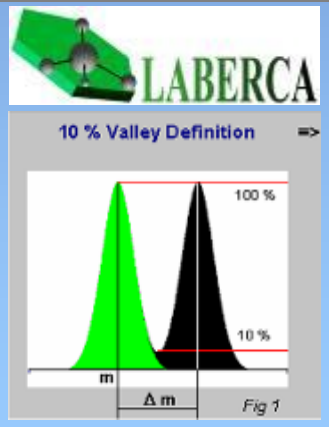
NORMAN's Workshop, Nov. 18-19th 2009, Amsterdam NL

HIGHLIGHTING AND IDENTIFICATION TECHNIQUE OF CHLORINATION BY-PRODUCTS OF ETHINYLESTRADIOL IN DRINKING WATER TREATMENT BY UNTARGETED PROFILING METHOD BY LC-HR(MS)^n

Gaël Gervais[a]; Emmanuelle Bichon[a]; Jean-Philippe Antignac[a]; Fabrice Monteau[a]; Valérie Ingrand[b]; Gaela Leroy[b], Laurianne Barritaud[b], Mathilde Chachignon[b], Pascal Roche[c], Luis Castillo[c] & Bruno LeBizec[a]



Laboratoire d'Etude des Résidus et Contaminants dans les Aliments
Ecole Nationale Vétérinaire de Nantes - France - www.laberca.org

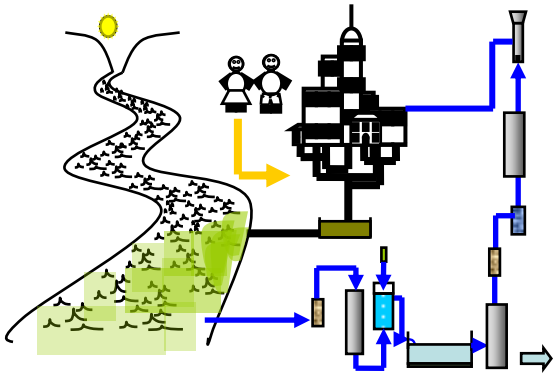
Contents

Introduction

Methodology

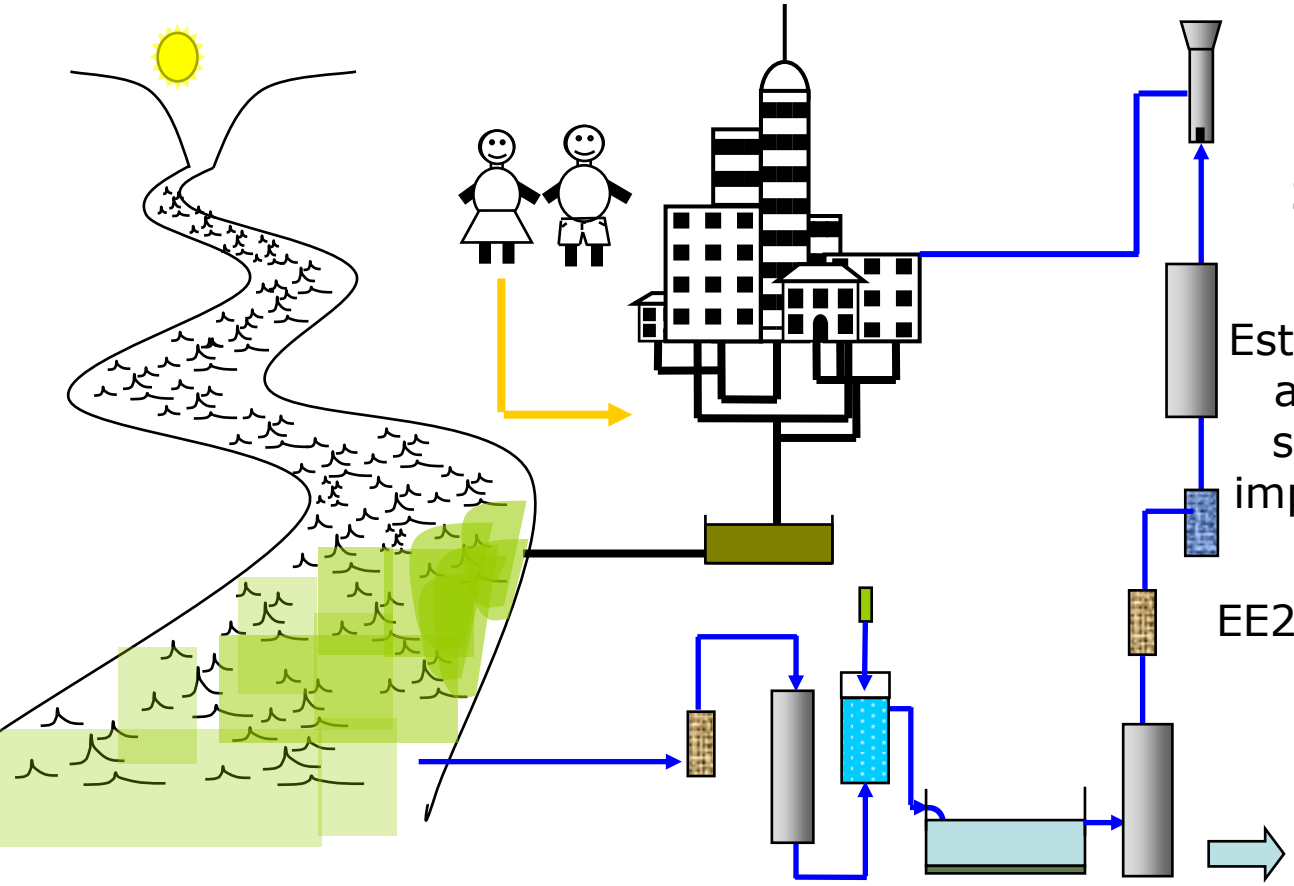
Results

Conclusion



Introduction

Endocrine Disruptors Compounds (EDCs) exposure is a major issue for Toxicology / Ecotoxicology / Food Safety Sciences



Particularly, EDC's in water

Steroid Hormones are very active EDC's

Estrogens (E1, EE2, E3& **EE2**) are commonly detected in surface waters and clearly implicated in fish feminization

EE2 disappears during Drinking water production

By-products (BPs) formation?

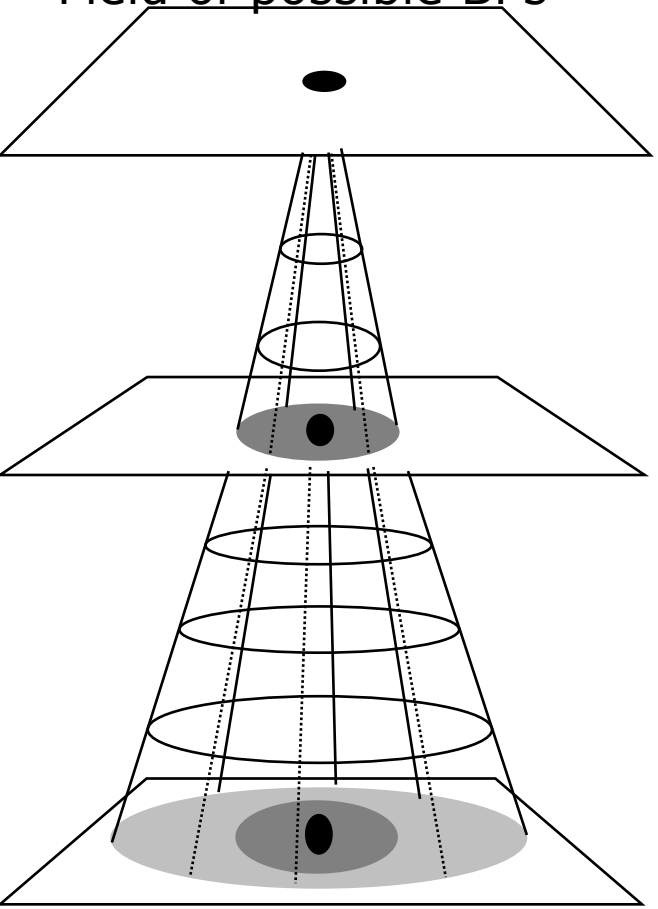
Toxicology

Treatments monitoring

Introduction:

