Interference from butanediols in a commercial ethylene glycol assay.

Northumbria Healthcare
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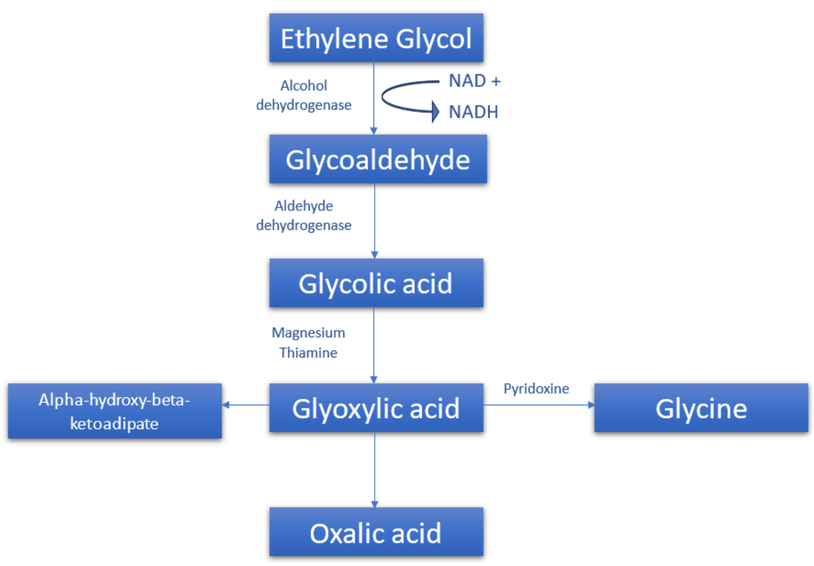
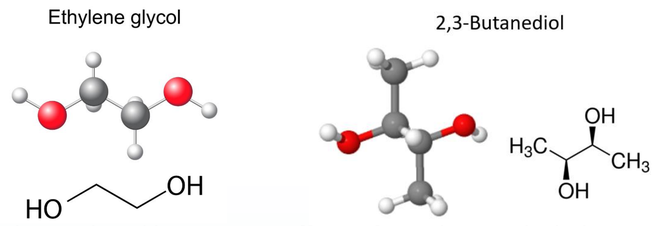
Introduction

Ethylene Glycol (1,2-ethandiol) (EG) is found most commonly in antifreeze, its ingestion may result in severe metabolic acidosis, CNS depression, cardiopulmonary compromise and renal insufficiency due to the production of glyoxylic acid and Oxalic acid during metabolism as shown below. Treatment must be started as soon as ingestion is suspected either with fomepizole or ethanol to block the conversion of EG to glycolaldehyde. The current standard assay for rapid testing of suspected EG poisoning uses the Catachem enzymatic method[5].



Following a patient case highlighting a discrepancy between EG measurements using the Catachem method, a Gas chromatography – mass spectrometer (GC-MS) glycol method at Northumbria Trust, and further testing on the same sample at City Assays, Black Country Pathology Services, Birmingham where it was measured using gas chromatography with flame ionisation detection (GC-FID), investigations were carried out.

Initial screen via the Catachem method found the sample positive for EG (>50mg/L) however EG was not detected in the sample using GC with either MS or FID detection at two different laboratories. Following a discussion with City Assays, 2,3-butanediol was suspected, this was confirmed via GC-MS at Northumbria where it was detected using the glycols assay.



2,3-butanediol is reported to be produced by persons suffering from chronic alcoholism and who may present with alcoholic ketoacidosis [1,2,3,4]. This may present with several symptoms in common with EG poisoning however the treatment varies significantly; with EG poisoning being administered fomepizole or ethanol urgently as an antidote, whereas alcoholic ketoacidosis cases may be administered saline and glucose.

This small study aimed to highlight the interference in the Catachem enzymatic ethylene glycol assay from 2,3-butanediol along with a range of other butanediol isomers.

Objectives

This small internal study at Northumbria NHS Trust was carried out to detect the cause of interference on the Catachem enzymatic assay to enable us to provide a service to detected potential false positive EG results in future case whilst still providing rapid results in line with the standards set out by National Poisons Information Service (NPIS) and the Association for Clinical Biochemistry (ACB)[7].

