Research Article

Drug Testing and Analysis

Received: 15 December 2010 Revised: 6 January 2011 Accepted: 10 January 2011 Published online in Wiley Online Library: 9 March 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.263

Digital image-based colourimetric tests for amphetamine and methylamphetamine

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

Digital image analysis was applied to the products of simple colourimetric tests for amphetamine and methylamphetamine. 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 each compound and a number of illicit amphetamine samples were analyzed to demonstrate the application of the technique. A wide linear range and low detection limit for both drugs under test facilitated the application to illicit samples. Good agreement with gas chromatographic quantification results was obtained for the illicit samples analyzed by this method. The results show great potential for use as a semi-quantitative field test for illicit drug compounds. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: digital images; RGB colour system; drug presumptive test; amphetamine; methylamphetamine; colourimetric detection

Introduction

Amphetamine and methylamphetamine are synthetic stimulants which affect the central nervous system.[1] They are increasingly abused worldwide and have caused serious health and social problems. Amphetamine is usually produced as the sulfate salt in the form of powder, paste, or tablet and methylamphetamine is usually produced as the hydrochloride salt in the form of powder or as a clear crystalline product. Both substances are controlled in the UK under the Schedule 2 of the Misuse of Drugs Act 1971.[2]

The United Nations International Drug Control Programme has published a manual for rapid testing methods for drugs of abuse which includes amphetamine and methylamphetamine and which is provided as a resource for the international law enforcement and narcotic laboratory community.[1] The suggested presumptive tests for amphetamine and methylamphetamine include the Marquis test, the Simon test, and the Simon test with acetone. Each of these tests has been widely used as qualitative presumptive tests in forensic science laboratories.

In this work we explore the potential value of extending the application of presumptive tests through the application of digital image-based analysis. Although the use of digital image analysis has been highlighted by previous researchers in the delivery of robust and rapid methods for analytical applications in the determination of elements such as iron (III), aluminium (III), and titanium (IV),[3–8] to the best of our knowledge there are no reports in the literature that reveal an application for drug detection which this work seeks to address. Digital image-based analysis thus provides a basis for a semi-quantitative analysis using presumptive tests only for both amphetamine and methylamphetamine. The developed methodology has demonstrated a potential to deliver a fast and direct semi-quantitative determination of the illicit drug. This has potential for operational value 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.

Digital image-based analysis is based on the analysis of Red Green Blue (RGB) basic colour data obtained from digital images.[4] Images generated by a digital camera are based on the use of charge-coupled devices,[4,9] where the reflected light from the coloured products of a colourimetric test would pass through and be detected by three different filters: red, green, and blue. After scaling and adjustment in order to compensate for variations in the condition of capture, results are obtained as individual RGB values, and the final colour is composed from the additive data of the three RGB filters. We can exploit the RGB values derived from the capture of digital images of colourimetric presumptive tests to calibrate the images on the basis concentration of illicit material present. This then provides a basis by which semi-quantitative analysis of illicit samples containing an unknown quantity of material can be undertaken. In previous studies, RGB values were measured with the image processing tool box in MATLAB,[5,10] Kylix version 3.0,[7,11] or Visual basic version 6.0.[6,8] RGB can also be measured using software such as Adobe Photoshop version 7.0 which was used in this work.

Materials and Methods

Materials

DL-Amphetamine sulfate and methylamphetamine hydrochloride were obtained from Sigma Chemical (St Louis, MO, USA). Concentrated sulfuric acid, glacial acetic acid, formaldehyde,

* *Correspondence to: Niamh Nic Daeid, Centre for Forensic Science, Department of Pure and Applied Chemistry, WestCHEM, University of Strathclyde, 204 George Street, Glasgow G1 1WX, UK. E-mail: n.nicdaeid@strath.ac.uk*

a *Centre for Forensic Science, Department of Pure and Applied Chemistry, WestCHEM, University of Strathclyde, 204 George Street, Glasgow G1 1WX, UK*

b *Department of Pure and Applied Science, Faculty of Science, and Trace Analysis and Biosensor Research Center, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand*

acetone and methanol (AR grade) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany), while acetaldehyde was obtained from Aldrich Chemical Co. Ltd (Dorset, England). Sodium carbonate anhydrous from Fisher Chemicals, Fisher Scientific UK Ltd (Loughborough, UK) and sodium nitroprusside dihydrate (>98.0%) from Fluka (Sigma-Aldrich Chemie, Steinheim, Germany) were used.

