

Comprehensive verification of new method “Ethanol as Internal Standard” for determination of volatile compounds in alcohol products by gas chromatography

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Recently proposed new method “Ethanol as Internal Standard” for determination of volatile compounds in alcohol products by gas chromatography is investigated from different sides. Results of experimental study from three different laboratories from Belarus and Russian Federation are presented.

Keywords: alcohol products; volatile compounds, ethanol, internal standard.

1. INTRODUCTION

The international regulation documents for quality and safety control of alcohol production [1, 2] prescribe determination of the following volatile compounds: acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, isobutyl alcohol, n-butanol, isoamyl alcohol. Results of the analysis are expressed in milligrams per litre (mg/L) of absolute alcohol (AA). Such analysis is carried out by the Internal Standard (IS) method. 1-pentanol and 2-pentanol are most commonly used as IS. This method ensures high data reliability. However, the procedure of introducing of an internal standard substance in the sample at the level of some ppm requires a high level of laboratory technicians and performing analyses. For this reason in some national standards the method of External Standard (ES) is used [3, 4]. Finally, to obtain quantitative values of analyzed volatile compounds in mg per litre of absolute alcohol, it is necessary to measure alcohol strength by volume (% v/v) of the analysed sample [1-4].

It was proposed [5] to use ethanol as IS for the analysis of alcohol products in order to increase the accuracy of measurements and obviate the need for the IS addition. The concentration of ethanol in this production can vary from 15% to 96%. The concentration of volatile compounds lies within the range from ppm in rectified alcohol to 30% for the intermediate alcohol products. As a result, the signals from ethanol and from impurities should be registered in a linear range [6]. Nowadays, the testing laboratories are equipped with modern instrumentation for the analysis of alcohol-containing products. These current-technology gas chromatographs have a linear range of registration of seven orders of magnitude that fully obeys the above requirement. Analysis of alcohol products in this case consists in the traditional procedure of determining the relative ratios of the detector response (Relative Response Factors - RRF) of analysed compounds with respect to ethanol by standard solutions and

then the subsequent use of these coefficients in the calculation of concentration of analysed volatile compounds. It should be noted that for modern chromatographs coefficients RRF are enough stable and can be tabulated [7].

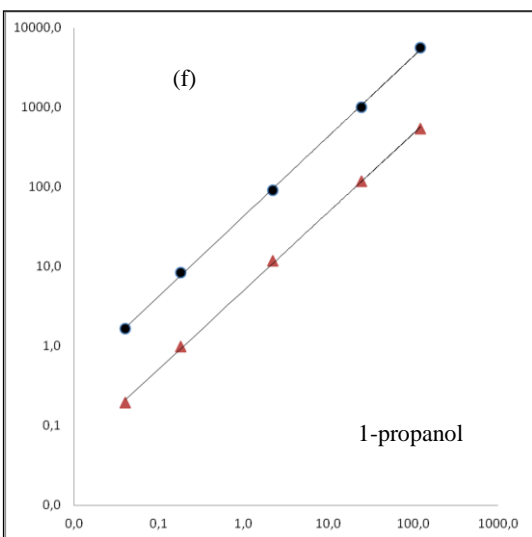
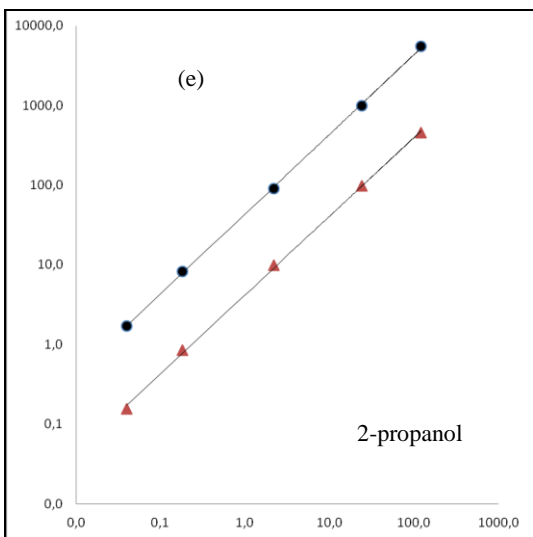
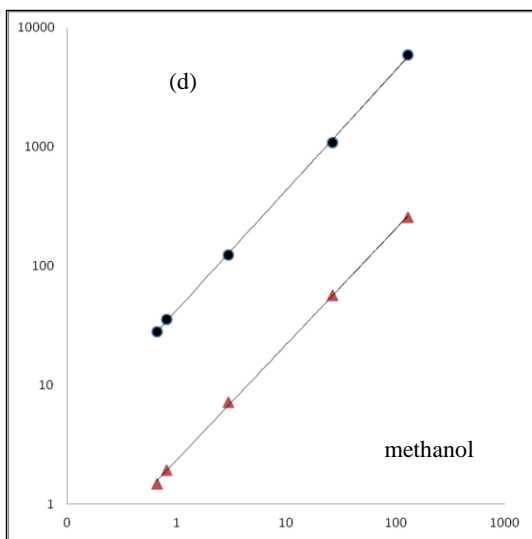
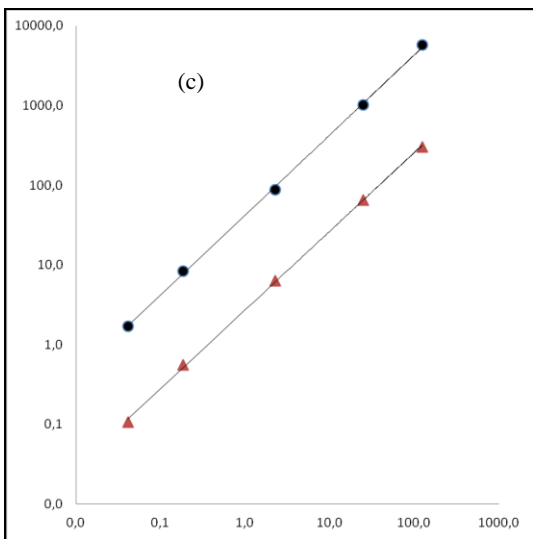
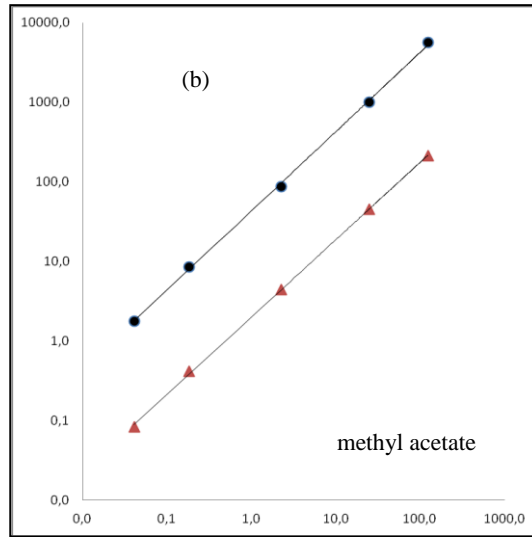
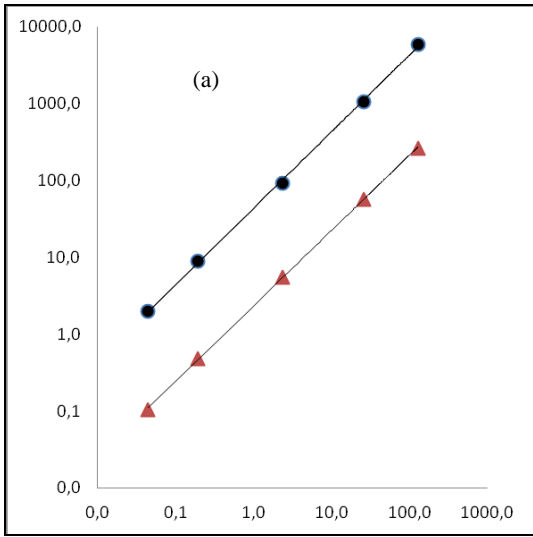
2. MATERIALS AND METHODS

