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Effectiveness of UV-Visible Spectroscopy (with Multi-Mode Plate Reader) and ATR-FTIR for the Discrimination of Black Marker Inks: A Chemometric Approach

فعالية التحليل الطيفي للأشعة فوق البنفسجية والمرئية (مع قارئ اللوحة متعدد الأوضاع) والأشعة تحت الحمراء لتحويل فورييه (ATR-FTIR) المُحلّلة بالوقت في تمييز أحبار قلم التحديد الأسود: بأسلوب القياس الكيميائي

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Abstract

In the ambit of Forensic examination of the questioned documents, writing instruments often serve as an essential tool in disclosing the legitimacy of a document. In the present study, a total of 18 marker samples including 9 permanent and 9 whiteboard (commonly known as dry erase) markers of black color of different brands were purchased from the local market of district Jhansi, Uttar Pradesh, India. UV-Visible spectroscopy with Multi-Mode Plate Reader and ATR-FTIR spectroscopy were used for the discrimination of samples. In the case of permanent marker inks, peaks near 390-405 nm and at 570-585 nm were found to be more prominent and differentiating than that of the whiteboard marker inks. Qualitative comparative analysis of the permanent and the whiteboard black marker ink samples showed 97.2% and 91.6% DP respectively based on their UV-Vis. spectral responses. FTIR spectroscopy of whiteboard markers confirmed the presence of characteristic peak at 1743 cm-1 (C=O stretch) together with a doublet in the region 2920-2850 cm-1 corresponding to asymmetric and symmetric C-H stretch in

Keywords: Forensic science; PCA; DFA; Marker ink discrimination; Document examination; Black marker inks; Photometric analysis.

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المستخلص

في نطاق الفحص الجنائي للوثائق موضع التحقيق، غالبًا ما تكون أدوات الكتابة بمثابة أداة أساسية في الكشف عن شرعية الوثيقة. في هذه الدراسة، تم شراء ما مجموعه 18 عينة من أقلام التحديد، بما في ذلك 9 أقلام بأحبار دائمة و9 أقلام تستخدم للسبورة البيضاء (المعروفة بإمكانية مسحها بمسحات جافة) ذات اللون الأسود لعلامات تجارية مختلفة من السوق المحلي لمنطقة جانسي، أوتار براديش، في الهند. تم استخدام التحليل الطيفي للأشعة فوق البنفسجية مع قارئ اللوحة متعدد الأوضاع والتحليل الطيفي ATR-FTIR لتمييز العينات. في حالة أحبار التحديد الدائمة، تم العثور على القمم القريبة من 390-405 نانومتر وعند 570-585 نانومتر لتكون أكثر بروزًا وتمييزًا من أحبار علامات السبورة البيضاء. أظهر التحليل المقارن النوعي لعينات حبر العلامة السوداء الدائمة وحبر السبورة البيضاء 97.2% و91.6% على التوالي استنادًا إلى الاستجابات الطيفية للأشعة فوق البنفسجية المرئية. أكد التحليل الطيفي FTIR لعلامات السبورة البيضاء وجود ذروة مميزة عند 1743 سم$^{-1}$ (امتدادC=O) مع وجود ثنائي في المنطقة 2850-2920 سم$^{-1}$ يتوافق مع امتداد C-H غير المتماثل والمتماثل في جميع

الكلمات المفتاحية: علوم الأدلة الجنائية، PCA، DFA، تمييز حبر أقلام التحديد، فحص المستندات، أحبار أقلام التحديد السوداء، التحليل الضوئي.

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almost all the whiteboard marker inks, whereas in case of permanent marker inks the former peak 1743 cm-1 was completely absent and the later appeared as a single peak around 2900 cm-1. The results obtained from ATR-FTIR was analyzed using multivariate techniques such as PCA and DFA. PCA was used for the segregation of samples into different groups, whereas DFA was used for the discrimination and classification of marker ink samples. DFA provided a correct classification of 88.9% of the marker ink samples analyzed by FTIR. It is concluded that Multi Mode Plate Reader can be used as a substantial tool for the discrimination of marker inks and was found to be fast and cost effective with high throughput. Moreover, ATR-FTIR results gave some additional information related to the chemical composition of the samples and provided better discrimination when coupled with chemometrics.

أحبار علامات السبورة تقريبًا، بينما في حالة أحبار العلامة الدائمة كانت الذروة السابقة 1743 سم$^{-1}$ غائبة تماماً وظهرت اللاحقة كقمة واحدة حوالي 2900 سم$^{-1}$. تم تحليل النتائج التي تم الحصول عليها من ATR-FTIR باستخدام تقنيات متعددة المتغيرات مثل PCA و DFA. تم استخدام PCA لفصل العينات إلى مجموعات مختلفة، في حين تم استخدام DFA للتمييز وتصنيف عينات حبر العلامة. قدمت DFA تصنيفًا صحيحًا لـ 88.9% من عينات حبر التحديد التي تم تحليلها بواسطة FTIR. تم التوصل إلى أنه يمكن استخدام Multi Mode Plate Reader كأداة أساسية لتمييز أحبار أقلام التحديد، وقد وجد أنها سريعة وفعالة من حيث التكلفة مع إنتاجية عالية. علاوة على ذلك، أعطت نتائج ATR-FTIR بعض المعلومات الإضافية المتعلقة بالتركيب الكيميائي للعينات وقدمت تمييزًا أفضل عندما تقترن بالقياسات الكيميائية.

1. Introduction

Chalk sticks were used as a writing instrument for writing over the blackboard, but later to counter difficulties in using the blackboard and the chalk stick, dry erase ink markers of different colors were used on whiteboards. Nowadays, the use of whiteboards and markers is a common practice in classroom teaching. The use of markers is not only limited to classroom teaching, rather there are various types of markers available in the market (such as dry- erase markers, wet- erase markers, alcohol resistant markers, sterile surgical markers, etc.) that can be used to serve the intended purpose of writing such as object labeling, photograph attestation, labeling goods for dispatch, marking sites for surgery and so on [1, 2]. But in general, markers can be classified into two categories i.e., temporary and permanent. A temporary marker, also known as a whiteboard or dry erase marker, is a highly used instrument for writing on the whiteboard, leaving a temporary mark which can be easily erased. Permanent markers are the most versatile markers usually, come up in dark colors and can be used on a variety of surfaces leaving a permanent mark or writing upon drying.

