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BELARUSIAN STATE UNIVERSITY
CHEMISTRY FACULTY
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QUANTITATIVE DETERMINATION OF VOLATILE COMPOUNDS IN ETHANOL OF PHARMACEUTICAL PREPARATIONS. METHOD VALIDATION

Master's thesis

specialty 1-31 80 06 «Chemistry for drug substances»

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Permitted to defend

«___»_____2022

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Doctor of Chemical Sciences

Minsk, 2022

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GENERAL DESCRIPTION OF WORK

ETHYL ALCOHOL, PHARMACOPEA, VOLATILE COMPOUNDS, METHANOL, ALDEHYDES, FUSEL OIL, GAS CHROMATOGRAPHY, INTERNAL STANDARD

The research, the results of which were included in the master's thesis, was carried out at the Institute of Nuclear Problems of the Belarusian State University within the framework of research work 3.4.04: «Development of new methods for precision determination of the qualitative and quantitative composition of a wide range of multicomponent matrices for biotechnology, including the pharmaceutical and food industries» (Belarusian State Program of Scientific Research «Convergence 2025» subprogram «Integration»).

The aim of this study was validation a modified internal standard method for analysis of rectified ethyl alcohol; to improve existing official (pharmacopeial) method of analysis of rectified ethyl alcohol.

The objects of the study are water-ethanol solutions (with ethanol volume concentration 96 %) of mixtures of volatile compounds: acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol.

Main results:

1. The method for direct determination of 9 volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol) in ethyl alcohol for pharmaceutical uses was developed and successfully validated.

2. The relative expended uncertainty of developed method did not exceed 6.1 %.

3. The method can be used for analysis other pharmaceutical substances containing ethyl alcohol.

The master's thesis consists of an introduction, a general description of the work, 3 chapters, a conclusion, 1 appendix and a bibliographic list.

Chapter 1 provides an analytical review of the literature on the subject of the thesis. Chapter 2 describes experiment, which was carried out during the study (preparation of solutions, conditions of gas chromatographic analysis and validation study). Chapter 3 presents results of validation study of the developed method.

The full text of the master's thesis is 43 pages, including 14 figures on 12 pages and 15 tables on 11 pages. The bibliographic list includes 30 titles on 3 pages.

INTRODUCTION

Ethyl alcohol (ethanol) is widely used in medicine and pharmaceutical production. In medical practice, ethyl alcohol is used mainly as an external antiseptic, disinfectant (active against viruses, gram-positive and gram-negative bacteria) and as a local irritant; Abroad, ethyl alcohol as an active ingredient is part of injectable preparations, it is used intravenously in acute methanol poisoning. Ethyl alcohol in various concentrations is widely used for the production and manufacture of tinctures, extracts and dosage forms for external use and it is also used as a solvent [1].

The most important indicators of the quality of alcohol include density, clarity, colour, acidity or alkalinity, absorbance, the content of chlorides, sulphates, heavy metals, methanol, aldehydes (acetaldehyde and 1,1-diethoxyethane), esters (methyl acetate and ethyl acetate), fusel oil (propan-1-ol, propan-2-ol, 2-methylpropan-1-ol, butan-1-ol and 3-methylbutan-1-ol), furfural, reducing substances, nonvolatile compounds, benzene and etc [2-6].

This study examines in detail such quality parameters of ethyl alcohol for pharmaceutical purposes as the content of organic volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol). The determination of these volatile compounds in ethanol is carried out predominantly by gas chromatography with a flame ionization detector (GC-FID) [2-6]. For quantitative calculations, methods such as the method of internal standard, the method of external standard, the method of additions are used.

The method of an internal standard has such disadvantages as the added error due to the pipetting of the internal standard, the inconvenience of the additional counting time required and the contamination of the sample by the internal standard.

The method of an external standard has such disadvantages as great dependence on the stability of the chromatographic detector system and the presence of matrix effects.

The method of standard additions has all disadvantages of internal and external standard methods (except matrix effect, cause this method often used for evaluation of matrix effect).

The scientists of laboratory of analytical research of Institute for Nuclear problems of Belarussian State University, department of an Analytical Chemistry of Belarussian State University and department of Physical-Chemical Methods of Products Certification of Belarusian State Technological University developed approach for the determination of volatile compounds in alcohol products, which is based on ethanol usage as internal standard (modified internal standard method) [7, 8].

The aims of this study were to validate a modified internal standard method for analysis of rectified ethyl alcohol and approbate for analysis of other pharmaceutical substances containing ethyl alcohol.

CHAPTER 1
LITERATURE REVIEW

1.1 The most common rectified ethyl alcohol contaminants

The qualitative and quantitative composition of ethyl alcohol from food raw materials depends on a large number of factors associated with the chosen technology for its production, storage and transportation of the final product [9-15].

Distinguish ethyl alcohol technical (hydrolytic), synthetic, food and pharmacopeial. Each of these species has its own quality standards [1].

The pharmaceutical industry uses rectified ethyl alcohol of the «Extra», «Lux» or «Alpha» brands, which has the lowest content of toxic impurities, obtained from food raw materials. Alcohol of the brand «Lux» is made from various types of grain, «Extra» – from various types of grain, a mixture of grain and potatoes, «Alpha» – from wheat, rye or a mixture thereof [1].

Analysis of literature data [9-15] showed that over 70 volatile substances can be present in raw alcohol, most of which are formed during the fermentation process, some are introduced with the feedstock, some are formed during distillation. The present volatile impurities are alcohols, carboxylic acids (the main representative is acetic acid), ethers (mainly diethyl ether and ethyl acetate), water, carbon dioxide, furfural, acetals (mainly 1,1-diethoxyethane, aldehydes (acetaldehyde, etc.). In the case with the presence of methyl alcohol and atomic oxygen in a mature mash or its distillation products, formaldehyde can be formed [1].

Volatile organic substances that are released from the mash during its distillation and have boiling points higher than those of ethyl alcohol, giving a cloudy (gray) shade to the water-alcohol solution, are referred to as a fusel oil. Fusel oil are isoamyl, 2-methylpropan-1-ol and propan-1-ol. The remaining compounds of fusel oil, acetic and other carboxylic acids, furfural and esters are present in significantly smaller amounts. The composition of fusel oil depends on the feedstock and its quality [1]. Compared to raw alcohol from grain crops and potatoes, raw alcohol from sugar beets contains relatively high amounts of propam-1-ol and 2-methylpropan-1-ol. Purification of raw alcohol by rectification from propam-1-ol and 2-methylpropan-1-ol is much more difficult than purification from 3-methylbutan-1-ol. For this reason, sugar beet is practically not used in the industrial production of pharmacopeial-quality ethyl alcohol. Most of the secondary and by-products of alcoholic fermentation have a harmful effect on the human body, and therefore the residual amount and composition of impurities affect the quality of rectified alcohol. Many of them not only worsen the organoleptic qualities of ethyl alcohol, but are also potent poisons. In particular, methyl alcohol and furfural are more than 80 times more toxic, propan-1-ol – 4 times, 2-methylpropan-1-ol – 8 times, 3-methylbutan-1-ol – 19 times more toxic than ethyl alcohol. Methanol is especially dangerous, the largest quantities of which are found in sugar beet mash, some fruits and berries. There is less methyl alcohol in a mature potato mash, much less in a cereal mash, and methanol is completely absent in a sugar mash saponify esters and convert them into salts of volatile acids [1].

The chemical structures of most common contaminants of ethyl alcohol from food raw materials are shown in Figure 1.1.

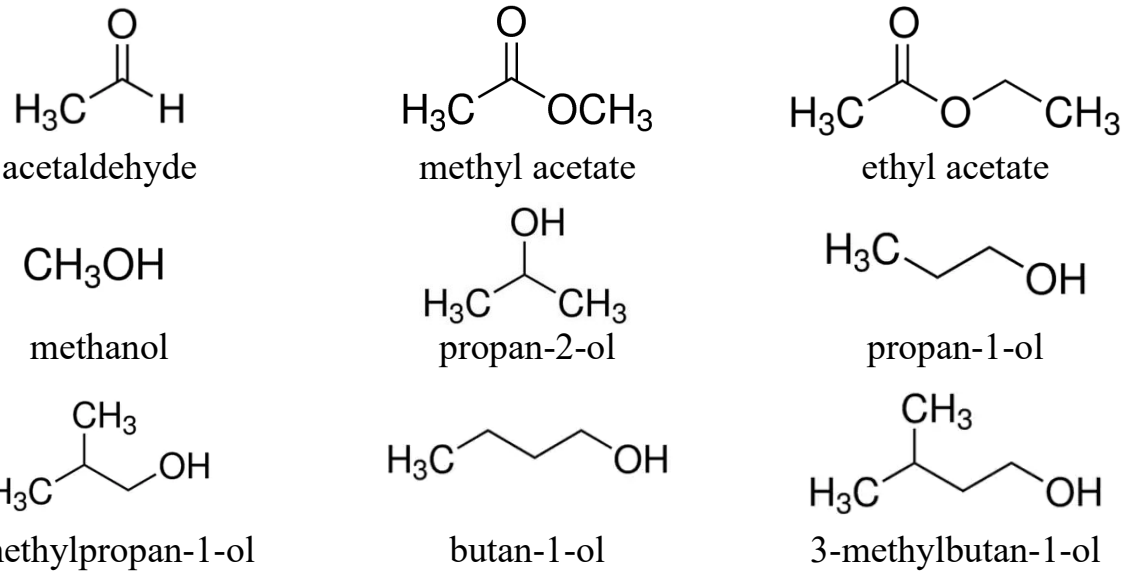


Figure 1.1 – The chemical structures of most common contaminants of ethyl alcohol from food raw materials

It is very important to use for pharmacopeial purposes alcohol produced from food raw materials. However, ethyl alcohol can be adulterated and have a synthetic origin.

In accordance with currently available technologies, synthetic ethyl alcohol is prepared by the hydration of ethylene. In turn, ethylene is prepared by the pyrolysis of oil gases, oil processing gases, naphtha, and petroleum gas oil. In the pyrolysis, propylene and butenes, in particular, butylene, are the main impurity compounds concomitant with ethylene. The hydration of these concomitant compounds results in the formation of propan-2-ol and butan-2-ol as the impurity compounds of synthetic alcohol [9, 16]. It is believed that acetone, which is present in synthetic alcohols, is formed by the partial oxidation of isopropanol the above impurity compounds in more detail. Thus, the acetone can be a characteristic of synthetic ethyl alcohol [17], but it can also occur in food and hydrolysis ethyl alcohols.

Also, undesirable compounds of ethyl alcohol include 2-butanone (methyl ethyl ketone), furfural, crotonaldehyde and etc. The chemical structures of some undesirable contaminants of ethyl alcohol are shown in Figure 1.2.

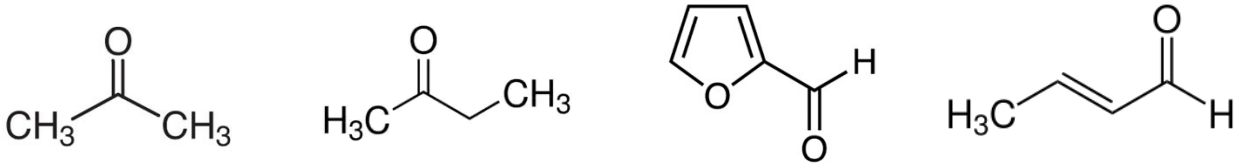


Figure 1.2 – The chemical structures of some undesirable contaminants of ethyl alcohol

1.2 World practice of a quality control of rectified ethyl alcohol

1.2.1 European Union and the United States of America

In European and United States Pharmacopeias the volatile compounds under consideration refer to the quality index «Volatile impurities» in case with European Pharmacopeia [2] and to «Organic Impurities» in case with United States Pharmacopeia [3].

Both Pharmacopoeias regulate the content of such volatile compounds as acetaldehyde (sum of acetaldehyde and acetal), benzene, methanol and other impurities (table 1.1).

Table 1.1 – Quality requirements for ethyl alcohol in European and United States Pharmacopeias

Compound	Limits, ppm v/v	Method of determination
Acetaldehyde (sum of acetaldehyde and acetal)	10	Standard addition method
Methanol	0.02	Standard addition method
Benzene	2	Standard addition method
Other volatiles	300	Internal standard method

Thus, both European and United States Pharmacopoeias require the preparation of at least 4 reference solutions, 1 test solution with internal standard compounds and 3 solutions with standard additions of each analyte separately.

In the European Union, there is another document regulating the content of volatile compounds in ethyl alcohol of agricultural origin, which can be used for pharmaceutical purposes – European Union Regulation (EC) No 110/2008 [4].

In accordance with this document, the content of such volatile compounds as methanol, ethyl acetate, acetaldehyde, 2-methylpropan-1-ol and furfural (table 1.2)

Table 1.2 – Quality requirements for ethyl alcohol in European Union Regulation (EC) No 110/2008

Compound	Limits, g/hL of 100 % vol. alcohol	Method of determination
Acetaldehyde	0.5	Internal standard method
Ethyl acetate	1.3	Internal standard method
2-methylpropan-1-ol	0.5	Internal standard method
Methanol	30	Internal standard method

1.2.2 Republic of Belarus

In the State Pharmacopeia of Republic of Belarus [5], the procedure of the determination of volatile compounds (acetaldehyde (sum of acetaldehyde and acetal), benzene, methanol and other impurities) is the same, as described in European and United States Pharmacopeias [2, 3]. However, the State Pharmacopeia of Republic of Belarus [5] separately describes the method of analysis of volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol) in ethyl alcohol (table 1.3).

Table 1.3 – Quality requirements for ethyl alcohol in State Pharmacopeia of Republic of Belarus

Compound	Limits, mg/L of 100 % vol. alcohol	Method of determination
Acetaldehyde	2	External standard method
Methanol	240	External standard method
Esters (methyl acetate and ethyl acetate)	10	External standard method
Fusel oil (sum of propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol)	6	External standard method

This method is based on the use of certified standard materials of ethyl alcohol solution in 3 levels of concentration and the external standard method is used for calculations.

