JOURNAL OF FORENSIC SCIENCES

*J Forensic Sci,* January 2012, Vol. 57, No. 1
doi: 10.1111/j.1556-4029.2011.01945.x
Available online at: onlinelibrary.wiley.com

**PAPER**

**CRIMINALISTICS**

*Dora Melucci,*[1] *M.S., Ph.D.; Dario Monti,*[1] *M.S.; Marcello D'Elia,*[2] *M.S.; and Giorgio Luciano,*[3] *Ph.D.*

# Rapid *In Situ* Repeatable Analysis of Drugs in Powder Form Using Reflectance Near-Infrared Spectroscopy and Multivariate Calibration

**ABSTRACT:** This study takes the first step toward *in situ* analysis of powder drugs which does not require any alteration of the samples. A fast, inexpensive analytical method based on reflectance near-infrared (NIR) spectrometry and multivariate calibration was applied. A diode-array fiber-optic portable spectrometer in the 900–1700 nm range was employed. Samples were laboratory-prepared ternary powders (diacetylmorphine, caffeine, and paracetamol). Partial least squares regression was applied. The choice of the standard samples for calibration and validation was performed through a D-optimal experimental design. The explained variance was higher than 90%, and the relative root mean square errors were <2%. The number of principal components (6) was very low when compared with the number of raw variables (356 absorbance values). Response plots showed slopes and intercepts were very close to optimal values. Correlation coefficients ranged between 0.909 and 0.989. The method here proposed proved to be competitive with Fourier transform NIR spectrometry.

**KEYWORDS:** forensic science, forensic chemistry, powder drugs, *in situ* repeatable analysis, near-infrared, partial least squares regression

One of the crimes most frequently faced by the State Police in the city of Bologna (Italy) is illegal possession and sale of street drugs in solid matrices. Among the narcotics and psychotropic substances seized and analyzed by this body during the year 2007, cannabis derivatives were most abundant (about 57% of the total seized), followed by cocaine (about 22%) and heroin (about 17%). As regards excipients found in the heroin samples seized, the Scientific Police of Bologna found paracetamol and caffeine almost in every case. The paracetamol content was found to be as high as $70\%_{w/w}$, while the maximum detected caffeine content was $60\%_{w/w}$.

Once confiscated, such samples must be analyzed. Requirements for analyses are nondestructivity, rapidity, accuracy, and ability to be carried out *in situ*. Nondestructivity is especially important because, from the legal point of view, it is a necessary condition for repeatability: the best condition for legal authorities is to have access to material evidence of a crime that can be made available whenever analysis is needed.

Modern instrumental analytical techniques, such as chromatography, mass spectrometry, and capillary zone electrophoresis, meet the demands for accuracy, but they do not meet the other demands:

- These techniques always alter samples (1–3).
- These techniques are not inexpensive or rapid: each analysis consumes both chemicals and time; moreover, to ensure that the analytical results are useful in the legal sense, copious official reports need to be written attesting to all experimental details relevant to each step of the entire experimental procedure carried out in the laboratory, and this takes time. Being able to analyze samples without altering them would be highly preferable.
- Application of these techniques always requires highly skilled operators: a chemist is needed to adequately manage the exhibits, ensuring that samples are taken correctly and the sampled aliquots opportunely dissolved. On the other hand, even personnel without professional competence in chemistry would be able to illuminate an exhibit with a reflectance near-infrared (NIR) probe and record the corresponding spectrum using a portable computer.
- Using these techniques, it is not easy to carry out analyses directly at the place where samples are seized.
- These techniques are very expensive, in terms of both instrumentation purchase and maintenance.

Besides validated instrumental analytical techniques, the State Police also commonly apply color tests which provide, *in situ*, a rapid overall identification of the active principles, thus enabling them to quickly determine whether they are dealing with a case of possession or trafficking in illegal drugs. However, according to the Scientific Police, color testing often gives false positives. Moreover, it does not provide any information about cutting agents. A rapid *in situ* method able to provide a rough analysis of both the active principles and cutting agents in solid drugs without altering samples is highly desirable. A possible candidate to meet such expectations is NIR spectrometry: NIR spectra can potentially be used as multivariate fingerprints of molecules (4,5); modern technology provides inexpensive, portable diffuse reflectance NIR spectrometers that use fiber-optic probes, making it possible to rapidly analyze powders without ever touching samples, simply shining a light on them.

[1]Department of Chemistry "Giacomo Ciamician," University of Bologna, Via Selmi 2, I-40126 Bologna, Italy.
[2]Gabinetto Regionale di Polizia Scientifica per l'Emilia Romagna, Via Volto Santo 3, I-40123 Bologna, Italy.
[3]Institute of Marine Science, National Council for Research, Via de Marini 6, I-16149 Genoa, Italy.

Received 23 Mar. 2010; and in revised form 20 Sept. 2010; accepted 8 Oct. 2010.

