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QUALIFICATION WORK

on the topic: **«DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FLUTICASONE PROPIONATE IN AN ANTI-ALLERGIC NASAL SPRAY »**

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ANNOTATION

A spectrophotometric method for the determination of fluticasone propionate in a nasal antiallergic spray has been developed. The method is based on the intrinsic light absorption of the drug in an alcohol medium at a wavelength of 237 nm. The method is linear, specific and correct, which confirms the suitability of the developed spectrophotometric method for further use in the pharmaceutical analysis of fluticasone propionate nasal spray.

The work consists of an introduction, three chapters, general conclusions and list of references used, which is composed of 30 sources. Contents of work posted on 42 pages and contains 6 tables, 12 figures and 1 schema.

Key words: fluticasone propionate, quality control, spectrophotometric determination, finished pharmaceutical products, nasal antiallergic spray.

АНОТАЦІЯ

Розроблено спектрофотометричний метод визначення флютиказону пропіонату в складі спрею назального протиалергічного. В основу методу покладено власне світлопоглинання препарату в спиртовому середовищі за довжини хвилі 237 нм. Методика є лінійною, специфічною та коректною, що підтверджує придатність розробленої спектрофотометричної методики для подальшого використання у фармацевтичному аналізі спрею назального з флютиказону пропіонатом.

Робота складається зі вступу, трьох розділів, загальних висновків та списку використаної літератури з 30 джерел. Зміст роботи викладено на 42 сторінках і містить 6 таблиць, 12 рисунків та 1 схему.

Ключові слова: флютиказону пропіонат, контроль якості, спектрофотометричне визначення, готові лікарські засоби, спрей назальний протиалергічний.

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List of abbreviations

AR - Allergic rhinitis

ARIA - Allergic rhinitis and its impact on asthma

DCs - Dendritic cells

FP - Fluticasone propionate

GC - Glucocorticoids

GINA - Global initiative for asthma

GR - Glucocorticoid receptor

INCSs - Intranasal glucocorticosteroids

LTRA - Leukotriene receptor antagonist

NSAID - Nonsteroidal anti-inflammatory drug

OTC - Over the counter

SHI - Statutory health insurance

INTRODUCTION

Actuality of topic. The prevalence of allergic rhinitis (AR) is increasing worldwide in both children and adults. It is associated with comorbidities like asthma, atopic dermatitis/eczema, allergic conjunctivitis, rhinosinusitis, sleep disturbance and eustachian tube dysfunction. AR does not lead to fatal outcomes but its effects on patient morbidity, quality of life and productivity add significant costs to health-care systems.

Recent years have shown the growing popularity and importance of pharmaceuticals for the treatment of allergic diseases, in particular, nasal sprays based on glucocorticosteroids. One of these drugs, fluticasone propionate in the form of an anti-allergic nasal spray, has become a popular means of controlling allergy symptoms.

The relevance of developing quality control methods for this drug lies in several key aspects:

- Efficacy and safety of treatment: Ensuring high quality of the product guarantees its effectiveness in controlling allergy symptoms and safety for patients. Quality control techniques help ensure the stability and correct composition of the product.
- Maintaining the stability of the drug: Given the sensitivity of glucocorticosteroids to storage conditions, it is important to have methods in place to detect any changes in the quality of the product during transport, storage and use.
- Regulatory requirements: Regulatory authorities set strict quality standards for pharmaceutical products. Compliance with these standards is mandatory in order to obtain permission to manufacture and market a product.
- Market competitiveness: In light of the growing competition in the pharmaceutical market, ensuring high quality and reliability of a product is key to maintaining and expanding its market position.

To date, monographs on finished medicinal products with fluticasone propionate are not available in the world's leading pharmacopoeias, so there is a need to develop affordable, modern methods of analysis that would meet the requirements of current legislation, international quality standards and pharmacopoeial requirements.

Therefore, the development and improvement of quality control methods for the glucocorticosteroid fluticasone propionate nasal spray is an urgent task aimed at ensuring the effectiveness and safety of treatment of patients with allergic diseases.

Purpose of work is to select the conditions for the spectrophotometric determination of fluticasone propionate in a nasal spray, considering the content of excipients.

Tasks of the research:

- to review the literature on the etiology of allergic rhinitis;
- to determine the range of medicines used in the treatment of allergic manifestations;
- to review the literature on the use of fluticasone propionate in pharmaceutical and medical practice;
- to review the existing methods of quality control of fluticasone propionate;
- to propose method of spectrophotometric determination fluticasone propionate in substance and finished medicines «Nasofan» nasal spray;
- to carry out statistical processing of the obtained results;
- to draw a conclusion on the possibility of using the proposed methods for the analysis of fluticasone propionate in the investigated dosage form.

The object of the research is fluticasone propionate in substance and finished medicines «Nasofan» nasal spray (series 100010098, manufactured by Teva Pharmaceutical Industries Ltd, Czech Republic), the active ingredient of which is fluticasone propionate at a dosage of 50.0 μg/dose.

The subject of the research is development of a spectrophotometric method for quality control of the active pharmaceutical ingredient of fluticasone propionate

nasal spray

Methods of the research: absorption spectrophotometry in the ultraviolet region of the spectrum, mathematical calculations for studying validation characteristics and statistical processing of the chemical experiment results.

The practical value of the results. The developed spectrophotometric method for the determination of fluticasone propionate can be used for further analysis of the active pharmaceutical component in finished medicinal products for identification and quantification.

Elements of scientific research. The conditions for the quantitative assessment of fluticasone propionate in the nasal spray by absorption spectrophotometry in the ultraviolet region were determined, the concentration of the active pharmaceutical ingredient, analytical wavelength, dissolution medium were selected, and the stability of solutions was studied.

Structure and scope of the qualification work. The work consists of an introduction, three chapters, general conclusions and list of references used, which is composed of 30 sources. Contents of work posted on 42 pages and contains 6 tables, 12 figures and 1 schema.

CHAPTER I
ETIOLOGY, PATHOGENESIS AND PRINCIPLES OF ALLERGIC RHINITIS TREATMENT
(literature review)

Allergic rhinitis is a condition where the nasal mucosa becomes inflamed due to exposure to allergens, leading to inflammation mediated by IgE. Clinically, it presents with four main symptoms: runny nose, sneezing, itching in the nose, and nasal congestion. It can also be linked to other conditions such as asthma, atopic dermatitis, and nasal polyps. Approximately 20-30% of the world population experiences allergic rhinitis, with 15% of them developing asthma. Diagnosis and treatment should adhere to the ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines for allergic rhinitis and the GINA (Global Initiative for Asthma) guidelines for asthma. Treatment typically involves a combination of allergen avoidance (where feasible), medications, and allergen immunotherapy. Intranasal corticosteroids are the most effective treatment for allergic rhinitis, with their sensory characteristics playing a significant role in patient compliance [1].

1.1. Etiology and pathogenesis of allergic rhinitis

Allergic rhinitis emerges as a symptomatic nasal disorder triggered by exposure to allergens, leading to inflammation mediated by IgE in the nasal membranes. Clinically, it presents with four primary symptoms: anterior or posterior rhinorrhea, sneezing, nasal itching, and nasal congestion. These symptoms often disrupt sleep, cause fatigue, mood changes, and compromise cognitive function, significantly affecting quality of life and productivity. Additional symptoms may include conjunctivitis, postnasal drip, Eustachian tube dysfunction, otitis media, sinusitis, and in children, dental malocclusions and facial deformities. Allergic rhinitis can be triggered by various allergens such as domestic mites, animals,

insects, pollen, molds, latex, tobacco smoke, and pollutants like automobile exhaust and certain medications like aspirin and other NSAIDs.

It is frequently associated with other conditions like asthma, atopic dermatitis, and nasal polyps, posing a significant global health challenge with substantial economic and societal burdens. Allergic rhinitis prevalence ranges from 1.4% to 39.7% in Western countries, affecting approximately 40% of the world's population with atopic tendencies. The indirect costs associated with allergic rhinitis-related absenteeism and decreased productivity surpass those of other common conditions such as migraine, diabetes, and asthma.

Various pharmacological interventions are available for treating allergic rhinitis, with intranasal corticosteroids recommended as the first-line therapy for moderate to severe cases, especially when nasal congestion is prominent. INCSs work by inhibiting the inflammatory response, reducing nasal mucosa permeability, inflammatory cell count, and mediator release [2].

Patient perception of sensory attributes like odor and taste plays a crucial role in their preference for currently available INCS medications. Unpleasant sensory experiences may decrease patient adherence to treatment protocols [3].

Previously, allergic rhinitis was classified into seasonal, perennial, and occupational types based on the timing of exposure, but this system was found lacking. The latest ARIA guidelines propose a new classification based on:

- Duration: Allergic rhinitis is now categorized as either "intermittent" or "persistent."
- Severity and Impact: Additionally, the severity of symptoms and their impact on the patient's quality of life are considered, leading to classification as either "mild" or "moderate-severe" (Figure 1.1)

