Application Note

Flavor and Fragrance

Analysis of Aldehydes in Beer by Agilent PAL3 Autosampler and 5977C GC/MSD

Authors

Yufeng Zhang and
Lay Peng Tan
Agilent Technologies Inc.

Abstract

This application note describes a method for quantitation of four aldehydes (hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal) responsible for off-flavors in beer using an Agilent 8890/5977C GC/MSD with an Agilent PAL3 (SPME) autosampler. The method used fully automated, solvent-free extraction and on-fiber derivatization. The aldehyde compounds were derivatized with the derivatization agent O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) using the on-fiber derivatization procedure. The derivatization agent was first adsorbed onto an Agilent 65 µm PDMS/DVB fiber. Next, the fiber was inserted into a 20 mL headspace vial containing a 2 mL beer sample with agitation at 60 °C for 30 minutes. Both extraction and derivatization procedures were automatically performed using the PAL3 autosampler. The method demonstrated excellent sensitivity, with detection limits of 0.0009 µg/L for hexanal, 0.52 µg/L for furfural, 0.015 µg/L for phenylacetaldehyde, and 0.003 µg/L for *trans*-2-nonenal. The limits of the quantification for the four compounds were 0.003, 1.72, 0.05, and 0.01 µg/L, respectively. The four aldehydes were successfully quantified in four beer samples purchased from a supermarket. Good repeatability was demonstrated with RSD < 4.9% based on three replicate injections of the four beer samples for all four aldehydes.

Introduction

Aldehydes are a critical group of compounds in beer that significantly influence its flavor and aroma profile. These volatile organic compounds, even at low concentrations, can impact undesirable sensory characteristics such as cardboard, grassy, or stale flavors, which negatively affect the overall quality and consumer acceptance of the product. The presence of aldehydes is often linked to oxidation processes during brewing, packaging, and storage, making them key indicators of beer freshness and stability.[1] Hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal are among the most recognized aldehydes responsible for off-flavors, with flavor thresholds of 350 µg/L, 15.157 mg/L, 105 µg/L, and 0.1 µg/L, respectively.[2]

Monitoring and controlling aldehyde levels in beer are essential for maintaining product consistency and ensuring a high-quality drinking experience. By accurately quantifying aldehydes, brewers can identify potential issues in their production processes, such as oxygen exposure or ingredient degradation, and implement corrective measures to minimize off-flavors. Additionally, understanding the aldehyde profile of beer can aid in the development of new brewing techniques and formulations that enhance flavor stability and shelf life.

Solid-phase microextraction (SPME) is a widely used, solvent-free sample preparation technique that operates on the principles of adsorption and desorption. It uses a fiber coated with an extractive phase to concentrate analytes from a sample. Various types of fibers are available, including PDMS, acrylate, carbon WR, DVB, and combinations of these sorbents, to address the differing polarities of analytes. SPME is extensively used in a range of analyses, including environmental characterization, food and flavor analysis, pharmaceutical studies, and forensics investigations. It is well suited for automated sample preparation, resulting in reduced preparation time per sample, minimized risk of human error, and the freeing up of lab personnel from repetitive tasks.

This application note demonstrates the use of a PAL3 RTC sampler with SPME tool and 8890/5977C GC/MSD for the analysis of the four aldehyde compounds in beer. The SPME sampling tool is equipped with its own read-and-write chip with preset parameters including the stationary phase and usage tracking. Both sample extraction and derivatization were automated with the PAL3 RTC sampler.

Experimental

Reagents and samples

Aldehyde standards (hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal) and derivatization reagent PFBHA were purchased from Sigma-Aldrich. HPLC-grade methanol was from Merck. The water was Milli-Q Ultrapure. Four different brands of beer were purchased from a local supermarket.

Standards preparation

A mixed stock solution consisting of 10 µg/mL hexanal, 10 µg/mL phenylacetaldehyde, 1,000 µg/mL furfural, and 1 µg/mL *trans*-2-nonenal was prepared in methanol.

A secondary mixed stock solution consisting of 100 µg/L hexanal, 100 µg/L phenylacetaldehyde, 10 µg/mL furfural, and 10 µg/L *trans*-2-nonenal was prepared in ultrapure water.

Using the secondary mixed stock solution, a series of calibration standards were prepared with a concentration range of 0.05 to 10 µg/L for hexanal, 5 to 1,000 µg/L for furfural, 0.1 to 50 µg/L for phenylacetaldehyde, and 0.025 to 5 µg/L for *trans*-2-nonenal.

Two milliliters of each calibration standard was added to a 20 mL headspace vial and immediately capped for analysis.

PFBHA derivatization reagent with a concentration of 60 mg/L was prepared by weighing 30.1 mg of powder PFBHA into a 500 mL volumetric flask and dissolving in ultrapure water up to the 500 mL mark. Ten milliliters of the 60 mg/L PFBHA solution was added to a headspace vial to be used for sample derivatization.

