

Forensic discrimination of blue ballpoint pen inks based on thin layer chromatography and image analysis

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Abstract

This article aims to provide a new and fast method for differentiation of inks on a questioned document. The data acquisition was carried out by designing specific image analysis software for evaluating thin layer chromatograms (TLC-IA).

The ink spot was extracted from the document using methanol and separated by TLC using plastic sheet silica gel 60 without fluorescent indicator, and a mixture of ethyl acetate, ethanol, and water (70:35:30, v/v/v) as mobile phase.

To discriminate between different pen inks, new software was designed on the basis of intensity profile of red, green, and blue (RGB) characteristic. In practice, after development of chromatogram, the chromatograms were scanned by ordinary office scanner, intensity profiles of RGB characteristics on the development straight of each sample were produced and compared with the mentioned software. RGB profiles of ballpoint inks from various manufacturers showed that the patterns in most cases were distinctly different from each other.

This new method allowed discriminating among different pen inks with a high reliability and the discriminating power of 92.8%. Blue ballpoint pen inks of 41 different samples available on the local market were successfully analyzed and discriminated.

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1. Introduction

Ink analysis is an important forensic procedure that can reveal useful information about questioned document. Modern inks contain many substances aiming to improve ink characteristics [1–3]. Obviously, the most important component is the coloring material, which comes in the form of dyes, pigments or their combination. Dyes are soluble in the liquid body of the ink, which is also known as the vehicle. On the other hand, pigments are finely ground multi-molecular granules that are insoluble in the vehicle. The vehicle, whose composition affects the flowing and drying characteristics of the ink, can consist of oils, solvents and resins. Other substances used for finely tuning the characteristics including driers, plasticizers, waxes, greases, soaps and detergents [4].

The wide array of materials used in inks, coupled with possible contamination from the writing surface confronts forensic ink chemists with a complex analytical challenge to carry out this type of analysis. But the aim of most analyses is to determine whether two pieces of written text originated from the same ink, therefore, comparison of different writing inks on a document is the main goal of the most investigations.

The techniques regarding the analysis of inks can be divided into non-destructive and destructive approaches. The non-destructive method, however, is preferable there is a more limited range of such techniques available to the forensic examiner. Among these, the use of IR absorption and luminescence [2] carried out in a video spectral comparator or a similar instrument is highly successful particularly for black inks. Other non-destructive techniques include Raman [5] and surface-enhanced resonance Raman spectroscopy (SERRS) [6], UV-vis micro-spectrophotometry [2], although the spectrum quality for last method is heavily sample-dependent.

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Removal of a small section of the ink line followed by solvent extraction of the ink opens up more avenues of analysis. Analytical techniques such as thin layer chromatography, high-performance liquid chromatography, infrared spectroscopy (IR), and capillary electrophoresis have been applied to the analysis of inks [7–13].

The chromatographic separation of ink into its constituent component dyes has proved a highly productive method, not only for the comparison of the inks but also for matching ink to a database of chromatograms. Thin layer chromatography (TLC) is widely used because it is rapid, inexpensive and minimally destructive of documents. In this method, qualitative evaluation of the chromatograms is often performed using the retention factor (R_f) values, but it is not accurate, especially when comparison of ink to a database of chromatograms is desired. Direct scanning of chromatograms can be performed using scanning densitometers, but unfortunately, it has low signal-to-noise ratio, due to large scanning area arranged initially as the diameter of the largest spot. Removing the zone for further analyzing by Fourier transform infrared spectroscopy (FT-IR) and X-ray fluorescence spectroscopy [14], gas chromatography/mass spectrometry (GC/MS)[15] can also be performed, but these approaches require sophisticated instruments.

Over the last two decades, an explosive growth took place in both the diversity of techniques and the range of applications of image processing. Recently, many solutions have been proposed for color image processing and analyzing, and interesting results have been reported concerning filtering, enhancement, restoration, edge detection, analysis, compression, preservation, manipulation, and evaluation of color images. The surge of emerging applications, such as single-sensor imaging, color-based multimedia, digital rights management, art, and biomedical applications, indicates that the demand for color imaging solutions will grow considerably [16]. These techniques have gained wide range applications in different scientific areas. For example there is digital restoration techniques that can be used to recover the original appearance of old paintings, with little chemical processing of the paintings' surfaces [17]. Also a novel real-time tracker based on color, texture and motion information was reported [18]. Recently the use of ordinary office scanner was reported using commercial software for image processing with objective of quantitative evaluation [19]. This method could not be employed to color and qualitative comparisons.

