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DETERMINATION OF CHROMATOGRAPHIC CONDITIONS FOR QUANTITATIVE ASSESSMENT OF ACTIVE COMPONENTS IN COMPLEX NASAL SPRAY AFTER MANUFACTURING AND EXPIRY DATE

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The aim of the work *is the development of chromatographic conditions, the study of the validation characteristics of the method of quantitative determination of phenylephrine hydrochloride, nitrofural, lidocaine hydrochloride and diphenhydramine hydrochloride, panthenol, povidone in the joint presence in the nasal spray by a complex method of liquid chromatography with UV detection. Evaluation of the quantitative content of active components after manufacturing and during the shelf life.*

Materials and methods. *Agilent 1260 liquid chromatographs, equipped with a diode-matrix detector from the company "Agilent technologies", USA. Chromatographic columns 250×4.6 mm in size, filled with octadecylsilyl silica gel for chromatography (Zorbax StableBond SB-Aq, Agilent company), mobile phase A – phosphate buffer solution pH 7.0 – acetonitrile P (1650:350), mobile phase B – acetonitrile P; elution mode – gradient; mobile phase flow rate – 1.0 ml/min; detection wavelengths – 220 nm (for panthenol, phenylephrine, povidone, diphenhydramine) and 235 nm (for nitrofural and lidocaine).*

Results*. Chromatographic separation conditions were developed for the co-presence determination of six target substances: panthenol, phenylephrine hydrochloride, nitrofural, povidone, lidocaine hydrochloride and diphenhydramine hydrochloride. The suitability of the technique for this task was confirmed by determining the validation characteristics. The methodology at the appropriate level is characterized by specificity, linearity, correctness and convergence in the range of application for panthenol (range 20.33–38.26 mg/ml, Δ_Z=0.93≤max Δ_Z=3.20, a=0.63≤max a=5.12, r=0.9978≥min r=0.9924), phenylephrine hydrochloride (range 1.70–3.21 mg/ml, Δ_Z=0.51≤max Δ_Z=3.20, a=0.15≤max a=5.12, r=0.9984≥min r=0.9924), nitrofural (range 0.137–0.257 mg/ml, Δ_Z=0.91≤max Δ_Z=3.20, a=0.032≤max a=5.12, r=0.9987≥min r=0.9924) povidone (range 20,44–38,50 mg/ml, Δ_Z=0.23≤max Δ_Z=3.20, a=2.33 ≤ max a=5.12, r=0.9942≥min=0.9924), lidocaine hydrochloride (range 6.80–12.81 mg/ml, Δ_Z=0.34≤max Δ_Z=3.20, a=0.66 ≤ max a=5.12, r=0.9988≥min r=0.9924), diphenhydramine hydrochloride (range 1.36–2.56 mg/ml, Δ_Z=0.20≤max Δ_Z=3.20, a=0.15≤max a=5.12, r=0.9980≥min r=0.9924). There are no significant changes when stored at 25 °C for 6 months.*

Conclusions*. An analytical method of quantitative determination of the component composition in an extemporaneous nasal spray by a complex method of high-performance liquid chromatography has been developed. The determined validation parameters confirm the correctness of the methodology. The chemical stability of the dosage form is observed for 6 months*

Keywords*: spray, phenylephrine hydrochloride, lidocaine hydrochloride, polyvinylpyrrolidone, panthenol, nitrofural, diphenhydramine hydrochloride, quantitative determination, liquid chromatography, stability*

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1. Introduction

The problem of acute inflammatory diseases of the upper respiratory tract, in particular acute rhinosinusitis (ARS), is one of the most relevant in modern clinical medicine. Mandatory pathogenetic and symptomatic treatment of ARS is carried out if necessary, in accordance with the existing clinical picture and the patient's condition by prescribing drugs of various pharmacological groups: analgesics, nonsteroidal anti-inflammatory drugs, antihistamine drugs, decongestants, in addition, herbal preparations with proven effectiveness, sodium chloride and glucocorticosteroids for intranasal use, antibacterial drugs, etc. [1].

Despite the wide range of industrially produced medicinal products, manufacturing in pharmacy conditions remains a popular practice throughout the world [2]. Among the extemporaneous medicinal products presented on the pharmaceutical market of Ukraine, the largest part consists of liquid dosage forms, of which otorhinolaryngological preparations are the priority link [3, 4].

Complex treatment of ARS requires the creation of combined medicines, which, in turn, requires the development of new methods of quality control. Complex nasal spray, the active pharmaceutical ingredients of which are phenylephrine hydrochloride, diphenhydr-

amine hydrochloride, lidocaine hydrochloride, nitrofural, panthenol, and povidone, belongs to over-the-counter extemporaneous drugs for symptomatic treatment, which is an important factor for quality treatment of ARS.

