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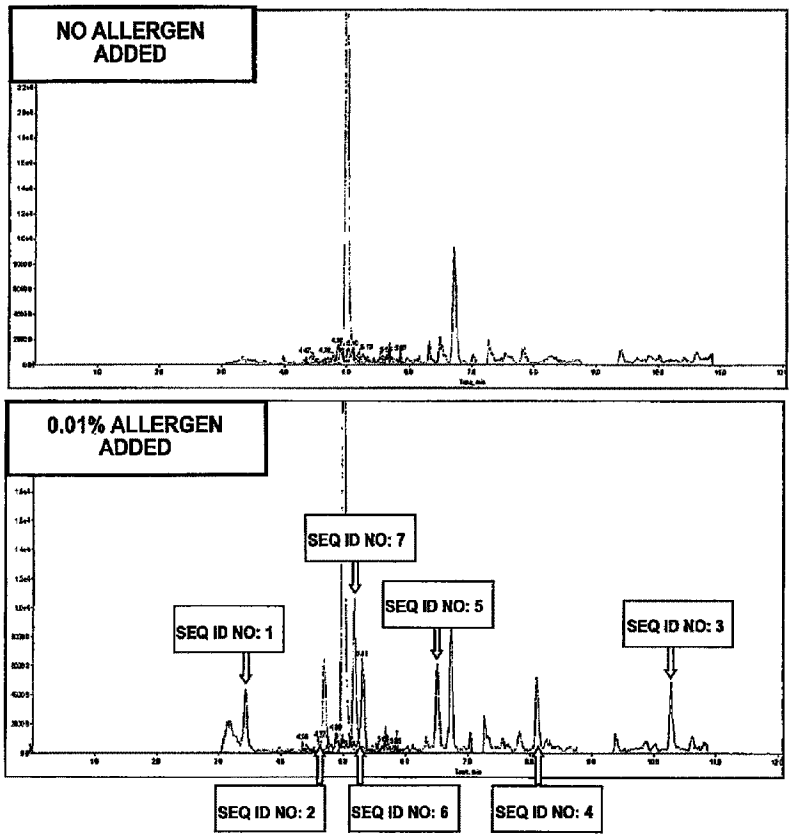

(57) Abrégé/Abstract:
A highly-sensitive-allergen-measurement method is provided. A method for detecting an allergen in a sample comprises treating the sample with a protease, and detecting the presence or absence of an allergen-derived polypeptide in the enzymatically treated sample by a chromatographic separation analysis, wherein the allergen is one or more members selected from the group consisting of buckwheat, crustacean, milk, egg and peanut.

ABSTRACT

A highly-sensitive-allergen-measurement method is provided. A method for detecting an allergen in a sample comprises treating the sample with a protease, and detecting the presence or absence of an allergen-derived polypeptide in the enzymatically treated sample by a chromatographic separation analysis, wherein the allergen is one or more members selected from the group consisting of buckwheat, crustacean, milk, egg and peanut.

NS0082

ALLERGEN DETECTION METHOD

Technical Field

[0001]

The present invention relates to a method for detecting an allergen in a sample.

Background Art

[0002]

Food allergy causes disadvantageous symptoms such as dermatitis, asthma, gastrointestinal dysfunction and anaphylactic shock due to immune responses triggered by food. Various kinds of food cause food allergy. Among them, many patients are allergic to seven food types of shrimp, crab, wheat, buckwheat, egg, milk and peanut, and they are likely to cause severe allergic symptoms.

[0003]

Even if a processed food product does not contain, as a raw material, any food that becomes the allergen, its final product may be contaminated with an allergen in some cases when a production line is shared with an allergen-containing processed food in a factory, or when an allergen is used in a process for manufacturing the raw material. Contamination with even a slight amount of allergen is dangerous for food allergic patients. A highly-sensitive-allergen-measurement method capable of detecting a trace amount of allergen in a sample such as a food

product is required.

[0004]

As the highly-sensitive-allergen-measurement method, Patent Literature 1 discloses a method for detecting the following allergen in a sample, including detecting a peptide having a specific sequence obtained by enzymatically cleaving the allergen by LC-MS/MS, wherein the allergen is selected from the group consisting of ovalbumin, lysozyme, casein, lactoglobulin, high molecular weight glutenin, low molecular weight glutenin, wheat protein, rye protein, oat protein, barley protein, mustard protein, sesame protein, macadamia nut protein, pistachio nut protein, brazil nut protein, walnut protein, peanut protein and hazelnut protein. Patent Literature 2 discloses a method for measuring a content of allergen in a composition, including forming an extract containing an allergen from a sample composition, and measuring the amount of allergen in the extract using LC-UV/MS or LC-MS.

Citation List

Patent Literature

[0005]

Patent Literature 1: JP 2014-525588 A

Patent Literature 2: JP 2013-539039 A

Summary of Invention

Technical Problem

[0006]

Detecting the presence of allergen (particularly buckwheat or crustacean) in a sample, such as a food product, with high sensitivity is needed.

Solution to Problem

[0007]

The present inventor found that, by detecting a specific amino acid sequence contained in each allergen, the presence of the allergen can be detected with high sensitivity.

[0008]

Accordingly, the present invention provides a method for detecting an allergen in a sample, the method comprising

treating the sample with a protease, and

detecting the presence or absence of an allergen-derived polypeptide in the enzymatically treated sample by chromatographic separation analysis,

wherein the allergen is one or more members selected from the group consisting of buckwheat, crustacean, milk, egg and peanut.

[0008A]

The present disclosure includes:

(1) A method for detecting an allergen in a sample, the method comprising treating the sample with a protease, and detecting the presence or absence of a buckwheat allergen-derived polypeptide in the protease-treated sample by chromatographic separation analysis, wherein the buckwheat allergen-derived polypeptide comprises any one or more of polypeptides

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consisting of amino acid sequences represented by SEQ ID NOs: 4, 6 and 7;

(2) The method according to (1), wherein the buckwheat allergen-derived polypeptide further comprises any one or more of polypeptides selected from the group consisting of amino acid sequences represented by SEQ ID NOs: 1, 2, 3, and 5;

(3) The method according to (1) or (2), wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 1 to 7 is detected;

(4) The method according to any one of (1) to (3), further comprising detecting the presence or absence of a crustacean allergen-derived polypeptide, wherein the crustacean allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 8 to 12;

(5) The method according to (4), wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 8 to 12 is detected;

(6) The method according to any one of (1) to (5), further comprising detecting the presence or absence of a milk allergen-derived polypeptide, wherein the milk allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 13 to 17;

(7) The method according to (6), wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 13 to 17 is detected;

(8) The method according to any one of (1) to (7), further comprising detecting the presence or absence of an egg allergen-derived polypeptide,

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wherein the egg allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 18 to 21;

(9) The method according to (8), wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 18 to 21 is detected;

(10) The method according to any one of (1) to (9), further comprising detecting the presence or absence of a peanut allergen-derived polypeptide, wherein the peanut allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 22 to 24;

(11) The method according to (10), wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 22 to 24 is detected;

(12) The method according to any one of (1) to (11), wherein the chromatographic separation analysis is liquid chromatography tandem mass spectrometry (LC-MS/MS); and

(13) The method according to any one of (1) to (12), wherein the protease is trypsin.

Advantageous Effects of Invention

[0009]

The present invention provides a

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highly-sensitive-allergen-detection method capable of detecting the presence of a trace amount of allergen (particularly, buckwheat or crustacean) in a sample such as a food product.

Brief Description of Drawings

[0010]

Fig. 1 is an LC-MS/MS analysis result on a trypsin digest of buckwheat allergen.

