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Direct SPME GC-MS determination of volatile congeners in wines without sample pre-treatment

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Abstract. In this study, the "Ethanol as an Internal Standard" method was employed for the SPME GC-MS quantification of volatile congeners in wines. This method is fast, cheap, and simple, as it requires no sample pre-treatment beyond dilution with water. A series of standard solutions containing 10 commonly encountered wine congeners was prepared gravimetrically to test the method's linearity and sensitivity. The proposed method was then compared to the traditional internal standard (IS) method, using 1-pentanol as the IS compound. Although the precision and linearity for some compounds were slightly lower when using the proposed method, these issues can be mitigated through further optimization of the SPME methodology. In terms of accuracy, the proposed method exhibited similar recoveries to the traditional IS method. Ten real wine samples (five white and five red) produced in Austria were simultaneously analysed using both IS methods for comparison. The results showed that over 90% of the obtained data (mean concentrations of individual congeners from both IS methods) did not differ significantly (p=0.05). Given its significant advantages, the "Ethanol as an Internal Standard" method is recommended for routine wine analysis.

1. Introduction

Volatile congeners are formed during the wine manufacturing process and play a crucial role in shaping the taste and aroma of the final product. These compounds are typically simple organic molecules with various functional groups, such as alcohols, aldehydes, ketones, esters, terpenes, and others. A key quality test for any wine product is the quantitative determination of volatile congeners, as they not only impact the wine's organoleptic properties but can also be harmful (e.g., methanol, acetaldehyde). Additionally, the profile of volatile compounds (i.e., their abundance and concentration) often serves as a "fingerprint" of the product, verifying its origin and quality [1,2].

1.1. Determination of volatile compounds

When it comes to spirit drinks, various distilled products, and pure ethanol, the determination of regulatory-monitored volatile substances is typically performed using gas chromatographic (GC) methods. This procedure is relatively simple because the absence of non-volatile substances in these samples allows their direct injection into the GC for subsequent quantification. GC methods are fast, relatively inexpensive, and highly efficient, making them a popular choice due to the wide range of sampling techniques, columns, and detectors available.

However, the situation is different for wines. Unlike distilled drinks, wines present a more complex matrix that includes non-volatile compounds. This complexity makes wine samples unsuitable for direct injection into a GC system.

1.2. GC analysis of wines

There are several approaches to overcome the challenge of direct injection of wine into a GC system. The least popular method involves distilling the wine to remove non-volatile compounds. Although this technique is still used for analysing wines, liquors, and other complex beverages, it is relatively complicated, labour-intensive, and time-consuming.

A more efficient approach to GC analysis of wine involves utilising the headspace above the sample. Since volatile compounds tend to evaporate in amounts inversely proportional to their boiling points, the air above the sample can be collected and injected either manually or with an autosampler, as in standard headspace GC analysis. There are various techniques to enhance GC

headspace sample preparation, such as saturating the sample with gas bubbles to increase volatility, heating, or using pressure differences.

Another effective solution is the use of solid-phase microextraction (SPME). This technique is used as a pre-concentration and sampling of volatile compounds either from the headspace or, less commonly in this context, directly from the sample matrix. However, direct extraction from the sample matrix is not suitable for wine due to the presence of non-volatile substances. SPME methods employ fibres coated with different sorbents, which can be polar, non-polar, or a combination of both, much like GC column coatings. This versatility makes SPME a universal and sensitive tool for analysing a wide range of substances. To enhance volatility and sensitivity in SPME methods, researchers often heat the sample, add salt, and optimize extraction time.

1.3. "Ethanol as an Internal Standard" method

The "Ethanol as an Internal Standard" method has been proposed as a modern and valuable tool for the direct quantification of volatile compounds in alcoholic products [3]. This method uniquely employs ethanol itself as the internal standard (IS) compound. While selecting the primary organic component of a sample as the IS is unconventional, extensive validation tests, experiments, and inter-laboratory studies have demonstrated the method's effectiveness for analysing volatile compounds in alcoholic products. The advantages of using the "Ethanol as an Internal Standard" method include:

- There is no need to add IS as ethanol is inherently present in the tested alcoholic sample.
- Concentration of the IS in mg/L of absolute alcohol (AA) units is known and is equal to ethanol density, 789300 mg/L; mg/L AA units are desired units for presenting volatile congeners concentrations
- There is no necessity to establish the ethanol content in any tested sample or measure the density of the sample.

