Ind. J. Phys. Anthrop & Hum. Genet. Vol. 40, No. 1, (2021) : 23-37

ATR-FTIR SPECTROSCOPY: A CONFIRMATORY AND NON-DESTRUCTIVE APPROACH FOR THE IDENTIFICATION AND DISCRIMINATION OF BLOOD STAINS FROM LOOK-ALIKE NON- BLOOD SUBSTANCES

SWEETY SHARMA, JASKIRANDEEP KAUR JOSSAN, KARAN SINGH AND RAJINDER SINGH

ABSTRACT

The analysis of body fluids is of utmost importance in forensic cases. Biological fluids contain DNA and it can be utilized to definitely identify the suspect or the victim. In the past, the analyses have mostly involved destructive techniques and provide false positive assignments on the identification of bloodstains. Of late, the use of ATR-FTIR spectroscopy has emerged as a potential tool for the analysis of body fluids as it provides confirmatory, rapid, facile, non- destructive and on-site identification and differentiation of body fluid stains. While some studies have reported correct identification of biological fluids using ATR-FTIR spectroscopy, in the actual crime scene cases, it is obvious that this evidence will be recovered along with various look-alike substances of bloodstains which can provide false positive results and hence create ambiguity in the interpretation of results. The current study mainly focused on the discrimination of blood from look-alike non-blood substances and to validate the vulnerability of ATR-FTIR spectroscopy. In the present study, 25 samples of look-alike non-blood substances which can provide false positive assignments using routine conventional presumptive (phenolphthalein and TMB test) tests and may be misclassified as blood owing to their similar appearance were analyzed. The ATR-FTIR spectra of all selected non-blood substances were analyzed against chemometric classification tools. The spectra of all non-blood substances were differentiated successfully based of visual comparison and further supported with chemometric tools. Not any of the selected substances were misclassified as blood due to their unique spectroscopic signatures.

Keywords: Forensics, ATR-FTIR spectroscopy, Blood identification, Chemometrics, Look-alike non-blood substances

---

Sweety Sharma, Department of Forensic Science, Punjabi University, Patiala, Punjab-147002, India, E-mail: sharmasweety511@gmail.com; **Jaskirandeep Kaur Jossan,** Department of Forensic Science, Punjabi University, Patiala, Punjab- 147002, India, E-mail: jsjossan@gmail.com; **Karan Singh,** Department of Forensic Science, Punjabi University, Patiala, Punjab-147002, India, E-mail: karankohli310@gmail.com; **Rajinder Singh (Corresponding Author),** Associate Professor, Department of Forensic Science, Punjabi University, Patiala, Punjab-147002, India, E-mail: rajinder_forensic@pbi.ac.in

INTRODUCTION

It is an important task to correctly identify body fluids at the crime scene, as they provide crucial pieces of DNA evidence that lead to the conclusive identification of an individual. Nonetheless, the identification of body fluids can prove to be an arduous process, since number of prevalent substances may be found at the scene of crime, which are often similar in appearance to a body fluid. Consequently, it is imperative to find a reliable and non-destructive method for the identification and discrimination of body fluids, especially blood, from other potential look-alike substances (Rosenblatt *et al.,* 2019).

Through many decades of research studies, various tests (presumptive and confirmatory) have been made available to identify the blood stains including polilight (alternative light source), phenolphthalein, benzidine, TMB, luminol, ABAcard® Hematrace®, and benzidine. These presumptive tests mainly depend on the mechanism of oxidation-reduction reaction, which ultimately leads to the sample destruction. Other limitations of these tests are: non-specificity, time-consuming, costly, ambiguous, and hazardous, and apparently results are subjective in nature. One of the major limitations of the conventional methods is their lack of selectivity, which resulted in false positive assignments, when actually sample is not present (Virkler and Lednev, 2009). Therefore, this may lead to added issues encompassing wasted resources, time, and money. A false positive result can arise due the interferents or look-alike substances that either a) are known to give a false positive result with conventional blood identification methods or b) which may be similar in physical appearance of blood. Attaining false-positive results or assignments from various substances can unnecessarily enhance the sample logjams in forensic laboratories and may be pernicious to a case (Casey *et al.*, 2020; Rosenblatt *et al.,* 2019). Hence, an alternative method to accurately identify blood stains at the crime scene would be tremendously advantageous to forensic science. Now a days, Raman and ATR-FTIR spectroscopy is attracting added attention in the field of forensic science due to its rapid, reliable, eco-friendly, and non-destructive nature (Muro *et al.*, 2015).

In the arena of forensic science, the Raman spectroscopy has been utilized as an effective tool for the confirmatory identification of various trace evidence such as drugs/illicit drugs (De Oliveira Penido *et al.*, 2016), gunshot residue (GSR) (Bueno *et al.*, 2012; Doty and Lednev, 2018), explosives (López-López and García-Ruiz, 2014), ink analysis (Buzzini and Suzuki, 2016; De Souza Lins Borba *et al*., 2015; Mohamad Asri et al., 2018), fibers (Casadio *et al*., 2010; Goodpaster and Liszewski, 2009), lipsticks (López-López *et al.*, 2014; Salahioglu and Went, 2012), nail paints (López-López *et al.*, 2015), and paint samples (Buzzini and Suzuki, 2016; Stewart et al., 2012). Lednev's and co-workers (2008 and later) have explored Raman spectroscopy for the analysis of various body fluids and other researchers have widened the field of body fluid analysis (McLaughlin and Lednev, 2015, 2014; Muro *et al.*, 2016; Sikirzhytskaya *et al.*, 2013, 2012; Sikirzhytski et al., 2012; Zapata *et al.,* 2015; Zou *et al.,* 2016).

