(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 August 2013 (08.08.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/116847 A1

(51) **International Patent Classification**:
G01N 33/53 (2006.01) ***C12Q 1/06*** (2006.01)
G01B 11/00 (2006.01)

(21) **International Application Number**:
PCT/US2013/024650

(22) **International Filing Date**:
4 February 2013 (04.02.2013)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/594,433 3 February 2012 (03.02.2012) US
61/618,245 30 March 2012 (30.03.2012) US

(71) **Applicant**: **CHARM SCIENCES, INC.** [US/US]; 659 Andover Street, Lawrence, MA 01843-1032 (US).

(72) **Inventors**: **JABOUR, John**; 15 Ian's Way, Farmington, NH 03835 (US). **SAUL, Steven, J.**; 11 Norfolk Road, Arlington, MA 02176 (US). **TESS, Mark, E.**; 38 Level Street, Merrimack, NH 03054 (US).

(74) **Agent**: **MEYER-LEON, Leslie**; IP Legal Strategies Group P.C., 101 River Road, Sudbury, MA 01776 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2013/116847 A1

(54) **Title**: EXTRACTION OF MYCOTOXINS

(57) **Abstract**: A method and composition for extracting an analyte from a test sample such as grain, so as to determine whether the test sample is contaminated with a toxin. The method is particularly useful for detecting the presence in a batch of grain of a mycotoxin, such as for example aflatoxin, ochratoxin, T2, zearalanone, vomitoxin (deoxynivalenol a/k/a DON), patulin and fumonisin. Extraction is performed with use of a composition that includes a proteinaceous material, such as albumin, as an extraction agent.

EXTRACTION OF MYCOTOXINS

Reference To Prior Applications

This application claims the benefit of U.S. Provisional Application No. 61/594,433, filed February 3, 2012 (hereby incorporated by reference) and U.S. Provisional Application No. 61/618,245, filed March 30, 2012 (hereby incorporated by reference).

BACKGROUND

The technical field of the invention relates to methods of extracting analytes, for example mytoxins, from a sample, such as, for example, grain, or, for example, maize.

Tests to detect one or more analytes in samples are known in the art. Some examples are described in U.S. Patent No. 5,985,675, issued November 16, 1999; U.S. Patent No. 6,319,466, issued November 20, 2001; U.S. Patent No. 7,410,808, issued August 12, 2008; International Publication Number WO 2006/089027, filed February 16, 2006; U.S. Patent No. 7,785,899, issued August 31, 2010; U.S. Patent No. 7,863,057, issued January 4, 2011 and International Patent Application Number PCT/US10/39113, filed June 18, 2010 the teachings of all of which are incorporated herein by this reference.

When chromatographic test strips, such as lateral flow test strips, are the testing medium, many sample matrices, such as solid or granular materials, require extraction of analyte into a liquid matrix prior to testing. For example, corn can be ground and the ground sample extracted with various combinations of solvents. Typical solvents include 70% methanol in a 2:1 ratio (2 milliliter per 1 gram of sample) and acetonitrile, ethanol or other concentrations of methanol, for example at 50%, 60%, or 80%. Depending on the use, such solvents can be relatively hazardous and costly. In addition, such solvents may require further dilution or buffering prior to application to a chromatographic medium or other testing medium, such as when using certain chromatographic test strips. Dilution can affect test sensitivity and, therefore, when higher sensitivity is desired, such as in jurisdictions, such as the European Union, which require higher sensitivity to certain toxins as compared to, for example, the United States, dilution can be undesirable. The solvent also may require adjustment depending on either or both the particular matrix from which the analyte is being extracted and the particular analyte being extracted and detected. That is, one solvent may not be a “one size fits all” but, instead require adjustment/optimization depending on the matrix and/or analyte of detection. We describe herein methods and compositions for performing relatively non-hazardous extractions of a variety of toxins, such as mycotoxins, from a sample. Examples of such non-hazardous extraction compositions include a variety of

high ionic strength compositions, including those with relatively abundant amine and carboxyl groups, such as protein, amino acid and polyethylene glycol based compositions.

SUMMARY

Aspects include a method for detecting an analyte, such as one or more mycotoxins, for example aflatoxin, ochratoxin, T2, zearalanone, vomitoxin (deoxynivalenol a/k/a DON), patulin and fumonisin, or other of a variety of mycotoxins and other toxins in a sample. The steps of the method can include: extracting the analyte from the sample to form an extract, the extracting including contacting the sample with a composition that includes substances with high ionic strength including substances with abundant amine and/or carboxyl groups such as amino acids and a variety of proteins (the extraction composition); contacting the extract with a labeled receptor to form a mobile phase, the labeled receptor characterized by an ability to bind to the analyte to provide, in the mobile phase, a labeled receptor-analyte complex and further characterized by an ability to provide a detectable signal when the labeled receptor is captured on a solid support; contacting the mobile phase with a first test area on a solid support, the first test area comprising a first test area capture agent immobilized on the solid support, said first test area capture agent configured to both capture labeled receptor unbound by the analyte from the sample and not capture the labeled receptor-analyte complex; and measuring the intensity of the detectable signal at the first test area, wherein the intensity of the detectable signal is related to the concentration of the analyte in the sample. The extraction composition can be provided to a user in a solution or can be in a solid form such as tablet, powder or other solid forms that can be dissolved in, for example, water. When provided to the user in tablet or powder form, for example, with instructions to add water or other available solvent, costs are reduced by the lowering of shipping weight. In addition, longer shelf life may be achieved when maintained in non-liquid form until prior to use. Aspects include mixing the dry extraction composition with a dry sample and adding a solvent, such as water, to the mixture to perform the extraction. After adding the solvent, the mixture can be shaken followed by allowing the solids to settle before utilizing the liquid layer (containing the extracted analyte if present) for testing.

When the extraction composition has been previously dissolved in liquid, the pH can be in the range of between pH 6 and pH 8.5. In one example a protein solution, including 2%-10%, for example, 5% protein, in buffered solution, for example 20mM sodium phosphate at pH 7.2, was used. In another examples 0.2 M amino acid solution, for example 0.2M arginine in water, at pH 7.2 were used.

Useful as an extraction agent is a composition that includes a proteinaceous material, which is understood to be a material or substance containing one or more proteins or fragments or constituents thereof, such as amino acids or digested components of proteins. Examples of proteinaceous materials include, alone or in combination, bovine collagen, bovine serum albumin, gelatin peptone, soy peptone, soy/casein, a digestive protein, and an enzymatic digest of proteins, e.g., Primatone® or Primatone® RL (a registered trademark of Kerry Group Services Ltd., Tralee County Kerry Ireland). Additional examples of useful proteinaceous materials include proteinaceous materials that are rich in amine and carboxyl groups, where by 'rich' is meant that the proteinaceous material contains a higher concentration of amine or carboxyl groups than the average concentration of such groups in proteins generally. Examples of useful amino acids include, alone or in combination, glycine and arginine.

Aspects include a chromatographic test strip, such as a chromatographic lateral flow test strip, such as a chromatographic lateral flow test strip including nitrocellulose and/or POREX® (Porex is a registered trademark of Porex Technologies Corp., Fairburn, GA), as a test medium, as the solid support. The test strip can include a test medium, for example a stationary phase membrane in contact or contacted with the mobile-phase composition and having a first end and a second end, wherein the membrane allows lateral capillary flow of the sample from the first end to the second end and has the test areas thereon. The test strip can also include a mobile phase membrane that is the same as, or different, from the stationary phase membrane.

In some aspects the labeled receptor comprises a labeled antibody, such as a polyclonal or monoclonal antibody. In other aspects the labeled receptor can be a labeled non-immunological receptor such as an enzyme. The labeled receptor can also be a combination of different receptors with differing affinities, such as differing affinities to the same analyte or affinity to different analytes. The label of the labeled receptor can be a colored particle, such as a gold particle.