The present study was recommended by *Research Center of Criminal Police of Iran* to develop a simple and easy method for discrimination of blue ballpoint pen inks and comparison of different writing inks on a document. The proposed method includes a new and fast data acquisition by designing new and specific image analysis software for evaluating thin layer chromatograms (TLC-IA) after scanning with ordinary office scanner, without in need of expensive instruments. This method allows one to compare two inks considering the R_f , color range and intensity of the separated ink components. The proposed procedure gives not only reliable results for ink, but also a

powerful tool for the comparative examination of similarly colored inks, paints, fibers and other materials of forensic interest.

2. Experimental

2.1. Sample preparation

Forty-one blue ballpoint pens were purchased from local market to form the most comprehensive collection of pens available (at the time of study) throughout Iran. The trademarks of collected samples are listed in Table 1.

A circle with 5 mm diameter was written uniformly by the pen on a paper, one fourth of it was punched out for extraction. For complete extraction of ink components from paper matrices, the samples were placed into 1 mL glass tubes separately, 0.1 mL methanol was added into each test tube and they were vigorously shaken for 1 min. Ink component was totally dissolved in methanol. Supernatant methanolic solutions were used for spots application on the TLC plate. A sample of blank paper of equal dimensions was also treated by the same manner.

Table 1
The list of studied blue ballpoint pens

No.	Commercial characteristics ^a
1	Cello pyramid 0.7 mm fine TC ball
2	OBA
3	AIHAO
4	Bic 01
5	Cenator
6	PARKER
7	A.T.CROSS FINE
8	Pelikan STICK 918
9	Marvy SB-10 1.0 mm
10	Bic 02
11	PIANO crystal
12	My pen 2001 PENS High Quality Bluce CE
13	AIBA
14	STAEDTLER Stick 430M A IRAN
15	Reynolds Medium 048 France
16	EIFEL Elegance
17	CASPION STICK 2001 M
18	STABILO liner 308
19	FABER-CASTELL 1.0 mm Medium (transparent)
20	BIC 08
21	Bocheng A-100
22	SCHNEIDER TOPS 505 M Germany
23	FIBER-CASTELL 1.0 mm Medium
24	MILAN PI 1 mm
25	Reform
26	PAPER = MATE FLEXGRIP ultra MED
27	PARKER UK
28	CANDID-DINI 2853
29	STABILO-galaxy 818 M
30	No name
31	No name
32	Zebra Rubber 101
33	SANFORD SAGA
34	Bensia
35	Girls
36	EUROPEN
37	PARS swiss Refill 606
38	STAEDTLER stick 430 M TBRITAIN
39	Lus HF 500
40	No name
41	STABILO bill 508

^a Purchased from local markets (May–June 2006).

2.2. Thin layer chromatography

TLC analyses were performed using Merck (Darmstadt, Germany) 20 cm × 20 cm silica gel 60 TLC plates without fluorescent indicator. The TLC plates were activated at 60 °C for 20 min and after cooling in desiccators were immediately used for spotting. The TLC plates were developed in a horizontal developing chamber. The solvent system includes: ethyl acetate/absolute ethanol/distilled water (70:35:30, v/v/v). Chromatographic development of plates was performed at room temperature for 40 min. All mobile phases were prepared daily with analytical grade chemicals (Merck) with volumes sufficient to supply the tank for each run. After development, the plates were air-dried. Separated compounds were visualized on the layer by their natural color and then plates were scanned with ordinary office scanner.

2.3. Hardware and software

An IBM compatible PC (Pentium IV) with a 2.6 GHz microprocessor, 256 MB random access memory (RAM) and a hard disk with 40 GB capacity for external storage was used for processing color images. The computer was equipped with an on-board graphic card (NViDiA Geforce 7300LE) and a scanner (CanoScan 4200F) was connected to computer for scanning (300 dpi) TLC plates as digital images. The images were saved as bmp files. Matlab (Version 6.5, The MathWorks, Inc.) was used to write a new program to process the previously saved images.

