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DEVELOPMENT AND VALIDATION OF METHODS FOR QUANTITATIVE DETERMINATION OF ACTIVE PHARMACEUTICAL SUBSTANCES IN NASAL SPRAY

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Intranasal administration of H_1-histamine receptor blockers may be a promising approach to the treatment of allergic rhinitis. Earlier, an original composition of a nasal spray containing fexofenadine hydrochloride and ammonium glycyrrhizinate and demonstrating a high level of therapeutic efficacy, was developed.

The aim of the study was to develop and validate a method of the quantitative determination of active pharmaceutical ingredients fexofenadine hydrochloride and ammonium glycyrrhizinate in a spray for intranasal administration.

Materials and methods. During the development and validation of the method of the fexofenadine hydrochloride and ammonium glycyrrhizinate quantitative determination in a nasal spray, the method of high performance liquid chromatography was used: a Dionex Ultimate 3000 UV chromatograph with a Luna C18 column (2) containing octadecylsilicagel with a 5 μm grain size as a sorbent. The analysis and validation procedures were performed in accordance with the requirements of the State Pharmacopoeia of the Russian Federation, the XIVth edition.

Results. The study showed that for the simultaneous quantitative determination of fexofenadine hydrochloride and ammonium glycyrrhizinate, the optimal elution regime is a gradient mode with a mobile phase containing 50 mmol/L potassium dihydrogen phosphate solution with methanol (45:55), which ensured the separation of the components in the 20 minutes interval. The validation procedures showed that the developed methodology correspond to all the criteria of validity in terms of the following indicators: correctness, precision, specificity and linearity in the analytical area.

Conclusion. The obtained results indicate the possibility of using the method of high-performance liquid chromatography in a gradient elution mode with a mobile phase of the composition of a 50 mmol/L solution of potassium dihydrogen phosphate with methanol (45:55) for the simultaneous quantitative determination of active pharmaceutical ingredients – fexofenadine hydrochloride and ammonium glycyrrhizinate as parts of a promising nasal spray for the allergic rhinitis treatment.

Keywords: allergic rhinitis; quantification; high performance liquid chromatography; fexofenadine hydrochloride; ammonium glycyrrhizinate

Abbreviations: AR – allergic rhinitis; API – active pharmaceutical ingredient; UV – ultraviolet; HPLC – high performance liquid chromatography; SPRF – State Pharmacopoeia of the Russian Federation

РАЗРАБОТКА И ВАЛИДАЦИЯ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ АКТИВНЫХ ФАРМАЦЕВТИЧЕСКИХ СУБСТАНЦИЙ В СПРЕЕ НАЗАЛЬНОМ

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Перспективным подходом к лечению аллергического ринита может стать интраназальное введение блокаторов H_1-гистаминовых рецепторов. Ранее был разработан оригинальный состав спрея назального, содержащего фексофенадина гидрохлорид и аммония глицирризинат, демонстрирующий высокий уровень терапевтической эффективности.
Цель состояла в разработке и валидации методики количественного определения активных фармацевтических субстанций фексофенадина гидрохлорида и аммония глицирризината в спрее для интраназального введения.
Материалы и методы. В ходе разработки и валидации методики количественного определения фексофенадина гидрохлорида и аммония глицирризината в спрее назальном применялся метод высокоэффективной жидкостной хроматографии: хроматограф с УФ детектором DionexUltimate 3000 с колонкой Luna C18 (2), содержащей в качестве сорбента октадецилсиликагель с зернением 5 мкм. Анализ и валидационные процедуры выполнялись в соответствии с требованиями Государственной Фармакопеи Российской Федерации XIV издания.
Результаты. Исследование показало, что для количественного определения при совместном присутствии фексофенадина гидрохлорида и аммония глицирризината оптимальным является градиентный режим элюирования с составом подвижной фазы 50 ммоль/л раствор калия дигидрофосфата и метанолом (45:55), который обеспечивал разделение компонентов смеси в интервале 20 минут. Валидационная оценка показала, что разработанная методика отвечает всем критериям валидности по показателям: правильность, прецизионность, специфичность и линейность в аналитической области.
Заключение. Полученные результаты свидетельствуют о возможности использования метода высокоэффективной жидкостной хроматографии в градиентном режиме элюирования с составом подвижной фазы 50 ммоль/л раствор калия дигидрофосфата с метанолом (45:55) для количественного определения активных фармацевтических субстанций – фексофенадина гидрохлорида и аммония глицирризината в составе перспективного спрея назального для лечения аллергического ринита.
Ключевые слова: аллергический ринит; количественное определение; высокоэффективная жидкостная хроматография; фексофенадина гидрохлорид; аммония глицирризинат
Список сокращений: АР – аллергический ринит; АФС – активная фармацевтическая субстанция; УФ – ультрафиолетовый; ВЭЖХ – высокоэффективная жидкостная хроматография; ГФ РФ – Государственная фармакопея Российской Федерации; х. ч. – химически чистый

INTRODUCTION

Allergic rhinitis (AR) is the most common disease resulting from the organism's hypersensitivity to various types of antigens. AR ranks the sixth place among the most common atopic diseases in the world, leading to a decrease of the quality of life and a deterioration of labor productivity, which negatively effects on the economic component of a human activity and requires significant financial investments, both personal and from the health care system [1, 2]. At the same time, the number of people diagnosed with allergic rhinitis is annually steadily increasing, which makes the development of effective and safe medicines for the treatment and prevention of AR relevant both for the patients themselves and for the state as a whole [3].

