Analysis of 'legal high' substances and common adulterants using handheld spectroscopic techniques

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Abstract

The identification of 'legal highs' is challenging as they often do not match their label claim and contain a wide range of impurities and/or adulterants. In addition, there is a need for techniques to be on-site, rapid and non-destructive. The feasibility of using the in-built algorithms of handheld near-infrared (NIR), Raman and attenuated total reflectance Fourier transform-infrared (ATR-FT-IR) spectroscopy for the identification of 'legal high' substances was investigated. Spectral libraries were constructed using three substances found in 'legal highs' (i.e., dextromethorphan, 2-aminoindane and lidocaine) and their 50:50 mixtures with caffeine. Model dilution mixtures with caffeine (i.e., 5 – 95% m/m) and seven 'legal high' Internet products were used to test the method. The 'legal high' constituents in most of the model mixtures were identified within a minimum range of 30 – 60% m/m for NIR, 20 – 75% m/m for Raman, and 41 – 85% m/m for ATR-FT-IR. This demonstrates that simple library mixtures could be used to identify test substances when the concentrations are variable. Below and above these levels, the test mixtures often correlated to the component in higher concentration. Collectively, the instruments identified the main constituents in the seven Internet products with varying correlation criteria. The NIR and ATR-FT-IR provided complementary information compared to Raman when carbohydrate cutting agents were added to the product, yet the Raman showed a high fluorescence signal for three products hindering identification. These initial studies indicate the suitability of three complementary techniques for rapid identification of 'legal high' products. Further development of spectral libraries, algorithms, and use of alternative Raman excitation wavelengths is needed to provide adequate tools for in-field analysis by non-experts.

Keywords: 'legal high', novel psychoactive substances, Raman, infrared, near infrared, handheld

1. Introduction

'Legal highs' or new psychoactive substances (NPS) are drugs of abuse (i.e., initially legal) that intend to mimic the effects of illegal drug substances. They include analogues of well-established drugs of abuse, psychoactive substances researched in the past, and pharmaceutical substances that are newly abused [1]. Since 2005, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has investigated over 300 NPS [2]. These products are easily accessible via the Internet, labelled with attractive names such as 'Trip' and 'Blurberrys', and advertised as 'research chemicals', 'bath salts' and/or 'not for human consumption' to surpass controlled drug regulations [3]. As they are advertised as legal alternatives to cocaine and ecstasy, they are often perceived as safe; however, there remains limited information on their pharmacology. In addition, they are often taken in combination with other drugs/alcohol or in repetitive doses within a short timeframe [4-6]. According to Corkery *et al.*, the reported deaths cases in the UK in which an NPS was implicated in a death rose from 10 in 2009 to 68 in 2012 [7].

A key issue associated with these substances is that they often do not comply with their label claim, but contain a wide range of drug mixtures and/or impurities that may or may not be psychoactive [3,8, 9-21]. This could result in dangerous consequences, such as neurotoxicity, when new derivatives are taken with other stimulants as cocktails [22]. In addition, the consumption of unknown psychoactive substances can impact on treatment. At present, there are no established protocols for treating those with NPS intoxications [23]. This is, in part, as a result of the varied chemical composition of these products, and the fact that little is known about the chronic use of these substances. In an effort to identify ambiguous new drugs such as these, samples have been collected from both patients admitted to hospital [8] and Amnesty bin samples [6]. In these circumstances, samples were transported from the hospital to laboratories for chemical analysis. This process is often time consuming and does

not give immediate feedback to those who would benefit in the field. This stimulates the need for simple and rapid techniques for the identification of these products where they are encountered.

Currently, the most common techniques used for analysis of NPS include wet chemical techniques such as chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy and hyphenated techniques (e.g., gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry) [3,8, 9-21, 24-26]. From these analyses, common adulterants identified in products have included stimulants such as caffeine; anaesthetics such as lidocaine, benzocaine and procaine; and inactive materials such as cellulose and talc to increase powder bulk.

There are limited studies on the use of laboratory-based solid-state techniques such as attenuated total reflectance Fourier transform-infrared (ATR-FT-IR) and Raman spectroscopy for the identification of 'legal high' products. Mainly, they have been used in conjunction with wet laboratory techniques to identify substances such as the phenethylamine 2C-N [27], 5,6-methylenedioxy-2-aminoindane (MDAI) [10], and the hydrochloride salts of buphedrone and pentedrone [28]. The use of Raman spectroscopy as an initial screening tool for NPS was investigated using (3,4-methylenedioxy)amphetamine [29], β-ketophenethylamines [30] and cathinone regioisomers [31], and showed promise for distinguishing drug analogues in seized samples. The examples above have been evaluated for lab-based instruments, which are not designed for in-field use.

Handheld instruments offer the advantage of carrying the laboratory to the sample. For example, electrochemical approaches are often a promising area for in-field detection as these techniques are readily transferred from the lab to the field. To this end, the use of a dropping mercury electrode was shown to detect mephedrone in urine [32], and more recently Smith *et al.* demonstrated the use of screen-printed electrochemical sensors for the detection

of cathinone derivatives [33,34]. On the other hand, portable instruments are also available for several solid-state spectroscopic techniques such as ATR-FT-IR, near-infrared (NIR) and Raman. NIR and Raman spectroscopy are rapid and non-destructive, where several different samples can be measured within minutes. ATR-FT-IR is also a rapid technique and uses smaller sample amounts (i.e., a few milligrams) than the other two techniques, yet sample recovery is often difficult when cleaning the internal reflection element (IRE) between uses. A number of studies report on the use of portable ATR-FT-IR and Raman instruments for the identification of drugs of abuse [35-39]; however, these are mainly limited to classical drugs of abuse such as heroin, cocaine and amphetamine. Mabbott *et al.* showed the feasibility of using surface enhanced Raman scattering (SERS) with a portable Raman instrument for the detection of pure mephedrone [40]. More recently, Tsujikawa *et al.* investigated a number of psychoactive drugs including 'legal highs' using portable NIR with various data pre-processing steps [41]. There are a limited number of studies in this area and in order to focus resources appropriately, the suitability of the various portable vibrational spectrometers available for the identification of 'legal high' substances and products has yet to be investigated.