Sample preparation

Beer samples were stored in a refrigerator at 4 to 6 °C prior to analysis. A 250 mL portion of the beer was poured into a clean plastic bottle and capped. The sample was degassed by hand shaking the capped bottle five times followed by opening the cap to release the carbon dioxide (CO_2). The degassing of the samples was performed a total of three times. A 2 mL degassed beer sample was transferred to a 20 mL headspace vial, which was immediately capped for analysis.

PAL3 RTC and GC/MSD parameters

The analysis parameters of the PAL3 RTC sampler are shown in Table 1.

Table 1. Agilent PAL3 autosampler and GC/MSD parameters for beer analysis.

Agilent PAL3 (SPME)	
Fiber Type	Agilent 65 µm PDMS/DVB (p/n 5610-5873)
Fiber Conditioning Temperature	250 °C
Preconditioning Time	5 min
Incubation Time	20 min
Incubation Temperature	60 °C
Derivatization Time	10 min
Agitator Speed	250 rpm
Sample Extraction Time	30 min
Sample Desorption Time	1 min
Post Conditioning Time	5 min
Gas Chromatograph	
Model	Agilent 8890 GC
GC Column	Agilent J&W DB-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI)
Column Pneumatics	Constant flow
Carrier Gas	Helium
Injector Mode	Splitless
Purge Flow to Split Vent	50 mL/min at 2 min
Inlet Temperature	250 °C
Injector Liner	Agilent Ultra Inert splitless liner (p/n 5190-4047)
Flow Rate	1.2 mL/min
Oven Temperature Program	60 °C for 2 min
	10 °C/min to 140 °C
	7 °C/min to 250 °C, hold 3.0 min
Equilibration Time	3 min
Mass Spectrometer	
Model	Agilent 5977C GC/MSD
Ionization Mode	EI, 70eV
Acquisition Mode	Scan
Scan Speed	N = 2
Scan Range	50 to 520 amu
GC Transfer Line Temperature	250 °C
Ion Source Temperature	230 °C
Quad Temperature	150 °C

Results and discussion

Compound identification and retention time confirmation

The 10 µg/L calibration standard was analyzed in full scan data acquisition mode, and the total ion chromatogram (TIC) is shown in Figure 1.

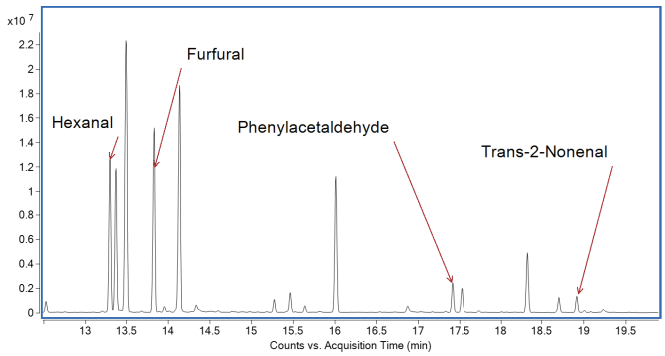

Figure 1. TIC of 10 µg/L aldehydes with Agilent SPME on-fiber PFBHA derivatization.

The data file for the 10 µg/L sample was processed using MassHunter Unknowns Analysis software. Automatic deconvolution of the data was performed using Unknowns Analysis to identify the components present exclusively in the sample. From the resulting list of components, the four target aldehydes were identified through library matching against the NIST 23 spectral library, achieving match scores above 80. The retention times (RTs) of the four aldehyde derivatives were identified as 13.299, 13.830, 17.419, and 18.914 minutes, respectively (Figures 2 to 5).

