TECHNICAL NOTE

Valerio Causin,[1] *Ph.D.; Rosario Casamassima,*[2]*; Carla Marega,*[1] *M.Sc.; Pietro Maida,*[2] *B.Sc.;*
Sergio Schiavone,[2] *Ph.D.; Antonio Marigo,*[1] *M.Sc.; and Antonino Villari,*[3] *Ph.D.*

The Discrimination Potential of Ultraviolet-Visible Spectrophotometry, Thin Layer Chromatography, and Fourier Transform Infrared Spectroscopy for the Forensic Analysis of Black and Blue Ballpoint Inks

ABSTRACT: The knowledge of the discriminating power of analytical techniques used for the differentiation of writing inks can be useful when interpreting results. Ultraviolet-visible (UV-VIS) spectrophotometry, thin layer chromatography (TLC), and diffuse reflectance Fourier transform infrared spectroscopy (FT-IR) were used to examine a population of 21 black and 12 blue ballpoint writing inks. Based on corroborative results of these methods, the discrimination power for UV-VIS, TLC, and FT-IR was determined to be 100% and 98% for the black and blue inks, respectively. Generally, TLC and UV-VIS can be used to differentiate the colorant components (i.e., dyes and some pigments) found in inks. As FT-IR can be utilized to identify some of the noncolorant components, it was determined to be an excellent complementary technique that can be implemented into an analytical scheme for ink analysis.

KEYWORDS: forensic science, questioned documents, inks, ultraviolet-visible spectroscopy, thin layer chromatography, Fourier transform infrared spectroscopy

Ink analysis can be a very important aspect in questioned documents examinations because it can be used to determine the authenticity or validity of a document. Therefore, it is of the utmost importance to have an accurate and discriminating protocol of analysis and also the knowledge of the significance and evidential value of a matching comparison. Inks for ballpoint pens are very popular and widely employed and so they are very frequently encountered in questioned document examinations. Many authors have proposed techniques, methods, and protocols to carry out analyses of such samples (1–13), but just a few have focused on the use of Fourier transform infrared (FT-IR) spectroscopy (14–16). Common examination techniques, such as thin layer chromatography (TLC), infrared luminescence, infrared reflectance, and visualization of inks in the ultraviolet (UV), visible (VIS), and near infrared (via filters and cameras) (17,18), compare questioned inks mainly on the basis of their color or dyestuff content. Similar information can be obtained by UV-VIS spectrophotometry. One of the purposes of this study was to propose a deconvolution procedure of UV-VIS absorption spectra to refine the qualitative comparisons that may be conducted and to assess the improvement in discriminating power resulting from this approach. The second objective was to show that FT-IR can be a very useful tool for the forensic examination of documents, providing additional information regarding chemical constituents

other than colorants. Inks are complex mixtures that must satisfy several requirements. For example, they should not dry out while in the pen, but it is necessary for them to fix quickly when applied to a substrate. As well, they must be fluid so as to allow the rotation of the ball on the tip of the pen, but at the same time, if too thin, they would tend to diffuse in the paper and to appear on both sides of the page. To calibrate all the desired features, a number of components appear in ink formulations, such as dyes, pigments, solvents, resins, viscosity adjustors, and ball lubricants. All the ingredients of the formula contribute to FT-IR spectra, so this technique is expected to be a useful one for a thorough characterization of writing inks. It should be remarked that when using this technique, though, special care should be exercised because it is necessary to consider the possible changes in the composition of the ink due to aging (15,19–24).

Thirty-three blue and black inks of very common ballpoint pens were analyzed by UV-VIS spectrophotometry, TLC, and diffuse reflectance FT-IR spectroscopy, so as to assess the discriminative potential of each technique and of a procedure that integrates all of them. Forensic document examiners are well aware about the possibilities of these techniques to distinguish between inks, but not many literature works report the discriminating power associated with a sequence of techniques such as that used in this work (6,14,16). An assessment of this datum is very useful when interpreting results and presenting them to Court.

