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Thin-Layer Chromatography of Inks—Efficiency of Separation of Ink Components

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There are numerous publications relating to the use of thin-layer chromatography (TLC) in the analysis of inks on documents. This paper compares the efficiencies of TLC separation of ink components when using several 'developing' solvents (solvent systems) commonly used for ink analysis in the forensic laboratory. The parameters chosen for this evaluation are the *shape of* and *distance between* chromatographic zones of separated dye components of ink, including components of Methyl Violet, a dye mixture often used in the manufacture of black, violet and blue ballpoint, fountain pen, and stamp pad inks. (This mixture typically consists of the dye Crystal Violet and its two or three homologues, which are triphenylmethane dyes of a similar chemical structure and hence similar physical and chemical properties, whose chemical structures differ only by one methylene [CH_2] group). The results obtained show that at least three solvent systems, one of which (ethyl acetate – isopropanol – water – acetic acid = 30:15:10:1) was developed and reported by this author in the 1980s, provide significantly improved TLC separation efficiency compared with the "Solvent System I" recommended in the current *SWGDOC Standard for Test Methods for Forensic Writing Ink Comparison.*

Introduction

In forensic cases, chemical analysis of inks is performed to determine if inks are of the same formulation (or of the same manufacturing batch) or of different compositions; to establish the authenticity of a document; to establish whether a document could have been produced on the purported date; or to determine the origin of a document. All inks are composed of colorants (dyes, pigments) and a carrier (vehicle). Ink vehicles consist of solvents, which are used to dissolve or disperse the colorants, and multiple other ingredients, such as thickeners, modifiers, lubricants, antiseptics, surfactants, by-products, *etc*. Most of the components of inks are organic compounds that can be separated and characterized by various methods of chromatography.

Chromatography was discovered in 1901 when a Russian botanist and biochemist, Michael S. Tswett, separated pigments from chlorophyll using self-made chromatographic columns packed with various adsorbents. This fundamental discovery marked a milestone in the development of chromatographic separation techniques that led to Nobel prizes in chemistry. Today, chromatography has become the main analytical method used in pharmaceutical, food analysis, petrochemical analysis (crude oil, gasoline, kerosene, etc.), biochemical research, environmental analysis (e.g., pesticides in drinking water), clinical analysis (e.g., therapeutic drug monitoring, metabolism disorders), toxicology, forensic, and doping analysis industries, along with many other areas. Thin-layer chromatography (TLC), which is a form of liquid chromatography, and gas chromatography-mass spectrometry (GC-MS) are two chromatographic methods that are widely used in analytical and forensic science laboratories all over the world.

There are numerous publications describing various procedures for the TLC analysis of writing, printing, typewriter, inkjet, and stamp pad inks (as well as toners whose colorants, which are dyes and pigments, have some solubility in the extraction solvent) on documents. TLC has been the principal analytical method employed by most laboratories in forensic ink analysis. The reason for this is that this method is comparatively simple, rapid, and cost efficient. Also, TLC allows the examiner to visually evaluate the qualitative and semi-quantitative composition of

ink dye components (of the inks compared) separated on the TLC plate [1].

In 1972, Brunelle and Pro offered a systematic approach to ink comparison and identification in which they used a solvent system consisting of ethyl acetate/ethanol/water = 70:35:30 as a developing solvent for the TLC separation of ballpoint ink dye components [2]. The Scientific Working Group for Forensic Document Examination (SWGDOC) Standard for Test Methods for Forensic Writing Ink Comparison lists this developing solvent as *"Solvent System I"* and recommends using it, along with other developing solvents that enable appropriate separation of ink dyes, for the TLC analysis of inks [3].

To perform a TLC analysis of ink on paper, the ink is chemically extracted from the ink-on-paper samples using an appropriate extracting solvent. The ink extract is applied, as a small spot, onto a glass plate pre-coated with a thin, white, chalk-like, layer of silica gel (termed the "stationary phase"). The TLC plate is then developed using a mixture of solvents (termed "developing solvent" or "liquid mobile phase"). As the plate develops, the solvent mixture diffuses up the plate by capillary action and carries the ink's components with it. Different colorant components of the ink will typically move at differing rates up the TLC plate due to their physical and chemical differences (specifically, due to differences in their partitioning behavior between the mobile and stationary phases), and will stop their migration at different points. Once the TLC plate is fully developed, the separated dye components will appear on the plate as a combination of colored spots. The combination of the separated dye (and other) components of the ink is called a TLC chromatogram. The obtained TLC chromatogram can then be compared with the TLC chromatograms of other ink samples (*e.g.*, those taken from an ink library) to determine if they match.

