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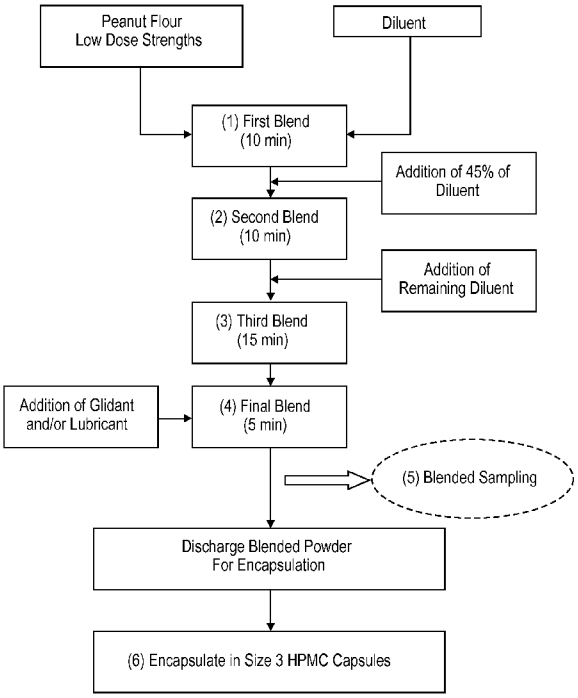
(54) Title: MANUFACTURE OF PEANUT FORMULATIONS FOR ORAL DESENSITIZATION

FIGURE 7



(57) Abstract: The present application relates to a method for managing the development and manufacturing process of a therapeutically effective formulation. Peanut proteins are characterized from peanut flour and encapsulated formulations made using the peanut flour for oral immunotherapy of peanut allergies.

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MANUFACTURE OF PEANUT FORMULATIONS FOR ORAL DESENSITIZATION

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/784,964, filed March 14, 2013, which application is incorporated herein by reference in its entirety.

[0002] This application is related to U.S. Provisional Application No. 61/784,863, filed March 14, 2013, entitled "Peanut Formulations and Uses Thereof" (Attorney Docket No. 43567-702.101), which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Allergies affect humans and companion animals and some allergic reactions (for example, those to insects, foods, latex, and drugs) can be so severe as to be life threatening.

[0004] Allergic reactions result when a subject's immune system responds to an allergen. Typically, there is no allergic reaction the first time a subject is exposed to a particular allergen. However, it is the initial response to an allergen that primes the system for subsequent allergic reactions. In particular, the allergen is taken up by antigen presenting cells (APCs; *e.g.*, macrophages and dendritic cells) that degrade the allergen and then display allergen fragments to T-cells. T-cells, in particular CD4+ "helper" T-cells, respond by secreting a collection of cytokines that have effects on other immune system cells. The profile of cytokines secreted by responding CD4+ T-cells determines whether subsequent exposures to the allergen will induce allergic reactions. Two classes of CD4+ T-cells (Th1 and Th2; T-lymphocyte helper type) influence the type of immune response that is mounted against an allergen.

[0005] The Th1-type immune response involves the stimulation of cellular immunity to allergens and infectious agents and is characterized by the secretion of IL-2, IL-6, IL-12, IFN-gamma, and TNF-beta by CD4+ T helper cells and the production of IgG antibodies. Exposure of CD4+ T-cells to allergens can also activate the cells to develop into Th2 cells, which secrete IL-4, IL-5, IL-10, and IL-13. IL-4 production stimulates maturation of B cells that produce IgE antibodies specific for the allergen. These allergen-specific IgE antibodies attach mast cell and basophil receptors, where they initiate a rapid immune response to the next exposure to allergen. When the subject encounters the allergen a second time, the allergen is quickly bound by these surface-associated IgE molecules, resulting in the release of histamines and other substances that trigger allergic reactions. Subjects with high levels of IgE antibodies are known to be particularly prone to allergies.

SUMMARY OF THE INVENTION

[0006] Provided herein is a method of making a low dose capsule formulation useful in the methods provided here, comprising, (a) mixing peanut flour and diluent in a first blend; (b) adding about 45% of diluent in a second blend; (c) adding remaining diluent in a third blend; (d) adding a glidant and/or lubricant in a final blend; and (e) encapsulating blended powder in a capsule. In one embodiment, the diluent of step (a) comprises starch or lactose, microcrystalline cellulose (Avicel®), or dicalcium phosphate. In another embodiment, the diluent of step (b) and/or (c) comprises starch, lactose, microcrystalline cellulose (Avicel®), or dicalcium phosphate. In another embodiment, the glidant of step (d) glidant of step (d) comprises colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), or combinations thereof. In another embodiment, the lubricant of step (d) comprises magnesium stearate. In one non-limiting example, the glidant comprises Cab-O-Sil. In one embodiment, step (d) comprises adding a glidant or a lubricant. In another embodiment, step (d) comprises adding a glidant and a lubricant. In another embodiment, the method further comprises sampling the blended mixture one or more times prior to encapsulation. In another embodiment, the dose comprises about 0.5 or about 1.0 mg peanut protein. In another embodiment of the described methods, step (d) further comprises passing the blended material through a mesh screen.

[0007] Provided herein is a method of making a higher dose capsule formulation useful in the methods provided here, comprising, (a) mixing peanut flour and diluent in a first blend; (b) discharging the blended material; (c) passing the blended material through a mesh screen and blending the screened material in a second blend; (d) adding in a glidant and/or lubricant in a third blend; and (e) encapsulating the blended powder. In one embodiment, the method optionally comprises sampling the blended material of step (d) one or more times prior to encapsulation. In yet another embodiment, the diluent of step (a) comprises starch, lactose or microcrystalline cellulose (Avicel®), or dicalcium phosphate. In another embodiment, the mesh screen of step (c) comprises a # 20 mesh screen. In another embodiment, the glidant of step (d) glidant of step (d) comprises colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), or combinations thereof. In another embodiment, the glidant of step (d) comprises Cab-O-Sil. In another embodiment, the lubricant of step (d) comprises magnesium stearate. In one embodiment, step (d) comprises adding a glidant or a lubricant. In another embodiment, step (d) comprises adding a glidant and a lubricant. In another embodiment, the dose comprises about 10, about 100 or about 475 mg peanut protein.

[0008] Provided herein is a method of making a capsule formulation useful in the methods provided here, comprising, passing peanut flour through a mesh screen; and encapsulating the blended powder. In one embodiment, the dose comprises about 475 mg peanut protein.

[0009] In any of such methods, the peanut flour may comprise characterized peanut proteins. In one embodiment, the peanut proteins comprise Ara h1, Ara h2 and Ara h6. The concentration of Ara h1, Ara h2 and Ara h6 may be characterized by RP-HPLC. In another embodiment, the concentration of Ara h1, Ara h2 and Ara h6 is at least an amount of a reference standard.

[0010] An encapsulated formulation produced by any of the methods described herein may be stable for at least about 3, 6, 9, 12, 18, 24, 36 or more months.

[0011] In one embodiment, the encapsulated formulation is stable at a temperature from about 2°C to about 8°C or from about 20°C to about 30°C.

[0012] In another embodiment, the encapsulated formulation is stable at a temperature of about 20°C, about 21°C, about 22.5°C, about 23°C, about 24°C, about 25°C, about 26°C, about 27.5°C, about 28°C, about 29°C,or about 30°C.

[0013] A capsule size that may be used to hold the formulations produced by the methods described herein may be, for example, size 3, 00 or 000. In one embodiment, the capsule comprises Hydroxypropyl Methyl Cellulose (HPMC).

[0014] The methods described herein may further comprise storing a formulation in a container means. Any suitable container means may be used to store the encapsulated formulations described herein. In one embodiment, the container means may be, for example, a bottle. A bottle may be, for example, an amber-colored bottle in order to minimize exposure of the encapsulated formulations to ultraviolet light. In another embodiment, the container means further comprises a dessicant packet to control moisture content of the container means.

INCORPORATION BY REFERENCE

[0015] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0017] Figure 1: Peanut flour extract at 214 nm using reversed phase HPLC. USDA Ara h standards, along with a 1 mg/mL BSA solution are also shown. The extracts are as follows: Top panel: Peanut flour, pH 8.2 extract; second panel: Ara h1 peak; third panel: Ara h2 peak; fourth panel: Ara h6 peak; bottom panel: 1 mg/ml BSA solution.

[0018] Figure 2: Chromatograph results from RP-HPLC analysis of 112FA02411 (GMP).

[0019] Figure 3: Chromatograph results from RP-HPLC analysis of 112FA02411 (Non GMP).

[0020] Figure 4: Chromatograph results from RP-HPLC analysis of 111FA36211 (Non GMP).

[0021] Figure 5: Total Protein Staining of Pooled and RP-HPLC Purified Ara h Proteins.

[0022] Figure 6: Immunoblots of Pooled and RP-HPLC Purified Ara h Proteins.

[0023] Figure 7: Blending Process Flow Diagram for Low Dose Capsules (0.5 mg and 1 mg).

[0024] Figure 8: Blending Process for the High Dose Capsules (at least 10 mg).

[0025] Figure 9: Chromatogram results from RP-HLPC analysis of 112FA02411 (GMP).

DETAILED DESCRIPTION OF THE INVENTION

[0026] Disclosed herein are systems and methods that isolate proteins from peanut flour, which may be used to manufacture pharmaceutical compositions for treatment of peanut allergies. The systems and methods utilize high pressure (phase) liquid chromatography (HPLC) to capture Ara h1, Ara h2 and Ara h6 from peanut flour.

[0027] During the past decade, much has been learned about allergens in peanut. Peanuts are commonly associated with severe reactions, including life threatening anaphylaxis. The current standard of care in management of food allergy is dietary avoidance of the food and education of the subject/family in the acute management of an allergic reaction. The burden of avoidance and constant fear of accidental exposure negatively impacts the health-related quality of life for both subjects and their families. Quality of life surveys indicate that families with children having food allergies have significant impact on food preparation, social activities, finding appropriate childcare, school attendance, and level of stress among other things.

[0028] Currently, the only treatment for peanut allergy is a peanut-free diet and ready access to self-injectable epinephrine. However, strict avoidance diets can be complicated due to difficulty in interpreting labels and by the presence of undeclared or hidden allergens in commercially prepared foods. Accidental ingestions are unfortunately common, with up to 50% of food-allergic subjects having an allergic reaction over a two-year period. Allergic reactions to peanut can be severe and life threatening; and peanut and/or tree nut allergies account for the vast majority of fatal food-induced anaphylaxis. This combination of strict avoidance diets, the high incidence of accidental exposures, and the risk of severe or even fatal reactions with accidental exposures adds a tremendous burden and stress on subjects and their families. Further complicating matters is the

fact that only about 20% of children will outgrow peanut allergy, meaning that the majority of people with peanut allergy will have it for the rest of their lives. If we couple the rising prevalence and increased consumption of peanut in Western countries with the facts that only approximately 1 in 5 will outgrow their allergy, that allergic reactions have the potential to be severe or even fatal, and that accidental exposures are common, developing an effective treatment for peanut allergy becomes even more imperative.

[0029] Specific immunotherapy for food allergy, in particular peanut allergy, in the forms of oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) has been studied in recent years and has demonstrated encouraging safety and efficacy results in early clinical trials, including beneficial immunologic changes. OIT has shown evidence for inducing desensitization in most subjects with immunologic changes over time indicating progression toward clinical tolerance.

[0030] Peanut OIT: In Jones *et al.*, peanut allergic children underwent an OIT protocol consisting of an initial dose escalation day, bi-weekly build-up (to 2 g) and daily maintenance phase followed by an OFC. After tolerating less than 50 mg peanut protein during an oral food challenge (OFC) at baseline, 27 of the 29 subjects ingested 3.9 g of peanut protein at the completion of OIT protocol.

[0031] Recently, Dr. Wesley Burks. (American Academy of Allergy, Asthma, and Immunology National Conference. Orlando, Florida, March 6, 2012) presented work showing that 10 children with PA completed an OIT protocol and underwent an oral food challenge (OFC) 4 weeks after cessation of oral intake of peanut to evaluate the development of clinical "sustained unresponsiveness". Three out of 10 subjects passed the OFC; the authors considered these subjects as clinically tolerant. Over the course of treatment, peanut IgE levels lower than 85 kU/L at a time point of 3 months into OIT was predictive of subjects who became immune tolerant.

[0032] A multi-center double-blinded randomized placebo-controlled study reported by Varshney, *et al.*, examined twenty-eight subjects. Three subjects withdrew early in the study because of allergic side effects. After completing up-dosing, a double-blind placebo-controlled food challenge was performed, in which all remaining peanut OIT subjects (n=16) ingested the maximum cumulative dose of 5000 mg (approximately 20 peanuts), whereas placebo subjects (n=9) could tolerate only a median cumulative dose of 280 mg (range, 0-1900 mg; $p < .001$). In contrast with the placebo group, the peanut OIT group showed reductions in skin prick test size ($P < 0.001$) and increases in peanut-specific IgG4 ($P < 0.001$). Peanut OIT subjects had initial increases in peanut-specific IgE ($P < 0.01$) but did not show significant change from baseline by the time of oral food challenge.

DEFINITIONS

[0033] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the inventions described herein belong. All patents and publications referred to herein are incorporated by reference.

[0034] The term "animal", as used herein, refers to humans as well as non-human animals, including, for example, mammals, birds, reptiles, amphibians, and fish. Preferably, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a primate, or a pig). An animal may be a transgenic animal.

[0035] The term "antigen", as used herein, refers to a molecule that elicits production of an antibody response (*i.e.*, a humoral response) and/or an antigen-specific reaction with T-cells (*i.e.*, a cellular response) in an animal.

[0036] The term "allergen", as used herein, refers to a subset of antigens which elicit the production of IgE in addition to other isotypes of antibodies. The terms "allergen", "natural allergen", and "wild-type allergen" may be used interchangeably. Preferred allergens for the purpose of the present invention are protein allergens.

[0037] The phrase "allergic reaction", as used herein, relates to an immune response that is IgE mediated with clinical symptoms primarily involving the cutaneous (*e.g.*, urticana, angiodema, pruritus), respiratory (*e.g.*, wheezing, coughing, laryngeal edema, rhinorrhea, watery/itching eyes), gastrointestinal (*e.g.*, vomiting, abdominal pain, diarrhea), and cardiovascular (*i.e.*, if a systemic reaction occurs) systems. For the purposes of the present invention, an asthmatic reaction is considered to be a form of allergic reaction.

