Bioanalysis

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Assay types with Bioanalysis

- Transporter assays:
  - VT
  - UPT
  - ML
- Metabolic studies
  - CYP inhibition
  - CYP induction
  - Clearance and MetID
- Miscelleneous
  - Solubility
  - Stability
  - NSB
  - Purity/Radiochemical Purity

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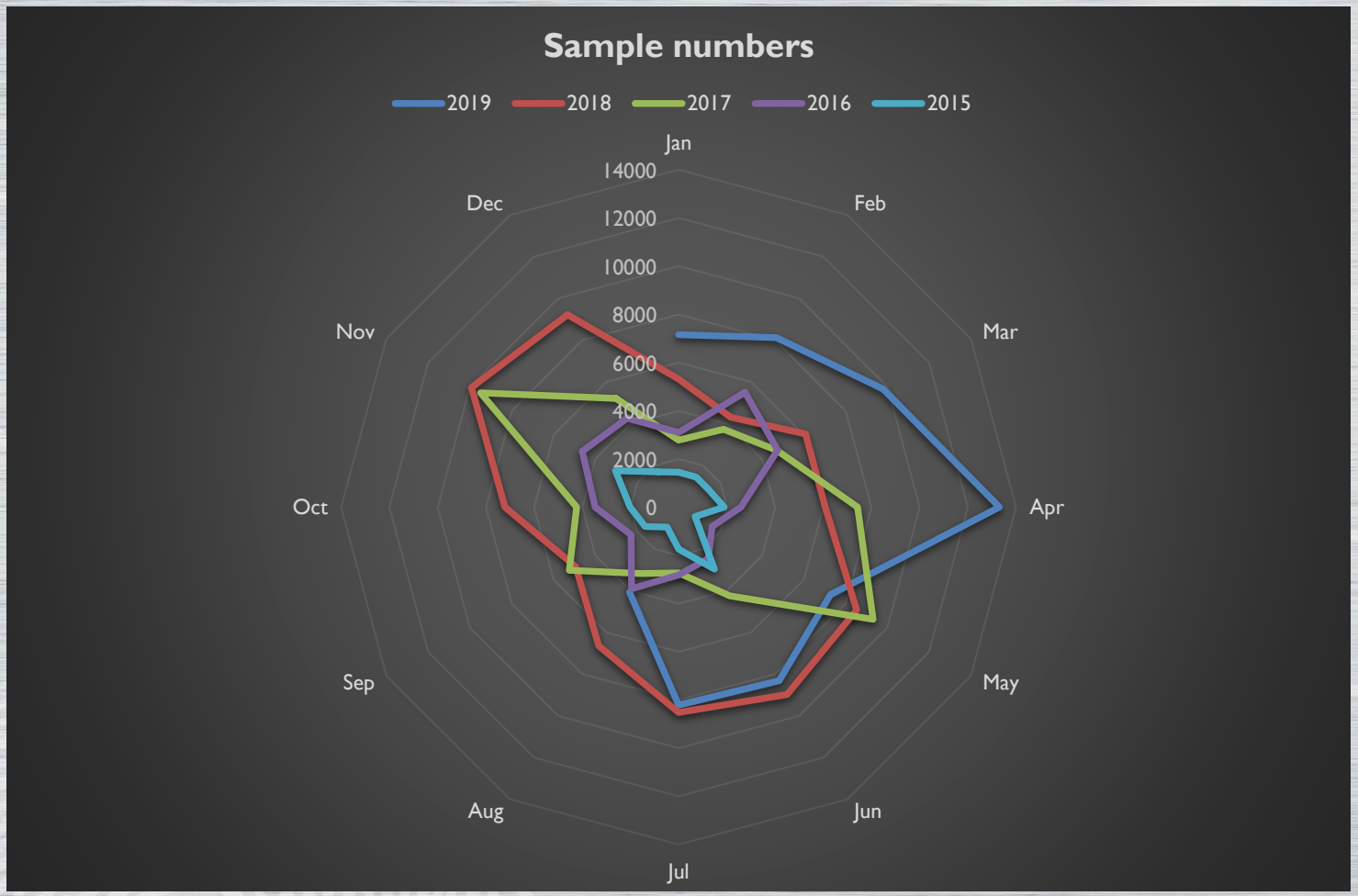
Growth in Sample numbers



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Automation

- Transporter assays:
  - VT – BSEP validated
  - ML - ongoing
  - UPT – in 2020
- Bioanalytical (used)
  - calibration
  - ISTD
  - LLE
  - LazWell
- Metabolic studies
  - From 2020
- Screen types
- Semi automated cell plating
- Production (cells and vesicles)

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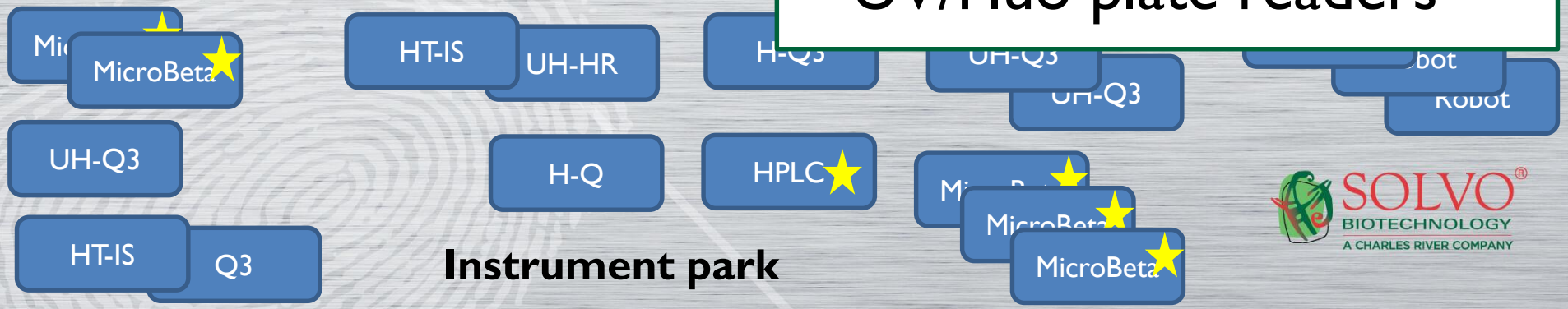
Team of Bioanalytics and Automation

- 12 FTEs, 2 consultants, 1 junior, 2 temps
  - 1 automation expert
  - 8 bioanalysts
  - 2 assistants
  - 1/2 RnD 1/2 Service SD
  - 2 engineers

- 2 Agilent UHPLC-QQQ
- 2 Thermo: UHPLC-QQQ and UHPLC-OrbiTrap
- 1 Sciex UHPLC-QTrap
- 1 Phytronix Luxon
- 1 LC-MS
- 1 RadioHPLC
- scintillation plate readers
- UV/Fluo plate readers