Aspects include extraction and/or detection of one or more analytes including one or more toxins, such as mycotoxins, for example, aflatoxin, vomitoxin (DON), fumonisin, T2, zearlanone, patulin and ochratoxin from a variety of solid matrices including feeds and grains such as barley, corn, corn flour, corn meal, corn gluten meal, corn germ meal, wheat, soybeans, dried distillers grain (DDGS), distiller's corn meal, corn germ meal, corn/soy

blend, cracked corn, hominy, oats, popcorn, rice, defatted rice bran, rough rice and milled rice, sorghum, and other similar matrices.

Aspects include providing an extraction composition that is compatible with a lateral flow test strip and can effectively and efficiently extract any of one or more of a variety mycotoxins from a variety of sample matrices. Such aspects include providing a standard extractant composition that can be used, with little or no alteration or adjustment, across a variety of matrices, analytes and/or tests.

Aspects include a composition that can both extract an analyte of interest and efficiently and effectively flow on a lateral flow test strip. Such aspects can include a composition that can both block binding sites on a chromatographic test strip membrane such as a nitrocellulose membrane and/or a POREX membrane and extract an analyte of interest. Such compositions can include, for example, bovine serum albumin (BSA) and/or polyethylene glycol (PEG). PEG based compositions can also be usefully combined with other materials such as other buffers, for example, POPSO (Piperazine-1,4-bis(2-hydroxy-3-propanesulfonic acid).

Aspects include providing an extraction composition that can be used to extract any of a number of toxins, such as mycotoxins, from any of a number of matrices, such as feed and grains, with little or no dilution or buffering prior to addition to a lateral flow test strip. Such aspects can include an extraction composition that also serves as an agent to block binding sites on a lateral flow test strip membrane, for example nitrocellulose.

Aspects include providing an extraction composition that can be used to extract any of a number of toxins, such as mycotoxins, from any of a number of matrices, such as feed and grains, with little or no dilution or buffering prior to addition to a lateral flow test strip. Such aspects can include an extraction composition that also serves as an agent to block binding sites on a lateral flow test strip membrane, for example nitrocellulose.

Aspects include providing an extraction composition that can be used extract one or more toxins from a sample for testing to detect one or more toxins in a single test such as a single lateral flow test strip.

Aspects include supplying the extraction composition, such as BSA, in dried form for mixing directly with the dry sample. Solvent, such as water, is added to the dry mixture and the mixture shaken before allowing the solid material to settle and testing the extract by sampling from the liquid layer above the solid layer. Alternatively, the extraction composition, such as BSA, can be dissolved in water prior to adding the dry sample. The

dry sample can also be first mixed with solvent, such as water, and then combined with the dry extraction composition.

DETAILED DESCRIPTION

Provided are compositions and methods for facilitating the extraction of small molecules, such as mycotoxins, from agricultural products such as a variety of grains, corns and feeds. Although not wishing to be constrained by theory, useful extraction compositions have been found to be a variety of materials with relatively high ionic strength, such as a variety of proteins, amino acids and polyethylene glycol. Many of the herein described compositions and methods include the use of materials with high ionic strength such as materials having one or multiple amine groups including a variety of protein and amino acid based compositions and materials. Useful compositions can include one or more proteins and/or amino acids. A variety of proteins can be usefully employed including alone or in combination bovine collagen, bovine serum albumin (BSA), gelatin peptone, soy peptone, soy/casein Primatone® and Primatone® RL. BSA can be particularly useful since it is relatively inexpensive, readily available and compatible with many lateral flow test strips. For example, BSA is useful to block binding sites on nitrocellulose when nitrocellulose is a component of a test strip. In addition, BSA can be useful to enhance the flow along a test strip. When milk is the matrix, the casein in the milk can block binding sites on nitrocellulose and, therefore, the importance of utilizing other blocking agents may be reduced. When milk, or other matrices that include agents that block binding sites on chromatographic membranes, are not tested, the sites may need to be blocked and, therefore, BSA can be useful. The properties of BSA allow a BSA based solution to be used on a test strip with little or no further dilution. For that reason, when BSA is used as an extracting agent, higher sensitivity to analyte may be achievable.

Proteins, such as BSA, can be used in a mixture, for example including a salt such as a phosphate salt, citrate salt and/or chloride salt. Other possibly useful ingredients include certain wetting agents, chelators and preservatives.

An extraction can take place using a variety of methods including combining the sample with the extraction composition, shaking the sample in a container, mixing the sample with a stirrer, or mixing the sample with a blender. Depending on the composition, an antifoaming agent may be useful. Other possible extraction include filtering to collect the extract, allowing sample to sit to form an extract layer above the ground sample, or centrifuging a portion of the sample to obtain an extract layer and sample layer such as in

cases in which an improved extract layer and sample layer is need, for example, if shaking and allowing to stand does not produce the desired separation.

The above described extraction methods can be useful to extract an analyte for detection in for a variety of detection methods and have been found particularly useful for extraction of one or more small molecules, such as one or more mycotoxins, in preparation for detection in lateral flow test strips. For lateral flow test strips, the sample extract can be tested directly or can be mixed with a dilution buffer. The dilution buffer can be used to allow a mobile phase to flow uniformly over the test strip. A mobile phase allows reconstitution of the dried reagents on the test strip. The extract can be diluted by a number of methods and a variety of possible dilution ratios of the extract with the dilution buffer. The dilution buffer can consist of, for example, phosphate buffer, or water. When the analyte is in sample liquid, such as fluid milk, the sample may not require dilution or extraction. When the sample is a solid, or semi-solid, and, therefore, must be combined with a liquid for test operation, the use of a composition for extraction, such as BSA and/or PEG based compositions, which can be added directly to a test strip without further dilution, is useful. Combinations with other materials, however, may nevertheless be desirable such as to alter the test sensitivity range or to allow consistency between samples.

It might also be desirable to, for example, extract the sample and then add the extract to a buffer prior to addition to a test strip. Although it might be possible to employ one step, in which the sample is extracted with BSA and the extract added to the test strip, extra BSA may be required to properly dilute the sample for effective test strip function. By minimizing the amount of BSA, and using the BSA only in an amount required to extract the analyte, test cost can be lowered. After extraction using the minimal amount of BSA required, the extract can be diluted with less costly (compared to BSA) dilution buffer prior to adding to the test strip.

Somewhat similarly, water soluble analytes, such as DON, may be extracted from samples using a composition that is partially or completely water. When testing for such analytes, however, it may still be useful to mix with a composition, such as a 5% BSA composition, prior to addition to a chromatographic test strip to block binding sites such as nitrocellulose binding sites, which might otherwise interfere with the operation.

Embodiments include using an extraction composition, such as BSA, in dried form and mixing the dried extraction composition directly with the dry sample. Solvent, such as water, can be added to the dry mixture and the mixture shaken before allowing the solid

material to settle and testing the extract by sampling from the liquid layer above the solid layer. Alternatively, the extraction composition, such as BSA, can be dissolved in water prior to adding the dry sample. The dry sample can also be first mixed with solvent, such as water, and then combined with the dry extraction composition.

The lateral flow assay test strip can include a support strip and a sample-absorbing matrix. The test device also can include a mobile-phase support attached to the support strip and in contact with the sample-absorbing matrix. In an example, a mobile-phase composition is disposed within or on the test device and has one or more labeled receptors, such as one or more gold labeled antibodies.

The mobile-phase composition can be applied prior to test operation, for example by spraying and drying onto a porous surface such as a polyethylene membrane. When exposed to a sample, the mobile-phase composition can be carried in the sample flow together with the sample. In test operation, the sample flows and, when a receptor is an antibody, the antibody binds to an analyte present in the sample to form an antibody-analyte complex. Alternatively, the mobile phase can be combined with sample prior to application to the test strip or other solid support. In this alternative embodiment, antibody can bind to analyte in the sample prior to contact with the test strip.