Recently, Rosenblatt *et al.,* (2019) worked on the method validation versus commonly encountered environmental interferents (EIs) of blood using Raman spectroscopy. Results showed that 24 substances of EIs were successfully discriminated using Raman spectroscopy and classification model that supports vector machines discriminant analysis. Another study was conducted by Casey *et al.*, (2020) for the identification of semen stains against the commonly found EIs of seminal fluid. A random forest algorithm was used for the differentiation of seminal fluids against commonly found EIs for the traces of seminal stains.

Certainly, Raman spectroscopy provides reasonable results in the examination of bodily fluid, but Raman spectroscopy is highly-expensive in comparison to FTIR spectroscopy (Quinn and Elkins, 2017). On the contrary, FTIR spectroscopy explicitly has a high potential for the detection of body fluid stains on different fabrics and further simulated substrates owing to its less penetration depth (Â10 µm) and surface sensitivity (Gregório *et al.,* 2017a; Quinn and Elkins, 2017; Zapata *et al.*, 2016). In the field of forensic science, ATR-FTIR spectroscopy has been utilized to analyze trace evidence such as gunshot residues (Bueno *et al*., 2013), hairs (Boll *et al.*, 2017; Manheim *et al.*, 2016), paints (Harkins et al., 1959), fibres (Goodpaster and Liszewski, 2009), inks (Causin et al., 2008; Lee *et al.*, 2018; Mohamad Asri *et al.*, 2018; Williamson *et al.*, 2016), cosmetics (Chophi *et al.*, 2019; G^{3}adysz *et al.*, 2017; Sharma *et al.*, 2019a, 2019b), and body fluids (De Wael *et al.*, 2008; Elkins, 2011; Gregório *et al.,* 2017b, 2017a; Orphanou, 2015; Quinn and Elkins, 2017; Sharma *et al.,* 2019; Sharma and Singh, 2019). ATR-FTIR is a confirmatory (qualitative and quantitative), non-destructive, sensitive, rapid, environment friendly technique. Furthermore, harmful reagents are not required in its due process (Elkins, 2011; Muro *et al.,* 2015; Orphanou, 2015; Quinn and Elkins, 2017).

In the present study, the differentiation of bloodstains from look-alike non-blood substances was carried out using ATR-FTIR spectroscopy and advance chemometrics. The detection of bloodstain is not invariably an easy assignment. Bloodstain possesses an often indistinct appearance with non-blood like substances, including fake blood, which could mislead in the identification process and forbid absolute confirmation required in aiding verdicts in the court of law without reasonable scientific doubt. Apparently, fake blood substances may possess a higher resemblance to bloodstain in physical appearance, and therefore it can be misused to fabricate the crime scene. In such cases, accurate detection of blood would be helpful to circumvent the application of expensive and redundant analysis of stains originating from non-biological sources (De Beijer *et al.*, 2018).

MATERIALS AND METHODS

Sample collection

Blood samples were collected from seventy-five (n=75) healthy adults by a well-qualified laboratory technician. Before collecting blood, consent of each volunteer

was taken. Blood was collected in dipotassium EDTA anticoagulant at a concentration of approximately 1.8 mg K2 EDTA per 2 ml of blood using vaccuete blood collection technique. A superficial vein of an individual was selected to withdraw the sample. The collected samples were stored at 2-4°C.

Collection of non-blood substances

25 samples of non-blood substances were purchased from different stores of Patiala and samples of fake blood were ordered from the e-commerce website (amazon.in). Detailed information of all collected samples is listed in Table-1.

Table-1: Detailed information of 25 substances analysed and chemical tests which may cause false positive results with these substances

Sample code	Number of samples	Sample name	Manufacturer	Amount	Chemical test that provides false positive results	
					Phenolphthalein	TMB
1	03	Fresh Beetroot juice	----	20 µl	+	-
2	03	Fresh Carrot juice	----	20 µl	+	+
3	03	Red Cough Syrup(Torex-dx)	Torque pharma Ghollumajra, Punjab	20 µl	-	-
4	03	Dettol liquid	Ravi Specialities Pharma Pvt Ltd	20 µl	-	-
5	03	Fake blood S1	ILH Halloween	20 µl	+	-
6	03	Fake blood S2	Party Toko	20 µl	+	-
7	03	Fake blood S3	Wanna Party	20 µl	+	+
8	03	Fake blood S4	PTC MART	20 µl	+	-
9	03	Hershey's Chocolate syrup	HersheysPvt. Ltd. Mandideep, Madhya Pradesh India	1 drop	+	-
10	03	Red Fruit Jam	Raisen, Madhya Pradesh. India	Arbitrary	-	-
11	03	Fresh Pomegranate Juice	-----	20 µl	-	-
12	03	Red Chilly Sauce	Capital foods pvt ltd Mumbai, Maharashtra	Arbitrary	-	-
13	03	Red drawing color	Camlin	Arbitrary	+	-
14	03	Red Dye (For clothes)	-----	20 µl	+	-
15	03	Red Ketchup	Tombo	Arbitrary	-	-
16	03	Red Marker Ink	Kokuyo Camlin Ltd Mumbai , Maharashtra	Arbitrary	-	-
17	03	Red Nail Paint	Hindustan Unilever limited	Arbitrary	-	-
18	03	Red Pen ink	Diamond Heritage, 16, Strand Road, 10th Floor, Office No 1015A, Kolkata, West Bengal	Arbitrary	+	-
19	03	Red Toothpaste	Hindustan unilever ltd	Arbitrary	-	-

20	03	Roohafza	Hamdard laboratories, New delhi	Arbitrary	-	-
21	03	Red Spray paint	Abro paints	Arbitrary	-	-
22	03	Liquid Vermillion	Meredith St, Mission Row Extension, Esplanade, Chowringhee North, Bow Barracks, Kolkata, West Bengal	Arbitrary	+	-
23	03	Alta	Shila Alta	20 µl	+	-
24	03	Red lipstick	Elle 18	Arbitrary	-	-
25	03	Savlon	ITC Limited	20 µl	-	-

Sample Preparation to Carry Out Validation Study

All seventy-five samples of blood were prepared on clean and sterile glass slides. Area of approximately 1×1 cm was marked on slides, and 50 µl of blood was deposited on the marked area and was allowed to dry at room temperature (25±5ºC) for 24 hours. By using a sterile spatula, the sample was scraped out and placed directly on the face of ATR crystal and was scanned 24 times at the resolution of 4 cm^{-1} in the range of 4000-600 cm^{-1} (MIR).

