

ETHANOL AS INTERNAL STANDARD FOR DETERMINATION OF VOLATILE COMPOUNDS IN SPIRIT DRINKS BY GAS CHROMATOGRAPHY

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Abstract

The new methodical approach of using ethanol as internal standard in gas chromatographic analysis of volatile compounds in spirit drinks in daily practice of testing laboratories is proposed. This method provides determination of volatile compounds concentrations in spirit drinks directly expressed in milligrams per liter (mg/L) of absolute alcohol according to official methods without measuring of alcohol strength of analyzed sample. The experimental demonstration of this method for determination of volatile compounds in spirit drinks by gas chromatography is described.

Introduction

According to the official methods (1-4) the accredited laboratories should determine the following volatile compounds in spirit drinks: acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, isobutyl alcohol, n-butanol, isoamyl alcohol. Concentrations of these compounds are expressed in milligrams per liter (mg/L) of absolute alcohol (AA). For calculation of concentrations the internal standard (IS) method is used (1-3, 5-7). It is proposed to use pentan-3-ol as IS. Some researchers (5,8) make calculation by means of the external standard (ES) method to avoid the introduction of another source of error, such as the addition of an internal standard.

To get quantitative values of impurities concentration per liter of absolute alcohol it is also required to measure alcohol strength of analyzed sample (1-4).

Early in paper (9,10) an idea was proposed to use main component (solvent) for determination of impurities concentration. It is possible at the present time to introduce this approach for routine practice of analytical laboratories due to modern GC with wide range of signal registration from flame ionization detector (FID). The linear range of modern FID is generally more than 10⁷. Signal registration from impurities compounds and from main component ethanol takes place without any distortions. In current paper experimental demonstration of this method for determination of volatile compounds in spirit drinks by gas chromatography is described.

Calibration of chromatograph includes the measuring of relative detector response factors for every analyzed compound relative to ethanol. Numeric values of relative detector response factors RF_i (1) are calculated from chromatographic data for standard solutions with known concentrations of analyzed compounds and may be expressed by the following equation:

$$RF_i = \frac{A_{Et}^{st}}{A_i^{st}} / \frac{\rho_{Et}}{C_i^{st}} = \frac{A_{Et}^{st} \cdot C_i^{st}}{A_i^{st} \cdot \rho_{Et}}, \quad [1]$$

where A_i^{st} and A_{Et}^{st} – peak areas for i -th compounds and ethanol respectively; C_i^{st} – concentrations for i -th compounds, expressed in mg/L (AA), ρ_{Et} = 789300 mg/L - density of ethanol (AA).

Concentration for i -th sample compound relative to absolute alcohol C_i [mg/L] is expressed by the following formula:

$$C_i = RF_i \times \frac{A_i}{A_{Et}} \times \rho_{Et}, \quad [2]$$

where A_i and A_{Et} – are the peak areas for i -th compound and ethanol respectively.

Standard and sample preparations

All individual standard compounds were purchased from Sigma-Fluka-Aldrich (Berlin, Germany). The standard solutions for graduation and sample solutions for researches were prepared by adding of individual standard compounds in ethanol-water mixture (96:4). Ethanol of high grade quality was purchased from Minsk-Kristall Winery and Distillery Plant (Minsk, Belarus).

Gas Chromatographic conditions

Analyses were carried out on the gas chromatograph Crystal5000 (JSC SDB Chromatec, Yoshkar-Ola, Russia) equipped with FID, a split/splitless injector, liquid autosampler, Unichrom software (New Analytical Systems Ltd., Minsk, Belarus), capillary column Rt-Wax, 60 m x 0.53 mm, phase thickness 1 μ m (Restek, Bellefonte, PA, USA). The oven temperature was: initial isotherm at 75 $^{\circ}$ C (9 min), raised to 155 $^{\circ}$ C at rate 7 $^{\circ}$ C/min with final isotherm of 155 $^{\circ}$ C (2.6 min). Carrier gas was nitrogen. Gas flow was 2.44 mL/min; injector temperature 160 $^{\circ}$ C; detector temperature 200 $^{\circ}$ C; injector volume 0.5 μ L and split ratio 1:20. This high split ratio was chosen to achieve good separation between peaks of 2-propanol and ethanol.