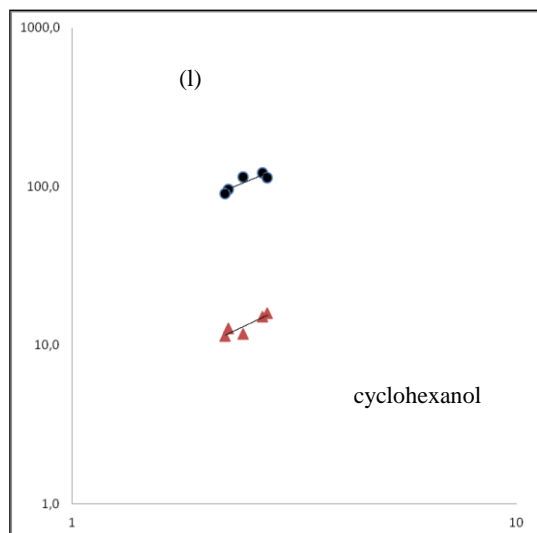
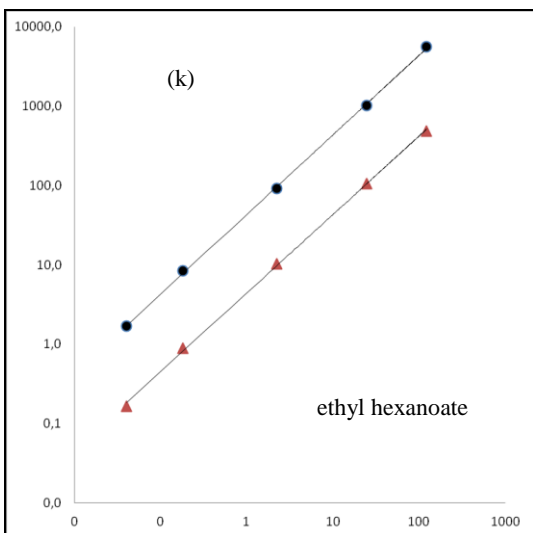
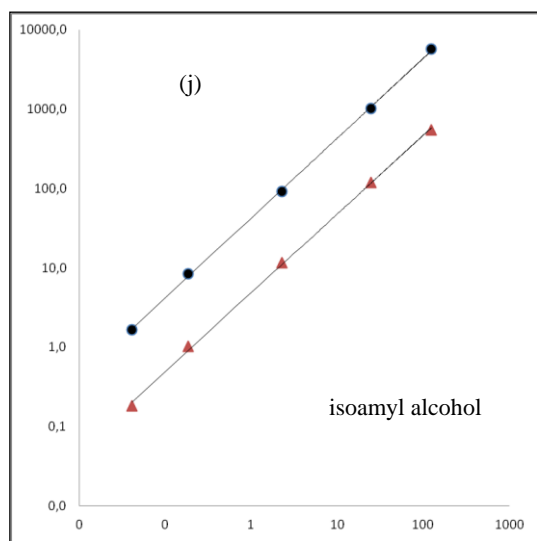
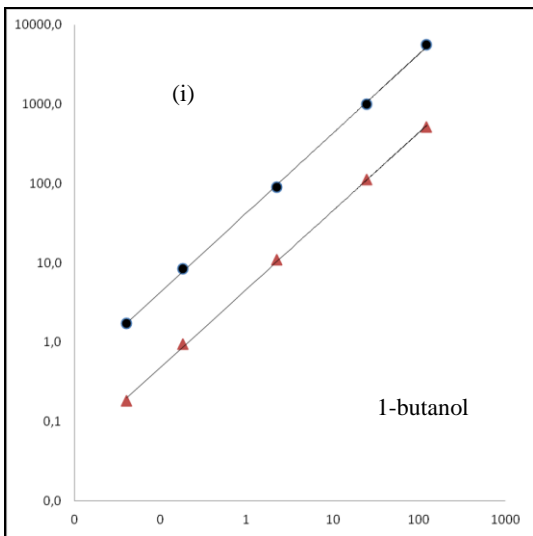
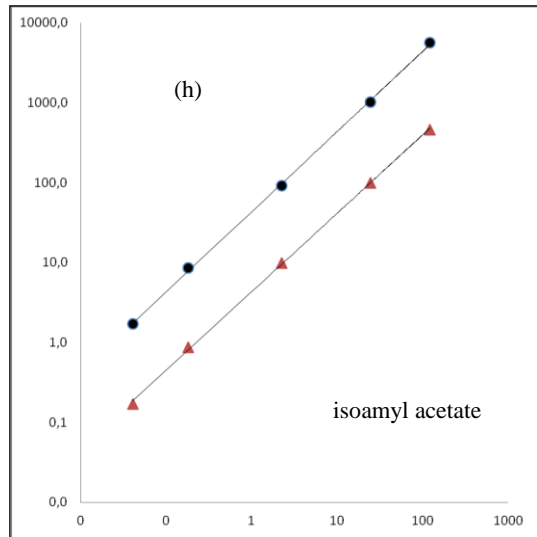
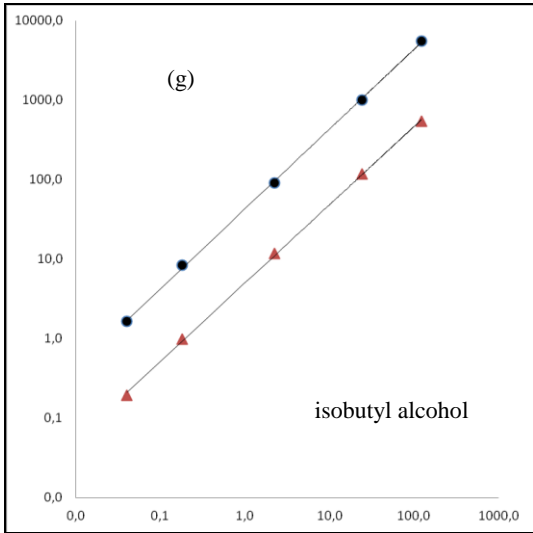
To continue comprehensive examination of the proposed method there were planned and carried out experimental studies with different gas chromatographs (GC) in three different test laboratories: Laboratory of analytical research (LAR) of Research Institute for Nuclear Problems of Belarusian State University (Minsk, Belarus), GC Crystal 5000 (JSC SDO “Chromatec”, Russia); in the Scientific centre “Vinodelie” (SCV) of North-Caucasian Zonal Research Institute of Horticulture and Viticulture (Krasnodar, Russia), GC Crystal 2000M (JSC SDO “Chromatec”, Russia); control laboratory (CL) of Branch of Joint Stock Company “Rosspirtprom” Wine and Distillery Plant “Cheboksary” (Cheboksary, Russia), GC HP6890 (Agilent Technologies, USA). All mentioned GC were equipped with flame ionization detector (FID). All individual standard compounds were purchased from Sigma-Fluka-Aldrich (Germany). The standard solutions were prepared by adding the individual standard compounds to the ethanol-water mixture (96:4) by gravimetric method according to ASTM D 4307 recommendations [8]. Concentrations of volatile compounds in prepared standard solutions were calculated according to the official method of measurement No. 253.0169/01.00258/2013, certified by Federal Agency for Technical Regulation and Metrology (Rosstandart).