Instrument park

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CAPEX NEWS

- **New High Throughput systems**
  - 5500 QTRAP: MS/MS/MS for quantification and structure elucidation
  - UHPLC for fast, selective and sensitive analyses
  - HT ion source: Luxon (LDTD-APCI) for ultra-fast 10 sec analysis time

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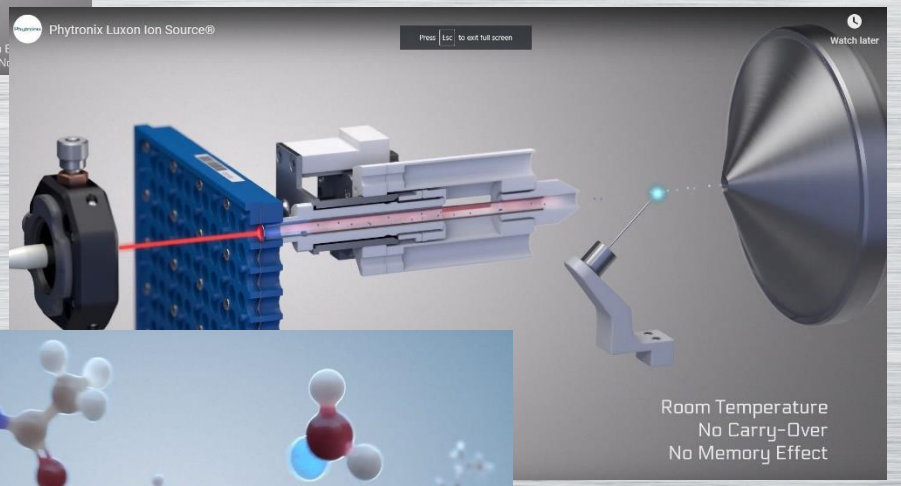
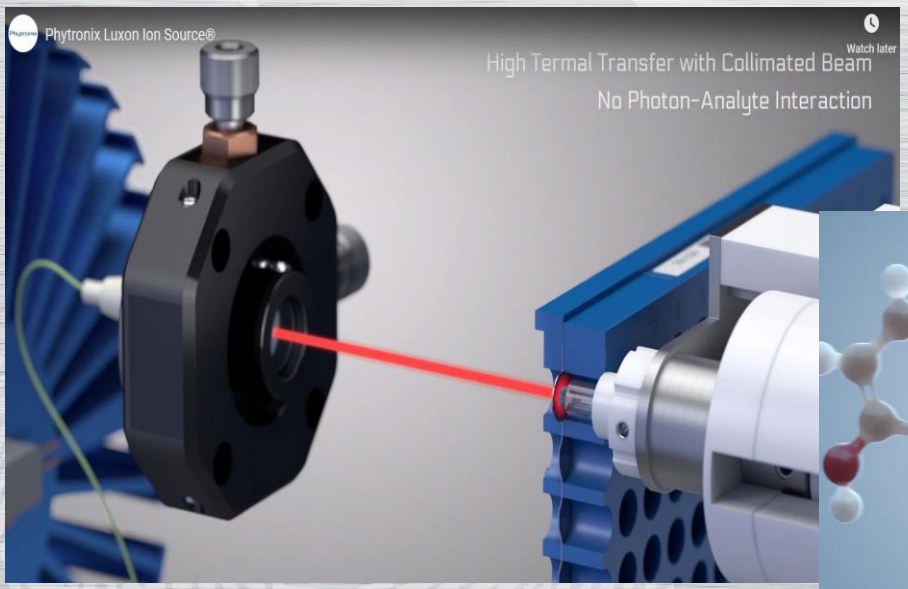
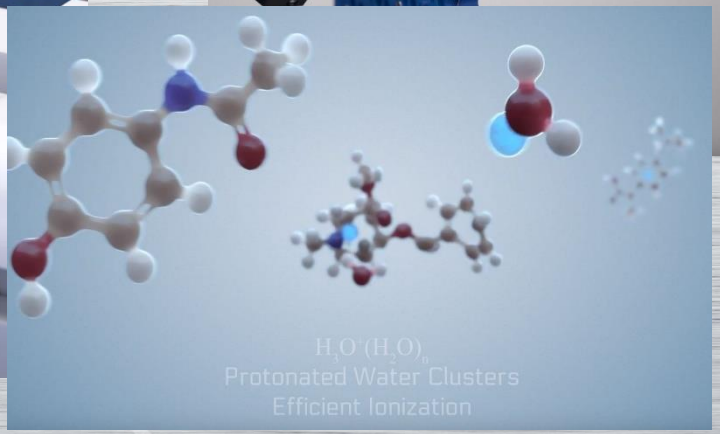
Phytronix LUXON







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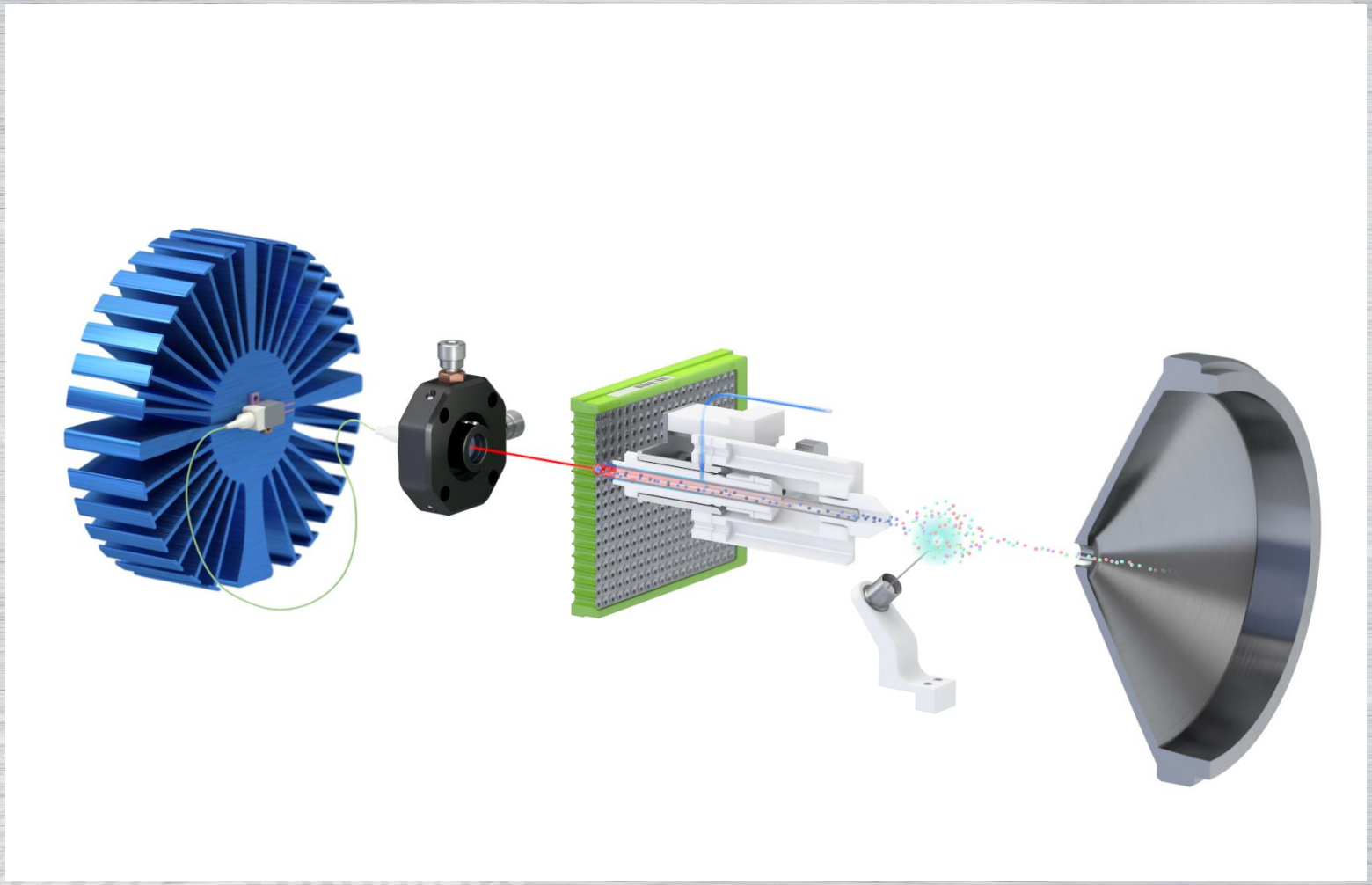
Phytronix LUXON



