FULL PAPER
THE ANALYST
www.rsc.org/analyst

# Application of SERS spectroscopy to the identification of (3,4-methylenedioxy)amphetamine in forensic samples utilizing matrix stabilized silver halides

**Bernd Sägmüller,[a] Bernd Schwarze,[b] Georg Brehm[a] and Siegfried Schneider*[a]**

[a] *Institut für Physikalische und Theoretische Chemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstrasse 3, 91058 Erlangen, Germany*
*E-mail: schneider@chemie.uni-erlangen.de*
[b] *Institut für Rechtsmedizin, Friedrich-Alexander-Universität Erlangen-Nürnberg, Universitätsstrasse 22, 91054 Erlangen, Germany*

***Received 18th June 2001, Accepted 28th August 2001***
***First published as an Advance Article on the web 17th October 2001***

A method based on surface-enhanced Raman scattering (SERS) spectroscopy was developed to meet the need for the reliable and rapid identification of illicit drugs such as the 'designer drug' XTC, preferably to increase the security of legal certificates. A matrix stabilized silver halide dispersion on a microtiter plate is used as the SERS-active substrate, providing an easy to use system for sample preparation and probing by means of a Raman microscope. The potential of the method is demonstrated by applying it to the identification of the psychoactive ingredients of drug containing tablets which were confiscated by the local police at techno-music events. The samples of interest were 26 different brands of XTC tablets and several pieces of evidence (powders) containing amphetamine. For reference, we show SERS and Raman spectra of pristine amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethamphetamine.

## Introduction

The amount of illicit drugs that are flooding European countries is large and expected to grow even more in the future. Furthermore, new kinds of drugs will be introduced to exploit new markets, their psychotrophic habitus dependent on upcoming fashions, *e.g.*, the present 'designer drugs' are related to techno-music events popular with today's youth. Therefore, forensic scientists need to be at least up-to-date with currently sold drugs or, even better, one step ahead by investigating compounds which promise, in view of their structure, a psychotrophic character and consequently are future candidates for legal prosecution.

It is essential that methods of identification are reliable and fast and produce unique results. Because of today's practice by lawyers of questioning the unambiguousness of the results of UV-VIS, mass and fluorescence spectroscopy, we present an application using surface-enhanced Raman scattering (SERS) spectroscopy. This method extends the arguments of an expert with results which are independent of existing analytical methods and confirm the expert's certificate by a third method, which is often considered necessary in repeated legal suits concerning drugs.

SERS is a form of vibrational spectroscopy that is able to identify analyte substances uniquely by their vibrational bands and is best suited where unique identification of the analytes is imperative. If characteristic ensembles of vibrational bands are recorded from analytes at comparatively low concentrations, SERS works very well and qualifies for trace analysis [1,2] where it overcomes the major disadvantage of conventional (pre-resonance) Raman spectroscopy, low sensitivity, and quenches effectively unwanted fluorescence of the sample such as is introduced by, *e.g.*, admixtures of flavors, food colors or a matrix, which leads to a more convincing vibrational spectrum.[3] To demonstrate the impressive amplification of this technique, surface-enhanced Raman spectra and surface-enhanced resonance Raman spectra of compounds diluted by several orders of magnitude have been reported as examples for the impressive amplification of the Raman signal by means of surface enhancement.[2,4] SERS spectra have been recorded on various types of surfaces which required, however, a complicated production procedure, *e.g.*, silver-island films,[6] hydrosols,[6] silver-films-over-nanospheres (AgFONs),[5] colloids[7] and photographic paper, which provides a silver surface after development of the silver halide crystals contained within the paper.[8] Only some of them are suitable for a reliable, quick and, with respect to the cost of preparation, reasonable method of analysis.

Efforts to identify illicit amphetamine by means of creating a reactive surface on a roughened silver surface have been undertaken;[1] the use of a fiber-optic probe for detection is also available.[9,10] Furthermore, the utilization of immunoassays for the identification of drugs has been reported by White *et al.*,[11] and there has been a more general effort at enzymatic detection using colloids as the substrate.[12] An analytical system applying a microtest plate in combination with silver or gold hydrosols was introduced by Bell and Spence.[13]

In this paper, we describe the application of SERS spectroscopy utilizing the combination of a silver halide dispersion combined with a microtiter plate. In contrast to the standard citrate system[7] of forming the active silver surface by chemical reduction, the use of a photolytically formed silver surface overcomes the problems arising from contamination with reaction products. This combination establishes a system suitable for a high analytical throughput reasonable for a forensic laboratory. We present spectra of exhibits confiscated by the local police without the need for further chemical derivatization of the exhibits, although one extraction step remains necessary.

