



The study of the matrix effect on the method of direct determination of volatile compounds in a wide range of alcoholic beverages

Siarhei Charapitsa^{a,*}, Svetlana Sytova^a, Anton Kavalenka^a, Lidia Sobolenko^{a,b},
Nikolai Kostyuk^b, Vladimir Egorov^b, Sergey Leschev^b, Sergey Vetokhin^c, Natalia Zayats^c

^a Institute for Nuclear Problems of Belarusian State University, 220030, Bobruyskaya Str., 11, Minsk, Belarus

^b Belarusian State University, 220030, Nezavisimosti Av., 4, Minsk, Belarus

^c Belarusian State Technological University, 220006, Sverdlova Str., 13a, Minsk, Belarus

ARTICLE INFO

Keywords:

Alcoholic beverages
Volatile compounds
Ethanol
Internal standard
Matrix effect

ABSTRACT

The results of experimental studies of the method of using ethyl alcohol as an internal standard for the direct determination of volatile compounds for a wide range of alcoholic and ethanol-containing products: whiskey, brandy, grappa, vodka, scotch, bourbon, brandy, calvados, sake, rum, gin, tequila and rectified ethyl alcohol 96% v/v are presented. The results, obtained for the developed method, were compared with the results, obtained for traditional internal standard method, using 2-pentanol as internal standard. The relative difference between developed and traditional methods aren't exceed $\pm 2.0\%$. Assessment of influence of matrix effects to the results, obtained for developed method, is performed using standard additions method with the following ANOVA analysis. Ways are proposed for performing interlaboratory study of the method under the patronage of the OIV to recognize the method for regulatory purposes at the international level.

1. Introduction

A lot of compounds known to be present in alcoholic beverages are critical to the quality of these beverages. Many of these compounds are already present in the raw materials or precursors, but many others are obtained from the various production processes, such as fermentation, boiling/distillation, maturation (especially in wood), pasteurization and so on. The balance of the compounds is very important; too many or too few specific volatiles can greatly affect the quality of the drink. Depending on manufacturing technology, alcoholic beverages have a characteristic composition of the volatile compounds (Buglass, 2011; Wiśniewska et al., 2016). In according to official analysis methods of EU (Commission regulation (EC) No 2870, 2000; OIV-MA-BS-14, R2009), USA (AOAC Official Method 972.10, 2005; AOAC Official Method 972.11, 2005), China (GB/T 11858, 2008), India (BIS IS 3752, 2009), Mexico (NOM-199-SCFI, 2017) the analysis of volatile compounds is performed by GC-FID with traditional method of internal standard. The traditional method of internal standard has such disadvantages as the added error due to the pipetting of the internal standard, the

inconvenience of the additional counting time required and the contamination of the sample by the internal standard. Interlaboratory study of traditional internal standard method was performed in 31 laboratories from 8 countries only for rum, whiskey, brandy, kirsch and grappa spirit drinks (Kelly et al., 1998). The method was also validated for analysis of rakia (Yilmaztekin & Cabaroğlu, 2011).

The authors developed approach for the determination of volatile compounds in alcohol products, which is based on ethanol usage as internal standard (Charapitsa et al., 2013, 2018). The developed method was validated in one laboratory (Charapitsa, Sytova, Korban, & Sobolenko, 2019) and passed interlaboratory study involving 9 laboratories from 4 countries (Charapitsa, Sytova, Korban, Sobolenko, Egorov, et al., 2019). The perspectives of determination of volatile compounds in alcohol products by GC-MS were also studied (Korban et al., 2019). In abovementioned studies the method was approbated for such alcohol beverages as whiskey, brandy, grappa, wine and rakia only.

The developed and traditional internal standard methods use single-point calibration with water-ethanol (60:40% v/v) solutions to establish calibration characteristics, by analogy, as is done in official analysis

* Corresponding author.

E-mail addresses: siarhei.charapitsa@gmail.com (S. Charapitsa), s_sytova@mail.ru (S. Sytova), anton.kavalenka@gmail.com (A. Kavalenka), lidia.sobolenko@gmail.com (L. Sobolenko), nnkostyuk@bsu.by (N. Kostyuk), egorvv@bsu.by (V. Egorov), leschev.sergey54@gmail.com (S. Leschev), veto@belstu.by (S. Vetokhin), zajatsni@mail.ru (N. Zayats).

<https://doi.org/10.1016/j.foodcont.2020.107528>

Received 1 June 2020; Received in revised form 30 July 2020; Accepted 31 July 2020

Available online 3 August 2020

0956-7135/© 2020 Elsevier Ltd. All rights reserved.

methods of EU (Commission regulation (EC) No 2870, 2000). Thus, the composition of the matrix of calibration solution and standard solutions, used for check linearity of response, may not correspond to the composition of the matrix of the test sample of alcoholic beverage. Ideally, the matrix-appropriate certified reference material (CRM) should be used for analysis (Thompson et al., 2006). However, the matrix-appropriate CRMs aren't available for all matrices of alcohol beverages, thus water-ethanol solutions are used as standard solutions. Since the influence of matrix effect on the detector response, analytical curves and calibration coefficients can distort the results of analysis, it is necessary to prove lack of influence of the matrix effect or find ways to eliminate it.

According to the single-laboratory validation guidelines (AOAC, 2002, pp. 1–38; Thompson et al., 2002) a test for general matrix effect can be made by applying the method of analyte additions (standard additions method). Standard solutions and matrix matched solutions are being prepared, and calibration results, obtained for both, be compared for significant difference. A lack of significance means that there is no detectable general matrix effect (Thompson et al., 2002). Also, the recovery parameter can be used for estimation of matrix effect. This approach for estimation of matrix effect is widely used for validation of different methods of analysis of a wide variety of objects: agricultural products (Dominguez et al., 2014; Uebelacker & Lachenmeier, 2011), drugs (Hall et al., 2012), alcoholic beverages (Isaac-Lam, 2016; Nalazek-Rudnicka & Wasik, 2017), biological liquids (Matuszewski, 2006; Saini et al., 2006), environmental objects (Borecka et al., 2014; Powley et al., 2005), etc.

In order to comparison results, obtained for both developed and traditional method, and to study matrix effect, the following alcoholic beverages and ethanol-containing products were selected as objects for research: whiskey, brandy, grappa, vodka, scotch, bourbon, brandy, calvados, sake, rum, gin, tequila and rectified ethyl alcohol 96% v/v.

2. Materials and methods

2.1. Reagents

High-purity rectified ethanol 96.0% v/v was supplied by Dyatlovo Distillery Plant Algon (Slonim, Belarus). Pure distilled and deionized water (conductivity ≤ 0.5 M Ω cm) was obtained from JSC Integral (Minsk, Belarus). The following chemical standards: acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 2-pentanol, 1-butanol, 3-methyl-1-butanol were purchased from Sigma Aldrich (Alcobendas, Madrid, Spain) with the highest purity available (more than 99%). Purities of volatiles were confirmed by the GC-FID analysis using the internal normalization method. The presence of water in the chemicals was tested by the GC-TCD analysis, which confirmed the absence of water.

2.2. Alcoholic beverages

The alcoholic beverages (bourbon, brandy, calvados, gin, grappa, rakia, rum, tequila, sake, scotch, vodka, whiskey) were purchased in commercial stores (Table 1).

