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Rapid Detection of Synthetic Cannabinoid Receptor Agonists Impregnated into Paper by Raman Spectroscopy

الكشف السريع عن منشطات مستقبلات القنب الاصطناعي المشبعة في الورق بواسطة مطياف رامان

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Abstract

The last decade has witnessed the emergence of new psychoactive substances that are analogues of classical drugs of abuse in order to escape the regulations surrounding the latter drugs. These drugs were of both herbal and synthetic origin and were advertised initially as 'legal highs'; thus, they were perceived as safe by users. Hence, upon their emergence, they were not controlled by the Misuse of Drugs Act 1971, which contributed to their popularity and increased sales online and within street markets. In 2016, the Psychoactive Substance Act introduced a blanket ban on all new psychoactive substances except for caffeine, alcohol, and nicotine. This in turn, contributed to the change in the sale of new psychoactive substances products that have been sold as concealed in different matrices, including herbal products, papers, fabrics, and textiles. Concealing drugs in paper has been very popular, especially since the drug product is of lightweight and can be sent via postal services. However, new psychoactive concealed in papers are toxic not only to the users; but also, to the person handling them (i.e. mail employees). One of the classes of new psychoactive substances that have been commonly concealed in papers and that have been linked to toxicity and hospitalization cases is synthetic cannabinoids. Therefore, there is a need to identify new

المستخلص

شهد العقد الماضي ظهور مؤثرات عقلية جديدة مماثلة للمخدرات التقليدية المُنتشر؛ بغية التحايل على الأنظمة المتعلقة بالمخدرات التقليدية. هذه المؤثرات ذات منشأ عشبي واصطناعي، وتم الإعلان عنها في البداية على أنها "مخدرات قانونية"؛ لذلك، كان المتعاطون يعتبرونها آمنة. وبالتالي، لم تخضع عند ظهورها لقانون إساءة استخدام المخدرات لعام 1971، مما أسهم في ازدياد شعبيتها وزيادة مبيعاتها عبر الإنترنت وفي الأسواق الشعبية. في عام 2016، فرض قانون المؤثرات العقلية حظراً شاملاً على جميع المؤثرات العقلية الجديدة باستثناء الكافيين والكحول والنيكوتين. وأسهم هذا الحظر بدوره في التغيير الذي شهده بيع منتجات المؤثرات العقلية الجديدة؛ حيث أصبحت تُباع مخفية وبطرق مختلفة، ويتضمن ذلك إخفاؤها في المنتجات العشبية، والورق، والأقمشة، والمنسوجات. أصبح إخفاء المخدرات في الورق شائعًا جدًا، خاصة أن المخدرات خفيفة الوزن يمكن إرسالها بواسطة البريد. وهذه المؤثرات العقلية الجديدة المخفية ضمن الورق سامة ليس فقط للمتعاطين؛ ولكن أيضًا للمتعاملين معها (مثل موظفي البريد). ويُعتبر القنب الاصطناعي أحد أنواع المؤثرات العقلية الجديدة التي كثيرًا ما يتم إخفاؤها في الورق والمرتبطة بحالات التسمم والعلاج في المستشفيات. وبالتالي، برزت الحاجة لكشف المؤثرات

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psychoactive substances concealed in papers non-destructively and rapidly to prevent toxicity linked to them. Handheld Raman spectroscopy offers this advantage as it is of lightweight and carries the sample to the matrix. Therefore, this work used handheld Raman spectroscopy for identifying synthetic cannabinoids concealed in papers using Raman spectroscopy combined with machine learning analytics. Synthetic cannabinoid and paper samples were measured non-destructively using a handheld Raman spectrometer equipped with a 1064 nm laser wavelength. Spectral data was exported into Matlab 2020b where machine learning analytics including identification and prediction was. The results showed that Raman spectroscopy could identify specific synthetic cannabinoids in papers that were either deposited on the surface of the paper or diffused inside the paper substrate. When machine learning analytics were applied to the Raman spectra of the papers, quantitative information was obtained regarding the amount of synthetic cannabinoid deposited on the paper surface. In summary, handheld Raman spectroscopy could identify and quantify synthetic cannabinoids on paper rapidly and non-destructively. Future work involves testing other classes of new psychoactive substance and applying deep learning analytics

