

Comparison of rapid detecting optical techniques for the identification of New Psychoactive Substances in ‘Legal High’ preparations

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Aims: Since 2008, new classes of designer drugs became more important in Germany. Substances and substance mixtures aiming at imitating classic drug effects are synthesized primarily in China and sold in preparations claiming to be e. g. bath salts, incense blends, room refreshers or as pure substances referred to as ‘research chemicals’. The substances, like aminoalkylindoles, piperazines or synthetic cathinone derivatives are called New Psychoactive Substances (NPS) and, in preparations, labelled ‘Legal Highs’. They are sold mainly via Online-Shops as ‘legal’ and ‘harmless’ products. A significant fraction of illicitly marketed NPS are scheduled in the German Narcotics Act (BtmG). Unfortunately, due to a decision of the European Court of Justice in 2014, newly surfacing non-listed substances cannot be treated as functional pharmaceuticals as defined in the medicines law anymore which makes it even more important to discriminate different substances in the same type of products. A safe presumptive detection method is required because the differentiation is necessary in field analysis of seized material in customs, traffic checks etc. We focused on the examination of two different field-suitable optical techniques with incomplete sample preparation requirements and high identification potential: Raman and infrared spectroscopy. **Methods:** Seized samples containing NPS were analysed with a portable Attenuated Total Reflection Infrared-Spectrometer (ATR-IR) system and different handheld Raman spectrometers from several manufacturers. **Results and Discussion:** NPS could be detected with portable spectrometers in authentic samples despite low concentrations and inhomogeneous distribution of substances in the matrix. Limits of detection and quality of the identification of the substances varied between both techniques. Different dark-coloured matrices like herbals caused problems concerning the excitation efficiency of the Raman laser. **Conclusion:** Combinations of portable Raman and infrared devices allow a reliable pre-analysis of NPS in ‘Legal High’ products and as pure substances. Infrared spectroscopy is more suitable for analysis of herbal blends and Raman spectroscopy can be used as a non-invasive technique due to its applicability on glass and plastic packaging.

1. Introduction

Rapid identification of an unknown substance outside of controlled laboratory environments is one of the most important challenges in forensics. Unknown samples may appear for example in the context of seizures at custom offices, at crime scene investigations and after seizure of clandestine drug laboratories. Despite traditional laboratory techniques such as GC-MS [1] are able to identify a huge number of substances, they require time-consuming sample preparation and a laboratory environment. In field, rapid and reliable pre-testing and identification of unknown substances is necessary to avoid hazards (e.g. toxic, explosive and health-damaging substances) for action forces as well as civilians and to get an initial hint for prosecution (narcotics, precursors).

Recently, classic narcotic substances have been complemented by an increasing number of newly synthesized substances, called ‘New Psychoactive Substances’ (NPS), imitating the forms of appearance and consume as well as the psychotropic effects of known drugs. The product range of NPS varies from pure substances referred to as ‘*Research Chemicals*’, to so called ‘*Legal Highs*’, describing ‘herbal blends’, ‘bath salts’ or ‘room refreshers’. All of these products are sold mainly via the internet [1].

As long as a NPS is not listed in the annexes (typically annex 2) of the German Narcotics Act (BtMG) its possession can be considered as legal [2]. In Germany, only a substance with known structure can be explicitly listed and controlled by law. This circumstance motivates the producers, predominantly located in China, to synthesize a large number of new substances. In many cases only a hydrogen atom of an existing substance is replaced by a halogen atom to generate a new, potent psychotropic substance (Fig. 1).

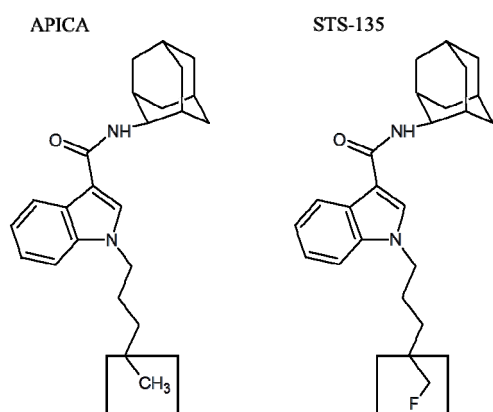


Fig. 1. Creation of a new substance through substitution. The substance STS-135 [*N*-(1-adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide], right-hand side] is evolved from the substance APICA [*N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide], left-hand side] via substitution of a hydrogen atom through a fluorine atom. Both substances have to be listed separately in Annex II of German Narcotic Act (BtMG).

The constantly growing number of new substances [3], the complexity of distribution pathways and the different forms of consumption cause new challenges for forensic science.

