Identification and Structure elucidation of transformation products of Emerging Contaminants by Advanced Mass Spectrometry Based Techniques

- **Dimitra A. Lambropoulou**
- Assist. Professor, Department of Chemistry, Aristotle University of Thessaloniki,
- Thessaloniki, 54124, Greece,
- **e-mail:** dlambro@chem.auth.gr, Tel.:+302310-997687, Fax: +30-2310-997687

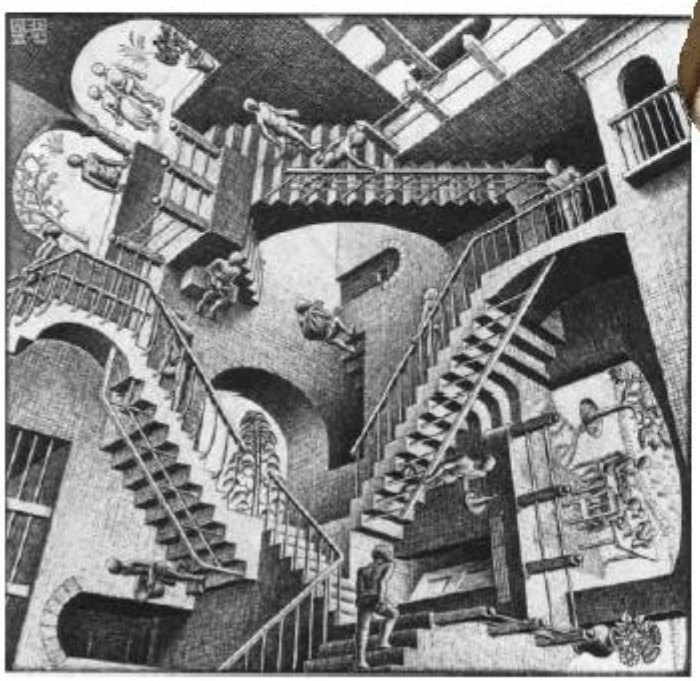
What Makes a "Contaminant of Emerging Concern"?

- **Over the last 15 years**, the focus of chemical pollution has definitely shifted from conventional ***"priority"*** pollutants, to so-called ***"emerging"*** or ***"new"*** unregulated contaminants. Concerns during this period about the potential health and ecological impacts of exposure to ***emerging contaminants* (ECs)** have led to the establishment of new, multi-stakeholder research and testing initiatives, committees, expert groups, newsletters, databases, etc., throughout the world.
- Up to date, despite these actions, the term ***"emerging contaminants"*** remains problematic and sometimes it is difficult to determine which chemicals should or should not be classified as ECs, because they represent a changing reality, dependent on perspective as well as timing.

EMERGING CONTAMINANTS

Emerging Contaminants ..

Emerging Contaminants …



… depend on one's perspective

What Makes a "Contaminant of Emerging Concern"?

Emerging contaminants **(ECs) include more than simply chemicals previously not known to occur in the environment.**

ECs also include chemicals already known to occur but now displaying new characteristics not previously suspected or recognized, such as those involving:

- ❑ origin or source (e.g., via sweat and dermal transfer)
- ❑ location (e.g., "out-of-place" chemicals; "chemical weeds")
- ❑ unusual concentrations or levels (e.g., enriched by sorption to plastics in oceans)
- ❑ transformation and fate pathways
- ❑ exposure routes
- ❑ biological effects pathways or endpoints

What Makes a "Contaminant of Emerging Concern"?

In general, ECs are a structurally diverse and heterogeneous group of chemical compounds, which have widely varying fate properties and adverse effects on environmental ecosystems and can be classified into the following categories:

- ***"new"* ECs,** which are chemicals that are recently manufactured and suddenly appear everywhere, and therefore, are not currently covered by existing regulations or legislation
- ***"old"* ECs,** which are the ones that were actually around for several decades, but simply were not under regular investigation or for which analytical methods did not exist until recently.
- ***"ECs within complex mixtures",*** such as industrial effluents, oil residues, hospital effluent, etc. of which either the mixture itself or newly identified (subgroups) of components within may be considered ECs.

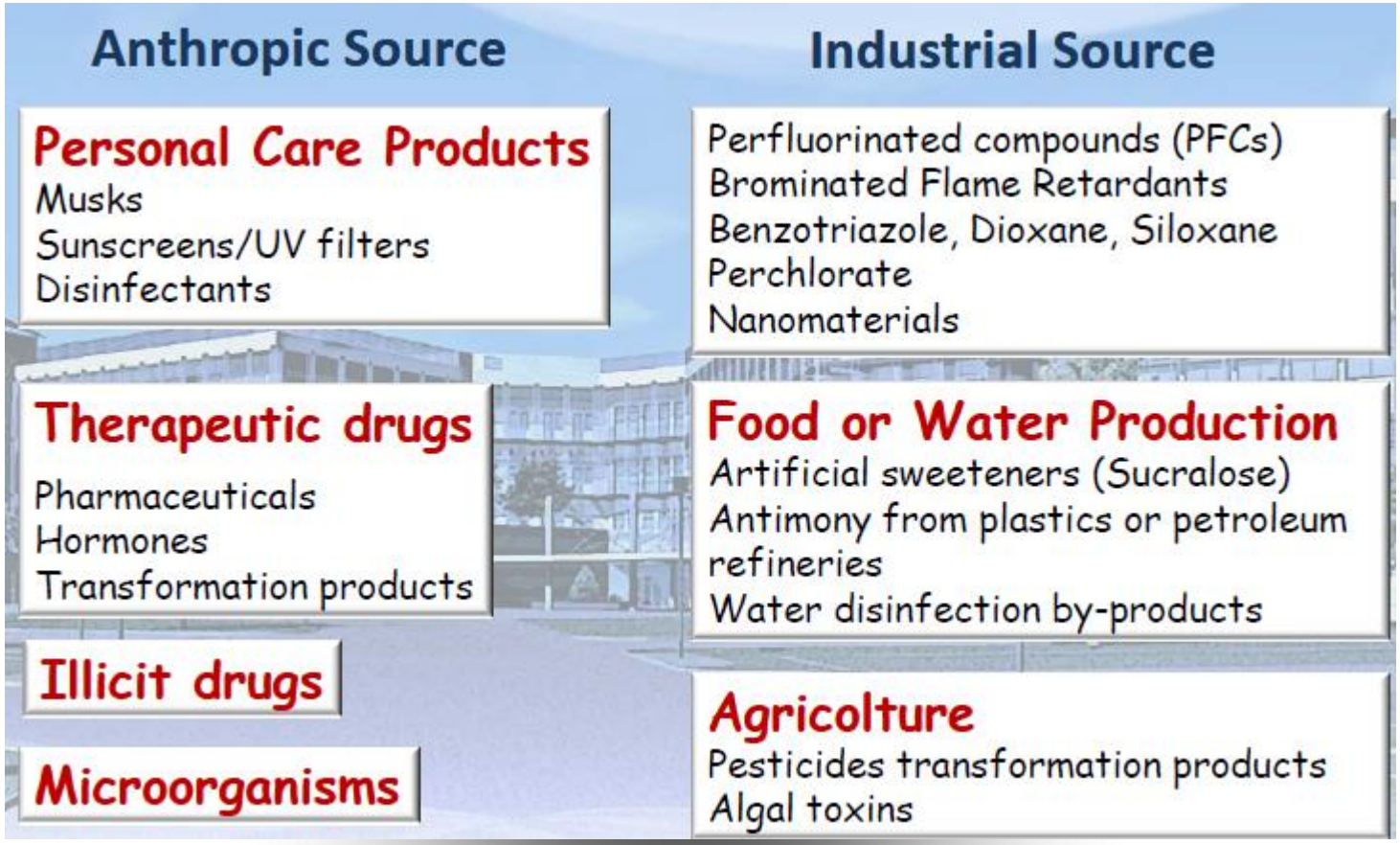
Fundamental Research Questions

Are ECs entering our environment?

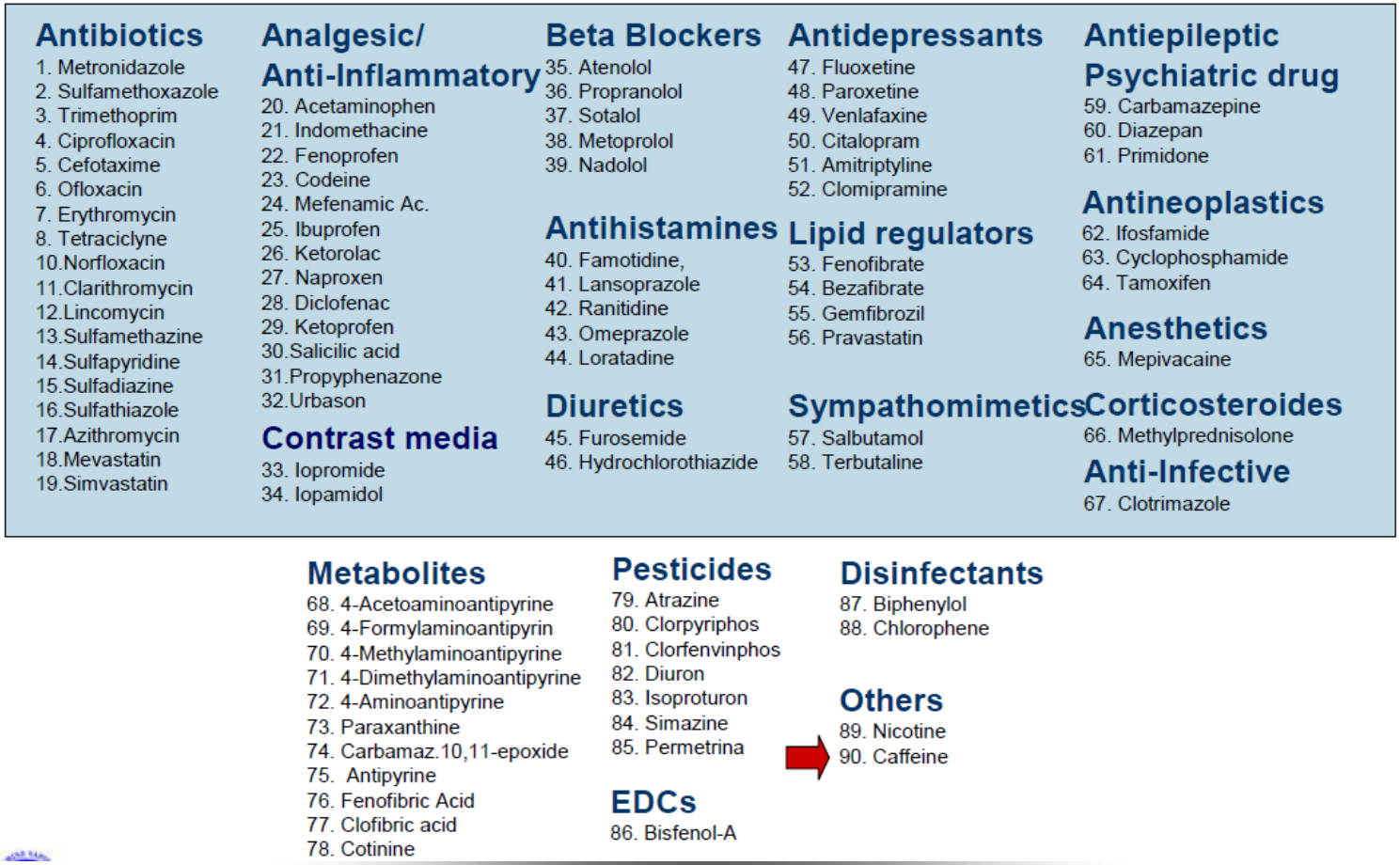
- *What are the sources (signatures)?*
- *What happens to them in the environment?*
- *Do they have adverse ecological health effects?*
- *Do unintended exposures pose a human health risk?*
- *How can we minimize their entry to the environment or remove them?*

Emerging pollutants

(Richardson and Ternes, *Anal. Chem.* 2011, 83, 4614)



Emerging pollutants



Why study these contaminants in the Environment?

- ✓ Used in **high quantities**
- ✓ **Heterogeneous group**
- ✓ Continuos **discharge**
- ✓ **Polar compounds** (generally small)
- ✓ Biologically **active substances**
- ✓ Complex **mixtures** - potential **toxic effects**

- ➢ **Massive and continuous use** (daily) – Pseudo-persistence
- ➢ **Lack of sensitive analytical methods** for most compounds & scarce knowledge on their presence in the environment
- ➢ Some (priority) compounds are **regulated** in environmental samples

Lack of data to perform a reliable Environmental Risk Assessment

Transformation Products of ECs?

- Despite the increasing number of published studies covering EC input, occurrence, fate and effects, there is still a lack of understanding and knowledge about these substances in the aquatic environment.

- Even more, we know almost nothing about the impacts of the environmental exposure to trace concentrations of their ***transformation products*** (TPs) **and/or *metabolites*,** but the detection of TPs in the environment is worrying.