Currently available pharmacological approaches to AR therapy involve the elimination of the main symptoms of the disease. For this purpose, both intranasal and systemic medicines are used. Medicines administered intranasally are represented by glucocorticosteroids, which are first-line drugs. It is also possible to inject decongestants and anticholinergics into the nasal cavity. H_1-histaminolytics, mast cell membrane stabilizers and leukotriene receptor antagonists are used systemically in the AR treatment [4]. Despite the sufficient level of effectiveness, in some cases, the use of intranasal glucocorticosteroids does not provide the necessary pharmacological safety requirements, which limits their daily use [5]. In this regard, repeated attempts to overcome the existing disadvantages of glucocorticosteroids, including the ones by creating rational combinations of drugs or their complete replacement by the medicines of an alternative pharmacotherapeutic group, were made. In the latter case, the intranasal use of H_1 – histamine receptor blockers is relevant, while in order to increase the therapeutic effect, it is possible to develop synergistic combinations based on non-sedative histamine blockers and other antiallergic agents [6]. A promising direction in the correction of allergic rhinitis can be considered the use of a combination of the H_1-histamine blocker of the latest generation – fexofenadine hydrochloride and an antiallergic herbal agent – ammonium glycyrrhizinate – as parts of the nasal spray being developed [7].

Fexofenadine is an active metabolite of terfenadine, an anti-allergic agent with an antihistamine action. Fexofenadine belongs to the latest generation of long-acting H_1-histamine receptor blockers, devoid of a pronounced sedative effect. Fexofenadine has a favorable medicine with the safety profile that is superior to that of the first generation antihistamines. The absence of the sedative effect makes it possible to use this medicine by various groups of the population and including the working hours, since the attention concentration, motor and cognitive functions are not impaired [8–12].

Ammonium glycyrrhizinate is one of the effective medicines obtained from the licorice extract. The ammonium salt of glycyrrhizic acid has proven its anti-inflammatory, antinocciptive, antiallergic, antiviral, antioxidant, immunostimulating and hepatoprotective activity [13–17].

In the previous study devoted to the experimental evaluation of the pharmacological efficacy of the combination of the active substances fexofenadine hydrochloride + ammonium glycyrrhizinate (the adjuvants were benzalkonium chloride, polyethylene oxide – 400, propylene glycol), the following factors were established. In the animals with AR, the intranasal administration of the test composition in terms of the severity of its action

was comparable to glucocorticosteroids; that implies the relevance of the further investigation of this combination from the perspective of the pharmaceutical analysis [18].

THE AIM of the study was to develop and validate a method of the quantitative determination of active pharmaceutical ingredients fexofenadine hydrochloride and ammonium glycyrrhizinate in a spray for intranasal administration.

MATERIALS AND METHODS

Test objects and materials for analysis

Based on the current trends in the field of qualitative and quantitative analysis of pharmacologically active compounds, as well as on the features of the pharmaceutical analysis of the compounds similar in structure (terfenadine and glycyrrhizic acid, respectively) to the target ones (fexofenadine hydrochloride and ammonium glycyrrhizinate), the method of high performance liquid chromatography had been chosen in this study [19, 20]. The following objects were used in the work: the substance of ammonium glycyrrhizinate (the content was 99.4%, fexofenadine CJSC «VIFITECH», Russia); the substance of hydrochloride (the content was 101.6%, Ind – Swift Laboratories Limited, India); the standard samples of fexofenadine hydrochloride (Sigma – Aldrich, USA); the standard samples of ammonium glycyrrhizinate (Sigma – Aldrich, USA); acetonitrile; water for chromatography; methanol (UHPLC Grade; Panreac, Spain), potassium dihydrogen phosphate. The methods was developed using the following equipment: a Dionex Ultimate 3000 chromatograph (Thermo Scientific, USA) equipped with a UV detector UVD-3000, a Luna C18 column (2) with a size of 150 x 4.6 mm (octadecylsilicagel 5 µm granulation) (Phenomenex, USA); OPN centrifuge – 3.02 (Russia); analytical balance Sartogosm, LV 210-a (Russia).

The temperature of the test samples was 20°C, the temperature of the chromatographic column was 30°C, and it was maintained by a thermostat. The sample volume was 20 µL injected by an autosampler. The detection was carried out spectrophotometrically at the wavelength of 234 nm.

Methods for quantitative analysis of active pharmaceutical ingredients (APIs)

A quantitative analysis of the APIs of the developed spray for intranasal administration was carried out in accordance with the requirements of the State Pharmacopoeia of the Russian Federation (the XIVth edition of the GPA.1.2.1.2.0001.15 «Chromatography», GPA.1.2.1.2.0005.15 «High performance liquid chromatography» and GPA.1.1.0012.15 «Validation of analytical methods»[1].

