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ARTICLES

Procedure for the Analysis of Sedative Drugs with the Use of an Array of Piezoelectric Sensors (Exemplified in the Determination of the Drug Corvalol)

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Abstract—A procedure was developed for the headspace analysis of sedative drugs with the use of a quartz piezoelectric microbalance array. The effects of the nature of film coatings in piezoelectric cell electrodes on the working weight range and the sorption properties of sorbent films were found. An array of six piezoelectric sensors with different response functions in the vapors of the drug Corvalol and its highly volatile constituents was proposed. The procedure is suitable for the quality assessment of other pharmaceuticals based on ethyl alcohol and natural peppermint oil.

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An increasing portion of adulterated drugs is a problem of considerable current interest. The adulteration level of some pharmaceuticals is 50% [1]. Along with the amount of medicinal agents supplied to drugstores, the quality of drugs plays an important role in solving the problem of the public provision of pharmaceuticals [2]. Therefore, inspection methods with the use of miniature, simple, and inexpensive measuring instruments are required to obtain real-time information without complex stages of sampling and sample preparation; these instruments should be accessible to small analytical laboratories and drugstores.

Modern physicochemical techniques are used for drug control and the determination of drug metabolites in the human body and environmental samples. The authentication and quality assessment of substances and pharmaceutical dosage forms are performed by chromatography and various spectrophotometric techniques including NMR spectroscopy and mass spectrometry [3, 4]. Chromatographic techniques are the most selective [5, 6]. Although the theory and practice of chromatographic analysis has been highly developed, it is often difficult to reliably identify substances in a multicomponent mixture based only on the retention characteristics. Thus, it is reasonable to use highly selective detectors and multidetector systems (two or three detectors with different sensitivities and selectivities) [7]. Complex mixtures of natural materials are currently analyzed by chromatography; in this case, the identification of individual components is often difficult to perform or impossible. An approach based on comparing chromatograms obtained under identical conditions using the principle of coincidence (fingerprints) is practicable. In this context, alternative spectroscopic and biological methods of analysis have been actively developed [8, 9]. Biological methods of analysis based on the use of various microorganism species are characterized by their reliability and a high reproducibility of the results [10]. However, microorganisms that respond to any changes in the environment (temperature, pressure, and oxygen content) are frequently used in analysis; because of this, the testing of drug preparations is not always correct [11].

Sensor methods of analysis, including the piezoelectric quartz crystal microbalance method, are in wide current use for the analysis of pharmaceuticals [12]. The design features of a piezoelectric detector (cell geometry), the rated frequency of the quartz wafer, the electrode material, and (to a greater extent) the properties of the electrode coating affect the sensitivity of microweighing and the selectivity of determination. The properties of modifier films on piezoelectric resonator electrodes are responsible for the disadvantages of the method: the low reproducibility of responses, the instability of signals, and the low selectivity of analyte sorption.

We used the drug Corvalol as a test material because its adulteration in Russia ranks fourth among all of the adulterated preparations and the use of a low-grade product can adversely affect human health and life [13]. A simple and reliable method for controlling this drug should be developed because of the increasing output of this drug in Russia; this is explained by the fact that this drug is economical for consumers (the imported analog Valocordin is three times more expensive).

An array of piezoelectric sensors with various electrode coatings was used for the test quality assessment of the Corvalol drug for monitoring the constant headspace phase of the drug.

The aim of this study was to choose sorption phases that are characterized by noticeable and different affin-

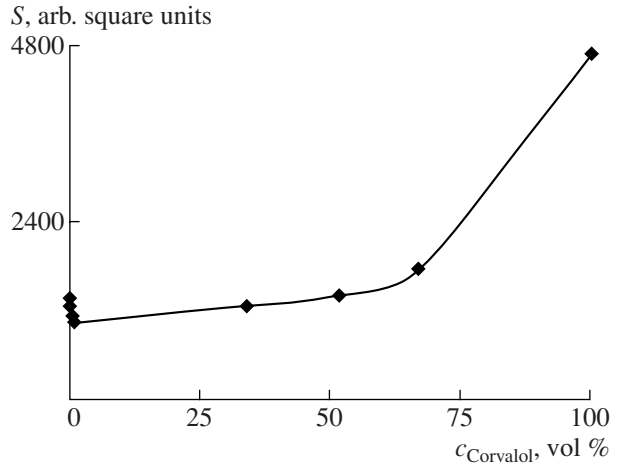

Fig. 1. Dependence of the surface areas of the visual imprints of the headspace phases on the concentration of Corvalol solutions.

