

Estradiol
Estriol
Estrone
17- α -Methyltestosterone
Progesterone
Testosterone



Method number: PV2001

Target concentration: 0.1 mg/m³

Procedure: Samples are collected open face by drawing workplace air through glass fiber filters. Samples are extracted with 4-mL methanol and analyzed by LC using an ultraviolet detector at 240 nm for 17- α -methyltestosterone, progesterone, and testosterone, and 280 nm for estradiol, estrone, and estriol.

Recommended sampling time and sampling rate: 240 min at 1.0 L/min (240 L)

Reliable quantitation limit:

| | |
|-----------------------------------|------------------------------|
| estradiol: | 5.4 $\mu\text{g}/\text{m}^3$ |
| estriol: | 7.1 $\mu\text{g}/\text{m}^3$ |
| estrone: | 4.6 $\mu\text{g}/\text{m}^3$ |
| 17- α -methyltestosterone: | 1.2 $\mu\text{g}/\text{m}^3$ |
| progesterone: | 3.1 $\mu\text{g}/\text{m}^3$ |
| testosterone: | 2.5 $\mu\text{g}/\text{m}^3$ |

Status of method: Partially evaluated method. This method has been subjected to established evaluation procedures of the Methods Development Team and is presented for information and trial use.

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February 2001 (Modified)

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1. General Discussion

1.1 Background

1.1.1 History

Air samples were received at SLTC collected on glass fiber filters requesting analysis for estradiol, estrone, estriol, 17- α -methyltestosterone, progesterone, and testosterone. The purpose of this study is to determine whether glass fiber filters are appropriate for the collection of these compounds, and to determine an appropriate analytical procedure.

In 1984, NIOSH published an evaluation of exposure to estradiol benzoate, progesterone, testosterone, β -estradiol, estrone, and testosterone propionate, in which they collected the air samples on glass fiber filters with analysis by LC with UV detection.¹ Workplace exposure monitoring for testosterone, and progesterone at Pharmacia & Upjohn uses collection of samples on glass fiber filters and analysis LC with ultraviolet detection at 242 nm.^{2,3} Other air borne steroids and hormones have been collected on glass fiber filters and analyzed by LC at SLTC.⁴ Based on these studies, collection on glass fiber filters and analysis by LC with an ultraviolet detector at two wavelengths, 240 and 280 nm, was chosen for estradiol, estrone, estriol, 17- α -methyltestosterone, progesterone, and testosterone. Estradiol, estriol, and estrone were analyzed at 280 nm, and 17- α -methyltestosterone, progesterone, and testosterone were analyzed at 240 nm, to obtain the maximum sensitivity. All of these analytes may be analyzed together at 270 nm, but the sensitivity is reduced.

This method was updated in February 2001 with new evaluation data that showed that the analytes are retained after 960 L of humid air is drawn through spiked filters (Appendix 1).

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

While these hormones are naturally occurring in the human body and, at the proper levels, are necessary for normal functioning. At elevated levels there can be toxic and carcinogenic effects because they are growth promoters. Estradiol, estrone, and estriol can be absorbed through skin, mucous membrane, and gastrointestinal tract.⁵ Estrogens, testosterone, and progesterone are used in varying amounts and combinations to treat menopausal symptoms. Estrogens can cause loss of libido and impotence in males and menstrual disorders in females.⁶ Estradiol, estrone, progesterone, and testosterone are confirmed animal carcinogens with neoplastigenic, tumorigenic, and teratogenic

¹ Lucas, A., Health Hazard Evaluation Report HETA 81-314-1435, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health: Cincinnati, OH, 1984.

² Portis, E., Progesterone in Air, Pharmacia & Upjohn, Kalamazoo, MI, unpublished work, 1994.

³ Portis, E., Testosterone in Air, Pharmacia & Upjohn, Kalamazoo, MI, unpublished work, 1994.

⁴ Eide, M., A Study of Melegestrol Acetate, Sulfamethazine, and Carbodox Extraction and Retention on Glass Fiber Filters and AA filters and the Extraction from Whatman 41 Filters, OSHA Salt Lake Technical Center: Salt Lake City, UT. Unpublished work, 1985.

⁵ Arky, R. *Physicians' Desk Reference*, 51 ed.: Montvale, NJ, 1997, p752.

⁶ Harrington, J.M., Occupational Exposure to Synthetic Estrogens, *Scand j Work Envirrn Health*, 8(1982), suppl 1, p167-171.

effects.^{7,8,9,10} Estradiol causes human and animal mutations. Estradiol has developmental reproductive effects.¹¹ Estriol is a suspected carcinogen with animal carcinogenic, neoplastigenic, tumorigenic, and teratogenic effects.¹² Estrone is a poison by intraperitoneal and subcutaneous routes. Estrone reproductive effects include inhibition of egg implantation, suppression of spermatogenesis, and impotence.¹³ 17- α -Methyltestosterone is a questionable human carcinogen, producing nonmalignant tumors in the liver. It is a poison by intraperitoneal route. It causes developmental abnormalities in the urogenital system.¹⁴ Progesterone is a poison by intravenous and intraperitoneal routes. It causes developmental abnormalities in the urogenital system. Progesterone effects on males include changes in spermatogenesis, prostate, seminal vesicle, Cowper's gland, and accessory glands, along with impotence and breast enlargement. Progesterone effects on females include changes in the menstrual cycle, uterus, cervix, and vagina.¹⁵ Testosterone is a poison by the intraperitoneal route. It causes developmental changes in the urogenital system.¹⁶

1.1.3 Workplace exposure¹⁷

Workers in the pharmaceutical industry are exposed to hormones in the production and formulation processes.

1.1.4 Physical properties and other descriptive information

Estradiol^{18,19,20}

| | | | |
|----------------|----------------------|--------------------|----------|
| CAS number: | 50-28-2 | IMIS number: | E319 |
| RTECS number: | KG2975000 | molecular weight: | 272.39 |
| melting point: | 178-179 °C | odor: | odorless |
| appearance: | white to pale yellow | λ_{\max} : | 280 nm |

⁷ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p131.

⁸ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p133.

⁹ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p189.

¹⁰ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p202.

¹¹ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p131.

¹² Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p267.

¹³ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p133.

¹⁴ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p732.

¹⁵ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p189.

¹⁶ Lewis, R.J., *Carcinogenically Active Chemicals*, Van Nostrand Reinhold: New York, 1991, p202.

¹⁷ Paul, M., *Occupational and Environmental Reproductive Hazards: A Guide for Clinicians*, Williams & Wilkins: Baltimore, MD, 1993, p290.

¹⁸ Lewis, R., J., *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold Co.: New York, 1993, p 473.

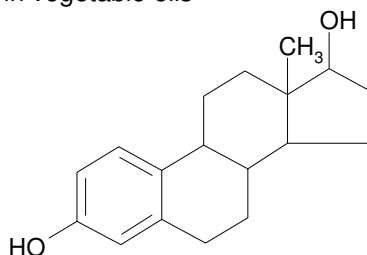
¹⁹ Budavari, S., *The Merck Index*, 12th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 1996, p 630.

²⁰ Lide, D., R., *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1992, p 7-15.

crystals
 synonyms: molecular formula: $C_{18}H_{24}O_2$
 Dihydrofollicular hormone; Dihydrofolliculin; Dihydroxestrin; Dihydrotheelin; Dimenformon; Diogyn; Estrace; Estraderm; (17 β)-Estra-1,3,5(10)-triene-3,17-diol; β -Estradiol; 3,17-Epidihydroxyestratriene; Estroclim; Evorel; Gynoeseryl; Macrodiol; Menorest; Oestrogel; Ovocyclin; Ovocyclin; Profoliol B; Progynon; System; Vagifem; Zumenon

solubility: alcohol, acetone, dioxane, fixed alkali hydroxides, sparingly soluble in vegetable oils

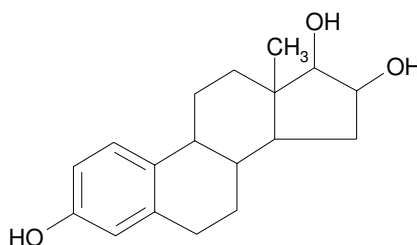
structural formula:



