17α-Ethinylestradiol in Water by LC-MS/MS - PBM

Parameter

17α-ethinylestradiol (EE2) in water.

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.

Method Summary

Centrifugation is used to remove suspended particulate. Samples are acidified with sulfuric acid, passed through a C18-based SPE column, and eluted with a polar solvent such as acetonitrile.

An internal standard is added and the samples are 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 Number	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, Drinking water, Seawater, Wastewater

Method Limitations

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

The BC Water Quality guideline applies to total EE2. If there are visible particles, a separate analysis of the centrifuged solids is required; the results of the aqueous and solids fractions can be summed together. Refer to EPA 1694 for details.

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. Non-volumetric glassware can be heated in a muffle furnace at 400ºC for two hours or solvent rinsed. Volumetric glassware should be solvent rinsed and never heated in an oven above 120ºC.

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

Sampling should be done by qualified personnel. Samples must be collected and stored such that degradation or alteration of the sample is minimized.

Collect samples in clean 1 L amber glass containers with PTFE-lined caps.

Preservation: Chlorinated samples must be preserved with or sodium thiosulfate (80 mg/L). No preservation is required for non-chlorinated samples, however 2-mercaptopyridine-1-oxide, sodium salt is recommended to inhibit microbial degradation.

Stability

Holding Time: Samples should be extracted as soon as possible, but must be extracted within 28 days. Extracts must be stored at 0°C or less and analyzed within 28 days after extraction.

Storage: Samples must be chilled during shipment and must not exceed 10°C during the first 48 hours after collection. In the laboratory, samples must be refrigerated at ≤ 6°C and protected from light until analysis.

Procedure

Sample Preparation:

Centrifuge samples with turbidity > 1 NTU to remove suspended particulate matter.

Samples are fortified with a surrogate and passed through solid phase extraction (SPE) disks containing octadecyl (C18) functional groups to extract EE2 and surrogate. The compounds 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 adding the internal standards.

Instrumental Analysis:

Transfer a portion of the reconstituted extract to an auto-sampler vial. Analyze by direct injection LC-ESI-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 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	Within 50-200% of initial calibration CVS
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
Lab Control Sample (LCS)	One per batch (max 20 samples)	70 – 130%
Lab Duplicates (DUP)	One per batch (max 20 samples)	20% 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. Preservation as per the Sample Handling and Preservation section is mandatory.
2. Sample holding times must be adhered to. Samples analyzed beyond the stated holding time must be qualified.
3. Tandem mass spectrometry is required to achieve sensitivity and selectivity. At least one MRM transition is required to be monitored (two or more are recommended).
4. If SPE is used, a surrogate such as Ethynylestradiol-d4 is required to be added to all samples and QC prior to extraction.
5. An internal standard, e.g. ^{13}C2-Ethynylestradiol is required to be added to all samples

prior to analysis.

6. Stated calibration requirements must be met. Calibration standards must be solvent-matched with samples unless equivalency is demonstrated.
7. Samples that exceed the calibration range must be diluted and re-analyzed, or reported as estimated or minimum values.
8. All stated Performance Requirements and Quality Control requirements must be met.

Apart from these limitations, and provided performance requirements are met, laboratories may introduce modifications to this method to improve quality or efficiency. Laboratories must disclose to their clients where modified or alternative methods are employed.

References

1. 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. 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. 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. Water Quality Guidelines for Pharmaceutically Active Compounds (PhACs): 17α-ethinylestradiol (EE2), Technical Appendix, British Columbia Ministry of Environment, September 2009.
5. 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

March 15, 2017 First version of method.