Achieving ultra-trace level detection limits for hormones in drinking water using a large-volume injection

Hitha P. P[1], Sashank Pillai[1] and Craig M. Butt[2]
[1]SCIEX, India; [2]SCIEX, USA

This technical note describes the ultra-trace level analysis of hormones in drinking water using only 20 mL of sample and a 300 µL injection volume. Using the QTRAP version of a SCIEX 7500 system, minimum reporting levels (MRLs) ranging from 0.1 to 4 ng/L were confirmed using a simple solid-phase extraction (SPE) sample preparation method (Figure 1). The UCMR3 MRL concentrations were used as the target MRLs. Laboratory fortified blank (LFB) samples spiked at 5 ng/L showed mean accuracies between 73% and 102% and mean precisions between 2.9% and 12% CV. MRL confirmation was performed using the prediction interval of results (PIR) technique.[1] Lower PIR values ranged between 59% and 97% and the upper PIR values ranged between 111% and 140%. Method applicability in real-world water samples was shown through MRL spikes in 2 packaged drinking waters.

Natural and synthetic hormones can enter aquatic environments through contaminated sewage treatment plant effluent.[2] If these waters are used for drinking water, their presence[4,5] might pose a significant human health risk through endocrine disruption.[6] Previous analytical methods for measuring hormones in drinking water have used large sample volumes (for example, 1 L), resulting in time-consuming sample preparation. In this technical note, we combine the sensitivity of the QTRAP version of the SCIEX 7500 system with a large-volume injection to obtain sub- to low-ng/L sensitivity with only 20 mL of sample volume.

Key benefits of the analysis of hormones in drinking water using the SCIEX 7500 system with large-volume injection

- **MRL confirmation at UCMR3 reporting levels:** Analysis of 7 replicate samples showed PIR limits between 50% and 150% for all analytes, confirming MRLs ranging from 0.1 to 4 ng/L
- **Accurate and precise quantitation in mid-level matrix spikes:** LFBs spiked at 5 ng/L showed mean accuracies ranging between 73% and 102% and mean precisions ranging between 2.9% and 12% CV
- **Simple SPE sample preparation method using only 20 mL of water:** Low sample volume decreased extraction time and might reduce sample storage and shipping costs
- **Good analyte retention and peak shape:** The Phenomenex Kinetex™ C8 column with a 13.2-min gradient program showed good chromatography even using the 300 µL injection volume.