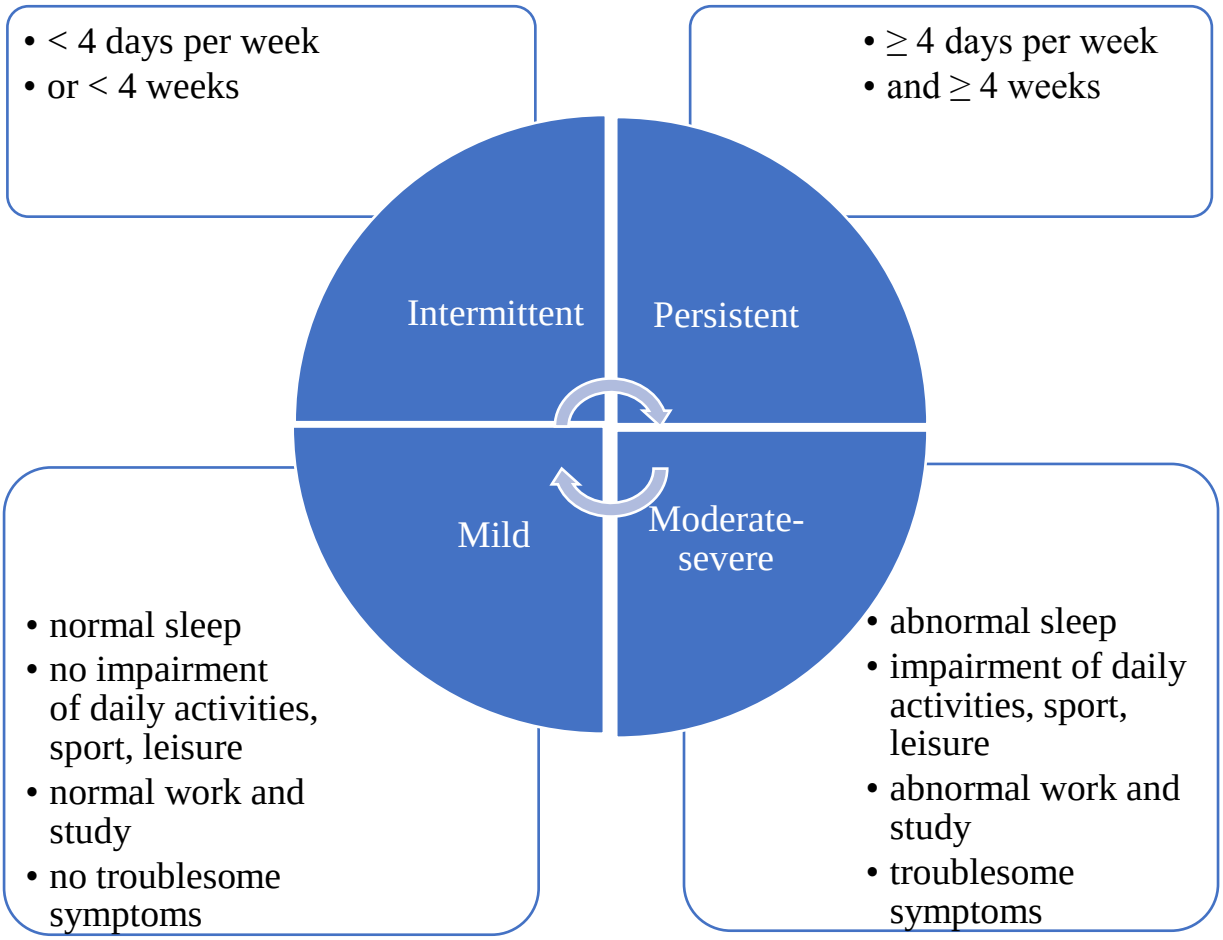


Figure 1.1. ARIA classification of allergy

Individuals experiencing intermittent allergic rhinitis typically present with symptoms such as sneezing, eye irritation, and watery nasal secretions. On the other hand, those with persistent allergic rhinitis commonly exhibit seromucous nasal secretions, postnasal drip, impaired sense of smell, nasal congestion, and may have comorbid conditions like asthma and chronic sinusitis. The Rhinoconjunctivitis quality of life questionnaire serves as a valuable tool for evaluating the severity of these symptoms [4].

During the sensitization phase in a Th2-favourable environment, barrier epithelial cells respond to allergen challenge. This generates cytokines that activate innate lymphoid type 2 cells and dendritic cells (DCs). DCs present allergenic peptides to naive T cells, which are influenced by cytokines secreted by type 2 lymphoid cells to differentiate into Th2 cells that produce IL-4/IL-13. They contact

naive B cells through CD40/CD40L interaction and induce their transition to plasma cells secreting IgE. IgE binds to FcεRI, present on mast cells and basophils, thereby enhancing its expression. After the next contact with the allergen, mast cells and blood basophils degranulate, releasing allergic mediators stored in the granules and newly synthesized lipid compounds (prostaglandins, leukotrienes) responsible for early allergy symptoms (vasodilation, vascular permeability, bronchoconstriction, etc.). In a more delayed phase, they also release several newly synthesized chemokines/cytokines. Together, they trigger an inflammatory response and the infiltration of other immune effector cells. When allergen exposure and subsequent epithelial damage persist, a chronic state of tissue damage and remodeling develops (figure 1.2) [5].

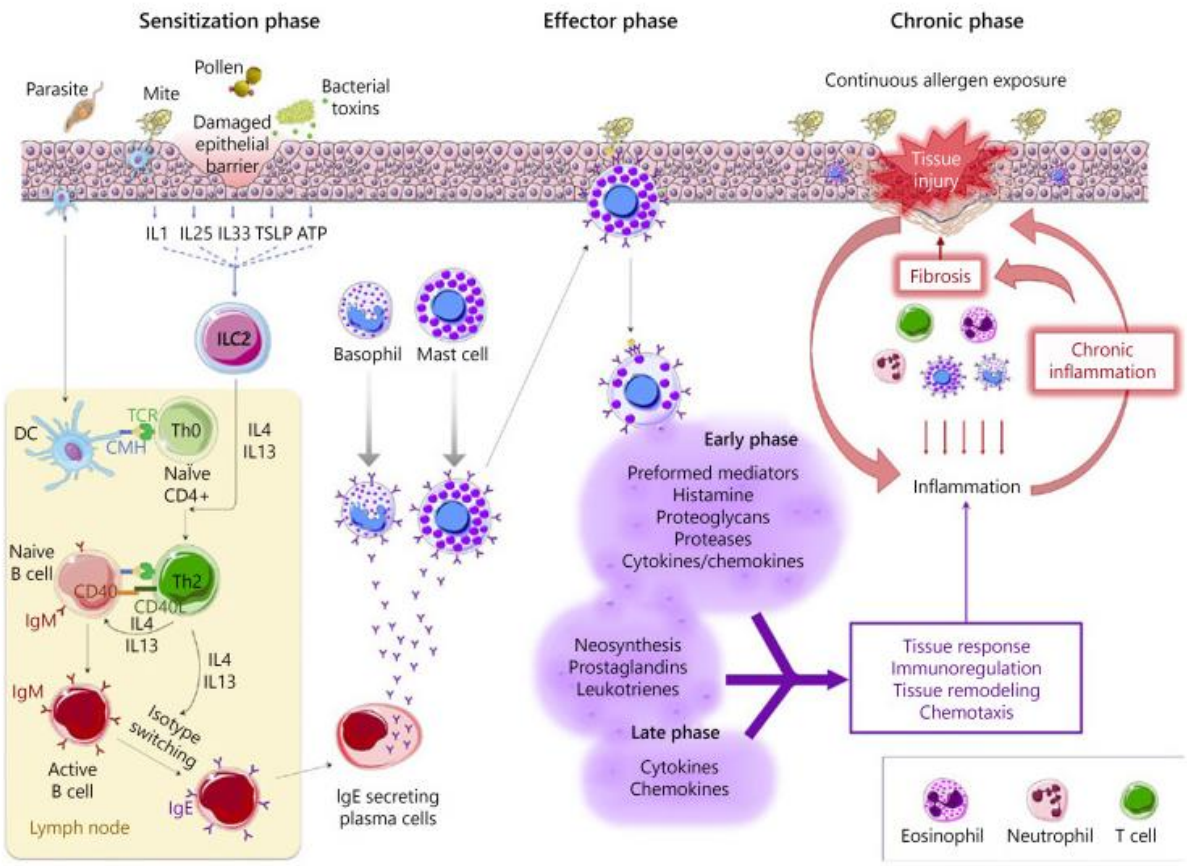


Figure 1.2. Mechanisms of allergic inflammation [6]

1.2. Treatment of allergic rhinitis

There are three main areas of allergic rhinitis therapy: elimination therapy, immunotherapy and drug therapy.

The goal of elimination therapy is to eliminate allergens and control the environment. In atopic disease, measures to eliminate allergens should be taken first.

Since it is often impossible to completely avoid contact with an allergen, methods have been developed to mechanically remove allergens from the surface of the nasal mucosa. To this end, nasal irrigation, various modifications of sinus rinsing, and endonasal sprays have been used. It should be borne in mind that all solutions used to eliminate the allergen from the nasal mucosa should be exclusively isotonic.

The most successful representatives of barrier therapy are saline sprays, the use of which leads to a significant reduction of allergen on the mucosal surface and to a decrease in the drug load in the treatment regimen.

Specific immunotherapy. The results of studies have convincingly proven the effectiveness of specific immunotherapy with allergens of grass, tree and shrub pollen, house dust mite antigens and cat dander. In order to minimise the risk of immunotherapy and increase the effectiveness of treatment, the question of the appropriateness of specific immunotherapy should be decided by an allergist or clinical immunologist [7].

1.2.1. Pharmacotherapy of allergic rhinitis

According to the specifications of many SHI pharmacotherapy consultants, OTC preparations should preferably be prescribed on a green prescription or should only be recommended. As a rule, the costs for nonprescription medicines are borne by the insured persons themselves. However, exceptions apply to seriously ill AR patients and should be considered so that these patients with severe disease can be treated under medical supervision.

Exceptions apply to OTC preparations which are used as the standard therapy for serious diseases for children up to the age of 12 and adolescents with developmental disabilities up to the age of 18 years.

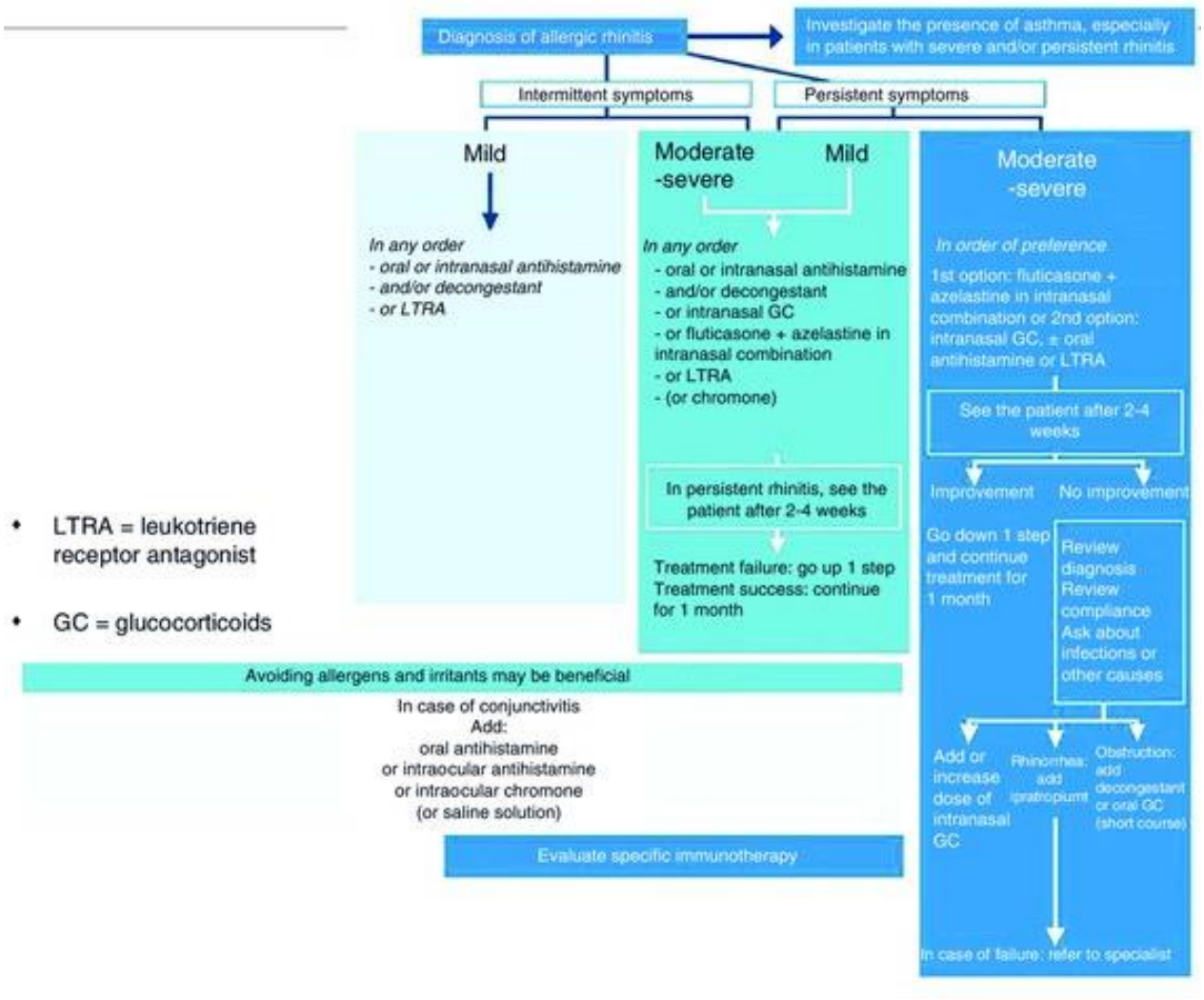
According to the OTC exemption list in Annex I of the Pharmaceutical Directive, the serious diseases in which nonprescription antihistamines can be prescribed for special cases are:

- only in emergency kits for treatment of bee, wasp, hornet venom allergies
- only for the treatment of severe, recurrent urticaria
- only in severe, persistent pruritus
- only for the treatment of severe allergic rhinitis,
- where topical nasal treatment with glucocorticosteroids is not sufficient.

In these cases, nonprescription antihistamines can also be the economic alternative, regardless of age.

Intranasal glucocorticosteroids (INCSs) are the gold standard in the pharmacological therapy of AR, as also outlined in the results of the Paris ARIA conference in 2019 [8].

In accordance with the mechanism of action and severity of allergic rhinitis, a stepwise treatment is performed, as shown in Scheme 1.1 [9].



Scheme 1.1. Stepwise treatment algorithm for allergic rhinitis

Intranasal corticosteroids (budesonide, ciclesonide, fluticasone propionate, fluticasone furoate, mometasone, triam-cinolone) are very powerful anti-inflammatory drugs that are effective in the treatment of AR and also of NAR (fluticasone propionate), in both adults and children and are the most effective medications for the control of AR symptoms. In most studies, INCS have shown greater effectiveness than the combined use of an oral antihistamine with a leukotriene receptor antagonist in the treatment of AR. The combination of a corticosteroid and an antihistamine (fluticasone propionate and azelastine) in an intranasal formulation (MP-AzeFlu) has demonstrated superior efficacy to the administration of each drug separately, with a very fast onset of action (5 min).This

formulation is currently indicated as first-line treatment for moderate-severe AR [10].

The use of short courses (1–3 weeks) of oral corticosteroids (prednisone, methylprednisolone, deflazacort) may be appropriate for the treatment of severe rhinitis that does not respond to other treatments [11].

Intranasal decongestants (phenylephrine, nafazoline, oxymetazoline, tramazoline, xylometazoline) can be used for short periods (≤7 days) in patients with nasal obstruction. Their use in children is not recommended.

There is scientific evidence (systematic reviews and metanalysis) that intranasal irrigation with saline is beneficial, and it is recommended in rhinitis and in CRS when used as single therapy or in combination with other treatments. There are also several therapies that are common to both AR and allergic asthma: allergen avoidance, leukotriene receptor antagonists (montelukast), monoclonal antibodies (anti-IgE or omalizumab and other biologics), and subcutaneous or sublingual specific immunotherapy (drops or tablets).

Allergen avoidance is an accepted strategy, although it is controversial in the treatment of respiratory allergic diseases. In the case of allergy to pet dander, cockroaches, fungi and occupational agents, the effect of avoidance seems to be more obvious, although in many cases this strategy is difficult to implement.

Anti-leukotrienes are less effective in monotherapy than oral antihistamines or intranasal corticosteroids [strong recommendation; low-quality evidence] in both adults and children. In combination, they can enhance treatment with antihistamines and intranasal corticosteroids. They are a good alternative first-line therapy in patients with coexisting allergic rhinitis and asthma.

Table 1.1.

Effect of drugs on symptoms of allergic rhinitis [12]

	Sneezing	Rhinorrhea	Nasal obstruction	Nasal itching	Eye symptoms
H1-antihistamines					
Oral	++	++	+	+++	++
Intranasal	++	++	+	++	0
Intraocular	0	0	0	0	+++
Corticosteroids					
Intranasal	+++	+++	+++	++	++
Chromones					
Intranasal	+	+	+	+	0
Intraocular	0	0	0	0	++
Decongestants					
Intranasal	0	0	++++	0	0
Oral	0	0	+	0	0
Anti-cholinergics	0	++	0	0	0
Anti-leukotrienes	0	+	++	0	++
Immunotherapy	+++	+++	+++	+++	+ to ++

1.2.2. The place of intranasal glucocorticoids in the treatment of allergic rhinitis

Intranasal glucocorticosteroids are extremely important in the treatment of patients with allergic rhinitis. They have a high anti-inflammatory activity associated with their inhibitory effect on inflammatory cells and their mediators, decreased microvascular permeability, increased synthesis of anti-inflammatory proteins, decreased eosinophil count, and inhibition of IgE production. These drugs also reduce the sensitivity of nasal mucosal receptors to histamine and mechanical stimuli, i.e. they also affect nonspecific nasal hyperreactivity. Depending on the severity of the clinical manifestations of allergic rhinitis and the presence of allergic

conjunctivitis, glucocorticosteroids are prescribed topically in the form of nasal sprays and eye drops, and much less often - orally (systemically).

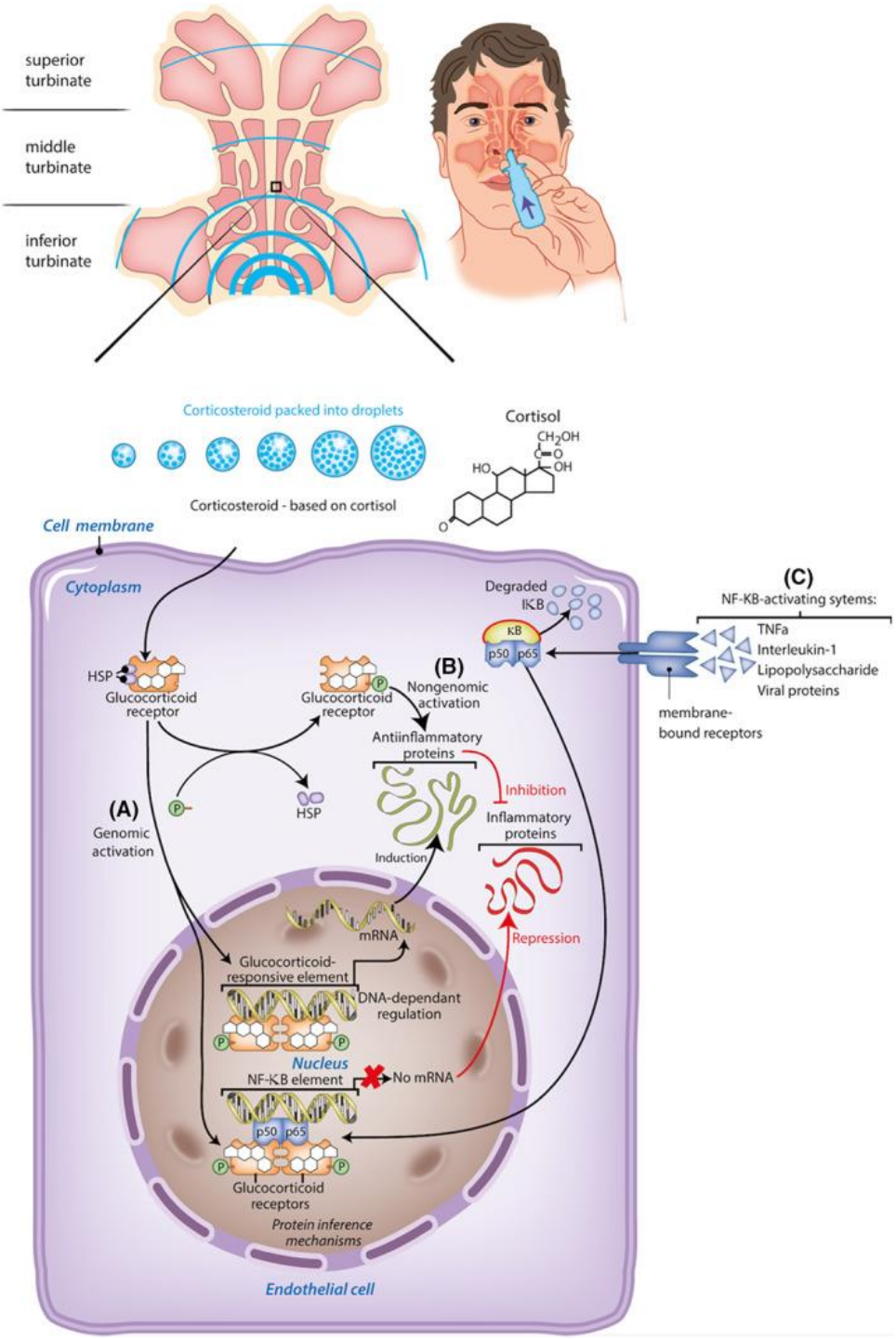


Figure 1.3. Mode of action of intranasal glucorticoids [13]

Figure 1.3 provides an outline of the mode of action. In response to allergic triggers, GCs impede the production and discharge of inflammatory agents, thereby

diminishing the migration of inflammatory cells into the nasal mucosa. Multiple mechanisms contribute to this effect. The primary mechanism involves GCs binding to the glucocorticoid receptor (GR) upon diffusion across the cell membrane. Subsequently, the GC/GR complex relocates to the nucleus and attaches to the DNA GC/GR complex (known as genomic activation). This process upregulates the transcription of genes responsible for anti-inflammatory proteins (termed transactivation) while concurrently suppressing the transcription of genes responsible for pro-inflammatory and immune proteins (referred to as transrepression). Additionally, the GC/GR complex interacts with other transcription factors, such as nuclear factor-κB, hindering the production of inflammatory proteins. Furthermore, through dissociation of the GC/GR complex, GC signaling activates membrane-associated receptors and second messengers (termed nongenomic activation) [14].

