Using TLC and GC-MS to Determine Whether Inks Came from the Same Manufacturing Batch

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Gas chromatography-mass spectrometry (GC-MS), being a well proven analytical method of determining the qualitative composition of multi-component systems, is effective for the analysis of inks, most of which are complex mixtures of chemical compounds. A combination of thin-layer chromatography (TLC), which analyzes ink colored components, and GC-MS, which analyzes ink noncolored components, demonstrates high discriminating power with regard to writing inks that can be distinguished neither by nondestructive techniques nor by TLC. Case examples are considered in which coupling of TLC and GC-MS allowed one to discriminate between ink indistinguishable by TLC and to determine that the inks on questioned documents came from the same manufacturing batch.

Introduction

Writing inks are multi-component mixtures of various organic substances (such as dyes, solvents, resins, modifiers, lubricants, thickeners, antiseptics, surfactants, and byproducts), most of which can be separated and characterized by chromatographic techniques.

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 in the pharmaceutical industry, food analysis, petrochemical analysis (e.g., crude oil, gasoline, and kerosene), biochemical research, environmental analysis (e.g., pesticides in drinking water), clinical analysis (e.g., therapeutic drug monitoring, metabolism disorders), toxicology, forensic and doping analysis, and in many other areas. Thin-layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) are 2 chromatographic methods that are most widely used in analytical and forensic science laboratories all over the world. The application of TLC, GC-MS, and other chromatographic techniques to the analysis of ink on documents has been reported in the literature (Aginsky 2000).

There are numerous publications describing various procedures for the TLC analysis of writing inks 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, for comparing inks, TLC allows the examiner to evaluate visually the qualitative and semi-quantitative composition of ink dye components separated on the TLC plate.

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 an eluent for the TLC separation of ballpoint ink dye components. The ASTM Standard Guide for Test Methods for Forensic Writing Ink Comparison E 1422-01 (2001) lists this eluent as “Solvent System 1” and recommends using it, along with other eluents that enable appropriate separation of ink dyes, for the TLC analysis of inks. Kelly and Cantu (1975) obtained a more effective separation of ink dye components by reducing the portion of water in the “Solvent System 1”: ethyl acetate/ethanol/water = 70:35:20. A similar solvent system consisting of ethyl acetate/isopropanol/water/acetic acid = 30:15:10:1 was routinely used by all government forensic science labs in Russia for the TLC analysis of colored components in ballpoint and other writing inks, stamp pad, typewriter ribbon inks, photocopier toners, and printing inks (Aginsky 1993).

Though TLC is an excellent method for discriminating between similarly colored inks that have been made using different dyes, the reported capabilities of this method for the identification of ink formula and for the determination of whether 2 or more entries were written with an ink of the same formula or batch are often over amplified. For example, Brunelle and Crawford (2003) recently wrote: "To identify the manufacturer and specific formulation of questioned inks, you must compare standard inks of known manufacturer and formulation simultaneous with the questioned inks using the same procedures (TLC and TLC densitometry)….While the above procedures are most commonly used and have withstood the test of the courts for the comparison and identification of inks, other methods may sometimes be helpful."

Although opinions of some ink experts may have been based on the results of the TLC chemical method alone, only partial information about the composition of ink can be obtained pertaining to ink dye components and, if present, fluorescent date tags. If an ink does not contain a unique component such as a date tag, then, as a rule, the results of the TLC analysis of an ink will not be sufficient for the reliable identification of the ink formulation or for determining that the compared entries were written with an ink of the same formulation.

