# Infrared versus Raman Spectroscopy for Characterisation of Cocaine and its Impurities in Human Fingernails

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## Introduction

Fingernails have previously shown the ability to accumulate substances such as endogenous compounds, drugs, impurities and metabolites (Rathi *et al.,* 2020). Therefore, fingernails act a drug depot and a suitable alternative biological matrix to blood and urine (Figure 1).
Infrared (IR) and Raman spectroscopy offer the advantage of rapid and non-destructive detection of drugs in biological matrices. IR and Raman are complimentary in analysing drug products and provide key information regarding a sample's chemical characteristics.
Pharmacologically active impurities are more Raman active, while pharmacologically inactive impurities are more IR active.

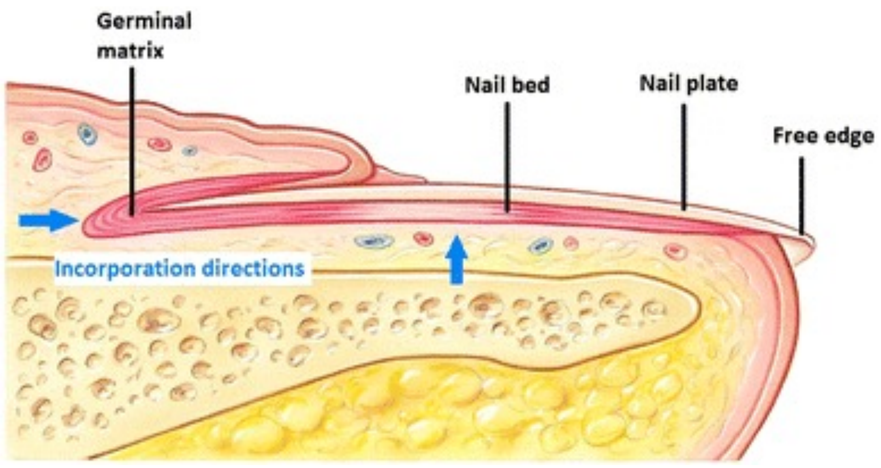


**Figure 1.** Sagittal section of fingertip, structure of fingernail (Marieb, 2012).

## Aim and Objectives

The aim of this study was to compare IR and Raman spectroscopic instruments for the characterisation of cocaine hydrochloride (HCl) and its impurities in human fingernails. The objectives were to:

- Explore the IR and Raman spectroscopic activity of fingernails, their endogenous compounds and drugs/impurities.
- Interpret IR and Raman spectra of un-spiked and spiked fingernails.
- Evaluate the potential of IR and Raman spectroscopy for the detection of drugs in fingernails.

## Materials and Methods

- Participants were recruited via LJMU's community site and provided a set of fingernail clippings.
- Ethical approval was provided from LJMU (PBS/2021-22/04).
- Each fingernail set was given an anonymous code and stored in a glass vial prior to analysis.
- Five sets of fingernails were spiked with cocaine HCl (COCN) or one of its impurities including: benzocaine (BEN), diltiazem HCl (DIL), levamisole HCl (LEV), lidocaine (LID) and procaine HCl (PRO).
- IR and Raman spectra of raw materials, un-spiked fingernails and spiked fingernails were collected non-destructively using the portable ATR-FTIR spectrometer and a portable Raman spectrometer (Figure 1) over a six-week period.
- Matlab 2019a was employed for spectral visualisation, interpretation and the application of machine learning algorithms (MLAs).

