APPLICATION NOTE

Gas Chromatography

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Optimized Method for the Simultaneous Analysis of Vicinal Diketones and Acetaldehyde in Beer

Introduction

Beer is one of the most commonly consumed alcoholic beverages, with a wide variety of taste profiles available. Thorough and robust analysis of the raw materials used in the brewing process, as well as the finished product, is paramount to ensure consistent product quality and taste. When brewing beer, brewers not only need to confirm the quality of the raw materials that are used, but also ensure that by-products of the fermentation process do not negatively impact the taste of the finished beer product.

Two vicinal diketone (VDK) compounds which can negatively impact the flavor of beer are 2,3-butanedione (diacetyl) and 2,3-pentanedione. VDKs are important by-products in the beer fermentation process, and are key indicators of beer maturity and quality. They are volatile in nature, and produce a butter/caramel like flavor which is considered unfavorable at high levels. The concentrations of VDK typically range from 1-50 ppb in lighter beers, but they can reach several hundred ppb or more in darker beers. The ability to characterize VDKs in beer products is an important tool in process control, quality assurance and product development.

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Acetaldehyde is a fermentation spillover product, which has significant effects on the flavor of beer. At low levels, it imparts an appealing crispness; but at higher levels, it produces a green apple flavor which is generally not desirable. Its content above the taste threshold value in beer can result in an unpleasant taste. Excellent quality beers have low levels of acetaldehyde under 3 to 8 ppm. The typical levels of acetaldehyde that are monitored are 1 to 20 ppm. Possible sources of acetaldehyde in beer include metabolism by yeast and undesired microbes, reduction of alanine, and the oxidation of ethanol by melanoidins and polyphenols.

Historically, breweries use two separate GCs, or two separate analytical runs in the testing of beers - one for the analysis of VDKs, and another for the analysis of acetaldehyde. Smaller breweries typically only purchase one GC due to budget limitations, and this can significantly reduce sample and finished product throughput. To switch modes, the analytical columns need to be switched out before each sample analysis, and this configuration poses a few risks, such as column breakage.

In this paper, an optimized method for the simultaneous analysis of VDK and acetaldehyde in beer was established using a PerkinElmer Clarus® 690 GC ECD/FID with a TurboMatrix™ HS-40. This method demonstrates results with good linearity and excellent detection limits.

Experimental

Sample Preparation

Samples were prepared by transferring the beer to a wide mouth beaker and sonicating briefly for full degassing. Another common method for degassing is to pour the beer through filter paper. After degassing, 5 mL of the beer sample was transferred to a headspace vial. The vial was sealed immediately, with the PTFE side of the septum facing toward the sample.

Instrumentation

A PerkinElmer Clarus 690 GC with a TurboMatrix HS-40 were used to perform these experiments. The Clarus 690 GC was configured with an electron capture detector (ECD) and a flame ionization detector (FID), and utilized PerkinElmer Elite-5 and BAC-1 columns installed in the injector via a two-hole ferrule. Nitrogen was used as the carrier gas for this study. The HS and GC conditions required for the analysis are listed in Table 1.

Calibration

The calibration curves were prepared by dissolving 2,3-butanedione (diacetyl), 2,3-pentanedione and acetaldehyde in 5 mL of DI water (Table 2 and 3). Each calibration standard was transferred to a separate headspace vial and capped with a PTFE/Silicone septum to ensure the lowest possible background levels.

Table 1. Analytical Parameters.