Samples of non-blood substances were prepared by depositing 1 drop of the sample on clean glass slides and kept to dry for 24 hours. When the liquid became completely dry, it was scrapped out using spatula and analyzed using the ATR-FTIR spectroscopy technique. After analyzing every sample, crystal face was cleaned safely with acetone (spectroscopic grade) to evade any form of contamination. Total 25 samples of non-blood substances were analyzed by FTIR and all samples were analyzed in triplicates to check the reproducibility. Therefore a total of 75 spectra of non- blood substances samples were generated.

Instrumentation

Samples were analyzed using Bruker Alpha, eco ATR-FTIR spectrometer. Operating parameters are given in Table-2.

Table-2: Operating parameters for the analysis of blood using ATR-FTIR spectroscopy

Parameters	Operating Parameters
Scans	24
Resolution	4 cm^{-1}
Detector	DLATGS
ATR crystal	ZnSe
Spectral processing software	OPUS
Software version	v 7.2

APPLIED CHEMOMETRIC METHODS

Principal Components Analysis (PCA)

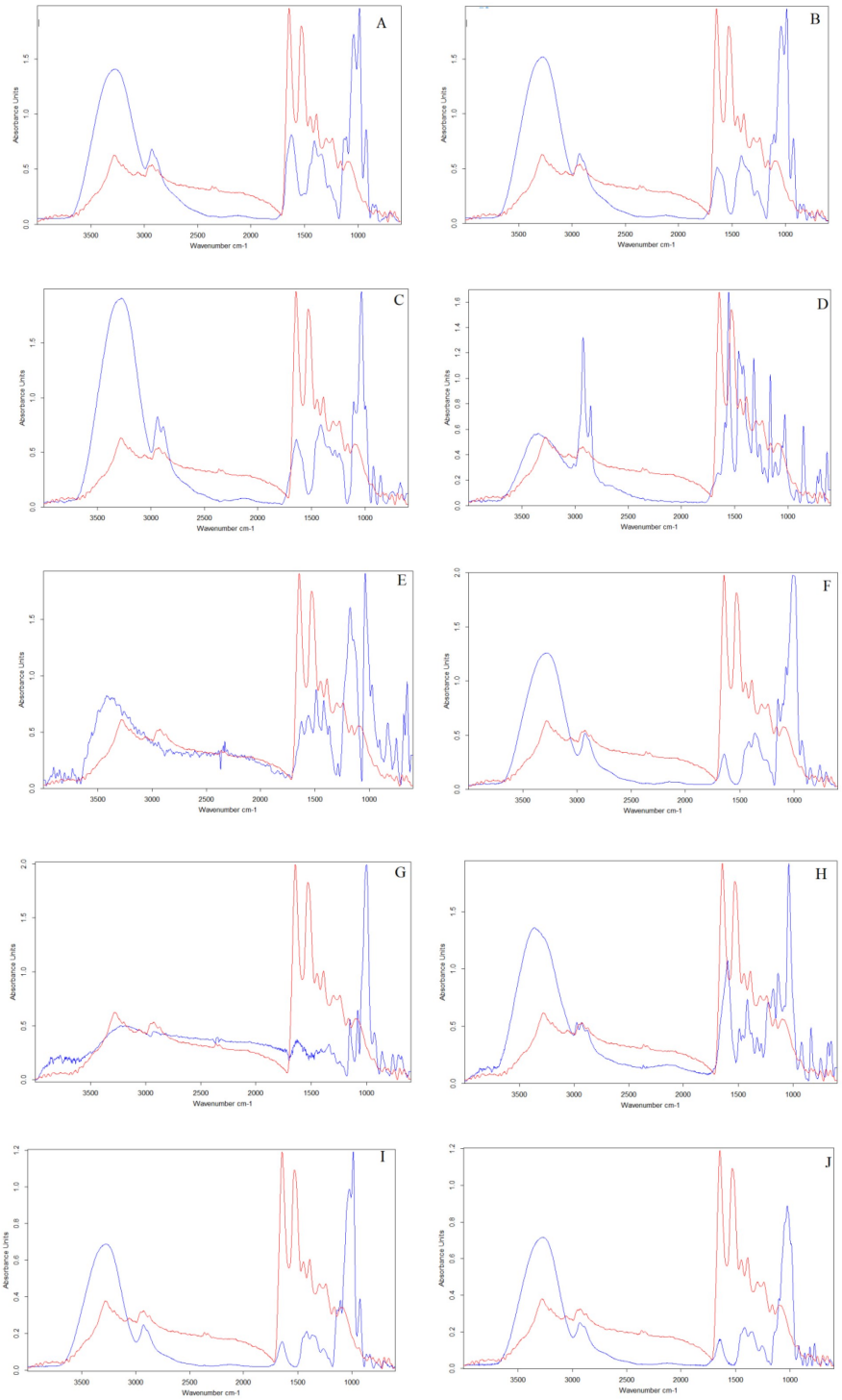
The principal component analysis was first coined by Pearson in 1901 and further developed independently by Hotelling in 1993 (Mishra *et al*., 2017). It is a pattern recognition statistical tool and can reduce the huge dimensional data into low dimensional data set using vector space transformation. PCA helps to present the significant information into few simpler plots known as loading and score plot. The algorithm used in PCA is to figure out the relationship among the highly correlated variables and hence, these correlated and orthogonal variables are known as principal components (Sehgal *et al.,* 2014).

