Method no.	PV2068
Matrix:	Air
Target Concentration:	0.03 mg/m^3 There is no OSHA PEL or ACGIH TLV for mestranol.
Procedure:	Samples are collected by drawing known volumes of air through FWS-B filters. The samples are extracted with isopropanol and analyzed by high performance liquid chromatography (HPLC).
Recommended air volume and sampling rate:	500 L at 2.0 L/min
Detection limit of the overall procedure (based on the recommended air volume):	0.6 µg/m^3
Status of method:	Stopgap method. This method has been only partially evaluated and is presented for information and trial use. It was developed for the simultaneous analysis of mestranol and norethindrone.

Date: July, 1987 (final) Chemist: Duane Lee

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1. General Discussion

1.1 Background

1.1.1 History of procedure

The OSHA Analytical Laboratory received a set of field samples requesting the analysis of mestranol and norethindrone. These air samples had been collected on FWS-B filters. This report describes the analytical procedure developed for the analysis of mestranol.

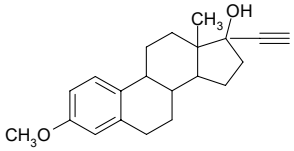
1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy).

There is sufficient evidence for the carcinogenicity of mestranol in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard mestranol as if it presented a carcinogenic risk to humans. (Ref. 5.1)

1.1.3 Potential workplace exposure

No estimate of worker exposure to mestranol could be found. Mestranol is not produced commercially in the United States. (Ref. 5.1)

1.1.4 Physical properties (Refs. 5.1-5.2)

Molecular weight:	310.42
Molecular formula:	$C_{21}H_{26}O_2$
CAS #:	72-33-3
Melting point:	150-151 °C
Solubility:	Practically insoluble in water; soluble in ethanol, acetone, diethyl ether, chloroform, and dioxane; slightly soluble in methanol
Synonyms:	3-Methoxy-19-norpregna-1,3,5(10)-trien-20-yn-17-ol; 17-ethynyl-3-methoxy-1,3,5(10)-estratrien-17-ol; 3-methylethynylestradiol; Conovid; Demulen; Enovid; Inostral; Menophase; Norinyl; Norquen; Ortho-Novin 2; Ovulen; Previson; Sequens; Sophia
Structure:	
Description:	White crystals (from methanol or acetone)

UV scan:

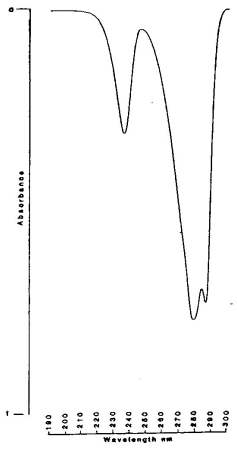


Figure 1a UV Scan
Mestranol in Acetonitrile

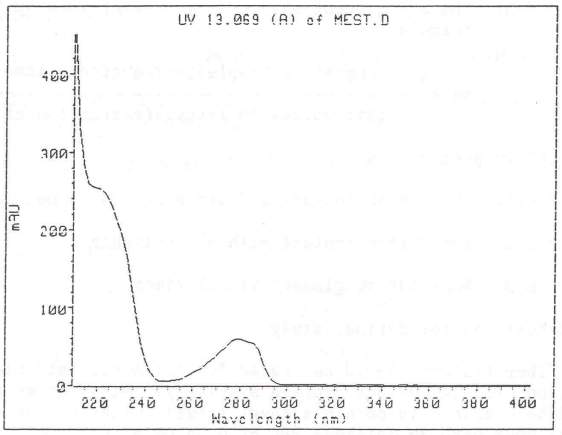


Figure 1b UV Scan
Mestranol in 60% Acetonitrile 40% Water

1.2 Limit defining parameters

The detection limit of the analytical procedure is 1.9 ng per injection. This is the amount of analyte which will give a peak whose height is approximately five times the baseline noise.

2. Sampling Procedure

2.1 Apparatus

2.1.1 Samples are collected by using a personal sampling pump that can be calibrated to within ±5% of the recommended flow rate with the sampling device in line.