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AB Sciex QTrap

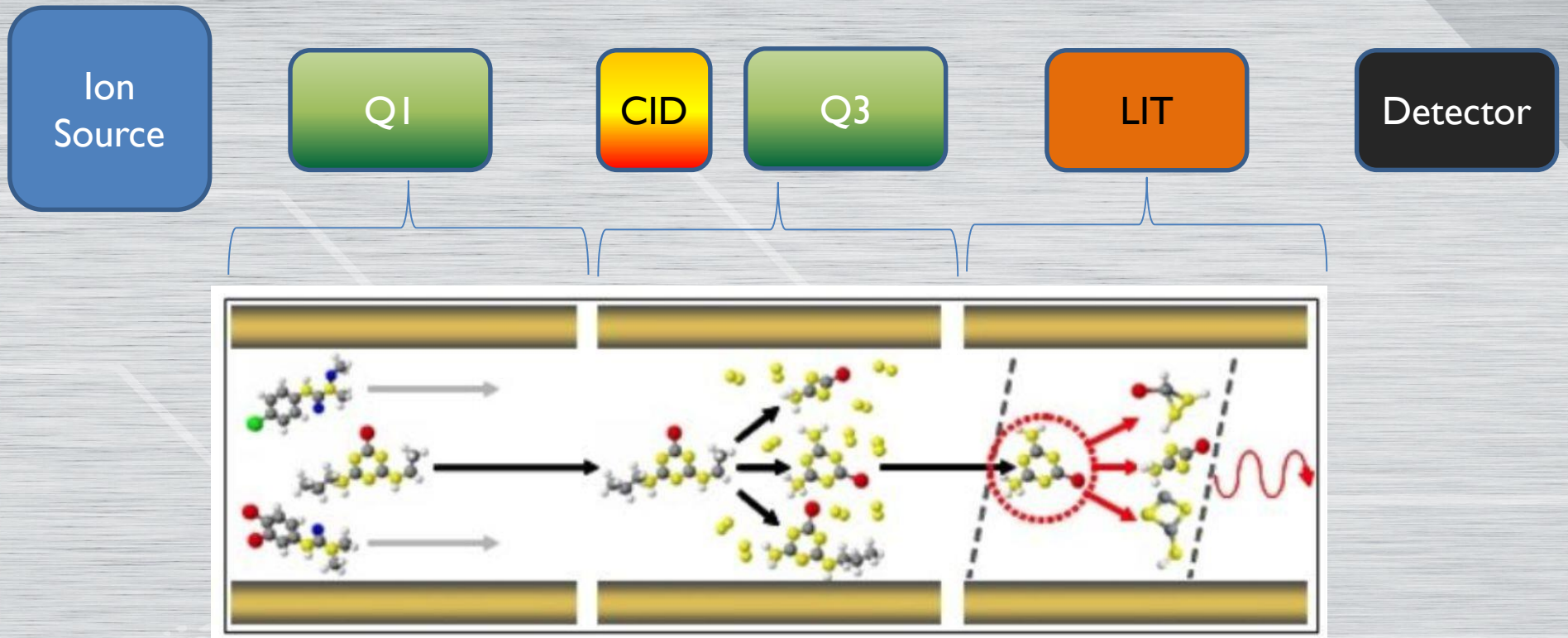

Figure 1. MRM3 for Quantitative Analysis by LC-MS. Analyte ion is first selected in the Q1 quadrupole, then fragmented in Q2 collision cell. Fragment ions are trapped then isolated in the linear ion trap, followed by excitation to perform the second fragmentation step. Second generation product ions are scanned out to the detector.

