NORMAN WORKSHOP ON CHEMICAL POLLUTANTS AND BIODIVERSITY
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Towards next generation environmental (bio)monitoring: State-of-the-art HRMS-based methodologies for broad coverage in chemical universe

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Introduction - environmental (bio)monitoring

Biomonitoring using wildlife

warning system on chemicals with potential hazardous properties
apex predators → ***ideal human simulator***
protection of environmental and human health (***One Health*** approach)

combined targeted and untargeted HRMS-based workflows

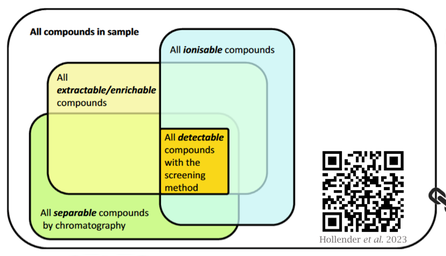
Analytical Challenges
- **Thousands** of chemicals of anthropogenic origin end up in the environment
- **Lack of reference standards**, molecular databases or spectral libraries

Sensitive analytical methodologies

Analytical Challenges
- **Low concentration levels** of chemicals in the environmental compartments
- **Sample complexity** (high matrix effects)

Additional separation dimension

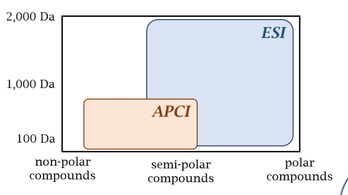
Analytical Challenges
- **Complex MS/MS data** produced by data-independent acquisition (**DIA**) modes
- **Few criteria for confident identification** with the established HRMS-data processing workflows
- Separation of **isobaric/isomeric** compounds which co-elute during the chromatographic analysis



Hollender *et al.* 2023

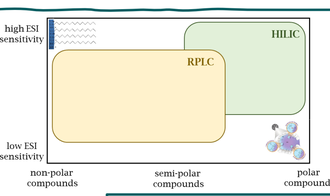
Established analytical methodologies based on analytes' **polarity**, **thermal stability**, **volatility** and **ionization efficiency**.

HRMS Instrumentation

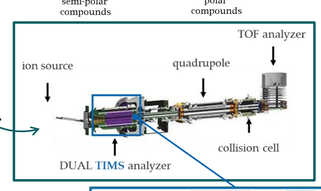


GC-APCI-QToF MS

LC-ESI-QToF MS



LC-VIP-HESI-TIMS-QToF MS



volatile non-polar

non-volatile polar, semi-polar

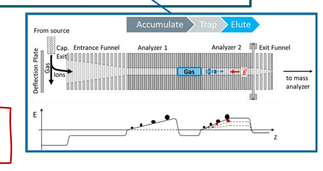
Vacuum Insulated Probe – Heated ESI

high-efficiency ceramic heater
vacuum insulation
cool fluid path
active exhaust

matrix effects
source contamination
memory effects in source

No loss and in-source fragmentation of thermally unstable compounds

3 to 5-times higher sensitivity for most of the targeted compounds using **VIP-HESI** source



Targeted HRMS-based workflows

NKUA Databases

>1,500 compounds with ***experimental CCS*** values!!!

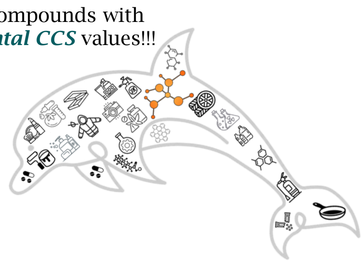
>2,500 organic micropollutants (*emerging contaminants and priority pollutants*) organized in 7 well-defined target datasets

Main chemical classes

LC-amenable chemicals
- Plant Protection Products (>900)
- Pharmaceuticals & Veterinary Drugs (>700)
  - Antibiotics (>50)
- Illicit drugs & New Psychoactive Substances (>500)
- Personal care products
- Industrial Chemicals (>150)
  - Benzothiazoles
  - Benzotriazoles
  - Flame Retardants
  - Quaternary ammonium compounds (QACs)
  - Surfactants
  - Tire additives
- Per- and Polyfluoroalkyl Substances (PFAS) (>60)
- Sweeteners
- Preservatives
- Steroids/Hormones

  +
- ***Transformation Products/ Metabolites (~200)***

GC-amenable chemicals
- Polycyclic Aromatic Hydrocarbons (PAHs)
- Polychlorinated Biphenyls (PCBs)
- Plant Protection Products
  - Organochlorine Pesticides (OCPs)
- Polychlorinated Naphthalenes (PCNs)
- Polybrominated Diphenyl Ethers (PBDEs)
- Explosives



