The Method for Direct Gas Chromatographic Determination of Acetaldehyde, Methanol, and Other Volatiles Using Ethanol as a Reference Substance: Application for a Wide Range of Alcoholic Beverages

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Abstract

The article presents the results of collaborative study of the method for gas chromatographic determination of alcoholic fermentation volatile by-products (acetaldehyde, methanol, methyl acetate, ethyl acetate, propan-2-ol, propan-1-ol, 2-methyl-propan-1-ol, butan-1-ol, and 3-methylbutan-1-ol) in alcoholic beverages with volume ethanol content in a range of 9.9–96.0% (wine, sake, liquor, tequila, vodka, grappa, bourbon, scotch, calvados, rakia, rum, brandy, whiskey, gin, and rectified ethyl alcohol). The zest of the method is the use of ethanol, present in beverages, as a reference substance for quantitative determination of mentioned components. Checking of precision data, obtained under repeatability conditions and under both repeatability and intermediate precision conditions, and trueness of test results, was carried out according to the ISO 5725–6. The relative difference between calibration coefficients (relative response factors for analyzed volatile compound relative to ethanol), obtained with an interval of 3 weeks, did not exceed 1.1%. Contents of acetaldehyde, methanol, and volatiles in 15 commercial alcoholic beverages analyzed by the developed method varied in the 1.84–677 mg L^{-1} of absolute ethanol, 3.25–12,394 mg L^{-1} of absolute ethanol, and 1.49–4243 mg L^{-1} of absolute ethanol intervals, correspondingly.

Keywords Alcoholic beverages · Reference substance · Acetaldehyde · Methanol · Volatiles · Ethanol

Introduction

Gas chromatography (GC) with flame-ionization detection (FID) is widely used for determination of volatiles in alcoholic beverages as an official method of analysis in countries of EU (Commission regulation (EC) No 2870 2000; OIV-MA-BS-14 2009), USA (AOAC Official Methods 972.10 2005; AOAC Official Methods 972.11 2005), China (GB/T 11,858 2008), India (BIS IS 3752 2009), Mexico (NOM-199-SCFI 2017), and Kenya (KS EAS 104 2014). This method has been known for 30 years (Reglero et al. 1986) and has been thoroughly investigated in international interlaboratory study for more than 20 years ago for 5 spirit drinks—whiskey, brandy, rum, grappa, and kirsch (Kelly et al. 1999). Later, the method was validated for analysis of rakia (Yilmaztekin and Cabaroglu 2011), fruit wines, beer, and rice wines (Kim et al. 2017). All of the above official methods of analysis and their additional studies use a traditional internal standard method to quantify mass concentrations of acetaldehyde, methanol, and other target volatile compounds. Since the area of this analysis is conservative, the traditional method of analysis has not undergone any changes and has been used for several decades.

Traditional method is based on use of the calibration characteristics—relative response factors (RRF) which express

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the sensitivity of a detector for an ith analyte relative to a chosen internal standard substance, which can be calculated according to the following equation

$$\text{RRF}_i^{\text{Trad}} = \frac{C_i^{\text{St}}}{A_i^{\text{St}}} \cdot \frac{A_{\text{IS}}^{\text{St}}}{C_{\text{IS}}^{\text{St}}}, \qquad (1)$$

where C_i^{St} and $C_{\text{IS}}^{\text{St}}$ are the mass concentrations of the ith volatile compound (analyte) and internal standard in standard solution, used for calibration, correspondingly, mg mg^{-1} (ratio mass of compound in mg to mass of standard solution in mg); A_i^{St} and $A_{\text{IS}}^{\text{St}}$ are the detector responses for the ith analyte and internal standard in standard solution, used for calibration, correspondingly, arbitrary units (a.u.).

The use of ratio of responses of analyte and internal standard compound allows to eliminate the impact of matrix effects and compensate for variability encountered in instrumental analysis. Pentan-3-ol, pentan-1-ol, 4-methylpentan-1-ol, methyl nonanoate, etc., are the most commonly used compounds as an internal standard. The official method for the analysis of volatile compounds in alcoholic beverages has capabilities that are associated with the means of its implementation, namely, the introduction of an internal standard substance into the sample. This procedure requires material, labor, and time costs associated with the need for manual sample preparation, additional calculations, and contamination of the analyzed sample.

In work (Charapitsa et al. 2013), it was an originally introduced method, based on the ethanol usage as a reference substance for analysis of volatile compounds in ethanol-containing products. The developed method does not have the disadvantages of the traditional method and is proposed as an alternative to the official one with the prospect of a complete replacement. According to this method, the reference substance was considered ethanol. This method turned all the traditional principles of using the internal standard method upside down. The ethanol is the substance, which always presents in alcohol products and has concentration and magnitude order more than concentration and magnitude order of analytes up to 6 orders. The implementation of this became possible thanks to the development of instrumental methods of analysis and high competition between manufacturers of chromatographic equipment in recent decades that has led to an increase in the accuracy characteristics of modern equipment. Thus, the linearity of the FID increased from 10^6 (Bayer 1986) until more than 10^7 (Feng et al. 2019).

These characteristics of FID make possible to apply a principally new approach to procedure and calculations in internal standard method. Thus, modern GC-FID can show analytical signals of alcoholic beverage samples with the same high accuracy, containing, for example, ethanol at a percentage level, while the concentration of most volatiles will be at the ppm level.

In the case with developed method, calibration characteristics $\text{RRF}_i^{\text{Eth}}$, which express the sensitivity of a detector for an ith analyte relative to ethanol, are calculated according to the following equation

$$\text{RRF}_i^{\text{Eth}} = \frac{C_i^{\text{St}}(*)}{A_i^{\text{St}}} \cdot \frac{A_{\text{Eth}}^{\text{St}}}{\rho_{\text{Eth}}}, \qquad (2)$$

where $C_i^{\text{St}}(*)$ is the concentration of the ith volatile compound in standard solution, used for calibration, expressed in mg L^{-1} of absolute alcohol (AA); $A_{\text{Eth}}^{\text{St}}$ is the detector response for ethanol in standard solution, used for calibration, a.u.; ρ_{Eth} *is* the density of ethanol, and $\rho_{\text{Eth}} = 789,300$ mg L^{-1}.

Thorough studies of the possibilities, advantages, and limitations of the developed method have been carried out over several years. The main results of the research are the next:

- the developed method was validated in one laboratory (single-laboratory study) (Charapitsa et al. 2019b);
- the developed method was investigated in international interlaboratory study (in 9 laboratories from 4 countries) (Charapitsa et al. 2019a);
- the influence of matrix effects to the results, obtained for developed method, was estimated for 13 ethanol-containing matrices (absence of matrix effect was proved) (Charapitsa et al. 2021).
- the perspectives of ethanol usage as a reference substance for the quantification of volatile compounds in alcoholic products by GC-MS-developed method were investigated (Korban et al. 2019, 2021).

