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The evaluation of dual laser handheld raman spectroscopy for identifying novel psychoactive substances

The evaluation of dual laser handheld Raman spectroscopy for identifying novel psychoactive substances

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Abstract

Novel psychoactive substances have been increasing over the last decade with more than 450 derivatives available on the market. The issue with novel psychoactive substances is much more complicated than their effects/side effects. Hence, these substances often contain mixtures of pharmacologically active/inactive impurities which interfere with their effects. The accelerated development of these substances (at a rate above once a week) urges the need to develop rapid and mobile techniques for their characterisation. Handheld Raman spectroscopy offers the advantage of being quick, non-destructive and specific to chemical entities within the measured analyte. One issue with the Raman signatures of analytes is associated with several variables including the laser wavelength that could be shorter (such as 532 - 785 nm) or longer wavelength lasers (such as 833 – 1064 nm). Using a longer wavelength laser decreases the fluorescence of the sample, but decreases peak resolution and thus limits the sensitivity of detection. Up-to-our knowledge the use of dual laser wavelength for identifying novel psychoactive substances has not been explored. Therefore, this work aims to evaluate the use of dual laser handheld Raman spectroscopy for identifying novel psychoactive substances.

Introduction

The last decade has witnessed the emergence of novel psychoactive substances (NPS) as analogues of classical drugs of abuse (such as amphetamine and cocaine) in order to escape the regulations surrounding them [1]. The first NPS derivative on the market was mephedrone (4-methyl methcathinone) which appeared on the UK

market in 2007 [2]. Since its emergence, mephedrone (meow meow, MKat) became popular due to its strong stimulant and subjective effects. It has been used at variety of scenes including festivals, parties, nightclubs and users' homes. Mephedrone was then followed by the appearance of numerous cathinone derivatives such as naphyrone, methylone, butylone, methylenedioxypyrovalerone, etc… Though cathinones offered strong stimulant and subjective effects, many side effects were associated with their use. However, the aforementioned side effects could not be attributed to the particular cathinones, the impurities present in them or the combination of drugs taken. Cathinones were banned in 2010 [3], and the ban was the first world's generic ban that was based on chemical structure [4]. Subsequently, new classes emerged such as aminoindans (amphetamine analogues) and phencyclidines (ketamine analogues). Phencyclidines were subsequently banned in 2012 [5], and newer derivatives of diverse classes started to appear on the market. Until 2015, the European Monitoring Centre for Drug and Drug Addiction-Early Warning system reported more than 450 NPS derivatives on the market [6].

The popularity of these products were attributed to several reasons: (1) they are often advertised as 'legal highs' and thus perceived as safe by the users, (2) they are cheaper than classical drugs of abuse, (3) they are easily purchased via the Internet and (4) they have the ability to produce unique subjective effects [1].

However, the issues associated with NPS products are much more complicated than their effects/side effects. Hence, these substances often contain impurities which can interfere with their effectiveness and toxicities. Impurities present could be pharmacologically active or inactive substances [7]. Reported impurities found in NPS products ranged between two and up to six impurities. Reported pharmacological active ingredients were: alternative NPS derivatives (for example 2-aminoindan instead of 5-iodo-2-aminoindan), benzocaine, caffeine, lidocaine, procaine and paracetamol. Furthermore, celluloses, sugars and talc were stated as pharmacological inactive ingredients present in NPS products [1].

The rapid and continuous emergence of these products, alongside their complex matrices, stimulates the need for developing rapid and mobile technologies for their identification. In this respect, the ideal technique would be rapid, non-destructive and

able to identify multiple ingredients present in a single product. Handheld spectroscopic techniques offer the advantage of being mobile, quick and inexpensive for identifying various drug products including NPS. More specifically, handheld Raman provides distinct signatures of the measured NPS products which corresponded to specific chemical constituents within a sample [8]. However, the Raman activity of the analyte measured is highly dependent on the interaction of the analyte with the laser and the laser wavelength used. Hence, fluorescence decreases with the use of longer wavelength lasers such as 1064 nm [8]. On the contrary, sensitivity is better with shorter wavelength lasers such as 785 nm. Consequently, an ideal approach to Raman measurements would incorporate the use of two lasers in order to achieve sensitive detection with low/no fluorescence [8]. Up-to-date, the use of dual laser Raman for identifying NPS products has not been explored.

