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The Detection of Biomarkers and Cocaine in Fingernails Using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

Megan Wilson[1], Jason Birkett[1], Iftikhar Khan[1], Ismail Abbas[2], Leung Tang[3] and Sulaf Assi[1]

[1]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, L3 3AF, UK.

[2]Faculty of Science, Lebanese University, Beirut, Lebanon.

[3]Chemical Analysis Group, Agilent Technologies, SK8 3GR, UK.

Email: m.wilson3@2019.ljmu.ac.uk

Abstract

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) provides portable and rapid analysis of biomarkers and drugs within fingernails. Nails offer a suitable alternative to traditional biological matrices and provide advantages such as non-invasive collection and small sample sizes. This work utilised ATR-FTIR for the detection of biomarkers and cocaine within fingernails. Fingernails were analyzed initially 'as received' to identify biomarkers such as carbohydrates, lipids, and proteins over the wavenumber range 650-4000 cm^{-1}. Spectra were collected for fingernails before and after spiking with cocaine hydrochloride. Measurements were taken at one week and up to six weeks. The results showed that FTIR spectra of fingernails had bands corresponding to carbohydrates at 926 and 1045 cm^{-1}, lipid bands at 2958, 2921 and 2851 cm^{-1}, and protein bands at 1644 and 1536 cm^{-1}. Analysis of cocaine-spiked fingernails demonstrated cocaine bands at 978 cm^{-1} (monosubstituted out-of-plane bending vibration), 1010 cm^{-1} (CO stretching), 1027 cm^{-1} (monosubstituted benzene ring stretching), 1055 cm^{-1} (CO and CN stretching), 1071 cm^{-1} (monosubstituted benzene ring stretching), and 1105 cm^{-1} (CO stretching). Principal component analysis (PCA) revealed discrete clusters within the PC scores of cocaine-spiked versus un-spiked fingernails. Findings showed that ATR-FTIR spectroscopy could characterize fingernails based on intrinsic components and identify the presence or absence of cocaine within them.

Introduction

The study of keratinized matrices, such as hair and fingernails, have become important in clinical and forensic toxicology due to their potential for drug accumulation. Keratinized matrices offer a greater window of detection for drugs over traditional body fluids such as blood and urine (1-3). Hair analysis has been used for detecting historic drug use in a range of forensic applications including suspicious deaths, and drug facilitated crimes (4,5). However, hair analysis is often subject to contamination by microorganisms and environmental dust/smoke (6-8).

Fingernail sampling is non-invasive, does not require medical personnel for its collection, and this makes it an ideal alternative matrix to hair. Fingernail sampling offer a further advantage in detecting a wide range of analytes due to nails' slow growth rate and higher drug accumulation. Chemical analysis of nails has been used for a variety of purposes such as forensic investigations (9), occupational (10), and environmental exposure (11). Fingernail analysis has also been used in the diagnosis of a variety of diseases (12) and, in the monitoring of medicines (antidepressants and benzodiazepines) and drugs of abuse (amfetamines, cocaine, and opiates) (13-16).

Many studies have applied analytical techniques for measurement of drugs of abuse in fingernails. These studies have focused on classical analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) and were semi-quantitative in nature (17, 18). However, both GC-MS and LC-MS required expensive and extensive extraction of the analytes prior to analysis. Considering these concerns, we propose a unique method for cocaine analysis based on ATR-FTIR spectroscopy, which is cost-effective, rapid, and requires minimum sample preparation. ATR-FTIR spectroscopy has become a crucial technique for forensic researchers and investigators to assess trace evidence alongside drugs, inks, explosives, biological fluids, and cosmetics (19-23).

Cocaine is Europe's most used illicit stimulant and remains in high supply and demand (24). Cocaine addicts must be monitored in a therapeutic or forensic/legal context, necessitating the use of multi-analyte methods that may identify several substances in a single run. This study presents a simple and sensitive ATR-FTIR method in conjunction with chemometric tools for the detection of constituents and drugs in fingernails.