Fig. 2 is an LC-MS/MS analysis result on a trypsin digest of crustacean allergen.

Fig. 3 is an LC-MS/MS analysis result on a trypsin digest of buckwheat allergen-containing wheat flour. Top; wheat flour with no buckwheat allergen added, bottom; wheat flour with a buckwheat allergen added.

Fig. 4 is an LC-MS/MS analysis result on a trypsin digest of crustacean allergen-containing wheat flour. Top; wheat flour with no crustacean allergen added, bottom; wheat flour with a crustacean allergen added.

Fig. 5 is an LC-MS/MS analysis result on a milk allergen.

Fig. 6 is an LC-MS/MS analysis result on an egg allergen.

Fig. 7 is an LC-MS/MS analysis result on a peanut allergen.

Fig. 8 is an LC-MS/MS analysis result on casein in bread with milk added.

Fig. 9 is an LC-MS/MS analysis result on Ara h1 or h3 in

bread with peanut added.

Description of Embodiments

[0011]

The present invention provides a method for detecting an allergen in a sample. According to the method of the present invention, one or more allergen members selected from the group consisting of buckwheat, crustacean, milk, egg and peanut can be detected. In a preferred embodiment, the allergen detected by the method of the present invention is one or more members selected from the group consisting of at least buckwheat and crustacean, more preferably one or more members selected from the group consisting of buckwheat and crustacean, still more preferably either buckwheat or crustacean. Alternatively, the allergen detected by the method of the invention includes at least buckwheat, and may be, for example, buckwheat alone, buckwheat and crustacean, or buckwheat, crustacean, milk, egg and peanut.

[0012]

In the present invention, examples of an object to be subjected to allergen detection include a food product, cosmetic, medicine, raw material thereof, and instrument used in a manufacturing process thereof; however, the examples are not limited thereto. Such an object subjected to a conventional pretreatment, for example, grinding, dissolution, suspension,

extraction, or a combination thereof, can be used as a sample for the method of the present invention. Alternatively, in a case where the object is an instrument, for example, its washing solution or wiped sample, or those subjected to grinding, dissolution, suspension, extraction etc., or a combination thereof can be used as a sample for the method of the present invention. A method for preparing the sample used in the method of the present invention is not limited to the above, and may include any method that can be used for preparing a sample for a protease treatment described below.

[0013]

In the method of the present invention, the prepared sample is treated with a protease. Examples of the protease used in the method of the present invention include trypsin, chymotrypsin, elastase and thermolysin, preferably trypsin. Conditions for the treatment may be appropriately selected depending on the type of enzyme. For example, in a case of trypsin, the conditions preferably include an enzyme concentration of 1000 to 20000 U, temperature of 25 to 45°C, pH of about 7 to 9 and period of time of 4 to 24 hours. The enzymatic treatment cleaves a protein molecule of the target allergen to produce polypeptides derived from the allergen. Accordingly, if the target allergen is contained in a sample, the enzymatically treated sample contains polypeptides derived from the target allergen. On the other hand, if the target allergen is not contained in a sample, the

enzymatically treated sample does not contain polypeptides derived from the target allergen.

[0014]

Accordingly, it is possible to determine the presence or absence of the target allergen in a sample by detecting the presence or absence of a target allergen-derived polypeptide in the sample treated with the protease described above.

[0015]

As to buckwheat, a cleavage product of a buckwheat 22 kDa protein molecule (SEQ ID NO: 25) can be detected as an allergen-derived polypeptide. In a preferred embodiment of the method of the present invention, in a case where the allergen is buckwheat, a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 1 to 7 described below is detected as an allergen-derived polypeptide.

VQVVGDEGR (SEQ ID NO: 1)

SVFDDNVQR (SEQ ID NO: 2)

GQILVVPQGFAVVLK (SEQ ID NO: 3)

EGLEWVELK (SEQ ID NO: 4)

NFFLAGQSK (SEQ ID NO: 5)

GFIVQAR (SEQ ID NO: 6)

NDDNAITSPIAGK (SEQ ID NO: 7)

In the method of the present invention, the presence or absence of any one or more of polypeptides consisting of the amino acid sequences represented by above-described SEQ ID NOs:

1 to 7 may be detected. Preferably, the presence or absence of all of these polypeptides is detected.

[0016]

As to crustacean such as crab and shrimp, a cleavage product of tropomyosin can be detected as an allergen-derived polypeptide. In a preferred embodiment of the method of the present invention, in a case where the allergen is crustacean, a polypeptides consisting of the amino acid sequence represented by any of SEQ ID NOs: 8 to 12 described below is detected as an allergen-derived polypeptide.

IQLLEEDLER (SEQ ID NO: 8)

MDALENQLK (SEQ ID NO: 9)

FLAEEADR (SEQ ID NO: 10)

IVELEEELR (SEQ ID NO: 11)

LAMVEADLER (SEQ ID NO: 12)

In the method of the present invention, the presence or absence of any one or more of polypeptides consisting of the amino acid sequences represented by above-described SEQ ID NOs: 8 to 12 may be detected. Preferably, the presence or absence of all of these polypeptides is detected.

[0017]

In a case where the allergen is buckwheat and crustacean, the allergen-derived polypeptide to be detected is preferably any one or more of polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 1 to 7 and any one or more

of polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 8 to 12, more preferably, all of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 1 to 12.

[0018]

As to milk, a cleavage product of casein or β-lactoglobulin (BLG) can be detected as an allergen-derived polypeptide. In a preferred embodiment of the method of the present invention, in a case where the allergen is milk, a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 13 to 17 described below is detected as an allergen-derived polypeptide. Among the following polypeptides, SEQ ID NOs: 13 to 15 are the amino acid sequences of casein-derived polypeptides, and SEQ ID NOs: 16 to 17 are the amino acid sequences of BLG-derived polypeptides.

FFVAPFPEVFGK (SEQ ID NO: 13)

YLGYLEQLLR (SEQ ID NO: 14)

NAVPITPTLNR (SEQ ID NO: 15)

VLVLDTDYK (SEQ ID NO: 16)

TPEVDDEALEK (SEQ ID NO: 17)

In the method of the present invention, the presence or absence of any one or more of polypeptides consisting of the amino acid sequences represented by above-described SEQ ID NOs: 13 to 17 is detected. Preferably, the presence or absence of all of these polypeptides is detected. As to casein, the presence

or absence of preferably any one or more of polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 13 to 15, more preferably all of these polypeptides is detected. As to BLG, the presence or absence of preferably any one or more of polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 16 to 17, more preferably all of these polypeptides is detected.

[0019]

As to egg, a cleavage product of ovalbumin can be detected as an allergen-derived polypeptide. In a preferred embodiment of the method of the present invention, in a case where the allergen is egg, a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 18 to 21 described below is detected as an allergen-derived polypeptide.

YPILPEYLQCVK (SEQ ID NO: 18)

ELINSWVESQTNGIIR (SEQ ID NO: 19)

LTEWTSSNVMEER (SEQ ID NO: 20)

HIATNAVLFFGR (SEQ ID NO: 21)

In the method of the present invention, the presence or absence of any one or more of polypeptides consisting of the amino acid sequences represented by above-described SEQ ID NOs: 18 to 21 is detected. Preferably, the presence or absence of all of these polypeptides is detected.

