POSTER NOTE

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Rapid LC method development for multiple drug classes by using a new four-channel HPLC system

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ABSTRACT

In this poster, we present a workflow for developing multiple LC methods for r drug classes by using a new four-channel HPLC, the Thermo Scientific™ Prel LX-4 MD™ HPLC Class I medical device. These analytical methods were chal by quantitation of four classes of drug compounds in plasma with good accura precision.

INTRODUCTION

The powerful combination of HPLC with mass spectrometry provides high qua analytical data to various scientific and research fields. With regard to LC/MS analysis being used in clinical research, LC/MS method development is crucia time-consuming due to the diversity and complexity of compounds. Developi methods for multiple drug classes provides greater challenges, in part, due to consideration of various HPLC columns, various mobile phases and different gradients that can be used. By using a new four-channel LC system, the LC r development can be done rapidly and efficiently. In this poster, four different L methods were developed and optimized rapidly for four classes of compound anticonvulsant compounds, 7 antidepressant compounds, 4 antipsychotic compounds, and 6 antiarrhythmic compounds) in plasma.

Figure 1. Thermo Scientific™ Prelude™ LX-4 MD HPLC and Endura MD™ mass spectrometer. Specific instruments used in this poster were pre-production versions.

INSTRUMENTS AND METHODS

New Four-Channel HPLC

A pre-production Prelude LX-4 MD™ HPLC system with four independent LC channels was tested (Figure 1).

- Each channel utilizes two syringe pumps (for binary operation) with volumes designed for single push per sample analysis. This reduced solvent use since the pumps only run during the method's run-time.
- The LC flows were channeled to a single mass spectrometer only while the compounds of interest were eluting off columns.
- Thermo Scientific™ Aria™ MX software staggered the injections on each channel so that the LC gradients ran in parallel but the elution windows never overlapped.

Mass Spectrometry

A prototype Thermo Scientific™ Endura MD™ mass spectrometer equipped with a Thermo Scientific™ Ion Max™ NG ion source and heated electrospray ionization (Thermo Scientific™ HESI II probe) sprayer was used throughout this test.

- A selected-reaction monitoring (SRM) transition was developed and monitored for each compound.
- Data were acquired and processed using Thermo Scientific™ Xcalibur™ software.

Sample Preparation

- Calibration standards and quality controls (QCs) were prepared by spiking panels of compounds into blank pooled plasma.
- Concentrations of individual compounds within each class were tailored to the different targeted analytical ranges (Table 1).
- The plasma was then processed by protein precipitation with 3x volumes of acetonitrile followed by dilution with aqueous. (All solvents are Fisher™ Optima™ grade).

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Table 1. Concentrations of Calibrators

	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Cal 7
ANTICONVULSANTS (µg/mL)							
Carbamazapine	60	30	12	6	3	1.2	0.6
Lamotrigine	60	30	12	6	3	1.2	0.6
Levetiracetam	300	150	60	30	15	6	3
Oxcarbazepine	300	150	60	30	15	6	3
Tiagabine	1.2	0.6	0.24	0.12	0.06	0.024	0.012
Topiramate	30	15	6	3	1.5	0.6	0.3
Zonisamide	300	150	60	30	15	6	3
ANTIDEPRESSANTS (ng/mL)							
Amitriptyline	2500	1250	500	250	125	50	25
Desipramine	2500	1250	500	250	125	50	25
Doxepin	1250	625	250	125	62.5	25	12.5
Imipramine	2500	1250	500	250	125	50	25
Trazodone	5000	2500	1000	500	250	100	50
Trimipramine	2500	1250	500	250	125	50	25
Venlafaxine	2500	1250	500	250	125	50	25
ANTIPSYCHOTICS (ng/mL)							
Clozapine	4000	2000	800	400	200	80	40
Quetiapine	4000	2000	800	400	200	80	40
Risperidone	400	200	80	40	20	8	4
9-Hydroxyrisperidone	400	200	80	40	20	8	4
ANTIARRHYTHMICS (ng/mL)							
Disopyramide	15000	7500	3000	1500	750	300	150
Flecainide	7500	3750	1500	750	375	150	75
Lidocaine	15000	7500	3000	1500	750	300	150
N-acetylprocainamide	15000	7500	3000	1500	750	300	150
Procainamide	15000	7500	3000	1500	750	300	150
Quinidine	15000	7500	3000	1500	750	300	150

Method development

MS Parameters: Ion source setting and selected-reaction monitoring (SRM) transition on Endura MD mass spectrometer were optimized for each compound by T connection: 400 µL/min of MP-A/MP-B (50/50) from LC and 10 µL/min of each compound (0.01 – 10 µg/mL) from syringe. Ion source parameters were listed in Table 2 and SRMs for each compound were listed in Table 3 and used in all following studies.