Number of new psychoactive substances reported to the EU Early Warning System, 2005–14

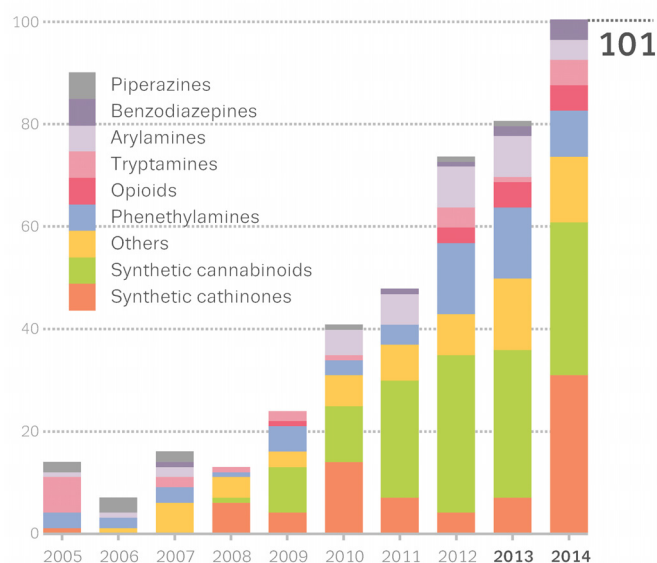


Fig. 2. EMCDDA statistics about the prevalence of NPS in Europe: The bars show the different substance classes and the number of newly reported substances within the EWS (European Early Warning System) per year. Especially the number of synthetic cannabinoids and cathinones is increasing tremendously from year to year [3].

An ideal pre-testing technique provides fast and reliable identification with a non-invasive simple sample-preparation. Techniques that are adequate for these demands can be located in laboratories, e.g. ambient MS techniques like *Desorption Electrospray Ionisation* (DESI) - or *Direct Analysis in Real Time* (DART) –Mass spectrometry [4], or be used in field like portable and handheld devices. Several systems have been fitted to in-field standards, Raman and FT-Infrared (IR) spectroscopy and Ion-mobility-spectrometry (IMS) to be mentioned in the first instance. Especially the optical techniques have been improved a lot in the last years.

FT-IR and Raman spectroscopy require little or no sample preparation and are able to deliver specific (structural) information about a substance or a mixture. In this study we show how FT-IR and Raman spectroscopy are applicable for the rapid identification and detection of NPS as pure substance and in authentic samples.

2. Material and Methods

2.1. Samples

Synthetic cannabinoids and cathinones were measured as pure substances to create reference spectra for the libraries or to compare different laser excitation wavelengths (Raman). Bath salts and herbal blends were measured as authentic samples from case work. Due to heating and burning by laser absorption, herbal blends were not measured using Raman spectroscopy. Measurement of herbal blends using FT-IR spectroscopy requires a fast extraction as pre-treatment [5].

Sample preparation: Herbal blends were prepared for the FT-IR measurement via the following scheme:

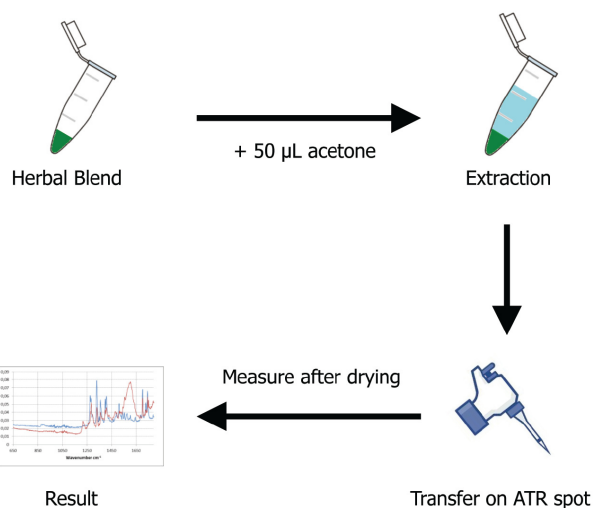


Fig. 3. Pre-Treatment for herbal blends (FT-IR): A small amount of the sample is placed in a reaction tube and $\sim 50 \mu\text{L}$ of acetone are added. After short shaking the extraction supernatant is transferred on the ATR diamond spot and measured after drying.

2.2. Portable/Handheld spectroscopic devices

FT-IR spectrometer: A portable FT-IR spectrometer (HazMatID™, Smiths Detection, Watford, UK), working with Attenuated Total Reflection (ATR) has been used to record the spectra. The sample was either pressed onto the ATR diamond cell with a mounting for direct analysis or pipetted on the spot and analysed after drying in case of acetone pre-extraction.

