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<td>Ryder, Alan G.; O’Connor, Gerard M.; Thomas J., Glynn</td>
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Quantitative analysis of cocaine in solid mixtures using Raman spectroscopy and chemometric methods.

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ABSTRACT

Near-Infrared (785 nm) excitation was used to obtain Raman spectra from a series of 33 solid mixtures containing cocaine, caffeine, and glucose (9.8-80.6 % by weight cocaine) which were then analysed using chemometric methods. Principal Component Analysis of the data was employed to ascertain what factors influenced the spectral variation across the concentration range. It was found that 98 % of the spectral variation was accounted for by three principal components. Analysis of the score and loadings plots for these components showed that the samples can be clearly classified on the basis of cocaine concentration. Discrimination on the basis of caffeine and glucose concentrations was also possible. Quantitative calibration models were generated using Partial Least Squares (PLS) algorithms which predicted the concentration of cocaine in the solid mixtures containing caffeine and glucose from the Raman spectrum with a root mean standard error of prediction (RMSEP) of 4.1 %. Caffeine and glucose concentrations were estimated with RMSEPs of 5.2 % and 6.6 % respectively. These measurements demonstrate the feasibility of using near-IR Raman spectroscopy for rapid quantitative characterisation of illegal narcotics.

Key words: Chemometrics; Raman; Spectroscopy; Forensic; Cocaine; Quantitative.

INTRODUCTION

IR spectroscopy has been one of the tools of choice for forensic identification of materials, primarily because of its ease of use and the availability of large searchable digital libraries of spectra. In the last decade, however, Raman spectroscopy has begun to emerge as a forensic tool.1 Compared to IR absorption spectroscopy, Raman spectroscopy has several particular advantages that have made the technique attractive for forensic analysis. Sample preparation is minimal in most cases, allowing for the non-destructive analysis of bulk or microscopic materials in-situ.2 The weak Raman signal from water allows spectra to be easily collected from aqueous solutions or moist materials including illegal narcotics.3 Raman analysis of complex biological materials such as human tissue is also possible without sample pre-treatment.4,5 Raman techniques can attain high spatial resolutions of ~ 1 µm which allow for the analysis of discrete micron-sized particles, as demonstrated by the determination of drug levels in individual cells 6,7 and the analysis of gunshot residues.8 These advantages have been exploited by several research groups that have employed Raman microscopy or fiber optic probes for the analysis and identification of explosive materials.9–14 Although the use of Raman spectroscopy for the identification of illegal narcotics is well established,3,15–17 very little work has been published on the use of Raman spectroscopy for the quantitative measurement of illegal drugs.

The application of Raman spectroscopy to quantitative analysis is advancing gradually, as the problems which have limited the use of the technique in the past 18 are reduced or
eliminated. In particular, the use of multivariate analysis techniques (Chemometrics) have been exploited to overcome problems associated with the complexities of light scattering from materials. Multivariate analysis methods are used to correlate (statistically) observed spectral changes with properties such as concentration and have permitted the quantitative analysis of various complex materials by Raman spectroscopy.19–23

We have demonstrated in a previous work the ability of Raman spectroscopy to accurately predict the concentration of cocaine when dispersed in a single dilutant, glucose.24,25 However, the types of dilutants found in seized drug samples vary enormously and thus present a significant problem for the analyst. For techniques like Raman spectroscopy, variations in the dilutant(s) content produce specific problems. First, the presence of fluorescent impurities can mask the Raman signal, thus making identification or quantitative measurement difficult or impossible. The general solution to this problem is to use excitation wavelengths in the UV or near-IR, with which fluorescence is minimised or prevented altogether.3,15-17 In this case we have employed 785 nm excitation, which reduces considerably the fluorescence interference, as demonstrated in our previous studies.24,25 The second major consideration is the Raman signal intensities of the dilutants compared to those of the illegal narcotics which are under investigation. If the Raman signal from the dilutant is weak with respect to the narcotic signal, then detection is relatively straightforward down to concentrations in the 10 % by weight range. Conversely, intense Raman signals from the dilutant(s) will mask signals from the target narcotics making quantitative measurements especially difficult. The existence of several dilutants in a drug mixture further exacerbates the problem of identification as well as making quantitative measurements more difficult. We have chosen a three component mixture as a model to assess the ability of Raman spectroscopy to obtain quantitative measurements from a complex mixture. Caffeine and glucose were chosen as typical examples of dilutants that could be mixed with illegal narcotics.