Like any other ink-based writing tool, markers do contain the three fundamental ingredients in their chemistry i.e., colorant, solvent, and polymer [3]. Apart from these three basic substances, several other chemicals such as metallic salts, surfactants, humectants, resins, plasticizers, and lubricants are also used to improve the properties of markers [4]. Each of these chemicals possesses distinctive properties and can be used to improve the color intensity, writability and stability of marker ink. These substances together regulate the flow and quality of the ink. Originally, xylene was used as a solvent but due to its toxicity and possible exposure to children other less toxic alternatives such as ethanol and isopropanol were used in the marker chemistry [5, 6]. Silicone polymers used in whiteboard marker prevent the pigment contact with the surface, whereas acrylic polymer is one of the essential substance used in permanent marker that facilitates the pigment to stick to a variety of surfaces which results in their multipurpose use. Xylene, toluene, and urethane resins are some key chemicals that impart permanent markers their characteristic ability to sustain on unusual surfaces and leave long-lasting marks behind.

Various analytical techniques have been utilized by different researchers to study the composition and characteristics of inks used by writers. These include the use of separation techniques such as Thin-Layer Chromatography (TLC) [7-9] and Capillary Electrophoresis [10] to analyze dyes and pigments present in the ink. GC-MS [11,12] and HPLC [13] were utilized to identify the volatile solvents used in the inks. Other methods, such as Infrared [14-16] and Raman [16, 17] spectroscopy were used to discriminate ballpoint pen inks on the basis of dyes and pigments present in markers. Mass Spectrometry coupled with different ionization techniques such as Laser Desorption Ionization (LDI) [18], Matrix-Assisted LDI [19], and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) [20] has also been used for ink analysis providing the elemental information of the sample under investigation.

LIBS technique is found to be effective in determining the chemical composition of various colours, brands and types of ink. The discriminatory power of this method was found to be highly effective [21]. LIBS when compared with Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) demonstrated significant differences i.e., discrimination of 99.8% and 100% between different batches of same brand as well as between different batches of paper as well as ink [22]. The combined techniques of LA-ICP-MS and LIBS technique for the characterization of inks and paper with minimal destruction proved effective in many cases as demonstrated in various research [23].

The combination of various techniques of VIS-reflectance Spectroscopy, TLC, Surface-enhanced Raman Spectroscopy enabled in the detection of all the organic and inorganic components found in the different brands of inks [24]. MALDI and LDI-MSI technique have been found to discriminate ink effectively surpassing the results of TLC [25-27].

Fourier transform infrared (FTIR) spectroscopy and particularly micro-FTIR can even differentiate the various spectra of individual inks in the questioned documents in a non-destructive way [28]. Raman spectroscopy is an effective nondestructive tool for forensically analyzing the inks on various documents. Due to the lack of time-consuming sample preparation stages, this method may quickly offer the chemical information about the ink. Raman spectroscopy has been utilized frequently for ink analysis and exhibits strong discrimination power. New publications on the detailed reviews of forensic uses of Raman spectroscopy emphasis in situ analysis of ink pigments and dyes in disputed documents [29].

XPS, LA-ICP-MS, TOF-SIMS and SEM technique have also been identified to effectively differentiate the components i.e., dyes and pigments of the writing inks as analyzed in various researches [30-32]. The HPLC technique has been proven to effectively discriminate between the various components of ink utilising various solvent systems as suggested by different researchers [33-36].

Marker ink pens are versatile writing instruments that enable users to write on usual (paper, whiteboard, cardboard etc.) and unusual (glass, metal, plastic, laminates, ceramic, skin etc.) surfaces as per their intended purposes. The use of marker pens has increased than ever before. Since ink pen markers are used to write on a variety of surfaces, they are prone to be used in illegal activities ranging from minor crimes such as writing threatening notes to heinous crimes such as terrorism (marking on the metallic or electronic surface of an IED). In such cases, forensic identification, classification and discrimination of marker inks play a significant role in the legal inquiry.

A number of studies have been carried out by different researchers focusing on the ballpoint pen, gel pen, stamp, and printer inks; however, the research is still very limited on marker pen inks. Therefore, the foremost aim of the present research work is to test the efficacy of ATR FTIR (non-destructive method) and UV-Visible spectroscopy (destructive method) for the discrimination of dry erase (whiteboard) and permanent markers based on the presence or absence of spectral bands.

2. Materials and Methods

2.1 Samples

In the present study, a total of 18 marker samples including 9 permanent and 9 whiteboard (commonly known as dry erase) markers of different brands were purchased from the local market of district Jhansi, Uttar Pradesh, India (Table 1). All the samples collected were black in color as it is conventional and gives good contrast over a wide range of surfaces. Separate rectangles of height 3 cm and length 4 cm were prepared using each marker pen on a standard 75 gsm A4 size paper manufactured in Gujrat, India by Millenium. All the rectangles were created simultaneously using normal hand pressure by a single person without overwriting. Methanol (AR grade) manufactured by HIMEDIA was used as an extracting solvent.

2.2 Sample Preparation for Multi-Mode Plate Reader Analysis

Four punches of diameter 5 mm were cut from the inked surfaces of each sample using a DP-52 perforateur manufactured by Kangaro Industries, Ludhiana, India. These punch-outs obtained from different samples were collected in separate Eppendorf tubes, each containing 1 ml methanol as extracting solvent. Now, in order to facilitate the extraction of marker ink components out of the paper substrate, each Eppendorf tube was then placed onto a vortex shaker for about 2 min. Four hundred µL solvent was pipetted out and loaded onto the micro-wells plate. All the samples were analyzed using flat bottom 96 wells microplate at 200-1000 nm. Blank samples were also prepared using a non-written area following the same procedure.

