Application note

Quantitative analysis of cocaine/levamisole samples by FT-Raman spectroscopy and chemometrics

Authors

Philippe Marion, Carole Miossec, Laura Guenoden, Jeanne Amargier, Martine Delcroix, Arnaud Vieillard, François Coudon, Service National de Police Scientifique, Laboratoire de Police Scientifique de Lille

Arnaud Di Bitetto,
Thermo Fisher Scientific

Introduction

Drug seizures have increased substantially in all the major northern European ports (Anvers, Rotterdam, Le Havre, and Dunkerque), and they are now considered one of the main drugs gateways into Europe. In France, cocaine seizures in particular have escalated significantly, especially in northern France, where an unprecedented 26.5 tons were seized between 2020 and 2021.

Cocaine is an alkaloid chemically extracted from coca leaves; the result is an illegal stimulant that is sold as a white crystalline powder. Cocaine appears in two main forms: a water-soluble salt that is usually injected or inhaled, and a water-insoluble free-base (crack), which is usually smoked.

In order to bulk up the product, and therefore increase profits, cocaine is frequently diluted (“cut”) with a wide variety of pharmacologically active chemicals. These are chosen for their physical or chemical resemblance to cocaine, their low cost, or their added physiological effects. The most frequently used additives include levamisole, phenacetin, caffeine, lidocaine, hydroxyzine, starch, and sugars such as mannitol, lactose, and glucose.

This application note focuses on levamisole, which is an imidazothiazole mainly used in veterinary medicine to deworm livestock, although it has increasingly been used as a cocaine cutting agent over the last decade.

In the context of police investigations, the characterization of a cocaine sample involves two steps: establishing the sample’s chemical form and then quantifying its purity. Fourier-transform infrared spectroscopy (FTIR) is currently used in French forensic laboratories to determine the chemical form of cocaine prior to the screening by GC-MS (gas chromatography mass spectrometry) and subsequent quantitative analysis by GC-FID (gas chromatography flame ionization detection).

In order to reduce analysis times and to ensure fast and effective policing, the Lille Forensic Police Laboratory, part of the Service National de Police Scientifique (SNPS) has developed a quantitative Raman spectroscopy technique that couples with chemometric methods to determine the concentrations of cocaine and levamisole in mixed powder samples.

Experimental

167 cocaine/levamisole samples from 38 different seizures, as well as an analytical reference standard of uncut cocaine, were used to develop the quantitative calibration model for both cocaine and levamisole. All samples were previously analyzed by GC-MS and GC-FID.

Samples were ground to a homogeneous powder using an agate mortar and pestle and then stored at room temperature prior to analysis.

Data was collected on a Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer equipped with a calcium fluoride beam splitter and a FT-Raman module (containing a 1064 nm laser) (Figure 1). A total of 60 scans were collected for each spectrum at 4 cm^{-1} resolution, with a total acquisition time of approximately 70 seconds. The laser power was set at 450 mW. For each sample, two spectra were acquired.

Samples were positioned in a 48-well plate and then automatically focused and analyzed using Thermo Scientific Array Automation Software. As the laser spot size is smaller than 60 microns, a very small quantity of sample is sufficient to carry out the analysis. Thermo Scientific™ OMNIC™ Software was used for instrument control, data acquisition, and data treatment.

To begin, spectra were compared against Thermo Scientific Raman libraries to determine the cocaine chemical form. Figure 2 shows the Raman spectra of both forms of cocaine (hydrochloride salt and base); the spectrum of levamisole hydrochloride is also shown.

Individual partial least squares (PLS) models were then developed for the quantitative analysis of cocaine and levamisole hydrochloride salts using Thermo Scientific™ TQ Analyst™ Software. PLS models use a statistical approach that examines the selected region(s) of the standard spectra to determine which areas vary statistically as a function of component concentration.

232 spectra were used as calibration standards and 104 spectra were used as validation standards to build both models. Concentration ranges of the components (quantified with the reference GC-FID method) are listed in Table 1.

In order to be consistent with the reference method, levamisole values will be reported from 2.0% to 68.0%. Both models used standard normal variate (SNV) pathlength treatments to mitigate spectral baseline shifting and intensity effects.

Components	Low	High
Cocaine	23.1%	100.0%
Levamisole	0.0%	68.0%

Table 1. Cocaine and levamisole concentration ranges used for calibration development.

Figure 1. Nicolet iS50 FTIR Spectrometer with the iS50 Raman Accessory (left). Available analysis templates (right).