TLC may help a forensic examiner to identify a writing ink and then determine whether the ink was or was not commercially available on the purported date of the writing. Since manufacturers are known to change old inks or introduce new ink formulations, it may be determined that an ink formulation was not in production on the purported date of the document. If the pattern of colorants in a questioned ink matches the pattern of colorants in the ink of a particular manufacturer in a reference library of inks, then the questioned ink can be said to come from that manufacturer, and the introductory date may be determined.

This paper compares the efficiencies of TLC separation of ink components when using several developing solvents (including *"Solvent System I"*) commonly used for ink analysis in the forensic laboratory. Two parameters chosen for this evaluation are the *shape of* and *distance between* chromatographic zones of separated dye components of ink, including components of Methyl Violet (MV), a dye mixture often used in the manufacture of black, violet and blue ballpoint, fountain pen, and stamp pad inks. These two parameters correspond to two key factors of a chromatographic resolution of components of a mixture: selectivity (the ability of the chromatographic system to 'chemically' distinguish between sample components, i.e. to separate these components) and separation efficiency (depends on the longitudinal diffusion of the molecules of the sample components in their chromatographic zones).

Methods and Materials

Ink Samples – Lines of the following five inks (designated as Inks *A* through *E*) were placed (drawn using steady pen pressure) on HP multipurpose white paper:

- Ink *A:* *Namiki (Pilot)* blue fountain pen ink (Japan),
- Ink *B:* *Private Reserve Black Magic Blue* fountain pen ink (USA),
- Ink *C:* A.T. Cross Company black rollerball ink (Cross Co. Japan, Ltd.),
- Ink *D:* OfficeMax® black ballpoint ink (made in China in 2003), and
- Ink *E:* Bic® black ballpoint ink (Bic Soft Feel Jumbo ballpoint pen, USA).

The above five inks, *A* through *E*, represent both water-based and oil-based inks. The main reason why these particular inks were selected for this study was because they have complex, multi-component compositions of their colorant (dye) components,[1] and one of the aims of this study was to determine whether TLC, using appropriate developing solvents, can be efficient enough to separate *all* of the dye components present in the each ink.

[1] Besides, inks *D* and *E* are the representatives of a massive population of ballpoint ink formulations of black, violet and blue colors available on the market that all contain components of Methyl Violet separable by TLC.

Sampling Device—A hypodermic needle-like apparatus, the Harris Micro-Punch™ (Electron Microscopy Sciences, Hatfield, PA), which removes *ca.* 0.5-mm samples (micro plugs) of paper and ink-on-paper. The bored out paper and ink samples are removed with a plunger.

Extracting Vessels—Grace 2-mL (12 x 32 mm) capped, screw thread, standard mouth clear glass vials (Grace Davison Discovery Science, USA).

TLC Materials and Procedure—Each of the inks sampled from paper (three 0.5-mm micro plugs) was extracted in dimethylformamide (about 2 microliters) for 15 minutes, and the obtained colored extract was applied using a capillary pipette onto a high performance (HP) TLC silica gel 60-F_{254} (10 x 10 cm) precoated glass plate (Merck, Germany). After application of the colored extracts of all five inks, *A* through *E*, the HPTLC plate was allowed to air dry and was then developed using one of the following developing solvents:

Solvent I: ethyl acetate / ethanol / water = 70:35:30 [2-4]

Solvent II: ethyl acetate / isopropanol / water / acetic acid = 30:15:10:1 [5-8][2]

Solvent III: butanol / isopropanol / water / acetic acid = 20:10:10:1 [9]

Solvent IV: butanol / ethanol / water / acetic acid = 60:10:20:0.5 [10, 11]

Resulting chromatograms were observed and photographed under daylight and UV light (254 and 365 nm).

