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

Ruth Marfil-Vega, PhD

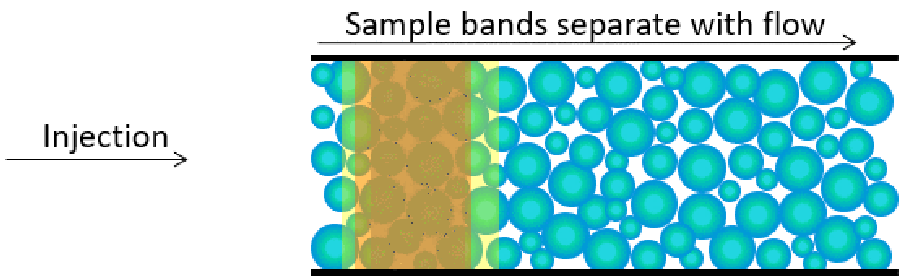
Senior Market Manager - Environmental

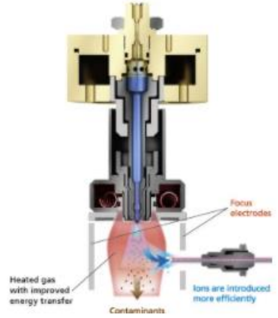
Topics for today's presentation: LC-MS/MS

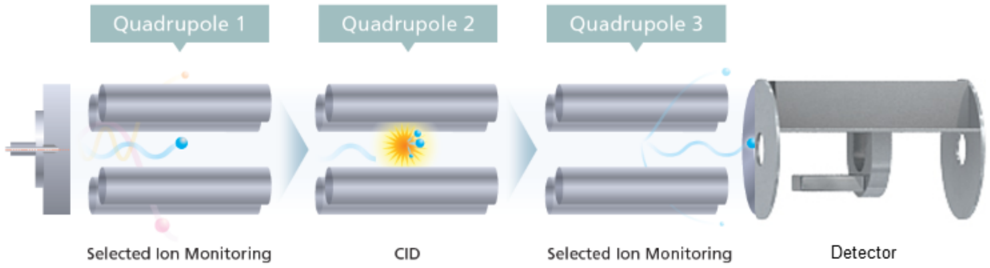
1. Presence in Environmental Labs.
2. Applications for protecting public health.
3. Myths about LC-MS/MS.

What is LC-MS/MS?

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry







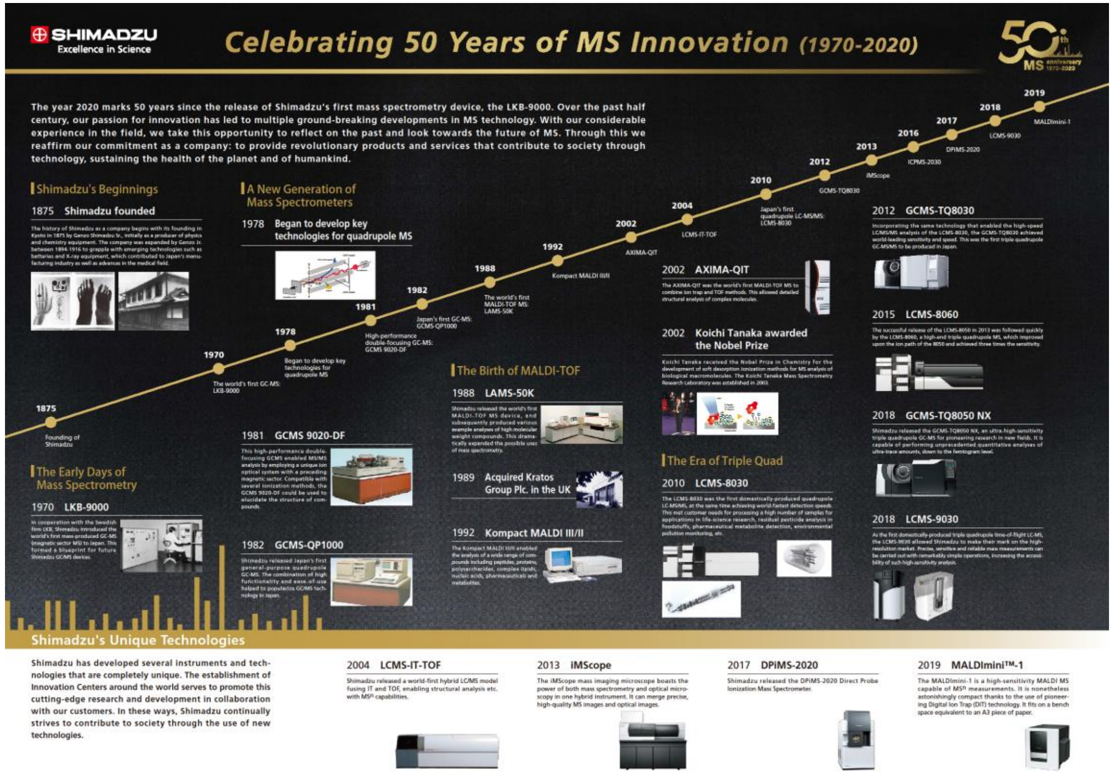
SHIMADZU

(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 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