Table 1- *Different Brands of Permanent and Whiteboard Marker Inks Used.*

Permanent Marker Inks		Whiteboard Marker Inks	
Sample Mark	**Manufacturer**	**Sample Mark**	**Manufacturer**
P1	Hauser	W1	Hauser
P2	Rorito	W2	Rorito
P3	Doms	W3	Doms
P4	Add Gel	W4	Add Gel
P5	Artline	W5	Artline
P6	Faber Castle	W6	Faber Castle
P7	Cello	W7	Cello
P8	Camlin	W8	Rotomac
P9	Luxar	W9	Korex

2.3 Instrument Used

2.3.1 Multi-Mode Plate Reader (MMPR): All the samples were analyzed on the basis of spectra obtained from an advanced spectral scanning Varioskan LUX MultiMode Microplate Reader manufactured by Thermo Fischer, Finland. It is controlled by SkanIt Software (version 2.4.5) that is equipped with data processing and reporting functions. The instrument provides measurement in the UV/VIS/NIR wavelength range and allows optimization of the measurement wavelengths and spectral scanning according to the application needs. The system is equipped with Xenon flash lamp as a light source that pulsed at 100 Hz and uses a photomultiplier tube as a detector to convert the emission light into electrical signals. The instrument comprises two monochromators that are serially connected for high spectral qualities minimizing leakage of undesired wavelengths, i.e., stray lights. Photometric measurements were taken through the 96 wells plate.

2.3.2 Universal Attenuated Total Reflectance (UATR) Fourier Transform Infrared (FTIR): FTIR spectra of all the samples were acquired using a non-destructive method involving Universal Attenuated Total Reflectance (UATR) accessory with spectrum two spectrometer manufactured by Perkin Elmer, Llantrisant (UK). Samples were scanned in the range of 4000 to 400 cm-1, with the accumulation of 32 scans at a spectral resolution of 4 cm-1. Each sample was subjected to baseline correction using the software Spectrum IR version 10.7.2.

2.4 Statistical Methods

All the statistical analysis except discrimination power was performed using Minitab® Statistical Software 21.4.0.0. PCA was used to understand the overall variability and potential overlap between the sample classes, whereas DFA was applied to ascertain the discrimination and classification of samples based on FTIR dataset.

2.4.1 Discrimination Power Analysis of Permanent and Whiteboard Marker Inks Using Multi-Mode Plate Reader

Discrimination Power (DP) is a potential method used for the pair-wise comparison of samples. In the present study, DP method was used to analyze the pair-wise similarities and dissimilarity among the photometric spectra of the samples. DP method was first employed by Smalldon and Moffat [37], and can be expressed mathematically as:

$$DP = \frac{\text{Total number of discriminated pairs of samples}}{\text{Total number of possible pairs of sample}} \times 100$$

The total number of possible sample pairs can be calculated using the formula [n (n-1)/ 2], where 'n' represents the total number of samples under investigation. All the samples were examined for both the intra-spectral and inter-spectral variations. Intra-spectral variation was studied between the samples of the same group whereas inter-spectral variation was studied between the two groups of samples.

2. 4. 2 Principal Component Analysis (PCA)

Principal Component Analysis is a widely used technique to reduce the dimensionality of multivariate datasets. The most common challenge with high dimensional data is their visualization and interpretation. PCA is a technique for reducing the amount of information in the data while preserving as much of the original variance as feasible. PCA generates new uncorrelated variables called principal components through a process of linear transformations of original data structure. These new variables are orthogonal and comes out of eigenvectors and eigenvalues of the covariance matrix of the data. Eigenvectors represent the direction of largest variation whereas, eigenvalues are measure of variance by each eigenvector in the dataset. PCA decomposes original data matrix X and replace it by T and P forming a covariance matrix, which can be mathematically expressed as

$$X = TP^T$$

Where, T is called the scores and has as many rows as the original data matrix and P are called the loadings and have as many columns as the original data matrix. The number of columns in the matrix T equals the number of rows in the matrix P [38-42].

2. 4. 3 Discriminant Function Analysis (DFA)

DFA is the most prevalent supervised pattern recognition technique used for the discrimination of multivariate data [43]. Its foundation is the creation of linear combination of predictor variables known as the discriminant function that can help in differentiating and classifying between two or more groups. It is a powerful method to determine whether a set of variables/predicters is effective in predicting category membership [44].

3. Results and Discussion

The results of the UV-Vis. spectroscopic analysis of both the permanent and the whiteboard black marker inks are shown in the Table 2. In order to avoid any spectral interference due to paper materials, photometric spectra of the samples were collected after their blank subtraction as shown in Fig 1.

Table 2 - *Different Wavelengths Observed in the Spectral Absorbance of Various Whiteboard and Permanent Black Marker Inks. The Symbol (√) Signifies the Presence of a Peak at Particular Wavelength Ranges.*

Samples	200-215 nm	225-240 nm	245-260 nm	275-290 nm	295-310 nm	340-355 nm	390-405 nm	570-585 nm	Others nm
P1			√		√		√	√	
P2			√		√		√	√	
P3					√		√	√	440 ,270
P4	√		√		√		√	√	
P5	√	√	√		√		√	√	
P6			√		√		√	√	780 ,760 ,735
P7	√	√	√		√				555 ,380
P8	√	√		√		√	√	√	
P9	√	√			√		√		630 ,510 ,480
W1	√	√	√	√		√	√		
W2	√	√	√	√		√			
W3	√	√	√	√			√		
W4	√	√		√		√	√		
W5	√	√		√		√	√		
W6	√	√	√	√					
W7	√	√	√		√	√			
W8	√	√	√		√	√			
W9	√	√	√		√				265