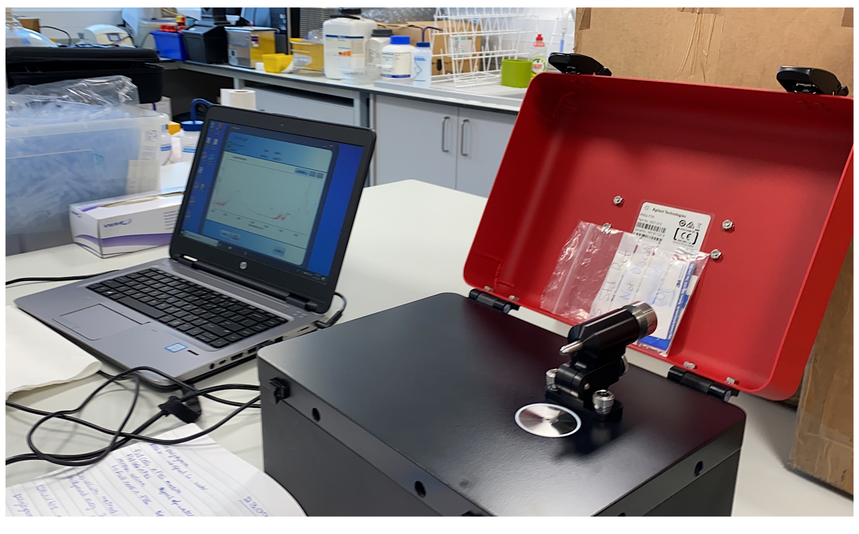

**Figure 2.** Portable ATR-FTIR spectrometer.

## Results and Discussion

Previous work has highlighted key Raman COCN bands at 848, 874 and 898 $cm^{-1}$ (C-C stretching of the tropane ring), 1004 (symmetric aromatic ring stretching), 1278 $cm^{-1}$ (C-N stretching), 1453 $cm^{-1}$ (Asymmetric $CH_3$ deformation), 1605 $cm^{-1}$ (C=C stretching) and 1712 $cm^{-1}$ (C=O stretching) (Santos *et al.,* 2022). However, fingernails are not highly Raman active, therefore the aforementioned bands were undetected. However, the two bands identified at 713 $cm^{-1}$ (rocking $CH_2$ in-plane) and 1087 $cm^{-1}$ (benzoic acid ring symmetric stretching/rocking $CH_2$), can be attributed to the deposition of COCN into the fingernails.
ATR-FTIR spectra (Figure 1b) showed key bands relating to COC at 500 $cm^{-1}$ (N-H stretching), 1728 $cm^{-1}$ (carbonyl group stretching), 1105 $cm^{-1}$ (C-O stretching) and 1071 $cm^{-1}$ (monosubstituted benzene stretching) that corresponded to COCN (Silvia *et al.,* 2018).

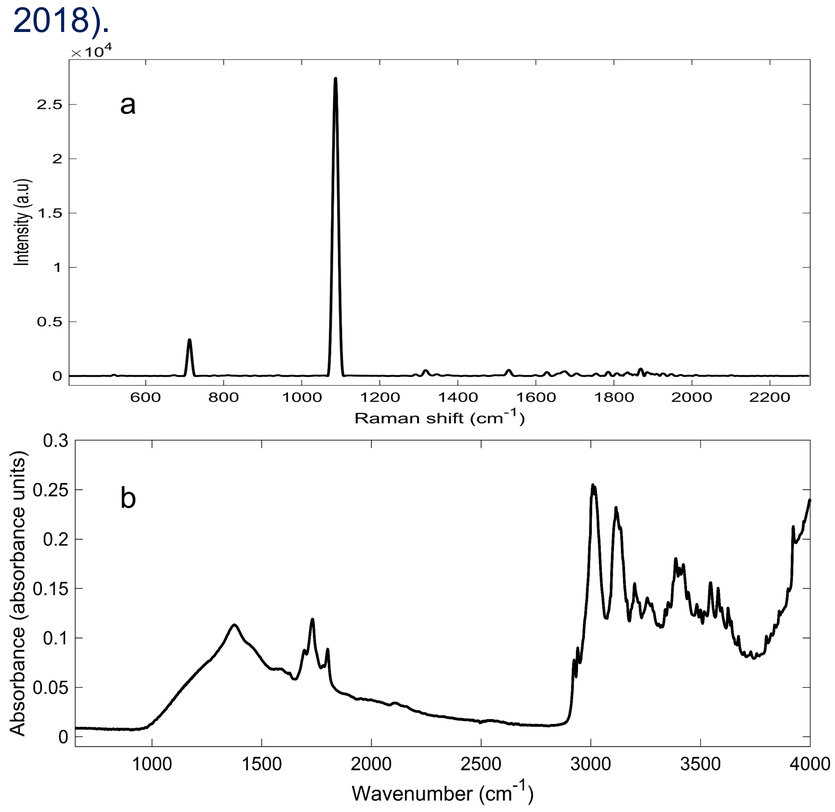


**Figure 3**. Fingernails spiked with COCN measured using a) Raman spectrometer and b) ATR-FTIR spectrometer

## Classification of Spiked Fingernails

When paired with MLAs, Raman spectroscopy demonstrated a higher level of specificity for COCN and its impurities in fingernails.
Principal component analysis (PCA) results showed that Raman spectroscopy were able to differentiate between the different sets of spiked fingernails.
PC1 demonstrated the highest variance among the data (42.8%) and showed scores relating to BEN, COCN, LID and PRO. PC2 showed the second highest variance (20.8%) and accounted for BEN, LEV and LID, and PC3 showed the third highest variance (6.8%) with scores relating to BEN. This is attributed to the incorporation of offset raster technology (ORS), which ensured that previous issues related to conventional Raman spectroscopy were limited (Geravand and Hashemi Nezhad, 2019). For example, the greater interrogation area improved the intensity and resolution of the Raman signal.

