

Will LC-MS/MS become the workhorse in environmental laboratories? Its applications for protecting public health.

Ruth Marfil-Vega, PhD

Senior Market Manager - Environmental

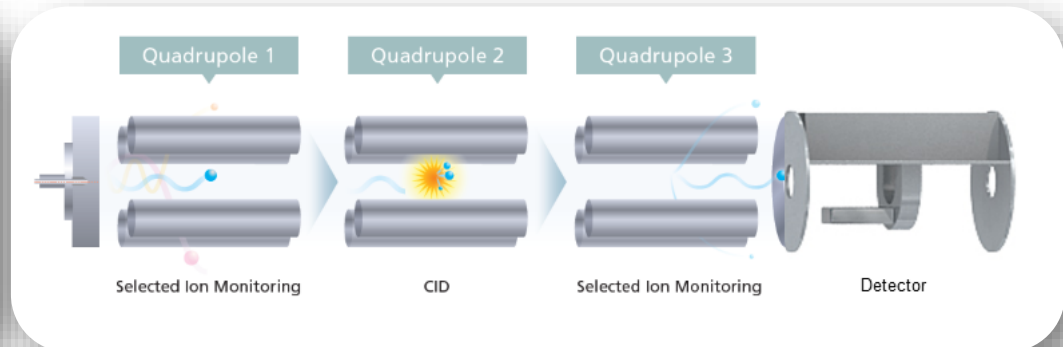
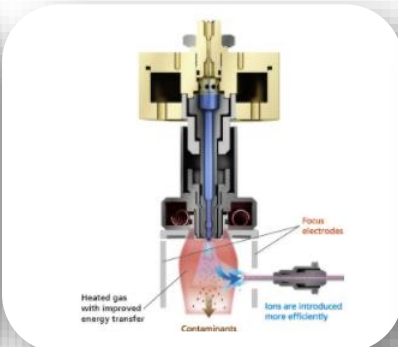
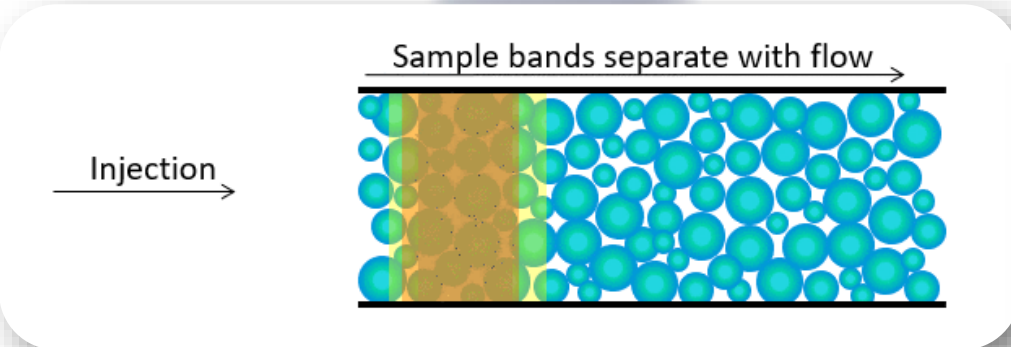
Topics for today's presentation: LC-MS/MS



1. Presence in Environmental Labs.
2. Applications for protecting public health.
3. Myths about LC-MS/MS.

What is LC-MS/MS?

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry



(LC)MS/MS History

Table 1. Historical Developments in MS

Investigator(s)	Year	Contribution
Thomson	1899–1911	First mass spectrometer
Dempster	1918	Electron ionization and magnetic focusing
Aston	1919	Atomic weights using MS
Stephens	1946	Time-of-flight mass analysis
Hipple, Sommer, and Thomas	1949	Ion cyclotron resonance
Johnson and Nier	1953	Double-focusing instruments
Paul and Steinwedel	1953	Quadrupole analyzers
Beynon	1956	High-resolution MS
Biemann, Cone, Webster, and Arsenault	1966	Peptide sequencing
Munson and Field	1966	Chemical ionization
Dole	1968	Electrospray ionization
Beckey	1969	Field desorption MS of organic molecules
MacFarlane and Torgerson	1974	Plasma desorption MS
Comisarow and Marshall	1974	FT-ICR MS
Yost and Enke	1978	Triple quadrupole MS
Barber	1981	Fast atom bombardment (FAB)
Tanaka, Karas, and Hillenkamp	1983	Matrix-assisted laser desorption/ionization
Fenn	1984	ESI on biomolecules
Chowdhury, Katta, and Chait	1990	Protein conformational changes with ESI MS
Mann and Wilm	1991	MicroESI
Ganem, Li, and Henion	1991	Noncovalent complexes with ESI MS
Chait and Katta	1991	Noncovalent complexes with ESI MS
Pieles, Zurcher, Schär, and Moser	1993	Oligonucleotide ladder sequencing
Henzel, Billeci, Stults, Wong, Grimley, and Watanabe	1993	Protein mass mapping
Siuzdak, Bothner, Fuerstenau, and Benner	1996–2001	Intact viral analysis

LC-MS/MS is a mature technique that has been widely commercialized for several decades.

SHIMADZU
Excellence in Science

Celebrating 50 Years of MS Innovation (1970-2020)

The year 2020 marks 50 years since the release of Shimadzu's first mass spectrometry device, the LKB-9000. Over the past half century, our passion for innovation has led to multiple ground-breaking developments in MS technology. With our considerable experience in the field, we take this opportunity to reflect on the past and look towards the future of MS. Through this we reaffirm our commitment as a company to provide revolutionary products and services that contribute to society through technology, sustaining the health of the planet and of humankind.

Shimadzu's Beginnings
1875 Shimadzu founded
The history of Shimadzu as a company begins with its founding in Kyoto in 1875 by inventors Kintaro Shimadzu, a pioneer in scientific, military and medical instruments. The company was established by Shimadzu and his associates, with continued by Shimadzu's lineage, featuring industry as well as advances in the medical field.

A New Generation of Mass Spectrometers
1978 Began to develop key technologies for quadrupole MS
1981 GCMS 9020-DF
This high-performance double-focusing GC-MS, the GCMS 9020-DF, featured a C18 bonded silica column and a quadrupole mass filter. It was the first GC-MS to be used for the analysis of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs).

The Birth of MALDI-TOF
1988 LAMS-50K
Shimadzu released the world's first MALDI-TOF MS, the LAMS-50K, which was the first MALDI-TOF MS to be used for the analysis of high-molecular weight compounds. This device laid the foundation for the general use of mass spectrometry.

The Early Days of Mass Spectrometry
1970 LKB-9000
In cooperation with the Swedish firm LKB, Shimadzu introduced the world's first mass spectrometer, the LKB-9000, which was the first mass spectrometer to be used for the analysis of organic compounds.

The Era of Triple Quad
1982 GCMS-OP1000
Shimadzu released the world's first general purpose quadrupole GC-MS, the GCMS-OP1000, which was the first GC-MS to be used for the analysis of organic compounds.

Shimadzu's Unique Technologies
Shimadzu has developed several instruments and technologies that are completely unique. The establishment of Innovation Centers around the world serves to promote this cutting-edge research and development in collaboration with our customers. In these ways, Shimadzu continually strives to contribute to society through the use of new technologies.

2004 LCMS-IT-TOF
Shimadzu released a world-first hybrid LCMS model using IT and TOF, enabling structural analysis etc. with MS/MS capabilities.

2013 IMScope
The IMScope mass imaging microscope boasts the power of both mass spectrometry and optical microscopy in one hybrid instrument. It can merge precise, high-quality MS images and optical images.