The method of high-performance liquid chromatography (HPLC) in various modifications is widely used to determine each of the components of the spray. For example, the method of liquid chromatography with UV detection is used to determine lidocaine hydrochloride in parenteral drugs [5], diphenhydramine hydrochloride in capsules and oral solutions [6, 7], phenylephrine hydrochloride in eye drops [8, 9], nitrofural in substance [10], dexpanthenol in the composition of artificial tears [11] and cosmetics [12] and povidone in the substance [13, 14]. The literature does not present HPLC methods for the simultaneous determination of panthenol, phenylephrine hydrochloride, nitrofural, povidone, lidocaine hydrochloride, and diphenhydramine hydrochloride, although the liquid chromatography method is proposed for the quantitative determination of such compounds as diphenhydramine hydrochloride and phenylephrine hydrochloride in a solution for oral use [15] and their mixtures with paracetamol and caffeine in tablets [16].

The aim of this study was the development of chromatographic conditions and the study of validation characteristics of the method of quantitative determination of panthenol, phenylephrine hydrochloride, nitrofural, povidone, lidocaine hydrochloride and diphenhydramine hydrochloride in the joint presence in the nasal spray by a complex method of liquid chromatography. Evaluation of the quantitative content of active components at release and during the shelf life.

2. Research planning (methodology)

Methodologically, the research logic was built as follows:

– determine the possibility of unification of the method of quantitative determination of active pharmaceutical ingredients of allergy spray [17], since 50 % of the component composition is identical;

– to select the parameters of the chromatographic system, study the physical and chemical properties of the active components, based on the results of the analysis of monographs of mono- and, if available, multi-component mixtures with target APIs, and various databases, such as: DataKnowledge of the European Directorate for the Quality of Medicines (EDQM) [18] choose chromatographic columns and mobile phases for the simultaneous determination of six components of the mixture;

– conduct validation studies in accordance with the tasks for which this technique was developed, guided by the requirements of current EP/SPhU publications [19, 20], recommendations of the ICH Q2 guidelines [21] and approaches proposed by leading scientists [22];

– study the term and conditions of storage of the dosage form in accordance with the current guidelines [23, 24].

3. Materials and methods

The object of the study was the extemporaneous medicinal product “Complex Nasal Spray”, produced by Pharmacy 431 (Logus), which is a multi-component liquid dosage form in a polymer container equipped with a dosing device, with the following composition: 0.025 g of phenylephrine hydrochloride; 0.02 g of diphenhydramine hydrochloride (diphenhydramine); 0.002 g of nitrofural; 0.1 g of lidocaine hydrochloride; 0.3 g each of panthenol and polyvinylpyrrolidone, water up to 10 ml.

Experimental studies were carried out during the 2022–2023 calendar years using liquid chromatographs – Agilent 1260 and 1290, equipped with diode-matrix detectors (Agilent Technologies, USA); analytical balance – Mettler Toledo MS104 (Mettler Toledo, Switzerland); chromatographic columns selected based on the results of the development – Zorbax StableBond SB-Aq (250*4.6 mm, 4.6 μm) of different serial numbers, Mettler Toledo SevenCompact 220 pH meter (Mettler Toledo, Switzerland). Sample preparation was carried out using measuring vessels of class A and reagents that meet the requirements of EP/SPhU.

In the course of research, reagents *potassium dihydrogen phosphate P, disodium hydrogen phosphate dihydrate P, phosphoric acid P, acetonitrile for chromatography P* manufactured by Sigma-Aldrich were used. The following were used as standard samples: D-panthenol (Sigma-Aldrich, lot. BCCJ9224, *P*=99.5 %), phenylephrine hydrochloride (PSS SPhU, p. 2, *P*=100,0 %), nitrofural (Supelco, lot. LRAD4452, *P*=99.6 %), povidone (Supelco, lot. LRAD4444, *P*=98.9 %), lidocaine hydrochloride (Supelco, lot. LRAD2221, *P*=99.9 %) diphenhydramine (Supelco, lot LRAD7254, *P*=99.9 %).

Draft methodology: determination is carried out by liquid chromatography (EP / SPhU, 2.2.29, 2.2.46, *current edition*).

Solvent: *water P.*

Buffer solution pH 7.0. 5.34 g of *disodium hydrogen phosphate dihydrate P* and 2.9 g of *potassium dihydrogen phosphate P* are dissolved in 1650 ml of *water P*, the solution is brought to pH 7.0 with *phosphoric acid P.*

Test solution: analyze the drug without sample preparation.

Initial solution A: about 25.0 mg of *nitrofural* (*RS, WRS or substances of a certain quality*), place in a volumetric flask with a capacity of 50.0 ml, add about 35 ml of *water P*, and heat until complete dissolution. The solution is cooled to room temperature, the volume of the solution is brought up to the mark with the same solvent and mixed thoroughly.

