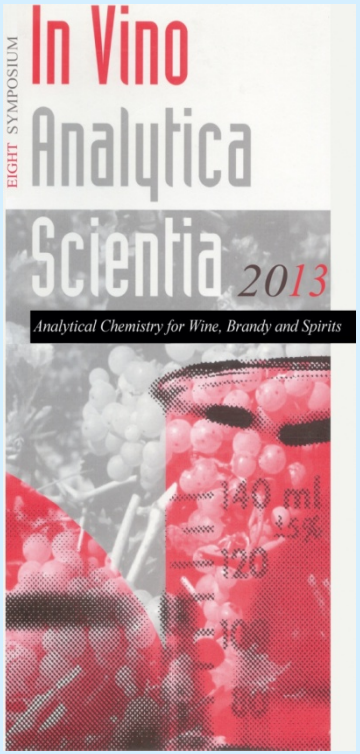

ETHANOL AS INTERNAL STANDARD FOR DETERMINATION OF VOLATILE COMPOUNDS IN SPIRIT DRINKS BY GAS CHROMATOGRAPHY

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BSU INP

Introduction

Over the world day-and-night according to the Official Methods the thousands accredited testing laboratories should determine the following 9 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 of absolute alcohol '**mg/L (AA)**'.

In accordance with Commission Regulation EC 2870-2000 and OIV (2009, v. 1-2) for quantitation the Internal Standard (**IS**) method is used. These documents propose to use pental-3-ol as IS.

Researchers from NIS make calculation by means of the External Standard (ES) method.

Finally, to get quantitative values of impurity concentrations in **mg/L (AA)** it is also required to measure alcohol strength (v/v concentration) of the analyzed sample.

Innovation

We propose the new methodical approach of using '**ethanol-as-IS'** in GC analysis of volatile compounds in spirit drinks in daily practice of analytical and testing laboratories**.**

We propose a significant simplification of the analysis. Namely, since **ethanol** is the main component in the alcohol products, we regard it as **IS** without introducing of any additional substances in the sample.

This method provides determination of volatile compound concentrations in spirit drinks expressed directly in **mg/L (AA)** without measuring the alcohol content in the analyzed sample.

The analysis of the experimental results show possibility of developing a **new international standard of measurement procedure**, which will allow increasing the data accuracy and considerably simplify the measurement procedure (*J. Agric. Food Chem.* ***2013****, 61, 2950-2956*).

Theoretical background

In our case the GC calibration includes measuring of relative detector response factors RRF_i for every analyzed compound relative to IS (**ethanol**).

RRF_i are calculated from the chromatographic data for standard solutions prepared by gravimetric method with known concentrations of analyzed compounds in **mg/L (AA)**. They may be expressed by the following equation:

$$RRF_i = RF_i / RF_{IS} = \frac{C_i^{st}(sol)}{A_i^{st}} / \frac{C_{IS}^{st}(sol)}{A_{IS}^{st}} = \frac{A_{IS}^{st} \cdot C_i^{st}(sol)}{A_i^{st} \cdot C_{IS}^{st}(sol)} = \frac{A_{IS}^{st} \cdot C_i^{st}}{A_i^{st} \cdot \rho_{Et}} \qquad (1)$$

where ρ_{Et} =789300 mg/L is the known density of ethanol.

Finally the concentration C_i of the *i*-th sample compound relative to **absolute alcohol** has the following form

$$C_i = RRF_i \cdot \frac{A_i}{A_{Et}} \cdot \rho_{Et} = C_i^{st} \cdot \frac{A_{Et}^{st}}{A_i^{st}} \cdot \frac{A_i}{A_{Et}} \qquad (2)$$

Validation. Gas Chromatographic conditions

There were a traditional GC conditions:

GC equipped with FID, a split/splitless injector;

- liquid autosampler;
- Unichrom software;
- capillary column Rt-Wax, 60 m x 0.53 mm, phase thickness 1 μm;
- initial isotherm at 75 °C (9 min), raised to 155 °C at rate 7 °C/min;
- with final isotherm of 155 °C (2.6 min);
- carrier gas was nitrogen;
- gas flow was 2.44 mL/min;
- injector volume 0.5 μL and split ratio 1:20.

Validation. Standard 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 8 volatile compounds reference ethanol-water solutions were gravimetrically prepared with known concentrations of volatile compounds.

- Validation of this method was been planed in accordance with ISO 5725.

