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Forensic Science International
106 (1999) 147–156

Forensic Science International

www.elsevier.com/locate/forsciint

Screening experiments of ecstasy street samples using near infrared spectroscopy

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Received 26 March 1999; received in revised form 15 September 1999; accepted 22 September 1999

Abstract

Twelve different sets of confiscated ecstasy samples were analysed applying both near infrared spectroscopy in reflectance mode (1100–2500 nm) and high-performance liquid chromatography (HPLC). The sets showed a large variance in composition. A calibration data set was generated based on the theory of factorial designs. It contained 221 *N*-methyl-3,4-methylenedioxyamphetamine (MDMA) samples, 167 *N*-ethyl-3,4-methylenedioxyamphetamine (MDE), 111 amphetamine and 106 samples without a controlled substance, which will be called placebo samples thereafter. From this data set, PLS-1 models were calculated and were successfully applied for validation of various external laboratory test sets. The transferability of these results to confiscated tablets is demonstrated here. It is shown that differentiation into placebo, amphetamine and ecstasy samples is possible. Analysis of intact tablets is practicable. However, more reliable results are obtained from pulverised samples. This is due to ill-defined production procedures. The use of mathematically pretreated spectra improves the prediction quality of all the PLS-1 models studied. It is possible to improve discrimination between MDE and MDMA with the help of a second model based on raw spectra. Alternative strategies are briefly discussed.

Keywords: Forensic science; Identification of illicit ecstasy samples; *N*-Methyl-3,4-methylendioxyamphetamine (MDMA); *N*-Ethyl-3,4-methylendioxyamphetamine (MDE); Amphetamine; Partial least squares (PLS)

1. Introduction

The illicit consumption of ecstasy tablets is a big problem in Germany. Stimulating drugs such as amphetamine and its derivatives are quite popular. This can mainly be

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0379-0738/99/$ – see front matter
PII: S0379-0738(99)00195-4

attributed to their distribution in the form of tablets. According to official investigations, the confiscated amounts of both amphetamine and ecstasy samples have increased in 1997 [1].

In 97% of the analysed preparations a single active substance is found. 47.5% of the samples contained *N*-methyl-3,4-methylendioxyamphetamine (MDMA, 'adam'), 42.7% *N*-ethyl-3,4-methylendioxyamphetamine (MDE, 'eve'), 6.5% amphetamine and 0.3% 3,4-methylendioxyamphetamine (MDA) and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB). In addition, these samples always include excipients necessary for tablet processing, and sometimes pharmaceutical agents as adulterants. Both the kind and amount of these substances vary over a broad range in typical ecstasy preparations.

Analysing confiscated samples is quite time-consuming. Usually one has to separate the active substance from its tablet matrix before identification is possible [2]. Near infrared (NIR) spectroscopy offers a new possibility for fast screening of street samples. This analytical technology is well established in food and agricultural industries, in clinical, biomedical and pharmaceutical research [3–6]. This is because NIR spectroscopy offers the possibility of rapid, precise and non-destructive measurements that are not restricted to a laboratory context [7].

In forensic science, only the identification of amphetamine and 3,4-methylendioxyamphetamine derivatives in complex matrices has been examined so far [8]. The focal point of this study was the successful identification of powdered ecstasy samples in complex (yet well defined) matrices using NIR spectroscopy. To avoid problems with inhomogenities or surface interferences neither tablets nor street samples were included in this study. In the work presented here, we extended the above study to intact tablet samples and validated the results with confiscated street samples.

2. Materials and methods

2.1. Confiscated samples

Ecstasy samples were obtained from the Landeskriminalamt Baden-Württemberg, an official investigation authority in Germany. Twelve different tablet sets were analysed, each consisting of ten samples. Seven sets contained MDMA–HCl (27–42% active substance, w/w), two sets MDE–HCl (14–37%) and two sets amphetamine sulfate (28–35%). One set was composed of placebo samples.

