



Rapid quantification of higher alcohols in wine, port wine and brandy by HS-GC-FID

Olena Shevchuk¹, Timon Huybrighs² and Cristina Fernandes¹

¹Sogrape Vinhos S.A., Rua 5 de Outubro, 4527, 4430-809 Avintes, Portugal

² PerkinElmer, Inc., Imperiastraat 8, B-1930 Zaventem, Belgium

Abstract. In response to the growing demand for rapid, precise, and efficient methods for the quantification of volatile compounds in alcoholic beverages, this study presents a novel approach for the determination of higher alcohols in wine, port wine, and brandy. An optimised headspace gas chromatography with flame ionisation detection (HS-GC-FID) method was used to quantify 9 compounds of interest including acetaldehyde, ethyl acetate, methanol, 2-butanol, n-propanol, isobutanol, allyl alcohol, n-butanol, and amyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol). The method uses a minimal sample volume of 10 μ L, requiring only 18 minutes analysis, and was found to be linear up to 800 mg/L for most compounds with an average relative standard deviation below 10% for repeatability tests at low concentrations. No significant difference in the concentrations of higher alcohols was observed when comparing the results of 9 different samples with the results obtained by the OIV method. The method with minimal sample volume required for routine quality control.

1. Introduction

The intricate composition of beverages such as wine, port wine, and brandy results from a complex interaction of numerous chemical compounds, each contributing distinctively to the sensory characteristics and overall quality of these beverages. In particular, wine production is based on the alcoholic fermentation of grape juice, an anaerobic process of sugar degradation carried out mainly by Saccharomyces cerevisiae. Key metabolites of alcoholic fermentation include not only ethanol, but also compounds such as higher alcohols and their acetates. diacetyl, acetoin, volatile fatty acids and their esters and acetaldehyde [1-3]. More than 1000 volatile compounds have been identified in wine, covering a wide spectrum of polarity, solubility, and volatility, with concentrations ranging from nanograms per litre to much higher levels, such as grams per litre [4]. Among these, higher alcohols, esters, and aldehydes are particularly important for influencing the aroma and flavour [2]. Compounds such as acetaldehyde, ethyl acetate, methanol, n-propanol, amyl alcohols, n-pentanol, and n-hexanol are frequently noted for their significant effects on aroma, flavour, and potential health effects [5].

Acetaldehyde, produced during fermentation by yeast and acetic acid bacteria, is a key intermediate in the metabolic pathway from ethanol [3, 5]. Moreover, acetaldehyde is the most abundant aldehyde in spirits. Although characterized by a fruity aroma, at higher concentrations it can lead to undesirable off-flavours, resulting in a very pungent and unpleasant taste. Acetaldehyde is harmful to human health, and has psychoactive and carcinogenic effects. It is included in the list of impurities due to its potential genotoxic and carcinogenic properties when in direct contact with tissues [3, 6].

Similarly, ethyl acetate, the most common ester in wine, is formed by the reaction between ethanol and acetic acid [1]. It contributes to the fruity and floral notes in young wines, enhancing their complexity. However, excessive ethyl acetate lead to off-flavours similar to nail polish remover, detracting from the wine's quality.

Methanol, present in trace amounts in wine, primarily derives from the hydrolysis of pectins in grape skins during fermentation [7, 8]. While methanol is a natural component of wine, its concentration must be carefully monitored due to its toxicological implications. Methanol can be converted into the highly toxic metabolite formic acid, which, when accumulated, can cause nerve damage, blindness, and respiratory failure [9]. Due to its toxic properties, the maximum concentration of methanol is regulated, for example, by EU regulations (ECC 1576/89) for spirits and by the International Organisation of Vine and Wine (OIV) for wines.

Higher alcohols such as n-propanol, amyl alcohols (including isoamyl alcohol and active amyl alcohol), npentanol, and n-hexanol are produced during fermentation and contribute to the fusel oil content in wine [1, 5]. These compounds play a crucial role in defining the wine's aroma and flavour profile. At low concentrations, they add complexity and desirable sensory attributes, but at higher levels, they can impart harsh, solvent-like characteristics [1].