Estriol^{21, 22, 23}

| | | | |
|----------------|--|--------------------|-------------------|
| CAS number: | 50-27-1 | IMIS: | E321 |
| RTECS number: | KG8225000 | molecular weight: | 288.39 |
| melting point: | 282 °C | λ_{max} : | 280 nm |
| appearance: | white crystals | molecular formula: | $C_{18}H_{24}O_3$ |
| odor: | odorless | | |
| synonyms: | Aacifemine; Colpogyn; Destriol; (16 α ,17 β)-Estra-1,3,5(10)-triene-3,16,17-triol; 1,3,5-Estratriene-3 β ,16 α ,17 β -triol; Follicular hormone hydrate; Hormomed; 16 α -Hydroxyestradiol; oestriol; Klimax E; Klimoral; Oekilp; Ortho-Gynest; Ovesterin; Ovestin; Ovo-Vinces; Theelol; Tridestrin; 3,16 α ,17 β -Trihydrox- $\Delta^{1,3,5}$ -estratriene; Trihydroxyestrin; Triovex | | |
| solubility: | alcohol, dioxane, chloroform, ether, vegetable oils, pyridine, fixed alkali hydroxide solutions | | |

structural formula:



Estrone^{24, 25, 26}

| | | | |
|-------------|---------|-------|------|
| CAS number: | 53-16-7 | IMIS: | E320 |
|-------------|---------|-------|------|

²¹ Lewis, R., J., *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold Co.: New York, 1993, p 474.

²² Budavari, S., *The Merck Index*, 12th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 1996, p 631.

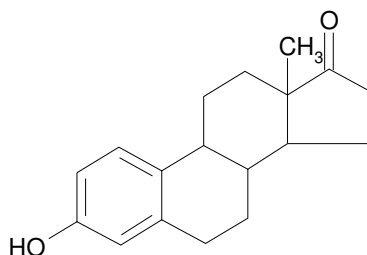
²³ Lide, D., R., *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1992, p 7-16.

²⁴ Lewis, R., J., *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold Co.: New York, 1993, p 474.

²⁵ Budavari, S., *The Merck Index*, 12th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 1996, p 632.

²⁶ Lide, D., R., *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1992, p 7-16

RTECS number: KG8575000 molecular weight: 270.37
 melting point: 254-256 °C λ_{\max} : 283-285 nm
 appearance: white crystals molecular formula: C₁₈H₂₂O₂
 odor: odorless
 synonyms: Crinovaryl; Cristallovaryl; Destrone; Disynformon; Endofolliculina; 1,3,5-Estratriene-3-ol-17-one; Estrol; Estrugenone; Estrusol; Femestron; Femidyn; Folikrin; Folipex; Folisan; Follestrine; Follicular hormone; Folliculin; Follidrin; Follicunodis; Glandubolin; Hiestrone; Hormofillin; Hormovarine; 3-Hydroxyestra-1,3,5(10)-trien-17-one; Kestron; Kolpon; Ketodestron; Ketohydroxyestron; Menformon; Oestron; Oestron; Oestroperos; Ovifollin; Perlatan; Theelin; Thelestrin; Thelykinin; Tokokin; Wynestron
 solubility: water, alcohol, acetone, chloroform, benzene, dioxane, pyridine, fixed alkali hydroxide solutions, slightly soluble in ether, vegetable oils
 structural formula:



17- α -Methyltestosterone^{27, 28, 29}

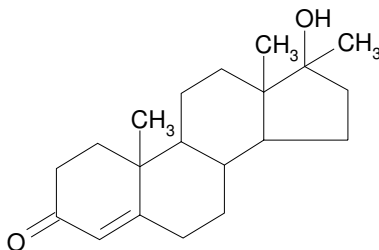
CAS number: 58-18-4 IMIS: M350
 RTECS number: BV8400000 molecular weight: 302.46
 melting point: 161-166 °C λ_{\max} : 242 nm
 appearance: white crystals molecular formula: C₂₀H₃₀O₂
 odor: odorless
 synonyms: Android; Glosso-Sterandryl; (17 β)-17-Hydroxy-17-methylandro-4-en-3-one; Metandren; 17 α -Methyl- Δ^4 -androsten-17 β -ol-3-one; Neohombreol-M; Nu-man; Orchisterone-M; Oreton Methyl; Perandren; Testred; Testhomona
 solubility: alcohol, methanol, ether and in other organic solvents, sparingly soluble in vegetable oils

²⁷ Lewis, R., J., *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold Co.: New York, 1993, p 781.

²⁸ Budavari, S., *The Merck Index*, 12th ed., Merck & Co. inc.: Whitehouse Station, NJ, 1996, p 1045.

²⁹ Lide, D., R., *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1992, p 7-13.

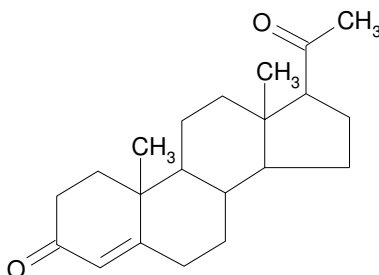
structural formula:



Progesterone^{30, 31, 32}

| | | | |
|----------------|--|--------------------|--|
| CAS number: | 57-83-0 | IMIS: | P446 |
| RTECS number: | TW0175000 | molecular weight: | 314.47 |
| melting point: | 129-130 °C | λ_{\max} : | 240 nm |
| appearance: | white crystals | molecular formula: | C ₂₁ H ₃₀ O ₂ |
| odor: | odorless | | |
| synonyms: | Corlutina; Corluvite; Corpus luteum hormone; Cyclogest; Gestiron; Gestone; Lipo-Lutin; Lutocyclin M; Lutogyl; Luteohormone; Lutromone; Pregn-4-ene-3,20-dione; Δ^4 -pregnene-3,20-dione; Progestasert; Progestin; Progestogel; Progestol; Progeston; Prolidon; Proluton; Syngesterone; Utrogestan | | |
| solubility: | alcohol, acetone, dioxane, conc H ₂ SO ₄ , sparingly soluble in vegetable oils | | |

structural formula:



Testosterone^{33, 34, 35}

| | | | |
|----------------|--|--------------------|--|
| CAS number: | 58-22-0 | IMIS: | T413 |
| RTECS number: | XA3066000 | molecular weight: | 288.39 |
| melting point: | 155 °C | λ_{\max} : | 238 nm |
| appearance: | white crystals | molecular formula: | C ₁₉ H ₂₈ O ₂ |
| odor: | odorless | | |
| synonyms: | Andro; Δ^4 -Androsten-17 β -ol-3-one; (17 β)-17-Hydroxyandrost-4-en-3-one; Mertestate; Oreton; Testoderm; Testolin; Testro AQ; Virosterone | | |
| solubility: | alcohol, ether, and other organic solvents | | |

³⁰ Lewis, R., J., *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold Co.: New York, 1993, p 965.

³¹ Budavari, S., *The Merck Index*, 12th ed., Merck & Co. inc.: Whitehouse Station, NJ, 1996, p 1335.

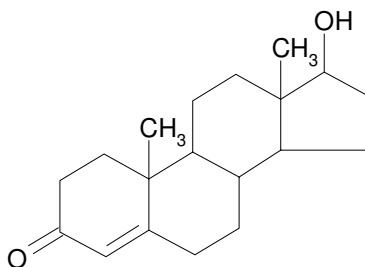
³² Lide, D., R., *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1992, p 7-21.

³³ Lewis, R., J., *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold Co.: New York, 1993, p 1125.

³⁴ Budavari, S., *The Merck Index*, 12th ed., Merck & Co. inc.: Whitehouse Station, NJ, 1996, p 1569.

³⁵ Lide, D., R., *CRC Handbook of Chemistry and Physics*, CRC Press: Boca Raton, FL, 1992, p 7-14.

structural formula:



This method was evaluated according to the OSHA SLTC “EVALUATION GUIDELINES FOR AIR SAMPLING METHODS UTILIZING CHROMATOGRAPHIC ANALYSIS”³⁶. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters.

1.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 4.8 µg/sample for estradiol, estriol, and estrone, and 4.0 µg/sample for 17-α-methyltestosterone, progesterone, and testosterone. These are the amounts spiked on a sampler that would produce a peak approximately 10 times the response for a sample blank. These spiked samplers were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (standard error of estimate and slope) for the calculation of the DLOP. The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The DLOP and RQL are listed in the table below.