ities to the headspace components of the Corvalol drug and an array of sensors, to develop an algorithm for measuring signals from individual elements, to form a total response of the array (a multidimensional analytical signal) as the kinetic "visual imprints" of an aroma, and to evaluate the reliability of decision making with respect to the quality of the drug based on the visual imprints of the test and reference samples.

EXPERIMENTAL

The AT-cut quartz piezoelectric resonators with a frequency of 10 MHz were modified by supporting sorbent solutions onto electrodes, which were degreased with ethanol. The volume of a modifier solution (concentration of 1.5 mg/cm^3) was regulated in accordance with the required sorbent weight. The headspace phases ($V_{headspace}$ = 2 cm^3) of the test drug, model solutions, distilled water, peppermint oil solutions, an artificial mint-flavoring agent, and ethanol were taken for analysis. The mass of a film after the thermal removal of a solvent (m_f) was calculated from the Sauerbrey equation for thin films [12]

$$m_f = \frac{\Delta F_f S}{K_f F_0^2},$$

where $\Delta F_f = F_0 - F_f$ is the change in the resonator frequency upon supporting a film, Hz; K_f is the calibration constant of the piezoelectric microbalance ($K_f = 2.3 \times 10^6$ m/Hz under normal conditions); F_0 is the oscillating frequency of the quartz wafer, MHz; and S is the surface area of the resonator electrodes (S = 0.2 cm^2).

The electrode modifiers were chosen from a database of quantitative and temporal sorption parameters of organic compounds from various classes on the films of stationary phases for gas chromatography or natural polymers and specific films with chemical reagents [14].

The sorbents were chosen in accordance with the nature of the main components of the headspace phase of the drug, which contained the vapors of water, ethanol, and the highly volatile components of peppermint oil (α-pinene, β-pinene, sabinene, 3-octanol, limonene, menthone, isomenthone, neomenthol, menthofuran, menthol, pulegone, piperitone, menthyl acetate, β-caryophyllene, and germacrene D) and mint essential oil (L-menthol, L-carvyl acetate, L-carvone, camphor, borneol, 1,8-cineol, and L-fenchone). The following nonpolar, medium-polarity, and polar chromatographic phases were used: beeswax, bee glue (propolis), polystyrene, Apiezon N, Apiezon L, squalane, polyethylene glycol adipate (PEGA), polyethylene glycol succinate (PEGS), polyethylene glycol sebacate (PEGSb), Triton X-100, polyethylene glycol 2000 (PEG 2000), and polyvinylpyrrolidone (PVP). The solvents of the sorption phases were acetone, toluene, chloroform, and hexane (analytical grade).

The efficiency of substance sorption by films was evaluated by the change in the oscillation frequency of a sensor (film resonator) ΔF_s (Hz) after establishing a thermodynamic equilibrium in sorbate vapors (in this case, the change of signals was ±(2–3) Hz/5 s) or upon reaching a maximum response ΔF_{max} (Hz) of the sensor.

The kinetics parameters of sorption were evaluated based on the times of full equilibrium and maximum sorption (τ_{max}, s) and the rate of change in the sensor oscillation frequency within the first 5–10 s after injecting vapors into the detection cell.

The experiment was performed in a six-sensor detection cell with a closed inlet [5]. The multidimensional analytical signal of the array of sensors was formed as kinetic visual imprints in accordance with an algorithm developed. The signals of the sensors were plotted on the axis of ordinates against time on the axis of abscissas. The surface area of visual imprints was a quantitative test of differences in the headspace compositions of the test substances with the retention of the template (geometry) of an image (Fig. 1). A program in Visual Basic was used to calculate the surface areas of the visual imprints. The program allowed us to calculate the surface area of a geometric figure of any complexity partitioned into n triangles, whose legs are the axes of a petal diagram.