2.2. Sample preparation and analysis

The NIR spectra were collected on a NIRsystems 6500 spectrophotometer (NIRSystems, Silverspring, MD) in diffuse reflective mode. The spectra were recorded at 2-nm intervals in the range from 1100 to 2500 nm. To reduce noise each spectrum was determined as an average of 32 scans. Both sides of the tablets as well as the powdered tablets, respectively, were analysed three times that way. The pack of the powdered samples was shaken between measurements in order to vary the packing of the powder in the vials (Fisher Scientific, Germany). A highly reflective ceramic

standard served as a reference. All spectra were log rationed against it. Near-Infrared Spectral Analysis Software (NSAS) version 3.30 (NIRSystems) was used for spectrophotometer diagnostics, data acquisition and calculation of the second derivatives of the spectra. All chemometrical treatments were performed using the multivariate analysis software package UNSCRAMBLER version 6.1 (Camo AS, Norway). All calculations used log (1/reflectance) data.

The analytical test method for determination of the composition of each individual tablet was high-performance liquid chromatography (HPLC). A liquid phase Chromato-Integrator D 2500 (Merck, Germany) with automatic injector LaChrom L 7200 set at 20 μl and UV/VIS detector L 4250 was used. A LiChrospher60 RP-select B column (5 μm, 125×4 mm) with precolumn (5 μm, 4×4 mm) was used for separation. The mobile phase consisted of 20 mmol potassium dihydrogenphosphate buffer, pH 3.0–acetonitrile (94:6, v/v). The flow-rate was 1.25 ml/min. The extinction wavelength was set at 220 nm for all substances.

An exact weight of powder was transferred to a 25-ml volumetric flask. A volume of 0.5 ml of a MDA–HCl solution (250 μg/ml in buffer) was added as internal standard. The solution was filled up to volume using phosphate buffer, sonicated for 10 min and filtered.

Individual solutions of MDMA, MDE and amphetamine in phosphate buffer with MDA as internal standard were prepared at five different concentration levels. The peak areas obtained were linearly related to the concentration.

2.3. Chemometrical treatments

As a result of scattering effects raw NIR reflectance spectra show large baseline shifts. In addition, the baseline offset is influenced by physical properties. The obtained absorption bands are highly overlapping and too complex for direct interpretation [9]. To minimise these problems second derivative spectra are often used for analysis.

In this study partial least squares (PLS) regression is applied to extract the relevant information from the complex spectra. Two different algorithms, PLS-1 and PLS-2 are available and extensively described in the literature [10–13]. PLS regression is a quantitative spectral decomposition technique that determines relevant variables automatically, and only these ones are included in the PLS models. Here, the relevance of each variable obtained was checked by full cross validation. PLS is believed to be the best multivariate regression method to remove spectral noise and other irrelevant information [14]. This regression method allows calibration even if the system is underdetermined or interferences are present. One obtains simplified plots which, in combination with background information on the original samples, allow the analyst to draw conclusions on relevant information.

2.4. Model data set

The training set for calibration was composed of 605 samples, 221 of which contained MDMA–HCl, 167 MDE–HCl and 111 amphetamine sulfate. In addition, there were 106 placebo samples containing either only excipients or excipients in combination with

commonly used adulterants. Full factorial designs were applied to vary the qualitative and quantitative composition of the samples [15,16]. The concentration values range between 0 and 56% (w/w) for MDMA–HCl and MDE–HCl and between 0 and 28% for amphetamine sulfate, respectively. Both powdered samples and intact tablets were included. The data were centred before calculation.

Quantitative prediction values were applied to derive a qualitative statement. We determined a so called cut-off value, a concentration limit that indicates whether the substance of interest is contained (by predicted concentration values greater than this value) or not. If the predicted values fall below this limit, the substance may be present in minor concentrations or not at all. Note that this is only a starting point for identification, since precise quantitative predictions can be obtained using different models based on data sets that only include a single substance.

