PHARMACEUTICAL SUBSTANCES : EMERGENT CONTAMINANTS OF THE AQUATIC SYSTEMS

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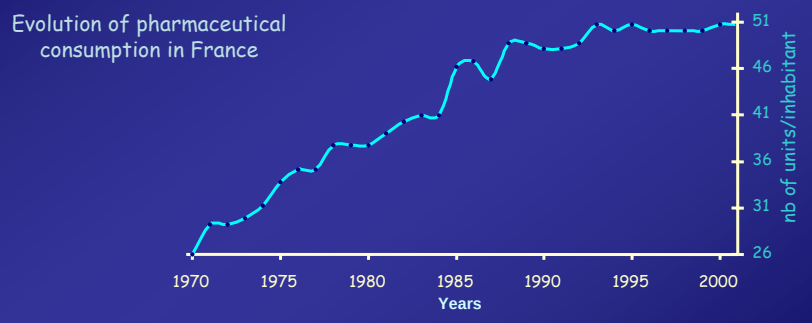
Study of pharmaceuticals in aquatic systems

- Widely used compounds, in constant increase :



Consumed quantities experessed as tons per year

Compounds	Therapeutic Class	UK (2000) (a)	Germany (1995-1997) (b)	Australia (c)	France (d)
Paracetamol	Analgesic	2000	-	295	2294
Aspirin	NSAID	770	> 500	20	880
Ibuprofen	NSAID	-	105-180	14	166
Erythromycin	Antibiotic	27	-	11	
Ketoprofen	NSAID	-	0,7	4	
Diclofenac	NSAID	26	75	4	39
Penicillin V	Antibiotic	22	140	9	

(a) (Webb, 2001)
(b) (Hirsch *et al.*, 1999 ; Ternes, 2001 ; Ternes *et al.*, 1998)
(c) (Khan et Ongerth, 2004)
(d) (Janex *et al.*, 2002)

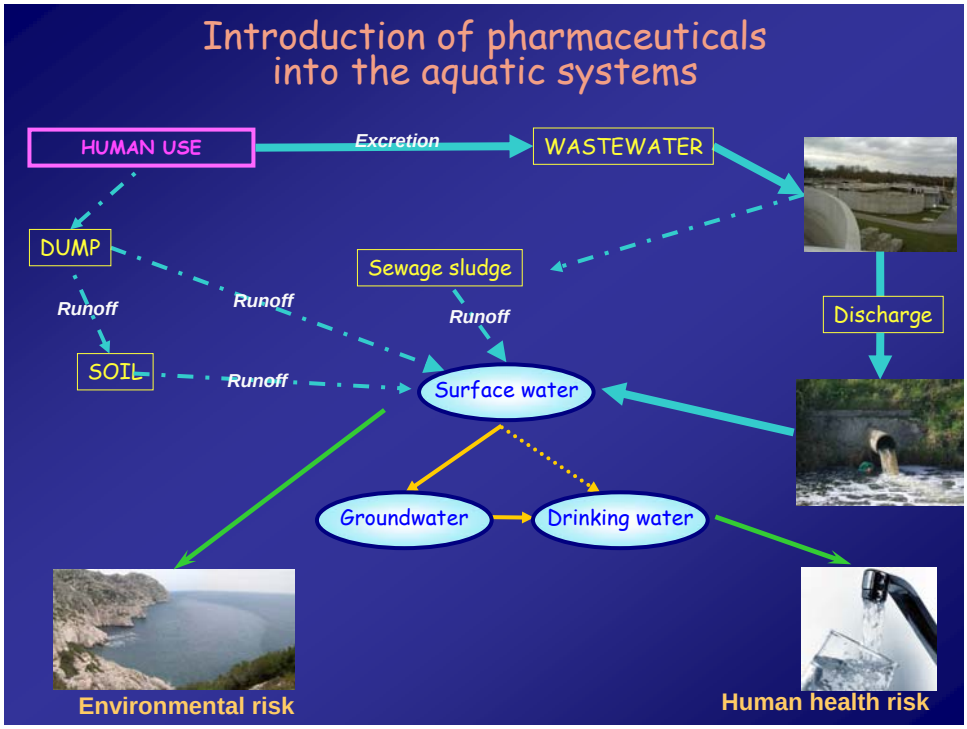
Study of pharmaceuticals in aquatic systems

