ANALYTICAL STRATEGIES FOR STEROID ANALYSIS IN WATER AT SUB PPT LEVELS

Emmanuelle Bichon, Stéphanie Christien, Soazig Elaudais, Fabrice Monteau, Jean-Philippe Antignac, Bruno Le Bizec

LABoratoire d'Étude des Résidus et Contaminants dans les Aliments
USC INRA 2013, École Nationale Vétérinaire de Nantes
BP 50707 - 44307 Nantes Cedex 3 - France
Fax : 33 2 40 68 78 78 - Tél : 33 2 40 68 78 80
e-mail : laberca@vet-nantes.fr

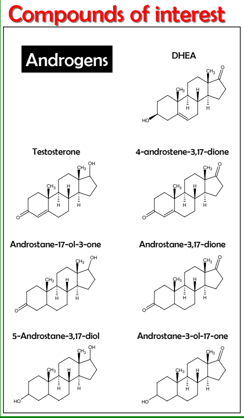
INTRODUCTION

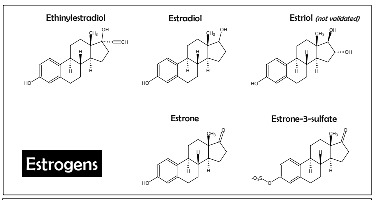
The occurrence of steroids in aquatic environment and their effects on normal endocrine function in aquatic organisms have been subjects of current concern. Several studies have shown that also birds, reptiles and mammals in polluted areas undergo alterations of the endocrine-reproductive system.

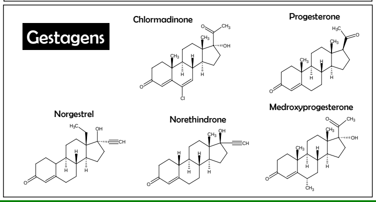
At present, a multitude of chemicals have shown to be endocrine disrupters. Among these, natural and synthetic estrogens are already effective at the lower ng/L. Their efficient control in environmental waters is made possible nowadays thanks to numerous analytical approaches available in the literature. This is the case for estradiol (E2), estrone (E1), estriol (E3) and ethinylestradiol (EE2) measurement. Estrone-3-sulfate (E1S) has also to be considered because of its stability in the environment. But less papers are concerned with androgens, gestagens and their phase I and phase II metabolites (in particular E1S).

Two analytical methodologies dealing with 29 steroids at low ppt levels, based on isotopic dilution are presented. The first one is dedicated to free steroids using two SPE preparation steps and a GC-MS/MS detection. The second method is focused on estrone-3-sulfate. Identification relies upon 2002/657/EC decision to confirm unambiguously the steroid presence even at ultra-trace levels (<1 ng.L-1).

Compounds of interest



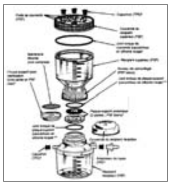




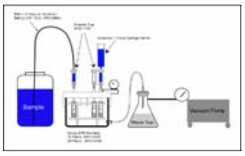
Analytical strategy for free steroids

250 mL water
↓
Internal standards (deuterated compounds)
↓
Filtration (optional)
↓
Solid-Phase Extraction C18
↓
Solid-Phase Extraction SiOH
↓
Derivatisation MSTFA/TMIS/DTE
↓
GC-MS/MS analysis (2 transitions per compound)

Module of filtration



Large volume adaptator on SPE system



GC-MS/MS Quattro micro (Waters)

LC-MS/MS Agilent 6410

Analytical strategy for estrone-3-sulfate

250 mL water
↓
Internal standard (boldenone sulfate d_3)
↓
Solid-Phase Extraction On weak anion exchange (MM4, Interchim, France)
↓
LC-MS/MS analysis (2 transitions per compound)

