Metodi innovativi di analitica per le miscele

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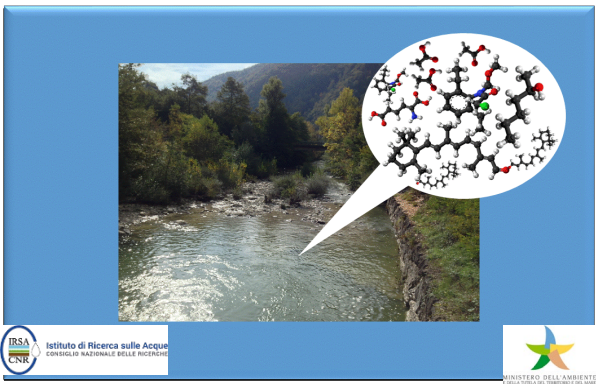
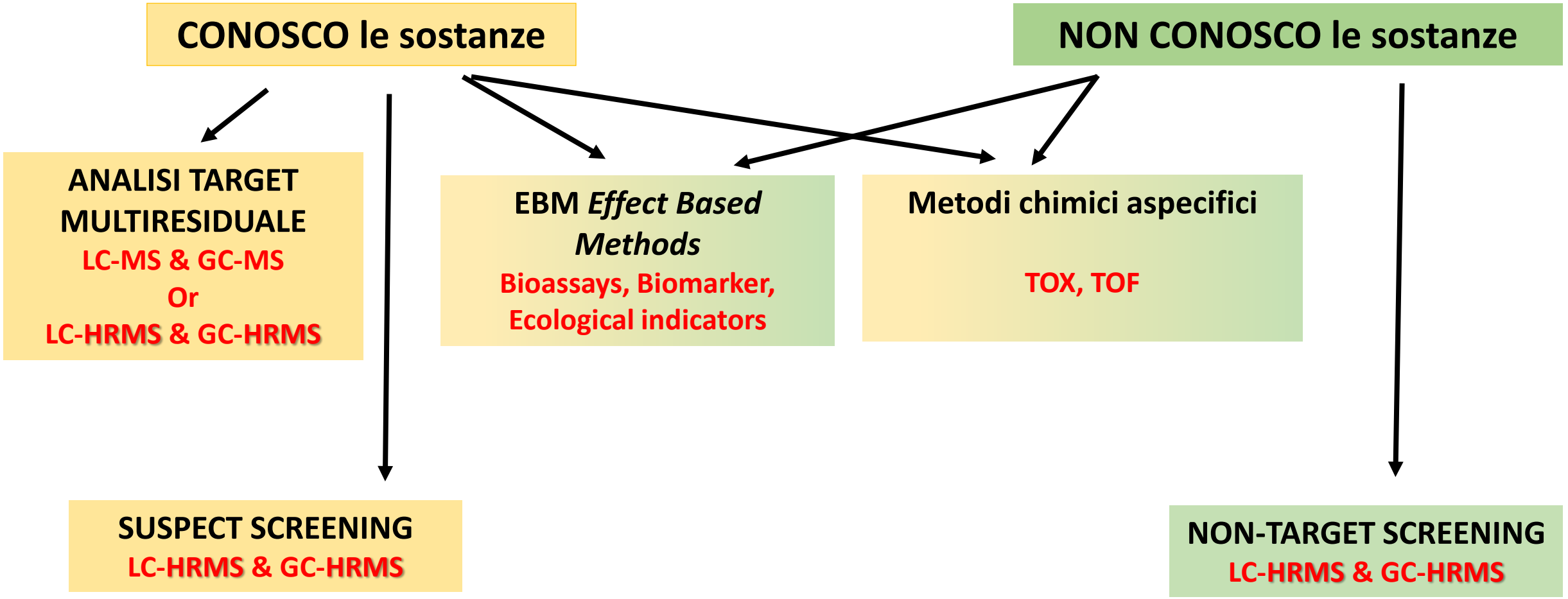


VALUTAZIONE DEGLI EFFETTI COMBINATI DELLE MISCELE DI SOSTANZE CHIMICHE

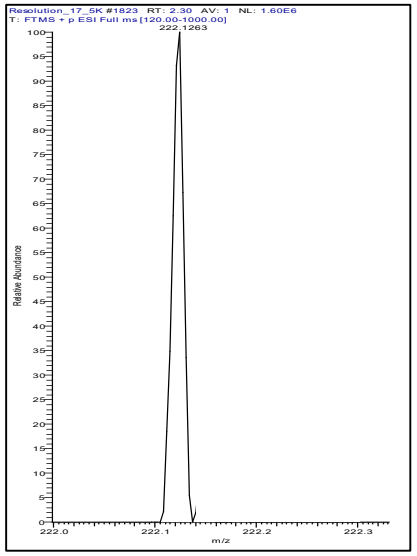
Digital Workshop

25 giugno 2020

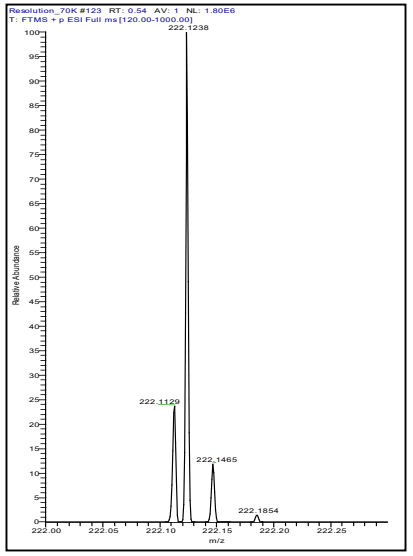
Strategie analitiche



HIGH RESOLUTION MASS SPECTROMETRY

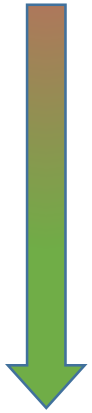


1) ion: m/z 222



1) ion: m/z 222.1129
2) ion: m/z 222.1236
3) ion: m/z 222.1465

Low resolution
LRMS



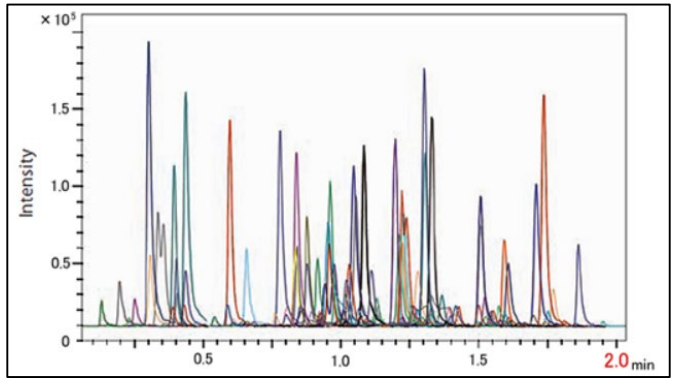
High resolution
HRMS
Orbitrap, QTOF...