It is clear that we cannot reference the reported SERS spectra to the samples and, concomitantly, to the exhibit labels given by the public prosecutor's office or by a complete description of

 DOI: 10.1039/b105321n

the sample's trademarks, in order to prevent this paper from being a quality assurance for illicit drugs currently in trade or use. For a distinction between the investigated tablets and powders, they are labeled XTC-*n* and POW-*n*, with *n* being a letter or a number, if listed sequentially.

## Experimental

### Chemicals

All chemicals were of analytical-reagent grade and were used as received (Sigma-Aldrich and Fluka, Germany). The water used was doubly distilled and the solvents were of spectroscopic grade (Fluka and Riedel-de Haën, Germany). All methanolic solutions were freshly prepared to ensure that the amine groups were not oxidized before use. All samples were stored under an inert atmosphere and chilled. The abbreviations used are as follows: amphetamine, AMP; methamphetamine, MAMP; 3,4-methylenedioxyamphetamine, MDA; 3,4-methylenedioxymethamphetamine, MDMA; and 3,4-methylenedioxyethamphetamine, MDE. For the molecular structures, see Fig. 1.

### Preparation of microtiter plates and generation of SERS-active surface

SERS-active surfaces were prepared from laboratory-made[14] gelatin based silver halide dispersions because of their unique properties when used in an automated way for the identification of a wide range of analytes. For example, the rheological properties are designed to allow spin- or drop-coating on different types of supporting materials and also to fill easily and reproducibly the bowls of microtiter plates applied in the design of high-throughput analytical systems. The dispersions were prepared by the dropwise addition (*ca.* 10 drops $min^{-1}$) of 30 ml of a 1.5 M aqueous solution of $AgNO_3$ into 20 ml of an aqueous solution of 160 mg KCl, 5.2 g of KBr and 2.0 g of gelatine (Type R 500041, Fluka). The reaction mixture was held at 38 °C while being smoothly stirred overnight and subsequently stored in darkness at 10 °C in a refrigerator.

The chilled and highly viscous dispersion was then cut into small pieces and treated with demineralized water to wash out the remaining potassium nitrate. For application, the dispersion can be liquefied again by warming it to 38 °C and stirring. At this temperature, 10 μl of the dispersion can be easily filled into a specific well of a microtiter plate. After drying overnight in complete darkness at room temperature, the plates can be stored at ambient temperature for later use. The reported composition was chosen because this dispersion generates microscopically flat surfaces in the well, exhibits a long shelf life of about 4 months and gives excellent signal-to-noise ratios of the recorded SERS spectra.[14] The SERS-active silver surface is formed *in situ* while the probing laser beam ($\lambda_{ex}$ = 514.5 nm) is focused to a narrow spot on the silver halide dispersion, thereby providing sufficiently high intensity to reduce the silver halides to elemental silver and chlorine radicals and cleave the crystal (photolytic generation of silver grains).[15] We believe that *in situ* formation of the silver surface produces optimal atomic roughness as demanded for a high SERS enhancement factor. Furthermore, application of the analyte prior to formation of the active surfaces protects the SERS-active centers created on them from becoming contaminated. The analyte molecules adsorb just at the time of the recording of the spectra at the freshly formed, clean silver spots.

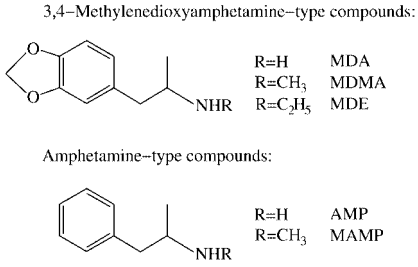


**Fig. 1** Molecular structures of the investigated compounds and abbrevations used.

### Recording of SERS spectra

The SERS spectra were recorded with a Renishaw Ramanscope 2000 using an air cooled argon ion laser for excitation at 514.5 nm wavelength. An Olympus LMPLFL long distance lens (focus at 12 mm distance from the lens surface) with 50-fold magnification was mounted in a Leica LMDM microscope. Thus, the laser power density in the focus of about 2 μm diameter can be as high as 1.75 mW $\mu m^{-2}$. The vibrational bands of polystyrene, from which the microtiter plates are made, do not interfere with the recorded spectra of the investigated compounds. Nevertheless, the strongest bands in the spectra of the polystyrene base can be used to calibrate the wavenumber scale, if necessary.

For the recording of SERS spectra, the microtiter plate is placed under the microscope lens and the laser is focused on the surface of the silver halide dispersion at the bottom of the well. A 10 μl volume of the analyte solution is applied quickly with a microliter syringe to avoid decomposition of the dry surface by the laser. The recording process is started immediately thereafter. During a period of at least 3 min, SERS spectra are repeatedly recorded while, at the same time, the focus of the probing laser beam is under optical surveillance and permanently readjusted to match the top of the swollen dispersion. The integration time of the CCD is limited to 10 s. For further evaluation, the spectra obtained are subject to a five-point baseline correction.