Therefore, this work aims to evaluate the application of dual laser handheld Raman for identifying drugs/impurities present in NPS products.

Methods

A total of 10 NPS products were purchased from four Internet websites (Table 1). Pharmacologically active and inactive constituents that are commonly present in NPS products were purchased from chemical suppliers.

Table 1. Details of the NPS products used in this study.

N	Product name	Label Claim	Substance(s) detected
P1	2AI	2-aminoindan	2-aminoindan MCC
P2	2AI	2-aminoindan	2-aminoindan MCC
P3	3-FMP	3-fluorophenmetrazine	2-aminoindan benzocaine, paracetamol
P4	4-FMPH	4-fluoromethylphenidate	MCC
P5	China White	methiopropamine	benzocaine
P6	Diclazepam	diclazepam	diclazepam MCC
P7	Doves	butylone	butylone

P8	Flubromazepam	flubromazepam	flubromazepam MCC
P9	Phenazepam	phenazepam	phenazepam MCC
P10	Poke	methiopropamine	methiopropamine

N: Product number, MCC: Microcrystalline cellulose

Three handheld Raman instruments were used in this study. Two of these instruments had mono-laser wavelengths that were 785 and 1064 nm respectively. The third instrument was equipped with a dual laser wavelength. All instruments used a laser output < 100 mW and were equipped with cooled charge-coupled device detector.

Samples were measured 'as received' without any treatment. Tablets were placed in direct contact with the sample holder and both sides of each tablet were measured. In addition, powders were measured via transparent glass vials. In all cases, three spectra were collected per sample such that each spectrum was the sum of one scan. For data analysis, spectra were exported into Matlab 2014b, where spectral comparison and principal component analysis (PCA) were applied.

Results and Discussion

The majority of the NPS products investigated in this study did not match their label claim. Hence, these products showed the presence of variable impurities which included both pharmacological active and inactive ingredients (Table 1). This confirmed the literature regarding the impurities present in NPS which could results in unpredicted health consequences [1]. The presence of inactive ingredients was not an issue in tablets-NPS products (P6, P8 and P9) which were expected to have excipients as part of their formulation. Hence, both P6 (diclazepam) and P8 (flubromazepam) contained MCC as an excipient. However, the presence of additional ingredients was not expected in powder-NPS products (P1-P5) which contained mixtures of pharmacologically active and inactive substances. For instance, P1 and P2 (2AI) were advertised as 100% pure; yet, both products contained a mixture 2-aminoindan and MCC (inactive substances). Additional products did not match their 'label claim' and contained pharmacological active ingredients. For instance, China

White was advertised as an NPS product containing methiopropamine, yet it showed to contain benzocaine as pharmacological active ingredient. Only one NPS product matched its label claim and was Doves which showed Raman spectra corresponding to butylone (its pharmacological active ingredient).

As the aforementioned NPS products were in mixture-form, the choice of handheld Raman spectroscopy was ideal for their characterisation. Hence, handheld Raman spectroscopy gave signatures of the analyte(s) of interest [8, 9]. These signatures corresponded to the Raman active species that were present at detectable concentration(s). The choice of the Raman laser wavelength in this case was critical [9]. Using a handheld Raman equipped with a 785 nm wavelength-laser the Raman signature of the NPS product corresponded mainly to the pharmacological active substances present in the product (such as diclazepam in P6 (diclazepam), and benzocaine in P5 (China White)) provided they were at a detectable concentration (Assi et al. 2013). Conversely, pharmacological inactive ingredients (such as MCC) fluoresced and masked the overall signal of the NPS product when measured using a 785 nm wavelength laser (Figure 1).

