Implementation of triple quadrupole LC-MS/MS complete kits on a high resolution mass spectrometer – limitations and benefits

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INTRODUCTION

Over the last decade, liquid chromatography coupled to mass spectrometry (LC-MS) and in particular triple quadrupole mass spectrometers (QQQ) have gained popularity and transitioned from research to routine clinical laboratories. Recently, we observe increasing demands for QQQ complete kits for small molecules running on high resolution instruments.

We report benefits and limitations faced during implementation of our ClinMass® LC-MS/MS complete kits on a Thermo Scientific™ Q Exactive™ Plus coupled to a Thermo Scientific™ Vanquish™ Duo system for Research Use Only.

PURPOSE

Besides Parallel Reaction Monitoring (PRM), which comes closest to the classic Multiple/Single Reaction Monitoring (MRM or SRM) from QQQ, the QE Plus offers additional acquisition modes shown in Figure 1.

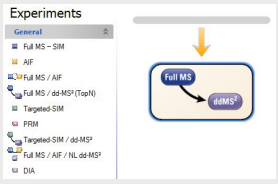

Figure 1: Acquisition modes of the QE Plus (FullMS-ddMS² selected)

Full Scan mode combined with data dependent fragmentation of detected parent ions (FullMS-ddMS²) enables to quantify on the parent ion trace and trigger high resolution fragment ion spectra. This approach also offers the option to retrospectively extract mass traces (within the set scan range) of substances which were not initially in focus of interest (new designer drugs, recently discovered metabolites etc.).

Targeted SIM (tSIM) would add additional sensitivity compared to FullMS, but exclude a retrospective mass extraction. This approach was not used for verification.

MATERIALS AND METHODS

Vitamin D measurements were carried out using the ClinMass® complete kit for 25-OH-Vitamin D2/D3 in serum/plasma (MS7000, RECIPE Chemicals + Instruments GmbH). The method is based on protein precipitation, followed by online SPE with subsequent isocratic separation.

Measurement of 15 Tricyclic Antidepressants (TCA) was performed with the ClinMass® TDM 200 Kit system for over 150 drugs in serum/plasma. The platform uses protein precipitation, followed by direct injection onto an analytical column with gradient separation (MS9000, MS9100, RECIPE Chemicals + Instruments GmbH).

Calibration and LLoQ testing were performed using ClinCal® serum calibrators included in the complete kits. For precision testing, ClinChek® serum controls at 2 levels were used (intra-assay: n = 5, inter-assay: 3 days, n = 15). Accuracy was evaluated using proficiency test samples (n = 5).

General:	
Runtime:	0 to 3 min
Polarity:	positive
Default charge state:	1
Inclusion:	on
MS²:	
Resolution:	17 500
AGC target:	5E+04
Maximum IT:	40 ms
Isolation window:	4.0 m/z
Fixed first mass:	-
(N)CE / stepped CE:	15, 20, 30

Full MS:	
Resolution:	70 000
AGC target:	1E+06
Maximum IT:	200 ms
Scan range:	250 to 350 m/z
dd-MS² / dd-SIM:	
Resolution:	17 500
AGC target:	5E+04
Maximum IT:	20 ms
Loop count:	1
TopN:	1
Isolation window:	4.0 m/z

dd-MS² / dd-SIM:	
Fixed first mass:	-
(N)CE / stepped nce:	-
Spectrum data type:	Centroid
dd Settings:	
Minimum AGC target:	1E+02
Intensity threshhold:	5E+03
Apex trigger:	-
Charge exclusion:	-
Peptide match:	-
Exclude isotopes:	-
Dynamic exclusion:	10.0 s

Table 1: Settings for Vitamin D (PRM, left) and TCAs (FullMS-ddMS², right); inclusion ‚on' for FullMS-ddMS²

Mass [m/z]	Start [min]	End [min]	(N)CE	(N)CE type	Comment
389.3685	2.70	2.90			D6-25-OH-Vit D3 - H2O
395.33084	2.75	2.95			25-OH Vit D2 - H2O
383.33084	2.73	2.85			25-OH Vit D3 - H2O

