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(54) **ANALYTICAL DEVICES FOR DETECTION OF LOW-QUALITY PHARMACEUTICALS**

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See application file for complete search history.

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(73) Assignee: **St. Mary's College**, Notre Dame, IN (US)

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6,847,451	B2	1/2005	Pugh
7,344,081	B2	3/2008	Tseng
7,885,444	B2	2/2011	Wang
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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 800 days.

OTHER PUBLICATIONS

(21) Appl. No.: **13/829,753**

Li et al., "A perspective on paper-based microfluidics: Current status and future trends", *Biomicrofluidics*, 2012, v. 6, pp. 011301-1-011301-13.*

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(65) **Prior Publication Data**

US 2014/0051173 A1 Feb. 20, 2014

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Related U.S. Application Data

Primary Examiner — Yelena G Gakh

(60) Provisional application No. 61/684,570, filed on Aug. 17, 2012.

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G01N 21/78 (2006.01)
G01N 21/84 (2006.01)

(57) **ABSTRACT**

A multilayer Paper Analytical Device (PAD) is provided for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product. A method for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product is also provided that employs a multilayer PAD. A kit is provided for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product that includes a multilayer PAD and instructions for using the kit.

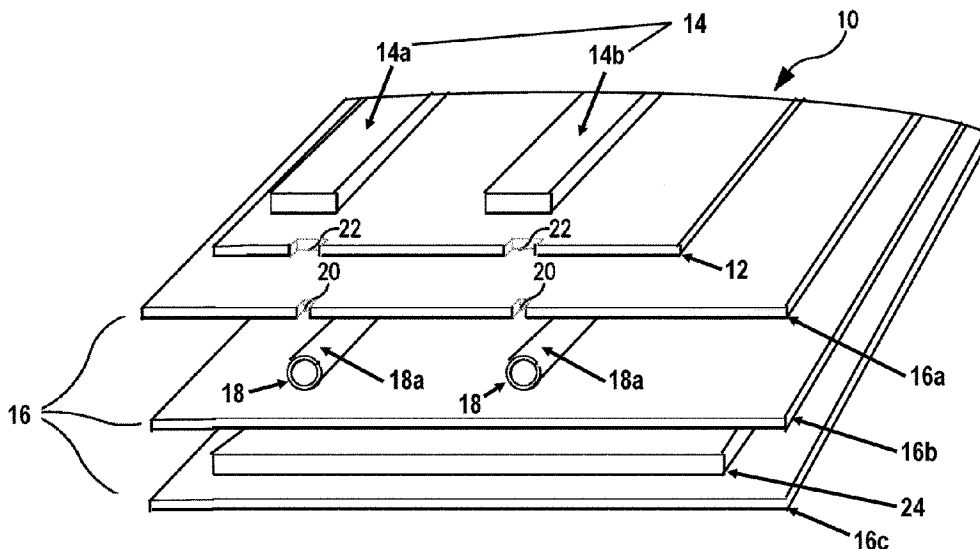
(52) **U.S. Cl.**

CPC **G01N 21/78** (2013.01); **G01N 21/8483** (2013.01); **Y10T 436/11** (2015.01)

(58) **Field of Classification Search**

CPC H01L 2224/97; G01N 33/552; G01N 33/54366; G01N 21/77; G01N 33/54386;

