

# Analysis of friction ridge evidence for trace amounts of paracetamol in various pharmaceutical industries by Raman spectroscopy

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## Article

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# Abstract

The detection of potentially harmful substances as well as those dangerous to life is a multifaceted challenge. On the one hand, it can directly save lives, on the other hand, it can help and improve the work of the police, increasing effectiveness of the investigation. The research carried out within the framework of this study is primarily aimed at identifying paracetamol in fingerprints, taking into account situations of direct contact of a person with paracetamol chronically or in a single high dose. The identification procedure presented, using Raman spectroscopy, aims to rapidly identify the xenobiotic after ingestion by the person - requires touching the tablet with the fingers, which we can name as touch evidence in forensic science investigation. In addition, the authors focus on the effect of additives present in drugs containing paracetamol as the main active ingredient. The results of the screening obtained will allow us to analyze the composition of drugs in terms of potentially toxic substances and their impact on the physicochemical activity of the active substance itself. The research methodology developed also allows for the quick detection of other substances dangerous to life and health, such as amphetamine, heroin, fentanyl, or morphine. In addition to the preventive function, it is also a new solution in the field of forensic methods.

## 1. Introduction

Raman spectroscopy is one of the most common methods used in forensic laboratories today [1-6]. The most recent reports in the literature indicate the unrivaled competitiveness of this method in forensic science due to its non-invasiveness. The overriding research priority when analyzing evidence is to use methods that will not damage the evidence. Of course, we are not always able to avoid the invasiveness of the method used, although Raman spectroscopy is completely unrivaled in this respect. The samples to be examined do not require any special preparation, so we can carry out "ad hoc" measurements even in the field if we have an environmental Raman – portable Raman spectroscopy. However, bearing in mind that the investigated Raman scattering effect is not strong i.e., only one photon per million incident photons is scattered, we must be guided by an excellent knowledge of the physical characteristics of this phenomenon, and fully adapt the technical parameters of the Raman apparatus to the analyzed samples. Given that light incident on the matter also undergoes other physical phenomena such as absorption, emission, refraction, and reflection. We need to adapt the Raman apparatus from the excitation source to the detection system in such a way as to redirect molecular spectroscopy observations to the observation of Raman scattering phenomena only. Raman spectroscopy has already been used to identify a wide variety of substances, especially drugs such as amphetamine, cocaine, ecstasy, or benzodiazepines [7-10], but we have no documentation so far that we can use this method in the identification of drugs and illegal substances in friction ridge. In contrast, we have support in the literature, the possibility of identifying substances directly from a fingerprint, despite the presence of other substances and the sweat-fat substance naturally occurring on our skin. They also describe that the spectra taken from the fingerprint were of similar quality to the reference spectra of the substances they selected. They also highlight the major problem of such identification - the localization of the substance on the trace [11,12].

Fingerprint examinations usually focus on latent fingerprints. These patterned deposits of sweat, skin cells, and other substances are one of the oldest forms of forensic evidence - concerning our research referred to as "touch evidence" relevant to toxicology and crime scene. In recent years, interest in the information that can be obtained from the chemical components overing or present in fingerprints has increased significantly. This applies to both the detection/disclosure of fingerprints and the possibility of obtaining further intelligence based on this type of evidence [13]. This directly confirms that the research area of forensic science is constantly changing. Forensic science must keep up not only with the developing crime but also with the pace of development of science and technology. The obstacle to this endeavor is imperfection of forensic techniques used to reveal and secure traces. We are talking here about forensic technical solutions that are characterized by unsatisfactory (and sometimes even insufficient) efficiency [14]. However, the implementation of new solutions in forensic practice must be subject to certain requirements. In this case, scientific and technological progress is not an exhaustive criterion. Only documented scientific knowledge may be used [15]. That is why it is so important to work on scientific standards that work not only to support reliable results from existing forensic analysis techniques, but also to help validate new ones. These efforts are intended to ensure that forensic techniques are ready to defy judicial scrutiny and provide the

best possible information. High standard of proof in criminal process demands that new type of evidence offered by prosecution in trial is reliable and verifiable, capable of providing a firm basis for making factual findings in the case.

This research work attempts to develop a test procedure of very high diagnostic value as a method to be used during evidence analysis. As part of the identification analysis of paracetamol, the possibility of identifying drugs and prohibited substances from the fingerprints of persons who came into contact with these substances was indicated. The diagnostic value of the presented method of basic identification analysis was precisely defined, using a reference analysis of a commonly available non-prescription drug - paracetamol.

Paracetamol, synthesized in 1878 by Harmon Northrop Morse, was ignored at the time due to its high toxicity. It regained popularity in 1948 thanks to the research of Brodie and Axelrod, who linked the occurrence of methemoglobinemia to acetanilide - of which paracetamol is a derivative [16, 17]. Acetanilide, despite its desirable antipyretic properties, caused methemoglobinemia, which means that it impaired the ability of hemoglobin to bind to and carry oxygen, which manifested as bruising, among other things. Further acetanilide derivatives such as phenacetin showed that aniline and p-Etoxyaniline were responsible for the impairment of these abilities, resulting from the deacetylation of these substances; however, another active compound resulting from the O-dealkylation of phenacetin was observed that was devoid of toxic effects, but still exhibited analgesic and antipyretic effects - p-hydroxy acetanilide, which is paracetamol [18-20].

Paracetamol was first launched in the U.S. in 1950 as Triogesic, a combination product because it also contained aspirin and caffeine but was withdrawn from the market by a false link to agranulocytosis. Paracetamol didn't hit the pharmaceutical market again until 1955 in the United States as Tylenol (only by prescription), a year later in Europe as Panadol and in 1961 it was available for sale in Poland. Deemed safe enough in therapeutic doses, it gained over-the-counter drug status in 1960 [21]. More than 100 years since the discovery of paracetamol and its widespread use for more than 50 years have not contributed to the understanding and elucidation of its exact mode of action, which includes several mechanisms. One of them, and attributed as the main one for paracetamol, is the inhibition of peroxidase (POX), resulting in the inhibition of prostaglandin synthesis (responsible for the formation of pain and fever) [18-20]. Paracetamol acts on many other levels responsible for the conduction of pain stimuli starting from tissue receptors, through the spinal cord to the thalamus and cortex. Among

Table 1. Biotransformation of paracetamol in the human body [24]

	TIME FROM TAKING THE DRUG	SYMPTOMS
PHASE I (may be asymptomatic)	30min – 24h	malaise, vomiting, feeling nauseous, pallor, weakness, increased sweating, gastrointestinal disturbances
PHASE II	24 - 72h	urinary excretion problem (kidney disorder), lethargy, pain in the right lower abdomen
PHASE III	72 - 96h	consciousness disturbance, skin yellowing, vomiting, metabolic acidosis, drop in blood pressure, cardiac arrhythmia, hypoxia, progressive liver damage
PHASE IV	4 days - 2 wks	progressively fulminating liver failure leads to death between 3 and 5 days after poisoning(if death does not occur, symptoms resolve)

One possible mechanism mentioned, in addition to the peripheral inhibition of COX activity, is inhibition of nitric oxide (NO) production via the L-arginine/NO pathway,

use of the paracetamol deacetylation metabolite in the cannabinoid system, or influence on the descending serotonergic pathways responsible for the suppression of pain stimuli [22]. The recommended daily dose for an adult is up to 4000 mg of paracetamol, taken at intervals, not more than 1 g of the substance at a time. Use as recommended - rarely or very rarely causes side effects, but they can appear most often in the form of malaise, vomiting, and nausea. We can observe side effects from most systems (table 1): cardiovascular system - thrombocytopenia, leukopenia; immune system - hypersensitivity reaction (from rash to anaphylactic shock); cardiovascular system - hypertension/hypotension; genitourinary system - renal dysfunction. The most serious

of all adverse effects of paracetamol is hepatotoxicity, which is observed with an intake of more than 10 g per day [22-23]. Biotransformation occurs in the liver, where about 5% of paracetamol is deacetylated, about 90% couples to glucuronic or sulfuric acid, and the remaining 5% is converted from NAPQI to mercapturic acid, unless glutathione reserves are depleted, more precisely, the sulfhydryl groups, the deficiency of which leads to hepatotoxicity [18-20].

