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An enzymatic method for acetaldehyde testing of alcoholic beverages

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Keywords

Beverage Analysis, Automated Discrete Analysis, Enzymatic Analysis

Goal

To describe an enzymatic method for acetaldehyde analysis in alcoholic beverages.

Introduction

Acetaldehyde (ethanal, CH_3CHO) is the second smallest aldehyde and is found in alcoholic beverages and many other foods, like yogurts, that are produced by fermentation processes. Yeasts and bacteria produce acetaldehyde as their metabolites. It is also naturally present in fruits like apples.

Aldehyde dehydrogenase (ALDH2) is the major enzyme responsible for oxidizing acetaldehyde into acetic acid. In 2009, the International Agency for Research on Cancer (IARC) concluded that consuming acetaldehyde with alcohol is carcinogenic to humans. Acetaldehyde is produced when the body breaks down ethanol. People with enhanced ALDH activity are exposed to an increased risk of esophageal cancer due to acetaldehyde's carcinogenicity.[1]

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The objective of this study is to develop and validate a rapid enzymatic method based on photometric UV-determination for acetaldehyde and compare it with a liquid chromatographic method. In the enzymatic reaction, acetaldehyde is quantitatively oxidized to acetic acid in the presence of aldehyde dehydrogenase (ALDH) and nicotinamide adenine dinucleotide (NAD^+). While acetaldehyde is oxidized, NAD^+ forms NADH. The principle of this enzymatic reaction is shown in Figure 1.

$$CH_3CHO + NAD^+ + H_2O \xrightarrow{ALDH} CH_3COOH + NADH + H^+$$

Figure 1. Enzymatic reaction for acetaldehyde.

Experimental

Materials and methods

Samples

Samples analyzed during the method validation phase were sourced from a variety of different alcoholic beverages: white wine, dessert wine, calvados, light rum, whiskey, beer, cider, sherry, and sparkling wine.

Red wine samples were also tested.

Sample pretreatment process

Samples were analyzed as shown in Tables 1, 2, and 3 without any sample pretreatment.

Enzymatic method and experiment

A Thermo Scientific™ Arena™ 20XT analyzer was used for the automated photometric determination. The Thermo Scientific™ Gallery™ and Gallery™ Plus discrete analyzers can also be used for this test.

The Thermo Scientific™ Acetaldehyde system kit was used for enzymatic analysis of acetaldehyde. The Acetaldehyde Standard was used for calibration or a self made solution was used.

This Acetaldehyde application measured all samples photometrically at 340 nm before and after the addition of ALDH enzyme. The amount of NADH formed is directly proportional to the amount of acetaldehyde consumed in reaction. The application consumed only 20 µL of sample in the reaction and first results were reported 10 minutes after starting the analysis.

HPLC method

In liquid chromatographic analysis (HPLC) acetaldehyde was determined by derivatization using the diphosphopyridine nucleotide (DPN) method. This method is a validated acetaldehyde method and is used routinely by the Alcohol Control Laboratory (ACL, Alko Inc.), an official alcohol quality control laboratory in Finland. The method is also accepted by International Organization of Vine and Wine (OIV).

Results and discussion

This paper discusses method validation tests for the enzymatic method as well as method correlation studies between the enzymatic and HPLC methods. For the validation work, specificity, selectivity, linearity, measurement range, detection and quantification limits, repeatability, accuracy, systematic error, sensitivity, measurement uncertainty, and recovery studies were performed. The main results are reported.

Sample pretreatment process

Acetaldehyde needs to be released from the matrix prior to analysis by adjusting the pH to 8. The acetaldehyde system kit contains a buffer reagent which is sufficient for adjusting the sample pH without the need for an added step. The advantage is that the evaporation of the liberated acetaldehyde from the samples is limited because the change in pH occurs in the cuvette immediately before the enzymatic reaction.

Red wine samples were treated using several methods, such as polyvinylpyrrolidone (PVP), polyvinylpolypyrrolidone (PVPP), and charcoal, but their correlation to HPLC as well as their recovery results were not comparable with other sample types. The use of a matrix calibrator was also studied. Red wines require further study, for example, the use of an application with a wine specific matrix calibrator or bias to adjust the results to the correct level. This Total Acetaldehyde method is not validated for red wine samples.

Recovery studies

One part of the method validation spiked samples with a known concentration of acetaldehyde and calculated the percentage of recovery from the spiked samples. Results of the studies in which 0 to 450 mg/L additions were analyzed are shown in Table 1.

Recoveries for spiked samples were between 96 to 102% for nine different sample types (white wine, dessert wine, calvados, light rum, whiskey, beer, cider, sherry, and sparkling wine). The alcohol content of the samples did not have an effect on acetaldehyde recoveries.