Sample Introduction	**TurboMatrix HS-40**
Needle Temperature	100 °C
Transfer Line Temperature	110 °C
Oven Temperature	70 °C
Thermostat Time	15 min
Pressurize Time	1.0 min
Inject Time	0.05 min
Withdraw Time	0.5 min
GC Cycle Time	8.7 min (PII = 8.8 min)
Operating Mode	Constant
Inject Mode	Time
Vial Vent	On
Column Pressure	28 psi
Transfer Line Temperature	Deactivated Fused Silica 0.32mm
Gas Chromatograph	**Clarus 690 GC**
GC Column	VDK = Elite 5 30M x 0.32mm x 1.0um AA = BAC 1 30M x 0.32mm x 1.8um
Headspace Connector	2-Hole Ferule
GC Injector	CAP with a 2 mm straight bore liner
Injector Temperature	225°C
Carrier Gas	Nitrogen
Carrier Gas Flow	3.0 ml/min (psi = 11.9)
Split Flow	5 ml/min
Oven Program Initial Temperature	45 °C
Hold Time	1.3 min.
Ramp 1	25 °C/min to 135 °C
Hold Time	0.0 min (4.9 min run time)
Sample Rate	3.125
Bunching Factor	1
Noise Threshold	VDK = 500; AA = 25
Area Threshold	VDK = 5000; AA = 100
Detectors	**ECD & FID**
Temperature	ECD = 150 °C; FID = 250 °C
N2 Makeup	30 ml/min
Attn	ECD = -6; FID = -6
Analytes	**Retention Time**
Butanedione	2.35 min (N_2)
Pentanedione	3.22 min (N_2)
Acetaldehyde	1.64 min (N_2)

Table 2. VDK Calibration points employed in this study.

Compound name	Level 1 (ppb)	Level 2 (ppb)	Level 3 (ppb)	Level 4 (ppb)
2,3-Butanedione (ppb)	9.6	24	48	120
2,3-Pentanedione (ppb)	5.57	13.9	27.8	69.6

Table 3. Acetaldehyde calibration points employed in this study.

Compound name	Level 1 (ppm)	Level 2 (ppm)	Level 3 (ppm)	Level 4 (ppm)
Acetaldehyde (ppm)	1	2	4	8

Results and Discussion

The chromatogram of a VDK calibration standard is shown in Figure 1.

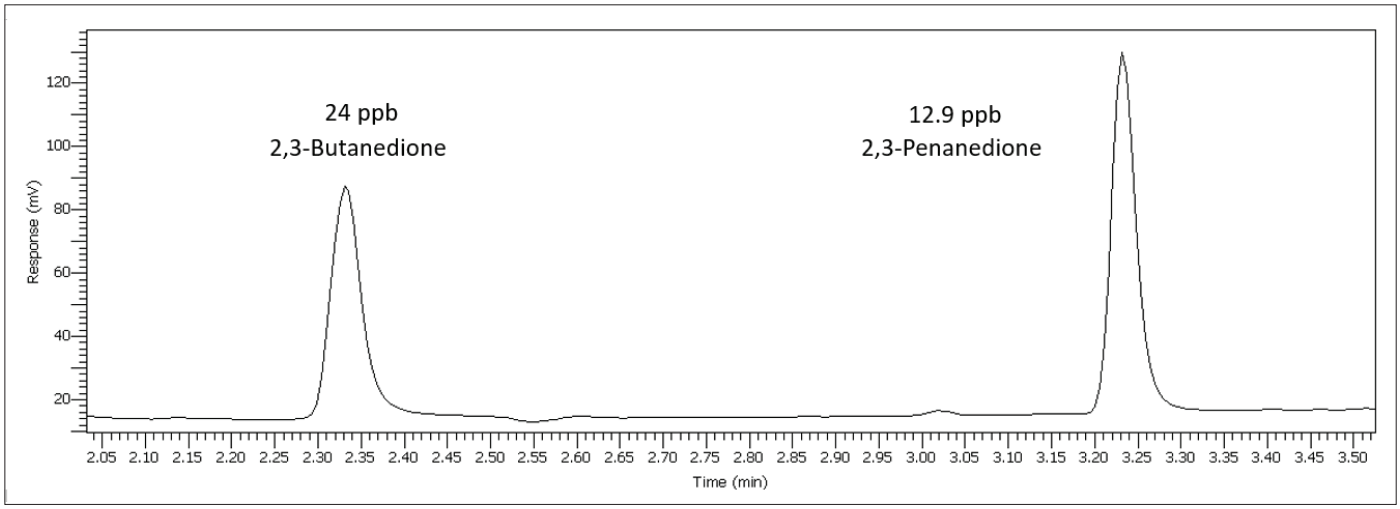

Figure 1. Chromatogram of 2,3-Butanedione and 2,3-Pentanedione using 0.05 min inject time.