To date, this method has been primarily tested for direct GC injection using FID [4] or MS [3] detectors. However, we aimed to expand its applicability. Specifically, we sought to evaluate the "Ethanol as an Internal Standard" method for SPME GC-MS quantification of volatile impurities in wines.

The primary objective of this experiment was to test the method's suitability for SPME GC-MS quantification of volatile compounds without any sample preparation. To enhance the study's value, we also included 1-pentanol as a traditional IS compound and compared the results obtained from both methods.

2. Materials and methods

2.1. GC instrument and measuring conditions

Measurements were performed on Agilent 7890A gas chromatograph coupled to 5875C MS detector. Separation of compounds was performed on 60 m*0.25 mm*1.4 µm DB-624 column (Agilent). The following oven program was used: initial isotherm of 40°C held for 3 min was then raised to 220°C at 30°C/min rate and final isotherm for 3 min. Total analysis time was 12 min. Helium was employed as a carrier gas with a constant column speed of 35 cm/s. Injections were performed manually with a manual SPME holder. Injector temperature was 200°C. Injections were performed in splitless mode (6 s).

MS detector was employed in a scan mode at a 29-400 m/z range. The exception was ethanol which was registered at its elution time window with M+1 ion (47 m/z). This allowed us not to saturate the MS detector with signal of ethanol which is presented in samples in high amounts.

2.2. Standard solutions and calibration

For the sake of calibration purposes and to check the linearity and sensitivity of two IS methods we have gravimetrically prepared a row of standard solutions of volatile compounds in 13% v/v water-ethanol matrix. After corresponding literature survey, ten most spread volatile substances found in wine were selected: 1-butanol, 1-hexanol, 2-propanol, 2-methyl-1-propanol (isobutanol), 3-methyl-1-butanol (isoamylol), acetaldehyde, ethyl acetate, linalool, methanol, octanoic acid.

Seven standard solutions containing abovementioned volatile compounds at 10-2500 mg/L AA concentrations (1-350 mg/L) were prepared by pipetting individual substances into 13% water-ethanol matrix and subsequent dilution of the obtained solution with the same matrix.

"Ethanol as an Internal Standard" method doesn't require any addition of the IS into tested sample. However, as we also used traditional IS method and prepared a solution of 1-pentanol in 13% v/v water-ethanol matrix. This solution was then added to measured wine samples.

Calibration was performed in the following way. Two methods of IS were used in this work: ethanol and 1-pentanol. For both methods a single-point calibration approach was used, i.e., calibration according to measurements of one standard solution. Solution used for calibration contained analysed compounds at concentrations around 600 mg/L AA. Calibration solution was measured four times under repeatability conditions. Relative response factors (RRF) were then calculated for each *i*-th analyte for both IS compounds according to the following common expression:

$$RRF_i = C_i / A_i * A_{IS} / C_{IS}, \quad (1)$$

where C is a concentration, mg/L AA, and A is the peak area, a.u.

2.3. Real wine samples analysis

Ten real wines produced in Austria were measured in this work, five red and five white wine samples. Measurements were performed in a following way: 50.0 µL of 1-pentanol standard solution were added to 4.00 mL of analysed wine. Then, 100 µL of the obtained solution was mixed with 400 µL of distilled water in a 5 mL glass

vial. Obtained mixture was stirred manually and SPME fibre was then immediately introduced into the headspace area of the sample (Figure 1). Extraction lasted for 5 minutes at a room temperature.

Figure 1. SPME process of a red wine sample.

2.4. Recovery tests

In order to evaluate accuracy of two methods we spiked four wine samples with different standard solutions (from i. 2.2). The spiked wines were then treated and measured by the same manner as written in paragraph 2.3. Because of the great variety of differences of initial and spiked concentrations of analytes recovery was calculated by using one of two following equations:

$$R_{i,\,Total} = (C_{i,\,Meas} - C_{i,\,0}) / C_{i,\,Add} * 100\% \qquad (2)$$

and

$$R_{i,\,Rel} = C_{i,\,Meas} / C_{i,\,Theor} * 100\%, \qquad (3)$$

where $C_{i,\,Meas}$ is measured concentration in spiked sample, $C_{i,\,0}$ is a concentration in original wine, $C_{i,\,Add}$ is added spiked concentration and $C_{i,\,Theor} = C_{i,\,0} + C_{i,\,Add}$.

Equation (2) was used when $C_{i,\,Add} / C_{i,\,0}$ ratio was within the 0.1-10 range. Otherwise, Eq. (3) was used.