The data, obtained in abovementioned single-laboratory (Charapitsa et al. 2019b) and interlaboratory (Charapitsa et al. 2019a) studies (the metrological characteristics of the method, such as repeatability, intermediate precision, reproducibility, trueness, uncertainty), was applicable to control the acceptability and trueness (according to the ISO 5725–6: 1994) of results of analysis of only model water–ethanol solutions. The results of estimation of metrological characteristics (relative standard deviation (RSD) of repeatability—RSD$_r$; RSD of intermediate precision—RSD$_{I(TO)}$; relative expanded uncertainty $P = 95\%$, with coverage factor $k = 2$—U; limit of detection—LOD; and limit of quantitation—LOQ) of the developed method, obtained in single-laboratory study (Charapitsa et al. 2019b) for concentration range from 2.10 to 5000 mg (L AA)$^{-1}$, are presented in Table 1.

The statistical data were insufficient for the application of the developed method for the analysis of real samples of alcohol-containing and ethanol-containing products with the accuracy and precision obtained for

Table 1 Metrological characteristics of the developed method

Compound	Measurement range of mass concentration, mg (L AA)$^{-1}$	RSD$_r$, %	RSD$_{I(TO)}$, %	U, % (P=95%, k=2)	LOD, mg (L AA)$^{-1}$	LOQ, mg (L AA)$^{-1}$
Acetaldehyde	From 2.10 to 10.0 incl	3.4	3.4	11.4	0.042	0.140
	From 10.0 to 5000 incl	2.2	2.2	7.5		
Methyl acetate	From 2.10 to 10.0 incl	5.1	5.1	13.6	0.016	0.053
	From 10.0 to 5000 incl	2.3	2.3	7.6		
Ethyl acetate	From 2.10 to 10.0 incl	5.0	5.0	13.6	0.016	0.054
	From 10.0 to 5000 incl	2.3	2.3	7.6		
Methanol	From 2.10 to 15.0 incl	1.9	1.9	9.9	0.016	0.054
	From 15.0 to 5000 incl	1.5	1.6	6.9		
Propan-2-ol	From 2.10 to 10.0 incl	3.5	3.5	11.5	0.043	0.140
	From 10.0 to 5000 incl	2.0	2.1	7.5		
Propan-1-ol	From 2.10 to 10.0 incl	5.1	5.1	13.7	0.017	0.058
	From 10.0 to 5000 incl	2.3	2.3	7.6		
2-Methylpropan-1-ol	From 2.10 to 10.0 incl	4.7	4.7	13.1	0.023	0.077
	From 10.0 to 5000 incl	2.1	2.1	7.4		
Butan-1-ol	From 2.10 to 10.0 incl	4.7	4.7	13.1	0.013	0.041
	From 10.0 to 5000 incl	2.1	2.1	7.4		
3-Methylbutan-1-ol	From 2.10 to 10.0 incl	4.7	4.7	13.1	0.018	0.059
	From 10.0 to 5000 incl	2.1	2.1	7.4		

model solutions. Questions were raised as to the possible effect of the matrix effects to the results, obtained for developed method. The study of the matrix effect on the method of direct determination of volatile compounds in a wide range of alcoholic beverages was carried out and statistical insignificance of the influence of the matrix effect on the results obtained by the developed method has been proved (Charapitsa et al. 2021). This conclusion made it possible to continue the study of the developed method and estimate accuracy of results, obtained for real samples of alcoholic and ethanol-containing products.

As the next and the most important step of long-term study of the developed method, the experimental study of the acceptability and trueness of the results (according to ISO 5725–6) obtained by the developed method in the analysis of real samples of alcoholic and ethanol-containing products was carried out. The suitability of the method for the determination of mass concentrations of 9 volatile compounds in alcoholic beverages with ethanol volume concentration in 10.0–96.0% range was estimated using the metrological characteristics, established by method validation in accordance with series of ISO 5725 (Table 1). During the study, 15 alcoholic samples were measured over 6 weeks under repeatability conditions and under both repeatability and intermediate precision conditions.

Material and Methods

Chemicals and Reagents

All chemical standards with their respective CAS numbers (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), 3-methylbutan-1-ol (123–51-3)) were provided by Sigma-Aldrich (Alcobendas, Madrid, Spain) with the highest purity available (more than 99%). Concentrations of impurities in volatile compounds were specified by the GC-FID (to detect of volatile impurities) and GC coupled with thermal conductivity detector (GC-TCD) (to detect of water) analysis using the internal normalization method. Rectified ethyl alcohol with volume concentration of ethanol 96.0% was provided by Dyatlovo Distillery Plant Algon (Slonim, Belarus). Pure distilled and deionized water (conductivity ≤ 0.5 MΩ·cm) was provided by JSC Integral (Minsk, Belarus).

Preparation of Standard Solutions

All standard solutions (SS) were prepared gravimetrically according to the (ASTM D4307 2015). SS were prepared in water–ethanol solution (WES) with ethanol

volume concentration 40%. WES was prepared by mixing of rectified ethyl alcohol and deionized water.

Standard solution *A* (SS-*A*) with concentrations of analytes about 5000 mg (L AA)$^{-1}$ was prepared by adding about 200 μg of the volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol) to WES. Standard solutions *B*, *C*, *D*, *E*, *F*, and *G* with concentrations of analytes about 500, 250, 200, 25.0, 10.0, and 2.0 mg (L AA)$^{-1}$, correspondingly, were prepared by diluting of SS-*A* with WES.

Alcoholic Beverages

The alcoholic beverages (wine, sake, liquor, tequila, vodka, grappa, bourbon, scotch, calvados, rakia, rum, brandy, whiskey, and gin) were purchased in commercial stores (Table 2).

Sample Preparation of Alcoholic Beverages

The samples of alcoholic beverages with high concentration of sugar (wine and liquor), declared by the manufacturer, were distilled according to the Appendix I (Commission regulation (EC) No. 2870 2000). Since the volume concentration of ethanol in wine sample was declared as range, the volume concentration of ethanol in this sample was determined according to the Appendix II (Commission regulation

(EC) No 2870 2000). The results of sample preparation are shown in Table 2.