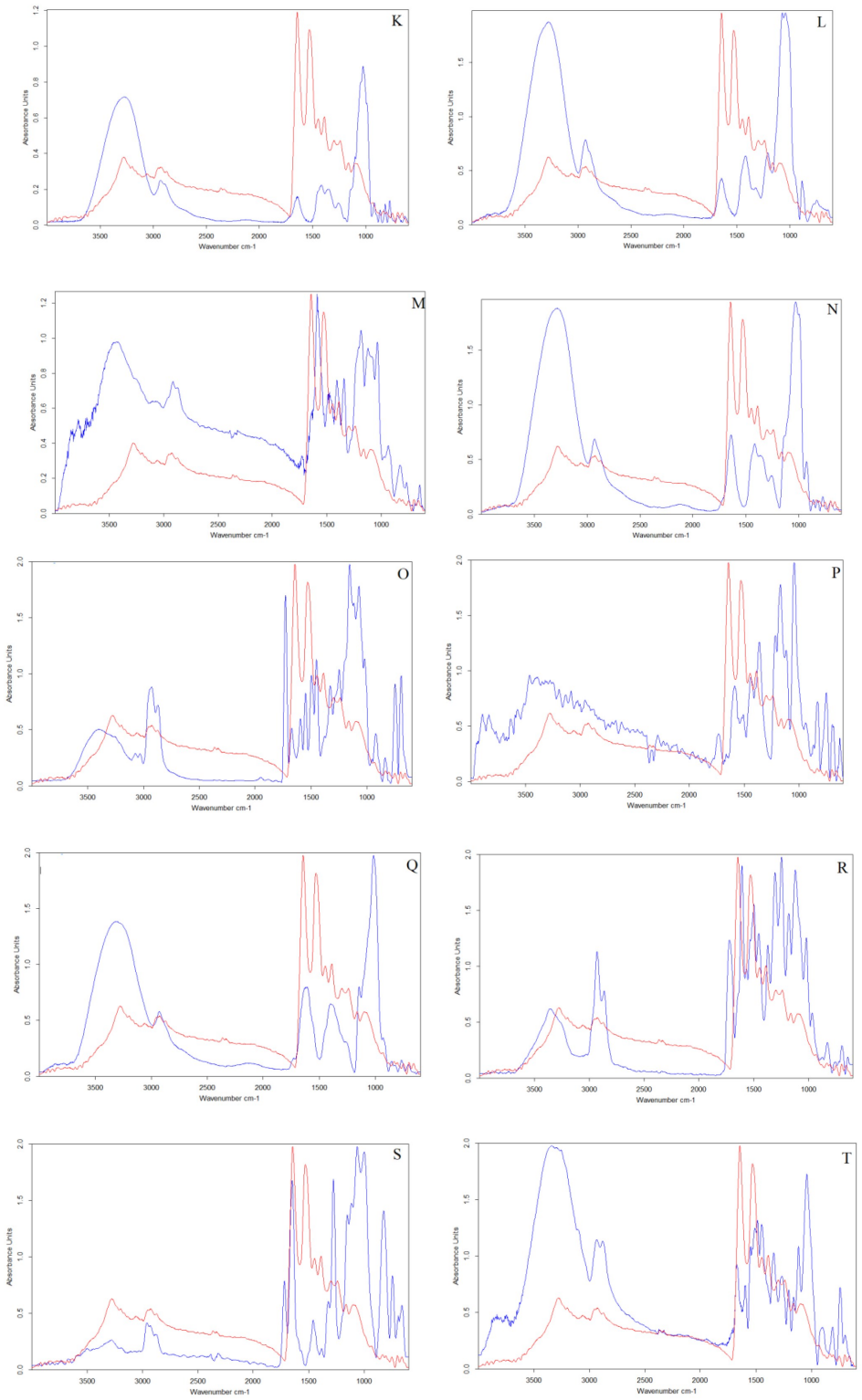
RESULTS AND DISCUSSION

When analyzing blood stains, there are multitude of reasons to get false positive assignments, depending upon the type of tests performed. To succefully exclude the substances which can cause false positive assignments, more selective test must be performed. To identify bloodstains accurately, each time the tested sample must be considered as a look-alike non blood substance. ATR-FTIR spectroscopy possesses the capability to identify and detect minute variations within and between the tested samples. This technique can easily differentiate among various chemical species which inherently make this technique suitable to provide comprehensive information when differentiating bloodstains and various substances which may cause false positive results.

Spectral comparisons of neat blood and various look-alike non-blood substances

Total 25 substances, as enumerated in Table-1, were selected and considered as potential false positive substances for the identification of blood due to the similar composition (juices, ketchup, etc.) or may be different composition (nail paint, vermillion, etc.). Figure-1 (A-Y) allows the visual discrimination between the blood and non-blood substances based on the collective features of peak that is position, shape, and intensity. Results suggested that look-alike substances or materials which are composed of proteins will also show the amide peaks (amide I and amide II). However, based on the intensity and shape they can easily differentiate the peaks originating from biological fluids. Further chemometric approach was applied to get the objective spectral interpretation in limited time domain, and to minimize the chances of error due to manual interpretation. The primary aim of applying chemometrics is to practice huge data sets and to extract the subjectivity for the interpretation of resultant spectra.


