



# Perspectives on using ethanol as an internal standard for the quantification of volatile compounds in alcoholic products by headspace SPME–GC/FID without sample preparation

Anton Korban<sup>1</sup> · Lucie Zavoralová<sup>1</sup> · Radomír Čabala<sup>1</sup> · Vladimir Egorov<sup>2</sup>

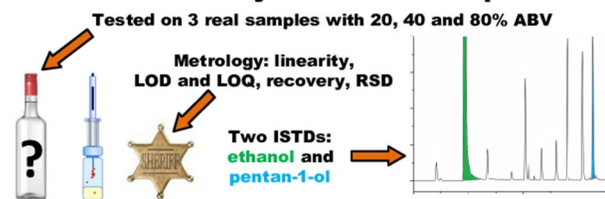
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## Abstract

For the first time, the beneficial “Ethanol as Internal Standard (IS)” method developed for the analysis of alcoholic products was tested in headspace mode of solid-phase microextraction gas chromatography with flame ionization detector (SPME–GC/FID) and compared with the traditional IS method. The extraction and separation conditions were optimized for the analysis of 10 volatile congeners prescribed in European legislation: acetaldehyde, methyl acetate, ethyl acetate, methanol, butan-2-ol, propan-1-ol, 2-methylpropan-1-ol (isobutanol), butan-1-ol, 1,1-diethoxyethane (acetal), and 3-methylbutan-1-ol (isoamylol). To evaluate the accuracy of the compared methods, three real alcoholic product samples with ethanol content of 20%, 40%, and 80% v/v were spiked with gravimetrically prepared standard solutions to calculate recoveries. The obtained data indicates that the suggested method can be employed as a robust and accurate tool for SPME analysis of alcoholic products with low ethanol content, such as wines. It was also observed that an increase in ethanol content in the matrix affects the accuracy of the “Ethanol as IS” method, potentially due to non-linearity of analyte extraction. However, in the case of wine sample, the “Ethanol as IS” method showed significantly better results compared to the traditional approach. The precision of the suggested method, evaluated using relative standard deviations (RSD) of obtained concentrations was found to be comparable to that of the traditional method.

## Graphical abstract

### SPME–GC/FID analysis of alcoholic products



**Keywords** Alcohols · Gas chromatography · Extraction · Volatile compounds · SPME

## Introduction

The determination of volatile congeners plays a vital role in quality control testing for all alcoholic products. International regulations mandate the quantification of volatile congeners using the GC–FID method [1–3]. Traditionally, this involves the utilization of an internal standard (IS) method.

In recent developments, the “Ethanol as IS” method has emerged as an advanced approach for quantifying volatile

✉ Radomír Čabala  
cabala@natur.cuni.cz

<sup>1</sup> Department of Analytical Chemistry, Charles University, Faculty of Science, Prague, Czech Republic

<sup>2</sup> Department of Analytical Chemistry, Belarusian State University, Chemistry Faculty, Minsk, Belarus

compounds in alcoholic products. This innovative method employs ethanol, the primary volatile organic compound, as the internal standard compound. Previous studies have highlighted its remarkable advantages, effectiveness, and suitability for routine implementation in analytical laboratories [4–6].

The analysis of sugar-containing alcoholic beverages, such as wines, liqueurs, or other spirit drinks, typically requires a sample preparation procedure due to the unsuitability of directly introducing such samples into the GC injection port. A common practice involves distilling the sample to separate sugars and other non-volatile compounds from the volatile compounds. Alternative approaches, including the use of headspace and solid-phase microextraction (SPME) methods, have also been explored [7–9]. SPME, as a solvent-free and cost-effective sample preparation technique, has gained popularity in the analysis of volatile compounds in alcoholic products.

In this study, the primary objective was to evaluate the suitability of the "Ethanol as IS" method for HS-SPME/FID analysis of volatile compounds in alcoholic products, marking the first application of this method in such a context. A comparison was made between the suggested method and the traditional IS method using pentan-1-ol.

