

The development of a method for the detection of β -hydroxybutyrate in post-mortem samples

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Introduction

Alcohol

- Alcohol is the 5th most common cause of death in the UK
- 45,000 deaths attributed to alcohol per annum in the EU

Chronic alcoholics

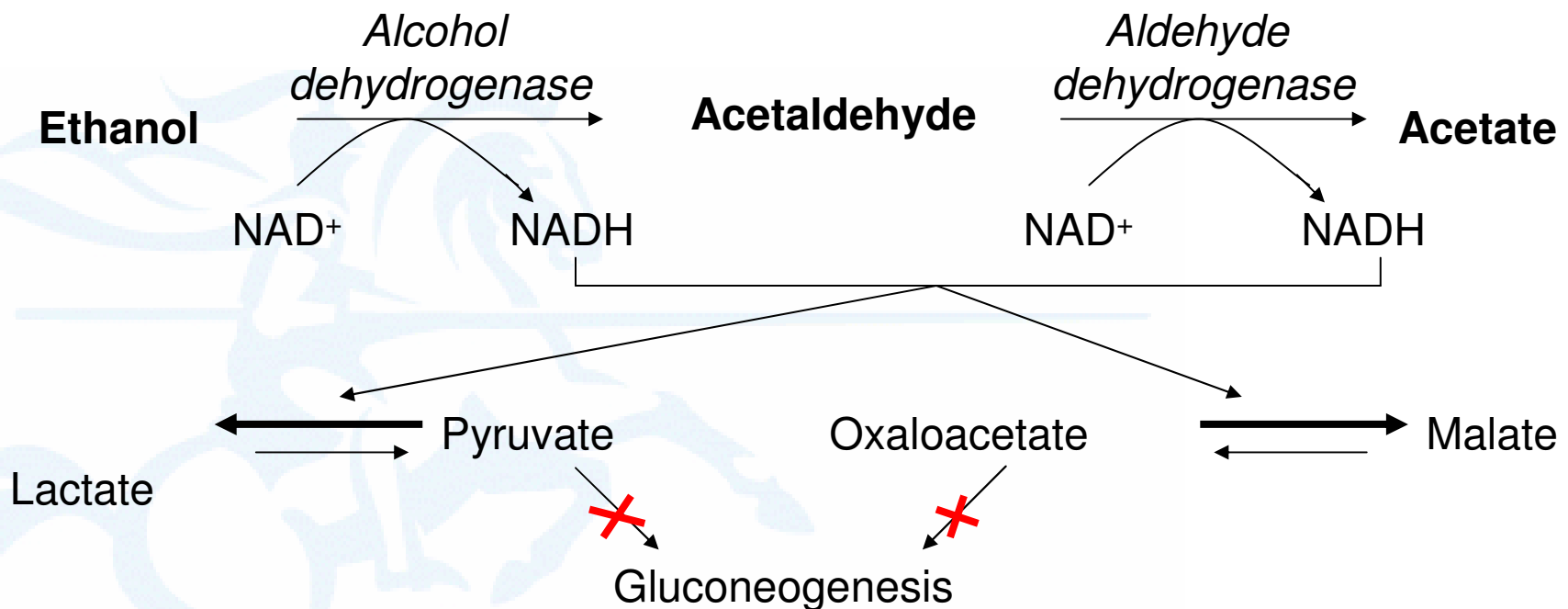
- Sudden and unexpected death
- Autopsy, histology, microbiology and toxicology don't determine cause
- Low or absent blood alcohol
- Fatty liver is frequently the only post-mortem finding
- Alcoholic ketoacidosis caused by circulating ketones may be the explanation and **BHB** a potential marker

Alcoholic ketoacidosis

Pathophysiology:

