

NON-ENZYMATIC COLORIMETRIC TEST STRIP AND METHOD FOR DETECTION OF ETHANOL VAPOUR CONTENT

FIELD OF THE INVENTION

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The invention relates to a non-enzymatic colorimetric test strip for detection of ethanol vapour content in a vapour sample and a method of detecting ethanol vapour content using the test strip.

BACKGROUND

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Various test kits and apparatus for detecting ethanol vapour content are available. Many of these test kits and apparatus utilize enzymes which react with ethanol vapour to produce a colour change, indicating the presence of ethanol vapour. Such test kits and apparatus are either too expensive or too complex for consumer use. Some of the test kits have limited shelf life under ambient conditions as the test kits contain enzymes. Some of these test kits may have structures that are more complexed and bulky, and this may cause awkwardness to use in public and do not allow for discreet use of the test kits in public. Some of the test kits may require contacting the sample with the test kits for a longer period of time to obtain the results.

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It is therefore desirable to provide a colorimetric test strip and a method for detection of ethanol vapour content that seeks to address at least one of the problems described hereinabove, or at least to provide an alternative.

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SUMMARY OF INVENTION

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In accordance with a first aspect of this invention, a non-enzymatic colorimetric test strip for detecting the presence of ethanol in a vapour sample is provided. The test strip comprising a substrate, the substrate having a first portion being an area on a major surface of the substrate coated with a substance defining a sample detection zone, leaving a region in the sample detection zone uncoated; a second portion being another area on the major surface of the substrate coated with a substance to define a control zone, leaving a region in the control zone uncoated; a first dye deposited and

immobilized onto the uncoated region of the sample detection zone to be in contact with the sample for detecting the presence of ethanol in the vapour sample by a colour change in the first dye; and a second dye deposited and immobilized onto the uncoated region of the control zone to be used as a control.

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In one embodiment, the sample detection zone and the control zone are in a spaced apart and adjacent configuration.

10 In one embodiment, the substance coated onto the first portion and the second portion of the substrate is wax. The wax is printed onto the first portion and the second portion of the substrate to form patterns, confining the first dye and the second dye in fixed areas within the respective sample detection zone and the control zone.

15 In accordance with a second aspect of this invention, a method of detecting the presence of ethanol in a vapour sample is provided. The method comprises placing a test strip of the present invention in close proximity to a vapour sample to be tested; allowing the vapour sample to come into contact with the first dye in the sample detection zone; capturing an image of the entire test strip using an image capture device; performing image analysis of the captured image to quantify the ethanol
20 vapour concentration based on the colour intensity change on the first dye in the sample detection zone relative to the second dye in the control zone; wherein a colour change of the first dye in the sample detection zone indicates the presence of ethanol in the vapour sample and the difference in the colour intensity of the first dye in the sample detection relative to the second dye in the control zone determines the
25 concentration of ethanol present in the vapour sample.

In one embodiment, the step of image analysis further comprises normalising the colour intensity measured of the first dye in the sample detection zone relative to the colour intensity of the second dye in the colour zone; and calculating and obtaining a
30 value of normalized colour intensity, R , of the first dye in the sample detection zone relative to the second dye in the control zone, wherein the value R is determined by the following equation (I):

$$\text{Normalized colour intensity, } R = (I_s / B_s) / (I_c / B_c) \quad (I)$$

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wherein

I_s denotes the colour intensity of the first dye in the sample detection zone,

B_s denotes the colour intensity of the white background near the sample detection zone,

5 I_c denotes the colour intensity of the second dye in the control zone,

B_c denotes the colour intensity of the white background near the control zone.

In one embodiment, the method further comprises comparing the value R to a calibration curve to determine the concentration of ethanol in the vapour sample.

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In one embodiment, the step of image analysis is performed by the image capture device. In another embodiment, the step of image analysis is performed by an image analysis device.