20 Claims, 3 Drawing Sheets



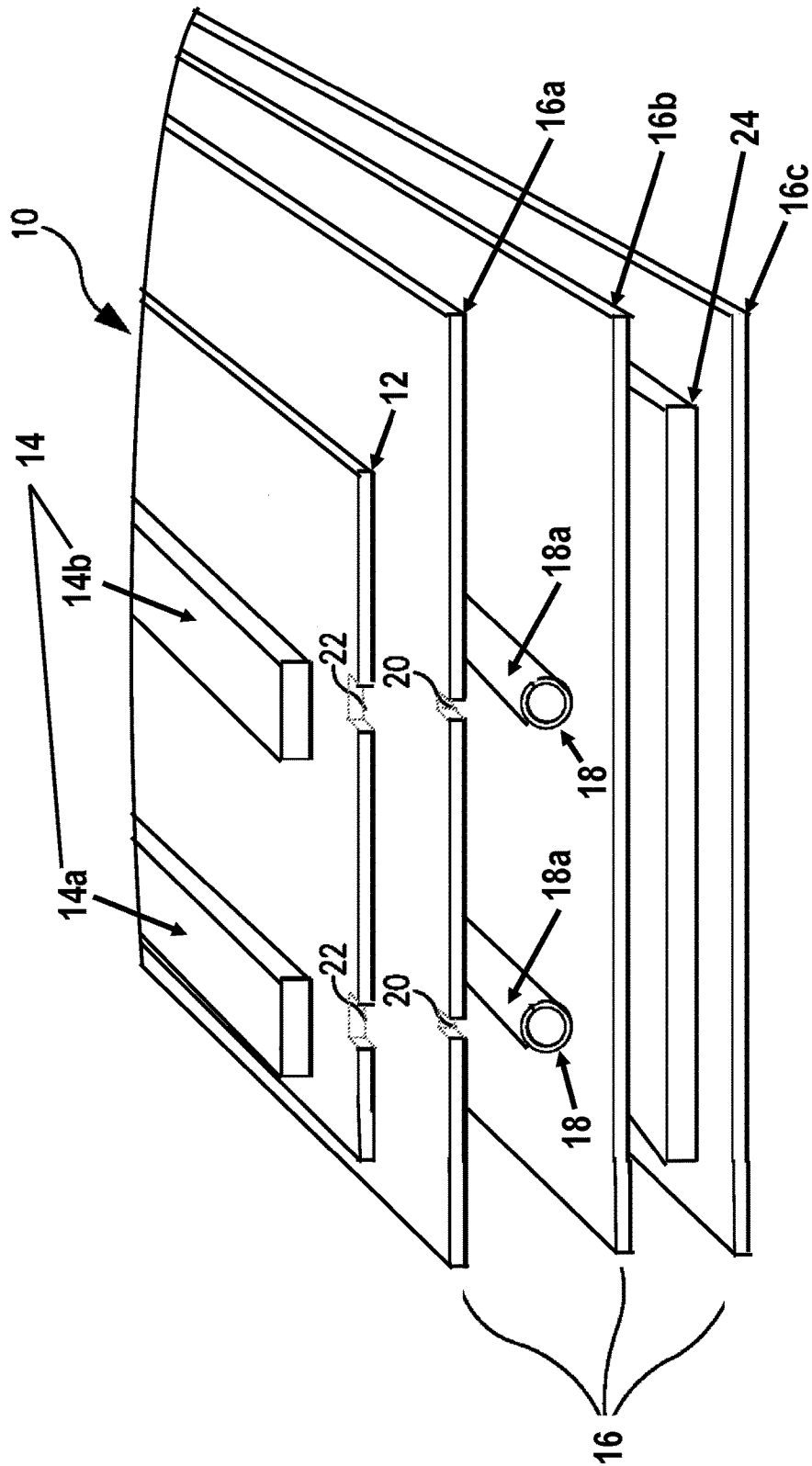


FIG. 1

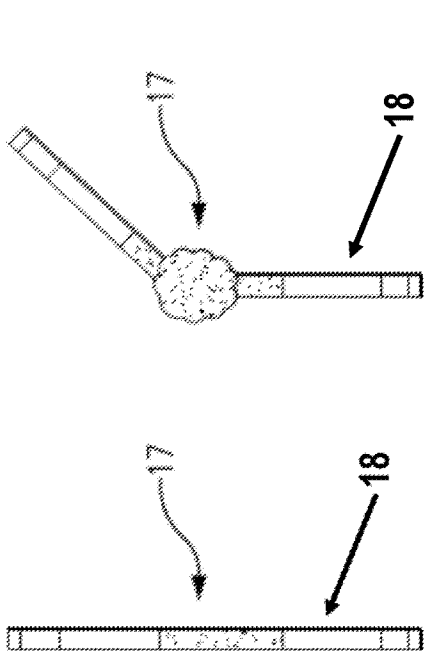


FIG. 3

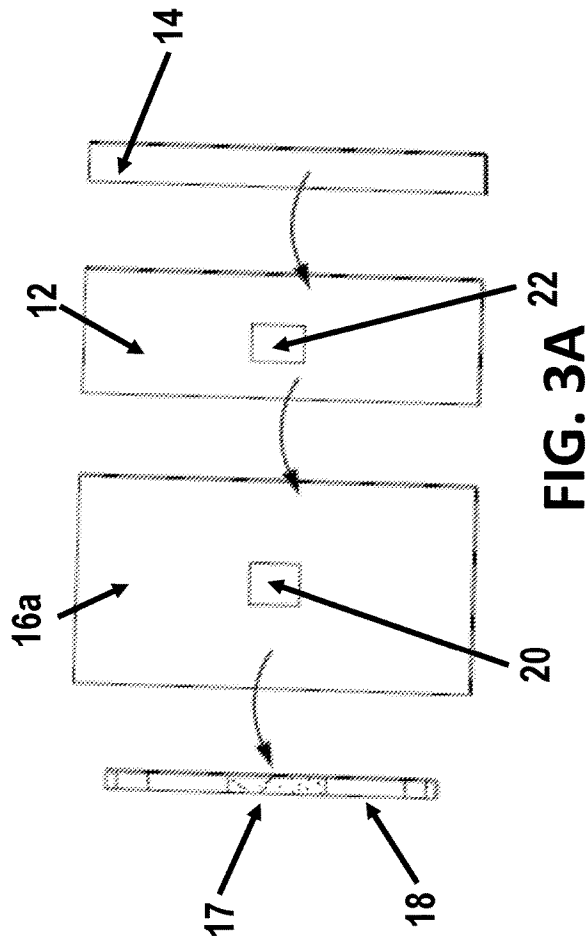


FIG. 3A

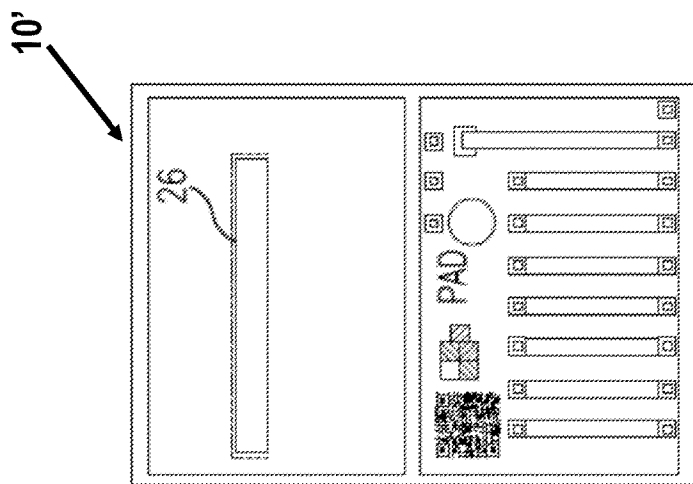


FIG. 2

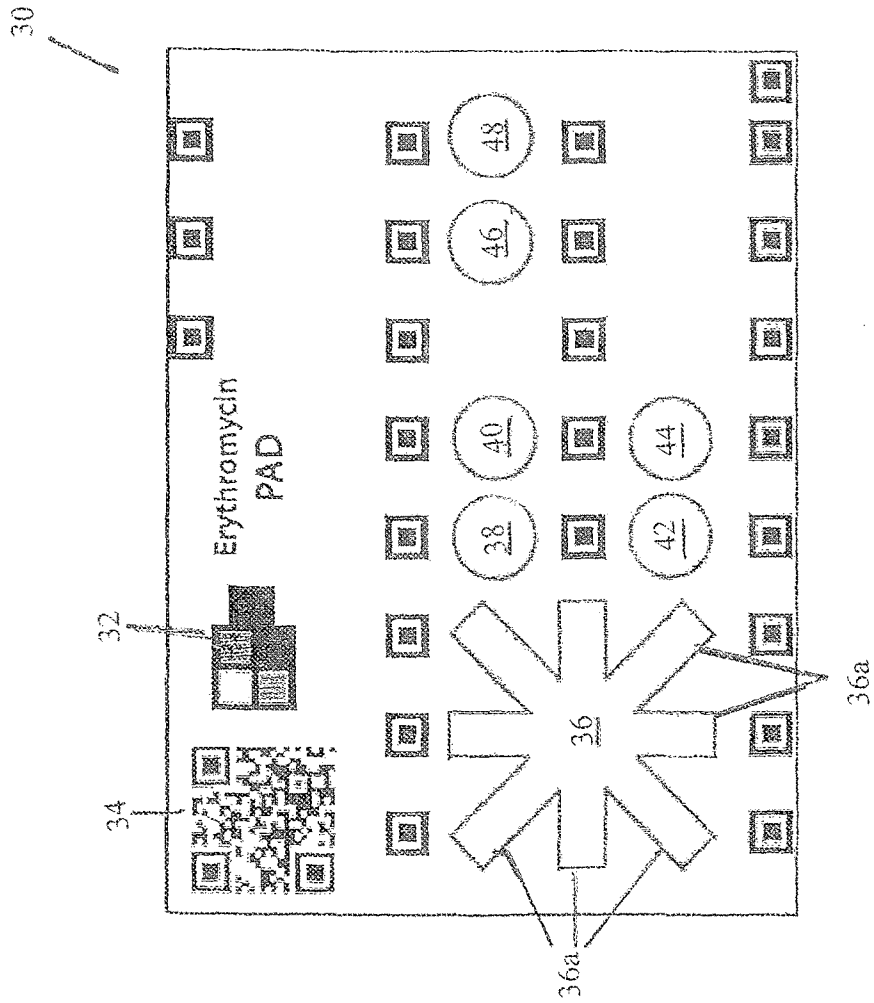


FIG. 4

ANALYTICAL DEVICES FOR DETECTION OF LOW-QUALITY PHARMACEUTICALS

FIELD OF THE INVENTION

The invention relates to user-friendly analytical devices, in particular, Paper Analytical Devices (PADs), for detection of at least two chemical components indicative of a low quality pharmaceutical product or dietary supplement, and a method of use thereof.

