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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 $\sim 1 \mu\text{m}$ 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 past¹⁸ 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

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 $\delta(\text{O}=\text{C}-\text{N})$ bending vibration at ~ 555 cm^{-1} and medium intensity bands at 484, 643 $\delta(\text{O}=\text{C}-\text{N})$, 740 and 800 cm^{-1} .²⁶ 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

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.

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

filename	% Cocaine	% Caffeine	% Glucose	filename	% Cocaine	% Caffeine	% Glucose
a23sp031	54.24	23.14	22.62	a23sp085	29.47	9.43	61.1
a23sp034	80.58	8.86	10.56	a23sp088	33.46	20.89	45.65
a23sp037	70.35	17.24	12.41	a23sp091	28.06	8.52	63.42
a23sp040	71.1	9.15	19.75	a23sp094	13.39	15.45	71.16
a23sp043	56.26	10.62	33.12	a23sp097	20.0	10.34	69.66
a23sp046	61.92	19.23	18.85	a23sp100	29.96	29.32	40.72
a23sp049	74.25	19.37	6.38	a23sp103	25.60	18.40	56.0
a23sp052	61.48	29.11	9.41	a23sp106	21.98	20.32	57.70
a23sp055	49.96	10.74	39.30	a23sp109	22.32	28.46	49.22
a23sp058	50.57	19.39	30.04	a23sp112	11.75	30.53	57.72
a23sp061	48.340	41.65	10.01	a23sp115	31.11	38.93	29.96
a23sp064	42.88	10.02	47.10	a23sp118	19.46	39.80	40.74
a23sp067	40.30	19.36	37.34	a23sp121	29.90	50.33	19.77
a23sp070	39.33	30.46	30.21	a23sp124	21.19	29.0	49.81
a23sp073	40.11	39.74	20.15	a23sp127	9.92	38.64	51.44
a23sp079	40.74	49.34	9.92	a23sp130	9.84	50.49	39.67
a23sp082	10.85	10.95	78.20				

Table 2: Results from PLS calibration models

Model	Cocaine		Caffeine		Glucose	
	A	B	A	B	A	B
RMSEP(%)	4.28	4.145	5.62	5.20	6.65	6.60
RMSEC(%)	3.97	3.74	4.92	4.55	5.78	5.73
Correlation(P)	0.977	0.982	0.90	0.936	0.94	0.958
Correlation(c)	0.98	0.978	0.925	0.916	0.957	0.944
Expl. X-variance	60(35)	56(38)	----	----	----	----
Expl. Y-variance	47(45)	60(33)	----	----	----	----