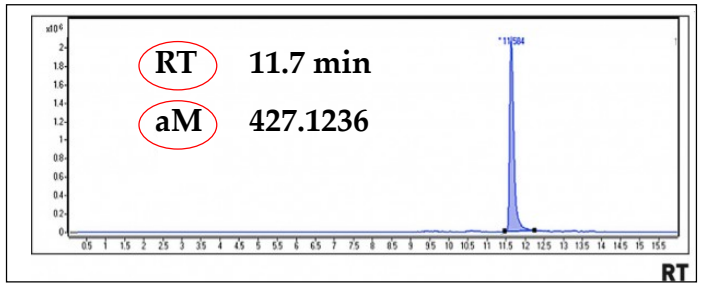
Molecular mass	Δm	N° chemical formula
236.1	± 0.1	1296
236.09	± 0.01	92
236.094	± 0.001	10
236.0949	± 0.0001	1

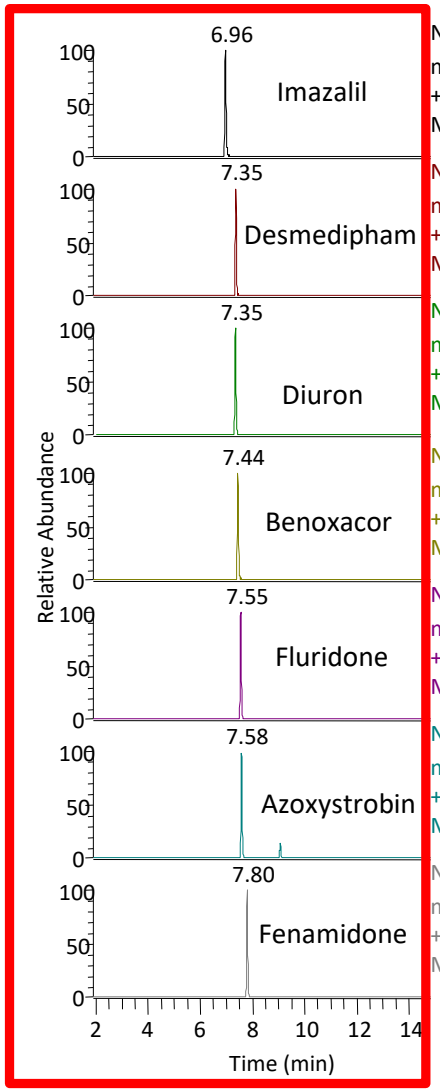
Carbamazepine
Exact Mass:
236.094955
$C_{15}H_{12}N_2O$

Gas/Liquid Chromatography-Mass Spectrometry: MS/MS confirmation

- Coupled with chromatography is the technique of choice to quantify molecules in mixtures;
- Separation technique and the detection is based on m/z and retention time (RT);







NL: 1.98E7
m/z= 297.0541-297.0571 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02

NL: 4.67E6
m/z= 301.1163-301.1193 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02

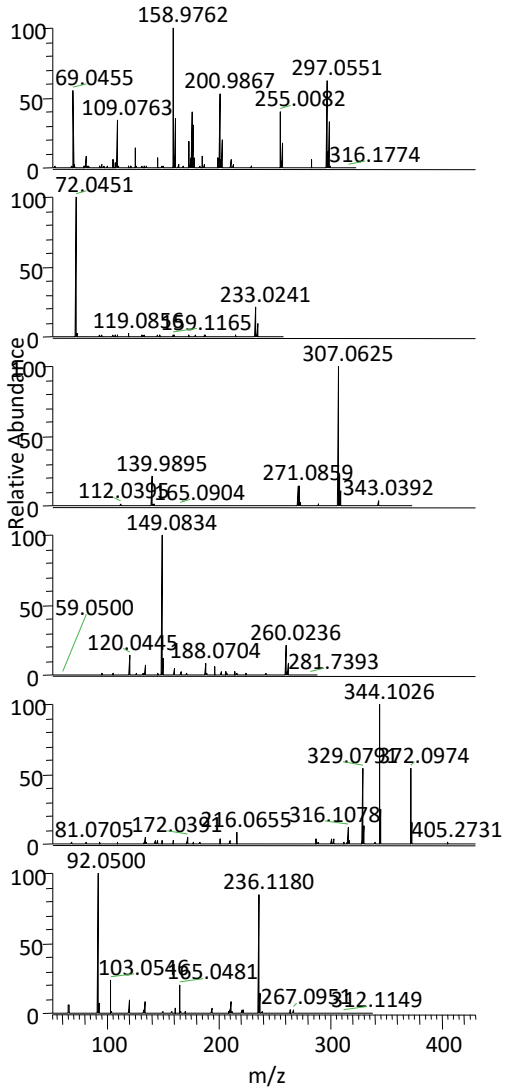
NL: 8.97E7
m/z= 233.0231-233.0255 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02

NL: 1.15E7
m/z= 260.0226-260.0252 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02

NL: 2.21E7
m/z= 330.1084-330.1118 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02

NL: 1.42E7
m/z= 404.1221-404.1261 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02

NL: 4.23E7
m/z= 312.1149-312.1181 F: FTMS
+ p ESI Full ms [50.00-750.00]
MS 4xloq_02



ANALISI TARGET MULTIRESIDUALE
Wide-Scope Target Analysis

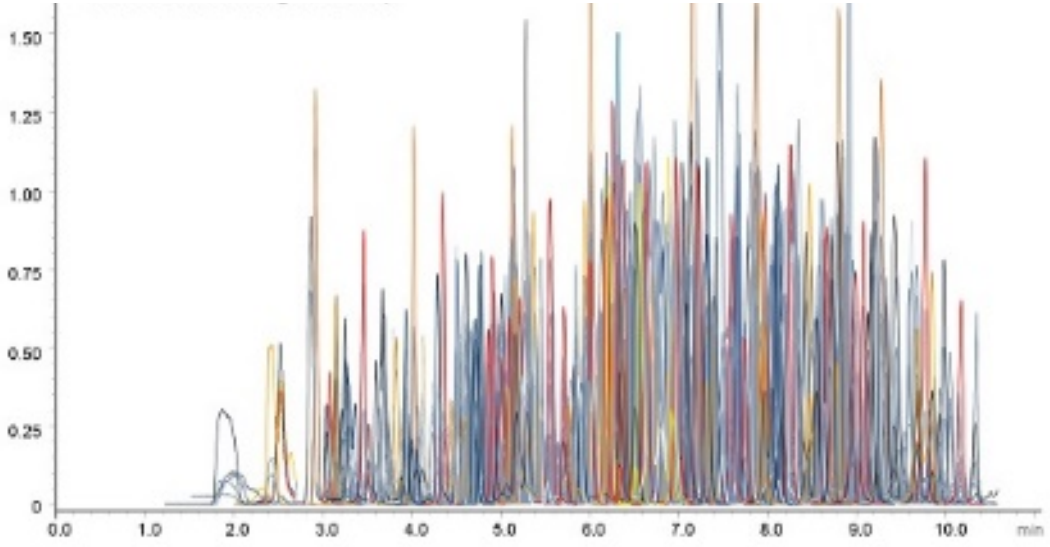
DISPONIBILITA' di standard
Metodo cromatografico validato
"one and done"

Wide-scope target screening of > 2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes

