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THE COMPONENT COMPOSITION OF DIAGNOSTIC POLLEN ALLERGEN EXTRACTS

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КОМПОНЕНТНИЙ СКЛАД ПИЛКОВИХ ДІАГНОСТИЧНИХ АЛЕРГЕННИХ ЕКСТРАКТІВ

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Резюме

Метою дослідження було визначити компонентний склад пилкових діагностичних алергенів, призначених для діагностики сенсибілізації у пацієнтів з алергічними захворюваннями, опосередкованими IgE-механізмом.

Матеріали та методи. Для вивчення компонентного складу нами були використані розчини екстрактів діагностичних алергенів МП «Імунолог» (Вінниця, Україна) – вільха, береза, амброзія, полин, грястиця, костриця лучна, соняшник, пажитниця, тимофіївка, тонконіг, жито, кукурудза, які є водно-сольовими розчинами білково-полісахаридних комплексів, виділених із відповідної сировини шляхом екстрагування з рідиною Еванс-Коха, які є водно-сольовими розчинами білково-полісахаридних комплексів, виділені із відповідної сировини екстракцією рідиною Еванса-Коха. Алерген був у рідкій формі з 2 мл алергену у флаконах з коричневого скла, 1 мл розчину якого містив 10 000 PNU алергену.

Електрофорез діагностичних розчинів алергенів проводили методом SDS-PAGE у колонках, рекомендованих Rockland Immunochemicals, Inc. (https://rockland-inc.com/sds-page.aspx). База даних www.allergen.org, офіційного сайту систематичної номенклатури алергенів, затверджена Всесвітньою організацією охорони здоров'я та Міжнародним союзом імунологічних товариств (ВООЗ / МСІТ), використовувалася для ідентифікації алергенних компонентів.

Результати і обговорення. Аналіз спектрограм діагностичних екстрактів алергену показує наявність основних та незначних компонентів у більшості аналізованих випадків. Аналіз спектрограми показав, що в білковому складі алергену берези є основний компонент Bet v1 з молекулярною масою 17 кДа, алерген вільхи – основний компонент Aln g1 з молекулярною масою 18 кДа, алерген жита містить основний компонент Sec c38 з молекулярною масою 13,5 кДа, алерген полину – основний компонент Art ab1 з молекулярною масою 25 кДа.

Спектрограма білкового складу діагностичного алергену *Festuca pratensis* показала основний компонент Fes p4 з молекулярною масою 60 кДа, алергену соняшника – основний компонент Hel a1 з молекулярною масою 34 кДа, алерген *Lolium perenne* містить основний компонент Lol p1 (27 кДа), алерген тимофіївки – основний компонент Phl p5 (32 кДа).

Спектрограма білкового складу діагностичного алергену *Poa pratensis* показала набір білкових молекул з різною молекулярною масою від 25 кДа до 90 кДа без чітких піків, і тому оцінити склад компонентів було неможливо. Спектрограма білкового складу діагностичного алергену кукурудзи містить основний компонент Zea m1 (25-35 кДа), алергену амброзії – мінорний компонент Amb a3 (11 кДа), але чітко відокремлений основний білок Amb a1 (38 кДа).

Спектрограма білкового складу діагностичного алергену граба показала основний компонент Car b1 (17 кДа), алергену *Dactylis glomerata* – основний компонент Dac g1 (32 кДа).

Крім того, багато екстрактів алергенів містило значну кількість баластних неалергенних білкових компонентів.

Ключові слова: алергія, алергенні екстракти, мажорні алергени, мінорні алергени.

Укр. пульмонол. журнал. 2020, № 2, С. 45–49.

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THE COMPONENT COMPOSITION OF DIAGNOSTIC POLLEN ALLERGEN EXTRACTS

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Abstract

The aim of our study was to determine the component composition of diagnostic pollen allergens, intended for the diagnosis of sensitization in patients with IgE-mediated allergic diseases.