Results and discussion

Once the gas chromatographic conditions had been optimized the satisfactory separation under these conditions has been achieved. Typical chromatogram of the used standard solutions is presented in Fig. 1-2.

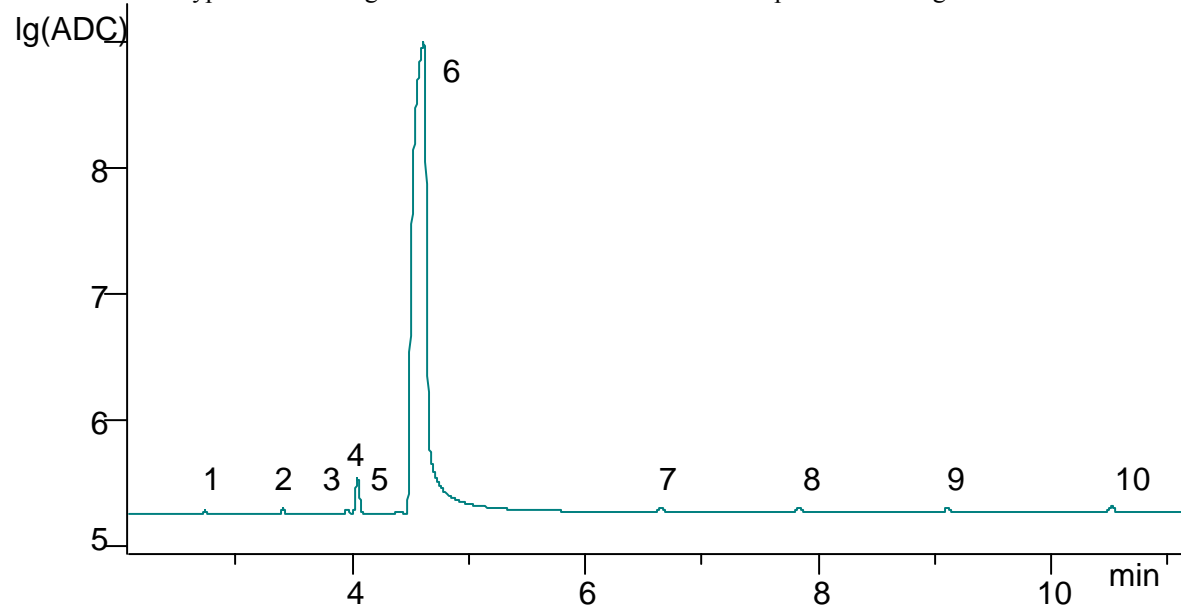


Fig.1. Typical chromatogram of standard ethanol-water (40% and 60%) solutions. To show the dominant compound ethanol and another minor compounds synchronously the logarithm scale of response signal is chosen. 1 - acetaldehyde, 2- methyl acetate, 3 - ethyl acetate, 4 - methanol, 5 - 2-propanol, 6 - ethanol, 7 - 1-propanol, 8 - isobutyl alcohol, 9 - n-butanol, 10- isoamyl alcohol .

