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# Method development and validation for the analysis of 20 hormones (including estrogens, androgens and progestagens compounds) in various aqueous matrices

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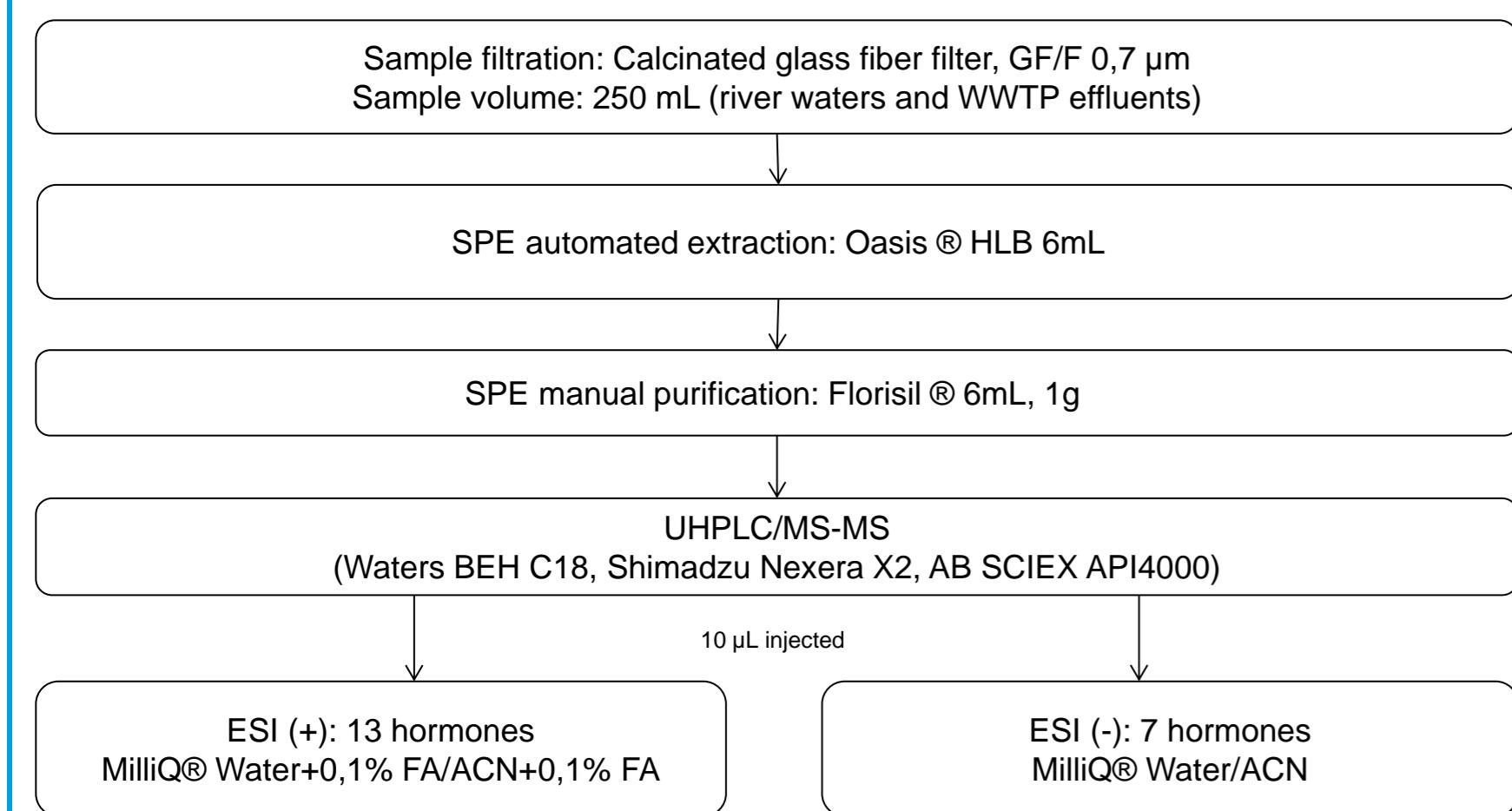
## INTRODUCTION

During the last twenty years, consumption of hormones compounds (natural hormones or synthetic analogues), mainly for human medicine, has considerably increased. Both androgens, progestogens and estrogens compounds are usually not entirely metabolized and reach aquatic environment mainly via effluents of wastewater treatment plants (WWTP). All of them can be considered as endocrine disrupting compounds because of undesirable effects in biota. Hence, a highly sensitive and selective analytical method is needed to identify and quantify these emerging contaminants in various environmental compartments, especially in surface waters and wastewaters.

