Research article

Drug Testing and Analysis

Received: 16 June 2011 Revised: 26 July 2011 Accepted: 26 July 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.346

Infrared attenuated total reflection (IR-ATR) spectroscopy for detecting drugs in human saliva

Kerstin M.-C. Hans, Susanne Müller and Markus W. Sigrist*

The consumption of drugs is of great concern worldwide. Various drug tests for humans have been developed but there is no compact and easy-to-use test device available yet for direct semi-quantitative drug testing in the field. We suggest using attenuated total reflection (ATR) infrared spectroscopy as a sensing method to analyze human saliva samples with respect to drugs. In this paper, we present ATR spectra in the infrared range between 2300 and 900 cm^{-1} as a first step towards such a device. We emphasize the common drug cocaine and its metabolites and investigate the problems of spectral interferences of selected diluents, masking agents, common medication, and soft drinks. Furthermore, spectra of saliva samples are recorded and a time-dependent change of the spectral signatures after alcohol consumption is presented. To the best of our knowledge, it is the first time that not only spectra of the drug of interest (cocaine) dissolved in water and in saliva but also spectra of interfering compounds possibly present in the saliva sample of a tested subject are discussed. This paper presents the most appropriate spectral range for strong cocaine absorption (including its metabolites) and minimum interference by the investigated substances. This spectral window is found to be between 1800 and 1710 cm^{-1}. In addition, we demonstrate the feasibility to identify cocaine in saliva at a concentration of 0.020 mg/ml with IR-ATR-spectroscopy without any separation or extraction procedures. For example, this technique could also be applied for drug detection in waste water. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: infrared spectroscopy; cocaine; metabolites; human saliva; evanescent field

Introduction

The consumption of illicit drugs such as cocaine or heroin is widely spread. An example of this fact is the study by Mura *et al.*[1] in France where an increase in the usage of drugs was observed among drivers under the age of 30 who were killed in road crashes. Driving under the influence of cocaine, for example, can lead to speeding, high-risk behaviour, poor impulse control, and loss of control of the vehicle.[2] Another example is workplace drug testing which is allowed in certain jobs. Often employers use saliva for the test since it is less invasive than urine or blood. The concern in this area is mirrored by the development of guidelines for workplace drug testing in oral fluid.[3] Hence, non-invasive on-site drug detection of impaired workers with an easy-to-use device is of great interest. Using saliva as a matrix simplifies the analysis even more; it is easier to collect than blood, plasma, or urine. In this paper we suggest a spectral region in the mid-infrared range for cocaine detection with minimal interferences and show first results of cocaine measurements in saliva using infrared- attenuated total reflection (IR-ATR) spectroscopy. Further investigations are aimed at building a portable sensor with a quantum cascade laser as a light source. The future combination with microfluidic techniques will enable transportation of the sample to the detection area and reduction of the sample volume.

Samples acquired in the field include different forms of cocaine like solid cocaine, cocaine.HCl, and cocaine dissolved in body fluids. For determining the origin of illicit cocaine samples, solid cocaine is investigated with a number of methods. IR spectroscopy on pure and diluted solid cocaine has been employed by Koulis *et al.*[4,5] and Lopéz-Artíguez *et al.*[6] Koçak *et al.*[7] used Fourier transform IR transflection spectroscopy to investigate solid cocaine.HCl. Furthermore, the differentiation between Pseudoecgonine and Ecgonine (Ecgonine is also metabolized by the body after cocaine consumption) is very helpful in determining the origin of illicit cocaine. A successful method for this differentiation is narrow bore capillary gas chromatography (GC).[8] Casale compared this technique with nuclear magnetic resonance (NMR) studies, GC in combination with mass spectroscopy and infrared spectroscopy on solid cocaine.[8] Kawase *et al.*[9] used terahertz-spectroscopy to detect solid cocaine in envelopes.

In a laboratory set-up, common methods for the detection of cocaine, heroin, and metabolites in body fluid samples are gas or liquid chromatography combined with mass spectrometry (GC-MS and LC-MS, respectively). These methods are the acknowledged standard in many toxicological laboratories.[10,11] Very often solid-phase extraction (SPE) is used to prepare these samples beforehand. The achieved detection limits are very low and reach down to 5 or even 1 ng/ml for certain drugs and metabolites in plasma, saliva, and urine.[12,13] However, the preparation of the samples is sophisticated and the apparatus is rather bulky and not easy to transport.

High performance liquid chromatography with diode array detection (HPLC-DAD) has a calculated limit of detection (LOD) for

---

* *Correspondence to: Markus W. Sigrist, ETH Zurich, Institute for Quantum Electronics, Laser Spectroscopy and Sensing Lab, Schafmattstrasse 16, 8093 Zurich, Switzerland. E-mail: sigristm@phys.ethz.ch*

ETH Zurich, Institute for Quantum Electronics, Zurich, Switzerland

morphine of 24 ng/ml, 32 ng/ml for codeine and 10 ng/ml for cocaine (Cocaethylene 10 ng/ml).[14]. Still SPE is used to prepare the plasma samples beforehand.

Drug analysis with Raman spectroscopy was done by several groups. Cocaine dissolved in ethanol was sensed by Burnett *et al.* They achieved a limit of detection of 6% *w/v* of cocaine dissolved in alcohol measuring through the glass of the bottles with Raman spectroscopy.[15] Shende *et al.*[16] used metal-doped sol-gels to separate the drugs and metabolites and detected them with surface enhanced Raman spectroscopy (SERS). Unfortunately the LOD for saliva samples is still rather high in the µg/ml range.

UV-spectroscopy was applied to detect one of the metabolites of cocaine in urine. After a liquid-liquid extraction, Sweeney *et al.* achieved a detection limit of 1.25 µg/ml for Benzoylecgonine.[17]

Ogert *et al.* employed a flow immunosensor to detect cocaine via fluorescence.[18] The stronger binding of the drug to immobilized antibodies compared to the binding of the fluorophore-labelled antigen was used. The detection limit was 5 ng/ml but no measurements have been reported for cocaine detection in body fluids. A similar technique was applied by Devine *et al.* who used an evanescent wave flow fluorometer and achieved limits of detection for cocaine and metabolites between 5 and 30 ng/ml.[19] Again, this data was only achieved with their own flow buffer and not for saliva or other body fluids.

Hitherto, the most often used technique in commercially available tests involves immunoassays. Immunoassays use the bonding of antigens (the substance of interest) and specially patterned antibodies which are localized on a surface to indicate the presence of a drug or its metabolite. However, many of those immunoassays still need to be improved with respect to reliability.[20] Depending on the test, a pretreatment of the sample might be necessary. This additional step provides another uncertainty in the outcome of the test depending on the skills of the persons involved in the pretreatment.

Since a direct, easy-to-use, semi-quantitative and compact test device for drug detection in human saliva is currently not available we suggest IR-ATR spectroscopy combined with microfluidics for the development of a hand-held test device that meets these challenges. Even a semi-quantitative test (as discussed later) can be of great use since it gives the testing person a better insight into the mental and physical condition of the tested person. As a first step towards such a device, we present infrared spectra of cocaine in water and human saliva. In addition, we explore possible spectral interferences with diluents, masking substances or common medicine in order to evaluate the most appropriate wavelength range.