Materials and methods. To study the component composition, we used solutions of extracts of diagnostic allergens MP "Immunolog" (Vinnytsia, Ukraine) – birch, alder, rye, wormwood, *Festuca pratensis*, sunflower, *Lolium perenne*, timothy grass, *Poa pratensis*, corn, ragweed, hornbeam, Dactylis glomerata, which were aqueous-saline solutions of protein-polysaccharide complexes isolated from the respective raw material by extraction with Evans-Koch liquid. The liquid allergen was formulated in 2 ml brown glass vials, 1 ml of solution containing 10,000 PNU of allergen. Electrophoresis of allergen diagnostic solutions was performed by SDS-PAGE in columns recommended by Rockland Immunochemicals, Inc. (https://rockland-inc.com/sds-page.aspx). The database of www.allergen.org, the official site for the systematic nomenclature of allergens approved by the World Health Organization and the International Union of Immunological Societies (WHO / IUCN), was used to identify allergenic components.

Results and discussion. Analysis of spectrograms of diagnostic allergen extracts demonstrated the presence of major and minor components in most of the analyzed cases. The protein composition of the birch allergen there were major component Bet v1 with a molecular mass of 17 kDa, alder allergen – major component Aln g1 with a molecular mass 18 kDa, rye allergen contains a major component Sec c38 with a molecular mass 13.5 kDa, wormwood allergen – major component Art ab1 with a molecular mass 25 kDa.

The spectrogram of the protein composition of the diagnostic allergen of *Festuca pratensis* showed major component Fes p4 with a molecular mass 60 kDa, sunflower allergen – major component Hel a1 with a molecular mass 34 kDa, *Lolium perenne* allergen contain major component Lol p1 (27 kDa), timothy grass allergen – major component Phl p5 (32 kDa).

The spectrogram of the protein composition of the diagnostic *Poa pratensis* allergen revealed a set of a protein molecules with a different molecular masses from 25 kDa to 90 kDa with no clear peaks, and therefore it was not possible to estimate the component composition. The spectrogram of the protein composition of the diagnostic corn allergen contained major component Zea m1 (25-35 kDa), ragweed allergen – minor component Amb a3 (11 kDa), but there was no clearly separated major protein Amb a1 (38 kDa).

The spectrogram of the protein composition of the diagnostic hornbeam allergen demonstrated a major component Car b1 (17 kDa), Dactylis glomerata allergen – major component Dac g1 (32 kDa).

In addition, a lot of the allergen extracts contained a significant amount of ballast non-allergenic protein components.

Key words: allergy, allergen extracts, major allergens, minor allergens.

Ukr. Pulmonol. J. 2020, 2:45–49.

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www.search.crossref.org
DOI: 10.31215/2306-4927-2020-108-2-45-49

Numerous guidelines for the management of patients with IgE-dependent allergic diseases indicate skin prick testing as a standard for diagnosis [1, 2, 3]. Despite the fact that the method is already more than 100 years old, it still remains the fastest, cheapest, reliable method for diagnosing the cause of patient sensitization. For diagnosis, extracts of allergens are used, which are administered intradermally to the patient by a standard lancet procedure [4].

One of the unsolved problems so far remains the standardization of solutions of allergen extracts. There is no universally accepted standard or universally accepted standardization procedure, which leads to different contents of major, minor, and ballast non-allergenic proteins in solution [5].

The *aim* of our study was to determine the component composition of pollen diagnostic allergens, intended for the diagnosis of sensitization in patients with IgE -mediated allergic diseases.

Materials and methods

To study the component composition, we used solutions of extracts of diagnostic allergens MP "Immunolog" (Vinnytsia, Ukraine) – birch (registration certificate № UA /15013/01/01), alder (registration certificate № UA/15013/01/01), rye (registration certificate № UA/15013/01/01), wormwood (registration certificate № UA/15013/01/01), *Festuca pratensis* (registration certificate № UA/15013/01/01), sunflower (registration certificate № UA/15013/01/01), *Lolium perenne* (registration certificate № UA/15013/01/01), timothy grass (registration certificate № UA/15013/01/01), *Poa pratensis* (registration certificate № UA / 15013/01/01), corn (registration certificate № UA/15013/01/01), ragweed (registration certificate № UA/15013/01/01), hornbeam (registration certificate № UA/15013/01/01), *Dactylis glomerata* (registration certificate № UA/15013/01/01) which were aqueous-saline solutions of protein-polysaccharide complexes isolated from the respective raw material by extraction with Evans-Koch liquid. The liquid allergen was formulated in 2 ml brown glass vials, 1 ml of solution containing 10,000 PNU of allergen. 1 PNU (Protein Nitrogen Unit) is an international unit used to determine the concentration of protein nitrogen in allergens, which is equal to 0.00001 mg of protein nitrogen.

