EDC WFD

Next step: Towards Interlaboratory Comparison and standardisation

7-9 September 2022

EDC WFD

- The aim of this ILC is to demonstrate the fitness for purpose of the optimised and validated methods in the project:
  - MS-based methods (GC or LC coupled to MS (TQD or HRMS)
  - Effect-based methods (ER Calux and A-YES)

- This ILC will allow to define performance characteristics of the methods in terms of repeatability within laboratories and reproducibility of the validated methods and support standardization process

Time schedule

by 30 th September 2022 The Organizer send the Protocol to the laboratories
by 2nd November 2022 The test materials will be sent to each involved laboratory
by 23rd November 2022 The involved laboratories will provide the results to ilc-empir@isprambiente.it
by 20th December 2022 A Preliminary statistical evaluation will be sent to the involved laboratories
By 15th February 2023 The Final Report will be sent to the involved laboratories. Plenary meeting for the presentation and discussion of the results

MATERIALS

- 2 materials representative of natural waters
- 1 QC as blank water
- Delivered as kits to be reconstituted by each laboratory
- Kit will be constituted of 4*1l water + SPM + DOC solution+ spike solution
- A written protocol + video will be delivered ⇒ the receipt

In order to formalize your pre-registration for this ILC, we kindly ask you to fill the following pre-registration form
https://forms.gle/dBYjMCDShkHJLfks7

New Deadline to register: 23th of September 2022

ILC contact persons

Paolo de Zorzi (ISPRA): paolo.dezorzi@isprambiente.it
Teemu Näykki (SYKE): teemu.naykki@syke.fi
Sabrina Barbizzi (ISPRA): sabrina.barbizzi@isprambiente.it

ISO/TC 147/SC 2/WG 84Estrogens using MS based methods

Determination of selected oestrogens in whole water samples — Method using solid phase extraction (SPE) followed by chromatography coupled to mass spectrometric detection

- Convenor and project leader: Sophie Lardy-Fontan (LNE, AFNOR)
- Support lead: Jochen Türk (IUTA, DIN)

- WG officially installed in T1 2022
- Standard to be published in T1 2024


QUESTIONS?
COMMENTS?
CONCERNS?

EDC WFD

Training workshop: Solutions to tackle WFD requirements for estrogen determination in water

7-9 September 2022

EDC WFD

BEFORE TO START

THIS TRAINING WILL BE REGISTERED

DOES ANYONE HAVE AN OBJECTION?

- This **Training/Workshop** aims:
  - to present the knowledge gained from the EDC-WFD project whose objective is to develop reliable and harmonized measurement methods for estrogens, which are key Endocrine Disrupting Chemicals (EDC), to comply with Water Framework Directive requirements

  - to accelerate the transfer of the most promising measurement methods and methodologies to interested parties: laboratories, PTproviders, researchers.

- The training workshop will cover all aspects of measurements from sampling to final method validation and will address both Mass spectrometry based methods as well as incoming Effect Based Methods (in vitro bioessays).

7th of September
Session 1

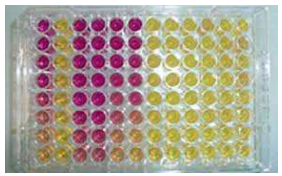
09:00 - 09:10: Welcome address
09:10 - 09:50: Presentation of the project and context
09:50 - 10:20: Issues and challenges related to estrogen analysis in relation to the WFD
10:20 - 11h00: Challenges related to sampling
11:00 - 11:15: Break
11:15 - 11:35: Overview of quantification strategy
11:35 - 12:15: Sample preparation

8th of September
Session 2

09:00 - 09:30: Discussion forum / debriefing from day 1
09:30 - 10:30: Mass spectrometry methods - Instrumental developments
10:30 - 10:45: Break
10:45 - 11:45: Achievements of Mass spectrometry based methods _ method performances and measurement reliability

11:45 - 12:00: Concluding remarks
12:00 - 12:15: Next step _ Towards Interlaboratory Comparison

9th of september
Session 3 dedicated to Effect Based Methods (EBM)



09:00 - 09:10 : Welcome address
09:10 - 09:40 : Presentation of the project and context
09:40 - 10:05 : Context and presentation of EBM methods versus MS based methods
10:05 - 10:40 : EBM protocols
10:40 - 11:15 : EBM data treatments
11:15 - 11:30 : Break
11:30 - 11:45 : Concluding remarks
11:45 - 12:00 : Next step : Towards Interlaboratory Comparison

About Session 1

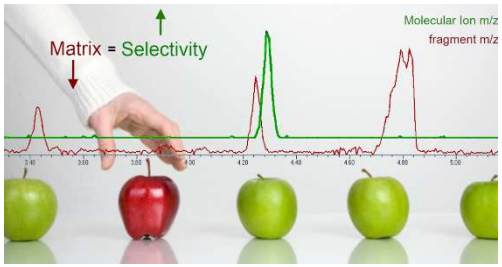
EDC WFD

Mass spectrometry methods - Instrumental developments

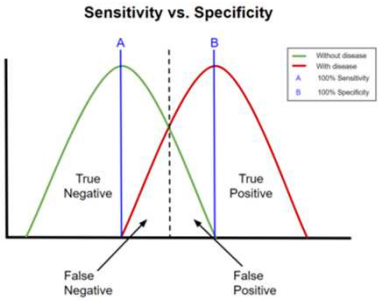
7-9 September 2022

EDC WFD

Mass Spectrometry methods – Instrumental developments



- Low concentration
- Separation
- Selectivity
- Sensitivity
- Matrix effect/background



Mass Spectrometry methods – Instrumental developments

Final organic extract after sample preparation

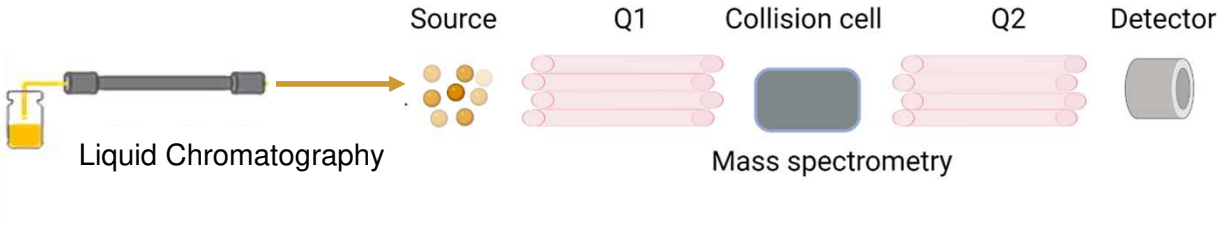
- LC/MSMS (QQQ) free estrogens

- LC/MSMS (QQQ) after derivatization
- LC/HRMS

- GC/MSMS (QQQ) after derivatization

- GC/HRMS

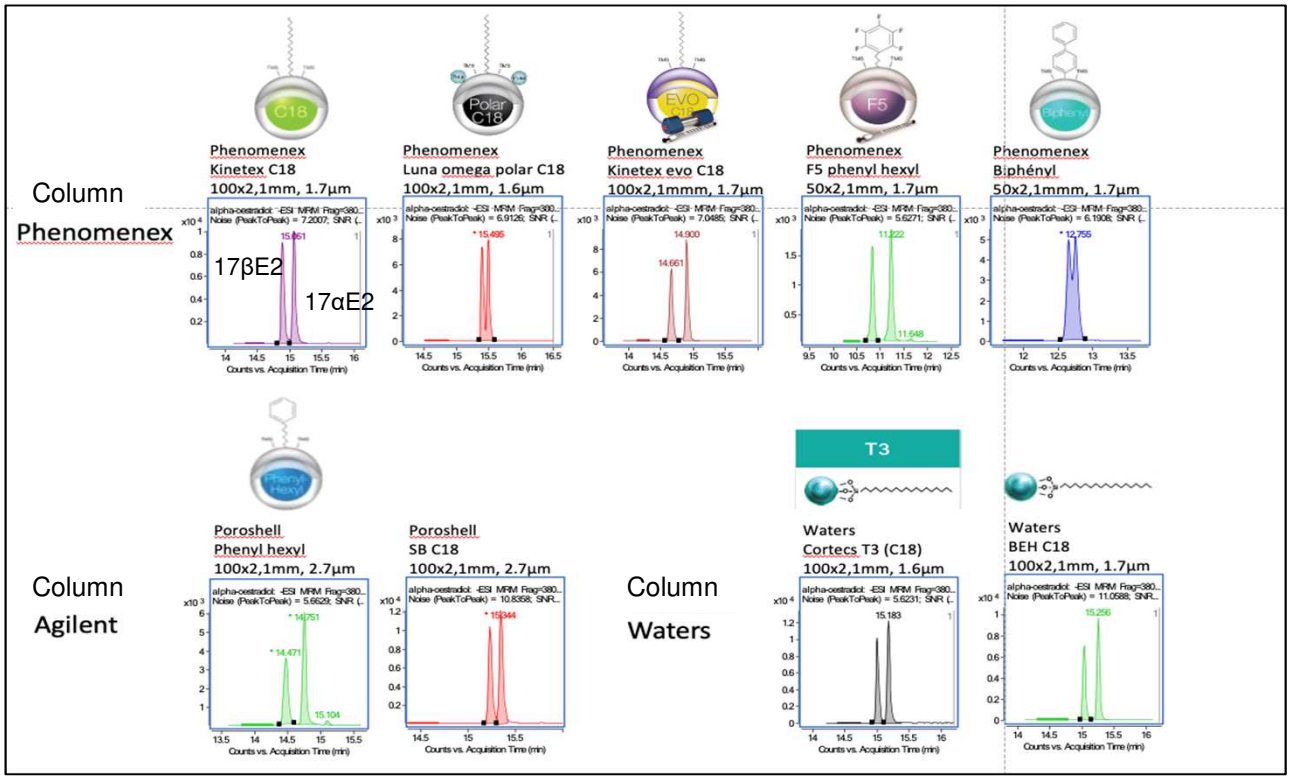
LC/MSMS


Source
Q1
Collision cell
Q2
Detector
Liquid Chromatography
Mass spectrometry

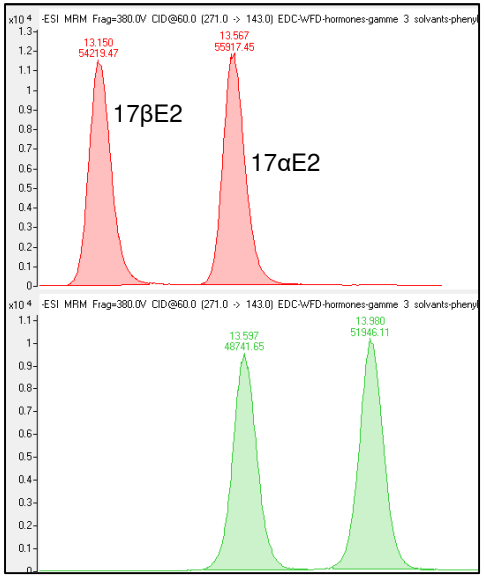
LC/MSMS

- LC parameter optimisation
  - Column
  - Mobile phase
  - Chromatographic separation
- MSMS parameter optimisation
  - MRM
  - Sensitivity, selectivity
- Summary and conclusion

LC parameter optimization : choice of column



LC parameter optimization : choice of column



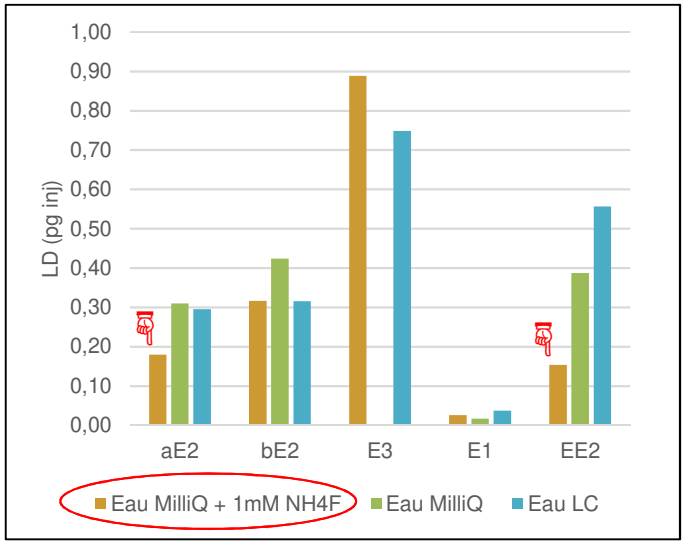
Column Poroshell Phenylhexyl 1.9µm

Column Poroshell Phenylhexyl 2.7µm

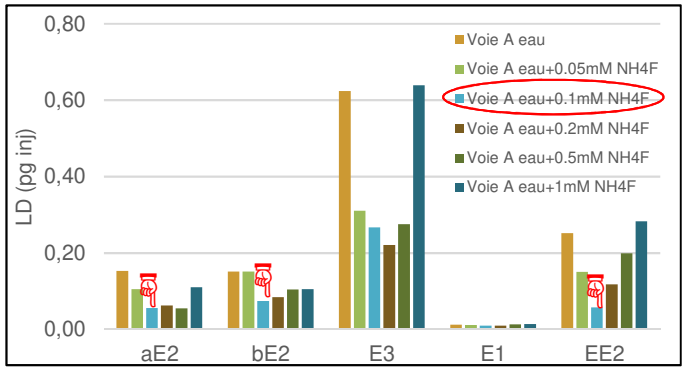
LC parameter optimization : mobile phase (composition)

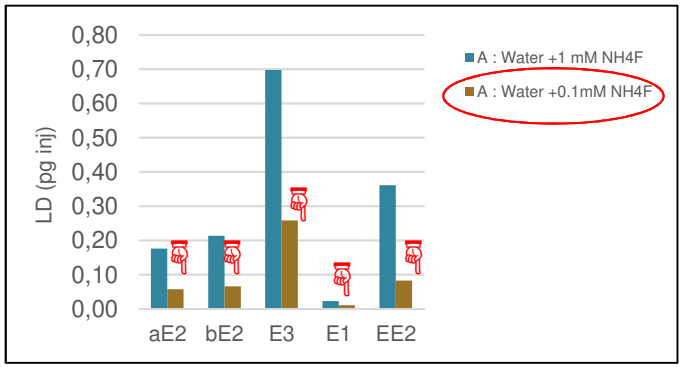
Influence of NH4F on the detection limit

- Colonne phenyl hexyl



- Colonne SB C18



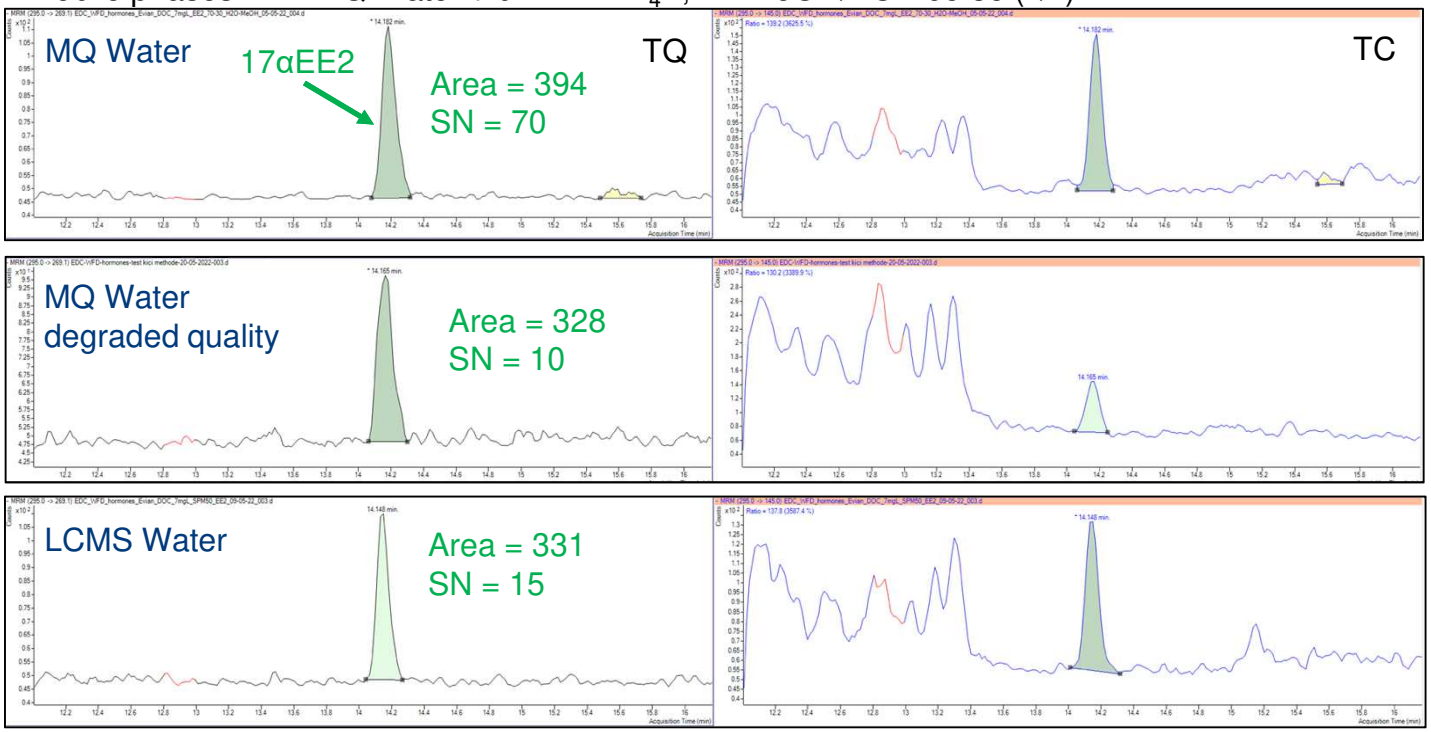


MQ Water + x% NH_4F
MeOH/ACN 65:35 (v/v)

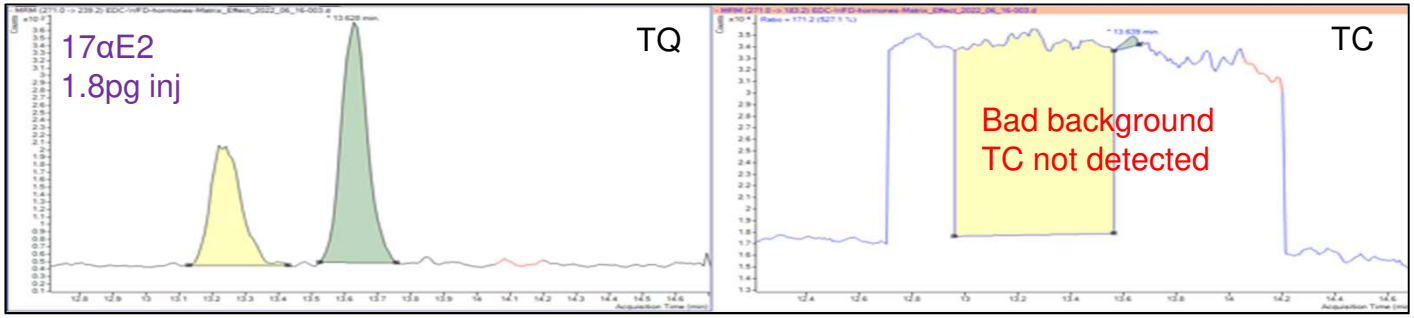
LC parameter optimization : mobile phase (quality)

17αEE2 : 0.9pg inj

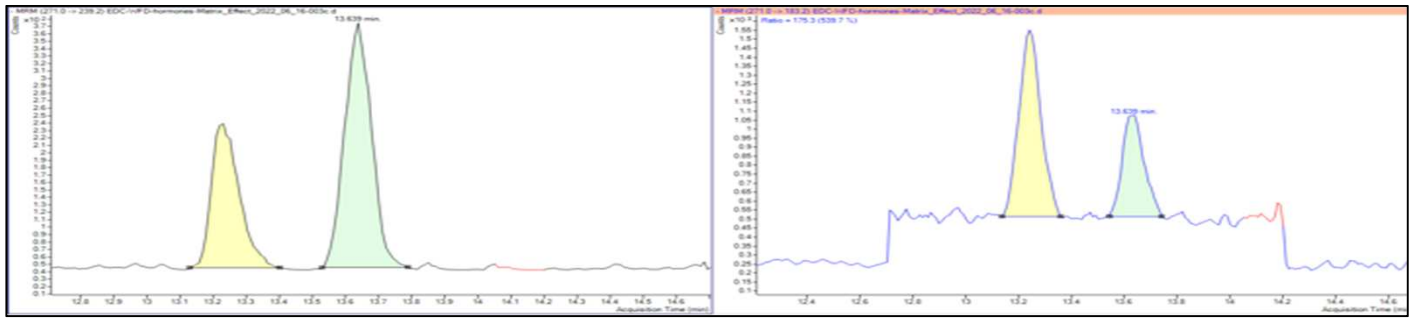
Mobile phases : A = MQ Water + 0.1mM NH_4F; B= MeOH/ACN 65:35 (v/v)



LC parameter optimization : mobile phase (quality)



old aqueous mobile phase (3 days)

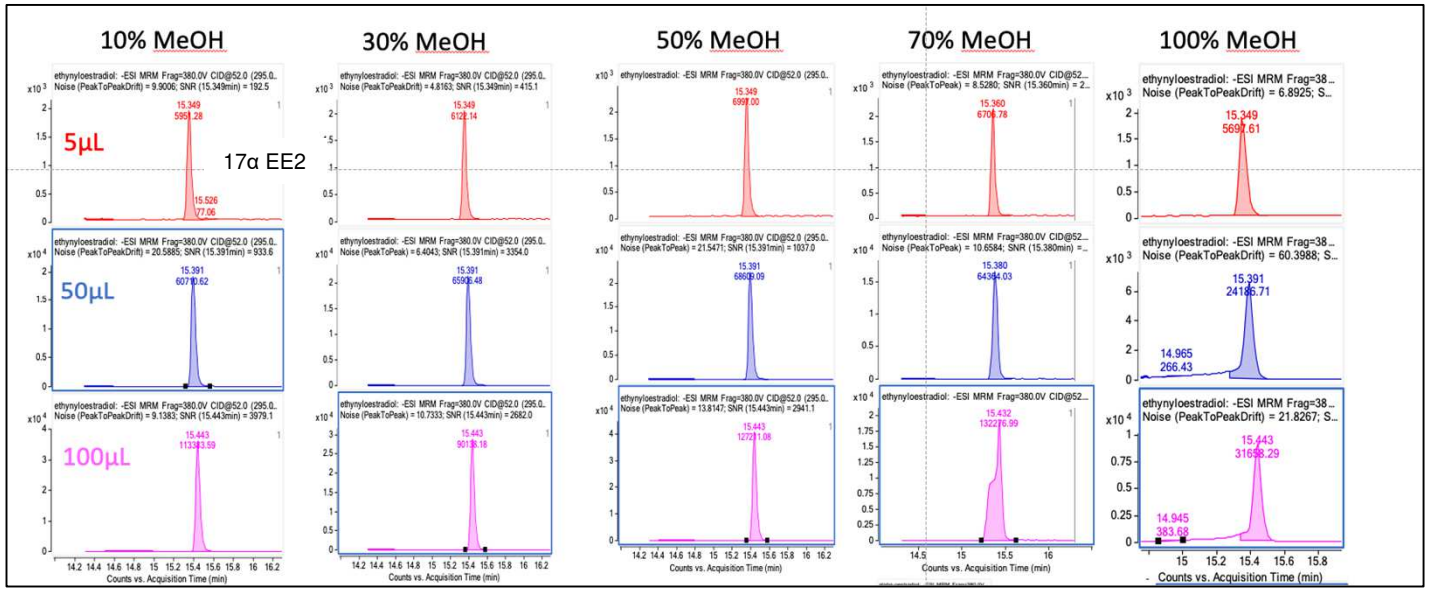


new aqueous mobile phase

MQ Water + 0.1mM NH_4F
MeOH/ACN 65:35 (v/v)

LC parameter optimization : injection solvent and volume

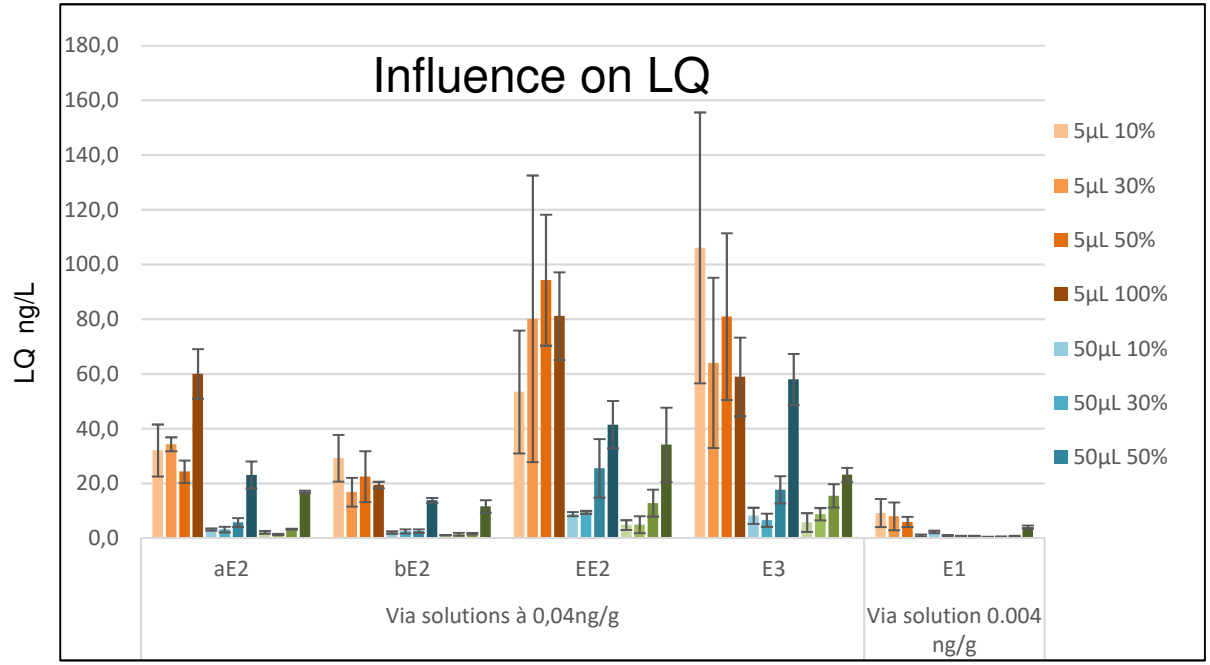
Influence on Peak shape



Peak shape of 17αEE2

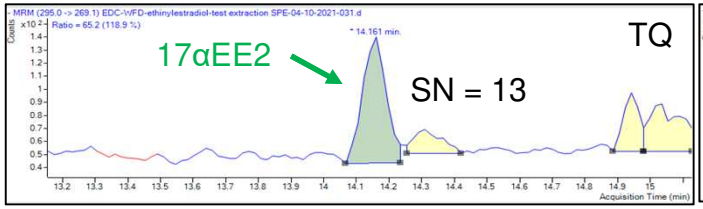
LC parameter optimization : injection solvent and volume

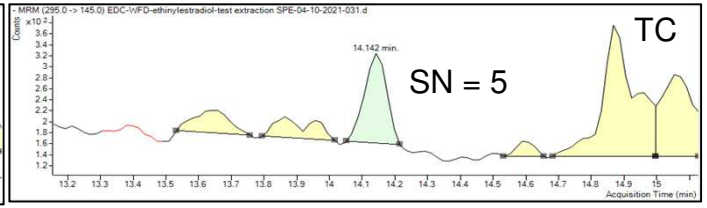
Mobile phases : A = MQ Water + 0.1mM NH_4F; B= MeOH/ACN 65:35 (v/v)



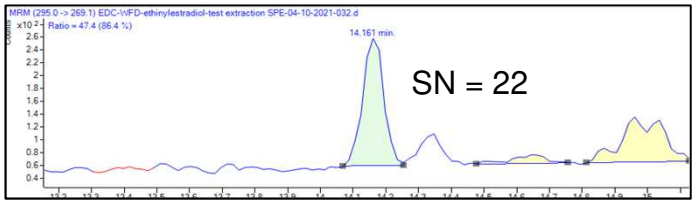
Best choice for lower LQ : 30% MeOH

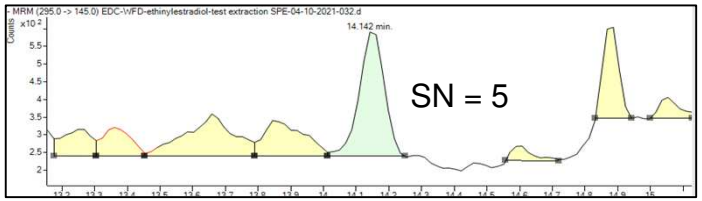
LC parameter optimization : injection solvent and volume



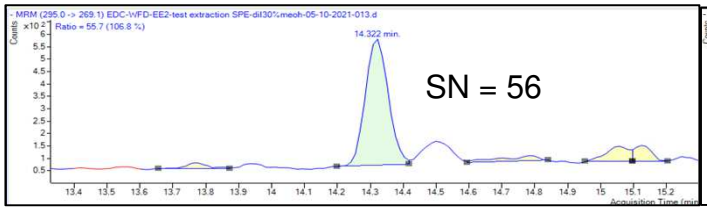


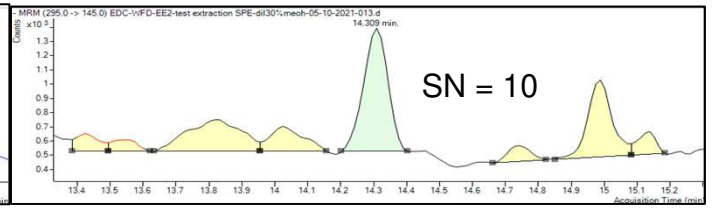
Organic extract, 100% MeOH, 5µL injected





Organic extract, 100% MeOH, 10µL injected





Organic extract, diluted 70:30 MQ Water/MeOH, 100µL injected

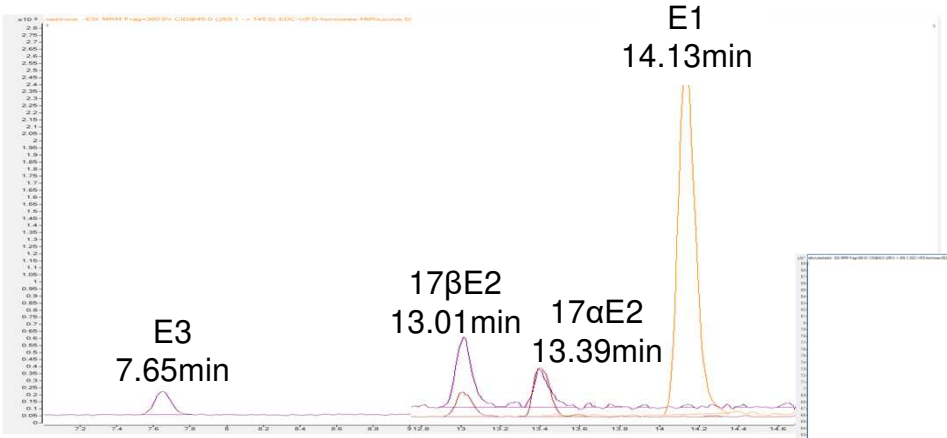
LC parameter optimization : optimized parameters

LC column	Poroshell 120 Phenylhexyl Acquity BEH C18 Waters Zorbax SB-Phenyl	Dp =1.9µm 2.1 x 100mm Dp=1.7µm 2.1 x 100mm Dp=1.8µm 2.1 x 100mm	Guard Filter 0.3µm Guard column Dp=1.9µm, 2.1x5mm Guard column 1.8µm, 2.1 x 5 mm	50°C 40°C 25°C
Mobile phase	MQ Water + 0.1mM NH_4F MQ Water + 0.3mM NH_4F MQ Water + 0.25mM NH_4F	MeOH/ACN 65:35 (v/v) MeOH/ACN 50:50 (v/v) MeOH		0.6cc/min 0.3cc/min 0.2cc/min
Solvent injection	MeOH (steroids multiresidue) and MQ water/MeOH 70:30 (v/v) (specific EE2) MQ Water + MeOH 65:35 (v/v) MeOH + MQ water 50:50 (v/v)			5µL, 100µL (17αEE2) 40µl 100µl 50 µl