2017 DPIMS-2020
Shimadzu released the DPIMS-2020 Direct Probe Ionization Mass Spectrometer.

2019 MALDImini™-1
The MALDImini™-1 is a high-sensitivity MALDI MS capable of MS/MS measurements. It is characterized by its extremely compact design thanks to the use of pioneering Digital Ion Trap (DIT) technology. It fits on a bench space equivalent to an A3 piece of paper.

2012 GCMS-TQ8030
Incorporating the same technology that enabled the high-speed LC/MS/MS analysis of the LCMS-8030, the GCMS-TQ8030 achieved world-leading sensitivity and speed. This was the first triple quadrupole GC/MS/MS to be produced in Japan.

2015 LCMS-8060
The successful release of the LCMS-8060 in 2015 was a landmark for the GCMS-8000 series. The GCMS-8060 achieved the world's highest sensitivity and speed. This was the first triple quadrupole GC/MS/MS to be produced in Japan.

2018 GCMS-TQ8050 NX
Shimadzu released the GCMS-TQ8050 NX, an ultra-high-sensitivity triple quadrupole GC-MS for proteomics research in new fields. It is capable of performing unique, ultra-sensitive analysis of ultra-low amounts, down to the femtomole level.

2018 LCMS-9030
As the first domestically produced triple quadrupole mass of flight (LC-MS), the LCMS-9030 was the first domestically produced triple quadrupole mass spectrometer. It is characterized by its extremely compact design thanks to the use of pioneering Digital Ion Trap (DIT) technology. It fits on a bench space equivalent to an A3 piece of paper.

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2002 AXIMA-QIT
The AXIMA-QIT was the world's first MALDI-TOF MS to combine ion trap and TOF methods. This allowed detailed structural analysis of complex molecules.

2002 Koichi Tanaka awarded the Nobel Prize
Koichi Tanaka received the Nobel Prize in Chemistry for his development of soft laser desorption/ionization (S-LDI) and biological macromolecules. The Koichi Tanaka Mass Spectrometry Research Laboratory was established in 2003.

2010 LCMS-8030
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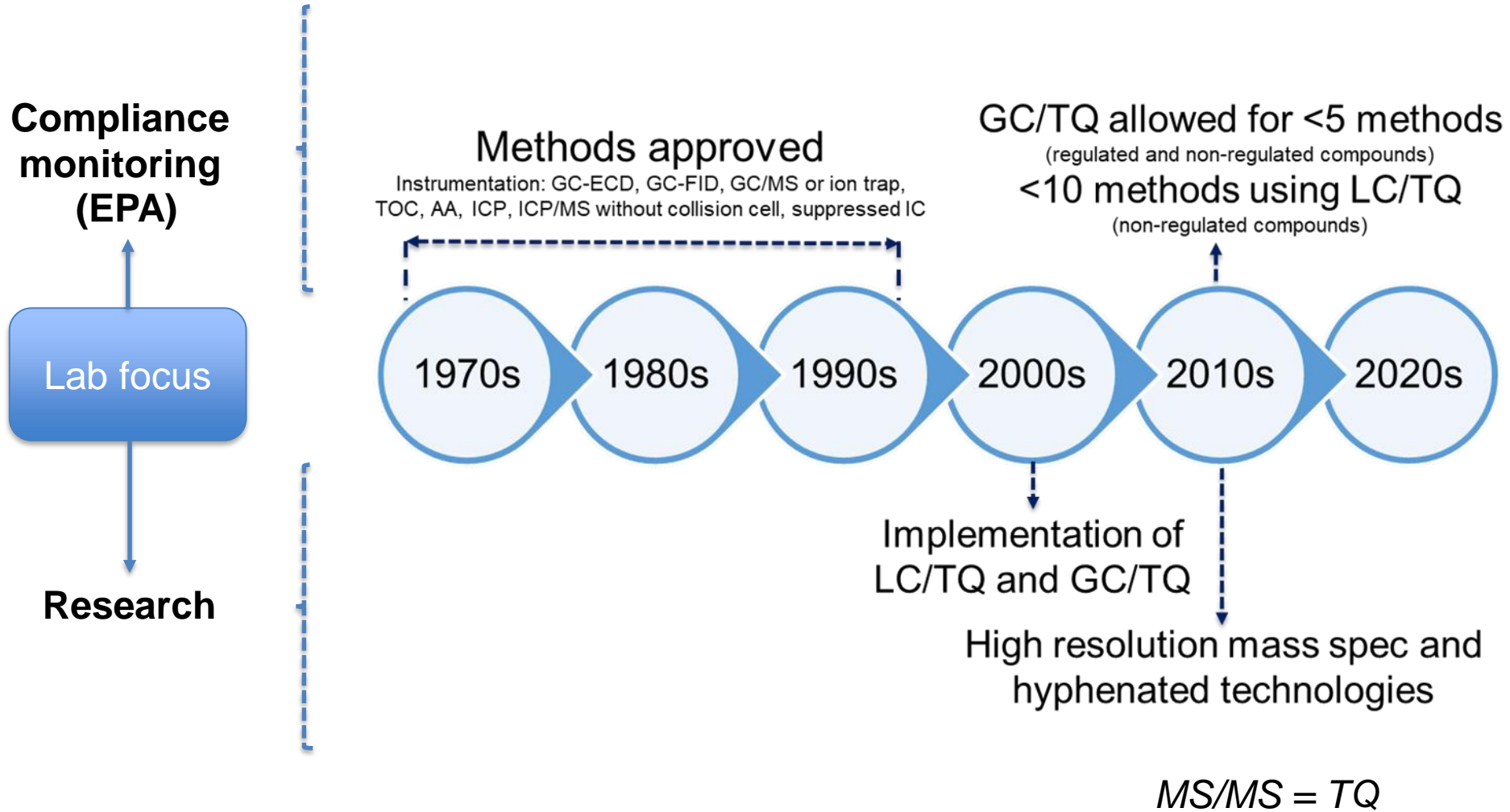
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https://www.shimadzu.com/an/news-events/celebrating_50_years_of/history.html

➔ **Chemistry Chronicles: A Mass Spec Timeline** (https://masspec.scripps.edu/research/pdf/90_art.pdf)

Why is LC-MS/MS less commonly used than other techniques?



Applications for protecting public health: **PFAS**

Safeguard Our Water from **PFAS**: Analytical Methods at a Glance

Method	EPA 537 & 537.1	ASTM D7979-19	ASTM D7968-19	EPA 8327 <small>TO BE PUBLISHED</small>	EPA 8328 <small>TO BE PUBLISHED</small> Meets DOD QSM	EPA 8329 <small>TO BE PUBLISHED</small>	EPA 533
Sample	Drinking Water	Ground/Surface/Waste Water Effluent	Soil Sediment Sludge	Ground/Surface/Waste Water Effluent	EPA 8327 + Soil, Sediment, Sludge	Soil Sediment Sludge	Drinking Water
Sample Preparation	Solid phase extraction (polymeric sorbent)	Cosolvation + direct injection	Solvent extraction + direct injection	Cosolvation + direct injection	Solvent extraction + solid phase clean-up	Direct injection	Solid phase extraction (anionic sorbent)
Quantitation	Internal standard calibration (1 MRM)	External calibration (2 MRMs + ion ratio) Isotopic dilution optional	External calibration (2 MRMs + ion ratio)	External calibration	Isotopic dilution (if analog available)	External calibration	Isotopic dilution
Targets	EPA 537 – 14 EPA 537.1 – 18	21	21	24 (EPA 537 + 10)	25 (EPA 8327 + GenX)	24 (EPA 537 + 10)	25 (Mostly outside EPA 537.1)
Shimadzu's Platform	Triple Quad LCMS-8045 or LCMS-8050	Triple Quad LCMS-8050 or LCMS-8060	Triple Quad LCMS-8050 or LCMS-8060	Triple Quad LCMS-8050 or LCMS-8060	Triple Quad LCMS-8045 or LCMS-8050	Triple Quad LCMS-8050 or LCMS-8060	Triple Quad LCMS-8045 or LCMS-8050