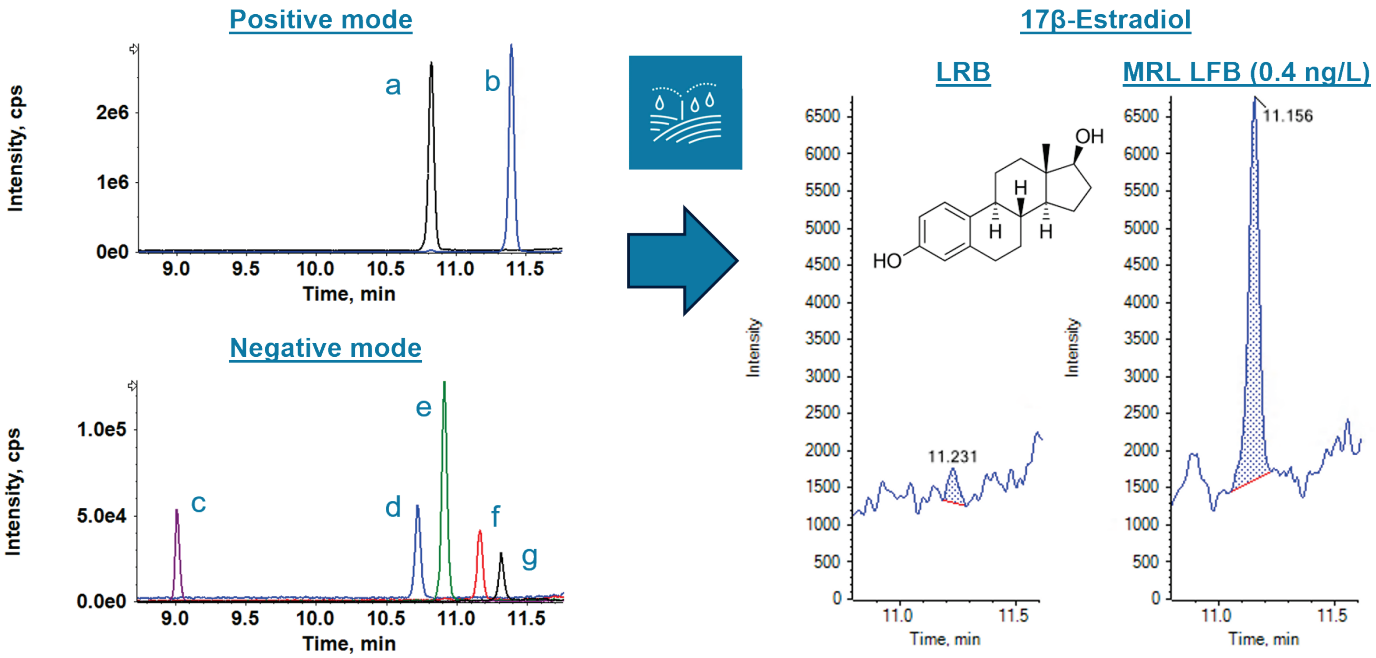

Figure 1. Overlaid extracted ion chromatograms (XIC) of a sample analyzed in positive and negative modes, a representative LRB and a MRL LFB sample spiked at 2 ng/L. Analysis using positive mode (top, left) separated 4-androstene-3,17-dione (a) and testosterone (b). Analysis using negative mode (bottom, left) separated estriol (c), equilin (d), estrone (e), 17β-estradiol (f) and 17β-ethynylestradiol (g).

Methods

Standard preparation: All analyte standards were purchased from commercial vendors as neat chemicals, except for androstenedione, which was purchased as a solution. Calibration standards were prepared in 90:10 (v/v), water/methanol at concentrations ranging from 0.25 to 100 pg/mL.

Sample preparation: A 20 mL aliquot of laboratory water was dechlorinated with sodium thiosulphate (80 mg/L) and preserved using 2-mercaptopyridine-1-oxide (65 mg/L) prior to fortification with the target analytes and surrogate standard (17β-ethynylestradiol-D4, final concentration 7 pg/mL). Samples were concentrated using a Phenomenex Strata™ C18-U SPE cartridge (500 mg, 6 cc, P/N: 8B-S002-HCH) that had been conditioned with 10 mL of methanol followed by 10 mL of water. After loading, the cartridges were washed with 5 mL of water, eluted with 4 mL acetonitrile and evaporated to near dryness under nitrogen gas in a 45°C water bath. After adding the internal standards, samples were reconstituted with 2 mL of 90:10 (v/v), water/methanol and transferred to autosampler vials for instrumental analysis.

Matrix spikes to evaluate method accuracy and precision: LFB samples (n=4) were spiked at 5 ng/L and processed through the sample preparation procedure to determine the method accuracy and precision. In addition, laboratory reagent blank (LRB) water samples (n=2) were prepared to evaluate the background contamination and to quantify bias introduced during sample preparation.

MRL confirmation: The MRL concentration was verified using methods similar to that presented in section 9.2.4 of the EPA 539 document. Briefly, 7 LFB samples were spiked at the UCMR3 reporting level and processed using the SPE sample preparation methods. Calculations to determine the PIR are presented in the results section.

Chromatography: Chromatographic separation was performed using the ExionLC AD system. Analytes were separated using a Phenomenex Kinetex C8 column (2.6 µm particle size, 100 x 2.1 mm, P/N: 00D-4497-AN) using the gradient conditions described in Table 1. Mobile phase A was water with 0.1mM ammonium fluoride and mobile phase B was methanol. The mobile phase flow rate was 0.400 mL/min, the injection volume was 300 µL and the column oven was set to 40°C.

Table 1. LC gradient conditions used for the analysis of hormones in drinking water using the QTRAP version of the SCIEX 7500 system.

Time	Flow rate (mL/min)	%A	%B
0	0.400	98	2
1.1	0.400	98	2
5.9	0.400	50	50
8.9	0.400	40	60
9.0	0.400	2	95
10.9	0.400	2	95
11.1	0.400	98	2
13.2	0.400	98	2

Mass spectrometry: The QTRAP version of the SCIEX 7500 system was used for the instrumental analysis using the OptiFlow Pro ion source with electrospray ionization under positive and negative polarity switching. Optimized source/gas and compound-specific parameters are summarized in Tables 2 and 3, respectively. The entrance potential (EP) was 10/-10 V for all compounds.

Data processing: Samples were acquired and processed using SCIEX OS software, version 2.1.6. Analyte responses were normalized to the corresponding internal standard response shown in Table 3.

Table 2. Source and gas parameters for the analysis of hormones in drinking water using the QTRAP version of the SCIEX 7500 system.