The objective of this work is to investigate three handheld spectroscopic methods (i.e., NIR, Raman and ATR-FT-IR) for the identification of 'legal high' substances in model mixtures and Internet products; and to propose a strategy for their use in monitoring products in the field by non-experts such as nurses, regulatory authorities and police. The study focuses on the 'legal high' substances dextromethorphan, 2-aminoindane, and lidocaine; and related products obtained from the Internet. The approaches to analyse the products are based on the instruments' in-built identification algorithms and spectral libraries composed of pure substances and mixtures with caffeine.

2. Experimental

2.1.Materials

Pure powder samples of two drugs commonly found in products available on 'legal high' websites, dextromethorphan hydrobromide (DXM) and 2-aminoindane hydrochloride (2AI) (Figure 1), were purchased from Sigma Aldrich. For commonly used adulterants and diluents, pure samples of benzocaine (BEN), caffeine (CAF), lactose (LAC), lidocaine hydrochloride (LID), microcrystalline cellulose (MCC), paracetamol (PAR), procaine hydrochloride (PRO) and talc (TAL) were purchased from Sigma Aldrich, Acros Organic and Fisher Scientific, and BDH laboratories. A total of seven 'legal high' products were purchased from the Internet and included five powders and two capsules. The label claim of the products indicated that they contained either an aminoindane derivative or DXM.

Model powder mixtures were prepared of 2AI/CAF, DXM/CAF and LID/CAF for use as method signatures and test samples. Powders composed of 50:50 2AI/CAF, DXM/CAF and LID/CAF were used for method signatures. Powder dilutions of the three mixture types containing various % m/m were constructed to have a known matrix for testing each instrument's in-built identification. Table 1 shows the three mixture types each containing twelve dilutions and includes Mixture 1 (2AI/CAF), Mixture 2 (DXM/CAF) and Mixture 3 (LID/CAF). The dilution mixtures were prepared by adding different amounts of pure CAF powder to each of 2AI, DXM and LID to get variable percentages in the range of 5 - 95% m/m. To test the selectivity of the instruments, a blank test set of both binary and ternary mixtures was prepared. The blank test sets were composed of BEN, MCC, PAR, PRO and TAL (i.e., common cutting agents and adulterants found in 'legal highs') in various percentages (Supplementary Data). The blank test set did not contain 2-AI, DXM, LID or CAF (i.e., the substances of interest in the three mixture types). The powders were weighed in their original vials using a Mettler Toledo balance capable of measurements from 0.01 mg

up to 220 g. The balance was enclosed in a safety cabinet (BIGNEAT F3-XIT). Each dilution was stored in a Kimble screw thread 4 ml glass vial with PTFE cap. To ensure uniformity of the mixtures within a vial, a VORTEX-GENIE2 (Scientific industries, Inc.) mixer was used to homogenise powders after the mixtures were prepared (10 min) and immediately before analysis (2 min).

2.2.Handheld Instrumentation

Three handheld instruments obtained from ThermoFisher Scientific Inc. (Wilmington, MA) were used and included the Thermo microPHAZIR RX NIR, Thermo TruScan RM Raman and Thermo TruDefender FT IR analyser. The Thermo microPHAZIR RX had a weight of 1.25 Kg, a battery life of six hours and operated in a temperature range of -5 to 40˚C. The spectral range of the instrument was 1600 – 2400 nm with a resolution of 11 nm. The Thermo TruScan RM had a weight of 0.9 Kg, a battery life of four hours and operated in a temperature range of -25 to 40˚C. It had a 785 nm laser excitation wavelength, an output of 250 mW, 0.2 - 2.5 mm spot size and a 2048 element silicon charge coupled device (CCD) detector. The spectral range of the instrument was 250 – 2875 cm^{-1} with a resolution of 8 – 10.5 cm^{-1}. The Thermo TruDefender FT analyser (with an internal reflection element (IRE)) had a weight of 1.3 Kg, a battery life of four hours and operated in a temperature range of -25 to 40˚C. The FT-IR utilised a broadband heated filament source and had a single-bounce diamond ATR with a DLTGS detector and ZnSe beamsplitter. The spectral range of the instrument was 650 – 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.3.Method

Powders were measured through the vials using the microPHAZIR and TruScan RM. A few milligrams from each vial were measured through direct contact with the instrument

nozzle via a crusher attachment when using the TruDefender FT analyser. The IRE was cleaned between measurements using a Kimwipe and methanol. The instruments used an algorithm that collected spectra until an appropriate S/N ratio was obtained.

Using the three instruments, signatures (i.e., high quality spectra) were taken for the pure substances and the 50:50 mixtures of 2AI/CAF, DXM/CAF and LID/CAF, and stored in the library as a method. At times, the Raman signatures took notably longer to collect than when using the other two instruments depending on the Raman activity of the material measured and the fluorescence generated by the sample. A number of test samples were compared against the generated methods and included the pure substances, powder dilution mixtures, a blank test set, and Internet products. When testing substances against the built methods, three scans were taken for each sample.

2.4.Identification using gas chromatography mass spectrometry (GC-MS)

The composition of the Internet products was confirmed using gas chromatography mass spectrometry with electron ionisation (GC-EI-MS). A Varian 240 GC-240 MS ion trap instrument was used in full scan mode (range 40 – 1000 m/z). The main constituents in the 'legal high' products were identified using a NIST library.

3. Results and Discussion

3.1.In-built identification method (or instrument algorithm)

The significance of the in-built identification algorithms, using the three instruments, is that they can give an immediate answer for the test product against library signatures.

The in-built algorithm of the microPHAZIR was based on the correlation in wavelength space (CWS) method [42], which compares the test substance spectrum (B) against the library signatures (A). This is made by calculating the correlation coefficient (r)

(Equation 1) between the standard normal variate-second derivative spectra (SNV-D2) of each test substance and the library signatures.

$$r_p = \sum(A_i - \overline{A})(B_i - \overline{B}) / \sqrt{\sum(A_i - \overline{A})^2 \sum(B_i - \overline{B})^2} \qquad \text{Equation 1}$$

An *r* value of 1 meant that the spectra were identical, whereas a negative *r* value showed dissimilarity [43, 44]. However, it is difficult to get a value of 1 in practice due to noise effects. An *r* value of 0.95 was used as a threshold [43], where ≥ 0.95 the tested substance was considered consistent with the library spectrum and < 0.95 indicated the two substances exhibited differences.

