TECHNICAL NOTE

Etienne Francios Van Zyl,[1] *ND. Anal. Chem. and Mercia Louw,*[1] *B.Sc. (Hon) Chem.*

The Differentiation of Illicit Methaqualone Tablet Formulations Using Principal Component and Soft Independent Modeling of Class Analogy Analysis of Their Near-Infrared Reflectance Spectra

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ABSTRACT: The viability of using principal component analysis (PCA) and soft independent modeling of class analogy analysis (SIMCA) of the near-infrared reflectance spectra of illicit methaqualone tablet formulations as an aid in sample differentiation was investigated.

Near-infrared spectra of ten different illicit species occurring on the South African market was obtained through direct diffuse reflectance measurements made on the tablet surfaces using a bidirectional fiber-bundle probe.

The learning set was analyzed using PCA and SIMCA and resolved in well-separated clusters thus making differentiation possible.

KEYWORDS: forensic science, criminalistics, principal component analysis, soft independent modeling of class analogy, near-infrared spectroscopy, illicit methaqualone tablet formulations, drug sample differentiation

Drug sample differentiation as to source is fast becoming an integral part of the analysis required from forensic science laboratories. Numerous approaches and protocols regarding the characterization or individualization of the different drug types have been suggested in the literature and are currently performed routinely at several international laboratories. All of these are aimed at describing the unique physical and/or chemical properties exhibited by the drug sample in order to generate a unique "profile" characterizing it. These characteristic "profiles" can then be used for sample-to-sample comparison, as well as for strategic purposes when used in conjunction with other intelligence [*1–3*].

Most of the techniques employed involve some type of sample manipulation. Most often manipulation includes a derivitization, followed by a chromatographic separation of major components, as well as minor impurities present, resulting in a unique peak-profile characterizing the sample [*1–5*].

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[1]Forensic Analysts, Forensic Science Laboratory, Pretoria, Republic of South Africa.

Experience however has shown that the uniqueness of the profiles thus obtained are influenced by human and instrumental variables introduced during sample preparation and measurement. Profiles generated at different facilities are usually not standardized, which may severely inhibit their national and international exchange, and therefore, their usefulness [*2*]. Consequently, reliable and standardized comparative analysis may require analysis at centralized facilities on a same analyst, same instrument, same day basis [*2*].

This paper reports on the use of FT-NIRS as a novel approach toward the reliable differentiation of illicit methaqualone containing tablet formulations that eliminates sample preparation, minimizes operator and instrumental variables and generates standardized outputs, which can rapidly be compared to other entries previously made.

Theory

The theory behind NIRS and its associated chemometrics, as well as numerous applications of the technique, have extensively been reported on in recent literature [*6–9*]. Application of NIRS in the pharmaceutical and forensic science fields has been described [*10,11*]. The fast identification of drugs for forensic science measurement was described by Kohn and Jeger using a fiber-optic probe and a FT-NIR spectrometer to determine 37 different drugs and associated substances [*11*].

The appeal of NIRS as a technique for drug sample differentiation is obvious. It is based on the sound fundamental principles of molecular spectroscopy, which is well known and understood. The NIR spectral region, which spans from 1100 to 2500 nm, records overtones and combination bands of the fundamental vibrations observed in the conventional MIR region, and has a common and equally high information content than the MIR spectral region [*11,12*].

Nearly all analytes of interest absorb in the NIR region primarily by virtue of the vibrational stretching activity of C-H, O-H, and N-H molecular bonds and their combination bands with other vibrational modes. NIR detection also is virtually universal [*12*].

The low absorbtivity of the NIR spectral region, by virtue of the higher associated energy, eliminates the need for sample preparation, which allows for direct sample measurement and leads to simple, fast, and non-destructive spectrum collection [*12*].