The aim of this work is to propose Raman spectroscopy to determine the potential for identifying a drug or prohibited substance in a dactyloscopy trace as a rapid identification method using non-invasive friction ridge analysis required in a life-threatening situation involving a drug user, whether intentionally or not - deliberate overdose - in the event of a suicide attempt. Through the execution of an evidence model, the execution of experimental reference, and evidence matrices as practical tests, the diagnostic value of the presented test procedure for the identification analysis of drugs and illegal substances was presented using the example of paracetamol present in friction ridge.

## 2. Material and methods

Table 2. Reference material - paracetamol and the xenobiotics used in the study (personal study).

Name of drug:	Active substance [mg per tablet]	Excipients	Tablet type	Application of the medicine	Additional information from the manufacturer	Country of manufacture
<b>Drug no. 1</b>	Paracetamol [500mg]	Carnauba wax, hypromellose, macrogol, povidone, gelatinized starch, croscarmellose sodium, stearic acid	Coated tablets	Fever relief, analgesic	None	Poland
<b>Drug no. 2</b>	Paracetamol [500mg]	Povidone K30, gelatinized corn starch, sodium carboxymethyl starch type A, stearic acid 50	Capsule-shaped tablets	Fever relief, analgesic	The drug contains sodium [less than 1 mmol sodium (23mg) per dose].	Czech Republic
<b>Drug no. 3</b>	Paracetamol [500mg] Hyoscine butylbromide[10mg].	Microcrystalline cellulose, potato starch, povidone K30, sorbitol, talc, magnesium stearate, colloidal silica anhydrous, carboxymethyl starch sodium, hypomellose, macrogol 6000	Coated tablets	Analgesic, diastolic	Indication for use for menstrual pains	Poland
<b>Drug no. 4</b>	Paracetamol [500mg]	Gelatinized starch, calcium carbonate, alginic acid, crospovidone Type A, povidone K-25, magnesium stearate, colloidal silica anhydrous, purified water. FRAMEWORK: Opadry White - YS-1-7003 (composition: titanium dioxide(E171), hydromellose2910, 3cp, hydromellose2910,6cp, macrogol 400, polysorbate 80), Carnauba wax, purified water.	Coated tablets	Fever relief, analgesic	A special formulation of excipients with the effect of accelerating the disintegration of the tablet, resulting in a faster release process compared to standard paracetamol tablets.	Ireland
<b>Drug no. 5</b>	Paracetamol [500mg]	Sorbitol (170mg), potato starch, povidone, magnesium stearate	Tablets	Fever relief, analgesic	None	Poland
<b>Drug no. 6</b>	Paracetamol [500mg]	CORE: Gelatinized corn starch, hydroxypropyl cellulose, talc, magnesium stearate SHELL: Opadry II Clear 85F29116: Polyvinyl alcohol, macrogol 335, talc	Coated tablets	Fever reduction, pain relief and minor/moderate severity	None	Bulgaria
<b>Drug no. 7</b>	Paracetamol [500mg] Codeine (codeine phosphate) [8mg] Caffeine [30mg].	Magnesium stearate, corn starch, gelatinized starch, potassium sorbate, povidone, talc,	Tablets	Fever relief, analgesic, symptomatic treatment of flu-like conditions	Indication for use in toothache	Germany

## 2.1 Bulk and powder of paracetamol

The material for the study consisted of 7 different preparations – drug no.1, drug no.2, drug no.3, drug no.4, drug no.5, drug no.6 and drug no.7 - belonging to the group of OTC drugs, (over the counter drug) i.e., generally available medicinal products which are sold without a prescription issued by a doctor. The active (therapeutic) substance is paracetamol in a dose of 500mg, the preparations come from different manufacturers, and have different compositions and purposes in use, depending on the additional substances, as shown in the table below. The material was tested in two forms, the original tablet and powdered.

The table 2 shows the list of active and additional substances included in the drug leaflets but without the percentages of each substance. The drugs were deliberately selected in terms of paracetamol dose, tablet form (film-coated tablets, effervescent tablets) suitable for crushing (no liquid forms of the drug).

The reference for the test substances was a reference form of paracetamol of the highest possible available purity class - pharmaceutical primary standard, purchased from Sigma-Aldrich. Product code - P0300000, Paracetamol, European Pharmacopoeia (EP) Reference Standard in powder form.

## 2.2 Fingerprint trail collection

Figure 1 illustrates the model of collecting a drug trace from a fingerprint - which in our case - is a fingerprint with traces of paracetamol. Paracetamol was incorporated into the friction ridges by brief contact with the drug under analysis - the friction ridge sample. The volunteer was one healthy female, who had abstained from food, drink, and hand hygiene procedures (for at least 1 hour before collection). The volunteer was given a tablet of the manufacturer's paracetamol and was asked to hold it carefully for 1 minute then the volunteer was asked to print her pointing finger - fingerprint lines onto a sterile microscope slide. Tests for subsequent paracetamol manufacturers and blank samples (without paracetamol) were performed one week apart using the same procedure - by taking the tablet in the hand, a fingerprint was then made on the slide from the fingers that came into contact with the tablet. The imprint left on the slide was subsequently observed using a stereoscopic microscope.

### Research permission and ethics declarations

The study was approved by the Ethics Committee of the University of Gdańsk. Full informed and written consent from the participants was obtained before the initiation of the study for study participation and for publication of the pictures used in the manuscript. The experimental protocol was approved by the University of Gdańsk and all methods were performed according to the relevant guidelines and regulations.

## 2.3 Methods

The identification analysis was carried out using the Raman spectrometer: LabRam Aramis Raman spectrometer from Horiba Jobin Yvon, equipped with an Olympus BX41 confocal microscope, a Synapse CCD camera from Horiba Jobin Yvon with a power supply, an XYZ table control joystick of the microscope with a power supply, a microscope illuminator for non-transparent samples and a transformer. Excitation was performed with the 632 nm wavelength of a Melles Griot helium-neon laser. Surface observations and measurements were carried out by using an x50 LWD magnification objective, D1 filter, 600 l/mm grating, hole - 50 µm.



The presence of paracetamol in the friction ridges was confirmed using a NIKON stereo microscope with wide-field observation.

## Results

Identification analysis using Raman spectroscopy of paracetamol in fingerprints was carried out on samples in the form of model evidence. The material analyzed consisted of fingerprints on the glass, made by a person who had direct contact with a drug containing the active substance paracetamol. The presence of paracetamol was initially confirmed using a stereoscopic microscope - figure 1F - where bright areas in the fingerprints, resembling crystals of the substance, were observed. Paracetamol accumulated in the friction ridge skin, which are rich in sweat-fatty substances. Final confirmation of these crystals as paracetamol was made using Raman spectroscopy.

### CHARACTERISTICS OF OTHER ACTIVE SUBSTANCES

In 3 of the 7 drugs selected for the study, we found the additional active substances shown in Table 3.