The quantification of these compounds is essential for several reasons. Firstly, their concentrations can serve as indicators of fermentation quality and potential spoilage. For instance, elevated levels of acetaldehyde and ethyl acetate can signal oxidative stress or microbial contamination. Secondly, understanding the concentration of these compounds helps to ensure the sensory quality of the wine, as both desirable and undesirable flavours are closely linked to their presence. Lastly, from a regulatory perspective, monitoring compounds such as methanol is crucial to ensure consumer safety. For example, the International Organisation of Vine and Wine (OIV) specifies 400 mg/L for red wines and 250 mg/L for white and rosé wines as maximum allowable concentrations for methanol. In addition to international standards, individual countries may have their own regulations governing the production and sale of alcoholic beverages. For example, the European Union has strict regulations on the permitted levels of various contaminants and additives in wine and spirits.

Traditional methods for quantifying these compounds, such as direct liquid injection gas chromatography (GC) and colorimetric methods, often face limitations in terms of sensitivity, specificity, and sample preparation time. Headspace gas chromatography coupled with flame ionisation detection (HS-GC-FID) has emerged as a robust and efficient technique for the rapid quantification of volatile compounds in complex matrices such as alcoholic beverages. This method offers several advantages, including minimal sample preparation, reduced matrix interferences, and high sensitivity for organic compounds.

2. Materials and methods

2.1. Chemicals

All standards at \geq 99% purity (acetaldehyde, ethyl acetate, methanol, 2-butanol, n-propanol, isobutanol, allyl alcohol, n-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and ethanol (solvent) were purchased from Sigma-Aldrich (Milan, Italy).

2.2. Sampling

In this study, a total of nine different samples were analysed, including red wine, white port and red port wines, as well as brandy. Additionally, four samples from different matrices - white port, pineau, dry rosé wine, and aromatised wine - were analysed. These samples were provided by the Bureau Interprofessionnel d'Études Analytiques (BIPEA), a European scientific organisation. BIPEA provides international interlaboratory ring tests to quantify compounds such as acetaldehyde, ethyl acetate, methanol, 2-butanol, n-propanol, isobutanol, allyl alcohol, n-butanol, and amyl alcohols across various beverage matrices.

2.3 Standard solutions and samples preparation

Samples and standard solutions were prepared by mixing 10 mL of the sample or standard solution with 1 mL of the internal standard solution. Then, 10 μ L of the resulting mixture was pipetted into a headspace flask. Calibration was performed using six levels, ranging from 0,50 to 800 mg/L, which were obtained by diluting the stock solution with a 20% (v/v) ethanol hydroalcoholic mixture. 4-Methylpentan-2-ol was selected as the internal standard (IS) because it is not typically present in the matrices analysed and demonstrates good chromatographic separation from the target compounds.

2.3. Chromatographic conditions

The samples and standards were analysed using a total vaporization technique employed within the static headspace method. 10 μ L of each standard or sample were thermostatted at 120°C for 15 minutes in headspace vials to vaporise the entire sample. Then 120 μ L of the headspace vapour was injected into the GC-FID system for analysis.

The analysis was performed on a PerkinElmer GC 2400 system equipped with an HS 2400 headspace sampler and a flame ionisation detector (FID). Method parameters included an injector temperature of 150°C, with helium as the carrier gas at a column flow rate of 2.5 mL/min and a split flow of 5 mL/min. Chromatographic separation was performed using an Elite-WAX ETR column (60 m x 0.32 mm I.D. x 1.0 μ m film thickness, PerkinElmer, Waltham, MA, USA). The column oven temperature programme began at 40°C, increased to 70°C, and then ramped up to 180°C. The FID temperature was maintained at 250°C.

Data acquisition and analysis were performed using the Simplicity Chrom software from PerkinElmer.

2.4. Method validation

The optimised method for determining the concentrations of nine volatile compounds using HS-GC-FID was validated according to the OIV (International Organisation of Vine and Wine) method validation guidelines, ensuring adherence to international standards in the wine industry [10, 11]. The validation process included the assessment of several critical parameters to confirm the method's reliability for routine use, such as linearity, limit of detection (LOD), limit of quantification (LOQ), reproducibility and accuracy.

