CENTENARY OF THE OIV
45th WORLD CONGRESS OF VINE AND WINE
14-18 OCTOBER 2024 FRANCE-DIJON
OIV

Available on iVES Conference Series vine & wine

DOI : https://doi.org/10.58233/72FFNcSH

Validation of a method for the determination of volatile compounds in spirituous beverages using contained ethanol as a reference substance

Siarhei Charapitsa[1], Svetlana Sytova[1], Lidziya Sabalenka[2], Alena Kavalenka[2], Anton Kavalenka[1], Alexander Kolesnov[3]

[1] Institute for Nuclear Problems of Belarusian State University, 220030, Bobruyskaya Str., 11, Minsk, Republic of Belarus
[2] New Analytical Systems Ltd., 220072, Nezavisimosti Ave, 70, Minsk, Republic of Belarus
[3] Peoples' Friendship University of Russia named after Patrice Lumumba, 117198, Miklukho-Maklaya Str., 6, Moscow, Russian Federation

Abstract. The article details a single-laboratory validation study on the gas chromatographic method for determining volatile by-products of alcoholic fermentation, such as acetaldehyde, methanol, methyl acetate, ethyl acetate, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, and 3-methylbutan-1-ol in beverages with ethanol content ranging from 9.9 to 96.0 % ABV (including wine, sake, liquor, tequila, vodka, grappa, bourbon, scotch, calvados, rakia, rum, brandy, whiskey, gin, and rectified ethyl alcohol). The method, validated in accordance with ISO 5725, utilizes ethanol present in the beverages as a reference substance for the quantitative determination of these compounds. Precision and accuracy of the method were evaluated under repeatability and intermediate precision conditions. The analysis of 15 commercial ethanol contained beverages using this method revealed acetaldehyde content ranging from 1.86 to 666 mg/L of absolute ethanol, methanol from 3.31 to 12.231 mg/L of absolute ethanol, and other volatiles from 1.52 to 4.221 mg/L of absolute ethanol. Additionally, the study employed the UniChrom software for processing chromatographic data, which significantly streamlined the analysis process. UniChrom facilitated the automated transfer of chromatographic data into Excel tables, enhancing data accuracy and consistency. Importantly, the validation and implementation of this method did not require any additional material, financial, or labor costs, making it an efficient and cost-effective solution for routine analytical settings.

1. Introduction

The accurate determination of volatile compounds in spirituous beverages is essential for ensuring product quality, compliance with regulatory standards, and consumer safety. Volatile compounds, including aldehydes, esters, alcohols, and other organic substances, contribute significantly to the flavor, aroma, and overall sensory profile of ethanol contained beverages including wines (ECB). However, the presence of these compounds in varying concentrations can also indicate the quality of the raw materials used, the conditions of fermentation and distillation, and the presence of possible contaminants.

Traditional methods for analyzing volatile compounds in ECBs often require the use of internal standards, which can introduce variability and increase the complexity of the analytical process. This complexity is especially pronounced when dealing with beverages that contain a wide range of alcohol content and other matrix components. Therefore, a robust, accurate, and simplified method for the determination of these compounds is of significant interest to both the beverage industry and regulatory bodies.

This study focuses on the validation of a gas chromatographic method that leverages the ethanol naturally present in spirituous beverages as a reference substance. By using ethanol as a reference substance, the method reduces the need for additional standard substances, minimizes potential sources of error, and simplifies the quantification process. This approach not only ensures more reliable and consistent results but also aligns with industry trends toward more sustainable and cost-effective analytical practices.

The method's validation follows the guidelines set forth by ISO 5725 guidelines [1], ensuring that it meets the required standards for precision, accuracy, linearity, and robustness. The study involves the application of this method across a variety of spirituous beverages, including whiskey, vodka, rum, tequila, and others, encompassing a wide range of ethanol concentrations and complex matrices. The results of this validation study demonstrate the method's effectiveness and its potential for widespread adoption in both quality control laboratories and research settings.

By providing a validated, reliable method for the determination of volatile compounds, this research contributes to the enhancement of analytical capabilities in the industry of ECBs. It offers a practical solution that can be easily integrated into routine quality control processes, ensuring that products meet the highest standards of quality and safety.

The aim of this study was to validate the proposed method for determining volatile compounds in spirituous beverages using contained ethanol as a reference substance and to compare it with the official GC-FID method of analysis in terms of precision, accuracy, limits of detection and quantification (LOD and LOQ), linearity, and robustness. The developed method was tested on a range of spirituous beverages to assess its effectiveness across different matrices.