In fact, NIR spectra are characterized by a lack of definite peaks; moreover, even if the spectrum from a pure substance may be used as fingerprint, in real samples, the copresence of several different substances make NIR spectra convolute and consequently, their use as fingerprints may be complicated. To address this fundamental issue, chemometric methodologies may be employed: multivariate statistics is required to extract useful analytical information from NIR spectra (6,7). Many recent publications (8–10) report good results with multivariate calibration based on the partial least squares regression method (PLSR) applied to spectral data; in some cases, powders containing some of the substances used here were analyzed (11,12): this is the reason why PLSR was adopted for data processing in this work as well.

In fact, inexpensive, portable NIR spectrometers are not Fourier transform instruments and consequently cannot work in reflectance above 1700 nm, where NIR spectra are particularly suitable for fingerprint identification. The present work seeks to demonstrate that in spite of the spectral range limitations, the method proposed is reliable enough to be useful for State Police investigations and competitive with FT-NIR (13). The rationale for using the 900–1700 nm range is that this choice makes it possible to use a very inexpensive, truly portable diode-array NIR spectrometer: it can be carried in a small bag and, together with a laptop computer, used to operate the spectrometer and run the data processing software.

After eventual validation, the main possible application of the method—the feasibility of which is demonstrated here—will be to perform a preliminary, rough screening of what pharmacological category a possible illicit powder belongs to. In fact, sophisticated methods are available for identifying and quantifying a sample once it has been brought to State-Police chemical laboratories; however, this takes time and at times promptness is a determining factor for the success of a legal investigation. If a simple, inexpensive method, like the one proposed here, were available, investigators could promptly decide—right at the site where the crime scene inspection or individual search has taken place—whether a powder is in fact an illicit drug, what kind of drug it is, whether the quantity exceeds the legal limit for possession, what cutting agents are present and their relative amounts. While the initial investigation should then be borne out by complete laboratory analysis, such method would jump-start the judiciary proceedings. All these details are important in reconstructing the history of a seized powder.

In the city of Bologna, heroin and cocaine are among the powder drugs most frequently seized. For this reason, these drugs were first considered as samples for testing the method proposed. Given that the pure solid standard of diacetylmorphine is cheaper and more readily available, heroin was chosen as the analyte for the present feasibility study. Once the feasibility of using reflectance NIR over a limited wavelength range to create a relevant PLS model for the analysis of powder samples has been demonstrated, cocaine, too, will be taken into account for analogous feasibility investigation.

Using PLSR to create a model requires having standard samples (objects) of accurately known composition. Usually, objects are real samples that have undergone analysis by validated methodologies independent of the analytical methodology on which chemometric calibration is based. However, in the present work, a different choice was made regarding preparation of objects: here they were obtained by mixing pure standards in precisely known percentages. The reason for this choice was practical: given the limited spectral range of the available DAD-NIR spectrometer, there was some uncertainty as to whether a significant signal would be observed and thus an adequate dynamic range did subsist before starting experimental work; moreover, there was the risk of investing *a priori* a great deal of time and money in analyzing real seized samples without *a posteriori* obtaining any useful analytical information from NIR spectra. On the contrary, simulation of real samples using reference samples prepared in the laboratory would provide an idea as to whether investigation of the proposed NIR-chemometric methodology is worthwhile. Once the model created from reference samples has been validated, creation of a more robust model based on real samples of known composition is to be hoped for: work in this direction is currently underway.

## Materials and Methods

### *Samples*

Prior to sample preparation, a Design of Experiments (DOE) was developed. Following the DOE, 13 solid standard mixtures were prepared to form the Training Set (samples for creation of the chemometric model), and 15 solid standard mixtures were prepared to form the Test Set (samples for validation of the created PLSR model).

All standard samples were obtained by weighing pure substances and mixing them according to the proportions indicated in the DOE. An analytical scale with $10^{-5}$-g minimum weight was used (Model SBC21; SCALTEC, Göttingen, Germany). In the case of NIR analyses, the 28 standard samples were subjected to a unique pretreatment involving preliminary homogenization in a mortar for few minutes; after that, samples were transferred to the bottom of vials and the NIR reflectance probe introduced for NIR-spectra acquisition. It must be pointed out that in the present feasibility work, sample mixing was necessary because they were prepared from pure standards; in fact, in the case of real standard samples and unknown samples, the samples will not need to be pretreated or even touched.

Each standard sample of the Training Set and the Test Set contained three components, those most frequently found in heroin samples seized on the streets of Bologna. These main components were diacetylmorphine (active narcotic principle), caffeine and paracetamol (adulteration excipients with pharmacological action, antagonist to the catastrophic health effects of heroin and aiming to reduce them).

Diacetylmorphine was purchased from S.A.L.A.R.S. S.p.A. (Como, Italy), while caffeine and paracetamol were purchased from Sigma (St. Louis, MO).

