

Correlation of ethanol concentrations in urine by enzymatic assay and headspace gas chromatography

Katherine Crompton*

Immunoanalysis Corporation, Pomona, CA, U.S.A.



Abstract

Blood Alcohol Concentration (BAC) and Urine Alcohol Concentration (UAC) are both reflections of the amount of alcohol present in an individual at the time of collection. The recommended conversion factor between UAC and BAC is approximately 1.33, although it has been reported that in the early absorption phase the UAC/BAC ratio may be less than 1.0; in the late absorption/distribution period the ratio varies between 1.3-1.4^[1]. This conversion factor needs to be taken into account when determining the quantitative correlation that exists between those urine samples analyzed using the enzymatic assay and a standard headspace gas chromatography (GC) method.

Objectives

The objectives of this study:

- evaluate the linearity of enzymatic assay (EA) for detecting ethanol in urine
- determine the quantitative correlation that exists between those urine samples analyzed using the enzymatic assay and a standard headspace gas chromatography (GC) method.

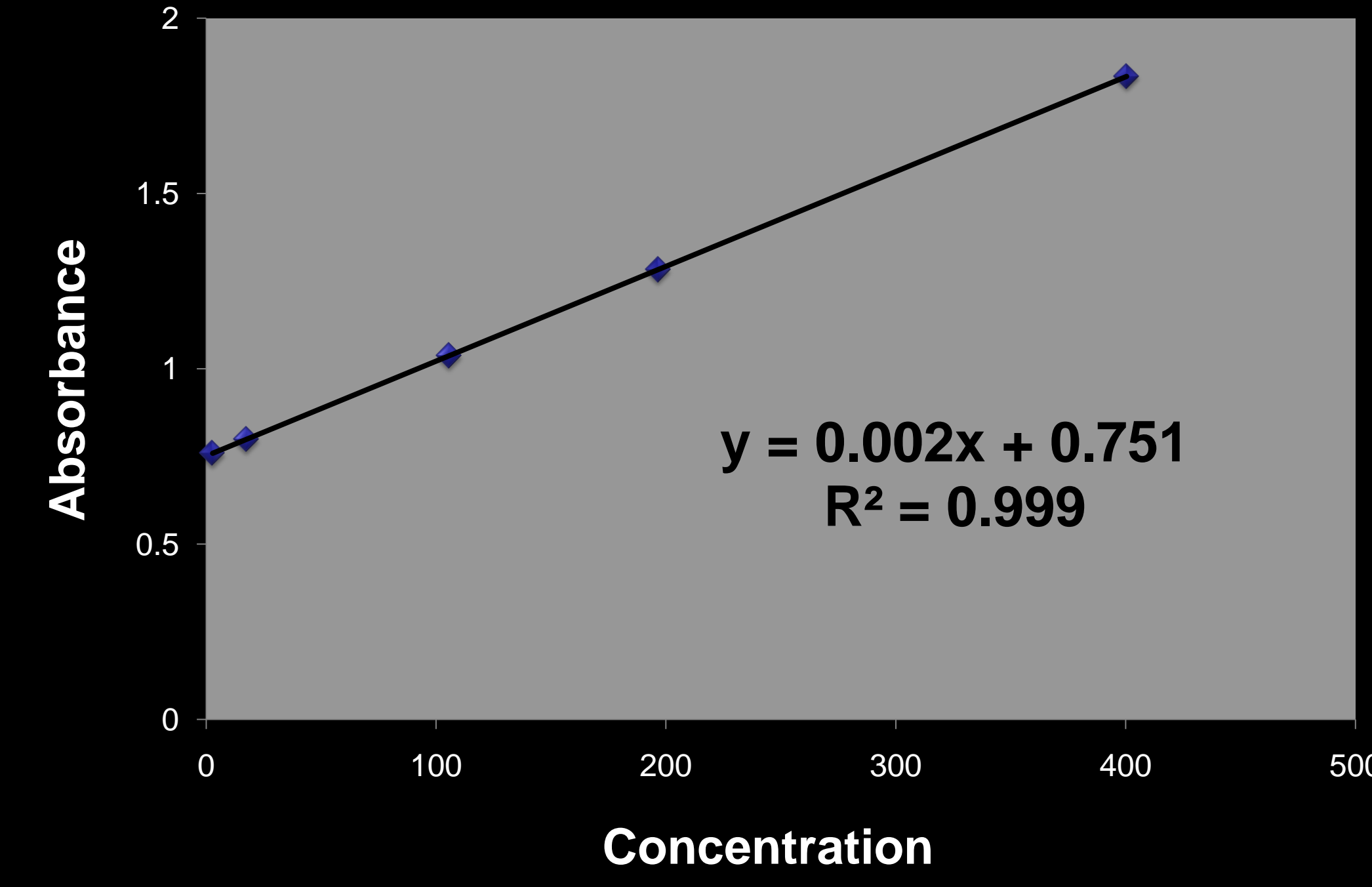
Methods

- Standards were purchased from Cerilliant (Round Rock, TX) at concentrations of 20, 100, 200, and 400mg/dL.
- Negative synthetic urine, drug standards, and authentic samples were diluted 1:10 with phosphate buffered saline (PBS).
- 10 µL sample volume was added to the microplate (EA) assay
- 100 µL of 0.6M Tris buffer with 0.1% Sodium (RA) was added to each well plus 100 µL of alcohol dehydrogenase + nicotinamide adenine dinucleotide (NAD) in Tris buffer (RE) were added immediately following sample addition
- The microplate was covered and incubated at room temperature for 30 minutes.
- The absorbance was measured with dual wavelength measurement s, 340nm and 620nm.

