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ORIGINAL PAPER

Proposals for the improving of the existing GC-FID methods for determination of methanol and volatile compounds in alcoholic beverages

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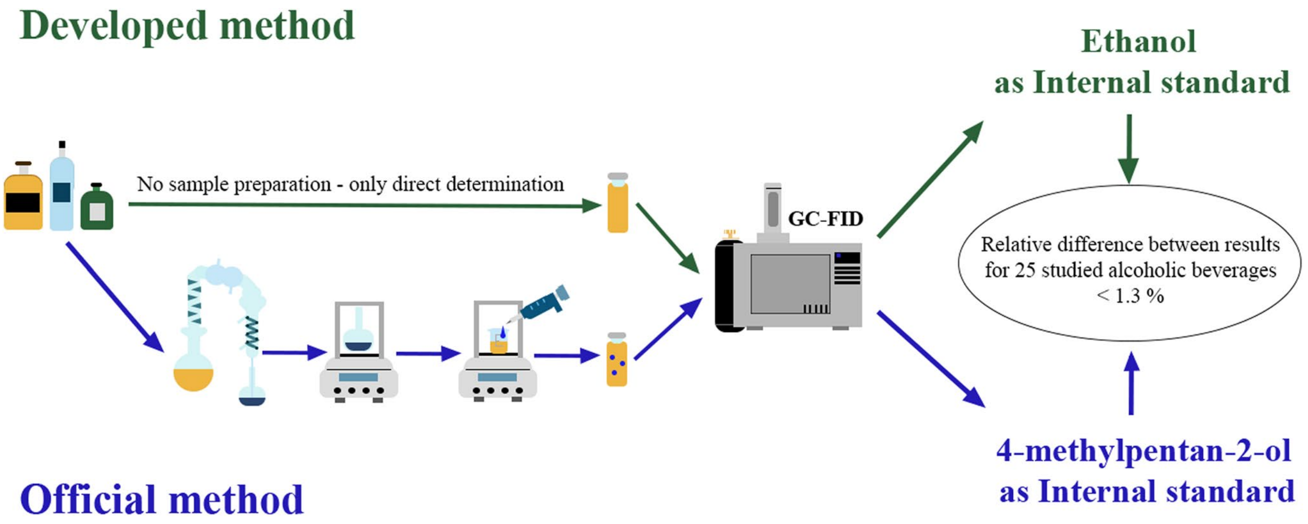
Abstract

In this work, proposals for improving the official gas chromatography flame ionization detector (GC-FID) methods for the determination of volatile congeners in alcoholic beverages are presented. The method based on the use of ethanol as an internal standard for the determination of 10 principal volatile compounds was developed, validated and compared with the official method in terms of analytical quality parameters (repeatability, intermediate precision, limits of detection and quantification, linearity and accuracy). The relative difference between results, obtained during test of 25 real samples of alcoholic beverages (rum, whiskey, bourbon, brandy, calvados, grappa, slivovice, tsikoudia, vodka, gin, grain spirit, liqueurs (herbal, limon, cherry, raspberry, sloe gin, egg and sambuca), vermouth, sake, nalewka, glühwein and rectified spirit), for the developed and official methods varies in the range from − 1.3 to 0.9% and statistically insignificant at 0.05 significance level (Student's test and ANOVA). The usage of the developed modified internal standard method allows to reduce minimal volume of sample from 200 to 1 mL and eliminate sources of uncertainty, associated with the sample preparation (distillation, density determination, and addition of an internal standard compound). The results of the conducted study show that the developed method can improve the official method, but additional interlaboratory study are required.

Graphical abstract



Keywords Alcoholic beverages · Commission Regulation (EC) No 2870/2000 · Ethanol · Internal standard · Methanol · Volatile compounds

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Extended author information available on the last page of the article

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Introduction

One of the most important parameters of the quality and safety of alcoholic products is the content of volatile compounds (congeners). Congeners are the volatile substances formed along with ethanol during fermentation, distillation and maturation of spirit drinks [1, 2]. The main volatile compounds under control are aldehydes (acetaldehyde and furfuraldehyde), acetal (acetal), esters (ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl lactate, ethyl octanoate and etc.), alcohols (methanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, hexan-1-ol, 2-phenylethanol and etc.). Determination of these volatile compounds in alcoholic beverage allows to confirm quality, safety and authenticity of alcoholic beverage.

