# The Effect Of Ethanol On Acetylsalicylic Acid Enzymatic Hydrolysis

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## PROBLEM

Co-ingestion of ethanol with various drugs can affect the magnitude of the action of the drug on the body as a function of dose. In some cases, there is a mechanistic link between ethanol and the drug. In other cases, ethanol can affect the rate of metabolic breakdown of the drug.

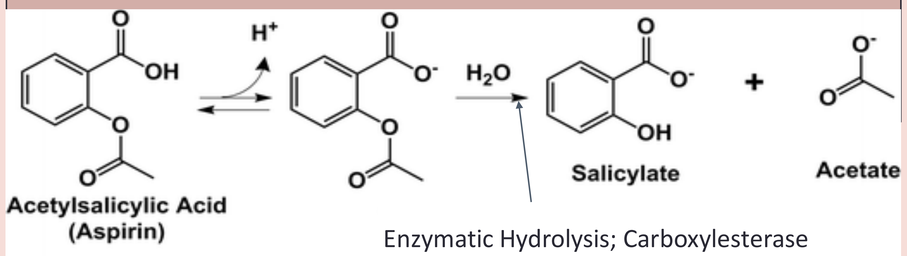
Aspirin is a popular over the counter pain reliever, and is one of the most commonly used drugs in the world, primarily because of the ease with which the drug can be made, its efficacy, and relative effectiveness. There are several potential side effects that may be associated with use, which can be exacerbated by the presence of ethanol.

Previous research has shown that the presence of ethanol causes an increase in the concentration of salicylic acid in the brain and other tissues of mice.

The mechanism of effect of ethanol on aspirin has not been elucidated.

## QUESTION

Does ethanol, in physiologically relevant concentrations, (e.g. <0.2 g/dL) interfere with the enzyme-catalyzed hydrolysis of acetylsalicylic acid? If so, what is the nature of that interference? (E.g. competitive, noncompetitive or uncompetitive?)




Enzymatic Hydrolysis; Carboxylesterase

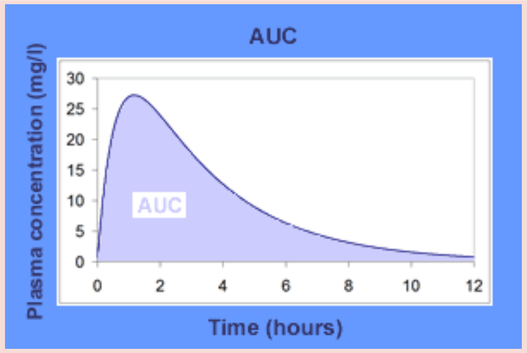
## HYPOTHESIS

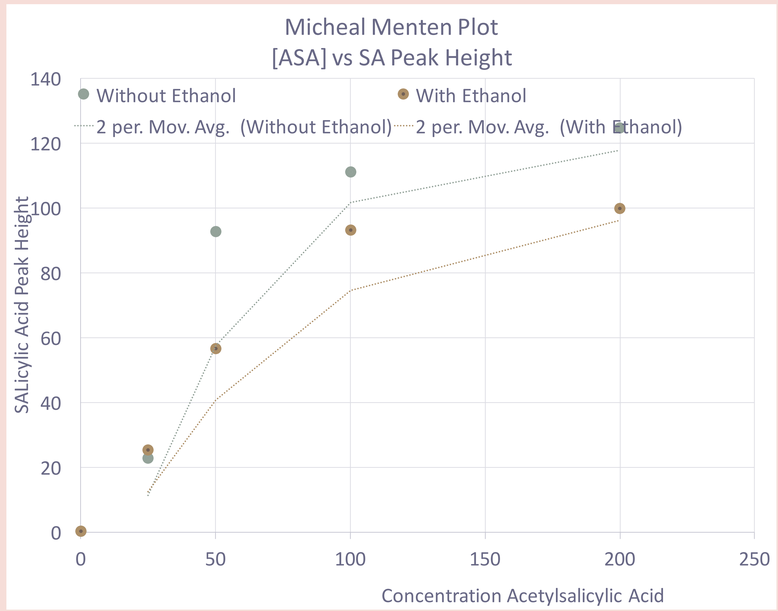
We have hypothesized that because of the structural similarity between ethanol and the –C-O- link in the acetyl ester of acetylsalicylic acid, ethanol acts as a competitive inhibitor in the carboxylesterase mediated hydrolysis reaction, thereby slowing the rate of aspirin hydrolysis in the body, with the resulting increase in half-life and area under the curve (AUC).