Table 1.2
DLOP and RQL

| Compound | Slope | SEE | DLOP µg/sample | DLOP µg/m ³ | RQL µg/sample | RQL µg/m ³ | Recovery % |
|-------------------------|--------|-------|-------------------|---------------------------|------------------|--------------------------|---------------|
| Estradiol | 29814 | 3889 | 0.34 | 1.4 | 1.3 | 5.4 | 98.2 |
| Estriol | 28813 | 4927 | 0.51 | 2.1 | 1.7 | 7.1 | 97.2 |
| Estrone | 25564 | 2780 | 0.33 | 1.4 | 1.1 | 4.6 | 99.1 |
| 17-α-Methyltestosterone | 165192 | 4763 | 0.087 | 0.36 | 0.29 | 1.2 | 97.9 |
| Progesterone | 133882 | 9931 | 0.22 | 0.93 | 0.74 | 3.1 | 98.9 |
| Testosterone | 191771 | 11471 | 0.18 | 0.75 | 0.60 | 2.5 | 98.8 |

³⁶ Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. *EVALUATION GUIDELINES FOR AIR SAMPLING METHODS UTILIZING CHROMATOGRAPHIC ANALYSIS*; OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 1999.

Table 1.2.1
Detection Limit of the Overall Procedure
for Estradiol

| mass per sample (μg) | area counts ($\mu\text{V}\cdot\text{s}$) |
|--------------------------------------|---|
| 1.2 | 61879 |
| 1.6 | 73812 |
| 2.0 | 84775 |
| 2.4 | 90518 |
| 2.8 | 107080 |
| 3.2 | 128541 |
| 3.6 | 134930 |
| 4.0 | 141871 |
| 4.4 | 157101 |
| 4.8 | 166864 |

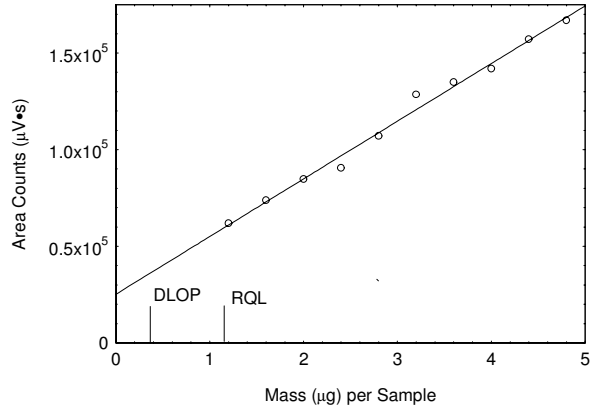


Figure 1.2.1 Plot of data to determine the DLOP/RQL for estradiol. ($Y = 29814X + 25280$)

Table 1.2.2
Detection Limit of the Overall Procedure
for Estriol

| mass per sample (μg) | area counts ($\mu\text{V}\cdot\text{s}$) |
|--------------------------------------|---|
| 1.2 | 48649 |
| 1.6 | 68686 |
| 2.0 | 89578 |
| 2.4 | 95632 |
| 2.8 | 102151 |
| 3.2 | 118298 |
| 3.6 | 125128 |
| 4.0 | 137529 |
| 4.4 | 149732 |
| 4.8 | 158645 |

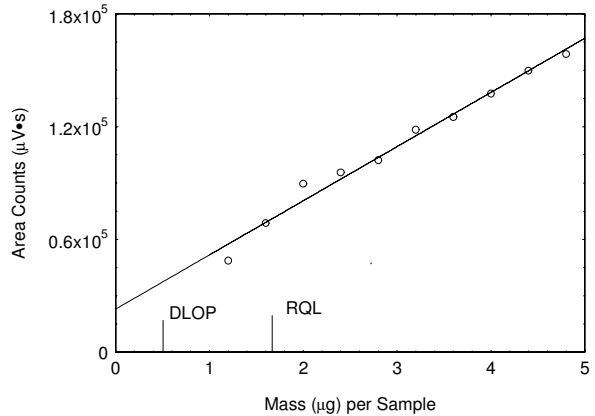


Figure 1.2.2 Plot of data to determine the DLOP/RQL for estriol. ($Y = 28813X + 22963$)

Table 1.2.3
Detection Limit of the Overall Procedure
for Estrone

| mass per sample (μg) | area counts ($\mu\text{V}\cdot\text{s}$) |
|--------------------------------------|---|
| 1.2 | 55600 |
| 1.6 | 64126 |
| 2.0 | 76329 |
| 2.4 | 87868 |
| 2.8 | 100103 |
| 3.2 | 107873 |
| 3.6 | 113854 |
| 4.0 | 122258 |
| 4.4 | 139880 |
| 4.8 | 149110 |

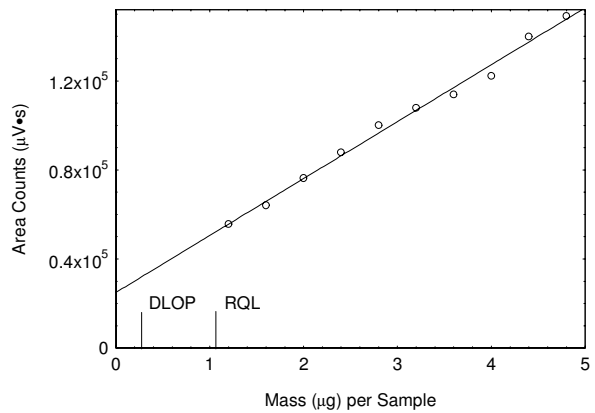


Figure 1.2.3 Plot of data to determine the DLOP/RQL for estrone. ($Y = 25564X + 25007$)

Table 1.2.4
Detection Limit of the Overall Procedure
for 17- α -Methyltestosterone

| mass per sample (μg) | area counts ($\mu\text{V}\cdot\text{s}$) |
|--------------------------------------|---|
| 0.4 | 101816 |
| 0.8 | 158834 |
| 1.2 | 237492 |
| 1.6 | 294083 |
| 2.0 | 367993 |
| 2.4 | 436341 |
| 2.8 | 499038 |
| 3.2 | 560057 |
| 3.6 | 624459 |
| 4.0 | 695956 |

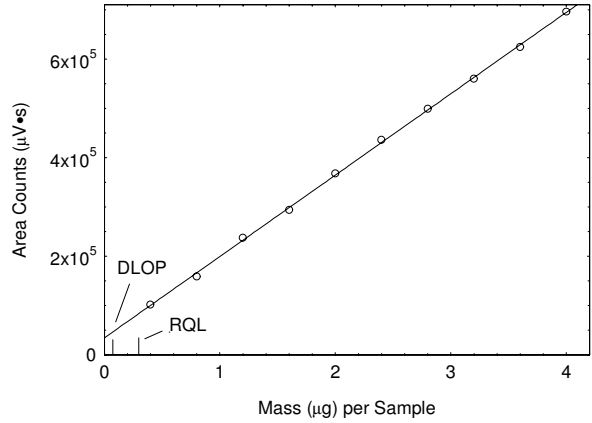


Figure 1.2.4 Plot of data to determine the DLOP/RQL for 17- α -methyltestosterone. ($Y = 165192X + 34184$)

Table 1.2.5
Detection Limit of the Overall Procedure
for Progesterone

| mass per sample (μg) | area counts ($\mu\text{V}\cdot\text{s}$) |
|--------------------------------------|---|
| 0.4 | 91229 |
| 0.8 | 138360 |
| 1.2 | 193359 |
| 1.6 | 249462 |
| 2.0 | 317502 |
| 2.4 | 340697 |
| 2.8 | 423923 |
| 3.2 | 455115 |
| 3.6 | 513319 |
| 4.0 | 575241 |

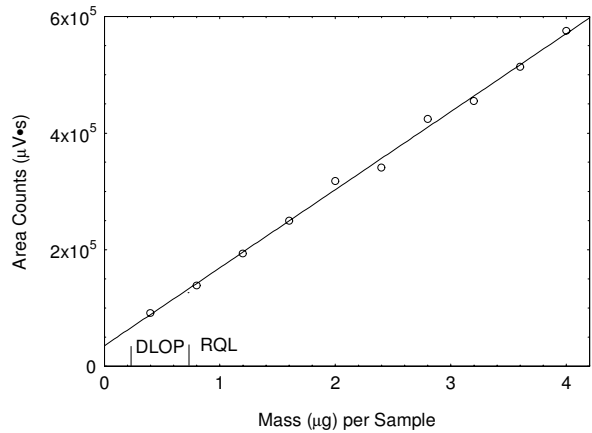


Figure 1.2.5 Plot of data to determine the DLOP/RQL for progesterone. ($Y = 133882X + 35281$)