According to the legislative acts [1, 2] the principal volatile substances of spirit drinks of viti-vinicultural origin under control are acetaldehyde, acetal, ethyl acetate, methanol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, butan-2-ol, 2-methylbutan-1-ol and 3-methylbutan-1-ol. Each of the listed principal volatile compounds can be determined in alcoholic beverages individually or in total by various methods: titration [3–5], sensor detection [6–8], spectroscopy [9–13]. However, the most common methods of determination of volatile compounds in alcoholic beverages are gas chromatography flame ionization detector (GC-FID) and gas chromatography mass spectrometry (GC–MS). The possibilities of GC-FID have been widely studied for many years [14–20], and the method itself is used at the legislative level in many countries [1, 2, 21, 22].

Quantitative calculations are performed as a rule using external standard [20, 23] and internal standard [14–19] methods. The method of standard additions can also be used for quantitative calculations, but it is usually used to check the trueness of the obtained results and evaluate the matrix effect [24].

Both the external standard method and the standard additives methods are affected by the stability of the chromatographic detector system. In turn, the external standard method can also be affected by the matrix effect. The standard addition method inherently requires the addition of a substance into the sample, which leads to dilution of the original sample. Thus, all these methods have a number of disadvantages that affect the accuracy and trueness of final analysis results [25]. Among the above methods, the internal standard method is the most preferred. The use of the internal standard method makes it possible to overcome the influence of the instability of the chromatographic system and the matrix effect. However, the addition of an internal standard substance leads to a change in the original sample.

In the case with the official method for the determination of volatile compounds in alcoholic beverages [1, 2] the internal standard method is used. The calibration coefficients—relative response factors (*RRF*) for analysed compounds are calculated according to the following formula

$$RRF_i^{IS} = \frac{A_{IS(C)}}{A_{i(C)}} \cdot \frac{C_{i(C)}}{C_{IS(C)}}, \tag{1}$$

where $A_{IS(C)}$ and $A_{i(C)}$ are the detector responses to the internal standard and i*th* volatile compound, obtained during measurement of solution, used for calibration, correspondingly, arbitrary units; $C_{IS(C)}$ and $C_{i(C)}$ are the concentrations of the internal standard and i*th* volatile compound in solution, used for calibration, correspondingly, μg/g.

As a result of performing measurements during the analysis procedure, results are expressed as the mass of the congener (in μg) per sample mass (in g), according to the to the following formula

$$C_{i(S)} = \frac{A_{i(S)}}{A_{IS(S)}} \cdot \frac{M_{IS}}{M_S} \cdot C_{IS(IS)} \cdot RRF_i^{IS}, \tag{2}$$

where $A_{IS(S)}$ and $A_{i(S)}$ are the detector responses to the internal standard and i*th* volatile compound, obtained during measurement of sample, correspondingly, arbitrary units; M_{IS} is the mass of a stock internal standard solution, added to sample, g; M_S is the mass of a sample, g; $C_{IS(IS)}$ is the concentration of the internal standard in a stock internal standard solution, μg/g.

However, according to the requirements of the legislative act [26] the final concentrations of analysed compounds should be expressed as mass of congener (in g) per 100 L of ethanol in the sample (absolute alcohol – AA) – g/hL AA. This concentration can be calculated according to the following formula

$$C^*_{i(S)} = \frac{C_{i(S)} \cdot \rho_S}{S_{Eth(S)} \cdot 100}, \tag{3}$$

where ρ_S is the density of sample, kg/m^3; $S_{Eth(S)}$ is the strength (volume ethanol concentration) of sample, %.

For this reason, the official method of analysis [1, 2] involves an additional procedure—the determination of the density and strength (volume ethanol content) of the sample. The determination of these parameters cannot be performed for the original sample of the alcoholic beverage. In accordance with the official methods [1, 2], it is necessary to use a distillate, which can be obtained as a result of the distillation of the original sample. This procedure requires at least 200 mL for the alcoholic beverage with the approximate alcoholic strength below 50% and at least 100 mL in case with alcoholic beverage with the approximate strength above 50% [1]. Thus, the sample to be analysed must undergo the following sample preparation steps: (1) distillation; (2)

determination of real alcoholic strength by volume by pycnometry; (3) addition of an internal standard compound. These sample preparation steps are the main sources of uncertainty in analysis (possible losses during distillation, dilution and contamination of the sample, accuracy of addition of the internal standard substance).

The use of a modified internal standard method overcomes the above disadvantages of both the internal standard method itself and the whole procedure of the analysis of alcoholic beverages [1, 2]. A modified internal standard method based on the use of the main compound of alcoholic beverages, ethanol, as an internal standard is proposed.