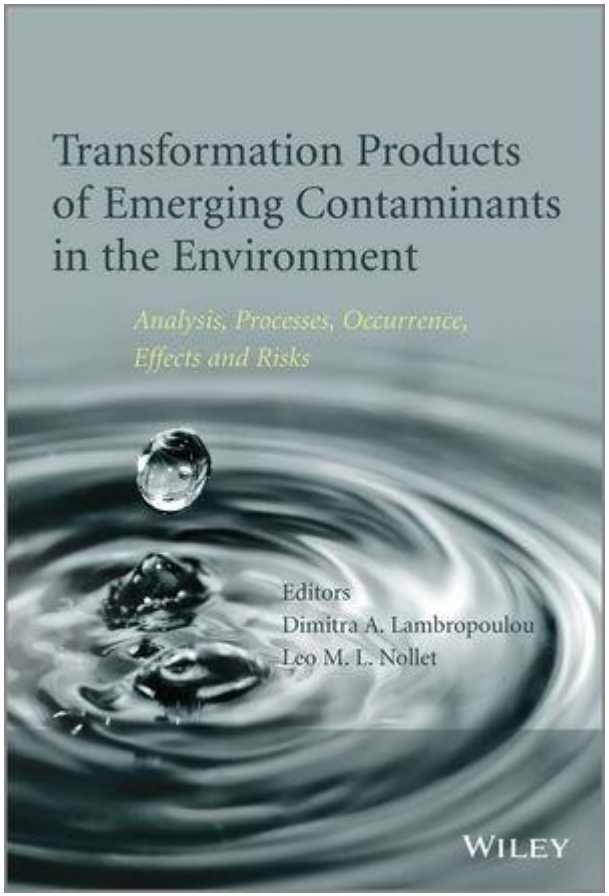
- TPs of ECs in aquatic environments are still rarely considered in water quality and chemical risk assessment, although they have been found in concentrations that are of concern.

- Since many different TPs can potentially be formed in the environment and analytical standards are typically lacking for these compounds, knowledge on the prevalence of TPs in aquatic environments is fragmentary.

Transformation Products (TPs)

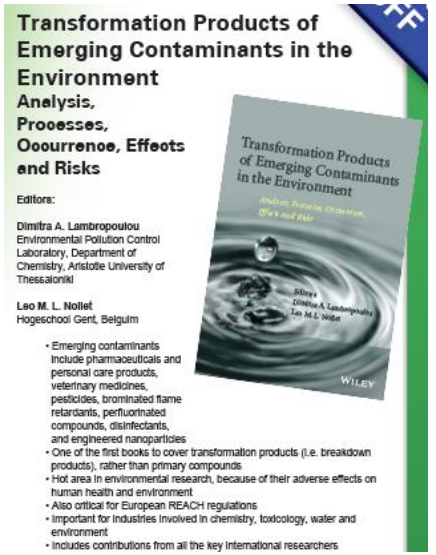
New emerging contaminants in the water cycle

Transformation Products of Emerging Contaminants in the Environment:
Analysis, Processes, Occurrence, Effects and Risks

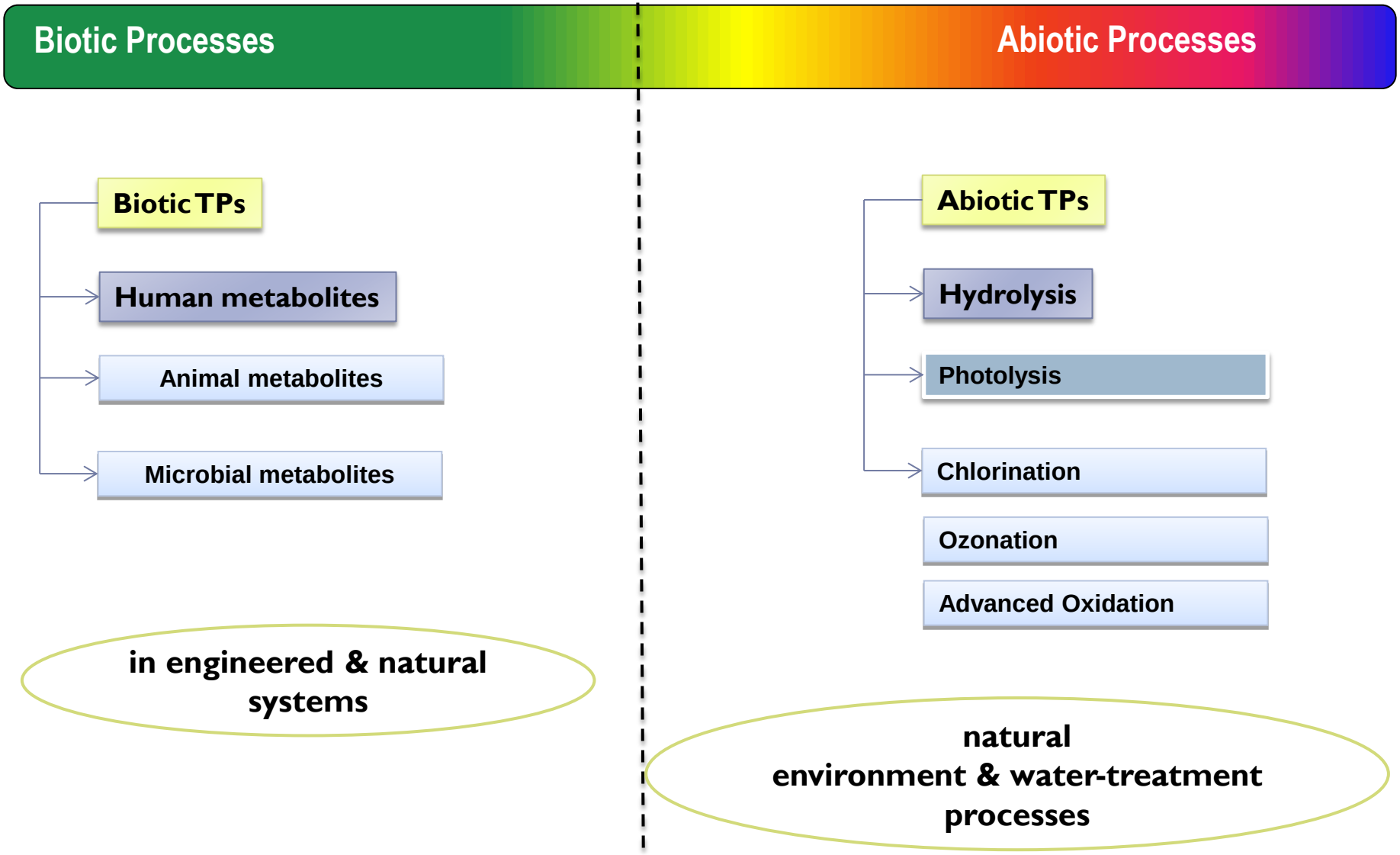


WILEY
WILEY-VCH
2014

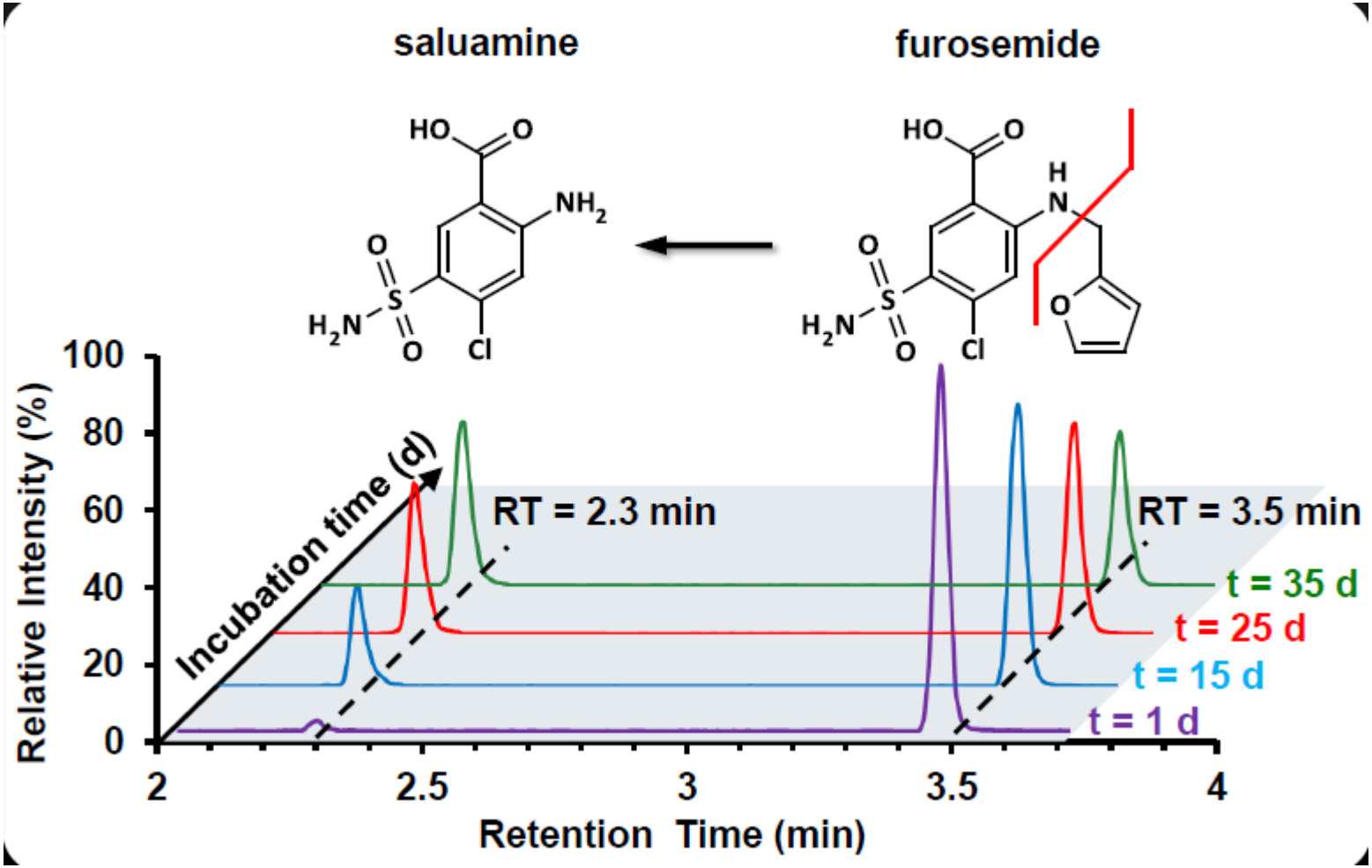
Dimitra A. Lambropoulou (Editor),
Leo M. L. Nollet (Editor)
ISBN: 978-1-118-33959-6
964 pages
February 2014



Classification of Transformation Products of ECs?

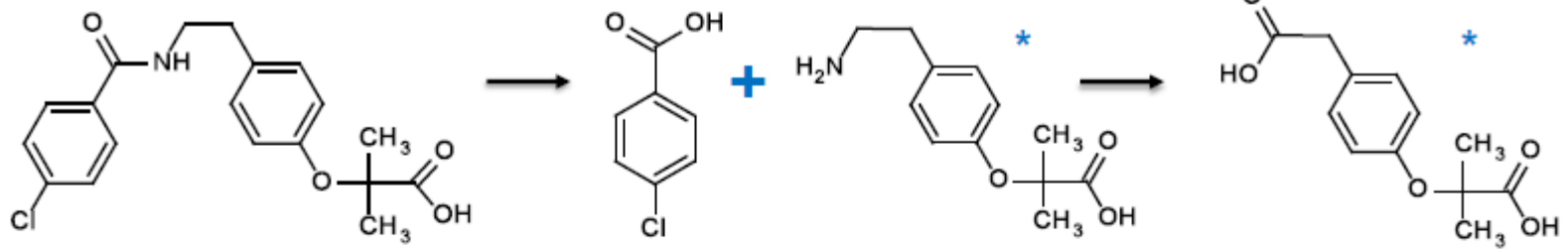


Transformation Products (TPs)