### *Experimental*

*DAD NIR*—The detector was a diode-array NIR spectrometer (EPP2000-NIR-InGaAs; StellarNet, Tampa, FL), operating in the 900–1700 nm range (11,100–5880 $cm^{-1}$) with optical resolution equal to 3.1 nm. Integration time was 90 ms.

For DAD-NIR measurements, a coaxial fiber-optic was used (internal diameter: 400 μm). The fiber formed a Y-connection that linked the light source and the detector to the terminal of 10-cm-long reflectance probe that was set put in contact with the solid sample. The sample was placed at the bottom of a brown glass vial whose internal diameter was equal to the external diameter of the probe (6 mm). The mass of the sample was 0.05 g; this value was chosen after a preliminary study on minimum sample mass required for repeatable spectra while keeping the sample composition constant. The minimum sample mass required for repeatability proved to be 10 mg; a value five times higher was chosen to keep the level of confidence above 99.99%.

The light source (model SL1; StellarNet) was a tungsten–kripton lamp as it has strong enough emission in the vis-NIR spectral

region to perform reliable measurements of apparent absorbance ($A = \log(P_0/P_R)$, where $P_0$ is the reflected-light power measured when a NIR-zero sample is illuminated with the probe and $P_R$ is the reflected-light power when a sample is illuminated.

Each NIR-spectra acquisition was run according to the following procedure. While keeping the light source off, a *Dark Zero* spectrum was recorded. Then, the source was switched on and the spectrum obtained from a $BaSO_4$ reference plate (model OPRSF5.5C; StellarNet) was recorded. In this manner, the $P_0$ value was obtained for all the wavelengths in the explored NIR range.

*FT NIR*—A commercial Antaris II FT-NIR Analyzer (Thermo Scientific, Milan, Italy) was used. The spectral range was 1000–2200 nm (10,000–4545 $cm^{-1}$). Resolution was 4 $cm^{-1}$ across the spectral range (0.6 nm at 1250 nm).

Fiber-optic sampling with the SabIR™ probe (Thermo Scientific) was employed for comparison with the DAD-NIR results.

### *Chemometric Methods*

*Spectra Preprocessing*—For the sake of comparison, spectra were used both raw and pretreated with the standard normal variate (SNV) method (14). In SNV pretreatment, the spectra are shifted and scaled using, for each spectrum, coefficients derived from that spectrum alone. For each spectrum, the mean and standard deviation of the $n$ absorbance values for the corresponding $n$ wavelengths of that spectrum alone are calculated. Absorbance values are transformed to ([absorbance−mean]/standard deviation). This correction is a normalization, which eliminates the very poor optical path repeatability inside the powder, a fact which depends heavily on particle size distribution and compactness as well as on the geometric measurement set-up (distance and angle of the probe vs. the explored surface). The scientific basis for normalization as pretreatment of spectroscopic data is reported in the literature and widely applied to spectral data ([14] and references therein). In fact, no significant difference was found between using normalized and raw spectra. For this reason, raw spectra and relevant chemometric models will be presented.

*Experimental Design*—Experimental design is a chemometric method used to plan experiments where the input variables are systematically varied within predefined ranges so that it is possible to estimate their effects on output variables (responses) and check for significance. The number of experiments and the way they are built depend on the objective and on operational constraints. A particular case of experimental design is the D-optimal design. Generated by the DOPT algorithm, it takes into account the multilinear relationships that exist between design variables and the constraints relevant to experimental regions (15,16).

In this work, the choice of the objects for Training Set and Test Set was performed through a D-optimal experimental design.

*Partial Least Squares Regression*—Univariate calibration is a well-known issue, routinely applied to spectrometric data sampled at a fixed wavelength. *Absorbance* versus *concentration* data are collected for standard samples, and linear regression is applied to calculate the calibration straight line that best fits the experimental data. To predict unknown samples, interpolation on calibration straight line is carried out. The whole procedure is based on validated equations (17). To apply univariate spectroscopic calibration, two necessary conditions must hold true:

- the analytes of interest must be characterized by specific absorption maxima;
- if several analytes are copresent, the corresponding absorption bands must not overlap.

In the case of NIR spectra, univariate calibration proves impossible because there is no detectable characteristic absorption band: this stems from the fact that NIR absorbance corresponds to overtones and combinations of molecular vibrations based in the mid-IR region of the spectrum (4). Hence, quantitative analysis based on NIR data requires multivariate calibration. The flip side of this is that multianalyte samples can be quantified through multivariate analysis, even when they give rise to overlapping absorption bands.

The input for multivariate calibration based on NIR data is a numerical matrix obtained as follows. The input matrix *lines* are relevant to *objects*, i.e., standard samples of accurately known composition. The input matrix *columns* are relevant to *variables*: independent variables ($x$) are *all* the *absorbance* recorded to obtain NIR spectra; dependent variables ($y$) are the *concentrations*.