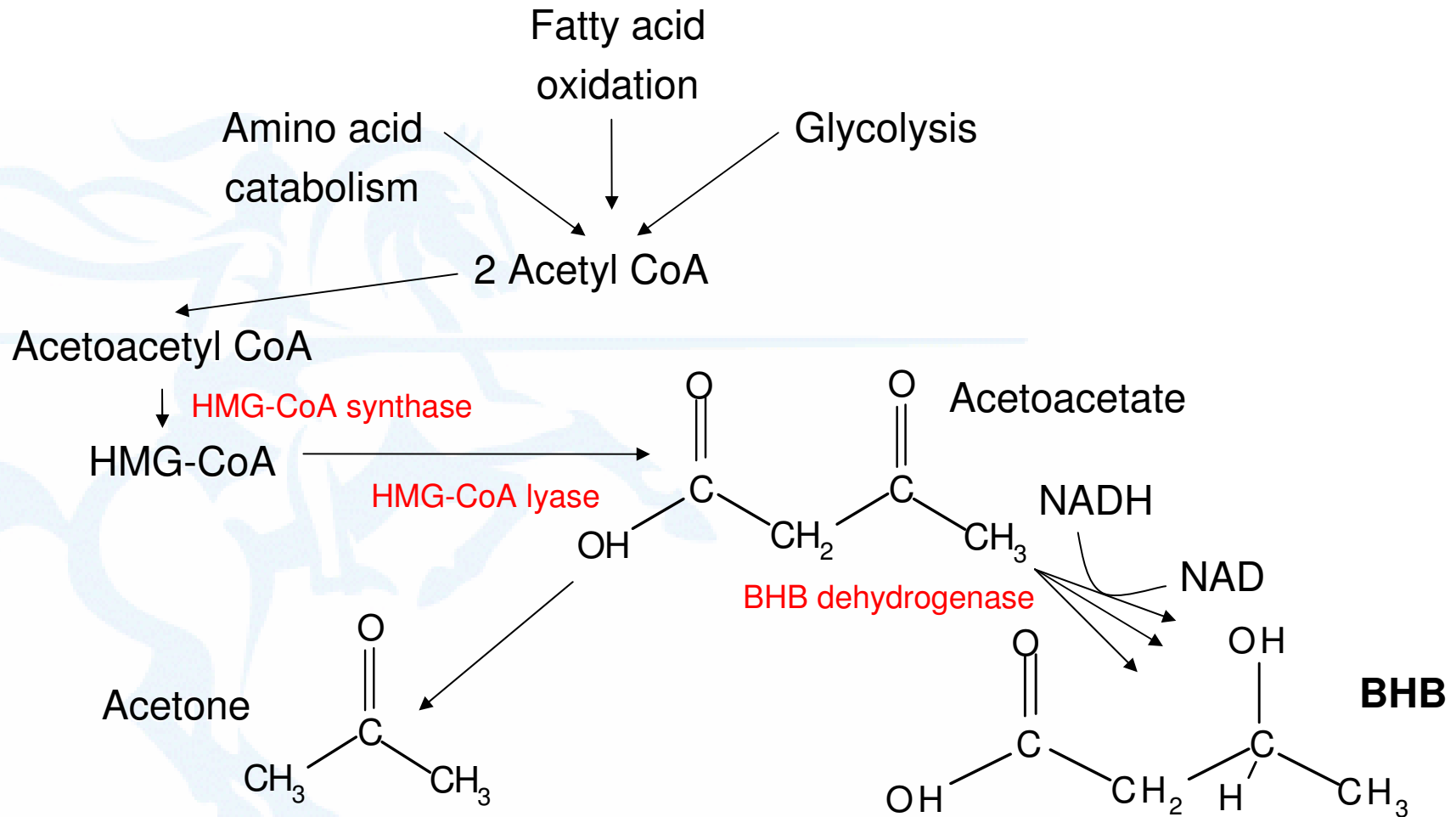
1. Starvation with glycogen store depletion
2. Elevation of NADH/NAD⁺ ratio secondary to alcohol metabolism
3. Depleted extracellular volume
4. The increase in cortisol, adrenaline, glucagon and growth hormone promotes free fatty acid release
5. Direct stimulation of lipolysis by alcohol
6. Decreased insulin production