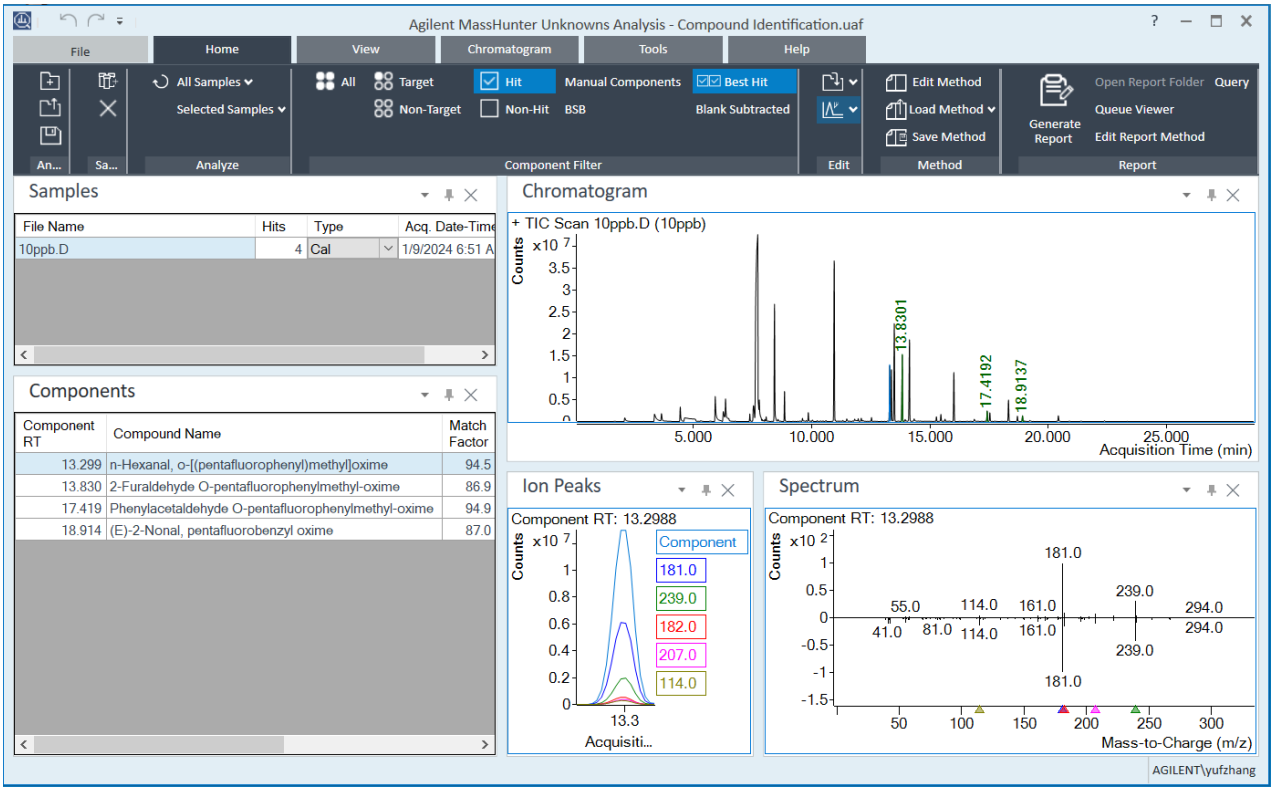

Figure 2. Hexanal derivative identified with an RT of 13.299 minutes.

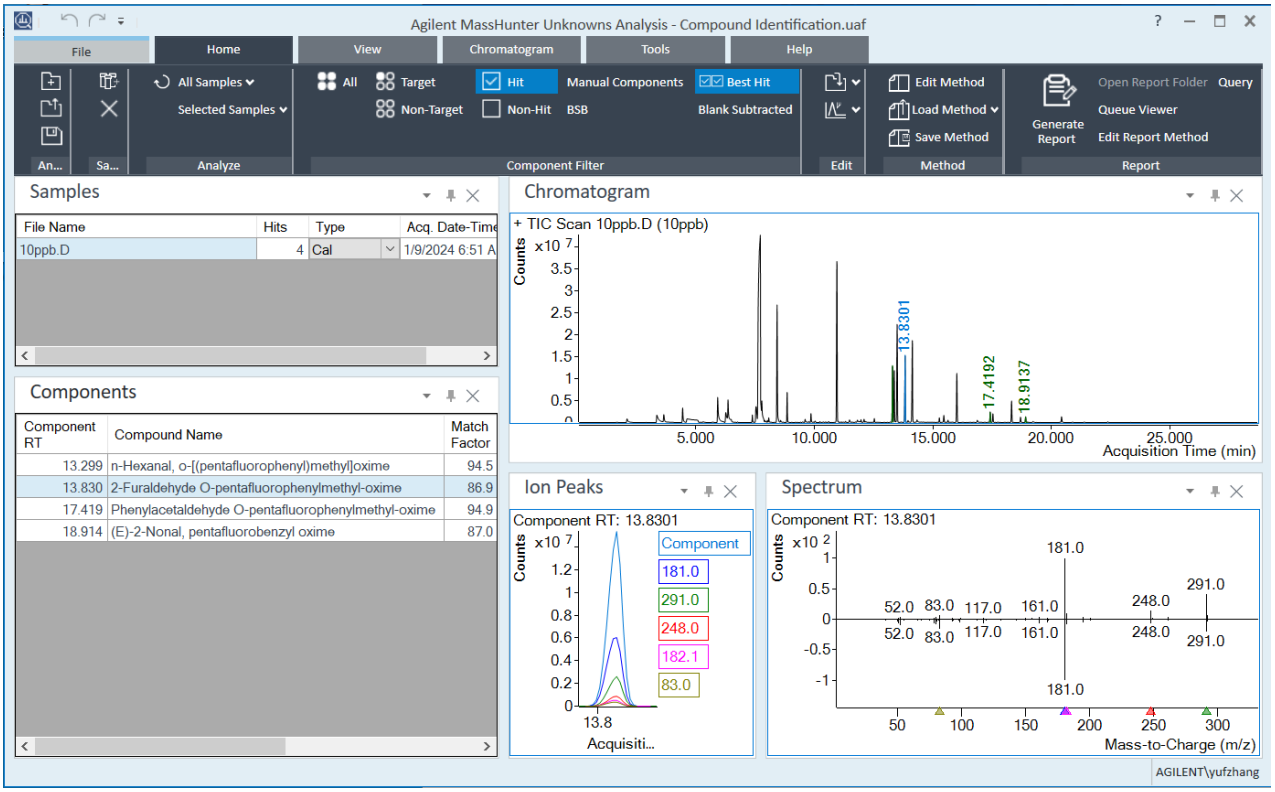

Figure 3. Furfural derivative identified with an RT of 13.830 minutes.