3. Results and discussions

3.1. Calibration, linearity and detection limits

After performing single-point calibration and establishing RRF values, seven standard solutions of 10 analytes were measured to evaluate the linearity of the two methods. Linearity functions were plotted by comparing the C_i/C_{IS} ratio (y-axis) with the A_i/A_{IS} ratio (x-axis).

The two IS methods exhibited different behaviours depending on the analyte. The "Ethanol as an Internal Standard" method produced linear curves with $R^2 > 0.999$ for the following compounds: acetaldehyde, methanol, 2-propanol, and linalool. Conversely, the traditional IS method showed linear curves for ethyl acetate, isobutanol, 1-butanol, isoamyl alcohol, and 1-hexanol. For each method, non-linear regression curves were observed for the remaining analytes. In most cases, the linearity was disrupted at the 2-3 highest concentration points. Since many of the analytes in the wine samples never reached such high concentrations, we decided to exclude these points from the calibration curves to improve the results. For future experiments, higher sample dilutions and the preparation of more diluted standard solutions are recommended, given the high sensitivity and efficiency of the SPME method.

Detection limits (LOD) and quantitation limits (LOQ) were evaluated by measuring the standard solution with the lowest analyte concentration 10 times under repeatability conditions and calculating the standard deviations (SD) of the results. For all 10 analytes, the traditional IS method using 1-pentanol yielded slightly better LOQs, averaging 2.4 ± 0.6 mg/L, compared to 3.2 ± 0.6 mg/L using the "Ethanol as an Internal Standard" method.

3.2. Wine samples analysis

Ten real wine samples were analysed using both IS methods simultaneously. The obtained analyte concentrations were then compared using Student's t-test for independent samples to determine whether there were any significant differences between the two methods. It was found that 8 out of 81 pairs of volatile concentration results (averages of 4 repeated measurements) did not support the hypothesis that the two IS methods produce no significant difference at $p = 0.05$. This means that for over 90% of the values, the “Ethanol as an Internal Standard” method yielded results that were not significantly different from those obtained using the traditional IS method with 1-pentanol.

We also evaluated the precision of the two methods by comparing the relative standard deviations (RSDs) of the results across the 4 repeated measurements. The results are presented in Figure 2 in the form of a box plot.

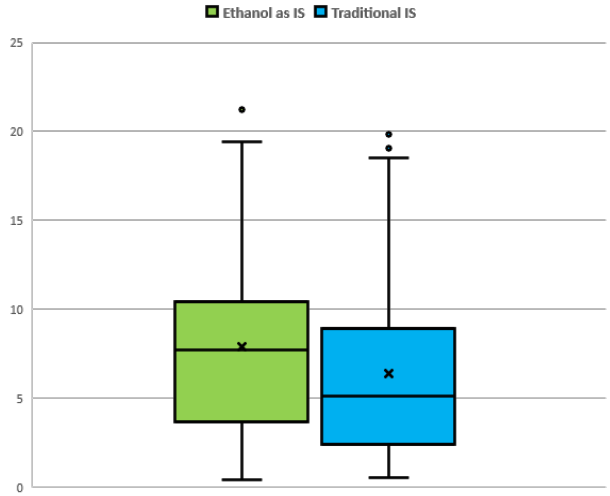

Figure 2. RSDs of results for two IS methods.

Analysis of the obtained results revealed that the “Ethanol as an Internal Standard” method was characterised with slightly worse precision in comparison with the traditional IS method.

Besides 10 analytes that were quantified in this work we also registered and identified 44 other volatile compounds in measured wine samples. This indicates satisfactory sensitivity of the used SPME methodology even despite the fact of absence heating the sample or adding salt as it is usually done while other SPME measurements to increase compounds volatility.

3.3. Recovery tests

Results from the recovery tests are presented below in a form of radar charts (spider net).

