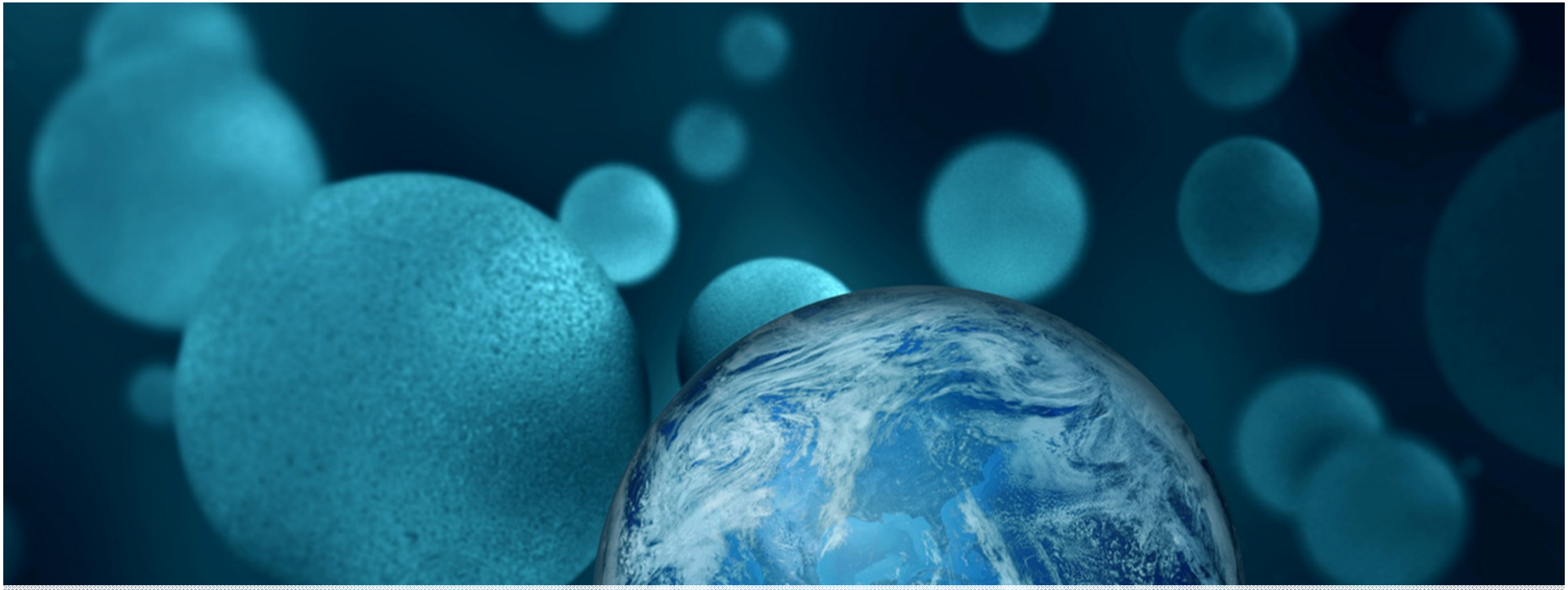

ThermoFisher SCIENTIFIC

Latest Omics Developments based on Orbitrap Mass Spectrometry

GOH Lin-Tang, PhD

Senior Manager (SEA)

The world leader in serving science

A Historical Review of (Bio)Mass Spectrometry

HISTORICAL COMMENTARY | SPECIAL FEATURE

NATURE METHODS | VOL.8 NO.8 | AUGUST 2011 | 633

A century of mass spectrometry: from atoms to proteomes

John R Yates III

Long before mass spectrometry became an important tool for cell biology, it was yielding scientific insights in physics and chemistry. Here is a brief history of how the technology has expanded from a tool for studying atomic structure and characterizing small molecules to its current incarnation as the most powerful technique for analyzing proteomes.

John R. Yates III is in the Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California, USA.
e-mail: jyates@scripps.edu

1999: The First Orbitrap Mass Analyzer Patent

US005886346A

United States Patent [19]

Makarov

[11] **Patent Number: 5,886,346**

[45] **Date of Patent: Mar. 23, 1999**

[54] **MASS SPECTROMETER**

[75] Inventor: **Alexander Alekseevich Makarov**, Manchester, United Kingdom

[73] Assignee: **HD Technologies Limited**, Manchester, United Kingdom

[21] Appl. No.: **930,568**

[22] PCT Filed: **Mar. 29, 1996**

[86] PCT No.: **PCT/GB96/00740**

§ 371 Date: **Sep. 29, 1997**

§ 102(e) Date: **Sep. 29, 1997**

[87] PCT Pub. No.: **WO96/30930**

PCT Pub. Date: **Oct. 3, 1996**

[30] **Foreign Application Priority Data**

Mar. 31, 1995 [GB] United Kingdom 9506695

[51] **Int. Cl.**[6] **H01J 49/42**

[52] **U.S. Cl.** **250/291**; 250/290

[58] **Field of Search** 250/291, 290, 250/292, 293, 295, 281, 282

[56]

U.S.

4,982,088 1/19

5,528,031 6/19

OTI

Blauth, E.W.: "Dyna
lishing Co., Amster

Primary Examiner–
Attorney, Agent, or
Crew

[57]

A mass spectromete
injection arrangem
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The electrodes (14,
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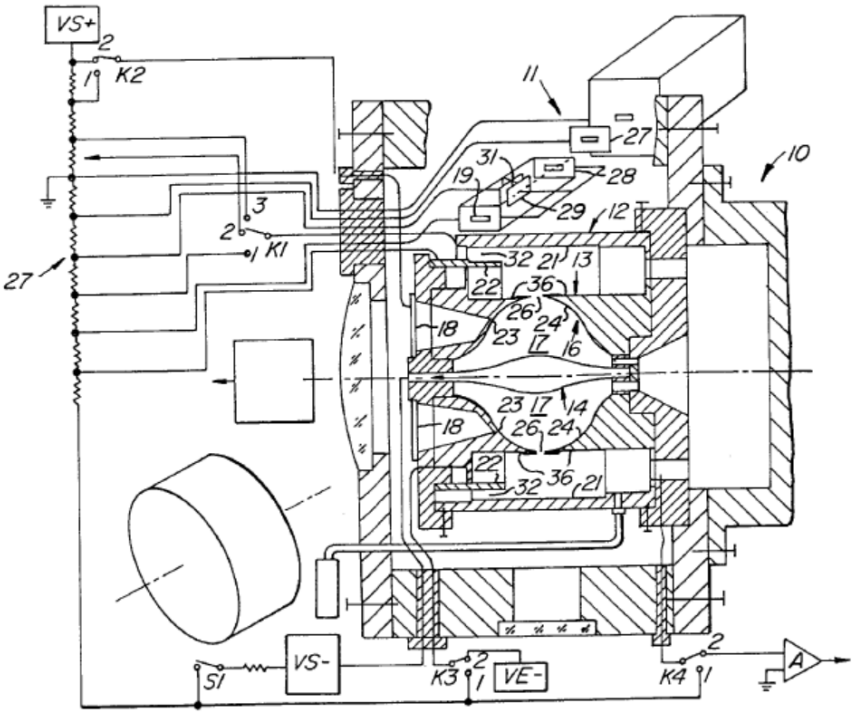
whereby ions can b
field for analysis.



22 Claims, 5 Drawing Sheets

ThermoFisher SCIENTIFIC

2000: The Foundation of a New Mass Analyzer

Anal. Chem. **2000,** *72,* 1156−1162

Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

Alexander Maka

HD Technologies L

This work describ employs trapping distribution of the tion of quadrupol absence of any achieved only du electrode. Orbiting tions along the ele $(m/z)^{-1/2}$. These current detection a using fast FT, simil trap design are pr 150 000 for ions demonstrated, alo wide mass range.

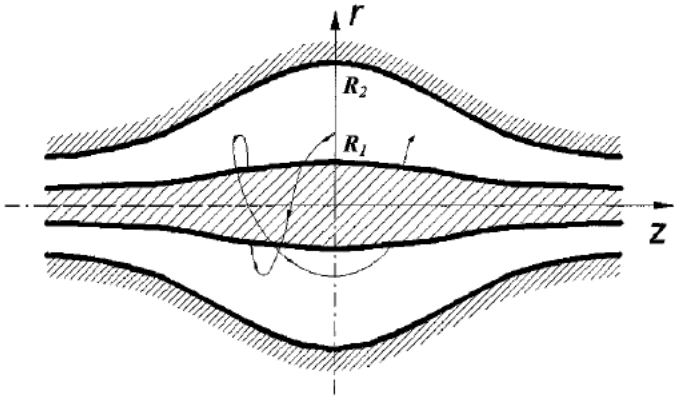


Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

current detection. However, it had been planned to derive the mass-to-charge ratio from the frequency of ion rotation. Due to the strong dependence of the rotation frequency on ion velocity and initial radius, this approach leads to poor mass resolution.

In this work, the concept of orbital trapping is freshly revised for application to mass analysis. A new type of mass analyzer is described which employs orbital trapping in an electrostatic field with potential distribution:[12,16,18]

could be deduced from eq 1:

$$z_{1,2}(r) = \sqrt{\frac{r^2}{2} - \frac{(R_{1,2})^2}{2} + (R_m)^2 \ln\left[\frac{R_{1,2}}{r}\right]} \qquad (2)$$

where index 1 denotes the central electrode, index 2 denotes the outer electrode, $z = 0$ is the plane of symmetry, and $R_{1,2}$ are the maximum radii of the corresponding electrodes.

Ion Trajectories. In the field (1), stable trajectories combine rotation around the central electrode with oscillations along the axis, resulting in an intricate spiral. Equation of motion in polar coordinates (r, φ, z) for ions with mass-to-charge ratio m/q are

$$\left.\begin{aligned} \ddot{r} - r\dot{\varphi}^2 &= -\frac{q}{m}\frac{k}{2}\left[\frac{(R_m)^2}{r} - r\right] && \text{(a)} \\ \frac{\mathrm{d}}{\mathrm{d}t}(r^2\dot{\varphi}) &= 0 && \text{(b)} \\ \ddot{z} &= -\frac{q}{m}kz && \text{(c)} \end{aligned}\right\} \qquad (3)$$

with initial conditions at the moment $t = 0$

$$r(0) = r_0 \qquad \dot{r}(0) = \dot{r}_0$$

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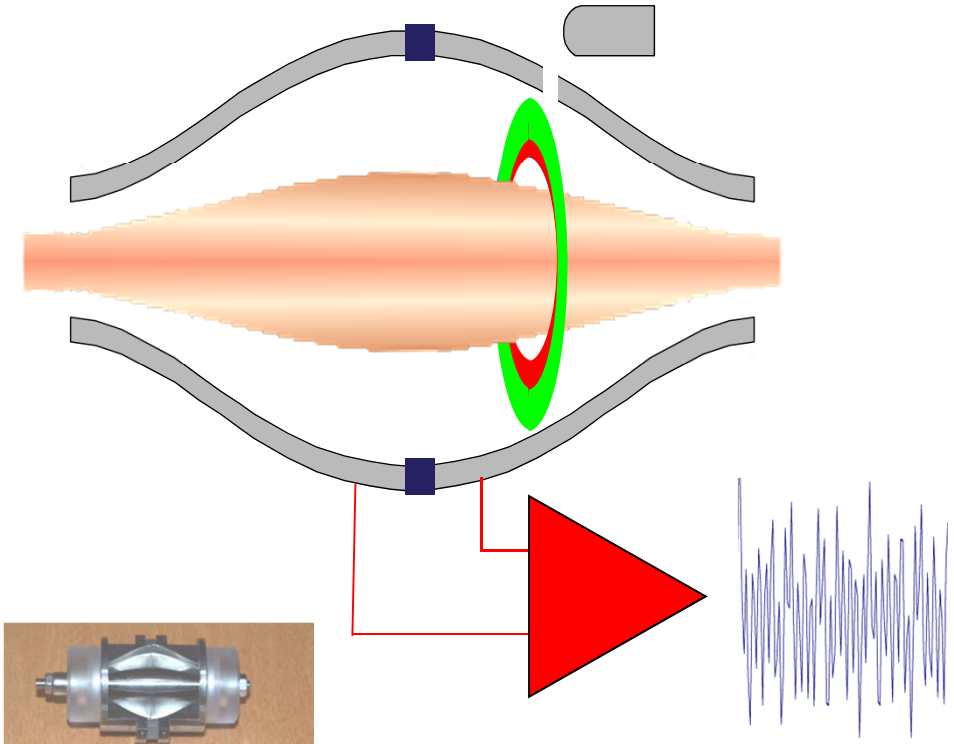
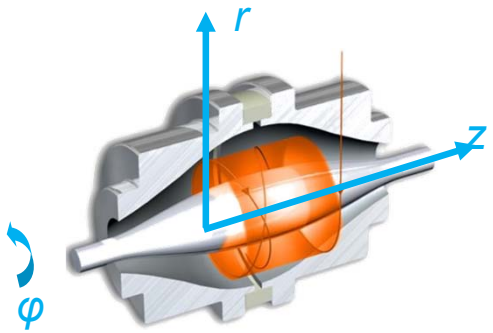
Orbitrap Mass Analyzer: Principle of Operation



$$\omega_z = \sqrt{\frac{k}{m/q}}$$



Hyper-logarithmic potential distribution: "ideal Kingdon trap"

$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

- Characteristic frequencies:
  - Frequency of rotation ω_φ
  - Frequency of radial oscillations ω_r
  - Frequency of axial oscillations ω_z

CONCLUSIONS

These results show that the orbitrap is a new and effective mass spectrometer which could potentially find its own unique niche. With mass resolution surpassed only by FT ICR, the orbitrap has the advantage of a much simpler and compact design.

To become useful for the main stream of mass spectrometric analysis, the orbitrap requires external collisional cooling and possibly external ion accumulation. These goals become the main priorities of further development work.

Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

ThermoFisher SCIENTIFIC

2003: The Beginnings were Basic and «UnExciting»

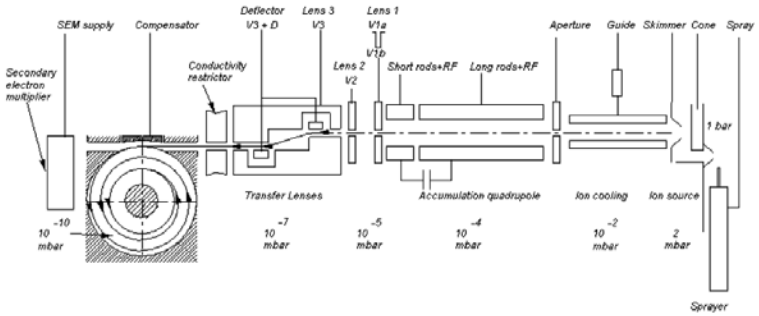
Anal. Chem. **2003,** *75,* 1699−1705

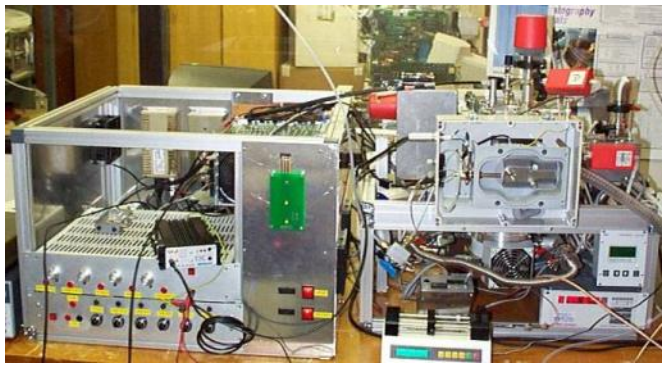
Interfacing the Orbitrap Mass Analyzer to an Electrospray Ion Source

Mark Hardman and Alexander A. Makarov*

Thermo Masslab Ltd., Crewe Road, Wythenshawe, Manchester, M23 9BE, U.K.