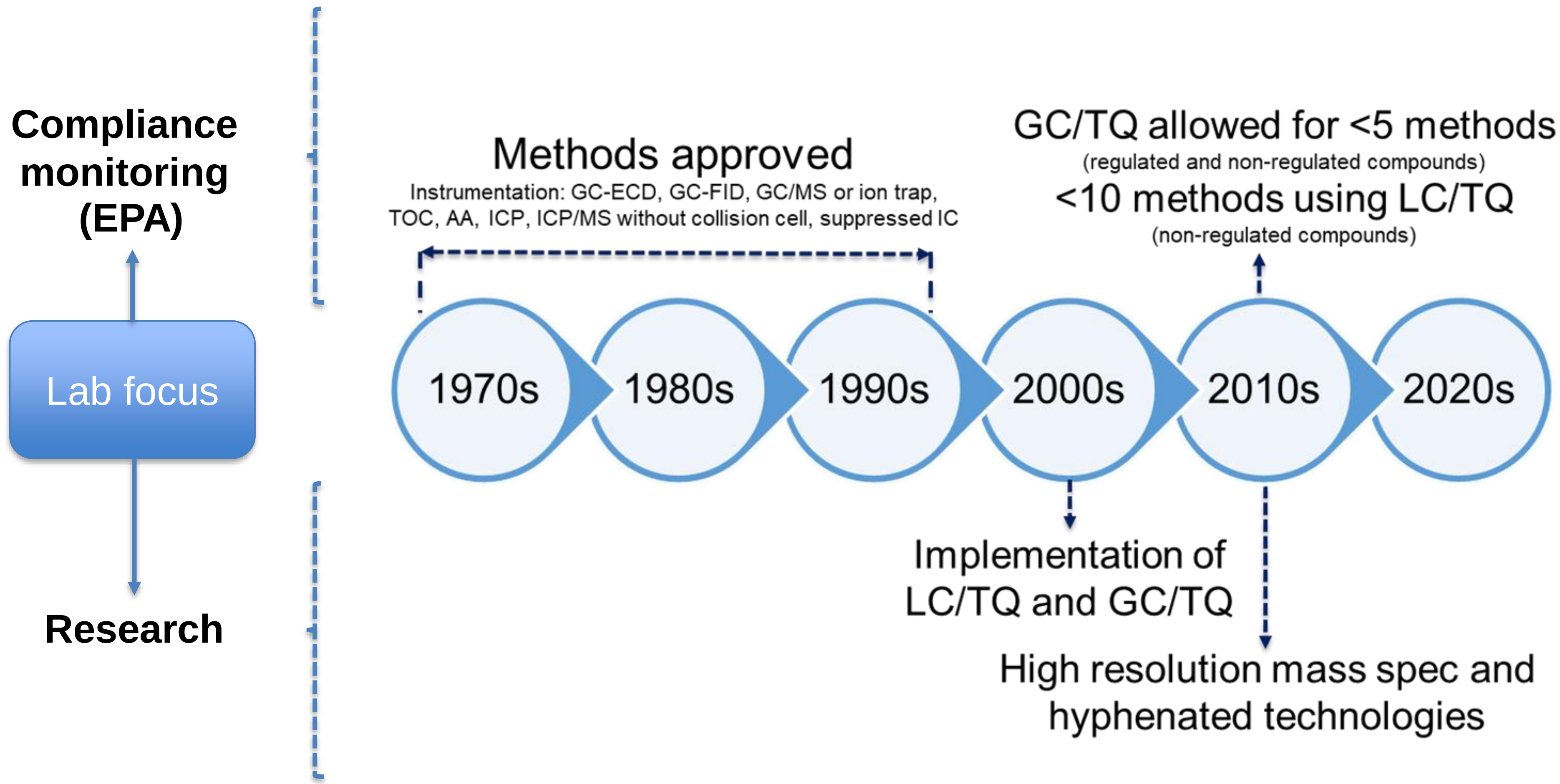
LC-MS/MS is a mature technique that has been widely commercialized for several decades.



https://www.shimadzu.com/an/news-events/celebrating_50_years/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?



MS/MS = TQ

SHIMADZU

A real life example

Analysis of estrogens in water

2003:
SPE + GC/MS with 2 steps derivatization

2007:
SPE + LC/MS/MS with and without derivatization

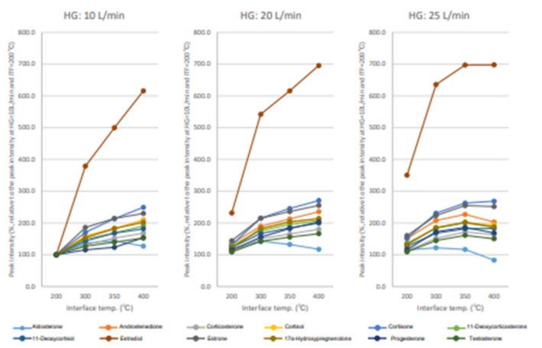
2013:
SPE + LC/MS/MS for UCMR3

2021:
Direct injection reaching lower detection limits

MOX and BSTFA
>16 h derivatization

Dansyl chloride
15 min derivatization

Direct injection
after SPE



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 (TO BE PUBLISHED)	EPA 8328 (TO BE PUBLISHED) Meets DOD QSM	EPA 8329 (TO BE PUBLISHED)	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

www.OneLabOneEarth.com

Shimadzu Scientific Instruments
7102 Riverwood Drive, Columbia, MD 21046
Ph: 410-381-1227 / 800-477-1227
www.ssi.shimadzu.com

Applications for protecting public health: Aquatic toxins

Cyanotoxins (EPA 544 and EPA 545)

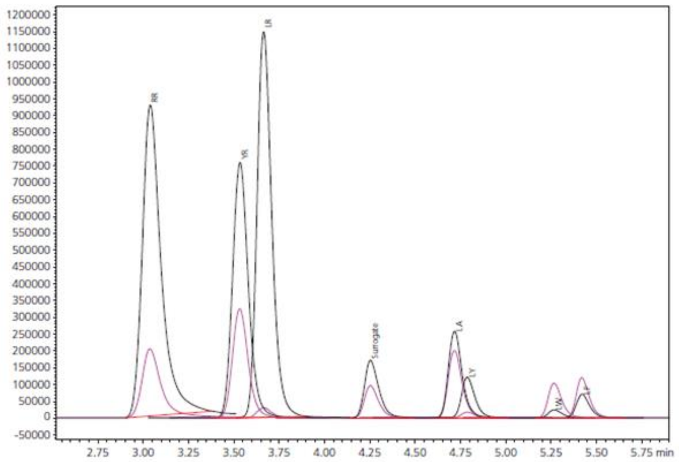


Figure 1. TIC of 100 ng/mL standard displaying target and reference ions for all compounds.

Microcystin	Quant MRM	Cal range	r^2	Lake Erie Spl (ng/mL)		Lake Erie Spl (ng/mL)	
		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)	Diarrheic 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.