2.3. Preparation of solvent (water-ethanol) standard solutions

All standard solutions (SS) were prepared gravimetrically according

to the (ASTM D4307-99, 2015 astm:2015). 2-Pentanol was used as the traditional internal standard.

Hydroalcoholic solution (HS) with ethanol concentration 40% v/v was prepared according to the item 5.13 of (Commission regulation (EC) No 2870, 2000) from rectified ethanol 96% v/v and deionized water.

Stock standard solution (SSS) was prepared according to the item 5.14.1 of (Commission regulation (EC) No 2870, 2000) by adding the volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol) to HS.

Internal standard solution (SS-P) was prepared according to the item 5.14.2 of (Commission regulation (EC) No 2870, 2000) by adding of 2-pentanol to HS.

Standard solution for calibration (SS-C) was prepared according to the item 5.14.3 of (Commission regulation (EC) No 2870, 2000) by adding of solutions SSS and SS-P to HS.

Standard solutions used to check linearity of the FID response SS-A, SS-B, SS-C, SS-D, SS-E, SS-F, SS-G and SS-H were prepared by analogy with item 5.14.6 of (Commission regulation (EC) No 2870, 2000) by adding aliquots of 10.0, 2.0, 1.0, 0.5, 0.4, 0.1, 0.05, 0.02 mL SSS, correspondingly, and 1.0 mL SS-P into separate 100 mL volumetric flasks containing approximately 80 mL of HS, and the content of the flasks were diluted to volume with HS and mixed thoroughly.

The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations.

The concentrations of volatile compounds with the corresponding uncertainties in the prepared standard solutions are shown in Table 2.

2.4. Preparation of matrix matched solutions

Matrix matched solutions (MMS) were prepared in alcoholic beverages' matrices using the standard addition method (AOAC, 2002, pp. 1–38; Thompson et al., 2002) gravimetrically according to the (ASTM D4307, 2015).

2.4.1. Preparation of matrix matched solutions with ethanol concentration about 40% v/v

In case with the beverages with ethanol concentration about 40% v/v (bourbon, brandy, calvados, gin, grappa, rakia, rum, tequila, scotch, whiskey) *matrix matched solutions* were prepared using SSS and SS-P. The vodka sample has matrix similar to the matrix of standard solutions, therefore, the *matrix matched solutions* for vodka were not prepared.

Matrix matched solutions MMS-A for each alcoholic beverage matrix were prepared by adding a 0.3 mL portion of each volatile compound (acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol) and 1.0 mL of SS-P into separate 100 mL volumetric flasks containing approximately 80 mL alcoholic beverage, and the content of the flasks were diluted to the volume with corresponding alcoholic beverage and mixed thoroughly. The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations. As a result, 10 *matrix matched solutions MMS-A* for abovementioned alcoholic beverage matrices with concentration of additions of volatiles about 5000 mg/L AA (Absolute Alcohol - AA) were prepared.

Matrix matched solutions MMS-B, MMS-C, MMS-D, MMS-E, MMS-F, MMS-G and MMS-H were prepared by adding aliquots of 2.0, 1.0, 0.5,

Table 1
The ethanol volume concentration in the alcoholic beverages and rectified ethanol.

Ethanol volume concentration in beverage, %												
Whiskey	Brandy	Rum	Gin	Grappa	Vodka	Tequila	Sake	Bourbon	Rakia	Scotch	Calvados	Ethanol
43.0	40.0	40.0	47.0	40.0	40.0	38.0	14.5	40.0	40.0	40.0	40.0	96.0

Table 2

The concentrations and uncertainties of concentrations of volatile compounds in the prepared standard solutions.

Compound	Concentration, mg/L AA							
	SS-H	SS-G	SS-F	SS-E	SS-D	SS-C	SS-B	SS-A
acetaldehyde	12.5 ± 0.25	27.0 ± 0.54	56.0 ± 1.1	225 ± 2.2	259 ± 2.5	580 ± 5.8	1136 ± 11.3	5511 ± 55
methyl acetate	10.8 ± 0.21	24.3 ± 0.48	51.3 ± 1.0	209 ± 2.0	240 ± 2.4	539 ± 5.3	1056 ± 10.5	5126 ± 51
ethyl acetate	13.0 ± 0.26	26.7 ± 0.53	54.1 ± 1.0	214 ± 2.1	245 ± 2.4	549 ± 5.4	1074 ± 10.7	5205 ± 52
methanol	13.9 ± 0.27	27.5 ± 0.55	54.6 ± 1.0	213 ± 2.1	244 ± 2.4	545 ± 5.4	1065 ± 10.6	5161 ± 51
2-propanol	11.4 ± 0.22	24.5 ± 0.49	50.9 ± 1.0	204 ± 2.0	235 ± 2.3	527 ± 5.2	1032 ± 10.3	5006 ± 50
ethanol	789300							
1-propanol	10.6 ± 0.21	23.7 ± 0.47	49.9 ± 0.9	203 ± 2.0	233 ± 2.3	525 ± 5.2	1028 ± 10.2	4991 ± 49
2-methyl-1-propanol	10.7 ± 0.21	23.9 ± 0.47	50.4 ± 1.0	205 ± 2.0	236 ± 2.3	530 ± 5.3	1038 ± 10.3	5041 ± 50
2-pentanol	543 ± 5.4	543 ± 5.4	543 ± 5.4	543 ± 5.3	543 ± 5.3	542 ± 5.2	542 ± 5.2	532 ± 5.3
1-butanol	10.5 ± 0.21	23.6 ± 0.47	49.8 ± 0.9	202 ± 2.0	233 ± 2.3	523 ± 5.2	1025 ± 10.2	4976 ± 49
3-methyl-1-butanol	11.2 ± 0.22	25.2 ± 0.50	53.2 ± 1.0	216 ± 2.1	249 ± 2.4	559 ± 5.5	1095 ± 10.9	5315 ± 53

0.4, 0.1, 0.05, 0.02 mL SSS, correspondingly, and 0.1 mL of SS-P into separate 10 mL volumetric flasks containing approximately 7.0 mL alcoholic beverage, and the content of the flasks were diluted to the volume with corresponding alcoholic beverage and mixed thoroughly. The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations. As a result, 70 *matrix matched solutions* for above-mentioned alcoholic beverage matrices with concentration of additions of volatiles in range 10–1000 mg/L AA were prepared.

2.4.2. Preparation of matrix matched solutions with ethanol concentration 96% v/v (rectified ethanol)

In case with the rectified ethanol, *matrix matched solutions* were prepared in 96.0% v/v ethanol.

Internal standard solution with ethanol concentration 96% v/v (SS-P-96%) was prepared by adding of a 7.0 mL portion of 2-pentanol into 100 mL volumetric flask, containing approximately 80 mL of 96.0% v/v ethanol.

Stock standard solution with ethanol concentration 96% v/v (SSS-96%) was prepared by adding approximately 5.0 mL portion of each volatile compound (acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol) into separate 100 mL volumetric flask containing approximately 40 mL 96.0% v/v ethanol, and the content of the flask was diluted to the volume with 96.0% v/v ethanol and mixed thoroughly.

Matrix matched solutions MMS-A, MMS-B, MMS-C, MMS-D, MMS-E, MMS-F, MMS-G and MMS-H for rectified ethanol were prepared by adding aliquots of 10.0, 2.0, 1.0, 0.5, 0.4, 0.1, 0.05, 0.02 mL SSS-96%, correspondingly, and 1.0 mL of SS-P-96% into separate 100 mL volumetric flasks containing approximately 80 mL alcoholic beverage, and the content of the flasks were diluted to the volume with corresponding alcoholic beverage and mixed thoroughly. The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations. As a result, 8 *matrix matched solutions* for rectified ethanol matrix with concentration of additions of volatiles in range 10–5000 mg/L AA were prepared.

