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# Rapid and semi-quantitative presumptive tests for opiate drugs

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ABSTRACT

Digital image analysis was applied to the products of simple colour presumptive tests for opiates. Adobe Photoshop software was used for colour analysis to obtain analytical data in the form of a Red Green Blue (RGB) value. Calibration curves were developed for morphine, codeine, and diamorphine hydrochloride and the developed tests successfully applied to seized heroin samples to demonstrate the application of the technique in a forensic case context. Good agreement with gas chromatographic quantification results was obtained for the illicit samples analysed and a wide linear range and low detection limit for all drugs under test facilitated the application to illicit samples. The results show great potential for use as a semi-quantitative field test for illicit drug compounds.



## 1. Introduction

Opiates are a group of drug compounds either derived directly from opium or prepared as a result of chemical modification of alkaloids extracted from opium and include morphine, codeine and diamorphine. The term is also more generally applied to other (including synthetic) compounds which provide a comparable pain relieving activity to morphine and related compounds [1]. The United Nations World Drug Report 2010 stated that while the global consumption of opiates remains relatively stable at 12.8–21.9 million people, there has been significant growth in the production of end use illicit compounds such as heroin on the Global illicit market [2].

The United Nations International Drug Control Programme has recommended four rapid testing methods for opiates, which are the Marquis, Mecke, Nitric acid, and Ferric sulfate tests [1]. Each of these tests have been widely used as qualitative presumptive tests in forensic science laboratories, however the Marquis and Nitric acid test are those most reported.

Opiates produce a reddish-purple product with the Marquis test reagents [1] while an initial orange coloured product is produced during the Nitric acid test, rapidly changing to red and then slowly to yellow to indicate the possible presence of morphine. The initial orange colour slowly changing to yellow indicates the presence of codeine and a change to green indicates the presence of diamorphine [1]. The Nitric acid test provides a potential differentiating test for morphine, codeine, and diamorphine, however it is recommended that it is used in tandem with the Marquis test for best results.

In this work, the potential value of extending the application of the Marquis and Nitric acid tests was investigated through the application of digital image based analysis to the developed coloured products. This has a significant potential for operational impact and value within forensic drug testing as in many cases drug seizures occur remote to analytical facilities and a rapid means of semi-quantitative determination of the target drug at point of seizure would be advantageous. The National Forensic Science Protocol for Scotland [3] specifically mentions the use of presumptive testing as a means of providing a sufficiency of evidence in certain drug cases. As such, the ability to provide semi-quantitative analysis rather than simple identification is advantageous. Secondly, in some developing countries, forensic science laboratories may not have access to a wide range of equipment which facilitates the quantification of drug samples, or field drug testing may be so far removed from laboratory facilities that quantification becomes impractical. A functional semi-quantitative test has obvious advantages in these cases. Finally, tests such as those proposed provide obvious and rapidly available point of seizure information for police intelligence purposes facilitating an agile operational response.

Digital image based analysis evaluates the RGB data (Red Green Blue basic colour data) obtained from digital images [4,5]. Within the digital camera, the reflected light from objects passes through

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and is detected by three different filters: Red, Green, and Blue. Results are obtained as individual RGB values, and the final colour is composed from the additive data of the three RGB filters. The RGB values can be exploited to produce a data set derived from the capture of digital images of colourimetric presumptive tests using standards of known concentration. This then provides a basis by which semi-quantitative analysis of illicit samples containing an unknown quantity of material can be undertaken.

The use of digital image analysis has been highlighted for analytical applications in the determination of elements such as iron (III), aluminium (III), and titanium (IV) [4,6–8,10,11]. RGB values were measured with the image processing tool box in Matlab's image processing tool box [6,7], Kylix version 3.0 [8,9], or Visual basic version 6.0 [10,11]. To the best of our knowledge, only our previous report reveals an application of these methods to the detection of illicit drug compounds (amphetamine and methylamphetamine) using Adobe Photoshop version 7.0 [12] which has also been previously reported as a means of analysing the colour intensity of hand scanner images [13]. In digital colourimetry, the colour of the products is obtained by the combination of both the individual RGB data and $I_{TOTAL}$, the total RGB intensity value, which has been demonstrated to contain information not included within the individual data [6].

This present work seeks to demonstrate the application of digital image analysis to the detection of opiates (morphine, codeine, and diamorphine) using the Marquis and Nitric acid tests.