Most importantly however, is the proven ability of the technique

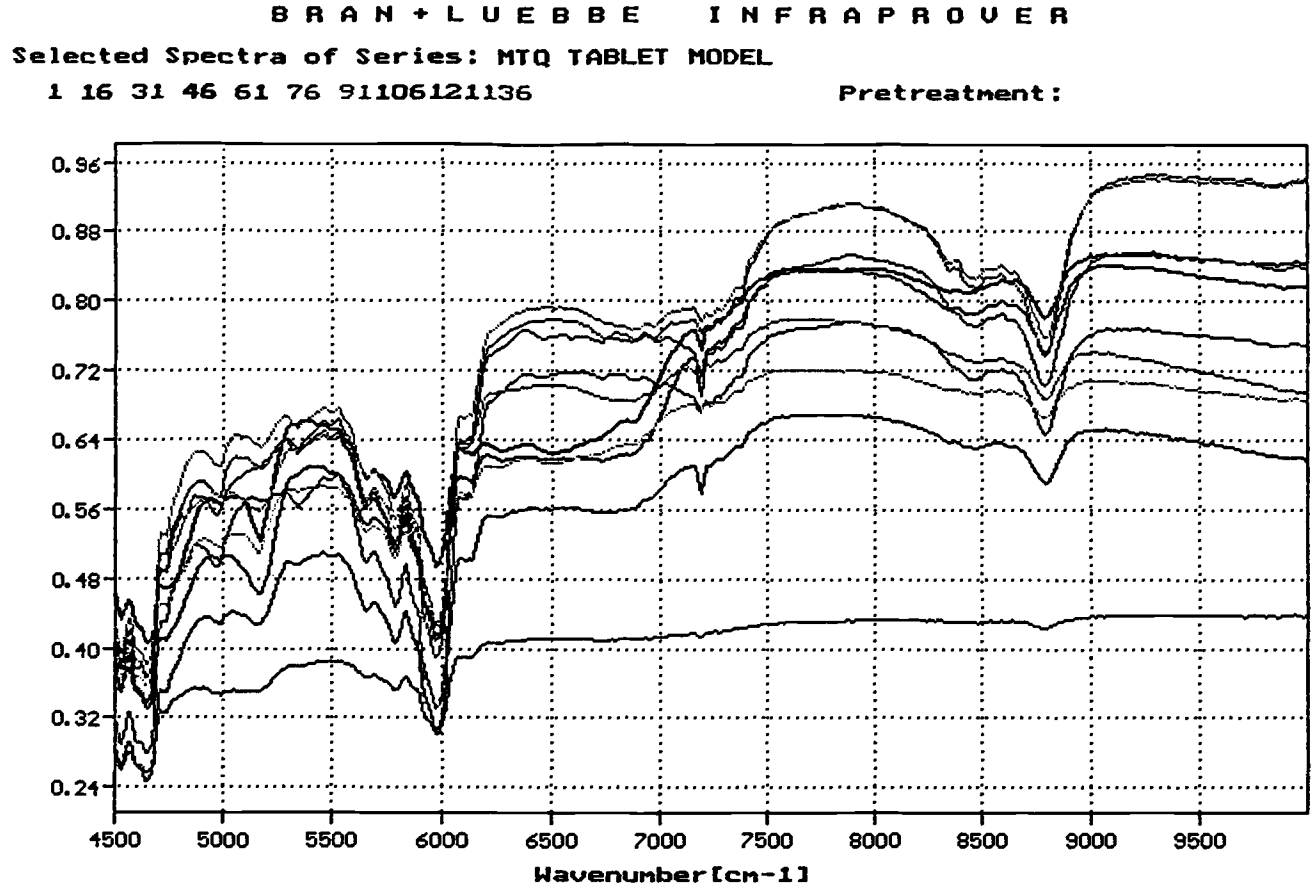

FIG. 1—*Diffuse reflectance spectra of ten species of illicit methaqualone containing tablet formulations shown without data pretreatment.*

to (when used in conjunction with the appropriate pattern-recognition chemometrics) reliably differentiate between very similar substances by exploiting subtle differences in the spectra of chemically similar materials [*9*].

For the purpose of this investigation, pattern recognition and cluster analysis were performed using the proprietary principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) software, which forms an integral part of the software supplied with the instrument.

