A paper-based colorimetric spot test for the identification of adulterated whiskeys

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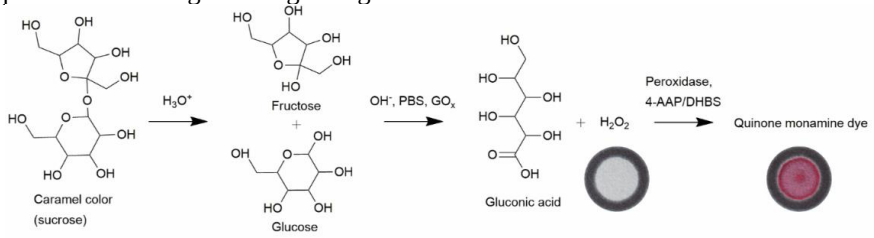
Abstract: *Paper-based analytical devices coupled with colorimetric detection were developed for point-of-need testing of adulterated whiskeys based on adding of caramel. An acid hydrolysis step was performed to generate glucose, which was then detected through an enzymatic assay. The pixel´s intensity was measured in different channel for comparison of analytical parameters like sensitivity and detectability levels. Principal component analysis demonstrated to be a powerful tool for screening at the crime investigation place, especially when amateur adulteration is used. The calibration curve in magenta channel and the mensuration of the brown color by pre-concentration in yellow channel were used to investigate sophisticated adulterations. This method was explored to analyze 47 samples of seized whiskeys by Brazilian Federal Police. The proposed approach allowed the identification of 90% (42) seized samples as adulterated samples. The final cost of the analysis was $0.02 and the volume necessary by analysis was 110 µl of sample.*

Key-Words*: colorimetric detection, whiskey, adulteration, caramel color, Principal Component Analysis*

Introduction: Paper-based analytical devices (PADs) have demonstrated great potentiality to be used as powerful tool for application in different research fields as environmental monitoring, clinic assays, forensics, food and beverage quality testing and adulteration assessments. The developed methods in PADs have showed high sensitivity and selectivity. The most popular instrumental techniques to analyze the authenticity of alcoholic beverages are high-performance liquid chromatography (HPLC)[1] and mass spectrometry (MS)[2], which require expensive instrumentation and large amount of chemicals and samples. The PADs are cheap, lightweight, globally affordable and they can be used for on-site assays with minimal sample/reagents consumption. The whiskey adulteration may be proceeded by different ways including simple dilution with tap water, whiskey from cheaper brands or even sugar cane spirits. In addition, the original color of authentic whiskey can be mimicked with the addition of caramel color. The latter is a good marker to detect adulteration because it is used to correct the color between fake and original samples. Counterfeiters use the caramel color to standardize the color intensity between fake and original whiskey. This step is used for customers do not see difference at naked-eye. Based on the the possibility to use a point-of-need device, which could be useful for crime scene investigation (CSI), this study presents an easy method using PADs with colorimetric detection and principal components analysis (PCA) for rapid screening of the authenticity of whiskey samples.

Experimental: The PADs were drawn in Corel Draw™ in a "spot test" layout fabricated using wax-print technique.[3] The diameter of the zone was 10 mm. The reactions were divided in some steps as present in the **Scheme 1**, first, the sample (10µL) was spotted follow by citric acid (5 µL) for hydrolysis. After hydrolysis step was added NaOH (5 µL) and phosphate buffer (5 µL) to neutralization and conditioner the medium for enzymatic reaction. Lastly, a solution with mixture of glucose oxidase (GOx)/peroxidase (PER) with 2-4-aminoantipyrine (4-AAP)/3,5-dichloro-2-hydroxybenzesulfonate (DHBS)(10µL). The GOx reacted with the glucose generated by hydrolysis of the caramel color/sucrose generating hydrogen peroxide. The hydrogen peroxide reacted with 4-AAP/DHBS in presence of PER generating a magenta color.



Scheme 1 Reaction scheme for the identification of caramel/sucrose and glucose in adulterated whiskeys, with spot tests showing the generation of a magenta color.

The capture of the colorimetric signal was performed after 25 minutes of the adding of enzyme/chromogenic solution, the device was scanned with resolution of 600 dpi. The captured image was analyzed in Corel Photo Paint™ through histogram tool in different color channels (red-green-blue (RGB), cyano-magenta-yellow-key (CMYK), magenta and yellow).