The calibration coefficients—relative response factors for analysed compound can be calculated according to the formula

$$RRF_i^{Eth} = \frac{A_{Eth(C)}}{A_{i(C)}} \cdot \frac{C^*_{i(C)}}{C^*_{Eth(C)}} = \frac{A_{Eth(C)}}{A_{i(C)}} \cdot \frac{C^*_{i(C)}}{\rho_{Eth}}, \tag{4}$$

where $A_{IS(C)}$ is the detector response to the ethanol, obtained during measurement of solution, used for calibration, arbitrary units; $C^*_{Eth(C)}$ and $C^*_{i(C)}$ are the concentrations of the ethanol and i*th* volatile compound in solution, used for calibration, correspondingly, g/hL AA; ρ_{Eth} is the density of anhydrous ethanol, $\rho_{Eth} = 78{,}927$ g/hL.

The concentration of volatile congener in the sample can be calculated using the modified internal standard method using the following formula

$$C^*_{i(S)} = \frac{A_{i(S)}}{A_{Eth(S)}} \cdot \rho_{Eth} \cdot RRF_i^{Eth}, \tag{5}$$

where $A_{Eth(S)}$ and $A_{i(S)}$ are the detector responses to the ethanol and i*th* volatile compound, obtained during measurement of sample, correspondingly, arbitrary units; ρ_{Eth} is the density of anhydrous ethanol, $\rho_{Eth} = 78{,}927$ g/hL.

Thus, in the case with the developed modified method of the internal standard, it is not necessary to perform sample preparation (add an internal standard compound) and set the values of its density and strength. The method also removes the restriction on the minimum volume of the sample and allows to analyse a sample with a volume of 1 mL.

Previously the method was successfully approbated for the individual determination of the methyl alcohol in alcoholic beverages [27]. The aims of the current study were to validate the developed method and compare it with the official methods of analysis [1, 2] in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, repeatability, intermediate precision and accuracy. The capabilities and possible problems of the simultaneous determination of 10 principal congeners by the developed and official methods were studied. The developed method was tested on samples of various types of spirit drink (rum, whiskey, bourbon, grain spirit, brandy, grappa, calvados, gin, vodka, slivovice, tsikoudia, bitter-tasting spirit drink, sake, tequila, distillates and liqueurs).

Material and methods

Reagents

All standards at ≥ 99% purity (acetaldehyde (CAS 75-07-0), ethyl acetate (CAS 141-78-6), acetal (CAS 105-57-7), methanol (CAS 67-56-1), butan-2-ol (CAS 78-92-2), propan-1-ol (CAS 71-23-8), 2-methylpropan-1-ol (CAS 78-83-1), butan-1-ol (CAS 71-36-3), 2-methylbutan-1-ol (CAS 123-51-3), 3-methylbutan-1-ol (CAS 123-51-3), 4-methylpentan-2-ol (CAS 108-11-2) and ethanol (CAS 64-17-5) were purchased from Sigma-Aldrich (Madrid, Spain).

Preparation of standard solutions

All standard and quality control (QC) solutions were prepared according to the procedure, described in the part III.2 item 5 of [1]. 4-methylpentan-2-ol was used as internal standard for the official method [1], and ethanol was used as internal standard for the developed method.

Preparation of alcoholic beverages samples

The alcoholic beverages: rum, whiskey, bourbon, grain spirit, brandy, grappa, calvados, gin, vodka, slivovice, tsikoudia, sake, tequila, vermouth, nalewka, glühwein, rectified spirit, cocktail and liqueurs, purchased from local markets, were distillated according to the Appendix I of Commission regulation EC [1]. The real alcoholic strength by volume of purchased samples was determined according to the Appendix II of Commission regulation EC [1] by Method A (pycnometry). Alcoholic beverage samples were prepared according to the procedure, described in the part III.2 item 8 of Commission regulation EC [1]. The results of determination of real strength of alcoholic beverages is shown in Table 1.

Instrumentation

A gas chromatograph model Crystal-5000.1, coupled with a flame ionization detector (Chromatec, Yoshkar-Ola, Mari El) was used for the chromatographic analyses. All the separations were carried out with a capillary column CP-WAX57CB 60 m × 0.25 mm × 0.4 µm film thickness (Agilent, Middelburg, Netherlands). A retention gap of 1 m × 0.32 mm was connected to the front of the column to improve the peak shape and GC conditions. Injections were made with autosampler in the split

Table 1 Declared by manufacturer and experimentally obtained strength of studied alcoholic beverages