The simplest multivariate calibration method is multiple linear regression (MLR) (18,19). The chemometric software calculates as many equations as the *dependent variables* to be determined; each equation relates one *dependent variable* to *all independent variables*. Taken together, the calculated equations form the *chemometric model*. The quality of the resulting models is measured by two types of root mean square errors (RMSE): RMSEC evaluates the ability of the model to *fit* experimental values (*calibration*), while RMSEP evaluates its ability to *predict* unknown values (*validation*).

In the case of spectroscopic data, MLR does not work, for two reasons:

- independent variables are strongly correlated;
- the number of *objects* is much smaller than the number of *variables*.

For multivariate calibration based on NIR spectra, PLSR may be applied. PLSR is a generalization of MLR. Few linear combinations of original $x$-values are calculated and used in the regression equation, maximizing correlation with $y$-values. In this way, insignificant and unstable information is discarded and only the most significant part of the $x$-variation is used for regression. The linear combinations of original variables are called principal components (PCs).

In this work, PLSR is applied with preprocessing that consists of interobject *centering*: each value in the input matrix is substituted with its difference versus the column-mean value. This preprocessing corrects for the baseline shift that usually affects NIR spectra in reflectance mode and is related to the low reproducibility of probe geometric position versus sample surface.

*Response Plots*—As the calibration model is an equation which cannot be displayed in two-dimension plots, other bidimensional plots are used to display the quality of the created model: *response plots.* These plots report *predicted* versus *measured* responses, both in *calibration mode* (relevant to Training Set) and in *validation mode* (relevant to Test Set).

The performance of a model is measured through the *slope* and the *intercept* of the *response plots*: the closer the slope is to unity and the closer the intercept is to zero, the better the model performance will be.

### *Software*

Experimental design was performed using Matlab® (The MathWorks, Natick, MA). Multivariate calibration was performed using The Unscrambler® (CAMO, Oslo, Norway). Plots were created using Origin® (Microcal, Northampton, MA).

## Results and Discussion

### *Experimental Design*

Tables 1 and 2 report the composition of the standard mixtures forming, respectively, the Training Set and Test Set. Concentration values are expressed as weight-over-weight percent. In the case of nonpure samples, numerical values are reported with four significant figures to obtain details regarding how close experimental values were versus values calculated by DOE.

### *PLSR Results Obtained with DAD-NIR*

Figure 1 reports DAD-NIR spectra relevant to pure analytes (Fig. 1*a*), Training Set (Fig. 1*b*), and Test Set (Fig. 1*c*), respectively.

Partial least squares regression results for diacetylmorphine, caffeine, and paracetamol are, respectively, reported in Fig. 2*a–c*, which are the *response plots* for the model created. In each reported response plot, the straight line (unity slope and null intercept) is the TARGET LINE, corresponding to perfect agreement between the experimental concentration of the considered analyte in standards (objects) and the concentration predicted by the created model. In all cases, the points reported in *response plots* are close

TABLE 1—*Composition of standard heroin-like powder mixtures forming the Training Set.*

| Sample number | Diacetylmorphine ($\%_{w/w}$) | Caffeine ($\%_{w/w}$) | Paracetamol ($\%_{w/w}$) |
|---|---|---|---|
| 1 | 0 | 0 | 100 |
| 2 | 0 | 25.04 | 74.96 |
| 3 | 0 | 55.02 | 44.98 |
| 4 | 0 | 94.93 | 5.07 |
| 5 | 0 | 100 | 0 |
| 6 | 30.15 | 34.80 | 35.05 |
| 7 | 35.04 | 29.95 | 35.01 |
| 8 | 45.05 | 54.95 | 0 |
| 9 | 49.98 | 0 | 50.02 |
| 10 | 49.67 | 50.33 | 0 |
| 11 | 54.99 | 0 | 45.01 |
| 12 | 94.97 | 0 | 5.030 |
| 13 | 100 | 0 | 0 |

TABLE 2—*Composition of standard heroin-like powder mixtures forming the Test Set.*

| Sample number | Diacetylmorphine ($\%_{w/w}$) | Caffeine ($\%_{w/w}$) | Paracetamol ($\%_{w/w}$) |
|---|---|---|---|
| 14 | 4.980 | 70.00 | 25.02 |
| 15 | 0 | 80.03 | 19.97 |
| 16 | 75.00 | 25.00 | 0 |
| 17 | 75.06 | 14.97 | 9.970 |
| 18 | 74.96 | 20.06 | 4.980 |
| 19 | 0 | 74.99 | 25.01 |
| 20 | 4.950 | 65.03 | 30.02 |
| 21 | 10.03 | 64.93 | 25.04 |
| 22 | 0 | 5.120 | 94.88 |
| 23 | 5.080 | 74.86 | 20.06 |
| 24 | 0 | 84.94 | 15.06 |
| 25 | 50.04 | 44.91 | 5.050 |
| 26 | 40.04 | 30.01 | 29.95 |
| 27 | 74.95 | 0 | 25.05 |
| 28 | 0 | 40.00 | 60.00 |