Comparison solution. About 62.0 mg of *phenylephrine hydrochloride* (*RS, WRS or substances of a certain quality*), 50.0 mg *diphenhydramine hydrochloride* (*RS, WRS or substances of a certain quality*), 250.0 mg *lidocaine hydrochloride* (*RS, WRS or substances of a certain quality*), 750.0 mg *panthenol* (*RS, WRS or substances of a certain quality*), and *povidone* (*RS, WRS or substances of a certain quality*) each, place in a volumetric flask with a capacity of 25.0 ml, add 10.0 ml of the initial solution A, about 10 ml of *water P* and dissolve. The solution is brought up to the mark with the same solvent and thoroughly mixed. The resulting solution is filtered through a filter with a pore diameter of no more than 0.45 μm.

Determination is carried out on a liquid chromatograph with a diode-matrix detector under the following conditions:

– chromatographic column 250×4.6 mm filled with *octadecylsilyl silica gel for chromatography P* with a particle size of 5 μm (for example, Zorbax SB-Aq or similar, for which the suitability of the chromatographic system is performed);

– mobile phase A: Add 350 ml of *acetonitrile P* to 1650 ml of buffer solution pH 7.0, mix and filter through a membrane filter with a pore diameter of no more than 0.45 μm;

– mobile phase B: *Acetonitrile P.*

The gradient program is presented in Table 1;

– flow rate – 1.0 ml/min;

– column temperature: 40 °C;

– wavelength detection:

a) 220 nm (for the determination of panthenol, phenylephrine, povidone and diphenhydramine);

b) 235 nm (for the determination of nitrofurazone and lidocaine);

– injection volume 2 μl;

– chromatography time: 32 min.

Table 1

Gradient elution program

Time, min	Mobile phase A, %	Mobile phase B, %
0	100	0
5	100	0
18	20	80
25	20	80
27	100	0
32	100	0

Alternately chromatograph the control solution, the reference solution, and the test solution.

The order of peaks and approximate retention times are presented in the Table 2.

A chromatographic system is considered suitable if:

– the degree of separation for the critical pair of peaks of phenylephrine and panthenol, calculated from the chromatogram obtained from the comparison solution, is at least 1.5;

– the symmetry coefficients of the peaks of panthenol, phenylephrine, nitrofural, polyvinylpyrrolidone and lidocaine, obtained on the chromatogram of the comparison solution, is no more than 2.0; for the peak of diphenhydramine – no more than 5.5;

– the relative standard deviation, calculated from the peak areas of panthenol, phenylephrine, nitrofural, polyvinylpyrrolidone, lidocaine and diphenhydramine obtained on chromatograms from the comparison solution, for 2 parallel injections, should be no more than 0.51 %; for 3 – no more than 1.34 %; for 4 – no more than 1.92 %; for 5 – no more than 2.37 %; for 6 – no more than 2.75 %;

– the efficiency of the chromatographic column, calculated from the polyvinylpyrrolidone peak, should be at least 1000.

Table 2

The order of peaks and approximate retention times

Peak output order	The name of the substance	Wavelength, nm	Approximate retention times, min	Relative retention time
1	Panthenol	220	3.03–3.90	≈0.21
2	Phenylephrine hydrochloride	220	3.36–4.33	≈0.23
3	Nitrofural	235	8.33–10.15	≈0.55
4	Polyvinylpyrrolidone	220	11.51–12.25	≈0.70
5	Lidocaine hydrochloride	235	16.40–17.63	=1
6	Diphenhydramine hydrochloride	220	22.78–25.58	≈1.42

The content of phenylephrine hydrochloride ($C_9H_{14}ClNO_2$) in terms of the volume of the dosage form should be from 0.0225 g to 0.0275 g; the content of nitrofural ($C_6H_6N_4O_4$) – from 0.0018 g to 0.0022 g; the content of lidocaine hydrochloride ($C_{14}H_{23}N_2OCl$) – from 0.09 g to 0.11 g; content of polyvinylpyrrolidone ($(C_6H_9NO)_n$) and panthenol ($C_9H_{19}NO_4$) – from 0.27 g to 0.33 g.

4. Research results

In the course of pharmaceutical development, the stability of solutions over time and the stability of the analytical technique to minor changes were investigated in accordance with the SPhU monograph/EP, 2.2.46 [19, 20], "*Methods of chromatographic separation*" [21].

The stability of the solutions in the autosampler for each of the components was investigated for 76 hours. The deviation of the peak areas compared to the starting point was evaluated. For each of the stability points, the deviation does not exceed σ_{max}=1.02 %.

To investigate the stability of the analytical method to minor changes, the possible conditions of the chromatographic system were simulated in accordance with the requirements of the monograph SPhU/EP, 2.2.46 [20]. To evaluate this parameter, comparison solutions and test solutions were chromatographed under changed conditions. Based on the obtained data, the sufficiency and variability of the selected parameters were evaluated to confirm the suitability and stability of the chromatographic system during the test. Data on changes in the retention time of the substances to be determined were used to establish approximate peak output limits. In the study of robustness, the change in the flow rate of the mobile phase (±0.1 ml/min), temperature (± 5 °C) was studied, and columns with different serial numbers were used.