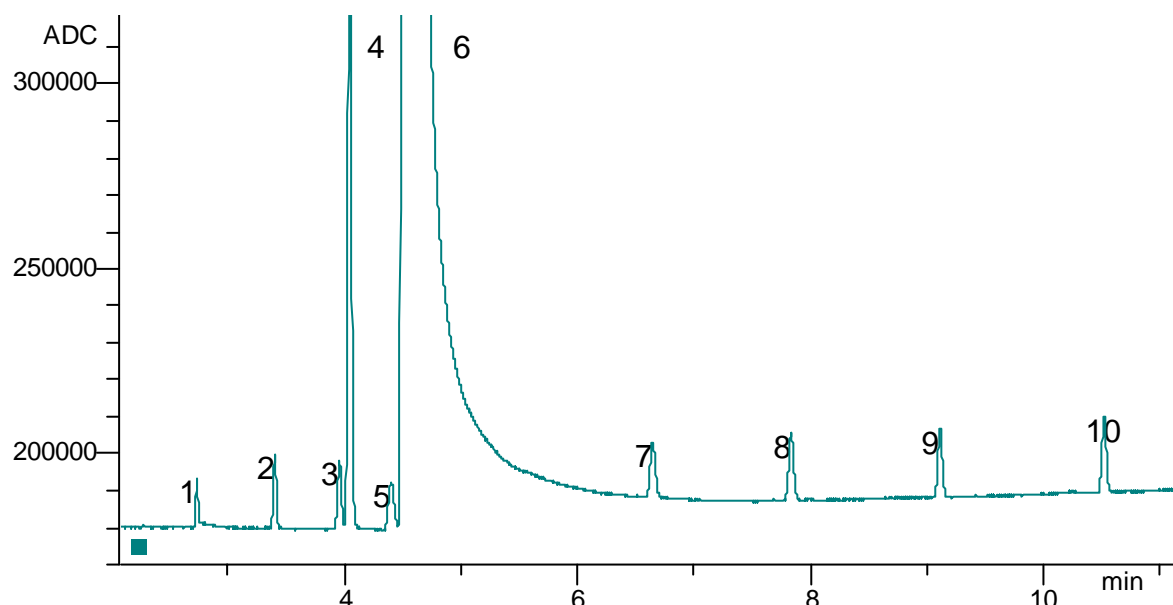


Fig.2. The same chromatogram as in Fig. 1, but linear scale of response signal is chosen.

Six standard ethanol-water (96:4) solutions were prepared to generate calibration curves. There were the following levels of volatile compounds concentrations: 13 mg/L, 20 mg/L, 500 mg/L, 1000 mg/L, 5000 mg/L and 20000 mg/L for methanol and 2 mg/L, 5 mg/L, 10 mg/L, 100 mg/L, 500 mg/L and 2000 mg/L for another eight volatile compounds. Every standard solution was injected three times. Analytical characteristics of the obtained calibration curves are presented in the Table 1. As can be seen in Table 1, correlation coefficients R^2 for all compounds are higher than 0.9996. Detection limits were determined by analysis of low level standards. The detection limits are between 0.235 mg/L for isobutyl alcohol and 0.394 mg/L for methanol.

Compound	Linear range (mg/L)	Slope	Correlation coefficient (R^2)	LOD* (mg/L)
acetaldehyde	2.24 - 1990	1.559	0.9996	0.289
methyl acetate	2.09 - 2000	1.517	0.9997	0.333
ethyl acetate	2.20 - 2094	1.247	0.9998	0.322
methanol	13.0 - 20045	1.377	0.9999	0.394
2-propanol	3.74 - 2033	0.914	0.9998	0.319
1-propanol	1.99 - 2094	0.809	0.9998	0.262
isobutyl alcohol	2.23 - 2000	0.674	0.9998	0.235
n-butanol	1.98 - 2000	0.737	0.9998	0.267
isoamyl alcohol	2.18 - 2073	0.681	0.9999	0.276

* limit of detection (LOD)

Table 1. Analytical characteristics of the obtained calibration graphs of volatile compounds in standard ethanol-water (96:4) solutions.

In order to study accuracy of the proposed methodical approach in the case of large ranges of volatile compounds concentrations 6 – 20000 mg/L for methanol and 1 – 2000 mg/L for another eight volatile compounds reference ethanol-water solutions were prepared with known concentrations of volatile compounds. Every reference solution was injected 30 (15 x 2) times. The repeatability in the worst case for lower concentrations 1 mg/L did not exceed 3.6 %. The obtained experimental results are presented in the Table 2.

Compound	Concentration according to certificate, (mg/L)	Concentration measured by IS method, (mg/L)	Relative discrepancy, %
acetaldehyde	1.158	1.129	-2.50
	5.137	5.182	0.88
	10.11	9.921	1.87
	99.64	93.86	-5.80
	497.6	481.1	-3.32
	1989	2037	2.42
methyl acetate	1.000	1.005	0.50
	5.000	5.121	2.42
	10.00	9.905	-0.95
	100.0	96.35	-3.65
	500.0	484.9	-3.02
	2000	2042	2.10
ethyl acetate	1.047	1.072	2.39
	5.234	5.374	2.67
	10.47	10.45	-0.19
	104.7	102.0	-2.58
	523.4	512.2	-2.14
	2093	2115	1.02
methanol	5.975	6.044	1.15
	53.07	53.51	0.83
	103.2	102.97	-0.22
	1005	988.1	-1.68
	5013	4987	-0.52
	20045	20118	0.36
2-propanol	2.636	2.645	0.34
	6.698	6.754	0.84
	11.78	11.77	-0.08
	103.0	101.0	-2.13
	509.0	503.2	-1.22
	2033	2047	0.69
1-propanol	1.047	0.997	-4.78
	5.234	5.223	-0.21
	10.21	10.23	0.20
	103.2	100.2	-3.10
	523.4	513.6	-1.87
	2094	2125	1.51
isobutyl alcohol	1.000	0.971	-2.90
	5.000	5.033	0.66
	10.00	9.82	-1.80
	100.0	97.7	-2.30
	500.0	491	-1.80
	2000	2032	1.60
n-butanol	1.000	0.991	-0.90
	5.000	5.061	1.22
	10.00	9.89	-1.10
	100.0	97.10	-2.90
	500.0	491.0	-1.80
	2000	2036	1.80