In an example, the test strip includes a stationary-phase support strip, which may be part of the same strip as the mobile-phase composition support strip, or on a separate strip in fluid flow contact with the first strip. A support strip can have a first membrane end in contact with the mobile-phase composition and a second membrane end that may be in contact with an optional disposal zone. Lateral-capillary flow of the sample is from the first membrane end to the second membrane end. The test strip can also be wholly or partially of a material, for example nitrocellulose, that can bind proteins. A variety of materials can be used in various portions of the strip including natural or synthetic materials including cellulosic materials such as paper, cellulose and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; glass fiber filter, for example WHATMAN® Fusion 5 membrane (Whatman® is a registered trademark of Whatman Paper Limited, Kent, England); cloth, both naturally occurring and synthetic; porous gels such as silica gel, agarose, dextran and gelatin; porous fibrous matrices; starch based materials, such as cross-linked dextran chains; ceramic materials; films of polyvinyl chloride and combinations of polyvinyl chloride-silica; POREX® (Porex® is a registered trademark of Porex Technologies Corp., Fairburn, GA) and the like. Generally, the material used in the flow

stream should allow liquid to flow on or through the strip. If a variety of materials are used they can be in fluid flow communication/contact or capable of being brought into fluid flow communication/contact. The strip should have sufficient inherent strength or additional strength can be provided by a supplemental support such as a plastic backing upon which porous or bibulous strip components are attached.

One or more test zones can be located on the test strip and may include a capture agent, such as a representative analyte or analogue thereof, which captures unbound labeled receptor, such as unbound labeled antibody. Examples of possible test zone capture agents include aflatoxin, or other toxins such as ochratoxin, DON, T2, patulin, zearlanone and fumonisin, depending on the analyte to be detected. Such a capture agent may be disposed on the test zone portion of the membrane for example by spraying. Prior to spraying, said capture agent can be conjugated to an attachment or carrier protein. Suitable attachment proteins are known to those skilled in the art to be proteins that bind readily to solid supports, such supports that include nitrocellulose. A useful attachment protein includes a carrier protein, i.e., a protein commonly used in conjunction with an immunogen, such as generally water soluble proteins with multiple accessible amino groups including albumin, e.g., bovine serum albumin (BSA), ovalbumin (OVA), keyhole limpet hemocyanin (KLH) and thyroglobulin (THG).

One or more optional control zones may also be on the test strip. The control zone may contain capture agent for the analyte receptor, such as an antibody with affinity to the analyte. Such capture agent can include antibody to the particular antibody, such as anti-species antibody, for binding with both analyte-bound antibody and excess unbound antibody. Alternatively, the control zone may be involved in an independent reaction that informs the user that the test is complete and includes consistent visual indicators, such as color development, for comparison to the test zone. The control zone can generate signal either on contact with sample or on contact with specific test material, such as labeled antibody, such as when the control zone includes an anti-species antibody or one of the several useful antibody capture agents known in the art including protein A, protein G or recombinant varieties of proteins A and G.

The lateral flow test device and method can also be in a sandwich assay format or, as described above, an inhibition/competitive format.

Lateral flow test results can be interpreted visually or by use of a reader, or analyzer, such as a ROSA® reader (ROSA® is a registered trademark of Charm Sciences, Inc.

Lawrence, MA), or Charm EZ® reader (Charm EZ® is a registered trademark of Charm Sciences, Inc. Lawrence, MA). Other reader/analyzer examples include fluorometers, luminometers, bar code readers, radiation detectors (such as scintillation counters), UV detectors, infrared detectors, electrochemical detectors or optical readers, such as spectrophotometers. The reader can be used to distinguish between one or more test zones and one or more control zones or simply to determine a relative change in the test zone. In one embodiment the reader is a ROSA reader. In a particular embodiment, the analyzer is an optical reader, e.g., the reader described in U.S. Patent No. 6,124,585, issued September 26, 2000, hereby incorporated by reference. In a quantitative test, the changes in the test areas, and, when a control zone is present, the extent of the difference between the control zone and test zone or test areas (test area and test zone are used interchangeably herein), can determine the test range detection level of analyte. To accurately and/or numerically assess the differences and the binding at the control zone and test zone, particularly in a quantitative assay, a reader is useful. The reader can also include, within its settings, various selectable calibration settings. Such calibration settings can be editable or changeable depending on the matrix being tested and/or the analyte being detected. In that way, for example, a standard curve can be adjusted to reflect the efficiency of extraction of a particular analyte from a particular matrix. Such an adjustable reader can be particularly useful to allow standardization of a surfactant based extraction solution, such as described herein, for use with a variety of matrices and a variety of analytes. Reader settings can also be adjusted automatically by reading test strip elements, for example by using EZ Compatible® (EZ Compatible® is a registered trademark of Charm Sciences, Inc. Lawrence, MA) in conjunction with a Charm EZ® reader as described in PCT/US2011/049170, filed August 25, 2011 ("Lateral Flow Assay Analysis") and PCT/US11/35576, Filed May 6, 2011 ("Device, System and Method for Transit Testing"), each of which is hereby incorporated by reference.

In a particular embodiment, the mobile phase contacts, or is put into contact with, a first test area on a solid support. The solid support can be configured to allow the mobile phase to flow from the first test area to a second test area on the solid support and, if a control zone is included, to the control zone. The first test area can include a capture agent immobilized on the solid support. The first test area capture agent will have greater binding affinity to the receptor than to the receptor-analyte complex. As a result of that differential in binding affinity, captured receptor in the test area will decrease as sample analyte

concentration increases. When there is a second test area, the second test area can also include a capture agent immobilized on the solid support. As with the first area capture agent, the second test area capture agent will have greater binding affinity to the receptor than to the receptor-analyte complex. The capture agent can be the same in each of the test areas and at the same or different concentrations in each area. The capture agents can also be different, for example with different binding characteristics to the receptor. The capture agents in different test areas can also be targeted to entirely separate receptors, such as when the test strip is designed to detect multiple analytes.

The receptor can be labeled with a label, such as a colored particle, that can be detected when the receptor is bound to the solid support via capture by the capture agent immobilized on the solid support. The intensity of the detectable signal, for example a visible signal, at the first and second test areas can be measured to determine a result. In an inhibition style test the strength (intensity) of the signals are inversely related to the concentration of analyte in the sample. The signal intensities can be observed visually or measured by an electronic test instrument. For example the intensity at each of the two test areas can be summed to determine a result that can relate to the concentration of an analyte in the sample.

Various suitable labels include chromogens, catalysts, fluorescent compounds, chemiluminescent compounds, radioactive labels, magnetic beads or magnetic particles, enzymes or substrates, vesicles containing signal producing substances, colorimetric labels, direct visual labels including colloidal metallic and metallic and non-metallic colored particles, dye particles, or organic polymer latex colored particles.

Additional embodiments for use in the methods set forth herein are set forth in U.S. Patent Application Serial No. 12/080,044, filed March 31, 2008 (hereby incorporated by reference).

Presence or absence tests, known in the art as qualitative tests, provide a yes or no result. Tests that detect the presence or absence of a target analyte above or below a certain threshold level are known as semi-quantitative tests. Tests that determine that a target analyte is present at a particular concentration, or within a range of concentrations, are known as quantitative tests.

Although, many of the herein examples and descriptions refer to detecting mycotoxins such as aflatoxin, zearalanone, patulin, DON, fumonisin and ochratoxin, other analytes can be detected and quantified in a variety of matrices using the herein disclosure.