15 In some embodiments, the vapour sample is a breath sample, a vapour from an alcoholic beverage or a vapour from an ethanol/water mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

20 The above advantages and features of an apparatus and method in accordance with this invention are described in the following detailed description and are shown in the drawings:

Figure 1 is a top plan view of the test strip in accordance with an embodiment of the
25 present invention.

Figure 2 is the test strip of Figure 1, after the dyes have been deposited and immobilized onto the sample detection zone and the control zone.

30 Figure 3 is a top plan view of an image of the test strip taken by an image capture device that is positioned substantially perpendicularly above the test strip after the sample detection zone was exposed to ethanol vapour, Figure 3 also illustrates the various parameters (I_s , B_s , I_c , B_c) of a measured colour intensity of the dye in the sample detection zone, the parameters are for calculation of the value of "normalized
35 colour intensity", R shown in equation (I).

Figure 4 illustrates an exemplary embodiment of using the test strip of the present invention in combination with an image capture device such as a smartphone, to detect the concentration of ethanol in an alcohol beverage and/or ethanol/water mixture.

Figure 5 is a graph showing a normalized colour intensity to methanol and ethanol vapour at 550 ppmv.

Figure 6 is a graph showing the normalized red intensity for various ethanol vapour concentration prepared from ethanol/water mixture and beer.

Figure 7 is a graph showing the values of normalized red intensity of the test strips after the test strips were exposed to breath samples containing 350 ppmv and 450 ppmv ethanol (based on fuel cell breathalyzer measurement). The values are shown as triangles in the graph.

Figures 8(a) and (b) are graphs showing the normalized red intensity for 550 ppmv of ethanol at (A) various temperatures; and (B) various humidity levels.

DETAILED DESCRIPTION

Various examples will now be described more fully with reference to the accompanying drawings in which some examples are illustrated.

In accordance with one aspect of the invention, there is provided a non-enzymatic colorimetric test strip for detecting the presence of ethanol in a vapour sample. Referring to Figure 1, the colorimetric test strip (101) comprises a substrate (102) having a first portion (103) and a second portion (104).

The first portion (103) of the substrate is an area on a major surface of the substrate (102). The said area of the first portion is coated with a substance to define a sample detection zone (105), leaving a region (106) in the sample detection zone uncoated. The second portion (104) of the substrate is another area on the same major surface of the substrate. The said area of the second portion is coated with the same

substance as the first portion to define a control zone (107), leaving a region (108) in the control zone uncoated.

The test strip further comprises a first dye (201) and a second dye (202). As shown in Figure 2, the first dye (201) is deposited and immobilized onto the uncoated region (106) of the sample detection zone (105). The second dye (202) is deposited and immobilized onto the uncoated region (108) of the control zone (107). Any suitable methods known in the art can be employed to deposit and immobilize the dyes onto the uncoated regions.

In one embodiment, the substance used to coat the first portion defining the sample detection zone (105) and the second portion defining the control zone (107) is wax. Other hydrophobic polymers may also be used without departing from the scope of the present invention. An example includes, but is not limited to, polydimethyl siloxane.

The first portion and the second portion are coated with the substance so as to confine the dyes (201, 202) within the uncoated regions (106, 108). It will be appreciated that other suitable wax-like substance may be used without departing from the scope of the present invention. Any suitable methods may be employed to coat the wax onto the substrate. In one embodiment, the wax is printed onto the substrate to form patterns and these patterns confine the dyes in fixed areas within the sample detection zone (105) and the control zone (107). The other areas of the substrate which are not coated with substance, usually the unprinted edges, are left blank as background control.

The substrate (102) can be made of any suitable material that is capable of providing structural support to the dyes deposited onto the substrate. Examples of suitable materials include, but are not limited to, nitrocellulose membrane and cellulose paper and plastic materials such as polyethylene terephthalate (PET). In one embodiment, the substrate is formed of polyethylene terephthalate (PET) material. Preferably, the substrate is formed of polyethylene terephthalate (PET) material and coated with a layer of silica.