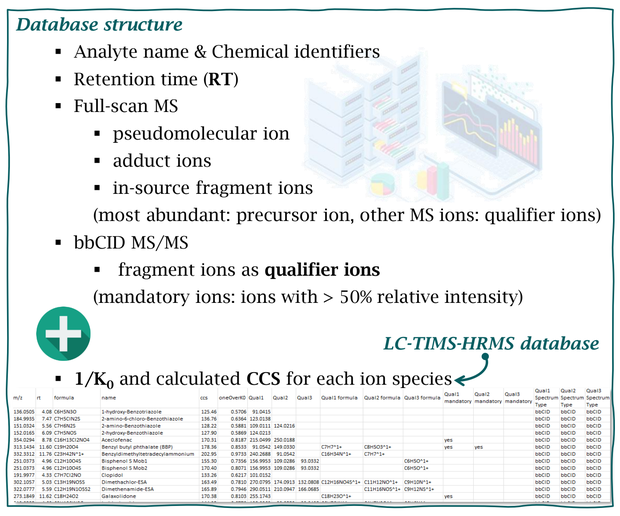
The databases are continuously being **updated**!
- new compounds of interest
- regulated compounds
- frequently identified chemicals
- literature

Database structure
- Analyte name & Chemical identifiers
- Retention time (**RT**)
- Full-scan MS
  - pseudomolecular ion
  - adduct ions
  - in-source fragment ions

  (most abundant: precursor ion, other MS ions: qualifier ions)
- bbCID MS/MS
  - fragment ions as **qualifier ions**

  (mandatory ions: ions with > 50% relative intensity)

LC-TIMS-HRMS database
- **$1/K_0$** and calculated **CCS** for each ion species



LC-HRMS GC-HRMS LC-TIMS-HRMS COMING SOON

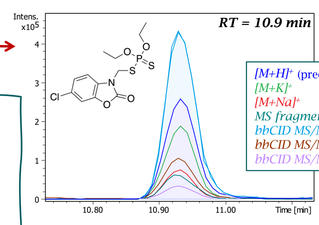
Wide-scope target analysis

Analyte Name	Formula	m/z expected	MRSQC	Δm/z [mDa]	ΔRT [min]	mSigma	Exp.Diag.Ions	ΔCCS [%]
Phosalone Mob1	$C_{12}H_{15}ClNO_4PS_2$	367.9941		-0.85	0.08	53.7	2	0.36
Phosalone Mob2	$C_{12}H_{15}ClNO_4PS_2$	367.9941		-0.63	0.09	68.5	2	0.28
Phosphamidon (Dimecron) Peak 1 Mob1	$C_{10}H_{19}ClNO_5P$	300.0762		0.09	0.04	23.8	2	0.70
Phosphamidon (Dimecron) Peak 2	$C_{10}H_{19}ClNO_5P$	300.0762		0.15	0.04	27.7	2	0.68
Phoxim	$C_{12}H_{15}N_2O_3PS$	299.0614		-0.63	0.09	16.6	2	0.26
Pirimicarb	$C_{11}H_{18}N_4O_2$	239.1503		0.24	0.03	15.6	2	0.24
Pirimiphos-methyl	$C_{11}H_{20}N_3O_3PS$	306.1036		-0.12	0.10	33.0	2	0.30
Profenophos	$C_{11}H_{15}BrClO_3PS$	374.9402		-0.92	0.06	75.5	2	0.26
Propachlor	$C_{11}H_{14}ClNO$	212.0837		-0.01	0.06	14.0	2	0.38
Propamocarb	$C_9H_{20}N_2O_2$	189.1598		0.05	0.01	21.1	3	-0.24
Propanil	$C_9H_9Cl_2NO$	218.0134		-0.61	0.06	9.7	2	-0.02
Propargite (NH4)	$C_{19}H_{30}NO_4S^{+}$	368.1890		-0.60	0.08	63.0	2	-0.07

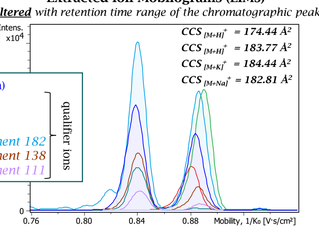
BRUKER ASQ *TASQ 2023b*

Phosalone

Extracted Ion Chromatograms (EICs)



Extracted Ion Mobilograms (EIMs)
(*filtered with retention time range of the chromatographic peak*)



IDENTIFICATION CRITERIA
Mass accuracy
Retention time
iSotopic profile
Qualifier ions