Table 3. List of additional active substances in the tested drugs

Additional active substances:	Formula:
Hyoscine butylbromide (scopolamine butylbromide)	$C_{21}H_{30}BrNO_4$
Codeine phosphate	$C_{18}H_{24}NO_7P$
Caffeine	$C_8H_{10}N_4O_2$

**SCOPOLAMINE BUTYLOBROMIDE** – drug no.3 - is an antimuscarinic

and anticholinergic drug used to treat abdominal spasm pain, renal colic, and bladder spasms. However, it does not directly affect the pain but prevents the occurrence of painful spasms [25].

**CODEINE (codeine phosphate)** – drug no.7 - morphine methyl ether from the group of weaker-acting opioid drugs - naturally occurring in opium, obtained from the opium poppy. Main action - analgesic (used in the treatment of pain moderate to severe)

and antitussive. Probably the analgesic effect is due to about 10 percent metabolization to morphine [26].

**CAFFEINE** – drug no.7 - a purine alkaloid - a substance found naturally in coffee beans and tea leaves. Analeptic effect causing stimulation of the cerebral cortex (abolition of fatigue and drowsiness, increased thinking efficiency). Acceleration of metabolism, arousal of gastric juices secretion, and diuretic effect [26].

Table 4 rearranges all the additives pointed out by the manufacturers that are present in the surveyed drugs, with an indication of the number of preparations in which the substance is repeated. The most common additive is magnesium stearate, which is present in as many as 5 out of 7 tested preparations. The structural formulas of these substances are shown in Figure 2.

### CHARACTERISTICS OF EXCIPIENTS

Table 4. Excipients in the tested drugs (from the drugs package leaflets).

Excipient Substance	Formula	Number of drugs in which it was present
Opadry White - YS-1-7003 (composition: titanium dioxide(E171), hydromellose2910, 3cp, hydromellose2910,6cp, macrogol 400, polysorbate 80)	Multi-component complex	1
Opadry II Clear 85F29116: Polyvinyl alcohol, macrogol 335, talc	Multi-component complex	1
Microcrystalline cellulose	$(C_6H_{10}O_5)_n$	2
Hydroxypropyl cellulose	HPC	1
Hypomellose	$C_{56}H_{108}O_{30}$	2
Carboxymethyl starch sodium	CMS-Na	1
Sodium carboxymethyl starch type A	CMS-Na	1
Croscarmellose sodium	NaCMC	1
Crospovidone Type A	$(C_6H_9NO)_n$	1
Colloidal silica anhydrous	$SiO_2$	2
Alginic acid	$(C_6H_8O_6)_n$	1
Stearic acid	$C_{18}H_{36}O_2$	2
Stearic acid 50	$C_{18}H_{36}O_3$	1
Magnesium stearate	$Mg(C_{18}H_{35}O_2)_2$	5
Macrogol	$C_{2n}H_{4n}+2O_{n+1}$	1
Macrogol 6000	$C_{2n}H_{4n}+2O_{n+2}$	1
Potassium sorbate	$C_6H_7KO_2$	1
Povidone	$(C_6H_9NO)_n$	3
Povidone K25	$(C_6H_9NO)_n$	1
Povidone K30	$(C_6H_9NO)_n$	2
Corn starch	$(C_6H_{10}O_5)_n$	1
Potato starch	$(C_6H_{10}O_5)_n$	2
Gelatinized starch	$(C_6H_{10}O_5)_n$	3
Gelatinized corn starch	$(C_6H_{10}O_5)_n$	2
Sorbitol	$C_6H_{14}O_6$	2
Talc	$Mg_3(OH)_2Si_4O_{10}$	3
Calcium carbonate	$CaCO_3$	1
Purified water	H O	2
Carnauba wax		2



Raman studies were initiated by preparing Raman spectra of the paracetamol reference - Figure 3. The peaks were assigned to the appropriate types of bonds and oscillations occurring on the Raman spectra of paracetamols - Table 5.

Table 5 Oscillation vibrations characteristic of paracetamol, present on the Raman spectrum.

	RAMAN SHIFT [cm <sup>-1</sup> ]	TYPE OF CHEMICAL BINDING	VIBRATIONS	INTENSITY
1	1168	C-O	STRETCHING	WEAK
2	1237	C-O	STRETCHING	WEAK
3	1279	C-O	STRETCHING	WEAK
4	1324	C-N	STRETCHING	STRONG
5	1371	C-H <sub>3</sub>	DEFORMATION	WEAK
6	1561	C=C	STRETCHING	WEAK
7	1610	C=C	STRETCHING	STRONG
8	1618	C=C	STRETCHING	STRONG
9	1648	C=O	STRETCHING	STRONG

Table 5 and Figure 3 were the basis for further spectroscopic analyses performed for Raman spectra of tablets and powders of paracetamol-containing drugs from different manufacturers. The analysis of the Raman spectra of tablets and drug powders containing paracetamol from different manufacturers was intended to perform a validation of the subsequent results of paracetamol identification in fingerprints using Raman spectroscopy. Figure 4 and Figure 5 present Raman spectra for tablets and powders of the drug paracetamol from different producers. These graphs confirm the presence of excipients in addition to the main paracetamol compound. We observe changes in the intensity of the Raman peaks of the paracetamol structure due, e.g., to the presence of additional Raman scatterers from other structures or to the presence of local concentrations of paracetamol in the analyzed pills. It was also noted that the form of the substance analyzed, i.e. whether it is a tablet or a powder (figure 4 and figure 5), does not affect the identification of paracetamol using Raman spectroscopy. The Raman spectrum does not change.

Through the analysis of the spectrum of paracetamol present in the fingerprints after contact with the tablet - Figures 6 and 7, it was observed that for the analyzed drugs: drug no. 1, drug no. 2, drug no. 5, drug no. 7 - additional substances slightly influenced the Raman spectrum of paracetamol. The presence of characteristic Raman scattering for the references was confirmed but differed significantly in intensity.

For the drug no.3 (extra active substance), drug no.4 (core) and drug no.6 (core), spectra differ from the reference. On the spectrum of drug no.3 – we do not see the following peaks on the spectrum: 1237,1279,1324, 1371 or 1648 cm<sup>-1</sup>. On the spectrum of drug no.4 we do not see the following peaks on the spectrum :1237, 1279, 1324, 1371, 1648 cm<sup>-1</sup> and on drug no.6 spectrum we do not see the following peaks on the spectrum: 1237, 1279,1324, 1371, or 1648 cm<sup>-1</sup>. Drug no.4 is a medicine which, due to its accelerated action and release of the active substance, has a special coating (Coating: Opadry /white YS-1-7003 (consisting of: Titanium dioxide (E171), Hypromellose 2910, 3cp, Hypromellose 2910, 6cp, Macrogol 400, polyorbate 80), Carnauba wax, Purified water). It is composed of a number of compounds (not analysed in our study), the presence of which probably influences the masking of the active substance. Drug no.6, has no additional active substances, but like drug no.4 is a film-coated tablet - has core and shell (SHELL: Opadry II Clear 85F29116: Polyvinyl alcohol, macrogol 35, talc).



The composition of the coating of drug no.4 and drug no.6 differs, except for one compound - macrogol, but in both cases it has been used in a different formulation (drug no.4 - Macrogol 400, drug no.6 - Macrogol 335). Drug no.7, despite the presence of two additives - codeine and caffeine - gives a spectrum characteristic of the active substance we are targeting - paracetamol (we see the following peaks on the spectrum: 1168, 1237, 1279, 1324, 1371, 1610, 1618 and 1648  $\text{cm}^{-1}$ ).