BACKGROUND OF THE INVENTION

User-friendly analytical devices such as Paper Analytical Devices (PADs) are known in the art as convenient and inexpensive means for assaying chemicals. As these devices contain all necessary reagents and do not require power, they are easy to operate in a field setting. U.S. Pat. No. 6,136,549 discloses systems for conducting spectrophotometric analysis which includes a chromatographic medium, such as an assay test strip, that is designed to be contacted with a test solution having activated magnetic particles. U.S. Pat. No. 6,770,487 discloses “dip stick” style paper-based diagnostic test devices, in which identifying information and the test result are machine-readable. U.S. Pat. No. 6,847,451 discloses apparatuses for determining the concentration of an analyte in a physiological sample, which include at least one light source, a detector array, means for determining whether a sufficient amount of sample is present on each of the plurality of different areas, and means for determining the concentration of the analyte based on the reflected light detected from those areas determined to have sufficient sample. U.S. Pat. No. 7,344,081 discloses a method of automatically detecting a test result of a probe zone of a test strip comprising capturing an image of a one-dimensional bar code and an image of at least one test strip from a scanning object, and determining a setting value for the at least one test strip based, at least in part, on said captured image of said bar code. U.S. Pat. No. 7,885,444 discloses a method for determining a response of each probe zone on a test strip by selecting an average pixel value of each section of reference white respectively adjacent to the image of a target line to serve as a reference for determining a color response of the target line. US Publication No. 2008/0012083 discloses an analytical system-on-a-chip that can be used as an analytical imaging device, for example, for detecting the presence of a chemical compound, which may also include software that can detect and analyze the output signals of the device. US Publication No. 2011/0189786 discloses a method of detecting the presence or absence of an analyte in a fluid sample. The method includes applying the sample to an inlet zone of a diagnostic system that includes a hydrophilic cotton loading thread to serve as a capillary to deliver a solute to a reagent testing zone, and detecting color change of reagent analyte interaction. US Publication No. 2011/0111517 discloses a paper-based microfluidic assay device comprising a porous, hydrophilic substrate; a fluid-impermeable barrier defining a boundary of an assay region and a boundary of a main channel region, the main channel region fluidically connected to the assay region; and a strip of conductive material disposed on the porous, hydrophilic substrate for detecting the concentration/flow of analyte. In a commercial embodiment, pSiFlow Technology Inc. provides a mobile testing and process management web infrastructure built around its Calibrated

Color Match (CCM) image processing technology that enables digital reading of color-based test strips using any mobile phone with a camera.

The World Health Organization (WHO) reports that many medications for sale in underdeveloped countries are of low quality, which either contain low concentrations of active ingredients that are not sufficient to treat the underlying condition, or contain substitute active ingredients that may have adverse effects on some patients, or have no active ingredients at all; or even contain toxic ingredients. Although the prevalence of such medications is difficult to measure, both the WHO and US Food and Drug Administration (FDA) estimate that 10-30% of all drugs in the developing world are low quality drugs. A consumer taking a low quality pharmaceutical product may die or experience other adverse medical effects from the underlying condition or from contaminants in the pharmaceutical product. The failure of treatment may be mistaken for resistant strains of the disease requiring much more rigorous treatment that can itself endanger the patient. Also, low quality medications that fail to cure the underlying condition may speed up development of actual resistance in pathogens.

Multiple factors contribute to the prevalence of low quality pharmaceutical products in developing countries. First of all, manufacturing and selling low quality pharmaceutical products are both easy and hugely lucrative, and low quality products can enter the supply chain at many points. Moreover, buyers and consumers cannot assess identity or quality of pharmaceutical products. Furthermore, manufacturing low quality pharmaceutical products is not a serious crime in many countries, and there is a low risk of detection from official agencies and organizations. Finally, the time, expertise, and expense required for testing pharmaceutical products is a particular barrier to effective post-market surveillance of pharmaceuticals in developing countries.

Some medical conditions arise not from a pathogen, but from a deficiency in an essential nutrient. For example, widespread iodine deficiency is a problem in many underdeveloped countries that is associated with developmental impairment in children. Fortification of table salt with potassium iodate or potassium iodide is a common route to address this problem. However, production and distribution methods for iodized table salt in many developing countries yield inadequate or inconsistent levels of iodine. Unfortunately, the time and expense of testing for iodized table salt deters manufacturers, distributors, and end-users from testing iodized table salt to determine iodine concentrations. Thus, a low-cost method of testing iodized table salt at the production facility or in the field is needed to determine whether the salt is adequately fortified with iodine within therapeutic concentrations recommended by the WHO.

Thus, there exist long-felt needs for a low-cost, easy-to-use, reliable, minimalistic chemical means of detecting low quality pharmaceutical products and dietary supplements such as iodized salt. These quality problems are also present for veterinary medications and nutritional supplements for animals. The present invention addresses these needs by providing an inexpensive, user-friendly, consistent analytical device capable of detecting various low quality pharmaceutical products and measuring levels of iodine in iodized salt.

SUMMARY OF THE INVENTION

The present invention provides an easy-to-use, inexpensive analytical device, typically a Paper Analytical Device (PAD), for detection or analysis of at least two chemical

components in a dosage formulation indicative of a low quality pharmaceutical product or dietary supplement. The analytical device typically comprises a multilayer PAD having one or more assay regions of porous, hydrophilic material in communication with a non-chemically interfering binder agent such as parafilm disposed adjacent at least to a hydrophobic layer. Vessels containing reagents are in registry with at least one assay region for which testing for a chemical component is desired. Rupture of a vessel wall establishes communication between corresponding assay regions and the reagent released by the ruptured vessel wall.

The vessel may be a separable tubular member insertable between two layers of the PAD. In this exemplary embodiment, the vessel wall is fabricated from a frangible material. Alternatively, the vessel may be integrally formed within the PAD, with the vessel wall including a rupture zone. The one or more vessels when ruptured are preferably in fluid communication with the assay region, e.g., directly or by a channel or other structure joining the two. In each example, rupture of the vessel wall releases the reagent contained by the vessel wall.

A porous, hydrophilic substrate such as a paper may be used for the assay regions. The device includes at least one or more of an information identification zone and a color calibration zone. Alternatively, at least one or more of the information identification zone and the color calibration zone may be integrated within the hydrophobic layer or layers.

The device may further include at least one electronically readable information zone that, after activation of the device, provides color information necessary for detection of the chemical components. The electronically readable information zone comprises alignment references for transforming or correcting a captured image of the PAD to facilitate analysis and processing of the color information to more accurately detect the at least two chemical components. The alignment references include a plurality of fiducial markers for orienting the captured image.

The chemical components to be detected by the whole PAD include at least one active ingredient and possibly one or more excipients. The active ingredient includes at least one of an anti-malarial, antibiotic, anti-parasitic, cox-inhibitor, analgesic, antipyretic, phosphodiesterase-5 inhibitors, anti-viral, anti-cancer or dietary supplement compound. The color information determines whether an inappropriate combination of active ingredient and excipient is present, indicative of a low quality pharmaceutical or dietary supplement product.

Also provided is a method for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product. The method comprises providing the multilayer analytical device as disclosed herein and disposing the product to be analyzed into assay regions. The device is activated in a manner such that the reagents contact the product to be analyzed in the assay regions to provide color information. In some cases, vessel rupture may establish communication between a reagent and the corresponding assay region. Generally, the activating of the device includes rupturing of the vessel wall sufficiently to enable release of the reagents therein onto the assay regions. The subsequently developed color information is analyzed to detect the presence or absence of the chemical components.

Additionally, a kit may be provided for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product. The kit comprises, but is not limited to, a multilayer analytical device as

disclosed herein and instructions for using the kit. The instructions include at least instructions for detecting the presence or absence of the at least two chemical components indicative of a low quality pharmaceutical product or dietary supplement using the kit. The instructions may be provided in hard copy, accessible via a link to a website or mobile application, accessible via a QR code or any combination thereof (including any equivalent and complementary instruction formats). The kit may also include a solvent that is present in an amount sufficient to dilute the pharmaceutical product to be analyzed to an analyzable concentration.