Sample	Rum	Whiskey	Bourbon	Grain spirit	Brandy
Declared strength %	40.0	40.0	43.0	40.0	40.0
Experimental strength, %	40.03 ± 0.06	39.97 ± 0.06	42.99 ± 0.06	39.98 ± 0.06	39.98 ± 0.06
Sample	Grappa	Calvados	Gin	Vodka	Slivovice
Declared strength %	40.0	40.0	47.0	40.0	45.0
Experimental strength, %	40.00 ± 0.06	40.00 ± 0.06	46.96 ± 0.06	39.99 ± 0.06	45.06 ± 0.06
Sample	Tsikoudia	Sake	Tequila	Vermouth	Nalewka
Declared strength %	38.0	14.5	38.0	15.0	18.0
Experimental strength, %	37.99 ± 0.06	14.51 ± 0.06	38.07 ± 0.06	15.00 ± 0.06	17.99 ± 0.06
Sample	Glühwein	Rectified spirit	Cocktail	Sambuca liqueur	Egg liqueur
Declared strength %	8.5	69.9	27.5	38.0	17.0
Experimental strength, %	8.52 ± 0.06	69.91 ± 0.06	27.52 ± 0.06	37.99 ± 0.06	16.97 ± 0.06
Sample	Herbal liqueur	Limon liqueur	Cherry liqueur	Raspberry liqueur	Sloe gin liqueur
Declared strength %	35.0	25.0	16.0	16.5	35.0
Experimental strength, %	34.99 ± 0.06	25.06 ± 0.06	15.97 ± 0.06	16.51 ± 0.06	34.96 ± 0.06

mode (30:1), and the injection volume was 1 µL. The temperature of injector was 170 °C. The oven was programmed for 75 °C for 9 min, increased by 5°/min to 130 ºC, then increased by 10°/min to 180 °C, followed by 5 min at the final temperature. The carrier gas was nitrogen. The temperature of FID was 280 °C. All samples were measured under repeatability conditions three times. Data acquisition and processing were controlled by UniChrom software (New Analytical Systems, Minsk, Belarus). The examples of chromatograms of standard solutions in the logarithmic scale are presented in Fig. 1.

The examples of chromatograms of alcoholic beverages in the logarithmic scale are presented in Figs. S.1-S.25 of Supplementary material.

Method validation

Both official and proposed methods were validated according to the guidelines for single-laboratory validation of methods of analysis by the International Union of Pure and Applied Chemistry (IUPAC) [28], the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [29] and Eurachem Guide [30].

Calibration

One-point calibration was performed according to the procedure described in the part III.2 item 8.4 of Commission regulation EC [1]. Relative response factors for both official

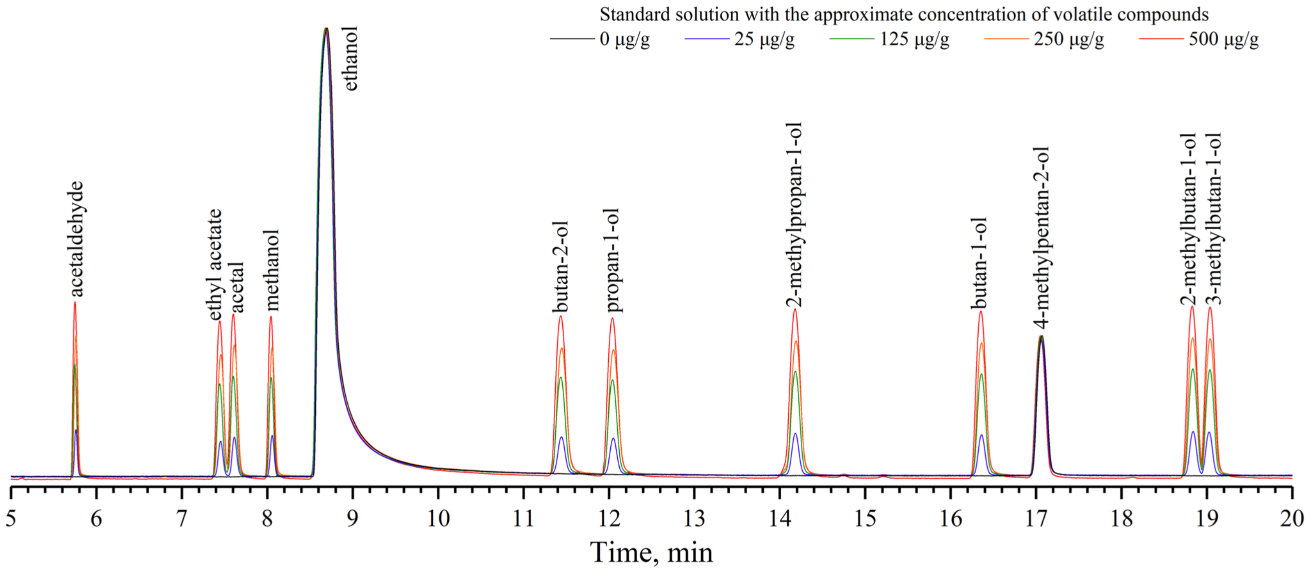

Fig. 1 Chromatograms of standard solutions in the logarithmic scale

and developed methods were calculated according to the formulas (1) and (4), correspondingly.