Applications for protecting public health: **Aquatic toxins**

Cyanotoxins (EPA 544 and EPA 545)

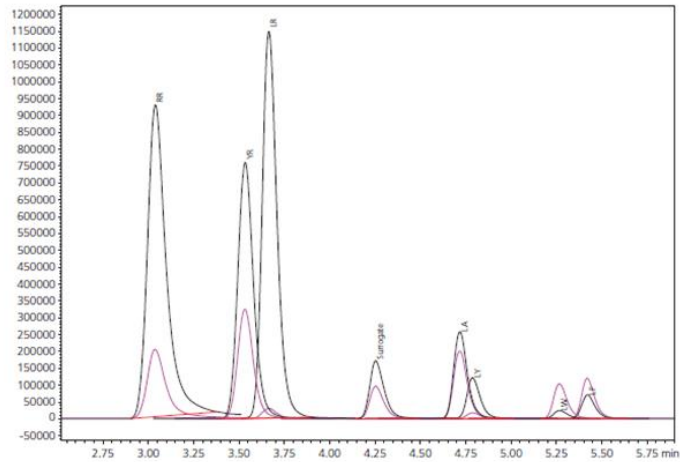


Figure 1. TIC of 100 ng/mL standard displaying target and reference ions for all compounds.

Microcystin	Quant MRM	Cal range ng/mL	r ²	Lake Erie Spl (ng/mL)		Lake Erie Spl (ng/mL)	
				Spike	Calc amt	Spike	Calc amt
RR	519.90>135.15	0.1 - 100	0.9915	1	0.937	50	49.3
YR	523.40>135.10	0.1 - 100	0.9993	1	1.012	50	48.2
LR	498.40>135.10	0.1 - 100	0.9994	1	0.993	50	48.3
LA	910.40>776.25	0.1 - 100	0.9977	1	0.951	50	45.6
LY	1002.50>135.25	0.5 - 100	0.9969	1	0.913	50	45.6
LW	1025.50>135.20	0.5 - 100	0.9979	1	0.894	50	45.4
LF	986.50>478.30	0.5 - 100	0.9985	1	0.943	50	45.4

Marine Toxins

Paralytic Shellfish Poisoning (PSP)	Diarrhetic Shellfish Poisoning (DSP)	Ciguatera Fish Poisoning (CFP)
Serious effects. Fatal toxic symptoms.	Diarrhea and/or vomiting. Not so serious conditions.	Fatal toxic symptoms (in the limited area)
LC-MS/MS in Japan & EU	MBA in Japan Fluorescence HPLC method in addition to MBA in EU and the USA (AOAC 2005.06 & 2011.02)	Review of regulatory frameworks
OA: 0.16 mg OA eq/ kg *1.	4 MU/g as MBA STX 0.8 mg STX eq /kg *1 (as 2 HCl)	

*1 CODEX STAN 292-2008.

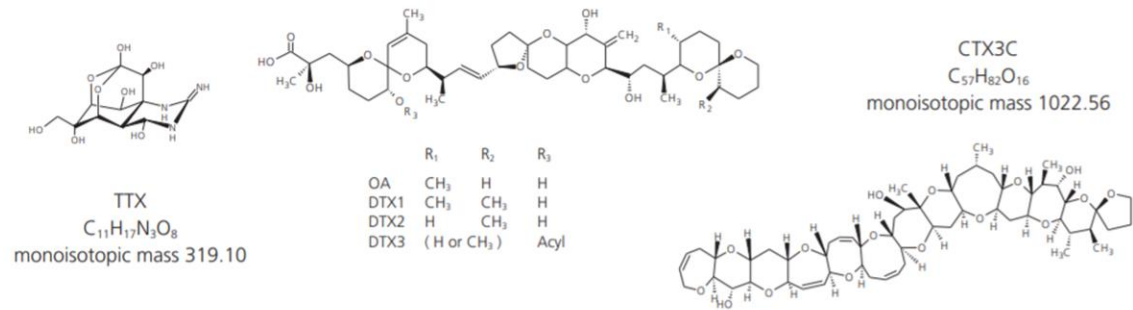
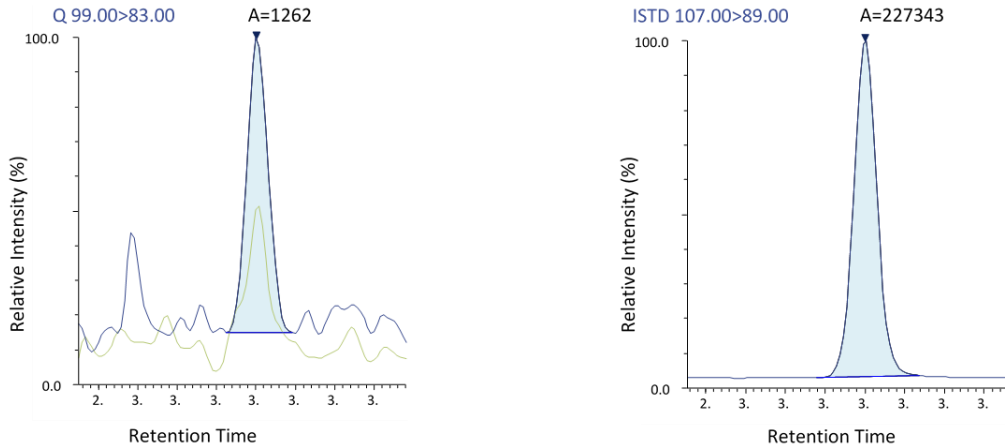


Figure 1. Structure of marine toxins

Applications for protecting public health: **Perchlorate**

EPA 6850: Perchlorate in Water, Soils and Solid Wastes



Chromatogram of perchlorate at 50 ppt (lowest concentration in calibration curve) using optimized conditions.

Robert English¹, Kristin Neir²
¹Shimadzu Scientific Instruments
²ALS Global, Houston, TX, USA

Validation of modified method EPA 6850 for analysis of perchlorate in both non-potable water and soil samples. The implemented modifications were within those allowed by EPA's guidelines.

