



Test for lactate (Vol. 4) Passes test

#### PURITY

Sodium content Not less than 2.5% and not more than 5.0%  
See description under TESTS

Total lactic acid Not less than 15% and not more than 40%  
See description under TESTS

Acid value Not less than 60 and not more than 130  
See description under TESTS

Ester value Not less than 90 and not more than 190  
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg  
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

## TESTS

### PURITY TESTS

Sodium content Stock Lanthanum Solution:  
Transfer 5.86 g of lanthanum oxide,  $\text{La}_2\text{O}_3$ , to a 100 ml volumetric flask, wet with a few ml of water, slowly add 25 ml of hydrochloric acid, and swirl until the material is completely dissolved. Dilute to volume with water, and mix.

Stock Sodium Solution:  
Use a solution containing 1 mg of Na in each ml (1000 mg/l Na). The solution may be obtained commercially or be prepared as follows: transfer 1.271 g of sodium chloride, previously dried at  $105^\circ$  for 2 h and accurately weighed, to a 500 ml volumetric flask, dilute to volume with water, and mix.

Standard Sodium Solutions:  
Transfer 10.0 ml of the Stock Lanthanum Solution to each of three 100 ml volumetric flasks. Using a microlitre syringe, transfer 0.20 ml of the Stock Sodium Solution to the first flask, 0.40 ml to the second flask, and 0.50 ml to the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0 and 5.0  $\mu\text{g}$  of Na per ml, respectively. Prepare these solutions fresh daily.

Sample Preparation:  
Transfer about 250 mg of the sample, accurately weighed, to a 30 ml beaker, dissolve with heating in 10 ml of alcohol, and quantitatively transfer the solution into a 25 ml volumetric flask. Wash the beaker with two 5 ml portions of alcohol, adding the washings to the flask, dilute to volume with

alcohol, and mix. Transfer 2.5 ml of the Stock Lanthanum Solution to a second 25 ml volumetric flask. Using a microlitre syringe, transfer 0.25 ml of the alcoholic solution of the sample to the second flask, dilute to volume with water, and mix.

Procedure:

Concomitantly determine the absorbance of each Standard Sodium preparation and of the Sample preparation at 589 nm, with a suitable atomic absorption spectrophotometer, following the operating parameters as recommended by the manufacturer of the instrument. Plot the absorbance of the Standard Sodium preparations vs. concentration of Na, in  $\mu\text{g}$  per ml, and from the curve so obtained determine the concentration C, in  $\mu\text{g}$  per ml, of Na in the Sample preparation. Calculate the quantity, in mg, of Na in the sample taken by the formula  $2.5 C$ .

Total lactic acid

Standard curve:

Dissolve 1.067 g of lithium lactate in sufficient water to make 1000.0 ml. Transfer 10.0 ml of this solution into a 100 ml volumetric flask, dilute to volume with water, and mix. Transfer 1.0, 2.0, 4.0, 6.0 and 8.0 ml of the diluted standard solution into separate 100 ml volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6 and 8  $\mu\text{g}$  of lactic acid per ml, respectively. Transfer 1.0 ml of each solution into separate test tubes, and continue as directed in the Procedure, beginning with "Add one drop of cupric sulfate TS ..." After colour development and reading the absorbance values, construct a Standard curve by plotting absorbance versus  $\mu\text{g}$  of lactic acid.

Test preparation:

Transfer about 200 mg of the sample, accurately weighed, into a 125 ml Erlenmeyer flask, add 10 ml of 0.5N ethanolic potassium hydroxide and 10 ml of water, attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 ml of water, and heat on a steam bath until no odour of alcohol remains. Add 6 ml of dilute sulfuric acid (1 in 2), heat until the fatty acids are melted, then cool to about 600, and add 25 ml of petroleum ether. Swirl the mixture gently, and transfer quantitatively to a separatory funnel. Collect the water layer in a 100 ml volumetric flask, and wash the petroleum ether layer with two 20 ml portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 ml of this solution into a second 100 ml volumetric flask, dilute to volume with water, and mix.

Procedure:

Transfer 1.0 ml of the Test preparation into a test tube, and transfer 1.0 ml of water to a second test tube to serve as the blank. Treat each tube as follows: Add one drop of cupric sulfate TS, swirl gently, and then add rapidly from a burette 9.0 ml of sulfuric acid. Loosely stopper the tube, and heat in a water bath at 900 for exactly 5 min. Cool immediately to below 200 in an ice bath for 5 min, add 3 drops of p-phenylphenol TS (on the day of use dissolve 750 mg of p-phenylphenol in 50 ml sodium hydroxide TS), shake immediately and heat in a water bath at 300 for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90° for exactly 90 sec, and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1 cm cell,

at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in  $\mu\text{g}$ , of lactic acid in the portion of the Test preparation taken from the Procedure by means of the Standard curve.

Acid value

Transfer about 1 g, accurately weighed, to a 100 ml Erlenmeyer flask, add 25 ml of alcohol, previously neutralized to phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1N sodium hydroxide to the first pink colour that persists for at least 30 sec. Calculate the acid value by the formula  $56.1 \times V \times N/W$ , in which V is the volume, in ml, and N is the normality of the sodium hydroxide solution, and W is the weight, in grams, of the sample taken. Retain the neutralized solution for the determination of Ester value.

Ester value

To the neutralized solution retained in the test for acid value add 10.0 ml of alcoholic potassium hydroxide solution prepared by dissolving 11.2 g of potassium hydroxide in 250 ml of alcohol and diluting with 25 ml of water. Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 hours. Cool, add 5 additional drops of phenolphthalein TS and titrate the excess alkali with 0.1N hydrochloric acid. Perform a blank determination using 10.0 ml of the alcoholic potassium hydroxide solution. Calculate the ester value by the formula  $56.1 (B - S) \times N/W$ , in which B - S represents the difference between the volumes of 0.1N hydrochloric acid required for the blank and the sample, respectively, N is the normality of the hydrochloric acid, and W is the weight, in grams, of the sample taken.