The linearity of the FID response was checked according to the part III.2 item 8.4 of Commission regulation EC [1]. The correlation coefficient R^2 was used for linearity assessment.

Concentration

The determination of the concentration by official method was performed according to the formulas (2) and (3). The determination of the concentration by the developed method was performed according to the formula (5).

LOQ and LOD

The determination of LOQ and LOD was carried out according to the item 6.2 of the Eurachem Guide [30], using following formulas

$$LOD = 3 \cdot \frac{s}{\sqrt{n}}, \tag{6}$$

$$LOQ = 10 \cdot \frac{s}{\sqrt{n}}, \tag{7}$$

where s is the standard deviation of concentrations, obtained during measurements of standard solution with the lowest concentration level of volatile compounds, μg/g; n is number of measurements, $n = 12$.

Precision

Precision was evaluated at two levels: repeatability and intermediate precision. Reproducibility was beyond the scope of the study, cause this parameter should be assessed by means of an interlaboratory study [29]. The repeatability and intermediate precision were studied analysing the standard solution at 4 concentration levels and alcoholic beverages. The repeatability was evaluated as the relative standard deviation (RSD) of three injections of standard solutions and alcoholic beverages under repeatability conditions. The intermediate precision was evaluated as the RSD of nine injections of standard solutions (3 injections/3 days of study).

Accuracy

The accuracy of the method was assessed at four concentration levels according to the ICH guideline [29]. Accuracy was estimated by recovery, which was calculated according to the following formula

$$Recovery = \frac{C}{\mu} \cdot 100\,\%, \tag{8}$$

where C and μ are the experimentally and gravimetrically obtained concentrations of volatile compound, correspondingly, μg/g (in case with the official method) or g/hL AA (in case with the developed method).

Comparison of the results

The relative difference between the results, obtained for both official and developed method, was calculated according to the following formula

$$\Delta = \frac{C^*(Dev) - C^*(Off)}{C^*(Off)} \cdot 100\,\%, \tag{9}$$

where $C^*(Dev)$ and $C^*(Off)$ are the concentrations of an analyte in the studied sample of alcoholic beverage, obtained by the developed and official methods, correspondingly, g/hL AA.

The comparison of results was also perfomed for each volatile compound at a 0.05 significance level, employing MS Excel (Microsoft, USA) for the statistical Student's test (t-Test: Paired Two Sample for Means) and ANOVA (Single factor) for both the official and developed methods. As a null hypothesis, the similarity between the concentration for the official and developed methods was taken.

Results and discussion

Method validation

Calibration

Calibration was performed before the start of measurements, and then controlled after every 5 samples, according to the procedure described in the part III.2 item 8.5 of Commission regulation EC [1]. The obtained calibration coefficients are presented in Table 2. The relative standard deviation of calibration coefficients varied from 0.5 to 1.1% in case of 4-methylpentan-2-ol and from 0.4 to 0.6% in case of ethanol.

Linearity, detection and quantification limits

LOD, LOQ, R^2 values and linearity range are reported in Table 3. Linearity was satisfactory ($R^2 > 0.999$) for all studied volatile compounds, with a calculated R^2 from 0.99965 to 0.99998 in the case with the official method and from 0.99967 to 0.99999 in the case with the developed method for the same analytical range of concentrations.

Table 2 Calibration coefficients, linearity parameters, LOD and LOQ values for the volatile compounds in standard solutions

Compound	Analytical range, µg/g	Official method				Developed method			
		RRF	R^2	*LOD*, µg/g	*LOQ*, µg/g	*RRF*	R^2	*LOD*, µg/g	*LOQ*, µg/g
Acetaldehyde	24.7–489	2.241	0.99968	0.46	1.53	1.296	0.99971	0.31	1.02
Ethyl acetate	24.4–490	1.946	0.99965	0.32	1.07	1.126	0.99967	0.34	1.12
Acetal	24.4–489	1.652	0.99988	0.43	1.44	0.956	0.99999	0.33	1.09
Methanol	27.8–493	2.154	0.99991	0.42	1.40	1.246	0.99999	0.21	0.69
Butan-2-ol	24.5–491	1.154	0.99998	0.35	1.15	0.667	0.99991	0.20	0.66
Propan-1-ol	24.5–491	1.210	0.99996	0.34	1.12	0.700	0.99996	0.16	0.52
2-methylpropan-1-ol	24.4–489	1.002	0.99998	0.31	1.02	0.579	0.99993	0.21	0.70
Butan-1-ol	24.5–491	1.101	0.99994	0.33	1.09	0.637	0.99996	0.23	0.78
2-Methylbutan-1-ol	24.5–491	1.003	0.99995	0.37	1.23	0.580	0.99996	0.27	0.91
3-Methylbutan-1-ol	24.5–492	1.019	0.99995	0.33	1.11	0.589	0.99996	0.28	0.92