	Samples							Theoretical Value	Mean Value	Mean Recovery	Std Dev	MDLc	MDLr	RSD	LOD
	A	B	C	D	E	F	G								
Soil (ug/kg)	0.915	0.774	1.067	0.993	0.947	1.015	1.015	1.0	0.96	96%	0.10%	0.302	0.300	10.01%	1.2
Water (ug/L)	0.123	0.128	0.103	0.098	0.106	0.116	0.124	0.1	0.11	114%	0.01%	0.036	0.036	10.04%	0.144

Perchlorate	Spiked Value	Sample	Sample	Sample	Sample	Avg	Std Dev	Precision	Recovery	Precision Limit	Recovery Limits
		1	2	3	4						
Soil (ug/kg)	100	88.2	88.4	88.6	88	88.3	0.25	0.3	88.30%	±15	85-115
Water (ug/L)	10	8.83	8.97	8.9	8.86	8.89	0.06	0.7	88.90%	±15	85-115

Applications for protecting public health: **Pesticides**

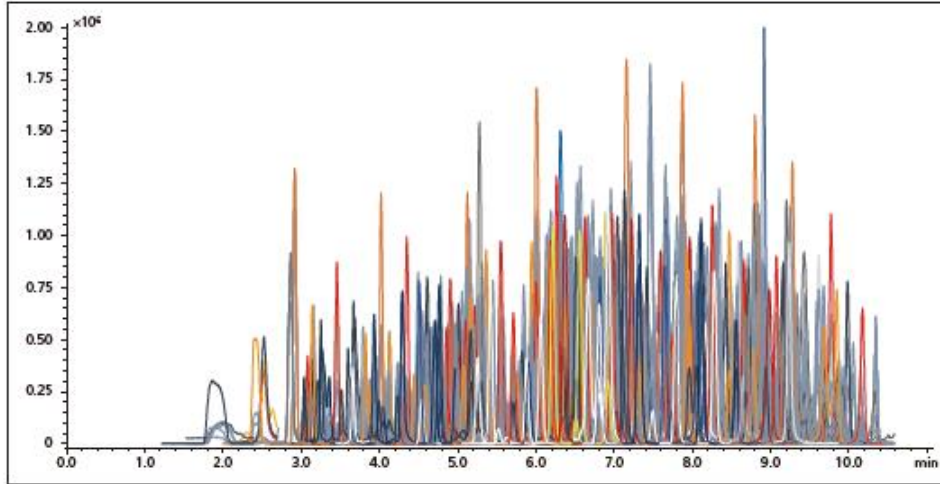


Fig. 1 MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg (Up to 3 MRMs per compound and 5 msec polarity switching time).

Application News

No. **C135**

Liquid Chromatography Mass Spectrometry

**Shimadzu Pesticide MRM Library
Support for LC/MS/MS**

David R. Baker, Alan Barnes, Neil Loftus
Shimadzu Corporation, UK

Application News

No. **C136**

Liquid Chromatography Mass Spectrometry

**Expanding Capabilities in Multi-Residue
Pesticide Analysis Using The LCMS-8060**

David R. Baker¹, Laëtitia Fages², Eric Capodanno², Neil Loftus¹
¹Shimadzu Corporation, UK; ²Phytocontrol, France

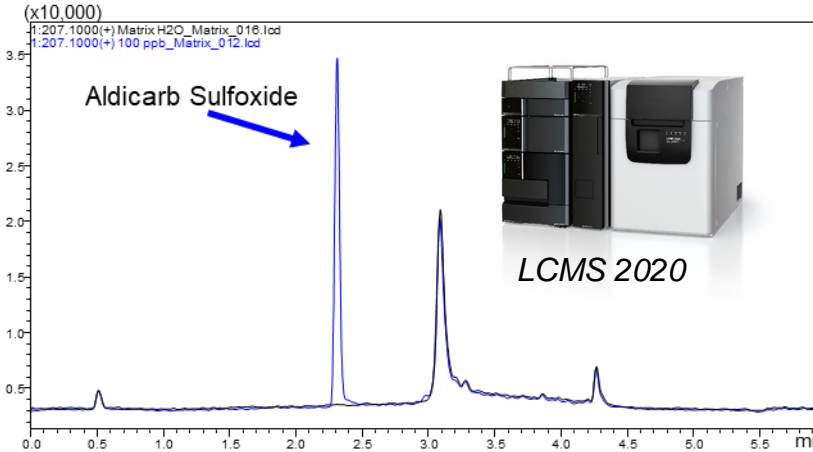
Quantification of Glyphosate, Glufosinate, and AMPA in Food via In-vial Addition of Pairing Agent

Uwe Oppermann¹, Stephane Moreau¹, Doriane Toinon²

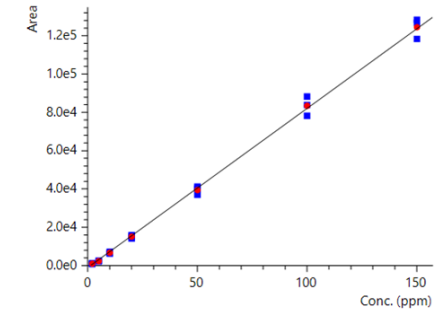
¹Shimadzu Europa GmbH, Germany

²Shimadzu Corporation, Japan

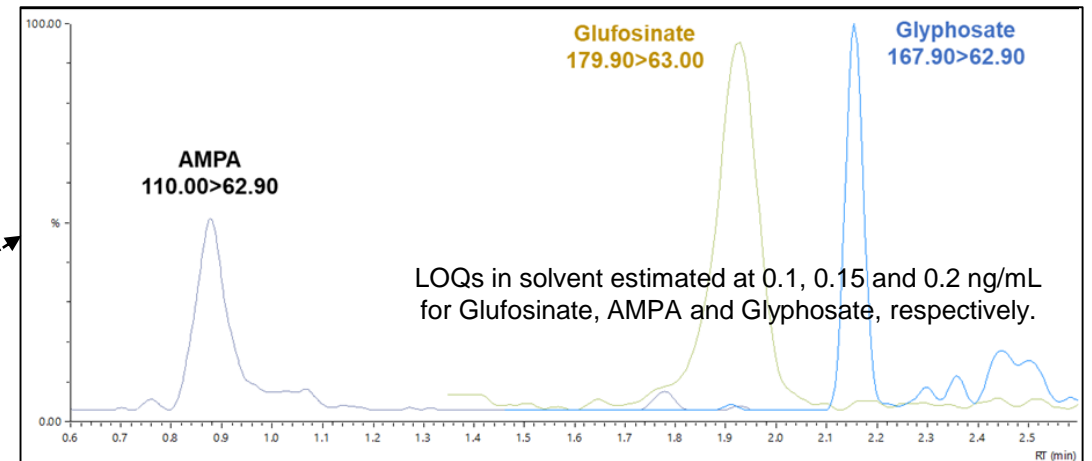
Alternatives for carbamates and glyphosate?



100 µg/L in tap water (blue), overlaid with blank (black).



Cal curve: 2 µg/L to 150 µg/L.
 $y=831.135x-1016.97$
 $R^2=0.9986, R=0.9993$



LOQs in solvent estimated at 0.1, 0.15 and 0.2 ng/mL for Glufosinate, AMPA and Glyphosate, respectively.

Applications for protecting public health: WBE

WBE: Wastewater Based Epidemiology

- A tool to provide real-time information on consumption of legal and illegal drugs of abuse by the population.
- It is expected to achieve more ambitious objectives such as establishing exposure to certain agents, incidence of specific diseases, and determination of some lifestyle consequences or environmental factors in populations.

Adapted from Lorenzo & Pico, Wastewater-based epidemiology: current status and future prospects, Current Opinion in Environmental Science & Health, Volume 9, 2019.