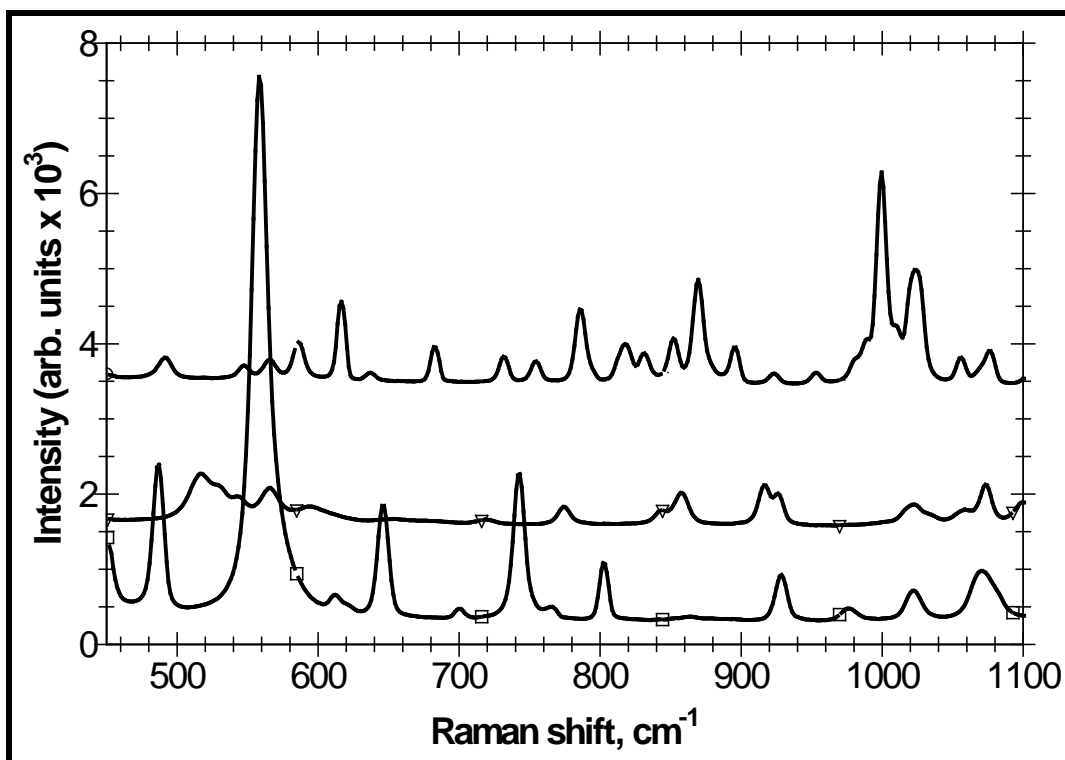


Figure 1: Raman spectra of cocaine (top), glucose (middle) and caffeine (bottom). All spectra at same scale, offset for clarity. The cocaine spectrum is offset by 3000 units and the glucose spectrum by 1000.

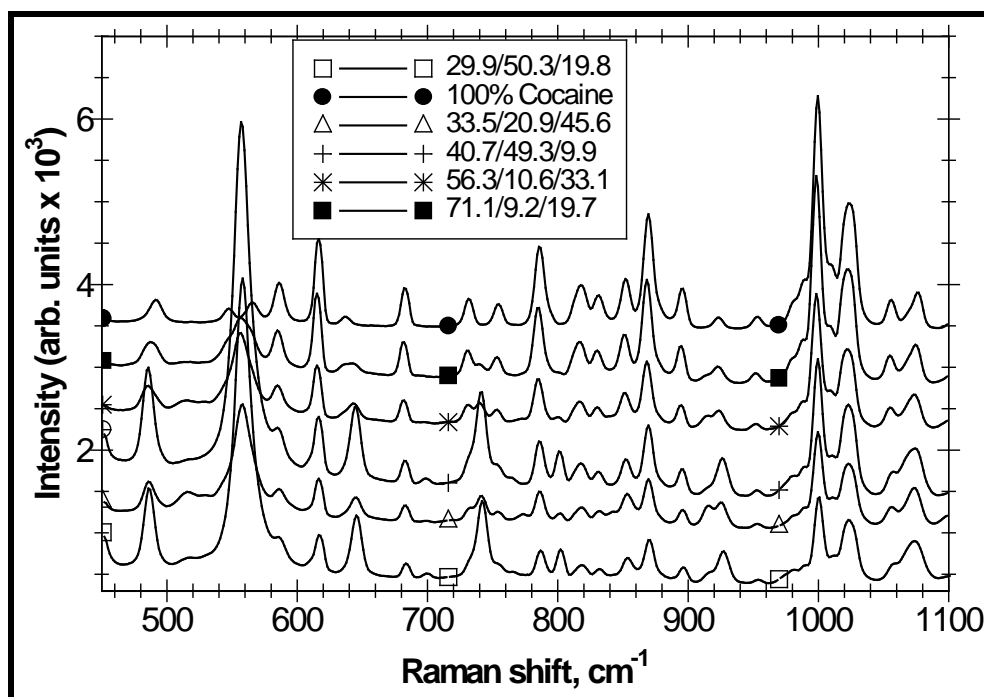


Figure 2 : Raman spectra of sample mixtures (offset for clarity). The spectra were obtained from samples with the following cocaine - caffeine - glucose concentrations: From top to bottom: 100-0-0; 71.1-9.2-19.7; 56.3-10.6-33.1; 40.7-49.3-9.9; 33.5-20.9-45.6; 29.9-50.3-19.8;

