Network of reference laboratories for monitoring of emerging environmental pollutants

NORMAN workshop

Occurrence, fate and effects of emerging pollutants in the environment – chemical analysis and toxicological assessment

Amsterdam, Nov 2012

Strategies for investigation of emerging contaminants (metabolites and TPs) in waters by LC-QTOF MS

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LARP

Laboratorio Análisis Residuos Plaguicidas

Emerging contaminants

- **Huge use** of synthetic organic chemicals in agriculture, public health, food, industry → **thousands potential contaminants**
- Some (priority) compounds are **regulated** in environmental samples
- Present concern on **emerging contaminants**, compounds which presence remains **unregulated** as they are not considered "classical" contaminants (e.g pharmaceuticals, PCPs, drugs abuse, most **metabolites and TPs**)

⇩

Efficient analytical **methods for screening** in order to detect and identify many emerging contaminants

⇩

Especial attention to **metabolites/TPs**, as many of them are unknown and there is still a lack of reference standards

Investigation of emerging contaminants & metabolites/TPs in waters by LC-QTOF MS

Why hybrid QTOF?

HR MS technique (accurate-mass full-spectrum acquisition)

⇩

Powerful technique for screening, identification/confirmation (and elucidation of unknowns)

⇩ ⇩

Accurate-mass product ion spectra in MS/MS experiments

⇩

Highly valuable for elucidation and confirmation purposes

MS^E acquisition (simultaneous LE & HE spectra; CID in collision cell)

⇩

Information on parent molecule (LE) and fragment ions (HE)

Investigation of emerging contaminants & metabolites/TPs in waters by LC-QTOF MS

Standards available

⇩

Information in database (exact mass, fragment ions, retention time, isotope pattern)

⇩

Information obtained
- **Mass accuracy (mass error)**
- **Isotope pattern (i-fit)**
- **Retention time**
- **Fragment ions, at HE acquisition**

Unequivocal identification at least two accurate-mass ions

(post)TARGET ANALYSIS

Analytes selection after MS acquisition

Long list of target compounds

Investigation of emerging contaminants & metabolites/TPs in waters by LC-QTOF MS

(post)TARGET ANALYSIS

Standards not available

⇩

Information in database
exact mass, theoretical isotope pattern, predicted Rt

⇩

Laborious and time-consuming task

Information obtained
- **Mass accuracy (mass error)**
- **Isotope pattern (i-fit)**
- **Fragment ions observed**
- **Justification fragments (mass fragmenter)**

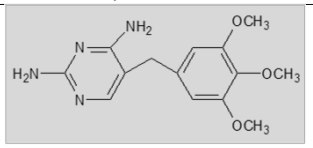
Confirmation with standards (later stage)

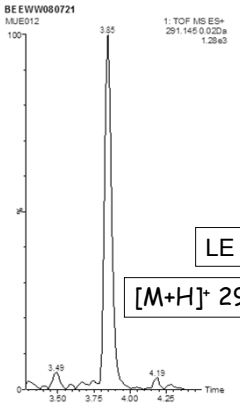
Tentative identification (highly reliable)

Contaminants Screening by Time-of-Flight Mass Spectrometry

- **Screening and identification (standards available)**

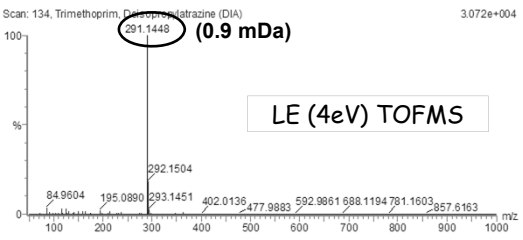
Trimethoprim (antibiotic)