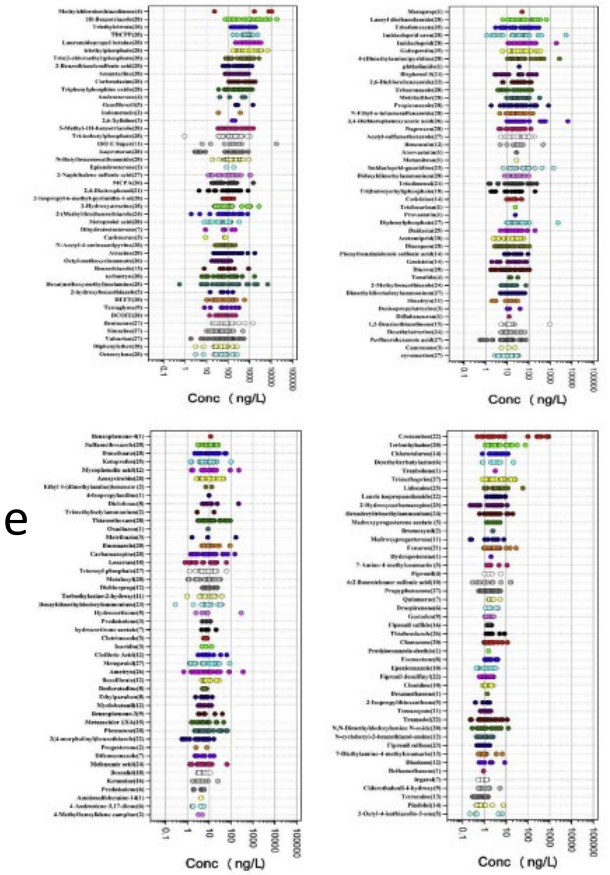
Pablo Gago-Ferrero[a,b,1], Anna A. Bletsou[a,1], Dimitrios E. Damalas[a], Reza Aalizadeh[a], Nikiforos A. Alygizakis[a], Heinz P. Singer[c], Juliane Hollender[c,d], Nikolaos S. Thomaidis[a,*]

MRM/SRM/targetedDIA

with triple quadrupole MS or HRMS



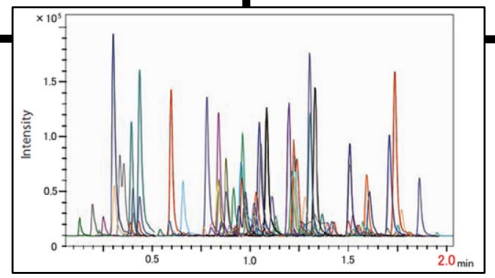
- Pesticidi
- Farmaci
- Droghe da abuso
- Composti industriali
- Sostanze dopanti
- Tensioattivi
- Metaboliti umani
- Prodotti di trasformazione



Cromatogramma di 646 pesticidi spiked into a mint extract at 0.010 mg/kg

Suspect Screening by HRMS

CAMPIONAMENTO ESTRAZIONE SEPARAZIONE CROMATOGRAFICA HRMS/MS

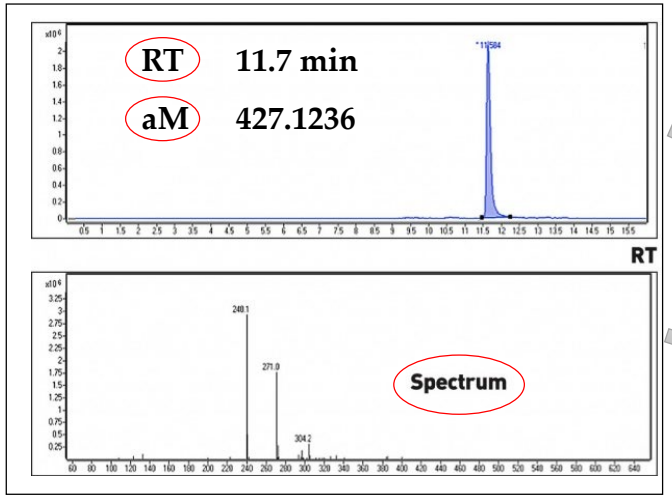


LISTA DEGLI ANALITI
RT & MS/MS

ANALISI TARGET

Assegnazione di un analita ad un picco

QUANTIFICAZIONE CON UNO STANDARD DI RIFERIMENTO



LISTA DI SOSPETTI
Formula elementare & struttura chimica

SUSPECT SCREENING by HRMS

Assegnazione di una gruppo di sospetti ad un picco in base alla massa esatta

Selezione di un sospetto
pattern isotopico , MS/MS, calc RT, proprietà chimico-fisiche, probabilità di ritrovamento

CONFERMA E QUANTIFICAZIONE CON UNO STANDARD DI RIFERIMENTO
o
SEMIQUANTIFICAZIONE

Suspect Screening by HRMS

Suspect screening and risk assessment of pollutants in the wastewater from a chemical industry park in China☆

Wei Liu [a,b], Hongye Yao [b], Wei Xu [a], Guangbing Liu [b], Xuebing Wang [a], Yong Tu [b], Peng Shi [a], Nanyang Yu [a], Aimin Li [a], Si Wei [a,*]

Standard solo per composti presenti
Ridotta sensibilità

- WWTP effluent of 9 chemical enterprises in a Chemical Industrial Park (CIP)
- MASS LIST
  - 148 High environmental hazards chemicals in the CIPs
  - List of Hazardous Chemicals for China (1796 chemicals)
  - AB SCIEX commercial database, including (1283 chemicals)

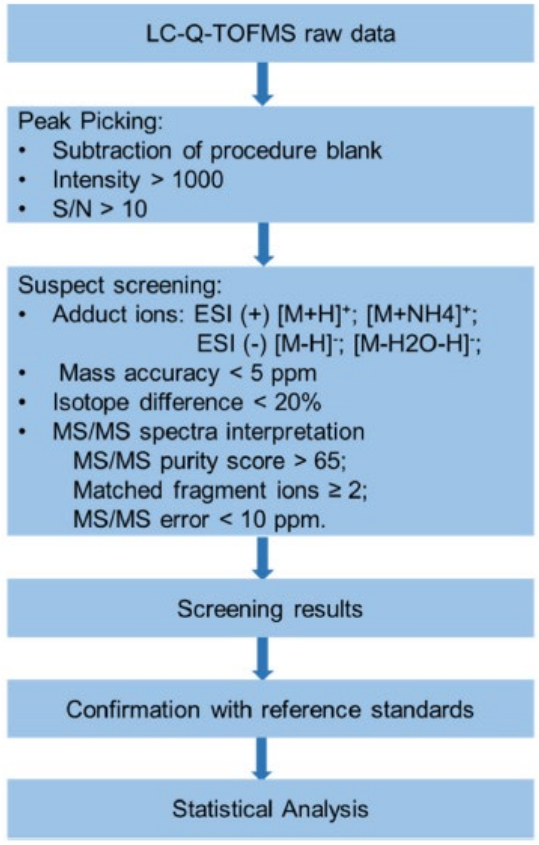


Fig. 1. Workflow for mass spectrometry data analysis.