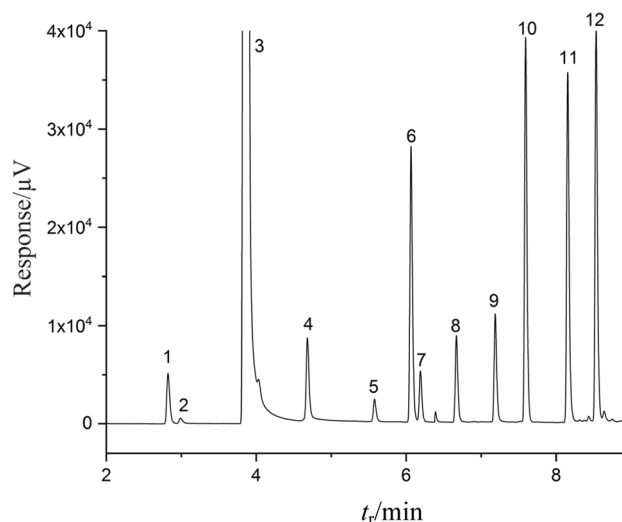
## Results and discussion

### Method optimization

The following SPME fibres from Sigma-Aldrich were tested: CAR/PDMS with 75  $\mu\text{m}$  film thickness (black), PDMS/DVB with 65  $\mu\text{m}$  film thickness (light blue), DVB/CAR/PDMS with 50/30  $\mu\text{m}$  film thickness (grey), PDMS with 100  $\mu\text{m}$  (red) and 30  $\mu\text{m}$  (yellow) film thickness. The model standard solution of 10 analysed volatile compounds was measured with each fibre in order to find the optimal one for this analysis. According to the obtained results, grey fibre of DVB/CAR/PDMS with 50/30  $\mu\text{m}$  film thickness was selected. The example of the chromatogram of standard solution "700" obtained on this fibre type is presented in Fig. 1. Although the black fibre provided larger overall responses, it was not selected due to much higher analytes peak areas resulting in poorer resolution of certain peaks, particularly for acetaldehyde and methanol.

### Linearity and sensitivity

Regulation [1] stipulates that calibration plot should be checked and the obtained linear plot should be characterised with a correlation coefficient ( $R$ ) of at least 0.99. To comply with this requirement, six prepared standard solutions were measured each in triplicate, and linear plot



**Fig. 1** The chromatogram of the prepared standard solution "700". 1—acetaldehyde, 2—methanol, 3—ethanol (IS #1), 4—methyl acetate, 5—propan-1-ol, 6—ethyl acetate, 7—butan-1-ol, 8—2-methylpropan-1-ol, 9—butan-2-ol, 10—acetal, 11—3-methylbutan-1-ol, 12—pentan-1-ol (IS #2)

graphs were generated by plotting the analyte to IS concentration ratio on the X-axis and the analyte to IS detector response ratio on the Y-axis. The calculated values of the correlation coefficient for each calibration curve are presented in Table 1. Based on the presented data, it can be inferred that both methods led to similar correlation coefficients. The required 0.99 value was not obtained only in case of acetaldehyde when the "Ethanol as IS" method was employed.

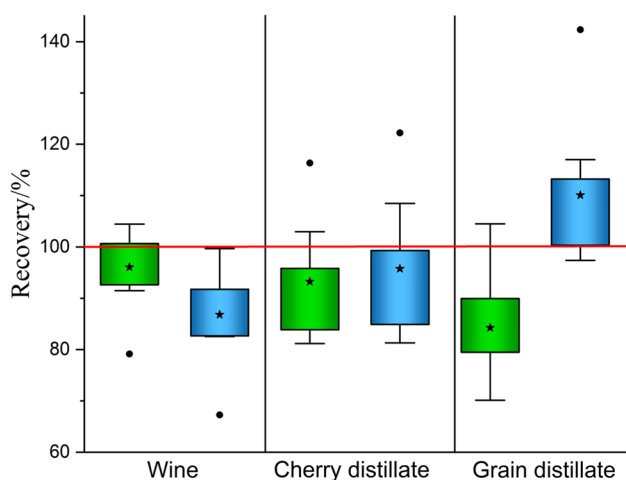
Sensitivity of the two methods was evaluated by calculating limits of detection (LODs) and quantification (LOQs) that are presented in Table 1. The LODs and LOQs values for ethanol were significantly lower compared to pentan-1-ol, except for methanol, which displayed slightly better results for the traditional IS method. This finding suggests that the use of ethanol as the internal standard may offer advantages in terms of higher sensitivity for most analytes.

It is hypothesized that the lower detection and quantification limits observed in the suggested method may be attributed to the elimination of sample pre-treatment steps. In contrast to the traditional method, which involves the addition of three equal pentan-1-ol solution aliquots of 30  $\text{mm}^3$  to the "50" standard solution, the suggested method does not require such additional manual procedures. The gravimetric sample preparation approach used in the traditional IS method introduces extra uncertainties, which could potentially contribute to higher LODs and LOQs values. By circumventing these additional steps, the suggested method may provide more accurate and precise results, leading to lower detection and quantification limits.