1.2.3 Russian Federation

In the State Pharmacopeia of Russian Federation [6] described the method of analysis of volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol) in ethyl alcohol (table 1.4).

Table 1.4 – Quality requirements for ethyl alcohol in State Pharmacopeia of Russian Federation

Compound	Limits, mg/L of 100 % vol. alcohol	Method of determination
Acetaldehyde	2	External standard method
Methanol	160	External standard method
Esters (methyl acetate and ethyl acetate)	10	External standard method
Fusel oil (sum of propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol)	5	External standard method

This method is based on the use of certified standard materials of ethyl alcohol solution in 3 levels of concentration and the external standard method is used for calculations.

1.3 Methods of determination of volatile compounds in ethyl alcohol

1.3.1 Standard addition method

The standard addition method analyzes an unknown sample and the same unknown sample spiked with a known amount of target compound, then uses the difference between detected peak areas (peak height) to determine quantity. This quantitative method is often used to analyze samples containing a target compound affected by the concentration of other compounds in the sample, such as odor compound analysis and headspace analysis [18, 19].

The illustration of principles of standard addition method is shown in Figure 1.3.

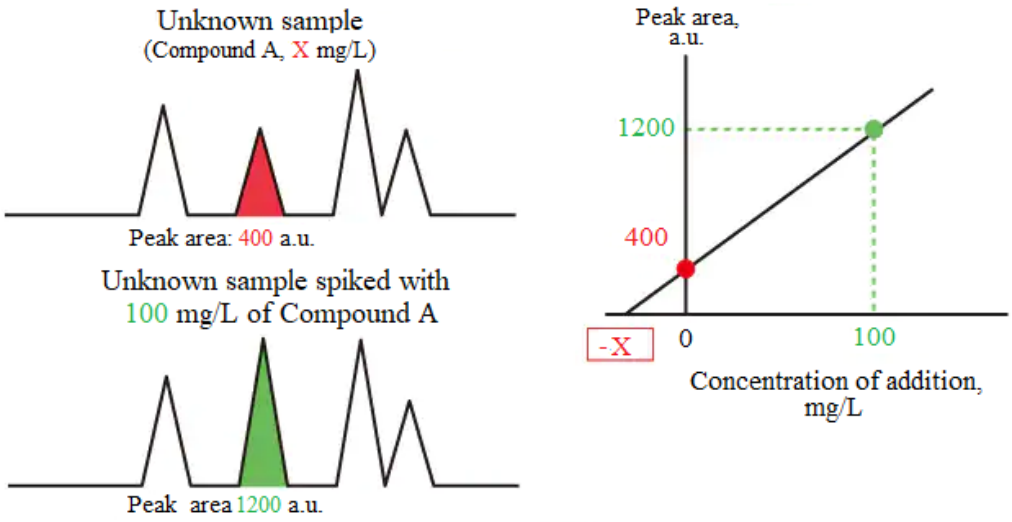


Figure 1.3 – The illustration of principles of standard addition method [19]

The concentration of analyte in in test solution (unknown sample) in standard addition method can be calculated according to the formula

$$C^i = C^a \times \frac{A^i}{A^{i+a} - A^i}, \tag{1.1}$$

where C^i – concentration of i-th analyte in test solution (unknown sample), expressed in concentration units;

C^a – concentration of addition of i-th analyte in test solution (unknown sample), expressed in concentration units;

A^i – the detector response for i-th analyte in test solution (unknown sample), measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

A^{i+a} – the detector response for i-th analyte in test solution (unknown sample) with addition of i-th analyte, measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

Advantages: Other compounds in the sample (matrix) can mitigate the effect (matrix effect) of changes in sample composition when introduced to a gas chromatograph.

Disadvantages: Extra work is required to add the target compound to the unknown sample. Because a target compound is added to the unknown sample (sometimes multiple quantities), rare samples cannot be used.

1.3.2 External standard method

The most commonly employed standardization method uses one or more external standards containing known concentrations of analyte. These standards are identified as external standards because they are prepared and analyzed separately from the samples [18, 19]. This method uses a standard sample of known concentration to prepare a calibration curve, then uses this curve to quantify compounds in an unknown sample.

The illustration of principles of external standard method is shown in Figure 1.4.

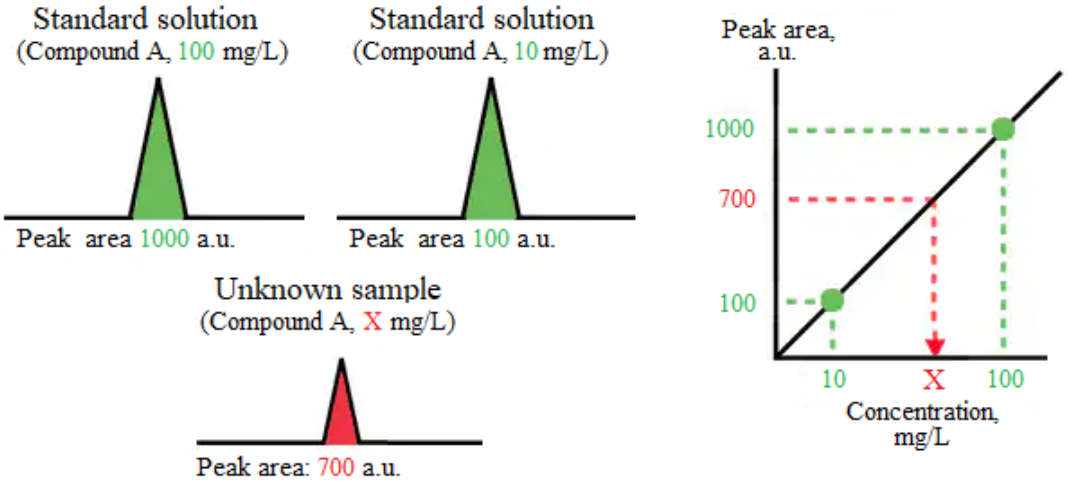


Figure 1.4 – The illustration of principles of external standard method [19]

The calibration coefficient in external standard method is Response Factor (*RF*), which can be obtained from linearity graph, using method of least squares or according to the formula

$$RF^i = \frac{C^i_{cal}}{A^i_{cal}}, \tag{1.2}$$

where C^i_{cal} – concentration of *i*-th analyte in calibration solution, expressed in concentration units;

A^i_{cal} – the detector response for *i*-th analyte in calibration solution, measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

The concentration of analyte in test solution (unknown sample) in external standard method can be calculated according to the formula

$$C^i = RF^i \cdot A^i, \tag{1.3}$$

where A^i – the detector response for *i*-th analyte in test solution (unknown sample), measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

Advantages: Quantitative analysis requires only separation and detection of the target compound.

Disadvantages: Sample injection volume errors carry over as errors in quantitative results.

1.3.3 Internal standard method

The internal standard method calculates the target compound concentration based on the relationship between the peak area ratio and concentration ratio of the target compound and an internal standard [18, 19].

Selecting the internal standard can be difficult as it must fulfill all the requirements:

- be separated almost completely from all compounds in the sample;
- be eluted close to the target compound;
- has similar chemical properties to the target compound (homologue, etc.);
- be chemically stable.

The illustration of principles of internal standard method is shown in Figure 1.5.

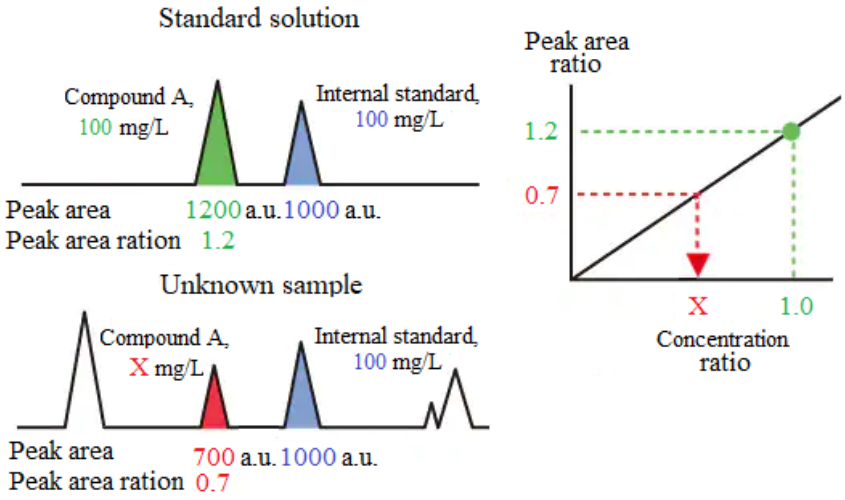


Figure 1.5 – The illustration of principles of internal standard method [19]

The calibration coefficient in internal standard method is Relative Response Factor (*RRF*), which can be obtained from linearity graph, using method of least squares or according to the formula

$$RRF_i^{IS} = \frac{C_{cal}^i}{A_{cal}^i} \cdot \frac{A_{cal}^{IS}}{C_{cal}^{IS}}, \tag{1.4}$$

where C_{cal}^i – concentration of *i*-th analyte in calibration solution, expressed in concentration units;

A_{cal}^i – the detector response for *i*-th analyte in calibration solution, measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

C_{cal}^{IS} – concentration of internal standard in calibration solution, expressed in concentration units;

A_{cal}^{IS} – the detector response for internal standard in calibration solution, measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

The concentration of analyte in test solution (unknown sample) in internal standard method can be calculated according to the formula

$$C^i = RRF_i^{IS} \cdot C^{IS} \cdot \frac{A^i}{A^{IS}}, \tag{1.5}$$

where A^i – the detector response for *i*-th analyte in test solution (unknown sample), measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

C^{IS} – concentration of internal standard in test solution (unknown sample), expressed in concentration units;

A^{IS} – the detector response for internal standard in test solution (unknown sample), measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

Advantages:

- quantity can be calculated as long as the target compound and internal standard are detected;
- concentration ratio is not dependent on injection volume, so this method compensates for injection volume errors;
- not susceptible to different sample densities caused by different sample compositions.

Disadvantages:

- requires a standard sample containing a known concentration of the target compound and the internal standard;
- the internal standard must be added to all unknown samples to obtain an accurate concentration.

1.3.4 Developed internal standard method

The modified internal standard method, based on the ethanol usage as a reference substance for analysis of volatile compounds in ethanol-containing products was developed in cooperation of laboratory of analytical research of Institute for Nuclear problems of Belarussian State University, department of an Analytical Chemistry of Belarussian State University and department of Physical-Chemical Methods of Products Certification of Belarusian State Technological University.

According to this method, as the internal standard was considered ethanol. This method turned all the traditional principles of using the internal standard method upside down. The ethanol is the substance, which always presents in alcohol products and has concentration and magnitude order more than concentration and magnitude order of analytes up to 6 orders. The implementation of this became possible thanks to the development of instrumental methods of analysis and high competition between manufacturers of chromatographic equipment in recent decades, that has led to an increase in the accuracy characteristics of modern equipment.

The illustration of principles of internal standard method is shown in Figure 1.6.

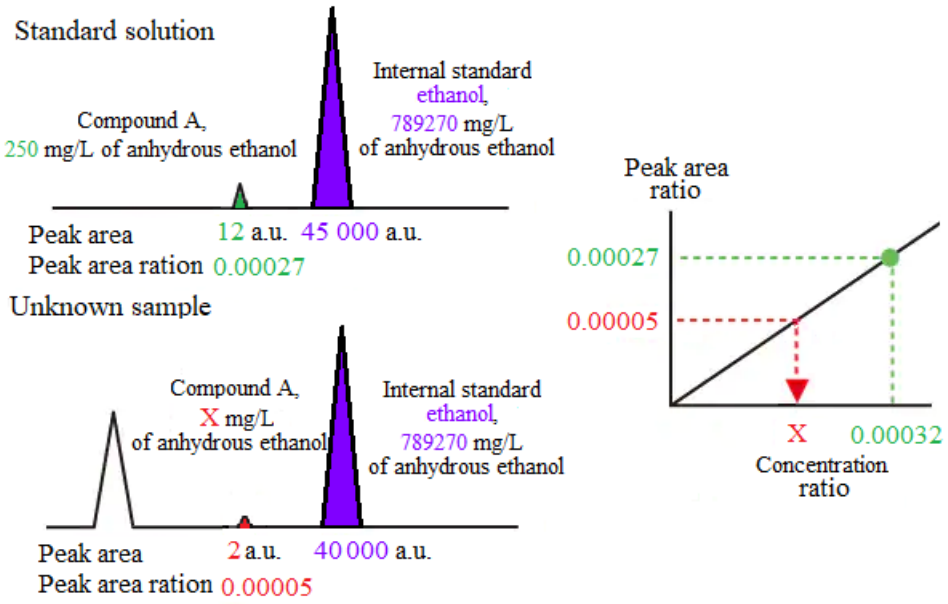


Figure 1.6 – The illustration of principles of modified internal standard method

The calibration coefficient in modified internal standard method is Relative Response Factor (*RRF*), which can be obtained from linearity graph, using method of least squares or according to the formula

$$RRF_i^{Eth} = \frac{C_{cal}^i}{A_{cal}^i} \cdot \frac{A_{cal}^{Eth}}{C_{cal}^{Eth}}, \tag{1.6}$$

where C_{cal}^i – concentration of *i*-th analyte in calibration solution, expressed in mg/L of anhydrous ethanol;

A_{cal}^i – the detector response for *i*-th analyte in calibration solution, measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

C_{cal}^{Eth} – concentration of ethanol in calibration solution, expressed in mg/L of anhydrous ethanol, which is the density of anhydrous ethanol, ρ^{Eth} = 789270 mg/L;

A_{cal}^{Eth} – the detector response for ethanol in calibration solution, measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

The concentration of analyte in test solution (unknown sample) in modified internal standard method can be calculated according to the formula

$$C^i = RRF_i^{Eth} \cdot \rho^{Eth} \cdot \frac{A^i}{A^{Eth}}, \tag{1.7}$$

where A^i – the detector response for i-th analyte in test solution (unknown sample), measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

ρ^{Eth} – concentration of ethanol in test solution (unknown sample), density of anhydrous ethanol, ρ^{Eth} = 789270 mg/L;

A^{Eth} – the detector response for ethanol in test solution (unknown sample), measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units.