Method

Catachem enzymatic EG method (cat no. C504-0A, Catachem Inc.) via a Beckman Coulter© AU5812 clinical chemistry analyser. Based on the affinity of the Glycerol Dehydrogenase from bacteria to catalyse the oxidation-reduction reaction of ethylene glycol in the presence of NAD. This two-point Kinetic procedure is read at 340nm and the increase in absorbance is directly proportional to the concentration of EG in the sample.

$$\text{Ethylene Glycol} + \text{NAD} \xrightarrow{\text{Glycerol Dehydrogenase}} \text{NADH} + \text{Glycoaldehyde} + H^+$$

For GC-MS analysis; The samples were derivatised using benzoyl chloride in an alkaline medium. The assay uses the Schotten Baumann reaction in which an acyl hydride reacts with an alcohol to produce an ester. Samples are then analysed using the Agilent Technologies 7890B GC system held at 80C for 1 minute, increased to 230C at 30C per minute, then to 310C at 10C per minute and held at 310C for 2 minutes. Detection via Agilent Technologies 5977B MSD uses Selective Ion Monitoring (SIM) at 70, 77, 105 and 123 between 4.4 and 7 minutes; 77, 105, 162 and 227 between 7 and 10 minutes and 70, 77, 105 and 149 from 10 to 16 minutes.
Stock standards were made in house using standards obtained from Sigma-Aldrich (Ethylene glycol; cat no. 324558; Diethylene glycol; cat no 93171; Triethylene Glycol; cat no. 95126; 1,4-Butanediol; cat no. 240559; 2,3-butanediol; cat no. B84904; 1,2-butanediol; cat no. 18930) in blank pooled plasma.
Spiked samples were made using blank plasma at concentrations of 500mg/L, 50mg/L and 5mg/L for the butanediol isomers 1,4-butanediol, 2,3-butanediol and 1,2-butanediol and ran via both ethylene glycol methods for comparison.
All spiked samples were also run via the Beckman Coulter© AU5812 DRI® Ethyl Alcohol Assay to check for alcohol results >100mg/L to rule out any possible further interference. This Kinetic method is based on the high specificity of alcohol dehydrogenase (ADH) for ethyl alcohol. In the presence of ADH and NAD, ethyl alcohol is readily oxidized to acetaldehyde and NADH. This enzymatic reaction is monitored spectrophotometrically at 340nm.

$$\text{Ethyl Alcohol} + \text{NAD} \xleftrightarrow{\text{ADH}} \text{NADH} + \text{Acetaldehyde}$$

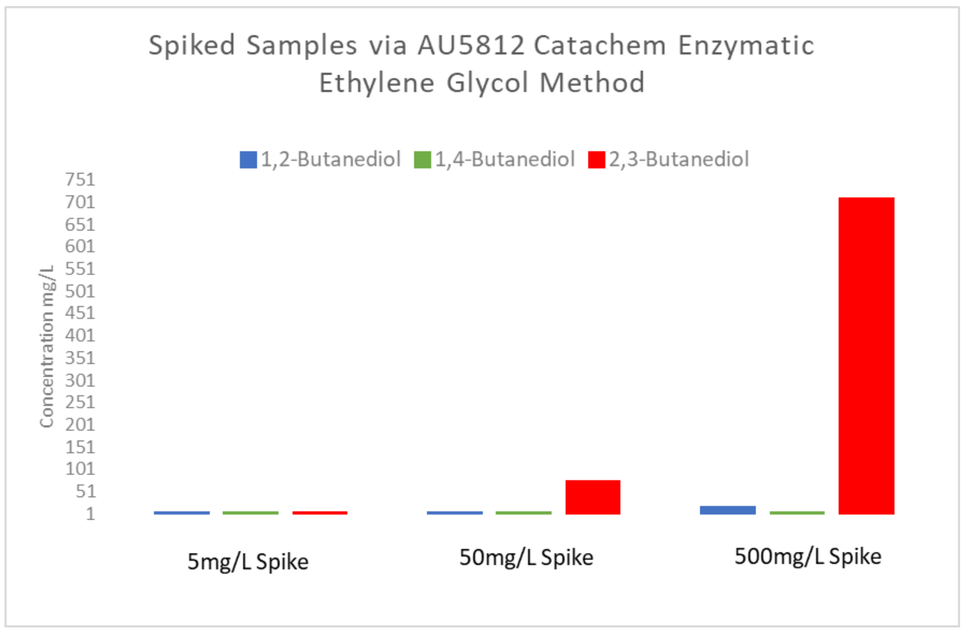
Results

All spiked samples were checked for alcohol as stated in the method. Results show all samples to be <100mg/L and therefore negative for alcohol (table 1).