[0020]

As to peanut, a cleavage product of Ara h1-3 can be detected

as an allergen-derived polypeptide. In a preferred embodiment of the method of the present invention, in a case where the allergen is peanut, a polypeptide consisting of the amino acid sequence represented by any of SEQ ID NOs: 22 to 24 described below is detected as an allergen-derived polypeptide. Among the following polypeptides, SEQ ID NOs: 22 to 23 are the amino acid sequences of Ara h1-derived polypeptides, and SEQ ID NO: 24 is the amino acid sequence of an Ara h3-derived polypeptide.

DLAFPGSGEQVEK (SEQ ID NO: 22)

VLLEENAGGEQEER (SEQ ID NO: 23)

SPDIYNPQAGSLK (SEQ ID NO: 24)

In the method of the present invention, the presence or absence of any one or more of polypeptides consisting of the amino acid sequences represented by above-described SEQ ID NOs: 22 to 24 is detected. Preferably, the presence or absence of all of these polypeptides is detected. As to Ara h1, the presence or absence of preferably any one or more of polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 22 to 23, more preferably all of these polypeptides is detected. As to Ara h3, the presence or absence of the polypeptide preferably consisting of the amino acid sequence represented by SEQ ID NO: 24 is detected.

[0021]

Accordingly, when the allergen is buckwheat, crustacean, milk, egg and peanut, the allergen-derived polypeptide to be

detected is preferably any one or more of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 1 to 7, any one or more of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 8 to 12, any one or more of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 13 to 17, any one or more of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 18 to 21, and any one or more of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 22 to 24; more preferably all of the polypeptides consisting of the amino acid sequences represented by SEQ ID NOs: 1 to 24.

[0022]

As a means for detecting the presence or absence of the target allergen-derived polypeptide in the sample treated with the protease described above, a chromatographic separation analysis is preferable. Examples of the chromatographic separation analysis include liquid chromatography-mass spectrometry such as liquid chromatography tandem mass spectrometry (LC-MS/MS) or liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS). Multiple reaction monitoring (MRM) using LC-MS/MS is preferable because it has high measurement accuracy (S/N ratio) and can detect multiple peptides at once.

[0023]

In the method of the present invention, as the chromatography

used for detection of the target allergen-derived polypeptide, liquid chromatography (LC) is preferable, and reversed phase liquid chromatography (RPLC) is more preferable. Also, the LC is preferably high performance liquid chromatography (HPLC), more preferably RP-HPLC. Examples of a carrier for RPLC include a carrier having a filler in which a hydrocarbon chain (preferably an octadecyl group) is bonded to silica gel or a polymer gel base material, such as a C18 column or C8 column. Any mobile phase (eluent) for the LC may be used as long as it is capable of individually separating the target allergen-derived polypeptides, and examples thereof includes, however is not limited to, a 100:0 to 0:100 (volume ratio) gradient solution of an aqueous solution of formic acid (A) and an aqueous solution of formic acid acetonitrile (B).

[0024]

In liquid chromatography-mass spectrometry, the eluate from the LC is subjected to mass spectrometry (for example, MS/MS, TOF/MS). Mass spectrometry can be performed, under the usual conditions used in peptide detection, using a publicly known mass spectrometer, for example, tandem quadrupole mass spectrometer or time-of-flight mass spectrometer. For example, multiple reaction monitoring (MRM) using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) is preferred. In mass spectrometry, each polypeptide in the eluate is separated according to the mass/charge (m/z).

For example, the presence or absence of the target polypeptide in a sample can be detected based on the measured m/z value by preliminarily making a database of the m/z value of the target polypeptide.

Examples

[0025]

Hereinafter, a more detailed description of the present invention is made with reference to Examples; however, the present invention is not limited to the following Examples.

[0026]

(Reagent)

Acetonitrile (Wako Pure Chemical Corporation, special grade, for HPLC)

Trypsin (Wako Pure Chemical Corporation, derived from porcine spleen, for biochemical analysis)

Iodoacetamide (IA) (Wako Pure Chemical Corporation, for biochemical analysis)

Dithiothreitol (DTT) (Wako Pure Chemical Corporation, for biochemical analysis)

Urea (Wako Pure Chemical Corporation, special grade)

Trifluoroacetic acid (TFA) (Junsei Chemical Co., Ltd., special grade)

(Buffer)

A: 0.1M DTT_0.5M Tris-HCl_4M urea (pH 8.2) buffer

B: 0.5M Tris-HCl_2M urea (pH 8.2) buffer

[0027]

Reference Example 1 Preparation of allergen-containing sample

0.5g of a specimen was collected in a 15 mL disposable test tube, and 5 mL of Buffer A (Test Examples 1 to 3) or Buffer B (Test Examples 4 to 5) was added, followed by shake extraction for 3 hours (Test Examples 1 to 3) or 5 hours (Test Examples 4 to 5). The obtained reaction product was centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected.

[0028]

Example 1 Analysis of trypsin digest by LC-MS/MS

1) Trypsin digestion

1 mg of allergen molecule or 0.25 mL of supernatant prepared in Reference Example 1 was collected in a polytube for 1.5 mL, and 0.25 mL of Buffer A (Test Examples 1 to 3) or Buffer B (Test Examples 4 to 5) was added for complete dissolution. To the obtained solution, 50 μL of 40 mg/mL DTT was added, followed by incubation at 37°C for 90 minutes. Subsequently, 50 μL of 40 mg/mL IA was added, followed by incubation at 37°C for 30 minutes in the absence of light. To the reaction solution, 600 μL of 50 mM sodium hydrogen carbonate was added, then 100 μL of a 10 mg/mL trypsin 50 mM sodium hydrogen carbonate solution was added, followed by incubation at 37°C for 16 hours (pH 7 to 9). After the reaction, 10 μL of TFA was added for inactivation

of trypsin.

[0029]

2) Desalting

OASIS HLB (3 cc, 60 mg; Waters) was conditioned with 1 mL of methanol and 2 mL of water. To this, the whole amount of the reaction solution obtained in 1) was dropwise added, followed by washing with 1 mL of water and subsequent elution with 1 mL of 60% acetonitrile.

[0030]

3) Multiple reaction monitoring (MRM) by LC-MS/MS

The eluate obtained in 2) was dried with nitrogen at 40°C, followed by dissolution in 0.2 mL of 25% acetonitrile. After the obtained solution was filtered, LC-MS/MS MRM was performed under the following conditions.

[0031]

(LC-MS/MS apparatus)

HPLC: Shimadzu Nexera X2

MS/MS: AB SCIEX QTRAP 5500

(HPLC conditions)

Column: Kinetik C 18 150 mm × 2.1 mm, particle diameter 2.6 μm

Column temperature: 50°C

Column flow rate: 0.3 mL

Eluent A: 0.1% formic acid; eluent B: 0.1% formic acid acetonitrile

Gradient: A:B 95:5 (0 min) → 40:60 (20 min) → 20:80 (50 min) → 95:5 (55 min) → 95:5 (90 min)

(Mass analysis conditions)

Ionization method: electrospray ionization method

Polarity: Positive

Spray voltage: 5500 V

Turbo heater temperature: 450°C

[0032]

Test Example 1 Detection of buckwheat allergen

Using the 22 kDa protein (SEQ ID NO: 25) as a target allergen molecule, target allergen-derived polypeptides were detected by LC-MS/MS MRM under the condition shown in Table 1. The result is shown in Fig. 1.