The PAD as described and shown herein may be provided in singular or plural quantities and may also be provided with one or more other PAD configurations, including, but not limited, to those PAD devices described and shown in co-owned U.S. patent application Ser. No. 13/566,915 and related applications.

The PAD disclosed and shown herein is readily used for analyzing the quality of a pharmaceutical or nutritional product, such as an iodized table salt.

BRIEF DESCRIPTION OF THE DRAWINGS

Further features of the invention, its nature and various advantages will be more apparent from the following detailed description, taken in conjunction with the accompanying drawings in which like reference characters refer to like parts throughout and in which:

FIG. 1 shows an exemplary multilayer paper analytical device (PAD).

FIG. 2 shows another exemplary multilayer paper analytical device (PAD).

FIG. 3 shows vessels **18** before and after breakage, while FIG. 3A shows the arrangement and placement of the vessels **18** of the exemplary multilayer paper analytical device of FIG. 1 or 2; these vessels are located behind the assay regions of the test strips **14**, so that when reagent **17** is released from the vessels **18**, the assay regions of the test strips **14** are properly wetted.

FIG. 4 shows an exemplary Erythromycin PAD.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, “paper analytical device” (or “PAD”) refers to a composition based on a porous hydrophilic material (such as paper) and comprising areas of hydrophobic barriers which define hydrophilic assay areas. The hydrophilic assay areas may include test reagents.

As used herein, “low quality pharmaceutical product” and “dietary supplement product” refers to products purported to be genuine products for treating a disease or disorder or providing a nutritional supplement but containing low concentrations of active ingredients that are not sufficient to treat purported use, a product containing substitute active ingredients that may have undesired side effects compared to the stated drug, a product containing active ingredients that have undergone degradation, or a product having no active ingredients at all. In addition, these terms include products that contain ingredients that should not be present in a genuine product of the stated type including ingredients that may be toxic.

As used herein, “active agent” refers to a chemical capable of being detected by an analytical device as disclosed herein, such as a PAD. The active agent may include chemically active ingredients, excipients or anticipated degradation products within a pharmaceutical formulation.

As used herein, a “camera device” refers to a device that contains a camera component. Exemplary camera devices are various types of digital cameras and scanners as well as mobile devices such as cell phones, smartphones or similar devices. When digital cameras are used, the images will be uploaded to a computer or other electronic device that is capable of transmitting the pictures to another location. Of course, when a picture or image is taken from a mobile phone, the phone already generally has the ability to forward the image, e.g., as a text message or as part of an e-mail.

The present invention provides an easy-to-use, inexpensive analytical device, such as a PAD, for detection of specific chemicals and/or chemical groups in an active ingredient, which is particularly capable of detecting various low quality pharmaceutical and dietary supplement products. One advantage of the present invention is that more than one active ingredient can be analyzed with the same device, which is especially useful in analyzing a combination drug. Moreover, in addition to verification of an appropriate active ingredient that should be present, it is also possible to screen for binders and fillers that should be present in the stated formulation, e.g., starch as a binder in acetaminophen tablets; and additionally, to screen for a multitude of likely substitute drugs, e.g., an antipyretic in place of expensive anti-malarial drugs, ampicillin in place of erythromycin, and binders or fillers such as gypsum or lactose in place of an active pharmaceutical.

Porous Hydrophilic Substrate

Typically, the analytical device of the invention comprises a porous, hydrophilic medium, preferably a paper such as a fast chromatography paper or an absorbant blotting paper. A suitable porous hydrophilic medium includes one that has a fast flow rate via capillary action, has enough absorption capacity to hold adequate amounts of reagent; has durability and stability such that it does not fall apart or fall over when wet; and is compatible with at least one of the methods used to fashion assay regions. Characteristics which should be considered when designing an analytical device of the invention, such as a PAD, include density, thickness, pH, basis weight, solvent flow-rate through fabricated substrate, compatibility with fabrication methods, pore size, and porosity. Examples of suitable materials for an analytical device of the invention, such as a PAD, include, but are not limited to nitrocellulose acetate, chromatography paper, cellulose acetate, cellulose paper, filter paper, tissue paper, writing paper, paper towel, cloth, and porous polymer film. In one embodiment, the substrate for a PAD is Whatman 3MM Chr chromatography paper, Ahlstrom 205, Ahlstrom 222, Ahlstrom 226, Ahlstrom 319, or Whatman No. 1 filter paper. In one preferred embodiment, Ahlstrom 319 filter paper is used.

Fabrication of the PAD and Assay Regions Thereupon

The final dimension of the PAD can have a length “L”, width “W” and height or depth “H” that can vary, depending on the number and size of assay regions and information zones needed on the PAD. The height or depth is determined by the chemistry required to detect various components of a particular low-quality pharmaceutical or dietary supplement product.

Several assay regions are defined on the analytical device of the invention, either by affixing individual strips or other shapes cut from paper onto an inert backing material, or by patterning a piece of paper with a hydrophobic barrier substantially permeating the thickness of the paper medium to define the boundaries of the desired assay regions. The hydrophilic assay regions or reaction areas with the boundaries defined by the hydrophobic area can be in any suitable

size or shape. The precise dimensions of the assay regions are determined by the type of reaction to be performed within each assay region, or for optimizing viewing results. Suitable shapes for reaction areas include rectangular lanes, circles (or “spots”), stars, squares, triangles, etc. Reaction areas can comprise multiple shapes. For example, a rectangular lane may culminate with a circle. The diameter of each circle can range from about 1.5 cm to about 0.3 cm. Preferably the circles are about 1.0 cm in diameter. The length of rectangular lanes can range from about 1 cm to the full length of the PAD, and the width can range from about 0.1 cm to the full width of the PAD.

The assay regions of the PAD can be produced in a number of ways that are known to one skilled in the art. For example, photolithography of a resist such as SU-8 can be used to produce hydrophobic regions within the hydrophilic paper medium according to the procedures laid out in US Publication No. 2011/0111517 A1, which is herein incorporated in its entirety. Alternately, the PAD can be fabricated using wax printing according to the procedures laid out in Lu, Y.; Shi, W.; Jiang, L.; Qin, J. Lin, B. *Electrophoresis* 2009, 30, p. 1497-1500 and Carrilho, E.; Martinez, A. W., Whitesides, G. M. *Anal. Chem.* 2009, 81, p. 7091-7095, which are herein incorporated in their entireties. In a preferred embodiment, an HP ColorQube printer is used to deposit wax ink, preferably black, in the desired regions of Ahlstrom 319 paper according to a template laid out in a computer program such as Adobe Illustrator; alternatively, the template may be stored and printed as an image file such as a PDF. The preferred paper type is too thick for wax deposition on one side of the paper to form the necessary continuous hydrophobic barrier, so wax ink must be printed on both sides of the paper, after which the paper is heated to 70-120° C. (preferably 100° C.) to allow the wax to melt through the paper and form a continuous hydrophobic barrier surrounding the desired assay region. For prototyping, a wax crayon can be applied heavily around the regions desired on both the front and back of the paper, after which the paper is heated to 70-120° C. (preferably 100° C.) to allow the wax to melt through the paper and form a continuous hydrophobic barrier surrounding the desired assay region.