Gas Chromatographic Analysis

All GC separations were performed on a gas chromatograph Crystal-5000.1 (JSC SDB Chromatec, Yoshkar-Ola, Russia), equipped with the autosampler, FID and TCD detectors. Data acquisition and processing were controlled by UniChrom software (New Analytical Systems Ltd, Minsk, Belarus). High purity nitrogen (99.999%) was employed as a carrier gas. The capillary column Rt-Wax, 60 m × 0.53 mm, film thickness 1.0 μm (Restek, Bellefonte, USA). The temperature of injector was 190 °C. The oven was programmed for 75 °C for 9 min, increased by 5° min^{-1} to 130 °C, then increased by 10° min^{-1} to 180 °C, followed by 5 min at the final temperature. The temperatures of FID and TCD were 280° and 150 °C, correspondingly. The split ratio was 10:1, and the injection volume was 0.8 and 1.0 μL. Volatile compounds were determined by direct injection of the SS and alcoholic beverages in the case with sake, tequila, vodka, grappa, bourbon, scotch, calvados, rakia, rum, brandy, whiskey, gin, and distillates of wine and liquor. All standard solutions SS and alcoholic beverage samples were measured under repeatability conditions two times. To assess the recovery of distillation for target volatile compounds, the original samples of wine and liquor were also directly

Table 2 The ethanol volume concentration and sugar mass concentration in the purchased alcoholic beverages

Alcoholic beverage	Ethanol volume concentration, declared by the manufacturer, %	Ethanol volume concentration, determined experimentally, %	Sugar mass concentration, declared by the manufacturer, g L^{-1}
Wine	9.0–13.0	9.9	50.0
Sake	14.5	N/D*	N/A**
Liquor	18.0	18.0	21.0
Tequila	38.0	N/D	N/A
Vodka	40.0	N/D	N/A
Grappa	40.0	N/D	N/A
Bourbon	40.0	N/D	N/A
Scotch	40.0	N/D	N/A
Calvados	40.0	N/D	N/A
Rakia	40.0	N/D	N/A
Rum	40.0	N/D	N/A
Brandy	40.0	N/D	N/A
Whiskey	43.0	N/D	N/A
Gin	47.0	N/D	N/A

*N/D, distillation was not carried out (it was not necessary); **N/A, the value was not declared by manufacturer

injected in GC-FID, equipped with the Split Precision Liner 5 mm × 8.0 × 105 filled with deactivate wool (Restek, Bellefonte, USA) in order to avoid the column contamination with non-volatile compounds, and measured twice under repeatability conditions.

Statistical Analysis

The experiment for checking of the results' acceptability, obtained with developed method for 15 ethanol-containing products, was carried out for 6 weeks by 3 operators. Calibration was performed every 3 weeks and obtained values of calibration characteristics were used for calculation of mass concentrations of volatile compounds in ethanol-containing products and SS, used for trueness checking, over the next 3 weeks. To ensure analytical stability and checking trueness of obtained results, quality control (QC) samples were injected after every 15 samples. All SS, except SS-C, were

used as QC samples in random order. Variation of intermediate precision factor and scheme of organization of experiment are shown in Table 3. The paired two-sample t-test and analysis of variance (ANOVA) of the experimental data obtained by GC-FID were performed using the MS Excel 2016 (Microsoft, USA).

Calculation

Calibration

The values of calibration coefficients in developed method were calculated according to Eq. (2). SS-C was used as calibration solution with volatile compound mass concentrations approximately 250 mg (L AA)$^{-1}$. The comparison of values of RRF, obtained under intermediate precision conditions, was performed for each volatile compound at 0.05

Table 3 Scheme of organization of the experiment

Checking of the acceptability of results, obtained under repeatability conditions						
Number of measurements, n	2					
Method of estimation	item 5.2.2 of (ISO 5725–6: 1994)					
Checking of the acceptability of results, obtained under both repeatability and intermediate precision						
Intermediate precision factor	Factor value					
Operator	1	2	3	1	2	3
Date of analysis	Aug, 27	Sept, 02	Sept, 09	Sept, 17	Sept, 24	Oct, 01
Date of calibration	Aug, 26			Sept, 16		
Injection volume, μL	1.0			0.8		
Method of estimation	Item 5.3.2.2 of (ISO 5725–6: 1994)					
Checking of the trueness of obtained results						
SS for checking trueness	SS-F	SS-E	SS-B	SS-D	SS-A	SS-G
Method of estimation	Item 7.2.3.1.3 of (ISO 5725–6: 1994)					

Table 4 Results of determination of RRF coefficients under intermediate precision conditions

Compound	1st calibration (Aug, 26)		2nd calibration (Sept, 29)		t-test: Paired two sample for means		ANOVA: Single factor	
					t_{cr} 6.31	P 0.05	F_{cr} 18.5	P 0.05
	RRF^{Eth}	R^2	RRF^{Eth}	R^2	$|t_{stat}|$	P_{stat}	F_{stat}	P_{stat}
Acetaldehyde	1.312	0.9994	1.316	0.9998	0.18	0.44	0.04	0.86
Methyl acetate	1.445	0.9997	1.455	0.9999	0.99	0.25	0.54	0.54
Ethyl acetate	1.074	0.9994	1.078	0.9999	1.00	0.25	1.22	0.38
Methanol	1.304	0.9995	1.310	0.9999	0.56	0.34	0.56	0.53
Propan-2-ol	0.765	0.9994	0.764	0.9999	0.26	0.42	0.08	0.80
Propan-1-ol	0.665	0.9992	0.658	0.9998	1.28	0.21	1.39	0.36
2-Methylpropan-1-ol	0.536	0.9993	0.537	0.9998	0.10	0.47	0.02	0.91
Butan-1-ol	0.615	0.9991	0.608	0.9998	0.68	0.31	0.47	0.56
3-Methylbutan-1-ol	0.560	0.9996	0.553	0.9998	0.85	0.28	0.68	0.50
Ethanol	1.000		1.000					

significance level, employing MS Excel 2016 (Microsoft, USA) for the statistical Student's test (t-test: paired two sample for means) and ANOVA (single factor). The results of calculations and statistical tests for RRF values are shown in Table 4.

Linearity

The values of linearity coefficient R^2 for all analytes were calculated using MS Excel 2016. The results of the linearity determination are shown in Table 4.

Calculation of the Mass Concentrations

The concentration of the ith volatile compound was determined according to the following equation:

$$C_{ik} = \text{RRF}_i^{\text{Eth}} \frac{A_{ik}}{A_{\text{Eth},k}} \cdot \rho_{\text{Eth}}, \tag{3}$$

where A_{ik} is the FID response for the ith volatile compound, obtained for kth measurement of ethanol-containing product or QC sample, a.u.; $A_{\text{Eth},k}$ is the detector response for ethanol, obtained for kth measurement of ethanol-containing product or QC sample, a.u.

Calculation of the Recovery for Distilled Samples

The values of the recovery of distillation process were estimated according to the following equation:

$$R_i = \frac{\overline{C}_i(\text{distilled})}{\overline{C}_i(\text{origin})} \cdot 100\%, \tag{4}$$

where C_{ik} (distilled) and C_{ik} (origin) are the arithmetic mean of the results for the ith volatile compound in distilled and original ethanol-containing product, correspondingly, mg $(\text{L AA})^{-1}$.

Checking of Acceptability of Test Results Obtained Under Repeatability Conditions

Checking of acceptability of test results obtained under repeatability conditions was carried out according to the item 5.2.2 (ISO 5725–6: 1994). If the condition (5) was met, then the arithmetic mean of the two results was taken as the final quoted result $\overline{C}_i$:

$$\text{RD} \leq 2.8 \cdot \text{RSD}_r, \tag{5}$$

$$\text{RD}_i = \frac{|C_{i1} - C_{i12}|}{\overline{C}_i} \cdot 100\%, \tag{6}$$

$$\overline{C}_i = \frac{1}{n} \sum_{k=1}^{n} C_{ik}, \tag{7}$$

where RD_i is relative difference between C_{i1} and C_{i2}, %; C_{i1} and C_{i2} are the first and the second results of calculation of mass concentration of the ith volatile compound in ethanol-containing product or QC sample, correspondingly, mg $(\text{L AA})^{-1}$; n is number of measurements, $n = 2$; $\overline{C}_i$ is the arithmetic mean of the results for the ith volatile compound, mg $(\text{L AA})^{-1}$.