[1] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

Preparation of standard sample solutions

The preparation of standard samples solutions was carried out as follows. An accurate weighed amount of a standard sample (30 mg for fexofenadine hydrochloride and 10 mg for ammonium glycyrrhizinate) was placed in a 10 ml volumetric flask. Then 5 ml of the mixture of methanol and 50 mmol/l potassium dihydrogen phosphate solution (55:45) was added, dissolved with stirring, and the volume of the flask was brought to the mark. 2 ml of the resulting solution was transferred into a 25 ml volumetric flask, and then the volume was brought to the mark with the same solvent. Before the injection, the solutions were filtered through a nylon filter with a pore size of 0.45 µm (Phenomenex, USA), discarding the first portions of the filtrate.

Preparation of test solution

Preparation of the test solution has undergone the following process. 2 ml of the medicines was transferred into a 25 ml volumetric flask, a mixture of methanol and 50 mmol/L potassium dihydrogen phosphate solution (55:45) was added to the mark, stirred and filtered through a nylon filter with a pore size of 0.45 µm (Phenomenex, USA), discarding the first portions of the filtrate (the test solution).

The calculation of the fexofenadine hydrochloride and ammonium glycyrrhizinate content in the spay in mg/ml was carried out according to the formula:

$$C_x = \frac{S_x \times a_{st} \times P \times W_x \times V_{st}}{S_{st} \times V_x \times W_{st} \times W_{st2} \times 100}, \quad (1)$$

where X is the content of the determined component, mg/ml; S_x is the area of the peak of the determined component of the test solution, mAU min, on the chromatogram; S_{st} is the area of the peak of the determined component of the standard solution, mAU min, on the chromatogram; V_x is the volume of the medicine aliquot, ml; a_{st} is the amount of the standard sample, g; P is a substance content in the standard sample solution, mg/ml; W_x is the volume of a volumetric flask taken to dilute the medicine, ml; W_{st}, W_{st2} are the volumes of volumetric flasks taken for dilution of the standard sample, ml.

The calculation of the content of the medicinal product components relative to the declared one was carried out according to the formula:

$$X = \frac{C_x \times 100}{L}, \quad (2)$$

where X is the content of the analyte relative to the declared one, %; C_x is the content of the analyte, mg/ml; L is the declared content of the substance in the nasal spray, mg/ml.

Preparation of solutions for validation assessment «linearity»

0.0300 g of a standard sample of fexofenadine hydrochloride was placed in a 10 ml volumetric flask, dissolved with stirring in 6 ml of the mixture of methanol

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and 50 mmol/L potassium dihydrogen phosphate solution (55:45), after which the volume of the flask was brought to the mark with the same composition.

The following amounts of the solution (1.2; 1.6; 2.0; 2.4; 2.8 ml, respectively) were transferred into volumetric flasks with a capacity of 25 ml and the volumes were brought to the mark with the same solvent.

Preparation of solutions for validation assessment according to the indicators «correctness» and «analytical area»

The model mixture was prepared with a content of fexofenadine hydrochloride and ammonium glycyrrhizinate in 60% relative to the declared spray (0.18 g of fexofenadine hydrochloride and 0.06 g of ammonium glycyrrhizinate per 100 ml of the medicine). Next, the obtained medicinal product was analyzed in accordance with the proposed methods.

At the same time, 2 ml of the solution of the obtained model mixture was placed into 25 ml volumetric flasks, then 0.4; 0.8 and 1.2 ml of a 0.3% solution of the standard sample of fexofenadine hydrochloride and a 0.1% solution of the standard sample of ammonium glycyrrhizinate to concentration levels of 80%, 100% and 120%, relative to the nominal, were added. After that, the volume of the flask was brought to the mark with the mobile phase in the ratio which was at the beginning of the analysis. Each dilution was repeated three times.

Statistical analysis

The results were processed by static methods using the Microsoft Excel v 13.0 software package with advanced statistical data analysis capabilities.

RESULTS AND DISCUSSION

Development of methods for simultaneous quantitative determination of fexofenadine hydrochloride and ammonium glycyrrhizinate in the nasal spray

The task of the present study was to develop HPLC – the methods of quantitative determination in the nasal spray. In the literature data, there is no HPLC methods for the simultaneous quantitative determination of fexofenadine hydrochloride and ammonium and glycyrrhizinate, however, methods for their individual determination have been described [21–24].

Preliminary studies on the development and optimization of the methods for the quantitative determination of the APIs of the nasal antiallergic spray, made it possible to establish the optimal aqueous component of the mobile phase, which additionally suppresses ionization. It is a solution of potassium dihydrogen phosphate at the concentration of 0.05–0.1 mol/l with a pH of 4.5–4.8. There was no pronounced difference when a 0.05 M – 0.1 M potassium dihydrogen phosphate solution was used. In order to minimize the potentially negative factors associated with the use of saline buffer solutions, a 50 mmol/L potassium dihydrogen phosphate solution was used.