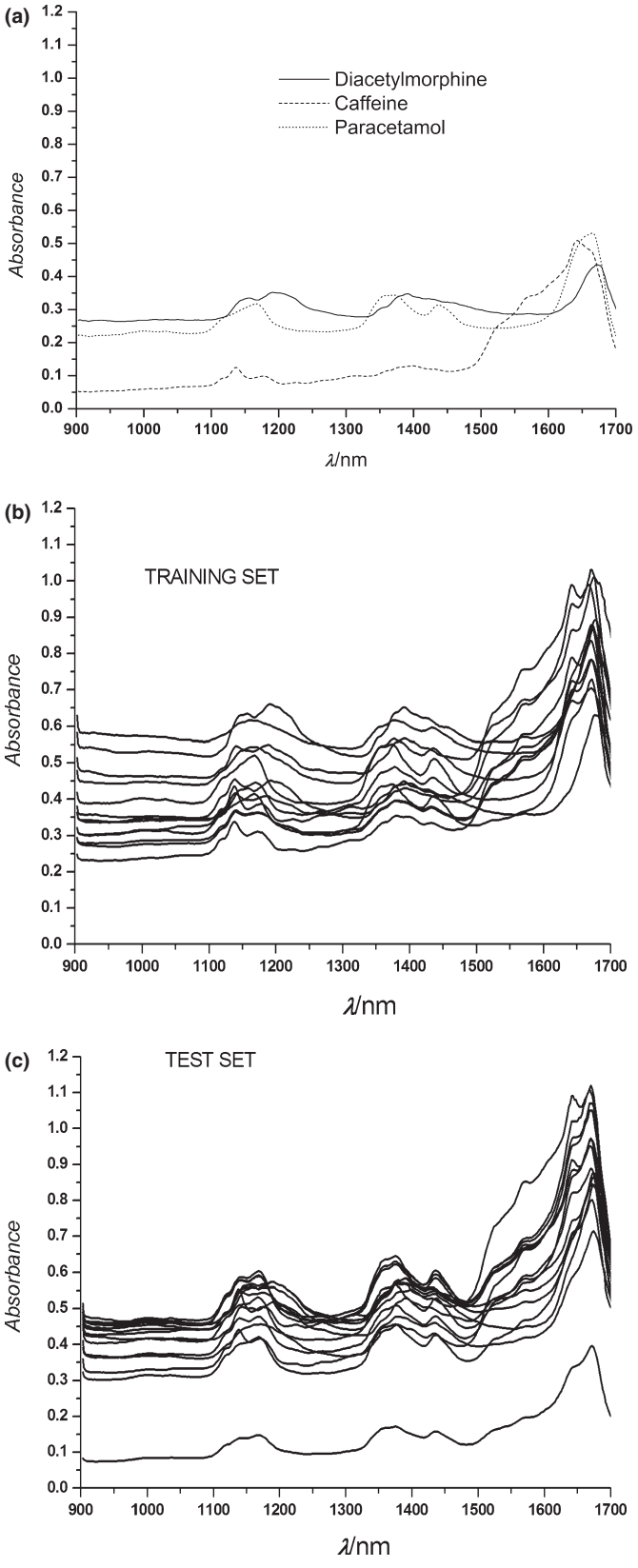


FIG. 1—*(a) DAD-NIR spectra: pure substances. (b) Training Set. (c) Test Set.*

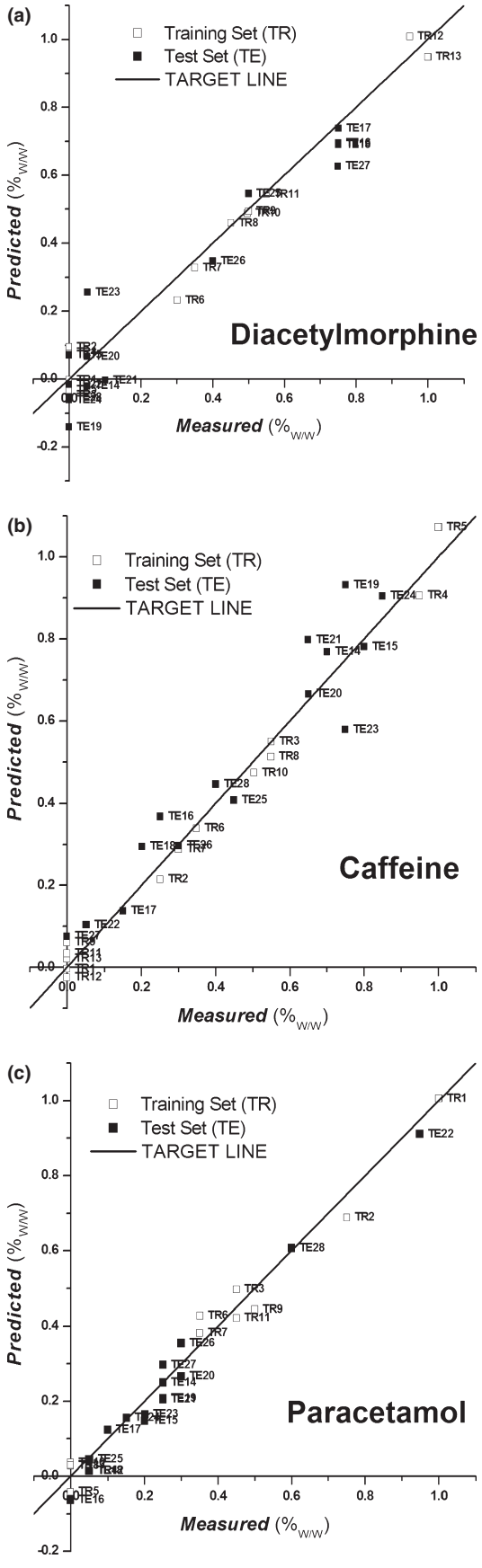


FIG. 2—*(a) Response plot for (a) diacetylmorphine, (b) caffeine, and (c) paracetamol, based on DAD-NIR data. Number of PCs: 6.*

to target line, thus demonstrating the good performance of the created PLS-regression model.

Statistical parameters are reported for the created model in Table 3, block A reporting (i) the number of components used by the model, (ii) the percentage of variance found, and (iii) the root mean square error in calibration and prediction (RMSEC and RMSEP). The error-related parameters are usually quoted with only one significant figure; however, to obtain greater information on the quality of the model, here RMSEC and RMSEP are reported with three significant figures. Table 3, block A shows very high variance, and this means that the PCs used to create the PLSR model hold a very high percentage of useful analytical information versus the original analytical variables. Moreover, the number of PCs is very low when compared to the number of raw variables: six PCs versus 356 absorbance values for each spectrum, obtained by a like number of diodes. Finally, calculated root mean square errors are low when compared with the corresponding concentration values, and this means good precision. Table 4, block A reports numerical details relevant to response plots. As the target line has null intercept and unitary slope, measured slopes and intercepts result very close to target values; moreover, correlation between *measured* and *predicted* values is close to the ideal unitary value. Hence, analysis of the response plots leads to the conclusion that the created PLS model performs well.

Besides response plots, another interesting output of the described PLSR processing is the regression coefficient plot (Fig. 3) reporting the superimposed regression coefficients for the three components of the samples. Figure 3 demonstrates the existence of the characteristic wavelengths, corresponding to curve maxima, thus indicating the model's ability to discriminate between various components of powder samples: different analytes have significantly different characteristic wavelengths. This finding is coherent with spectra relevant to pure analytes (Fig. 1*a*).