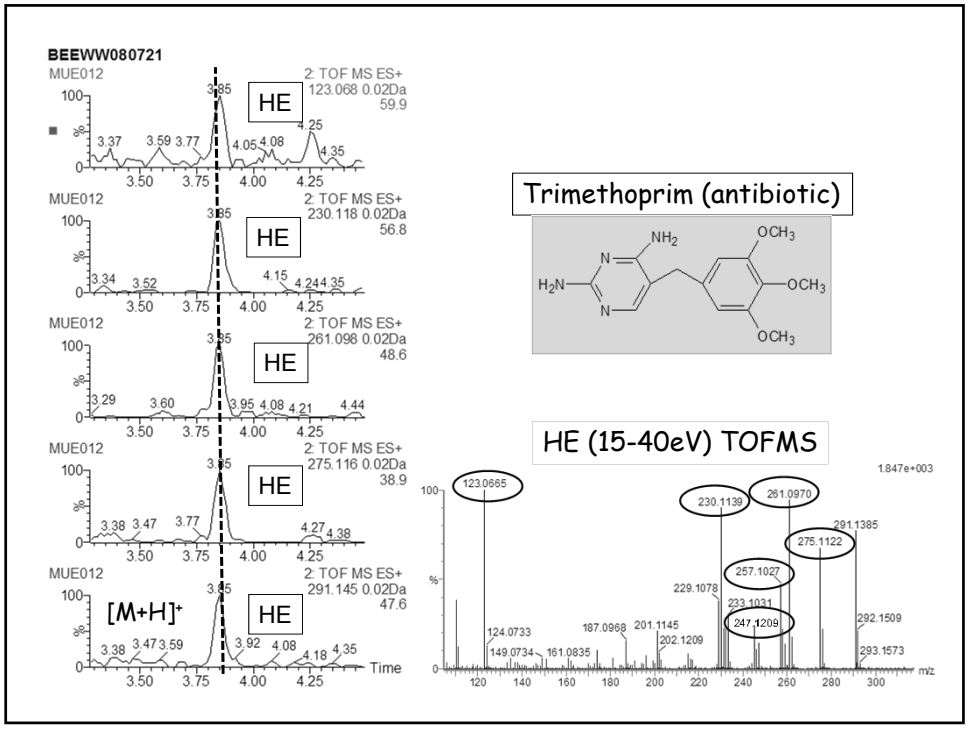
LE

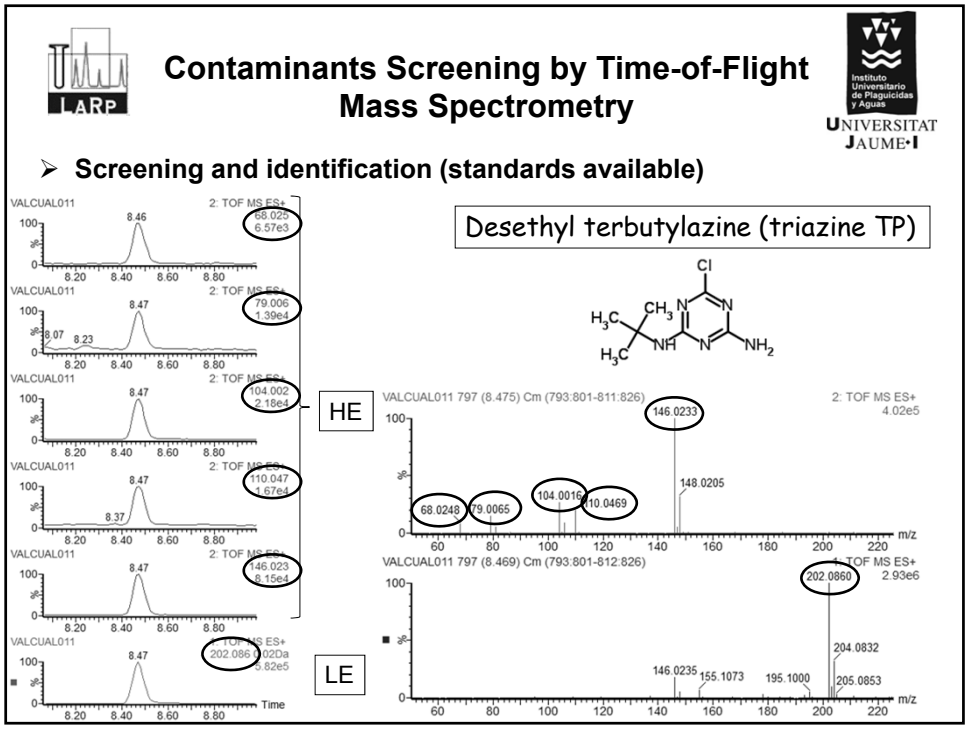
$[M+H]^+$ 291.1457



LE (4eV) TOFMS

ΔRt (sample-standard)= 0.07 min


BEEWW080721
MUE012
2: TOF MS ES+
123.068 0.02Da
59.9
HE
230.118 0.02Da
56.8
261.098 0.02Da
48.6
275.116 0.02Da
38.9
291.145 0.02Da
47.6
[M+H]+
Time
Trimethoprim (antibiotic)
HE (15-40eV) TOFMS
123.0665
230.1139
261.0970
291.1385
275.1122
257.1027
229.1078
233.1031
247.1209
292.1509
293.1573
124.0733
149.0734
161.0835
187.0968
201.1145
202.1209
m/z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Contaminants Screening by Time-of-Flight Mass Spectrometry
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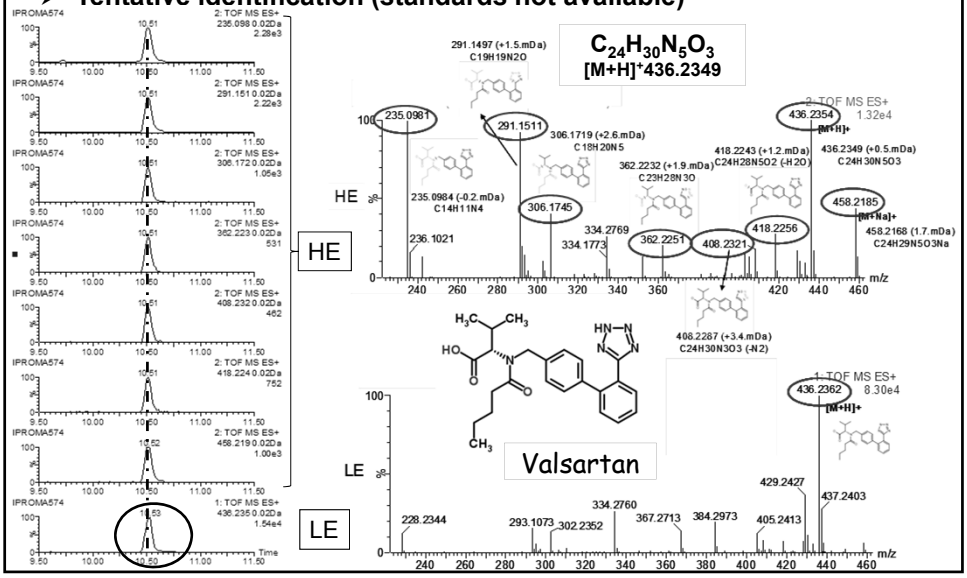
Screening and identification (standards available)
VALCUAL011
2: TOF MS ES+
68.025
6.57e3
79.006
1.39e4
104.002
2.16e4
110.047
1.67e4
146.023
8.15e4
202.086
5.82e5
Time
Desethyl terbutylazine (triazine TP)
HE
LE
VALCUAL011 797 (8.475) Cm (793:801-811:826)
4.02e5
146.0233
148.0205
68.0248
79.0065
104.0016
110.0469
m/z
VALCUAL011 797 (8.469) Cm (793:801-812:826)
2.93e6
202.0860
204.0832
146.0235
155.1073
195.1000
205.0853

