A rapid, high-throughput mass spectrometry method for the quantitation of acetaldehyde in beer

Quick and sensitive quantitation of aldehydes in fermentation samples using the SCIEX Triple Quad 6500 + MS/MS system paired with high-throughput acoustic injection (Echo® MS)

Jonas Rombout[1,2], Lieselotte Vermeersch[1,2], Paul RS Baker[3], Adrian M Taylor[3], Kevin J Verstrepen[1,2]

[1] VIB Laboratory for Systems Biology, VIB-KU Leuven Center for Microbiology, Leuven, 3001, Belgium [2] CMPG Laboratory of Genetics and Genomics, Department M2S, KU Leuven, Leuven, 3001, Belgium [3] SCIEX, Framingham, MA USA

This technical note demonstrates the ability of the SCIEX Echo® MS Triple Quad 6500+ system[1] to sensitively quantify acetaldehyde in fermentation samples. Acetaldehyde is a central intermediate in the conversion of glucose to ethanol by yeast. It is one of the most abundant aldehydes in beer and affects aroma and flavor stability. Currently, acetaldehyde in beverages can be measured in different ways, including gas chromatography[2-4], HPLC[5] or enzymatic/chemical photometric assays[6-8]. While photometric assays are a popular high-throughput method for measuring acetaldehyde in beverages, their sensitivity is not always sufficient to quantify small concentration differences, partly because of interference of the sample's color with the spectrophotometric readout of the technique[6]. Compared to enzymatic assays, GC-MS is a more sensitive alternative, with sample acquisition speeds of around 10 min per sample[3].

The method described in this technical note, using the SCIEX Echo® MS system, demonstrates excellent sensitivity for beer sample analysis over a wide concentration range of acetaldehyde at acquisition speeds up to 300 times faster than GC-MS methods. Furthermore, the described sample preparation method accommodates a fast and high-throughput workflow, with the opportunity to expand the measurements to other aldehydes with minimal adjustments.

Key features for acetaldehyde analysis using the Echo® MS system

- Quick and easy sample preparation in multi-well plates exploits the high-throughput acoustic sample injection capability of the Echo® module, which promotes small sample volumes and prevents cross-contamination.
- The MS/MS method developed on the SCIEX Triple Quad 6500+ system facilitates sensitive screening of acetaldehyde in beer samples at an acquisition speed of 1 sample per 2.5 seconds.
- Multiple Reaction Monitoring (MRM) allows for both quantitative analysis and qualitative verification of acetaldehyde in the sample.