- Toxicity studies on several compounds :
  - Endocrine disruptors ?
    (paracetamol, inhibition of vitellogenin in vitro, Miller, 1999)
  - Metabolic effects ?
    (AINS, CYP 450 activation)
    (Fluoxetine, ACHE activity inhibition)

- Occurrence in aquatic systems :
  - Wastewater
  - Surface water
  - Drinking water

⇒ Emerging contaminants

Introduction of pharmaceuticals into the aquatic systems



Aims of study

Analytical tools

Environmental field

Various compounds :
- Different chemical groups
- Range of concentrations depending on uses

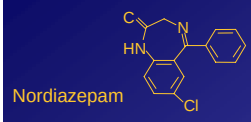
Several matrices :
(surface water, WWTP effluents, sludge, sediment, particles, biota…)

Contamination monitoring ?
- Contamination range
- Which compounds

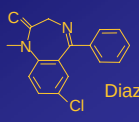
Sources ?
- Identification
- Input quantification

Material and Methods

Studied compounds

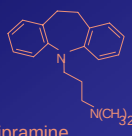


Nordiazepam

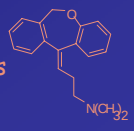


Diazepam
Valium®

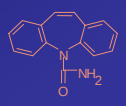
Antidepressives



Imipramine

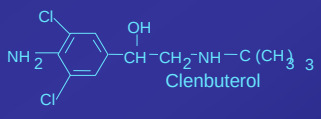


Doxepin

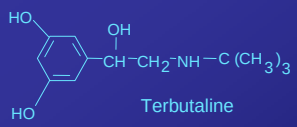


Tegretol® Carbamazepine

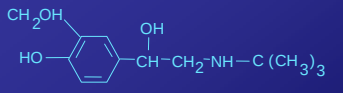
Bronchodilatators



Clenbuterol

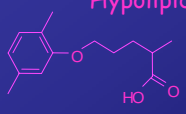


Terbutaline



Salbutamol

Hypolipidic

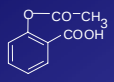


Gemfibrozil

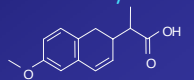
Lipur®

Studied compounds

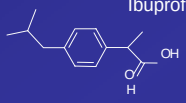
Aspirin



Naproxen *Apranax®*

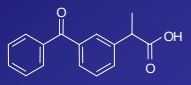


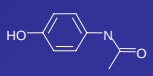
Ibuprofen *Nurofen ®*



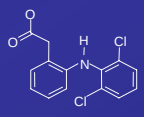
Non Steroidal Anti-inflammatory drugs (NSAIDs)

Ketoprofen *Ketum®*



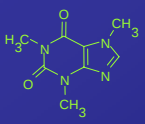


Paracetamol *Doliprane®*

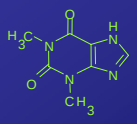


Voltarène® Diclofenac

Caffeine



Theophylline



Bronchodilatators
Psychoanaleptics

Lab developped methods

Water

Dissolved Phase

Particulate Phase

Sediment

Organisms

Microwave Assisted Extraction

Solid Phase Extraction

GC-MS Analysis

1st internal standards

2nd internal standards

Natural water
Filtration on glass fiber filter
(0.7µm pore size)

Suspended matter
Freeze dried
Microwave extraction (30W, 10 min.)
30 ml (acetonitrile/ water pH 2; 70/30; v/v)
Filtration on glass fiber cotton
Evaporation of acetonitrile under vacuum heated system (Rapidvap ®)
Adjustment to 50ml with milliQ water (pH 2)

Dissolved phase
Adjustment to pH 2 with HCl

Conditionning of OASIS MCX 60 mg cartridges
ethyl acetate, milliQ water (pH 2)

