinical study that featured co-administration of itraconazole, paroxetine, cimetidine, moxifloxacin, and valproic acid with a proprietary drug candidate. Various methods involving protein-precipitation and liquid-liquid extraction were employed utilizing direct-analysis or dilution of supernatant as well as evaporation with nitrogen followed by reconstitution of analyte in organic solvent.

RESULTS

- Accuracies and precision better than ± 15% (± 20% at LLOQ).
- Demonstrated selectivity and stability in the presence of co-administered drug.

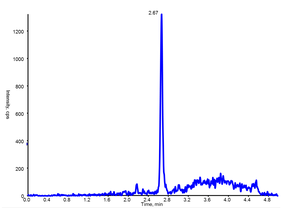


Figure 1: Itraconazole

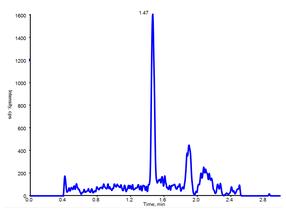


Figure 2: Paroxetine

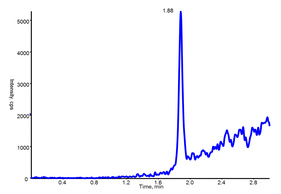


Figure 3: Cimetidine

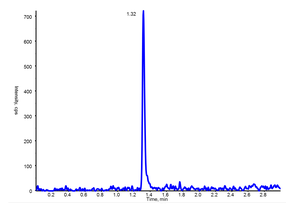


Figure 4: Moxifloxacin

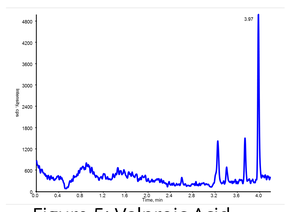


Figure 5: Valproic Acid

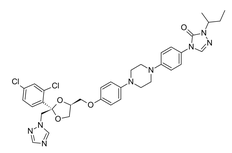
METHODS

Itraconazole:

Extraction
- Samples extracted with 8:2 ACN:H2O followed by direct injection of 100 µL supernatant
- Dynamic range: 2.00 – 800 ng/mL

HPLC Parameters
- MPA: H2O + 0.1% formic acid
- MPB: ACN + 0.1% formic acid
- Flow rate: 0.7 mL/min
- Agilent Pursuit 5 Diphenyl Column

Mass Spectrometry
- Sciex QTRAP® 5500 or 6500
- ESI in positive ion mode
- MRM Transitions:
  - Itraconazole: 705.7 → 392.3
  - Ketoconazole-D_8: 539.1→ 82.3



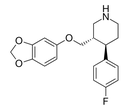
Structure 1: Itraconazole

Paroxetine:

Extraction
- Acetonitrile precipitation
- Supernatant evaporated under nitrogen at 40°C and reconstituted in 6:4 H2O:ACN
- Dynamic range: 0.0500 – 50.0 ng/mL

HPLC Parameters
- MPA: H2O + 0.1% formic acid
- MPB: ACN + 0.1% formic acid
- Flow rate: 0.7 mL/min
- Millipore Sigma
- Discovery® HS C18 Column

Mass Spectrometry
- Sciex QTRAP® 5500 or 6500
- ESI in positive ion mode
- MRM Transitions:
  - Paroxetine: 330.6 → 192.1
  - Paroxetine-D_6: 335.4 → 197.2



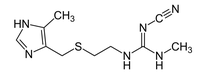
Structure 2: Paroxetine

Cimetidine:

Extraction
- Samples extracted via liquid-liquid extraction with ethyl acetate
- Supernatant evaporated under nitrogen at 40°C and reconstituted in 1:1 H2O:MeOH
- Dynamic range: 5.00 – 1000 ng/mL

HPLC Parameters
- MPA: 1 mM Ammonium Acetate
- MPB: Methanol
- Flow rate: 0.7 mL/min
- Synergi™ Polar-RP Column

Mass Spectrometry
- Sciex API 4000 operating in MRM mode
- ESI in positive ion mode
- MRM Transitions:
  - Cimetidine: 253.0 →159.1
  - Cimetidine-D_3: 256.5 → 162.1