[0038] The phrase "anaphylactic allergen", as used herein, refers to a subset of allergens that are recognized to present a risk of anaphylactic reaction in allergic individuals when encountered in its natural state, under natural conditions. For example, for the purposes of the present invention, pollen allergens, mite allergens, allergens in animal danders or excretions (*e.g.*, saliva, urine), and fungi allergens are not considered to be anaphylactic allergens. On the other hand, food allergens, insect allergens, and rubber allergens (*e.g.*, from latex) are generally considered to be anaphylactic allergens. Food allergens are particularly preferred anaphylactic allergens for use in the practice of the present invention. In particular, legumes (peanuts), tree nut allergens (*e.g.*, from walnut, almond, pecan, cashew, hazelnut, pistachio, pine nut, brazil nut), dairy allergens (*e.g.*, from egg, milk), seed allergens (*e.g.*, from sesame, poppy, mustard), soybean, wheat, and seafood allergens (*e.g.*, from fish, shrimp, crab, lobster, clams, mussels, oysters, scallops, crayfish) are anaphylactic food allergens according to the present invention. Particularly interesting anaphylactic allergens are those to which reactions are commonly so severe as to create a risk of death.

[0039] The phrase "anaphylaxis" or "anaphylactic reaction", as used herein, refers to a subset of allergic reactions characterized by mast cell degranulation secondary to cross-linking of the high-affinity IgE receptor on mast cells and basophils induced by an anaphylactic allergen with subsequent mediator release and the production of severe systemic pathological responses in target organs, *e.g.*, airway, skin digestive tract, and cardiovascular system. As is known in the art, the severity of an anaphylactic reaction may be monitored, for example, by assaying cutaneous reactions, puffiness around the eyes and mouth, vomiting, and/or diarrhea, followed by respiratory reactions such as wheezing and labored respiration. The most severe anaphylactic reactions can result in loss of consciousness and/or death.

[0040] The phrase "antigen presenting cell" or "APC", as used herein, refers to cells which process and present antigens to T-cells to elicit an antigen-specific response, *e.g.*, macrophages and dendritic cells.

[0041] When two entities are "associated with" one another as described herein, they are linked by a direct or indirect covalent or non-covalent interaction. Preferably, the association is covalent. Desirable non-covalent interactions include, for example, hydrogen bonding, van der Walls interactions, hydrophobic interactions, magnetic interactions, etc.

[0042] The phrase "decreased anaphylactic reaction", as used herein, relates to a decrease in clinical symptoms following treatment of symptoms associated with exposure to an anaphylactic allergen, which can involve exposure via cutaneous, respiratory, gastrointestinal, and mucosal (*e.g.*, ocular, nasal, and aural) surfaces or a subcutaneous injection (*e.g.*, via a bee sting).

[0043] The term "epitope", as used herein, refers to a binding site including an amino acid motif of between approximately six and fifteen amino acids which can be bound by an immunoglobulin (*e.g.*, IgE, IgG, etc.) or recognized by a T-cell receptor when presented by an APC in conjunction with the major histocompatibility complex (MHC). A linear epitope is one where the amino acids are recognized in the context of a simple linear sequence. A conformational epitope is one where the amino acids are recognized in the context of a particular three dimensional structure.

[0044] An allergen "fragment" according to the present invention is any part or portion of the allergen that is smaller than the intact natural allergen. In preferred embodiments of the invention, the allergen is a protein and the fragment is a peptide.

[0045] The phrase "immunodominant epitope", as used herein, refers to an epitope which is bound by antibody in a large percentage of the sensitized population or where the titer of the antibody is high, relative to the percentage or titer of antibody reaction to other epitopes present in the same antigen. In one embodiment, an immunodominant epitope is bound by antibody in more than 50% of the sensitive population, more preferably more than 60%, 70%, 80%, 90%, 95%, or 99%.

[0046] The phrase "immunostimulatory sequences" or "ISS", as used herein, relates to oligodeoxynucleotides of bacterial, viral, or invertebrate origin that are taken-up by APCs and activate them to express certain membrane receptors (*e.g.*, B7-1 and B7-2) and secrete various cytokines (*e.g.*, IL-1, IL-6, IL-12, TNF). These oligodeoxynucleotides contain unmethylated CpG motifs and when injected into animals in conjunction with an antigen, appear to skew the immune response towards a Th1-type response. See, for example, Yamamoto *et al.*, *Microbiol. Immunol.* 36:983, 1992; Krieg *et al.*, *Nature* 374:546, 1995; Pisetsky, *Immunity* 5:303, 1996; and Zimmerman *et al.*, *J. Immunol.* 160:3627, 1998.

[0047] As used herein, the terms "comprising," "including," and "such as" are used in their open, non-limiting sense.

[0048] The term "about" is used synonymously with the term "approximately." As one of ordinary skill in the art would understand, the exact boundary of "about" will depend on the component of the composition. Illustratively, the use of the term "about" indicates that values slightly outside the cited values, *i.e.*, plus or minus 0.1% to 10%, which are also effective and safe. In another embodiment, the use of the term "about" indicates that values slightly outside the cited values, *i.e.*, plus or minus 0.1% to 5%, which are also effective and safe. In another embodiment, the use of the term "about" indicates that values slightly outside the cited values, *i.e.*, plus or minus 0.1% to 2%, which are also effective and safe.

[0049] "Isolated" (used interchangeably with "substantially pure") when applied to polypeptides means a polypeptide or a portion thereof, which has been separated from other proteins with which it naturally occurs. Typically, the polypeptide is also substantially (*i.e.*, from at least about 70% to about 99%) separated from substances such as antibodies or gel matrices (polyacrylamide) which are used to purify it.

Formulations

[0050] Formulations described herein include one or more active ingredients. Active ingredients may be isolated from peanut flour which may be obtained from any source such as, for example, the Golden Peanut Company. The peanut flour may be from about 10% to about 15%, or about 12% defatted peanut flour milled from lightly roasted peanuts. The peanut flour may be, in some instances, released by a supplier after standard analysis of content and microbiology, and may be stable for 9-12 months under refrigeration. The peanut flour may be formulated, encapsulated and tested prior to administration to a subject.

[0051] For analysis of the peanut flour, bulk substance (BS) and final formulation, a reverse phase HPLC assay (RP-HPLC) has been developed that separates three peanut flour protein allergens: Ara h1, Ara h2 and Ara h6. This assay forms the basis for identity and content testing

at release and during stability. The reverse phase-HPLC assay may be utilized as an identification assay and to monitor lot-to-lot consistency and stability of the peanut allergens acceptable for production of the Characterized Peanut Allergen formulation.

[0052] Additional characterization of the protein allergens may also be performed using Enzyme Linked Immunosorbent Assays (ELISA) and gel analysis.

[0053] Peanuts and peanut flour are common foods and additives found in many food formulations. The intended clinical use for Characterized Peanut Allergen identified by the present inventors is found in relatively small quantities (0.5 to 4000 mg/dose) compared to quantities contained in food and may be delivered via the same route as orally ingested peanut-containing products.

[0054] Formulations described herein may be tested in a multi-center, placebo-controlled study to demonstrate the safety and efficacy of Characterized Peanut Allergen in subjects from about 4 to about 26 years of age with moderate-to-severe clinical reactions to peanut ingestion. Subjects with significant concomitant health conditions, uncontrolled asthma, or prior admission to an intensive care unit due to anaphylaxis may be excluded. Standard anti-allergy medications (e.g., antihistamines, oral corticosteroids, *etc.*) may be allowed on maintenance and while up-dosing with Characterized Peanut Allergen (CPA).

[0055] A formulation comprising Characterized Peanut Allergen (CPNA), may include peanut protein (comprising peanut allergen proteins Ara h1, Ara h2 and Ara h6) formulated with a one or more diluents, one or more glidants, one or more lubricants and, optionally, one or more filling agents, in graduated doses, comprising capsules containing about 0.5 mg, about 1 mg, about 10 mg, about 100 mg and about 1000 mg each of peanut protein. Each capsule may be opened and the content mixed into taste-masking food immediately prior to administration.

[0056] An active pharmaceutical ingredient is initially sourced as raw peanuts, Arachis hypogaea, a member of the legume family. Raw peanuts may be procured from multiple farming sources, where the shelled, raw peanuts are processed into 12% defatted roasted peanut flour (PF). The PF may be comprise a certificate of analysis (CofA) for further processing under cGMP conditions.

[0057] Formulation, fill and testing of the CPNA capsules may be performed at a cGMP contract manufacturing site. Under cGMP manufacturing conditions, the protein flour (PF), which is comprised of approximately 50% peanut protein w/w, is mixed with one or more diluents, one or more glidants and one or more lubricants.

[0058] In one embodiment, a composition comprises one or more diluents. "Diluents" for use in the formulations include, but are not limited to, alginic acid and salts thereof; cellulose derivatives such as carboxymethylcellulose, methylcellulose (*e.g.*, Methocel®), hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose (*e.g.*, Klucel®), ethylcellulose (*e.g.*, Ethocel®),

microcrystalline cellulose (*e.g.*, Avicel®); silicified microcrystalline cellulse; microcrystalline dextrose; amylose; magnesium aluminum silicate; polysaccharide acids; bentonites; gelatin; polyvinylpyrrolidone/vinyl acetate copolymer; crosspovidone; povidone; starch; pregelatinized starch; tragacanth, dextrin, a sugar, such as sucrose (*e.g.*, Dipac®), glucose, dextrose, molasses, mannitol, sorbitol, xylitol (*e.g.*, Xylitab®), lactose (*e.g.*, lactose monohydrate, lactose anhydrous, etc.); dicalcium phosphate; a natural or synthetic gum such as acacia, tragacanth, ghatti gum, mucilage of isapol husks, polyvinylpyrrolidone (*e.g.*, Polyvidone® CL, Kollidon® CL, Polyplasdone® XL-10), larch arabogalactan, Veegum®, polyethylene glycol, waxes, sodium alginate, a starch, *e.g.*, a natural starch such as corn starch or potato starch, a pregelatinized starch such as Colorcon (Starch 1500), National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®; a cross-linked starch such as sodium starch glycolate; a cross-linked polymer such as crospovidone; a cross-linked polyvinylpyrrolidone; alginate such as alginic acid or a salt of alginic acid such as sodium alginate; a clay such as Veegum® HV (magnesium aluminum silicate); a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth; sodium starch glycolate; bentonite; a natural sponge; a surfactant; a resin such as a cation-exchange resin; citrus pulp; sodium lauryl sulfate; sodium lauryl sulfate in combination starch; and combinations thereof. In one embodiment, the formulation comprises microcrystalline cellulose or starch 1500. In another embodiment, the formulation comprises microcrystalline cellulose and starch 1500.

[0059] Suitable glidants (anti-caking agents) for use in the solid dosage forms described herein include, but are not limited to, colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), and combinations thereof. In one embodiment, the composition comprises Cab-O-Sil.

[0060] Suitable lubricants for use in the solid dosage forms described herein include, but are not limited to, stearic acid, calcium hydroxide, talc, corn starch, sodium stearyl fumerate, alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, magnesium stearate, zinc stearate, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol or a methoxypolyethylene glycol such as Carbowax™, PEG 4000, PEG 5000, PEG 6000, propylene glycol, sodium oleate, glyceryl behenate, glyceryl palmitostearate, glyceryl benzoate, magnesium or sodium lauryl sulfate, and combinations thereof. In one embodiment, the composition comprises magnesium stearate. In another embodiment, the composition comprises sodium stearyl fumerate.

[0061] In some embodiments, a formulation may further comprise one or more filling agents. "Filling agents" include compounds such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose,

dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and combinations thereof.

[0062] Ingredients described herein may be mixed according to, for example, the processes illustrated in Figures 7 and 8. Mixed formulations may be subsequently encapsulated as 0.5, 1, 10, 100 mg, 475 mg, and 1000 mg of peanut protein in size 3, 00 or 000. Hydroxypropyl Methyl Cellulose (HPMC) capsules. Compatibility studies may evaluate combinations of the peanut flour with one or more of the excipients, which may have in some instances, GRAS recognition. The diluent provides the opportunity to formulate the low and high doses to contain adequate volume for dispersal from the opened capsule. The glidant and lubricant add flowability to the PF such that the capsule is easily emptied of flour by the subject or practitioner at time of administration. For clinical trials, the capsules may be bulk packed into a container means such as, for example, bottles. In some instances, the container means may be treated to prevent (partially or fully) exposure to light. For example, a container means may be amber-colored. A container means may also, in some instances, contain a dessicant to prevent (partially or fully) exposure to moisture during shipping and storage. At the time of use, capsule(s) containing CPNA may be opened and the content mixed into taste-masking food immediately prior to administration.

[0063] In order to standardize the delivery of peanut protein allergens, a cGMP manufactured Characterized Peanut Allergen (CPNA) formulation has been developed. The protein content of the formulation is critical from two aspects. First, the total protein delivered should be consistent among batches, and second, the proportion of critical individual allergens should be controlled.

[0064] Total protein content of the bulk substance and final formulation release may be quantified using protein determination methods described herein which address current issues in the industry: namely, prior to the present application establishing the absolute or relative amounts of individual peanut protein allergens in the peanut flour is more problematic and has not been controlled. .

[0065] Peanut protein is comprised of several individual protein allergens typically detectable by polyacrylamide gel electrophoresis and immunoblotting using allergen specific polyclonal antisera from allergic humans or immunized animals. Of these proteins, based on immunoblot, reactivity against crude peanut extracts by human sera from peanut allergic humans, and *in vitro* histamine release from sensitized basophils, Ara h1, Ara h2 and Ara h6 have been identified as allergenic peanut protein allergens, with Ara h2 and Ara h6 contributing the majority of the allergenic activity of crude peanut extract.

[0066] Prior to the present application peanut allergen proteins have typically been fractionated from crude peanut extracts by size exclusion chromatography (SEC) or polyacrylamide gel electrophoresis. These techniques may present a relative view into the spectrum of Ara proteins, but

do not provide the resolution and sensitivity needed to compare individual peanut allergen expression among peanut flour lots, nor possible changes in protein structure over time. In order to address these limitations, the present inventors have developed a reverse phase HPLC (RP-HPLC) method to enhance the resolution and allow physical separation of peanut allergens Ara h1, Ara h2 and Ara h6.

[0067] An assay was developed for the determination and characterization of Ara h1, 2 and 6 allergenic proteins in roasted peanut flour. A simple single stage extraction procedure was modified using Tris buffer at pH 8.2, followed by centrifugation and filtration. Samples are prepared at 100 mg/mL and extracted at 60°C for 3 hours. The final neat filtrate is suitable for direct analysis by HPLC.