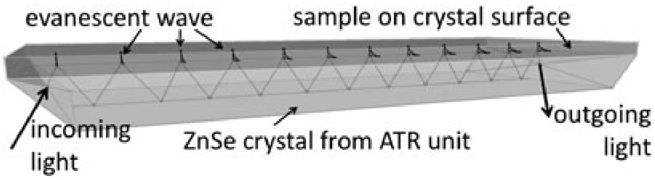

Figure 1. Scheme of the generation of evanescent waves on the surface of a crystal. The light enters the crystal from the left side and propagates via total reflection through the crystal. At each total reflection an evanescent wave is generated and penetrates the sample on the surface of the crystal. The outgoing light on the right side is then attenuated according to the absorbance of the sample under investigation.

Experimental method

ATR spectroscopy is an important tool in optical spectroscopy for investigating the absorption pattern of a sample. The basic principle involves the creation of an evanescent wave by total reflectance in a crystal (Figure 1). The attenuation of this evanescent field by the absorption in the sample on the crystal surface is then detected. Since the evanescent field decays exponentially the penetration depth into the sample is rather short (e.g. 1–2 µm for water in the mid-infrared). These small optical path lengths allow the analysis of strongly absorbing substances without reducing the detector signal beyond detectability.

The spectra were recorded with a commercial Fourier-Transform-Infrared (FTIR) spectrometer (Paragon 1000 PC from Perkin Elmer) combined with the matching ATR unit from Perkin Elmer. The ATR unit consists of four mirrors, which guide the light into and out of the ZnSe crystal. It is a multireflectance device with exchangeable top plates which provides the opportunity to choose between 11 and 12 reflections. The FTIR spectrometer was tuned to a resolution between 1 and 4 cm^{-1} as indicated in the captions of the individual plots.

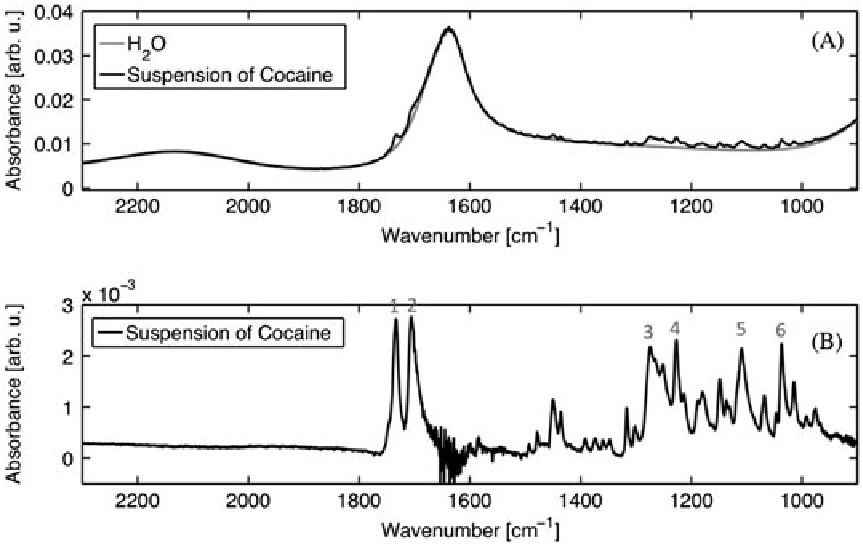

Figure 2. Spectra of a suspension of cocaine and water. (A) Spectrum of the suspension in black and spectrum of demineralized water in grey. (B) Difference in absorbance between the two graphs above. The graph depicts the characteristic absorbance of cocaine flakes in water. Concentration of cocaine in water was approximately 1.3 mg/ml and the spectral resolution of the FTIR was 1 cm^{-1}. The main peaks are numbered for further reference in Table 1.

Table 1. Wavelength position of selected cocaine absorption peaks. Position of cocaine absorption peaks 1–6 (cf. Figure 2 for assignments) as reported in literature in comparison to this work. The relative heights of the peaks differ from the shown spectrum in Figure 2 but also vary between the sources. The table shows the good agreement of the presented data with the literature. (app.: approximate estimated peak position, nl: not listed)

Peak 1 in cm^{-1}	Peak 2 in cm^{-1}	Peak 3 in cm^{-1}	Peak 4 in cm^{-1}	Peak 5 in cm^{-1}	Peak 6 in cm^{-1}	Ref.
app. 1736	app. 1714	app. 1268	nl	app. 1107	nl	[7]
1734.5	1705.9	1272.6	1227.7	1107.5	1035.6	[4]
1733.7	1706.7	nl	nl	nl	nl	[5]
1729.9	app. 1710	app. 1278	app. 1230	1109.4	1027.5	[29]
1728–1725	1711–1706	app. 1263	app. 1232	app. 1110	app. 1020	[6]
1734	1706	1274	1227	1110	1037	this paper

The measured attenuation of the signal enables the calculation of the absorption by taking the wavelength dependence of the penetration depth of the evanescent wave into the sample into account. As explained by Popov and Lavrent'ev,[21] the penetration depth depends on the wavelength used for the analysis and on the refractive index of both the sample and the crystal. The absorbance data in the recorded spectra shown below have all been corrected based on the dampening rate of the evanescent field given by Popov and Larent'ev[21] and the refractive index of water retrieved from Downing and Williams.[22] The calculated penetration depth of one reflection was divided by the maximum of the penetration depth. This normalization factor N was used to calculate the corrected absorbance as:

$$A(\tilde{\nu}) = -\frac{\frac{(\log_{10}(I(\tilde{\nu})/I_0(\tilde{\nu})))}{N \cdot r}}{\left(\tilde{\nu} \cdot n_{ZnSe} \cdot 2 \cdot \pi \sqrt{0.5 - \left(n_{H_2O}(\tilde{\nu})/n_{ZnSe}\right)^2}\right)} \quad (1)$$

with A the absorbance, $I_0(\tilde{\nu})$ the intensity of the outgoing light without sample on the surface of the crystal, $I\ (\tilde{\nu})$ the outgoing light with the sample on the crystal surface (Figure 1), N the normalization factor, r the number of reflections, $\tilde{\nu}$ the wavenumber, n_{ZnSe} the refractive index of zinc selenide and $n_{H_2O}\ (\tilde{\nu})$ the refractive index at wavenumber $\tilde{\nu}$ of water according to Downing and Williams.[22]

The drug cocaine and its metabolites were purchased from Lipomed with the approval of the cantonal drug control Zurich. All other tested samples were bought in commercial shops like supermarkets, health food shops and pharmacies. Most of the samples were in powder form. It was therefore easy to weigh and dilute them with demineralized water for creating a liquid with the desired concentration. Samples pressed in pills were mashed and their powder was treated as described above.

Results and discussion

Spectra of cocaine, metabolites, diluents, masking substances, and common medicine in aqueous solutions

An important drug for quick on-the-road-side tests is cocaine. Cocaine has a very characteristic absorbance in the mid infrared (Figure 2) which might enable the tester to quantify the cocaine in the human body fluid sample. Another example is heroin, which exhibits characteristic absorbance features between 1800 and 750 cm^{-1} as well.[23] Heroin can be found in saliva up to a few hours after consumption and, when smoked, reaches concentrations as high as 20 µg/ml in the first few minutes after administration.[24] Codeine also absorbs characteristically in the interval from 1700 to 1000 cm^{-1}.[25] In the following, we focus on the drug cocaine. Since cocaine and heroin share many diluents,[26–28] most results will be helpful for heroin investigations as well.

In Figure 2, a typical infrared spectrum of a solution, sol, or suspension is depicted. In Figure 2A, the absorbance of the suspension consisting of cocaine both solved in water and in the form of solid flakes suspended in water is shown in black. The absorbance is plotted and not the absorption coefficient since the measurements were not quantitative and therefore stating the true absorption coefficient would be misleading. The grey coloured graph represents the absorbance of water in this spectral range. The difference between the suspension and the demineralized water is very small due to the high absorbance of water in the mid-infrared even though the concentration of cocaine was very high with approximately 1.3 mg/ml. Figure 2B depicts the difference between the absorbance of the suspension and the water, thus representing the characteristic absorbance of cocaine flakes in water. In the following graphs the water background is always subtracted if not stated otherwise.