### Preparation of samples

The confiscated XTC-containing tablets are normally consumed in halves to maintain the ecstatic effect. At an average weight of about 300 mg per tablet, approximately 100–150 mg are taken for the extraction procedure. The samples are treated with 1 ml of aqueous saturated sodium carbonate solution in an ultrasonic bath to disintegrate the tablet completely and to free the drug-base. Extraction is subsequently performed with 1 ml of cyclohexane.

If only smaller fractions of a tablet are found, the disintegration procedure can be performed with reduced volumes of the aqueous solutions and extraction solvent to provide sufficiently high concentrated cyclohexane solutions. The minimum applicable volume in this experimental setup is 10 μl determined by the parameters of the microtiter plate. In this work we used the exhibits with masses as described above and thus refer always to the amount of the active ingredient which half a tablet delivers to 1 ml of cyclohexane. However, only 10 μl of these solutions were used for analysis in all experiments.

After separation of the phases in a centrifuge, the organic phase (cyclohexane) is applied without further treatment in volumes of 10 μl per well on the microtiter plate. The same procedure is applied to the reference compounds, which were received as the corresponding hydrochlorides. Depending on the kind of tablet or powder, several different compounds are admixed with the active ingredient(s), *e.g.*, for dilution of the drug, for stabilization of the tablet to enhance the galenic or for increased esophagic torpidity for better gastric resorption to achieve an accelerated entactogenic effect. In the case of

amphetamine powder, which is often contaminated with reaction products from its synthesis, either solvents or precursors, we had to assure ourselves that the drug base was completely set free and that the accompanying additives, *e.g.*, sugar, fatty acids, tablet base, food colors, caffeine or pharmaceutical admixtures, remained preferentially in the extracted residual phase.

Fortunately, cyclohexane extracts the amine drug compounds but leaves most of the unwanted compounds in the residue. This profitable property was not found when using solvents with a comparatively high vapor pressure such as diethyl ether or ethyl acetate or solvents with excellent solvation properties such as dioxane or tetrahydrofuran. In a few cases, a cellulose type residue, which had to be removed by filtration, was found in the cyclohexane extract after a standing time of ~1 h. Especially when treating colored tablets, spectroscopically disturbing amounts of accompanying food colors are often found which are very likely to obscure the spectra. We found that most of them do not migrate into the cyclohexane phase.

## Results and discussion

### Raman and SERS spectra of the reference compounds

To understand better the SERS spectra of the investigated samples, we first recorded the preresonance Raman spectra of the reference compounds. Following the nomenclature of Fig. 1, the spectra of the amphetamines AMP and MAMP are shown in Fig. 2. Methylation of the amino group of AMP has only a minor effect on the general appearance of the Raman spectra in the investigated region and it reduces in this region the number of clearly distinguishable bands. The four strongest bands remain unchanged with respect to both location and relative intensity. Small, but significant, differences show up in the location and shape of several bands of lower intensity, thereby allowing a spectroscopic differentiation between the two species. Also, the four strongest bands in the preresonance Raman spectra of both compounds are conserved also in the SERS spectra (Fig. 3), thus providing evidence that the spectra are characteristic for the investigated compounds and not perturbed by impurities or reaction byproducts. The relative intensities of the 1610, 1590 and 1000 $cm^{-1}$ bands are changed in favor of a 1600 $cm^{-1}$ band upon adsorption on the Ag surface. This provides evidence that the phenyl ring is close enough to the surface to experience orientation dependent amplification of its various modes. Introduction of a methyl group on the amino nitrogen reduces the intensity of the 1095 and 1140 $cm^{-1}$ bands and increases the intensity of the bands at 1300, 1370 and 1420 $cm^{-1}$ to a considerable extent. All these bands are related to the C–H bending vibrations of the methyl group and therefore are important for the distinction between the two amphetamine species.

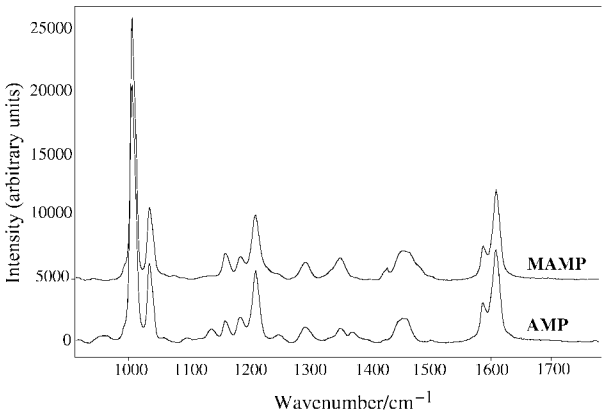


**Fig. 2** Preresonance Raman spectra ($\lambda_{exc}$ = 514.5 nm) of the neat free bases of amphetamine (AMP) and methamphetamine (MAMP). The MAMP spectrum has been baseline corrected and shifted vertically for better visualization.