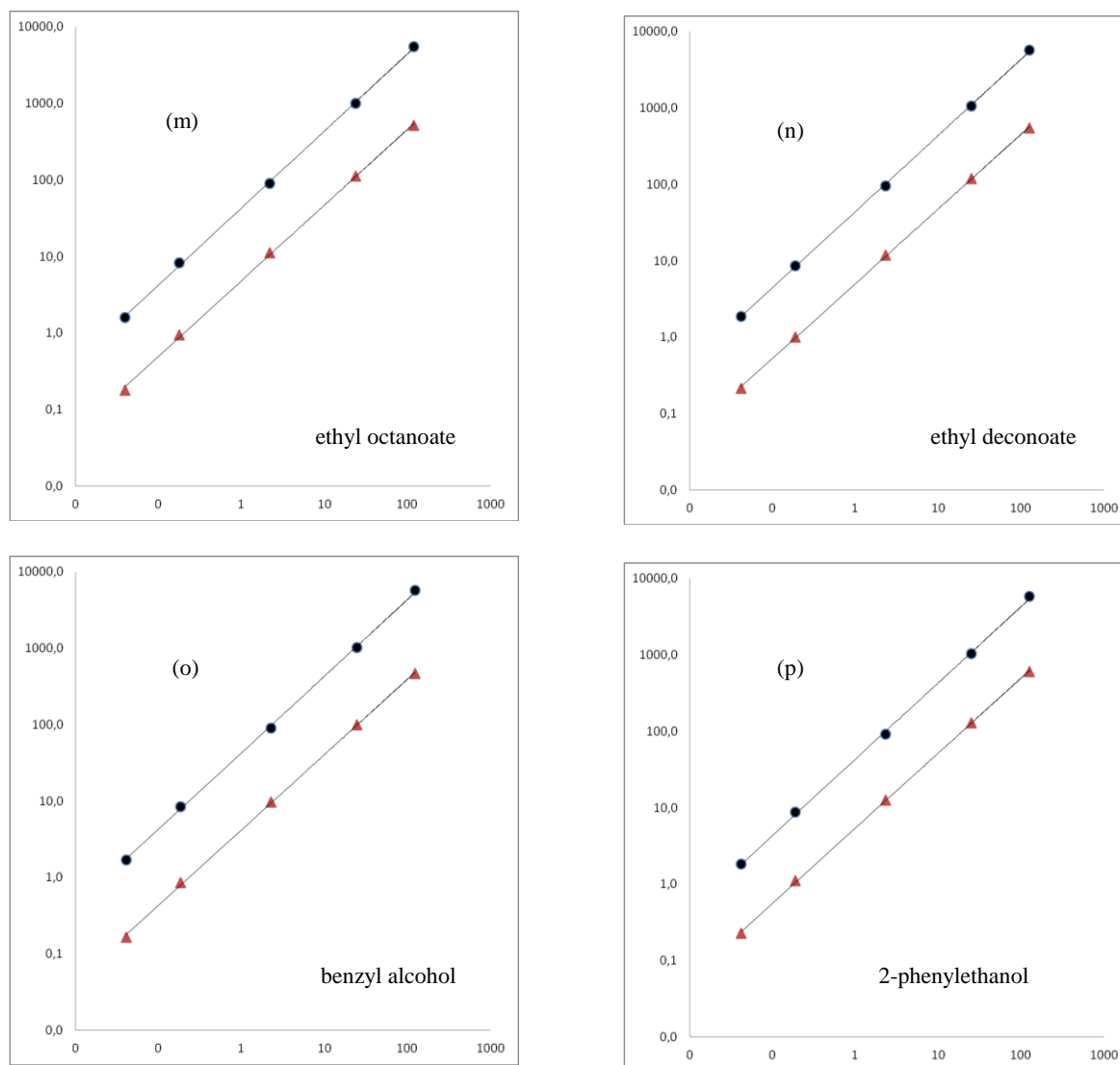
3. RESULTS AND DISCUSSION

The first series of experimental research has been performed in SCV on the GC Crystal 2000M. Content of volatile compounds in the first series of the prepared standard solutions was chosen like in cognac and brandy products. Cyclohexanol was added as IS. Concentrations of volatile compounds in the solutions prepared by gravimetric method and calculated concentrations on the base of measured raw data are given in Table 1. The analysis of the experimental data presented in Table 1 shows that the value of relative bias in the determination of the volatile compound concentrations in experiments in the whole range of concentrations for all fifteenth examined compounds does not exceed 10%.

For illustrative purposes the experimental data are presented in Figure 1 and Figure 2 for the following main analyzed components: acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, isobutyl alcohol, isoamyl acetate, 1-butanol, isoamyl alcohol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, benzyl alcohol and 2-phenylethanol. The presented graphs show the linear dependence of the detector response (triangle marked) and concentration (circle marked) in mg/L (AA) on the amount of the examined component coming directly to the detector.







● – concentration, mg/L (AA), ▲ – response x 10, pC, horizontal axis – amount, pg

Figure 1. Experimental results on demonstration of the following compounds: (a) – acetaldehyde, (b) – methyl acetate, (c) – ethyl acetate, (d) – methanol, (e) – 2-propanol, (f) – 1-propanol, (g) – isobutyl alcohol, (h) – isoamyl acetate, (i) – 1-butanol, (j) – isoamyl alcohol, (k) – ethyl hexanoate, (l) – cyclohexanol, (m) – ethyl octanoate, (n) – ethyl decanoate, (o) – benzyl alcohol and (p) – 2-phenylethanol.

The analysis of the experimental data shows that the relative bias between the experimentally measured concentrations calculated in accordance with proposed method using ethanol as IS and the values of concentrations assigned during the preparation by gravimetric method for all analyzed fifteen components in the five analyzed solutions does not exceed 7,7 %. At the same time the relative bias between measured concentrations calculated in accordance with traditional IS method using cyclohexanol as IS and traditional ES method the values of concentrations assigned during the preparation by gravimetric method for all analyzed fifteen components in the five analyzed solutions does not exceed 6,6 % and 25,9 %, respectively.

The second series of experimental research has been performed in LAR and in CL on the GC Crystal 5000 and HP6890, respectively. To demonstrate the reliability of the proposed method the standard ethanol-water (96:4) solution with initial volatile compounds concentration about 4000 mg/L (AA) was analyzed after dilution with water in the ratios 1:1, 1:9, 1:99, 1:1999 and 1:9999.

Experimental results are presented in Table 2 and Table 3. Illustrative presentation of obtained experimental data are in the Figure 3–6.