Ketoacidosis and alcohol abuse



- This leads to increased dependence on fatty acids and amino acids for energy

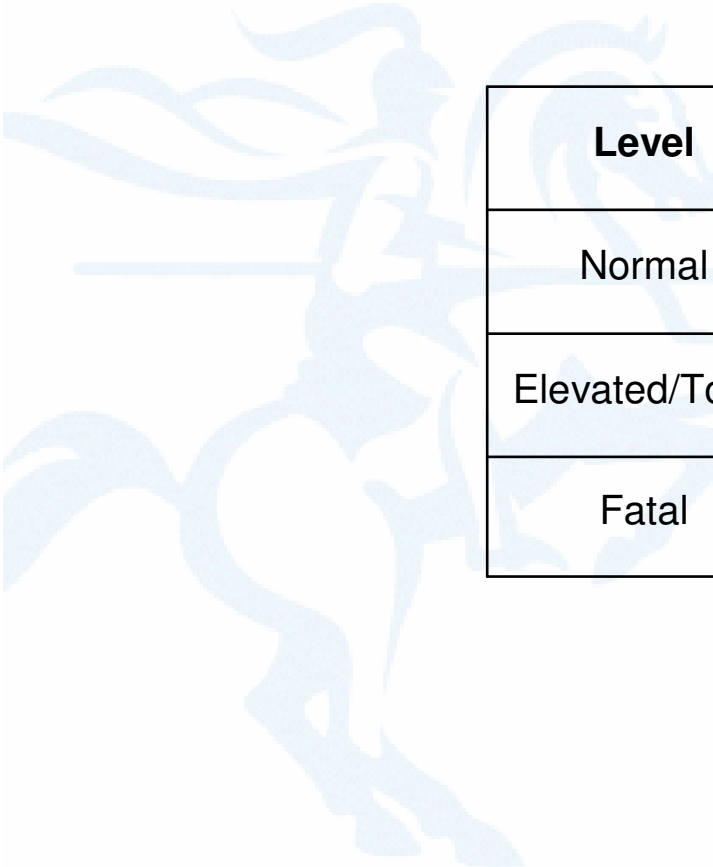
Production of ketone bodies



Rationale for Measurement

- Acetone is neutral and doesn't contribute to acidosis.
- Acetone production is spontaneous whereas production of BHB is NADH dependant.
- The extent of post mortem conversion of acetoacetate to acetone is not known.
- Both acetoacetate and acetone are unstable in blood.
- Decomposition can result in the production of other alcohols which can interfere with analysis of acetone.
- The ratio of BHB to acetoacetate is significantly higher in patients with AKA (6:1) than DKA (3:1).

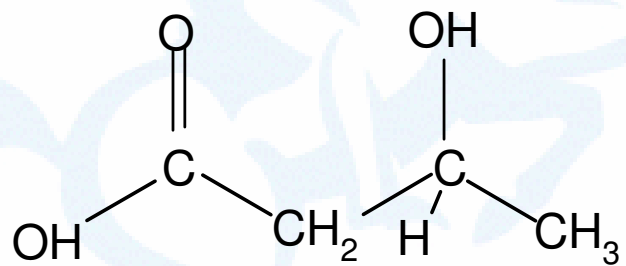
Normal, elevated and fatal ranges for β -hydroxybutyrate



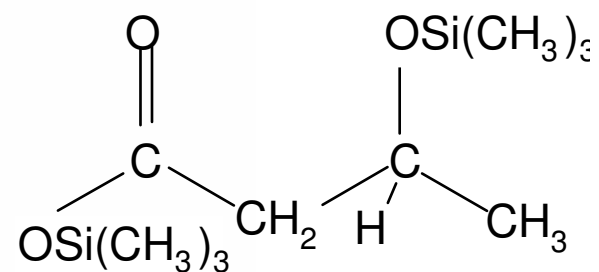
Level	Range (mg/L)	Range (μ M/L)
Normal	≤ 52	≤ 500
Elevated/Toxic	52 – 260	500 – 2500
Fatal	≥ 260	≥ 2500

Methods

- BHB is a small volatile compound; suitable for gas chromatographic analysis.
- BHB has two -OH groups, making it highly suitable for silyl derivatisation.