PCA is a data reduction technique, whereby spectral data can efficiently be compressed to achieve acceptable computing times. So-called principal components are extracted, which are orthogonal relative to one another, and can still sufficiently reproduce the spectra. Each spectrum can be reconstructed from a sum over the product of the principal components (also known as factors) and loadings and a residual spectrum. The loadings are the weightings of each original spectrum for each principal component. The spectrum is represented by a point in factor space, spanned by the principal components. The principal components and loadings are calculated so that the first principal components include the features with the widest variances of all the spectra, and the higher factors gradually fade into noise. This ensures that an information loss is virtually ruled out by this method of data reduction [*13*].

Experimental

Apparatus

The near-infrared spectra of all samples were obtained using a Bran + Luebbe Infraprover, a Fourier Transform (FT) instrument. Measurements were made at a scan resolution of 25 cm^{-1}. Five one second scans, averaged and ratioed to a reference spectrum, constituted one measurement.

Cluster analysis and pattern recognition was done using the proprietary PCA and SIMCA capabilities of the Bran + Luebbe Infraprover Software, Version—4.0, supplied with the instrument.

Materials

The samples tested were actual illicit methaqualone containing tablets seized between 1991 and 1992 and submitted to this laboratory for analysis. The tablets were seized under unconnective circumstances and all main groups, that is, species from different cases, differed with regard to their physical appearance. In-species however all tablets exhibited the same macroscopical physical appearance, such as imprint pattern, color, shape, etc.

All that was known about the chemical composition of the tablets was that all contained methaqualone, either as the base or salts thereof.

Procedure

A learning set, also called calibration set or training set, of spectra were obtained by collecting 15 surface measurements from randomly selected tablets of each main group or species. Measurements were made employing diffuse reflectance using a bidirectional fiber-bundle probe. No sample preparation other than a dusting of the surfaces to be measured was performed.