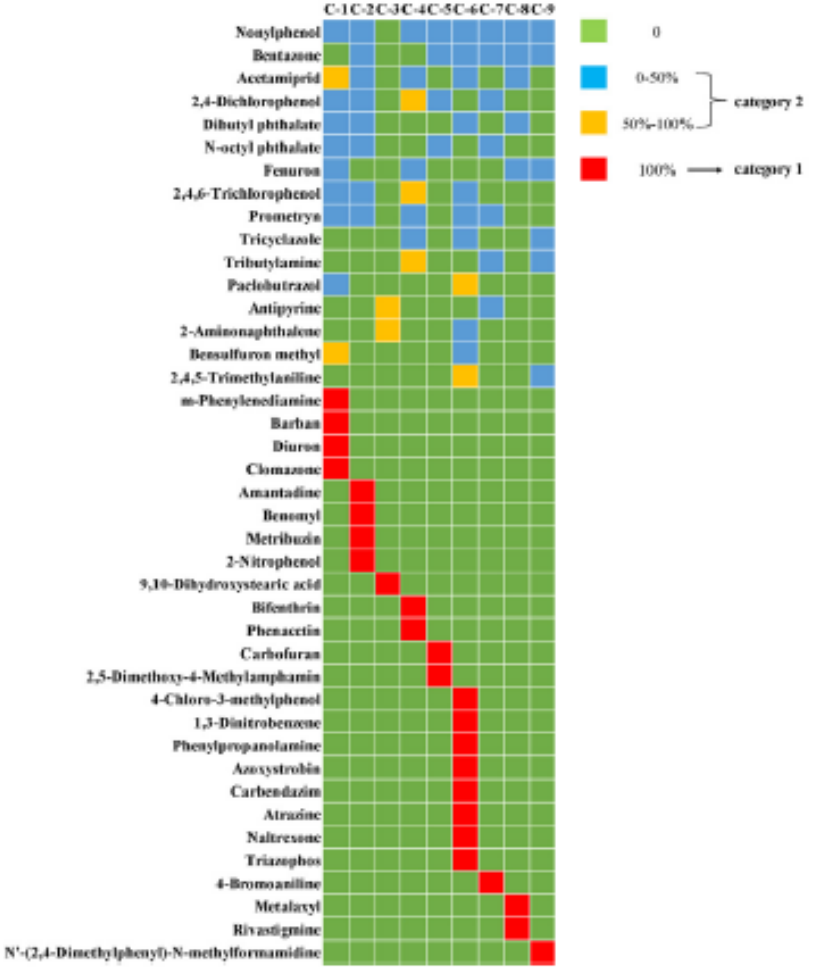
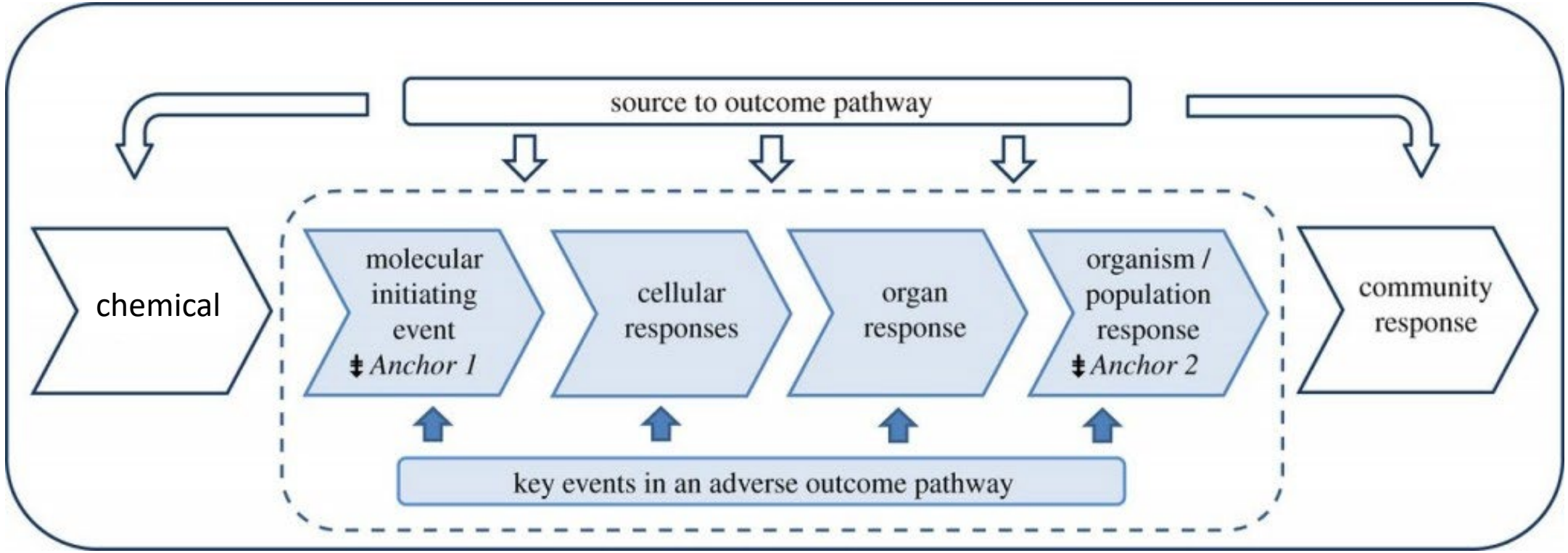


Fig. 5. Characteristic pollutants of nine chemical enterprises in the CIPs.

EBM Effect Based Methods



In vitro bioassays

Ecological indicators

Biomarker / In vivo bioassays

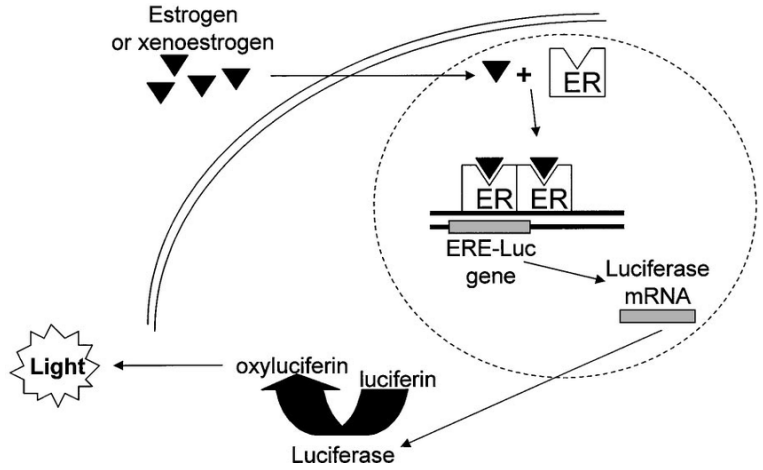
Effect Based Methods

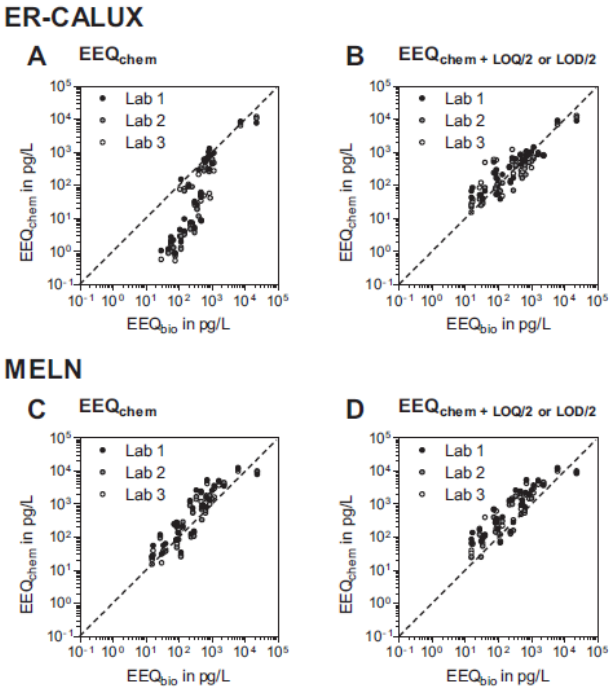
Molecular Initiating Event **(MIE)**

- **Covalent interaction with DNA or proteins**
- **Binding to hormone receptors (e.g. ER, AR, AhR, GR, PR, TR)**
- **Competition with hormones for transport protein binding**
- **Inhibition of enzymes**