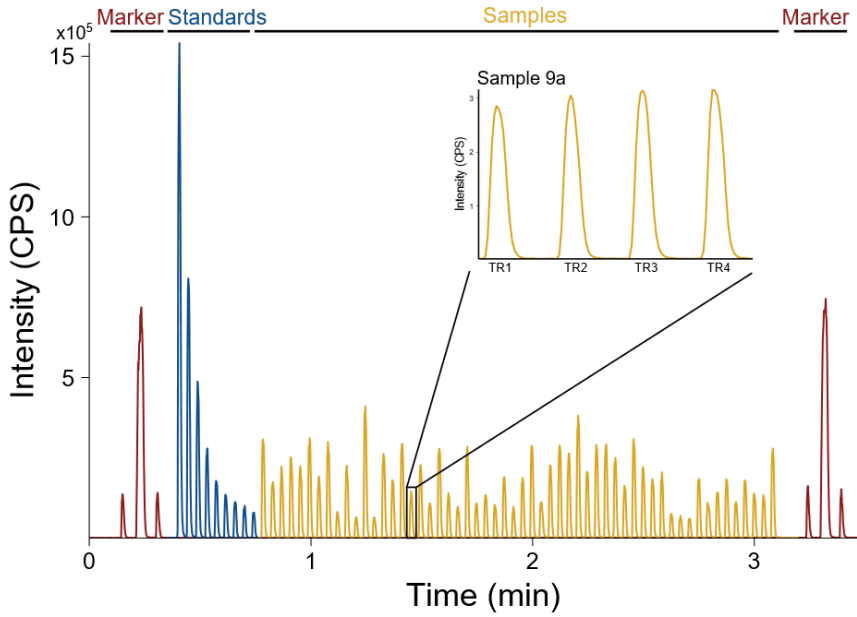

***Figure 1**: Total Ion Chromatogram (TIC) of an Echo® MS run with marker samples (red), calibration standards (blue), and fermentation samples (gold). The marker sample injections are used to align the timing file with the first sample. The intensity is shown in counts per second (CPS). **Insert** shows the technical repeatability of the system, where sample 9a was derivatized once and injected 4 times. Samples were acquired at a speed of 1 sample per 2.5 sec.*

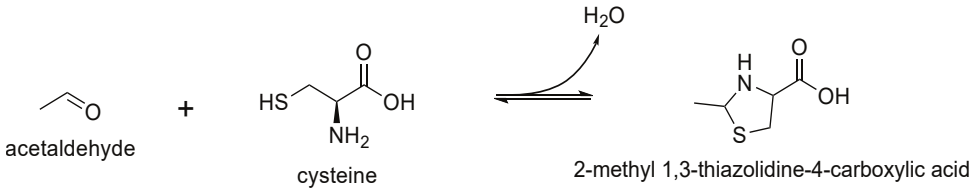

***Figure 2**: Reaction mechanism of acetaldehyde with cysteine as proposed by (Baert et al., 2015)[13]*

Different yeast strains and species are often employed in industrial beer fermentation processes to convert sugars into ethanol and carbon dioxide. Apart from these compounds, a range of other molecules are produced, especially flavor compounds, which are important for the final product characteristics and quality. Acetaldehyde is one of the most abundant aldehydes in beer, influencing aroma and flavor stability. Depending on final acetaldehyde levels and the product matrix, acetaldehyde's aroma can range from pleasant fruity to unwanted green apple-like or grassy[9-12]. Therefore, when screening or developing novel brewing strains, it is important to accurately measure the total amount of acetaldehyde produced.

Methods

Sample preparation: Beer samples (generated during fermentation experiments with engineered strains of *Saccharomyces cerevisiae* in wort) were diluted in a multi-well plate with LC-MS grade water (Biosolve) and L-cysteine stock solution (1 g/L in LC-MS grade water, Merck) to a final L-cysteine concentration of 0.2 g/L and a final sample dilution rate of 1:50 in a total volume of 150 µL. Aldehydes react with L-cysteine, forming a non-volatile 2-substituted 1,3-thiazolidine-4-carboxylic acid[13] (**Figure 2**). To promote the formation of these cysteinated aldehydes, samples were heated for 10 min at 50°C in a shaking heat block and cooled on ice for 5 minutes. 50 µL of the derivatized sample was transferred to each well of an Echo® MS qualified 384 well plate (Labcyte, p/n: C74290), which was centrifuged for 5 min at 3000 rpm and subsequently shaken for 1 min at 1350 rpm in a plate shaker. The samples were then analyzed using the Echo® MS system, equipped with a SCIEX Triple Quad 6500+ tandem mass spectrometer.

A series of 8 acetaldehyde standards was prepared using the standard addition method[14-15]. Acetaldehyde concentrations between 0 and 1.2 ppm were spiked in a 1:50 diluted fermentation matrix. The matrix used for the preparation of standards was produced by a reference strain of *S. cerevisiae,* fermenting a specific wort batch that was kept the same throughout all fermentation experiments. Standard addition was chosen as a calibration method to account for the matrix effect generated by complex fermentation samples (which can contain up to hundreds of different compounds). Subsequent derivatization and analysis of these standards were performed as described above.

Acoustic ejection: Using the Echo® module of the SCIEX Echo® MS system, an 80 nL sized droplet was ejected from every well of the plate into the Open Port Interface (OPI), which connects the Echo®'s acoustic ejection system to the MS's ionization source via a capillary. SP mode was used for ejection, and droplet ejections were inter-spaced with a delay of 2.5 sec. The mobile phase consisted of 70% (v/v) acetonitrile (VWR) in LC-MS grade water spiked with 0.1% (v/v) formic acid (VWR) and had a constant flow rate of 400 µL/min.