Raman spectrometer: Three Raman spectrometers have been used in this study with two different laser wavelengths. All devices had handheld size and could be used in point-and-shoot mode. The following table shows the technical specifications of three devices:

Tab.1. Technical Details of the Raman Handheld Devices.

| | Mira M-1 | Mira M-2 | Progeny™ |
|------------------------|-------------------------------------|-------------------------------------|-----------------------------|
| Producer | Metrohm, Filderstadt, Germany | Metrohm, Filderstadt, Germany | Rigaku, Sevenoaks, UK |
| Wavelength | 785 nm | 1064 nm | 1064 nm |
| Max. Laser Power | 75 mW | 400 mW | 490 mW |
| Scan Range | 400 - 2300 cm ⁻¹ | 400 - 2300 cm ⁻¹ | 200 - 2500 cm ⁻¹ |
| Resolution (FWHM) | 12 - 14 cm ⁻¹ | 12 - 14 cm ⁻¹ | 8 - 11 cm ⁻¹ |
| Detector | Linear CCD Array | Cooled InGaAs | Cooled InGaAs |
| Measuring Technique | Orbital Raster Scan (ORS) | Orbital Raster Scan (ORS) | Focal |

The spectra were processed using the software Panorama (Labcognition, Cologne, Germany) and displayed using Spekwin32 [6].

3. Results and Discussion

3.1. Raman spectroscopy

Quality of the detection and identification of several NPS using Raman spectroscopy was dependent of the applied laser excitation wavelength. Systems using 785 nm as excitation wavelength generate more intense signals due to the higher sensitivity of CCD detectors for that spectral range and the dependence of the intensity of Raman scattering from the wavelength [7] (Fig. 4, 8 and 9).

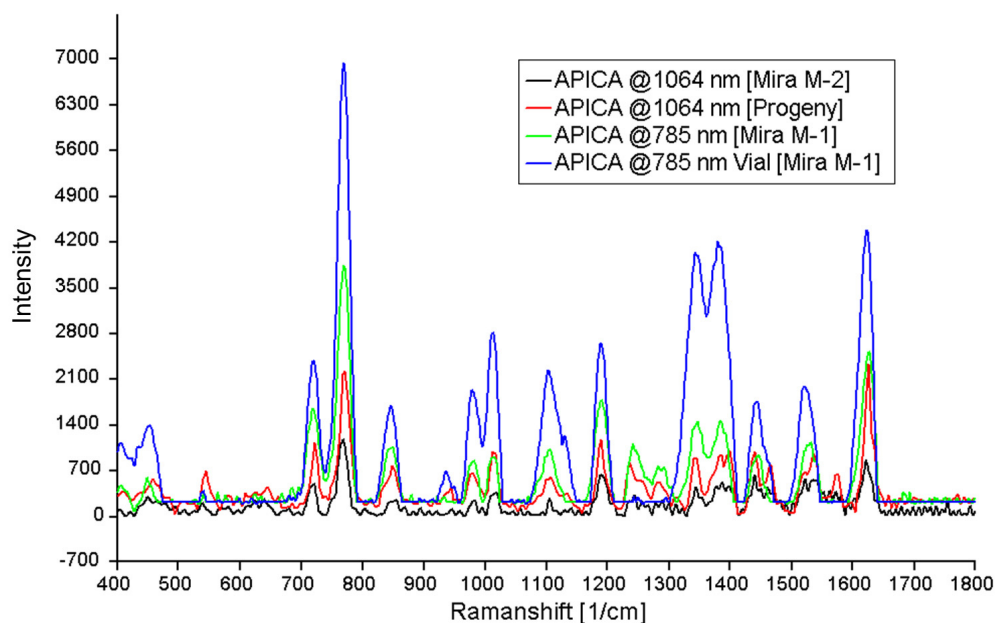


Fig. 4. The synthetic cannabinoid APICA as pure substance recorded with two different wavelengths (1064 nm and 785 nm) and three Raman devices devices (in brackets: Mira M-1, Mira M-2, Progeny). One spectrum ('Vial', green) was recorded inside the Vialholder. Spectra recorded with 785 nm show more intense signals due to higher detector sensibility. This substance does not cause fluorescence effects. Varieties between the spectra result from different spectral resolution and post-processing effects.

For some substances, the 785 nm wavelength can induce intense fluorescence during excitation. Due to the higher intensity of fluorescence signals, the weaker Raman signals can be suppressed. Excitation with 1064 nm can avoid this problem because this wavelength induces significantly less fluorescence [8] (Fig. 5, 6, 7, 9 and 10).