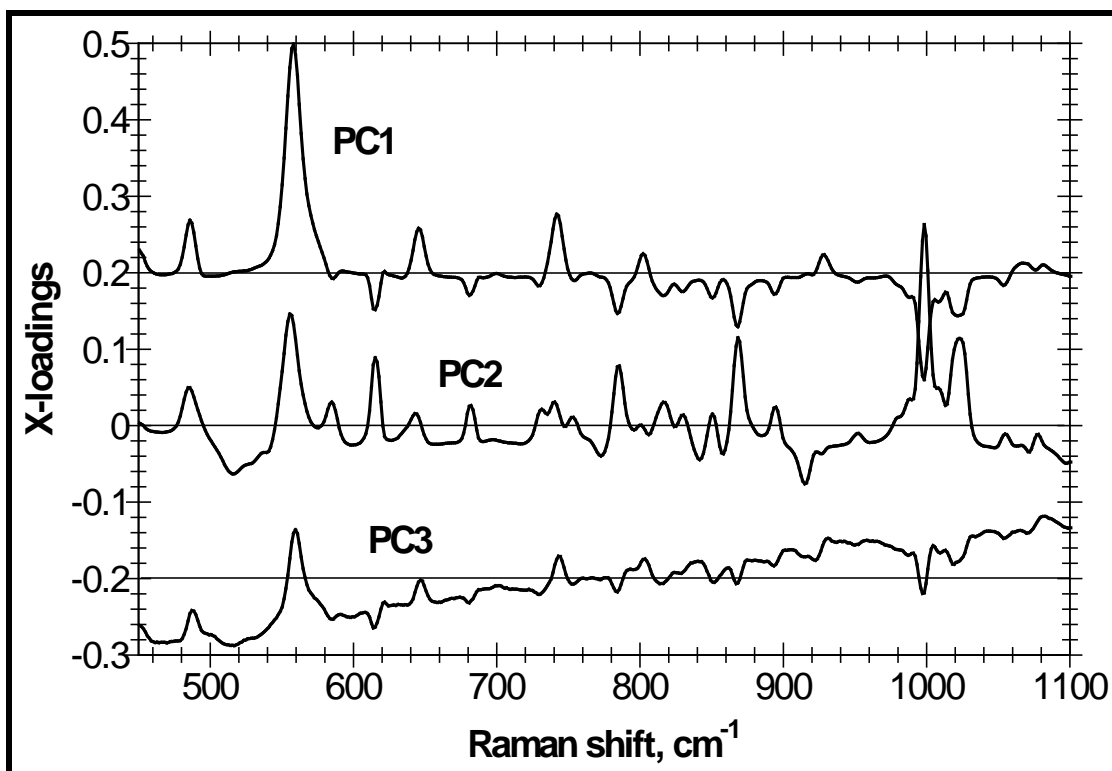


Figure 3: X-loadings plots for all three components. PC1 is offset by +0.2, and PC3 by -0.2. Same scale for all plots.

