Oslo, 20 June 2017

Determination of fluoroquinolones in fish tissues, biological fluids and environmental waters

H. Ziarrusta, N. Val, H. Dominguez, L. Mijangos, A. Prieto, A. Usobiaga, N. Etxebarria, O. Zuloaga, M. Olivares.

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Universidad del País Vasco
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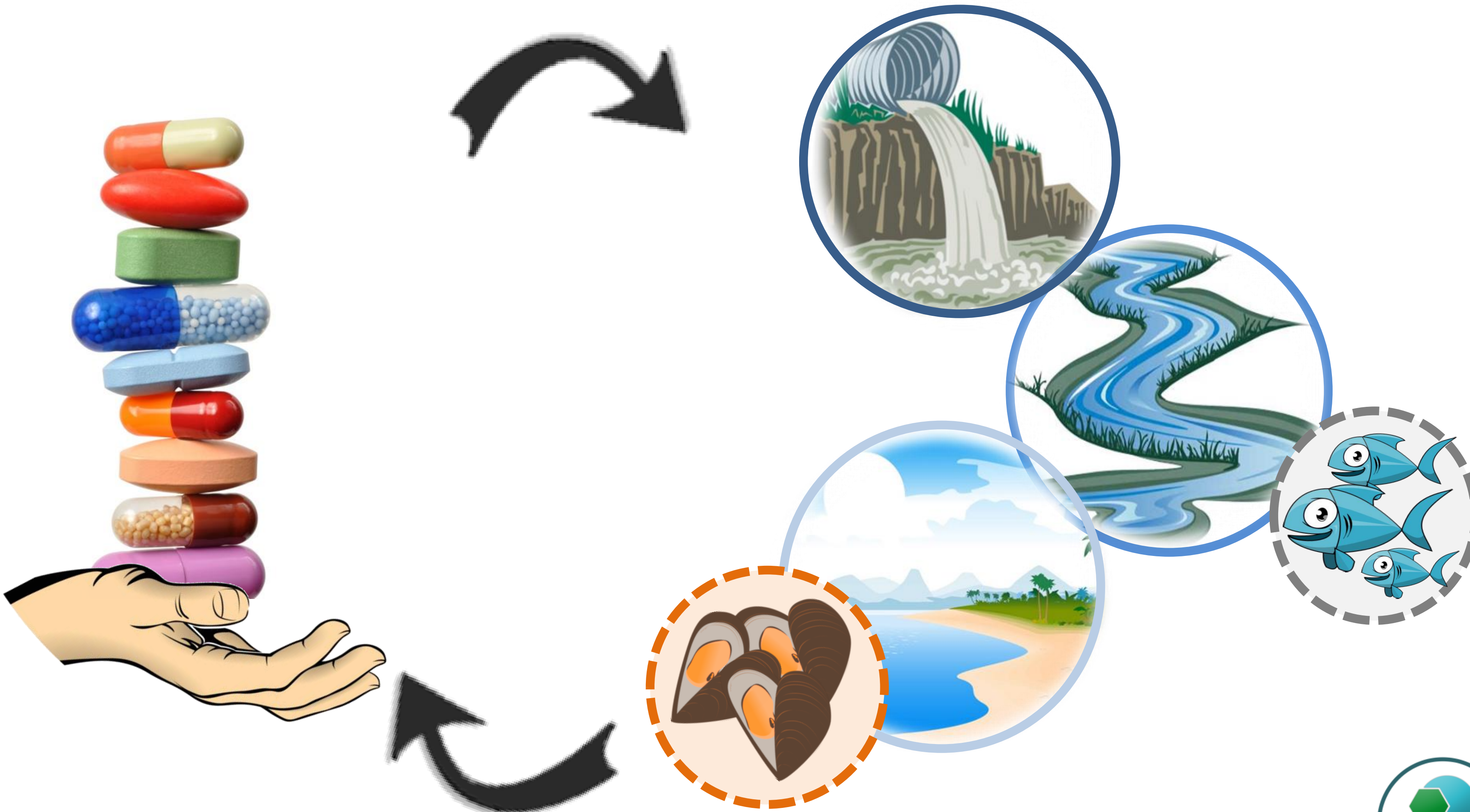
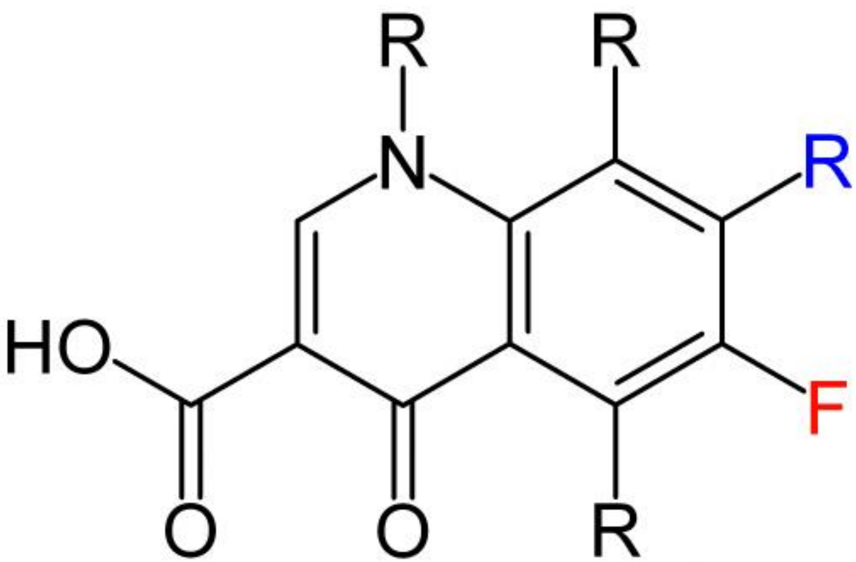
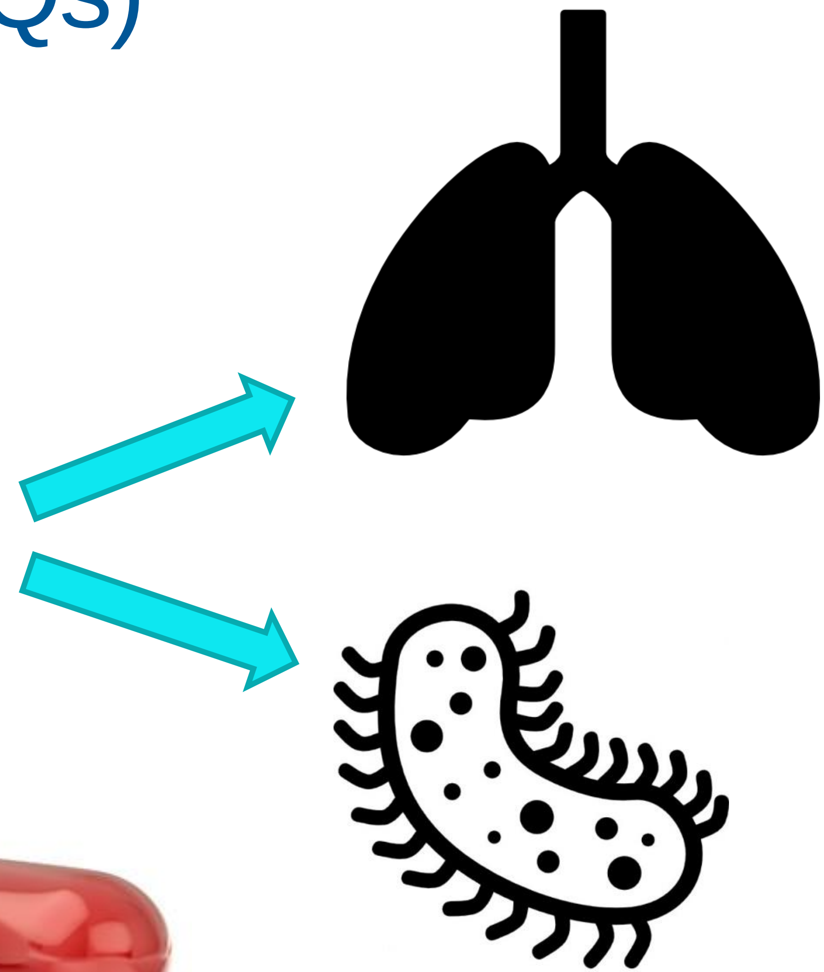
Pharmaceuticals