CCS value matching

LC-TIMS-HRMS data

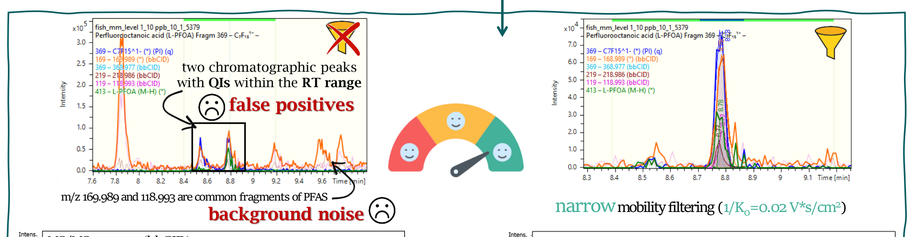
4D-target analysis

Wide-scope target analysis

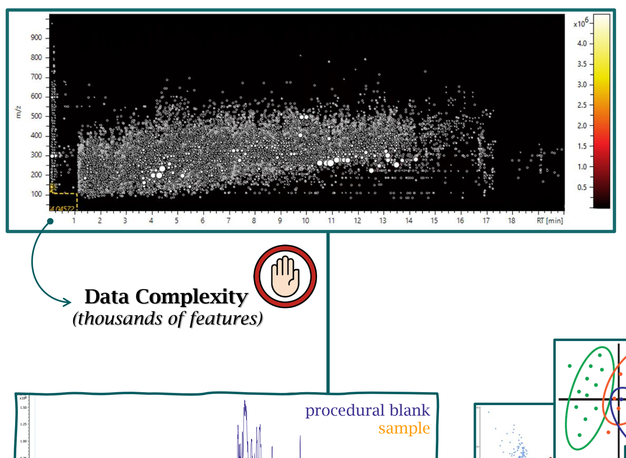
Gago-Ferrero *et al.* 2020

Added value of TIMS in targeted workflows
- ✓ Additional ID criterion.
- ✓ Higher-quality MS and MS/MS spectra in DIA mode.
- ✓ Reduction of false positives & negatives.
- ✓ Increased reliability in the targeted results.
- ✓ Reduced time for data review.

Effect of ion mobility filtering

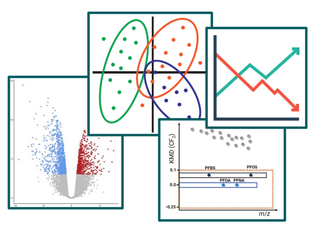


Diamanti *et al.* 2020 · Badry *et al.* 2020 · Nikolopoulou *et al.* 2022 · Gkotsis *et al.* 2022 · Gkotsis *et al.* 2023 · Ng *et al.* 2023 · Movalli *et al.* 2023



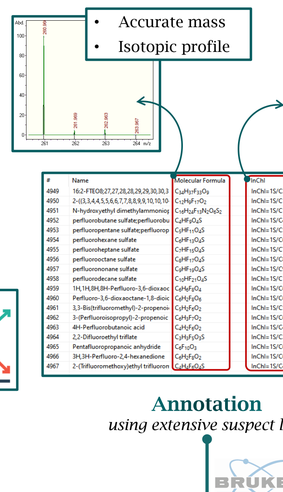
Blank Subtraction

Data Complexity
(thousands of features)



Prioritization
based on statistical differences/trends of sub-groups (e.g. volcano plot, PCA), their structural similarity (e.g. KMD plot: PFAS)