The uncertainty of the analytical method characterizes the error of performing the experiment. The total uncertainty of the method (Δ_{As}) according to [19] for the components panthenol (1.54 %), phenylephrine hydrochloride (1.72 %), nitrofural (2.26 %), povidone (1.49 %), lidocaine hydrochloride (1.69 %) and diphenhydramine (1.82 %) does not exceed the criterion maxΔ_{As}=3.2 %.

During the validation of the analytical method for determining the components of the complex nasal spray: panthenol, phenylephrine hydrochloride, nitrofurazone, povidone, lidocaine hydrochloride and diphenhydr-

amine – the suitability of the chromatographic system was studied for each of the specified active substances; specificity; linearity, correctness and precision. The research was carried out using model mixtures with known concentrations of target analytes in final solutions in the range of application of the analytical technique:

– for panthenol – 20.33–38.26 mg/ml;
– phenylephrine hydrochloride – 1.70–3.21 mg/ml;
– nitrofural – 0.137–0.257 mg/ml;
– povidone – 20.44–38.50 mg/ml;
– lidocaine hydrochloride – 6.80–12.81 mg/ml;
– diphenhydramine hydrochloride – 1.36–2.56 mg/ml.

To check the suitability of the chromatographic system, a sufficient number of parallel chromatograms of the comparison solution were obtained.

The results of the comparison of the suitability of the chromatographic system obtained by two analysts are shown in Table 3.

To confirm the specificity of the analytical method and identify the analytes of the substances to be determined, the chromatograms obtained during the injection of the control solution, mobile phase, reference solutions (solutions of individual substances and the combined solution) and the test solution were compared (Fig. 1, *a–j*).

The linearity and correctness of the analytical method for each of the analytes were evaluated using model solutions in the range of its application [19]. To check the convergence, the results of control of model solutions at a concentration level of about 100 % analyzed by two analysts on different days using two different columns were compared (Table 4).

Stability was studied based on general requirements [23, 24]. In storage conditions: temperature (25±2) °C; and relative humidity (60±5) **%**. During the test period, the appearance of the dosage form was assessed visually, and the quantitative content of active substances – by chromatographic control methods. The results of tests of the stability of the samples for 6 months under long-term storage conditions are shown in Table 5.

Table 3

The results of studying the suitability of the chromatographic system

No.	Parameter		Value	Result	
				Analyst 1	Analyst 2
1	The degree of separation of the peaks of panthenol and phenylephrine		≥1.5	2.01	2.41
2	Symmetry coefficients	PNT[1]	≤2.0	1.56	1.27
		FE[2]		1.60	1.77
		NF[3]		1.11	1.04
		PVP[4]		1.04	0.87
		LD[5]		1.21	1.32
		DF[6]	≤5.5	5.01	4.49
3	Relative standard deviation	PNT	for 2 parallel injections no more than 0.51 %	0.34	0.10
		FE		0.34	0.07
		NF		0.41	0.37
		PVP		0.31	0.20
		LD		0.34	0.09
		DF		0.38	0.06
System efficiency calculated from the polyvinylpyrrolidone peak			≥1000	1611	2251
Conclusion				Corresponds	Corresponds

Note: PNT – panthenol; FE – phenylephrine hydrochloride; NF – nitrofural; PVP – povidone; LD – lidocaine hydrochloride; DF – diphenhydramine

Table 4

Results of determination of parameters of linearity, correctness and convergence

Parameter	Value						Criterion (90–110 %), *n*=15	Conclusion
	PNT[1]	FE[2]	NF[3]	PVP[4]	LD[5]	DF[6]		
Linearity								
b	112.6	2759.7	4743.1	347.9	927.91	3932.8	n/a	n/a
a	51.7	297.86	118.99	603.72	455.99	432.88	n/a	n/a
max *a*, %	0.6	0.15	0.032	2.33	0.66	0.15	≤5.12 %	Corresponds
r	0.9978	0.9984	0.9987	0.9942	0.9988	0.9980	≥0.9924	Corresponds
Correctness								
Z_{min}, %	95.92	97.11	97.90	95.36	96.22	95.89	90 %	Corresponds
Z_{max}, %	100.32	102.52	106.16	102.38	100.21	100.70	110 %	Corresponds
Z_{aver}, %	97.88	99.66	101.23	99.72	98.26	97.96	95–105 %	Corresponds
Convergence								
Relative standard deviation, S_z, %	0.46	0.51	0.91	0.23	0.34	0.20	–	–
Relative confidence interval, Δz, %=$t_{(95\,\%,5)}S_z$	0.927	1.028	1.834	0.463	0.685	0.403	≤maxΔ_{4s}, %=3.2 %	Corresponds