Results and Discussion

Figures 1 through 4 show the results of the TLC separation of the dye components of the above five inks, *A* through *E*, using the developing solvents designated as Solvent I through Solvent IV.[3]

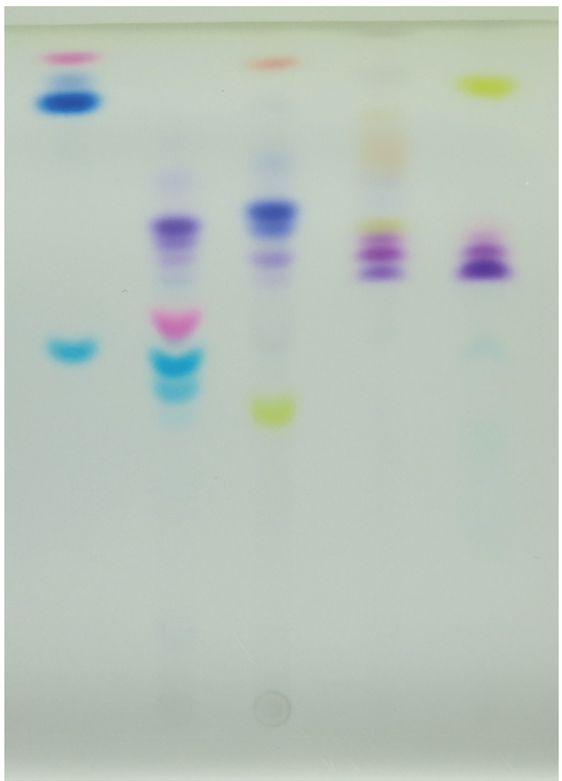

Figure 1. The thin-layer chromatogram obtained for Inks *A* through *E* (left to right) using Solvent I as the developing solvent (photographed under daylight).

[2] The author has been using this developing solvent for the TLC analysis of ink since the early 1980s [6].
[3] To minimize the influence of different ink concentrations on the results of the comparative TLC analyses of each ink, as equal as possible amounts of the ink were applied on all TLC plates prepared for the chromatographic separation of the inks' components using the developing solvents I through IV. Besides, to check the repeatability of the results obtained, each developing solvent was used to chromatograph the above five inks on three separate TLC plates.

