



## Comprehensive Investigation of Organic Micro-pollutants in Waters by Gas and Liquid Chromatography Coupled to QTOF MS

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GLP-Certified Laboratory Pesticide Residue Analysis

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## Analytical Challenges in Environmental Research




**Thousands** of environmental (organic) contaminants of very different physico-chemical characteristics


**Metabolites and Transformation/Degradation Products**

Investigation of **new (unknown) contaminants** potentially dangerous for the environment

Reliable **detection, identification** and accurate **quantification** in samples




## State-of-the-art in environmental analytical chemistry



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- **Well-established analytical (quantitative) methods for many priority contaminants**
  - Quantitative analysis based on **tandem MS** (GC & LC)
  - Good sensitivity and selectivity: low LODs and LOQs
- **Need to advance in HRMS strategies** for investigation of large number of compounds
  - Wide-scope screening methodologies (target, suspect, non-target)

## Ideal screening of organic contaminants?



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- **Universal** → detect all potential contaminants in the samples
- **Rapid**
- **Little sample handling**
- Reliable **identification** of compounds detected
- If possible, accurate **quantification**

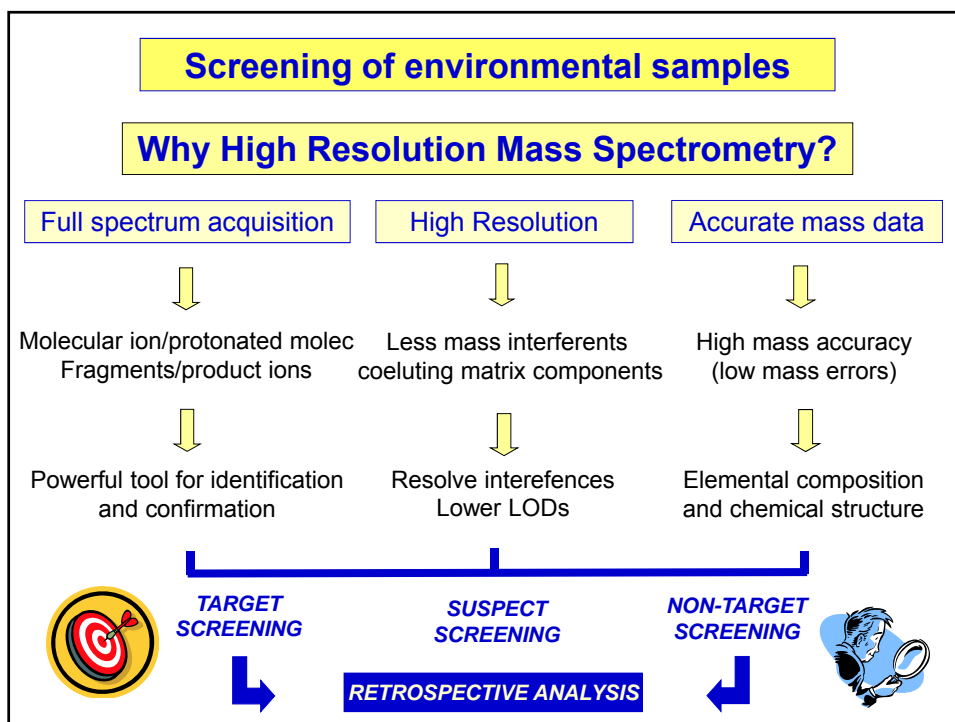
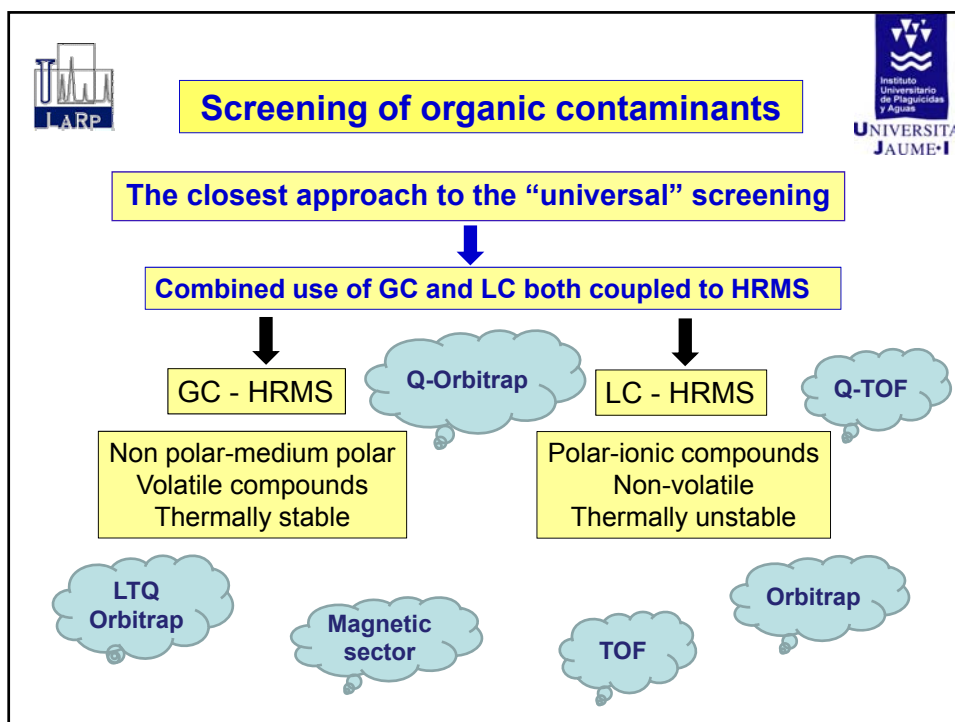


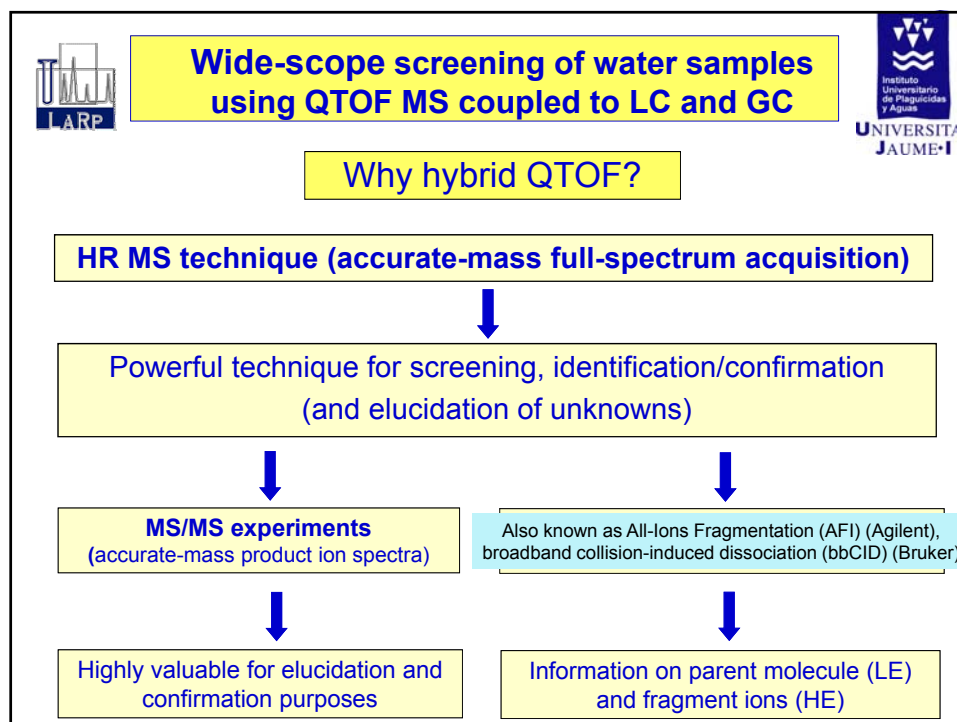
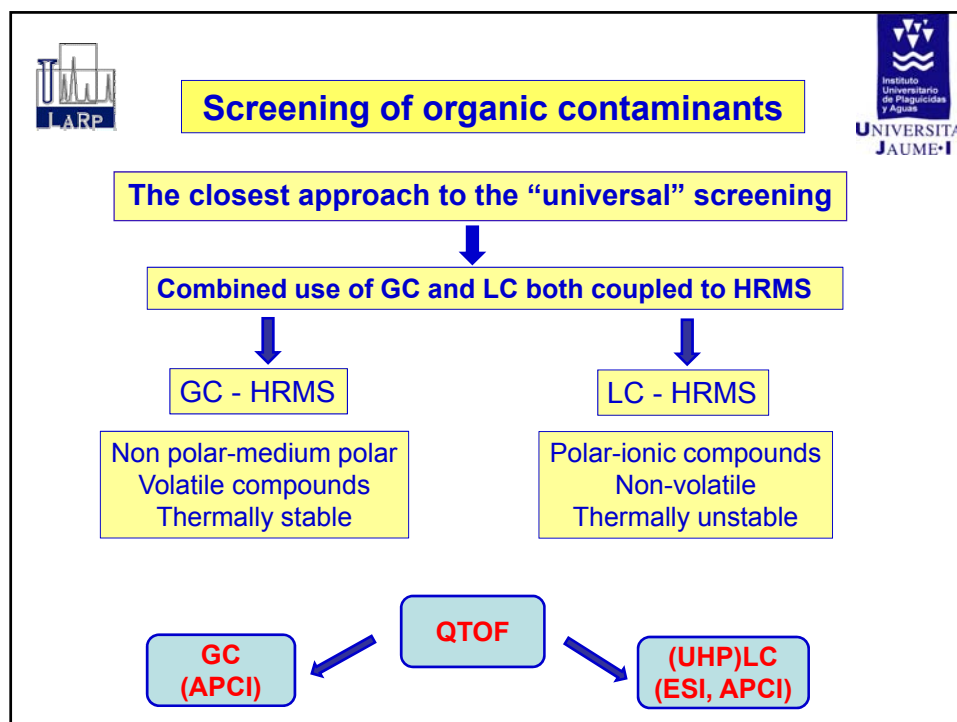
**This ideal situation that can not be reached**

