46th World Congress of Vine and Wine
OIV International Organisation of Vine and Wine
Intergovernmental Organisation
Available on IVES Conference Series vine & wine

DOI : https://doi.org/10.58233/BvQRaKco

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

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

[1] Institute for Nuclear Problems of Belarusian State University, Bobruyskaya Str., 11, 220030, Minsk, Belarus
[2] New Analytical Systems Ltd, Nezavisimosti Ave 70, 220072, Minsk, Belarus
[3] RUDN University, Miklukho-Maklaya Str., 6117198, Moscow, Russia

Abstract. The paper proposes an algorithm for validating a modified internal standard method using ethyl alcohol contained in the test sample as a reference substance. It is shown that the method can be validated based on experimental data previously obtained in the laboratory during validation of the standard method in accordance with current standards. As a result, no additional financial, material, or labor costs are required to validate the proposed method. The new approach has been proposed for standardization as an official OIV method of analysis, and is currently under consideration as draft resolution OENO-SCMA 24-756 “Method for determination of volatile compounds in spirituous beverages of vitivinicultural origin using contained ethanol as a reference substance”.

1. Introduction

The method for the determination of volatile compounds in spirituous beverages from grapes or other plant raw materials using ethanol as a reference substance (RS) was proposed firstly in 2013 [1]. Its quintessence is to use ethanol as RS for the analysis of an alcohol product in order to increase the accuracy of measurements and to obviate the need for the IS addition because ethanol is contained in the sample in any case. The advantages of this method also include the absence of the need to measure the strength of the analyzed sample: the concentration of volatile impurities is calculated based on measurements directly in units of g/100 L of anhydrous alcohol (g/100 L AA) or mg/L of anhydrous alcohol (mg/L AA).

This method is suitable for the determination by gas chromatography in spirits produced from grapes, wines and other alcohol-containing products of a number of volatile substances, in particular, such as acetaldehyde (ethanal), ethyl acetate, 1,1-diethoxyethane (acetal), methanol, butan-2-ol, propan-1-ol (n-propanol), isobutyl alcohol (2-methylpropan-1-ol), butan-1-ol (n-butanol), active amyl alcohol (2-methylbutan-1-ol), isoamyl alcohol (3-methylbutan-1-ol) with an analyte content from 0.2 g/100 L AA upto 1500 g/100 L AA.

Since 2013, new method has undergone comprehensive testing and validation on wide range of alcoholic beverages as well as other alcohol-containing products. Firstly, validation of the method for alcoholic beverages was performed in the single laboratory [2]. Then some interlaboratory study was carried out [3]. The developed method was tested across a variety of compounds, demonstrating improved detection and quantification limits compared to the official method [4, 5].

The problem considered in the work is how one can do validation of the method for the determination of volatile compounds in spirituous beverages using contained ethanol as a reference substance on the basis of archived experimental data. Such archived data (chromatograms) can be taken from analysis of an alcoholic beverage samples by the Commission Regulation EC2870/2000 method using internal standard pentan-3-ol or other internal standards. The external standard (ES) method is applied in many laboratories in a number of countries. And the data obtained by the ES method can also be recalculated by the new method using ethanol as an internal standard. Each laboratory working in this field has a large amount of such data. When performing any analysis, modern gas chromatographs register all component peaks in the sample including the peak of ethanol.

Thus, the discussed method can be easily validated within one laboratory on the basis of the data which was previously obtained by traditional method. No other measurements or manual procedures except those indicated in standards [6] are required.

2. Archived experimental data

The description of the validation of the proposed method is based on experimental data obtained earlier in the testing laboratory during the validation of the method in accordance with regulation EC2870/2000 [6]. These data include the chromatograms of 5 standard solutions (SSs) with known certified values of concentration of volatile compounds and 4 spiritous beverages such as brandy, raki, wine and whiskey.

Standard solutions of acetaldehyde, methyl acetate, ethyl acetate, acetal, methanol, propan-2-ol, n-propanol, 2-methylpropan-1-ol, n-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol in water-ethanol solution with a volume content of ethyl alcohol (*ABV*, %) of 40 % have been used at 5 concentration levels of the compounds: “0.1” (19-31 µg/g), “0.5” (95-152 µg/g), “1.0” (181-288 µg/g), “1.5” (278-443 µg/g), “2.0” (372-592 µg/g). The density of 40 % water-ethanol solution was 948.06 g/L. The certified values of concentration of the volatile compounds in SSs in units of µg/g are presented in Table 1.

Table 1. The certified values of concentration of the compounds in SSs in units of µg/g for 5 concentration levels.

Compound	“0.1”	“0.5”	“1.0”	“1.5”	“2.0”
acetaldehyde	19.2	95.7	181	278	372
methyl acetate	30.7	152	288	443	592
ethyl acetate	27.7	138	260	400	535
acetal	24.9	124	233	359	480
methanol	21.6	108	203	312	418
2-butanol	22.4	111	210	323	432
n-propanol	21.8	108	204	314	420
2-methylpropan-1-ol	21.3	106	200	308	412
n-butanol	22.3	111	210	322	431
2-methylbutan-1-ol	23.1	115	217	333	446
3-methylbutan-1-ol	22.1	110	208	320	428

These values of concentration have been recalculated in the units of mg/L AA (AA – anhydridous alcohol) taking in account the ethanol content in SSs. Thus, 5 concentration levels in units of mg/L AA are the following: “0.1” – from 45 to 73, “0.5” – from 227 to 361, “1.0” – from 428 to 683, “1.5” – from 658 to 1049, “2.0” – from 881 to 1404 (Table 2).