Contaminants Screening by Time-of-Flight Mass Spectrometry

- **Tentative identification (standards not available)**



Investigation of emerging contaminants & metabolites/TPs in waters by LC-QTOF MS

RETROSPECTIVE ANALYSIS

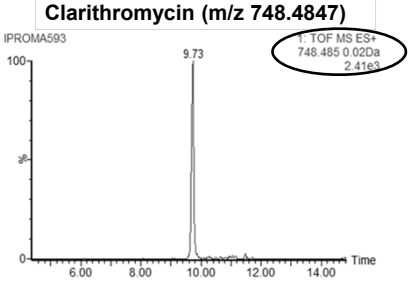
⇩

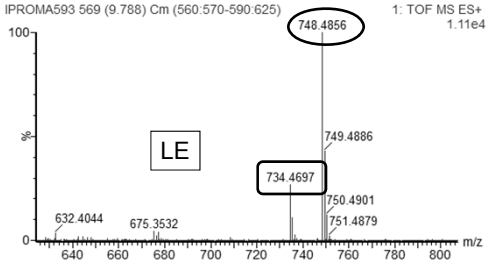
Data re-evaluation to investigate any compound at any time without additional sample analysis

Retrospective investigation of pharmaceuticals´ metabolites in surface waters and wastewaters

➢ Retrospective analysis of pharmaceutical metabolites

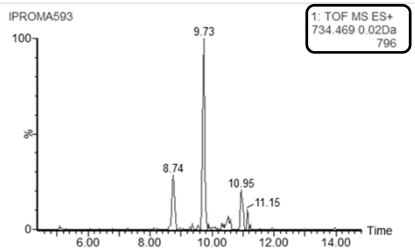
Clarithromycin (m/z 748.4847)

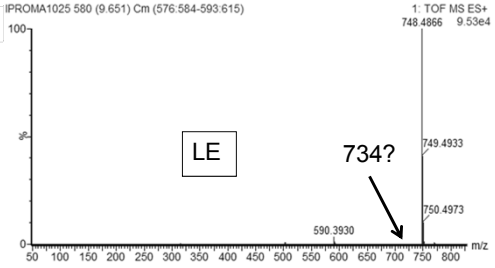




Effluent wastewater sample

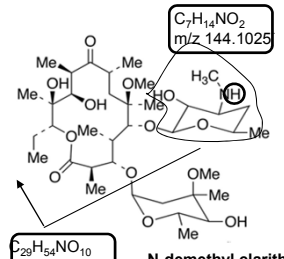
N-demethyl clarithromycin (m/z 734.4691)