Table 1.2.6
Detection Limit of the Overall Procedure
for Testosterone

| mass per sample (μg) | area counts ($\mu\text{V}\cdot\text{s}$) |
|--------------------------------------|---|
| 0.4 | 127538 |
| 0.8 | 195308 |
| 1.2 | 270978 |
| 1.6 | 353728 |
| 2.0 | 410892 |
| 2.4 | 494119 |
| 2.8 | 595561 |
| 3.2 | 655697 |
| 3.6 | 718464 |
| 4.0 | 823370 |

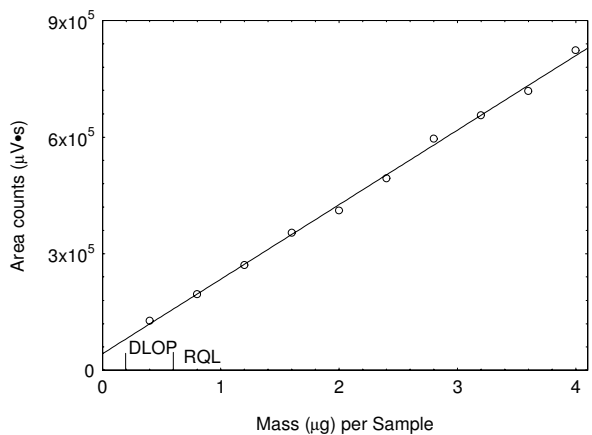


Figure 1.2.6 Plot of data to determine the DLOP/RQL for testosterone. ($Y = 191771X + 42669$)

Below are chromatograms of the RQL levels for each analyte.

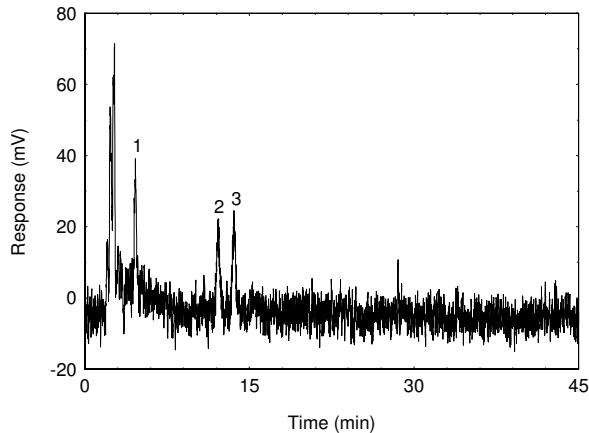


Figure 1.2.7 Chromatogram of the RQL of estradiol, estrone, and estriol at 280 nm. (1 = estriol; 2 = estrone; 3 = estradiol)

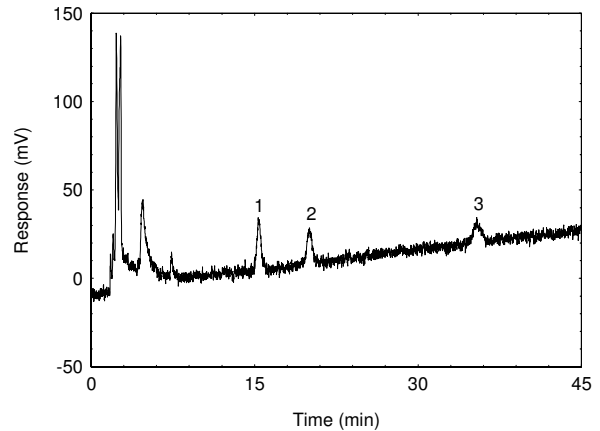


Figure 1.2.8 Chromatogram of the RQL of 17- α -methyltestosterone, progesterone, and testosterone at 240 nm. (1 = testosterone; 2 = 17- α -methyltestosterone; 3 = progesterone)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

- 2.1.1 Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within $\pm 5\%$ of the recommended flow rate.
- 2.1.2 Samples are collected on 37-mm diameter binderless glass fiber filters, type A/E. These are placed into three-piece cassettes and sampled open faced.

2.2 Reagents

None required

2.3 Technique

- 2.3.1 Immediately before sampling, remove the top piece and the end plug from the cassette.
- 2.3.2 Attach the cassette to the sampling pump so that it is in an approximately vertical position with the inlet facing up during sampling. Position the sampling pump, cassette and tubing so it does not impede work performance or safety.
- 2.3.3 Air being sampled should not pass through any hose or tubing before entering the cassette.
- 2.3.4 After sampling for the appropriate time, remove the sample, and replace the top piece and the end plug. Wrap each sample end-to-end with a Form OSHA-21 seal.
- 2.3.5 Submit at least one blank sample with each set of samples. Handle the blank sampler in the same manner as the other samples except draw no air through it.
- 2.3.6 Record sample volumes (in liters of air) for each sample, along with any potential interferences.
- 2.3.7 Ship any bulk samples separate from the air samples.

2.3.8 Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples in a refrigerator.

2.4 Extraction efficiency

The extraction efficiencies of estradiol, estriol, estrone, 17- α -methyltestosterone, progesterone, and testosterone were determined by liquid-spiking glass fiber filters with the analyte at 0.1 to 2 times the target concentration. These samples were stored overnight at ambient temperature and then extracted and analyzed. The mean extraction efficiency over the studied range was 100.1% for estradiol, 99.8% for estriol, 100% for estrone, 99.9% for 17- α -methyltestosterone, 99.9% for progesterone, and 100.2% for testosterone. The wet extraction efficiency was determined at 1 times the target concentration by liquid spiking the analyte onto glass fiber filters which had 240-L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) drawn through them immediately before spiking. The mean recovery for the wet samples was 99.7% for estradiol, 99.5% for estriol, 99.8% for estrone, 99.4% for 17- α -methyltestosterone, 99.6% for progesterone, and 99.8% for testosterone.

Table 2.4.1
Extraction Efficiency (%) of Estradiol

| <u>level</u> | | <u>sample number</u> | | | | | | mean |
|-----------------------|--------------------------|----------------------|-------|-------|-------|-------|-------|-------|
| \times target concn | μg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 2.66 | 98.5 | 101.1 | 100.2 | 101.0 | 98.0 | 98.6 | 99.6 |
| 0.5 | 13.3 | 99.6 | 101.2 | 100.1 | 101.0 | 101.1 | 100.1 | 100.5 |
| 1.0 | 26.6 | 99.2 | 99.6 | 99.9 | 101.3 | 101.0 | 101.1 | 100.4 |
| 2.0 | 53.2 | 99.4 | 99.0 | 98.7 | 101.1 | 101.3 | 100.2 | 100.0 |
| 1.0 (wet) | 26.6 | 99.6 | 99.5 | 99.1 | 100.2 | 99.9 | 99.8 | 99.7 |

Table 2.4.2
Extraction Efficiency (%) of Estriol

| <u>level</u> | | <u>sample number</u> | | | | | | mean |
|-----------------------|--------------------------|----------------------|-------|-------|-------|-------|-------|-------|
| \times target concn | μg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 2.48 | 101.1 | 99.8 | 95.8 | 101.2 | 98.2 | 100.0 | 99.4 |
| 0.5 | 12.4 | 98.4 | 101.3 | 100.2 | 99.5 | 99.0 | 99.4 | 99.6 |
| 1.0 | 24.8 | 100.1 | 101.1 | 101.1 | 101.1 | 100.2 | 100.2 | 100.6 |
| 2.0 | 49.6 | 99.3 | 99.1 | 98.3 | 98.2 | 101.0 | 99.9 | 99.3 |
| 1.0 (wet) | 24.8 | 99.3 | 99.2 | 100.1 | 98.9 | 100.0 | 99.2 | 99.5 |

Table 2.4.3
Extraction Efficiency (%) of Estrone

| <u>level</u> | | <u>sample number</u> | | | | | | mean |
|-----------------------|--------------------------|----------------------|-------|-------|-------|-------|-------|-------|
| \times target concn | μg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 2.64 | 98.6 | 100.1 | 98.9 | 101.1 | 101.0 | 101.1 | 100.1 |
| 0.5 | 13.2 | 101.1 | 100.3 | 99.5 | 100.2 | 100.1 | 98.9 | 100.0 |
| 1.0 | 26.4 | 99.7 | 100.0 | 100.1 | 99.9 | 100.3 | 101.0 | 100.2 |
| 2.0 | 52.8 | 99.7 | 99.0 | 99.1 | 99.1 | 101.3 | 100.3 | 99.8 |
| 1.0 (wet) | 26.4 | 99.6 | 99.7 | 100.2 | 99.3 | 99.9 | 99.8 | 99.8 |