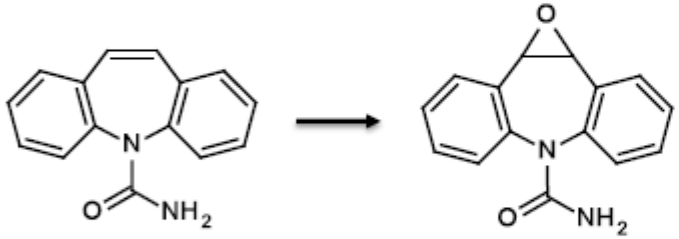


Proposed Transformation pathways

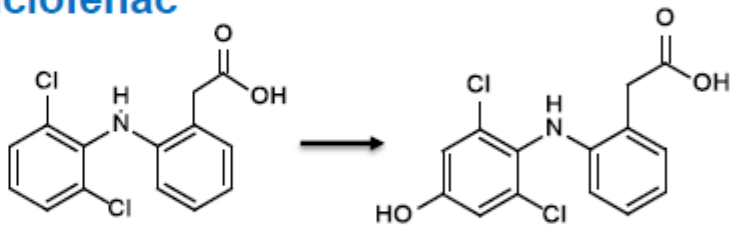
Bezafibrate



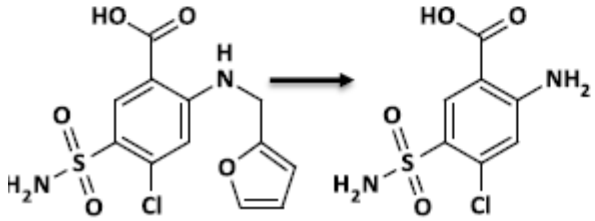
Carbamazepine



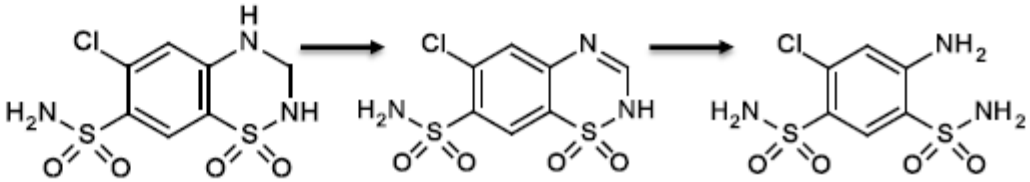
Diclofenac



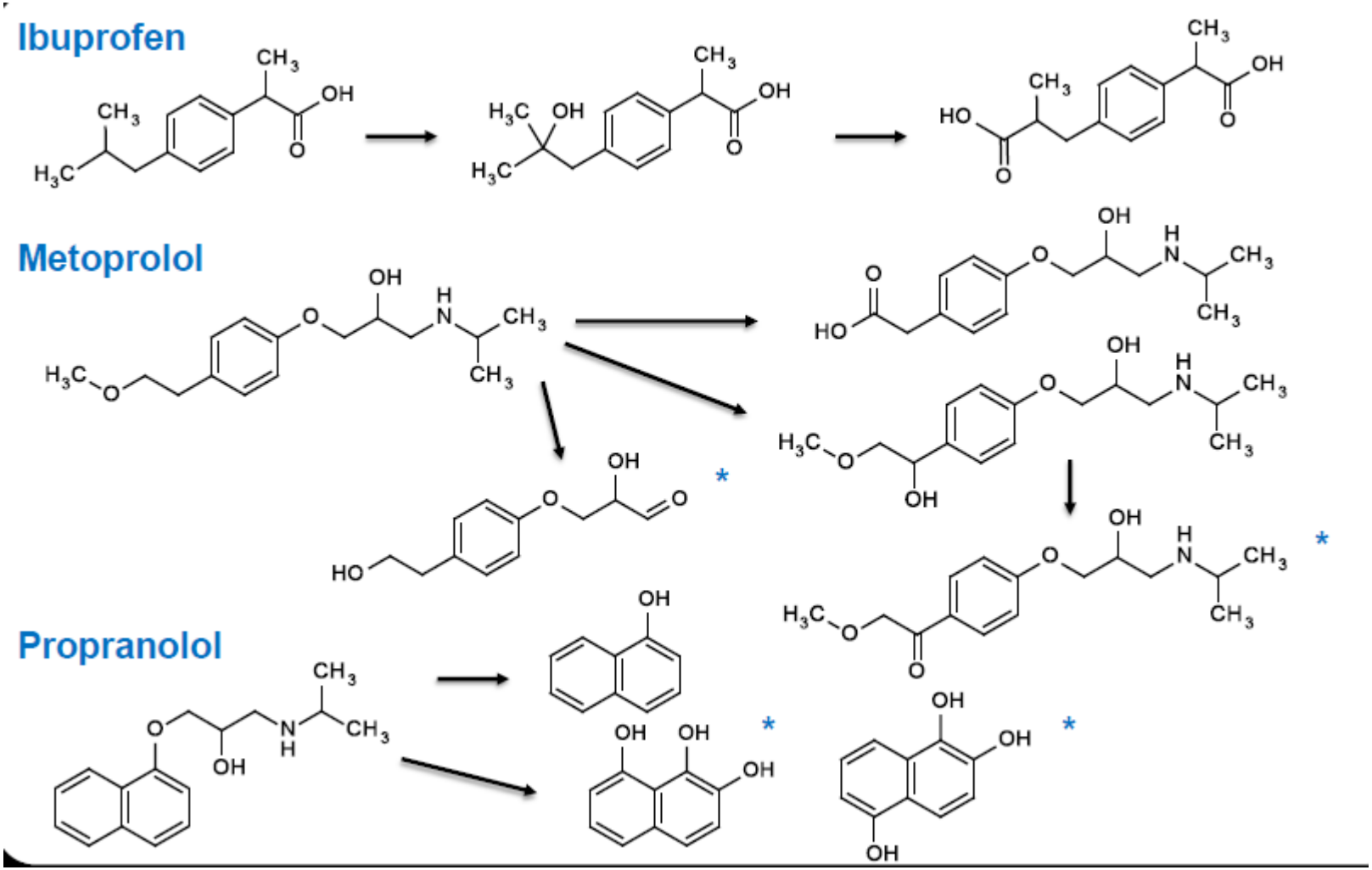
Furosemide



Hydrochlorothiazide



Proposed Transformation pathways



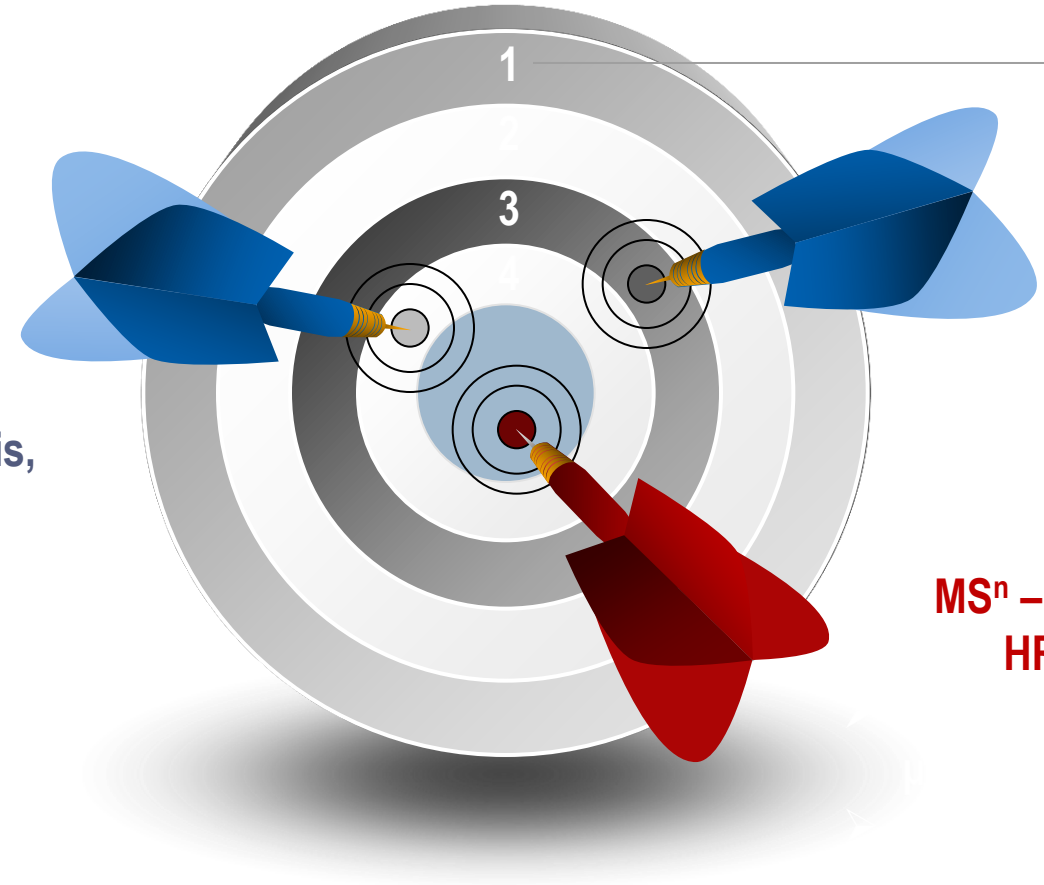
Identification and Structure elucidation of TPs

Lab experiments:

Hydrolysis, Photolysis,

Photocatalysis,

Biodegradation



"Exact masses"

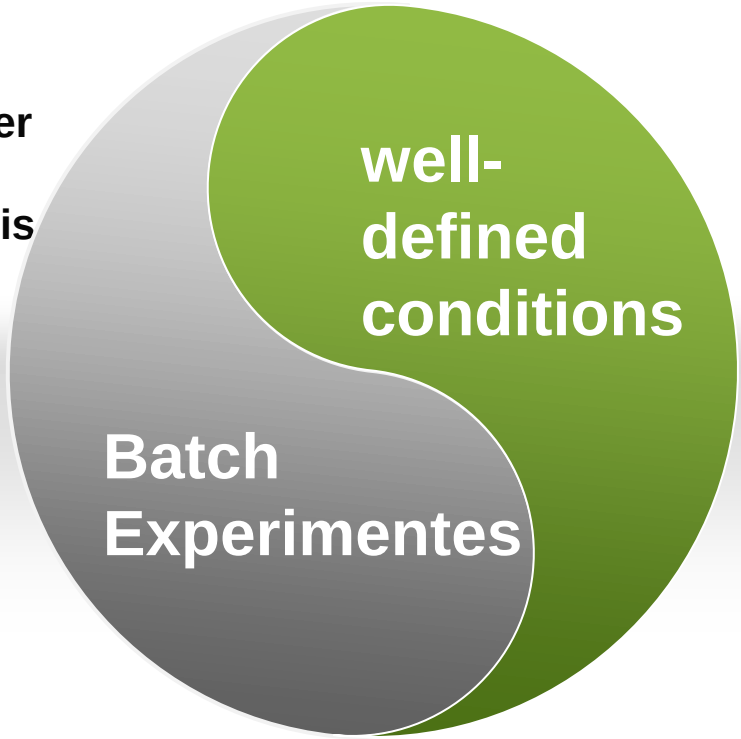
Chemical composition

MS^n – Fragmentation
HR-MS, Q-MS

Identification of TPs in WWTs, underground water, natural water, drinking water

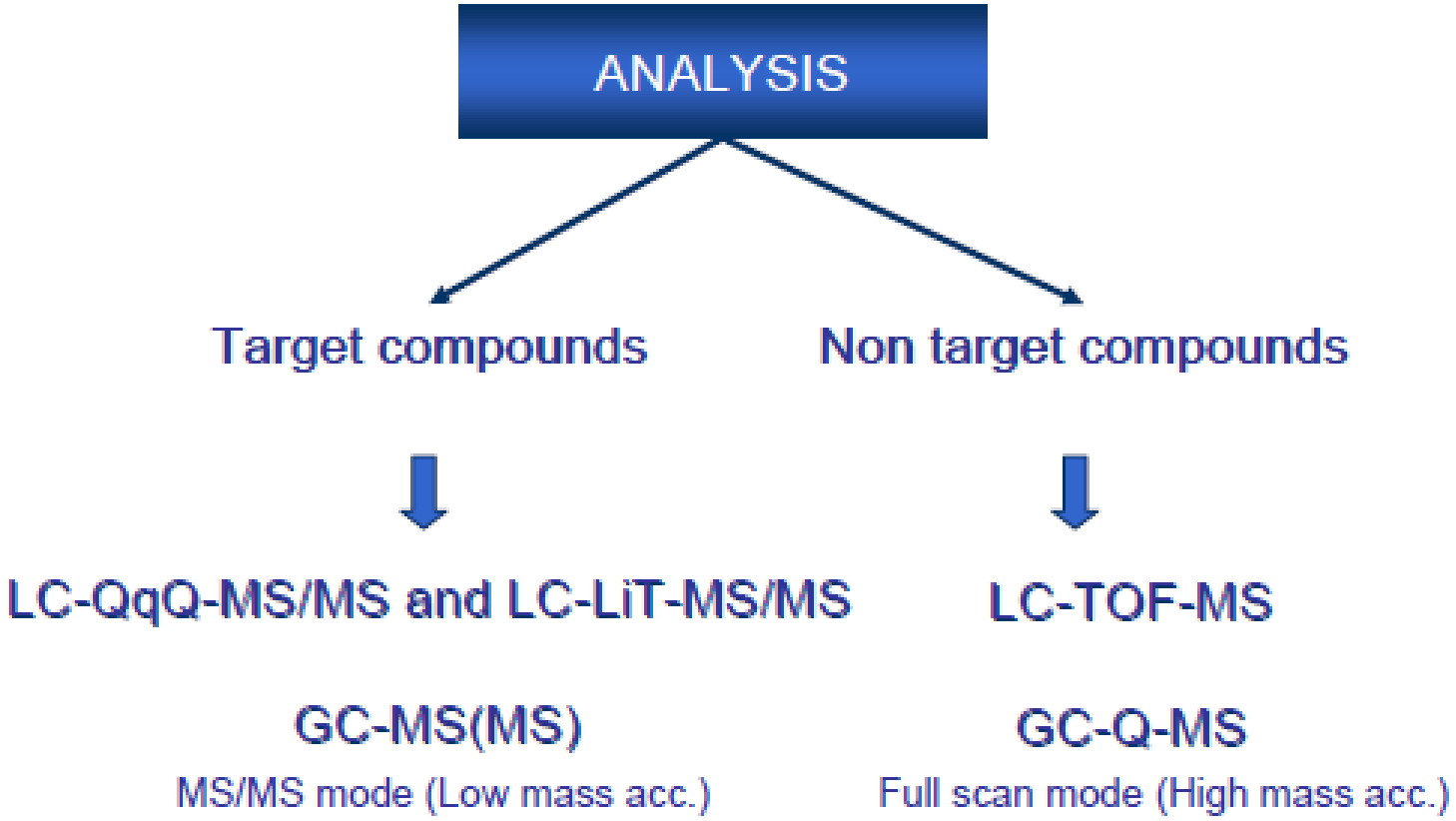
Identification approaches – laboratory studies

Simulation of the transformation processes in batch experiments under well-defined conditions with appropriate controls is a very common first approach for the identification of TPs.