Fluoroquinolones (FQs)





Fluoroquinolones: effects

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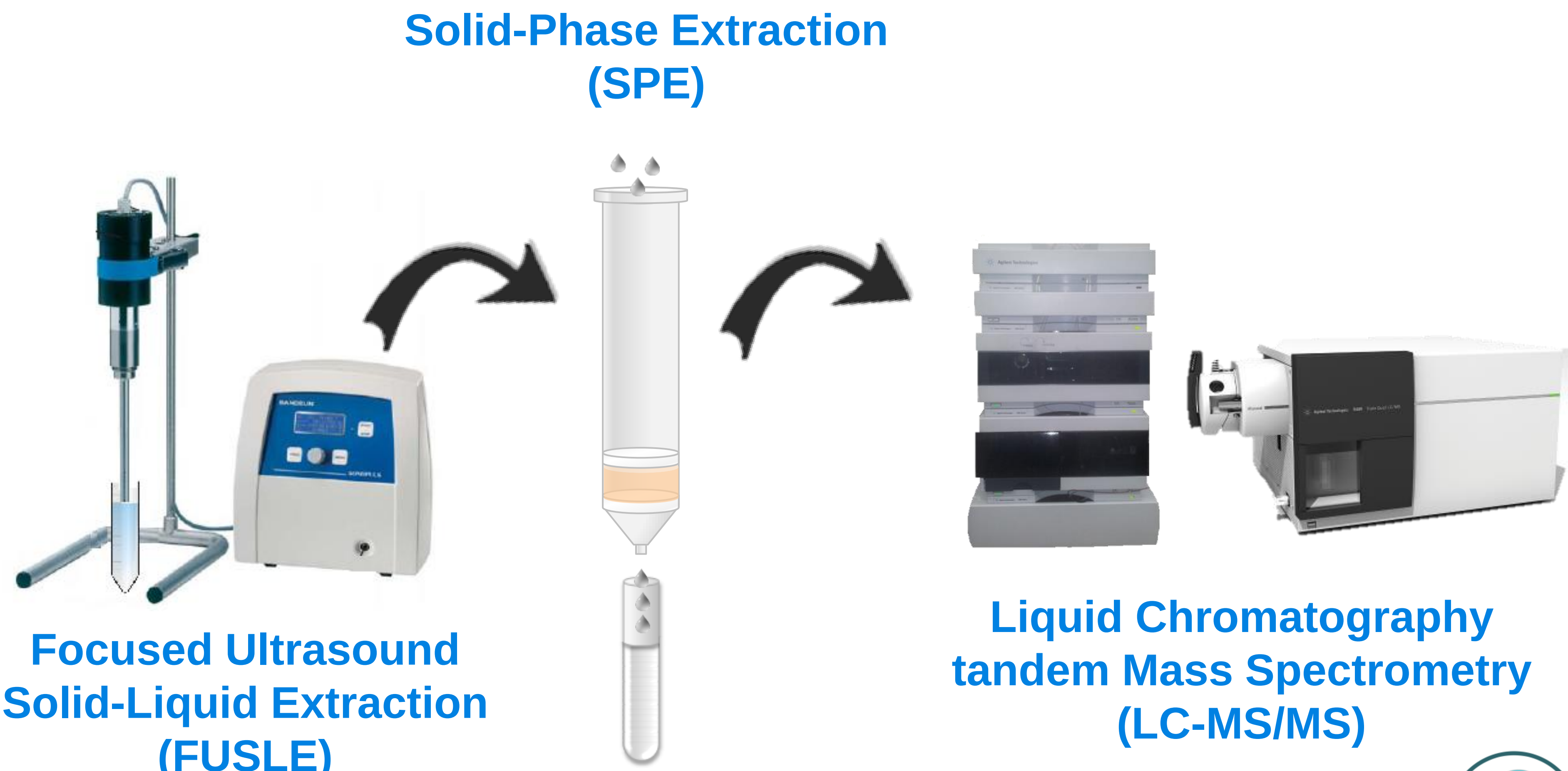
Fluoroquinolones: method development

Lack of analytical methods

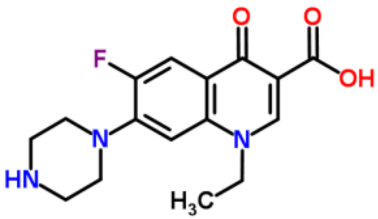
Objectives

1. Optimization LC-MS/MS analysis of FQs
2. Optimization of the extraction: fish tissue/biofluids + environmental water
3. Application to real samples

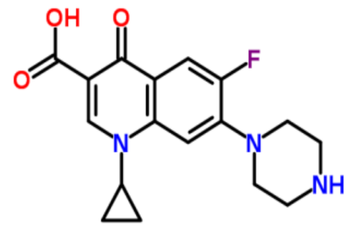
Experimental part



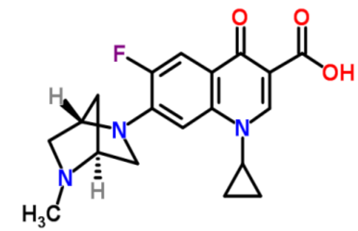
Fluoroquinolones



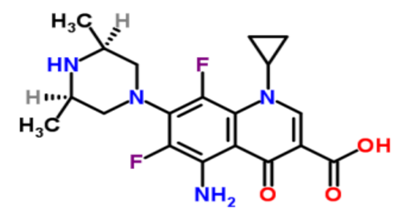
Norfloxacin (NORF)



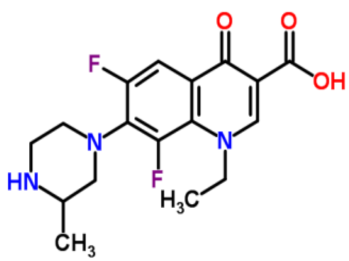
Enofloxacin (ENO)



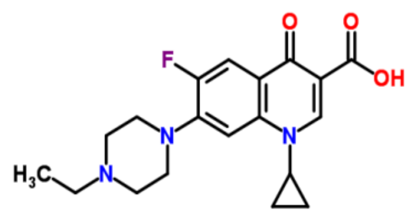
Pefloxacin (PEFLO)



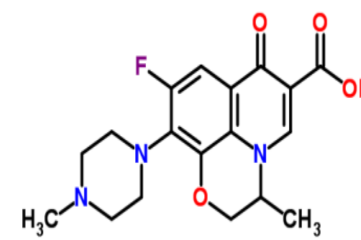
Danofloxacin (DANO)