Both the TruScan RM and the TruDefender FT analyser operated using Bayesian theorem. The TruScan RM operated using the same engines except that it started with calculating a probability value (PVAL) [45, 46] for the test substance spectrum against a selected library signature (Equation 2).

$$P(A|B) = P(B|A)P(A)/P(B) \qquad \text{Equation 2}$$

Where *P (A|B)* is the conditional probability of *A* such that *B* exists; it is also known as the posterior probability.

P (B|A) is the conditional probability of *B* such that *A* exists; it is also known as the likelihood.

P (A) is the prior or marginal probability of *A*.

P (B) is the prior or marginal probability of *B*; this acts as a normalising constant.

A PVAL ≥ 0.05 meant that the test substance spectrum was considered consistent with the selected library signature. A PVAL below 0.05 meant that the test substance and library spectrum exhibited differences. If the PVAL was below 0.05, the instrument then worked in discovery mode where the test spectrum was compared against all library signatures and gave a consistent match or percentage correlation to a library signature(s). The percentage correlation was not quantitative, but indicated how much of the molecular signal from the test substance correlated to the library signature(s). No upper percentage threshold was used as it depended on the number of signatures matching the test spectrum. Correlation values < 10% were discarded due to the noise effects of these portable instruments, which is greater than their bench-top counterparts. The TruDefender FT analyser operated under this algorithm but reported the percentage correlation without giving a PVAL.

To summarise, the NIR and ATR-FT-IR algorithms compared the test spectrum to all library signatures, whereas the Raman algorithm compared the spectrum to a specific signature first. Additionally, the Raman and ATR-FT-IR's screen display showed both the associated spectra and correlation value; the NIR did not display spectra, but showed only the numerical correlation value.

3.2.Analysis of pure substances and dilution mixtures

3.2.1. Substance selection

For this preliminary study, substances that were commercially available in large quantities were selected enabling the preparation of a variety of powder mixtures. The substances used to construct the model mixtures (i.e., DXM, 2-AI, LID and CAF) (Figure 1) were selected to evaluate a range of 'legal' substances currently available from Internet sources. DXM, an active pharmaceutical ingredient found in many cough suppressants, is known to cause hallucinations and phencyclidine (PCP) - like behavioural effects at high doses [47].

Traditionally, this substance was abused by ingesting large quantities of cough suppressant, but more recently DXM has become available on Internet sites in powder form. DXM abuse continues to increase worldwide and it is particularly popular amongst teenagers [48,49]. 2-AI is a conformationally rigid analogue of amphetamine and is one of the popular aminoindanes along with 5,6-methylenedioxy-1-aminoindane (MDAI) and 5-iodo-2-aminoindane (5-IAI) [22,50]. It continues to be available on a wide range of Internet sites and has been used as a commercially available substitute for MDAI and 5-IAI and also used in branded products [51]. LID was also used, as this substance, although traditionally used to cut cocaine due to its numbing effect, is now commonly used to cut 'legal high' products [3, 12, 52]. CAF, a stimulant, was chosen as the diluent because it is one of the major adulterants encountered in NPS [3, 52].

3.2.2. MicroPHAZIR RX (NIR)

The pure substances and 50:50 mixtures were consistent with their NIR signatures above the threshold (0.95) (Supplementary information). For the dilution mixture results (Table 2), Mixtures 1 (2AI/CAF) and 2 (DXM/CAF) were consistent with (i.e., values ≥ 0.95 shown in bold) their corresponding 50:50 library signature in the range of 29.9 – 59.9% m/m (Mixtures 1D4 – 1D6) and 30.6 – 60.1% m/m (Mixtures 2D4 – 2D6), respectively. The range was larger for Mixture 3 (LID/CAF), which showed consistency with the 50:50 library signature in the range of 30.2 – 70.1% m/m (Mixtures 3D4 – 3D7).

Concentrations of drug below and above these values had NIR spectra that were either consistent with a single substituent or did not correlate to a library signature at or above the 0.95 threshold. For example, at ≤ 10.0% m/m 2AI, the spectra were consistent with the CAF signature. At concentrations ≥ 85.3% m/m 2AI, the dilutions' spectra were consistent with the 2AI signature. The remaining dilutions which included Mixture 1D3 and Mixtures 1D7 – 1D9 (20.0% m/m and 69.9 – 80.1% m/m) did not result in an r value at or above the threshold (i.e., $r \geq 0.95$). Similarly, for Mixture 2, concentrations ≤ 9.8% m/m DXM, the

dilutions' spectra were consistent with the CAF signature. At concentrations ≥ 75.2% m/m DXM, the dilutions' spectra were consistent with the DXM signature. The remaining dilutions, which included Mixture 2D3 and 2D7 (20.0% m/m and 70.0% m/m), resulted in *r* values below the threshold. On the other hand, the spectra from Mixture 3 dilutions with ≤ 20.0% m/m and ≥ 95.1% m/m LID, were consistent with the CAF and LID signatures, respectively. Between 75.1 – 89.7% m/m the Mixture 3 dilutions' spectra resulted in no correlations above the threshold. In summary, the dilutions' mixtures were consistent with the pure and 50:50 signatures when the dilution mixture concentration was close to the signature concentrations, yet identification of substances at the 0.95 threshold proved difficult for intermediate concentrations such as those for Mixtures 2D3 and 2D7. Although some *r* values were below the chosen threshold, all samples did correlate to an appropriate library signature with the smallest *r* value of 0.8606. Thus, for mixture analysis, consideration should be made for possibly lowering the threshold as correlations are made to a single library signature and not a combination of signatures as will be seen with the TruScan RM and TruDefender ATR-FT-IR.

3.2.3. TruScan RM (Raman)

The pure substances and 50:50 mixtures were consistent with their Raman signatures at or above the threshold (PVAL ≥ 0.05) with the exception of LID, which correlated (100%) to its signature in discovery mode (Supplementary information). For the dilution mixtures, the TruScan RM algorithm compared each mixture against their corresponding 50:50 library signature first. For PVALs below 0.05, the spectrum was then compared against all library signatures in discovery mode and gave a % correlation (Table 2). For all dilution mixtures, the spectra were either consistent with the 50:50 library signature (PVAL ≥ 0.05) or showed a % correlation in discovery mode.