[0068] The HPLC separation utilizes a reversed phase separation using a wide pore 300 Å silica column with a bonded butyl stationary phase. A binary gradient may be employed based upon 0.1% trifluoroacetic acid and acetonitrile. The mobile phase may be compatible with mass spectrometry. Detection may be accomplished with a UV detector at 214 nm, as sensitivity may be reduced with detection at 280 nm.

[0069] Specificity of the method may be determined by comparing the retention times and peak patterns of the whole peanut extract with Ara h proteins. The principle Ara h protein peaks, in some instances, may not resolve as discrete entities, but rather may appear as ensembles of many similar proteins. Thus, the Ara h1, Ara h2 and Ara h6 allergens may appear as clusters of peaks within a retention time region. Accordingly, the relative amount of a particular Ara h protein is then determined as the percentage of the total area within a defined elution region. Chromatographic resolution of the various regions is assessed, and the method may be useful for comparison of subtle differences in these regional patterns for different lots and sources of peanut flour proteins, and stability of the formulation.

[0070] A representative example chromatographic series at 214 nm is shown in Figure 1 comparing the crude extract (top panel) with profiles from purified Ara h1, Ara h2 and Ara h6 proteins and BSA.

[0071] RP-HPLC method pre-qualification may be assessed by comparing three independent preparations of a single peanut flour lot, by comparing the results of replicate assays performed by two different analysts on two different days, or by comparing the results of independent preparations of different lots of peanut flour on the same or different days.

[0072] Precision may be estimated by performing the extraction of a single sample in triplicate, and analyzing the results according to the proposed method (see, *e.g.*, **Table 1**). Triplicate extractions and determinations of a single lot of peanut flour are conducted; reported values are

percent area of each Ara h species. Integration of the peaks may be performed by using forced integration events on a data system (*e.g.*, ChemStation), or manual integration. The precision for these triplicate independent preparations of a single lot of peanut flour may range from about 1.1% relative standard deviation (RSD) for Ara h6 to about 18.3% for Ara h1. The higher (% RSD) for Ara h1 may be associated with integrating the Ara h1 shoulder from the subsequent larger cluster.

Table 1: RP-HPLC Method Precision

Peanut Flour Lot #	% Area		
	Ara h2	**Ara h6**	**Ara h1 (shoulder)**
112FA02411	12.20	6.36	7.41
	11.95	6.30	9.68
	12.30	6.22	10.72
Average	12.15	6.30	9.27
Std Dev	0.1762	0.0718	1.6955
%RSD	**1.45%**	**1.14%**	**18.29%**

[0073] A second precision method compares the results obtained by two different analysts performing the assay on two different days. Each value presented represents the average of duplicate injections. **Table 2** provides exemplary results of a comparison of the percent area values and extractable protein content of three peanut flour lots, by two different analysts on different days. Comparison of the quantitative results obtained from these assays yields Ara h values that agree between 86% to 107%; total protein content may agree within 95% - 102%. The percent of the match between the two analysts may also be presented.

Table 2: RP-HPLC Method Precision

Peanut Flour Lot #	% Area					
	Ara h2		**Ara h6**		**Ara h1 (shoulder)**	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
111FA36111	10.60	12.29	5.59	5.77	8.82	9.73
% Match	86.31		96.76		90.70	
111FA36211	10.65	12.02	5.48	5.67	11.14	9.36
% Match	88.58		96.63		118.97	

112FA02411	10.62	12.15	5.93	6.30	9.91	9.27
% Match	87.40		94.12		106.92	
Values are the average of two injections						

[0074] Analysis of various PF lots may be used to demonstrate that the expression of Ara h1, Ara h2 and Ara h6 is consistent, both individually and relative to each other across lots of peanut flour. This assay may also form the basis for identity and content testing at release and during stability determination.

[0075] The assay was be conducted and analyzed by a second cGMP manufacturer. The HPLC profiles (see, *e.g.*, **Figure 2, Figure 3 and Figure 4**), total protein and percentage of each allergen within the total protein (see, *e.g.*, **Table 3**) are generally consistent between the assays performed by both laboratories using the same peanut flour lots (allows bridging of the data).

Table 3 Comparison for Ara h Proteins and Total Extractable Protein Content

Peanut Flour Lot	**% Area**			**% Protein**
	Ara h2 (shoulder)	**Ara h6 (shoulder)**	**Ara h1 (shoulder)**	
111FA36111	11.40	5.29	9.79	11.26
111FA36211	11.20	5.67	9.85	11.03
112FA02411 (Non GMP)	11.31	5.68	10.41	10.23
112FA02411 (GMP)	10.58	5.79	10.16	10.25

RP-HPLC Confirmation Studies

[0076] To confirm that the RP-HPLC peak profile actually separates and identifies Ara h1, Ara h2 and Ara h6, material isolated from each peak may be further characterized by SDS polyacrylamide gel electrophoresis using, for example, a 4-20 Novex Tris-HCl pre-cast gel (see, *e.g.*, **Figure 5**). Additional gels may be transferred to polyvinylidene difluoride (PVDF) membranes, processed for immunoblotting and may be reacted with Ara h1, Ara h2 or Ara h6 chicken antisera and developed with horse radish peroxidase conjugated goat anti-chicken IgG using, for example, an assay method described by de Jong *et al.* (*EMBO J.*, 1988; 7(3): 745–750). It should be noted that while extracts may be derived from roasted peanut flour, the antisera may be generated against Ara h proteins purified from raw peanut extracts. The antisera react with both the control Ara h proteins derived from raw peanuts and from the isolated Ara h proteins obtained from roasted peanut extracts (see, *e.g.*, **Figure 6**).

[0077] The immunoblots show that the material isolated from each of the three principle HPLC peaks was reactive with the appropriate peanut protein specific antisera, and that the molecular weight of the immunoreactive proteins corresponded to the protein molecular weights as reported in the literature (Koppelman *et al.* 2010). It was determined that the Ara h proteins extracted from the peanut flour are not sensitive to heating to 60°C. Additional confirmatory experiments may be conducted; these assays may be used to establish the most appropriate stability indicating assay that provides the greatest sensitivity to changes occurring during long-term storage. However, the early immunoblot data described herein indicate that the reported RP-HPLC method will track the individual peanut proteins among peanut flour lots.

Source and Testing of the Peanut Flour

[0078] Peanut Flour (PF) for use in a formulation described herein may be sourced from any reliable producer including, but not limited to, the Golden Peanut Company (GPC) which manufactures peanut flour and peanut oil (a byproduct of defatting the roasted peanuts).

[0079] A GPC manufacturing facility may be audited by an internationally recognized certification body for food safety programs (*e.g.*, Intertek Labtest (UK) Limited). The audit may focus on compliance with the British Retail Consortium Food Standard (BRC) Global Standards for Food Safety. The BRC Global Standards are a leading global safety and quality certification program, used throughout the world by over 17,000 certificated suppliers in 90 countries through a network of over 80 accredited and BRC recognized Certification Bodies. The BRC Global Standards are widely used by suppliers and global retailers. They facilitate standardization of quality, safety, operational criteria and manufacturers' fulfillment of legal obligations. They also help provide protection to the consumer. There were no major or critical non-conformity findings during the most recent audit.

[0080] The PF may be about 12% defatted peanut flour milled from lightly roasted peanuts. The PF may be by the supplier after standard analysis of content and microbiology, and is identified as stable for 9 months under refrigeration.

Incoming Raw Material Release Testing for PF

[0081] The PF raw material may be tested for appearance, identify, total protein content and moisture content prior to release for cGMP production (see, e.g., **Table 4**). The PF may be stored under controlled conditions at 2-8°C.

Table 4: Raw Material Testing for PF

Assay	Method	Acceptance Criteria
Appearance Powder/Color	Visual	Fine powder Tan color
Identity	RP-HPLC	Comparable to Reference Chromatogram
Protein Content	Nitrogen Content by AOCS Combustion Method for Determination of Crude Protein (AOCS Official Method Ba 4e-93)	Report Results
Moisture	Loss on Drying (LOD) USP <921>	Report Results

Formulation Excipients

[0082] **Table 5** provides exemplary excipients that may be used in a formulation described herein. Other excipients that may be used in a formulation described herein are provided elsewhere in the description.

[0083] Exemplary intended dosage form include, for example, a Hydroxypropyl Methyl Cellulose (HPMC) based capsule; the strength of the dosage form may be about 0.5 mg, about 1 mg. about 10 mg, about 100 mg, about 475 mg, or about 1000 mg of peanut protein. The peanut protein itself, in some instances, may be a cohesive material without inherent flow properties conducive to conventional pharmaceutical manufacturing processes. Thus, inactive pharmaceutical ingredients (excipients) may be added to the formulation so the peanut flower may be developed into a proper pharmaceutical dosage form with flow characteristics to enhance both manufacturing and also delivery of the dosage form.

[0084] Compatibility studies may be conducted to evaluate combinations of peanut flour with exemplary excipient categories (diluent, glidant and lubricant). The excipients may have GRAS recognition or be shown to be safe in pharmaceutical formulations. The diluent provides the opportunity to formulate the low and high doses to contain adequate volume for dispersal from the opened capsule. The glidant and lubricant add flowability to the PF such that the capsule is easily emptied of flour by the subject.

[0085] As per **Table 5** each of the excipients under consideration are designated as USP, NF or USP-NF.

Table 5: Excipients Under Consideration

Functionality	Excipient	Manufacturer (Trade Name)	Grade	Description
Diluents	Lactose Monohydrate	Foremost (Lactose 316/Fast-Flo)	NF	Simple Organic Diluent (Monohydrate)
	Lactose Anhydrous	Kerry/Sheffield (Lactose DT)	NF	Simple Organic Diluent (Anhydrous)
	Mannitol	Roquette (Pearlitol 300DC)	NF	Simple Organic Diluent
	Microcrystalline Cellulose	FMC (Avicel pH102)	NF	Complex Organic Diluent
	Partially Pregelatinized Corn Starch	Colorcon (Starch 1500)	USP/NF	Complex Organic Diluent
	Silicified Microcrystalline Cellulose	JRS Pharma (PROSOLV HD90)	USP	Complex Organic/Inorganic Co-processed Diluent
	Dicalcium Phosphate	Innophos (DiTab)	NF	Inorganic Diluent
Glidant	Colloidal Silicon Dioxide	Cabot (Cab-O-Sil M5P)	USP	Glidant/Anticaking Agent
	Talc	Ultra Chemicals (Ultra Talc 4000)	USP	Glidant/Anticaking Agent
Lubricants	Magnesium Stearate (vegetable source)	Mallinckrodt	USP	Lubricant
	Sodium Stearyl Fumarate	JRS Pharma (Pruv)	USP	Lubricant
Capsule Shell	White Opaque HPMC Capsule Shell	Capsugel (V-Caps)	n/a	Vegetable Source Capsule Shell
Capsule Coloring Agents	Pigment Blends	V54.9041 V18.9221 V41.9071	TBD	Representative of final capsule shell color
	Caramel Color	Sensient	TBD	Colorant for matching placebo blends

Formulation of the Characterized Peanut Allergen

[0086] Peanut flour (containing peanut allergen proteins Ara h1, Ara h2 and Ara h6) may be formulated with a bulking and a flow agent in graduated doses, comprising capsules containing 0.5 mg, 1 mg, 10 mg, 100 mg and 1000 mg each of peanut protein.

Low Dose Capsules (0.5 mg and 1 mg)

[0087] **Figure 7** and **Table 6** outline the proposed blending process for the low dose capsules, which include the 0.5 mg peanut protein and 1 mg peanut protein capsules.

[0088] Provided herein is a method of making a low dose capsule formulation useful in the methods provided here, comprising, (a) mixing peanut flour and diluent in a first blend; (b) adding about 45% of diluent in a second blend; (c) adding remaining diluent and/or lubricant in a third blend; (d) adding a glidant in a final blend; and (e) encapsulating blended powder in a capsule. In one embodiment, the diluent of step (a) comprises starch, lactose or microcrystalline cellulose (Avicel®), or dicalcium phosphate. In another embodiment, the diluent of step (b) and/or (c) comprises starch, lactose or microcrystalline cellulose (Avicel®), or dicalcium phosphate. In another embodiment, the glidant of step (d) glidant of step (d) comprises colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), or combinations thereof. In another embodiment, the glidant of step (d) comprises Cab-O-Sil. In another embodiment, the lubricant of step (d) comprises magnesium stearate. In another embodiment, the method further comprises sampling the blended mixture one or more times prior to encapsulation. In another embodiment, the dose comprises about 0.5 or about 1.0 mg peanut protein. In one embodiment, the method optionally comprises sampling the blended material of step (d). In one embodiment, step (d) comprises adding a glidant or a lubricant. In another embodiment, step (d) comprises adding a glidant and a lubricant.

Table 6: Proposed Operation Steps for Low Dose Capsules (0.5 mg and 1 mg)

Operation Step	Equipment Type	Details	Comments
1	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size Use of intensifier bar dependent on uniformity results from developmental batches
2	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size
3	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size
4	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size
5	Sample Thief	TBD	Thief length and chamber size appropriate for blender size and analytical sample size

			requirements
6	Encapsulator	Dosing Disk / Auger	Encapsulation method TBD based on fill weight variation assessments in developmental batches

High Dose Capsules (10 mg, 100 mg and 475 mg)

[0089] **Figure 8** and **Table 7** outline the proposed blending process for the high dose capsules, which include the 10 mg peanut protein, 100 mg peanut protein and 475 mg peanut protein capsules.

[0090] Provided herein is a method of making a high dose capsule formulation useful in the methods provided here, comprising, (a) mixing peanut flour and diluent in a first blend; (b) discharging the blended material; (c) passing the blended material through a mesh screen and blending the screened material in a second blend; (d) adding in a glidant and/or lubricant in a third blend; (e) encapsulating the blended powder. In one embodiment, the method optionally comprises sampling the blended material of step (d) one or more times prior to encapsulation. In yet another embodiment, the diluent of step (a) comprises starch, lactose or microcrystalline cellulose (Avicel®), or dicalcium phosphate. In another embodiment, the mesh screen of step (c) comprises a # 20 mesh screen. In another embodiment, the glidant of step (d) glidant of step (d) comprises colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), or combinations thereof. In another embodiment, the glidant of step (d) comprises Cab-O-Sil. In another embodiment, the lubricant of step (d) comprises magnesium stearate. In one embodiment, step (d) comprises adding a glidant or a lubricant. In another embodiment, step (d) comprises adding a glidant and a lubricant.