BHB

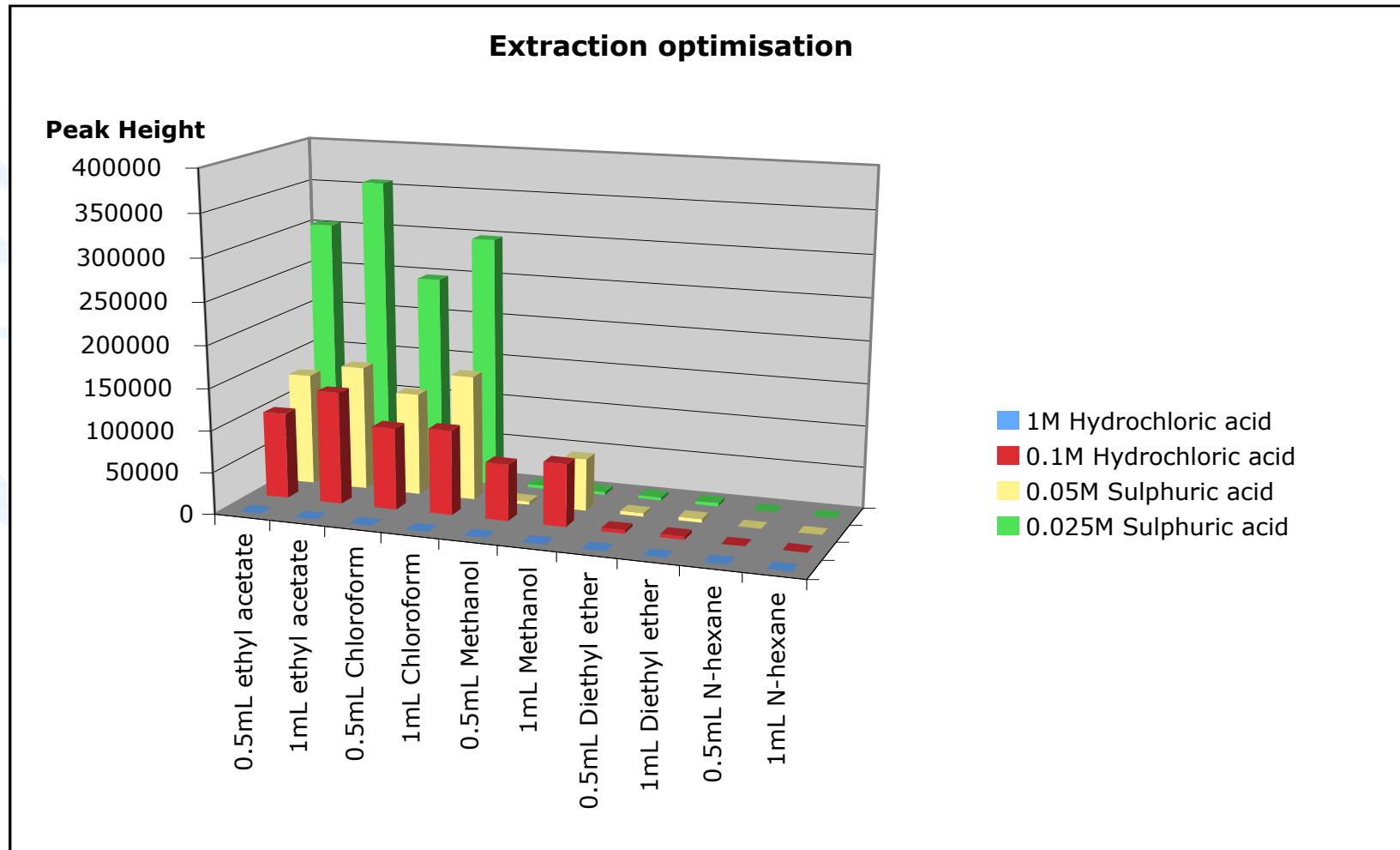


BHB tri-methyl-silyl derivative

Results: extraction development

- Protein precipitation ✗
- Salt displacement ✗
- Liquid/liquid extraction:
 - Strength of acid
 - Volume of acid
 - Solvent
 - Evaporation

Results: extraction development



Extraction details

Into a 2mL tube add:
100 μ L blank blood, 100 μ L BHB standard
100 μ L of internal standard, GHB-D6