[1]Dipartimento di Scienze Chimiche dell'Università, via Marzolo 1, 35131 Padova, Italy.
[2]Reparto Carabinieri Investigazioni Scientifiche, S.S. 114 Km. 6,400 – 98128 Tremestieri, Messina, Italy.
[3]Dipartimento Farmaco Chimico Facoltà di Farmacia dell'Università, viale Annunziata, 98123 Messina, Italy.
Received 24 Mar. 2007; and in revised form 20 Oct. 2007; accepted 8 Mar. 2008.

Materials and Methods

Samples and Samples Preparation

A total of 33 ballpoint pens (21 black and 12 blue) from different manufacturers and of different models were randomly acquired

CAUSIN ET AL. • ANALYSIS OF INKS 1469

from several stationery shops in the Messina region. Lines were drawn with the pens on unlined sheets of the same batch of A4, white, 80 g/m^2 office paper (GBR, Padova, Italy). Right after the deposition, the ink coming from lines c. 1 cm long was extracted with 40 μL of absolute ethanol (Carlo Erba, Rodano, Italy) in a glass vial. To check the role of sampling on analytical results, another extraction with ethanol was performed directly from the pen ink reservoir, obtaining indistinguishable data if compared with the samples extracted from the lines on paper. Blank paper was also treated with ethanol with the same procedure used for ink lines, to quantify the matrix effect.

UV-VIS

Samples for UV-VIS spectrometry were prepared by transferring 10 μL of ink extract in another vial and diluting to 0.5 mL with ethanol. A Lambda 3 spectrophotometer (Perkin Elmer, Waltham, MA) was employed. The spectral range used in the acquisition of data was 250–900 nm. No spectral features were observed for any sample between 780 and 900 nm, i.e., in the near-infrared region of the acquired spectrum. The data were processed with Perkin Elmer Computerized Spectroscopy Software. Deconvolution of multiple peaks with Gaussian functions was carried out with Peak-Fit software (Systat, San Jose, CA) just in the visible range of the spectra, i.e., in the interval 490–660 nm. Quartz cells, with an optical path of 1 cm and 0.5 mL volume (Hellma, Müllheim, Germany), were employed.

TLC

TLC analyses were performed using as the eluent mixture 1-butanol, 1-propanol, water, and acetic acid (10:5:5:0.5). Five microliter of each extract were spotted on high-performance TLC silica gel plates without fluorescence indicator (Merck, Whitehouse Station, NJ) and developed in a horizontal developing chamber (Camag, Muttenz, Switzerland).

Diffuse Reflectance Infrared Spectroscopy

FT-IR spectra were obtained by a Nicolet Protégé 460 (Thermo Scientific, Waltham, MA) equipped with a diffuse reflectance accessory using the procedure described by Merrill and Bartick (25). Ten microliter of ethanol extracts of the ink was allowed to evaporate in a microsample cup (diameter: 3 mm; depth: 2 mm). A paper blank was obtained and its spectrum was used to subtract spectral interference due to the paper substrate. All samples were analyzed by collecting 256 scans at 4 cm^{-1} resolution, from 4000 to 450 cm^{-1}. Omnic software (Thermo Scientific) was used for data processing, i.e., subtraction of paper, CO_2, and H_2O signals and baseline normalization.

Results and Discussion

UV-VIS

UV-VIS spectra contain information mainly related to the colorants (dyes and pigments) content in the ink formulation, even though a contribution from other components such as solvents or resins is possible, especially in the UV region. UV-VIS spectroscopy can be a suitable technique for an initial screening. The samples analyzed in this study were differentiated according to the qualitative appearance of their absorption spectra in the visible range into five and three groups, respectively, in the case of black
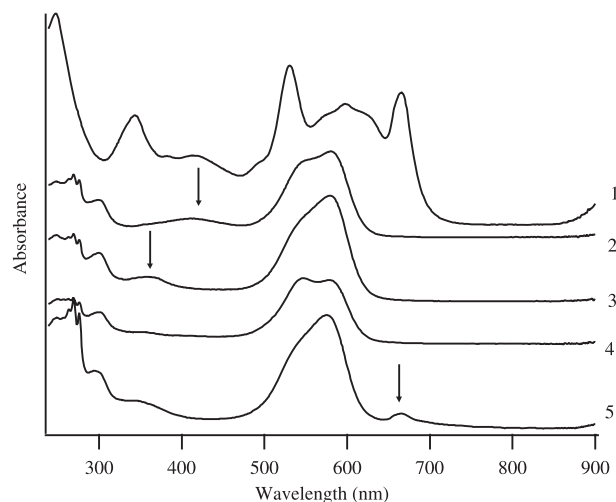