A qualitative test of the total analytical signal—a change in the geometry of a visual imprint or considerable deviations of signals along the main axes—was chosen individually for each particular test material (Fig. 2). An inconsistency between the visual imprints of samples suggests a qualitative and quantitative change in the headspace composition.

Distilled water, the solutions of ethanol and Corvalol with various concentrations, peppermint oil, mint essential oil, and commercial Corvalol preparations

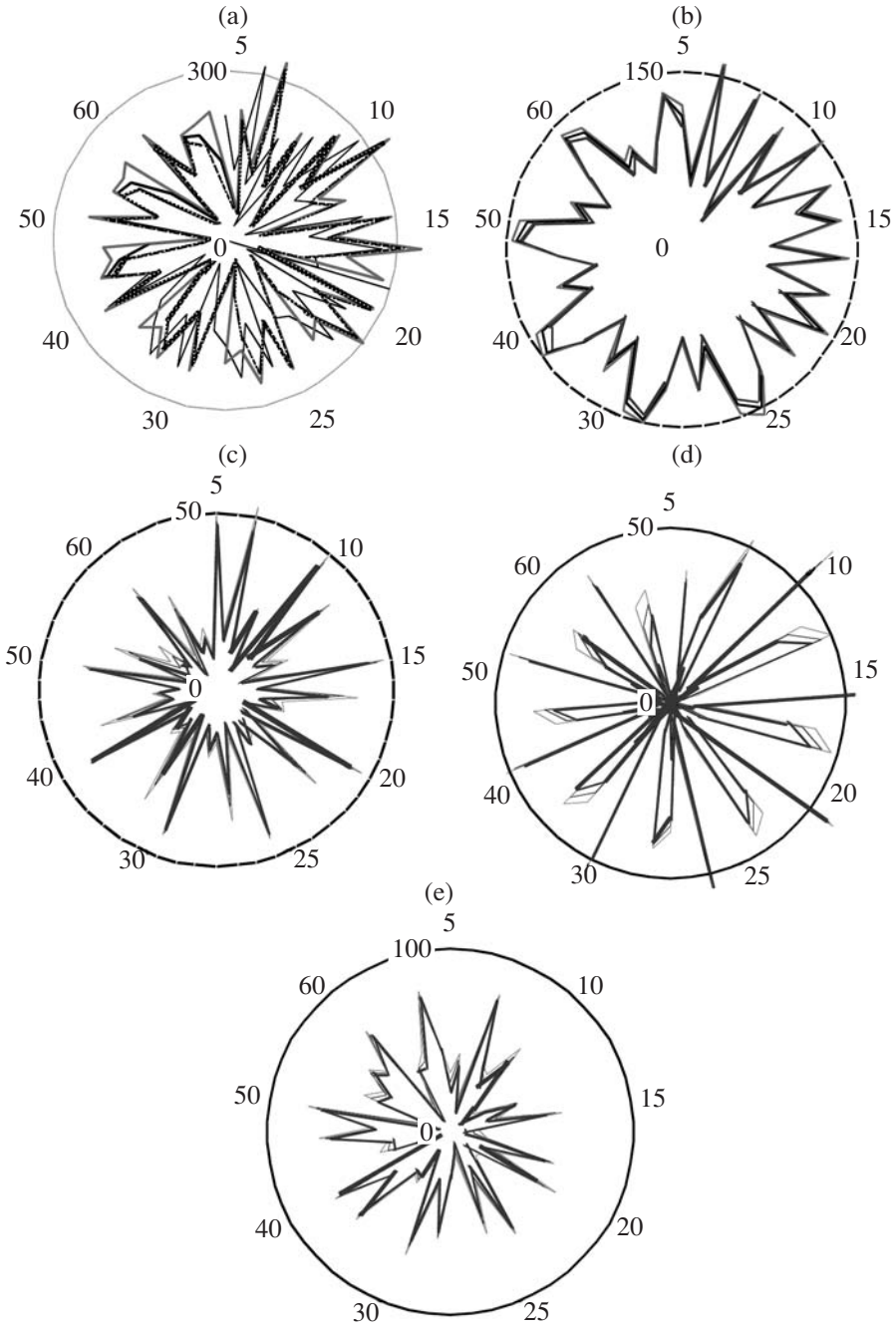

Fig. 2. Changes in the average multidimensional analytical signal of a detector based on six sensors with consideration for confidence intervals in the headspace vapors of (a) a standard Corvalol drug sample, (b) ethanol, (c) natural peppermint oil, (d) mint essential oil, and (e) Corvalol drug diluted by a factor of 1.5.

purchased from retail outlets were used as the test samples in the model experiment.