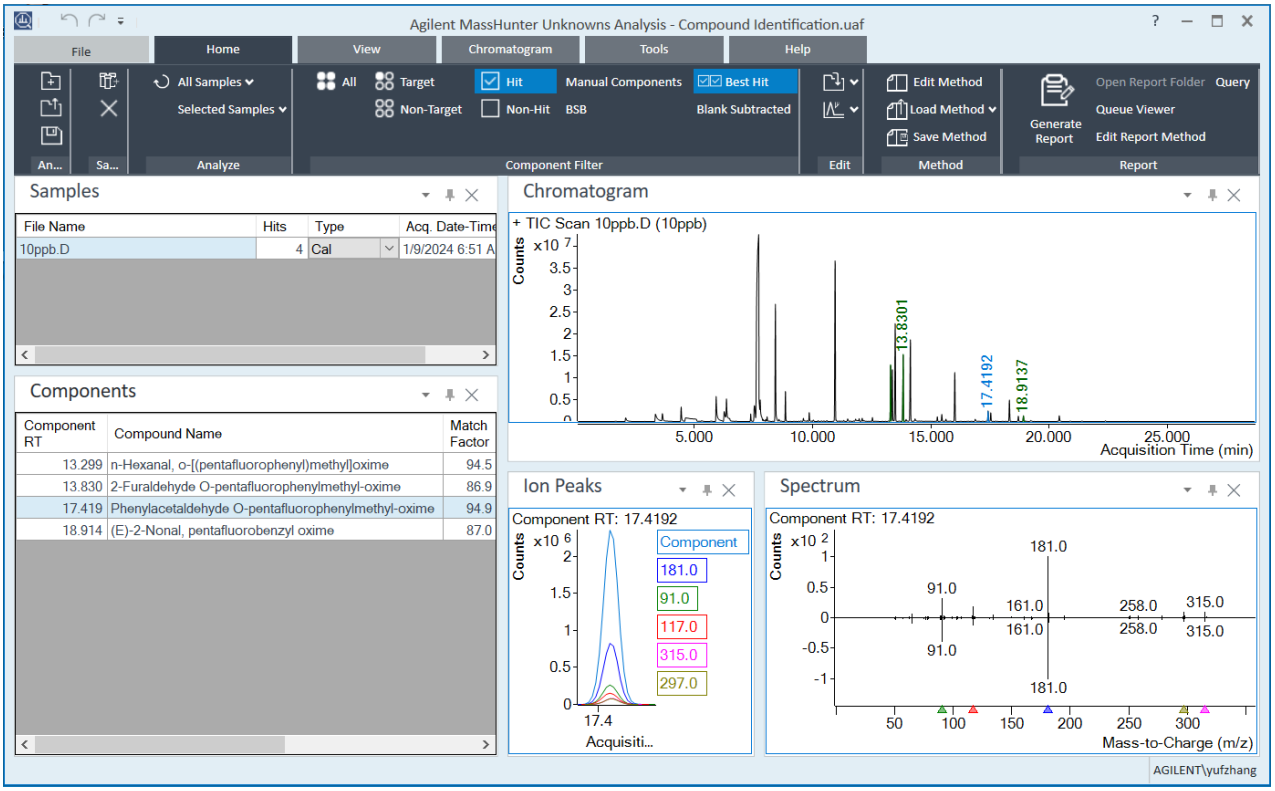

Figure 4. Phenylacetaldehyde derivative identified with an RT of 17.419 minutes.