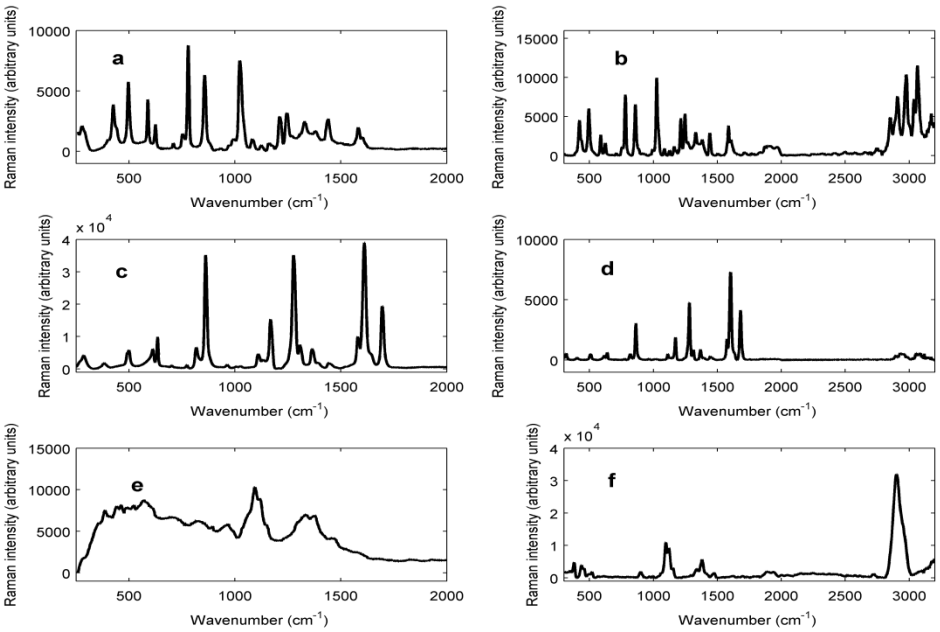

Figure 1 Raw Raman spectra of standards of (a) 2-aminoindan hydrochloride measured using a handheld Raman equipped with 785 nm wavelength laser, (b) 2-aminoindan hydrochloride measured using a handheld Raman equipped with dual wavelength laser, (c) benzocaine measured using a handheld Raman

equipped with 785 nm wavelength laser, (d) benzocaine measured using a handheld Raman equipped with dual wavelength laser, (e) MCC measured using a handheld Raman equipped with 785 nm wavelength laser and (f) MCC measured using a handheld Raman equipped with a dual wavelength laser.

The movement towards a longer wavelength lasers (such as 1064 nm) overcame the fluorescence issue but decreased the peak resolution; thus, interfering with the sensitivity of detection (Assi et al. 2013). Therefore, the choice of a dual laser Raman instrument within the near infrared range (NIR) range would offer the best of both lasers; i.e. increased peak identification and sensitivity. Figure 1 shows the raw Raman spectra of pure 2-aminoindan hydrochloride, benzocaine and MCC measured with a monolaser (785 nm wavelength) and a dual laser wavelength Raman (700 – 1100 nm). For all three materials, the range of the spectra acquired by both lasers had a wider range up to 3200 cm^{-1}. The latter range between 2800 and 3200 cm^{-1} corresponded to the CH and OH peaks. The aforementioned range could easily distinguish between the three raw materials (i.e. 2-aminoindan, benzocaine and MCC). This was an advantage over the monolaser Raman (both 785 nm and 1064 nm) which showed Raman signatures up to 2000 cm^{-1} only. Up to 2000 cm^{-1}, both the mono- and dual-lasers showed similar peak scattering. However, the peak resolution and the overall spectral quality was better using the dual laser. This impacted the classification of the NPS products which hugely corresponded to their main constituents (Figure 2).