RESULTS AND DISCUSSION

Table 1 summarizes the quantitative kinetic parameters of sorption of the highly volatile components of the test preparation (water vapor, ethanol, and peppermint oil) from the headspace phases of the model solutions on the modifier films of the piezoelectric resonator electrodes.

We found that a propolis film exhibited an increased equivalent affinity to water and ethanol vapors; for this reason, this sorbent cannot be used for evaluating the degree of dilution of alcohol-containing preparations. Apiezon N and Apiezon L films are hydrophobic; the vapors of ethanol and peppermint oil give significant

Table 1. Quantitative (ΔF_{max}, Hz) and kinetic (τ_{max}, s) parameters of the sorption of Corvalol and ethanol vapors (the volume fraction of the main component was 98%), m_f = 10–15 μg

Film	Ethanol		Corvalol		Film	Ethanol		Corvalol	
	ΔF_{max}	τ_{max}	ΔF_{max}	τ_{max}		ΔF_{max}	τ_{max}	ΔF_{max}	τ_{max}
Squalane	5	5	12	5	PEGA	88	10	149	10
Polystyrene	4	5	7	5	Triton X-100	116	5	235	5
Beeswax	7	5	9	10	PEGSb	154	80	300	55
Propolis	32	5	75	5	PVP	188	55	329	65
Apiezon L	93	40	48	45	PEGS	100	5	133	5
Apiezon N	87	35	42	35	PEG 2000	156	100	244	15

Table 2. Metrological characteristics of the responses of sensors with optimum-weight films in the vapors of a standard Corvalol sample, distilled water, and ethanol (m_f = 15 ± 5 μg, n = 3; P = 0.95)

Parameter	Film					
	Triton X-100	PEGA	PEG 2000	PEGS	PEGSb	PVP
Corvalol drug						
$x^* \pm \Delta x$	250 ± 5	144 ± 5	249 ± 7	139 ± 7	300 ± 1	329 ± 8
Δ%	1.9	4.3	2.8	5.0	0.4	2.3
Ethanol						
$x^* \pm \Delta x$	110 ± 4	87 ± 5	153 ± 6	97 ± 2	163 ± 6	140 ± 4
Δ%	2.6	6.0	4.1	3.2	4.0	3.0
Distilled water						
$x^* \pm \Delta x$	71 ± 1	27 ± 5	29 ± 4	48 ± 3	65 ± 4	147 ± 5
Δ%	2.0	1.8	6.0	5.9	5.8	3.5

Note: x^* is the average analytical sensor signal $\Delta\bar{F}_{max}$, Hz.

and distinguishable signals. However, the composition of these sorbents is always different, because Apiezons are lubricating greases produced from heavy petroleum fractions. This considerably decreases the metrological reliability of the results of measurements and can result in errors and an incorrect evaluation of analytical results upon replacing a spent film, especially, from a new batch of the grease. In the quartz piezoelectric resonators loaded with squalane, polystyrene, and beeswax films, the analytical signal was 4–12 kHz. This frequency change corresponds to a noise response level; therefore, these coatings were not used in the subsequent experiment. Noises were mainly due to an imperfect drive circuit and a pressure produced upon injecting the headspace phase of samples into the cell.

The following sorbents were chosen for the subsequent experiment: Triton X-100, PEG 2000, PEGA, PEGS, PEGSb, and PVP. Triton X-100, PEGA, PEGS, PEGSb, and PVP were dissolved in acetone, and PEG 2000 was dissolved in ethanol. They are characterized by high sorption parameters and the reproducibility of responses and a low baseline drift of sensors in the headspace vapor of the Corvalol drug (Tables 1 and 2).