In contrast to the amphetamine spectra, the introduction of methyl and ethyl groups on the amino nitrogen of the MD series causes significant shifts of some band positions in the preresonance Raman spectra (Fig. 4). The substituent-related shifts are less pronounced in the corresponding SERS spectra (Fig. 5). Comparing the SERS spectra of the MD series, one finds little difference in the band patterns in the region between 900 and 1400 $cm^{-1}$, which can be used to distinguish between the different MD species.

For empirical pattern detection we selected a small number of significant bands with a view to automation of the detection procedure. Since the five strongest bands between 1250 and

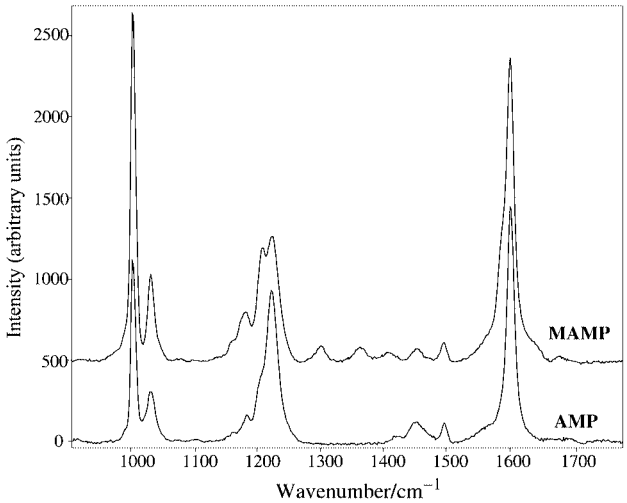


**Fig. 3** SERS spectra ($\lambda_{exc}$ = 514.5 nm) of $10^{-3}$ M methanolic solutions of the free base of the amphetamines. The spectra have been baseline corrected and the MAMP spectrum has been shifted vertically for better visualization.

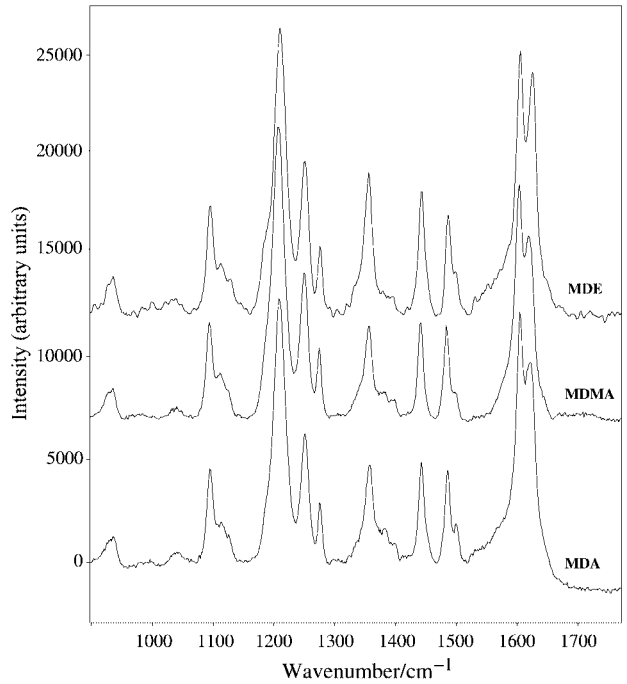


**Fig. 4** Preresonance Raman spectra ($\lambda_{exc}$ = 514.5 nm) of the neat free bases of 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethamphetamine (MDE). The MDMA and MDE spectra have been shifted vertically for better visualisation.

1550 $cm^{-1}$ have similar relative intensities, a distinction between the three derivatives is more difficult on the basis of the SERS spectra than on the basis of the preresonance Raman spectra. However, a distinction can be achieved by looking in detail at the whole region between 900 and 1650 $cm^{-1}$, where C–H and C–C vibrations of the skeleton are superimposed on those of the methyl or ethyl group. As discussed earlier, the shoulders related to the N–H bending mode decrease in intensity on going from MDA to MDE.

We propose to use the concepts applied in combination with pattern recognition to achieve the identification and differentiation of the illicit drugs in an automated procedure. To this end, the spectral region between 900 and 1640 $cm^{-1}$ is divided into 10 segments (termed A–K in Fig. 5). From the integrated band intensities the relative contributions of each segment are calculated and used as criteria (see below).