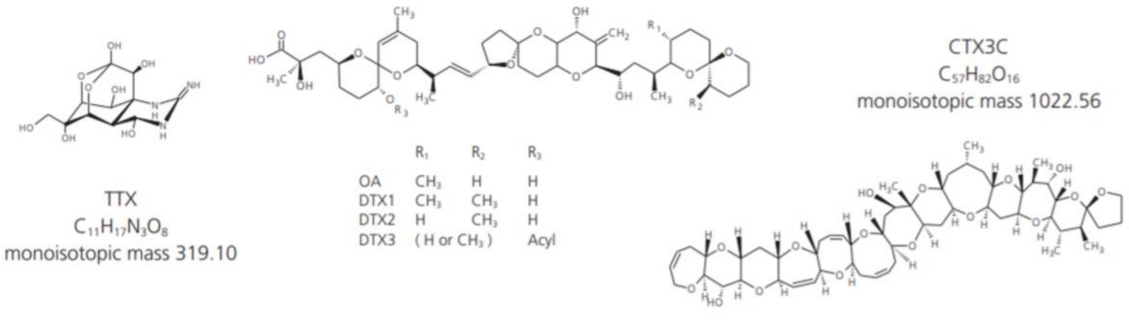
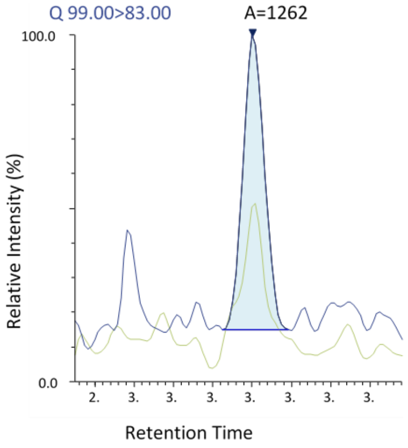


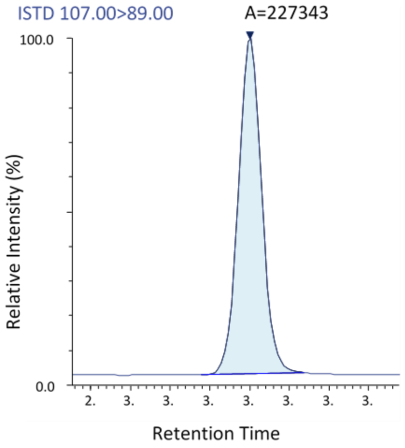
Figure 1. Structure of marine toxins

SHIMADZU

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 English[1], Kristin Neir[2]
[1]Shimadzu Scientific Instruments
[2]ALS 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	Mean	Mean					
	A	B	C	D	E	F	G	Value	Value	Recovery	Std Dev	MDLc	MDLr	RSD	LOD
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 1	Sample 2	Sample 3	Sample 4	Avg	Std Dev	Precision	Recovery	Precision Limit	Recovery Limits
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

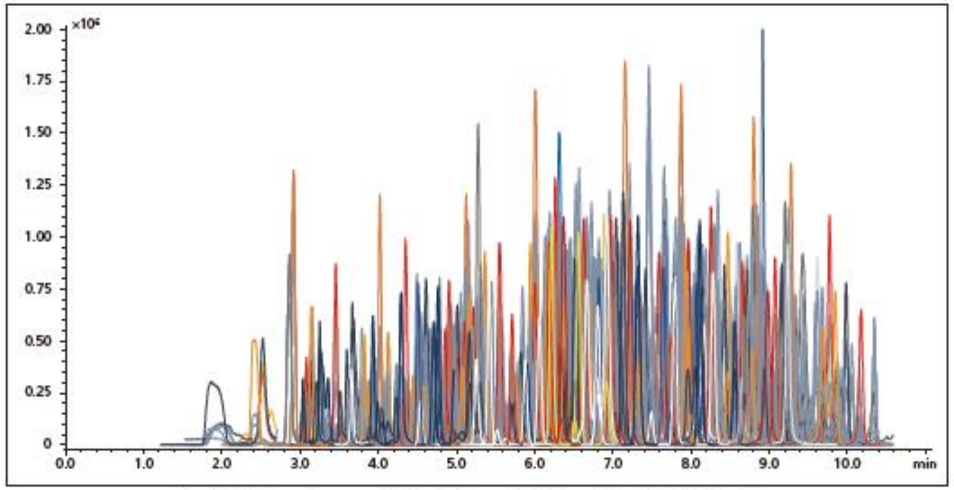


Fig. 1 MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg (Up to 3 MRMs per compound and 5 msec polarity switching time).

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

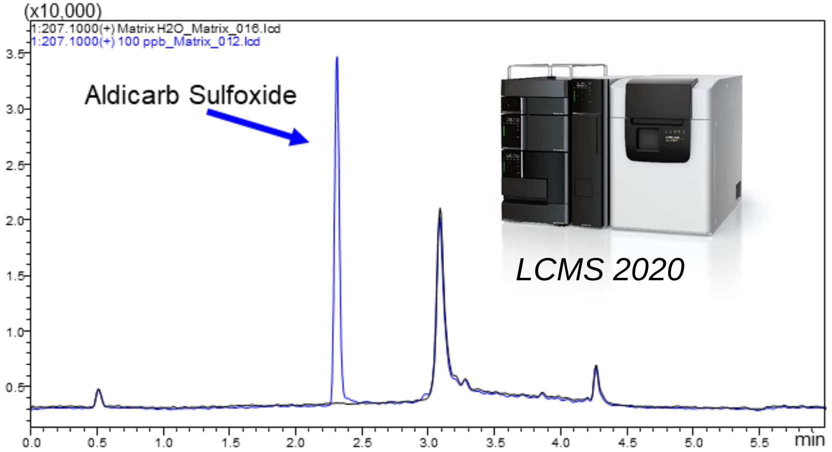
Liquid Chromatography Mass Spectrometry

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

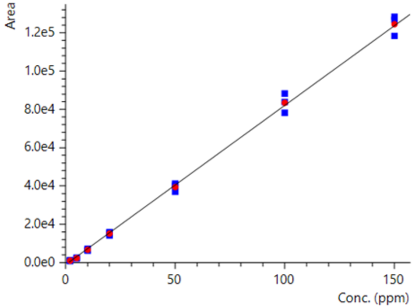
David R. Baker[1], Laëtitia Fages[2], Eric Capodanno[2], Neil Loftus[1]
[1]Shimadzu Corporation, UK; [2]Phytocontrol, France