Analytical performances in GC-MS/MS

Monitored steroids in one aquisition run

Molécules	Type	Transition 1	Collision T1 (eV)	Transition 2	Collision T2 (eV)	T_R (min)*
Fraction androgènes-progestagènes-estrogènes						
lynestrol	AR	356,3>341,3	5	356,3>209,2	5	21,63
5β-androstan-3α,17α-diol	AR	256,2>241,2	8	346,3>241,2	8	22,87
5β-androstan-3 β,17α-diol	AR	256,2>241,2	8	346,3>241,2	8	23,16
5β-androstane-3,17-dione	AR	432,4>417,4	5	432,4>275,2	15	23,49
5α-androstan-3α,17α-diol	AR	346,3>241,2	8	331,3>241,2	5	23,51
5β-androstane-3on-17β-ol	AR	434,4>405,4	5	434,4>195,1	12	23,79
5β-androstane-3β,17β-diol d_3	EI	349,3>259,2	5			24,87
androstérone	AR	434,4>419,4	5	434,4>239,2	10	24,89
5β-androstane-3β,17β-diol	AR	421,4>255,2	8	331,3>241,2	5	24,93
Etiocholanolone d_5	AR	439,4>424,4	5			25,06
Etiocholanolone	AR	434,4>419,4	5	419,4>329,3	5	25,14
5α-androstan-3α,17β-diol d_3	EI	349,3>244,2	8			25,30
5β-androstane-3α,17β-diol	AR	346,3>256,2	5	256,2>241,2	8	25,34
5α-androstan-3α,17β-diol	AR	256,2>241,2	8	346,3>256,2	5	25,35
5α-androstan-3 β,17α-diol	AR	421,4>255,2	8	436,4>241,2	15	26,06
DHEA	AR	432,4>417,4	12	432,4>327,3	15	26,42
Epiandrosterone	AR	434,4>419,4	5	434,4>329,3	5	26,59
5α-androstan-3 β,17β-diol d_3	EI	424,4>258,2	8			26,91
5α-androstan-3 β,17β-diol	AR	421,4>255,2	8	436,4>241,2	15	26,96
5α-androstane-3,17-dione	AR	432,4>417,4	12	432,4>290,2	10	26,97
17α-testostérone-d_3	EI	435,4>209,2	12			27,04
17α-testostérone	AR	432,4,>209,2	12	432,4>327,3	15	27,08
17a-estradiol	AR	416,4>285,2	12	416,4>326,3	5	27,25
Dihydrotestostérone	AR	434,4>195,1	12	434,4>405,4	5	27,31
4-androstène-3,17-dione-d_3	EI	433,4>418,4	5			27,58
Estrone	AR	414,4>399,3	5	414,4>309,3	20	27,64
4-androstène-3,17-dione	AR	430,4>209,2	15	430,4>415,4	8	27,64
17ß-testostérone-d_3	EI	435,3>209,2	12			27,90
17ß-testostérone	AR	432,3>209,2	12	432,3>327,3	15	27,95
17ß-estradiol-d_3	EI	419,3>329,3	5			27,97
17ß-estradiol	AR	416,3>285,2	12	416,3>326,1	5	28,02
Noréthindrone	AR	442,4>427,4	5	442,3>302,3	5	29,43
Ethinylestradiol d4	EI	444,4>429,4	5			30,08
Ethinylestradiol	AR	425,4>193,1	15	285,2>205,2	10	30,13
Norgestrel	AR	456,3>301,3	20	456,4>194,1	20	30,26
Estriol	AR	504,4>386,3	5	504,4>386,3	5	31,60
Progestérone d9	EI	469,4>454,4	5			31,90
Progestérone	AR	460,4>445,4	10	460,4>157,1	15	32,00
Mégestrol-d_3	EI	561,4>471,4	5			33,56
Megestrol	AR	558,4>236,2	20	558,4>453,4	20	33,60
Médroxyprogestérone-d_3	EI	563,4>333,3	10			33,87
Médroxyprogestérone	AR	560,4>328,3	20	560,4>315,3	18	33,92
Medrogestone	AR	484,4>469,4	5	484,4>157,1	15	33,95
Chlormadinone	AR	578,4>143,1	20	578,4>473,3	15	36,20

