SHORT COMMUNICATION

Single-Laboratory Validation of a Gas Chromatographic Method of Direct Determination of Volatile Compounds in Spirit Drinks: Need for an Improved Interlaboratory Study

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Background: **The quality and safety control of an alcoholic drink is mainly the establishment of its chemical content, particularly the quantity of volatile compounds.** ***Objective:*** **A single-laboratory validation of a gas chromatographic method of direct determination of volatile compounds in spirit drinks was conducted. The discussed method applies ethanol, the major volatile component of an alcoholic beverage, as an internal standard. Possible algorithms of method validation based on interlaboratory study were proposed and described.** ***Methods:*** **Seven standard solutions of the following volatile compounds were prepared gravimetrically in 40% (v/v) water–ethanol solution: acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, isobutanol, 1-butanol, and isoamylol. Each sample was measured with the proposed method 30 times in repeatability conditions.** ***Results:*** **Flame ionization detector response was linearly correlated with assigned concentrations at a range of 2 to 5000 mg/L of absolute alcohol (AA) with coefficients of determination (R^2) more than 0.995 for all analyzed components. Repeatability ($RSD_r \leq 4.5\%$; $RSD_r \leq 2.0\%$), reproducibility ($RSD_R \leq 5.0\%$; $RSD_R \leq 2.0\%$), and trueness (relative bias $\leq 2.6\%$; relative bias $\leq 1.4\%$) were obtained for low (10–25 mg/L AA for methanol and 2–10 mg/L AA for other volatiles) and high (25–5000 mg/L AA for methanol and 10–5000 for other volatiles) ranges of concentrations, correspondingly.** ***Conclusions:*** **The method increases the reliability of measurements and eliminates manual procedures of internal standard addition into both calibration standard solutions and spirit drinks.**

T he quantification of volatiles in various alcoholic beverages is performed with GC methods. Values of concentrations of volatile compounds are presented in micrograms per gram units (1). It is important to note that legislative documents establish final presentation of volatile compound concentrations in milligrams per liter absolute alcohol (AA; or grams/hectoliter AA) units (2–4). To present concentrations in these quantity dimensions, one should perform an additional procedure of measuring the alcohol by volume content in the test sample.

Methods

The current study is a further exploration of our previously published "Ethanol as Internal Standard" method (5–6). It consists of the determination of Relative Response Factor (RRF) for ethanol. First, response factor for i-th volatile and ethanol are determined according to the following formula:

$$Response\,Factor_i = \frac{Concn_{i/eth}^{st}}{Area_{i/eth}^{st}}, \qquad (1)$$

where $Concn_{i/eth}^{st}$ is the concentration of i-th volatile or ethanol in milligrams per liter AA units in standard solution (SS); and is $Area_{i/eth}^{st}$ flame-ionization detection (FID) responses for i-th volatile or ethanol, arbitrary units, in SS. The RRFs are then calculated according to the following expression:

$$RRF_i^{eth} = \frac{Response\,Factor_i}{Response\,Factor_{eth}} = \frac{Concn_i^{st}}{Area_i^{st}} \cdot \frac{Area_{eth}^{st}}{\rho_{eth}}, \qquad (2)$$

where $\rho_{eth} = 789\,300$ mg/L is ethanol density.

The test spirit sample is directly injected and analyzed under usual conditions. The concentration of i-th volatile is determined according to the following formula:

$$Concn_i^{test} = RRF_i^{eth} \cdot \frac{Area_i^{test}}{Area_{eth}^{test}} \cdot \rho_{eth}, \qquad (3)$$

where $Area_i^{test}$ and $Area_{eth}^{test}$ are FID responses for i-th volatile and ethanol, correspondingly, in arbitrary units, in the test sample. The concentrations obtained according to Equation 3 have dimensions of milligrams per liter AA that fully suits international requirements.

Experimental Design of Single-Laboratory Validation

The single-laboratory validation was conducted in the Laboratory of Analytical Research of the Institute for Nuclear

Problems of Belarusian State University. Used equipment and reagents are described in previous works (5–6).

To proceed a single-laboratory validation of the method, seven SSs were prepared gravimetrically in 40% (v/v) water–ethanol matrix. The contents of the prepared SS are shown in Table 1. The concentrations of volatiles in the prepared samples were chosen so that they could overlap those in most alcoholic drinks and distillates. Thus, quantity profiles of the samples SS-3 and SS-2 are close to those in vodka and rectified 96% ethyl alcohol from agricultural origin, SS-A is close to distillate fractions, and the others correspond to the rest of the alcoholic beverages.