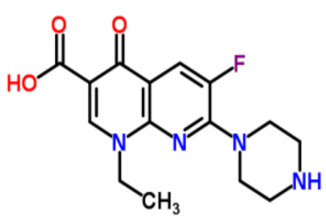
Ofloxacin (OFLO) and Levofloxacin (LEVO)



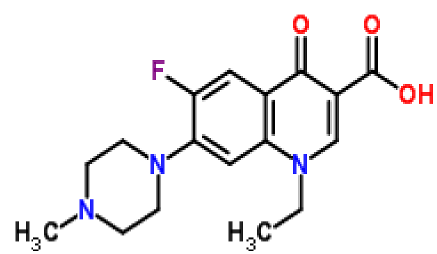
Ciprofloxacin (CIPRO)



Sparfloxacin (SPAR)



Lomefloxacin (LOME)

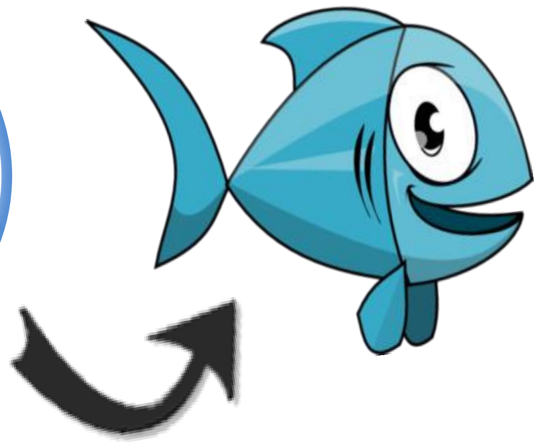


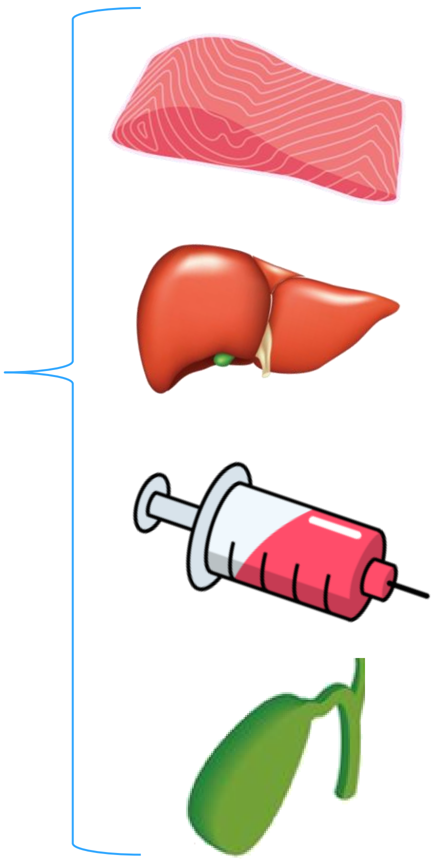
Enrofloxacin (ENRO)

Environmental samples

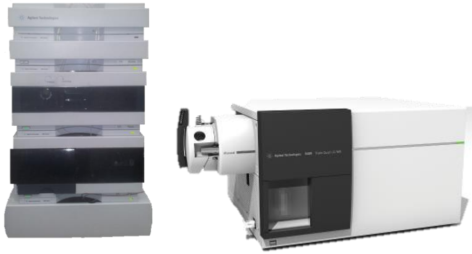
Environmental waters

Fish tissues and biofluids



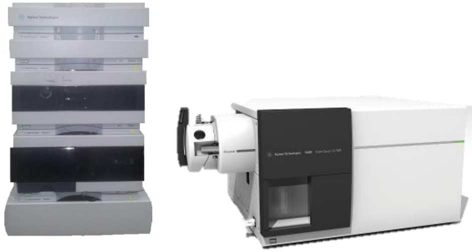


Results and Discussion



1. Optimization LC-MS/MS analysis of FQs
2. Optimization of the extraction: fish tissue/biofluids + environmental water
3. Application to real samples

Optimization of LC-MS/MS



- Column temperature: 35 ºC
- Flow rate: 0.3 mL/min
- Gradient
- Max. injection volume: 3µL

Chromatographic parameters

- Capillary voltage: 3000 V
- Nebulizer pressure: 30 psi
- Drying gas flow: 8 L/h
- Drying gas temp.: 300 ºC

Electrospray ionization parameters

- Fragmentor and collision energy

MS/MS parameters

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Fragmentor and collision energy

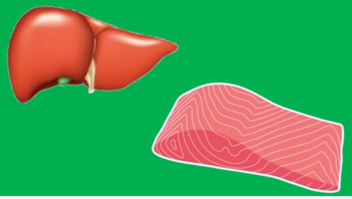
Analyte	SRM transitions
NORF	320 (104 V) → 302/231/282 (17/41/29 eV)
ENO	321 (104 V) → 303/232/204 (17/37/45 eV)
PEFLO	334 (104 V) → 316/290/233 (17/13/25 eV)
OFLO/LEVO	362 (104 V) → 318/261/344 (17/25/17 eV)
CIPRO	332 (104 V) → 314/231/288 (17/41/13 eV)
DANO	358 (104 V) → 340/82/255 (21/45/41 eV)
LOME	352 (104 V) → 265/308/334 (21/13/17 eV)
ENRO	360 (104 V) → 342/316/286 (17/17/37 eV)
SPAR	393 (104 V) → 349/292/375 (17/21/17 eV)

Results and Discussion

1. Optimization LC-MS/MS analysis of FQs
2. Optimization of the extraction: fish tissue/biofluids + environmental waters
3. Application to real samples