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AB Sciex QTrap

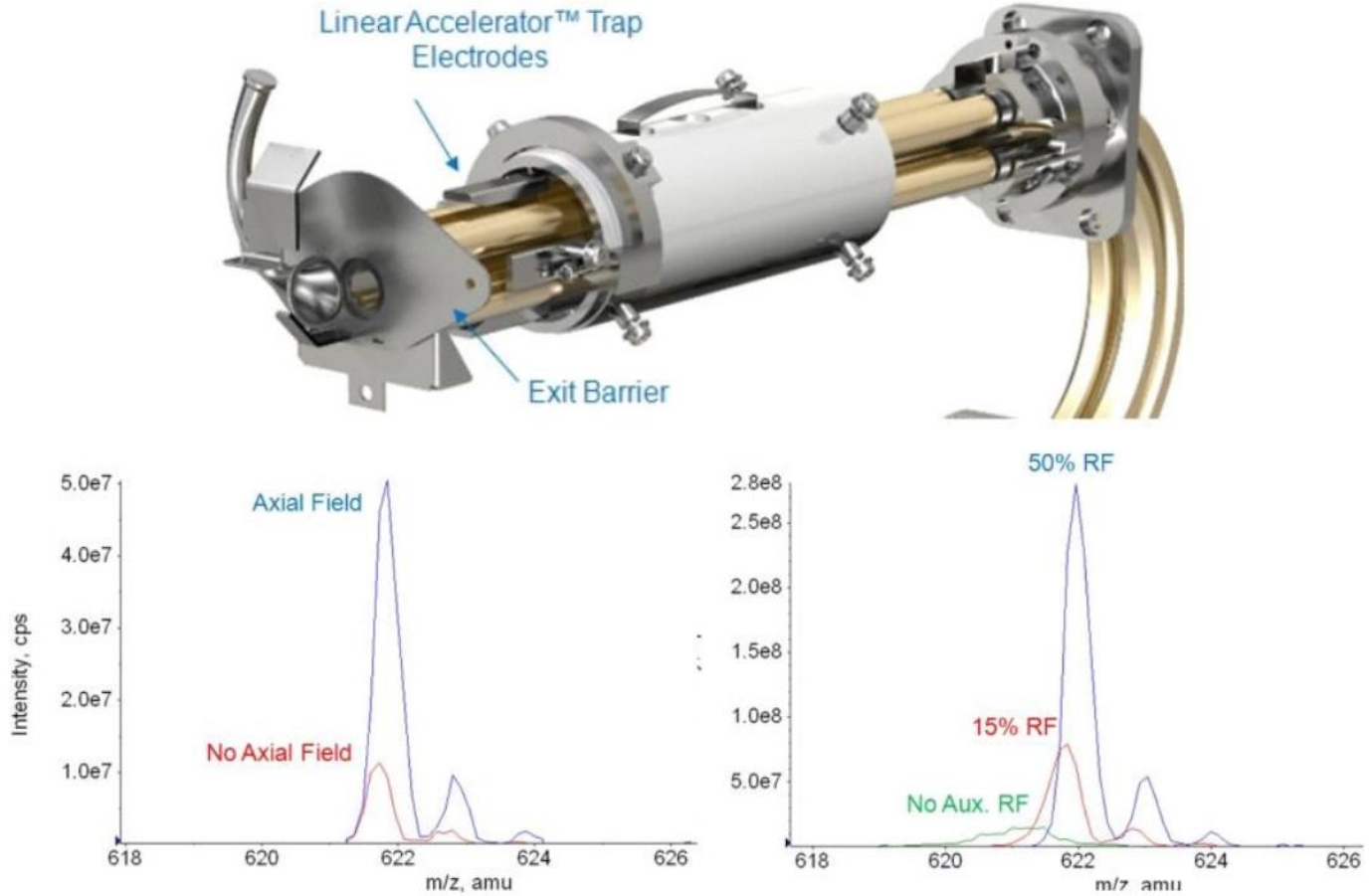

Figure 4. Linear Accelerator™ Trap Innovations for Sensitivity. Addition of electrodes in this new trap design (top) significantly improves the sensitivity of trap scanning by moving the trapped ions into the extraction region before axial ejection from the trap (bottom left). Further sensitivity gains are achieved by the addition of RF to the exit barrier of the trap (bottom right).

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AB Sciex QTrap

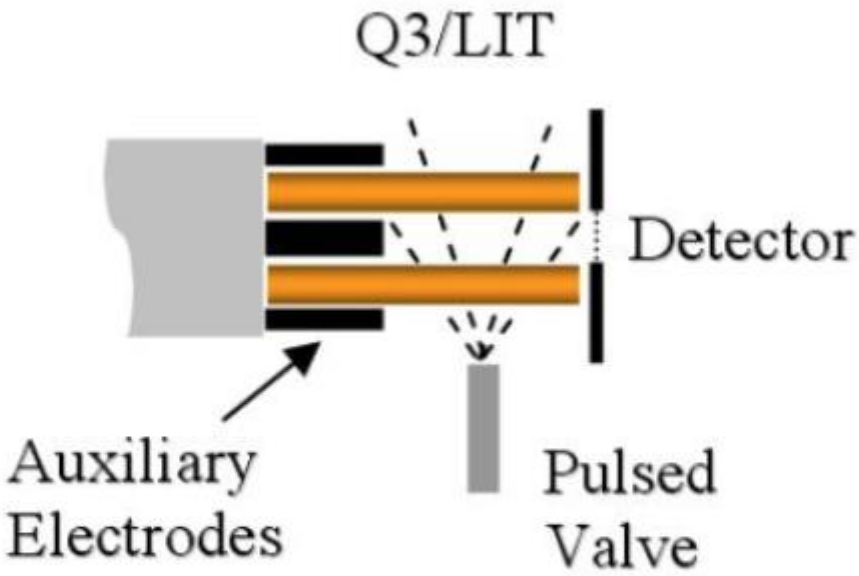

Figure 2. Pulsed Gas Valve. Gas is introduced into the linear ion trap using a high speed pulsed gas valve which rapidly increases LIT pressure and reduces required excitation time for ion trap fragmentation.

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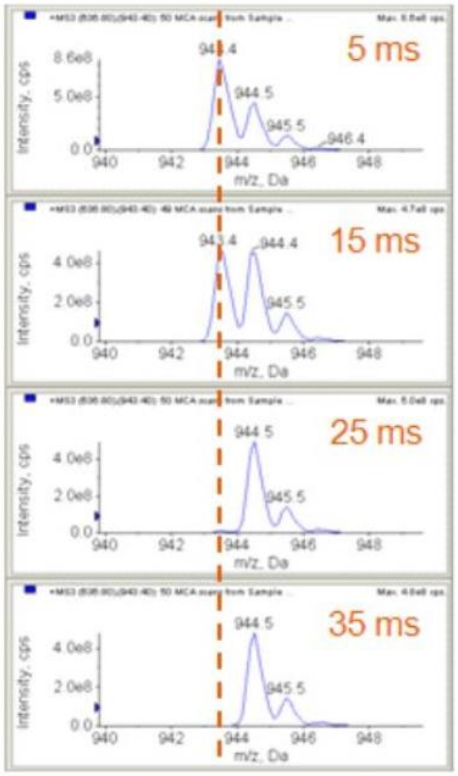
AB Sciex QTrap



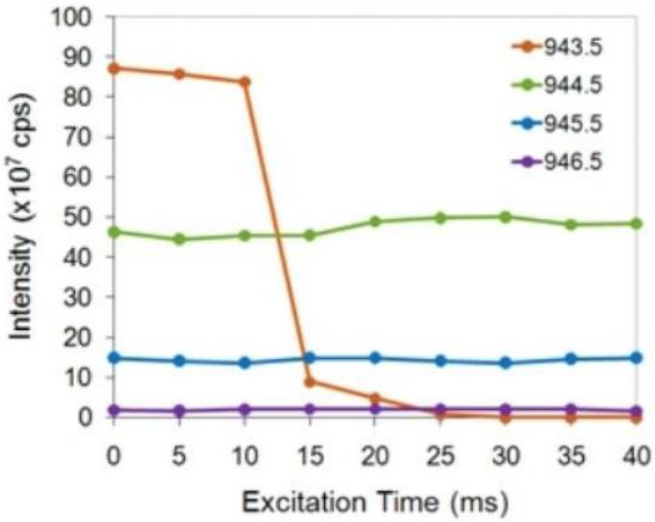


Figure 3. Single Frequency Excitation for Highest Selectivity. Narrow band excitation is used to specifically excite and fragment just the C12 isotope of the ion isolated in the LIT (left). This isotope can be fragmented to completion with no impact on the nearby C13 isotopes (right).