ENVIRONMENTAL
Science & Technology

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Viewpoint

Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19

Aaron Bivins, Devin North, Arslan Ahmad, Warish Ahmed, Eric Alm, Frederic Been, Prosun Bhattacharya, Lubertus Bijlsma, Alexandria B. Boehm, Joe Brown, Gianluigi Buttiglieri, Vincenza Calabro, Annalaura Carducci, Sara Castiglioni, Zeynep Cetecioglu Gurol, Sudip Chakraborty, Federico Costa, Stefano Curcio, Francis L. de los Reyes, III, Jeseth Delgado Vela, Kata Farkas, Xavier Fernandez-Casi, Charles Gerba, Daniel Gerrity, Rosina Girones, Raul Gonzalez, Eiji Haramoto, Angela Harris, Patricia A. Holden, Md. Tahmidul Islam, Davey L. Jones, Barbara Kasprzyk-Hordern, Masaaki Kitajima, Nadine Kotlarz, Manish Kumar, Keisuke Kuroda, Giuseppina La Rosa, Francesca Malpei, Mariana Mautus, Sandra L. McLellan, Gertjan Medema, John Scott Meschke, Jochen Mueller, Ryan J. Newton, David Nilsson, Rachel T. Noble, Alexander van Nuijs, Jordan Peccia, T. Alex Perkins, Amy J. Pickering, Joan Rose, Gloria Sanchez, Adam Smith, Lauren Stadler, Christine Stauber, Kevin Thomas, Tom van der Voorn, Krista Wigginton, Kevin Zhu, and Kyle Bibby*

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

High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry

Keng Tiong Ng^{a,1}, Helena Rapp-Wright^{a,b,1}, Melanie Egli^a, Alicia Hartmann^{a,c}, Joshua C. Steele^{d,h,j}, Juan Eduardo Sosa-Hernández^e, Elda M. Melchor-Martínez^e, Matthew Jacobs^b, Blánaid White^b, Fiona Regan^b, Roberto Parra-Saldivar^e, Lewis Couchman^f, Rolf U. Halden^{d,h,i,j}, Leon P. Barron^{a,g,*}

^a Dept. Analytical, Environmental & Forensic Sciences, King's College London, 150 Stamford Street, London, SE1 9NH, United Kingdom

^b DCU Water Institute and School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland

^c Hochschule Presentius, Limburger Straße 2, Idstein, Hessen, Germany

^d Biodesign Center for Environmental Health Engineering, The Biodesign Institute, Arizona State University, 1001 S. McAllister Avenue, Tempe, AZ 85287-8101, USA

^e Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, Nuevo Leon 64849, Mexico

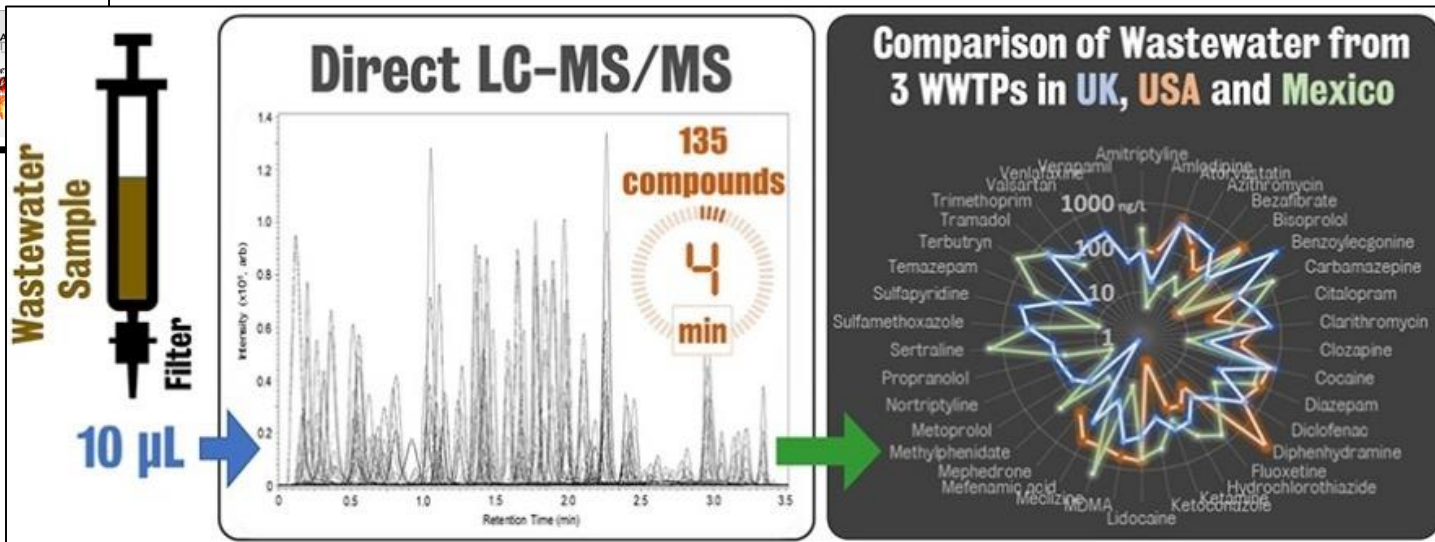
^f Analytical Services International, St George's University of London, London, United Kingdom

^g Environmental Research Group, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom

^h School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, Arizona, USA

ⁱ OneWaterOneHealth, Arizona State University Foundation, 1001 S. McAllister Avenue, Tempe, AZ 85287-8101, USA

