17α-Ethinylestradiol (EE2) in Water by LC/MS/MS - PBM

Parameter

17α-ethinylestradiol (EE2) in water, dissolved or total

Analytical Method

Analysis by Liquid Chromatography tandem-mass spectrometry (LC-MS/MS).

Introduction

17α-ethinylestradiol (EE2) is a synthetic hormone and a derivative of estradiol, the major endogenous estrogen in humans. Used in almost all formulations of birth control pills, it has become ubiquitous in the environment due to its resistance to degradation and tendency to accumulate in organic matter. EE2 is an endocrine disrupting compound (EDC).

This method is applicable to the quantitative determination of EE2 and other endocrine disruptors such as 17β-estradiol and bisphenol A in water.

BC CSR standards for EE2 are applicable to the dissolved fraction of EE2 in water, however the BC Water Quality Guidelines for EE2 are applicable to total EE2 in water. Different analytical procedures are required for dissolved or total EE2, as outlined in the procedure.

Method Summary

For Dissolved EE2, Centrifugation is used to remove suspended particulate (filtration is not permitted due to the potential for extraction of EE2 by the filtration process). Samples are acidified with sulfuric acid, passed through a C18-based solid phase extraction (SPE) column, and eluted with a polar solvent such as acetonitrile.

For Total EE2, SPE may be utilized to extract and elute both the dissolved and particulate fraction of a sample (for up to approximately 1% solids), or the particulate fraction may be separated by filtration or centrifugation and extracted separately, with extracts or final test results combined to determine the total EE2 concentration in the sample.

An internal standard is added to the extract, which is analyzed by LC-MS/MS.

This method is performance-based. Laboratories may adopt alternative options to improve performance or efficiency if all stated performance requirements and prescribed (mandatory) elements are met.

MDL(s) and EMS Analyte Codes

Analyte	CAS No.	Approx. MDL (µg/L)	EMS Analyte Code
17α-ethinylestradiol	57-63-3	0.0005	defined on request

EMS Method Code(s)

***Refer to EMS Parameter Dictionary on the ministry website for all current EMS codes.

Matrix

Freshwater, Groundwater, Seawater, Wastewater.

Method Limitations

EE2 has a relatively high octanol/water partition coefficient (Kow ≈ 4). Therefore, it binds rapidly to organic matter. Samples to be analyzed for dissolved EE2 must not be filtered prior to analysis as this may result in a low bias.

EE2 has a solubility of approximately 9 mg/L in water at room temperature. Solubility decreases as ionic strength increases. Therefore, this method is not suitable for EE2 concentrations above the solubility limit.

Interferences and Precautions

Contamination from personal care products and medications used by laboratory staff is possible, therefore it is important to take precautions to avoid contamination of the samples, e.g. wear protective gloves and clothing.

All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by DI water. Glassware can be heated in a muffle furnace at 400ºC for two hours or solvent rinsed.

Solid phase extraction media may be a source of interferences. The analysis of method blanks can provide important information regarding the presence or absence of such interferences. Each brand and lot of SPE devices should be tested to ensure that contamination does not preclude analyte identification and quantitation.

Contamination of the analytical system can occur after high level samples are analyzed. Analysts should be aware of the degree of carryover that occurs on their instrument system,

and should take appropriate steps to prevent the occurrence of false positives.

Sample Handling and Preservation

Sample Containers: Collect samples in clean amber glass containers with PTFE-lined caps (consult laboratory for volume requirements; 1L is typical).

Preservation: Chlorinated samples must be preserved with sodium thiosulfate (~80 mg/L is recommended, to neutralize up to ~15 mg/L of free chlorine), or with ascorbic acid (~50 mg/L). No preservation is required for non-chlorinated samples, however addition of 2-mercaptopyridine-1-oxide, sodium salt (~65 mg/L) is recommended as an antimicrobial preservative to extend hold times. Protect from light (Ref: EPA 539 & EPA-820-R-10-008).

