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TECHNICAL NOTE

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Quantification of the Amphetamine Content in Seized Street Samples by Raman Spectroscopy

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ABSTRACT: A Raman spectroscopy method for determining the drug content of street samples of amphetamine was developed by dissolving samples in an acidic solution containing an internal standard (sodium dihydrogen phosphate). The Raman spectra of the samples were measured with a CDD-Raman spectrometer. Two Raman quantification methods were used: (1) relative peak heights of characteristic signals of the amphetamine and the internal standard; and (2) multivariate calibration by partial least squares (PLS) based on second derivative of the spectra. For the determination of the peak height ratio, the spectra were baseline corrected and the peak height ratio ($h_{\text{amphetamine at } 994\,\text{cm}^{-1}}/h_{\text{internal standard at } 880\,\text{cm}^{-1}}$) was calculated. For the PLS analysis, the wave number interval of 1300–630 cm^{-1} (348 data points) was chosen. No manual baseline correction was performed, but the spectra were differentiated twice to obtain their second derivatives, which were further analyzed. The Raman results were well in line with validated reference LC results when the Raman samples were analyzed within 2 h after dissolution. The present results clearly show that Raman spectroscopy is a good tool for rapid (acquisition time 1 min) and accurate quantitative analysis of street samples that contain illicit drugs and unknown adulterants and impurities.

KEYWORDS: forensic science, amphetamine, seized street samples, quantification, Raman spectroscopy

The number of drug samples seized by the police or the customs and suspected to contain controlled substances is constantly increasing. As a consequence, there is a significant need for rapid and accurate analysis methods with low running costs to reduce analysis costs in criminal laboratories.

Traditionally, seized drug samples are analyzed by qualitative spot tests and different chromatographic (HPLC, GC) or hyphenated chromatographic-spectroscopic methods, e.g., GC-MS. Vibrational spectroscopy is much less extensively used, although those methods, such as FTIR (1,2) and especially Raman spectroscopy (3–8), are potentially suitable for forensic drug analysis. Raman spectroscopy produces information-rich spectra and, in most cases, sample preparation is minimal, allowing for the nondestructive analysis of tablets, powders, and liquids *in situ*. The water signal is very weak in Raman spectra and thus spectra can easily be collected from moist materials. Moreover, Raman spectroscopy, with a typical acquisition time of a few seconds, is a rapid method compared with for example, chromatographic methods with a typical acquisition time of minutes.

Quantitative analysis of pharmaceutical compounds in various dosage forms has been performed using Raman spectroscopy (9–11). Some reports of the use of Raman spectroscopy for the identification of illicit drugs have been published (3–8) and they show that this represents a good tool for identification of illicit drugs. Only a few reports, however, have been published on the use of Raman spectroscopy for the quantitative analysis of illicit drugs. For example, Bell et al. (12,13) have shown that Raman spectroscopy can be used for profiling of seized ecstasy tablets. In this study, Raman spectra indicated that the *N*-methyl-3,4-methylendioxyamphetamine (MDMA) content varied in different tablets and also within one tablet, but the authors were not able to calculate the absolute MDMA content of the tablets. Ryder et al. (14,15) demonstrated Raman's potential for the quantitative analysis of cocaine. Test samples were prepared in a laboratory by mixing known amounts of cocaine, glucose, and caffeine. However, seized drug samples rarely consist of just one or two known adulterants, and the type of diluents can vary widely. In reality, the variation of adulterants creates specific problems for quantitative analysis. An example of this is the presence of fluorescent impurities, which can cause high background fluorescence and mask the Raman signal, making the quantitative analysis difficult or impossible. This problem can be avoided by using excitation wavelengths in the near-IR region, where the impact of fluorescence is minimized.

The objective of this investigation was to develop a new Raman spectroscopy-based method for quantification of illicit drug samples. In particular, a method for determining the drug content of street samples of amphetamine was developed. The specific aim was to determine whether dissolving of the sample and addition of an internal standard might solve the problems related to the quantitative analysis of street samples. Two quantification methods were used: (1) direct use of an internal standard and relative peak heights and (2) multivariate calibration by partial least squares (PLS). The advantages and disadvantages of the two methods are discussed below.

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Materials and Methods

Materials

Sodium dihydrogen phosphate monohydrate was purchased from Merck (Darmstadt, Germany), Pure DL-amphetamine sulfate was purchased from Sigma-Aldrich (St. Louis, MO). Lactose monohydrate was purchased from Merck, caffeine from Sigma-Aldrich, and Koffisal® from Nycomed (Roskilde, Denmark). All reagents were of analytical grade and used as purchased. Amphetamine powders seized by the Finnish police were used for the method evaluation.