The orbitrap mass analyzer employs the trapping of pulsed ion beams in an electrostatic quadro-logarithmic field. This field is created between an axial central electrode and a coaxial outer electrode. Stable ion trajectories combine rotation around the central electrode with harmonic oscillations along it. The frequencies of axial oscillations and hence mass-to-charge ratios of ions are obtained using fast Fourier transform of the image current detected on the two split halves of the outer electrode. This work proves that such a trap could be coupled to a continuous, electrospray, ion source. Such a coupling necessitated the development of an rf-only quadrupole for external accumulation of ions and their injection in very short (<1 μs) ion bunches. Along with good sensitivity, this mass spectrometer provides mass resolving power up to 150 000 fwhm, mass accuracies within a few parts per million, and relative mass range up to 8-fold. The maximum number of ions available for analysis is limited by the space-charge capacity of the accumulation quadrupole.





Courtesy of A Makarov

ThermoFisher SCIENTIFIC

2005: The First Hybrid Orbitrap MS

JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2005; **40**: 430–443
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jms.856

JMS

SPECIAL FEATURE:
PERSPECTIVE

The Orbitrap: a new mass spectrometer

Qizhi Hu,[a] Robert J. Noll,[a] Hongyan Li,[a] Alexander Makarov,[b] Mark Hardman[c] and R. Graham Cooks[a]*

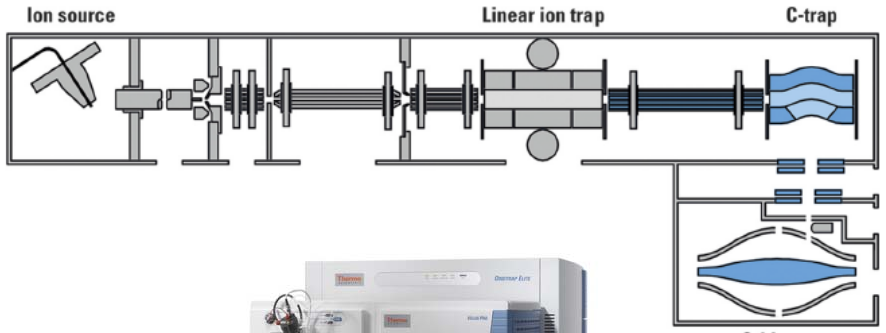
[a] Purdue University, Chemistry Department, West Lafayette, IN 47907, USA
[b] Thermo Electron (Bremen), Hanna-Kunath-Str. 11, Bremen 28199, Germany
[c] Thermo Electron (San Jose) 355 River Oaks Parkway, San Jose, CA 95134 USA

Received 4 February 2005; Accepted 15 March 2005

Research areas such as proteomics and metabolomics are driving the demand for mass spectrometers that have high performance but modest power requirements, size, and cost. This paper describes such an instrument, the Orbitrap, based on a new type of mass analyzer invented by Makarov. The Orbitrap operates by radially trapping ions about a central spindle electrode. An outer barrel-like electrode is coaxial with the inner spindlelike electrode and mass/charge values are measured from the frequency of harmonic ion oscillations, along the axis of the electric field, undergone by the orbitally trapped ions. This axial frequency is independent of the energy and spatial spread of the ions. Ion frequencies are measured non-destructively by acquisition of time-domain image current transients, with subsequent fast Fourier transforms (FFTs) being used to obtain the mass spectra.

In addition to describing the Orbitrap mass analyzer, this paper also describes a complete Orbitrap-based mass spectrometer, equipped with an electrospray ionization source (ESI). Ions are transferred from the ESI source through three stages of differential pumping using RF guide quadrupoles. The third quadrupole, pressurized to less than 10^{-3} Torr with collision gas, acts as an ion accumulator; ion/neutral collisions slow the ions and cause them to pool in an axial potential well at the end of the quadrupole. Ion bunches are injected from this pool into the Orbitrap analyzer for mass analysis. The ion injection process is described in a simplified way, including a description of electrodynamic squeezing, field compensation for the effects of the ion injection slit, and criteria for orbital stability. Features of the Orbitrap at its present stage of development include high mass resolution (up to 150 000), large space charge capacity, high mass accuracy (2–5 ppm), a mass/charge range of at least 6000, and dynamic range greater than 10.[3]

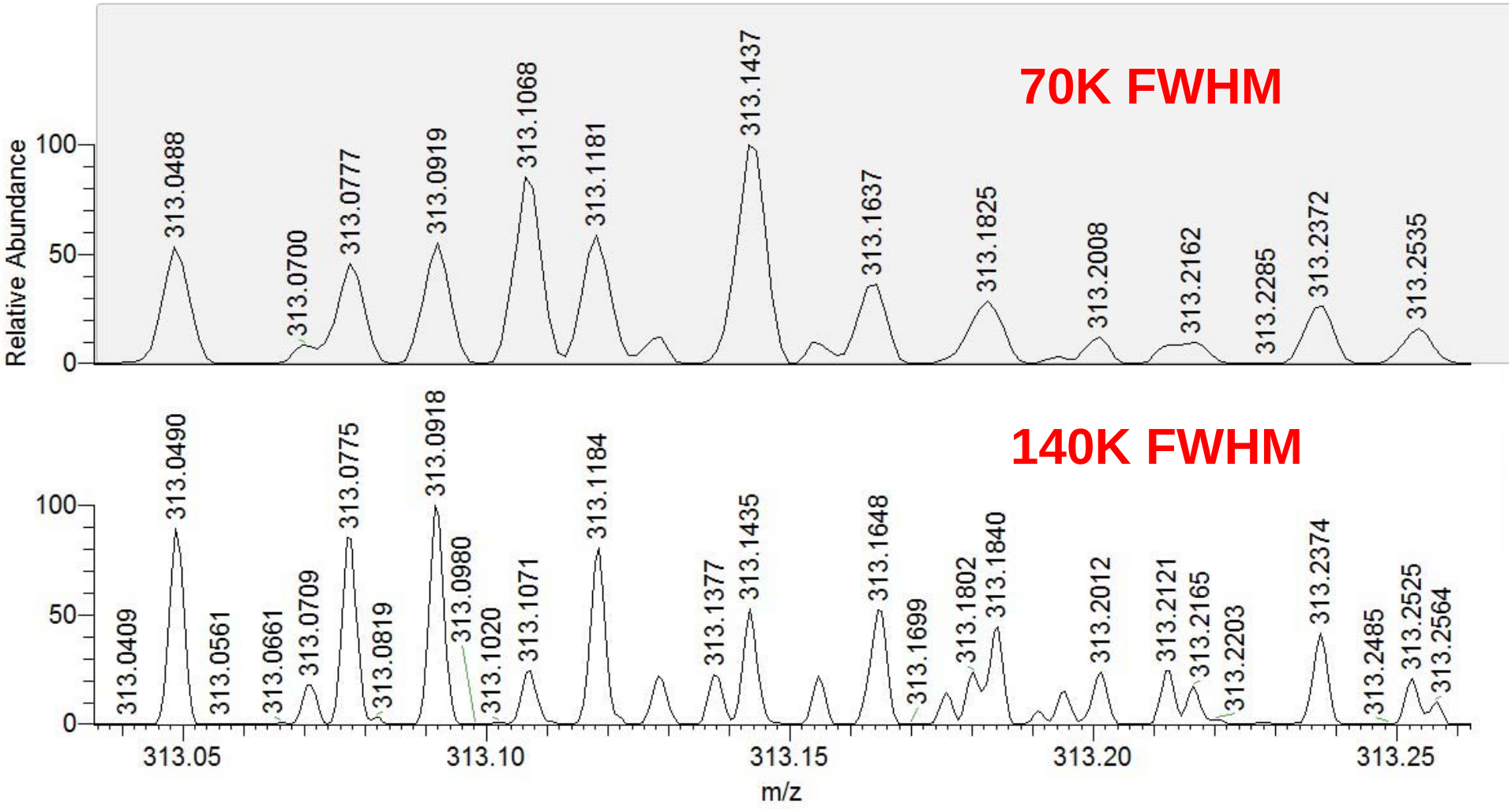
Applications based on electrospray ionization are described, including characterization of transition-metal complexes, oligosaccharides, peptides, and proteins. Use is also made of the high-resolution capabilities of the Orbitrap to confirm the presence of metaclusters of serine octamers in ESI mass spectra and to perform H/D exchange experiments on these ions in the storage quadrupole.



ThermoFisher SCIENTIFIC

Mass Resolution: The Most Direct Approach to Deal with Complexity

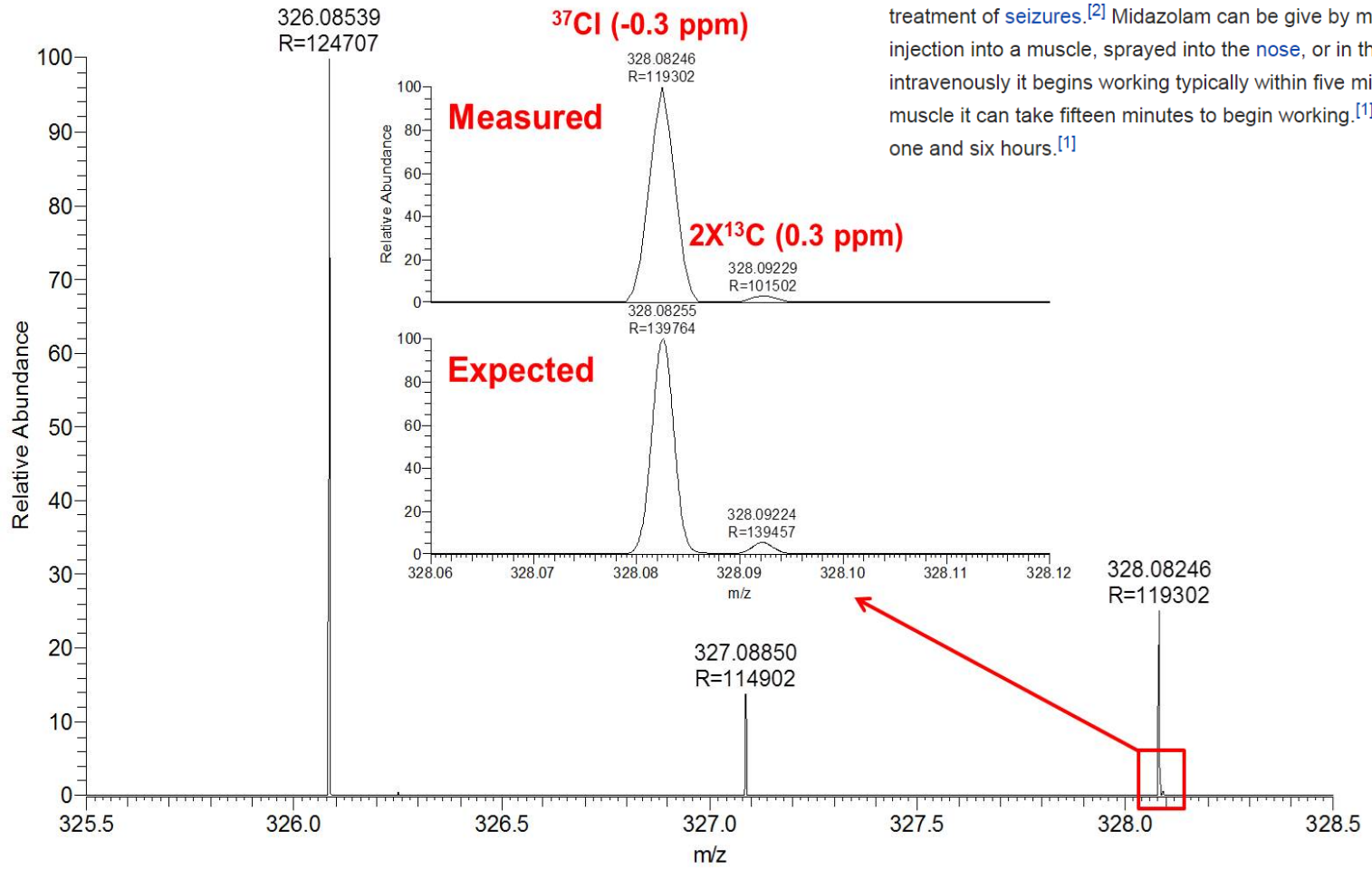
Protonated AFB1: $C_{17}H_{13}O_6$; m/z = 313.071215



ThermoFisher SCIENTIFIC

Unparallel Discriminating Power: Midazolam Mystery

Midazolam, marketed under the trade names **Versed** among others, is a medication used for anesthesia, procedural sedation, trouble sleeping, and severe agitation.[1] It works by making people sleepy, decreasing anxiety, and causing a loss of ability to create new memories.[1] It is also useful for the treatment of seizures.[2] Midazolam can be give by mouth, intravenously, by injection into a muscle, sprayed into the nose, or in the cheek.[1][2] When given intravenously it begins working typically within five minutes, when injected into a muscle it can take fifteen minutes to begin working.[1] Effects last for between one and six hours.[1]



ThermoFisher SCIENTIFIC

2006: Success on the GO!

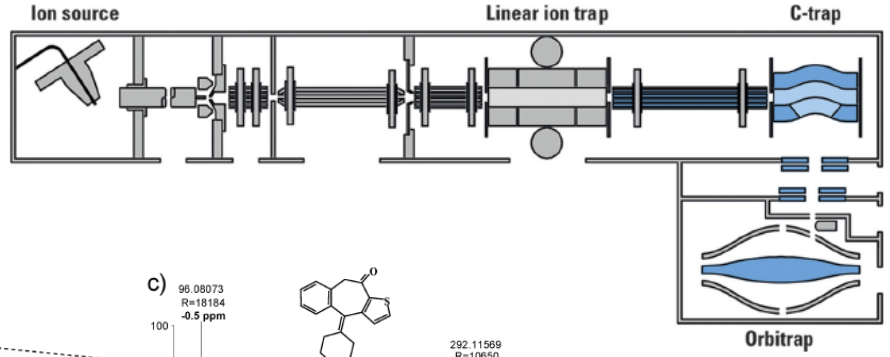
Anal. Chem. **2006,** *78,* 2113−2120

Accelerated Articles

Performance Evaluation of a Hybrid Linear Ion Trap/Orbitrap Mass Spectrometer

Alexander Makarov,* Eduard Denisov, Alexander Kholomeev, Wilko Balschun, Oliver Lange, Kerstin Strupat, and Stevan Horning

Thermo Electron (Bremen) GmbH, Hanna-Kunath-Strasse 11, Bremen 28199 Germany



Design and performance of a novel hybrid mass spectrometer is described. It couples a linear ion trap mass spectrometer to an orbitrap mass analyzer via an r trapping quadrupole with a curved axis. The latter i pulsed ion beams into a rapidly changing electric fi the orbitrap wherein they are trapped at high k energies around an inner electrode. Image current tion is subsequently performed after a stable electro field is achieved. Fourier transformation of the acq transient allows wide mass range detection with resolving power, mass accuracy, and dynamic range entire instrument operates in LC/MS mode (1 spect s) with nominal mass resolving power of 60 000 and automatic gain control to provide high-accuracy measurements, within 2 ppm using internal stan and within 5 ppm with external calibration. The maxi resolving power exceeds 100 000 (fwhm). Rapid, mated data-dependent capabilities enable real-tim quisition of up to three high-mass accuracy M spectra per second.

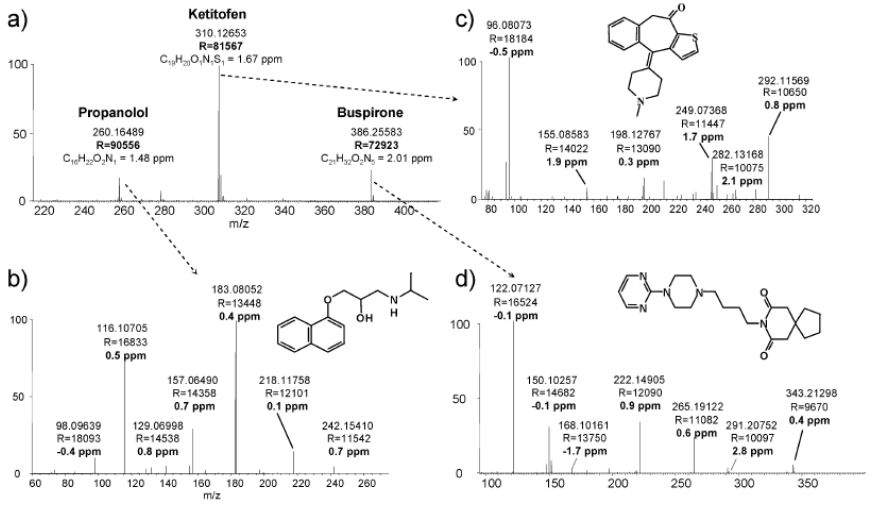


Figure 9. Example of data-dependent acquisition with external mass calibration for a sample containing small molecules, with one high-resolution mass spectrum recorded of the precursors at $R = 60\,000$ and $N = 500\,000$ (a) followed by three data-dependent MS/MS spectra at $R = 7500$, $N = 30\,000$, (b) for precursor at $m/z = 260$, (c) for precursor at $m/z = 310$, and (d) for precursor at $m/z = 386$.