3. Results and discussion

3.1. Proceeding

Three different PLS1-models based on second derivative spectra have been calculated. The statistical parameters of the models obtained are shown in Table 1. These models were validated with various external laboratory test sets. They turned out to be fit for identification of ecstasy samples in intact tablet samples. A reliable cut-off value for correct identification was determined at 5% (w/w) active compound. This corresponds to 15 mg substance.

Transferability to 'real' street samples of the calculated models (based on laboratory samples) was investigated. The ingredients of the analysed street samples vary over a wide range. Most common excipients were lactose, starch, cellulose, sorbitol and colloidal silicon dioxide. As admixtures caffeine, MDA–HCl and colouring agents were often present. All sample sets could be identified correctly after consideration of some general aspects to be discussed in the following.

3.2. Quality of confiscated tablets

Confiscated street samples often show large inhomogenities. This is due to non-standardised production procedures. The basic mixtures for tablet processing are often

Table 1
Description of the PLS-1 models used for identification of street samples (calibration set with 605 samples)

Statistical parameters	Amphetamine model	MDMA model	MDE model
Principal components	5	3	4
Slope	0.9998	1.0006	0.9998
Offset	−0.0055	−0.0179	−0.0095
Correlation	0.9713	0.9909	0.9809
RMSEP (%)[a]	1.59	2.10	2.20

[a] RMSEP: root mean square error of prediction.

not mixed carefully. In many cases, the heterogeneity of the tablet matrix is visible to the naked eye. A typical NIR feature is shown in Fig. 1a. Six original NIR spectra of a typical confiscated tablet (active substance: 31% MDMA–HCl) are rendered, with each

