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Rapid and semi-quantitative presumptive tests for opiate drugs

Aree Choodum[a,1], Niamh Nic Daeid[b,*]

[a] Department of Applied Science, Faculty of Science, and Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand
[b] Centre for Forensic Science, Department of Pure and Applied Chemistry, WestCHEM, University of Strathclyde, 204 George Street, Glasgow G1 1WX, UK

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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

* Corresponding author. Tel.: +44 141 5484700; fax: +44 141 5482532.
E-mail addresses: aree.c@psu.ac.th (A. Choodum), n.nicdaeid@strath.ac.uk (N. Nic Daeid).
[1] Tel.: +66 07428 8102; fax: +66 07444 6681.

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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.

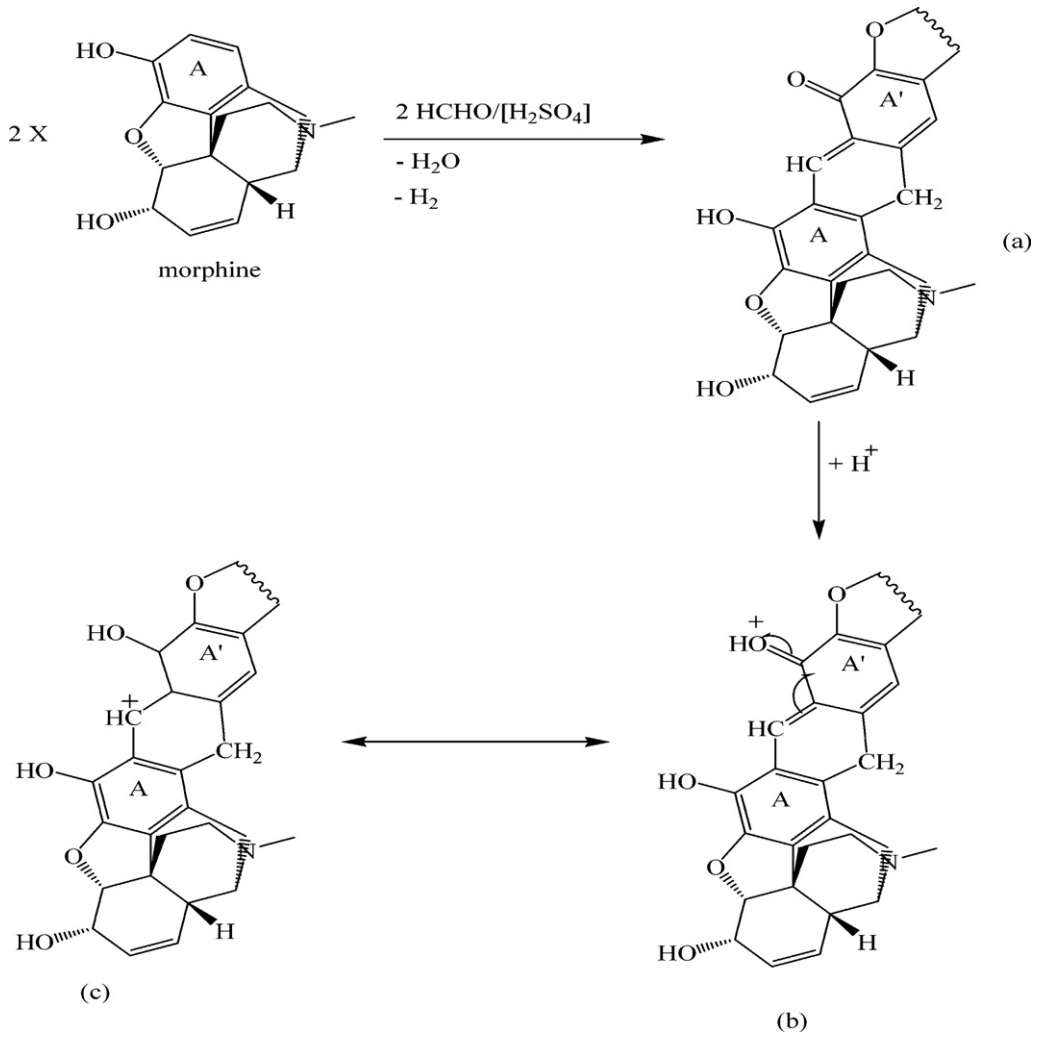
2.4. Heroin street sample

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.



Scheme 1.

3. Results and discussion

3.1. Colourimetric presumptive testing of opiates

3.1.1. Marquis test

Opiates provided a purple coloured product after 3 min due to the formation of oxonium–carbenium salts (Scheme 1) [15]. Two molecules of opiate (for example of morphine in Scheme 1) and two molecules of formaldehyde condense in the presence of concentrated sulfuric acid to the dimeric product (a) in Scheme 1, which is protonated to oxonium–carbenium salts (b/c) in Scheme 1 [15].