^j AquaVitas, LLC, 9260 E. Rainree Dr., Ste 140, Scottsdale, AZ 85260, USA



Myths about LC/MS/MS - Method development

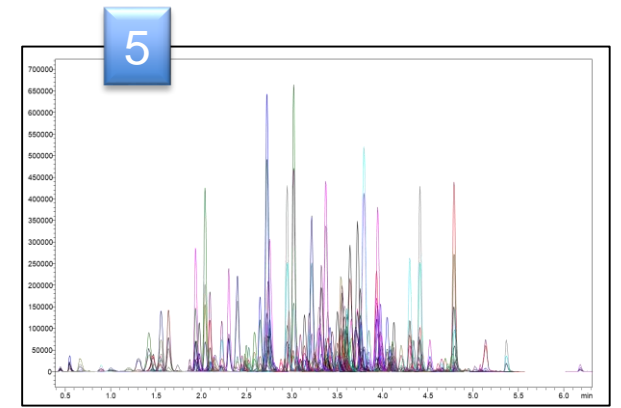
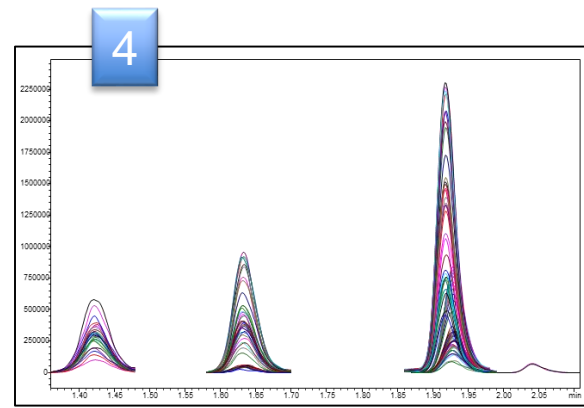
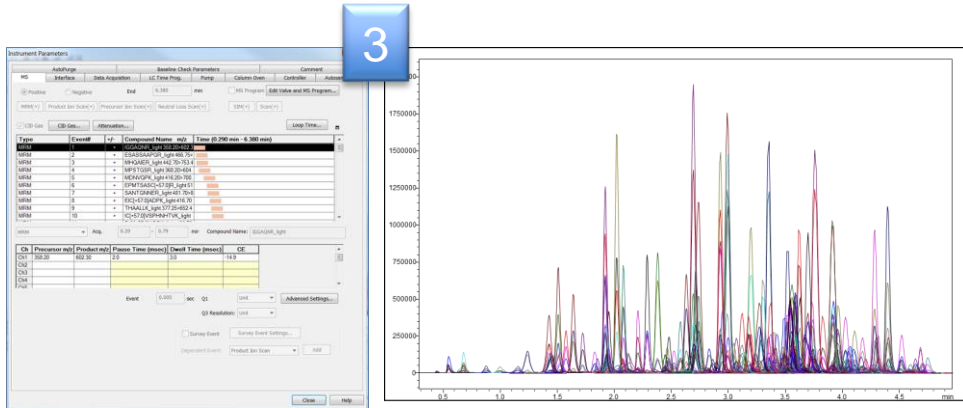
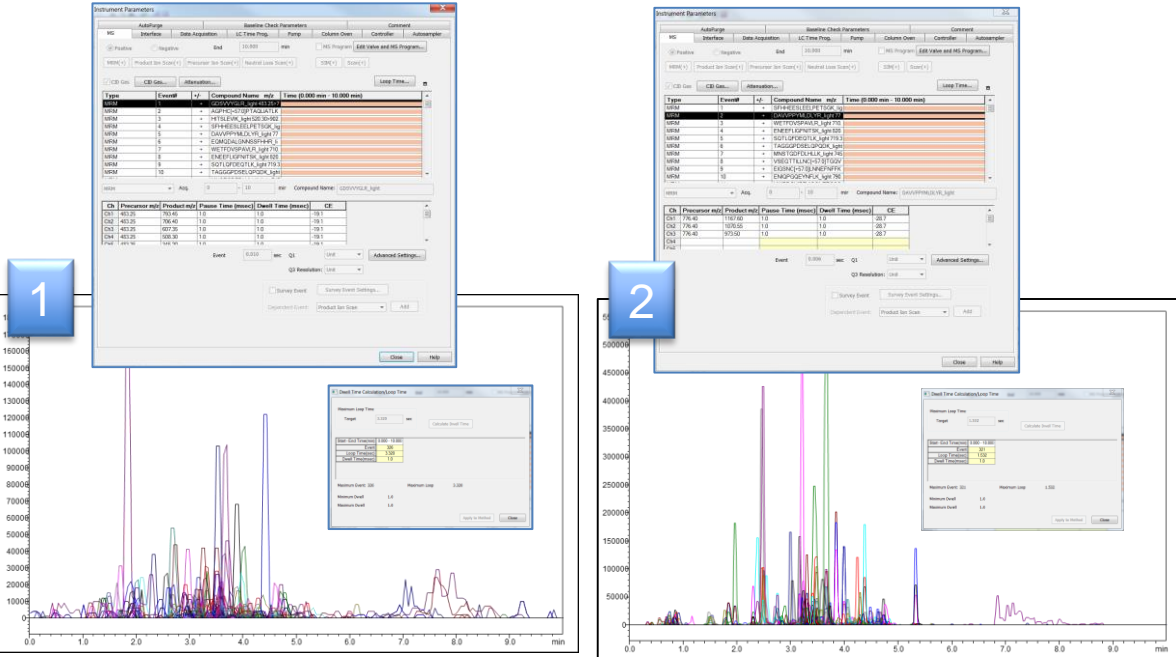


It's complex.



Software tools help with the creation of MRMs.

1. Initial MRMs screen
2. MRMs selection based on RT and intensity
3. Confirmation scheduled MRMs
4. Collision Energy Optimization
5. Method confirmation



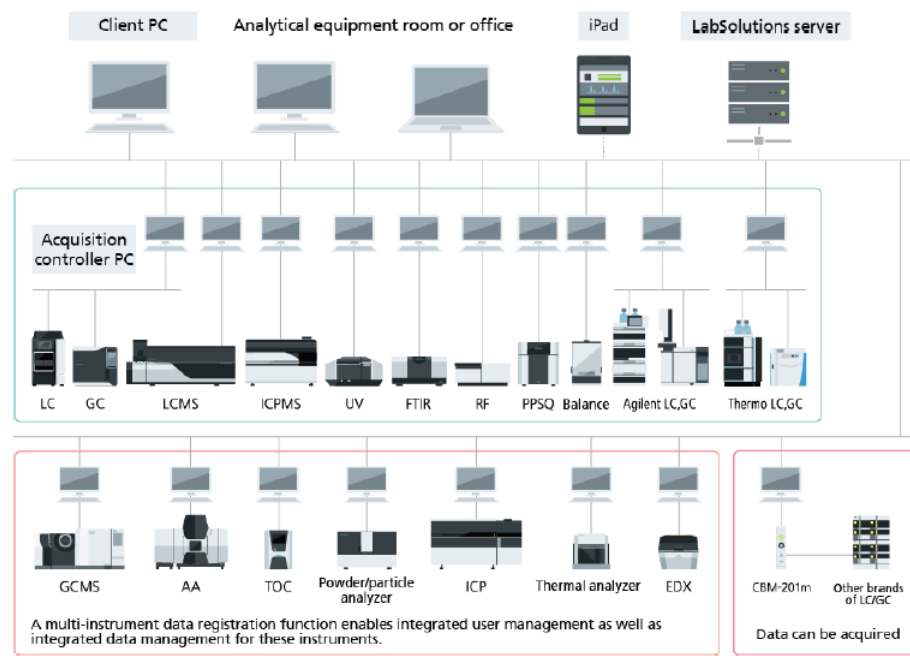
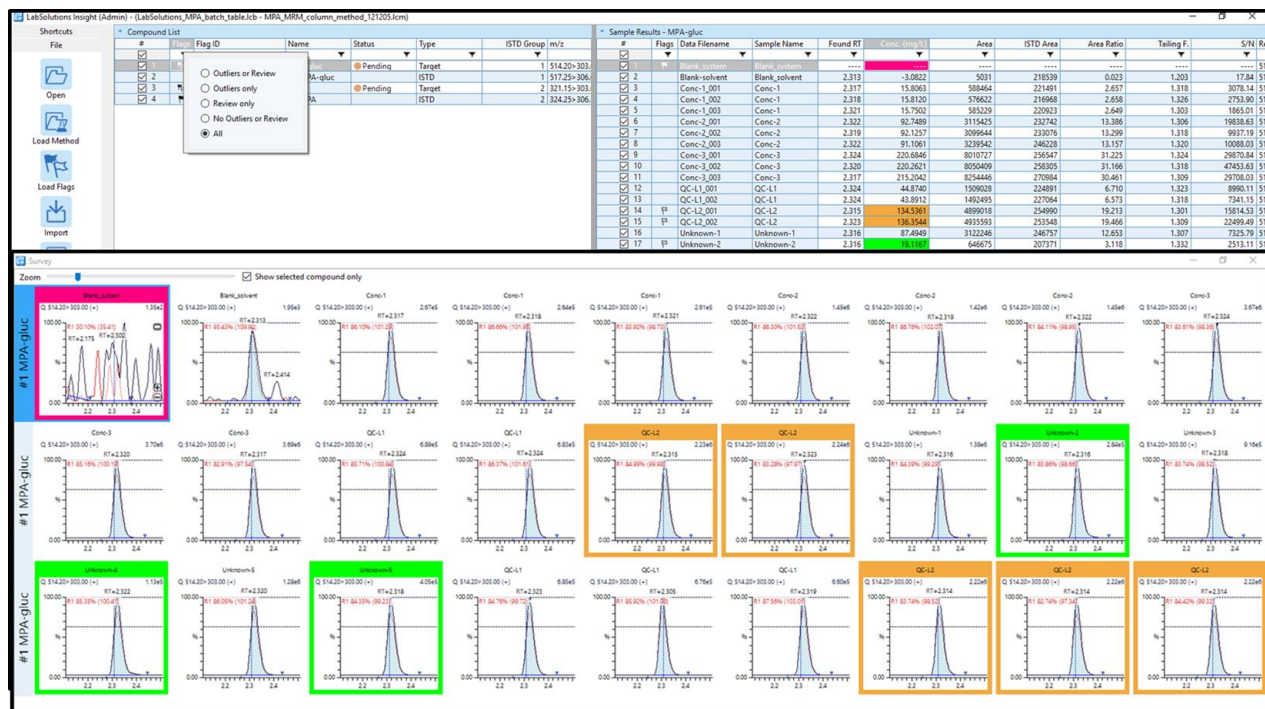
Myths about LC/MS/MS – Data Processing



It's tedious and LC/MS/MS cannot be connected to CDS.



