Application Note 10418

Determining Vicinal Diketones in Beer Using Valve-and-Loop Headspace Analysis

Andrea Caruso, Thermo Fisher Scientific, Milan, Italy

Key Words

Chromeleon CDS, flavors, food quality, gas chromatography, TRACE 1310 GC, TriPlus 300 headspace autosampler

Goal

To determine and quantitate vicinal diketones in lager beer using the Thermo Scientific™ TriPlus™ 300 headspace autosampler, the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software.

Introduction

Diketones, such as diacetyl (2,3-pentanedione) or 2,3-butanedione, are naturally occurring products in some alcoholic beverages and foods. They are important ingredients in beer aroma and are characterized by their buttery flavor. In lager beer they are considered off-flavors, hence the importance of carefully monitoring their content in these beverages.

In 1999, the European Brewery Convention issued a method[1] for the determination of 2,3-butanedione and 2,3-pentanedione in beers via headspace gas chromatography.

One of the critical points of this method is the need to incubate the samples at 35 °C. Most sampling systems cannot achieve this low temperature without employing cryogenic systems. In this study, analysis is performed without a cryogenic system, using a TriPlus 300 headspace autosampler and a TRACE 1310 GC, equipped with an Instant Connect Electron Capture Detector (ECD) module controlled by Chromeleon CDS software.

The TriPlus 300 HS oven can operate at a constant temperature of 35 °C without any cryogenic devices.

Experimental

The experiments described here use a TriPlus 300 headspace autosampler and the TRACE 1310 GC. The data are collected and processed using Chromeleon 7.2 CDS software. The system is equipped with one Instant Connect Split/Splitless (SSL) injector with a dedicated headspace liner (P/N 453A1335) and an Instant Connect ECD detector. The column used is a Thermo Scientific™ TR-WAX 60 m x 0.25 mm x 0.5 µm (P/N 260W235P). The standards used for calibration are acquired from Sigma-Aldrich. For testing purposes, several lager beers from different producers were analyzed.

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Samples and standards preparation

Prior to the analysis, beer must be decarbonated by stirring in a large beaker at room temperature for 20 minutes on a magnetic agitator.

Prepare the standard and stock solutions as follows:

The internal standard used is 2,3-hexanedione.

- Hexanedione stock solution: Weigh 0.25 g of 2,3-hexanedione and add absolute ethanol to 100 mL in a volumetric flask.
- Internal standard solution: Add 5 ml of the hexanedione stock solution to a volumetric flask and add water to 250 mL.
- Butanedione stock solution: Weigh 1 g of 2,3-butanedione and add absolute ethanol to 100 mL.
- Butanedione standard solution: Add 10 mL of butanedione stock solution to a volumetric flask and add water to 1 L.
- Pentanedione stock solution: Weigh 1 g of 2,3-pentanedione and add absolute ethanol to 100 mL in a volumetric flask.
- Pentanedione standard solution: Add 10 mL of pentanedione stock solution to a volumetric flask and add water to 1 L.

Calibrations

Prepare the calibration stock standards as follows:

- Prepare six 100 mL volumetric flasks and add the following quantities of solutions (mL):

Flask number	1	2	3	4	5	6
Vicinal diketone conc. mg/L	0	5	10	15	20	25
Hexanedione stock solution	2	2	2	2	2	2
Butanedione standard	0	5	10	15	20	25
Pentanedione standard	0	5	10	15	20	25

The calibration points are prepared in a beer matrix. The beer used must have been previously tested and contain a concentration of vicinal diketones below 0.05 mg/L.

- Add the six standards to 20 mL crimp top vials with 3.5 g of ammonium sulfate, 5 mL of sample beer, 50 µL of solution from each flask. Close the vial with a magnetic cap and agitate on a vortex for about 1 minute.

Vial number	1	2	3	4	5	6
Vicinal diketone conc. mg/L	0	0.05	0.1	0.15	0.2	0.25
Ammonium sulfate (g)	3.5	3.5	3.5	3.5	3.5	3.5
Lager beer (mL)	5	5	5	5	5	5
Stock standard (µL)	50	50	50	50	50	50
From Stock calibration flask	1	2	3	4	5	6

Sample preparation

The samples for analysis are prepared as follows:

To a 20 mL crimp-top vial, add 3.5 g of ammonium sulfate, 5 mL of sample beer and 50 µL of internal standard solution. Close the vial with a magnetic cap and agitate on a vortex for about 1 minute.

