METHOD STATEMENT

Determinand:
Steroids by GC-MSMS with negative chemical ionisation

Matrix:
Ground waters, Saline and Surface Waters

Principle of Method:
The compounds of interest are extracted from filtered aqueous matrix by solid phase extraction (SPE) and the extracts are further cleaned up by means of another SPE procedure utilising a cartridge with a molecular imprinted polymer (MIP) stationary phase that has a class selective affinity for steroidal oestrogens. The isolated determinands are then derivatised with Pentafluorobenzoyl Chloride (PFBCl) and MSTFA and analysed by GC-MSMS with negative chemical ionisation (NCI) using methane as the reagent gas. Quantification is based upon an internal standardisation procedure

Sampling and Sample Preparation:
Samples should be taken in an STL116 bottle. This is a 500ml amber glass bottle with 1.5ml of 30%HCl and 0.125g Copper Nitrate as a preservative.

Samples are stored between 5 ± 3°C prior to analysis.

In-house stability data shows that the analytes are stable for 14 days when stored under these conditions.

Interferences:
MS/MS is an extremely selective technique and interferences should only be encountered very rarely, however in theory, any compound which is extracted by the procedure, which has a chromatographic retention time similar to the compound of interest and which produces both precursor and product ions similar to that of the compounds in question, may interfere

Performance of Method:

Precision, Bias and Limit of Detection

Compound	LOD (ng/L)	Calibration Range (µg/l)	Low Standard		High Standard	
			Bias	RSD	Bias	RSD
Estrone	0.293	0.0003 – 0.03	2.39%	2.81%	1.46%	2.91%
17-β-Estradiol	0.021	0.00002 – 0.002	5.75%	15.3%	4.24%	5.04%
17-α-Ethynylestradiol	0.030	0.00002 – 0.002	6.64%	13.9%	7.65%	14.2%

Matrix Spike Recoveries

Compound	Surface Water		Ground Water		Saline Water	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Estrone	97.3%	4.72%	97.2%	4.19%	97.3%	4.81%
17-β-Estradiol	102.6%	5.78%	104.7%	6.09%	104.3%	7.44%
17-α-Ethynylestradiol	106.2%	15.8%	106.76%	12.9%	105.3%	13.3%

Uncertainty of Measurement

The Uncertainty of Measurement has been calculated following the procedure given in GOP 5.4N.

Compound	Relative UoM (%)	Minimum UoM (ug/L)
Estrone	12.2	0.0000946
17-β-Estradiol	14.2	0.0000125
17-α-Ethynylestradiol	37.8	0.0000210

References:
In-house method.