2. Materials and methods

2.1. Reagents and test samples

All chemical standards, along with their respective CAS numbers, were supplied by Sigma-Aldrich (Alcobendas, Madrid, Spain) with a purity exceeding 99 %. The chemicals include acetaldehyde (75-07-0), methyl acetate (79-20-9), ethyl acetate (141-78-6), methanol (67-56-1), propan-2-ol (67-63-0), propan-1-ol (71-23-8), 2-methylpropan-1-ol (78-83-1), butan-1-ol (71-36-3), and 3-methylbutan-1-ol (123-51-3). Impurity concentrations in these volatile compounds were determined using GC-FID for volatile impurities and GC with a thermal conductivity detector (GC-TCD) for water detection, employing the internal normalization method. Rectified ethyl alcohol (96.0 % ethanol by volume) was sourced from Dyatlovo Distillery Plant Algon (Slonim, Belarus). Pure distilled and deionized water (conductivity ≤ 0.5 MΩ·cm) was provided by JSC Integral (Minsk, Belarus).

2.2. Preparation of standard solutions

All standard solutions (SS) were prepared gravimetrically following ASTM D4307 guidelines [2]. These solutions were made in a water-ethanol solution (WES) with a 40 % ABV ethanol concentration, achieved by mixing rectified ethyl alcohol with deionized water. Standard solution A (SS-A) was prepared by adding approximately 200 μg of each volatile compound (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol) to the WES, resulting in analyte concentrations of around 5000 mg/L AA. Standard solutions B, C, D, E, F, and G, with analyte concentrations of approximately 500, 250, 200, 25.0, 10.0, and 2.0 mg/L AA, respectively, were prepared by serially diluting SS-A with WES.

2.3. Preparation of test samples

Samples of ECBs with high sugar content, such as wine and liquor, as declared by the manufacturer, were distilled following the procedure outlined in Appendix I of Commission Regulation (EC) No 2870 [3]. For the wine sample, where the ethanol concentration was provided as a range, the exact ethanol volume concentration was determined according to the method specified in Appendix II [3]. The results of the sample preparation are presented in Table 1.

Table 1. Strength by volume of test samples.

Sample	Strength of sample, % ABV	
	declared	experimental
Bourbon	40	40±0.06
Brandy	40	40±0.06
Calvados	40	40±0.06
Gin	47	47±0.06
Grappa	40	40±0.06
Liquor	18	18±0.06
Rakia	40	40±0.06
Rum	40	40±0.06
Sake	14.5	14.5±0.06
Scotch	40	40±0.06
Tequila	38	38±0.06
Vodka	40	40±0.06
Whiskey	43	43±0.06
White wine dry	9.0-13.0	9.9±0.06

2.4. Instrumentation

Chromatographic analyses were conducted using a Crystal-5000.1 gas chromatograph (JSC SDB Chromatec, Yoshkar-Ola, Russia), equipped with an autosampler, flame ionization detector (FID), and thermal conductivity detector (TCD). A capillary column Rt-Wax, 60 m × 0.53 mm I.D. × 1.0 μm film thickness (Restek, Bellefonte, USA) was used for separations. The injector temperature was set at 190°C, with the oven programmed to start at 75°C (held for 9 min), followed by a ramp of 5°C/min to 130°C, and then 10°C/min to 180°C, with a 5-minute hold at the final temperature. FID and TCD temperatures were maintained at 280°C and 150°C, respectively. Nitrogen (99.999 % purity) served as the carrier gas, with a split ratio of 10:1, and injection volumes of 1.0 μL. Volatile compounds were analyzed by direct injection for ECB such as sake, tequila, vodka, grappa, bourbon, scotch, calvados, rakia, rum, brandy, whiskey, gin, and by injecting distillates in the case of wine and liquor. All standard solutions and beverage samples were measured twice under repeatability conditions. Data acquisition and processing were managed using UniChrom software (New Analytical Systems Ltd, Minsk, Belarus).