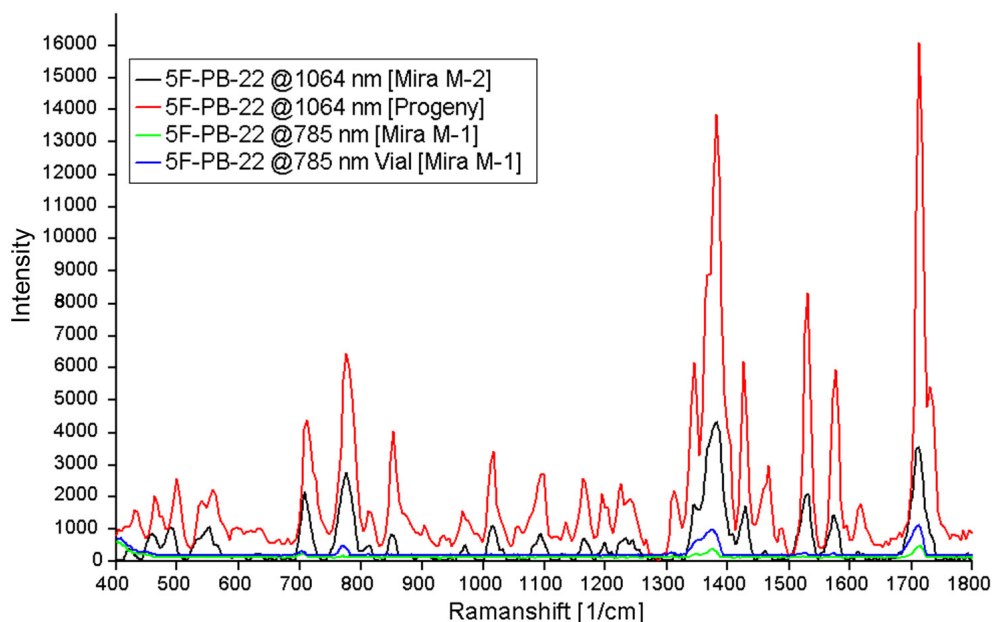


Fig. 5. The synthetic cannabinoid 5F-PB-22 as pure substance recorded with two different wavelengths (1064 nm and 785 nm) and three Raman devices (in brackets: Mira M-1, Mira M-2, Progeny). Spectra recorded with 1064 nm show more signals due to fluorescence effects at 785 nm. Varieties between the 1064 nm spectra result from different spectral resolution and post-processing effects.

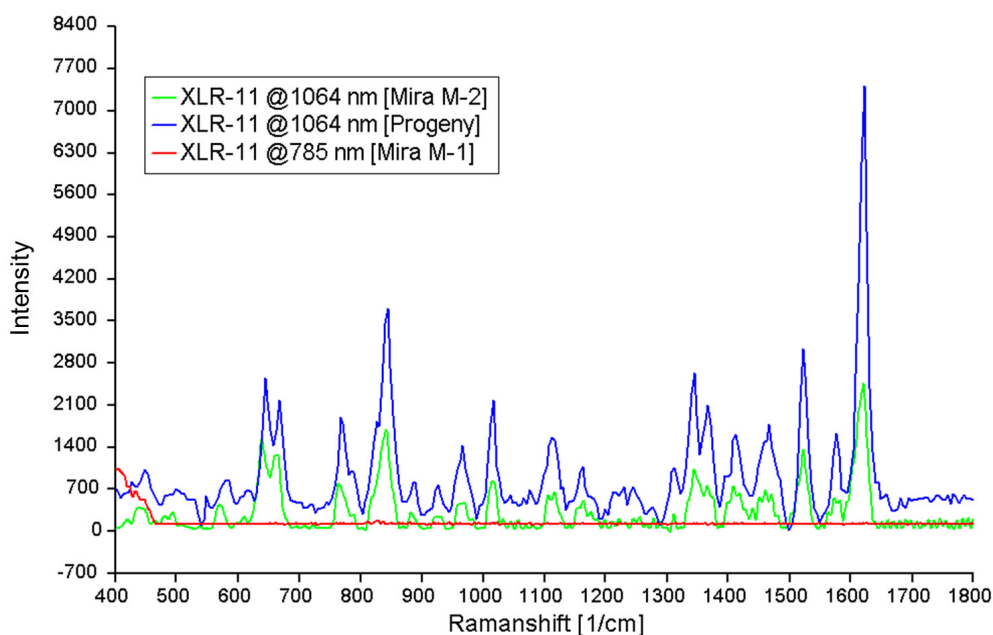


Fig. 6. The synthetic cannabinoid XLR-11 as pure substance recorded with two different wavelengths (1064 nm and 785 nm) and three Raman devices (in brackets: Mira M-1, Mira M-2, Progeny). Only spectra recorded with 1064 nm show enough signal for identification due to fluorescence effects at 785 nm. Varieties between the 1064 nm spectra result from different spectral resolution and post-processing effects.