Interferenze da antagonisti

No selettività: metodi di screening





EEQ (17b-estradiol-equivalent)

REGOLAMENTO (UE) 2017/644 DELLA COMMISSIONE

del 5 aprile 2017

che stabilisce i metodi di campionamento e di analisi per il controllo dei livelli di diossine, PCB diossina-simili e PCB non diossina-simili in alcuni prodotti alimentari e che abroga il regolamento (UE) n. 589/2014

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Effect-based and chemical analytical methods to monitor estrogens under the European Water Framework Directive

Metodi chimici aspecifici

TOX (Total Organic Halides):

composti organici alogenati (Cl, Br, I):

- assorbibili (AOX)
- strippabili (POX)
- estraibili con solvente (EOX)

No identificazione della sostanza (no selettività)
Bassa sensibilità
Veloci, economici

TOF (Total Organic Fluorine)

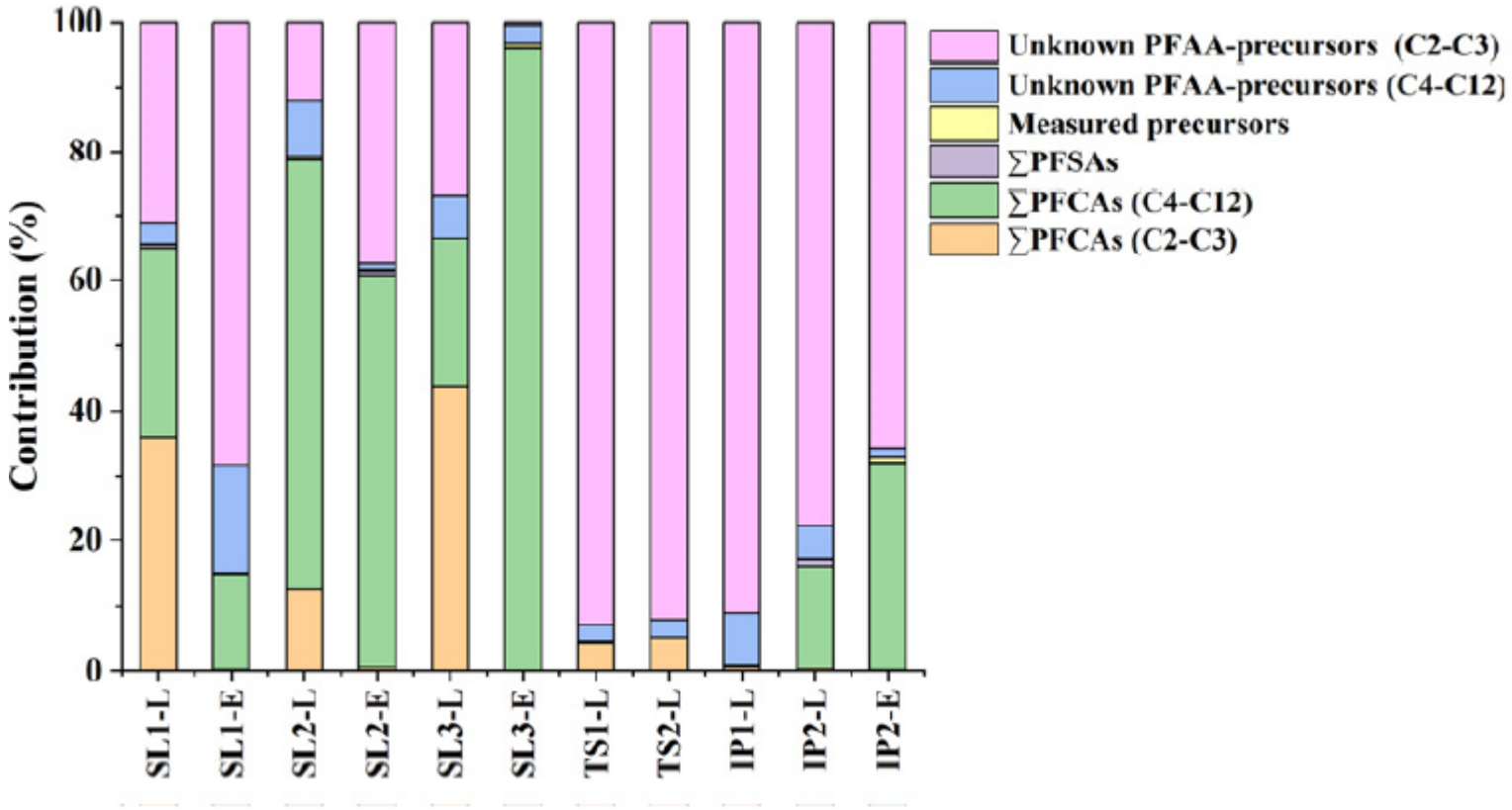
sostanze organiche con F (PFAS Per- and Poly-fluoroalkyl Substances):

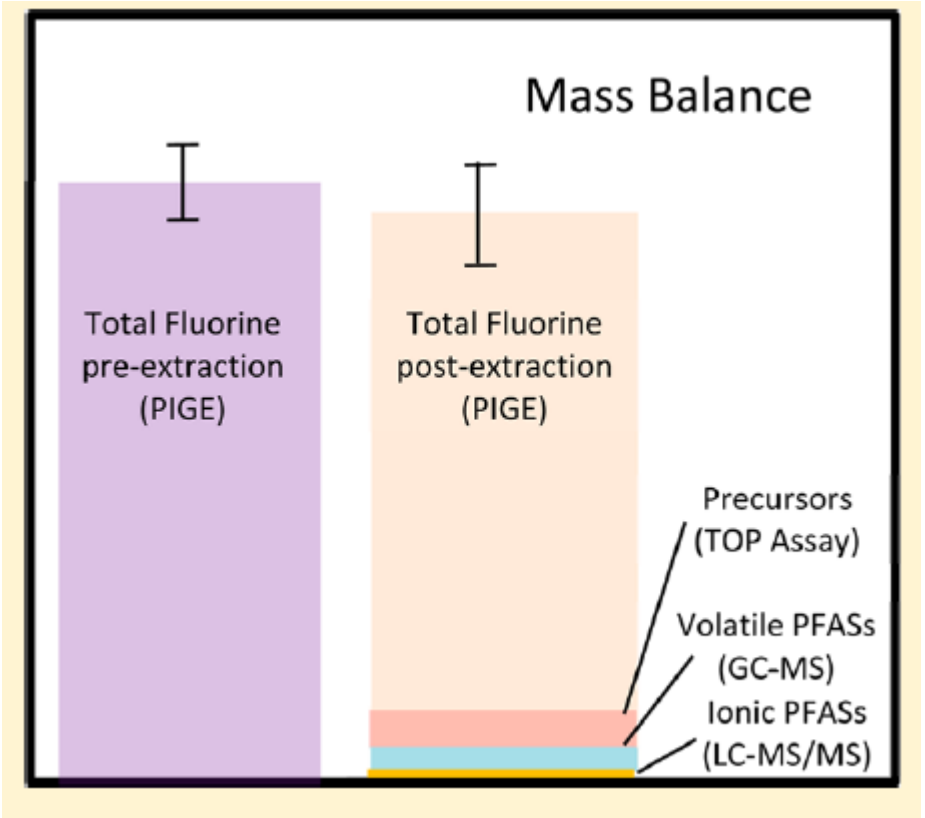
- CIC (Combustion Ion Chromatography) per EOF/AOF (extractable/ adsorbable organic fluorine)
- PIGE (Particle-induced gamma ray emission) spetroscopy
- 19F NMR (Fluorine-19 nuclear magnetic resonance spectroscopy)