The “cut and paste” method requires the lanes to be cut from the hydrophilic paper medium and adhered to a relatively strong backing, mylar plastic for example, using an adhesive. This method does not require the application of a hydrophobic agent in order to define the hydrophilic reaction areas. As a result, the chance of bleed-over of hydrophobic agent into the lanes is eliminated. The lanes can be cut using any precise cutter, such as an exacto-knife or craft cutter.

Referring to FIG. 1, an exemplary device 10 that employs the “cut and paste” method is shown as a multilayer device employing a non-chemically interfering binder agent such as parafilm 12 to attach unadulterated test strips 14 to at least one laminate backing 16 (as shown herein, parafilm 12 attaches test strips 14 to first laminate backing 16a). One or more vessels 18 are disposed between first and second laminate backings 16a and 16b with each vessel having a wall 18a for containment of an unstable or corrosive reagent 17 therein. FIGS. 3 and 3A illustrate the vessel before (FIG. 3) and after (FIG. 3A) being ruptured which releases the reagent into the assay region for testing of the pharmaceutical product. Vessels 18 may be independent tubular members as shown that are placed in registry with release openings 20 provided in first laminate backing 16a and in further registry with corresponding release openings 22 in parafilm 12. Release openings 20 and 22 are in registry with

those test strips **14** for which testing for a particular chemical compound and/or active agent is desired. For example, test strip **14a** may be employed for detecting acetaminophen or vitamin C, while test strip **14b** may be employed for detecting starch or vitamin C.

A substrate **24** which may be a paper layout may be provided between second laminate backing **16b** and a third laminate backing **16c**, which paper layout includes at least one or more of at least one information identification zone (e.g., at least one electronically readable information zone such as a bar code, QR code or set of fiducial marks) and at least one color calibration zone that are readily observed during use of the device. The information identification zone contains information about the PAD-type, serial number and fabrication date and allows for remote identification of the PAD. The color calibration zone allows for accurate computer analysis of PAD images taken under varying light conditions. Fiducial markers aid in orienting captured images so that an image software can correct or transform the captured image. It is understood that one or more of the information identification zone, the color calibration zone and the fiducial markers may be provided on any of the backings **16** and that these features may be incorporated on separate layers of device **10** to augment the interchangeability of the layers with one another (for example, providing substrate **24** with the fiducial markers and integrating the information identification and color calibration zones on one or more backings **16**).

It is understood that laminate backings **16** are not limited to the quantity shown, and that fewer or additional laminate backings may be employed as may be amenable for successful testing of detectable chemicals and active agents. It is also understood that the layers shown herein are exemplary, and that the layers may be placed in any order amenable to the successful practice of the device.

Referring further to FIG. 2, a related device **10'** employs the "cut and paste" method in which the vessels and test strips are integrated with a grate **26** on which a chemical or active agent (e.g., in the form of a pill) may be applied. Grate **26** may comprise emery paper, cardboard or any other suitable material or structure for deriving a suitable testing sample while ensuring a portable and easily stored device.

When vessels **18** are independent members insertable between laminate backings **16a** and **16b**, vessel wall **18** may be fabricated from a frangible composite or comparable or equivalent material. Although vessels **18** are shown herein as independently insertable members, it is understood that one or more vessels may be integrated with one or both of laminate backings **16a** and/or **16b**. For example, either laminate backing **16a** or **16b** may be fabricated to include a vessel integral therewith, thereby obviating the need for two laminate backings to house a vessel therebetween. In this example, release openings **20** may be replaced by frangible zones (not shown) that are in registry with release openings **22** in parafilm **12** and release reagent **17** upon rupture thereof. Also, one or more insertable vessels may be incorporated with one or more vessels integral with device **10**, which may be desirable when testing of different chemicals will be conducted at different times and/or in different locations. Such a configuration may be desirable when employing different reagents having different storage properties.

Vessels **18** stably house those reagents either which may degrade over time when exposed to ambient conditions such that long-term storage is achieved or otherwise damage the test strip such that device integrity is preserved before activation. Referring further to FIGS. 3 and 3A, vessels **18**

can be loaded with unstable or corrosive reagents (illustrated generally as reagent **17**) that are readily released upon compromise of vessel wall **18a**. The objects in FIG. 3A are illustrated from left to right in the order of a vessel **18** containing reagent **17**, a laminate backing **16a** (a hydrophobic layer) that has a release opening **20**, a parafilm **12** (or other non-chemically interfering binder agent) that also has a release opening **22**, and a test strip containing assay regions **14**. Because vessels **18** are built into the PAD, they can be broken on-site to release the reagent as needed. Precise lane control as to test strips **14** is thereby maintained while ensuring device requiring minimal packaging and thereby minimal cost.

Reagents and Reagent Deposition

The analytical device also contains at least one assay reagent in each of the assay regions. In one embodiment, a hydrophobic barrier defines independent isolated assay regions of various shapes and at least one of the assay regions includes a reagent or precursor thereof that is capable of identifying a component that should not be present in the pharmaceutical product.

Various reagents or reagent forming precursors can be optionally loaded into the reaction areas. The reagents or precursors can be loaded into the reaction area individually by hand, or via an automated process. The reagents may be pre-loaded as liquid solutions or suspensions or may be loaded into the vessels. Examples of reagent materials suitable for use in the analytical device of invention such as a PAD include, but are not limited to, Folin-Ciocalteu, potassium hexacyanoferrate(II) trihydrate, iodine-potassium iodide reagent, universal indicator, ferric chloride, triiodide, triiodide-starch complex, soluble starch; cationic, anionic, and neutral pH indicators; barium chloride, sodium rhodizonate, potassium hexacyanoferrate(II), NaOH, tosic acid, potassium carbonate, citric acid, copper sulfate, sodium tetraphenylborate, cobalt thiocyanate, ammonium molybdate, nitroaniline, 1,2-naphthaquinone-4-sulfonate, dimethylglyoxime, and paradimethylaminobenzaldehyde. These reagents may be deposited from aqueous solution or from organic solution. For wax printed PADs, acetonitrile is the preferred organic solvent because the wax barriers are not affected by the acetonitrile. Surprisingly, many colorimetric reagents plateau at particular concentrations so that adding additional reagents will not enhance color results. Thus, the upper limit on the amount of reagents added is more or less determined by the PAD's loading capacity. The volume of the reagent loaded onto the PAD can range from about 2 to about 100 microliters, or from about 10 to about 50 microliters, or preferably from about 20 to about 30 microliters.

Reagents may be deposited on the surface of the analytical device in many ways that will be familiar to those skilled in the art, including but not limited to the use of: microcapillary pipettes and droppers, single- or multi-channel automatic pipetting devices, rods that can capture a droplet of solution or "frog" type depositors that perform this function with multiple rods simultaneously, dipping or spraying equipment, or solution deposition robots.

General Method of Use

The invention also provides a method for detecting the presence or absence of a chemical and/or a chemical functional group in a composition, or for quantifying the amount of at least one chemical in a composition, or for comparing the amounts of two chemicals present in the composition. This method comprises providing a paper analytical device of the invention; disposing the composition into the assay region in a manner such that it contacts the assay reagent or reagents in the assay region; and analyzing the assay region

to detect the presence or absence of the chemical and/or the chemical functional group in the composition or to detect the amount of the targeted chemical or chemicals present in the assay region or to compare the amounts of two chemicals present in the composition.