Workflows developed for automating data process and review and increasing sample throughput. Connectivity to same software platform as other instruments. available.



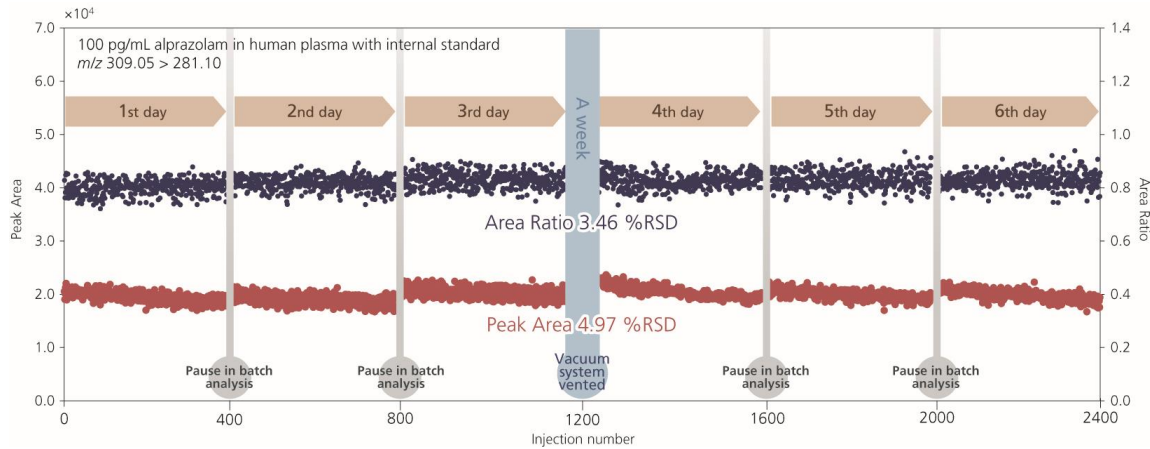
A multi-instrument data registration function enables integrated user management as well as integrated data management for these instruments.

Data can be acquired

Myths about LC/MS/MS – Sensitivity



It's too sensitive and signal fluctuates over time.



Sensitivity allows for achieving lower detection limits. Good laboratory practices and the use of internal standards help with maintaining robustness.

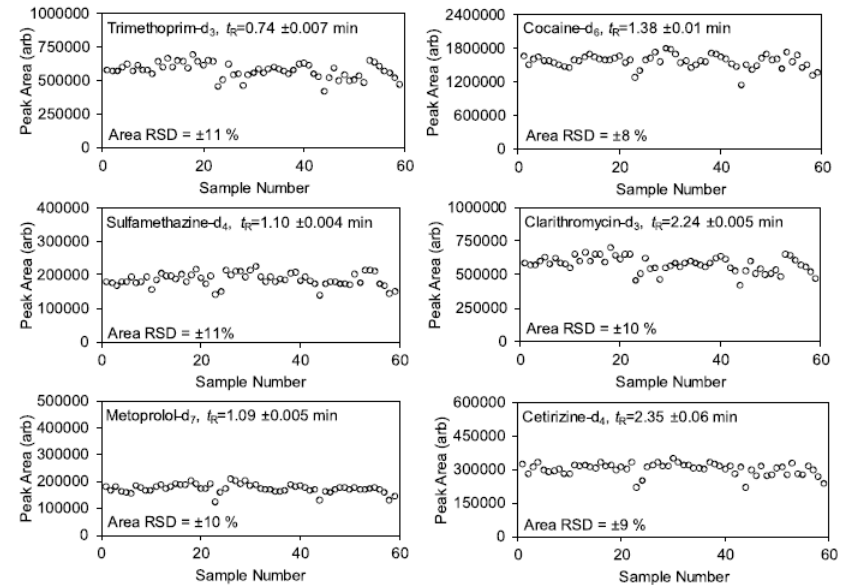
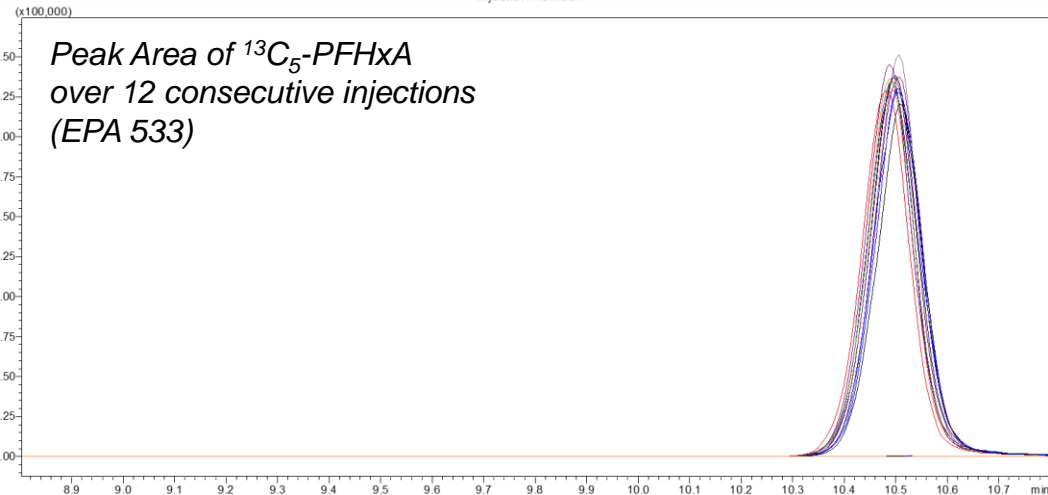


Fig. 3. Peak area and retention time stability for selected SIL-IS over a sequence of $n = 59$ spiked London wastewater samples (500 ng L^{-1}) and measured using direct LC-MS/MS analysis over a total batch analysis time of 6.4 h.