Table 2. The certified values of concentration of the compounds in SSs in units of mg/L AA for 5 concentration levels.

Compound	“0.1”	“0.5”	“1.0”	“1.5”	“2.0”
acetaldehyde	45.6	227	428	658	881
methyl acetate	72.7	361	683	1049	1404
ethyl acetate	65.7	326	617	948	1268
acetal	58.9	293	553	850	1138
methanol	51.3	255	481	740	990
2-butanol	53.0	264	498	766	1024
n-propanol	51.6	256	484	744	996
2-methylpropan-1-ol	50.5	252	474	729	976
n-butanol	52.9	263	497	764	1022
2-methylbutan-1-ol	54.7	272	514	790	1058
3-methylbutan-1-ol	52.5	261	493	758	1014

The *ABV* values of the spiritous beverages were 34.4 % in brandy, 48.0 % in raki, 18.1 % in wine and 41.0 % in whiskey. The density of the beverages in units g/L were: 956.48 of brandy, 934.02 of raki, 975.60 of wine and 946.40 of whiskey.

The chromatograms of the SSs and the spiritous beverages are shown in Figures 1 and 2, where the number 6 is a mark of ethanol peak which is proposed as RS and the number 10 is a mark of pentan-3-ol peak which is traditional IS.

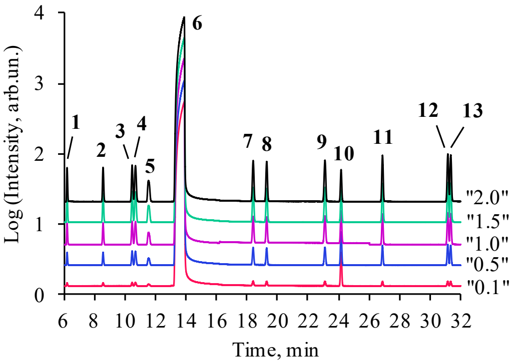

Figure 1. Chromatograms of the SSs at levels “0.1”, “0.5”, “1.0”, “1.5”, “2.0”. 1 – acetaldehyde, 2 – methyl acetate, 3 – ethyl acetate, 4 – acetal, 5 – methanol, 6 – ethanol (RS), 7 – butan-2-ol, 8 – n-propanol, 9 – 2-methylpropan-1-ol, 10 – pentan-3-ol (added IS), 11 – n-butanol, 12 – 2-methylbutan-1-ol, 13 – 3-methylbutan-1-ol.

The archived experimental data include three chromatograms of each SSs and two chromatograms of each spiritous beverages.

All samples of the SSs and beverages were with addition of aproximately 220 µg/g pentan-3-ol (traditional IS). The exact values of pentan-3-ol concentration are:

- in SS “0.1” is 229 μg/g (542 mg/L AA);
- in SS “0.5” is 228 μg/g (540 mg/L AA);
- in SS “1.0” is 226 μg/g (536 mg/L AA);
- in SS “1.5” is 228 μg/g (540 mg/L AA);
- in SS “2.0” is 226 μg/g (536 mg/L AA);
- in the brandy sample is 187 μg/g (520 mg/L AA);
- in the raki sample is 231 μg/g (450 mg/L AA);
- in the wine sample is 214 μg/g (1154 mg/L AA);
- in the whiskey sample is 224 μg/g (518 mg/L AA).

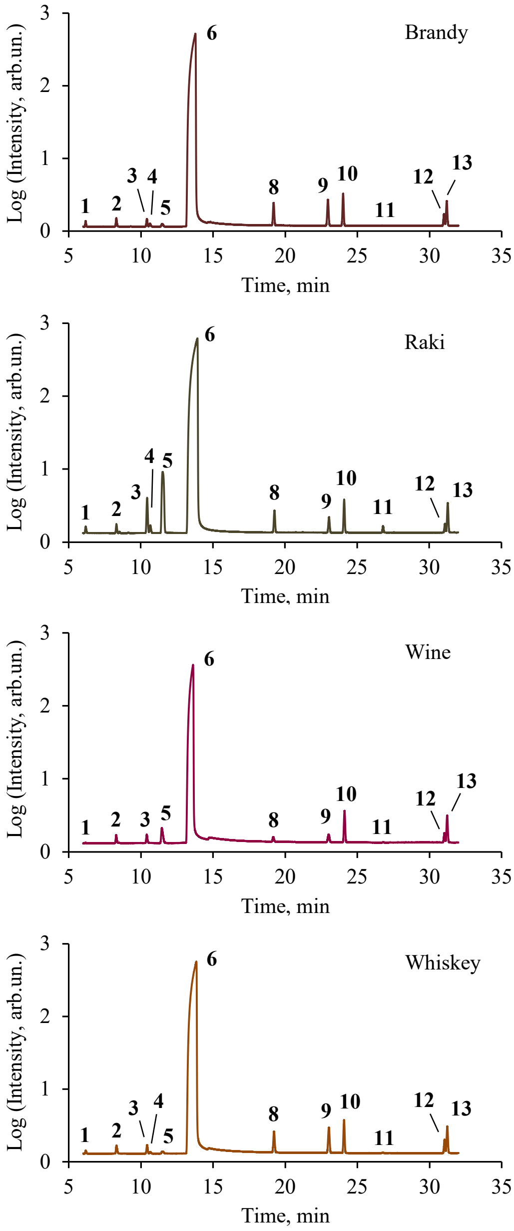

Figure 2. Chromatograms of spiritous beverages. 1 – acetaldehyde, 2 – methyl acetate, 3 – ethyl acetate, 4 – acetal, 5 – methanol, 6 – ethanol, 8 – n-propanol, 9 – 2-methylpropan-1-ol, 10 – pentan-3-ol, 11 – n-butanol, 12 – 2-methylbutan-1-ol, 13 – 3-methylbutan-1-ol.