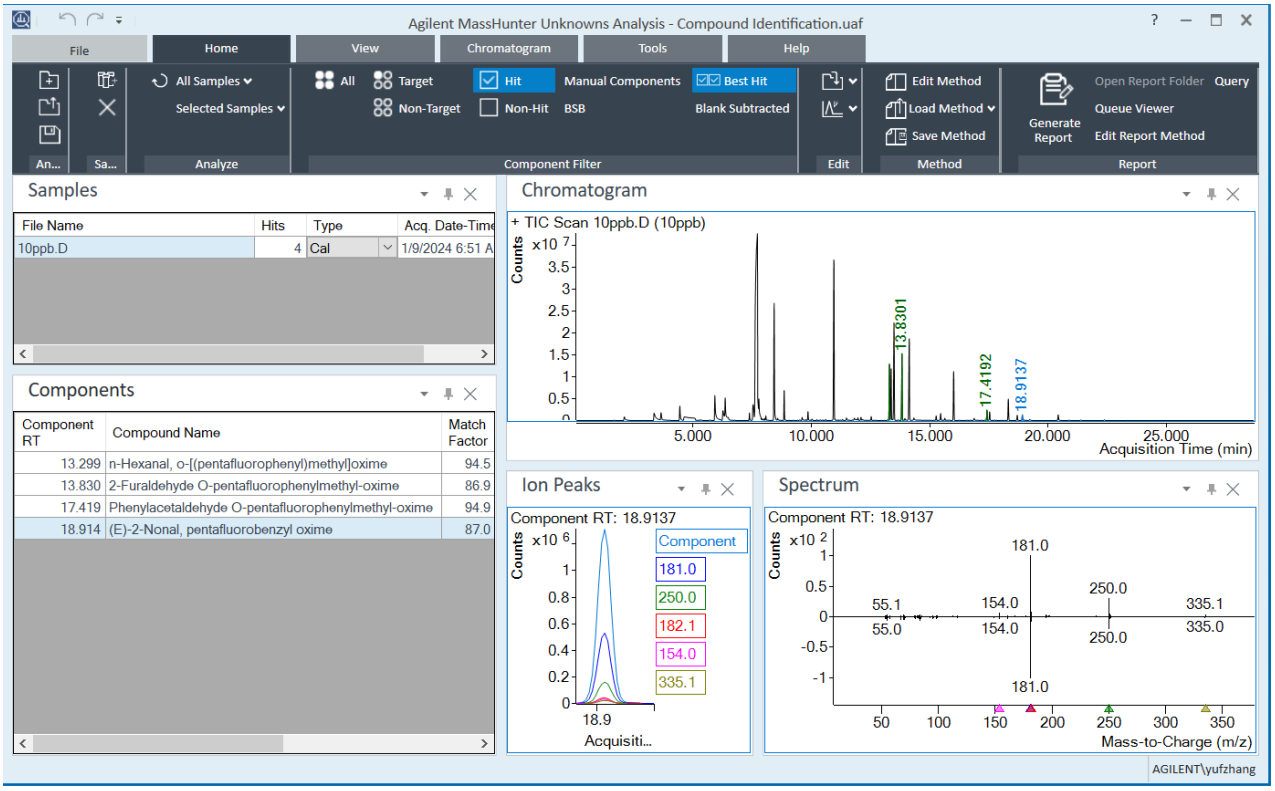

Figure 5. *trans*-2-Nonenal derivative identified with an RT of 18.914 minutes.

Calibration curves

Based on the response of the calibration standard solutions, the calibration curves were plotted for the four aldehyde derivatives. The results are shown in Table 2 and Figures 6 to 9.

Table 2. The calibration range and R^2 for the four aldehydes.

No.	Compound Name	Calibration Range (µg/L)	R^2
1	Hexanal	0.05 to 10	0.999
2	Furfural	5 to 1,000	0.998
3	Phenylacetaldehyde	0.1 to 50	0.996
4	*trans*-2-Nonenal	0.025 to 5	0.998

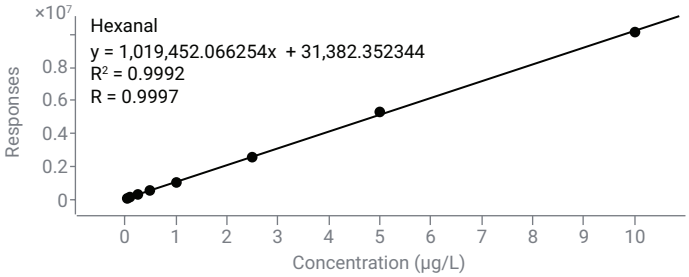

Figure 6. Calibration curve for hexanal 0.05 to 10 µg/L.

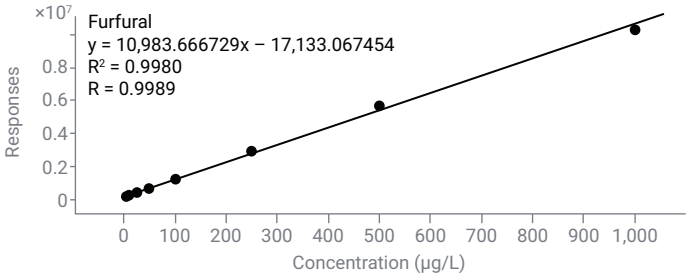

Figure 7. Calibration curve for furfural 5 to 1,000 µg/L.

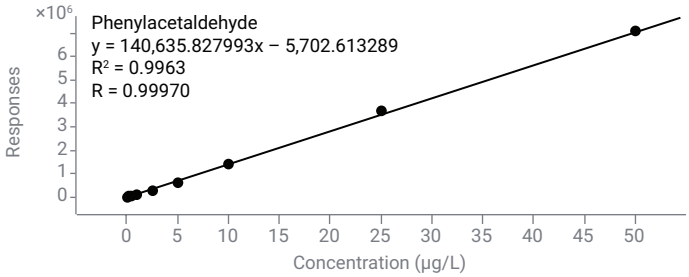

Figure 8. Calibration curve for phenylacetaldehyde 0.1 to 50 µg/L.

