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Exemplary performance data of a Triple Quadrupole Mass Spectrometer in a Simulated Clinical LDT Workflow

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ABSTRACT

Purpose: We have demonstrated the capability of LCMS systems to create LDT methods using compound optimization function provided by the instrument control software.

Methods: Thermo Scientific™ TSQ Quantis™ MD and Thermo Scientific™ TSQ Altis™ MD utilize syringe infusion of organic compounds to create the MS methods in analyzing other chemicals in similar class. Create methods were tested for accuracy and imprecision of the results.

Results: MS compound optimization was performed with representative target compounds of clinical samples. Resulting optimized parameters were used in creating an example method. MS source parameters were adapted from built-in values in the instrument control SW. Testing with LDT methods demonstrated exemplary performance in accuracy and imprecision.

INTRODUCTION

A typical approach for clinical analysis starts with creating an LDT methods for analytes of interest. We have demonstrated the capability of LCMS systems to create methods using compound optimization function provided by the instrument control software.

Organic compounds were used to test capability and suitability of the MS system for analyzing other chemicals in similar class.

MS compound optimization was performed by syringe infusion of test samples. Resulting optimized parameters were used in creating Instrument Methods. MS source parameters were adapted from built-in values in the instrument control SW, depending on LC flow values. Manual adjustment of source position allows optimizing desired performance in robustness or sensitivity.

MATERIALS AND METHODS

Sample Preparation

1. Immunosuppressant Samples:

Ascomycin, Tacrolimus, Sirolimus, Everolimus, Cyclosporin A and Cyclosporin D standards for optimization were prepared from stock solutions in acetonitrile. Recipe® ClinCal® Whole Blood Calibrators and Controls for Immunosuppressants were prepared according to recommended procedures. Samples were processed by precipitation with ZnSO4 and methanol containing internal standard followed by centrifugation.

2. Steroid Samples:

Internal standard solutions from Estrone-2,3,4-13C3 and Testosterone-2,3,4-13C3 were prepared from stock solutions in acetonitrile. Custom Calibrators and Controls for Steroids were extracted with MTBE and reconstituted in methanol/water.

Test Method(s)

Three TSQ Quantis MD and three TSQ Altis MD sytems were tested with compound optimization function provided by Instrument Control Software (ICSW). Standard samples were infused with external syringe. Resulting parameters from optimization were used to create LDT methods.

1. Immunosuppresants LC:

Vanquish MD HPLC with Thermo Scientific™ Hypersil GOLD™ C8 (50 x 2.1 mm, 5 µm) at 0.8 mL/min flow; Mobile phase A: 0.1% formic acid and 10 mM of ammonium formate in water; Mobile Phase B: 0.1% formic acid and 10 mM of ammonium formate in methanol.

2. Steroids LC:

Vanquish MD HPLC with Thermo Scientific™ Accucore™ aQ (100x2.1mm, 2.6µm) at 0.25 mL/min flow; Mobile phase A; 0.5 mM Ammonium fluoride (NH4F) in water; Mobile Phase B; methanol.

Data Analysis

Thermo Scientific™ TraceFinder™ LDT was used to submit sample batches to instruments and to process data.

Accuracy and Imprecision were calculated from replicate injections of QC samples in HESI and APCI ionization mode.

RESULTS: Immunosuppresants

Accuracy and Imprecision of QC samples were tested with five injections of each level.

Figure 1. Example Chromatogram and Spectra of Immunosuppresants: Tacrolimus, Sirolimus, Everolimus and Cyclosporin A, and their spectrum.

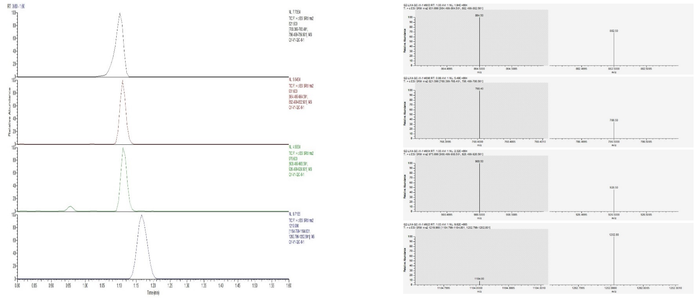


Table 1. Examples of Accuracy and Imprecision to show Inter- and Intra- System performance for testing Immunosuppresants with three Quantis MD (Q1, Q2 and Q3) and three Altis MD (A1, A2 and A3) running with various HPLCs (V1, V2 and V3).

V1A1	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9996	0.54	1.09	1.32
Everolimus	0.9983	6.32	2.28	2.52
Sirolimus	0.9978	3.65	1.45	3.13
Tacrolimus	0.9982	1.94	1.91	1.31

V2A1	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9998	1.51	0.59	1.15
Everolimus	0.9981	1.83	2.65	1.77
Sirolimus	0.9971	10.24	5.48	2.14
Tacrolimus	0.9986	0.9	1.38	1.97

V3A1	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9997	1.44	1.79	0.69
Everolimus	0.9991	5.25	3.34	2.41
Sirolimus	0.9978	4.66	4.34	3.15
Tacrolimus	0.9991	2.26	2.09	1.58

V1A2	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9997	0.79	0.96	1.23
Everolimus	0.9987	7.99	2.51	3.35
Sirolimus	0.9984	2.98	3.75	3.55
Tacrolimus	0.999	3.26	1.36	2.42

