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DISCRIMINATION OF BLACK BALLPOINT PEN INKS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

(Diskriminasi Pen Mata Bola Berdakwat Hitam Menggunakan Kromatografi Cecair Berprestasi Tinggi)

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Abstract

In this study, thirteen types of black ballpoint pen inks of three major brands were analyzed using high performance liquid chromatography (HPLC). Separation of the ink components was achieved using Bondapak C-18 column with gradient elution using water, ethanol and ethyl acetate. The chromatographic data obtained at wavelength 254.8nm was analyzed using agglomerative hierarchical clustering (AHC) and principle component analysis (PCA). AHC was able to group the inks into three clusters. This result was supported by PCA, whereby distinct separation of the three different brands was achieved. Therefore, HPLC in combination with chemometric methods may be a valuable tool for the analysis of black ballpoint pen inks for forensic purposes.

Keywords: ballpoint inks, principle component analysis, HPLC, chemometric

Abstrak

Dalam kajian ini, tiga belas jenis dakwat pen mata bulat daripada tiga jenama di analisis menggunakan kromatografi cecair prestasi tinggi (HPLC). Pemisahan komponen-komponen dakwat diperolehi menggunakan turus "Bondapak C-18" secara elusi berperingkat mengunakan kombinasi air, etanol dan etil asetat. Data kromatografi yang diperolehi pada panjang gelombang 254.8 nm dianalisis menggunakan pengelompokan hierarki agglomerative, (AHC) dan analisis komponen prinsip, (PCA). AHC membolehkan sample dakwat dikelaskan kepada tiga kelompok. Hasil daripada analisis AHC disokong oleh PCA yang juga menunjukkan pemisahan yang jelas bagi tiga kelompok mengikut jenama. Oleh itu, gabungan di antara HPLC dengan kaedah kemometrik merupakan teknik yg berguna untuk analisis dakwat pen mata bulat bagi tujuan forensik.

Kata kunci: dakwat pen mata bulat, analisis komponen prinsip, HPLC, kimometrik

Introduction

Over recent years, technological developments have rapidly expanded the range of pen designs and ink composition used throughout the world [1].Quill and nib pens have given way to other classes of writing instruments such as the ballpoint, roller ball and gel pens which have different requirements for the properties of their writing inks [2]. These inks may contain many substances aiming to improve ink characteristic [3]. Although all black ballpoint pen inks may look the same, there can be some important differences in their chemical composition, constituent and substances. This study emphasizes on black ballpoint pen inks available in Malaysian market as these pens are often used in the occasion of signing important documents. Black ballpoint pen inks are also used in documents with significant financial value like checks, insurance claims, wills, contract and tax return [4]. The examination of inks is often performed to evaluate the authenticity of these document. A ballpoint pen ink consists of synthetic dyes in (acidic and/or basic), pigments (organic and/or inorganic) and a range of additives [5]. Inks of similar color may consist of different dye composition and are frequently the subjects of forensic examinations. The inks can be discriminated by non-destructive or destructive methods [3, 6] depending on whether a sample needs to be taken

from the document, a process that would alter it. Destructive methods include high performance liquid chromatography (HPLC), thin layer chromatography (TLC), capillary electrophoresis (CE), gas chromatography/mass spectroscopy (GC/MS) and fourier transform infrared (FTIR) Spectroscopy [2,3,6]. Non-destructive methods include scanning electron microscopy (SEM), video spectral comparative (VSC) and Raman Spectroscopy [3,6,7]. In this study, HPLC was used as this method is able to provide detailed information regarding the components of the inks. The data obtained was subjected to chemometric methods as these techniques allow the extraction of more information based on the similarities and differences among samples in a data set [8]. The application of chemometric techniques such as agglomerative hierarchical clustering (AHC) and principle component analysis (PCA) helps in the interpretation of complex chromatographic data sets to better understanding of the studied subject. The goal of AHC is to identify relatively similar, that is, homogeneous, group of objects (ink samples) in the space of measured features. Combination of PCA and AHC could be used to investigate the measurable variability between the same colors of pens of different brands by matching the ink's components provided in the chromatograms.