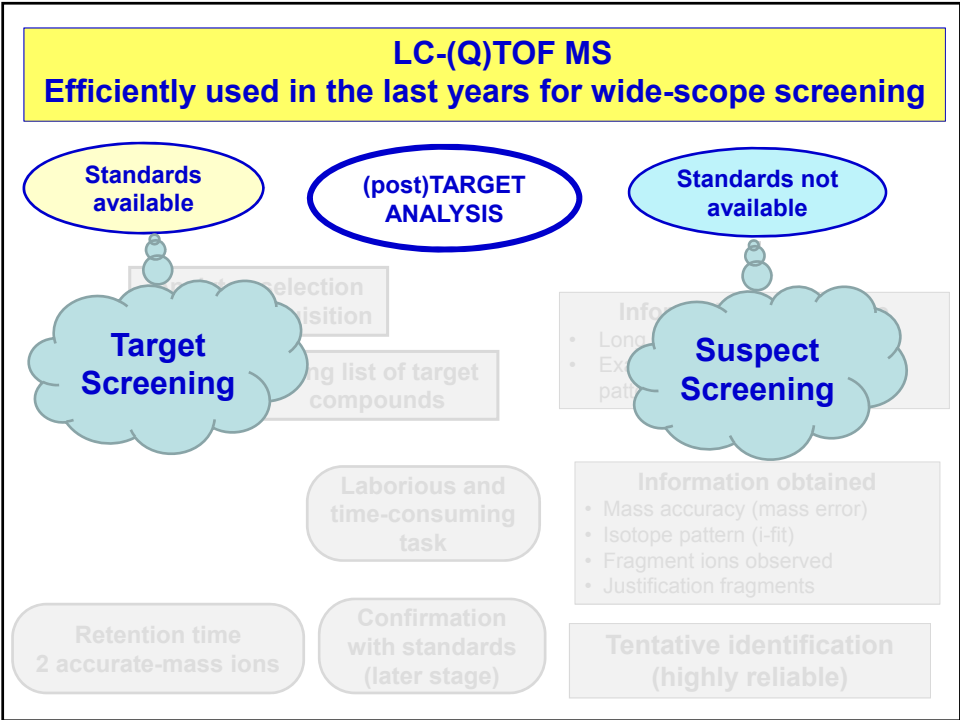
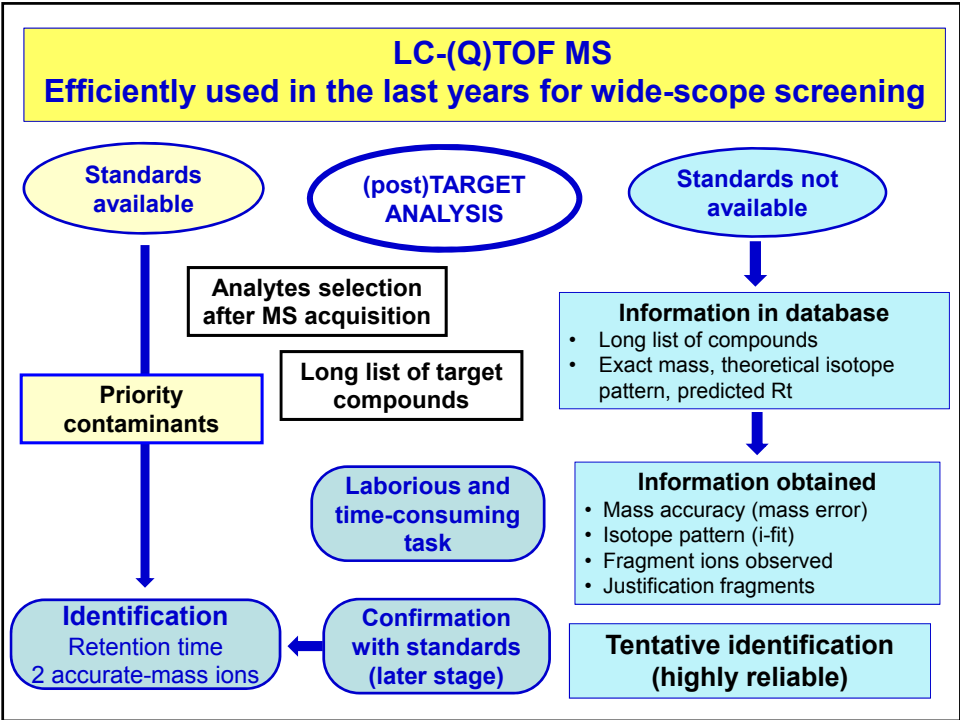
Sample treatment

Measurement

Some compounds require specific conditions  
(e.g. highly volatile, highly polar/ionic)







**What about GC-QTOF?  
Is possible to use this technique similarly to LC-QTOF?**

- EI is the “queen” of GC ionization techniques: robust source, standardized mass spectra, commercially available libraries, but the extensive fragmentation may become its **Achilles’ heel**
- The **lack of molecular ion** in EI mass spectra may be a **handicap** when developing mass spectrometry methods for both target and screening

```

graph TD
    A[EI] --> B[Widely used and accepted]
    B --> C[Extensive fragmentation]
    C --> D[Lack/low abundance of molecular ion is a handicap]
    D --> E[Target GC-MS/MS]
    D --> F[Screening GC-TOFMS]
  