EXPERIMENTAL

Cocaine hydrochloride was supplied by Sigma-Aldrich, UK, and was used as received. The dilutant materials anhydrous D-glucose (BDH), and caffeine (Aldrich) were reagent grade. Sample mixtures (10-30 mg total weight) were made up by mixing known weights of drug and dilutant, followed by grinding in an agate mortar and pestle to ensure sample homogeneity by thorough mixing of components. The mixtures were transferred to clean stainless steel hexagonal sample holders with an internal diameter of ~ 2 mm and tamped into place. The sample set [Table 1] covered a representative range of concentrations to demonstrate the feasibility of using chemometric techniques to provide a quantitative analysis method.

For near-IR Raman spectroscopy the excitation source was a Ti:Sapphire solid state laser (Spectra Physics 3900s), tuned to emit at 785 nm and optically pumped using an Argon ion laser (Spectra Physics 2017). A 785 nm narrow-band interference filter (CVI Laser Corporation, Albuquerque, NM) was used to remove non-resonant emissions. A modified Quantitative analysis of cocaine in solid mixtures using Raman spectroscopy and chemometric methods. A.G. Ryder, G.M. O'Connor, and T.J. Glynn, Journal of Raman Spectroscopy, 31(3), 221-227, (2000).
Nikon Optiphot metallurgical microscope with a Nikon M-plan x20 power objective (N.A.= 0.4) was used to focus the laser light on the sample, with a spot size of 10 µm. The incident power at the sample was ~ 6 mW. A holographic filter (HSNF-785 Kaiser Optical Inc.) was used to attenuate the elastically scattered laser light. The light was dispersed using a 0.5 m grating spectrometer (SPEX 500M) onto a liquid nitrogen cooled 512 x 512 pixel back-illuminated CCD detector (Princeton Instruments) operating at -120 °C. A spectral range of ~650 cm\(^{-1}\) and a resolution of ~ 4 cm\(^{-1}\) was obtained with the spectrometer operating in first order. All spectra were recorded at a set interval of 450-1100 cm\(^{-1}\) (510 data points). The exposure time was set at 30 seconds for all samples. The spectra were uncorrected for instrument and detector responses. The choice of spectral range was governed by two considerations: the experimental setup (limited by the CCD array and spectrometer dispersion) and a desire to work with a complex spectral region with many vibrational modes, in order to assess the capability of chemometrics and Raman spectroscopy.

Three Raman spectra at different surface locations were recorded for each sample. Features in the spectra caused by cosmic rays incident on the detector were manually removed using the EasyPlot software package (ver. 3.00-7, Spiral Software+MIT). The three spectra per sample were then added together, averaged, and smoothed over a five-point average before chemometric analysis. Unscrambler (V6.11b, CAMO, Trondheim, Norway) multivariate analysis software was used for quantitative analysis. All calculations were performed on a Pentium 200 MHz, 32 Mbytes RAM IBM-compatible personal computer (Windows 95 operating system) with all calculations taking between 1 and 3 minutes to complete.

RESULTS AND DISCUSSION

The Raman spectra of pure cocaine, caffeine and glucose displayed in Fig. 1 show large differences in Raman intensity. The caffeine spectrum is dominated by the intense \(\delta(O=\text{C-N})\) bending vibration at ~555 cm\(^{-1}\) and medium intensity bands at 484, 643 \(\delta(O=\text{C-N})\), 740 and 800 cm\(^{-1}\). The cocaine spectrum is composed of well defined bands, the most intense of which is the 996 cm\(^{-1}\) band while the glucose spectrum is composed of much weaker bands which display some variation in position and size when repeatedly sampled. The mean and standard deviation values (in parentheses) of all points in each spectrum: 535 (412) cocaine, 627 (984) caffeine, and 272 (157) glucose, are an indication of the relative intensities. These variations in Raman intensity play a critical role in determining the ability to identify the cocaine in a mixture and therefore gauge its concentration accurately. The spectra illustrated in Fig. 2 show that even at relatively high concentrations of cocaine (~30 %), if the caffeine concentration is high (50 %) then it can be difficult to identify all the cocaine bands. The spectra also illustrate how small the contribution of the glucose is relative to the other two compounds.