Note: PNT – panthenol; FE – phenylephrine hydrochloride; NF – nitrofural; PVP – povidone; LD – lidocaine hydrochloride; DF – diphenhydramine

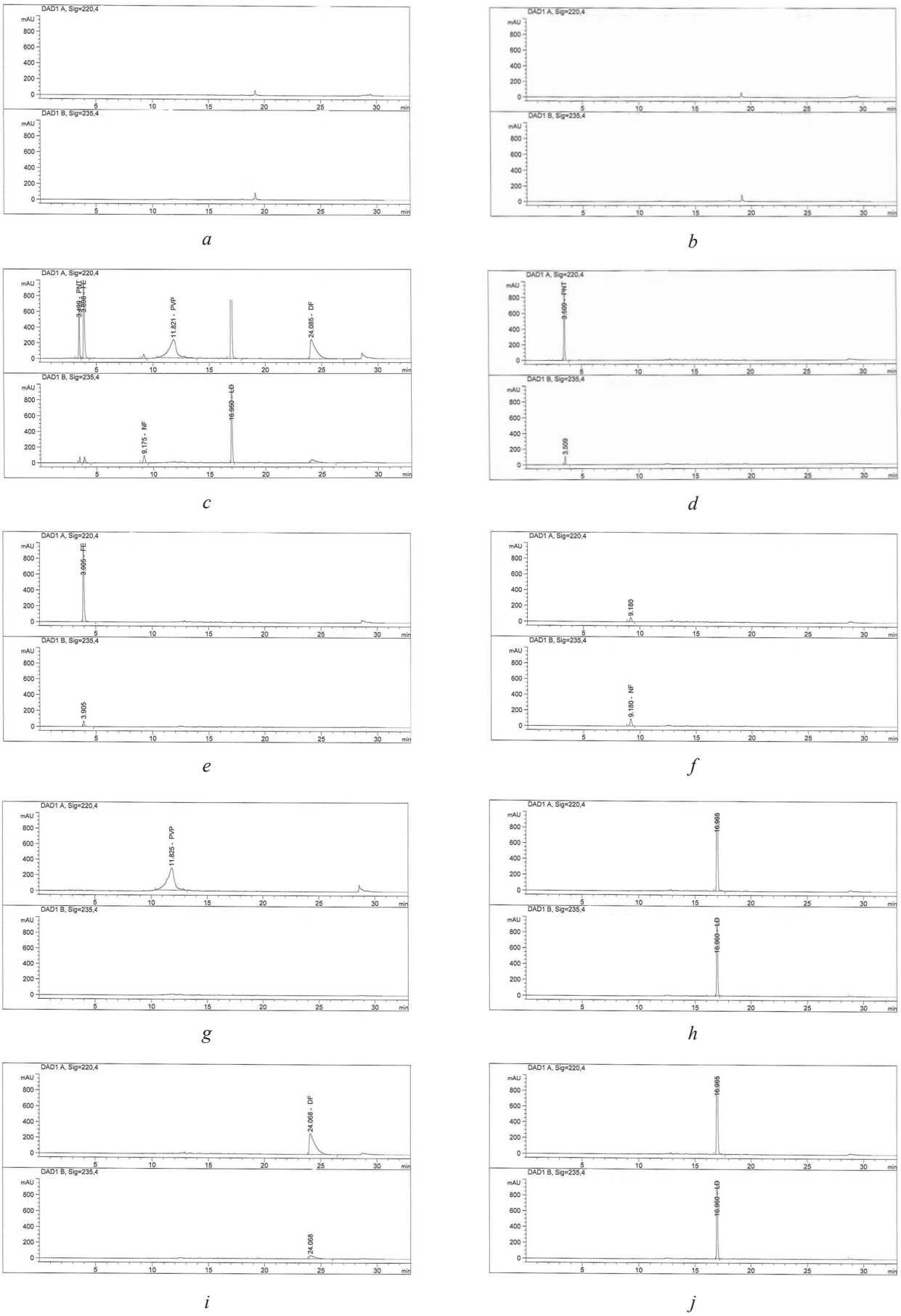

Fig. 1. Typical chromatogram of: *a* – the control solution; *b* – mobile phase; *c* – comparison solution; *d* – comparison solutions: panthenol; *e* – phenylephrine hydrochloride; *f* – nitrofural; *g* – povidone; *h* – lidocaine hydrochloride; *i* – diphenhydramine hydrochloride; *j* – of the test solution

Table 5

The results of studying the stability of the medicinal product under long-term storage conditions (temperature (25±2) °C; humidity (60±5) % RH)