The mid-infrared spectrum of cocaine was reported previously. Koulis *et al*. analyzed solid cocaine base[4,5] and the Sigma Aldrich Library contains a spectrum of cocaine.HCl solved in Nujol.[29] Lopez-Artiguez *et al*.[6] analyzed cocaine.HCl and the effects of diluents on the infrared spectra of the powder. Koçak *et al*. analyzed solid cocaine.HCl with infrared transflection spectroscopy.[7] However, an accurate comparison is not possible since not all peaks in those references are given with their exact position. In Table 1 the positions of the main peaks in Figure 2 are compared with literature data. Peak positions that were estimated by us from referenced spectra are marked by the abbreviation app. for approximately. The absorption strength in the spectra varies between the sources.

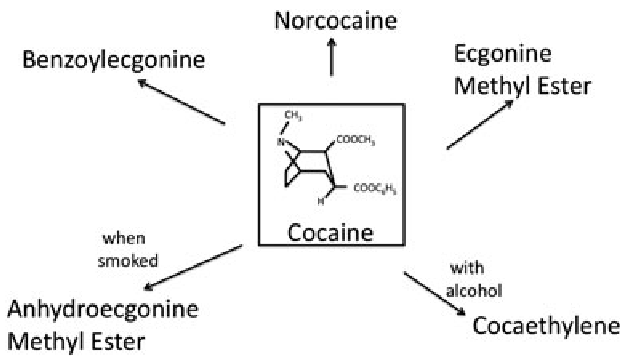

Figure 3. Metabolites of cocaine according to Cone *et al*.[30] Cocaine gets metabolized to benzoylecgonine, norcocaine, and ecgonine methyl ester. If cocaine is smoked anhydroecgonine methyl ester is metabolized and if alcohol is consumed alongside cocaine, cocaethylene is metabolized in addition.

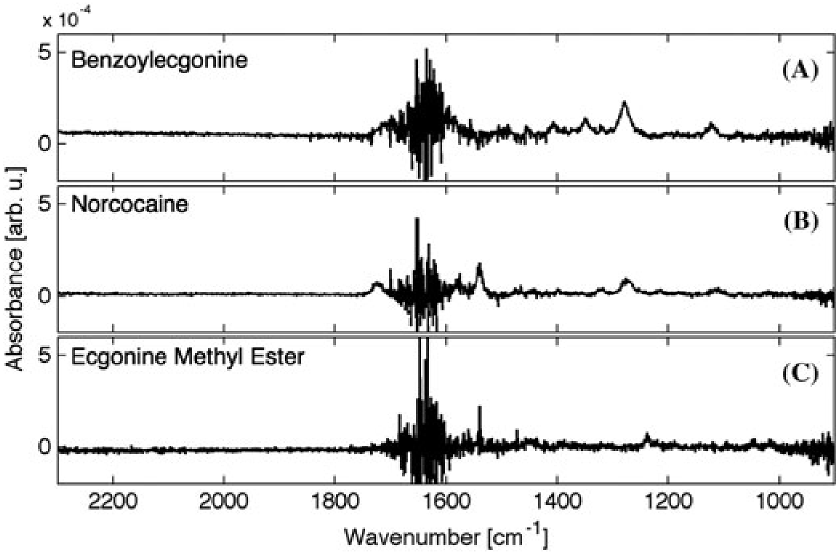

Figure 4. Spectra of selected metabolites of cocaine. Metabolites were measured in water. The liquid water background was subtracted after the measurement. (A) shows the absorption spectrum of benzoylecgonine; (B) norcocaine; and (C) ecgonine methyl ester. The spectral resolution of the FTIR was 1 cm^{-1}.

This variation is partly due to the presence of a solvent or due to the fact that cocaine free base or cocaine. HCl were analyzed. Nevertheless, the comparison between the spectra shows that the measurements presented in this paper are within the range of data variations in the published literature.

Whenever a drug is consumed, the body starts to metabolize it and therefore similar molecules (metabolites) like the introduced drug are produced by the body. In the case of cocaine the first metabolites are benzoylecgonine, norcocaine, and ecgonine methyl ester. When cocaine is smoked anhydroecgonine methyl ester is metabolized and if alcohol is consumed alongside cocaine cocaethylene is metabolized in addition. These pathways have been described by Cone *et al.*[30] and are depicted in Figure 3.

Previous research shows that metabolites do not only occur in urine but also in saliva.[31–33] The most common techniques for determining the concentrations are mass spectrometry combined with liquid or gas chromatography. Jenkins *et al.* found a mean concentration of 50 ng/ml of benzoylecgonine in the saliva of test persons 2 h after the administration of a single dose of approximately 40 mg cocaine.[24] Jufer *et al.* detected concentrations up to 3000 ng/ml in saliva.[31] For on-the-road-side-testing, it is thus necessary not only to measure cocaine but also its metabolites since the effect on the body can stem from both the metabolites and cocaine itself. In Figures 4 and 5, the spectra of some metabolites are depicted. The vertical scales of the spectra differ considerably which is most likely due to the fact that cocaethylene was found to be very insoluble in water whereas the other metabolites are much more soluble. This results in a spectral analysis of a suspension of cocaethylene in water rather than of a solution. The ATR technique might not be the best choice when solid particles in liquids are present since the particles sediment on top of the crystal surface where they have two effects: First, since the evanescent

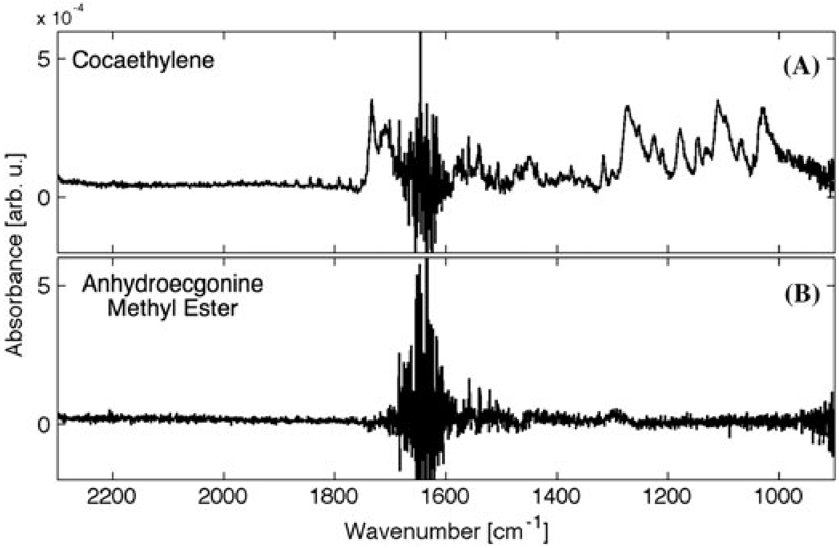

Figure 5. Spectra of selected metabolites of cocaine. Metabolites were measured in water. The liquid water background was subtracted after the measurement. (A) depicts cocaethylene particles in water; (B) depicts the spectrum of anhydroecgonine methyl ester. The spectral resolution of the FTIR was 1 cm^{-1}.