The *LOD* values ranged from 0.31 to 0.46 µg/g in case with the official method and from 0.16 to 0.34 µg/g in case with the developed method. The *LOQ* values ranged from 1.02 to 1.53 µg/g in case with the official method and from 0.52 to 1.12 µg/g in case with the developed method. The LOD and LOQ values obtained in the current study for the developed method were largely lower than those reported in validation studied of the official method for the determination of volatile congeners in alcoholic beverages [24, 31, 32]. The values obtained were closest to the data presented in a recent validation study [19].

Precision and accuracy study

Precision (repeatability and intermediate precision) and accuracy tests were performed at four different concentrations for each volatile compound and the results are shown in Table 3.

The repeatability ($n = 3$) values varied from 0.2 to 2.2% in the case with the official method and from 0.1 to 1.4% in the case with the developed method. The intermediate precision ($n = 9$) values varied from 0.7 to 2.5% in the case with the official method and from 0.2 to 1.7% in the case with the developed method. In general, the developed method demonstrates the values of the standard deviation lower than the official one. The comparison with previous reported results of validation studies of the official method [15, 19] showed, that the developed method has better or close precision parameters.

The accuracy ($n = 9$) values varied from 99.0 to 102.2% in the case with the official method and from 98.9 to 101.2% in the case with the developed method. The obtained results are fully in accordance with acceptable recovery requirements described in AOAC guideline [33].

Alcoholic beverages study

The results of the measurements of alcoholic beverages samples are shown in Tables 4 and 5.

Quality control was performed after every 10 samples, using QC solutions, according to the procedure described in the part III.2 item 8.5 of Commission regulation EC [1]. The analysis

Table 3 Results of precision and accuracy study for official and developed methods

Compound	Repeatability, %		Intermediate precision, %		Recovery, %	
	Official method	Developed method	Official method	Developed method	Official method	Developed method
Acetaldehyde	0.6–2.2	0.4–1.4	1.2–2.5	0.9–1.6	100.0–101.6	99.6–100.6
Ethyl acetate	0.5–1.4	0.3–0.7	1.4–2.4	0.8–1.7	99.0–100.7	98.9–99.8
Acetal	0.4–1.2	0.1–0.9	0.8–2.2	0.2–1.7	99.8–100.8	99.6–100.0
Methanol	0.2–1.3	0.3–1.1	1.0–1.8	0.3–0.9	100.6–101.5	100.2–100.7
Butan-2-ol	0.3–1.4	0.2–1.0	0.8–1.9	0.3–1.1	100.4–102.1	100.2–101.1
Propan-1-ol	0.4–0.9	0.1–0.7	0.7–1.8	0.3–0.8	100.6–101.9	100.3–100.9
2-Methylpropan-1-ol	0.3–1.0	0.2–0.8	0.7–1.7	0.3–0.9	99.6–102.2	99.5–101.2
Butan-1-ol	0.5–1.0	0.1–0.9	0.7–1.7	0.5–0.9	99.8–101.7	99.7–100.7
2-Methylbutan-1-ol	0.5–1.0	0.2–0.7	0.7–2.0	0.5–1.3	100.4–101.7	99.8–100.7
3-Methylbutan-1-ol	0.5–1.2	0.1–0.7	0.7–1.7	0.5–1.3	100.1–101.5	99.8–100.5

Table 4 Results of measurements of vodka, rum, gin, grain spirit, grappa, calvados, whiskey, bourbon, brandy, tsikoudia, tequila, slivovice and rectified spirit