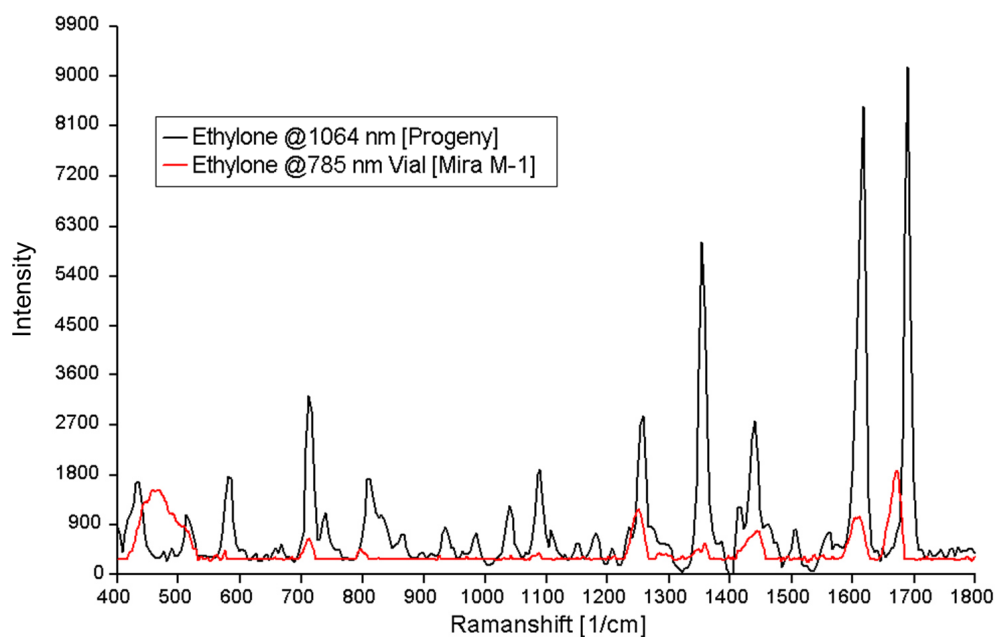


Fig. 7. The synthetic cathinone ethylone as pure substance recorded with two different wavelengths (1064 nm and 785 nm) and two Raman devices devices (in brackets: Mira M-1, Progeny). Spectra recorded with 1064 nm present more signals due to fluorescence effects at 785 nm.

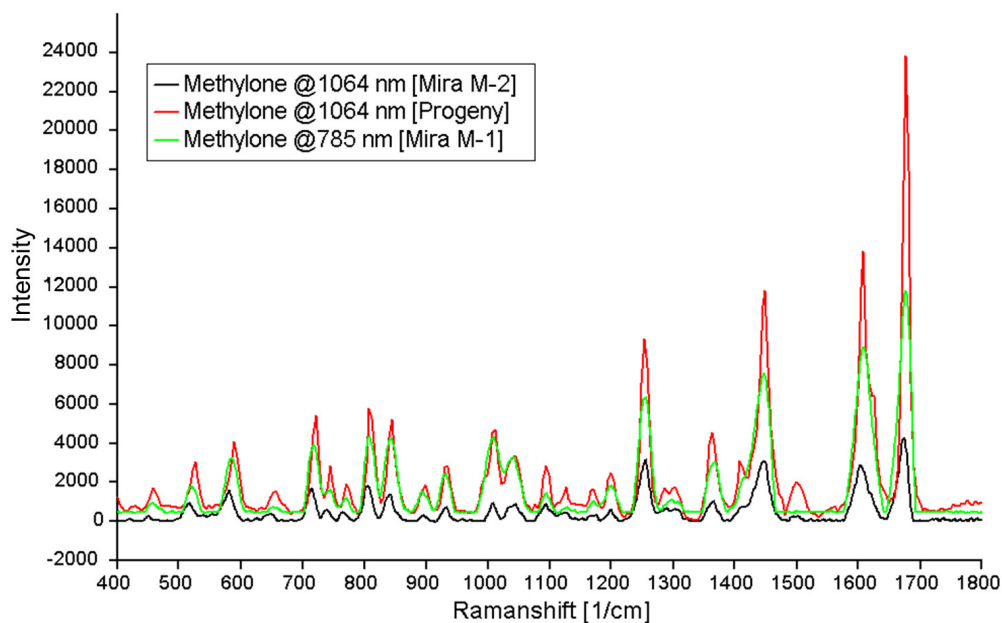


Fig. 8. The synthetic cathinone methylone as pure substance recorded with two different wavelengths (1064 nm and 785 nm) and three Raman devices (in brackets: Mira M-1, Mira M-2, Progeny). Spectra recorded with 1064 nm (Progeny) and 785 nm are comparable. Variations between the 1064 nm spectra result from different spectral resolution and post-processing effects.

Despite fluorescence, post processing of raw data can cause identification problems as well. Most handheld systems use automatic baseline correction or other algorithms for spectrum correction. This can lead to deletion of small signals in case of bad signal-to-noise ratios.

Application of Raman spectroscopy on herbal blends is not possible because the dark plant material absorbs too much energy of the laser beam leading to altering of the sample.