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Traditional LCMS vs. LDTD (Phytronix LUXON)

	Analyte Properties	Buffers	Sample prep		Analysis time	
			Instr1 LCMS	Instr2 LDTD	Instr1 LCMS	Instr2 LDTD
Compound A	Mw 👍	MeOH/water	Dilution		4 min	**15 sec**
	LLOQ ☹	KH pH 7.4	Dilution			
	~~Structure~~	HBSS pH 7.4	Dilution			
	~~Phys-chem properties~~	Assay mix (glucose)	Dilution	LLE		

Instr1: LCMS
Agilent 6460A QQQ
Agilent 1260A LC

Instr2: LDTD
Phytronix LUXON
Sciex QTrap 5500

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Traditional LCMS vs. LDTD (Phytronix LUXON)

	Instrument1 LCMS	Instrument2 LDTD
Calibration	Linear	
LLOQ	41nM	4.5nM
Accuracy%	-15-15%	-8.5-6.3%
CV%	<20%	<15%
Run time	84min	5.2min

Instr1: LCMS
Agilent 6460A QQQ
Agilent 1260A LC

Instr2: LDTD
Phytronix LUXON
Sciex QTrap 5500

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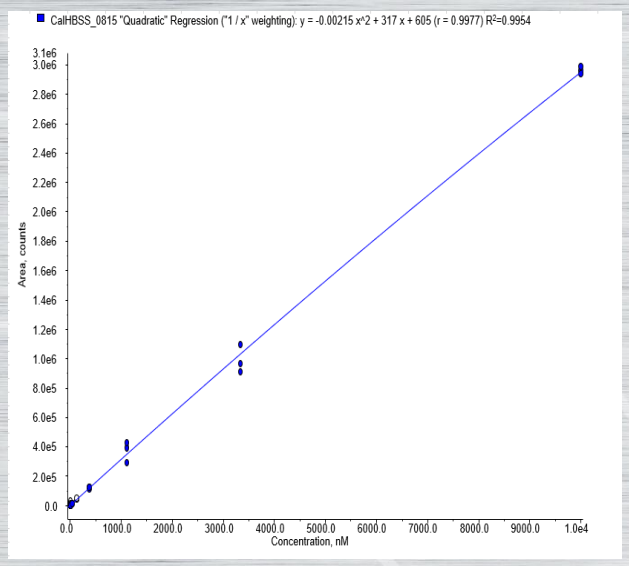
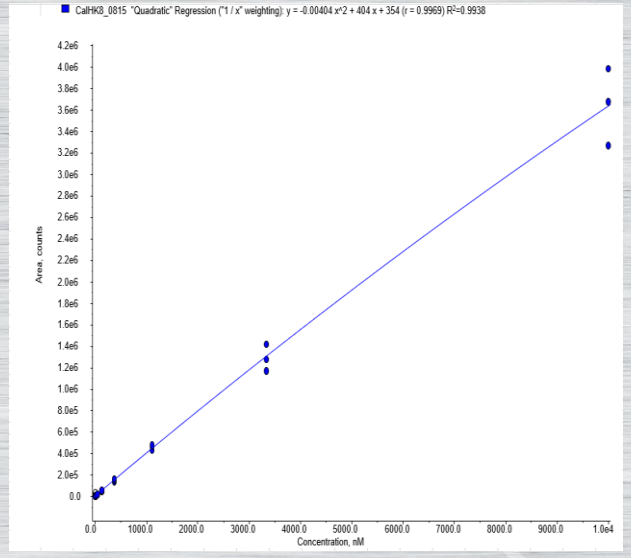
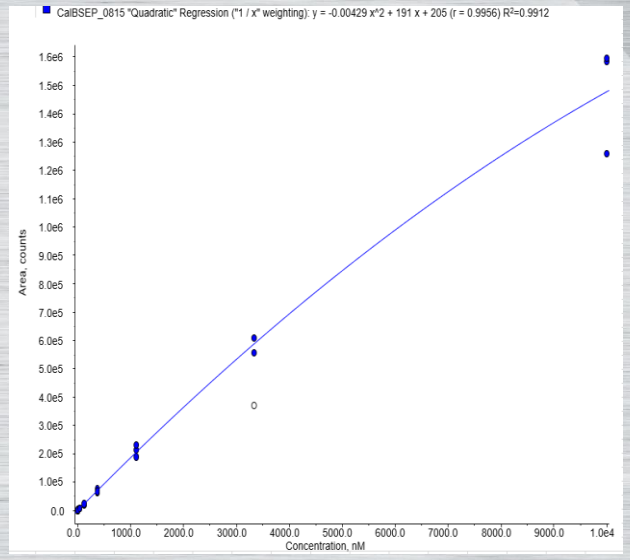
Compound B in different matrices







HBSS
LLOQ: 4.57 nM

KH pH 8.0
LLOQ: 1.52 nM

BSEP
LLOQ: 4.57 nM

Analysis time per sample: **7sec**

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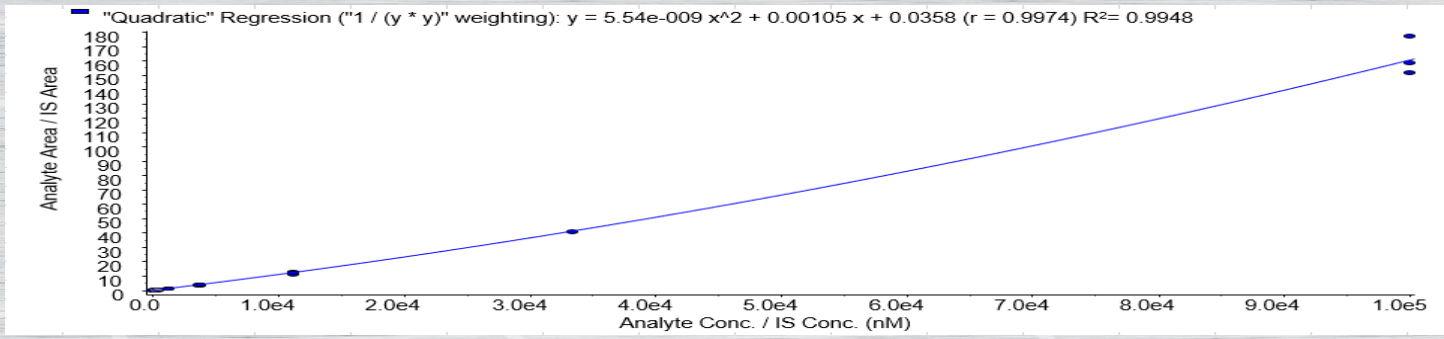
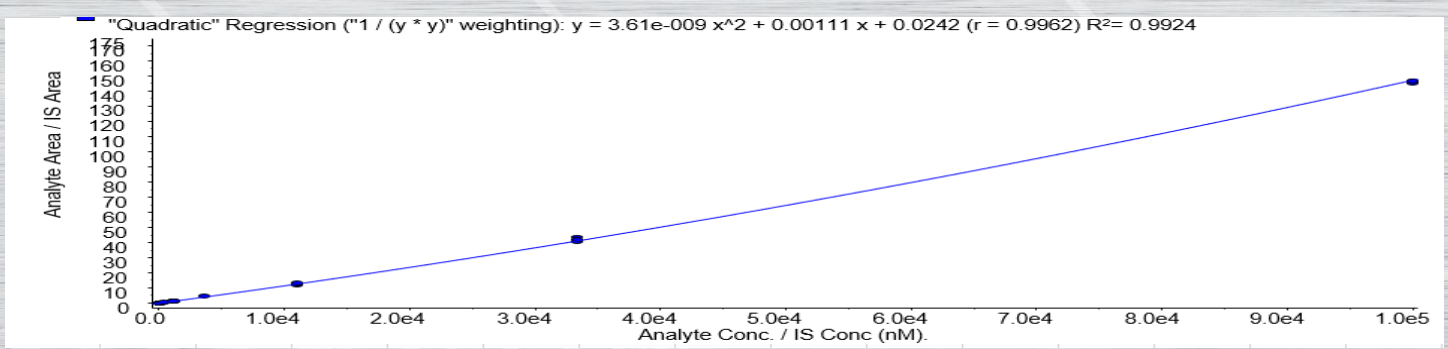
Compound C in different matrices with IS