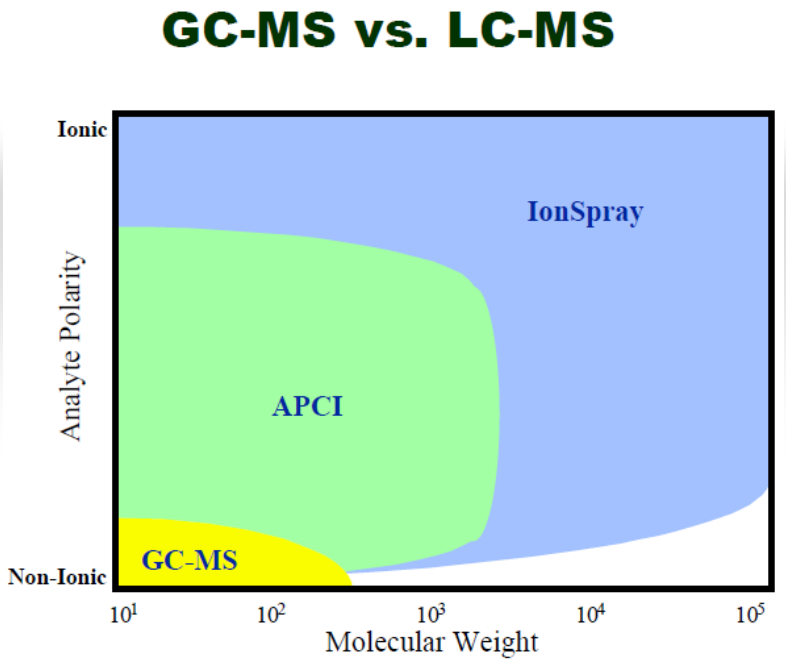
Batch experiments can be applied under biotic and abiotic conditions at high concentrations of the parent ECs.

Flow chart in environmental analysis



Identification approaches – analytical techniques

Nowadays, liquid chromatography (LC) coupled to MS (LC-MS) using a variety of mass analyzers is the technique of choice for the investigation of ECs and TPs in environmental samples.
LC is a suitable chromatographic technique for polar, thermolabile compounds, thus for the identification of TPs, which are generally more polar than their parent molecules.



Flow chart of screening procedure of transformation products (TPs)

There are various workflows in the literature

for the identification of TPs,

depending indispensably on the instrumentation and

the available software

- E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner,M. Ruff, H.P. Singer, et al., Identifying small molecules via high resolution mass spectrometry: communicating confidence, Environ. Sci. Technol. 48 (2014) 2097–2098.
- Aurea C. Chiaia-Hernandez & Emma L. Schymanski & Praveen Kumar & Heinz P. Singer & Juliane Hollender Suspect and nontarget screening approaches to identify organic contaminant records in lake sediments, Anal Bioanal Chem, September 2014.
- C. Hug, N. Ulrich, T. Schulze, W. Brack, M. Krauss, Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening, Environ. Pollut. 184 (2014) 25–32.
- Dimitra A. Lambropoulou (Editor), Leo M. L. Nollet (Editor), Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks, ISBN: 978-1-118-33959-6, 964 pagesFebruary 2014

Flow chart of screening procedure of transformation products (TPs)

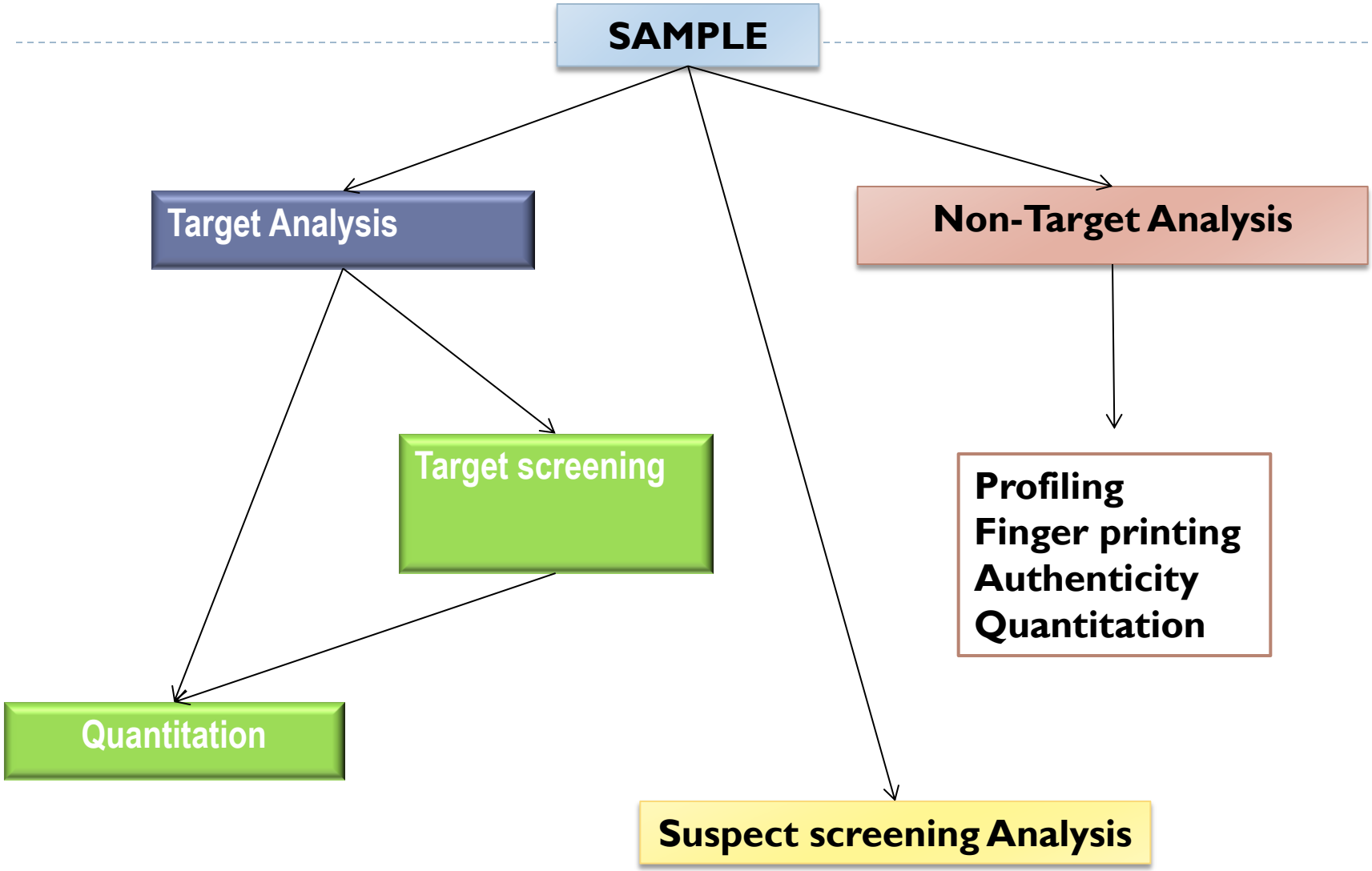
There are various workflows in the literature for the identification of TPs, depending indispensably on the instrumentation and the available software

(a) target analysis, which is based on the determination of already known TPs, and identification is carried out with standard solutions;

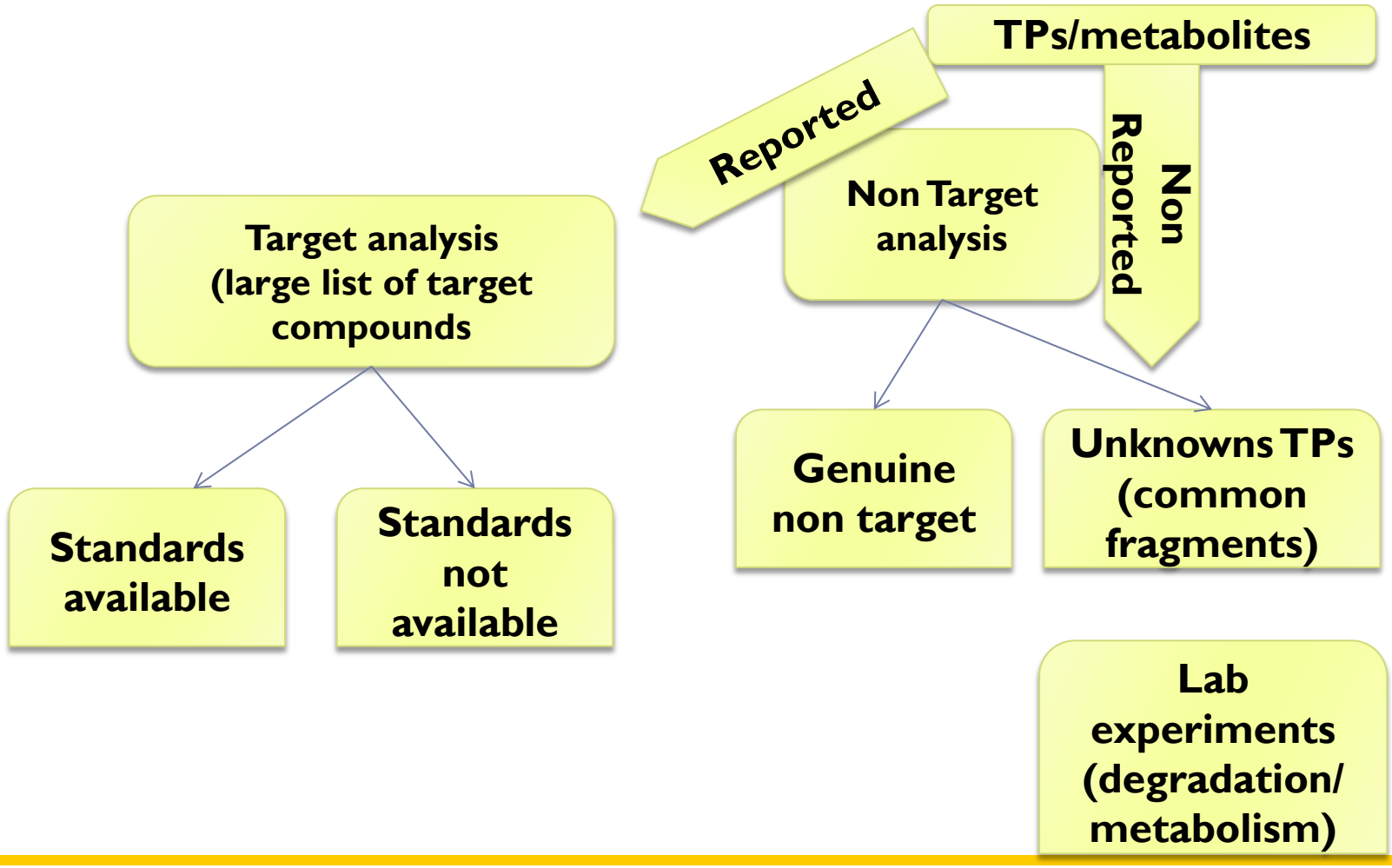
(b) suspect screening, with a list of possible TPs assembled from the literature or from prediction models, and the samples are screened for those candidates; and,

(c) non-target screening, with identification of novel TPs being carried out with sophisticated post-acquisition data tools and supplementary analytical techniques.