Table 7: Proposed Operation Steps for High Dose Capsules (10 mg, 100 mg and 475 mg)

Operation Step	Equipment Type	Details	Comments
1	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size
2	Sieve	U.S STD #20 (850 μm)	Promote blend uniformity
3	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size

Operation Step	Equipment Type	Details	Comments
4	Diffusion Blender	V-Blender	Blender shell size TBD based on batch size
5	Sample Thief	TBD	Thief length and chamber size appropriate for blender size and analytical sample size requirements
6	Encapsulator	Dosing Disk / Auger	Encapsulation method TBD based on fill weight variation assessments in developmental batches

Control of the Bulk Substance

[0091] Exemplary proposed specifications for formulated Bulk Substance are summarized in **Table 8.**

Table 8: Proposed Specifications for Bulk Substance

Attribute		Method	Acceptance Criteria
General	Appearance Powder/Color	Visual	TBD
	Moisture	LOD	Report Results
Identity	Presence of Ara h1, Ara h2 and Ara h6 proteins	Reverse Phase HPLC	Comparable to Reference Chromatogram Report percent area of Ara h1, Ara h2 and Ara h6
Strength (Assay)	Total Protein Determination	Nitrogen Content by AOCS Combustion Method for Determination of Crude Protein (AOCS Official Method Ba 4e-93)	Low doses (0.5 and 1 mg): Target protein concentration ± 15% High doses (10, 100 and 475 mg): Target protein concentration ± 10%
Safety	Bioburden	Microbiological Limits USP <61> Microbial Enumeration	Total Aerobic Microbial Count: NMT 1000 CFU/g

Attribute		Method	Acceptance Criteria
		USP <62> Specified Microorganisms	Total Yeasts & Molds Count: NMT 100 CFU/g *E. coli*: Absent *S. aureus*: Absent *P. aeruginosa*: Absent Salmonella species: Absent

Bulk Stability Testing

[0092] The formulation may be filled into capsules within 24 hours of blending.

Formulation

Overview of Chemistry and Manufacturing Composition

[0093] Peanut flour (containing peanut allergen proteins Ara h1, Ara h2 and Ara h6) may be formulated with a bulking and a flow agent in graduated doses, comprising capsules comprising about 0.5 mg, about 1 mg, about 10 mg, about 100 mg, about 475 mg, or about 1000 mg each of peanut protein with one or more diluents, one or more glidants, one or more lubricants. Optionally one or more filling agents may be added. Each capsule may be opened and the content mixed into taste-masking food immediately prior to administration.

[0094] Non-animal capsules that meet global Pharmaceutical standards may be used for the formulations described herein. In one non-limiting embodiment, HPMC capsules from Capsugel may be used.

[0095] In another non-limiting embodiment, capsules may be color coded to distinguish the different doses Matching color-coded placebo capsules may also be produced.

Table 9: Exemplary Dosage Forms

	Peanut Protein Dose	Capsule Size
1	0.5 mg	3
2	1 mg	3
3	10 mg	00
4	100 mg	00
5	475 mg	000

[0096] The final excipient composition of the formulation may be determined after completion of the ongoing compatibility study with the different excipients (see **Table 5**).

Manufacturing Process

[0097] Encapsulation method/equipment may be determined based on fill weight variation assessments in developmental batches. In-process controls may include periodic weight checks.

Control of the Formulation

[0098] Exemplary release specifications of the formulations are presented in **Table 10.**

Table 10: Proposed Specifications for the Formulation

Attribute		Method	Acceptance Criteria
General	Appearance Powder/color	Visual	TBD
	Capsule Integrity	Visual	Intact capsules with no visible signs of cracking. Capsules open easily without breaking
	Content Uniformity	USP <905>	Meets USP <905> requirements
	Deliverable Mass	% Weight Delivered	Report results
	Moisture	Loss on Drying (LOD) USP <921>	Report Results
Identity	Presence of Ara h1, Ara h2 and Ara h6 proteins	Reverse Phase HPLC	Comparable to reference chromatogram and Report percent area of Ara h1, Ara h2 and Ara h6
Strength (Assay)	Protein Content	Nitrogen Content by AOCS Combustion Method for Determination of Crude Protein (AOCS Official Method Ba 4e-93)	Low doses (0.5 and 1 mg): Target protein concentration ± 15% High doses (10 and 100mg): Target protein concentration ± 10%

Attribute		Method	Acceptance Criteria
Safety	Bioburden	Microbiological Limits USP <61> Microbial Enumeration USP <62> Specified Microorganisms	Total Aerobic Microbial Count: NMT 1000 CFU/g Total Yeasts & Molds Count: NMT 100 CFU/g *E. coli*: Absent *S. aureus*: Absent *P. aeruginosa*: Absent *Salmonella* species: Absent

Appearance

[0099] Appearance assessments may be performed on the bulk substance (*e.g.*, formulation during one or more preparation steps and/or of the final mixture prior to encapsulation) and the formulation. Assessment of the appearance may include, for example, consists of visually inspecting the container against a white background illuminated by a full spectrum light.

Content Uniformity

[00100] Content uniformity (CU) of capsules may be performed according to USP standards. Content uniformity may be based on a total protein nitrogen content combustion assay. The intent is to identify a combustion instrument with the sensitivity to enable assaying individual capsules at all doses.

Deliverable Mass

[00101] The capsule deliverable mass may be evaluated by weighing capsules, and emptying the contents, and weighing the emptied capsules. The % delivered amount may then be calculated.

Moisture Content

[00102] Moisture content may impact the stability of proteins, and understanding the changes in moisture content over time is useful for understanding changes in the formulation that may, in some instances, lead to shorter shelf life. For peanut flour filled capsules, moisture content may be measured using Loss on Drying (LOD) determinations according to the USP. Conditions for the LOD may be determined based on the excipients requirements and requirements for the peanut flour.

Identity (RP-HPLC)

[00103] RP-HPLC may be used to confirm identity of the PF, BS and final formulation. Samples may be analyzed according to the methods described in more detail in the related

application entitled "Peanut Formulations and Uses Thereof", filed the same day herewith (Attorney Docket No. 43567-702.101), which is incorporated herein by reference in its entirety, and the resulting chromatograms may be compared to the example chromatogram provided in the test method (See, e.g., **Figure 9**).

[00104] A positive identification of peanut flour may be confirmed if the sample chromatogram matches the chromatogram provided in the method. If a positive indication is not confirmed, a lot of peanut flour may be discarded as sub-standard. Absence of active in placebos may be confirmed by demonstrating that no peaks elute between 12 and 35 minutes in the chromatography.

Total Extractable Protein

[00105] A similar approach to the determination of total extractable protein in peanut flour may be used for the determination of total extractable protein in the capsule formulations. The approach may be evaluated for all strengths. In brief, capsule contents may be emptied, weighed, and analyzed by RP-HPLC. Chromatographic analysis of peanut flour samples extracted using this procedure produce a chromatographic "fingerprint" that is unique to peanut flour extracts. The region of the samples that elute between approximately 12 minutes and 35 minutes may be integrated. The total area integrated may be quantitated against a BSA standard. The total extractable protein content may then calculated using the following equation.

$$\text{Mg/g protein} = \frac{R_u}{R_s} \times C_{STD} \times \frac{V_{Sample}}{Wt_{Sample}}$$

where:

R_u = Total Ara h Protein Peak Area *or* Ara h Species Peak Area in the Working Sample;

R_s = Average BSA Peak Area in all Working Standards *CSTD* = BSA Working Standard Concentration (mg/mL);

V_{Sample} = Total Diluent Volume of the Working Sample (10.0 mL); and

Wt_{Sample} = Weight of peanut flour sample (g).

Apparent Ara h1, Ara h2 and Ara h6 Protein Ratios

[00106] Chromatographic analysis of samples extracted using the RP-HPLC method may produce a chromatographic "fingerprint" that is unique to peanut flour extracts, and relative ratios of regions corresponding to Ara h1, Ara h2, and Ara h6 (see, *e.g.*, **Figure 1**). The protein content of each of these regions (mg/g) may be quantitated according to the equation provided above. Relative percent content of total protein for each region is then calculated according to the equation below.

$$\text{Ara h\%} = \frac{\textit{Ara h PeakArea}}{\textit{Total / protein PeakArea}} \times 100$$

Protein Content

[00107] Protein content in filled capsules may be determined in the same manner as that of the peanut flour (AOCS Official Method Ba 4e-93). Since the accurate protein content determinations may be dependent on the nitrogen content of the sample, no excipients containing nitrogen may be used in the formulation. The method is based on the Dumas method and is based on the combustion of the crude protein in pure oxygen, and measurement of the nitrogen gas that is evolved. The method that may be used may be AOCS Official Method Ba 4e-93. The AOCS Method Definition and Scope are provided below.

[00108] Briefly, this method describes a generic combustion method for the determination of crude protein. Combustion at high temperature in pure oxygen frees nitrogen, which is measured by thermal conductivity detection and then converted to equivalent protein by an appropriate numerical factor. This is an alternative method to the mercury catalyst Kjeldahl method and has two advantages: 1) less time is needed for nitrogen determination, and 2) hazardous and toxic chemicals are not utilized.

Stability Testing

[00109] Formulations may be stored at 2-8°C. To assess accelerated and long-term stability, formulations may be tested according to the frequency and specifications described in **Table 11 and Table 12.** Testing for appearance/color, moisture, identity and strength may be performed at all timepoints, and the bioburden may be performed annually at 12, 24, and 36 months.

Table 11A: Stability Protocol Testing Scheme for a Formulation

Temperature	**5°C +/-3°C**	**25°C +/-2°C 60%RH**
Testing Frequency	1,3,6,9,12,18,24,36 months	1,3,6,9,12,18,24,36 months

[00110] Tables 11B – 11F provide data obtained by testing stability of various formulations at 5°C.

Table 11B

Stability Condition: 5°C Characterized Peanut Allergen, 475 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	10.18	8.5	9.67	9.31
		Report Area % Ara h2	9.48	9.89	10.88	8.93
		Report Area % Ara h6	5.89	5.16	5.32	4.21
		Report the ratio of Ara h2/h6	1.61	1.92	2.05	2.12

Table 11C

Stability Condition: 5°C Characterized Peanut Allergen, 100 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	7.97	10.33	10.51	9.64
		Report Area % Ara h2	8.81	8.78	9.01	8
		Report Area % Ara h6	4.17	3.92	4.27	3.61
		Report the ratio of Ara h2/h6	2.11	2.24	2.11	2.22

Table 11D

Stability Condition: 5°C Characterized Peanut Allergen, 10 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	6.66	7.71	9.36	7.11
		Report Area % Ara h2	10.95	9.75	9.54	10.16
		Report Area % Ara h6	5.93	5.8	5.55	5.51
		Report the ratio of Ara h2/h6	1.85	1.68	1.72	1.84

Table 11E

Stability Condition: 5°C Characterized Peanut Allergen, 1.0 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	7.35	7.43	8.54	7.65
		Report Area % Ara h2	16.11	14.39	12.31	12.94
		Report Area % Ara h6	7.14	6.56	5.77	6.36
		Report the ratio of Ara h2/h6	2.26	2.19	2.13	2.03

Table 11F

Stability Condition: 5°C Characterized Peanut Allergen, 0.5 mg Capsule							
Specifications			Stability Intervals				
Test	Method	Acceptance Criteria	Initial	1 Month	3 Month	6 Month	9 Month
Identification (HPLC)	TM-074	Report Area % Ara h1	7.12	8.25	8.3	8	6.09
		Report Area % Ara h2	19.37	14.76	15.26	16.15	20.78
		Report Area % Ara h6	8.77	8.69	8.9	8.8	10.38
		Report the ratio of Ara h2/h6	2.21	1.7	1.71	1.84	2.00

[00111] Tables 11G – 11K provide data obtained by testing stability of various formulations at 25°C.

Table 11G

Stability Condition: 25°C Characterized Peanut Allergen, 475 mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Month	3 Month	6 Month
Identification (HPLC)	TM-074	Report Area % Ara h1	10.18	8.26	10.1	9.93
		Report Area % Ara h2	9.48	9.86	10.48	9.77
		Report Area % Ara h6	5.89	5.09	5.25	4.41
		Report the ratio of Ara h2/h6	1.61	1.94	2	2.22

Table 11H

Stability Condition: 25°C Characterized Peanut Allergen, 100 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	7.97	9.92	10.42	9.75
		Report Area % Ara h2	8.81	8.32	9.4	8.04
		Report Area % Ara h6	4.17	4.18	4.28	3.6
		Report the ratio of Ara h2/h6	2.11	1.99	2.2	2.23

Table 11I

Stability Condition: 25°C Characterized Peanut Allergen, 10 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	6.66	7.99	9.47	7.26
		Report Area % Ara h2	10.95	10.77	10.23	10.11
		Report Area % Ara h6	5.93	5.81	4.99	5.83
		Report the ratio of Ara h2/h6	1.85	1.85	2.05	1.73

Table 11J

Stability Condition: 25°C Characterized Peanut Allergen, 1.0 mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	7.35	7.63	8.24	7.74
		Report Area % Ara h2	16.11	12.59	12.97	12.89
		Report Area % Ara h6	7.14	6.55	5.81	6.05
		Report the ratio of Ara h2/h6	2.26	1.92	2.23	2.13

Table 11K

Stability Condition: 25°C Characterized Peanut Allergen, 0.5 mg Capsule							
Specifications			Stability Intervals				
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Month**	**3 Month**	**6 Month**	**9 Month**
Identification (HPLC)	TM-074	Report Area % Ara h1	7.12	8.22	7.95	7.83	6.16
		Report Area % Ara h2	19.37	9.49	16.3	16.28	20.92
		Report Area % Ara h6	8.77	15	8.76	8.2	9.47
		Report the ratio of Ara h2/h6	2.21	1.58	1.86	1.99	2.21

Table 12A: Stability Protocol Specifications for a Formulation

Attribute		Method	Acceptance Criteria
General	Appearance Powder/color	Visual	TBD
	Capsule Integrity	Visual	Intact capsules with no visible signs of cracking. Capsules open easily without breaking
	Moisture	Loss on Drying (LOD) USP <921>	Report Results
Identity	Presence of Ara h1, Ara h2 and Ara h6 proteins	Reverse Phase HPLC	Comparable to reference chromatogram and report percent area of Ara h1, Ara h2 and Ara h6
Strength (Assay)	Protein Content	Nitrogen Content by AOCS Combustion Method for Determination of Crude Protein (AOCS Official Method Ba 4e-93)	Low doses (0.5 and 1 mg): Target protein concentration ± 15% High doses (10 and 100 mg): Target protein concentration ± 10%
Safety	Bioburden*	Microbiological Limits USP <61> Microbial Enumeration USP <62> Specified Microorganisms	Total Aerobic Microbial Count: NMT 1000 CFU/g Total Yeasts & Molds Count: NMT 100 CFU/g *E. coli*: Absent *S. aureus*: Absent *P. aeruginosa*: Absent *Salmonella* species: Absent

*Bioburden may be measured at release and annually.