Based on the experimental results in the model solutions, we found that:

• the highly volatile components of the headspace phase of the Corvalol drug cannot be determined with the use of one sensor because of the competitive sorption of all mixture components; in this case, a signal due to the sorption of individual substances responsible for the quality of the drug cannot be separated from the overall sensor response;

• a system of sensors with sorbent films of different polarity should be used for the headspace analysis of the Corvalol drug.

The analytical signal of six sensors simultaneously exposed to the vapor of a test sample is a matrix of responses measured at a certain point in time. The arrangement of resonators in an array depends on the kinetic parameters of the interaction of sorbates with sorbents and on the time taken to reach a maximum response for each particular sensor. The analytical signal of several units that detect sorption interactions (sorbate weights) in the thin sorbent film–multicomponent gas mixture system is a multidimensional matrix of the form

$$\begin{cases} \Delta F_1 = a_1^1 c_1 + a_2^1 c_2 + \ldots + a_n^1 c_n \\ \Delta F_2 = a_1^2 c_1 + a_2^2 c_2 + \ldots + a_n^2 c_n, \\ \vdots \\ \Delta F_j = a_1^j c_1 + a_2^j c_2 + \ldots + a_n^j c_n, \end{cases}$$

where ΔF_1, …, and ΔF_n are the signals of jth sensors (Hz); a_1^1, a_2^1, …, and a_n^1; a_1^1, a_1^2, …, and a_1^j are the sensitivities of microweighing of the 1st, …, and jth sensors to the vapors of the 1st, …, and nth components of the mixture (Hz m^3 mg^{-1}); and c_1, …, c_n are the component concentrations in a near-sensor space (mg/m^3).

To find the concentrations of individual components in an ideal case, the number of sensors should be equal to the number of components and each sensor should be much more highly selective for a component than for the other (i.e., the sensitivities a_1a_2, …, $a_1^j a_2^j$ are not equal). The problem of high selectivity can be solved with the use of chemosorbents; however, sensors with a chemolayer are suitable for only a single use. Nonexpendable sensors exhibit (equivalent) cross selectivity for sample components. For this reason, it is difficult to identify and determine all of the components of a test mixture. These solutions are not always required. In rapid analysis, it is sufficient to evaluate the degree of conformity with a standard, for example, for the aroma of a product or the smell of water, air, etc. That is, it is of importance to perform an integrated assessment of the test sample. For this purpose, it is sufficient to adjust the array of sensors (to choose coatings) to the main classes of compounds (either the most toxic compounds or compounds responsible for the quality of the sample). A decrease in the number of sensors simplifies the treatment of the matrix of responses. A method for the representation of multidimensional analytical signals consists in the visualization (the construction of petal diagrams based on the signals of all sensors with consideration of the interaction time).

The kinetic visual imprint of the responses in a headspace phase was chosen as the analytical signal of an array of sensors. To obtain this imprint, we used an integral algorithm for measuring sensor signals with a 5-s time interval in a certain sequence and the formation of a circle diagram. The algorithm developed allowed us to obtain visual imprints with a low degree of identity and to find insignificant differences in the composition for various samples, including the headspace phase of the Corvalol drug. To make a decision on the result of testing, the standard visual imprints of smells should be obtained. A comparison of the resulting diagrams with standards allows us to evaluate changes in not only the qualitative, but also the quantitative composition of the headspace phase of the test preparation. The following factors affect the reliability level of this evaluation:

(1) the metrological reliability of visual imprints, which predominantly depends on the nature and stability of modifier films;

(2) the reproducibility of the aromas of the test and standard materials from sample to sample, which depends on the quality and type of raw materials, the level of production, and the technology;

(3) the constant qualitative and quantitative composition of the headspace phase taken from a sample using repeated discrete gas extraction and injected into the detection cell.

The choice of a standard substance based on some characteristics remains an incompletely solved problem in the practice of food and pharmaceutical analysis and in the analysis of other multicomponent products. We statistically processed the sensor signals obtained in the vapors of the standard Corvalol drug and test samples. We calculated the main metrological characteristics of the sorption of the test preparations (confidence intervals, dispersions, and relative errors) and visualized the average analytical signal of the array with a confidence interval (Fig. 2). We proposed using the reproducibility of the geometry of a visual imprint with an error of no higher than 20% as a reliability criterion for this imprint. This allowed us to make a more correct decision on the degree of conformity between the visual imprints of the test and standard samples. For all of the test samples, the sensor responses were reliably reproduced with $\Delta = 2$–15%.