Materials and Methods

Chemicals and Reagents

Ethanol 95% was purchased from Merck (Darmstadt, Germany), Ethyl acetate and acetone were obtained from System (ChemAR).

Samples

Thirteen black ballpoint pens of three major brands consisting of different types were purchased from local stores (Table 1).

Table 1. Types of pens from three major brands

Sample	Brand	Type
CbH1	CARERA	Carera B3 II
CbH2	CARERA	Carera B7
CbH3	CARERA	Carera B8
CbH4	CARERA	Carera B6
SbH1	STABILO	Stabilo galaxy 818 F
SbH2	STABILO	Stabilo liner 308 F
SbH3	STABILO	Stabilo liner 308 M
SbH4	STABILO	Stabilo galaxy 818 XF
SbH5	STABILO	Stabilo Point 88
PbH1	PILOT	Pilot BP – SF
PbH2	PILOT	Pilot BP – SM
PbH3	PILOT	Pilot supergrip M
PbH4	PILOT	Pilot supergrip F

Sample preparation
Ink samples were obtained by cutting 1.0 cm of each ink barrel. Each ink barrel was placed into different vial and 5 ml of ethanol was added into each vial. The sample was sonicated for about 15minutes and the solution was analyzed by HPLC.

High Performance Liquid Chromatography (HPLC)
The gradient elution employed for HPLC separation is shown in the Table 2. The column used was µ Bondapak C-18, 30 cm x 3.9mm. Detection was done by a dual wavelength UV monitoring at 254.8 nm.

Table 2. Gradient elution for HPLC system

Time range	Solvent Composition	Flow Rate	Temperature
0 min – 8 min	water-ethanol-ethyl acetate (55:40:5)	1.0ml/min	27 ˚C
8 min – 13 min	water-ethanol-ethyl acetate (40:40:20)	1.0ml/min	27 ˚C
13 min – 25 min	water-ethanol-ethyl acetate (35:40:25)	1.5ml/min	27 ˚C

Chemometric analysis of the HPLC data
The selected retention times for each sample were subjected to AHC and PCA using XLSTAT 2012 software.

Result and Discussion

Fig. 1 shows a chromatogram obtained from one of the ink samples. Major components selected (based on retention times) in black ballpoint pen inks are shown in the Table 3.

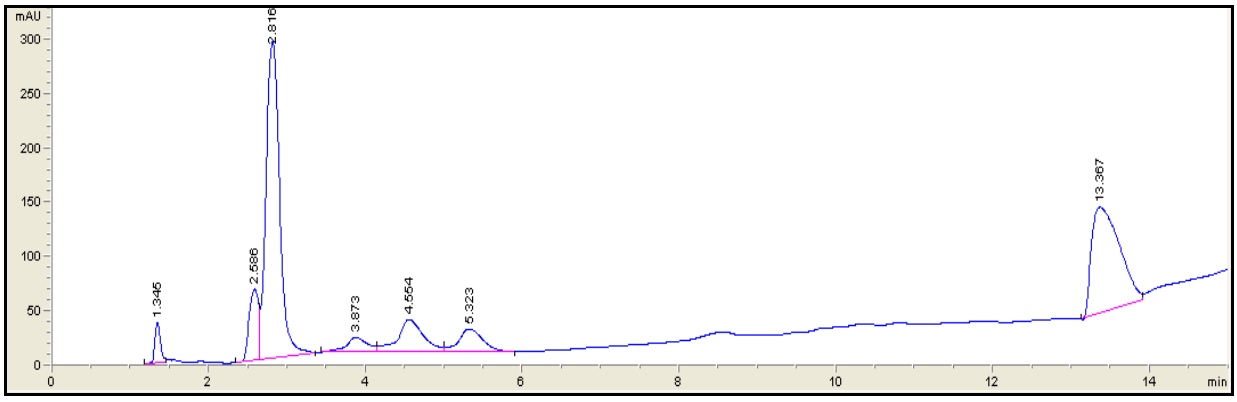

Figure 1. Chromatogram of ink sample (PbH4)