3.1.2. Nitric acid test

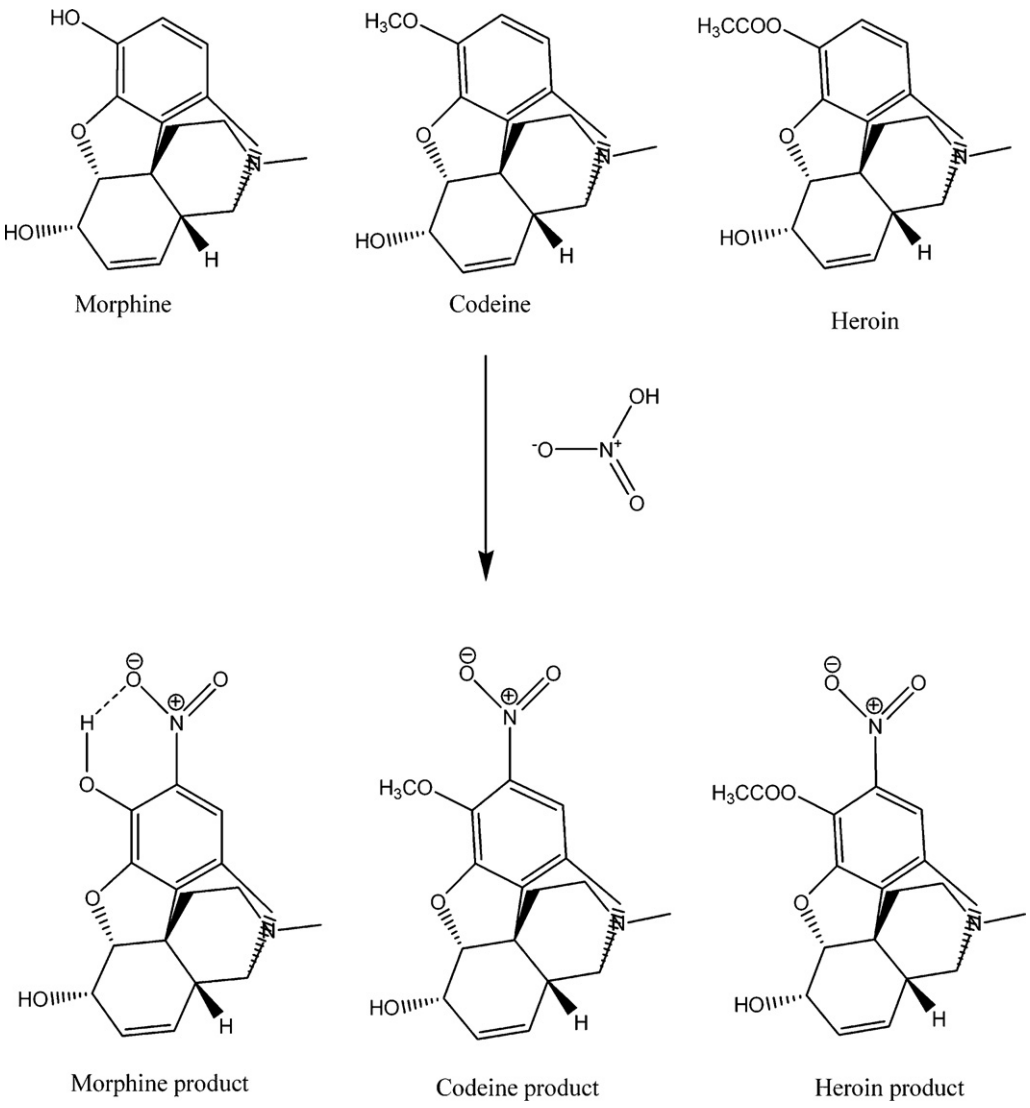
The reaction of concentrated nitric acid with opiates results in the formation of the appropriate coloured nitroproduct [15]. Morphine, codeine, and diamorphine are nitrated at C-2 (Scheme 2). The nitroproduct of morphine forms a hydrogen bond between the nitro group and hydroxyl group, while this is impossible for the O-substituted derivatives, *i.e.* codeine and diamorphine [15]. This resulted in the formation of a different coloured product for each compound. The reaction product for morphine rapidly changed from orange to red and then slowly to yellow. The initial orange product generated in the presence of codeine slowly changed to yellow and then to light green in the presence of diamorphine [1]. Solvation of the drug compounds in methanol did not alter the colour reactions observed.

It was found that a higher volume of nitric acid was required for the reaction with the O-substituted derivatives of morphine in order to produce visible nitroproducts (400 μL compared to 200 μL for morphine). A longer reaction time of 5 min was also required for the reaction with diamorphine to go to completion compared to 3 min for codeine and morphine. This was presumably due to the larger O-substituted group (CH_3CO group) at the C-3 position of diamorphine compared to the smaller of CH_3 and H for codeine and morphine, respectively.

3.2. Digital images analysis for quantification of opiates

The analytical (RGB) data ranging from 0 to 255 for each channel (RGB) were obtained from a standard trichromatic response of a digital image [4,6,8,10]. That is, if the image returned from the camera was black, the user would obtain a R, G, B value of 0, 0, 0, respectively, and similarly an R, G and B value of 255, 255, 255 would be obtained for a white image. The colours obtained from each of the three channels are subtractive colours and selectively absorb certain wavelengths of light, thus affecting the observed colour [5,11].

A simple system was set up for photographing the coloured products obtained from the colourimetric test of opiates as previously described. Each colourimetric reaction was undertaken six times for each standard solution and each sample analysed. This allowed any variation in precision due to the experimental method



Scheme 2.

to be accounted for including any inhomogeneity of illumination, curvature of the glass test tubes [11] or any variation of colour formation. No inconsistency of colour from any of the sequential images was observed (0.17 to 4.93% RSDs).

3.2.1. Individual RGB value of coloured products

3.2.1.1. Marquis test. The Marquis test produced a violet to purple-reddish coloured product for opiates which darkened on increasing concentration. RGB values (I_R, I_G, and I_B) obtained from Adobe Photoshop were related to the concentrations of the compounds and are illustrated in Fig. 1. Morphine and codeine exhibited similar relationships to the individual RGB values obtained illustrating a similar intensity of the three colour components at each concentration. Diamorphine exhibited significant different individual RGB intensities however, all of the opiates tested demonstrated linearity at concentrations below 0.5 mg mL^{-1}.

The molecular absorption of coloured products was also investigated by calculating the absorbance at each concentration using Eq. (1):

$$A_X = -\log\frac{I_X - I_{X,b}}{I_{X,w} - I_{X,b}} = -\log\frac{(I_X)_c}{(I_{X,w})_c} = -\log\ R_X \quad (1)$$

where for each colour (R,B,G), A_X is the absorbance of X, I_X is the intensity of X, $I_{X,b} = 0$, $I_{X,w} = 255$, and R_X is the reflectance of light X and C is the concentration of X [13].