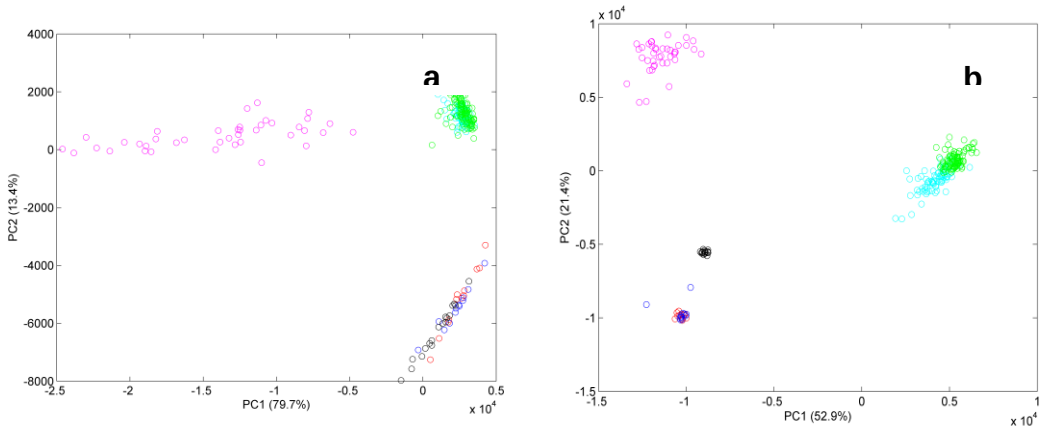

Figure 2 PCA score plots of the NPS products labelled as 4-FMPH (magenta), diclazepam (cyan), flubromazepam (green), 2-aminoindan (red and blue) and 3-

FMP (black) measured using handheld Raman instruments equipped with (a) 1064-nm laser wavelength and (b) dual laser wavelength.

Hence, the dual laser wavelength Raman showed better discrimination (as four individual clusters) than the monolaser wavelength Raman (three clusters). The clusters obtained using the dual laser wavelength Raman incorporated: cluster 1 (P4: 4-FMPH), cluster 2 (P1 and P2: 2AI), cluster 3 (P3: 3-FMP) and cluster 4 (P6: diclazepam and P8: flubromazepam). Nevertheless, using the monolaser wavelength Raman only three clusters were obtained and corresponded to cluster 1 (4-FMPH), cluster 2 (P6: diclazepam and P8: flubromazepam) and cluster three (P1 and P2: 2AI, and P3: 3-FMP). P6 (Diclazepam) and P8 (flubromazepam) were both tablets and shared a common inactive ingredient which was MCC. The clustering between P1, P2 (2AI) and P3 (3-FMP) was not expected as P3 (3-FMP) contained three additional ingredients to the 2-aminoindan hydrochloride, which were: MCC, benzocaine and paracetamol. Thus, the overlap encountered between the three aforementioned products could be attributed to the poor spectral resolution and overlapping features of the spectra acquired using the handheld Raman equipped with 1064 nm wavelength laser.

Consequently, the choice of the dual laser Raman was ideal for acquiring NPS products' spectra which contained multiple impurities (both pharmacologically active and inactive). The NPS products' signatures matched constituents present in these products which included both active and inactive constituents. For instance, China White consisted of pure benzocaine and showed Raman spectrum corresponding to benzocaine (Figure 3 a and b). Additionally, diclazepam and flubromazepam showed corresponding peaks to MCC (main inactive ingredient) in addition to their main pharmacological active ingredients peaks (Figure 3 c and d).