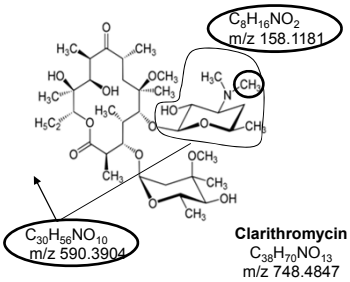


Clarithromycin reference standard

➢ Retrospective analysis of pharmaceutical metabolites

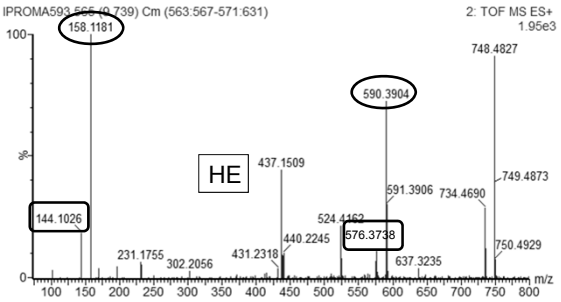


N-demethyl clarithromycin
$C_{37}H_{68}NO_{13}$
m/z 734.4691

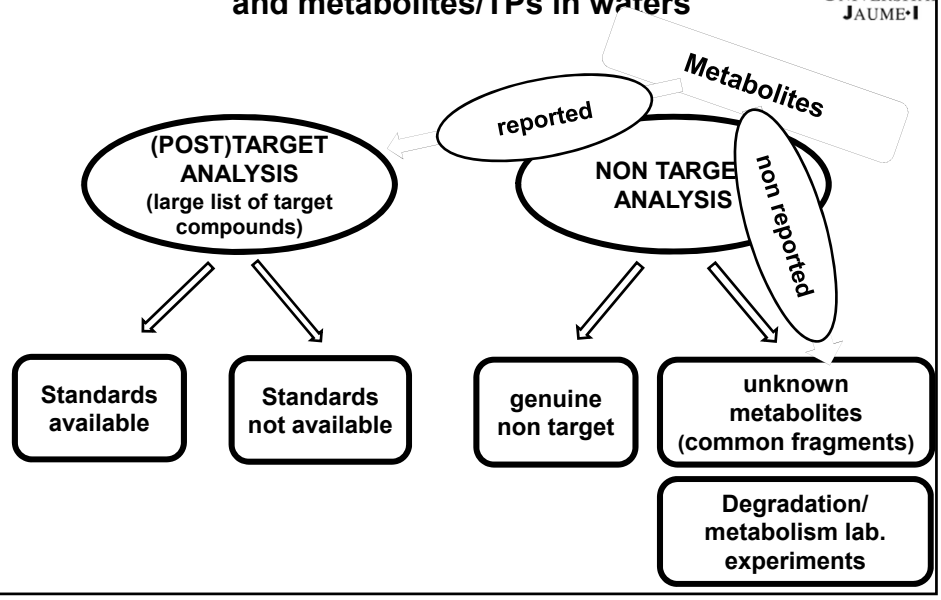


Clarithromycin
$C_{38}H_{70}NO_{13}$
m/z 748.4847

Other (reported) metabolites detected:
Clopidogrel carboxylic acid, fenofibric acid,……



Potential of (UHP)LC-QTOF MS for investigation of emerging contaminants and metabolites/TPs in waters

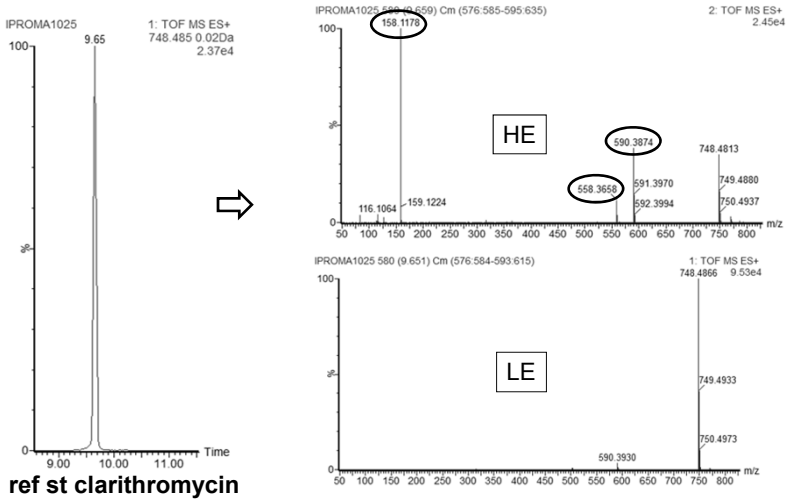


Contaminants Screening by Time-of-Flight Mass Spectrometry

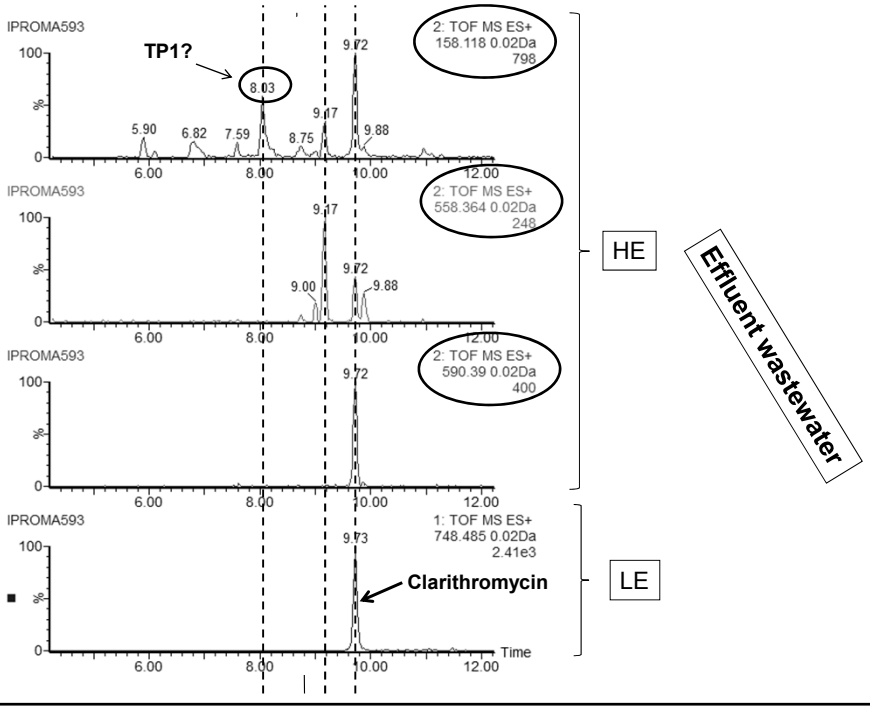
- **Genuine non target analysis**
  - ✓ No selection of analytes
  - ✓ Searching for any sample component that might be "relevant"
  - ✓ Analytical challenge (little success in the environmental field)
    - Complex unknown sample matrices
    - Low analyte concentrations
    - Many peaks in the TIC (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