The relationship between the concentration of the drug compounds and the absorbance are presented in Fig. 2. The relations were similar to those commonly reported using spectrophotometric methods.

The highest sensitivity was observed for morphine and codeine and was obtained using the green colour component. Green is a complementary colour of purple and thus a high absorbance of this colour was expected given the presumptive test colour was purple. For diamorphine, the highest sensitivity was obtained from the blue colour component again highlighting the difference between the colour reactions obtained across the three opiate compounds.

3.2.1.2. Nitric acid test. The relationship between the intensities of each RGB colour component and the concentration of opiates used with the Nitric acid test are shown in Fig. 3 and those of the calculated absorbance are presented in Fig. 4. The results for the intensity and absorbance of the blue component (I_B and A_B) reflect the fact that the presumptive test product is yellow-orange in colour and as such the highest absorbance is observed in its complementary

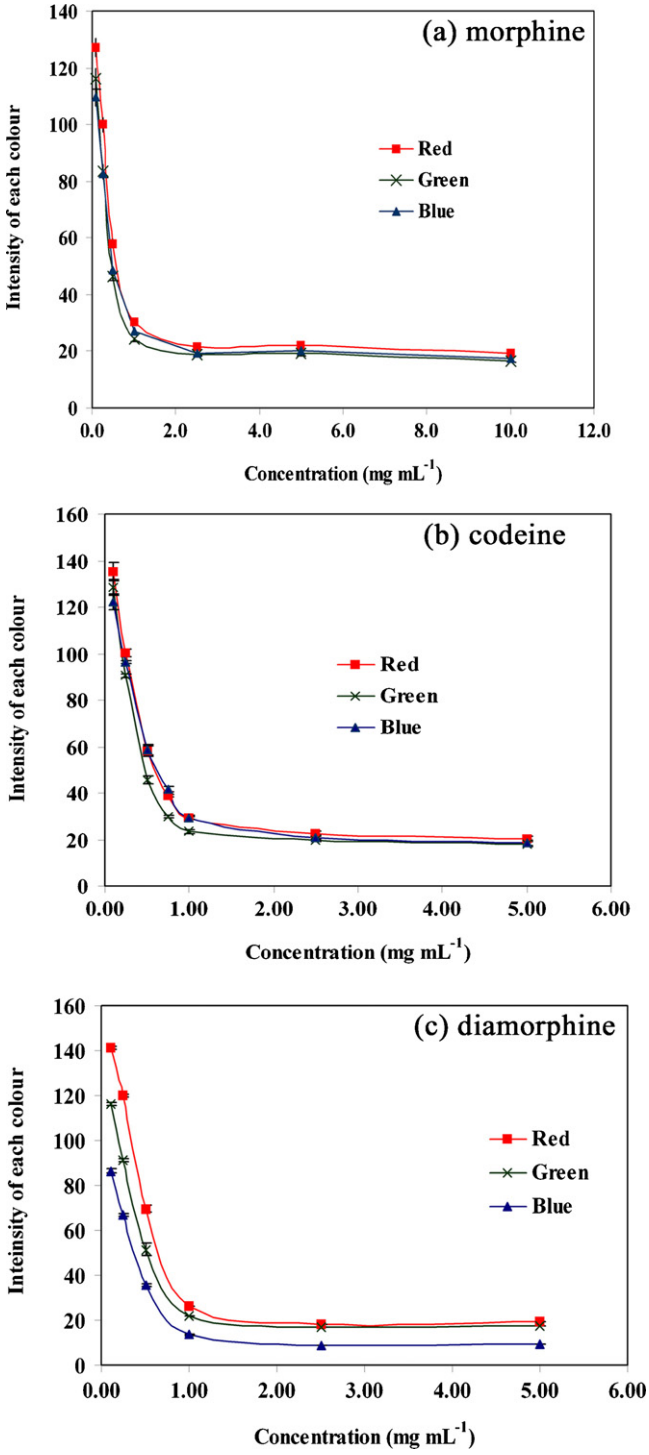


Fig. 1. Relationship between the intensity of each colour and the concentration of (a) morphine (b) codeine (c) diamorphine obtained from the Marquis test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