3.1. Calibration

The calibration was performed using a one-point calibration method. The calibration solution was a standard solution C (SS-C) with a volatile compound concentration of 250 mg/L. The calibration coefficient – relative response factor for the volatile compounds – was calculated using the following formula.

$$RRF^{Eth} = \frac{A_{Eth(C)}}{A_{(C)}} \cdot \frac{C^{*}_{(C)}}{C^{*}_{Eth(C)}} = \frac{A_{Eth(C)}}{A_{(C)}} \cdot \frac{C^{*}_{(C)}}{\rho_{Eth}}, \qquad (1)$$

where $A_{(C)}$ is the detector response to the volatile compound, obtained during measurement of SS-C, arbitrary units; $A_{Eth(C)}$ is the detector response to the ethanol, obtained during measurement of SS-C, arbitrary units; $C^{*}_{Eth(C)}$ and $C^{*}_{(C)}$ are the concentrations of the ethanol and volatile compound in SS-C, correspondingly, mg/100 mL of anhydrous alcohol (AA); ρ_{Eth} is the density of anhydrous ethanol, ρ_{Eth} = 789300 mg/L.

3.2. Determination of concentration

The concentration of methanol (in mg/L AA) was calculated according to the following equation

$$C^{*} = RRF^{Eth} \cdot \frac{A}{A_{Eth}} \cdot \rho_{Eth}, \qquad (2)$$

where A is the detector response to the volatile compound, arbitrary units; A_{Eth} is the detector response to ethanol, arbitrary units.

3.3. Method validation

3.3.1. Outliers

Cochran's and Grubbs' tests were conducted to identify and eliminate outliers. Initially, an upper-tail Cochran test was applied to compare interlaboratory variances, followed by two-tailed single and paired Grubbs' tests, in accordance with ISO 5725-2 guidelines [1].

3.3.2. Linearity

The linearity of the method was evaluated using the coefficient of determination (R^2) for all volatile compounds, with all results exceeding 0.995.

3.3.3. Accuracy and precision

The precision of the method was assessed in terms of repeatability (intraday) and intermediate precision (interday) following ISO 5725-2 guidelines [1]. Repeatability was evaluated as the relative standard deviation (RSD) from two injections of standard solutions under repeatability conditions. Intermediate precision was determined as the RSD from two injections of standard solutions, conducted over 15 days with variations in time and operators. The accuracy of the method was assessed in terms of recovery, following the ISO 5725-4 guidelines [1].

3.3.4. LOD and LOQ

The determination of *LOD* and *LOQ* was carried out according to the IUPAC Technical report [4] using following formulas

$$LOD = 3 \cdot s, \qquad (3)$$

$$LOQ = 3 \cdot LOD, \qquad (4)$$

where s is the standard deviation of volatile compound concentration, obtained during 30 independent determinations at the lowest concentration level of volatile compound (SS-3), mg/L AA.

3.3.5. Uncertainty

The standard uncertainty, u, of the results was determined following the guidelines provided by Eurolab [5] and Eurachem [6], using the specified formula (5).

$$u = \sqrt{s^{2}_{I(TO)} + s^{2}_{\hat{\delta}} + u^{2}_{ref} + \hat{\delta}^{2}}, \qquad (5)$$

where $s_{I(TO)}$ is the standard deviation of intermediate precision, mg/L AA; s_{δ} standard deviation of bias, mg/L AA; u_{ref} is the uncertainty of concentrations of standard solutions, mg/L AA; $\hat{\delta}$ is the method bias, mg/L AA.

3.4. Test samples

3.4.1. Checking of acceptability of test results obtained under repeatability conditions

The acceptability of test results obtained under repeatability conditions was verified according to ISO 5725-6 guidelines [1]. If the specified condition (section 5.2.2 ISO 5725-6 guidelines [1]) was satisfied, the arithmetic mean of the two results was taken as the final reported value.

3.4.2. Checking of acceptability of test results obtained under both repeatability and intermediate precision conditions

The acceptability of test results obtained under both repeatability and intermediate precision conditions was assessed in accordance with ISO 5725-6 guidelines [1]. If the specified condition (section 5.3.2.2 ISO 5725-6 guidelines [1]) was met, the results obtained under different intermediate precision conditions were deemed acceptable, and the grand mean of the arithmetic means of the two final quoted results was used.

3.4.3. Checking of trueness of obtained test results

The trueness of the test results was evaluated in accordance with ISO 5725-6 guidelines [1]. If the specified condition (section 7.2.3.1.3 of ISO 5725-6 guidelines [1]) was met, the trueness of the obtained results was deemed acceptable.

4. Results and discussion

During the validation phase of the method, outliers were detected and removed at the 500 mg/L AA and 5000 mg/L AA levels for 3-methylbutan-1-ol, which were subsequently excluded to ensure the accuracy and reliability of the data. Calibration and linearity assessment results are shown in Table 2, indicating all linearity coefficients exceeded 0.999, confirming the detector's response linearity across all tested concentration levels.