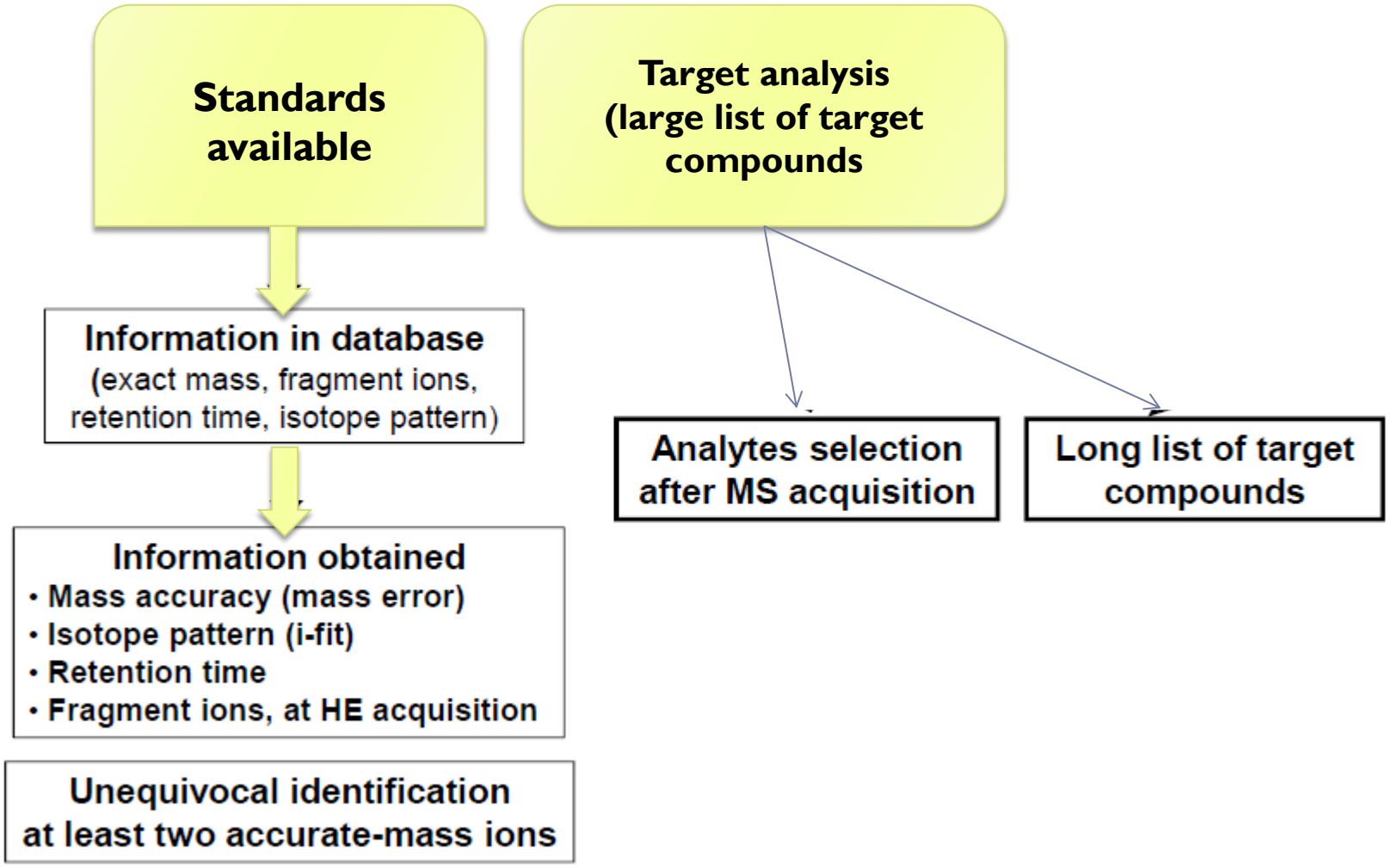
Target or Non-target analysis workflow



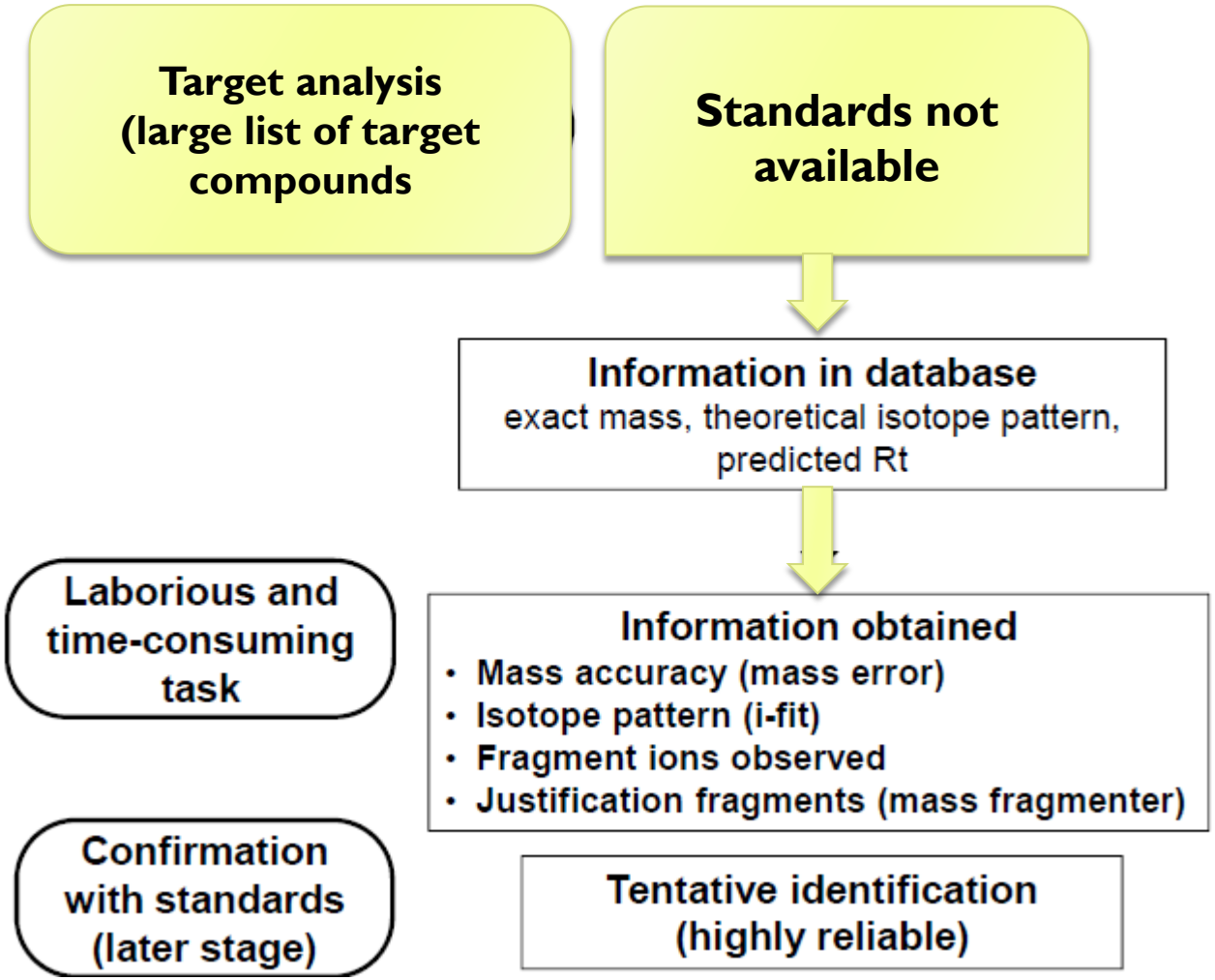
Identification of TPs



Identification of TPs



Identification of TPs



Identification of TPs

- **Genuine non target analysis**
  - ✓ No selection of analytes
  - ✓ Searching for any sample component that might be "relevant"
  - ✓ Analytical challenge (little success in the environmental field)
    - Complex unknown sample matrices
    - Low analyte concentrations
    - Many peaks in the TIC (commonly no abundant peaks for environmental pollutants)
    - Selection of "relevant " components to be investigated ????
    - From the information obtained (accurate-mass full-spectrum)
      - Assignment of the empirical formula
      - Searching in chemical data bases (Reaxys, ChemSpider)
      - Assignment of the chemical structure

Mass analyzers commonly employed

quadrupole-linear ion trap

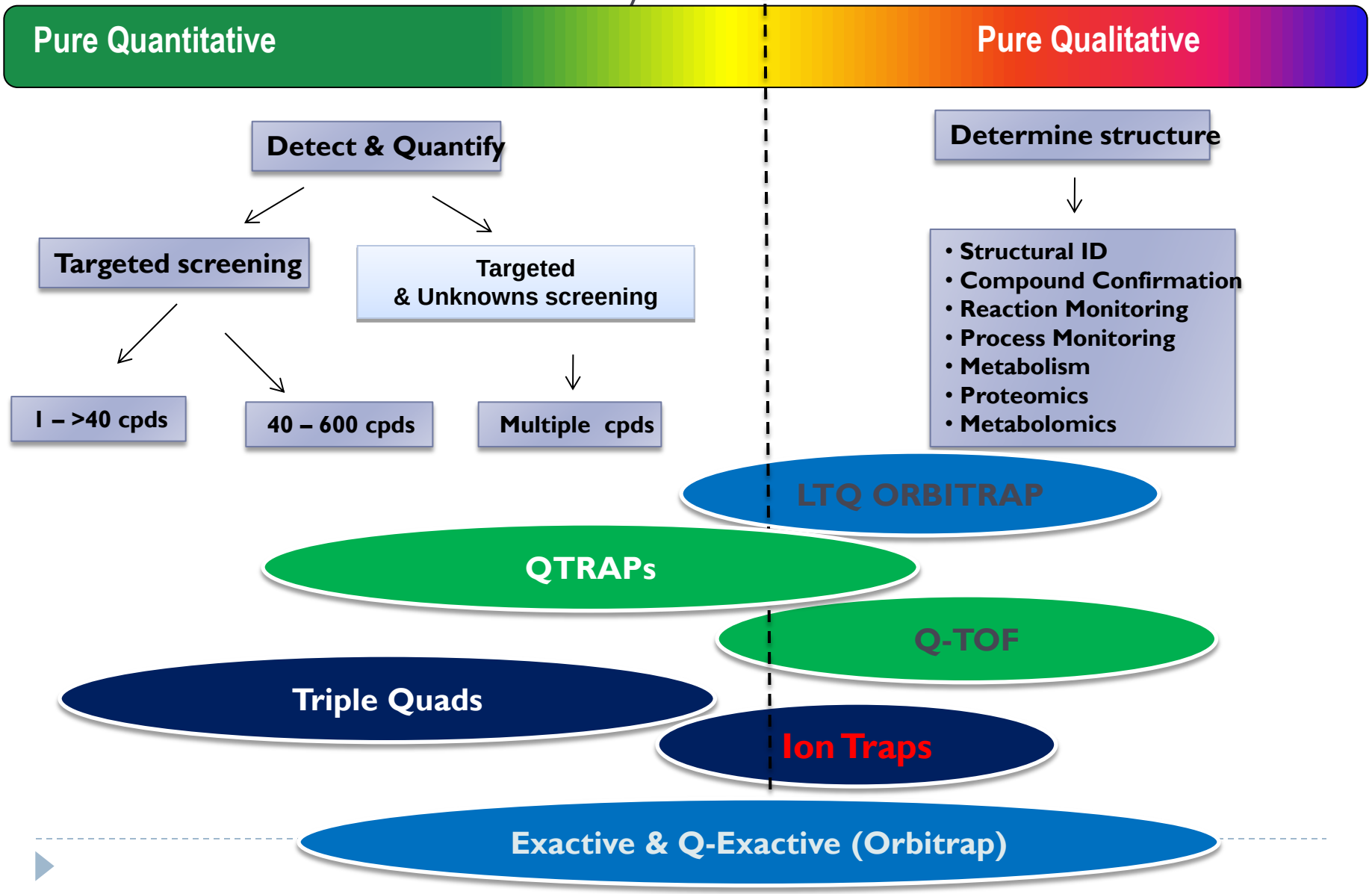
time-of-flight

linear ion trap-Orbitrap
or
quadrupole-Orbitrap

triple quadrupole

quadrupole
time-of-flight

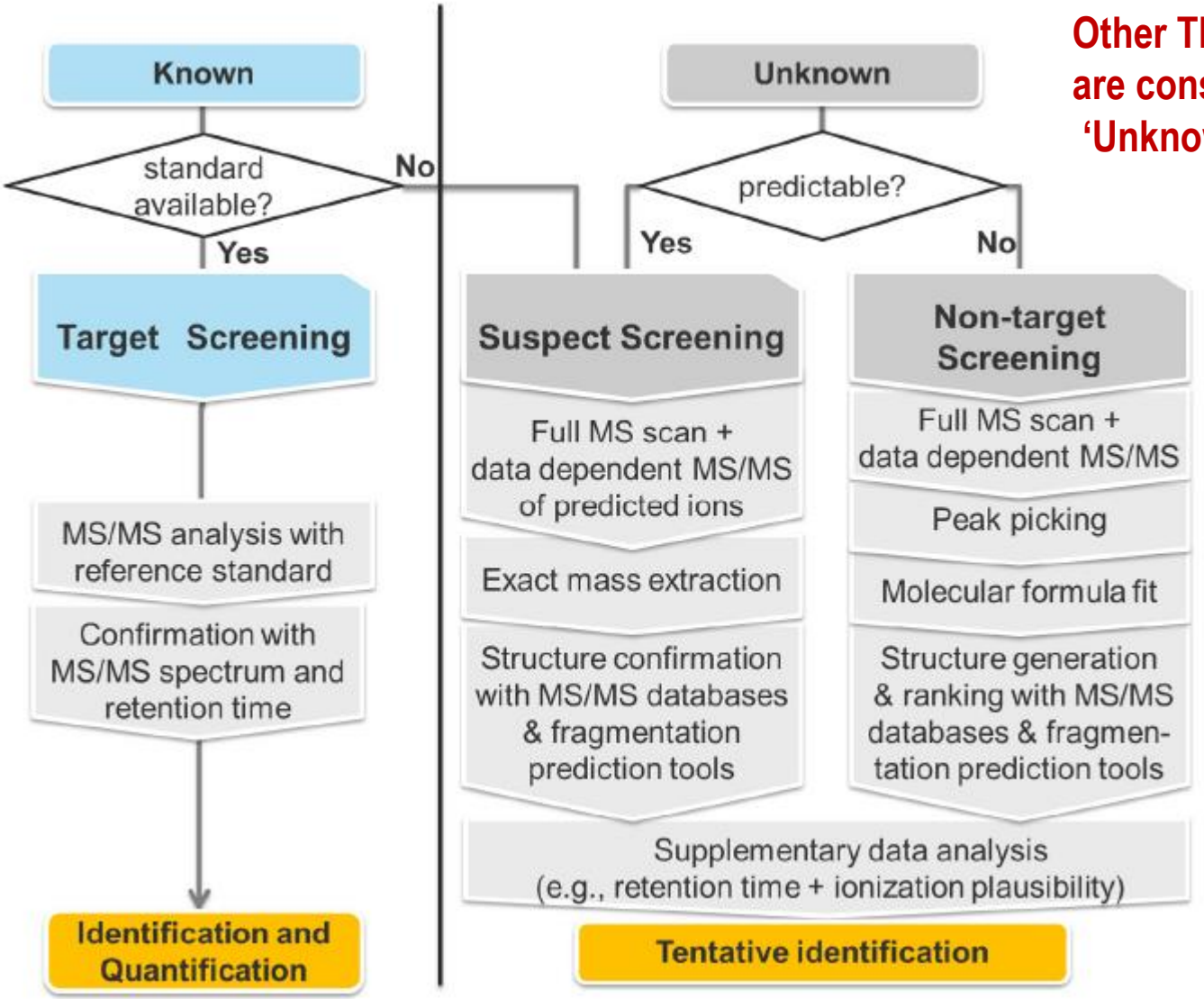
Which LCMS Analyzer Do I Choose?