2.4.1. Linearity

The linearity of the method was evaluated using a calibration curve based on five different concentrations of the standards, with all analyses conducted in triplicate. The linear range was determined by plotting the ratio of the chromatographic peak area of each standard to the area of the internal standard (4-methyl-2-pentanol) against the concentration ratio. Linearity was expressed by the square coefficient of determination (r^2).

2.4.2. Limit of detection and limit of quantification

The limit of detection (LOD) is the smallest concentration of an analyte in a test sample that can be reliably distinguished from zero. The LOD was determined by identifying the lowest concentration at which a chromatographic peak is observed with a height three times greater than the baseline noise. The limit of quantification (LOQ) is defined as the lowest concentration on the calibration curve that achieves a signal-to-noise ratio of 10.

2.4.3. Reproducibility and accuracy

To assess the method's reproducibility, both intra-day and inter-day precision measurements were conducted. Intra-day precision was determined using 10 consecutive injections (n = 10) of standard compounds in a wine solution, while inter-day precision was assessed by performing three injections on three different days (n = 9). Precision was evaluated at two different concentration levels and expressed as the relative standard deviation (RSD %).

The accuracy of the method was assessed by comparing the results of nine different samples with those obtained using the OIV method. Additionally, participation in the BIPEA interlaboratory proficiency testing program was used to evaluate accuracy, with results being analysed through Z-score.

3. Results and discussion

Several analytical methods, including standardised ones, are available in the literature for the analysis of higher alcohols in alcoholic beverages. Gas chromatography (GC) is commonly used, with either direct liquid injection or headspace injection for sample introduction. This report, describes a headspace (HS) GC-FID method specifically utilising the total vaporization technique, also known as the full evaporation technique, for headspace sample preparation.

In this method, 10 μ L of the sample volume is introduced into the headspace vial. During thermostatting in the HS oven, the sample fully evaporates, leaving only the gas phase in the vial. As a result, no equilibrium is established between the sample and gas phases, allowing a single calibration method to be applied across different sample types.

The HSGC-FID conditions were optimised by adjusting several experimental parameters, including peak area, resolution, peak symmetry, and separation. The optimum values for these are shown in Table 1.

Table 1. Parameters values for HSGC-FID.

Headspace conditions				
Oven temperature	120°C			
Needle temperature	125°C			
Transfer line temperature	135°C			
HS pressure	25 psi			
Thermostatting time	15,0 min			
Pressurization time	2,0 min			
Injection time	0,04 min			
GC cor	ditions			
Injector temperature	150°C			
Injector pressure	21 psi			
Split flow	5 mL/min			
Column	Elite-WAX ETR, 60 m x 0.32 mmID x 1.0 µm df			
GC oven program	40°C, 3°C/min to 70°C, 15°C/min to 180°C			
GC run time	18 min			
FID temperature	250°C			
FID air flow	400 mL/min			
FID H2 flow	30 mL/min			
FID make-up gas (N2) flow	25 mL/min			

3.1. Validation of the method

The proposed HS-GC-FID method was validated for the determination of nine compounds in wine, port wine, and brandy. The calibration standards were selected based on historical data from Portuguese wines, port wines, and brandy.

The signal-response of the GC-FID system was evaluated at five concentration levels using the calibration standard mixtures prepared in 20% (v/v) of ethanol. Analysis was performed in triplicate for every concentration level, and calibration curves were established for each of the considered compounds.

3.1.1. Calibration and Linearity

Calibration plots were created by plotting the relative analyte-to-IS peak area ratio against the relative analyteto-IS concentration ratio. The correlation coefficients (r²) obtained for each compound were always equal to or greater than 0.995, indicating excellent linearity. For example, acetaldehyde had a correlation coefficient of 0.9997, and ethyl acetate had a correlation coefficient of 0.9978. The RSE for the calibration standards was below 10%, indicating high precision of the calibration curve.