[0033]

[Table 1]

DETECTED POLYPEPTIDE	MRM TRANSITION	
	Q1	Q3
VQVVGDEGR (SEQ ID NO: 1)	479.5	632.1
		533.0
SVFDDNVQR (SEQ ID NO: 2)	540.0	746.2
		631.1
GQILVVPQGFAVVLK (SEQ ID NO: 3)	784.5	1057.8
		958.6
EGLEWVELK (SEQ ID NO: 4)	551.6	674.3
		488.1
NFFLAGQSK (SEQ ID NO: 5)	506.6	750.4
		603.2
GFIVQAR (SEQ ID NO: 6)	395.9	586.2
		473
NDDNAITSPIAGK (SEQ ID NO: 7)	658.2	786.4
		673.2

[0034]

Test Example 2 Detection of crustacean allergen

Using tropomyosin (SEQ ID NO: 26) as a target allergen molecule, target allergen-derived polypeptides were detected by LC-MS/MS MRM under the conditions shown in Table 2. The result is shown in Fig. 2.

[0035]

[Table 2]

DETECTED POLYPEPTIDE	MRM TRANSITION	
	Q1	Q3
IQLLEEDLER (SEQ ID NO: 8)	629.2	1016.6
		903.4
MDALENQLK (SEQ ID NO: 9)	531	815.2
		744.1
FLAEEADR (SEQ ID NO: 10)	475.5	690.2
		619.1
IVELEEELR (SEQ ID NO: 11)	565.1	917.5
		788.4
LAMVEADLER (SEQ ID NO: 12)	573.6	1033.6
		831.4

[0036]

Test Example 3 Detection of buckwheat allergen and crustacean allergen from food composition

From wheat flour to which 0.01% by mass of buckwheat or crustacean (shrimp powder) was added, an allergen-containing sample was prepared according to Reference Example 1. The obtained sample was subjected to trypsin digestion, desalting and LC-MS/MS MRM according to Example 1, with the result that the target allergen-derived polypeptides were detected. The same analysis was performed using wheat flour to which no buckwheat or crustacean was added as a control. The allergen-derived polypeptides detected and conditions of

LC-MS/MS analysis for buckwheat and crustacean were the same as those described above for Tables 1 and 2, respectively. The measurement results are shown in Figs. 3 and 4.

[0037]

Test Example 4 Detection of milk, egg and peanut allergens

An allergen-containing sample was prepared according to Reference Example 1 from each of whole milk powder, whole egg powder and peanut flour. Each sample was subjected to trypsin digestion, desalting and LC-MS/MS MRM according to 1) to 3) in Example 1, with the result that the target allergen-derived polypeptides were detected. The target allergen molecules and detected allergen-derived polypeptides for each of the whole milk powder, whole egg powder and peanut flour are shown in Tables 3 to 5. All allergens of the whole milk powder, whole egg powder and peanut flour could be detected by LC-MS/MS analysis (Figs. 5 to 7).

[0038]

[Table 3]

MILK ALLERGEN

TARGET ALLERGEN MOLECULE	DETECTED POLYPEPTIDE	MRM TRANSITION	
		Q1	Q3
CASEIN	FFVAPFPEVFGK (SEQ ID NO: 13)	692.9	920.3
			991.3
	YLGYLEQLLR (SEQ ID NO: 14)	634.3	249.2
			991.3
	NAVPITPTLNR (SEQ ID NO: 15)	598.3	158.3
			911.4
β-LACTOGLOBULIN (BLG)	VLVLDTDYK (SEQ ID NO: 16)	533.3	853.4
			-
	TPEVDDEALEK (SEQ ID NO: 17)	623.5	819.4
			-

[0039]

[Table 4]

EGG ALLERGEN

TARGET ALLERGEN MOLECULE	DETECTED POLYPEPTIDE	MRM TRANSITION	
		Q1	Q3
OVALBUMIN	YPILPEYLQCVK (SEQ ID NO: 18)	733.4	1092.7
			979.5
	ELINSWVESQTNGIIR (SEQ ID NO: 19)	930.0	888.5
			1017.5
	LTEWTSSNVMEER (SEQ ID NO: 20)	791.4	951.4
			1052.5
	HIATNAVLFFGR (SEQ ID NO: 21)	673.4	223.2
			1095.6

[0040]

[Table 5]

PEANUT ALLERGEN

TARGET ALLERGEN MOLECULE	DETECTED POLYPEPTIDE	MRM TRANSITION	
		Q1	Q3
Ara h1	DLAFPGSGEQVEK (SEQ ID NO: 22)	688.8	930.5
			300.2
	VLLEENAGGEQEER (SEQ ID NO: 23)	786.9	989.5
			804.4
Ara h3	SPDIYNPQAGSLK (SEQ ID NO: 24)	695.4	977.5
			700.4

[0041]

Test Example 5 Detection of milk and peanut allergens from food composition

Bread to which either whole milk powder or peanut flour was added (added bread; additive amount of 0.84% by mass) and bread to which neither milk nor peanut was contained (non-added bread) were produced. The added bread and non-added bread were mixed to prepare 100-fold diluted added bread (additive amount of whole powdered milk and peanut flour of 0.0084% by mass). The concentration of a milk or peanut allergen (soluble protein of peanut containing casein and Ara h) contained in the obtained 100-fold diluted added bread was measured by ELISA (with lower limit of quantification of 1 ppm). The measurement result is shown in Table 6.

[0042]

[Table 6]

ALLERGEN	NON-ADDED BREAD (ppm)	ADDED BREAD (ppm)	100-FOLD DILUTED ADDED BREAD (ppm)
MILK	N.D.	625	6.5
PEANUT	N.D.	745	8.7

[0043]

Next, allergen-containing samples were prepared, according to Reference Example 1, from non-added bread and 100-fold diluted added bread. The obtained samples were subjected to trypsin digestion, desalting and LC-MS/MS MRM according to Example 1,

with the result that the target allergen-derived polypeptides were detected. As objects to be detected, casein-derived polypeptides (SEQ ID NOs: 13 to 15) were selected for milk, and Ara h1- or h3-derived polypeptides (SEQ ID NOs 22 to 24) were selected for peanut.

[0044]

As a result, peaks of the milk allergen (casein) and peanut allergen (Ara h1 or h3) could be confirmed with sufficient sensitivity for the 100-fold diluted added bread (Figs. 8 and 9). Furthermore, even when the LC-MS/MS MRM measurement was carried out similarly except for using a 500-fold diluted added bread, it was confirmed that the S/N of the peaks was 10 or more also. On the other hand, no peak was confirmed for the non-added bread. Therefore, according to the method of the present invention, an allergen can be detected with measurement sensitivity equivalent to that of ELISA (with lower limit of quantification of 1 ppm).

CLAIMS:

1. A method for detecting an allergen in a sample, the method comprising

treating the sample with a protease, and

detecting the presence or absence of a buckwheat allergen-derived polypeptide in the protease-treated sample by chromatographic separation analysis,

wherein the buckwheat allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 4, 6 and 7.

2. The method according to claim 1, wherein the buckwheat allergen-derived polypeptide further comprises any one or more of polypeptides selected from the group consisting of amino acid sequences represented by SEQ ID NOs: 1, 2, 3, and 5.

3. The method according to claim 1 or 2, wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 1 to 7 is detected.

4. The method according to any one of claims 1 to 3, further comprising detecting the presence or absence of a crustacean allergen-derived polypeptide, wherein the crustacean allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 8 to 12.

5. The method according to claim 4, wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 8 to 12 is detected.

6. The method according to any one of claims 1 to 5, further comprising detecting the presence or absence of a milk allergen-derived polypeptide, wherein the milk allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 13 to 17.

7. The method according to claim 6, wherein the presence or absence of all of the polypeptides consisting of amino acid sequences

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represented by SEQ ID NOs: 13 to 17 is detected.

8. The method according to any one of claims 1 to 7, further comprising detecting the presence or absence of an egg allergen-derived polypeptide, wherein the egg allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 18 to 21.