It is well known that ink dyes constitute only a part of ink composition. Thus, for ballpoint inks, colorants (ink dyes) never represent more than a quarter of the ink composition. The remaining part of an ink's composition consists mainly of noncolored viscous liquid and solid substances such as solvents, resins, modifiers, by-products (impurities and micro impurities) which constitute more than 75% of the mass of ink contained in a ballpoint pen cartridge and more than 50% of the mass of an "old" ink that has dried on paper. Many of these substances can be analyzed by GC-MS, which is a well proven analytical method of determining the qualitative composition of multicomponent systems. Though the use of GC-MS in forensic ink analysis is recommended by the ASTM Standard Guide for Test Methods for Forensic Writing Ink Comparison E 1422-01 (2001), this method appears to be rarely used. One of the reasons for this may be that some ink chemists believe that after about 1 year of being placed on paper, ballpoint inks will no longer contain any ingredient that could be detected and identified by GC-MS. This assumption ignores that:

- High boiling solvents typically used for manufacturing ballpoint inks ***never*** evaporate ***completely*** from an ink placed on paper. Even very old ballpoint inks contain the residues of their volatile components (phenoxyethanol or similar high boiling solvents) in amounts sufficient for their GC-MS detection and identification (Aginsky 1993, 1996, 2002; Gaudreau and Luc Brazeau 2002; La Porte, Wilson, Cantu, Mancke, and Fortunato 2004).
- Many solid ink ingredients (some of the substances and by-products used in the process of manufacturing ink), at temperatures typically used in the injector of a gas chromatograph (average 250 ^{0}C), have structural stability in the vapor phase and have vapor pressure that is sufficient for their GC-MS analysis. In other words, many ink noncolored components, which are either solids or high boiling viscous liquids, are vaporizable at high temperatures used in the injectors of gas chromatographs; therefore, these substances can be easily separated and characterized by GC-MS (Aginsky 1996).

Generally, the GC-MS analysis of noncolored ink components provides much more information on individualizing ballpoint ink composition than the TLC analysis of the ink's dye components. First, this occurs because the number of noncolored ink components that can be separated and characterized (identified) by GC-MS is usually significantly greater than the number of colored ink components analyzed by TLC. Secondly, the high separation efficiency and selectivity of GC-MS enables separation, detection, and unambiguous qualitative identification of many noncolored ingredients of ink-on-paper, including such minor components as by-products and other impurities that are often present in ink.

Methods and Materials

Ink Samples

Lines of Pilot, Pentel, Bic (USA), Bic (France), and Zebra red ballpoint inks were placed on HP multipurpose white paper. The Pilot inks used

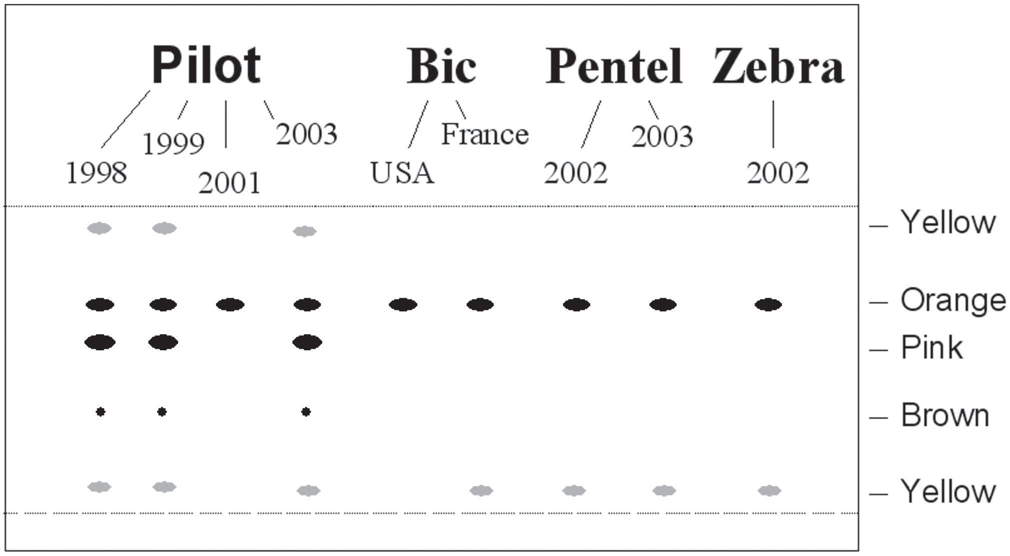

Figure 1. The thin-layer chromatogram obtained for the 9 red ballpoint inks analyzed shows there are 2 groups of inks that the brand cannot be discriminated by TLC. The amount of dye components in the analyzed inks varies from 1 orange dye in the Bic (USA) and Pilot-2001, up to 5 dye components of orange, pink, yellow (2 dyes), and brown (minor component) colors, in 3 Pilot inks manufactured in 1998, 1999, and 2003 respectively.