Compositions Suitable for Analysis

The chemicals to be detected can be in any suitable formulation, including tablets, pills, solids, or powders. Other suitable formulations include liquids, such as suspensions, syrups, or solutions of medications. In some instances, a solid formulation can be used directly with the PAD, by swiping or rubbing the formulation onto the PAD at a specific location(s). In other instances, a solid formulation must be diluted into a liquid solution or suspension in order to be used with the specific PAD. Liquid formulations may be added directly to the PAD, or may be further diluted and then added to the PAD. In some instances, a formulation may be used both directly and also as a dilution on the same PAD.

The PADs can be used to detect low quality human and animal pharmaceutical products, including classes of treating agents such as anti-malarials (artemether, lumifantrine), beta lactam antibiotics (ampicillin, amoxicillin), cox-inhibitors, antiparasitic drugs (albendazole, mebendazole, ivermectin), antipyretics (aspirin, acetaminophen) phosphodiesterase inhibitors (sildenafil citrate), and anti-virals (ostamivir phosphate). They can also be used to analyze foodstuffs that have been supplemented or fortified with micronutrients (iodine, iron, zinc, vitamin C) or with medications (diethylcarbamazine citrate). However, other classes of active agents are also contemplated, such as NSAIDs (ibuprofen), analgesics (lidocaine), HMG-CoA reductase inhibitors (statins), ace-inhibitors (quinapril), macrolide antibiotics (erythromycin), anti-anxiety medications (alprazolam), hi-polar disorder and schizophrenia medications (olanzapine), anemia medications (epoetin alfa), and anti-retrovirals (abacavir), etc. Specific PADs and their application are disclosed herein.

Deposition of the Composition to be Analyzed on the PAD and Activation of the PAD Tests

In one embodiment, a drug tablet may be broken in half and rubbed on the surface of the paper in the assay regions, or a rough surface such as a piece of wire or plastic mesh or sandpaper may be used to assist in forming powdered material on the assay regions. Alternatively, a solid formulation may be crushed or ground to powder, or a capsule containing powdered material may be opened, and the powder may be spread on the assay region using a paddle or spatula. Such deposition of solid material may be carried out with the aid of an assisting device, such as a plastic mesh or plate pierced with holes in regions that correspond to the locations of the assay regions. In order to deposit a controlled amount of the composition to be analyzed, a straight-edge may be drawn across the top surface of the assisting device to pack the composition within the holes of the assisting device, after which the assisting device is lifted from the PAD. The composition may be disposed within the assay region by placing a solution or suspension containing the composition drop-wise onto the desired region of the paper analytical device. Alternatively, the composition may be disposed within the assay region by dipping part of the PAD into the solution or suspension of the composition and allowing the composition to move into the assay region with the resulting capillary flow.

In the preferred embodiment, the pharmaceutical product is applied directly to the vessel rupture zones where communication with the reagents forms a solution. The remain-

ing pharmaceutical product is diluted with solvent and dropped onto the other assay regions.

General Method of Analysis of the Test Results

Once the composition has been applied to the assay regions, the disposing of the solvent into the assay regions typically causes a colorimetric change in each region that can be analyzed to detect the presence/absence of the chemical and/or the chemical functional group in the composition, to quantify the amount of the targeted chemical, or to compare the amounts of two chemicals present in the composition.

The hydrophobic regions can also define control regions within the hydrophilic paper medium. For example, a timer region may be included in order to alert the user when the test has completed. The timer region may comprise a color-generating reaction in which one component travels up the lane with the solvent flow and creates a color when it encounters another component at the top of the lane, or it may comprise other timing mechanisms such as delay of the solvent flow by a deposited reagent such as sugars, surfactants, or polymers. Additionally, the PAD may include positive or negative control regions. A negative control may be included in order to verify that the PAD has not become contaminated during storage or use or that the solvent used to develop the colors does not interfere with a color generating reaction. A positive control may be included to show that the reagents in a test lane are still viable, or it may be used as a standard for the image analysis software as disclosed hereinbelow. The PAD may also contain assay regions whose only function is to demonstrate that the user has complied with instructions for correct use of the PADs, or assay regions whose function is to demonstrate that the PAD is an authentic device and not a counterfeit.

Information Identification Zone

The paper analytical device contains at least one electronically readable information zone, which provides information necessary for determining the outcome of the test performed on the PAD based on images obtained by a camera device. The information zone typically includes appropriate information that is electrically readable per se or after being photographed or otherwise imaged electronically. Such information may include an identification tag such as a two-dimensional bar code (e.g., a QR code), color standards, and/or fiducial or alignment marks.

Each PAD can be imprinted with a two-dimensional barcode such as a Quick Response (QR) barcode that contains the type and serial number of the PAD so that a PAD test can be uniquely identified and the necessary color processing steps to perform the test can be automatically determined, which provides a simple and inexpensive way to uniquely identify the PAD, in addition to providing pertinent information for perspective distortion correction and subsequent color analysis. Depending on the application, other information can also be encoded in the two-dimensional code image. A key task of the image analysis software is the perspective correction or transformation of distorted images, which transforms an image captured at an unknown standoff and optical axis position to a canonical coordinate system in which regions to be analyzed for subsequent color characterization are expressed. The origin and basis vectors for this coordinate system can be automatically calculated from the position of "finder marks" or fiducial marks on the QR code. In some embodiments, each PAD may also contain one or more additional fiducial markers such as "finder squares" or rectifiers to eliminate angle and 3D distortions of the PAD's photographed image. The identification zone can be placed anywhere on the PAD.

Preferably, the identification zone can be printed on the PAD prior to application of hydrophobic regions and the identification zone is located on an upper corner of the PAD.

Color Calibration Zone

In analyzing a PAD, the color content of specific regions of the PAD will be analyzed to automatically determine the test result. This removes human subjectivity in color interpretation. However, PAD images may be captured under different ambient lighting conditions and their global effect on PAD color distributions must be suppressed. Thus, it is important to perform color calibration using the color calibration zone on the PAD, which consists of different colored sub-regions, including a white region and a black region. Image analysis software can be used to compare the extracted colors in the PAD image's color calibration zone to known values to identify the specific color correction methods needed. One such method is white balancing, in which the overall brightness of the image is adjusted to force the white square in the PAD image to have a pure white color value. The calibration zone can be in any suitable shape, including rectangles, squares, circles, or triangles. The sub-regions can be in any suitable shape, including rectangles, squares, circles or triangles. Preferably, the color calibration zone is a rectangle region and the sub-regions are different colored squares. The color calibration zone can be printed onto the PAD prior to or after application of hydrophobic regions. The calibration zone can be placed anywhere on the PAD. Preferably, the calibration zone is printed on the PAD prior to application of hydrophobic regions and is located on an upper corner of the PAD.

In the preferred embodiment, the PAD of the invention comprises one information zone having a color calibration zone, another information zone having multiple fiducial marks, and yet another information zone comprising a QR code or other identification tag.

The method further comprises providing a camera device, capturing an image of the PAD that has reacted with the composition using the camera device, and providing an image analysis software capable of using information provided by the information zone and the image of the test result in order to identify and quantify a colorimetric change within the assay region of the paper analytical device shown in the captured image. In the preferred embodiment of the method of the invention, the capture image contains a two-dimensional bar code such as a QR code and one or more fiducial markers. The image software identifies the QR code region, separates the image of the PAD's assay regions from background present in the picture, scales, rotates, and performs geometrical transformations on the captured PAD image based on the QR code and the one or more fiducial markers, aligns the PAD assay regions with stored images in the database, reads test results from pre-specified locations in the stored assay regions, and classifies the test results. The method of the invention further comprises compiling a database of the captured images of the paper analytical devices and the computed test outcomes, wherein the two-dimensional barcode is a QR code that allows for automated identification of a specific PAD including the PAD-type, serial number and fabrication date.