TOP assay (total oxidizable precursor) per PFAS ossidabili (precursori dei PFAA)

Metodi chimici aspecifici: PFAS

PFAS in carta e tessuti (analisi target+TOP assay+PIGE)
(Robel et al., Environ Sci Technol 2017)



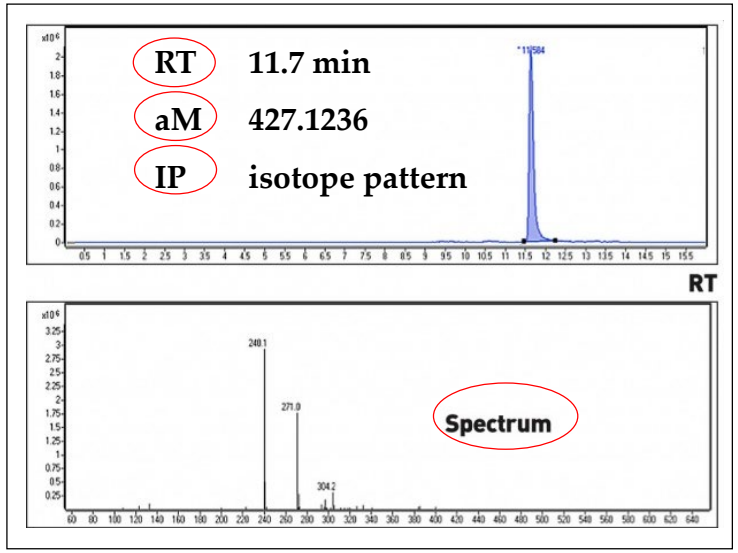


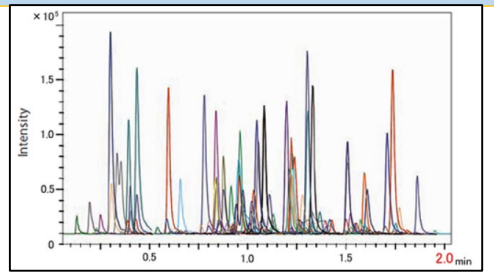
PFAS in percolati di discarica (analisi target + TOP assay)
(Wang et al., Sci Total Environ 2020)

NON-TARGET SCREENING (NTS) by HRMS:

GC o LC-HRMS: «Unknown Unknowns»

CAMPIONAMENTO | ESTRAZIONE | SEPARAZIONE CROMATOGRAFICA | HRMS/MS





Elemental formula proposal (via aM)

Structure proposal by similarity comparison with known molecules:

- molecular mass
- MS/MS spectrometric fragmentation
- Hydrophobicity/polarity (cal RT)

Reconciliation with in-silico predictions and environmental behaviour

X

Suspect selection

CONFERMA E QUANTIFICAZIONE CON UNO STANDARD DI RIFERIMENTO

Livelli confidenza

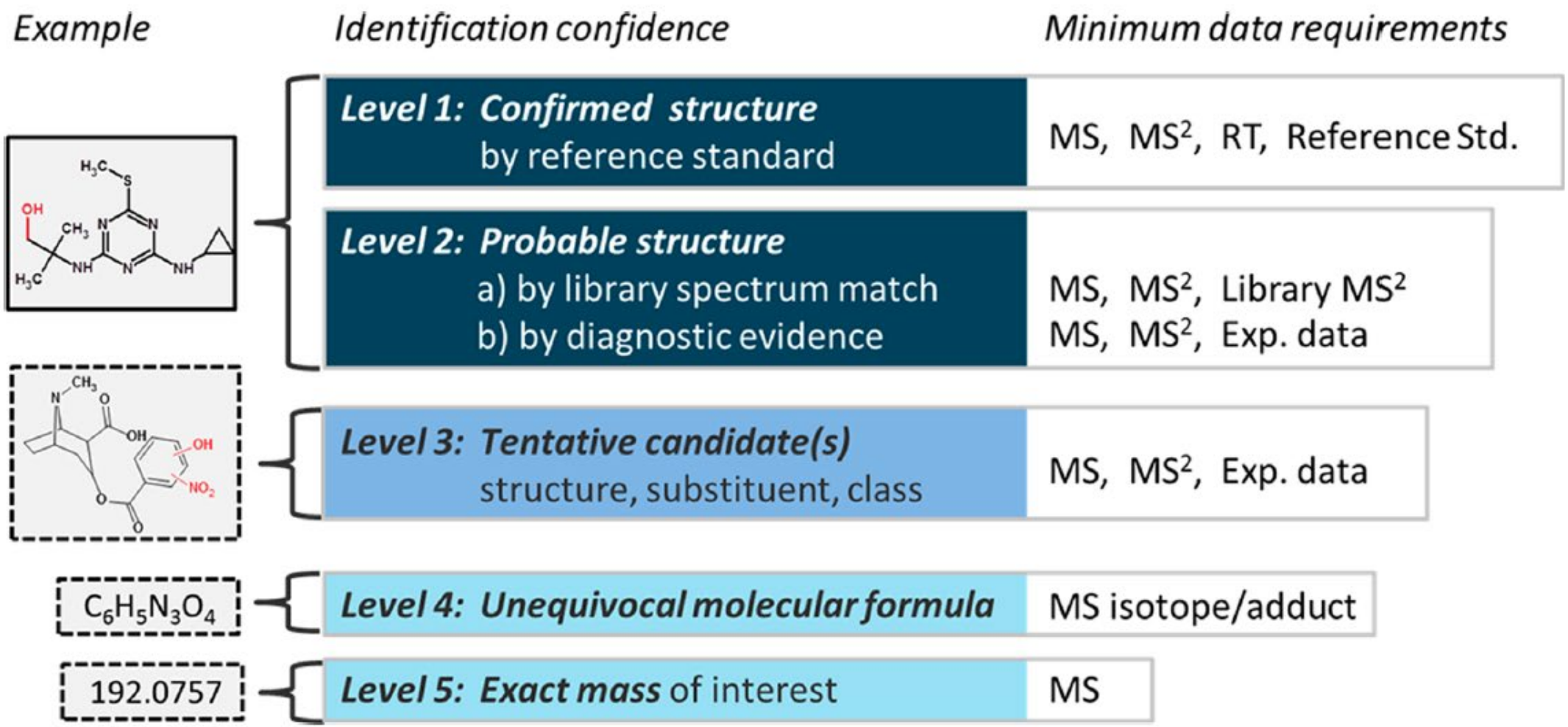


Figure 1. Proposed identification confidence levels in high resolution mass spectrometric analysis. Note: MS^2 is intended to also represent any form of MS fragmentation (e.g., MS^e, MS^n).