3. Software for data processing

The archived experimental data have been processed using the software: Excel, Chromatec Analytic 3.1 (Chromatec, Russia), Agilent ChemStation B.04.03 (Agilent Technologies Inc., USA), UniChrom 5.1 (New Analytical Systems Ltd., Belarus). The obtained results of the data processing with the different software are the very close (indistinguishable).

4. The results of synchronous analysis of archived data using traditional IS pentan-3-ol and proposed ethanol as RS

4.1. The calibration and the linearity testing

The values of calibration coefficients in establishing the values of the relative response factors RRF_i^{p3} (IS is pentan-3-ol) and RRF_i^{eth} (ethanol as RS) were determined by the single-point calibration method based on measurement results of the SS “1.0”, performing calculations for each compound according to equations (1) and (2).

$$RRF_i^{\mathrm{p3}} = \frac{\tilde{C}_{i,\text{"1.0"}}^{certified} \cdot \sum_{k=1}^{n} \left(A_{i,k,\text{"1.0"}} / A_{\mathrm{p3},k,\text{"1.0"}} \right)}{\tilde{C}_{\mathrm{p3},\text{"1.0"}}^{certified} \cdot \sum_{k=1}^{n} \left(A_{i,k,\text{"1.0"}} / A_{\mathrm{p3},k,\text{"1.0"}} \right)^2}, \qquad (1)$$

$$RRF_i^{\mathrm{eth}} = \frac{C_{i,\text{"1.0"}}^{certified} \cdot \sum_{k=1}^{n} \left(A_{i,k,\text{"1.0"}} / A_{\mathrm{eth},k,\text{"1.0"}} \right)}{\rho_{\mathrm{eth}} \cdot \sum_{k=1}^{n} \left(A_{i,k,\text{"1.0"}} / A_{\mathrm{eth},k,\text{"1.0"}} \right)^2}, \qquad (2)$$

where $A_{i,k,\text{"1.0"}}$ is the detector response to the *i*-th volatile compound, obtained during measurement *k* of SS “1.0”; $A_{\mathrm{p3},k,\text{"1.0"}}$ is the detector response to pentan-3-ol, obtained during measurement *k* of SS “1.0”; $A_{\mathrm{eth},k,\text{"1.0"}}$ is the detector response to ethanol, obtained during measurement *k* of SS “1.0”, $\tilde{C}_{i,\text{"1.0"}}^{certified}$ is the certified concentration of the *i*-th volatile compound in SS “1.0”, μg/g; $\tilde{C}_{\mathrm{p3},\text{"1.0"}}^{certified}$ is the certified concentration of pentan-3-ol in SS “1.0”, μg/g; $C_{i,\text{"1.0"}}^{certified}$ is the certified concentration of the *i*-th volatile compound in SS “1.0”, mg/L AA; ρ_{eth} is the density of anhydrous ethanol, ρ_{eth} = 789270 mg/L; *n* is the total number of repeated measurements of SS “1.0”.

The FID response linearity has been checked by successively analysing in triplicate each of the SSs for 4 concentration levels “0.1”, “0.5”, “1.5” and “2.0”. If pentan-3-ol has been used as IS, linear plots are obtained when:

- X-axis is certified concentration of the *i*-th volatile compound in SS_j (in μg/g) / certified concentration of pentan-3-ol (in μg/g);

- Y-axis is a detector response to the *i*-th compound (peak area), obtained in the result of measurement of SS_j / detector response to the pentan-3-ol (peak area), obtained in the result of measurement of SS_j.

If ethanol has been used as RS, linear plots are obtained when:

- X-axis is concentration of the *i*-th volatile compound in SS_j (mg/L AA)/ density of anhydrous ethanol (789270 mg/L);
- Y-axis is a detector response to the *i*-th compound (peak area), obtained during measurement of SS_j / detector response to the ethanol (peak area), obtained during measurement of SS_j.

The found values of *RRF* and the results of linearity testing for the both methods are represented in Table 3.

Table 3. The values of the calibration coefficients *RRF* and the parameters of linearity R^2.

Compound	IS: pentan-3-ol		RS: ethanol	
	RRF^{p3}	R^2	RRF^{eth}	R^2
acetaldehyde	2.139	0.99988	1.229	0.99991
methyl acetate	2.650	0.99988	1.522	0.99989
ethyl acetate	1.892	0.99990	1.087	0.99991
acetal	1.418	0.99990	0.815	0.99995
methanol	2.030	0.99990	1.166	0.99998
2-butanol	1.099	0.99993	0.631	0.99999
n-propanol	1.130	0.99995	0.649	0.99999
2-methylpropan-1-ol	0.954	0.99993	0.548	0.99998
n-butanol	1.025	0.99993	0.589	0.99998
2-methylbutan-1-ol	0.940	0.99995	0.540	0.99999
3-methylbutan-1-ol	0.948	0.99991	0.545	0.99997

The values of the coefficient of determination R^2 for all compounds for the both methods were more than 0.999 (Table 3). Thus, linearity is excellent in the both methods.