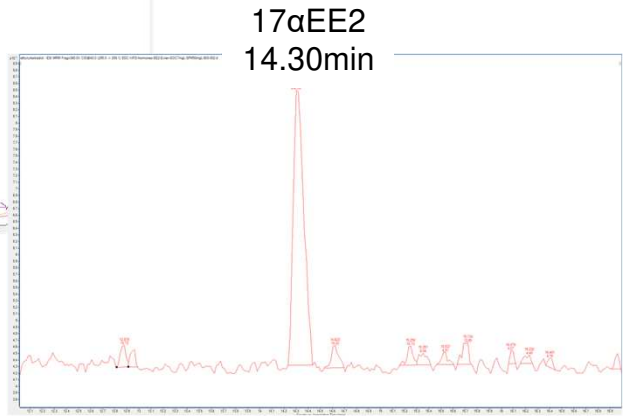
LC parameter optimization : one example of optimized parameters

Mobile phases : A = MQ Water + 0.1mM NH_4F; B = MeOH/ACN 65:35 (v/v)

Column Poroshell 120 Phenylhexyl : Dp = 2.1x100mm, 1.9µm



Multiresidue method : 1-1.8pg injected

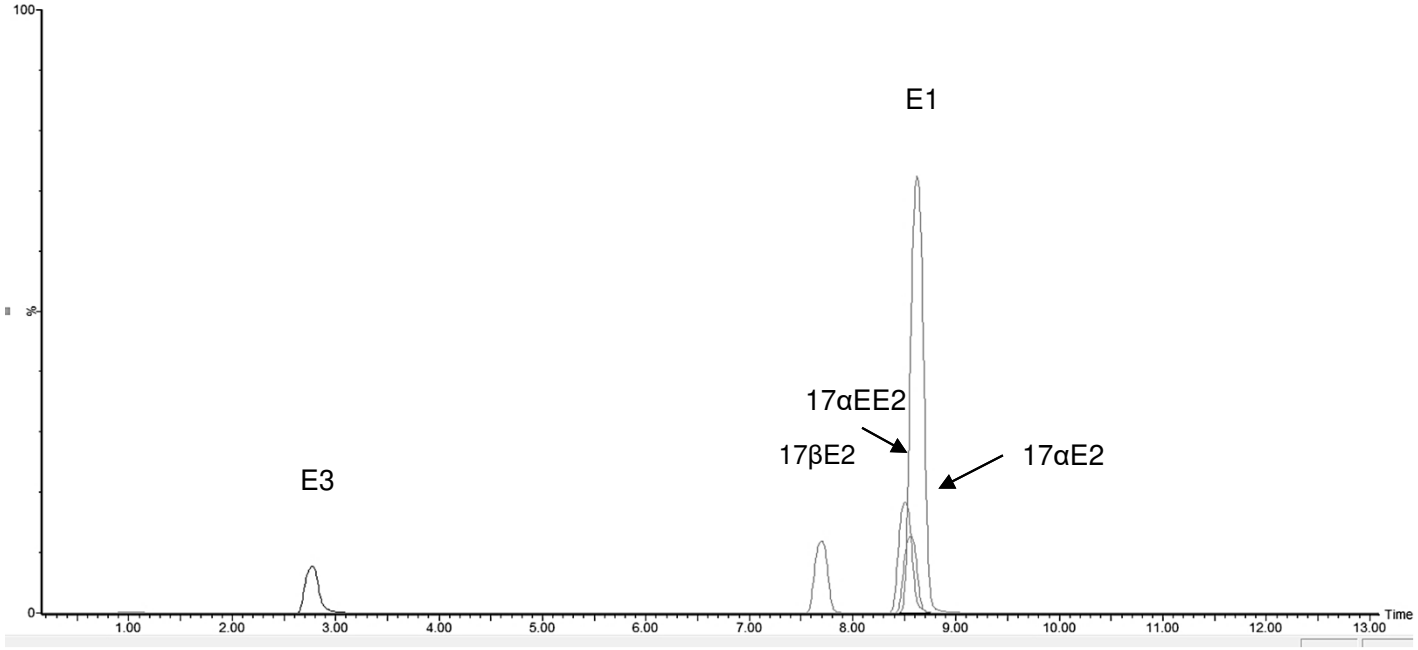


Specific 17aEE2 method : 0.6pg injected

LC parameter optimization : one example of optimized parameters

Mobile phases : A = MQ Water + 0.3mM NH_4F; B = MeOH/ACN 50:50 (v/v)

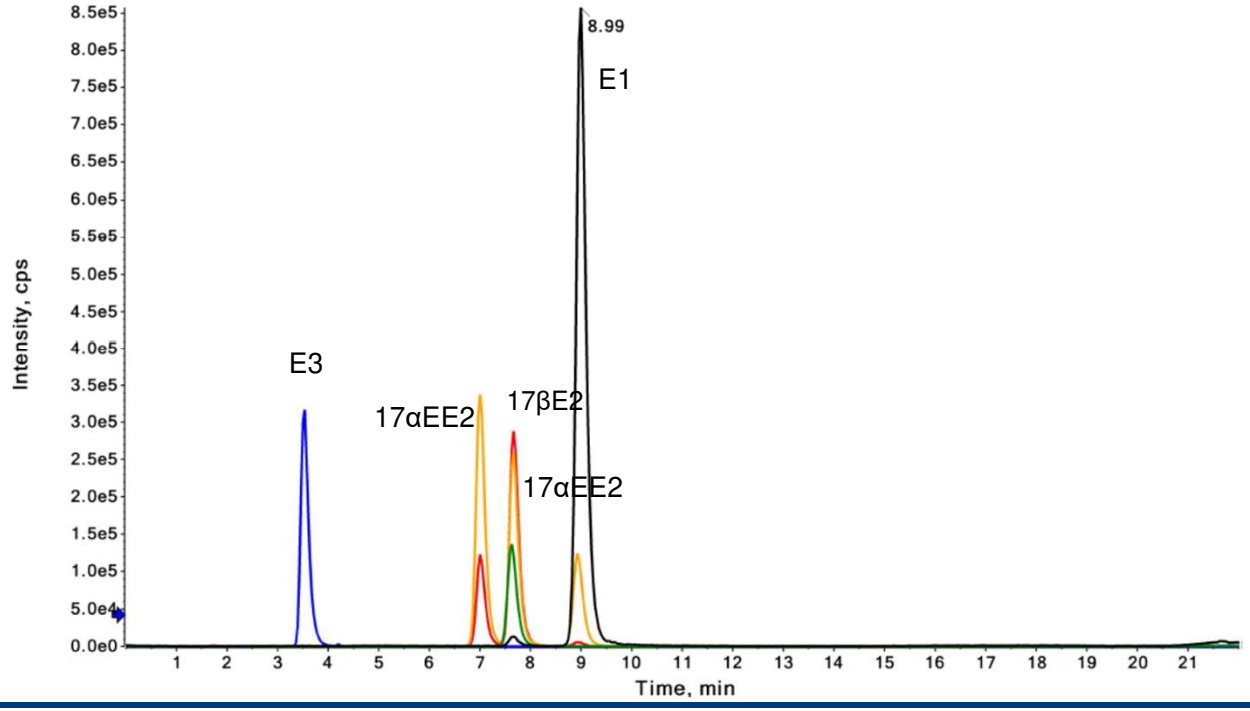
Column Acquity BEH C18 Waters : Dp = 2.1x100mm, 1.7µm



LC parameter optimization : one example of optimized parameters

Mobile phases : A = MQ Water + 0.25 mM NH_4F; B = MeOH

Column Zorbax SB-Phenyl: Dp = 2.1x100mm, 1.8 µm



MS/MS parameter optimization

Parameters to be optimised in mass spectrometry

- Source parameters (ionisation) : selectivity/sensitivity (S/N)
  - Ionisation mode (free estrogens : ESI -)
  - Flow (depending on instruments)
  - Temperature (depending on instruments)

- MRM of compounds : TQ, TC, TQ/TC
  - Area
  - Intensity
  - S/N

- Dwell time

MS/MS parameter optimization (source parameters)

Method 1 example on Agilent Technologies 6495

Method	Estrogen	Labelled estrogen	Sample injection
Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-$^{13}C_3$, 17αE2-d_2, 17βE2-$^{13}C_3$, E3-d_2, E1-d_4, 17βE2-d_4, 17αEE2-d_4	100% MeOH 5µL injected
Estrogen 17αEE2 specific	17αEE2	17αEE2-d_4	70:30 MQ water/MeOH, v/v 100µL injected

Parameter	Value
Acquisition mode	MRM
Ionisation mode	Electrospray Ionisation (ESI) negative
Gas temperature and flow	120°C, 16 L.min^{-1}
Nebulizer pressure	40 psi
Capillary voltage	3.5kV
Sheat gas temperature and flow	375°C, 12 L.min^{-1}
Collision gas (nitrogen 99,9990%) pressure	Fixed by the defaut value system
Nozzle voltage	300V

MS/MS parameter optimization (source parameters)

Method 2 example on TQS Micro Waters

Method	Estrogen	Labelled estrogen	Sample injection
Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-$^{13}C_3$ 17βE2-d_5, E3-$^{13}C_3$, 17αEE2-d_4	65% Water/35% MeOH 40µL injected

Parameter	Value
Acquisition mode	MRM
Ionisation mode	Electrospray Ionisation (ESI) negative
Gas temperature and flow	650°C, 1200 L.h^{-1}
Capillary voltage	2.0 kV
Gas cone flow	50 L.h^{-1}

MS/MS parameter optimization (source parameters)

Method 3 example on AB TSQ Sciex 6500

Method	Estrogen	Labelled estrogen	Sample injection
Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-d_2, 17αE2-d_2, 17βE2-$^{13}C_2$, E3-d_2, 17αEE2-$^{13}C_2$	50% Water/50% MeOH 50µL injected

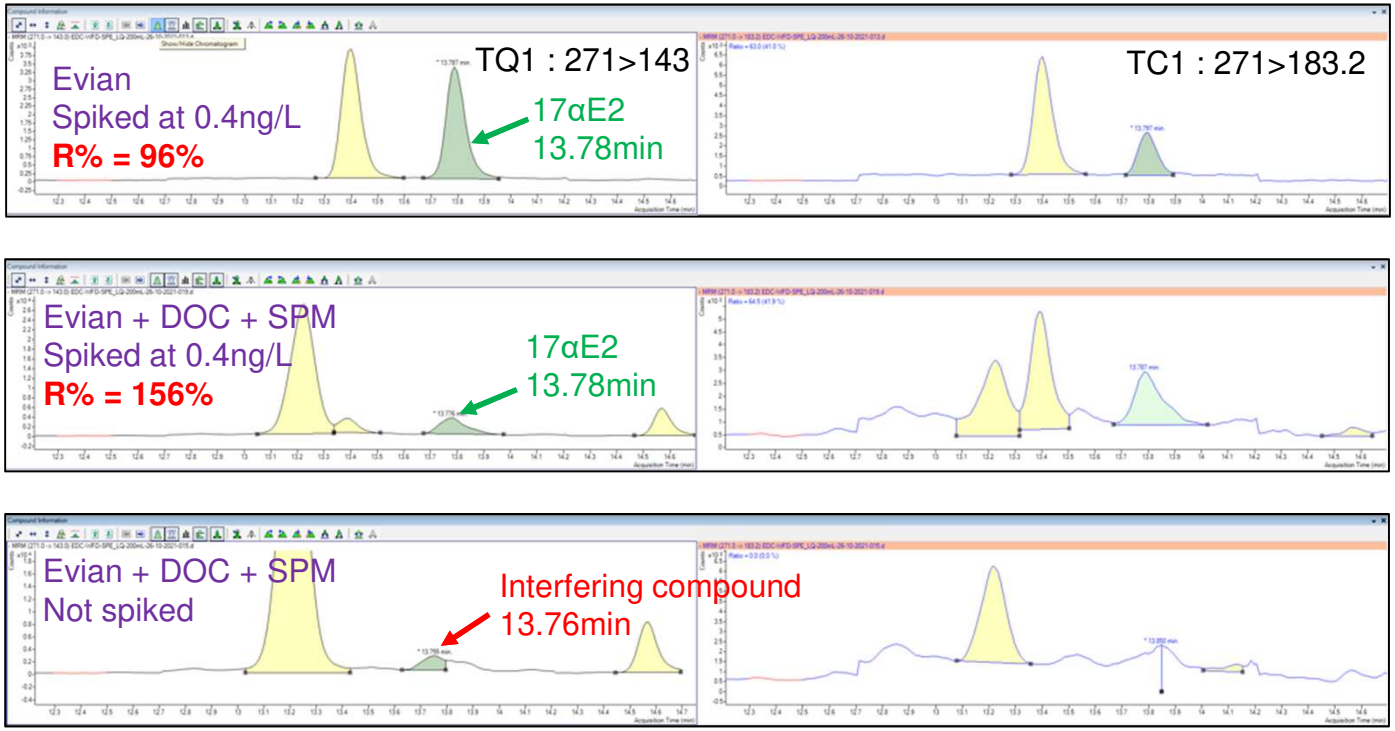
Parameter	Value
Acquisition mode	MRM
Ionisation mode	Electrospray Ionisation (ESI) negative
Source temperature	600°C
Capillary voltage	4500V
CAD	12psi
Curtain gas	20 psi
Csource gas	70psi
Exhaust	70psi

MS/MS parameter optimization : MRM choice

- Choice of MRMs on standard solutions
  - Area
  - Intensity
  - S/N
- First list of potential interesting MRMs
- Test of these MRMs on « real » sample extract (impact of the matrix in terms of sensitivity and selectivity)

MS/MS parameter optimization : MRM choice (interfering compounds)

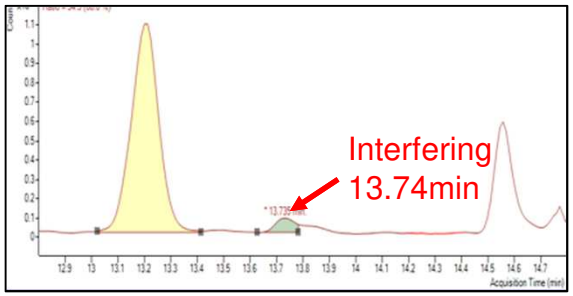
Evian Water / Evian Water + DOC 7mg/L + SPM 50mg/L (17αE2 at 0.4ng/L)
Loss of sensitivity and selectivity



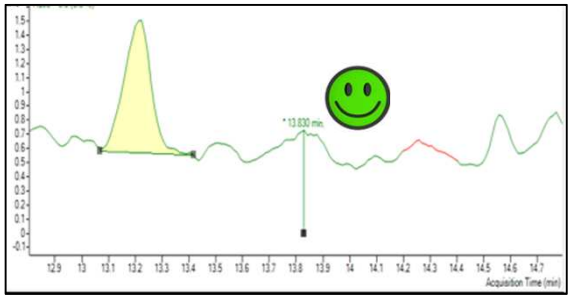
MS/MS parameter optimization : MRM choice (interfering compounds)

New tested transitions

Tested MRM 2 (TQ) : 271>145.3



Tested MRM 3 (TQ) : 271>239.2



Evian + DOC + SPM, not spiked

New optimised transitions
17αE2
TQ3 : 271>239.2
TC1 : 271>183.2

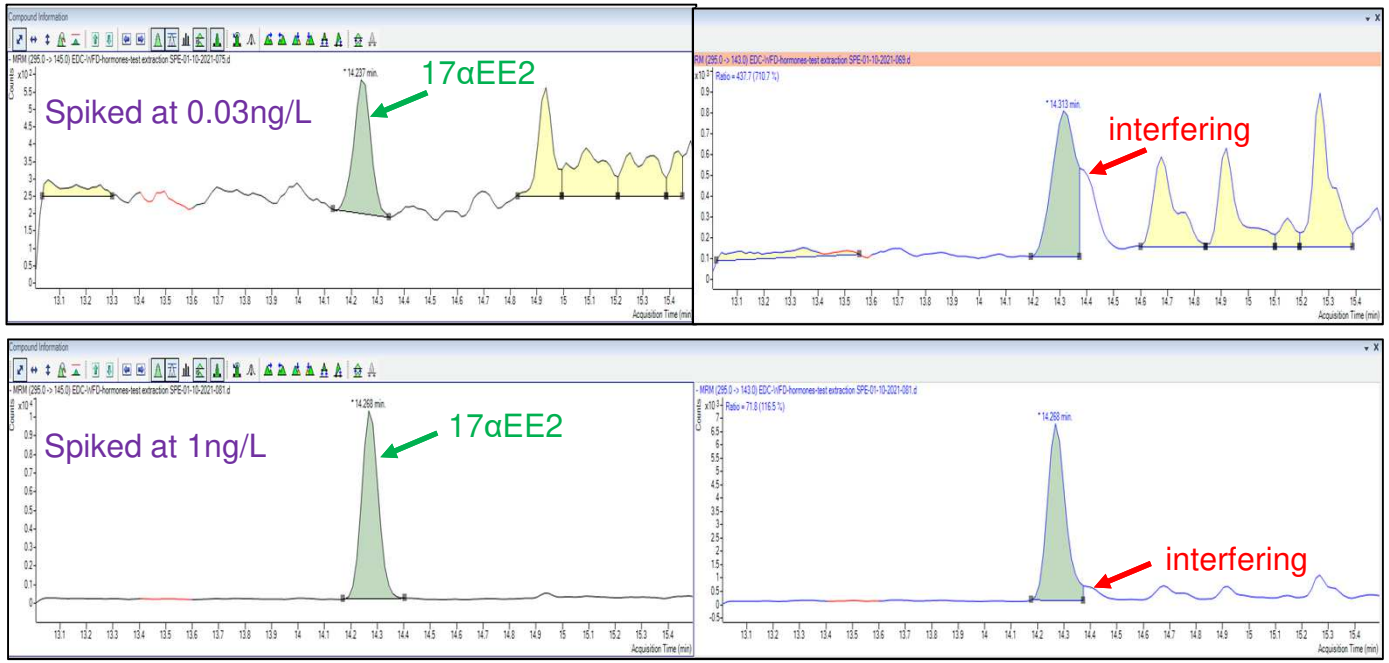
Evian Water + DOC + SPM spiked at 0.4ng/L
recovery = 95 ± 7%

MS/MS parameter optimization : MRM choice (interfering compounds)

Evian + DOC 7mg/L + SPM 50mg/L (300mL extracted on SPE cartridge OASIS HLB 200mg with purification step), 17αEE2 for example

TQ 1 : 295>145

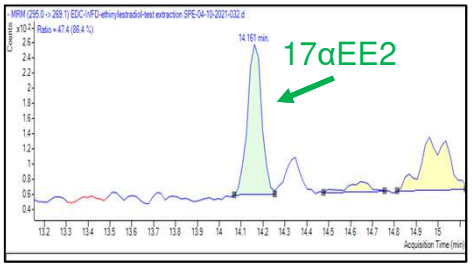
TC 1 : 295>143



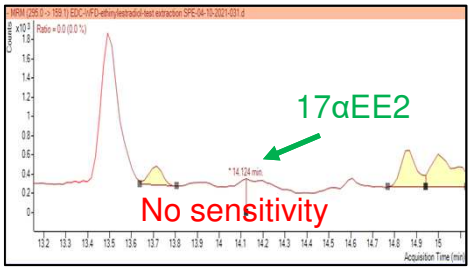
MS/MS parameter optimization : MRM choice (interfering compounds)

1- New tested transitions

Tested MRM 1 : 295>269.1

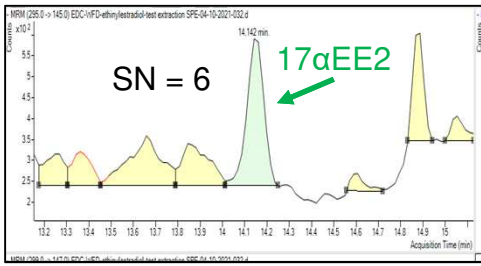


Tested MRM 2 : 295>159.1

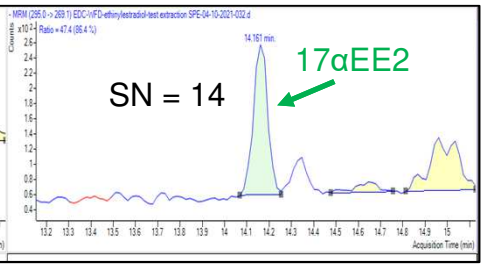


2- Comparison of the intensity of MRM

TQ 1 : 292>145



Tested MRM 1 : 295>269.1



New optimised MRM
17αEE2

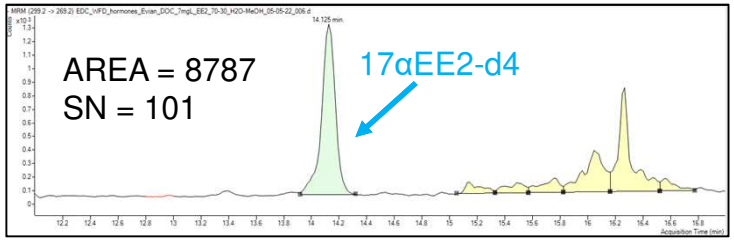
TQ2 : 295>269.1
TC2 : 295>145

Spiked at 0.03ng/L

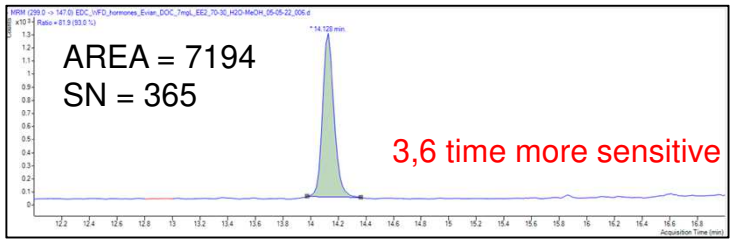
MS/MS parameter optimization : MRM choice (S/N parameter)

17αEE2-d4 : 10pg inj

MRM1 = 299.2>269.2



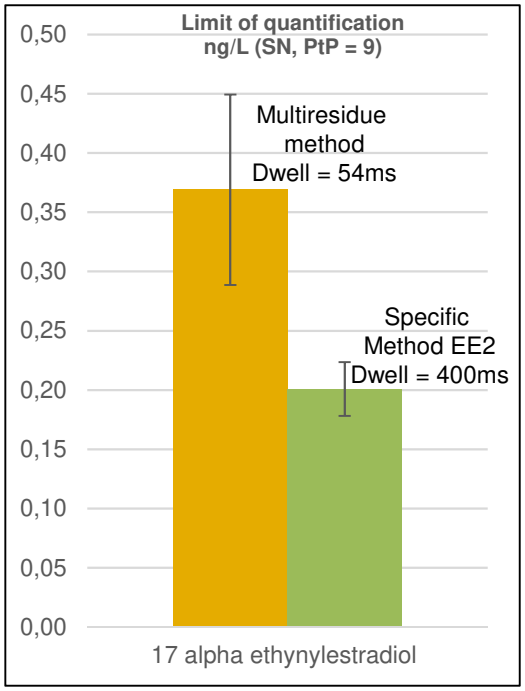
MRM2 = 299>147

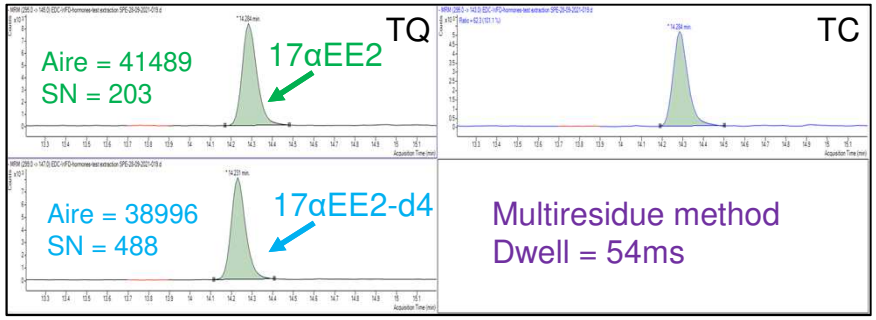


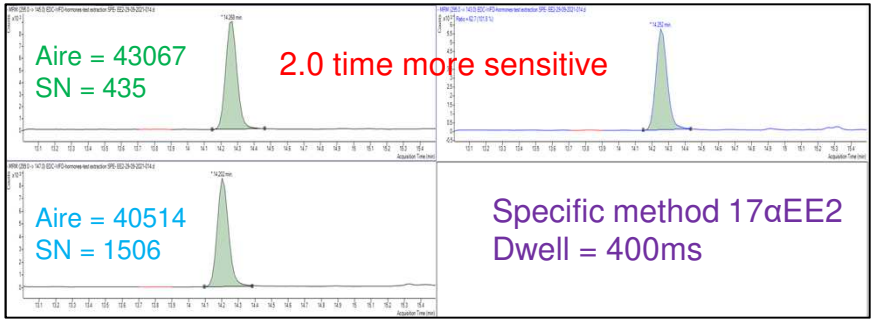
TQ 17αEE2-d4 = 299>147

MS/MS parameter optimization : Dwell time (LQ)

Evian + DOC 7mg/L + SPM 50mg/L spiked at 10ng/L (30mL extracted on SPE cartridge OASIS HLB 200mg with purification step), 17αEE2 example







MS/MS parameter optimization : Optimized parameters

Method 1 example on Agilent Technologies 6495

Compound	MRM-TQ (collision energy)	MRM-TC (collision energy)	Dwell time (ms)	Retention time (min)
E1	269.1>145 (45)	269.1>142.9 (60)	70	14.40
E1-$^{13}C_3$	272.2>148.1 (48)		70	14.40
E1-d_4	273>147 (45)		70	14.35
17αE2	271.0>239.2 (48)	271.0>183.2 (50)	50	13.62
17αE2-d_2	273.2>147.1 (52)	273.2>241.2 (40)	50	13.59
17βE2	271.0>183.2 (50)	271.0>143.0 (60)	90	13.23
17βE2-$^{13}C_3$	274.2>148.1 (52)		90	13.23
17βE2-d_4	275.0>147.0 (50)		90	13.16
E3	287.2>142.9 (50)	287.2>171 (32)	200	7.79
E3-d_2	289.2>147 (50)		200	7.78
17αEE2 (specific method)	295.0>269.1 (40)	295.0>145 (52)	400	14.14
17αEE2-d_4 (specific method)	299.0>147 (52)		200	14.11

MS1 and MS2 are fixed to wide resolution, the fragmentor and cell accelerator voltage value are set to 380V and 2V

MS/MS parameter optimization : Optimized parameters

Method 2 example on TQS Micro Waters

Compound	Cone Voltage (Tc)	MRM-TQ (collision energy)	MRM-TC (collision energy)	Dwell time (ms)	Retention time (min)
E1	50	269.0>145.1 (38)	269.0>159.0 (38)	17	8.62
E1-$^{13}C_3$	40	272.0>148.0 (38)	272.0>162.10 (34)	17	8.62
17αE2	40	271.0>145.0 (38)	271.0>183.0 (38)	17	8.49
17βE2	40	271.0>145.0 (38)	271.0>183.0 (38)	17	7.70
17βE2-d_5	40	276.2>147.1 (40)	276.2>187.2 (40)	17	7.60
E3	40	287.0>145.0 (38)	287.2>171 (32)	17	2.77
E3-$^{13}C^3$	40	290.2>148.1 (38)	290.2>174.1 (32)	17	2.75
17αEE2	40	295.0>145.0 (40)	295.0>159.0 (40)	17	8.55
17αEE2-d_4	40	299.0>147.0 (40)	299.0>161.1 (34)	17	8.48

MS1 and MS2 are fixed 0,5 uma resolution
17αE2 quantify with 17βE2-d_5

MS/MS parameter optimization : Optimized parameters

Method 3 example on AB Sciex TSQ 6500

Compound	CFX	**MRM-TQ** (collision energy)	**MRM-TC** (collision energy)	**Dwell time (ms)**	**Retention time (min)**
E1	-15	269.0>145.0 (-50)	269.0>159.0 (-50)	100	9.02
E1-d_2	-11	271.0>147.0 (-52)	271.0>161.10 (-52)	100	9.03
17αE2	-15	271.0>145.0 (-52)	271.0>143.0 (-66)	100	7.01
17αE2-d_5	-7	273.0>147.0 (-54)	273.0>145.0 (-61)	100	6.99
17βE2	-15	271.0>183.0 (-54)	271.0>145.0 (-52)	100	7.71
17βE2-$^{13}C_2$	-15	273.2>185.0 (-52)	276.2>147.0 (-54)	100	7.68
E3	-11	287.0>171.0 (-48)	287.2>145 (-52)	100	3.58
E3-d_2	-15	289.0>173.1 (-52)	289.2>147.1 (-54)	100	3.56
17αEE2	-9	295.0>145.0 (-54)	295.0>143.0 (-62)	100	7.67

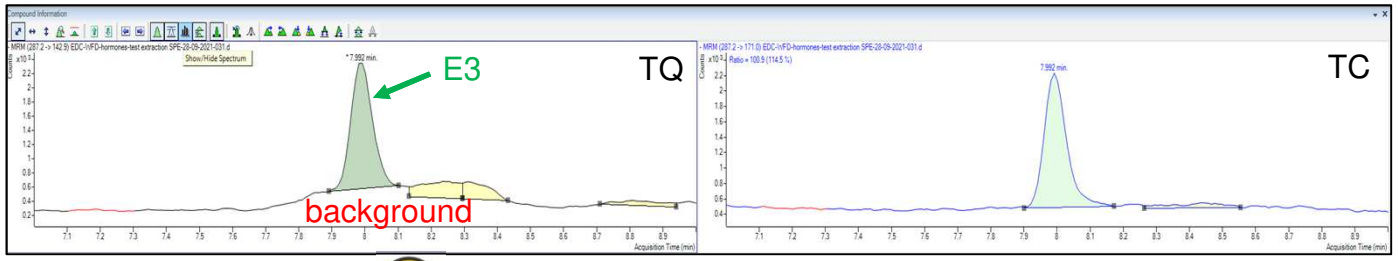
MS1 and MS2 are fixed 0,5 Da resolution

Matrix effect : Impact of sample preparation on quality of analysis

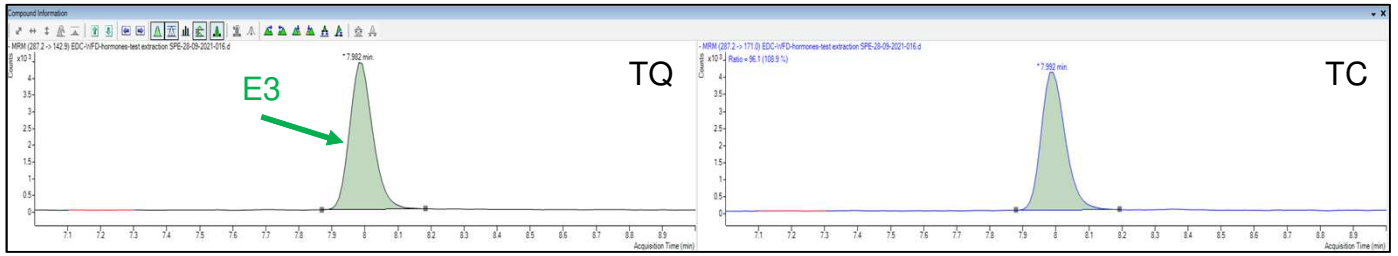
- THE BEST OPTIMIZATION FOR MS/MS CONDITIONS IS NOT SUFFICIENT
- SAMPLE PREPARATION AND PURIFICATION IS REALLY NEEDED TO REACH THE LOWER REQUESTED LQ

Matrix effect : Impact of sample preparation on quality of analysis

Evian + DOC 7mg/L + SPM 50mg/L spiked at 10ng/L (30mL extracted on SPE cartridge OASIS HLB 200mg)