in this work were manufactured in the following years: 1998, 1999, 2001, and 2003. The Pentel inks were manufactured in 2002 and 2003. The Zebra ink was manufactured in 2002.

Sampling Devices

Harris 0.5-mm Uni-Cores (Shunderson Communications, Inc., Canada) hypodermic-needle-sized hole punches, used to remove micro plugs of ink-on-paper about the size of a typewritten period. The bored-out ink samples are removed with a plunger.

Extracting Vessels

Alltech 100-microliter glass vials with cone-shaped interior.

TLC Materials and Procedure

The inks sampled from paper (2 to 4 micro plugs) were extracted in chloroform (5 microliters), and the colored extracts were applied on the high performance (HP) TLC silica gel 60-F_{254} (10 x 10 cm) precoated glass plates (Merck, Germany). The plates were developed in ethyl acetate/isopropanol/water/acetic acid = 30:15:10:1.

GC-MS

The inks sampled from paper (2 to 3 micro plugs), as well as paper blanks, were extracted in chloroform (3 microliters), and the extracts were analyzed using an Agilent 6850 gas chromatograph interfaced with an Agilent 5973N mass selective detector and equipped with a split/splitless injection system.

GC Conditions and MS parameters

Column: HP-5MS, 30 m x 0.25 mm ID x 0.25-micrometer film thickness (cross-linked 5%-phenyl-95%-dimethylpolysiloxane)

Carrier: helium (column flow 1 mL/min)

Oven program: isothermal for 1 min at 35 °C, program 15 °C/min to 220 °C and hold for 7 minutes

Injection: 1 microliter, splitless, T=260 °C

Purge on time: 1 minute

GC-MS transfer line: 280 °C

Tune: autotune

Solvent delay: 5 minutes

Scan range: 39 - 220 atomic mass units (amu)

Results and Discussion

In the thin-layer chromatogram obtained for 9 red ballpoint inks (Figure 1), the eluting solvent was efficient and selective to completely separate all dye components present in the inks analyzed. The amount of dye components in the analyzed inks varies from 1 orange dye in the Bic (USA) and Pilot-2001, up to 5 dye components of orange, pink, yellow (2 dyes), and brown (minor component) colors, in 3 Pilot inks manufactured in 1998, 1999, and 2003, respectively. The chromatogram (Figure 1) clearly illustrates the difficulties that any examiner can face when using TLC for ink comparison and formula identification, namely:

- The same ink manufacturer can use different dye mixtures for producing different ink formulations of similar color or different batches of the same ink formulation. For example, the Pilot red ballpoint ink manufactured in 2001 contains only 1 dye, while each of the Pilot red ballpoint inks manufactured in 1998, 1999, and 2003 contains a mixture of the same 5 dye components.
- Different ink manufacturers using exactly the same dyes may produce different ink formulations used in pens with different brand names. Among the 9 inks analyzed, the brand of 2 groups of inks could not be discriminated by TLC. Given that, within each group, the inks contain the same dyes: 1 group comprises the Bic (USA) and Pilot-2001 inks that contain 1 and the same orange dye; and the other group includes the Pentel-2002, Pentel-2003, Bic (France), and Zebra-2002 inks that contain the same 2 dyes of orange and yellow colors.