Add 1mL ethyl acetate and
100 μ L of 0.025M sulphuric acid



Mix for 10 minutes and centrifuge for 5 minutes



Transfer solvent to a GC vial and evaporate to dryness on **LOW** heat



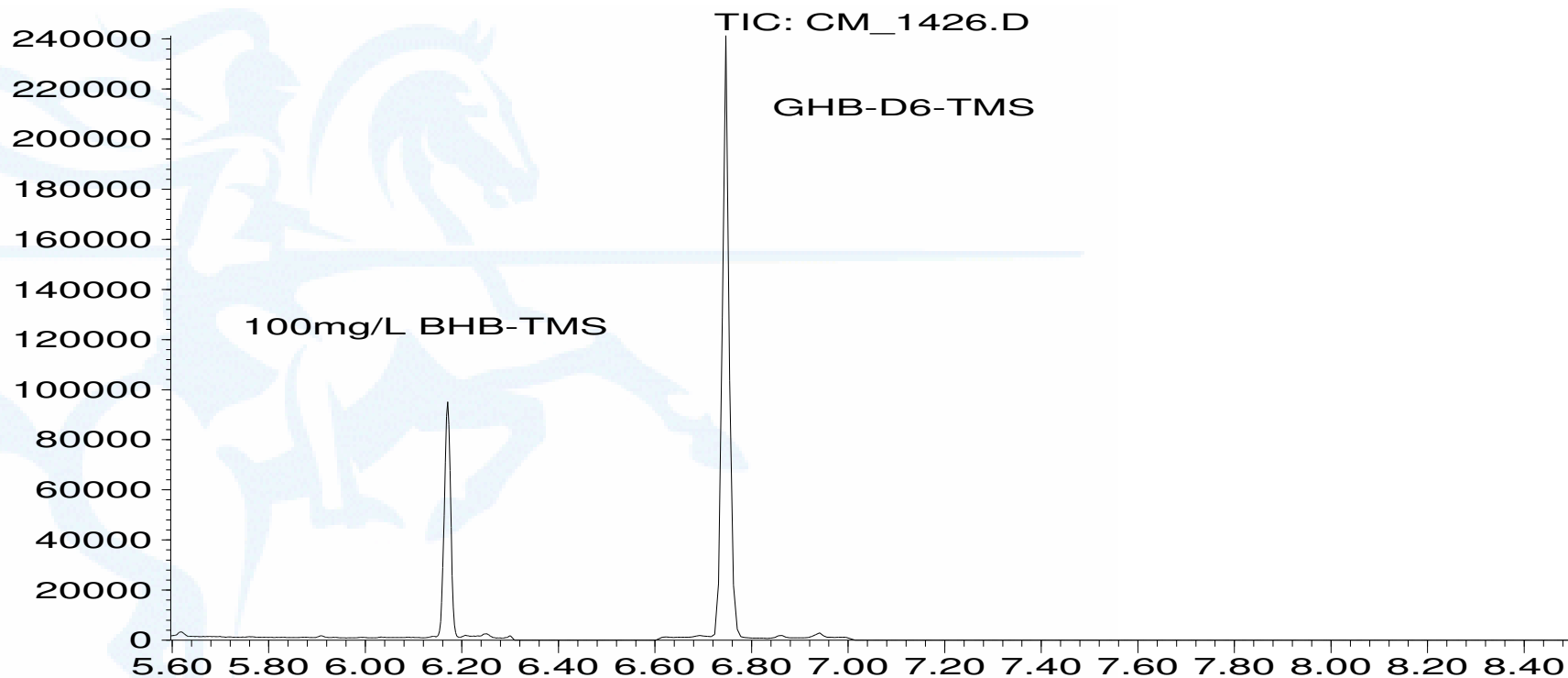
Reconstitute in 25 μ L derivatisation reagent and 75 μ L acetonitrile
Heat for 60 minutes and inject.