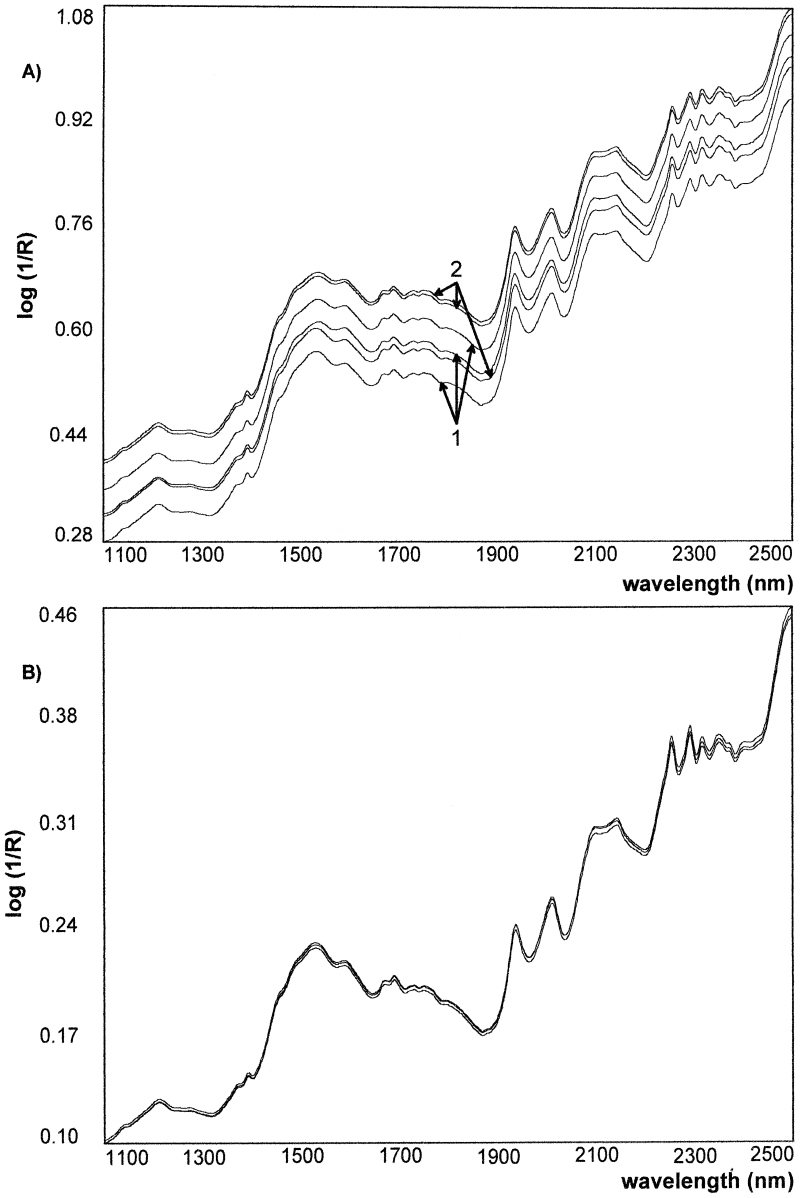

Fig. 1. (a) Original raw NIR spectra of a typical confiscated tablet (active compound: 31% MDMA–HCl). Both tablet sides (marked with 1 or 2) were measured three times. (b) Original raw NIR spectra of this tablet after pulverisation. The pack in the vials was shaken between the three measurements.

tablet side measured three times. The measured spectra vary over a wide range. Prediction values derived from these spectra will lead to concentration values with high standard deviations. Not surprisingly, this problem can be avoided by pulverisation of the samples. This step minimises particle size effects and surface interferences, and homogenises the mixture of the ingredients. The obtained NIR spectra of the powdered tablet sample are shown in Fig. 1b. The three spectra shown are closely similar and will lead to nearly identical prediction values. Note that if the production procedures were standardised NIR spectroscopy would be able to identify ecstasy in *intact* tablet samples.

3.3. Mathematical pretreatment

Physical properties and scattering effects influence the appearance of NIR spectra. Some of these sources of variation in the data can be reduced by grinding procedures or averaging replicate measurements as described above. Spectral pretreatment with calculations of derivatives is a common method to further improve the performance of the spectra. Second derivatives enhance spectral resolution and compensate for baseline shifts [17]. Generally, the application of second derivative spectra deteriorates the signal-to-noise ratio by about a factor of two per derivation. The derivatives were calculated with a boxcar smooth of 10-nm width and a central difference gap of 0 nm. Models based on second derivatives are less prone to errors caused by factors not represented in the calibration set. This is especially important for analysis of street samples. Using these models a better discrimination between placebo and verum samples is achieved. Table 2 shows the discriminatory power of the models predicting a placebo test set. Some of the predicted values are negative. These are the uncorrected values generated by the mathematical models used for analysis of our data set. In the context of concentration values, negative values indicate the absence of a substance in question. Mathematically pretreated data lead to more uniform prediction values as expressed by the standard deviations. The maximum mean value for this placebo set, applying the second derivatives, is about 2.4%, which is clearly below the previously determined cut-off value. Using raw spectra for identification of active compounds generates a mean value above the cut-off, here about 7% MDE–HCl. The cut-off would have to be raised or false identification of placebo samples as MDE-samples would occur. Fig. 2 illustrates the details of the procedure. Following this strategy, 100% of both confiscated powdered samples and laboratory samples have been identified correctly, compared to 92% of the intact confiscated tablets.

Table 2
Prediction concentration values for a confiscated placebo test set (ten pulverised samples)

Predicted concentrations values for	Raw, original spectra	Second derivative spectra
MDMA–HCl	−1.4%±0.7%	−3.5%±0.2%
MDE–HCl	7.0%±0.5%	0.9%±0.3%
Amphetamine sulfate	−0.2%±0.3%	2.4%±0.2%

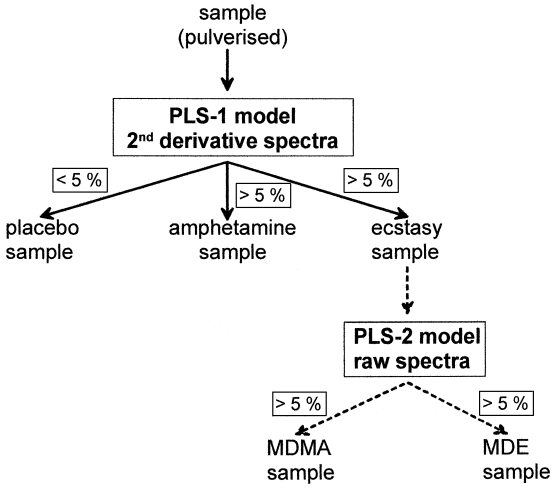

Fig. 2. Procedure.