Table 2.4.4
Extraction Efficiency (%) of 17- α -Methyltestosterone

| level | | sample number | | | | | | mean |
|-----------------------|--------------------------|---------------|-------|-------|-------|-------|-------|-------|
| \times target concn | μg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 2.5 | 100.5 | 98.9 | 99.5 | 101.4 | 99.9 | 99.4 | 99.9 |
| 0.5 | 12.5 | 100.7 | 99.7 | 99.4 | 100.3 | 100.2 | 100.2 | 100.1 |
| 1.0 | 25.0 | 99.8 | 99.5 | 99.3 | 99.1 | 99.0 | 99.0 | 99.3 |
| 2.0 | 50.0 | 100.3 | 100.5 | 100.8 | 100.3 | 100.1 | 100.1 | 100.4 |
| 1.0 (wet) | 25.0 | 99.9 | 100.1 | 99.2 | 99.5 | 99.3 | 98.6 | 99.4 |

Table 2.4.5
Extraction Efficiency (%) of Progesterone

| level | | sample number | | | | | | mean |
|-----------------------|--------------------------|---------------|-------|-------|-------|-------|-------|-------|
| \times target concn | μg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 2.6 | 98.1 | 100.2 | 101.1 | 101.1 | 100.0 | 100.2 | 100.1 |
| 0.5 | 13.0 | 99.8 | 99.8 | 99.6 | 100.2 | 99.9 | 100.1 | 99.9 |
| 1.0 | 26.0 | 100.2 | 99.9 | 100.2 | 100.1 | 99.9 | 99.7 | 100.0 |
| 2.0 | 52 | 99.7 | 99.3 | 99.3 | 99.4 | 99.4 | 99.9 | 99.5 |
| 1.0 (wet) | 26.0 | 99.6 | 99.2 | 99.5 | 99.8 | 99.9 | 99.7 | 99.6 |

Table 2.4.6
Extraction Efficiency (%) of Testosterone

| level | | sample number | | | | | | mean |
|-----------------------|--------------------------|---------------|-------|-------|-------|-------|-------|-------|
| \times target concn | μg per sample | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 | 2.32 | 98.3 | 101.0 | 101.2 | 100.2 | 100.0 | 100.1 | 100.1 |
| 0.5 | 11.6 | 100.1 | 100.3 | 100.2 | 100.1 | 100.1 | 100.2 | 100.2 |
| 1.0 | 23.2 | 101.3 | 100.1 | 100.0 | 100.3 | 100.4 | 100.0 | 100.4 |
| 2.0 | 46.4 | 99.9 | 101.2 | 99.7 | 99.2 | 99.8 | 100.3 | 100.0 |
| 1.0 (wet) | 23.2 | 99.6 | 99.9 | 99.4 | 99.8 | 99.5 | 100.4 | 99.8 |

2.5 Retention efficiency

Six glass fiber filters were spiked with one of the analytes, allowed to equilibrate for 6 h, and then were placed into a three-piece cassette with another glass fiber filter, and with a spacer in-between the filters. The amounts spiked on the filters were 53.2 μg (0.222 mg/m^3) estradiol, 49.6 μg (0.207 mg/m^3) estriol, 52.8 μg (0.22 mg/m^3) estrone, 50.0 μg (0.200 mg/m^3) 17- α -methyltestosterone, 52.0 μg (0.217 mg/m^3) progesterone, and 46.4 μg (0.193 mg/m^3) testosterone. The cassettes had 240 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 $^{\circ}\text{C}$) pulled through them at 1 L/min. The samples were extracted and analyzed. The mean retention efficiency was 99.0% estradiol, 99.6% estriol, 98.3% estrone, 99.5% 17- α -methyltestosterone, 99.4% progesterone, and 98.3% testosterone. There was 0% found on the backup glass fiber filter for all analytes.

Table 2.5.1
Retention Efficiency (%) of Estradiol

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|-------|-------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 96.7 | 98.5 | 98.7 | 99.1 | 100.1 | 101.0 | 99.0 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 96.7 | 98.5 | 98.7 | 99.1 | 100.1 | 101.0 | 99.0 |

Table 2.5.2
Retention Efficiency (%) of Estriol

| section | sample number | | | | | | mean |
|---------|---------------|-------|-------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 99.2 | 102.1 | 101.0 | 98.3 | 98.0 | 99.1 | 99.6 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 99.2 | 102.1 | 101.0 | 98.3 | 98.0 | 99.1 | 99.6 |

Table 2.5.3
Retention Efficiency (%) of Estrone

| section | sample number | | | | | | mean |
|---------|---------------|-------|-------|-------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 96.4 | 101.1 | 100.0 | 100.2 | 96.2 | 96.0 | 98.3 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 96.4 | 101.1 | 100.0 | 100.2 | 96.2 | 96.0 | 98.3 |

Table 2.5.4
Retention Efficiency (%) of 17- α -Methyltestosterone

| section | sample number | | | | | | mean |
|---------|---------------|-------|------|------|------|-------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 98.8 | 101.6 | 98.6 | 99.0 | 98.8 | 100.3 | 99.5 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 98.8 | 101.6 | 98.6 | 99.0 | 98.8 | 100.3 | 99.5 |

Table 2.5.5
Retention Efficiency (%) of Progesterone

| section | sample number | | | | | | mean |
|---------|---------------|------|-------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 99.7 | 99.3 | 101.1 | 99.8 | 98.4 | 98.0 | 99.4 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 99.7 | 99.3 | 101.1 | 99.8 | 98.4 | 98.0 | 99.4 |

Table 2.5.6
Retention Efficiency (%) of Testosterone

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 98.5 | 98.3 | 99.5 | 98.7 | 97.4 | 97.6 | 98.3 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 98.5 | 98.3 | 99.5 | 98.7 | 97.4 | 97.6 | 98.3 |

2.6 Sample storage

Nine glass fiber filters were each spiked with 26.6 µg (0.111 mg/m³) of estradiol, 24.8 µg (0.103 mg/m³) of estriol, 26.4 µg (0.110 mg/m³) of estrone, 25.0 µg (0.100 mg/m³) of 17- α -methyltestosterone, 26.0 µg (0.108 mg/m³) of progesterone, and 23.2 µg (0.0967 mg/m³) of testosterone. They were allowed to equilibrate for 4 h, then 240 L of air with an absolute humidity of 15.7 milligrams of water per liter of air (about 80% relative humidity at 22.2 °C) was drawn through them. They were sealed and stored at room temperature in a drawer. Three samples were analyzed immediately. Three more were analyzed after 7 days of storage and the remaining three after 14 days of storage. The amounts recovered, which are corrected for extraction efficiency, indicate good storage stability for the time period studied.

Table 2.6.1
Storage Test for Estradiol
(% Recovery)

| time (days) | sample number | | | mean |
|-------------|---------------|------|-------|------|
| | 1 | 2 | 3 | |
| 0 | 99.6 | 99.5 | 100.2 | 99.8 |
| 7 | 98.9 | 99.8 | 99.5 | 99.4 |
| 14 | 99.1 | 98.6 | 96.0 | 97.9 |

Table 2.6.2
Storage Test for Estriol
(% Recovery)

| time (days) | sample number | | | mean |
|-------------|---------------|-------|-------|-------|
| | 1 | 2 | 3 | |
| 0 | 99.3 | 100.1 | 99.2 | 99.5 |
| 7 | 100.1 | 100.2 | 100.1 | 100.1 |
| 14 | 99.1 | 99.2 | 96.2 | 98.2 |

Table 2.6.3
Storage Test for Estrone
(% Recovery)

| time (days) | sample number | | | mean |
|-------------|---------------|-------|------|------|
| | 1 | 2 | 3 | |
| 0 | 99.6 | 100.2 | 99.8 | 99.9 |
| 7 | 100.5 | 99.3 | 99.2 | 99.7 |
| 14 | 97.8 | 98.9 | 95.0 | 97.2 |