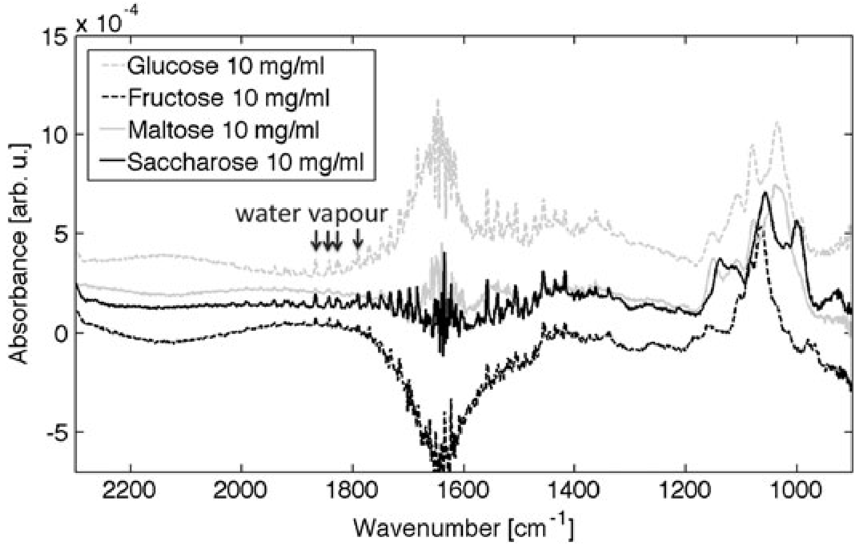

Figure 6. Absorbance of sugars in solution. The concentration is 10 mg/ml and the spectral resolution of the FTIR was 2 cm^{-1}. The difference in the absorption at 1650 cm^{-1} might be due to small temperature differences of the water or due to the interaction of the sugar molecules with the water. The graphs depict the sugar absorbance of the solution after the water background subtraction. The dashed grey graph shows glucose, the grey maltose, the dashed black fructose, and the solid black saccharose. The vertical arrows mark examples for water vapour absorption which interfere with the measurement.

field is maximum at the crystal surface, the solid particles contribute more to the absorption than the solution. Secondly, the solid flakes displace the liquid which again increases the contribution of the flakes in the spectra. As a result, spectra acquired from liquids with solid particles cannot be used for quantitative analysis of the data when recorded via ATR spectroscopy. However, the recorded spectra still give qualitative information on the absorption of the metabolites. Comparing the spectra of the metabolites with the cocaine spectrum of Figure 2 indicates that there are many similarities in the spectra and that it might be possible to measure cocaine and some of its metabolites simultaneously.

Very often sugars are used to dilute drugs since they are cheap and easily available. Hence, potential interferences between sugar spectra and the spectrum of the drug of interest need to be investigated. In Figure 6, the absorbance of glucose, fructose, maltose, and saccharose diluted in water at a concentration of 10 mg/ml is shown. A concentration this high was chosen to ensure that all absorption lines in the spectra could be detected by the FTIR spectrometer. Their major absorbance is between 1200 and 1000 cm^{-1}. The peak or dip at 1650 cm^{-1} might be linked with the interaction between the sugar molecules and the water or might be caused by small temperature differences between the measurements of water and the sugar sample (which were performed separately one after the other). Temperature changes in water can be responsible for small variations in water absorption as explained by Freda *et al.*[34] Narrow peaks from water vapour absorption are visible throughout the spectra. These interferences occur due to the evaporation of water from the sample. They will not be relevant in an enclosed lab-on-a-chip sensor since the channels are

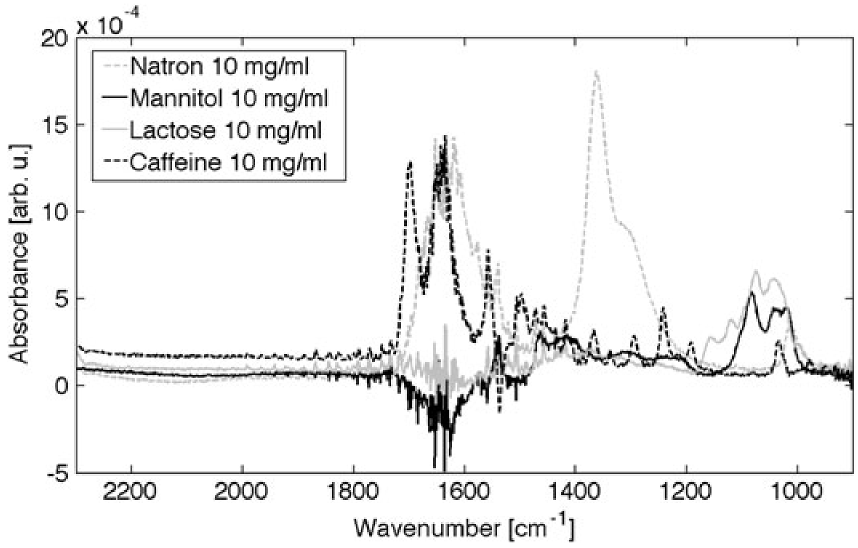

Figure 7. Spectra of selected diluents for drugs. The water background is subtracted from the spectra. The natron (sodium bicarbonate) spectrum is depicted in the dashed grey graph, mannitol in black, lactose in grey, and caffeine with a dashed black line. The spectral resolution of the FTIR was 2 cm^{-1}.

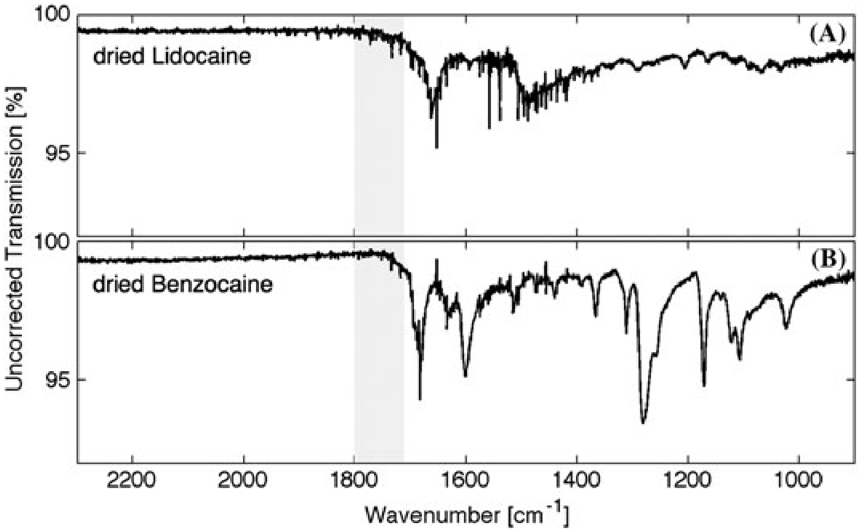

Figure 8. Spectra of (A) lidocaine and (B) benzocaine as examples for narcotics used as diluent. The samples were solved in water and than dried onto the crystal surface. The spectral resolution of the FTIR was 1 cm^{-1}. The light grey area marks the suggested interval for cocaine detection.

closed. The comparison of various sugar spectra with the cocaine spectrum (Figure 2) implies that no interferences with the main cocaine absorption peaks (1800–1700 cm^{-1}) occur.