2.4.3. Preparation of matrix matched solutions with ethanol concentration 14.5% v/v (sake)

In case with the sake, *matrix matched solutions* were prepared in 14.5% v/v hydroalcoholic solution.

Hydroalcoholic solution with ethanol concentration 14.5% v/v (HS-14.5%) was prepared from rectified ethanol 96% v/v and deionized water.

Internal standard solution with ethanol concentration 14.5% v/v (SS-P-14.5%) was prepared by adding of a 1.0 mL portion of 2-pentanol to HS-14.5%.

Stock standard solution with ethanol concentration 14.5% v/v (SSS-14.5%) was prepared by adding a 0.9 mL portion of each volatile

compound (acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol) and 1.0 mL of SS-P into separate 100 mL volumetric flask containing approximately 80 mL HS-14.5%, and the content of the flask was diluted to the volume with 14.5% v/v ethanol and mixed thoroughly. The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations.

Matrix matched solutions MMS-A, MMS-B, MMS-C, MMS-D, MMS-E, MMS-F, MMS-G and MMS-H for sake matrix were prepared by adding aliquots of 10.0, 2.0, 1.0, 0.5, 0.4, 0.1, 0.05, 0.02 mL SSS-14.5%, correspondingly, and 1.0 mL of SS-P-14.5% into separate 100 mL volumetric flasks containing approximately 80 mL sake, and the content of the flasks were diluted to the volume with sake and mixed thoroughly. The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations. As a result, 8 *matrix matched solutions* for rectified ethanol matrix with concentration of additions of volatiles in range 10–5000 mg/L AA were prepared.

2.5. Preparation of the alcoholic beverages test samples

In case with the beverages with ethanol concentration about 40% v/v (bourbon, brandy, calvados, gin, grappa, rakia, rum, tequila, scotch, whiskey) 0.1 mL of SS-P was added into separate 10 mL volumetric flasks containing approximately 9.0 mL alcoholic beverage sample, and the content of the flasks was diluted to the volume with corresponding alcoholic beverage and mixed thoroughly. In case with the rectified ethanol 96% v/v 0.1 mL of SS-P-96% was added into separate 10 mL volumetric flasks containing approximately 9.0 mL rectified ethanol, and the content of the flasks was diluted to the volume with rectified ethanol and mixed thoroughly. In case with the sake 0.1 mL of SS-P-14.5% was added into separate 10 mL volumetric flasks containing approximately 9.0 mL sake, and the content of the flasks was diluted to the volume with sake and mixed thoroughly. The weight of the flasks, each component added and the total final weight of contents were recorded and used in following calculations of concentrations.

2.6. Analysis

Analysis was performed using a gas chromatograph Crystal-5000.1 (JSC SDB Chromatec, Yoshkar-Ola, Russia), equipped with the auto-sampler, FID and TCD detectors. All the separations were carried out with a capillary column Rt-Wax, 60 m × 0.53 mm, phase thinness 1.0 μm (Restek, Bellefonte, USA). The injections were made in the split mode (12:1), and the injection volume was 1 μL. The temperature of injector was 190 °C. The oven was programmed for 75 °C for 9 min, increased by 5°/min to 130 °C, then increased by 10°/min to 180 °C, followed by 5 min at the final temperature. The temperatures of FID and TCD were

260° and 160 °C, correspondingly. Volatile compounds were determined by direct injection of the alcoholic drink. All standard solutions SS, matrix matched solutions MMS and alcoholic beverage test samples were measured under repeatability conditions three times.

The example of obtained chromatograms for SS and alcoholic beverage test samples are shown in Figs. 1 and 2, correspondingly.

3. Calculation

3.1. Calibration

The single-point calibration was done according to the item 8.4 of (Commission regulation (EC) No 2870, 2000) with the usage of SS-C.

3.1.1. Calibration (2-pentanol as internal standard)

Values of RRF_i^{Trad} (Relative Response Factor – RRF) for each i -th volatile compound were determined according to item of 9.1 (Commission regulation (EC) No 2870, 2000) by Eq. (1):

$$RRF_i^{Trad} = \frac{C_i^{SS-C}(mg/mg)}{A_i^{SS-C}} \cdot \frac{A_{2-pentanol}^{SS-C}}{C_{2-pentanol}^{SS-C}(mg/mg)}, \quad (1)$$

where C_i^{SS-C} and $C_{2-pentanol}^{SS-C}$ are the mass concentrations of the i -th volatile compound and 2-pentanol in SS-C, correspondingly, expressed in mg/mg (ratio mass of compound in mg to mass of SS-C in mg) units; A_i^{SS-C} and $A_{2-pentanol}^{SS-C}$ are the detector responses for the i -th volatile and 2-pentanol in SS-C, correspondingly, a.u. (arbitrary units).

3.1.2. Calibration (ethanol as internal standard)

The values of RRF_i^{Eth} for each volatile compound were determined according to the following Eq. (2):

$$RRF_i^{Eth} = \frac{C_i^{SS-C}(mg/L AA)}{A_i^{SS-C}} \cdot \frac{A_{Eth}^{SS-C}}{\rho_{Eth}}, \quad (2)$$

where C_i^{SS-C} is the concentration of the i -th volatile compound in SS-C, expressed in mg/L AA units; A_{Eth}^{SS-C} is the detector response for ethanol in SS-C, a.u.; ρ_{Eth} is the density of ethanol, $\rho_{Eth} = 789300$ mg/L.

The results of RRFs calculations, obtained for both traditional and developed methods, are shown in Table S1 of Supplementary materials.

3.2. Calculation of concentrations in the test samples

3.2.1. Calculation of concentrations in the test samples (2-pentanol as internal standard)

The concentration of i -th volatile in j -th alcohol beverage test sample in mg/mg units was determined according to item 9.1.2 of (Commission regulation (EC) No 2870, 2000) by Eq. (3):

$$C_i^{Trad}(j)(mg/mg) = RRF_i^{Trad} \frac{A_i^j}{A_{2-pentanol}^j} \cdot C_{2-pentanol}^j(mg/mg), \quad (3)$$

where A_i^j and $A_{2-pentanol}^j$ are the detector responses for i -th volatile and 2-pentanol in the j -th alcohol beverage test sample, correspondingly, a.u.; $C_{2-pentanol}^j$ is the concentration of 2-pentanol in the j -th alcohol beverage test sample, expressed in mg/mg (ratio mass of compound in mg to mass of SS-C in mg) units.

To obtain the results of the study of the sample, according to the traditional method of the internal standard in the legislatively required value of mg/L AA, it is necessary to determine the volume concentration of ethyl alcohol H^j and the density ρ^j of the j -th alcohol beverage test sample. Concentration of i -th volatile compound in mg/L AA units was determined according to item 9.2 of (Commission regulation (EC) No 2870, 2000) by Eq. (4):

$$C_i^{Trad}(j)(mg/L AA) = \frac{C_{2-pentanol}^j(mg/mg)}{H^j/100\%} \cdot \rho^j, \quad (4)$$

where H^j is the volume concentration of ethyl alcohol in the j -th alcohol beverage test sample, % v/v; ρ^j is the density of the j -th alcohol beverage test sample, expressed in mg/L units.