Mixtures 1 (2AI/CAF), 2 (DXM/CAF) and 3 (LID/CAF) were consistent with (i.e., values shown in bold in Table 2) a mixture of both components in the range of 20.0 – 74.6% m/m 2AI (Mixtures 1D3 – 1D8), 20.0 – 80.1% m/m DXM (Mixtures 2D3 – 2D9) and 20.0 – 89.7% m/m LID (Mixtures 3D3 – 3D11), respectively. These results are much improved compared to the MicroPHAZIR (NIR), where correlations to a mixture occurred for a smaller % m/m range. Most of the dilution mixtures correlated to library signatures in discovery mode. For example, Mixture 1D3 (20.0% m/m) was initially compared to the 50:50 2AI/CAF signature resulting in a PVAL of 0.0118 which was below the designated threshold. The algorithm then compared the Mixture 1D3 spectrum to all library signatures, which correlated to 2AI/CAF (82%) and CAF (18%). Figure 2a shows a representative Raman screen display for Mixture 1D3 when using discovery mode. By comparing the spectra, the test spectrum shows the CAF O=C-N bending vibration at 555 cm^{-1} [53] and 2AI C-C chain vibrations (700-900 cm^{-1}).

Concentrations of drug below and above the ranges mentioned above had Raman spectra that were correlated with a single substituent in discovery mode, and were consistent with the component in highest concentration. The 1D dilution mixtures ≤ 10.0% m/m 2AI (Mixtures 1D1 and 1D2) and ≥ 80.1% m/m 2AI (Mixture 1D9 - 1D12) gave spectra that were consistent with the signatures of CAF and 2AI respectively. For the 2D dilution mixtures, ≤ 9.8% m/m DXM (Mixtures 2D1 and 2D2) and ≥ 84.8% m/m DXM (Mixture 2D10) were consistent with the CAF and DXM signatures, respectively. The 3D dilution mixtures ≤ 10.5% m/m LID (Mixtures 3D1 and 3D2) and ≥ 95.1% m/m LID (Mixture 3D12) were consistent with the CAF and LID signatures, respectively. In summary, the Raman instrument was able to identify the main constituent(s) of each dilution mixture either by obtaining a PVAL ≥ 0.05 or % correlation in discovery mode. As the TruScan RM algorithm can

correlate a spectrum to more than one library signature in discovery mode, this improved identification for the dilutions mixtures in comparison to the MicroPHAZIR (NIR).

3.2.4. TruDefender ATR-FT-IR

All the pure substances were consistent with their own signature with values of 100%, while the 50:50 mixtures correlated to the mixture signature or the individual signatures (Supplementary information).

Mixtures 2 (DXM/CAF) and 3 (LID/CAF) were consistent with (i.e., values in bold) a mixture of both components in the range of 30.6 – 89.8% m/m DXM (Mixtures 2D4 – 2D11) and 41.0 – 85.1% m/m LID (Mixtures 3D5 – 3D10), respectively (Table 2). For Mixture 1 (2AI/CAF) dilutions, those in the range of 20.0 – 85.3% m/m 2AI (Mixtures 1D3 - 1D10) did not correlate to any library signatures, except for 40.3% m/m 2AI (Mixture 1D5), which correlated to the signatures of 2AI (56%) and CAF (30%). Figure 2b shows a representative ATR-FT-IR spectrum for 1D3. Although the spectrum shows absorptions from characteristic vibrations such as NH_2 and C-H stretching (i.e., 3200 to 2500 cm^{-1}), no correlation was found using the algorithm. The ATR-FT-IR in-built algorithm performed the worst out of all three techniques when analysing these intermediate 1D mixtures. Upon inspection of the IR spectra for 2AI and CAF, they both showed strong infrared absorption and had a similar distribution of absorption peaks in the fingerprint area, yet no strong distinctive peaks outside of this area. This suggests the similarity between the spectra resulted in these two compounds being difficult to identify in a mixture in the absence of an exactly matching library signature. This may have implications when using this method for analysing other amphetamine-like substances mixed with CAF.

The 1D dilutions ≤ 10.0% m/m 2AI (Mixtures 1D1 and 1D2) and ≥ 90.0% m/m 2AI (Mixtures 1D11 and 1D12) were consistent with the signatures of CAF and 2AI, respectively.

A study investigating a 10% m/m MDAI mixture with CAF using lab-based ATR-FT-IR, showed similar results where the powder matched to the CAF signature exclusively (r = 0.995) [10]. For 2D dilutions ≤ 20.0% m/m DXM (Mixture 2D1 - 2D3) and ≥ 95.2% m/m DXM (Mixture 2D12), the spectra were consistent with the signatures of CAF and DXM respectively. Similarly, dilutions with ≤ 30.2% m/m LID (Mixtures 3D1 - 3D4) were consistent with the CAF signature. Dilutions with ≥ 89.7% m/m LID (Mixture 3D11 and 3D12) gave no correlation, as CAF is a stronger Raman scatterer than LID.

In summary, the ATR-FT-IR experiments were successful for detecting the main constituents for most dilutions of the 2D and 3D mixtures. Furthermore, the instrument was able to identify DXM/CAF mixtures in a larger % m/m range than the other two instruments. However, in the case of Mixture 1D, correlations only occurred when the concentrations were very similar to the library signatures (i.e., pure or 50/50 mixture). The ATR-FT-IR instrument was only successful for identifying CAF in the 1D1 and 1D2 mixtures, CAF and 2AI in the 1D5 mixture and 2AI in the 1D11 and 1D12 mixtures. This was likely due as a result of the similarity between the absorption spectra of CAF and 2AI. In contrast, LID/CAF mixtures at high LID concentrations did not correlate to any library signature(s).

3.3.Application to 'legal high' products

The contents of the 'legal high' products were initially verified using GC-EI-MS, and the main constituents with corresponding major ion peaks for each product are shown in Table 3. Product 1 was labelled as 2AI but was composed of two constituents not on its label claim, CAF (m/z 194) and methyl phenidate (m/z 84). Products 2-6 contained a main constituent that was stated on their label claim. For example, products 2 and 3 were purchased as the products 'Blurberry' and 'Pink champagne' and were confirmed to match part of their label claim, 2AI (m/z 133) and CAF (m/z 194). The major ion peak for the DXM

products 4-6 was seen at m/z 271. Product 7 was labelled as DXM, but was confirmed to contain CAF as the active ingredient.

The three spectroscopic instruments' in-built algorithms were at times complementary in identifying the main constituents in the 'legal high' products (Table 3). The main constituents of all products were identified with one or two algorithms, however the correlation values were varied. One exception was methylphenidate in P1, which was not identified using the spectroscopic instruments as this reference was not present in the library.