Sample deposition; drying under vacuum

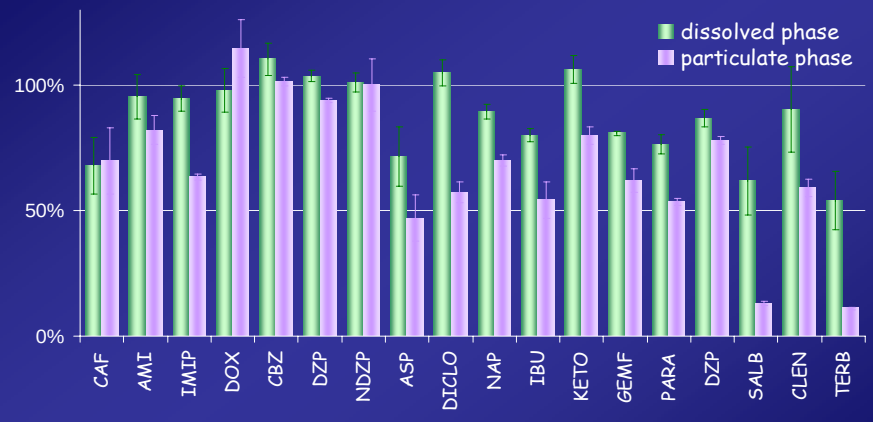
Elution
4 ml ethyl acetate; 4 ml ethyl acetate-acetone (50:50; v:v)
4 ml ethyl acetate-acetone (50:50; v:v) with NH_4OH (3%)

Evaporation under nitrogen

GC-MS injection for neutral compounds

Incubation with MSTFA (30 µl, 35 min. at 65°C)
GC-MS injection for acidic compounds

Recoveries



Dissolved phase : Recoveries between 64-110% (RSDs < 15%)
Particulate phase : Recoveries between 61-115% (SALB, TERB), (RSDs < 15%)

Environmental limits of detection

	Tap water (1l)	Surface water (1l)	Marine water (1l)	Particles (0.1-0.5 g)
amitryptilin	0.7	2.2	2.6	4.2
aspirin	0.2	2.1	2.1	2.6
cafeine	1.5	2.5	2.3	4.3
carbamazepine	0.8	1.4	2.2	5.2
clenbuterol	0.6	0.3	1.2	4.0
diazepam	0.4	1.4	1.9	5.3
nordiazepam	0.4	1.4	1.9	6.1
diclofenac	0.9	0.7	2.6	2.4
doxepine	0.7	2.1	2.4	7.9
gemfibrozil	0.1	0.3	1.2	2.1
ibuprofen	0.1	0.1	1.7	3.2
imipramine	0.7	1.2	1.6	9.5
ketoprofen	0.3	0.7	1.8	4.1
naproxen	0.1	1.0	2.1	1.5
paracetamol	5.3	8.5	7.2	6.7
salbutamol	0.6	0.5	1.2	4.1
terbutaline	0.6	0.3	0.8	2.1
(ng/l ou ng/g)	0.1-5.3	0.1-8.5	0.8-7.2	1.5-9.5

→ Applicability to natural samples

Study of pharmaceuticals in aquatic systems

Seine Estuary

Loire Estuary
1 sampling campaign (Surface waters)

- Surface waters

Gironde Estuary
2 sampling campaigns (Surface waters)
Estuary + Garonne river
Characterization of sources
Impact on Seine Bay

Adour Estuary
2 sampling campaigns (river + estuary)

Environmental field : Results and Discussion

Concentration levels in Atlantic coast estuaries

Important variability depending the estuary anthropisation level

	GIRONDE	LOIRE	ADOUR	SEINE
aspirin	<2 - 4	<2 - 27	8 - 28	<2 - 196
caffeine	<1 - 5	<1 - 73	<1 - 2	40 - 860
diclofenac	<2 - 4	<2 - 6	8 - 23	8 - 380
gemfibrozil	<2 - 4	<2 - 5	<2 - 9	3 - 126
ibuprofen	<2 - 3	<2 - 9	14 - 37	5 - 610
ketoprofen	<2 - 3	<2 - 9	<2	3 - 78
naproxen	<2 - 7	<2 - 8	1 - 6	3 - 185
carbamazepine	<1 - 5	<1 - 228	<1 - 8	3 - 164