Validated compounds

Compound	LOD (ng/L)	LOQ (ng/L)
5β-androstane-3α,17α-diol	1.9	2.8
5β-androstane-3β,17α-diol	0.7	1.9
5β-androstane-3,17-dione	0.3	0.6
5α-androstane-3α,17α-diol	0.7	9.0
5β-androstane-3on-17β-ol	0.4	0.6
androsterone	0.1	0.5
5β-androstane-3β,17β-diol	0.3	0.3
etiocholanolone	0.1	0.2
5α-androstane-3α,17β-diol	1.6	1.8
5α-androstane-3β,17α-diol	0.2	0.6
DHEA	0.2	0.3
epiandrosterone	0.2	2.0
5α-androstane-3β,17β-diol	0.9	12.4
5α-androstane-3,17-dione	0.6	16.2
17α-testosterone	0.1	0.2
17α-estradiol	0.1	0.2
dihydrotestosterone	0.5	0.7
estrone	0.5	1.1
4-androstene-3,17-dione	0.2	0.3
17β-testosterone	0.1	0.3
17β-estradiol	0.1	0.1
norethindrone	0.2	0.3
ethinylestradiol	0.2	0.7
norgestrel	0.4	0.4
progesterone	0.8	1.2
megestrol	6.5	9.2
medroxyprogesterone	0.1	0.2
chlormadinone	1.6	1.8

Analytical performances in LC-MS/MS

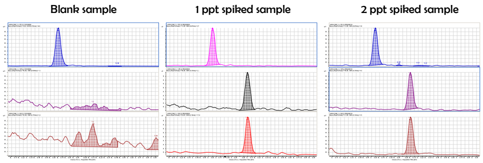
Identification at ppt level (2 transitions monitored)

Blank sample | 1 ppt spiked sample | 2 ppt spiked sample

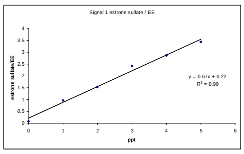
Boldenone sulfate d_3 (IS) 368.3>353.3

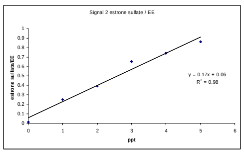
Estrone-3-sulfate signal 2 349.2>145.2

Estrone-3-sulfate signal 1 349.2>269.2



Linearity 0-5 ppt range on spiked samples





Unknown samples

Illustration with 4-androstene-3,17-dione

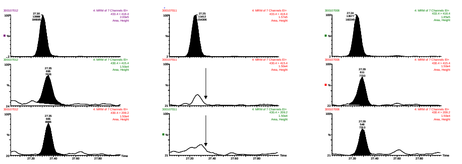
Diagnostic signals

0.4 ppt spiked sample | Blank sample | unknown sample

Internal standard (IS) 4-androstene-3,17-dione d_3

4-androstene-3,17-dione signal 1

4-androstene-3,17-dione signal 2

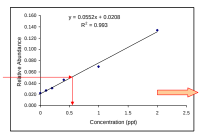


Unambiguous Identification

Analyte : 4-androstene-3,17-dione				Spiked sample		Iunknown sample
Matrix : water						
Sample reference				Spike 1 ppt		Unknown
RT IS (min)				27.618		27.646
RT Analyte (min)				27.650		27.691
RT Analyte / RT Etalon				1.001		1.002
Compliant SD RT	+/- (%)	0.5		0.996	1.006	IN
Abundance IS				63408		53235
Abundance signal 1 (BP)	331	>	241	6029		3176
Abundance signal 2	421	>	255	4432		2524
Ratio (signal 2 / signal 1)				0.735		0.795
Compliance Ratio	+/- (%)	20		0.588	0.882	IN
Number of identification points						5.0
Conclusion						IDENTIFY

Quantification

Relative Abundance = $\frac{\text{signal 1}}{\text{IS}}$ = 0.051



CONCLUSION

Two validated analytical methodologies dealing with 29 steroids at low ppt levels, based on isotopic dilution are presented.

The first one is dedicated to free steroids using two SPE preparation steps and a GC-MS/MS detection. The acquisition is carried out in SRM mode on a Quattro Micro instrument (Waters, two transitions followed per compound). In this way, DHEA, 4-androstenedione, testosterone and their main metabolites (androstanediols, etiocholanolone, androsterone), estrone, estradiol, ethinylestradiol, progesterone and various contraceptive gestagens are monitored.

The second method is focused on estrone-3-sulfate for which a specific combination between a SPE preparation and a LC-MS/MS detection (Gemini column, Phenomenex, Agilent 6410 LC-MS/MS system) has been developed.

Identification relies upon 2002/657/EC decision to confirm unambiguously the steroid presence even at ultra-trace levels (<1 ng.L-1).

These methodologies are currently used for the endocrine disruptors monitoring in water organised in the field of the quadriennal National French Plan for Health - Environment (PNSE - Plan National Santé Environnement) which will be drawing to a close at the end of 2008.

Poster presented during the 4th NORMAN Workshop