Experimental

This work comprised ATR-FTIR spectroscopic analysis of 19 sets (11 males and eight females) of fingernails from individuals of adult age and different ethnicities, and with various diets, medicine/drug use, and lifestyles (Table 1). Fingernail clippings were collected alongside a checklist regarding their medicines use and lifestyle. No intervention was made by the researchers in terms of collecting the fingernail clippings, considering the procedure as an everyday activity. Ethical approval was granted from the two institutions that held the study being: Liverpool John Moores University in the UK (PBS/2021-22/04) and the Lebanese University in Lebanon (2022-0104).

One fingernails set was placed in a glass vial and spiked with 50 mg of cocaine hydrochloride. The vial was vortexed for 2 minutes, coating the fingernail in the cocaine. Measurements were taken at weekly intervals over a six-week period. Spectral measurements were made using the Agilent 4500 ATR-FTIR spectrometer over the wavenumber range of 650-4000 cm^{-1} and spectral resolution of 4 cm^{-1}. Three spectra were collected per fingernail such that each spectrum was the sum of 64 scans.

Spectra were exported into Matlab 2019a, for spectral interpretation, spectral quality evaluation, and analysis. For spectral interpretation, the absorption bands of cocaine hydrochloride and fingernails were evaluated. The number of peaks (N), wavenumber range, maximum peak intensity/position, and signal to noise ratio (SNR) were all considered while evaluating spectral quality (Table 2). Principal component analysis (PCA) was applied to the nail spectra to visualize (1) patterns among the different participants' fingernails, and (2) patterns among cocaine-spiked and un-spiked fingernails. For PCA analysis, PC scores and loading plots were evaluated. PC scores demonstrated the patterns of clusters among the different fingernails, and PC loadings justified the importance of the clusters. PC loading plots showed the key functional groups that expressed the highest variance among the data. In this respect, PC1 expressed the highest variance, PC2 the second highest variance and PC3 the third highest variance. For accuracy and precision of the PCA model, clusters were inspected for type I and type II errors. Type I error was encountered when scores of the same fingernail set were not grouped together; whereas type II error was seen when a score of one fingernail set was grouped with a different set.

Results and Discussion

The use of ATR-FTIR for fingernails inspection showed many advantages in forensic analysis. First, the collection procedure was non-invasive, non-destructive, needed small sample size (1-3 mm), and required minimal intervention from the researcher.

Second, FTIR spectra provided unique identifiable features relating to endogenous constituents within nails. These identifiable features can be used to understand individuals' diet, disease state, drug/medicines' use and/or lifestyle characteristics. Third, previous studies have shown that spectral data of nails could highlight the presence of some physical and/or psychological conditions. For example, conditions such as anxiety, depression and diabetes affect the nail's keratin, and this is observed through the nail's appearance (12). Individuals who suffer from chronic depression or systematic diseases are likely to possess discolored nails, nail plate alterations, and/or brittle fingernails (12). Disfiguration to the nail plate is often the consequence of altered trace element levels during chronic depression. Research has highlighted a strong association between decreased levels of nail constituents and depression (12). Moreover, by optimizing the method for a portable device, it could be used on site at airports, police stations, hospitals, and crime scenes.

This study aimed to complement previous studies by exploring the effects of different diets and lifestyles on the IR spectra of fingernails. Moreover, it assessed deposition of cocaine in nails upon chronic exposure over a period of six weeks. This in turn enabled discrimination of chronic dealing with cocaine that has been of interest in forensic investigations. In this respect, it has been important to distinguish individuals suspected of possessing cocaine for a 'one-use' from those who possess drugs chronically with intent to supply.

FTIR Band Assignments of Fingernails

FTIR spectra of the fingernail sets (n = 19) showed bands corresponding to the absorbances of key endogenous constituents usually present in fingernails (Figure 1). These constituents included proteins, lipids, carbohydrates, and amino acids (25). The identified bands were assigned vibrational bands (Table 2).

