

5.2.04

AOAC Official Method 975.61 Lasalocid in Feeds

Spectrofluorometric Method

First Action 1975

Final Action 1977

A. Principle

Lasalocid is extracted from pH 4.7 solution with ethyl acetate, fluorescent impurities are removed by acid and alkali treatments, and compound is determined fluorometrically, correcting for nonspecific fluorescence by complexing with H_3BO_3 . Monensin and ethoxyquin do not interfere.

B. Apparatus

Spectrofluorometer.—With 10 mm fused quartz cells. Excitation and emission wavelengths, ca 310 and 419 nm, respectively. Accurately determine peak excitation and emission wavelengths using standard solution I, following manufacturer's directions. *Do not change wavelength settings between readings.*

For routine setting and checking of instrument, use standard solution I.

Adjust settings to compensate for decreased intensity with age from dulled reflecting surfaces and lamp.

C. Reagents

(a) *Acetate buffer solution*.—pH 4.7. Dissolve 5.0 g $NaCH_3COO$ in ca 50 mL H_2O , adjust to pH 4.7 with CH_3COOH , and dilute to 100 mL with H_2O .

(b) *Ethyl acetate*.—Must have ca 0 fluorescence. If necessary, purify as follows: Elute 1 gal. (4 L) $CH_3COOC_2H_5$ through 9–10 cm (od) column packed with ca 100 cm silica gel (activated desiccant, 100–200 mesh, Grade H, W.R. Grace & Co., Davison Chemical Division, 10 E. Baltimore St, PO Box 2117, Baltimore, MD 21203, USA) topped with 5 cm layer of $NaHCO_3$. Redistil eluate from all-glass apparatus with 60 cm jacketed distilling column, discarding first and last 10%. To redistil ethyl acetate, add 40% aqueous NaOH and mix briefly. Follow with anhydrous Na_2SO_4 and shake again. (Ratio of ethyl acetate:40% NaOH: Na_2SO_4 is 1000:50:200 or multiple thereof.)

(c) *Methanolic boric acid solution*.—Dissolve 20.0 g H_3BO_3 in methanol and dilute to 500 mL with methanol. Prepare fresh daily.

(d) *Lasalocid standard solutions*.—(1) *Standard solution I*.—Dissolve 30.0 mg Lasalocid Reference Standard (available from Alpharma, Inc., One Executive Dr, Fort Lee, NJ 07024, Tel: +1-201-947-7774; www.alpharma.com) in ethyl acetate and dilute to 100 mL with ethyl acetate. Pipet 4 mL into 100 mL volumetric flask, dilute to volume with ethyl acetate, and mix. Pipet 25 mL final dilution into 50 mL glass-stoppered centrifuge tube containing 2.4 mL pH 4.7 buffer. Shake mechanically 25–30 min and centrifuge 10 min at 2000 rpm. Pipet 2 mL clear ethyl acetate extract into 100 mL volumetric flask, dilute to volume with ethyl acetate, and mix. (2) *Standard solution II*.—Pipet additional 2 mL clear ethyl acetate extract into another 100 mL volumetric flask containing 10 mL methanolic H_3BO_3 solution, dilute to volume with ethyl acetate, and mix.

D. Extraction

(a) *Feeds*.—Accurately weigh ca 4 g ground test portion into 50 mL glass-stoppered centrifuge tube containing 2.4 mL pH 4.7 buffer. Turn and shake tube by hand to wet uniformly. Immerse tube