Table 2. Retention time, calibration range and correlation coefficient of the proposed method (n=3).

Compound	RT (min)	Range (mg/L)	Correlation Coefficient (r ²)	RSE (%)
Acetaldehyde	4.70	10 - 250	0.9997	1.84
Ethyl Acetate	8.38	5-400	0.9978	2.11
Methanol	8.56	10-600	0.9997	1.06
2-butanol	12.25	1 - 10	0.9961	3.39
n-propanol	12.60	20-75	0.9996	1.34
Isobutanol	13.73	10 - 500	0.9999	1.41
Allyl alcohol	14.15	0.50 - 5	0.9957	7.11
n-butanol	14.69	0.50 - 5	0.9971	7.64
Amyl alcohols	15.67	100 - 800	0.9998	1.05

3.1.2. Limit of detection and limit of quantification

The LOD values ranged from 0.12 mg/L for n-butanol to 9.04 mg/L for amyl alcohols. The LOQ values ranged from 0.41 mg/L for n-butanol to 30.13 mg/L for amyl alcohols. These values indicate the method's sensitivity and its ability to detect and quantify low concentrations of the analytes. For instance, the LOD for acetaldehyde was 0.69 mg/L, and the LOQ was 2.29 mg/L, demonstrating the method's capability to detect even small amounts of this compound.

Table 3. Limits of quantification (LOQ) and limits of detection (LOD) of the proposed method (n = 3).

Analyte	LOD (mg/L)	LOQ (mg/L)
Acetaldehyde	0.69	2.29
Ethyl Acetate	1.59	5.32
Methanol	5.75	19.18
2-butanol	0.24	0.81
n-propanol	1.66	5.53
Isobutanol	3.05	10.18
Allyl alcohol	0.16	0.50
n-butanol	0.12	0.41
Amyl alcohols	9.04	30.13

3.1.3. Reproducibility and accuracy

Repeatability (intra-day precision) and reproducibility (inter-day precision) were assessed by analyzing two samples with different concentrations. The intra-day precision was less than 10% for all analytes, with values ranging from 0.12% for n-butanol to 8.96% for amyl alcohols. The inter-day precision ranged from 0.32% for n-butanol to 9.34% for amyl alcohols. These precision values did not exceed 10%, indicating the method's reliability. For example, the intra-day precision for methanol was 2.53%, and the inter-day precision was 3.53%, showing consistent results over multiple days.

Repeatability and reproducibility were assessed by analysing two samples with different concentrations from the interlaboratory study. The repeatability, or intra-day precision, was evaluated as the relative standard deviation of ten injections and was less than 10% for all analytes, whereas the reproducibility (inter-day precision) was calculated analysing the samples over a period of 3 days. The intra-day precision ranged from 0.12% for n-butanol to 8.96% for amyl alcohols and the inter-day precision to 0.32% for n-butanol to 9.34% for amyl alcohols. Precision values did not exceed 10% for the compounds analysed indicating the method's reliability.

3.1.4. Accuracy

The method's accuracy was validated by comparing the results with those obtained using the OIV method. The comparison showed no significant differences, indicating that the proposed method is accurate and reliable (Table 5 and 6).

The interlaboratory evaluation study for wine samples, expressed in mg/L, provided additional validation (Table 7). For example, the concentration of acetaldehyde in White Port was 44 mg/L with a Z-score of 0.87, indicating good agreement with the expected value. Similarly, the concentration of ethyl acetate in Pineau was 19 mg/L with a Z-score of -1.63, demonstrating the method's accuracy across different wine types.

Analyte	Concentration (mg/L)	Intra-day precision (%)	Inter-day precision (%)
Acetaldehy	25	1.28	1.09
de	50	0.55	0.68
Ethyl	30	1.96	6.41
Acetate	70	3.31	4.43
	175	3.18	5.64
Methanol	350	2.53	3.53
	1	0.12	1.34
2-butanol	5	0.51	1.42
	30	0.50	0.72
n-propanol	50	0.89	1.47
	60	0.44	1.67
Isobutanol	250	0.37	0.90
Allvl	0.50	0.26	0.61
alcohol	2.50	0.24	0.69
	1.50	0.12	0.32
n-butanol	3.00	0.23	1.23
Amvl	100	6.90	6.29
alcohols	300	8.96	9.34

Table 4. Precision results for the proposed method.