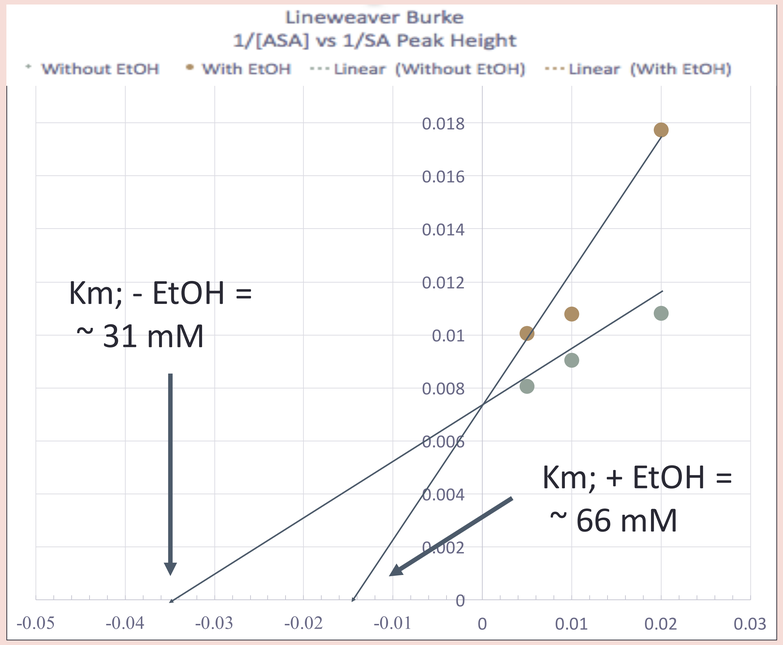
## METHODS

HPLC Analysis: An HPLC method was developed for Acetylsalicylic Acid, Salicylic Acid and Acetaminophen using a 4 x 200 mm C18 RP column, with diode array detection. The mobile phase consisted of Acetonitrile and 0.1M Sodium Acetate pH 4.0; 15:85, increasing to 40:60 over 14 min., with a 2 min hold, then returning to 15:85 over 2 min. with a 5 min equilibration period. Overall run time was 21 min. Absorbance at 210 and 270 nm was monitored and recorded.

Enzymatic Assay:
An enzyme assay using carboxylesterase (porcine liver, Sigma) was modified for microbial volumes. Each individual replicate contained 100 mM EPPS buffer at pH 8.0, Ethanol (0.2 g/dL), substrate (acetylsalicylic acid) at 0, 25, 50, 100, and 200mM and internal standard (100 ug/mL APAP) in a final volume of 100 uL. The mixture was heated at 25C for 45 minutes and then terminated by the addition of 100 uL acetonitrile. An ~ 100 uL aliquot of the acetonitrile-aqueous solution was transferred to a GCMS autosampler vial microbial insert (150 uL capacity) and reserved for HPLC analysis.




## METHODS CONTINUED

Peak heights corresponding to enzymatic reaction product (salicylic acid) and internal standard (APAP) were measured and utilized to determine the reaction velocity corresponding to each substrate concentration, and were plotted as a "Substrate Velocity Plot" corresponding to the Michaelis-Menten Equation. The inverse values (1/V and 1/S) were plotted in a "Lineweaver-Burk" plot, in which the X-axis intercept approximates -1/Km, and the Y-axis intercept approximates 1/Vmax. (Km is the "Michaelis Menten Constant" corresponding inversely to the binding of substrate to the active site of an enzyme, and Vmax corresponds to the maximal velocity of a particular enzyme system).

## RESULTS

Our experimental results generated a similar Y-axis intercept with or without the presence of ethanol, suggesting that there was no effect of ethanol on the actual functional capability of the enzyme. However, the presence of ethanol did cause a significant increase in the Km, indicating that ethanol affected the ability of the enzyme to bind the substrate.

## CONCLUSIONS

In our experiment, the presence of ethanol affected the ability of the carboxyltransferase enzyme to metabolize aspirin. This effect could reasonably be expected to increase both the half-life and AUC of aspirin in cases of co-ingestion.

## FUTURE WORK

Because of the extensive involvement of the carboxylase enzyme in the metabolism of multiple drugs, we anticipate further experiments on the effects of ethanol on the metabolism of (e.g.) sympathomimetic amines (cocaine and methylphenidate) opiates (meperidine) and similarly structurally related compounds.

## REFERENCES

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