[00112] Tables 12B – 12K provide data obtained by assessing stability and characteristics of various formulations at 5°C and 25°C at various time points.

Table 12B

Stability Conditions: 5°C; 0.5 mg capsule							
Specifications			Stability Intervals				
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**	**9 Mo**
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 99%; RSD: 0.4%	Average: 99%; RSD: 0.5%	Average: 100%; RSD: 0.3%	Average: 99%; RSD: 0.1%	Average: 99%; RSD: 0.6%
Assay	TM-085	Target protein concentration ±15%	91%	88%	92%	101%	88%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Comparable	Comparable	Comparable	Comparable	Comparable
		Report Area % Ara h1	7.12	8.25	8.3	8	6.09
		Report Area % Ara h2	19.37	14.76	15.26	16.15	20.78
		Report Area % Ara h6	8.77	8.69	8.9	8.8	10.38
		Report the ratio of Ara h2/h6	2.21	1.7	1.71	1.84	2.00
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	3.83 %	4.00%	4.50%	6.40%	5.40%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species	Meets Acceptance Criteria	NA	NA	NA	NA

Stability Conditions: 5°C; 0.5 mg capsule							
Specifications			Stability Intervals				
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo	9 Mo
		are absent					

Table 12C

Stability Condition: 25°C/60%RH; 0.5 mg capsule							
Specifications			Stability Intervals				
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo	9 Mo
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 99% RSD: 0.4%	Average: 100% RSD: 0.5%	Average: 99% RSD: 0.4%	Average: 100% RSD: 0.3%	Average: 99% RSD: 0.7%
Assay	TM-085	Target protein concentration ±15%; (85-115% label claim)	91%	90%	90%	98%	82%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Comparable	Comparable	Comparable	Comparable	Comparable
		Report Area % Ara h1	7.12	8.22	7.95	7.83	6.16
		Report Area % Ara h2	19.37	9.49	16.3	16.28	20.92
		Report Area % Ara h6	8.77	15	8.76	8.2	9.47
		Report the ratio of Ara h2/h6	2.21	1.58	1.86	1.99	2.21
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	3.83 %	3.70%	4.20%	4.10%	4.60%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S.*	Meets Acceptance Criter	NA	NA	NA	NA

Stability Condition: 25°C/60%RH; 0.5 mg capsule							
Specifications			Stability Intervals				
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo	9 Mo
		aureus, *P. aeruginosa* and Salmonella species are absent	ia				

Table 12D

Stability Condition: 5°C: Characterized Peanut Allergen, 1.0 mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.3%	Average: 100%; RSD: 0.4%	Average: 99%; RSD: 0.6%	Average: 99%; RSD: 0.3%
Assay	TM-085	Target protein concentration ±15%; (85-115% label claim)	101%	90%	86%	94%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Comparable	Comparable	Comparable	Comparable
		Report Area % Ara h1	7.35	7.43	8.54	7.65
		Report Area % Ara h2	16.11	14.39	12.31	12.94
		Report Area % Ara h6	7.14	6.56	5.77	6.36
		Report the ratio of Ara h2/h6	2.26	2.19	2.13	2.03

Stability Condition: 5°C: Characterized Peanut Allergen, 1.0 mg Capsule						
Specifications			**Stability Intervals**			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	5.02%	5.20%	5.70%	6.20%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent	Meets Acceptance Criteria	NA	NA	NA

Table 12E

Stability Condition: 25°C/60%RH; Characterized Peanut Allergen, 1.0mg Capsule						
Specifications			**Stability Intervals**			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD:	Average: 100%; RSD:	Average: 99%; RSD:	Average: 100%; RSD:

Stability Condition: 25°C/60%RH; Characterized Peanut Allergen, 1.0mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
			0.3%	0.3%	0.2%	0.3%
Assay	TM-085	Target protein concentration ±15%; (85-115% label claim)	101%	90%	87%	94%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Comparable	Comparable	Comparable	Comparable
		Report Area % Ara h1	7.35	7.63	8.24	7.74
		Report Area % Ara h2	16.11	12.59	12.97	12.89
		Report Area % Ara h6	7.14	6.55	5.81	6.05
		Report the ratio of Ara h2/h6	2.26	1.92	2.23	2.13
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	5.02%	5.00%	5.80%	6.10%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent	Meets Acceptance Criteria	NA	NA	NA

Table 12F

Stability Condition: 5°C; Characterized Peanut Allergen, 10mg Capsule						
Specifications			**Stability Intervals**			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.2%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%
Assay	TM-085	Target protein concentration ±10% (90-110% label claim)	95%	93%	96%	98%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Comparable	Comparable	Comparable	Comparable
		Report Area % Ara h1	6.66	7.71	9.36	7.11
		Report Area % Ara h2	10.95	9.75	9.54	10.16
		Report Area % Ara h6	5.93	5.8	5.55	5.51
		Report the ratio of Ara h2/h6	1.85	1.68	1.72	1.84
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	4.90%	5.60%	5.40%	5.50%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratorie	Total Aerobic Microbial Count: NMT 1000 cfu/g;	Meets Acceptance Criteria	NA	NA	NA

Stability Condition: 5°C; Characterized Peanut Allergen, 10mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
	s	Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent				

Table 12G

Stability Condition: 25°C/60%RH; Characterized Peanut Allergen, 10mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.2%	Average: 100%; RSD: 0.2%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%
Assay	TM-085	Target protein concentration ±10% (90-110% label claim)	95%	93%	93%	97%
Identification (HPLC)	TM-074	Comparable to reference	Comparabl	Comparabl	Comparabl	Comparabl

Stability Condition: 25°C/60%RH; Characterized Peanut Allergen, 10mg Capsule						
Specifications			Stability Intervals			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
		chromatogram	e	e	e	e
		Report Area % Ara h1	6.66	7.99	9.47	7.26
		Report Area % Ara h2	10.95	10.77	10.23	10.11
		Report Area % Ara h6	5.93	5.81	4.99	5.83
		Report the ratio of Ara h2/h6	1.85	1.85	2.05	1.73
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	4.90%	4.60%	5.20%	5.20%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent	Meets Acceptance Criteria	NA	NA	NA

Table 12H

Stability Condition: 5°C; Characterized Peanut Allergen, 100mg Capsule						
Specifications			**Stability Intervals**			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.2%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%
Assay	TM-085	Target protein concentration ±10% (90-110% label claim)	99%	95%	99%	99%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Comparable	Comparable	Comparable	Comparable
		Report Area % Ara h1	7.97	10.33	10.51	9.64
		Report Area % Ara h2	8.81	8.78	9.01	8
		Report Area % Ara h6	4.17	3.92	4.27	3.61
		Report the ratio of Ara h2/h6	2.11	2.24	2.11	2.22
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	4.02%	3.70%	4.40%	4.40%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratorie	Total Aerobic Microbial Count: NMT 1000 cfu/g;	Meets Acceptance Criteria	NA	NA	NA

Stability Condition: 5°C; Characterized Peanut Allergen, 100mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
	s	Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent				

Table 12I

Stability Condition: 25°C; Characterized Peanut Allergen, 100mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.4%
Assay	TM-085	Target protein concentration ±10% (90-110% label claim)	99%	96%	98%	97%
Identification (HPLC)	TM-074	Comparable to reference	Comparabl	Comparabl	Comparabl	Comparabl

Stability Condition: 25°C; Characterized Peanut Allergen, 100mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
		chromatogram	e	e	e	e
		Report Area % Ara h1	7.97	9.92	10.42	9.75
		Report Area % Ara h2	8.81	8.32	9.4	8.04
		Report Area % Ara h6	4.17	4.18	4.28	3.6
		Report the ratio of Ara h2/h6	2.11	1.99	2.2	2.23
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	4.02%	4.00%	4.40%	4.80%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent	Meets Acceptance Criteria	NA	NA	NA

Table 12J

Stability Condition: 5°C; Characterized Peanut Allergen, 475mg Capsule						
Specifications			**Stability Intervals**			
Test	**Method**	**Acceptance Criteria**	**Initial**	**1 Mo**	**3 Mo**	**6 Mo**
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.0%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.0%	Average: 100%; RSD: 0.1%
Assay	TM-085	Target protein concentration ±10% (90-110% label claim)	90%	94%	96%	96%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Compar-able	Compar-able	Compar-able	Compar-able
		Report Area % Ara h1	10.18	8.5	9.67	9.31
		Report Area % Ara h2	9.48	9.89	10.88	8.93
		Report Area % Ara h6	5.89	5.16	5.32	4.21
		Report the ratio of Ara h2/h6	1.61	1.92	2.05	2.12
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	4.00%	3.60%	3.70%	3.90%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100	Meets Acceptance Criteria	NA	NA	NA

Stability Condition: 5°C; Characterized Peanut Allergen, 475mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
		cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent				

Table 12K

Stability Condition: 25°C; Characterized Peanut Allergen, 475mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
Appearance (n=10)	Visual	White opaque capsule containing white to off-white fine granular powder*	Conforms	Conforms	Conforms	Conforms
Deliverable Mass	TM-086	>95%*	Average: 100%; RSD: 0.0%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%	Average: 100%; RSD: 0.1%
Assay	TM-085	Target protein concentration ±10% (90-110% label claim)	90%	95%	96%	95%
Identification (HPLC)	TM-074	Comparable to reference chromatogram	Compar-able	Compar-able	Compar-able	Compar-able
		Report Area % Ara h1	10.18	8.26	10.1	9.93
		Report Area % Ara h2	9.48	9.86	10.48	9.77
		Report Area % Ara h6	5.89	5.09	5.25	4.41
		Report the	1.61	1.94	2	2.22

Stability Condition: 25°C; Characterized Peanut Allergen, 475mg Capsule						
Specifications			Stability Intervals			
Test	Method	Acceptance Criteria	Initial	1 Mo	3 Mo	6 Mo
		ratio of Ara h2/h6				
Loss on Drying (@130°C for 2 hours)	USP <731>	Report Results	4.00%	3.80%	4.10%	4.50%
Microbial Limits / Specified Microorganisms	USP <61> and <62> Quality Chemical Laboratories	Total Aerobic Microbial Count: NMT 1000 cfu/g; Total Combined Yeasts & Molds Count: NMT 100 cfu/g; *E.coli*, *S. aureus*, *P. aeruginosa* and Salmonella species are absent	Meets Acceptance Criteria	NA	NA	NA

Placebo

[00113] Placebo may consist of the defined mixture of excipients without the PF. Placebo may be filled in the same color-coded capsules as the active formulation.

Table 13: Placebo Release Specification

Attribute		Method	Acceptance Criteria
General	Appearance Powder/color	Visual	TBD
	Capsule Integrity	Visual	Intact capsules with no visible signs of cracking. Capsules open easily without breaking
	Content Uniformity	USP <905>	Meets USP <905> requirements
	Deliverable Mass	% Weight Delivered	Report results

Attribute		Method	Acceptance Criteria
	Moisture	Loss on Drying (LOD) USP <921>	Report Results
Identity	Absence of Ara h1, Ara h2 and Ara h6 proteins	Reverse Phase HPLC	No peaks detected in the elution region of PF
Strength (Assay)	Protein Content	Nitrogen Content by AOCS Combustion Method for Determination of Crude Protein (AOCS Official Method Ba 4e-93)	No protein detected
Safety	Bioburden	Microbiological Limits USP <61> Microbial Enumeration USP <62> Specified Microorganisms	Total Aerobic Microbial Count: NMT 1000 CFU/g Total Yeasts & Molds Count: NMT 100 CFU/g *E. coli*: Absent *S. aureus*: Absent *P. aeruginosa*: Absent *Salmonella* species: Absent

Methods of Use

[00114] The pharmaceutical compositions prepared using the methods described herein may be used to compare various lots of peanut proteins for consistency of product.

[00115] Peanuts and peanut flour are common foods and additives found in many food products. The intended clinical use for Characterized Peanut Allergen (CPA) is found in relatively small quantities (0.5 to 4000 mg/dose) compared to quantities contained in food and will be delivered via the same route as orally ingested peanut-containing products.

[00116] Currently, preclinical studies exploring treatment modalities in food allergy animal models are limited. The principle model for induction of peanut allergy in mice is to expose mice by oral gavage to peanut proteins in the form of peanut butter, ground roasted peanuts, or purified peanut proteins, in combination with cholera toxin. After 3 to 6 weekly exposures the mice are challenged to demonstrate an allergic response. Mice may be challenged by intraperitoneal injection with sub-lethal doses of with a formulation described herein and scored for reaction

severity. The intent is to demonstrate that the principle elicitors of anaphylaxis are specific Ara h proteins, rather than a combination of all peanut proteins. In an immunotherapy protocol, mice are treated with whole peanut extract, extract depleted of Ara h proteins, or with purified Ara h proteins alone. Upon challenge post treatment, changes in body temperature, symptom score and mouse mast cell protease-1 release mice may be assessed. Mice that are desensitized to further challenge may be treated with an entire extract or the Ara h protein combination.

[00117] The cellular requirements underlying peanut induced anaphylaxis may be determined explored in wild-type C57BL/6, B-cell deficient, CD40L-deficient, mast cell deficient or FcεRI ε-chain-deficient mice sensitized to peanut proteins. After intraperitoneal challenge with a formulation described herein, anaphylaxis is assessed by measurement of antigen-specific immunoglobulins (Igs), overall symptom score, body temperature, vascular permeability, mast cell mediator release and anaphylactic reactions. The B-cell, mast cell and CD40L deficient mice may be sensitized to peanut proteins as shown by production of IgE, and Th2-associated cytokines. The FcεRI ε-deficient mice may experience anaphylaxis albeit somewhat less severe than the wild-type animals.

[00118] In a model of esophago-gastro-enteropathy induced by long term feeding of peanuts to sensitized mice described by Mondoulet *et al.*, 2012, epicutaneous immunotherapy with a formulation described herein may lessen the severity of gastro-intestinal lesions. (Mondoulet *et al.*, 2012).

[00119] Data obtained from these models, which may demonstrate one or more of the hallmarks of human food allergic reactions, and are to be considered with respect to variability of human food allergy.

[00120] Provided herein is a method of identifying a composition for treatment for desensitization of peanut allergy in a subject, comprising: (a) determining the concentrations of Ara h1, Ara h2 and Ara h6 in a composition of peanut flour by RP-HPLC; (b) comparing the concentrations to the concentrations of a reference standard; and (c) identifying a composition for desensitization of peanut allergy in a subject, wherein the sample contains at least the concentrations of Ara h1, Ara h2 and Ara h6 of the reference standard.