Mass spectrometry: Data were acquired on a SCIEX Triple Quad 6500+ system using an MRM method that measured 2 transitions in positive ion mode. One transition was used to quantitate the cysteinylated aldehyde (quantifier ion), while the other transition was monitored simultaneously to ensure correct identification of the analyte (qualifier ion)[13,16,17]. The spray voltage was set to 4000V, and the source temperature was 350°C. Ion source gas 1 was 90 psi, gas 2 was 70 psi. The curtain gas was 30 psi. MRM transitions and compound-specific parameters that were used, such as collision energy (CE),

***Table 1:** MRM transitions of 2-methyl-1,3-thiazolidine-4-carboxylic acid (further referred to as 2M-TZCA) with resp. optimized ESI ionization source settings.*

Compound	Formula	Ion Type	$[M+H]^+$	DP	Q1	Q3	CE	CXP
2M-TZCA	$C_5H_9NO_2S$	Qualifier	148.4	60	148.4	84.90	23	15
2M-TZCA	$C_5H_9NO_2S$	Quantifier	148.4	60	148.4	58.98	40	15

declustering potential (DP) and cell exit potential (CXP), are listed in (**Table 1**).

Data processing: Peak area integration and analysis was carried out in the Analytics module of the SCIEX OS software suite, using the MQ4 algorithm which performed automatic baseline correction. The noise percentage was set to 40%, the baseline subtraction window to 2.00 min and the minimum peak height to 100. After analysis, data was visualized using custom-made R-scripts.

Results and Discussion

Optimization of sample preparation protocol

An issue commonly associated with direct-injection MS/MS (i.e., the absence of a chromatography step) is reduced sensitivity due to matrix components, hindering efficient ionization of the compound of interest[19-20]. However, we observed that ionization efficiency and signal intensity could be drastically improved by

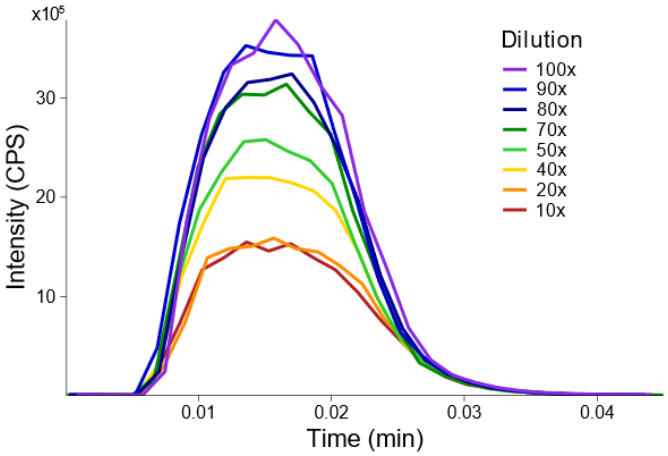

***Figure 3**: Total Ion Chromatograms (not smoothed) of different dilutions of a reference sample, derivatized with L-cysteine after dilution.*

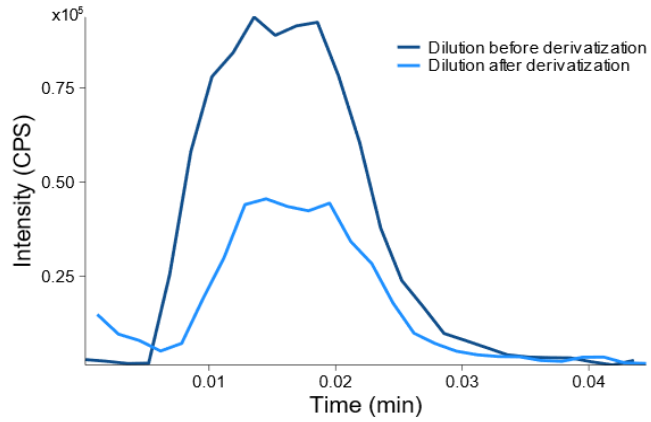

***Figure 4**: Total Ion Chromatograms (not smoothed) of a reference sample, diluted 1:50 before and after derivatization with L-cysteine.*