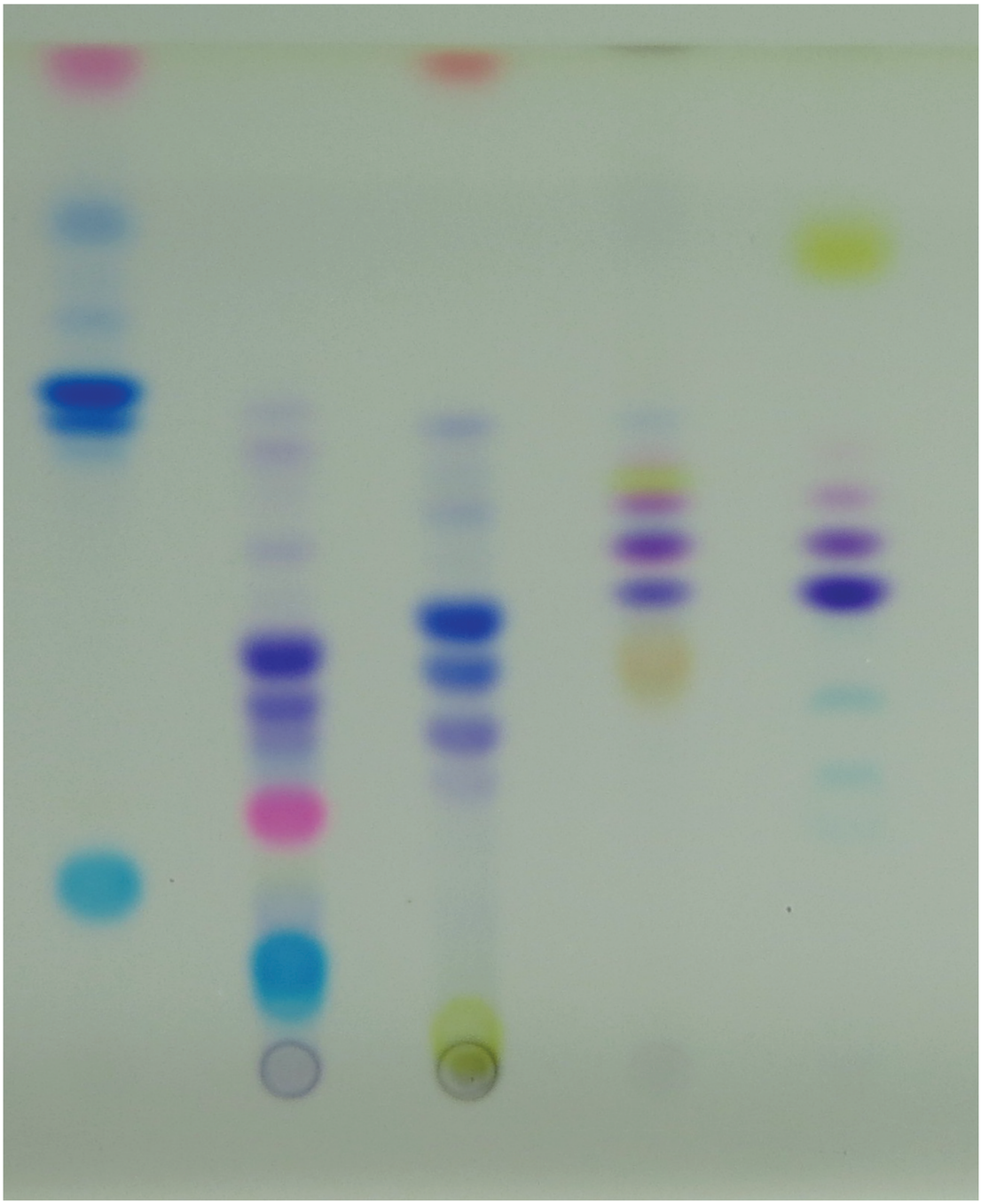

Figure 2. The thin-layer chromatogram obtained for Inks *A* through *E* (left to right) using Solvent II as the developing solvent (photographed under daylight).