[00121] The method may, in some instances, further comprise administering a composition described herein to a subject, wherein the composition comprises at least the concentrations of Ara h1, Ara h2 and Ara h6 of the reference standard.

[00122] The method may be used to compare lots of peanut flour and, in some instances, exclude peanut flour from use in a composition or method described herein where the sample does not contain at least the reference standard amount of Ara h1, Ara h2 and Ara h6.

[00123] While preferred embodiments have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may now occur to those skilled in the art without departing from the embodiments. It should be understood that various alternatives to the embodiments described herein may be employed in practicing the embodiments. It is intended that the following claims define the scope of the embodiments and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of making a low dose capsule formulation of peanut flour comprising characterized peanut proteins, comprising:

(a) mixing peanut flour and diluent in a first blend;

(b) adding about 45% of diluent in a second blend;

(c) adding remaining diluent in a third blend;

(d) adding a glidant and/or lubricant in a final blend; and

(e) encapsulating blended powder in a capsule.

2. The method of claim 1, wherein the diluent of step (a) comprises starch.

3. The method of claim 1, wherein the diluent of step (b) and/or (c) comprises starch, lactose, microcrystalline cellulose (Avicel®), or dicalcium phosphate.

4. The method of claim 1, wherein the glidant of step (d) is selected from the group consisting of colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), and combinations thereof.

5. The method of claim 1, wherein said glidant comprises Cab-O-Sil.

6. The method of claim 1, wherein the lubricant of step (d) comprises magnesium stearate.

7. The method of claim 1, wherein step (d) comprises adding a glidant or a lubricant.

8. The method of claim 1, wherein step (d) comprises adding a glidant and a lubricant.

9. The method of claim 1, further comprising sampling the blended mixture prior to encapsulation.

10. The method of claim 1, wherein the dose comprises about 0.5 or about 1.0 mg peanut protein.

11. The method of claim 1, wherein step (d) further comprises passing the blended material through a mesh screen.

12. Provided herein is a method of making a capsule formulation, comprising,

(a) mixing peanut flour and diluent in a first blend;

(b) discharging the blended material;

(c) passing the blended material through a mesh screen and blending the screened material in a second blend;

(d) adding in a glidant and/or lubricant in a third blend; and

(e) encapsulating the blended powder.

13. The method of claim 12, further comprising optionally sampling the blended material of step (d) one or more times prior to encapsulation.

14. The method of claim 12, wherein the dose comprises about 10, about 100, or about 475 mg peanut protein.

15. The method of claim 12, wherein the diluent of step (a) comprises starch, lactose, microcrystalline cellulose (Avicel®), or dicalcium phosphate.

16. The method of claim 12, wherein the mesh screen of step (c) comprises a # 20 mesh screen.

17. The method of claim 12, wherein the glidant of step (d) is selected from the group consisting of colloidal silicon dioxide (Cab-O-Sil), talc (*e.g.*, Ultra Talc 4000), and combinations thereof.

18. The method of claim 12, wherein said glidant comprises Cab-O-Sil.

19. The method of claim 12, wherein the lubricant of step (d) comprises magnesium stearate.

20. The method of claim 12, wherein step (d) comprises adding a glidant or a lubricant.

21. The method of claim 12, wherein step (d) comprises adding a glidant and a lubricant.

22. Provided herein is a method of making a capsule formulation useful in the methods provided here, comprising,

(a) mixing peanut flour, diluent, glidant and/or lubricant;

(b) discharging the blended material;

(c) passing the blended material through a mesh screen; and

(d) encapsulating the blended powder.

23. The method of claim 22, wherein the dose comprises about 10 or about 100 mg peanut protein.

24. The method of any one of claims 1-23, wherein the peanut flour comprises characterized peanut proteins.

25. The method of claim 24, wherein the peanut proteins comprise Ara h1, Ara h2 and Ara h6.

26. The method of claim 25, wherein the concentration of Ara h1, Ara h2 and Ara h6 is characterized by RP-HPLC.

27. The method of claim 26, wherein the concentration of Ara h1, Ara h2 and Ara h6 is at least an amount of a reference standard.

28. The method of any one of claims 1-27, wherein the encapsulated formulation is stable for at least about 3, 6, 9, 12, 18, 24, 36 or more months.

29. The method of any one of claims 1-28, wherein the encapsulated formulation is stable at a temperature from about 2°C to about 8°C or from about 20°C to about 30°C.

30. The method of claim 29, wherein the encapsulated formulation is stable at a temperature of about 25°C.

31. The method of any one of claims 1-30, wherein the capsule size is 3, 00 or 000.

32. The method of any one of claims 1-31, wherein the capsule comprises Hydroxypropyl Methyl Cellulose (HPMC).

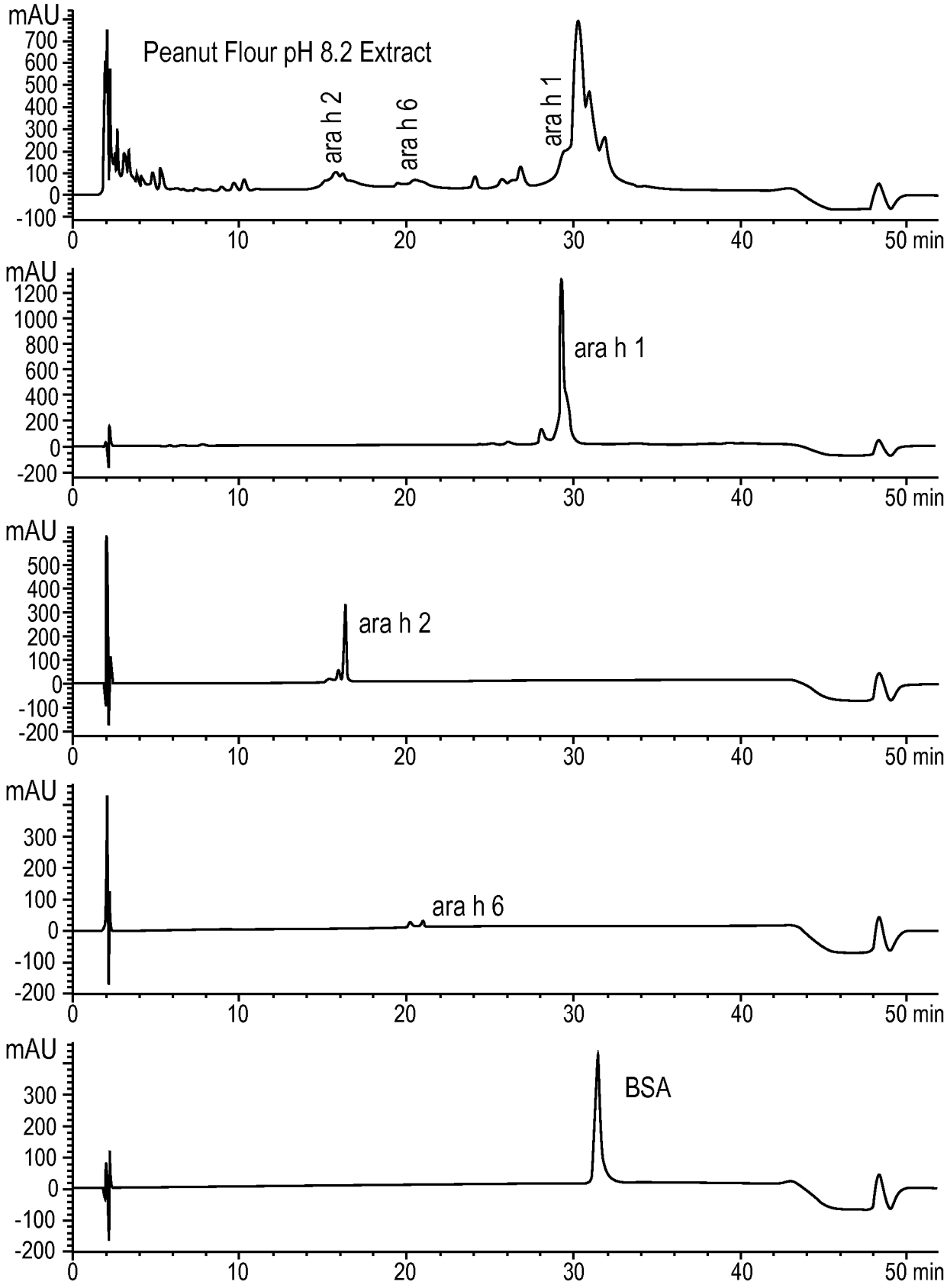
33. The method of any one of claims 1-32, further comprising storing the formulation in a container means.

34. The method of claim 33, wherein the container means is a bottle.

35. The method of claim 34, wherein the bottle is an amber-colored bottle.

36. The method of claim 33, wherein the container means further comprises a dessicant packet to control moisture content of the container means.

FIGURE 1


mAU
Peanut Flour pH 8.2 Extract
ara h 2
ara h 6
ara h 1
50 min
mAU
ara h 1
50 min
mAU
ara h 2
50 min
mAU
ara h 6
50 min
mAU
BSA
50 min

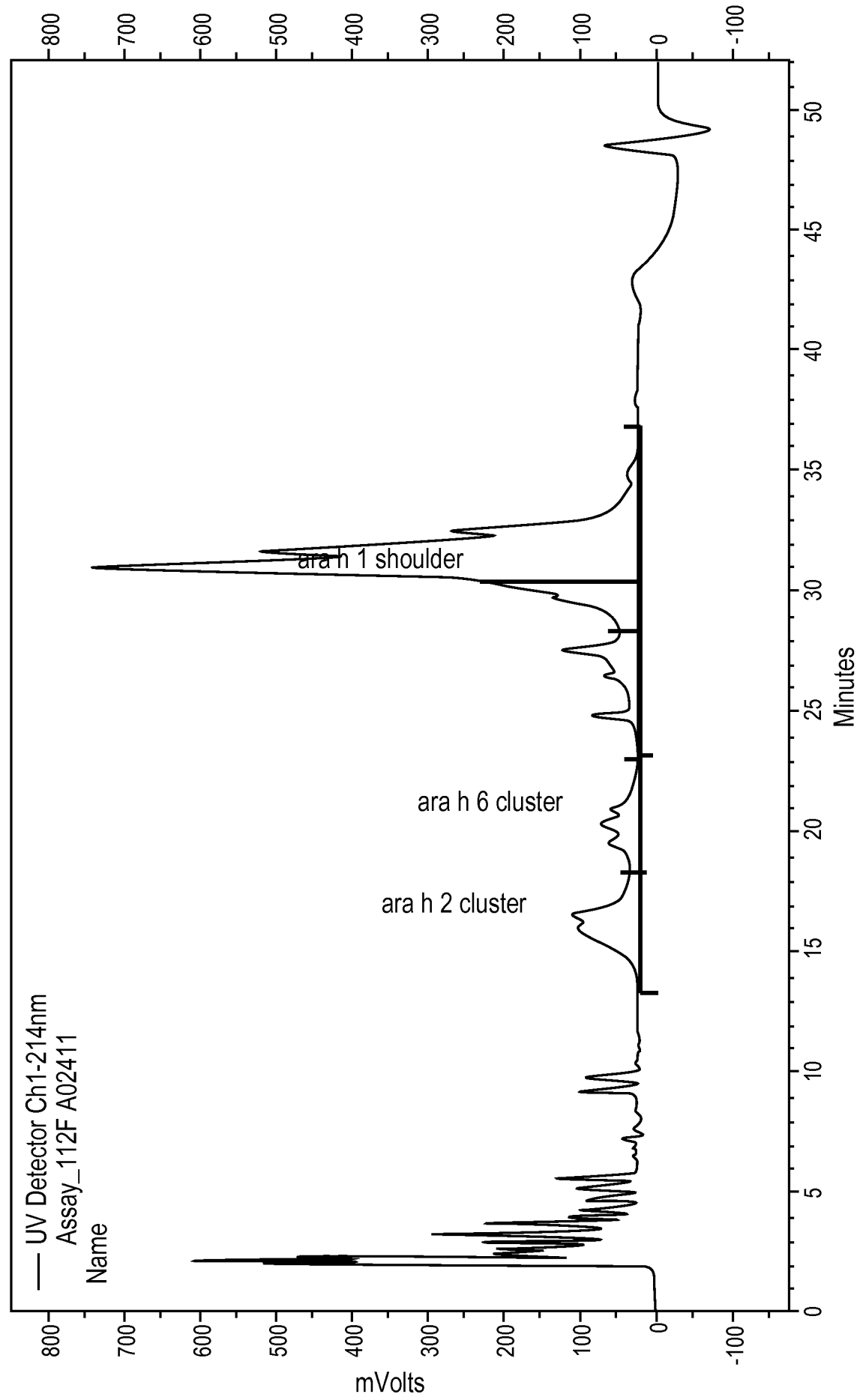

UV Detector Ch1-214nm
Assay_112F A02411
Name
ara h 1 shoulder
ara h 6 cluster
ara h 2 cluster
mVolts
Minutes
800
700
600
500
400
300
200
100
0
-100
0
5
10
15
20
25
30
35
40
45
50