Raman spectra for the individual substances were measured in the Raman shift range 0-2000  $\text{cm}^{-1}$ , and the area determined to be characteristic of paracetamol is 1100-1800  $\text{cm}^{-1}$ . In the analysed Raman shift range 1100-1800  $\text{cm}^{-1}$ , we see for drug no.3, drug no.4 and drug no.6 the absence of characteristic peaks for paracetamol.

In terms of the following of 1200-1220  $\text{cm}^{-1}$  we observe two peaks with similar parameters for drug no.4, drug no.6 (despite the different compositions of the envelopes) or additional substances present (drug no.3), the spectra in the given range are very similar. Drug no.3 has butylbromide hyoscine as an additional active substance, which prevents us from identifying paracetamol to any great extent. The visible peaks (1609,1633) belong to hyoscine [25].

## Statistical Model for the Identification of Paracetamol in Friction Ridge Skin

The interval of interest for the Raman spectra were between 50 and 1900 $\text{cm}^{-1}$ . Data processing and plot has been performed by using R language for statistical computing [27] and the package ChemoSpec. Raman spectra were baseline corrected with asymmetric least squares method [28], normalized and aligned, since different acquisition settings and equipment were employed to simulate the method interoperability (supplementary figure S1). Robust Principal Component Analysis (rPCA) was performed to find the components which explain as much of the dataset variance as possible and Hierarchical Cluster Analysis (HCA) to group samples.

## Results and discussion for Statistical Model

The scree plot in figure 8 suggests to limit the PCA to the first three principal components. Indeed, as is possible to observe from supplementary figure S2, a clear clustering occurs. Fig. 8b shows the score plot, in which the first two principal components (PC1, PC2) have been reported. Ellipses represent the 95% confidence interval for a sample belonging to a specific category. It is possible to observe that most of the differentiation between paracetamol-containing samples (both as fingerprints and as pure raw substance) and the other (fingerprints without paracetamol and fingerprints with other chemical compounds) occurs mostly in the first principal component (PC1), which explains 47% of the total dataset variance. Indeed, it is interesting to see that the PC1 loading plot overlaps with the analytical standard of paracetamol Raman spectra, suggesting the physical meaning of this component (Fig. 8c).

PC2 and PC3 may represent other substances (e.g., excipients) present in some samples, or a linear combination of their Raman spectra. PC4 loading plot has been reported to confirm that limiting the analysis to the first three PCs is sufficient, being the latter one to be considered as a background noise.

Since "noise" has been removed by the rPCA analysis, by selecting the most important PCs (PC1, PC2, PC3), HCA has been carried out on the PCA scores, to group observations into clusters. Fig 9 reports the dendrogram based on the Euclidean distance between samples as distance matrix, using the Ward clustering method. Thus, by cutting the dendrogram tree at the highest height, it is possible to clearly distinguish the paracetamol-containing samples, to the blank ones; moreover, a clear separation exists between the raw paracetamol samples and the fingerprints-containing ones.

## Conclusions

With the development of civilization, there has, of course, been the development of chemistry, pharmacy, and, consequently, an enormous increase in the number of industrial, and environmental poisonings. Forensic toxicology is confronted with the problem of rapid identification of drugs or illegal substances. It is therefore very important to develop new methods of toxicological analysis. The purpose of the toxicological analysis is to reveal the toxic substance in biological material. Current toxicological analyses in use are invasive. They mainly consist of an analysis of biological material such as blood, urine, and tissues of the xenobiotic. Of course, the type of xenobiotic and the type of evidence will determine the choice of a particular method. The research objective is to propose Raman spectroscopy for the determination of the substance identification potential of a dactyloscopy trace as a fast identification method for dactyloscopy traces (and a possible claim by the drug producer). Depending on the drug producer, the above studies confirm the identifiability of the active substance of the preparation taken. Preparations with a complex structure or with additional excipients make the identification of the substance considerably more difficult, but do not make it completely impossible.

In this study, we investigated an automated procedure for the Raman spectra pre-treatment and analysis, which can be applied to the analysis of fingerprints. CA (Cluster Analysis) analysis can be used to effectively identify substances (e.g., paracetamol) from complex mixtures (e.g., drugs), even in fingerprints, by comparing the PC loadings with analytical targets. HCA successfully clustered and paracetamol-containing from other drug samples, resulting in a useful tool for the rapid screening of fingerprints in forensic science.

This study is a preliminary analysis of the possibility of identifying active substances in drugs using Raman spectroscopy. We demonstrate the considerable potential of this method for fast toxicological analysis, without requiring invasive action on the patient. In addition, it should be made clear that the methods used in toxicology must have developed procedures according to the principles of scientific analysis of evidence. This work presents an innovative approach to toxicological analysis, where fingerprint evidence containing the second piece of xenobiotic evidence is taken into consideration. A transparent presentation of the toxicological procedure as a scientific method will make it applicable to other laboratories and allow the results obtained to be compared between laboratories, which is very relevant in international investigations.

## Declarations

### Data availability section

The datasets generated and/or analysed during the current study are available in the Gdańsk University of Technology repository, [https://mostwiedzy.pl/pl/open-research-data/paracetamol-in-friction-ridge-skin-raman-spectra-paracetamol-reference-and-from-drugs-2023,407122411411215-0?\\_share=aa9b00484e8a2c20](https://mostwiedzy.pl/pl/open-research-data/paracetamol-in-friction-ridge-skin-raman-spectra-paracetamol-reference-and-from-drugs-2023,407122411411215-0?_share=aa9b00484e8a2c20)

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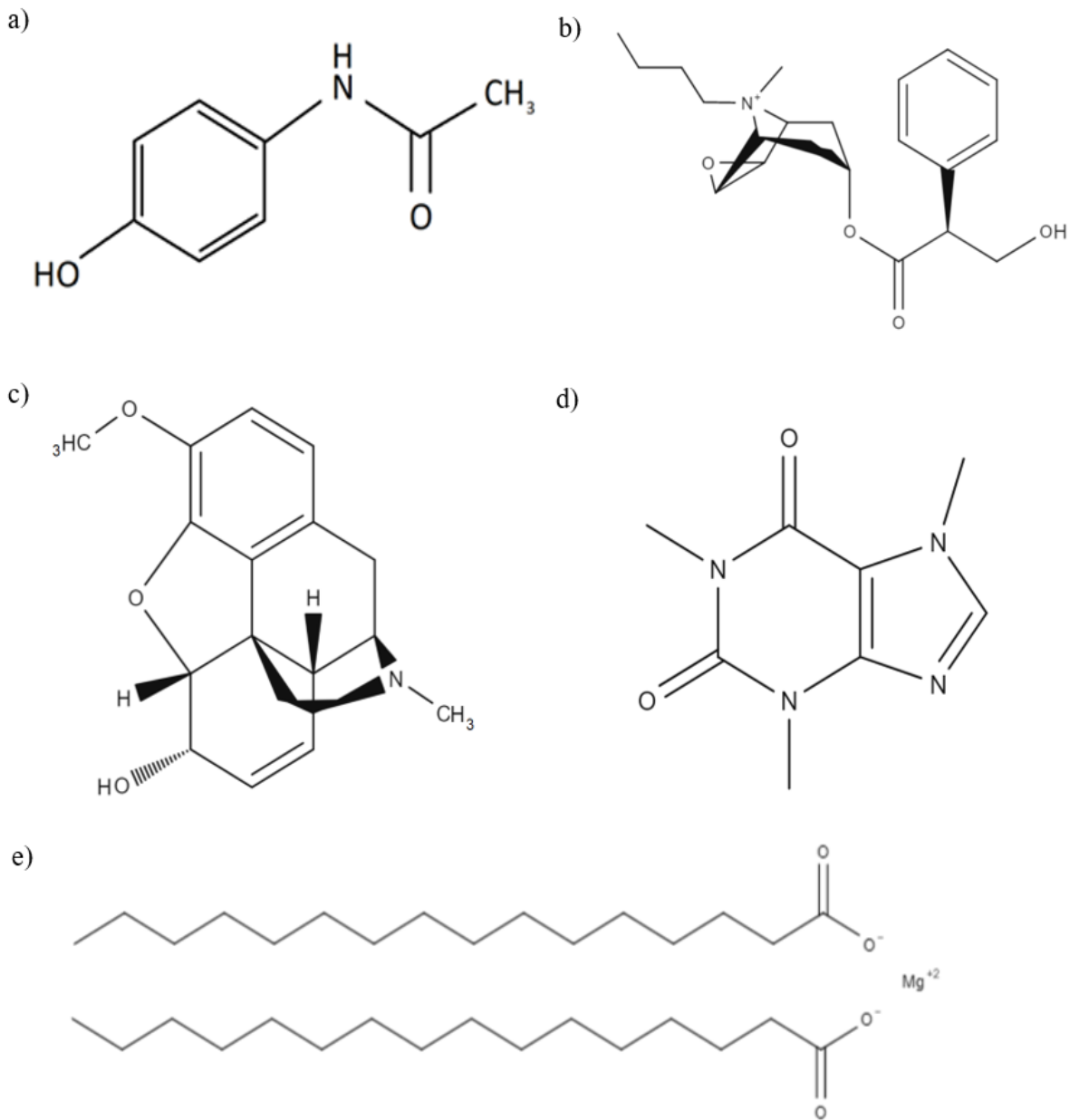
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## Figures