Spectral Quality

Table 3 shows the IR spectra quality of the fingernail sets (n = 19). In this respect, fingernail sets showed strong (n = 7) or medium (n = 12) IR activity. Considering the four evaluation parameters, the number of bands (N) ranged from 19-25 bands for fingernail sets with strong IR activity, and from 16-25 bands for fingernail sets with medium IR activity. Having more IR bands allowed better discrimination between the fingernails sets. This in turns is advantageous especially in early disease diagnosis where pattern recognition between healthy and diseased fingernails can be established (12). Despite differences in the number of bands, all fingernail sets shared the same maximum band position (between 1630 – 1650 cm^{-1}) that corresponded to the nails' proteins. The absorbance intensity of the protein band was in the range 0.02–0.25 absorbance units. It is worth noting that the absorbance of 0.02 was seen for an individual with anxiety (LJMUWN1), and this confirmed the findings of Cashman and Sloan's (2010) study, which suggested that psychological conditions affected the nail's keratin (12). In addition, S/N varied between nails and was seen in the range of 50.5-130 for fingernails with strong IR activity, and in the range of 5.4 – 43.3 for fingernails with medium IR activity. Low S/N was seen for fingernails with high moisture content, this could be attributed to the individuals' diet.

Fingernails Classification

PCA analysis was often applied to large dataset to reduce dimensionality of data and to increase interpretability without information loss (Figure 2). During this analysis, spectral data was classified into the subspaces of PC scores and PC loading plots. The PC scores showed patterns of clusters among the different fingernails; whereas the PC loadings indicated which absorbances were important for each PC loading. When applied to the 19 sets of fingernails, the first three PC scores captured 93.1% of the variance but showed overlap among the fingernail scores apart from two fingernail sets. The latter two sets (sets 5 and 6) were from individuals of Arab ethnicity, and this demonstrated the ability of IR to classify participants' characteristics. This finding would be beneficial in diseases' management where personalized therapy is required.

Detection of Cocaine in Nails

The ability of ATR-FTIR to detect cocaine has been evaluated through the analysis of fingernail clippings spiked with cocaine hydrochloride for a period of six weeks. Table 4 shows the main bands attributed to cocaine hydrochloride that were featured in the spectra of fingernails. The key band attributed to the benzene ring stretching at 1072 cm^{-1} was featured in the nails' spectra in weeks 1-6. This further confirmed that nails act as drug depot i.e. accumulating drugs over time. This latter finding supported previous literature that identified historic drug use through the analysis of nails as an alternative matrix. A greater window of detection could be attributed to how the drug is deposited into the nail through the blood supply. In this sense, drugs are deposited by diffusion into the germinal matrix and nail bed, which grows at a rate of 3.47 mm per month (1). As the nail grows, the drug is deposited horizontally and vertically into the nail, and this explains drug detection of up to six months after consumption.

In the specific case of cocaine, cocaine has high affinity to proteins that results in cocaine-protein interactions (28). These interactions are reliant on the concentration of the cocaine consumed/exposed to (28). Therefore, individuals who are chronically exposed to cocaine, are more likely to have higher concentrations of cocaine within the fingernails and more cocaine-protein interactions. As previously mentioned, fingernails are composed of a laminated keratinized structure of alpha keratin (29). Keratins are a group of structural fibrous proteins that are often found in ectodermal tissues, hair, and nails (29). Unlike other structural proteins, such as collagen and elastin, which are found in bones and teeth, keratin has sulphur-rich amino acids, cysteine, and disulfide links (30). Therefore, cocaine has a high affinity for proteins within the fingernail and is easily deposited.