FIGURE 2

FIGURE 3

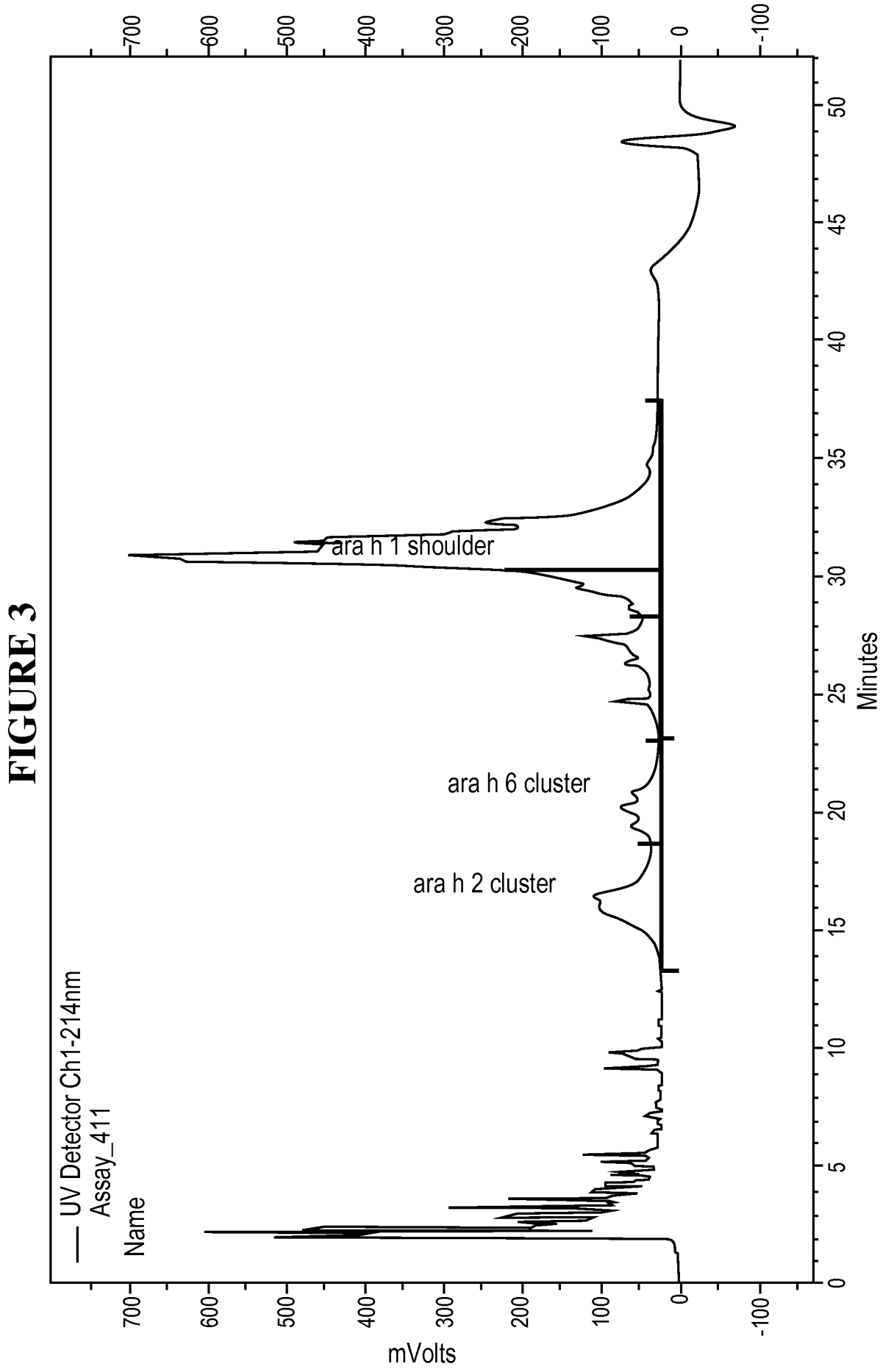

UV Detector Ch1-214nm
Assay_411
Name
ara h 1 shoulder
ara h 6 cluster
ara h 2 cluster
mVolts
Minutes
700
600
500
400
300
200
100
0
-100
0
5
10
15
20
25
30
35
40
45
50

FIGURE 4

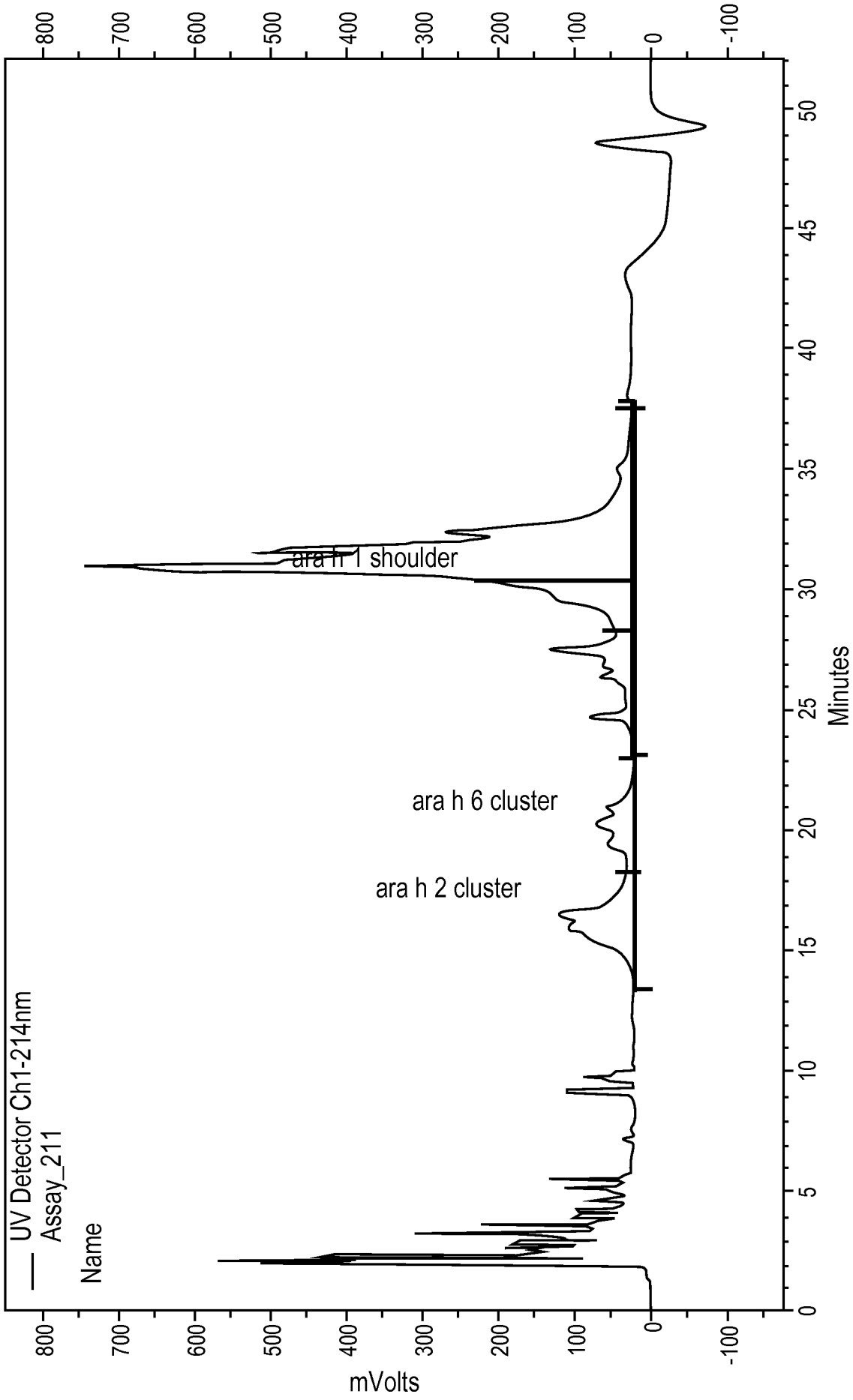
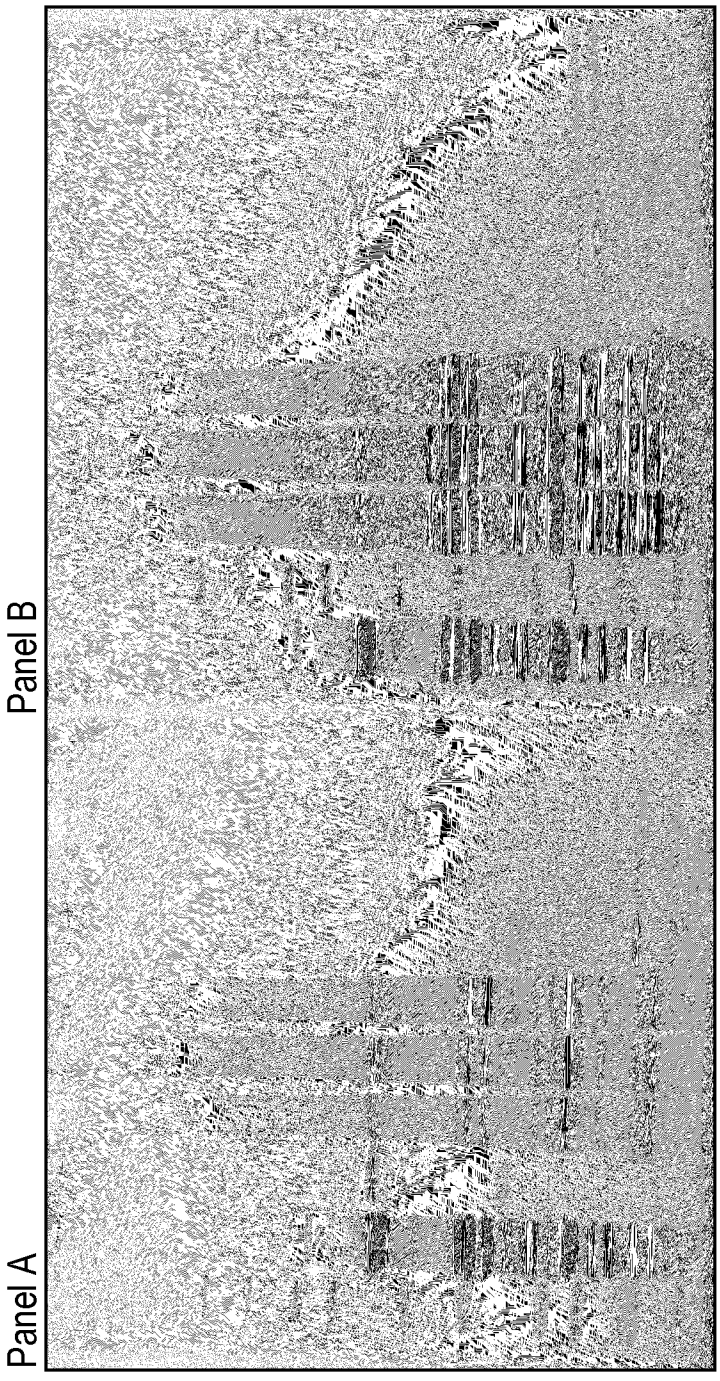

UV Detector Ch1-214nm
Assay_211
Name
ara h 1 shoulder
ara h 6 cluster
ara h 2 cluster
mVolts
Minutes
800
700
600
500
400
300
200
100
0
-100
0
5
10
15
20
25
30
35
40
45
50

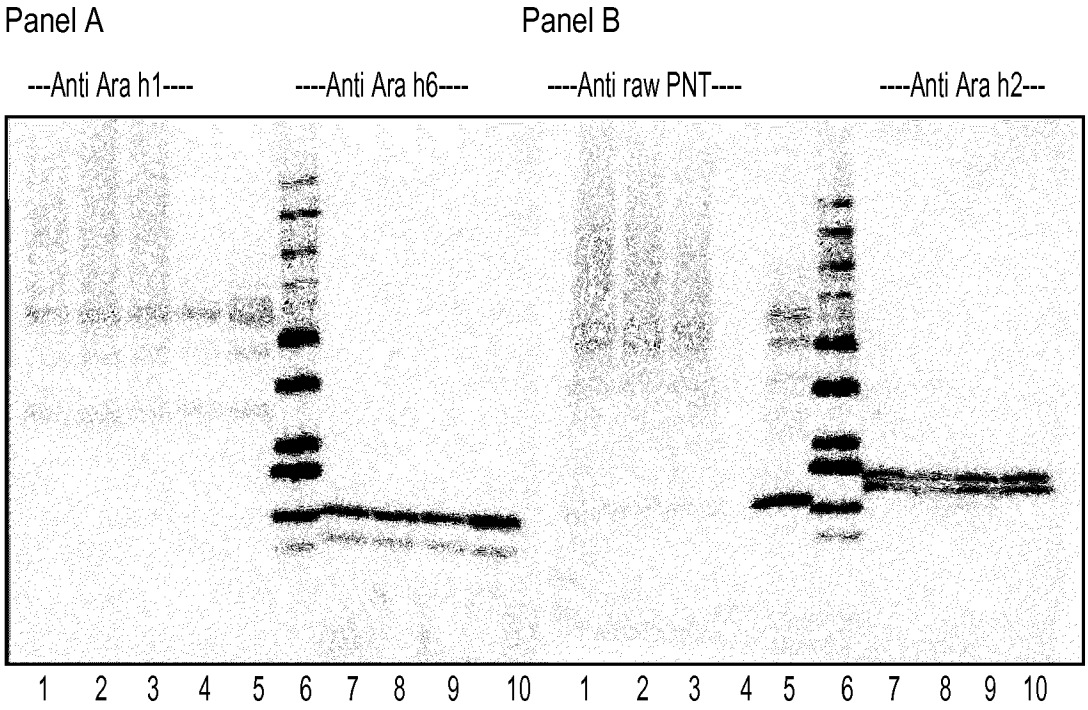
FIGURE 5



Lanes – Panel A
1 = Markers
2 = Total extract
3 = Ara h1 control
4 = Ara h1 5C
5 = Ara h1 40C
6 = Ara h1 60C
7 = Ara h6 control
8 = Ara h6 5C
9 = Ara he 40C
10 = Ara h6 60C

Lanes – Panel B
1 = Total Extract
2 = Markers
3 = Pooled Ara h 5C
4 = Pooled Ara h 40C
5 = Pooled Ara h60c
6 = Empty
7 = Ara h2 control
8 = Ara h2 5C
9 = Ara h2 40C
10 = Ara h2 60C

FIGURE 6



Lanes – Panel A

1 = Ara h1 5C
2 = Ara h1 40C
3 = Ara h1 60C
4 = Ara h1 control
5 = Total extract
6 = Markers
7 = Ara h6 5C
8 = Ara h6 40C
9 = Ara he 60C
10 = Ara h6 control

Lanes – Panel B

1 = Pooled Ara 5C
2 = Pooled Ara 40C
3 = Pooled Ara 60C
4 = Empty
5 = total extract
6 = Markers
7 = Ara h2 5C
8 = Ara h2 40C
9 = Ara h2 60C
10 = Ara h2 control