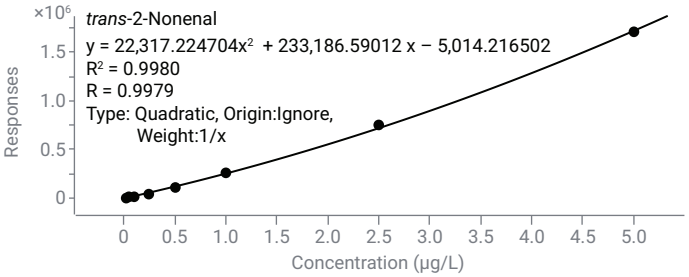

Figure 9. Calibration curve for *trans*-2-nonenal 0.025 to 5 µg/L.

Quantification results of beer samples

Based on the calibration curve setup, quantification was performed for the four aldehydes in the four brands of beer samples that were tested. The quantification results are summarized in Tables 3 to 6. All the aldehydes in the four beer samples were below their respective flavor thresholds. Among the four brands of beer samples that were tested, Brand 4 contained the lowest levels of aldehydes, with 0.45 µg/L hexanal, 6.26 µg/L furfural, 6.64 µg/L phenylacetaldehyde, and 0.031 µg/L *trans*-2-nonenal.

Table 3. Quantification results of hexanal in four brands of beer samples.

No.	Beer	Hexanal Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	0.67	0.67	0.69	0.68	1.7
2	Brand 2	1.53	1.54	1.61	1.56	2.8
3	Brand 3	0.99	1.03	1.04	1.02	2.6
4	Brand 4	0.45	0.46	0.45	0.45	1.3

Table 4. Quantification results of furfural in four brands of beer samples.

No.	Beer	Furfural Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	18.45	18.75	18.45	18.55	0.9
2	Brand 2	49.46	49.39	52.27	50.37	3.3
3	Brand 3	24.81	26.53	25.04	25.46	3.7
4	Brand 4	6.10	6.37	6.30	6.26	2.2

Table 5. Quantification results of phenylacetaldehyde in four brands of beer samples.

No.	Beer	Phenylacetaldehyde Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	11.07	10.41	10.05	10.51	4.9
2	Brand 2	8.36	8.26	8.07	8.23	1.8
3	Brand 3	8.72	8.63	8.92	8.76	1.7
4	Brand 4	6.84	6.59	6.48	6.64	2.8

Table 6. Quantification results of *trans*-2-nonenal in four brands of beer samples.

No.	Beer	Trans-2-Nonenal Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	0.034	0.035	0.034	0.034	1.7
2	Brand 2	0.061	0.063	0.063	0.062	2.8
3	Brand 3	0.034	0.034	0.035	0.034	1.7
4	Brand 4	0.030	0.031	0.031	0.031	1.3

With three replicate injections for each beer sample, the concentration %RSDs were calculated to be below 4.9%. The TIC overlays of the three replicate injections are shown in Figures 10 to 13.

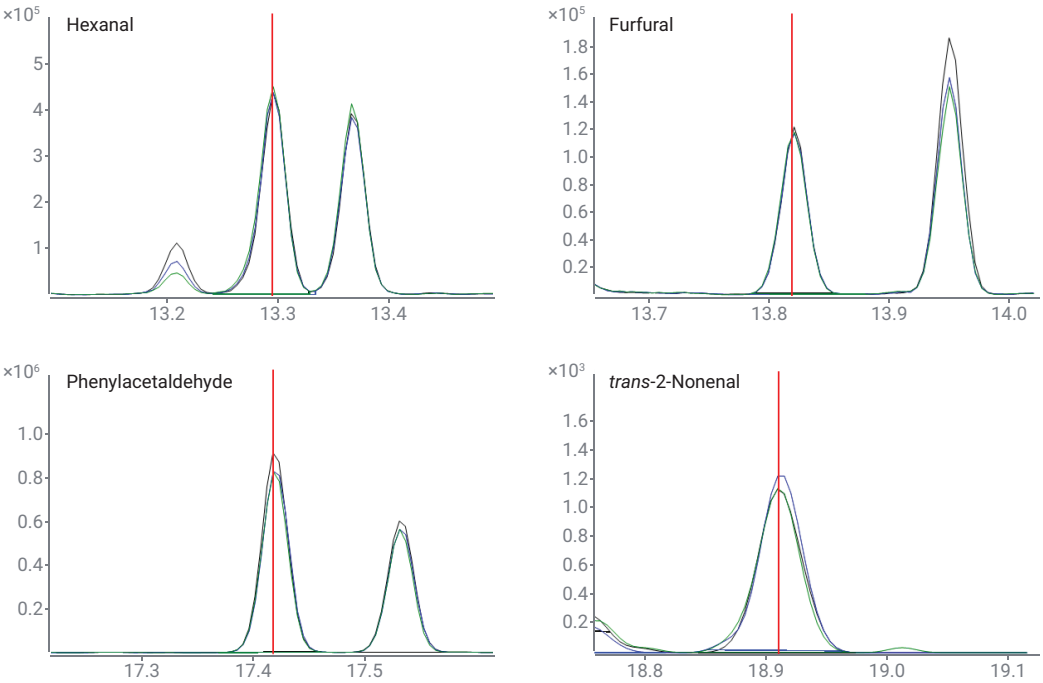

Figure 10. TIC overlay of the four aldehydes in the Brand 1 beer sample.