To visualise patterns among the spectra of the cocaine spiked fingernails' spectra over the six weeks period, PCA models were applied. The first three PCs contributed to 96.7% of the variance among the data (Figure 4). PC scores indicated three distinct clusters yet an overlap within and between the cluster. Hence, cluster one that contributed to the highest variance among the data showed scores corresponding to weeks five and six. This indicated that cocaine deposited over longer duration of time and that was further confirmed by the PC1 loading that showed bands corresponding to cocaine. Cluster two showed the second highest variances among the data and contained scores of weeks one and three in addition to one score for week four, four scores for week five and three scores for week six. This in turn showed type II error

where the scores of weeks four, five and six were clustered with weeks one and three. Cluster three showed scores with the least variance and that corresponded to week four with one score of week five. This could be attributed to the change resulting from the interaction of cocaine with the nail's proteins; however, further research is required for confirming such interaction. Nonetheless, the pattern of clustering has shown that PCA was successful in monitoring the depot.

Conclusion

ATR-FTIR spectroscopy offered a rapid and portable technique for the detection of endogenous constituents and cocaine within fingernails. The results demonstrated that the IR spectra of the fingernail sets indicated individuals' characteristics and disease status that influenced the protein constituents featured in the IR spectra. Furthermore, the length of exposure to cocaine, influenced the depositing of the drug within the fingernail and increased the likelihood of cocaine-protein interactions. Subsequently, chronic exposure increased the probability of drug detection and provided useful information for court when investigating possession with intent to supply. In the future a multivariate quantitative approach (e.g. principal component regression) would be ideal to track the changes in cocaine concentration within the fingernail overtime.

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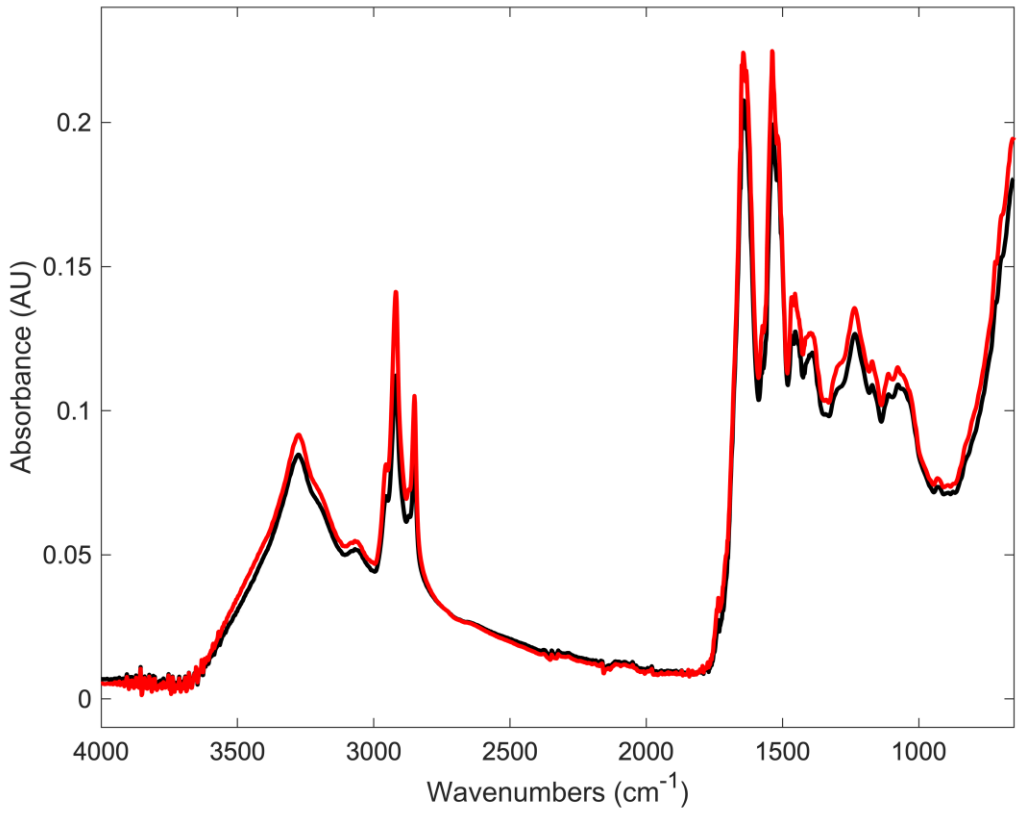

Figure 1. Raw IR spectra of the fingernails sets one (black) and set nine (red) measured using the portable Agilent 4500 ATR-FTIR spectrometer.