By-products Information

Field of possible BPs



Experiments

Water + EE2 + NaOCl

↓

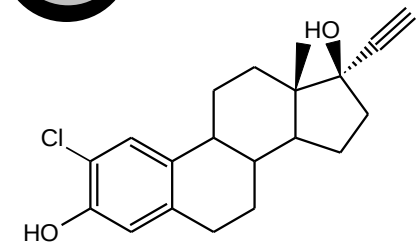
Water + EE2 + NaOCl + Salts

↓

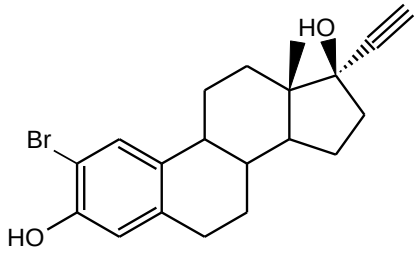
Real conditions:

Water + EE2 + NaOCl + Salts + NOM + Humic acids + Fulvic aids+ Colloids…

Targets



4-Cl-EE2; 2,4-Cl_2-EE2

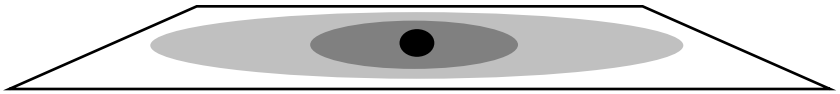


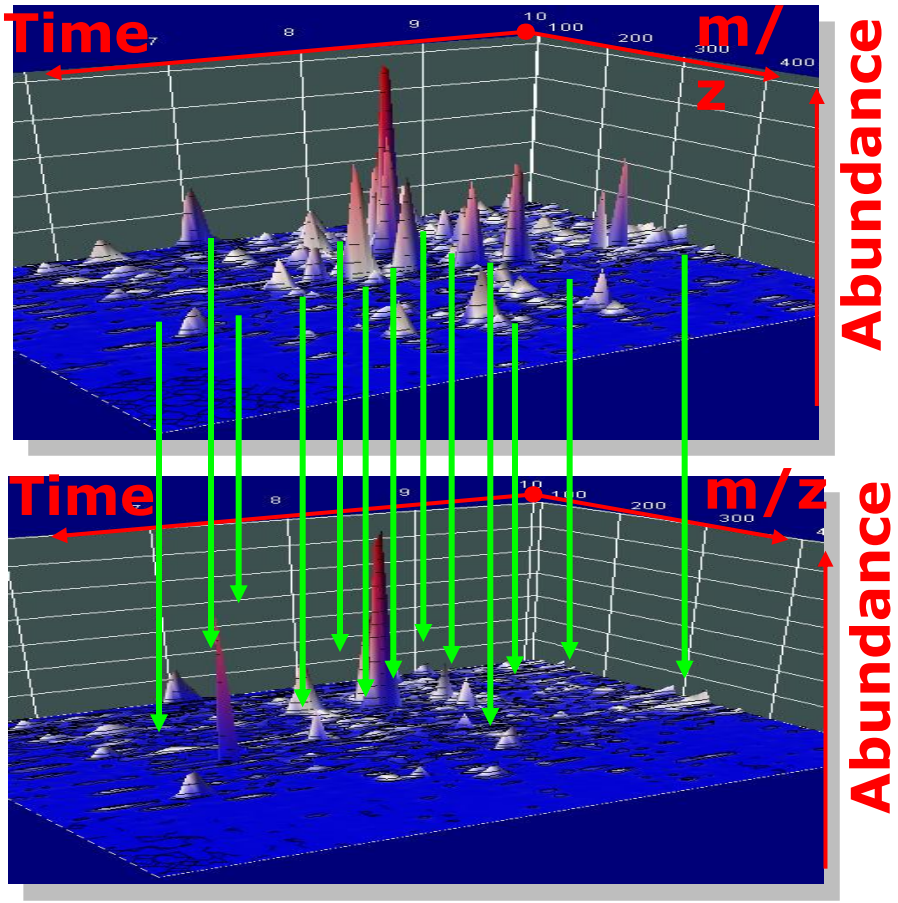
4-Br-EE2; 2,4-Br_2-EE2; 2-Br-4-Cl-EE2…

?

Exhaustiveness! Of BPs

Introduction:





Sampling

Classical treatment applied to EE2-spiked real water

Extraction:

Non selective extraction by SPE for polar & apolar compounds

Liquid chromatography:

Non selective chromatographic gradient from 0% to 100% Organic

High Resolution Mass spectrometry:

Use of Full Scan mode

Exhaustiveness of m/z

- **Comparison of groups of samples (Treated Vs. Untreated)**
- **Computational tool for data processing "fingerprints"**

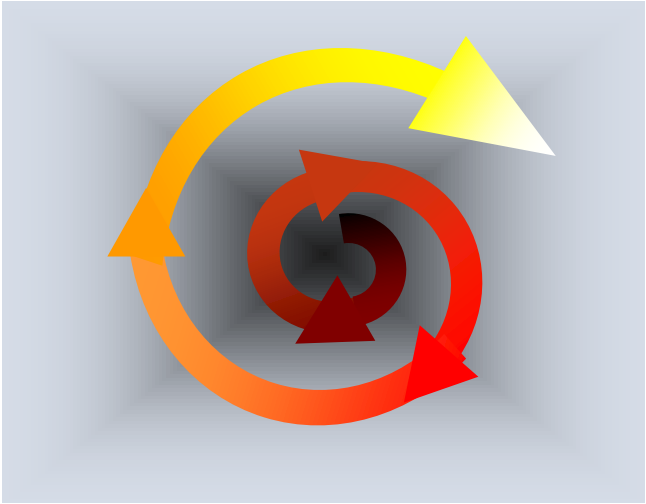
Contents

Introduction

Methodology

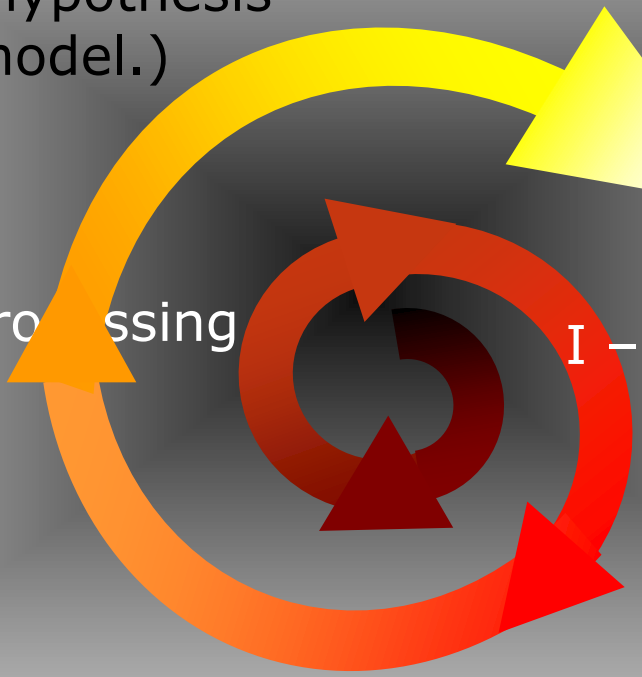
Results

Conclusion



Methodology for BPs identification

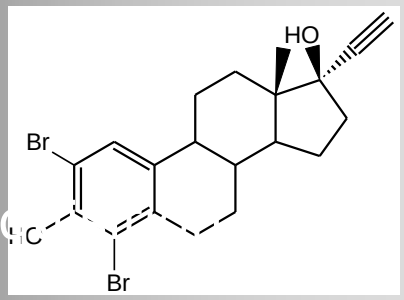
V – Structural hypothesis (lit./frag./model.)



II – Data pro ssing

I – Data ac

III



measurement

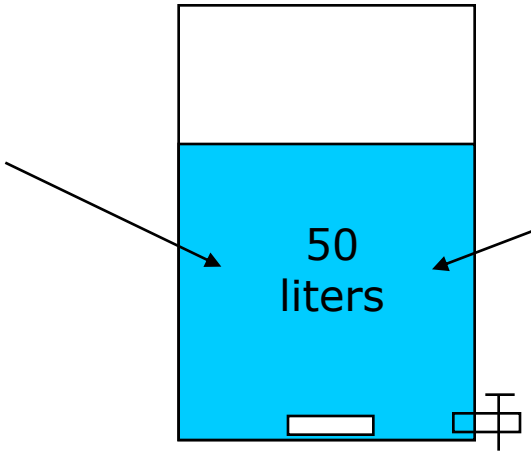
IV – raw formula determination

"From shadow to light"

Methodology: sampling

Real water from DWTP

free from EE2



Spiking:

5 mg/L in Ultra Pure Water

Chlorination:

NaOCl

Gr.1 "treated":

EE2-spiked (5 ppb) chlorinated (0.8 mg/L)
3 contact times:

- 10 min.
- 30 min.
- 120 min.