9. The method according to claim 8, wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 18 to 21 is detected.

10. The method according to any one of claims 1 to 9, further comprising detecting the presence or absence of a peanut allergen-derived polypeptide, wherein the peanut allergen-derived polypeptide comprises any one or more of polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 22 to 24.

11. The method according to claim 10, wherein the presence or absence of all of the polypeptides consisting of amino acid sequences represented by SEQ ID NOs: 22 to 24 is detected.

12. The method according to any one of claims 1 to 11, wherein the chromatographic separation analysis is liquid chromatography tandem mass spectrometry (LC-MS/MS).

13. The method according to any one of claims 1 to 12, wherein the protease is trypsin.

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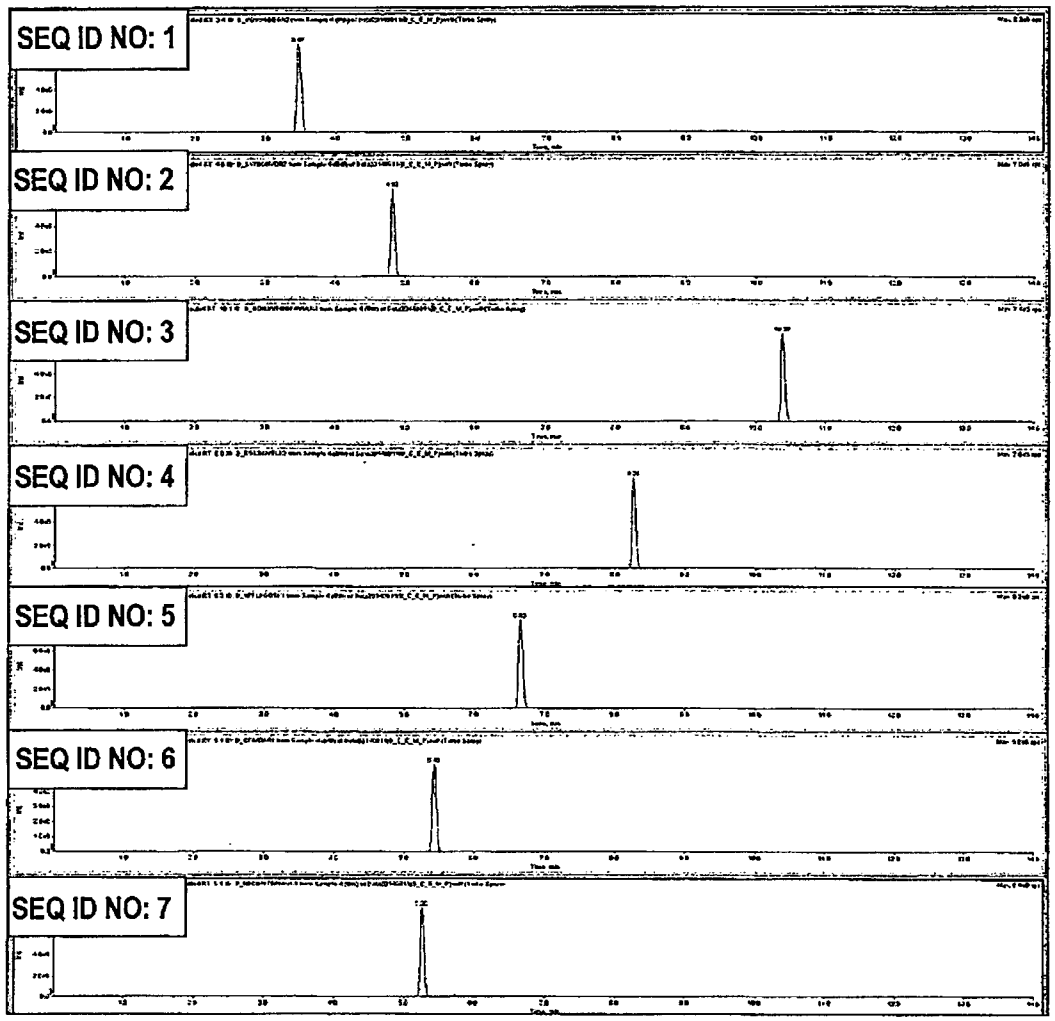
NS0082

[Drawing]

[Fig. 1]