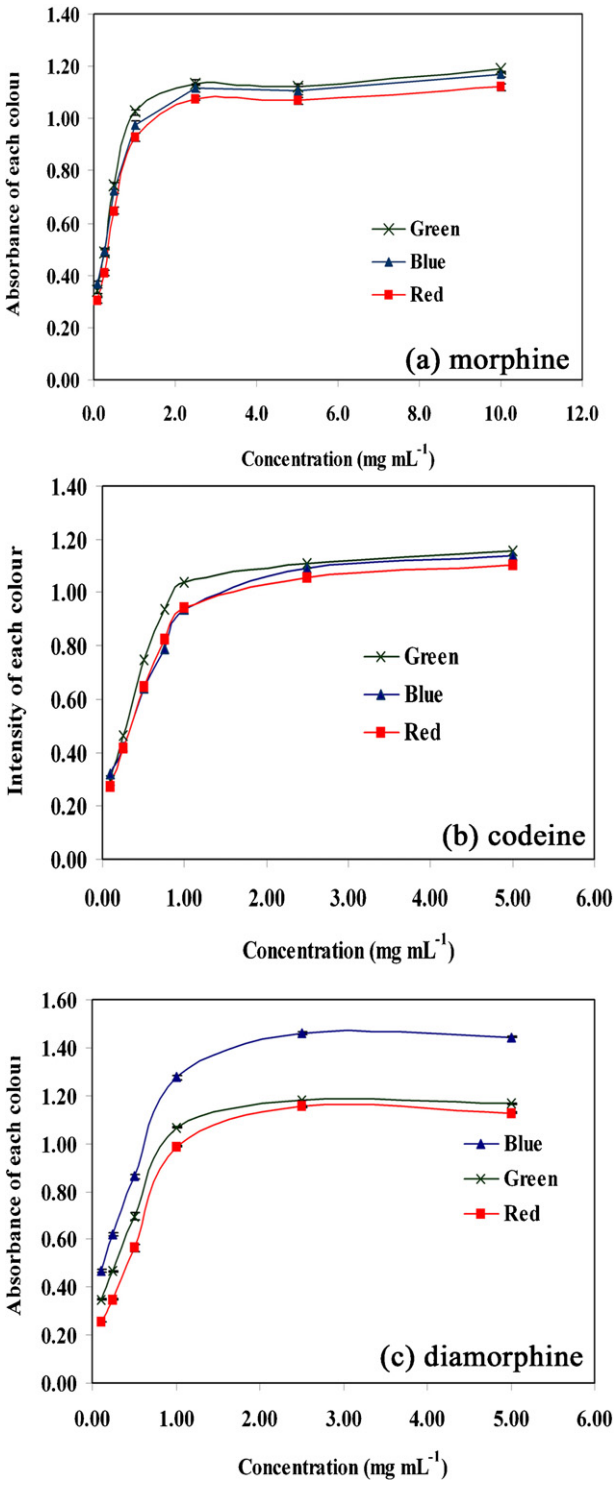


Fig. 2. Relationship between the absorbance of each colour and the concentration of (a) morphine (b) codeine (c) diamorphine obtained from the Marquis test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

colour (blue). Observations of the relationship profiles for the specific opiates are different with morphine differing markedly from codeine and diamorphine. This is reflected in the variations of the colour of the reaction products formed, for example the intensity of the green colour component (I_G) for morphine decreased with increasing concentration where as more constant values were obtained for codeine and diamorphine.

3.2.2. *Total intensities and total absorbance of coloured products*

In digital colourimetry, the colour of the products is obtained by the combination of RGB data. The relationship between the total intensity, the total absorbance and the drug concentration were also investigated. The total intensity, I_{TOTAL}, is defined as $I_R + I_G + I_B$, while total absorbance, A_{TOTAL}, is given by $A_R + A_G + A_B$. The relationship of I_{TOTAL} and A_{TOTAL} with drug concentration are presented in

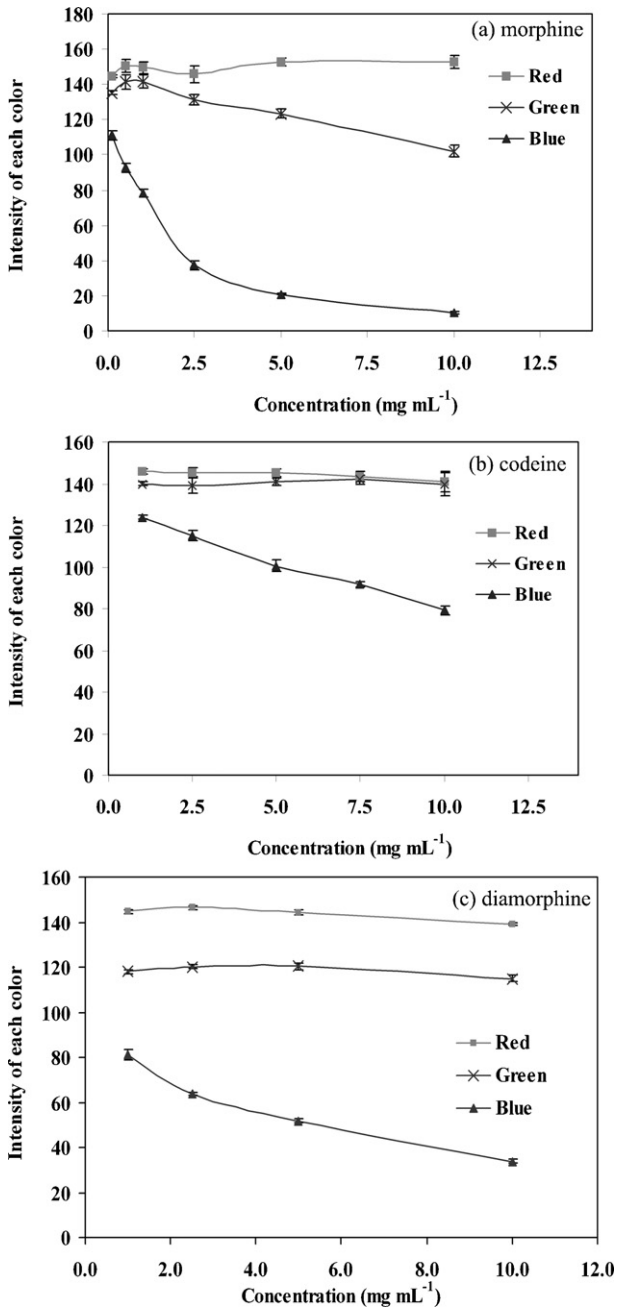


Fig. 3. Relationship between the intensity of each colour and the concentration of (a) morphine (b) codeine (c) diamorphine obtained from the Nitric acid test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