Gr.2 "witnesses":

EE2-spiked & not-chlorinated

EE2-unspiked & chlorinated

EE2-unspiked & not-chlorinated

Ultra pure water

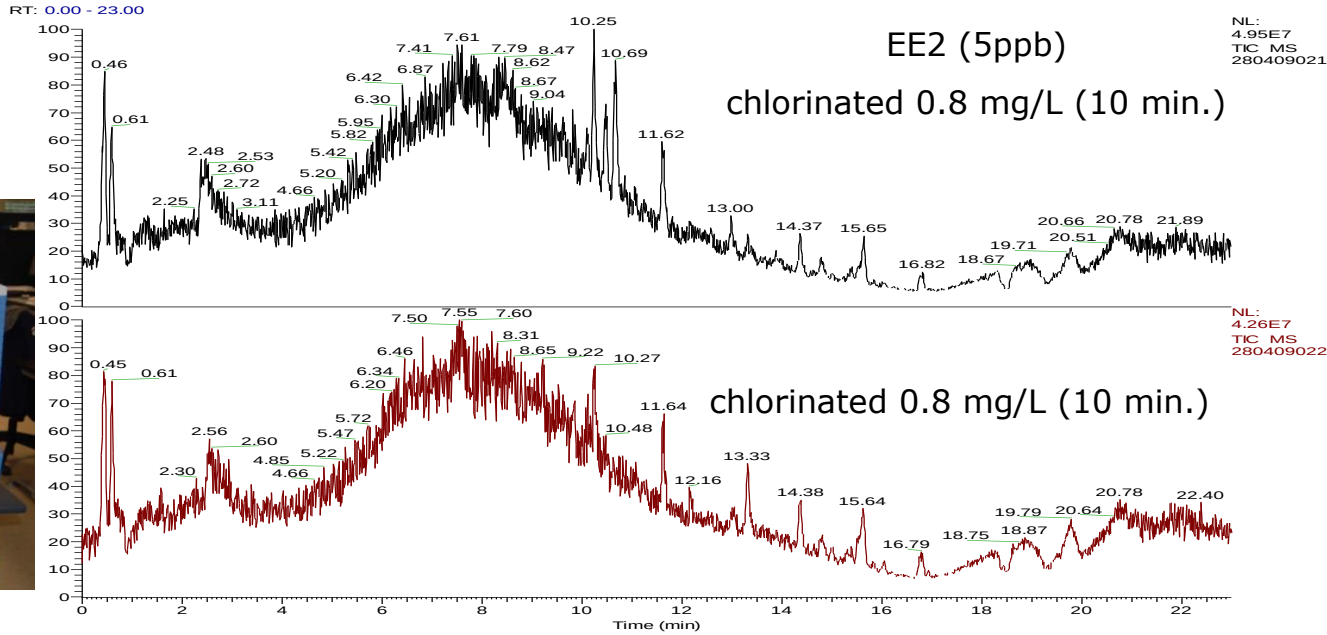
Each sample extracted twice

Each extract injected twice

=12 fingerprints

=16 fingerprints

Methodology: I-Data acquisition



Data acquisition of samples:

Extraction: SPE on Oasis HLB cartridge (500 mL -> 100 µL)

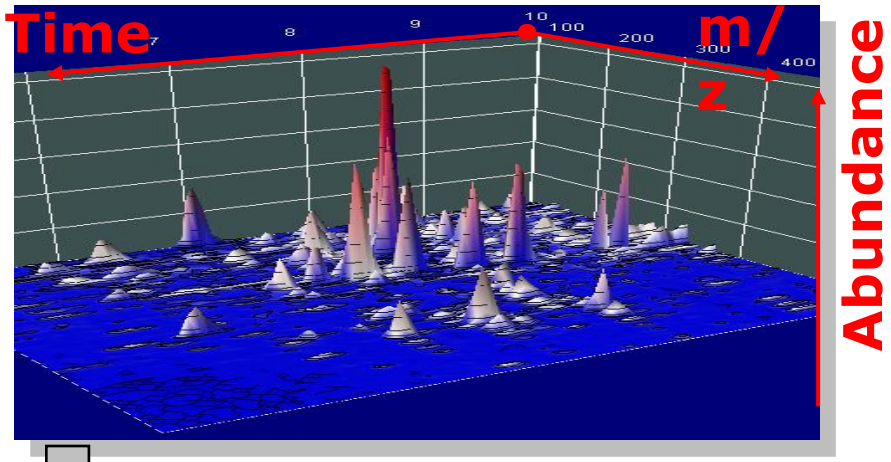
HPLC: C18 50*2.1 mm (3µm)

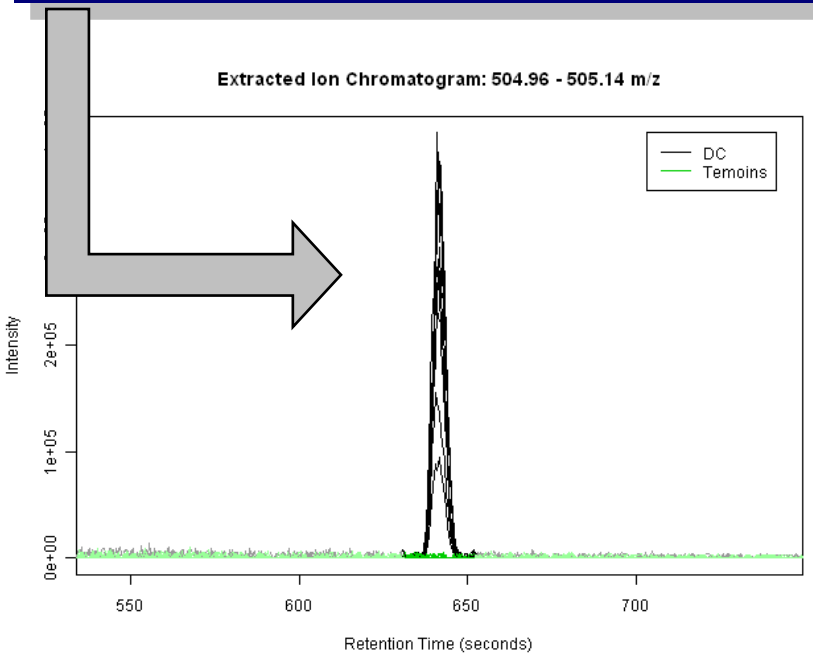
UPW + 0.1% Acet. Acid / Acetonitrile

(HR)MS: LTQ-Orbitrap in ESI-

Full scan mode 70-800 Da

Methodology: II-Data processing





XCMS software:

Compares fingerprints of samples (m/z; R_t & abundance) inside the group of samples and between groups.