Other common diluents of drugs are sodium bicarbonate (natron), mannitol, and caffeine. Their spectra are depicted in Figure 7, again after the subtraction of the water background. Caffeine has a stimulating effect like cocaine, which disguises the dilution of the drug to a consumer. Natron is a very common diluent since it is used to synthesize *crack*. *Crack* is synthesized by cooking cocaine and natron, which in combination has an enhanced effect on the human body.[35] Since natron is very often also used as baking soda and since caffeine and the other diluents occur in other (legal) contexts as well, a potential interference of their spectra with the spectrum of the drug of interest needs to be taken into account. However, as only caffeine exhibits a major absorption at 1700 cm^{-1}, which decays very quickly towards larger wavenumbers, spectral interferences with the cocaine spectrum between 1800 and 1710 cm^{-1} are negligible.

An important issue with respect to spectral interference concerns compounds with carbon atoms double bonded to an oxygen atom. Examples are caffeine, lidocaine, and benzocaine. Caffeine was discussed in the previous paragraph. The other two examples are narcotics which are often used as adulterants.[26] We recorded spectra of these substances by suspending them in water followed by drying on the ATR crystal. As the spectra in Figure 8 imply, the spectral features closest to the interval of interest occur around 1683 cm^{-1} for benzocaine and around 1658 cm^{-1} for lidocaine. Hence, spectral interferences with the cocaine spectrum between 1800 and 1710 cm^{-1} are minimal. This could be advantageous in comparison to Raman spectroscopy, which shows difficulties to identify cocaine in the presence of benzocaine due to overlapping spectra.[37]

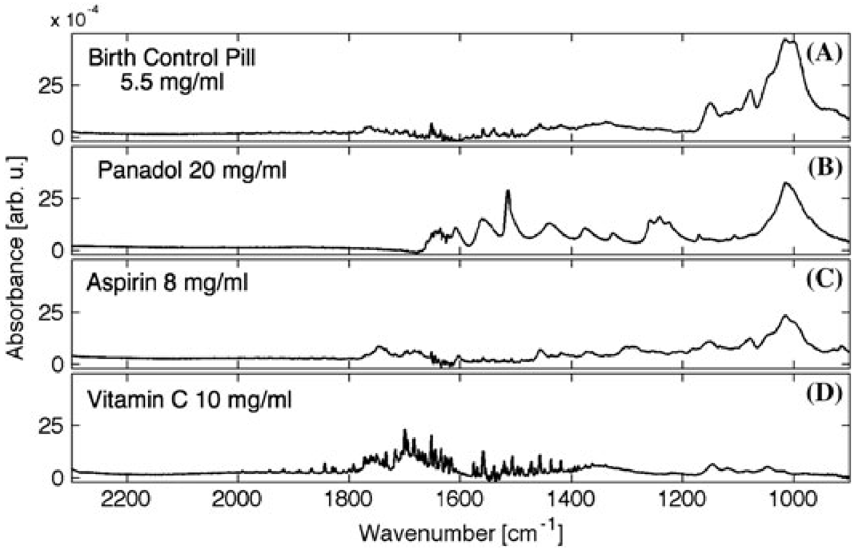

Figure 9. Spectra for selected common medicine. The medicine in pill form was mashed and diluted with water. Most medicine did not solve completely. The water background was subtracted. (A) The spectrum of the birth control pill (active ingredient Drospirenone; brand name: Yasmin) with a spectral resolution of 2 cm^{-1}; (B) Panadol (active ingredient paracetamol; spectral resolution: 2 cm^{-1}); (C) Aspirin (active ingredient acetylsalicylic acid; spectral resolution: 2 cm^{-1}); (D) Vitamin C (spectral resolution: 1 cm^{-1}).

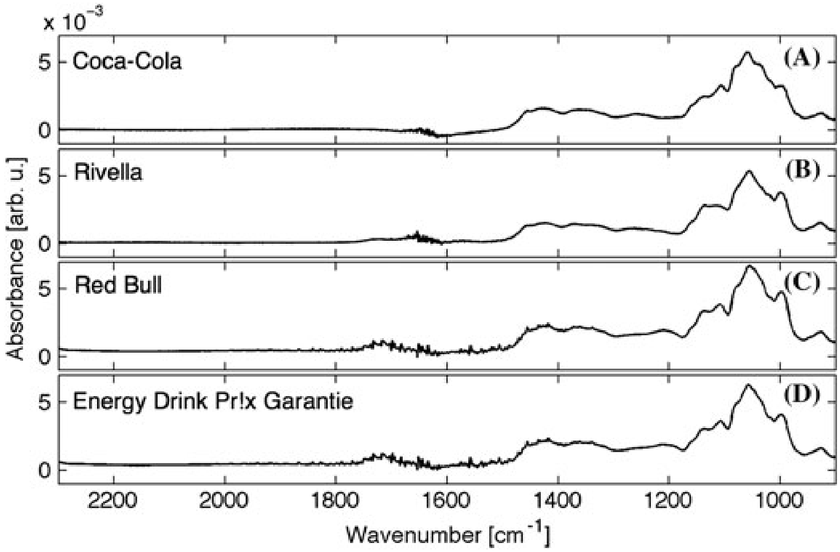

Figure 10. Spectra of common soft drinks with and without caffeine. The water background is subtracted. (A) spectrum of Coca-Cola (spectral resolution: 1 cm^{-1}); (B) Rivella (spectral resolution: 1 cm^{-1}); (C) Red Bull (spectral resolution: 2 cm^{-1}); and (D). a low cost energy drink (spectral resolution: 2 cm^{-1}).

Some sources claim that pain killers can be used as masking substance for disguising the abuse of drugs.[38] Figure 9 depicts the recorded spectra of the widely used pain killers Panadol with the active ingredient paracetamol and Aspirin with the active ingredient acetylsalicylic acid. In addition the spectrum of Vitamin C is shown as a component of many pain killers or cold medications. Finally the spectrum of a birth control pill with the active ingredient drospirenone (brand name: Yasmin) is plotted. Again the major absorption of this medication can be found between 1200 and 1000 cm^{-1}. It has to be taken into account that most medicine only dissolved poorly and therefore solid particles remained in the liquid forming a suspension. Hence, the same problems occurred as discussed previously in the paragraph on metabolites. Again, the major absorption of the medicine does not interfere spectrally with the cocaine absorption peaks between 1800 and 1700 cm^{-1}.

Since it is generally unknown what people may have consumed before the drug test, common soft drinks were analyzed as well. In Figure 10, the spectra of Red Bull, Coca-Cola, Rivella, and another energy drink are depicted. As expected, the major absorption features in these spectra origin from sugar absorptions between 1200 and 1000 cm^{-1} and therefore there is no spectral interference with the first two peaks of the cocaine spectrum in Figure 2.

Many other soft drinks, diluents and masking substances were analyzed, but since their major absorbance was not in the interval between 1800 and 1710 cm^{-1} their spectra are not depicted.