Sample Preparation

Sodium Dihydrogen Phosphate Standard Solution—Amphetamine samples were dissolved in acidic solution in order to improve their solubility. Sodium dihydrogen phosphate was added as an internal standard. The solution was prepared by dissolving sodium dihydrogen phosphate monohydrate (25.0024 g) in 25 mL of distilled water and by adding 50 mL of 2 M hydrochloric acid. The molarity of the resulting (pH 1.3) solution was 2.4 M.

Amphetamine Samples—Seized amphetamine powder (150 mg) was dissolved in 600 μL of sodium dihydrogen phosphate standard solution. The solution was filtered before Raman measurements through a Spartan cellulose membrane (13 mm, 0.45 μm) filter (Schleicher&Schuell, Dassel, Germany).

Lactose, Caffeine, and Koffisal® Samples—Lactose, caffeine, and Koffisal® samples were prepared by dissolving the substance (150 mg) in 600 μL of sodium dihydrogen phosphate standard solution. The solution was filtered before Raman measurements through a Spartan cellulose membrane (13 mm, 0.45 μm) filter.

Raman Spectra

The Raman spectra of the samples were measured with a CDD-Raman (CCD, charge-coupled device) spectrometer (RAMSTAS) developed by VTT Electronics (Oulu, Finland) (16). In the present study, the acquisition time for amphetamine spectra was 1 min and the power of the 830 nm laser at the sample was 100 mW. The diameter of the measurement spot was 0.5 mm. The acquisition interval was 0–2000 cm^{-1} (1084 data points). Two spectral bands were chosen for the determination: one for the amphetamine sample (994 cm^{-1}) and another for the internal standard (880 cm^{-1}). The spectra were baseline corrected and the peak height ratio ($h_{\mathrm{amphetamine}}/h_{\mathrm{internal\ standard}}$) was calculated.

For the PCA and PLS analysis, the wave number interval of 1300–630 cm^{-1} (348 data points) was chosen. No manual baseline correction was performed, but the spectra were differentiated twice to obtain their second derivatives, which were further analyzed. The second derivatives of the 76 spectra were treated as independent variables and the amphetamine concentrations of the same samples as determined by HPLC as dependent variables. The predicting capacity and best number of the latent variables of the PLS models were assessed by cross-validation. In the cross-validation, one-fifth of the samples were randomly selected and removed from the model and used to test the model. This was repeated 1000 times. The best rank was selected to be the one that gives, on average, the best prediction. The maximum coefficient of determination for the prediction (i.e., Q^2) was used as a criterion for the rank.

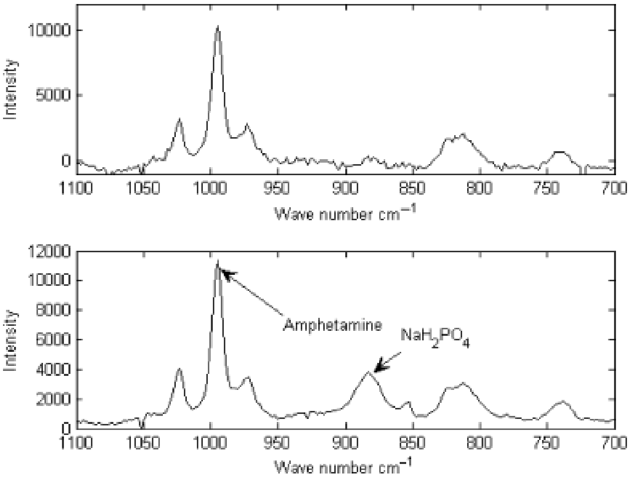

FIG. 1—*Raman spectrum of (a) amphetamine solution alone and (b) the same sample with a sodium dihydrogen phosphate internal standard.*

Reference Method for Quantification of Amphetamine

The LC method used was modified from Law et al. (17). Sample preparation consisted of dissolving 20–40 mg of homogenized material in 10 mL of water, followed by sonication and filtration. The LC was an Agilent 1100 series instrument equipped with a diode array detector and a Zorbax Sil 87 150 mm × 4.6 mm (5 μm) column (Agilent Technologies, Santa Clara, CA). LC separation was performed in the isocratic mode, using a mobile-phase flow rate of 1.5 mL/min and UV detection at 258 nm. Mobile-phase (pH 8.9) components consisted of methanol (810 mL), 1 M ammonium nitrate (15 mL), 2 M ammonia (30 mL), and water (45 mL). The expanded relative uncertainty of measurement in the concentration range of 0.1–100 wt% was ± 5.2% (level of confidence 95%). The method has been accredited according to EN ISO/IEC 17025 by FINAS.