Optimization of the extraction

2.1 Fish tissues: liver and muscle

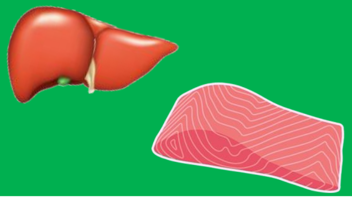


2.2 Fish biofluids: plasma and bile

2.3 Environmental waters: seawater, estuarine water and WWTP water

Optimization of the extraction

2.1 Fish tissues: liver and muscle

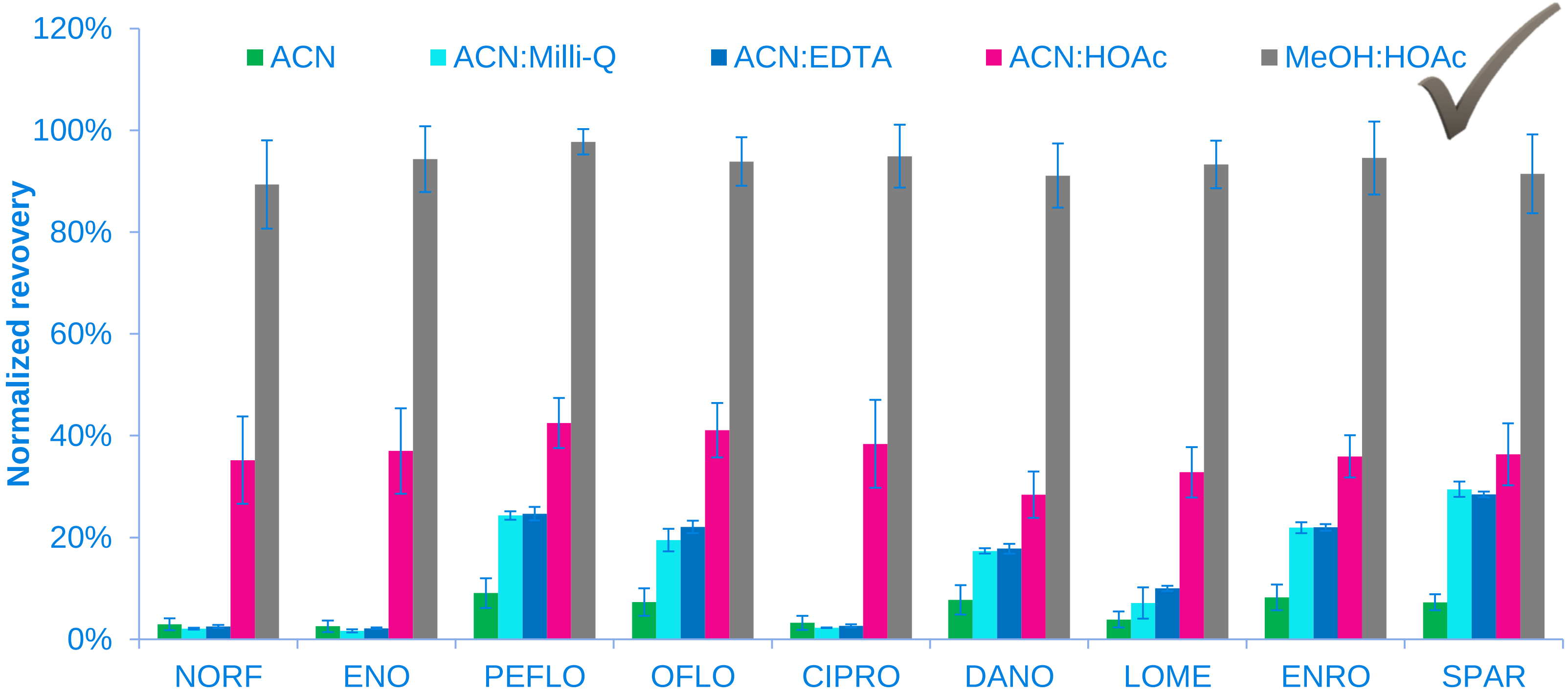


2.2 Fish biofluids: plasma and bile

2.3 Environmental waters: seawater, estuarine water and WWTP water

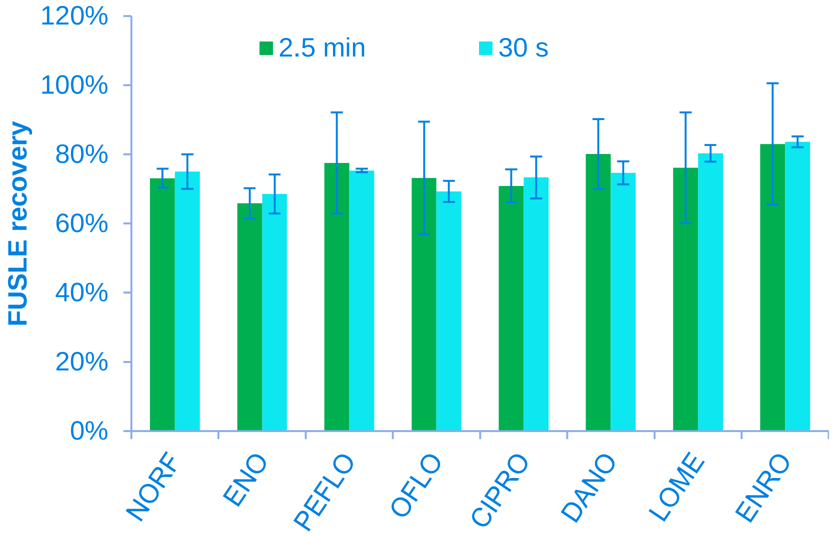
Fish tissues: FUSLE

- Extraction solvent



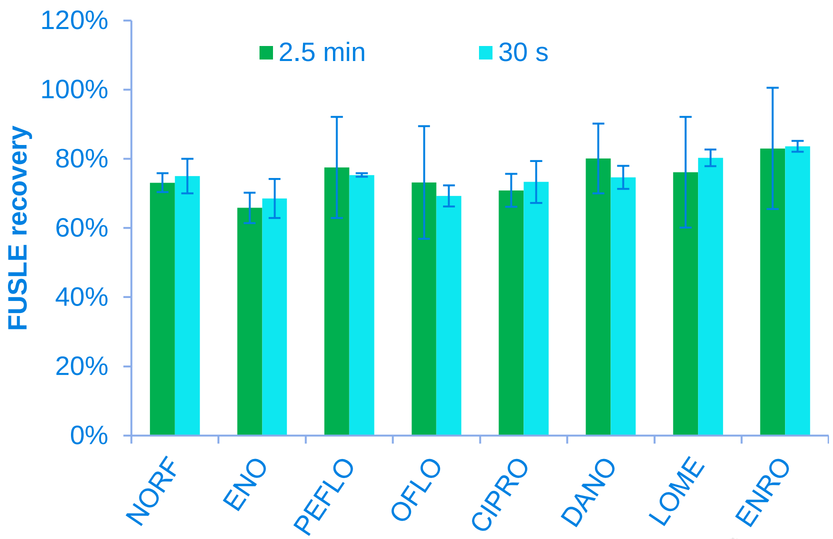
Fish tissues: FUSLE

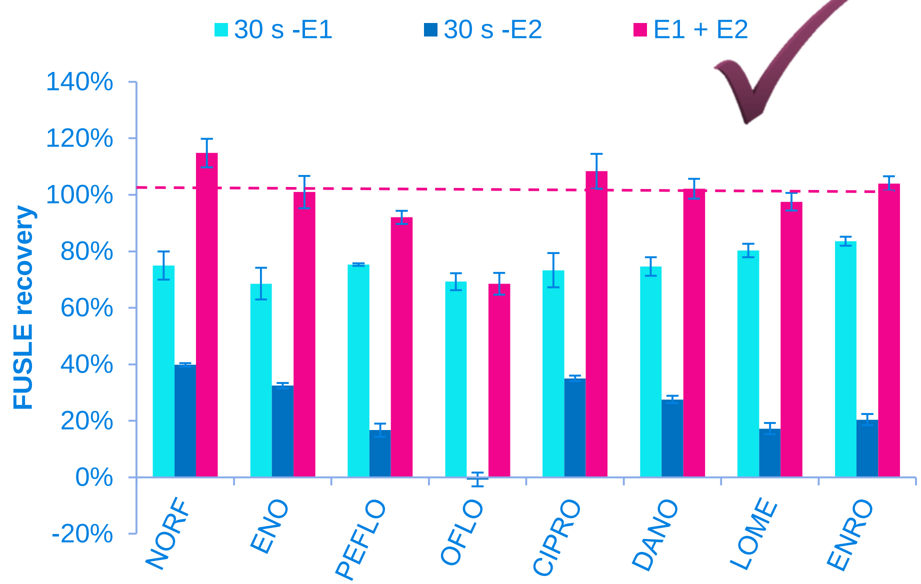
- Extraction time



Fish tissues: FUSLE

- Extraction time

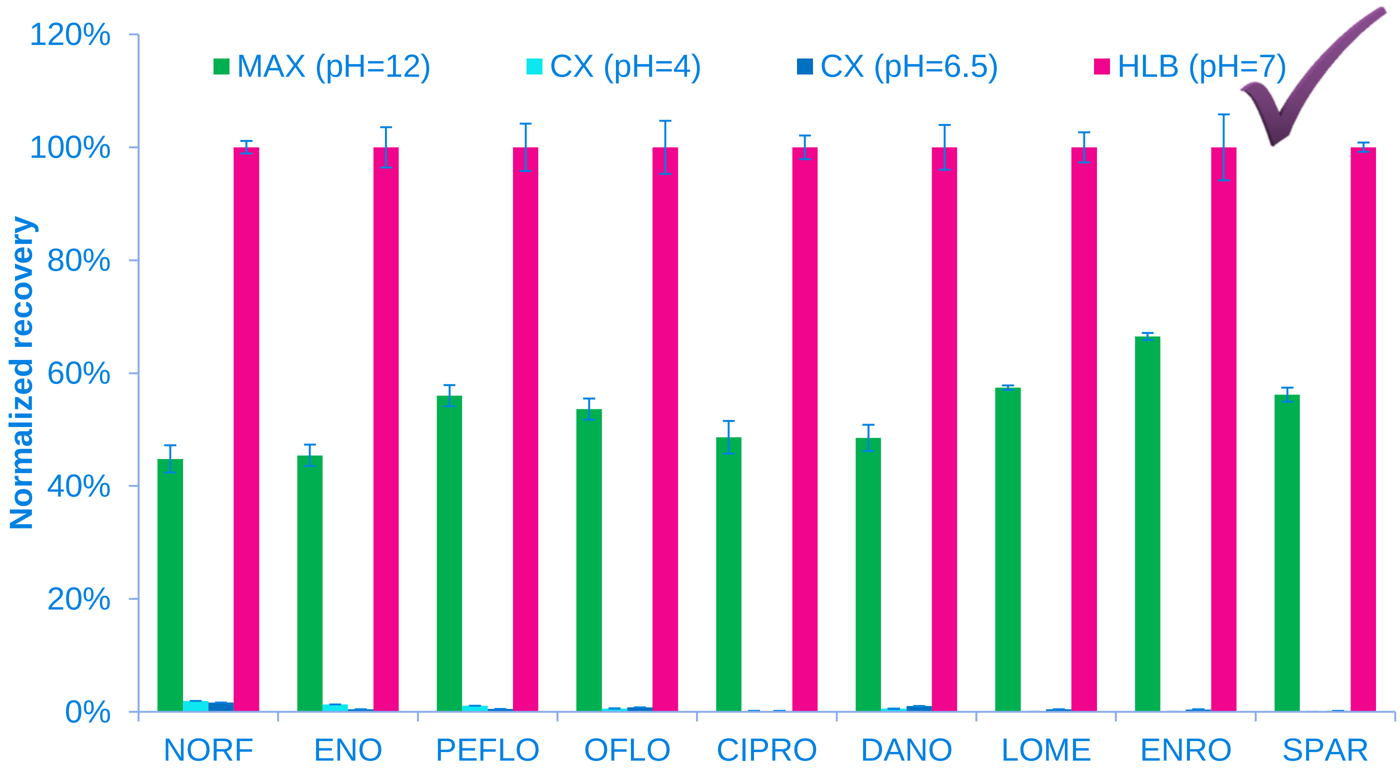




Optimum: 30 s x 2

Solid-Phase Extraction (SPE)