The spectra were normalized to compensate for vertical shift

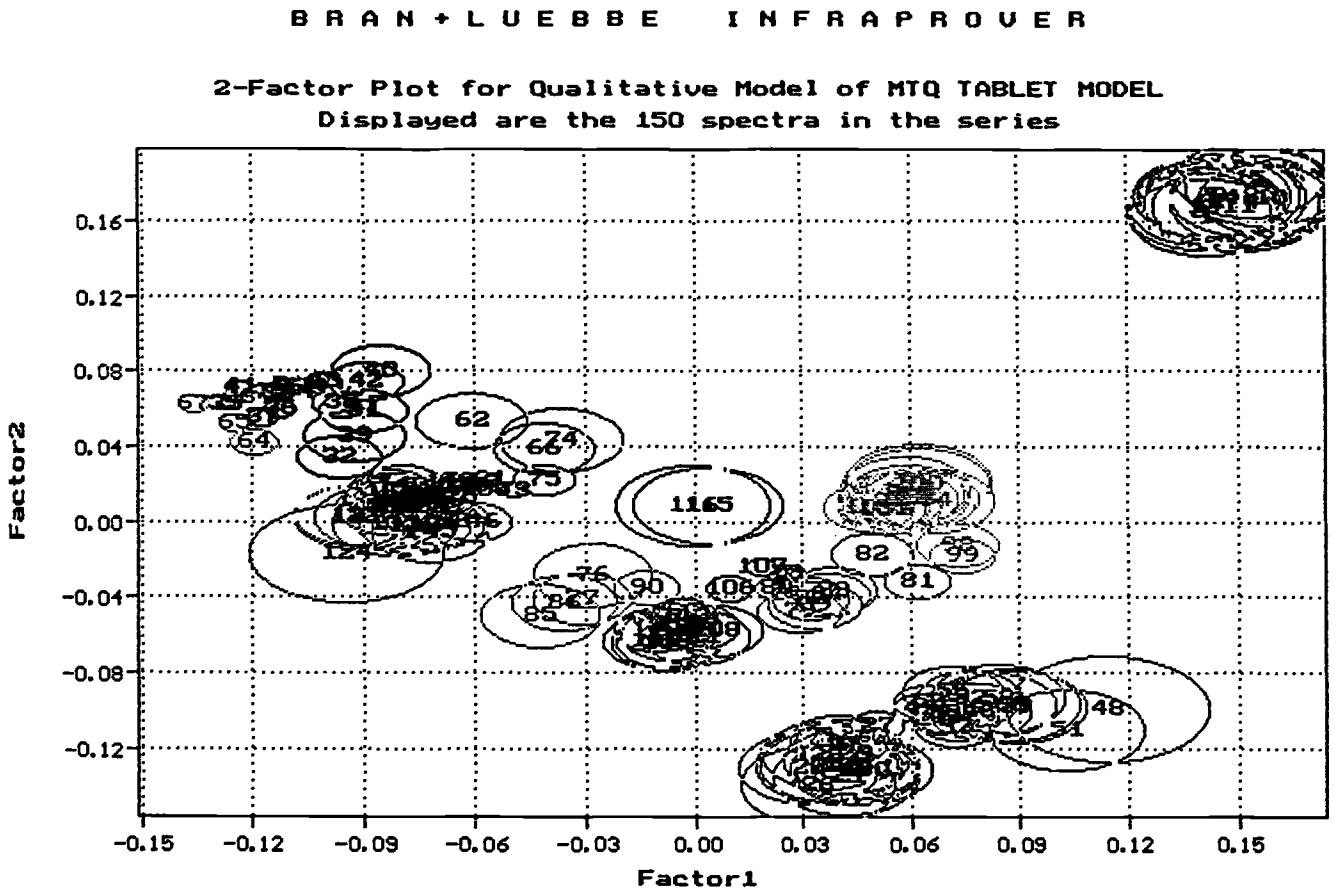

FIG. 2—*Two-factor plot of the 150 spectra in the learning set shown without any data pretreatment.*

by scaling between 0 and 1 after which it was presented for cluster analysis using PCA and SIMCA. The cluster radii were calculated using the two principal factors exhibiting the lowest correlation.

The learning set was internally validated. During validation the connectivity (do the individual measurements form a coherent area?), uniformity (how homogeneous is the distribution of the individual measurements within a given cluster?), convexivity (is there any danger of a substance class enveloping another?), risk of interference and relationship between the volume of the classes are considered.

Sample differentiation was achieved through the direct measurement of whole tablets from similar origin but not used during compilation of the learning set. The measured spectra of these "unknown" samples were then subjected to the same data pretreatment and data reduction sequence as spectra in the learning set before comparison to entries contained therein.

Results and Discussion

One of each of the spectra collected from the ten different species are shown in Fig. 1. The corresponding two-factor plot after PCA and SIMCA are shown in Fig. 2.

The same spectra, after normalization, are shown in Fig. 3 with the corresponding and much improved two-factor plot in Fig. 4. From the two-factor plot shown in Fig. 4 it is clear that, after being normalized, the spectra of all species investigated can be well separated using cluster analysis. The achieved separations are based on subtle differences in the spectra, thus indicating subtle differences in the NIR active content of the different species.

A cluster is understood to mean the "cloud" of points of a class. Tolerance spheres around each spectrum are calculated in order to connect the points with one another. With the exception of measurements 98 and 99, all species show a tight and well defined grouping in-species. The tolerance spheres of measurements 98 and 99 do not overlap with the tolerance spheres of the other measurements of the same class. This indicates that very slight differences exist between these two spectra and others of the same class. Visual investigation of the tablet measured during 98 and 99 showed a rather inhomogeneous composition which most likely gave rise to the differences in their spectra and thus the observed bad grouping.

All "unknowns" presented were, without exception, either correctly identified as belonging to a defined species, or correctly failed as not belonging to the defined species.

The difference between the original spectrum and the spectrum reconstructed by the factors and loadings constitutes the "residual" spectrum. When the residuum is added up over the wavenumber the residual is obtained. The average residual of the learning set is determined and multiplied by eight to give the allowed residual. The actual observed averaged residual (0.000,67) never exceeded the allowed maximum residual (0.0053), thus all spectra were still similar enough so as to qualitatively classify all tablets as methaqualone containing.