Even after dilution with water in the ratio 1:999, the difference between the measured concentrations of all compounds and their values calculated using the gravimetric method does not exceed 7.8 %. With the dilution 1:9999 there are peaks of methanol and ethanol only. Other compounds are significantly less than the level of detection. But even in this case the relative discrepancy of measured concentrations of methanol does not exceed 6.6%.

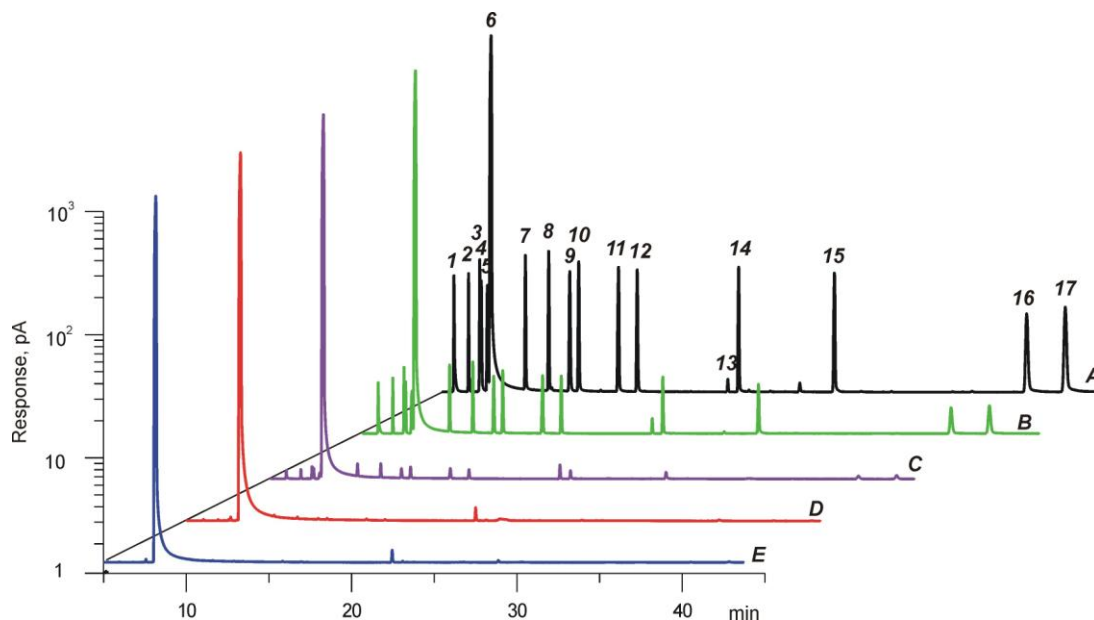
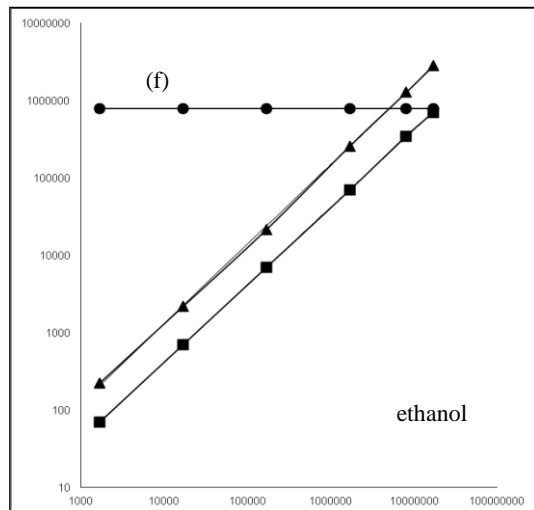
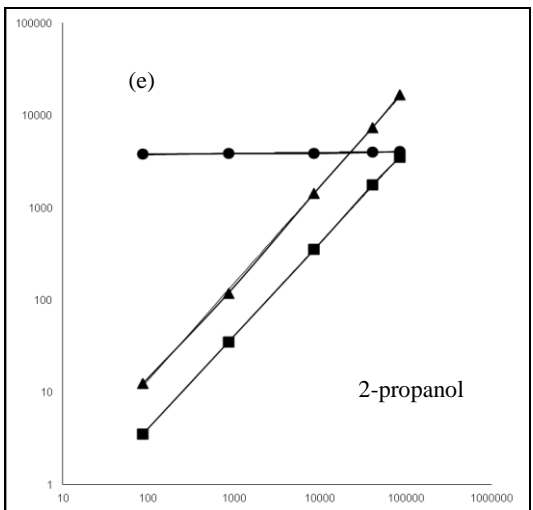
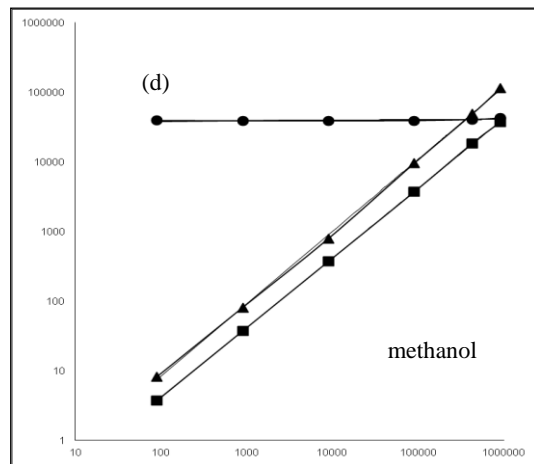
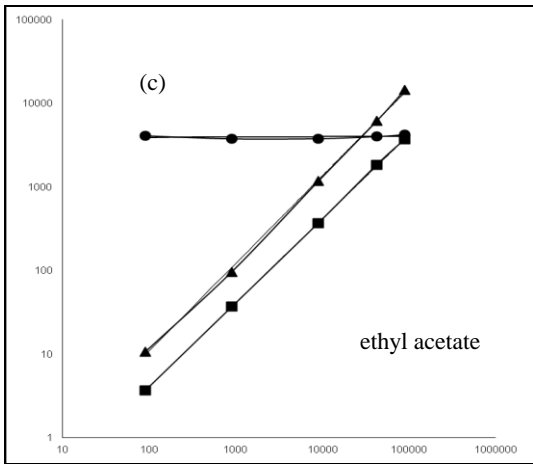
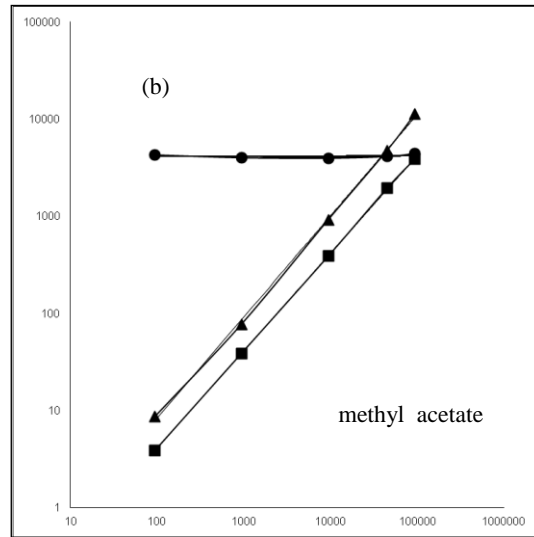
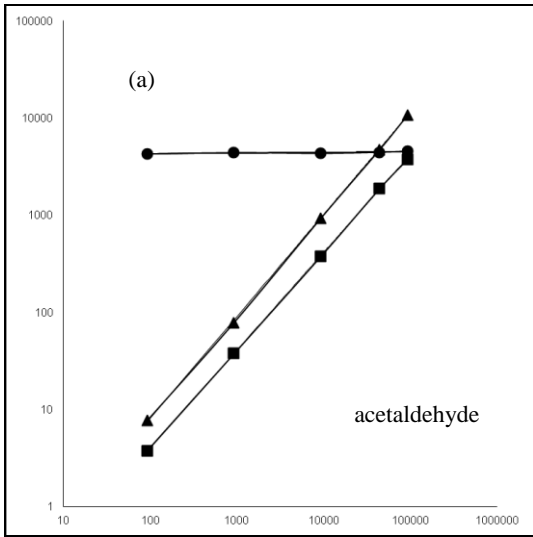
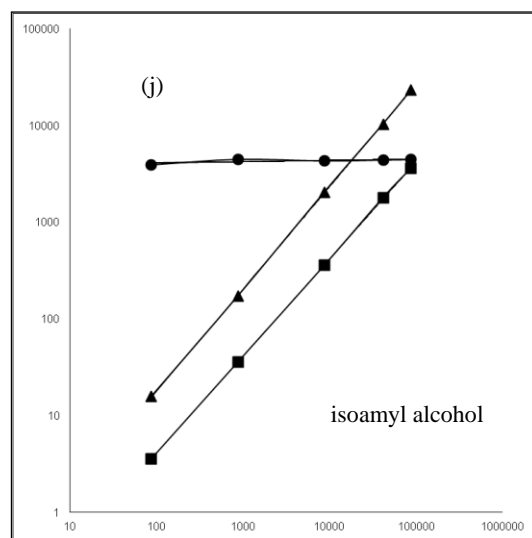
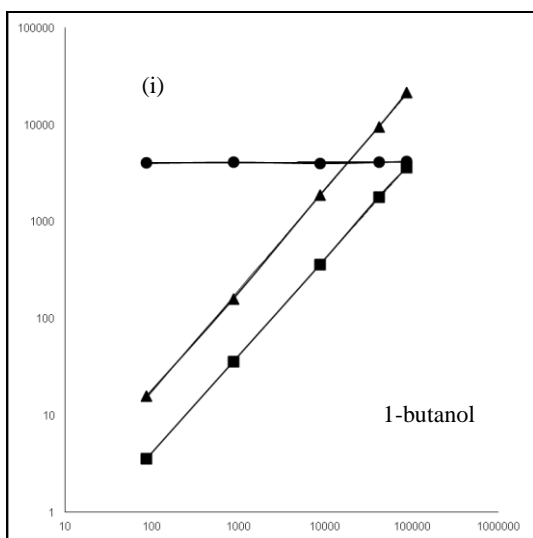
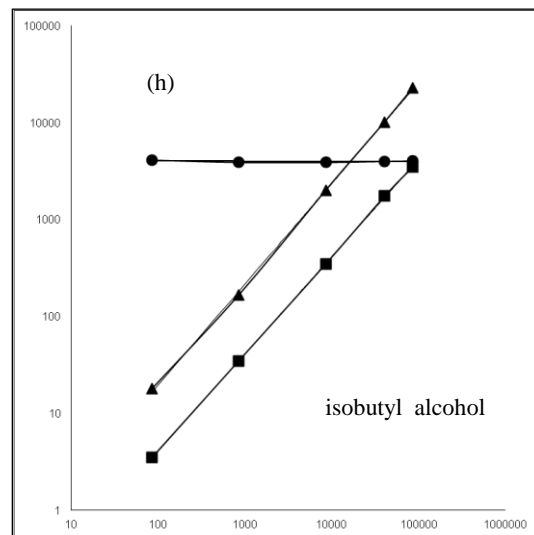
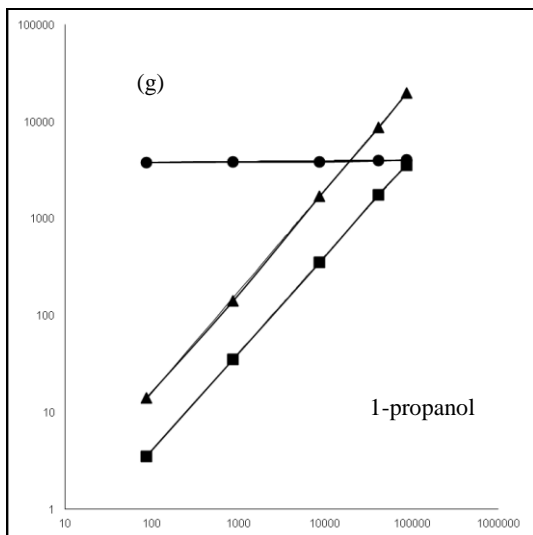