Table 2. Mass spectrometer global settings on Endura MD™

	Spray Voltage (Positive)	Sheath Gas (Arb)	Aux Gas (Arb)	Sweep Gas (Arb)	Ion Trans Tube Temp	Vaporizer Temp	Collision Gas Pressure
Setting	3000 V	45	15	2	350 °C	400 °C	2.0 mTorr

LC Method development

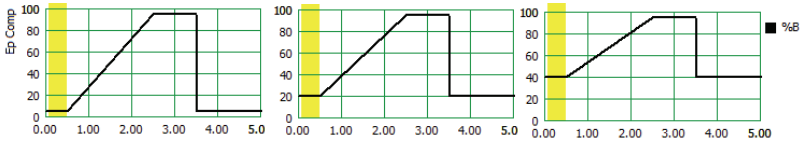
Step 1: Prepare mobile phase and install four different HPLC columns[1,2] on the four-channel Prelude LX-4 MD™ HPLC system.

Mobile Phase	MP-A: 5mM ammonium formate with 0.05% formic acid in H_2O MP-B: 5mM ammonium formate with 0.05% formic acid in methanol
HPLC columns	Thermo Scientific™ Accucore™ Phenyl-Hexyl, 2.6 µm, 50x2.1 mm, Thermo Scientific™ Hypersil™ GOLD, 1.9 µm, 50 x 2.1 mm, Thermo Scientific™ Hypersil™ GOLD aQ, 1.9 µm, 50 x 2.1 mm Thermo Scientific™ Accucore™ PFP, 2.6 µm, 50 x 2.1 mm

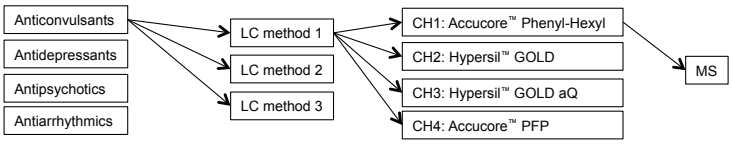
Table 3. SRMs for each compound and isotope-labelled internal standards (IS)