Electrophoresis of allergen diagnostic solutions was performed by SDS-PAGE in columns recommended by Rockland Immunochemicals, Inc. (https://rockland-inc.com/sds-page.aspx). The results were scanned and processed using Image Studio Lite ver. 5.2 with the subsequent construction and analysis of spectrograms using GelAnalyzer software ver. 19.1. The database of www.allergen.org, the official site for the systematic nomenclature of allergens approved by the World Health Organization and the International Union of Immunological Societies (WHO / IUCN), was used to identify allergenic components.

The study of the component composition of diagnostic allergens using polyacrylamide gel electrophoresis was performed in the laboratory of "R-Biopharm AG" (Darmstadt, Germany).

Results

For this purpose, by the technique of polyacrylamide gel electrophoresis, electrophoregrams of the following allergens were obtained: birch, alder, rye, wormwood, *Festuca pratensis*, sunflower, *Lolium perenne*, timothy grass, *Poa pratensis*, corn,ragweed, hornbeam, *Dactylis glomerata*.

Electrophoregrams and control molecular masses are shown in Fig.1.

Using photometric analysis, a spectrogram of the protein composition of the control masses of the protein fractions was formed. Later all the spectrograms of individual allergens were further compared with it to determine their component composition (Fig. 2).

The spectrogram of the protein composition of the diagnostic birch allergen is shown in Fig. 3.

Analysis of the spectrogram showed that in the protein composition of the birch allergen there were major component Bet v1 with a molecular mass of 17 kDa (mark 2), minor component Bet v3 (24 kDa, mark 1).

The spectrogram of the protein composition of the diagnostic alder allergen is shown in Fig. 4.

Analysis of the spectrogram showed that in the protein composition of the allergen there were major component Aln g1 with a molecular mass 18 kDa (mark 2). In addition, the spectrogram contained non-allergenic ballast protein components with a molecular mass 27-35 kDa (mark 1) and 8-12 kDa (mark 3), and in this spectrum could be a minor component Aln g4 with a molecular mass 7 kDa.

The spectrogram of the protein composition of the diagnostic allergen of rye is shown in Fig. 5.

Analysis of the spectrogram showed that the protein composition of the allergen contained a major component Sec c38 with a molecular mass 13.5 kDa (mark 4), minor components Sec c5 (30 kDa, mark 2) and Sec c20 (70 kDa, mark 1). In addition, non-allergenic ballast protein components with masses about 18-20 kDa were present (mark 3).

The spectrogram of the protein composition of the diagnostic wormwood allergen is shown in Fig. 6.

Analysis of the spectrogram showed that in the protein composition of the allergen there was a major component Art ab1 with a molecular mass 25 kDa.

The spectrogram of the protein composition of the diagnostic allergen of *Festuca pratensis* is shown in Fig. 7

Analysis of the spectrogram showed that in the protein composition of the allergen there was a major component Fes p4 with a molecular weight 60 kDa (mark 1). In addition, non-allergenic ballast protein components with masses about 35 kDa (mark 2), 22-23 kDa (mark 3) and 12-15 kDa (mark 4) were present.

The spectrogram of the protein composition of the diagnostic sunflower allergen is shown in Fig. 8.

Analysis of the spectrogram showed that the protein composition of the sunflower allergen contained a major component Hel a1 with a molecular mass 34 kDa (mark 3), minor components Hel a2 (14.7 kDa) (mark 4) and Hel a6 (42 kDa) (mark 2). In addition, the spectrogram contained non-allergenic ballast protein with a molecular mass about 80-90 kDa (mark 1).

The spectrogram of the protein composition of the diagnosticallergen *Lolium perenne* shown in Fig. 9.

.Analysis of the spectrogram showed that the protein composition of *Lolium perenne* allergen contained a major