Traditionally, intranasal glucocorticosteroids are used in the treatment of patients with allergic rhinitis, as their efficacy and sufficient safety have been proven in many randomised, double-blind, placebo-controlled trials. The use of intranasal glucocorticosteroids provides pronounced anti-inflammatory effects directly on the mucous membranes of the nose, eyes and bronchi with minimal systemic manifestations. When prescribed to patients with arterial rhinitis, topical glucocorticosteroids have a pronounced therapeutic effect, reducing nasal congestion, itching, sneezing, and rhinorrhoea. Currently, modern topical glucocorticosteroids containing mometasone furoate, fluticasone propionate or furoate, and less commonly beclomethasone dipropionate and budesonide are used to treat allergic rhinitis. Modern topical forms of glucocorticosteroids are highly effective and cause minimal adverse effects, but they should be prescribed with caution to patients with immunosuppression, severe bacterial, fungal and viral (herpes) infections [15].

1.2.3. Pharmacological properties of fluticasone propionate

Fluticasone propionate (FP) is a topically active corticosteroid with established efficacy in seasonal and perennial AR. Its pharmacodynamic and pharmacokinetic properties, combined with its safety profile, have positioned FP as a leading medication in the market. The rapid and extensive uptake of FP by airway tissue, its strong affinity for the GR, and its minimal systemic bioavailability contribute to its impressive therapeutic and safety profile.

The propionate ester side chain of FP makes it highly lipophilic, which is crucial for its pharmacological characteristics. This lipophilicity allows FP to bind rapidly and strongly to tissues, leading to more prolonged retention compared to hydrophilic molecules like budesonide and hydrocortisone. The potency and therapeutic efficacy of FP are further enhanced by its high affinity for the GR.

Studies have shown that FP has a high selectivity and affinity for the GR receptor, as demonstrated by its association rate constant and low dissociation constant. Compared to dexamethasone, FP has a significantly lower equilibrium dissociation constant, indicating a stronger binding affinity. The relative receptor affinity of FP to the human GR is also much higher than that of dexamethasone.

These characteristics of FP, including its rapid association, high receptor affinity, and prolonged half-life of the FP-GR complex, support its clinical efficacy. The long half-life also allows for once-daily dosing schedules, enhancing the practicality of FP in the treatment of allergic rhinitis [16].

Fluticasone propionate has a pronounced anti-inflammatory effect, but its systemic activity is minimal when administered intranasally. The drug does not inhibit or inhibits to a very small extent hypothalamic-pituitary-adrenal function. After intranasal administration of fluticasone propionate (at a dose of 200 μg/day) for 24 hours, no significant change in plasma cortisol AUC is observed compared to placebo.

With intranasal administration of fluticasone propionate (200 μg/day), C_{max} in blood plasma is undetectable in most patients (less than 0.01 ng/ml). The level of

direct absorption of the drug from the nasal cavity is insignificant. The total systemic absorption of the drug, including the part of the dose that is swallowed, is also insignificant [17].

FP has a large volume of distribution - approximately 318 litres. Binding to blood proteins is moderately high - 91%. The active pharmaceutical ingredient of spray or drops is rapidly excreted from the systemic circulation, mainly by hepatic metabolism as an inactive carboxyl metabolite via cytochrome P450 CYP3A4. Caution should be exercised when co-administered with strong CYP3A4 inhibitors, such as ketoconazole and ritonavir, due to the potential increase in systemic exposure of fluticasone propionate.

The main route of drug elimination is intestinal excretion, mainly in the form of unchanged unabsorbed substance. Renal clearance of fluticasone propionate is very low (less than 0.2%) [18].

CONCLUSION TO CHAPTER I

1. A literature review was conducted on the etiology, pathogenesis of allergic rhinitis, classification and principles of treatment depending on the type of allergic rhinitis.

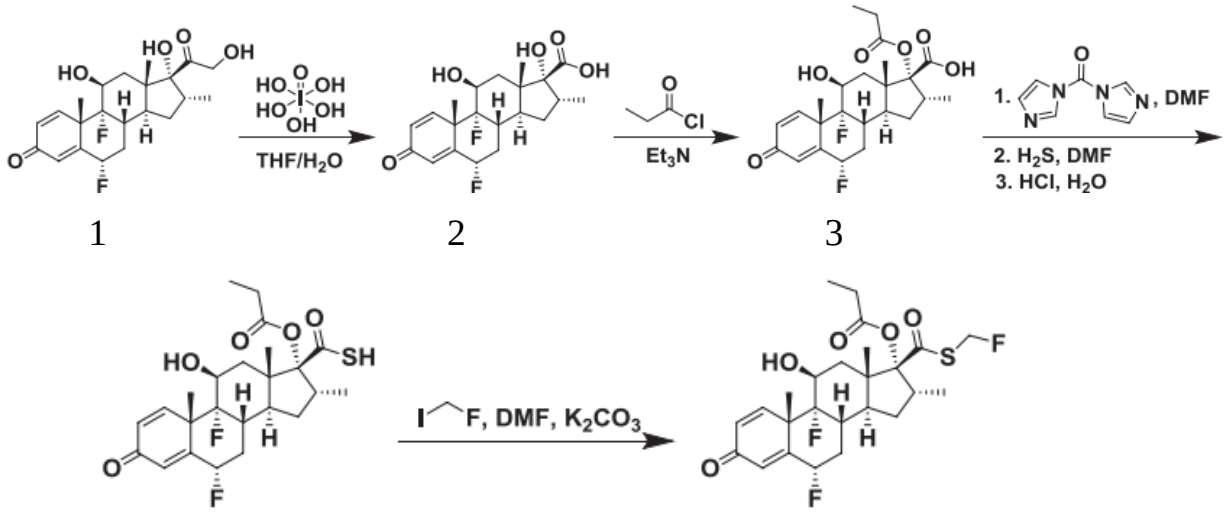
2. The published information demonstrates that worldwide the preference in the treatment of allergic rhinitis is given to drugs from the group of glucocorticosteroids, the mechanism of action of this group of drugs and the advantages of intranasal corticosteroids are presented.

3. According to the literature, fluticasone preparations, in particular fluticasone propionate, unlike all drugs of the intranasal glucocorticoid group, do not affect cortisol levels, have a long-lasting pharmacological effect and high efficacy in eliminating all symptoms of allergic rhinitis.

CHAPTER II
FLUTICASONE PROPIONATE: PHARMACEUTICAL ASSORTMENT, SYNTHESIS AND CURRENT ANALYTICAL METHODS

2.1. Syntesis of fluticasone propionate

Synthesis of fluticasone started with oxidation of 21-hydroxypregnan-20 one derivative (1), which with periodic acid in aqueous tetrahydrofuran produced intermediate carboxylic acid (2). It was readily 17α-acylated, without concomitant acylation, by reaction with excess propionyl chloride and triethylamine to produce propionate (3), which reacted rapidly with N,N′-carbonyldiimidazole in dimethylformamide to produce species, which, with H_2S in dimethylformamide and further acidic hydrolysis, produced the unstable carbothioic acid derivative (4). Reaction of the obtained carbothioic acid with fluoroiodomethane is a key synthetic stage of flumethasone synthesis. Chloromethyl carbothioate was prepared from carbothioate salt by alkylation with chloroiodomethane in dimethylformamide to produce fluticasone propionate (5) [19].

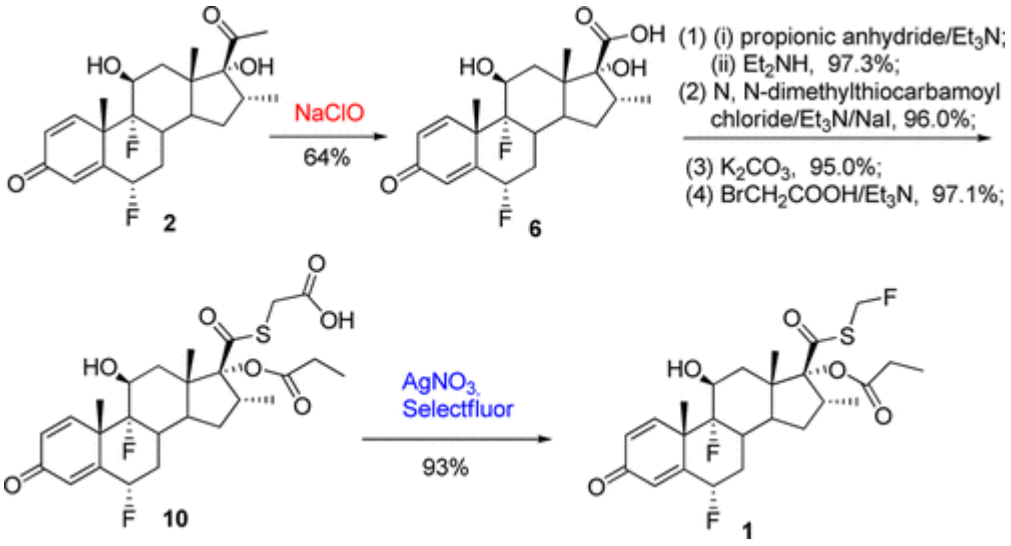


1 2 3

4 5

A new process for the preparation of fluticasone propionate is proposed, in which compound 2 is used as a starting material for the production of compound 6 using NaClO or NaBrO, which are much cheaper than H_5IO6, as an oxidant. In

addition, the toxic, expensive and polluting ICH_2F was replaced by $AgNO_3$ and selective fluorine in the decarboxylating fluorination [20].



2.2. Fluticasone propionate pharmaceuticals market

The global fluticasone propionate inhalers market size was USD 4044.74 million in 2021 [21].

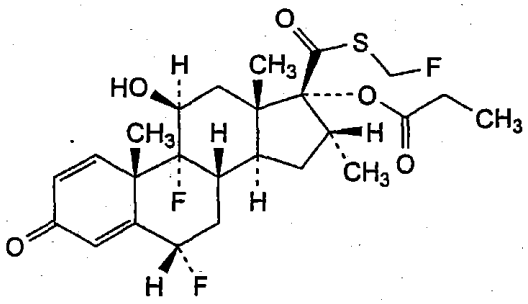
Most fluticasone propionate medicinal products are available in the form of nasal sprays (figure 2.1).

Figure 2.1. Assortment of fluticasone propionate nasal sprays

The nasal sprays consist of microfine fluticasone propionate suspended in an aqueous solution, designed for topical application to the nasal mucosa using a metered, atomizing spray pump.