Apparatus, software, and photographic procedure

The Canon EOS 20D digital camera was used (22.5 × 15.0 mm, 12-bit RGB CMOS sensor). 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 with the camera set to automatic focus, automatic white balance, automatic sensitivity (where the ISO speed was automatically set within 100 to 400) and captured in the single image mode. Each image with a size of 2.55 MB (3504 × 2336-pixel) was recorded as a JPEG (24-bit) 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.

Colourimetric presumptive test methods

Amphetamine sulfate was tested using the Marquis test and the Simon test with acetone, while methylamphetamine hydrochloride was tested using the Marquis and Simon tests. Two hundred microlitres 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 optimized to determine the volumes of various reagents which needed to be added to produce the darkest colour reaction for the least concentrated solution. The resultant colours were photographed after 3 min. Each presumptive test was repeated 6 times. The linear range was investigated for both amphetamine sulfate (0.10 to10 mg ml^{-1}) and methylamphetamine hydrochloride (0.10 to 5 mg ml^{-1}). The average intensities of the RGB 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 [12] and precision was expressed as the percentage relative standard deviation for each colour from the six images analyzed.

Marquis test

The Marquis test was used for both compounds under test. Two reagents were required: 2.5% *v/v* formaldehyde in glacial acetic acid (reagent 1A) and secondly, concentrated sulfuric acid (reagent 1B).[1] The optimized conditions were used for all test solutions as follows: reagent 1A (50 µL) was first added to the drugs solution followed by reagent 1B (1 ml). The solution was then mixed by shaking and left to stand for 3 min prior to photography.

The Simon test

The Simon test was used for testing methylamphetamine hydrochloride. Two reagents were required: 10% *v/v* acetaldehyde in aqueous sodium nitroprusside solution (1% *w/v*) (reagent 2A) and 2% *w/v* sodium carbonate in water (reagent 2B).[1] The optimized conditions were used for all test solutions as follows: reagent 2A (100 µl) was first added to the drugs solution followed by reagent 2B (800 µl). The solution was then mixed by shaking and left to stand for 3 min prior to photography.

The Simon test with acetone

The Simon test with acetone was used for testing amphetamine sulfate. Two reagents were required: 1% *w/v* sodium nitroprusside in aqueous acetone (5% *v/v*) (reagent 3A) and 2% *w/v* sodium carbonate in water (reagent 2B) were used.[1] The optimized conditions were used for all test solutions as follows: reagent 3A (100 µl) was first added to the drugs solution followed by reagent 2B (800 µl). The solution was then mixed by shaking and left to stand for 3 min prior to photography.

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 sample quantification. Separation was undertaken using a HP-5 MS capillary column (30 m length × 0.25 mm id × 0.25 µm film thickness). Samples (1 µl) were analyzed with a split ratio of 25 to 1 and a carrier gas (high-purity-grade helium) flow rate of 1.0 ml min^{-1}. The column was kept at 100 °C for 1 min, increasing to 260 °C at a rate of 20 °C min^{-1} and held for 3 min. The inlet and transfer line were kept at 260 °C and 280 °C, respectively. The instrument was calibrated for amphetamine sulfate in the range of 0.1 to 10 mg ml^{-1} where n=6 for each calibration standard injected.

Analysis of amphetamine street samples

Five different seized samples and 15 sub-samples from the same seized sample were investigated using the Marquis test. Amphetamine (20 µg) from each sample was extracted with 2 ml of methanol and sonicated for 15 min. The supernatant was analyzed using and both the Marquis test and gas chromatography-mass spectrometry (GC-MS).