FIGURE 7

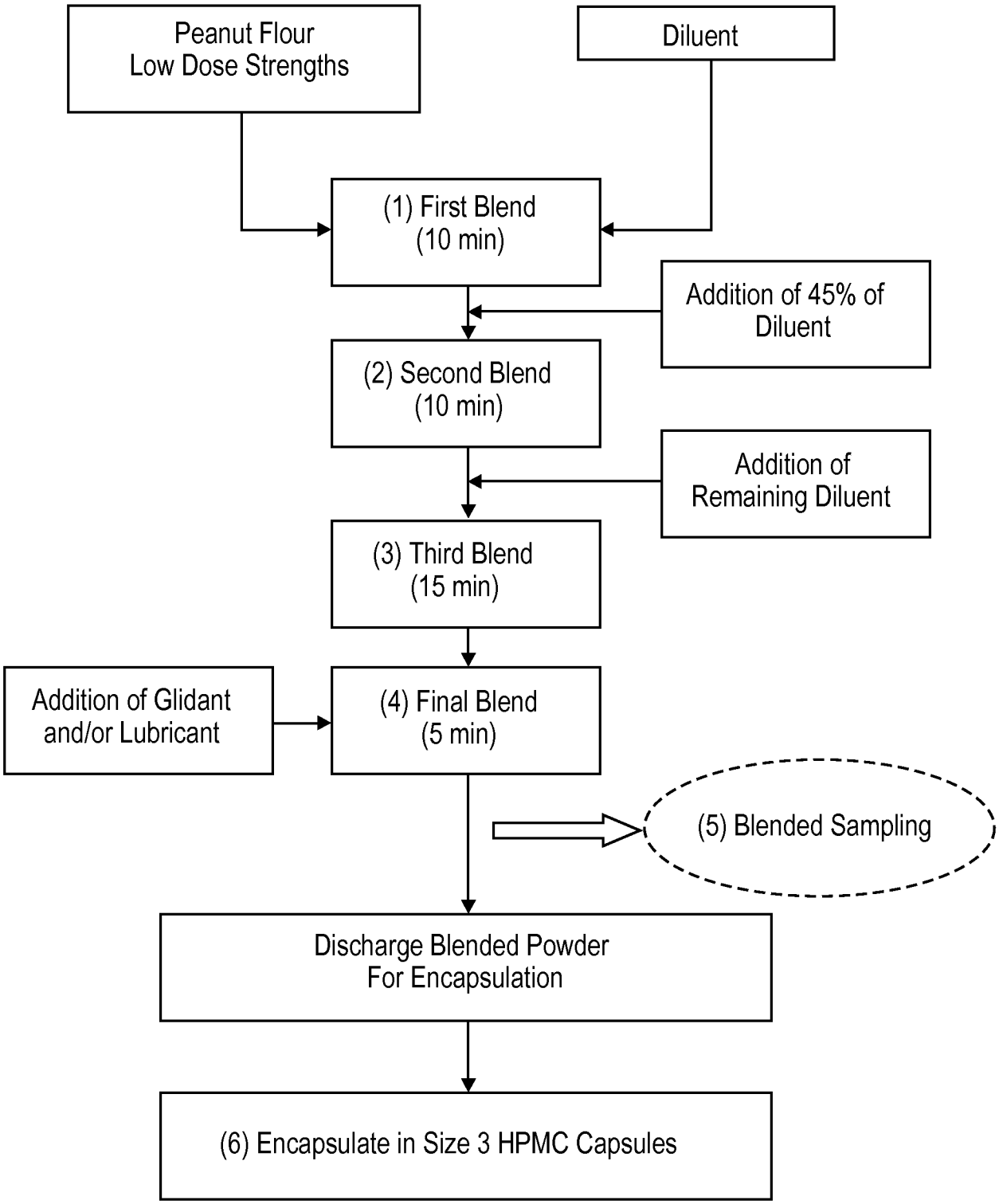
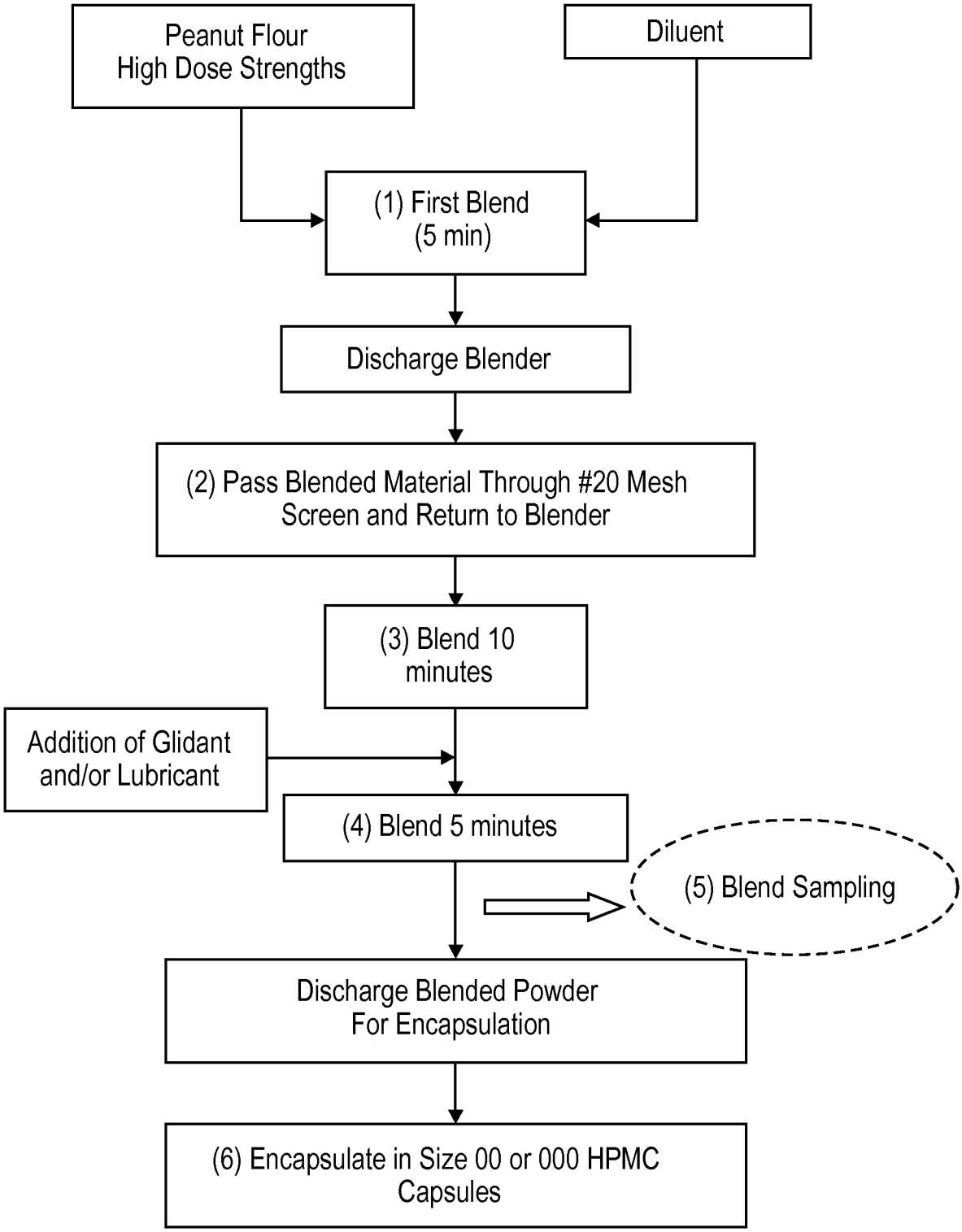

Peanut Flour
Low Dose Strengths
Diluent
(1) First Blend
(10 min)
Addition of 45% of
Diluent
(2) Second Blend
(10 min)
Addition of
Remaining Diluent
(3) Third Blend
(15 min)
Addition of Glidant
and/or Lubricant
(4) Final Blend
(5 min)
(5) Blended Sampling
Discharge Blended Powder
For Encapsulation
(6) Encapsulate in Size 3 HPMC Capsules

FIGURE 8


Peanut Flour
High Dose Strengths
Diluent
(1) First Blend
(5 min)
Discharge Blender
(2) Pass Blended Material Through #20 Mesh
Screen and Return to Blender
(3) Blend 10
minutes
Addition of Glidant
and/or Lubricant
(4) Blend 5 minutes
(5) Blend Sampling
Discharge Blended Powder
For Encapsulation
(6) Encapsulate in Size 00 or 000 HPMC
Capsules

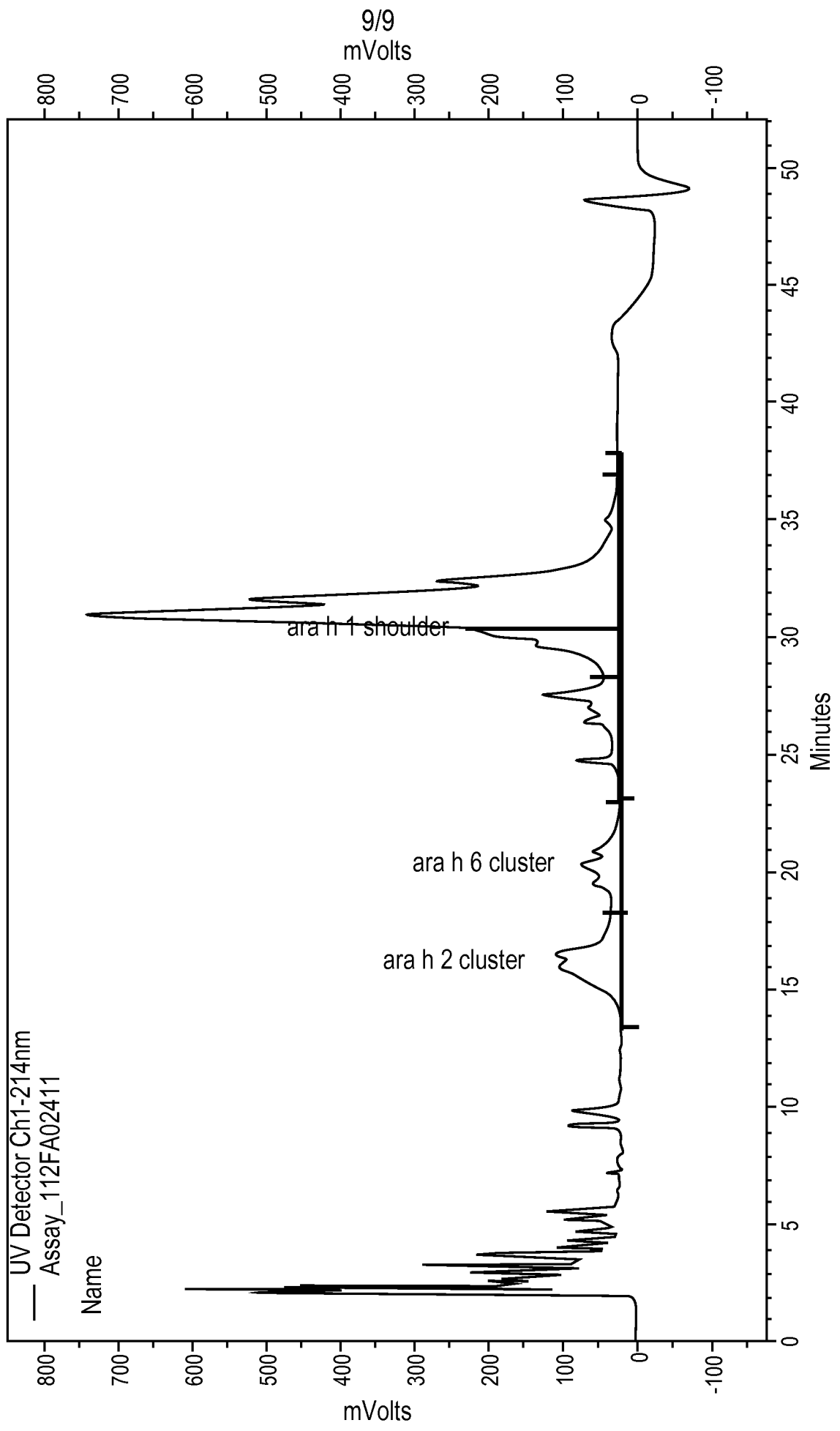

mVolts
800
700
600
500
400
300
200
100
0
-100
Minutes
0
5
10
15
20
25
30
35
40
45
50
ara h 1 shoulder
ara h 6 cluster
ara h 2 cluster
UV Detector Ch1-214nm
Assay_112FA02411
Name

FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/24401

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/35; A61J 3/00; C07K 16/16 (2014.01)
USPC - 424/134.1, 275.1, 810
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 38/00, 39/00, 39/35, 47/38; A61J 3/00; C07K 16/16, 16/28, 14/415 (2014.01)
USPC: 424/134.1, 275.1, 400, 810; 530/370, 403, 868, 536/23.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; Google; ProQuest; peanut allergy, peanut proteins, ara h1, ara h2, ara h6, capsule, diluents, lubricants, glidants, microcrystalline cellulose, starch, dicalcium phosphate, colloidal silicon dioxide, talc, magnesium stearate, sodium stearyl fumerate, reverse-phase HPLC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/123759 A1 (CLARK, A et al.) September 20, 2012; page 2, lines 28-30; page 3, lines 10-15; page 4, lines 11-12; page 5, lines 9-16; page 13, lines 36-38	12-23, 24/12-23, 25/24/12-23, 26/25/24/12-23, 27/26/25/24/12-23
Y	WO 2012/001074 A2 (THAKUR, J et al.) January 05, 2012; page 5, paragraph 1; page 1, paragraph 3; page 6, 'process A'; page 7, lines 2-3; page 7, lines 17-18; page 7, lines 20-21	12-23, 24/12-23, 25/24/12-23, 26/25/24/12-23, 27/26/25/24/12-23
Y	SINGH, H. Developing RP-HPLC method for detection of peanut allergens. October, 2011. In: AACC International Annual Meeting. October 16-19, 2011. Retrieved from the internet: <URL: http://www.aaccnet.org/meetings/Documents/2011Abstracts/p11ma199.htm>; abstract; title	26/25/24/12-23, 27/26/25/24/12-23
Y	BERNARD, H. Identification of a new natural Ara h 6 isoform and of Its proteolytic product as major allergens in peanut. Journal of Agricultural and Food Chemistry. 2007. Vol. 55. pages 9663–9669; page 9664, right column, 'results'; page 9665, right column, last paragraph; page 9666, left column, lines 1-2; figure 2	26/25/24/12-23, 27/26/25/24/12-23
Y	US 3809767 A (SAIR, L et al.) May 07, 1974; column 30, lines 26-30	16, 24/16, 25/24/16, 26/25/24/16, 27/26/25/24/16

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 2014 (01.07.2014)	21 JUL 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/24401

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2008/0317878 A1 (LI, XM et al.) December 25, 2008; paragraphs [0007], [0015], [0098]; abstract; claims 1, 11	1-23, 24/1-23, 25/24/1-23, 26/25/24/1-23, 27/26/25/24/1-23
A	US 2004/0234548 A1 (CAPLAN, MJ) November 25, 2004; paragraphs [0008], [0083]; abstract; claims 1, 6, 8	1-23, 24/1-23, 25/24/1-23, 26/25/24/1-23, 27/26/25/24/1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/24401

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 28-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.