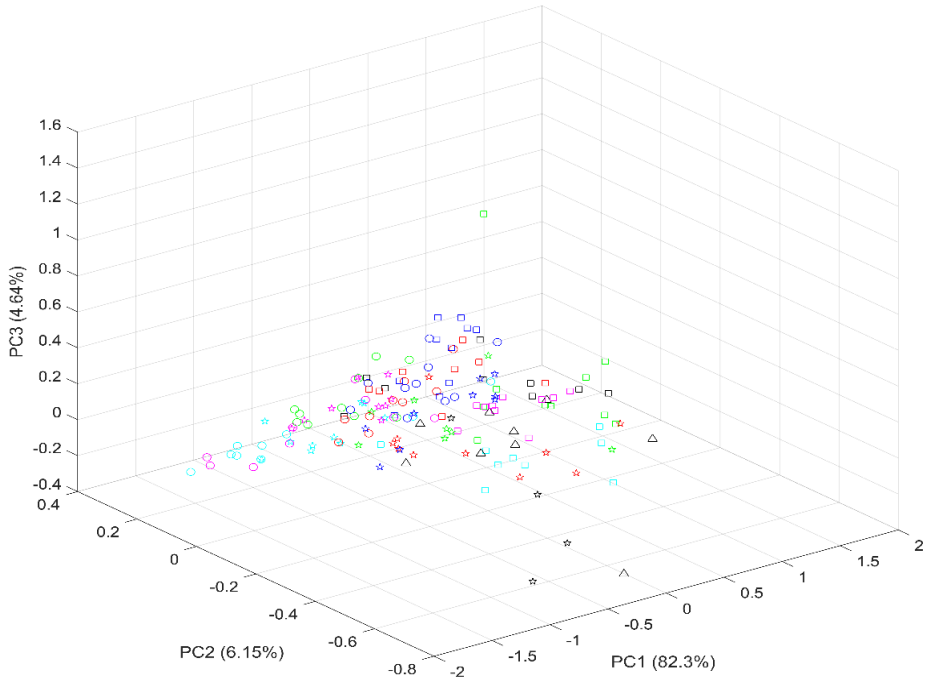

Figure 2. PC scores plot of the FTIR spectra of the fingernail sets (n = 19) measured using the portable Agilent 4500 ATR-FTIR spectrometer. Nail set one (black circle), nail set two (blue circle), nail set three (red circle), nail set four (green circle), nail set five (magenta circle), nail set six (cyan circle), nail set seven (black square), nail set eight (blue square), nail set nine (red square), nail set ten (green square), nail set eleven (magenta square), nail set twelve (cyan square), nail set thirteen (black pentagon), nail set fourteen (blue pentagon), nail set sixteen (red pentagon), nail set seventeen (magenta pentagon), nail set eight (cyan pentagon), nail set nineteen (black triangle).