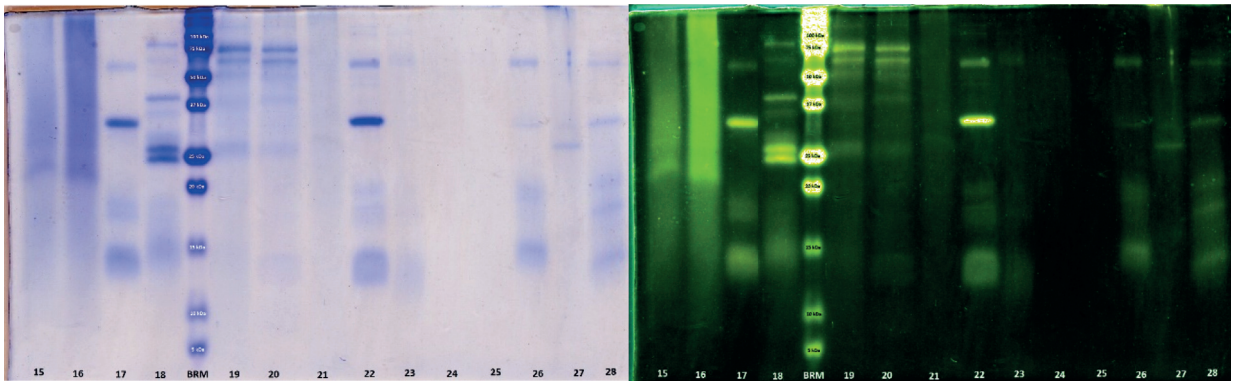

Fig. 1. Electrophoregrams of diagnostic allergens and control masses of protein fractions before (left) and after (right) computer processing for analysis

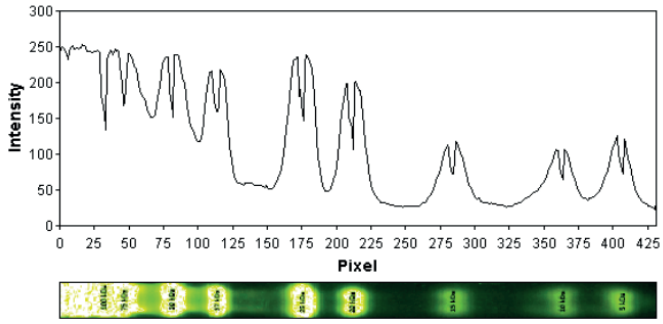

Fig. 2. Spectrogram of the protein composition of the control masses of protein fractions

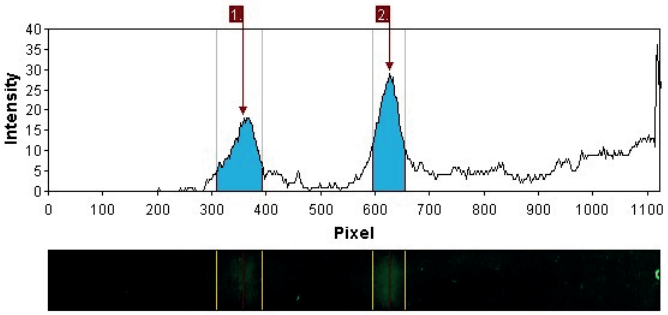

Fig. 3. Spectrogram of the protein composition of the birch diagnostic allergen

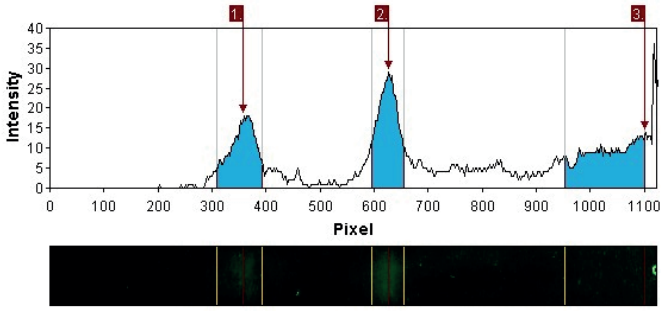

Fig. 4. Spectrogram of protein composition of diagnostic alder allergen

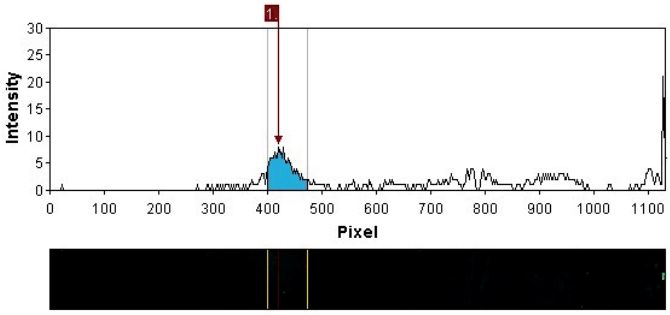

Fig. 6. Spectrogram of the protein composition of the diagnostic wormwood allergen