Method	Concentration of volatile compound in alcoholic beverage, g/hL AA												
	Vodka	Rum	Gin	Grain spirit	Grappa	Calvados	Whiskey	Bourbon	Brandy	Tsikoudia	Tequila	Slivovice	Rectified spirit
Acetaldehyde													
Official	0.0504	4.51	0.170	3.30	18.1	9.01	8.68	7.04	7.65	24.3	2.52	11.4	0.0497
Developed	0.0501	4.54	0.172	3.33	18.0	9.04	8.60	7.08	7.64	24.5	2.53	11.4	0.0495
Δ, %	−0.7	0.7	0.8	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.9	0.5	0.5	−0.4
Ethyl acetate													
Official	0	14.5	0	8.47	28.9	58.3	58.9	64.5	39.6	26.6	12.6	90.7	2.52
Developed	0	14.6	0	8.54	28.8	58.5	58.4	64.9	39.6	26.8	12.7	91.2	2.51
Δ, %	–	0.7	–	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.8	0.3	0.5	−0.4
Acetal													
Official	0	0.300	0	1.10	1.02	9.15	7.49	7.98	6.61	11.3	0.955	9.66	0.433
Developed	0	0.303	0	1.11	1.01	9.18	7.42	8.03	6.60	11.4	0.958	9.71	0.432
Δ, %	–	0.7		0.9	−0.7	0.3	−0.8	0.6	−0.2	0.9	0.3	0.5	−0.4
Methanol													
Official	2.18	2.22	0.416	11.0	41.4	91.0	13.2	8.84	29.7	75.5	146	1055	0.605
Developed	2.17	2.23	0.419	11.1	41.2	91.3	13.0	8.89	29.7	76.1	146	1060	0.603
Δ, %	−0.7	0.7	0.8	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.8	0.3	0.5	−0.4
Butan-2-ol													
Official	0	0.188	0.154	0	2.02	5.85	0	0	0.0408	0.0	0.139	16.5	0
Developed	0	0.190	0.155	0	2.01	5.87	0	0	0.0408	0.0	0.139	16.6	0
Δ, %	–	0.8	0.9	–	−0.6	0.3	–	–	−0.2	–	0.3	0.5	–
Propan-1-ol													
Official	0	17.5	0	30.2	21.9	31.2	29.7	22.4	27.4	28.4	33.9	401	0
Developed	0	17.6	0	30.5	21.8	31.3	29.5	22.6	27.4	28.7	34.1	403	0
Δ, %	–	0.7	–	0.9	-0.6	0.3	-0.9	0.6	-0.2	0.9	0.3	0.5	–
2-Methylpropan-1-ol													
Official	0	21.2	0	74.3	34.7	51.3	152	64.1	101	23.5	49.8	44.1	0
Developed	0	21.4	0	74.9	34.4	51.5	151	64.5	101	23.7	50.0	44.3	0
Δ, %	–	0.7	–	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.9	0.3	0.5	–
Butan-1-ol													
Official	0	0.233	0	0.642	0.565	15.0	0.578	0.856	0.313	1.95	0.913	3.95	0
Developed	0	0.235	0	0.648	0.561	15.0	0.573	0.861	0.312	1.96	0.915	3.97	0
Δ, %	–	0.8	–	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.8	0.3	0.5	–
2-Methylbutan-1-ol													
Official	0	16.0	0	93.4	34.7	56.9	127	121	68.6	40.1	46.3	36.3	0
Developed	0	16.1	0	94.2	34.5	57.1	125	122	68.5	40.4	46.4	36.5	0
Δ, %	–	0.7	–	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.9	0.3	0.5	–

Table 4 (continued)

Method	Concentration of volatile compound in alcoholic beverage, g/hL AA												
	Vodka	Rum	Gin	Grain spirit	Grappa	Calvados	Whiskey	Bourbon	Brandy	Tsikoudia	Tequila	Slivovice	Rectified spirit
3-Methylbutan-1-ol													
Official	0	49.1	0	268	117	209	360	346	282	136	158	124	0
Developed	0	49.5	0	270	117	209	357	348	282	137	159	125	0
Δ, %	–	0.7	–	0.9	−0.6	0.3	−0.9	0.6	−0.2	0.8	0.3	0.5	–

continued if the recoveries for each congener were between 90 and 110%. In all cases the recoveries for both official and developed methods had acceptable values. The chromatograms of alcoholic beverages are presented in Figs. S.1–S.25 of Supplementary material The most abundant volatile compounds, which were found in all the studied samples, are acetaldehyde and methanol (25 of 25 samples). The remaining congeners were found in the following number of samples: ethyl acetate (18), acetal (18), propan-1-ol (18), 2-methylpropan-1-ol (16), 3-methylbutan-1-ol (16), 2-methylbutan-1-ol (15) and butan-2-ol (7). All 10 studied volatile congeners were obtained in 6 alcoholic beverages: rum, grappa, calvados, brandy, tequila and slivovice. Such alcoholic beverages as grain spirit, whiskey, bourbon, tsikoudia, cocktail and sake had 9 volatile congeners (all studied except butan-2-ol). Other samples contained from 2 to 8 volatile compounds.

The comparison of the results obtained for both official and developed showed that the relative difference between the values of concentrations is less than ± 1.5%. This comparison was also performed for each volatile compound at a 0.05 significance level, employing the statistical Student's test (t-Test: Paired Two Sample for Means) for the means comparison for obtained concentrations. As an alternative approach the ANOVA (Single factor) was used to confirm these results, considering the normal distribution of the data and employing a 0.05 significance level. Both tests confirmed that the difference between the means, obtained for both methods for all the studied samples is statistically insignificant. The results of the statistical analysis are presented in Tables S.1 – S.25 of the Supplementary material.

