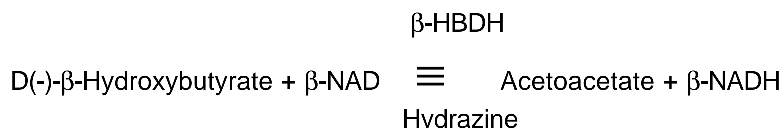
Determination of the Concentration and Molecular Weight of (D)-(-)-β-HYDROXYBUTYRIC ACID

PRINCIPLE:



Abbreviations used:
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
β-HBDB = β-Hydroxybutyrate Dehydrogenase
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 8.5, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 8.5 at 25EC
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.5 at 25°C with 1 M HCL.)

B. 1.0 M Hydrazine Hydrate Solution (Hydrazine)
(Prepare 20 ml by adding 1 ml of Hydrazine Hydrate, Sigma Prod. No. H-0883 to 5 ml of 1 M HCl. Mix and dilute to 20 ml with deionized water. The pH of this solution should be approximately 8.5 at 25°C.)

C. 15.1 mM β-Nicotinamide Adenine Dinucleotide Solution (β-NAD)
(Dissolve the contents of one 20 mg vial of β-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120 in the appropriate volume of deionized water.)

D. 0.4 mM D-(-)-β-Hydroxybutyric Acid Solution (HBA)
(Immediately before use, dissolve approximately 50 mg of D-(-)-β-Hydroxybutyric Acid, Sodium Salt, Sigma Prod. No. H-0265 in 100 ml of deionized water. Further dilute 10:1 with deionized water.)

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REAGENTS:

E. β-Hydroxybutyrate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 25-50 units/ml of β-Hyroxybutyrate Dehydrogenase, Sigma Prod. No. H-6126 in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

		Test	Blank
Reagent A (Buffer)		1.00	1.00
Reagent B (Hydrazine)		1.00	1.00
Reagent C (β-NAD)	0.10	0.10	
Reagent D (HBA)		1.00	------
Deionized Water		------	1.00

Mix by inversion and equilibrate to 25°C using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both the Test and Blank. Then add:

	Test	Blank
Reagent E (Enzyme Solution)	0.04	0.04

Immediately mix by inversion and monitor the increase in A_{340nm} until the reaction is complete (approx. 40-60 minutes). Record the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

$\Delta A = A_f - A_i$ $\quad$ A_i = Initial Absorbance
A_f = Final Absorbance

$$\mu\text{moles D(-) HBA/ml Reaction Mixture} = \frac{(\Delta A\text{Test} - \Delta A\text{Blank})(3.14)(df)}{(6.22)(1)}$$

D(-)HBA = D-(-)-β-Hydroxybutryic Acid
df = Dilution factor
3.14 = Total volume (in milliliters) of assay
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
1 = Volume (in milliliters) of D(-) HBA

CALCULATIONS: (continued)

$$\text{Apparent Molecular Weight} = \frac{\mu\text{g of D(-)HBA/ml Reaction Mixture}}{\mu\text{moles of D(-)HAB/ml Reaction Mixture}}$$

$$\text{\% Purity} = \frac{\text{Theoretical Molecular Weight}}{\text{Apparent Molecular Weight}}$$

Theoretical Molecular Weight = 126.1

FINAL ASSAY CONCENTRATIONS:

In a 3.14 ml reaction mix, the final concentrations are 32 mM Tris, 319 mM hydrazine hydrate, 0.48 mM β-nicotinamide adenine dinucleotide, oxidized form, 1-2 units of β-hydroxybutyrate dehydrogenase, and varying amounts of (D)-(-)-β-hydroxybutyric acid.

REFERENCE:

Williamson, D.H. and Mellanby, J. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U.) 2nd ed., Vol. IV, 1836-1839

NOTES:

1. β-Hydroxybutyrate Dehydrogenase Unit Definition: One unit will oxidize 1.0 μmole of D-β-hydroxybutyrate to acetoacetate per minute at pH 7.8 at 37°C.

2. This assay is based on the cited references.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.