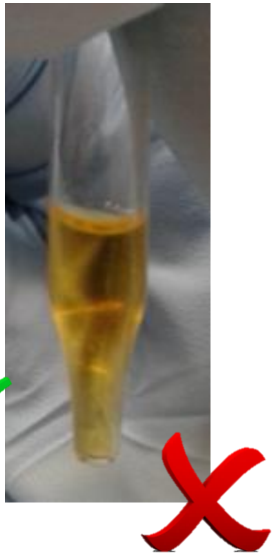
- Sorbent phase



Liver: dirty extracts

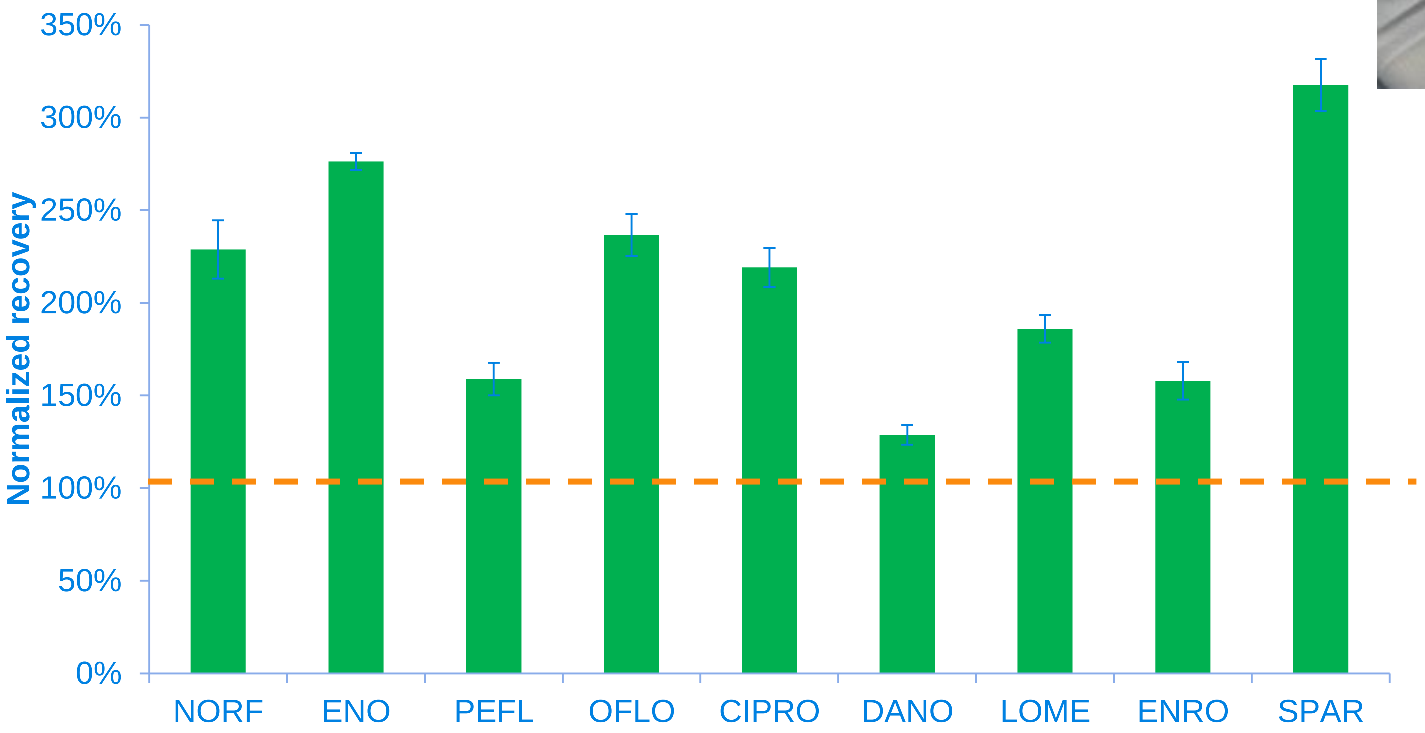
Muscle

Liver

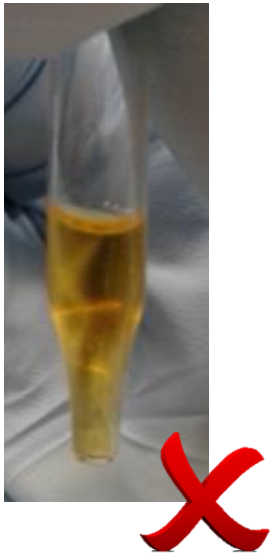


Liver: dirty extracts

- Positive matrix effect



Liver



Liver: extra step to remove lipids

- Clean-up strategies

Liver: extra step to remove lipids

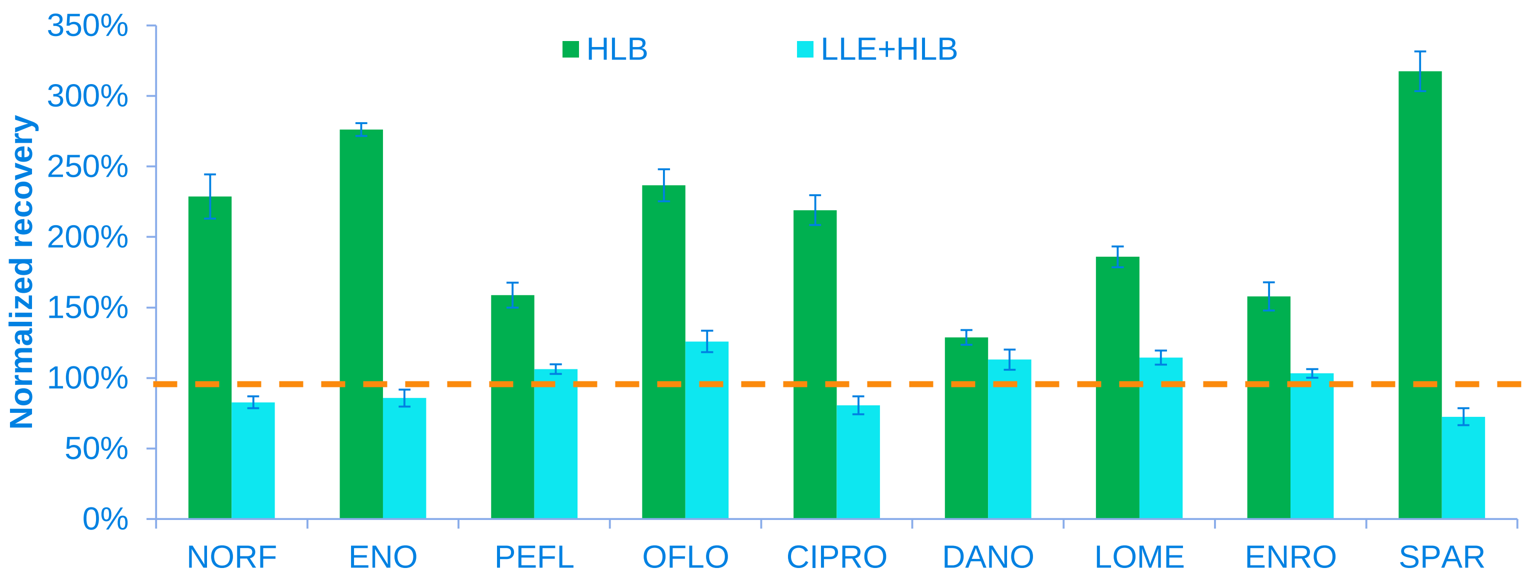
- Clean-up strategies
  1. Increase HLB phase

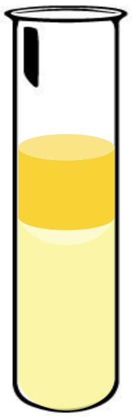
Liver: extra step to remove lipids

- Clean-up strategies
  1. Increase HLB phase ✗
  2. Florisil-HLB ✗

Liver: extra step to remove lipids

- Clean-up strategies
  1. Increase HLB phase ✗
  2. Florisil-HLB ✗
  3. Liquid-liquid extraction (LLE) prior to HLB ✓





Optimization of the extraction