To improve the metrological reliability of a multidimensional analytical signal, a sensor with a Triton X-100 film was removed from the detector. In this case, the retention of the individual geometry of visual imprints was evaluated in the test samples (Figs. 3a and 3b). It was found that a five-sensor detector afforded significant analytical signals for all types of the test samples, which were distinguished with a high degree of reliability. The inconsistency between the visual imprints of a Corvalol sample (sample 2) and a standard sample (sample 1) (Figs. 3a and 3b) suggests either adulteration or violation of the storage time and conditions.

The dilution of a standard sample of the Corvalol drug with water changed the surface area of the visual imprint. This change can serve as a sign of drug adulteration as a result of the use of ethanol solutions with lower concentrations. The decrease of the signals of PVP and PEGA film sensors was most adequate to the degree of dilution of a Corvalol sample with water. A decrease in the signals of sensors with these films in the headspace vapor of the test preparations unambiguously indicates a decrease in the ethanol content of the preparations. A shift of the maximum sensor signals along the axis of time by more than 5–10 s was evaluated as a change in the qualitative composition of the headspace phase, in particular, upon replacing natural mint extracts by synthetic components.

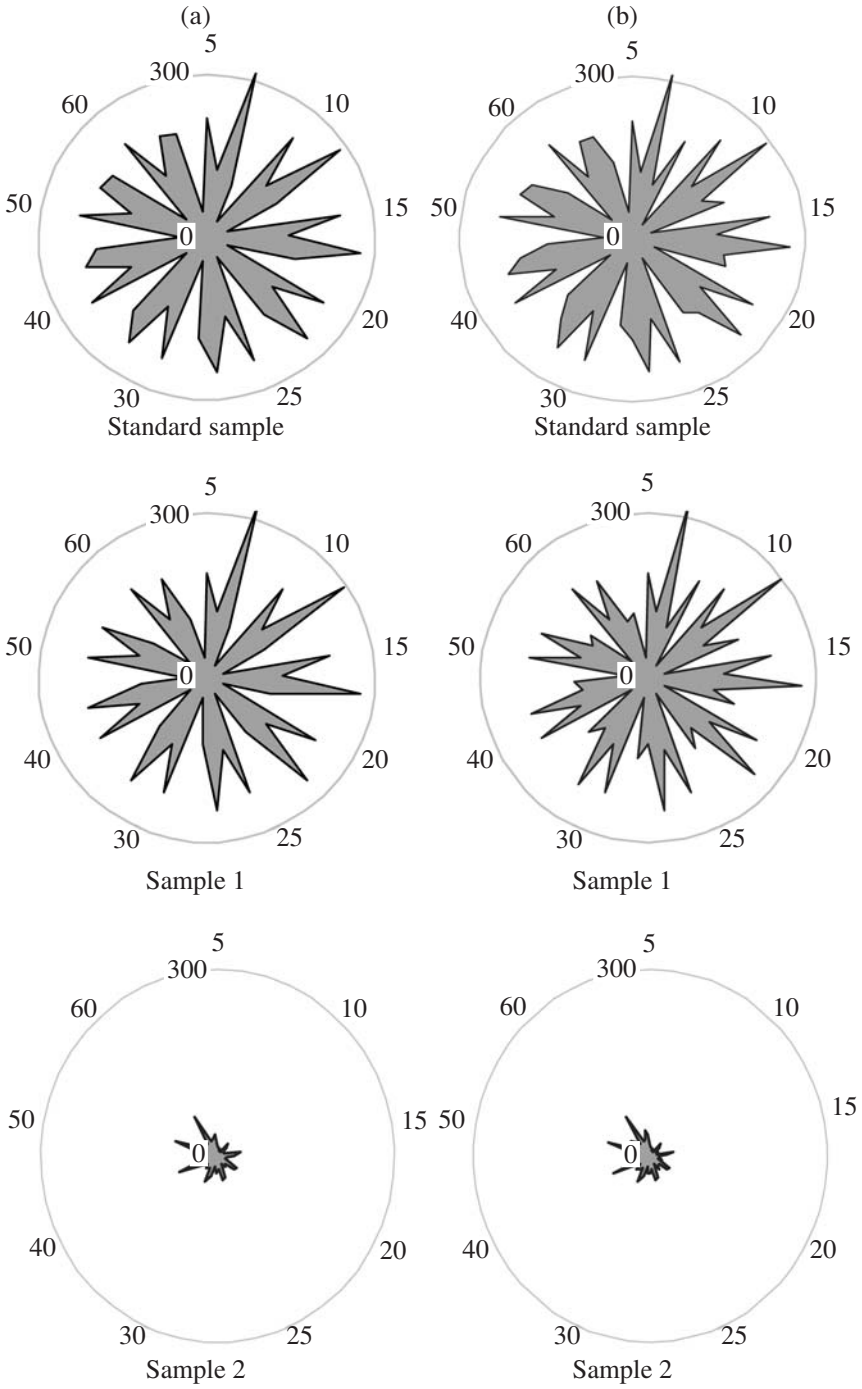

Fig. 3. Averaged visual imprints of the headspace phases constructed based on the signals of (a) five and (b) six sensors for the reference (a standard sample) and test (samples 1 and 2) samples of the Corvalol drug.

The following tentative limits, which were evaluated using a computer program, were accepted for differences between the visual imprints of the samples: The degrees of identity of the visual imprints of the test and standard samples from 100 to 80% were evaluated to be consistent with a standard (+). The degrees of identity from 80 to 60% were evaluated to be conditionally consistent with a standard (±). The degree of identity of a visual imprint lower than 60% suggested adulteration, dramatic violation of storage conditions, or a product stored for a time longer than the shelf life, and it was evaluated to be inconsistent with a standard (–). Chemometrics methods (cluster analysis and the main component method) were used for the treatment of multidimensional analytical signals from an array of sensors in order to compare the compositions of head-