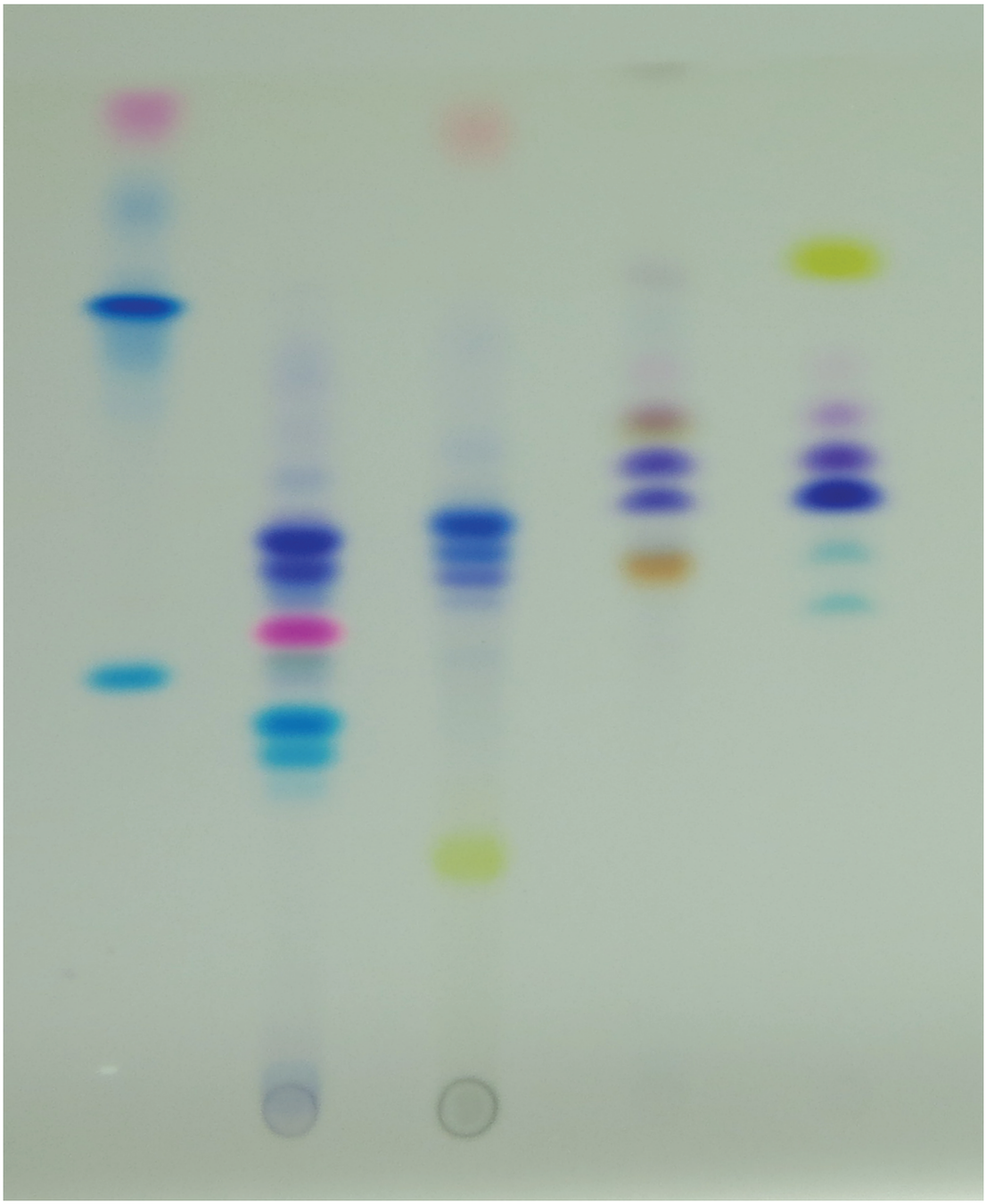

Figure 3. The thin-layer chromatogram obtained for Inks *A* through *E* (left to right) using Solvent III as the developing solvent (photographed under daylight).