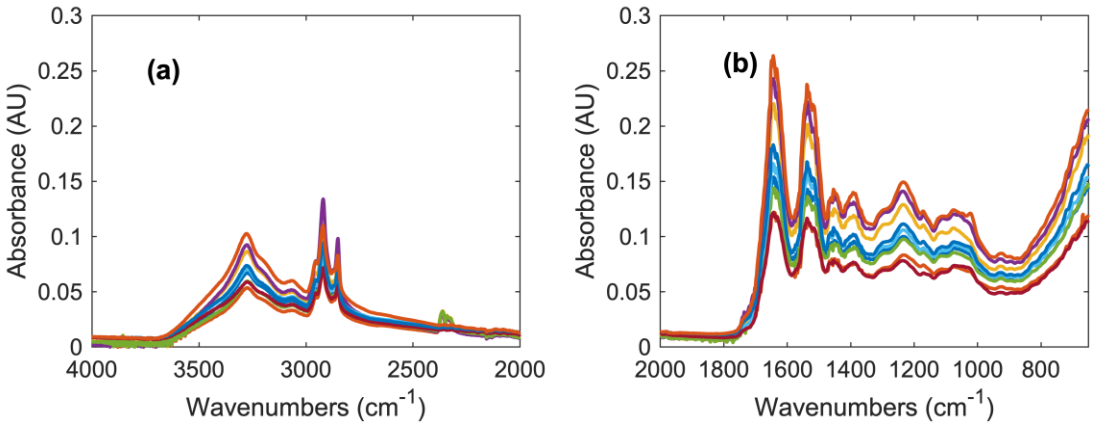

Figure 3. Raw FTIR spectra of fingernails spiked with cocaine for a period of six weeks measured using the portable Agilent 4500 ATR-FTIR spectrometer.

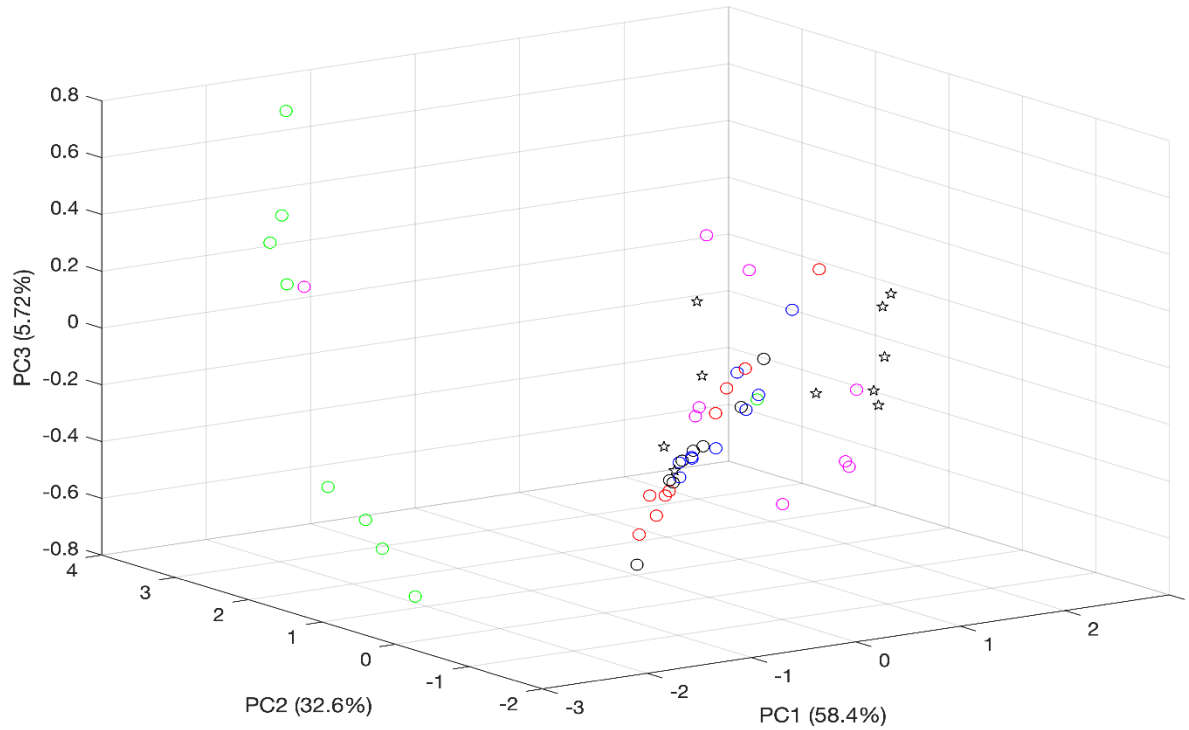

Figure 4. PC scores plot of fingernail clippings spiked with cocaine for one to six weeks using the portable Agilent 4500 ATR-FTIR spectrometer. Week one of spiking (black circle), week two of spiking (blue circle), week three of spiking (red circle) week four of spiking (green circle), week five of spiking (magenta circle), and week six of spiking (black pentagon).