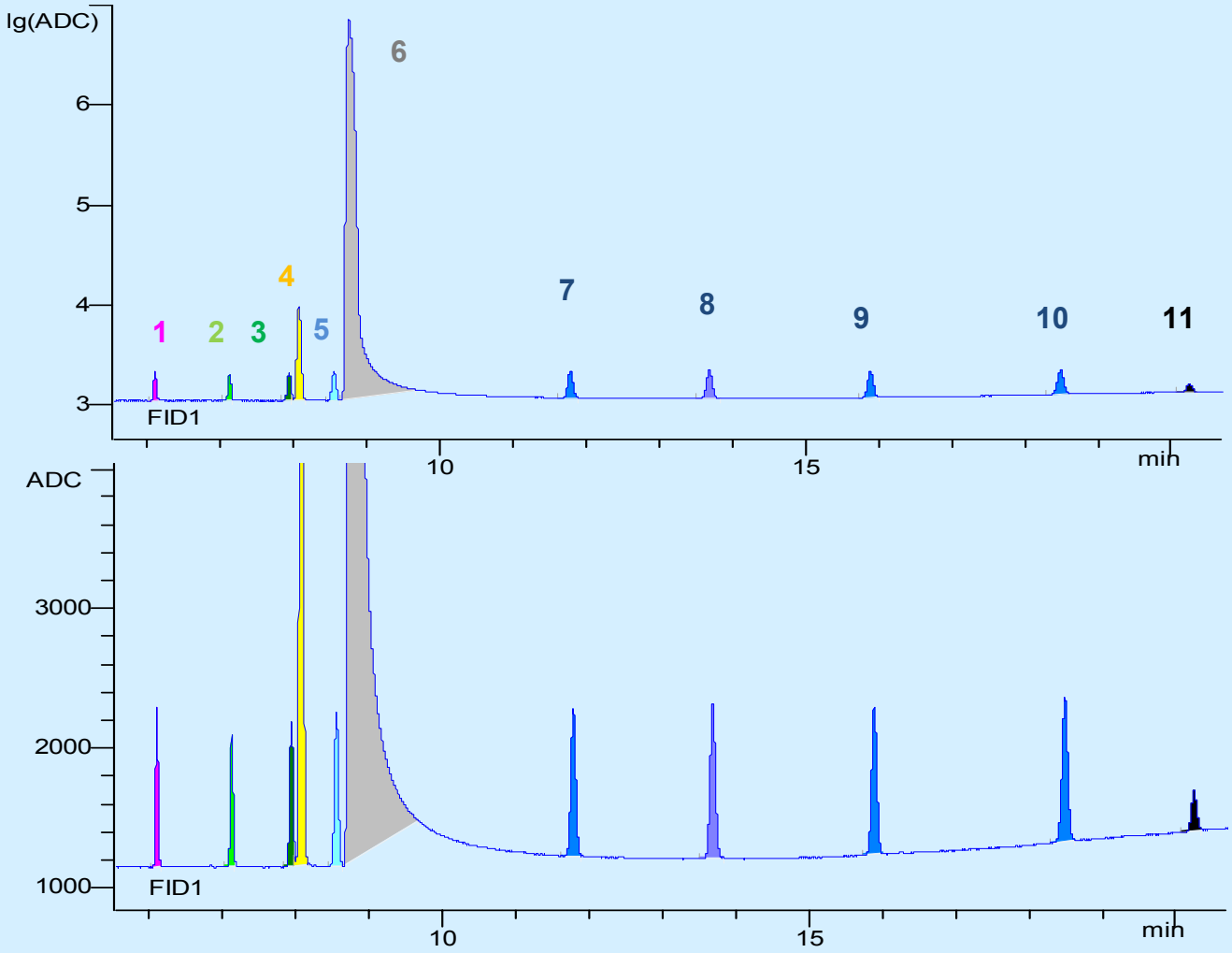
Every reference solution was injected 30 (15 x 2) times.

Validation. Characteristics of standard solutions

Table 1. Concentrations of analyzed volatile compounds are expressed in **mg/L (AA)**. 1-pentanol was introduced as traditional **IS**.

Compound	Concentration, mg/L (AA)							Relative error, % (P=0,95)
	VC-1	VC-2	VC-3	VC-4	VC-5	VC-6	VC-7	
acetaldehyde	4275	1096	111	56,2	11,2	2,22	1,13	± 3 %
methyl acetate	4397	1128	114	57,8	11,5	2,29	1,17	± 3 %
ethyl acetate	4173	1070	108	54,9	10,9	2,17	1,11	± 3 %
methanol	41995	10774	1092	555,5	113,3	24,96	14,3	± 3 %
2-propanol	3991	1025	105	54,1	12,1	3,69	2,70	± 3 %
1-propanol	4012	1029	104	52,8	10,5	2,08	1,06	± 3 %
isobutyl alcohol	3975	1020	103	52,3	10,4	2,06	1,05	± 3 %
n-butanol	4071	1044	106	53,5	10,7	2,11	1,08	± 3 %
isoamyl alcohol	4071	1044	106	53,5	10,7	2,11	1,08	± 3 %
1-pentanol (IS)	27,1	27,1	27,1	27,1	27,1	27,13	27,13	± 3 %

Validation. Chromatograms



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
11 – 1-pentanol (IS)

Fig.1. Typical chromatogram of standard ethanol-water (40% vs 60 %) solutions. To show the dominant component ethanol and another compounds synchronously the logarithm scale of response signal is chosen.

Validation. Response factors

Table 2. Analytical characteristics of the obtained calibration graphs

Compound	1-pentanol as IS		ES		Ethanol as IS		LOD* (mg/L)
	RRF	Correlation coefficient, R^2	RF (mg/L)/ (pA*min)	Correlation coefficient, R^2	RRF	Correlation coefficient R^2	
acetaldehyde	2,396	0,9997	266,1	0,9997	1,710	0,9997	0,344
methyl acetate	2,491	0,9997	276,7	0,9996	1,779	0,9999	0,683
ethyl acetate	1,757	0,9997	195,1	0,9997	1,254	0,9999	0,322
methanol	2,133	0,9998	236,9	0,9997	1,523	0,9999	0,231
2-propanol	1,400	0,9998	155,5	0,9997	0,999	0,9999	0,119
ethanol	1,413	N/A	155,5	N/A	1	N/A	N/A
1-propanol	1,179	0,9997	130,9	0,9996	0,841	0,9999	0,222
isobutyl alcohol	1,018	0,9998	113,0	0,9997	0,727	0,9999	0,178
n-butanol	1,117	0,9999	124,1	0,9998	0,798	0,9999	0,189
isoamyl alcohol	1,030	0,9999	114,4	0,9998	0,735	0,9999	0,179
1-pentanol	1	N/A	110,1	N/A	0,708	N/A	0,271

* limit of detection (LOD)