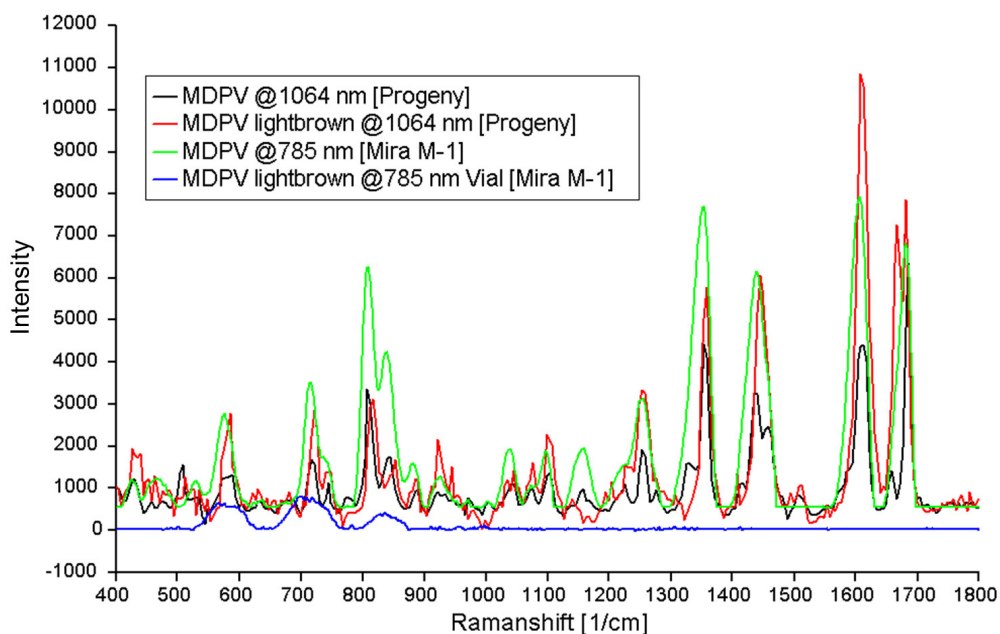


Fig. 9. The synthetic cathinone MDPV as pure substance and mixed with dye, recorded with two different wavelengths (1064 nm and 785 nm) and three Raman devices (in brackets: Mira M-1, Mira M-2, Progeny). One spectrum ('Vial', blue) was recorded inside the vial holder. Spectra of the pure substance recorded with 785 nm exhibit more intense signals due to higher detector sensibility. This substance does not cause fluorescence effects. In case of the dyed substance (lightbrown), the additive causes high fluorescence which leads to signal suppression (blue) at 785 nm. Variations between the spectra result from different spectral resolution and post-processing effects.

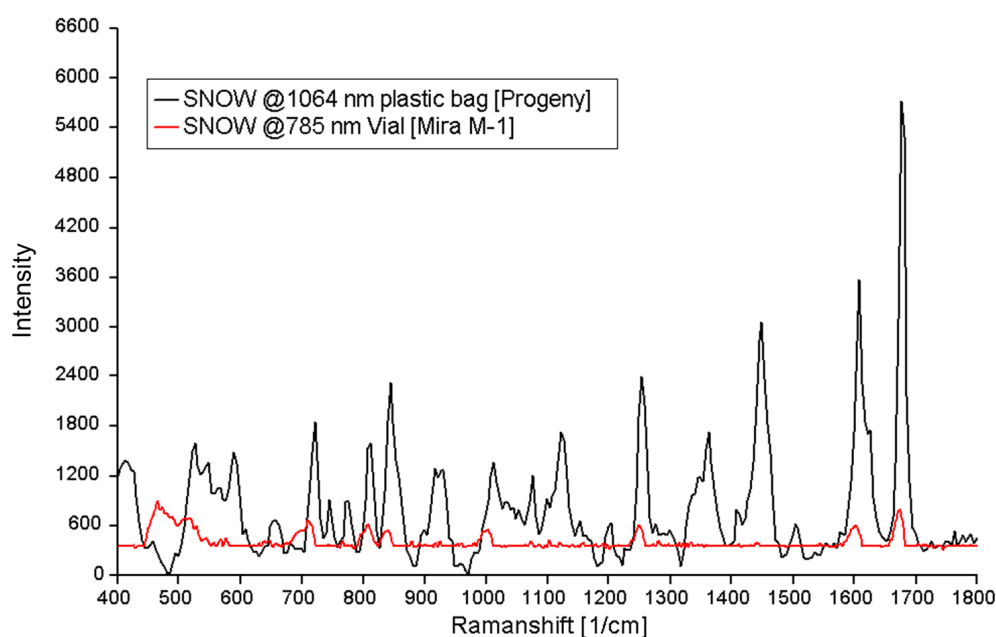


Fig. 10. The bathsalt mixture 'SNOW', containing lidocaine and methylone, recorded with two different wavelengths (1064 nm and 785 nm) and two Raman devices (in brackets: Mira M-1, Progeny). Spectra recorded with 1064 nm contain more signals to identify methylone as active component, due to fluorescence effects at 785 nm.