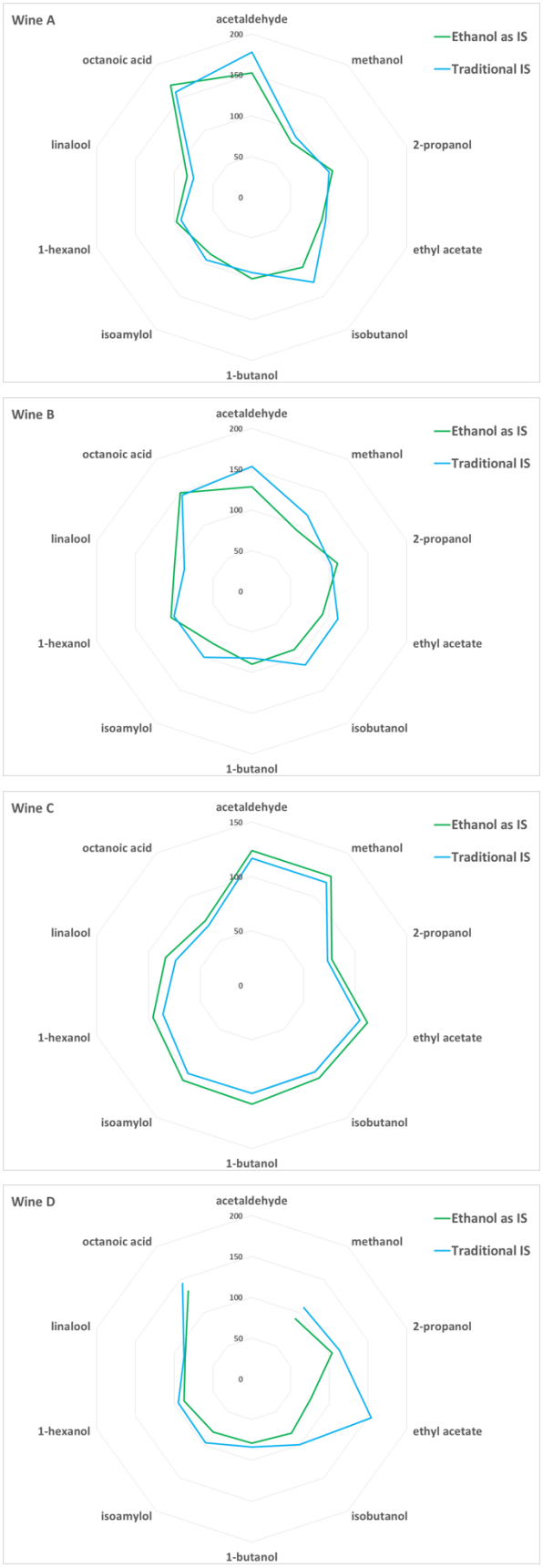

Figure 3. Results of the recovery tests for 4 wine samples.

The results presented in Figure 3 clearly indicate that the recoveries for both IS methods are very similar. However, results for acetaldehyde in the wine D sample are missing due to an observed anomaly. It appears that a possible interaction between reactive acetaldehyde and one or more components in wine D led to unusually high recovery values for acetaldehyde: 408% for the "Ethanol as an Internal Standard" method and 490% for the traditional IS method. Given these extreme deviations, we excluded these results from the chart.

4. Conclusions

This work represents the first attempt to quantify volatile compounds in wine using the SPME GC-MS method without any sample preparation, except for dilution with water. The proposed "Ethanol as an Internal Standard" method enables the direct quantification of any volatile compound present in wine in mg/L AA units. This eliminates the need for sample pre-treatment procedures such as IS solution preparation, addition to the test sample, sample density measurement, or volumetric ethanol content determination. The absence of these steps makes the method highly attractive from a practical standpoint, as it saves time, labour, and materials.

In this experiment, we conducted key metrological tests of the suggested method and compared it with the traditionally employed IS method. Although the "Ethanol as an Internal Standard" method showed slightly lower precision and higher detection limits compared to the traditional method, it remained well-suited for the determination of volatile compounds. In over 90% of cases, the results obtained with the proposed method were not significantly different from those obtained with the traditional IS method. Recovery tests also showed comparable results between the two methods. Given the significant advantages of the "Ethanol as an Internal Standard" method, it has strong potential to become a universal approach for SPME GC-MS wine analysis.

To further improve the method's precision and linearity for certain compounds, we plan to optimise the SPME process, such as adjusting the extraction time and water dilution ratio. Unlike many other SPME methods, adding salt or heating the sample is not necessary, as our simple methodology effectively detects not only major compounds but also many others. The absence of extensive sample pre-treatment, aside from water dilution, makes this method a powerful and accessible tool for the safety and quality control of wines.

5. References

1. G. D. Dumitriu, N. L. De Lerma, C. I. Zamfir, V. V. Cotea, R. A. Peinado, LWT, 86, 643-651 (2017)
2. A. Ziółkowska, E. Wąsowicz, H. H. Jeleń, Food Chem., 213, 714–720 (2016)

3. A. Korban, S. Charapitsa, R. Cabala, L. Sobolenko, V. Egorov, S. Sytova, Food Chem., 338, 128107 (2020)

4. S. Charapitsa, S. Sytova, A. Korban, L. Sobolenko, J. AOAC Int., 102, 2 (2019)