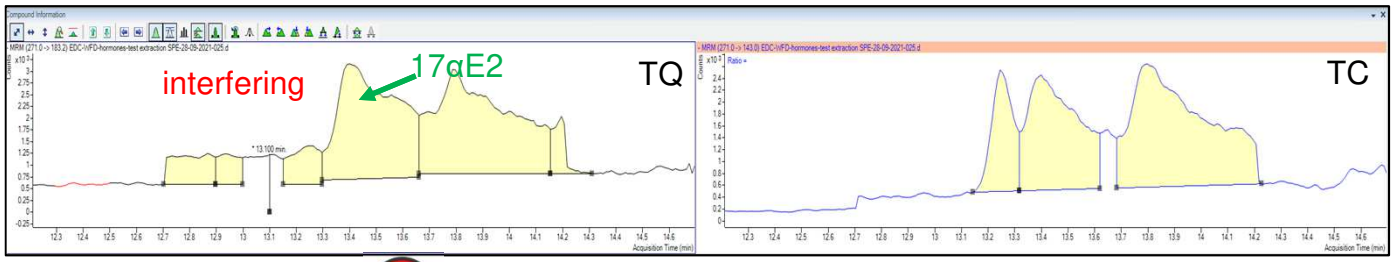
Without purification step 😐



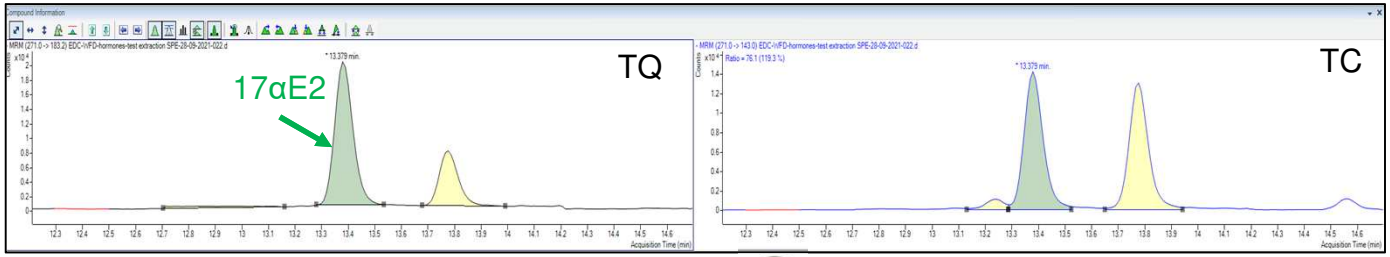
With purification step (Supelclean LC-NH_2, 500mg) 😊

Matrix effect : Impact of sample preparation on quality of analysis

Evian + DOC 7mg/L + SPM 50mg/L spiked at 10ng/L (30mL extracted on SPE cartridge OASIS HLB 200mg)



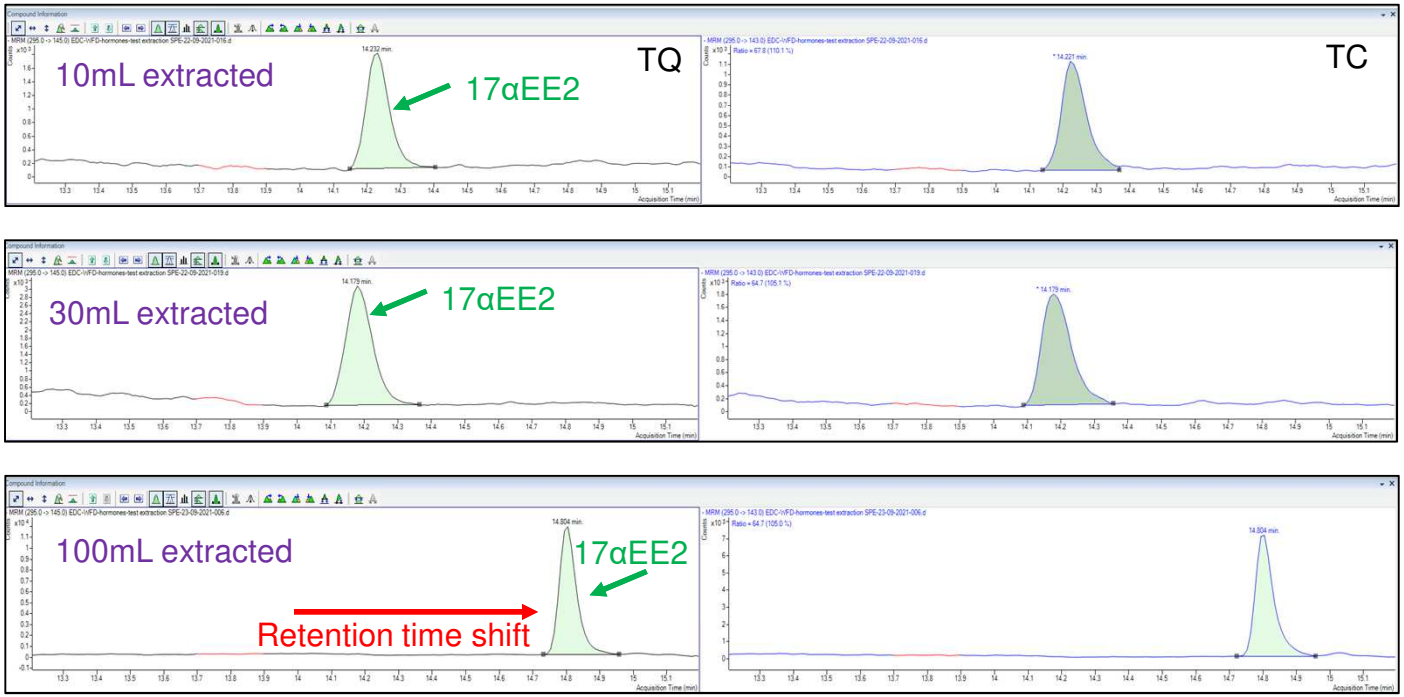
Without purification step



With purification step (Supelclean LC-NH_2, 500mg)

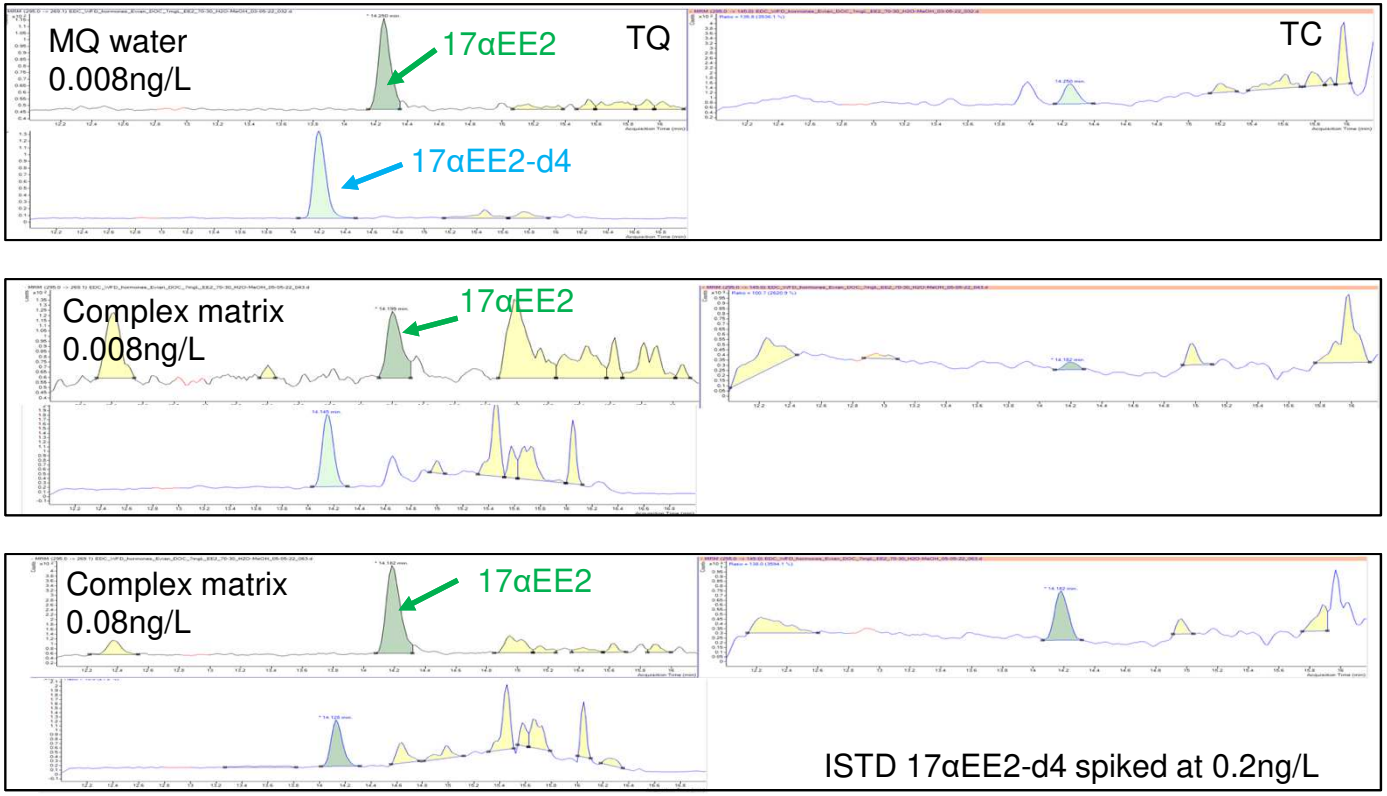
Matrix effect : Impact of sample preparation on quality of analysis

Evian + DOC 7mg/L + SPM 50mg/L spiked at 10ng/L (extracted on SPE cartridge OASIS HLB 200mg, without purification step)



Matrix effect : Low high complex matrices/Low high spike level

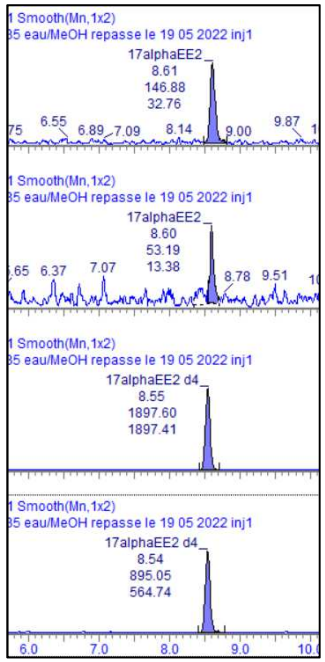
Method 1 example on Agilent Technologies 6495, Column Poroshell 120 Phenylhexyl : Dp = 2.1x100mm, 1.9µm



Matrix effect : Low high complex matrices/Low high spike level

Method 2 example on TQS Micro Waters, Column Acquity BEH C18 Waters : Dp = 2.1x100mm, 1.7µm

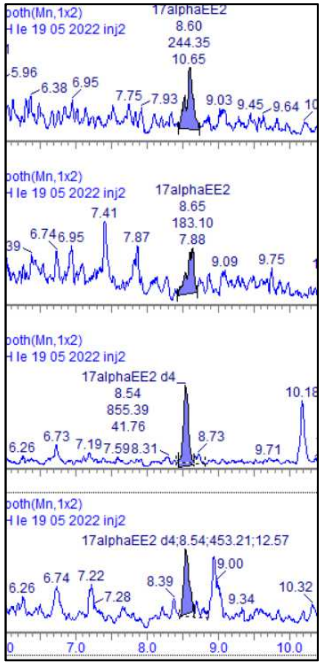
Lowest point of the calibration curve



17αEE2
≈ 2.5 pg/inj

17αEE2-d4
≈35 pg/inj

LQ in complex matrix (DOC 7 mg/L, SPM 50mg/L+ spike new EQS)

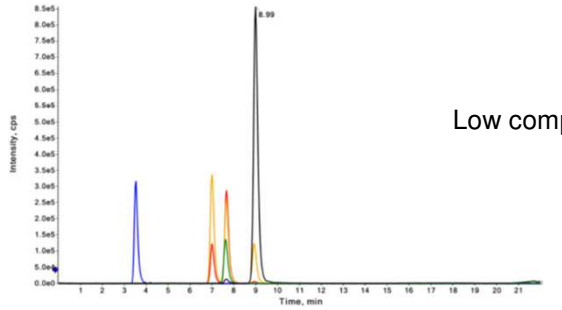


17αEE2
≈ 10 pg/inj

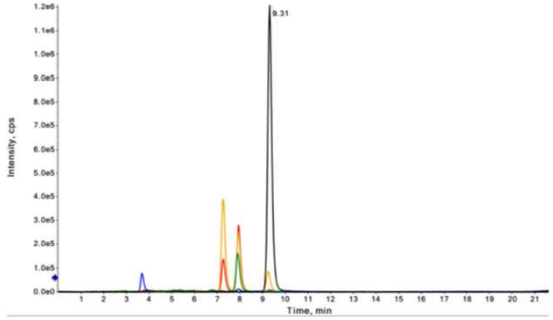
17αEE2-d4
≈35 pg/inj

Matrix effect : Low high complex matrices/Low high spike level

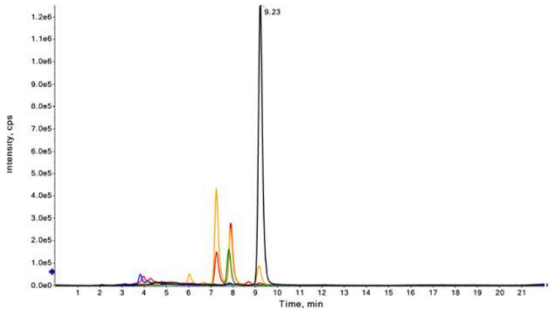
Method 3 example on AB Sciex TSQ 6500, Column Zorbax SB-Phenyl: Dp = 2.1x100mm, 1.8 µm



Low complex matrix/high or low spike level



Low complex matrix/low spike level



High complex matrix/low spike level

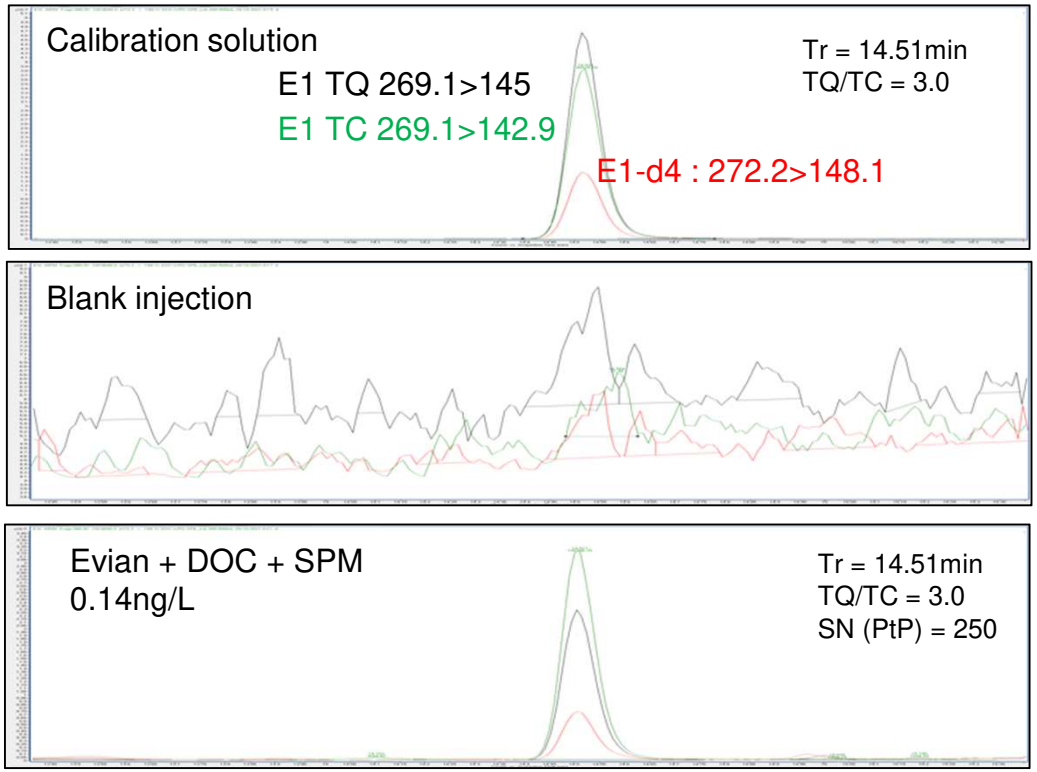
Identification and quality criteria

☞Quality criteria for identification and quantification of a compound

- Retention time
- Quantification transition TQ
- Confirmation transition TC
- Ratio of TQ/TC transitions
- Signal to noise ratio S/N = 9 quantification
- Isotope dilution
- Injection blank

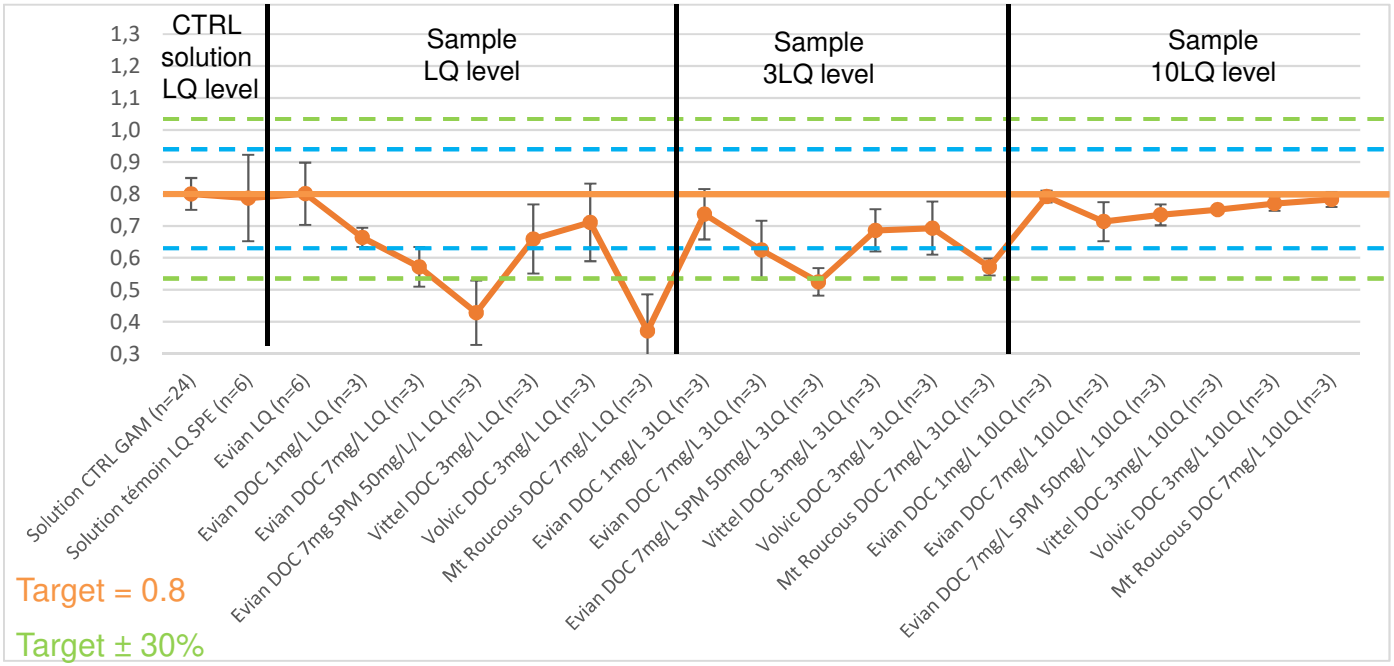
Identification and quality criteria

Quality criteria, E1 example



Identification and quality criteria (TQ/TC)

Ratio of TQ/TC transitions, 17αE2, (LQ level = 0.1ng/L)

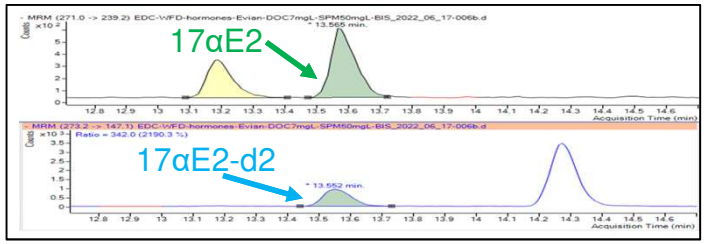


Target = 0.8

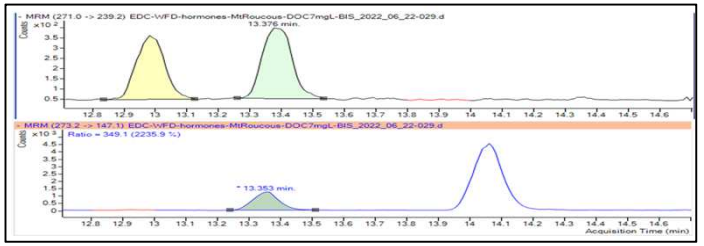
Target ± 30%

Target ± 20%

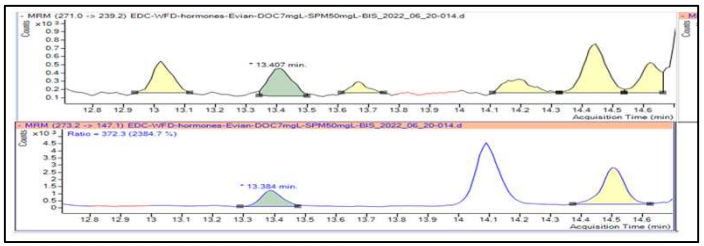
Identification and quality criteria (isotopic dilution)



Std solution	Area	SN
17αE2	1950	56
17αE2-d2	7150	286



MQ water	Area	SN
17αE2	2306	51
17αE2-d2	7120	180

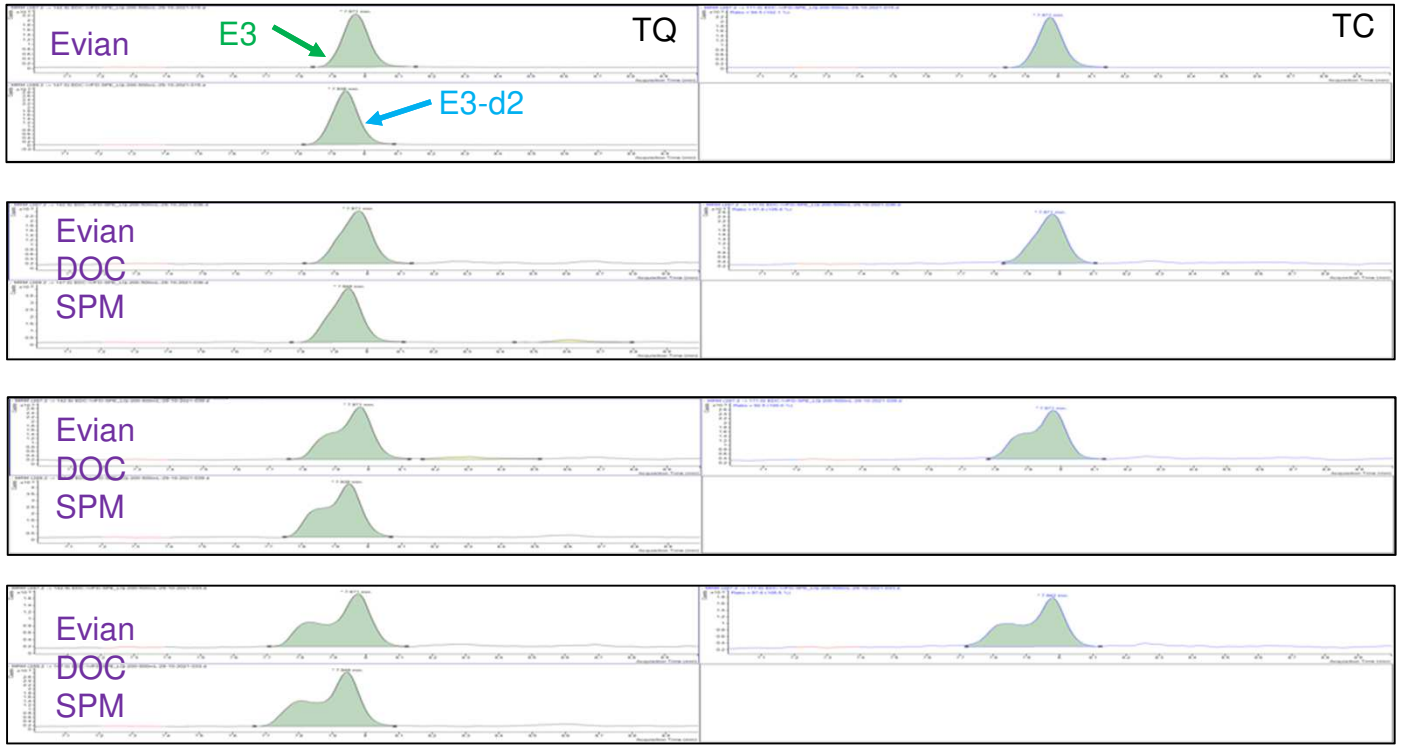


Complex matrix	Area	SN
17αE2	1840	6
17αE2-d2	6852	16

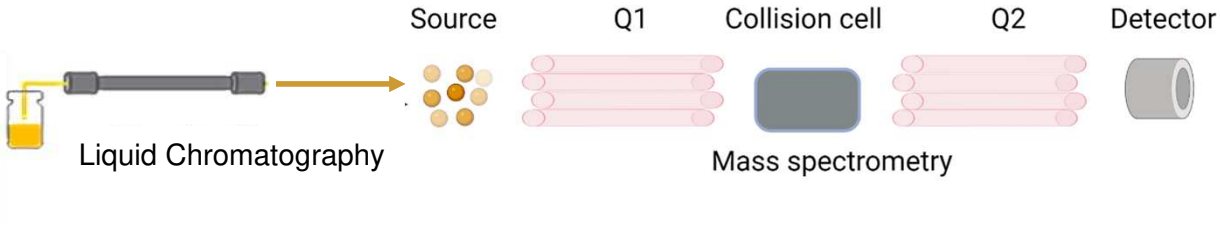
Same loss of sensitivity for 17αE2 and 17αE2-d2, ISTD quantification ⇒ OK

Identification and quality criteria (isotopic dilution)

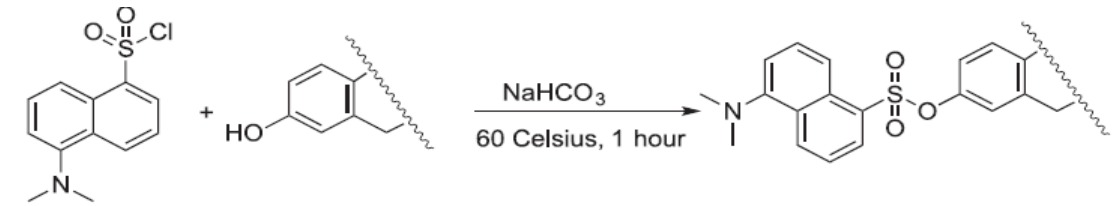
E3 examples of chromatographic peak shapes



LC/MSMS with derivatisation

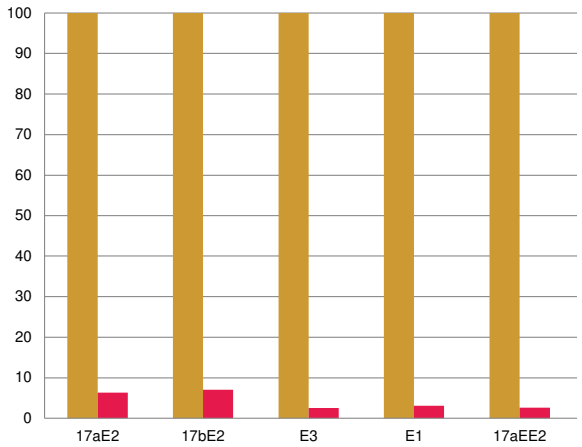


LC/MSMS : dansylation



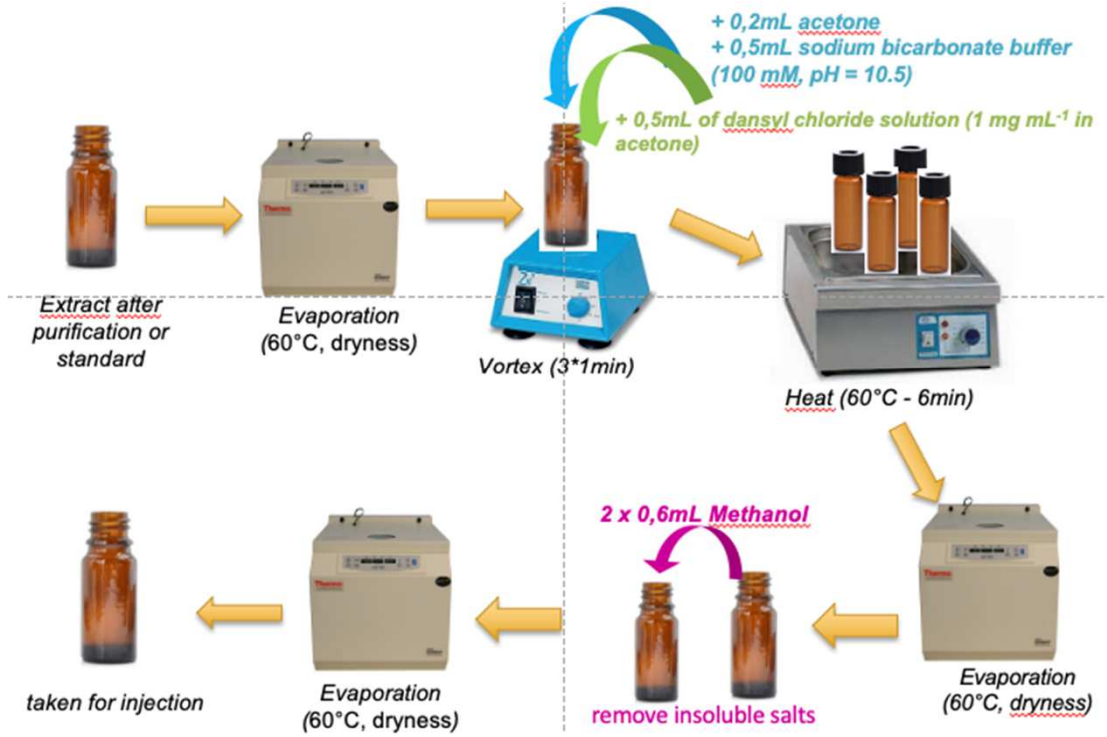
dansyl chloride estrogen estrogen derivative

Bussy et al (2017)



Switch from ESI $^-$ to ESI $^+$ to increase sensitivity

LC/MSMS : dansylation



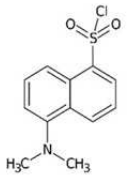
LC/MSMS : derivatisation

Journal of Chromatography A, 1534 (2018) 43–54

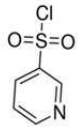
Trace analysis of estrogenic compounds in surface and groundwater by ultra high performance liquid chromatography-tandem mass spectrometry as pyridine-3-sulfonyl derivatives☆

Alex Glineur[a,b], Bruno Barbera[a,b], Katherine Nott[b], Philippe Carbonnelle[b], Sébastien Ronkart[b], Georges Lognay[a], Eva Tyteca[a,*]

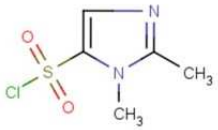
[a] AgroBioChem Department, Laboratory of Analytical Chemistry, University of Liège, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium
[b] La Société Wallonne des Eaux, Rue de la Concorde 41, 4800 Verviers, Belgium



Dansyl chloride



PS-Cl



DMIS-Cl

Derivatization reagent used :
Pyridine-3-Sulfonyl chloride (PS-Cl)