Results and discussion

Colourimetric testing of amphetamine and methylamphetamine

The Marquis test

Both amphetamine sulfate and methylamphetamine hydrochloride provided an initial orange-coloured product which turned to a brown/orange product after 3 min due to the formation of a stable carbenium ion.[13] The carbenium ion is formed when carbonium ion of formaldehyde reacts with the aromatic ring in the presence of acid and is stabilized by addition of a second aromatic ring to produce the coloured product.[13]

The Simon test

The Simon test provided a blue-coloured product on reacting with methylamphetamine hydrochloride due to the formation of a Simon-Awe complex.[13] Acetaldehyde reacts with the secondary amine to produce an enamine which subsequently reacts with sodium nitroprusside to produce an immonium salt. The immonium salt is hydrolyzed to a Simon-Awe complex to give a blue-coloured product.[13]

The Simon test with acetone

The Simon test with acetone provided a purple-coloured product in the presence of amphetamine sulfate. The reaction mechanism is analogous to that with methylamphetamine hydrochloride; however the replacement of acetaldehyde with acetone causes the reaction to be selective to primary rather than secondary amines.[13]

Digital images analysis

The analytical (RGB) data available from a digital image obtained from a standard trichromatic response (8-bit channel), produces a value ranging from 0 to 255 for each channel [4–5, 7–8]. That is, if the image returned from the camera was black, the user would obtain an 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 referred to as subtractive colours and selectively absorb certain wavelengths of light, thus affecting the observed colour.[4,8]

A simple system was set up for photographing the coloured product obtained from colourimetric test of amphetamine and methylamphetamine as previously described. Each colourimetric reaction was undertaken six times for each standard solution and each sample analyzed. This allowed any variation in precision due to the experimental methods to be accounted for including inhomogeneity of illumination and curvature of the glass test tubes[6] or any variation of colour formation. The obtained sensitivities and linear ranges of both samples of amphetamine sulfate and methylamphetamine hydrochloride produced acceptable percentage relative standard deviations.

Digital image analysis with the Marquis test (orange-brown-coloured product)

The Marquis test provided orange-brown-coloured products for both amphetamine sulfate and methylamphetamine hydrochloride. Digital images of the coloured products were analyzed using Adobe Photoshop version 7.0 as previously described. These provided RGB intensity values (I_R, I_G, and I_B) which are related to concentration and are illustrated in Figure 1. Both drugs demonstrated the same relationship to the RGB values obtained and demonstrated linearity at low concentrations. These results were similar to those previously reported for iron.[14] The colour intensity can also be directly related to the absorbance at each concentration using Eqn 1:

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

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.[14]