4.2. The calculation of the values of volatile compound concentration

The values of measured concentration of volatile compounds have been calculated using equations (3) in the traditional method with pentan-3-ol IS and using equation (4) in the new method with ethanol as RS:

$$\tilde{C}^{measured}_{i,k,j} = RRF^{\mathrm{p3}}_{i} \cdot \tilde{C}^{certified}_{\mathrm{p3},j} \cdot A_{i,k,j} / A_{\mathrm{p3},k,j}, \qquad (3)$$

$$C^{measured}_{i,k,j} = RRF^{\mathrm{eth}}_{i} \cdot \rho_{\mathrm{eth}} \cdot A_{i,k,j} / A_{\mathrm{eth},k,j}, \qquad (4)$$

where $A_{i,k,j}$ is the detector response to the *i*-th volatile compound, obtained during measurement *k* of sample *j*; $A_{\mathrm{p3},k,j}$ is the detector response to pentan-3-ol, obtained during measurement *k* of sample *j*; $A_{\mathrm{eth},k,j}$ is the detector response to ethanol, obtained during measurement *k* of sample *j*; $\tilde{C}^{certified}_{\mathrm{p3},j}$ is the certified concentration of the pentan-3-ol in sample *j*, µg/g; $\tilde{C}^{measured}_{i,k,j}$ is measured concentration of the *i*-th volatile compound in the sample *j*, µg/g; $C^{measured}_{i,k,j}$ is measured concentration of the *i*-th volatile compound in sample *j*, mg/L AA.

Then, the average values of measured concentration have been calculated in the case of 3-pentanol as IS $\langle\tilde{C}\rangle^{measured}_{i,j}$ in units of µg/g and in the case of ethanol used as RS $\langle C\rangle^{measured}_{i,j}$ in units of mg/L AA. These values $\langle\tilde{C}\rangle^{measured}_{i,j}$ have been recalculated in the units of mg/L AA taking in account the ethanol content in the sample and density of the sample:

$$\left\langle C\right\rangle^{measured}_{i,j} = \frac{\left\langle\tilde{C}\right\rangle^{measured}_{i,j} \cdot \rho_{beverage}[\mathrm{kg/m^3}]}{(ABV/100\%)\cdot 1000}. \qquad (5)$$

$$\left\langle C\right\rangle^{measured}_{i,j}[\mathrm{g/100\ L\ AA}] = 10 \cdot \left\langle C\right\rangle^{measured}_{i,j}[\mathrm{mg/L\ AA}] \qquad (6)$$

4.3. The estimation of precision, accuracy, LOD and LOQ

The values of relative standard deviation ($RSD_{i,j}$, %) and accuracy ($bias_{i,j}$,%) are determined according equations (7) and (8) [7, 8] :

$$RSD_{i,j} = SD_{i,j} \cdot 100\% / \left\langle C\right\rangle^{measured}_{i,j}, \qquad (7)$$

$$bias_{i,j} = \frac{\left\langle C\right\rangle^{measured}_{i,j} - C^{certified}_{i,j}}{C^{certified}_{i,j}} \cdot 100\%, \qquad (8)$$

where $SD_{i,j}$ is standard deviation of the *i*-th volatile compound in sample *j*, mg/L AA.

The results of precision and accuracy checking are presented in Tables 4 and 5.

In accordance with the Eurachem manual [9] the limits of detection *LOD* and quantitation *LOQ* are calculated using the equations (9) and (10):

$$LOD = 3 \cdot \frac{SD_{\text{"0.1"}}}{\sqrt{n}}, \qquad (9)$$

$$LOQ = k_Q \cdot \frac{SD_{\text{"0.1"}}}{\sqrt{n}}, \qquad (10)$$

where $SD_{\text{"0.1"}}$ is the standard deviation of a volatile compound at minimal concentration level (SS "0.1"); *n* is the number of measurements of SS "0.1", $n = 3$; k_Q is a coefficient taken = 10.

Table 4. The values of the *RSD* (%) obtained by the both methods of data analysis and the differences between them for SSs.

Compound	*RSD* (%) at IS: pentan-3-ol				*RSD* (%) at RS: ethanol				Δ*RSD*, (%)			
	"0.1"	"0.5"	"1.5"	"2.0"	"0.1"	"0.5"	"1.5"	"2.0"	"0.1"	"0.5"	"1.5"	"2.0"
acetaldehyde	4.8	1.1	0.6	0.4	4.8	1.0	0.5	0.4	0.0	0.1	0.1	0.0
methyl acetate	1.3	0.3	0.2	0.2	1.2	0.2	0.3	0.2	0.1	0.1	-0.1	0.0
ethyl acetate	1.1	0.2	0.2	0.1	1.2	0.4	0.3	0.1	-0.1	-0.1	-0.1	0.0
acetal	1.0	0.1	0.5	0.3	0.9	0.0	0.4	0.4	0.1	0.1	0.1	-0.1
methanol	1.0	0.5	0.2	0.1	1.0	0.5	0.1	0.1	0.0	0.0	0.1	0.0
2-butanol	0.4	0.3	0.1	0.1	0.4	0.3	0.1	0.1	0.0	0.0	0.0	0.0
n-propanol	0.4	0.2	0.3	0.1	0.5	0.1	0.1	0.1	0.0	0.0	0.2	0.0
2-methyl-propan-1-ol	1.4	0.2	0.2	0.1	1.5	0.1	0.1	0.1	-0.1	0.1	0.1	0.0
n-butanol	1.3	0.1	0.1	0.1	1.3	0.1	0.1	0.1	0.0	0.0	0.0	0.0
2-methyl-butan-1-ol	0.4	0.1	0.1	0.1	0.5	0.0	0.1	0.1	-0.1	0.1	0.0	0.0
3-methyl-butan-1-ol	0.5	0.5	0.5	0.2	0.4	0.5	0.3	0.2	0.1	0.0	0.2	0.0

Table 5. The values of *bias* (%) obtained by the both methods of data analysis and the differences between their absolute values for SSs.