Advantages:

- there is no need to apply an internal standard, which reduces material, labor and time costs when testing samples of alcoholic products;
- method allows calculation the concentration of volatile impurities directly from gas chromatographic measurements in the legally required dimension of mg/L of anhydrous ethanol.

CHAPTER 2
EXPERIMENTAL

2.1 Materials and methods

2.1.1 Reagents

All chemical standards with their corresponding CAS numbers: acetaldehyde (75-07-0), methyl acetate (79-20-9), ethyl acetate (141-78-6), methanol (67-56-1), propan-2-ol (67-63-0), propan-1-ol (71-23-8), 2-methylpropan-1-ol (78-83-1), butan-1-ol (71-36-3), 3-methylbutan-1-ol (123-51-3), bornyl acetate (76-49-3), menthol (89-78-1) and tridecanol (112-70-9) were provided by Sigma-Aldrich (Alcobendas, Madrid, Spain) with the highest purity available (more than 99%). Concentrations of impurities in volatile compounds were specified by the GC-FID (to detect of volatile impurities) and GC coupled with thermal conductivity detector (GC-TCD) (to detect of water) analysis using the internal normalization method. Rectified ethyl alcohol with volume concentration of ethanol 96.0 % was provided by Dyatlovo Distillery Plant Algon (Slonim, Belarus). Pure distilled and deionized water (conductivity ≤ 0.5 MΩ·cm) was provided by JSC Integral (Minsk, Belarus). The drug «Urolesan» was purchased at a pharmacy.

2.1.2 Preparation of solutions

2.1.2.1 Preparation of stock solution A

The stock solution A (with approximate concentrations of volatile compounds 1,000 mg/L AA and 10,000 mg/L AA for methanol) was prepared by adding of the volatile compounds (acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol) to rectified ethyl alcohol 96.6 %. The weight of the flasks, each compound added and the total final weight of contents were recorded and used in following calculations of concentrations.

2.1.2.2 Preparation of stock solution A

The calibration solutions (CS) 1, 2 and 3 (with approximate concentrations of volatile compounds 20, 10 and 5 mg/L AA, correspondingly and 250, 125 and 60 mg/L AA for methanol) were prepared by mixing of stock solution A and rectified ethyl alcohol. The weight of the flasks, each compound added and the total final weight of contents were recorded and used in following calculations of concentrations.

2.1.2.3 Preparation of standard solutions

The standard solutions (SS) 1, 2 and 3 (with approximate concentrations of volatile compounds 20, 10 and 5 mg/L AA, correspondingly) were prepared by mixing of stock solution A and rectified ethyl alcohol. The weight of the flasks, each

compound added and the total final weight of contents were recorded and used in following calculations of concentrations.

The concentrations of volatile compounds with the corresponding uncertainties in the prepared solutions are shown in Table 2.1.

Table 2.1 – The mass concentrations and uncertainties of concentrations of volatile compounds in the prepared solutions

Compound	Concentration ± standard uncertainty of concentration, mg/L AA						
	Stock solution A	*CS*-1	*CS*-2	*CS*-3	*SS*-1	*SS*-2	*SS*-3
acetaldehyde	1027±5.3	25.7±0.13	12.9±0.06	6.69±0.03	24.6±0.12	12.8±0.06	6.75±0.03
methyl acetate	1143±5.6	27.4±0.13	13.1±0.06	6.16±0.03	26.1±0.13	12.9±0.06	6.24±0.03
ethyl acetate	1006±5.3	24.1±0.13	11.5±0.06	5.42±0.03	23.0±0.12	11.4±0.06	5.49±0.03
methanol	10030±17	249±0.39	124±0.21	63.0±0.14	238±0.37	122±0.21	63.6±0.16
propan-2-ol	930±5.3	23.7±0.13	12.1±0.06	6.44±0.03	22.7±0.12	11.9±0.06	6.50±0.03
propan-1-ol	925±5.3	22.2±0.13	10.6±0.06	4.99±0.03	21.1±0.12	10.5±0.06	5.05±0.03
2-methylpropan-1-ol	943±5.5	22.6±0.13	10.8±0.06	5.08±0.03	21.5±0.13	10.7±0.06	5.14±0.03
butan-1-ol	928±5.3	22.2±0.13	10.6±0.06	5.00±0.03	21.2±0.12	10.5±0.06	5.06±0.03
3-methylbutan-1-ol	906±5.5	21.7±0.13	10.4±0.06	4.89±0.03	20.7±0.12	10.2±0.06	4.94±0.03

2.1.3 Analysis conditions

Analysis was performed using a gas chromatograph Crystal-5000.1, equipped with the autosampler, FID and TCD detectors. All the separations were carried out with a capillary column Rt-Wax, 60 m × 0.53 mm, 1.0 μm (Restek, Bellefonte, USA). The injections were made in the split mode (12:1), and the injection volume was 1μL. The temperature of injector was 190°C. The oven was programmed for 75°C for 9 min, increased by 5°/min to 130°C, then increased by 10°/min to 180°C, followed by 5 min at the final temperature. The temperatures of FID and TCD were 260° and 160°C, correspondingly.

The examples of chromatograms, obtained for rectified ethyl alcohol, CS and SS are showed in Figures 2.1 and 2.2.

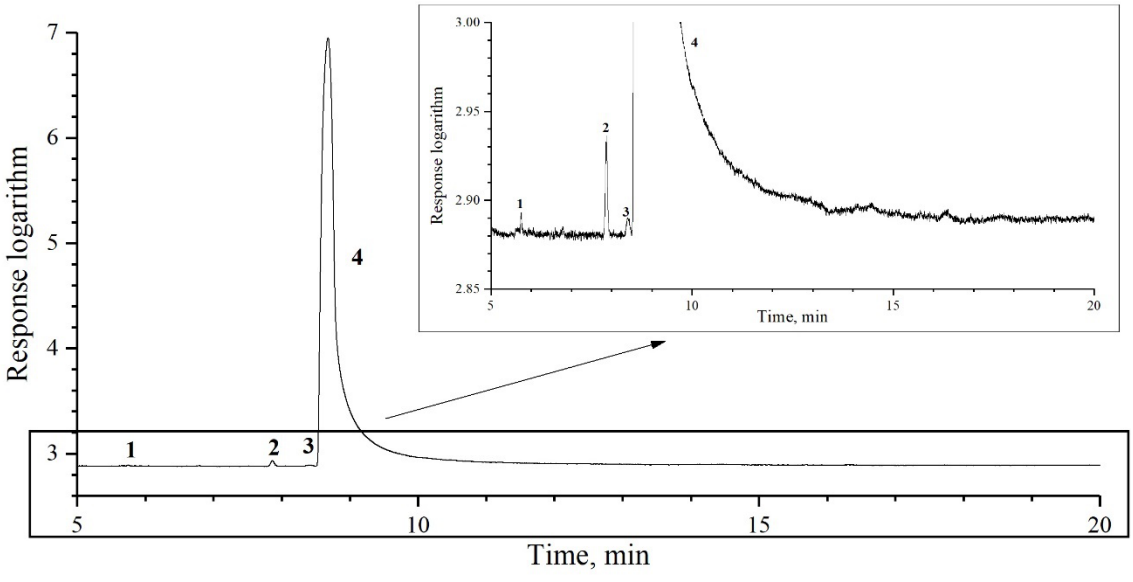


Figure 2.1 – The chromatogram of rectified ethyl alcohol in the logarithmic scale.
1 - acetaldehyde; 2 – methanol; 3 – propan-2-ol; 4 – ethanol.

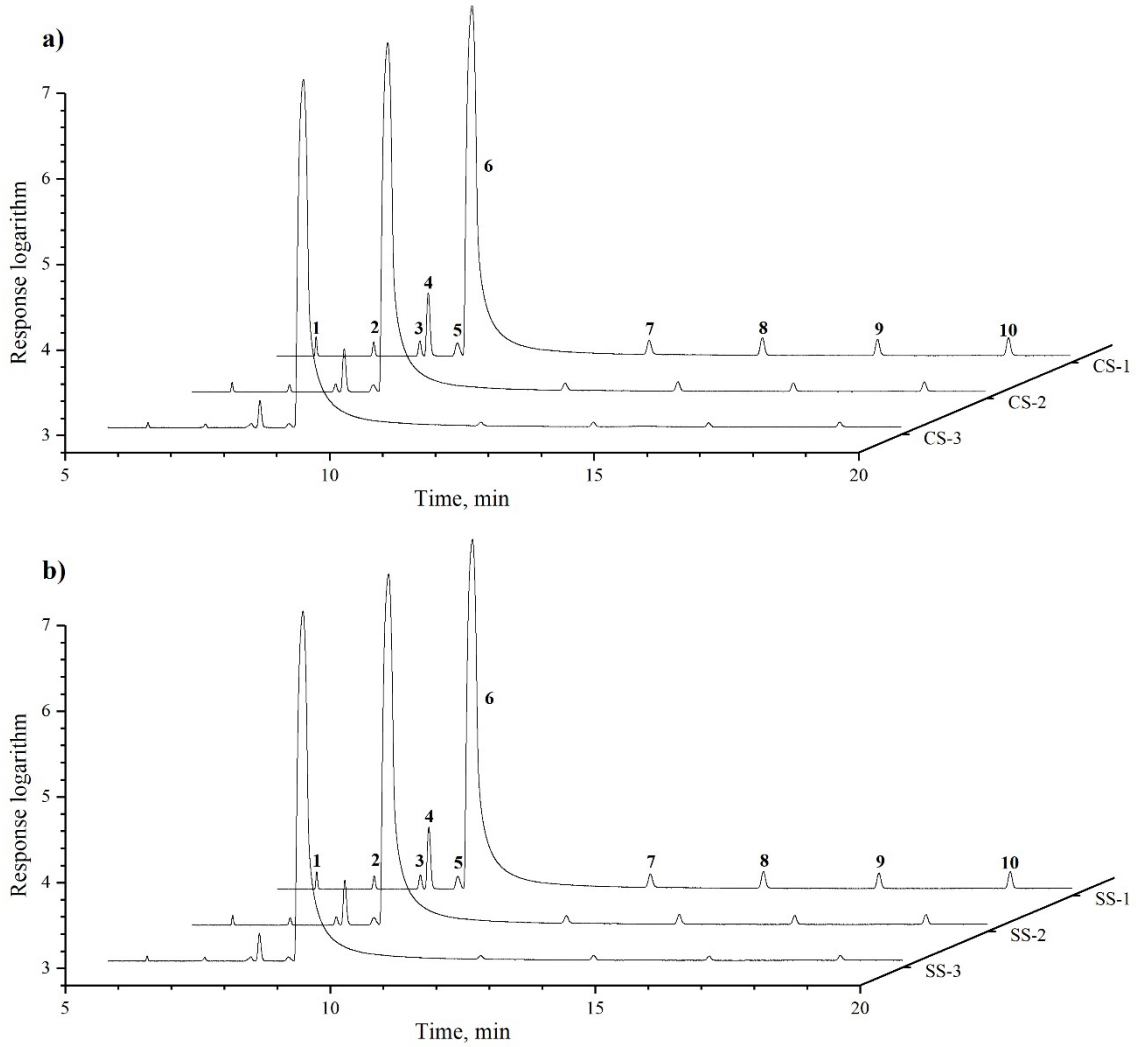


Figure 2.2 – The chromatograms of prepared calibration (a) and standard solutions (b) in the logarithmic scale. 1 - acetaldehyde; 2 – methyl acetate; 3 – ethyl acetate; 4 – methanol; 5 – propan-2-ol; 6 – ethanol; 7 – propan-1-ol; 8 – 2-methylpropan-1-ol; 9 – butan-1-ol; 10 – 3-methylbutan-1-ol

2.2 Validation study

The single-laboratory validation study of the method: statistical analysis of the obtained results, namely precision parameters evaluation (repeatability, intermediate precision and trueness), was carried out according to the number of standards [21-24], the ICH guidelines [25] and guides [26-29]. Reproducibility, which refers to the use of an analytical procedure in different laboratories, was beyond the scope of the present study.

2.2.1 Raw data processing

2.2.1.1 Calibration

The values of RRF_i^{Eth} for each volatile compound were also determined according to method of least squares [30], using all calibration solutions.

The dependence of the ratio of the detector response to the *i*-th volatile compound to the detector response to ethanol on the ratio of the mass concentration (in mg/L of anhydrous alcohol) of the *i*-th volatile compound to the density of anhydrous ethanol (in mg/L) is characterized as line

$$y^i = a^i + b^i \cdot x^i, \tag{2.8}$$

where

$$y^i = \frac{A^i}{A^{Eth}}, \tag{2.9}$$

$$x^i = \frac{C^i}{\rho_{Eth}}, \tag{2.10}$$

where A^i – measured detector response to the *i*-th volatile compound (measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

A^{Eth} – measured detector response to the ethanol (measurement units depend on the estimated parameter, for example, the magnitude of the peak area), peak area units;

C^i – mass concentration of the *i*-th volatile compound in the calibration solution, mg/L AA;

a^i and b^i – regression coefficients, which are calculated in accordance with the expressions

$$a^i = \frac{\sum_{k=1}^{M}\sum_{j=1}^{N}\left(x_j^i(k)\right)^2 \sum_{j=1}^{N} y_j^i(k) - \sum_{k=1}^{M}\sum_{j=1}^{N} x_j^i(k) \sum_{k=1}^{M}\sum_{j=1}^{N} x_j^i(k) \cdot y_j^i(k)}{MN\sum_{k=1}^{M}\left(\sum_{j=1}^{N}\left(x_j^i(k)\right)^2 - \left(\sum_{j=1}^{N} x_j^i(k)\right)^2\right)}, \tag{2.11}$$

$$b^i = \frac{N\sum_{k=1}^{M}\sum_{j=1}^{N} x_j^i(k) \cdot y_j^i(k) - \sum_{k=1}^{M}\sum_{j=1}^{N} x_j^i(k) \sum_{j=1}^{N} y_j^i(k)}{MN\sum_{k=1}^{M}\sum_{j=1}^{N}\left(x_j^i(k)\right)^2 - \left(\sum_{j=1}^{N} x_j^i(k)\right)^2}, \tag{2.12}$$

where N – number of measurements of the *k*-th calibration solution, $N = 2$;

M – number of calibration solutions, $M = 3$.