HBSS
LLOQ: 45.72 nM



KH pH 8.0
LLOQ: 45.72 nM

Analysis time per sample: **12sec**

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Phytronix LUXON

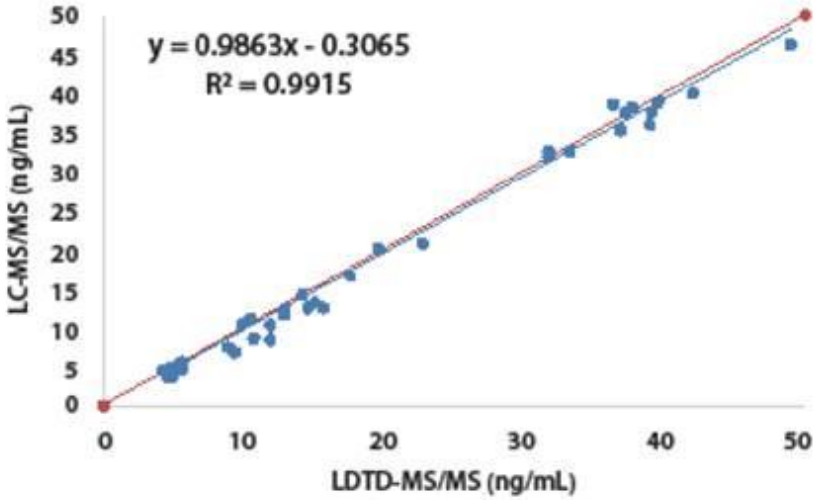
Clinical Applications

IMMUNOSUPPRESSANTS - ANTI-RETROVIRAL - THERAPEUTIC DRUGS - ETC.

The Luxon Ion Source® is a robust, rapid and precise solution with your Sciex mass spectrometer for clinical applications.

It is capable of completing the quantification of hundreds of samples, in less than 30 minutes, of a multitude of molecules in various matrices such as :

- Testosterone in plasma
- Cholesterol in serum
- Cotinine in human plasma
- Biomarker creatinine in serum
- Immunosuppressant drugs



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Advantages of LDTD over a traditional LC-MS

Pros:

- No Carry over
- No LC system
- Less matrix effect
- Low sample volumes (1-5ul/well)
- Ultra-Fast analysis (16x)
- Significantly lower LLOQ

Cons:

- Longer method development due to sample preparation steps
- Automated system for LLE, LazWell pipetting
- Costly LazWell plates

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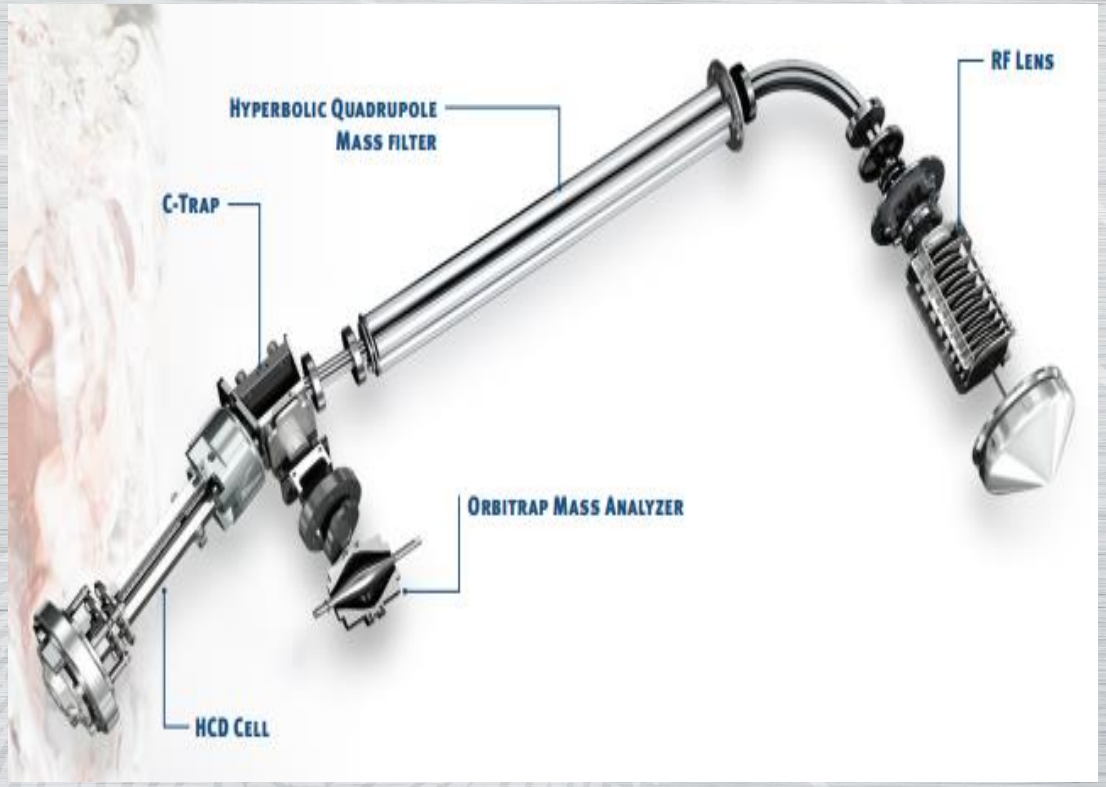
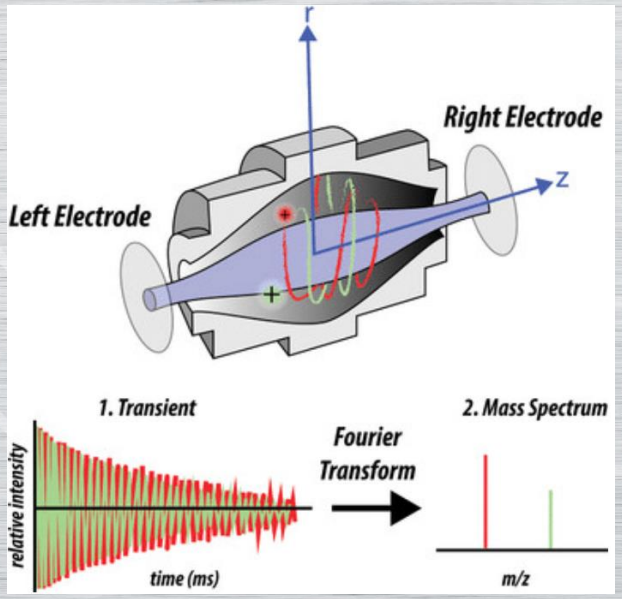
Thermo Scientific Q-Exactive Focus Mass spectrometer