It is a well known fact that there is a small number of commercially available dyes that are often used by different ink manufacturers. For this reason, compared inks of different formulations that contain the same dyes may easily be misidentified as the inks of the same formulation. In this connection, it becomes evident that when an examiner tries to determine whether 2 or more entries were written with the ink of the same formula or identify a questioned ink formulation (and determine the year when this formulation first came into existence), the chemical analysis should not be restricted by the TLC analysis of ink colored components and fluorescing components. Coupling TLC with GC-MS allows one to obtain much more information about the composition of the ink analyzed. GC-MS may establish that compared inks contain the same unique combination of components.

Generally, as far as the analysis of ballpoint ink is concerned, GC-MS has a better discriminating power than TLC. Thus, all different ink formulations in the above mentioned 2 groups of the red ballpoint ink brands that could not be discriminated by TLC (because the inks contain the same dyes) were reliably differentiated by GC-MS. Moreover, the 3 Pilot red ballpoint inks manufactured in 1998, 1999, and 2003, which also could not be discriminated by TLC (Figure 1), were easily differentiated by GC-MS (Figure 2).

The Pilot-1998 and Pilot-1999 inks contain the same liquid components: the residue of benzyl alcohol and phenoxyethanol (Figure 2). However, the ink manufacturer used different solid components: the Pilot-1998 ink contains diphenoxyethane, and the Pilot-1999 ink contains N-butylbenzenesulfonamide. The difference in the qualitative composition of solid noncolored components of the inks indicates that probably different resins were used in the manufacturing of these 2 inks. Comparing the results of the TLC and GC-MS analyses of the Pilot-1998 and Pilot-1999 inks, one can conclude that these inks represent either 2 manufacturing batches of the same ink formulation or 2 different formulations in which the same dyes and solvents were used.

Case Example 1

One of the key issues in a civil case was whether 2 documents dated in 1972 and 1977 were indeed signed in 1972 and 1977 or were these documents signed simultaneously and several years after 1977. The expert retained by the plaintiff compared the blue ballpoint inks on the questioned documents using TLC and came to a conclusion that the similarity of ink formulation used in the documents dated in 1972 and 1977 was an indication of simultaneous preparation of the documents.

This author, retained by the defendant, conducted the TLC and GC-MS analyses of the same blue ballpoint inks used to produce the signatures on the 1972 and 1977 documents. The TLC analysis showed similarity of the dye compositions in the inks. The GC-MS analysis, however, showed significant differences in the compositions of the inks' noncolored components (Figure 3).

The ink on the 1977 document contains only 1 solvent (phenoxyethanol), while the ink on the 1972 document contains 3 solvents: unidentified aliphatic alcohol (component "C"),