Figure. 2. Chromatograms of standard solutions A-E from Table 1. 1 – acetaldehyde, 2 – methyl acetate, 3 – ethyl acetate, 4 – methanol, 5 – 2-propanol, 6 – ethanol, 7 – 1-propanol, 8 – isobutyl alcohol, 9 – isoamyl acetate, 10 – 1-butanol, 11 – isoamyl alcohol, 12 – ethyl hexanoate, 13 – cyclohexanol, 14 – ethyl octanoate, 15 – ethyl decanoate, 16 – benzyl alcohol, 17 – 2-phenylethanol.

Table 2. The experimental data from LAR. The measured concentrations of analyzed volatile compounds and ethanol, presented according to the degree of dilution with water.

sample (dilution)	measured concentration mg /L (AA) (relative bias,%) [concentration under certificate mg /L (AA) / mg /L (sol)] [response x10, pC}									
	amount, pg compound									
	acetaldehyde	methyl acetate	ethyl acetate	methanol	2-propanol	ethanol	1-propanol	isobutyl alcohol	1-butanol	isoamyl alcohol
A (No)	4556 (6,6) [4275/37 68] {10720} 91899	4436 (0,9) [4397/ 3875] {11276} 94524	4253 (1,9) [4173/ 3678] {14420} 89710	42586 (1,4) [41995/ 37017] {115328} 902864	4112 (3,0) [3991/ 3518] {16655} 85806	N/A N/A [789300/ 695748] {2825852} 16969460	4076 (1,6) [4012/ 3 536] {19676} 86253	4049 (1,9) [3975/ 3504] {22784} 85466	4174 (2,5) [4071/ 3588] {21330} 87522	4458 (9,5) [4071/ 3588] {23143} 87522
B (1:1)	4451 (4,1) [4275/ 1884] {4732,6} 43761	4127 (-6,1) [4397/ 1938] {4741} 45012	4018 (- 3,7) [4173/ 1839] {6157} 42719	40462 (-3,7) [41995/ 18509] {49525} 429935	4000 (0,2) [3991/ 1759] {7323} 40860	N/A N/A [789300/ 347874] {1277251} 8080695	3973 (-1,0) [4012/ 1768] {8668} 41073	4007 (0,8) [3975/ 1752] {10190} 40698	4096 (0,6) [4071/ 1794] {9462} 41677	4412 (8,4) [4071/ 1794] {10353} 41677
C (1:9)	4340 (1,5) [4275/ 377] {931,6} 9190	3961 (-9,9) [4397/ 388] {918,7} 9452	3780 (-9,4) [4173/ 368] {1169} 8971	39043 (-7,0) [41995/ 3702] {9647} 90286	3875 (-2,9) [3991/ 352] {1432} 8581	N/A N/A [789300/ 69575] {257842} 1696946	3868 (-3,6) [4012/ 354] {1704} 8625	3904 (-1,8) [3975/ 350] {2004} 8547	4012 (-1,4) [4071/ 359] {1870} 8752	4318 (6,1) [4071/ 359] {2045} 8752
D (1:99)	4406 (3,1) [4275/ 37,7] {78,57} 919	4002 (- 9,0) [4397/ 38,8] {77,10} 945	3762 (-9,8) [4173/ 36,8] {96,66} 897	38645 (-8,0) [41995/ 370,2] {793,5} 9029	3866 (-3,1) [3991/ 35,2] {118,8} 858	N/A N/A [789300/ 6958] {21427} 169695	3862 (-3,7) [4012/ 35,4] {141,33} 863	3903 (-1,8) [3975/ 35,0] {166,6} 855	4107 (0,9) [4071/ 35,9] {159,1} 875	4479 (10,0) [4071/ 35,9] {171,9} 875
E (1:999)	4280 (0,1) [4275/ 3,77] {7,74} 91,9	4292 (-2,4) [4397/ 3,88] {8,60} 94,5	4107 (-1,6) [4173/ 3,68] {10,7} 89,7	38764 (-7,7) [41995/ 37,02] {80,7} 903	3818 (-4,3) [3991/ 3,52] {12,4} 85,8	N/A N/A [789300/ 696] {2173} 16969	3820 (-4,8) [4012/ 3,54] {14,2} 86,3	4140 (4,1) [3975/ 3,50] {17,9} 85,5	4024 (-1,2) [4071/ 3,59] {15,8} 87,5	3937 (-3,3) [4071/ 3,59] {15,8} 87,5
F (1:9999)	N/A	N/A	N/A	39210 (-6,6) [41995/ 3,702] {8,12} 90,3	N/A	N/A N/A [789300/ 69,6] {223} 1697	N/A	N/A	N/A	N/A





● – concentration, mg/L (AA) ■ – concentration, mg/L (sol) ▲ – response x 10, pC, horizontal axis – amount, pg

Figure 3. Experimental results on demonstration of the following compounds: (a) – acetaldehyde, (b) – methyl acetate, (c) – ethyl acetate, (d) – methanol, (e) – 2-propanol, (f) – 1-propanol, (g) – isobutyl alcohol, (h) – 1-butanol, (j) – isoamyl alcohol. The first line (circle marked) is concentration of the analysed compound expressed in mg per litre of absolute alcohol. The second line (triangle marked) and the third ones (square marked) are the detector response versus the amount of the compound and the concentration in mg per litre of solution, respectively.

