

## 5.1.28

### AOAC Official Method 980.36 Melengestrol Acetate (MGA) in Feed Supplements

Gas Chromatographic Method  
First Action 1980  
Final Action 1988

#### A. Principle

MGA is extracted from aqueous slurry of supplement with hexane, partitioned from hexane into aqueous methanol, and then from aqueous methanol into  $\text{CH}_2\text{Cl}_2$ . After evaporation of  $\text{CH}_2\text{Cl}_2$ , dried extract is transferred to alumina column with hexane, and eluted with  $\text{CHCl}_3$ -hexane solution. MGA in eluate is determined by GC. MGA can be assayed in cattle feed supplements containing 0.125 mg/lb to 1.00 mg MGA/lb supplement (0.28–2.2 mg/kg).

#### B. Apparatus

(a) *Extractor*.—Liquid-liquid (Ace Glass No. 6840-96 or equivalent).

(b) *Gas chromatograph*.—Tracor Model MT-220 (replacement Model 540, Tracor Instruments, Inc.) or equivalent, with  $^{63}\text{Ni}$  pulsed electron affinity detector, 0.6 m (2 ft) 3 mm id glass column packed with 1% OV-17 on 100–200 mesh Gas-Chrom Q (Alltech-Applied Science Laboratories, Inc.), and Tracor 1.0 mV recorder with chart speed of 0.5 in. (12.7 mm)/min. *Operating conditions*.—Linde 99.996% high purity nitrogen, or equivalent, carrier gas 50 mL/min; purge, off; temperatures—injector 235 C, detector 300 C, column 225 C; detector pulse height 60 V, pulse interval 300 ms, sensitivity  $80 \times 10^{-11}$  AFS.

(c) *Rotary-evaporator*.—Valley Electromagnetics (One Wolfer Park, Spring Valley, IL 61362, USA) or equivalent, **976.36B(m)** (see 23.1.07).

(d) *Chromatographic tube*.—18 500 mm, fitted with coarse porosity fritted glass disc and Teflon stopcock (Fischer and Porter Co., or equivalent).

#### C. Reagents

(a) *Aluminum oxide*.—Woelm acid, anionotropic, activity grade 1 for column chromatography (ICN Pharmaceuticals or equivalent).

(b) *Cholesteryl chloroacetate (CCA) internal standard solution*.—(1) *Stock solution*.—500 g/mL. Dissolve 125 mg (Aldrich Chemical Co.; C7680-9) in 250 mL absolute ethanol-hexane (5 + 95). (2) *Working solution I*.—50 g/mL. Pipet 10 mL stock solution and dilute to 100 mL with absolute ethanol-hexane. (3) *Working solution II*.—5 g/mL. Pipet 10 mL stock solution and dilute to 1 L with absolute ethanol-hexane (5 + 95).

(c) *Medroxyprogesterone acetate (MAP) extraction standard solution*.—(1) *Stock solution*.—2 mg/mL. Dissolve 200 mg (Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001 USA) in 100 mL absolute alcohol (requires overnight shaking). (2) *Working solutions*.—16, 20, 30 and 60 g/mL. Pipet 8, 10, 15 and 30 mL aliquots stock solution and dilute to 1 L with absolute alcohol for use with 0.125–0.250, 0.250–0.450, 0.450–0.750, 0.750–1.00 mg MGA/lb samples, respectively.

(d) *Melengestrol acetate (MGA)*.—(1) *Stock solution*.—25 g/mL. Dissolve 100 mg Reference Standard (Pharmacia & Upjohn Co., 100 Route 206 North, Peakpack, NJ 07977, USA, Tel: +1-888-768-5501) in 100 mL absolute alcohol. Dilute 5.0 mL to 200 mL with hexane. (2) *Working*

*solution*.—1.25 g/mL. Dilute 10 mL stock solution to 200 mL with absolute alcohol-hexane (5 + 95).

(e) *Gas chromatography reference solution*.—CCA, 5 g/mL; MAP, 2.4 g/mL; MGA, 0.125 g/mL. Pipet 10 mL each CCA (50 g/mL) and MGA (1.25 g/mL) and 4 mL MAP (60 g/mL) and dilute to 100 mL with hexane.

(f) *Solvents*.—Distilled-in-glass hexane and  $\text{CH}_2\text{Cl}_2$ , pesticide and gas chromatography grade (Burdick & Jackson Laboratories, or equivalent).

(g) *Solvent partition solution*.—Mix 0.25% aqueous  $\text{Na}_2\text{SO}_4$  solution with methanol (30 + 70).

#### D. Extraction

Grind dry test samples to pass 1 mm screen. Thoroughly mix test sample and place ca 15 g test portion, weighed to nearest 0.01 g, into extractor, and pipet 10 mL appropriate extraction standard solution, **C(e)**, for level MGA being assayed. Rinse test portion to bottom of extractor with 30 mL  $\text{H}_2\text{O}$ . Place magnetic stirring bar in extractor. Fill to side arm with hexane and insert solvent return tube in position. Attach extractor to condenser and 500 mL receiving flask containing 100 mL hexane and several SiC boiling chips. Extract test portion 3 h by heating receiving flask enough to have rapid reflux (20 mL/min) at condenser while stirring vigorously. It is imperative that reflux rate be fast enough to completely extract test portion in 3 h. 250 watt Glas-Col heating mantle operated at 120 V will do this. Control emulsions by regulating stirring rate. Test portion must be stirred vigorously and continuously. Up to 30 mL additional  $\text{H}_2\text{O}$  may be added through condenser to aid stirring action, if necessary. Let apparatus cool, set receiving flask aside, and transfer extractor contents to 1 or 2 L separator. Let phases separate clearly; discard aqueous (lower) layer and transfer hexane layer to 2 L round-bottom flask and roto-evaporate just to dryness. Dissolve residue in 100 mL solvent partition solution, **C(g)**.