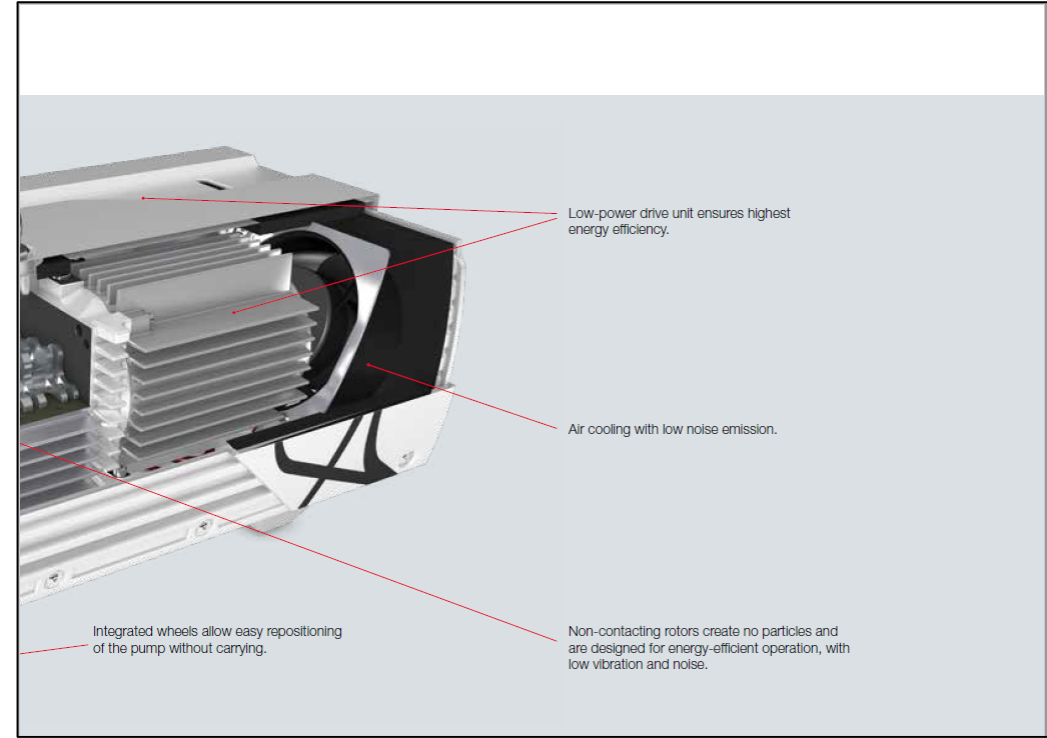
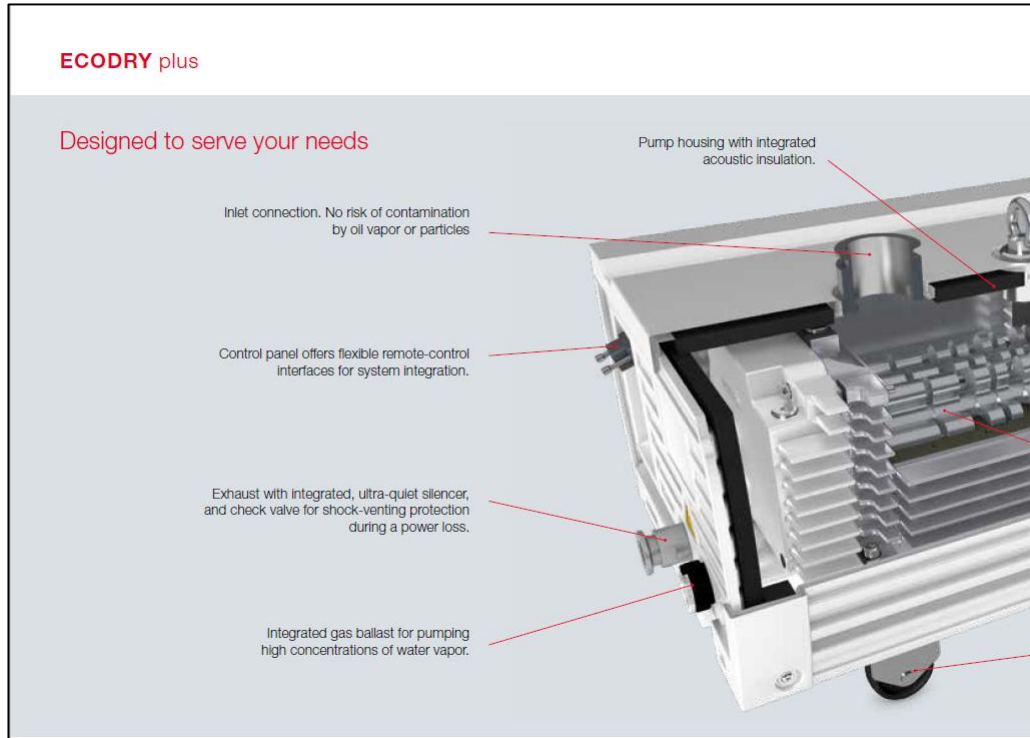
Myths about LC/MS/MS – Vacuum



Vacuum systems are scary!



Turbo pumps rarely fail. Newer dry pumps are easier to maintain (and make less noise!).



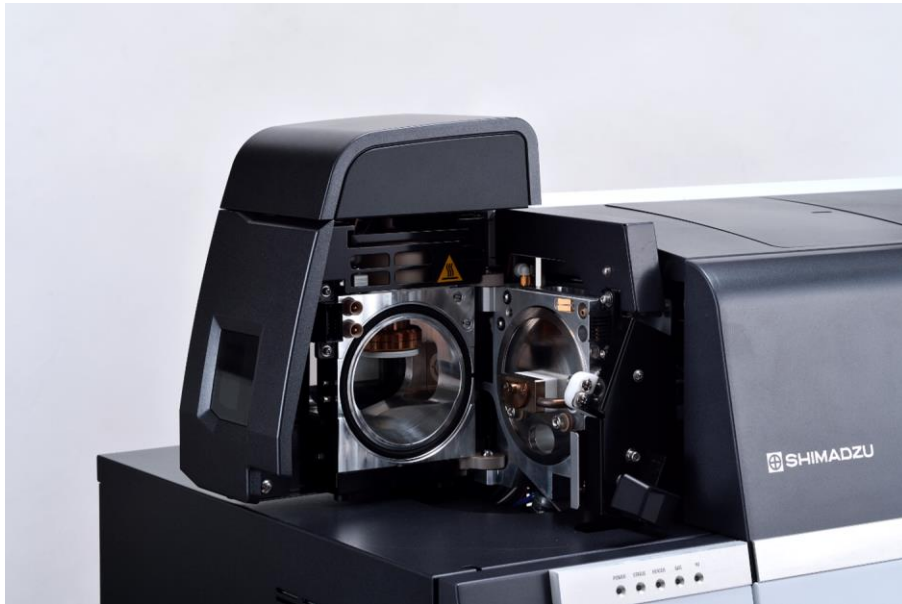
Myths about LC/MS/MS – Maintenance



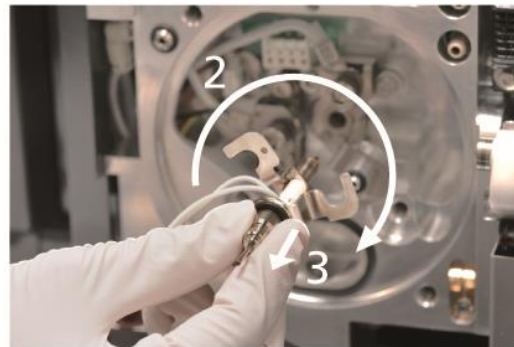
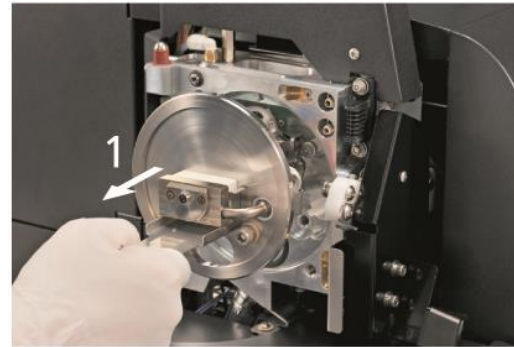
I need to break vacuum to maintain the system.



Routine maintenance can be done by the analyst without the need to break vacuum.



Source cleaning



Desolvation line replacement



ESI Capillary source replacement

Take home messages



- LC-MS/MS are common instruments in environmental labs.
- Broad range of applications are suitable for LC-MS/MS analysis. Productivity is increased.
- Good laboratory practices and latest technology developments ease operations.



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rmmarfilvega@shimadzu.com