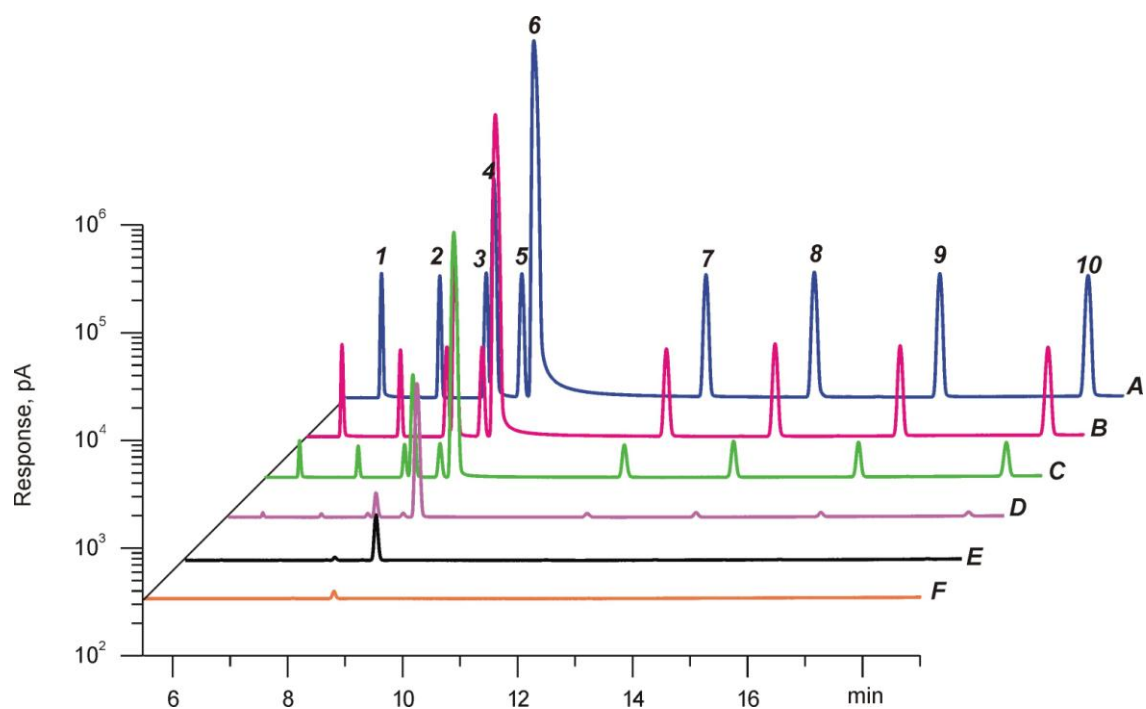
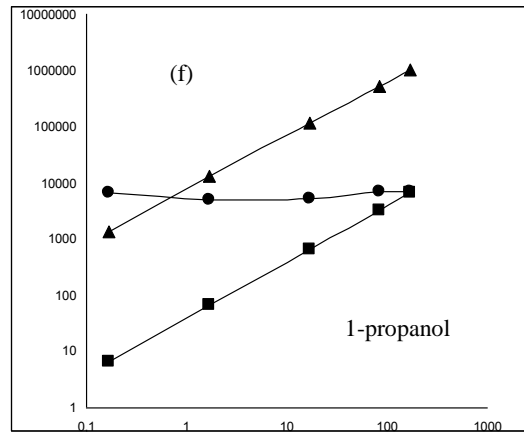
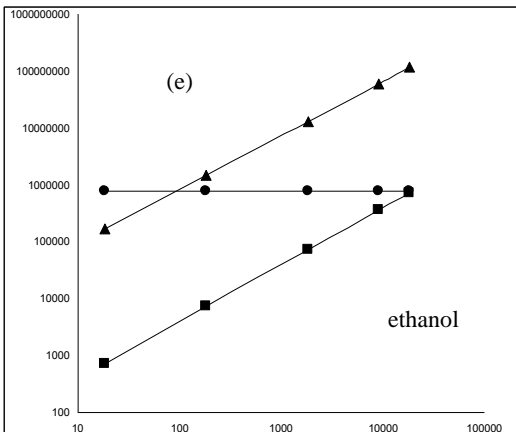
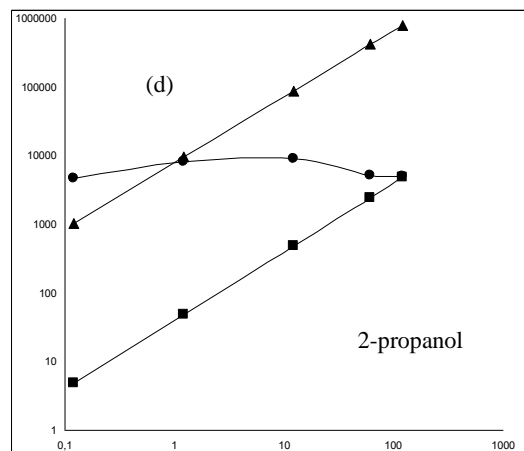
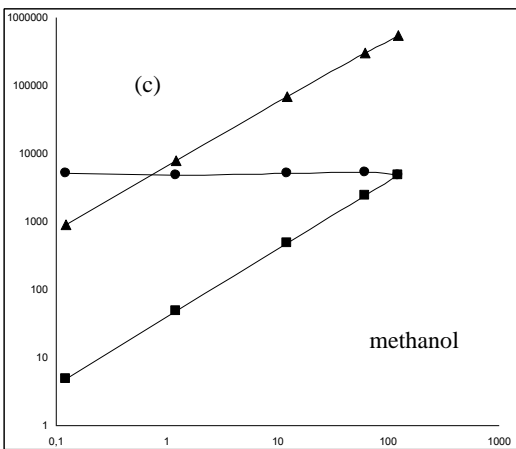
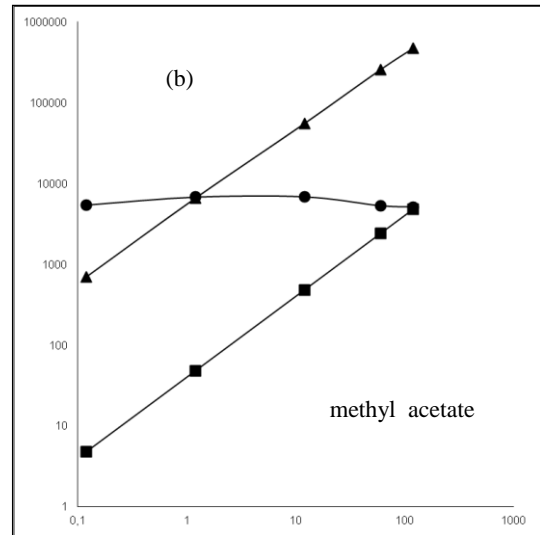
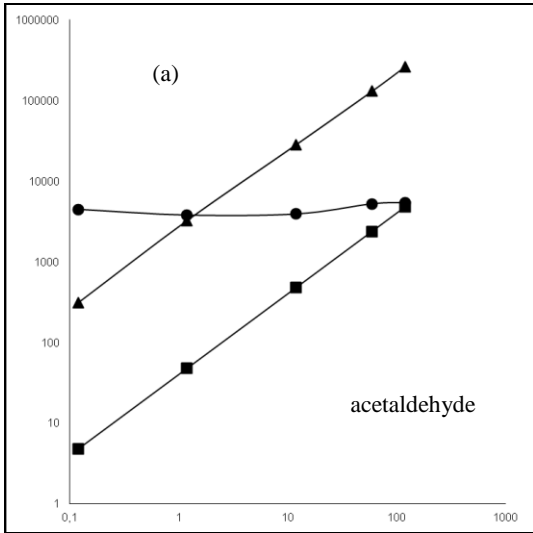
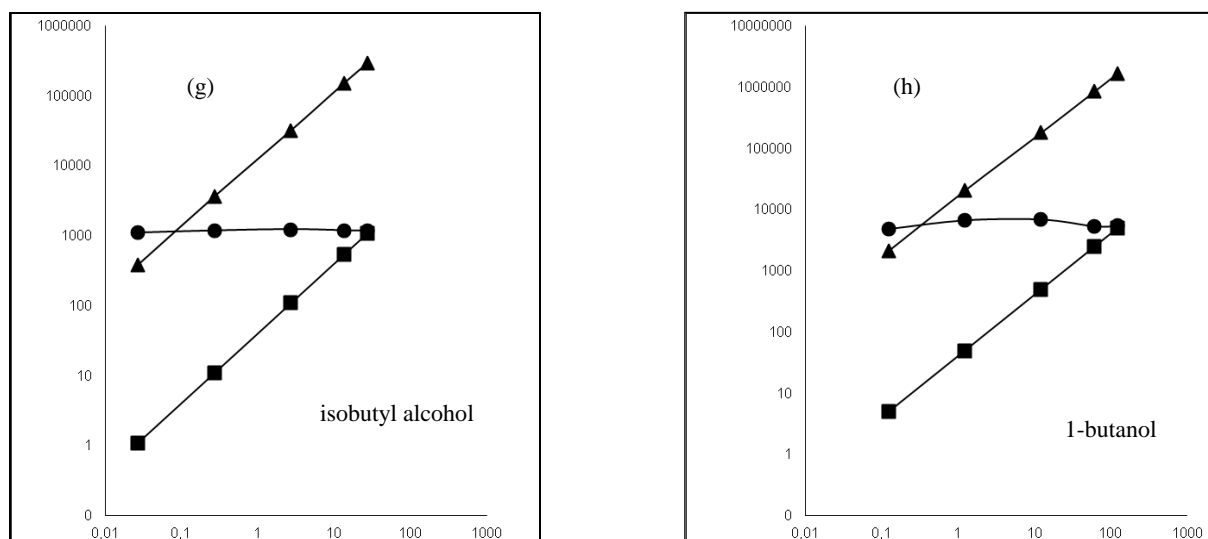


Figure 4. Chromatograms of standard solutions A-F from Table 2. 1 – acetaldehyde, 2 – methyl acetate, 3 – ethyl acetate, 4 – methanol, 5 – 2-propanol, 6 – ethanol, 7 – 1-propanol, 8 – isobutyl alcohol, 9 – n-butanol, 10 - isoamyl alcohol

Table 3. The experimental data from CL. The measured concentrations of analyzed volatile compounds and ethanol, presented according to the degree of dilution with water.

sample (dilution)	compound							
	acetaldehyde	methyl acetate	methanol	2-propanol	ethanol	1-propanol	isobutyl alcohol	n-butanol
A (No)	5170	5200	5291	5242	789300	7196	1171	5324
	4756	4784	4868	4823	726156	6620	1077	4898
	5405	5127	4840	5091	789300	7157	1157	5358
	4,5	-1,4	-8,5	-2,9	N/A	-0,5	-1,2	0,6
	119	120	122	121	18154	166	27	122
	262640	473048	551182	785344	117394309	1038326	289264	1635733,8