**Table 1** The obtained correlation coefficients (*R*) for both IS methods and indication, whether the value suits regulation requirements (+) or not (-) and limits of detection and quantification for both IS methods expressed in mg/dm<sup>3</sup> of absolute alcohol (AA) units

Compound	Correlation coefficient		Limit of detection mg/dm <sup>3</sup> AA		Limit of quantification mg/dm <sup>3</sup> AA	
	Ethanol as IS	Traditional IS	Ethanol as IS	Traditional IS	Ethanol as IS	Traditional IS
Acetaldehyde	0.986 –	0.996 +	4.0	11.1	13.4	37.0
Methanol	0.996 +	0.994 +	19.0	15.0	63.5	50.0
Methyl acetate	0.995 +	0.994 +	18.2	23.2	60.8	77.2
Propan-1-ol	0.997 +	0.998 +	4.6	16.2	15.2	54.0
Ethyl acetate	0.995 +	0.996 +	9.0	21.7	29.9	72.4
Butan-1-ol	0.998 +	0.999 +	7.3	16.9	24.2	56.5
Isobutanol	0.998 +	0.998 +	4.9	17.1	16.3	57.0
Butan-1-ol	0.998 +	0.999 +	3.5	15.7	11.6	52.2
Acetal	0.992 +	0.996 +	12.3	14.7	41.1	48.9
Isoamylol	0.997 +	1.000 +	6.5	14.0	21.6	46.5
Mean			8.9 (4.1) <sup>a</sup>	16.6 (2.6)	29.7 (13.7)	55.2 (8.6)

<sup>a</sup>Half width of confidence interval (95%)



**Fig. 2** Recovery values for the “Ethanol as IS” (green) and the traditional IS (blue) methods presented in a form of box plots. Stars show the average values and dots outside boxes are outliers (colour figure online)

## Recovery

Recovery was calculated in order to evaluate the accuracy of two used methods. Three real alcoholic samples of various matrixes and profile of volatile compounds were measured. They were: wine sample [19.5% alcohol by volume (ABV)], cherry distillate (42.5% ABV) and homemade grain distillate (81% ABV). Each alcoholic product sample was spiked with the “700” standard solution and pentan-1-ol standard solution. The obtained recovery values for all ten analysed volatile compounds are presented in Fig. 2 in a form of box plots.

Analysis of the data presented in Fig. 2 yields the following conclusions. In the case of analysing wine sample, the “Ethanol as IS” method demonstrated compliance with the legislative requirements for recovery levels for all analytes, except for acetal (an outlier point for both methods). In contrast, the traditional IS method exhibited significantly poorer results, with 6 out of 10 recoveries falling outside the acceptable  $\pm 10\%$  limit. The calculated relative standard deviations (RSDs) for all analytes for the “Ethanol as IS” method and the traditional IS method were  $2.4 \pm 0.7\%$  and  $3.2 \pm 1.0\%$ , respectively, indicating similar precision of the two methods. As it was earlier mentioned, acetal showed the least satisfactory results for both methods among all the compounds analysed. This discrepancy can potentially be attributed to undesirable chemical reactions between acetal and acids or other compounds present in the wine matrix.

Cherry distillate exhibited poorer results for both methods, as neither method met the recovery limits requirement for five out of ten analytes. The repeatability was also inferior for both methods, with RSD values of  $8.5 \pm 2.9\%$  and  $10.4 \pm 3.7\%$  for the “Ethanol as IS” and the traditional IS methods, respectively.

Grain distillate yielded the worst results overall. The “Ethanol as IS” method achieved  $\pm 10\%$  recovery values for only two analytes, compared to six for the traditional method. The RSD values were  $5.6 \pm 2.1\%$  and  $5.8 \pm 2.6\%$  for the suggested and conventional methods, respectively. The poor results for grain distillate can be attributed to the high ethanol content in the matrix, which undoubtedly influences the sorption processes of the SPME fibre. One possible solution could be to dilute the sample with water to decrease the ethanol content and improve the accuracy of the measurements.