The substrate (102) forming the test strip is typically elongated. In the exemplary embodiment shown in Figure 1, the substrate is substantially rectangular and planar. It will be appreciated that other shapes and sizes of the substrate, such as circular,

square, non-planar, etc. may be utilised without departing from the scope of the present invention.

5 The sample detection zone (105) and the control zone (107) may be of any suitable shapes and dimensions. Suitable shape includes, but is not limited to, rectangular, square, circular, oval or other shape. In the exemplary embodiment shown in Figure 1, the sample detection zone (105) and the control zone (107) are substantially square. The sample detection zone (105) and the control zone (107) are in a spaced apart configuration, leaving a gap (109) between the two zones. Preferably, the sample
10 detection zone (105) and the control zone (107) are positioned in a spaced apart and adjacent configuration.

The uncoated regions (106, 108) in the sample detection zone and the control zone for the deposition of the dyes may be of any suitable shapes and sizes. The uncoated
15 regions may be rectangular, square, circular, oval, or other shape. The uncoated regions may be of the same or different shape as the sample detection zone and the control zone. The uncoated regions should have a size that is smaller than the respective sample detection zone and the control zone but sufficient for receiving the dyes, and sufficient to provide a clear visual and optical detection and analysis of the
20 vapour sample. The uncoated region (106) in the sample detection zone may be of the same or different shape and size as the uncoated region (108) in the control zone. In the exemplary embodiment shown in Figure 1, the uncoated regions (106, 108) are both circular in shape and are of the same size. The uncoated regions (106, 108) are positioned substantially in the center of the respective sample detection zone (105)
25 and the control zone (107).

The dyes (201, 202) are deposited and immobilized onto the uncoated regions (106, 108) of the sample detection zone and the control zone. The first dye (201) in the sample detection zone (105) is provided to be in contact with the vapour sample for
30 detecting the presence of ethanol in the vapour sample. A change in the colour of the dye indicates the presence of ethanol in the vapour sample. The dyes used in the present invention are organic solvatochromic dyes. Any suitable organic solvatochromic dyes may be used including, but are not limited to, Nile red dye and Reichardt's dye. Preferably, the dyes are Nile red dyes.

The colorimetric test strip of the present invention can be used to detect ethanol vapour in breath, and from alcoholic beverage and ethanol/water mixture. The test strip has a linear colorimetric response to ethanol vapour and can detect ethanol vapour over a range of concentration between 0-600 part per million volume (ppmv).

- 5 The test strip is suitable for detecting ethanol vapour in breath near the DUI limit of 350 ppmv.

10 In one embodiment, the test strip of the present invention is fabricated by providing a substrate, and coating the substrate with silica. The substrate has a first portion and a second portion. The first portion is wax printed to form patterns, defining a sample detection zone, with the wax covering a substantial area of the first portion, leaving a region in the first portion uncoated. The second portion is also wax printed to form patterns, defining a control zone, with the wax covering a substantial area of the second portion, leaving a region in the second portion uncoated. The uncoated regions
15 in the sample detection zone and the control zone are provided for receiving the dyes. The patterns formed by the wax immobilized the dyes within the uncoated regions. In an exemplary embodiment as shown in Figure 2, the dyes are deposited in the uncoated regions, positioned in the center of the wax-confined zones. The dyes are deposited and dried by evaporation of the solvent at room temperature. In one
20 embodiment, the dyes are dried for about 15 to 25 mins, preferable about 20 min. After the dyes are dried, the test strip is ready for use.

In a second aspect of the present invention, a method of detecting the presence of ethanol in a vapour sample is provided. The method comprises placing a test strip as
25 described hereinabove in close proximity to a vapour sample to be tested, and allowing the vapour sample to come into contact with the sample detection zone.