2.1.2 Mine Safety Appliances (MSA) membrane filter type FWS-B (PVC) 5.0 micron 37-mm diameter, or equivalent.

2.1.3 Backup pad, 37-mm diameter, Millipore AP10, MF support pad, or equivalent.

2.1.4 Filter holder, 37-mm polystyrene cassette, Millipore M000037AO, or equivalent.

2.2 Reagents

None

2.3 Sampling technique

2.3.1 Assemble the filter in the two-piece cassette holder and close firmly. The filter is supported by the backup pad. Secure the cassette holder together with tape.

2.3.2 Attach the outlet of the filter cassette to the personal sampling pump inlet with flexible tubing. Air being sampled should not pass through any hose or tubing prior to entering the sampler.

2.3.3 Attach the sampler vertically in the employee's breathing zone in such a manner that it does not impede work performance.

2.3.4 After sampling for the appropriate time, remove the sampling device and reinstall the end-plugs on the cassettes.

2.3.5 Wrap each sample end-to-end with an OSHA seal (Form 21).

2.3.6 Submit at least one blank for each set of samples. The blank should be handled in the same manner as the samples, except no air is drawn through it.

2.3.7 Record the air volume (in liters of air) for each sample, and list any possible interferences.

2.3.8 Bulk samples submitted for analysis must be sent in a separate container.

2.4 Extraction efficiency

Twelve FWS-B filters were each spiked with 15.1 µg of mestranol along with norethindrone. Three of the filters were extracted in 3 mL of isopropanol by shaking for 30 min and then analyzed. The results are listed in Table 2.4.

Table 2.4
Extraction Efficiency

sample	amount spiked, µg	amount found, µg	recovered %
Ex1	15.1	14.6	96.7
Ex2	15.1	14.9	98.7
Ex3	15.1	15.5	102.6
$\bar{x}$			99.3

2.5 Retention efficiency

To the remaining nine filters from above, 500 L of humid air (80% relative humidity) was drawn through each filter. Three of the filters were extracted with 3 mL of isopropanol by shaking for 30 min and then analyzed. The results are listed in Table 2.5.

Table 2.5
Retention Efficiency

sample	amount spiked, µg	amount found, µg	recovered %
R1	15.1	15.5	102.6
R2	15.1	15.3	101.3
R3	15.1	15.2	100.7
$\bar{x}$			101.5

2.6 Sample storage

The remaining six samples from above were stored. Three of the samples were stored at ambient temperature in a drawer, and three were stored in a refrigerator. After six days of storage, the samples were extracted with 3 mL of isopropanol by shaking for 30 min. and then analyzed. The results are given in the tables below.

Table 2.6.1
Ambient Storage

sample	amount spiked, µg	amount found, µg	recovered %
SA1	15.1	14.8	98.0
SA2	15.1	14.6	96.7
SA3	15.1	15.3	101.3
$\bar{x}$			98.7

Table 2.6.2
Refrigerated Storage

sample	amount spiked, µg	amount found, µg	recovered %
SR1	15.1	14.9	98.7
SR2	15.1	15.2	100.7
SR3	15.1	15.3	101.3
$\bar{x}$			100.2

2.7 Recommended air volume and sampling rate

2.7.1 The recommended air volume is 500 L.

2.7.2 The recommended flow rate is 2.0 L/min.

2.8 Interferences

It is not known if any compounds will interfere with the collection of mestranol.

2.9 Safety precautions

2.9.1 Attach the sampling equipment in such a manner that it will not interfere with work performance or employee safety.

2.9.2 Follow all safety practices that apply to the work area being sampled.

3. Analytical Procedure

3.1 Apparatus

3.1.1 A balance capable of weighing to the nearest tenth of a milligram. A Mettler HL52 balance was used in this evaluation.

3.1.2 Mechanical shaker.

3.1.3 A high performance liquid chromatograph (HPLC) equipped with a fluorescence detector, a manual or an automatic injector, and a strip chart recorder. A system that included a Waters autosampler (WISP), a Waters model 6000-A pump, a Kratos model 980G fluorescence detector, and a Varian strip chart recorder was used in this evaluation.

3.1.4 HPLC column capable of separating norethindrone from any interferences. A (25 cm × 4.6 mm i.d.) Alltech C18 (10 micron) column was used in this evaluation.