Quantification of Glyphosate, Glufosinate, and AMPA in Food via In-vial Addition of Pairing Agent
Uwe Oppermann[1], Stephane Moreau[1], Doriane Toinon[2]
[1]Shimadzu Europa GmbH, Germany
[2]Shimadzu Corporation, Japan

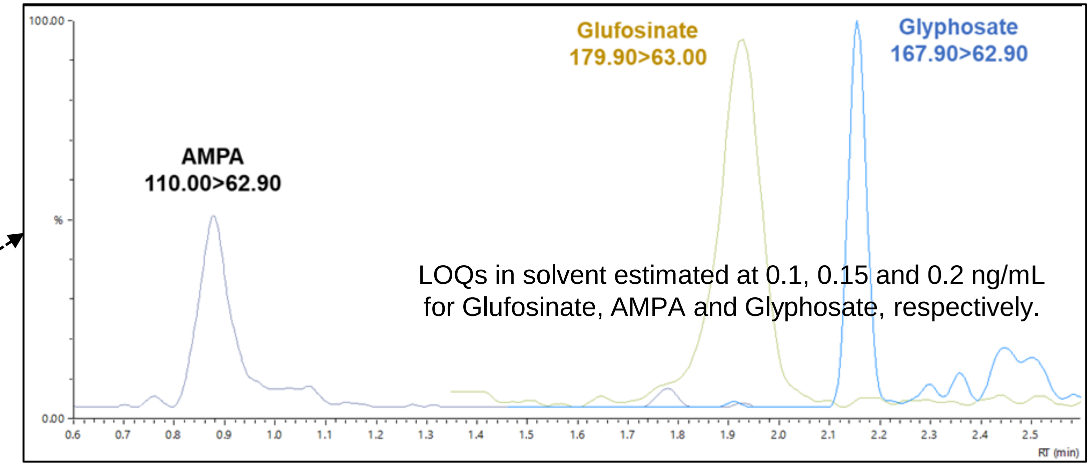
Alternatives for carbamates and glyphosate?



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
R2=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.

SHIMADZU

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
pubs.acs.org/est
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*

Journal of Hazardous Materials 398 (2020) 122933

Contents lists available at ScienceDirect

Journal of Hazardous Materials

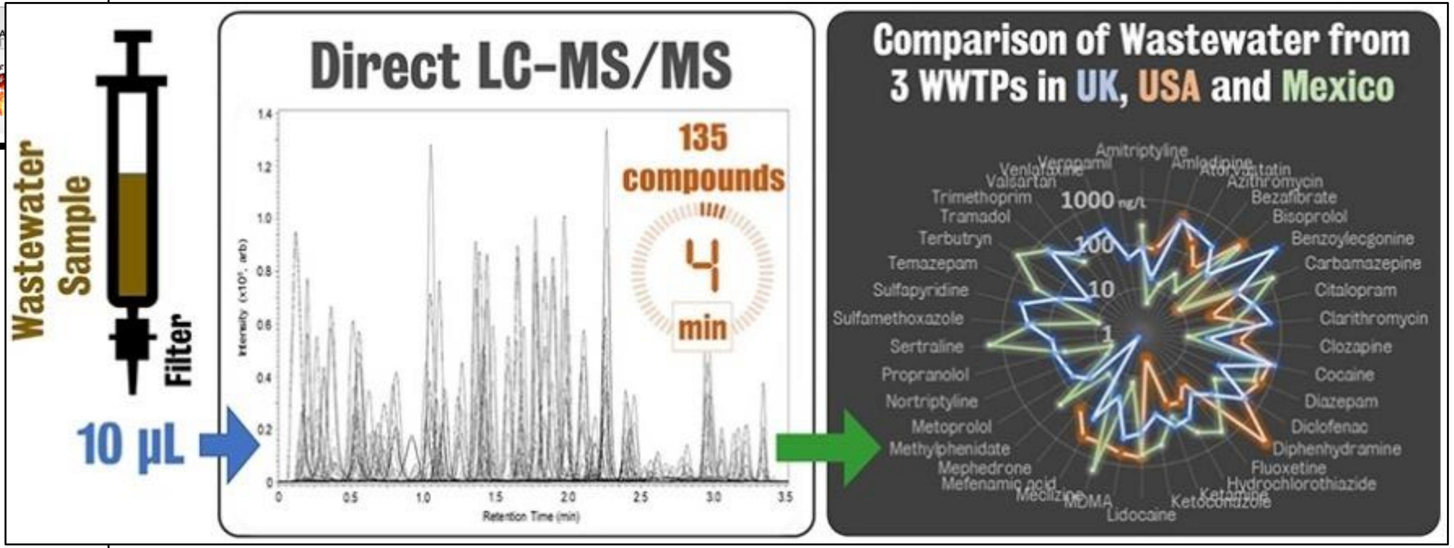
journal homepage: www.elsevier.com/locate/jhazmat

ELSEVIER

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

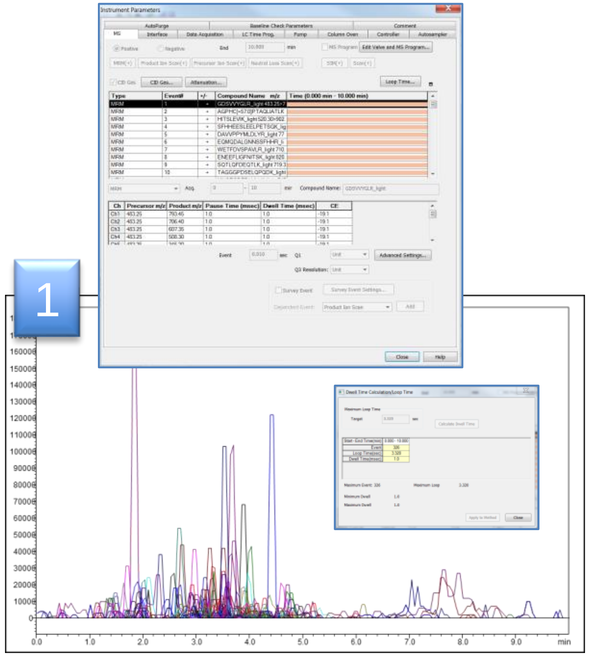
Keng Tiong Ng[a,1], Helena Rapp-Wright[a,b,1], Melanie Egli[a], Alicia Hartmann[a,c], Joshua C. Steele[d,h,j], Juan Eduardo Sosa-Hernández[e], Elda M. Melchor-Martínez[e], Matthew Jacobs[b], Blánaid White[b], Fiona Regan[b], Roberto Parra-Saldivar[e], Lewis Couchman[f], Rolf U. Halden[d,h,i,j], Leon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