3.2. FT-Infrared spectroscopy

FT-Infrared spectroscopy can be used to identify two or more components in different concentrations in a mixture via spectrum subtraction. After recording the main spectrum, one identified component spectrum e.g. that of a cutting agent like caffeine in high concentration, can be subtracted from the original spectrum. A second library search using the so-called residual spectrum can yield a second, lower concentrated component. This procedure can be repeated as long as there is enough spectral information left in the residual spectrum (Fig. 11 and 12).

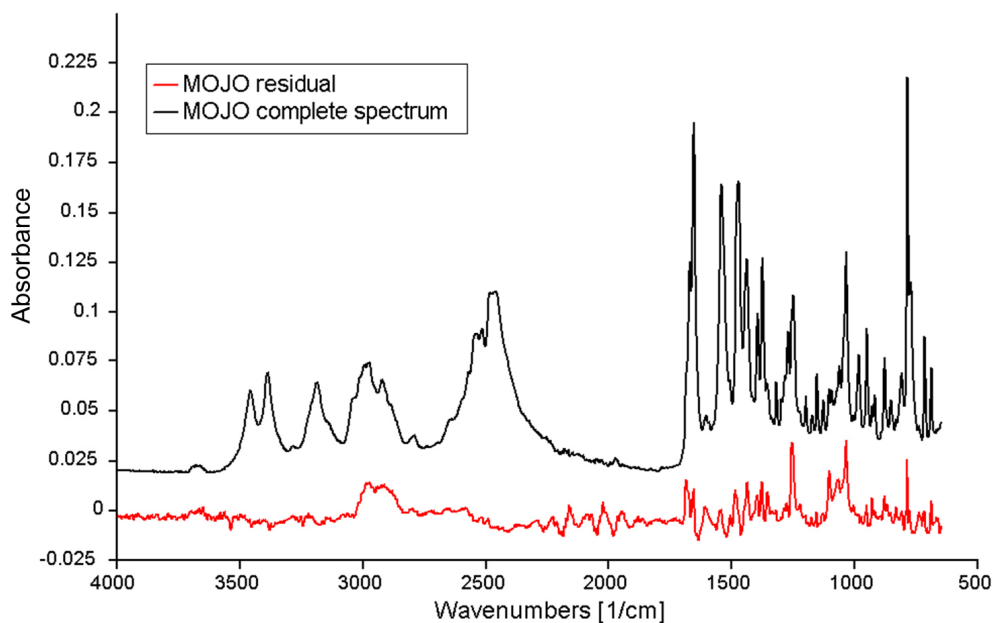


Fig. 11. Complete and residual FT-IR spectrum of the bath salt product ‚MOJO‘, containing lidocaine and MDPV. The complete spectrum (black) yields lidocaine as main component (library search). Subtraction of the lidocaine reference spectrum leads to the residual spectrum (red).

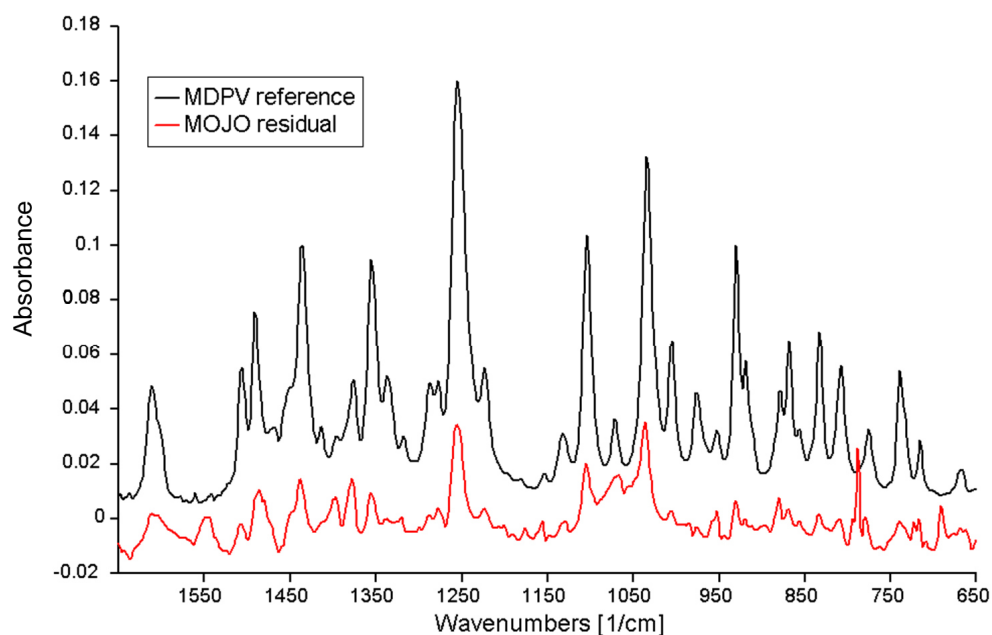


Fig. 12. Fingerprint section of the ‚MOJO‘ residual spectrum (red). Second library search yields MDPV (black) as second minor component.