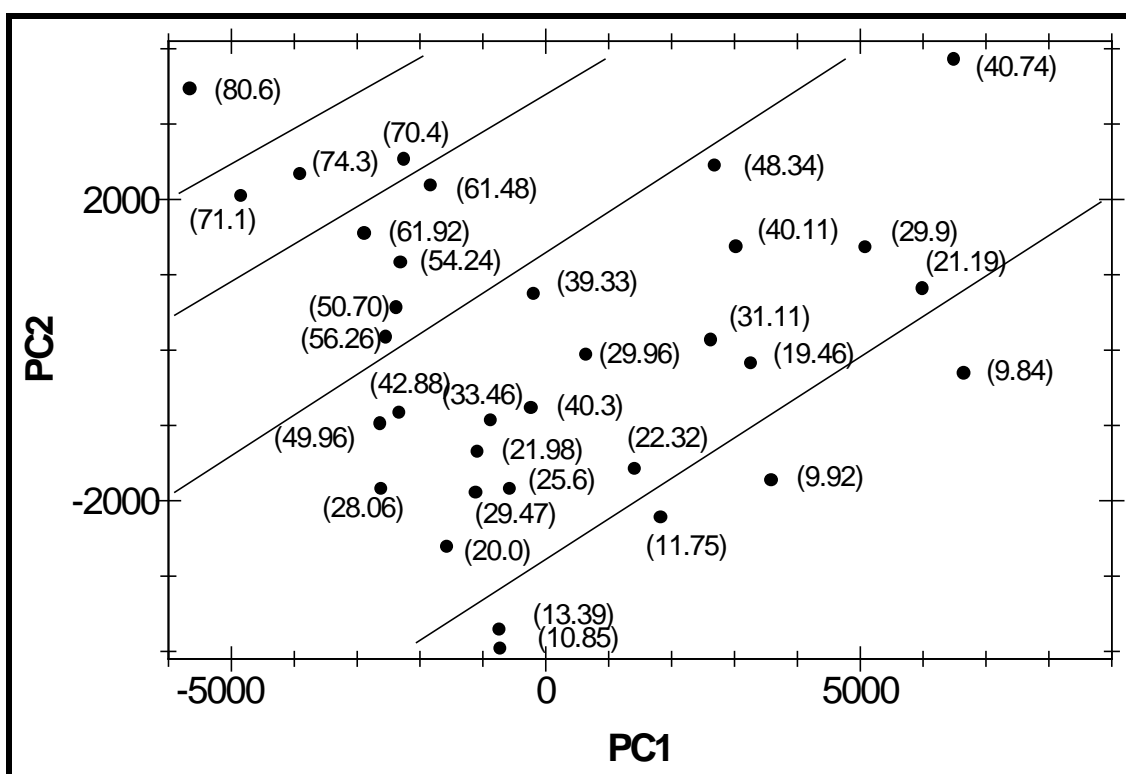


Figure 4: Score plot for PC1 vs PC2, with cocaine concentration given in parentheses.

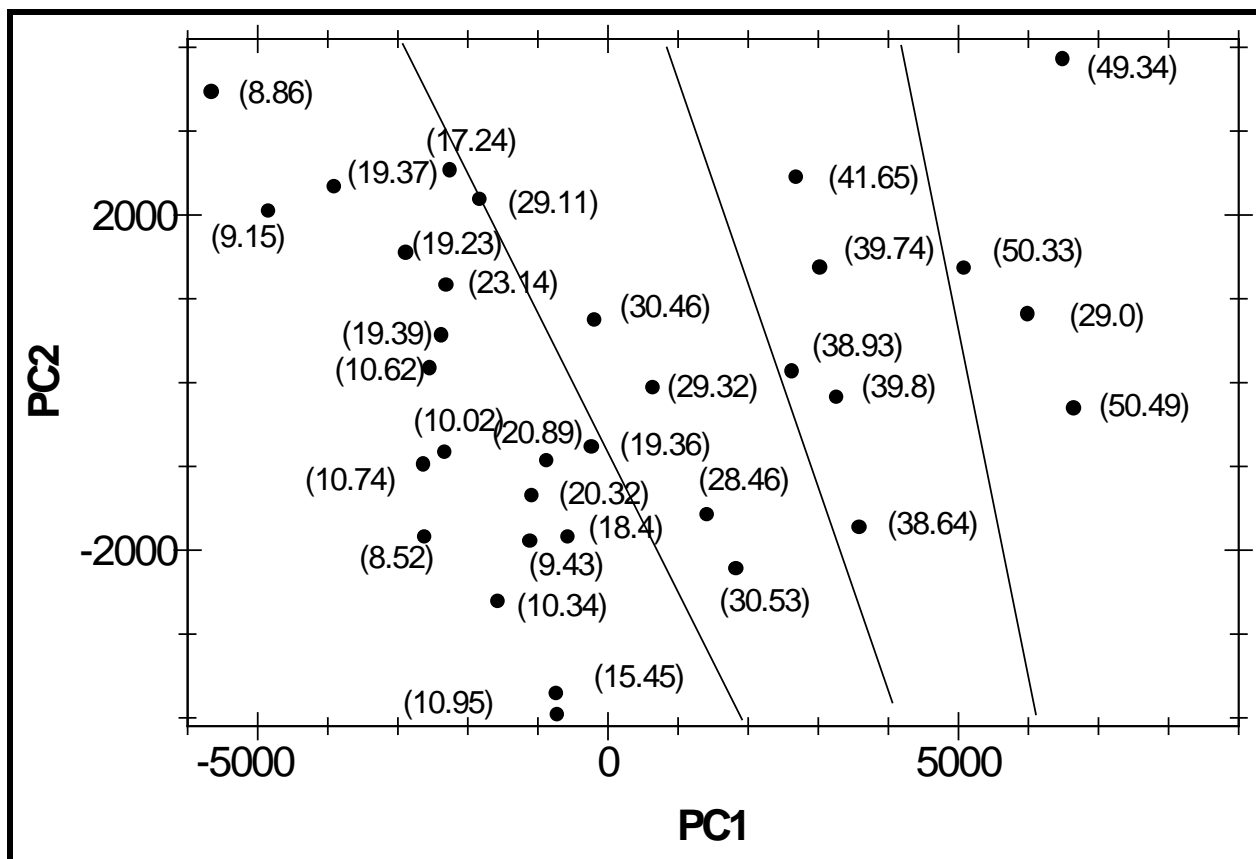


Figure 5: PC1/PC2 score plot with the caffeine concentration given in parentheses.