When the vapour sample comes into contact with the sample detection zone, the dye in the sample detection zone absorbs the ethanol vapour and changes its colour. This
30 is due to a change in polarity of the dye in response to ethanol. The step of contacting the vapour sample with the test strip involves only contacting the vapour sample with the sample detection zone. For this reason, the gap (109) between the sample detection zone (105) and the control zone (107) should not be too narrow. The gap (109) should be broad enough to avoid the vapour sample from coming into contact
35 with the control zone (107) when the test strip is in used. Preferably, the vapour

sample comes into contact with the sample detection zone for about 1-2 minutes, more preferably about 1 minute.

After the vapour sample contacted with the sample detection zone, an image of the entire test strip consisting of the sample detection zone, the control zone and white background of the test strip is captured. This is done using an image capture device. Any suitable image capture device may be used without departing from the scope of the present invention. Such image capture device includes, but is not limited to, camera, smartphone with an image capture unit, electronic notebook with an image capture unit or other mobile devices with image capture units. In order for the image capture device to capture the entire image of the test strip consisting of the sample detection zone, the control zone and the white background of the test strip, the test strip should be of a size sufficient for a clear image of the dyes in the sample detection zone and the control zone to be taken and for the entire image of the test strip to be taken by the image capture device.

Figure 3 shows an image of the test strip taken by the image capture device that is positioned perpendicularly above the test strip. The image shows the difference in the colour intensities of the sample detection zone and the control zone. The distance between the test strip and the image capture device should be sufficient to allow an image with good resolution to be taken. In one embodiment, the minimum distance between the test strip and the image capture device is about 8 to 12 cm, preferably about 8 to 9 cm. It will be appreciated that the distance may varies, depending on the type of image capture device that is used, as well as the size of the test strip that is used.

The captured image of the test strip is then analysed to determine the colour intensity of the first dye in the sample detection zone.

In one embodiment, the analysis of the captured image of the test strip is carried out using the image capture device configured to process and analyse the captured image. The image capture device may include a processor, a software code or a mobile application configured to calculate and determine the colour intensity of the first dye in the sample detection zone.

In another embodiment, the image captured by the image capture device is transmitted to an image analysis device for analysis. The image analysis device, preferably a computing device or microcomputer, is operable to analyse the image captured by the image capture device to determine the colour intensity of the first dye in the sample detection zone. The image analysis device may comprise a processor, a software code or computer program configured to calculate and determine the colour intensity of the first dye in the sample detection zone.

In the embodiments described hereinabove, the processor, the software code or the computer program performs an image analysis to quantify the ethanol vapour concentration based on the colour intensity change of the first dye in the sample detection zone relative to the second dye in the control zone. The control zone is the portion of the test strip which is not exposed to the vapour sample. The value obtained from the image analysis is then compared to a calibration curve to determine the concentration of ethanol in the vapour sample. A colour change of the first dye in the sample detection zone indicates the presence of ethanol in the vapour sample. The difference in the colour intensity of the first dye in the sample detection relative to the colour intensity of the second dye in the control zone determines the concentration of ethanol in the vapour sample.

The image analysis further comprises normalising the colour intensity measured of the first dye in the sample detection zone relative to the colour intensity of the second dye in the control zone. The analysis further comprises calculating and obtaining a value of "normalized colour intensity", R , of the first dye in the sample detection zone relative to the second dye in the control zone, wherein the value R is determined by the following equation (I):

$$\text{Normalized colour intensity} = (I_s / B_s) / (I_c / B_c) \quad (I)$$

wherein

I_s denotes the colour intensity of the first dye in the sample detection zone,

B_s denotes the colour intensity of the white background near the sample detection zone,

I_c denotes the colour intensity of the second dye in the control zone,

B_c denotes the colour intensity of the white background near the control zone.

A colour change of the first dye in the sample detection zone is indicated by the value of "normalized red colour intensity", *R*. The white background on the test strip is used
5 as a reference to minimize any differences in brightness between the sample detection zone and the control zone due to shadow/lighting position.