Parameter	Value
Polarity	Positive/negative
Curtain gas	40 psi
CAD gas	9 psi
Ion spray voltage	2000/-2000 V
Temperature	450°C
GS1	45 psi
GS2	85 psi

Table 3. Compound-specific MRM parameters and internal standard assignment for the analysis of hormones in drinking water using the SCIEX 7500 system. Quantifier transitions are designated by "1" and qualifier transitions are designated by "2".

Compound	Internal standard	Polarity	Q1 (m/z)	Q3 (m/z)	CE (V)	CXP (V)	Q0D (V)
16α-Hydroxyestradiol (Estriol)_1	Estriol-D2	Negative	286.9	171	-47	-19	-50
16α-Hydroxyestradiol (Estriol)_2	Estriol-D2	Negative	286.9	145	-52	-14	-75
17β-Estradiol_1	Estradiol-D4	Negative	270.9	145	-51	-17	-70
17β-Estradiol_2	Estradiol-D4	Negative	270.9	143	-65	-15	-80
Equilin_1	Estradiol-D4	Negative	266.9	143.1	-41	-14	-60
Equilin_2	Estradiol-D4	Negative	266.9	223	-45	-13	-60
Estrone_1	Estradiol-D4	Negative	268.9	145.2	-48	-8	-70
Estrone_2	Estradiol-D4	Negative	268.9	143.1	-66	-15	-60
17α-Ethynylestradiol_1	Estradiol-D4	Negative	294.9	145.1	-51	-13	-30
17α-Ethynylestradiol_2	Estradiol-D4	Negative	294.9	143.0	-65	-16	-30
Testosterone_1	Testosterone-D5	Positive	289.2	109.1	31	12	0
Testosterone_2	Testosterone-D5	Positive	289.2	97.1	29	12	35
4-Androstene-3,17-dione_1	Testosterone-D5	Positive	287.2	97.1	29	13	20
4-Androstene-3,17-dione_2	Testosterone-D5	Positive	287.2	109.1	36	10	20
17α-Ethinylestradiol-D4 (SUR)	n/a	Negative	298.9	145.2	-68	-12	-30
Testosterone-D5 (IS)	n/a	Positive	294.2	100.1	29	15	0
Estriol-D2 (IS)	n/a	Negative	288.9	173.2	-48	-19	-50
Estradiol-D4 (IS)	n/a	Negative	274.9	147.1	-72	-10	-70

Chromatographic separation of isomer pairs

Chromatographic separation of the compounds was achieved using the Phenomenex Kinetex C8 column. The mobile phase conditions and gradient program used are described in Table 1. Critically, the LC method chromatographically separated hormone isotope pairs, including testosterone/4-andro-3,17-dione, estrone/17β-estradiol and equilin/estrone. This ensured that there was no interference from the M+2 precursor ion.

Sensitivity, accuracy, precision and linearity of solvent calibration standards

Performance of the SCIEX 7500 system was determined through triplicate injections of the solvent-based calibration standards (Table 4). Using the 300 µL injection volume, limit of quantitation (LOQ) concentrations observed for the 7 hormones ranged from 0.25 to 5 ng/L. Estimated in-sample LOQs ranged from 0.025 to 0.5 ng/L, considering the 10-fold SPE concentration factor. High levels of accuracy (90%–101%) and precision (%CV <7.0%) for all analytes were shown at the LOQ concentration. The linear dynamic range spanned 2–3 orders of magnitude with r values >0.997 for all compounds. The sensitivity of the QTRAP version of the SCIEX 7500 system for the steroid hormones allows for the use of only 20 mL of sample to reach sub-ng/L detection limits.

Table 4. LOQ, mean LOQ accuracy (%), mean LOQ precision (%CV), linearity range (ng/L) and regression coefficient (r) for steroid hormones analytes in the solvent standards.

Compound	LOQ (ng/L)	Mean LOQ accuracy (%)	Mean LOQ precision (%CV)	Linearity range (ng/L)	Regression coefficient (r)
Estriol	*1.0*	*96*	*6.2*	*1–100*	*0.999*
17β-Estradiol	*2.5*	*98*	*6.4*	*2.5–100*	*0.999*
Equilin	*5.0*	*101*	*7.0*	*5–100*	*0.999*
Estrone	*2.5*	*101*	*5.6*	*2.5–100*	*0.999*
17α-Ethynylestradiol	*5.0*	*90*	*6.5*	*5–100*	*0.997*
Testosterone	*0.25*	*100*	*3.6*	*0.25–100*	*0.999*
4-Androstene-3,17-dione	*1.0*	*95*	*1.4*	*1–100*	*0.999*

Evaluation of 5 ng/L matrix spikes for accuracy and precision

Method performance was evaluated in LFBs (n=4) that were spiked at 5 ng/L and processed through the SPE extraction and instrumental analysis procedures. Mean accuracy ranged from 73% (testosterone) to 102% (17α-ethynylestradiol) and mean precision ranged from 2.9% (equilin) to 12% (testosterone), see Table 5 for summarized results. The data quality acceptance criteria that are typical for drinking water analysis require accuracy within ±30% of the nominal value and %CV <20%. The observed results exceed these criteria and none of the analytes were detected in the LRB samples.