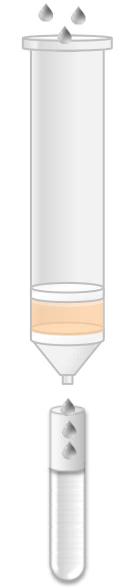
2.1 Fish tissues: liver and muscle

2.2 Fish biofluids: plasma and bile  

2.3 Environmental waters: seawater, estuarine water and WWTP water

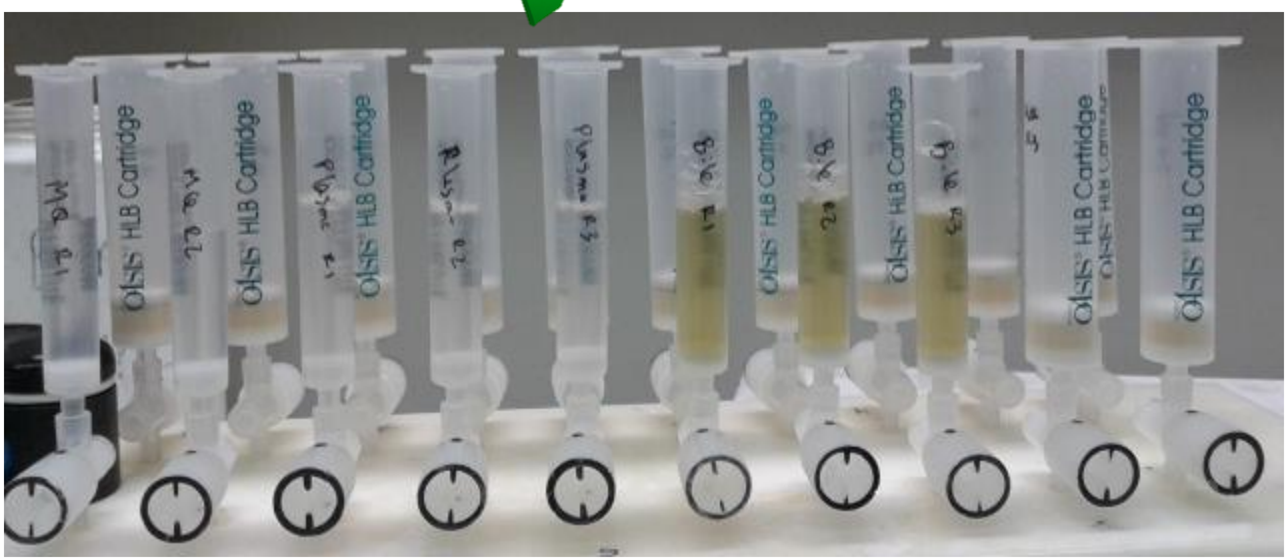
Biofluids: SPE

- HLB: signal suppression



Biofluids: SPE

- HLB: signal suppression
- MIPs cartridges



Optimization of the extraction

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RP-SPE (Oasis HLB)

- Seawater ✓
- Estuarine and effluent: dirty extracts
  - Signal suppression (> 50 %)

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- Estuarine and effluent: dirty extracts
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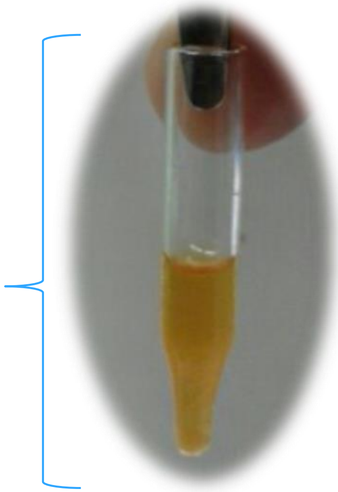
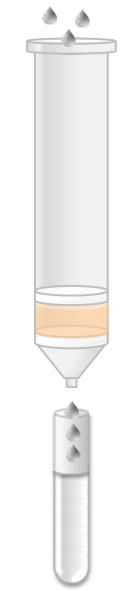
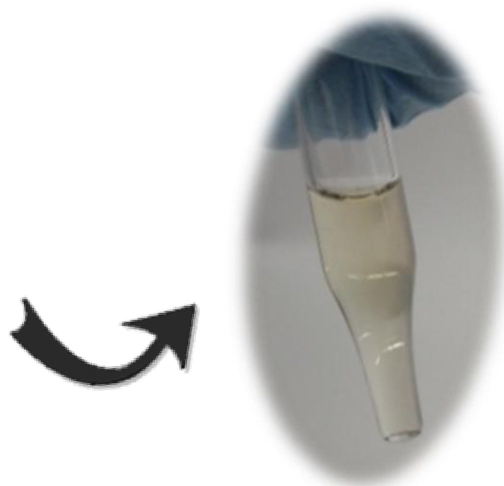