Other possible target analytes include hormones, vitamins, drugs, metabolites and their receptors and binding materials, antibodies, peptides, protein, allergens, fungicides, herbicides, pesticides and plant, animal and microbial toxins may be determined using the present methods and apparatuses. Other analytes that may be determinable by this disclosure include antibiotics, such as beta-lactams, cephalosporins, erythromycin, sulfonamides, tetracyclines, nitrofurans, quinolones, vancomycin, gentamicin, amikacin, chloramphenicol, streptomycin and tobramycin, toxins, and drugs of abuse, such as opioids and the like, as well as the metabolites of any of the above listed possible target analytes.

Although much of the description herein relates to use of the extraction compositions for extracting analytes for detection using lateral flow type devices and tests, it will be appreciated that the extraction compositions described herein may also be useful to extract analytes, such as toxins, prior to detection in other test formats, for example ELISA assays, radiobinding assays such as those available from Charm Sciences, Inc. (Lawrence, MA) and known as the Charm II assays, and other detection methods and tests.

Numerous embodiments and advantages of this disclosure have been set forth in the foregoing description. Many of the novel features are captured in the following claims. The disclosure, however, is illustrative only, and modifications by one of skill in the art may be made with the present disclosure without departing from the scope of the invention.

Examples

Within the tables, T1 is test line 1 result; T2 is test line 2 result; C is Control line result; RR Conc is the concentration provided by the ROSA Reader (using a preprogrammed algorithm); Result is Rosa Reader result. The ROSA Reader is programmed to provide a result and RR concentration. The result is calculated from a comparison of T1 and T2 with C using an algorithm. The "spread" is the difference between the result for a negative control (NC) result and the result with the particular analyte concentration. The RR Conc is determined by the ROSA Reader through a calculation that associates the Result with a concentration for the particular toxin and matrix. "ppb" = "parts per billion".

Example 1: Previously Dissolved BSA (Extraction Composition) Mixed With Dry Sample

In Tables 1-3 the ROSA reader was calibrated using results from extractions using 70% methanol and, therefore, RR Conc is relevant. For Tables 4-14, the RR Conc results are not calibrated and, therefore, serve only as a relative indicator of detection (the concentration is not accurate because the reader is not calibrated).

Table 1 results are from an experiment using a 70% methanol extraction solution. The original sample was 1000 ppb fumonisin B1, B2 and B3 in corn and the sample was diluted to an in-assay concentration of 10.6 ppb. % cross-reactivity is a ratio of the RR Conc for a cross-reacting analyte (in Tables 1-3 fumonisin B2 and B3) with the RR Conc for B1. A lower cross-reactivity percentage indicates greater specificity to the analyte of detection, which in Tables 1-3 is fumonisin B1. It can be desirable to have cross-reactivity, such as when detection of the cross-reacting substances is desired. Cross-reactivity is, however, undesirable when the cross-reacting substance is not to be detected and, therefore, is a test interference. Results are in parts per trillion. For example, the RR Conc result in Table 1, for in-assay 10.6 ppb B1 is an RR Conc for the sample of 0.933 ppm, or 933 ppb.

Table 1

Conc		T1	T2	C	RR Conc	Result
10.6 ppb B1		2710	4513	2445	0.9	-2333
		3107	3451	2223	1	-2112
		2672	4027	2176	0.9	-2347
	AVE	**2830**	**3997**	**2281**	**0.933**	**-2264**
	%CV	9%	13%	6%	6%	6%
10.6 ppb B2		4581	4951	2380	0.2	-4772
		4166	4196	2074	0.3	-4214
		3543	4505	2123	0.4	-3802
	AVE	**4097**	**4551**	**2192**	**0.300**	**-4263**
	%CV	13%	8%	7%	33%	11%
% Cross reactive, B1 = 100%					**32%**	
10.6 ppb B3		3548	4735	2485	0.55	-3313
		3408	4734	2673	0.75	-2796
		3211	4834	2293	0.5	-3459
	AVE	**3389**	**4768**	**2484**	**0.600**	**-3189**
	%CV	5%	1%	8%	22%	11%
% Cross reactive, B1 = 100%					**64%**	

Table 2 results are from an experiment using a 5% BSA extraction solution. The original sample was 1000 ppb and the sample was diluted to an in-assay concentration of 22.7 ppb. Results show higher RR Conc as is appropriate given the higher concentration (22.7 ppb) and similar cross-reactivity.

Table 2

Conc		T1	T2	C	RR Conc	Result
22.7 ppb B1		1990	4016	2936	2.3	-134
		1924	3323	2641	2.5	35
		1875	3379	2700	2.6	146
	AVE	**1930**	**3573**	**2759**	**2.467**	**16**
	%CV	3%	11%	6%	6%	
22.7 ppb B2		2988	4282	2635	1	-2000
		2827	4077	2508	1.1	-1888
		2744	4482	2558	1	-2110
	AVE	**2853**	**4280**	**2567**	**1.033**	**-1999**
	%CV	4%	5%	2%	6%	6%
% Cross reactive, B1 = 100%					**42%**	
22.7 ppb B3		2554	3893	2986	2	-475
		2757	3990	2846	1.6	-1055
		2279	3770	2581	1.7	-887
	AVE	**2530**	**3884**	**2804**	**1.767**	**-806**
	%CV	9%	3%	7%	12%	37%
% Cross reactive, B1 = 100%					**72%**	

Table 3 results are from an experiment using a 5% BSA extraction solution. The original sample was 1000 ppb and the sample was diluted to an in-assay concentration of

10.6 ppb. Results show similar detection levels as with the methanol extraction at 10.6 ppb and similar cross-reactivity.

Table 3

Conc		T1	T2	C	RR Conc	Result
10.6 ppb B1		3018	5064	2961	1	-2160
		2847	3393	2071	1	-2098
		3375	3562	2404	1	-2129
	AVE	**3080**	**4006**	**2479**	**1.000**	**-2129**
	%CV	9%	23%	18%	0%	1%
10.6 ppb B2		3242	4743	2194	0.45	-3597
		3361	4650	1832	0.3	-4347
		3286	4588	2085	0.45	-3704
	AVE	**3296**	**4660**	**2037**	**0.400**	**-3883**
	%CV	2%	2%	9%	22%	10%
% Cross reactive, B1 = 100%					**40%**	
10.6 ppb B3		2921	4029	2339	0.95	-2272
		3123	4379	2364	0.75	-2774
		3251	4678	2234	0.5	-3461
	AVE	**3098**	**4362**	**2312**	**0.733**	**-2836**
	%CV	5%	7%	3%	31%	21%
% Cross reactive, B1 = 100%					**73%**	

Tables 4-14 include results from tests using a variety of extraction compositions that include a variety of protein and amino acid based extraction solutions as indicated within

each table. All protein solutions were five percent (5%) protein in 20 mM NaPO4 at pH 7.2. Amino acid and other solution are 0.2M. NC results are for a negative control. ND results are for samples with zero detected aflatoxin in a sample by a reference method (depending on the limit of detection of the reference method it is possible some aflatoxin is present in ND samples). The data in table 4 shows a "spread" of 6391 between the NC result (in some cases, as in table 4, the average of two NC results) and result with a sample originally spiked at 90 ppb aflatoxin which was diluted to an in-assay concentration of approximately 5.2 ppb (90/4/4.3).