Using the microPHAZIR CWS in-built algorithm, all products did correlate to a library signature, yet only one product correlated at a value above 0.95. This sample was labelled as DXM (P7) and correlated to the CAF signature (r = 0.9646). This agreed with the GC-EI-MS analysis, which gave a major ion peak at m/z 194 corresponding to CAF. Products 1, 2 and 3 had correlations with CAF (0.835), 2AI/CAF (0.6767) and 2AI/CAF (0.3965), respectively, which were the major constituents found using GC-EI-MS. Although the correlation values were below 0.95 (i.e., indicating they were not an identical match), they do correlate to appropriate signatures as these products most likely contained other substituents. As mentioned previously with the dilution mixtures, it may be considered to lower the correlation coefficient threshold when identifying 'legal high' substances using this instrument as products are often a mixture of ingredients. Products 4-6 also gave r values below 0.95 ranging from 0.4265-0.5012 for MCC, which most likely was used as a cutting agent. Overall, NIR takes into account both the physical properties as well as the chemical constituents of a substances [54] and may be advantageous when comparing products suspected to be from the same source.

The TruScan RM probability in-built algorithm gave correlations for three products, which were labelled as DXM (P4 – P6). The products correlated to DXM in discovery mode from 87-88%. Figure 3a shows the Raman spectra of P4 with library correlations to DXM

(87%) and isobutyl chloroformate (2%), where isobutyl chloroformate was discarded as the % correlation was less than 10%. These products were confirmed to contain DXM using GC-EI-MS (Table 3). Product 1 gave Raman scattering; however, did not correlate with any signature in discovery mode. Although this product contained CAF (m/z 194) confirmed using GC-EI-MS, it also contained methylphenidate, which possibly masked the CAF Raman signal. On the other hand, products 2, 3 and 7 showed a large fluorescence background with little discernable peaks indicating the products were cut with a highly fluorescent substance. For example, the Raman spectrum of Pink champagne (P3) shows a broad featureless spectrum with no library correlation found (Figure 3c). The use of a longer wavelength can reduce fluorescence, which may be needed for analysing certain 'legal high' products. In some circumstances, when a Raman active drug is present in high concentration the Raman scattering may still be seen with minimum fluorescence despite the presence of impurities. This was observed for seized cathinone samples that were cut by calcium carbonate and other impurities [30].

The TruDefender probability algorithm correlations varied markedly from the previous two algorithms. Whereas P4 – P6 were consistent with DXM using the TruScan RM, these products correlated to other existing libraries in the instrument as mixtures of starch, maltodextrin, amylose and amylopectin. An example of this is shown in Figure 3b where the FT-IR spectrum of P4 and its library correlations are represented. This indicates that the DXM present in these products was not pure and the additives used were likely carbohydrate in nature. Carbohydrates (e.g., starch) contain many polar C-O and O-H bonds, which are strong infrared absorbers and weak Raman scatterers. These results were similar to those seen for the NIR analysis, where these samples matched to the carbohydrate MCC. The use of the TruScan RM would be advantageous for quick identification of 'legal high' products that contain bulking agents of this nature. Products 3 and 7 had the highest

correlation to CAF, which was confirmed using GC-EI-MS. These products also correlated to the LID/CAF signature, but as no LID was found using GC-EI-MS, this seemed to be a false positive due to the CAF being a stronger Raman scatterer in that particular Model mixture. For Pink champagne (P3), the product correlated to CAF at 72%, but LID/CAF at 3%, thus the latter value was discarded (i.e., below 10%) (Figure 3d). The presence of CAF in 'legal high' products, either in pure or mixed form, using ATR-FT-IR has been previously reported [10]. Product 2, Blurberry, produced an ATR-FT-IR signal, but the results were non-reproducible and correlated to a range of signatures including 2AI/CAF, LID/CAF, DXM/CAF, and CAF. Although the product was vortexed before each reading, the product may have retained some heterogeneity impacting on the reproducibility of the signal. Product 1 showed many peaks in the fingerprint area, but due to the presence of methyl phenidate, no match was found.

4. Conclusion

The use of a library method composed of pure and 50:50 mixtures for three handheld instruments shows promise for identification of 'legal high' substances. A summary of the instruments' performance for both dilution mixtures and 'legal high' Internet products is shown in Table 4. For the dilution mixtures, the % m/m range where a mixture was successfully identified was different for the three instruments, with the TruScan RM (Raman) performing the best for all mixtures with a minimum range of 20.0 – 74.6% m/m. The identification of mixtures using the MicroPHAZIR (NIR) was limited as the algorithm correlated to only a single library signature and not a combination as with the other instruments. Outside these ranges, the powder mixtures were in most cases correlated to the pure substance in highest concentration in the default and/or discovery mode, the exceptions being 2AI/CAF (1D) and LID/CAF (3D) mixtures analysed by ATR-FT-IR. This

demonstrates that simple library mixtures could be used to identify substances when the concentrations are variable. When analysing the Internet products, most active ingredients in the products were identified with one or two instruments, however the correlation values were varied. The three techniques were affected differently by the presence of cutting agents and adulterants in the 'legal high' products. Both NIR and ATR-FT-IR were sensitive to the presence of carbohydrate cutting agents, whereas the Raman was not. However, three products showed a high fluorescence signal during Raman analysis, which hindered identification. Thus, future efforts should focus on building more extensive NPS libraries, examination of thresholds used for NIR, evaluation of a lower energy wavelength for Raman analysis to reduce fluorescence, and investigation of alternative algorithms.

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Conflict of interest

The authors declare no conflict of interest.