```

**Atmospheric Pressure Chemical Ionization in GC(APCI)-MS**

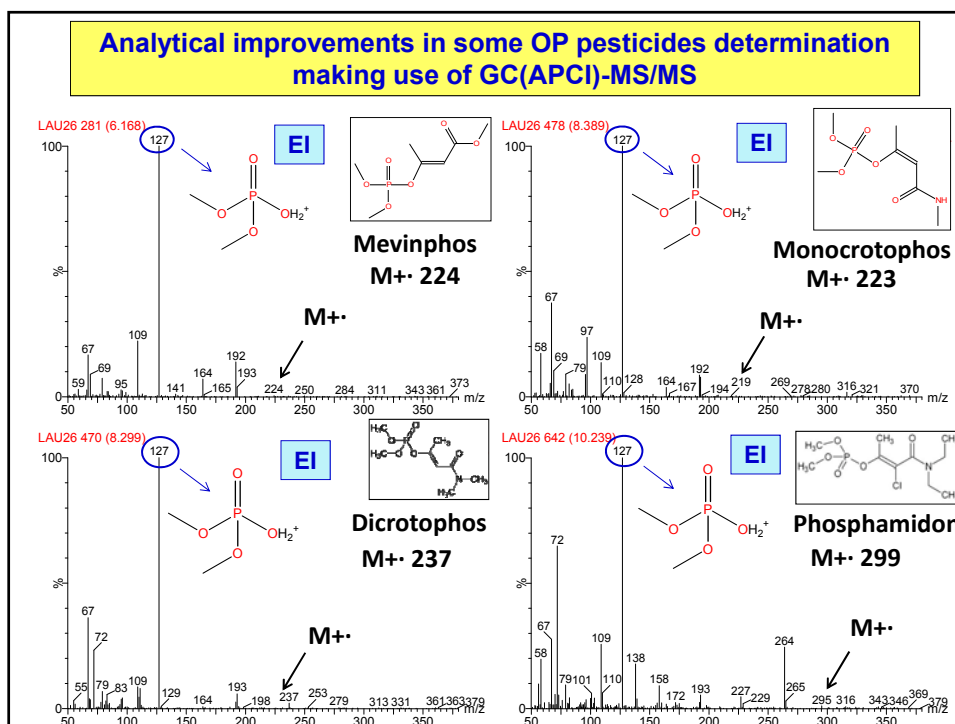
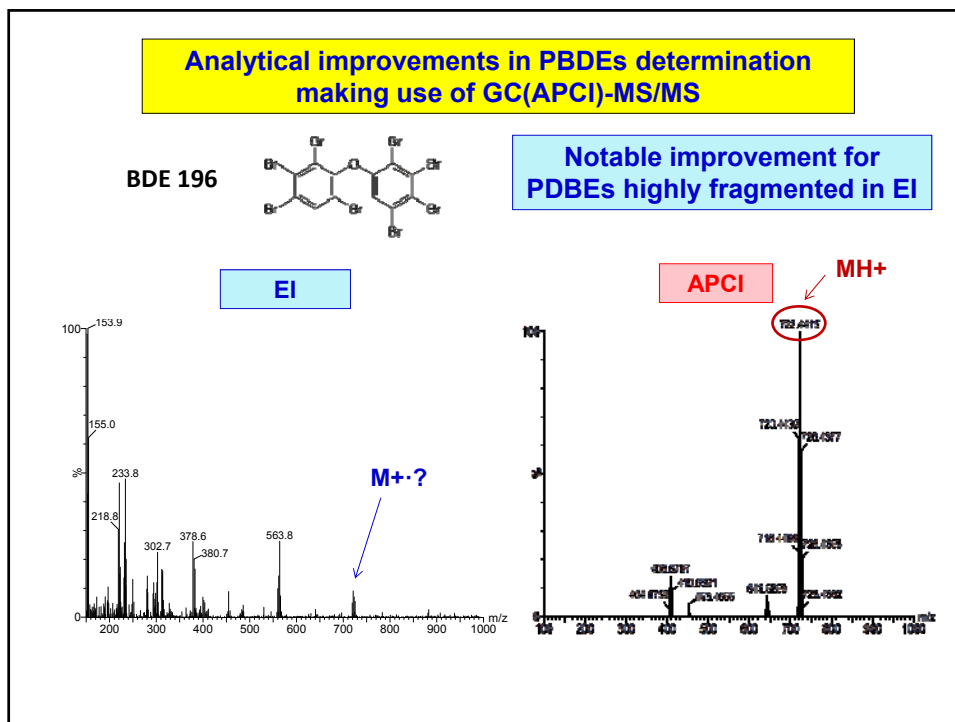
↓ **SOFT IONIZATION SOURCE**

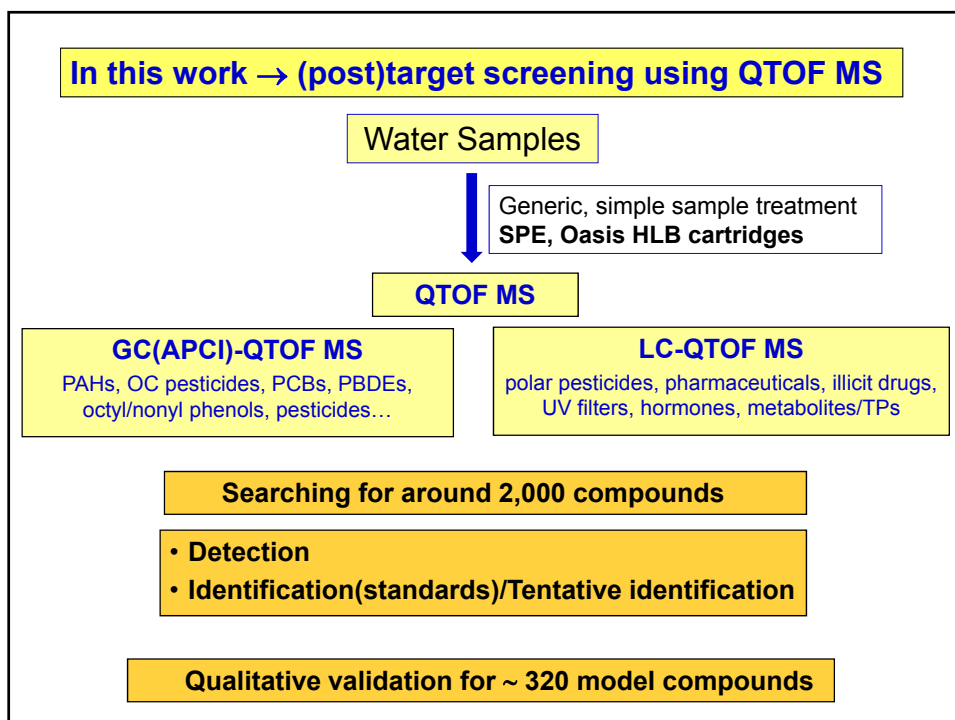
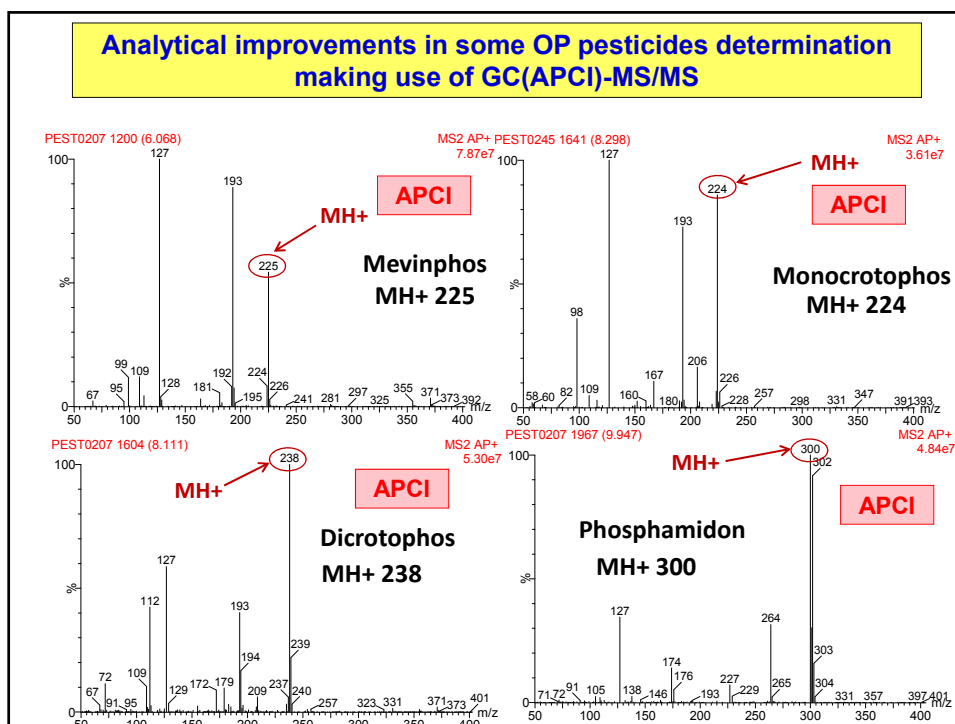
- Very **low fragmentation** of the molecule is observed, compared to EI
- The **M+·** or **[M+H]+** commonly is the **base peak** of the spectrum
- This APCI source allows us to couple GC to a **novel tandem mass spectrometers**
  - GC-MS/MS
  - GC-QTOF MS