Stability

Holding Time: Samples should be extracted as soon as possible, but must be extracted within 7 days, or within 28 days if preserved with 2-mercaptopyridine-1-oxide. Extracts must be analyzed within 30 days after extraction (Ref: EPA 539 & EPA-820-R-10-008).

Storage: Sample temperature should be chilled to ≤ 10°C immediately after sampling and during transit to the laboratory. In the laboratory, samples and extracts must be refrigerated at ≤ 6°C. Avoid freezing of samples to prevent sample breakage. Storage of extracts at ≤ -10°C is recommended. Samples and extracts must be protected from light until analysis (EE2 is subject to photodegradation).

Procedure

Sample Preparation for Dissolved EE2 Analysis:

If Dissolved EE2 analysis is required, centrifuge any samples with visibly evident turbidity (~ 10 NTU) to physically separate suspended particulate matter prior to analysis of the clarified supernate (do not filter). All samples may optionally be centrifuged, which may be necessary for direct injection methods.

Sample Preparation for Total EE2 Analysis:

If Total EE2 analysis is required, shake samples well prior to sub-sampling. If the amount of particulate matter is low enough to prevent clogging of the SPE cartridge, (labs may establish a suitable limit, which may not exceed 1% by estimation), then a representative portion of the sample is extracted in total by SPE, using the elution solvent to extract the particulate fraction. For samples with higher levels of particulate matter, separate the particulate matter from the aqueous phase by filtration (with a glass fiber filter) or centrifugation, with particulate matter extracted separately by Soxhlet or an alternative solvent extraction technique. The dissolved and particulate fractions may be combined prior to analysis, or may be analyzed separately with the results combined to determine total EE2 concentration in the sample. Refer to EPA Methods 1694 or 1698 for further guidance.

Sample Extraction by SPE:

Fortify samples with a surrogate and pass them through solid phase extraction (SPE) disks containing octadecyl (C18) functional groups to extract EE2 and surrogate. Test analytes are eluted from the solid phase with a small amount of methanol. The extract is concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1 mL volume with 50:50 methanol:water after addition of internal standards.

Instrumental Analysis:

Transfer a portion of the reconstituted extract to an auto-sampler vial. Analysis by direct injection LC/MS/MS (GC-MS/MS is also acceptable, but derivatization is necessary to improve stability).

Initial calibrations must be five points or more (no more than one outlying point may be excluded). Stored calibrations may be used until the Calibration Verification Standard fails to meet acceptance criteria.

Refer to EPA 539 for detailed operating conditions suitable for this test. General guidance is provided as follows:

HPLC Parameters:

Mobile Phase A: methanol
Mobile Phase B: ammonium hydroxide (0.2%)
Flow Rate: 0.2 mL/min
Injection Volume: 50 µL
Column Temperature: 35°C
Column: C18, 2.1 x 150 mm, 3.5 µm

MS/MS Parameters:

Sample Introduction: Electrospray ionization (ESI)
Polarity: Negative
Capillary Voltage: 3 kV
Source Temperature: 120°C
Desolvation Gas Temperature: 350°C
Desolvation Gas Flow: 15 L/min
Cone gas Flow: 0.8 L/min
Extractor Lens: 2 V
RF Lens: 0.1 V

MRM Transitions:

17α-ethinylestradiol: 295.1 → 144.7
17α-ethinylestradiol-d4 (surrogate): 299 → 144.7
^{13}C2-Ethynylestradiol (internal standard): 297 → 144.7

Performance Requirements

Any analytical method options selected for this analysis must meet or exceed the performance requirements specified below.

Accuracy and Precision requirements are distinct from daily QC requirements, and apply to measures of long term method performance (averages and standard deviations). Achievement of these requirements is to be demonstrated during initial and ongoing method re-validation studies. For Initial Validations, averages of at least 8 Lab Control Samples or RMs must be assessed. Ongoing Re-validations (performance reviews) should assess QC data encompassing longer timeframes (e.g. 6 months to 1 year). A minimum frequency of 2 years is recommended for Ongoing Re-validations.