Table 3. Major components selected for chemometric analysis

Retention time range	Assigned component name
1.2 – 1.5 min	Component A
2.1 – 2.3 min	Component B
2.3 – 2.6 min	Component C
2.6 – 2.9 min	Component D
3.6 – 3.9 min	Component E
4.3 – 4.6 min	Component F
5.0 – 5.3 min	Component G
5.3 – 5.6 min	Component H
11.8– 12.0 min	Component I
12.0 – 15.0 min	Component J

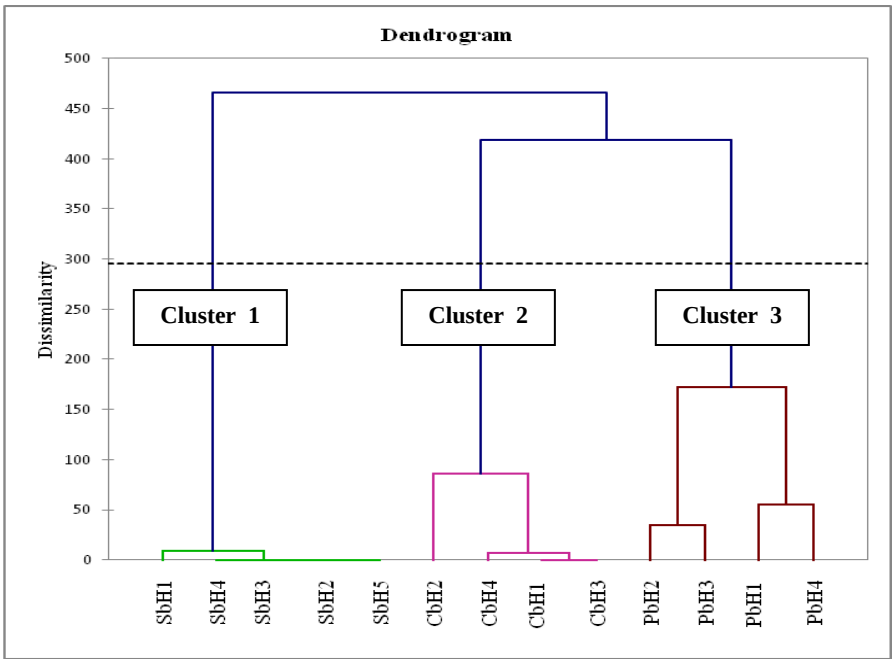

Figure 2. Dendrogram of inks samples based on HPLC data.

Agglomerative Hierarchichal Clustering (AHC)

AHC is a process of subdividing a set of entities into subsets in which the members are similar to each other, but different from members of other subsets. A shown in the dendogram in Figure 2, three clusters representing the three brands of inks were obtained. The various types of inks from Stabilo pens were grouped in cluster 1. Cluster 2 and 3 are formed by inks from Carera and Pilot pens, respectively. Therefore, the AHC analysis on the HPLC chromatogram data indicates that this approach is suitable to classify inks of different brands.