Each sample was measured 30 times under repeatability conditions according to a previous work (7). Calibration was made according to Equation 2 by measurement of SS-C sample. The linearity of FID was checked, and R^2 coefficients are presented in Table 2. The value of LOQ appears to be the lowest level of volatile concentration at which the performance is acceptable for a typical application (8). The "SS-3" solution was used for this aim (*see* Table 1). The obtained LOQs are presented in Table 2.

It is necessary to note that the important feature of prepared SS in comparison with purchased spirit drinks is the possibility to establish the accuracy profile of the investigated method.

Thus, relative biases were obtained for all compounds according to the following common equation:

$$Relative\,bias_{il},\% = \frac{\frac{1}{N}\sum_{J=1}^{N}(Concn_{ilj} - Concn_{il}^{st})}{Concn_{il}^{st}} \times 100\% \quad (4)$$

The obtained values are presented in Table 2. It is shown that the maximal absolute value did not exceed 3%. The maximum values of relative standard deviation were calculated and compared with those obtained during interlaboratory study (1) in Table 3.

In addition, the same procedure was conducted for a real spirit drink sample. A bottle of a famous-brand whiskey was purchased in a commercial source and measured 30 times without any preliminary preparations. The values of relative standard deviations were calculated and presented in Table 4.

The Proposals to Collaborative Interlaboratory Study

In accordance with the requirements of ISO/IEC 17025 (8), the method must be validated before its application in routine laboratory practice. It is important to notice that the discussed method can be validated based on data obtained experimentally

Table 1. The concentrations of volatile components in the prepared standard solutions

| Compound | Concentration, mg/L AA | | | | | | |
	SS-3	SS-2	SS-1	SS-D	SS-C	SS-B	SS-A
Acetaldehyde	3.93 ± 0.12	11.0 ± 0.33	48.6 ± 1.5	132 ± 3.5	223 ± 4.5	436 ± 4.4	4336 ± 43
Methyl acetate	1.03 ± 0.02	9.74 ± 0.19	51.4 ± 1.0	177 ± 4.2	242 ± 4.8	487 ± 4.9	5533 ± 55
Ethyl acetate	1.11 ± 0.02	9.49 ± 0.19	49.7 ± 1.0	237 ± 5.2	233 ± 4.7	476 ± 4.8	6813 ± 68
Methanol	18.5 ± 0.56	27.7 ± 0.83	71.7 ± 2.2	189 ± 5.0	271 ± 5.4	526 ± 5.3	5741 ± 57
2-propanol	2.75 ± 0.08	11.9 ± 0.36	55.6 ± 1.7	139 ± 3.7	258 ± 5.2	512 ± 5.1	5774 ± 58
Ethanol	789 300						
1-propanol	1.27 ± 0.03	10.7 ± 0.21	56.5 ± 1.1	189 ± 5.1	265 ± 5.3	532 ± 5.3	6010 ± 60
Isobutanol	1.45 ± 0.03	11.3 ± 0.23	58.9 ± 1.2	195 ± 4.6	275 ± 5.5	553 ± 5.5	6214 ± 62
1-butanol	1.72 ± 0.03	11.0 ± 0.22	56.6 ± 1.1	187 ± 5.1	264 ± 5.3	532 ± 5.3	5996 ± 60
Isoamylol	1.95 ± 0.04	11.6 ± 0.23	59.2 ± 1.2	196 ± 4.8	276 ± 5.5	556 ± 5.6	6244 ± 62

Table 2. Relative response factors, least-squares correlation coefficients, limits of quantification (mg/L AA), and relative biases (%)

| Component | RRF | R^2 | LOQ | Relative bias, % | | | | | |
				SS-3	SS-2	SS-1	SS-D	SS-B	SS-A
Acetaldehyde	1.402	0.9984	0.30	−2.6	−0.6	−1.4	0.4	−0.5	−0.3
Methyl acetate	1.583	0.9962	0.23	0.7	−0.3	−1.0	0.8	−0.2	−0.5
Ethyl acetate	1.308	0.9959	0.30	−1.7	−0.2	0.6	0.6	0.0	−0.5
Methanol	1.252	0.9961	0.33	−1.0	−0.1	−1.2	0.2	0.0	−0.2
2-propanol	0.861	0.9971	0.25	−2.0	−0.2	−0.1	0.9	−0.3	−0.5
Propanol-1	0.689	0.9982	0.25	−2.0	−0.8	−0.2	0.1	−0.2	−0.1
Isobutanol	0.574	0.9965	0.16	−2.0	−0.3	−0.3	0.2	0.1	−0.3
1-butanol	0.619	0.9980	0.26	−2.0	−0.3	−0.1	0.2	0.0	−0.1
Isoamylol	0.584	0.9990	0.27	−2.0	−0.3	−0.1	0.3	0.0	0.0