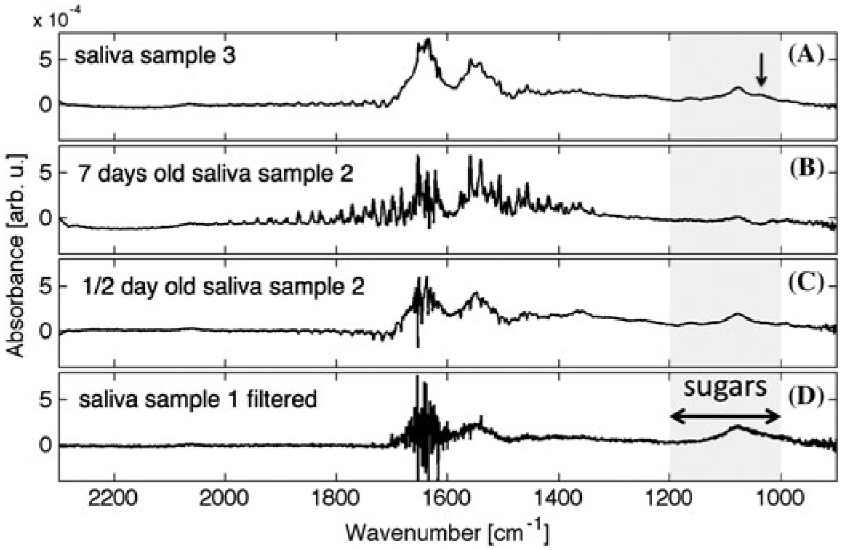

Figure 11. Infrared spectra of saliva from a fasting person acquired at different days and measured at different times. The spectra of saliva sample 1 ((D); spectral resolution: 1 cm^{-1}), sample 2 ((B) and (C); spectral resolution: 2 cm^{-1}) and sample 3 ((A); spectral resolution: 4 cm^{-1}) are shown. The different samples were acquired at different days but always on an empty stomach. (B) and (C) are depicted to emphasize the change in the spectrum over time. The vertical arrow shows a peak only occurring in one saliva spectrum and the horizontal arrow and the underlaid grey area mark the interval of the major absorbance of sugar molecules in solution. The water background was subtracted from all spectra.

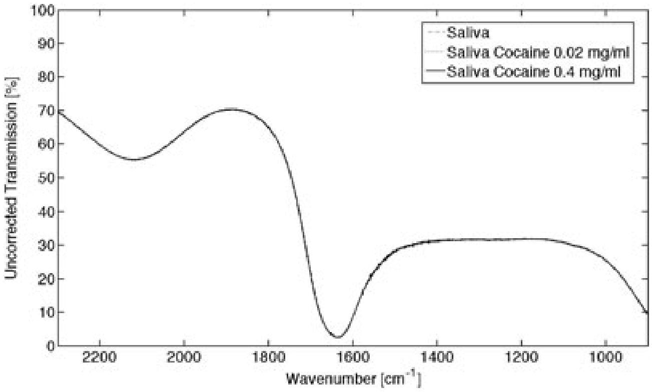

Figure 12. Uncorrected Transmission spectra of pure saliva from a fasting person and saliva spiked with cocaine. The graphs show the transmission spectrum of saliva (light grey dashed), of saliva with cocaine (0.02 mg/ml; black dotted) and of saliva spiked with cocaine (0.4 mg/ml; black). The graphs practically coincide, i.e. show no significant difference in the transmission pattern. The resolution of the FTIR was 1 cm^{-1}.

Saliva spectra

If human saliva is used as a biological matrix, interferences between the drug spectrum and the spectrum of pure saliva of the person under suspicion needs to be considered. Saliva from a fasting person was taken as the simplest saliva spectrum. Saliva samples were taken from volunteers. Figure 11 depicts saliva spectra of only one volunteer but even then small variations were found between different samples.

Repeating patterns are visible in the absorption spectra like, for example, the absorption of sugar between 1100 and 1000 cm^{-1} (Figure 6) emphasized by the horizontal arrow and the light grey background in Figure 11. The two broad peaks around 1650 and 1550 cm^{-1} are most likely due to proteins. However, clear differences in the absorption strength are visible at the wavelength ranges mentioned. Sometimes even additional absorption features appear like in the spectrum depicted in Figure 11A, emphasized by the vertical arrow. Major changes in the absorbance of saliva occur when saliva is stored for several days in the fridge as demonstrated with the second and third spectrum. The major changes are in the range where most likely sugar and protein absorption dominate. Proteins degenerate with time and naturally occurring bacteria in saliva might be linked with the reduction of the sugar content. A detailed study on the extent of variations in the infrared spectra of saliva taken from various human volunteers is the subject of further investigations.

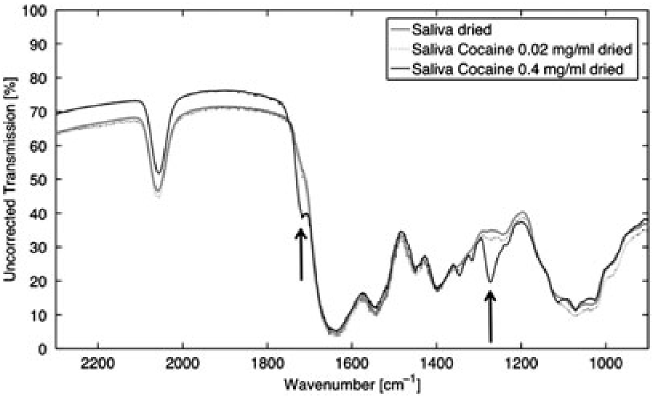

Figure 13. Uncorrected Transmission spectra of pure dried saliva from a fasting person and saliva spiked with cocaine. The graphs show the transmission spectrum of dried saliva (grey), of dried saliva with cocaine (0.02 mg/ml; black dotted) and of dried saliva spiked with cocaine (0.4 mg/ml; black solid). The characteristic change in transmission of the cocaine is now clearly visible (indicated by the arrows). The resolution of the FTIR was 1 cm^{-1}.

The limit of detection for cocaine in saliva is rather high since the strong water absorption is hindering a sensitive detection (Figure 12). This serious deficit of measuring cocaine in saliva can be partly overcome by evaporating the saliva and measuring the dried sample (Figure 13). A similar dried-sample-technique using transmission FTIR-spectroscopy to detect thiocyanate in saliva was used by Schultz *et al.*[36] Unfortunately, the improved detection limit for cocaine involves less accuracy in terms of quantity. This results in an only semi-quantitative analysis of the cocaine concentration in the chosen fluid because the saliva never dries in the same pattern on the ZnSe crystal. The limit of detection was estimated by comparing spectra of saliva spiked with cocaine and without cocaine. The lowest concentration of cocaine in saliva where the characteristic absorption pattern of cocaine is still visible is at a concentration of 0.02 mg/ml of cocaine (Figure 13). This LOD around 0.020 mg/ml represents a good first step in sensing cocaine in saliva since cocaine concentrations in saliva can get as high as 0.5 mg/ml after a single cocaine dose (40 mg cocaine base, smoked administration).[24]

It is obvious that in real life not all test persons have an empty stomach. In addition, the influence of cigarettes or mouthwashes on the composition of the saliva needs to be analyzed. We investigated the change in the infrared spectra of saliva under different circumstances. One example is the influence of the mouthwash Meridol on the saliva spectrum.

The test person was asked to give a saliva sample before rinsing the mouth with Meridol. Before acquiring the sample, the mouth was rinsed with water. Samples were extracted by spitting into a glass tube. After rinsing the mouth with the mouthwash the human subject was asked to rinse the mouth with water again and then give the second sample. The infrared spectra of both samples are depicted in Figure 14. The saliva spectrum of the fasting person is shown in Figure 14C, while Figure 14B represents the situation after the mouthwash. In Figure 14A, the spectrum of the mouthwash itself is depicted. The major absorption of the mouthwash Meridol is between 1200 and 1000 cm^{-1}. This is the spectral range in which the absorption of the middle grey spectrum increased the most. In addition, the absorbance is reduced between 1700 and 1500 cm^{-1}.

Since it is likely that protein absorption takes place in this spectral range, the remains of the mouthwash in the oral cavity might be conducive to thin the saliva of the test person and therefore decrease the protein content. The observed differences in the saliva spectra that are related to the mouthwash Meridol do not hinder cocaine detection in saliva because the major spectral interferences between cocaine, saliva, and Meridol occur between 1700 and 1000 cm^{-1}, but not between 1800 and 1710 cm^{-1}.