Ions statistically different between groups are listed by order of relevance (p-value)

The software creates Extracted Ion Chromatograms (EIC) for each relevant ion

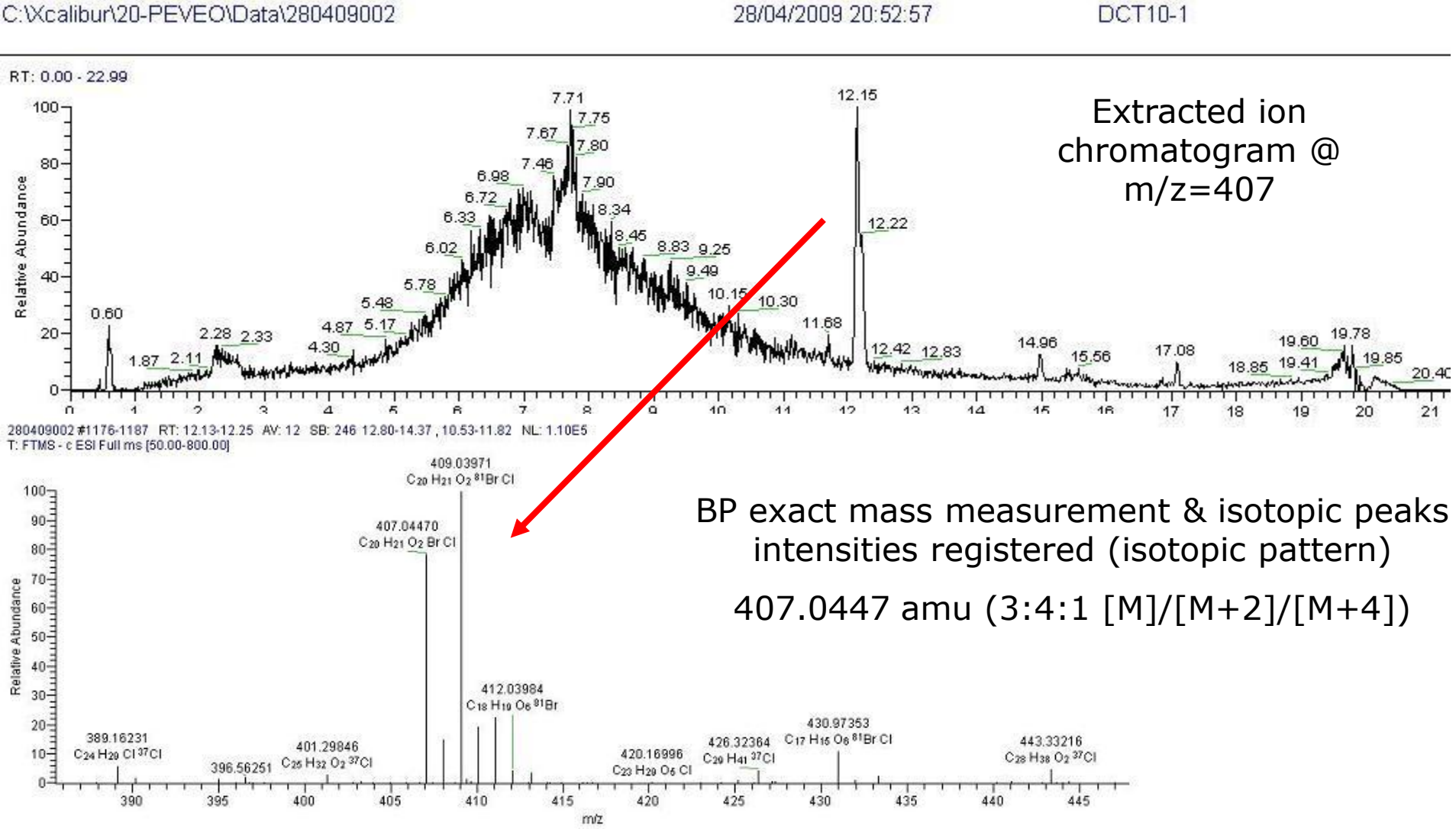
Visual confirmation of ions relevance

List of ions that are different (over- or under-expressed) between "treated" and "untreated" samples

Methodology:

III-Exact mass measurement & isotopic pattern

M407T730 : m/z = 407 & R_t = 730 sec (12.15min) in EE2-spiked chlorinated samples



Extracted ion chromatogram @ m/z=407

BP exact mass measurement & isotopic peaks intensities registered (isotopic pattern)

407.0447 amu (3:4:1 [M]/[M+2]/[M+4])

Methodology: IV-Raw formula selection

407.0447 amu (3:4:1 [M]/[M+2]/[M+4])

Rt (min.)	observed m/z	raw formula	theoretical m/z	delta(mmu)	R	RDB eq.
12.15	407.0447	C20H22O2ClBr	407.0419	2.8	0.9994	9
		C21H26OBrCl	407.0784	-33.7	0.9993	8
		C18H24O4Cl3	407.0892	-44.5	0.9788	6
		C20H19BrCl2	406.9973	47.4	0.9449	10
		C19H22O3Cl3	407.0954	-50.7	0.9792	5
		C18H14O4ClBr	406.9689	75.8	0.9992	11
		C18H18OCBr2	406.9650	79.7	0.8951	9

Criteria of selection:

Limited number of elements: C, N,O,H, Br,Cl, P, S,

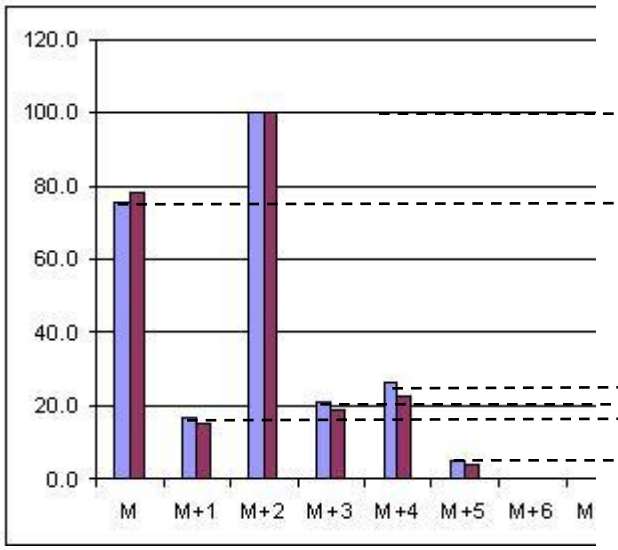
mass precision: Δmmu<5 mmu

Isotopic pattern: R^2 highest possible

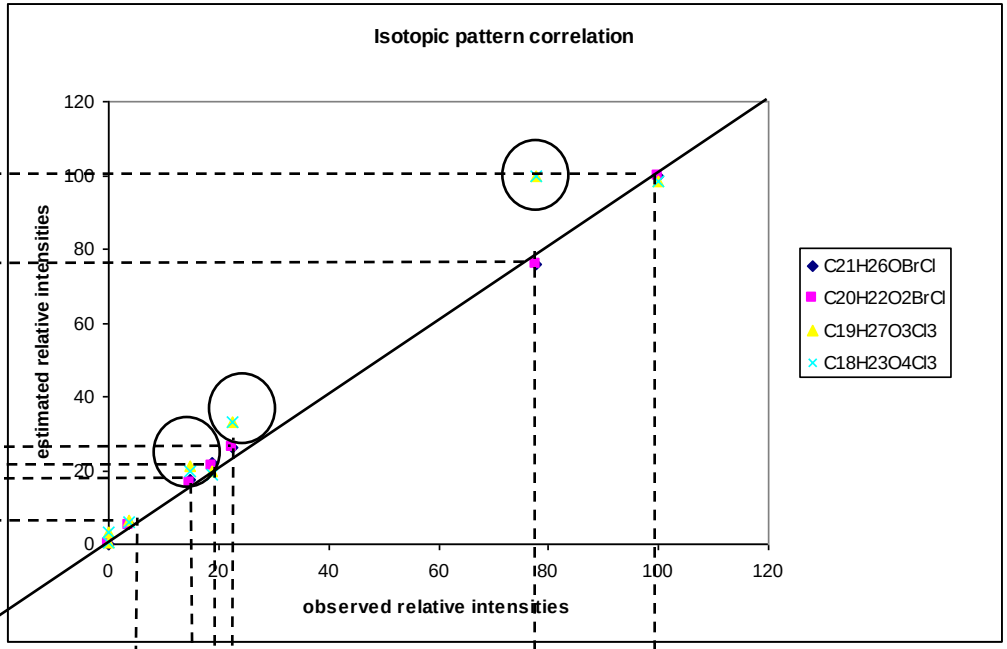
RDBeq. Close to EE2 (9)