Schymanski, et al., 2014

NON-TARGETED METHOD with HRMS
NTS + Suspect screening

Quantificazione in base a standard o semiquantificazione basata su standard di composti con struttura simile
Suspect list+ analiti inattesi
Analisi retrospettiva

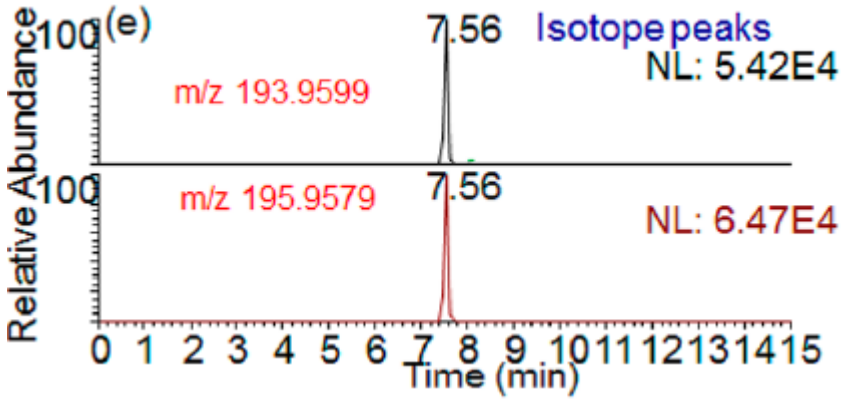
- STRATEGIE DI *FILTERING*
- Suspect Screening via Mass Lists
- Isotopic patterns
- Diagnostic fragments or Neutral losses searching
- Homologous series searching

Isotopic patterns (Brominated compounds)

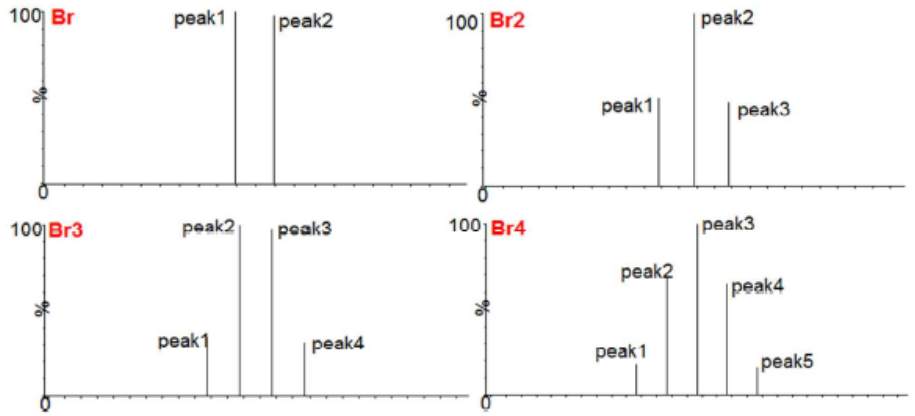
analytical chemistry
Article
pubs.acs.org/ac

Untargeted Identification of Organo-Bromine Compounds in Lake Sediments by Ultrahigh-Resolution Mass Spectrometry with the Data-Independent Precursor Isolation and Characteristic Fragment Method

Hui Peng,[*,†] Chunli Chen,[†] David M. V. Saunders,[†] Jianxian Sun,[†] Song Tang,[‡] Garry Codling,[†] Markus Hecker,[†,‡] Steve Wiseman,[†] Paul D. Jones,[†,‡] An Li,[⊗] Karl J. Rockne,[$] and John P. Giesy[*,†,§,∥,⊥,#,○]



Il bromo ha due isotopi, 79Br e 81Br in un rapporto di circa 1: 1. Ciò significa che un composto contenente 1 atomo di bromo avrà due picchi molecolari con uno spazio di 2 m/z unità tra loro e con altezze quasi uguali.



1593 formule elementari corrispondenti a composti organobromurati (la maggior parte non conosciuti)
Intensità di risposta da 10 a 1000 volte più elevate di quella dei PBDE

Diagnostic fragments (PFAS)

analytical chemistry
Article
pubs.acs.org/ac

Discovery of C_5–C_{17} Poly- and Perfluoroalkyl Substances in Water by In-Line SPE-HPLC-Orbitrap with In-Source Fragmentation Flagging

Yanna Liu, Alberto Dos Santos Pereira,† and Jonathan W. Martin*

36 new PFAS in WWTP effluent

Environmental Science & Technology
Article
Cite This: Environ. Sci. Technol. 2018, 52, 5830−5840
pubs.acs.org/est

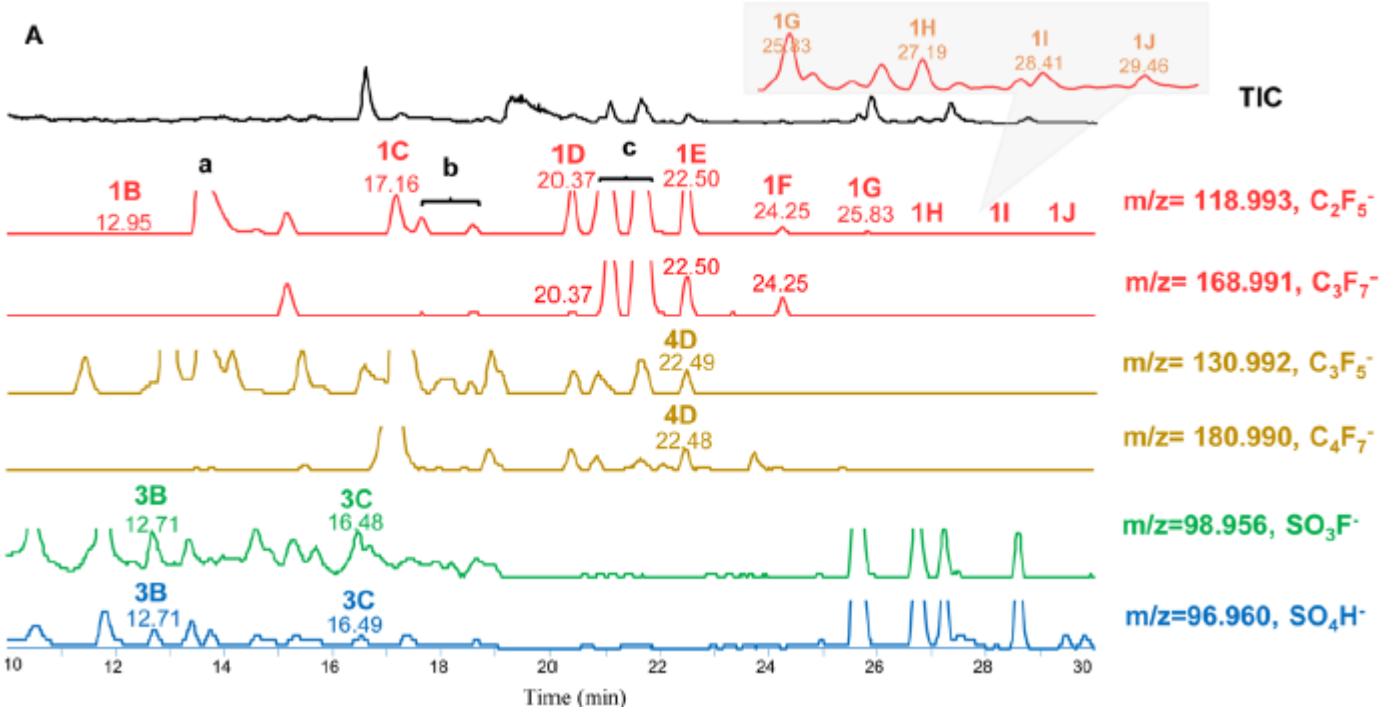
Nontarget Mass Spectrometry Reveals New Perfluoroalkyl Substances in Fish from the Yangtze River and Tangxun Lake, China