Compound	*bias* (%) at IS: pentan-3-ol				*bias* (%) at RS: ethanol				Δ|*bias*|, (%)			
	"0.1"	"0.5"	"1.5"	"2.0"	"0.1"	"0.5"	"1.5"	"2.0"	"0.1"	"0.5"	"1.5"	"2.0"
acetaldehyde	-3.1	-1.7	0.3	-0.4	-3.3	-1.4	0.2	0.3	-0.1	0.3	0.0	0.0
methyl acetate	-4.9	-3.4	-0.4	-1.0	-5.0	-3.1	-0.4	-0.3	-0.1	0.3	-0.1	0.7
ethyl acetate	-6.0	-2.9	-0.3	-0.9	-6.2	-2.6	-0.3	-0.2	-0.1	0.3	-0.1	0.7
acetal	-3.1	-1.7	-0.1	-1.1	-3.2	-1.4	-0.2	-0.4	-0.1	0.3	-0.1	0.7
methanol	2.7	-0.3	0.1	-1.1	2.6	0.0	0.0	-0.3	0.1	0.3	0.1	0.7
2-butanol	-1.6	-1.0	0.3	-0.7	-1.8	-0.7	0.3	0.1	-0.1	0.3	0.1	0.6
n-propanol	-1.4	-0.5	0.5	-0.4	-1.6	-0.2	0.4	0.3	-0.1	0.3	0.1	0.1
2-methyl-propan-1-ol	-1.7	-1.2	0.5	-0.4	-1.8	-0.9	0.4	0.4	-0.1	0.3	0.1	0.0
n-butanol	0.0	-0.9	0.7	-0.3	-0.1	-0.6	0.6	0.4	-0.1	0.3	0.1	-0.1
2-methyl-butan-1-ol	0.1	-0.5	0.6	-0.2	-0.1	-0.2	0.5	0.6	0.0	0.3	0.1	-0.4
3-methyl-butan-1-ol	0.0	-0.8	0.9	0.0	-0.1	-0.5	0.9	0.7	-0.1	0.3	0.1	-0.7

Table 6. The values of limits *LOD* and *LOQ* in units of mg/L AA.

Compound	IS: pentan-3-ol		RS: ethanol	
	LOD	*LOQ*	*LOD*	*LOQ*
acetaldehyde	3.63	12.1	3.65	12.2
methyl acetate	1.57	5.22	1.45	4.85
ethyl acetate	1.17	3.89	1.23	4.09
acetal	1.00	3.33	0.91	3.02
methanol	0.92	3.07	0.92	3.07
2-butanol	0.35	1.17	0.36	1.20
n-propanol	0.36	1.21	0.41	1.36
2-methyl-1-propanol	1.22	4.05	1.29	4.29
n-butanol	1.21	4.04	1.17	3.89
2-methyl-1-butanol	0.38	1.26	0.47	1.56
3-methyl-1-butanol	0.45	1.43	0.34	1.15

The results of the *LOD* and *LOQ* limits assessment are presented in Table 6.

It was revealed that the *RSD, bias, LOD* and *LOQ* values determined using ethanol as the RS do not differ significantly from the data obtained by the traditional method with pentan-3-ol as IS (Tables 4-6).

So, the differences between *RSD* do not exceed 0.2 % (Table 4) and the variances between the absolute values of *bias* do not surpass 0.7 % (Table 5).

The values of limits *LOD* and *LOQ* are very close in the both methods (Table 6). It is demonstrated strong metrological performance parameters without compromising analytical quality.

4.4. Synchronous analysis of archived data of spiritous beverages

The values of measured concentration of volatile compounds have been calculated using equations (3) and

(5) in the traditional method with pentan-3-ol IS and using equation (4) in the new method with ethanol as RS. The average values of measured concentration of volatile compounds determined by the both methods of data analysis for analized beverage samples and the relative differences between them (in %) are shown in Table 7.

Table 7. The average values of measured concentration in units mg/L AA obtained by the both methods of data analysis for different beverages.

Compound	*C* (mg/L AA) at IS: pentan-3-ol				*C* (mg/L AA) at RS: ethanol				$\Delta C\times 100\%/(C_{average})$, %			
	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey
acetaldehyde	18.7	116	96.7	53.5	18.7	116	96.8	53.7	-0.3	-0.4	-0.2	-0.4
methyl acetate	470	24.7	228	234	471	24.8	228	235	-0.3	-0.4	-0.2	-0.4
ethyl acetate	401	939	149	171	402	943	150	171	-0.3	-0.4	-0.2	-0.4
acetal	-	116	50.5	25.3	-	117	50.6	25.4	-	-0.4	-0.2	-0.4
methanol	1199	5183	82.2	68.9	1203	5203	82.3	69.2	-0.3	-0.4	-0.2	-0.4
2-butanol	-	-	-	-	-	-	-	-	-	-	-	-
n-propanol	141	297	399	353	141	298	400	354	-0.3	-0.4	-0.2	-0.4
2-methylpropan-1-ol	249	169	423	408	250	170	424	409	-0.3	-0.4	-0.2	-0.4
n-butanol	6.60	55.5	2.54	3.30	6.62	55.7	2.54	3.31	-0.3	-0.4	-0.2	-0.4
2-methylbutan-1-ol	218	77.0	133	147	219	77.3	133	147	-0.3	-0.4	-0.2	-0.4
3-methylbutan-1-ol	864	379	361	370	866	381	361	372	-0.3	-0.4	-0.2	-0.4

Table 8. The values of the *RSD* (%) obtained by the both methods of data analysis and the differences between them for beverage samples.