Agricultural watershed
Important cities and high rivers flows
⇒ LOW CONCENTRATION LEVELS

thropic pressure
Dax) during summer

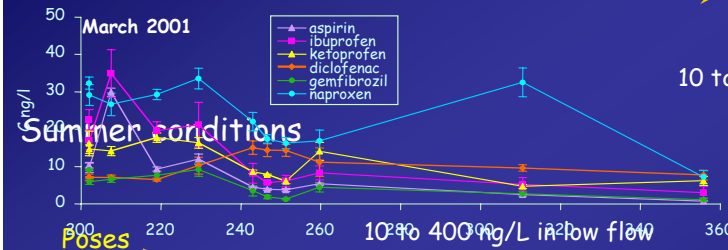
ION LEVELS

SEINE ESTUARY as the MOST CONTAMINATED

Specific studies of the Seine Estuary

Winter conditions

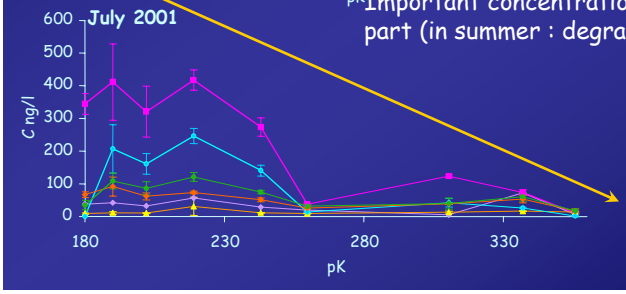
Poses → Seine Bay



10 to 40 ng/L in high flow

Summer conditions

Poses

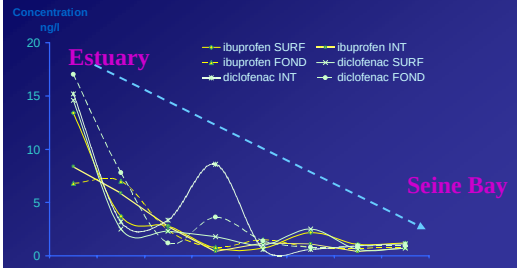


10 to 400 ng/L in low flow

Important concentration decrease in downstream part (in summer : degradation phenomena)

Seine Bay

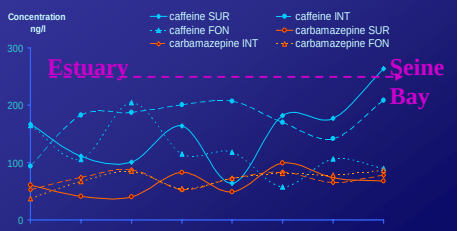
Contamination in the Seine Bay



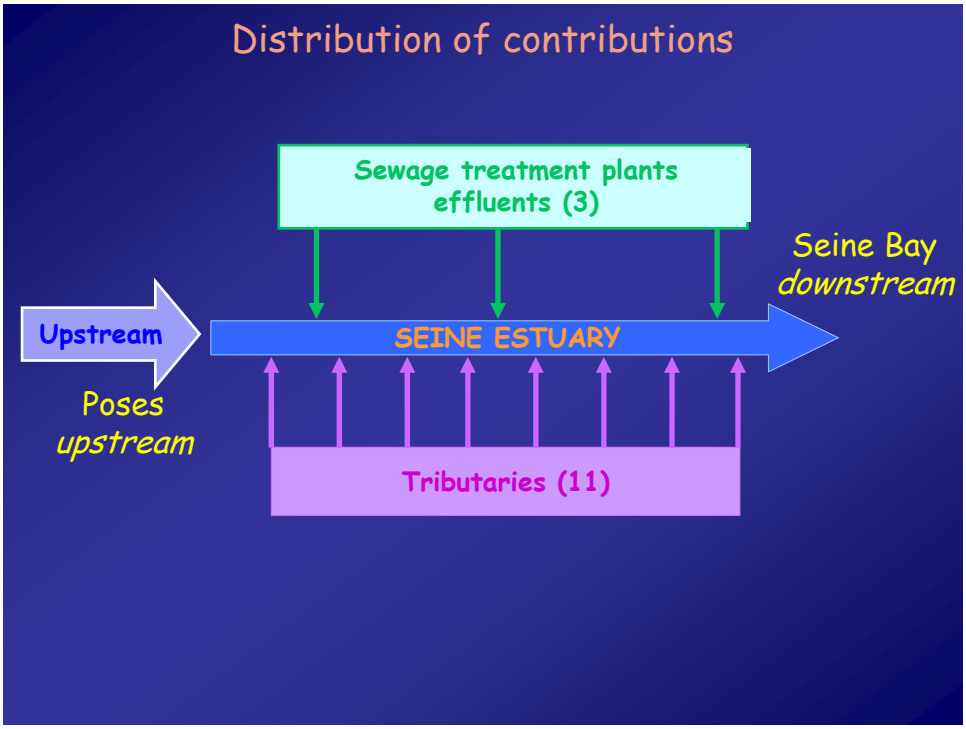
Degradation phenomena occur in the Seine Bay