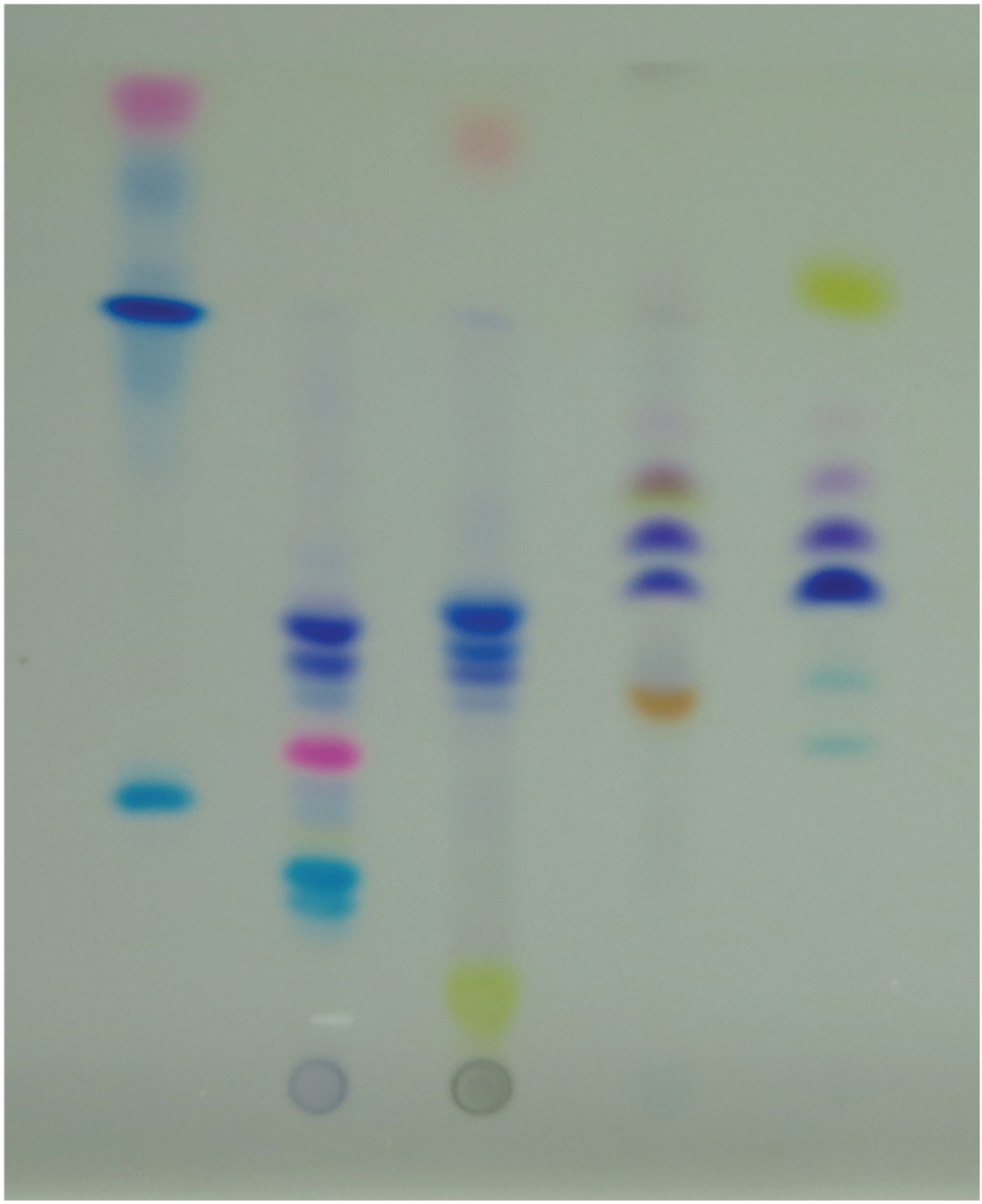

Figure 4. The thin-layer chromatogram obtained for Inks *A* through *E* (left to right) using Solvent IV as the developing solvent (photographed under daylight).

Table 1. The results of the TLC separation of the dye components of the five inks, designated as Inks *A* through *E*, using four developing solvents, designated as Solvents I through IV.

Ink	Number of separated dye components			
	Solvent I	***Solvent II***	***Solvent III***	***Solvent IV***
A	4	6	4	4
B	8	12	11	10
C	9	10	10	9
D	7	9	8	9
E	6	7	7	7

The results of the TLC separation of the dye components of Inks *A* through *E* shown in Figures 1 through 4 are summarized in Table 1 above.

The following inferences can be made based on the results of the TLC separations of the dye components of the inks analyzed in this work:

- **Solvents II, III and IV provide a significantly better separation efficiency than Solvent I**. In particular, Figures 1 through 4 show that Solvents II, III and IV provided a well-defined separation of most of the dye components (with chromatographic zones of regular shape – round or ellipsoid), while Solvent I failed to completely separate most of the dye components whose chromatographic zones are either overlapped (partially or completely) or are very close to each other. In addition, some of the chromatographic zones of the dye components on the TLC chromatograms obtained using Solvent I are distorted and thus have irregular shape.
- **Solvents II, III and IV provide a significantly better selectivity of the TLC analysis than Solvent I:** as follows from Figures 1 through 4 and Table 1, each of the Solvents II, III and IV separated more dye components present in the five inks analyzed versus Solvent I.

Additionally, the comparison of the *shape of* and *distance between* the chromatographic zones of separated dye components of the five inks, including the chromatographic zones of the four MV homologues in Inks *D* and *E* shown in Figures 1 through 4 indicates that Solvent II provided somewhat better separation efficiency and selectivity than Solvents III and IV.

As an example, Figure 5 shows a juxtaposition of the TLC chromatograms obtained for Ink *E* using the above developing solvents.

Figure 5 shows that Solvents II, III and IV provide a significantly better separation of the MV homologues that Solvent I does. And, as mentioned above, the comparison of the *shape of* and *distance between* the chromatographic zones of the four MV homologues indicates that Solvent II provides a slightly better separation efficiency versus Solvents III and IV.

Finally, it is noteworthy that a freshly prepared Solvent II ($S_{II\text{-}fresh}$ in Figure 5) shows almost the same separation efficiency for the four MV homologues as (just slightly better than) the same Solvent II prepared 32 days prior to the day of the TLC analysis ($S_{II\text{-}old}$). This evidences that Solvent II is stable enough to be used for the TLC analysis of ink for at least 4 weeks after its preparation.