العقلية الجديدة المخفية في الورق بشكل غير إتلافي وبسرعة من أجل الوقاية من السمية المرتبطة بها. توفر مطيافية رامان المحمولة هذه الميزة؛ حيث إنها خفيفة الوزن وتحمل العينة إلى المصفوفة. بناءً على ذلك، استخدمت هذه الدراسة مطيافية رامان المحمولة لكشف القنب الاصطناعي المخفي في الورق؛ حيث تم استخدام مطيافية رامان جنبًا إلى جنب مع تحليل البيانات بواسطة تعلم الآلة. وتم قياس عينات القنب الاصطناعي والورق بشكل غير إتلافي باستخدام مطيافية رامان المحمولة المجهزة بأشعة ليزر ذات طول موجة 1064 نانومتر. ومن ثم تم تصدير البيانات الطيفية إلى برنامج (MATLAB R2020b) حيث يتم إجراء تحليل البيانات بواسطة تعلم الآلة بما في ذلك الكشف والتنبؤ. وأظهرت النتائج قدرة مطيافية رامان على كشف أنواع معينة من القنب الاصطناعي في الورق، وهي تلك المترسبة على سطح الورق أو المنتشرة داخل الركيزة الورقية. عند تطبيق تحليل البيانات بواسطة تعلم الآلة على أطياف رامان من الورق، تم الحصول على معلومات كمية حول كمية القنب الاصطناعي المترسبة على سطح الورق. وخلاصة القول، يمكن لمطيافية رامان المحمولة كشف القنب الاصطناعي على الورق وتحديد كميته بسرعة ودون إحداث تلف. كما تنطوي الأعمال المستقبلية على اختبار أنواع أخرى من المؤثرات العقلية الجديدة وتطبيق تحليل البيانات بواسطة التعلم العميق.

1. Introduction

Raman spectroscopy is based on the inelastic scattering resulting from the interaction of monochromatic light with the analyte of interest. Raman scattering reveals molecular information from which structure and chemical information can be extracted to characterise a wide range of chemical compounds. The advantages of using handheld Raman spectroscopy comprise that it is non-destructive, requires little or no sample preparation, and can measure through glass and transparent packaging [1-2].

Raman spectroscopy has been previously applied to the analysis of drugs of abuse and NPS such as amphetamine, benzodiazepines, cannabinoids, cocaine and opiates, which includes different polymorphs and salts forms [3-9]. Moreover, illicit drug residues impregnated into a variety of matrices had been detected using Raman spectroscopy which enabled law enforcement and border control to analyse suspicious materials containing drug compounds without being in direct contact with the substance [3,6, 10-14].

The matrices mentioned above included paper and cloth that could be sent by postal deliveries including fast parcels. Handling these parcels has been repeatedly reported to impact public health. For example, hospitalisation cases have been reported and attributed to prison staff-handling fast parcels [15]. Hence to minimise the adverse events related to handling parcels of NPS-impregnated papers, it is important to identify these parcels on-site. Handheld Raman spectroscopy offers this advantage. Moreover, the selectivity of Raman to chemical substrates offers a unique signature for every analyte. In face of these emerging challenges, various researchers have developed methods to detect substances impregnated into matrices such as alcohol and textiles [3,6,11]. The aforementioned

Table 1. *PLSR values for AKB-48, caffeine and procaine*

Substance	RMSEC (mg/mL)	r^2_{cal}	RMSEP (mg/mL)	r^2_{pre}
AKB-48	0.752	0.982	0.812	0.975
Caffeine	1.160	0.917	1.609	0.905
Procaine	0.450	0.994	0.587	0.990