3.4. Differentiation of structurally similar substances

Due to strong variations in the calibration matrix, to admixtures of adulterants, and to the spectral smoothening by the application of derivative spectra, the developed models only classify samples as either ecstasy, amphetamine or placebo. Therefore, the calculated models represent a kind of filter which can be used to rapidly screen confiscated samples. In addition, a precise differentiation between MDE and MDMA is possible after positive identification of ecstasy in a sample. To achieve this, one can return to the raw spectra with their benefits. Noted above, derivative spectra, in contrast to raw spectra are smoothed. In this study this may cause problems with the differentiation between structurally very similar substances. MDMA and MDE share the same fundamental structure, differing only in a CH_2-group. Once ecstasy has been positively identified, it turned out to be possible to distinguish between both substances using a separate raw spectra model (here PLS-2) for prediction. Again, the cut-off value was determined at 5% (w/w) active compound. The prediction values for the test set shown in Fig. 1a and b are given in Table 3.

Using the derivative models, the obtained predictions clearly indicate the presence of MDMA–HCl, but the predicted MDE–HCl concentration also exceeds the fixed cut-off value. Taken together, these results establish that ecstasy is contained in the sample set. Choosing the raw model afterwards, the presence of MDE–HCl can definitively be excluded. MDMA–HCl is again identified by prediction values well above the cut-off value. Note that the models based on second derivatives have superior performance as filters and, therefore, have to be relied upon to identify ecstasy in the first place (see Table 2). Applying this step for differentiation between MDMA–HCl and MDE–HCl, the active substance could be correctly identified for 92% of the powdered and 83% of the intact confiscated samples. As discussed above, the top value of 92% can most probably be attributed to the presence of various unidentified admixtures in street

Table 3
Prediction values using a pulverised test set, containing an average of 31% MDMA–HCl as active substance[a]

	MDMA–HCl		MDE–HCl	
	Second derivative spectra	Raw spectra	Second derivative spectra	Raw spectra
Mean value	21.6%	22.0%	6.8%	0.1%
Standard deviation	0.6%	0.9%	0.4%	0.7%

[a] The results of the derivative models (a) indicate the presence of MDMA–HCl and/or MDE–HCl (predictions exceed cut-off value). Choosing the raw model (b) in a second step, the presence of MDE–HCl can then definitively be excluded.

samples. The discrepancy in predictive power for intact and powdered samples is due to the factors discussed in Section 3.2. Laboratory samples comparable to the test set were 100% correctly identified both as tablets and as powdered samples.

3.5. Alternative approaches

To corroborate these findings, we examined two additional data sets. First, a laboratory data set containing only MDMA–HCl and excipients was selected. In this set, quality and quantity of excipients varied over a wide range as prescribed by factorial designs. A PLS-1 model of the derivative spectra was calculated. The regression plot is shown in Fig. 3. Three principle components are necessary to describe the variation in the data matrix, which is mainly caused by different kinds of tablet excipients. This model offers an excellent possibility to predict MDMA–HCl in both tablet and pulverised laboratory samples. As a second set, we used all confiscated tablets containing MDMA–HCl for regression. A number of 11 principal components was found to reproduce the significant variation in the data set. This number is a good measure for how complex and different confiscated ecstasy samples are composed. Table 4 combines the statistical data for comparison. Applying models based on confiscated tablets for identification of street samples, one needs more than three times as many principle components as when using those based on factorial designs to reproduce the latent data information equally well. Reducing the principle component number to three (comparable to the laboratory model) the analyst obtains very poor results with high root mean square errors of prediction.