Quality indicator	Method	Requirements		Storage period						
				0	30 days	60 days	90 days	120 days	150 days	180 days
Description	Visually	Transparent liquid from light yellow to yellow colour. The formation of turbidity and sediment is not allowed		Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds	Corresponds
Quantitative definition	HPLC (2.2.29, 2.2.46)	PNT[1]	0.27 g to 0.33 g	0.324	0.324	0.326	0.322	0.321	0.326	0.320
		FE[2]	0.0225 g to 0.0275 g	0.0251	0.0251	0.0250	0.250	0.249	0.252	0.251
		NF[3]	0.0018 g to 0.0022 g	0.00182	0.00181	0.00181	0.00180	0.00182	0.00183	0.00180
		PVP[4]	0.27 g to 0.33 g	0.324	0.325	0.325	0.322	0.326	0.324	0.323
		LD[5]	0.09 g to 0.11 g	0.110	0.110	0.110	0.109	0.102	0.108	0.110
		DF[6]	0.018 g to 0.022 g	0.0208	0.0209	0.0208	0.0205	0.207	0.209	0.208

Note: PNT – panthenol; FE – phenylephrine hydrochloride; NF – nitrofural; PVP – povidone; LD – lidocaine hydrochloride; DF – diphenhydramine

5. Discussion of research results

The practice of separating substances from their mixture is widely used in the pharmaceutical industry. Most often, such methods are used to determine organic impurities of substances or compositional compositions of organic substances, which are usually similar in their structures, have similar chemical properties, and therefore show similar abilities to the phenomena of sorption-desorption – the phenomenon on which the method of chromatographic separation is based. The separation of multicomponent mixtures of active substances, which differ in chemical composition and properties, requires a more detailed analysis. In the literature, it was not possible to find methods and techniques that would allow the separation of the target active components under the conditions of one technique, therefore we were faced with an interesting task-challenge – to choose the parameters of the chromatographic system in such a way as to ensure the separation of all 6 active substances under the conditions of one method.

Since complex nasal spray and allergy spray have a common part of active components, in order to verify the possibility of unification of the method of determining their quantitative content, the method of quantitative determination was tested, which was previously developed and validated to solve the problem of determining the active substances – phenylephrine hydrochloride, diphenhydramine (diphenhydramine) and nitrofural (nitrofurazone) – in a spray for allergies [17]. According to the results of the approbation, the merging of the chromatographic peaks of lidocaine and phenylephrine was observed, which prevented their evaluation. In addition, under the specified conditions, the retention of peaks on the sorbent was observed in the chromatogram of only four of the six declared substances.

The parameters of the previously defined chromatographic system for assessing the content of active components in allergy spray were adapted to define a new task [17]. For this, an additional search and analysis of monographs was conducted on APIs – lidocaine [25] and its hydrochloride [26, 27], panthenol and its derivatives [28, 29], as well as povidone [13, 14].

Conducting a theoretical search to determine the parameters of separation and options for their possible changes were called to fulfill the main task – to determine the possibility of quantitative assessment of active substances in common conditions with a compatible presence. At the same time, during the development, an ambitious goal was set – to achieve separation conditions with the possibility of quantitative assessment of all declared components within the framework of one analysis to prevent overloading of control of the quantitative content of individual components by separate methods. Achieving this goal would provide a reduction in quality control time, significant savings in analyst time for testing, savings in reagents, consumables, and other resources.

As in the case of the development of chromatographic conditions for determining the quantitative content of APIs in an allergy spray [17], when choosing chromatographic columns, attention was paid to the physical properties of the substances to be determined and the recommended information on conducting research on the reproduction of pharmacopoeial methods, given in the database of the EDQM [18]. In the course of pharmaceutical development, a number of chromatographic columns were tested, which are recommended for individual component mixtures, namely: LiChrospher RP Select B (250*4.6 mm, 4 μm) [7, 18], LiChrospher 100 RP18 (250*4.6 mm, 4 μm) [10, 18], XTerra Shield RP18 (150*3.9 mm, 4.6 μm) [18, 26], Zorbax Eclipse XDB-C18 (150*4.6 mm, 5 μm) and Zorbax StableBond SB-Aq (250*4.6 mm, 4.6 μm) – during pharmaceutical development. Among the tested columns, the Zorbax StableBond SB-Aq column (250*4.6 mm, 4.6 μm) turned out to be optimal, and it was used in further tests.

With the verification of the possibility of performing the analysis by the method of determining the active substances in the allergy spray, the combination of a 0.1 % aqueous solution of trifluoroacetic acid and methanol was first tested as mobile phases in the gradient elution mode [17]. However, the composition of the mobile phases and the gradient program were not suitable for the component composition of the nasal complex spray.

Due to the fact that, although not significantly, the solvents methanol and acetonitrile differ in density, it was suggested to replace methanol with acetonitrile. However, the desired result from the separation of lido-

caine and phenylephrine peaks was not achieved. As a result, the parameters of the chromatographic system were significantly changed: as the mobile phase, it was decided to use a 0.01 M aqueous solution of potassium dihydrogen phosphate with a LiChrosphere 250*4 mm column with a particle size of 5.0 μm, the flow rate of the mobile phase and the temperature of the column thermostat were left unchanged – 1.2 ml/min and 40 °C, respectively. The elution options that were used are isocratic without adding an organic component and gradient, combining 0.01 M phosphate buffer solution and acetonitrile.