RMSEC: root mean square error of calibration; RMSEP: root mean square error of prediction, r^2_{cal}: correlation coefficient value of the calibration set, r^2_{val}: correlation coefficient value of the validation set.
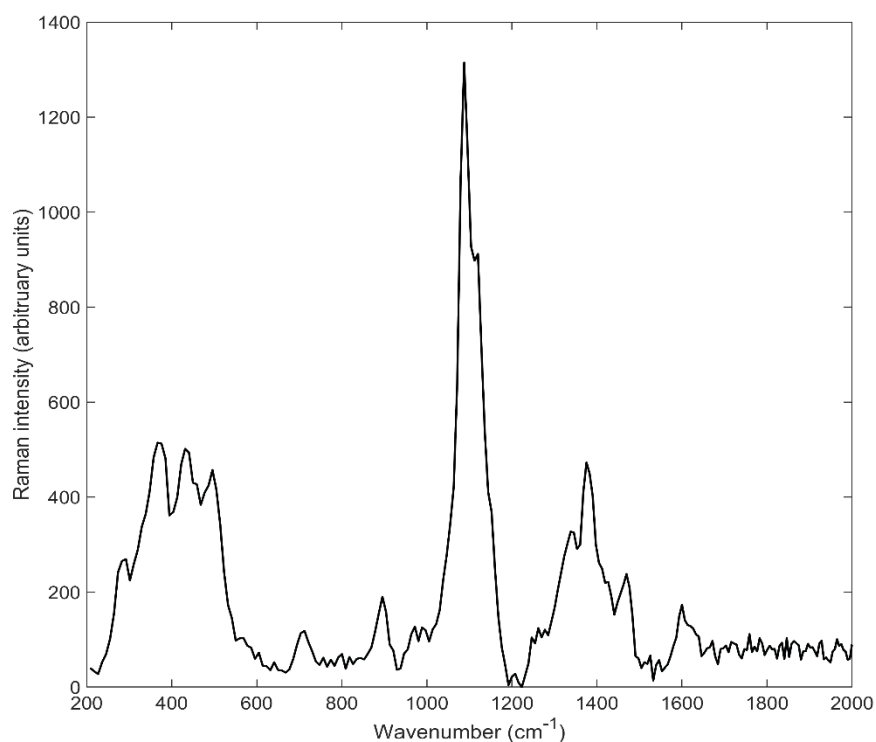

Figure 1. *Raman spectrum of blank paper 80 g/m² measured using the Rigaku handheld FT-Raman spectrometer.*

studies showed Raman was successful in detecting cocaine hydrochloride impregnated in textiles and alcoholic beverages. This research builds on existing research in detecting synthetic cannabinoid receptor agonists (SCRAs) impregnated in paper using handheld Raman spectroscopy.

2. Materials and methods

2.1 Standards and chemicals

Three SCRAs including AB-FUBINACA, AKB-48, and 5F-PB-22 were purchased from Sigma Aldrich. Four common impurities in NPS products benzocaine, caffeine, lidocaine and procaine were purchased from Sigma Aldrich. Ethanol of analytical grade (purity ~99.8%) was purchased from Sigma Aldrich. Five different densities of paper were purchased (75 g/m², 80 g/m², 90 g/m², 100 g/m² and 120 g/m²) from Navigator and were used for impregnating SCRAs and common impurities.

2.2 Instrumentation

Raman measurements were collected using

the handheld Rigaku FirstGuard 1064 nm Raman spectrometer equipped with a thermoelectric cooling, charge-coupled device detector, and 1064 nm laser wavelength. Each spectrum was the sum of 8 scans with 8 seconds exposure time and 490 mW laser power. Spectra were collected over the wavenumber range of 200 – 2000 cm^{-1}.

2.3 Procedure

The three SCRAs and four cutting agents were dissolved in ethanol at four different concentrations (10, 15, 20 and 25 mg/mL) and impregnated into five different densities of paper (75, 80, 90, 100 and 120 g/m^2) through soaking. Paper samples of each density were cut into 1 cm^2 segments and then soaked in different solutions at ambient room temperature. After drying, ten spectra were collected for

each paper such that the position/side of the paper was changed after each spectrum.