FIG. 1—*The four groups of UV/VIS absorbance spectra detected in black ink samples. Arrows indicate the bands that characterized groups 2, 3, and 5.*

and blue inks. Figures 1 and 2 show the different recorded spectra. For the black inks, group 1 samples produced a very unique spectrum, while groups 2, 3, 4, and 5 had a very similar pattern (Fig. 1). The differences between samples of these latter groups were in the presence of minor bands at 411, 360, and 666 nm, respectively, in groups 2, 3, and 5. The minor peaks in group 4 were absent. Similar findings were observed for blue inks. Group 1 samples produced a markedly different spectrum, while group 2 could be differentiated on the basis of the presence of a minor peak at 650 nm, which was absent in group 3 (Fig. 2). In the UV range, the spectrum was richer in spectral features and allowed for a further differentiation rather than for a broad classification as that made possible considering just the visible spectral interval.

The discriminative power (DP) of the techniques employed in this work was evaluated according to Smalldon and Moffat (26) as

$$DP = \text{(number of discriminated sample pairs)}$$
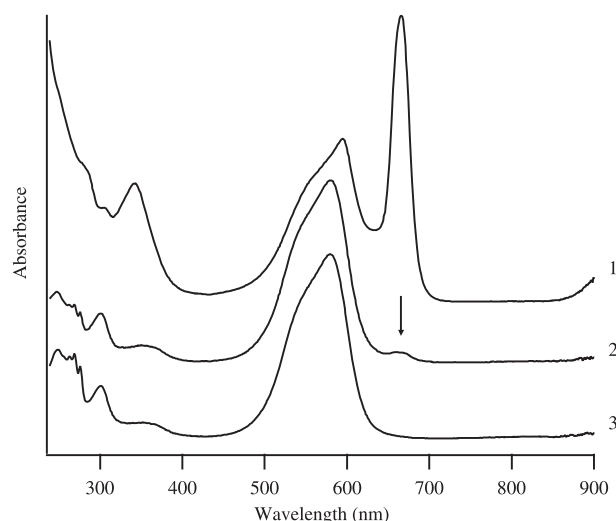$$/\text{(number of possible sample pairs)}$$

FIG. 2—*The three groups of UV/VIS absorbance spectra detected in blue ink samples. The arrow indicates the band that characterized group 2.*
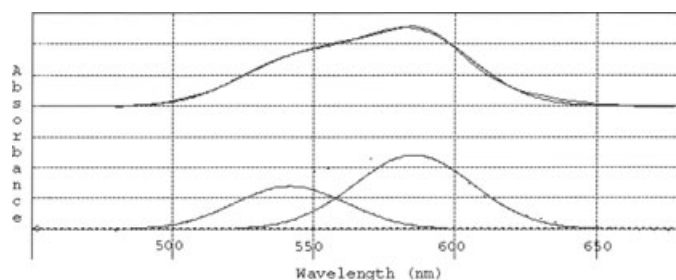
FIG. 3—*Deconvolution of the main peak in the visible absorption spectrum of sample 2.*

If n is the total number of samples, the number of possible sample pairs was $[n \cdot (n-1)]/2$, i.e., $(21 \cdot 20)/2 = 210$ and $(12 \cdot 11)/2 = 66$, respectively, for black and blue inks.