Ionisation Mode : ESI$^+$

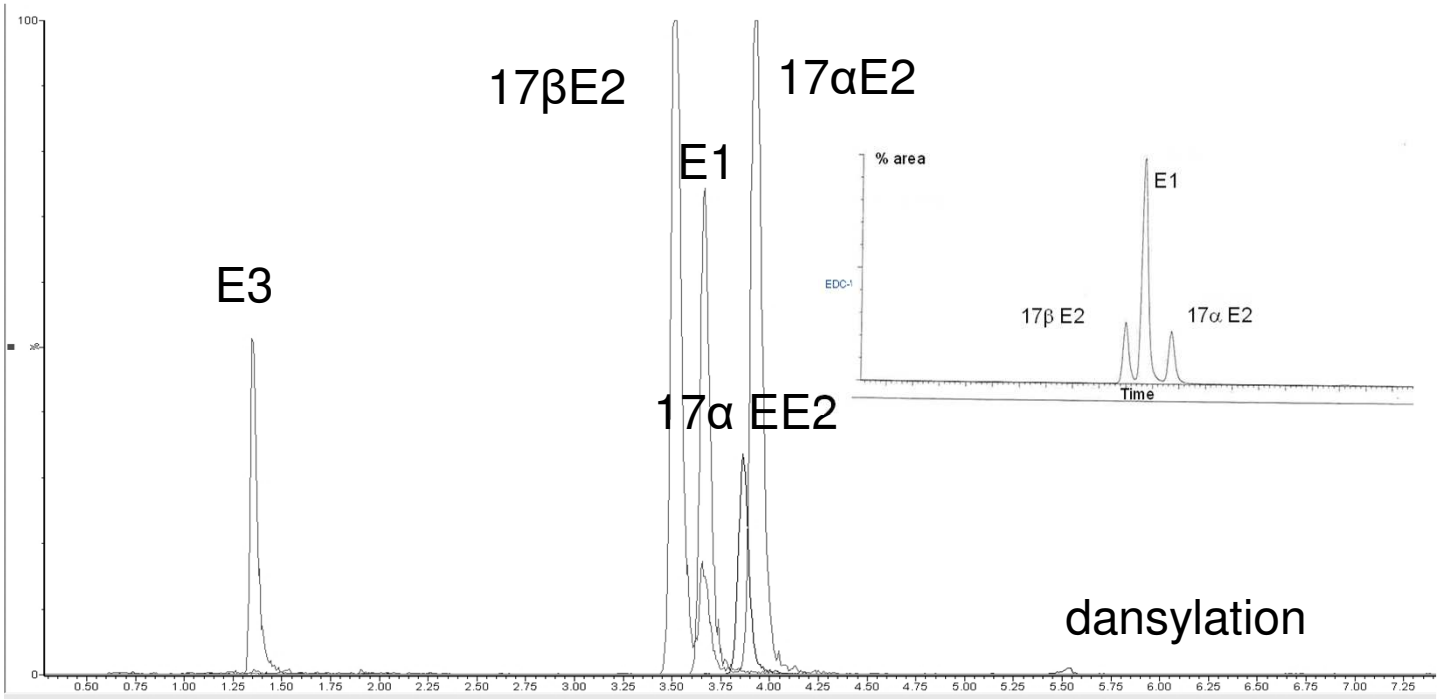
LC parameter optimization : optimized parameters

LC column	Cortecs Shield RP18 Agilent InfinityLab Poroshell 120 Phenylhexyl	Dp=1.6µm 2.1 x 100mm (dansylated estrogens) Dp=2.7µm 2.1 x 100mm (derivatised estrogens)	Guard Filter 0,2µm N/A	40°C 40°C
Mobile phase	MQ Water + 0,1% Formic acid (dansylated estrogens) MQ Water + 0,01% Formic acid (derivatisated estrogens)	ACN + 0,1% Formic acid ACN		0.3cc/min 0.3cc/min
Solvent injection	MQ Water + ACN 35:65 (v/v) (dansylated estrogens) MQ Water + ACN 75:25 (v/v) (derivatized estrogens)			20µl,100µl 20 µl

LC parameter optimization : one example of optimized parameters

Mobile phases : A = MQ Water + 0.1% HCOOH; B = ACN + 0.1% HCOOH

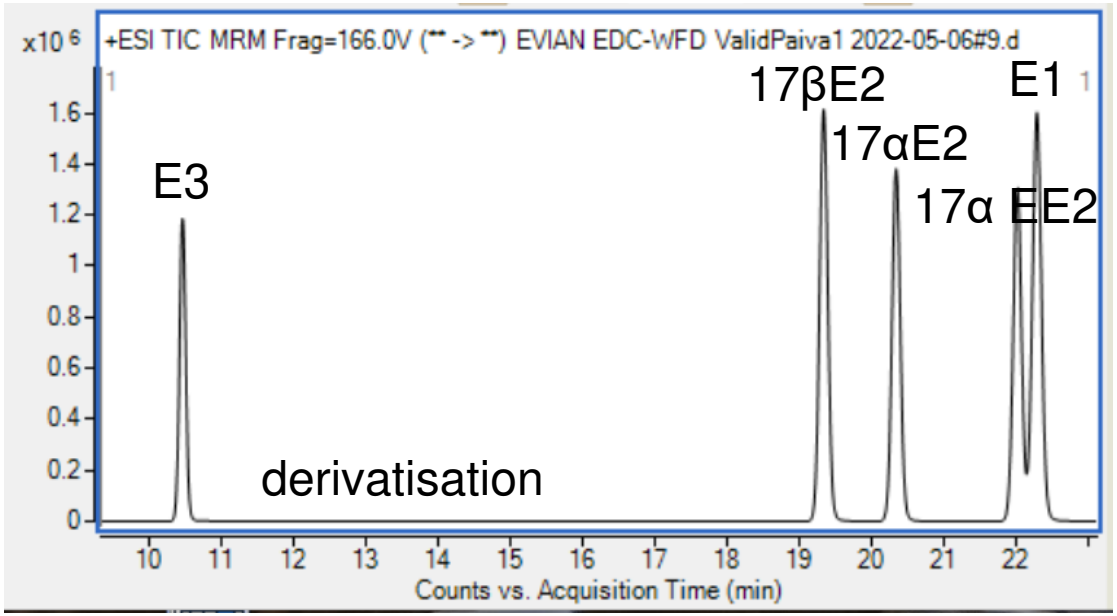
Column Cortecs Shield RP18 Waters Dp= 2.1x100mm, 1.6µm



LC parameter optimization : one example of optimized parameters

Mobile phases : A = MQ Water + 0.01% HCOOH; B = ACN

Column Poroshell 120 Phenylhexyl : Dp = 2.1x100mm, 2.7µm



EE2 and E1 <u>do not</u> interfere in MS/MS so co-elution is OK!

MS/MS parameter optimization (source parameters)

Method 1 with dansyladed estrogens : example on TQS Micro Waters

Method	Estrogen	Labelled estrogen	Sample injection
Dansylated Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-$^{13}C_3$, 17βE2-d_5, E3-$^{13}C_3$, 17αEE2-d_4	35 % Water/65% ACN without formic acid 20µL injected

Parameter	Value
Acquisition mode	MRM
Ionisation mode	Electrospray Ionisation (ESI) positive
Gas temperature and flow	650°C, 1200 $L.h^{-1}$
Capillary voltage	3.5kV
Cone gaz flow	50 $L.h^{-1}$

MS/MS parameter optimization (MSMS parameters)

Method 1 with dansyladed estrogens : example on TQS Micro Waters

Compound	Cone voltage (Tc)	MRM-TQ (collision energy)	MRM-TC (collision energy)	Dwell time (ms)	Retention time (min)
Dansylated E1	40	504.3>156.0 (54)	504.3>171.5 (34)	18	3.33
Dansylated E1-$^{13}C_3$	40	507.3>156.0 (56)	507.3>171.1 (32)	18	3.33
Dansylated 17αE2	40	507.3>156.0 (56)	507.3>171.1 (36)	18	3.55
Dansylated 17βE2	40	507.3>156.0 (56)	507.3>171.1 (32)	18	3.22
Dansylated 17βE2-d_5	40	511.3>156.0 (56)	511.3>171.1 (36)	18	3.17
Dansylated E3	40	522.2>171.1 (38)	522.2>156.1 (56)	18	1.32
Dansylated E3 $^{13}C_3$	24	525.3>156.1 (54)	525.3>171.1 (34)	18	1.33
Dansylated 17αEE2	50	530.3>156.1 (56)	530.3>171.1 (56)	18	3.49
Dansylated 17αEE2-d_4	30	534.3>156.0 (56)	534.3>171.1 (36)	18	3.46

MS1 and MS2 are fixed 0.5 uma resolution,
Quantification of 17αE2 with 17βE2d_5

MS/MS parameter optimization (source parameters)

Method 2 with derivatisation estrogens : example on Agilent G6495C QQQ

Method	Estrogen	Labelled estrogen	Sample injection
Derivatised Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-$^{13}C_3$, 17βE2-$^{13}C_3$, E3-$^{13}C_3$, 17αEE2-$^{13}C_2$	MQ Water + ACN 75:25 20µL injected

Parameter	Value
Acquisition mode	dMRM
Ionisation source and mode	Agilent jet stream Electrospray Ionisation (ESI), positive ion mode
Gas temperature and flow	250°C, 14 L/min
Capillary voltage	4kV
Sheat gas temperature and flow	350°C, 11 L/min

MS/MS parameter optimization (MSMS parameters)

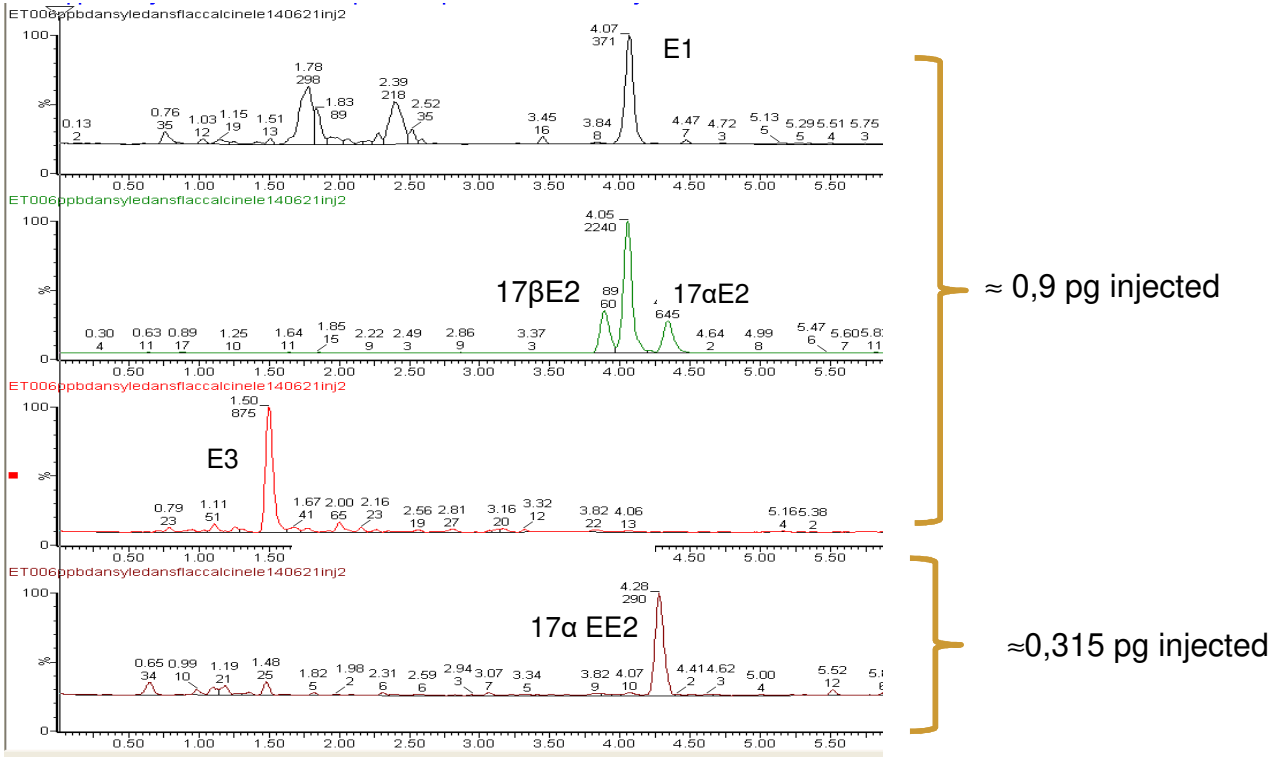
Method 2 with derivatisation estrogens : example on Agilent G6495C QQQ

Compound	MRM-TQ	MRM-TC	Retention time (min)
Derivatised E1	412>348	412>185 412>270	22.3
Derivatised E1-$^{13}C_3$	415>351	415>351 415>273	22.3
Derivatised 17αE2	414>350	414>213 414>272	20.3
Derivatised 17βE2	414>350	414>213 414>272	19.3
Derivatised 17βE2-$^{13}C_3$	417>216	417>275 417>353	19.3
Derivatised E3	430>288	430>146 430>366	10.4
Derivatised E3 $^{13}C_3$	433>291	433>149 433>369	10.4
Derivatised 17αEE2	438>213	438>160 438>157 438>374	22.0
Derivatised 17αEE2-$^{13}C_2$	440>213	440>160 440>376	22.0

MS1 and MS2 are respectively fixed at wide and unit resolution,

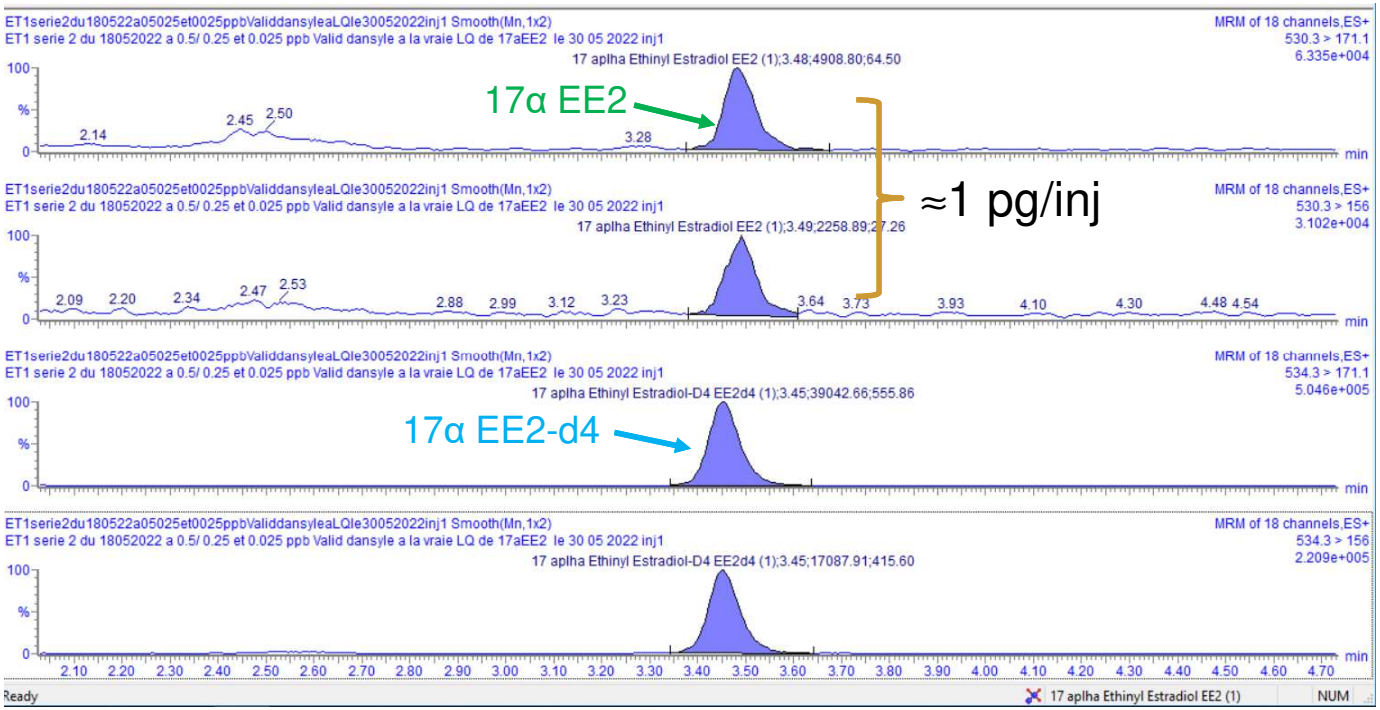
Matrix effect : Low high complex matrices/Low high spike level

Dansylation



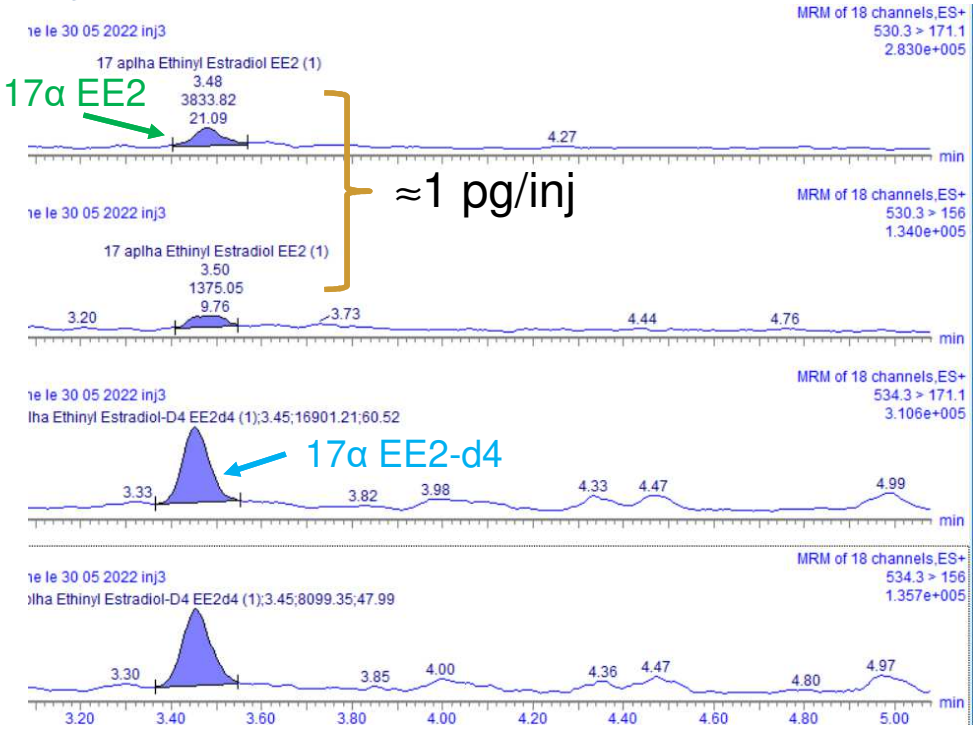
Matrix effect : Low high complex matrices/Low high spike level

Lowest point of the calibration curve (eq to 1/3 new EQS), dansylation



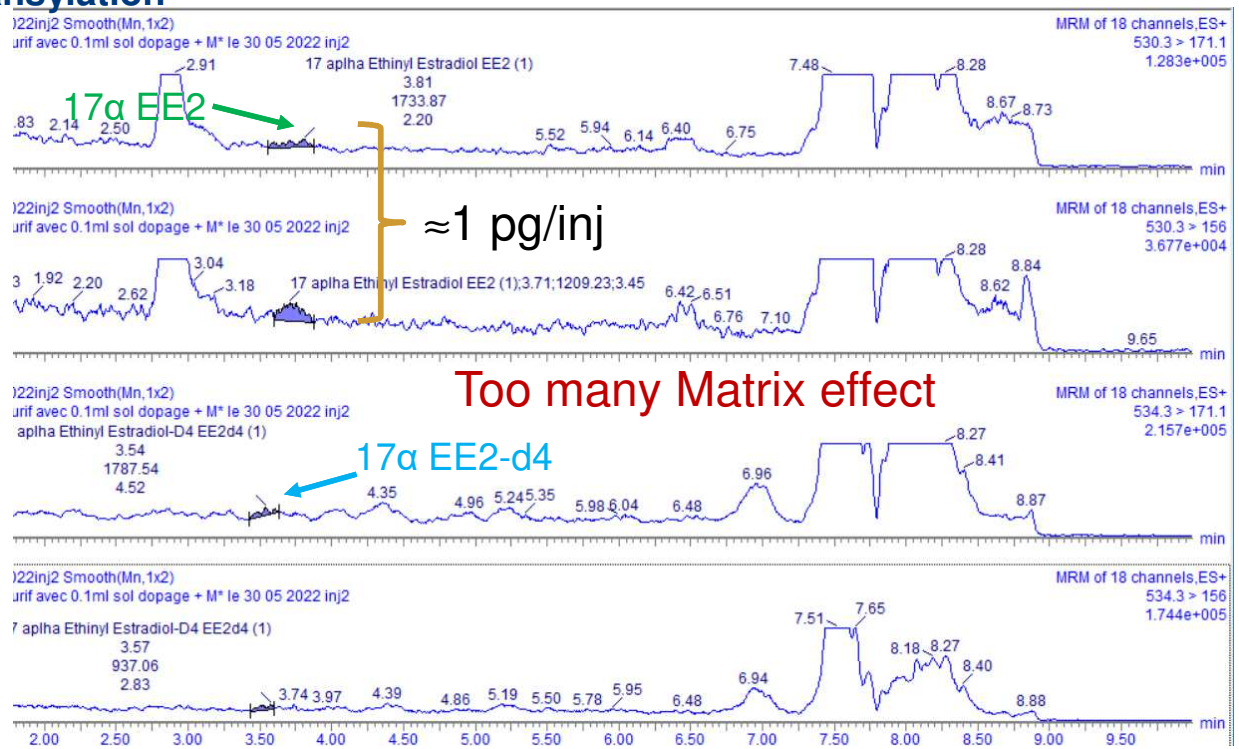
Matrix effect : Low high complex matrices/Low high spike level

Evian water with DOC 1mg/L (spiked before extraction eq to 1/3 new EQS)
Dansylation



Matrix effect : Low high complex matrices/Low high spike level

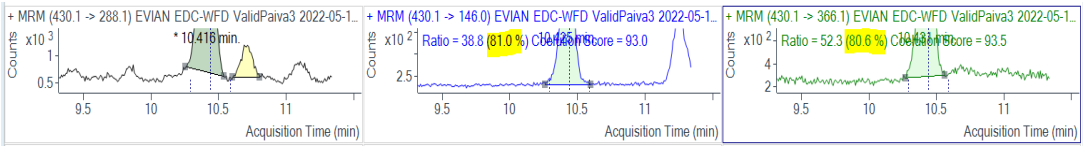
Water with DOC 7mg/L (spiked before extraction eq to 1/3 new EQS)
Dansylation



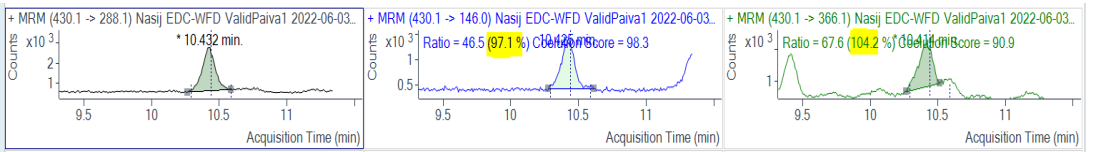
Matrix effect : Low high complex matrices/Low high spike level

Derivatisation

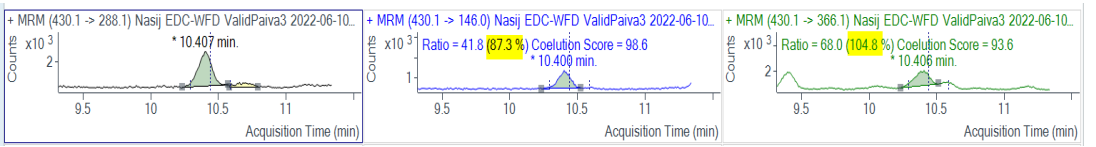
EVIAN + DOC 7mg/L
+ 50 mg/L **SPM**
0.18 ng/L **E3** (LOQ)



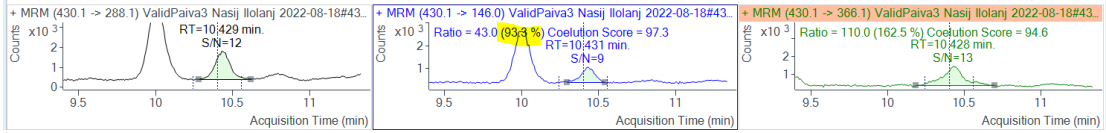
Näsijärvi Lake Water
0.18 ng/L **E3** (LOQ)



Näsijärvi Lake Water
+ DOC 10 mg/L
0.18 ng/L **E3** (LOQ)



Ilolanjoki River water
0.08 mg/L **E3**



- SPM caused clear, barely acceptable interference at the Quantifier 430 → 288 at LOQ level.
- EVIAN and Lake water with DOC: No issues.
- Quantifier seems to have been selected correctly because it provides combination of highest sensitivity and no interferences in natural lake and river water samples.

Matrix effect : Low high complex matrices/Low high spike level

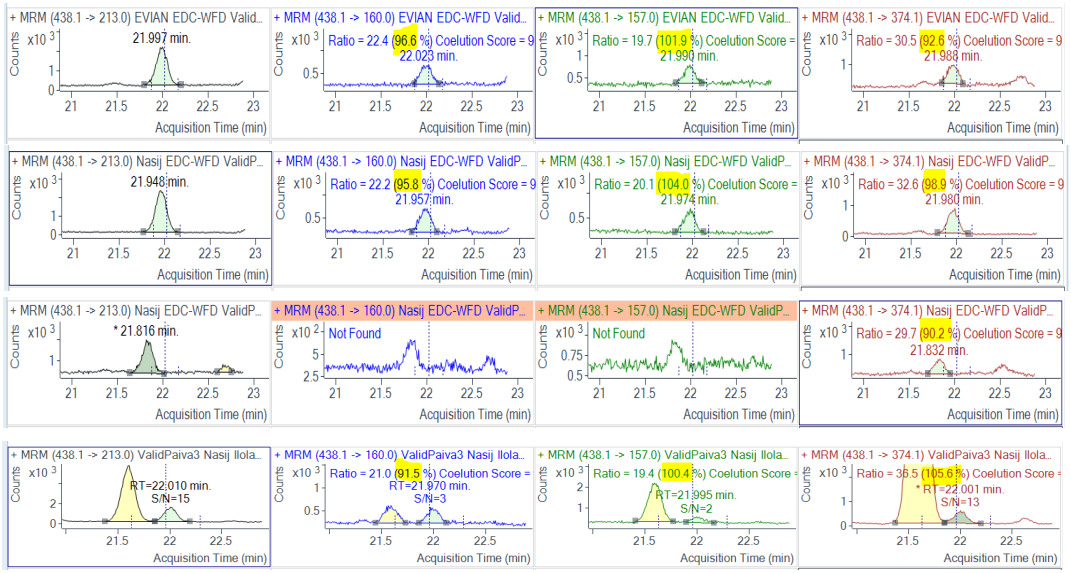
Derivatisation

EVIAN + DOC 7mg/L
+ 50 mg/L **SPM**
0.09 ng/L **EE2** (LOQ)

Näsijärvi Lake Water
0.09 ng/L **EE2** (LOQ)

Näsijärvi Lake Water
+ DOC 10 mg/L
0.09 ng/L **EE2** (LOQ)

Ilolanjoki River water
0.08 ng/L **EE2**



- All three qualifiers (438 → 160, 438 → 157, and 438 → 374) perform equally well but the 3rd one (438 → 374) provides the best sensitivity and may be the only that is one above detection limit if signal intensity is compromised at LOQ.

Tips and Tricks (as examples - not exhaustive)

◉ Change of pre-filter and pre-column between each round of analysis

◉ Rinsing the chromatographic column after each set of analyses with 90% of organic phase and 10% of aqueous phase at 0,3ml/min during 15min

◉ Change the mobile phase regularly :

- 0.1mM NH4F water phase
- 1mM NH4F stock solution has a shelf life of several weeks
- the organic phase (65/35 MeOH/ACN) cannot be kept after use, stored and reused : the experiment showed a strong impact of an old organic phase on the signal response with a decrease in S/N

◉ Ultra pure water quality with quality to be monitored : impact on the background if degraded quality

◉ Blank injection : one injection blank (MeOH) at least between each sample and two blank after the higher level of calibration standard solution

◉ Standard flushing of the injection needle with methanol

CONCLUSION FOR LC-MS/MS : THE TARGETED LQ CAN BE REACHED FOR ALL THE COMPOUNDS

WHAT TO DO TO HAVE GOOD PERFORMANCE?

- Careful optimisation of MS conditions
- Careful and precise optimization of chromatographic separation
- Track cross contamination
- Use high quality criteria
- Use ISTD quantification
- Be aware of the importance of sample preparation especially purification

LC/HRMS

Triple TOF

Q exactive Orbitrap

Mass Spectrometry methods – LC-ESI-HRMS (Triple ToF)

Method 1 : AB Sciex Triple ToF 6600 with Agilent 1290 infinity II

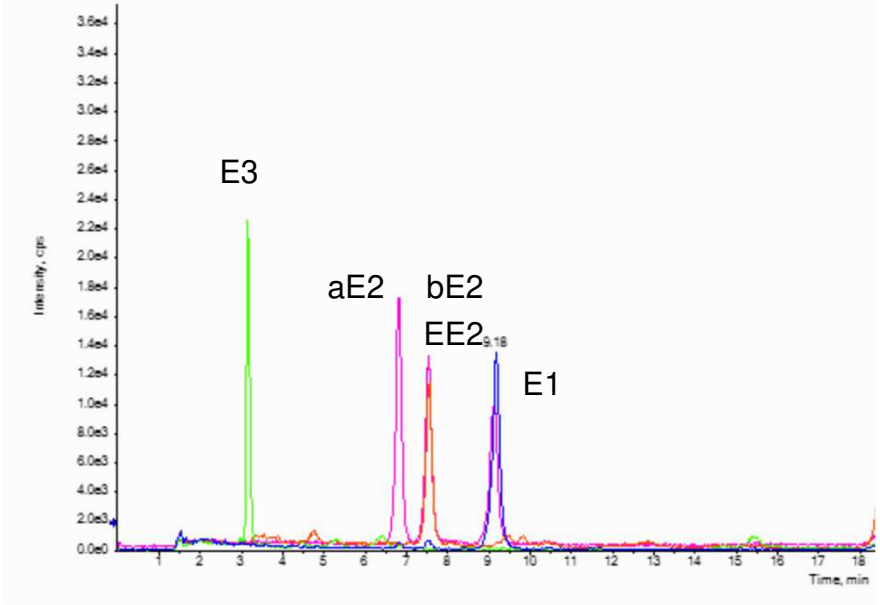
- Acquisition in ESI negative mode
- Mass range: 80 – 350 Da
- Scan rate: 1Hz
- Extraction of exact masses for quantification
- Injection volume: 10 µl

Mass Spectrometry methods – LC-ESI-HRMS (Triple ToF)

Method	Estrogen	Labelled estrogen	Sample injection
Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-d_2, 17αE2-d_2, 17βE2-$^{13}C_2$, E3-d_2, 17αEE2-$^{13}C_2$	50% Water/50% MeOH 50µL injected

Parameter	Value
Acquisition mode	MRM
Ionisation mode	Electrospray Ionisation (ESI) negative
Source temperature	600°C
Capillary voltage	4500 V
CAD	12 psi
Curtain gas	20 psi
Source gas	90 psi
Exhaust	90 psi

Mass Spectrometry methods – LC-ESI-HRMS (Triple ToF)



- The same chromatographic separation as low-res LC-MS/MS but less sensitive (factor 10)

Mass Spectrometry methods – LC-ESI-HRMS (Q Exactive Orbitrap)

Method 2 : Dionex 3000 UHPLC with Q Exactive Orbitrap

- Acquisition in ESI positive mode with Dansyl Chloride derivatization
- Scan mode : Full scan
- Scan range : 450-600 m/z
- Resolution : 35000
- AGC target : 3.e6
- Maximum inject time : 100ms

Mass Spectrometry methods – LC-ESI-HRMS (Q Exactive Orbitrap)

Chromatographic separation is achieved with Waters BEH C18 2.1 x 100 mm 1.7 µm UPLC column at 40 °C.