Another example for the various circumstances a person under suspicion might be is the influence of alcohol. It is likely that people that will be drug tested are under the influence of alcohol since these people draw special attention to themselves. Very often drugs are consumed alongside alcohol. It is possible to correlate the blood alcohol level with the alcohol level in saliva[39,40] which is why it is important to ensure that there are no interferences between the characteristic absorption of the saliva of a drunk person and the absorption of cocaine. Figure 15C depicts again the saliva spectrum of a fasting person. The mouth was again rinsed with water beforehand and the sample was obtained by having the test person spit into a tube. Figure 15B

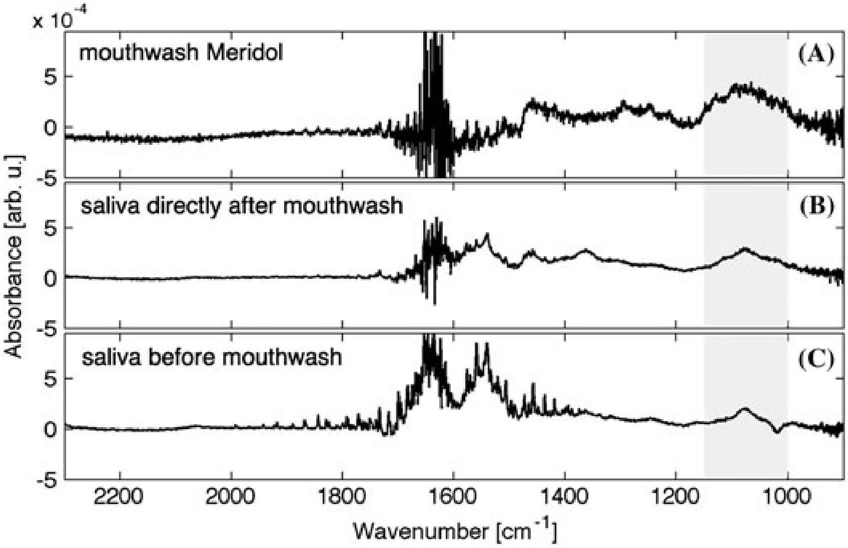

Figure 14. Spectrum of saliva from a fasting person (C) and after rinsing the oral cavity with Meridol (B). (A) The spectrum of the mouthwash Meridol itself is depicted. The major absorption of Meridol is marked with light grey background in all three graphs. The major change in the saliva spectrum after the rinsing is emphasized with the same grey background. The water background was subtracted from the spectra and the resolution of the FTIR was 1 cm^{-1}.

shows the saliva spectrum directly after the alcohol consumption and after rinsing the oral cavity with water. Figure 15A depicts the saliva spectrum 2 h after the alcohol consumption. It is evident that the amplitude of the characteristic alcohol peak has substantially decreased and that the interference of the sugar absorbance is gaining importance. This result demonstrates that alcohol can be detected in saliva by IR-ATR spectroscopy, including its temporal evolution which reflects the decrease in the blood alcohol level. No major interferences for the detection of cocaine in saliva occur in the important range between 1800 and 1710 cm^{-1}.

The influence of other substances (e.g. coffee and energy drinks) on the saliva spectrum were investigated as well. Their spectra are not depicted since no major interferences were recorded between 1800 and 1710 cm^{-1}. In addition, no further metabolites of the investigated or consumed substances could be found.

Conclusion

Our studies performed in aqueous solutions indicate that several important metabolites of cocaine can spectroscopically be accessed simultaneously as their characteristic absorption ranges coincide. The detailed investigation of spectral interferences by substances that are often consumed in combination with illicit drugs, notably diluents and adulterants, masking agents, common medications and drinks, suggests that the most appropriate spectral region for cocaine detection is between 1710 and 1800 cm^{-1}. As our first measurements on actual human saliva

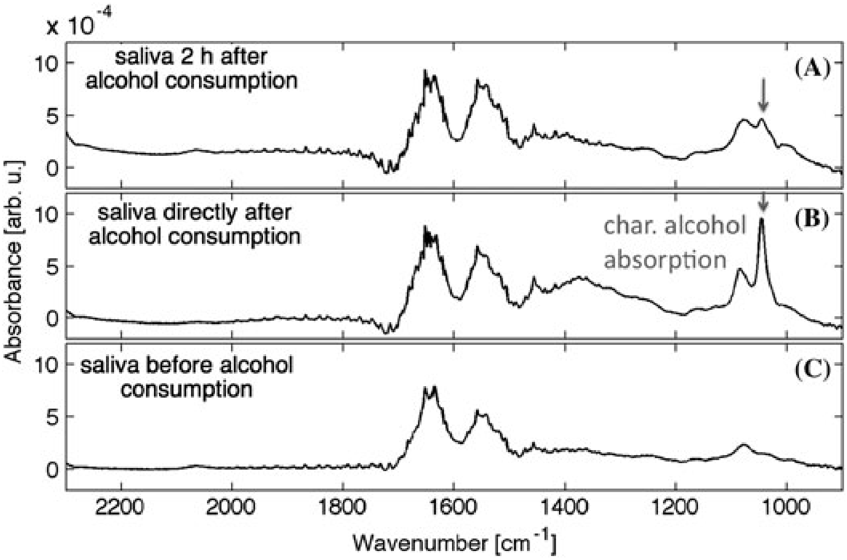

Figure 15. Infrared spectrum of saliva of a fasting person (C), directly after (B) and 2 h after (A) the consumption of alcohol. The characteristic alcohol absorption is marked with an arrow in the spectra. A change in the alcohol absorption is very well visible. The water background was subtracted from the spectra (spectral resolution: 4 cm^{-1}).

samples indicate, this statement is also true for saliva even though those spectra crucially depend on the time and condition of the sampling, this fact will be explored further with more test persons in the near future. The influence of mouthwash and of alcohol consumption on saliva spectra could clearly be observed, yet again without major interference in the important cocaine absorption range (1710 to 1800 cm^{-1}) mentioned above. The current limit of detection for cocaine in saliva using the dried sample technique (to decrease the influence of strong water absorption) is 0.02 mg/ml if the person is tested on an empty stomach. This is achieved without any further sample preparation such as separation or extraction procedures. Although this limit corresponds to concentrations actually occurring in real-life situations, it appears feasible to considerably lower it, for example, by using a quantum cascade laser as light source, a detector with higher responsivity and an improved design of the interaction zone between laser beam and sample resulting in an enhanced evanescent field absorption. A detection platform like described above could be useful for sensing other drugs in body fluids (e.g. heroin in saliva) as well.

Acknowledgements

The financial support by the Swiss government research initiative Nano-Tera (Project IrSens: Swiss National Science Foundation (SNSF), Project number: 20NAN1_123589) and ETH Zurich is gratefully acknowledged.

References

[1] P. Mura, C. Chatelain, V. Dumestre, J. M. Gaulier, M. H. Ghysel, C. Lacroix, *et al*. Use of drugs of abuse in less than 30-year-old drivers killed in a road crash in France: A spectacular increased for cannabis, cocaine and amphetamine. *Forensic Sci. Int.* 2006, *160*, 168.

[2] F. J. Couper, B. K. Logan. Drugs and Human Performance Fact Sheets. National Highway Traffic Safety Administration, Washington DC, 2004.

[3] G. Cooper, C. Moore, C. George, S. Pichini. Guidelines for European workplace drug testing in oral fluid. *Drug Test. Analysis* 2011, *3*, 269.

[4] C. V. Koulis, K. J. Hymes, J. L. Rawlins. A new infrared spectral library of controlled and noncontrolled drug standards using internal reflection spectroscopy. *J. Forensic Sci.* 2000, *45*, 876.