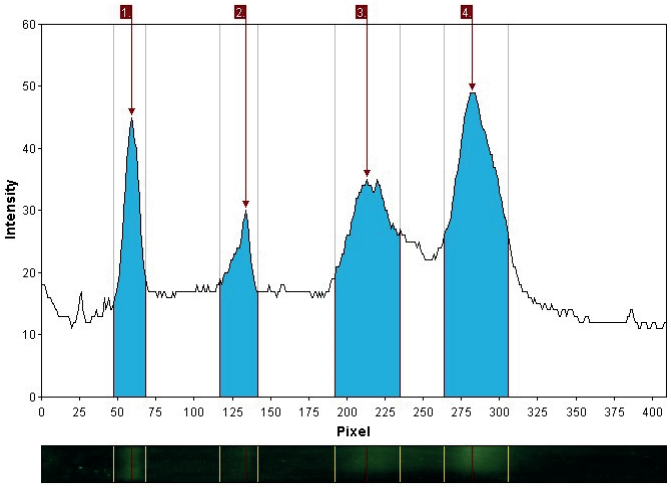

Fig. 5. Spectrogram of the protein composition of the diagnostic rye allergen

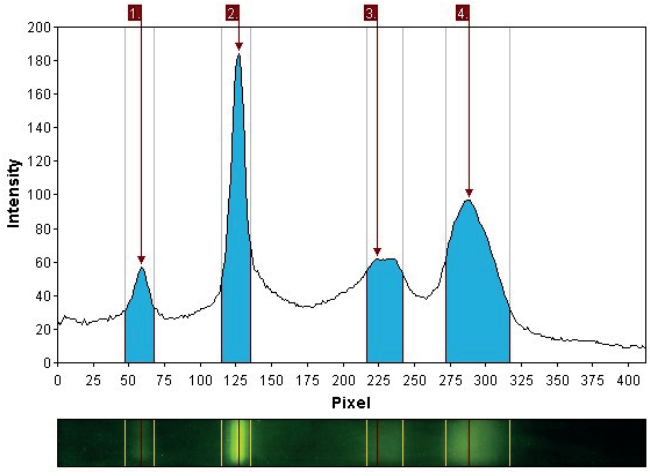

Fig. 7. Spectrogram of the protein composition of the diagnostic allergen Festuca pratensis

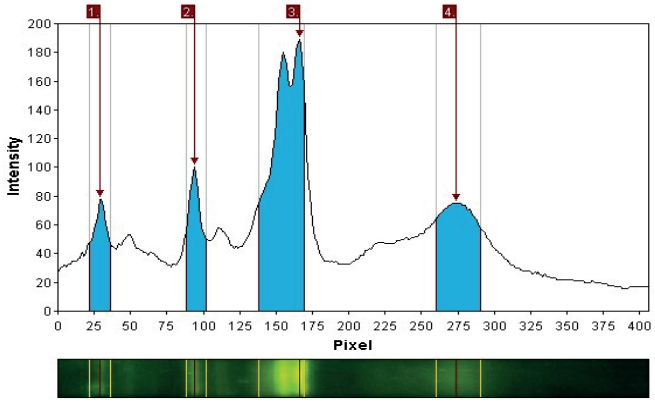

Fig. 8. Spectrogram of the protein composition of the diagnostic sunflower allergen

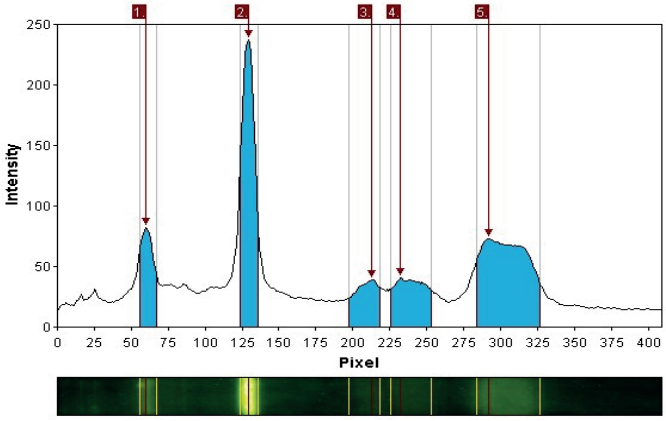

Fig. 9. Spectrogram of the protein composition of the diagnostic Lolium perenne allergen