ANTICONVULSANTS	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	isotope-labelled IS	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
carbamazapine	237.1	192.1	26	101	carbamazepine-d10	247.3	204.2	23	121
carbamazapine	237.1	194.1	22	101	carbamazepine-d10	247.3	202.2	36	121
lamotrigine	256.0	145.0	42	192	lamotrigine-13C-15N4	261.1	214.0	28	165
lamotrigine	256.0	211.0	29	192	lamotrigine-13C-15N4	261.1	144.9	41	165
Levetiracetam	171.0	69.1	32	54	levetiracetam-d6	177.2	132.1	17	50
Levetiracetam	171.0	126.1	17	54	levetiracetam-d6	177.2	160.4	6	50
oxcarbazepine	253.1	180.1	36	103	oxcarbazepine-13C6	259.2	242.1	15	107
oxcarbazepine	253.1	236.0	18	103	oxcarbazepine-13C6	259.2	186.1	33	107
tiagabine	376.1	149.0	28	140	tiagabine-d4	382.2	253.1	21	154
tiagabine	376.1	247.0	21	140	tiagabine-d4	382.2	152.1	28	154
zonisamide_POS	213.1	77.1	34	83	zonisamide-13C6_POS	219.1	138.1	17	84
zonisamide_POS	213.1	132.1	18	83	zonisamide-13C6_POS	219.1	108.0	32	84
ANTIDEPRESSANTS									
amitriptyline	278.2	191.1	28	108	Amitriptyline-d3	281.3	233.1	19	120
amitriptyline	278.2	233.2	23	108	Amitriptyline-d3	281.3	191.1	28	120
desipramine	267.2	193.1	39	92	desipramine-d3	270.3	75.1	20	98
desipramine	267.2	208.1	25	92	desipramine-d3	270.3	208.1	25	98
doxepin	280.2	165.1	59	112	doxepin-d3	283.3	202.1	39	126
doxepin	280.2	202.1	39	112	doxepin-d3	283.3	141.1	29	126
imipramine	281.2	86.1	20	98	imipramine-d3	284.3	89.1	20	106
imipramine	281.2	193.1	42	98	imipramine-d3	284.3	61.1	44	106
trazodone	372.2	148.1	35	162	trazadone-d6	378.2	182.1	26	172
trazodone	372.2	176.1	26	162	trazadone-d6	378.2	150.1	36	172
trimipramine	295.2	100.1	20	94	trimipramine-d3	298.3	103.1	20	115
trimipramine	295.2	193.1	43	94	trimipramine-d3	298.3	61.1	41	115
venlafaxine	278.2	121.2	30	103	venlafaxine-d4	284.3	64.2	30	100
venlafaxine	278.2	215.2	22	103	venlafaxine-d4	284.3	121.0	30	100
ANTIPSYCHOTICS									
clozapine	327.1	192.1	45	116	clozapine-d4	331.2	272.1	26	163
clozapine	327.1	270.1	25	116	clozapine-d4	331.2	192.1	46	163
quetiapine	384.2	221.1	41	170	quetiapine-d8	392.3	258.1	25	156
quetiapine	384.2	253.1	28	170	quetiapine-d8	392.3	226.1	40	156
risperidone	411.3	110.3	58	175	risperidone-d4	415.3	195.2	31	175
risperidone	411.3	191.2	31	175	risperidone-d4	415.3	114.1	52	175
9OH-Risperidone	427.3	207.1	29	142	9-hydroxyrisperidone-d4	431.3	211.1	29	149
9OH-Risperidone	427.3	179.1	44	142	9-hydroxyrisperidone-d4	431.3	114.1	45	149
ANTIARRHYTHMICS									
disopyramide	340.2	195.1	32	105					
disopyramide	340.2	239.1	20	105					
flecainide	415.2	301.0	35	171					
flecainide	415.2	398.1	25	171					
Lidocaine	235.2	58.4	32	114					
Lidocaine	235.2	86.3	26	114					
N-acetylprocainamide	278.2	120.2	35	128					
N-acetylprocainamide	278.2	205.1	23	128					
procainamide	236.2	120.2	32	109					
procainamide	236.2	163.1	22	109					
quinidine	325.2	172.1	36	157					
quinidine	325.2	184.1	31	157					

Step 2: Program multiple LC method with different MP-B% as initial gradient such as 5%, 20% and 40% MP-B



Step 3: Run a batch of four drug classes with three LC methods on each channel of four-channel Prelude LX-4 MD. Therefore, each drug class was injected twice into all four channels (CH1, CH2, CH3 and CH4) and separated by three LC methods.



Step 4: Review chromatogram of four drug classes separated by different HPLC column and different LC method with respect to retention time, the baseline separation and peak shape. Table 3 lists the retention time of anticonvulsant compounds and Accucore™ PFP column was chosen as the best one of four columns.

Table 3. Retention time of anticonvulsant compounds separated by four different HPLC columns with three different LC gradients. Note: two RTs designates split peak and italic number indicates asymmetric and/or broad peak.