The residual standard deviation (the standard deviation of the difference between the experimental $y_j^i(k)_{\exp}$ and calculated values $y_j^i(k)_{calc}$ was calculated by the formula

$$S_0^i = \sqrt{\frac{\sum_{k=1}^{M}\sum_{j=1}^{N}\left(y_j^i(k)_{\exp} - y_j^i(k)_{calc}\right)^2}{MN-2}}. \quad (2.13)$$

The significance of the coefficient a^i for the i-th volatile compound was checked according to Student's statistical test

$$t_a^i = \frac{|a^i|}{S_a^i}, \quad (2.14)$$

where S_a^i – standard deviation of the regression coefficient value a^i, which was calculated according to the formula

$$S_a^i = S_0^i \sqrt{\frac{\sum_{k=1}^{M}\sum_{j=1}^{N}\left(x_j^i(k)\right)^2}{MN\sum_{k=1}^{M}\sum_{j=1}^{N}\left(x_j^i(k)\right)^2 - \left(\sum_{j=1}^{N}x_j^i(k)\right)^2}}. \quad (2.15)$$

The resulting value t_a^i is compared with the Student's coefficient t_{table} = 2,77 with a confidence level P = 95% and the number of degrees of freedom $f = MN - 2 = 6 - 2 = 4$.

Since the obtained values $t_a^i \leq t_{table}$, a conclusion is made about the insignificance of the coefficient a^i, the line of the calibration graph passes through the origin of coordinates and is determined by the functional dependence

$$y_{corr}^i = b_{corr}^i \cdot x^i, \quad (2.16)$$

where b_{corr}^i – corrected slope for the i-th volatile compound, which was calculated according to the formula

$$b_{corr}^i = \frac{\sum_{k=1}^{M}\sum_{j=1}^{N}x_j^i(k)\cdot y_j^i(k)}{\sum_{k=1}^{M}\sum_{j=1}^{N}\left(x_j^i(k)\right)^2}. \quad (2.17)$$

The values of RRF_i^{Eth} for each volatile compound in case with least square method can be calculated according to the formula

$$RRF_i^{Eth} = \frac{1}{b_{corr}^i}. \quad (2.18)$$

The calculation of the residual standard deviation $S_{0_{corr}}^i$, which characterizes the scatter of the results of experimental data relative to the plotted curve, was carried out according to the formula

$$S^i_{0_{corr}} = \sqrt{\frac{\sum_{k=1}^{M}\sum_{j=1}^{N}\left(y^i_j(k)_{\exp} - y^i_j(k)_{calc}(corr)\right)^2}{MN-1}}, \quad (2.19)$$

where $y^i_j(k)_{calc}(corr)$ – corrected value of the ratio of the detector response to the *i*-th volatile compound to the detector response to ethanol from the ratio of the mass concentration (in mg/L of anhydrous alcohol) of the *i*-th volatile compound to the density of anhydrous ethanol (in mg/L) calculated by formula (2.16).

The calculation of the standard deviation S_x, mg/L of anhydrous ethanol, for the mass concentration of the *i*-th volatile compound in the *k*-th calibration solution is carried out according to the formula

$$S^i_x(k) = \frac{S^i_{0_{corr}}(k)}{b^i_{corr}} \sqrt{\frac{1}{N} + \frac{\left(\overline{y}^i(k)\right)^2}{\left(b^i_{corr}\right)^2 \sum_{k=1}^{M}\left(x^i(k)\right)^2}}, \quad (2.20)$$

where

$$\overline{y}^i(k) = \frac{1}{N}\sum_{j=1}^{N} y^i_j(k). \quad (2.21)$$

The relative standard deviation for the ratio of the mass concentration of the *i*-th volatile compound in the *k*-th calibration solution $S^i_{x_{rel}}(k)$ was calculated by the formula

$$S^i_{x_{rel}}(k) = \frac{S^i_x}{x^i(k)} \cdot 100\ \%. \quad (2.22)$$

As a standard for the stability of the calibration curve *K*, the error limits of the concentration value of the *i*-th volatile compound determined from the calibration curve were taken

$$K^i(k) = t_{p,f} \cdot \frac{S^i_x}{x^i(k)} \cdot 100\ \%, \quad (2.23)$$

where $t_{p,f}$ = 2,306 – Student's distribution coefficient at degrees of freedom $f = MN - 1 = 3 \cdot 3 - 1 = 8$ (P = 95 %).

2.2.1.2 Concentration

The concentration of *i*-th volatile was determined according to the following equation

$$C_i^{Eth}(j) = RRF_i^{Eth} \frac{A_i^j}{A_{Eth}^j} \cdot \rho_{Eth}, \quad (2.24)$$

where A_i^j and A_{Eth}^j – the detector response for *i*-th volatile and ethanol in the *j*-th SS, a.u.

2.2.2 Statistical analysis

2.2.2.1 Outliers

Cochran's and Grubbs' tests were performed to detect and eliminate outliers. Firstly, an upper-tail Cochran test was performed according to the item 7.3.3 of ISO 5725-2 [21] for the comparison of the interlaboratory variances. Secondly, a two-tailed single Grubbs' and a paired Grubbs' tests were applied according to the to the item 7.3.4 of ISO 5725-2 [21].

2.2.2.2 Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) of each individual volatile compound were estimated according to the item 6.2 of Eurachem Guide [27] by usage *SS*-3 as the solution with low impurities concentrations. The *SS*-3 was measured 10 times under repeatability conditions. The LOD and LOQ of *i*-th volatile were determined according to the following equations

$$LOD_i = 3 \cdot \sqrt{\frac{\frac{1}{n-1}\sum_{k=1}^{n}\left(C_{ik}^3 - \overline{C}_i^3\right)^2}{n}}, \quad (2.25)$$

$$LOQ_i = 10 \cdot \sqrt{\frac{\frac{1}{n-1}\sum_{k=1}^{n}\left(C_{ik}^3 - \overline{C}_i^3\right)^2}{n}}, \quad (2.26)$$

where *n* – number of replicate observations, *n* = 30;

C_{ik}^3 – assigned value of concentration for *i*-th volatile for *k*-th measurement of *SS*-3 obtained by formula (2.24), mg/L AA;

$\overline{C}_i^3$ – average value of concentration for *i*-th volatile in *SS*-3, mg/L AA.

2.2.3 Precision

The repeatability variance (within-days variance) s_r^2 was determined according to the following formula (item 7.4.5.1 of ISO 5725-2 [21])

$$s_{r_{ij}}^2 = \frac{\sum_{l=1}^{t}\sum_{k=1}^{n}\left(C_{ijkl} - \overline{C}_{ijl}\right)^2}{t\left(n-1\right)}, \quad (2.27)$$

where *t* – number of days, *t* = 15,

n – number of replicate observations, $n = 2$,
C_{ijkl} – assigned value of concentration for i-th volatile in j-th test sample for k-th measurement in l-th day, mg/L AA,
$\overline{C}_{ijl}$ – average value of concentration for i-th volatile in j-th test sample in l-th day, mg/L AA.

Relative standard deviation of repeatability RSD_r was determined according to the following formula

$$RSD_{r_{ij}} = \frac{s_{r_{ij}}}{\overline{\overline{C}}_{ij}} \cdot 100\ \%, \tag{2.28}$$

where $\overline{\overline{C}}_{ij}$ – average value of concentration for i-th volatile in j-th test sample among 15 days of measurement, mg/L AA.

The limit of repeatability r was determined according to the following formula (item 4.1.4 of ISO 5725-6 [24])

$$r_{ij} = 2.8 \cdot RSD_{r_{ij}}. \tag{2.29}$$

The between-days variance (intermediate precision) s_d^2 was determined according to the following formula (item 7.4.5.2 of ISO 5725-2 [21])

$$s_{d_{ij}}^2 = \frac{\sum_{l=1}^{t}\left(\overline{C}_{ijl} - \overline{\overline{C}}_{ij}\right)^2}{t-1} - \frac{s_{r_{ij}}^2}{n}. \tag{2.30}$$

The intermediate precision variance was determined according to the following formula (item 7.4.5.5 of ISO 5725-2 [21])

$$s_{I(TO)_{ij}}^2 = s_{r_{ij}}^2 + s_{d_{ij}}^2. \tag{2.31}$$

Relative standard deviation of repeatability $RSD_{I(TO)}$ was determined according to the following formula

$$RSD_{I(TO)_{ij}} = \frac{s_{I(TO)_{ij}}}{\overline{\overline{C}}_{ij}} \cdot 100\ \%. \tag{2.32}$$

The limit of repeatability $r_{I(TO)}$ was determined according to the following formula (item 4.1.4 of ISO 5725-6 [24])

$$r_{I(TO)_{ij}} = 2.8 \cdot RSD_{I(TO)_{ij}}. \tag{2.33}$$

2.2.4 Trueness

For the estimation of trueness, the bias values were calculated in accordance with item 4.7.2 of ISO 5725-4 [23]

$$\delta_{ij} = \frac{\overline{\overline{C}}_{ij} - \mu_{ij}}{\mu_{ij}} \cdot 100\ \%, \tag{2.34}$$

where μ_{ij} – certified value of concentration for *i*-th volatile in *j*-th test sample, mg/L AA.

The variation of the estimate of the laboratory bias was determined according to the following formula (item 4.7.2 of ISO 5725-4 [23])

$$s_{\delta_{ij}} = \sqrt{\frac{s^2_{I(TO)_{ij}} - ((n-1)/n)s^2_{r_{ij}}}{t}}. \tag{2.35}$$

The 95% confidence interval of the bias was calculated according to the following formula (item 4.7.2 of ISO 5725-4 [23])

$$\hat{\delta}_{ij} - A_{ij}s_{I(TO)_{ij}} \le \delta_{ij} \le \hat{\delta}_{ij} + A_{ij}s_{I(TO)_{ij}}, \tag{2.36}$$

where

$$A_{ij} = 1.96\sqrt{\frac{n((s_{I(TO)_{ij}} / s_{r_{ij}})^2 - 1) + 1}{tn(s_{I(TO)_{ij}} / s_{r_{ij}})^2}}. \tag{2.37}$$

2.2.5 Uncertainty

The standard uncertainty *u* of the method was calculated according to the [28, 29] guidelines using the following formula (item 1.2.2 of Eurolab technical report [29])

$$u_{ij} = \sqrt{s^2_{I(TO)_{ij}} + s^2_{\delta_{ij}} + u^2_{ref_{ij}} + \hat{\Delta}^2_{ij}}, \tag{2.38}$$

where u_{ref} – uncertainty of the assigned value, calculated according to the [29], mg/L AA.

The expanded uncertainty *U* (*P* = 0.95) was calculated according to the following formula

$$U_{ij} = k \cdot u_{ij}, \tag{2.39}$$

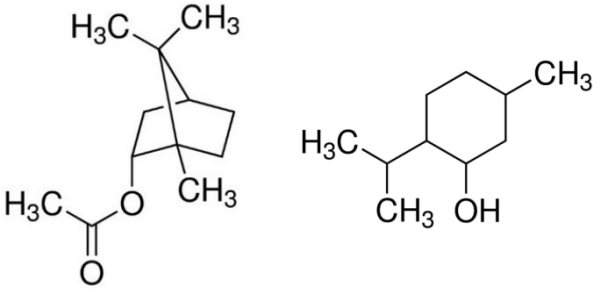
where *k* = 2 is the coverage factor (item 2.3.3 of EURACHEM/CITAC Guide [28]).

2.3 Method approbation study

Method approbation study was carried out using the drug «Urolesan».

The drug «Urolesan» is used in the treatment and prevention of disorders and diseases of the kidneys and urinary tract, as well as for the dissolution of kidney and gallstones. This drug has dosage form as drops. The certificate of quality is performed at Appendix B.

The most important parameters of quality of this drug are content of bornyl acetate and menthol. The chemical structures bornyl acetate and menthol are shown in Figure 2.3.



bornyl acetate menthol

Figure 2.3 – The chemical structures of bornyl acetate and menthol.

These substances can be determined using GC-FID. The internal standard method is used for quantitative calculations. Tridecanol is usually used as an internal standard (IS).

2.3.1 Preparation of solutions

2.3.1.1 Preparation of water-ethanol solution

The water-ethanol solution with ethanol volume concentration 68.0 % (WES) was prepared by mixing of rectified ethyl alcohol and deionized water.

2.3.1.2 Preparation of calibration solution U

The calibration solution U was prepared by adding of bornyl acetate, menthol and tridecanol to WES. The weight of the flasks, each compound added and the total final weight of contents were recorded and used in following calculations of concentrations. The concentrations of bornyl acetate, menthol and tridecanol were 1950.2, 2019.1 and 2024.6 mg/L AA, correspondingly.

2.3.1.3 Preparation of sample

The sample for analysis was prepared by adding of tridecanol to Urolesan sample. The concentration of tridecanol was 1985.2 mg/L AA.