An experimental study of the chromatography of fexofenadine hydrochloride and ammonium glycyrrhizinate in the mixtures of a 50 mmol/l potassium dihydrogen phosphate solution with methanol, made it possible to conclude that methanol and its mixtures with water easily dissolved the components of the medicine[2].

The baseline drift inherent in the gradient elution mode did not influenced the calculation of separation results. To shorten the analysis time, improve separation and decrease the viscosity of the mobile phase, the temperature of the chromatographic column was increased to 30°C. The optimized conditions for the chromatographic determination are presented in Table 1.

A typical nasal spray chromatogram is shown in Fig. 1.

At the same time, chromatography of the standard samples solutions of fexofenadine hydrochloride, ammonium glycyrrhizinate, as well as the solutions of the adjuvants in the composition of a nasal spray (benzalkonium chloride, polyethylene oxide – 400, propylene glycol) and the mobile phase, was carried out. Under the proposed conditions of the chromatographic determination, a reliable separation of the components is carried out with a certain baseline drift characteristic of the gradient elution mode, within 20 minutes.

To assess the suitability of the chromatographic system, the solutions of the nasal spray and standard samples of fexofenadine hydrochloride and ammonium glycyrrhizinate were sequentially analyzed in six replicates to determine retention times, asymmetry factors, resolution coefficients, and the efficiency of the chromatographic system.

The main characteristics of the fexofenadine hydrochloride and ammonium glycyrrhizinate peaks on the chromatograms of the nasal spray solution and standard sample solutions, are presented in Tables 2 and 3.

The presented results indicate that the retention times of the two main peaks on the chromatogram of the nasal spray solution match with the retention times of the peaks on the chromatograms of the standard samples solutions of fexofenadine hydrochloride and ammonium glycyrrhizinate.

The areas of the peaks obtained in the analysis of six consecutive injections of the standard samples solutions were used to calculate the value of the relative standard deviation (RSD). The results are shown in Table 4.

As follows from the presented results, the relative standard deviation of the peak areas of fexofenadine hydrochloride and ammonium glycyrrhizinate obtained by repeated administrations of the same standard solutions, does not exceed 2%. That matches the suitability requirements of the chromatographic system.

[2] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I – IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

Table 1 – Conditions for chromatographic determination

Time, min	Elution mode	
	Methanol, %	50 mmol/l potassium dihydrogen phosphate solution, %
0	55	45
10	55	45
30	95	5

Table 2 – Characteristics of APIs peaks on nasal spray chromatograms

Component of spray	Characteristics of chromatograms			
	t_R, min	A_s	R_s	N
Fexofenadine hydrochloride	6.2±0.2	Not more than 1.2	Not less than 1.5	2800
Ammonium glycyrrhizinate	16.4±0.2	Not more than 1.4	Not less than 1.5	30000

Table 3 – Characteristics of peaks of fexofenadine hydrochloride and ammonium glycyrrhizinate on chromatograms of standard sample solution

Component of spray	Characteristics of chromatograms			
	tR, min	A_s	R_s	N
Fexofenadine hydrochloride	6.2±0.2	Not more than 1.4	Not less than 1.5	2900
Ammonium glycyrrhizinate	16.4±0.2	Not more than 1.3	Not less than 1.5	31000

Table 4 – Evaluation of reproducibility of-peak areas on chromatograms of standard solutions of fexofenadine hydrochloride and ammonium glycyrrhizinate

Analyte	Injection replication, n	Peak area, mAU×min	RSD,%
Fexofenadine hydrochloride	1	33.12	Xm = 33.16 S^2 = 0.005667 SD = 0.07528 RSD = 1.25%
	2	33.26	
	3	33.08	
	4	33.24	
	5	33.18	
	6	33.10	
Ammonium glycyrrhizinate	1	8.13	Xm = 8.12 S^2 = 0.002417 SD = 0.04916 RSD = 0.82%
	2	8.04	
	3	8.12	
	4	8.18	
	5	8.16	
	6	8.10	

Table 5 – Initial data for evaluating of linearity methods in relation to fexofenadine hydrochloride

№	Concentration of fexofenadine hydrochloride standard solution, %	Peak area, mAU×min (3 sequential injections mean)
1	0.0144	19.90
2	0.0192	28.10
3	0.0240	33.16
4	0.0288	39.98
5	0.0336	48.26

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Table 6 – Initial data for evaluating of linearity methods in relation to ammonium glycyrrhizinate

№	Concentration of ammonium glycyrrhizinate standard solution, %	Peak area, mAU×min (3 sequential injections mean)
1	0,0048	5,01
2	0,0064	6,18
3	0,0080	8,12
4	0,0096	9,04
5	0,0112	10,45

Table 7 – Results of precision assessment of methods for quantitative determination of fexofenadine hydrochloride and ammonium glycyrrhizinate (replication level)