The table 2 also presents the results of the evaluation of the LOD and LOQ.

Overall, the low LOD and LOQ values across all volatile compounds confirm that the method is well-suited for the comprehensive analysis of spirituous beverages. This is particularly important for ensuring compliance with safety standards and for maintaining the sensory quality of the beverages.

Metrological characteristics evaluation results are presented in Table 3, with the highest expanded uncertainty value reaching 10.3 % (for propan-1-ol) at the lower concentration level, and 4.8 % (for acetaldehyde) at the upper concentration level.

Table 2. Results of calibration, linearity checking and LOQ / LOD determination.

Compound	RRF^{Eth}	R^2	*LOD,* mg/L AA	*LOQ,* mg/L AA
Acetaldehyde	1.258	0.9994	0.042	0.140
Methyl acetate	1.557	0.9997	0.016	0.053
Ethyl acetate	1.070	0.9994	0.016	0.054
Methanol	1.253	0.9995	0.120	0.399
Propan-2-ol	0.804	0.9994	0.043	0.144
Propan-1-ol	1.000	0.9992	0.017	0.058
2-Methylpropan-1-ol	0.681	0.9993	0.023	0.077
Butan-1-ol	0.571	0.9991	0.012	0.041
3-Methylbutan-1-ol	0.617	0.9996	0.018	0.059

Table 3. Metrological characteristics of the validated method.

Compound	Measurement range of concentration, mg/L AA	Repeatability limit, *r, %*	Intermediate precision limit, $r_{I(TO)}$, %	Expanded uncertainty, *U*, % (*P* = 95%, *k* = 2),
Acetaldehyde	from 5.28 to 13.5 incl.	9.4	9.4	8.3
	from 13.5 to 4969 incl.	5.8	5.8	4.8
Methyl acetate	from 2.09 to 10.5 incl.	13.9	13.9	10.2
	from 10.5 to 5073 incl.	6.2	6.2	4.6
Ethyl acetate	from 2.08 to 10.4 incl.	13.8	13.8	10.1
	from 10.4 to 5052 incl.	6.1	6.1	4.5
Methanol	from 16.41 to 24.8 incl.	5.2	5.2	7.1
	from 24.8 to 5074 incl.	3.9	4.2	5.0
Propan-2-ol	from 4.21 to 12.6 incl.	9.5	9.5	7.8
	from 12.6 to 5072 incl.	5.3	5.7	4.4
Propan-1-ol	from 2.13 to 10.7 incl.	14.0	14.0	10.3
	from 10.7 to 5163 incl.	6.2	6.2	4.6
2-Methylpropan-1-ol	from 2.08 to 10.5 incl.	12.9	12.9	9.5
	from 10.5 to 5059 incl.	5.8	5.8	4.3
Butan-1-ol	from 2.09 to 10.5 incl.	12.9	12.9	9.6
	from 10.5 to 5063 incl.	5.7	5.7	4.3
3-Methylbutan-1-ol	from 2.14 to 10.7 incl.	12.8	12.8	9.4
	from 10.7 to 5203 incl.	5.7	5.7	4.3

In addition to the comprehensive analysis of volatile compounds, rigorous validation procedures were conducted to ensure the reliability and accuracy of the method used. The checking of acceptability of test results obtained under repeatability conditions confirmed that the method consistently produced stable and reproducible results when measurements were repeated under the same conditions. Furthermore, the evaluation of test results obtained under both repeatability and intermediate precision conditions demonstrated that the method maintained its precision even when variables such as time and operator were altered, indicating robust performance across different conditions.

Moreover, the checking of trueness of the obtained test results was performed to ensure that the method accurately measured the true concentration of volatile compounds in the samples. The results from these assessments were all within acceptable limits, indicating that the method is both precise and accurate

The results of test samples' analysis are presented in Table 4.

Table 4. Results of analysis of test samples.

Sample	Acetaldehyde	Methyl acetate	Ethyl acetate	Methanol	Propan-2-ol	Propan-1-ol	2-Methylpropan-1-ol	Butan-1-ol	3-Methylbutan-1-ol
Bourbon	98.3	-*	611	114	1.79	181	722	8.58	3267
Brandy	160	-	354	342	4.94	262	1138	3.31	3227
Calvados	89.4	-	631	958	5.17	335	545	166	2259
Gin	2.28	-	1.71	4.36	3.71	-	-	-	10.6
Grappa	221	-	452	439	2.68	209	325	5.45	1113
Liquor	23.1	-	3.04	6.88	-	1.54	-	-	50.4
Rakia	107	46.8	1190	12231	11.1	4221	454	42.3	1306
Rectified ethanol	2.54	-	-	3.31	2.63	-	-	-	-
Rum	34.8	-	260	13.8	7.43	327	49.0	4.50	229
Sake	45.4	-	137	24.7	-	638	198	19.7	738
Scotch	36.9	-	215	48.1	2.28	579	565	2.98	534
Tequila	29.1	-	124	1714	6.11	362	522	10.1	1702
Vodka	1.86	-	-	9.70	1.52	-	-	-	-
Whiskey	63.0	-	303	67.4	3.02	581	564	3.35	729
White wine dry	666	-	548	406	12.1	157	334	11.4	1791