Mobile phase A : H_2O, 0.1% FA
Mobile phase B : ACN, 0.1% FA

Parameter	Value
Acquisition mode	Full scan
Ionisation mode	Electrospray Ionisation (ESI) positive with dansylation
Sheath gas flow rate	30 L/min
Aux gas flow rate	5
Sweep gas flow rate	3
Spray voltage	24.5kV
Capillary temperature	350°C
Aux gas heater temperature	400°C

Mass Spectrometry methods – LC-ESI-HRMS (Q Exactive Orbitrap)

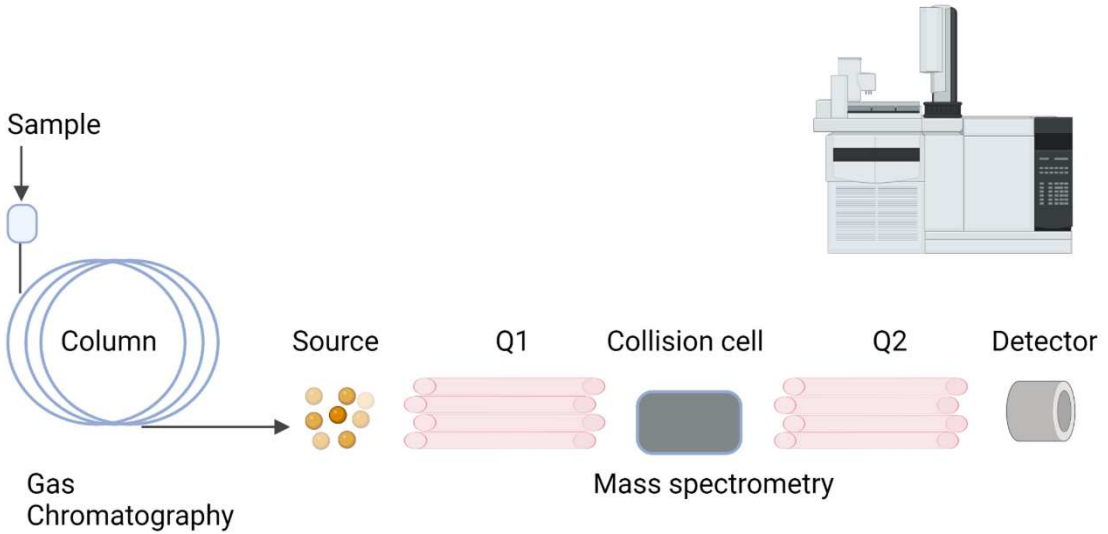
Dansyl Chloride derivatization performed and for quantification m/z values of analytes and their corresponding IS m/z values are given below:

	Analyte	Analyte Ion (m/z)	Internal Standart (IS)	IS Ion (m/z)
1	17α-estradiol	506.237	17α-estradiol-d_2	508.245
2	17β-estradiol	506.237	17β-estradiol-d_5	511.267
3	17-ethinylestradiol	530.237	17-ethinylestradiol-d_4	534.260
4	Estriol	522.231	Estriol-d_3	525.249
5	Estrone	504.221	Estrone-$^{13}C_3$	507.231

CONCLUSIONS FOR LC-HRMS

LOWEST PERFORMANCES (HIGHEST LOQ) THAN WITH LC-MSMS

GC/MSMS


Sample
Column
Source
Q1
Collision cell
Q2
Detector
Gas
Chromatography
Mass spectrometry

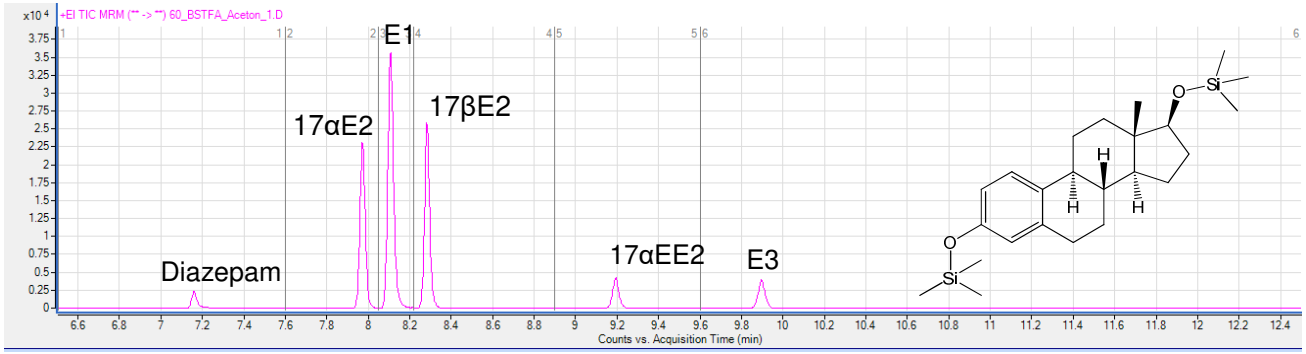
GC/MSMS

- GC parameter optimisation
  - Derivatisation agent
  - Volume of injection
  - Temperature program (chromatographic separation)
  - Injection mode
  - Flow

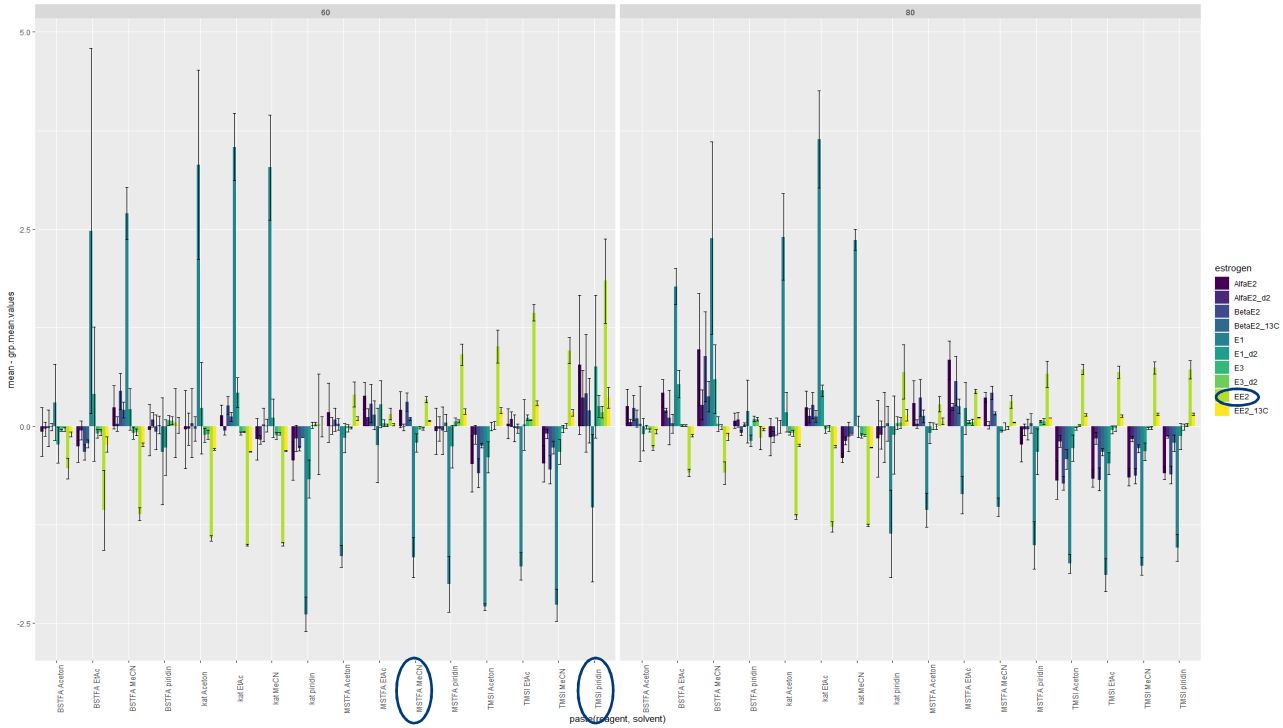
- MSMS parameter optimisation
  - MRM
  - temperature of MS transfert line, MSMS source
  - Collision energy
  - Dwell time
  - Sensitivity, selectivity

Optimisation 1: Combination of derivatisation agent

Tested Combinations	T = 60 °C					T = 80 °C			
Derivatisation agent	TMSI	BSTFA	MSTFA	BSTFA+ catalyst		TMSI	BSTFA	MSTFA	BSTFA+ catalyst
Pyridine	**x**	x	x	x	Pyridine	**x**	x	x	x
EtAc	x	x	**x**	x	EtAc	x	x	**x**	x
Aceton	x	x	x	x	Aceton	x	x	x	x
MeCN	x	x	x	x	MeCN	x	x	x	x

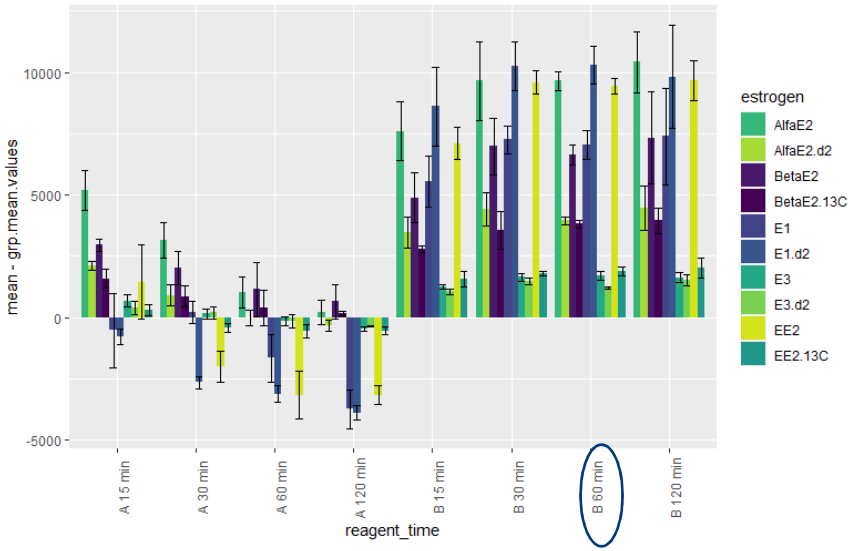


Optimisation 1: Combination of derivatisation agent



Optimisation 2: Final derivatisation agent. temperature and time

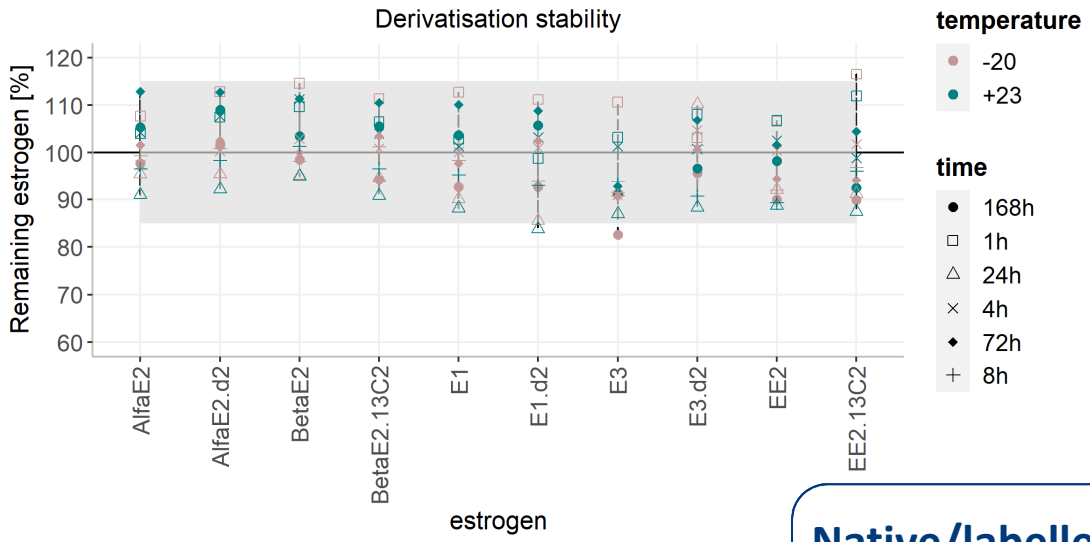
A	MSTFA + EtAc			
T °C	60	70	80	90
15 min	x	x	x	x
30 min	x	x	x	x
60 min	x	x	x	x
120 min	x	x	x	x
B	TMSI + Pyridine			
T °C	60	70	80	90
15 min	x	x	x	x
30 min	x	x	x	x
60 min	x	x	x	x
120 min	x	x	x	x



Optimal parameters

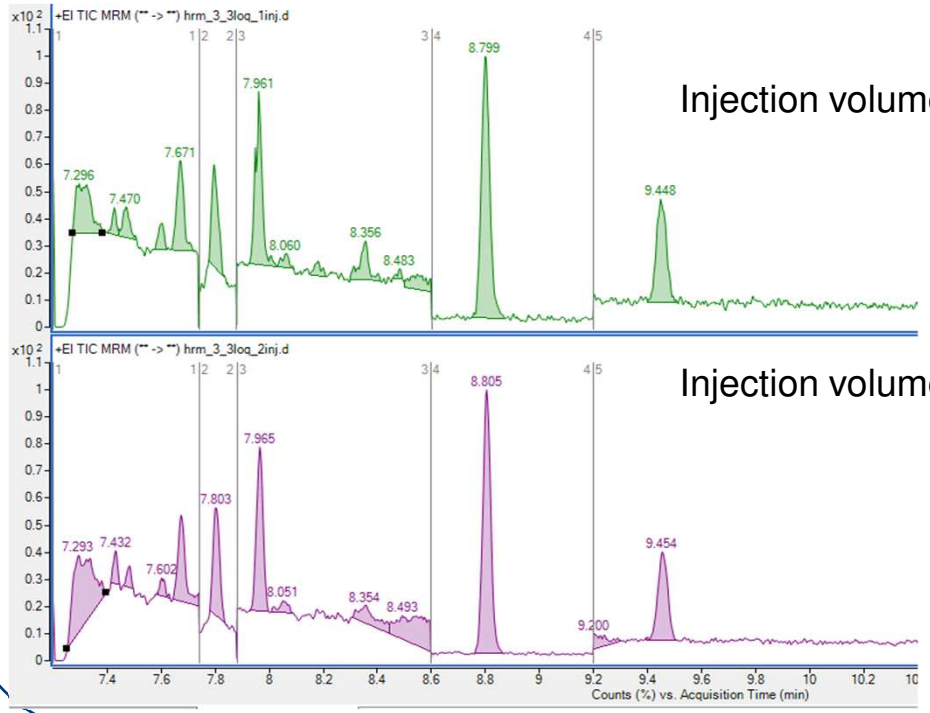
TMSI + Pyridine
T = 90 °C
t = 60 min

Optimisation 3: Stability of derivatised extracts during instrumental analysis

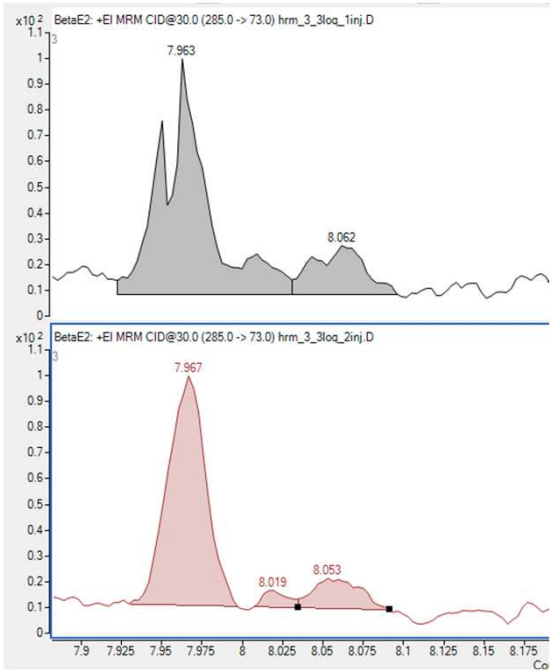


Native/labelled estrogens stable at +23 and -20 °C within 7 days

GC parameter optimization : injection solvent and volume



GC parameter optimization : injection solvent and volume



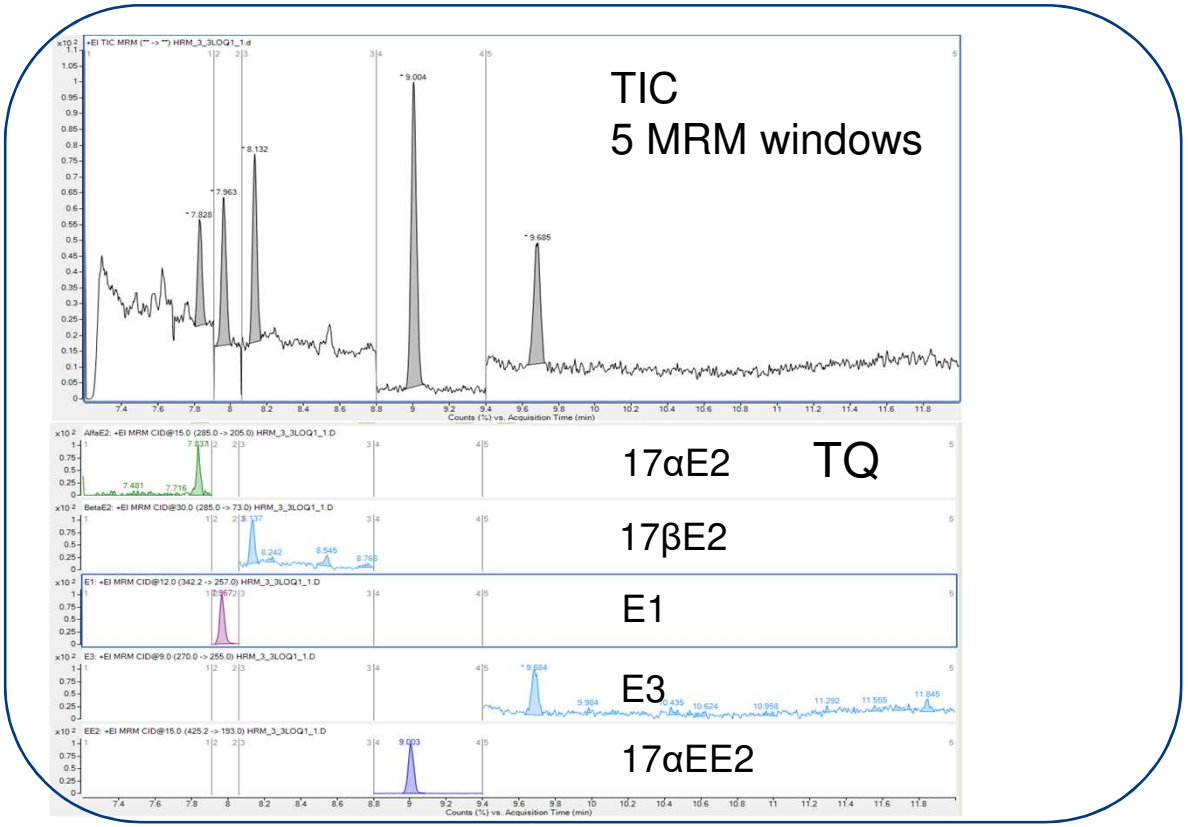
Injection volume = 1 µL
17βE2

Injection volume = 2 µl
17βE2

GC parameter optimization : optimized parameters

	Gas Chromatography
GC column	Agilent Technologies DB-5 MS capillary column. 30 m × 0.25 mm × 0.25 µm
Mobile phase	H_2 operated under constant flow (1 mL min^{-1}) at an average velocity of 26 cm sec^{-1}
Sample solvent	TMSI (25µL) and Pyridine (25µL) Sample volume = 50 µL
Injection volume	2 µL
Temperature program	145 °C (0 min). 30 °C/min to 290 °C (6 min). 30 °C/min to 310°C (0.5 min). total run: 12min
Time windows	A window per compound together with corresponding labelled estrogen. total No. windows: 5

GC parameter optimization : optimized parameters



MS/MS parameter optimization : MRM choice

- Choice of MRMs on standard solutions
  - Area
  - Intensity
  - S/N
- First list of potential ineresting MRMs
- Test of these MRMs on « real » sample extract (impact of the matrix in terms of sensitivity and selectivity)

MSMS parameter optimization : optimized parameters

Method

Method	Estrogen	Labelled estrogen	Sample injection
Estrogen multi-residue	E1, 17αE2, 17βE2, 17αEE2, E3	E1-d_2. 17αE2-d_2. 17βE2-$^{13}C_2$. 17αEE2-$^{13}C_2$. E3-d_2	2µL

Parameter	Value
Acquisition mode	MRM
Ionisation mode	EI mode at 70 eV
Injection mode	spitless mode at 250 °C (purge-off time. 2 min)
MS Transfer line	280 °C
Source temperature	250 °C
Collision gas (nitrogen 99.9990%) pressure	1.5 mL min^{-1}

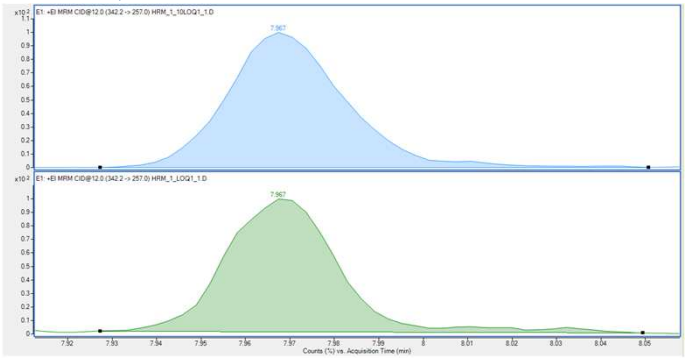
MSMS parameter optimization : optimized parameters

Compound	**MRM-TQ** (CE[V]/DT[ms])	**MRM-TC1** (CE[V]/DT[ms])	**MRM-TC2** (CE[V]/DT[ms])	**Retention time** [min]
E1	342.2>257 (60, 12)	342.2>244 (60, 15)	218>203 (30, 9)	7.97
E1-d_2	344.2>259 (30, 12)	344.2>245.8 (30, 12)		7.97
17αE2	285.1>72.9 (60, 30)	285.0>205.0 (30, 15)	416.2>285.1 (30, 9)	7.84
17αE2-d_2	287.2>207.0 (30, 15)	418.3>287.2 (30, 15)		7.84
17βE2	285.0>73.0 (30, 30)	416.2>285.0 (60, 9)	416.2>326.0 (30, 3)	8.35
17βE2-$^{13}C_3$	287.2>207.0 (30, 15)	418.3>287.0 (30, 9)		8.35
E3	270.0>255.0 (60, 9)	311.0>285.0 (60, 15)	296.3>281.0 (30, 12)	9.69
E3-d_2	298.0>283.0 (30, 9)	506.3>416.0 (30, 3)		9.69
17αEE2	425.2>193.0 (60, 15)	440.2>425.2 (30, 3)	205.0>115.0 (30, 15)	9.01
17αEE2-d_4	442.3>427.3 (30, 3)	427.2>194.8 (50, 15)		9.01

MS1 and MS2 are fixed to unit resolution.

Matrix effect : Low high complex matrices/Low high spike level

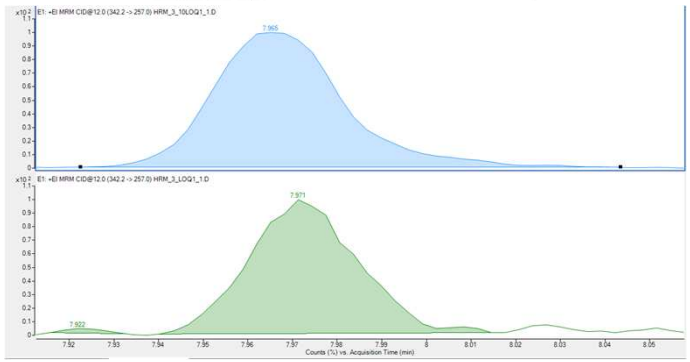
E1 – TQ



10LOQ

LOQ

Evian +
DOC 1 mg/L



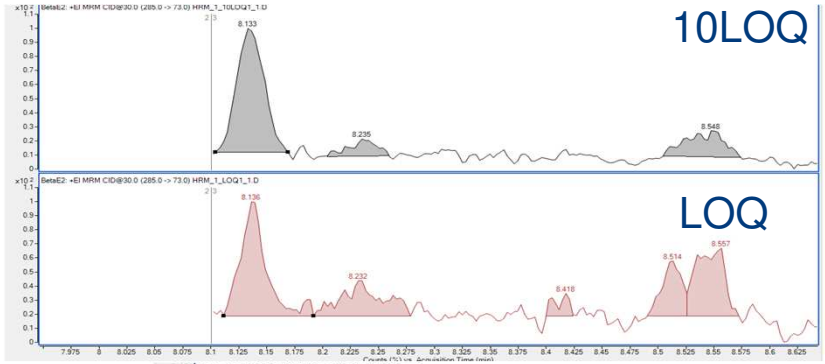
10LOQ

LOQ

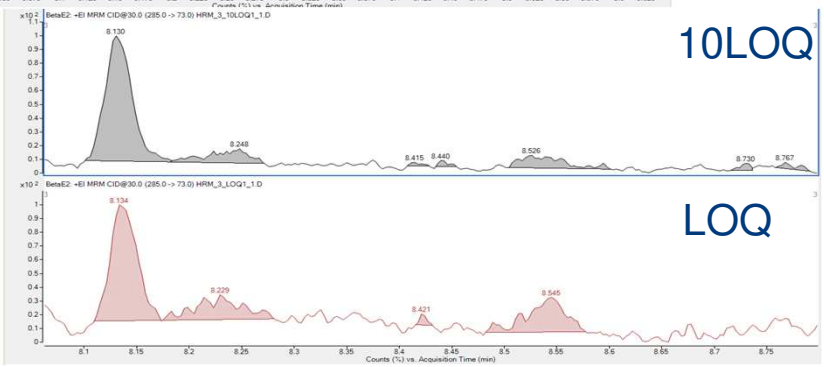
Evian +
DOC 7 mg/L +
SPM 50 mg/L

Matrix effect : Low high complex matrices/Low high spike level

βE2 – TQ



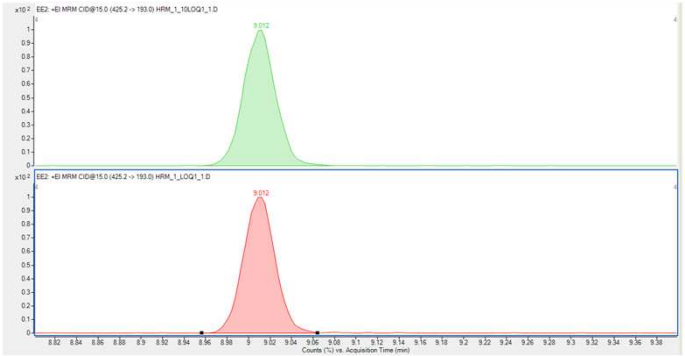
Evian +
DOC 1 mg/L



Evian +
DOC 7 mg/L +
SPM 50 mg/L

Matrix effect : Low high complex matrices/Low high spike level

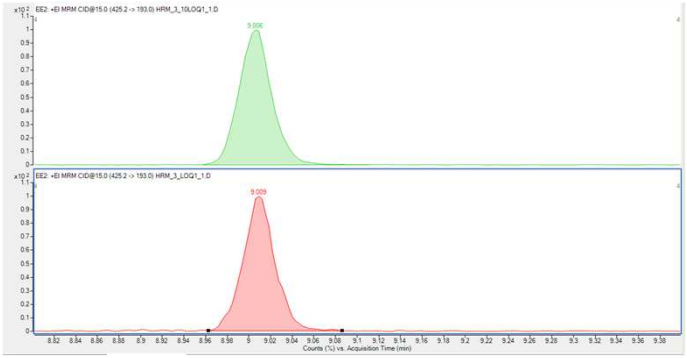
EE2 – TQ



10LOQ

LOQ

Evian +
DOC 1 mg/L



10LOQ

LOQ

Evian +
DOC 7 mg/L +
SPM 50 mg/L

CONCLUSION FOR GC-MS/MS: THE TARGETED LOQ CAN BE REACHED FOR αE2, β E2, and E1 but not for EE2 and E3

WHAT TO DO TO HAVE GOOD PERFORMANCE?

- Optimised derivatisation conditions
- Importance of sample preparation especially purification
- Optimisation of chromatographic separation and MS conditions
- Track cross contamination
- Use ISTD quantification

GC/HRMS

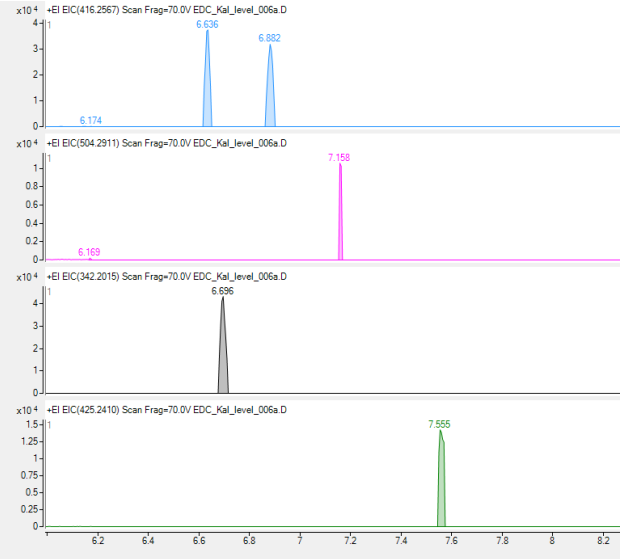
Mass Spectrometry methods – GC-HRMS

Agilent 7250 qToF with Agilent 8890 GC

	Gas Chromatography
GC column	Agilent Technologies DB-5 MS capillary column. 30 m × 0.25 mm × 0.25 µm
Mobile phase	He operated under constant flow (1 ml min^{-1})
Sample solvent	TMSI (50 µL) and Acetone (50 µL) Sample volume = 100 µL (60 min at 80°C)
Injection volume	1 µL
Temperature program	145°C (0 min) - 40 K/min to 205°C - 2 K/min to 240°C - 40 K/min to 245°C. Total run: 26.5 min

Mass Spectrometry methods – GC-HRMS

Agilent 7250 qToF with Agilent 8890 GC



- The same chromatographic separation as low-res GC-MS/MS and comparable sensitivity
- For routine laboratories too expensive 500 k€ (HRMS) vs.150 k€ (low-res GC-MS)

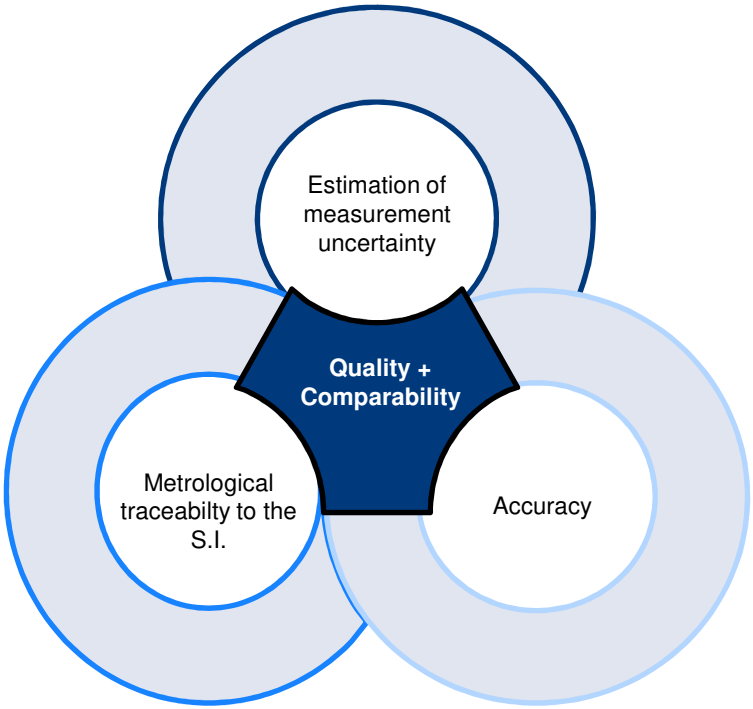

QUESTIONS?
COMMENTS?
CONCERNS?