The presence (and abundance) of the **M+·** or **[M+H]+** facilitates rapid, wide-scope, and more sensitive **screening**

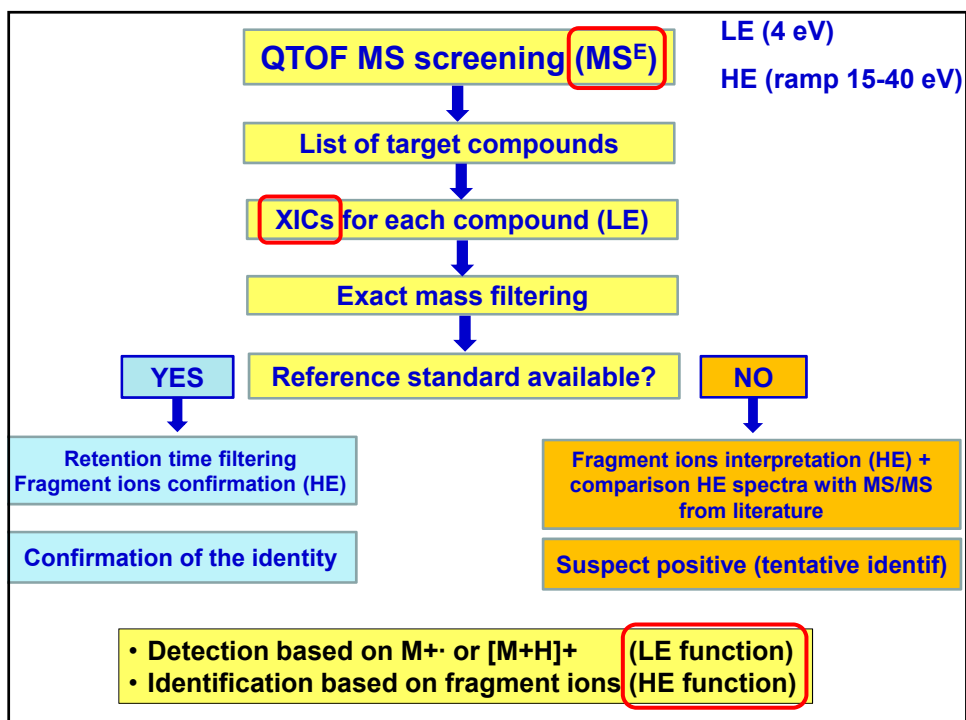
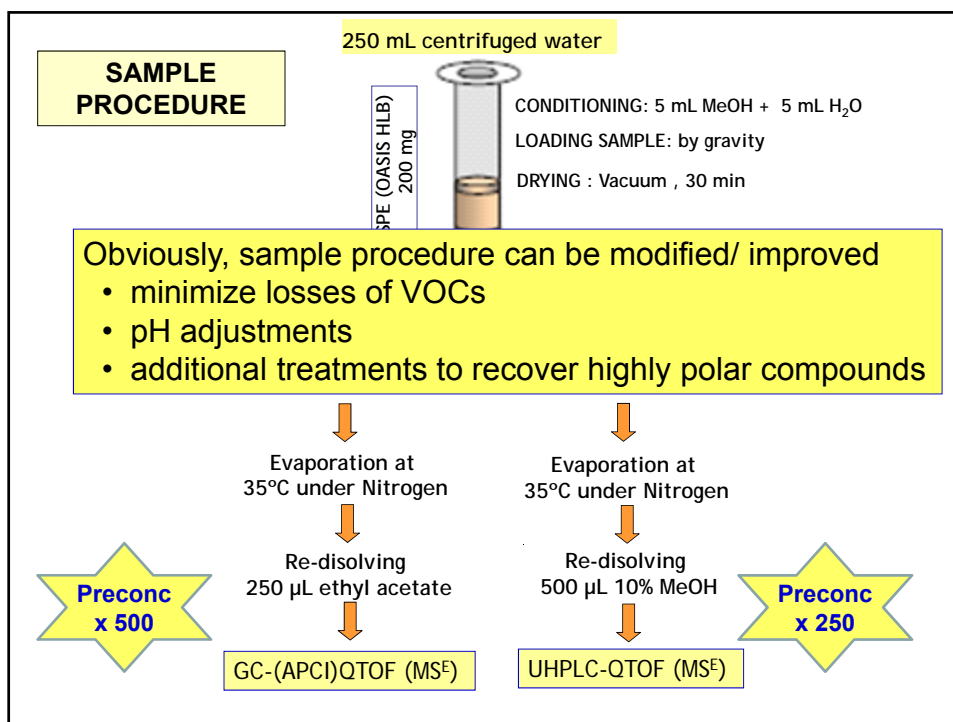
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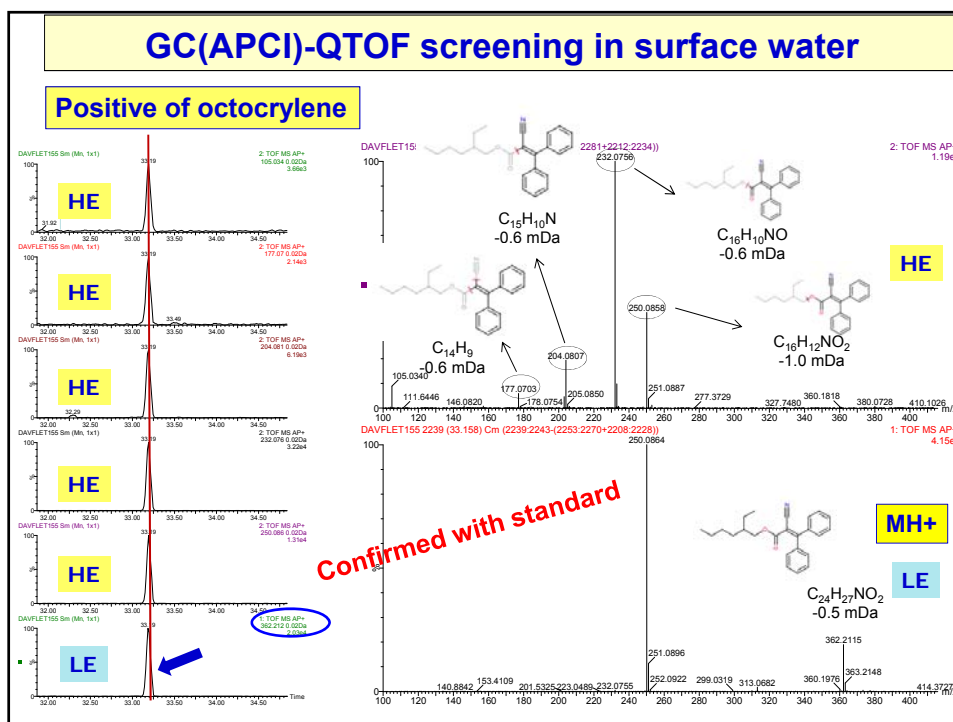
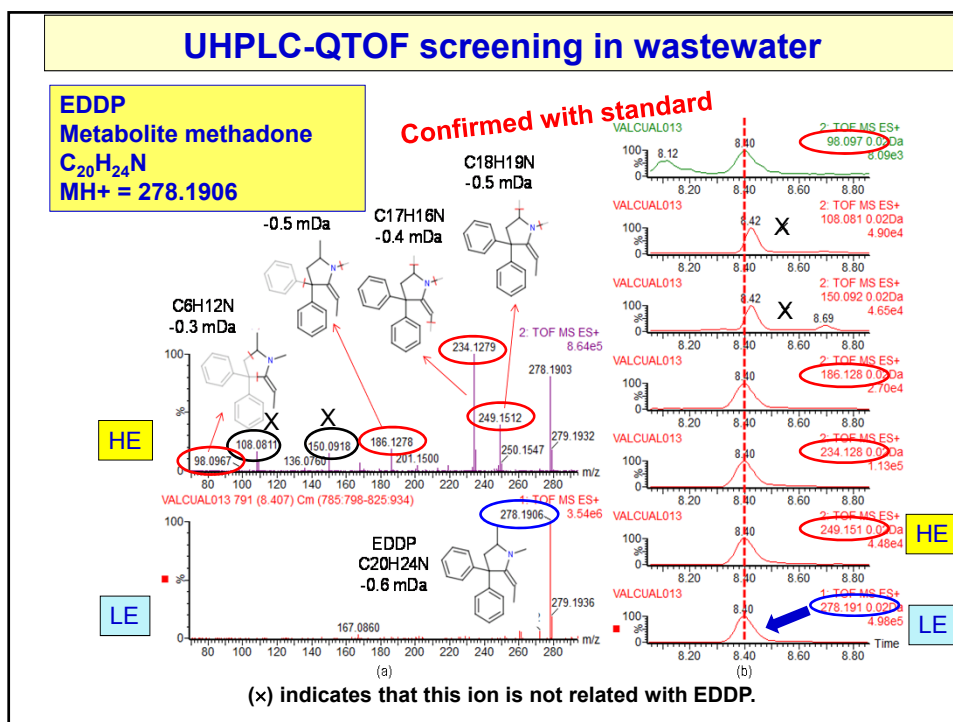
Detection **based on molecular ion** searching is more **sensitive and specific**











