A New Possible Alternative Colorimetric Drug Detection Test for Fentanyl

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Abstract
A new colorimetric test using eosin Y has been developed to detect fentanyl and fentanyl mixed with cocaine and hydrocodone. This assay can be performed in solution or on a solid support, such as a piece of paper. While fentanyl can be tested with the established and currently used Marquis reagent and cocaine with the Modified Scott test, eosin Y may be an attractive alternative or complimentary test for drug identification.

Keywords: Fentanyl; Colorimetric; Sensor

Introduction
Law enforcement and correctional officers use colorimetric spot tests to make a quick on site presumptive identification when a potential drug is seized from a suspect. Modern instrumental methods for drugs of abuse have yet to replace wet chemical colorimetric assays for rapid lab and field screening of suspected material [1-15]. Instrumental techniques can be time intensive and costly, requiring technical expertise in addition to being location-bound. Alternatively, simple colorimetric assays (i.e. “spot tests”) offer speed, simplicity of operation, portability, and affordability [12-15]. The stability and versatility of these spot tests enable lab scientists to “triage” samples for additional drug analysis, as well as providing quick answers to law enforcement officers or crime scene analysts in the field. A number of spot tests, e.g. Marquis, Duquenois-Levine, and Scott, utilize an array of reagents with various handling requirements [12-15].

These tests often use caustic reagents, such as strong acids or bases [12-15]. While demonstrating impressive analytical power, these spot tests are often characteristic for a class of compounds relying on the reactivity of a specific chemical functional group. Fentanyl is an opioid that has been used in palliative care because it is more potent than morphine [16]. Many overdoses and deaths have been associated with fentanyl when mixed with other cocaine or opioids like hydrocodone and heroin [17]. Forensic scientists, law enforcement, and correctional officer’s use color spot tests to conduct presumptive testing of these analytes [18]. Herein we introduce eosin Y, a new spot test device for lab and potential field use. Eosin Y can be used as a mix-and-measure assay or a spot test on paper providing a stable color change for the rapid detection of fentanyl and a mixture with cocaine or hydrocodone.

Methods and Procedures:
a. Mix and Measure Assay: Marquis and Modified Scott Test: Tests A (Marquis) and G (Modified Scott) were purchased from the Safariland Group (NIK Public Safety), and the procedure was performed as per operator instructions included with the NIK test kit. Eosin Y was dissolved at 400mM either in pH=7 phosphate buffer or pH=5 acetate buffer to yield a 150 μM stock solution. 150 μL of the eosin Y solution was placed into wells of a 96 well optical bottom plate. 150 µL aliquots of fentanyl and fentanyl/cocaine/hydrocodone (10mg/ml) solution were added to the eosin Y wells. Color changes were instantaneous, and the plates were immediately scanned with a desktop scanner.
b. Paper Assay: Eosin Y was dissolved in a print solution adapted from a previously reported formulation. The formulation was added to the print cartridge in a commercial desktop printer and then printed on paper [19].
c. Drugs: Fentanyl, cocaine, and hydrocodone were purchased from Sigma Aldrich with a DEA license.

Results and Discussion
Figure 1 shows the results of when fentanyl was tested with the NIK-A test (Marquis Test) and cocaine with NIK-G test (Modified Scott Test). With the NIK-A test, fentanyl immediately afforded an orange color that darkened and changed to brown over 3-minutes. When cocaine was tested in the NIK-G test, the final result was a top pink layer over a bottom blue layer. These results agree well with the published color charts from the Safariland Group. Figure 2 shows a scanned image of fentanyl
and fentanyl mixed with hydrocodone and cocaine in eosin Y in a mix and measure assay. When compared to the control (water), the color changes are visible to the naked eye in phosphate buffer (eosin Y, pH= 7) and acetate buffer (eosin Y, pH=5), indicating that fentanyl can be easily discriminated in eosin Y and identified when compared to a control, such as water which readily dissolves fentanyl.

**Figure 1:** Results of NIK-A and NIK-G from the test kit. NIK-A with fentanyl shows a color change to orange and then to brown. NIK-G shows a bi-phasic color change with top layer pink and bottom layer blue in the presence of cocaine.

Figure 2: Scanned images of 96-well plate excerpts showing eosin Y reacting with fentanyl, hydromorphone, cocaine and the mixtures leading to distinct visible color changes in two different buffers (phosphate and acetate).

**Figure 3:** Eosin Y printed on paper with a commercial desktop printer. Fentanyl changes the paper to a darker pink and hydromorphone changes the color to a violet. Cocaine and water washed the sensor away.

**Conclusion**

Eosin Y has excellent potential for use in the field. The dye is “inactive” until use. Other practical benefits of this assay include ease-of-use, low sample volume requirements, and the use of safe and non-toxic reagents. The data shown in the mix and measure assay reveals a reasonably high specificity among fentanyl, and common mixing agents for fentanyl, like cocaine and hydromorphone. The paper assay is more limited as it does not allow cocaine to react with the sensor. However, fentanyl and hydromorphone interact well with the paper assay and lead to visible color changes. Research is ongoing with other controlled substances and cutting agents to explore the utility of eosin Y as a drug sensor. In conclusion, the eosin Y test could present an alternative or complimentary spot test to the commercially available NIK Tests.

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