	Retention Time (min) in LC Method 1: (Gradient 5%-95% MP-B)							
Anticonvulsants Compound	CH1 Accucore™ Phenyl-Hexyl 1st Injection	CH1 Accucore™ Phenyl-Hexyl 2nd Injection	CH2 Hypersil™ GOLD 1st Injection	CH2 Hypersil™ GOLD 2nd Injection	CH3 Hypersil™ GOLD aQ 1st Injection	CH3 Hypersil™ GOLD aQ 2nd Injection	CH4 Accucore™ PFP 1st Injection	CH4 Accucore™ PFP 2nd Injection
carbamazapine	2.51	2.52	2.46	2.46	2.4	2.4	2.39	2.38
lamotrigine	2.19	2.3	2.22	2.22	2.07	2.07	2.37	2.36
Levetiracetam	0.67	1.83	1.51/1.62	1.49/1.61	1.48/1.6	1.36/1.57	1.64	*1.64*
oxcarbazepine	2.39	2.42	2.37	2.37	2.32	2.31	2.31	2.31
tiagabine	2.58	2.57	2.54	2.54	2.47	2.47	2.59	2.59
zonisamide	0.79/1.84	2.1	1.96	1.94	1.93	1.93	2.02	2.02
	Retention Time (min) in LC Method 2: (Gradient 20%-95% MP-B)							
	CH1 Accucore™ Phenyl-Hexyl 1st Injection	CH1 Accucore™ Phenyl-Hexyl 2nd Injection	CH2 Hypersil™ GOLD 1st Injection	CH2 Hypersil™ GOLD 2nd Injection	CH3 Hypersil™ GOLD aQ 1st Injection	CH3 Hypersil™ GOLD aQ 2nd Injection	CH4 Accucore™ PFP 1st Injection	CH4 Accucore™ PFP 2nd Injection
carbamazapine	2.33	2.31	2.31	2.3	2.21	2.2	2.17	2.15
lamotrigine	2.03	1.97	1.99	1.91	1.76	1.68	2.13	2.11
Levetiracetam	*1.52*		*1.48*	*1.4*	*1.23*	*1.01*	*1.26*	*1.15*
oxcarbazepine	2.2	2.18	2.19	2.16	2.09	2.07	2.07	2.05
tiagabine	2.41	2.41	2.4	2.39	2.29	2.29	2.46	2.45
zonisamide	1.74	1.65	1.7	1.62	1.59	1.47	1.64	1.55
	Retention Time (min) in LC Method 3: (Gradient 40%-95% MP-B)							
	CH1 Accucore™ Phenyl-Hexyl 1st Injection	CH1 Accucore™ Phenyl-Hexyl 2nd Injection	CH2 Hypersil™ GOLD 1st Injection	CH2 Hypersil™ GOLD 2nd Injection	CH3 Hypersil™ GOLD aQ 1st Injection	CH3 Hypersil™ GOLD aQ 2nd Injection	CH4 Accucore™ PFP 1st Injection	CH4 Accucore™ PFP 2nd Injection
carbamazapine	1.95	1.89	1.93	1.87	1.78	1.74	NA	1.67
lamotrigine	1.59	1.47	1.54	1.4	1.17	0.8	NA	1.61
Levetiracetam	*0.83*	*0.67*	*0.8*	*0.65*	*0.64*	*0.54*	NA	0.5
oxcarbazepine	1.78	1.69	1.75	1.68	1.6	1.53	NA	1.53
tiagabine	2.08	2.04	2.06	2.03	1.92	1.91	NA	2.16
zonisamide	1.33	0.84	1.28	0.8	1.02	0.67	NA	NA

Step 5: Optimize LC gradient further with respect to the peak shape of each compound in different LC methods. For example, Table 3 lists that all compounds except levetiracetam have good peak shape in LC method 1 and LC method 3 can achieve a good peak shape of levetiracetam. As shown in Figure 2, a gradient jump from 5% to 40% of MP-B was added in LC method 1 (Figure 2 right) which improved the peak shape of levetiracetam and achieved the baseline separation of oxcarbazepine isomers as well as remaining of other compounds' peak shapes.

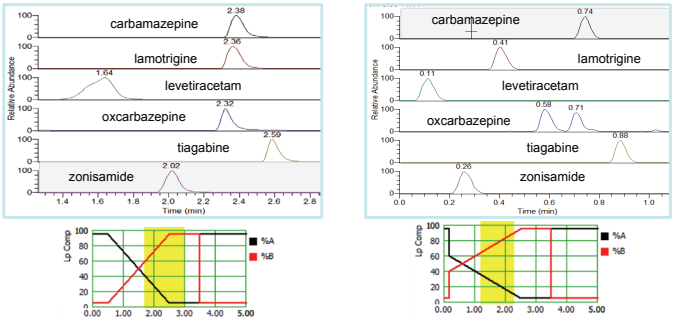


Figure 2. TIC of anticonvulsant compounds separated by Hypersil™ GOLD, 1.9 µm, 50 x 2.1 mm with two different LC gradients (Corresponding gradient profile are shown under each chromatograph).

Step 6: Finalize LC/MS methods by running plasma samples with respect to the biomatrix impact on HPLC column separation, mass spec sensitivity and ion expression, etc. As shown in Table 4, accuracies were better than 84% and precisions expressed as %RSD were better than 14.7% across all compounds and all concentrations of four drug classes.

Therefore, four LC methods were developed successfully for four drug classes (anticonvulsants, antidepressants, antipsychotics, and antiarrhythmics) and summarized in Figure 3.

Table 4. Accuracies and Precisions (as %RSD) of QCs analyzed over three runs with six replicates in each run.