Validation. Linearity for methanol

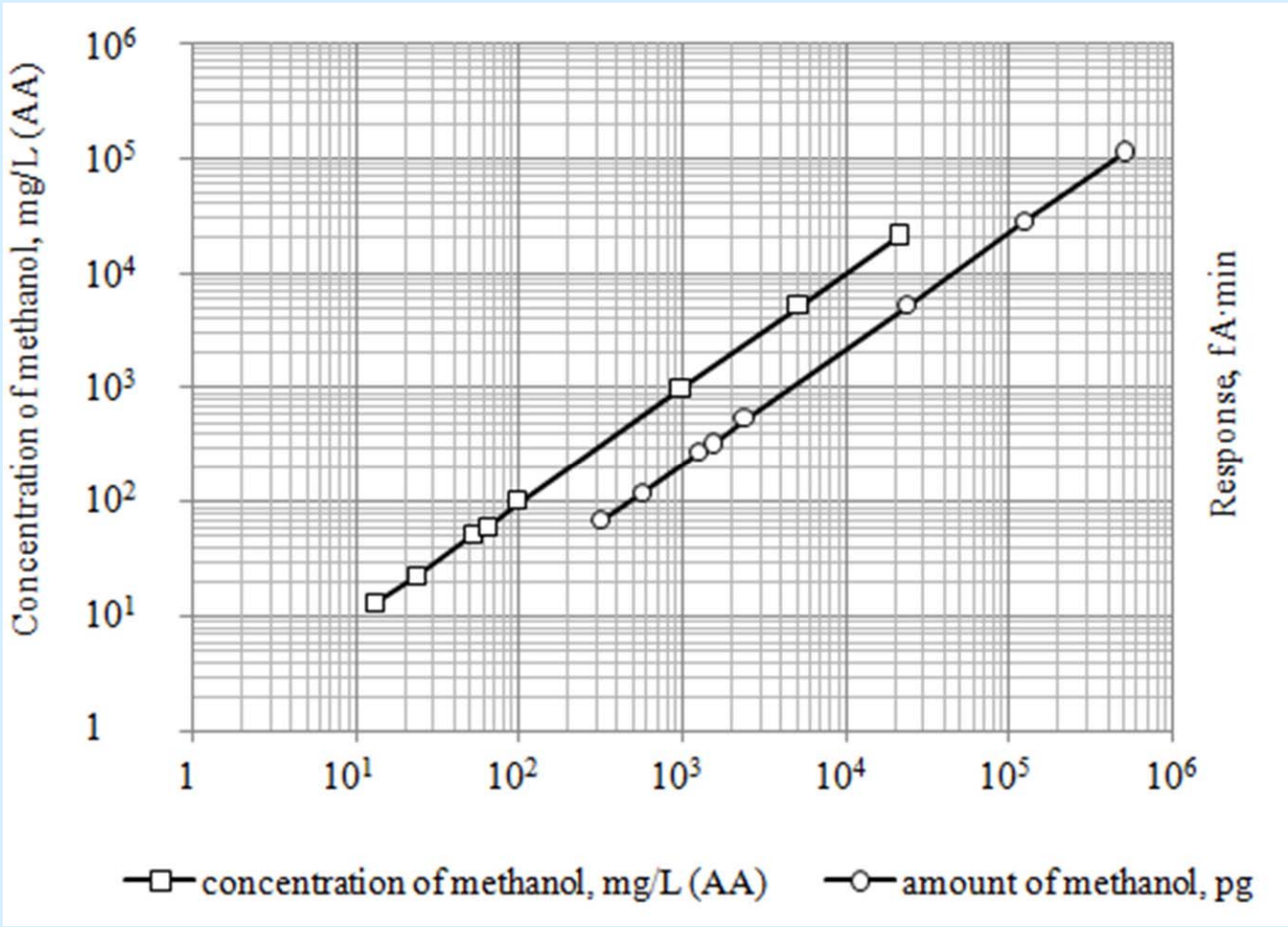


Fig. 2. Analytical characteristics of the obtained calibration graphs for methanol.