2.4 Data analysis

The obtained spectra were analysed using Matlab 2020b. For spectral identification, the Raman spectra for each measured substance were inspected against tables of functional groups from the literature [6]. For quantitative analysis, partial least square regression was applied (PLSR) which predicted the concentration of the SCRA and/or impurity in the impregnated paper based on multiple variables. PLSR reduced the dimensionality of the data according to variance among the data [17]. Hence, data with the least important variables were rejected. This is done by finding factors that capture variance among the data such that factors are added one at each time. The first
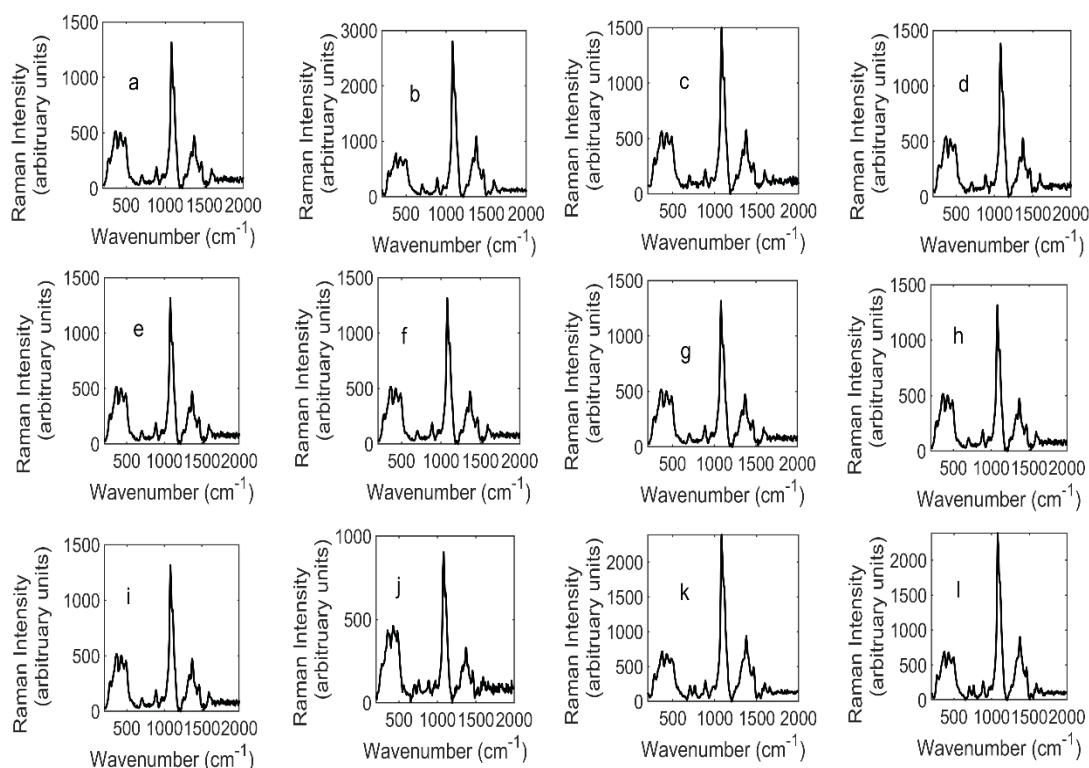

Figure 2. *Raw Raman spectra of (a) 5F-PB-22 impregnated into paper at 10 mg/mL (b) 15 mg/mL (c) 20 mg/mL (d) 25 mg/mL, AB-FUBINACA 22 impregnated into paper at (e)10 mg/mL (f) 15 mg/mL (g) 20 mg/mL (h) 25 mg/mL and AKB-48 22 impregnated into paper at (i) 10 mg/mL (j) 15 mg/mL (k) 20 mg/mL (l) 25 mg/mL measured by the handheld Rigaku FT- Raman spectrometer.*

PLSR factor captures the maximum variance among the data, the second captured the second maximum variance and so on. PLSR is defined by the following equations [17]:

$$X = T.P + E \quad \textit{Equation 1}$$
$$C = T.q + f \quad \textit{Equation 2}$$

X and Y are the matrices of predictors (n x m matrix of predictors) and responses (n x p of responses). T and U represent projections of X and Y scores, respectively. P and q represent orthogonal loading matrices for the projected X and Y scores, and E and f are the error terms for the predicted and responses matrix.