By a comparison based on a qualitative analysis of UV-VIS absorption spectra, 96% and 79% of the black and blue inks, respectively, could be distinguished. Roux et al. reported a DP of 0.83 for both blue and black inks (6). A quantitative approach for the comparison of these samples was also applied. The major peak in the visible part of black groups 2, 3 and 4 and blue groups 2 and 3 was deconvoluted by two Gaussian functions. These functions were integrated in the 490–660 nm interval and their areas, denominated A_1 and A_2, respectively, were computed. The mass-independent ratio $R = A_1/A_2$ was defined and was used for comparing the various inks within the same category. If A_1 and A_2 separately, instead of the ratio R, were used, they would have been dependent on the size of the sample. As it was not possible to rigorously control the quantity of ink extracted for each sample, this approach was not feasible and the use of the mass independent ratio was necessary. Ten replicates of selected samples, i.e., 1, 2, and 8, were run to assess the repeatability of the measurements. The relative standard deviation observed in such experiments was 5%. An example of the results of the deconvolution procedure is shown in Fig. 3. No further discrimination was attained for blue inks by this quantitative approach, and just three more black couples of samples could be differentiated, increasing the DP to 98%. Despite the marginal improvement in the DP, the application of a quantitative treatment of UV-VIS spectra can be useful as it yields a numerical parameter that can help the Courts with a more objective result for interpretation.

TLC

TLC is the most widespread method utilized for the forensic analysis of writing inks. In this study, TLC was applied with the objective of refining the discrimination within the main groups individuated on the basis of the qualitative analysis of the visible absorption spectrum. Different discriminative performances were observed, as demonstrated in Figs. 4 and 5. For example, all the samples of the black inks in groups 1 and 4 could be discriminated, but a lower fraction of the possible pairs of samples within groups 2 and 3, 67% and 89%, respectively, displayed differentiable chromatographic patterns. For blue inks, 90% or more of the possible pairs in each group could be discriminated. Other studies on the forensic analysis of inks reported DPs of 0.9 (14) and 0.99 (6). Only in the case of group 2 of the black inks, a much lower DP was observed. Although by TLC all the components of the ink formula are separated, just colored or fluorescent compounds can be visibly detected, so this method is also strictly linked to the quality and quantity of pigments and dyes used for the manufacture of the
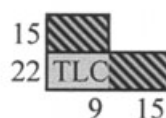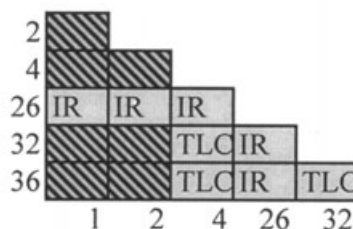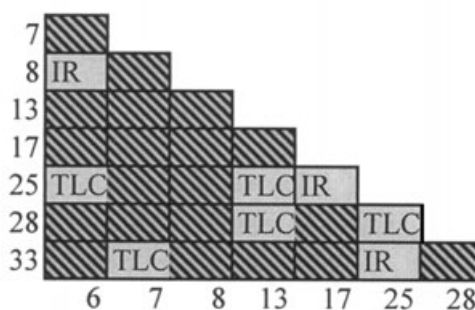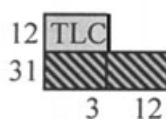

FIG. 4—*Differentiation of blue inks by FT-IR and TLC. Shaded squares represent couples of sample distinguishable by both methods, gray squares represent pairs distinguishable just by the indicated method.*

ink. This may explain why in group 2 of black inks, a poorer than expected discrimination was obtained. A change in solvent system could indeed have improved the discriminating power, but a technique for detecting solvents and resins, in addition to colorant components, was used.