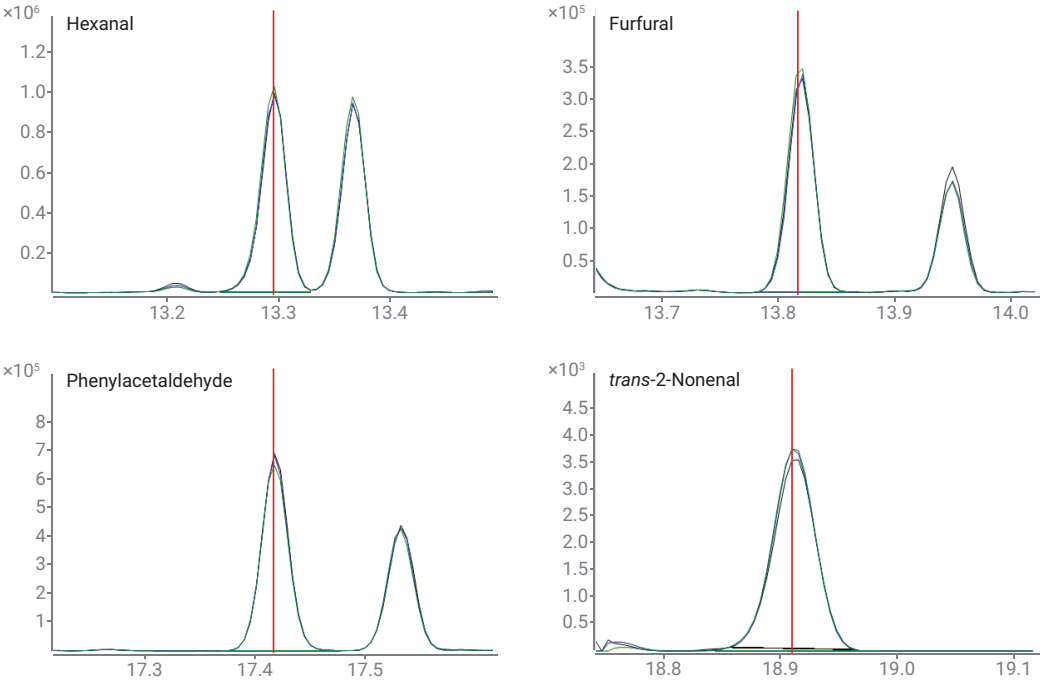

Figure 11. TIC overlay of the four aldehydes in the Brand 2 beer sample.

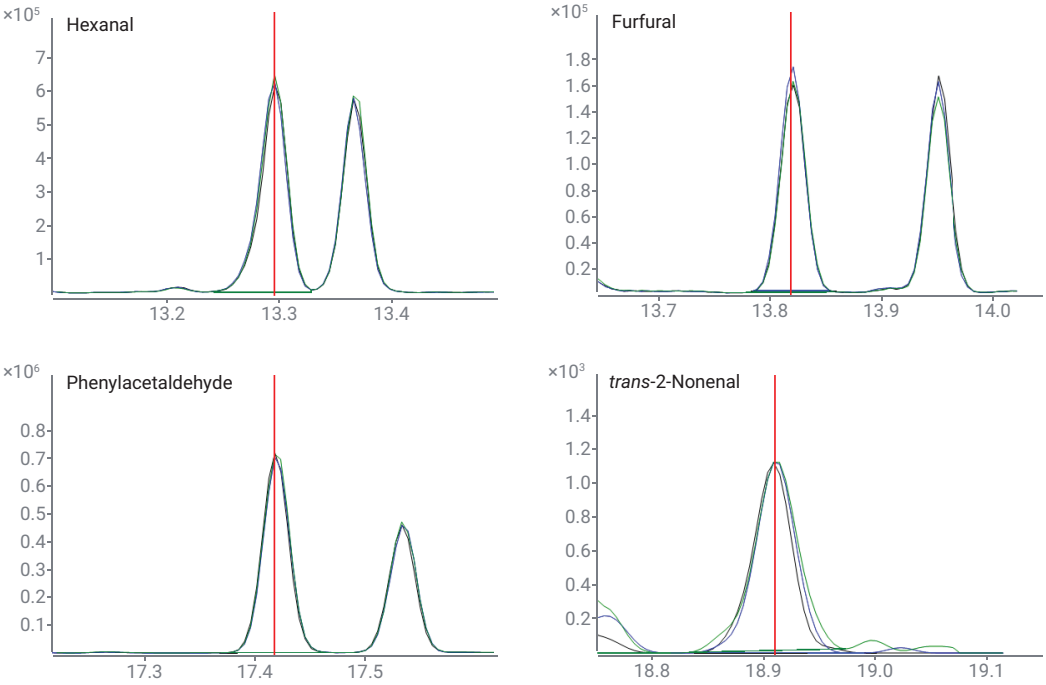

Figure 12. TIC overlay of the four aldehydes in the Brand 3 beer sample.