**Figure 1**

Conceptual research scheme: A – Original blister with pills – Drug no.1; B – Brief contact with drug; C – Leaving a fingerprint on a glass surface; D – Observation under stereoscopic microscope; E – Friction ridge with small bright crystal from the pill observed under microscope; F – Picture made under stereoscopic microscope- bright crystals are the correspondent of paracetamol.



**Figure 2**

Structural formulas of the active substance paracetamol (a) and of the additives in the drug: scopolamine butylobromide (b), codeine (codeine phosphate) (c), caffeine (d), which are also the active substances and the repeating additive: Magnesium stearate (e) in 5 of the 7 drugs analyzed.



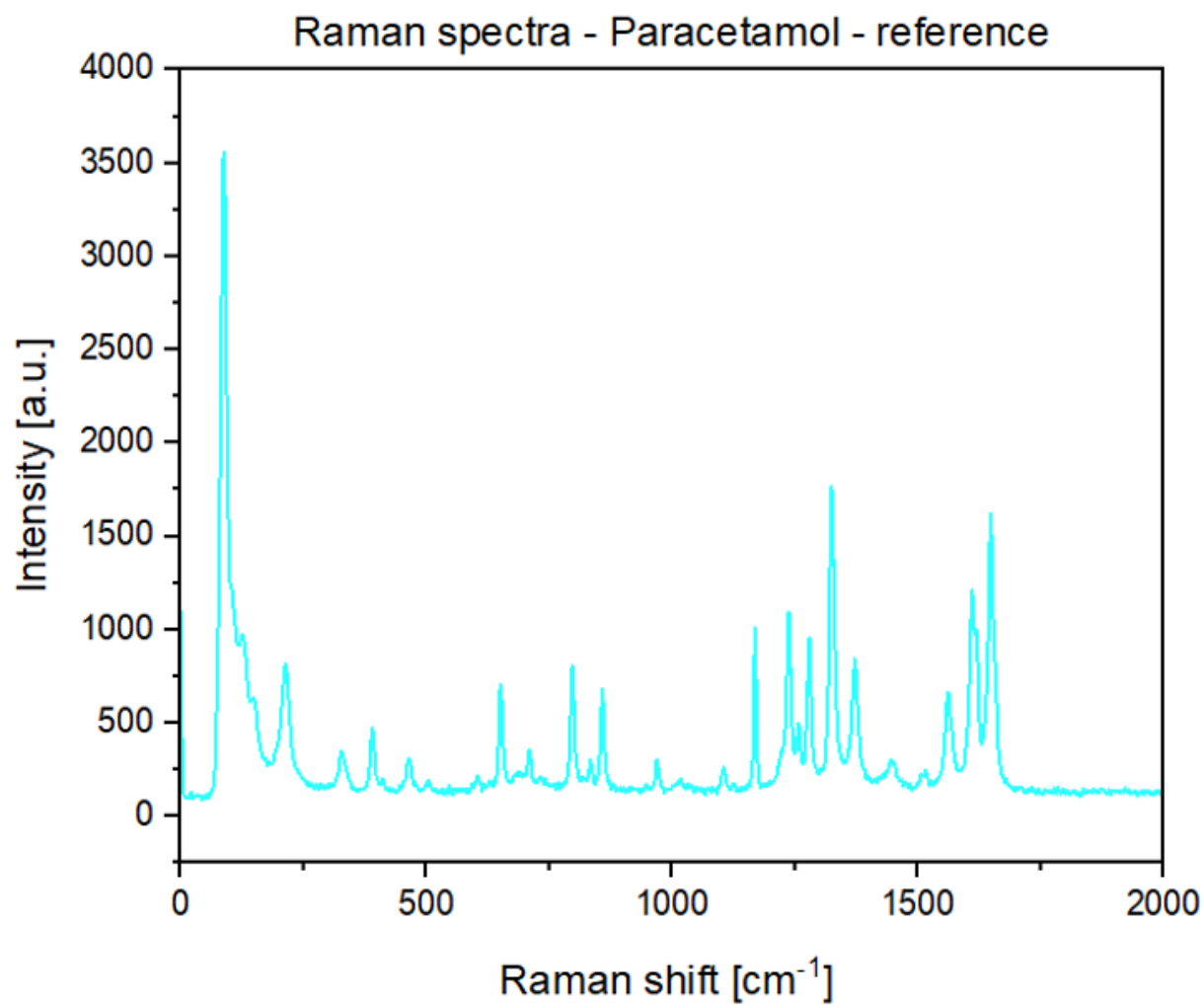


Figure 3

Raman spectrum of paracetamol reference.