choosing an appropriate dilution strategy, both in terms of ratio and timing during the derivatization reaction. Different dilutions of a reference sample were measured to determine which dilution level was optimal for achieving the highest possible signal intensity (**Figure 3**). In general, a higher dilution ratio resulted in higher signal intensity. However, to obtain good technical repeatability, we opted for a 1:50 dilution ratio since this dilution allows more accurate pipetting during sample preparation while preventing the loss of acetaldehyde due to evaporation as much as possible. Furthermore, a 1:50 dilution ratio still allows the detection of acetaldehyde concentrations on the lower end of the spectrum, since higher dilution ratios could push the fermentation samples below the detection limit of the system.

Apart from the sample dilution ratio, the timing of sample dilution was investigated. The same reference sample was measured in both conditions to verify whether signal intensity differs between diluting the sample before Vs. after derivatization. Results showed that dilution before derivatization leads to higher signal intensity (**Figure 4**), most likely because of a more efficient reaction between acetaldehyde and L-cysteine, with less matrix interference. Diluting the sample before derivatization increases the relative concentration of the L-cysteine, which promotes the reaction direction towards the cysteinylated complex, thus reaching equilibrium at a higher relative 2M-TZCA concentration[17].

Construction of calibration curve for acetaldehyde

A total of 8 standards were prepared by adding increasing amounts of acetaldehyde to a 1:50 diluted control sample and subsequently derivatizing using L-cysteine. These derivatized standards were measured on the Echo® MS system in the same batch as the fermentation samples. Since the fermented matrix already contains a certain amount of acetaldehyde, the area

***Table 2:** Overview of final acetaldehyde concentrations in standards, prepared by standard addition of acetaldehyde in a 1:50 diluted control sample, with their corresponding measured area values averaged over 3 technical replicates and corresponding standard deviation.*

[Acetaldehyde] (ppm)	Area	SD
0	*3.43E+04*	*2.05E+03*
0.0188	*4.95E+04*	*1.08E+03*
0.0375	*5.97E+04*	*1.36E+03*
0.0750	*7.93E+04*	*2.40E+03*
0.1500	*1.24E+05*	*4.54E+03*
0.3000	*2.12E+05*	*5.84E+03*
0.6000	*3.75E+05*	*2.62E+04*
1.2000	*7.08E+05*	*4.24E+04*

values of the standards were corrected by subtracting the area of the control standard without added acetaldehyde. The exact concentrations of the standards are noted in **Table 2**.

A calibration curve (**Figure 5**) was constructed usingthe data above to quantify acetaldehyde produced by novel, engineered yeast strains. Standard addition was performed in a 1:50 diluted fermentation sample matrix of a reference strain of *S. cerevisiae*. Since engineered variants of this reference strain are not expected to alter the matrix drastically, the calibration curve can be considered reliable for use with unknown fermentation samples prepared with an identical dilution ratio. The calibration curve, measured over 3 replicate injections per standard, is linear over the reported concentration rangev (R^2=0.9999), allowing for accurate acetaldehyde quantification in samples containing between 0.94 and 60 ppm of acetaldehyde before dilution. Since commercial beers are generally expected to contain acetaldehyde levels between 4-15 ppm[11,18], our method promises excellent accuracy for the quantitation of samples over a wide range of acetaldehyde concentrations.

High-throughput quantification of acetaldehyde in fermented samples

The production of acetaldehyde by genetically engineered strains of *S. cerevisiae* was evaluated by performing fermentation experiments with these strains and subsequently diluting and derivatizing each fermentation sample in duplicate. **Figure 6** shows the acetaldehyde concentration (ppm) detected in one batch of fermentation samples. Correct identification and detection of acetaldehyde were confirmed by monitoring the ion ratio of the quantifier and qualifier transition. Integration of the quantifier peak is used to calculate the acetaldehyde concentration in each sample.