ThermoFisher SCIENTIFIC

2007: Addition of HCD to Complement Ion Trap CID

NATURE METHODS | VOL.4 NO.9 | SEPTEMBER 2007 | 709

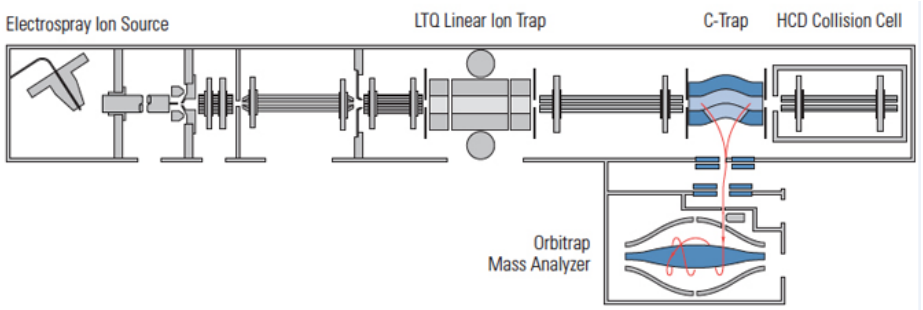
Higher-energy C-trap dissociation for peptide modification analysis

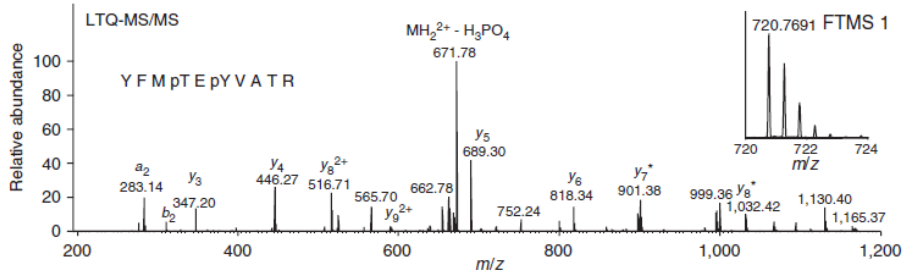
Jesper V Olsen[1], Boris Macek[1], Oliver Lange[2], Alexander Makarov[2], Stevan Horning[2] & Matthias Mann[1]

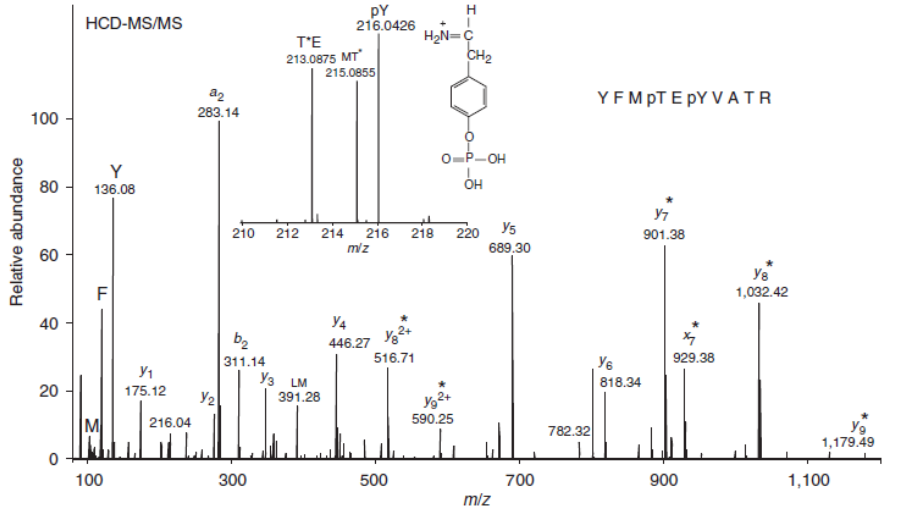
Peptide sequencing is the basis of mass spectrometry–driven proteomics. Here we show that in the linear ion trap–orbitrap mass spectrometer (LTQ Orbitrap) peptide ions can be efficiently fragmented by high-accuracy and full-mass-range tandem mass spectrometry (MS/MS) via higher-energy C-trap dissociation (HCD). Immonium ions generated via HCD pinpoint modifications such as phosphotyrosine with very high confidence. Additionally we show that an added octopole collision cell facilitates *de novo* sequencing.

[1]Department for Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Am Klopferspitz 18, D-82131 Martinsried, Germany. [2]Thermo Fisher Scientific (Bremen) GmbH, Hanna-Kunath-Strasse 11, D-28199 Bremen, Germany. Correspondence should be addressed to S.H. (stevan.horning@thermofisher.com) or M.M. (mmann@biochem.mpg.de).

RECEIVED 19 MARCH; ACCEPTED 30 JULY; PUBLISHED ONLINE 26 AUGUST 2007; DOI:10.1038/NMETH1060







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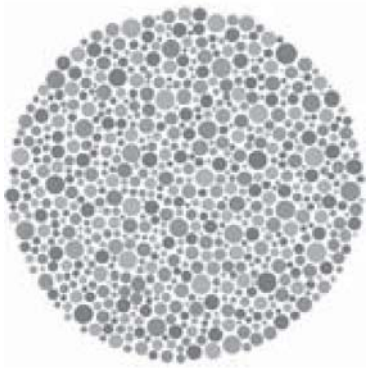
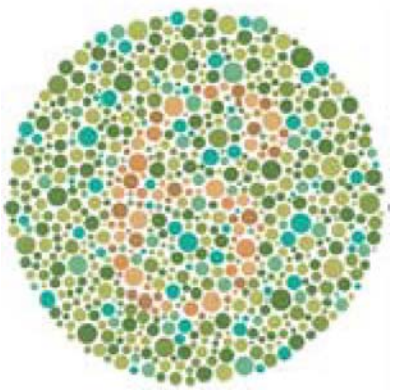
Moving Beyond Qualitative Proteomics

Problem: Quantitative information about expression level of a protein is essential to understanding its biological role in response to change or disease.

Qualitative

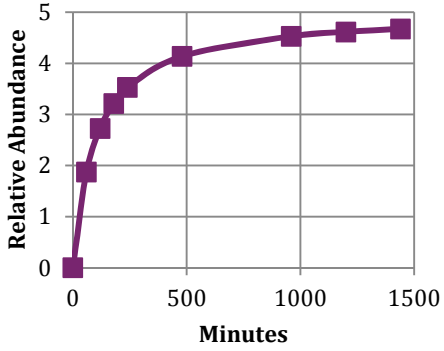


Quantitative

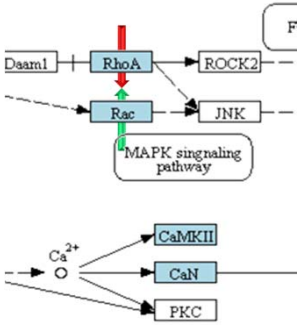


Add another dimension to any experiment by determining the relative abundance of each identified protein

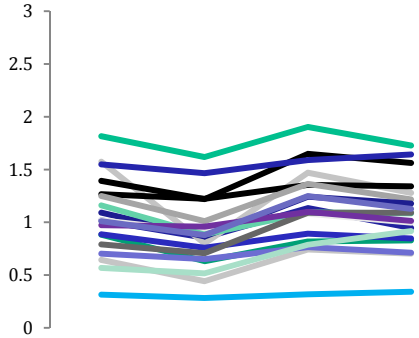
Alterations in expression can reveal a meaningful biological pattern not apparent in a pure identification experiment, which provides only a list of detected proteins



Changes with time



Changes with treatment

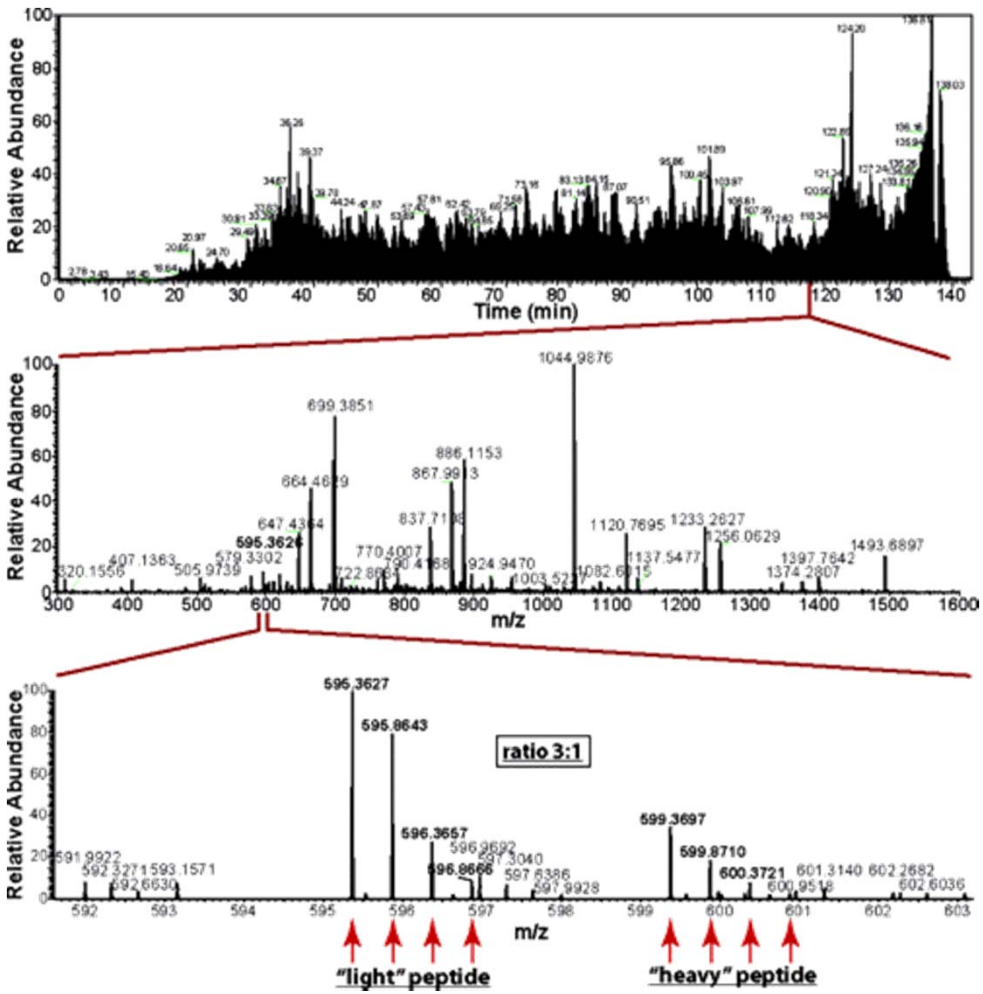


Changes with cell line

ThermoFisher SCIENTIFIC

2008: Enabling Absolute SILAC Experiments

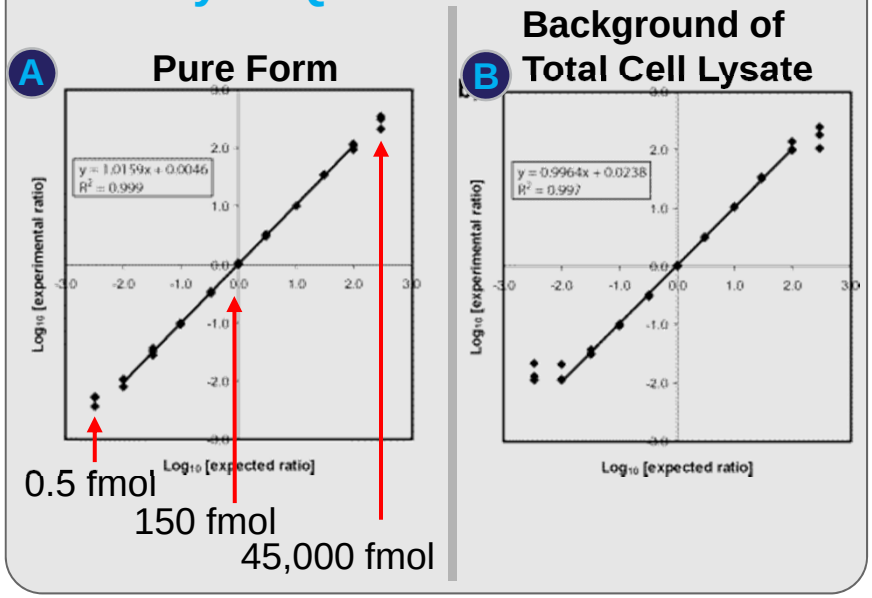
S. Hanke, H. Besir, D. Oesterhelt, M. Mann
J. Proteome Res. **2008,** 7 (3), 1118–1130.



Experiment requires:

- ✓ *Resolving power*
- ✓ *Mass accuracy*
- ✓ *Dynamic Range*
- ✓ *Parallel MS/MS*
- ✓ *Scan Speed*
- ✓ *Sensitivity*

Linearity of Quantitation of a Protein



ThermoFisher SCIENTIFIC

2008: Introduction of ETD on Hybrid Orbitrap MS

research articles Journal of proteome research

A Proteomics Grade Electron Transfer Dissociation-Enabled Hybrid Linear Ion Trap-Orbitrap Mass Spectrometer

Graeme C. McAlister,[†] W. Travis Berggren,[⊥] Jens Griep-Raming,[∥] Stevan Horning,[∥] Alexander Makarov,[∥] Doug Phanstiel,[†] George Stafford,[§] Danielle L. Swaney,[†] John E. P. Syka,[§] Vlad Zabrouskov,[§] and Joshua J. Coon*[,†,‡]

Departments of Chemistry and Biomolecular Chemistry, University of Wisconsin, Madison, Wisconsin 53706, Thermo Fisher Scientific, San Jose, California 95134, Thermo Fisher Scientific, Bremen, Germany, and WiCell Research Institute, Madison, Wisconsin 53706

Received April 08, 2008

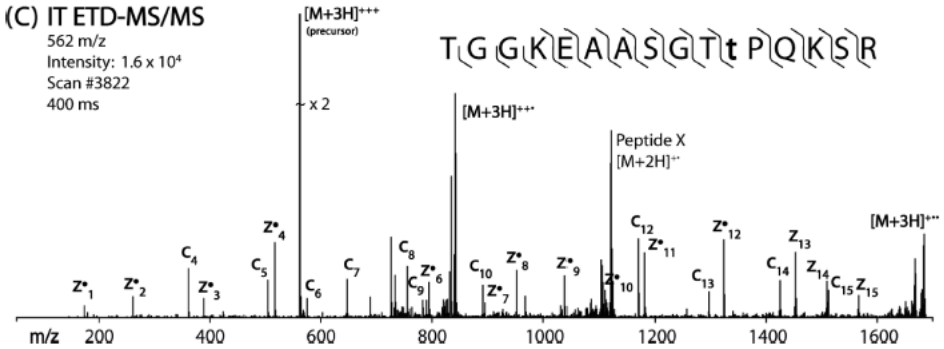


Figure 5. The full-MS spectrum of a SILAC-labeled peptide pair is shown in panel A. The orbitrap affords excellent mass resolution and accuracy so that the pair is easily distinguished from one another. Panel B displays the selected ion chromatograms for these two species. The ETD-MS/MS spectrum was acquired using the ion trap and is presented in panel C. From this spectrum, we can easily deduce sequence and localize the site of phosphorylation (t), and from the selected ion chromatogram (panel B), we conclude this phosphorylation site is upregulated ~5-fold.