2.3. Physicochemical properties of fluticasone propionate

Fluticasone propionate



(*S*)-fluoromethyl-6α, 9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)-androsta-1,4-diene-17β-carbothioate

$C_{25}H_{31}F_3O_5S$ M.м. 500.6

Fluticasone propionate is white or almost white powdered substance. Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

2.4. Methods of fluticasone propionate analysis

According to the monographs of the world's leading pharmacopoeias, despite the widespread use and demand for fluticasone propionate, there are no monographs for the finished drug for intranasal administration.

The analysis can be performed based on the physicochemical properties of the molecule.

A pharmacopoeial, rapid and accurate method of identification is the instrumental method of infrared absorption spectrophotometry.

The resulting spectrum of the test sample is compared with the reference spectrum or the spectrum of a standard sample: both spectra should correspond in terms of absorption intensity at the same wavenumber.

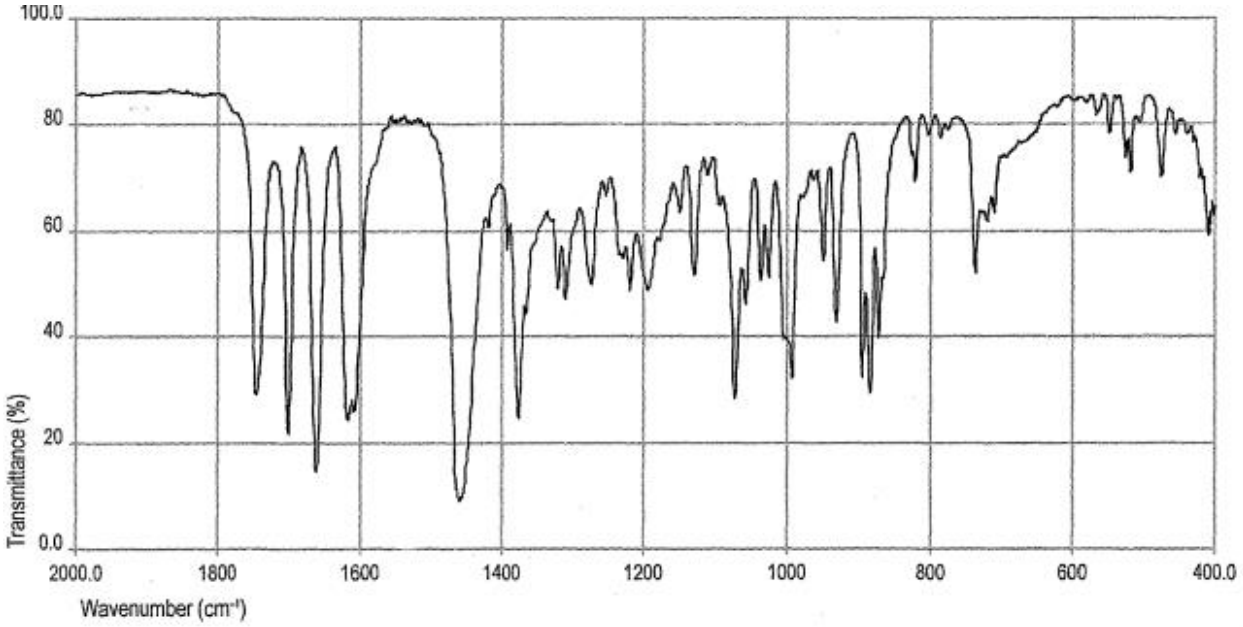


Figure 2.2. Reference infrared spectrum of fluticasone propionate [22].

To confirm the structure of the compound obtained as a result of the synthesis, the NMR method was proposed, which allows to identify the compound and determine the presence of impurities (figure 2.3-2.4).

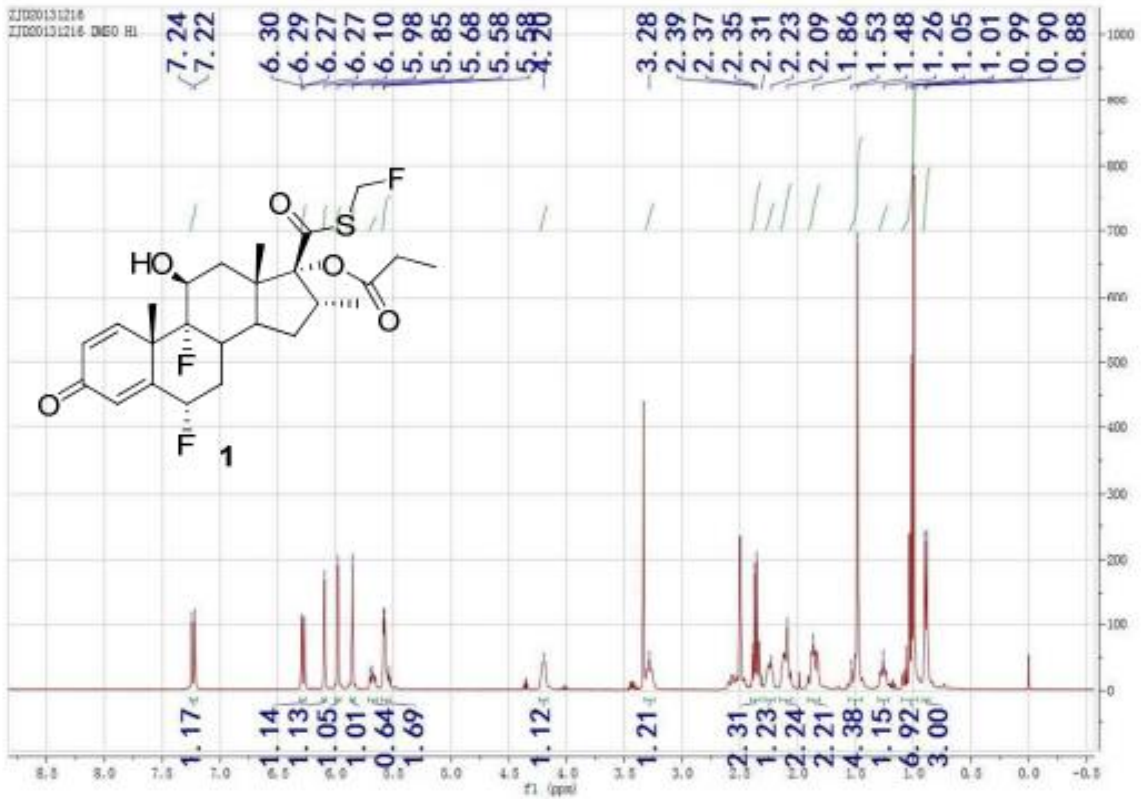


Figure 2.3. ^{1}H NMR-spectra of fluticasone propionate

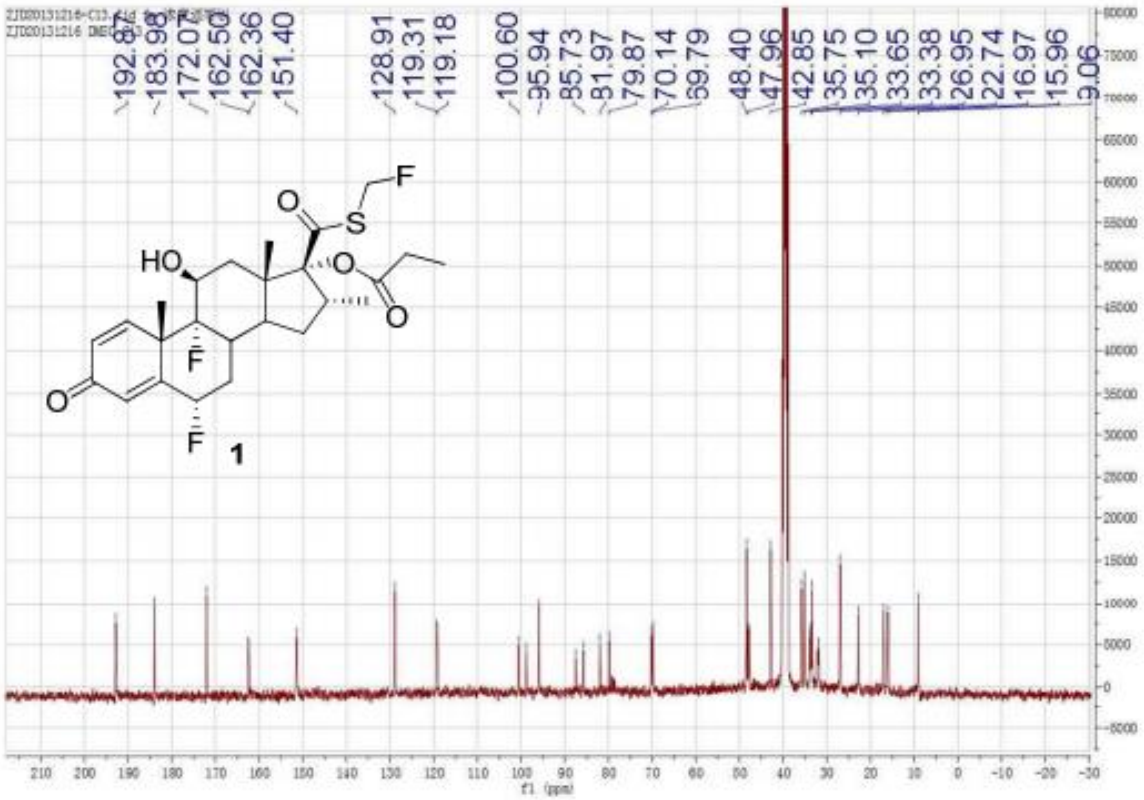


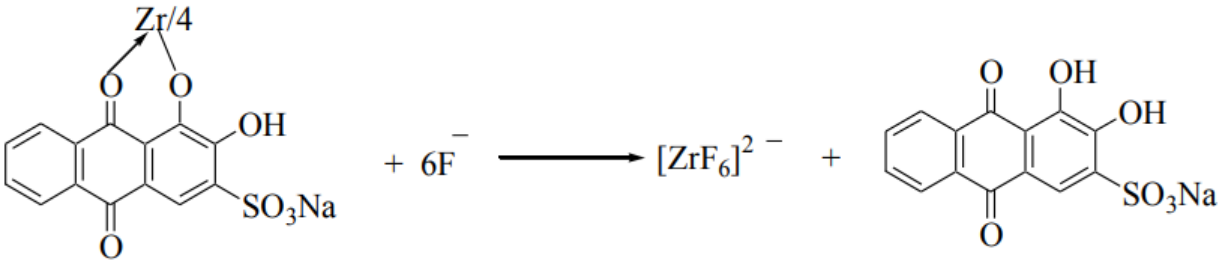
Figure 2.4. ^{13}C NMR-spectra of fluticasone propionate

According to its chemical structure, fluticasone propionate is an 11-β-hydroxysteroid, a corticosteroid, a fluorinated steroid, a steroid ester, a 2-furoate ester, a thioester and a 3-oxo-delta (1), delta (4)-steroid. It is a derivative of fluticasone and androstane hydride.