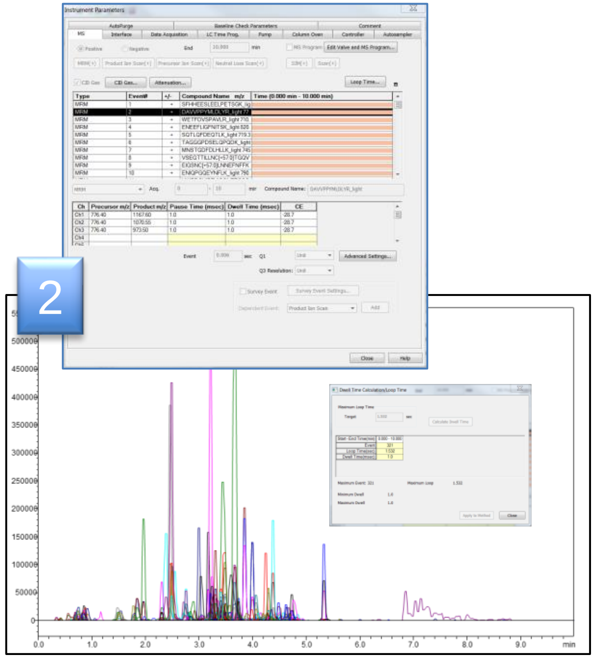
[a] Dept. Analytical, Environmental & Forensic Sciences, King's College London, 150 Stamford Street, London, SE1 9NH, United Kingdom
[b] DCU Water Institute and School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland
[c] Hochschule Fresenius, Limburger Straße 2, Idstein, Hessen, Germany
[d] Biodesign Center for Environmental Health Engineering, The Biodesign Institute, Arizona State University, 1001 S. McAllister Avenue, Tempe, AZ 85287-8101, USA
[e] Tecnologico de Monterrey, Escuela de Ingenieria 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. Raintree Dr., Ste 140, Scottsdale, AZ 85260, USA



KT Ng et al., J Hazardous Materials, 398, 2020, 122933

Myths about LC/MS/MS - Method development

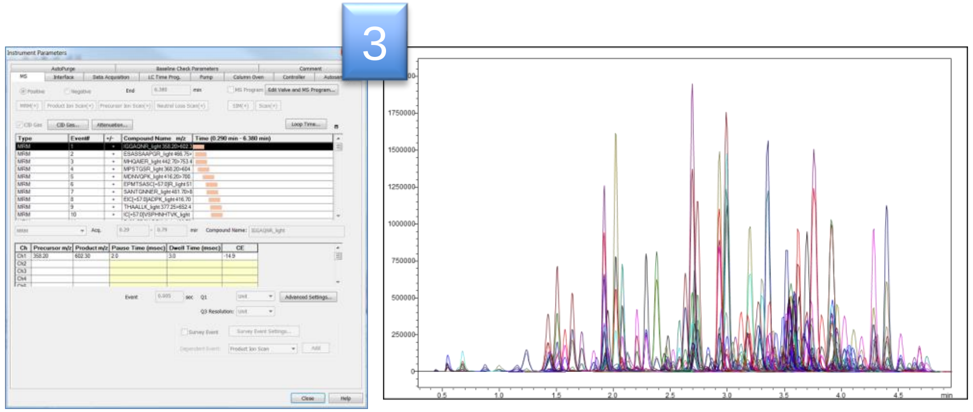


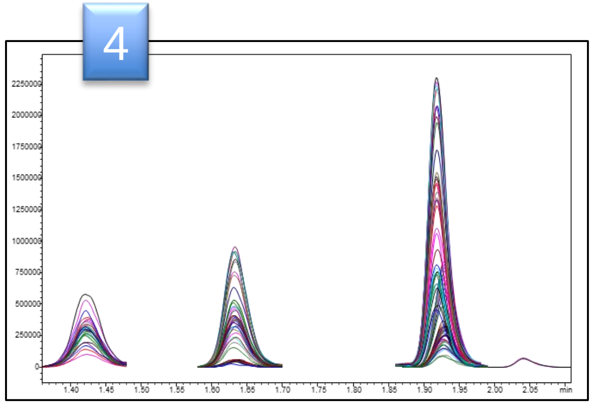


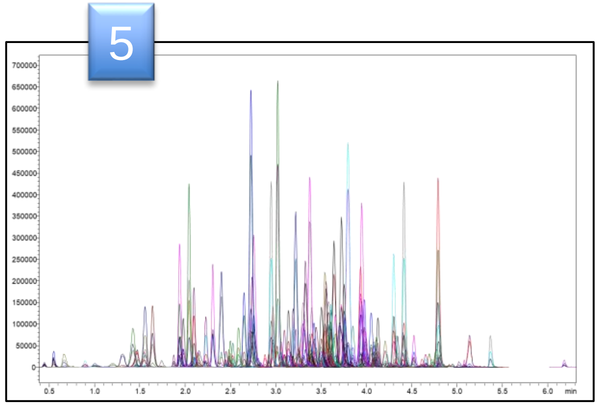
It's complex.