Mass [m/z]	Start [min]	End [min]	(N)CE	(N)CE type	Comment
278.19033	3.1	3.6	30	NCE	Amitriptyline
315.16225	3.3	3.8	25	NCE	Clomipramine
327.1371	2.9	3.4	45	NCE	Clozapine
267.18558	2.3	2.7	23	NCE	Desipramine
280.16959	2.2	2.6	30	NCE	Doxepin
281.20123	2.9	3.4	22	NCE	Imipramine
278.19033	2.7	3.2	40	NCE	Maprotiline
301.1466	3.15	3.6	25	NCE	Norclomipramine
313.12145	1.3	1.8	40	NCE	Norcloazpine
266.15394	1.4	1.9	30	NCE	Nordoxepin
281.20123	2.7	3.0	40	NCE	Nortrimipramine
264.17468	2.5	3.0	25	NCE	Nortriptyline
295.21688	3.2	3.7	24	NCE	Trimipramine
281.20916	3.1	3.6	30	NCE	D3-Amitriptyline
318.18108	3.3	3.8	25	NCE	D3-Clomipramine
331.16221	2.9	3.4	45	NCE	D4-Clozapine
270.20441	2.3	2.7	20	NCE	D3-Desipramine
283.18842	2.2	2.6	30	NCE	D3-Doxepin
284.22006	2.9	3.4	22	NCE	D3-Imipramine
283.22171	2.7	3.2	40	NCE	D5-Maprotiline
304.16543	3.15	3.6	25	NCE	D3-Norclomipramine
321.17166	1.3	1.8	40	NCE	D8-Norclozapine
269.17277	1.4	1.9	30	NCE	D3-Nordoxepin
267.19351	2.5	3.0	25	NCE	D3-Nortriptyline
298.23571	3.2	3.7	24	NCE	D3-Trimipramine

Table 2: Inclusion lists for Vitamin D and TCAs (all analytes: species +H, charge state 1, polarity positive, no MSX)

A Thermo Scientific™ Q Exactive™ Plus coupled to a Thermo Scientific™ Vanquish™ Duo system for Research Use Only was used. We show PRM data for Vitamin D and FullMS-ddMS² data for TCAs. Method settings are shown in Table 1 and Table 2. Verification data from a Thermo Scientific™ Endura™ coupled to a Thermo Scientific™ Transcend™ II system was used for performance comparison.

RESULTS AND DISCUSSION

Results for Vitamin D in PRM concerning precision, accuracy and LLoQ are shown in Table 3 and Figure 2.

	LLoQ [µg/L]		Intra-assay precision [%]		Inter-assay precision [%]		Accuracy [%] INSTAND 290 proficiency test	
	QE Plus	Endura	QE Plus	Endura	QE Plus	Endura	QE Plus	Endura
Vitamin D2	4.9	2.1	3.6 / 6.9	4.5 / 3.1	6.4 / 9.3	10.0 / 8.3	-	-
Vitamin D3	4.5	2.1	4.8 / 3.8	2.2 / 2.8	5.5 / 6.7	4.3 / 3.0	81.4 - 102	102 - 103