Therefore, the drug produces steroid cycle reactions. When the drug is dissolved in concentrated sulfuric acid and then water is added, a cherry colour with green-brown fluorescence appears. After the addition of chloroform and shaking, the lower layer turns yellow and the upper layer green.

The presence of fluorine in the structure of fluticasone propionate can be determined after combustion in a flask with oxygen by reaction with a solution of

alizarin in the presence of zirconium nitrate in hydrochloric acid:



As a result of the interaction, a soluble complex compound of zirconium and fluoride is formed; the red-violet color of the reagent solution turns yellow.

In addition to chemical reactions, scientists around the world are developing physicochemical methods for the determination of fluticasone propionate in substances, medicinal products and human biological fluids for pharmaceutical, toxicological and forensic analysis.

It was proposed to determine the individual solution of the drug by UV absorption spectrophotometry in the UV spectrum in the wavelength range of 200-400 nm against the background of a reference 70:30 (v/v) methanol/water solution. The maximum absorbance for fluticasone propionate solution in a methanol/water mixture was detected at 237 nm [23].

Figure 2.5 shows the electron sputtering spectra of positive ions of a solution of fluticasone propionate in methanol/water, showing an ion at 501.3 corresponding to $(M+H)^+$ and that at 1001.1 to $(M_2+H)^+$ ions.

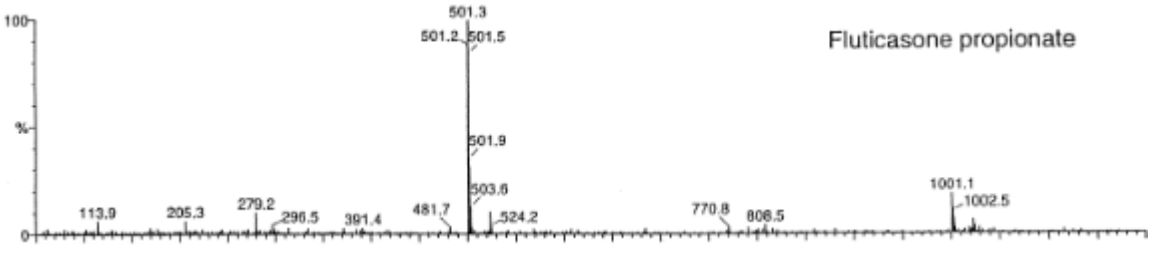


Figure 2.5. Positive ion electrospray mass spectra of fluticasone propionate

A novel method utilizing micellar electro kinetic chromatography was developed and validated for the precise quantification of fluticasone propionate in

nasal spray. This method employed a fused-silica capillary (50 μm i.d.; effective length, 40 cm) and a background electrolyte consisting of 25 mM borate and 25 mM anionic detergent SDS solution at pH 9. The capillary temperature was controlled at 35°C, and a voltage of 20 kV was applied during analysis. Injection was conducted using the hydrodynamic mode at 50 mbar for 6 s, with detection performed at 238 nm. Linearity within the concentration range of 2–80 μg/mL (r2 = 0.9956) was established. Specificity and stability-indicating capability were verified through forced degradation studies, including mass spectrometry analysis, which confirmed the absence of excipient interference. Limits of detection and quantitation were determined as 0.56 and 2 μg/mL, respectively. Comprehensive method validation demonstrated satisfactory accuracy, precision, and robustness. Application of the proposed method for quantitative analysis of FP nasal sprays yielded comparable results to those obtained using a validated reversed-phase liquid chromatographic method, with no significant differences observed ($P > 0.05$) [24].

In the study [25], liquid chromatography with a UV detector operating at a wavelength of 236 nm was utilized. A C18 column was employed for separation. Isocratic elution was conducted using a mixture of acetonitrile and water (60:40, v/v). The analytical method validation was conducted following ICH guidelines, covering selectivity, range, linearity, accuracy, detection limit, quantitation limit, precision, robustness, and solution stability. The method demonstrated selectivity and specificity. The precision and accuracy of the assay (100 ± 5.0%) were acceptable within the range of 50-150% of the analytical concentration of fluticasone propionate at the target concentration of 0.060 mg/mL. Linearity (0.9958) was achieved across the range of 0.03 to 0.09 mg/mL for active pharmaceutical ingredient.

CONCLUSION TO CHAPTER II

1. The synthesis routes of fluticasone propionate, which include economic and environmental components, are considered in accordance with the modern requirements of green chemistry and international requirements for the synthesis of pharmaceutical products.

2. The physicochemical properties of the compound are discussed, the formula of the drug, constants and properties are given.

3. A literature review of modern methods for the analysis of fluticasone propionate in the substance and finished medicinal products, including chemical methods based on the presence of a steroid cycle in the molecular structure, as well as physicochemical, in particular spectral and chromatographic, based on the physicochemical properties of the compound, was carried out.

CHAPTER III
DEVELOPMENT OF METHODS FOR DETERMINATION OF FLUTICASONE PROPIONATE IN NASAL SPRAY

The object of the study is a dosage form – «Nasofan» nasal spray (series 100010098, manufactured by Teva Pharmaceutical Industries Ltd, Czech Republic), which contains:

Figure 3.1. Visual appearance of the investigational drug «Nazofan»

active ingredient: fluticasone propionate*;*
1 dose contains fluticasone propionate 50 μg*;*
excipients: glucose anhydrous, microcrystalline cellulose and carmellose sodium (Avicel RC 591), phenylethyl alcohol, benzalkonium chloride solution, polysorbate 80, purified water.

It is indicated for the symptomatic treatment of allergic rhinitis.

Materials and methods. In the analysis of the selected medicine, tests were performed in accordance with the requirements of the European Pharmacopoeia for nasal sprays: determination of «Uniformity of mass of single-dose preparations», identification and quantification of the active pharmaceutical ingredient of the spray «Nazofan» by spectrophotometric method, standard method.

3.1. Mass uniformity of the nasal spray

The «Uniformity of mass» test is mandatory for quality control of nasal sprays. For this purpose, the test was carried out in accordance with the general article of the European Pharmacopoeia «Nasal drops and liquid nasal sprays» [26].

To conduct the study, the medicinal product is taken in its original packaging, a dose is released and discarded. After at least 5 seconds, shake the container for 5 seconds, release the dose and discard it. Repeat this operation three more times. After that, the container is weighed, the dose is released, and the container is weighed again. The mass of the individual dose is calculated as the difference of the two masses.

The delivered masses of 10 samples must be determined by difference weighing. A preparation meets the specification if not more than 2 single values deviate by more than 25% and none by more than 35% from the mean value.

The homogeneity of the active substance content was determined by the calculation and weight method (2.9.40) [27] by the formulas:

Weight of an individual unit (g) = weight of the container with the medicinal product (before the dose is sprayed (g) - weight of the container with the medicinal product (after the dose is sprayed (g)

Average weight of an individual dose (g) = total weight of 10 doses / 10

Deviation (%) = (Weight of an individual unit (g) - Average weight of an individual dose (g)) · 100 / Average weight of an individual dose (g)

The results of the calculations are shown in Table 3.1.

Table 3.1.

The «Uniformity of mass» test for «Nazofan» nasal spray

№	Weight of an individual unit, g	Deviation from the average weight, %	№	Weight of an individual unit, g	Deviation from the average weight, %.
1	0,11033	-7,03337	6	0,10305	0,02910
2	0,10069	2,31859	7	0,10247	0,59177
3	0,09998	3,00737	8	0,09860	4,34614
4	0,10632	-3,14319	9	0,10687	-3,67676
5	0,10295	0,12612	10	0,09951	3,46333
Average weight of an individual dose, g				0,10308	
Standard deviation, %				0,00374	

3.2. Selection of conditions for the determination of fluticasone propionate by UV-spectrophotometry

3.2.1. Determination of the analytical wavelength

To determine the wavelength that is analytical for the identification of the compound and suitable for the quantitative determination of fluticasone propionate, the absorption spectrum of a solution of fluticasone propionate in ethanol was analyzed according the requiments of the European Pharmacopoeia «2.2.25. Absorption spectrophotometry,ultraviolet and visible» [28] in the wavelength range from 200 nm to 400 nm (Fig. 3.1).

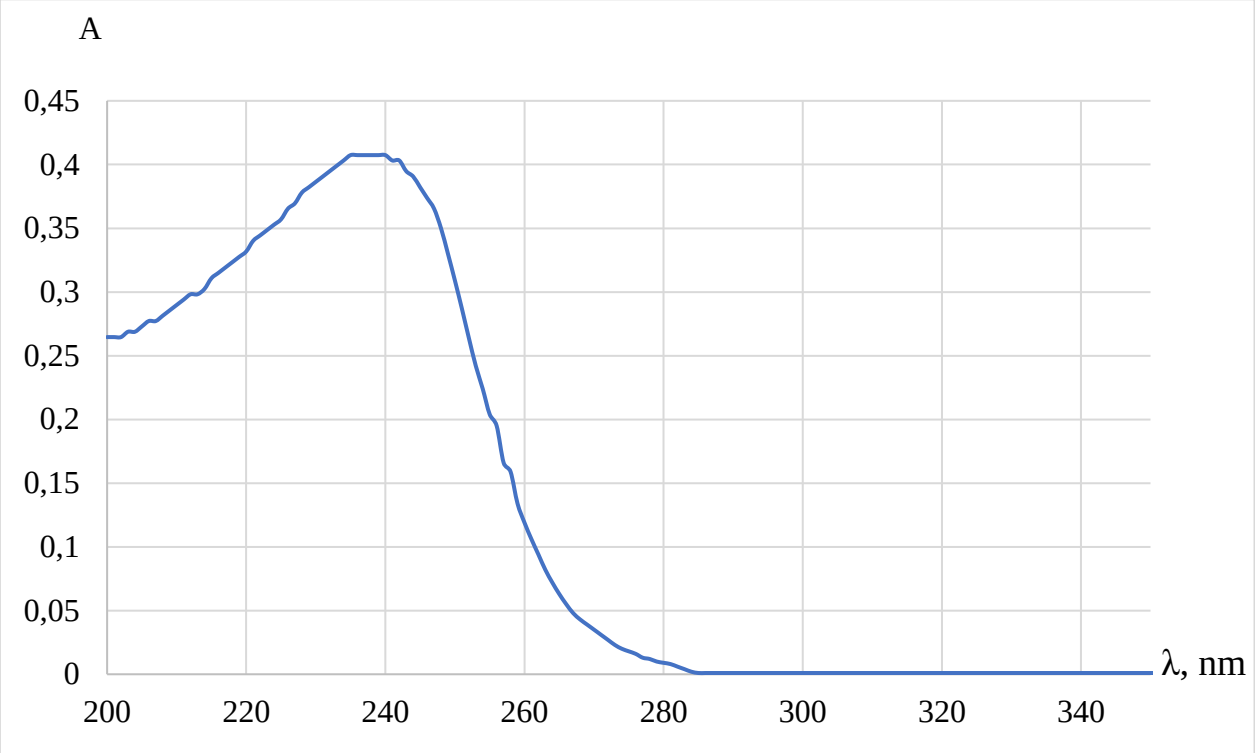


Figure 3.1. Absorption spectrum of 10 µg/ml of fluticasone propionate alcohol solution

There is one clear absorption maximum in the spectrum at 237 nm, which is analytical and can be used to identify the compound in the substance and the finished drug product.