Checking of Acceptability of Test Results Obtained Under Both Repeatability and Intermediate Precision Conditions

Checking of acceptability of test results obtained under both repeatability and intermediate precision conditions was carried out according to the item 5.3.2.2 (ISO 5725–6: 1994). If the condition (8) was met, then results, obtained under different intermediate precision conditions, are considered acceptable and the grand mean of arithmetic mean of the two final quoted results can be used.

$$\text{CD}_{0.95} \leq \sqrt{\left(2.8 \cdot \text{RSD}_{I(TO)}\right)^2 - \frac{\left(2.8 \cdot \text{RSD}_r\right)^2}{2}}, \tag{8}$$

$$\text{CD}_{0.95} = \frac{\overline{C}_{i1} - \overline{C}_{i2}}{\overline{\overline{C}}_i} \cdot 100\%, \tag{9}$$

$$\overline{\overline{C}}_i = \frac{1}{2} \sum_{l=1}^{2} \overline{C}_{il}, \tag{10}$$

where $\text{CD}_{0.95}$ is the relative critical difference, %; $\overline{C}_{i1}$ and $\overline{C}_{i2}$ are two final quoted results of calculation of mass concentration of the ith volatile compound in ethanol-containing product or QC sample, obtained under the lth intermediate precision condition, correspondingly, mg $(\text{L AA})^{-1}$; $\overline{\overline{C}}_i$ is the grand mean of arithmetic means of the two final quoted results for the ith volatile compound, mg $(\text{L AA})^{-1}$.

Checking of Trueness of Obtained Test Results

Checking of trueness of obtained test results was carried out according to the item 7.2.3.1.3 (ISO 5725–6: 1994). If the condition (11) was met, then trueness of obtained results was considered as acceptable.

$$\frac{\left|\overline{C}_{il} - \mu_i\right|}{\mu_i} \cdot 100\% \leq 2 \cdot \sqrt{\text{RSD}_{I(TO)}^2 - \frac{\text{RSD}_r^2}{2}}, \tag{11}$$

Table 5 Results of determination of RRF coefficients with one GC-FID instrument for 7 years

Compound	RRF^{Eth}, obtained in year							RSD, % for 2013–2020 years	RSD, % for 2016–2020 years
	2013[1]	2016[2]	2017[3]	2018[4]	2019[5]	2020[6]	Current study		
Acetaldehyde	1.627	1.451	1.309	1.402	1.313	1.391	1.314	8.1	4.4
Methyl acetate	1.591	1.562	1.477	1.583	1.727	1.482	1.450	6.1	6.6
Ethyl acetate	1.305	1.201	1.082	1.308	1.125	1.064	1.076	9.1	8.3
Methanol	1.449	1.217	1.264	1.252	1.291	1.251	1.307	5.9	2.5
Propan-2-ol	0.962	0.865	0.806	0.861	0.880	0.794	0.765	7.8	5.6
Propan-1-ol	0.852	0.676	0.686	0.689	0.729	0.676	0.662	9.3	3.4
2-Methylpropan-1-ol	0.708	0.579	0.576	0.574	0.597	0.570	0.537	9.2	3.5
Butan-1-ol	0.772	0.645	0.621	0.619	0.654	0.614	0.612	8.8	2.8
3-Methylbutan-1-ol	0.715	0.630	0.574	0.584	0.587	0.572	0.557	9.1	4.3

1— Charapitsa et al. 2013; 2—Charapitsa et al. 2016; 3—Charapitsa et al. 2018; 4—Charapitsa et al. 2019b; 5—Charapitsa et al. 2019a; 6—Charapitsa et al. 2021

where $\overline{C}_{il}$ is the arithmetic mean of the results, obtained under the lth intermediate precision condition, for the ith volatile compound, mg (L AA)$^{-1}$; μ_i is the reference value (calculated as a result of gravimetrical preparation of SS)
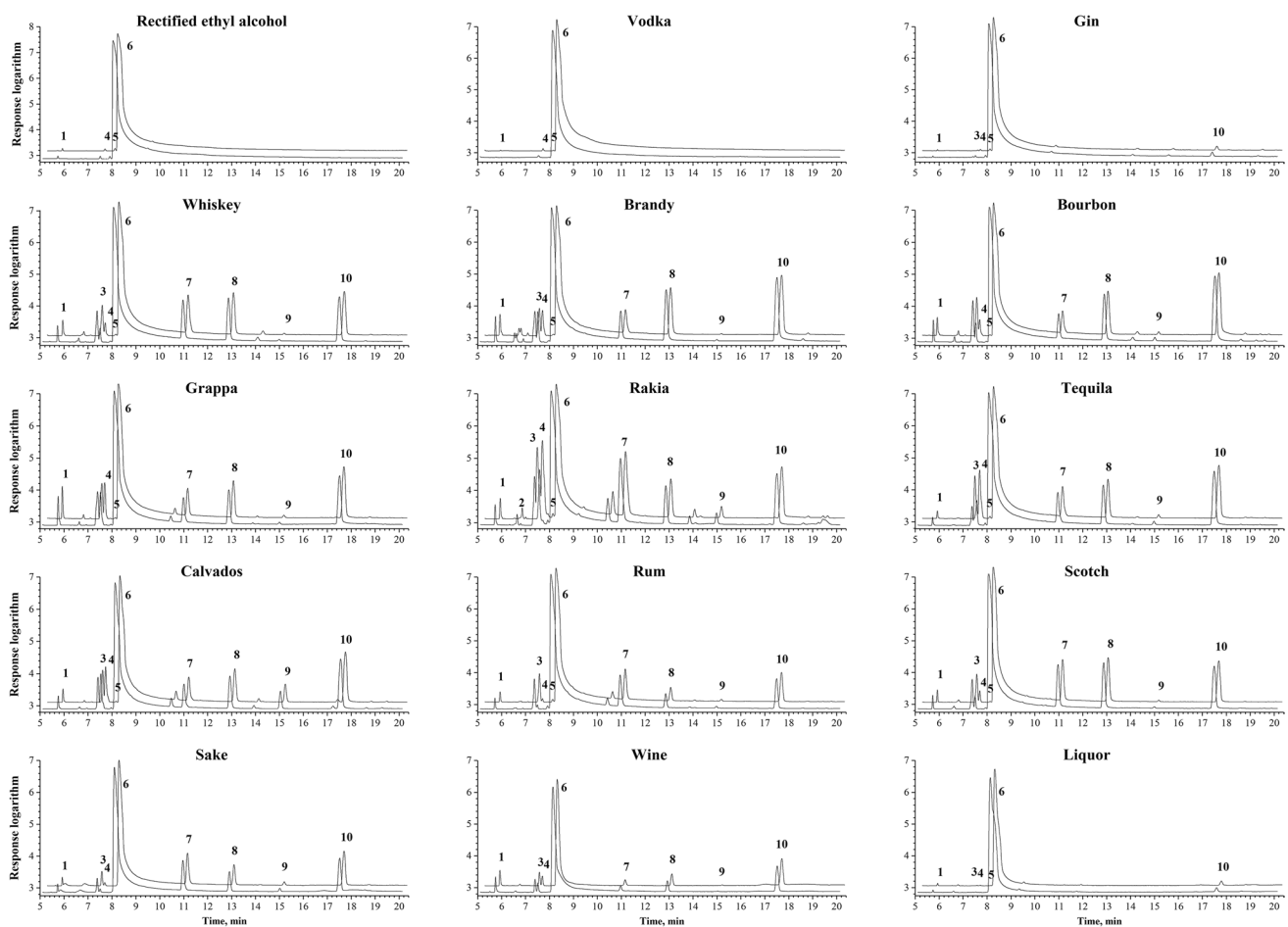