FT-IR Spectroscopy

FT-IR spectroscopy is a technique that can be used to analyze noncolorant components found in writing inks. Although it can be difficult to positively assign all the bands in a complex FT-IR spectrum, detection of solvents or resins employed as ingredients brings about an increased potential for discrimination. As previously discussed for TLC, FT-IR spectroscopy was used to discriminate the inks within their groups, individuated by visible absorption spectroscopy. A number of bands have proven useful for this purpose. Most of the samples, except group 1 of both black and blue inks, displayed the peaks at 1296, 1094, and 1055 cm^{-1}, typical of
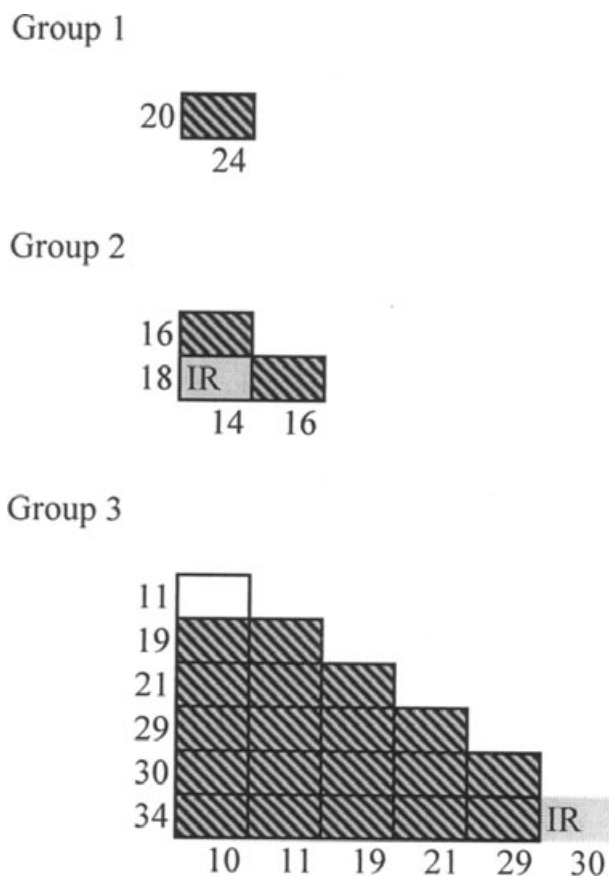
Group 1

Group 2

Group 3

FIG. 5—*Differentiation of blue inks by FT-IR and TLC. Shaded squares represent couples of sample distinguishable by both methods, gray squares represent pairs distinguishable just by the indicated method, white squares represent samples indistinguishable by both techniques.*
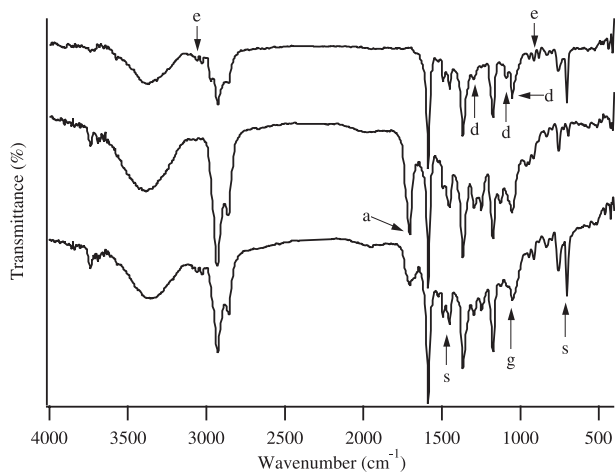

FIG. 6—*Examples of FT-IR spectra obtained for samples 10 (top), 34 (middle), and 25 (bottom). Diagnostic bands are indicated by arrows. a = acid or ester, d = dye, e = epoxy resin, g = ethylene glycol, s = solvents (styrene and benzyl alcohol).*