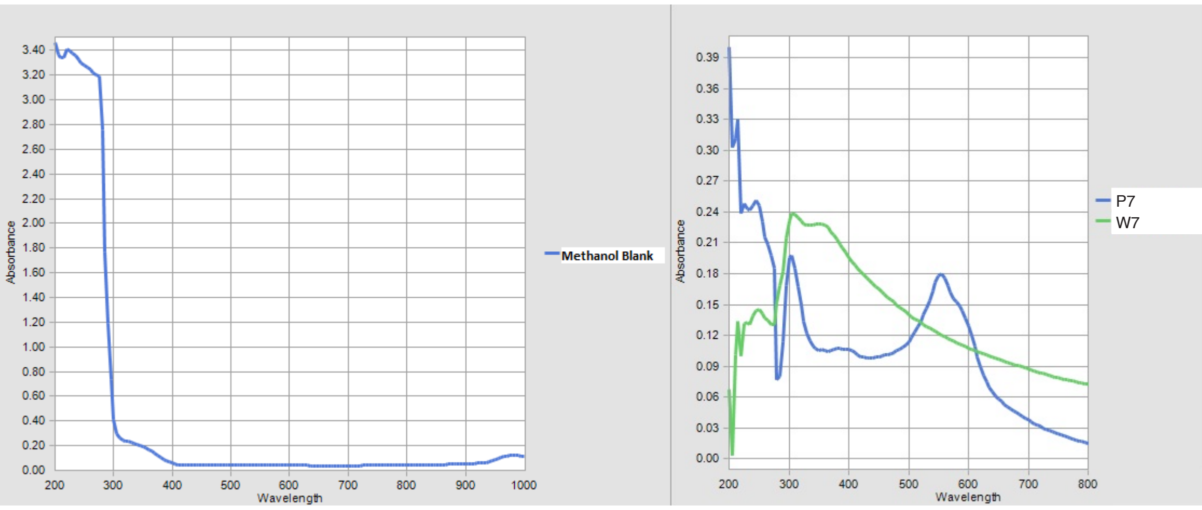

Figure 1 - *UV-Visible Spectral Responses of Methanol Blank together with Cello Permanent (P7) and Cello Whiteboard (W7) after Blank Subtraction.*

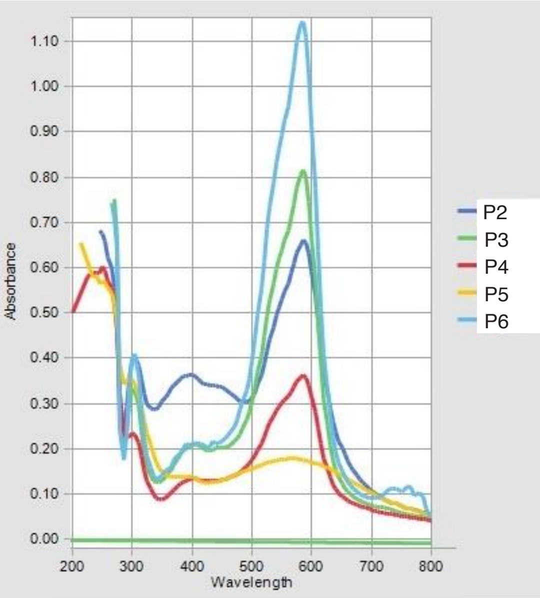

Figure 2 - *UV-Visible Spectral Absorbance of Black Permanent Marker Inks of Five Different Brands.*

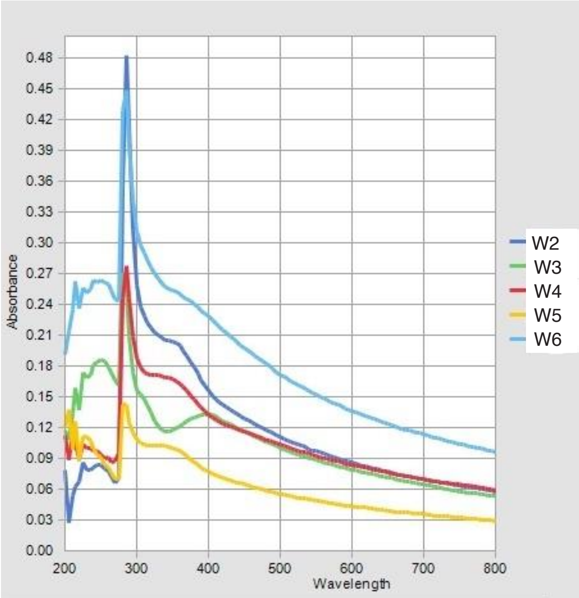

Figure 3 - *UV-Visible Spectral Absorbance of Black Whiteboard Marker Inks of Five Different Brands.*

For permanent marker inks, two characteristic peaks were found common in most of the samples, one being the sharp and low intensity peak observed between 295–310 nm and the other being the mountain top with high intensity peak observed between 570-585 nm (mostly at 585 nm) as shown in Fig 2. Only Samples P1 and P2 were found similar showing common spectral absorbances at four different wavelength ranges. For whiteboard marker inks, a sharp and intense peak between 275-290 nm was observed in all the samples except W7, W8 and W9. The middle UV region (200–300 nm) was found to be the most differential region for whiteboard marker inks as large number of peaks were found only in this region as shown in Fig 3. In addition, any extra peak other than the usual peaks of whatever low intensity may add up to the information and can lead to better discrimination of the samples.

In the present study, both the intra-spectral and inter-spectral variations among the permanent and whiteboard marker inks were studied and presented in Table 3 and 4 respectively. The results of the intra-spectral variations between the groups of permanent and whiteboard marker inks showed 97.2% and 91.6% DP respectively. The inter-spectral variations between the samples of permanent and whiteboard marker inks showed 100% DP.