3. Results and Discussion

3.1 Spectral interpretation of the paper

Spectral interpretation of paper (Figure 1) was conducted over four regions being: 250 – 550 cm^{-1}, 550 – 950 cm^{-1}, 950 – 1200 cm^{-1} and 1200 – 1600 cm^{-1}. The first spectral range (250 – 550 cm^{-1},) showed several medium-intensity bands due to skeletal bending modes involving C-C (276 cm^{-1}), C-O-C (358 cm^{-1}), O-C-C (432 cm^{-1}) and O-C-O (487 cm^{-1}) functional groups.

The second spectral range 550 – 950 cm^{-1} showed two weak bands widely spaced were observed at 655 and 809 cm^{-1} and were attributed to the out-of-plane bending of C-C-C, C-O-C, O-C-O and C-C-O. The third range (950 – 1200 cm^{-1}) showed a very strong band at 1072 cm^{-1} and was attributed to the symmetrical stretching of C-C and C-O functional groups. In the fourth range (1200 – 1600 cm^{-1}) several medium-intensity bands were observed in the Raman spectrum and are attributed to C-C-H (1255 cm^{-1}), O-C-H (1331 cm^{-1}), C-O-H (1455 cm^{-1}) and H-C-H bending (1597 cm^{-1}).
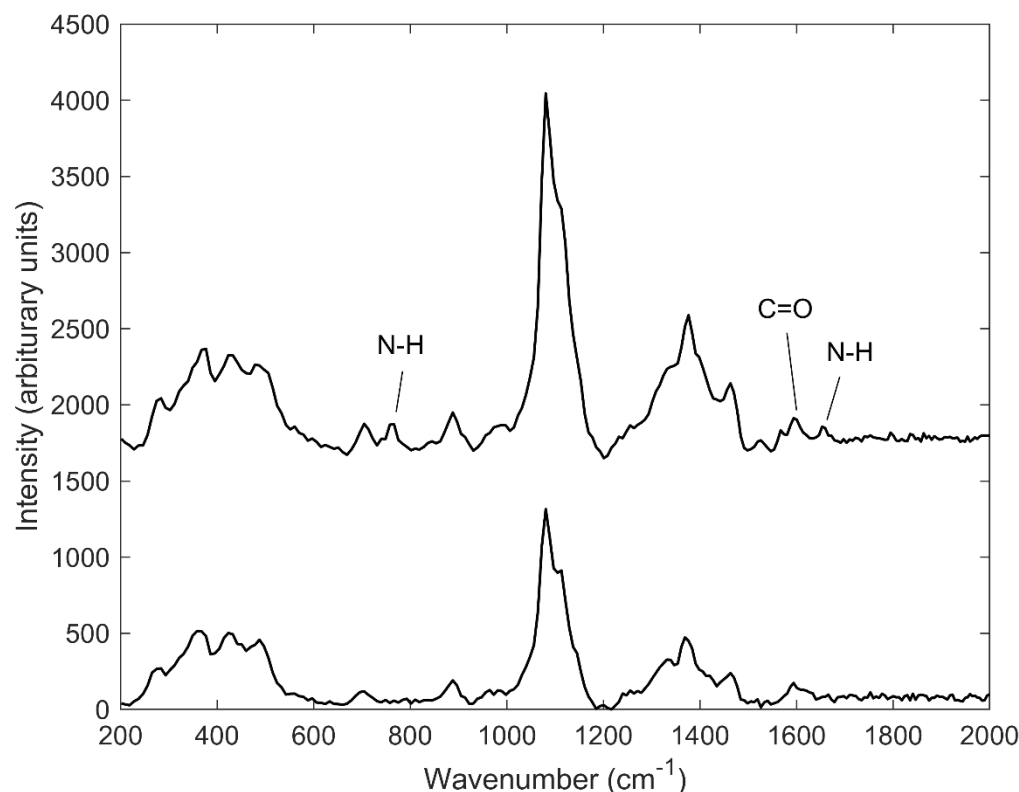