3.2.2. Calculation of concentrations in the test samples (ethanol as internal standard)

The concentration of i -th volatile was determined according to Eq. (5):

$$C_i^{Eth}(j) = RRF_i^{Eth} \frac{A_i^j}{A_{Eth}^j} \cdot \rho_{Eth}, \quad (5)$$

where A_{Eth}^j is the detector response for ethanol in the j -th alcohol beverage test sample, a.u.

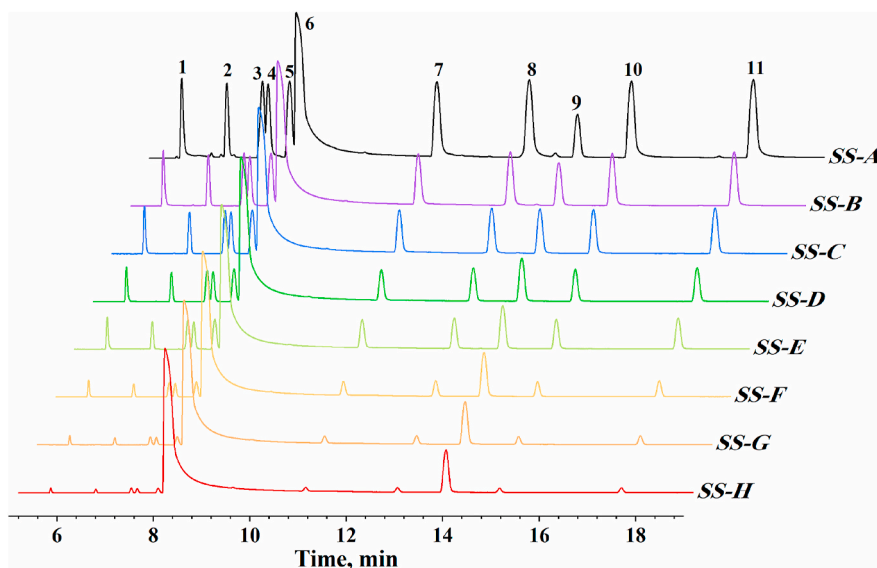


Fig. 1. The chromatograms of prepared standard solutions in the logarithmic scale. 1 - acetaldehyde; 2 - methyl acetate; 3 - ethyl acetate; 4 - methanol; 5 - 2-propanol; 6 - ethanol; 7 - 1-propanol; 8 - 2-methyl-1-propanol; 9 - 2-pentanol; 10 - 1-butanol; 11 - 3-methyl-1-butanol.

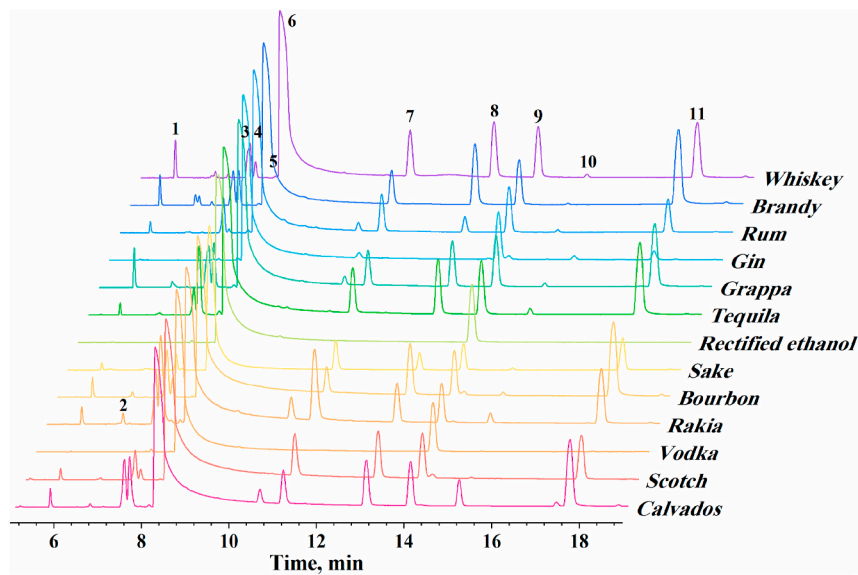


Fig. 2. The chromatograms of the ethanol containing beverages in the logarithmic scale. 1 - acetaldehyde; 2 - methyl acetate; 3 - ethyl acetate; 4 - methanol; 5 - 2-propanol; 6 - ethanol; 7 - 1-propanol; 8 - 2-methyl-1-propanol; 9 - 2-pentanol, 10 - 1-butanol; 11 - 3-methyl-1-butanol.

The concentrations, obtained according to Eq. (5), have dimensions of mg/L AA. Developed method “Ethanol as Internal Standard” doesn’t require to determine the volume concentration of ethyl alcohol, density or any other characteristic of test sample.

The results, obtained for alcoholic beverages and rectified ethanol for both methods are shown in Table 3 and in more detail in Table S3 of Supplementary materials.

3.2.3. Recovery

Values of *Recovery* for each volatile compound in standard solutions were determined according to Eq. (6):

$$Recovery_i(SS) = \frac{C_{gi}^{SS}}{C_{ci}^{SS}} \cdot 100\%, \quad (6)$$

where C_{gi}^{SS} and C_{ci}^{SS} are the calculated and experimentally obtained value of the concentration of *i*-th volatile compound in the standard solution, correspondingly, mg/L AA.

The expected values of recovery for concentration orders 10–200 mg/L AA vary from 85 to 110%, 200–1000 mg/L AA – from 90 to 105%, 5000 mg/L AA – from 95 to 105% (AOAC, 2012, p. 1) The results of recovery calculation for standard solutions are shown in Fig. 3 and in more detail in Table S2 of Supplementary materials.

3.2.4. Comparison of traditional and developed methods

The relative difference between the results, obtained for both tradi-

tional and developed methods, was calculated according to Eq. (7):

$$\Delta_{i, methods}^{(j)} = \frac{\bar{C}_i^{Eth}(j) - \bar{C}_i^{2-pentanol}(j)}{\bar{C}_i^{2-pentanol}(j)} \cdot 100\%, \quad (7)$$

where $\bar{C}_i^{2-pentanol}(j)$ and $\bar{C}_i^{Eth}(j)$ are the average values of concentrations of *i*-th volatile compound in the *j*-th alcohol beverage test sample, calculated using traditional and developed methods, correspondingly, mg/L AA.

The comparison of results also was performed for each volatile compound at 0.05 significance level, employing MS Excel 2016 for the statistical Student’s test (*t*-Test: Paired Two Sample for Means) and ANOVA (Single factor) for both developed and traditional method.

The results of the comparison of traditional and developed methods, are shown in Table 4 and in more detail in Table S3 of Supplementary materials.

3.3. Linearity

The linearity in the concentration range 10–5000 mg/L AA for the standard solutions and matrix matched solutions was calculated using MS Excel 2016. The results of the linearity determination are shown in Table 5.

Table 3

The results, obtained for alcoholic beverage samples for both developed and traditional methods.