Table 3: Verification results for Vitamin D in PRM mode

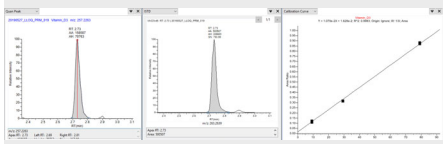

Figure 2: 25-OH-Vitamin D3 from PRM processing method

	LLoQ [µg/L]		Intra-assay precision [%]		Inter-assay precision [%]		Accuracy [%] GTFCh TCA proficiency test	
	QE Plus	Endura	QE Plus	Endura	QE Plus	Endura	QE Plus	Endura
Amitriptyline	0.78	0.95	1.4 / 1.6	1.4 / 1.8	4.0 / 2.5	6.9 / 6.3	96 - 98	101 - 102
Clomipramine	0.98	0.91	5.2 / 2.9	1.1 / 1.1	3.9 / 2.5	3.7 / 6.6	111 - 112	102 - 109
Clozapine	3.02	1.84	1.1 / 0.7	1.3 / 1.0	2.1 / 1.3	5.6 / 4.4	-	-
Desipramine	0.86	0.88	0.7 / 2.3	1.3 / 0.9	2.3 / 1.6	6.0 / 6.2	107 - 109	100 - 102
Doxepin	1.70	0.89	0.3 / 1.0	2.0 / 2.0	2.4 / 1.6	8.5 / 8.4	102 - 104	97 - 103
Imipramine	0.70	0.90	0.4 / 1.4	1.7 / 1.7	1.7 / 1.9	4.4 / 4.6	108 - 109	100 - 101
Maprotiline	1.11	0.93	0.3 / 1.2	2.3 / 2.4	1.7 / 1.7	4.8 / 5.2	108 - 109	102 - 107
Norclomipramine	1.12	4.50	0.8 / 0.6	1.7 / 2.3	2.0 / 1.4	3.6 / 4.6	105 - 108	92 - 97
Norclozapine	2.32	1.44	1.0 / 1.2	2.5 / 2.1	1.8 / 1.2	5.9 / 6.1	-	-
Nordoxepin	0.71	0.86	0.5 / 1.1	2.6 / 2.2	1.3 / 1.3	5.8 / 4.5	100 - 104	97 - 102
Nortrimipramine	0.64	-	1.6 / 2.9	-	3.0 / 2.6	-	-	-
Nortriptyline	0.86	0.88	0.8 / 0.8	2.7 / 1.4	2.0 / 1.1	6.0 / 6.9	104 - 107	94 - 99
Trimipramine	0.85	0.86	4.1 / 3.6	1.7 / 2.1	5.8 / 6.9	6.7 / 7.7	97 -101	100 - 104

Table 4: Verification results for TCAs in FullMS-ddMS² mode

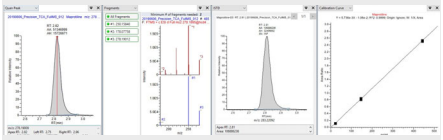

Figure 3: Maprotiline from TCA FullMS-ddMS² processing method

Results for TCAs in FullMS-ddMS² concerning precision, accuracy and LLoQ are shown in Table 4 and Figure 3.

Normaprotiline and Protriptyline could not be quantified in FullMS-ddMS², due to co-elution and identical parent ions. It should, however, be noted that they showed excellent results in PRM. The isobaric and usually also co-eluting substances Nortrimipramine and Imipramine could be quantified in FullMS-ddMS², due to outstanding chromatographic separation by the Vanquish™ system.

SUMMARY AND CONCLUSION

- LC-MS/MS ClinMass® complete kits can easily be implemented on a Q Exactive™ instrument
- Results from the Q Exactive™ Plus and the Endura™ are comparable in terms of sensitivity, accuracy and precision and meet research laboratory requirements
- In PRM mode, quantitation can be performed on a fragment (quantifier), therefore method characteristics are nearly identical with SRM/MRM mode, excluding even more interferences due to high resolution of the fragment ion

- **Benefits** from using FullMS-ddMS² compared to MRM/SRM mode at a triple quadrupole:
  - Improved identification/selectivity due to high resolution fragment ion spectra for all substances of interest
  - No limitation in extraction options from chosen scan range, inclusion of new substances in quantitation method possible for already acquired data
  - One single instrument for routine quantitation of small molecules and screening, confirmation and characterization of unknown substances

- **Limitations** of the high resolution instrument:
  - No fragment ions < 50 m/z can be detected
  - Polarity switching within one method not feasible due to high switching times of the Orbitrap™ mass analyzer

ACKNOWLEDGEMENTS

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