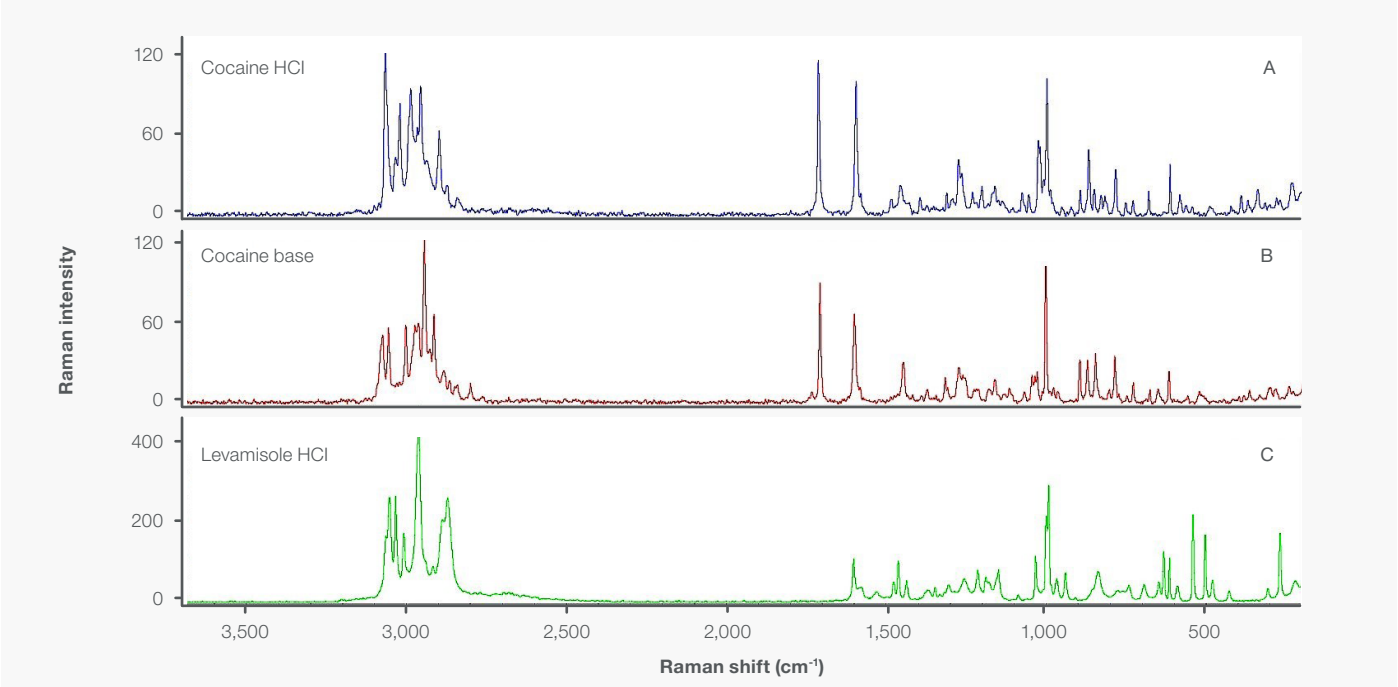

Figure 2. Raman spectra of (A) cocaine hydrochloride salt, (B) cocaine base, and (C) levamisole hydrochloride salt.

Results and discussion

Both PLS models show low root mean square error of calibration (RMSEC) and good correlation to the reference method data. The correlation coefficient and RMSEC are indications of how well the component concentrations of the calibration standards are predicted by the calibration model. Ideally, the correlation coefficient should have a value close to one, and the RMSEC should approach the standard error of the reference technique (5%). The factors that represent independent sources of variation from the concentration and spectral information are ranked by the amount of variation in the data that they explain.

Table 2 shows how well the PLS models quantify cocaine and levamisole in powder mixtures.

Additionally, comparison of the root mean square error of prediction (RMSEP) to the RMSEC can provide a good indication of how accurately the model predicts samples that are not in the calibration. The RMSEP is computed with an independent set of validation samples that were withheld from the calibration.

Another test of model robustness is the root mean square error of cross validation (RMSECV). This diagnostic model sequentially removes a specified number of standards from the calibration set (in this case, 10 standards were removed during each calibration), calibrates the method, and then uses the new calibration model to quantify the standards that were removed from the calibration set. This is repeated until all the standards in the calibration set have been quantified as validation standards.

A good result for model accuracy is that both the RMSECV and the RMSEP are less than 1.5 times the RMSEC. This is the case for both PLS models discussed here.

The calibration curve for cocaine in Figure 3 demonstrates good correlation between the calculated (FT-Raman) and reference (GC-FID) values with a correlation coefficient of 0.982 and an RMSEC of 2.09. The calibration curve for levamisole in Figure 4 also demonstrates good correlation between the calculated and reference values with a correlation coefficient of 0.990 and an RMSEC of 1.67.

The predicted residual error sum of squares (PRESS) plots (Figures 5 and 6) rank the variation factors and show their associated variations. Each time a factor is added, and represents useful information to the calibration model, RMSECV and PRESS values decrease.