Nitrogen rules

Methodology: IV-Raw formula selection



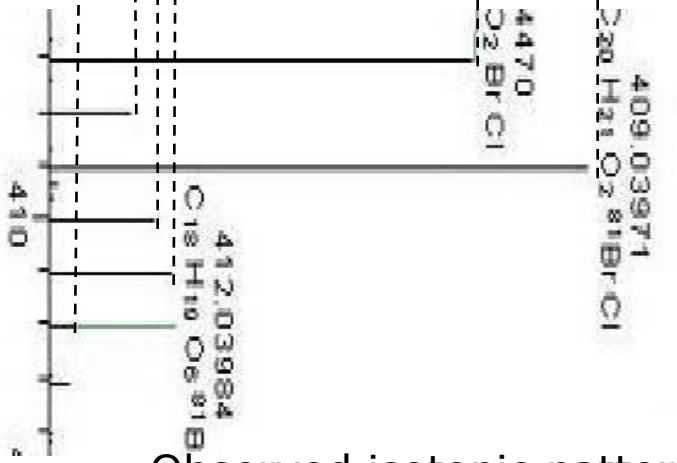
Theoretical pattern



R=1

Correlation coefficient as high as possible

Especially with halogenated compounds

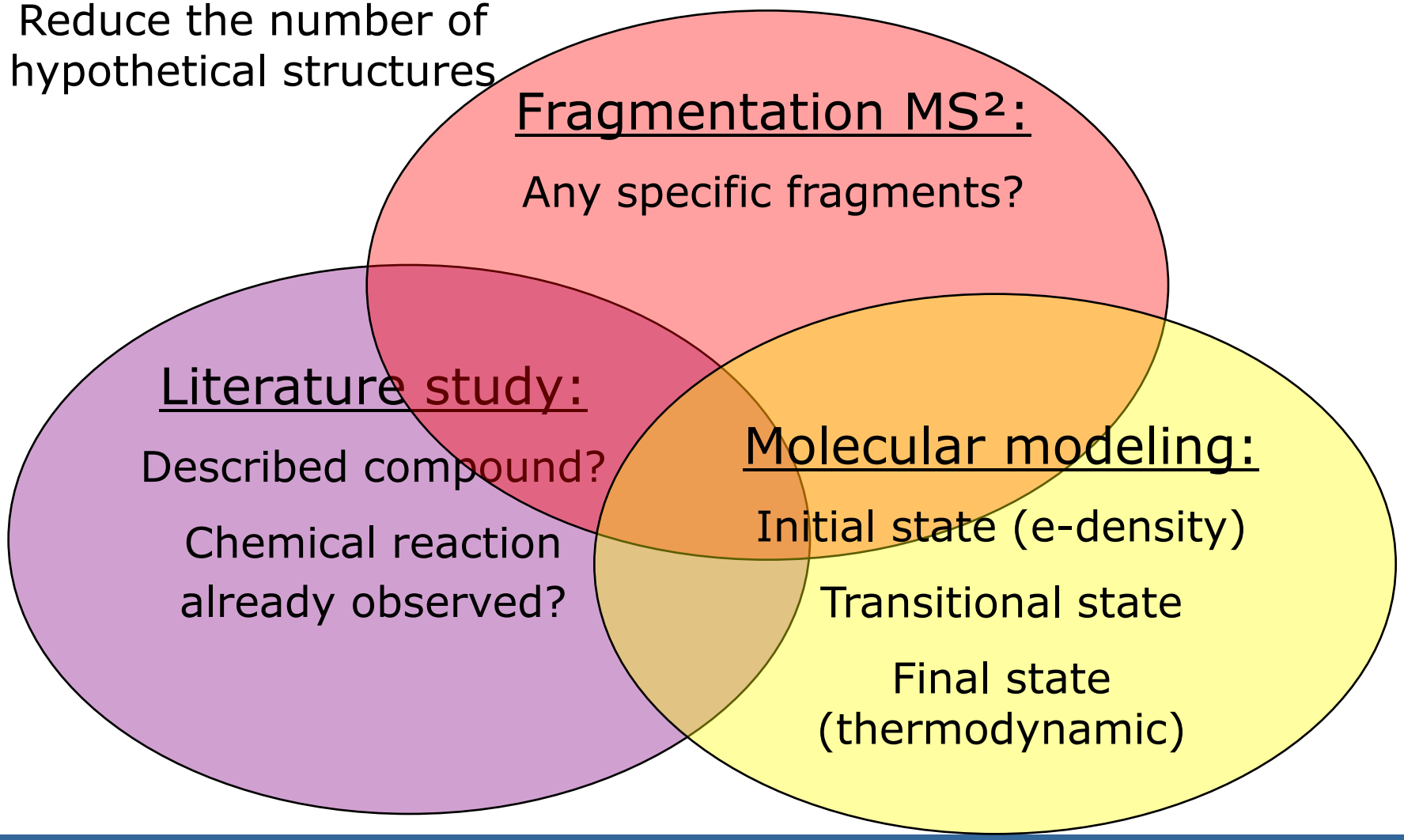


Observed isotopic pattern

Methodology: V-Structural elucidation

Propose a structure

Reduce the number of hypothetical structures



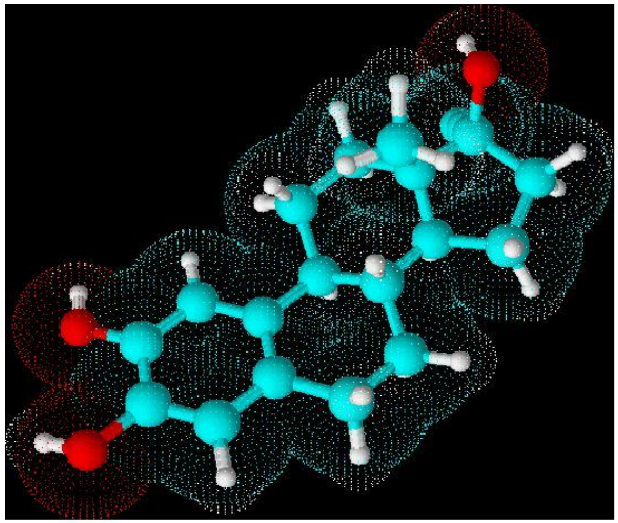
Contents

Introduction

Methodology

Results

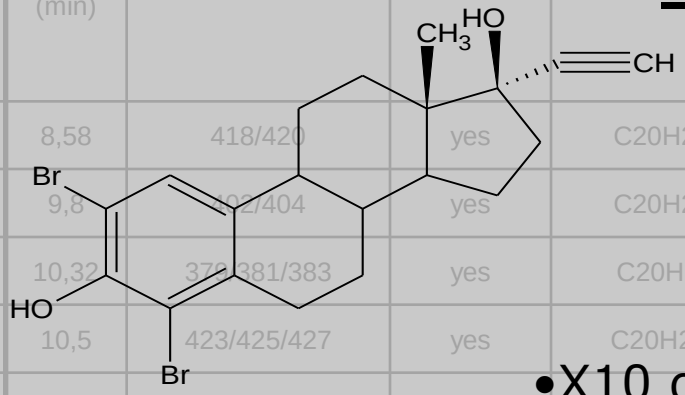
Conclusion

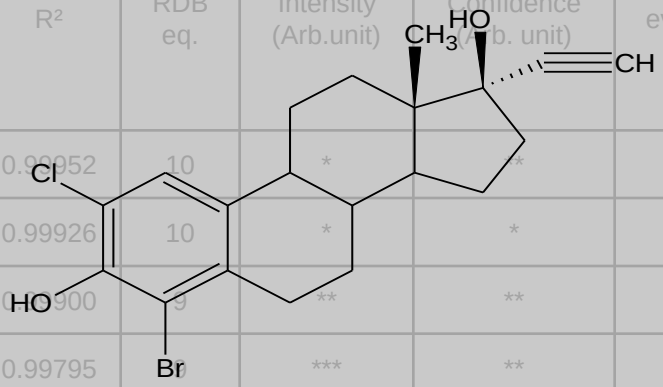


Results:

Highlighted ions & raw formulae selected

X10 & X9





- X10 decreased along the treatment. This is in accordance with disappearance kinetic (*Lee, 2009*)
- Bromination of EE2 already observed (*Nakamura, 2006; Lee, 2009; Flores, 2008*)
- ortho positions favored for halogenation (mol. Model.)