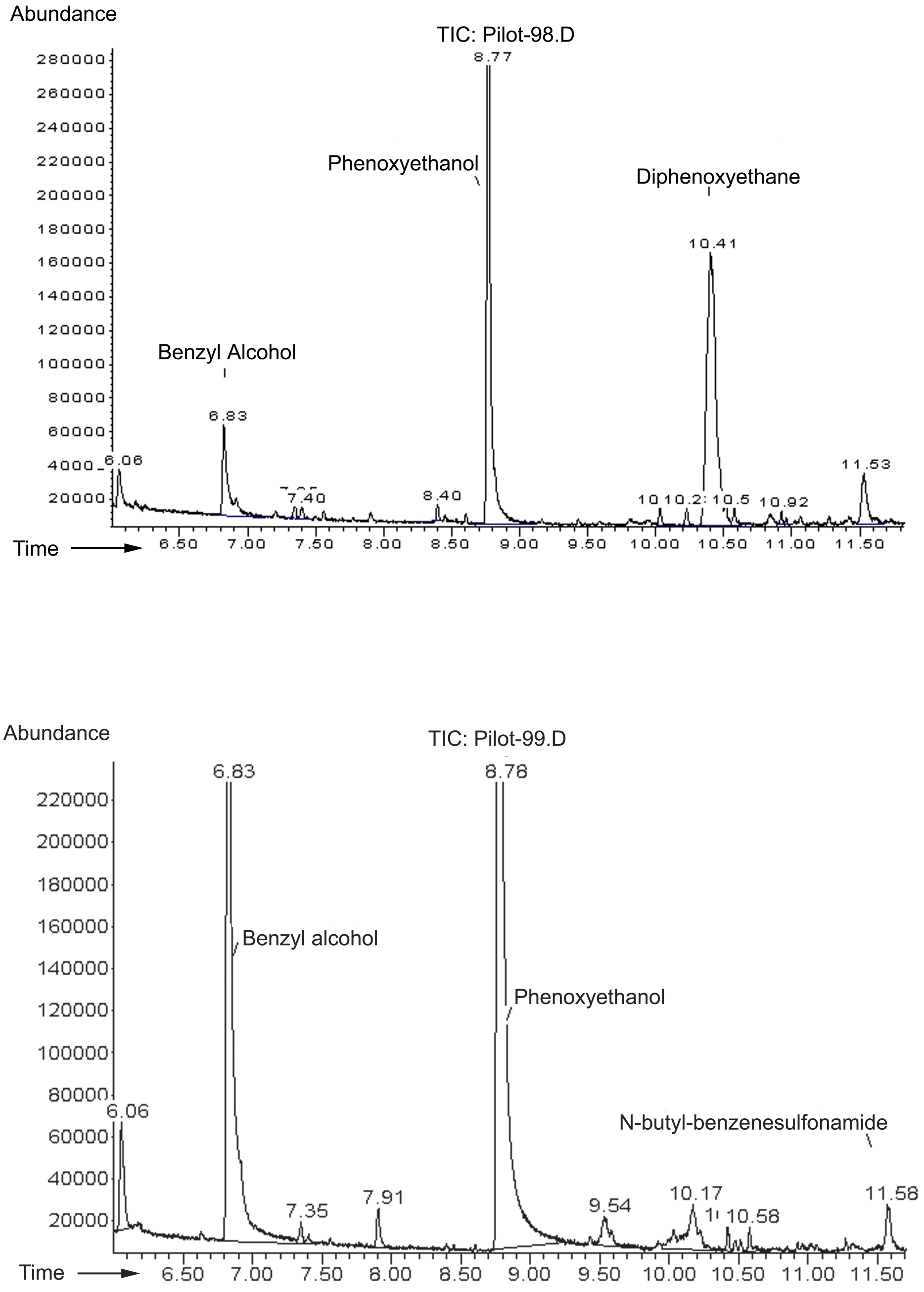

Figure 2. Total ion chromatograms of the Pilot red ballpoint inks manufactured in 1998 (upper chromatogram) and 1999 (lower chromatogram).