## Raman and SERS spectra of the drug samples

Near-infrared (NIR) Raman spectroscopy would normally be recommended for the investigation of XTC tablets and amphetamine powders (or the solutions thereof) because of the large amount of fluorescence induced by shorter wavelength excitation. However, a severe limitation, when working with exhibits in the form of tablets or powders, are the strong vibrational bands originating from accompanying compounds. This is illustrated in Fig. 6, where a comparison between an NIR Raman spectrum of an XTC tablet (trace A), the drug as its corresponding hydrochloride salt (trace B), the free base of the drug (trace C) and the SERS spectrum (trace D) is given. Referring to trace A, NIR Raman spectroscopy allows the investigation of many kinds of exhibits mostly without the need to consider fluorescence, but it produces a complete overlay of the vibrational contributions of all compounds present in the sample. It is immediately obvious, therefore, that the NIR Raman spectrum is overcrowded by a large number of bands, which easily leads to misinterpretation. The same is found for the preresonant Raman spectra of the cyclohexane extract itself, which consists mainly of the solvent spectrum with a high fluorescence background and/or a surplus of bands originating from traces of sugars, fatty acids or stretching agents which do migrate into the non-polar phase. After evaporation of the solvent, no preresonant Raman spectra of analytical value can be recorded on the residue because of the increased fluorescence background at visible excitation wavelengths.

More clarity in the spectrum is achieved by investigating the corresponding salt (trace B) or the free base (trace C), but the preparation of the drug leading to this form is a tedious and time-consuming extraction and purification procedure.

In view of the fact that the SERS-active surfaces are generated in an approximately neutral environment, reference is made throughout this paper to the analyte in the form of the free base. Although providing essentially the same amount of information as the preresonance Raman spectrum, the SERS spectrum from the sample is achieved much more easily by applying the crude extract on the surface, and one obtains a spectrum essentially free of disturbing contributions (trace D). The structure specific spectral features are reproducible; therefore, identification of the analyte is possible.

Fig. 7 compares the SERS spectra recorded from MDMA- and AMP-containing tablets and powders and gives an impression of the distinction capabilities of this SERS detection procedure. Trace A displays the spectrum of the extract of an MDMA hydrogensulfate containing tablet (XTC-E), which has already been shown in Fig. 6 (trace D) and discussed in comparison with the spectra of the pristine drug and the SERS spectrum of the free base (see Fig. 5). Trace B shows the spectrum recorded from the extract of a tablet named XTC-X, which is nearly identical with the SERS spectra from the powder extractions (traces C–F). By comparison with the spectra displayed in Fig. 3, one can identify the active ingredient as an amphetamine. The band pattern around 1200 $cm^{-1}$ provides evidence for the methyl-substituted derivative MAMP.

One of the problems encountered during an automated analysis of vibrational spectra is that band positions can shift slightly owing to a false wavenumber calibration and peak

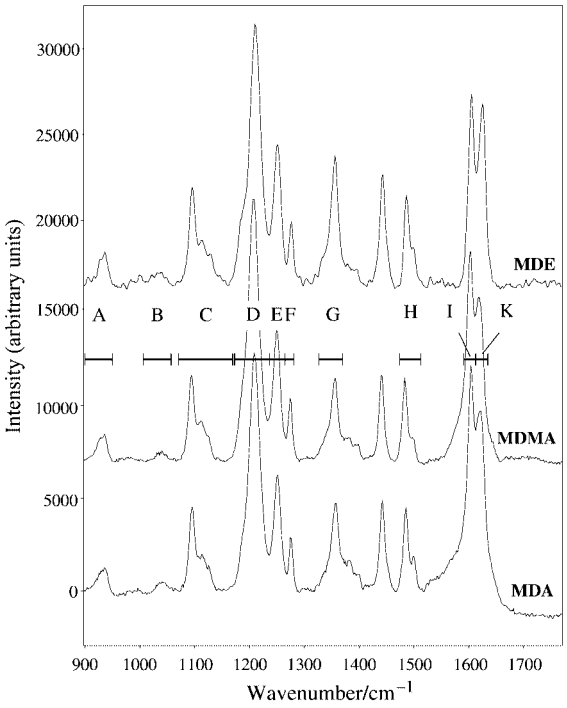


**Fig. 5** SERS spectra ($\lambda_{exc}$ = 514.5 nm) of $10^{-3}$ M methanolic solutions of the free bases of the 3,4-methylenedioxyamphetamines. The spectra have been baseline corrected and the MDMA and MDE spectra shifted vertically for better visualization. The segments A–K indicate the integration boundaries for the calculation of the relative band intensities used for identification.