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List of Tables

Table 1 Details of the dilution mixtures prepared[1]

Mixture number	Mixture type	Drug content (% m/m)	Caffeine content (% m/m)
Mixture 1D1	2AI/CAF	4.9	95.1
Mixture 1D2	2AI/CAF	10.0	90.0
Mixture 1D3	2AI/CAF	20.0	80.0
Mixture 1D4	2AI/CAF	29.9	70.1
Mixture 1D5	2AI/CAF	40.3	59.7
Mixture 1D6	2AI/CAF	59.9	40.1
Mixture 1D7	2AI/CAF	69.9	30.1
Mixture 1D8	2AI/CAF	74.6	25.4
Mixture 1D9	2AI/CAF	80.1	19.9
Mixture 1D10	2AI/CAF	85.3	14.7
Mixture 1D11	2AI/CAF	90.0	10.0
Mixture 1D12	2AI/CAF	95.1	4.9
Mixture 2D1	DXM/CAF	5.5	94.5
Mixture 2D2	DXM/CAF	9.8	90.2
Mixture 2D3	DXM/CAF	20.0	80.0
Mixture 2D4	DXM/CAF	30.6	69.4
Mixture 2D5	DXM/CAF	39.9	60.1
Mixture 2D6	DXM/CAF	60.1	39.9
Mixture 2D7	DXM/CAF	70.0	30.0
Mixture 2D8	DXM/CAF	75.2	24.8
Mixture 2D9	DXM/CAF	80.1	19.9
Mixture 2D10	DXM/CAF	84.8	15.2
Mixture 2D11	DXM/CAF	89.8	10.2
Mixture 2D12	DXM/CAF	95.2	4.8
Mixture 3D1	LID/CAF	5.0	95.0
Mixture 3D2	LID/CAF	10.5	89.5
Mixture 3D3	LID/CAF	20.0	80.0
Mixture 3D4	LID/CAF	30.2	69.8
Mixture 3D5	LID/CAF	41.0	59.0
Mixture 3D6	LID/CAF	60.4	39.6
Mixture 3D7	LID/CAF	70.1	29.9
Mixture 3D8	LID/CAF	75.1	24.9
Mixture 3D9	LID/CAF	79.7	20.3
Mixture 3D10	LID/CAF	85.1	14.9
Mixture 3D11	LID/CAF	89.7	10.3
Mixture 3D12	LID/CAF	95.1	4.9

[1]D; dilution number, AI: aminoindane hydrochloride, CAF: caffeine, DXM: dextromethorphan hydrobromide, LID: lidocaine hydrochloride, %m/m: percentage mass per mass.

Table 2 Mean comparison values of the dilution mixtures using hand-held NIR, Raman and ATR-FT-IR spectroscopy[1]

Substance	NIR		Raman		ATR-FT-IR	
	library signature	*r* value[2]	library signature	PVAL[3] or correlation (%)[4]	library signature(s)	correlation (%)[4]
Mixture 1D1	CAF	**0.9871**	CAF	**100***	CAF	**100**
Mixture 1D2	CAF	**0.9752**	CAF	**100***	CAF	**100**
Mixture 1D3	CAF	0.8988	2AI/CAF	**82***	None	0
			CAF	**18***		
Mixture 1D4	2AI/CAF	**0.9947**	2AI/CAF	**0.3159**	None	0
Mixture 1D5	2AI/CAF	**0.9746**	2AI/CAF	**0.3717**	2AI	**56**
					CAF	**30**
Mixture 1D6	2AI/CAF	**0.9477**	2AI	**73***	None	0
			CAF	**27***		
Mixture 1D7	2AI	0.8866	2AI/CAF	**0.4228**	None	0
Mixture 1D8	2AI	0.9218	2AI/CAF	**0.3929**	None	0
Mixture 1D9	2AI	0.8862	2AI	**97***	None	0
Mixture 1D10	2AI	**0.9845**	2AI	**95***	None	0
Mixture 1D11	2AI	**0.9823**	2AI	**100***	2AI	**100**
Mixture 1D12	2AI	**0.9981**	2AI	**100***	2AI	**100**
Mixture 2D1	CAF	**0.9842**	CAF	**100***	CAF	**100**
Mixture 2D2	CAF	**0.9578**	CAF	**100***	CAF	**100**
Mixture 2D3	DXM/CAF	0.8909	DXM/CAF	**89***	CAF	**97**
			CAF	**11***		
Mixture 2D4	DXM/CAF	**0.9487**	DXM/CAF	**0.0480**	DXM/CAF	**100**
Mixture 2D5	DXM/CAF	**0.9708**	DXM/CAF	**0.4447**	DXM/CAF	**100**
Mixture 2D6	DXM/CAF	**0.9485**	DXM/CAF	**0.1943**	DXM/CAF	**88**
					DXM	**11**
Mixture 2D7	DXM	0.9320	DXM	**83***	DXM	**68**
			DXM/CAF	**16**	DXM/CAF	**31**
Mixture 2D8	DXM	**0.9501**	DXM	**83***	DXM/CAF	**75**
			DXM/CAF	**16***	DXM	**24**
Mixture 2D9	DXM	**0.9587**	DXM	**87***	DXM/CAF	**70**
			DXM/CAF	**12***	DXM	**28**
Mixture 2D10	DXM	**0.9912**	DXM	**93***	DXM	**80**
					DXM/CAF	**19**
Mixture 2D11	DXM	**0.9928**	DXM	**96***	DXM/CAF	**86**
					DXM	**13**
Mixture 2D12	DXM	**0.9979**	DXM	**100***	DXM	**100**
Mixture 3D1	CAF	**0.9940**	CAF	**100***	CAF	**100**
Mixture 3D2	CAF	**0.9914**	CAF	**100***	CAF	**100**
Mixture 3D3	CAF	**0.9797**	LID/CAF	**0.0453**	CAF	**100**
Mixture 3D4	LID/CAF	**0.9721**	LID/CAF	**0.0552**	CAF	**95**
Mixture 3D5	LID/CAF	**0.9959**	LID/CAF	**0.2515**	LID/CAF	**100**
Mixture 3D6	LID/CAF	**0.9751**	LID/CAF	**0.0796**	LID/CAF	**100**
Mixture 3D7	LID/CAF	**0.9581**	LID/CAF	**0.2131**	LID/CAF	**100**
Mixture 3D8	LID/CAF	0.9386	LID/CAF	**0.2545**	LID/CAF	**100**
Mixture 3D9	LID	0.8606	LID/CAF	**55***	LID/CAF	**90**
			LID	**27***		
Mixture 3D10	LID	0.9067	LID	**79***	LID/CAF	**100**
			LID/CAF	**20***		
Mixture 3D11	LID	0.9374	LID	**87***	None	0
			LID/CAF	**12***		
Mixture 3D12	LID	**0.9848**	LID	**100***	None	0

[1]D; dilution number, AI: aminoindane hydrochloride, CAF: caffeine, DXM: dextromethorphan hydrobromide, LID: lidocaine hydrochloride. The numbers in bold indicate a match for the test substance.