Characteristic functional groups and their associated IR peaks observed for whiteboard and permanent marker inks are listed in Table 5. Based on the peaks observed in the IR spectra, both the whiteboard and the permanent marker inks were examined and classified. Samples were analyzed non-destructively on the piece of paper, C-H and O-H stretching were found common in each of the samples at group frequencies between 2925-2900 cm-1 and 3335-3315 cm-1 respectively, corresponding to the cellulose bands. Minor differences were observed in the relative intensity and forms of the peaks because all the samples analyzed were only black in color. FTIR spectroscopy of whiteboard markers confirmed the presence of a doublet in the region 2920-2850 cm-1 (corresponding to asymmetric and symmetric C-H stretch) in almost all the whiteboard marker inks, which in the case of permanent marker inks appeared as a single peak around 2900 cm-1. Also, a peak at 1642 cm-1 corresponding C=C stretch and a medium peak at 1743 cm-1 indicative to acids or esters, were found particularly in whiteboard marker inks shown in Fig 5. For permanent marker inks, the occurrence of C-H stretch at 2900 cm-1; O-H bend at 1315 cm-1; C-N stretch at 1027 cm-1 and C-C stretch (in ring) at 1585 cm-1 correspond to the presence of aromatic compounds as shown in Fig 4.

The results obtained from IR spectroscopy were subjected to PCA to obtain clustering information of species from multiple wavebands. The values of variance explained by all the principal components (PCs) are shown in Table 6. For the analysis, only first four factors i.e., PC1, PC2, PC3 and PC4 were considered because of their eigenvalues found

Table 3 - *Intra-spectral Variations among the Different Permanent and Whiteboard Marker Inks.*

Samples for Intra-spectral variation	* n	Total number of =pairs n(n-1)/2	Discriminating pairs (total no.)	Non-Discriminating Pairs (Total no.)	Discrimination Power (DP)
Whiteboard	9	36	33	3	91.6%
Permanent	9	36	35	1	97.2%

* n = Total no. of samples

Table 4 - *Inter-spectral Variations among the Different Permanent and Whiteboard Marker Inks.*

Samples for inter variations	Total no of samples $n = n_1 + n_2$	Total number of pairs = $n_1 * n_2$	Discriminating pairs (total no.)	Non-Discriminating Pairs (Total no.)	Discrimination Power (DP)
Whiteboard (n_1) and Permanent (n_2)	18	81	81	0	100%

* n = Total no. of samples

Table 5 - *Characteristics Bands Observed in the IR Spectra of Permanent and Whiteboard Marker Inks.*

Whiteboard Marker Inks		Permanent Marker Inks	
Peak (cm^{-1})	**Functional Group Assignment**	**Peak (cm^{-1})**	**Functional Group Assignment**
3320	O-H stretching	3330	O-H stretching
2918	Asymmetric C-H stretch (alkyl)	2900	C-H stretch (alkyl)
2853	Symmetric C-H stretch	1585	C-C stretch (ring)
1743	Aliphatic -COOR (ester)	1315	O-H bending
1642	C=C stretch	1427	O-H stretch
1428	C-O bend	1161	C-O stretch
1102	C-O stretch	1027	C-N stretch amine
895	C-H bend	871	C-H bend