Because of the fast and easy sample preparation and derivatization method, measurements could be completed swiftly after the end of the fermentations, increasing the sample throughput and decreasing the possible loss of acetaldehyde due to evaporation. Furthermore, the combination of this specific derivatization method with the Echo® MS system allows for high technical repeatability, with standard deviations of separately derivatized replicates ranging from 0.16 to 16 ppb for an average measured acetaldehyde concentration of 147 ppb in the samples (before correction of the 1:50 dilution factor).

A 1:50 dilution of the samples was needed to overcome reduced sensitivity because of matrix components. Therefore, the measured acetaldehyde concentrations are low, ranging from ppb to low ppm values. However, the need for dilution does not pose an issue because of the system's high sensitivity for the compound, thus being able to detect these low concentrations accurately.

A specific control strain of *S. cerevisiae* and a control sample of unfermented wort (first two bars and the last bar, respectively, in **Figure 6**), with expected low acetaldehyde contents, showed concentrations within the expected range of 2-5 ppm. Furthermore, meaningful differences between the different samples could be found and were positively related to values for beer fermentations mentioned in the literature[18]. The range of

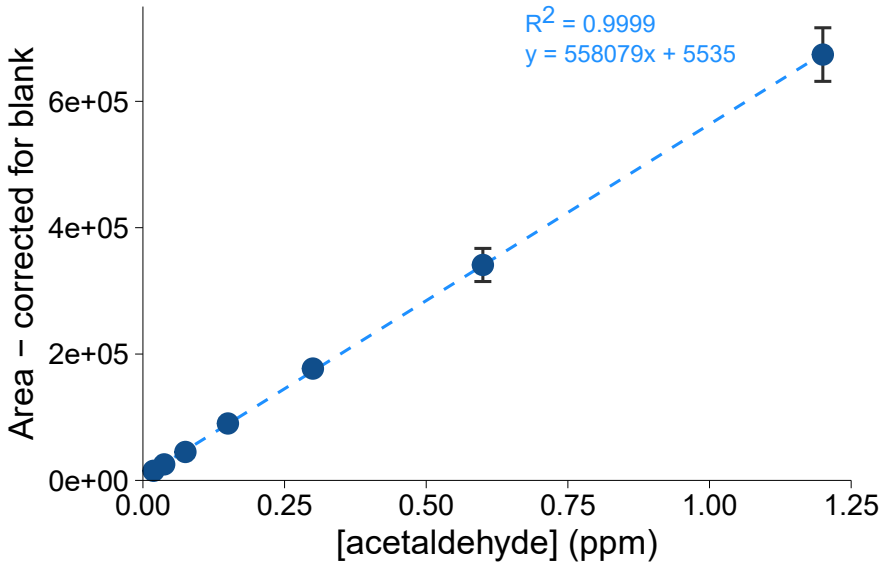

Figure 5: *Calibration curve of derivatized acetaldehyde standards, prepared using standard addition. The measured area values were subtracted with the area value of the unspiked blank.*