Flow chart of screening procedure of transformation products (TPs).

'Known' TPs have been confirmed or confidently identified before

Other TPs are considered 'Unknown'



A.A. Bletsou et al./Trends in Analytical Chemistry 66 (2015) 32–44


TARGET ANALYSIS

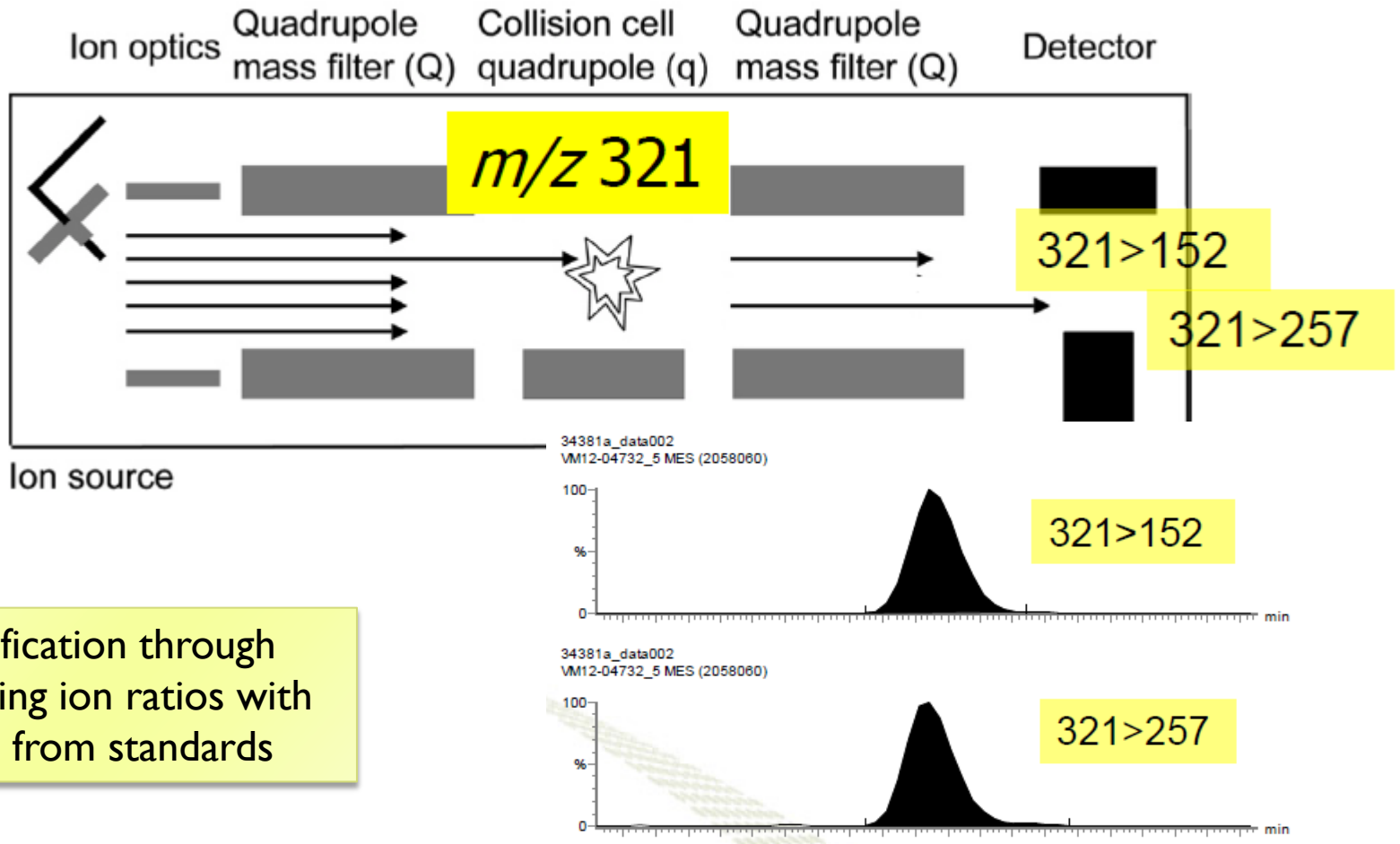
Targeted analysis using LC-MS/MS

Having two analysers increases the selectivity that ensures interfering peaks from other analytes or matrix are rarely observed

- Less isobaric interferences
- Lower limits of detection become achievable
- Direct injection of aqueous samples
- Provides a greater degree of confidence for identification
- Most common variant is the triple quadrupole

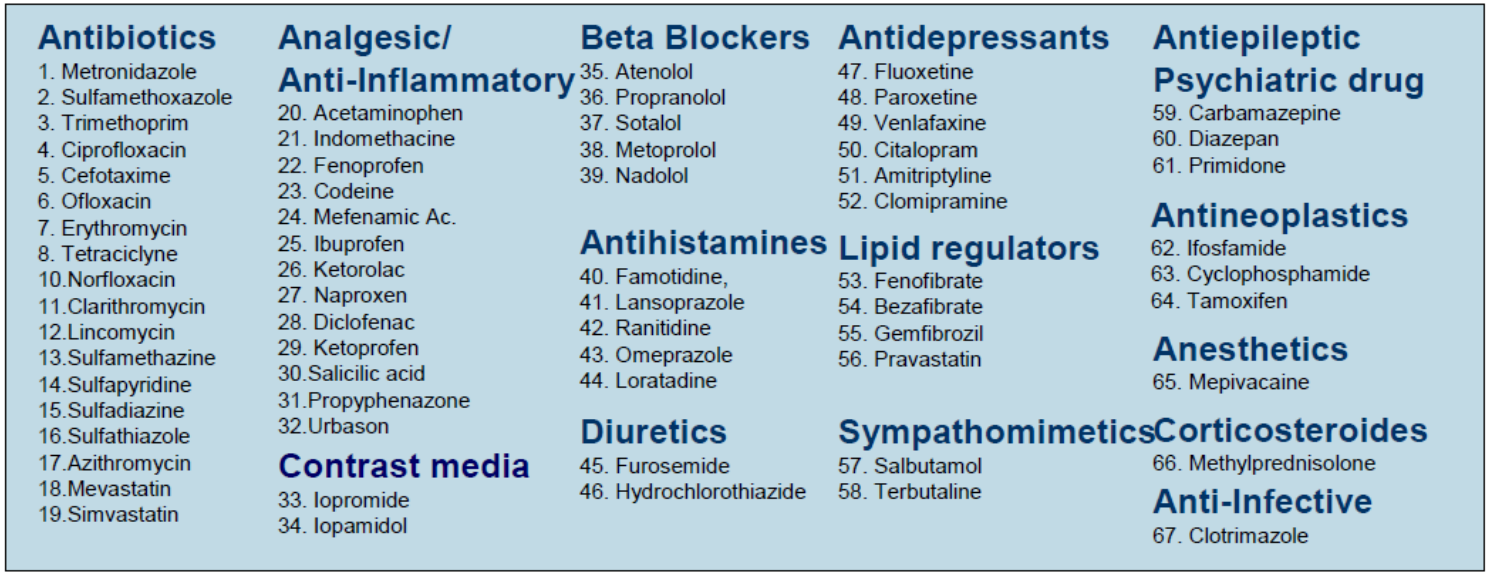
Targeted analysis using LC-MS/MS

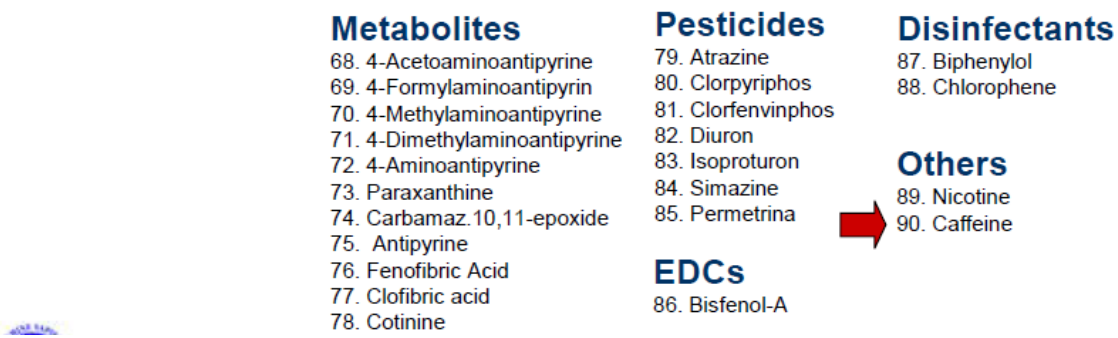
MS/MS selected reaction monitoring (SRM)



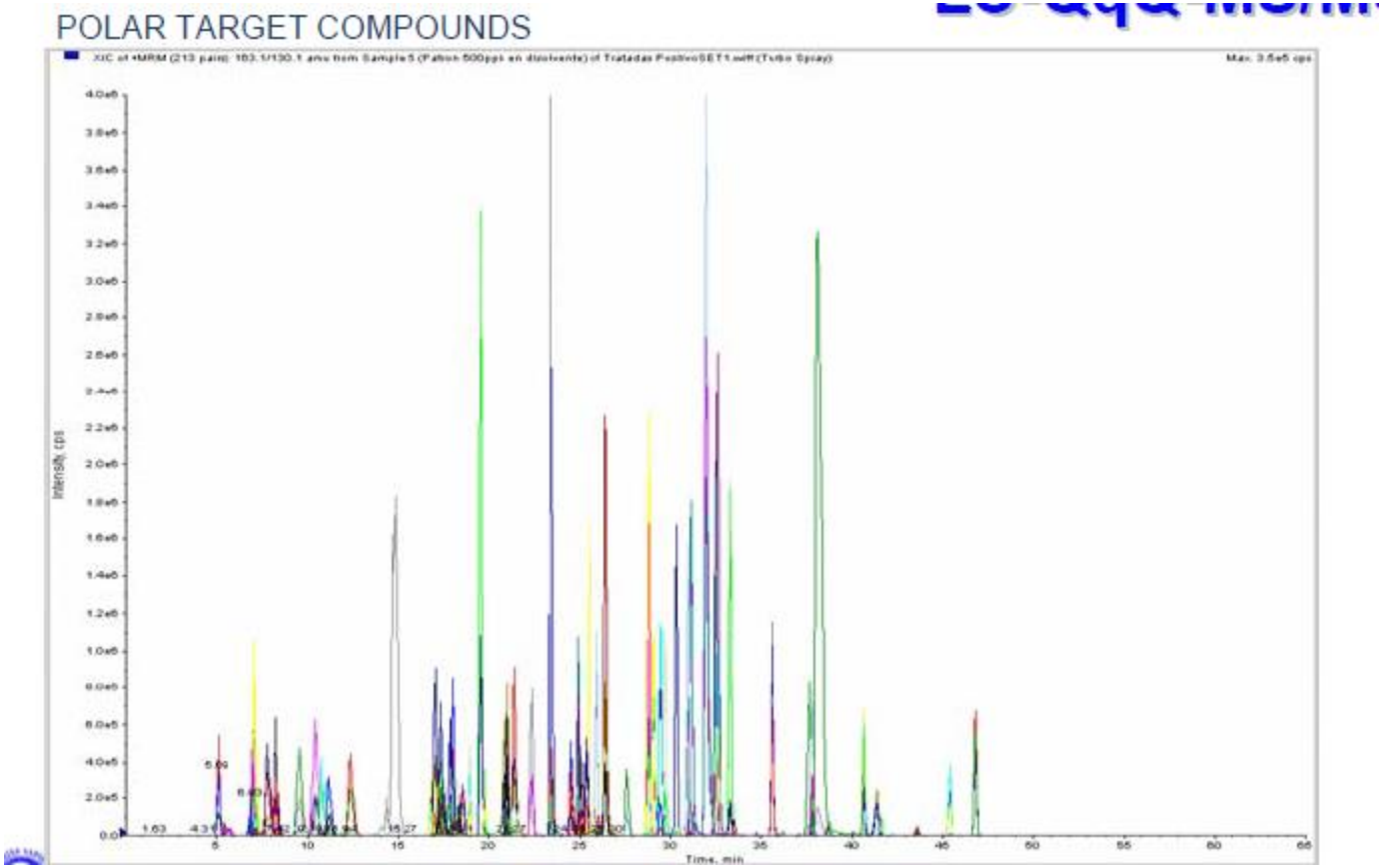
Identification through comparing ion ratios with those from standards

Target analysis





Target analysis



Target analysis

Identification criteria for the SRM method by LC-QqQ-MS/MS

Compound	t_R (min) (%RSD)	Product Ion (m/z) SRM 1	Product Ion (m/z) SRM 2	[SRM2]/[SRM1] (%RSD)
Furosemide	3.7 (1.5)	205, $[M - H - CO_2SO_2NH_2]^-$	285, $[M - H - CO_2]^-$	0.9 (5)
Clofibric Acid	3.8 (0.7)	127, [	$H - C_6H_4ClO]^-$	0.2 (8)
Bezafibrate				0.7 (3)
Hydroclhlorothiazi				0.9 (3)
Ketoprofen				
Naproxe				
Diclofe				
Fenopr				
Indomet				
Mefenamic				
Ibuprofen				
Diuron				0.2 (8)
Chlorophene	12.2 (0.2)			0.6 (11)
Gemfibrozil	12.3 (0.4)	121, $[M - H - CO_2C_5H_{12}]^-$	127, $[M - H - C_8H_9OH]^-$	**0.1 (8)**

SRM METHOD

IDENTIFICATION CRITERIA:

- ✓ t_R
- ✓ 2 SRM transitions per compound
- ✓ SRM2/SRM1 ratio

Limitations of targeted approach

- Need reference standards
- Need to program methods with RTs of analytes and specific transitions to monitor
- The targeted approach will fail to detect other contaminants present in the sample
- Unable to go back and “mine” the data later

Introducing a new screening solution for targeted and non-targeted analysis using HR-MS

Advantages of HR-MS screening?