Results and Discussion

Figure 1 shows the spectrum of amphetamine solution with (Fig. 1*b*) and without the internal standard (Fig. 1*a*). Amphetamine has a group of three bands at 1050–950 cm^{-1} and a signal-

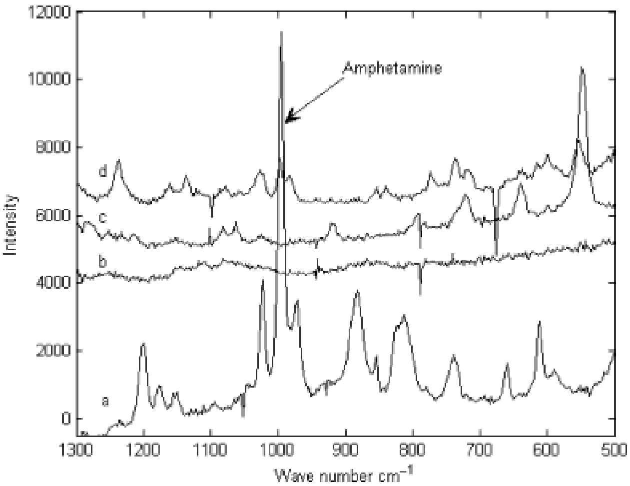

FIG. 2—*Spectra of solutions containing common used diluents and adulterants in seized street samples (b, lactose; c, caffeine; and d, Koffisal). In addition, the spectrum of an amphetamine solution with sodium dihydrogen phosphate is shown (a).*

TABLE 1—*Amphetamine content in seized powder samples as determined by Raman spectroscopy (peak height and PLS model) and by LC.*

Sample Numbers	Sample Code	Amount of Amphetamine (wt%)		
		Peak Height	PLS	LC
1–6	A	25.6 ± 1.6	23.9 ± 1.2	24
7–12	B	18.0 ± 0.4	17.6 ± 0.3	18
13–18	C	55.5 ± 2.3	56.5 ± 1.4	56
19–23	D	53.4 ± 0.7	52.3 ± 1.3	53
24–31	E	58.9 ± 0.2	57.7 ± 2.5	58
32–40	F	46.7 ± 1.2	47.7 ± 1.0	48
41–47	G	25.2 ± 1.5	26.6 ± 0.7	27
48–55	H	27.4 ± 0.5	27.2 ± 0.9	27
56–62	I	7.1 ± 0.2	8.6 ± 1.6	8
63–70	J	69.7 ± 0.4	68.6 ± 2.0	69

PLS, partial least squares.

free area in the wave number range 850–950 cm^{-1}. The internal standard possesses a characteristic band at 880 cm^{-1}. The characteristic bands of amphetamine at 994 cm^{-1} and sodium dihydrogen phosphate at 880 cm^{-1} were used for the identification and quantification of amphetamine. The ratio of amphetamine peak at 994 cm^{-1} to that at 1023 cm^{-1} was calculated in order to ensure that the quantitation is accurate (nothing hidden underneath). This ratio varied from 0.2 to 0.22 for samples that were stored for less than 2 h. However, the ratio varied from 0.1 to 0.6 for samples with a storage time of 22 h.

In addition to amphetamine, the spectra of some commonly used diluents and adulterants, lactose, caffeine, and Koffisal®, were measured. Figure 2 shows that these adulterants do not emit any signal at the examined wave numbers and they do not interfere with the determination of amphetamine. Adulterants selected are commonly used in amphetamine mixtures in Finland.

Amphetamine was quantified using powder samples from 10 independent seizures in Finland (Table 1). Two different quantification criteria, i.e., the band height method and PLS, are compared here. Traditionally, sophisticated data evaluation methods are not utilized due to the fact that Raman spectra, in most cases, contain sharp peaks that are clearly separated. Thus, in many cases the peak height or peak area of one characteristic Raman signal is used. The amphetamine content in the samples was calculated using a standard curve showing the peak height ratio ($h_{\mathrm{amphetamine}}/h_{\mathrm{internal\ standard}}$) as a function of amphetamine concentration in the solution ($y = 2.8925x - 0.0144$, $r^2 = 0.9992$). The amphetamine solutions for the standard curve were prepared using pure, commercially available amphetamine. Table 1 illustrates that these results corresponded with those of the LC method.