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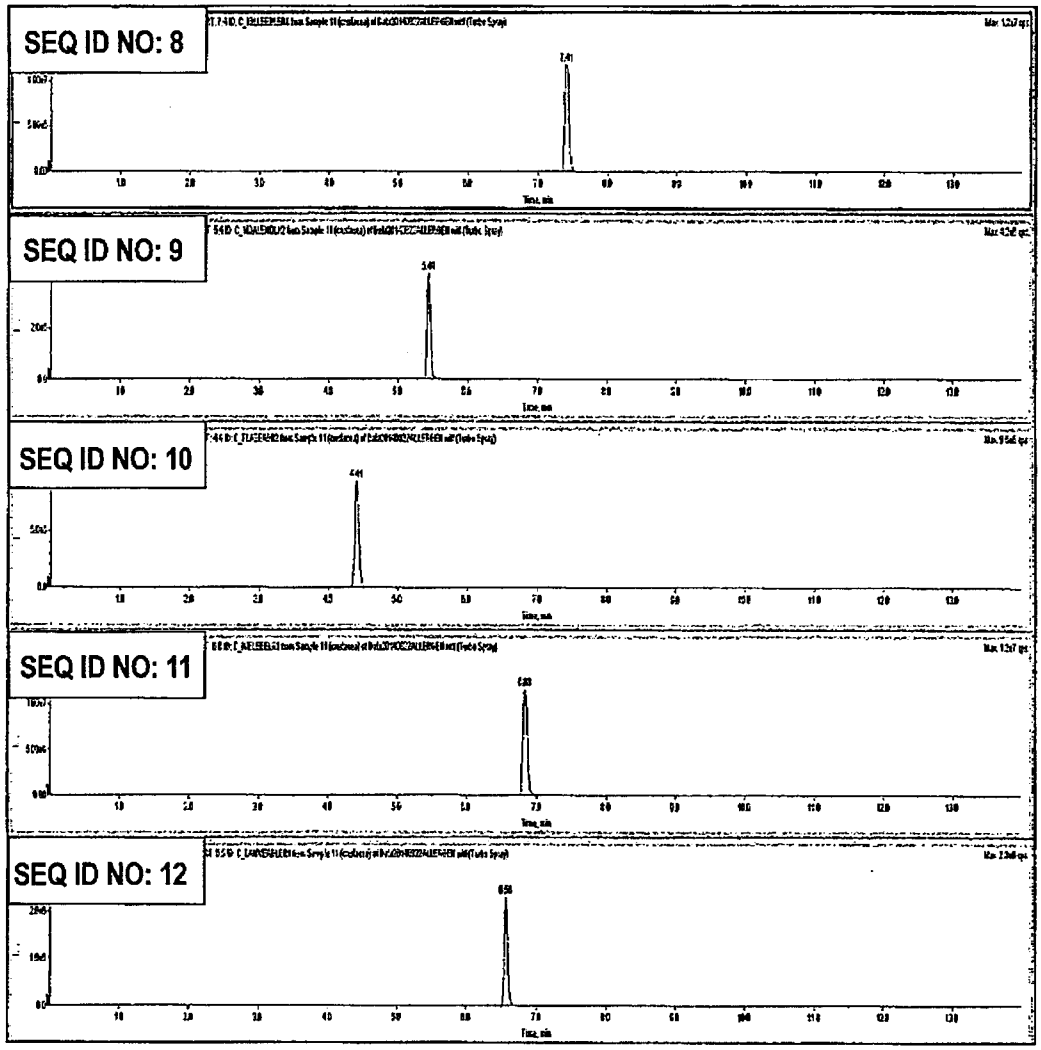
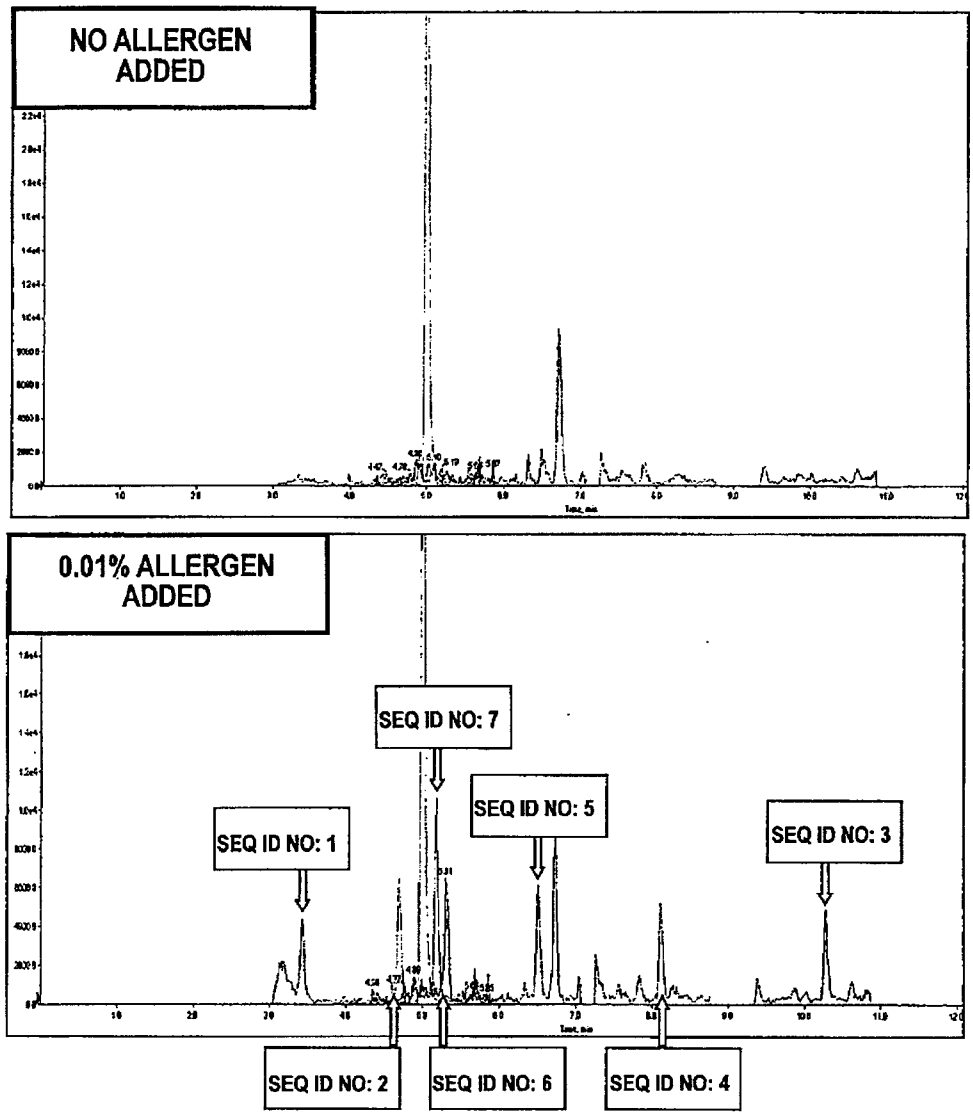
[Fig. 2]



[Fig. 3]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0.01% ALLERGEN ADDED
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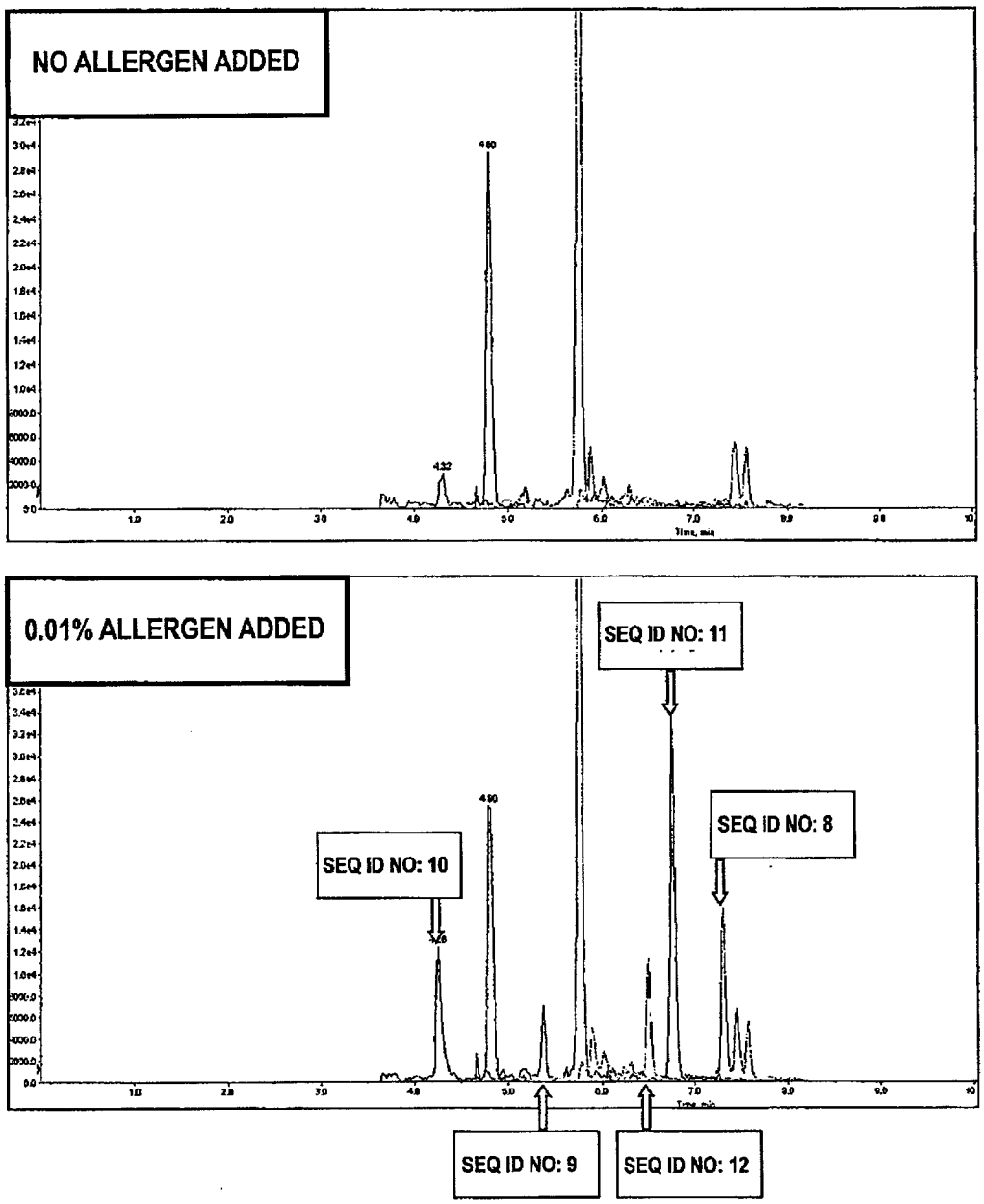
SEQ ID NO: 5
SEQ ID NO: 1
SEQ ID NO: 3
SEQ ID NO: 2
SEQ ID NO: 6
SEQ ID NO: 4