Validation of the Identification process

Code	Rt (min)	m/z	adduct	Raw formula	Δ (ppm)	R^2	RDB eq.	Intensity (Arb.unit)	Confidence (Arb. unit)	evolution
X9	12,15	407/409/411	yes	C20H22O2BrCl	0.307	0.99909	9	*	***	--
X10	12,42	451/453/455	yes	C20H22O2Br2	0.774	0.99937	9	***	***	--

Results:

Highlighted ions & raw formulae selected

Code	Rt (min)	m/z	adduct	Raw formula	Δmmu	R^2	RDB eq.	Intensity (Arb.unit)	Confidence (Arb. unit)	evolution
X1	8,58	418/420	yes	C20H22O4NBr	0.900	0.99952	10	*	**	+
X2	9,8	402/404	yes	C20H22O3NBr	1.005	0.99926	10	*	*	+
X3	10,32	379/381/383	yes	C20H22O3Cl2	26.569	0.99900	9	**	**	=
X4	10,5	423/425/427	yes	C20H22O3BrCl	0.262	0.99795	9	***	**	=
X5	10,7	467/469/471	yes	C20H22O3Br2	0.221	0.99915	9	****	***	=
X6	10,85	543/545/547	no	C22H26O6Br2 (adduct) C20H22O6N2Br2	0.166 25.086	0.99706 0.99713	9 10	**	*	+
X7	11,62	501/503/505/507	yes	C20H21O3ClBr2	0.324	0.99717	9	*	*	-
X8	11,74	545/547/549/551	yes	C20H21O3Br3	0.607	0.99886	9	***	**	---
X9	12,15	407/409/411	yes	C20H22O2BrCl	0.307	0.99909	9	*	***	--
X10	12,42	451/453/455	yes	C20H22O2Br2	0.774	0.99937	9	***	***	--

Results:

Discussion of X3,X4 and X5 compounds

$$C_{20}H_{22}O_3Br_XCl_y$$

(with **x** & **y = 0, 1, 2** & **x + y=2**)

One more Oxygen, where?

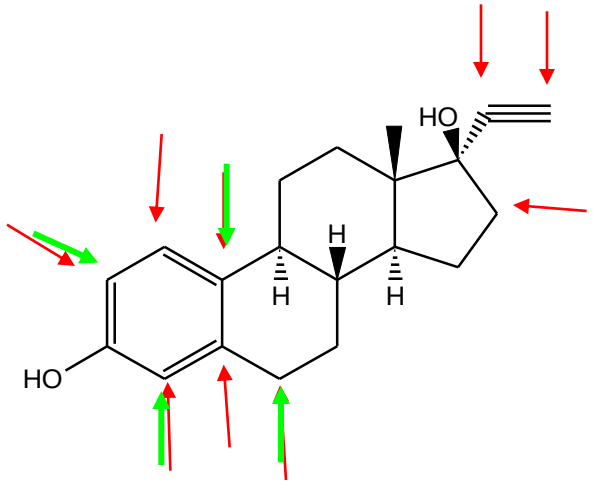
2 halogens, Where?

Literature study:

Oxidation in position 2, 4, 6 & 10

Halogenation is an electrophilic substitution on the A-ring

No reactions on the ethinyl moiety



Results:

Discussion of X3,X4 and X5 compounds

$$C_{20}H_{22}O_3Br_XCl_y$$

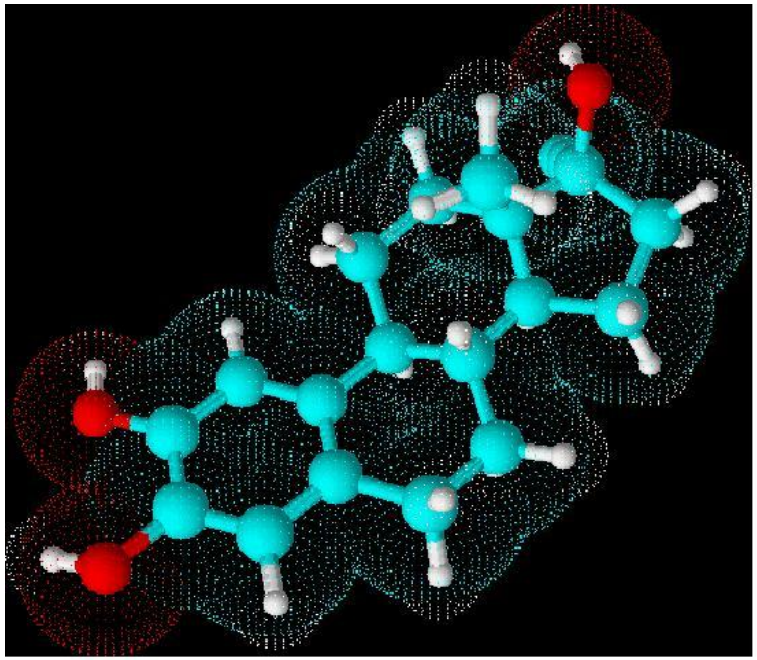
(with **x** & **y = 0, 1, 2** & **x + y=2**)

One more Oxygen, where?

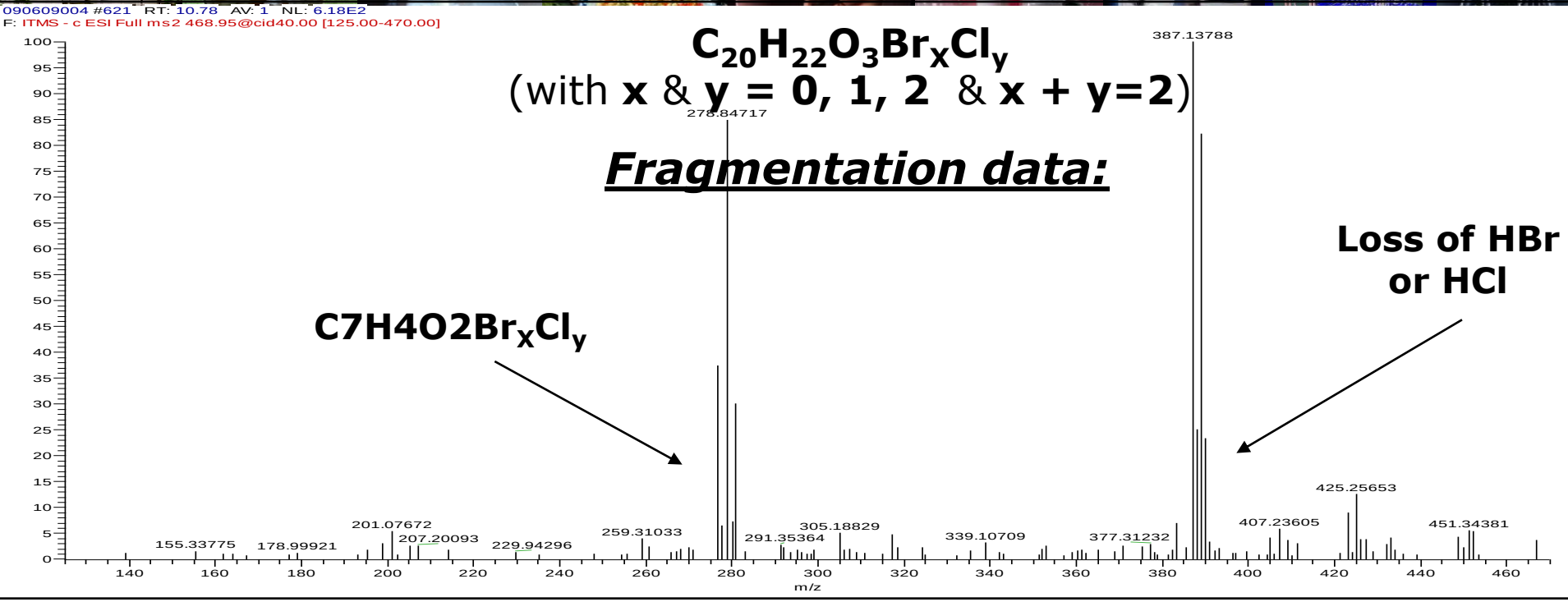
2 halogens, Where?