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EThcD: The Best of Both Worlds

Journal of proteome research

Technical Note

pubs.acs.org/jpr

Terms of Use

Unambiguous Phosphosite Localization using Electron-Transfer/ Higher-Energy Collision Dissociation (EThcD)

Christian K. Frese,[†,‡] Houjiang Zhou,[†,‡] Thomas Taus,[§] A. F. Maarten Altelaar,[†,‡] Karl Mechtler,[§,∥] Albert J. R. Heck,[*,†,‡] and Shabaz Mohammed[*,†,‡]

[†]Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

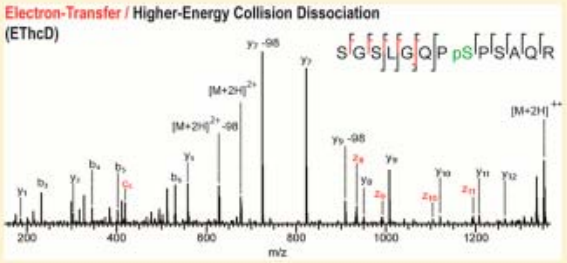
[‡]Netherlands Proteomics Centre, Padualaan 8, 3584 CH Utrecht, The Netherlands

[§]Research Institute of Molecular Pathology (IMP), Dr. Bohrgasse 7, A-1030 Vienna, Austria

[∥]Institute of Molecular Biotechnology (IMBA), Vienna, Austria

Supporting Information

ABSTRACT: We recently introduced a novel scheme combining electron-transfer and higher-energy collision dissociation (termed EThcD), for improved peptide ion fragmentation and identification. We reasoned that phosphosite localization, one of the major hurdles in high-throughput phosphoproteomics, could also highly benefit from the generation of such EThcD spectra. Here, we systematically assessed the impact on phosphosite localization utilizing EThcD in comparison to methods employing either ETD or HCD, respectively, using a defined synthetic phosphopeptide mixture and also using a larger data set of Ti^{4+}-IMAC enriched phosphopeptides from a tryptic human cell line digest. In combination with a modified version of phosphoRS, we observed that in the majority of cases EThcD generated richer and more confidently identified spectra, resulting in superior phosphosite localization scores. Our data demonstrates the distinctive potential of EThcD for PTM localization, also beyond protein phosphorylation.



KEYWORDS: *electron transfer dissociation, ETD, HCD, EThcD, phosphorylation site localization, phosphoRS*

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2011: Fully Featured & Enhanced Hybrid Orbitrap (Elite)

Author's Choice

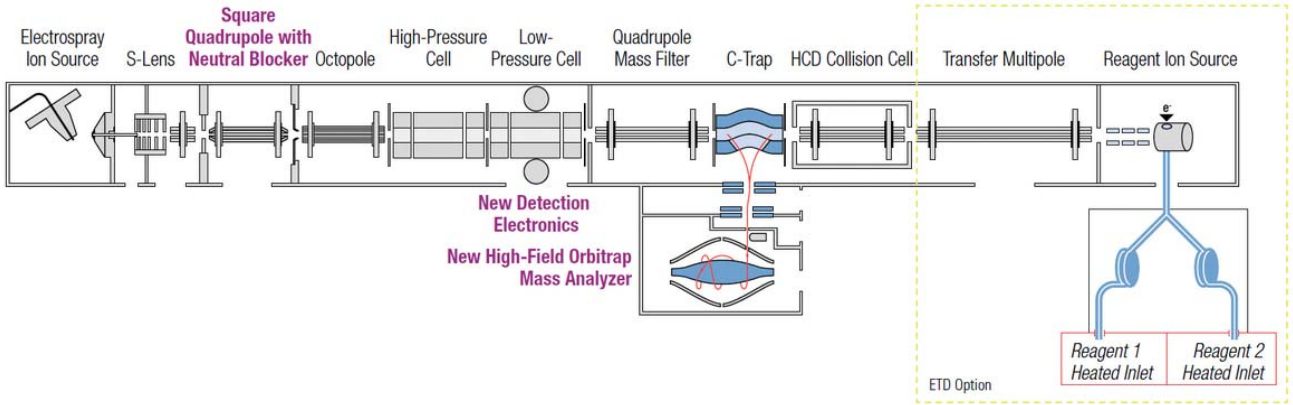
Deep and Highly Sensitive Proteome Coverage by LC-MS/MS Without Prefractionation*

Suman S. Thakur‡¶, Tamar Geiger‡¶, Bhaswati Chatterjee‡, Peter Bandilla‡, Florian Fröhlich§, Juergen Cox‡, and Matthias Mann‡‖

In-depth MS-based proteomics has necessitated fractionation of either proteins or peptides or both, often requiring considerable analysis time. Here we employ long liquid chromatography runs with high resolution coupled to an instrument with fast sequencing speed to investigate how much of the proteome is directly accessible to liquid chromatography-tandem MS characterization without any prefractionation steps. Triplicate single-run analyses identified 2990 yeast proteins, 68% of the total measured in a comprehensive yeast proteome. Among them, we covered the enzymes of the glycolysis and gluconeogenesis pathway targeted in a recent multiple reaction monitoring study. In a mammalian cell line, we identified 5376 proteins in a triplicate run, including representatives of 173 out of 200 KEGG metabolic and signaling pathways. Remarkably, the majority of proteins could be detected in the samples at sub-femtomole amounts and many in the low attomole range, in agreement with absolute abundance estimation done in previous works (Picotti *et al.* Cell, 138, 795–806, 2009). Our results imply an unexpectedly large dynamic range of the MS signal and sensitivity for

expressed in a given cellular state (6).

Notwithstanding these successes, an intrinsic challenge in MS-based proteomics remains the large "dynamic range" of protein abundance levels; at least four orders of magnitude in yeast (7, 8) and even larger in human cells. In the standard "shotgun" proteomics strategy the enzymatic digestion of proteins to peptides followed by liquid chromatography tandem mass spectrometry (LC MS/MS)[1] further compounds the complexity and dynamic range challenges (9, 10). For in-depth analysis of very complex mixtures such as those represented in total cell lysates, at least one step of protein or peptide fractionation is therefore always employed before LC MS/MS. However, each additional fractionation step is accompanied by corresponding increases in the required starting material and in the required measurement time. Furthermore, because of the very high sensitivity of modern mass spectrometers, peptides and proteins can easily be found in several adjacent biochemical fractions, diminishing the contribution of classical biochemical fractionation to achieving deep coverage of the proteome. In contrast, LC is in principle



ThermoFisher SCIENTIFIC

2011: Introduction of Quadrupole-Orbitrap MS Platform

⌘ *Author's Choice*

Mass Spectrometry-based Proteomics Using Q Exactive, a High-performance Benchtop Quadrupole Orbitrap Mass Spectrometer*[S]

Annette Michalski‡, Eugen Damoc§, Jan-Peter Hauschild§, Oliver Lange§, Andreas Wieghaus§, Alexander Makarov§, Nagarjuna Nagaraj‡, Juergen Cox‡, Matthias Mann‡¶, and Stevan Horning§¶

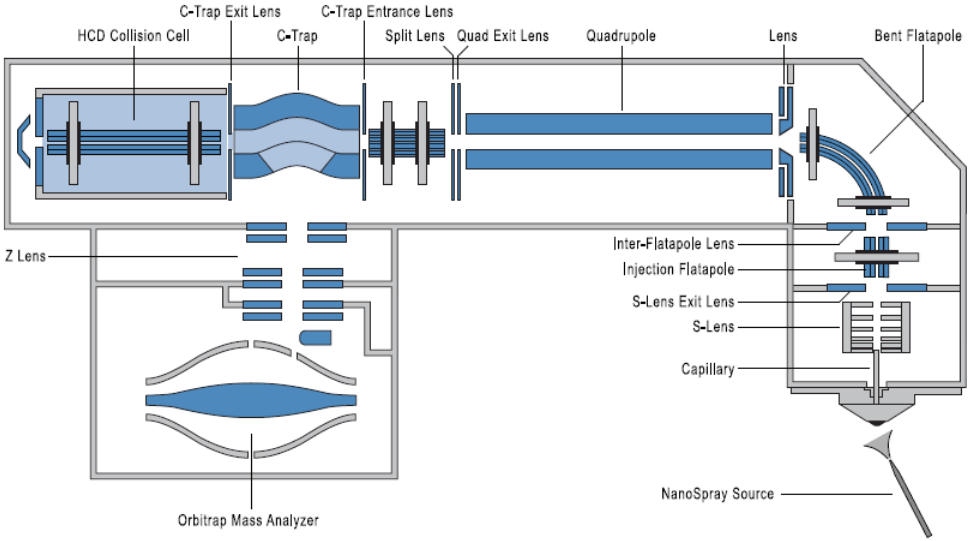
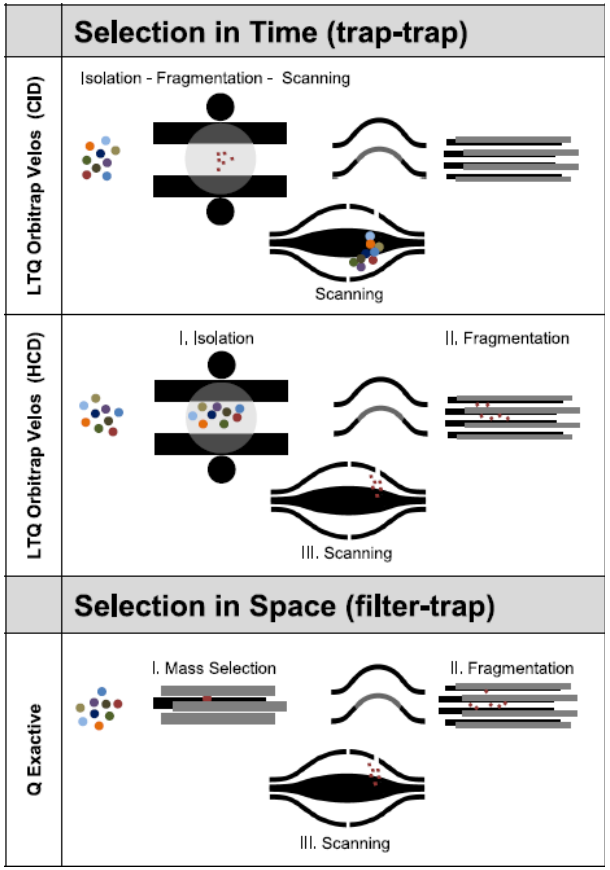


FIG. 2. **Construction details of the Q Exactive.** This instrument is based on the Exactive platform but incorporates an S-lens, a mass selective quadrupole, and an HCD collision cell directly interfaced to the C-trap. Note that the drawing is not to scale.



ThermoFisher SCIENTIFIC

2011: A Superior Fully-Benchtop Orbitrap MS Platform

Rapid Commun. Mass Spectrom. **2013**, *27*, 157–162
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6437

Comparison of the LTQ-Orbitrap Velos and the Q-Exactive for proteomic analysis of 1–1000 ng RAW 264.7 cell lysate digests

Liangliang Sun, Guijie Zhu and Norman J. Dovichi*
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

RATIONALE: There is interest in extending bottom-up proteomics to the smallest possible sample size. We investigated the performance of two modern mass spectrom cell lysate digests.
METHODS: An ultra-performance liquid chrom a Q-Exactive mass spectrometer was used for p
RESULTS: For 1–1000 ng RAW 264.7 cell lysate d more peptides than the LTQ-Orbitrap Velos (high due to its faster scan rate and higher resolution. LTQ-Orbitrap Velos were compared. HCD pr RAW 264.7 cell lysate digests with MASCOT dat were also compared and comparable protein gro
CONCLUSIONS: The Q-Exactive outperformed RAW 264.7 cell lysate digests in terms of obtained

Table 2. Identification results of 1–1000 ng RAW 264.7 cell lysate digest after analysis with the Q-Exactive and LTQ-Orbitrap Velos (HCD) in duplicate runs*

	Protein groups	Peptides	Peptide spectrum matches (PSMs)	MS/MS	Identification rate (%)	MASCOT significance threshold (FDR<1%#)
			Q-Exactive			
1000 ng	1382	5570	13895	33797	41.11	0.017
500 ng	1310	5471	13347	32001	41.71	0.018
100 ng	810	2975	6883	17341	39.69	0.016
50 ng	663	2111	4049	11017	36.75	0.0093
10 ng	344	970	1830	5233	34.97	0.0070
5 ng[a]	200/242	426/588	755/992	2550/3952	29.61/25.10	0.0019/0.0059
1 ng[a]	22/81	48/147	79/247	457/1497	17.29/16.50	0.020/0.020
			LTQ-Orbitrap Velos (HCD)			
1000 ng	1255	5023	12493	33695	37.08	0.0050
500 ng	913	3651	9148	23469	38.98	0.0030
100 ng	617	2251	4602	11384	40.43	0.0035
50 ng	382	1184	2324	7505	30.97	0.0020
10 ng	219	515	877	2942	29.81	0.0010
5 ng	132	282	480	2133	22.50	0.0010
1 ng	48	77	116	672	17.26	0.0030

*Results were from MASCOT database searching.
[a]Two kinds of instrument methods (shown in Table 1) were used for the analysis of 1 ng and 5 ng cell lysate digests. The first number was from a maximum injection time for MS/MS of 120 ms and an intensity threshold of 1.00E+05, and the second number was from a maximum injection time for MS/MS of 250 ms and an intensity threshold of 1.00E+04.
#FDR ranged from around 0.7% to 0.9% for 5–1000 ng cell lysate digest data. For 1 ng cell lysate digest data, the FDR was 0.

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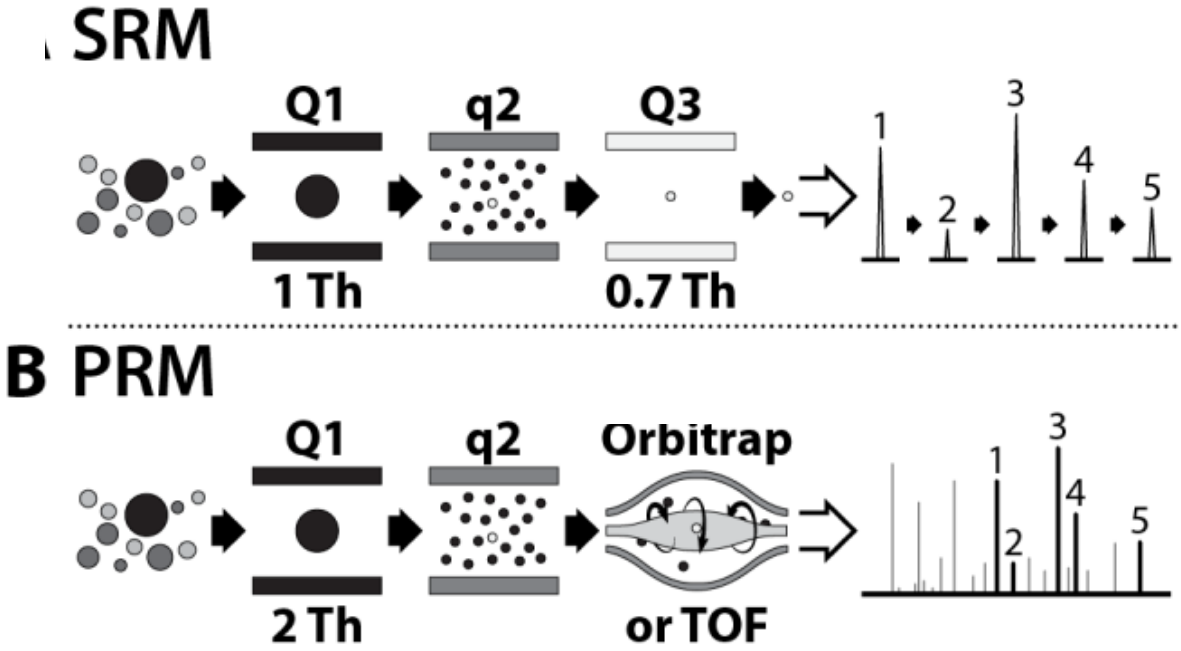
QqQ MRM vs Orbitrap-based HR-MSMS (PRM)