Analysis

Recommended Conditions	
TriPlus 300 Headspace Autosampler	
Oven Temperature:	35 °C
Manifold Temperature:	40 °C
Transfer Line Temperature:	60 °C
Equilibration time:	40 min, shaking on high
Pressurization Mode:	Pressure, 1 bar
Pressure Equilibration Time:	0.3 min
Loop Filling Mode:	Pressure, 0.5 bar, equilibration time, 0.2 min
Loop Size:	1 ml
Injection Mode:	Standard, injection time, 0.2 min
Purge:	After injection for 1 min at 100 mL/min
Vial Venting:	On
TRACE 1310 GC	
Liner:	Dedicated headspace liner (P/N 453A1335)
Carrier Gas:	Helium, constant flow, 3 mL/min
Column Type:	TR-WAX 60 m x 0.25 mm x 0. 5µm (P/N 260W235P)
Column Oven:	Initial 60 °C, hold 1 min. Ramp at 15 °C/min up to 150 °C. Hold 2 min
Instant Connect SSL Injector	
Inlet temperature:	160 °C
Mode:	Split flow, 60 mL/min, split ratio, 20:1.
Instant Connect ECD	
Temperature:	180 °C
Makeup Gas:	Nitrogen, 15 mL/min

Calculations

Calculate the relative peak areas for butanedione and pentanedione to that of hexanedione as follows:

RPA butanedione = A butanedione / A hexanedione

RPA pentanedione = A pentanedione / A hexanedione
(Where RPA = relative peak area, A = Area)

Then calculate the calibration factor of the analyte as follows:

For both analytes plot a graph of the added diketone amount versus the RPA.

Provided that the coefficient of correlation of the curve is > 0.99, the slope of the curve is the calibration factor (Fd) of the analyte.

The concentration of butanedione and pentanedione into the sample is calculated as follows:

Butanedione mg/L = RPA butanedione * Fd
Pentanedione mg/L = RPA pentanedione * Fd

Results and Discussion

Figures 1 and 2 are the added amount vs RPA plots for butanedione and pentanedione, respectively.

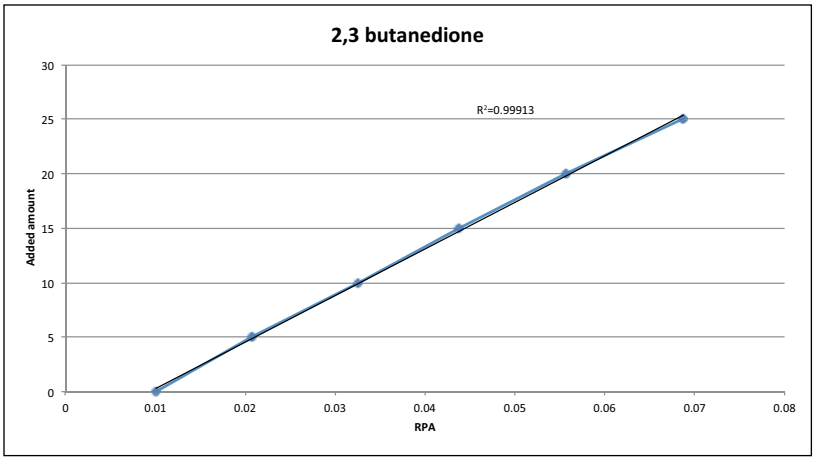

Figure 1. Butanedione added amount vs RPA plot.

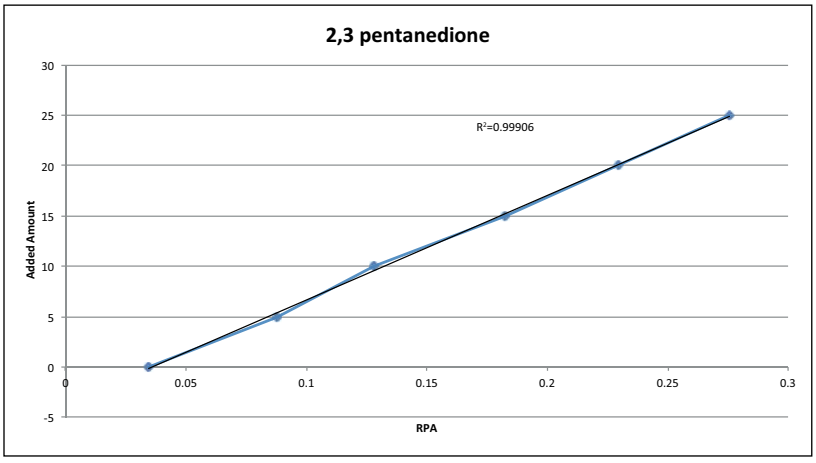

Figure 2. Pentanedione added amount vs RPA plot.

The chromatogram of one calibration point at 0.1 mg/L concentration is shown in Figure 3.