triarylmethane (15) (Fig. 6). A band at *c.* 1700 cm^{-1}, indicative of the presence of acids or esters, was found to be particularly diagnostic (Fig. 6). On the basis of the presence or absence of this peak, Wang et al. (15) operated a subdivision of the inks they
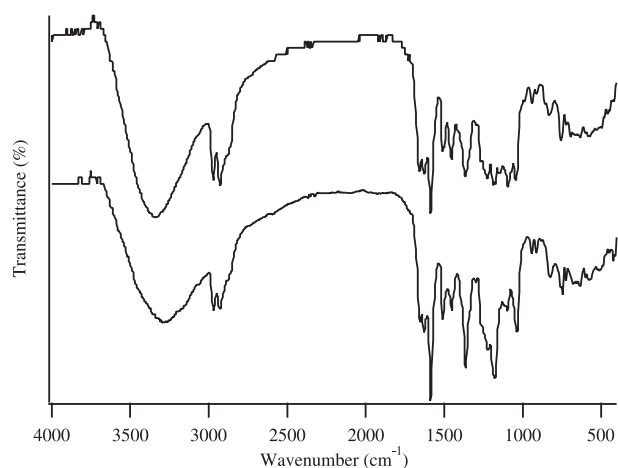

FIG. 7—*FT-IR spectra obtained for samples 24 (top) and 20 (bottom).*

studied into two main groups. Bands originated by epoxy resins (15) appeared for some samples at 914 and 3062 cm^{-1} and helped in their differentiation (Fig. 6). Also, doublets were located at about 700 and 1475 cm^{-1} that were ascribed to the presence of solvents such as styrene or benzyl alcohol (16) (Fig. 6). Ethylene glycol contributed with quite broad peaks between 1050 and 1150 cm^{-1}.

Although for some signals as those just described an assignment was possible, in some other cases the complex appearance of the spectrum prevented a straightforward and unambiguous interpretation. Figure 7 shows the spectra pertaining to the two inks included in the blue group 2. Although very similar in the high and low wavenumber regions, a marked diversity exists in the position and relative intensities of the peaks located in the range 1000–1300 cm^{-1}. It was not possible to single out the component creating this difference, but nevertheless it was determined to be discriminating.

It has been widely reported in the literature that writing inks, when they are placed on a substrate, undergo an aging process (15,19–24) that consists mainly of the evaporation of volatile components, oxidation of some of the constituents of the formulation, and the hardening of the resins. This is expected to bring about changes in the FT-IR spectra of inks as the time goes by, as studied by Humecki (19,24) and Wang et al. (15). Humecki (19) found that the aging of ink influenced the intensity of the OH band at 3330 cm^{-1}, of the CH band at 2950 cm^{-1} and of the C=O band at about 1700 cm^{-1}. An increase in the intensity of the C=O signal and a decrease in the OH and CH bands were reported in a time span of 20 years. Wang et al. (15), on the other hand, observed a decrease in the absorption bands at 1584, 1494, 1360, 1296, 1245, 1094, and 1055 cm^{-1} attributed to the changes in the epoxy resin and the volatilization of solvents. The only signal which almost disappeared as a consequence of aging was that at 1245 cm^{-1} (15), while for all the other cases just a decrease in intensity was reported. The differentiation of our samples by FT-IR was based on a qualitative comparison of spectra that took into account the presence or absence of absorption bands. Differences in the intensity of bands were considered significant only when very large and macroscopic alterations in the relative intensities of adjacent peaks were observed. Because in the sampling procedure of our samples, their age was not rigorously controlled, the intensity ratios used in the aforementioned works (15,19) were not considered for the differentiation of the samples studied in this work, as they could have been altered by aging. Moreover none of the other mentioned
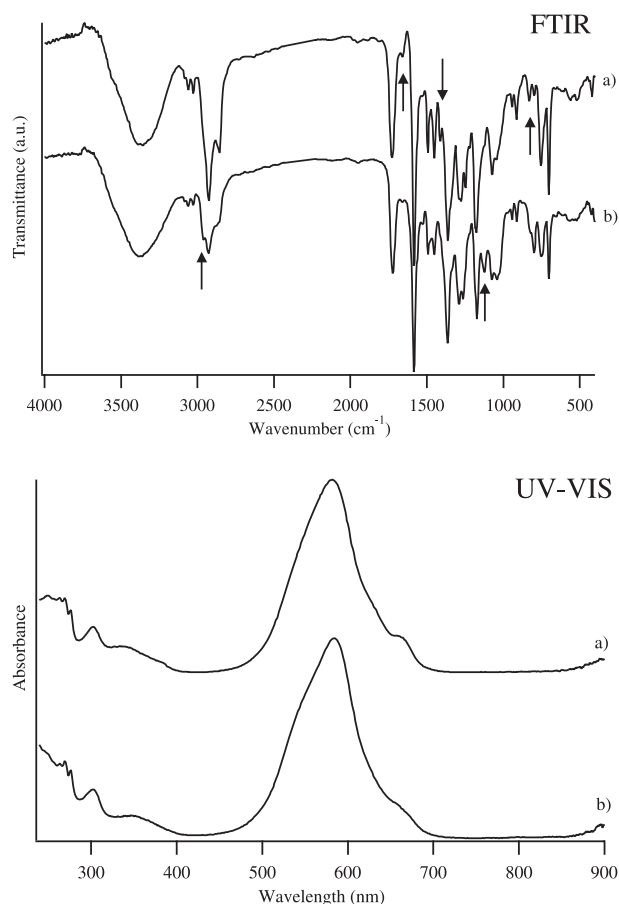
FIG. 8—*UV-VIS and FT-IR spectra obtained for samples (a) 14 and (b) 18. UV-VIS patterns were indistinguishable, while differences in the FT-IR spectra are shown by the arrows.*