Fig. 1 The chromatograms of samples of ethanol-containing products, obtained under repeatability conditions: 1—acetaldehyde; 2—methyl acetate; 3—ethyl acetate; 4—methanol; 5—propan-2-ol; 6—ethanol; 7—propan-1-ol; 8—2-methylpropan-1-ol; 9—butan-1-ol; 11—3-methylbutan-1-ol
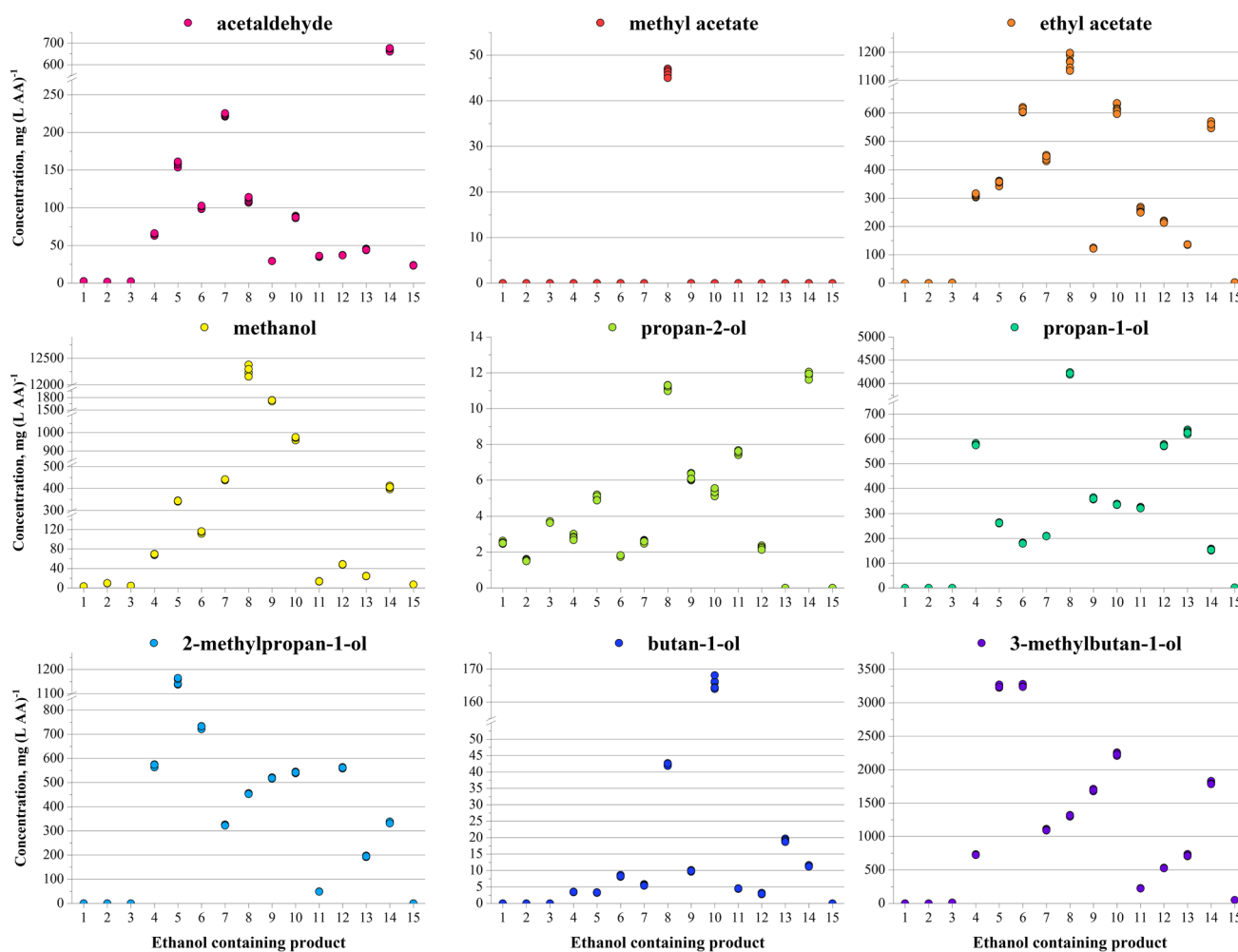
Fig. 2 The results of determination of mass concentrations of volatile compounds under both repeatability and intermediate precision conditions in samples of ethanol-containing products: 1—rectified ethyl alcohol; 2—vodka; 3—gin; 4—whiskey; 5—brandy; 6—bourbon; 7—grappa; 8—rakia; 9—tequila; 10—calvados; 11—rum; 12—scotch; 13—sake; 14—wine; 15—liquor

of mass concentration of the ith volatile compound in QC sample.

Results and Discussion

Results of Calibration

The results of determination of the RRFs, obtained under both repeatability and intermediate precision conditions (time, operator, injection volume), are shown in Table 4. The paired two-sample t-test and ANOVA confirmed that the difference between the means, obtained under intermediate precision conditions, is statistically insignificant. The relative difference between mean values of RRF did not exceed 1.1%.

The values of linearity coefficient R^2 for all analytes in the range of mass concentrations from 2.10 to 5000 mg (L AA)$^{-1}$, obtained for the 1st and 2nd calibration procedures, were more than 0.999 (Table 4).

The obtained results of calculations of calibration coefficients under both repeatability and intermediate precision conditions seem to be close to each other in case with using instrument from the same manufacturer (Rome et al. 2012). Review of previously published results of calibrations (Charapitsa et al. 2013; 2016; 2018; 2019a, b; 2021) shows that this pattern can be a promising topic for further research. The values of RRFs, which have been obtained for 7 years of studying the developed method (Charapitsa et al. 2013; 2016; 2018; 2019a, b; 2021) with one gas chromatograph, are performed in Table 5. The comparison of values of RRF^{Eth} showed that such volatile compounds, as acetaldehyde, methyl acetate, ethyl

acetate, and methanol, have values above 1 and in the case with propan-1-ol, propan-2-ol, 2-methylpropan-1-ol, and 3-methylbutan-1-ol—bellow 1. This pattern is explained by different sensitivity of FID to ethanol and abovementioned volatile compounds.