[Fig. 4]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SEQ ID NO: 8
SEQ ID NO: 10
SEQ ID NO: 9
SEQ ID NO: 12

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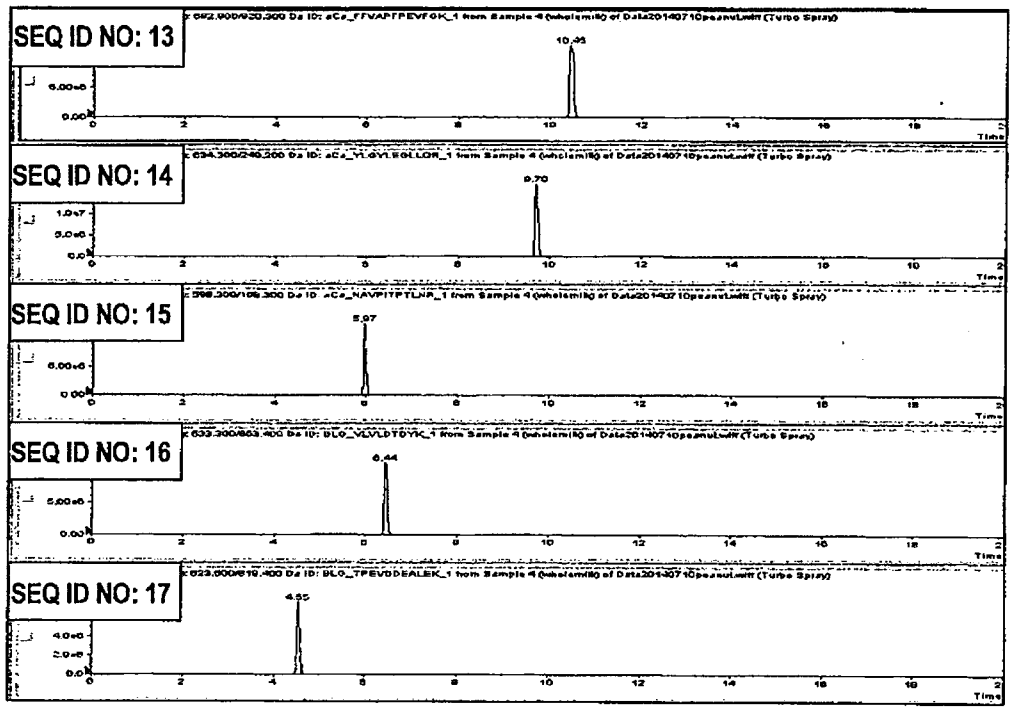
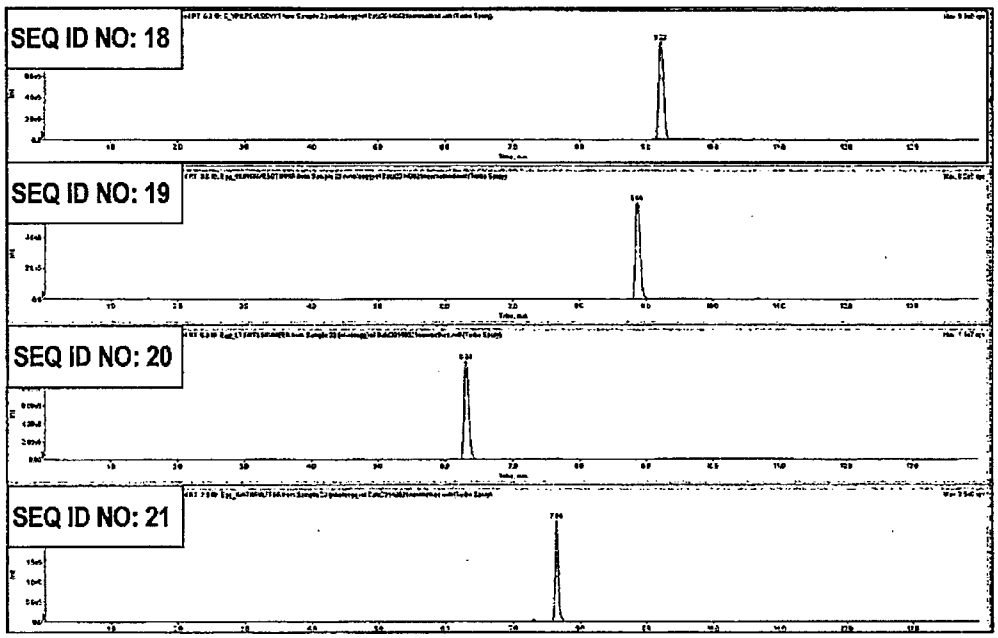
[Fig. 5]