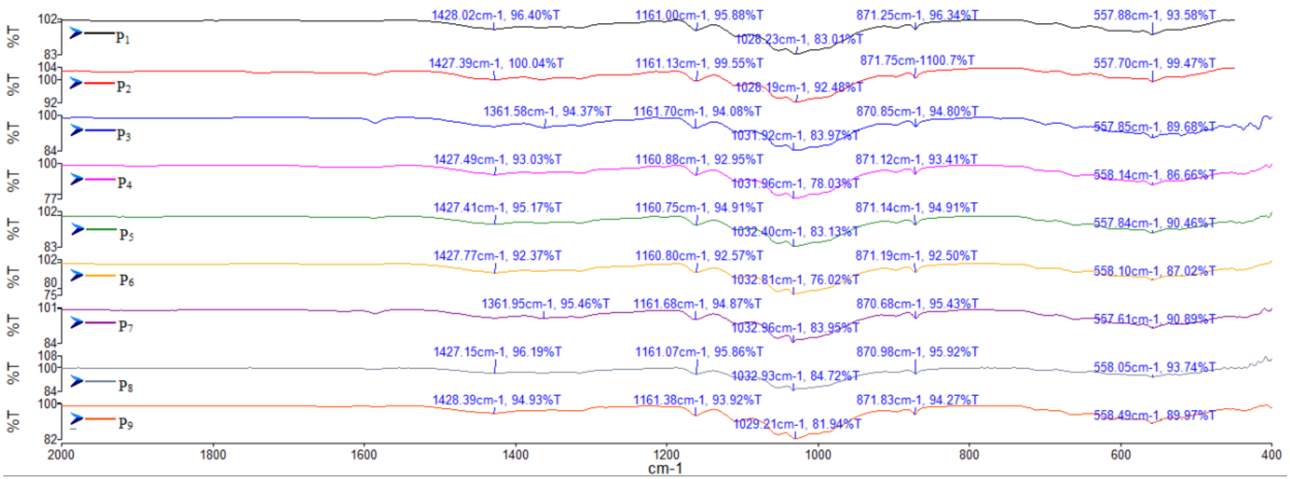

Figure 4 - *Infrared Spectra of Different Permanent Black Marker Inks.*

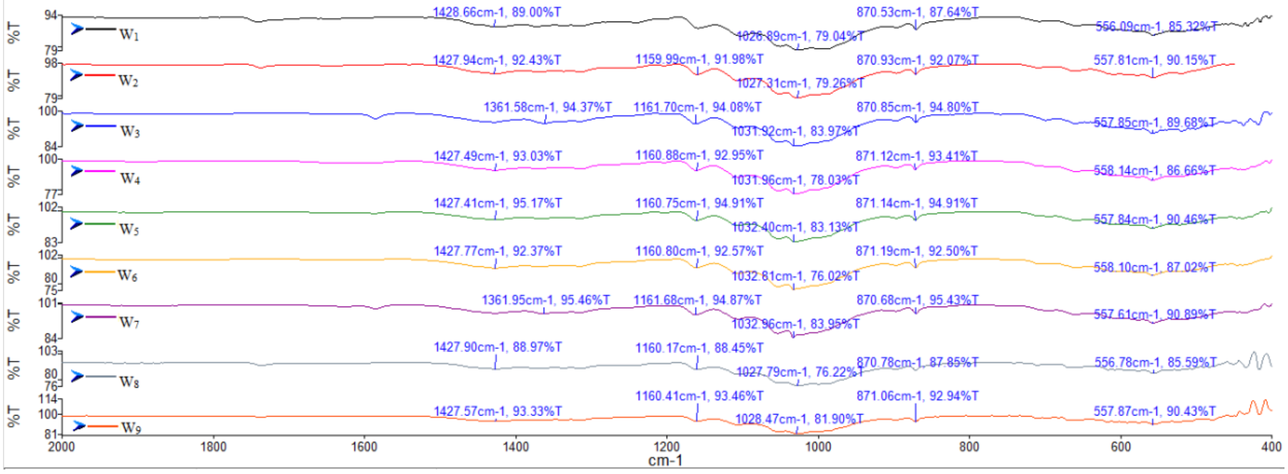

Figure 5 - *Infrared Spectra of Different Whiteboard Black Marker Inks.*

grater than 1. The aggregate variance explained by the four PCs was found 99.0% (PC1 for 86.4%, PC2 for 7.0%, PC3 for 3.7% and PC4 for 1.80%), shown in Table 6.

Prior to the discriminant function analysis, all the samples were clustered into different groups based on PC2 and PC1 (showing maximum variability) loadings as shown in Fig 6. All the samples were clustered into four different groups which was further substantiated by the hierarchical clustering dendrogram (shown in Fig 7) for their proper classification.

Table 6 - *The Total Variance Explained for the Permanent and Whiteboard Black Marker Inks.*

Components	1	2	3	4	5	6	7	8	9	10
Eigenvalue	201.79	16.37	8.57	4.32	0.79	0.48	0.38	0.35	0.15	0.09
(%) Proportion	86.4	7.00	3.70	1.80	0.30	0.20	0.20	0.20	0.10	0.000
(%) Cumulative	86.4	93.4	97.1	99.0	99.3	99.5	99.7	99.8	99.9	99.9

Table 7 - *Tests of Equality of Group Means.*

	Wilks' Lambda	F	df1	df2	Sig.
PC1	0.031	147.497	33	14	0.000
PC2	0.468	5.296	3	14	0.012
PC3	0.790	1.238	3	14	0.333
PC4	0.896	0.543	3	14	0.661