Designed for routine applications, the Thermo Scientific™ Q-Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer combines high resolution accurate mass (HRAM) analysis, and highly selective quantitative and qualitative analysis in a single affordable system.





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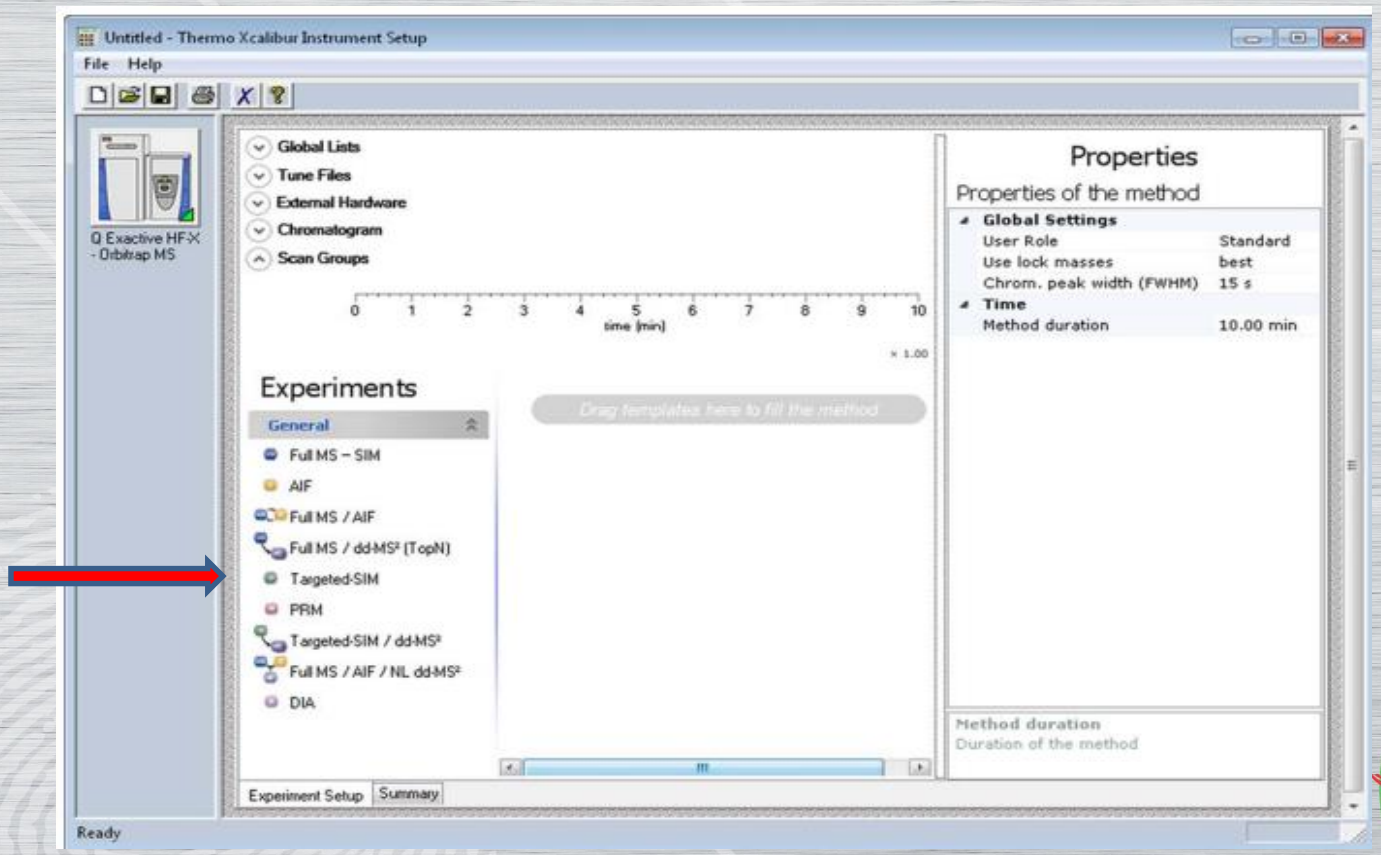
Quantitative Analysis with an OrbiTrap Q-Exactive Focus

- The Q-Exactive Focus MS offers quantitative analyses at up to 6 orders of magnitude of linear dynamic range.
- Easy to use drag-and drop Method editor with several experiment types



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Quantitative Analysis with an OrbiTrap Q-Exactive Focus

Instead of SRM or MRM the Q-Exactive Focus uses PRM experiment type for MS2

PRM

Parallel Reaction Monitoring (PRM) experiment comprises of targeted MS/MS scans of parent ions from an inclusion list.

The inclusion list contains the Precursors. The Q-Exactive Focus acquires full MS2 spectra, hence the data contains more information than a simple Triple Quad MS2 spectrum. Any product ion can be used for quantitation and confirmation from raw data.

m/z CLOUD

Thermo "m/z cloud" online database can be used for confirmation based on real MS2 spectra for 8,859 analytes and 24,387 spectral trees.

