ESTROGENIC COMPOUNDS 5044

FORMULAE: Table 1 MW: Table 1 CAS: Table 2 RTECS: Table 2

METHOD: 5044, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 March 2003**

OSHA: Table 2
NIOSH: Table 2
ACGIH: Table 2

PROPERTIES: Table 1

Synonyms: Table 2

SAMPLING

SAMPLER: FILTER, PTFE (37-mm, 2-µm)

FLOW RATE: 1 L/min

VOL.-MIN: 150 L
-MAX: 1000L

SHIPMENT: Ship at ambient temperature.

SAMPLE STABILITY: 30 days at 25 °C [1]

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: Not determined

BIAS: Not determined

OVERALL PRECISION(S_{rT}): Not determined

ACCURACY: Not determined

MEASUREMENT

TECHNIQUE: HPLC, UV detection

ANALYTE: See Table 1

EXTRACTION: Methanol, extraction overnight at room temperature

INJECTION VOLUME: 25 µL

MOBILE PHASE: 60% acetonitrile / 40% water @ 26 °C, 1.75 mL/min

COLUMN: C18 reversed phase, 150 x 4.6-mm, 5-µm; in-line pre-filter, 2.0- µm

DETECTOR: UV/Vis at 200 nm and 237 mµ

CALIBRATION: Standards in methanol

RANGE: β-Estradiol 0.3 to 44 µg/sample
Estrone 0.2 to 64 µg/sample
Progesterone 0.5 to 64 µg/sample
β-Estradiol 3-Benzoate 0.5 to 64 µg/sample [1]

ESTIMATED LOD: Table 3

PRECISION (S_r):

β-Estradiol	0.040
Estrone	0.039
Progesterone	0.030
β-Estradiol 3-Benzoate	0.032

@ 0.5 - 64 µg/sample [1]

APPLICABILITY: This method has not been used to evaluate field air samples. The method is based on a preliminary investigation analyzing field wipe samples collected during a health hazard evaluation at a facility producing birth control pills [1]. Appendix 1 contains information on recovery of analytes from wipe samples.

INTERFERENCES: Any compound that elutes at the same HPLC retention times may interfere.

OTHER METHODS: No validated methods were found.

REAGENTS:

1. Water, distilled, deionized, degassed
2. Acetonitrile, HPLC grade, degassed.*
3. Methanol, HPLC grade.*
4. β-Estradiol (Sigma E8875 or equivalent)*
5. Estrone (Sigma E9750 or equivalent)*
6. Progesterone (Sigma P0130 or equivalent)*
7. β-Estradiol 3-Benzoate (Sigma E8515 or equivalent)*
8. Stock standard solutions: place approximately 40 mg of each analyte, weighed to 0.001 mg, in separate 20-mL vials and dissolve in 20-mL methanol.
9. Estrogen mixed standards: combine 4 mL of each stock standard solution in a 20-mL vial. Prepare serial dilutions of this mixture in methanol to 0.05 µg/mL.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Filters: PTFE-laminate, 37-mm, 2-µm pore size (SKC Inc., Cat No. 225-17-07 or equivalent), cellulose spacer ring, 37-mm OD, 32-mm ID (SKC Inc., Cat. No. 225-23 or equivalent) in a 37-mm cassette filter holder.
2. Culture tubes, PTFE-lined screw cap, 16-mm x 100-mm.
3. Vials, auto-sampler, 4-mL, with PTFE-lined septa.
4. Pipets, volumetric (0.5- to 20-mL)
5. Vials, PTFE-lined screw cap, 20-mL.
6. Forceps.
7. Hamilton syringes, 50-µL and 100-µL.
8. Syringe, disposable, 10-mL.
9. Syringe filter, PTFE, 0.45-µm
10. HPLC with integrator and autosampler; UV/Vis detectors in series (200-nm and 237-nm); C18 column, 150x4.6-mm (Alltima; Alltech Associates Inc., State College PA, Cat. No. 88053, or equivalent); in-line pre-filter, 2.0 µm (Opti-Solv; Optimize Technologies, or equivalent).
11. Rotator, rotating tube shaker (Labquake-Thermolyne or equivalent).

SPECIAL PRECAUTIONS: Acetonitrile and methanol are flammable and health hazards. Methanol is a cumulative poison. Estrogenic compounds may be carcinogens [3]. Handle in a well ventilated hood and wear protective clothing.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Take personal samples at 1 L/min for a total sample size of 150 to 1000 L.
3. Pack filter cassettes securely for shipment (unrefrigerated).
4. Store samples at ambient temperature upon receipt at the laboratory.

SAMPLE PREPARATION:

5. Remove each filter from the cassette holder, roll with forceps and place in a 16 x 100 mm culture tube.
6. Add 4.0 mL methanol to each tube.
7. Prepare media and reagent blanks in the same manner.
8. Cap each tube tightly and place on rotator. Rotate $\geq$8 hr at room temperature.
9. Filter extracts, if necessary, using a disposable syringe fitted with a 0.45-µm PTFE filter into a clean vial.
10. Transfer all extracts to labeled autosampler vials.

CALIBRATION AND QUALITY CONTROL:

11. Calibrate daily with at least six working standards over the range of interest.
    a. Prepare serial dilutions of the stock mixture in the range of 0.08 to 80 µg/mL
    b. Analyze with samples and blanks (steps 15 and 16).
    c. Prepare a calibration graph (peak area vs mass of analyte, µg per sample).