Compound (QC-Hi/QC-M/QC-Low)	QC-Hi		QC-M		QC-Low	
	Acc	%RSD	Acc	%RSD	Acc	%RSD
ANTICONVULSANTS (µg/mL)						
Carbamazapine (60/6/1.2)	2.59	5.79	-0.86	2.20	-5.37	3.54
Lamotrigine (60/6/1.2)	3.76	4.65	-0.68	7.09	8.89	8.61
Levetiracetam (300/30/6)	3.65	5.55	-0.45	3.95	-1.55	4.91
Oxcarbazepine (300/30/6)	0.93	2.83	2.64	7.04	-3.52	8.03
Tiagabine (1.2/0.12/0.024)	6.65	8.79	7.87	10.9	11.3	14.7
Topiramate (30/3/0.6)	-0.89	4.91	-2.36	4.48	-0.07	7.71
Zonisamide (300/30/6)	-14.4	5.60	-2.24	6.60	-2.62	4.59
ANTIDEPRESSANTS (ng/mL)						
Amitriptyline (2500/250/50)	5.40	12.20	-4.07	9.24	2.22	8.96
Desipramine (2500/250/50)	0.11	8.16	3.49	9.22	0.09	9.32
Doxepin (1250/125/25)	-5.48	13.3	-1.86	8.88	0.47	10.4
Imipramine (2500/250/50)	1.18	8.34	0.66	10.50	-1.03	8.47
Trazodone (5000/500/100)	2.10	3.34	-4.66	7.75	10.9	8.97
Trimipramine (2500/250/50)	5.83	6.59	2.41	8.65	4.94	9.41
Venlafaxine (2500/250/50)	5.65	7.41	-3.10	10.0	6.02	9.26
ANTIPSYCHOTICS (ng/mL)						
Clozapine (4000/400/80)	2.73	5.94	-5.96	7.06	5.02	10.4
Quetiapine (4000/400/80)	1.88	5.52	-5.66	4.94	2.46	5.08
Risperidone (400/40/8)	0.68	3.19	-3.76	3.46	11.8	6.58
9-Hydroxyrisperidone (400/40/8)	1.84	6.71	-4.47	7.87	7.07	5.14
ANTIARRHYTHMICS (ng/mL)						
Disopyramide (15000/1500/300)	-6.99	7.99	-1.80	8.16	-7.00	8.49
Flecainide (7500/750/150)	4.93	7.60	-7.70	6.65	-5.89	8.81
Lidocaine (15000/1500/300)	-10.6	7.63	-2.22	8.36	-11.7	10.4
N-acetylprocainamide (15000/1500/300)	-1.62	10.2	-12.07	7.97	-1.31	6.50
Procainamide (15000/1500/300)	-5.22	7.89	0.36	8.07	-16.0	11.0
Quinidine (15000/1500/300)	2.99	5.81	4.95	5.80	NA	NA

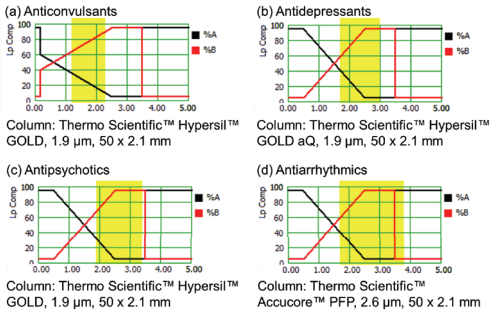


Figure 3. HPLC gradients, columns and data acquisition windows used for analysis. Mobile phases are 5 mM ammonium formate with 0.05% formic acid in water (A) and methanol (B) for all methods.

CONCLUSIONS

A novel workflow on rapid LC method development was demonstrated by developing LC/MS methods for four disparate drug classes for the Prelude LX-4 MD four-channel HPLC system..

Multiple HPLC columns and multiple LC methods were simultaneously tested and provided information for the choice of HPLC column and further optimization of LC gradients. Symmetric peak and baseline separation were readily achieved for all analyzed compounds.

Bioanalysis of all compounds in plasma showed good accuracy and precision over the course of evaluation.

REFERENCES

1. Thermo Scientific™ App Note of Development and Validation of Methods for Chemotherapy Drugs on the New Prelude SPLC LC-MS/MS System.
2. Thermo Scientific™ App Note of Evaluation of a Liquid Chromatography Tandem Mass Spectrometry Analytical Method for the Quantification of a Panel of Antiepileptic Drugs and their Metabolites in Human Plasma.

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