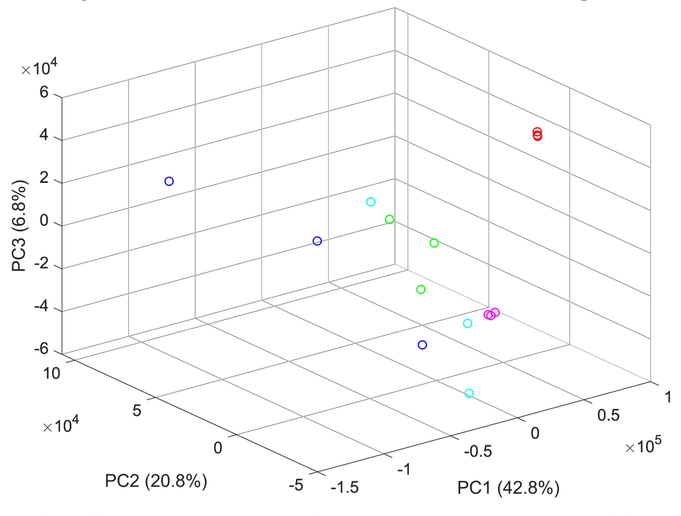


**Figure 4.** PCA scores plot of the Raman spectra of fingernails spiked with BEN (blue), COCN (red), LEV (green), LID (magenta), and PRO (cyan).

IR spectroscopy did not show the same level of specificity as Raman. This is demonstrated in figure 4. No clear clusters can be observed for the sets of spiked fingernails. This can be attributed to IR identifying chemical structures and when paired with PCA was unable to differentiate between the different sets of spiked fingernails. For example, LID and PRO share similar chemical characteristics such as benzene ring, ester bond and amide functional groups. The same can be said for COCN and DIL, which shared a benzene ring, ester bond and a nitrogen group. LEV also shares a benzene ring functional group and is chemically similar to DIL.

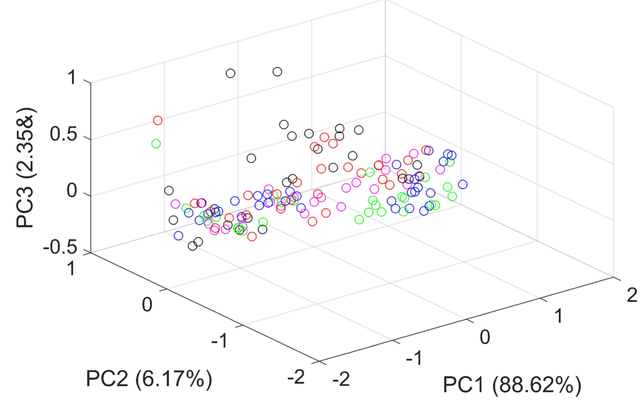


**Figure 4.** PCA scores plot of the ATR-FTIR spectra of fingernails spiked with BEN (black), COCN (green), LEV (magenta), LID (blue) and PRO (red).

Self-organising maps (SOM) confirmed PCA outcomes as seen in figure 5. No clear groups can be identified through the sample hit matrix, therefore suggesting that IR spectroscopy was not able to differentiate between the different sets of spiked fingernails.

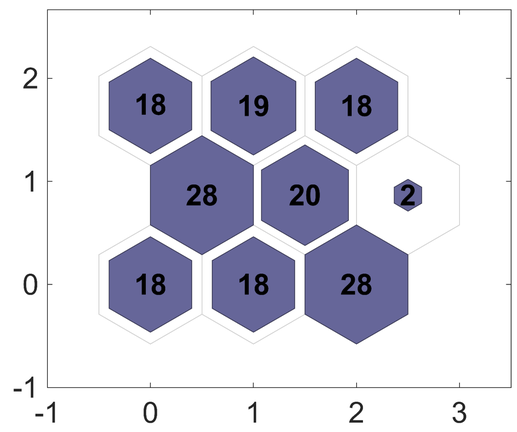


**Figure 5.** SOM sample hit map for ATR-FTIR spectra of spiked fingernails.

## Conclusions and Further Work

The results showed that IR and Raman spectroscopy could detect COCN in human fingernails. When paired with MLAs, Raman spectroscopy showed a higher level of specificity and was able to differentiate between the different sets of spiked fingernails. However, as fingernails show poor Raman activity, future work will look at developing a surface-enhanced Raman method for the detection of COCN and its impurities in fingernails. Furthermore, future will look at near-infrared spectroscopy as a complimentary technique, for detection of physiochemical properties. Future work will focus on a longer spiking duration, to increase the number of drug-protein interactions and drug deposition.

## References

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