The estimation of relative standard deviation of relative response factors, obtained during 2013–2020 years of method studying, showed that its value did not exceed 9.3%. The RRF^{Eth} for methanol demonstrates the best repeatability ($RSD_{RRF} = 5.9\%$). Since the instrument was repaired in 2015, the values of RRF^{Eth}, obtained during 2016–2020 years of studying, were estimated separate from results, obtained in 2013 (Table 5). In this case, RSD of RRF^{Eth} did not exceed 8.3%, and in the case with methanol, 2.5%.

The high reproducibility of the values of RRF^{Eth} for modern GC-FID can significantly increase the time interval between instrument calibrations. In perspective, for each GC-FID model, the values of RRF^{Eth} can be tabulated.

Results of Measurements of Alcoholic Beverages and Rectified Ethyl Alcohol

The chromatograms of samples of ethanol-containing products, obtained under repeatability conditions ($n = 2$), are shown in Fig. 1. The results of calculations of mass concentrations (the arithmetic mean of the two results under repeatability conditions) of volatile compounds in ethanol-containing products, obtained under repeatability and intermediate

Table 6 The grand mean values of mass concentrations $\overline{\overline{C}}$ and uncertainties of mass concentrations $U\left(\overline{\overline{C}}\right) k = 2$, $P = 95\%$ of volatile compounds, obtained for alcoholic beverages and rectified ethyl alcohol

Compound	$\overline{\overline{C}} \pm U\left(\overline{\overline{C}}\right)$, mg (L AA)$^{-1}$ $k = 2$, $P = 95\%$				
	Rectified ethyl alcohol	Vodka	Gin	Whiskey	Brandy
Acetaldehyde	2.54 ± 0.3	1.85 ± 0.2	2.33 ± 0.3	64.0 ± 4.8	159 ± 12
Methyl acetate	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ethyl acetate	0 ± 0	0 ± 0	1.67 ± 0.2	310 ± 24	355 ± 27
Methanol	3.29 ± 0.3	9.63 ± 1.0	4.38 ± 0.4	69.0 ± 4.8	343 ± 24
Propan-2-ol	2.53 ± 0.3	1.54 ± 0.2	3.67 ± 0.4	2.80 ± 0.3	5.04 ± 0.6
Propan-1-ol	0 ± 0	0 ± 0	0 ± 0	578 ± 44	263 ± 20
2-Methylpropan-1-ol	0 ± 0	0 ± 0	0 ± 0	571 ± 42	1155 ± 85
Butan-1-ol	0 ± 0	0 ± 0	0 ± 0	3.45 ± 0.5	3.29 ± 0.4
3-Methylbutan-1-ol	0 ± 0	0 ± 0	10.7 ± 0.8	728 ± 54	3241 ± 240
Compound	$\overline{\overline{C}} \pm U\left(\overline{\overline{C}}\right)$, mg (L AA)$^{-1}$ $k = 2$, $P = 95\%$				
	Bourbon	Grappa	Rakia	Tequila	Calvados
Acetaldehyde	101 ± 7.6	223 ± 17	111 ± 8.3	29.3 ± 2.2	87.8 ± 6.6
Methyl acetate	0 ± 0	0 ± 0	46.3 ± 3.5	0 ± 0	0 ± 0
Ethyl acetate	612 ± 47	443 ± 34	1167 ± 89	123 ± 9.4	616 ± 47
Methanol	113 ± 7.8	439 ± 30	12,252 ± 845	1727 ± 119	971 ± 67
Propan-2-ol	1.76 ± 0.2	2.60 ± 0.3	11.2 ± 0.8	6.16 ± 0.7	5.31 ± 0.6
Propan-1-ol	181 ± 14	209 ± 16	4216 ± 320	360 ± 27	337 ± 25.6
2-Methylpropan-1-ol	729 ± 54	324 ± 24	454 ± 34	519 ± 38	542 ± 40
Butan-1-ol	8.39 ± 1.1	5.50 ± 0.7	42.3 ± 3.1	9.90 ± 1.3	166 ± 12
3-Methylbutan-1-ol	3257 ± 241	1101 ± 81	1309 ± 97	1696 ± 126	2233 ± 165
Compound	$\overline{\overline{C}} \pm U\left(\overline{\overline{C}}\right)$, mg (L AA)$^{-1}$ $k = 2$, $P = 95\%$				
	Rum	Scotch	Sake	Wine	Liquor
Acetaldehyde	35.6 ± 2.7	37.1 ± 2.8	44.9 ± 3.4	668 ± 50	23.5 ± 1.8
Methyl acetate	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ethyl acetate	258 ± 20	216 ± 16	136 ± 10	557 ± 42	2.98 ± 0.4
Methanol	13 ± 0.9	48 ± 3.3	24 ± 1.7	406 ± 28	6.84 ± 0.7
Propan-2-ol	7.54 ± 0.9	2.25 ± 0.3	0 ± 0	11.9 ± 0.9	0 ± 0
Propan-1-ol	324 ± 25	574 ± 44	628 ± 48	155 ± 12	1.53 ± 0.2
2-Methylpropan-1-ol	49 ± 3.6	561 ± 42	194 ± 14	335 ± 25	0 ± 0
Butan-1-ol	4.51 ± 0.6	2.94 ± 0.4	19.2 ± 1.4	11.4 ± 0.8	0 ± 0
3-Methylbutan-1-ol	225 ± 17	528 ± 39	718 ± 53	1804 ± 134	51.0 ± 3.8

precision conditions with Eq. (7), are shown in Fig. 2 and Table 6. The concentrations of analyzed volatile compounds in distillate samples showed the recovery values from 95.9 to 101.0% and from 98.4 to 99.8% for wine and liquor samples, correspondingly.

The acetaldehyde was found in 15 samples with its concentration varying from 1.84 to 677 mg/(L AA)$^{-1}$; methyl acetate was found in 1 sample with its concentration varying from 45.0 to 47.0 mg/(L AA)$^{-1}$; ethyl acetate was found in 13 samples with its concentration varying from 1.63 to 1198 mg/(L AA)$^{-1}$; methanol was found in 15 samples with its concentration varying from 3.25 to 12,394 mg/(L AA)$^{-1}$; propan-2-ol was found in 13 samples with its concentration varying from 1.49 to 12.1 mg/(L AA)$^{-1}$; propan-1-ol was found in 12 samples, its concentration varied from 1.49 to 4243 mg/(L AA)$^{-1}$; 2-methyl propan-1-ol was found in 11 samples with its concentration varying from 48.4 to 1165 mg/(L AA)$^{-1}$; butan-1-ol was found in 11 samples with its concentration varying from 2.79 to 168 mg/(L AA)$^{-1}$;

and 3-methylbutan-1-ol was found in 13 samples with its concentration varying from 10.6 to 3284 mg/(L AA)$^{-1}$.