B (1:1)	5170	5200	5291	5242	789300	7196	1171	5324
	2378	2392	2434	2412	363078	3310	539	2449
	5231	5292	5288	5243	789300	7187	1174	5311
	1,2	1,8	-0,1	0,0	N/A	-0,1	0,2	-0,3
	59,5	59,8	60,8	60,3	9077,0	82,8	13,5	61,2
	131420,7	256031	309192	422395	60259800	534562	150959	831805
C (1:9)	5170	5200	5291	5242	789300	7196	1171	5324
	476	478	487	482	72616	662	108	490
	3936	6849	5107	9092	789300	5219	1216	6861
	-1,0	-1,8	0,8	-1,3	N/A	-0,5	-1,5	-0,8
	11,9	12,0	12,2	12,1	1815,4	16,6	2,7	12,2
	28419	55123	70780	89072	12827790	116146	31552	176935
D (1:99)	5170	5200	5291	5242	789300	7196	1171	5324
	47,6	47,8	48,7	48,2	7261,6	66,2	10,8	49,0
	3815	6781	4919	8321	789300	5075	1167	6648
	0,5	1,3	0,0	-0,2	N/A	0,1	0,0	-0,2
	1,19	1,20	1,22	1,21	181,54	1,66	0,27	1,22
	3267	6580	7903	9631	1499539	13284	3594	20158
E (1:999)	5170	5200	5291	5242	789300	7196	1171	5324
	4,76	4,79	4,87	4,83	726,88	6,63	1,08	4,90
	4474	5388	5233	4717	789300	6553	1090	4803
	-13,5	3,6	-1,1	-10,0	N/A	-8,9	-7,0	-9,8
	0,119	0,120	0,122	0,121	18,172	0,166	0,027	0,123
	0,119	0,120	0,122	0,121	18,172	0,166	0,027	0,123





