



Metrology for monitoring endocrine disrupting compounds under the Water Framework Directive

Mass spectrometry developments



21-22 February 2023



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







WFD

EDC

[≋]

LC/HRMS





Q exactive Orbitrap





Mass Spectrometry methods – LC-ESI-HRMS

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

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

LC/HRMS



- Triple TOF
- Q exactive Orbitrap

Not enough sensitive





EDC

[≋]

WFD

LC/MSMS (ESI-)



EDC 🗊

WFD





LC/MSMS

 LC parameter optimisation Column Mobile phase Chromatographic separation

 MSMS parameter optimisation MRM Sensitivity, selectivity



LC parameter optimization : choice of column



EDC 🛞 WFD

LC parameter optimization : mobile phase (composition)

Influence of NH4F on the detection limit



Colonne phenyl hexyl

Colonne SB C18



EDC

[ജ]

MQ Water + x% NH₄F MeOH/ACN 65:35 (v/v)

10 EDC-WFD

LC parameter optimization : mobile phase (quality)

17αEE2 : 0.9pg inj

Mobile phases : A = MQ Water + 0.1mM NH₄F; B = MeOH/ACN 65:35 (v/v)



11 EDC-WFD

February 2023

EDC

[≋]

VFD

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 for EE2



LC parameter optimization : one example of optimized parameters

Mobile phases : A = MQ Water + 0.1mM NH₄F; B = MeOH/ACN 65:35 (v/v) Column Poroshell 120 Phenylhexyl : Dp = 2.1x100mm, 1.9µm



13 EDC-WFD

Parameters to be optimized 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

Owell time

MS/MS parameter optimization (source parameters)

Method 1 example on Agilent Technologies 6495

Method	Estrogen		Labelled estrogen	Sample injection		
Estrogen multi-residue	Ε1, 17αΕ2, 17βΕ2, 17αΕΕ2, Ε3		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 17 α EE2 specific	17αΕΕ2		17αEE2-d ₄	70:30 MQ water/MeOH, v/v 100μL injected		
Parameter	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 : MRM choice

Ochoice 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)



Evian + DOC + SPM, not spiked



New tested transitions

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

EDC

[≋]

/FD

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), 17 α EE2 example



EDC 🗊

WFD

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
- IMPORTANCE OF ISOTOPIC DILUTION

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)





21 EDC-WFD

February 2023

EDC 🗊

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)







22 EDC-WFD

February 2023

EDC

[∭]

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

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



February 2023

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

EDC 🗊

WFD

Same loss of sensitivity for $17\alpha E2$ and $17\alpha E2$ -d2, ISTD quantification \implies OK

Identification and quality criteria (isotopic dilution)

E3 examples of chromatographic peak shapes









EDC

[≋]

/FD

LC/MSMS with derivatisation ESI+



EDC

[≋]

WFD



LC/MSMS : dansylation





Switch from ESI - to ESI + to increase sensitivity

EDC

[≋]

VFD

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-CI

DMIS-CI

Derivatisation reagent used : Pyridine-3-Sulfonyl chloride (PS-CI)

EDC

Ionisation Mode : ESI+

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



29 EDC-WFD

February 2023

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 do not interfere in MS/MS so co-elution is OK!

ED

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



32 EDC-WFD



• 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

PQuality 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



EDC 🖲 WFD

CONCLUSION FOR LC-MS/MS:

LC-MS/MS* ALLOWS TO REACH THE FORMER EQS LEVELS FOR ALL THE ANALYTES AND THE NEW EQS LEVELS ESPECIALLY FOR EE2



* Valid for the instruments tested in the project

36 EDC-WFD





EDC 🗊

WFD

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	х	х	х	Pyridine	x	х	х	х
EtAc	х	x	x	x	EtAc	х	x	x	x
Aceton	х	х	х	х	Aceton	х	х	х	х
MeCN	х	х	х	х	MeCN	х	х	х	х



EDC

[≋]

FD

GC parameter optimization : choice of derivatisation agent

Optimisation 2: Final derivatisation agent. temperature and time

Α	MSTFA + EtAc					
Т °С	60	70	80	90		
15 min	х	х	х	х		
30 min	х	х	х	х		
60 min	х	х	х	х		
120 min	х	х	х	х		
В	TMSI + Pyridine					
Т°С	60	70	80	90		
15 min	х	х	х	х		
30 min	х	х	х	х		
60 min	х	х	х	х		
120 min	х	х	х	х		



EDC

Optimal parameters

TMSI + Pyridine T = 90 °C t = 60 min

GC parameter optimization : optimized parameters



41 EDC-WFD MS/MS parameter optimization : MRM choice

Ochoice 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)

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



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



EDC-WFD 44

CONCLUSION FOR GC-MS/MS:

GC-MS/MS* ALLOWS TO REACH THE FORMER EQS LEVELS FOR ALL THE ANALYTES



* Valid for the instruments tested in the project

45 EDC-WFD



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



EDC 🕑 WFD

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)

ED(

CONCLUSION* :

- LC/HRMS NOT ENOUGH SENSITIVE
- LC-MS/MS : FORMER AND NEW EQS REACHED FOR ALL THE COMPOUNDS (ESI- AND ESI+)
- GC-MS/MS : FORMER EQS REACHED FOR ALL THE COMPOUNDS
- GC/HRMS : PERFORMANCES SIMILAR TO THOSE OF GC-MS/MS

WHAT TO DO TO HAVE GOOD PERFORMANCE ?

- Careful optimization of MS conditions (in relation with matrix interferences)
- Careful and precise optimization of chromatographic separation
- Track cross contamination
- Use high quality criteria
- Use ISTD quantification (especially in relation with matrix interferences)
- Be aware of the importance of sample preparation especially purification

* Valid for the instruments tested in the project