Component	Peak area, mAU × min	Found, mg/ml	Metrological characteristics
Fexofenadine hydrochloride	32.91	2.98	$\bar{x}$ = 2.92 S^2 = 0.00188 SD = 0.04336 RSD = 0.72% $\Delta\bar{x}$ = ±0.05 $\bar{\varepsilon}$ = ±1.56% $\bar{x} \pm \Delta\bar{x}$ = 2.92 ± 0.05 mg/ml
	31.86	2.88	
	32.13	2.91	
	31.76	2.87	
	32.27	2.92	
	32.76	2.96	
Ammonium glycyrrhizinate	8.36	1.03	$\bar{x}$ = 1.00 S^2 = 0.002657 SD = 0.05154 RSD = 0.86% $\Delta\bar{x}$ = ±0.05 $\bar{\varepsilon}$ = ±5.40% $\bar{x} \pm \Delta\bar{x}$ = 1.00 ± 0.05 mg/ml
	8.02	0.99	
	7.44	0.92	
	8.52	1.05	
	7.86	0.97	
	8.56	1.05	

Note: The peak area of the solution of the fexofenadine hydrochloride standard sample = 33.16 mAU×sec; the peak area of the solution of the ammonium glycyrrhizinate standard sample = 8.12 mAU×sec

Table 8 – Results of intra-laboratory precision assessment of methods for quantitative determination of fexofenadine hydrochloride and ammonium glycyrrhizinate

Fexofenadine hydrochloride					
Analyst 1		Analyst 2		Metrological characteristics	
Peak area, mAU × min	Found, mg/ml	Peak area, mAU × min	Found, mg/ml	Analyst 1	Analyst 2
32.91	2.98	32.64	2.96	$\bar{x}$ = 2.92 S^2 = 0.00188 SD = 0.04336 RSD = 1.48% $\bar{x} \pm \Delta\bar{x}$ =2.92 ± 0.05 mg/ml	$\bar{x}$ = 2.98 S^2 = 0.001987 SD = 0.04457 RSD = 1.49% $\bar{x} \pm \Delta\bar{x}$ =2.98 ± 0.05 mg/ml
31.86	2.88	33.42	3.03		
32.13	2.91	33.04	3.00		
31.76	2.87	32.08	2.91		
32.27	2.92	33.12	3.01		
32.76	2.96	32.54	2.95		
T_{calc} = 2,04 < t (95%; 10); F_{calc} = 1,06 < F (95%; 5; 5) – the differences between the results obtained are random					
Ammonium glycyrrhizinate					
Analyst 1		Analyst 2		Analyst 1	Analyst 2
8.36	1.03	8.84	1.08	$\bar{x}$ = 1.00 S^2 = 0.002657 SD = 0.05154 RSD = 5.15% $\bar{x} \pm \Delta\bar{x}$ = 1.00 ± 0.05 mg/ml	$\bar{x}$ = 1.02 S^2 = 0.003107 SD = 0.055737 RSD = 5.45% $\bar{x} \pm \Delta\bar{x}$ = 1.02 ± 0.06 mg/ml
8.02	0.99	8.56	1.04		
7.44	0.92	8.12	0.99		
8.52	1.05	8.92	1.09		
7.86	0.97	7.49	0.91		
8.56	1.05	7.84	0.95		
$t_{calc.}$ = 0,64 < t (95%; 10); $F_{calc.}$ = 1,17 < F (95%; 5; 5) the differences between the results obtained are random					

Note: Analyst 1: the peak area of the solution of the standard sample of fexofenadine hydrochloride = 33.16 mAU×sec; the peak area of the solution of the standard sample of ammonium glycyrrhizinate = 8.12 mAU×sec; Analyst 2: the peak area of the solution of the standard sample of fexofenadine hydrochloride = 33.04 mAU×sec; the peak area of the solution of the standard sample of ammonium glycyrrhizinate = 8.21 mAU×sec.

Table 9 – Scheme of preparation of model mixture solutions with adjuvants of API standard samples solutions

Analyte	Added as a model mixture, mg	Added as a standard sample amount, mg	The total calculated content of the component after dilution mg/ml	Concentration level relative to nominal,%
Fexofenadine hydrochloride	3,6	1,2	0,192	80
	3,6	2,4	0,240	100
	3,6	3,6	0,288	120
Ammonium glycyrrhizinate	1,2	0,4	0,064	80
	1,2	0,8	0,080	100
	1,2	1,2	0,096	120

Table 10 – The results of assessing the correctness of the method for the quantitative determination of fexofenadine hydrochloride and ammonium glycyrrhizinate

Additive added, mg	Additive found, mg	Detection rate, %	Characteristics calculated on the basis of the detection rate value
		Fexofenadine hydrochloride	
1.20	1.26	104.84	$\bar{x}$ = 100.52% SD = 3.76 RSD = 3.74%
1.20	1.15	95.74	
1.20	1.18	98.07	
2.40	2.29	95.39	
2.40	2.44	101.69	
2.40	2.52	104.95	
3.60	3.69	102.54	
3.60	3.53	98.07	
3.60	3.72	103.36	
		Ammonium glycyrrhizinate	
0.4	0.36	90.00	$\bar{x}$ = 95,79% SD = 7.51 RSD = 7.84%
0.4	0.34	85.00	
0.4	0.36	90.00	
0.8	0.82	102.50	
0,8	0.85	106.25	
0,8	0.78	97.50	
1,2	1.14	95.00	
1,2	1.09	90.83	
1,2	1.26	105.00	