#### E. Solvent Partition

Quantitatively transfer extract in 500 mL receiving flask to 500 mL separator. Rinse 2 L flask with 100 mL solvent partition solution, **C(g)**, into receiving flask and then quantitatively transfer receiving flask contents into same separator. Gently shake funnel by inverting 15 times, let solvent layers separate clearly, and drain lower layer into 1 L separator containing 200 mL  $\text{CH}_2\text{Cl}_2$ . Repeat rinsing flasks, transfers, and partitioning with 3 or more 100 mL portions solvent partition solution. Vigorously shake combined extracts in 1 L separator 15 s, let phases separate clearly, and drain lower ( $\text{CH}_2\text{Cl}_2$ ) layer into original 500 mL receiving flask. Repeat partition into  $\text{CH}_2\text{Cl}_2$  2 more times, using 40 mL  $\text{CH}_2\text{Cl}_2$  each time. Roto-evaporate combined extracts to near dryness. Remove residual  $\text{H}_2\text{O}$  with 2 separate additions of 10 mL absolute alcohol and roto-evaporate solvent. Remove residual alcohol with two 10 mL portions hexane, roto-evaporating each portion. Dissolve residue in 10 mL hexane.

#### F. Alumina Column Chromatography

Prepare column by slurring 20 g aluminum oxide in  $\text{CHCl}_3$  and transfer with  $\text{CHCl}_3$  wash bottle to tube, **B(d)**, containing ca 100 mL  $\text{CHCl}_3$ . While column drains, let alumina settle and add 5 g  $\text{Na}_2\text{SO}_4$  to column while letting  $\text{CHCl}_3$  slowly drain. Use ca 100–150 mL total  $\text{CHCl}_3$  in this step. In this and following steps, it is important to let each portion of solvent added drain to top of  $\text{Na}_2\text{SO}_4$  layer before adding next portion.  $\text{CHCl}_3$  must contain 0.5–1.0% alcohol. Rinse column with 10–15 mL hexane, followed by three 50 mL hexane

washes. While column drains, quantitatively transfer extract to column with four 10 mL portions and one 75 mL portion hexane. Discard all washings collected to this point. Elute MGA with five 50 mL portions CHCl<sub>3</sub>-hexane (33 + 67) solvent, using each portion to rinse flask containing extract; collect 250 mL eluate in 250 mL volumetric flask.

#### G. Determination

Thoroughly mix column eluate and pipet aliquot for analysis as follows: 0.125–0.250 mg MGA/lb (0.28–0.55 mg/kg), 40 mL; 0.250–0.450 mg MGA/lb (0.55–0.99 mg/kg), 30 mL; 0.450–0.750 mg MGA/lb (0.99–1.65 mg/kg), 20 mL; 0.750–1.00 mg MGA/lb (1.65–2.2 mg/kg), 10 mL, and evaporate to dryness in 50 mL glass-stoppered round-bottom flask, using warm (45–60 C) hot plate or water bath and stream of N<sub>2</sub> or air. When aliquot approaches dryness, add ca 5 mL absolute alcohol and evaporate to remove residual H<sub>2</sub>O. Repeat addition of alcohol if necessary until aliquot dries completely. Dissolve in 10.0 mL 5 g/mL CCA working solution II, **C(b)**(3). Inject 3 L each of test portion and standard into gas chromatograph operated as in **B(b)**. Order of elution, min: MAP, 4–6; MGA, 6–8; CCA, 8–11. Make several injections to achieve reproducible (<±5%) peak heights or areas depending on type and condition of gas chromatograph. It is imperative that GC response be linear. Check linearity using MGA concentrations 0.5 and 2.0 times GC reference solution, **C(e)**, concentration (0.0625, 0.125, and 0.25 g/mL). Keep CCA concentration constant.

#### H. Calculations

Calculate mg MGA/lb (mgMGA/kg) test sample:

$$\text{MGA/lb, mg} = R \quad R \quad 1.25 \quad (250/\text{mL aliquot}) \quad (0.4536/W)$$

$$\text{MGA, mg/kg} = \frac{R \quad R \quad 1.25 \quad (250/\text{mL aliquot})}{W}$$

where  $R$  and  $R$  = peak height ratios of MGA/CCA in test portion and CCA/MGA in standard, respectively; 1.25 = g MGA in 10 mL standard solution, **C(e)**; 250 = mL eluate collected; mL aliquot = 10–40 mL aliquot taken; 0.4536 = conversion factor to obtain mg/lb;  $W$  = g test sample extracted. MAP is added to test portion prior to extraction to indicate magnitude of extraction efficiency and cleanup losses. Reassay test portions showing <85% MAP recovery after reasons for low MAP recovery have been determined.

$$\begin{aligned} & \text{MAP recovered, \%} \\ & = R_1 \quad R_2 \quad 24 \quad (100/M) \quad (250/\text{mL aliquot}) \end{aligned}$$

where  $R_1$  and  $R_2$  = peak height ratios of MAP/CCA in test portion and CCA/MAP in standard, respectively; 24 = g MAP in 10 mL standard solution, **C(e)**;  $M$  = g MAP added to test portion; and (250/mL aliquot) are defined above.

Reference: *JAOAC* **63**, 425(1980).

CAS-2919-66-6 (melengestrol acetate)