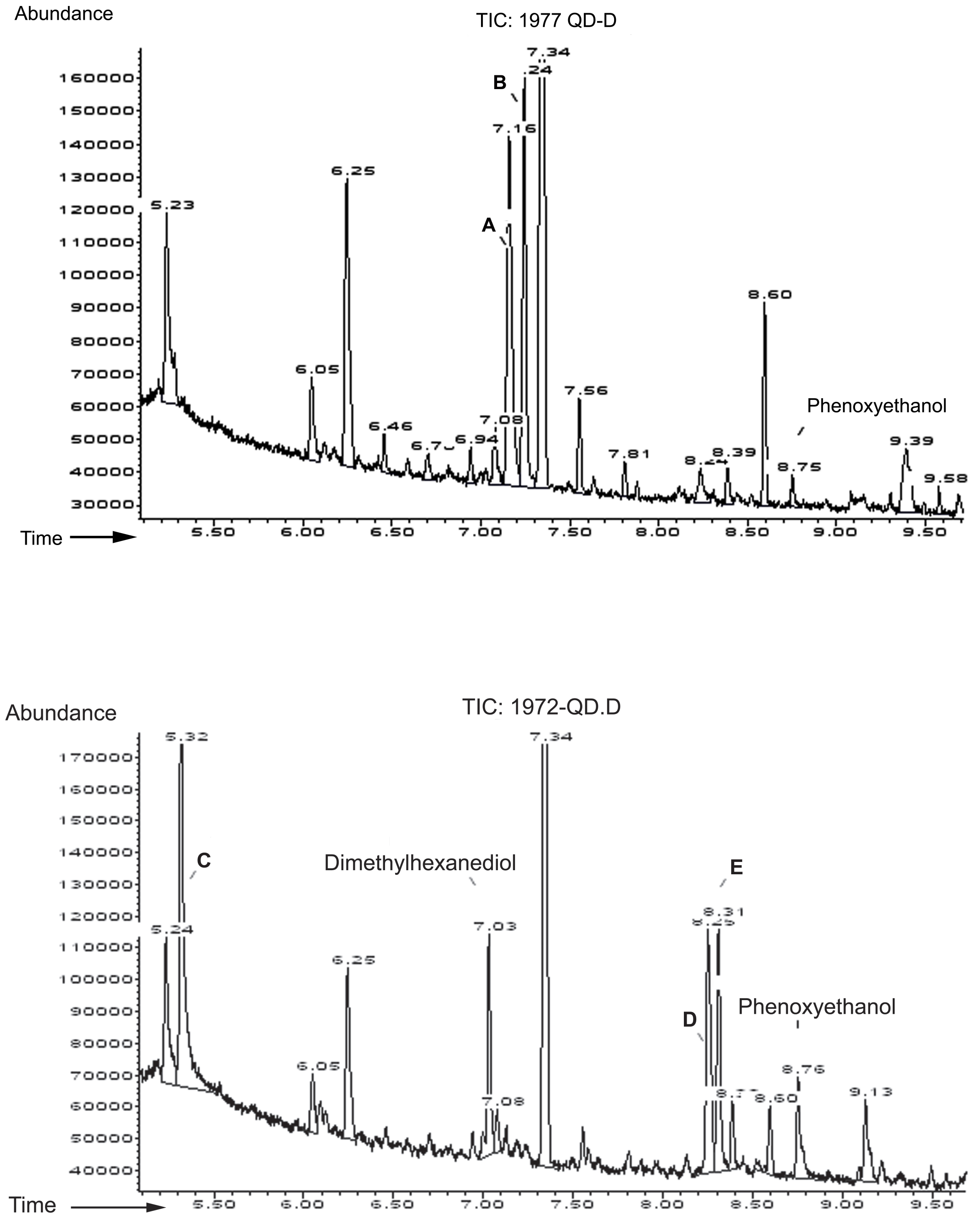

Figure 3. Total ion chromatograms of the blue ballpoint inks used to produce the signatures on the 1977 and 1972 documents. The upper chromatogram is for the 1977 document showing (A) 1-isocyanato-2-methylbenzene and (B) 2-methylbenzeneamine. The lower chromatogram is for the 1972 document showing (C) an unidentified aliphatic alcohol, (D) 2,4-dimethylbenzeneamine, and (E) 2,4-dimethylphenyl isocyanate.