V1A3	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9994	2.15	1.41	1.48
Everolimus	0.9993	5.95	2.22	1.67
Sirolimus	0.9984	7.13	5.69	3.97
Tacrolimus	0.9992	2.42	1.77	2.15

V1A3	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9991	2.83	4.43	2.1
Everolimus	0.9988	5.47	2.47	2.2
Sirolimus	0.9973	5.48	4.91	4.16
Tacrolimus	0.9978	3.48	3.32	1.68

V1A3	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9986	1.25	1.46	1.76
Everolimus	0.9987	7.07	2.74	2.66
Sirolimus	0.9981	8.18	5.76	1.98
Tacrolimus	0.999	2.98	1.72	2.18

V1Q1	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.998	6.08	6.17	5.03
Everolimus	0.9974	7.7	4.78	3.93
Sirolimus	0.9972	6.67	5.51	3.64
Tacrolimus	0.9977	4.77	2.95	1.92

V2Q1	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9998	3.32	3.35	2.84
Everolimus	0.9991	4.91	3.31	3
Sirolimus	0.9981	4.71	3.81	2.62
Tacrolimus	0.9978	3.36	3.01	1.25

V3Q1	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9996	0.27	0.51	1.01
Everolimus	0.9989	3.7	0.57	1.3
Sirolimus	0.9983	2.37	1.1	0.83
Tacrolimus	0.9976	1.79	1.43	0.49

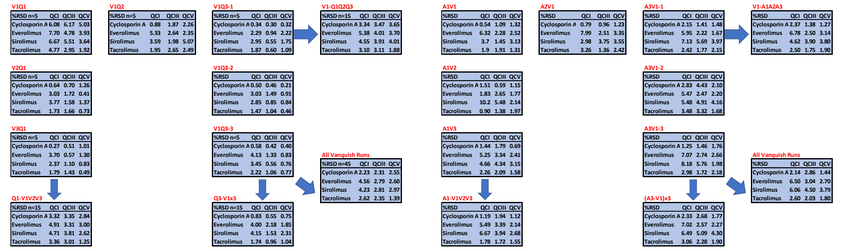
V1Q2	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9973	0.88	1.87	2.26
Everolimus	0.9995	5.33	2.64	2.35
Sirolimus	0.9985	3.59	1.98	5.07
Tacrolimus	0.9986	1.95	2.65	2.49

V1Q3	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9998	0.34	0.3	0.32
Everolimus	0.9989	2.29	0.94	2.22
Sirolimus	0.9984	2.95	0.55	1.75
Tacrolimus	0.9981	1.87	0.6	1.09

V1Q3	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9996	0.5	0.46	0.21
Everolimus	0.9994	3.03	1.49	0.91
Sirolimus	0.9986	2.85	0.85	0.84
Tacrolimus	0.9988	1.47	1.04	0.46

V1Q3	R^2	QC I %RSD	QCIII %RSD	QCV %RSD
CyclosporinA	0.9997	0.58	0.42	0.4
Everolimus	0.999	4.13	1.33	0.83
Sirolimus	0.998	3.45	0.56	0.76
Tacrolimus	0.9983	2.22	1.06	0.77

Table 2. Examples of performance: Summary of RSD values in combinations of instruments for testing Immunosuppresants with three Quantis MD (Q1, Q2 and Q3) and three Altis MD (A1, A2 and A3) running with various HPLCs (V1, V2 and V3).



RESULTS: Steroids

Accuracy and Imprecision of QC samples were tested with five injections of each level.

Table 3. Table 1. Accuracy and Imprecision in combinations of instruments for testing Steroids with Quantis MD (Q1) and Altis MD (A2) in HESI and APCI ionization mode.

HESI V3Q1	LOQ (ng/mL)	R^2	QC1 %RSD	QC2 %RSD	QC3 %RSD
Estrone	0.03	0.9986	3.36	2.42	1.74
Testosterone	0.01	0.9992	1.07	0.24	0.25

APCI V3Q1	LOQ (ng/mL)	R^2	QC1 %RSD	QC2 %RSD	QC3 %RSD
Estrone	0.03	0.9978	5.88	1.18	1.76
Testosterone	0.01	0.9986	1.60	7.00	4.61

HESI V1A2	LOQ (ng/mL)	R^2	QC1 %RSD	QC2 %RSD	QC3 %RSD
Estrone	0.03	0.9995	1.20	0.84	1.20
Testosterone	0.01	0.9989	0.55	0.74	0.32

APCI V1A2	LOQ (ng/mL)	R^2	QC1 %RSD	QC2 %RSD	QC3 %RSD
Estrone	0.03	0.9989	2.60	1.36	1.47
Testosterone	0.01	0.9986	3.52	3.70	3.82

CONCLUSIONS

- MS compound optimization was performed by syringe infusion of test samples from analytical standards. Resulting optimized parameters were used in creating example methods. MS source parameters were adapted from a built-in function of ICSW, determined from LC flow values.
- We have demonstrated that adequate methods for accuracy and imprecision were created by instruments built-in functions for immunosuppressant and steroid samples, in support of LDT.
- Results from organic compounds demonstrated the capability and suitability of the MS systems for LDT workflow.

TRADEMARKS/LICENSING

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