Table 4

5% Primatone RL

NC	T1	T2	C	Result
	3934	4154	2318	-3452
	3698	4078	2022	-3732
AVE	**3816**	**4116**	**2170**	**-3592**
ND	3733	4049	2005	-3772
5.2 ppb	1078	2397	3137	2799
Spread				6391

Table 5

5% Bovine collagen

NC	T1	T2	C	Result
	4614	4526	2357	-4426
	4194	4430	2325	-3974
AVE	**4404**	**4478**	**2341**	**-4200**
ND	4436	4709	2401	-4343
5.2 ppb	738	2163	3936	4971
Spread				9171

Table 6

85% Casein,15%soy

	T1	T2	C	Result
NC	3847	4399	2571	-3104
ND	3575	3809	1917	-4343
5.2 ppb	957	2471	3503	3578
Spread				6682

Table 7

5% Gelatin Peptone

	T1	T2	C	Result
NC	4494	4555	2072	-4905
ND	4066	3990	2011	-4034
5.2 ppb	1400	2947	3815	3283
Spread				8188

Table 8

5% Soy Peptone

	T1	T2	C	Result
NC	4419	4159	2176	-4226
ND	4263	4224	2171	-4145
5.2 ppb	1176	2460	3183	2730
Spread				6956

Table 9

0.2M Glycine pH 7.2

	T1	T2	C	Result
NC	4337	4510	2059	-4729
ND	3820	4151	2232	-3507
5.2 ppb	1234	2566	3157	2514
Spread				7243

Table 10

0.2M Arginine pH 7.2

	T1	T2	C	Result
NC	4088	4155	1799	-4645
ND	3901	4115	1812	-4392
5.2 ppb	664	1945	3091	3573
Spread				8218

Table 11

0.2M Diaminopropane pH 7.2

	T1	T2	C	Result
NC	3707	4062	2239	-3291
ND	3791	4043	2311	-3212
5.2 ppb	**1014**	**2488**	**3272**	**3042**
Spread				6333

Table 12

0.2M Na Phos pH 7.2

	T1	T2	C	Result
NC	4294	4218	2205	-4102
ND	4121	4077	2306	-3586
5.2 ppb	1727	3038	3838	2911
5.2 ppb	1590	2784	3390	2406
AVE	**1659**	**2911**	**3614**	**2659**
Spread				6761

Table 13

0.02M NA PO4 pH 7.2

	T1	T2	C	Result
NC	3072	3551	1801	-3021
5.2 ppb	1579	2648	3109	1991
Spread				5012

Table 14

0.02M Arginine pH 7.2

	T1	T2	C	Result
NC	3883	4136	1960	-4099
ND	3994	4210	2029	-4146
5.2 ppb	1401	2689	3143	2196
Spread				6295

Tables 15 and 16 include results from tests using a BSA based extraction composition. The composition included 5% protein (BSA) in 0.1M Na PO4 at pH 7.4 and 0.08% KATHON®(Rohm And Haas Company, Philadelphia PA). Table 15 in-assay concentrations of aflatoxin in the sample are at 0, 2.16, 5.6, 11, 19.5, 90.1 parts per billion (PPB) (concentrations shown on far left of table). As can be seen, the difference between the result at 0 ppb and the various concentrations (the spread) grows as the concentration of aflatoxin in the sample is increased. Table 16 results are from a test sample that was diluted 4.3 fold. As a result, the in-assay concentrations are effectively decreased by 4.3 from the concentration shown on the left of the table (for example 90.1 is actually an in-test concentration of 20.95 (90.1/4.3).

Table 15: 1x

		T1	T2	C	RR Conc	Result
0		3886	3773	2359	0	-2941
		3785	3082	1844	0	-3179
		3421	3323	2002	1	-2740
		3862	3919	2431	0	-2919
	AVE	**3739**	**3524**	**2159**	**0**	**-2945**
	%CV	6%	11%	13%	200%	6%
2.16		2688	2714	2542	7	-318
		2920	2589	2365	5	-779
		3266	2825	2715	5	-661
		3254	3204	2944	6	-570
	AVE	**3032**	**2833**	**2642**	**6**	**-582**

		T1	T2	C	RR Conc	Result
	%CV	9%	9%	9%	17%	34%
5.6		1984	2408	2847	19	1302
		2124	2297	2730	16	1039
		2232	2703	2989	16	1043
		2357	2540	3150	20	1403
	AVE	**2174**	**2487**	**2929**	**18**	**1197**
	%CV	7%	7%	6%	12%	15%
11		915	1642	2750	50	2943
		1459	1956	2945	30	2475
		1329	2144	3065	42	2657
		993	1662	2827	52	2999
	AVE	**1174**	**1851**	**2897**	**44**	**2769**
	%CV	22%	13%	5%	23%	9%
19.5		972	1644	3038	67	3460
		1007	1752	3055	63	3351
		755	1438	2505	47	2817
		799	1440	2775	62	3311
	AVE	**883**	**1569**	**2843**	**60**	**3235**
	%CV	14%	10%	9%	15%	9%
90.1		289	950	3643	150	6047
		251	964	3633	150	6051
		88	734	3145	150	5468
		136	908	3678	150	6312
	AVE	**191**	**889**	**3525**	**150**	**5970**
	%CV	50%	12%	7%	0%	6%

Table 16: 4.3x

Conc		T1	T2	C	RR Conc	Result
0		3778	4268	1923	0	-4200
		4050	4229	2122	0	-4035
		3858	3715	1923	0	-3727
		4002	4287	2302	0	-3685
	AVE	**3922**	**4125**	**2068**	**0**	**-3912**
	%CV	3%	7%	9%	0%	6%
2.16		4094	4378	2494	0	-3484
		4139	4679	2726	0	-3366
		4128	4498	2637	0	-3352
		3524	4132	2357	0	-2942
	AVE	**3971**	**4422**	**2554**	**0**	**-3286**
	%CV	8%	5%	6%	0%	7%
5.6		3745	4267	2665	1	-2682
		3819	3614	2338	1	-2757
		4005	4250	2723	1	-2809
		3809	4268	2641	1	-2795
	AVE	**3845**	**4100**	**2592**	**1**	**-2761**
	%CV	3%	8%	7%	0%	2%
11	5	2743	3114	2495	5	-867
	5	2811	3647	2691	4	-1076
	5	3664	4027	3018	2	-1655
	5	3157	3573	2895	4	-940
	AVE	**3094**	**3590**	**2775**	**4**	**-1135**
	%CV	14%	10%	8%	34%	32%
19.5	5	2715	3672	3572	14	757
	5	2837	3810	3540	11	433
	5	2475	3281	3312	15	868

Conc		T1	T2	C	RR Conc	Result
	5	2080	3053	3015	15	897
	AVE	**2527**	**3454**	**3360**	**14**	**739**
	%CV	13%	10%	8%	14%	29%
90.1	5	700	1736	3815	150	5194
	5	616	1539	3342	124	4529
	5	832	1967	3977	150	5155
	5	684	1824	3708	150	4908
	AVE	**708**	**1767**	**3711**	**144**	**4947**
	%CV	13%	10%	7%	9%	6%

Example 2: Dry BSA (Extraction Composition) Mixed With Dry Sample and the Combination Mixed with Water (Evaluation of Charm ROSA WET Aflatoxin Quantitative Test for Feed and Grain (AFQ-WET)

50 grams corn was combined with dry BSA extraction composition and the combination mixed with 150 mL water (amount of water 3x weight of extraction composition). The mixture was shaken vigorously for 1-2 minutes and then allowed to settle for 1 minute to obtain sample extract. Sample tested was from liquid extract layer above settled solid layer.

First quantitation range: 0-20 ppb aflatoxin.

0.300 mL sample extract was added to 0.300 mL AFQ-B dilution buffer (3.5% BSA in 0.1M $NaPO_4$ (AFQ-B dilution buffer is a product of Charm Sciences, Lawrence, MA) and mixed.

Second quantitation range: 20-100 ppb.

0.300 mL extract was added to 1.0 mL AFQ-B dilution buffer and mixed.