Figure 3. *Raman spectra of blank paper (bottom) and AKB-48 (top) impregnated into paper at 25 mg/mL concentration measured in the wavenumber region 200-2000 cm^{-1}. Only functional groups attributed to the AKB-48 were labelled.*
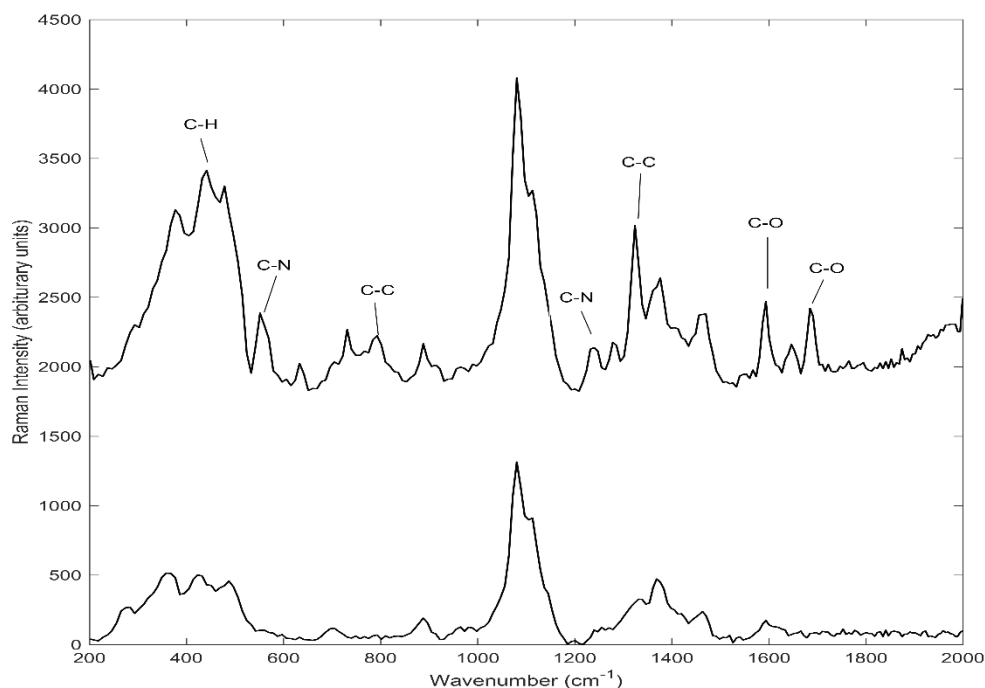
Figure 4. *Raman stacked spectra of blank paper (bottom) and caffeine (top) impregnated into paper at 25 mg/mL concentration measured in the wavenumber region 200 -2000 cm⁻¹. Only functional groups attributed to caffeine were labelled.*

3.2 Spectral interpretation of SCRA impregnated into the paper

Of the three SCRAs impregnated into paper (Figure 2), only AKB-48 was detected in concentrations of 20 mg/mL and above. AKB-48 spectra (Figure 3) showed three sharp peaks (one weak and two medium) attributed to two functional groups at 650 (NH of amide), 1650 (CO of amide) and 1680 (CO of amide) cm⁻¹.

The aforementioned two peaks were seen at 1680 (medium sharp) and 650 cm⁻¹ (weak sharp), respectively. An additional medium sharp peak at 1650 cm⁻¹ was attributed to the stretching of the C=O functional group of carbonyl amide.

3.3 Spectral interpretation of cutting agents impregnated into the paper

Caffeine impregnated into paper was detected in concentrations of 15 mg/mL and above. At 15 mg/mL functional groups attributed to caffeine were observed at 790, 1312 and 1597 cm⁻¹ attributing to C-C stretching (790 and 1312 cm⁻¹) and out-of-phase stretching of C=O (1597 cm⁻¹) peaks. At 20 mg/mL three more peaks were observed attributing to pyrimidine ring deformation (C-N) at 550 cm⁻¹, secondary amine symmetrical stretching (C-N) at 1311 cm⁻¹ and in-phase stretching of carbonyl amide (C=O) at 1680 cm⁻¹. Caffeine impregnated into paper is shown in Figure 4.