Analytical details

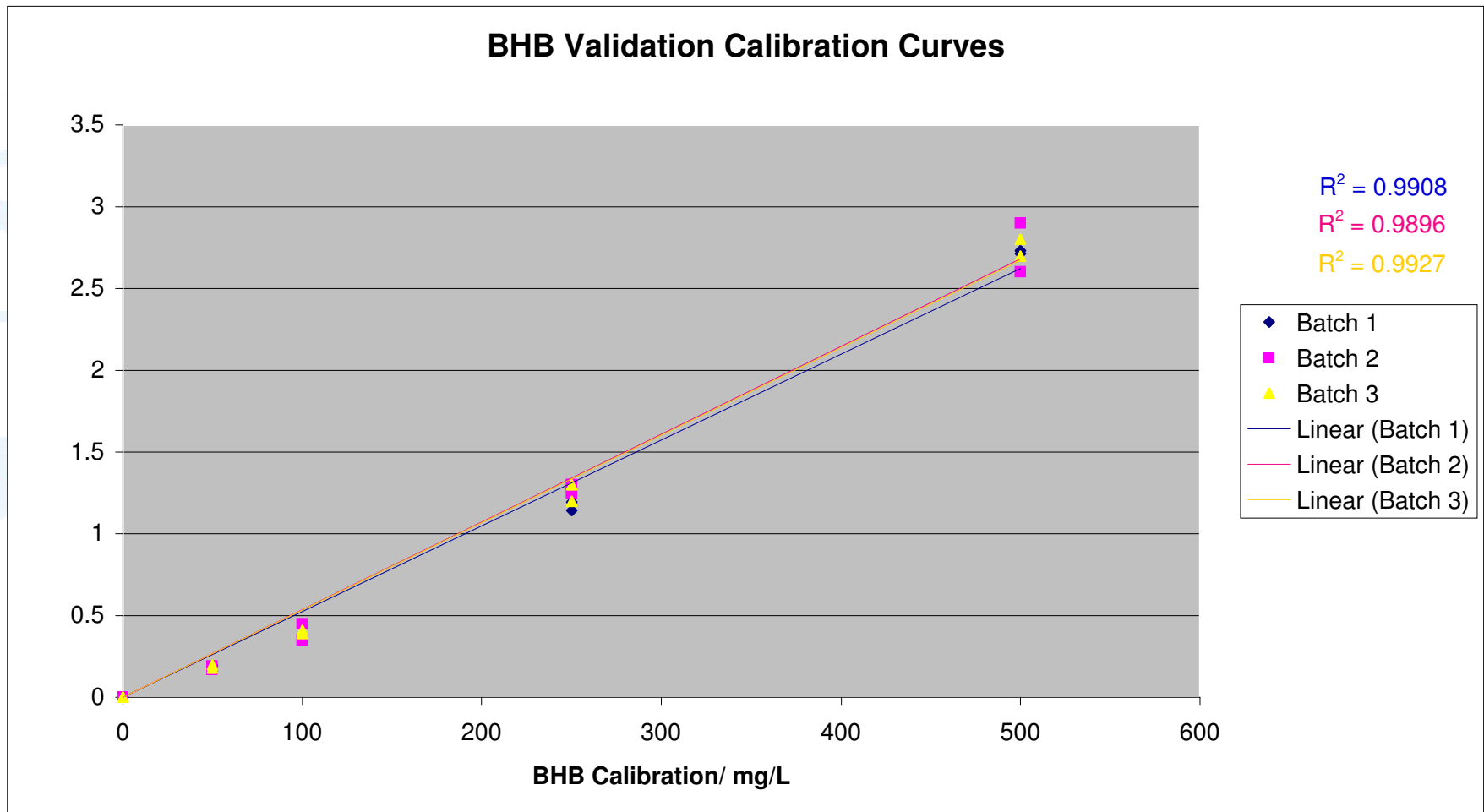
- Agilent GC-MS: HP 5890 GC coupled to a HP 5971 MS
- Carrier gas: Helium
- Electron impact ionisation
- Column: HP-5MS (30m x 0.25mm i.d., 0.25 μ m)
- Initial column temperature of 60°C, held for 2 minutes, ramped at 20°C/minute to 180°C and held for 1 minute
- 9 minute run time
- Ions monitored for BHB-TMS: **147**, 117, 191 and 91
- Ions monitored for GHB-D6-TMS: **239**, 73 and 240

Chromatograms

Abundance



Validation



Conclusions

- A method has been developed that allows detection and quantification of BHB in post-mortem samples.
- It is linear over the range needed: 50mg/L to 500mg/L (levels associated with fatality from literature).
- Due to its polarity BHB is highly water soluble. This resulted in a low recovery of 20%, but the assay is fit for purpose, with sufficient sensitivity.