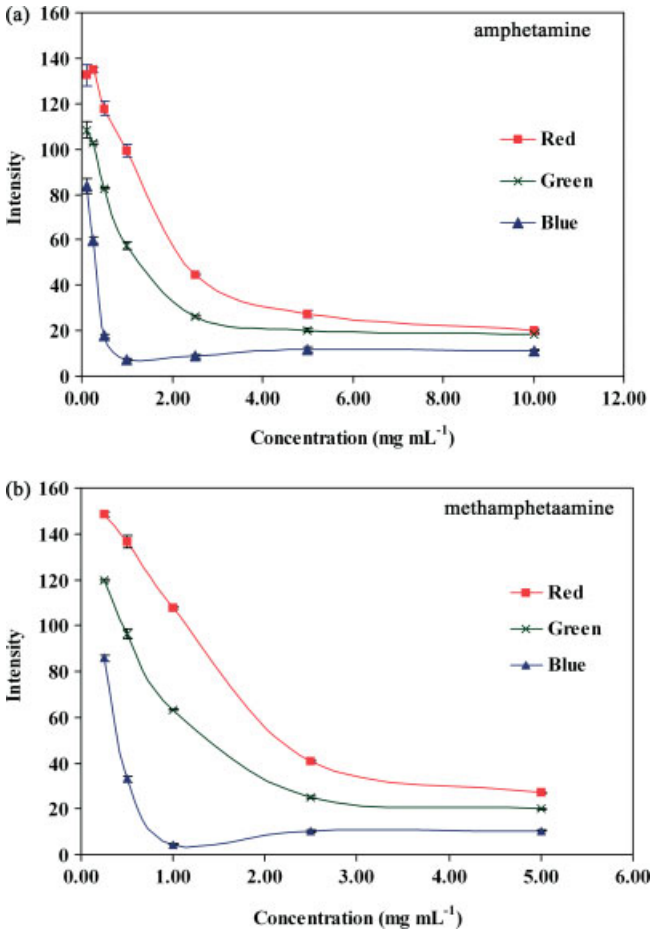

Figure 1. Relationship between the intensity of each colour and the concentration of (A) amphetamine (B) methylamphetamine with the Marquis test.

The derived relationship between the concentration of both drugs and the absorbance are presented in Figure 2 and the logarithmic relationship for each drug is presented in Figure 3 The obtained relations were similar with those commonly reported using spectrophotometric methods.

The highest absorbance (lowest intensity) observed for both amphetamine sulfate and methylamphetamine hydrochloride was obtained using the blue colour component; I_B. Blue is a complementary colour of orange and thus a high absorbance of this colour is expected given the presumptive test colour was orange-brown. The linear relationship between the known standard drug concentrations and the RGB component intensities, calculated absorbances and in some cases the logarithmic values of the RGB component intensities together with the linear ranges for each of these relationships are presented in Table 1.

The results illustrate the relationships and concentration ranges within which a calibrated and repeatable linear relationship was obtained between the digital image intensities and the products of the presumptive colour test reactions.

Digital image analysis with the Simon test (blue-coloured product)

The Simon test produced a blue-coloured product with methylamphetamine hydrochloride. The relationships between the

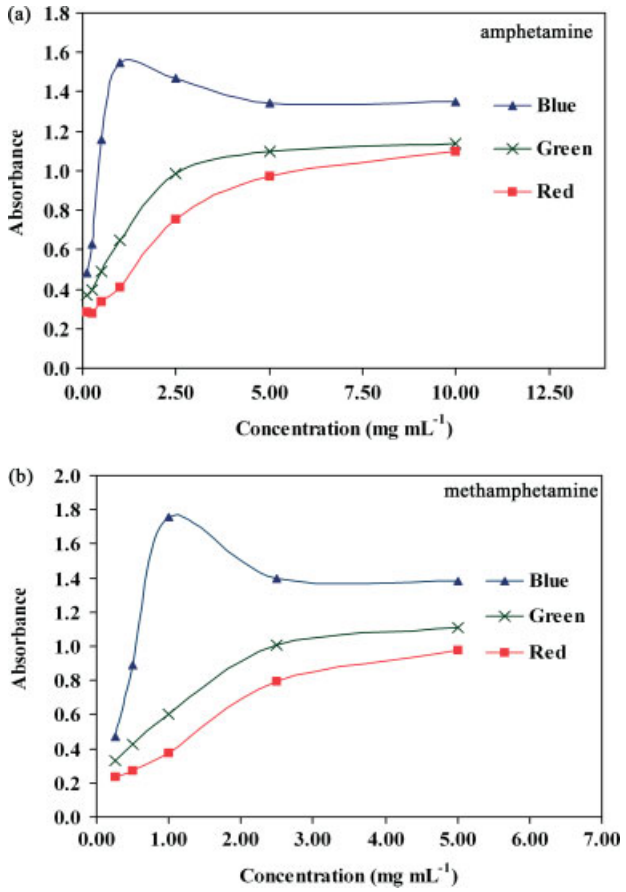

Figure 2. Relationship between the absorbance of each colour and the concentration of (A) amphetamine (B) methylamphetamine with the Marquis test.