* – means, that volatile compound wasn't detected in sample

5. Conclusions

The validation of the method for determining volatile compounds in spirituous beverages using ethanol as a reference substance has established a reliable analytical tool tailored to the specific needs of the beverage industry. The method’s robustness and consistency, even in the presence of varying operational conditions, make it a strong candidate for routine analytical use. Its ability to handle diverse matrices with high precision underscores its versatility, potentially setting a new standard in the quality control of ECBs.

Moreover, the method's integration into laboratory workflows is straightforward, requiring no additional resources beyond standard practices. This efficiency, coupled with the accurate detection and quantification capabilities demonstrated during the study, ensures that the method can support both regulatory compliance and the enhancement of product quality across various types of spirituous beverages.

The findings from this study also highlight the method's applicability to a broad spectrum of ECBs, making it a valuable addition to the arsenal of analytical techniques available to the industry. The removal of outliers during validation further affirms the method's reliability, ensuring that results are both accurate and repeatable. As such, this method represents a significant advancement in the analytical capabilities available for the assessment of volatile compounds in ECBs.

The method under discussion can be conveniently validated within a single laboratory using data previously acquired during the validation of the conventional internal standard method. This approach not only simplifies the validation process but also leverages existing data, reducing the need for extensive new measurements. No additional measurements or manual procedures are necessary beyond those specified in the relevant standards [3, 7-8] or in any other GC-FID method for the determination of volatile compounds in ECBs of any strength.

The streamlined nature of this validation process ensures that laboratories can quickly and efficiently integrate the method into their existing workflows without incurring additional costs or requiring specialized training. Moreover, the use of ethanol as a reference substance aligns with current trends towards more sustainable and cost-effective analytical practices, further enhancing the method's appeal. Examples of such validations, which demonstrate the method’s robustness and applicability across a variety of settings, are detailed in publications [9-11]. These studies highlight the method's effectiveness and its potential for widespread adoption in routine quality control and research applications within the wine and spirituous beverage industry.

6. Conflicts of interest

The authors declare no conflict of interest.

7. References

1. ISO 5725, Accuracy (trueness and precision) of measurement methods and results - Parts 1-6 (International Organization for Standardization, Geneva, 2019)
2. ASTM D4307, Standard Practice for Preparation of Liquid Blends for Use as Analytical Standards (2004)
3. Commission Regulation (EC) No 2870/2000 laying down Community reference methods for the analysis of spirits drinks (2000)
4. IUPAC Technical Report, M. Thompson, S. L. R. Ellison, R. Wood, Pure Appl. Chem., 74, 5 (2002)
5. EUROLAB Technical Report No. 1/2006: Measurement Uncertainty Revisited: Alternative Approaches to Uncertainty Evaluation
6. EURACHEM/CITAC Guide CG 4 (2012): Quantifying Uncertainty in Analytical Measurement
7. OIV-MA-AS312-03A Methanol (GC) (2014)
8. OIV-MA-AS315-27 Analysis of volatile compounds in wines by gas chromatography (2016)
9. S. Charapitsa, S. Sytova, A. Kavalenka, L. Sabalenka, M. Zayats, V. Egorov, S. Leschev, A. Kolesnov, BIO Web Conf. 68, 04001 (2023)
10. L. Sabalenka, S. Charapitsa, S. Sytova, A. Kavalenka, D. Yushkevitch, M. Zayats, S. Leschev, J. Food Meas. Charact. 17, 3207–3217 (2023)
11. S. Charapitsa, S. Sytova, A. Korban, L. Sobolenko, V. Egorov, S. Leschev, M. Zakharov, R. Čabala, R. Busarova, I. Shestakovich, A. Tolstouhova, S. Ondroušek, J. Vávra, M. Yilmaztekin, T. Cabaroglu, BIO Web Conf. 15, 02030 (2019)