### *PLSR Results Obtained with FT-NIR*

The same data processing discussed for DAD-NIR data was then applied to FT-NIR data, and the results are reported in Tables 3 and 4, blocks B and C.

The optimal number of PCs recommended by the software for FT-NIR data is 3. In this case, created model shows good performance (Table 3, block B). However, these results are slightly worse than those obtained in the case of DAD-NIR (Table 3, block A). In particular, poor results were observed for all analytes in validation mode, and for paracetamol, even in calibration.

Tables 3 and 4 (block C) report FT-NIR results for the same number of PCs as for DAD-NIR data. In fact, performance is comparable to the DAD-NIR-based model (Tables 3 and 4, block A), and in some cases even worse, particularly in validation mode and especially for diacetylmorphine.

## Conclusions and Perspectives

A very inexpensive methodology based on a portable DAD-NIR reflectance spectrometer in the 900–1700 nm range, and PLSR calibration by reference standard samples prepared in the laboratory, has been tested and compared with FT-NIR methodology. Three-component samples were examined (diacetylmorphine, caffeine, and paracetamol). The key advantages of the proposed method are low-cost, *in situ* analysis, no sample alterations, portability, and rapidity.

It has been demonstrated that even using a very inexpensive DAD-NIR spectrometer—*per se* unable to explore the fingerprint NIR range covered by expensive FT-NIR spectrometers—it is

TABLE 3—*Partial least squares regression results: explained variance and root mean square errors.*

| Method | Analyte | Variance explained (calibration) (%) | Variance explained (validation) (%) | RMSEC ($\%_{w/w}$) | RMSEP ($\%_{w/w}$) |
|---|---|---|---|---|---|
| A | Diacetylmorphine | 98.0 | 92.8 | 0.0481 | 0.0880 |
| DAD-NIR | Caffeine | 98.9 | 90.9 | 0.0358 | 0.0924 |
| Number of PCs: 6 | Paracetamol | 98.1 | 97.4 | 0.0431 | 0.0378 |
| B | Diacetylmorphine | 97.5 | 89.0 | 0.0540 | 0.109 |
| FT-NIR | Caffeine | 97.2 | 91.3 | 0.0567 | 0.0904 |
| Number of PCs: 3 | Paracetamol | 89.2 | 88.8 | 0.103 | 0.0784 |
| C | Diacetylmorphine | 99.52 | 86.3 | 0.0235 | 0.122 |
| FT-NIR | Caffeine | 99.8 | 90.8 | 0.0154 | 0.0929 |
| Number of PCs: 6 | Paracetamol | 99.7 | 88.5 | 0.0178 | 0.0795 |

PCs, principal components; RMSEC, root mean square error in calibration; RMSEP, root mean square error in prediction.