**Screening applied to 33 water samples (12 GW, 12 SW, 9 EWW) from Mediterranean Spanish region (Valencia and Castellón)**

78 pesticides (metabolites/TPs); 24 pharmaceuticals (metabolites/TPs)  
4 drugs abuse; 4 preservatives; 5 UV-filters; 2 sweeteners  
3 PAHs; 3 musks; 2 X-ray agents; 1 antimicrobial; 2 insect repellents

**The most frequently detected**

**Pesticides**

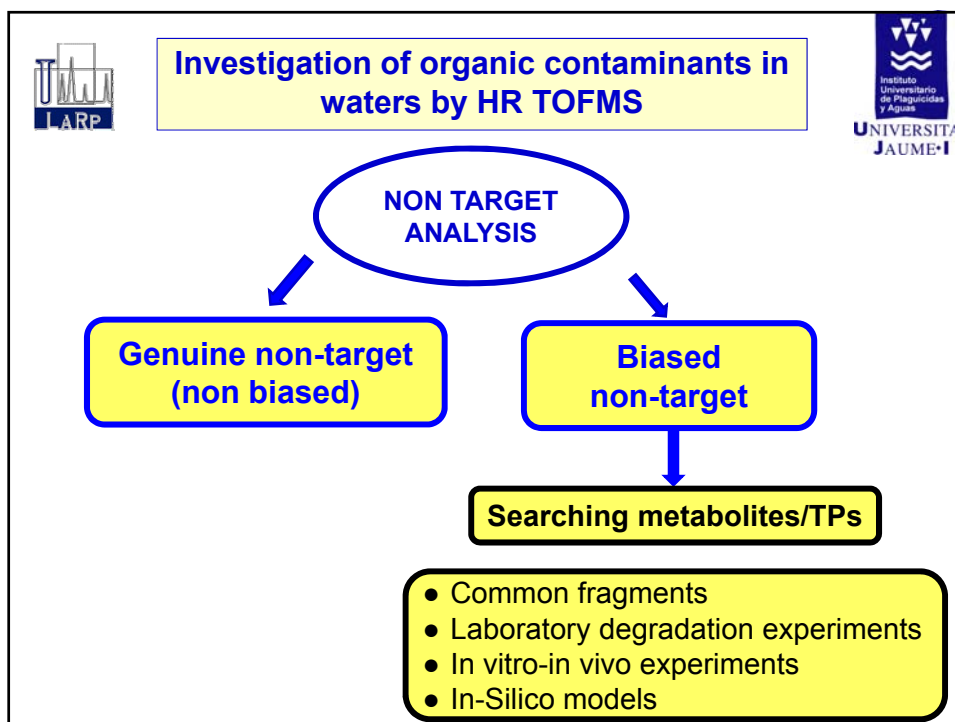
Triazine herbicides (particularly, terbuthylazine and terbutryn)  
Insecticides diazinon and chlorpyrifos-ethyl  
Fungicides thiabendazol, carbendazim and propiconazole

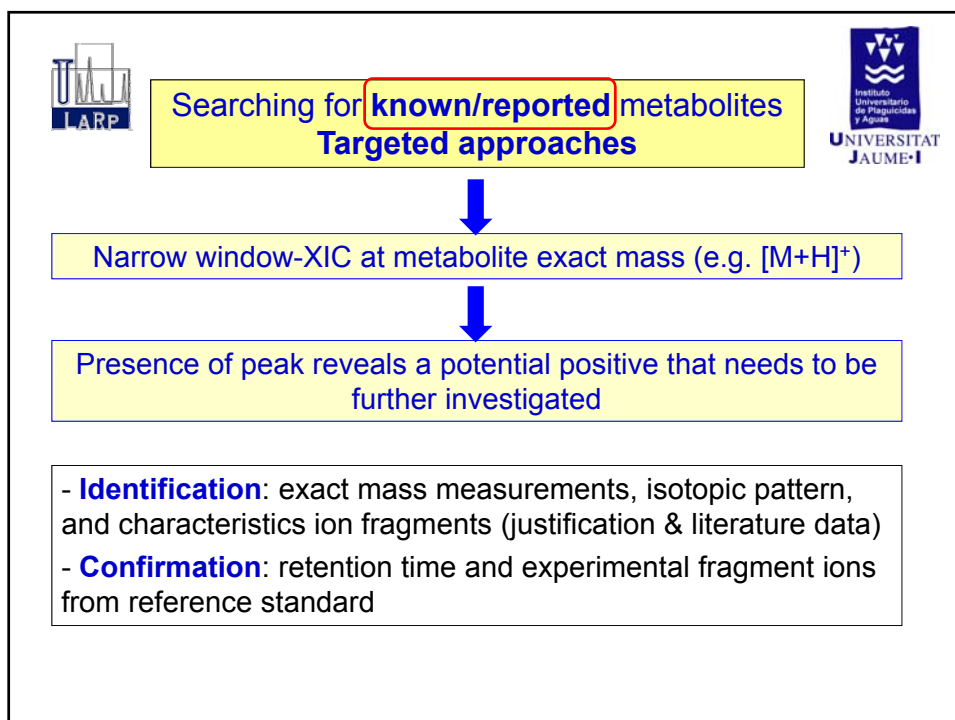
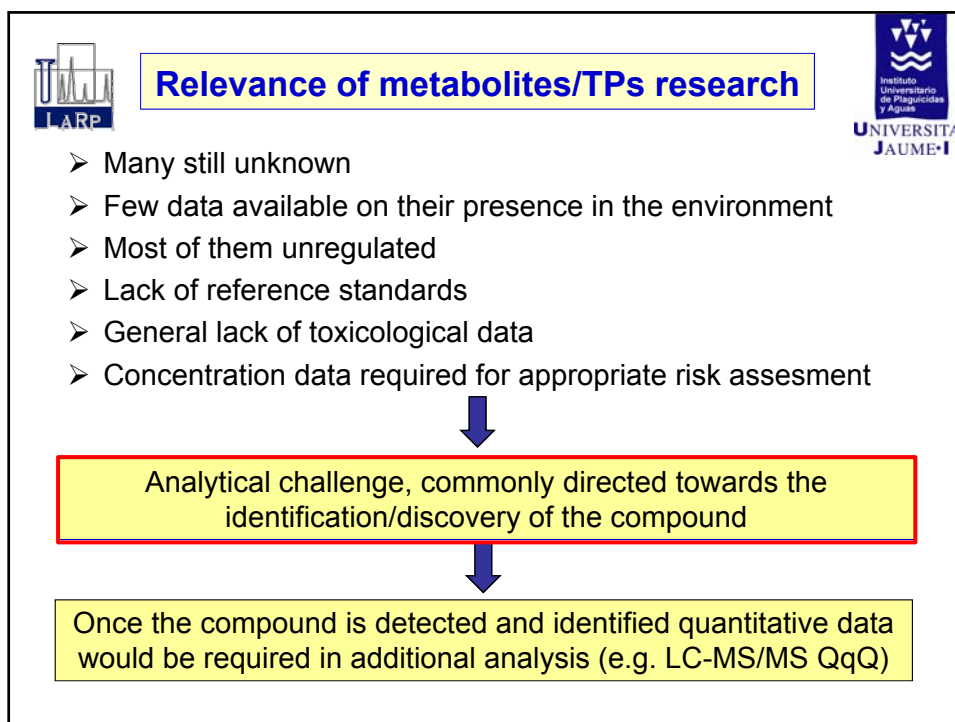
**Pharmaceuticals**