Results and discussion: The pixels intensity in RGB, CMYK, yellow and magenta color channels of 47 seized whiskey sample of 4 different whiskey brands and original samples of the whiskeys was utilized for analyses by PCA. The PCA showed powerful tool for a fast screening between fake-whiskey and original samples. **Figure 1** show PCA graph, just PCA was able identify 75% (35 samples) seized sample as adulterated. The seized samples that by PCA tool was not identify as adulterated were submitted the comparison with calibrate curve. The calibrate curves were performed with range concentrate between 0 – 650 µL/L of caramel color in original whiskey. In the search of limit of detection (LOD) lower was make a study about the sensibility of each color channel (RGB, CMYK and magenta). The comparison of sensibility was performed by slope of each calibration curve analyzed in a different color channel (RGB, CMYK and magenta). The magenta channel shown the color channel with higher incline as can be noted in **Figure 2**. The Ballantines whiskey had the follow slope results 0.044 (RGB), 0.063 (CMYK) and 0.098 (Magenta) (slope units are in A.U. per caramel color concentration (µL/L)). The Ballantines whiskey was example but all whiskeys brand had the same behavior. The LOD Ballantine's brand in magenta channel was 79 µL/L of caramel color in original whiskey. The 12 seized samples that did not identify as adulterated by PCA were compared the pixels intensity with calibration curve in magenta color channel of each whiskey brand. The calibrate calibration curve identified more 4 samples as adulterated.

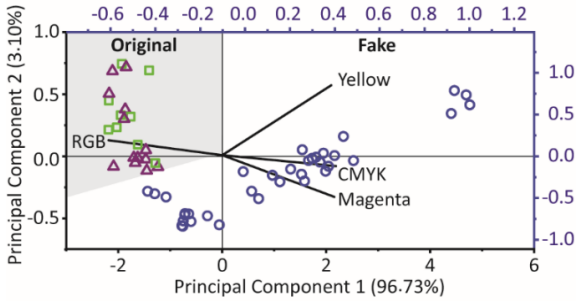


Figure 1 Principal Component Analysis (PCA) for screening of seized whiskey samples. The blue circles and purple triangles are sized samples, identified by PCA as fake whiskey and original whiskey respectively, as indicated by the white and grey shaded areas. The green squares are original whiskeys from 6 different brands (Red Label, Black Label, Jack Daniels, Ballantines, Chivas Regal and White Horse).

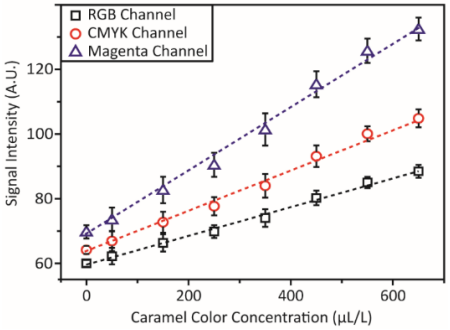


Figure 2 Change in signal intensity on addition of caramel color to Ballantines whiskey, using the Magenta (triangle), CMYK (circle) and RGB (square) color channels for analysis.

Caramel color is used as additive to make the correction of color in fake whiskey from this information was performed the comparison of the brown color between seized and original whiskeys. In the PADs was spotted 100 µL of samples only and waited the drying. The pixels intensity of spot was measured in yellow channel color. The comparison of the brown color shown that more 3 samples presented difference between original and adulterated whiskey. The method was able to identify 42 (90%) seized samples as adulterated. The cost final of the method was $ 0.02 per analysis. The 10 random seized samples of 2 different brands that by our method were identified as adulterated were send to official laboratory to make analysis to identify adulteration by caramel color/sucrose. The official method is based in a titration being necessary 50 mL for analysis. The official analysis shown that 6 samples were adulterated, 4 samples the results of analysis was lower than limit of quantify.

Conclusion: The protocol using PADs, colorimetric detection and PCA showed a great tool to identify adulteration in whiskey based in caramel color/sucrose as marker. These devices can be easily used by non-expert staff in the field, being possible the same staff make simultaneous analysis, using samples volume 500x lower than official method in Brazil and the cost of $0.02. This method identified that 90% of the samples provided by Federal Police had differences in brown color or concentration of caramel color/sucrose.

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