A
B
C
D
E
F
G
H
I
J
Absorbance Units
Wavenumber cm-1


K
L
M
N
O
P
Q
R
S
T
Absorbance Units
Wavenumber cm-1

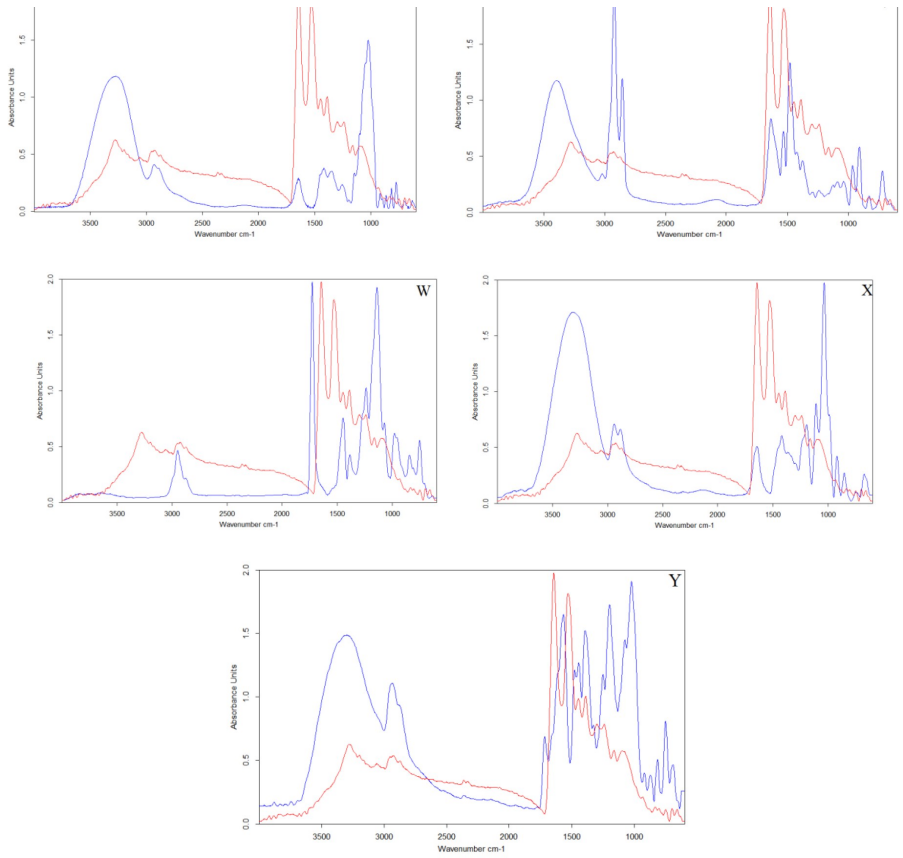


Figure-1: Overlaid ATR-FTIR spectra of neat blood and blood look alike substances which may cause false positive results (A) Beetroot juice (B) Carrot juice (C) Cough syrup (D) Dettol (E) Fake blood.1 (F) Fake blood.2 (G) Fake blood.3 (H) Fake blood.4 (I) Harshey syrup (J) Jam (K) Pomegranate juice (L) Red tooth paste (M) Red alta (N) Chilly sauce (O) Drawing color (P) Red dye (Q) Ketchup (R) Red marker (S) Red nail paint (T) Red pen ink (U) Rooh afza drink (V) Savlon (W) Spray paint (X) Stamp ink (Y) Vermillion

Discrimination between blood and non-blood substances using PCA model

3-D PCA model, as shown in Figure-3, was constructed to assess the differences and similarities between two groups (Group1- blood; Group 2- non-blood substances) via clustering in the generated plot. Model was built using 8 principle components (Table-2). PCA is considered as an unsupervised clustering method, constructed on the basis of differences in peak position (wavenumber cm^{-1}), shape and intensity. These disparities permit the model to create specific variables which are known as principle components (PCs). PCs are used to exhibit the qualities whichever more or less similar to the spectral data (blood and non-blood substances) present. As shown in Table-2 and Figure-2, Initial 2 PCs showed largest variance in the dataset, therefore, initial PCs were selected to

get the desired results in the scatter plot. However, Kaiser Criterion test (scree plot in Figure- 2) is conducted to extract the number of PCs used to construct the model. Cumulative variance of 79% was observed in the dataset with three PCs; 43%, 27%, and 9%, for PC1, PC2, and PC3, respectively. The discriminating power achieved was 100% for the current study.

Table-2: Eigen values of principle components

	PC-1	PC-2	PC-3	PC-4	PC-5	PC-7	PC-8
Eigen values	18.13987	11.51059	3.797253	2.726094	1.620334	1.057281	0.6616053

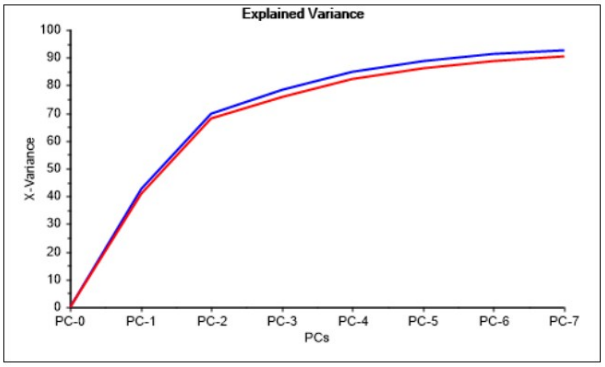


Figure-2: Scree plot to select number of PCs

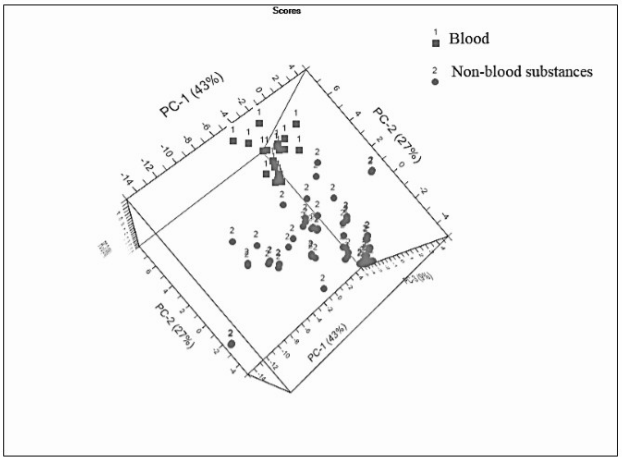


Figure-3: PCA score plot to discriminate the blood and look-alike non-blood substrates