Prior to carrying out Principal Component Analysis (PCA) or regression processes, the spectral data was treated with a Multiplicative Scatter Correction (MSC) for common offset. PCA was carried out using full cross-validation (leave one out method) with all variables...
equally weighted at a value of one. The data thus evaluated showed that 98 % of all the
spectral variation could be accounted for by a total of three principal components, the loadings
plots of which are shown in Fig. 3. The first principal component, PC1, with an explained
spectral-variance of 68 %, indicates a direct correlation between the concentrations of cocaine
and caffeine. The positive peaks match the vibrational modes of caffeine while the negative
peaks correspond to the most intense peaks in the Raman spectrum of cocaine. The second
component, PC2, with an explained spectral-variance of 27 % describes the changes in
glucose concentration relative to the combined concentration of cocaine and caffeine. In this
case the positive features in the plot correspond to the vibrational modes of cocaine and
caffeine, and the negative peaks to those of glucose. The loading plot for PC3 (spectral-
variance of 3 %) indicates a gradual increase from low to high wavenumber and the sharp
peaks (in position and relative sign) are equivalent to those in the PC1 loadings plot. The
slope of the graph correlates with the baseline slope of the mean spectrum for all the samples,
which has a slope of -0.52 intensity units per cm$^{-1}$. The slope in baseline is most probably
caused by variation in sample morphology as concentration varies, particularly with regard to
glucose, although an instrumental function cannot be discounted. In any effect for
quantitative measurements, PC3 will have minimal impact.

These assumptions are supported by analysis of the PC1/PC2 score plots [Fig. 4, 5 & 6] which show clearly the differentiation of samples according to the concentrations of the
three components. The lines on the plots separate the samples according to concentration. In
Fig. 4 cocaine concentration is shown to be negatively correlated with PC 1 and positively
correlated with PC 2. Fig. 5 shows a larger positive correlation between caffeine
concentration and PC1 while the positive correlation with PC 2 is much less than in Fig. 4.
These results are in agreement with the observed loading plots in Fig. 3 for these two
components. The score plot in Fig. 6, where the samples are grouped by the concentration of
glucose, shows an inverse linear correlation with PC 2, which again is evident from the
loading plot for PC 2 where the negative peaks correspond to the vibrational bands of glucose.

One feature of these score plots is the fact that sample outliers are not always the same
for each component. When the samples are ranked by cocaine concentration [Fig. 4] one
sample (a23sp124, underlined in plots) is perfectly fitted, while when grouped by caffeine
[Fig. 5] or glucose concentration [Fig. 6] is an obvious outlier. This may be due to its
relatively high glucose concentration of 49.8 %. It has been observed that the variations in
peak positions, intensities and band resolution of the glucose components in the spectra are
much greater than for cocaine or caffeine. Morphological changes due to the mixing process
may be the cause of this variation. Alternatively, variations in the crystalline composition of
the glucose at the sampling spot may be the cause; we have observed in this laboratory that it
is often difficult to obtain reproducible Raman spectra from glucose. This illustrates a
potential problem in employing these methods for forensic use on street samples, namely,
variations in cutting agent quality.

The last component, PC3, with an explained x-variance of 3 % seems to represent a
gradual increase in baseline intensity as wavenumber increases. PC3 correlates reasonably
well with the mean value of each Raman spectrum (mean calculated before smoothing and
MSC operations). The PC2/PC3 score plot in Fig. 7 illustrates this very clearly, with the
samples distributed along the PC2 axis according to glucose concentration (not shown) and
dispersed along the PC3 axis according to the mean value of the Raman spectrum. From this 
PCA analysis, it is evident that only two components would be necessary for a more 
quantitative analysis based on PLS.

Quantitative prediction models were generated using Partial Least Squares methods for 
multiple variables with the object of estimating the concentrations of all three compounds in 
the mixtures. Table 2 gives the results obtained from two PLS models both of which were run 
with full cross-validation on mean centred data that was weighted at 1.0 for all variables. 
Model A which uses the same data set (and pre-treatment) as that employed for the PCA 
analysis yielded a Root Mean Square Error of Prediction (RMSEP) of 4.3 % for cocaine. For 
model B, the first derivative (Savitsky-Golay routine over a five point range with a second 
order polynomial) of the spectral data (identical to that used for model A) was taken. The 
PLS model (model B) with this data set was run under identical conditions to model A, 
produced slight improvements in the RMSEP(C) values. The greatest improvement is for the 
caffeine component where there is a ~0.4 % improvement in RMSEP(C) values. The best 
results in both cases were obtained using two principal components, which give explained Y 
variances of 92-93 % for both models. Fig. 8 shows the predicted versus measured cocaine 
concentration for the calibration and validation samples for model B. There is a significant 
degree of scatter about the regression line but the correlation coefficient is high at 0.98 
indicating a good linear relationship. The two lines parallel to the centre regression line 
indicate a ± 10 % boundary. Several other data pre-treatment methods were examined but 
none proved to yield better RMSEC(P) values. Methods such as normalisation, second-order 
derivitisation, and Fast Fourier Transform were tried but the values for RMSEP were all 
greater than the values given in table 2. The use of test sets (random, 13 samples) instead of 
cross validation was examined but no significant variation in the RMSEC (P) values was 
observed.