Conclusion

This paper compared the efficiencies of TLC separation of ink components when using several developing solvents commonly used for ink analysis in the forensic laboratory. It is found that at least three solvent systems, one of which (ethyl acetate – isopropanol – water – acetic acid = 30:15:10:1) was developed and reported by this author in the 1980s, provide significantly improved TLC separation efficiency when compared with the "Solvent System I" recommended in the current *SWGDOC Standard for Test Methods for Forensic Writing Ink Comparison*.

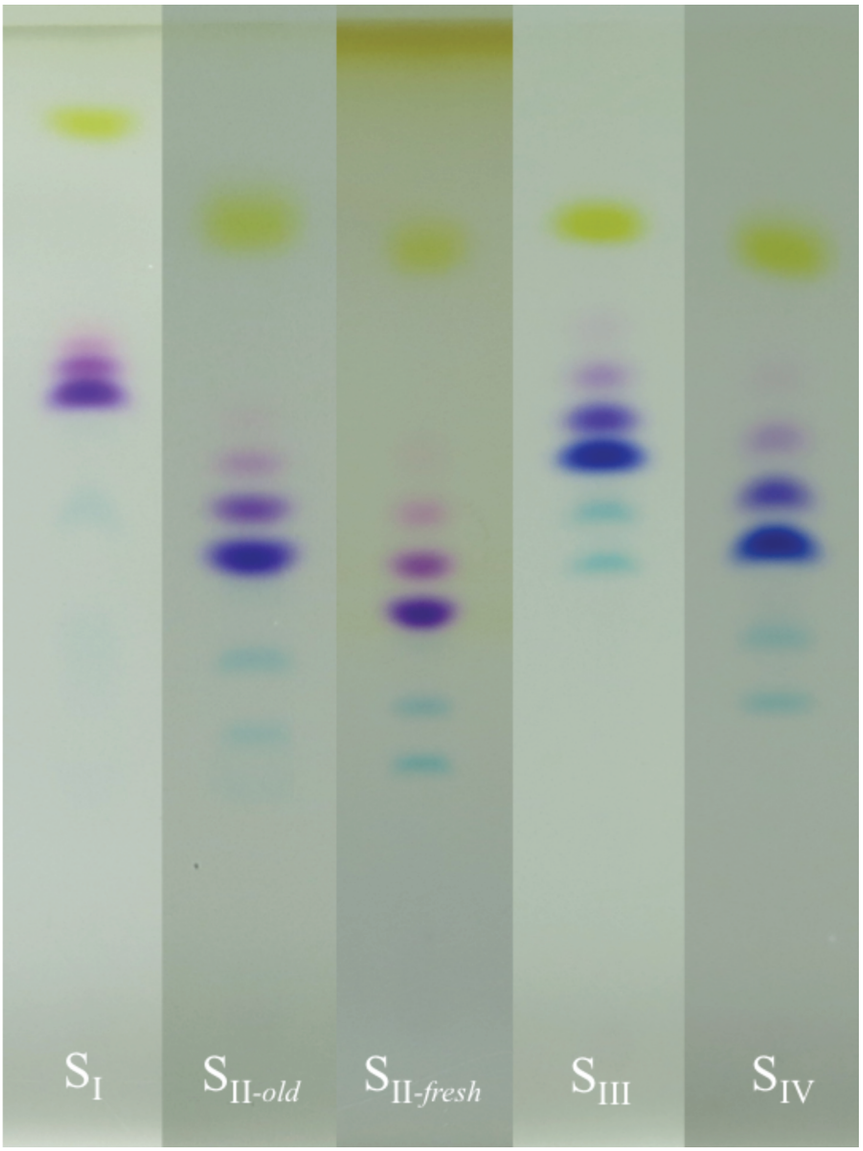

Figure 5. Five thin-layer chromatograms obtained for Ink *E* using the following developing solvents (photographed under daylight):

S_I - Solvent I (prepared about 1 hour prior to the TLC analysis)

$S_{II\text{-}old}$ - Solvent II (prepared 32 days prior to the day of the TLC analysis)

$S_{II\text{-}fresh}$ - Solvent II (prepared about 1 hour prior to the TLC analysis)

S_{III} - Solvent III (prepared about 1 hour prior to the TLC analysis)

S_{IV} - Solvent IV (prepared about 1 hour prior to the TLC analysis)

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