The calibration curves were plotted as the peak area versus the amount of the analyte. The determination coefficients (r^2) were better than 0.998, showing the reliability of the analysis in the range of 10 to 120 ppb for 2,3-butanedione, and 6 to 70 ppb for 2,3-pentanedione (Figures 2 and 3). Tables 4 and 5 summarize the results for retention time, linearity, and signal to noise (S/N).

Testing for the quantitation of acetaldehyde is a high-volume test performed in breweries and food inspection institutions. Analysis time and resolution are two critical factors owing to the similar retention time between acetaldehyde and ethanol in beer. The PerkinElmer Elite BAC-1 column shows superior properties to separate acetaldehyde and ethanol, as shown in Figure 4.

The calibration curve for acetaldehyde is plotted as the peak area versus the amount of analyte. The determination coefficients (r^2) was over 0.9999, showing the reliability of the analysis (Figure 5). Table 5 summarizes the results for retention time, linearity and signal to noise (S/N).

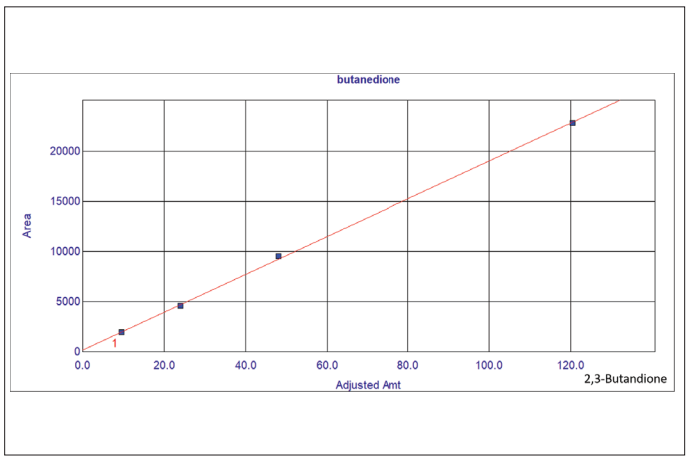

Figure 2. Calibration curve of 2,3-Butanedione R-squared 0.9996.

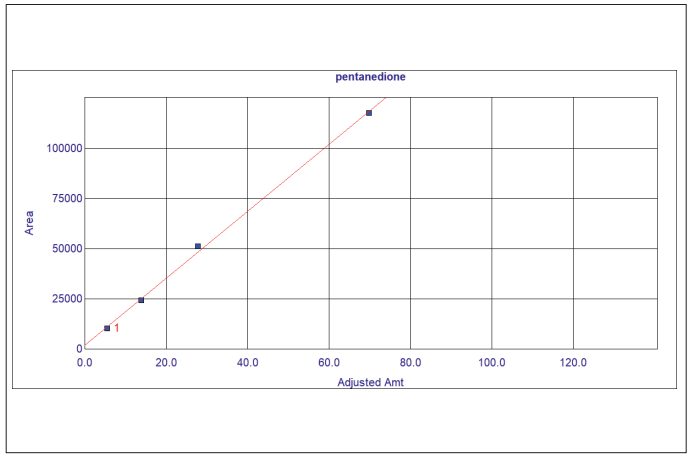

Figure 3. Calibration curve of 2,3-Pentanedione R-squared 0.999.

Table 4. Results for retention time, reporting limit and linearity of VDK.

Compound Name	RT	Reporting Limit	Linearity	
	(min)	(S/N RMS at 25 ppb)	Slope	r^2
2,3-Butanedione	2.35	350	188.29	0.9996
2,3-Pentanedione	3.22	4390	1663	0.9985

Table 5. Results for retention time, reporting limit and linearity of Acetaldehyde.