Compound	*RSD* (%) at IS: pentan-3-ol				*RSD* (%) at RS: ethanol				Δ*RSD*, (%)			
	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey
acet-aldehyde	6.4	1.0	3.6	1.1	6.7	2.9	3.5	1.8	-0.3	-1.9	0.1	-0.8
methyl acetate	1.3	1.1	1.7	0.6	1.0	0.9	1.6	0.2	0.3	0.2	0.1	0.5
ethyl acetate	6.5	2.3	2.2	0.2	6.2	0.3	2.3	0.6	0.3	2.0	-0.1	-0.4
acetal	-	1.9	0.7	2.2	-	0.0	0.6	3.0	-	1.9	0.1	-0.8
methanol	0.3	2.1	0.2	3.2	0.0	0.2	0.3	2.4	0.3	1.9	-0.1	0.8
2-butanol	-	-	-	-	-	-	-	-	-	-	-	-
n-propanol	0.3	2.0	0.6	1.0	0.0	0.1	0.7	0.2	0.3	1.9	-0.1	0.8
2-methylpropan-1-ol	0.1	2.3	0.5	0.9	0.4	0.4	0.5	0.1	-0.3	1.9	0.0	0.8
n-butanol	6.6	0.8	1.4	2.5	6.9	1.1	1.5	1.7	-0.3	-0.3	-0.1	0.8
2-methylbutan-1-ol	0.7	2.1	0.3	2.5	1.0	0.1	0.3	1.7	-0.3	2.0	0.0	0.8
3-methylbutan-1-ol	1.1	2.1	0.7	1.4	1.4	0.1	0.8	0.6	-0.3	2.0	-0.1	0.8

It is visible from the Table 7, that the values of concentration obtained in the cases of the data processing with IS pentan-3-ol and RS ethanol have not signifficant differences. So, the variances do not surpass 0.4 %.

The values of *RSD* of volatile compound concentrations for the samples of beverages were found using equation (7) and they are given in the Table 8. It is revealed that the absolute values of differences between the *RSD* obtained by the IS methods with pentanol-3-ol and ethanol do not exceed 0.3 % for wine, 2.0 % for raki, 0.1 % for brandy and 0.8 % for whiskey.

5. The results of archived data analysis using external standard method and proposed ethanol as RS

5.1. The determination of response factors and the linearity testing at external standard method

The method of absolute calibration is applied in many laboratories in a number of countries, for example, Belarus, Russia, Kazakhstan and others. In this case, the number of standard solutions can be 3 or more.

In this case, standart solutions are used with the certified concentration values in units of mg/L, and the strength of SSs is approximately the same as in the analized sample. Therefore, to demonstrate the ability of new ethanol RS method in comparison with external standart method, the values of volatile compound concentration of analyzed archive data can be recalculated in units of mg/L according to equation (11).

$$\tilde{\tilde{C}}_{i,j}[\text{mg/L}] = \tilde{C}_{i,j}[\mu\text{g/g}]\cdot \rho_j[\text{g/L}]/1000. \qquad (11)$$

So, 5 concentration levels in units of mg/L are: “0.1” – from 18 to 29, “0.5” – from 91 to 145, “1.0” – from 171 to 273, “1.5” – from 263 to 420, “2.0” – from 352 to 652.

When the method of external standard is applied, the values of response factors *RF* and coefficients of determination R^2 are calculated by the multiple-points lineal regression based on the measurement results of all SSs according to equations (12) and (13):

$$RF_i = \frac{\sum_{j=1}^{l}\sum_{k=1}^{n}\left(\tilde{\tilde{C}}^{certified}_{i,j}\cdot A_{i,k,j}\right)}{n\cdot\sum_{j=1}^{l}\left(\tilde{\tilde{C}}^{certified}_{i,j}\right)^2}, \qquad (12)$$

$$R_i^2 = 1 - \frac{\sum_{j=1}^{l}\sum_{k=1}^{n}\left(A_{i,k,j} - RF_i\cdot\tilde{\tilde{C}}^{certified}_{i,j}\right)^2}{\sum_{j=1}^{l}\sum_{k=1}^{n}\left(A_{i,k,j} - \frac{1}{l\cdot n}\sum_{j=1}^{l}\sum_{k=1}^{n}A_{i,k,j}\right)^2}, \qquad (13)$$

where $A_{i,k,j}$ is the detector response to the *i*-th volatile compound, obtained during measurement *k* of SS$_j$; $\tilde{\tilde{C}}^{certified}_{i,j}$ is the certified concentration of the *i*-th volatile compound in SS$_j$ in units mg/L; *n* is the total number of repeat measurements of SS$_j$; *l* is the number of levels of concentrations (*l* = 5 for the assay of SSs “0.1”, “0.5”, “1.0”, “1.5”, “2.0” in this study); RF_i is a response factor value for the *i*-th volatile compound in units (peak area units)/(mg/L). It is important to note, that coefficients *RF* in different methods can have the dissimilar units: (peak area units)/(mg/L), (peak area units)/(μg/g), (peak area units)/(mg/L AA) et al., in contrast to unitless coefficients *RRF*.