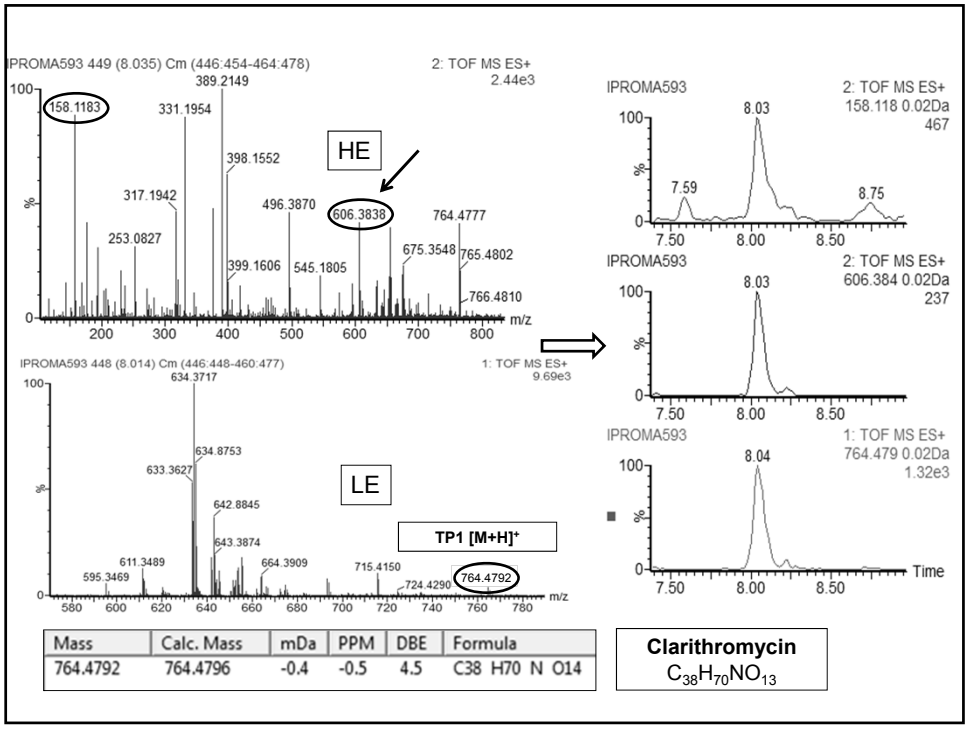
Contaminants Screening by Time-of-Flight Mass Spectrometry

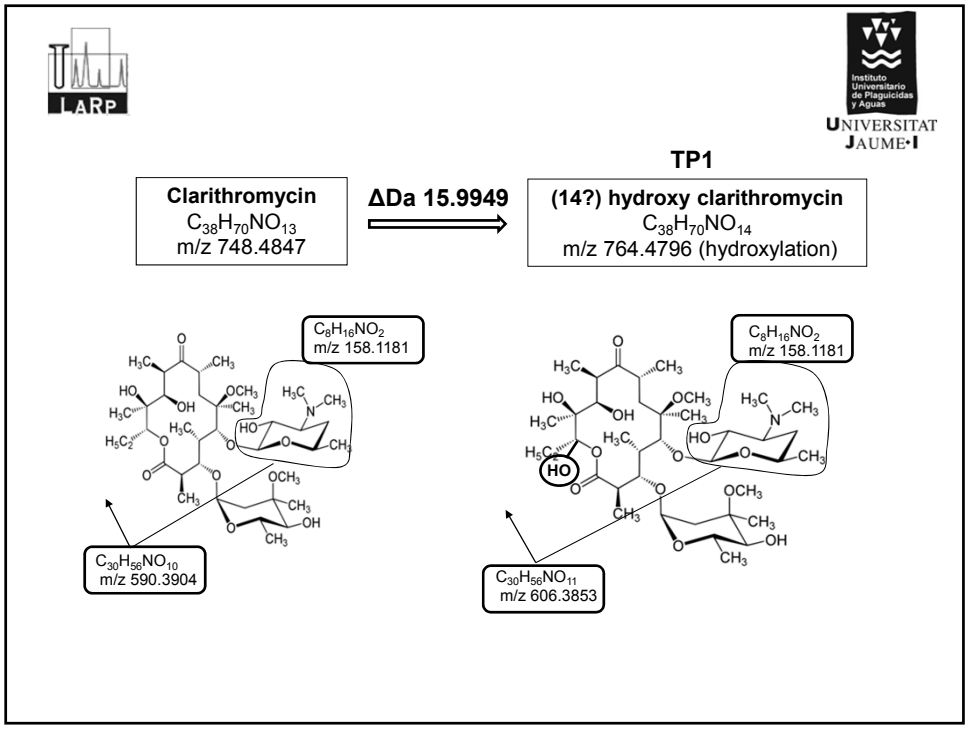
- **Searching for metabolites (common fragments)**