Conclusion

Given the fact that NIR measurements can be made without sample preparation and in seconds, makes NIRS an efficient tool to collect standardized and reliable spectral information regarding

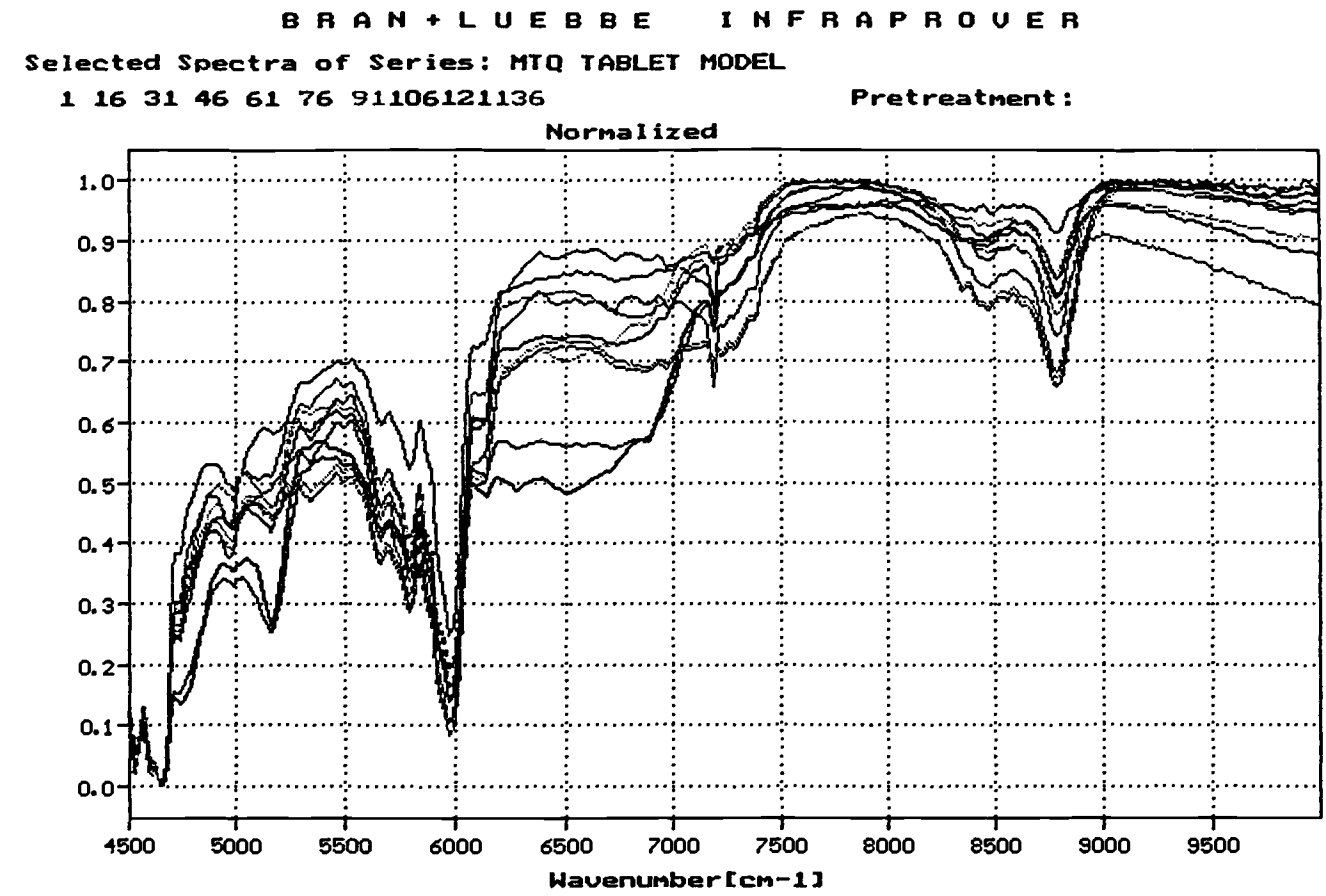

FIG. 3—*Diffuse reflectance spectra of ten species of illicit methaqualone containing tablet formulations shown after normalization.*