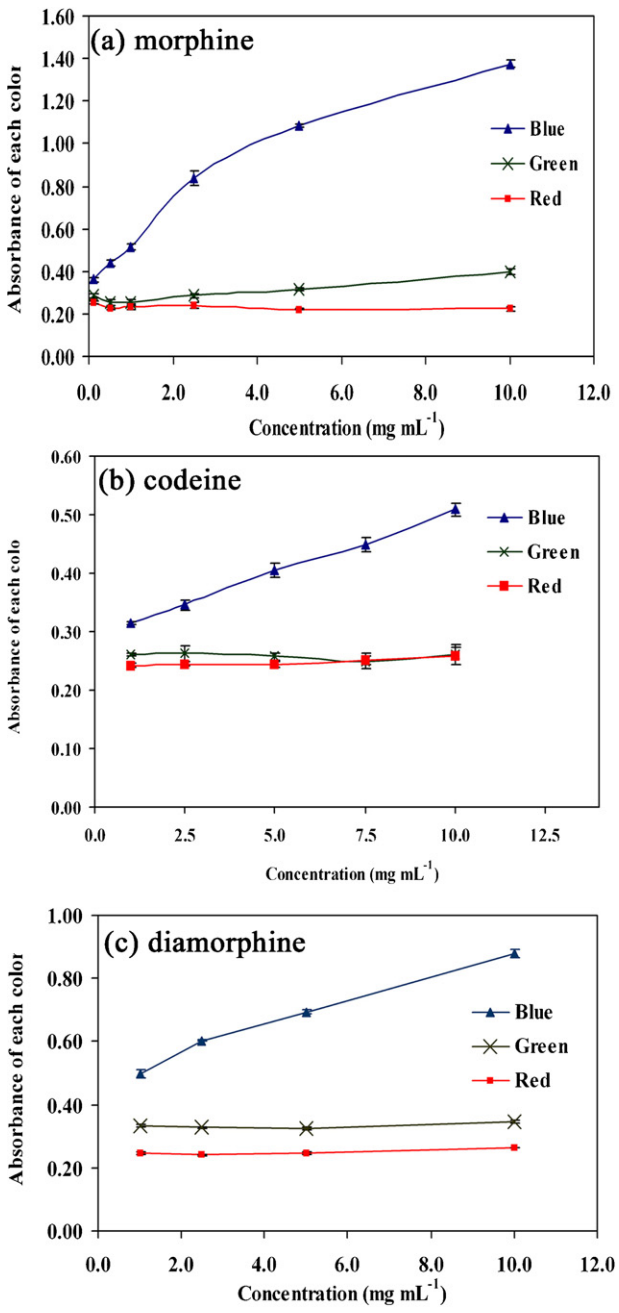


Fig. 4. Relationship between the absorbance of each colour and the concentration of (a) morphine (b) codeine (c) diamorphine obtained from the Nitric acid test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Fig. 5a and b for the Marquis test and Fig. 5c and d for the Nitric acid test. The linear range and their calibration equations were shown in Table 1.

Total RGB intensity and calculated absorbance obtained from the Marquis test demonstrated similar results for morphine, codeine and diamorphine at concentrations below 1 mg mL^{-1}. Significantly different results were obtained with diamorphine at higher concentrations. The Nitric acid test demonstrated significantly different results for all drugs over the investigated concentrations which confirms the discriminating ability of this test for the opiates under investigation.

The total RGB results are important when colours are compared since multivariate analysis of colour data has indicated that I_{TOTAL} contains information not included within the individual data [6]. Thus, the use of I_{TOTAL} and A_{TOTAL} for quantification could reduce the number of variables required from individual RGB data. However, in some cases, the sensitivity and linearity of I_{TOTAL} may be less than the individual RGB data reducing the test's sensitivity [6].

3.3. Analytical performance

The sensitivity, linear range, limit of detection, and precision in term of % RSD, were calculated for the analysed compounds and are presented in Tables 2 and 3. The relationship between absorbance and concentration demonstrated better linearity in general, while the total absorbance and intensity values provided better

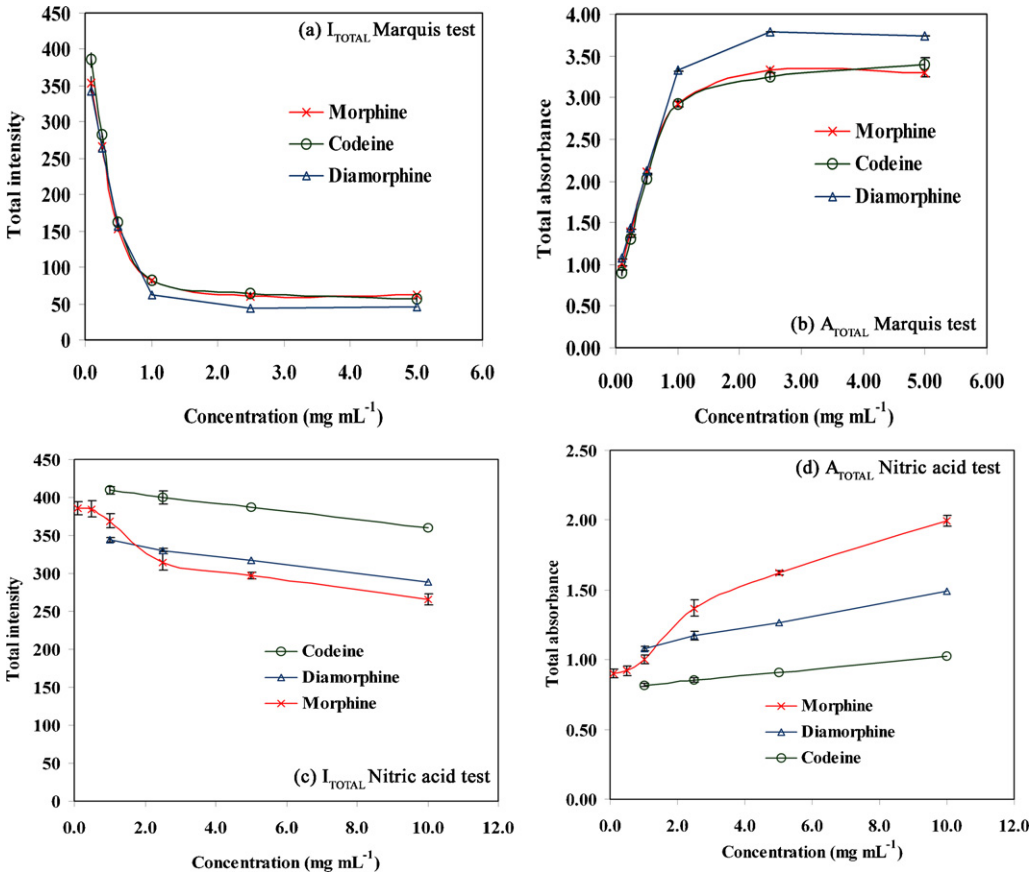


Fig. 5. Relationship between the total intensity (I_{TOTAL}) and the total absorbance (A_{TOTAL}) with the concentration of morphine, codeine, diamorphine (a) I_{TOTAL} from the Marquis test (b) A_{TOTAL} from the Marquis test (c) I_{TOTAL} from the Nitric acid test (d) A_{TOTAL} from the Nitric acid test.