Validation. Linearity for all other 8 compounds

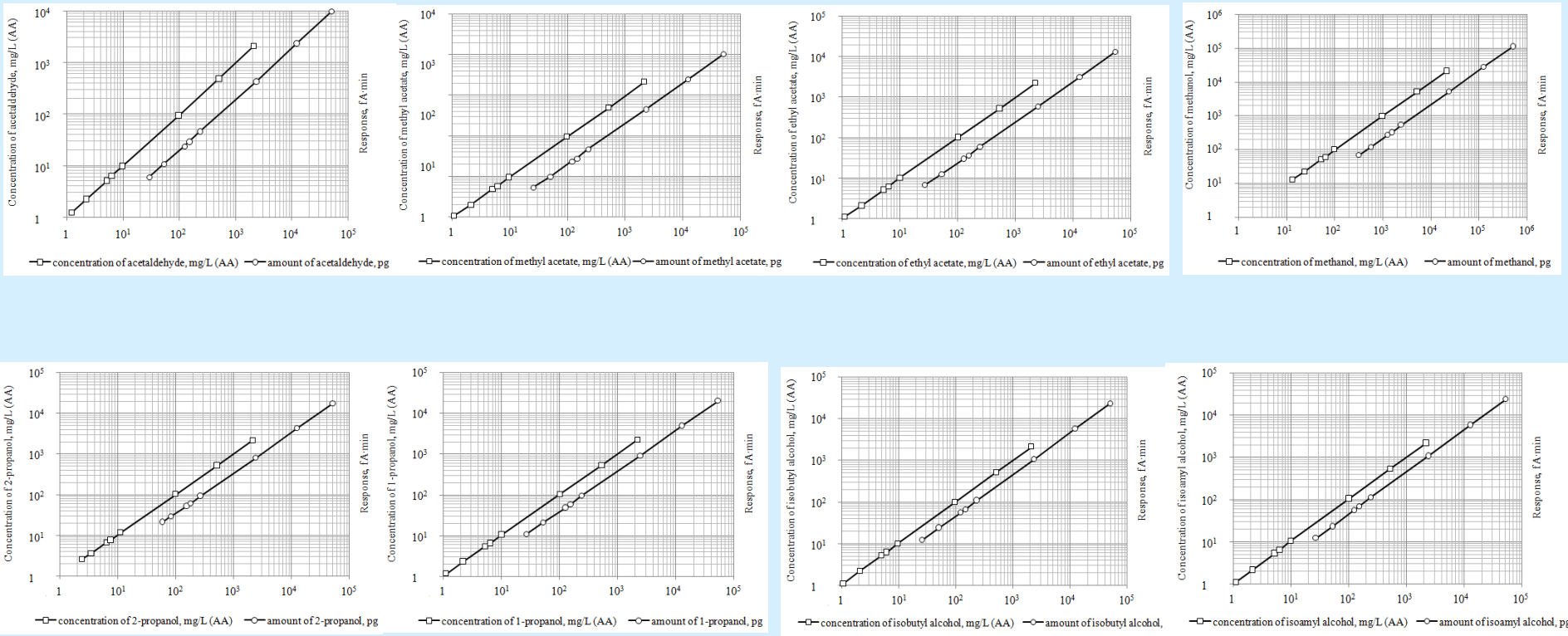


Fig. 3. Analytical characteristics of the obtained calibration graphs for **all other 8 compounds.**

Validation. Metrological characteristics of ‘ethanol-as-IS’ method

№ St Sol	acetaldehyde			methyl acetate			ethyl acetate			methanol			2-propanol			1-propanol			isobutyl alcohol			n-butanol			isoamyl alcohol		
	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %	C st, u(C), mg/l	C exp, S(TO), mg/l	Δ, u, %
1	1,23 0,024	1,23 0,07	-0,1 6,1	1,08 0,001	1,08 0,04	-0,5 3,5	1,13 0,002	1,10 0,05	-2,3 5,2	13,39 0,376	12,82 0,22	-4,2 5,4	2,45 0,20	2,55 0,07	4,0 9,6	1,13 0,001	1,15 0,07	1,8 6,3	1,08 0,001	1,08 0,05	-0,3 4,4	1,08 0,001	1,07 0,07	-0,7 6,3	1,12 0,001	1,08 0,06	-3,8 7,0
2	2,25 0,025	2,26 0,09	0,2 4,3	2,11 0,005	1,99 0,08	-5,4 6,7	2,20 0,005	2,08 0,07	-5,8 6,7	23,72 0,382	22,29 0,14	-6,0 6,3	3,50 0,20	3,58 0,10	2,2 6,8	2,21 0,005	2,30 0,10	3,8 5,8	2,11 0,005	2,17 0,09	2,6 5,3	2,11 0,005	2,19 0,13	4,0 7,5	2,19 0,005	2,11 0,13	-3,5 7,1
3	5,16 0,03	5,10 0,10	-1,0 2,2	5,04 0,01	4,90 0,08	-2,9 3,3	5,27 0,01	5,08 0,11	-3,5 4,1	53,15 0,38	51,28 0,19	-3,5 3,6	6,49 0,20	6,55 0,08	1,0 3,5	5,29 0,01	5,36 0,13	1,4 2,9	5,05 0,01	5,11 0,10	1,2 2,3	5,03 0,01	5,15 0,11	2,3 3,3	5,22 0,01	5,24 0,17	0,4 3,4
4	6,44 0,03	6,25 0,11	-2,9 3,4	6,34 0,01	5,75 0,11	-9,2 9,4	6,62 0,01	6,16 0,07	-7,0 7,0	66,17 0,38	59,71 1,01	-9,8 9,9	7,81 0,20	7,53 0,08	-3,5 4,5	6,65 0,01	6,41 0,16	-3,6 4,3	6,34 0,01	6,12 0,09	-3,6 3,9	6,32 0,01	6,14 0,12	-2,9 3,5	6,57 0,01	6,30 0,19	-4,0 5,0
5	9,75 0,03	9,81 0,31	0,7 3,4	9,68 0,01	9,56 0,26	-1,2 3,0	10,11 0,01	10,01 0,22	-1,0 2,4	99,70 0,39	99,78 0,55	0,1 0,7	11,21 0,20	11,50 0,10	2,7 3,4	10,15 0,01	10,51 0,16	3,5 3,8	9,69 0,01	9,98 0,13	3,1 3,4	9,66 0,01	10,07 0,12	4,3 4,5	10,03 0,01	10,38 0,13	3,5 3,7
6	96,65 0,15	94,06 1,44	-2,7 3,1	97,38 0,12	95,68 1,77	-1,7 2,6	101,8 0,13	101,1 1,57	-0,7 1,7	980,5 0,85	990,3 1,86	1,0 1,0	100,5 0,23	101,2 0,31	0,7 0,8	102,2 0,12	103,3 0,17	1,1 1,1	97,47 0,12	98,97 0,25	1,5 1,6	97,18 0,13	99,28 0,20	2,2 2,2	100,9 0,12	103,6 0,38	2,7 2,7
7	506,0 0,98	486,8 5,79	-3,8 4,0	510,5 0,90	491,6 12,5	-3,7 4,5	533,5 0,96	520,7 9,93	-2,4 3,1	5129 7,46	5124 11,8	-0,1 0,3	521,3 0,90	515,6 0,57	-1,1 1,1	535,6 0,90	530,8 0,55	-0,9 0,9	511,0 0,88	507,0 0,95	-0,8 0,8	509,5 0,93	506,0 1,82	-0,7 0,8	529,1 0,90	526,2 2,50	-0,5 0,7
8	2085 2,77	2080 20,9	-0,2 1,1	2104 2,25	2113 10,1	0,4 0,7	2198 2,46	2202 5,29	0,2 0,3	21128 11,05	21130 20,0	0,0 0,1	2144 2,08	2139 5,04	-0,2 0,3	2207 2,10	2206 2,28	-0,1 0,2	2106 2,14	2107 3,61	0,1 0,2	2099 2,43	2104 6,81	0,2 0,4	2180 2,12	2187 11,0	0,3 0,6