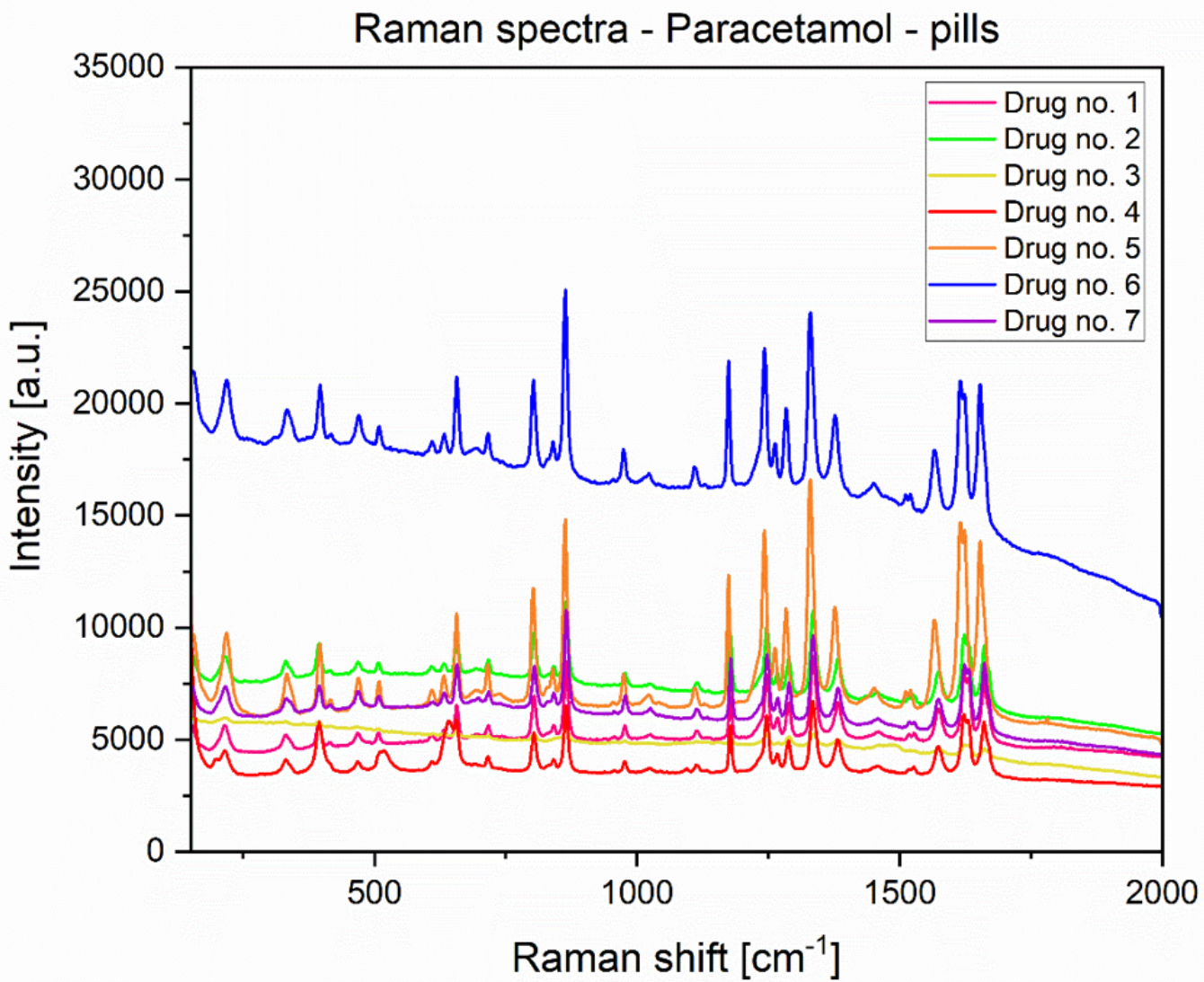


Figure 4

Raman spectra – Paracetamol – pills.



## Raman spectra - Paracetamol - powders

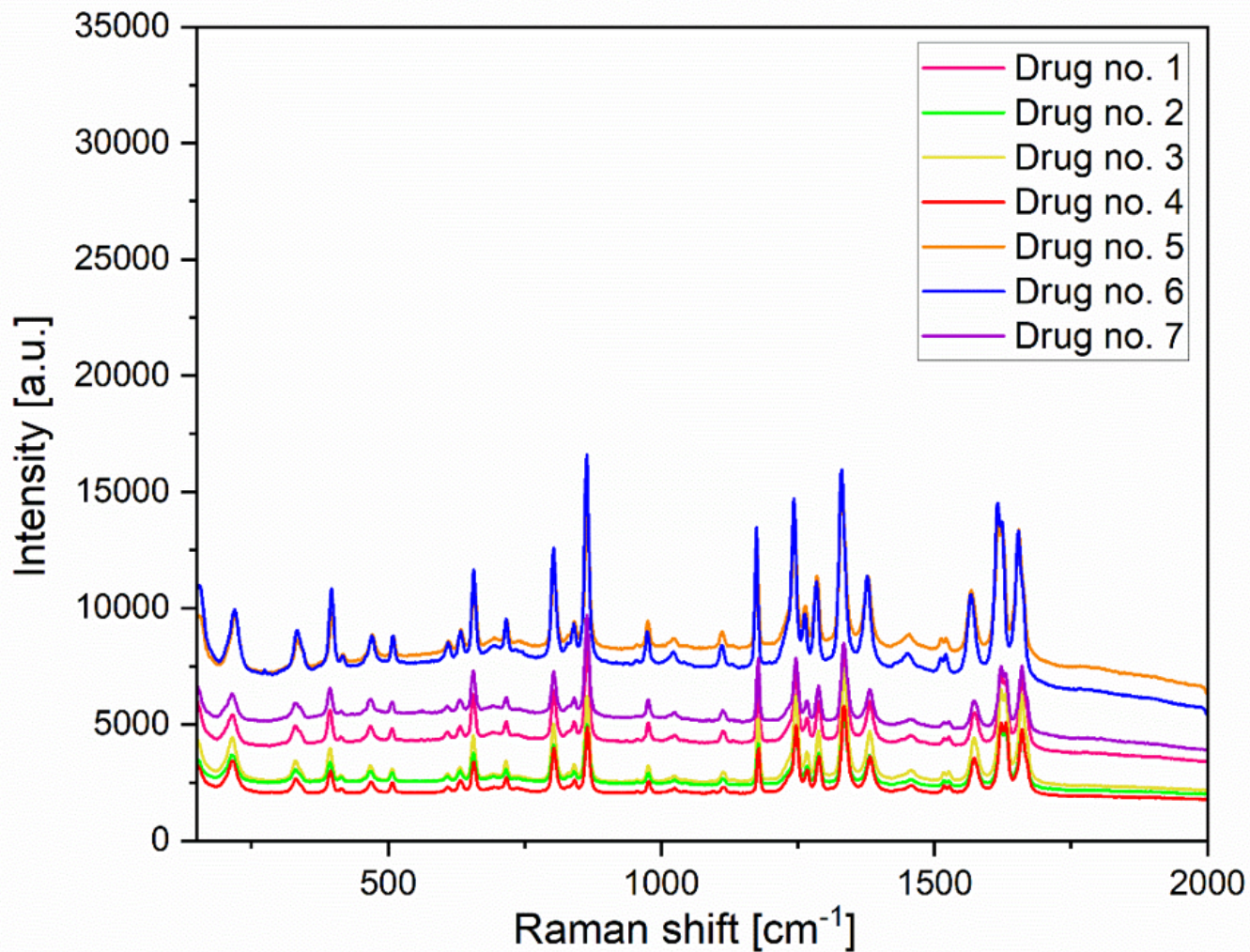


Figure 5

Raman spectra – Paracetamol – powders.