Analysis of herbal blends can be performed using Infrared spectroscopy. Due to low concentrated NPS in herbal blends and inhomogeneous mixing, it is necessary to pre-treat the samples (as shown Fig. 3) to concentrate the active agent. Using this fast extraction the fingerprint area can be resolved (Fig. 13).

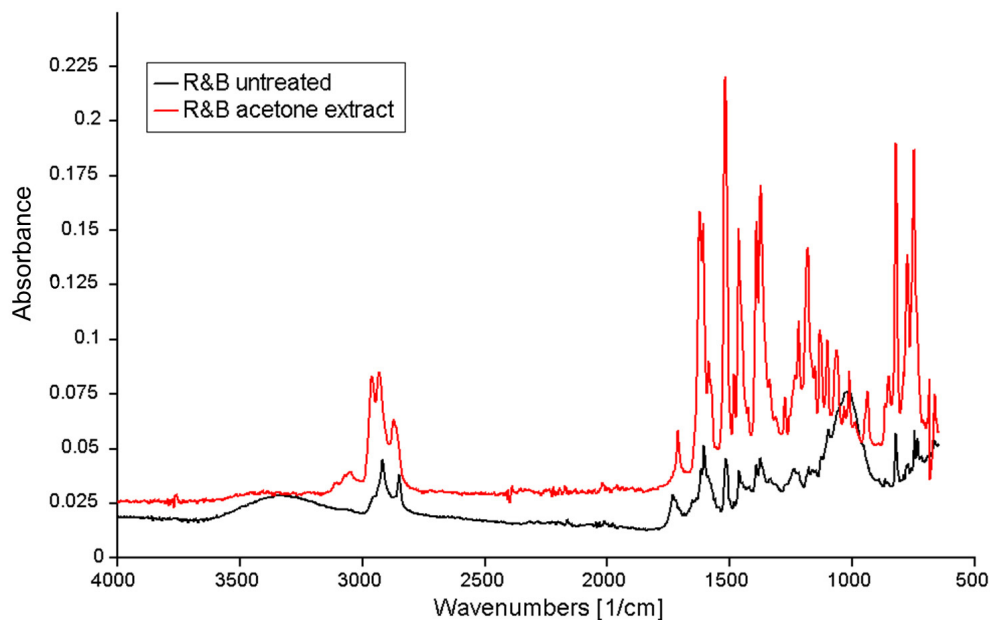


Fig. 13. Two spectra of the herbal blend 'R&B', recorded without (black) and after acetone extraction (red). The fingerprint region is partly overlaid due to matrix effects of the herbal blend, an identification of the active substance is not possible. After acetone extraction the fingerprint region is resolved and JWH-210 can be identified as active synthetic cannabinoid.

4. Conclusions

In-field identification of unknown substances is a challenge in forensic sciences. Here we show that several fast detecting techniques promise to offer fast and reliable results without complex sample preparation requiring a laboratory environment. Most established tests like immunoassay-based drug tests are not applicable to NPS due to the fast growing and changing of the substances in this field. Optical techniques provide structural information about a substance and, due to an expandable library, can be applied on a huge range of substances.

Raman spectroscopy is a technique which can be applied on liquid, including aqueous, and solid samples. Raman spectroscopy allows to measure samples in packaging like glass bottles and plastic bags without any further sample preparation. Dark-coloured samples like herbal blends or dyed preparations may cause problems due to absorption and sample decomposition. Low concentrated substances may cause problems in detection as well, this problem can be handled using spectrum subtraction or mixture analysis but this option is pending in most of the handheld systems.

(FT-)Infrared spectroscopy can be used for a wide range of different samples. Multiple components can be identified using spectrum subtraction even in lower concentrations. Herbal blends can be analysed via pre-extraction of the active substance. Aqueous samples cause severe problems due to a spectrum overlay of water. FT-IR spectroscopy requires removal of a small part of the samples and cannot be applied on packed samples.

Combination of both techniques, Raman and Infrared spectroscopy, can be used best to cover the expanding field of narcotics and NPS. In case of Raman spectroscopy it is recommendable to focus on 1064 nm systems because a lot of NPS cause fluorescence with 785 nm systems. Using only one technique requires expertise of the operator regarding the scopes of Raman and Infrared spectroscopy (Tab. 2).

Tab. 2. Applicability of FT-IR and Raman spectroscopy on *Legal High products* and *Research Chemicals*.

| | FT-IR | Raman (@785 nm) | Raman (@1064 nm) |
|---------------------------------|-------|-----------------|------------------|
| Research Chemicals | ++ | - | ++ |
| Bath Salts | + | + | + |
| Herbal Blends | + | -- | -- |
| Liquid Samples | - | + | + |
| Packed Samples (plastic, glass) | -- | ++ | ++ |

5. Acknowledgements

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6. References

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