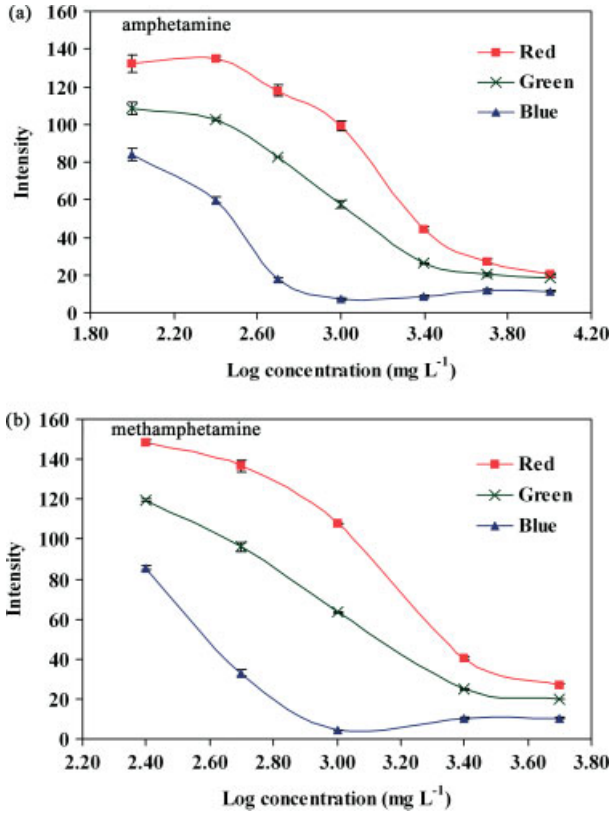

Figure 3. Relationship between the intensity of each colour and the log concentration of (A) amphetamine (B) methylamphetamine with the Marquis test.

intensities of each RGB colour component and the concentration of the drugs under test are shown in Figure 4A and those of calculated absorbance are presented in Figure 4B. 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 blue in colour and as such little change in the values of these intensities is expected.

A linear relationship between the drug concentration was obtained for the intensity of the green component (2.0 to 6.0 mg ml^{-1}) and the absorbance of the red and green component (1.0 to 6.0 mg ml^{-1}). A linear relationship was also demonstrated between the logarithm of the drug concentration (1.0 to 6.0 mg ml^{-1}) and the red and green component intensities.

Digital image analysis with the Simon test with acetone (purple-coloured product)

Amphetamine sulfate was reacted with the Simon test reagents with acetone to produce a purple-coloured product. As a consequence the blue and to a lesser extent the red components

Table 1. Calibration equation of amphetamine sulfate and methylamphetamine hydrochloride with the Marquis test

	Amphetamine			Methylamphetamine		
Parameter*	Calibration curve equation	R^2	Linear range (mg mL^{-1})	Calibration curve equation	R^2	Linear range (mg mL^{-1})
I_R and C	$y = -(39 \pm 2)x + (140 \pm 3)$	0.9923	0.25–2.50	$y = -(48 \pm 2)x + (159 \pm 2)$	0.9974	0.25–2.50
I_G and C	$y = -(58 \pm 4)x + (115 \pm 2)$	0.9904	0.10–1.00	$y = -(74 \pm 7)x + (136 \pm 5)$	0.9911	0.25–1.00
I_B and C	$y = -(166 \pm 2)x + (100.7 \pm 0.7)$	0.9998	0.10–0.50	–	–	–
A_R and C	$y = (0.21 \pm 0.01)x + (0.22 \pm 0.01)$	0.9952	0.25–2.50	$y = (0.26 \pm 0.02)x + (0.15 \pm 0.02)$	0.9926	0.25–2.50
A_G and C	$y = (0.26 \pm 0.01)x + (0.35 \pm 0.02)$	0.9922	0.10–2.50	$y = (0.30 \pm 0.02)x + (0.28 \pm 0.02)$	0.9941	0.25–2.50
A_B and C	–	–	–	$y = (1.71 \pm 0.02)x + (0.04 \pm 0.01)$	0.9999	0.25–1.00
I_R and log C	$y = -(59 \pm 1)x + (277 \pm 3)$	0.9996	0.25–1.00	–	–	–
I_G and log C	$y = -(77 \pm 2)x + (289 \pm 7)$	0.9981	0.25–2.50	$y = -(96 \pm 4)x + (352 \pm 11)$	0.9968	0.25–2.50