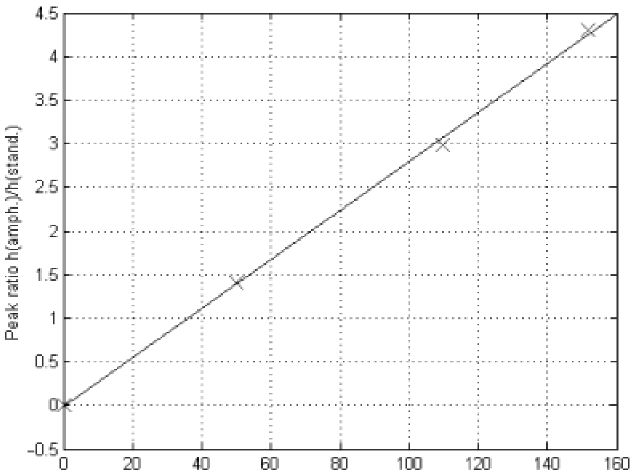

FIG. 3—*Amphetamine content as determined by a Raman spectrometer, in three samples as a function of storage time of the sample. Symbols are explained in Table 1.*

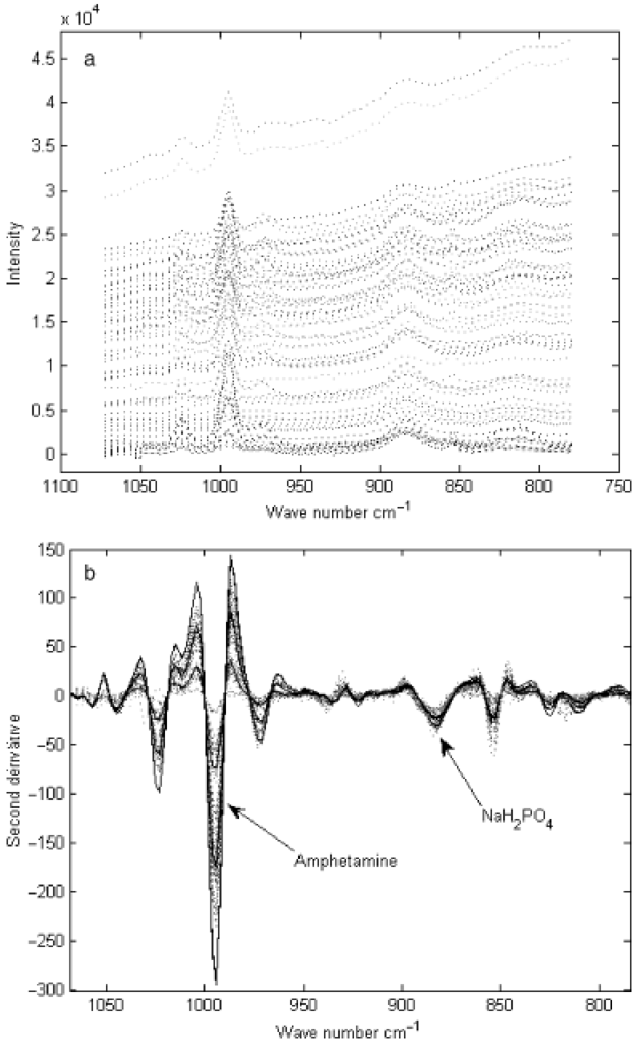

FIG. 4—*(a) Raw spectra of the seized street samples shown in Table 1 for partial least squares calibration. (b) Second derivative of the spectra for samples shown in Table 1. The differentiation eliminates the base line drift caused by fluorescence.*

The effect of the storage time of a dissolved amphetamine sample on its spectra was also investigated. The storage time at room temperature was varied from 10 min to 22 h (1300 min). Figure 3 clearly shows that the concentration of amphetamine remains constant during the first 2 h, but the concentration at 22 h is higher than the initial value. These results indicate that samples should be run before 2 h.