Table 2.6.4
Storage Test for 17- α -Methyltestosterone
(% Recovery)

| time (days) | sample number | | | mean |
|-------------|---------------|-------|------|------|
| | 1 | 2 | 3 | |
| 0 | 99.8 | 99.5 | 99.3 | 99.5 |
| 7 | 97.9 | 100.2 | 97.5 | 98.5 |
| 14 | 92.1 | 89.9 | 93.4 | 91.8 |

Table 2.6.5
Storage Test for Progesterone

| time (days) | sample number | | | mean |
|-------------|---------------|------|------|------|
| | 1 | 2 | 3 | |
| 0 | 99.6 | 99.8 | 99.7 | 99.7 |
| 7 | 99.9 | 99.4 | 99.1 | 99.5 |
| 14 | 96.6 | 96.9 | 93.2 | 95.6 |

Table 2.6.6
Storage Test for Testosterone

| time (days) | sample number | | | mean |
|-------------|---------------|-------|-------|-------|
| | 1 | 2 | 3 | |
| 0 | 99.6 | 99.8 | 100.4 | 99.9 |
| 7 | 100.4 | 101.1 | 99.8 | 100.4 |
| 14 | 94.4 | 93.4 | 91.2 | 93.0 |

2.7 Recommended air volume and sampling rate.

Based on the data collected in this evaluation, 240-L air samples should be collected at a sampling rate of 1.0 L/min.

2.8 Interferences (sampling)

2.8.1 It is not known if any compounds will severely interfere with the collection of estradiol, estriol, estrone, 17- α -methyltestosterone, progesterone, and testosterone on the glass fiber filter.

2.8.2 Suspected interferences should be reported to the laboratory with submitted samples.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

3.1.1 A liquid chromatograph equipped with an ultraviolet detector. A Waters 600E controller, 490E ultraviolet detector, and 717 autosampler was used in this evaluation.

3.1.2 An LC column capable of separating the analyte from any interferences. The column used in this study was a Bakerbond C18, 5- μ m particle, 25-cm long with 4.6-mm i.d.

3.1.3 An electronic integrator or some suitable method of measuring peak areas. A Waters Millennium³² data system was used in this evaluation.

3.1.4 Four milliliter glass vials with PTFE-lined caps.

3.1.5 A 100- μ L syringe or other convenient size for sample injection.

3.1.6 Pipets for dispensing the extracting solvent.

3.1.7 Volumetric flasks - 10-mL and other convenient sizes for preparing standards.

3.2 Reagents

3.2.1 Methyl alcohol, HPLC grade. Fisher Optima methyl alcohol, 99.9% min (lot 992075) was used in this evaluation.

3.2.2 Deionized water. A Millipore Milli-Q water purification system was used for this evaluation.

3.2.3 Phosphoric acid. JT Baker Bakeranalyzed phosphoric acid 85.9%, (lot D25821) was used in this evaluation.

3.2.4 β -Estradiol, reagent grade. Sigma β -estradiol, 99% min (lot 98H0953) was used in this evaluation.

3.2.5 Estriol, reagent grade. Sigma estriol, 99% min (lot 18H0385) was used in this evaluation.

3.2.6 Estrone, reagent grade. Acros estrone, 99%+ (lot A002906801) was used in this evaluation.

3.2.7 17- α -Methyl testosterone, reagent grade. Sigma 17- α -methyl testosterone, 99% min (lot 41H0140) was used in this evaluation.

3.2.8 Progesterone, reagent grade. Sigma progesterone, 99% min (lot 128H0456), was used in this evaluation.

3.2.9 Testosterone, reagent grade. Sigma testosterone, 99% min (lot 67H0276), was used in this evaluation.

3.2.10 The LC mobile phase was 65:35:0.2 methyl alcohol:water:H₃PO₄.

3.3 Standard preparation

3.3.1 At least two separate stock standards are prepared by diluting a known quantity of estradiol, estriol, estrone, 17- α -methyl testosterone, progesterone, and testosterone with methanol. The concentration of the stock standards were 266 and 330 $\mu\text{g/mL}$ estradiol, 248 and 460 $\mu\text{g/mL}$ estriol, 264 and 520 $\mu\text{g/mL}$ estrone, 250 and 840 $\mu\text{g/mL}$ 17- α -methyltestosterone, 260 and 324 $\mu\text{g/mL}$ progesterone, and 232 and 420 $\mu\text{g/mL}$ testosterone.

3.3.2 Dilutions of these stock standards were prepared to bracket sample concentrations. The range of the standards used in this study was approximately from 0.1 to 50 $\mu\text{g/mL}$ for each hormone.

3.4 Sample preparation

3.4.1 Sample cassettes are opened and the glass fiber filter is placed in a 4-mL vial.

3.4.2 The filter is extracted with 4 mL of methyl alcohol.

3.4.3 The vials are sealed immediately and extracted for 30 minutes using a laboratory shaker.

3.5 Analysis

3.5.1 Liquid chromatograph conditions.

Injection size: 20 μL
Column: Bakerbond C-18, 5- μm particle, 25-cm long with 4.6-mm i.d.
Mobile phase: 1 mL/min 65:35:0.2 methyl alcohol:water:H₃PO₄
Detector: UV at 240 and 280 nm

3.5.2 Peak areas are measured by an integrator or other suitable means.

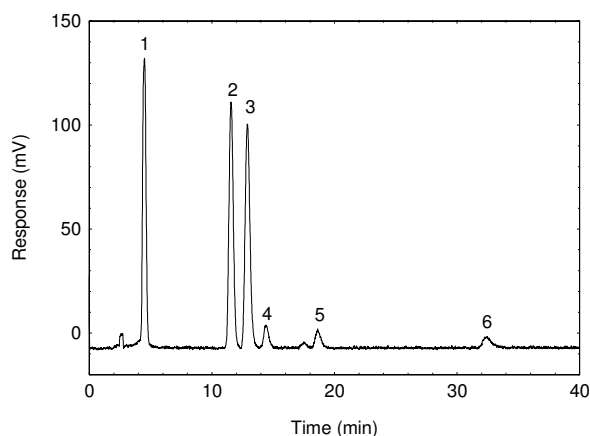


Figure 3.5.1 A chromatogram at 280 nm of 13.3 $\mu\text{g/mL}$ estradiol, 12.4 $\mu\text{g/mL}$ estriol, 13.2 $\mu\text{g/mL}$ estrone, 12.5 $\mu\text{g/mL}$ 17- α -methyl testosterone, 13.0 $\mu\text{g/mL}$ progesterone, and 11.6 $\mu\text{g/mL}$ testosterone. Key: (1) estriol, (2) estrone, (3) estradiol, (4) testosterone, (5) 17- α -methyltestosterone, (6) progesterone.

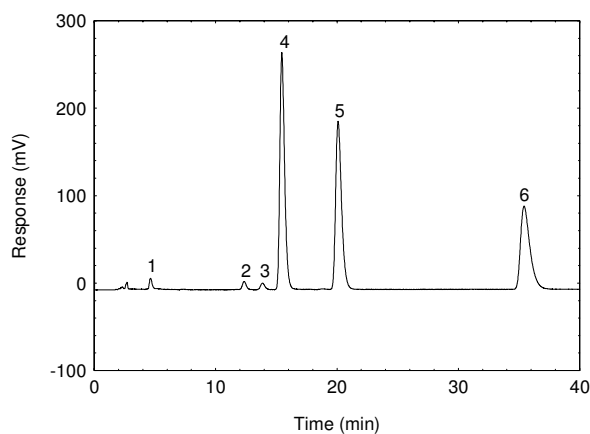


Figure 3.5.2 A chromatogram at 240 nm of 13.3 $\mu\text{g/mL}$ estradiol, 12.4 $\mu\text{g/mL}$ estriol, 13.2 $\mu\text{g/mL}$ estrone, 12.5 $\mu\text{g/mL}$ 17- α -methyl testosterone, 13.0 $\mu\text{g/mL}$ progesterone, and 11.6 $\mu\text{g/mL}$ testosterone. Key: (1) estriol, (2) estrone, (3) estradiol, (4) testosterone, (5) 17- α -methyltestosterone, (6) progesterone.