PARALLEL REACTION MONITORING FOR HIGH RESOLUTION AND HIGH MASS ACCURACY QUANTITATIVE, TARGETED PROTEOMICS

Amelia C. Peterson, Jason D. Russell, Derek J. Bailey, Michael S. Westphall, Joshua J. Coon[†]

Departments of Chemistry and Biomolecular Chemistry, and Genome Center of Wisconsin, University of Wisconsin – Madison, Madison, WI, USA

[†]Corresponding author: 425 Henry Mall, Madison, WI, 53706; tel (608) 263-1718; jcoon@chem.wisc.edu



- *Serial* monitoring
- ***Parallel*** monitoring

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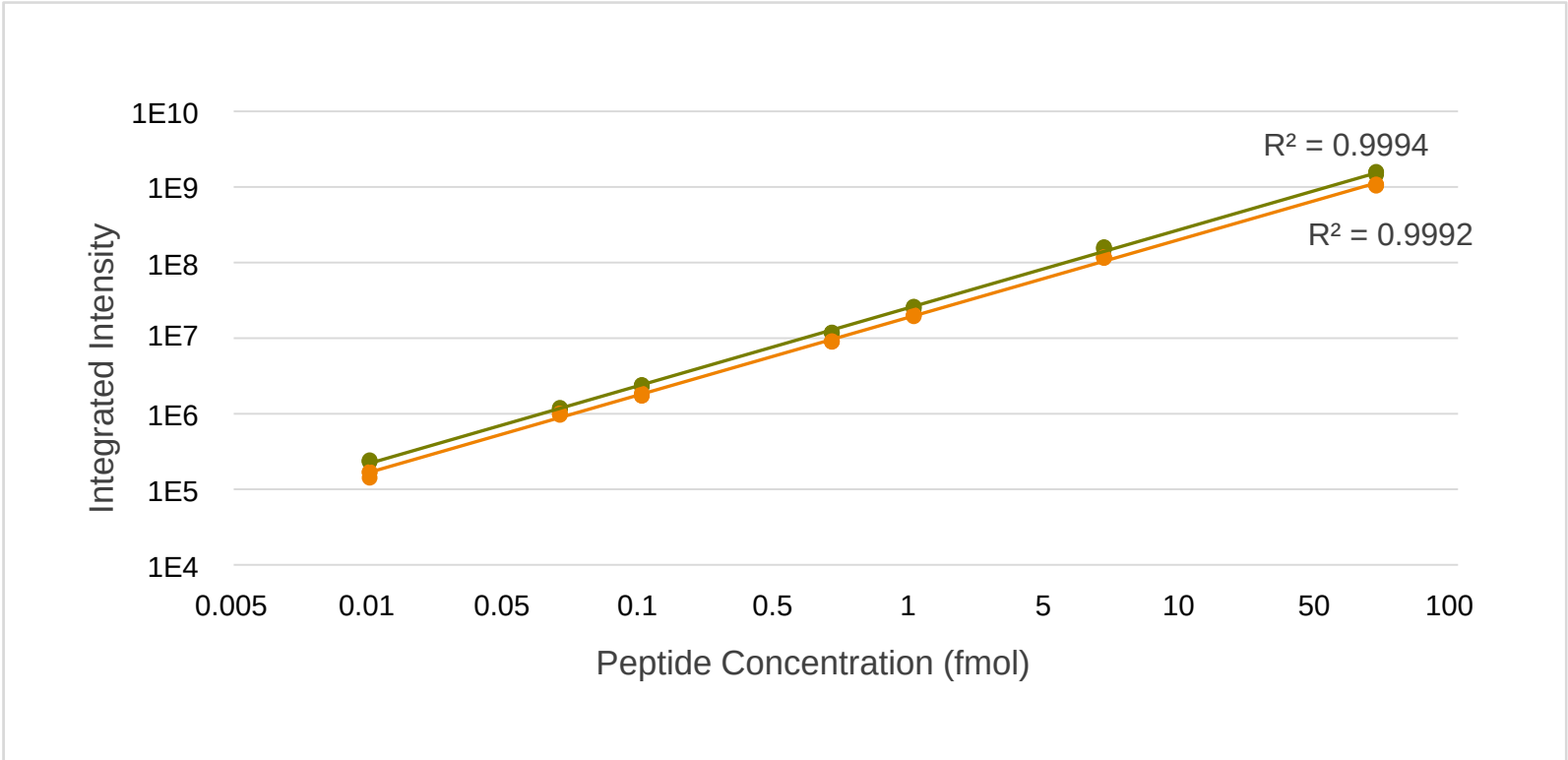
Linearity & CV of PRM



Peptide	CV (0.05 fmol)	CV (0.01 fmol)
GISNEGQNASIK (2 amu)	2.19%	9.70%
GISNEGQNASIK (0.7 amu)	2.09%	2.03%

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2014: Recent HRMS Comparison Study by US FDA

J. Am. Soc. Mass Spectrom. (2014) 25:1285–1294
DOI: 10.1007/s13361-014-0880-5

RESEARCH ARTICLE

Mass Accuracy and Isotopic Abundance Measurements for HR-MS Instrumentation: Capabilities for Non-Targeted Analyses

Ann M. Knolhoff, John H. Callahan, Timothy R. Croley

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, HFS-707, College Park, MD 20740, USA

U.S. Department of Health and Human Services

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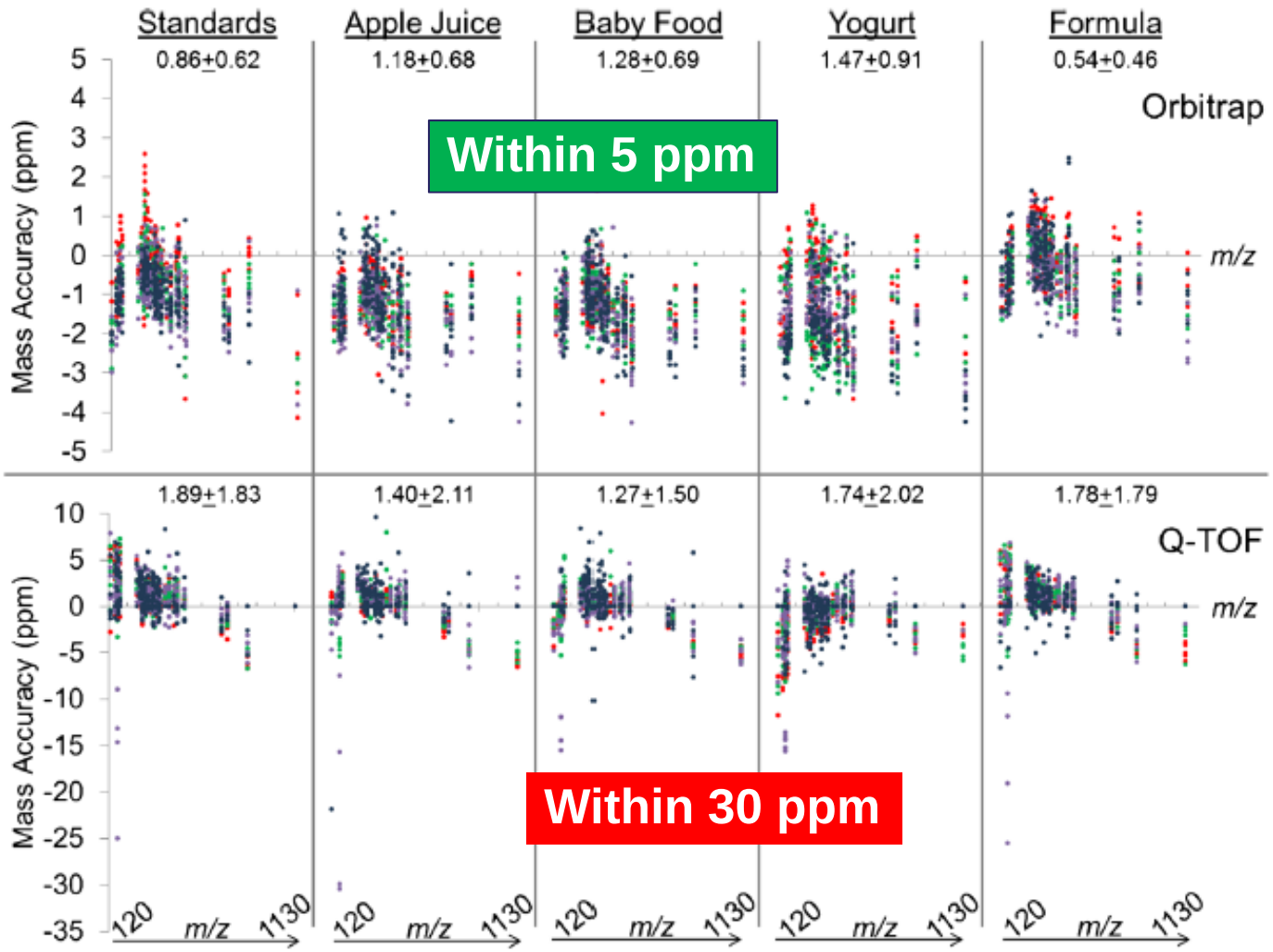
Critical Parameter #1: Mass Accuracy



Detection of 48 compounds (antibiotics, toxins, pesticides, drugs etc) in various food matrices.

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Critical Parameter #2: Isotopic Abundance/Pattern

Table 1. Average Absolute Isotope Ratio Deviation Values

pg on column	Standards	Apple juice	Baby food	Yogurt	Formula
A + 1					
Q-Exactive, Overall: 1.69 ± 2.30					
10	1.95 ± 2.26	3.17 ± 3.27	3.67 ± 3.33	3.21 ± 2.83	2.18 ± 1.69
100	2.61 ± 4.81	1.95 ± 1.98	1.91 ± 2.19	1.95 ± 1.87	2.10 ± 2.08
500	0.86 ± 0.96	1.07 ± 1.05	1.07 ± 1.18	1.26 ± 1.47	1.18 ± 1.36
2000	1.02 ± 1.79	0.75 ± 0.96	0.89 ± 1.34	0.74 ± 0.97	0.66 ± 0.89
MaXis, Overall: 5.01 ± 7.53					
10	9.20 ± 7.07	13.47 ± 9.06	15.30 ± 11.03	11.78 ± 7.62	11.49 ± 9.44
100	4.85 ± 6.66	7.78 ± 13.99	6.79 ± 7.02	6.94 ± 7.91	5.99 ± 6.25
500	3.05 ± 6.45	5.22 ± 9.58	3.30 ± 3.85	3.23 ± 3.79	3.33 ± 4.34
2000	1.77 ± 2.36	2.79 ± 6.28	2.13 ± 3.13	1.88 ± 2.56	2.03 ± 2.62
A + 2					
Q-Exactive, Overall: 1.59 ± 4.33					
10	5.31 ± 18.09	3.36 ± 5.42	4.38 ± 9.08	5.15 ± 6.56	6.44 ± 5.03
100	1.75 ± 3.01	1.93 ± 2.91	2.24 ± 4.60	1.70 ± 2.37	1.57 ± 1.86
500	1.03 ± 1.26	0.91 ± 0.62	0.86 ± 0.59	1.05 ± 0.81	1.22 ± 1.94
2000	0.81 ± 1.05	0.86 ± 1.20	0.73 ± 0.56	0.82 ± 0.57	0.74 ± 0.53
MaXis, Overall: 3.67 ± 6.47					
10	10.96 ± 9.71	12.89 ± 6.70	19.43 ± 38.22	11.21 ± 5.68	14.92 ± 7.62
100	3.55 ± 4.75	6.09 ± 6.85	6.73 ± 7.02	4.67 ± 4.46	5.22 ± 5.24
500	2.13 ± 3.14	4.02 ± 7.02	3.02 ± 3.17	3.01 ± 4.27	2.78 ± 3.38
2000	1.24 ± 2.06	2.23 ± 4.56	1.69 ± 2.36	1.68 ± 2.57	1.94 ± 3.21

Values listed are the average ± standard deviation for the calculated absolute isotope ratio deviation for all compounds for A + 1 and A + 2.

Superior HRAM Attributes in Complex Matrix Analysis

Analytica Chimica Acta 853 (2015) 415–424

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Quantitative performance of liquid chromatography coupled to Q-Exactive high resolution mass spectrometry (HRMS) for the analysis of tetracyclines in a complex matrix

Morgan Solliec, Audrey Roy-Lachapelle, Sébastien Sauvé *

Department of Chemistry, Université de Montréal, Montréal, QC, Canada

Impeccable Mass Stability at High Mass Accuracy

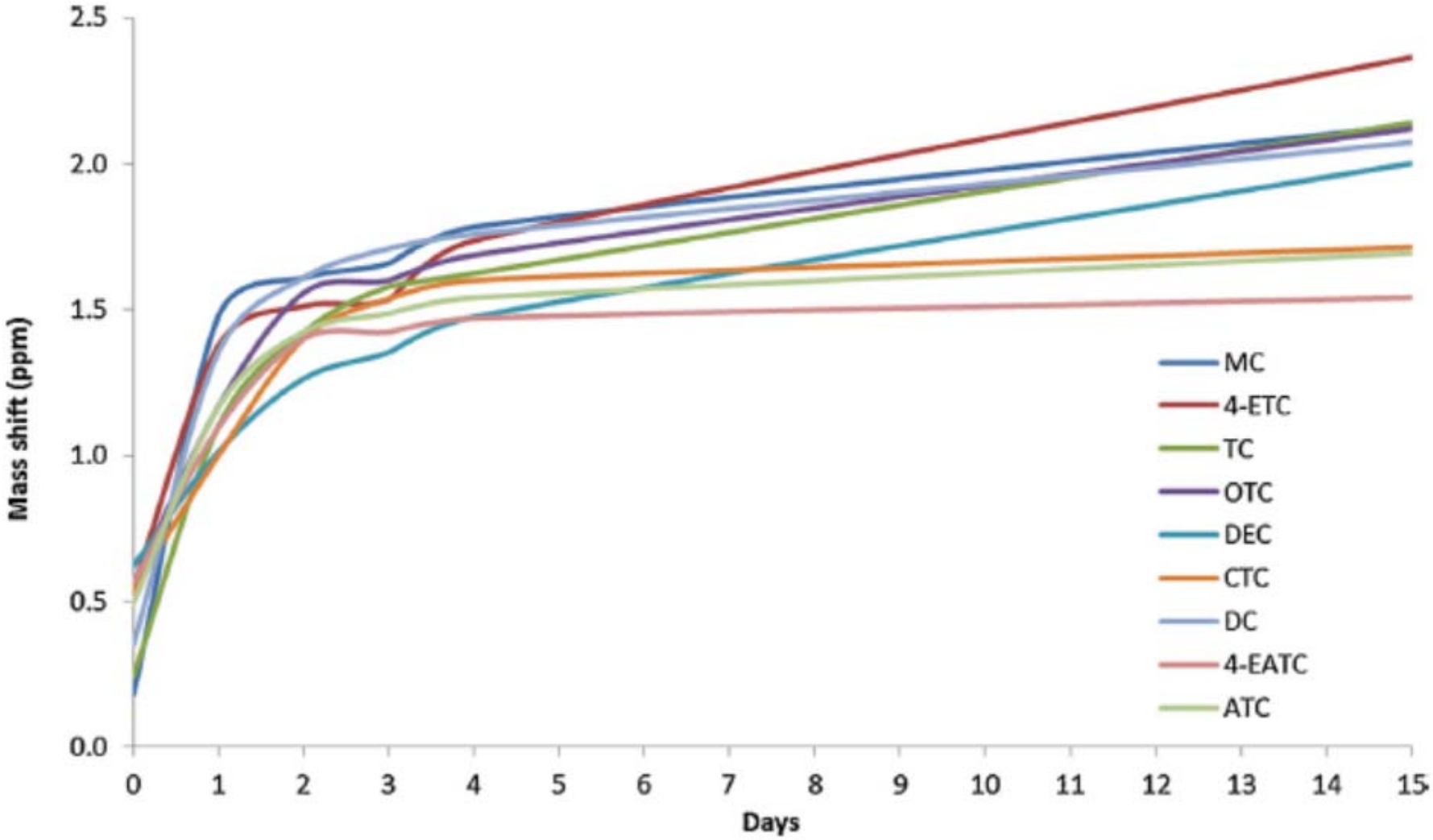

Fig. 4. Mass accuracy stability of TCs measured in FS without lock mass (250 $\mu g L^{-1}$; $n=3$).