Yanna Liu,† Manli Qian,‡ Xinxin Ma,§ Lingyan Zhu,§ and Jonathan W. Martin*,†,∥

330 new PFAS (4 new PFAS class) in fish liver

in-source fragmentation flagging scans:

- $[C_2F_5]^-$ (m/z 118.992)
- $[C_3F_7]^-$ (m/z 168.988)
- $[SO_4H]^-$ (m/z 96.959)
- $[Cl]^-$ (m/z 34.9)……



Homologous series searching (Surfactants)

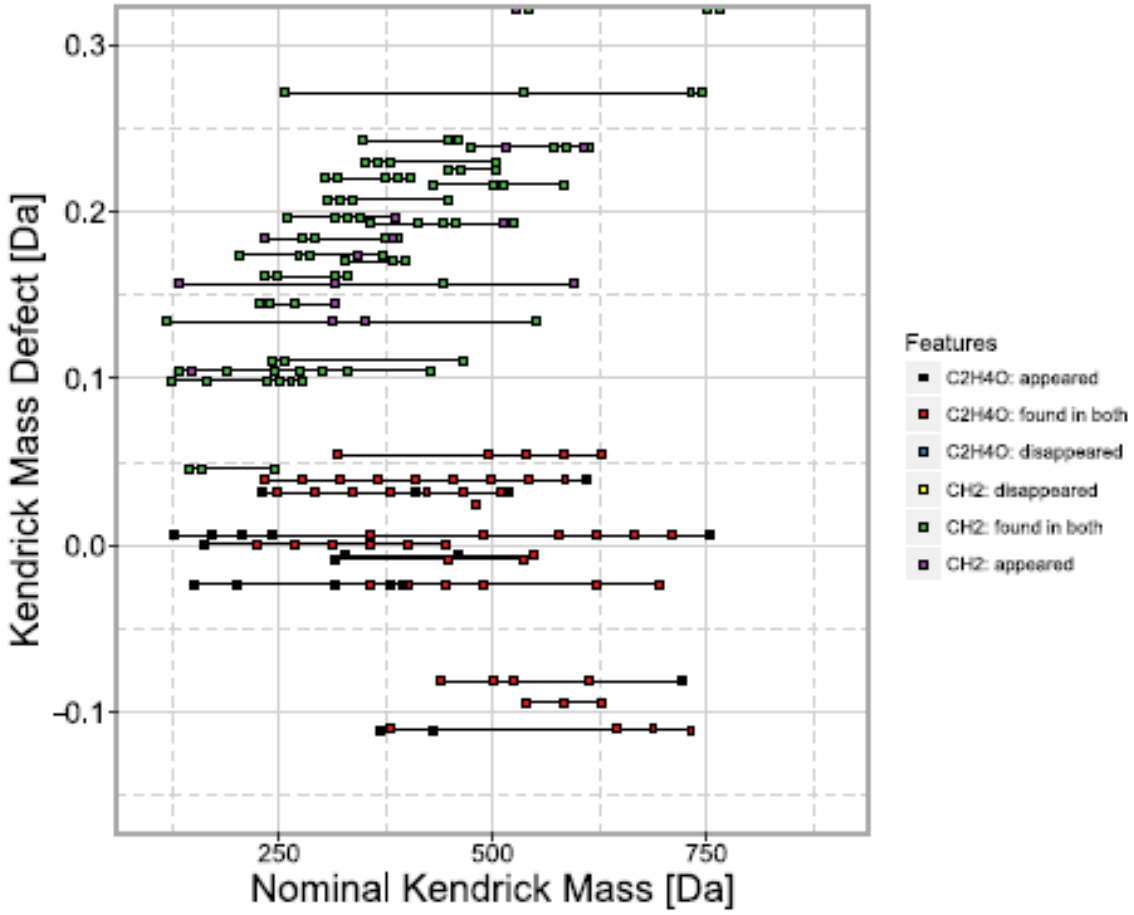
Contents lists available at ScienceDirect

Chemosphere

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

A non-targeted high-resolution mass spectrometry data analysis of dissolved organic matter in wastewater treatment

Yaroslav Verkh [a], Marko Rozman [a, b], Mira Petrovic [a, c, *]



Riconoscimento di serie di composti omologhi **attraverso l'uso di** Kendrick mass defect plots

Serie omologa è una serie di composti con lo stesso gruppo funzionale e proprietà chimiche simili in cui i membri della serie differiscono per il numero di unità ripetitive che contengono (es **glicole polietilenico** (**PEG**) $H\text{-}(O\text{-}CH_2\text{-}CH_2)_n\text{-}OH$)

Kendrick Mass (KM) = m/z * (Nominal (round) M_{unit}/ M_{unit})
KMD (Kendrick Mass Defect)=Nominal (round) KM– (KM)

Quadro riassuntivo

Tecnica analitica	Vantaggi	Limiti
Wide-Scope Target Analysis	• **Selettività / Specificità** • **Sensibilità / Accuratezza**	• Misura solo i **composti che si cercano** • **Laboriosa** attività di **validazione**
Suspect Screening	• **Ampia lista** di sostanze monitorate • **Validazione solo** per composti presenti • **Semi-quantificazione** • **Analisi retrospettiva**	• Misura solo i **composti attesi** • Necessità di **standard** per la conferma definitiva e la quantificazione
Effect Based Methods *In vitro biassays*	• Misura gli **effetti** integrati • **NO standard** singolo composto	• Ridotta informazione sulla **composizione** della miscela (NO selettività) • **Non** ancora completamente **validato**
Metodi chimici aspecifici	• Misura il **totale** dei composti di una miscela • **NO standard** singolo composto	• Ridotta informazione sulla **composizione** della miscela (NO selettività) • **ridotta sensibilità**
Non-target screening	• Misura anche i **composti inattesi** • **Semiquantificazione** • **Analisi retrospettiva** • ***Filtering***	• **Non** ancora completamente **validato** • Necessità di **standard** per la conferma definitiva e la quantificazione