Table 3.   The maximums of repeatability and reproducibility (%) values obtained during single-laboratory validation and during interlaboratory study (1)

Component	Single-laboratory validation				Interlaboratory study	
	RSD_r, Range 1[a]	RSD_R, Range 1[a]	RSD_r, Range 2[b]	RSD_R, Range 2[b]	RSD_r	RSD_R
Acetaldehyde	2.9	3.2	1.7	1.6	15.8	26.2
Methyl acetate	4.5	5.0	2.0	2.0	NA[c]	NA
Ethyl acetate	4.4	4.7	1.7	2.0	2.4	7.6
Methanol	0.9	1.1	0.9	1.5	5.6	11.8
2-propanol	2.8	3.6	1.7	1.9	NA	NA
Propanol-1	4.5	4.3	1.7	1.7	3.4	6.1
Isobutanol	2.7	3.4	1.3	1.4	1.4	8
1-butanol	4.5	4.7	0.9	1.6	11.2	15.7
Isoamylol	4.4	4.1	1.2	1.7	1.8	7.3

[a]   10–25 mg/L AA for methanol and 2–10 mg/L AA for other volatiles.

[b]   25–5000 mg/L AA for methanol and 10–5000 mg/L AA for other volatiles.

[c]   Not available.

Table 4.   Volatile compounds concentrations (mg/L AA), repeatability (RSD_r, %), and within-laboratory precision (RSD_R, %) obtained for purchased whiskey sample

Component	Concentration	RSD_r	RSD_R
Acetaldehyde	57.9	0.66	1.62
Methyl acetate	12.3	2.09	2.36
Ethyl acetate	371	1.25	1.66
Methanol	65.5	0.51	0.56
2-propanol	3.09	2.27	2.86
Propanol-1	621	0.14	0.17
Isobutanol	623	0.37	0.41
1-butanol	3.59	3.01	2.84
Isoamylol	778	0.17	0.21

during traditional method usage in accordance with previous works (2–3). No more measurements except those indicated in those works (2–3) are required.

Compared with the previously mentioned study, a set of SSs should be prepared with the addition of traditional internal standard compound. Such important characteristics as trueness (relative biases) and robustness of the discussed method can be evaluated only through the measurements of an artificially prepared SS. Eventually, the basic metrological characteristics can be determined for both traditional and discussed methods.

The interlaboratory study can also be conducted during the analysis of purchased alcoholic beverages, as it was done in a previous study (1). The latter are suggested to be the most widespread alcoholic beverages available at commercial sources. However, compared with a previous study (1), the list of purchased samples should include vodka and rectified 96% ethyl alcohol to expand the borders of method application into lower volatile concentrations.

It is important to note that LGC (formerly the Laboratory of the Government Chemist) regularly prepares certified reference material, such as LGC5100 Whisky-Congeners for the world market. Before the reference material can be sold, the interlaboratory comparison is undertaken. During this, the 10 mL aliquots of commercially available whiskey are measured by 16 profile authoritative laboratories. Thus, this procedure can be also used for method validation by contacting LGC with a request of data presentation from these 16 laboratories. It is highly likely that each laboratory prepares SS for calibration, linearity checking, and quality control tests. According to a previous study (9), LGC is allowed to request not only measurement results but also accompanied technical and experimental information, including calibration process data. This data would be completely sufficient for the purpose of "Ethanol as Internal Standard" validation.

Conclusions

The results of a single-laboratory validation of the "Ethanol as Internal Standard" method are presented. It was shown that this method is simpler and more accurate compared with the traditional internal standard method (2–3). The linearity, precision, and trueness of the method were not worse than those of the traditional method. Two ranges of volatile compound concentrations were studied, and corresponding metrological tests have been undertaken for both. Several probable procedures of interlaboratory study of the discussed method were proposed. It was shown that the whole procedure of the interlaboratory experiment can be very close to that undertaken earlier during the testing of a method that was afterward the bedrock of a European legislation.

Acknowledgments

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