A more powerful data evaluation scheme is the use of multivariate analysis. Here, PLS models were developed as a comparison with the traditional evaluation criteria described above. In PLS, the second derivatives of the spectra were used because differentiating eliminates the baseline drift caused by fluorescence (Fig. 4*a* and *b*). A PLS score plot was used for identification of the outliers (Fig. 5). It appeared that the outlying samples numbered as 71–76 are spectra of standard

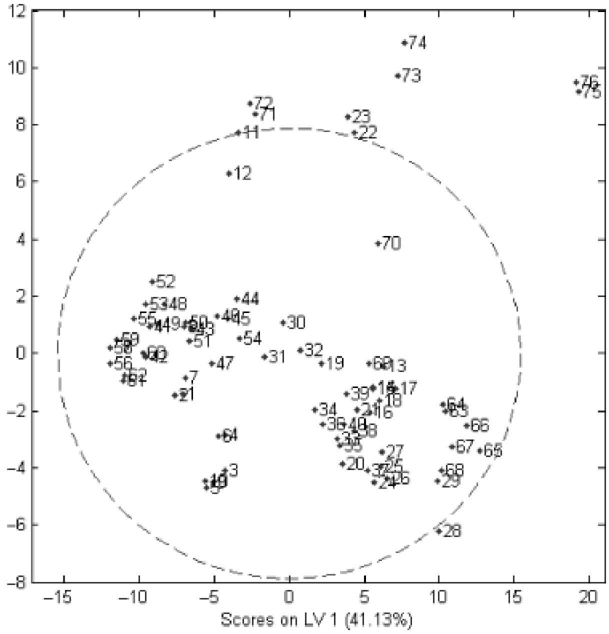

FIG. 5—*Partial least squares score plot. Numbers refer to the samples shown in Table 1.*

solutions prepared from pure commercial amphetamine. In PLS, the whole spectrum is considered and thus also the adulterants or other impurities common in seized street drug samples can affect the results. The standard samples do not contain any impurities and correspondingly, the spectra lack in this kind of information. Thus, pure compounds cannot be used as standards for PLS because of their significant difference from the street samples. In the traditional peak height method, this problem is eliminated due to fact that only one signal is used. Other outliers (11, 12, 22, 23, 70) in Fig. 4 are the samples with a storage time of 22 h.

A quantitative prediction model with the object of estimating the amphetamine concentration in samples was generated using the PLS method. Six latent variables and three sets of data were used in the calculations. In the first set, X1 is a 76 × 150 matrix in which each row is the second derivative of a spectrum and Y1 is a vector of 76 concentration values of respective samples. Similarly, in the second set, X2 (70 × 150) is a subset of X1 (without standard samples 71–76) and Y2 (70 × 1), respectively, is a subset of Y1. In the third set, X3 (50 × 150) is X2 without samples having a storage time of 22 h (samples 7, 8, 11, 12, 19, 22, 23, 30, 31, 33–40, 68–70, 71–76), and Y3 (50 × 1) conserves the concentrations, respectively.

The predictability of PLS was investigated by cross-validation. The predictability is very good when the six standards are discarded (X2, Y2) and is even slightly improved when the samples with a storage time of 22 h are discarded (X3, Y3) (Table 2). With cross-validation, the number of latent variables (rank) was also optimized. In order to be able to compare the results, rank = 6 was used for all calculations, and this value fits in optimal rank's standard deviation from the 1000 repeated cross-validations. The PLS calibration of (X3, Y3) is shown in Fig. 5. Table 1 shows the results obtained by the PLS model. These are in good agreement with the LC results and with Raman results determined by the peak height method. It is interesting to note that the long storage time does not affect the PLS results as strongly as it impacts on the traditional peak height method (Fig. 6).

TABLE 2—*Results of the cross-validation and analysis of the optimal number of latent variables.*

Set (Five Parts)	Rank	Rank ± SD	R^2	Q^2	SD (Q^2)
X1,Y1	6	5.6 ± 2.3	91.13	76	3
X2, Y2	6	6.9 ± 2.1	99.03	96.8	0.4
X3, Y3	6	4.9 ± 1.2	99.37	97.0	0.3

R^2, coefficient of determination; Q^2, coefficient of prediction; rank, number of latent variables.

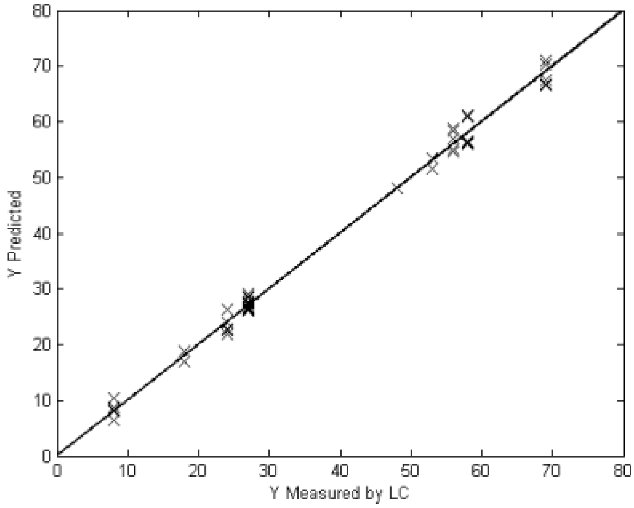

FIG. 6—*Correlation plot of the partial least squares model. Predicted concentrations versus the measured concentrations are shown.*