Aflatoxin-corn assay was run at 5.61 ppb, 11 ppb, 20.6 ppb (run twice, once with results compared to a dose-response curve set for the 0-20 ppb curve and second time, after dilution in buffer, with reference to a 20 - 100 ppb dose response curve.) 21 test strips were run at each concentration including zero control and positive control 20 ppb). All 21 strips were 0 for negative control and positive at 20 ppb with positive control (result range 14-25 ppb with mean of 18 ppb, standard deviation of 3 and %CV of 16.7%. 0 was result for 0

20/21 strips with one strip registering 1. At 5.61 ppb the result range was 5-8 ppb with a mean of 6.2 ppb, a standard deviation of 0.9. At 11 ppb the result range was 9-12 with a mean of 11 ppb a standard deviation of 1 and %CV of 9.1%. At 20.6 ppb the result range was 18-27 ppb, the mean was 22 ppb, the standard deviation was 2 and %CV was 9.1%.

The 20.6 ppb result range, on the 100 ppb dose response curve (sample diluted additional 4.3x from above), was 10-27. The one 10 ppb result was out of range. The mean was 19 ppb, the standard deviation was 4 ppb and the %CV was 21.1%. The 93.2 ppb sample result range was 70-102 with a mean of 84, a standard deviation of 8 and %CV of 9.5%.

1. Time required for completion of an analysis:

The test kit is capable of analyzing a single sample in less than 30 minutes with a pre-ground sample.

Extraction and Sample Preparation:	4	minutes
AFQ-WET Test Procedure:	5	minutes
Reader Interpretation:	0.5	minutes
Total Time:	9.5	minutes

2. Comparative accuracy of test kits on corn samples naturally contaminated with Aflatoxin.

Comparative accuracy of test kit was conducted on naturally contaminated corn. Samples were ground using a Bunn G3 grinder and passed through a 20-mesh sieve. Material that did not pass through the 20-mesh sieve were ground using a Perten LabMill 3100 with a 20-mesh sieve, at which time both portions were combined and mixed on an inversion mixer for at least 24 hours. Sample aliquots of 50 g were obtained throughout the entire sample at each concentration. HPLC analysis was conducted on 21 samples over a 3 day period. AFQ-WET analysis was conducted by 3 operators each testing 7 samples on a unique lot of test strips according to the operator's manual.

Table 17: HPLC Analysis of Corn

		5 ppb	10 ppb	20 ppb	100 ppb
Day 1	**1**	4.90	9.92	22.2	90.0
	2	5.88	11.1	17.9	90.1
	3	7.00	11.0	24.8	90.7
	4	5.40	11.85	21.7	88.2
	5	7.38	10.0	18.4	107.2
	6	5.38	11.0	21.5	97.0
	7	5.66	10.2	19.9	95.8

		5 ppb	10 ppb	20 ppb	100 ppb
Day 2	**1**	5.07	10.9	19.6	97.3
	2	6.01	10.45	22.3	83.2
	3	5.60	10.88	22.8	117.5
	4	5.23	10.7	22.7	88.1
	5	5.44	14.02	19.9	86.1
	6	5.65	10.4	21.5	96.1
	7	5.64	11.1	21.6	100.4
Day 3	**1**	5.52	11.77	19.8	86.6
	2	5.07	10.29	19.9	100.9
	3	4.93	10.9	19.3	92.2
	4	6.13	12.87	18.7	87.2
	5	5.32	10.28	18.8	82.3
	6	5.33	10.77	19.1	93.2
	7	5.32	10.4	19.4	86.3
	Average	5.61	11.0	20.6	93.2
	Std Dev	0.62	1.0	1.8	8.4
	% CV	11%	9%	9%	9%

Table 18: AFQ-WET Analysis of Corn

		5 ppb	10 ppb	20 ppb	100 ppb
	MATRIX	**00**	**00**	**00**	**01**
Analyst 1	**1**	4	9	19	99
	2	5	9	19	96
Lot 001A	**3**	6	12	22	85
	4	5	9	23	90
	5	4	9	20	81
	6	5	10	15	98
	7	3	9	18	107
Analyst 2	**1**	5	11	18	84
	2	5	13	20	86
Lot 001B	**3**	7	14	18	81
	4	6	11	22	74
	5	6	11	18	78
	6	6	11	22	88
	7	5	12	20	110
Analyst 3	**1**	5	13	23	109
	2	6	12	22	81

Lot 001C	**3**	6	12	20	93
	4	6	11	21	96
	5	6	12	21	80
	6	7	13	22	97
	7	7	11	21	120
	Average	5.48	11.1	20.2	92.0
	Std Dev	1.03	1.5	2.0	12.2
	% CV	19%	14%	10%	13%

Acceptable Ranges:

ppb	Lower	To	Upper
5.61	2.81	to	8.42
11.0	6.2	to	15.8
20.6	12.3	to	28.8
93.2	63.4	to	123.0

Example 3: Suggested additional commodities.

Additional commodity testing was conducted on non-detect samples, according to the methods used in Example 2. Samples were ground using a Bunn G3 grinder (Bunn-O-Matic Corporation, Springfield, Illinois) and passed through a 20-mesh sieve. Material that did not pass through the 20-mesh sieve were ground using a Perten LabMill (Perten Instruments®, Hägersten, Sweden) and passed through a 20-mesh sieve, at which time both portions were combined and mixed on a Turbula® mixer (Willy A. Bachofen Ag, Muttenz, Switzerland) for 4 hours. Sample aliquots of 50 g were obtained throughout the entire sample and fortified with an aflatoxin reference standard to prepare 5 and 20 ppb samples AFQ-WET analysis was conducted by one operator testing 5 samples at each concentration. Validations were completed for the following additional commodities passing all specifications:

Table Set 19:

	Barley			Corn Flour		
	ND	**5 ppb**	**20 ppb**	**ND**	**5 ppb**	**20 ppb**
	0	7	23	0	3	22
	0	6	22	0	3	25
	0	7	24	0	4	22
	0	7	25	0	5	23
	0	5	23	0	3	22
Average	0	6	23	0	4	23
Std dev	0	1	1	0	1	1
%CV	NA	14%	5%	NA	25%	6%

	Corn Germ Meal		
	ND	5 ppb	20 ppb
	0	4	17
	0	3	19
	0	4	17
	1	3	14
	1	3	18
Average	0	3	17
Std dev	1	1	2
%CV	137%	16%	11%

	Corn Meal		
	ND	5 ppb	20 ppb
	0	5	19
	0	6	21
	0	5	16
	0	4	22
	0	6	21
Average	0	5	20
Std dev	0	1	2
%CV	NA	16%	12%

	Corn/Soy Blend		
	ND	5 ppb	20 ppb
	1	5	17
	0	6	17
	0	6	19
	0	5	19
	1	5	18
Average	0	5	18
Std dev	1	1	1
%CV	137%	10%	6%

	DDGS		
	ND	5 ppb	20 ppb
	1	4	16
	1	4	17
	1	5	18
	0	5	17
	1	5	18
Average	1	5	17
Std dev	0	1	1
%CV	56%	12%	5%

	Hominy		
	ND	5 ppb	20 ppb
	0	3	17
	0	3	17
	0	4	18
	0	4	14
	0	3	16
Average	0	3	16
Std dev	0	1	2
%CV	NA	16%	9%

	Oats		
	ND	5 ppb	20 ppb
	0	4	14
	1	4	16
	0	3	15
	0	3	18
	0	3	18
Average	0	3	16
Std dev	0	1	2
%CV	224%	16%	11%

	Popcorn		
	ND	5 ppb	20 ppb
	0	5	25
	0	5	23
	1	5	20
	1	5	21
	0	5	19
Average	0	5	22
Std dev	1	0	2
%CV	137%	0%	11%

	Rice Bran Defatted		
	ND	5 ppb	20 ppb
	1	3	15
	0	3	15
	1	3	14
	1	3	14
	1	4	17
Average	1	3	15
Std dev	0	0	1
%CV	56%	14%	8%

	Rough Rice		
	ND	5 ppb	20 ppb
	0	3	17
	0	5	18
	0	3	18
	1	4	18
	0	3	16
Average	0	4	17
Std dev	0	1	1
%CV	224%	25%	5%