features of interest



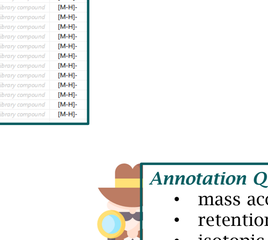
#	Name	Molecular Formula	InChI	Neutral Ma...	RT [min]	add CCS	Library Compound	Main Ne...
4949	16:2-FTEOB;2,2,2,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10	$C_{18}H_{17}F_{19}O_3$	InChI=1S/C34H37F33O9/c35-19(36,...	1216.19106	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4950	2-((3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)oxy)ethan-1-ol	$C_{12}H_9F_{17}O_2$	InChI=1S/C12H9F17O2/c13-5(14,1-2-...	508.03111	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4951	N-hydroxyethyl-dimethylammonium perfluorooctanesulfonate	$C_{12}H_{14}F_{17}NO_4S$	InChI=1S/C12H14F17NO4S/c1-31(...	631.08682	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4952	perfluorobutane sulfate;perfluorobutanesulfonic acid	$C_4HF_9O_4S$	InChI=1S/C4HF9O4S/c5-1(6,3(9,10)1...	315.94518	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4953	perfluoropentane sulfate;perfluoropentanesulfonic acid	$C_5HF_{11}O_4S$	InChI=1S/C5HF11O4S/c6-1(7,2(8,9)...	365.94199	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4954	perfluorohexane sulfate	$C_6HF_{13}O_4S$	InChI=1S/C6HF13O4S/c7-1(8,2(9,10)...	415.93880	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4955	perfluoroheptane sulfate	$C_7HF_{15}O_4S$	InChI=1S/C7HF15O4S/c8-1(9,2(10,1...	465.93560	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4956	perfluorooctane sulfate	$C_8HF_{17}O_4S$	InChI=1S/C8HF17O4S/c9-1(10,3(13,...	515.93241	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4957	perfluorononane sulfate	$C_9HF_{19}O_4S$	InChI=1S/C9HF19O4S/c10-1(11,2(12...	565.92921	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4958	perfluorodecane sulfate	$C_{10}HF_{21}O_4S$	InChI=1S/C10HF21O4S/c11-1(12,3(1...	615.92602	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4959	1H,1H,8H,8H-Perfluoro-3,6-dioxaoctane-1,8-disulfonic acid	$C_6H_4F_{12}O_8S_2$	InChI=1S/C6H4F12O8S2/c7-3(8,1(19...	594.01383	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4960	Perfluoro-1,6-dioxaoctane-1,8-dioic acid	$C_6HF_{11}O_6$	InChI=1S/C6HF11O6/c7-3(8,1(5(18)...	321.97236	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4961	3,3-Bis(trifluoromethyl)-2-propenoic acid	$C_5H_2F_6O_2$	InChI=1S/C5H2F6O2/c6-4(7,8)2(1-3...	207.99590	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4962	3-(Perfluoroisopropyl)-2-propenoic acid	$C_6H_2F_7O_2$	InChI=1S/C6H3F7O2/c7-4(5(8,9)10)...	240.00213	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4963	4H-Perfluorobutanoic acid	$C_4H_2F_6O_2$	InChI=1S/C4H2F6O2/c5-1(6)3(7,8)4...	195.99590	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4964	2,2-Difluoroethyl triflate	$C_3H_3F_5O_3S$	InChI=1S/C3H3F5O3S/c4-2(5)1-11-...	213.97231	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4965	Pentafluoropropanoic anhydride	$C_6F_{10}O_3$	InChI=1S/C6F10O3/c7-3(8,5(12,13)...	309.96878	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4966	3H,3H-Perfluoro-2,4-hexanedione	$C_6H_2F_{10}O_2$	InChI=1S/C6H2F10O2/c7-4(8,5(12,13...	257.99270	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-
4967	2-(Trifluoromethoxy)ethyl trifluoromethanesulfonate	$C_4H_4F_6O_4S$	InChI=1S/C4H4F6O4S/c5-3(6,7)14-...	261.97345	0.00	add CCS	Click to assign MS/MS library compound	[M-H]-

Annotation
using extensive suspect lists

BRUKER Met *MetaboScape 2023b*

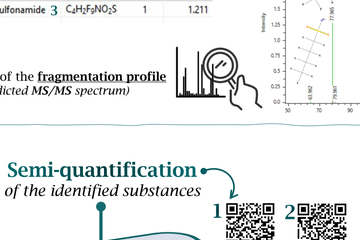
Annotation Quality
- mass accuracy (precursor ion)
- retention time fit
- isotopic pattern quality
- MS/MS spectra matching
- matching of CCS value

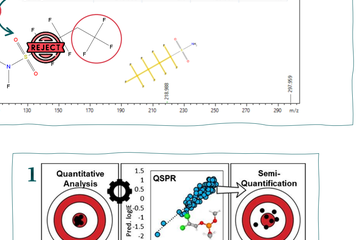
Evaluation
of the annotated results



BioTransformer 3.0

Semi-quantification
of the identified substances





Take home messages

- ✓ **Environmental (bio)monitoring** covering a **broad chemical space** can be achieved using **HRMS** combined with complementary **chromatographic** and **ionization** techniques.
- ✓ **Comprehensive** and **sensitive** analytical methodologies should be established, combining **targeted** and **untargeted** post-acquisition data treatment workflows.
- ✓ Ion Mobility Spectrometry (**IMS**) incorporated in the established LC-HRMS techniques enhances the **confidence level** in chemicals' identification.
- ✓ Simultaneous monitoring of **metabolites/transformation products** holds significant importance, as these compounds may reveal increased concentration levels and/or toxicity in comparison to their parent chemicals.

HRMS-based environmental (bio)monitoring proves to be a powerful tool for regulators, providing valuable insights into the presence of thousands of chemicals.

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