● – concentration, mg/L (AA) ■ – concentration, mg/L (sol) ▲ – response x 10, pC, horizontal axis – amount, pg

Figure 5. Experimental results on demonstration of the following compounds: (a) – acetaldehyde, (b) – methyl acetate, (c) – methanol, (d) – 2-propanol, (e) – ethanol, (f) – 1-propanol, (g) – isobutyl alcohol, (h) – isoamyl acetate, (i) – 1-butanol. (The first line (circle marked) is concentration of the analysed compound expressed in mg per litre of absolute alcohol. The second line (triangle marked) and the third ones (square marked) are the detector response versus the amount of the compound and the concentration in mg per litre of solution, respectively.

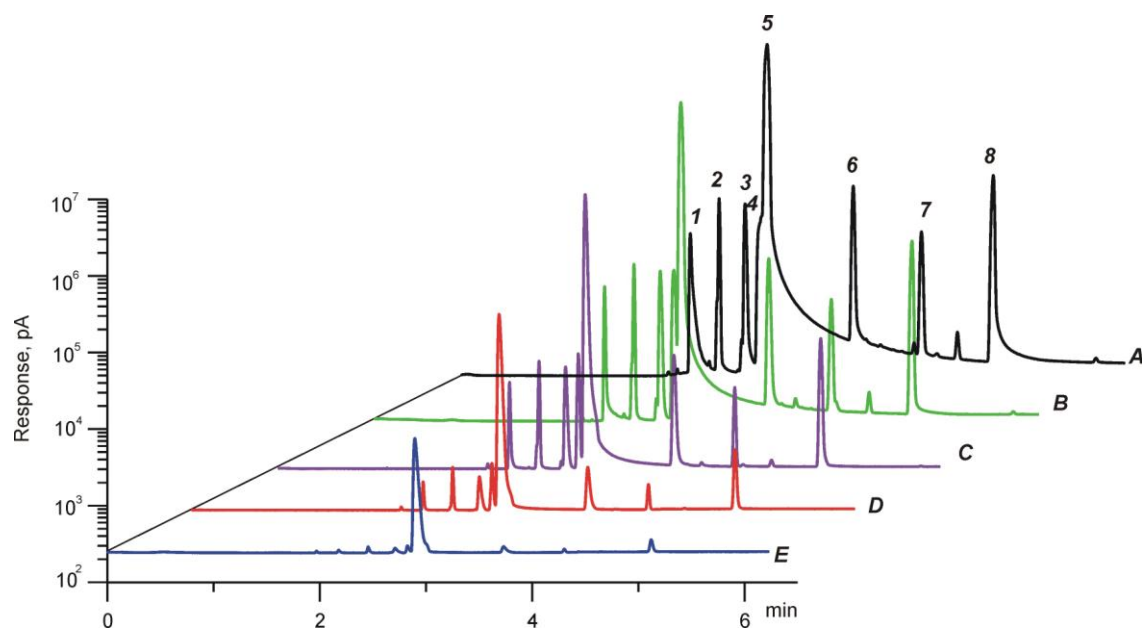


Figure 6. Chromatograms of standard solutions A-F from Table 3. 1 – acetaldehyde, 2 – methyl acetate, 3 – methanol, 4 – 2-propanol, 5 – ethanol, 6 – 1-propanol, 7 – isobutyl alcohol, 8 – 1-butanol.

It is important to note that at present time practically all manufactures produce GC with a wide linear dynamic range of FID and with high stable technical parameters. For illustration in the Table 4 are presented experimental coefficients RRF from different 13 laboratories. Coefficients RF for ethanol are presented in the bottom line. These coefficients RRF and RF were obtained during adjustment works of new GC in control laboratories of distillery plants.

Table 4. Values of coefficients RRF_i^{Et} and RF for ethanol from 13 different laboratories.

compound	RRF (relative to ethanol)													RRF average	RSD, %
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13		
acetaldehyde	1,114	1,532	1,584	1,403	1,506	1,636	1,524	1,672	1,596	1,622	1,301	1,627	1,648	1,52	10,6
methyl acetate	1,485	1,770	1,625	1,523	1,722	1,901	1,767	1,929	1,544	1,712	1,541	1,591	1,806	1,69	8,7
ethyl acetate	1,178	1,101	1,101	1,125	0,940	1,164	1,102	1,207	1,050	1,228	1,085	1,305	1,117	1,13	7,9
methanol	1,302	1,294	1,297	1,351	1,335	1,337	1,425	1,325	1,286	1,414	1,347	1,449	1,215	1,34	4,8
2-propanol	0,972	0,953	1,002	0,968	0,916	0,927	0,921	0,975	0,997	0,803	0,861	0,962	0,955	0,94	5,9
1-propanol	0,775	0,814	0,783	0,748	0,763	0,760	0,802	0,757	0,773	0,803	0,717	0,852	0,704	0,77	5,1
isobutanol	0,620	0,641	0,631	0,631	0,642	0,650	0,686	0,609	0,695	0,664	0,604	0,708	0,552	0,64	6,5
1-butanol	0,646	0,655	0,657	0,673	0,690	0,705	0,699	0,685	0,760	0,718	0,640	0,772	0,610	0,69	6,8
isoamylol	0,581	0,595	0,599	0,613	0,656	0,648	0,686	0,616	0,671	0,660	0,607	0,715	0,586	0,63	6,6
	response factor (RF), pC														
ethanol	144,67	85,21	91,39	172,88	102,35	67,36	142,48	79,23	42,47	79,37	8046,72	130,36	693,52	759,8	289,0
	RF average, pC														

Values RF are varied in wide range from minimum value 42,47 pC from Lab 9 to maximum, value 693,52 pC from Lab 13. At the same time the relative standard deviations for values RRF experimentally obtained in different 13 laboratories do not exceed 10,6 % for acetaldehyde and do not exceed 8,7 % for all other analyzed volatile compounds. This fact allows us to use averaged values RRF for the primary estimating measurements of volatile compounds in alcohol products without graduation procedure of GC.

Thousands of analytical and testing laboratories all over the world carry out gas chromatographic analysis of volatile compounds in spirit drinks every day. Their employees may validate proposed new method in actual practice, making sure its simplicity, accessibility and effectiveness in everyday practice. The obtained results show the possibility of developing a new international standard of measurement procedure, which will allow increase the data accuracy and will considerably simplify the measurement procedure.

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