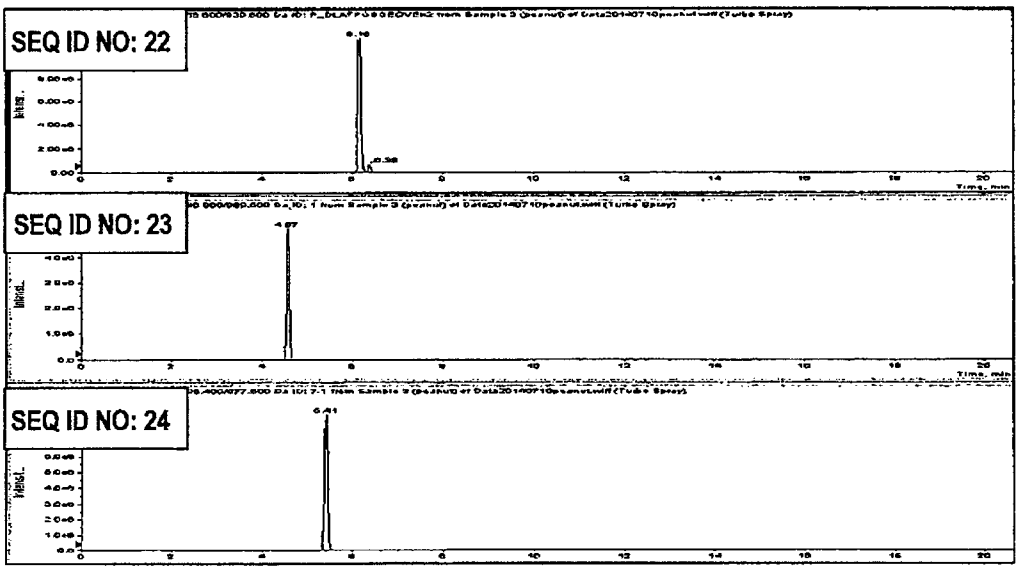

SEQ ID NO: 13
10.46
SEQ ID NO: 14
9.70
SEQ ID NO: 15
5.07
SEQ ID NO: 16
6.44
SEQ ID NO: 17
4.55
Time

NS0082

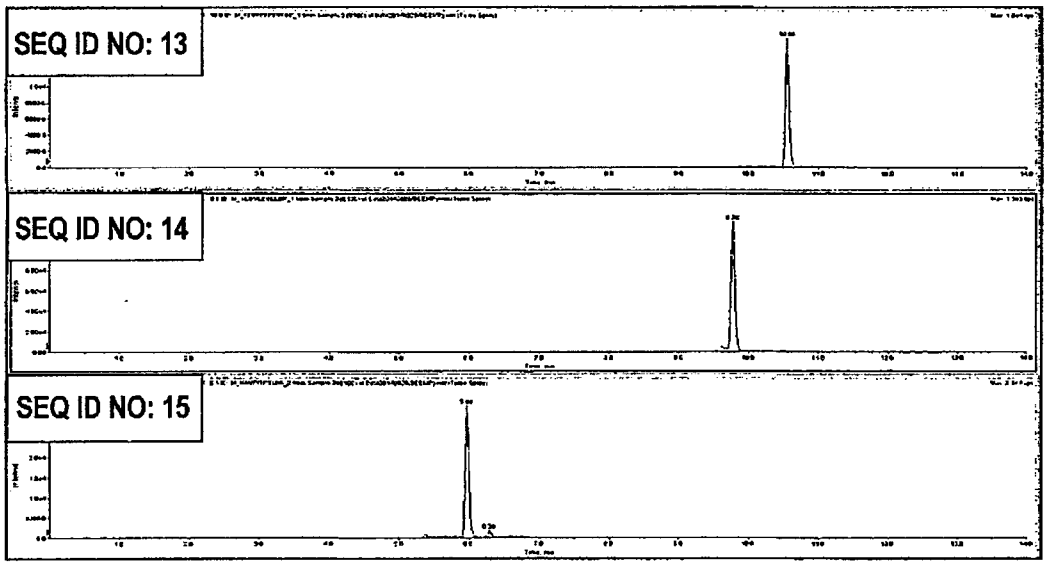
[Fig. 6]



[Fig. 7]



[Fig. 8]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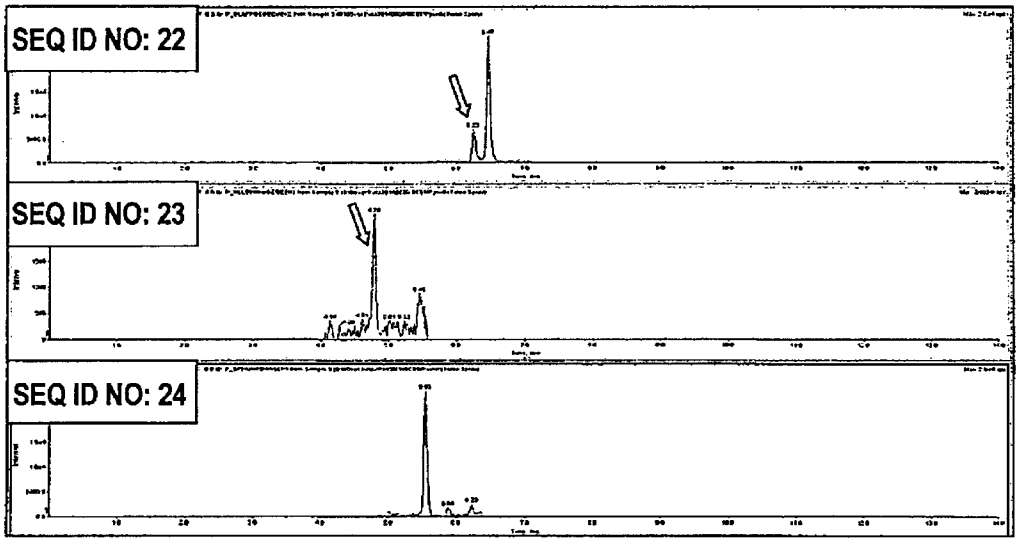
SEQ ID NO: 15

[Fig. 9]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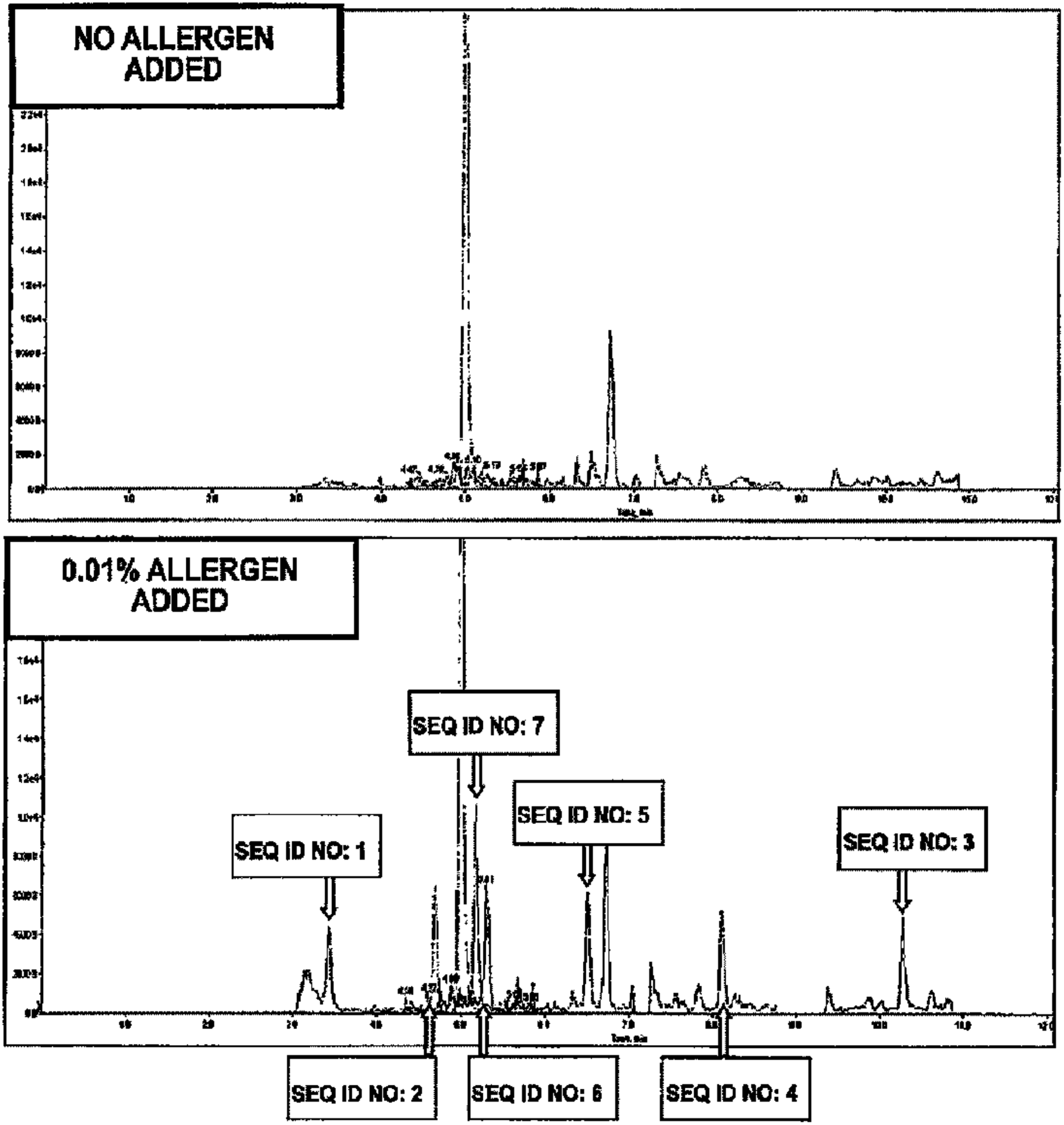
SEQ ID NO: 23
SEQ ID NO: 24


NO ALLERGEN ADDED
0.01% ALLERGEN ADDED
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SEQ ID NO: 1
SEQ ID NO: 5
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SEQ ID NO: 4