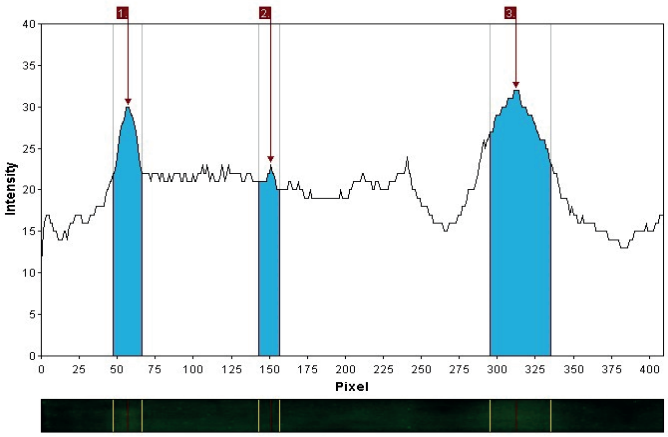

Fig. 10. Spectrogram of the protein composition of the diagnostic timothy grass allergen

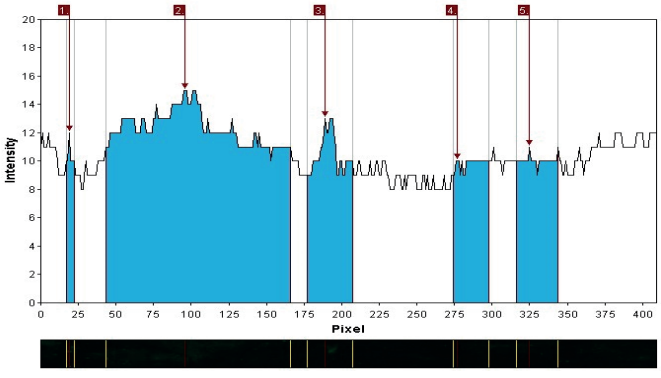

Fig. 11. Spectrogram of the protein composition of the diagnostic Poa pratensis allergen

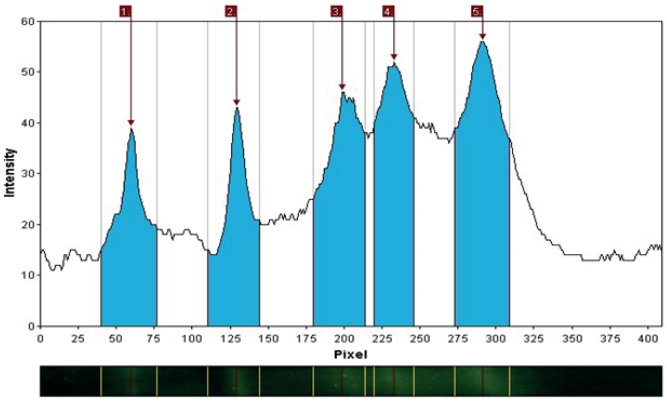

Fig. 12. Spectrogram of the protein composition of the diagnostic corn allergen

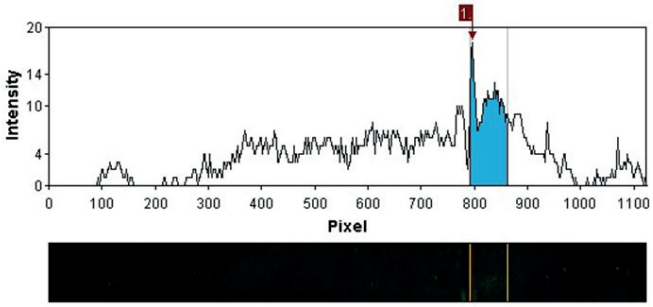

Fig. 13. Spectrogram of the protein composition of the diagnostic ragweed allergen

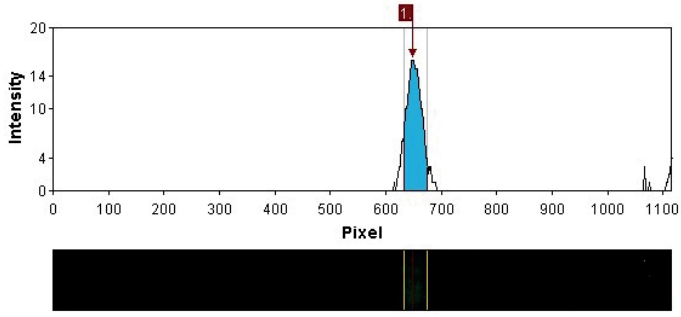

Fig. 14. Spectrogram of the protein composition of the diagnostic hornbeam allergen