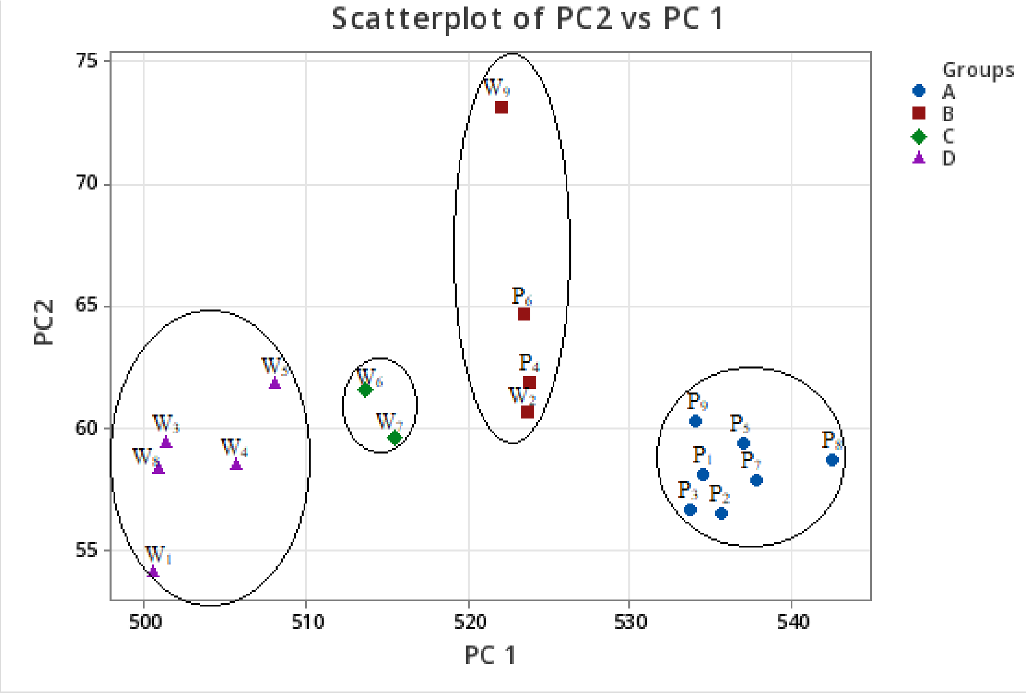

Figure 6 - *Scatter Plot of PC1 and PC2.*

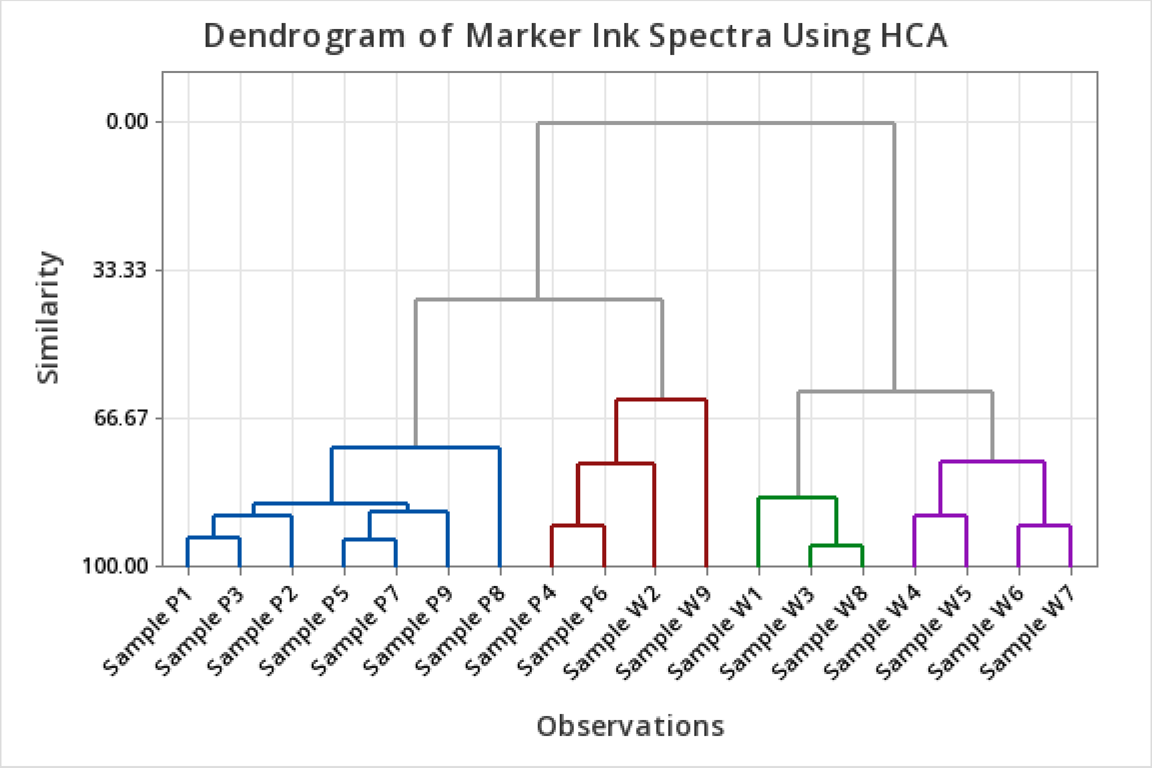

Figure 7 - *Plot of Hierarchical Clustering Dendrogram of Permanent and Whiteboard Marker Ink Samples.*

Table 8 - *Summary of Canonical Discriminant Functions.*

	Eigenvalues			Wilks' Lambda			
Function	**1**	**2**	**3**	**Test of Function(s)**	**1 through 3**	**2 through 3**	**3**
Eigenvalue	59.257	1.932	0.158	Wilks' Lambda	0.005	0.295	0.863
% of Variance	96.6	3.1	0.3	Chi-square	69.173	15.891	1.909
% Cumulative	96.6	99.7	100	df	12	6	2
Canonical Correlation	0.992	0.812	0.370	Sig.	0.000	0.014	0.385

The classification of samples was assessed using DFA with cross validation as shown in Table 9. The scores of first four principal components were used as independent variables. The two independent variables PC1 and PC2 were found potential and significantly contributing to the discriminant function ($p < 0.05$) as shown in Table 7. In our case the homogeneity of variances was not met. However, we conclude that the groups do differ in their covariance matrices considering the small sample size and no outliers in the data.

The first three canonical discriminant functions were used in the analysis, in which the first function was found highest discriminating with eigenvalue around 59.26 and canonical correlation 0.992. The other two functions were found less discriminative as compared to the first one, in which the second function was more discriminating than the third one with eigenvalue 1.932 and canonical correlation 0.812 as shown in Table 8. The first two discriminant dimensions with Wilks' Lambda values of 0.005 and 0.295 respectively, were found statistically significant ($p < 0.05$). The smaller the Wilks' Lambda value, the grater the discriminatory ability of the function.

From Table 9, the classification of actual group columns A, C and D matches with the column of predicted group with a proportion of 1. This shows that the samples contained in groups A, C and D were correctly classified. Only in column B, 2 samples appeared to be misclassified with a proportion of 0.5. The cross-validation result showed that the two samples W2 and W9 were misclassified as shown in Table 10. In actual group, the minimum square distance for W2 and W9 was 8.802 and 10.21, whereas in cross-validated group it was found 14.511 and 463.39 respectively, predicting the samples to be assigned in group C for correct classification. Overall, the classification of the samples tested by cross-validation showed that 88.90% of these classifications were correct.

Table 9 - *Summary of Classification with Cross-validation.*

Predicted Group	Actual Group			
	A	B	C	D
A	7	0	0	0
B	0	2	0	0
C	0	2	2	0
D	0	0	0	5
Total N	7	4	2	5
N correct	7	2	2	5
Proportion	1.00	0.50	1.00	1.00

The combined-group plot of canonical discriminant function scores is shown in Fig 8. The individual discriminant scores of permanent and whiteboard marker samples were shown in blue circles and red triangles. The x-axis shows that the function 1 best discriminates the samples with most of the permanent markers on one side and the whiteboard markers on the other side, while the y-axis shows that function 2 separates the two categories with lower differences than function 1.

Van der Werf et al. [45] analyzed twenty-four permanent marker inks obtained from different manufacturers using PyGC-MS and were able to discriminate them on the basis of binder composition. Sharma et al. [46] used Diffuse Reflectance UV-Visible Spectroscopy for the discrimination and comparison of twenty-four marker pens (12 permanent and 12 whiteboard) containing blue inks and observed discriminating power (DP) of 95.45% for

Table 10 - *Summary of Misclassified Samples.*

Samples	Actual Group	Cross-validated Group	Group	Square Distance		Probability	
				Actual Group	Cross-validated Group	Actual Group	Cross-validated Group
W2	B	C	A	71.372	137.674	0.00	0.00
			B	8.802	89.827	0.89	0.00
			C	12.925	14.511	0.11	1.00
			D	67.230	84.290	0.00	0.00
W9	B	C	A	72.44	579.66	0.00	0.00
			B	10.21	608.53	1.00	0.00
			C	38.94	463.39	0.00	1.00
			D	103.19	583.85	0.00	0.00