⇒ decrease of concentrations

High concentration for some compounds (carbamazepine and caffeine)



Distribution of contributions



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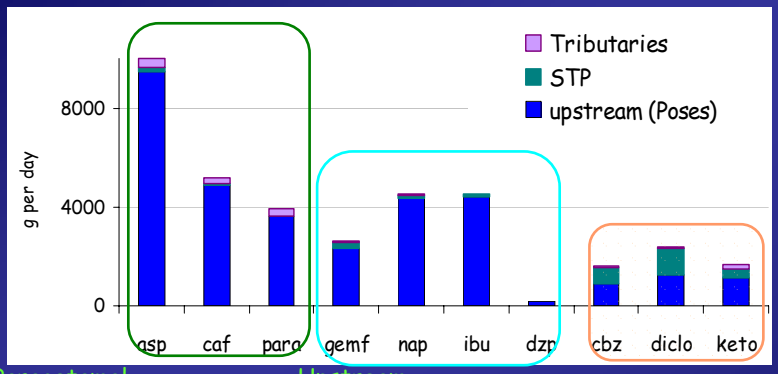


Paracetamol

Upstream 93%

Trib. 7%

0% STP

Upstream 52%

Trib. 1%

Upstream 96%

3% STP

Diclofenac

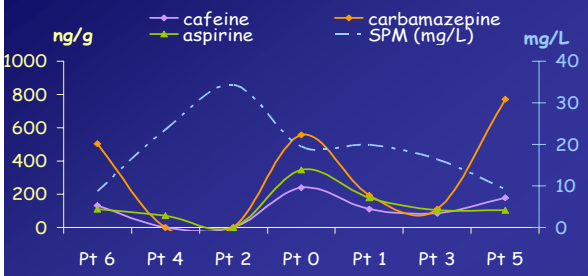
STP 46%

Trib. 2%

- High consumed compounds
- Most persistent compounds and low STP efficiency
- Input dependent of population and degradation in aquatic systems

⇒ Important role played by STP discharges.

Particulate Matter : Mediterranean sea

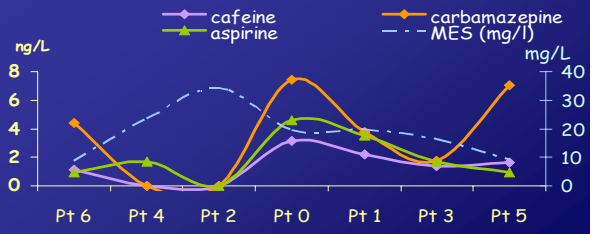


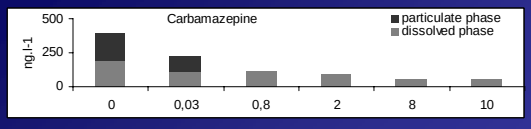
Particles can show high concentrations :
Up to 800 ng/g for carbamazepine

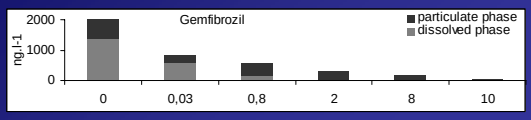
Low Amount of particules

⇨ Negligible in comparison to dissolved phase (Max : 8 ng/L)