sensitivity and a wider linear range than the individual RGB values for all drug compounds with the nitric test.

3.4. Heroin street sample analysis

An illicit heroin sample was tested with the Marquis test and Nitric acid test using the developed method. The colour changes were similar to those obtained from diamorphine hydrochloride for both tests. The sample was quantified using the presumptive test method and the results compared with the data obtained from GC–MS quantification and are presented in Table 4.

The relationship between I_R and the concentration, C, provided the most reliable and accurate results between the digital images and the target drug concentration and clearly demonstrates the correlation between the accurately determined concentration of diamorphine in the heroin sample and the semi-quantitative method. Example images and the chromatogram obtained from the illicit sample are presented in Fig. 6.

Table 1
Calibration equations of total intensity and absorbance (y: I_{TOTAL} and A_{TOTAL}; x: C: concentration (mg mL^{-1})).

Drugs	Relationships	Linear range	Calibration equation	
			Equation	R^2
Morphine				
Marquis	I_{TOTAL} and C	0.1–0.5	$y = -(496 \pm 34)x + (398 \pm 11)$	0.9954
	A_{TOTAL} and C	0.1–0.5	$y = (2.8 \pm 0.1)x + (0.72 \pm 0.04)$	0.9984
Nitric acid	I_{TOTAL} and C	0.5–2.5	$y = -(35.4 \pm 0.9)x + (403 \pm 1)$	0.9994
		2.5–10	$y = -(6.5 \pm 0.2)x + (331 \pm 1)$	0.9993
	A_{TOTAL} and C	0.5–2.5	$y = (0.23 \pm 0.02)x + (0.80 \pm 0.03)$	0.9944
		2.5–10	$y = (0.083 \pm 0.007)x + (1.18 \pm 0.04)$	0.9933
Codeine				
Marquis	I_{TOTAL} and C	0.1–0.5	$y = -(551 \pm 57)x + (433 \pm 19)$	0.9894
	A_{TOTAL} and C	0.1–0.5	$y = (2.85 \pm 0.04)x + (0.60 \pm 0.02)$	0.9997
Nitric acid	I_{TOTAL} and C	1.0–10	$y = -(5.32 \pm 0.3)x + (414 \pm 2)$	0.9925
	A_{TOTAL} and C	1.0–10	$y = (0.023 \pm 0.001)x + (0.794 \pm 0.008)$	0.9896
Diamorphine				
Marquis	I_{TOTAL} and C	0.1–0.5	$y = -(462 \pm 22)x + (385 \pm 7)$	0.9977
	A_{TOTAL} and C	0.1–1.0	$y = (2.52 \pm 0.04)x + (0.83 \pm 0.03)$	0.9994
Nitric acid	I_{TOTAL} and C	1.0–10	$y = -(6.0 \pm 0.4)x + (348 \pm 2)$	0.9933
	A_{TOTAL} and C	1.0–10	$y = (0.045 \pm 0.002)x + (1.04 \pm 0.01)$	0.9960

Table 2
Analytical performance of the proposed method from the Marquis test.

Drugs	Relationship[a]	Linear range (mg mL^{-1})	Sensitivity (unit: mg mL^{-1})	Linearity (R^2)	LOD[b] (mg mL^{-1})	% RSD	Accuracy (mg mL^{-1})	
							Known	Experiment
Morphine	I_R and C	0.1–0.5	172 ± 3	0.9996	0.0173 ± 0.0003	2.53–3.67	0.25	0.25 ± 0.01
	I_G and C	0.1–0.5	172 ± 18	0.9886	0.09 ± 0.01	1.41–4.02	0.25	0.28 ± 0.06
	I_B and C	0.1–0.5	151 ± 11	0.9941	0.067 ± 0.005	2.38–4.09	0.25	0.27 ± 0.05
	A_R and C	0.1–0.5	0.87 ± 0.07	0.9940	0.067 ± 0.005	0.72–3.70	0.25	0.23 ± 0.04
	A_G and C	0.1–0.5	1.01 ± 0.02	0.9996	0.0165 ± 0.0003	0.53–4.11	0.25	0.25 ± 0.01
	A_B and C	0.1–0.5	0.89 ± 0.02	0.9992	0.0238 ± 0.0007	1.08–3.50	0.25	0.24 ± 0.02
	I_{TOTAL} and C	0.1–0.5	496 ± 34	0.9954	0.058 ± 0.004	2.40–2.87	0.25	0.26 ± 0.04
	A_{TOTAL} and C	0.1–0.5	2.8 ± 0.1	0.9984	0.035 ± 0.001	1.65–3.70	0.25	0.24 ± 0.02
Codeine	I_R and C	0.1–0.5	191 ± 17	0.9922	0.076 ± 0.007	1.86–3.80	0.75	[c]
	I_G and C	0.1–0.5	204 ± 20	0.9909	0.082 ± 0.008	1.12–4.00	0.75	[c]
	I_B and C	0.1–0.5	158 ± 6	0.9987	0.031 ± 0.001	0.72–4.19	0.75	[c]
	A_R and C	0.1–0.75	0.85 ± 0.04	0.9962	0.065 ± 0.003	1.11–4.19	0.75	0.73 ± 0.03
	A_G and C	0.1–0.75	0.99 ± 0.07	0.9911	0.099 ± 0.007	0.85–3.95	0.75	0.73 ± 0.04
	A_B and C	0.1–1.0	0.70 ± 0.03	0.9939	0.099 ± 0.004	0.72–3.67	0.75	0.76 ± 0.04
	I_{TOTAL} and C	0.1–0.5	551 ± 57	0.9894	0.089 ± 0.009	0.98–3.98	0.75	[c]
	A_{TOTAL} and C	0.1–0.5	2.85 ± 0.04	0.9997	0.0138 ± 0.0002	0.95–3.80	0.75	[c]
Diamorphine	I_R and C	0.1–0.5	182 ± 16	0.9924	0.075 ± 0.006	0.44–3.80	0.75	[c]
	I_G and C	0.1–0.5	161 ± 1	0.9999	0.0073 ± 0.0001	0.37–3.29	0.75	[c]
	I_B and C	0.1–0.5	123 ± 2	0.9998	0.0119 ± 0.0002	0.39–1.35	0.75	[c]
	A_R and C	0.1–1.0	0.82 ± 0.02	0.9982	0.061 ± 0.002	0.22–2.92	0.75	0.67 ± 0.02
	A_G and C	0.1–1.0	0.80 ± 0.02	0.9980	0.065 ± 0.002	0.26–2.05	0.75	0.75 ± 0.03
	A_B and C	0.1–1.0	0.89 ± 0.03	0.9980	0.065 ± 0.002	0.44–1.91	0.75	0.71 ± 0.02
	I_{TOTAL} and C	0.1–0.5	462 ± 22	0.9977	0.041 ± 0.002	0.69–3.28	0.75	[c]
	A_{TOTAL} and C	0.1–1.0	2.52 ± 0.04	0.9994	0.0364 ± 0.0006	0.44–1.91	0.75	0.71 ± 0.01