ref st clarithromycin




IPROMA593 449 (8.035) Cm (446:454-464:478)
2: TOF MS ES+
2.44e3
HE
IPROMA593 448 (8.014) Cm (446:448-460:477)
1: TOF MS ES+
9.69e3
LE
TP1 [M+H]+
IPROMA593
2: TOF MS ES+
158.118 0.02Da
467
IPROMA593
2: TOF MS ES+
606.384 0.02Da
237
IPROMA593
1: TOF MS ES+
764.479 0.02Da
1.32e3
Time
Mass | Calc. Mass | mDa | PPM | DBE | Formula
764.4792 | 764.4796 | -0.4 | -0.5 | 4.5 | C38 H70 N O14
Clarithromycin
C38H70NO13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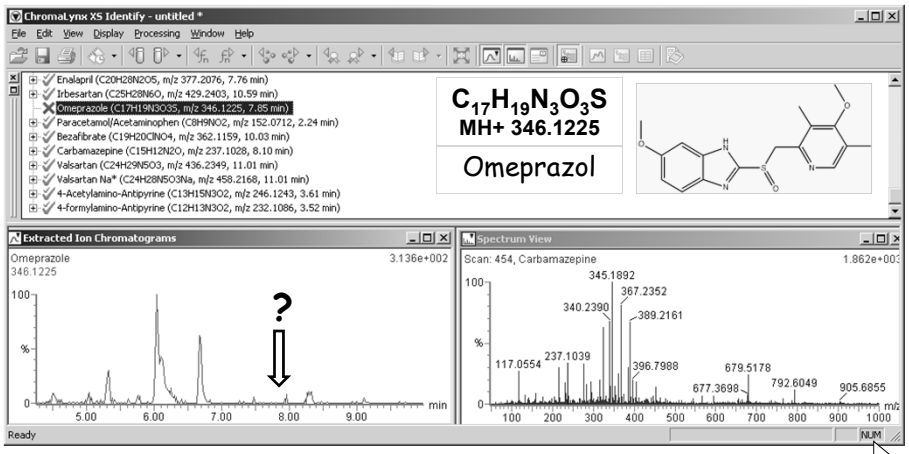
TP1
Clarithromycin
C38H70NO13
m/z 748.4847
ΔDa 15.9949
(14?) hydroxy clarithromycin
C38H70NO14
m/z 764.4796 (hydroxylation)
C8H16NO2
m/z 158.1181
C30H56NO10
m/z 590.3904
C8H16NO2
m/z 158.1181
C30H56NO11
m/z 606.3853

Contaminants Screening by Time-of-Flight Mass Spectrometry

- **Screening (no positives?) → laboratory degradation experiments**



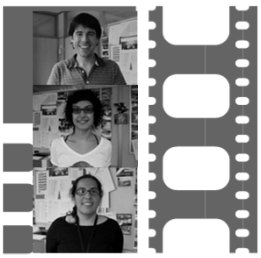
Degradation?