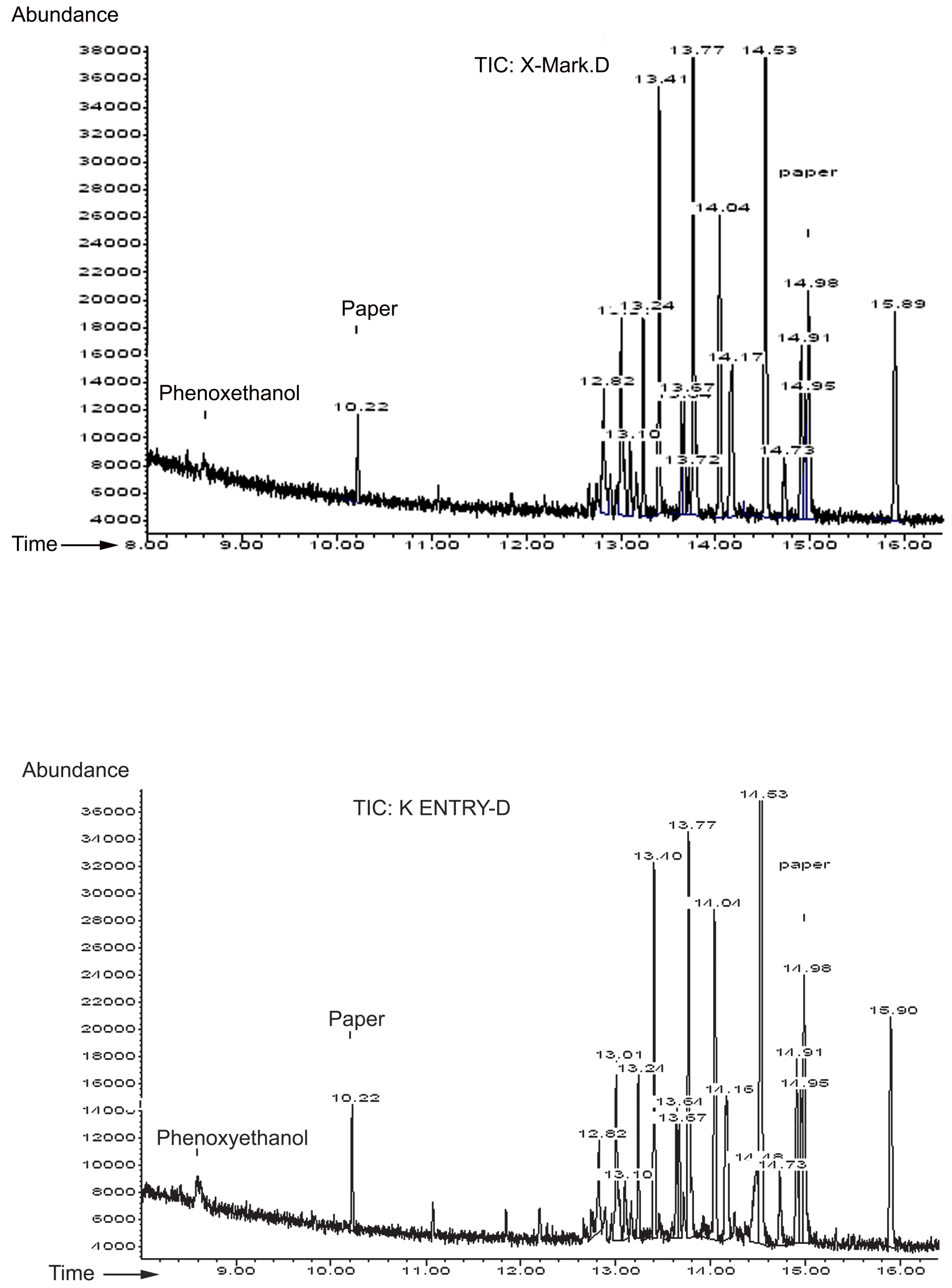

Figure 4. Total ion chromatograms of the blue ballpoint ink used to produce the "X" mark (upper chromatogram) and the remainder of the writing (lower chromatogram) on the questioned document. The GC-MS analysis showed that the compared inks have the same unique composition of noncolored components.

dimethylhexanediol and phenoxyethanol. In addition, these inks contain different solid components: A and B in the ink on the 1977 document, and D and E in the ink on the 1972 document. Therefore, the results of the TLC and GC-MS analyses show that the compared inks contain similar dyes but have completely different compositions of their liquid (solvents) and solid noncolored components. These results clearly show that the inks on the 1972 and 1977 documents represent 2 different formulations.

Case Example 2

In the second case, the key issue was to determine whether the "X" mark in response to a question on the Application for Employment dated in 1992 and the remainder of the writing on the document were written simultaneously, or whether the "X" mark was written recently—at least 10 years later than the remainder of the writing on the document.

Samples were taken of the blue ballpoint ink used to produce the questioned "X" mark and the remainder of the writing on the document. The samples were then analyzed by TLC and GC-MS. The TLC analysis showed similarity of the dye compositions in the ink samples. The GC-MS analysis showed that the compared inks have the same unique composition of noncolored components (Figure 4).