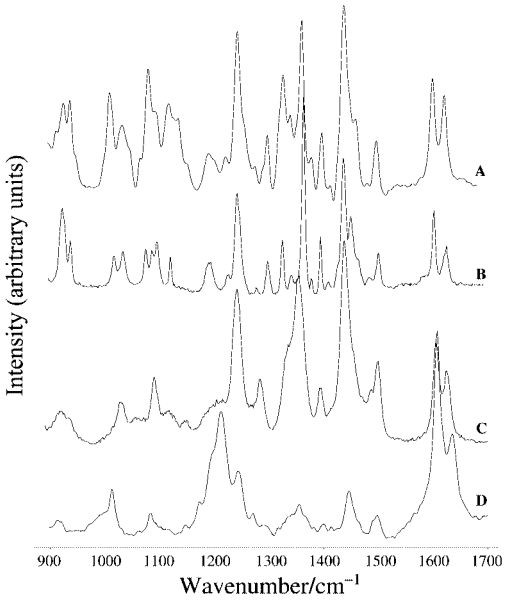


**Fig. 6** Comparison of Raman spectra of MDMA recorded from different types of samples: Trace A: NIR Raman spectrum of an XTC-E tablet. Trace B: Raman spectrum of MDMA.HCl. Trace C: Raman spectrum of the free MDMA base. Trace D: SERS spectrum of the extract of the XTC-E-tablet. $\lambda_{exc}$ = 1064 nm for trace A, 514.5 nm otherwise.

intensities can vary owing to background effects. In SERS spectra, both peak position and relative intensity can vary with the nature of the SERS-active surface present, the nature of the chemical environment and the wavelength of the exciting laser radiation. Therefore, we propose as a means of identification the comparison of the relative band intensities integrated over selected spectral segments, which are defined on the basis of the spectra of reference compounds recorded under identical conditions. In the following, we want to demonstrate the degree of confidence that can be achieved by applying such a procedure for the detection of MDMA in illicit tablets. The regions of integration are shown graphically in Fig. 5 and are specified in Table 1.

To quantify the extent of possible systematic errors in the determination of the individual band intensities of both sample and reference and the expected linear relation between the sample and reference readings of the individual bands of each tablet, the correlation coefficient $r$:

$$r = \frac{n\sum_{i=0}^{n} x_i y_i - \sum_{i=0}^{n} x_i \sum_{i=0}^{n} y_i}{\sqrt{\left[n\sum_{i=0}^{n} x_i^2 - \left(\sum_{i=0}^{n} x_i\right)^2\right]\left[n\sum_{i=0}^{n} y_i^2 - \left(\sum_{i=0}^{n} y_i\right)^2\right]}} \quad (1)$$

is used.

The average relative contributions of the different spectral segments A–K were determined from 20 SERS spectra of the reference and from the extracts of 20 illicit tablets. All spectra were recorded and processed in the same manner. We determined the correlation coefficient between the 40 intensity readings for each segment and found the resulting values (*c.f.* Table 1) to be fairly close to zero, thus proving the absence of a linear systematic error.[16]

To visualize the confidence in the relation between the samples and the reference, a plot of the relative band intensities of the sample *versus* those of the mean of the reference is shown in Fig. 8. In this graph, the bisecting line denotes a perfect match between a sample SERS spectrum and the selected reference. The ratio of the medians shows a very good match between the scattered values of the integrated band areas of the samples and the references. We decided for the median to express the 'central tendency' of the measures, because it is often a more realistic measure than the mean.[16] Segment B covers the breathing vibration of the phenyl ring system, the band intensity of which is depressed in the spectra of MDMA. Even if the

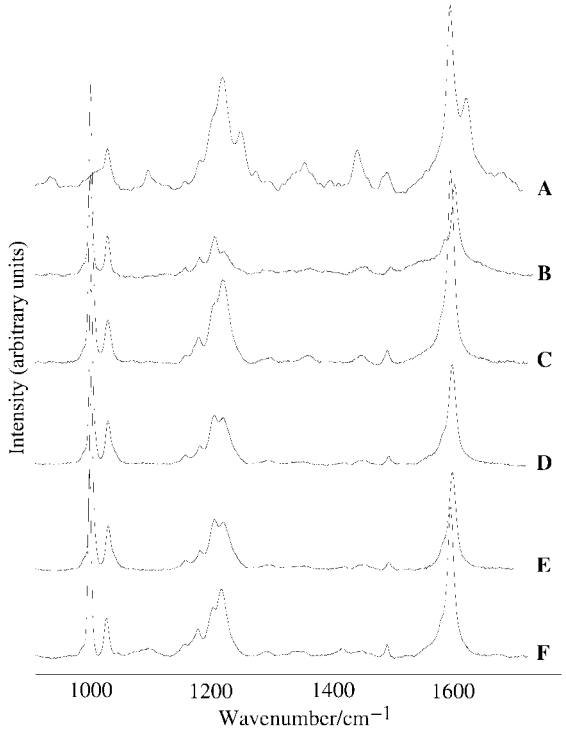


**Fig. 7** SERS spectra of different types of illicit drugs. Trace A: XTC-E tablet, Trace B: XTC-X tablet. Traces C–F represent SERS spectra of different powder exhibits.