3.2.2. Study of the light absorption of ethanol solutions of fluticasone propionate at 237 nm in accordance with the basic law

For quantitative evaluation, for the maximum light absorption at 237 nm, the compliance of fluticasone propionate solutions with the Bouguer-Lambert-Behr law was studied (Figure 3.2).

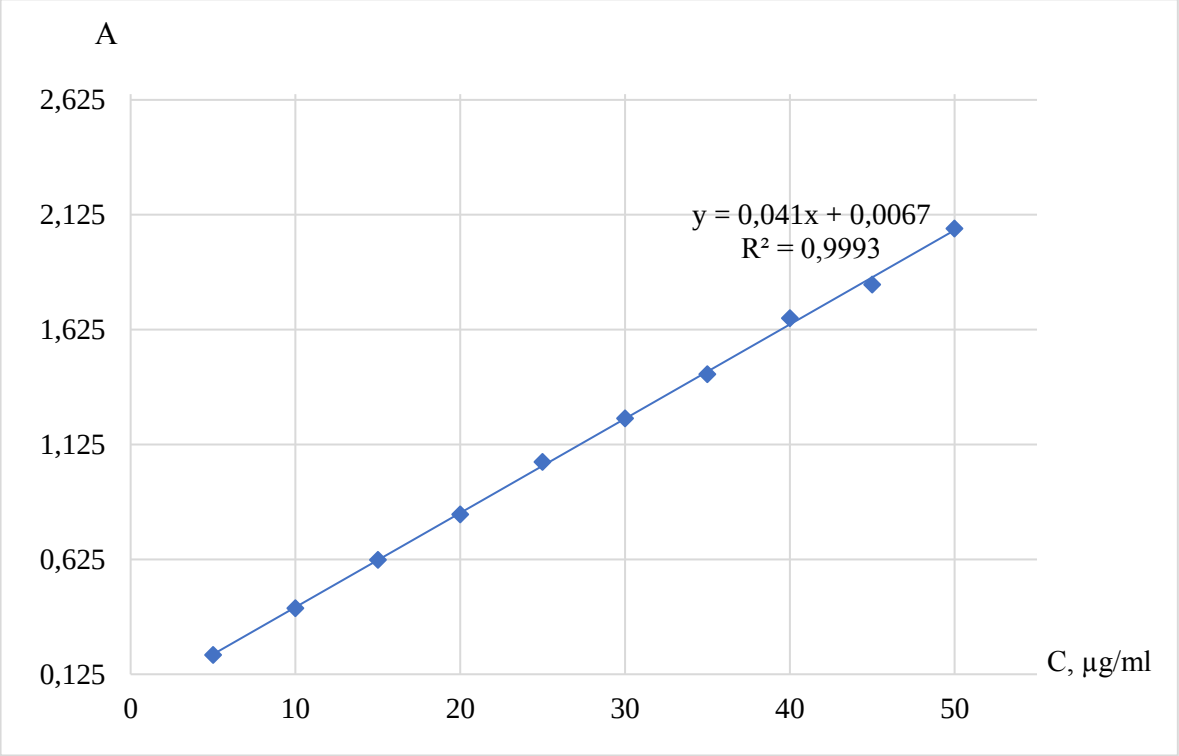


Figure 3.2. Subordination of fluticasone propionate solutions to the Bouguer-Lambert-Behr law at 237 nm

From the data obtained (Fig. 3.2), it can be seen that the compliance with the Bouguer-Lambert-Beer law is observed within the concentration of fluticasone propionate alcohol solution from 5 µg/ml to 50 µg/ml.

3.2.3. Determination of the stability of fluticasone propionate alcohol solution over time

For further use of the methodology, the stability of the solutions over time was studied. The stability of the solutions was determined for 1 hour with an interval of 10 minutes by measuring absorption on an Evolution 60s spectrophotometer in the «Kinetics» mode (Table 3.3, Fig. 3.3).

Table 3.3

Study of absorption changes of the solution with time at 237 nm

Solution	Time, min.						
	0	10	20	30	40	50	60
0,001% ethanol solution of fluticasone propionate	0,414	0,413	0,415	0,414	0,414	0,415	0,415

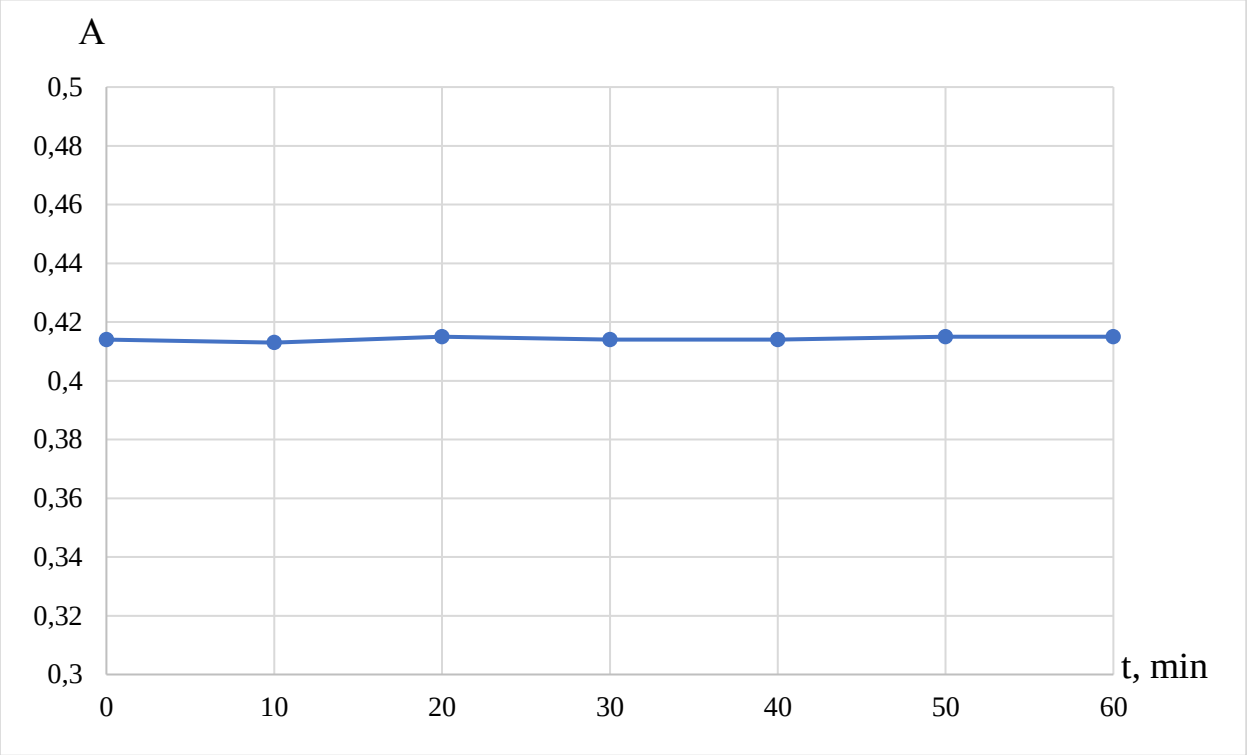


Figure 3.3: Study of the change in absorption with time of 0.001% solution of fluticasone propionate at 237 nm

The results of the study of the change in optical density over time showed that the solutions are stable for 1 hour.

3.2.4. Method of fluticasone propionate spectrophotometric determination in spray dosage form

The test is carried out in accordance with the requirements of the European Pharmacopoeia, 2.2.25

Test solution. Place a spray weight (10 doses) equivalent to 500.0 μg of fluticasone propionate in a 10 ml volumetric flask, bring the volume of ethanol to the mark and mix.

Reference solution. Place 50.0 mg of fluticasone propionate standard sample in a 100 mL volumetric flask, dissolve in 50 mL of ethanol, bring the volume of the solution to the mark with the same solvent and mix.

Measure the absorption of the test solution and the reference solution using a spectrophotometer at 237 nm in a cuvette with a layer thickness of 10 mm, using ethanol as a compensation solution.

Calculation.

The content of fluconazole propionate (X) in a dose of the drug in the form of a spray, in μg, is calculated by the formula:

$$X = \frac{A_1 \cdot m_0 \cdot 10 \cdot P \cdot m_{av.dose} \cdot 1000}{A_0 \cdot m_{spray} \cdot 100},$$

where: A_1 - absorption of the test solution;

A_0 - absorption of the reference solution;

P - the content of the main substance in fluticasone propionate standard sample, %;

m_0 - weight of the sample of fluticasone propionate standard sample, mg;

m_{spray} - weight of the spray sample taken for analysis, mg;

$m_{av.dose}$- average weight of the spray dose, mg.

3.2.5. Results of the quantitative determination of fluticasone propionate in the studied spray

Before quantification of fluticasone propionate in the test spray, the spectral characteristics of the placebo solution were studied to exclude the influence of excipients on the absorption results at 237 nm (Fig. 3.4).