Conclusions

This study with limited sample amount demonstrates that Raman spectroscopy offers a potential for the quantitative analysis of seized street drug samples. Dissolving the sample and adding an internal standard makes this possible even if the sample contains unknown adulterants and impurities. Calculations can be performed by either a traditional calibration method or by using PCA and PLS methods. Both methods produced results accurate enough for routine forensic analysis. In conclusion, Raman spectroscopy appears to be a potential candidate to be added in future analytical toolboxes for forensic applications.

References

1. Cheng WC, Poon NL, Chan MF, Phil M. Chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets seized in Hong Kong. J Forensic Sci 2003;48:1249–59.
2. Praisler M, Van Bocxlaer J, De Leenheer A, Massart DL. Chemometric detection of thermally degraded samples in the analysis of drugs of abuse with gas chromatography-Fourier-transform infrared spectroscopy. J Chromatogr A 2002;962:161–73.
3. Hodges CM, Akhavan J. The use of Fourier transform Raman spectroscopy in the forensic identification of illicit drugs and explosives. Spectrochimica Acta 1990;46A:303–7.
4. Kuptsov AH. Applications of Fourier transform Raman spectroscopy in forensic science. J Forensic Sci 1994;39:305–18.
5. Sands HS, Hayward IP, Kirkbride TP, Bennet R, Lacey RJ, Batchelder DN. UV-excited resonance Raman spectroscopy of narcotics and explosives. J Forensic Sci 1998;43:509–13.

6. Tsuchihashi H, Katagi M, Nishikawa M, Tatsuno M, Nishioka H, Nara A, Nishio E, Petty C. Determination of metamphetamine and its related compounds using Fourier transform Raman spectroscopy. Appl Spectrosc 1997;51:1796–9.
7. Faulds K, Smith WE, Graham D, Lacey RJ. Assessment of silver and gold substrates for the detection of amphetamine sulphate by surface enhanced Raman spectroscopy (SERS). Analyst 2002;127:282–6.
8. Carter JC, Brewer WE, Angel SM. Raman spectroscopy for the *in situ* identification of cocaine and selected adulterants. Appl Spectrosc 2000;54:1876–81.
9. Orkoula MG, Kontoyannis CG, Markopoulous CK, Koundourellis JE. Quantitative analysis of liquid formulations using FT-Raman spectroscopy and HPLC. The case of diphenhyrdamine hydrochloride in Benadryl®. J Pharm Biomed Anal 2006;41:1406–11.
10. Mazurek S, Szostak R. Quantitative determination of diclofenac sodium and aminophylline in injection solutions by FT-Raman spectroscopy. J Pharm Biomed Anal 2006;40:1235–42.
11. Szostak R, Mazurek S. Quantitative determination of acetylsalicylic acid and acetaminophen in tablets by FT-Raman spectroscopy. Analyst 2002;127:144–8.
12. Bell SEJ, Burns DT, Dennis AC, Speers JS. Rapid analysis of ecstasy and related phenethylamines in seized tablets by Raman spectroscopy. Analyst 2000;125:541–4.
13. Bell SEJ, Burns DT, Dennis AC, Matchett LJ, Speers JS. Composition profiling of seized ecstasy tablets by Raman spectroscopy. Analyst 2000;125:1811–5.
14. Ryder AG, O'Connor GM, Glynn TJ. Identifications and quantitative measurements of narcotics in solid mixtures using near-IR Raman spectroscopy and multivariate analysis. J Forensic Sci 1999;44:1013–9.
15. Ryder AG, O'Connor GM, Glynn TJ. Quantitative analysis of cocaine in solid mixtures using Raman spectroscopy and chemometric methods. J Raman Spectrosc 2000;31:221–7.
16. Niemelä P, Suhonen J. Rugged fiber-optic Raman probe for process monitoring applications. Appl Spectrosc 2001;55:1337–40.
17. Law B, Gill R, Moffat AC. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous ethanol eluent. J Chromatogr 1984;301:165–72.

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