Compound Name	RT	Reporting Limit	Linearity	
	(min)	(S/N RMS at 2 ppm)	Slope	r^2
Acetaldehyde	1.64	146	2244	0.9999

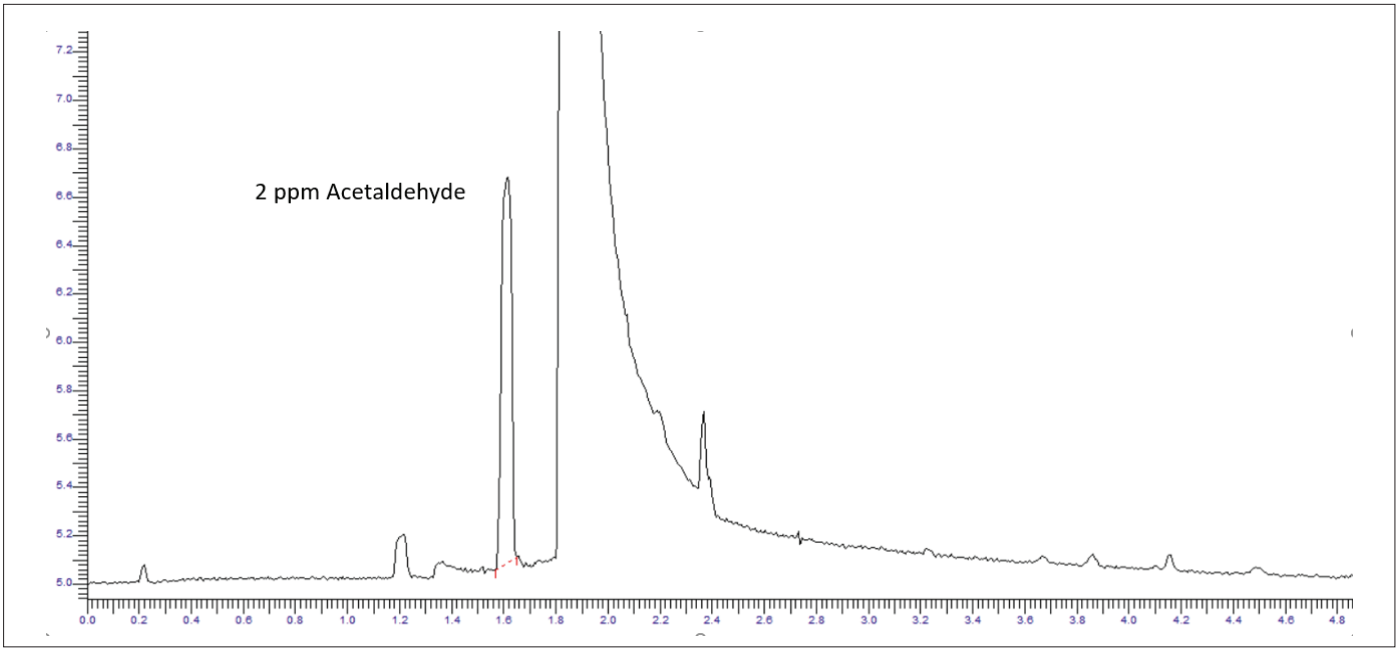

Figure 4. Chromatogram of Acetaldehyde at 2ppm at 0.05 inject time.

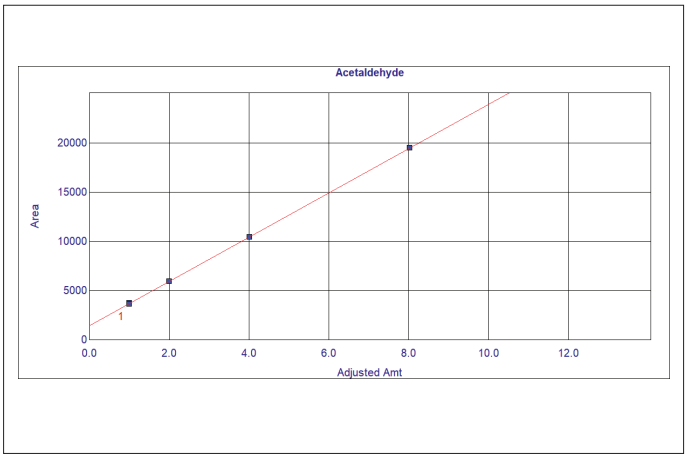

Figure 5. Calibration curve of Acetaldehyde. R-squared 0.999914.

Summary

The results obtained in this experiment demonstrate that the Clarus® 690 GC with ECD/FID, used in conjunction with the TurboMatrix™ HS-40, offers a solution for the simultaneous determination of VDKs and acetaldehyde in beer, providing the performance and stability.

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