Animal Feed Matrix Challenge: Orbitrap *vs* TOF MS

Food Additives & Contaminants: Part A, 2015
http://dx.doi.org/10.1080/19440049.2015.1023742

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Analysis of veterinary drug and pesticide residues in animal feed by high-resolution mass spectrometry: comparison between time-of-flight and Orbitrap

María Luz Gómez-Pérez, Roberto Romero-González, José Luis Martínez Vidal and Antonia Garrido Frenich*

Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería, Agrifood Campus of International Excellence, Almería, Spain

(*Received 22 December 2014; accepted 24 February 2015*)

The use of medium–high-resolution mass spectrometers (M–HRMS) provides many advantages in multi-residue analysis. A comparison between two mass spectrometers, medium-resolution (MRMS) time-of-flight (TOF) and high-resolution (HRMS) Orbitrap, has been carried out for the analysis of toxic compounds in animal feed. More than 300 compounds belonging to several classes of veterinary drugs (VDs) and pesticides have been determined in different animal feed samples using a generic extraction method. The use of a clean-up procedure has been evaluated in both instruments, and several validation parameters have been established, such as the matrix effect, linearity, recovery and sensitivity. Finally, both instruments have been used during the analysis of 18 different feed samples (including chicken, hen, rabbit and horse). Some VDs (sulfadiazine, trimethoprim, robenidine and monensin sodium) and one pesticide (chlorpyrifos) have been identified. In general, better results were obtained using the Orbitrap, such as sensitivity (1–12.5 μg kg^{-1}) and recovery values (60–125%). Moreover, this analyser had several software tools, which reduced the time for data processing and were easy to use, performing quick screening for more than 450 compounds in less than 5 min. However, some disadvantages such as the high cost and a decrease in the number of detected compounds at low concentrations must be taken into account.

Keywords: animal feed; pesticide; veterinary drug; TOF; Orbitrap

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Quantitative Comparative Study: Orbitrap MS *vs* QqQ

Food Additives and Contaminants
Vol. 28, No. 10, October 2011, 1424–1437

Taylor & Francis
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Quantitative analysis of mycotoxins in cereal foods by collision cell fragmentation-high-resolution mass spectrometry: performance and comparison with triple-stage quadrupole detection

V.M.T. Lattanzio[a]*, S. Della Gatta[a], M. Godula[b] and A. Visconti[a]

[a]*National Research Council of Italy, Institute of Sciences of Food Production, Via Amendola, 122/O, 70126 Bari, Italy;*
[b]*Thermo Fisher Scientific Praha, Slunečná 27 Praha 10, CZ-100 00, Czech Republic*

(*Received 29 March 2011; final version received 30 May 2011*)

A liquid chromatography-high-resolution mass spectrometry (LC-HRMS) method for the simultaneous determination of aflatoxins (B_1, B_2, G_1, G_2), ochratoxin A, deoxynivalenol, zearalenone, T-2 and HT-2 toxins in wheat flour, barley flour and crisp bread was developed. Mycotoxin fragmentation patterns obtained by high-energy collision dissociation (HCD) were investigated to obtain quantitative and confirmatory information (two characteristic masses per mycotoxin) using Orbitrap™-based high-resolution mass spectrometry. LC-HRMS (full-scan) detection carried out by HCD allows the monitoring of the pseudo-molecular ion and an additional characteristic fragment (for each mycotoxin) with mass accuracy in the range 0.1–3.9 ppm, meeting current European regulatory requirements for LC-MS confirmatory analysis. A sample preparation procedure based on polymeric solid-phase extraction cartridges was applied, allowing recoveries higher than 74% for nine mycotoxins, with a relative standard deviation lower than 13%. Detection limits in the range 0.5–3.4 $\mu g\,kg^{-1}$ were obtained for three cereal matrices. A critical comparison between the proposed method and a validated method based on triple quadrupole mass spectrometry showed similar performance in terms of detection limits, recoveries and repeatability, and matrix effects. Based on an efficient sample extraction and clean-up, the LC-HCD-HRMS method reported here represents a reliable and robust alternative tool for mycotoxin analysis in food matrices as compared with well-established triple quadrupole-based approaches.

Keywords: LC/MS; in-house validation; mycotoxins; *Fusarium*; aflatoxins; ochratoxin A; zearalenone; bakery products; cereals

Quantitative Comparative Study: Orbitrap MS *vs* QqQ

Table 5. Comparison of recovery and repeatability values obtained in durum wheat flour, wheat- and rye-based crisp bread by using LC-HRMS and LC-MRM methodologies after SPE clean up.

		Recoveries, % (RSDr, %)								
		DON	AFG_2	AFG_1	AFB_2	AFB_1	HT-2	T-2	ZEN	OTA
Spiking level ($\mu g\,kg^{-1}$):		300	0.4	1.2	0.4	2	20	20	30	1.2
Wheat flour	MRM	95 (2)	n.d.	82 (4)	84 (6)	89 (4)	95 (4)	92 (4)	95 (9)	74 (7)
	HRMS	102 (5)	90 (8)	89 (0)	95 (2)	81 (6)	104 (4)	98 (6)	76 (6)	97 (9)
Wheat crisp bread	MRM	100 (0)	n.d.	106 (5)	85 (10)	102 (6)	107 (2)	108 (6)	84 (5)	101 (3)
	HRMS	104 (0)	102 (5)	104 (4)	80 (2)	102 (2)	105 (1)	103 (1)	85 (1)	93 (2)
Rye crisp bread	MRM	95 (3)	91 (7)	79 (2)	85 (7)	77 (3)	97 (2)	91 (3)	96 (7)	82 (2)
	HRMS	105 (1)	93 (2)	95 (6)	93 (8)	87 (4)	100 (3)	95 (3)	101 (9)	74 (13)

Table 6. Comparison of detection limits in durum wheat flour, barley flour and wheat- and rye-based crisp bread by using LC-HRMS, with and without HCD, and LC-MRM methodologies after SPE clean-up.

	Detection limits ($\mu g\,kg^{-1}$)											
	Wheat flour			Barley flour			Crisp bread (wheat based)			Crisp bread (rye based)		
	HRMS	HCD-HRMS	MRM	HRMS	HCD-HRMS	MRM	HRMS	HCD-HRMS	MRM	HRMS	HCD-HRMS	MRM
DON	0.2	1.6	3.9	0.2	1.8	10.3	0.3	3.4	29.0	0.5	2.3	59.2
AFG_2	0.1	1.5	0.1	0.1	0.5	0.2	0.1	0.2	0.4	0.1	0.5	1.9
AFG_1	0.1	0.6	0.2	0.1	1.1	0.7	0.2	0.1	0.7	0.3	1.2	2.6
AFB_2	0.1	0.7	0.3	0.1	0.5	0.3	0.1	0.2	0.4	0.1	0.5	1.1
AFB_1	0.1	1.0	0.3	0.1	1.0	0.5	0.1	0.4	0.5	0.1	1.6	1.1
HT-2	0.3	1.7	0.3	0.2	2.5	1.1	0.3	1.0	0.5	0.4	1.7	1.7
T-2	0.2	1.0	0.2	0.2	0.5	0.5	0.3	2.9	0.5	0.5	1.6	0.9
ZEN	0.8	1.0	2.8	0.3	1.4	4.0	0.4	1.0	2.2	1.6	2.3	5.8
OTA	0.2	1.4	0.1	0.6	1.9	0.3	0.5	0.4	0.1	0.5	2.9	0.4

2015: Quadrupole-Orbitrap MS Quantifies like a QqQ

Analytica Chimica Acta 856 (2015) 54–67

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journal homepage: www.elsevier.com/locate/aca

Reliability of veterinary drug residue confirmation: High resolution mass spectrometry versus tandem mass spectrometry

A. Kaufmann *, P. Butcher, K. Maden, S. Walker, M. Widmer

Official Food Control Authority of the Canton of Zurich, Fehrenstrasse 15, 8032 Zürich, Switzerland

ABSTRACT

Confirmation of suspected residues has been a long time domain of tandem triple quadrupole mass spectrometry (QqQ). The currently most widely used confirmation strategy relies on the use of two selected reaction monitoring signals (SRM). The details of this confirmation procedure are described in detail in the Commission Decision 93/256/EC (CD). On the other hand, high resolution mass spectrometry (HRMS) is nowadays increasingly used for trace analysis. Yet its utility for confirmatory purposes has not been well explored and utilized, since established confirmation strategies like the CD do not yet include rules for modern HRMS technologies.

It is the focus of this paper to evaluate the likelihood of false positive and false negative confirmation results, when using a variety of HRMS based measurement modes as compared to conventional QqQ mass spectrometry. The experimental strategy relies on the chromatographic separation of a complex blank sample (bovine liver extract) and the subsequent monitoring of a number of dummy transitions respectively dummy accurate masses. The term "dummy" refers to precursor and derived product ions (based on a realistic neutral loss) whose elemental compositions ($C_xH_yN_zO_dCl_e$) were produced by a random number generator. Monitoring a large number of such hypothetical SRM's, or accurate masses inevitably produces a number of mass traces containing chromatographic peaks (false detects) which are caused by eluting matrix compounds. The number and intensity of these peaks were recorded and standardized to permit a comparison among the two employed MS technologies. QqQ performance (compounds which happen to produce a response in two SRM traces at identical retention time) was compared with a number of different HRMS[1] and HRMS[2] detection based modes. A HRMS confirmation criterion based on two full scans (an unfragmented and an all ion fragmented) was proposed. Compared to the CD criteria, a significantly lower probability of false positive and false negative findings is obtained by utilizing this criterion.

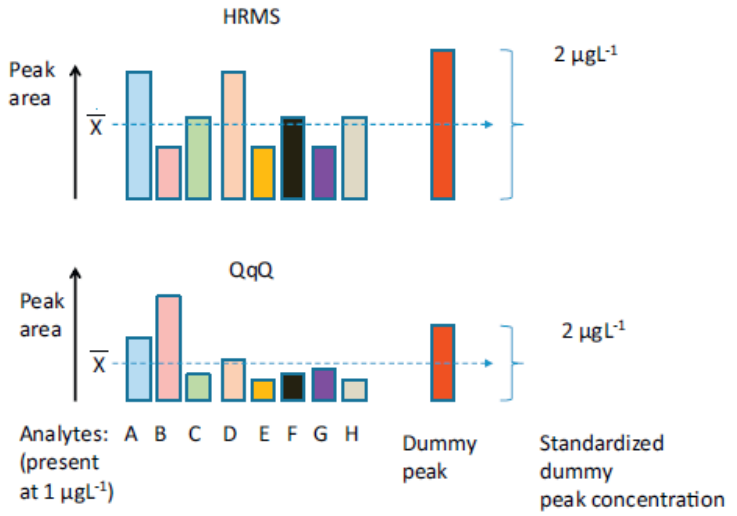

Fig. 1. The standardization process used to make QqQ and HRMS peak areas comparable. The dummy peak area is divided by the average response produced by eight veterinary drugs present at 1 µg L^{-1} in mixed standard solution.

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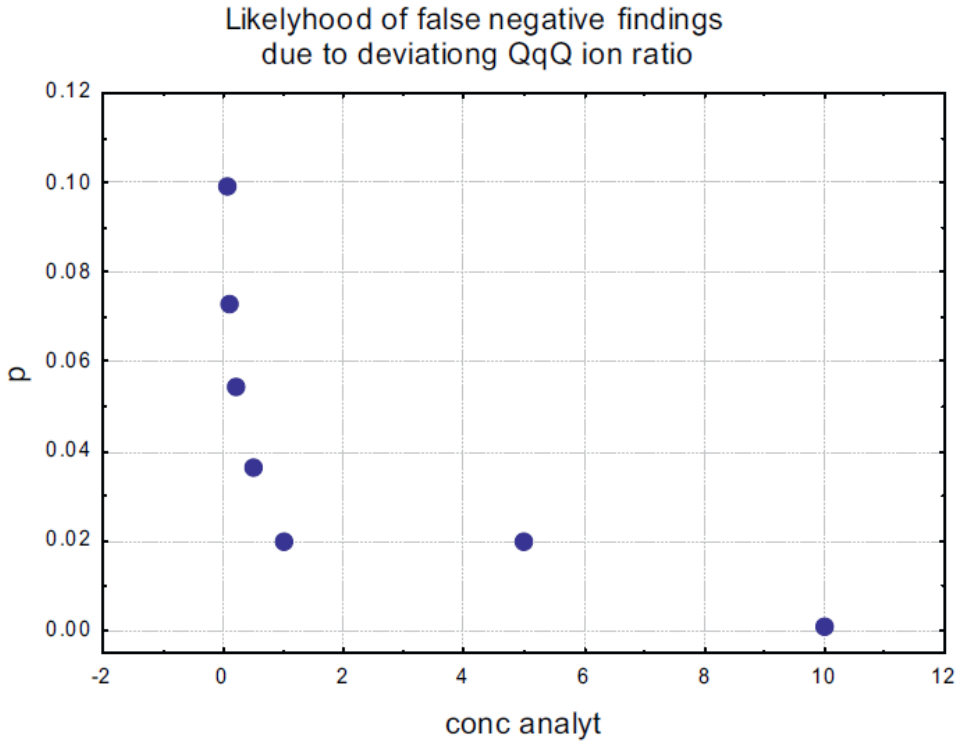
Assessment of False Negative Detection by QqQ and Q-Orbi

Table 8
Number of false negative findings obtained when analyzing a liver extract spiked with different concentrations of a total of 42 vet. drugs. HRMS data was confirmed by the proposed criterion, while QqQ data was evaluated according to the CD

Conc. $\mu g\,L^{-1}$	QqQ (CD)	HRMS
1	19	9
5	9	4
50	0	0

An important conclusion from this work is the fact that QqQ instrument based SRM sensitivity has tremendously increased over the last decade, while the selectivity of detection has remained virtually unchanged. Yet it makes less and less sense to proceed further in this direction. This has been realized by a number of instrument vendors which are actively promoting selectivity enhancing devices (e.g., ion mobility). An alternative, less tuning intensive strategy, is the use of HRMS. In the future, HRMS technology is not only expected to be produce more sensitivity but also more selectivity by the availability of even higher mass resolving instrumentations.

The HRMS confirmation criteria proposed in this paper does not rely on ion ratio and permits the monitoring of additional product ions which may finally lead to the acceptance or rejection of the confirmation hypothesis. The obtained data permits the conclusion that the use of a precursor ion and a single product ion can be sufficient for a successful confirmation. This is certainly an advantage over current unit mass resolving MS/MS instrumentation, since confirmation of poorly fragmenting analytes becomes more feasible.



ThermoFisher SCIENTIFIC

Orbitrap-based Validated Method: Qual-Quan in Single Run

Analytica Chimica Acta 810 (2014) 45–60

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Targeted analysis of multiple pharmaceuticals, plant toxins and other secondary metabolites in herbal dietary supplements by ultra-high performance liquid chromatography–quadrupole-orbital ion trap mass spectrometry

Lukas Vaclavik*, Alexander J. Krynitsky, Jeanne I. Rader

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, 5100 Paint Branch Parkway, HFS-717, College Park, MD 20740, USA

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US FBI Rapid Screening Method for DoB by LC_Q-Orbitrap

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Journal of Chromatography B

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Rapid screening for drugs of abuse in biological fluids by ultra high performance liquid chromatography/Orbitrap mass spectrometry☆

Eshwar Jagerdeo*, Jason E. Schaff

Federal Bureau of Investigation Laboratory, Quantico, VA 22135, United States

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ABSTRACT

We present a UPLC®-High Resolution Mass Spectrometric method to simultaneously screen for nineteen benzodiazepines, twelve opiates, cocaine and three metabolites, and three "Z-drug" hypnotic sedatives in both blood and urine specimens. Sample processing consists of a high-speed, high-temperature enzymatic hydrolysis for urine samples followed by a rapid supported liquid extraction (SLE). The combination of ultra-high resolution chromatography with high resolution mass spectrometry allows all 38 analytes to be uniquely detected with a ten minute analytical run. Limits of detection for all target analytes are 3 ng/mL or better, with only 0.3 mL of specimen used for analysis. The combination of low sample volume with fast processing and analysis makes this method a suitable replacement for immunoassay screening of the targeted drug classes, while providing far superior specificity and better limits of detection than can routinely be obtained by immunoassay.