In one embodiment, the image analysis software is provided on the camera device for processing the captured image in situ. Alternatively, the image analysis software may be provided on a network server such that the captured image is processed by sending the picture to the network server that performs the analysis and transmits the results back to the camera device.

Controls

The hydrophobic regions can also define control regions within the hydrophilic paper medium. For example, a timer region may be included in order to alert the user when the test has completed. The timer region may comprise a colorimetric indicator. Additionally, the PAD may include positive or negative control regions. A negative control may be included in order to verify the purity of the reaction solvent. A positive control may be included in order to verify the presence (or absence) of the chemical to be detected. The control substrates, if any, may be included in the paper medium at the time the other colorimetric reagents are added to the paper medium. The PAD may also contain hydrophilic regions for titrations and/or reverse titrations, as well as user compliance lanes for improving the accuracy of the quantitative analysis of the chemicals.

Kits

The PADs may be packaged in kits providing a user with all of the materials necessary for using the PAD. For example, the kit may contain a solvent (such as deionized water or ethanol), a plastic micropipette, weighing paper, and a cotton swab. Instructions may be provided in hard copy, accessible via a link to a website or mobile application, accessible via a QR code or any combination thereof (including any equivalent and complementary instruction formats). The PADs comprising test reagents may be subjected to degradation due to temperature, light, or moisture which may affect the accuracy of the tests performed. As a result, the PADs may be individually packaged and sealed in light- and moisture-resistant packets. Additionally, the packets may be packaged with a desiccant in order to maintain a specific moisture level, and remove excess moisture. Another embodiment of the invention is a kit for detecting the presence/absence of a chemical and/or a chemical functional group in a composition, quantifying a chemical in a composition, or measuring the relative amounts of two materials present in a composition. Typically, the kit includes a PAD as disclosed herein; a solvent sufficient to saturate the paper assay device; and instructions for detecting the presence/absence of a chemical and/or a chemical functional group in a composition, quantifying a chemical in a composition, or measuring the relative amounts of two materials present in a composition. Preferably, the solvent is one that is sufficient to dissolve or suspend the composition containing the chemical and/or the chemical functional group to be analyzed. Typically, the kit contains a dish to hold the solvent and a spatula swab, or pipette for applying the composition onto the PAD.

The exemplary "cut and paste" devices **10** and **10'** described hereinabove are conducive for use with a variety of PAD embodiments in a kit. FIG. 4, for example, shows an exemplary embodiment of an Erythromycin PAD **30** comprising a color calibration zone **32**, an identification zone **34**, reaction areas as well as a timer region. PAD **30** includes a "red flag" wheel **36** having a plurality of spokes **36a** wherein each spoke is customizable with different chemicals to test for unique excipients and counterfeiting agents. The specific type of chemical in each spoke can be selected depending upon where the test is being conducted as well as to anticipate the types of counterfeit drugs that may be in use in such areas. If a spoke turns a different color when activated, such color change is indicative of the presence of a counterfeiting agent. Reaction areas **38**, **40**, **42**, **44**, **46** and **48** work in concert to perform a quantitative and qualitative assessment of the active ingredient and possible counterfeiting agents. In an exemplary PAD **30**, HCl and Bromocresol green at area **38** works in conjunction with HCl and Methyl

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yellow at area 40 to help determine the concentration of Erythromycin. Ninhydrin at area 42 detects counterfeiting agents with primary or secondary amines. Sodium nitroprusside-acetylaldehyde at area 44 detects the presence of amines and counterfeiting agents. At area 46, phenol treated paper over a capillary containing sulfuric acid detects and differentiates between certain reducing sugars when activated colorimetrically. At area 48, ferric iron in glacial acetic acid and concentrated sulfuric acid, contained in a glass capillary under glass fiber paper, detects the presence of macrolide rings when activated. It is understood that testing is not constrained by the examples disclosed herein. For instance, wheel 36 and spokes 36a thereof may be customizable along with the reaction areas to proactively combat changes in counterfeiting efforts. As counterfeiters recognize the testing patters of a PAD 30 and alter their products according, PAD 30 can be customized with alternative testing reagents in anticipation of the alteration of counterfeit products.

Lastly, the color changes of the PAD regions of interest are identified to determine a qualitative or qualitative test result. While the precise amount of active agent may not be identified, the brightness or intensity of the assaying region can provide some indication of the relative amount of active agent that is present in the product tested.

Example

An illustration of the invention and a comparison of its advantages over other devices appears below.
The Problem:

During the development of the Erythromycin PAD, it became necessary to create a new method for PAD fabrication. The paper had to be unadulterated, and the PAD had to be compatible with organic solvents. It was also necessary to develop a method of stabilizing certain reagents for long term storage. To this end, the Cut & Paste/Capillary Method was developed.

The PADs are developed as business-card sized testing systems that can help determine the authenticity of a suspicious pharmaceutical. Paper, or another absorbant material, is impregnated with a colorimetric reagent(s) that will indicate when a particular chemical or functional group is present.

The assay regions of the PAD were produced in a number of ways that are known to one skilled in the art:
SU-8 Method:

The paper medium is saturated with a crosslinking photoresist and then exposed to UV radiation in a pattern, creating hydrophobic and hydrophilic zones thereby.
Wax Method:

A modified inkjet printer places hydrophobic barriers of black wax to create lanes. The PAD is then heated to ensure full permeation through the paper.
Cut & Paste/Capillary Method:

The multilayer cut and paste design uses parafilm to attach unadulterated test strips to a laminate backing (e.g., as shown and described herein with respect to FIG. 1). Capillaries are loaded with unstable or corrosive reagents that are built into the PAD so that they can be broken on-site to release the reagent (e.g., as shown and described herein with respect to FIGS. 3 and 3A).

Cost Comparison:

Cost of reagents and labor were assumed to be approximately equal and therefore ignored for the purposes of this analysis.

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TABLE 1

SU-8:	
5	Paper: \$0.07 per PAD (4,800 PADs per 100 sheet pack - \$290.00) SU-8 photo resist: \$1.315 per PAD Total: \$1.36 per PAD Wax:
Paper: \$0.09 per PAD (3,200 PADs per 100 sheet pack - \$290.00) Monochrome Wax Printing: \$0.03 per PAD	
10	Total: \$0.12 per PAD Cut & Paste:
Paper: \$0.013 per PAD (18,728 PADs per 100 sheet pack - \$290.00) Laminate: \$0.032 per PAD (450 PADS per 50 sheet pack - \$14.85) Printer Paper: \$0.002 per PAD (4,500 PADs per 500 sheets pack - \$10.00) Parafilm: \$0.034 per PAD (2,602 PADs per 250' x 4" pack - \$89.10)	
15	Total: \$0.08 per PAD

TABLE 2

Observed Assets and Limitations			
	SU-8	Wax	Cut & Paste/Capillary
ASSETS	Precise lane control	Somewhat bio-degradable	Very versatile
25	Rigid	Unadulterated paper	Can use multiple types of absorbant material
	Insensitive to most temperature conditions		Precise lane control
			Unadulterated paper Rigid Can be easily scaled up Needs less packaging Insensitive to most temperature conditions Lowest cost
LIMITATIONS	Cannot be used for acid sensitive analytes	Cannot use organic solvents	Not biodegradable
35	One type of paper	One type of paper	More involved fabrication method
	Not biodegradable	Lack of precise lane control	
	Adulterated paper	Not rigid ("floppy")	
45	More involved fabrication method	Needs significant packaging	
	More difficult to scale up	Sensitive to very warm temperature conditions	
	Needs significant packaging Relatively higher cost		

55 For the sake of brevity, it should be understood that certain structures and functionality, or aspects thereof, of embodiments of the presently disclosed invention that are evident from the illustrations of the figures have not been necessarily restated herein. Also, additional features relating to the use of the present invention can be found in U.S. patent application Ser. No. 13/566,915, filed Aug. 3, 2012, the entire disclosure of which is expressly incorporated herein by reference herein.