[a] I = colour intensity, A = colour absorbance calculated using Eq. (1), C is the concentration of the prepared standards.
[b] LOD = $y_B + 3S_B$ [14].
[c] Out of linear range.

Table 3
Analytical performance of the proposed method from the nitric test.

Drugs	Relationship[a]	Linear range (mg mL^{-1})	Sensitivity (unit: mg mL^{-1})	Linearity (R^2)	LOD[b] (mg mL^{-1})	% RSD	Accuracy (mg mL^{-1})	
							Known	Experiment
Morphine	I_B and C	0.10–2.5	30 ± 2	0.9904	0.38 ± 0.03	1.70–4.68	0.25	0.28 ± 0.15
	I_G and C	1.0–10	4.2 ± 0.3	0.9912	1.36 ± 0.09	1.94–3.12	0.25	[c]
	A_B and C	0.10–2.5	0.199 ± 0.008	0.9966	0.227 ± 0.009	0.78 –4.32	0.25	0.32 ± 0.09
	A_G and C	1.0–10	0.0154 ± 0.0009	0.9935	1.17 ± 0.07	2.67–4.93	0.25	[c]
	I_{TOTAL} and C	0.5–2.5	35.4 ± 0.9	0.9994	0.107 ± 0.003	2.42–3.28	0.25	[c]
		2.5–10	6.5 ± 0.2	0.9993	0.44 ± 0.01	1.49–3.28	0.25	[c]
	A_{TOTAL} and C	0.5–2.5	0.23 ± 0.02	0.9944	0.33 ± 0.02	3.21–4.28	0.25	[c]
		2.5–10	0.083 ± 0.007	0.9933	1.3 ± 0.1	1.10–4.28	0.25	[c]
Codeine	I_B and C	1.0–10	4.89 ± 0.2	0.9928	1.10 ± 0.05	0.67–2.80	7.5	7.3 ± 0.4
	A_B and C	1.0–10	0.0214 ± 0.0006	0.9977	0.61 ± 0.02	0.98–2.97	7.5	7.1 ± 0.2
	I_{TOTAL} and C	1.0–10	5.32 ± 0.3	0.9925	1.10 ± 0.06	0.60–2.52	7.5	7.0 ± 0.4
	A_{TOTAL} and C	1.0–10	0.023 ± 0.001	0.9896	1.29 ± 0.08	0.95–2.84	7.5	6.9 ± 0.5
Diamorphine	I_B and C	2.5–10	3.9 ± 0.3	0.9948	1.17 ± 0.08	0.24–2.88	1.25	[c]
	A_B and C	2.5–10	0.0370 ± 0.0004	0.9999	0.166 ± 0.002	0.41–2.43	1.25	[c]
	I_{TOTAL} and C	1.0–10	6.0 ± 0.4	0.9933	1.19 ± 0.07	0.17–0.91	1.25	1.6 ± 0.5
	A_{TOTAL} and C	1.0–10	0.045 ± 0.002	0.9960	0.91 ± 0.04	0.28–1.32	1.25	1.4 ± 0.4

[a] I = colour intensity, A = colour absorbance calculated using Eq. (1), C is the concentration of the prepared standards.
[b] LOD = $y_B + 3S_B$ [14].
[c] Out of linear range.

Table 4
Heroin street sample results.

Sample no.	GC–MS (%)	Digital image analysis of Marquis test (%)[a]							
		I_R and C	I_G and C	I_B and C	A_R and C	A_G and C	A_B and C	I_{TOTAL} and C	A_{TOTAL} and C
1	27.9	28.2	36.1	41.3	30.6	36.4	39.4	33.7	35.4
2	26.1	25.4	32.3	37.9	27.9	31.2	34.5	30.4	30.9
3	25.3	24.9	31.9	37.5	27.2	31.2	34.8	30.0	30.9

[a] I = colour intensity, A = colour absorbance calculated using Eq. (1), C is the concentration of the prepared standards.
The GC–MS linear range was determined as 0.10–5 mg mL^{-1} with good linearity (R^2 = 0.9976) and good precision (% RSD < 3.56). The calibration graph of $y = 270.62x - 25.586$ from 0.10 to 2.5 mg mL^{-1} (R^2 = 0.9971) was used.