EDC WFD

Achievements of Mass spectrometry based methods: method performances and measurement reliability

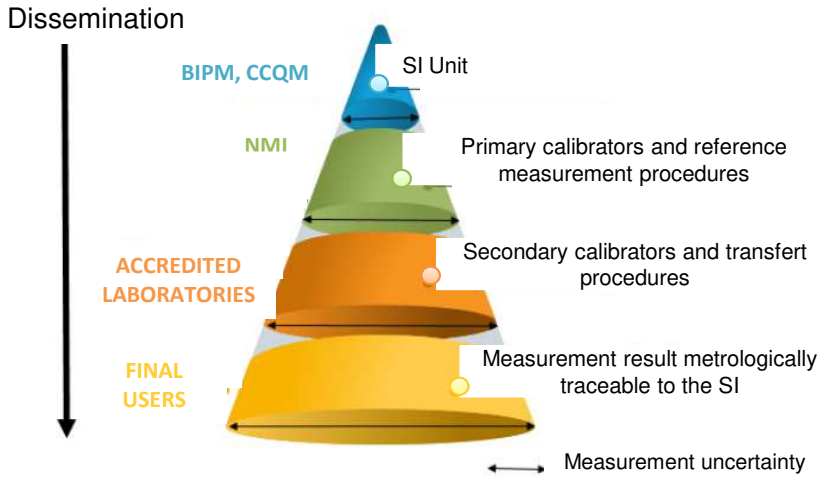
7-9 September 2022

EDC WFD


Estimation of measurement uncertainty
Quality + Comparability
Metrological traceabilty to the S.I.
Accuracy

Why is metrological traceability important?

Metrological traceability gives you confidence and assurance that your measurements results are right.



BIPM: Bureau international des poids et mesures, CCQM: Comité consultatif pour la quantité de matière, NMI: National Metrology Institute

METROLOGICAL TRACEABILITY

METROLOGICAL TRACEABILITY

ISO/IEC 17025:2017

6.5.1 The laboratory shall establish and maintain metrological traceability of its measurement results by means of a documented unbroken chain of calibrations, each contributing to the measurements uncertainty, linking them to an appropriate reference.

6.5.2 The laboratory shall ensure that measurement results are traceable to the International System of Units (SI) through:

a) calibration provided by a competent laboratory; or

b) certified values of certified reference materials provided by a competent producer with stated metrological traceability to the SI; or

c) direct realization of the SI units ensured by comparison, directly or indirectly, with national or international standards.

METROLOGICAL TRACEABILITY

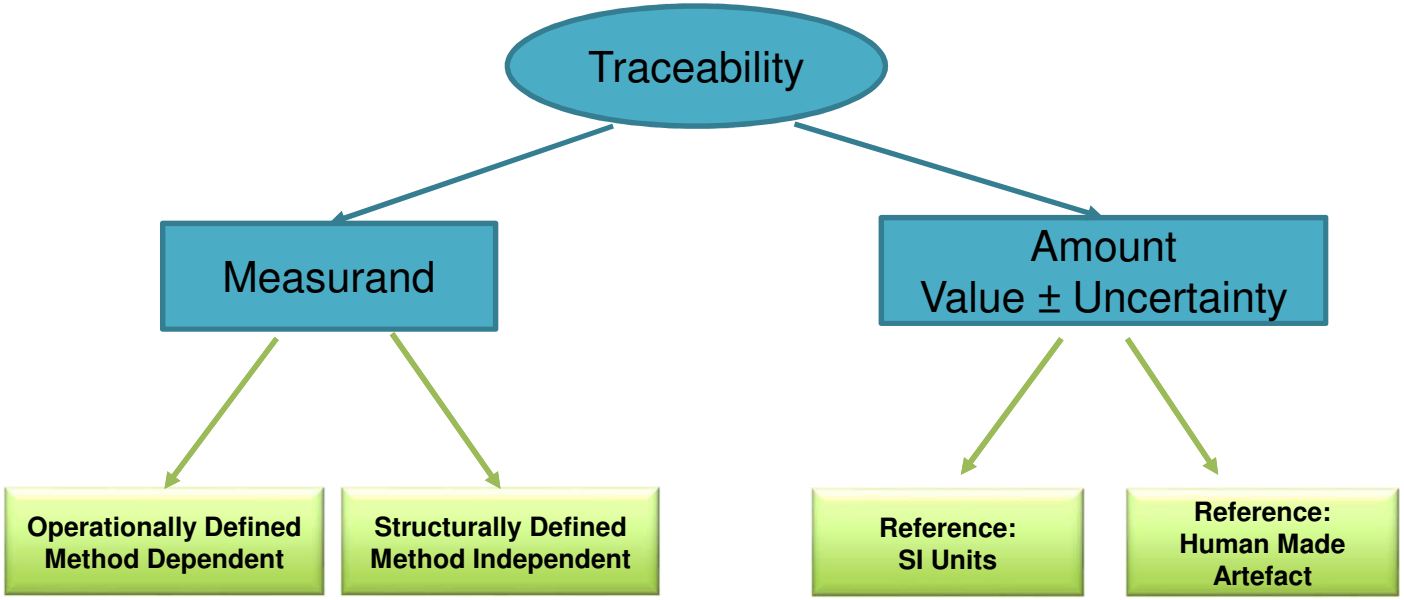
ISO/IEC 17025:2017

6.5.3 When metrological traceability to the SI units is not technically possible, the laboratory shall demonstrate metrological traceability to an appropriate reference, e.g.:

a) certified values of certified reference materials provided by a competent producer;

b) results of reference measurement procedures, specified methods or consensus standards that are clearly described and accepted as providing measurement results fit for their intended use and ensured by suitable comparison.

METROLOGICAL TRACEABILITY



METROLOGICAL TRACEABILITY

Definitions

Reference Material:

Material, sufficiently homogeneous and stable with respect to one or more specified **properties**, which has been established to be fit for its intended use in a measurement process.

Properties can be quantitative or qualitative, e.g. identity of substances or species.

Certified Reference Material:

Reference material characterized by a **metrologically valid procedure** for one or more specified properties, accompanied by an RM **certificate** that provides the **value** of the **specified property**, its associated **uncertainty**, and a statement of **metrological traceability**

METROLOGICAL TRACEABILITY

Lack of CRM to establish traceabilty: only one available for 17bE2.

5 CRM were developped produced by TUBITAK UME to fill the gap in this field:

- ✓ 17α-estradiol (solid standard)
- ✓ 17β-estradiol (solid standard)
- ✓ 17α-ethinylestradiol (solid standard)
- ✓ Estriol (solid standard)
- ✓ Estrone (solid standard)

To be used in the validation
To be used in the ILC
Artefacts could become commercially available after the end of the project


QUESTIONS?
COMMENTS?
CONCERNS?

METHOD VALIDATION

What are we talking about ?

- Validation: verification, where the specified requirements are adequate for an intended use (VIM, 2012)

Note 1 to entry: This process is used to asses that a method is fit for its intended purpose. It includes:

establishing the performance characteristics, advantages and limitations of a method and the identification of the influences which may change these characteristics, and the extent of such changes;

a comprehensive evaluation of the outcome of this process with respect to the fitness for purpose of the method.

- This fitness-for-purpose shall be demonstrated through experimental, well documented evidence

Experimental Design (philosophy)

It summarizes the in-house validation strategy for the MS-methods and EBMs optimized within the Project. All the partners will validate their methods in accordance with CEN/TS 16800:2020.

The scope of each method will describe the measurands, the matrices and application range

- Common design for all the partners

Comparability

- Metrologically robust
- Sustainable

Our strategy

METHOD VALIDATION (WITHIN LABORATORY)

❑ Validation will be performed according to the following standards:

Identification ISO 21253-1	+	Performance characteristics CEN/TS 16800: 2021 Level 1	+	Uncertainty estimation EN ISO 11352 GUM approach

❑ With respects to their intended scope:

	MS Based Methods	Effect Based Methods
Measurand	E1, 17αE2, 17βE2, 17αEE2, E3	E2 eq
Unit	µg/L	µg E2 eg /L
Matrix	Surface water and ground water Some partners will investigate drinking water and marine water	
Matrix characteristics	Whole water up to 50 mg/L of SPM, DOC level up to 7 mg/L	
Concentration range	1/3 EQS to tens of ng/L	

Performance characteristics

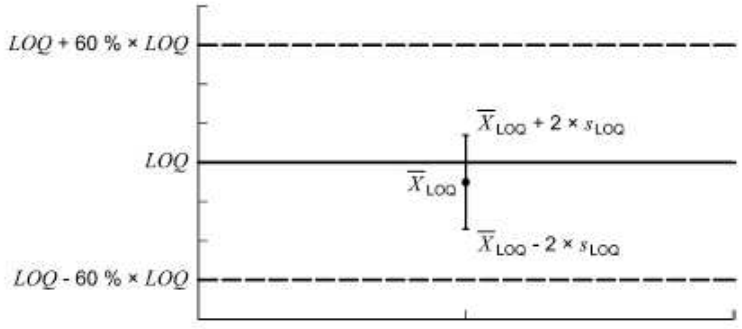
	MS based Methods
Calibration	X + calibration range
Application range	X
Detection limit	
Quantification limit	
Verified quantification limit	X
Selectivity	X
Sensitivity	X
Trueness	
Bias	X
Method recovery	Absolute and relative
Precision	X repeatability and intermediate precision (days, operators)
Measurement uncertainty	X

LIMIT OF QUANTIFICATION (VERIFIED)

- Experimental verification under intermediate precision condition of the estimated LOQ is essential
- To establish LOQ-V based on an estimated LOQ, **the level of accepted accuracy should be defined.**

$$X_{LOQ} - 2 \times s_{LOQ} > LOQ - 0{,}6 \times LOQ \quad (10)$$

$$X_{LOQ} + 2 \times s_{LOQ} < LOQ + 0{,}6 \times LOQ \quad (11)$$



EMA set at 60%

MATRIX SELECTION

Definition

Influence parameter: :intrinsic characteristic of the matrix, independent of the analyte concentration, a variation of which is liable to modify the analytical result [ISO/TS 21231:2019]

Representative matrix : sample for which all the intrinsic characteristics are characteristic of a type of water or the source of a group of samples [ISO/TS 21231:2019]

Representative validation matrix : matrix used to assess probable analytical performance with respect to other matrices, or for matrix-matched calibration, in the analysis of broadly similar commodities. [FDA]

Representative validation matrix

To be aligned with the scope !

- Type of waters :
  - **Inland surface waters**
  - **Ground waters**
  - Drinking water
- Influence parameters:

	MS based Methods / targeted substances
SPM	x
Organic content as DOC	x
Ionic strenght	x
Chlorine for tap water	
pH	no

Representative validation matrix

To be aligned with the scope !

Displaying of natural representative blank matrix

Representative validation matrix

➢ 3 common synthetic representative matrix

- ✓ Evian water + DOC: 1 mg/L
- ✓ Evian water + DOC: 7 mg/L
- ✓ Evian water + DOC: 7 mg/L + SPM 50 mg/L

➢ 3 representative matrix chosen by each laboratory: synthetic or natural

Panel of matrix implemented

- ✓ Tap water (high level chlorine)
- ✓ Evian water + DOC 3 mg/L
- ✓ Vittel water + DOC 3 mg/L
- ✓ Lake Water “native” (DOC 10 mg/L and 3 mg/L SPM)
- ✓ Lake Water + spiked DOC 5 mg/L
- ✓ Lake Water + spiked DOC 10 mg/L
- ✓ Evian water+ spiked DOC 14 mg/L+SPM 50mg/L
- ✓ Surface water with DOC 5.71 mg/L
- ✓ Surface water with DOC 5.84 mg/L
- ✓ Vittel DOC 3 mg/L
- ✓ Volvic DOC 3 mg/L
- ✓ Mont Roucous DOC 7mg/L
- ✓ Tap water DOC 10 mg/ L
- ✓ Canal water DOC 12 mg/L
- ✓ Saskia - Source „Leissling“ DOC 3 mg/L

Method validation experimental

- ✓ 6 matrix: 3 common + 3 customized (in intermediate precision conditions)

  3 levels of concentrations: LOQ, 3LOQ, 10LOQ

  Triplicates

  ME% at 3LOQ

  R% at 3 LOQ

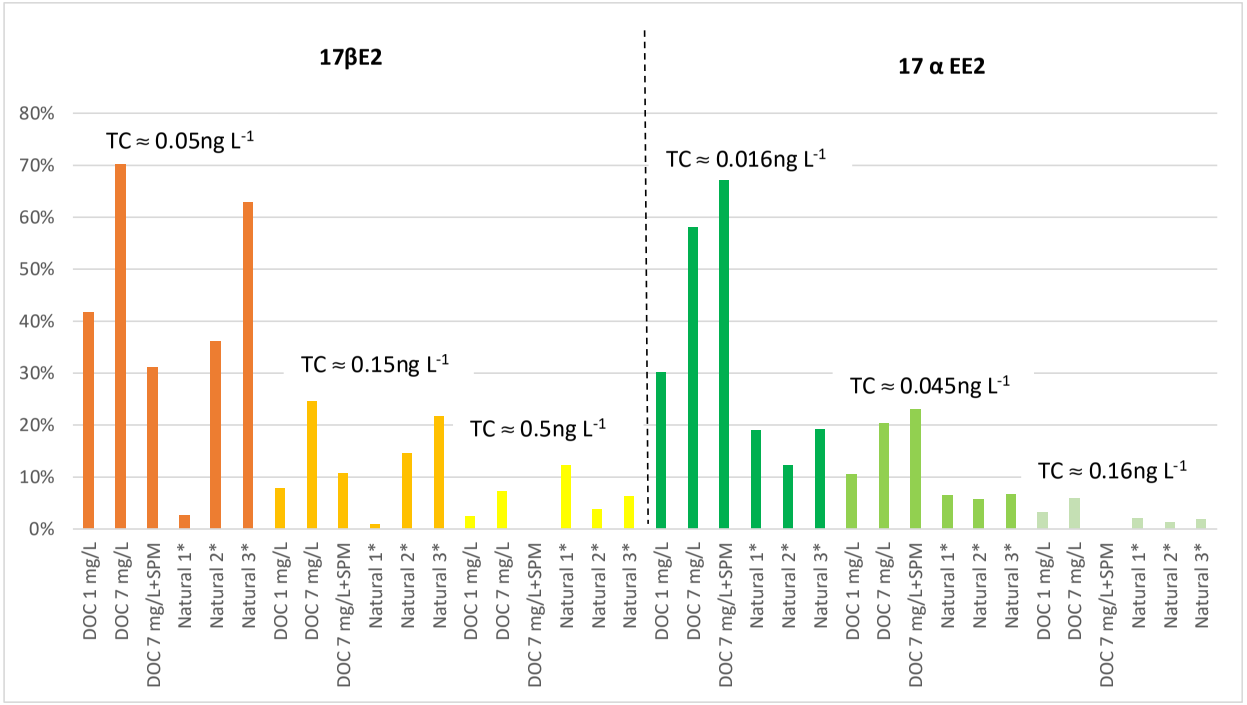
  6 calibrations (in intermediate precision conditions)


QUESTIONS?
COMMENTS?
CONCERNS?

Blank matrix

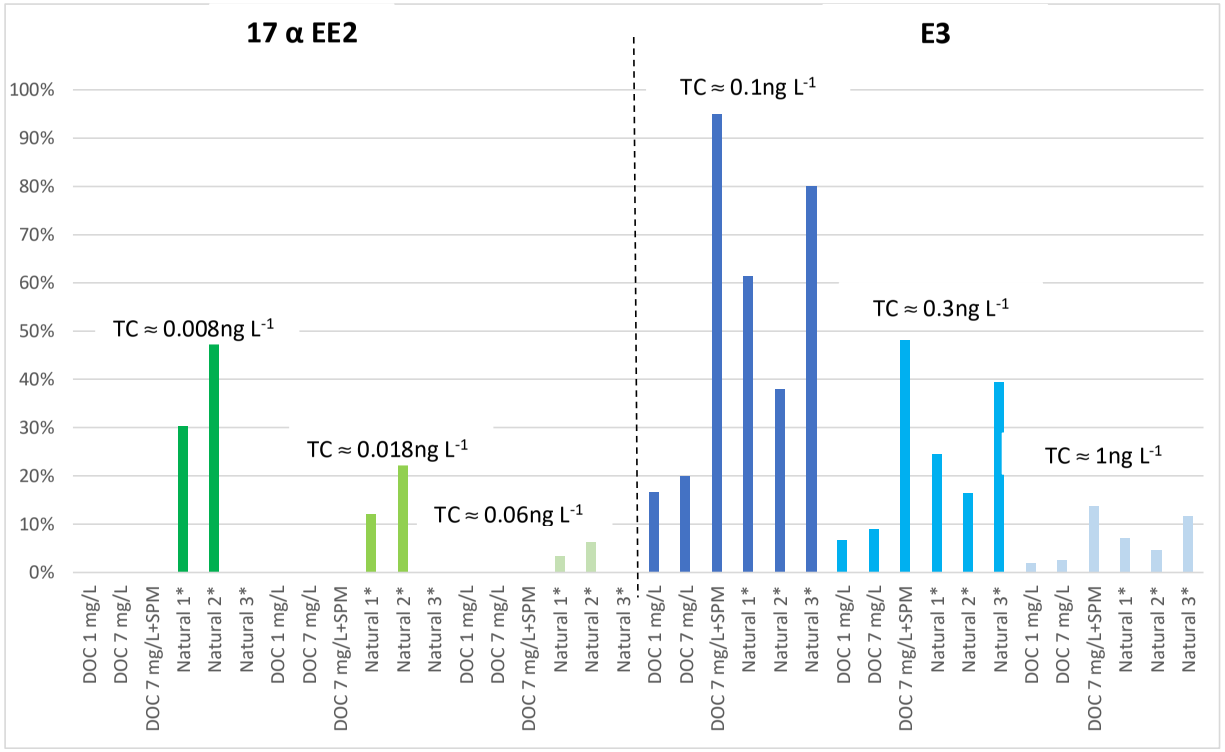
ISSUE OF BLANK/BACKGROUND

Ratio of the blank/targeted concentration (TC) %



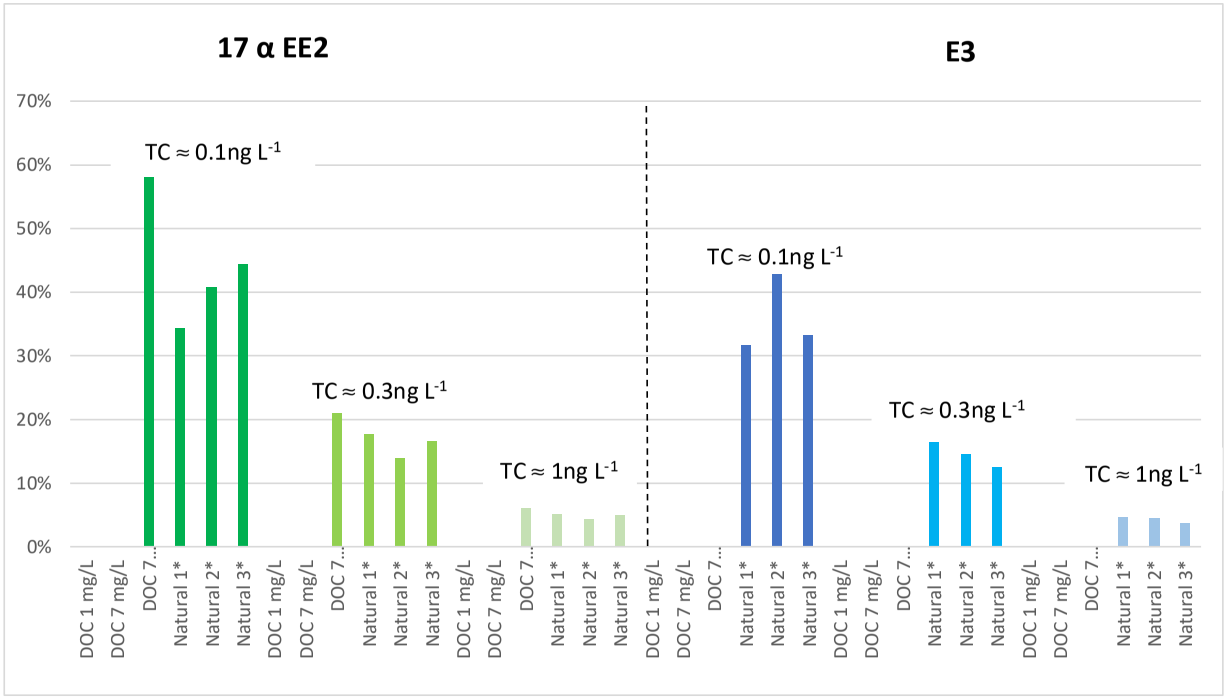
ISSUE OF BLANK/BACKGROUND

Ratio of the blank/targeted concentration (TC) %

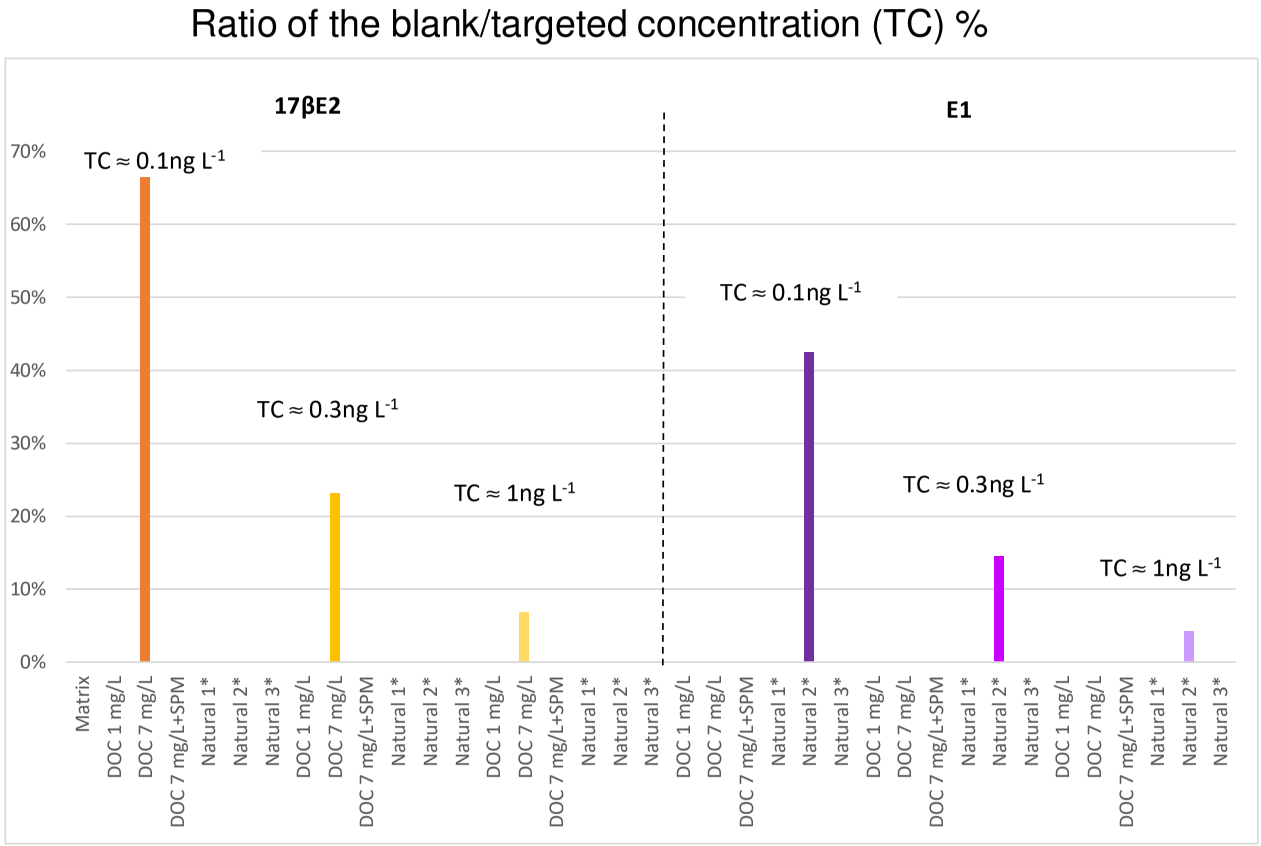


ISSUE OF BLANK/BACKGROUND

Ratio of the blank/targeted concentration (TC) %

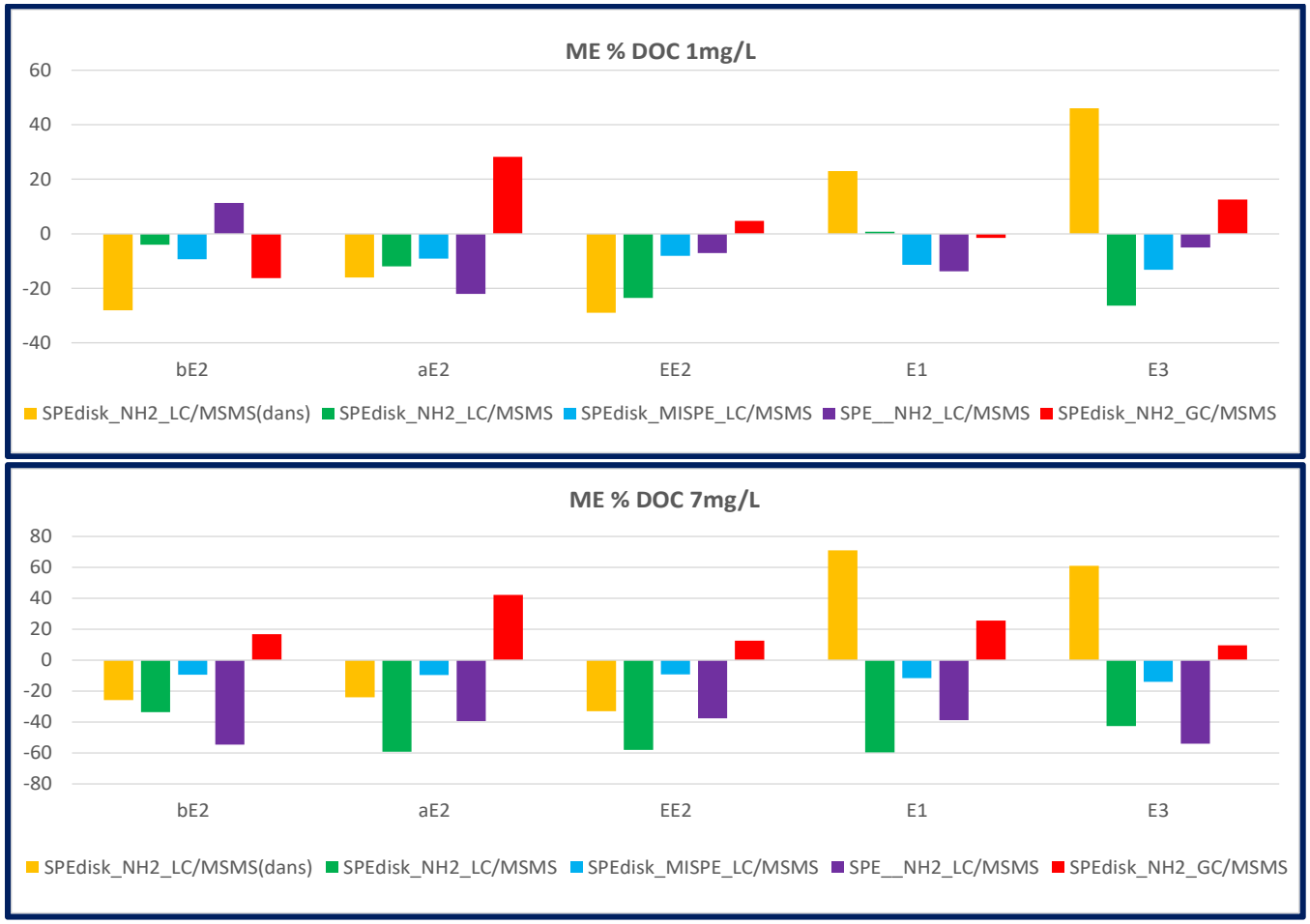


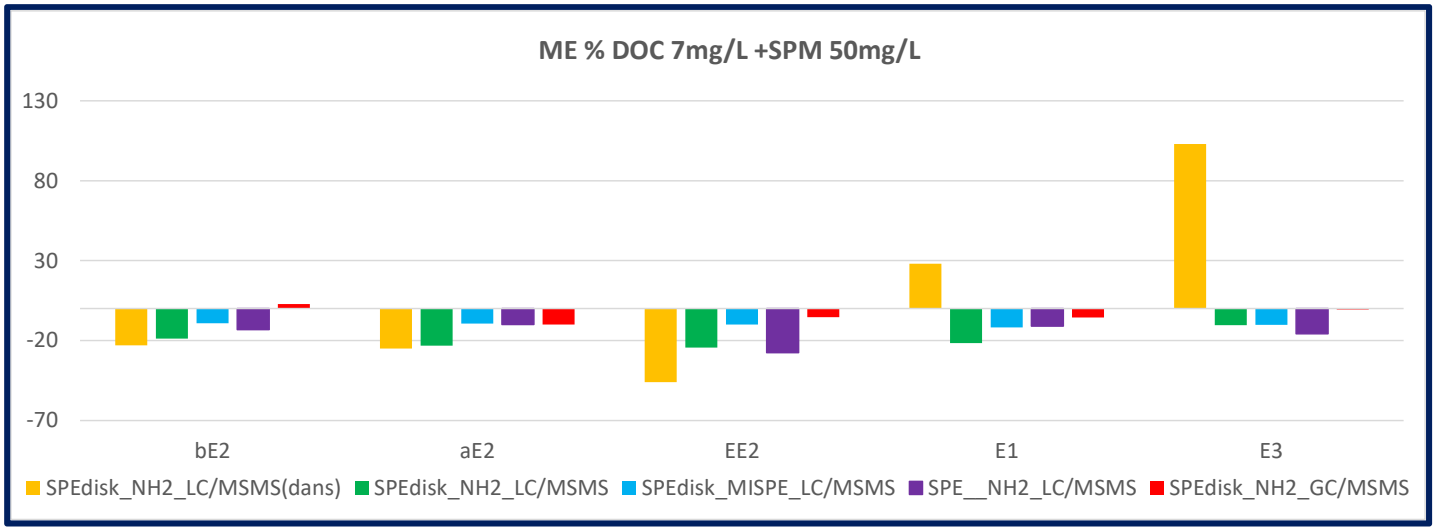
ISSUE OF BLANK/BACKGROUND




QUESTIONS?
COMMENTS?
CONCERNS?