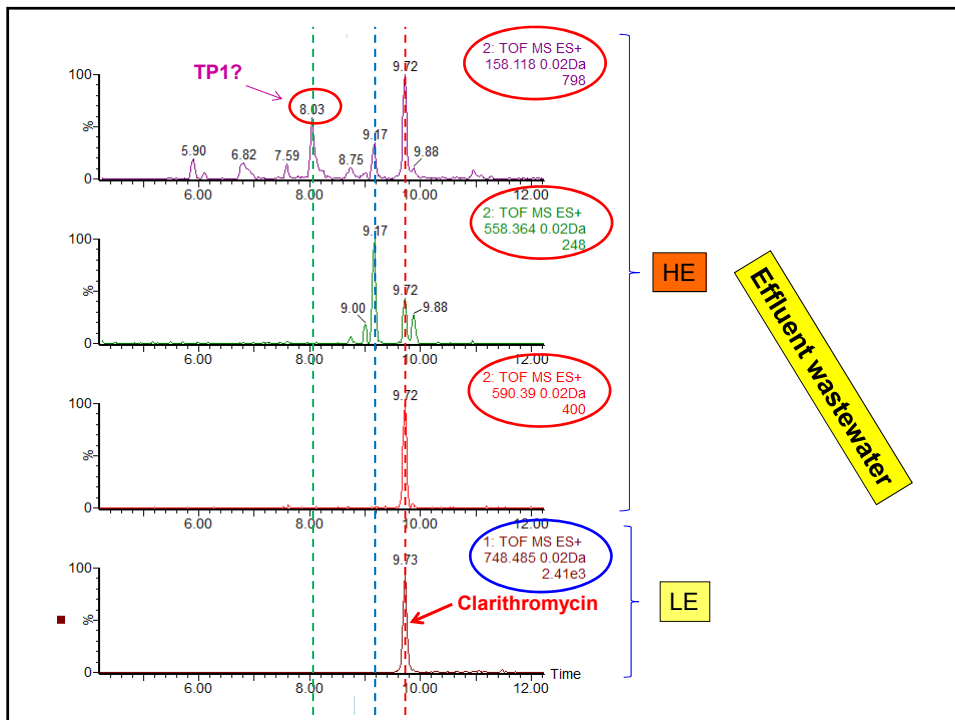
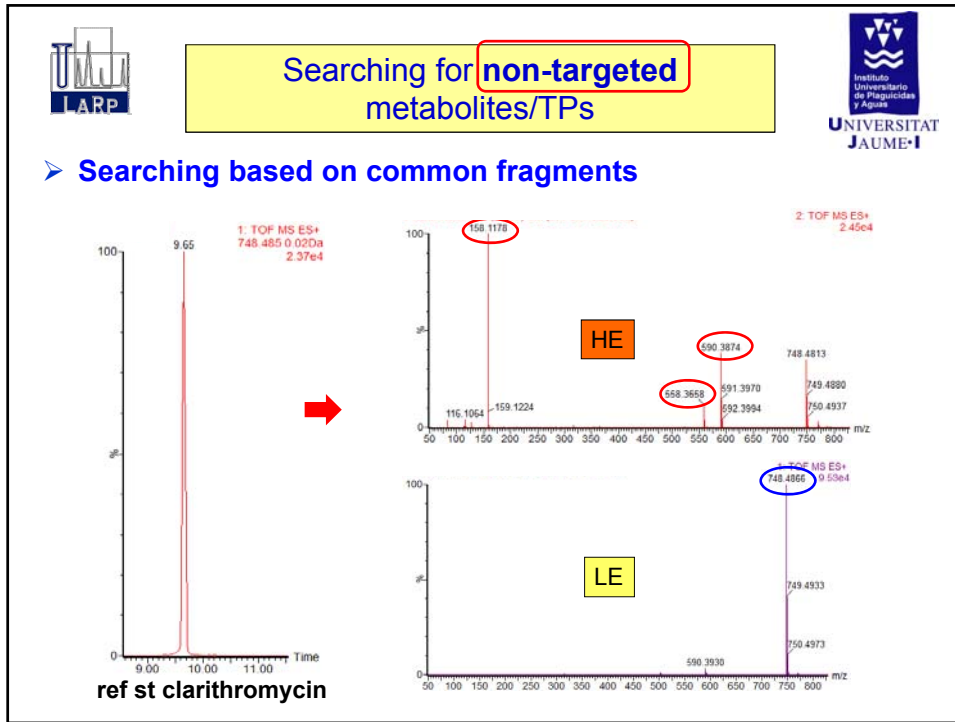
Antibiotic ofloxacin  
Anti-inflammatory/analgesic diclofenac  
Angiotensin II receptor antagonists valsartan and irbesartan  
Antidepressant venlafaxine  
Anti-epileptic carbamazepine

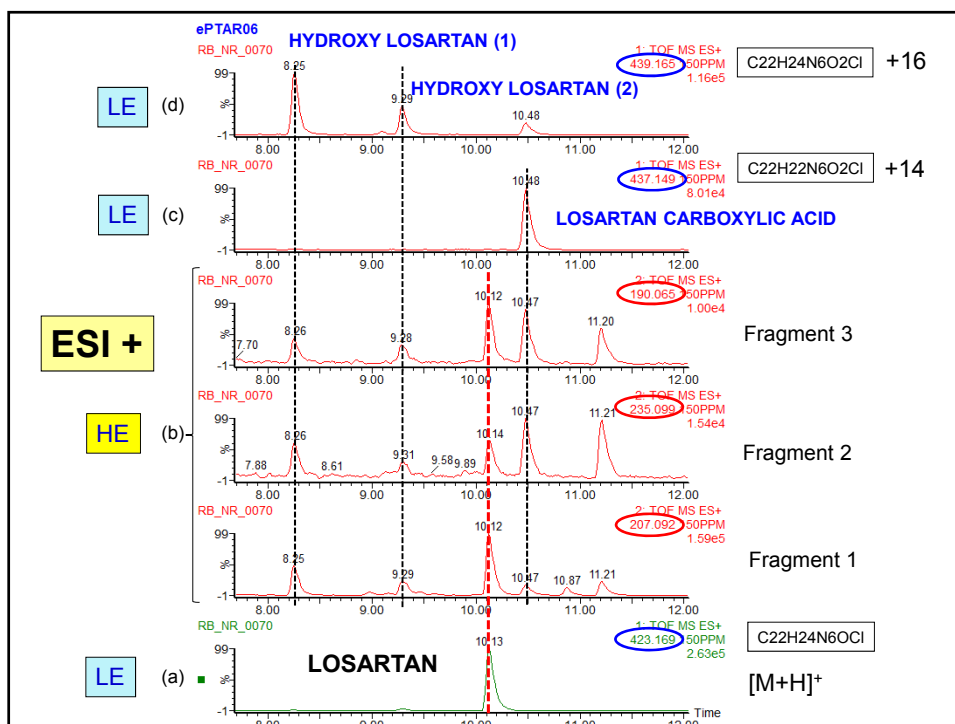
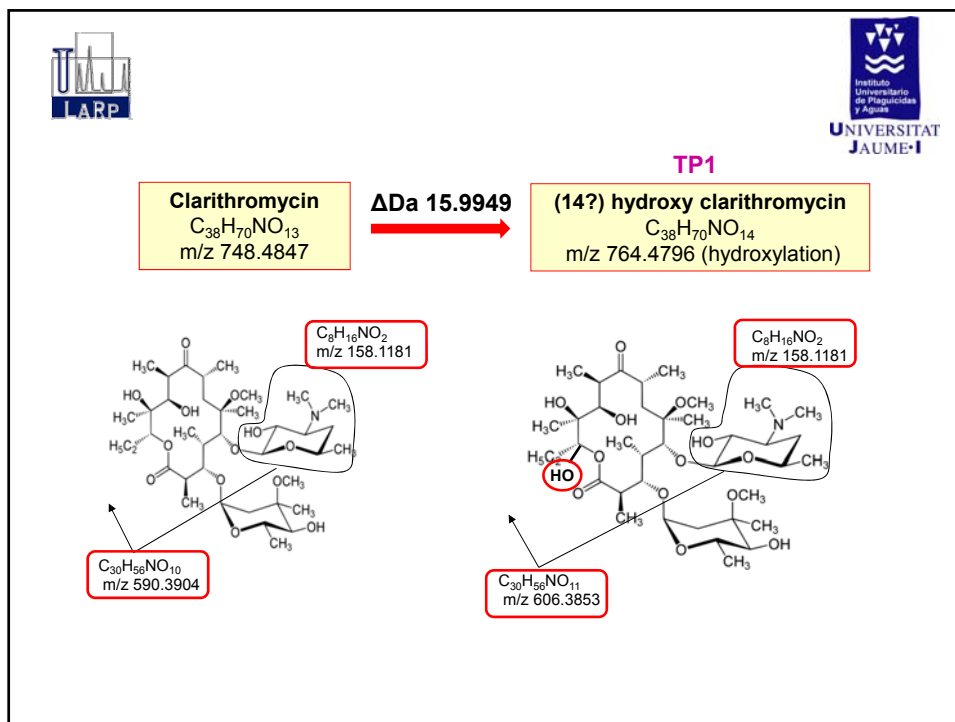
**Others**