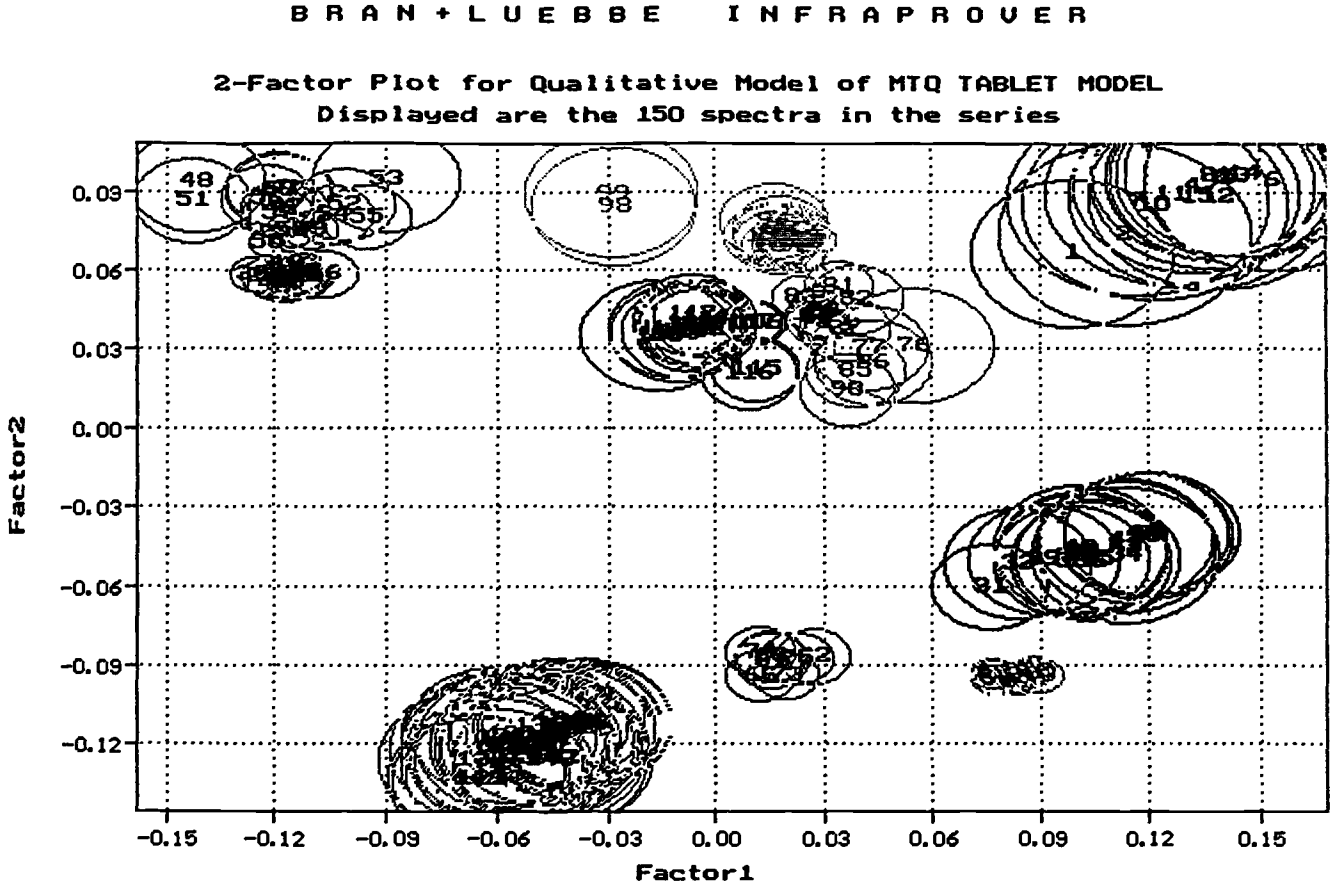

FIG. 4—*Two-factor plot of the 150 spectra contained in the learning set shown after normalization.*

the character of the total NIR active content of illicit tablet formulations, whilst preserving sample integrity to the fullest.

The limited results presented indicate that NIRS coupled with data analysis techniques such as PCA and SIMCA can successfully be employed for rapid differentiation between illicit methaqualone containing tablet formulations. We believe that similar results will be obtained for powdered drugs and we intend to do further experimental work regarding the differentiation of cocaine and heroin sources using NIRS.

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Address requests for reprints or additional information to
E. F. van Zyl
Forensic Science Laboratory
Private Bag X620
Pretoria
0001
Republic of South Africa