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





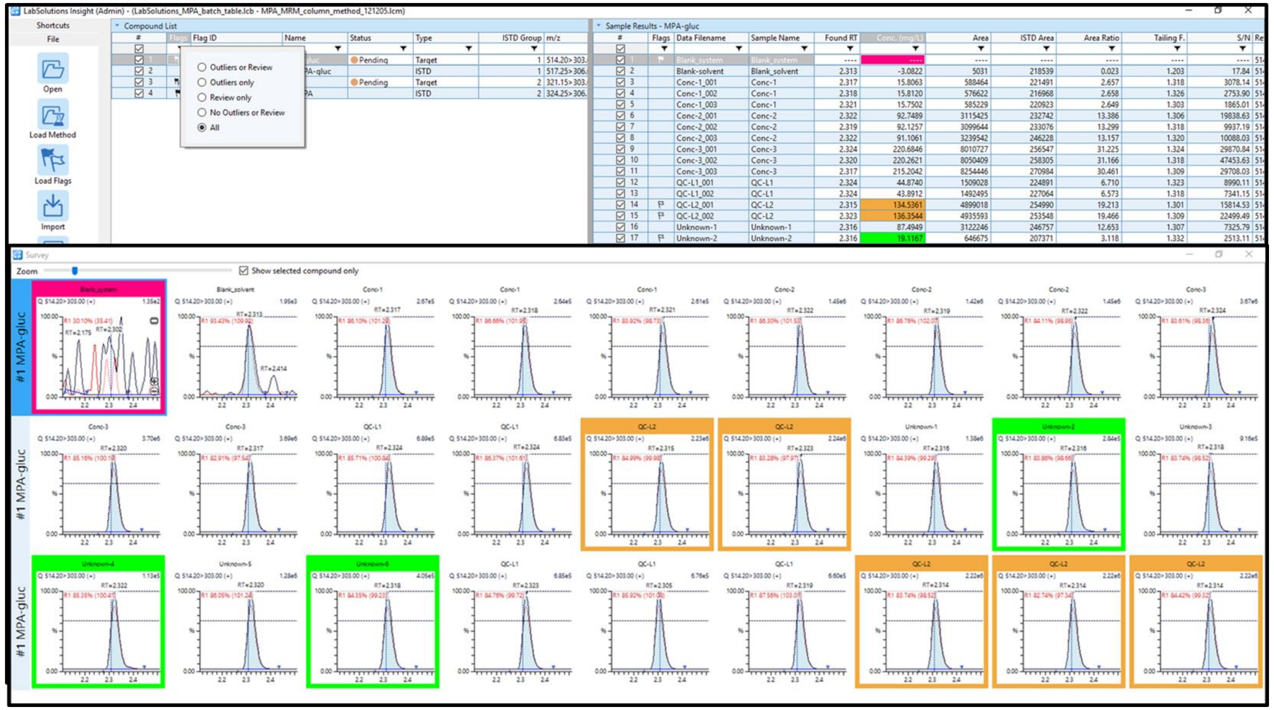


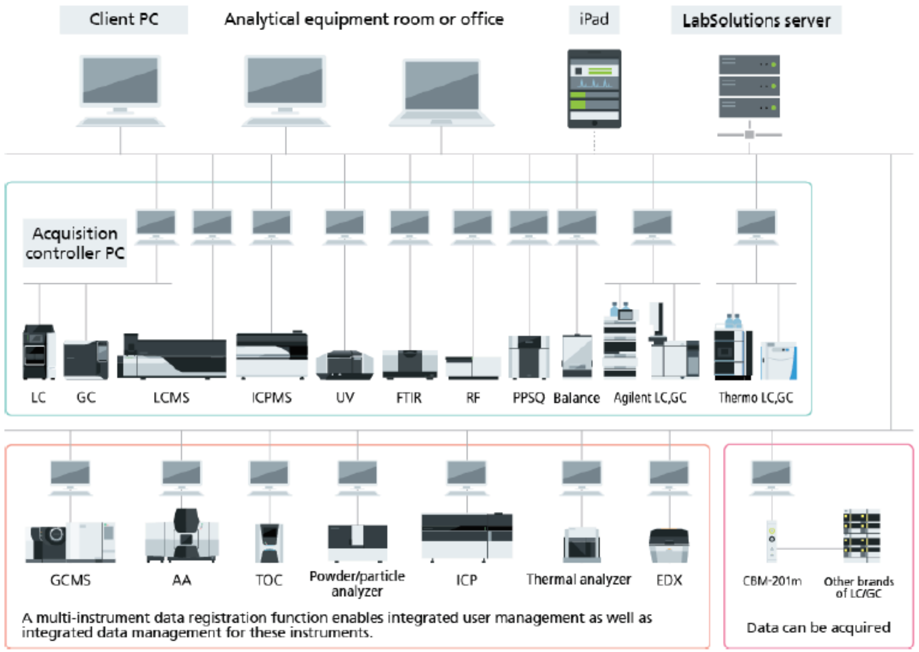
SHIMADZU

Myths about LC/MS/MS – Data Processing

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

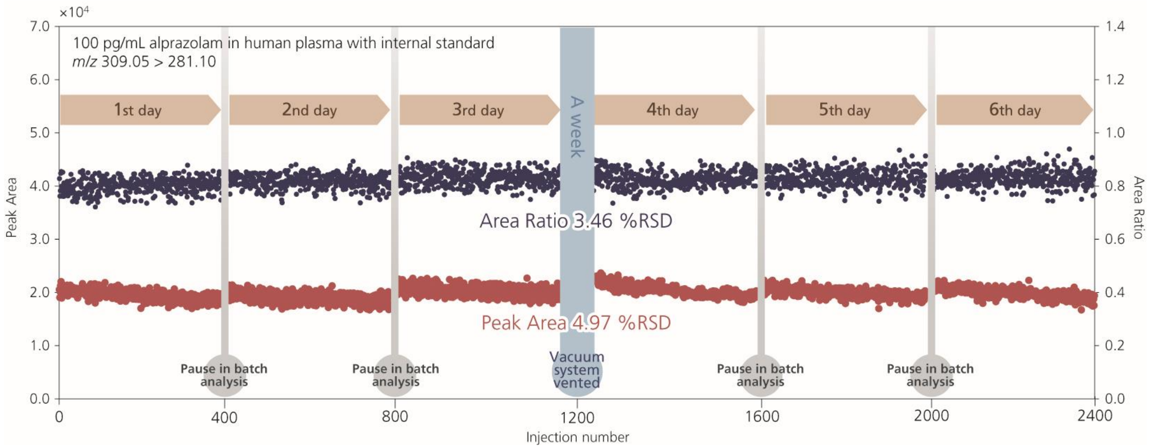
Workflows developed for automating data process and review and increasing sample throughput.
Connectivity to same software platform as other instruments. available.