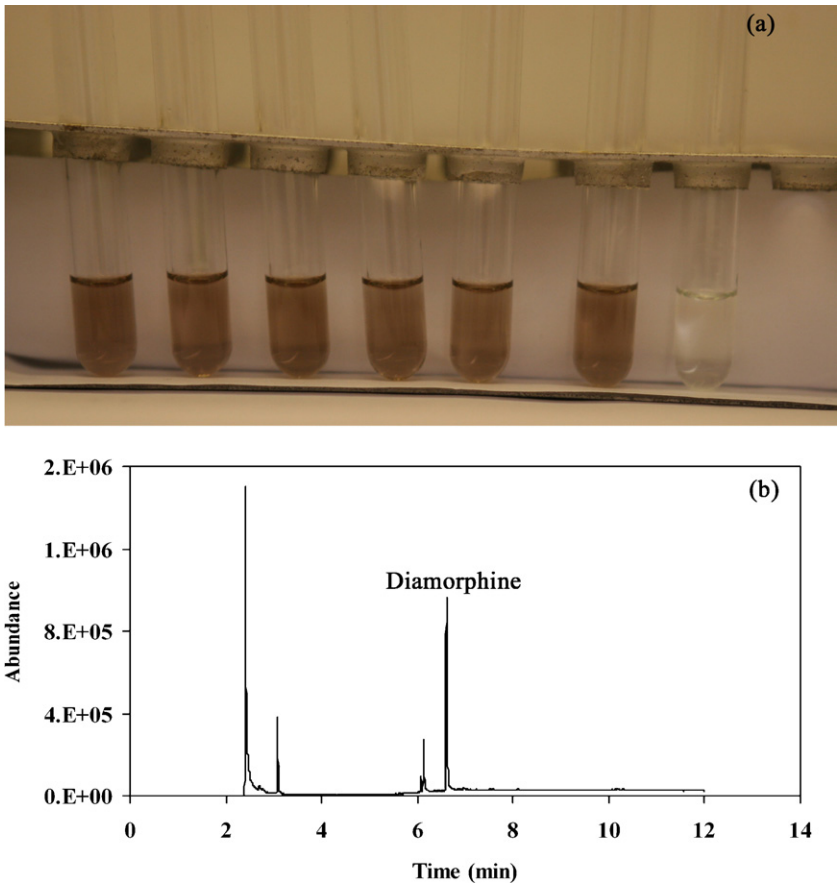


Fig. 6. Example of (a) the image obtained from a heroin street sample with the Marquis test (b) the chromatogram of the same extracted heroin sample analysed using GC–MS.

4. Conclusion

Digital image analysis of coloured test products of standard presumptive test has demonstrated a significant potential for the development of an accurate, rapid, portable and economically viable semi-quantitative test for the analysis of controlled substances. The methodology devised in this work is based on well known colourimetric presumptive tests and has been demonstrated to be reliable and accurate for the analysis of opiates.

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References

[1] United Nations International Drug Control Programme, Rapid testing methods of drugs of abuse. Manual for use by national law enforcement and narcotics laboratory personnel, 1994, United Nation: Vienna.
[2] World Drug Report 2010. 2010, United Nations Office on Drugs and Crime Vienna.
[3] The National Forensic Science Protocol for Scotland, http://www.copfs.gov.uk/Publications/2007/05/Protocol-National-Forensic-Science-Protocol-COPFSPolice (accessed 31.08.11).
[4] L. Byrne, J. Barker, G. Pennarun-Thomas, D. Diamond, S. Edwards, TrAC Trends in Analytical Chemistry 19 (8) (2000) 517.
[5] P.M. Epperson, J.V. Sweedler, R.B. Bilhorn, G.R. Sims, M.B. Denton, Analytical Chemistry 60 (1988) 327A.
[6] A. Lopez-Molinero, D. Lian, D. Sipiera, R. Falcon, Microchemical Journal 96 (2) (2010) 380.
[7] L.M. Goddijn, M. White, Estuarine, Coastal and Shelf Science 66 (3–4) (2006) 429.
[8] E.d.N. Gaiao, V.L. Martins, W.d.S. Lyra, L.F.d. Almeida, E.C.d. Silva, M.C.U. Arajo, Analytica Chimica Acta 570 (2) (2006) 283.
[9] W. Silva Lyra, V.B. dos Santos, A.G.G. Dionzio, V.L. Martins, L.F. Almeida, E. Nbrega Gaio, P.H.G.D. Diniz, E.C. Silva, M.C.U. Arajo, Talanta 77 (5) (2009) 1584.
[10] N. Maleki, A. Safavi, F. Sedaghatpour, Talanta 64 (4) (2004) 830.
[11] Y. Suzuki, M. Endo, J. Jin, K. Iwase, M. Iwatsuki, Analytical Sciences 22 (2006) 411.
[12] A. Choodum, N. Nic Daeid, Drug Testing and Analysis 3 (5) (2011) 277.
[13] M. Kompany-Zareh, M. Mansourian, F. Ravaee, Analytica Chimica Acta 471 (1) (2002) 97.
[14] J.N. Miller, J.C. Miller, Statistic and Chemometric for Analytical Chemistry, 5th ed., Pearson Education Limited, Essex, 2005.
[15] K.-A. Kovar, M. Laudszun, Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursors Chemicals, Pharmazeutisches Institut der Universitat Tubingen, Germany, 1989, Available at: http://www.unodc.org/pdf/scientific/SCITEC6.pdf (last accessed 06/01/11).