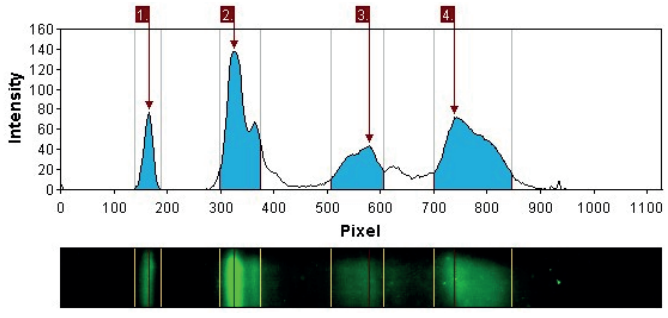

Fig. 15. Spectrogram of the protein composition of the diagnostic Dactylis glomerata allergen

component Lol p1 (27 kDa, mark 2) and minor components Lol p4 (57 kDa) (mark 1), Lol p11 (16 kDa, mark 4), Lol p2-Lol p3 (molecular weight 11 kDa, mark 5). However, the spectrogram showed a peak of non-allergenic protein with mass about 19-20 kDa (mark 3).

The spectrogram of the protein composition of the diagnostic timothy grass allergen is shown in Fig. 10.

Analysis of the spectrogram showed that in the protein composition of the timothy grass allergen there was a major component Phl p5 (32 kDa, mark 2) and minor components Phl p4 (55 kDa, mark 1), Phl p2 (10-12 kDa, mark 3).

The spectrogram of the protein composition of the diagnostic *Poa pratensis* allergen is shown in Fig. 11.

Analysis of the spectrogram showed that the diagnostic *Poa pratensis* allergen was a set of a protein molecules with a different molecular masses from 25 kDa to 90 kDa (marks 1-5) with no clear peaks, and therefore it was not possible to estimate the component composition.

The spectrogram of the protein composition of the diagnostic allergen of corn is shown in Fig. 12.

Analysis of the spectrogram showed that in the protein composition of the corn allergen there was a major component Zea m1 (25-35 kDa, mark 2) and a minor component Zea m12 (14 kDa, mark 5). In addition, the spectrogram contained non-allergenic ballast protein with a molecular mass about 60 kDa (mark 1), 20 kDa (mark 3), 18 kDa (mark 4).

The spectrogram of the protein composition of the diagnostic ragweed allergen is shown in Fig. 13.

Analysis of the spectrogram showed that the protein composition of the ragweed allergen contains a minor component Amb a3 (11 kDa, mark 1), but there was no clearly separated major protein Amb a1 (38 kDa).

The spectrogram of the protein composition of the diagnostic hornbeam allergen is shown in Fig. 14.

Analysis of the spectrogram showed that in the protein composition of the hornbeam allergen there was a major component Car b1 (17 kDa, mark 1).

The spectrogram of the protein composition of the diagnostic *Dactylis glomerata* allergen is shown inFig. 15.

Analysis of the spectrogram showed that in the protein composition of the *Dactylis glomerata* allergen here was a major component Dac g1 (32 kDa, mark 2) and minor components Dac g4 (60 kDa, mark 1), Dac g2 and Dac g3 (11 kDa and 14 kDa, mark 4). In addition, the spectrogram contained non-allergenic ballast protein with a molecular mass about 19 kDa (mark 3).

Discussion

Most diagnostic allergen extracts have been found to contain major and some minor protein components. However, not all important major components have been identified (eg, Amb a1). This may be important for the diagnosis of sensitization in patients with IgE-dependent diseases, because in the presence of sensitivity to these components (and according to studies, it can be up to 75% of patients) the diagnostic results may not be completely reliable.

In addition, a lot of the allergen extracts contains a significant amount of ballast non-allergenic protein components, which are not important in skin testing, however, affect the total amount of PNU, which is the criterion of standardization of allergens. Currently, the procedure of standardization of diagnostic allergens, approved in a number of Eastern European countries, including Ukraine, requires correction and transition to standardization of the component composition of extracts.

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