## MATERIAL AND METHOD

### Method synoptic

Initially based on previous estrogenic analytical method [1]

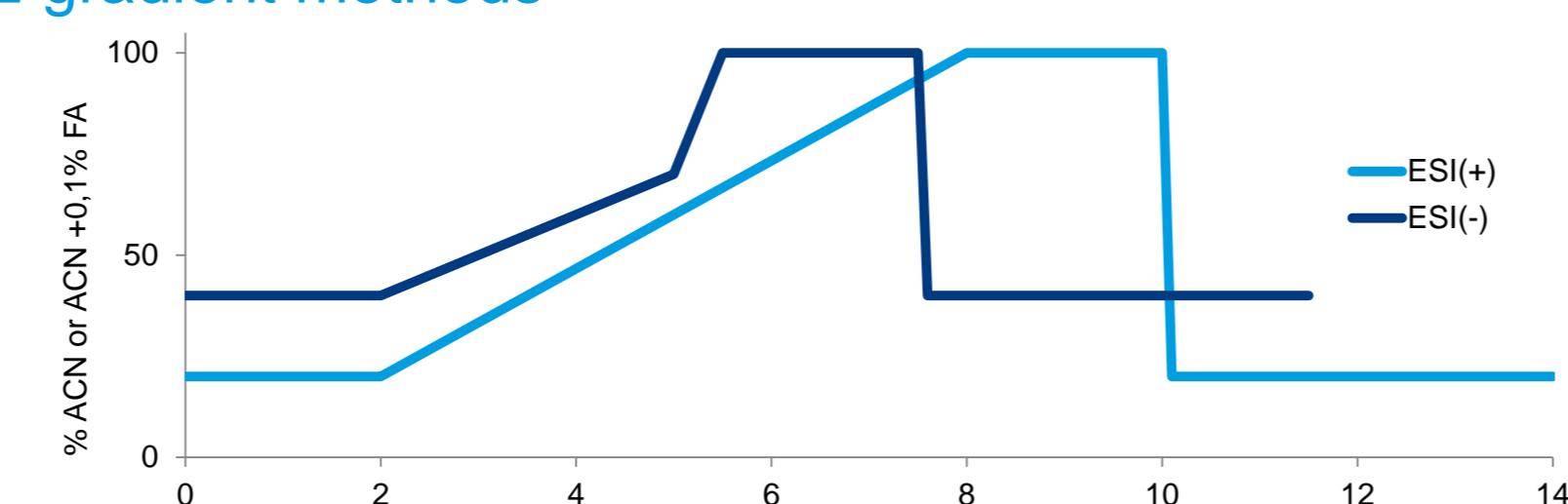


### UHPLC/MS-MS parameters

#### Apparatus:

- UHPLC: Shimadzu Nexera 2
- Column: Waters® BEH C18 100 x 2,1 mm, 1,7 µm
- Mass spectrometer: AB SCIEX API 4000 TQ

#### 2 gradient methods



#### 2 ESI-MS-MS methods:

- 2 ionization modes : ESI positive for androgens and progestagens, ESI negative for estrogens
- Source parameters see following table
- Acquisition methods: MRM mode, parameters are listed against. Confirmation criteria are described in the EU council decision [2]

#### Source parameters

	ESI(+)	ESI(-)
Nebulizer gas (PSI)	60	50
Turbo gas (PSI)	30	60
Curtain gas (PSI)	30	10
Collision gas (PSI)	10	8
Temperature (°C)	500	400
Ion spray voltage (V)	+4500	-4500

#### Quantitation method:

- Internal calibration
- Use of molecular analogue labelled with stable(s) isotope(s) (D and/or <sup>13</sup>C) as internal standard (IS)