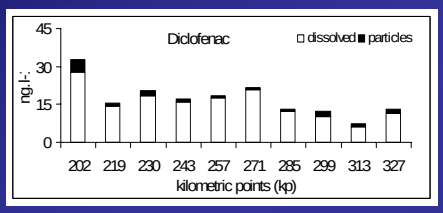
⇨ But dissemination role

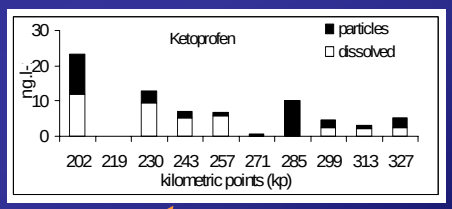


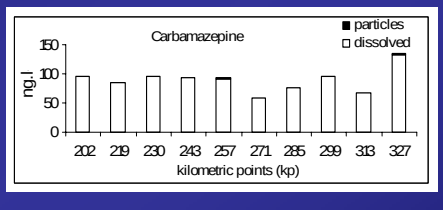




Gironde estuary : high SPM







Seine estuary : moderate SPM

CONCLUSION

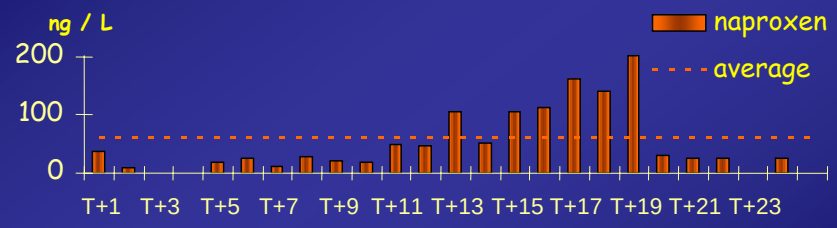
- Real contamination of aquatic media
  - rivers, estuaries, marine waters
- Contamination mainly in dissolved phase
- Contamination of particles non negligible (dissemination role?)
- Important variability of the contamination (seasons, use, treatments …) related to number of humans and type of treatments

PERSPECTIVES

- Integrative sampling tools (POCIS : Polar Organic Chemical Integrative Sampler)
- Role of particles
- Transfer to organisms
- Products of transformation?

LIMITS of discrete sampling

- No integrative view : dependent on sampling frequency

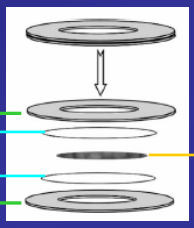


Intra-day variability in STP Effluent (Rouen, June 2005)

Development of Integrative sampling

Use of POCIS device

Access to the time weighted average concentration over extended periods



For calculating concentrations : $Cw = \frac{Cs \times Ms}{Rs \times d}$ *(Alvarez. 1999)*

Cw : Concentration in water
Qs : Quantity in POCIS device
Ms : Masse of sorbent
T : Time of exposure
Rs : Sampling rate

Laboratory determination for each analyte

POCIS uptake rate experiments

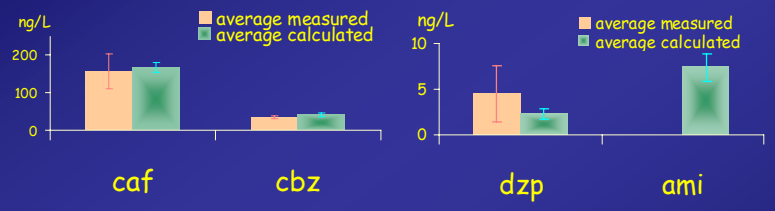
- Water renewal each day
- Stirring by magnetic stir bar

Analysis : - Water
- Sorbent

Experimental conditions

- Analyte concentration : 0.5 and 5 µg/L
- Time of exposure : 7 and 14 days
- Water temperature : 15 and 21°C
- Water salinity : 0 and 35 PSU

RESULTS : 3 days exposure
Comparison measurements / calculations

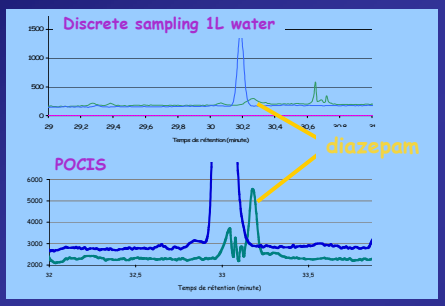


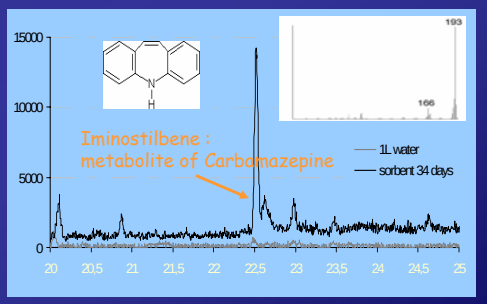
⇒ Results very similar between measured and calculated values (AMI detected only in sorbent)

4 compounds found in POCIS sorbent
Triplicate : RSDs <20%

Quantitative and qualitative interest :

- Quantification of compound present in quantities below detection limit (discrete sampling) : accumulation effect
- Identification of non target compounds





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Acknowledgments

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Région Aquitaine for Anne Togola PhD funding

And thank you for your attention …