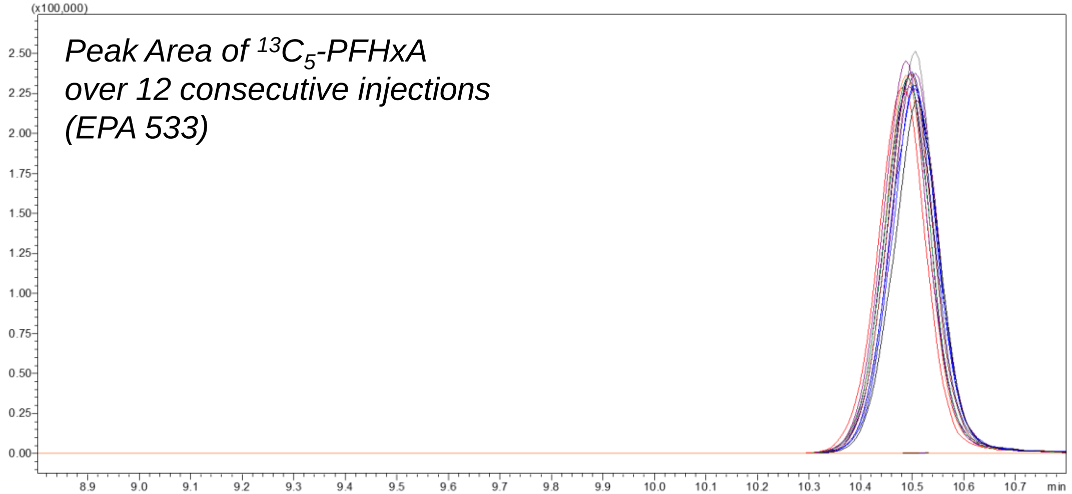




Myths about LC/MS/MS – Sensitivity

It's too sensitive and signal fluctuates over time.





Peak Area of $^{13}C_5$-PFHxA over 12 consecutive injections (EPA 533)

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

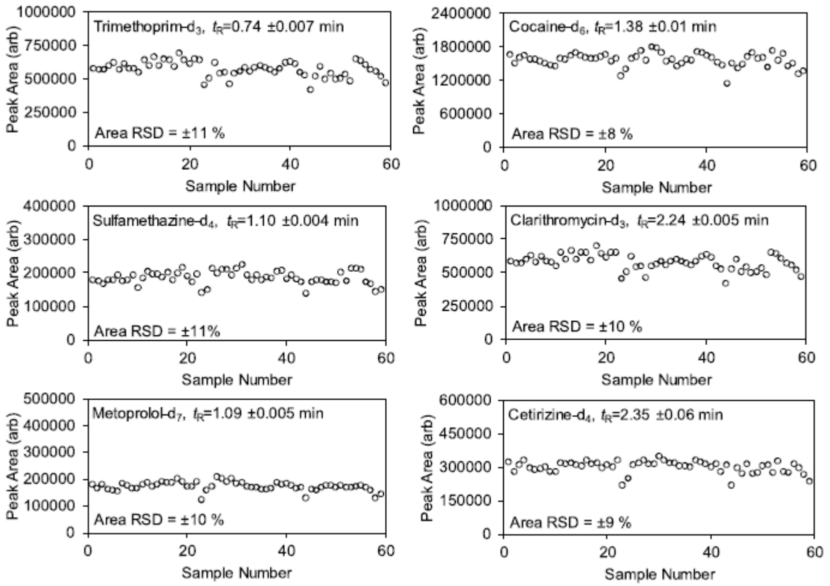


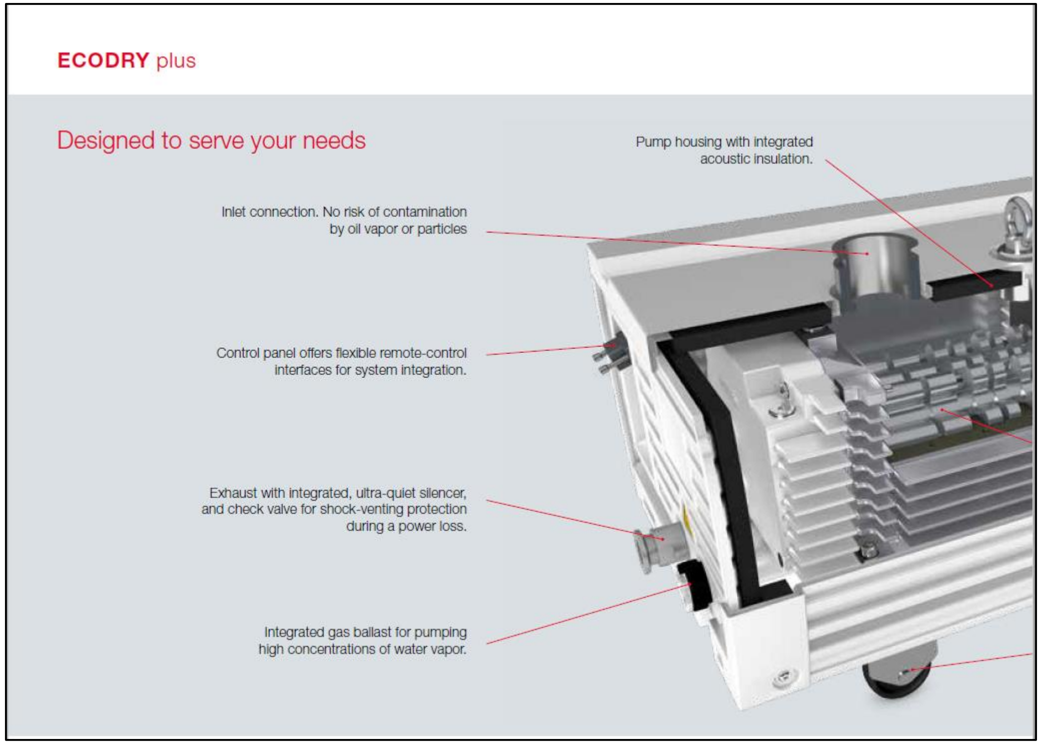
Fig. 3. Peak area and retention time stability for selected SIL-IS over a sequence of $n = 59$ spiked London wastewater samples (500 ng L^{-1}) and measured using direct LC–MS/MS analysis over a total batch analysis time of 6.4 h.