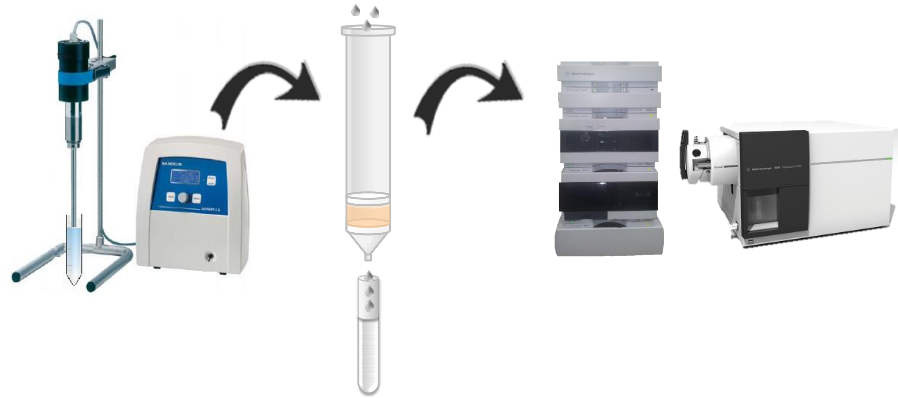
- Solution:

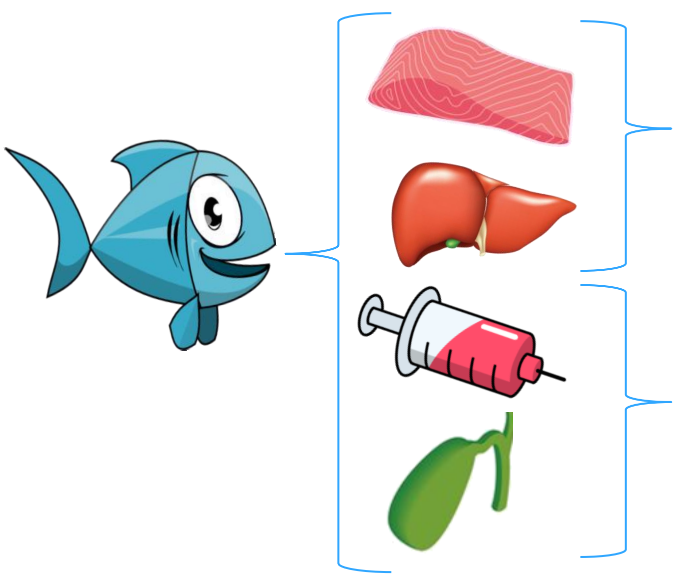
WAX + HLB

- Cleaner extracts and less signal suppression (< 33 %)



Method validation



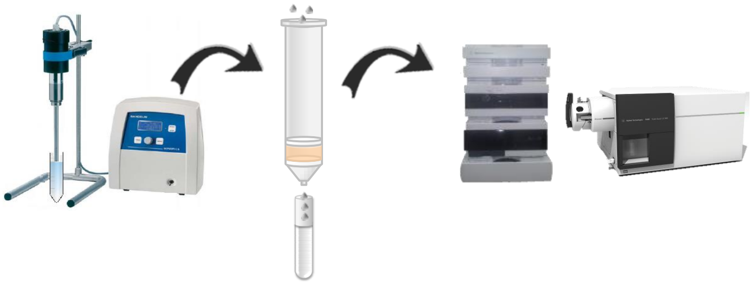


75 ng/g

200 ng/g

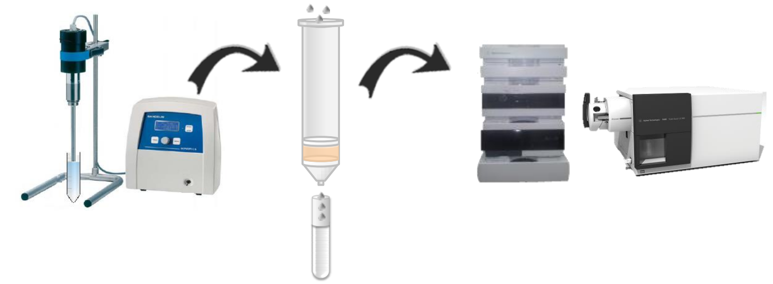
75 ng/L

Method validation



- Linearity
  - R^2: 0.997-0.9997
- Limits of detection
  - LOD < 2 ug/mL
  - LOQ < 5 ug/mL
- Precision
  - RSD < 15 %
- Accuracy

Method validation

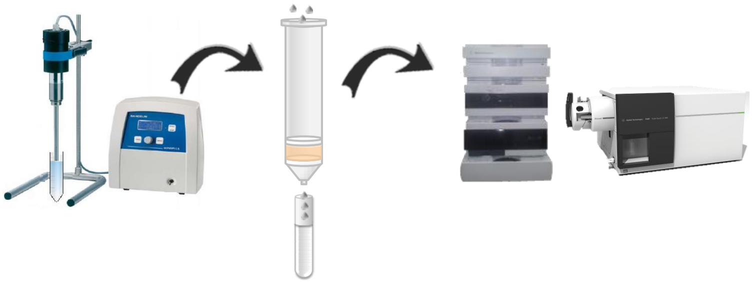


- Apparent recoveries: 80 - 126 %

Analyte	Fish tissues		Fish biofluids		Environmental waters		
	Liver	Muscle	Plasma	Bile	Seawater	Estuarine	Effluent
NORF[a]	107	96	93	96	90	84	93
ENO[a]	89	83	95	104	80	82	83
PEFLO[b]	103	89	91	97	101	109	109
OFLO[b]	96	94	110	105	105	93	125
CIPRO[a]	92	92	96	97	87	85	109
DANO[b]	104	92	115	94	112	109	118
LOME[b]	82	94	103	106	106	120	126
ENRO[b]	90	94	100	101	101	97	99
SPAR[a]	14	162	109	70	67	54	59

[a]Corrected with [$^{2}H_{8}$]–CIPRO. [b]Corrected with [$^{2}H_{5}$]–ENRO.