65 It is to be understood that the presently disclosed invention is not to be limited to the exact configurations as illustrated and described herein. To those of ordinary skill in the art, one or more inventions will be understood to be

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contemplated from the present application. Accordingly, all expedient modifications readily attainable by one of ordinary skill in the art from the disclosure set forth herein, or by routine experimentation there from, are deemed to be within the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. A multilayer paper analytical device for detection of at least two chemical components indicative of a low quality pharmaceutical product or dietary supplement, comprising: one or more assay regions in communication with a non-chemically interfering binder agent disposed adjacent at least one hydrophobic layer; and

one or more vessels each having a wall that houses one or more reagents in registry with at least one assay region for which testing for a chemical component is desired; wherein the binding agent and hydrophobic layer provide a fluid path such that rupture of a vessel wall establishes fluid communication between the one or more reagents released by the ruptured vessel wall and a corresponding assay region along the fluid path;

wherein each assay region is configured to receive a sample of a suspected a low quality pharmaceutical product or dietary supplement such that, after activation of the device by rupturing of a vessel wall in registry with the assay region, the reagent wets the assaying region for reaction with the sample.

2. The device of claim 1, wherein the at least one vessel is fabricated from a rupturable material.

3. The device of claim 1, wherein the one or more vessels are integral with at least one assay region and the binder agent is parafilm.

4. The device of claim 1, wherein the one or more vessels when ruptured are in fluid communication with the at least one assay region.

5. The device of claim 1, further comprising an information identification zone and a color calibration zone that includes reference colors to assist in processing of the color information obtained from the assay regions after activation of the device to more accurately detect the at least two chemical components.

6. The device of claim 1, further comprising at least one optically readable information zone that, after activation of the device, provides color information necessary for detection of the at least two chemical components, and wherein the least one optically readable information zone comprises alignment references for transforming or correcting a captured image of the paper analytical device to facilitate analysis and processing of the color information to more accurately detect the at least two chemical components.

7. The device of claim 6, wherein the alignment references include a plurality of fiducial markers for orienting the captured image.

8. The device of claim 6, wherein the hydrophilic substrate includes one or more user compliance regions.

9. A method for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product, which comprises:

providing a multilayer analytical device according to claim 1;

disposing the pharmaceutical product to be analyzed into assay regions;

activating the device in a manner such that the reagents contact the product to be analyzed in the assay regions to provide color information; and

analyzing the color information to detect the presence or absence of the at least two chemical components.

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10. The method of claim 9, wherein the pharmaceutical or dietary supplement product to be analyzed is disposed upon the assay regions by depositing the product onto the device or by applying a dilution containing the product onto the device;

wherein the activating of the device includes rupturing of the vessel wall sufficiently to enable release of the reagents therein to establish communication with the assay regions; and

wherein disposing the product into the assay region and activating the device cause a color change that can be analyzed to detect the presence or absence of the chemical components in the product.

11. The method of claim 9, wherein the device further includes a color calibration zone that includes reference colors to assist in processing of the color information obtained from the assay regions after activation of the device to more accurately detect the at least two chemical components, and the method further comprises automating the color analyzing by:

capturing an image of the device using a camera device; and

providing image analysis software capable of recognizing and quantifying a color change within the assay regions of the device that is shown in the captured image.

12. The method of claim 11, which further comprises: repeating the detection for a plurality of pharmaceutical and dietary supplement products; and compiling a database of the captured images of the analytical devices, including time stamping and geotagging of the captured images.

13. The method of claim 9, wherein the chemical components to be detected include an active ingredient and an excipient, and:

the presence of one of the chemical components determines that the pharmaceutical or dietary supplement product contains an insufficient amount of active ingredient;

the absence of one of the chemical components determines that the product does not contain an appropriate active ingredient;

the absence of one of the chemical components determines that the product does not contain an appropriate excipient; or

the presence of one of the chemical components determines that the product contains an inappropriate excipient.

14. The method of claim 9, wherein the chemical components to be detected include an active ingredient and an excipient, wherein the active ingredient includes at least one of an anti-malarial, antibiotic, anti-parasitic, cox-inhibitor, analgesic, antipyretic, anti-viral, anti-cancer and dietary supplement compound and wherein the color information determines whether an inappropriate combination of active ingredient and excipient is present, indicative of a low quality pharmaceutical or dietary supplement product.

15. The method of claim 9, wherein the chemical components to be detected include an active ingredient and an excipient, wherein the active ingredient includes at least one of a pharmaceutical or dietary supplement compound and wherein the color information determines whether an inappropriate combination of active ingredient and excipient is present, indicative of a low quality pharmaceutical or dietary supplement product.

16. A kit for detection of at least two chemical components indicative of a low quality pharmaceutical or dietary supplement product, the kit comprising:

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a multilayer analytical device according to claim 1; instructions for using the kit; and a solvent that is present in an amount sufficient to dilute the product to be analyzed to an analyzable concentration.

17. The device of claim 1, further comprising at least one of an identification tag.

18. The device of claim 1, further comprising a color calibration zone that includes reference colors to assist in processing of the color information obtained from the assay regions after activation of the device to more accurately detect the at least two chemical components.

19. The device of claim 1, further comprising user compliance regions that include a timer region that indicates when the test is completed.

20. A multilayer paper analytical device for detection of at least two chemical components indicative of a low quality pharmaceutical product or dietary supplement, comprising: one or more assay regions in communication with a non-chemically interfering binder agent disposed adjacent at least one hydrophobic layer; one or more vessels each having a wall that houses one or more reagents in registry with at least one assay region for which testing for a chemical component is desired; wherein the binding agent and hydrophobic layer provide a fluid path such that rupture of a vessel wall establishes

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fluid communication between the one or more reagents released by the ruptured vessel wall and a corresponding assay region along the fluid path; wherein each assay region is configured to receive a sample of a suspected a low quality pharmaceutical product or dietary supplement such that, after activation of the device by rupturing of a vessel wall in registry with the assay region, the reagent wets the assaying region for reaction with the sample; at least one optically readable information zone which after activation of the device provides color information necessary for detection of the at least two chemical components, wherein the at least one optically readable information zone comprises alignment references for transforming or correcting a captured image of the paper analytical device to facilitate analysis, wherein the alignment references include a plurality of fiducial markers for orienting the captured image; a color calibration zone that includes reference colors to assist in processing of the color information obtained from the assay regions after activation of the device to more accurately detect the at least two chemical components; and a two-dimensional barcode.

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