isoamyl alcohol	1.036	1.003	-3.19
	5.182	5.169	-0.25
	10.37	10.21	-1.54
	104.0	101.0	-2.60
	518.0	510.0	-1.58
	2073	2110	1.78

Table 2. Experimental measured concentrations of volatile compounds in reference ethanol-water (96:4) solutions.

The concentrations of analyzed volatile compounds calculated according to IS method are expressed directly in milligrams per liter (mg/L) of absolute alcohol. It is not necessary to do additional measurements of alcohol strength in this case and potential error in value of ethanol concentration is eliminated from resulting formula. There is significant simplification of total measurement procedure.

Verification of method stability against dilution of testing samples was been done by the next way. Three reference ethanol-water solutions were analyzed after dilution with water in ratio 1:1 and 1:3. The obtained results are presented in Table 3.

Compound	Concentration according to certificate, (mg/L)	Measured concentration after dilution 1:1, (mg/L)	Relative discrepancy, %	Measured concentration after dilution 1:3, (mg/L)	Relative discrepancy, %
acetaldehyde	10.11	10.34	2.2	10.50	3.8
	99.64	97.28	-2.4	97.40	-7.3
	497.6	483.3	-2.9	473.1	-4.9
methyl acetate	10.00	10.25	2.5	9.78	-2.2
	100.0	92.76	-7.2	89.17	-10.8
	500.0	463.7	-7.3	452.4	-9.5
ethyl acetate	10.47	10.18	-2.8	10.63	1.6
	104.7	100.0	-4.5	95.46	-8.8
	523.4	489.6	-6.5	477.3	-8.8
methanol	103.2	97.99	-5.0	95.18	-7.8
	1005	921.9	-8.3	904.1	-10.0
	5013	4654	-7.2	4514	-9.9
2-propanol	11.80	11.63	-1.2	10.56	-10.4
	103.2	97.86	-5.2	93.13	-9.7
	509.4	479.5	-5.9	463.7	-9.0
1-propanol	10.21	10.36	1.5	10.01	-1.9
	102.1	98.00	-4.0	96.23	-5.7
	523.4	482.9	-5.8	483.2	-7.7
isobutyl alcohol	10.00	10.42	4.2	10.35	3.5
	100.0	96.87	-3.1	94.32	-5.7
	500.0	480.1	-4.0	471.5	-5.7
n-butanol	10.00	10.17	1.7	9.98	-0.2
	100.0	97.02	-3.0	95.21	-4.8
	500.0	482.9	-3.4	475.2	-5.0
isoamyl alcohol	10.37	11.28	8.8	10.35	-0.2
	103.7	103.0	-0.6	99.52	-4.0
	518.2	509.0	-1.8	500.6	-3.4

Table 3. Experimental measured concentrations of volatile compounds in reference ethanol-water (96:4) solutions after dilution with water in ratio 1:1 and 1:3.

Estimated volume of relative accuracy for proposed method followed from analysis of the obtained experimental in accordance with (11) does not exceed 11 %.

Conclusion

The goal of this work is to show possibility of the new methodical approach of using ethanol as internal standard in gas chromatographic analysis of volatile compounds in spirit drinks in daily practice of analytical and testing laboratories.

Thousands of testing laboratories over the world every day carry out gas chromatographic analysis of volatile compounds in spirit drinks. They may test this approach in their real practice. It is important to note that there is no need to perform any additional measurements. This method could be tested while performing current measurements with existing instrumentation and calculations could be done in parallel according to two different methods: using traditional ES method (4) or according to (1) with pentan-3-ol as IS and using ethanol as IS.

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