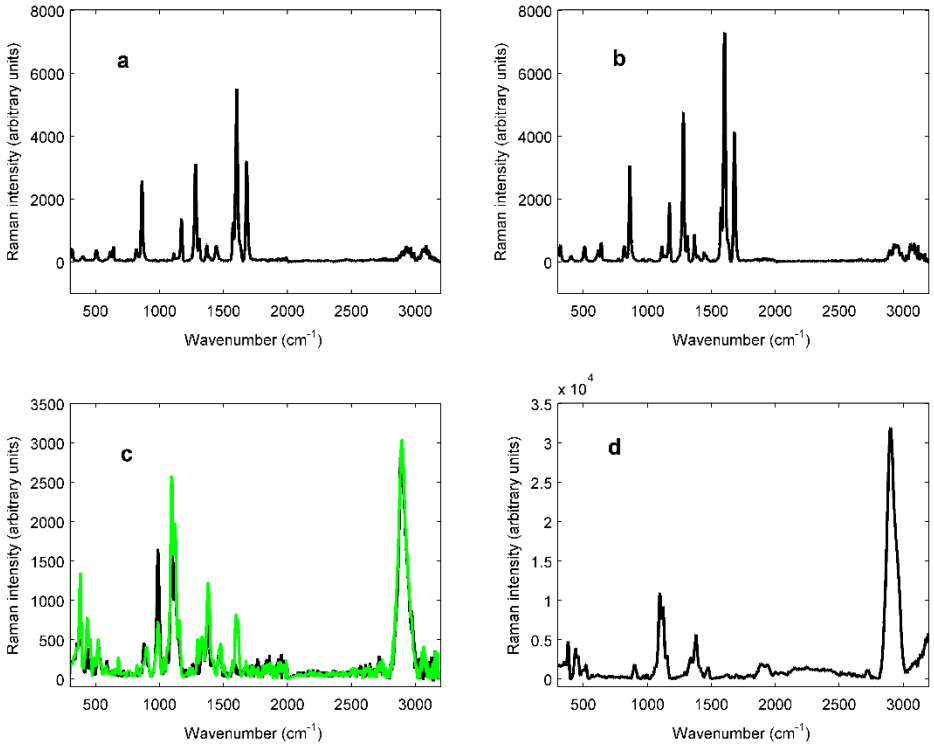

Figure 3 Raw Raman spectra of (a) China white NPS product, (b) standard benzocaine, (c) diclazepam and flubromazepam NPS products and (d) standard MCC measured using a handheld Raman spectrometer equipped with a dual wavelength laser.

Also, P3 (3-FMP) showed spectral features corresponding to 2-aminoindan, benzocaine and paracetamol (Figure 4 c, e and f). Additionally, 2-aminoindan products showed peaks corresponding to both 2-aminoindan hydrochloride and MCC (Figure 4 a, b, and d).

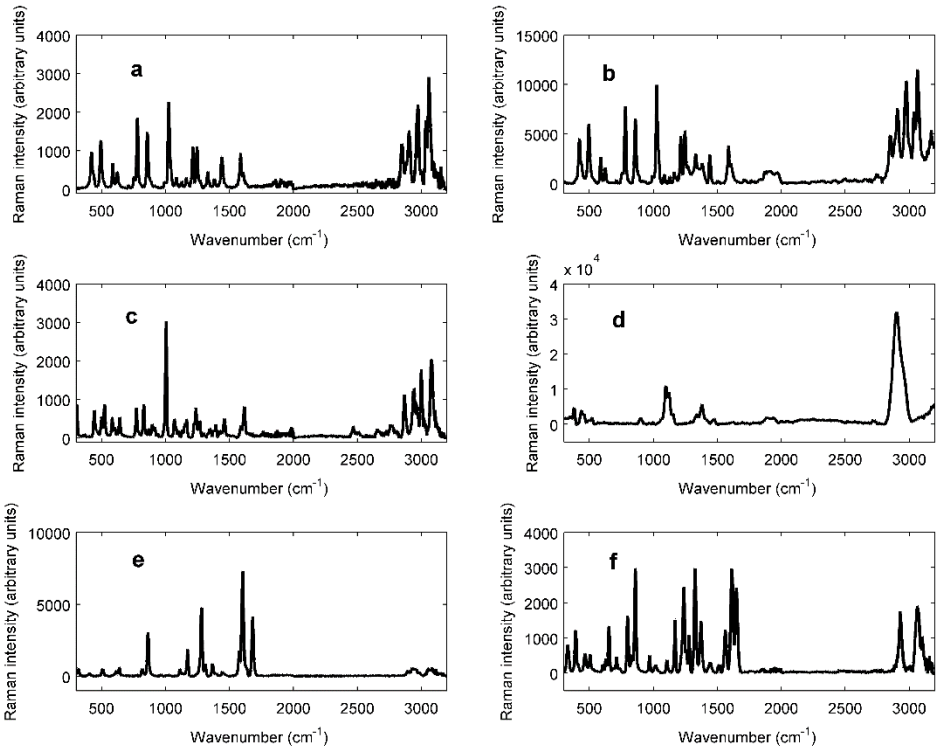

Figure 4 Raw Raman spectra of (a) 2-aminoidan NPS product, (b) standard 2-aminoindan, (c) 3-FMP NPS products and (d) standard MCC, (e) standard benzocaine and (f) standard paracetamol measured using a handheld Raman spectrometer equipped with a dual wavelength laser.

Conclusion

Although NPS products are advertised as highly pure, they often contain a mixture of pharmacologically active/inactive ingredients which result in unpredicted consequences. The use of handheld Raman spectroscopy equipped with dual laser wavelength showed to be accurate in identifying both pharmacologically active/inactive ingredients present in NPS products. Future work involves the quantification of multiple ingredients present in NPS products using handheld Raman equipped with dual laser wavelength.

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