12. Determine the desorption efficiency and recovery in the range of interest for each lot of filters used for sampling.
    a. Prepare four tubes at each of three levels plus three media blanks.
    b. Add known amounts of the analyte or analyte mixture in methanol to the filters.
    c. Allow to stand ≥2 hr for solvent evaporation.
    d. Prepare samples (steps 6 through 10) and analyze together with standards (steps 15 and 16).
    e. Determine recovery.
13. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph and recovery graph are in control.

MEASUREMENT:

14. Set HPLC according to manufacturer's instructions and conditions on page 5044-1.
15. Inject sample aliquot manually or with autosampler.
16. Measure peak areas.

CALCULATIONS:

17. Determine the mass in µg of analytes found on the sample filter, W, and on the average blank filter, B.
18. Calculate concentration, C, of analytes in the air volume sampled, V (L):

$$C = \frac{W - B}{V}, mg/m^3$$

NOTE: µg/L ≡ mg/m^3

EVALUATION OF METHOD:

This method has been used to analyze field-generated wipe samples [2]. It was not evaluated with laboratory-generated air samples. LOD/LOQ, extraction efficiency, sampling stability and storage stability studies were performed with laboratory-spiked filters.

LOD/LOQ values (in µg/filter) for β-Estradiol were 0.8/2.5; for Estrone, 0.2/0.5; for Progesterone, 0.5/1.7; and for β-Estradiol 3-Benzoate, 0.5/1.7.

Extraction efficiency for β-Estradiol ranged from 94 to 103%; for Estrone, 95 to 104%; for Progesterone; 97 to 102%; for β-Estradiol 3-Benzoate, 95 to 101%. An additional recovery study using Wash 'n Dri® wipes gave extraction efficiencies of 97 to 100%.

The stability of the analytes on the filter media (static efficiency) was evaluated by drawing air through spiked filters. Recoveries ranged from 94 to 102% for β-Estradiol; for Estrone, from 96 to 101%; for Progesterone, from 99 to 102%; and for β-Estradiol 3-Benzoate, from 92 to 101%.

Recovery after 30-day storage at 25 °C was 93% for β-Estradiol, 94% for Estrone, 98% for Progesterone, and 111% for β-Estradiol 3-Benzoate.

REFERENCES:

[1] Mathews ES and Neumeister CE [2000]. Backup Data Report for Method 5044, Estrogenic Compounds by HPLC (unpublished), NIOSH, DART.
[2] Neumeister CE [1983]. Analytical Report for Sequence 1400. Cincinnati, OH: National Institute of Occupational Safety and Health (unpublished).
[3] Sixth Annual Report on Carcinogens [1991]. USDHHS/PHS, National Toxicology Program

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TABLE 1. PROPERTIES

Compound	Formula	M.W.	M.P. (^{o}C)	UV Max (nm)
β-Estradiol	$C_{18}H_{24}O_2$	272	173-179	206
Estrone	$C_{18}H_{22}O_2$	270	254-256	198
Progesterone	$C_{21}H_{30}O_2$	314	127-131	237
β-Estradiol-3-Benzoate	$C_{25}H_{28}O_3$	376	191-196	201

TABLE 2. GENERAL INFORMATION

Compound	Synonyms	CAS	RTECS	Exposure Limit
β-Estradiol	dihydroxyfollicular hormone dihydroxyestrin oestra-1,3,5(10) triene-3,17-betadiol	50-28-2	KG2975000	None Specified
Estrone	3-hydroxyestra-1,3,5(10)-trien-17-one 1,3,5-estratrien-3-ol-17-one oestrone folliculin	53-16-7	1009137ES	None Specified
Progesterone	pregn-4-ene-3,20-dione luteohormone Corlutin	57-83-0	TW0175000	None Specified
β-Estradiol-3-Benzoate	estradiol benzoate oestradiol monobenzoate (17β)-estra-1,3,5(10)-triene-3,17-diol 3-benzoate Benovocylin	50-50-0	KG4050000	None Specified

TABLE 3. LOD/LOQ DETERMINATION

Compound	LOD µg/filter	LOQ µg/filter
β-Estradiol	0.8	2.5
Estrone	0.2	0.5
Progesterone	0.5	1.7
β-Estradiol 3-Benzoate	0.5	1.7

APPENDIX 1: PREPARATION OF SPIKES ON WIPES:

Wipe media (Wash 'n Dri® Moist Disposable Towelettes) were opened and spread to evaporate to dryness. Dried towelettes were rolled and inserted into 16x100-mm PTFE-lined screw cap tubes. A Hamilton syringe was used to place measured amounts of stock solution in methanol (50 µL containing 40 µg of each analyte) on each wipe. After allowing the spikes to evaporate, 8 mL of methanol was added to each tube, and the tubes were capped and rotated overnight at room temperature to extract the spiked analyte from the wipes.

EFFICIENCY OF EXTRACTION:

Compound	Efficiency of Extraction (%) (n=6)	S_r
β-Estradiol	100	1.4
Estrone	97	3.1
Progesterone	99	0.82
β-Estradiol 3-Benzoate	99	0.76