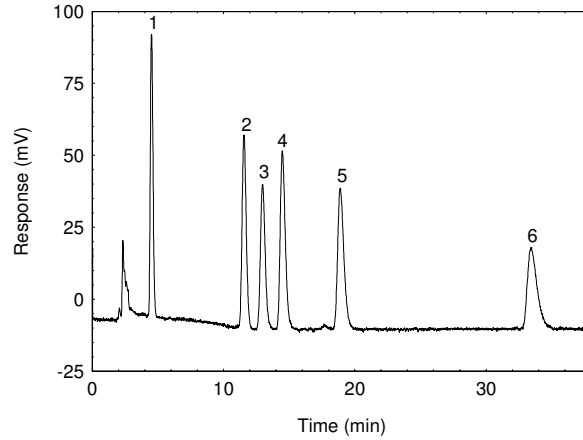


Figure 3.5.3 A chromatogram at 270 nm of 13.3 µg/mL estradiol, 12.4 µg/mL estriol, 13.2 µg/mL estrone, 12.5 µg/mL 17-α-methyltestosterone, 13.0 µg/mL progesterone, and 11.6 µg/mL testosterone in methanol. Key: (1) estriol, (2) estrone, (3) estradiol, (4) testosterone, (5) 17-α-methyltestosterone, and (6) progesterone.

3.5.3 An external standard calibration method is used. A calibration curve can be constructed by plotting the response of the injections versus micrograms of analyte per standard. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

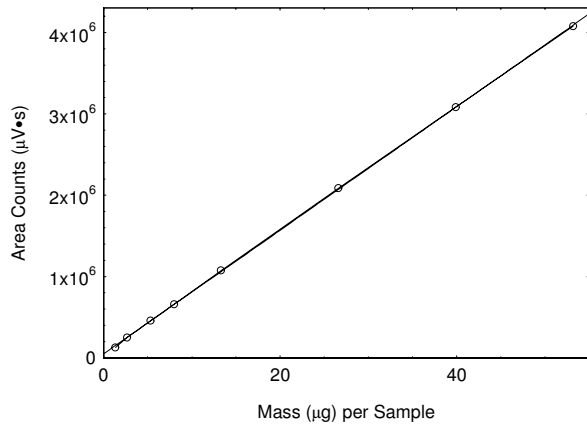


Figure 3.5.4 Calibration curve of estradiol. ($Y = 7.59 E4 x + 5.15 E4$.)

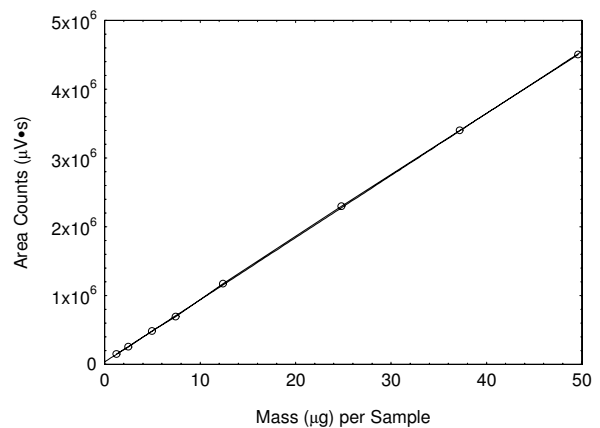


Figure 3.5.5 Calibration curve of estriol. ($Y = 9.03E4x + 3.53 E4$).

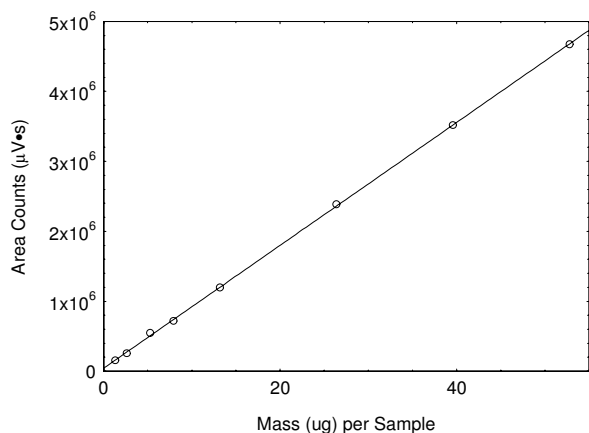


Figure 3.5.6 Calibration curve of estrone. ($Y = 8.78E4x + 4.35E4$).

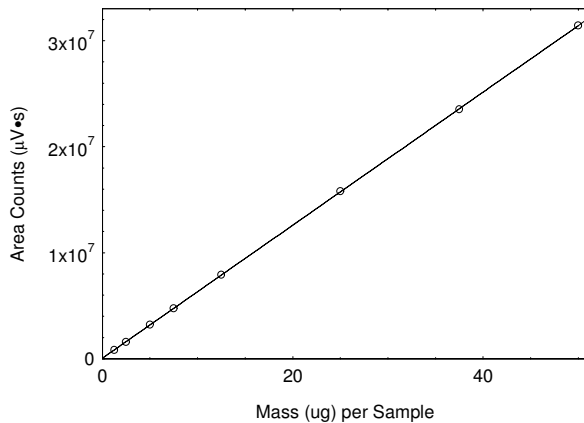


Figure 3.5.7 Calibration curve of 17- α -methyltestosterone. ($Y = 6.27E5x + 5.70E4$).

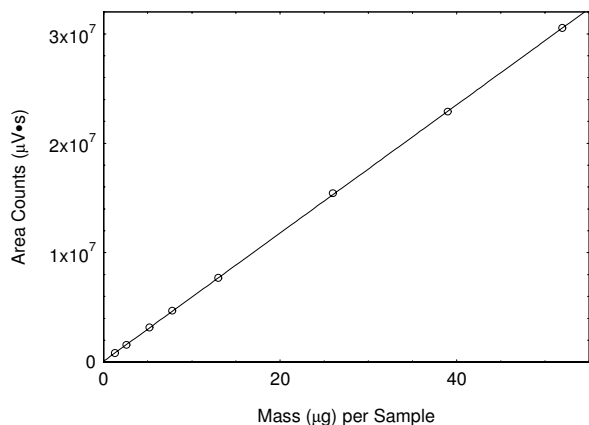


Figure 3.5.8 Calibration curve of progesterone. ($Y = 5.86E5x + 7.64E4$).

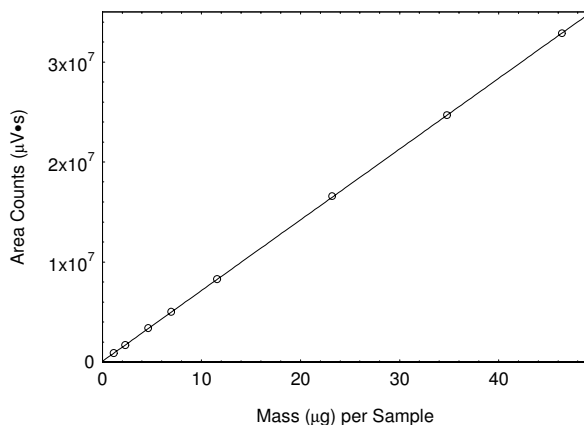


Figure 3.5.9 Calibration curve of testosterone. ($Y = 7.08E5x + 6.88E4$).

3.6 Interferences (analytical)

- 3.6.1 Any compound that produces a LC response and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.
- 3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by mass spectrometry or by another analytical procedure. The mass spectra in Figures 3.6.1 through 3.6.6 were from the NIST spectral library. The air and bulk samples received at SLTC were compared to this NIST library when they were confirmed. The instrument used was an HP 5973 Mass Selective Detector on an HP 6890 GC. The column used was a 30-meter 0.32 mm i.d. capillary column coated with 0.25- μ m film thickness of DB-5-MS. The temperature program was 150 $^{\circ}$ C for 2 min then increased at 10 $^{\circ}$ C/min to 290 $^{\circ}$ C and held for 20 min.

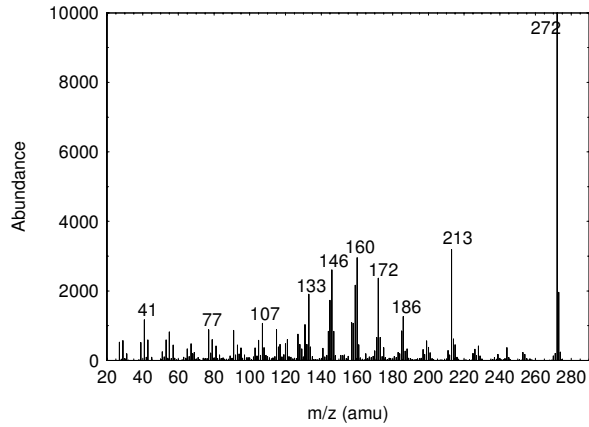


Figure 3.6.1 The mass spectrum of estradiol.