The values of measured concentration of volatile compounds in SSs at the absolute calibration method have been calculated using equations (14) and (15):

$$\tilde{\tilde{C}}^{measured}_{i,k,j}[\text{mg/L}] = \left(1/RF_i\right)\cdot A_{i,k,j}, \qquad (14)$$

$$C^{measured}_{i,k,j}[\text{mg/L AA}] = \frac{\tilde{\tilde{C}}^{measured}_{i,k,j}[\text{mg/L}]\cdot 100}{ABV_j}. \qquad (15)$$

The values of relative standard deviation *RSD*, accuracy (*bias*), limits *LOD* and *LOQ* are determined by the same equations (7), (8), (9) and (10) in the all methods. The found values of *RF,* R^2, *LOD* and *LOQ* for the absolute calibration method are given in Table 9.

The comparison of R^2 values which obtained in the method with ethanol as RS (Tables 3) and the data found in the absolute calibration method (Table 9) is testify that coefficient of linearity is much greater at method with ethanol as RS for all compounds. The limits *LOD* and *LOQ* in the both methods do not differ significantly (Table 6 and 9).

Table 9. The values of calibration coefficients *RF*, parameters of linearity R^2, limits *LOD* and *LOQ* obtained by external standard method

Compound	$RF\cdot 10^{5*}$	R^2	LOD^{**}	LOQ^{**}
acetaldehyde	7.48	0.9996	3.56	11.9
methyl acetate	6.00	0.9995	1.45	4.84
ethyl acetate	8.42	0.9995	1.31	4.38
acetal	1.12	0.9994	0.99	3.29
methanol	7.85	0.9996	0.81	2.72
2-butanol	1.45	0.9996	0.46	1.53
n-propanol	1.42	0.9995	0.31	1.03
2-methylpropan-1-ol	1.68	0.9996	1.21	4.05
n-butanol	1.56	0.9995	1.27	4.24
2-methylbutan-1-ol	1.71	0.9996	0.42	1.39
3-methylbutan-1-ol	1.70	0.9994	0.42	1.39

* units of $RF\cdot 10^5$ are (peak area units)/(mg/L) (for 40 % v/v);

** units of *LOD* and *LOQ* are mg/L AA.

The received data from *RSD* and accuracy (*bias*) testing in the ES method and in the ethanol IS are shown in the Table 10 as maximum values established for different SSs.

Table 10. The maximums of the absolute values of *RSD* and *bias* obtained for SSs by the method of external standard (ExS) and the method with IS ethanol.

Compound	RSD^{max} (%)		$bias^{max}$ (%)	
	ExS	IS eth.	ExS	IS eth.
acetaldehyde	4.7	4.8	3.5	3.3
methyl acetate	1.6	1.2	4.6	5.0
ethyl acetate	1.7	1.1	5.9	6.2
acetal	1.9	0.9	2.9	3.2
methanol	1.6	1.0	2.8	2.6
2-butanol	1.7	0.4	1.9	1.8
n-propanol	1.7	0.5	1.9	1.6
2-methyl-propan-1-ol	1.6	1.5	2.1	1.8
n-butanol	1.7	1.3	1.3	0.6
2-methyl-butan-1-ol	1.7	0.5	1.0	0.6
3-methyl-butan-1-ol	1.8	0.5	1.5	0.9

5.2. Synchronous analysis of archived data of spiritous beverages by the ES method and using ethanol as RS

The values of measured concentration of volatile compounds in spiritous beverage with strength of the beverage $ABV_{beverage}$ have been calculated in the external standard method using equations (16) and (17):

$$\tilde{\tilde{C}}^{measured}_{i,k,beverage}[\text{mg/L}] = \left(1/RF_i\right)\cdot A_{i,k,beverage}, \qquad (16)$$

$$C^{measured}_{i,beverage}[\text{mg/L AA}] = \frac{\tilde{\tilde{C}}^{measured}_{i,beverage}[\text{mg/L}]\cdot 100}{ABV_{beverage}}. \qquad (17)$$

The average values of these concentration and the relative differences between them and values found using RS ethanol (in %) are shown in Table 11. The values of *RSD* of volatile compound concentration for the samples of beverages are represented in Table 12.

Table 11. The average values of measured concentration obtained for beverages by ES method by RS ethanol.

Compound	*C* (mg/L AA), ES method				*C* (mg/L AA), RS ethanol				$\Delta C\times 100\%/(C_{average})$, %			
	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey
acetaldehyde	18.6	115	96.0	53.3	18.7	116	96.8	53.7	-0.9	-0.8	-0.8	-0.8
methyl acetate	470	25	228	235	471	24.8	228	235	-0.3	-0.1	-0.2	-0.2
ethyl acetate	400	941	149	171	402	943	150	171	-0.4	-0.3	-0.3	-0.3
acetal	-	116	50.4	25.3	-	117	50.6	25.4	-	-0.3	-0.3	-0.3
methanol	1196	5183	81.9	68.8	1203	5203	82.3	69.2	-0.6	-0.4	-0.5	-0.4
2-butanol	-	-	-	-	-	-	-	-	-	-	-	-
n-propanol	140	296	396	351	141	298	400	354	-1.0	-0.8	-0.9	-0.9
2-methylpropan-1-ol	247	168	420	406	250	170	424	409	-1.0	-0.8	-0.9	-0.9
n-butanol	6.55	55.2	2.51	3.28	6.62	55.7	2.54	3.31	-0.8	-1.0	-0.3	-0.3
2-methylbutan-1-ol	216	76.5	131	146	219	77.3	133	147	-1.2	-1.0	-1.1	-1.1
3-methylbutan-1-ol	855	376	357	367	866	381	361	372	-1.4	-1.2	-1.3	-1.3

Table 12. The values of the *RSD* obtained for beverages by ES method by RS ethanol.