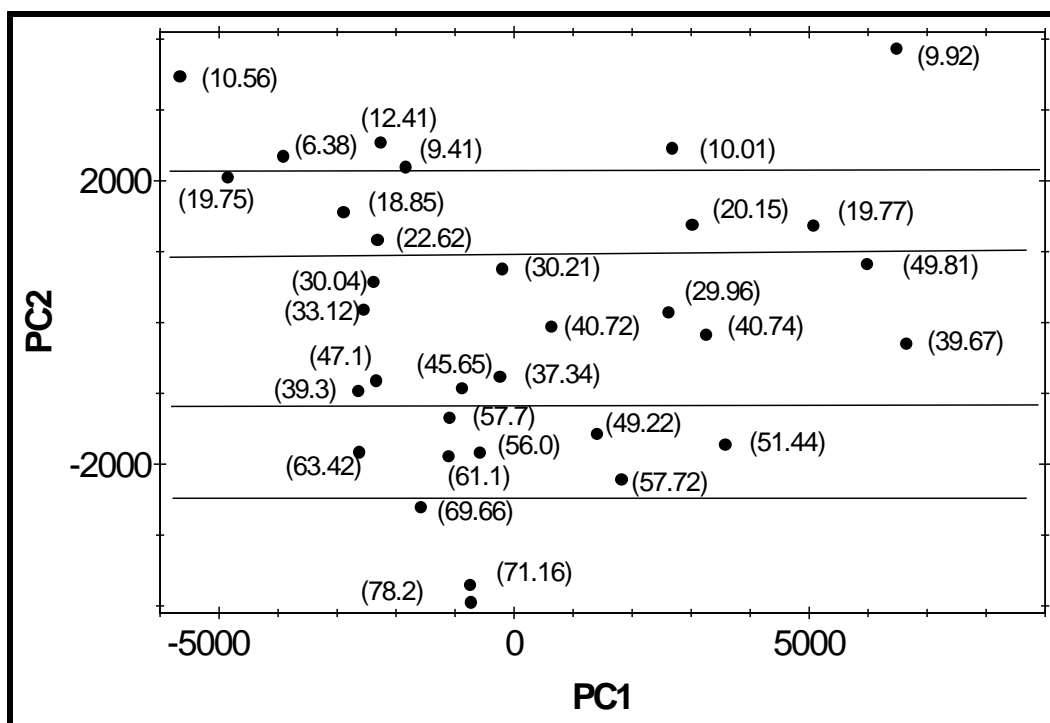


Figure 6: PC1/PC2 score plot with the glucose concentration given in parentheses.