* ***I = colour intensity, A = colour absorbance calculated using equation 1, C is the concentration of the prepared standards.***

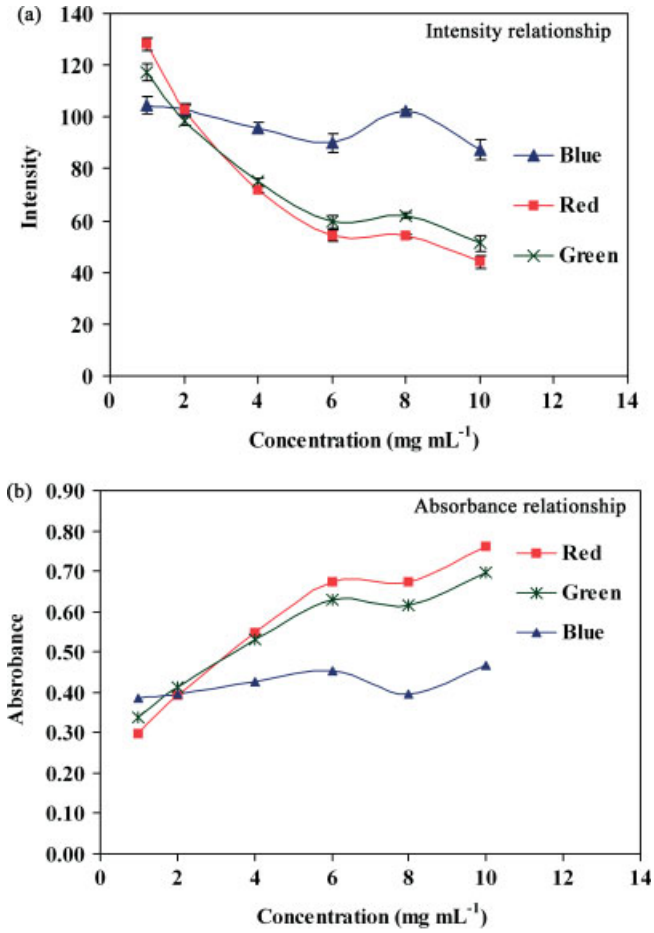

Figure 4. Relationship between (A) intensity and (B) absorbance of each colour and the concentration of methylamphetamine with the Simon test.

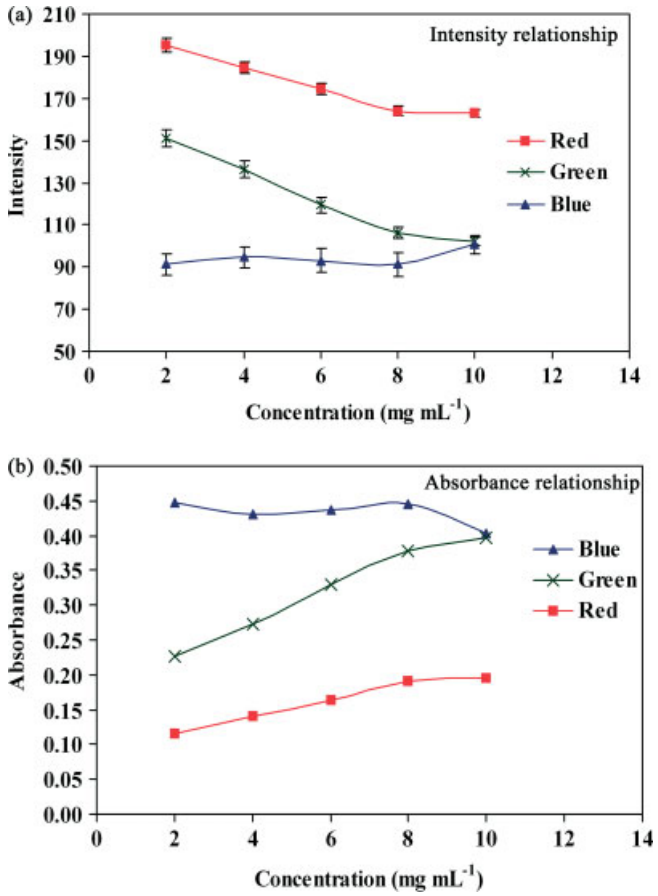

Figure 5. Relationship between (A) intensity and (B) absorbance of each colour and the concentration of amphetamine with the Simon test with acetone.