### MRM acquisition parameters

Compound	Retention Time (min)	Transitions (Quantification and Confirmation)		MS-MS parameters			
		Parent ion	Daughter ion	Type	DP (V)	EP (V)	CE (V)
Cortisol	3,60	363	121	Q	76	33	8
	3,65	363	91	C	76	89	6
Cortisol-D4	3,59	367	121	C	66	31	21
	3,67	367	97	C	66	47	18
Cortisone	3,60	361	163	Q	61	33	10
	3,61	361	91	C	68	69	16
Cortisone-D8	3,58	368	168	C	65	35	16
	3,69	373	163	C	46	87	5
Dexamethasone	4,08	393	91	C	65	13	24
	397	377	97	C	45	13	22
Dexamethasone-D4	4,06	397	359	C	45	17	24
	398	398	119	C	37	9	8
Testosterone	4,71	289	99	C	36	31	6
	293	98	96	C	76	31	18
Testosterone-D4	4,69	293	110	C	35	20	9
	297	109	99	C	63	37	9
Norethindrone	4,73	305	91	C	65	65	16
	306	91	91	C	65	68	16
Norethindrone-D6	4,71	305	91	C	51	16	20
	307	91	91	C	45	10	19
Androstenedione	4,92	287	97	C	66	35	8
	287	109	109	C	45	33	18
Androstenedione-D7	4,90	294	100	C	76	37	20
	294	111	109	C	37	20	9
Drospirenone	5,06	367	97	C	10	37	20
	388	325	97	C	89	6	18
Drospirenone-D4	5,05	371	91	C	74	43	17
	388	325	97	C	61	21	20
Epitestosterone	5,07	288	109	C	68	37	19
	294	109	109	C	35	17	9
Epitestosterone-D5	5,05	294	113	C	77	38	24
	291	255	113	C	45	19	25
Levonorgestrel	5,18	313	91	C	71	63	16
	313	109	91	C	56	16	16
Levonorgestrel-D6	5,16	319	87	C	76	55	16
	319	91	91	C	61	21	20
Megetrol Acetate	5,71	388	267	C	25	18	20
	389	325	267	C	61	21	20
13C-Megestrol Acetate-D3	5,70	315	99	C	46	35	8
	324	100	99	C	76	37	18
Progesterone	5,73	315	99	C	27	16	18
	324	113	99	C	37	22	22
Progesterone-D9	5,70	324	123	C	70	43	17
	345	93	93	C	45	11	16
Medroxyprogesterone*	5,37	291	123	C	45	19	25
	291	255	123	C	45	19	25
Androsterone*	5,51	271	95	C	45	10	17
	271	147	95	C	90	56	7
Estradiol	0,60	287	145	C	-110	-54	-7
	287	171	145	C	-50	-15	-13
Estradiol-D2	0,60	288	147	C	-95	-54	-13
	270	145	145	C	-95	-54	-19
β-Estradiol	1,80	270	183	C	-95	-54	-7
	270	183	183	C	-95	-54	-9
β-Estradiol-D2	1,80	273	185	C	-90	-58	-19
	273	185	185	C	-90	-58	-19
α-Estradiol	2,20	270	183	C	-95	-54	-7
	270	183	183	C	-95	-54	-9
α-Estradiol-D2	2,20	273	185	C	-95	-54	-19
	273	185	185	C	-95	-54	-19
α-Ethinylestradiol	2,50	295	154	C	-55	-54	-7
	295	159	154	C	-55	-54	-13
α-Ethinylestradiol-D4	2,40	299	147	C	-90	-54	-7
	299	147	147	C	-90	-54	-7
Estrone	2,70	269	145	C	-85	-54	-7
	269	145	145	C	-85	-54	-25
Estrone-D4	2,70	273	147	C	-90	-56	-7
	273	147	147	C	-90	-56	-7
Diethylstilbestrol	3,30	267	251	C	-93	-32	-6
	267	251	251	C	-93	-32	-6
Diethylstilbestrol-D8	3,22	275	259	C	-75	-38	-145
	275	259	259	C	-75	-38	-20
Dienestrol	3,54	265	95	C	-100	-42	-13
	265	249	95	C	-100	-42	-13
Dienestrol-D6	3,77	271	95	C	-90	-38	-17
	271	255	95	C	-90	-38	-17

\*Medroxyprogesterone and Androsterone are respectively assigned to IS testosterone-D3 and progesterone-D3

## METHOD PERFORMANCES

### Estimated limits of quantitation