## 2. Materials and methods

### 2.1. Chemicals

Diamorphine hydrochloride and morphine tartrate (BPC 1959) were obtained from Macfarlan Smith Limited (Edinburgh, Scotland). Codeine hydrochloride (Fluka), concentrated sulfuric acid, glacial acetic acid, formaldehyde, concentrated nitric acid, and methanol (AR grade) were all purchased from Sigma–Aldrich Company Ltd, Dorset, UK.

### 2.2. Photographic system

A Canon EOS 20D digital camera (22.5 mm × 15.0 mm, 12-bit RGB CMOS sensor) was used throughout the experiments. In order to establish a calibration curve for each drug, a series of test tubes each containing a known amount of the drug under test and the test reagents were photographed against a white background to eliminate any potential interferent colours. Once the presumptive test reaction was complete, six photographs were taken for each experiment The camera was set to automatic focus, automatic white balance, automatic sensitivity (where the ISO speed was set within 100–400) and captured in single image mode. Each image was 2.55 MB (3504 × 2336-pixel) and was recorded as a JPEG (24-bits) on a Lexar 2 GB 80X Professional CF (compact flash) card.

Images were transferred to a computer using Microsoft Photo Editor (Microsoft XP). The average colour intensity of Red, Green, and Blue of each colour product in each test tube were obtained using the "Crop" tool and the "Histogram" in Adobe Photoshop (version 7.0). The data were transferred into an Excel (version 12.2.6) spreadsheet for subsequent data analysis.

### 2.3. Colourimetric presumptive test methods

Morphine tartrate, codeine hydrochloride and diamorphine hydrochloride were diluted in methanol to the required concentration. Two hundred microliters of each drug standard in methanol was transferred to a test tube and the appropriate test reagents added sequentially. In each case, the presumptive test reaction was firstly optimised to determine the volumes of various reagents required to produce the darkest colour reaction for the least concentrated solution. The resultant colours were photographed after 3 min, except the Nitric acid test of diamorphine hydrochloride which required 5 min for the colour to stabilise.

Each presumptive test was repeated 6 times. The linear range was investigated in the range of 0.10 to10 mg mL$^{-1}$ for all drug compounds. The average intensities of the Red, Green and Blue colours from each of the 6 images obtained for each standard solution were investigated using Adobe Photoshop and a calibration graph was prepared for each colour. The limit of detection for both drug compounds was calculated [14] and precision was expressed as the percentage relative standard deviation of the intensity for each colour from the 6 images analysed.

#### 2.3.1. Marquis test

Two reagents are required for the Marquis test, 2.5% (v/v) formaldehyde in glacial acetic acid (reagent 1A) and concentrated sulfuric acid (reagent 1B) [1]. The optimised conditions were used for all test solutions as follows: Reagent 1A (50 μL) was firstly added to the drug solution followed by reagent 1B (400 μL). The solution was then mixed by shaking and left to stand for 3 min prior to photography.

#### 2.3.2. Nitric acid test

Concentrated nitric acid is the only reagent used in the Nitric acid test. After an appropriate volume of concentrated nitric acid (200 μL for morphine and 400 μL for both codeine and diamorphine) was transferred to each drug solutions, the colour change was noted before mixing. The solutions were photographed after 3 min post mixing for the morphine and codeine samples, and after 5 min for diamorphine.

### 2.4. Heroin street sample

A seized heroin sample was analysed using the Marquis and Nitric acid test. Heroin (5 μg) was extracted with 2 mL of methanol and sonicated for 10 min. The supernatant was analysed using both presumptive tests and gas chromatography–mass spectrometry (GC–MS). All extractions and analysis were repeated in triplicate.

### 2.5. Gas chromatography–mass spectrometry analysis

An Agilent 6850 gas chromatograph (Agilent Technologies Incorporated, Palo Alto, California) equipped with mass spectrometer Model 5975C and 6850 Series injector was used for comparison and confirmation. The HP-5MS capillary column (30 m length × 0.25 mm id × 0.25 μm film thicknesses) was used. Diamorphine hydrochloride standard solutions (1 μL) in methanol were injected and analysed with a split ratio of 25 to 1 with carrier gas (high-purity-grade helium) at a flow rate of 1.0 mL min$^{-1}$. The column was kept at 200 °C for 1 min, then increased at a rate of 20 °C min$^{-1}$ to 300 °C and held for 6 min. The inlet and transfer line were constantly kept at 260 °C and 280 °C, respectively. The mass spectrometer was operated in the electron ionization mode at 70 eV. Mass spectra were obtained in the full scan mode (50–550 amu). Chromatographic separation was monitored in TIC mode.

The linear range for diamorphine hydrochloride was calibrated between 0.1 and 10 mg mL$^{-1}$ with 6 replicate injections for each concentration.