Table 1: DRI Ethyl Alcohol results from spiked samples run via Beckman Coulter© AU5812.

	Average Alcohol (AU) of Pooled plasma mg/L		
Compound	**5 mg/L**	**50 mg/L**	**500 mg/L**
1,2-Butanediol	12.52	13.10	9.91
1,4-Butanediol	11.47	13.04	22.42
2,3-Butanediol	22.42	15.12	9.91

1,4- and 1,2- butanediol showed no cross reactivity with the Catachem enzymatic method via the AU5812 as shown in the graph below.



All Samples at low (5mg/L) levels were not seen to interfere with the Catachem assay and were not picked up by the GC-MS (table 2). However, 2,3-butanediol showed significant cross reactivity with the Catachem enzymatic method at levels >50mg/L as seen in the graph above.

All Peaks for all isomers of butanediol and EG were seen in the Standard curve on the GC-MS with distinguished peaks at different retention times (RT). EG was not detected in any of the spiked samples as expected and the isomers present were seen at the 50mg/L and 500mg/L levels (table 2).

Table 2: Results for the Agilent GC-MS method for the spiked samples of butanediol isomers.

Compound	Ions detected	Retention Time	5 mg/L	50 mg/L	500 mg/L
1,2-Butanediol	77, 105, 193, 227	7.00	-	79.74	647.20
1,4-Butanediol	77, 105, 176, 193, 298	8.60	-	67.43	582.32
2,3-Butanediol	77, 105, 147, 176, 210	6.62	-	18.64	157.05
Ethylene Glycol	77, 105, 227, 270	6.44	-	-	-

Discussion

Research shows there is a potential for high alcohol levels in a patient with chronic alcoholism, which may cause the presence of 2,3-butanediol[(1,2,3)]. This leads to the potential for 2,3-butanediol to cause false positive EG results via the Catachem enzymatic analysis as represented in the graph above. In turn this has the potential to cause the incorrect diagnosis of EG poisoning instead of chronic alcoholism and ultimately lead to unnecessary invasive treatment or providing ethanol to an already intoxicated patient which may exacerbate symptoms.

The Standards created show that it is possible to distinguish separate peaks for EG and 2,3-butanediol via the GC-MS method using the Schotten-Baumann derivatisation at levels >10mg/L. This will be useful to be able rule out any potential false positive ethylene glycol results in those chronic alcoholics with 2,3-butanediol presence.

Conclusion

Potential interference has been reported in the rapid Catachem EG method analysis of samples from persons suffering from chronic alcoholism who may present with alcoholic ketoacidosis, and have several symptoms in common with EG poisoning, due to the natural production of 2,3-butanediol. Due to the difference in treatment requirements for ethylene glycol poisoning compared to alcoholic ketoacidosis, but the necessity, as set out by the NPIS and ACB[6], to provide a result within 4 hours of suspected EG poisoning, it is a requirement to have a rapid screen. A revised protocol has been put in place within the Northumbria trust, who analyse suspected EG poisoning cases for the North East area; that for EG samples between 50-400mg/L analysed via the Catachem method, results are issues with a caveat indicating potential interference, but that treatment for EG must be continued until otherwise informed. The samples are then analysed by the Agilent GC-MS method within the Toxicology department to confirm the findings and rule out any potential interference from 2,3-butanediol, the next working day.

Recommendations

Changes have been made to the screening for any sample with an EG of between 50 and 400 mg/L to be referred for GC-MS analysis, this should continue to be followed unless patient history suggests otherwise. Further research should be continued to highlight any possible difference in symptoms, history or biochemical results that may highlight potential for interference, to allow for correct and timely management of treatment.

References

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