were reflected by the product, while the green component was absorbed and this is reflected in the data presented in Figure 5.

The resultant linear relationships of the amphetamine sulfate concentration and intensity and absorbance of the red and green component colours were between 2.0 to 8.0 mg ml^{-1}.

Method validation

System performance including the sensitivity (slope of the calibration graph), linear range, and limit of detection of the proposed method is presented in Table 2.

Amphetamine street sample analysis

Six unrelated seized samples of amphetamine sulfate were extracted and analyzed using GC-MS and the digital image analysis of the results of the Marquis presumptive test as previously described. Fifteen separate sub samples of one of the seized samples were also analyzed. The GC-MS was calibrated in the range of 0.10 to 1.0 mg ml^{-1} amphetamine sulfate in methanol and demonstrated good linearity (R^2 = 0.9997) and %RSD < 3. The data is presented in Table 3 and clearly demonstrates the correlation between the accurately determined concentration of amphetamine sulfate in the samples and the devised semi-quantitative method. The relationship between I_G and log C demonstrates the most reliable and accurate relationship between the interpreted digital images and the target drug concentration. The analysis of the seized samples S2, S3, S4, and S5 by GCMS revealed a number of interferences (e.g. caffeine) within the illicit drug mixtures; however the results of the determination of the amphetamine percentage using the developed method and that obtained by GC-MS were very comparable. This suggests that the applied colourimetric tests are not influenced by interferants within the street sample.

Conclusion

Digital image analysis of coloured test products has demonstrated a significant potential for the development of an accurate, rapid, portable, and economically viable semi-quantitative tests 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 amphetamine sulfate and methylamphetamine hydrochloride.

Acknowledgements

The authors would like to thank the Development and Promotion of Science and Technology Talents Project (DPST) supported by Royal Thai Government for the scholarship for Aree Choodum, Dr Anna Cadogan for kindly providing the digital camera, and

Table 2. System performance of the proposed method

Drugs	Test	Relationship	Linear range (mg mL^{-1})	LOD* (mg mL^{-1})	% RSD	Accuracy (mg mL^{-1}) Known	Accuracy (mg mL^{-1}) Experiment
amphetamine	*Marquis*	I_R and C	0.25–2.50	0.33 ± 0.02	0.77–3.43	0.75	0.9 ± 0.1
		I_G and C	0.10–1.00	0.14 ± 0.01	0.21–3.21	0.75	0.85 ± 0.06
		I_B and C	0.10–0.50	0.0111 ± 0.0001	2.47–4.68	0.75	**
		I_R and log C	0.25–1.00	0.012	0.77–3.43	0.75	0.83
		I_G and log C	0.25–2.50	0.001	0.21–3.21	0.75	0.80
	Simon (acetone)	I_R and C	2.0–8.0	0.137 ± 0.001	0.98–1.71	5.00	5.3 ± 0.3
		I_G and C	2.0–8.0	0.44 ± 0.01	2.02–3.12	5.00	5.0 ± 0.1
methylamphetamine	*Marquis*	I_R and C	0.25–2.50	0.189 ± 0.007	0.28–1.97	0.75	0.74 ± 0.07
		I_G and C	0.25–1.00	0.153 ± 0.01	0.33–2.30	0.75	0.77 ± 0.06
		I_G and log C	0.25–2.50	0.001	0.33–2.30	0.75	0.69
	Simon	I_G and C	2.0–6.0	1.0 ± 0.1	1.79–4.19	5.00	4.9 ± 0.4
		I_R and log C	1.00–6.00	1.01	1.50–4.20	5.00	4.67
		I_G and log C	1.00–6.00	1.01	1.79–4.19	5.00	4.75

***I*= colour intensity, C is the concentration of the prepared standards,** * LOD = $y_B + 3S_B$ [12] ** out of linear range.