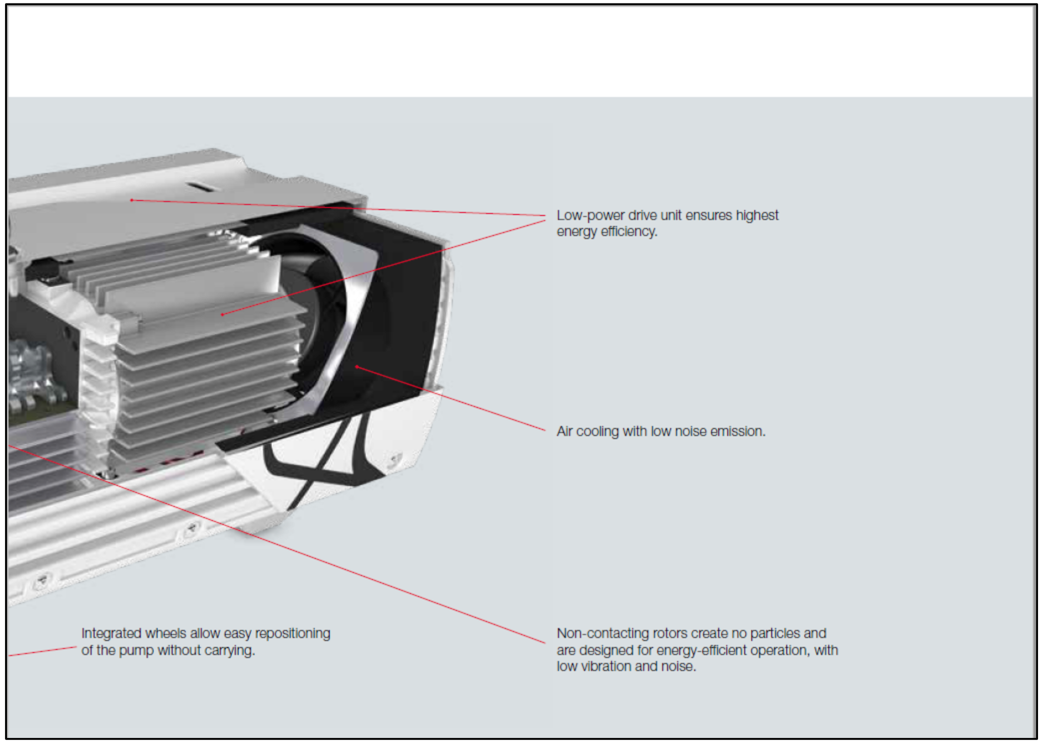
KT Ng et al., J Hazardous Materials, 398, 2020, 122933

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!).



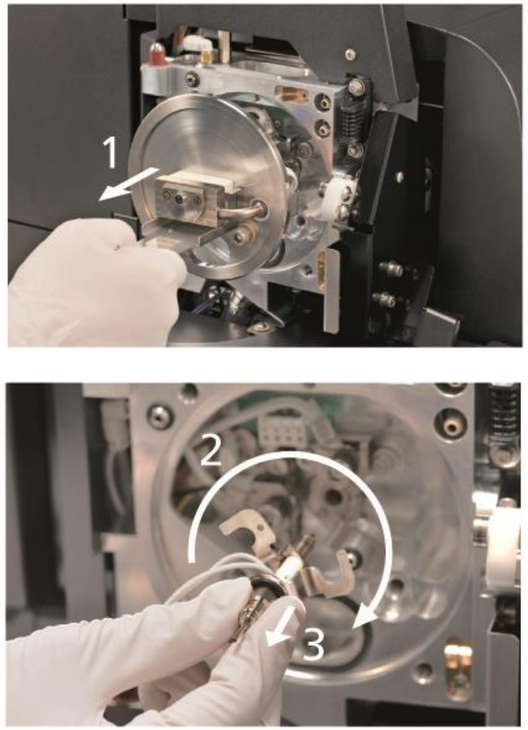


Myths about LC/MS/MS – Maintenance

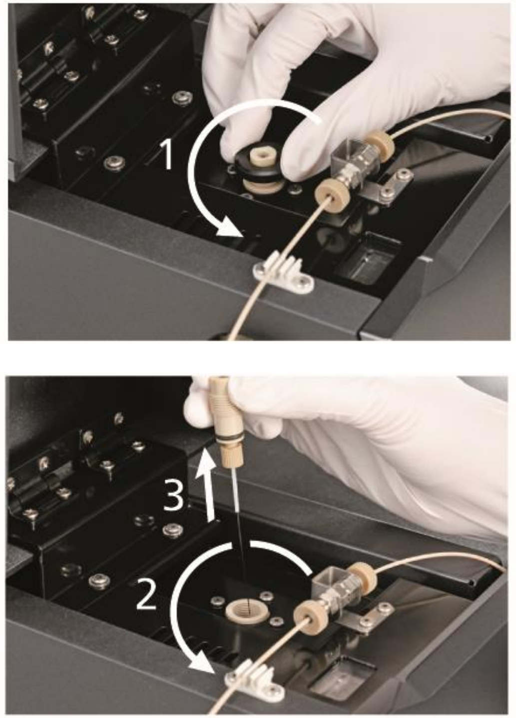
I need to break vacuum to maintain the system.

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.

Visit OneLabOneEarth.com

Ruth Marfil-Vega, PhD
rmmarfilvega@shimadzu.com