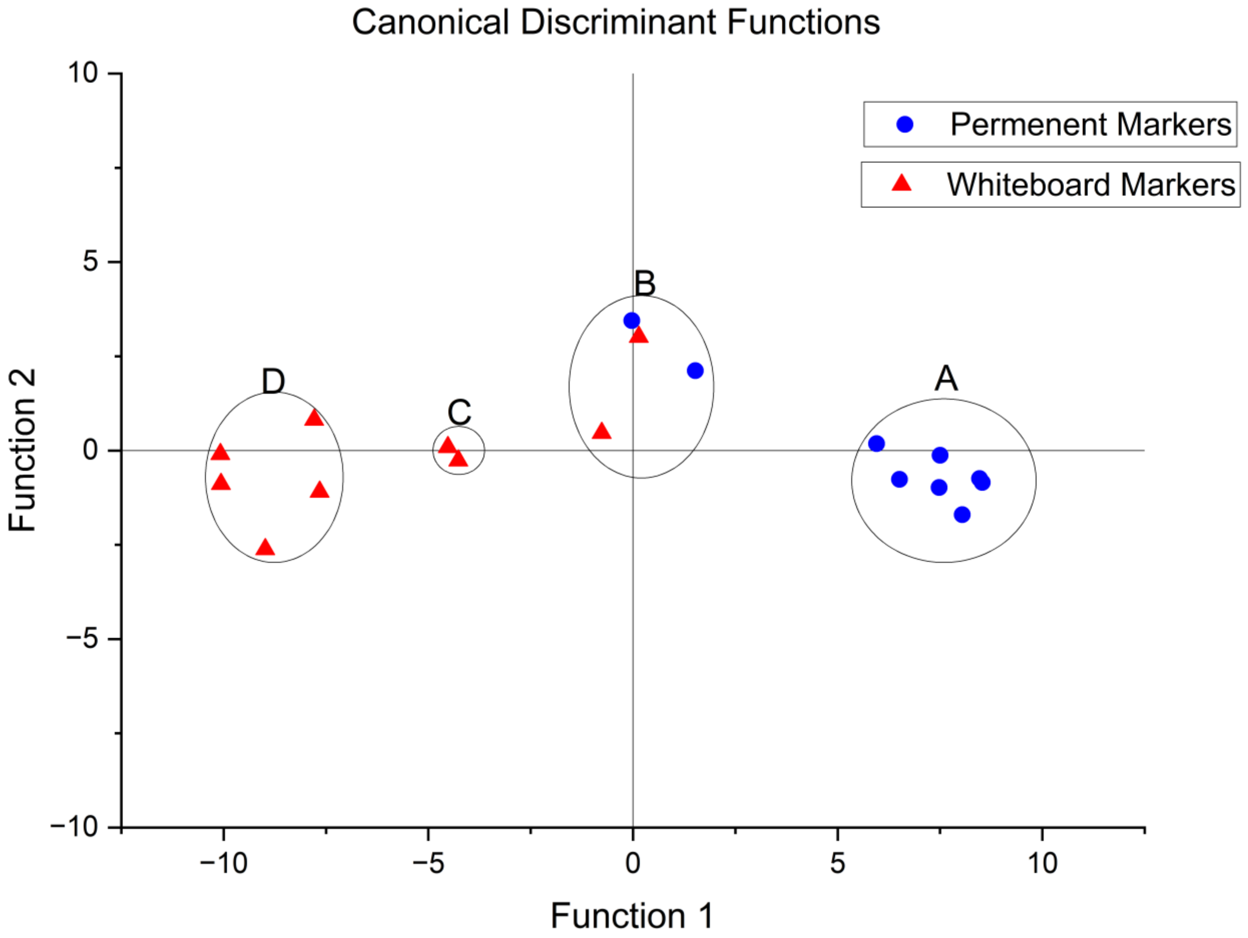

Figure 8 - *Combined-group Plot of Canonical Discriminant Functions.*

permanent marker inks only. Brinskelle et al. [47] used forty-five surgical marker pens to analyze bacterial transmission from them using sonication and culture methods and suggested the single use of markers to reduce transmission risk. Aweb et al. [48] analyzed permanent and non-permanent markers inks of four different colors using Fourier Transform Infrared Spectroscopic technique and differentiated them based on the diagnostic peaks observed in IR spectra. The findings of the present study correlate favorably well with the findings of Sharma et al. [46] and further support the concept of using UV-Vis-NIR spectroscopy and FTIR as substantial tools for the classification and discrimination of marker inks.

4. Conclusion

The present research work provides a precocious solution to the marker ink examination that can play a significant role in their legal inquiry as far as classification and discrimination are concerned. One of the most striking findings of the present study is that the inter-spectral variation among the two groups of marker inks (i.e., whiteboard and permanent marker inks) gave 100% DP. Furthermore, the pair-wise comparison of the results of permanent marker inks obtained from MMPR gave 97.2% DP, whereas whiteboard marker inks gave 91.6% DP. The differentiation of the marker samples by MMPR was based on a qualitative comparison of spectra taking into account the presence and absence of absorption bands. The versatility of the MMPR instrument makes it an ideal tool for analyzing various forensic exhibits. It can be used for a fast, cost-effective, and high throughput analysis.

Furthermore, the universal attenuated total reflectance ATR-FTIR spectroscopy as a non-destructive method requires no pretreatment, and clearly indicates the presence of saturated hydrocarbons and acids or esters as the main constituent in almost all the whiteboard marker inks. The IR spectra of permanent marker inks show the presence of aliphatic hydrocarbons as the chief constituent and did not show any traces of toluene and xylene, the most concerned chemicals due to EN 17-3 regulations. The analysis of results obtained from ATR-FTIR coupled with chemometric is proved better in their classification and discrimination. In the present study, PCs conjoined with DFA gave 88.9% correct classification of marker ink samples.

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Conflicts of Interests

The authors declare no conflicts of interest.

Author Contributions

Pawan Gupta conceived the idea and performed experimental work; Anu Singla designed the experiment; Baljeet Yadav contributed to data analysis and Vartika Khare supported in proof reading of the manuscript.

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