Figure-4 shows the combined loading plot of PC1 and PC2. The loading plot is divided into three regions; Region I- 4000-1740 cm^{-1}; where PC1 and PC2 both show positive correlation except few ranges that is 3523-3101 cm^{-1}, where PC2 shows negative correlation. Region II-1713-1150 cm^{-1}; where PC2 shows positive correlation whereas PC1 shows negative correlation except few points (1673-1511 and 1434-1352 cm^{-1}). Region III- 1418-600 cm^{-1}; where majority of PC1 shows positive correlation and PC2 shows negative correlation.

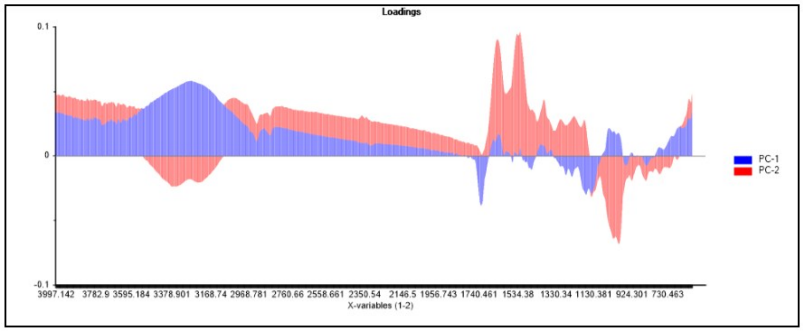


Fig. 4 PC1 and PC2 loading plot

The value of accuracy, specificity, precision, and sensitivity was calculated using given formulas and resulted with 100% accuracy with 0% rate of false-positive and negative values.

Accuracy = TP+ TN/[TP+ TN+ (FP)+FN]× 100

Specificity = TN/TN + FP × 100

Precision = TP (TP+ FP) × 100

Sensitivity = TP/ [TP + FN] × 100

False positive rate= FP/ (TP+ FN) × 100

False negative rate = FN/ (TN+FP) × 100

[TP- True positive; TN- True negative; FP-False positive; FN- False negative]

CONCLUSIONS

The reliable, rapid and accurate identification of bloodstains at the crime scene is of paramount importance. Herein, we conducted the systematic work of determining the influence of look-alike non-blood substances on the efficiency of bloodstain identification. This study demonstrated that with the use of ATR-FTIR spectroscopy, in combination with chemometric methods, twenty-five non-blood substances could easily be differentiated from blood, which either provide false positive assignment with presumptive tests (phenolphthalein and TMB), or may be similar to a bloodstain at the crime scene. The visible spectral variance between blood and non-blood substances was significantly demonstrated using ATR-FTIR spectroscopy, which is a fast, non-destructive, and highly selective approach. Moreover, ocular spectral interpretation is augmented with the error and thus entails subjectivity in the results. Chemometric method, however, was integrated to provide objective interpretation of the spectral data.

Overall, this technique has the potential to become a reliable, rapid, confirmatory, and non-destructive alternative to presumptive methods for blood detection, which can provide on-site confirmation and differentiation of bloodstains from look-alike non blood substances.

ACKNOWLEDGEMENTS

The authors would sincerely like to thank University Grants Commission (UGC), Ministry of Human Resource Development, Government of India for financial assistance for providing laboratory facilities in the Department of Forensic Science, Punjabi University, Patiala.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Boll, M.S., Doty, K.C., Wickenheiser, R. and I.K, Lednev, 2017. Differentiation of hair using ATR FT-IR spectroscopy: A statistical classification of dyed and non-dyed hairs. *Forensic Chem*., 6:1–9.https://doi.org/10.1016/j.forc.2017.08.001

Bueno, J., Sikirzhytski, V. and I.K,Lednev, 2013. ATR-FTIR Spectroscopy for Gunshot Residue Analysis: Potential for Ammunition Determination, *Anal. Chem.*, 85 (15): 7287-7294. https://doi.org/10.1021/ac4011843

Bueno, J., Sikirzhytski, V. and I.K, Lednev, 2012. Raman Spectroscopic Analysis of Gunshot Residue Offering Great Potential for Caliber Differentiation. *Anal. Chem*., 84:4334–4339. https://doi.org/10.1021/ac203429x

Buzzini, P. and E. Suzuki, 2016. Forensic applications of Raman spectroscopy for the in situ analyses of pigments and dyes in ink and paint evidence. *J. Raman Spectrosc*., 47:16–27. https://doi.org/10.1002/jrs.4818

Casadio, F., Leona, M., Lombardi, J.R. and R. Van Duyne, 2010. Identification of Organic Colorants in Fibers, Paints, and Glazes by Surface Enhanced Raman Spectroscopy. *Acc. Chem. Res*., 43:782–791. https://doi.org/10.1021/ar100019q

Casey, T., Mistek, E., Halámková, L., I.K. Lednev, 2020. Raman spectroscopy for forensic semen identification: Method validation vs. environmental interferences. *Vib. Spectrosc*., 109:103065. https://doi.org/10.1016/j.vibspec.2020.103065

Causin, V., Casamassima, R., Marega, C., Maida, P., Schiavone, S., Marigo, A. and A.Villari, 2008. The Discrimination Potential of Ultraviolet-Visible Spectrophotometry, Thin Layer Chromatography, and Fourier Transform Infrared Spectroscopy for the Forensic Analysis of Black and Blue Ballpoint Inks. *J. Forensic Sci*., 53:1468–1473. https://doi.org/10.1111/j.1556-4029.2008.00867.x