The value, *R* obtained from the image analysis is then compared to a calibration curve to determine the concentration of ethanol in the vapour sample. This step can be
10 carried out by the image capture device or the image analysis device. The method may further include presenting a message via a user interface in the image capture device or the image analysis device, wherein the message includes results of the analysis of the vapour sample. It will be appreciated that other means for presenting the results of the analysis of the vapour sample to a user may be employed without
15 departing from the scope of the present invention.

The method of the present invention is able to detect ethanol vapour content over a range of concentration between 0-600 ppmv. The method is capable of providing an analytical colorimetric response in less than 2 minutes, preferably in about 1 minute.
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In one embodiment, the test strip and the method of the present invention is used to determine the authenticity of alcohol beverage. The present invention is able to directly detect ethanol concentration without interference from common adulterant in alcohol beverages, such as methanol. Figure 4 shows an exemplary embodiment of using the
25 test strip of the present invention in combination with an image capture device to detect the concentration of ethanol in an alcohol beverage and/or ethanol/water mixture. As illustrated in Figure 4, the test strip (101) is placed over the open top of a bottle (401) containing the vapour sample (402). Only the portion of the test strip with the sample detection zone (105) is placed over the open top of the bottle (401). After
30 exposing the sample detection zone (105) to the vapour in the bottle for a relatively short period of time, about 1-2 minutes, preferably 1 minute, the test strip (101) is removed from the top of the bottle (401). An image of the entire test strip (101) is then captured using an image capture device (403), such as a smartphone as shown in Figure 4. The captured image is then analysed to quantify the ethanol vapour
35 concentration based on the colour intensity change on the first dye in the sample

detection zone relative to the second dye in the control zone. Based on the value of the concentration of ethanol obtained, one can determine whether the alcohol beverage is authentic or not.

- 5 Figure 5 shows that the technology of the present invention has minimized colour change upon exposure to methanol in comparison to ethanol at same concentration of 550 ppmv. This shows that even when the alcohol beverage was adulterated with methanol, the technology of the present invention is still able to determine the original ethanol concentration in the sample.

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In another embodiment, the test strip and the method of the present invention is applied to determine the progress of food/beverage fermentation process by detecting the ethanol emitted by the fermentation of the food products. The technology of the present invention is able to quantify the concentration of ethanol used as food
15 preservatives or food additives.

In one embodiment, the dye used in the method of the present invention is Nile red dye. Upon exposure to the vapour sample, the vapour's polarity changes the dipole moment of the Nile red dye in the sample detection zone. This results in the colour
20 change of the dye from purple to pink. The pink colour is more intense when the dye in the sample detection zone is exposed to ethanol at higher concentration.

The colorimetric test strip and the method of the present invention have several advantages. The test strip is a low cost portable ethanol sensor. The test strip can
25 detect ethanol vapour at relatively low vapour concentration, and can differentiate ethanol level below or above DUI limit from breath. The test strip does not require any battery or electrical power. It can be used in conjunction with an image capture device including, but not limited to, camera, smartphone with an image capture unit, electronic notebook with an image capture unit or other mobile devices with image capture unit.
30 As both the test strip and the image capture device are portable, this allows ethanol content of a vapour sample to be determined relatively quickly on-site.

The test strip uses a dye that is a non-enzymatic and for this reason, the method can be applied without relying on enzymatic reaction. The test strip is not susceptible to
35 enzyme inactivity and has a relatively long reagent shelf life under ambient conditions.

The test strip uses a single-component dye for the detection of ethanol vapour in a vapour sample instead of using an array of dyes. This reduces the cost of fabrication. The present invention incorporates a control zone in the test strip and this allows normalization to eliminate background lighting interference in one time image taking.

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To facilitate a better understanding of the present invention, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention. One skilled in the art will recognize that the examples set out below are not an exhaustive list of the embodiments of this invention.

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EXAMPLES

Example 1

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Various ethanol vapour samples were prepared from ethanol/water mixture and beer diluted in water stored inside a closed bottle. In this test, a test strip with Nile red dyes is used. The test was carried out by placing the test strip over the open top of the bottle containing the vapour samples. Only the portion of the test strip consisting of the sample detection zone is placed above the bottle. After exposing the sample detection zone to the vapour sample, an image of the test strip was captured using a smartphone.