Table 1. Details of participants' characteristics including age, biological sex, history of medicine use, long-term medical conditions, diet, lifestyle, and cosmetics' use.

Unique Identification Code	Age Range (Years)	Biological Sex	Ethnicity	Long-term Conditions	Medicines	Smoke	Vitamins, Supplements and Drugs	Medicines, vitamins, Supplements and Drugs Six Months	Diet	Exercise	Nail cosmetics
LJMUMWN1	40-64	F	White	Anxiety and asthma	Fostair inhaler	No	vitamin D	Mirtazapine	No	No	No
LJMUMWN2	18-24	M	White	No	No	Yes, smokes weed daily	2C-B, cocaine, helium, ketamine, LSD, MDMA, magic mushrooms, poppers, weed, Xanax.	Cocaine, ketamine, and weed	No	Yes	No
LJMUMWN3	40-64	M	White	No	No	No	No	No	No	No	No
LJMUMWN4	18-24	F	White	No	No	Yes, every couple of weeks	No	Weed	Low fat diet	No	Yes, nail polish
LJMUMWN5	40-64	F	Arab	No	Loratadine, omeprazole, and mebeverine.	Yes, daily	Multivitamins, biotin, collagen, hyaluronic acid	Loratadine, omeprazole, and mebeverine.	No	Yes	Yes, hand cream daily, nail polish
LJMUMWN6	40-64	M	Arab	No	No	No	No	No	No	No	No
LJMUMWN7	18-24	M	White	No	No	Yes, smokes weed daily	Cocaine, helium, ketamine, MDMA, and weed	Cocaine and weed	No	No	No
LJMUMWN8	40-64	M	White	Prostate cancer	Lansoprazole	No	Lansoprazole and vitamin D	vitamin D	No	No	Yes, hand cream daily
LJMUMWN9	40-64	F	White	Anxiety and asthma	Fostair inhaler	No	vitamin D	Mirtazapine	No	No	No
LJMUMWN10	18-24	F	Arab	No	Aspirin 75mg during winter	No	Cod liver oil tablets with omega 3, vitamins A and D, Perfectil	No	No	No	Nail polish, vegetable glycerin and essential oils
LJMUMWN11	18-24	F	Arab	No	Roaccutane	No	Roaccutane	No	No	No	Yes, nail polish and hand cream
LJMUMWN12	40-64	F	Arab	No	Loratadine, omeprazole, and mebeverine.	Yes, daily	Multivitamins, biotin, collagen, hyaluronic acid	Loratadine, omeprazole, and mebeverine.	No	Yes	Yes, hand cream daily, nail polish
LJMUMWN13	18-24	F	White	No	Microgynon once daily	No	Multivitamins and biotin	Multivitamins and biotin	No	No	Yes, gel nails every two weeks

LJMUMWN14	40-64	M	White	No	No	No	No	No	No	No	No
LJMUMWN15	40-64	M	White	No	No	No	No	No	No	No	No
LJMUMWN16	40-64	M	White	No	No	No	No	No	No	No	No
LJMUMWN17	40-64	F	Arab	No	Loratadine, omeprazole, and mebeverine.	Yes, daily	Multivitamins, biotin, collagen, hyaluronic acid	Loratadine, omeprazole, and mebeverine.	No	Yes	Yes, hand cream daily, nail polish
LJMUMWN18	18-24	M	White	Phenylketonuria	No	No	Multivitamins and Phlexy-10	Multivitamins and Phlexy-10	Yes, low protein diet. Most protein comes from supplementation.	Yes, 6 times a week	No
LJMUMWN19	18-24	M	White	No	No	No	Creatine monohydrate	Creatine monohydrate, ketamine and weed	No	Yes, 4 times a week	No