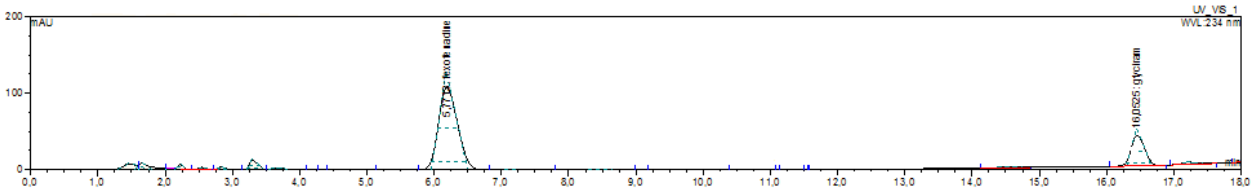

Figure 1 – Test solution chromatogram

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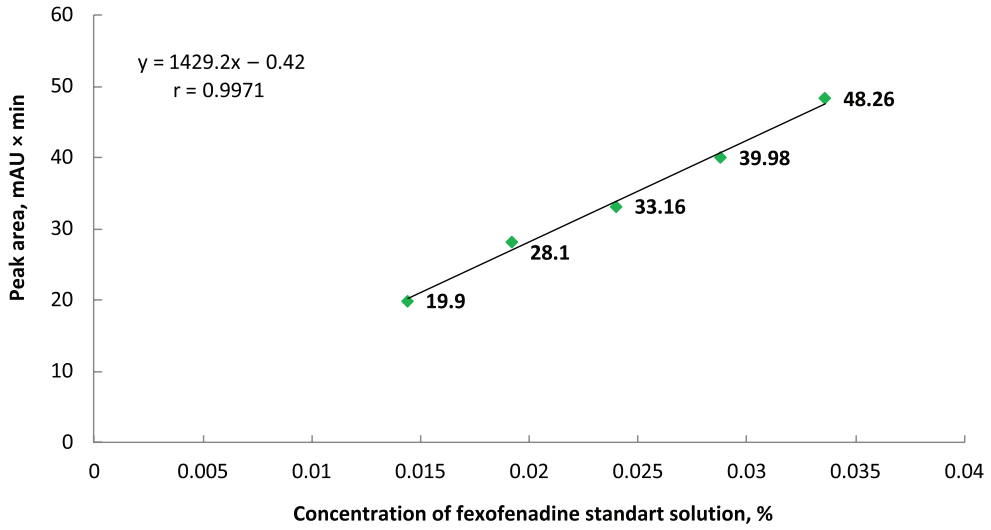

Figure 2 – Dependence graph of fexofenadine hydrochloride peak area on its concentration

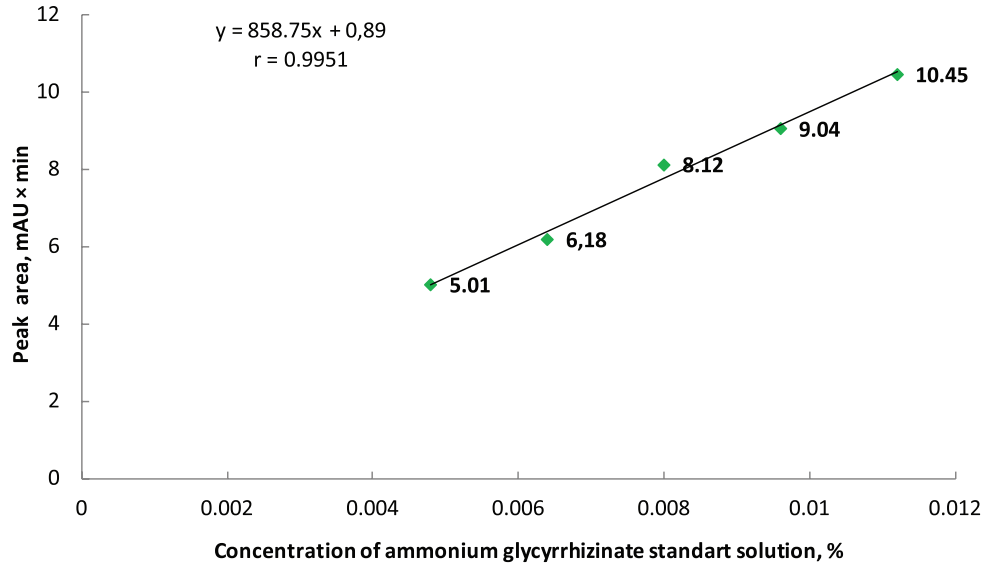

Figure 3 – Dependence graph of ammonium glycyrrhizinate peak area on its concentration

Thus, the characteristics of the peaks on the chromatograms of both the nasal spray solution and the standard sample solutions match the suitability parameters of the chromatographic system. They are: the efficiency of the chromatographic column, calculated from the peaks of the analytes, is not less than 2000 theoretical plates, the asymmetry factors of the peaks are in the range of 0.8 up to 1.5; the relative standard deviation of the peaks areas of the determined substances does not exceed 2%.