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Figure 6 shows the normalized red intensity for 0 to 550 ppmv ethanol vapor (each concentration was repeated 3 times). The normalized red intensity is linearly corresponding to the concentration as shown by the linear regression line equation $y = 0.0002x + 0.9936$, and the R^2 value of the line is 0.9892 (y is normalized red intensity, x is ethanol concentration). The results for the beer samples overlap with the ethanol-water mixture samples, indicating that the test strip can be used to detect ethanol from real alcoholic beverages. From Figure 6, it can be seen that the value (R) of the normalized red intensity increases with higher ethanol vapour concentration. The DUI limit of 350 ppmv (denoted as Z in Figure 6) is within the dynamic range.

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Example 2

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This example illustrates the use of the test strip of the present invention in detecting alcoholic content in a breath sample. Test strip with Nile red dyes was used in this example. This application of the test strip has been validated by commercial fuel cell breathalyzer.

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The alcohol concentration in the breath sample was first measured using a commercial fuel cell breathalyzer (such as Alcoscan 9000). The values (R) of the normalized red intensity were obtained from the dye in the sample detection zone of the test strips which were exposed to breath samples containing 350 ppmv and 450 ppmv alcohol respectively (based on the fuel cell breathalyzer measurement). The values obtained are shown as triangles in Figure 7. The triangles fall in the correct range of the normalized red intensity obtained previously in Figure 6. This result validates the usage of the test strip for detection of alcohol in breath samples near the DUI limit (of 350 ppmv). Furthermore, ethanol vapour concentration in breath samples can also be determined by comparing the values R to a calibration curve.

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Example 3

The ethanol vapour detection was tested at 550 ppmv under various temperature (4 – 50 °C) and various humidity (25 – 60%RH). The results obtained are shown in Figures 8(a) and 8(b). The results show that similar readings are obtained for the same vapour concentration under different temperatures and humidity levels. These results suggest the robustness of the test strip and method of the present invention for the detection of ethanol vapour under various temperatures and humidity levels.

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Although an embodiment of the present invention have been shown and described, the present invention is not limited to the described embodiment. Instead, it would be appreciated by those skilled in the art that changes may be made to the embodiments without departing from the scope of the invention, the scope of which is set forth in the following claims.

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CLAIMS

1. A non-enzymatic colorimetric test strip for detecting the presence of ethanol in a vapour sample, the test strip comprising:

5 a substrate, the substrate comprising:

a first portion being an area on a major surface of the substrate coated with a substance defining a sample detection zone, leaving a region in the sample detection zone uncoated;

10 a second portion being another area on the major surface of the substrate coated with a substance to define a control zone, leaving a region in the control zone uncoated;

a first dye deposited and immobilized onto the uncoated region of the sample detection zone to be in contact with the sample for detecting the presence of ethanol in the vapour sample by a colour change in the first dye;
15 and

a second dye deposited and immobilized onto the uncoated region of the control zone to be used as a control,

wherein the first dye and the second dye are each a single-component dye.

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2. The test strip according to claim 1, wherein the first dye and the second dye are organic solvatochromic dyes.

25 3. The test strip according to claim 2, wherein the first dye and the second dye are Nile red dyes.

4. The test strip according to claim 1, wherein the sample detection zone and the control zone are in a spaced apart configuration.

30 5. The test strip according to claim 1, wherein the sample detection zone and the control zone are in a spaced apart and adjacent configuration.

6. The test strip according to claim 1, wherein the substance coated onto the first portion and the second portion of the substrate is wax.

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7. The test strip according to claim 6, wherein the wax is printed onto the first portion and the second portion of the substrate to form patterns to confine the first dye and the second dye in fixed areas within the respective sample detection zone and the control zone.