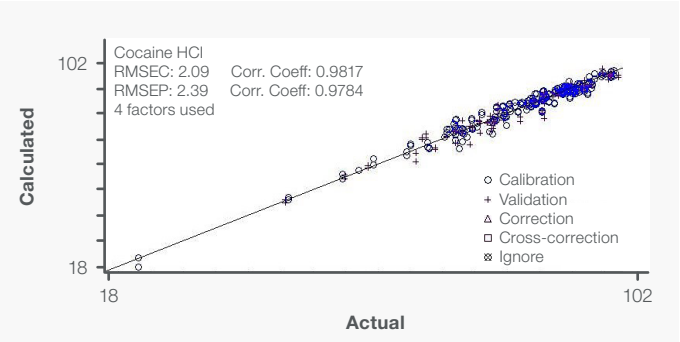

Figure 3. Calibration curve for cocaine with independent validation (+) samples.

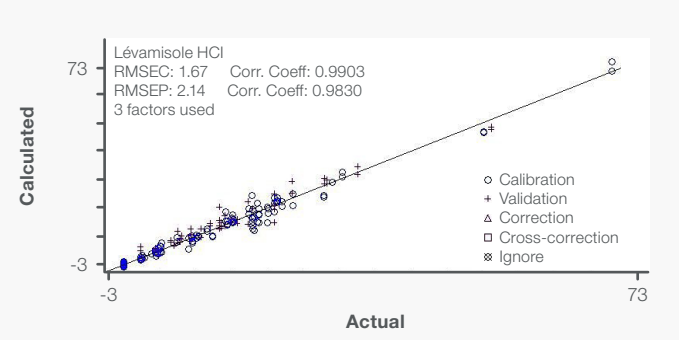

Figure 4. Calibration curve for levamisole with independent validation (+) samples.

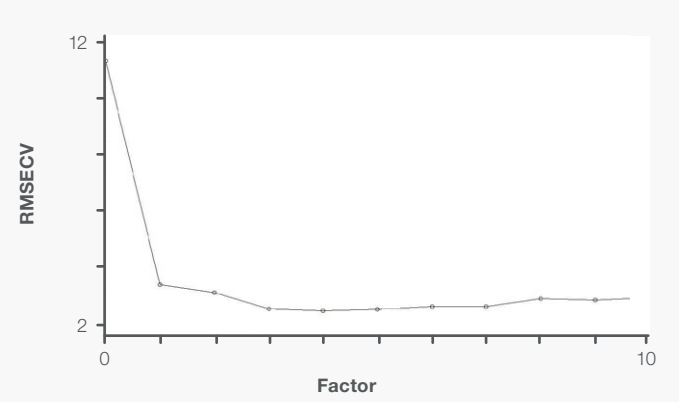

Figure 5. PRESS plot for cocaine PLS model.

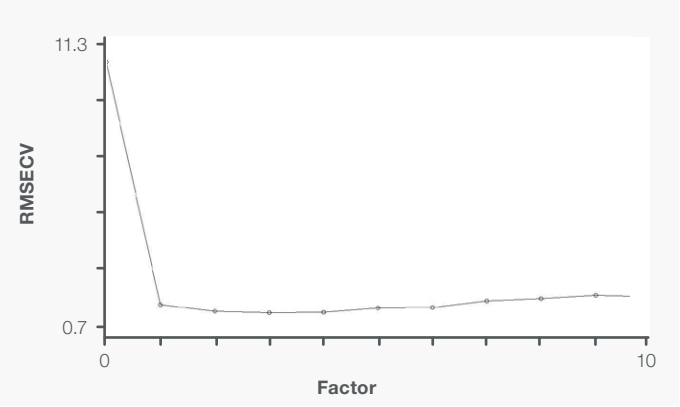

Figure 6. PRESS plot for levamisole PLS model.

PLS model	Factors	RMSEC	Correlation coefficient	RMSECV	RMSEP
Cocaine	4	2.09	0.9817	2.39	2.39
Levamisole	3	1.67	0.9903	1.89	2.14

Table 2. Summary of calibration results for PLS models.

In order to evaluate the accuracy of this new method in comparison with the reference method (GC-FID), 89 samples were analyzed by the two methods at the same time.

For cocaine, all differences between results were below 4.0% and deviations were even below 2.0% for 81.0% of the samples. For levamisole, all differences between results were below 2.6% and deviations were below 2.0% for 96.6% of the samples.

Conclusions

The Nicolet iS50 FTIR Spectrometer, equipped with the iS50 Raman Accessory, was used along with proprietary software packages to provide an innovative solution that can simultaneously determine a cocaine sample's chemical form (hydrochloride salt or base) and quantify its cocaine and levamisole concentrations.

This non-destructive method was successfully applied to the quantification of a hundred samples and provided results that were comparable to those obtained by the laboratory reference method (GC-FID).

Sample preparation is faster and easier, making it an ideal method for the routine determination of cocaine purity within the short deadlines required by police authorities. This analytical method could also be adapted to other cocaine cutting agents, such as phenacetin or caffeine.

References

1. UNODC, Cocaine - A spectrum of products, Cocaine Insights 2, UNODC, Vienna, October 2021
2. Quantitative Analysis of Wheat Flour Using FT-NIR, Application note AN52269, Thermo Scientific

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