Compound	Retention time, min	Number of results	The value of concentration, obtained for both developed/traditional* methods, mg/L AA			
			Minimal	Maximal	Median	Average
acetaldehyde	5.80	13	0.7/0.8*	216/219	65.3/65.6	76.2/76.4
methyl acetate	6.75	1			55.7/55.8*	
ethyl acetate	7.50	11	2.3/2.2	1326/1325	269/268	391/392
methanol	7.63	13	3.2/3.3	11766/11748	52.2/52.3	1188/1185
2-propanol	8.07	12	0.7/0.8	12.2/12.2	4.1/4.1	4.7/4.7
1-propanol	11.15	11	0.6/0.6	4451/4444	344/344	709/708
2-methyl-1-propanol	13.06	11	0.2/0.2	1244/1240	551/542	496/496
1-butanol	15.18	11	0.4/0.4	154/154	5.9/6.0	22.8/22.8
3-methyl-1-butanol	17.71	11	25.7/25.5	3592/3589	1203/1219	1502/1501

* Methyl acetate was found only in the rakia sample.

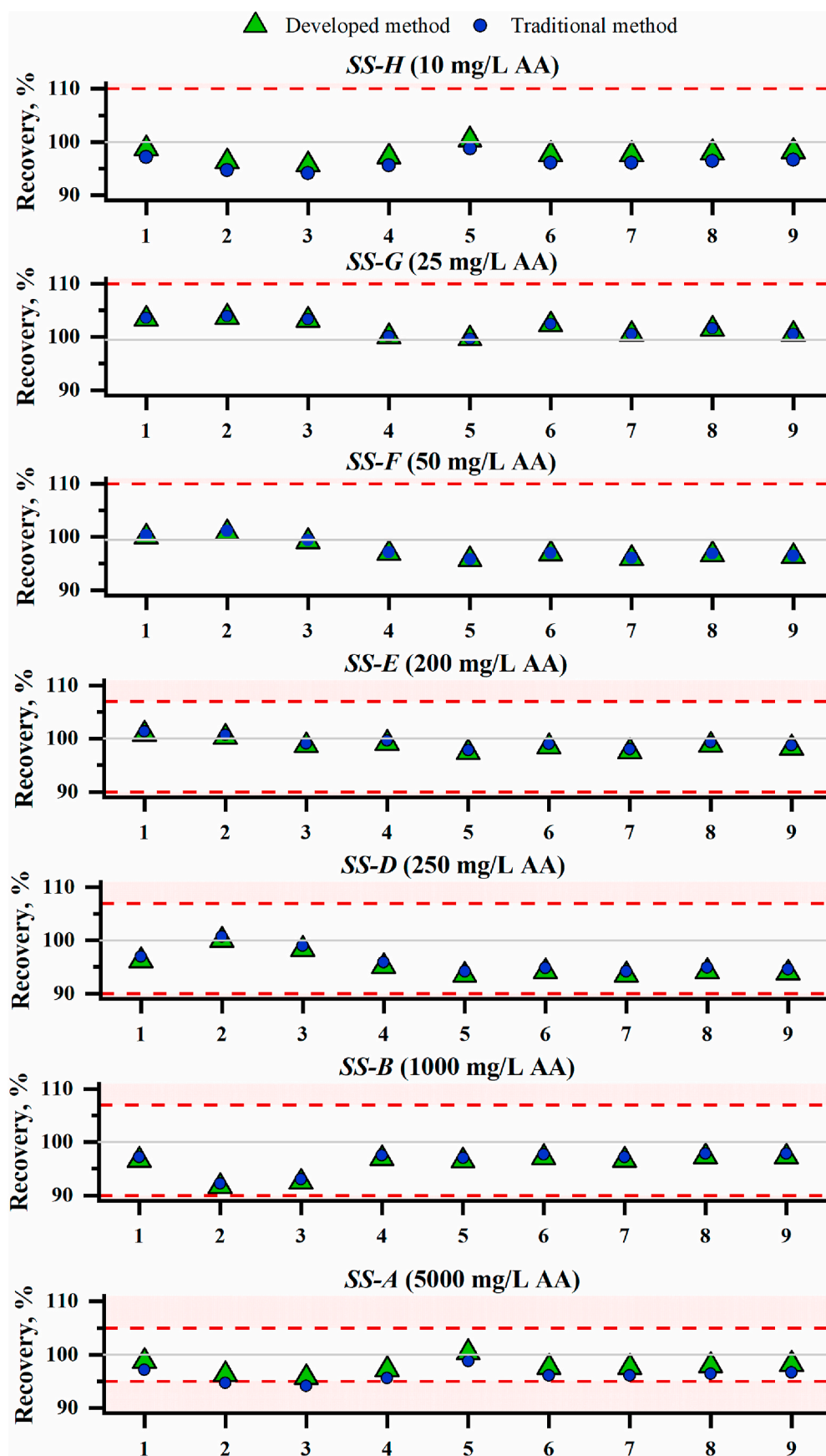


Fig. 3. The values of recovery of the concentrations of the standard solutions, obtained for both developed and traditional methods. 1 - acetaldehyde; 2 - methyl acetate; 3 - ethyl acetate; 4 - methanol; 5 - 2-propanol; 6 - 1-propanol; 7 - 2-methyl-1-propanol; 8 - 1-butanol; 9 - 3-methyl-1-butanol.

Table 4

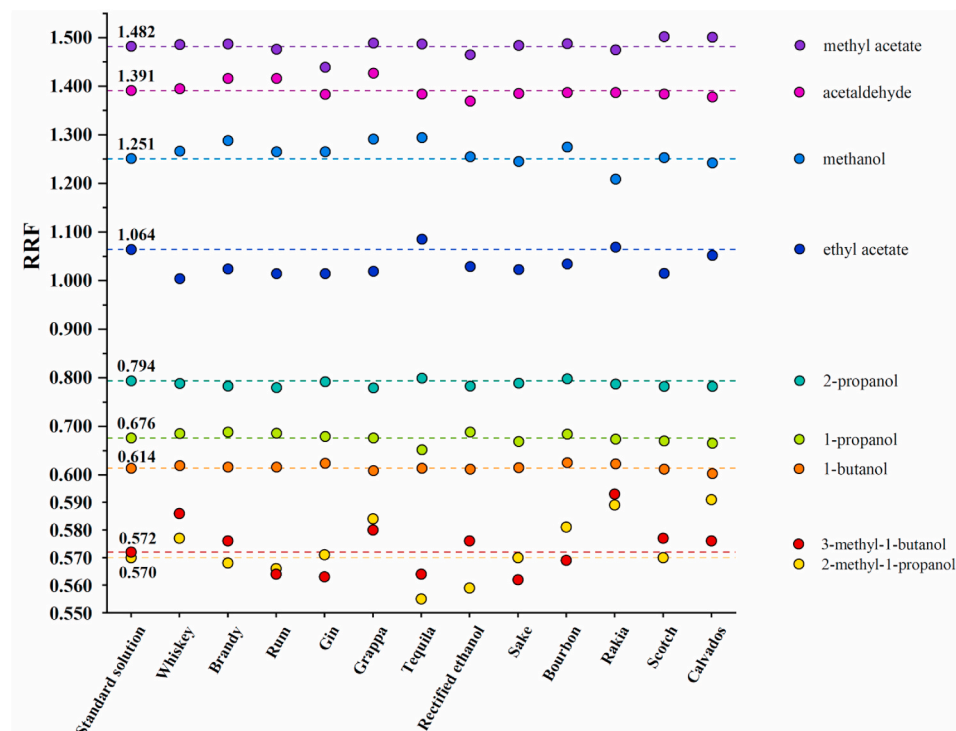
The relative difference between the concentrations, obtained for both developed and traditional methods for the alcoholic beverages and rectified ethanol.