Conclusions

A GC-FID method was developed and in-house validated for simultaneous analysis of 10 volatile congeners in alcoholic beverages. The modified internal standard method makes it possible to eliminate the sources of uncertainty that appear during sample preparation in accordance with the official method of analysis. The developed method can be validated by any laboratory without performing additional measurements on the basis of already existing measurements performed during the validation of official methods of analysis [1, 2].

The developed method can also be validated for a number of volatile congeners in alcoholic products and ethanol containing products in general. Official methods of analysis of alcoholic beverages [34] or ethanol containing products (in the pharmaceutical, cosmetic and perfumery industries) can become simpler, faster, easier, cheaper, more reliable and robust. The obtained results can be an occasion for launching the interlaboratory study of the developed modified internal standard GC-FID method in order to improve official methods of analysis and make them cheaper and faster.

Table 5 Results of measurements of liqueurs, cocktail, nalewka, vermouth, sake and glühwein

Method	Concentration of volatile compound in alcoholic beverage, g/hL AA											
	Liqueur							Cocktail	Nalewka	Vermouth	Sake	Glühwein
	Herbal	Sloe gin	Limon	Raspberry	Cherry	Egg	Sambuca					
Acetaldehyde												
Official	3.66	0.112	2.51	3.15	0.969	0.689	0.420	4.29	4.07	3.05	2.90	1.65
Developed	3.68	0.113	2.53	3.12	0.974	0.694	0.424	4.24	4.10	3.06	2.86	1.64
Δ, %	0.4	0.6	0.8	− 1.0	0.6	0.8	0.8	− 1.3	0.9	0.6	− 1.1	− 0.6
Ethyl acetate												
Official	1.35	0	0	3.18	26.6	0	0	8.40	7.44	0	4.70	5.59
Developed	1.35	0	0	3.15	26.7	0	0	8.30	7.51	0	4.65	5.56
Δ, %	0.4	–	–	− 1.1	0.6	–	–	− 1.2	0.9	–	− 1.1	− 0.5
Acetal												
Official	0.141	0	0	0.502	0.875	0	0	1.90	0.673	0	0.862	0.614
Developed	0.141	0	0	0.497	0.880	0	0	1.87	0.678	0	0.852	0.611
Δ, %	0.4	–	–	− 1.0	0.5	–	–	− 1.3	0.8	–	− 1.1	− 0.6
Methanol												
Official	1.95	2.05	2.91	12.7	0.977	0.975	0.232	7.73	16.8	1.75	1.82	2.53
Developed	1.96	2.07	2.94	12.6	0.982	0.981	0.234	7.63	16.9	1.76	1.81	2.51
Δ, %	0.4	0.5	0.8	− 1.1	0.5	0.7	0.8	− 1.2	0.9	0.6	− 1.0	− 0.6
Propan-1-ol												
Official	0.250	0	0	0	0	0.249	0.244	5.31	0.322	0.594	48.6	6.93
Developed	0.251	0	0	0	0	0.250	0.246	5.24	0.325	0.598	48.1	6.89
Δ, %	0.4	–	–	–	–	0.7	0.8	− 1.2	0.9	0.6	− 1.1	− 0.5
2-Methylpropan-1-ol												
Official	0.0988	0	0	0	0	1.495	0	10.5	0.364	0	15.4	12.2
Developed	0.0992	0	0	0	0	1.505	0	10.3	0.368	0	15.2	12.2
Δ, %	0.4	–	–	–	–	0.7	–	− 1.2	0.9	–	− 1.1	− 0.6
Butan-1-ol												
Official	0	0	0	0	0	0	0	0.191	0	0	1.46	0
Developed	0	0	0	0	0	0	0	0.189	0	0	1.45	0
Δ, %	–	–	–	–	–	–	–	− 1.3	–	–	− 1.0	–
2-Methylbutan-1-ol												
Official	0.129	0	0	0	0	0.812	0	7.02	0	0	14.8	17.3
Developed	0.130	0	0	0	0	0.816	0	6.93	0	0	14.6	17.2
Δ, %	0.4	–	–	–	–	0.6	–	− 1.3	–	–	− 1.1	− 0.6
3-Methylbutan-1-ol												
Official	0.461	0	0	0	0	9.91	0	49.9	0.345	0	56.4	50.7
Developed	0.463	0	0	0	0	9.97	0	49.2	0.348	0	55.8	50.4
Δ, %	0.4	–	–	–	–	0.7	–	− 1.2	0.8	–	− 1.1	− 0.6

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Data availability Data is avaiable in the Supplementary material.

Declarations

Conflict of interest The authors declare no competing interests.

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