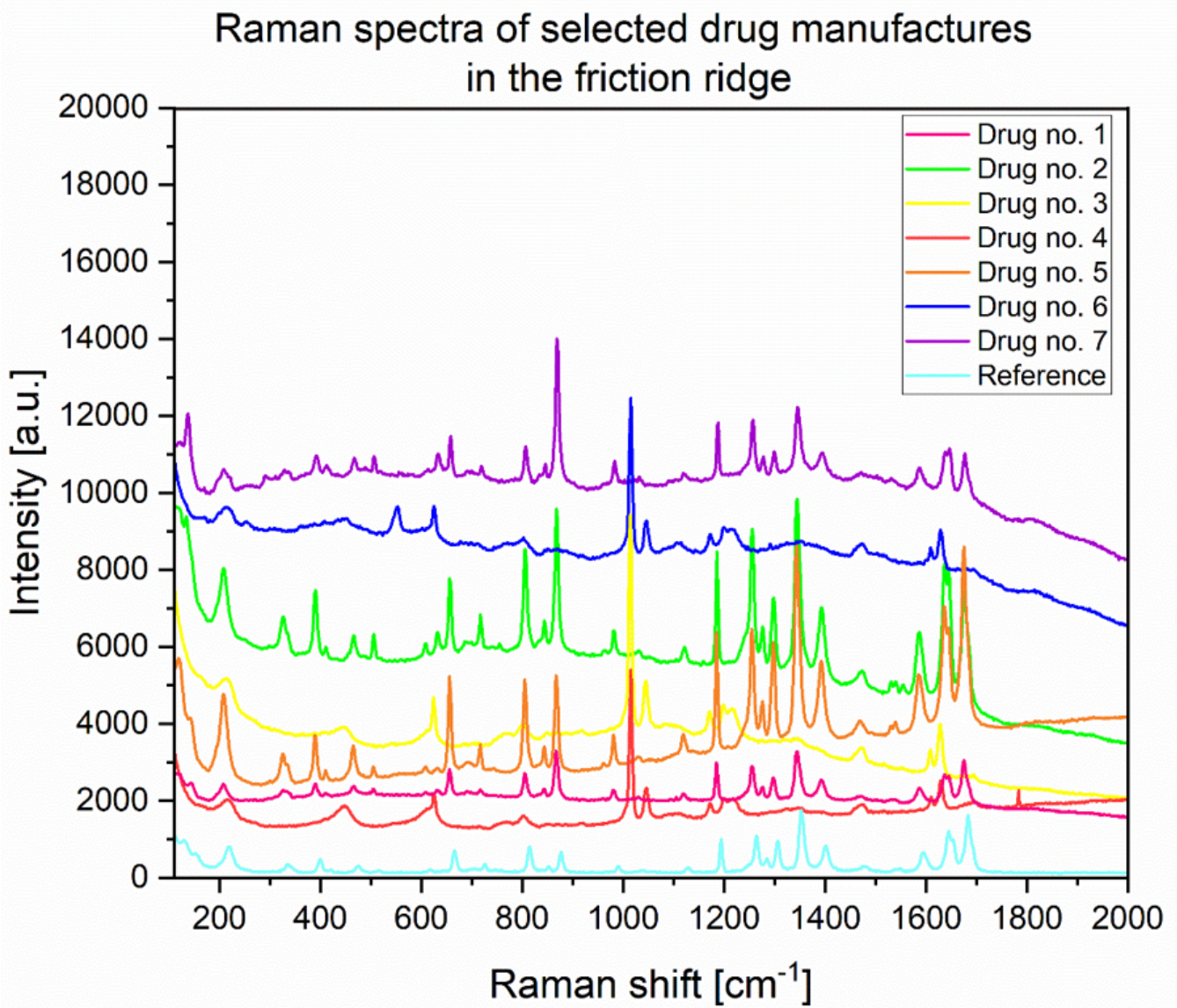


Figure 6

Raman spectra of selected drug manufactures in the friction ridge.

## Raman spectra of selected drug manufactures in the friction ridge

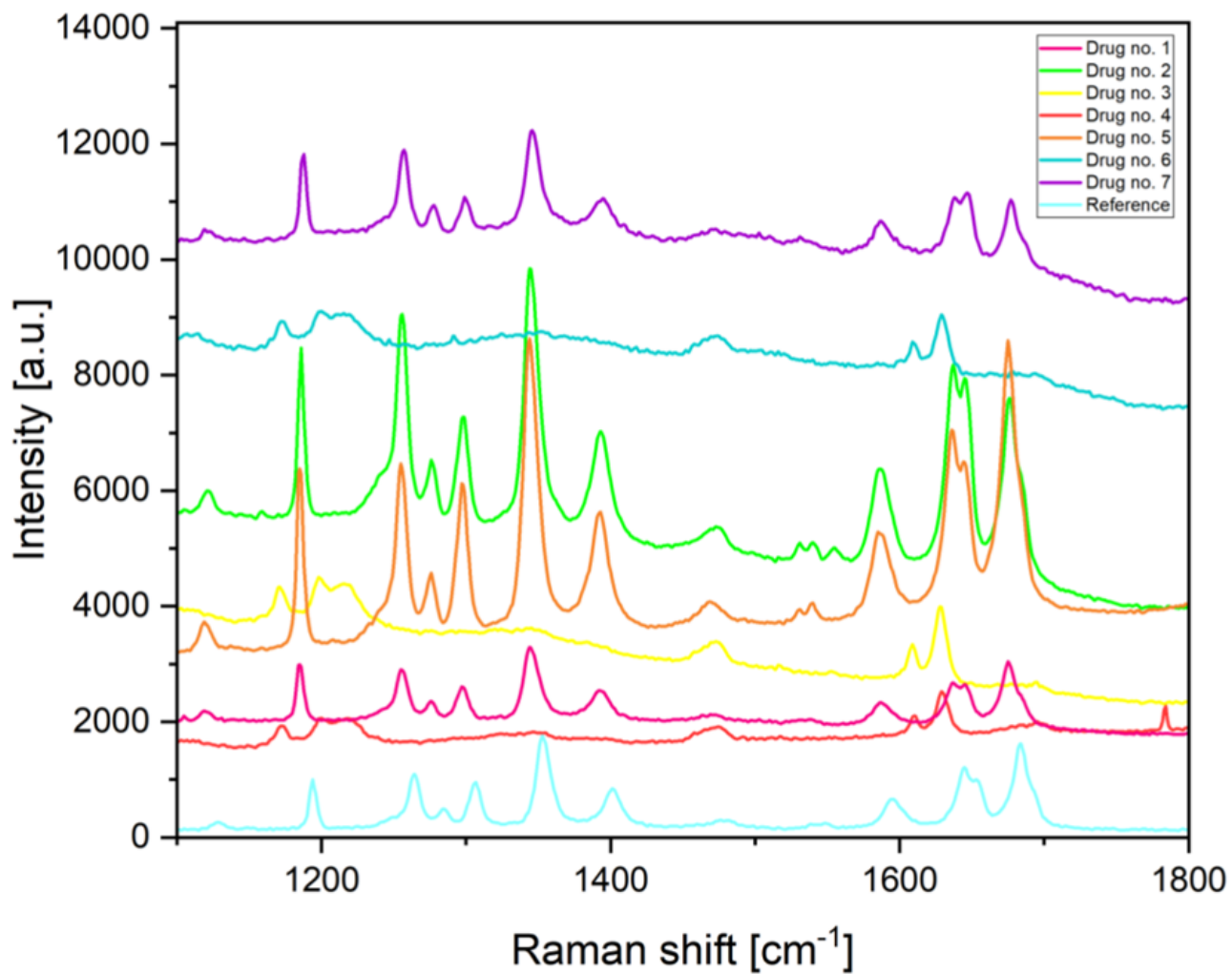
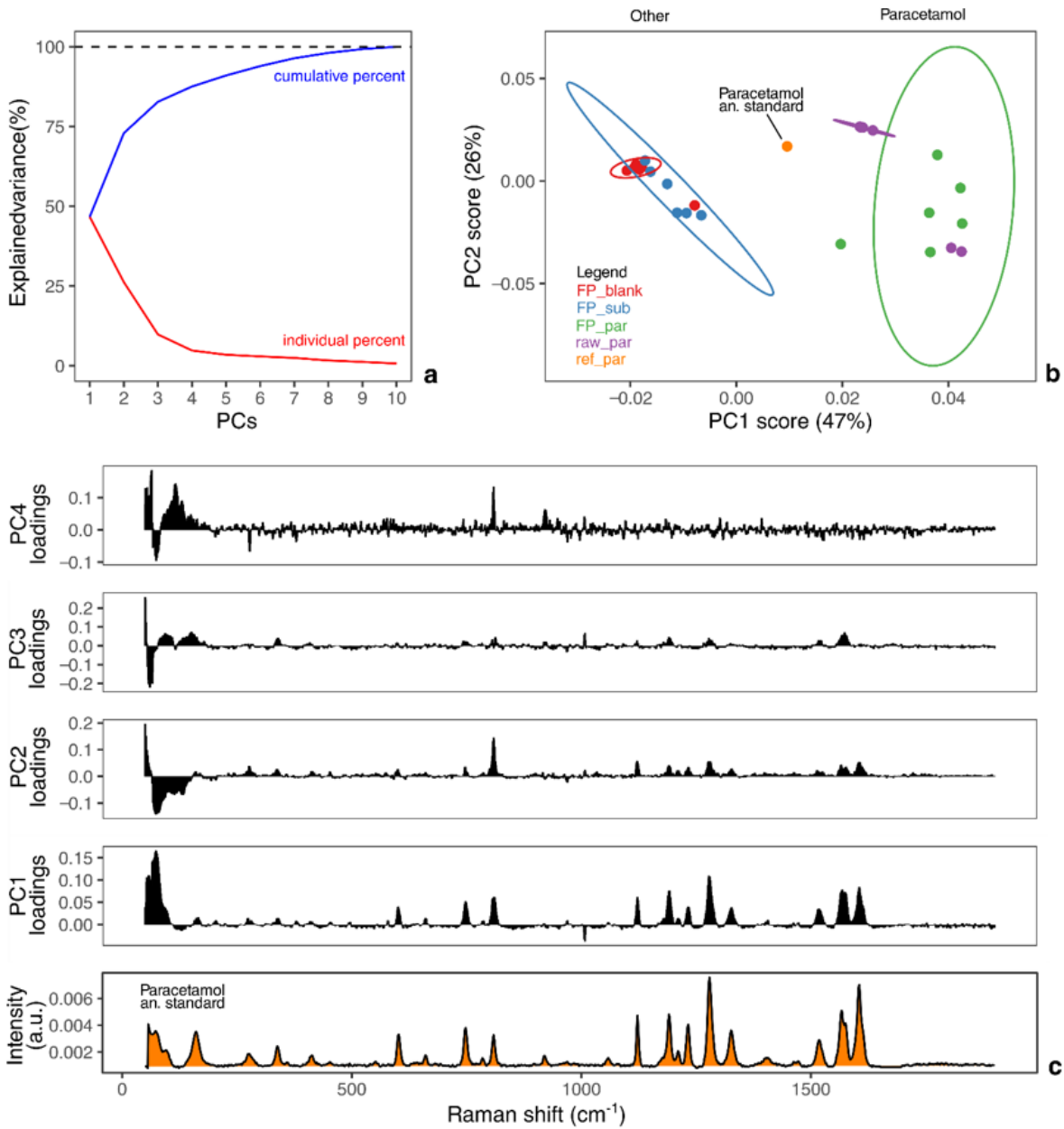