Matrix effects

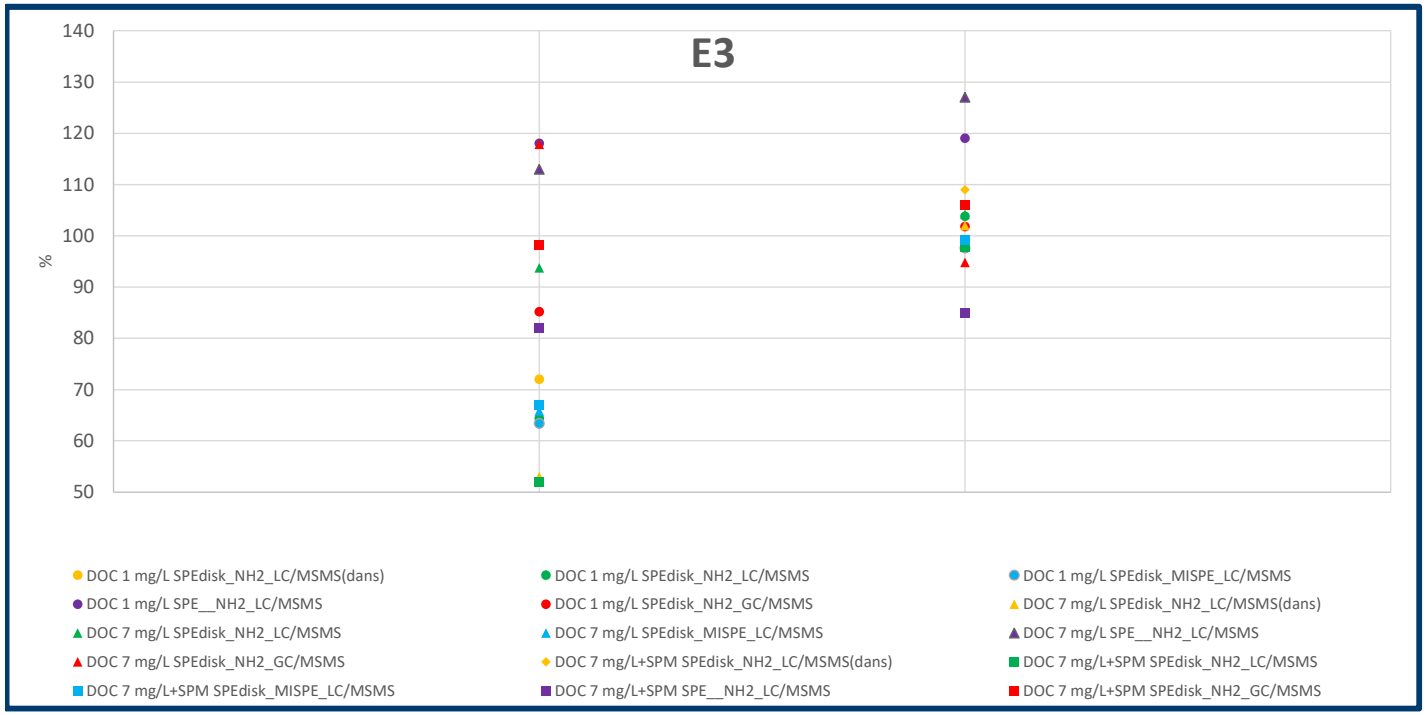

ME % DOC 1mg/L
60
40
20
0
-20
-40
bE2
aE2
EE2
E1
E3
SPEdisk_NH2_LC/MSMS(dans)
SPEdisk_NH2_LC/MSMS
SPEdisk_MISPE_LC/MSMS
SPE__NH2_LC/MSMS
SPEdisk_NH2_GC/MSMS
ME % DOC 7mg/L
80
60
40
20
0
-20
-40
-60
-80
bE2
aE2
EE2
E1
E3
SPEdisk_NH2_LC/MSMS(dans)
SPEdisk_NH2_LC/MSMS
SPEdisk_MISPE_LC/MSMS
SPE__NH2_LC/MSMS
SPEdisk_NH2_GC/MSMS



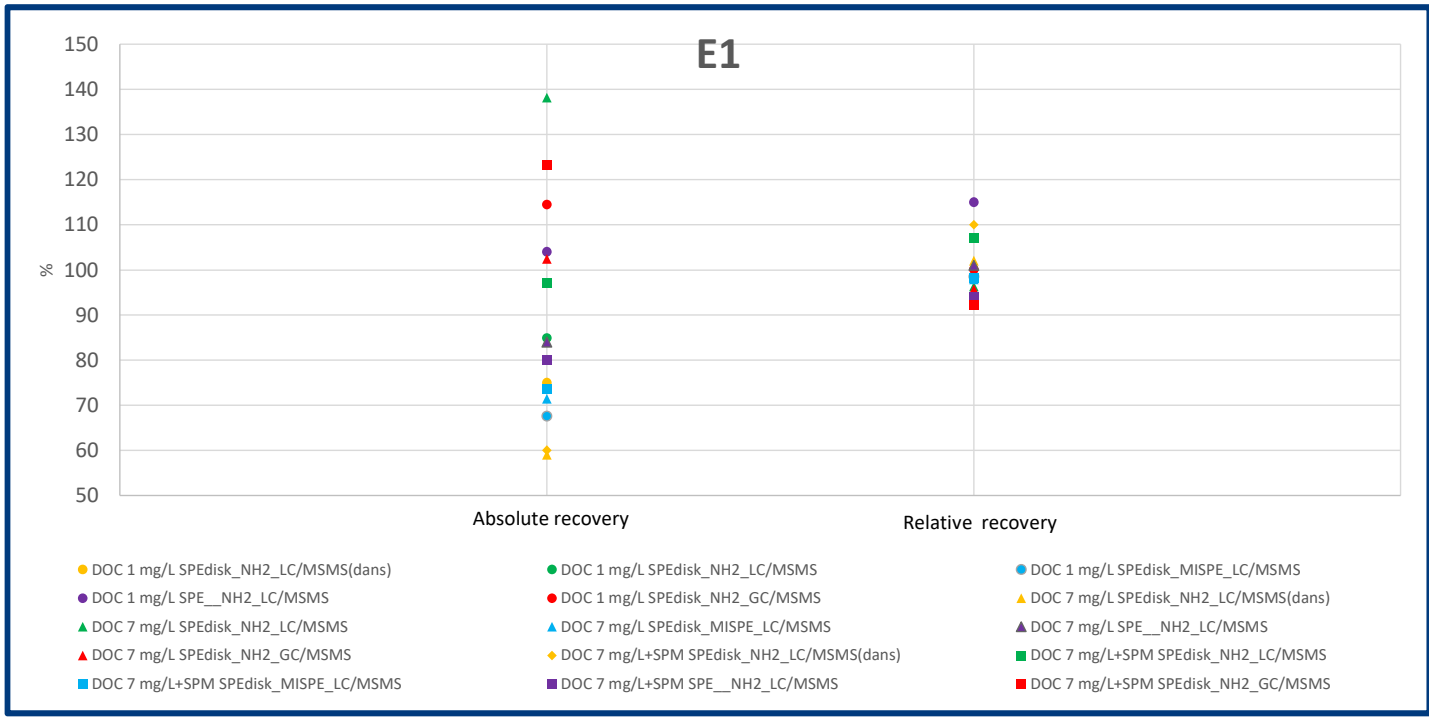
ME% MISPE LC MSMS ≈ ME% GC MSMS < ME% LC/MSMS

Purification limits ME% ⇒ MISPE >> LC NH2

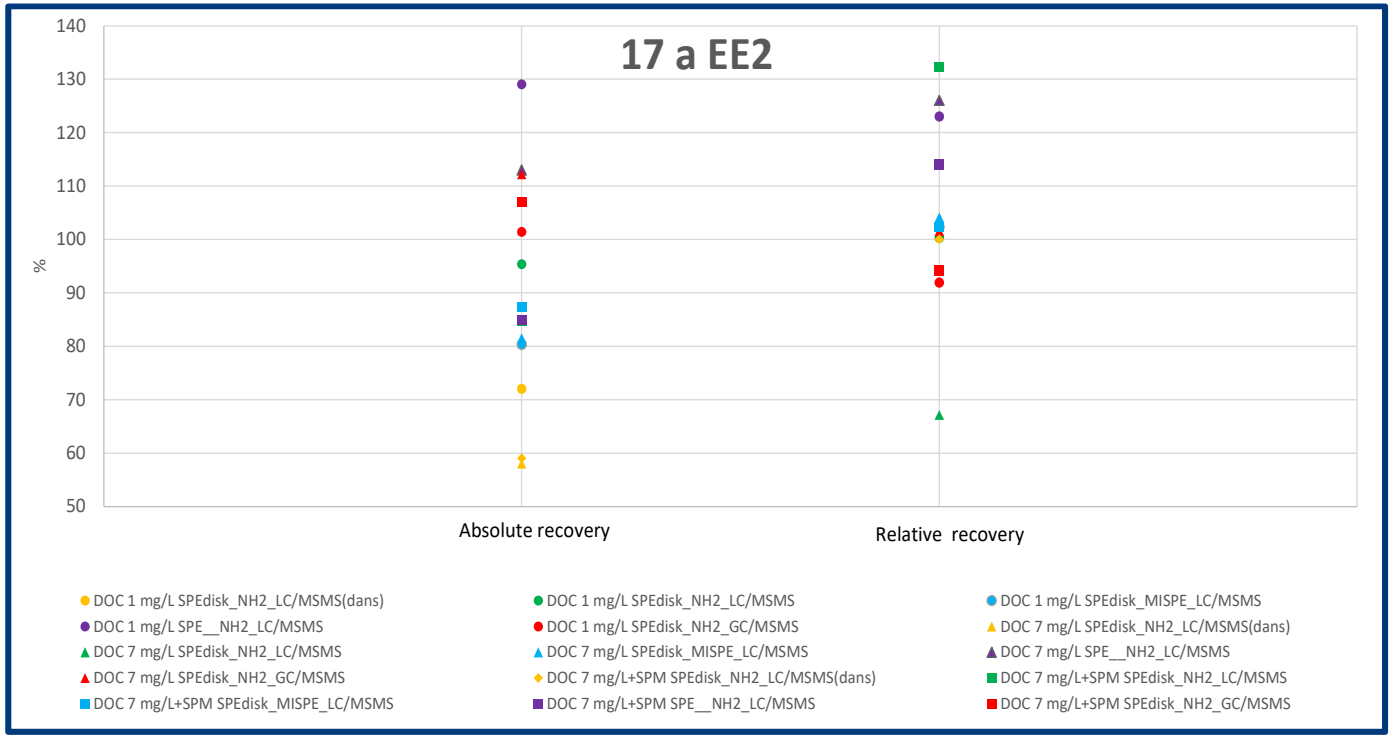
Recoveries



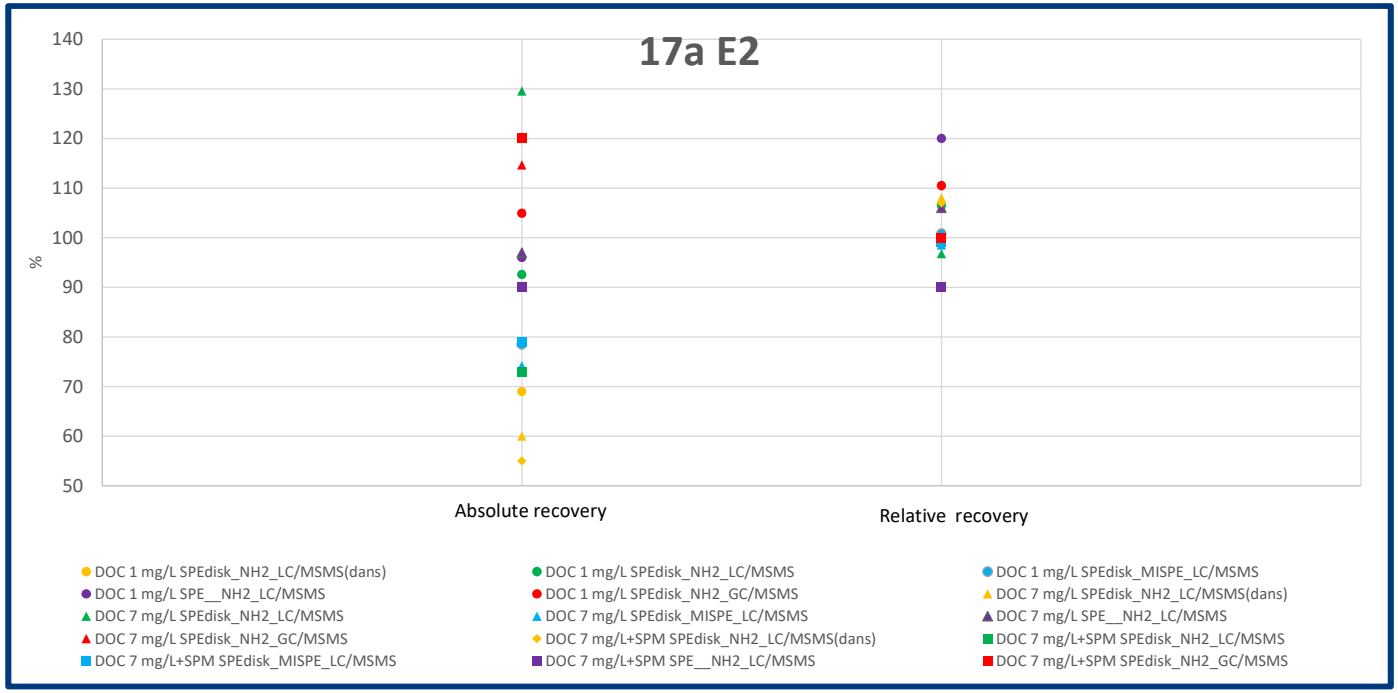
Abs R% > 50% ∀ matrix & methods
ID/MS suitable to reliable measurements



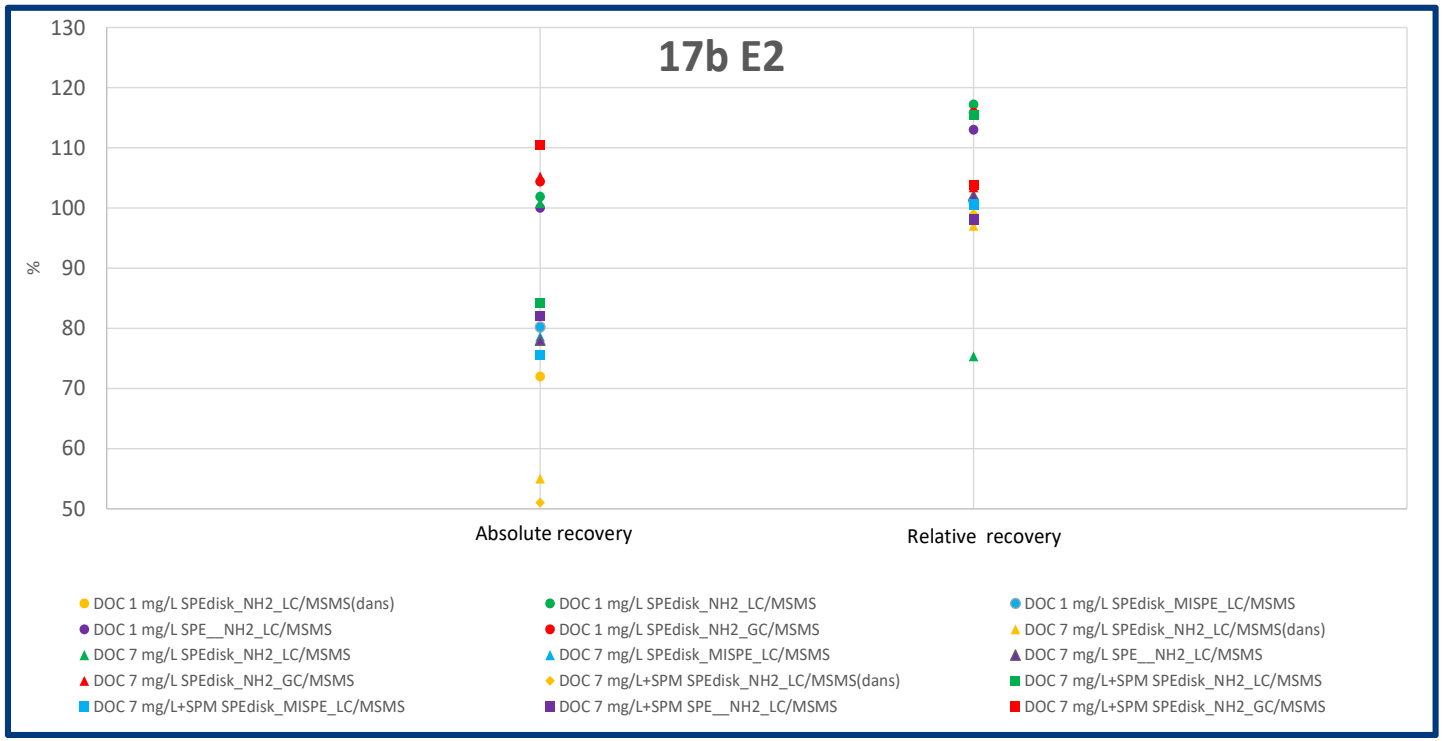
Abs R% > 50% ∀ matrix & methods
ID/MS suitable to reliable measurements



Abs R% > 50% ∀ matrix & methods
ID/MS suitable to reliable measurements



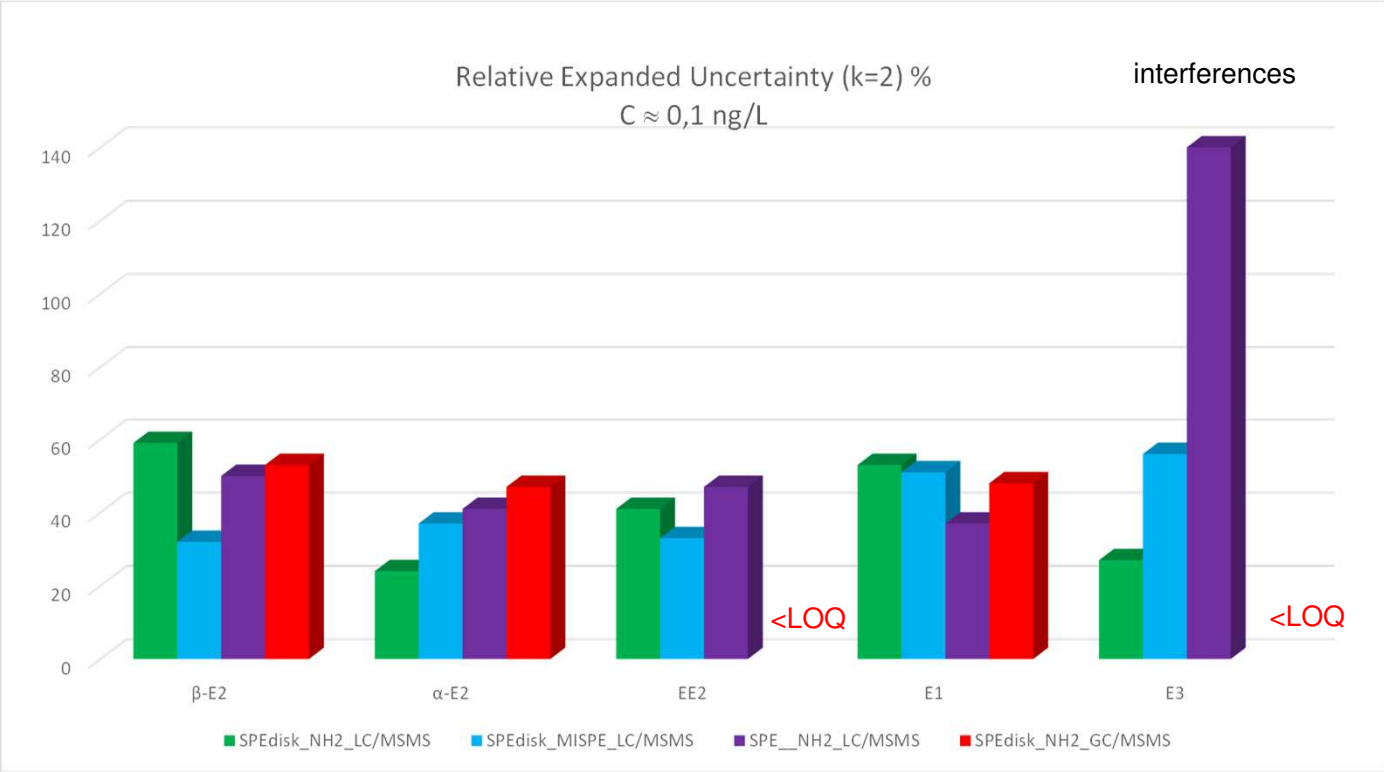
Abs R% > 50% ∀ matrix & methods
ID/MS suitable to reliable measurements



Abs R% > 50% ∀ matrix & methods
ID/MS suitable to reliable measurments


QUESTIONS?
COMMENTS?
CONCERNS?

Uncertainties


Relative Expanded Uncertainty (k=2) %
C ≈ 0,1 ng/L
interferences
140
120
100
80
60
40
20
0
<LOQ
<LOQ
β-E2
α-E2
EE2
E1
E3
SPEdisk_NH2_LC/MSMS
SPEdisk_MISPE_LC/MSMS
SPE__NH2_LC/MSMS
SPEdisk_NH2_GC/MSMS

Accuracy
LOQ

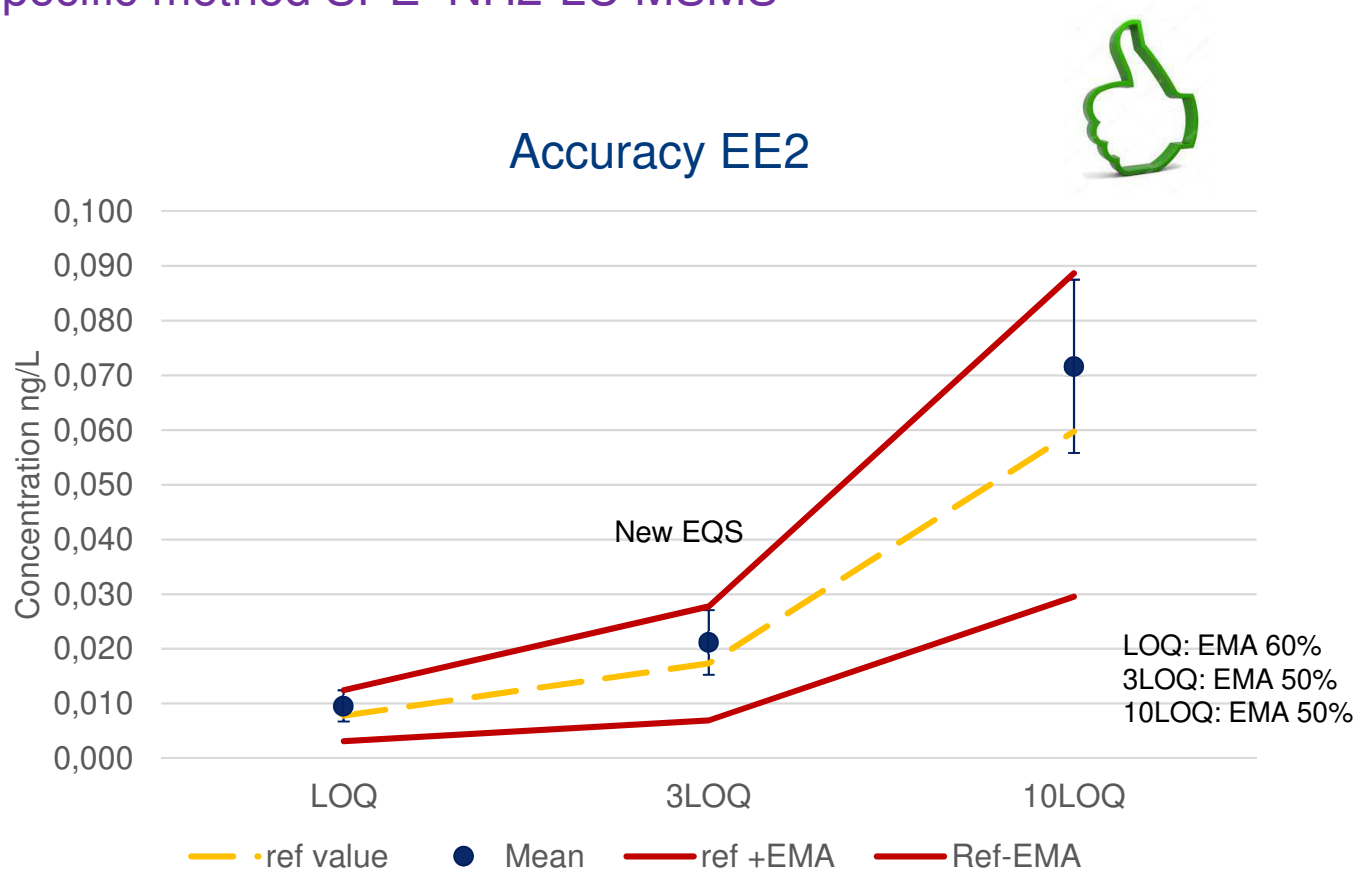
- Concerning GC/MSMS, the LOQ are highest compared to LC/MSMS but the robustness towards ME is more important

- Methods based on HRMS either coupled with GC or LC do not increase the sensitivity and will not solve the issue of the WFD requirements

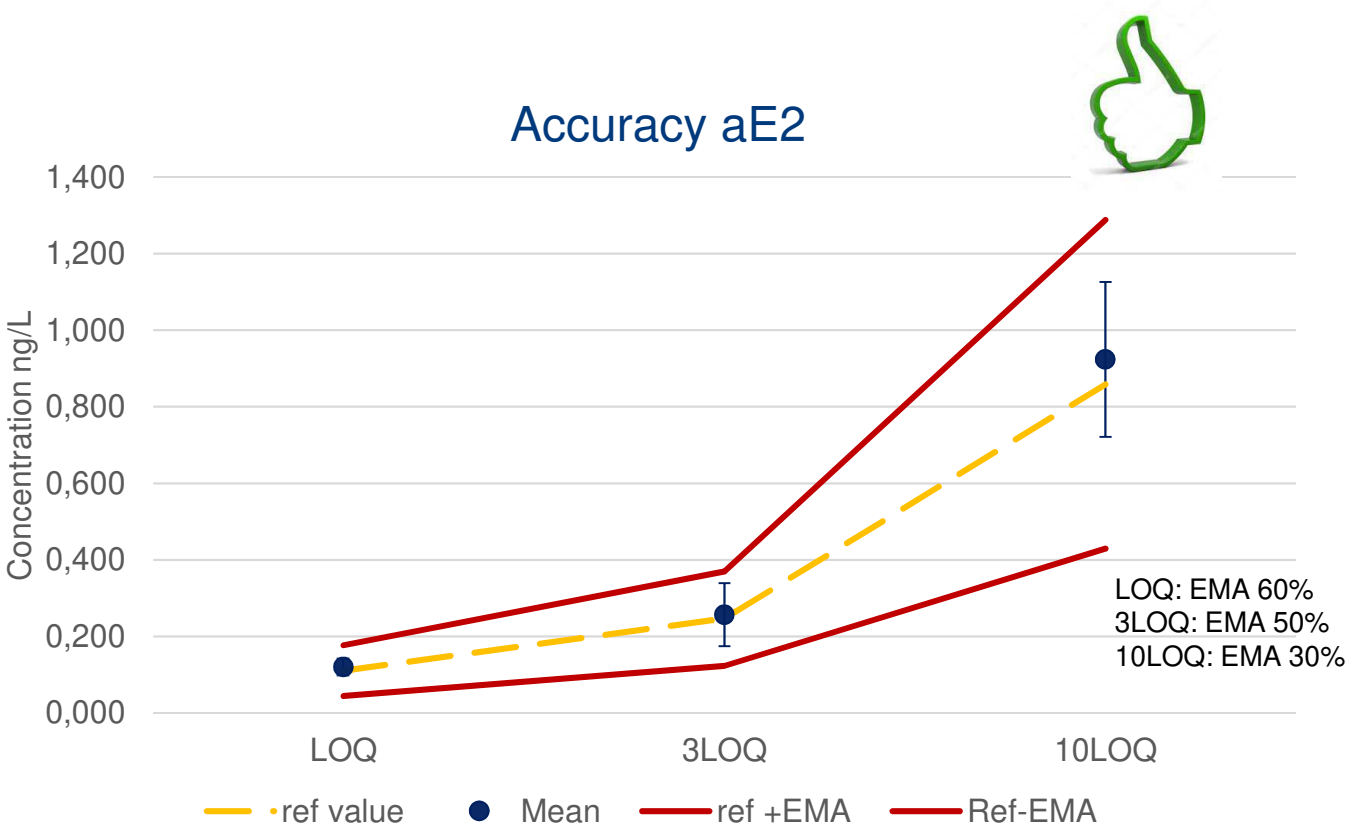
- All the methods developped by LC MSMS reach LOQ 0,1 ng/L (with the EMA set at 60%)

- Some of the developped methods have the potencies to tackle new EQS and 1/3 EQS for EE2 notably