Table 3. Amphetamine street sample results

Sample no.	GCMS results (%)	Digital image analysis of Marquis test (%) I_R and C	I_G and C	I_G and log C
1	24.3	20.8	**	23.8
2*	18.8	21.0	19.2	19.4
3 (1)*	22.4	29.2	21.0	24.3
3 (2)	26.7	25.9	**	23.9
4	23.1	23.3	**	21.0
5	23.7	20.0	**	20.0
6	23.1	23.0	**	21.7
7	24.2	22.4	**	20.8
8	22.7	23.4	**	21.9
9	25.2	25.3	**	24.8
10	19.0	19.1	**	18.0
11	24.2	21.1	**	20.6
12	25.4	25.4	**	23.2
13	25.8	22.4	**	21.1
14	19.8	19.0	**	17.6
15	19.4	19.8	**	18.5
S2	8.4	7.6	8.6	8.3
S3	10.2	14.0	10.7	12.4
S4	5.1	**	6.2	5.6
S5	3.9	**	3.4	3.3

1 to 15 are street sample from S1 seized.
* Samples were diluted with 4 ml, while others diluted with 2 ml methanol.
** the concentration obtained from street sample was out of the linear range. (1) and (2) are the repeated analysis from the same sample.

the Centre for Forensic Science, Department of Pure and Applied Chemistry, University of Strathclyde where the research was carried out. We would also like to express our thanks to the referees of this work for their insightful comments which have helped us in clarifying the manuscript

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*, United Nations: Vienna, **1994**.
[2] Forensic Services Scottish Police Sevice Authority, Type of Drugs. Available at: http://www.spsa-forensics.police.uk/services/drugs/types_drugs [November 2010].
[3] C. W. Earle, M. E. Baker, M. B. Denton, R. S. Pomeroy, *Trends Anal. Chem.* **1993**, *12*, 395.
[4] L. Byrne, J. Barker, G. Pennarun-Thomas, D. Diamond, S. Edwards, *Trends Anal. Chem.* **2000**, *19*(8), 517.
[5] A. Lopez-Molinero, D. Linan, D. Sipiera, R. Falcon, *Microchem. J.* **2010**, *96*(2), 380.
[6] Y. Suzuki, M. Endo, J. Jin, K. Iwase, M. Iwatsuki, *Anal. Sci.* **2006**, *22*, 411.
[7] E. N. Gaiao, V. L. Martins, W. S. Lyra, L. F. Almeida, E. C. de Silva, M. C. U. Araújo, *Anal. Chim. Acta* **2006**, *570*(2), 283.
[8] N. Maleki, A. Safavi, F. Sedaghatpour, *Talanta* **2004**, *64*(4), 830.
[9] P. M. Epperson, J. V. Sweedler, R. B. Bilhorn, G. R. Sims, M. B. Denton, *Anal. Chem.* **1988**, *60*, 327A.
[10] L. M. Goddijn M. White, *Estuar. Coast. Shelf S.* **2006**, *66*(3), 429.
[11] W. Silva Lyra, V. B. dos Santos, A. G. G. Dion?zio, V. L. Martins, L. F. Almeida, E. Nobrega Gaiao, P. H. G. D. Diniz, E. C. Silva, M. C. U. Araujo, *Talanta* **2009**, *77*(5), 1584.
[12] J. N. Miller J. C. Miller, *Statistic and Chemometric for Analytical Chemistry*, 5th ed. Pearson Education Limited: Essex, **2005**.
[13] 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 [6 January 2011].
[14] M. Kompany-Zareh, M. Mansourian, F. Ravaee, *Anal. Chim. Acta* **2002**, *471*(1), 97.