Method no.:	PV2070
Matrix:	Air
Target Concentration:	0.03 mg/m3 There is no OSHA PEL or ACGIH TLV for norethindrone.
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/m3
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 norethindrone and mestranol.

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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 norethindrone and mestranol. These air samples had been collected on FWS-B filters. This report describes the analytical procedure developed for the analysis of norethindrone.

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

There is limited evidence for the carcinogenicity of norethindrone in animals. (Ref. 5.1)

1.1.3 Potential workplace exposure

No estimate of worker exposure to norethindrone could be found. Norethindrone is not produced in the United States. (Ref. 5.3)

1.1.4 Physical properties (Ref. 5.1 and 5.2)

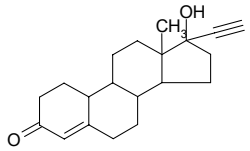
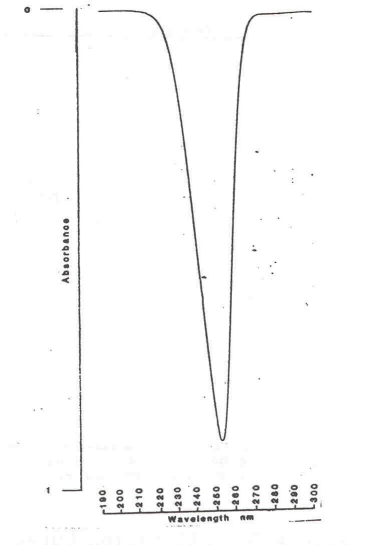
Molecular weight:	298.4
Molecular formula:	$C_{20}H_{26}O_2$
CAS #:	68-22-4
Melting point:	203-204 °C
Solubility:	Soluble in ethanol, acetone, chloroform, pyridine, and dioxane.
Synonyms:	17-hydroxy-19-norpregn-4-en-20-yn-3-one; anhydroxynorprogesterone; 19-norethisterone; norpregneninolone; Conceplan; Mini-Pe; Noriday; Anovule
Structure:	
Description:	White crystalline powder with a slightly bitter taste.
UV scan:	

Figure 1 UV Scan
Norethindrone in Acetonitrile

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 from air samples.

2.4. Extraction efficiency

Twelve FWS-B filters were each spiked with 15.5 ug of norethindrone along with mestranol. 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

amount spiked, µg	amount found, µg	recovered, %
15.5	15.6	100.6
15.5	15.1	97.4
15.5	15.2	98.1
	$\bar{x}$	98.7

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

amount spiked, µg	amount found, µg	recovered, %
15.5	15.3	98.7
15.5	15.4	99.4
15.5	15.3	98.7
	$\bar{x}$	98.9

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

amount spiked, µg	amount found, µg	recovered, %
15.5	15.1	97.4
15.5	15.1	97.4
15.5	15.1	97.4
	$\bar{x}$	97.4

Table 2.6.2
Refrigerated Storage

amount spiked, µg	amount found, µg	recovered, %
15.5	15.1	97.4
15.5	15.2	98.1
15.5	15.2	9.1
	$\bar{x}$	97.9

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 norethindrone.

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 an ultraviolet (UV) detector, a manual or an automatic injector, and a strip chart recorder. A Waters system that included a WISP autosampler, model 6000-A pump, and a model 440 fixed wavelength detector 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.

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

3.3 Standard preparation

Stock standards were prepared by weighing 15 mg of norethindrone, 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/H2O 70:30
Flow rate:	1 mL/min
Detector:	Ultraviolet detector (Waters 440) Wavelength = 254 nm
Retention time:	6.4 min
Injection volume:	20 µL/injection

3.5.2 Chromatogram:

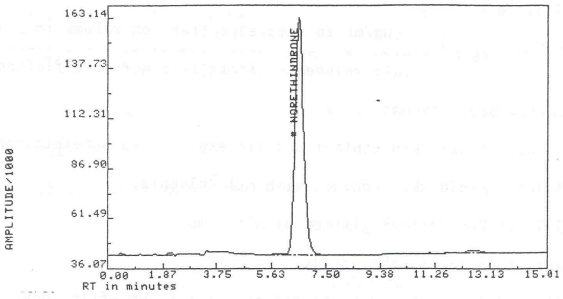


Figure 2 Chromatogram

3.6 Interferences

3.6.1 Any collected compound having a similar retention time and absorbs at 254 nm 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 norethindrone in a sample is determined from the calibration curve.

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

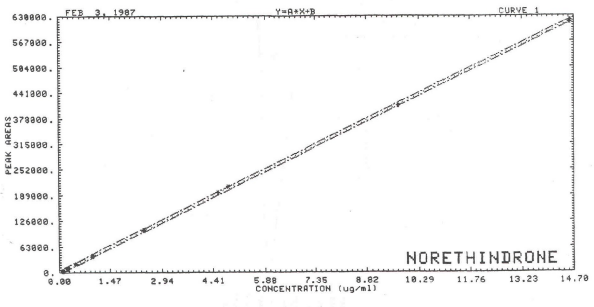


Figure 3 Calibration Curve

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

3.8 Safety precautions

3.8.1 Avoid skin contact and air exposure to norethindrone.

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 detection limit.

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. 441-460.

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

5.3 IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 6, International Agency for Research on Cancer, Lyon, 1974, pp. 179-189.