The confiscated samples show a large variety in composition which can mainly be attributed to admixtures and colouring agents. They seem to be inappropriate for calibration of models to be used for identification. We conclude that the chosen way of calibration based on well-defined laboratory samples and factorial designs offers a better approach to obtain reliable results.

4. Conclusion

In this study the successful use of NIR spectroscopy for identification of ecstasy and amphetamine in street samples was demonstrated. All confiscated sample sets could be

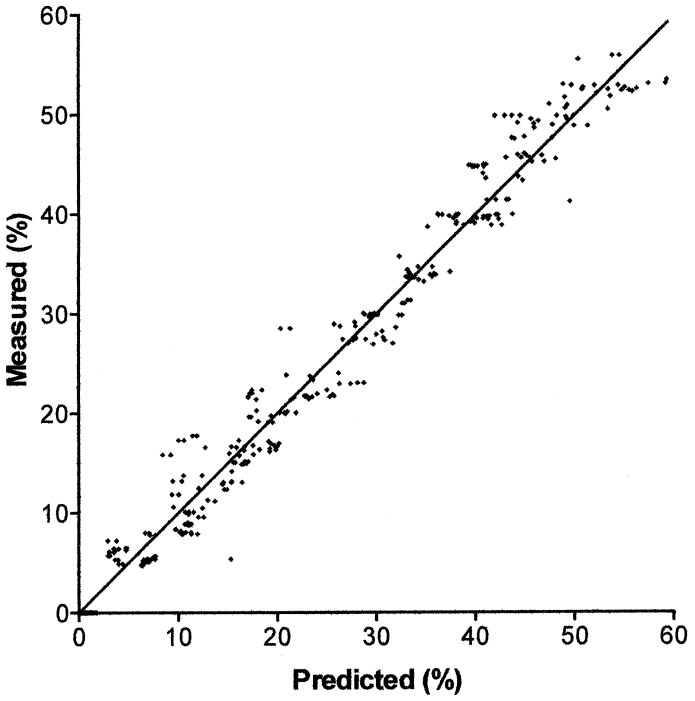

Fig. 3. Regression plot for MDMA–HCl.

identified correctly after some general considerations are taken into account: because of poor production quality, homogenisation of tablets through pulverisation significantly improves prediction values. Mathematical data pretreatment also often leads to better predictions. Models based on second derivatives are more tolerant with respect to effects, not represented in the calibration set. Therefore, they constitute an efficient filter for the screening of samples. However, having established the presence of ecstasy in a sample, differentiation between the structurally very similar 3,4-methylendioxyamphetamine derivatives is more successfully achieved applying models based on raw spectra.

Laboratory samples are better suited for model calculation. Confiscated samples show very variable composition. Using these samples for model calibration, one obtains highly complex models that are too difficult to interpret. After careful construction of an

Table 4
Comparison of the complexity of the data (PLS-1 models based on powdered samples and second derivative spectra)

Data sets	Numbers of PC[a]	Slope	Offset (%)	Correlation	RMSEP (%)
Laboratory MDMA samples	3	1.000	0.0	0.989	2.6
Confiscated MDMA samples	11	0.990	0.1	0.978	2.5
Confiscated MDMA samples	3	0.936	1.5	0.856	6.3

[a] PC: Principal components.

optimum model and thorough validation including numerous sources of variation, it could be shown that NIR spectroscopy is a reliable tool for screening experiments.

Acknowledgements

The authors would like to thank Dr. Schneider (Landeskriminalamt Baden-Württemberg, Germany) for making confiscated samples available. Many thanks are due to Dr. M. Ebeling (Hoffmann–La Roche, Switzerland) for his collaboration on this work. The authors are grateful to Foss GmbH (Hamburg, Germany) for donation of the NIRSystems 6500 spectrophotometer applied in this work.

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