[2] *r* value: correlation coefficient value

[3] PVAL: probability value of the test substance spectrum against the selected library spectrum. A PVAL above 0.05 indicates that the test spectrum is consistent with the 50:50 library signature; the asterisk (*) indicates a PVAL < 0.05 where the algorithm then compared the test substances to other signatures in discovery mode.

[4]Correlation (%): indicates how much the test spectrum is similar to the matching library signature(s).

Table 3 Mean comparison values of the 'legal high' products using hand-held NIR, Raman and ATR-FT-IR spectroscopy; and corresponding GC-EI-MS results[1]

Product	Label claim	NIR		Raman		ATR-FT-IR		GC-EI-MS	
		library signature	*r* value[2]	library signature	correlation (%)[3]	library signature	correlation (%)[3]	match	Major ion peak (m/z)
P1	2AI	CAF	0.8535	None	0	None	0	CAF	194
								Methyl phenidate	84
P2	Blurberry: Aminoindane, Caffeine, Cola vera, Aminoacid complex	2AI/CAF	0.6767	None	0	Non-reproducible		2AI	133
								CAF	194
P3	Pink champagne: Aminoindane, Caffeine, Cola vera, Aminoacid complex	2AI/CAF	0.3965	None	0	CAF	**72**	CAF	194
								2AI	133
P4	DXM	MCC	0.4568	DXM	**87**	Starch, Maltodextrin;	**65**	DXM	271
						Amylose, Amylopectin	**12**		
P5	DXM	MCC	0.4265	DXM	**88**	Starch, Maltodextrin;	**60**	DXM	271
						Amylose, Amylopectin	**13**		
P6	DXM	MCC	0.5012	DXM	**88**	Starch, Maltodextrin;	**57**	DXM	271
						Amylose, Amylopectin	**14**		
P7	DXM	CAF	**0.9646**	None	0	CAF	**58**	CAF	194
						LID/CAF	**26**		

[1]P: product, 2AI: 2-aminoindane hydrochloride, CAF: caffeine, DXM: dextromethorphan hydrobromide, LID: lidocaine, MCC: microcrystalline cellulose. The numbers in bold indicate the test substance is consistent with the library signature.

[2] *r* value: correlation coefficient value

[3]Correlation (%): indicates how much the test spectrum is similar to the library signature(s).

Table 4 A summary of the instruments' performance for both dilution mixtures and 'legal high' Internet products using hand-held NIR, Raman and ATR-FT-IR spectroscopy.

Handheld technique	**Analysis of dilution mixtures**	**Range where a mixture was identified for the dilution mixtures**	**Analysis of 'legal high' products**	**Identification results for 'legal high' products**
MicroPHAZIR NIR	Appropriate library correlations were found for all mixtures; not all above the 0.95 threshold	*r* values ≥ 0.95 threshold: 1D 29.9 – 59.9% m/m 2D 30.6 – 60.1% m/m 3D 30.2 – 70.1% m/m	Appropriate library correlations were found for all products; not all above the 0.95 threshold	Active ingredients were identified in P1-3 and P7. Cutting agents were identified in P4-6.
TruScan RM Raman	Appropriate library correlations were found for all mixtures using the default or discovery mode.	PVAL ≥ 0.05 or % correlation: 1D 20.0 – 74.6% m/m 2D 20.0 – 80.1% m/m 3D 20.0 – 89.7% m/m	Appropriate library correlations were found for only 3 out of 7 products; fluorescence masked the Raman signal in 3 products.	Active ingredients were identified in P4-6. No cutting agents were identified.
TruDefender ATR-FT-IR	Appropriate library correlations were found for all 2D mixtures, but no correlations for some 1D and 3D mixtures.	% correlation: 1D 40.3% m/m 2D 30.6 – 89.8% m/m 3D 41.0 – 85.1% m/m	Appropriate library correlations were found for 4 out of 7 products; other products did not correlate to a signature, were non-reproducible, or resulted in a false positive.	Active ingredients were identified in P3 and P7. Cutting agents were identified in P4-6.

Figure Captions

Figure 1: The chemical structures of (a) dextromethorphan, (b) 2-aminoindane, (c) lidocaine, and (d) caffeine.

Figure 2: Screen display from handheld instruments showing a) a Raman spectrum of Mixture 1D3 (2AI/CAF 20:80% m/m) (black line) compared against the signatures of 2AI/CAF (82%) (red line) and CAF (18%) (blue line), and b) an ATR-FT-IR spectrum of Mixture 1D3 (2AI/CAF 20:80% m/m) with no match found.

Figure 3: Screen display from handheld instruments showing a) a Raman spectrum of DXM P4 (black line) compared against the signatures of DXM (87%) (red line) and isobutyl chloroformate (2%) (blue line), b) an ATR-FT-IR spectrum of DXM P4 (black line) compared against the signatures of starch/maltodextrin (65%) (red line) and amylose/amylopectin (12%) (blue line), c) a Raman spectrum of Pink champagne P3 (black line) with no match found, and d) an ATR-FT-IR spectrum of Pink champagne P3 (black line) compared against the signatures of CAF (72%) (red line) and LID/CAF (3%) (blue line).