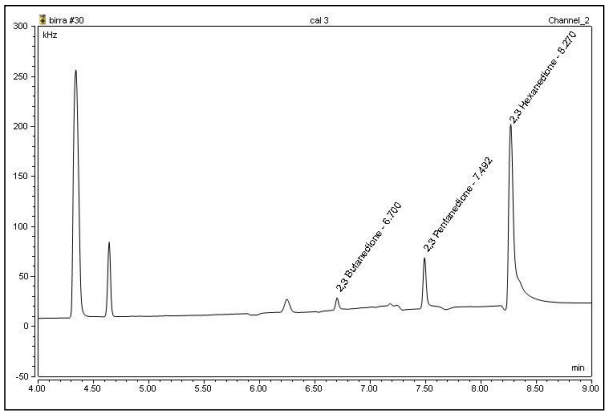

Figure 3. Calibration point chromatogram at 0.1 mg/L.

Beers from different brands were tested. All of the analyzed lager beers had a diketone content lower than 0.05 mg/L.

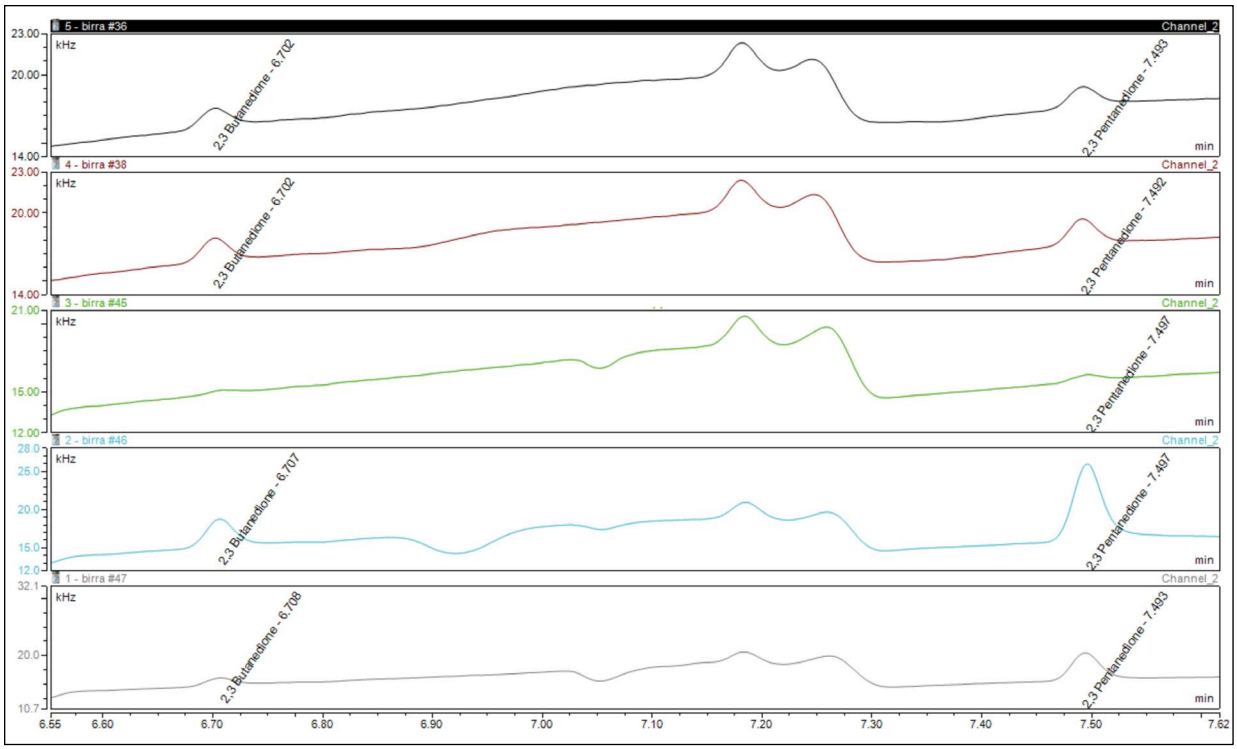

Figure 4. Chromatograms of different brands of beer.

Conclusion

The system comprised of a TRACE 1310 GC and TriPlus 300 headspace autosampler controlled by Chromeleon 7.2 CDS software is a valid and reliable solution for the quality analysis of beer.

The 120-position tray guarantees throughput and enables weekend-long unattended analysis.

The 35 °C headspace oven temperature allows the analysis to be performed at nearly room temperature, matching the recommended temperature of the method without employing complex and expensive cryogenic devices or increasing the time between two successive analyses to allow the cooling of the system.

The inertness of the entire sample path and high-flow purging capability eliminate any carryover effect, ensuring the highest sample integrity and consistency and reliability of results.

The optional barcode capabilities of the autosampler and the accurate auditing capabilities of Chromeleon CDS software also ensure the highest data quality and traceability.

References

1. Vicinal Diketones in Beer: Gas Chromatographic Method, *Analytica-EBC*, **1999**. 9.24.2

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Africa +43 1 333 50 34 0
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