- Over recent years use of high resolution mass spectrometry has gained in popularity as a screening tool in the environmental sector

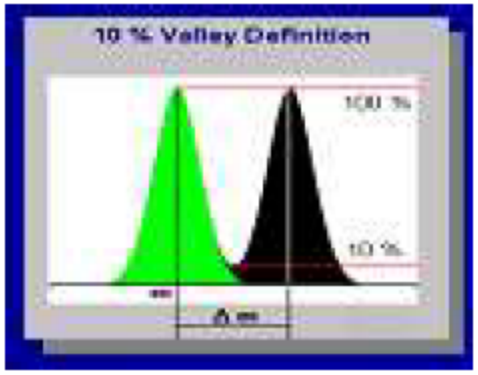
  - ✓ **Ability to perform non-targeted analysis**
  - ✓ **Ability to perform historical (retrospective) data review**
  - ✓ **Ability to perform full spectral analysis**
  - ✓ **Ability to screen for larger number of compounds and adducts**
  - ✓ **Increased specificity in complex matrices**
  - ✓ **Elucidation of unknowns ?**

Why high resolution system ?

- Definition of resolution
  - → Resolution is the capacity to differentiate 2 masses
  - → R=m/Δm

    m : mass of the first peak

    Δm : difference of mass between two consecutive peaks

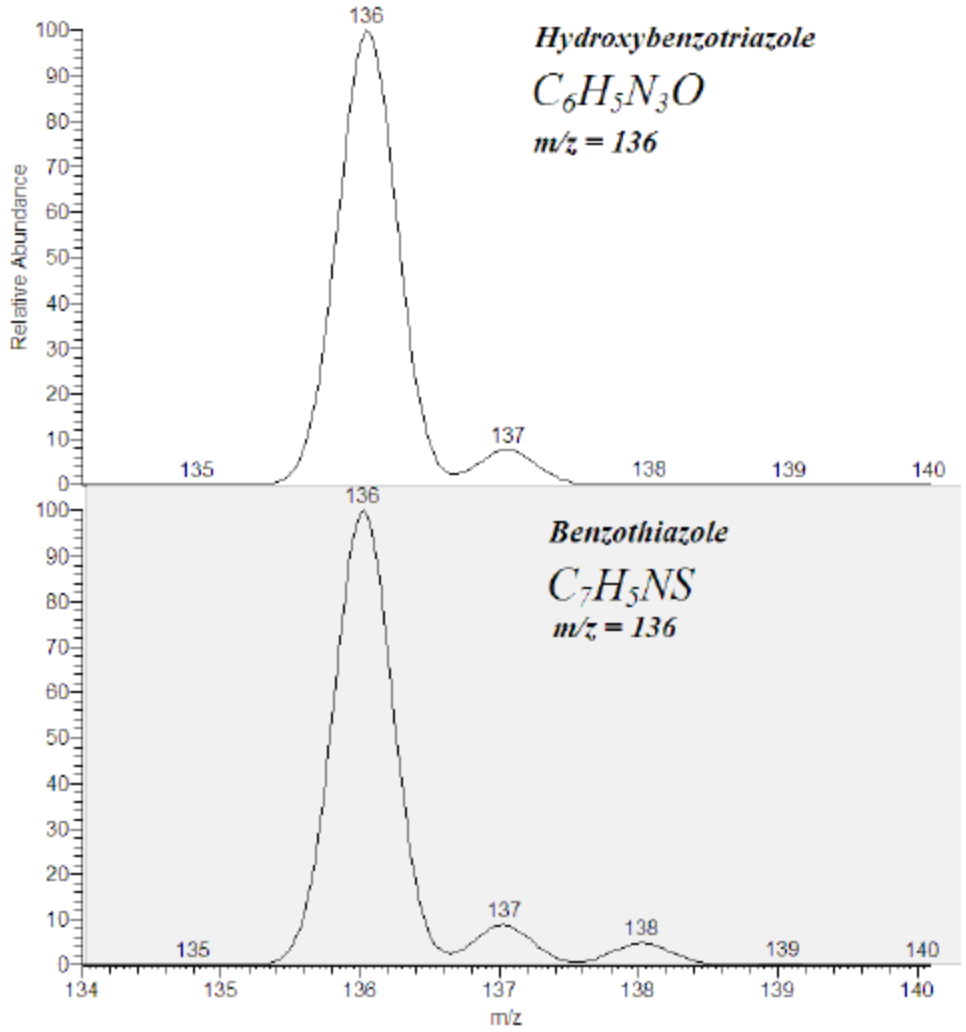


- LC-MS low resolution is not a good tool for screening :
  - → No or few spectral databases in LC-MS
  - → Mass spectrum too simple to be specific
  - → Not able to dissociate 2 molecules with the same unit mass

Why high resolution system ?

- Example
  - No dissociation of the 2 compounds

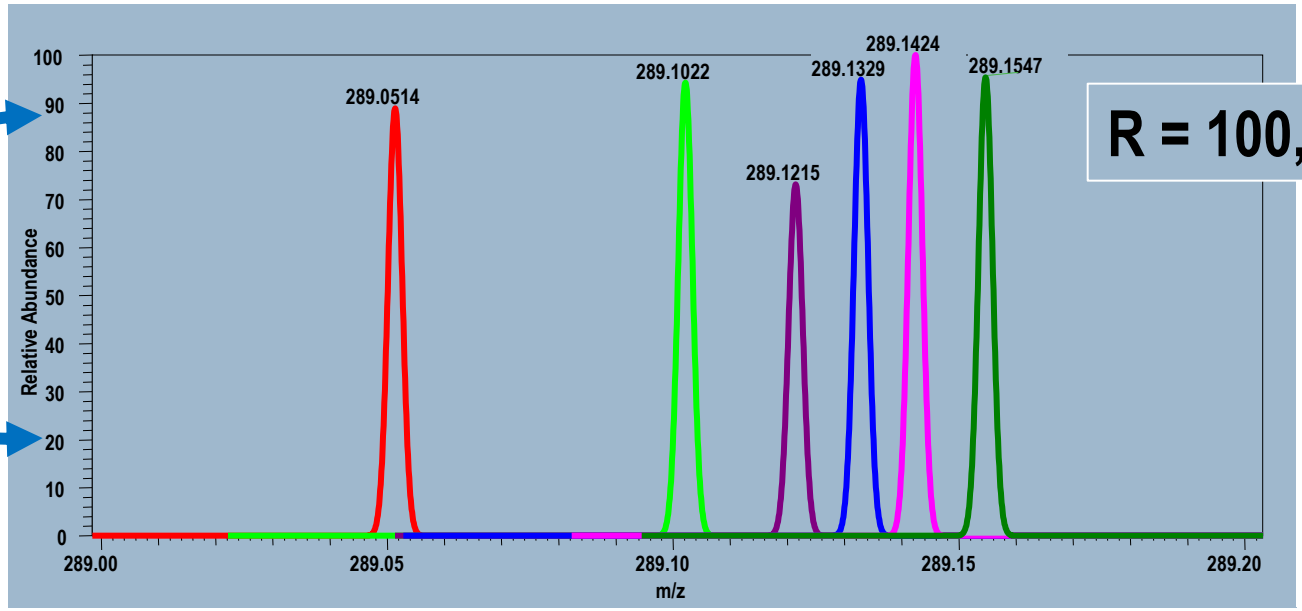
Need to work with high resolution systems



Exact Mass and Isobaric Compounds

Element	Exact Mass
H	1.007825
C	12.000000
N	14.003074
O	15.994915

Is a simultaneous measurement possible?

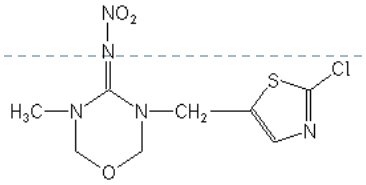


Yes, at high resolution !

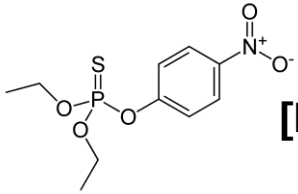
Resolution (FWHM)

Thiamethoxam

$[M+H]^+$ = 292.02656

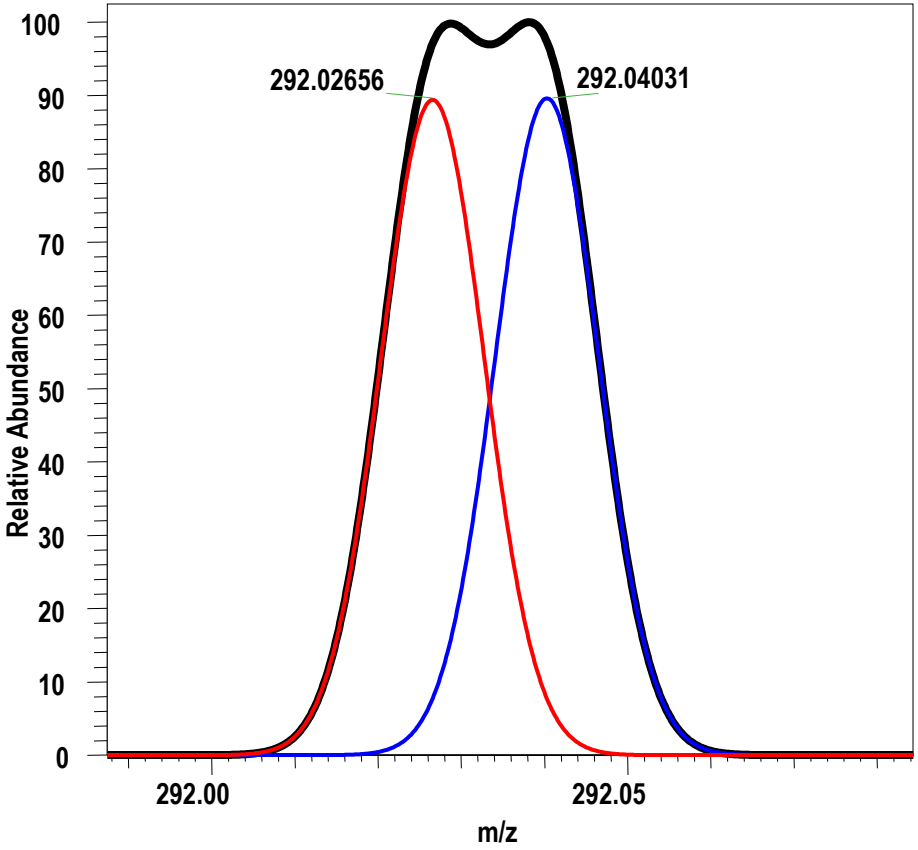


Δm
0.0138 amu



Parathion

$[M+H]^+$ = 292.04031



Δm (Parathion and Thiamethoxam) is 0.0138 amu

R = m / Δm
R = 292 / 0.0138

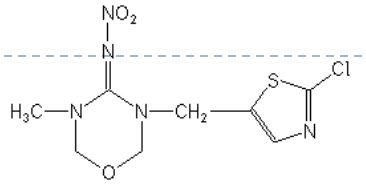
R = 21,160

Mix 1:1

Resolution (FWHM)