[5] C. V. Koulis, J. A. Reffner, A. M. Bibby. Comparison of transmission and internal reflection infrared spectra of cocaine. *J. Forensic Sci.* 2001, *46*, 822.

[6] M. López-Artíguez, A. Cameán, M. Repetto. Unequivocal identification of several common adulterants and diluents in street samples of cocaine by infrared spectroscopy. *J. Forensic Sci.* 1995, *40*, 602.

[7] A. Koçak, J. P. Lucania, S. L. Berets. Some advances in fourier transform infrared transflection analysis and potential applications in forensic chemistry. *Appl. Spectrosc.* 2009, *63*, 507.

[8] J. F. Casale. Detection of pseudoecgonine and differentiation from ecgonine in illicit cocaine. *Forensic Sci. Int.* 1990, *47*, 277.

[9] K. Kawase, Y. Ogawa, H. Minamide, H. Ito. Terahertz parametric sources and imaging applications. *Semicon. Sci. Technol.* 2005, *20*, S258.

[10] A. Holmgren, P. Holmgren, F. C. Kugelberg, A. W. Jones, J. Ahlner. Predominance of illicit drugs and poly-drug use among drug-impaired drivers in Sweden. *Traffic Inj Prev* 2007, *8*, 361.

[11] E. Gallardo, M. Barroso, J. A. Queiroz. LC-MS: A powerful tool in workplace drug testing. *Drug Test. Analysis* 2009, *1*, 109.

[12] W.-L. Wang, W. D. Darwin, E. J. Cone. Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. *J. Chromatogr. B: Biomed. Appl.* 1994, *660*, 279.

[13] M. Concheiro, A. de Castro, Ó. Quintela, A. Cruz, M. López-Rivadulla. Confirmation by LC-MS of drugs in oral fluid obtained from roadside testing. *Forensic Sci. Int.* 2007, *170*, 156.

[14] P. Fernández, L. Morales, C. Vázquez, A. M. Bermejo, M. J. Tabernero. HPLC-DAD determination of opioids, cocaine and their metabolites in plasma. *Forensic Sci. Int.* 2006, *161*, 31.

[15] A. D. Burnett, H. G. M. Edwards, M. D. Hargreaves, T. Munshi, K. Page. A forensic case study: the detection of contraband drugs in carrier solutions by Raman spectroscopy. *Drug Test. Analysis* 2010. DOI: 10.1002/dta.169

[16] C. Shende, F. Inscore, P. Maksymiuk, S. Farquharson. Ten-minute analysis of drugs and metabolites in saliva by surface-enhanced Raman spectroscopy. *Proc. SPIE* 2005, *6007*, 60070S.

[17] W. Sweeney, L. R. Goldbaum, N. T. Lappas. Detection of benzoylecgonine urine by means of UV spectrophotometry. *J. Anal. Toxicol.* 1983, *7*, 235.

[18] R. A. Ogert, A. W. Kusterbeck, G. A. Wemhoff, R. Burke, F. S. Ligler. Detection of cocaine using the flow immunosensor. *Anal. Lett.* 1992, *25*, 1999.

[19] P. J. Devine, N. A. Anis, J. Wright, S. Kim, A. T. Eldefrawi, M. E. Eldefrawi. A fiber-optic cocaine biosensor. *Anal. Biochem.* 1995, *227*, 216.

[20] J. M. Walsh, R. Flegel, D. J. Crouch, L. Cangianelli, J. Baudys. An evaluation of rapid point-of-collection oral fluid drug-testing devices. *J. Anal. Toxicol.* 2003, *27*, 429.

[21] V. Y. Popov, V. V. Lavrent'ev. Role of the penetration depth in perturbed total internal reflection spectroscopy. *Zhurnal Prikladnoi Spektroskopii* 1980, *32*, 336.

[22] H. D. Downing, D. Williams. Optical constants of water in the infrared. *J. Geophys. Res.* 1975, *80*, 1656.

[23] NIST National Institute of Standards and Technology. Available at: http://webbook.nist.gov/chemistry/ 2009 May 11.

[24] A. J. Jenkins, J. M. Oyler, E. J. Cone. Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. *J. Anal. Toxicol.* 1995, *19*, 359.

[25] Sigma-Aldrich. The Aldrich Library of FT-IR Spectra, 2nd Edn, Vol. *3*. Aldrich, Milwaukee, WI (USA), 1997.

[26] L. A. King. Drug content of powders and other illicit preparations in the UK. *Forensic Sci. Int.* 1997, *85*, 135.

[27] I. Barni Comparini, F. Centin, A. Pariali. Simultaneous determination of narcotics, adulterants and diluents in street samples by means of gas chromatography with capillary columns. *J. Chromatogr, A* 1983, *279*, 609.

[28] M. J. West, M. J. Went. Detection of drugs of abuse by Raman spectroscopy. *Drug Test. Analysis* 2010. DOI: 10.1002/dta.217

[29] R. J. Keller (ed.). The Sigma Library of FT-IR Spectra Edition I. Sigma Chemical Company, Inc., Milwaukee, WI (USA), 1986.

[30] E. J. Cone, M. Hillsgrove, W. D. Darwin. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. *Clin. Chem.* 1994, *40*, 1299.

[31] R. Jufer, S. L. Walsh, E. J. Cone, A. Sampson-Cone. Effect of repeated cocaine administration on detection times in oral fluid and urine. *J. Anal. Toxicol.* 2006, *30*, 458.

[32] E. T. Moolchan, E. J. Cone, A. Wstadik, M. A. Huestis, K. L. Preston. Cocaine and metabolite elimination patterns in chronic cocaine users during cessation: Plasma and saliva analysis. *J. Anal. Toxicol.* 2000, *24*, 458.

[33] R. Dams, R. E. Choo, W. E. Lambert, H. Jones, M. A. Huestis. Oral fluid as an alternative matrix to monitor opiate and cocaine use in substance-abuse treatment patients. *Drug Alcohol Depend.* 2007, *87*, 258.

[34] M. Freda, A. Piluso, A. Santucci, P. Sassi. Transmittance fourier transform infrared spectra of liquid water in the whole mid-infrared region: Temperature dependence and structural analysis. *Appl. Spectrosc.* 2005, *59*, 1155.

[35] W. Schmidbauer, J. vom Scheidt. Handbuch der Rauschdrogen. Fischer, Frankfurt am Main, 1998.

[36] C. P. Schultz, M. Khalique Ahmed, C. Dawes, H. H. Mantsch. Thiocyanate levels in human saliva: Quantitation by fourier-transform infrared spectroscopy. *Anal. Biochem.* 1996, *240*, 7.

[37] J. Chance Carter, W. E. Brewer, S. M. Angel. Raman spectroscopy for the in situ identification of cocaine and selected adulterants. *Appl. Spectrosc.* 2000, *54*, 1876.

[38] J. Gombos. The Drug Testing FAQ - Fooling the Bladder Cops. 1996. Available at: http://cocaine.org/drugtestfaq/index.html 2009 July 09.

[39] J. Ruz, A. Fernandez, M. D. Luque de Castro, M. Valcarcel. Determination of ethanol in human fluids. II. Determination of ethanol in urine, breath and saliva. *J. Pharmaceut. Biomed. Anal.* 1986, *4*, 559.

[40] L. C. Degutis, R. Rabinovici, A. Sabbaj, R. Mascia, G. D'Onofrio. The saliva strip test is an accurate method to determine blood alcohol concentration in trauma patients. *ACAD Emerg. Med.* 2004, *11*, 885.