Table 5. Mean accuracy (%) and precision (%CV) for LFB samples spiked at 5 ng/L (n=4) for the analysis of hormones in drinking water.

Compound	Mean accuracy (%)	Mean precision (%CV)
Estriol	84	7.0
17β-Estradiol	94	3.4
Equilin	84	2.9
Estrone	89	2.2
17α-Ethynylestradiol	102	3.4
Testosterone	*73*	*12*
4-Androstene-3,17-dione	*88*	*5.5*

MRL confirmation at the UCMR3 reporting levels

The MRL confirmation has been prescribed in some EPA methods, including the initial demonstration of capability experiments in EPA Method 539 for the analysis of hormones in drinking water. The procedure involves spiking 7 replicate LFB samples at the target reporting levels and processing through the extraction and instrumental methods. MRLs are confirmed if the calculated PIR recovery values are between 50% and 150% using the equation below. For this technical note, the target MRLs were equivalent to the UCMR3 reporting levels (Table 6).

$$PIR\ (upper\ or\ lower) = \frac{Mean \pm\ HR_{PIR}}{Fortified\ concentration} \times 100\%$$

In this equation, HR_{PIR} represents the half range for the PIR and was calculated as,

$$HR_{PIR} = 3.963s$$

HR_{PIR} = Half range for the PIR

s = The standard deviation of replicate analyses

3.963 = Constant value for 7 replicates

Chromatograms for the LRB and MRL LFB samples are shown in Figures 1 and 2. In the MRL LFB samples, mean recoveries of the hormones ranged from 86% to 118% with %CVs ranging from 4.6% to 8.7% (Table 6). These experimentally measured mean recovery and precision values were used to calcuate the PIR. Considering all 7 hormone analytes, the lower PIR values were between 59% and 97% and the upper PIR values were between 111% and 140%. Therefore, the MRLs were verified at sub- to low-ng/L URMR3 reporting levels.

To demonstrate the method applicability in real-world water samples, 2 different packaged drinking water samples were purchased from a local store and spiked at the UCMR3 MRL concentration. These are typically referred to as laboratory fortified sample matrix (LFSM) samples in some EPA methods. The LFSM samples were processed and instrumentally analyzed identically to the MRL LFB samples. The 2 samples were extracted individually but instrumentally analyzed a total of 7 times to obtain statistics for accuracy and precision (duplicate #1 was injected 3 times and duplicate #2 was injected 4 times). The