Validation of developed methods

The criterion for evaluating the analytical methods is its validation. The validation of the HPLC analysis method for the quantitative determination of the components of the nasal spray was carried out in accordance with State Pharmacopoeia of the Russian Federation of the XIVth edition; to solve practical issues of implementing validation procedures, ~~we~~ the authors of the article were guided by the literature data[3].

The specificity of the methods for determining fexofenadine hydrochloride and ammonium glycyrrhizinate in the nasal spray was confirmed by the correspondence of the retention times of the main peaks on the test solution chromatogram and the peaks of the standard samples solutions of fexofenadine hydrochloride and ammonium glycyrrhizinate. The analysis of the model mixture consisting of nasal spray excipients confirmed the absence of irrelevant chromatographic peaks in the domain of peaks output of fexofenadine hydrochloride and ammonium glycyrrhizinate[4].

[3] Validacija analiticheskih metodik dlja proizvoditelej lekarstv [Validation of analytical methods for drug manufacturers]. Edited by V.V. Beregovykh. M. Litterra, 2008:132.

[4] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

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The linearity relative to fexofenadine hydrochloride and ammonium glycyrrhizinate, was established using standard solutions. The concentration range of solutions included the proposed analytical area of the methods – from 80 to 120% of each component[5].

The solutions were chromatographed three times under the above-listed conditions; the results are shown in Table 5.

The obtained data were used to plot the dependence graph of the peak area on the concentration of fexofenadine hydrochloride (Figure 2).

A linear regression analysis of the results obtained by the least squares method made it possible to establish the dependence of the fexofenadine hydrochloride peak area on its concentration. It is linear and is described by the equation y = 1429 (± 264.2) x – 0.42; the correlation coefficient is 0.9971, and the free term of the equation is statistically insignificant, which is important for confirming the correctness of the method.

The linearity of the methods with respect to ammonium glycyrrhizinate was carried out in a similar manner. The solutions for chromatography were obtained by diluting the initial 0.1% solution of the ammonium glycyrrhizinate standard sample. The results of the determination are presented in Table 6.

The calibration graph based on the obtained data is shown in Fig. 3.

The analysis of the obtained dependence showed that it is described by a linear equation in the form of y = b × x + a, where b = 858.75 ± 204.59. The free term of the equation is 0.89, but its statistical significance is missing. The correlation coefficient is 0.9951, which meets the requirements (≥0.98)[6].-

Thus, the obtained results indicate a satisfactory linearity of the methods for determining fexofenadine hydrochloride and ammonium glycyrrhizinate.

The precision of the methods was evaluated by analyzing a sample of the nasal spray in a six-fold repetition (the replication level). To assess the intra-laboratory precision, the analysis of the test sample was carried out by another analyst on other days using the same equipment. The results of the precision assessment are presented in Tables 7 and 8.

The obtained results indicate the satisfactory precision of the proposed methods for the quantitative determination of the components of the developed nasal spray at the levels of replication and intra-laboratory precision.

It was found out that the average content of fexofenadine hydrochloride in the test sample of the nasal spray is 2.92 ± 0.05 mg/ml (97% of the declared; relative determination error ± 1.56%); ammonium glycyrrhizinate is 1.00 ± 0.05 mg/ml (100% of the declared value; relative determination error ± 5.4%).

Given that the nasal spray is multicomponent, the correctness of the methods was checked using the standard addition method.[7]

The scheme for obtaining solutions with adjuvants is shown in Table 9.

The results of determining the correctness of the methods are presented in table 10.

As follows from the obtained results, the detection rate of the added additives of fexofenadine hydrochloride was in the range from 95 to 105%, of ammonium glycyrrhizinate – from 85 to 106.25% with an RSD value of no more than ±3.74% and ±7.84%, respectively, which meets the requirements[8]. Thus, the proposed methods is characterized by the satisfactory correctness.

The analytical range of the method relative to the nominal concentration of the analytes in the nasal spray was from 80% to 120%.

The method of high performance liquid chromatography, along with other methods, is known to be increasingly used in both qualitative and quantitative analyses of active pharmaceutical ingredients. This method acquires particular relevance in the course of the analysis of pharmacologically active compounds combinations presented in one dosage form.

According to *Ibrahim F. A., et al., 2019* and using high performance liquid chromatography with an UV detection, it is possible to successfully identify and quantify the active components in combinations of moxifloxacin (a synthetic antibacterial agent of the fluoroquinolone group) with glucocorticosteroids intended for the systemic use – dexamethasone and prednisolone. Moreover, in this study, the authors used an original approach from the area of «green chemistry» without the use of toxic organic solvents: as an eluent in the isocratic determination mode, a mixture of ethanol:water in the ratio of 90:10 was used [25].