During isocratic elution, retention of 2 out of 6 declared chromatographic peaks was observed, which did not meet the stated goals. The use of the gradient started with the initial conditions of 100 % buffer solution with a gradual increase in the content of the organic component to 80 % to determine the approximate target ratios of the aqueous and organic components of the mobile phases, optimal for the retention of substances on the sorbent of the chromatographic column, however, only 4 out of 6 were identified under these conditions target peaks. And, since the obtained result did not solve the set problems and would require the development of an additional methodology for the quantitative assessment of the remaining components, it was decided to continue the tests on the selection of parameters.

Another variant of the parameters for verification was a pH 7.0 buffer solution (5.34 g of disodium hydrogen phosphate and 2.9 g of potassium dihydrogen phosphate in 1.5 L of water for chromatography) with the gradient program described above. However, during use, we encountered the problem that the salts of the buffer solution, when the volume of the organic component – acetonitrile – was increased, began to salt out on the device, which made further work impossible. Even though the separation of all 6 target substances was observed, we were forced to look for other variants of the chromatographic separation parameters.

Using the same gradient program, the use of a phosphate buffer solution was proposed, but with a twofold increase in the concentration of potassium dihydrogen phosphate salts to 0.02 M. However, this choice was unsuccessful in the test because phenylephrine, diphenhydramine, and polyvinylpyrrolidone were not supported by the data conditions.

Thus, attention was focused on the refinement of mobile phases and chromatographic parameters using a phosphate buffer solution of pH 7.0. But already using the column Zorbax StableBond SB-Aq (250*4.6 mm, 4.6 μm).

To prevent negative phenomena such as salting out of phosphate salts, 82.5 % phosphate buffer solution pH 7.0 and 17.5 % acetonitrile were used to prepare mobile phase A. To prevent stratification of the organic and aqueous components, mobile phase A must be filtered through a membrane filter with a pore diameter of no more than 0.45 μm already after mixing.

Since the determined substances have differences in their physical and chemical properties, which affect the ability to sorption-desorption processes on the stationary phase sorbent, the use of the gradient elution mode is proposed. The gradient program was developed considering one of the key tasks – to ensure the separation of chromatographic peaks of all substances to be determined under the conditions of one chromatographic system.

The proposed elution program managed to provide the necessary separation. However, several important points should be noted.

A critical pair of chromatographic peaks, the separation of which should be monitored when determining the suitability of a chromatographic system, is panthenol and phenylephrine, which have retention times of about 3–4 min and a separation factor of ≈2.0 (criterion≥1.5).

The criterion for peak symmetry of all components, except for diphenhydramine, is defined as ≤2.0. For diphenhydramine, the peak symmetry factor is defined as sufficient at the level of ≤5.5. The values of this indicator are defined as compromise, as they contribute to the simultaneous determination of all six organic components of the dosage form under the conditions of one chromatographic system. The reproducibility and stability of this indicator has been determined and confirmed experimentally during pre-validation and validation work, so it can be applied. However, the consequences of going beyond the specified parameters were not determined, so these criteria are recommended for control when determining the suitability of a chromatographic system.

Among the wavelengths proposed by monographs on substances and dosage forms, the optimal choice for detecting panthenol, phenylephrine, polyvinylpyrrolidone and diphenhydramine is 220 nm, and for nitrofurazone and lidocaine – 235 nm.

The injection volume is determined to be optimal at the level of 2 μl. This makes it possible to carry out control without over-dilution, which always introduces additional uncertainty into the methodology. At the same time, an additional complication is that the analyzed dosage form has a concentration range of active substances from 30 mg/ml to 0.2 mg/ml. Thus, the introduction of additional re-dilution operations will not allow determination under the conditions of one chromatographic system and will require the development of several analytical methods for determining the quantitative content of active substances in the complex nasal spray.

The creation of a single method of quality control of six components of the spray significantly reduces the time for conducting research. The proposed method contributes to the “greenness of the technique” by limiting the use of toxic and flammable solvents and responsible handling of resources from non-renewable sources. At the same time, the use of a single method helps to reduce the amount of harmful waste that requires special disposal.