Thus, acetaldehyde and methanol are the only volatile compounds (out of purposefully analyzed) that are present in all studied samples of alcoholic and ethanol-containing products. Samples of vodka, gin, and rectified ethyl alcohol contained the least numbers of volatile compounds (3–4 out of 9 analyzed) with a concentration that can be detected by GC-FID.

Acceptability and Trueness of Test Results

The results of checking of acceptability of test results, obtained under repeatability and under both repeatability and intermediate precision conditions, are shown in Figs. 3 and 4, correspondingly.

The gray lines in Figs. 3 and 4 show the critical values (calculated according to the ISO 5725–6: 1994), used for checking of acceptability of test results for mass concentrations of the order of less than 15 mg (L AA)$^{-1}$ for methanol
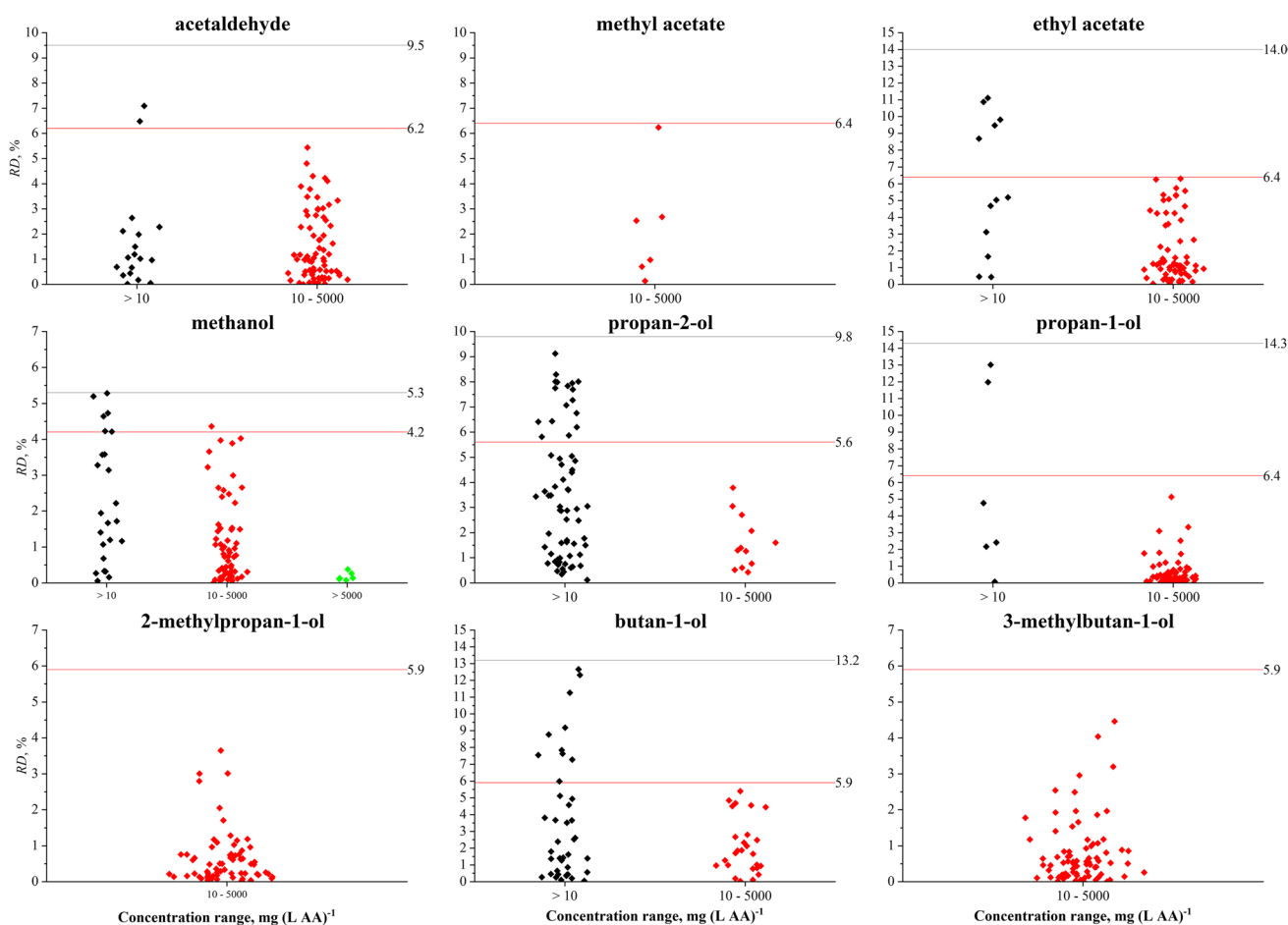

Fig. 3 The results of checking of acceptability of test results obtained under repeatability conditions: gray line—control value for range of concentrations 0–10 mg (L AA)$^{-1}$; red line—control value for range of concentrations 10–5000 mg (L AA)$^{-1}$
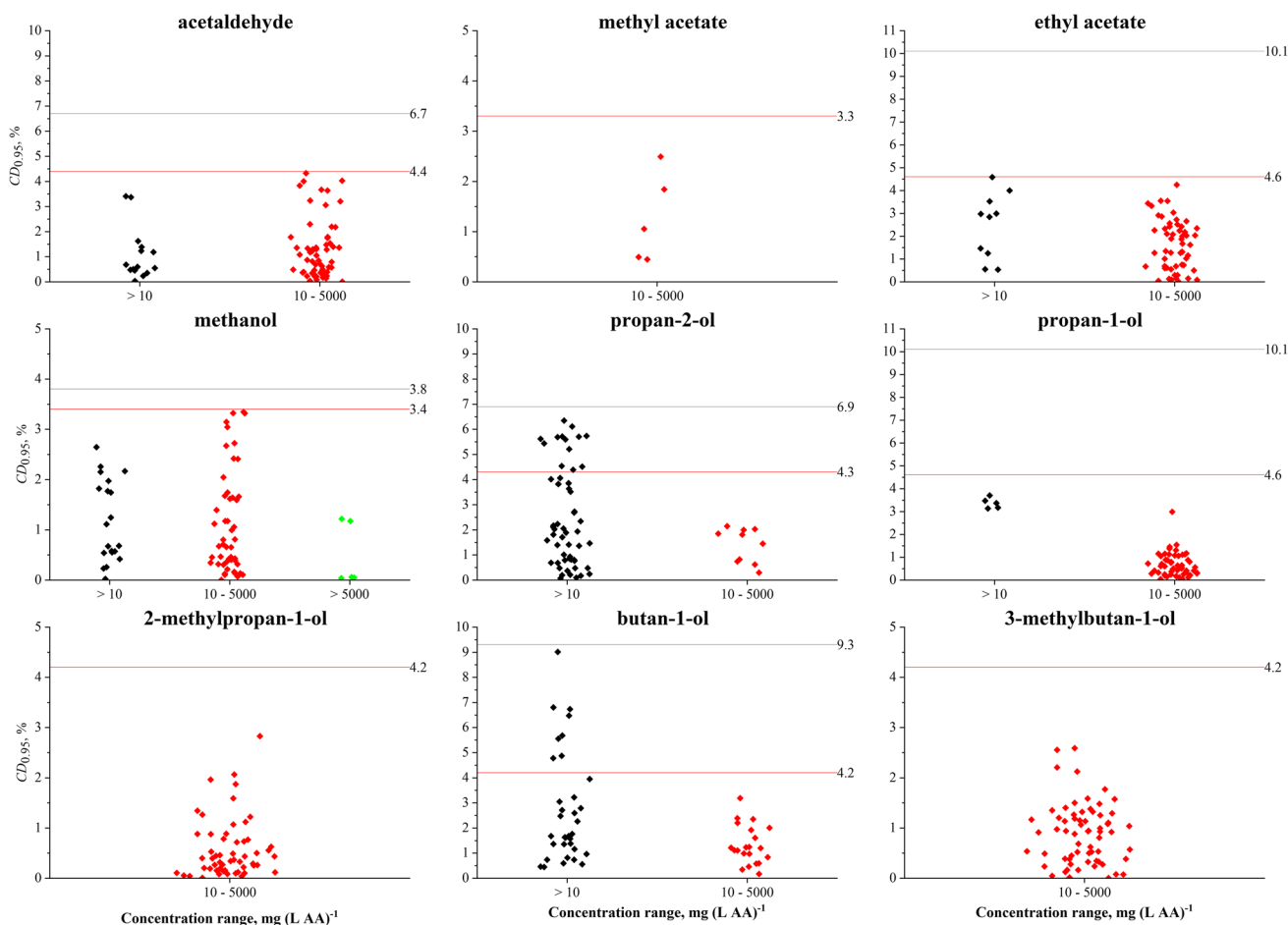
Fig. 4 The results checking of acceptability of test results obtained under both repeatability and intermediate precision conditions: gray line—control value for range of concentrations 0–10 mg (L AA)$^{-1}$; red line—control value for range of concentrations 10–5000 mg (L AA)$^{-1}$