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4. Conclusion

In the presented work, a simple and rapid GC–FID method for the determination of the principal volatile compounds in alcoholic beverages has been proposed. The developed method was optimised, and 9 volatile compounds characteristic of alcoholic beverages were quantified in only 18 min.

Using the full evaporation technique offers the advantage of using a single calibration method for different sample types. The fast quantitative determination was achieved by applying the optimised GC-FID conditions, which allowed for good chromatographic separation of the nine target compounds (Fig. 1). Additionally, this method showed good linearity and good precision in the range of approximately 0.50 mg/L to 800 mg/L. Results for different matrices are reported and validated in the interlaboratory study, including wines, and port wines. Further validation steps, such as recovery, specificity, and robustness, should be performed for the additional method validation,

On the basis of the obtained results, the developed GC-FID method can be proposed as a fast and simple quality control procedure available to the industry in order to support the technological choices and improve the winemaking practices and the spirit drink production.

Sample Name	Acetaldehyde (mg/L)		Ethyl Acetate (mg/L)		Methanol (mg/L)	
	Our results	OIV method	Our results	OIV method	Our results	OIV method
Red Wine 1	79.53	60.29	56.87	54.8	177.9	172.83
Red Wine 2	79.95	60.89	58.85	57.85	186.0	184.02
White Port wine	79.27	77.235	31.63	34.33	157.2	151.86
Red Port wine 1	104.44	96.57	100.12	124.285	172.18	176.225
Red Port wine 2	32.41	32.78	43.61	49.62	218.34	226.51
Brandy 1	234.81	230.15	342.0	340.12	139.45	138.56
Brandy 2	37.74	36.47	37.55	34.85	295.41	257.04
Brandy 3	34.29	37.22	34.86	33.255	269.71	249.02

Table 5. Sample analysis results for acetaldehyde, ethyl acetate and methanol for the optimised HS-GC-FID method and OIV method, expressed in mg/L.

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Sample Name	n-propano	l (mg/L)	Amyl Alcohols (mg/L)		
	Our results	OIV method	Our results	OIV method	
Red Wine 1	28.77	29.85	266.71	260.24	
Red Wine 2	31.06	32.16	278.42	282.51	
White Port wine	58.45	48.56	311.55	294.03	
Red Port wine 1	57.31	48.23	392.6	382.85	
Red Port wine 2	49.47	43.92	322.2	325.5	
Brandy 1	152.49	151.85	954.1	955.48	
Brandy 2	107.87	94.055	699.5	591.06	
Brandy 3	97.81	88.595	633.6	590.29	

Table 6. Sample analysis results for n-propanol and amyl alcohols for the optimised HS-GC-FID method and OIV method, expressed in mg/L.

Table 7. Results of the Interlaboratory evaluation study for wine samples, expressed in mg/L.

	White	e Port ^a	Pineau ^a		Dry rosé wine ^a		Aromatized wine ^a	
Compound	Our results	Z-score	Our results	Z-score	Our results	Z-score	Our results	Z-score
Acetaldehyde	44	0.87	21	0.25	69	0.97	49	1.9
Ethyl Acetate	19	-1.63	19	-1.08	32	0.13	30	-0.84
Methanol	134	-0.8	90	-0.9	78	0.24	40	0.47
2-butanol	-	-	-	-	4.7	-1.14	0.7	1.4
n-propanol	54	-1	25	-1	32	0.5	18	1
Isobutanol	93	-1	115	-1.14	27	1.5	15	2
n-butanol	1.1	0.54	1.4	0.76	12.4	1.82	-	_
Amyl alcohols	325	-0.56	423	-1.32	174	1.42	100	1.67

^a Declared by BIPEA



Figure 1. GC-FID chromatogram of standard solution.

5. References

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