Another study conducted by the authors' team of *Al-Sanea M. M. et al, 2021,* showed that the method of high performance liquid chromatography makes it possible to quantitatively determine active substances in widespread combinations of antihypertensive medicines: hydrochlorothiazide + olmesartan medoxomil and hydrochlorothiazide + fosinopril-sodium. It should be noted that in this work, isocratic determination mode with a mobile phase based on potassium dihydrogen phosphate + orthophosphoric acid (pH = 3) with the addition of acetonitrile and methanol, made it possible not only to qualitatively and quantitatively determine the target compounds, but also to identify a number of specific impurities, for example, chlorothiazide, which is

[5] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

[6] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

[7] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

[8] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. [Electronic resource]. Access mode: http: // http://femb.ru/femb/ pharmacopea.php

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a product of the dehydrogenation reaction of hydrochlorothiazide [26].

Considering a widespread use of the HPLC method, as well as its high analytical characteristics, it is not surprising that HPLC is widespread in the pharmaceutical analysis of anti-allergic medicines and their combinations. So, *Shamshad N., et al., 2021* showed that it is possible to successfully identify and quantify cetirizine in the presence of chloroquine and pyrimethamine, using an isocratic elution mode with a mixture of methanol: water (70:30) and UV detection [27]. Furthermore, *Shamshad & Mirza, 2021* demonstrated the possibility of determining cetirizine in the presence of diclofenac sodium [28]. Loratadine can be successfully identified in combination with pseudoephedrine using a methanol: water (90:10) mixture as an eluent in an isocratic mode [29].

In addition, high performance liquid chromatography makes it possible to separate the substances with antiallergic properties from their metabolites (including those exhibiting a pharmacological activity), which was shown by *Sebaiy & Ziedan, 2019*. In this work, the authors identified and quantified loratadine and its active metabolite desloratadine (which is also an antiallergic drug that blocks histamine H_1 receptors) when eluted with a mixture of methanol + phosphoric acid (85:15) in an isocratic mode and detection with a UV spectrophotometric unit [30].

The methods for the qualitative and quantitative determination of fexofenadine hydrochloride in one dosage form with montelukast-sodium and ambroxol hydrochloride in isocratic elution mode with a mixture of methanol:water (70:30) and UV detection, are known [31].

Thus, based on the literature data, in this study for the qualitative and quantitative determination of the active substances of fexofenadine hydrochloride and ammonium glycyrrhizinate, the method of high performance liquid chromatography with UV detection was used. In the course of the work it was shown that due to the different solubility of the target substances, the isocratic elution mode does not allow achieving optimal separation of the components with an analysis duration of less than 30 minutes, which determined the use of a gradient mode. It should be noted that similar conditions of changing the analysis mode have been described in the literature. So, by *Leistner & Holzgrabe, 2021*, when analyzing impurities to the baclofen substance, a gradient mode was used, since the existing impurities for this substance are represented by sparingly soluble zwitter-ions [32] 11 impurities of the ivabradine substance [33] and a combination of pharmacologically active compounds of paracetamol and methionine [34] were investigated by the same approach.

The further course of the study showed that the developed analysis method is reproducible and matches all validity requirements, which is especially important in the analysis of medicines combinations. As *Narula & Pal, 2021* indicate the validation assessment of the analytical methods is a necessary step in the creation of rational methods for the medicines analysis and occupies one of the leading places in the course of their development [35]. There are cases when the optimal analytical methods (diazepam, metformin) did not meet the requirements of validity and, accordingly, could not be used in practical application [36]. In this regard, the developed method of the simultaneous determination of fexofenadine hydrochloride and ammonium glycyrrhizinate in an antiallergic nasal spray is a suitable analytical tool for a pharmaceutical analysis.

CONCLUSION

For the determination of fexofenadine hydrochloride and ammonium glycyrrhizinate using HPLC in a gradient elution mode, the selection of optimal conditions has been carried out and the methods has been developed. The results of the validation assessment showed that the developed methods matches the suitability parameters: it is correct, precise, specific and linear in the analytical field, which confirms its applicability for confirming the quantitative determination of fexofenadine hydrochloride and ammonium glycyrrhizinate in a medicine. It has been experimentally found out that during HPLC analysis, the average content of fexofenadine hydrochloride in the developed nasal spray with antiallergic action is 2.92±0.05 mg/ml (the relative determination error ±1.56%), ammonium glycyrrhizinate is 1.00±0.05 mg/ml (the relative determination error ±5.40%).

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Mikhail V. Larskiy – carrying out the experimental part of the work, preparation a preliminary version of the manuscript; Anastasia E. Pozdnyakova – review of literary sources on the topic of research, conducting the experimental part of the work, preparation a preliminary version of the manuscript; Zara D. Khadzhieva – development of the research concept, approval of the final version of the manuscript; Dmitry I. Pozdnyakov – statistical processing of the obtained results, preparation of the preliminary version of the manuscript.

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