

5.2.01

AOAC Official Method 982.44 Bacitracin in Premix Feeds Liquid Chromatographic Method First Action 1982

A. Principle

Bacitracin is extracted from feed into acidified organic solvent system. Extract is centrifuged, and supernate is analyzed by ion-suppressed reversed-phase LC with photometric detection at 254 nm.

B. Reagents and Apparatus

(a) *Liquid chromatograph*.—Hewlett-Packard Model 1084-A (replaced by HP1050 or HP1090) equipped with UV photometric detector. Operating conditions: flow rate 2.0 mL/min; detector wavelength 254 nm; 20 L loop injection valve (Valco Instruments Co., Inc., PO Box 55603, Houston, TX 77255, USA; Nos. ECGW and SC 20 CW), or equivalent equipment; ambient temperature.

(b) *Chromatographic column*.—15 cm 4.6 mm id, containing 5 m Supelcosil LC-8 reverse phase packing. Use column for bacitracin analysis only.

(c) *Phosphate–EDTA buffer*.—pH 4.5. Dissolve 13.6 g KH_2PO_4 and 2.5 g EDTA in 1 L H_2O .

(d) *Phosphate buffer*.—pH 6.0. Dissolve 1.5 g K_2HPO_4 and 8.5 g KH_2PO_4 in 1 L H_2O .

(e) *Solvent systems*.—Measure volume indicated (Table 982.44) with graduated cylinder (except where noted otherwise) into 100 mL volumetric flask and dilute to volume with H_2O .

(f) *Mobile phase*.—Mix 59% (v/v) B solvent (Table 982.44) with 41% (v/v) A solvent. Mix, and adjust pH to 6.8 with NaOH. Slight adjustment to percent volume of B solvent may be required to obtain desired separation.

C. Preparation of Standard

(a) *Drying of standard*.—(Caution: Bacitracin is very hygroscopic. Dry standard day before use and store in desiccator over night.) Accurately weigh 130–140 mg bacitracin reference standard (Alpharma, Inc.) into tared (= A) 50 mL volumetric flask. Dry standard 3 h at 60 °C under vacuum at <5 mm pressure. Remove from oven and place in desiccator to cool. Reweigh (= B). Amount bacitracin standard = $B - A$.

(b) *Preparation of standard solution*.—(Note: Store standards under refrigeration if not analyzed within 3 h of preparation. Preferably, prepare standard, store in refrigerator >30 min before analysis, and remove from refrigerator just before analysis.) *Standard solution 1*.—Dissolve bacitracin standard in 50 mL volumetric flask with ca 20 mL extracting solvent (Table 982.44)

Table 982.44. Formulation of solvent mixtures

Solvent	A solvent	Volume % B solvent	Extracting solvent
CH_3CN	0	40	28
Methanol	0	12	28
Phosphate–EDTA buffer	20	20	0
Phosphate buffer ^a	0	0	3
Concd phosphoric acid ^a	0	0	1.2

^a Use volumetric pipet.

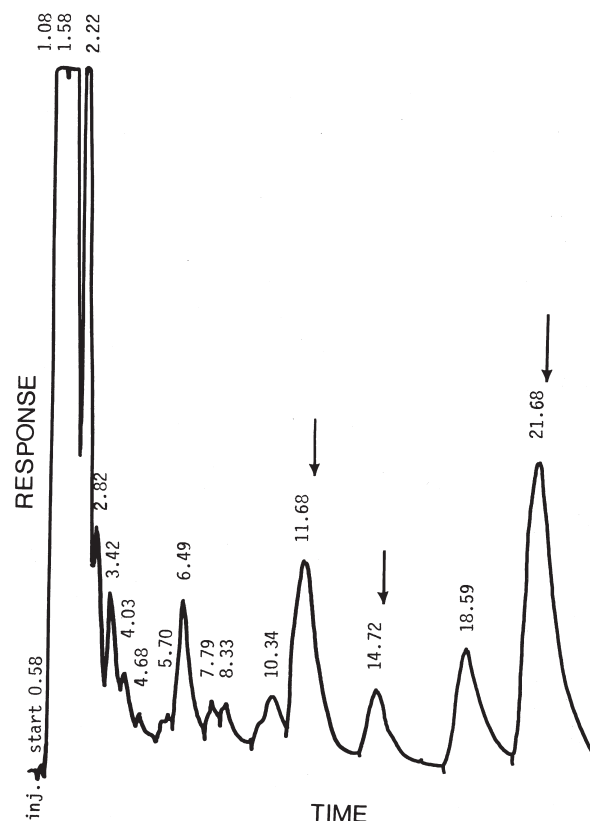


Figure 982.44. Liquid chromatogram of bacitracin active components (indicated by arrows).

and dilute to volume. Prepare following dilutions from this solution. *Standard solution 2*.—Pipet 20 mL standard solution 1 into 25 mL volumetric flask; dilute to volume with extracting solvent. *Standard solution 3*.—Pipet 15 mL standard solution 1 into 25 mL volumetric flask; dilute to volume with extracting solvent.

D. Extraction

Accurately weigh test portion of ground feed containing ca 6000 units bacitracin activity into 125 mL Erlenmeyer. Add 50 mL extracting solvent with volumetric pipet and extract with wrist-action shaking >5 min. Centrifuge 10 mL portion of extract 2–3 min at 2000–3000 rpm. Use clear supernate for assay. (Note: Store extracted test portion solution under refrigeration if not analyzed within 3 h. Preferably, prepare test portion solutions, store in refrigerator >30 min before analysis, and remove from refrigerator just before analysis.)

E. Determination

Inject clear supernate from centrifuged feed and standard solutions into chromatograph, starting with standard solution, then 2 test solutions, and then another standard solution, until all test and standards have been injected. Measure and total peak heights of the 3 active component peaks (Figure 982.44) for test (PH) and standard (PH) solutions.

Calculate response line for standards, using least squares linear fitting of following equation:

$$PH = m(P) + b$$

2006 AOAC INTERNATIONAL

where PH = peak heights of standard solutions 1, 2, and 3; P = potency of standard solution in units/50mL for standard solutions 1, 2 and 3; m , b = least squares determined slope and intercept.

Determine bacitracin content of feed from:

$$\text{Bacitracin / lb, g} = \frac{0.01080(PH - b)}{(m \text{ test portion weight})}$$

where $0.01080 = 453.6 \text{ (g/lb)}/42\,000 \text{ (units/g bacitracin)}$.

$$\text{Bacitracin / kg} = \frac{2.2 \cdot 0.01080 (PH - b)}{m \text{ test portion weight}}$$

Reference: *JAOAC* **65**, 1178(1982).

CAS-1405-87-4 (bacitracin)