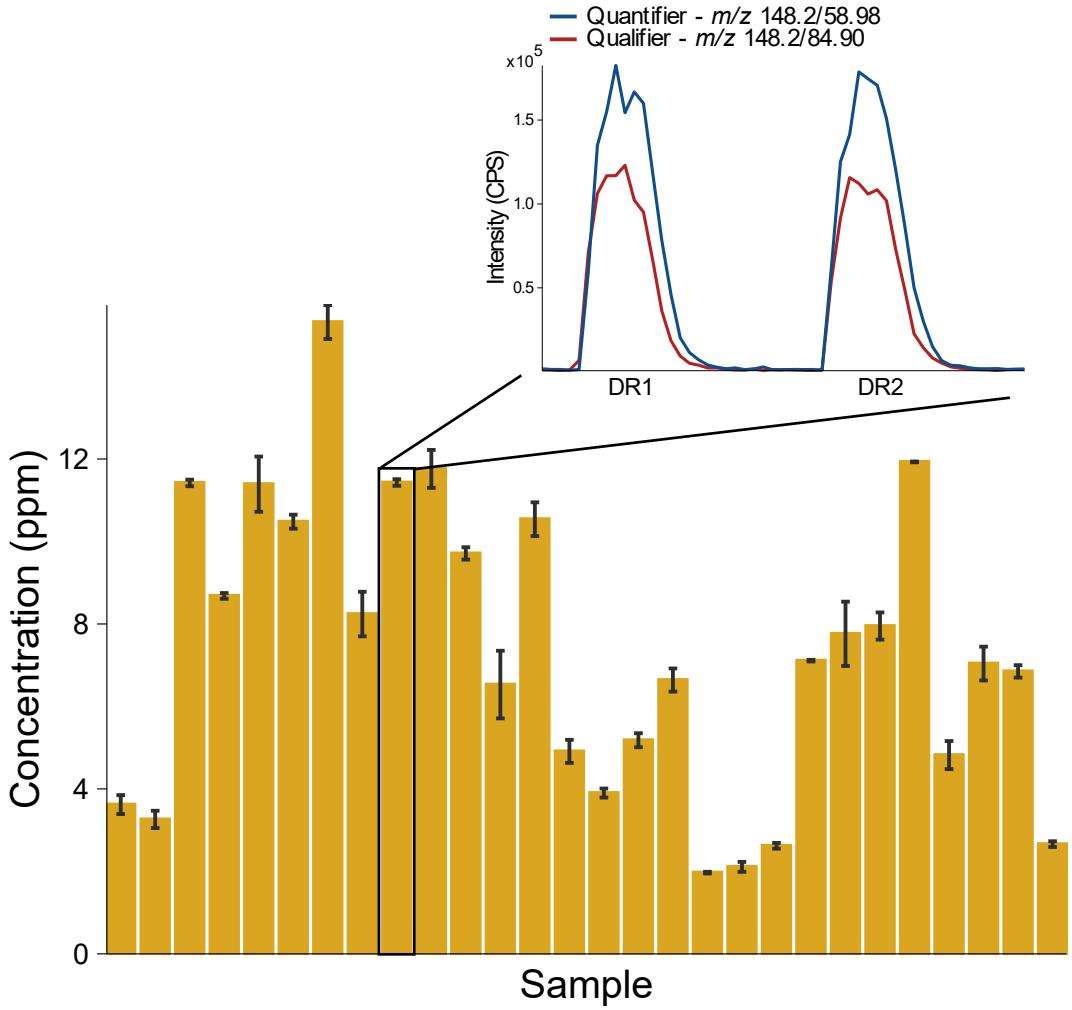

Figure 6: *Acetaldehyde concentrations (ppm) of different fermentation samples using a variety of engineered S. cerevisiae strains, corrected for matrix dilution before derivatization and measurement. The insert shows the extracted-ion chromatogram (XIC, not smoothed) for two separately derivatized replicates (DR) of sample 9, with the quantifier shown in blue, the qualifier in red. Error bars signify standard deviation of two separately derivatized replicates.*

concentrations found in our fermentation samples here, using the L-cysteine derivatization method in combination with the Echo® MS system, corresponds well with the range of 4-15 ppm acetaldehyde content found in commercial beer samples.

A main advantage of the Echo® MS system is the possibility to measure acetaldehyde accurately and quickly without carryover, which allows for samples with low acetaldehyde contents to be measured directly after highly concentrated samples without a meaningful increase in standard deviation. When looking at the obtained concentrations and standard deviations of the batch of samples, ordered chronologically based on injection and measurement, we clearly see little to no carryover from sample to sample (**Figure 6**).

Conclusions

- The Echo® MS system enables fast, high-throughput sample measurements without compromising sensitivity of the detection and quantitation of the compounds of interest.
- The currently used derivatization protocol provides easy sample preparation and high repeatability.
- The combination of the system's sensitivity and sample dilution protocol supports measurements of acetaldehyde concentration over a wide linear range with limited matrix effects.

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