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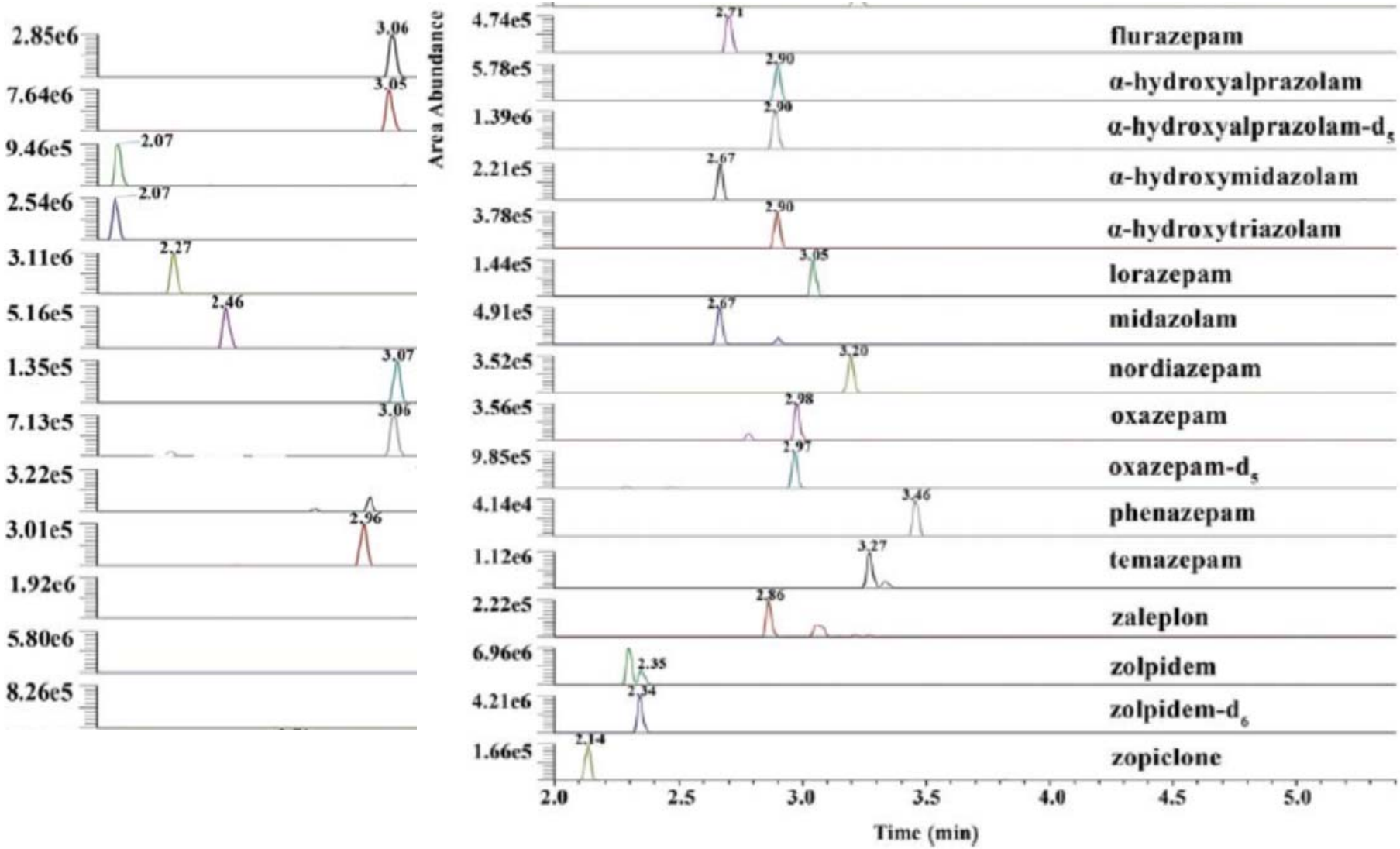

Fig. 2. List of benzodiapines and internal standards extracted from whole blood and urine at 1 ng/mL.

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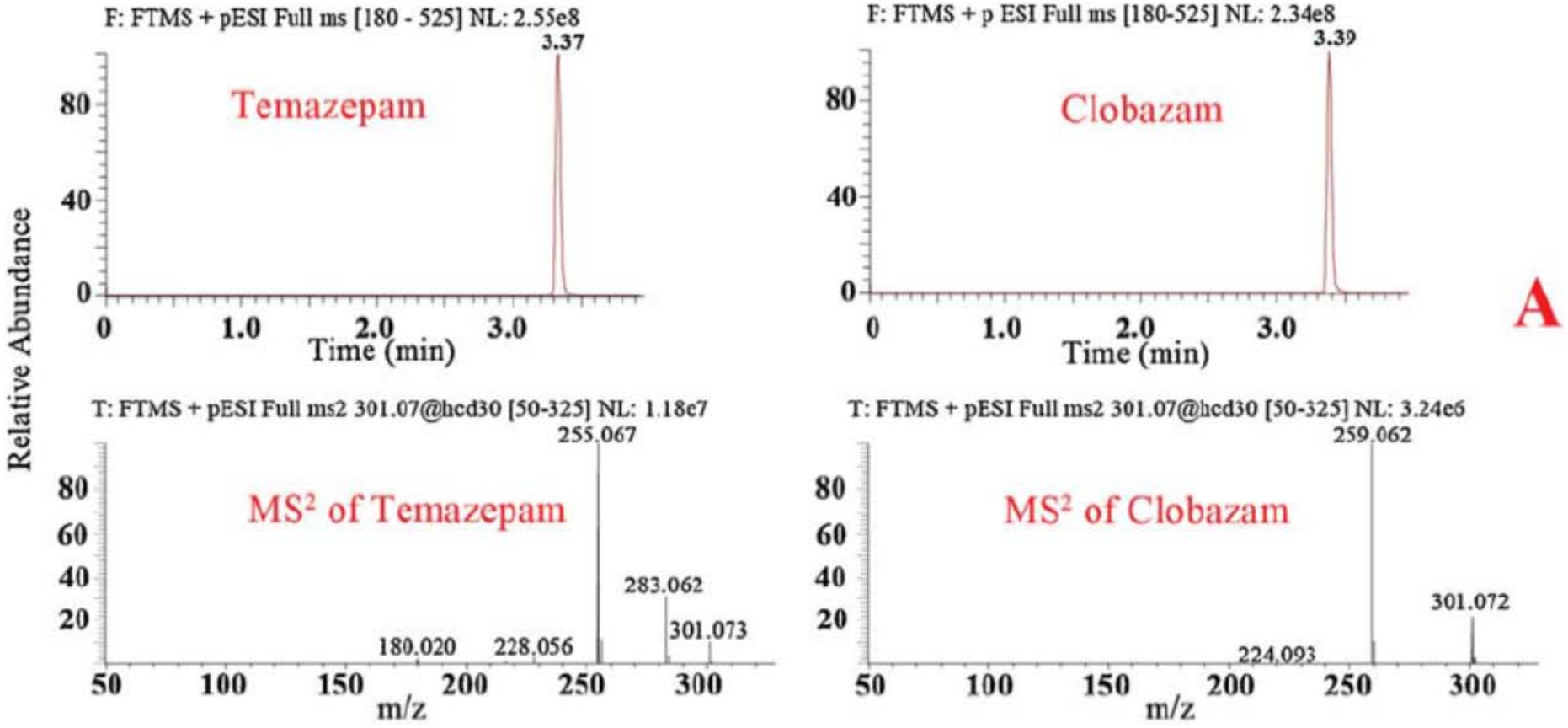
US FBI Rapid Screening Method for DoB by LC_Q-Orbitrap

E. Jagerdeo, J.E. Schaff / J. Chromatogr. B 1027 (2016) 11–18



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Orbitrap MS for Forensic & Clinical Toxicology Screening

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Abstract

LC-high resolution (HR)-MS well established in proteomics has become more and more important in bioanalysis of small molecules over the last few years. Its high selectivity and specificity provide best prerequisites for its use in broad screening approaches. Therefore, Orbitrap technology was tested for developing a general metabolite-based LC-HR-MS/MS screening approach for urinalysis of drugs necessary in clinical and forensic toxicology. After simple urine precipitation, the drugs and their metabolites were separated within 10 min and detected by a Q-Exactive mass spectrometer in full scan with positive/negative switching, and subsequent data dependent acquisition (DDA) mode. Identification criteria were the presence of accurate precursor ions, isotopic patterns, five most intense fragment ions, and comparison with full HR-MS/MS library spectra. The current library contains over 1900 parent drugs and 1200 metabolites. The method was validated for typical drug representatives and metabolites concerning recovery, matrix effects, process efficiency, and limits showed acceptable results. The applicability was tested first for cardiovascular drugs, which should be screened for in poisoning cases and for medication adherence of hypertension patients. The novel LC-HR-MS/MS method allowed fast, simple, and robust urine screening, particularly for cardiovascular drugs showing the usefulness of Orbitrap technology for drug testing.

Orbitrap MS for Forensic & Clinical Toxicology Screening

Table 5
Excerpt of a TraceFinder search result file of an LC–HR-MS/MS analysis of an authentic urine sample presenting the compounds, proposed formula, calculated and measured masses, errors, and identification criteria (isotopic pattern, IP; fragment ion, FI; library spectrum, LS), and the number of confirmation points.

Compound name	Proposed formula	Calculated exact mass, *m/z*	Measured accurate mass, *m/z*	Mass error, ppm	IP	FI	LS	Confirmation points
Amlodipine	$C_{20}H_{25}ClN_2O_5$	409.1525	409.1531	1.53	Pass	Pass	Pass	3 out of 3
Amlodipine –M artifact (cleavage product)	$C_{12}H_{12}O_2NCl$	238.0629	238.0633	1.59	Pass	Pass	Pass	3 out of 3
Amlodipine –M artifact (dehydro-deamino-carboxy-glucuronide-cleavage product)	$C_{26}H_{30}ClNO_{12}$	584.1529	584.1535	0.98	Pass	Pass	Pass	3 out of 3
Amlodipine-M (dehydro-deamino–HOOC–)	$C_{20}H_{20}ClNO_7$	422.1001	422.1008	1.63	Pass	Pass	Pass	3 out of 3
Amlodipine-M (dehydro-deamino–HOOC–glucuronide)	$C_{26}H_{28}ClNO_{13}$	598.1322	598.1327	0.82	Pass	Pass	Pass	3 out of 3
Amlopidine-M (dehydro-)	$C_{20}H_{23}ClN_2O_5$	407.1368	407.1373	1.23	Pass	Pass	Pass	3 out of 3
Amlopidine-M (dehydro-deamino-deethyl-)	$C_{18}H_{18}ClNO_5$	364.0946	364.0945	−0.49	Pass	Pass	Pass	3 out of 3
Amlopidine-M (dehydro-deamino-deethyl-glucuronide)	$C_{24}H_{26}NO_{11}$	540.1267	540.1278	2.01	–	Pass	Pass	2 out of 3
Candesartan	$C_{24}H_{20}N_6O_3$	441.1669	441.1675	1.23	Pass	Pass	Pass	3 out of 3
Candesartan neg	$C_{24}H_{20}N_6O_3$	439.1524	439.1528	1.03	Pass	Pass	Pass	3 out of 3
Candesartan-M (*O*-deethyl-)	$C_{22}H_{16}N_6O_3$	413.1356	413.1359	0.69	Pass	Pass	Pass	3 out of 3
Candesartan-M (*O*-deethyl-) neg	$C_{22}H_{16}N_6O_3$	411.1211	411.1212	0.41	Pass	–	–	1 out of 3
Hydrochlorothiazide neg	$C_7H_8ClN_3O_4S_2$	295.9570	295.9571	−0.05	–	Pass	Pass	2 out of 3
Metoprolol	$C_{15}H_{25}NO_3$	268.1907	268.1908	0.62	–	Pass	Pass	2 out of 3
Metoprolol-M (–COOH glucuronide)	$C_{20}H_{29}NO_{10}$	444.1864	444.1868	1.03	Pass	–	–	1 out of 3
Metoprolol-M (*O*-demethyl-)	$C_{14}H_{23}NO_3$	254.1750	254.1749	−0.32	–	Pass	Pass	2 out of 3
Metoprolol-M (glucuronide)	$C_{21}H_{33}NO_9$	444.2228	444.2232	1.03	–	Pass	Pass	2 out of 3
Metoprolol-M (hydroxy-)	$C_{15}H_{25}NO_4$	284.1856	284.1859	1.06	Pass	Pass	Pass	3 out of 3
Metoprolol-M (*N*-oxide)	$C_{15}H_{25}NO_4$	284.1856	284.1859	1.07	Pass	–	Pass	2 out of 3
Torasemide	$C_{16}H_{20}N_4O_3S$	349.1328	349.1328	−0.22	Pass	–	Pass	2 out of 3
Torasemide neg	$C_{16}H_{20}N_4O_3S$	347.1183	347.1182	−0.21	Pass	Pass	Pass	3 out of 3
Torasemide-M (–COOH ring)	$C_{16}H_{18}N_4O_5S$	379.1070	379.1069	−0.36	Pass	Pass	Pass	3 out of 3
Torasemide-M (–COOH ring) neg	$C_{16}H_{18}N_4O_5S$	377.0925	377.0924	−0.11	Pass	–	–	1 out of 3
Urapidil	$C_{20}H_{29}N_5O_3$	388.2343	388.2343	0.14	–	–	–	0 out of 3
Urapidil neg	$C_{20}H_{29}N_5O_3$	386.2196	386.2199	0.55	Pass	Pass	Pass	3 out of 3
Urapidil-M (hydroxy-)	$C_{20}H_{29}N_5O_4$	404.2292	404.2294	0.50	Pass	Pass	Pass	3 out of 3
Urapidil-M (*N*-demethyl-)	$C_{19}H_{27}N_5O_3$	374.2186	374.2190	0.95	Pass	Pass	Pass	3 out of 3

Doping Control Screening based on Orbitrap MS

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Development and validation of an open screening method for diuretics, stimulants and selected compounds in human urine by UHPLC–HRMS for doping control

A. Jiménez Girón[a,*], K. Deventer[b], K. Roels[b], P. Van Eenoo[b]

[a] Department of Analytical Chemistry, University of Extremadura, 06006 Badajoz, Spain
[b] Doping Control Laboratory (DoCoLab), Ghent University (UGent), Department of Clinical Chemistry, Microbiology and Immunology, Technologiepark 30, B-9052 Zwijnaarde, Belgium

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ABSTRACT

A new doping control screening method for the analysis of diuretics and stimulants using ultra high pressure liquid chromatography–high resolution Orbitrap mass spectrometry has been developed. The screening was performed in full scan MS with scan-to-scan polarity switching which allowed to detect more than 120 target analytes. Sample preparation was limited to 10-fold dilution of the urine into the internal standard solution followed by injection. Total run time per sample was 10 min. Validation of the method yielded detection limits for diuretics between 25 and 250 ng mL^{-1} and for stimulants between 5 and 500 ng mL^{-1}. The screening method has been implemented in routine doping control.