3. Results and discussion

3.1. Optimization of TLC conditions

Selection of proper extraction solvent to remove of ink from a paper will affect the resulting TLC. Pyridine is the reported solvent used with ballpoint pen inks [8]. In this work, the extraction was performed with different solvents (for details see Table 2) using various extraction modes as follows:

- Immersion of paper into solvent and simple agitation for 1 min.
- Immersion of paper into solvent and ultra-sound assisted extraction.
- Immersion of paper into solvent and micro-wave assistance extraction.

The obtained results reveal that immersion of paper into methanol or pyridine and simple agitation could result complete extraction of inks from paper and no further

Table 2

List of solvents used for the extraction of ink components from paper

Solvent	Solubility of ink colors
Ethyl acetate	Slightly
Ethanol	Slightly
Acetic acid	Slightly
Acetone	Slightly
Butanol	Slightly
1,2-Dichloroethane	Slightly
Butyl acetate	Slightly
Tetrachloroethane	Slightly
Acethyl acetate	Slightly
Cyclohexan	Slightly
Methanol	Soluble*
Pyridine	Soluble

* The chosen extraction solvent was shown as bold.

Table 3
Different solvent systems used to plate development

Solvent system	Ratio	Spot capacity
Butanol:ethanol:H ₂ O	50:15:10	9
Ethyl acetate:cyclohexan:methanol:NH ₃	70:15:10:5	5
Ethyl acetate: Butanol: NH ₃	60:35:5	10
Ethyl acetate:ethanol:H₂O	70:35:30	15*
Toluene:acetone:ethanol:NH ₃	30:60:7:2	5

* The chosen solvent system was shown as bold.

improvement was observed using ultra-sound or micro-wave. Methanol was chosen by considering the safety of the solvent.

The choice of the plate (stationary phase) and mobile phase was made regarding the nature of the sample. Many types of TLC plates were examined and found that silica gel plates provided better resolution of dye spots. Because of easy handling, plastic sheet silica gel is preferred in these studies. Many mobile phase systems were investigated in this work and also reported in literature [8], among them five mobile phases were selected (see Table 3 for details) and found that ethyl acetate/ethanol/distilled H₂O (70:35:30, v/v/v) was effective in separating nearly all dye mixtures. In this system, the spot capacity more than 15 was achieved, so it was the solvent system of choice for this study. A typical chromatogram was shown in Fig. 1.

To confirm complete separation of all component in the studied sample, two-dimensional (2D) TLC was carried out using various solvent system, the results obtain proved that above mentioned one-dimensional (1D) TLC is able to sufficient separation.

3.2. Image processing software

For the discrimination of blue ballpoint pen inks, the components of each sample were firstly separated by TLC. The

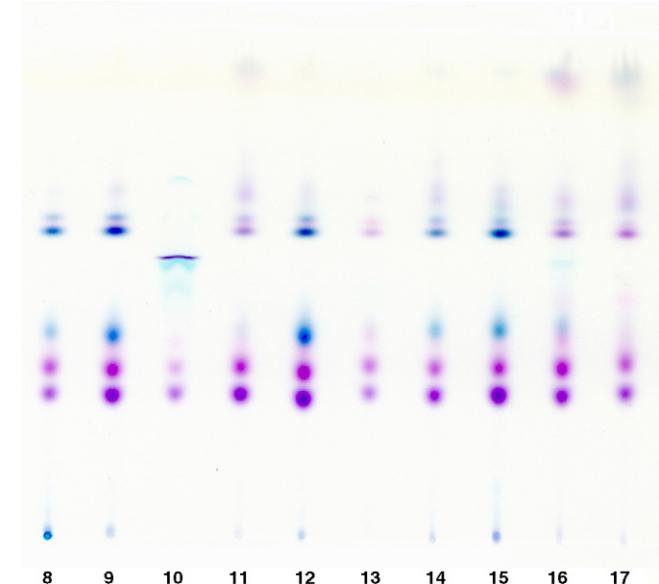


Fig. 1. Typical thin layer chromatograms of 10 different inks samples at optimum conditions.

essential step is the identification of spots from each sample and their relative abundance. The latter is a very important step and usually requests more sophisticated instruments. In this work chromatograms were scanned by a simple official scanner for color image generating of spots corresponding to each sample. Colors of an image are described in the red, green, and blue (RGB) space, where colors are represented by their RGB components in an orthogonal Cartesian space. Our proposed algorithm computes the similarity measure between two RGB color images corresponding to the ink chromatograms. This algorithm can be divided into four stages as follows.