4–5 min in 70 C water bath. Cool to room temperature and add 25 mL ethyl acetate by pipet. Stopper tube and shake briefly but vigorously by hand to disperse test portion. If necessary, break up lumps with narrow-tip spatula or glass rod. Stopper tube and shake mechanically 25–30 min. Centrifuge 10 min at 2000 rpm. Pipet 15 mL clear ethyl acetate extract into 50 mL centrifuge tube. Add 2 mL 1.5M HCl and shake 10 min. Centrifuge 10 min at 2000 rpm. Pipet 10 mL clear ethyl acetate extract into another 50 mL glass-stoppered centrifuge tube and add 0.5 mL 40% NaOH solution. Shake briefly by hand, add 2 g anhydrous Na_2SO_4 , and shake again. Centrifuge 10 min at 2000 rpm. If ethyl acetate solution is not clear or fine particles are present at surface, swirl tube gently by hand and recentrifuge. Pipet 2 mL ethyl acetate layer into 100 mL volumetric flask without disturbing aqueous alkaline solution, dilute to volume with ethyl acetate, and mix. Designate as Test solution I. Pipet another 2 mL aliquot ethyl acetate layer into second 100 mL volumetric flask containing 10 mL methanolic H_3BO_3 solution, dilute to volume with ethyl acetate, and mix. Designate as Test solution II.

Pipet 25 mL ethyl acetate into 50 mL glass-stoppered centrifuge tube containing 2.4 mL pH 4.7 buffer and proceed as above, beginning "Stopper tube and shake briefly". Designate final solutions as Reagent blank solution I and Reagent blank solution II.

(b) *Premixes*.—Accurately weigh 2.00 g 15% premix and transfer into 500 mL volumetric flask. Add exactly 250 mL ethyl acetate and shake 25 min on mechanical shaker. Centrifuge aliquot, and dilute with ethyl acetate as in (a) to obtain Test solution I and Test solution II (complex) containing ca 0.24 g lasalocid/mL ethyl acetate. Proceed with fluorescence measurements as in E. (*Note*: Omit treatment with pH 4.7 buffer for both premix and standard.)

E. Determination

Set excitation and emission wavelengths of apparatus at maximum. Adjust instrument with standard solution I, C(d)(1), in cell to microammeter reading of 0.400. Check this reference point before and after each reading, using same cell for all reference readings. Because of decomposition in UV, discard and replace standard solution I after every second reading. Measure fluorescence at 419 nm in order: Test solution I (U_1), Standard solution I (S_1), Reagent blank solution I (R_1), Test solution II (U_2), Standard solution II (S_2), and Reagent blank solution II (R_2). If reading of standard solution I drifts, adjust gain to initial setting. If drift is beyond 0.393–0.407, recheck readings of all solutions.

Although fluorescence response of standard is linear from 0.12 to 0.48 g/mL, concentration of lasalocid in test solution should be $\pm 25\%$ of that of standard solution.

F. Calculations

(a) Mg/kg lasalocid Na in feed =

$$\frac{[(U_1 \ R_1) \ (U_2 \ R_2)] \ D \ S}{[(S_1 \ R_1) \ (S_2 \ R_2)] \ W \ 0.96}$$

where U , S_1 , S_2 , and R are defined in E; D = dilution factor (25/100/2 = 1250); S = concentration of lasalocid in Standard solution I (= 0.24 g/mL); W = g test portion; and 96 = percent recovery. When $S = 0.24$ g/mL, $W = 4.00$ g, and R_1 and $R_2 = 0$,

$$\text{Mg/kg lasalocid Na in feed, \%} = \frac{(U_1 - U_2) \cdot 78.1}{S_1 - S_2}$$

(b) Lasalocid Na in premix, \% =

$$\frac{[(U_1 - R_1) - (U_2 - R_2)] \cdot D \cdot S}{[(S_1 - R_1) - (S_2 - R_2)] \cdot W \cdot 10000}$$

where U , S_1 , S_2 , and R are defined in E; D = dilution factor (250 / 50 = 5); S = concentration of lasalocid in

Standard solution I (= 0.24 g/mL); W = g test portion. When S = 0.24 g/mL, W = 2.00 g, and R_1 and R_2 = 0,

$$\text{Mg/kg lasalocid Na in 15\% premix} = \frac{(U_1 - U_2) \cdot 150000}{S_1 - S_2}$$

Reference: *JAOAC* **58**, 507(1975).

CAS-25999-31-9 (lasalocid)