2.3.2 Analysis conditions

Analysis was performed using a gas chromatograph Crystal-5000.1, equipped with the autosampler and FID detector. All the separations were carried out with a capillary column Rt-Wax, 60 m × 0.53 mm, 1.0 μm (Restek, Bellefonte, USA). The injections were made in the split mode (3.3:1), and the injection volume was 1 μL. The temperature of injector was 200°C. The oven was programmed for 70°C for 1 min, increased by 10°/min to 130°C, followed by 10 min at this temperature, then increased by 22°/min to 240°C, followed by followed by 5 min at the final temperature. The temperature of FID was 240°C.

The examples of chromatograms, obtained for calibration solution U and sample are showed in Figures 2.4 and 2.5.

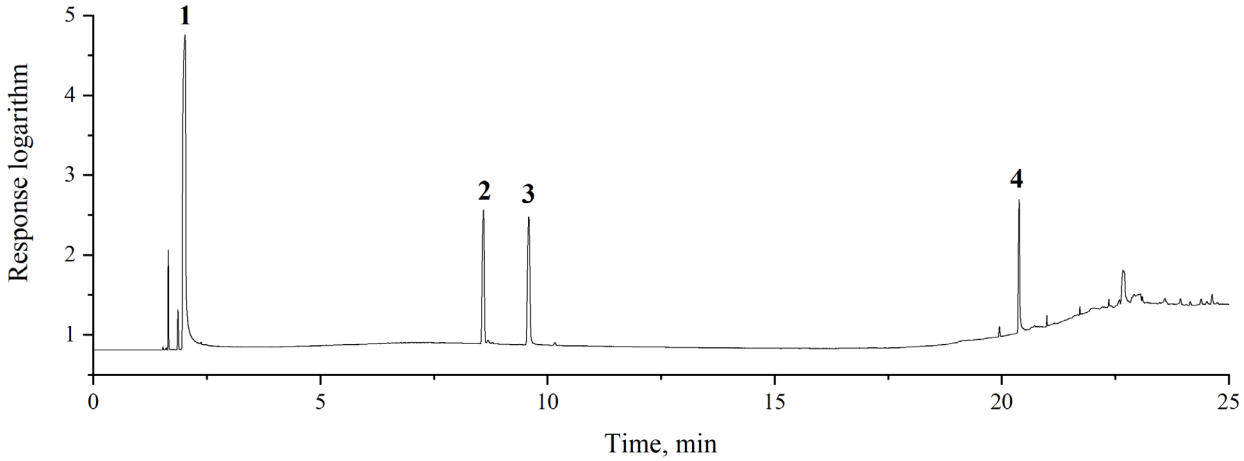


Figure 2.4 – The chromatogram of calibration solution U in the logarithmic scale.
1 - ethanol; 2 – bornyl acetate; 3 – menthol; 4 – tridecanol.

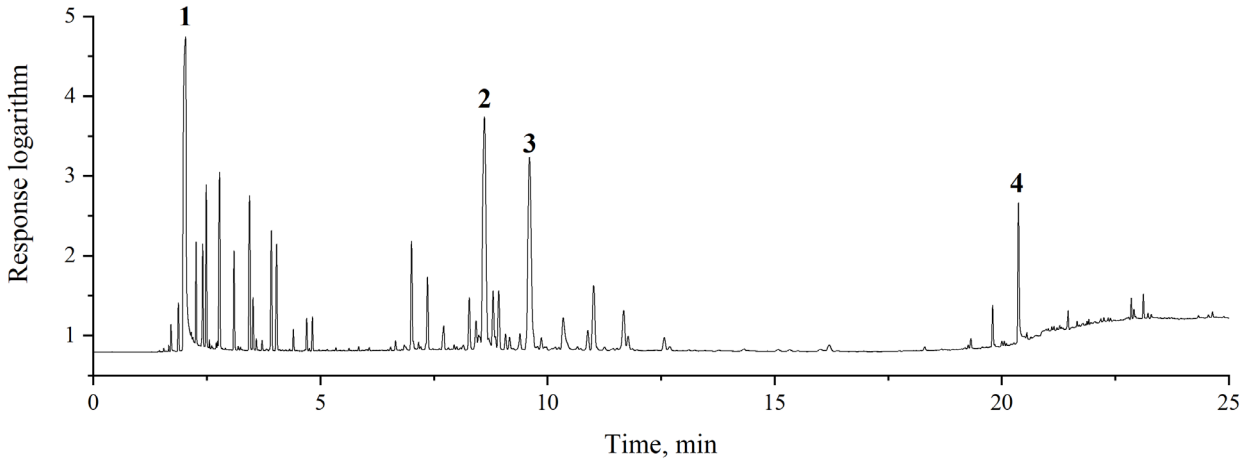


Figure 2.5 – The chromatogram of Urolesan sample in the logarithmic scale.
1 - ethanol; 2 – bornyl acetate; 3 – menthol; 4 – tridecanol.

2.3.3 Calibration

The calibration coefficients were calculated for traditional internal standard method and developed internal standard method using equations (1.4) and (1.6), correspondingly.

2.3.4 Concentration

The concentrations of analytes in sample, expressed in mg/L AA, were calculated for traditional internal standard method and developed internal standard method using equations (1.5) and (1.7), correspondingly.

The recalculation from mg/L AA to mg/mL was carried out according to the formula

$$C_{T,D}^{i} = \frac{C_{T,D^*}^{i}}{C^{Eth}} \cdot 100\ \%, \tag{2.40}$$

where $C_{T,D}^{i}$ – concentration of *i*-th analyte in sample, calculated using traditional method or developed method by formula, expressed in mg/mL;

C_{T,D^*}^{i} – concentration of *i*-th analyte in sample, calculated using traditional method by formula (1.5) or using developed method by formula (1.7), expressed in mg/mL;

C^{Eth} – concentration of ethanol in sample, according to the certificate of quality (Appendix B), expressed in % v/v.

2.3.5 Comparison of obtained results

The relative difference between the results, obtained for both the traditional and developed methods, Δ, %, was calculated using the formula

$$\Delta^{i} = \frac{C_{D}^{i} - C_{T}^{i}}{C_{D}^{i}} \cdot 100\ \%, \tag{2.41}$$

where C_{D}^{i} – concentration of *i*-th analyte in sample, calculated using developed method by formula (1.7), expressed in mg/mL;

C_{T}^{i} – concentration of *i*-th analyte in sample, calculated using traditional method by formula (1.5), expressed in mg/mL.

The relative bias between the results, obtained for the traditional and developed methods, δ, %, was calculated using the formula

$$\delta_{T,D}^{i} = \frac{C_{T,D}^{i} - \mu^{i}}{\mu^{i}} \cdot 100\ \%, \tag{2.42}$$

where $C_{T,D}^{i}$ – concentration of *i*-th analyte in sample, calculated using traditional method or developed method by formula, expressed in mg/mL;

μ^{i} – concentration of *i*-th analyte in sample, according to the certificate of quality (Appendix B), expressed in mg/mL.

CHAPTER 3
RESULTS AND DISCUSSION

3.1 Results of validation method study

3.1.1 Calibration and linearity

The results of calibration, based on use of multi-point calibration are presented in table A.1. As a result of the study, it was shown that the obtained values $t_a^i \leq t_{table}$. Thus, a conclusion is made about the insignificance of the coefficient a^i, the line of the calibration graph passes through the origin of coordinates and is determined by the functional dependence $y^i = b^i \cdot x^i$. The signal-response of the GC-FID system was evaluated at three concentration levels across a range of 4.99–25.7 mg/L AA for acetaldehyde, methyl acetate, ethyl acetate, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol and at three concentration levels across a range of 63.0–249 mg/L AA for methanol.

Calibration plots were made by plotting the relative analyte-to-IS peak area ratio against the relative analyte-to-IS concentration ratio, and the linearity was evaluated by the squared correlation coefficient (R^2). The plotted calibration graphs are shown in Figure A.1. The values of squared correlation coefficient R^2 were more than 0.999 for all studied volatile compounds.

3.1.2 LOQ and LOD

The results of *LOQ* and *LOD* calculations are presented in table 3.1.

Table 3.1 – The results of *LOQ* and *LOD* determination

Compound	*LOQ*, mg/L AA	*LOD*, mg/L AA
acetaldehyde	0.167	0.557
methyl acetate	0.188	0.626
ethyl acetate	0.138	0.462
methanol	0.494	1.646
propan-2-ol	0.210	0.700
propan-1-ol	0.171	0.569
2-methylpropan-1-ol	0.141	0.470
butan-1-ol	0.185	0.617
3-methylbutan-1-ol	0.130	0.432

3.1.3 Outliers

There were not detected statistical outliers in obtained results (tables 3.2 – 3.4).

Table 3.2 – Outliers test results for acetaldehyde, methyl acetate and ethyl acetate

Day t	acetaldehyde						methyl acetate						ethyl acetate					
	SS-3		SS-2		SS-1		SS-3		SS-2		SS-1		SS-3		SS-2		SS-1	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
1	6.77	6.70	13.1	12.8	24.4	24.7	6.17	6.24	12.6	12.9	26.1	25.4	5.45	5.46	11.7	11.4	23.1	22.6
2	6.96	6.77	12.9	12.9	24.7	25.1	6.32	6.29	13.2	13.0	25.2	26.2	5.48	5.30	11.8	11.4	23.0	23.5
3	6.81	6.79	12.7	12.5	24.7	24.5	6.46	6.27	13.2	12.7	26.2	26.3	5.47	5.45	11.4	11.4	23.1	23.6
4	6.83	6.82	13.0	13.2	24.8	24.5	6.17	6.17	12.9	12.7	26.2	25.5	5.50	5.68	11.4	11.3	23.1	23.0
5	6.60	6.87	12.6	12.5	24.6	25.0	6.32	6.33	12.9	12.5	26.5	26.1	5.50	5.51	11.4	11.2	23.0	23.0
6	6.86	6.78	12.7	12.8	24.8	24.3	6.28	6.24	13.0	13.0	25.8	26.2	5.49	5.42	11.3	11.4	23.1	23.1
7	6.89	6.73	12.6	12.5	23.9	24.4	6.07	6.12	12.8	12.9	26.2	26.0	5.49	5.55	11.4	11.4	23.0	23.1
8	6.82	6.64	12.8	12.5	24.8	25.1	6.10	6.21	12.5	13.0	26.2	25.8	5.51	5.48	11.5	11.4	22.9	22.9
9	6.64	6.78	12.7	12.7	24.4	24.7	6.22	6.17	12.5	12.9	26.0	26.2	5.49	5.62	11.4	11.6	22.9	23.0
10	6.83	6.87	12.8	12.8	24.5	25.0	6.07	6.11	12.7	13.0	25.8	26.2	5.47	5.49	11.4	11.4	23.0	23.1
11	6.59	6.78	12.8	13.0	24.5	24.4	6.05	6.27	12.7	13.0	25.2	26.1	5.49	5.49	11.4	11.4	23.1	23.0
12	6.66	6.68	12.7	12.9	24.1	24.2	6.10	6.25	12.8	13.0	25.2	26.3	5.49	5.51	11.4	11.2	23.0	23.1
13	6.79	6.72	12.4	12.9	24.6	25.1	5.98	6.31	12.7	12.8	26.1	26.1	5.49	5.49	11.3	11.4	23.0	22.9
14	6.55	6.79	12.6	12.8	23.9	24.4	5.86	6.24	12.7	13.0	26.0	25.4	5.50	5.42	11.4	11.3	22.9	22.9
15	6.78	6.74	12.7	12.8	24.5	23.9	6.17	6.28	12.9	12.9	26.0	26.2	5.57	5.48	11.4	11.4	23.1	23.0
Grubbs																		
$\overline{\overline{C}}$	6.76		12.8		24.5		6.19		12.8		26.0		5.49		11.4		23.0	
S	0.063		0.151		0.278		0.089		0.113		0.213		0.047		0.083		0.124	
G_p	1.633		2.069		1.375		1.896		2.278		1.482		2.159		2.449		2.338	
G_1	1.430		1.426		1.548		1.630		1.280		1.448		2.065		1.221		1.673	
$G_{crit\ 1\%}$	2.806																	
$G_{crit\ 5\%}$	2.549																	
Cohran																		
max S^2	0.036		0.131		0.185		0.070		0.153		0.608		0.017		0.102		0.144	
C	0.237		0.368		0.164		0.360		0.234		0.235		0.301		0.435		0.359	
$C_{crit\ 1\%}$	0.471																	
$C_{crit\ 5\%}$	0.575																	