3.2.1. Stage 1: color image normalization

A function of the input images was computed that is invariant to confounding scene properties but is discriminative with respect to desired scene information. A number of invariants were described in literature [20]. The simplest example is a normalized-RGB image. The normalized-RGB color vector at each pixel depends on the spectral reflectance of the corresponding surface patch but not its orientation, which makes it useful for material-based segmentation. Normalized-RGB is invariant to local illumination and viewing geometry. The Normalized-RGB color components (r, g, b) are calculated as follows:

$$r = \frac{R}{R + G + B} \quad g = \frac{G}{R + G + B} \quad b = \frac{B}{R + G + B} \quad (1)$$

3.2.2. Stage 2: computing color image profile

Color provides many cues [21]. The well-known color descriptor is the RGB color histogram [22], which was for tracking in various occasions [23,24]. There are also other potential features like the color moments [25], MPEG-7 color descriptors [26], and color correlograms [27]. In this study, the RGB color profile was selected to describe the color properties of inks.

The intensity profile of an image is the set of intensity values taken from regularly spaced points along a line segment in an image. For points that do not fall on the center of a pixel, the intensity values are interpolated. The intensity profile was computed for each r, g and b images along with the line passing through the center of the image on the chromatographic development straight of each ink spot. The intensity profile of typical chromatogram was shown in Fig. 2.

3.2.3. Stage 3: correlation of color image profiles

In this stage the intensity profiles were considered as sequences and the normalized cross-correlation of sequences were computed. The cross-correlation is a measure of similarity of two signals, commonly used to find features in an unknown signal by comparing it to a known one. It is a function of the relative shift between the signals and sometimes it is called the sliding dot product [28,29]. For example, consider two real valued functions f and g that differ only by a shift along the x -axis. One can calculate the cross-correlation to figure out how much g must be shifted along the x -axis to make it identical to f . The formula essentially slides the g function along the x -axis,

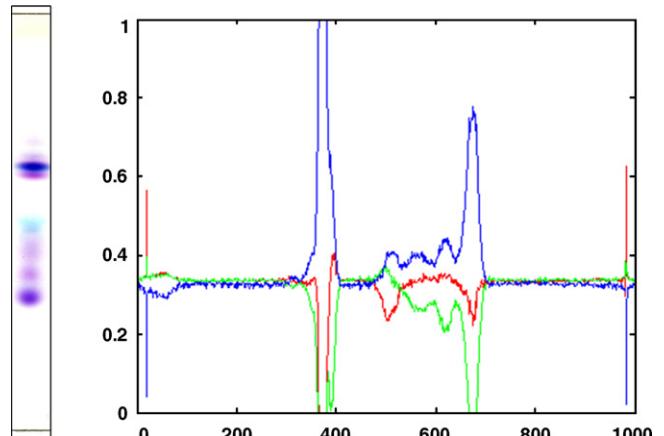


Fig. 2. RGB characteristic of an ink after TLC.

calculating the integral of their product for each possible amount of sliding. When the functions match, the value of cross-correlation is maximized. For discrete functions f and g the cross-correlation is defined as (2a):

$$C_{fg}(m) = \sum_n f(n+m)g(n) \quad (2a)$$

$$C_{fg}(m) = \sum_n \frac{(f(n+m) - \bar{f})(g(n) - \bar{g})}{\sigma_f \sigma_g} \quad (2b)$$

For image-processing applications in which the brightness of the images can vary due to lighting and exposure conditions, the images can be first normalized. This is typically done by subtracting the mean and dividing by the standard deviation then calculating dot product of two normalized vectors. So Eq. (2b) is obtained where \bar{f} and \bar{g} are the mean of the two series f and g and σ_f and σ_g are standard deviation of f and g . It is necessary to mention that the result sequence is normalized so the cross-correlations at zero lag are identically 1.0.

To compare the r image components, the cross-correlation between intensity profiles of r image components of two inks was computed by Eq. (2b). Then we have found the maximum cross-correlation of sequences that were denoted as cr . The above procedure was repeated for g and b components and finally three cr , cg and cb values were calculated which represent the similarity of r , g and b profiles, respectively.