F: female; M: Male; 2C-B: 4-Bromo-2,5-dimethoxyphenethylamine; Fostair inhaler: is an antiasthma containing beclometasone dipropionate/ formoterol fumarate dihydrate 100/6 µg per actuation pressurised inhalation solution; LSD: Lysergic acid diethylamide; MDMA: 3,4-methylene-dioxy-methamfetamine; Perfectil is multivitamin brand sold in the UK, Phlexy-10: amino acid supplements, Roaccutane: isotretinoin; Xanax: alprazolam.

Table 2. Vibrational band assignment of endogenous constituents within human fingernails measured using the portable Agilent 4500 ATR-FTIR spectrometer.

Compound Name	Band Position (cm^{-1})	Band Assignment
Carbohydrates (total) (25, 26)	900 – 1180	C=C bending of alkenes
Carbohydrates (structural) (25, 26)	1188 – 1485	C-H bending of alkanes
Lipids (25)	2770 – 3000	Symmetric stretching of CH_2 and CH_3
Proteins (25, 27)	1600 – 1700	C=O stretching vibrations of amide amide I and amide II In-plane bending of the N-H and stretching of C-N bonds
Amino acid (25)	3400	Broad N-H stretching

Table 3. Spectral quality of the fingernail sets (n = 19) when measured using the portable Agilent 4500 ATR-FTIR spectrometer.

Sample Number	Sample Name	N	Maximum Band Position (cm^1)	Maximum Band Intensity	S/N Ratio	IR Activity
1	LJMUMWN1	16	1642.47	0.02	43.3	M
2	LJMUMWN2	20	1644.34	0.19	14.1	M
3	LJMUMWN3	19	1644.34	0.18	21.6	M
4	LJMUMWN4	19	1644.34	0.18	59.5	S
5	LJMUMWN5	21	1653.66	0.18	4.1	M
6	LJMUMWN6	19	1653.66	0.19	9.6	M
7	LJMUMWN7	23	1635.02	0.24	8.1	M
8	LJMUMWN8	21	1644.34	0.23	29.5	M
9	LJMUMWN9	22	1642.47	0.22	24	M
10	LJMUMWN10	22	1644.34	0.25	130	S
11	LJMUMWN11	21	1644.34	0.19	18.5	M
12	LJMUMWN12	26	1648.07	0.21	5.4	M
13	LJMUMWN13	25	1642.47	0.14	121	S
14	LJMUMWN14	23	1648.07	0.19	13.6	M
15	LJMUMWN15	25	1648.07	0.17	10	M
16	LJMUMWN16	25	1642.47	0.18	30	S
17	LJMUMWN17	21	1644.34	0.21	50.5	S
18	LJMUMWN18	22	1642.47	0.19	22	S
19	LJMUMWN19	20	1644.34	0.19	122	S

S: Strong; M: Medium; IR: infrared; N: Number of bands; S/N: signal to noise ratio.
The maximum band intensity unit is absorbance units.
Absorption range for all fingernails was 650-4000 cm^{-1}.

Table 4. Vibrational band assignment of cocaine hydrochloride within spiked fingernails measured using the portable Agilent 4500 ATR-FTIR spectrometer.

Compound Name	Band Position (cm^{-1})	Band Assignment
Cocaine hydrochloride	1728	C=O stretching
	1712	C=O stretching
	1265	C-O stretching
	1230	C-O stretching
	1105	C-O stretching
	1071	Monosubstituted benzene stretching
	1026	C-O stretching
	729	Monosubstituted benzene ring out-of-plane bending