## Conclusion

This paper presents the first application of the “Ethanol as IS” method in headspace SPME-GC/FID analysis for quality control of alcoholic products. The method was compared to the traditional IS method in terms of correlation coefficients, limits of detection and quantification, precision, and accuracy. The suggested method exhibited similar correlation coefficients and demonstrated significantly improved LODs and LOQs in comparison with the traditional method. The precision, as indicated by the RSD, was similar for both methods across all analysed samples. Accuracy assessment involved spiking three real alcoholic samples with ABV ranging from 20 to 80%. The results revealed that the “Ethanol as IS” method outperformed the traditional IS method in wine analysis, providing superior accuracy. For the 40% ABV sample, both methods showed similar accuracy. However, the “Ethanol as IS” method was found to be less suitable for grain distillate samples, possibly due to the high ethanol content in the matrix, which could impact sorption processes. Nevertheless, given the widespread use of SPME methods for volatile analysis in wine, the “Ethanol as IS” method holds great potential as a robust and effective tool for such analyses. Further studies should focus on enhancing the precision of the method and addressing the challenges associated with analysing alcoholic products with higher ABV values.

## Experimental

### Instrument parameters

A Shimadzu GC-2010 chromatograph equipped with FID was used for chromatographic measurements. Injections were performed with a standard SPME holder. Samples were injected into the GC system at 210 °C in the split (1:15) mode. Rxi-624 Sil MS capillary column (30 m length, 0.25 mm ID, and 1.4 µm phase thickness) was used for separation. The selected column allowed satisfactory separation of all examined volatile compounds, ethanol and pentan-1-ol. Hydrogen obtained from NM-600 hydrogen generator (VICI DBS, Italy) was employed as the carrier gas (99.9999% purity) at a 35 cm/s speed; column flow was 1.89 cm<sup>3</sup>/min, purge flow was 4.1 cm<sup>3</sup>/min.

The following oven temperature program was used: 40 °C for 3 min, rising by 20 °C/min to 160 °C, hold for 1 min. The FID was operated at a temperature of 250 °C. Example chromatogram of solution 700 is presented in Fig. 1.

### Preparation of standard solutions

The following common volatile compounds stated in EC legislation [1] were analysed: acetaldehyde, methyl acetate, ethyl acetate, methanol, butan-2-ol, propan-1-ol, 2-methylpropan-1-ol (isobutanol), butan-1-ol, acetal (1,1-diethoxyethane), and 3-methylbutan-1-ol (isoamylol). Pentan-1-ol was employed as a traditional IS. All chemicals mentioned above and ethanol with 96.4% ABV were of more than 99.5% GC purity (Sigma-Aldrich or Merck). Deionised water (18 MΩ cm) was used for the preparation of calibration solution. Standard solutions were prepared gravimetrically by adding individual substances into ethanol or water–ethanol matrix of the desired ABV value. Six standard solutions of ten volatile compounds in 40% ABV matrix were gravimetrically prepared: “50”, “200”, “650”, “700”, “2000”, and “4000”. Numbers shows the target concentration of analytes in mg/dm<sup>3</sup> AA units.

### Sample analysis

Each sample was analysed according to the following procedure. 100 mm<sup>3</sup> of the tested sample were mixed with 300 mm<sup>3</sup> of deionised water in a 5 cm<sup>3</sup> glass vial. Then, 13.3 mm<sup>3</sup> of pentan-1-ol standard solution were added to the measured sample. SPME extraction performed for 5 min at a room temperature.

### Metrological parameters

The requirements of the EC 2870/2000 to the calibration plot were evaluated by calculating correlation coefficients. Each of six calibration solutions was measured in triplicate. Limits of detection and quantification were calculated as prescribed in the Eurachem guide [10]. According to the guide solution with low analyte concentrations should be measured ten times under repeatability conditions. However, in case of a headspace method, measuring the same solution for so many times could lead to significant evaporation of analytes and thus to incorrect results. That is why the experiment was modified in order to avoid this undesirable process. For this, three equal aliquots of the solution “50” with the lowest analytes concentration were prepared (i.e. adding water and pentan-1-ol solution). Each of three samples was measured three times under repeatability conditions thus resulting in total nine measurements. The standard deviation among nine analyte concentration values was then multiplied by 3 and 10 to calculate LOD and LOQ, respectively. Recovery was also calculated according to the Eurachem guide as the ratio of experimentally obtained and theoretically calculated analyte concentration after the analyte was spiked [10]. Measurements for recovery were also performed in triplicate.

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**Data availability** These data are collected by the experiment. The data that support the plots within this paper and other findings of this study are available from the corresponding authors upon reasonable request.

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