TABLE 4—*Partial least squares regression results: response-plots parameters.*

| Method | Analyte | Slope (calibration) | Slope (validation) | Intercept (calibration) ($\%_{w/w}$) | Intercept (validation) ($\%_{w/w}$) | Correlation (calibration) | Correlation (validation) |
|---|---|---|---|---|---|---|---|
| A | Diacetylmorphine | 0.980 | 0.951 | 0.00714 | 0.0880 | 0.980 | 0.928 |
| DAD-NIR | Caffeine | 0.989 | 0.967 | 0.00379 | 0.0924 | 0.989 | 0.909 |
| Number of PCs: 6 | Paracetamol | 0.981 | 1.00 | 0.00579 | −0.0151 | 0.981 | 0.974 |
| B | Diacetylmorphine | 0.975 | 0.763 | 0.00898 | 0.0649 | 0.975 | 0.890 |
| FT-NIR | Caffeine | 0.972 | 0.832 | 0.00948 | 0.0310 | 0.972 | 0.913 |
| Number of PCs: 3 | Paracetamol | 0.892 | 0.773 | 0.0329 | 0.106 | 0.892 | 0.888 |
| C | Diacetylmorphine | 0.995 | 0.742 | 0.00171 | 0.0910 | 0.995 | 0.863 |
| FT-NIR | Caffeine | 0.998 | 0.882 | 0.000700 | 0.0320 | 0.998 | 0.908 |
| Number of PCs: 6 | Paracetamol | 0.997 | 0.894 | 0.000990 | 0.0308 | 0.997 | 0.885 |

PCs, principal components.

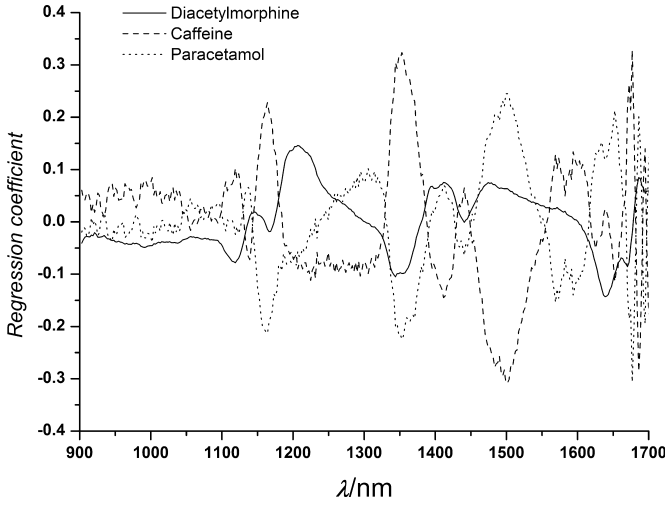


FIG. 3—*Regression coefficients plot, based on DAD-NIR data. Number of PCs: 6.*

possible to create a chemometric model for calibration in the case of ternary powders containing the active principle and cutting agents found in heroin-like samples. The proposed DAD-NIR methodology is competitive with FT-NIR, and in some cases provided even better results.

Recently, a significant change has been seen in the composition of heroin samples seized by the State Police in Bologna: heroin samples now on the criminal market have a significant content in monoacetylmorphine (up to 10%$_{w/w}$) and, at the same time, a lower diacetylmorphine content (rarely over 15%$_{w/w}$). This is most likely related to the birth of new gangs of organized crime. Hence, as a perspective work is planned for a new tetracomponent experimental design using diacetylmorphine, monoacetylmorphine, caffeine, and paracetamol.

To analyze the wide variety of real samples, further in-depth studies are needed, especially using real samples as standards, after determining their exact composition by validated techniques. Moreover, to use the proposed method as both a qualitative and quantitative tool for any real sample, the quantitative chemometric procedure proposed here must be preceded by a qualitative chemometric procedure capable of classifying drugs: work is currently underway on use of the SIMCA classification method in pursuit of this goal.

Finally, it must be pointed out that no useful analytical method exists without evaluating basic figures of merit such as detection limit, accuracy, etc. In the case of univariate calibration, classical statistics provides straightforward methods for calculating these parameters (17). As for multivariate analysis, calculation of the basic figures of merit is complicated and as yet, there is no universal agreement (20,21) although this aspect stands beyond the aim of the present preliminary feasibility study.