Table 3.3 – Outliers test results for methanol, propan-2-ol and propan-1-ol

Day t	methanol						propan-2-ol						propan-1-ol					
	SS-3		SS-2		SS-1		SS-3		SS-2		SS-1		SS-3		SS-2		SS-1	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
1	63.7	63.3	122.3	122.2	238.3	238.1	6.72	6.27	12.0	12.3	23.1	22.9	5.15	5.31	10.5	10.5	21.2	21.3
2	63.2	62.2	122.4	122.2	237.3	238.0	6.39	6.35	12.0	12.2	23.2	23.2	5.01	5.11	10.4	10.5	21.4	21.3
3	62.0	62.2	121.9	122.6	239.1	239.0	6.48	6.19	12.0	11.9	22.8	22.3	5.24	5.26	10.4	10.6	21.2	21.3
4	63.2	63.7	121.9	122.3	238.5	238.0	6.27	6.61	11.5	11.6	22.7	22.8	5.23	5.07	10.6	10.9	21.7	21.3
5	62.4	60.8	122.0	122.5	237.8	238.3	6.44	6.32	11.7	11.9	22.7	23.4	5.01	4.99	10.6	10.5	21.3	21.4
6	63.4	62.5	123.0	122.4	237.5	237.5	6.77	6.38	11.7	12.1	22.6	22.3	5.12	5.06	10.9	10.6	21.4	21.4
7	64.0	64.1	122.6	121.6	237.2	236.5	6.40	6.56	12.0	11.9	23.0	22.8	4.98	5.06	10.4	10.6	21.0	21.1
8	64.1	63.8	122.7	122.5	237.8	236.5	6.40	6.32	12.6	11.8	22.2	21.9	5.06	5.04	10.5	10.5	21.6	21.1
9	63.7	64.4	123.1	121.2	236.7	237.2	6.44	6.67	12.4	12.1	22.5	22.5	4.94	5.09	10.5	10.4	21.0	21.1
10	63.9	64.0	120.1	122.4	238.0	238.4	6.52	6.68	11.9	11.7	22.6	22.9	5.08	4.98	10.6	10.5	21.6	21.1
11	64.0	63.7	122.3	122.1	238.2	236.5	6.57	6.62	12.1	12.0	23.5	23.4	5.06	5.04	10.4	10.5	21.3	21.0
12	64.1	63.7	121.8	122.1	236.6	236.3	6.63	6.64	12.4	11.9	22.2	22.0	4.96	5.00	10.6	10.6	21.1	21.0
13	63.9	63.7	121.9	122.1	237.8	236.9	6.49	6.40	11.9	12.1	22.7	22.7	5.09	5.07	10.5	10.5	21.0	21.2
14	63.7	63.5	121.4	122.0	238.3	237.3	6.42	6.42	11.4	11.5	23.0	23.1	4.95	5.00	10.3	10.2	21.0	21.0
15	64.0	63.9	123.0	121.9	238.0	238.2	6.51	6.53	12.3	12.3	22.5	22.7	4.98	4.97	10.4	10.5	21.2	20.9
Grubbs																		
$\overline{\overline{C}}$	63.4		122.1		237.7		6.48		12.0		22.7		5.06		10.5		21.2	
S	0.760		0.356		0.663		0.097		0.242		0.387		0.086		0.119		0.165	
G_p	0.817		1.538		2.087		1.618		1.370		1.844		2.186		2.122		1.787	
G_1	2.393		2.540		1.806		1.488		2.089		1.817		1.022		2.001		1.337	
$G_{crit\ 1\%}$	2.806																	
$G_{crit\ 5\%}$	2.549																	
Cohran																		
max S^2	1.296		2.460		1.367		0.100		0.275		0.203		0.013		0.049		0.137	
C	0.454		0.400		0.318		0.292		0.395		0.384		0.239		0.334		0.288	
$C_{crit\ 1\%}$	0.471																	
$C_{crit\ 5\%}$	0.575																	

Table 3.4 – Outliers test results for 2-methylpropan-1-ol, butan-1-ol and 3-methylbutan-1-ol

Day t	2-methylpropan-1-ol						butan-1-ol						3-methylbutan-1-ol					
	SS-3		SS-2		SS-1		SS-3		SS-2		SS-1		SS-3		SS-2		SS-1	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
1	5.11	5.03	10.6	11.0	21.6	21.3	5.06	5.05	10.5	10.6	21.4	21.1	5.04	4.88	10.4	10.1	20.3	20.7
2	5.13	5.15	10.6	10.7	21.3	21.5	5.02	5.04	10.5	10.3	21.2	21.3	5.01	4.93	10.6	10.4	20.5	20.6
3	5.00	5.14	10.7	10.7	21.7	21.2	5.09	5.07	10.5	10.7	21.4	21.6	5.04	4.97	10.3	10.5	20.7	20.9
4	5.16	5.14	10.7	10.8	21.8	21.7	4.88	5.01	10.7	10.1	21.2	21.6	4.95	4.95	10.3	10.5	20.8	20.6
5	5.17	4.91	10.7	10.6	21.2	21.4	5.15	5.18	10.3	10.5	21.5	21.1	4.95	4.89	10.3	10.2	21.0	20.7
6	5.19	5.13	10.4	10.5	21.2	21.6	4.98	4.91	10.5	10.7	21.4	21.2	4.94	4.92	10.2	10.3	21.0	20.8
7	5.17	5.14	10.3	10.5	21.4	21.6	5.05	5.20	10.6	10.8	20.9	21.6	5.10	4.90	10.2	10.2	21.0	20.8
8	5.06	5.19	10.6	10.7	21.5	21.4	5.13	4.87	10.3	10.3	21.4	21.5	4.97	4.99	10.2	10.2	21.1	20.7
9	5.01	5.14	10.7	10.7	21.6	21.3	5.33	4.93	10.6	10.8	21.5	21.3	4.92	4.97	10.3	10.3	20.9	20.9
10	5.11	5.03	10.7	10.6	21.4	21.6	5.10	5.17	10.6	10.6	21.2	21.2	4.96	4.86	10.2	10.2	20.7	20.7
11	5.16	5.10	10.7	10.6	21.4	21.3	5.14	5.00	10.6	10.8	20.9	21.2	5.01	4.97	10.2	10.3	20.9	20.7
12	5.15	5.20	10.5	10.7	21.5	21.3	5.11	4.91	10.4	10.8	21.6	21.3	4.95	4.88	10.3	10.3	20.7	20.9
13	5.15	5.15	10.5	10.4	21.6	21.6	5.06	5.12	10.7	10.5	21.3	21.1	4.97	4.86	10.3	10.2	20.7	20.9
14	5.16	5.09	10.6	10.5	21.5	21.6	5.05	5.11	10.8	10.6	21.3	21.0	4.89	4.91	10.3	10.3	20.8	20.7
15	5.16	5.14	10.5	10.7	21.6	21.6	5.02	5.33	10.8	10.6	21.4	21.1	4.96	4.89	10.4	10.3	20.9	20.6
Grubbs																		
$\overline{\overline{C}}$	5.12		10.6		21.5		5.07		10.6		21.3		4.95		10.3		20.8	
S	0.042		0.110		0.118		0.072		0.136		0.123		0.035		0.092		0.127	
G_p	1.306		1.758		2.275		1.471		1.126		1.526		1.600		2.503		1.102	
G_1	1.886		1.753		1.693		1.734		2.274		1.977		1.354		1.105		2.500	
$G_{crit\ 1\%}$	2.806																	
$G_{crit\ 5\%}$	2.549																	
Cohran																		
max S^2	0.033		0.073		0.135		0.080		0.152		0.192		0.019		0.033		0.080	
C	0.446		0.382		0.334		0.366		0.315		0.295		0.337		0.262		0.207	
$C_{crit\ 1\%}$	0.471																	
$C_{crit\ 5\%}$	0.575																	

3.1.4 Precision, trueness and uncertainty

The accepted values of the precision parameters of the method are presented in table 3.5.

Table 3.5 – The accepted values of metrological characteristics of the method

Compound	Standard solution	General mean, $\bar{\bar{C}}$, mg/L AA	Limit of repeatability, r, %	Limit of intermediate precision, $r_{I(TO)}$, %	Variation of the estimate of the bias, s_δ, mg/L AA	Border of confidence interval of the laboratory bias, mg/L AA		Relative expanded uncertainty U (P = 0.95, k = 2), %
						left	right	
acetaldehyde	3	6.76	4.1	4.9	0.025	-0.042	0.055	4.1
	2	12.8	3.3	4.7	0.048	-0.087	0.101	4.0
	1	24.5	3.1	4.4	0.086	-0.219	0.120	3.9
methyl acetate	3	6.19	5.1	6.5	0.031	-0.102	0.019	5.3
	2	12.8	4.5	5.1	0.047	-0.182	0.003	4.5
	1	26.0	4.4	4.8	0.088	-0.341	0.005	4.3
ethyl acetate	3	5.49	3.1	3.9	0.016	-0.029	0.035	3.6
	2	11.4	3.0	3.6	0.031	-0.040	0.082	3.5
	1	23.0	2.0	2.5	0.043	-0.047	0.124	2.9
methanol	3	63.4	1.9	3.8	0.209	-0.593	0.226	3.0
	2	122.1	1.5	1.5	0.128	-0.382	0.122	1.3
	1	237.7	0.6	1.0	0.190	-0.641	0.104	1.0
propan-2-ol	3	6.48	6.4	7.6	0.037	-0.091	0.054	6.0
	2	12.0	5.0	7.4	0.073	-0.105	0.182	5.9
	1	22.7	2.3	5.2	0.105	-0.122	0.291	4.6
propan-1-ol	3	5.06	3.3	5.7	0.025	-0.030	0.067	4.9
	2	10.5	2.6	4.1	0.035	-0.016	0.123	3.9
	1	21.2	2.3	3.2	0.053	-0.020	0.189	3.4
2-methylpropan-1-ol	3	5.12	3.8	4.4	0.017	-0.056	0.010	4.1
	2	10.6	2.9	4.1	0.035	-0.107	0.030	3.9
	1	21.5	2.1	2.6	0.043	-0.142	0.025	3.2
butan-1-ol	3	5.07	6.6	7.7	0.029	-0.052	0.060	6.1
	2	10.6	4.7	5.9	0.048	-0.025	0.161	5.1
	1	21.3	2.7	3.1	0.049	-0.023	0.169	3.4
3-methylbutan-1-ol	3	4.95	3.5	4.0	0.014	-0.025	0.031	3.8
	2	10.3	2.5	3.5	0.029	-0.009	0.105	3.7
	1	20.8	2.1	2.7	0.044	-0.012	0.160	3.3

3.2 Results of method approbation study

3.2.1 Calibration

The results of calibration coefficients calculations are presented in table 3.6.

Table 3.6 – The results of calibration

Compound	Calibration coefficient, obtained for traditional internal standard method, RRF^T(RSD*)	Calibration coefficient, obtained for developed internal standard method, RRF^D(RSD*)
bornyl acetate	0.793(1.5)	0.457(0.4)
menthol	0.867(0.9)	0.500(1.1)

* RSD – relative standard deviation, expressed in %

3.2.2 Concentration

The results of calculation of concentrations of analytes are presented in table 3.7.

Table 3.7 – The concentrations of analytes in sample «Urolesan»

Compound	Concentration, obtained using traditional internal standard method, C_T, mg/L AA	Concentration, obtained using developed internal standard method, C_D, mg/L AA	Relative difference between results, Δ, %
bornyl acetate	35515	36176	-1.8
menthol	13247	13495	-1.9

3.2.3 Bias of the results

The biases of the results of calculation of concentrations of analytes are presented in table 3.8.

Table 3.8 – The concentrations of analytes in sample «Urolesan»

Compound	Concentration, declared in certificate of quality, μ, mg/mL	Concentration, obtained using traditional internal standard method, C_T, mg/mL	Bias of concentration, obtained using traditional internal standard method, δ_T, %	Concentration, obtained using developed internal standard method, C_D, mg/mL	Bias of concentration, obtained using developed internal standard method, δ_D, %
bornyl acetate	24.4	24.15	-1.0	24.60	0.8
menthol	9.1	9.01	-1.0	9.18	0.8

The results obtained show that the developed method is suitable for the analysis of ethanol-containing drugs and is not inferior in accuracy to the traditional internal standard method. It should be noted that the results obtained by the developed method are closer to the true value (Table 3.8) compared to the traditional method.

CONCLUSIONS

An analytical method for direct determination of mass concentrations of 9 volatile compounds: acetaldehyde, methyl acetate, ethyl acetate, methanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol by GC-FID was developed and validated for rectified ethyl alcohol and ethanol containing products, with satisfactory performance.

According to the results of measurements of gravimetrically prepared standard solutions precision, accuracy, uncertainty, linearity and limits of quantification were estimated. Thus, the method showed satisfactory precision with repeatability limit ranging from 0.6 % to 6.6 %, intermediate precision limit ranging from 1.0 % to 7.7%, good linearity ($R^2 \geq 0.999$) and accuracy with biases ranging from -0.7% to 0.6%. Analysis of trueness showed that laboratory bias is insignificant at the significance level $\alpha = 5$ %. The *LOQs* were found to be in the range from 0.130 to 0.494 mg/L AA and the *LODs* between 0.432 and 1.646 mg/L AA. The values of relative expanded uncertainty ($P = 95$ %, $k = 2$) were found to be in the range from 1.0 to 6.1 %.

Approbation of the method performed on a real pharmaceutical ethanol-containing product "Urolesan" showed that the developed method allows obtaining results close to the true values of the concentration of analytes in solution and is simpler and faster.

The robustness of the method and its selectivity allowed its application to the measurement of volatile compounds of rectified ethyl alcohol and ethanol containing products. All of these results show that this method is suitable for routine determination of volatile compounds for the quality control of ethyl alcohol samples by both manufacturers and control laboratories.