Sample	$\Delta^{methods}, \%$								
	acetaldehyde	methyl acetate	ethyl acetate	methanol	2-propanol	1-propanol	2-methyl-1-propanol	1-butanol	3-methyl-1-butanol
Whiskey	-0.2	-	-0.3	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
Brandy	0.3	-	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Rum	-0.1	-	-0.2	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1
Gin	0.9	-	0.8	0.9	0.9	0.9	0.9	1.0	1.0
Grappa	-1.3	-	-1.4	-1.3	-1.3	-1.3	-1.4	-1.4	-1.3
Tequila	1.8	-	1.6	1.8	1.7	1.7	1.7	1.7	1.7
Rectified ethanol	-1.6	-	-	-1.7	-1.6	-	-	-	-
Vodka	0.2	-	-	0.2	0.1	-	-	-	-
Sake	-0.5	-	-0.5	-0.4	-	-0.5	-0.5	-0.5	-0.5
Bourbon	-0.9	-	-0.9	-0.8	-0.9	-0.8	-0.9	-0.9	-0.8
Rakia	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2
Scotch	0.7	-	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Calvados	0.2	-	0.1	0.2	0.1	0.2	0.1	0.1	0.2

Table 5

The coefficients of determination, obtained using the standard solutions and matrix matched solutions.

Sample	R^2								
	acetaldehyde	methyl acetate	ethyl acetate	methanol	2-propanol	1-propanol	2-methyl-1-propanol	1-butanol	3-methyl-1-butanol
Standard solutions	0.99996	0.99983	0.99991	0.99999	0.99991	0.99999	0.99999	0.99999	0.99999
Whiskey	0.99998	0.99972	0.99979	0.99999	0.99989	0.99979	0.99983	0.99994	0.99989
Brandy	0.99979	0.99977	0.99997	0.99954	0.99999	0.99974	0.99998	0.99993	0.99991
Rum	0.99988	0.99996	0.99961	0.99999	0.99997	0.99990	0.99999	0.99996	1.00000
Gin	0.99998	0.99997	1.00000	0.99991	0.99999	0.99997	0.99999	1.00000	0.99984
Grappa	0.99996	0.99985	0.99995	0.99983	0.99992	0.99998	0.99994	0.99997	0.99991
Tequila	0.99998	0.99981	0.99994	0.99994	0.99996	0.99993	0.99998	0.99992	0.99990
Rectified ethanol	0.99998	0.99979	0.99999	0.99999	0.99999	0.99989	0.99991	0.99990	0.99992
Sake	0.99989	0.99991	0.99999	0.99993	0.99998	0.99989	0.99995	0.99998	0.99997
Bourbon	0.99992	0.99996	0.99999	0.99999	0.99997	0.99999	0.99999	0.99992	0.99989
Rakia	0.99997	0.99998	0.99997	0.99931	0.99990	0.99998	0.99997	0.99999	0.99999
Scotch	0.99990	0.99998	0.99995	0.99999	0.99998	0.99992	0.99970	0.99995	1.00000
Calvados	0.99998	0.99999	0.99990	0.99982	0.99988	0.99999	0.99995	0.99988	0.99998

**Fig. 4.** The values of RRF, obtained using the prepared standard and matrix matched solutions.

3.4. Matrix effect study

3.4.1. Calculation of calibration coefficients

The values of $RRF_i^{Eth(Matrix)}(j)$ for each volatile compound in matrix matched calibration solution *MMS-C* were determined according to Eq. (8):

$$RRF_i^{Eth(Matrix)}(j) = \frac{C_i^{MMS-C(j)} \cdot \bar{A}_{Eth}^{MMS-C(j)}}{\bar{A}_i^{MMS-C(j)} - \bar{A}_i \cdot \rho_{Eth}}, \quad (8)$$

where $C_i^{MMS-C(j)}$ is the concentration of the *i*-th volatile compound in matrix matched calibration solution of *j*-th alcohol beverage test sample *MMS-C* in mg/L AA units; $\bar{A}_i^{MMS-C(j)}$ and \bar{A}_i^j are the average values of detector responses for the *i*-th volatile compound in matrix matched calibration solution of *j*-th test sample *MMS-C* and *j*-th test sample solution with no additions of volatile compounds, correspondingly, a.u.; $\bar{A}_{Eth}^{MMS-C(j)}$ is the average value of detector responses for ethanol in matrix matched calibration solution of *j*-th test sample *MMS-C*, a.u.

The results of the determination of RRFs values for the matrix matched solutions are shown in Fig. 4 and in more detail in Table S4 of Supplementary materials.

3.4.2. Recovery

The values of *Recovery* for each volatile compound in the matrix matched standard solutions were determined according to Eq. (9):

$$Recovery_i(MMS) = \frac{C_{a_i}^{MMS}}{C_i^{MMS}} \cdot 100\%, \quad (9)$$

where $C_{a_i}^{MMS}$ and C_i^{MMS} are the calculated and experimentally obtained value of the concentration of the addition of *i*-th volatile compound in the matrix matched solution, correspondingly, mg/L AA.

The results of the recovery calculation matrix matched solutions are shown in Fig. 5 and in more detail in Table S5 of Supplementary materials.

3.4.3. The study of the matrix effect

The matrix effect was determined for each volatile compound by the means of the statistical comparison calibration coefficients obtained for the standard solution and for 12 ethanol-containing matrices with ethanol concentration from 14.5 to 96.0% v/v. This comparison was performed for each volatile compound at 0.05 significance level, employing MS Excel 2016 for the statistical Student's test (*t*-Test: Paired Two Sample for Means) for the means comparison for obtained calibration coefficients. As a null hypothesis the similarity between the calibration coefficients for the standard solution and matrix matched solutions was taken. As an alternative approach to the estimation of the matrix effect ANOVA (Single factor) was used to confirm these results, considering the data normal distribution and employing 0.05 significance level. In this case calibration coefficients, obtained for the standard and matrix matched solutions were considered as the independent samples. The results, obtained for statistical Student test and ANOVA Single factor analysis for comparison of calibration coefficients are shown in Table S4 of Supplementary materials.

4. Results and discussion

The comparison of the results obtained for both developed and traditional methods as in the case of standard solutions (Fig. 3), and in the case of alcohol beverages and rectified ethyl alcohol samples (Table 4) showed that the relative difference between the values of concentrations and recovery is less than $\pm 2\%$.

The paired two-sample *t*-Test and ANOVA confirmed, that the difference between the means, obtained for both methods for all the studied samples is statistically insignificant (Table S2 of Supplementary

material).

The values of recovery, obtained for standard solutions using ethanol as internal standard for concentration orders 10–200 mg/L AA varied from 92.8 to 103.7%, 200–1000 mg/L AA – from 91.7 to 100.9%, 5000 mg/L AA – from 95.7 to 100.4%. The values of recovery, obtained for standard solutions using 2-pentanol as internal standard for concentration orders 10–200 mg/L AA varied from 96.5 to 106.2%, 200–1000 mg/L AA – from 92.2 to 101.4%, 5000 mg/L AA – from 94.1 to 98.8%.

The estimation of the matrix effect was presented in the study of its influence on the values of the RRF (Fig. 4) and recovery (Fig. 5), obtained for the matrix matched solutions. The comparison of the RRFs, obtained for the standard solutions and matrix matched solutions using *t*-Test and ANOVA confirmed, that the difference between the means obtained with both methods for all the studied samples is statistically insignificant.