Chophi, R., Sharma, S. and R. Singh, 2019. Forensic analysis of red lipsticks using ATR-FTIR spectroscopy and chemometrics. *Forensic Chem*., 100209. https://doi.org/10.1016/j.forc.2019.100209

De Beijer, R.P., De Graaf, C., Van Weert, A., Van Leeuwen, T.G., Aalders, M.C.G. and A. Van Dam, 2018. Identification and detection of protein markers to differentiate between forensically relevant body fluids. *Forensic Sci. Int*., 290:196–206. https://doi.org/10.1016/j.forsciint.2018.07.013

De Oliveira Penido, C.A.F., Pacheco, M.T.T., Lednev, I.K. and L. Silveira Jr., 2016. Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse. *J. Raman Spectrosc*., 47:28–38. https://doi.org/10.1002/jrs.4864

De Souza Lins Borba, F., Saldanha Honorato, R. and A. De Juan, 2015. Use of Raman spectroscopy and chemometrics to distinguish blue ballpoint pen inks. *Forensic Sci. Int*., 249:73–82. https://doi.org/10.1016/j.forsciint.2015.01.027

De Wael, K., Lepot, L., Gason, F. and B. Gilbert, 2008. In search of blood—Detection of minute particles using spectroscopic methods. *Forensic Sci. Int*., 180:37–42. https://doi.org/10.1016/j.forsciint.2008.06.013

Doty, K.C. and I.K. Lednev, 2018. Raman spectroscopy for forensic purposes: Recent applications for serology and gunshot residue analysis. *TrAC Trends Anal. Chem.* 103:215–222. https://doi.org/10.1016/j.trac.2017.12.003

Elkins, K.M, 2011. Rapid Presumptive "Fingerprinting" of Body Fluids and Materials by ATR FT-IR Spectroscopy. *J. Forensic Sci*., 56:1580–1587. https://doi.org/10.1111/j.1556-4029.2011.01870.x

G^{3}adysz, M., Król, M. and P. Koœcielniak, 2017. Differentiation of red lipsticks using the attenuated total reflection technique supported by two chemometric methods. *Forensic Science International*. 280: 130-138. https://doi.org/10.1016/j.forsciint.2017.09.019

Goodpaster, J. V. and E.A. Liszewski, 2009. Forensic analysis of dyed textile fibers. *Anal. Bioanal. Chem*., 394:2009–2018. https://doi.org/10.1007/s00216-009-2885-7

Gregório, I., Zapata, F. and C. García-Ruiz, 2017a. Analysis of human bodily fluids on superabsorbent pads by ATR-FTIR. *Talanta*., 162:634–640. https://doi.org/10.1016/j.talanta.2016.10.061

Gregório, I., Zapata, F. and C. García-Ruiz, 2017b. 2017b. Statistical approach for ATR-FTIR screening of semen in sexual evidence. *Talanta*., 174:853–857. https://doi.org/10.1016/j.talanta.2017.07.016

Harkins, T.R., Harris, J.T. and O.D. Shreve, 1959. Identification of Pigments in Paint Products by Infrared Spectroscopy. *Anal. Chem*., 31:541–545. https://doi.org/10.1021/ac50164a025

Lee, L.C., Liong, C.Y. and A.A. Jemain, 2018. Effects of data pre-processing methods on classification of ATR-FTIR spectra of pen inks using partial least squares-discriminant analysis (PLS-DA). *Chemom. Intell. Lab. Syst*., 182: 90–100. https://doi.org/10.1016/j.chemolab.2018.09.001

López-López, M. and C. García-Ruiz, 2014. Infrared and Raman spectroscopy techniques applied to identification of explosives. *TrAC Trends Anal. Chem.*, 54:36–44. https://doi.org/10.1016/j.trac.2013.10.011

López-López, M., Özbek, N. and C. García-Ruiz, 2014. Confocal Raman spectroscopy to trace lipstick with their smudges on different surfaces. *Talanta.,* 123:135–139. https://doi.org/10.1016/j.talanta.2014.02.025

López-López, M., Vaz, J. and C. García-Ruiz, 2015. Confocal Raman spectrocopy for the analysis of nail polish evidence. *Talanta* 138:155–162. https://doi.org/10.1016/j.talanta.2015.02.031

Manheim, J., Doty, K.C., McLaughlin, G. and I.K. Lednev, 2016. Forensic Hair Differentiation Using Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) Spectroscopy. *Appl. Spectrosc*., 70:1109–1117. https://doi.org/10.1177/0003702816652321

McLaughlin, G. and I.K. Lednev, 2015. In Situ Identification of Semen Stains on Common Substrates via Raman Spectroscopy. *J. Forensic Sci*. 60:595–604. https://doi.org/10.1111/1556-4029.12708

McLaughlin, G. and I.K. Lednev, 2014. A modified Raman multidimensional spectroscopic signature of blood to account for the effect of laser power. *Forensic Sci. Int*., 240:88–94. https://doi.org/10.1016/j.forsciint.2014.04.021

Mishra, S., Sarkar, U., Taraphder, S., Datta, S., Swain, D., Saikhom, R., Panda, S., and M. Laishram, 2017. Principal Component Analysis. *Int. J. Livest. Res.,* 1. https://doi.org/10.5455/ijlr.20170415115235

Mohamad Asri, M.N., Mat Desa, W.N.S. and D. Ismail, 2018. Source Determination of Red Gel Pen Inks using Raman Spectroscopy and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy combined with Pearson's Product Moment Correlation Coefficients and Principal Component Analysis. *J. Forensic Sci.*, 63:285–291. https://doi.org/10.1111/1556-4029.13522