Table 1. Recovery % from different alcoholic beverage samples.

Sample	White Wine 5523	Sweet Wine 3842	Calvados 3960	Light Rum 4108	Whisky 3596	Beer 4111	Cider 4115	Sherry 5525	Sparkling Wine 7583
Acetaldehyde Spike (mg/L)	Recovery (%)								
0	0	0	0	0	0	0	0	0	0
50	97.9	100.4	102.5	103.7	102.2	96.7	96.5	90.21	100.4
100	102.1	98.6	102.3	103.4	108.8	97.2	98.9	94.90	97.7
150	104.6	97.5	100.5	102.9	102.5	95.2	98.1	98.09	95.9
200	101.2	99.6	101.5	103.5	100.9	95.4	98.8	96.69	91.5
250	98.3	100.1	99.4	102.9	101.0	94.9	97.6	97.24	96.0
300	100.3	98.3	100.8	102.1	101.6	96.6	95.9	96.72	95.3
350	101.1	100.8	99.4	100.2	102.2	98.0	98.2	98.03	99.4
400	101.6	102.6	100.0	101.3	100.5	97.6	97.7	93.20	98.1
450	106.4	104.5	100.0	99.6	104.0	97.9	100.9	98.28	97.8
Average (%)	101	100	101	102	102	97	98	96	97

Method comparison studies

The same samples were analyzed in parallel with enzymatic and HPLC methods.

Acetaldehyde concentrations for nine different samples analyzed by both enzymatic and HPLC methods are shown in Table 2.

The acetaldehyde concentration varied from 10.2 to 125.6 mg/L using the enzymatic method. Most of the samples were analyzed in the primary analysis range without the need for an automated dilution.

With these sample types, no systematic error was found when the method was compared to the validated HPLC method.

As shown in this study, the automated enzymatic acetaldehyde method correlates well with HPLC results for all sample types tested.

Table 2. Method correlation studies with the enzymatic and HPLC methods.

Sample	Sample Type	Enzymatic Method (mg/L)	HPLC Method (mg/L)	Bias (mg/L)
3594	White wine	41.8	40.8	1.0
3595	Dessert wine	125.6	128.0	–2.4
3596	Whiskey	10.2	11.6	–1.4
5520	White wine	40.3	37.9	2.4
5523	White wine	19.7	17.9	1.8
5522	White wine	22.9	21.0	1.9
5519	White wine	49.5	47.1	2.4
5524	White wine	29.9	26.0	3.9
5521	White wine	31.9	29.3	2.6

Acetaldehyde quantitation from different sample types

White wine, light rum, whiskey, sherry, cognac, and calvados were analyzed using the enzymatic method.

Acetaldehyde concentrations in the alcoholic beverage samples varied from 5.7 to 124.6 mg/L as shown in Table 3.

The enzymatic method was fast, accurate, and user friendly. Theoretical analysis time from start to final results was less than 30 minutes for all these samples.

Table 3. Original acetaldehyde concentration analyzed using the enzymatic method.

Sample	Acetaldehyde (mg/L) by Enzymatic Method
1. Calvados 3960	33.0
2. Calvados 3961	83.9
3. Light Rum 4108	6.7
4. Whiskey 4109	8.1
5. White Wine 5519	49.5
6. White Wine 5520	40.3
7. White Wine 5521	31.9
8. White Wine 5522	22.9
9. White Wine 5523	19.7
10. White Wine 5524	29.9
11. Sherry 5525	124.6
12. Sherry 5598	123.4
13. White Wine 5599	25.5
14. Cognac 5600	46.1
15. Whiskey 5601	57.7
16. Calvados 5637	104.9
17. Light Rum 5638	5.7
18. White Wine 5639	37.2
19. White Wine 5640	67.3
20. White Wine 5641	53.7

Conclusion

The enzymatic method correlated very well with the liquid chromatography method as shown in this study. When spiked samples were analyzed, very good recoveries were found from 96 to 102%. In the sample analysis, acetaldehyde concentration was found to vary between 10.2 to 125.6 mg/L and therefore most samples are analyzed in the primary analysis range without the need for an automated dilution. The method was found to be linear up to 500 mg/L and the limit of detection and quantification for the method were 1.3 and 1.6 mg/L. Samples were analyzed without a separate sample pH adjustment. Red wine analytics require more investigation.

Enzymatic determination of acetaldehyde provides a rapid, user-friendly way of analyzing acetaldehyde from alcoholic beverages like white wine, dessert wine, calvados, light rum, whiskey, beer, cider, sherry, and sparkling wine.

References

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