The values of recovery, obtained for matrix matched solutions for additions concentration ranges 10–200 mg/L AA varied from 82.9 to 109.9%, 200–1000 mg/L AA – from 90.1 to 107.0%, 5000 mg/L AA – from 95.1 to 104.8% (Fig. 5 and in more detail in Table S5 of Supplementary materials).

The results of determinations of the RRFs, obtained for the matrix matched solutions, showed, that the values of RRFs are very close to each other despite being defined for the different matrices. Summarizing these results with our previous observations of the similarity of the RRFs for the similar instruments (Charapitsa, Sytova, Korban, Sobolenko, Egorov, et al., 2019), authors are sure that this fact confirmed the findings of the RRF studies (Rome et al., 2012) and bring closer to proving the possibility of tabulating coefficients to significantly simplify the analysis of the alcoholic beverages in future.

5. Conclusions

The statistical analysis of the results, obtained in work, showed, that the influence of the matrix effect on the results obtained with developed method is statistically insignificant ($P < 0.05$). Thus, the developed method can be used for determination of concentrations of volatile compounds in ethanol-containing products with ethanol volume concentration from 14.5% to 96.0%. These results suggest that the method can be implemented not only in food testing laboratories, but also in pharmaceuticals, since according to European Pharmacopeia and legislative acts (Second Commission Directive 82/434/EEC, 1982) it is necessary to control the content of volatile components (for example, methanol) in ethyl alcohol, used as a raw material and ethanol-containing end-products of pharmaceutical, cosmetic and perfumery industry. It is expected that the influence of the matrix effect is insignificant in the entire range of ethanol volume concentrations in alcoholic beverages, which is planned to be studied in future works.

High efficiency and wide international testing of the method using ethanol as an internal standard can serve as the basis for initiating, in the established manner, interlaboratory study on the basis of specialized international organizations, for example, the International Organization for Standardization (ISO), the Codex Alimentarius Commission or the International Organization of Vine and Wine (OIV) for the purpose of its subsequent approval as a standardized reference method on the international level.

CRedit authorship contribution statement

Siarhei Charapitsa: Conceptualization, Project administration, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Svetlana Sytova:** Software, Data curation, Writing - review & editing, Funding acquisition. **Anton Kavalenka:** Software, Data curation, Writing - review & editing, Visualization. **Lidia Sobolenko:** Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Nikolai Kostyuk:** Resources, Writing - review & editing, Funding acquisition. **Vladimir**

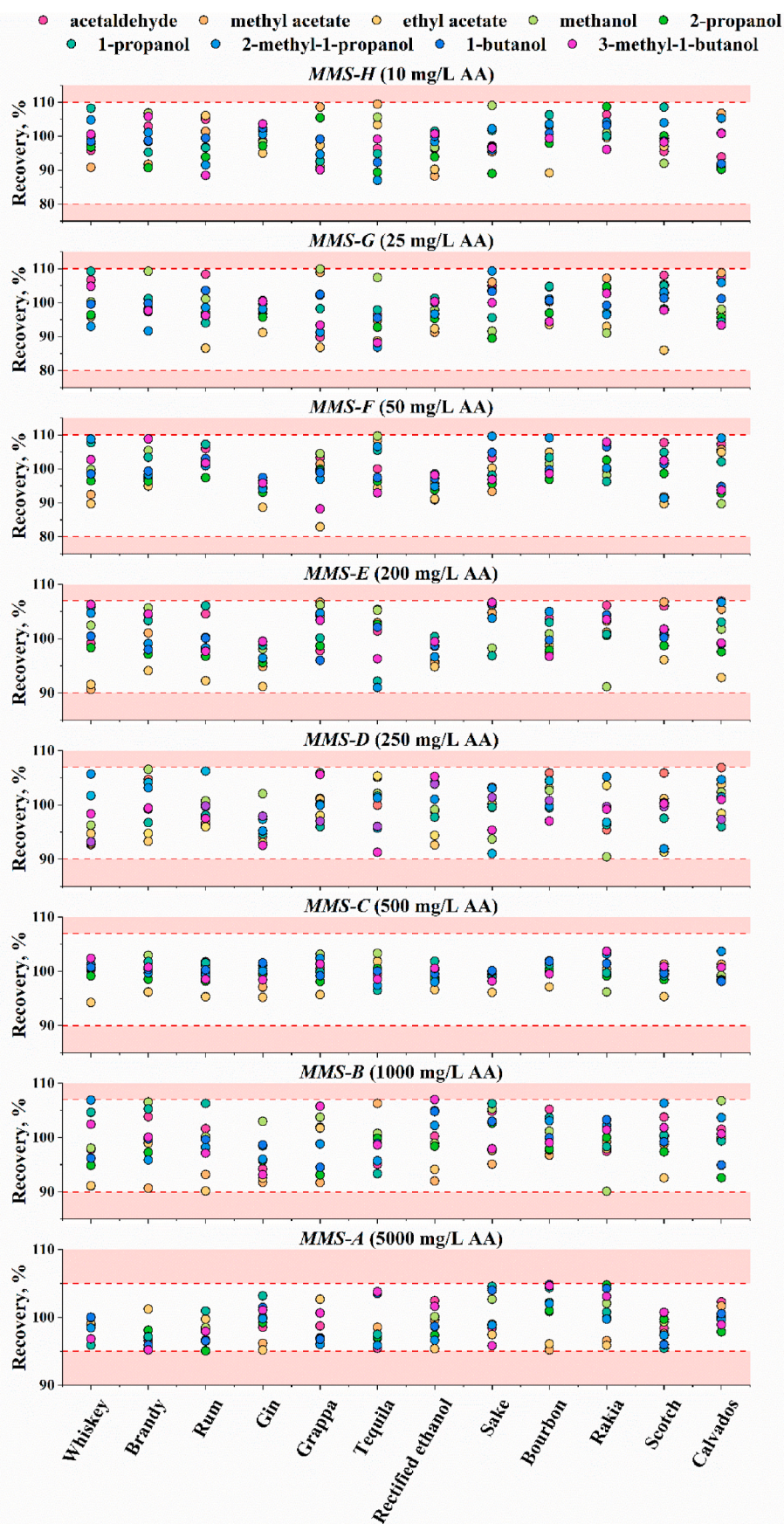


Fig. 5. The values of recovery, obtained for the matrix matched solutions.

Egorov: Conceptualization, Supervision, Writing - review & editing.
Sergey Leschev: Conceptualization, Supervision, Writing - review & editing.
Sergey Vetokhin: Supervision, Funding acquisition.
Natalia Zayats: Validation, Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

The work was done within the grant 3.08.5 “To optimize methods for the precise determination of the qualitative and quantitative composition of a wide range of multicomponent substances for biotechnology” of the Belarusian State Program of Scientific Research “Convergence-2020” and was supported by the World Federation of Scientists in the National Scholarship program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2020.107528>.