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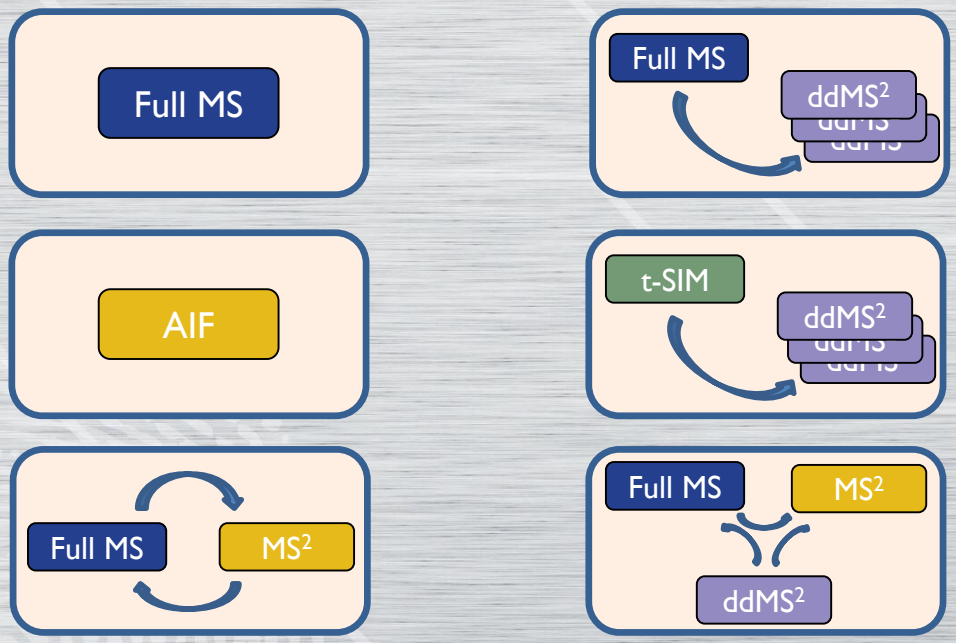
Metabolomics analysis with a Q-Exactive Focus

- Because of the High Resolution Accurate Mass feature, the Q-Exactive Focus can be used for targeted and untargeted metabolomics experiments also.
- Flexible instrument experiment types for a wide range of metabolomics applications



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Metabolomics analysis with a Q-Exactive Focus

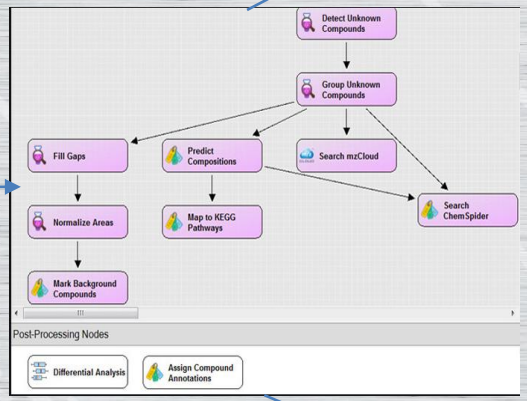
- Excellent software support for Metabolomics experiments evaluation with online and editable offline database as well

ChemSpider
The free chemical database

m/z CLOUD

Mass Frontier

BioCyc



Summary

- Quality bioanalysis of transporter assay samples
  - Efflux, Uptake, VT
  - B-Clear, HepatoPac, Proximal Tubule Cell ML, Caco-2
  - Fit-for-purpose scientific validation
- Metabolism
  - CYP inhibition and induction
  - Metabolic profile and identification
- High throughput
  - Assay and sample preparation automation
  - HT ion source for fast turnaround
- Technologies beyond triple quads
  - QTrap, OrbiTrap analyzers for quantification, qualitative information and structure elucidation

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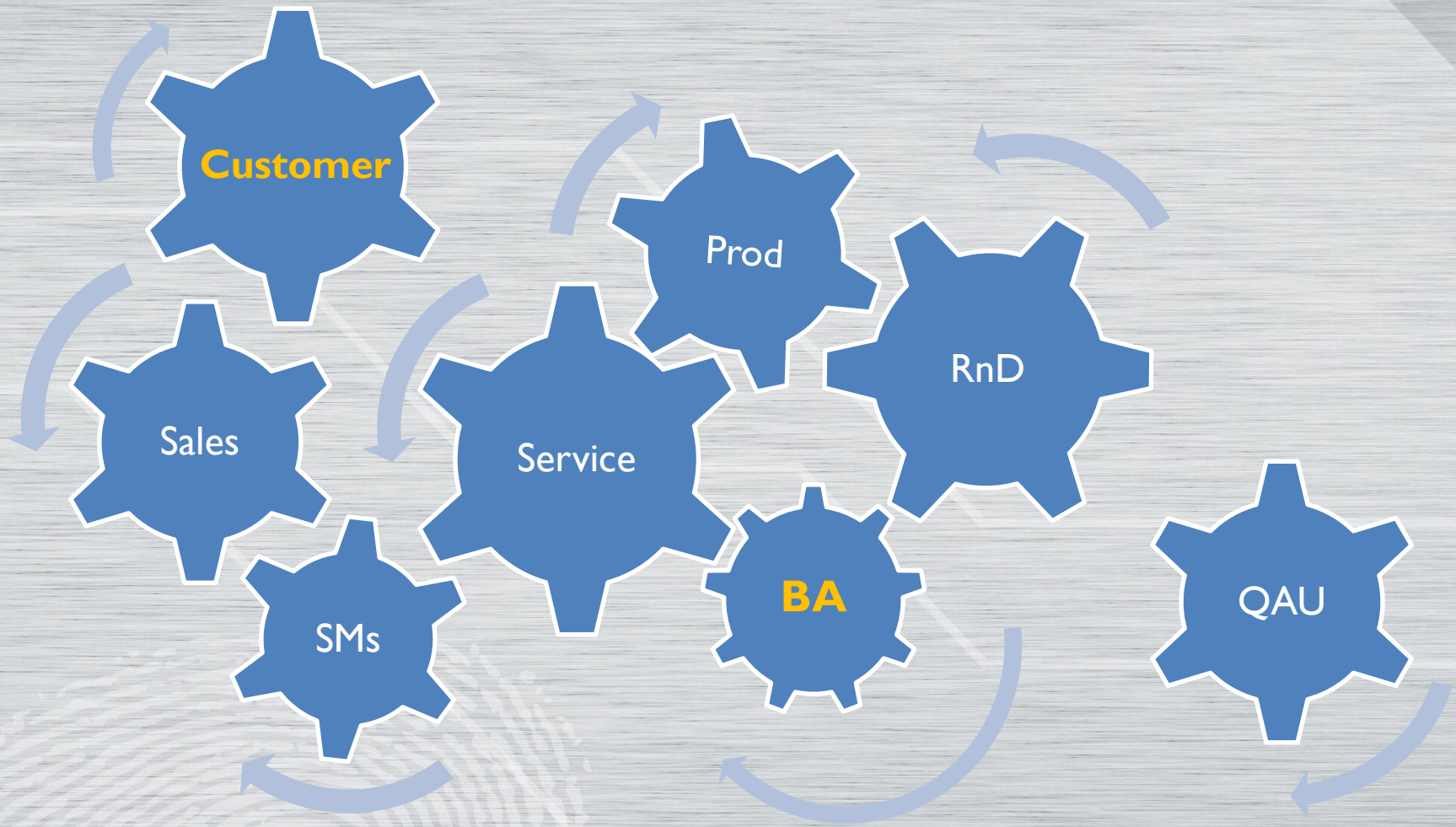
With you, for safe and potent medicines . . .



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