Table 3. The analysis of experimental data shows that the value of ***relative uncertainty u*** in the determination of the impurities concentration in experiments in the whole range of concentrations for all examined impurities ***does not exceed 10%***.

Validation. Method was certificated in Rosstandart

001340

ФЕДЕРАЛЬНОЕ АГЕНТСТВО
ПО ТЕХНИЧЕСКОМУ РЕГУЛИРОВАНИЮ И МЕТРОЛОГИИ
(Росстандарт)
Федеральное государственное унитарное предприятие
«Уральский научно-исследовательский институт метрологии»
(ФГУП «УНИИМ»)
Государственный научный метрологический институт

СВИДЕТЕЛЬСТВО
об аттестации методики (метода) измерений

№ 253.0169/01.00258/2013

Методика измерений массовой концентрации летучих компонентов в водке и спирте
наименование методики, включая наименование измеряемой величины, и, при необходимости,
этиловом методом газовой хроматографии
объекта измерений, дополнительных параметров и реализуемый способ измерений
предназначенная для измерений массовой концентрации летучих компонентов в водке и
область использования
спирте этиловом методом газовой хроматографии в лаборатории аналитических

исследований НИИ ЯП БГУ.

разработанная Научно-исследовательским учреждением "Институт ядерных проблем"
наименование и адрес организации (предприятия), разработавшей методику
Белорусского Государственного Университета (НИИ ЯП БГУ).

220030 Беларусь, г. Минск, ул. Бобруйская, д. 11

и содержащаяся в документе "Определение летучих компонентов в водке и спирте
обозначение и наименование документа, содержащего методику, год утверждения, число страниц
этиловом методом газовой хроматографии. Методика измерений"

Методика аттестована в соответствии с ФЗ № 102 "Об обеспечении единства измерений" и ГОСТ Р 8.563-2009.

Аттестация осуществлена по результатам метрологической экспертизы материалов по
теоретических и (или) экспериментальных исследований
разработке методики измерений и экспериментальных исследований

В результате аттестации методики измерений установлено, что методика измерений
нормативно-правовой документ в области обеспечения единства измерений (при наличии) и ГОСТ Р 8.563
соответствует требованиям, предъявляемым ГОСТ Р 8.563-2009

Показатели точности измерений приведены в приложении на 2 л.

Зам. директора по качеству **Ю.С.Бессонов**

Зав. лабораторией **Е.В. Осинцева**

Дата выдачи 12.07.2013

Рекомендуемый срок пересмотра методики измерений: 12.07.2018

М.П.

Россия, 620000, г. Екатеринбург, ул. Красноармейская, 4
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МЕТРОН

013400

Federal Agency
for Technical Regulation and Metrology
(Rosstandart)
The Federal State Unitary Enterprise
"Ural Scientific-Research Institute of Metrology"
(Federal State Unitary Enterprise "UNIIM")
State Scientific Institute of Metrology

CERTIFICATE
of certification procedure (method)

№ 253,0169/01.00258/2013

Method of measurement of the mass concentration of volatile compounds in alcohol drinks
name of method, including the name of the measured values and, if appropriate, measurement object,
by Gas Chromatography
additional parameters and implemented method for measuring
designed for measuring of the mass concentration of volatile components in alcohol drinks by
are of use
gas chromatography in the Laboratory of Analytical Research of INP BSU.

developed by the Research Institute "Institute of Nuclear Problems" of Belarusian State University (INP BSU)
name and address of the organization (enterprise) developed the method

220030 Belarus, city of Minsk, Bobruiskaya Str., 11

and contained in the document "Determination of volatile compounds in alcohol drinks by gas chromatography"
designation and name of the document containing the method, year of approval, number of pages

Method is certified in accordance with the Federal Law № 102 "On ensuring the uniformity of measurements" and GOST R 8.563-2009.
Certification carried out on the results of metrological examination of theoretical and experimental materials
development of measurement techniques and (or) experimental studies
As a result of evaluation of the measurement procedure is established that the method meets the
legal document in the area of traceability (if available) and GOST R 8.563
requirements of GOST R 8.563-2009.

Performance measurement accuracy are given in Appendix on 2 pages.

Deputy Director — Yu.S.Bessonov

Head of Laboratory — E.V.Osintseva

Date of issue — 12.07.2013

Recommended for revision of the measurement procedure — 12.07.2018

Russia, 620000, City of Ekaterinburg, Krasnoarmeiskaya Str., 4
Tel.: (343) 350-26-18, fax: (343) 350-20-39. E-mail: uniim@uniim.ru

Prospection

How to introduce this new method '**ethanol-as-IS**' in the daily practice ?

There is only one working way: to propose for customers more easy, attractive and effective way for laboratory business..

Thousands of testing laboratories over the world day-and-night carry out gas chromatographic analysis of volatile compounds in spirit drinks.

They may test this new method in their real practice. It is important to note that there is no need to perform any additional measurements.

