CYANIDE IN TISSUE, PROCESSED MEATS, AND SERUM

Contents

DETERMINATIVE METHOD

A. INTRODUCTION

Theory

Cyanide ion reacts with p-Nitrobenzaldehyde to form an active reductant, a cyanohydrin, which reacts with o-dinitrobenzene to form a highly colored purple compound. The reaction is specific both for p-nitro or p-cyano benzaldehyde and the cyanide ion. Cyanide ion is regenerated in the second reaction and thus acts as a catalyst, increasing the sensitivity of the method. The detection limit for cyanide ion is approximately 0.5 ppm.

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B. EQUIPMENT

Apparatus

a. Culture tubes, borosilicate, 10 x 75 mm, Scientific Products, T-1285-2, or equivalent.

b. Centrifuge tube, screw-cap, 15 mL, Corning 25310, or equivalent.

c. Volumetric flask, 100 mL, Kimax 28013, or equivalent.

d. Centrifuge, Damon/IEC, clinical, or equivalent.

e. Tissuemizer, Tekmar model SDT, shaft number 10N, or equivalent.

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C. REAGENTS AND SOLUTIONS

Reagent and Solution List

a. p-Nitrobenzaldehyde, Sigma N-6126, or equivalent, 0.1M in methyl cellosolve (ethylene glycol monomethyl ether).

b. o-Dinitrobenzene, Sigma D-2638, or equivalent, 0.1M in methyl cellosolve (ethylene glycol monomethyl ether).

c. Methyl cellosovle (ethylene glycol monomethyl ether), Sigma E-5378, or equivalent.

CAUTION: Toxic, combustible, harmful if inhaled or absorbed through the skin.

d. Sodium hydroxide, reagent grade, 0.5M in H_2O, Sigma 925-30, or equivalent.

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D. STANDARDS

1. Source	Potassium cyanide, reagent grade, Fisher Scientific Company, P-226, or equivalent.
2. Preparation of Standards	a. Stock solution, 100 mg CN/100 mL. b. Reference solution A, 0.05 mg CN/100 mL. c. Reference solution B, 0.25 mg CN/100 mL.

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E. EXTRACTION PROCEDURE

1. Sample Extraction

a. Weigh 1.0 g of serum into a 15 mL screw-top disposable centrifuge tube. The sample should be weighed with minimum contact with laboratory equipment, especially metals, and disposable or *clean* labware is imperative to avoid extraneous color development. Add 5 mL 0.1M p-nitrobenzaldehyde.

NOTE: If serum cannot be separated from the tissue sample furnished or if processed meat is to be analyzed, homogenize 2.5 g of lean tissue or 3.0 g of processed meat with 5.0 mL H_2O and weigh 1.0 g of resulting fluid into the centrifuge tube, as for serum.

b. Shake tube for 2 min and add 5.0 mL 0.1M o-dinitrobenzene.

c. Shake tube for 2 min and and centrifuge for 5 min at approximately 3000 rpm.

d. Transfer 1.0 mL of supernatant liquid to a 10 x 75 mm culture tube using a disposable pipet, carefully avoiding any solid material. (If solid material cannot be avoided, filter supernatant using a fast paper such as Reeve Angel 802, or equivalent.)

e. Add 0.2 mL 0.5M NaOH. Mix by holding the tube with the thumb and index finger of one hand and flicking the tube with the index finger of the other hand.

f. Observe the tube for 1 min. Note any purple color. Place in the dark for 15 min and again examine for purple color.

g. If any color other than purple develops, such as brown or yellow brown, repeat the analysis using labware that is known to be clean.

h. If any other color develops, or other color develops in repeated analysis, submit the sample for confirmation by GC/MS.

2. Quality Control

Each time the analysis is performed, the following control samples should be analyzed:

a. Reagent blank (1.0 mL H_2O, 5 mL 0.1M p-nitrobenzaldehyde, 5 mL 0.1M o-dinitrobenzene, and 0.2 mL 0.5M NaOH).

b. Tissue blank (1.0 g of serum or slurry from blank sample, 5 mL 0.1M p-nitrobenzaldehyde, 5 mL 0.1M o-dinitrobenzene, and 0.2 mL 0.5M NaOH).

c. 0.5 ppm fortified standard (1.0 mL reference solution A, 1.0 g of serum or slurry from blank sample, 5.0 mL 0.1M p-nitrobenzaldehyde, 5.0 mL 0.1M o-dinitrobenzene, and 0.2 mL 0.5M NaOH).

d. 2.5 ppm fortified standard (1.0 mL reference solution B, 1.0 g of serum or slurry from blank sample, 5.0 mL 0.1M p-nitrobenzaldehyde, 5.0 mL 0.1M o-dinitrobenzene, and 0.2 mL 0.5M NaOH).

e. If no color develops in 2.c or 2.d, analyze a fortified reagent standard: 1.0 mL H_2O, 5.0 mL 0.1M p-nitrobenzaldehyde, 5.0 mL 0.1M o-dinitrobenzene, and 0.2 mL 0.5M NaOH).