CONCLUSIONS

This study has demonstrated that by using PCA, complex mixtures comprising of 
cocaine, dispersed in two solid dilutants can be differentiated on the basis of cocaine 
concentration. The PCA study also showed that the mixtures could be ranked according to the 
concentration of either dilutant. This qualitative procedure demonstrates the usefulness of 
Raman spectroscopy and chemometric methods as a possible screening method for the 
analysis of illegal drug samples. PLS was employed for a more quantitative approach and, 
after suitable calibration, it is possible to determine the concentration of cocaine in a three 
component system with a reasonable level of accuracy. The PLS methods produced 
calibration plots with RMSEP values of ~ 4 % which may be accurate enough for routine 
forensic analysis.

However, the study also demonstrates the need for large training sets in order to 
achieve a reasonable level of accuracy (< 5 %). This is one of the largest obstacles facing the 
application of chemometric methods and Raman spectroscopy to routine quantitative forensic 
applications. Although it is not practical to create large training sets for each possible narcotic 
formulation, there may be situations where the approach is viable, specifically in the analysis 

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221-227, (2000).
of tablet formulations where the range of mixing agents may be limited. Further work is being conducted, examining methods for improving the accuracy of Raman spectroscopic/chemometric methods for illegal narcotic analysis.

**ACKNOWLEDGEMENTS**

This work was supported by the ‘Science & Technology Against Drugs’ Programme of Forbairt, the National Science and Technology Agency of Ireland, and by a Forbairt Post-doctoral Fellowship to Dr. A. Ryder.

Table 1
Composition (by weight) of samples used for chemometric analysis.

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<th>% Cocaine</th>
<th>% Caffeine</th>
<th>% Glucose</th>
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<td>a23sp031</td>
<td>54.24</td>
<td>23.14</td>
<td>22.62</td>
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<td>a23sp034</td>
<td>80.58</td>
<td>8.86</td>
<td>10.56</td>
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<td>a23sp037</td>
<td>70.35</td>
<td>17.24</td>
<td>12.41</td>
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<td>9.15</td>
<td>19.75</td>
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<td>a23sp043</td>
<td>56.26</td>
<td>10.62</td>
<td>33.12</td>
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<td>a23sp046</td>
<td>61.92</td>
<td>19.23</td>
<td>18.85</td>
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<td>a23sp049</td>
<td>74.25</td>
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<td>49.96</td>
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<td>49.34</td>
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<td>a23sp082</td>
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Table 2: Results from PLS calibration models

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<th>Caffeine</th>
<th>Glucose</th>
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<tr>
<td>RMSEP(%)</td>
<td>4.28</td>
<td>4.145</td>
<td>5.62</td>
</tr>
<tr>
<td>RMSEC(%)</td>
<td>3.97</td>
<td>3.74</td>
<td>4.92</td>
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<tr>
<td>Correlation(P)</td>
<td>0.977</td>
<td>0.982</td>
<td>0.90</td>
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<tr>
<td>Correlation(c)</td>
<td>0.98</td>
<td>0.978</td>
<td>0.925</td>
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<tr>
<td>Expl. X-variance</td>
<td>60(35)</td>
<td>56(38)</td>
<td>---</td>
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<tr>
<td>Expl. Y-variance</td>
<td>47(45)</td>
<td>60(33)</td>
<td>---</td>
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**Figure 1:** Raman spectra of cocaine (top), glucose (middle) and caffeine (bottom). All spectra at same scale, offset for clarity. The cocaine spectrum is offset by 3000 units and the glucose spectrum by 1000.

**Figure 2:** Raman spectra of sample mixtures (offset for clarity). The spectra were obtained from samples with the following cocaine - caffeine - glucose concentrations: From top to bottom: 100-0-0; 71.1-9.2-19.7; 56.3-10.6-33.1; 40.7-49.3-9.9; 33.5-20.9-45.6; 29.9-50.3-19.8;
Figure 3: X-loadings plots for all three components. PC1 is offset by +0.2, and PC3 by -0.2. Same scale for all plots.

Figure 4: Score plot for PC1 vs PC2, with cocaine concentration given in parentheses.


**Figure 5:** PC1/PC2 score plot with the caffeine concentration given in parentheses.

**Figure 6:** PC1/PC2 score plot with glucose concentration given in parentheses.
Figure 7: PC2/PC3 score plot with mean value of spectrum in parentheses (some values omitted for clarity).

Figure 8: Predicted vs. measured cocaine concentration for PLS calibration model B.
REFERENCES