APPENDIX A
THE RESULTS OF CALIBRATION

Table A.1 – The results of calibration

acetaldehyde														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*b*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$8.47 \cdot 10^{-6}$	$5.30 \cdot 10^{-6}$	$-4.34 \cdot 10^{-7}$	0.681	$3.00 \cdot 10^{-7}$	$2.64 \cdot 10^{-7}$	1.648	0.663	1.507	$3.48 \cdot 10^{-7}$	$3.79 \cdot 10^{-7}$	4.5	11.5
	2	$8.47 \cdot 10^{-6}$	$5.37 \cdot 10^{-6}$											
CS-2	1	$1.63 \cdot 10^{-5}$	$1.04 \cdot 10^{-5}$									$4.03 \cdot 10^{-7}$	2.5	6.3
	2	$1.63 \cdot 10^{-5}$	$1.10 \cdot 10^{-5}$											
CS-1	1	$3.26 \cdot 10^{-5}$	$2.15 \cdot 10^{-5}$									$4.93 \cdot 10^{-7}$	1.5	3.9
	2	$3.26 \cdot 10^{-5}$	$2.20 \cdot 10^{-5}$											
methyl acetate														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*b*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$7.81 \cdot 10^{-6}$	$4.59 \cdot 10^{-6}$	$3.09 \cdot 10^{-7}$	0.577	$2.98 \cdot 10^{-7}$	$2.46 \cdot 10^{-7}$	1.256	0.589	1.698	$3.42 \cdot 10^{-7}$	$4.19 \cdot 10^{-7}$	5.4	13.8
	2	$7.81 \cdot 10^{-6}$	$4.73 \cdot 10^{-6}$											
CS-2	1	$1.66 \cdot 10^{-5}$	$1.00 \cdot 10^{-5}$									$4.48 \cdot 10^{-7}$	2.7	7.0
	2	$1.66 \cdot 10^{-5}$	$1.02 \cdot 10^{-5}$											
CS-1	1	$3.47 \cdot 10^{-5}$	$2.06 \cdot 10^{-5}$									$5.46 \cdot 10^{-7}$	1.6	4.0
	2	$3.47 \cdot 10^{-5}$	$1.99 \cdot 10^{-5}$											
ethyl acetate														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*b*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$6.87 \cdot 10^{-6}$	$6.37 \cdot 10^{-6}$	$4.83 \cdot 10^{-7}$	0.874	$2.92 \cdot 10^{-7}$	$2.41 \cdot 10^{-7}$	2.003	0.895	1.118	$3.92 \cdot 10^{-7}$	$3.16 \cdot 10^{-7}$	4.6	11.8
	2	$6.87 \cdot 10^{-6}$	$6.35 \cdot 10^{-6}$											
CS-2	1	$1.46 \cdot 10^{-5}$	$1.37 \cdot 10^{-5}$									$3.38 \cdot 10^{-7}$	2.3	6.0
	2	$1.46 \cdot 10^{-5}$	$1.32 \cdot 10^{-5}$											
CS-1	1	$3.05 \cdot 10^{-5}$	$2.74 \cdot 10^{-5}$									$4.12 \cdot 10^{-7}$	1.3	3.5
	2	$3.05 \cdot 10^{-5}$	$2.68 \cdot 10^{-5}$											
methanol														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*B*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$7.98 \cdot 10^{-5}$	$6.04 \cdot 10^{-5}$	$2.23 \cdot 10^{-6}$	0.744	$1.60 \cdot 10^{-6}$	$1.38 \cdot 10^{-6}$	1.614	0.753	1.328	$1.97 \cdot 10^{-6}$	$1.89 \cdot 10^{-6}$	2.4	6.1
	2	$7.98 \cdot 10^{-5}$	$6.00 \cdot 10^{-5}$											
CS-2	1	$1.57 \cdot 10^{-4}$	$1.21 \cdot 10^{-4}$									$2.02 \cdot 10^{-6}$	1.3	3.3
	2	$1.57 \cdot 10^{-4}$	$1.20 \cdot 10^{-4}$											
CS-1	1	$3.15 \cdot 10^{-4}$	$2.36 \cdot 10^{-4}$									$2.45 \cdot 10^{-6}$	0.8	2.0
	2	$3.15 \cdot 10^{-4}$	$2.36 \cdot 10^{-4}$											

Continuation of Table A.1

propan-2-ol														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*B*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$8.16 \cdot 10^{-6}$	$1.05 \cdot 10^{-5}$	$9.14 \cdot 10^{-7}$	1.190	$5.89 \cdot 10^{-7}$	$5.28 \cdot 10^{-7}$	1.730	1.231	0.812	$7.44 \cdot 10^{-7}$	$4.40 \cdot 10^{-7}$	5.4	13.9
	2	$8.16 \cdot 10^{-6}$	$1.02 \cdot 10^{-5}$											
CS-2	1	$1.53 \cdot 10^{-5}$	$1.95 \cdot 10^{-5}$									$4.70 \cdot 10^{-7}$	3.1	7.9
	2	$1.53 \cdot 10^{-5}$	$1.95 \cdot 10^{-5}$											
CS-1	1	$3.00 \cdot 10^{-5}$	$3.72 \cdot 10^{-5}$									$5.63 \cdot 10^{-7}$	1.9	4.8
	2	$3.00 \cdot 10^{-5}$	$3.57 \cdot 10^{-5}$											
propan-1-ol														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*B*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$6.44 \cdot 10^{-6}$	$8.94 \cdot 10^{-6}$	$6.16 \cdot 10^{-7}$	1.341	$5.46 \cdot 10^{-7}$	$4.51 \cdot 10^{-7}$	1.366	1.370	0.730	$6.40 \cdot 10^{-7}$	$3.37 \cdot 10^{-7}$	5.2	13.4
	2	$6.44 \cdot 10^{-6}$	$8.62 \cdot 10^{-6}$											
CS-2	1	$1.37 \cdot 10^{-5}$	$1.98 \cdot 10^{-5}$									$3.61 \cdot 10^{-7}$	2.6	6.8
	2	$1.37 \cdot 10^{-5}$	$1.94 \cdot 10^{-5}$											
CS-1	1	$2.86 \cdot 10^{-5}$	$3.88 \cdot 10^{-5}$									$4.39 \cdot 10^{-7}$	1.5	3.9
	2	$2.86 \cdot 10^{-5}$	$3.87 \cdot 10^{-5}$											
2-methylpropan-1-ol														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*B*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$6.34 \cdot 10^{-6}$	$1.08 \cdot 10^{-5}$	$1.56 \cdot 10^{-7}$	1.745	$7.04 \cdot 10^{-7}$	$5.81 \cdot 10^{-7}$	0.269	1.753	0.571	$7.09 \cdot 10^{-7}$	$2.91 \cdot 10^{-7}$	4.6	11.8
	2	$6.34 \cdot 10^{-6}$	$1.06 \cdot 10^{-5}$											
CS-2	1	$1.35 \cdot 10^{-5}$	$2.43 \cdot 10^{-5}$									$3.12 \cdot 10^{-7}$	2.3	6.0
	2	$1.35 \cdot 10^{-5}$	$2.46 \cdot 10^{-5}$											
CS-1	1	$2.82 \cdot 10^{-5}$	$4.95 \cdot 10^{-5}$									$3.81 \cdot 10^{-7}$	1.4	3.5
	2	$2.82 \cdot 10^{-5}$	$4.86 \cdot 10^{-5}$											
butan-1-ol														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*B*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$6.19 \cdot 10^{-6}$	$9.68 \cdot 10^{-6}$	$4.38 \cdot 10^{-7}$	1.536	$4.34 \cdot 10^{-7}$	$3.59 \cdot 10^{-7}$	1.223	1.557	0.642	$4.95 \cdot 10^{-7}$	$2.29 \cdot 10^{-7}$	3.7	9.5
	2	$6.19 \cdot 10^{-6}$	$9.48 \cdot 10^{-6}$											
CS-2	1	$1.31 \cdot 10^{-5}$	$2.11 \cdot 10^{-5}$									$2.45 \cdot 10^{-7}$	1.9	4.8
	2	$1.31 \cdot 10^{-5}$	$2.13 \cdot 10^{-5}$											
CS-1	1	$2.75 \cdot 10^{-5}$	$4.24 \cdot 10^{-5}$									$2.99 \cdot 10^{-7}$	1.1	2.8
	2	$2.75 \cdot 10^{-5}$	$4.26 \cdot 10^{-5}$											
3-methylbutan-1-ol														
level, *k*	measurement, *j*	*X*	*Y*	*a*	*B*	S_0	S_a	t_a	b_{corr}	*RRF*	$S_{0(corr)}$	S_x	S_x, %	*K*, %
CS-3	1	$6.19 \cdot 10^{-6}$	$1.09 \cdot 10^{-5}$	$7.22 \cdot 10^{-7}$	1.662	$5.90 \cdot 10^{-7}$	$4.87 \cdot 10^{-7}$	1.482	1.697	0.589	$7.08 \cdot 10^{-7}$	$3.01 \cdot 10^{-7}$	4.9	12.5
	2	$6.19 \cdot 10^{-6}$	$1.06 \cdot 10^{-5}$											
CS-2	1	$1.31 \cdot 10^{-5}$	$2.37 \cdot 10^{-5}$									$3.21 \cdot 10^{-7}$	2.4	6.3
	2	$1.31 \cdot 10^{-5}$	$2.22 \cdot 10^{-5}$											
CS-1	1	$2.75 \cdot 10^{-5}$	$4.66 \cdot 10^{-5}$									$3.92 \cdot 10^{-7}$	1.4	3.7
	2	$2.75 \cdot 10^{-5}$	$4.61 \cdot 10^{-5}$											

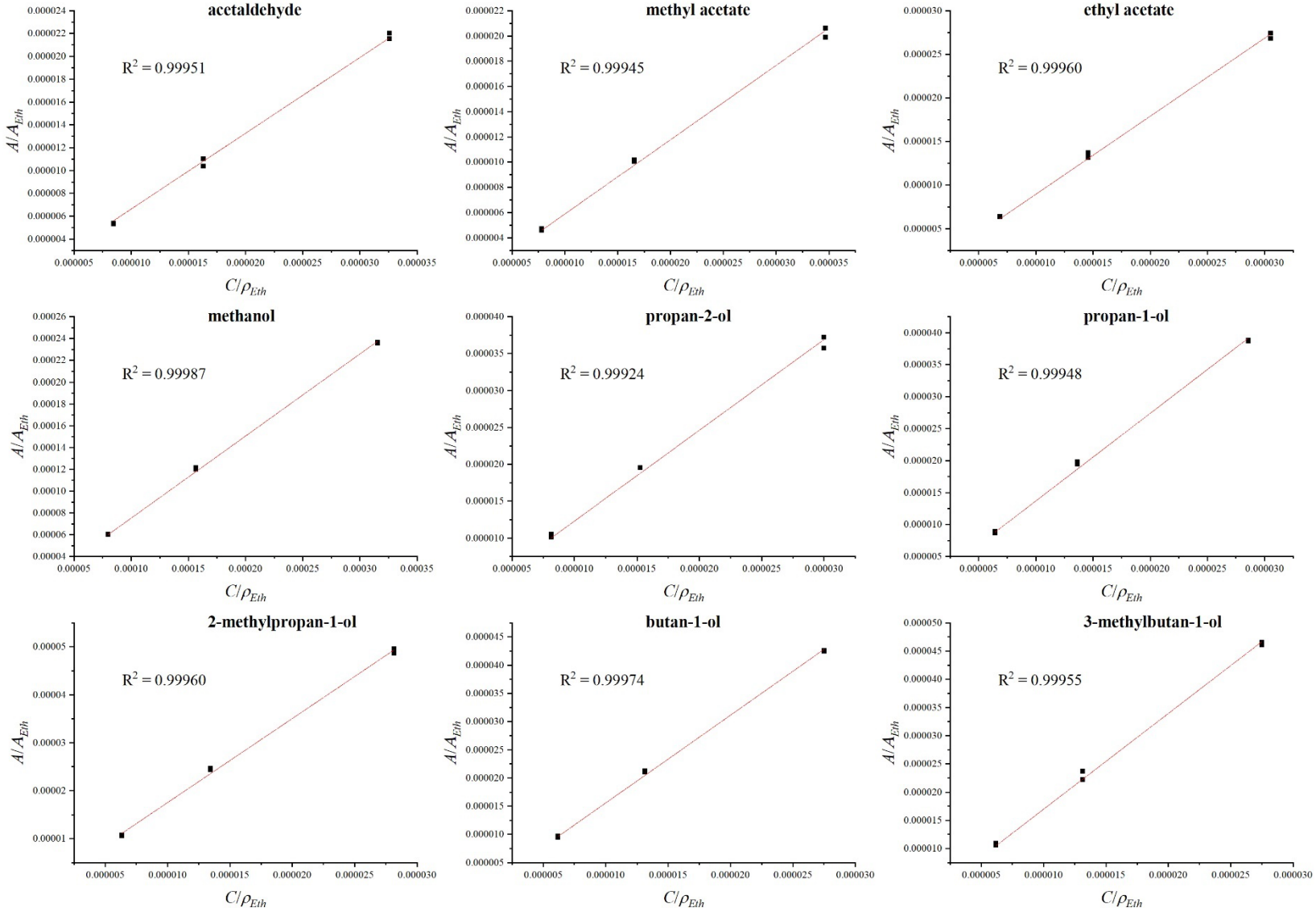
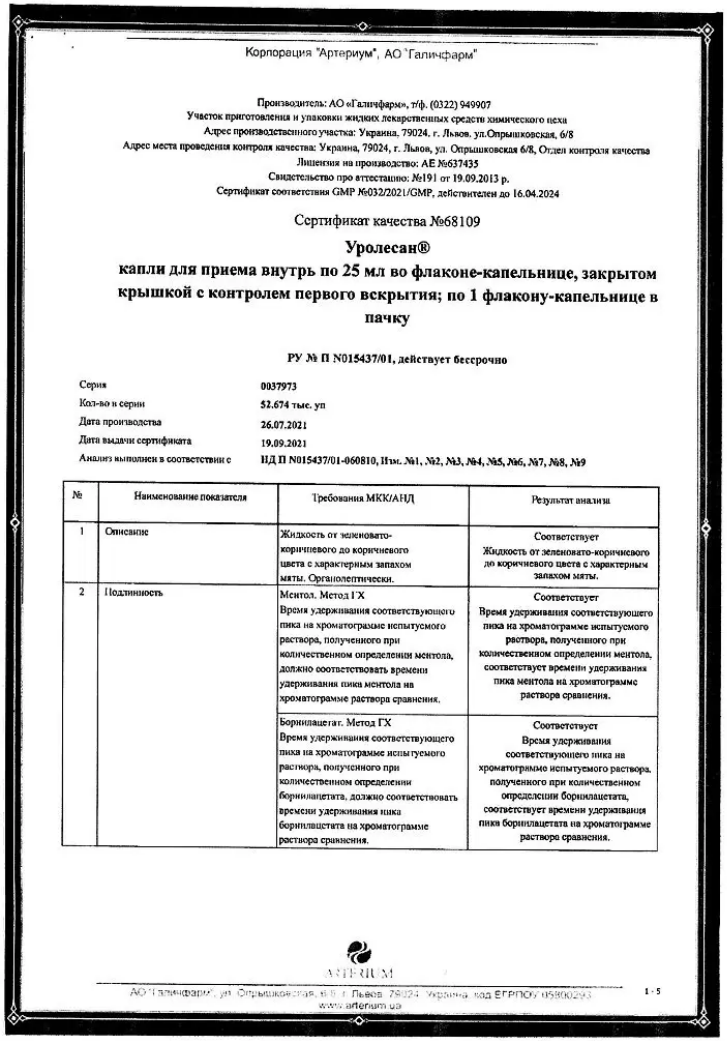