*Acknowledgments*

The authors wish to thank Matteo Alessandri for the precious experimental work carried out in starting up instrumentation and software for multivariate analysis of experimental data. Funding was provided by the Italian Ministry of University (FFO, Ordinary Fundamental Fund).

## References

1. Boleda MR, Galceran MT, Ventura F. Trace determination of cannabinoids and opiates in wastewater and surface waters by ultra-performance

liquid chromatography-tandem mass spectrometry. J Chromatogr A 2007;1175:38–48.
2. Feng J, Wang L, Dai I, Harmon T, Bernert JT. Simultaneous determination of multiple drugs of abuse and relevant metabolites in urine by LC-MS-MS. J Anal Toxicol 2007;31:359–68.
3. Costa JL, Wang AY, Micke GA, Maldaner AO, Romano RL, Martins-Júnior HA, et al. Chemical identification of 2,5-dimethoxy-4-bromoamphetamine (DOB). Forensic Sci Int 2007;173:130–6.
4. Jee RD. Near-infrared spectroscopy. In: Moffat AC, Osselton MD, Widdop B, editors. Clarke's analysis of drugs and poisons, Vol. 1. U.K.: Pharmaceutical Press, 2003; 346–57.
5. Ozaki Y, Katawa S, Heise HM, Siesler HW. Near-infrared spectroscopy. principles, instruments, applications. Weinheim, Germany: Wiley-VCH, 2002.
6. Kramer R. Chemometric techniques for quantitative analysis. New York, NY: Marcel Dekker Inc., 1998.
7. Cen H, He Y, Huang M. Combination and comparison of multivariate analysis for the identification of orange varieties using visible and near infrared reflectance spectroscopy. Eur Food Res Technol 2007;225:699–705.
8. Xie L, Ying Y, Yinga T. 401 Chemometrics methods for identification of transgenic tomatoes using visible and near-infrared diffuse transmittance technique. J Food Eng 2007;82:395–401.
9. Causin V, Marega C, Carresi P, Schiavone S, Marigo A. A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement and differential scanning calorimetry for tracing the source of illegal drugs. Forensic Sci Int 2006;164:148–54.
10. Baratieri SC, Barbosa JM, Freitas MP, Martins JA. Multivariate analysis of nystatin and metronidazole in a semi-solid matrix by means of diffuse reflectance NIR spectroscopy and PLS regression. J Pharm Biomed Anal 2006;40:51–5.
11. Dou Y, Sun Y, Ren Y, Ren Y. Artificial neural network for simultaneous determination of two components of compound paracetamol and diphenhydramine hydrochloride powder on NIR spectroscopy. Anal Chim Acta 2005;528:55–61.
12. Berntsson O, Danielsson LG, Johansson MO, Folestad S. Quantitative determination of content in binary powder mixtures using diffuse reflectance near infrared spectrometry and multivariate analysis. Anal Chim Acta 2000;419:45–54.
13. Moros J, Kuligowski J, Quintás G, Garrigues S, De la Guardia M. New cut-off criterion for uninformative variable elimination in multivariate calibration of near-infrared spectra for the determination of heroin in illicit street drugs. Anal Chim Acta 2008;630:150–60.
14. Davies AMC, Fearn T. Back to basics: the ''final'' calibration. Spectrosc Eur 2007;19:15–8.
15. Box GEP, Hunter WG, Hunter JS. Statistics for experimenters: an introduction to design, data analysis, and model building. New York: John Wiley & Sons, 1978.
16. Neubauer MG, Pace RG. D-optimal (0,1)—weighing designs for eight objects. Linear Algebra Appl 2010;432:2634–57.
17. Miller JC, Miller JN. Statistics and chemometrics for analytical chemistry, 4th edn. Harlow, U.K.: Pearson/Prentice Hall, 2005.
18. Wold S, Sjöström M, Eriksson L. PLS-regression: a basic tool of chemometrics. Chemo Intell Lab Syst 2001;58:109–30.
19. Næs T, Isaksson T, Fearn T, Davies T. A user-friendly guide to multivariate calibration and classification. Chichester, U.K.: NIR Publications, 2002.
20. Boqué R, Faber NKM, Rius FX. Detection limits in classical multivariate calibration models. Anal Chim Acta 2000;423:41–9.
21. Zhang L, Garcia-Munoz S. A comparison of different methods to estimate prediction uncertainty using Partial Least Squares (PLS): a practitioner's perspective. Chemom Intell Lab Syst 2009;97:152–8.

Additional information and reprint requests:
Dora Melucci, M.S., Ph.D.
Department of Chemistry ''Giacomo Ciamician''
University of Bologna
Via Selmi 2
I-40126 Bologna
Italy
E-mail: dora.melucci@unibo.it