3.2.4. Stage 4: computing the similarity

The weighted mean of cr , cg and cb was computed by Eq. (3) and a scalar score between 0 and 1 was obtained as a similarity measure between two RGB color images corresponding to the ink chromatograms. In Eq. (3) the weights w_1 , w_2 and w_3 are adjustable but in the experiments they are equal to 1. Fig. 3 shows the similarity of two inks calculated by mentioned method.

$$\text{Score} = \frac{w_1 \times cr \times w_2 \times cg + w_3 \times cb}{w_1 + w_2 + w_3} \quad (3)$$

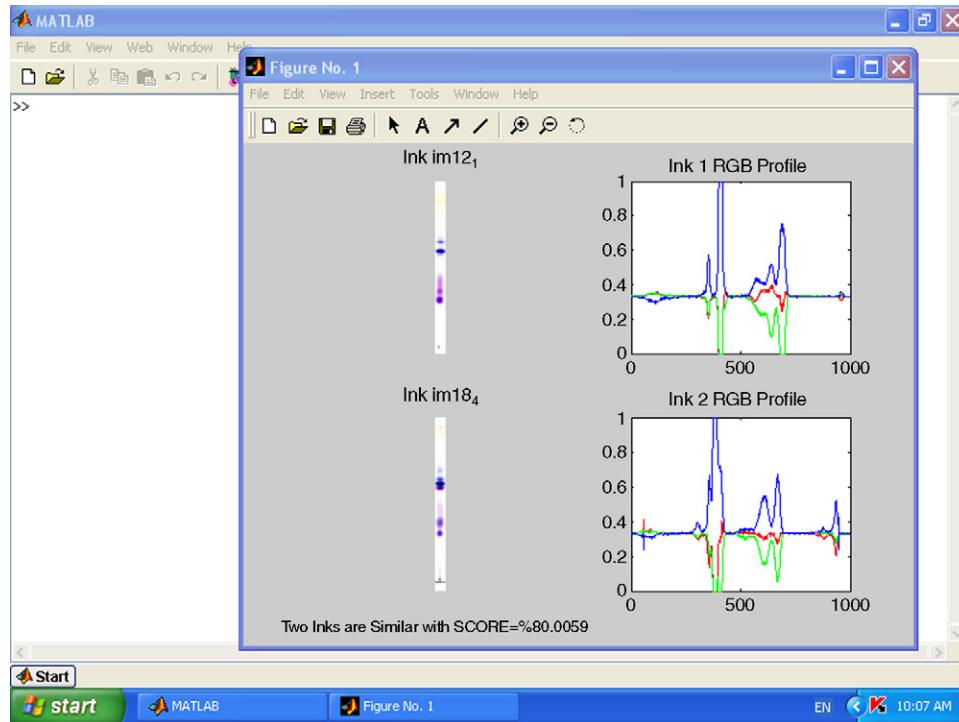


Fig. 3. Screen shot of Matlab software running: show the similarity of two inks as score (%).

3.3. Differentiated analysis

The ability of proposed method to differentiate between various blue ballpoint pens was evaluated by comparing the similarity of different inks according to Eq. (3). For this purpose, similarity for repeated sampling ($n = 5$) of each 41

ballpoint inks were calculated. The mean and minimum values were 94.5% and 88.7%, respectively. These score values were selected as a criterion of similarity: whereas the ink pairs with the score value more than 94.5% considered as similar, the pairs with the values between 88.7% and 94.5% were considered as uncertain similar and those values less than 88.7% were