Compound	*RSD* (%), ES method				*RSD* (%), RS: ethanol				Δ*RSD*, (%)			
	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey	Wine	Raki	Brandy	Whiskey
acet-aldehyde	7.0	3.0	3.9	1.6	6.7	2.9	3.5	1.8	0.3	0.1	0.4	-0.2
methyl acetate	0.7	1.0	2.0	0.0	1.0	0.9	1.6	0.2	-0.3	0.1	0.4	-0.2
ethyl acetate	5.9	0.2	1.9	0.4	6.2	0.3	2.3	0.6	-0.3	-0.1	-0.4	-0.2
acetal	-	0.2	1.1	2.8	-	0.0	0.6	3.0	-	0.1	-0.4	-0.2
methanol	0.4	0.1	0.1	2.6	0.0	0.2	0.3	2.4	0.3	-0.1	-0.1	0.2
2-butanol	-	-	-	-	-	-	-	-	-	-	-	-
n-propanol	0.4	0.0	0.3	0.4	0.0	0.1	0.7	0.2	0.3	-0.1	-0.4	0.2
2-methylpropan-1-ol	0.7	0.3	0.1	0.3	0.4	0.4	0.5	0.1	0.3	-0.1	-0.4	0.2
n-butanol	7.2	1.2	1.1	1.9	6.9	1.1	1.5	1.7	0.3	0.1	-0.3	0.2
2-methylbutan-1-ol	1.3	0.0	0.1	1.9	1.0	0.1	0.3	1.7	0.3	-0.1	-0.3	0.2
3-methylbutan-1-ol	1.7	0.0	0.4	0.8	1.4	0.1	0.8	0.6	0.3	-0.1	-0.4	0.2

It's revealed that the absolute values of differences between the *RSD* obtained by the method of external standard and new method with RS ethanol do not exceed 0.3 % for wine, 0.3 % for wine, 0.1 % for raki, 0.4 % for brandy and 0.2 % for whiskey.

It should be noted that the values of the concentration of volatile compounds in alcoholic beverages during absolute calibration cannot be calculated without determining the strength of the alcoholic beverage. Thus, the accuracy of determining the density and strength of each analyzed alcohol-containing sample critically affects the accuracy of determining the content of volatile compounds in them when appling the external standard method. However, when working with the internal standard method using ethanol as a reference substance, determining the strength of the analyzed sample is not required and does not affect the accuracy of the results obtained.

6. Conclusion

New method offers a fast, cost-effective and accurate additional tool to the traditional procedure described in Commission Regulation EC2870/2000. The proposed approach enables the reprocessing and analysis of different chromatographic data, significantly enhancing its efficiency. Proposed innovative validation strategy reduces time and costs, ensuring reliable performance across diverse alcoholic matrices. Consequently, the modified method represents a powerful and practical tool for quality control, regulatory compliance, and routine analysis in the alcoholic beverage industry. Following the materials of the work done, everyone can validate the new method in their own laboratory by using of existing archived experimental data and ensure that the method works and is effective. Determining volatile compounds in spirituous beverages of different origin has never been so easier.

7. References

1. S.V. Charapitsa, A.N. Kavalenka, N.V. Kulevich, N.M. Makoed, A.L. Mazanik, S.N. Sytova, N.I. Zayats, Y.N. Kotov, J. Agric. Food Chem., **61**, 2950-2956 (2013)
2. S.V. Charapitsa, S.N. Sytova, A.L. Korban, L.N. Sobolenko, J. AOAC Int., **102**, 669-672 (2019)
3. S. Charapitsa, S. Sytova, A. Korban, L. Sobolenko, V. Egorov, S. Leschev, M. Zakharov, R. Čabala, R. Busarova, I. Shestakovich, A. Tolstouhova, S. Ondroušek, J. Vávra, M. Yilmaztekin, T. Cabaroglu, BIO Web Conf., **15**, 02030 (2019)
4. S. Charapitsa, S. Sytova, A. Kavalenka, L. Sobolenko, N. Kostyuk, V. Egorov, S. Leschev, S. Vetokhin, N. Zayats, Food Control., **120**, 107528 (2021)
5. S. Charapitsa, S. Sytova, A. Kavalenka, L. Sobolenko, M. Zayats, V. Egorov, S. Leschev, I. Melsitova, S. Vetokhin, N. Zayats, J. Food Composition. Analysis, **114**, 104772 (2022)
6. Commission regulation (EC) No 2870/2000
7. ISO 5725-2: 1994. Accuracy (trueness and precision) of measurement methods and results. Part 2
8. ISO 5725-4: 1994. Accuracy (trueness and precision) of measurement methods and results. Part 4
9. Eurachem guide “The fitness for purpose of analytical methods, a laboratory guide to method validation and related topics (2nd ed.)” (2014)
10. Guide quantifying uncertainty in analytical measurement (3rd ed.) Eurachem (2012)
11. Commission regulation (EC) No 110/2008