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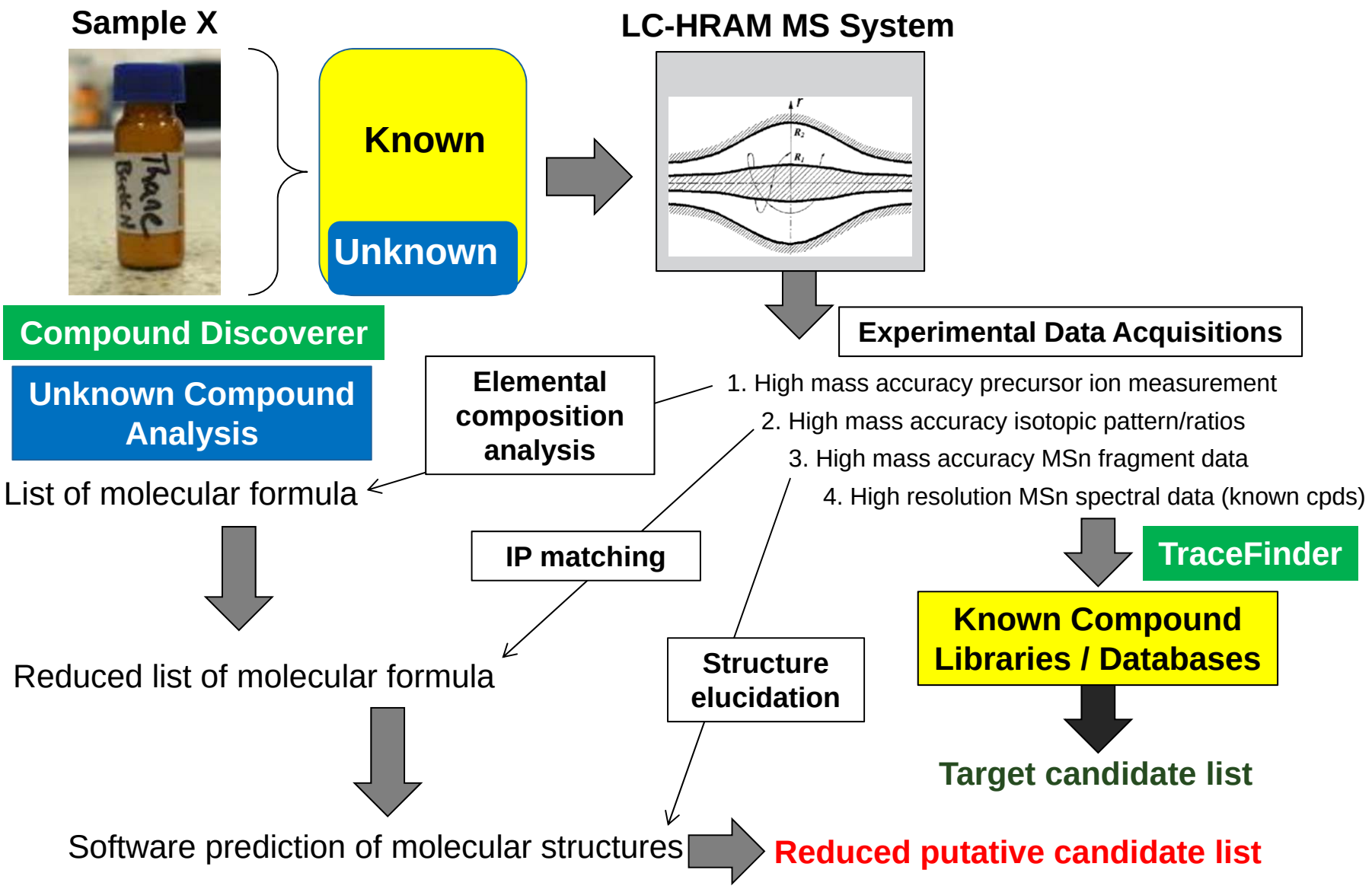
Doping Control Screening based on Orbitrap MS

Table 1
Retention times and standard deviation ($n = 10$), theoretical masses, detection limits and matrix effects for the two diagnostic ions (ME1 and ME2, respectively) for the investigated compounds.

No.	Compound	Chemical formula	$t_R \pm$ std (min)	Ion 1	m/z	Ion 2	m/z	LOD (ng mL^{-1})	ME 1 (%)	ME 2 (%)
Diuretics										
1	2-Amino-4-chloro-1,3-benzenedisulphonamide (ACB)	$C_6H_8ClN_3O_4S_2$	2.45 ± 0.01	$[M-H]^-$	283.95720	$[M-H]^{-i}$	285.95425	125	−11.6	−11.5
2	Acetazolamide	$C_4H_6N_4O_3S_2$	2.90 ± 0.03	$[M-H]^-$	220.98085	$[M+H]^+$	222.99541	50	−17.0	−19.7
3	Althizide	$C_4H_6N_4O_3S_2$	6.01 ± 0.01	$[M-H]^-$	381.97622	$[M+NH_4]^+$	401.01732	50	19.3	−36.3
4	Amiloride	$C_6H_8ClN_7O$	4.56 ± 0.03	$[M+H]^+$	230.05516	$[M+H]^{+i}$	232.05221	25	−7.5	9.5
5	4-Amino-6-trifluoromethyl-benzene-1,3-disulphonamide (ATFB)	$C_{25}H_{38}O_8$	3.05 ± 0.01	$[M-H]^-$	317.98355	$[M+NH_4]^+$	337.02465	125	−14.5	−21.8
6	Bemithizide	$C_7H_8F_3N_3O_4S_2$	6.51 ± 0.01	$[M-H]^-$	400.01980	$[M+NH_4]^+$	419.06090	50	7.8	−22.1
7	Bendroflumethiazide	$C_{15}H_{14}F_3N_3O_4S_2$	6.43 ± 0.01	$[M-H]^-$	420.03051	$[M+NH_4]^+$	439.07161	50	18.0	−28.1
8	Bumetanide	$C_{17}H_{20}N_2O_5S$	6.32 ± 0.01	$[M-H]^-$	363.10202	$[M+H]^+$	365.11657	250	14.6	20.7
9	Canrenone	$C_{23}H_{27}O_3$	7.79 ± 0.01	$[M+H]^+$	341.21112	$[M+Na]^+$	363.19307	25	−2.9	25
10	Chlortalidone	$C_{14}H_{11}ClN_2O_4S$	5.79 ± 0.01	$[M-H]^-$	337.00553	$[M-H]^{-i}$	339.00258	25	0.5	7.4
11	Chlorothiazide	$C_7H_6ClN_3O_4S_2$	2.89 ± 0.02	$[M-H]^-$	293.94155	$[M+NH_4]^+$	312.98265	125	5.7	−56.0
12	Clofenamide	$C_6H_7ClN_2O_4S_2$	2.82 ± 0.01	$[M-H]^-$	268.94630	$[M-H]^{-i}$	270.94335	125	−6.2	−4.7
13	Clopamide	$C_{14}H_{19}ClN_3O_3S$	6.30 ± 0.01	$[M-H]^-$	344.08411	$[M-H]^{-i}$	346.08116	25	11.1	10
14	Cyclopenthiazide	$C_{13}H_{18}ClN_3O_4S_2$	6.65 ± 0.01	$[M-H]^-$	378.03545	$[M+NH_4]^+$	397.07650	50	4.1	−8.3
15	Diclofenamide	$C_6H_6Cl_2N_2O_4S_2$	4.68 ± 0.01	$[M-H]^-$	302.90733	$[M-H]^{-i}$	304.90438	25	6.9	3.1
16	Epitizide	$C_{10}H_{11}ClF_3N_3O_4S_3$	5.92 ± 0.01	$[M-H]^-$	423.94795	$[M+NH_4]^+$	442.98910	50	−20.5	24.9
17	Ethacrynic acid	$C_{13}H_{14}Cl_2O_4$	6.50 ± 0.01	$[M-H]^-$	301.00399	$[M+H]^+$	303.01854	50	9.9	−8.7
18	Furosemide	$C_{12}H_{11}ClFN_2O_5$	5.30 ± 0.01	$[M-H]^-$	329.00044	$[M-H]^{-i}$	330.99749	125	9.1	13.8
19	Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	2.98 ± 0.01	$[M-H]^-$	295.95720	$[M-H]^{-i}$	297.95425	50	−34.3	−38.2
20	Hydroflumethiazide	$C_8H_8F_3N_3O_4S_2$	3.61 ± 0.01	$[M-H]^-$	329.98356	$[M+NH_4]^+$	349.02466	25	15.3	−14
21	Indapamide	$C_{16}H_{16}ClN_3O_3S$	6.55 ± 0.01	$[M-H]^-$	364.05281	$[M+H]^+$	366.06737	50	17.7	−14.0
22	Mebutizide	$C_{13}H_{20}ClN_3O_4S_2$	6.73 ± 0.02	$[M-H]^-$	380.05110	$[M+NH_4]^+$	399.09220	125	7.0	−5.0
23	Mefruside (I.S.)	$C_{13}H_{19}ClN_2O_5S_2$	6.50 ± 0.02	$[M+H]^+$	383.04967	$[M-H]^-$	381.03511			
24	Mefruside-6-oxo metabolite	$C_{13}H_{17}ClN_2O_6\ S_2$	6.00 ± 0.01	$[M-H]^-$	395.01437	$[M-H]^{-i}$	397.01143			
25	Metolazone	$C_{16}H_{16}ClN_3O_3S$	6.37 ± 0.01	$[M-H]^-$	364.05281	$[M+H]^+$	366.06737	125	24.7	−3.1
26	Piretanide	$C_{17}H_{18}N_2O_5S$	6.10 ± 0.01	$[M-H]^-$	361.08637	$[M+H]^+$	363.10092	50	12.6	−34.2
27	Polythiazide	$C_{11}H_{13}ClF_3N_3O_4S_3$	6.42 ± 0.01	$[M-H]^-$	437.96360	$[M+NH_4]^+$	457.00471	50	9.7	−23.4
28	Probenecide	$C_{13}H_{19}NO_4S$	6.30 ± 0.01	$[M-H]^-$	284.09620	$[M+H]^+$	286.11076	50	18.9	−8.2
29	Quinethazone	$C_{10}H_{12}ClN_3O_3S$	4.33 ± 0.01	$[M-H]^-$	288.02115	$[M-H]^{-i}$	290.01856	125	68.4	11.5
30	Spironolactone	$C_{24}H_{32}O_5S$	7.79 ± 0.01	$[M+H\text{-}HSCOCH_3]^+$	341.21112	–	–	25	−2.9	–
31	Spironolactone metabolite[a]	$C_{23}H_{32}O_4S$	7.39 ± 0.01	$[M+H]^+$	405.20941	$[M+OAc]^-$	463.21645	25	13.5	39
32	Torasemide	$C_{16}H_{20}N_4O_3S$	6.53 ± 0.01	$[M-H]^-$	347.11833	$[M+H]^+$	349.13289	50	40.0	1.5
33	Triamterene	$C_{12}H_{11}N_7$	6.33 ± 0.01	$[M+H]^+$	254.11487	–	–	5	−7.0	–
34	Trichloromethiazide	$C_8H_8Cl_3N_3O_4S_2$	5.16 ± 0.01	$[M-H]^-$	377.89490	$[M-H]^{-i}$	379.89195	50	15.3	13.2
35	Xipamide	$C_{15}H_{15}ClN_2O_4S$	6.11 ± 0.01	$[M-H]^-$	353.03683	$[M+H]^+$	355.05138	125	−3.2	−22.5
Stimulants										
1	3,3-Diphenylpropylamine	$C_{15}H_{17}N$	6.90 ± 0.02	$[M+H]^+$	212.14338	$[M+Na]^+$	234.12532	250	−9.6	−36.1
2	4-Methyl-2-Hexaneamine	$C_7H_{17}N$	5.40 ± 0.03	$[M+H]^+$	116.14338	–	–	50	−16.0	–
3	Adrafinil	$C_{15}H_{15}NO_3S$	6.70 ± 0.08	$[M+Na]^+$	312.06648	$[M-H]^-$	288.06999	250	26.9	−29.7
4	Amfepramone	$C_{13}H_{19}NO$	5.80 ± 0.02	$[M+H]^+$	206.15394	–	–	50	−11.3	–
5	Amfepramone metabolite 1[b]	$C_{11}H_{17}NO$	5.34 ± 0.01	$[M+H]^+$	180.13829	$[M+Na]^+$	202.12024			
6	Amfepramone metabolite 2[c]	$C_{13}H_{21}NO$	5.65 ± 0.01	$[M+H]^+$	208.16959	$[M+Na]^+$	230.15154			
7	Amineptine	$C_{22}H_{28}NO_2$	6.97 ± 0.01	$[M+H]^+$	338.21146	$[M-H]^-$	336.19690	500	1.3	−24.9
8	Amineptine metabolite C5[d]	$C_{20}H_{23}NO_2$	6.68 ± 0.01	$[M+H]^+$	310.18016	$[M-H]^-$	308.16560	500	−33.1	−6.1

A. Jiménez Girón et al. / Analytica Chimica Acta 721 (2012) 137–146

HRAM Workflow for Known Screening & Unknown Analysis



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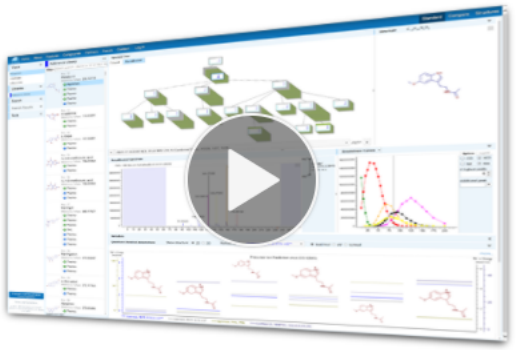

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Search

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compounds	trees	spectra	QM models	

(+ added in the last 14 days)

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Regularly Updated mzCloud™ & Statistics Report

Current mzCloud Statistics

Number of compounds: 7,408

Number of spectral trees: 11,533

	Raw	Filtered	Recalibrated
Positive	8,329	8,329	8,329
Negative	3,204	3,204	3,204

Number of spectra: 2,628,325

Number of spectra in positive ESI: 2,126,075

	Raw	Filtered	Recalibrated
MS^1	53,122	27,499	27,499
MS^2	460,779	167,736	167,746
MS^3	217,087	76,045	76,045
MS^4	280,772	103,933	103,933
MS^5	163,598	61,308	61,308
MS^6	36,765	13,774	13,774
MS^7	6,705	2,511	2,511
MS^8	843	322	322
MS^9	78	30	30

Compound classes

	Compounds	Trees	Spectra
Therapeutics/Prescription Drugs	1,025	2,004	294,257
Drugs of Abuse/Illegal Drugs	903	1,329	160,236
Sports Doping Drugs	193	313	34,684
Steroids/Vitamins/Hormones	21	49	10,268
Endogenous Metabolites	1,198	2,153	907,909
Natural Products/Medicines	78	133	115,562
Natural Toxins	64	96	132,335
Counterfeit Drug (Therapeutic)	71	116	11,297
Extractables/Leachables	169	322	43,201
Pesticides/Herbicides	533	993	107,836
Excipients/Additives/Colorants	119	209	81,309
Illegal Additives	53	98	9,204
Personal Care Products/Cosmetics	65	105	32,344
Textile Chemicals/Auxiliary/Dyes	13	28	6,126
Industrial Chemicals	289	506	59,604
Perflourinated Hydrocarbons	20	34	2,066
Nanomaterials	0	0	0

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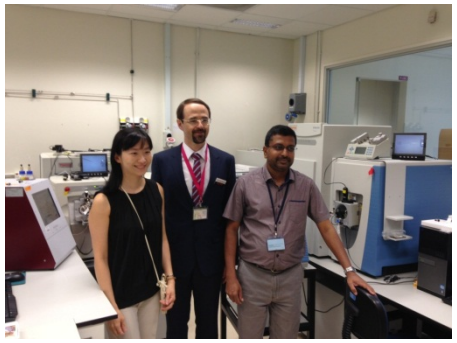

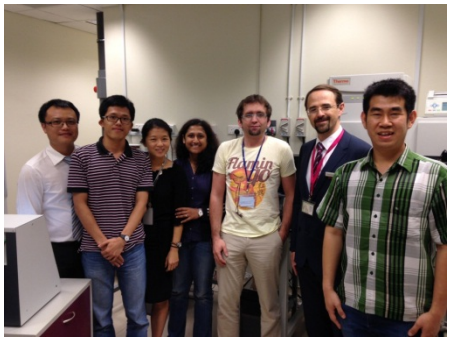

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