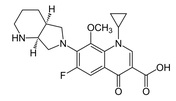
Structure 3: Cimetidine

Moxifloxacin:

Extraction
- Acetonitrile precipitation, followed by dilution in H_2O
- Dynamic range: 25.0 - 25000 ng/mL

HPLC Parameters
- MPA: H2O + 1% formic Acid
- MPB: ACN +1% Formic Acid
- Flow Rate: 0.7 mL/min
- Discovery® HS C18 Column

Mass Spectrometry
- Sciex API 4000 operating in MRM mode
- ESI in positive ion mode
- MRM Transitions:
  - Moxifloxacin: 402.2 →261.2
  - Moxifloxacin-$^{13}C,D_3$: 256.5 → 162.1



Structure 4: Moxifloxacin

Valproic Acid:

Extraction
- Methanol (MeOH) precipitation, followed by a dilution in H2O + 0.1% Formic Acid
- Dynamic Range: 3.00 - 150 µg/mL

HPLC Parameters
- MPA: 10 mM Ammonium Formate (aq)
- MPB: MeOH
- Flow rate: 0.5 mL/min
- Phenomenex Luna® C8 Column

Mass Spectrometry
- Sciex QTRAP® 5500
- ESI in negative ion mode
- MRM Transitions:
  - Valproic Acid: 143.0 Single Ion Monitoring (SIM)
  - Valproic Acid-d_6: 149.0 SIM

Structure 5: Valproic Acid

Methodology	HPLC-MS/MS				
Analyte	Itraconazole	Paroxetine	Cimetidine	Moxifloxacin	Valproic Acid
Matrix	Human Plasma				
Internal Standard (IS)	Ketoconazole-D_8	Paroxetine-D_6	Cimetidine-D_3	Moxifloxacin-$^{13}C,D_3$	Valproic Acid-D_6
Extraction Method	Acetonitrile Precipitation		Liquid-Liquid Extraction with Ethyl Acetate	Acetonitrile Precipitation	Methanol Precipitation
Validated Range	2.00-800 ng/mL	0.0500-50.0 ng/mL	5.00-1000 ng/mL	25.0-25000 ng/mL	3.00-150 µg /mL
Calibration Model	Linear 1/x^2 Regression				
Precision (% CV)					
Intrabatch	0.9% to 6.3%	1.2% to 12.2%	2.3% to 7.0%	3.2% to 9.3%	2.1% to 5.6%
Interbatch	4.4% to 9.8%	1.7% to 16.8%	3.0% to 9.1%	3.5% to 8.6%	2.2% to 7.2%
Accuracy (% Bias)					
Intrabatch	-12.0% to 3.3%	-8.2% to -4.7%	-3.2% to 12.0%	-6.0% to -1.1%	-6.0% to -1.3%
Interbatch	-12.0% to -6.7%	-7.2% to 1.3%	0.0% to 12.0%	-7.6% to -1.6%	-5.0% to 3.0%
Analyte Recovery	54.5% to 57.7%	67.1% to 68.7%	48.7% to 52.2%	76.1% to 82.7%	102% to 106%
Benchtop Stability	Up to 6 hours				
Freeze-Thaw Cycles	Up to 4 cycles				
Processed Sample Stability	Demonstrated 24 hours re-injection stability of processed sample extracts			Greater than 29 hours	Greater than 31 hours
Long Term Stability	93 days at -70°C	28 days at -70°C	134 days at -70°C	94 days at -70°C	103 days at -70°C

Table 1: Validation Summary

CONCLUSIONS

- Methods were developed and validated for simultaneous analysis of proprietary drug candidates with itraconazole, paroxetine, cimetidine, moxifloxacin, and valproic acid by HPLC-MS/MS.
- Methods are robust, simple, selective, and rapid with a run time of 6.2 - 6.8 minutes.
- Validated stability and selectivity for analysis of a novel drug candidate in the presence of the co-administered drugs in compliance with the 2018 FDA Guidance for the Validation of Bioanalytical Methods.
- Successfully applied to analyze clinical pharmacokinetic study samples:
  - 5084 plasma samples analyzed across five analytes
  - 93% batch passing rate
  - Performed within a 1 month turnaround after receipt of final shipment
  - Demonstrated reproducibility upon incurred sample repeat (ISR) analysis