Accuracy Requirement: Laboratories must demonstrate method accuracy (measured as average recovery) of 80-120% or better for Lab Control Samples or Certified Reference Materials at concentrations above ten times the MDL.

Precision Requirement: Laboratories must demonstrate method precision equal to or better than 15% relative standard deviation for clean matrix spikes at concentrations above ten times the MDL.

Sensitivity Requirement: Where possible, the method should support Reporting Limits (and MDLs) that are less than 1/5 of applicable numerical standards. The method is not fit-for-purpose if an MDL exceeds a guideline, standard, or regulatory criteria against which it will be used for evaluation of compliance.

Quality Control

Summary of QC Requirements

QC Component	Minimum Frequency	Minimum Data Quality Objectives
Internal Standard Area Checks	All samples and QC	Peak area counts for all internal standards in all injections must be 50-150% of the initial calibration (average or mid-point) or initial CVS (Ref: EPA 539)
Surrogate Compounds	All samples and QC	70 – 130%
Calibration Verification Standard (CVS) – 2nd source	1 per initial calibration	85 – 115% recovery
Method Blank (MB)	One per batch (max 20 samples)	Less than reported DL
Laboratory Control Sample (LCS)	One per batch (max 20 samples)	70 – 130%
Laboratory Duplicate (DUP)	One per batch (max 20 samples)	30% RPD [or within 2x reported DL for low level results]
Matrix Spike (MS) or Reference Material (RM)	One per batch (max 20 samples)	60 – 140%

Continuing Calibration Verification (CCV)	At least every 12 hours (max 20 samples), and at end of each batch.	80 – 120% recovery for mid-level standards
If DQOs are not met, repeat testing or report qualified test results. DQOs do not apply to MS results where sample background exceeds spike amount.		

Prescribed Elements

The following components of this method are mandatory:

1. Tandem mass spectrometry is required to achieve sensitivity and selectivity objectives (LC/MS/MS or GC/MS/MS). At least two MRM transitions are required to be monitored. Labs must define appropriate criteria for confirmation of analyte identity by secondary transitions.
2. A suitable surrogate (Ethynylestradiol-d4 is recommended) is required to be added to all samples and QC prior to analysis. If solid phase extraction (or alternate extraction technique) is utilized, the surrogate must be added prior to extraction.
3. An internal standard, e.g. ^{13}C2-Ethynylestradiol, is required to be added to all samples prior to analysis.
4. Test results for EE2 must clearly indicate whether they are applicable to Total or Dissolved EE2 concentrations. Sample preparation procedures prior to Dissolved and Total EE2 analysis must be followed as described.
5. Stated calibration requirements must be met. Calibration standards must be solvent-matched with samples unless equivalency is demonstrated.
6. If this method is utilized for seawaters or brine samples, method validation for that matrix must be conducted prior to use using Matrix Spikes and/or Reference Materials.
7. All stated Performance Requirements and Quality Control requirements must be met.
8. Sample container materials, preservation, storage, and hold time requirements may not be modified. Samples analyzed beyond the stated holding time must be qualified. Refer to latest version of "BC MOE Sample Preservation and Hold Time Requirements" for updates.

Apart from these limitations, and provided performance requirements are met, laboratories may introduce modifications to this method to improve quality or efficiency.

References

1. EPA Method 539, Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS), United States Environmental Protection Agency Office of Water, November 2010.
2. EPA Method 1694, Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS, United States Environmental Protection Agency Office of Water, December 2007.
3. EPA Method 1698, Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS, United States Environmental Protection Agency Office of Water, December 2007.
4. BC MOE Water Quality Guidelines for Pharmaceutically Active Compounds (PhACs), 17α-ethinylestradiol (EE2), Technical Appendix, British Columbia Ministry of Environment, September 2009.
5. EPA-820-R-10-008, Stability of Pharmaceuticals, Personal Care Products, Steroids, and Hormones in Aqueous Samples, POTW Effluents, and Biosolids, United States Environmental Protection Agency Office of Water, September 2010.

Revision History

Sept 15, 2017 First version added to BC Lab Manual in support of 2017 CSR updates.