Everybody could test and validate '**ethanol-as-IS**' method while performing current measurements with existing instrumentation and calculations could be done in parallel according to the traditional way with addition of 'pental-3-ol' as IS and using '**ethanol-as-IS**'.

Road map. Detailed description of ‘ethanol-as-IS’ in Internet

Ethanol as Internal Standa

unichrom.com/vodka/ethanol-solutione.shtml

::: UniChrom

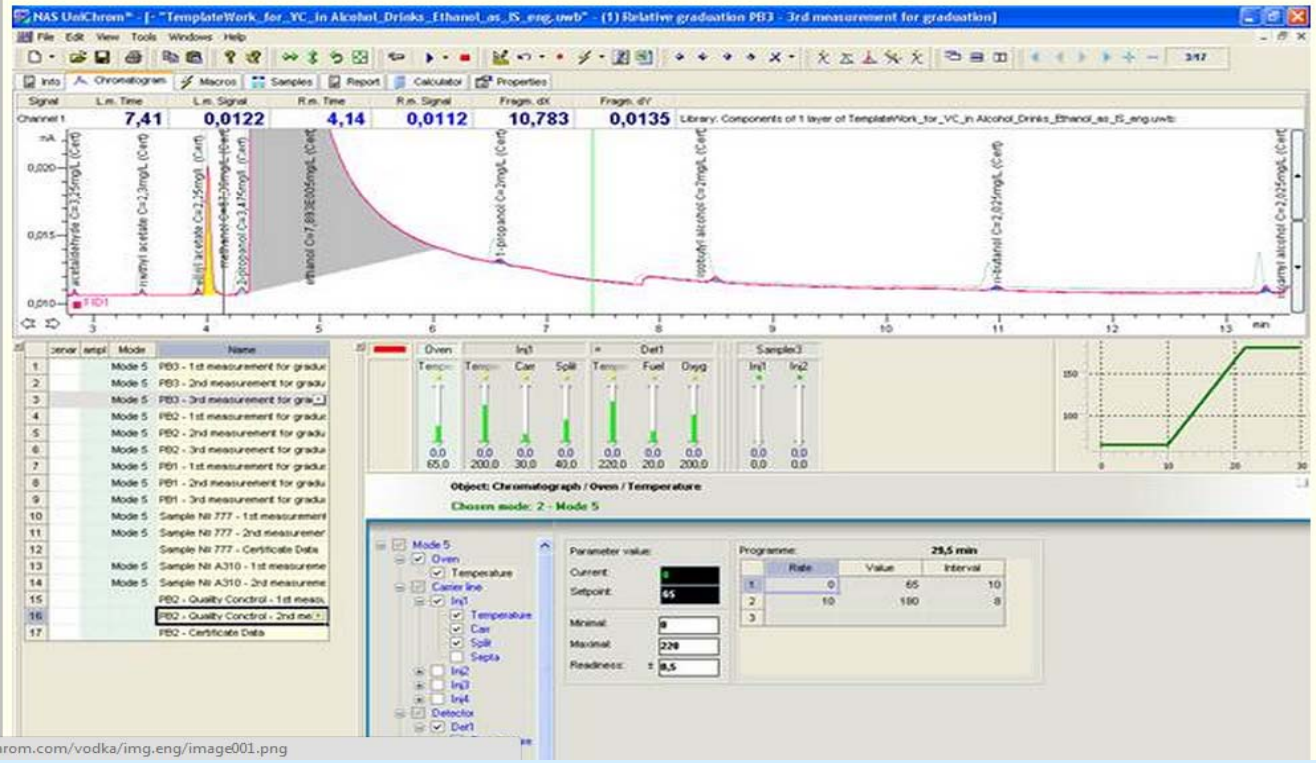
BEL RUS DEU ENG

Main Page / Products / Solutions / Ethanol as Internal Standard for Determination of Volatile Compounds in Alcohol Drinks by Gas Chromatography with UniChrom Datasystem

< Back

Ethanol as Internal Standard for Determination of Volatile Compounds in Alcohol Drinks by Gas Chromatography