bands were very diagnostic for differentiation in our samples, so the effect of aging on the DP of FT-IR estimated by us can be excluded.

Figures 4 and 5 reveal very satisfactory DP values, ranging from 80% to 82% of black groups 2 and 3, respectively, to 95% of blue group 1.

Conclusion

Although a number of works exist on the forensic analysis of writing inks, data about the discrimination potential of the techniques or sequence of techniques are still not widely available. This work was aimed at showing that by applying three very common analytical methods, UV-VIS spectrophotometry, TLC, and FT-IR spectroscopy, differentiation of most of the ballpoint inks commonly found on the market could be achieved. These techniques were in fact used on 21 black and 12 blue samples and just in one case, between blue samples 10 and 11, no difference could be detected (Figs. 4 and 5). This corresponds to an overall DP of the sequence of the techniques equal to 100% and 98% for black and blue inks, respectively. Knowledge of the DP of an analysis is of great help to the forensic scientist when it comes to interpreting results (27,28). It has been shown that FT-IR spectroscopy is particularly interesting as a complementary technique to TLC and UV/VIS spectroscopy because FT-IR spectra are due to resins and solvents, not just due to colorant components. Figure 8 shows an example of samples that could not be differentiated by UV-VIS and TLC, but that had different FT-IR spectra. Deconvolution of visible spectra was also performed, so as to be able to quantify the comparisons made. It is very important to have a quantitative and more objective parameter when reporting results to the Court.