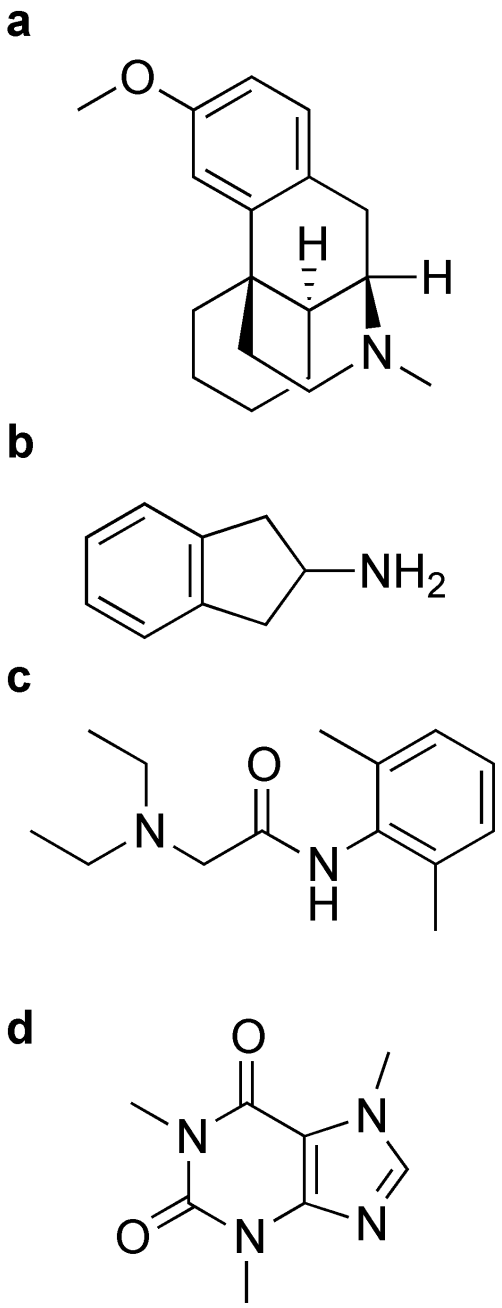
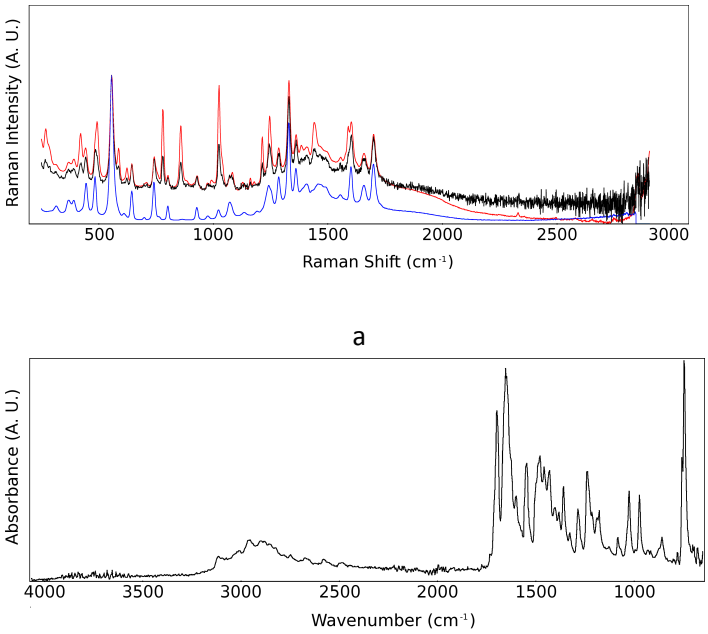


Figure 1: The chemical structures of (a) dextromethorphan, (b) 2-aminoindane, (c) lidocaine, and (d) caffeine.



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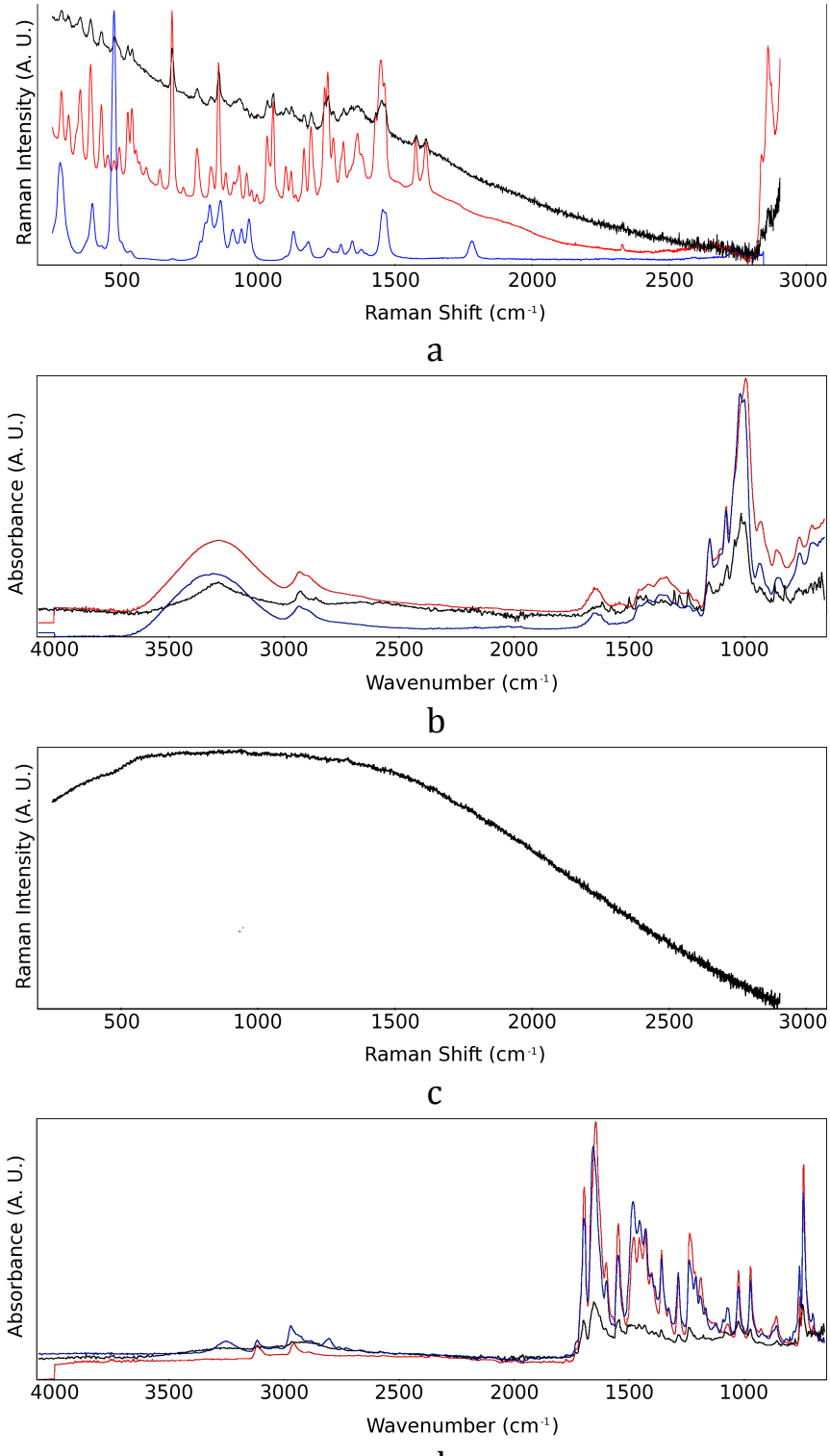

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