Method validation



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Results and Discussion

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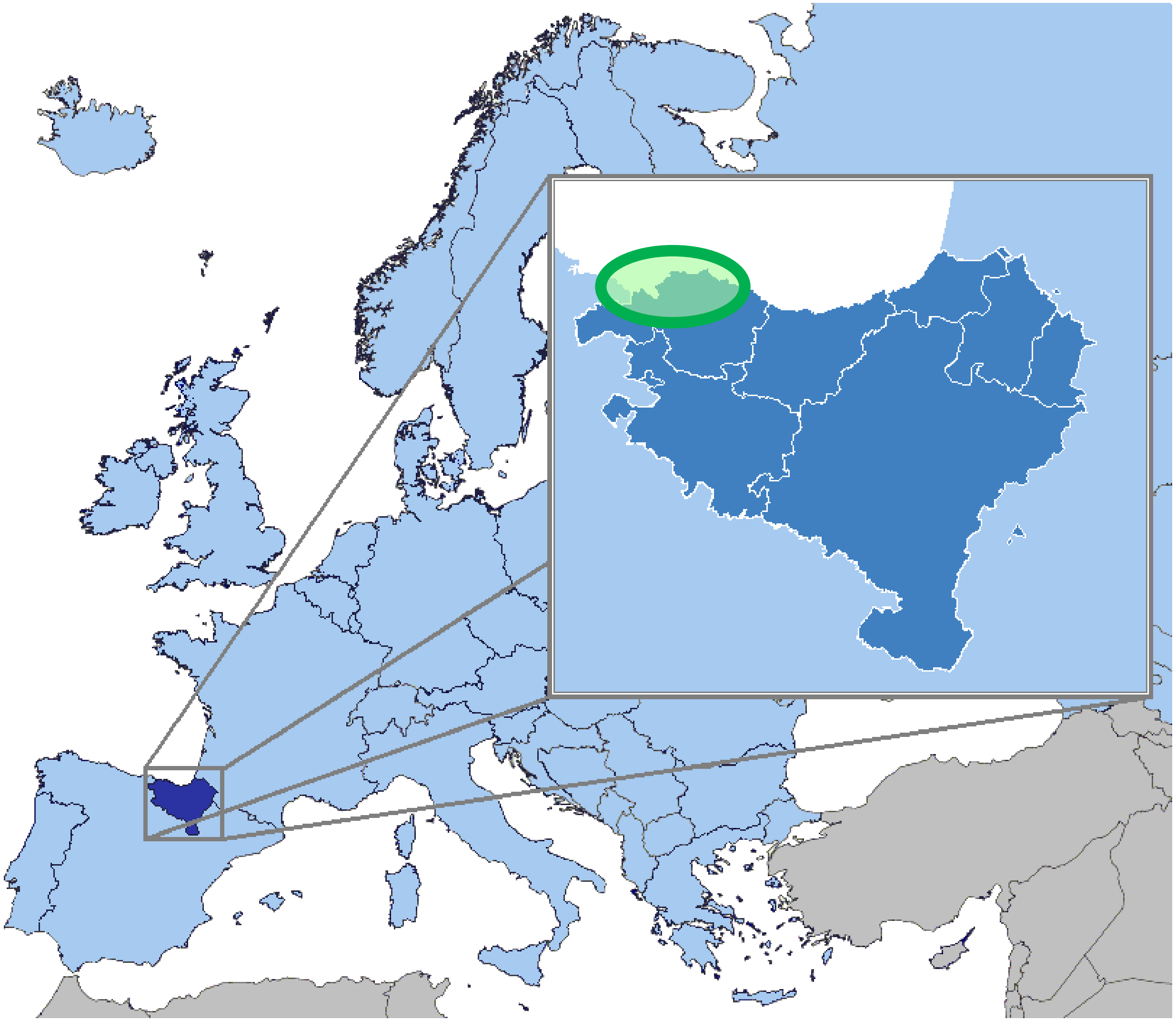
Real Samples from Biscay Coast



Real Samples from Biscay Coast

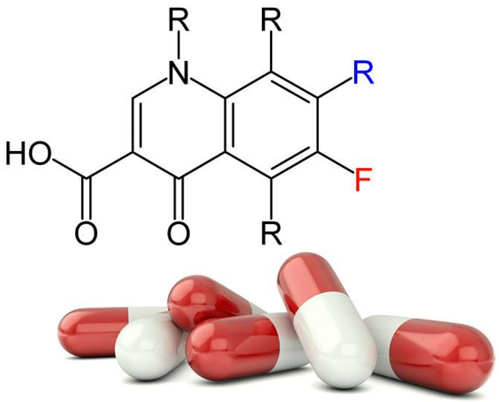
Real Samples from Biscay Coast

- WWTP effluent
  - 143 ng/L NORF
  - 160 ng/L CIPRO
  - 234 ng/L OFLO/LEVO
- Estuarine water
  - 44 ng/L NORF
  - 79 ng/L CIPRO
  - 278 ng/L OFLO/LEVO
- Grey mullet liver
  - 4 ng/g OFLO/LEVO
- Seawater < LOQ

Conclusions

Conclusions

- Optimization of 10 FQs
  - Correction with labelled analogues



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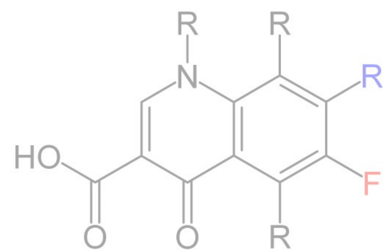
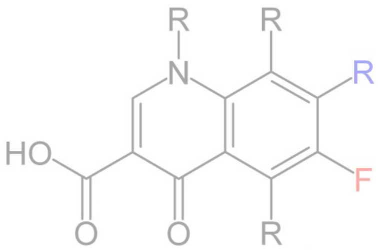


- Optimization of 10 FQs
  - Correction with labelled analogues

- 7 matrices with different clean-up strategies:
  - Fish muscle and seawater: RP-SPE
  - Fish liver: LLE + RP-SPE
  - Fish bile and plasma: MIPs
  - Estuarine and effluent waters: WAX + RP-SPE

Conclusions



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- Real samples from the Biscay Coast:
  - CIPRO, NORF and OFLO/LEVO
  - Water and fish liver samples

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Tussen takk!


FAAFETAI LAVA
MATONDI
DHANYAVADALU
KHOB CHAI
ANI KIÉ
GAMSA HAMNIDA
TRUGÉRÉ
TAPADH LEAT
ABOUÏ NGAN
ENKOSI
SOBODI
WAAD MAHADSANTAHAY
SAGOLUN
MÈSI
KIA ORA
MURAKOZE
KÖSZÖNÖM
NANNI
MAURUURU
AKPÉ
MERCI
TAKK
MERKZI
SHUKRIYA
SULPÁY
XIÈXIE
GRATIAS AGO
MANANA
ESKERRIK ASKO
DANKE
BLAGODARIA
DÍKY
NOUARI
AGUYJÉ
MEHARBANI
ASANTE SANA
TAIKU
RAHMAT
DANK U WEL
HVALA
GRÀCIES
RAHMAT
RAHMAT
KE YA LEBOHA
GRANDMERCÉ
AKIBA
TERIMA KASIH
MALO TE OFA
DANKON
GRAZIE
O SHEUN
KYAY TZU TIN PA TE
TESEKKUR EDIREM
TACK
SUKRIYA
DANKI
KOP KHUN KHA
TAK
SPAS
MAHALO
WELALIN
NANDRI
FOFO
VINAKA
DZIAKUJU
GO RAIBH MAITH AGAT
SHUKRAN
TANEMIRT
MOTASHAKKERAM
WOPILA
ARIGATO
KIITOS
WAITA
NGIYABONGA
BIREPO
MSAPO
DANKIE
BUZNYG
MATONDO
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FALEMINDERIT
ORKUN
MISAOTRA
AABHAR
WADO
GRATZIAS
GRACIÑAS
HVALA
DANKEWOL
EFHARISTO
CHNORAKALOUTIOUN
DHANYABAAD
AABHAR
TUALUMBA
BARKAL
NAGODE
TENKI
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DIDI MADLOBA
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RAHMET

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