References

1. Smalldon KW. The comparison of ink in dyestuffs using minimal quantities of writing. J Forensic Sci Soc 1969;9:151–2.
2. Laing DK, Isaacs MDJ. The comparison of nanogram quantities of ink using visible microspectrometry. J Forensic Sci Soc 1983;23:147–54.
3. Tappolet JA. The high performance thin layer chromatography (HPTLC). Its application to the examination of writing inks. Forens Sci Int 1983;22:99–109.
4. Totty RN, Ordidge MR, Onion LJ. A comparison of the use of visible microspectrometry and high performance thin layer chromatography for the discrimination of aqueous inks used in porous tip and roller ball pens. Forens Sci Int 1985;28:137–44.
5. Olson LA. Color comparison in questioned document examination using microspectrophotometry. J Forensic Sci 1986;31:1330–40.
6. Roux C, Novotny M, Evans I, Lennard C. A study to investigate the evidential value of blue and black ballpoint pen inks in Australia. Forens Sci Int 1999;101:167–76.
7. Rodger C, Dent G, Watkinson J, Smith E. Surface-enhanced resonance raman scattering and near-infrared Fourier transform raman scattering as in situ probes of ink jet dyes printed on paper. Appl Spectrosc 2000;54:1567–76.
8. Claybourn M, Ansell M. Using raman spectroscopy to solve crime: inks, questioned documents and fraud. Sci Justice 2000;40:261–71.
9. Kher AA, Green EV, Mulholland MI. Evaluation of principal component analysis with high-performance chromatography and photodiode array detection for the forensic differentiation of ballpoint pen inks. J Forensic Sci 2001;46:878–83.
10. Thanasoulias NC, Parisis NA, Evmiridis NP. Multivariate chemometrics for the forensic discrimination of blue ballpoint pen inks based on their vis spectra. Forens Sci Int 2003;138:75–84.
11. Wilson JD, LaPorte GM, Cantu AA. Differentiation of black gel inks using optical and chemical techniques. J Forensic Sci 2004;49:364–70.
12. Bugler JH, Buchner H, Dallmayer A. Characterization of ballpoint pen inks by thermal desorption and gas chromatography-mass spectrometry. J Forensic Sci 2005;50:1209–14.
13. Payne G, Wallace C, Reedy B, Lennard C, Schuler R, Exline D, et al. Visible and near-infrared chemical imaging methods for the analysis of selected forensic samples. Talanta 2005;67:334–44.
14. Tsutsumi K, Ohga K. Analysis of writing ink dyestuff by TLC and FT-IR and its application to forensic science. Anal Sci 1998;14:269–74.
15. Wang J, Luo GA, Sun SQ, Wand ZQ, Wang YJ. Systematic analysis of bulk blue ballpoint pen ink by FTIR spectrometry. J Forensic Sci 2001;46:1093–7.
16. Zięba-Palus J, Kunicki M. Application of the micro-FTIR spectroscopy, raman spectroscopy and XRF method examination of inks. Forens Sci Int 2006;158:164–72.
17. Ellen D. The scientific examination of questioned documents. Methods and techniques. London: Taylor and Francis, 1997.
18. Kelly JS, Lindblom BS, editors. Scientific examination of questioned documents, 2nd ed. Boca Raton, FL: Taylor and Francis, 2006.
19. Humecki H. Experiments in ballpoint ink aging using infrared spectroscopy. In: Proceedings of the international symposium on questioned documents. FBI Academy, Quantico, VA. Washington: U.S. Government Printing Office, 1985;131–5.
20. Brunelle RL, Cantu AA. A critical evaluation of current ink dating techniques. J Forensic Sci 1987;32:1522–36.
21. Brunelle RL. Ink dating—the state of the art. J Forensic Sci 1992;37:113–24.
22. Aginsky VN. Some new ideas for dating ballpoint inks—a feasibility study. J Forensic Sci 1993;38:1134–50.
23. Aginsky VN. A microspectrophotometric method for dating ballpoint inks — a feasibility study. J Forensic Sci 1995;40:475–8.
24. Brunelle RL, Crawford KR Advances in the forensic analysis and dating of writing ink. Springfield, IL: C.C. Thomas, 2003.
25. Merrill RA, Bartick EG. Analysis of ballpoint pen inks by diffuse reflectance infrared spectroscopy. J Forensic Sci 1992;37:528–41.

26. Smalldon KW, Moffat AC. The calculation of discrimination power for a series of correlated attributes. J Forensic Sci 1973;13:291–5.
27. Causin V, Marega C, Carresi P, Schiavone S, Marigo A. A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement and differential scanning calorimetry for tracing the source of illegal drugs. Forens Sci Int 2006;164:148–54.
28. Causin V, Marega C, Carresi P, Schiavone S, Marigo A. A quantitative differentiation method for plastic bags by wide angle X-ray diffraction for tracing the source of illegal drugs. Forens Sci Int 2007;168:37–41.

Additional information and reprint requests:
Dr. Valerio Causin, Ph.D.
Dipartimento di Scienze Chimiche
Università di Padova
Via Marzolo 1
35131 Padova
Italy
E-mail: valerio.causin@unipd.it