Molecular modeling:

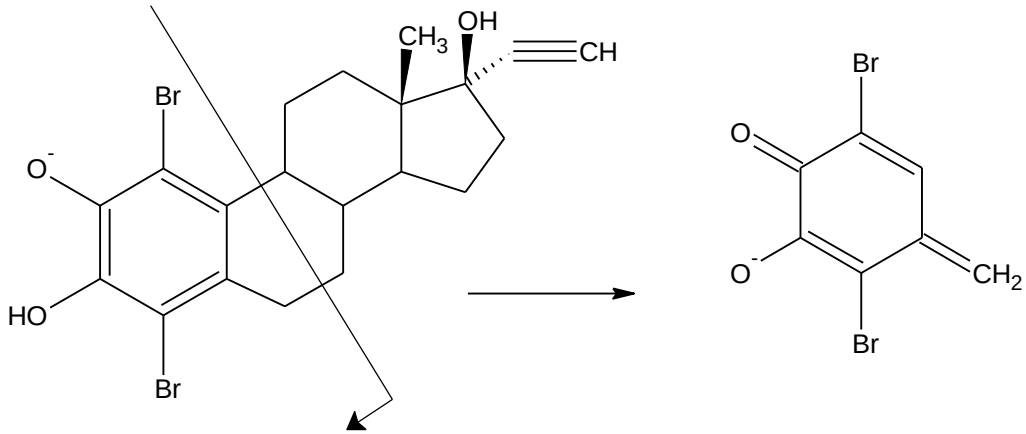
- Transitional state favored halogenations on the A-ring
- Final state favored halogenations on ethinyl group
- Oxidation favorable in C2 position



Additional Hydroxyl-group position on EE2	1	2	4	6	10
Heat of formation, $\Delta H°_f$ (kcal)	-82,94	-85,67	-83,06	-82,56	-60,23



With previous information, only one possibility

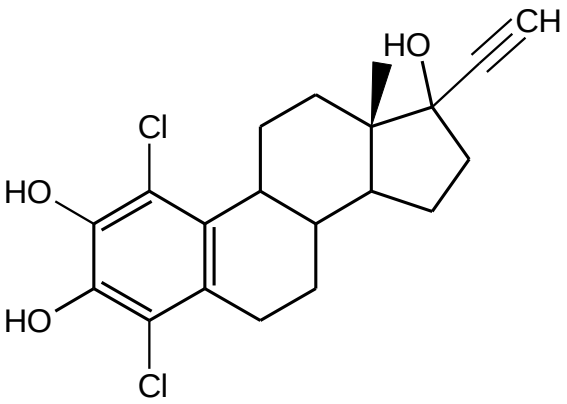


Results:

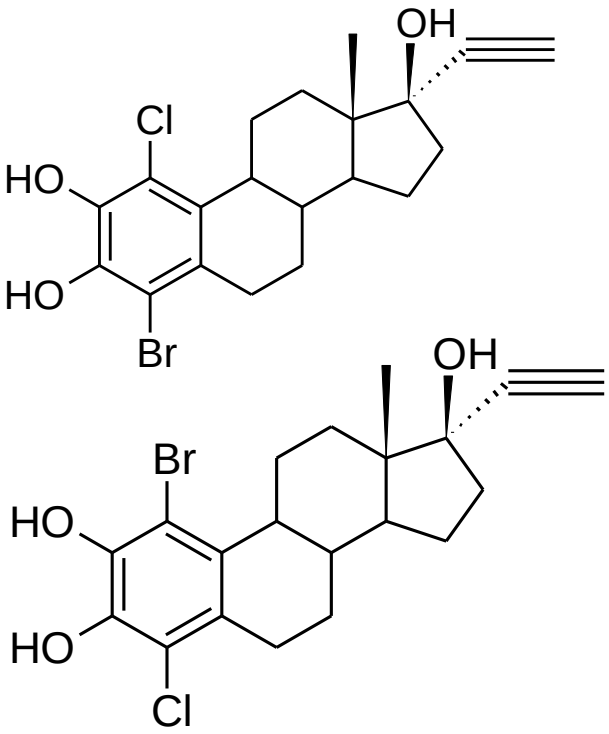
Discussion of X3,X4 and X5 compounds

X3,X4 & X5

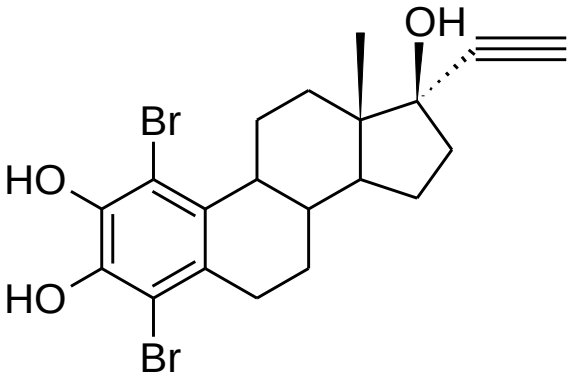
$C_{20}H_{22}O_3Br_XCl_y$
(with **x** & **y = 0, 1, 2** & **x + y=2**)



m/z = **379**/381/383



m/z = 423/**425**/427



m/z = 467/**469**/471

Main By-products of EE2

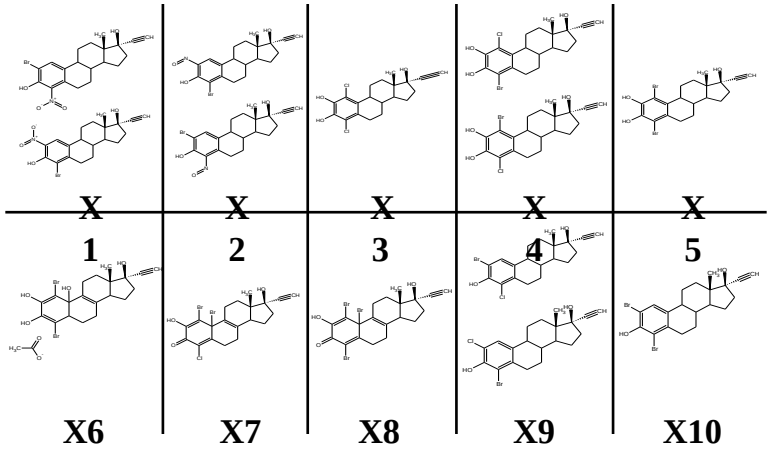
Contents

Introduction

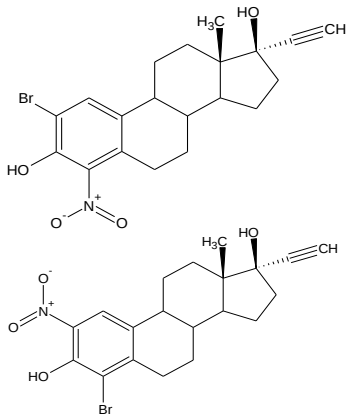
Methodology

Results

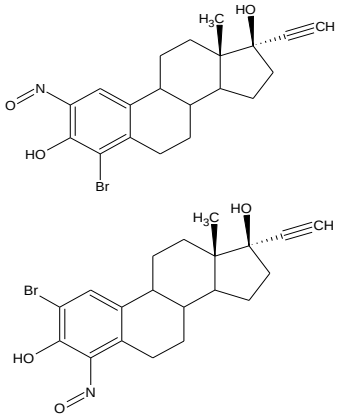
Conclusion



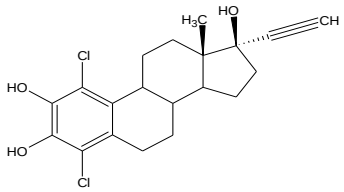
Conclusion



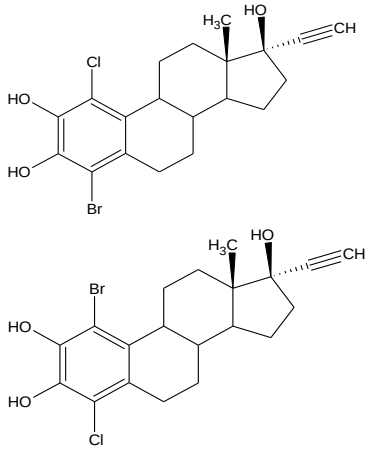
X1



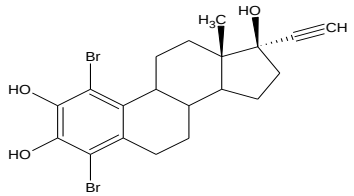
X2



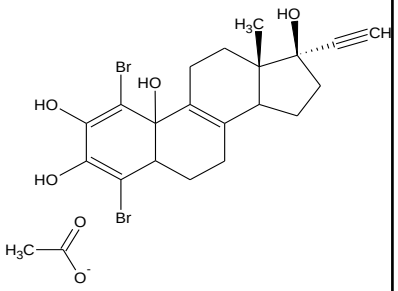
X3



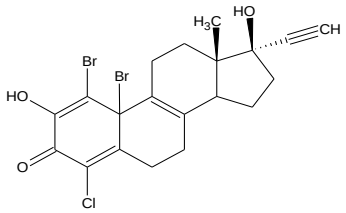
X4



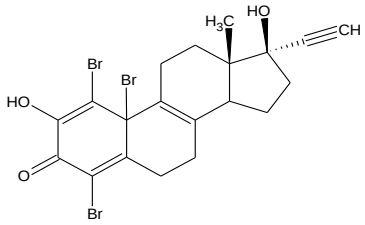
X5



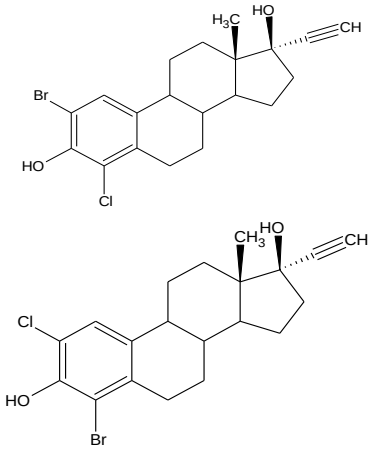
X6



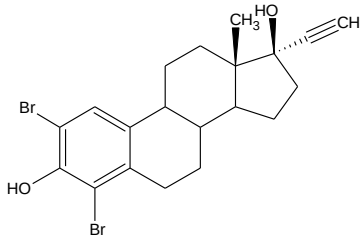
X7



X8



X9



X10

Conclusion

Perspectives:

- Develop targeted analysis of identified BPs to reach observable amounts (<1 ng/L)
- Confirm these BPs in DWTP by (LC-MS/MS)
- Apply this identification process to other kind of treatment (ozonation, etc.)
- Apply this identification process to other EDCs (Estrone-sulfate, etc.)
- Evaluate BPs Toxicity (probably lower than EE2)

LABERCA:
Antignac Jean-Philippe
Bichon Emmanuelle
Monteau Fabrice
Le Bizec Bruno

CAE:
Ingrand Valérie
Leroy Gaëla
Barritaud Lauriane
Chachignon Mathilde

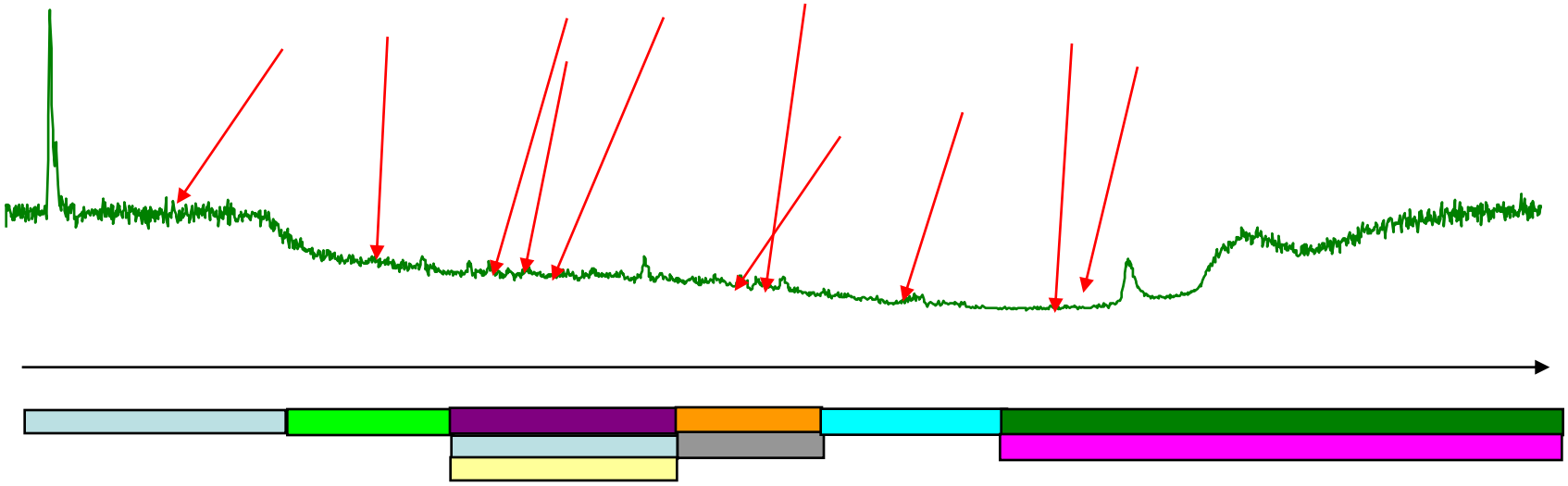
Anjou-Recherche:
Roche Pascal
Castillo Luis
Bourdin Delphine

Thank you!

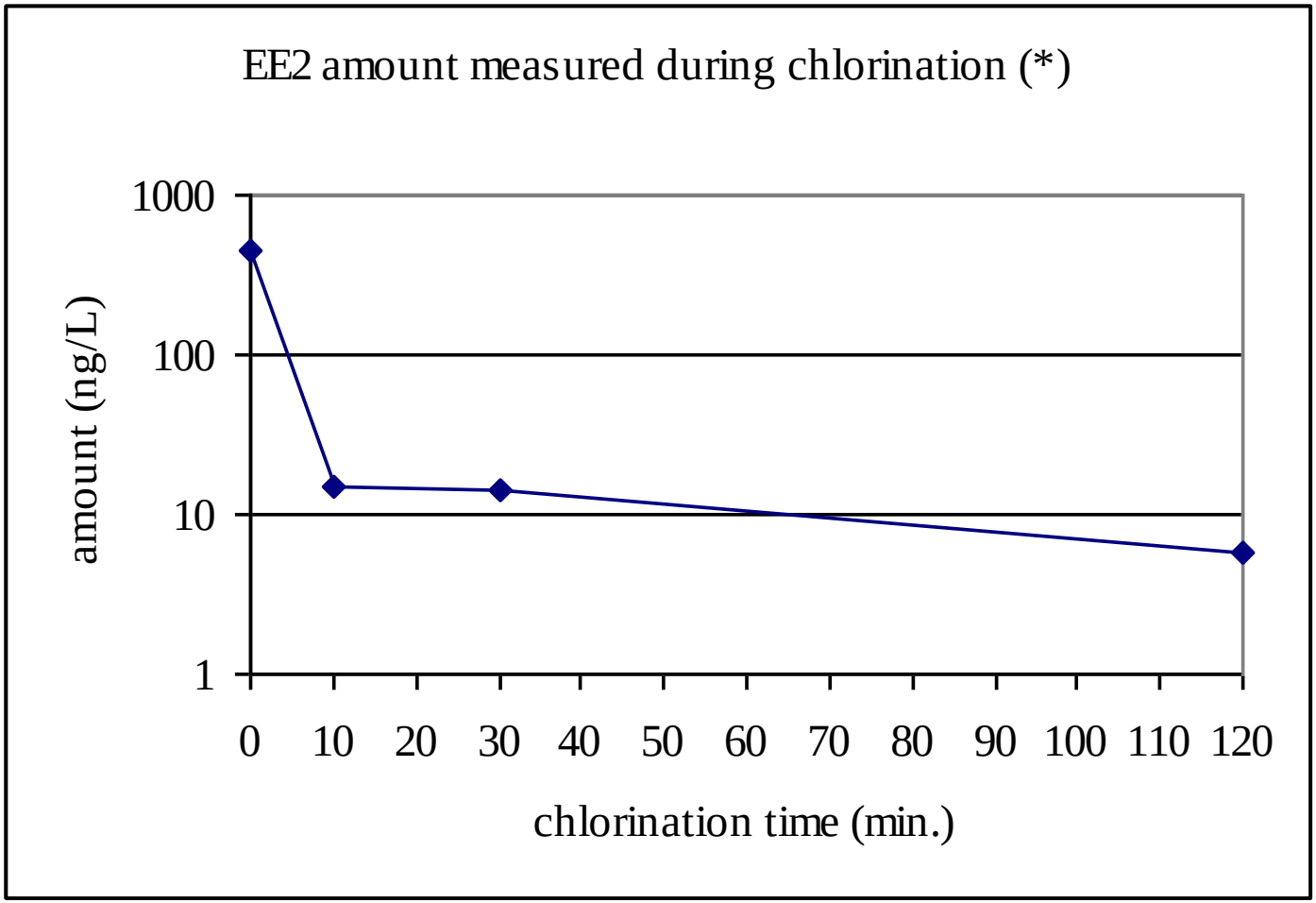
Techniques for BPs Identification:

Structural elucidation

If XCMS highlights 10 ompounds



Create 10 MS/MS functions centered on the isotopi pattern of the parent ion with a 6 Da « Iso-width »



*data from CAE, VEOLIA Environment