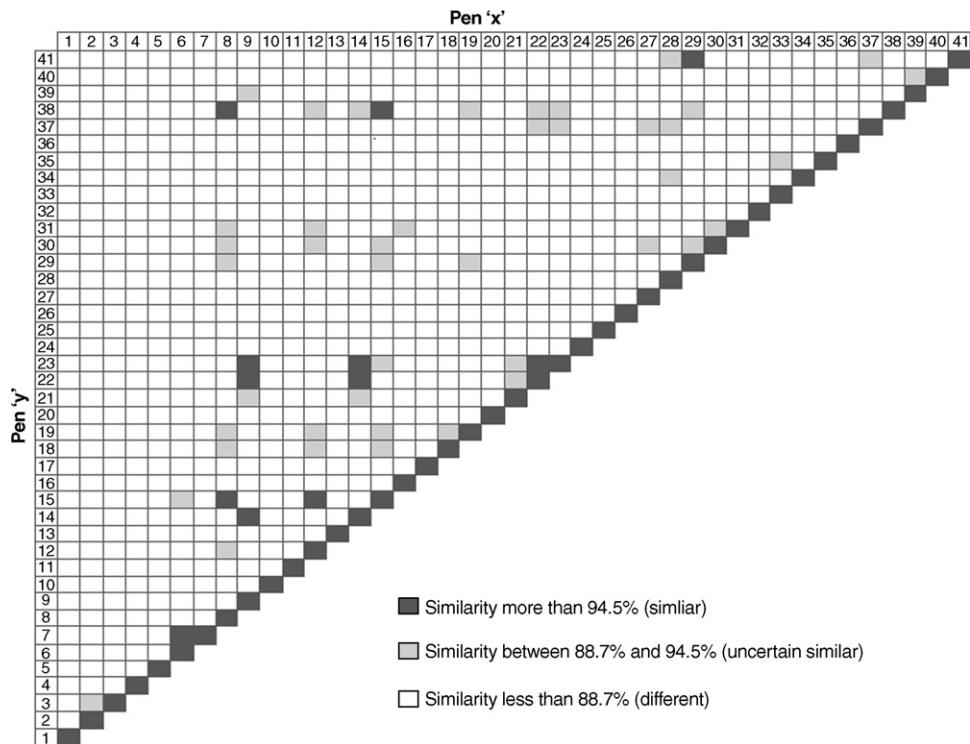


Fig. 4. All possible combination of comparing inks with TLC-IA.

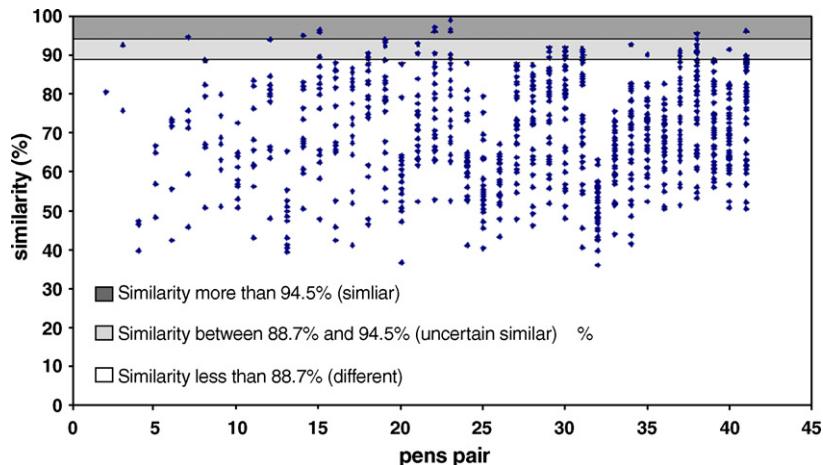


Fig. 5. Distribution of similarity between all possible pen pairs analyzed with TLC-IA was shown in three different zones.

considered as non-similar (different). The comparison between all possible binary combinations of 41 studied inks, which has 820 cases, were carried out and similarity scores were calculated using Eq. (3). The obtained results were illustrated in Figs. 4 and 5. These results reveal that, from the 820 samples, the 761 pen-pair samples were differentiated using TLC-IA method, while only 59 samples were not differentiated and were considered as either similar ($n = 12$) or uncertain similar ($n = 47$).

In order to check the possibility to differentiate the examined inks by the proposed method the discriminating power (DP) was calculated for all pairs of the pens. DP is defined as a ratio of the number of differentiated pairs of samples with respect to the total number of all possible combinations. The number of pairs for a sample number of n was achieved as follows [30]:

$$\text{Number of pairs} = \frac{n(n - 1)}{2} \quad (4)$$

In this method, the DP was achieved 92.8%, which confirm that the proposed method is able to differentiate a significant number of pen-pair samples.

4. Conclusions

We have described a fast and straightforward method for evaluating thin layer chromatograms after scanning with ordinary office scanner. New and effective software was designed based on the intensity profile of RGB characteristic. After chromatographic development, the chromatograms were scanned, intensity profiles of RGB characteristic on the development straight of each sample were produced and compared by the mentioned software. RGB profile of inks from various manufacturers and countries showed patterns, which were in most cases distinctly different from another. This new method allowed discrimination between different pen inks with high reliability. Using the proposed method we have successfully analyzed and discriminated 41 blue ballpoint pen inks of different trade marks available on the local market at the time of study. In this method DP was achieved 92.8%, which confirm it is able to differentiate a significant number of pen-pair samples.

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