Figure A.1 – The linearity graphs for studied volatile compounds

APPENDIX B
CERTIFICATE OF QUALITY OF DRUG «UROLESAN»



Корпорация "Артериум", АО "Галичфарм"

Производитель: АО «Галичфарм», т/ф. (0322) 949907
Участок приготовления и упаковки жидких лекарственных средств химического цеха
Адрес производственного участка: Украина, 79024, г. Львов, ул.Опрышковская, 6/8
Адрес места проведения контроля качества: Украина, 79024, г. Львов, ул. Опрышковская 6/8, Отдел контроля качества
Лицензия на производство: АЕ №637435
Свидетельство про аттестацию: №191 от 19.09.2013 р.
Сертификат соответствия GMP №032/2021/GMP, действителен до 16.04.2024

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РУ № П N015437/01, действует бессрочно

Серия	0037973
Кол-во в серии	52.674 тыс. уп
Дата производства	26.07.2021
Дата выдачи сертификата	19.09.2021
Анализ выполнен в соответствии с	НД П N015437/01-060810, Изм. №1, №2, №3, №4, №5, №6, №7, №8, №9

№	Наименование показателя	Требования МКК/АНД	Результат анализа
1	Описание	Жидкость от зеленовато-коричневого до коричневого цвета с характерным запахом мяты. Органолептически.	Соответствует Жидкость от зеленовато-коричневого до коричневого цвета с характерным запахом мяты.
2	Подлинность	Ментол. Метод ГХ Время удерживания соответствующего пика на хроматограмме испытуемого раствора, полученного при количественном определении ментола, должно соответствовать времени удерживания пика ментола на хроматограмме раствора сравнения.	Соответствует Время удерживания соответствующего пика на хроматограмме испытуемого раствора, полученного при количественном определении ментола, соответствует времени удерживания пика ментола на хроматограмме раствора сравнения.
		Борнилацетат. Метод ГХ Время удерживания соответствующего пика на хроматограмме испытуемого раствора, полученного при количественном определении борнилацетата, должно соответствовать времени удерживания пика борнилацетата на хроматограмме раствора сравнения.	Соответствует Время удерживания соответствующего пика на хроматограмме испытуемого раствора, полученного при количественном определении борнилацетата, соответствует времени удерживания пика борнилацетата на хроматограмме раствора сравнения.

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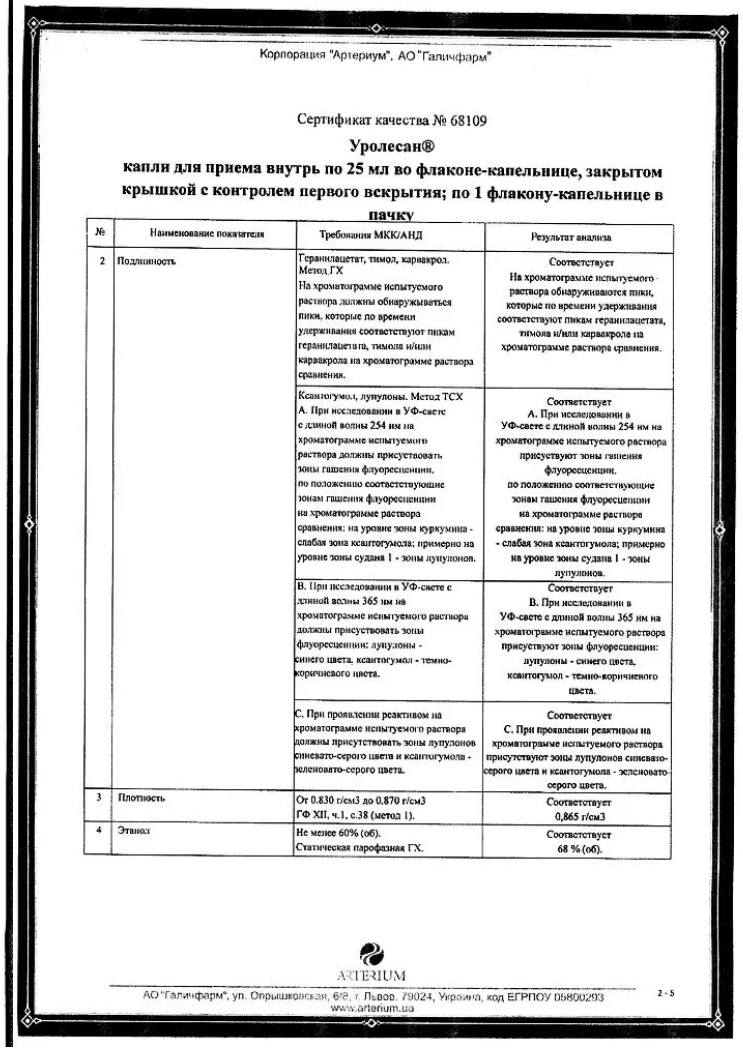

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№	Наименование показателя	Требования МКК/АНД	Результат анализа
2	Подлинность	Геранилацетат, тимол, карвакрол. Метод ГХ На хроматограмме испытуемого раствора должны обнаруживаться пики, которые по времени удерживания соответствуют пикам геранилацетата, тимола и/или карвакрола на хроматограмме раствора сравнения.	Соответствует На хроматограмме испытуемого раствора обнаруживаются пики, которые по времени удерживания соответствуют пикам геранилацетата, тимола и/или карвакрола на хроматограмме раствора сравнения.
		Ксантогумол, лупулоны. Метод ТСХ А. При исследовании в УФ-свете с длиной волны 254 нм на хроматограмме испытуемого раствора должны присутствовать зоны гашения флуоресценции, по положению соответствующие зонам гашения флуоресценции на хроматограмме раствора сравнения: на уровне зоны куркумина - слабая зона ксантогумола; примерно на уровне зоны судана 1 - зоны лупулонов.	Соответствует А. При исследовании в УФ-свете с длиной волны 254 нм на хроматограмме испытуемого раствора присутствуют зоны гашения флуоресценции, по положению соответствующие зонам гашения флуоресценции на хроматограмме раствора сравнения: на уровне зоны куркумина - слабая зона ксантогумола; примерно на уровне зоны судана 1 - зоны лупулонов.
		В. При исследовании в УФ-свете с длиной волны 365 нм на хроматограмме испытуемого раствора должны присутствовать зоны флуоресценции: лупулоны - синего цвета, ксантогумол - темно-коричневого цвета.	Соответствует В. При исследовании в УФ-свете с длиной волны 365 нм на хроматограмме испытуемого раствора присутствуют зоны флуоресценции: лупулоны - синего цвета, ксантогумол - темно-коричневого цвета.
		С. При проявлении реактивом на хроматограмме испытуемого раствора должны присутствовать зоны лупулонов сине-серого цвета и ксантогумола - зеленовато-серого цвета.	Соответствует С. При проявлении реактивом на хроматограмме испытуемого раствора присутствуют зоны лупулонов сине-серого цвета и ксантогумола - зеленовато-серого цвета.
3	Плотность	От 0.830 г/см3 до 0.870 г/см3 ГФ XII, ч.1, с.38 (метод 1).	Соответствует 0,865 г/см3
4	Этанол	Не менее 60% (об). Статическая парофазная ГХ.	Соответствует 68 % (об).

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№	Наименование показателя	Требования МКК/АНД	Результат анализа
5	Объем содержимого упаковки	Должен соответствовать требованиям. ГФ XI.	Соответствует 25 мл
6	Микробиологическая чистота	Общее количество аэробных бактерий не более 10000 в1 мл. ГФ XII, ч.1, с.160 Категория 3Б.	Соответствует Общее количество аэробных бактерий: <200 в 1 мл.
		Общее количество грибов не более 100 в 1 мл. ГФ XII, ч.1, с.160 Категория 3Б.	Соответствует Общее количество грибов : <50 в 1 мл.
		Энтеробактерий и некоторых других грамотрицательных бактерий не более 100 в 1 мл. ГФ XII, ч.1, с.160 Категория 3Б.	Соответствует Энтеробактерий и некоторых других грамотрицательных бактерий : <10 в 1 мл
		Отсутствие E.coli в 1 мл. ГФ XII, ч.1, с.160 Категория 3Б.	Соответствует Отсутствует E.coli в 1 мл.
		Отсутствие Salmonella в 10 мл. ГФ XII, ч.1, с.160 Категория 3Б.	Соответствует Отсутствует Salmonella в 10 мл.
		Отсутствие Staphylococcus aureus в 1 мл. ГФ XII, ч.1, с.160 Категория 3Б.	Соответствует Отсутствует Staphylococcus aureus в 1 мл.
7	Количественное определение	Ментол - не менее 7,2 мг/мл. Метод ГХ	Соответствует 9,1 мг/мл.
		Борнилацетат - не менее 16 мг/мл. Метод ГХ.	Соответствует 24,4 мг/мл.
		Сумма флавоноидов, в пересчете на гиперозид, - не менее 0,2 мг/мл. Метод абсорбционной спектрометрии в видимой области спектра.	Соответствует 0,7 мг/мл

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№	Наименование показателя	Требования МКК/АНД	Результат анализа
8	Упаковка	По 25 мл во флакон-капельницу оранжевого стекла, укупоренный пробкой и крышкой пластмассовой. На флакон наклеивают этикетку. Каждый флакон вместе с пробкой-капельницей и инструкцией по применению вкладывают в пачку. По 25 мл во флакон-капельницу оранжевого стекла, укупоренный пробкой-капельницей и укупорочно-навинчиваемой крышкой с контролем первого вскрытия, или укупорочно-навинчиваемой крышкой с логотипом Корпорации «Артериум» с контролем первого вскрытия. На флакон наклеивают этикетку. Каждый флакон вместе с инструкцией по применению вкладывают в пачку.	Соответствует По 25 мл во флаконе-капельнице оранжевого стекла, укупоренный пробкой-капельницей и укупорочно-навинчиваемой крышкой с логотипом Корпорации «Артериум» с контролем первого вскрытия. На флакон наклеена этикетка. Каждый флакон вместе с инструкцией по применению вложен в пачку.
9	Маркировка	На этикетке на русском языке указывают: торговое наименование препарата с предупредительной маркировкой ® и торговое наименование препарата на английском языке без предупредительной маркировки ®, лекарственную форму, условия хранения, объем в миллилитрах, регистрационный номер, номер серии, дату выпуска и дату окончания срока годности «До». Дополнительно указывают логотип КорпорацииАртериум» (на латинице).	Соответствует На этикетке на русском языке указано: торговое наименование препарата с предупредительной маркировкой ® и торговое наименование препарата на английском языке без предупредительной маркировки ®, лекарственная форма, условия хранения, объем в миллилитрах, регистрационный номер, номер серии, дата выпуска и дата окончания срока годности «До». Указан логотип Корпорации «Артериум» (на латинице).

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№	Наименование показателя	Требования МКК/АНД	Результат анализа
9	Маркировка	На пачке на русском языке указывают: наименование производителя АО «Галичфарм» и его адрес, торговое наименование препарата с предупредительной маркировкой ® и торговое наименование препарата на английском языке без предупредительной маркировки®, «Группировочное наименование: Душицы обыкновенной травы экстракт +Клещевины обыкновенной семян масло+Моркови дикой семян экстракт +Мяты перечной листьев масло+Пихты масло+Хмеля солодий экстракт», лекарственную форму, объем в миллилитрах, качественный и количественный состав препарата на 1 мл. «Хранить в недоступном для детей месте», номер серии, дату изготовления, дату окончания срока годности «До», номер регистрационного удостоверения, условия хранения, условия отпуска, штриховой код, голограмму, номенклатурный код ТМЦ согласно информационной системы,техническую информацию полиграфического производителя. Дополнительно указывают логотип Корпорации «Артериум» (на латинице). На пачке допускается нанесение средства идентификации лекарственного препарата. На пачке допускается нанесение стикера контроля первого вскрытия.	Соответствует На пачке на русском языке указано: наименование производителя АО «Галичфарм» и его адрес, торговое наименование препарата с предупредительной маркировкой ® и торговое наименование препарата на английском языке без предупредительной маркировки®, «Группировочное наименование: Душицы обыкновенной травы экстракт +Клещевины обыкновенной семян масло+Моркови дикой семян экстракт +Мяты перечной листьев масло+Пихты масло+Хмеля солодий экстракт », лекарственную форму, объем в миллилитрах, качественный и количественный состав препарата на 1 мл, «Хранить в недоступном для детей месте», номер серии, дата изготовления, дата окончания срока годности «До», номер регистрационного удостоверения, условия хранения, условия отпуска, штриховой код, голограмма, номенклатурный код ТМЦ согласно информационной истемы,техническая информация полиграфического производителя логотип Корпорации «Артериум» (на латинице). На пачке нанесено средство идентификации лекарственного препарата. На пачке нанесен стикер контроля первого вскрытия.

Срок годности: 2.00 г. Годен до: 06.2023

Хранение: **В оригинальной упаковке при температуре не выше 25°С.**

Заключение: **Соответствует требованиям НД П N015437/01-060810, Изм. №1, №2, №3, №4, №5, №6, №7,№8, №9 «Страна импортер: Российская Федерация»**

Начальник ОКК Павлюк Инесса Витальевна

Разрешено к реализации

«Этим я свидетельствую, что приведенная выше информация является достоверной и точной. Данная серия продукции была произведена (включая упаковку и маркировку) [illegible] указанном участке и процесс контроль ее качества в соответствии с требованиями GMP,установленными [illegible] регуляторным органом [illegible] в соответствии со спецификациями, которые включены в регистрационное досье. Процессы производства, контроля и анализа были оценены и установлено соответствие GMP»

Уполномоченное лицо по качеству Кирцей Яна Владимировна

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