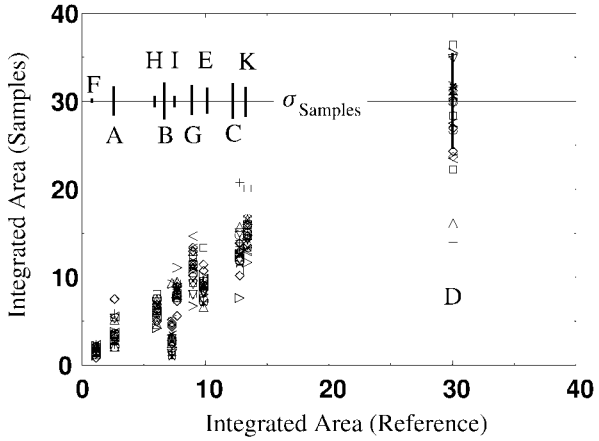


**Fig. 8** Comparison of the integrated and normalized band areas determined for 23 different tablet samples (as indicated by different symbols) *versus* the mean of the integrated areas of 20 measurements of a $10^{-2}$ M methanolic solution of an MDMA reference (applied volume 10 μl). The thick lines represent the standard deviations of the band areas of the tablet samples. The integration band limits are defined in Table 1.

**Table 1** Limits of integration as used for construction of Fig. 8[ab]

| Segment | Range/cm$^{-1}$ | $\sigma$ (tab.[a]) | $\sigma$ (ref.[b]) | Ratio $\sigma$[c] | Correlation coeff.[d] | Ratio median[e] |
|---|---|---|---|---|---|---|
| A | 900–950 | 1.62 | 0.65 | 2.5 | −0.0766 | 0.75 |
| B | 1005–1050 | 2.4 | 2.3 | 1.0 | 0.0946 | 0.37 |
| C | 1075–1170 | 2.3 | 1.41 | 1.6 | 0.0750 | 0.98 |
| D | 1171–1233 | 5.58 | 1.4 | 4.0 | 0.0196 | 1 |
| E | 1234–1269 | 1.47 | 0.67 | 2.2 | 0.0001 | 0.9 |
| F | 1270–1283 | 0.38 | 0.32 | 1.2 | −0.2125 | 0.64 |
| G | 1318–1371 | 1.91 | 1.32 | 1.4 | −0.1880 | 0.79 |
| H | 1470–1506 | 1.00 | 0.92 | 1.1 | 0.1631 | 0.94 |
| I | 1591–1610 | 1.01 | 0.56 | 1.8 | −0.0479 | 0.95 |
| K | 1611–1640 | 1.6 | 1.4 | 1.1 | −0.2369 | 0.92 |

Standard deviations of the integrated band areas in these regions: [a] of SERS spectra of the tablets and [b] of 20 SERS spectra of the reference compound MDMA. [c] Ratio of the standard deviations determined from the samples and the references. [d] Correlation coefficient for a linear relationship $r$ of 20 references and samples. [e] Ratio of the median of 20 normalized sample and reference integrated band areas.

**Table 2** Correlation coefficients between the sample and reference readings for each tablet

| Tablet | $r_1$ | $r_2$ | Tablet | $r_1$ | $r_2$ |
|---|---|---|---|---|---|
| XTC-1 | 0.95 | 1 | XTC-11 | 0.97 | 0.96 |
| XTC-2 | 0.96 | 0.98 | XTC-12 | 0.97 | 0.97 |
| XTC-3 | 0.93 | 0.95 | XTC-13 | 0.94 | 0.99 |
| XTC-4 | 0.78 | 0.96 | XTC-14 | 0.97 | 0.97 |
| XTC-5 | 0.96 | 0.93 | XTC-15 | 0.93 | 0.88 |
| XTC-6 | 0.96 | 0.98 | XTC-16 | 0.97 | 0.99 |
| XTC-7 | 0.96 | 0.95 | XTC-17 | 0.97 | 0.99 |
| XTC-8 | 0.64 | 0.92 | XTC-18 | 0.96 | 0.99 |
| XTC-9 | 0.96 | 0.97 | XTC-19 | 0.96 | 0.96 |
| XTC-10 | 0.97 | 0.98 | XTC-20 | 0.99 | 0.99 |
| AMP-1 | 0.82 | 0.92 | Fraudulent | 0.26 | 0.76 |
| AMP-2 | 0.75 | 0.73 | | | |
| *Reference solutions*— | | | | | |
| MDMA reference | 0.97 | 1 | AMP reference | −0.03 | 0.72 |

For calculation of $r_1$ all segments are used; for $r_2$ only the segments C, F, G, H and K are used. The segments are defined in Table 1. The references are $10^{-2}$ M methanolic solutions.