3.1.5 An electronic integrator, or some other suitable method for measuring detector response. The Hewlett-Packard 3357 Laboratory Data System was used in this evaluation.

3.1.6 Vials, 4-mL with Teflon-lined septum.

3.1.7 Volumetric flasks and pipets.

3.2 Reagents

3.2.1 HPLC grade acetonitrile (ACN).

3.2.2. HPLC grade water. A Millipore Milli-Q system was used to prepare the water for this evaluation.

3.2.3. HPLC grade isopropanol (IPA).

3.2.4. Mestranol, Sigma Chemical Company, St Louis, MO.

3.3 Standard preparation

Stock standards were prepared by weighing 15 mg of mestranol, placing in 25-mL volumetric flasks, and diluting to volume with isopropanol. Dilutions of the stock standards were made by pipet to obtain working range standards. Stock and dilute standards were stored in a freezer.

3.4 Sample preparation

3.4.1 Transfer the FWS-B filters to separate 4-mL WISP vials.

3.4.2 Pipet 3.0 mL of isopropanol into each vial and seal with a Teflon-lined septum.

3.4.3 Shake the vials for 30 minutes.

3.5 Analysis

3.5.1 Instrument conditions

Column:	Alltech C18 10-µm, (25 cm × 4.6 mm i.d.)
Eluent:	ACN/H_2O 70:30
Flow rate:	1 mL/min
Detector:	Fluorescence detector (Kratos 980G)
	Excitation = 215 nm
	Emission = 290 nm (no filter)
Retention time:	12.3 min
Injection volume:	20 µL/injection

3.5.2 Chromatogram

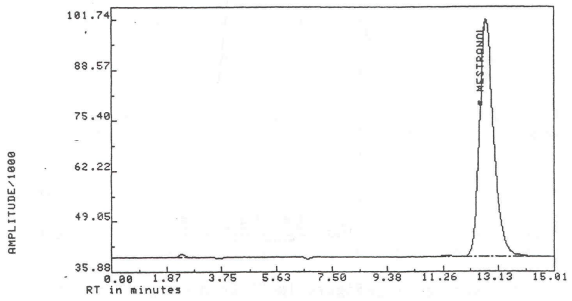


Figure 2 Chromatogram

3.6 Interferences

3.6.1 Any collected compound having a similar retention time and fluoresces is an interference.

3.6.2 HPLC conditions may be varied to circumvent an interference.

3.6.3 Retention time alone is not proof of chemical identity. Analysis by other means should be sought whenever possible for confirmation of identity.

3.7 Calculations

3.7.1 A calibration curve is constructed by plotting detector response versus standard concentration.

3.7.2 The concentration of mestranol in a sample is determined from the calibration curve.

3.7.3 The air concentration is then determined by the following formula.

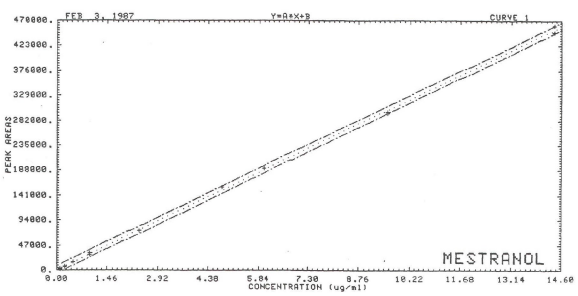


Figure 3 Calibration Curve

$$mg/m^3 = \frac{(mg/mL \text{ in sample})(\text{extraction volume in mL})}{(\text{air volume in liters})(\text{extraction efficiency})}$$

3.8 Safety precautions

3.8.1 Avoid skin contact and air exposure to mestranol.

3.8.2 Avoid skin contact with all solvents.

3.8.3 Wear safety glasses at all times.

4. Recommendations for further study

Glass fiber filters should be tested for extraction efficiency, retention efficiency, and storage stability. Also, other procedures should be examined to obtain a lower target concentration since a company does have an internal PEL of 0.05 μg/m^3.

5. References

5.1 IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 21, International Agency for Research on Cancer, Lyon, 1979, pp. 257-278.

5.2 Merck Index, Tenth Edition, Merck & Co., Inc, Rahway, N.J. 1983.