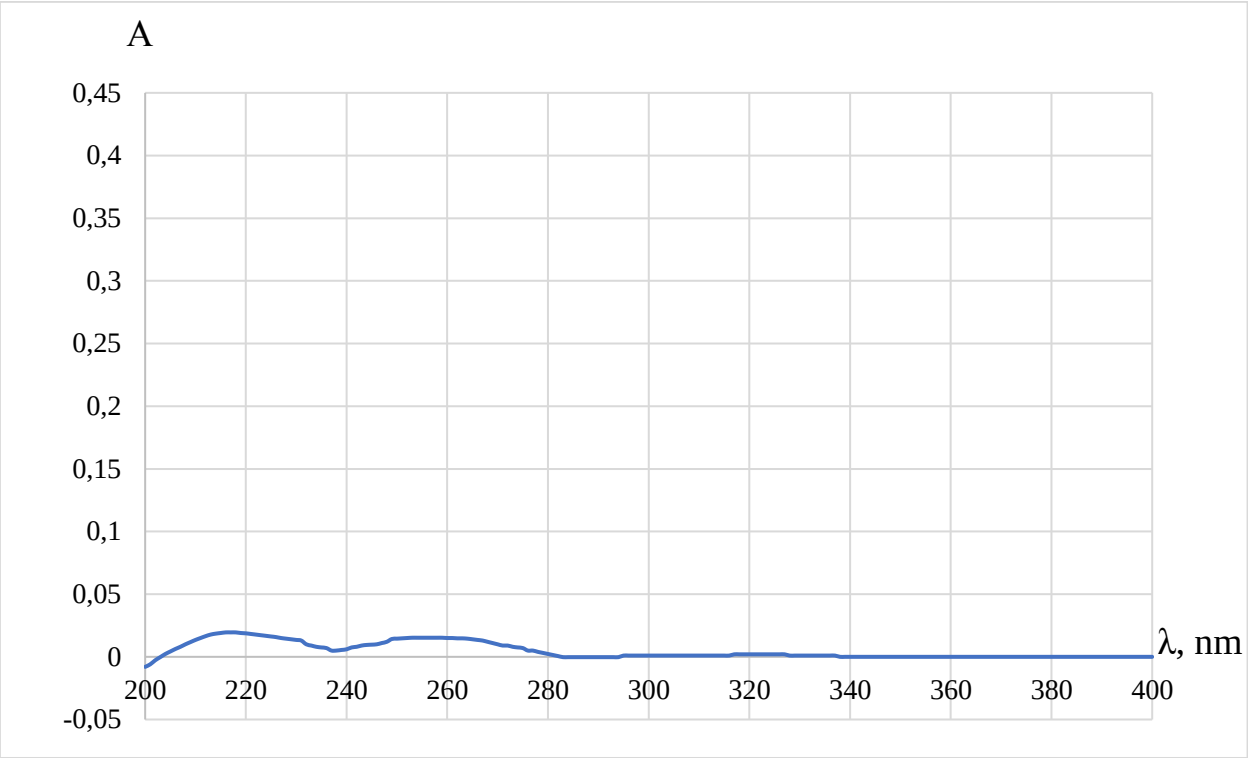


Figure 3.4. Absorption spectrum of placebo solution

Figure 3.4 shows that there are specific excipient light absorption maxima at 220 nm and in the 260 nm range, which is consistent with the literature that the excipient benzalkonium chloride absorbs light at 260±5 nm. However, the spectrum shows that at the maximum selected for the quantitative determination of fluticasone propionate, excipients do not contribute to the absorption, i.e. do not interfere with the determination of the active pharmaceutical ingredient by the proposed method.

For the quantitative determination of fluticasone propionate in «Nasofan» nasal spray and statistical processing of the results, the determination was performed for 6 parallel determinations.

The resulting spectrum of the test drug in an alcohol solution is shown in Figure 3.5.

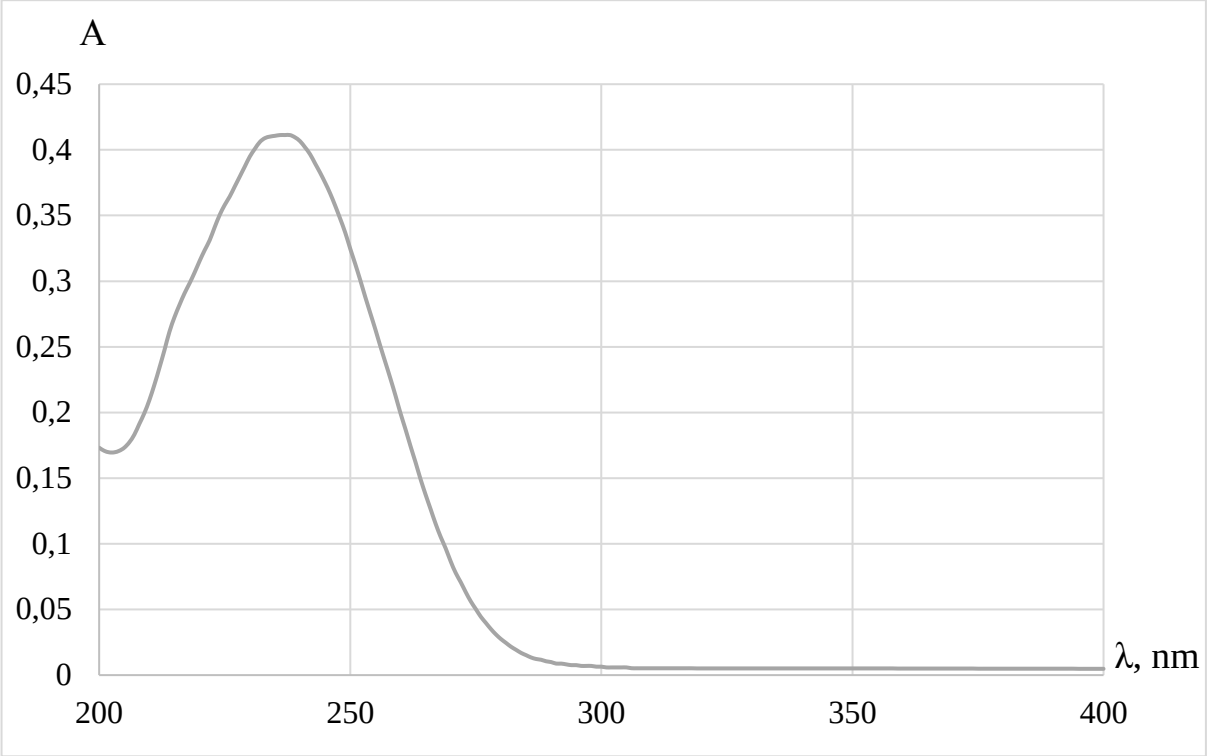


Figure 3.5. Absorption spectrum of an alcohol solution of «Nasofan» nasal spray

Table 3.4.

Results of the quantitative spectrophotometric determination of fluticasone propionate in «Nasofan» nasal spray

№ визна-чення	Absorption of the test solution	Absorption of the standard sample solution	Average weight of the spray, g	Content of fluticasone propionate in the drug dose, µg	Average content of fluticasone propionate in the drug dose, µg
1	0,417	0,415	0,10308	50,28	50,22
2	0,419			50,52	
3	0,416			50,16	
4	0,419			50,52	
5	0,413			49,80	
6	0,415			50,04	

The obtained results were subjected to statistical processing in accordance with the requirements of the State Pharmacopoeia of Ukraine, general article «5.3.N.1. Statistical analysis of chemical experiment results^{N}» [29, 30]:

Number of degrees of freedom:

$$\nu = n-1$$

The average value of the sample:

$$\bar{x} = \frac{\sum_i^n xi}{n}$$

Dispersion:

$$S^2 = \frac{\sum_i^n xi - n\,\bar{x}^2}{\nu}$$

Standard deviation:

$$S = \sqrt{s^2}$$

Relative average deviation:

$$S_r = \frac{s}{\bar{x}}$$

Relative standard deviation:

$$RSD = S_r \cdot 100\% ;$$

Standard deviation of the average result:

$$S_{\bar{x}} = \frac{s}{\sqrt{n}}$$

Relative standard deviation of the average result:

$$S_{\bar{x},r} = \frac{S_{\bar{x}}}{\bar{x}}$$

Relative standard deviation of the average result, in percent:

$$RSD_{\bar{x}} = S_{\bar{x},r} \cdot 100\%$$

Limit values of the confidence interval of a single measurement result:

$$Xi \pm \Delta X = x_i \pm t(P_2; \nu) \cdot s = x_i \pm t(95\%, 5) \cdot s$$

Limit values of the confidence interval of the mean result:

$$\bar{X}_i \pm \Delta X = \bar{x} \pm \frac{t(P2; \nu) * s}{\sqrt{6}}$$

The relative uncertainty of the average result:

$$\bar{\varepsilon} = \frac{\bar{X}_{i \pm \Delta X}}{\bar{X}} \cdot 100\%$$

The relative uncertainty of a particular definition:

$$\varepsilon = \frac{Xi \pm \Delta X}{\bar{X}} \cdot 100\%$$

The metrological characteristics of the average result of the spectrophotometric method for the quantitative determination of fluticasone furoate in spray are given in Table 3.5.

Table 3.5.

Metrological characteristics of the mean result of the spectrophotometric method for the quantitative determination of fluticasone propionate in the nasal spray

X	$\bar{X}$	ν	$X - \bar{X}$	S^2	S	Sx	ΔX	$\Delta\bar{X}$	$\bar{\varepsilon}$	ε
50,28	50,22	5	0,06	0,0792	0,28	0,11	0,23	0,09	0,19	0,46
50,52			0,30							
50,16			-0,06							
50,52			0,30							
49,80			-0,42							
50,04			-0,18							

Thus, the relative uncertainty of the mean result of the quantitative determination of fluticasone propionate in «Nasofan» nasal spray indicates the possibility of using the spectrophotometric method for the determination of the active pharmaceutical ingredient in this dosage form.

CONCLUSION TO CHAPTER III

1. The possibility of using the spectrophotometric method for the determination of fluticasone propionate in the substance and finished drug product in the form of a spray has been considered and demonstrated. The advantages of the method are that it does not require special sample preparation, is specific and sensitive.

2. With a single analysis, fluticasone propionate can be identified and quantified by ultraviolet and visible absorption spectrophotometry (standard method).

3. The results of the spectrophotometric determination of fluticasone propionate in Nasofan nasal spray demonstrate that other excipients do not contribute to the absorption at the selected wavelength, the results of quantitative determination are reliable, coincide with the data specified by the manufacturer, and are metrologically validated.

GENERAL CONCLUSION

1. Fluticasone propionate is a topical glucocorticosteroid that eliminates clinical manifestations of allergic rhinitis, improving the quality of life of patients.

2. Ensuring the quality of the medicinal product is one of the main tasks of the pharmaceutical industry, so it is necessary to control the quality, according to the current legislation, at each stage of the "life cycle" of the medicinal product. This requires improvement and development of methods that meet modern requirements, are accessible and pharmacopoeial.

3. It is proposed to identify and quantify fluticasone propionate in the substance and the medicinal product in the form of a nasal spray by absorption spectrophotometry in the ultraviolet region, using the standard method in the analytical wavelength of 237 nm.

4. The data of spectrophotometric determination of the spray demonstrate that excipients do not interfere with the determination of fluticasone propionate, and the results of quantitative determination of the active pharmaceutical ingredient (50.22 μg/dose) in «Nasofan» nasal spray coincide with the data specified by the manufacturer.

5. The obtained results demonstrate that the methodology is linear, specific, and correct, which confirms the suitability of the developed spectrophotometric methodology for further use in the pharmaceutical analysis of fluticasone propionate in nasal spray.

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