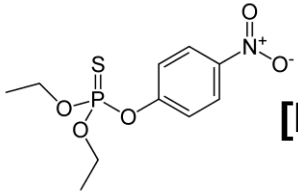
Thiamethoxam

$[M+H]^+$ = 292.02656



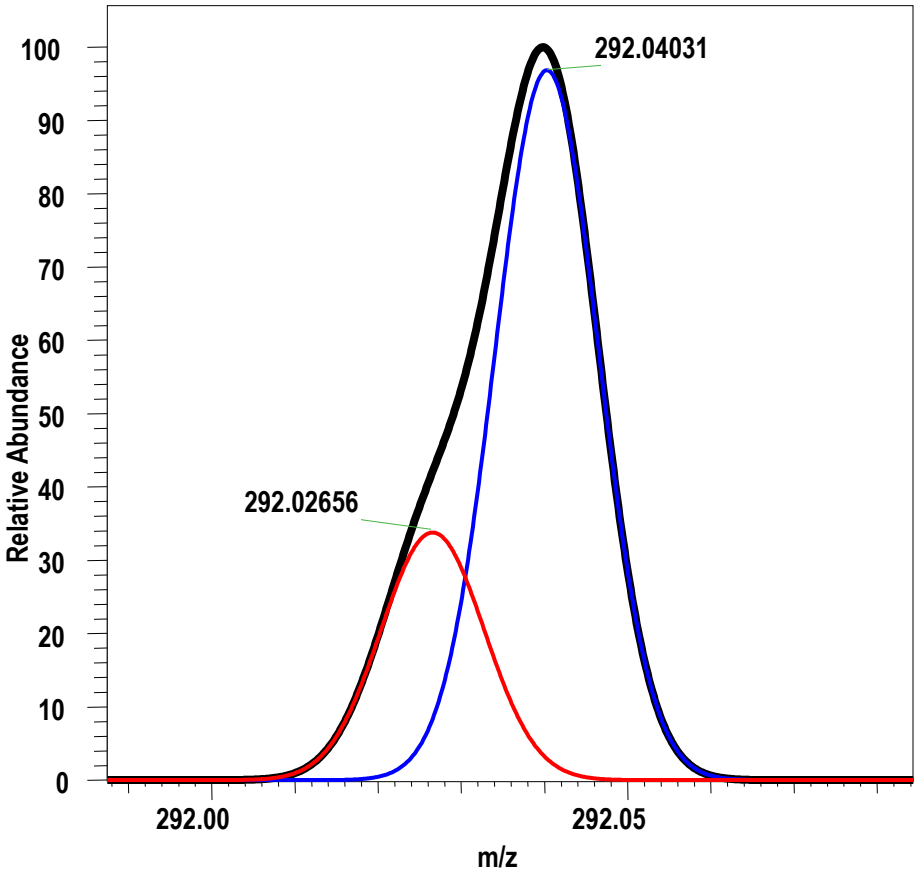
Δm

0.0138 amu



Parathion

$[M+H]^+$ = 292.04031



Δm (Parathion and Thiamethoxam) is 0.0138 amu

R = m / Δm
R = 292 / 0.0138

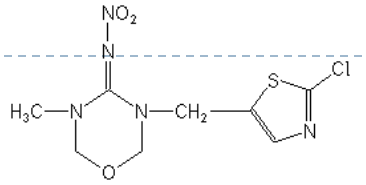
R = 21,160

Mix 1:3

Resolution (FWHM)

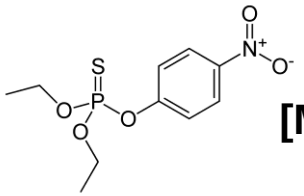
Thiamethoxam

$[M+H]^+$ = 292.02656



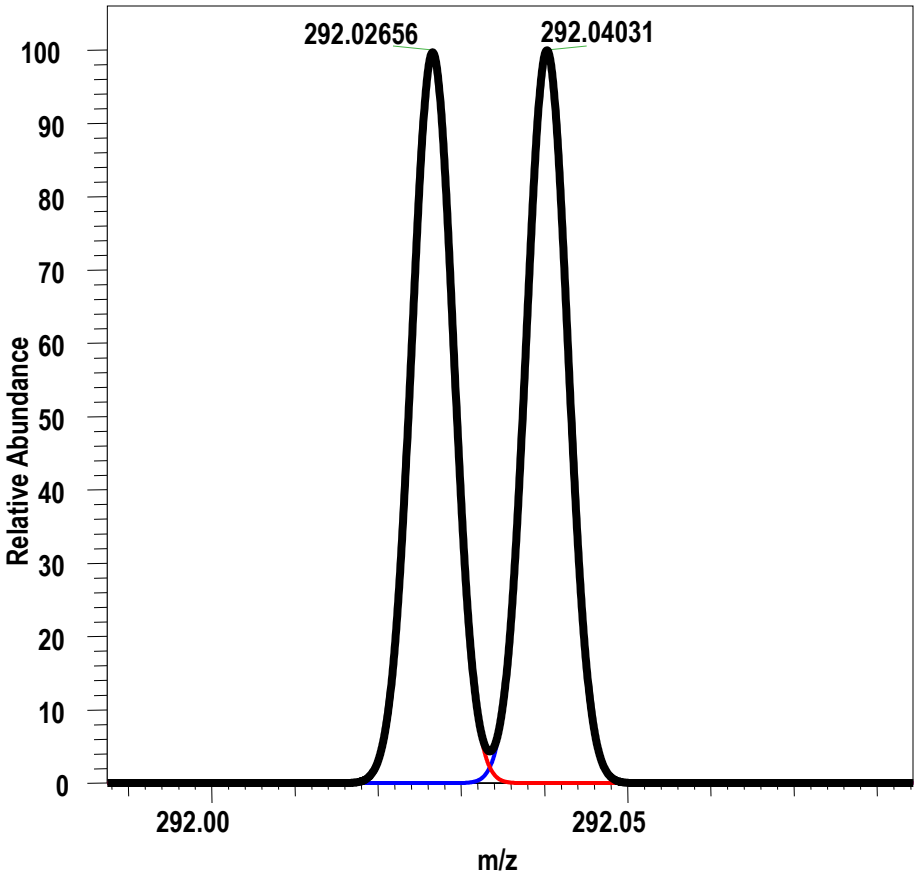
Δm

0.0138 amu



Parathion

$[M+H]^+$ = 292.04031



Δm (Parathion and Thiamethoxam) is 0.0138 amu

R = m / Δm
R = 292 / 0.0138
R = 21,160

Measured at R = 50,000

Mix 1:1

NON TARGET SCREENING

Trends in non-targeted analysis

- **Transfer of methods from specific methodologies to those providing data for comparison with databases**
- **An alternative so-called "non-targeted" approach**
  - LC-HRMS
    - Database searching via mass measurements
  - LC-HRMS/MS
    - Also provides spectral library searching
  - Non-targeted acquisition but initial data processing tends to be still targeted…

Non-targeted acquisition

- **Use of "high resolution" instruments**
  - Time of flight (ToF) or orbitrap mass analysers
  - Full spectral information
  - High mass resolving powers and mass resolution
    - Specifications vary significantly
  - Good mass accuracy
  - Good sensitivity through improved ion optics
  - Variable acquisition speeds

High Resolution instruments

Holcapek *et al*. (2012). *J. Chromatogr. A* 1259: 3

Comparison of ToF with orbitrap

Mass analyser	Resolving power ($x10^3$)	Mass accuracy (ppm)	Acquisition speed (Hz)
Q	3-5	Low	2-10
IT	4-20	Low	2-10
Tof	10-60	1-5	10-100
Orbitrap	100-240	1-3	1-5

Q, ToF and orbitrap also include common hybrid configurations with Q or LIT as the first mass analyser providing MS/MS or MS*n* capabilities

Data processing for screening

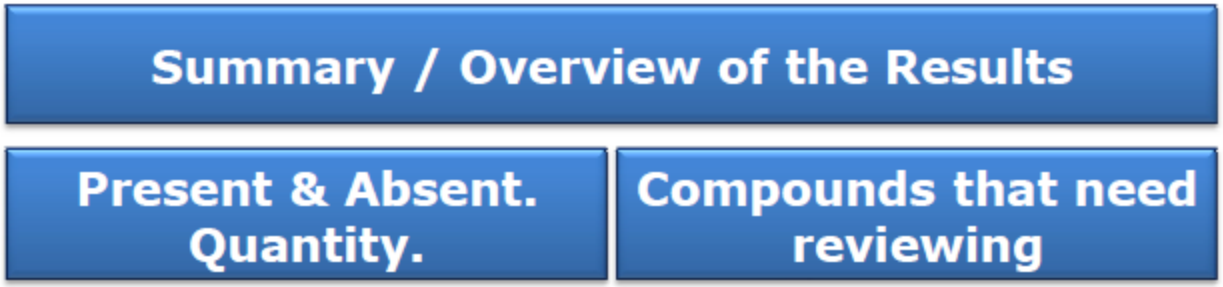
- Peak detection by extracting those ions matched with entries in a database
  - Can be psuedo molecular ions and fragments
- Recognition is based upon measurement of:
  - Accurate mass
  - Isotope pattern
  - Retention time (if available)
  - A response threshold
- Results are reported as a “hit list” with or without creating chromatographic peaks

Non target screening

- To be effective data processing must be automated and quick
- Minimise false negatives whilst generating a manageable number of false detects
  - ❖ Apply tolerances on response threshold, retention time and isotopic fit and the presence of a second diagnostic ion
- It requires more computing power and data management/storage than that traditionally associated with LC-MS analyses using QqQ instruments

Non-targeted workflow

- Obtain a summary of the identified compounds that are present (and absent) and determine concentration
- Provide a list of all compounds that meet user criteria (retention time, accurate mass measurement of precursor and fragments, adducts found, isotope ratios, user-defined limits)
- Provide a list of spurious results (e.g. RT & accurate mass measurement shifted, isotope ratios questionable..)



Looking for Unknown Components…?

-

…Use a Filter Approach

Suspect screening

- Suspect screening is the technique of choice for the identification of TPs, when the confirmation of the analytes with a reference standard is impossible, **but molecular formula and structure of suspected molecules can be predicted**
- In suspect screening, **an important step of the identification workflow is the prediction of possible TPs using computational (in silico) prediction tools.**

Suspect screening - Computational (in silico) prediction tools

- Commercially available or freely accessible programs have been applied in the prediction step on environmental analysis

- ✓ University of Minnesota - Pathway Prediction System (UM-PPS: http://eawag-bbd.ethz.ch/)
- ✓ CATABOL (http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity/catabol-301c.aspx);

- (CATABOL and UM-PPS predict microbial metabolic reactions based on biotransformation rules)

- ✓ PathPred (http://www.genome.jp/tools/pathpred/);

- (PathPred is a multi-step reaction prediction server for biodegradation pathways of xenobiotic compounds and biosynthesis pathways of secondary metabolites) and,

- ✓ Meteor (http://www.lhasalimited.org/products/meteor-nexus.htm).

- Meteor was built based on mammalian biotransformation reactions of common functional groups and allows prediction of the most probable TPs, providing in parallel relevant literature references.

The prediction system should be properly selected by considering the organism or the system where TPs are formed.

To conclude

Despite great progress in the last years, the identification of suspects and non target TPs is still an analytical challenge since software and methods to predict fragmentation patterns, ionization behavior, and retention time are still under development.

Furthermore, the lack of comprehensive mass spectral libraries for high-accuracy MS/MS and the limited comparability between collision-induced dissociation (CID) and higher energy collision dissociation (HCD) spectra make the identification of unknown compounds more challenging.

To conclude

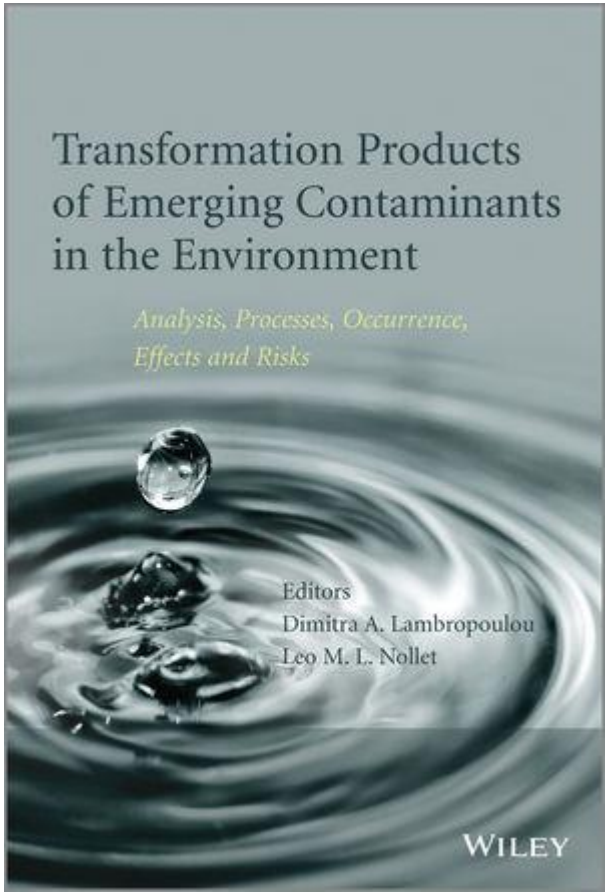
Commercial software such as Mass Frontier and Mass Fragmenter are available to predict mass spectral fragments using different fragmentation rules, but they need a lot of improvement.

Advancement of predictive models and computer tools is urgently required

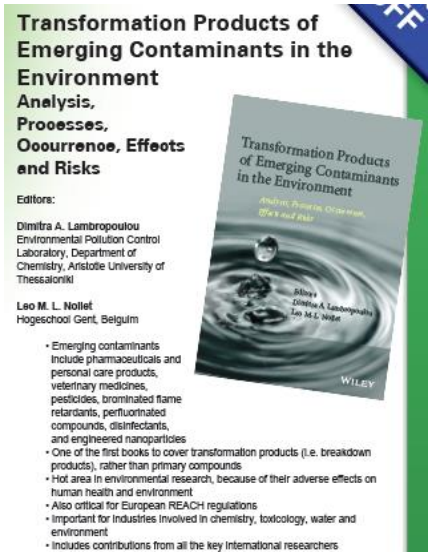
together with innovative analytical tools, spectral databases, multivariate tools, (pattern recognition) and biodiagnostic tools (omics)

Identification & Structure elucidation strategy employing HR-MS, complementary techniques and advanced software tools is promising

Transformation Products of Emerging Contaminants in the Environment:
Analysis, Processes, Occurrence, Effects and Risks



Dimitra A. Lambropoulou (Editor),
Leo M. L. Nollet (Editor)
ISBN: 978-1-118-33959-6
964 pages
February 2014




Thank you for
your attention!!!