In the course of experimental work, the stability of the solutions in the autosampler of the chromatograph was determined for at least 76 hours. The obtained values of the results of the robustness tests indicate that changes in the chromatographic conditions within the limits proposed by the general monograph [19] do not have a significant effect on the suitability of the chromatographic

system. Also, based on the obtained robustness study data, the parameters for checking the suitability of the chromatographic system, which should be checked when determining the suitability of the chromatographic system, are defined:

– the degree of separation for the critical pair of peaks of phenylephrine and panthenol, calculated from the chromatogram obtained from the comparison solution, is at least 1.5;

– the symmetry coefficients of the peaks of panthenol, phenylephrine, nitrofural, polyvinylpyrrolidone and lidocaine, obtained on the chromatogram of the comparison solution, is no more than 2.0; for the peak of diphenhydramine – no more than 5.5;

– the relative standard deviation, calculated from the peak areas of panthenol, phenylephrine, nitrofural, polyvinylpyrrolidone, lidocaine and diphenhydramine obtained on chromatograms from the comparison solution, for 2 parallel injections, should be no more than 0.51 %; for 3 – no more than 1.34 %; for 4 – no more than 1.92 %; for 5 – no more than 2.37 %; for 6 – no more than 2.75 %;

– the efficiency of the chromatographic column, calculated from the polyvinylpyrrolidone peak, should be at least 1000.

The order of output of the target peaks was confirmed as follows: 1 – panthenol (RTT≈0.21); 2 – phenylephrine (RTT≈0.23); 3 – nitrofural (RTT≈0.55); 4 – povidone (RTT≈0.70); 5 – lidocaine (RTT=1.00); 6 – diphenhydramine (RTT≈1.42). The relative retention times were calculated relative to the lidocaine peak retention time, which was taken as 1.

The complete uncertainty of the method of analysis of each of the components as a whole ensures sufficient measurement accuracy, since Δ_{AS} % does not exceed the critical parameter maxΔ_{AS} 3.20 % [19].

The specificity was confirmed by comparing the chromatograms obtained from the corresponding solutions (Fig. 1, *a–j*) at both analytical wavelengths: 220 nm and 235 nm.

There are no additional peaks on the chromatograms of the control (blank) solution and the mobile phase, the retention times of which would coincide with the retention times of the target peaks of panthenol, phenylephrine, nitrofural, polyvinylpyrrolidone, lidocaine, and diphenhydramine. Peak separation is sufficient. The most critical is the separation of the chromatographic peaks of panthenol and phenylephrine. The retention times of the target peaks on the chromatograms of the comparison solutions of individual substances coincide with the retention times of the corresponding peaks on the chromatograms obtained from the test solution and the reference solution. Deviations of absolute retention times do not exceed ±2 %.

Thus, the analytical technique is characterized by specificity.

During the study of linearity, the linear dependence of the signal response on the concentration for each of the target compounds was confirmed (Table 2). Correlation coefficients (r>0.9924) and the angular coefficient ($|a|$, %≤5,12 %) in the studied ranges for analytes that were studied in the range of 70–130 % of the nominal concentration [22] meet the requirements [19, 20].

Relative standard deviation (S_z, %) and their relative confidence intervals (Δ_z, %), calculated for the results obtained by two analysts, meet the proposed acceptance criteria (maxΔ_{As}, %=3.2 %) (Table 2).

Thus, the analytical method of determining the active substances in the complex nasal spray is characterized by proper linearity, correctness and precision. The method is correct and can be applied to solve the given problem.

During the trial period, control over critical quality indicators that can affect the quality and functional characteristics of extemporaneous medicines [23, 24] was carried out: the appearance of the dosage form was assessed visually, the quantitative content of active substances was assessed by chromatographic control methods. During the study of stability in the conditions of long-term tests at a temperature of (25±2) °C and a relative humidity of air (60±5) %, no "significant changes" were detected, the test samples showed little variability of the results and were within the specifications. Thus, the drug should be stored at a temperature not higher than 25 °C for no more than 6 months.

Study limitations. The proposed method of liquid chromatography cannot be used to determine traces of the drug in the human body during toxicological analysis, and the correctness of the method has not been proven when changing auxiliary substances or introducing other components into the composition.

Prospects for further research. Refinement of the parameters of chromatographic separation for the possibility of controlling the quantitative content of active substances in the medicinal form when replacing components with similar ones in terms of action, considering "green chemistry".

6. Conclusions

According to the results of the tests, the authors proposed an analytical method for determining the quantitative content of six active substances of an organic nature, which are present together in the complex nasal spray of extemporaneous production (Pharmacy 431/Logus): panthenol, phenylephrine hydrochloride, nitrofural (nitrofurazone), polyvinylpyrrolidone (povidone), lidocaine hydrochloride and diphenhydramine hydrochloride (diphenhydramine). The method is characterized by sufficient specificity, the analytes do not have a negative effect on the determination of each other. The methodology for each of the analytes is characterized by linearity, correctness and convergence of results in the range of application. The method is resistant to minor changes, the comparison solutions are stable for at least 76 hours. The total uncertainty for each of the components is within the defined limits of acceptance. The shelf life of the complex nasal spray has been confirmed for six months at a temperature not higher than 25 °C.

Therefore, the proposed method is suitable for performing the task of quantitative assessment of the content

of active pharmaceutical ingredients in the extemporaneous medicinal product "Complex Nasal Spray" and can be recommended for implementation for routine control.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Data availability

Data will be provided upon reasonable request.

Use of artificial intelligence technologies

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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