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8. The test strip according to claim 1, wherein the uncoated region in the sample detection zone has the same dimension as the uncoated region in the control zone.

9. The test strip according to claim 1, wherein the uncoated region in the sample detection zone and the first dye are positioned in the center of the sample detection zone.

10. The test strip according to claim 1, wherein the uncoated region in the control zone and the second dye are positioned in the center of the control zone.

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11. The test strip according to claim 1, wherein the uncoated region in the sample detection zone and the uncoated region in the control zone are circular in shape.

12. The test strip according to claim 1, wherein the substrate is made of material selected from the group consisting of nitrocellulose membrane, cellulose paper and plastic material including polyethylene terephthalate.

13. The test strip according to claim 12, wherein the substrate is made of polyethylene terephthalate material, coated with a layer of silica.

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14. A method of detecting the presence of ethanol in a vapour sample, the method comprising:

placing a test strip in close proximity to a vapour sample to be tested, the test strip comprising:

30 a substrate having a sample detection zone, a control zone and a white background;

a first dye deposited and immobilized onto a region within the sample detection zone; and

35 a second dye deposited and immobilized onto a region within the control zone,

wherein the first dye and the second dye are each a single-component dye;

allowing the vapour sample to come into contact with the first dye in the sample detection zone;

5 capturing an image of the entire test strip using an image capture device;
performing image analysis of the captured image to quantify the ethanol vapour concentration based on the colour intensity change on the first dye in the sample detection zone relative to the second dye in the control zone;

10 wherein a colour change of the first dye in the sample detection zone indicates the presence of ethanol in the vapour sample and the difference in the colour intensity of the first dye in the sample detection relative to the second dye in the control zone determines the concentration of ethanol present in the vapour sample.

15 15. The method according to claim 14, wherein the step of image analysis further comprises:

normalising the colour intensity measured of the first dye in the sample detection zone relative to the colour intensity of the second dye in the colour zone; and

calculating and obtaining a value of normalized colour intensity, R, of the first dye in the sample detection zone relative to the second dye in the control zone,
20 wherein the value R is determined by the following equation (I):

$$\text{Normalized colour intensity, } R = (I_s / B_s) / (I_c / B_c) \quad (I)$$

wherein

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I_s denotes the colour intensity of the first dye in the sample detection zone,

B_s denotes the colour intensity of the white background near the sample detection zone,

I_c denotes the colour intensity of the second dye in the control zone,

30 B_c denotes the colour intensity of the white background near the control zone.

16. The method according to claim 15, wherein a colour change of the first dye in the sample detection zone is indicated by the value of normalized red colour intensity, R.

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17. The method according to claim 16, further comprises:
comparing the value R to a calibration curve to determine the concentration of ethanol in the vapour sample.
- 5 18. The method according to claim 15, wherein the step of image analysis is performed by the image capture device.
19. The method according to claim 15, wherein the method further comprises:
transmitting the captured image to an image analysis device for analysis,
10 wherein the step of transmitting is carried out by the image capture device, configured to transmit data to the image analysis device.
20. The method according to claim 19, wherein the step of image analysis is performed by the image analysis device.
- 15 21. The method according to claim 18 or 20, wherein the image capture device or the image analysis device comprises a processor configured to calculate and obtain the value of normalized colour intensity R, of the first dye in the sample detection zone relative to the colour intensity of the second dye in the control zone.
- 20 22. The method according to claim 15, wherein the method further comprises:
presenting a message via a user interface, wherein the message includes results of the analysis of the vapour sample.
- 25 23. The method according to claim 14 or 15, wherein the vapour sample is a breath sample.
24. The method according to claim 14 or 15, wherein the vapour sample is vapour from an alcoholic beverage.
- 30 25. The method according to claim 14 or 15, wherein the vapour sample is vapour from an ethanol/water mixture.
26. The method according to claim 24, wherein the method further comprises:
35 determining, depending on the value of the concentration of ethanol obtained, if the beverage is authentic.