Benzoylcegonine (the main metabolite of cocaine)  
Tonalide (musk) and octocrylene (UV filter)








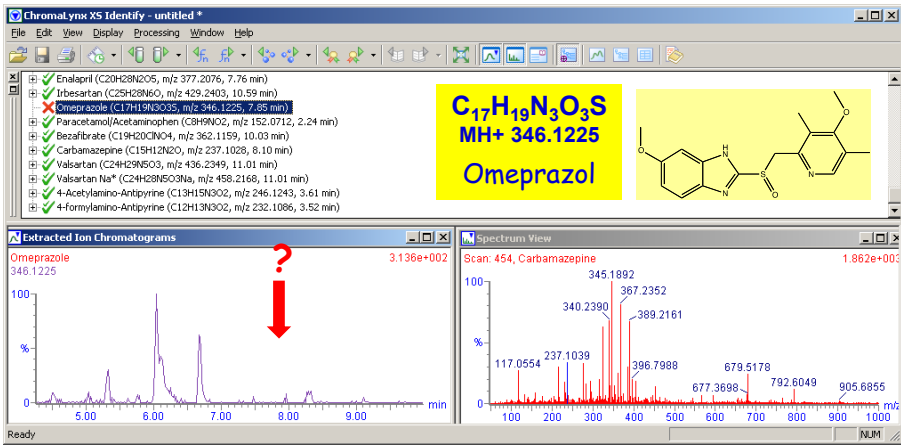




Searching for **non-targeted** metabolites/TPs



➤ **Screening (no positives?) → laboratory degradation/metabolism experiments**




**Degradation/Metabolism studies of Omeprazol: Identification of TPs and metabolites**

**Surface water spiked 500 ng/mL**

- ✓ CHLORINATION (NaOCI)
- ✓ PHOTOLYSIS (Suntest)
- ✓ HYDROLYSIS (darkness)

3 healthy volunteers → dosis 40 mg omeprazol



**Urine sampling**

- Control (before adm)
- 15 min
- 1 h
- 3.5 h
- 6.5 h
- 9 h
- 15 h
- 24 h

Sampling aliquots (defined time intervals)

**Analysis**  
UPLC-ESI-(Q)TOF MS

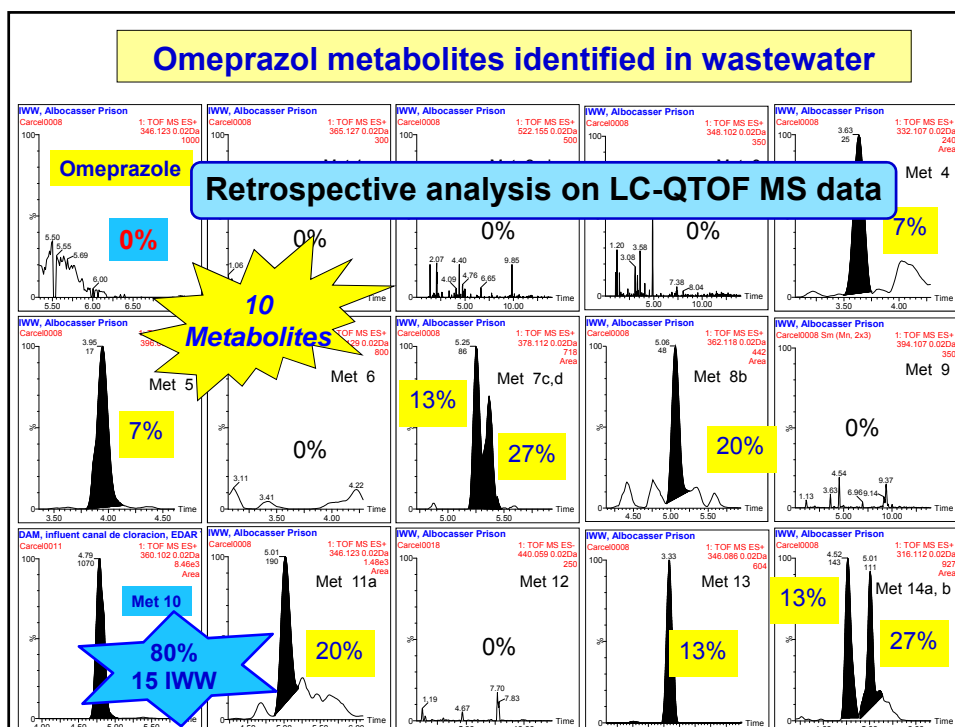
→ MSE


Low energy (LE)  
High energy (HE)

Additional injection in QTOF MS/MS may be required


↳

**Data Processing:**  
MetaboLynx → XICs (control & sample)





### Degradation experiments for cocaine and its major metabolite benzoylecgonine

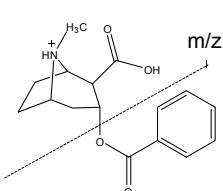


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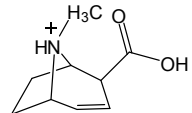
**Photo-degradation laboratory experiments**

- Searching for TPs (common fragments)