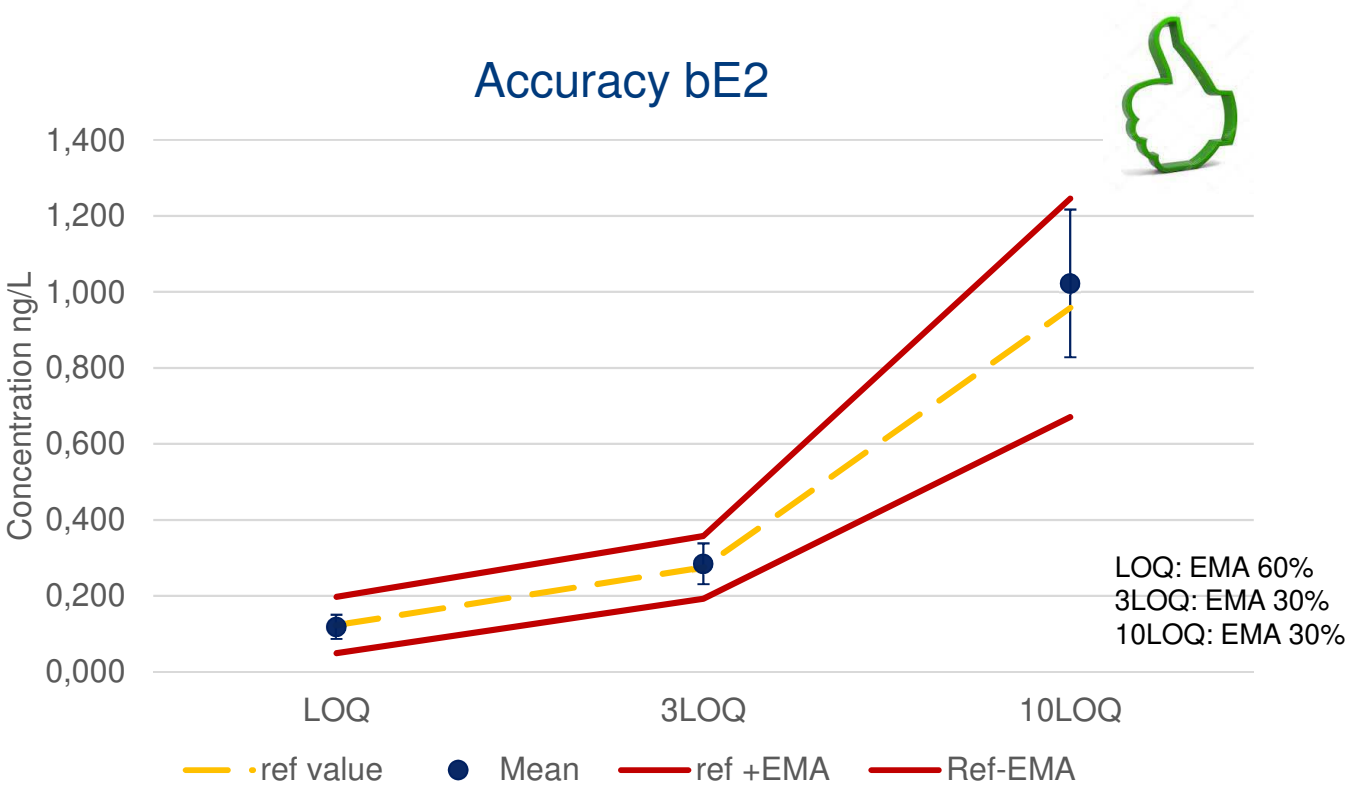
Specific method SPE–NH2-LC MSMS



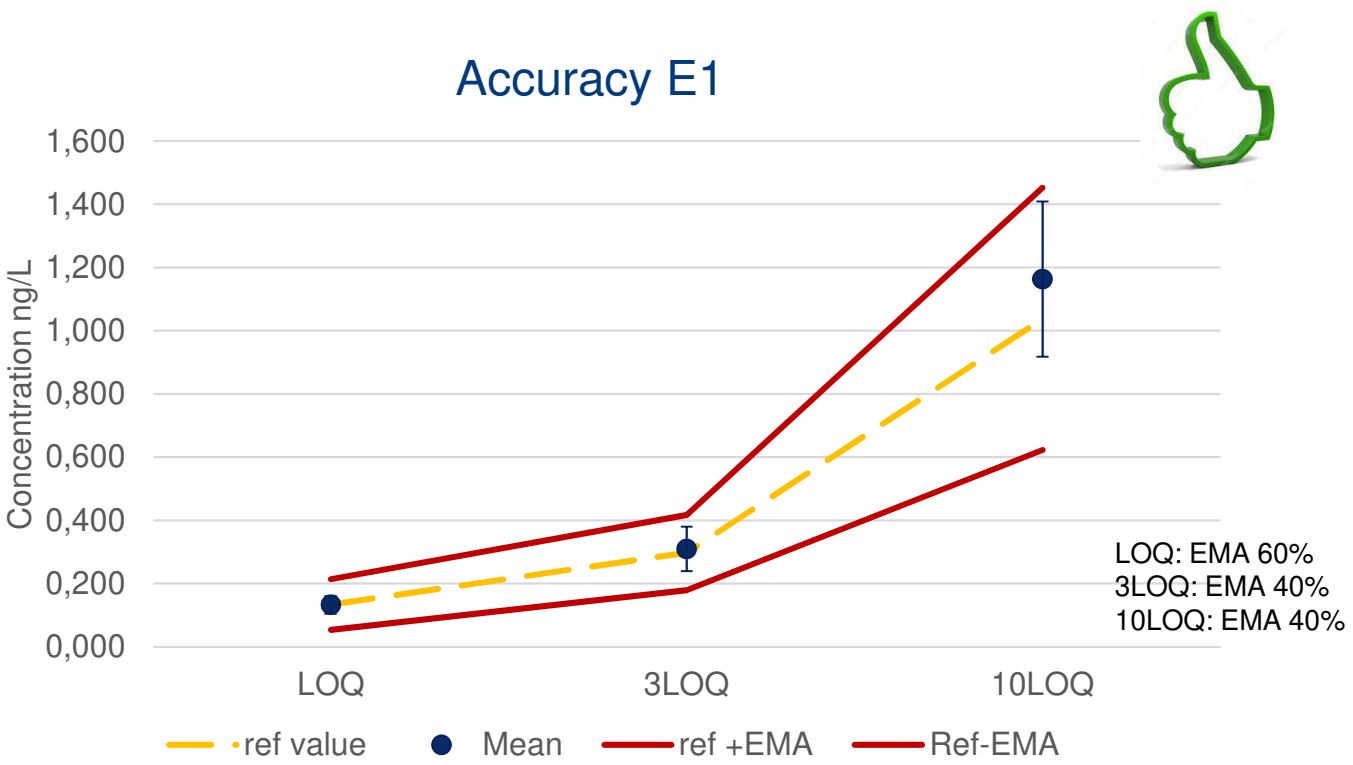
method SPE–NH2-LC MSMS



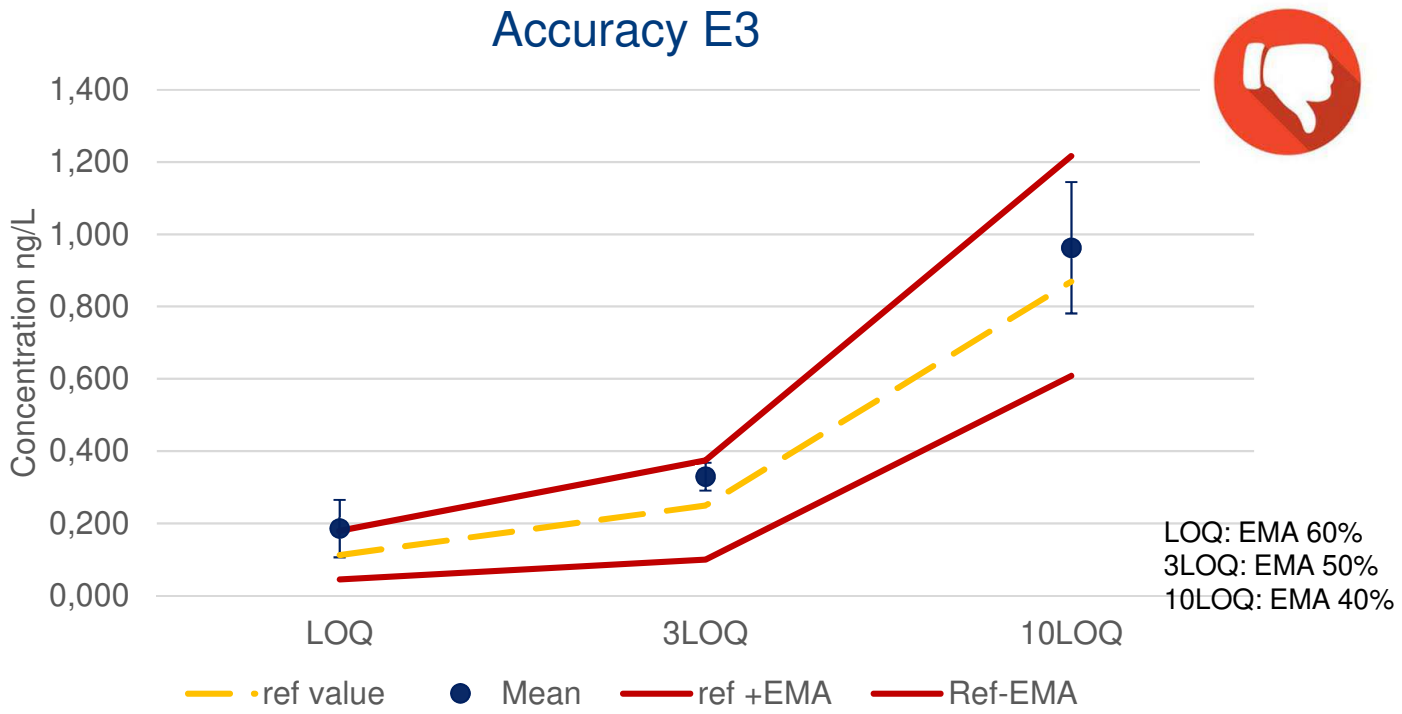
method SPE–NH2-LC MSMS



method SPE–NH2-LC MSMS

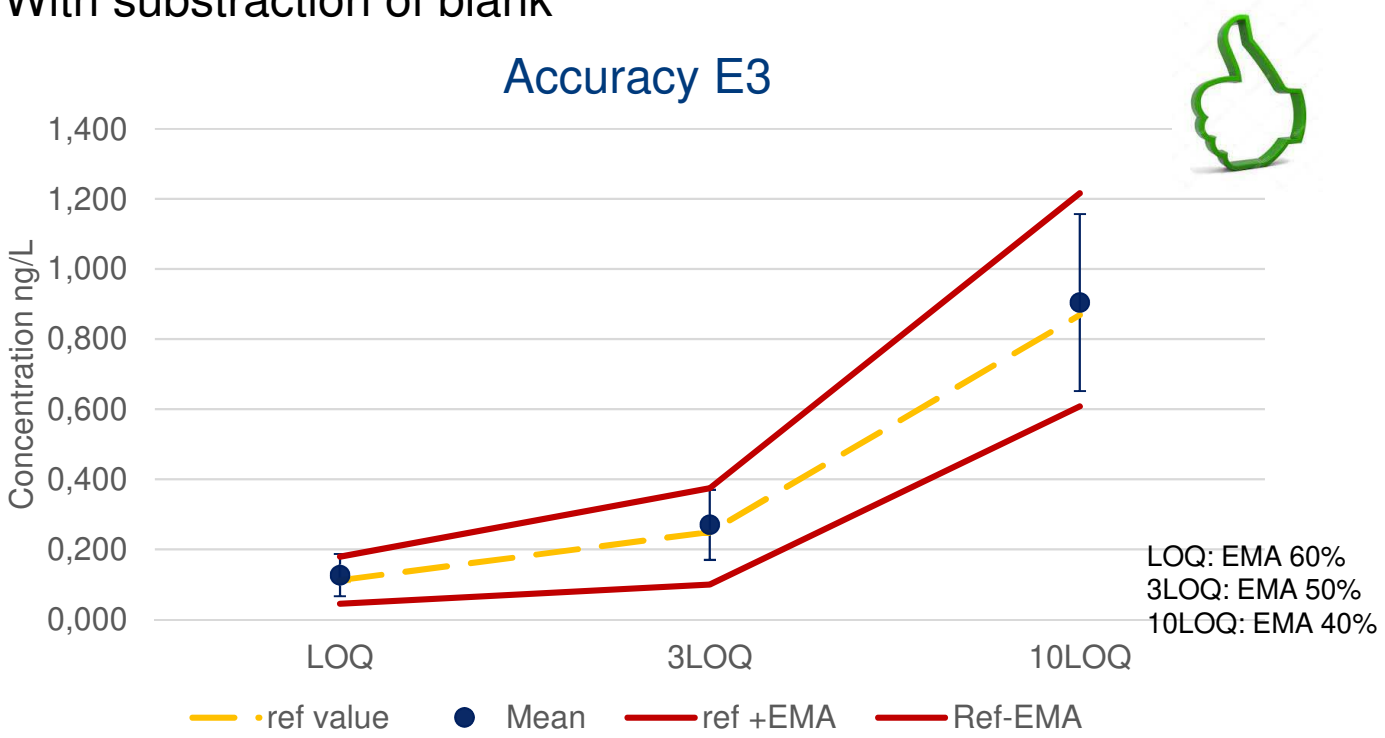


method SPE–NH2-LC MSMS

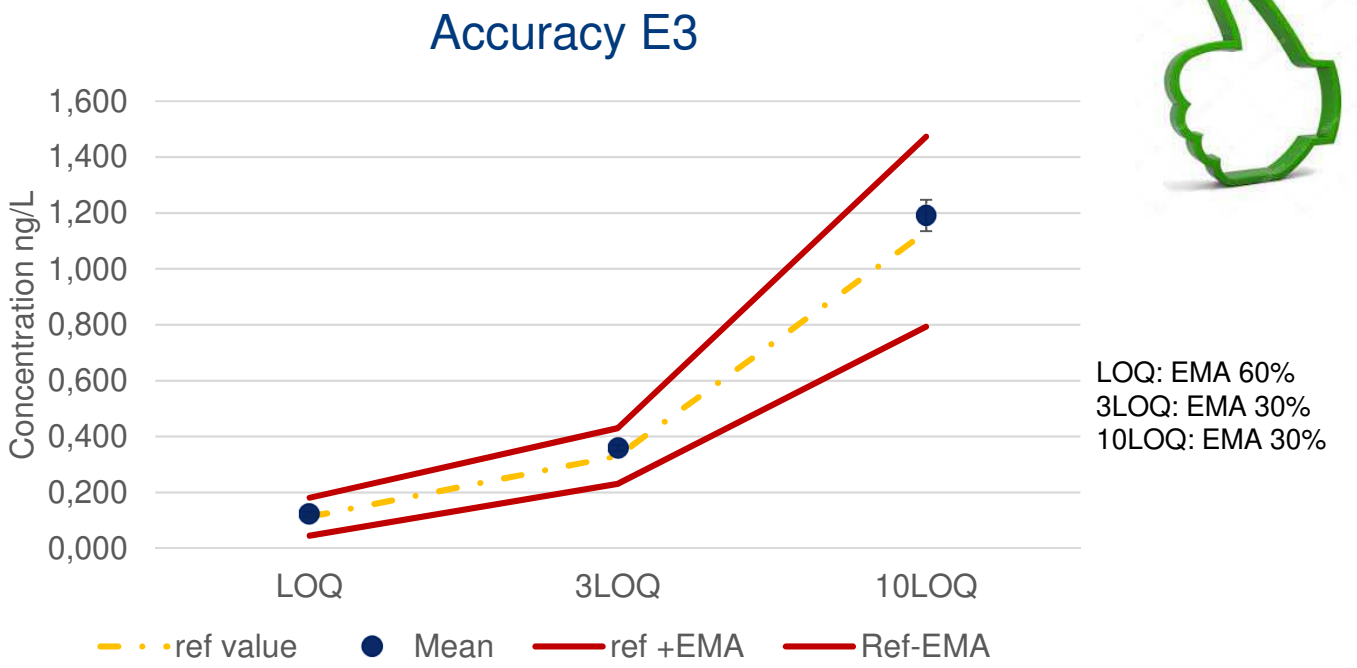


method SPE–NH2-LC MSMS

With substraction of blank

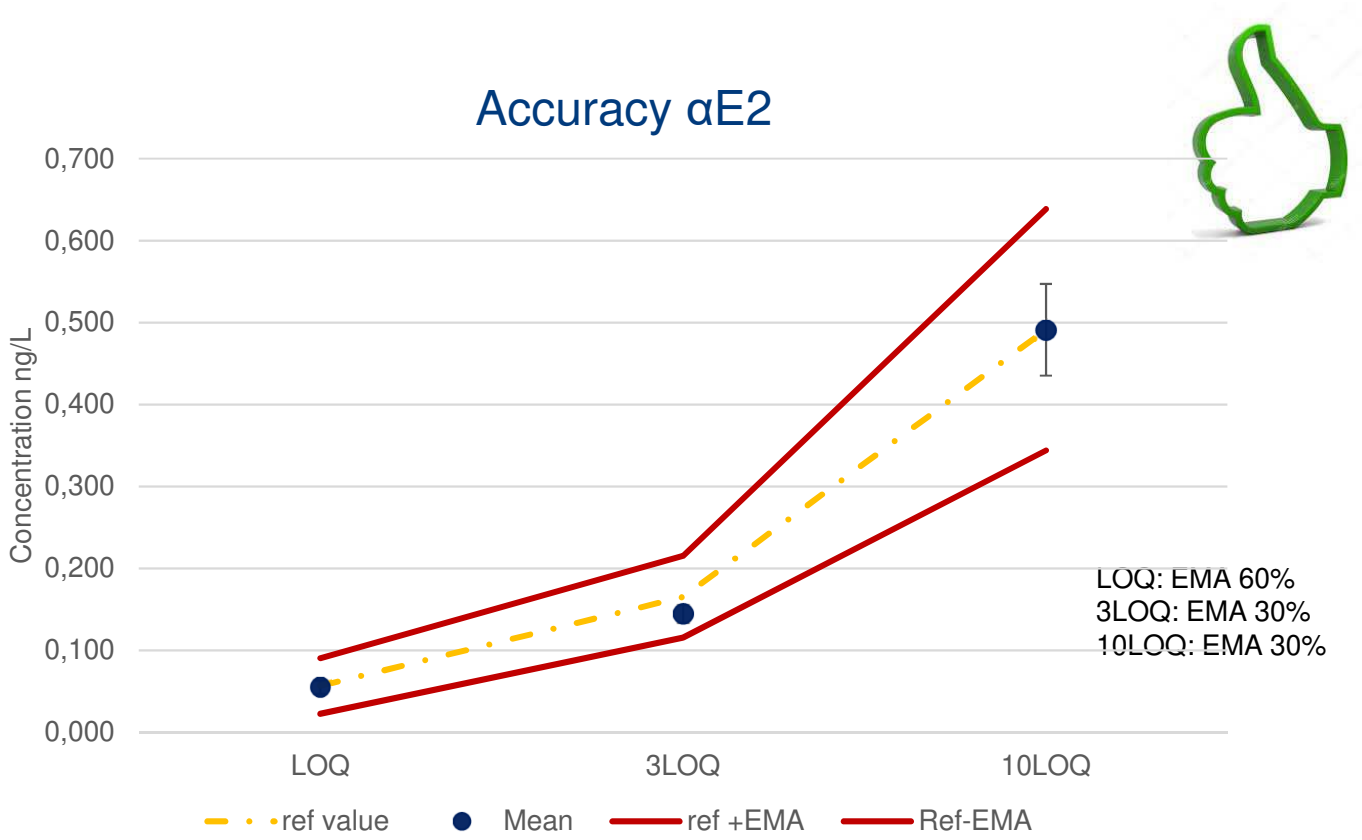


Method SPEdisk–NH_2-LC MSMS

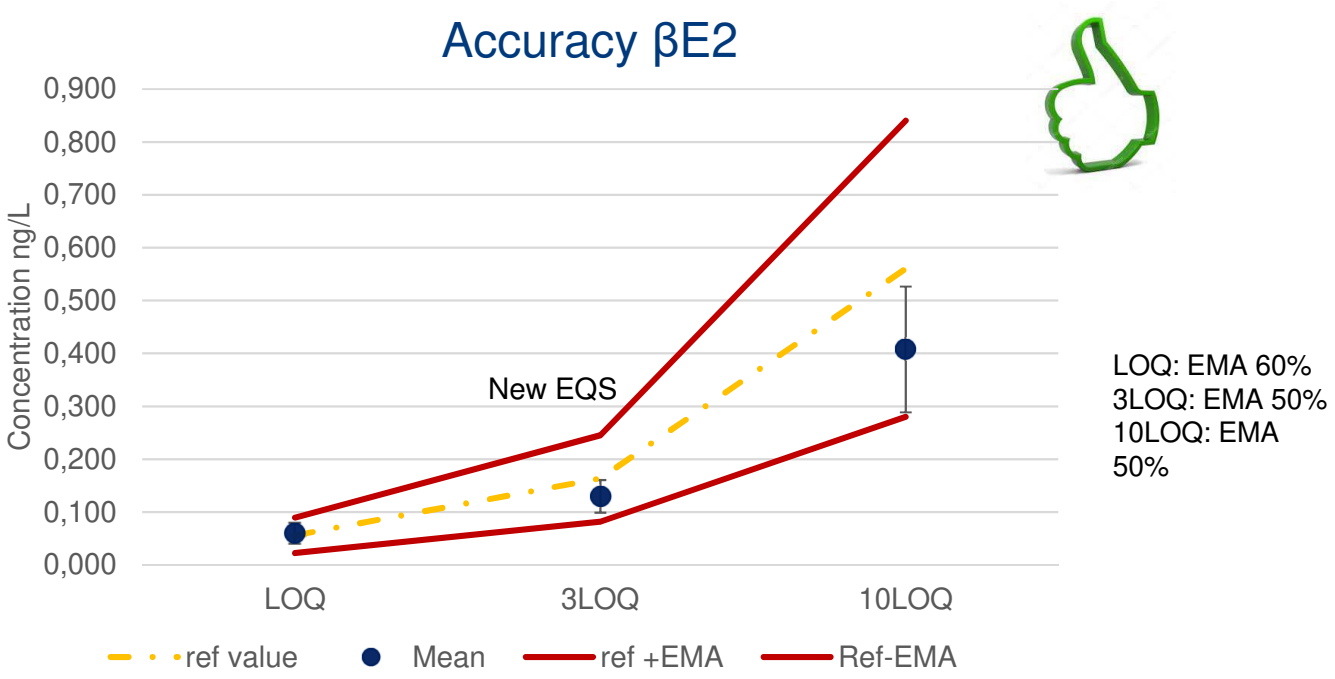


LOQ: EMA 60%
3LOQ: EMA 30%
10LOQ: EMA 30%

Method SPEdisk–NH_2-LC MSMS

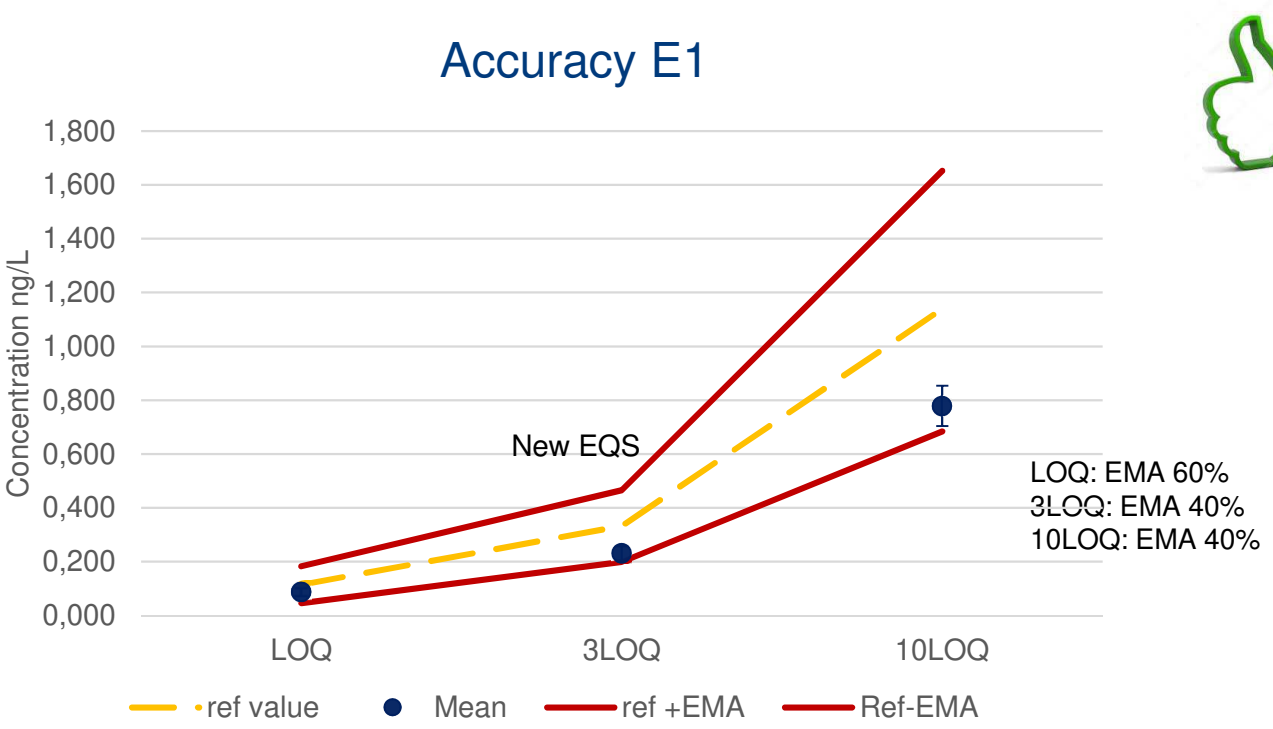


Method SPEdisk–NH_2-LC MSMS



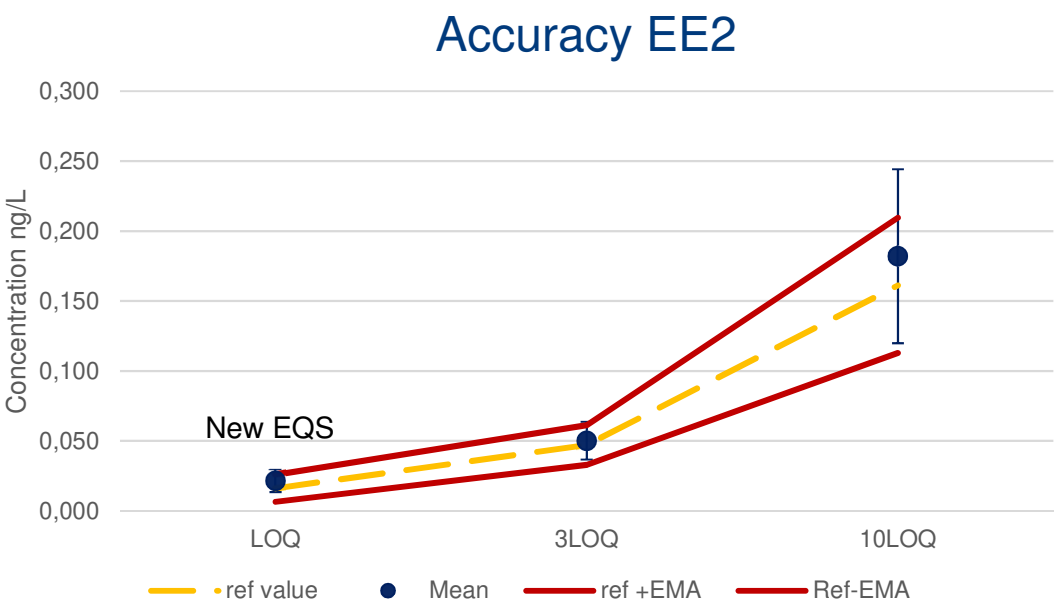
LOQ: EMA 60%
3LOQ: EMA 50%
10LOQ: EMA 50%

Method SPEdisk–NH_2-LC MSMS



LOQ: EMA 60%
3LOQ: EMA 40%
10LOQ: EMA 40%

Method SPEdisk–NH_2-LC MSMS

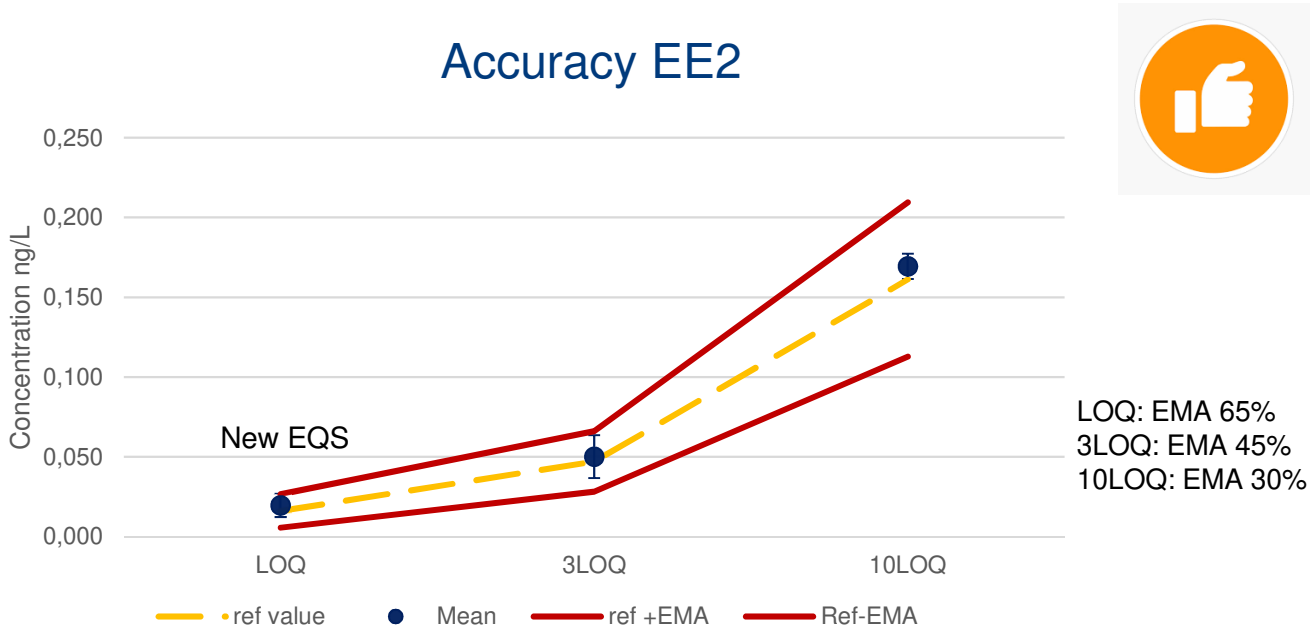


LOQ: EMA 60%
3LOQ: EMA 30%
10LOQ: EMA 30%

- For 10 LOQ we should have repeated matrix 3 (due to contamination of SPM) we have made a underestimation of the impact. If we delete these data <<<EMA 30%
- For LOQ we do not validate our first level at 60% but at 85 % ⇒ significance of background level at the tested LOQ

Method SPEdisk–NH_2-LC MSMS

Accuracy EE2 For Matrix with background <50% of the LOQ



LOQ: EMA 65%
3LOQ: EMA 45%
10LOQ: EMA 30%

Method SPEdisk–NH_2-LC MSMS (dans)

Validated method on a restrictive scope ground water/surface water up to DOC 3mg/L and SPM 50 mg/L


QUESTIONS?
COMMENTS?
CONCERNS?

RECOMMANDATIONS

« GOLD STANDARD »
&
GOOD PRACTICE

Ultratraces estrogens measurements < 0,1ng/L

⇒ New dimension

Every detail counts

Generalities

- Advanced chemists/operators
- Dedication of material to estrogens measurements

- Glassware shall be prefered to any other type of material
- Cleaning with detergents/solvents/calcination shall be implemented + shorten time of storage before uses
- Caution has to be paid with reusable material as it could increase risks of cross contamination + losses due to sorption
- Solvents including laboratory water even with high purity could be a problem because of interferences: should be checked regularly
- Gravimetric control

Generalities

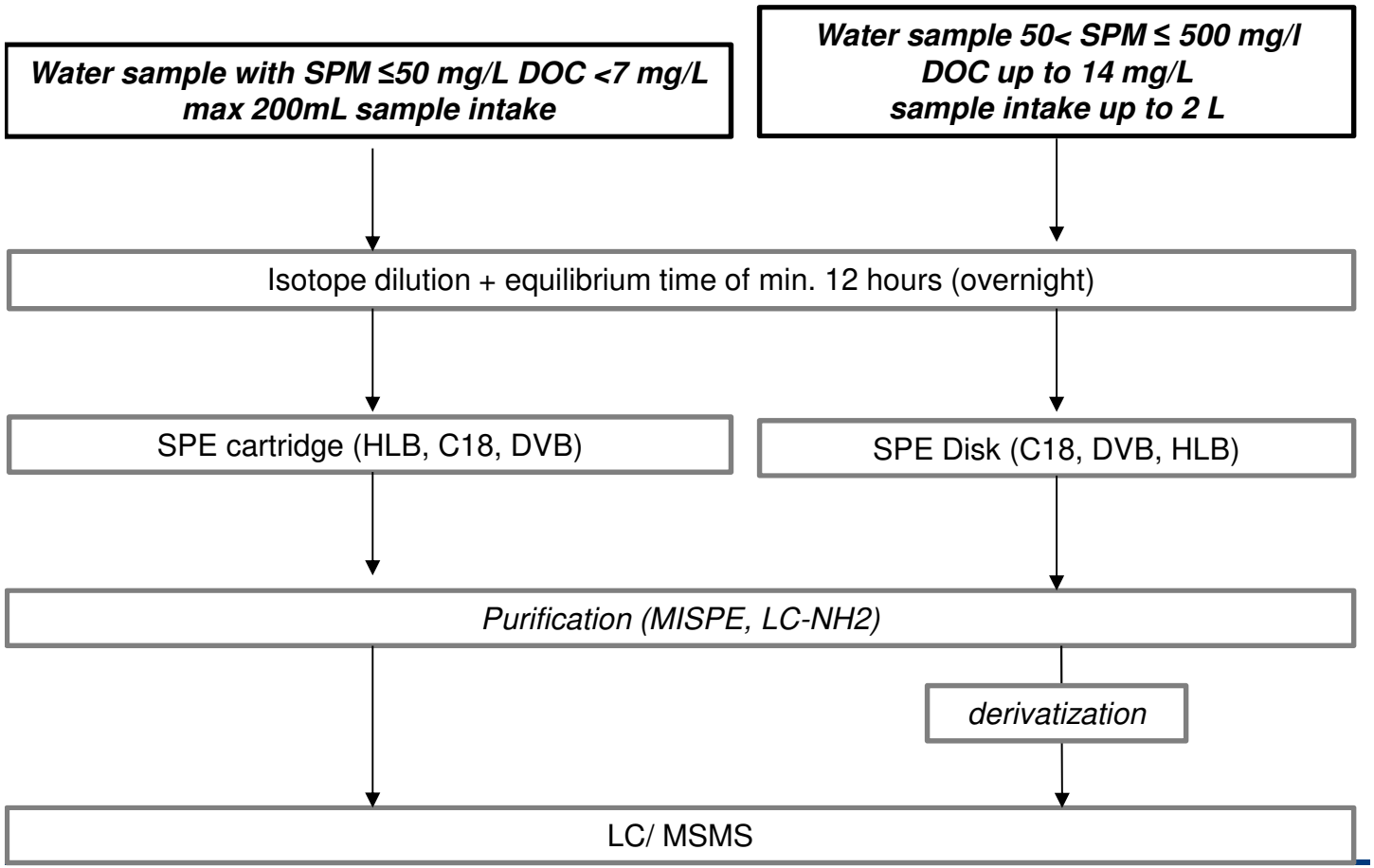
- 17 a E2 shall be introduced in the method

- ID/MS shall be implemented to reliable quantification
- Inclusion of tracers is recommended

- Validation (LOQ) in real representative matrix shall be the reference approach

- Validation based on defined EMA shall be prefered to S/N approach as being more robust

- QA/QC approach shall be rigorous and implemented to cover the overall procedure :
  - Method blank shall be systematically implemented in each series
  - Instrumental blanks shall be included in each run to prevent cross contamination
  - Positive QC at the LOQ shall be included in each series to attest of the efficiency of the sample preparation
  - Positive QC at the IQL/LOQ shall be included in each runs to attest on the intrinsic sensitivity of the instrument and the absence of drift


Water sample with SPM ≤50 mg/L DOC <7 mg/L
max 200mL sample intake
Water sample 50< SPM ≤ 500 mg/l
DOC up to 14 mg/L
sample intake up to 2 L
Isotope dilution + equilibrium time of min. 12 hours (overnight)
SPE cartridge (HLB, C18, DVB)
SPE Disk (C18, DVB, HLB)
Purification (MISPE, LC-NH2)
derivatization
LC/ MSMS

LC/MSMS

- Implementation of prefilter + guard column is recommended
- Dedication of instrument is recommended
- Rigorous preventing cleaning shoud be implemented
- Automatic integration should be used with caution
- Avoid multiple injection of same blank


QUESTIONS?
COMMENTS?
CONCERNS?