Table 3. Comparative evaluation of the results of testing samples by various techniques

Sample	Evaluation by weighing vapors	n_D^{20}	pH	Evaluation in accordance with GOST (State Standard) 5631-79
Standard Corvalol sample	"+"	1.3597	7.3	"+"
Standard sample diluted by a factor of 1.5	"–"	1.3487	5.8	"–"
Standard sample diluted by a factor of 3	"–"	1.3463	5.8	"–"
Corvalol, sample 1	"+"	1.3597	7.0	"+"
Corvalol, sample 2	"+"	1.3595	7.0	"+"
Corvalol, sample 3	"–"	1.3845	6.5	"–"
Valoserdin	"±"	1.3640	6.9	"±"
Valocordin	"±"	1.3622	7.1	"±"
Aqueous solution of peppermint oil	"–"	1.3490	3.0	"–"

space phases over the Corvalol drug, to evaluate the reproducibility of signals in the vapors of a sample, and to identify various samples. The accuracy of decision making with respect to testing Corvalol samples was checked by refractometry and pH-metry (Table 3). It was found that changes in the composition of the headspace phase of the Corvalol drug estimated from the signals of an array of sensors correlated with other quantitative characteristics of the samples (n_D^{20} and pH).

The samples of the Valoserdin and Corvalol drugs, which are similar in composition (both contain peppermint oil), were identical in terms of the results of analysis by piezoelectric microweighing. However, based on the results obtained by all of the methods, a sample of the Valocordin drug was inconsistent with the standard chosen; this was explained by the fact that it contains a mixture of peppermint oil and hop oil. Roughly adulterated preparations (Corvalol samples diluted with water or model ethanol solutions with mint essential oil added) were unambiguously evaluated to be inconsistent with a standard (–).

The procedure developed for testing the Corvalol drug does not imply the detailed identification of all of the constituents of an aroma, which are responsible for the specific smell of the product. However, it allows one to control the constant composition of the headspace phase of the drug and concentration ratios between aroma-forming components. This characteristic can be used to assess the quality of the Corvalol drug or to determine possible reasons for changes in the composition due to rough adulteration, dilution, the use of artificial flavoring agents, and a violation of storage length and conditions.

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