Results

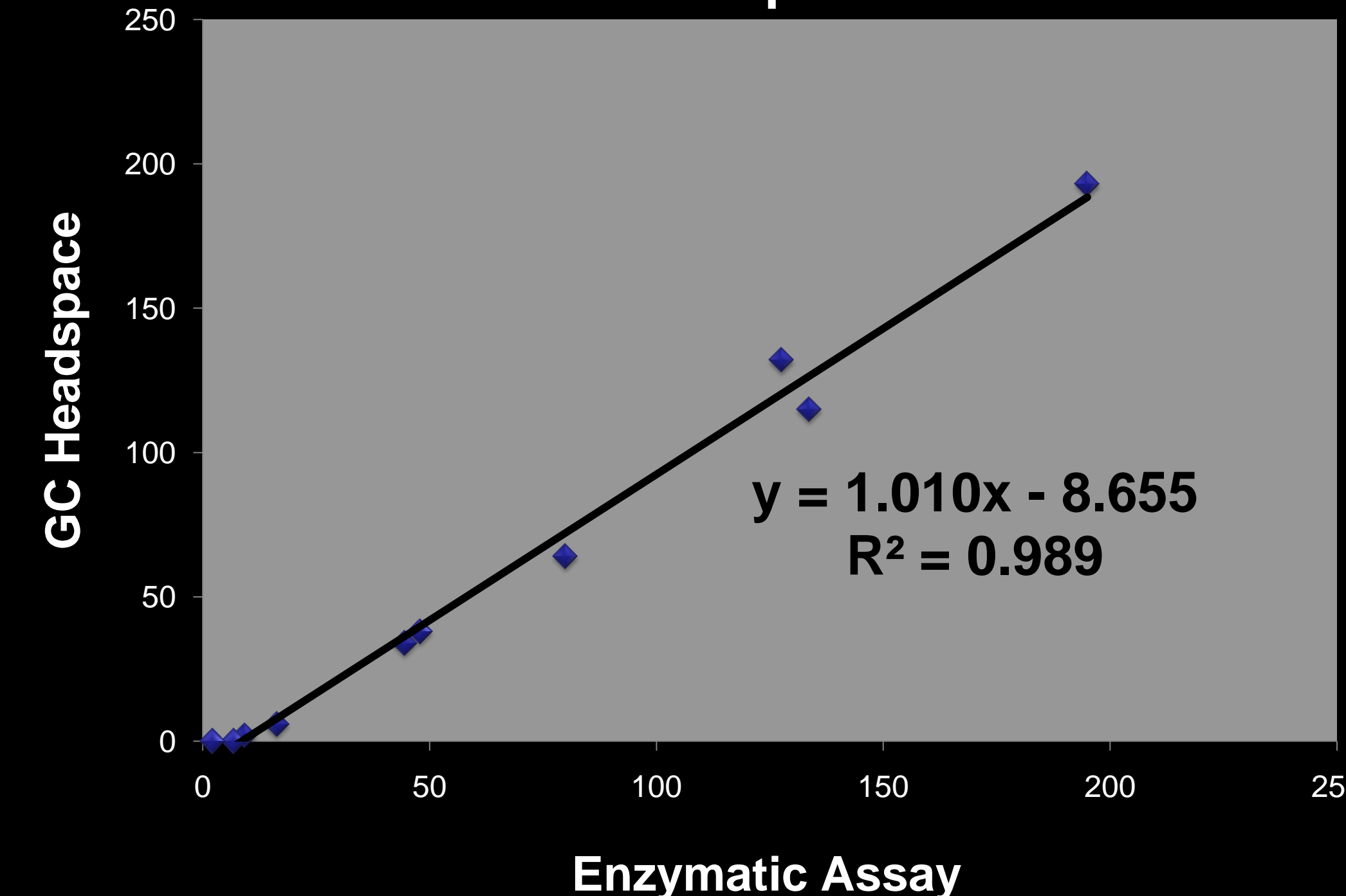
The linearity of the standard curve over the range of 20 to 400 mg/dL was $r^2 = 0.999$. The intraday precision testing (n=8) resulted in coefficient of variation (CVs) all below 4%, and the interday precision testing (n=5) resulted in CVs all below 10%.

Concentration versus Absorbance



Ten specimens were collected from individuals two hours after social alcohol intake, and were analyzed using the EA method and by headspace GC, producing a correlation coefficient of $r^2 = 0.989$ between the two assays.

Quantitative Correlation of enzymatic assay versus headspace GC



The concentration of ethanol in the authentic samples ranged from zero to 195 mg/dL.

Summary

The enzymatic assay consistently produced results with good linearity, excellent precision and a high quantitative correlation with a headspace gas chromatography method.

Based on the results of this study, this enzymatic assay would be a highly suitable choice for accurately measuring the concentration of ethanol in urine samples.

This method is currently being applied by Immunoanalysis client laboratories.

References

1. Jones AW. Urine as a biological specimen for forensic analysis of alcohol and variability in the urine-to-blood relationship. *Toxicol Rev.* 2006;25(1):15-35.



Urine

SOFT, Oklahoma City, 2009