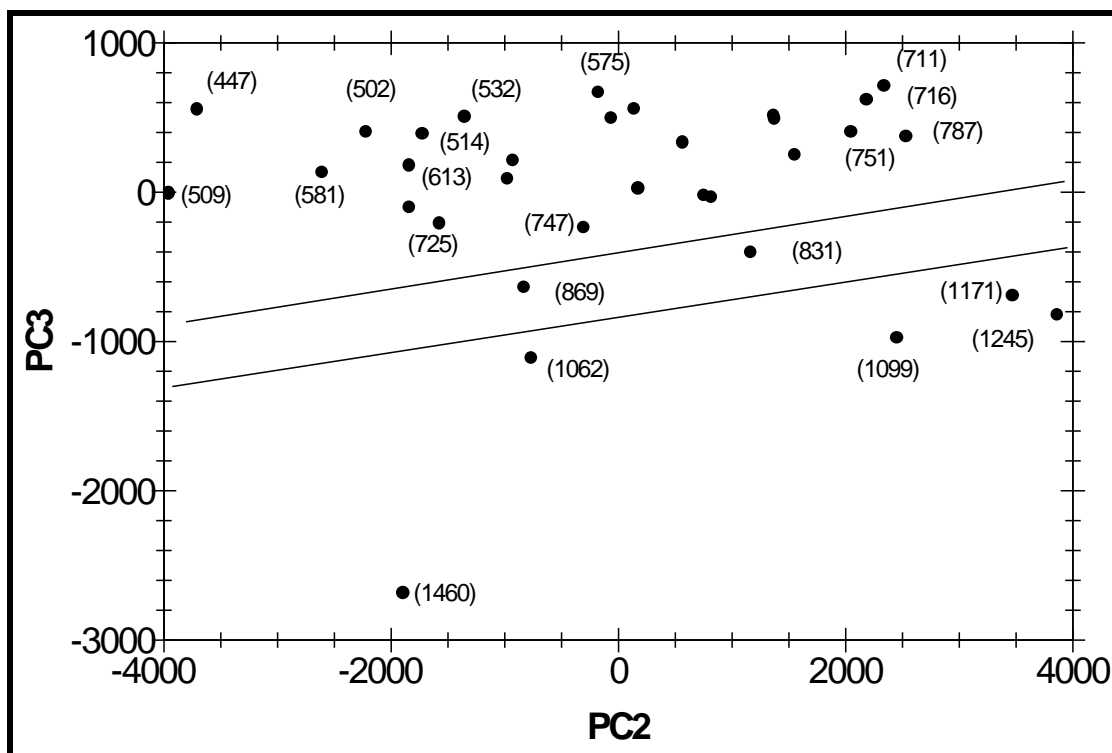


Figure 7: PC2/PC3 score plot with mean value of spectrum in parentheses (some values omitted for clarity)

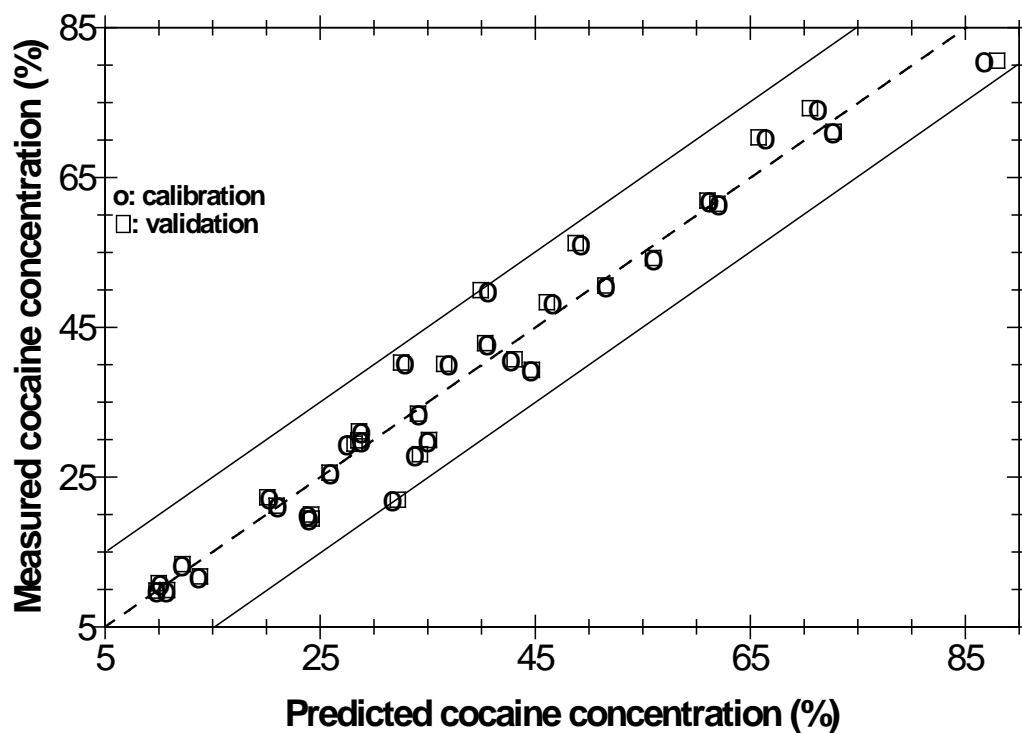


Figure 8: Predicted vs. measured cocaine concentration for PLS calibration model B.

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