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

Surface water spiked 500 ng/mL

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

3 healthy volunteers → dosis 40 mg omeprazol

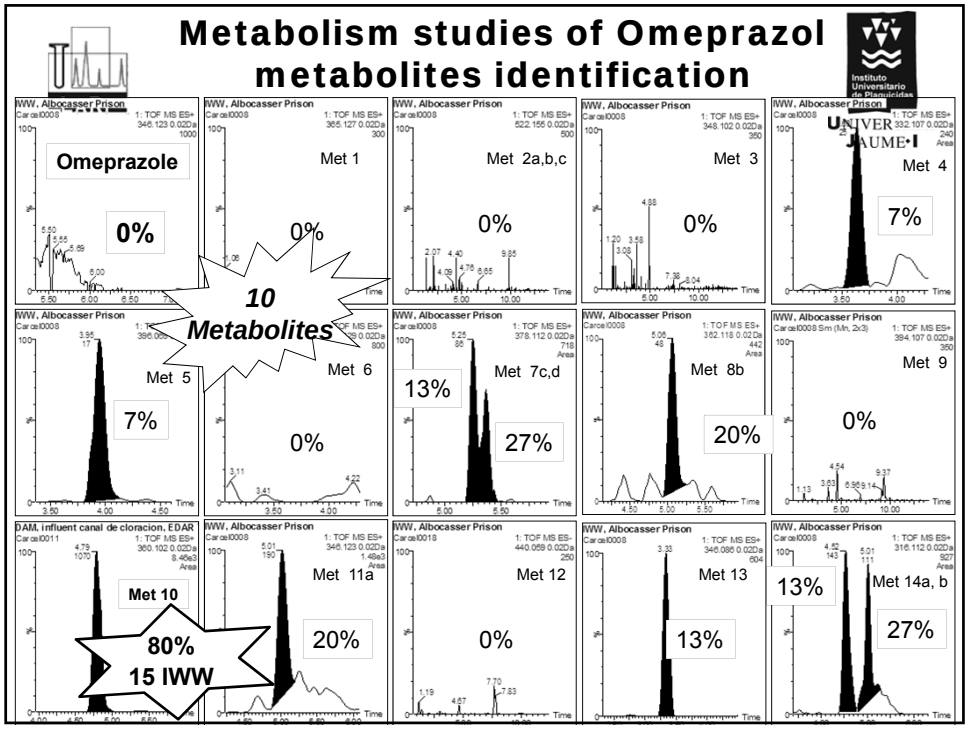


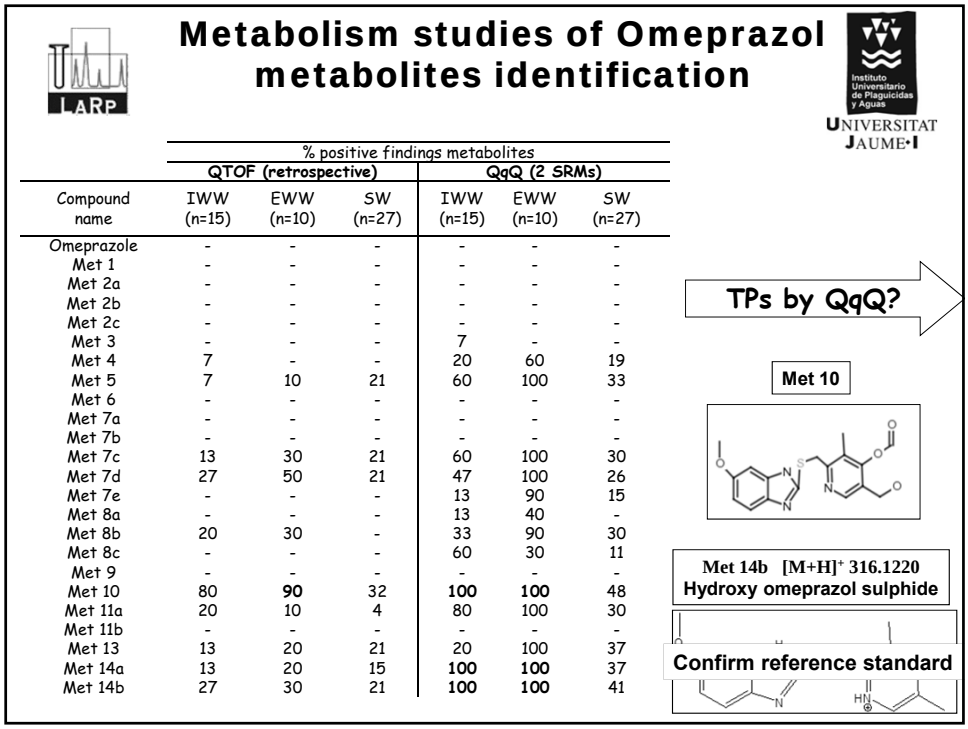
Urine sampling
- **Control (before adm)**
- **15 min**
- **1 h**
- **3.5 h**
- **6.5 h**
- **9 h**
- **15 h**
- **24 h**

Sampling aliquots (defined time intervals)

Analysis
UPLC-ESI-(Q)TOF MS ⇒ **MS^E** → Low energy (LE); High energy (HE) fragments

Data Processing:
MetaboLynx ⇒ XICs (control & sample)





	% positive findings metabolites					
	QTOF (retrospective)			QqQ (2 SRMs)		
Compound name	IWW (n=15)	EWW (n=10)	SW (n=27)	IWW (n=15)	EWW (n=10)	SW (n=27)
Omeprazole	-	-	-	-	-	-
Met 1	-	-	-	-	-	-
Met 2a	-	-	-	-	-	-
Met 2b	-	-	-	-	-	-
Met 2c	-	-	-	-	-	-
Met 3	-	-	-	7	-	-
Met 4	7	-	-	20	60	19
Met 5	7	10	21	60	100	33
Met 6	-	-	-	-	-	-
Met 7a	-	-	-	-	-	-
Met 7b	-	-	-	-	-	-
Met 7c	13	30	21	60	100	30
Met 7d	27	50	21	47	100	26
Met 7e	-	-	-	13	90	15
Met 8a	-	-	-	13	40	-
Met 8b	20	30	-	33	90	30
Met 8c	-	-	-	60	30	11
Met 9	-	-	-	-	-	-
Met 10	80	**90**	32	**100**	**100**	48
Met 11a	20	10	4	80	100	30
Met 11b	-	-	-	-	-	-
Met 13	13	20	21	20	100	37
Met 14a	13	20	15	**100**	**100**	37
Met 14b	27	30	21	**100**	**100**	41

TPs by QqQ?