Interesting features concern the caffeine bands at 450, 515, 790, 1207, 1312, 1597 and 1680 cm⁻¹ and are assigned to methylene rocking (C-H), pyrimidine ring deformation (C-N), skeletal vibrations (C-C), symmetrical stretching of a secondary amine (C-N), indazole ring symmetrical stretching (C-C) and out-of-phase (C=O) and in-phase stretching of carbonyl amide (C=O), respectively.

Procaine impregnated into paper was detected in 20 mg/mL and above concentrations. Four functional groups were identified in the Raman spectra
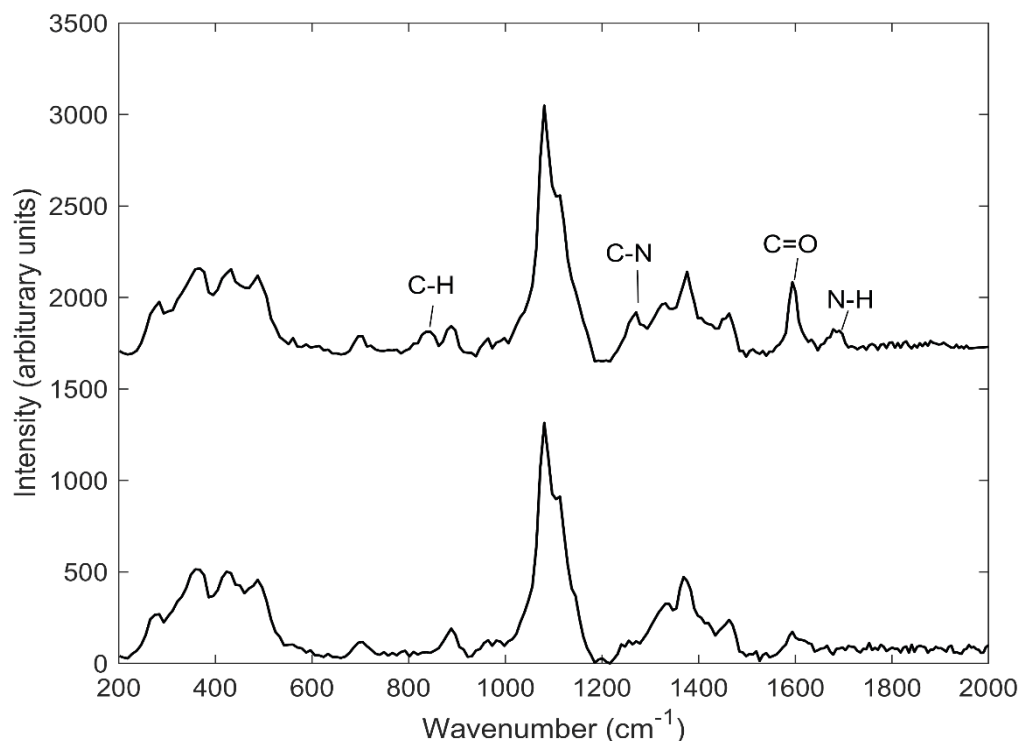
Figure 5. *Raman stacked spectra of blank paper (bottom) and procaine (top) impregnated into paper at 25 mg/mL concentration measured in the wavenumber region 200 -2000 cm⁻¹. Only functional groups attributed to the drugs are labelled.*
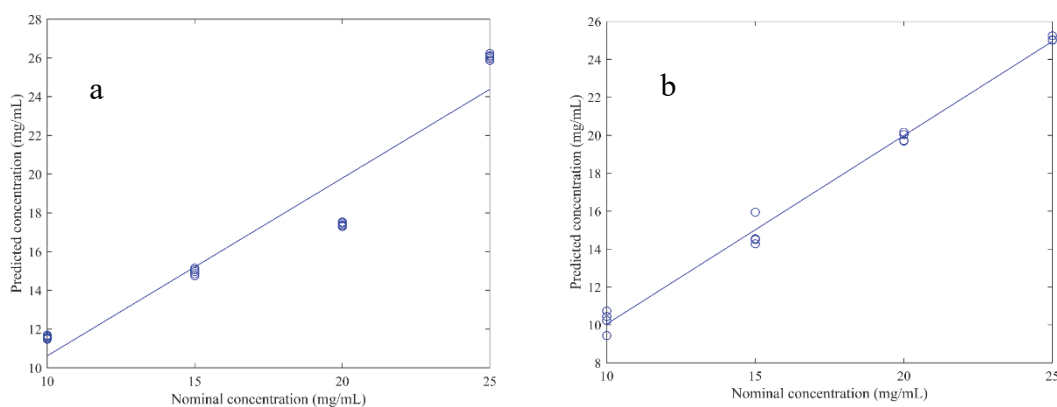