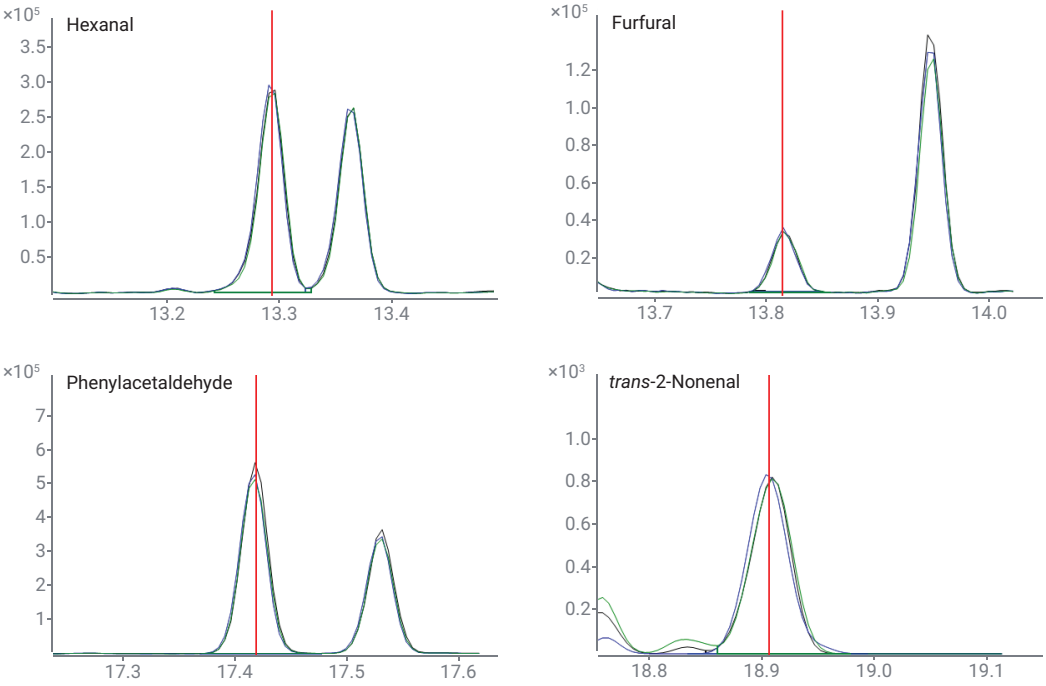

Figure 13. TIC overlay of the four aldehydes in the Brand 4 beer sample.

Determination of detection limits

Using the lowest calibration concentration of each compound, the signal-to-noise ratio (S/N) in full scan mode was calculated. The limit of quantification (LOQ) was determined at a S/N of 10, and the limit of detection (LOD) was determined at a S/N of 3. A summary of the LOQ and LOD results is presented in Table 7.

Table 7. LOQ and LOD of the aldehydes.

Aldehyde	S/N	LOQ (µg/L)	LOD (µg/L)
Hexanal (0.05 µg/L)	161	0.003	0.0009
Furfural (5 µg/L)	29	1.72	0.52
Phenylacetaldehyde (0.1 µg/L)	20	0.05	0.015
trans-2-Nonenal (0.025 µg/L)	24	0.01	0.003

Conclusion

This application note describes the quantitative analysis of four aldehydes (hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal) responsible for off-flavors in beer using an Agilent 8890/5977C GC/MSD with a PAL3 (SPME) autosampler. This method offers the advantages of full automation, rapid analysis, solvent-free extraction, and on-fiber derivatization. With this automated solution, excellent sensitivity was demonstrated for the detection of hexanal (0.0009 µg/L), furfural (0.52 µg/L), phenylacetaldehyde (0.015 µg/L), and *trans*-2-nonenal (0.003 µg/L). Four different brands of beer were analyzed, with hexanal detected in the range of 0.45 to 1.56 µg/L, furfural in the range of 6.62 to 50.37 µg/L, phenylacetaldehyde in the range of 6.64 to 10.51 µg/L, and *trans*-2-nonenal in the range of 0.031 to 0.062 µg/L. Good repeatability was demonstrated with RSD < 4.9% based on three replicate injections of the four beer samples for all four aldehydes.

References

1. Aguiar, D.; *et al.* Assessment of Staling Aldehydes in Lager Beer under Maritime Transport and Storage Conditions. *Molecules* **2022**, *27(3)*, 600.
2. Moreira, M. T. G.; *et al.* Aldehyde Accumulation in Aged Alcoholic Beer: Addressing Acetaldehyde Impacts on Upper Aerodigestive Tract Cancer Risks. *Int. J. Mol. Sci.* **2022**, *23(22)*, 14147.

www.agilent.com/chem/5977c

DE58984949

Printed in the USA, July 18, 2024
5994-7633EN

Agilent
Trusted Answers