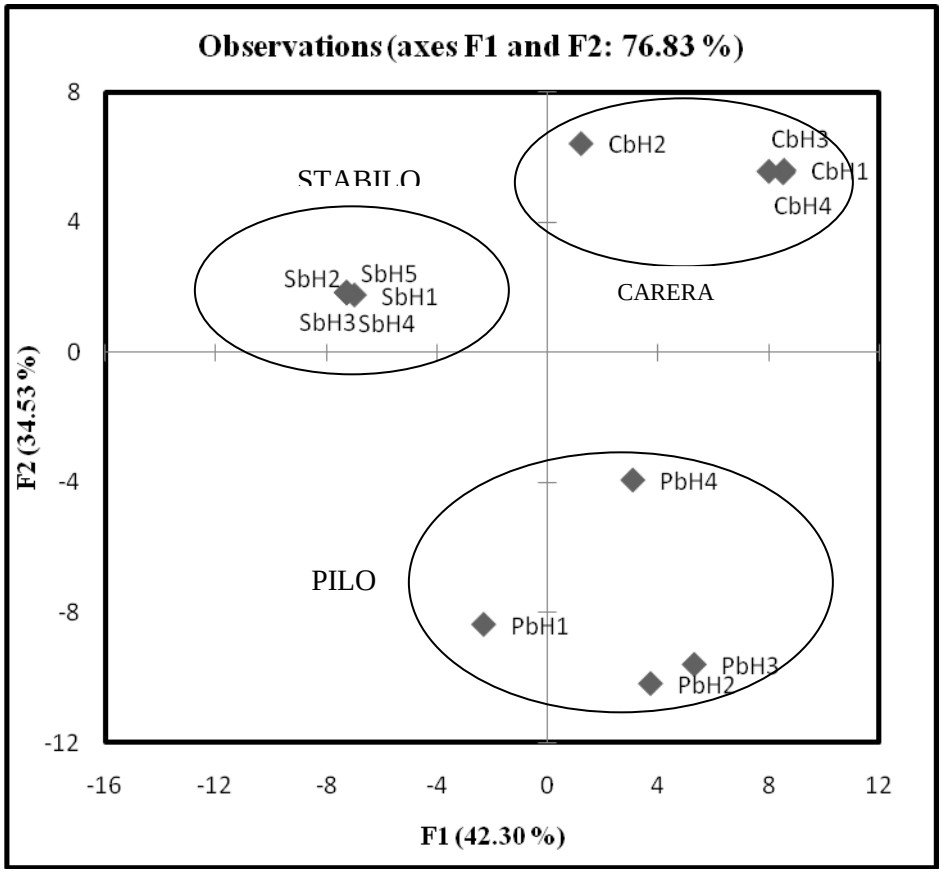

Figure 3. Score plot of F1 versus F2

Principal component analysis (PCA)

Principal component analysis provides information on the most meaningful parameters that describe the whole data sets rendering data reduction with a minimum loss of original information [9]. The PCA technique allowed the identification of an association between variables, thus reducing the dimensionality of the data sets. It is a powerful technique for pattern recognition that attempts to explain the variance of a large set of inter-correlated variables and transform them into a smaller set of independent (uncorrelated) variables (principal components). Based on the eigenvalue criterion, only the PCs with eigenvalue greater than one are considered important. This criterion is based on the fact that the average eigenvalue of the autoscaled data is just one [10].

PCA analysis, applied to HPLC inks sample datasets, resulted in three latent factors explaining 76.83% of the total variance. Figure 3 shows the PCA scores plot obtained showing a clear separation of ink samples from different brands for the first two principle components (F1 and F2). All the Stabilo inks are clustered in the left region of the

plot. Most of the Carera inks are grouped in the upper-right region, white Pilot inks are clustered together in the lower-right part of the plot. The results are strongly supported the AHC analysis discussed earlier.

Conclusion

In this study, the application of chemometric techniques (agglomerative hierarchical clustering and principle component analysis) on HPLC data can successfully evaluate the variations of thirteen types of black ballpoint pen inks of different brands. These results suggest that the HPLC profile of inks of different brands differ due to their similarities and differences in the chemical compositions. AHC grouped the various types of inks into three clusters of similar ink component's characteristic of each brand. The results concurred with the PCA. Combination of HPLC and chemometric techniques provided a more objective interpretation of the results for the analysis of black ballpoint pen inks for forensic purposes.

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