Met 10

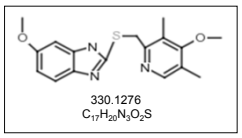
Met 14b [M+H]⁺ 316.1220
Hydroxy omeprazol sulphide

Confirm reference standard

Metabolism studies of Omeprazol TPs identification

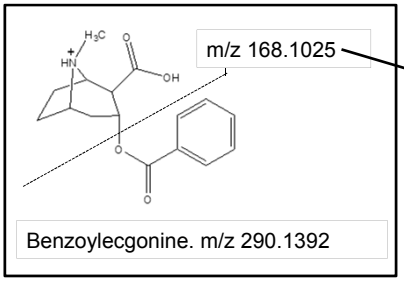
Compound name	% positive findings TPs					
	QTOF (retrospective)			QqQ (2 SRMs)		
	IWW (n=15)	EWW (n=10)	SW (n=27)	IWW (n=15)	EWW (n=10)	SW (n=27)
Omeprazole	-	-	-	-	-	-
TP 1	-	-	-	-	-	11
TP 2	-	-	-	-	-	-
TP 3	-	-	-	-	-	-
TP 4	-	-	-	-	10	7
TP 5	-	-	-	-	**90**	26
TP 6	-	-	-	-	-	-
TP 7-10	-	-	-	-	-	-
TP 11-12	-	-	-	-	-	-
TP 13	-	-	-	7	50	11
TP 14	-	-	-	-	-	-
TP 15-16	-	-	-	-	-	-
TP 17	-	-	-	-	-	-

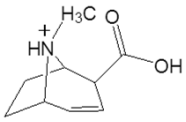
TP 5



Contaminants Screening by Time-of-Flight Mass Spectrometry

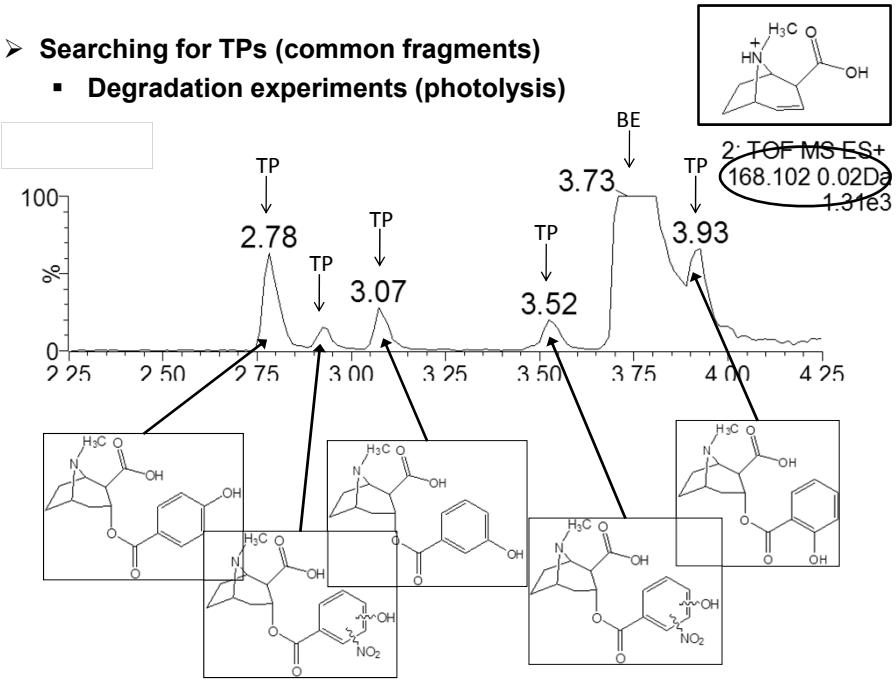
- **Searching for TPs (using common fragments)**





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

- **Searching for TPs (common fragments)**
  - **Degradation experiments (photolysis)**



CONCLUSIONS

- **LC-QTOFMS** is a powerful analytical tool to investigate emerging contaminants and metabolites/TPs in the aquatic environment:
  - large-scope (post) target screening of organic contaminants including manyTPs (around 1,000 organic contaminants are included at present in our laboratory → database contains around 3,000 entries)
  - Simultaneous acquisition of LE and HE spectra → valuable information on parent molecule and fragments → highly reliable identification
  - Can be efficiently made without standards → tentative identification → standards required in the final step of confirmation
  - Retrospective analysis, at any time
  - Combination of laboratory degradation experiments and QTOF MS measurements is highly valuable for investigation of TPs
  - Genuine non target analysis still has many limitations for elucidating true unknowns

Thank you for your time

Acknowledgements

- **IUPA LC-QTOF team**
  - **Juanvi Sancho (leader)**
  - **María Ibáñez**
  - **Ramón Díaz**
  - **Lubertus Bijlsma**
  - **Emma Gracia**
  - **Clara Boix**
- **Waters**
  Xevo-QTOF MS instrument
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