	Sorghum		
	ND	5 ppb	20 ppb
	1	4	21
	0	4	18
	0	5	20
	0	5	19
	0	4	22
Average	0	4	20
Std dev	0	1	2
%CV	224%	12%	8%

	Soybeans		
	ND	5 ppb	20 ppb
	0	6	19
	0	4	16
	0	5	17
	0	4	18
	0	4	17
Average	0	5	17
Std dev	0	1	1
%CV	NA	19%	7%

	Wheat		
	ND	5 ppb	20 ppb
	0	3	16
	0	4	18
	0	4	16
	0	3	15
	0	4	16
Average	0	4	16
Std dev	0	1	1
%CV	NA	15%	7%

Example 4: Fuminisin extracted from Corn with dry bovine serum albumin

Table 20: FUMQ-WET-001

Samples: Naturally contaminated Corn Reference Material Validated by HPLC. 10x of 10x Dilution from a 3x Extraction Ratio

Conc ppb	Com#	T1	T2	C	RR Conc	Result	Recal
ND	5	4494	4177	1083	0	-6505	0
NC	5	4657	4110	1089	0	-6589	0
NC	5	4923	4608	1365	0	-6801	0
NC	5	4802	4526	1304	0	-6720	0
NC	AVE	**4719**	**4355**	**1210**	**0**	**-6654**	**0**
NC	%CV	4%	6%	12%	0%	2%	0%
NC	%Inh/Pts	100%	100%	100%		0	
NC							
540	5	3140	3634	1861	1	-3052	600
500	5	3635	3985	2407	2	-2806	650
500	5	3457	3931	2285	2	-2818	650
500	5	3412	3826	2213	2	-2812	650
500	AVE	**3411**	**3844**	**2192**	**2**	**-2872**	**638**
500	%CV	6%	4%	11%	29%	4%	4%
500	%Inh/Pts	72%	88%	181%		3782	
500							
1040	5	3116	3557	2267	3	-2139	950

Conc ppb	Com#	T1	T2	C	RR Conc	Result	Recal
1000	5	3101	3612	2412	3	-1889	1000
1000	5	3182	3767	2583	3	-1783	1100
1000							
1000	AVE	**3133**	**3645**	**2421**	**3**	**-1937**	**1017**
1000	%CV	1%	3%	7%	0%	9%	8%
1000	%Inh/Pts	66%	84%	200%		4717	
1000							
2170	5	2625	3355	2686	6	-608	1900
2000	5	2520	3187	2578	6	-551	2000
2000	5	2260	2839	2245	6	-609	1900
2000							
2000	AVE	**2468**	**3127**	**2503**	**6**	**-589**	**1933**
2000	%CV	8%	8%	9%	0%	6%	3%
2000	%Inh/Pts	52%	72%	207%		6064	
2000							
4730	5	1850	2765	3206	18	1797	5700
5000	5	1971	2827	3201	16	1604	5300
5000	5	1987	2942	3218	16	1507	5000
5000							
5000	AVE	**1936**	**2845**	**3208**	**17**	**1636**	**5333**
5000	%CV	4%	3%	0%	7%	9%	7%
5000	%Inh/Pts	41%	65%	265%		8290	
5000							
1040	5	3414	3991	2596	2	-2213	900
1000	5	3234	3728	2535	3	-1892	1000
1000	5	3122	3676	2381	3	-2036	1000
1000	5	3351	3966	2698	3	-1921	1000
1000	AVE	**3280**	**3840**	**2553**	**3**	**-2016**	**975**
1000	%CV	4%	4%	5%	18%	7%	5%
1000	%Inh/Pts	70%	88%	211%		4638	
1000							
2170	5	2675	3301	2668	6	-640	1900
2000		2731	3500	2756	6	-719	1800
2000	5	2844	3598	2617	5	-1208	1500
2000	5	2787	3525	2839	6	-634	1900
2000	AVE	**2759**	**3481**	**2720**	**6**	**-800**	**1775**
2000	%CV	3%	4%	4%	9%	34%	11%
2000	%Inh/Pts	58%	80%	225%		5854	
2000							
4730	5	2059	2992	3257	15	1463	4900
5000	5	1720	2614	2857	15	1380	4800
5000	5	1732	2521	2872	16	1491	5000
5000	5	1750	2633	2803	14	1223	4400

Conc ppb	Com#	T1	T2	C	RR Conc	Result	Recal
5000	AVE	**1815**	**2690**	**2947**	**15**	**1389**	**4775**
5000	%CV	9%	8%	7%	5%	9%	6%
5000	%Inh/Pts	38%	62%	244%		8043	
5000							
9460	5	1337	2231	3199	25	2830	8900
10000	5	1442	2457	3404	25	2909	9300
10000	5	1364	2409	3337	25	2901	9200
10000	5	1255	2186	3136	25	2831	9000
10000	AVE	**1350**	**2321**	**3269**	**25**	**2868**	**9100**
10000	%CV	6%	6%	4%	0%	2%	2%
10000	%Inh/Pts	29%	53%	270%		9522	
10000	Corrected by dilution factor						
9460	5	2219	3021	4011	25	2782	8800
10000	5	1575	2204	3220	25	2661	8300
10000	5	2207	2887	4050	25	3006	9700
10000	5						
10000	AVE	**2000**	**2704**	**3760**	**25**	**2816**	**8933**
10000	%CV	18%	16%	12%	0%	6%	8%
10000	%Inh/Pts	42%	62%	311%		9470	
10000	Corrected by dilution factor						

Example 5: Zearalonone extracted from Corn with dry bovine serum albumin

Table 21: ZEARQ-WET-001

Samples: Naturally contaminaed Corn Reference Material Validated by HPLC. 10x of 10x Dilution from a 3x Extraction Ratio

Conc	Com#	T1	T2	C	RR Conc	Result	Recal
ND	5	4085	4054	1174	0	-5791	14
ND	5	4531	4386	1165	0	-6587	0
ND	5	4554	4349	1101	0	-6701	0
ND	5	4921	4888	1507	0	-6795	0
ND	AVE	**4523**	**4419**	**1237**	**0**	**-6469**	**4**
ND	%CV	8%	8%	15%	0%	7%	200%
ND	%Inh/Pts	100%	100%	100%		0	
ND							
99	5	3474	3849	1970	11	-3383	87
100	5	3590	3966	2264	17	-3028	103
100	5	3482	3809	1966	11	-3359	88
100	5	3476	3706	2081	17	-3020	103
100	AVE	**3506**	**3833**	**2070**	**14**	**-3198**	**95**
100	%CV	2%	3%	7%	25%	6%	9%
100	%Inh/Pts	78%	87%	167%		3271	
100							

Conc	Com#	T1	T2	C	RR Conc	Result	Recal
254	5	2450	3110	2634	115	-292	278
250	5	2926	3572	2888	91	-722	242
250	5	2697	3388	2838	108	-409	268
250	5	2658	3274	2664	97	-604	252
250	AVE	**2683**	**3336**	**2756**	**103**	**-507**	**260**
250	%CV	7%	6%	5%	10%	38%	6%
250	%Inh/Pts	59%	75%	223%		5962	
250							
1004	5	1178	2023	3630	892	4059	982
1000	5	1359	2420	4119	1064	4459	1094
1000	5	1127	1945	3417	782	3762	906
1000	5	1089	2121	3654	907	4098	992
1000	AVE	**1188**	**2127**	**3705**	**911**	**4095**	**994**
1000	%CV	10%	10%	8%	13%	7%	8%
1000	%Inh/Pts	26%	48%	300%		10563	
1000							
ND	5	4419	4197	1108	0	-6400	1
ND	5	4328	3965	955	0	-6383	1
ND	5	4250	4113	1234	0	-5895	11
ND							
ND	AVE	**4332**	**4092**	**1099**	**0**	**-6226**	**4**
ND	%CV	2%	3%	13%	0%	5%	133%
ND	%Inh/Pts	96%	93%	89%		243	
ND							
99	5	4124	4601	2781	1	-3163	97
100	5	3628	3994	2176	1	-3270	92
100	5	3687	4071	2166	1	-3426	86
100							
100	AVE	**3813**	**4222**	**2374**	**1**	**-3286**	**92**
100	%CV	7%	8%	15%	0%	4%	6%
100	%Inh/Pts	84%	96%	192%		3182	
100							
254	5	2911	3965	3277	7	-322	276
250	5	2688	3361	2655	6	-739	241
250	5	2624	3439	2817	7	-429	266
250							
250	AVE	**2741**	**3588**	**2916**	**7**	**-497**	**261**
250	%CV	5%	9%	11%	9%	44%	7%
250	%Inh/Pts	61%	81%	236%		5972	
250							
1004	5	1182	2255	3740	25	4043	978
1000	5	1074	1818	3346	25	3800	915
1000	5	1214	2185	3594	25	3789	912
1000							