Benzoylcocaine. m/z 290.1392

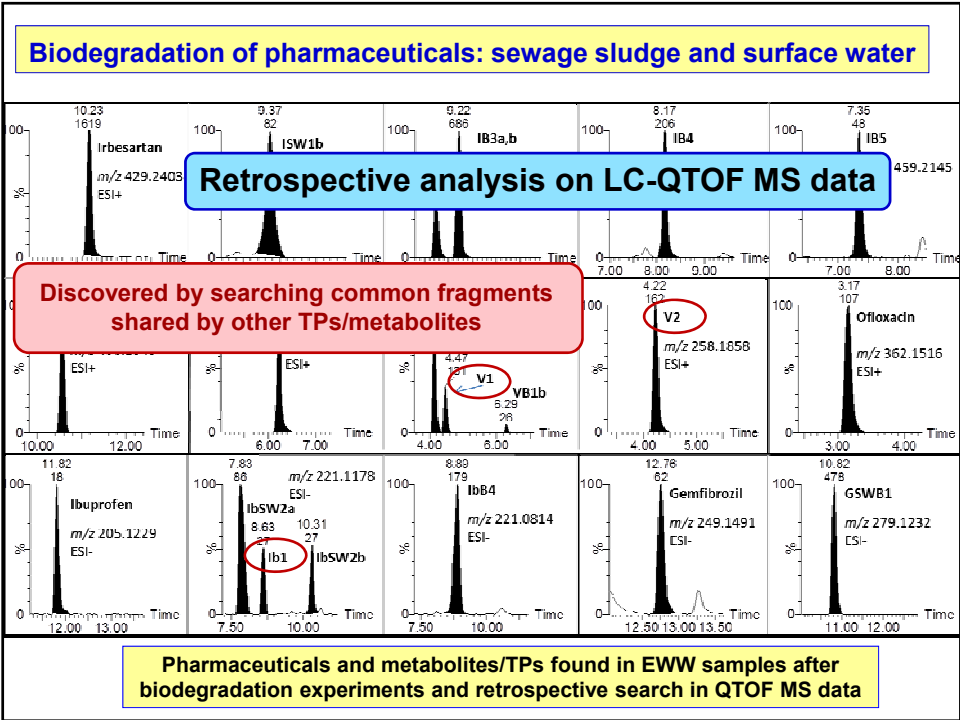
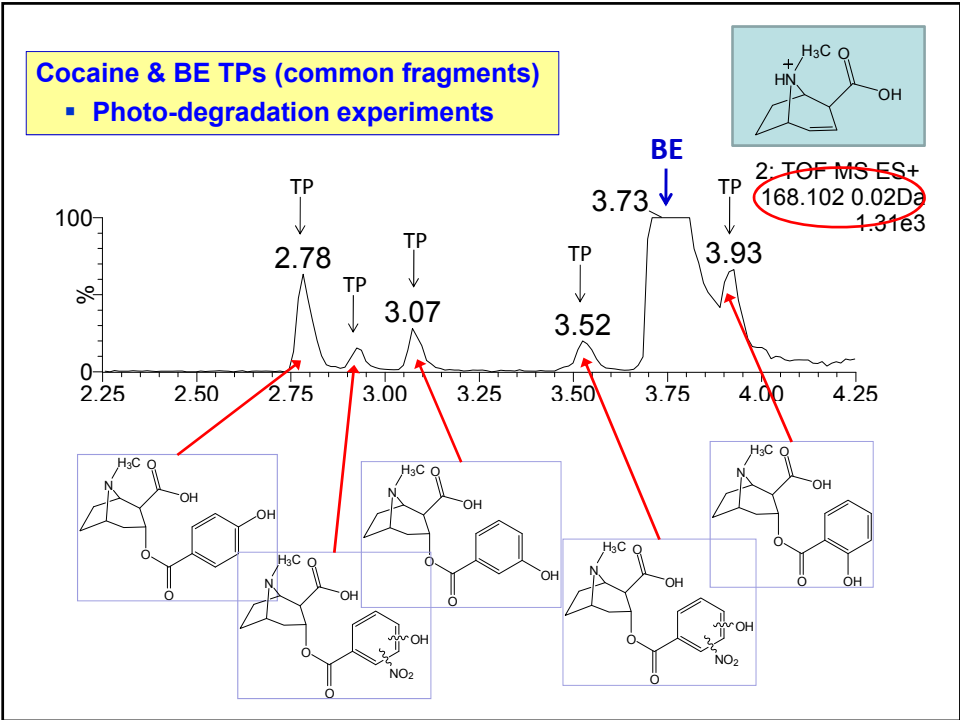



m/z 168.1025




**Fragment m/z 168.1025 characteristic for various TPs of BE**







Searching for **non-targeted** metabolites/TPs



➤ **Software for predicting metabolites**

**Knowledge-Based Systems (“expert systems”)**

- ✓ MetabolExpert
- ✓ META
- ✓ Meteor
- ✓ MetaDrug
- ✓ TIMES (Tissue Metabolism Simulator)
- ✓ SyGMa (Systematic Generation of potential Metabolites)
- ✓ **UM-PPS (University of Minnesota Pathway Prediction System)**
- ✓ **EAWAG-BBD/PPS**
- ✓ JChem Metabolizer

↑

Built on from UM Biocatalysis/Biodegradation Database  
Predicting general environmental metabolism/degradation

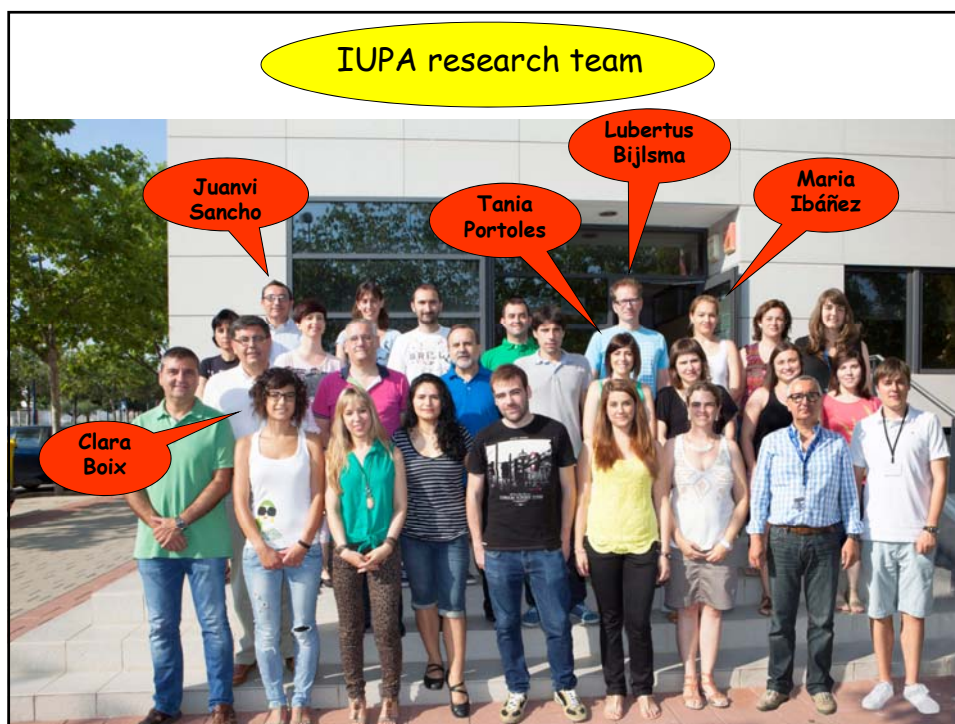
**CONCLUSIONS**

- **Combined use of GC and LC** coupled to **QTOF MS**: powerful approach for **large-scope screening** of organic contaminants in the environment
- Both **GC (APCI)** and **LC (ESI, APCI)** can be efficiently coupled to QTOF MS using a **single instrumental platform**
- **Accurate-mass full-spectrum data** provided by TOFMS allow easily increase the number of compounds searched: **retrospective analysis**
- **Metabolites and TPs** can be discovered in the water using different approaches, as searching for common fragments, in-silico prediction tools or laboratory experiments
- The **relevance of EI source in GC** must not be ignored (GC-EI-Orbitrap)
- Despite advances, still many **“difficult” compounds** (e.g. highly volatile and highly polar/ionic) **require specific methods** with different sample treatment and measurement conditions

## CONCLUSIONS

### Challenges/Trends

- **Improvement in sensitivity** required in environmental applications: new generation instruments?
  - NPS, such as MDPV, mephedrone and methylene were found in IWW of some European cities at low ng/L (by LC-MS/MS QqQ)
  - However, remained undetected by LC-QTOF MS
- Need to built and unify **large databases** of compounds of interest, including metabolites/TPs (empirical formula, exact mass, fragments observed, Rt predicted, etc)
- Need to improve and to widen (**accurate**) **mass spectral libraries**
- **Instrumentation improvements**: ion mobility, NMR, GCxGC, LC-LC, etc





Thank you for your attention!



Questions

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