Figure 7

Raman spectra (range 1100-1800 cm<sup>-1</sup>) of selected drug manufactures in the friction ridge.



**Figure 8**

(a) scree plot, (b) score plot (FP blank - fingerprint blank sample, FP sub - fingerprint after contact with substance other than paracetamol, FP par - fingerprint after contact with paracetamol, raw par - powders/tablets, ref par - paracetamol reference) and (c) loading plot, with reported the paracetamol standard Raman spectra as reference, for the rPCA.

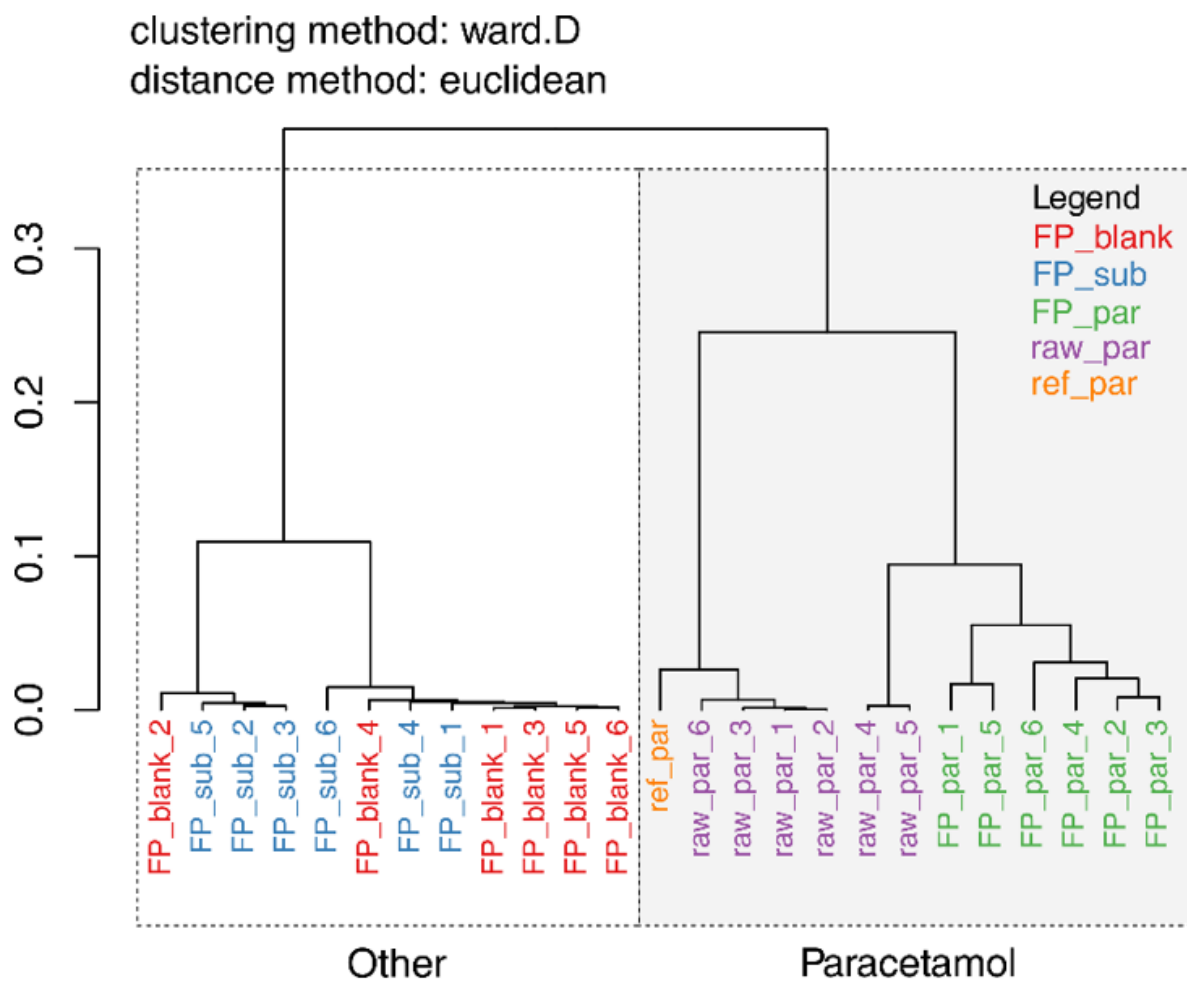


Figure 9

The dendrogram based on the Euclidean distance between samples as a distance matrix.

## Supplementary Files

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