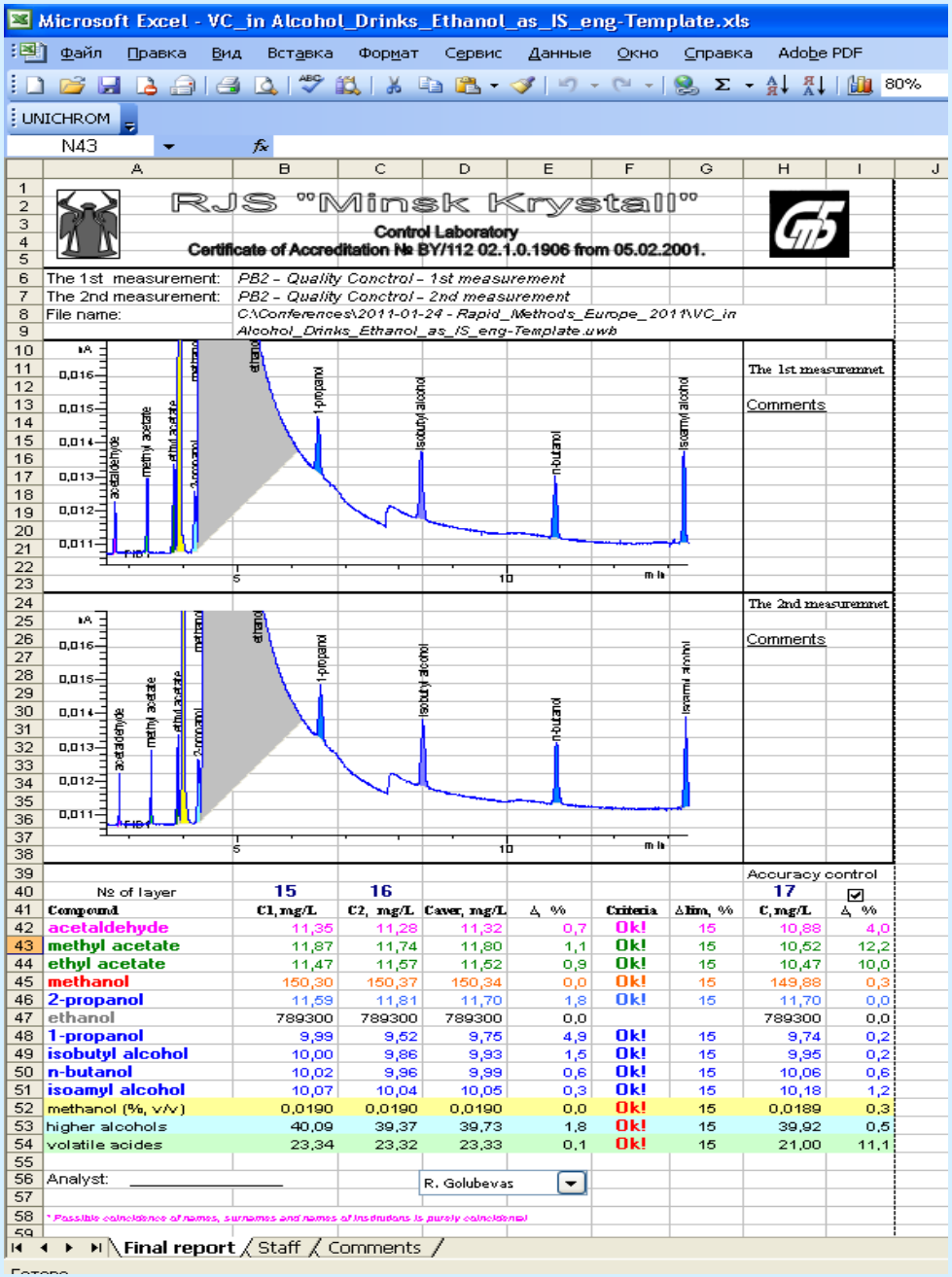
There are the following prepared templates TemplateWork_for_VC_in Alcohol_Drinks_Ethanol_as_IS_eng.uwb and TemplateReport_for_VC_in Alcohol_Drinks_Ethanol_as_IS_eng.xls for illustration how this method is working.



unichrom.com/vodka/img.eng/image001.png

Detailed description is here: www.unichrom.com/vodka/ethanol-solutione.shtml

Road map. Unification of final report generation

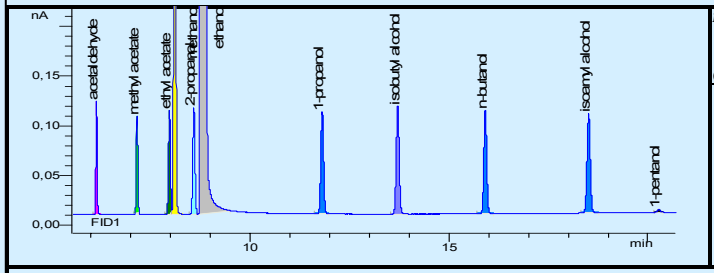


Generation of final report of any official documents with help of OLE Automation technology.

Road map. Traditional ‘1-pentanol-as-IS’ and ‘ethanol-as-IS’

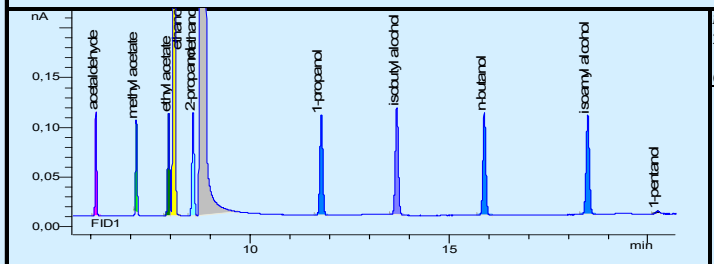
Bobruisk Hydrolysis Plant
Control Laboratory
Certificate of Accreditation № BY/112 02.2.0.2948 to 29.12.2014.
Chekhova Str., 54, Bobruisk, 213827, Mogilev region, Belarus

The 1st mesurement: *SS-6 (1000 mg/L AA) - 1st measurement*
The 2nd measurement: *SS-6 (1000 mg/L AA) - 2nd measurement*
File name: *\\Black\documents\IS_and_ES_and_Ethanol-as-ISTD_rus\IS_and_ES_and_Ethanol-as-ISTD.uwb*



Ethanol_as_ISTD
1st measurement

Comments:



Ethanol_as_ISTD
2nd measurement

Comments:

				Reproducibility			Trueness				
No of layer	3	4					5				
Compound	C1, mg/L	C2, mg/L	Caver, mg/L	Δ, %	Criteria	Δ lim, %	C,mg/L	Δ, %	Criteria	Δ lim, %	
acetaldehyde	1163,04	1162,25	1162,64	0,1	Ok!	10	1096,40	6,0	Ok!	10	
methyl acetate	1224,67	1122,11	1173,39	8,7	Ok!	10	1127,70	4,1	Ok!	10	
ethyl acetate	1144,40	1142,64	1143,52	0,2	Ok!	10	1070,30	6,8	Ok!	10	
methanol	11408,93	11382,99	11395,96	0,2	Ok!	10	10774,00	5,8	Ok!	10	
2-propanol	1066,65	1064,46	1065,56	0,2	Ok!	10	1025,00	4,0	Ok!	10	
ethanol	789300	789300	789300	0,0	Ok!		789300	0,0	Ok!		
1-propanol	1063,86	1063,16	1063,51	0,1	Ok!	10	1029,00	3,4	Ok!	10	
isobutyl alcohol	1063,98	1059,30	1061,64	0,4	Ok!	10	1019,60	4,1	Ok!	10	
n-butanol	1090,73	1092,01	1091,37	0,1	Ok!	10	1044,20	4,5	Ok!	10	
isoamyl alcohol	1088,60	1092,13	1090,36	0,3	Ok!	10	1044,20	4,4	Ok!	10	
methanol (%, v/v)	1,4391	1,4358	1,4374	0,2	Ok!	10	1,3590	5,8	Ok!	10	
higher alcohols	5373,83	5371,05	5372,44	0,1	Ok!	10	5162,00	4,1	Ok!	10	
volatile acides	2369,07	2264,76	2316,91	4,5	Ok!	10	2198,00	5,4	Ok!	10	
1-pentanol	28,27	28,15	28,21	0,4	Ok!	10	27,13	4,0	Ok!	10	

Analyst _____ *Makoed S.F*

Head of Lab _____ *Leskovets O.P.*

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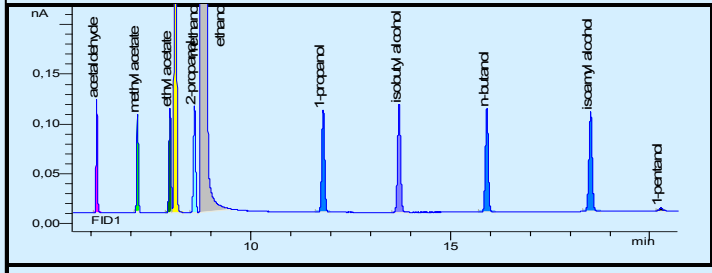
Bobruisk Hydrolysis Plant
Control Laboratory
Certificate of Accreditation № BY/112 02.2.0.2948 to 29.12.2014.
Chekhova Str., 54, Bobruisk, 213827, Mogilev region, Belarus

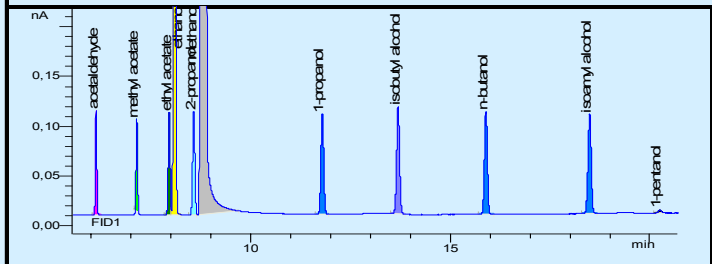
The 1st mesurement: *SS-6 (1000 mg/L AA) - 1st measurement*
The 2nd measurement: *SS-6 (1000 mg/L AA) - 2nd measurement*
File name: *\\Black\documents\IS_and_ES_and_Ethanol-as-ISTD_rus\IS_and_ES_and_Ethanol-as-ISTD.uwb*



Pentanol_as_ISTD
1st measurement

Comments:

Ethanol, % (v/v)
96



Pentanol_as_ISTD
2nd measurement

Comments:

Ethanol, % (v/v)
96

				Reproducibility			Trueness				
No of layer	3	4					5				
Compound	C1, mg/L	C2, mg/L	Caver, mg/L	Δ, %	Criteria	Δ lim, %	C,mg/L	Δ, %	Criteria	Δ lim, %	
acetaldehyde	1152,69	1156,53	1154,61	0,3	Ok!	10	1096,40	5,3	Ok!	10	
methyl acetate	1213,60	1116,43	1165,02	8,3	Ok!	10	1127,70	3,3	Ok!	10	
ethyl acetate	1133,99	1136,79	1135,39	0,2	Ok!	10	1070,30	6,1	Ok!	10	
methanol	11305,46	11324,98	11315,22	0,2	Ok!	10	10774,00	5,0	Ok!	10	
2-propanol	1014,75	1059,09	1036,92	4,3	Ok!	10	1025,00	1,2	Ok!	10	
ethanol	#ССЫЛКА!	789300	#ССЫЛКА!	########	#ССЫЛКА!		789300	#ССЫЛКА!	#ССЫЛКА!		
1-propanol	1011,99	1057,68	1034,84	4,4	Ok!	10	1029,00	0,6	Ok!	10	
isobutyl alcohol	1012,14	1011,73	1011,94	0,0	Ok!	10	1019,60	-0,8	Ok!	10	
n-butanol	1037,69	1086,53	1062,11	4,6	Ok!	10	1044,20	1,7	Ok!	10	
isoamyl alcohol	1035,71	1086,69	1061,20	4,8	Ok!	10	1044,20	1,6	Ok!	10	
methanol (%, v/v)	1,4260	1,4285	1,4272	0,2	Ok!	10	1,3590	5,0	Ok!	10	
higher alcohols	5112,28	5301,73	5207,01	3,6	Ok!	10	5162,00	0,9	Ok!	15	
volatile acides	2347,60	2253,23	2300,41	4,1	Ok!	10	2198,00	4,7	Ok!	15	
1-pentanol	27,13	27,13	27,13	0,0	Ok!	10	27,13	0,0	Ok!	10	
1-pentanol	0,25	0,25									

Analyst _____ *Makoed S.F*

Head of Lab _____ *Leskovets O.P.*

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Generation in parallel two reports: traditional ‘**1-pentanol-as-IS**’ and ‘**ethanol-as-IS**’