Muro, C.K., Doty, K.C., Bueno, J., Halámková, L. and I.K. Lednev, 2015. Vibrational Spectroscopy: Recent Developments to Revolutionize Forensic Science. *Anal. Chem.*, 87:306–327. https://doi.org/10.1021/ac504068a

Muro, C.K., Doty, K.C., De Souza Fernandes, L. and I.K. Lednev, 2016. Forensic body fluid identification and differentiation by Raman spectroscopy. *Forensic Chem.*, 1:31–38. https://doi.org/10.1016/j.forc.2016.06.003

Orphanou, C.M, 2015. The detection and discrimination of human body fluids using ATR FT-IR spectroscopy. *Forensic Sci. Int.*, 252:e10–e16. https://doi.org/10.1016/j.forsciint. 2015. 04. 020

Quinn, A.A. and K.M. Elkins, 2017. The Differentiation of Menstrual from Venous Blood and Other Body Fluids on Various Substrates Using ATR FT-IR Spectroscopy. *J. Forensic Sci.*, 62: 197–204. https://doi.org/10.1111/1556-4029.13250

Rosenblatt, R., Halámková, L., Doty, K., Oliveira, E. and I. Lednev, 2019. Raman spectroscopy for forensic bloodstain identification: Method validation vs. environmental interferences. *Forensic Chem.*, 16:100175. https://doi.org/10.1016/j.forc.2019.100175

Salahioglu, F. and M.J.Went, 2012. Differentiation of lipsticks by Raman spectroscopy. *Forensic Sci. Int.*, 223:148–152. https://doi.org/10.1016/j.forsciint.2012.08.018

Sehgal, S., Singh, H., Agarwal, M., Bhasker, V. and Shantanu, 2014. Data analysis using principal component analysis, 2014. International Conference on Medical Imaging, m-Health and Emerging Communication Systems (MedCom).45–48. https://doi.org/10.1109/MedCom.2014.7005973

Sharma, S., Chophi, R., Kaur, H. and R. Singh, 2019a. Differentiation of Cosmetic Foundation Creams Using Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy: A Rapid and NonDestructive Approach in Trace Evidence Analysis. *J. Forensic Sci.*,65(3):751-761. https://doi.org/10.1111/1556-4029.14257

Sharma, S., Chophi, R., Kumar, R., Sharma, V. and R. Singh, 2019b. Differentiation of locally manufactured Kajal by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy supported by chemometric analysis. *Forensic Sci. Int.*, 303:109930. https://doi.org/10.1016/j.forsciint.2019.109930

Sharma, S., Chophi, R. and R. Singh, 2019. Forensic discrimination of menstrual blood and peripheral blood using attenuated total reflectance (ATR)-Fourier transform infrared (FT-IR) spectroscopy and chemometrics. *Int. J. Legal Med.*, 134: 63-77. https://doi.org/10.1007/s00414-019-02134-w

Sharma, S. and R. Singh, 2019. Detection and discrimination of seminal fluid using attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectroscopy combined with chemometrics. *Int. J. Legal Med.,* 134: 411-432. https://doi.org/10.1007/s00414-019-02222-x

Sikirzhytskaya, A., Sikirzhytski, V. and I.K. Lednev, 2012. Raman spectroscopic signature of vaginal fluid and its potential application in forensic body fluid identification. *Forensic Sci. Int.*, 216:44–48. https://doi.org/10.1016/j.forsciint.2011.08.015

Sikirzhytskaya, A., Sikirzhytski, V., McLaughlin, G. and I.K. Lednev, 2013. Forensic Identification of Blood in the Presence of Contaminations Using Raman Microspectroscopy Coupled with Advanced Statistics: Effect of Sand, Dust, and Soil. *J. Forensic Sci.,* 58:1141–

1148. https://doi.org/10.1111/1556-4029.12248

Sikirzhytski, V., Sikirzhytskaya, A. and I.K. Lednev, 2012. Multidimensional Raman spectroscopic signature of sweat and its potential application to forensic body fluid identification. *Anal. Chim. Acta.,* 718:78–83. https://doi.org/10.1016/j.aca.2011.12.059

Stewart, S.P., Bell, S.E.J., Armstrong, W.J., Kee, G. and S.J. Speers, 2012. Forensic examination of multilayer white paint by lateral scanning Raman spectroscopy. *J. Raman Spectrosc*., 43: 131–137. https://doi.org/10.1002/jrs.2982

Virkler, K. and I.K.Lednev, 2009. Blood species identification for forensic purposes using Raman spectroscopy combined with advanced statistical analysis. *Anal. Chem.,* 81(18): 7773-7777. https://doi.org/10.1021/ac901350a

Williamson, R., Raeva, A. and J.R.Almirall, 2016. Characterization of Printing Inks Using DART-Q-TOF-MS and Attenuated Total Reflectance (ATR) FTIR. *J. Forensic Sci*., 61:706–714. https://doi.org/10.1111/1556-4029.13107

Zapata, F., de la Ossa, M.Á.F. and C. García-Ruiz, 2016. Differentiation of Body Fluid Stains on Fabrics Using External Reflection Fourier Transform Infrared Spectroscopy (FT-IR) and Chemometrics. *Appl. Spectrosc*., 70:654–665. https://doi.org/10.1177/0003702816631303

Zapata, F., Fernández de la Ossa, M.Á. and C. García-Ruiz, 2015. Emerging spectrometric techniques for the forensic analysis of body fluids. *TrAC Trends Anal. Chem*., 64:53–63. https://doi.org/10.1016/j.trac.2014.08.011

Zou, Y., Xia, P., Yang, F., Cao, F., Ma, K., Mi, Z., Huang, X., Cai, N., Jiang, B., Zhao, X., Liu, W. and X. Chen, 2016. Whole blood and semen identification using mid-infrared and Raman spectrum analysis for forensic applications. *Anal. Methods*., 8:3763–3767. https://doi.org/10.1039/C5AY03337C