standard deviations do not vary significantly, the ratio of the medians reveals significant differences in the integrated areas of the samples and the references. Noteworthy in this regard is the standard deviation calculated for the sample readings. They are usually less than twice those of the reference compounds. The only exception is region D, which covers a vibration of type 19 according to Varsányi.[17] The large scatter in this relative intensity could be related to a large variation of the enhancement factor with the strongly disturbed adsorption geometry of MDMA on the Ag surface or with the variable contribution of shifted C–H bending vibrations of substituents. However, if all band areas of the illict drugs are taken into account, the standard deviations are within a range that allows automatic detection of the active ingredient of designer drug exhibits, no matter whether it is a highly contaminated powder or a tablet.

## Conclusion

SERS spectroscopy was applied successfully for the forensic analysis of so-called 'designer drugs'. Illicit drugs, tablets and powders were investigated utilizing a sampling technique on microtest plates, allowing rapid detection of the active ingredient using a commercial Raman microscope. The method presented in this paper is suitable for high-throughput analysis and permits the storage of the sample for later off-site processing. With normal effort, as usual in routine forensic analysis, the active substance is eluted from various forms of the drugs, thereby diminishing to a large extent the signal contributions of admixtures which disturb the interpretation of NIR Raman or conventional IR spectra.

A further reduction of the relative signal contribution by small amounts of additives carried within the cyclohexane phase is found whenever the heat of adsorption of these compounds is smaller than that of the active drug (competitive adsorption at the SERS-active surface). Alternatively, the fluorescence of adsorbed impurities such as food colors will be quenched.

Comparison of the SERS spectra of reference compounds (pristine MD- and AMP-type drugs) with those of contemporary forensic exhibits reveal a nearly perfect match. The limits of detection (micrograms of analyte per well) found in this study suggest that procedure can be developed into a commercial trace analytical XTC detection system.

## Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie is gratefully acknowledged. We thank Professor Dr R. Singer (Institut für Werkstoffwissenschaften II der Universität Erlangen-Nürnberg) for granting access to the Raman microscope. Legal support by Dr I. Wolff of the Bundesopiumstelle, Bonn, and technical support by Dr C. Lehner, Bruker Optik GmbH, is also gratefully acknowledged.

## References

1 R. A. Sulk, R. C. Corcoran and K. T. Carron, *Appl. Spectrosc.*, 1999, **53**, 280.
2 K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari and M. S. Field, *Chem. Rev.*, 2000, **99**, 2957.
3 S. De, A. Pal, N. Jana and T. J. Pal, *Photochem. Photobiol., A*, 2000, **131**, 111.
4 D. Graham, W. E. Smith, A. M. T. Linacre, C. H. Munro, N. D. Watson and P. C. White, *Anal. Chem.*, 1997, **69**, 4703.
5 R. P. Van Duyne, J. C. Hulteen and D. A. Treichel, *J. Chem. Phys.*, 1993, **99**, 2101.
6 J. A. Creighton, C. G. Blatchford and M. G. Albrecht, *J. Chem. Soc., Faraday Trans. 2*, 1979, **75**, 790.
7 P. C. Lee and D. J. Meisel, *J. Phys. Chem.*, 1982, **86**, 3391.
8 H. Gliemann, U. Nickel and S. Schneider, presented at the 51st Annual Conference of the Society for Imaging Science and Technology, Portland, Oregon, 1998.
9 J. C. Carter, W. E. Brewer and M. S. Angel, *Appl. Spectrosc.*, 2000, **54**, 1876.
10 K. T. Carron and R. C. Corcoran, *PCT Int. Appl.*, 1998, Appl. No. 514667.
11 P. C. White, S. Athmani, W. E. Smith and D. A. Sadler, *PCT Int. Appl.*, 1999, Appl. No. 854009.
12 X. Dou, T. Takama, Y. Yamaguchi, H. Yamamoto and Y. Ozaki, *Anal. Chem.*, 1997, **69**, 1492.
13 S. E. J. Bell and S. J. Spence, *Analyst*, 2001, **126**, 1.
14 B. Saegmuelle, G. Brehm and S. Schneider, *Appl. Spectrosc.*, 2000, **54**, 1849.
15 G. Haase and R. Matejec, in *Die Photographische Empfindlichkeit*, ed.W. F. Berg, F. Dörr, J. Eggers, G. Haase, E. Klein and R. Matejec, Frankfurt am Main, 1968, ch. 9.3, pp. 1333–1421.
16 *Analytical Chemistry*, ed. R. Kellner, J.-M. Mermet, M. Otto and H. M. Widmer, Wiley-VCH, Weinheim, New York, 1997.
17 G. Varsányi, *Vibrational Spectra of Benzene Derivatives*, Academic Press, New York, 1969.