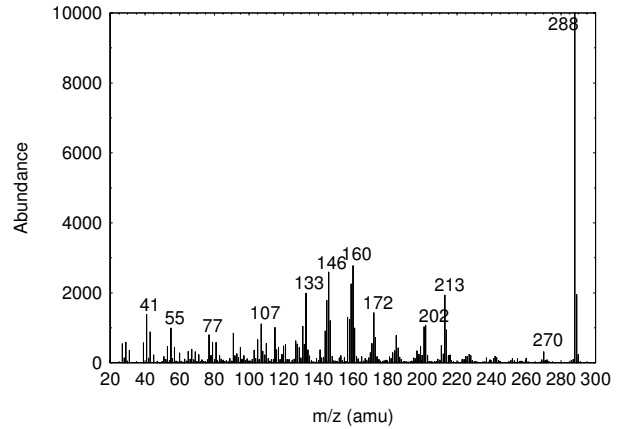


Figure 3.6.2 The mass spectrum of estriol.

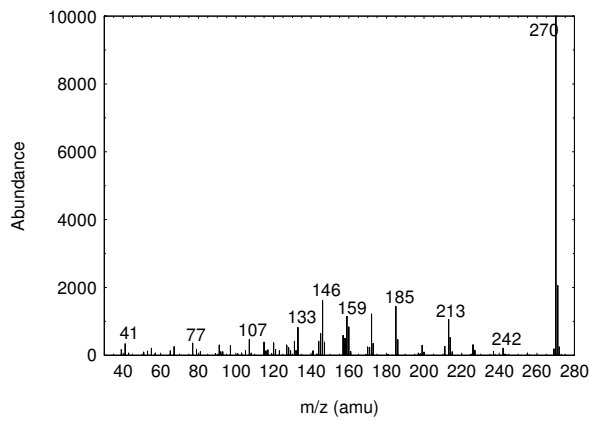


Figure 3.6.3 The mass spectrum of estrone.

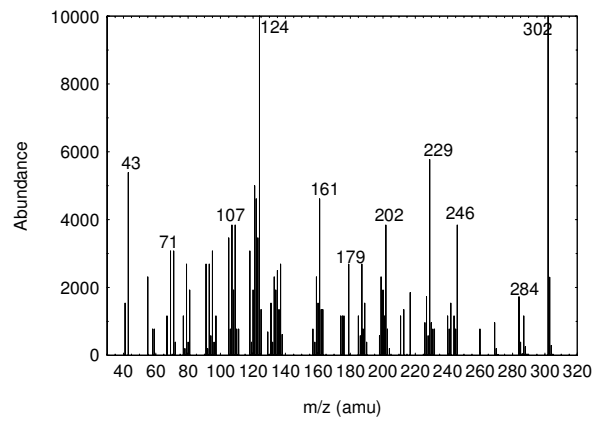


Figure 3.6.4 The mass spectrum of 17- α -methyltestosterone.

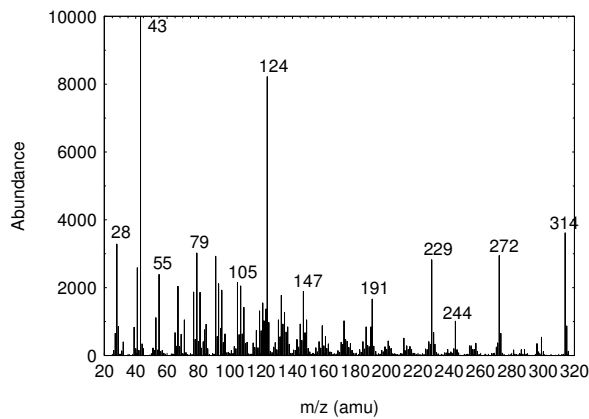


Figure 3.6.5 The mass spectrum of progesterone.

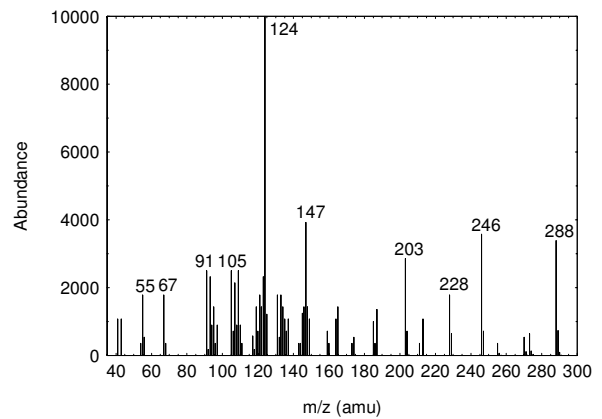


Figure 3.6.6 The mass spectrum of testosterone.

3.7 Calculations

The amount of each analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$C_M = \frac{M}{VE_E}$$

where C_M is concentration by weight (mg/m³)
 M is micrograms per sample (µg/mL corrected for blank × 4-mL extraction volume)
 V is liters of air sampled
 E_E is extraction efficiency, in decimal form

4. Recommendations for Further Study

Collection and reproducibility studies need to be performed to make this a validated method.

Appendix 1

Additional Evaluation Data (February 2001)

Samples were taken in the field following the sampling protocol for nuisance dust, 2 L/min for 960 liters. A retention study for the hormones was performed at this higher flow rate and air volume to see if there was any loss of sample.

Retention efficiency at 2 L/min and 960 liters

Six glass fiber filters were spiked with one of the analytes, allowed to equilibrate for 4 h, and then were placed into a three-piece cassette with another glass fiber filter, and with a spacer in-between the filters. The amounts spiked on the filters were 51.6 µg estradiol, 53.0 µg estriol, 55.5 µg estrone, 53.2 µg 17-α-methyltestosterone, 45.2 µg progesterone, and 52.2 µg testosterone. The cassettes had 960 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) pulled through them at 2 L/min. The samples were extracted with methanol and analyzed by LC using the same column and conditions used for the other tests in this method. The mean retention efficiency was 98.8% estradiol, 95.3% estriol, 97.5% estrone, 98.5% 17-α-methyltestosterone, 97.8% progesterone, and 96.4% testosterone. There was 0% found on the backup glass fiber filter for all analytes.

Table A1
Retention Efficiency (%) of Estradiol at 2 L/min and 960 liters

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 98.1 | 99.1 | 98.7 | 97.7 | 99.2 | 99.8 | 98.8 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 98.1 | 99.1 | 98.7 | 97.7 | 99.2 | 99.8 | 98.8 |

Table A2
Retention Efficiency (%) of Estriol at 2 L/min and 960 liters

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 94.1 | 94.4 | 95.9 | 95.8 | 95.9 | 95.7 | 95.3 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 94.1 | 94.4 | 95.9 | 95.8 | 95.9 | 95.7 | 95.3 |

Table A3
Retention Efficiency (%) of Estrone at 2 L/min and 960 liters

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 94.6 | 96.8 | 97.2 | 98.9 | 97.8 | 99.6 | 97.5 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 94.6 | 96.8 | 97.2 | 98.9 | 97.8 | 99.6 | 97.5 |

Table A4
Retention Efficiency (%) of 17- α -Methyltestosterone at 2 L/min and 960 liters

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 99.8 | 96.7 | 96.6 | 99.7 | 99.6 | 98.7 | 98.5 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 99.8 | 96.7 | 96.6 | 99.7 | 99.6 | 98.7 | 98.5 |

Table A5
Retention Efficiency (%) of Progesterone at 2 L/min and 960 liters

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 97.4 | 97.9 | 96.8 | 97.8 | 99.4 | 97.6 | 97.8 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 97.4 | 97.9 | 96.8 | 97.8 | 99.4 | 97.6 | 97.8 |

Table A6
Retention Efficiency (%) of Testosterone at 2 L/min and 960 liters

| section | sample number | | | | | | mean |
|---------|---------------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| front | 96.3 | 95.2 | 96.9 | 97.5 | 97.1 | 95.4 | 96.4 |
| rear | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 96.3 | 95.2 | 96.9 | 97.5 | 97.1 | 95.4 | 96.4 |