and 10 mg (L AA)$^{-1}$ for other volatiles. The red lines in Figs. 3 and 4 show the critical values, used for checking of acceptability of test results for mass concentrations from 15

to 5000 mg (L AA)$^{-1}$ for methanol and 10 to 5000 mg (L AA)$^{-1}$ for other volatiles. In the case when the concentration of the volatile compound was more than 5000 mg (L AA)$^{-1}$

Table 7 The results of checking of trueness of obtained test results

Compound	$\frac{|\overline{C}-\mu|}{\mu} \cdot 100\%$						Control value for concentration in range	
	SS-*G*	SS-*F*	SS-*E*	SS-*D*	SS-*B*	SS-*A*	0–10.0 mg (L AA)$^{-1}$	10.0–5000 mg (L AA)$^{-1}$
Acetaldehyde	1.2	1.7	0.3	2.3	0.1	0.2	4.8	3.1
Methyl acetate	1.7	1.0	0.3	0.9	1.1	2.6	7.2	3.3
Ethyl acetate	2.3	0.8	0.9	1.0	1.1	1.3	7.1	3.3
Methanol	1.9	0.3	0.8	0.9	0.6	0.9	2.7	2.4
Propan-2-ol	1.9	1.9	2.0	0.4	2.4	1.9	4.9	3.1
Propan-1-ol	0.6	1.9	0.4	1.6	0.8	0.4	7.2	3.3
2-Methylpropan-1-ol	0.8	2.3	0.3	0.3	0.5	0.9	6.6	3.0
Butan-1-ol	0.5	0.01	0.1	0.7	0.5	1.0	6.6	3.0
3-Methylbutan-1-ol	2.3	1.1	0.4	0.4	1.3	0.3	6.6	3.0

(methanol in rakia), the results of calculations for RD and $CD_{0.95}$ were shown in Figs. 3 and 4 as green dots, but the acceptability of the results was not checked, due to the lack of data for the critical values for the assessment. The statistical analysis of the obtained measurement results confirmed that all results can be considered acceptable. The results of checking of trueness of obtained test results are shown in Table 7. The results of checking of trueness of obtained test results fully meet the acceptance criteria set forth (ISO 5725–6: 1994).

Conclusions

An analytical method for direct determination of mass concentrations of methanol, acetaldehyde, and volatiles by GC-FID was developed and validated for 15 ethanol-containing products, with satisfactory performance. The results obtained are an experimental confirmation of the suitability of the developed method for the analysis of 9 volatile compounds in a wide range of ethanol-containing matrices. The procedure is characterized by an absence of sample preparation (in the case with sugar-free ethanol-containing products), and the developed method allows for routine use in food testing and pharmaceutical laboratories. The expanded relative uncertainty ($k = 2$, $P = 0.95$) values for methanol in mass concentration ranging from 2.10 to 15.0 mg $(L\ AA)^{-1}$ and from 15.0 to 5000 mg $(L\ AA)^{-1}$ are 9.9 and 5.9%, correspondingly. The expanded relative uncertainty ($k = 2$, $P = 0.95$) values for acetaldehyde in mass concentration ranging from 2.10 to 10.0 mg $(L\ AA)^{-1}$ and from 10.0 to 5000 mg $(L\ AA)^{-1}$ are 11.4 and 7.5%, correspondingly. The expanded relative uncertainty ($k = 2$, $P = 0.95$) values for other volatile compounds in mass concentration ranging from 2.10 to 10.0 mg $(L\ AA)^{-1}$ and from 10.0 to 5000 mg $(L\ AA)^{-1}$ are 13.7 and 7.6%, correspondingly. This values of expanded relative uncertainty demonstrate the high accuracy and trueness of the method, which can be an occasion for initiating interlaboratory study on the basis of specialized international organizations, for example, the International Organization for Standardization (ISO) and the Codex Alimentarius Commission or the International Organization of Vine and Wine (OIV) for the purpose of its subsequent approval as a standardized referee method on the international level.

The noticed opportunity of tabulation of calibration coefficients makes it possible to improve the traditional methods of analysis, based on GC-FID measurements. It can allow to exclude calibration procedure from analysis or make only one calibration at the first analysis on new instrument. This opportunity can make analysis cheaper, faster, and less laborious. The study of possibility of

tabulation of RRFs needs to be carried out in a larger number of laboratories (full-scale interlaboratory study) for collecting more statistics for various GC-FID instruments.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable.

Conflict of Interest Siarhei Charapitsa declares that he has no conflict of interest. Svetlana Sytova declares that she has no conflict of interest. Anton Kavalenka declares that he has no conflict of interest. Lidia Sobolenko declares that she has no conflict of interest. Yauheni Shauchenka declares that he has no conflict of interest. Nikolai Kostyuk declares that he has no conflict of interest. Vladimir Egorov declares that he has no conflict of interest. Sergey Leschev declares that he has no conflict of interest. Sergey Vetokhin declares that he has no conflict of interest. Natalia Zayats declares that she has no conflict of interest. Sergey Tsimbalaev declares that he has no conflict of interest. Alexander Kolesnov declares that he has no conflict of interest.

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