References

- AOAC. (2002). *AOAC guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals*. Gaithersburg, MD, USA: AOAC International, 2002.
- AOAC. (2012). *Appendix F: Guidelines for standard method performance requirements*. Gaithersburg, MD, USA: AOAC International, 2012.
- AOAC Official Method 972.10. (2005). *Alcohol (higher) and ethyl acetate in distilled liquors. Alternative gaschromatographic method*. Gaithersburg, MD, USA: AOAC International, 2002.
- AOAC Official Method 972.11. (2005). In *Methanol in distilled liquors. Gas chromatographic method*. Gaithersburg, MD, USA: AOAC International, 2002.
- ASTM D4307-99. (2015). *Practice for preparation of liquid blends for use as analytical standards*. Philadelphia, PA, USA.
- BIS IS 3752. (2009). *Alcoholic drinks – methods of test* (p. 2005). New Delhi: Bureau of Indian Standards, 2005.
- Borecka, M., Białk-Bielińska, A., Siedlewicz, G., Stepnowski, P., & Ksenia, P. (2014). The influence of matrix effects on trace analysis of pharmaceutical residues in aqueous environmental samples. In *GeoPlanet: Earth and planetary sciences* (Vol. 14, pp. 1–16). https://doi.org/10.1007/978-3-319-03683-0_1.
- Buglass, A. J. (2011). In A. J. Buglass (Ed.), *Handbook of alcoholic beverages: Technical, analytical and nutritional aspects*. John Wiley & Sons.
- Charapitsa, S. V., Kavalenka, A. N., Kulevich, N. V., Makoev, N. M., Mazanik, A. L., Sytova, S. N., Zayats, N. I., & Kotov, Y. N. (2013). Direct determination of volatile compounds in spirit drinks by gas chromatography. *Journal of Agricultural and Food Chemistry*, 61(12), 2950–2956. <https://doi.org/10.1021/jf3044956>.
- Charapitsa, S., Sytova, S., Korban, A., Boyarin, N., Shestakovich, I., & Čabala, R. (2018). The establishment of metrological characteristics of the method “Ethanol as Internal Standard” for the direct determination of volatile compounds in alcoholic products. *Journal of Chemical Metrology*, 12(1), 59–69. <https://doi.org/10.25135/jcm.14.18.02.063>.
- Charapitsa, S. V., Sytova, S. N., Korban, A. L., & Sobolenko, L. N. (2019). Single-laboratory validation of a gas chromatographic method of direct determination of volatile compounds in spirit drinks: Need for an improved interlaboratory study. *Journal of AOAC International*, 102(2), 669–672. <https://doi.org/10.5740/jaoacint.18-0258>.
- Charapitsa, S., Sytova, S., Korban, A., Sobolenko, L., Egorov, V., Leschev, S., Zakharov, M., Čabala, R., Busarova, R., Shestakovich, I., Tolstouhova, A., Ondrousek, S., Vavra, J., Yilmaztekin, M., & Cabaroglu, T. (2019). Interlaboratory study of ethanol usage as an internal standard in direct determination of volatile compounds in alcoholic products. *BIO Web of Conferences*, 15, 1–8. <https://doi.org/10.1051/bioconf/20191502030>.
- Commission regulation (EC) No 2870/2000 laying down Community reference methods for the analysis of spirits drinks. <https://eur-lex.europa.eu/eli/reg/2000/2870/oj>.
- Dominguez, A., Placencia, F., Cereceda-Balic, F., Fadie, X., & Quiroz, W. (2014). Analysis of tomato matrix effect in pesticide residue quantification through QuEChERS and single quadrupole GC/MS. *Chilean Journal of Agricultural Research*, 74, 148–156. <https://doi.org/10.4067/S0718-58392014000200004>.
- GB/T 11858. (2008). *Vodka* (p. 10). Beijing: National Standards of People's Republic of China, 2008.
- Hall, T. G., Smukste, I., Bresciano, K. R., Wang, Y., McKearn, D., & Savage, R. E. (2012). Identifying and overcoming matrix effects in drug discovery and development. In *Tandem mass spectrometry-applications and principles* (Vol. 18, pp. 390–419).
- Isaac-Lam, M. (2016). Determination of alcohol content in alcoholic beverages using 45 MHz benchtop NMR spectrometer. *International Journal of Spectroscopy*, 1–8. <https://doi.org/10.1155/2016/2526946>, 2016.
- Kelly, J., Chapman, S., Brereton, P., Bertrand, A., Guillou, C., & Wittkowski, R. (1998). Gas chromatographic determination of volatile congeners in spirit drinks: Interlaboratory study. *Journal of AOAC International*, 82, 1375–1388.
- Korban, A., Charapitsa, S., Čabala, R., Sobolenko, L., & Sytova, S. (2019). The perspectives of ethanol usage as an internal standard for the quantification of volatile compounds in alcoholic products by GC-MS. *Journal of Mass Spectrometry*. <https://doi.org/10.1002/jms.4493>.
- Matuszewski, B. K. (2006). Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. *Journal of Chromatography B*, 830(2), 293–300. <https://doi.org/10.1016/j.jchromb.2005.11.009>.
- Nalazek-Rudnicka, K., & Wasik, A. (2017). Development and validation of an LC-MS/MS method for the determination of biogenic amines in wines and beers. *Monatshefte Für Chemie - Chemical Monthly*, 148(9), 1685–1696. <https://doi.org/10.1007/s00706-017-1992-y>.
- NOM-199-SCFI. (2017). *Bebidas alcohólicas-Denominación, especificaciones físicoquímicas, información comercial y métodos de prueba. NORMA Oficial Mexicana*.
- OIV-MA-BS-14. (2009). *Determination of the principal volatile substances of spirit drinks of viti-vinicultural origin*.
- Powley, C., George, S., Ryan, T., & Buck, R. (2005). Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrices. *Analytical Chemistry*, 77(19), 6353–6358. <https://doi.org/10.1021/ac0508090>.
- Rome, K., McIntyre, A., & Macclesfield, A. (2012). Intelligent use of relative response factors in gas chromatography-flame ionisation detection. *Chromatography Today*, 5, 52–56.
- Saini, G., Wani, T. A., Gautam, A., Varshney, B., Ahmed, T., Rajan, K., Pillai, K., & Paliwal, J. (2006). Validation of the LC-MS/MS method for the quantification of mevalonic acid in human plasma and determination of the matrix effect. *Journal of Lipid Research*, 47, 2340–2345. <https://doi.org/10.1194/jlr.D600018-JLR200>.
- Second Commission Directive 82/434/EEC of 14 May 1982 on the approximation of the Laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products.
- Thompson, M., Ellison, S., & Wood, R. (2002). Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure and Applied Chemistry*, 74(5), 835–855. <https://doi.org/10.1351/pac200274050835>.
- Thompson, M., Ellison, S. L. R., & Wood, R. (2006). The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). *Pure and Applied Chemistry*, 78(1), 145–196. <https://doi.org/10.1351/pac200678010145>.
- Uebelacker, M., & Lachenmeier, D. (2011). Quantitative determination of acetaldehyde in foods using automated digestion with simulated gastric fluid followed by headspace gas chromatography. *Journal of Automated Methods & Management in Chemistry*, 1, 13. <https://doi.org/10.1155/2011/907317>.
- Wiśniewska, P., Śliwińska, M., Dymerski, T., Wardencki, W., & Namieśnik, J. (2016). The analysis of raw spirits – a review of methodology. *Journal of the Institute of Brewing*, 122(1), 5–10. <https://doi.org/10.1002/jib.288>.
- Yilmaztekin, M., & Cabaroglu, T. (2011). Confirmatory method for the determination of volatile congeners and methanol in Turkish raid according to European union regulation (EEC) No. 2000R2870: Single-Laboratory validation. *Journal of AOAC International*, 94, 611–617. <https://doi.org/10.1093/jaoac/94.2.611>.