Conc	Com#	T1	T2	C	RR Conc	Result	Recal
1000	AVE	**1157**	**2086**	**3560**	**25**	**3877**	**935**
1000	%CV	6%	11%	6%	0%	4%	4%
1000	%Inh/Pts	26%	47%	288%		10346	
1000							

CLAIMS

1. A method for extracting one or more analytes from a dry test sample comprising the steps of:

a) mixing the sample with a composition comprising a proteinaceous material to form an admixture;

b) providing conditions for the admixture to separate into a settled layer and a liquid layer, and

c) collecting at least a portion of said liquid layer, wherein said at least a portion of said liquid layer is an extract containing said analyte.

2. The method of claim 1, wherein, in said mixing step, said composition comprising a proteinaceous material is dry and is mixed with the sample to form a dry admixture, and wherein said method further comprises adding a solvent to the dry admixture to form a wet admixture.

3. The method of any of claims 1-2, wherein the proteinaceous material is an amino acid.

4. The method of any of claims 1-2, wherein the proteinaceous material is an albumin.

5. The method of claim 4, wherein said albumin is bovine serum albumin.

6. The method of claim 4, wherein said albumin is porcine albumin.

7. The method of any of claims 1-2, wherein the proteinaceous material is Primatone RL.

8. The method of any of claims 1-2, wherein the proteinaceous material is a collagen.

9. The method of any of claims 1-2, wherein the proteinaceous material is a peptone.

10. The method of claim 9, wherein the proteinaceous material is gelatin peptone.

11. The method of claim 9, wherein the proteinaceous material is soy peptone.

12. The method of any of the preceding claims, wherein at least one of the analytes is a mycotoxin.

13. The method of claim 12, wherein the at least one of the analytes is fumonisin.

14. The method of claim 12, wherein the at least one of the analytes is ochratoxin.

15. The method of claim 12, wherein the at least one of the analytes is zearlanone.

16. The method of claim 12, wherein the at least one of the analytes is aflatoxin.

17. The method of any one of the preceding claims wherein the test sample comprises a grain.

18. The method of claim 17, wherein the grain comprises corn.

19. The method of claim 17, wherein the grain comprises maize.

20. The method of claim 17, wherein the grain comprises distiller's grain.

21. The method of claim 17, wherein the grain comprises a rice.

22. The method of any one of the preceding claims wherein the composition comprising the proteinaceous material is in the form of a tablet.

23. The method of any one of claims 1-21, wherein the composition comprising the proteinaceous material is in the form of a hydratable solid.

24. The method of any one of claims 1-21, wherein the composition comprising the proteinaceous material is in the form of a powder.

25. The method of any one of the preceding claims wherein the composition comprising the proteinaceous material further comprises a preservative.

26. The method of any one of the preceding claims wherein the composition comprising the proteinaceous material further comprises KATHON®.

27. The method of any one of the preceding claims wherein the composition comprising the proteinaceous material further comprises a salt.

28. A method for detecting whether a mycotoxin analyte is present in a dry test sample, comprising:

a) preparing an extract containing said mycotoxin analyte according to the method of any of the preceding claims;

b) contacting the extract with a labeled receptor to form a mobile phase, the labeled receptor characterized by an ability to bind to the analyte to provide, in the mobile phase, a labeled receptor-analyte complex and further characterized by its ability to provide a detectable signal when the labeled receptor is captured on a solid support;

c) contacting the mobile phase with a first test area on a solid support, the first test area comprising a first test area capture agent immobilized on the solid support, said first test area capture agent configured to capture labeled receptor unbound by the analyte from the sample and not to capture the labeled receptor-analyte complex; and

d) measuring the intensity of the detectable signal at the first test area, wherein the intensity of the detectable signal is related to the concentration of the analyte in the sample.

29. The method of claim 28, wherein said solid support comprises nitrocellulose.

30. A composition comprising a proteinaceous material for use in the method of any one of the preceding claims.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/24650

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53; B01D 11/00; C12Q 1/06 (2013.01)
USPC - 530/422; 436/501, 18; 422/68.1
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): B01D 1/00, 11/00, 11/04, 15/00; G01N 1/04, 30/0, 33/53, 33/543, 33/57, 33/52; C12Q 1/00, 1/02, 1/04, 1/24 (2013.01)
USPC: 530/412, 422, 413, 427, 362, 363, 356; 210/633, 639, 656, 767, 773, 511, 908; 436/501, 536, 541, 8, 15, 16, 17, 18; 422/68.1

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; Google Scholar; DialogPRO; ACS; extract*, isolat*, detect*, assay* analyte, sample, solvent, pre*mix, admix*, mix*, proteinaceous, mycotoxin, albumin, 'BSA,' dry, dried, solid, lyophili*, collagen, peptone, primatone

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0081808 A1 (BURMEISTER, J et al.), March 26, 2009; abstract; paragraphs [0019], [0039], [0041], [0048], [0101]	1, 4/1, 5/4/1 ----- 2, 3/1, 3/2, 4/2, 5/4/2, 6/4/1, 6/4/2, 7/1, 7/2, 8/1, 8/2, 9/1, 9/2, 10/9/1, 10/9/2, 11/9/1, 11/9/2
Y	WO 2003/006994 A1 (NASIR, MS et al.), January 23, 2003; abstract; page 6, line 23 to page 7, line 9; page 9, lines 9-18; page 13, lines 8-14	2, 3/2, 4/2, 5/4/2, 6/4/2, 7/2, 8/2, 9/2, 10/9/2, 11/9/2
Y	US 7097983 B2 (MARKOVSKY, RJ et al.), August 29, 2006; abstract; column 14, lines 15-20	3/1, 3/2
Y	WO 1992/14136 A1 (ETTER, J et al.), August 20, 1992; page 19, lines 21-34; page 19, line 35 to page 20, line 11	6/4/1, 6/4/2
Y	WO 2009/040364 A1 (FANNES, FJH), April 2, 2009; page 10, line 32 to page 11, line 18	8/1, 8/2
Y	WO 2011/097248 A2 (MAY, E et al.), August 11, 2011; page 7, lines 1-7; page 24, lines 16-23	9/1, 9/2, 10/9/1, 10/9/2, 11/9/1, 11/9/2
Y	SCHLAEGER, EJ. The Protein Hydrolysate, Primatone RL, Is A Cost-Effective Multiple Growth Promoter Of Mammalian Cell Culture In Serum-Containing And Serum-Free Media And Displays Anti-Apoptosis Properties. J Immunol Methods. August 14, 1996, Vol. 194, No. 2, pp 191-9, PMID: 8765172.	7/1, 7/2

☐ 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
09 April 2013 (09.04.2013)	23 APR 2013
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/US13/24650

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.: 12-30
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.