The ink used to produce both the "X" mark and the remainder of the writing on the document has a very complex composition of its noncolored components (stilbene amino derivatives and other aromatic compounds) that can be considered as a unique "chemical fingerprint" of this ink. The evident coincidence of the "chemical fingerprints" of the compared ink samples, being coupled with the TLC results, unambiguously proves that the compared ink samples represent the ink of the very same composition, that is, the ink of the same manufacturing batch. This result indicates simultaneous making of all writings on the document, including the "X" mark. Using the same pen (or a different pen containing the ink of the very same composition) used in 1993 to mark the "X" 10 years later would be unlikely. Theoretically, the same person could use the same pen after 10 years; however, the probability of this happening is extremely small.

In *Case Example 2*, a definite conclusion that the inks compared represent the ink of the same manufacturing batch was possible. This ink contains a lot of chromatographically separable organic ingredients that, in the aggregate, formed a unique combination—a complex mixture of numerous colored and noncolored organic substances present in the ink in a certain, measurable relative proportion. This "chemical fingerprint" is so complex that it is practically improbable that it can be coincidentally duplicated either by an ink of a different formulation or by the ink of the same formulation but of a different manufacturing batch. If one were to assume what is very unlikely to happen in practice, namely, that another batch of ink was manufactured using the very same major and minor ingredients, even then the "chemical fingerprints" of the inks representing these 2 expectedly identical batches should be *a priori* different. This takes place because no technology, at least in chemical industry, is ideal. As acknowledged by ink manufacturers, variations between batches should produce measurable differences. These differences between batches occur due to inevitable variations of various parameters of the technological process. Some of these variations are:

- an amount of each ingredient added to the reaction mixture;
- the chemical purity of these ingredients (the presence of by-products and contaminants);
- the chemical composition of ink resins (including nonreacted chemical substances used for synthesizing the resin, molecular-mass distributions of ink resins' components, and a composition of primer and minor proprietary additives to ink resins)[1]; and
- fluctuations of the temperature and the duration of various processes in the ink manufacturing.

Depending on the composition of ink, batch variations are more or less pronounced to be detected chromatographically. There is no universal rule that could be applicable to any case in which an examiner would need to determine whether the similarity found between the inks being compared is enough to come to a scientifically sound conclusion that these inks belong to the same manufacturing batch. In some cases, a key feature can be a unique component present in the inks being compared. In other cases, the complex composition of an ink, like that considered in *Case Example 2* above, can show that such a composition is so unique that its duplication is practically impossible. There are inks, however, that are not complex in composition and are far from being unique. If this is the case, then it may be impossible not only to determine that the compared inks (matching at the applied level of analysis) came from the same manufacturing batch, but also to determine if these inks are of the same formulation.

In any case, a minimum requirement that should be met to avoid scientifically unsound conclusions based upon the ink comparison testing is that the examiner should have substantial knowledge about ink manufacturing and should be highly proficient in using chromatographic methods to obtain maximum useful information about the composition of the inks that are to be compared.

Conclusion

TLC enables characterization of only a small part of ink composition; therefore, in most cases, this method should be used in combination with GC-MS, which is the well-proven analytical method that combines the efficient separation ability of capillary gas chromatography and the sensitive and selective detectability and molecular identification ability of mass spectrometry. GC-MS can provide the examiner with valuable information about ink ingredients no matter how long the ink has been on the paper.

The experimental data obtained in this work shows powerful capabilities of GC-MS for ink comparison and ink formula identification. It has also been shown with an example that GC-MS, coupled with TLC, can determine that compared inks have the same unique composition that characterizes these inks as belonging to the same manufacturing batch.

Footnote

[1] Batch-to-batch variations (mainly, due to variations in the quality of ink resins that, as known, play a key role in ink aging) significantly change the rate of ink aging. Thus, with experimental data, it has been shown that 2 inks of the same formulation but of different manufacturing batches would age at significantly different rates (Aginsky, 1996).

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