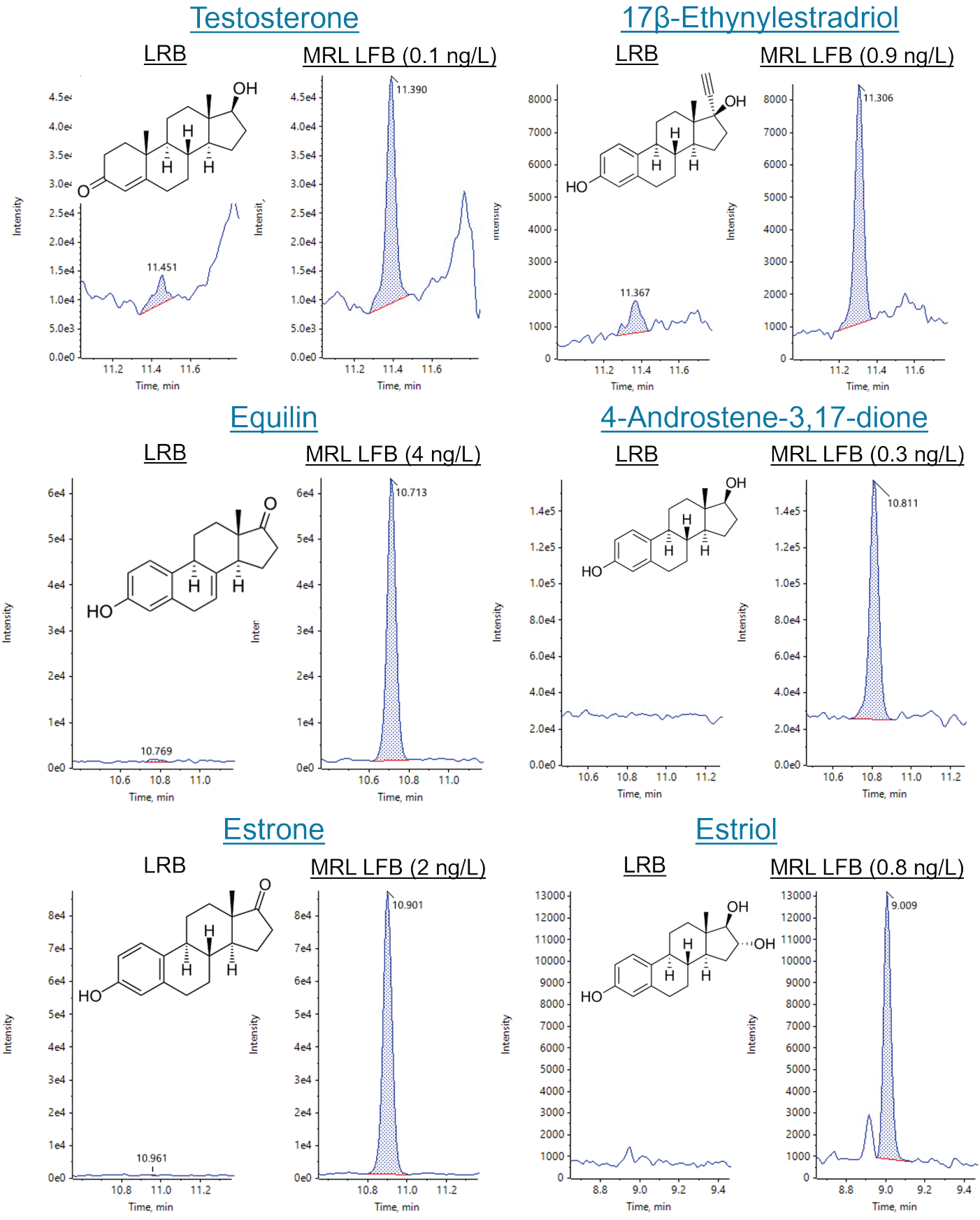

Figure 2. XICs of the LRB (left) and LFB (right) samples at the UCMR3 MRL concentration. Chromatograms are shown for testosterone, 17β-ethynylestradiol, equilin, 4-androstene-3,17-dione, estrone and estriol.

Table 6. MRL spikes in LFB and LFSM samples at the UCMR3 MRL concentration. LFB samples were prepared as 7 individual replicate samples that were injected once each. LFSM samples were comprised of 2 different packaged water samples that were injected a total of 7 times.

		LFB MRL samples (n=7)				LFSM MRL samples (n=2)	
Analyte	UCMR3 concentration (ng/L)	Mean recovery (%)	Mean precision (%CV)	Lower PIR (%)	Upper PIR (%)	Mean recovery (%)	Mean precision (%CV)
Testosterone	*0.1*	*94*	8.5	*62*	*126*	*95*	*6.6*
4-Androstene-3,17-dione	*0.3*	*96*	8.7	*63*	*129*	*97*	*8.2*
17β-Estradiol	*0.4*	*97*	8.6	*64*	*130*	*97*	*9.9*
Estriol	*0.8*	*87*	*7.0*	*63*	*111*	*86*	*5.3*
17α-Ethynylestradiol	*0.9*	*118*	4.6	*97*	*140*	*112*	*5.3*
Estrone	*2.0*	*101*	7.2	*72*	*130*	*103*	*5.5*
Equilin	*4.0*	*86*	7.8	*59*	*112*	*85*	*7.9*

mean accuracy was between 85% and 112% and mean precision (%CV) was between 5.3% and 9.9%. Overall, these results demonstrate the ability of the SCIEX 7500 system to analyze steroid hormones in drinking water with sensitivity, accuracy and precision using only 20 mL of sample, a simple SPE sample preparation method and 300 µL injection volume.

Conclusions

This technical note demonstrated the analysis of hormones in drinking water using the QTRAP version of the SCIEX 7500 system with a 20 mL of sample and a 300 µL injection volume.

- The low-volume, simple SPE method reduced extraction time and might reduce sample storage and shipping costs
- The MRLs were confirmed at concentrations ranging from 0.1 to 4 ng/L using the PIR technique
- LFB samples spiked at 5 ng/L showed good accuracy and precision. Mean accuracies ranged between 73% and 102% and mean precisions ranged between 2.9% and 12% CV.
- The Phenomenex Kinetex C8 column and gradient program used resulted in symmetric peak shape and good chromatographic separation even using the 300 µL injection volume

References

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
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