Figure 6- *PLSR calibration models of the Raman spectra of (a) caffeine and (b) procaine HCl impregnated into paper.*

of procaine (Figure 5) and included methylene (C-H) (850 cm⁻¹), carbonyl ester (C=O) (1597 cm⁻¹), tertiary (C-N) (1311 cm⁻¹) and aromatic amine (N-H) (1674 cm⁻¹).

3.4 Quantification of AKB-48 using PLSR

AKB-48 was the only SCRAs derivative detected impregnated into paper using Raman spectroscopy, so a PLSR model was developed only for AKB-48. The model validity was assessed with RMSEC, RMSEP, r^2_{cal} and r^2_{pre}. The calibration model for AKB-48 showed RMSEC and r^2_{cal} values of 0.752 mg/mL and 0.982, respectively. The prediction model showed similar values to the calibration model with

RMSEP and r^2_{pre} values of 0.812 mg/mL and 0.975, respectively. The random pattern for the residual plot and high r^2 values indicate a good PLSR model performance.

3.5 Quantification of caffeine and procaine using PLSR

For the two cutting agent compounds that were detected impregnated into paper using Raman spectroscopy, PLSR models were developed to quantify caffeine and procaine concentrations. The model validity was assessed with RMSEC, RMSEP, r^2_{cal}, and r^2_{pre} with VIP-selecting variables under 5 factors having the best performance. These results demonstrated that the PLSR models for the aforementioned cutting agents had a good performance. For the caffeine calibration model dataset, it showed RMSEC and r^2_{cal} of 1.160 mg/mL and 0.917. The RMSEP and r^2_{pre} were similar to the calibration set of 1.609 mg/mL and 0.905. This further confirmed the robustness of the models.

The best performance of PLSR for cutting agents used was procaine. The RMSEC and r^2_{cal} of the calibration set were 0.450 mg/mL and 0.994, respectively. The RMSEP and r^2_{pre} were 0.5872 mg/mL and 0.990 with a random pattern for the residual plot, indicating a good PLSR model performance.

4. Conclusions

In summary, the use of Raman spectroscopy equipped with a 1064 nm laser wavelength has proven to be successful in detecting 40% of the evaluated samples impregnated into paper. Where detected, key functional groups such as carbonyl and amide groups were seen in the spectra of the measured samples. The sensitivity was a challenge as the limit of detection of Raman spectroscopy was above 15 or 20 mg/mL depending on the substance. However, Raman spectroscopy showed to be quan-

titative around these concentrations. Hence, when PLSR was applied to the Raman spectra of the three detected drugs accurate and precise results were obtained with r^2 values in the range of 0.90 – 0.99 and RMSE values in the range of 0.44 – 1.6 mg/mL. Future work involves improving the sensitivity of quantification of substances impregnated in a paper by either using surface offset Raman spectroscopy or transmission Raman spectroscopy. The latter two techniques can detect drugs beyond the surface of the paper and hence are preferred for quantification where the bulk of the sample is needed.

Conflict of interest

Source of funding

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