OSAC 2022-S-0017 Standard Guide for Microspectrophotometry in Forensic Fiber Analysis

Trace Materials Subcommittee
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OSAC Proposed Standard

OSAC 2022-S-0017 Standard Guide for Microspectrophotometry in Forensic Fiber Analysis

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Standard Guide for Microspectrophotometry in Forensic Fiber Analysis

1. Scope

1.1 This guide is intended to assist forensic science practitioners (FSPs) with procedural recommendations for conducting spectral measurements of color on single fiber samples using ultraviolet (UV), visible (VIS), near infrared (NIR), or fluorescence emission analyses, and on comparing samples on the basis of these measurements. Spectral measurement of color (referred to as color measurement in this document) by microspectrophotometry is part of a broader analytical scheme.

1.2 This guide primarily focuses on color measurements within the visible spectral range but includes some details concerning measurements in the UV and NIR spectral ranges. The particular method(s) employed by each FSP depends upon available equipment, FSP training (Practice E2917, Practice WK78748), sample suitability, and sample size.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 This standard is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practice E2917), and demonstrated proficiency to perform forensic casework.

1.5 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.

1.6 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 *ASTM Standards:*[1]

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

E284 Terminology of Appearance

E620 Practice for Reporting Opinions of Scientific or Technical Experts

E805 Practice for Identification of Instrumental Methods of Color or Color-Difference Measurement of Materials

E1459 Guide for Physical Evidence Labeling and Related Documentation

E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

E1732 Terminology Relating to Forensic Science

E2224 Guide for Forensic Analysis of Fibers by Infrared Spectroscopy

E2227 Guide for Forensic Examination of Dyes in Textile Fibers by Thin-Layer Chromatography

E2228 Guide for Microscopical Examination of Textile Fibers

E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs

E3255 Practice for Quality Assurance of Forensic Science Service Providers Performing Forensic Chemical Analysis

WK78747 Guide for Forensic Examination of Fibers

[1] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.
[2] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

WK78748 Practice for a Forensic Fiber Training Program

2.2 Other Standards:

ISO 17025[2] General Requirement for the Competence of Testing and Calibration Laboratories

3. Terminology

3.1 *Definitions*—For definitions of fiber-associated terminology used in this guide, see Terminologies E1732.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aperture, n*—an opening in an optical system that controls the amount of light passing through a system. (E1732)

3.2.2 *dichroism, n*—the property of exhibiting different colors, especially two different colors, when viewed along different axes by plane polarized light. (E1732)

3.2.3 exclusionary difference, n—A difference in one or more characteristics between compared items that is sufficient to determine that the compared items did not originate from the same source, are not the same substance, or do not share the same composition or classification. (OSAC Preferred Term – Lexicon)

3.2.3.1 Discussion—What is sufficient depends on the performance and limitations of the method used on the material in question.

3.2.4 *metameric samples*—two or more samples that appear to have the same color under one type of illumination but can appear dissimilar under different lighting conditions, or two or more samples that appear to be the same color under all lighting conditions, yet their reflectance/transmittance spectral curves are different. (E1732)

3.2.5 *spectral resolution, n*—measure of the ability to distinguish between adjacent peaks in a spectrum; it is usually determined by measuring peak width at half the maximum value of the peak height or full-width half-maximum (FWHM). (E1732)

3.2.5.1 *Discussion*—Spectral resolution is not to be confused with spatial resolution [the smallest features that can be resolved in the field of view of the microspectrophotometer (MSP) camera or eyepieces or can be used to refer to the smallest spectral sampling area of the MSP].

4. Summary of Guide

4.1 This guide covers the collection and comparison of spectra from the UV, VIS, and NIR ranges obtained from fibers and can be applied to different models of microspectrophotometers (MSPs). This guide is not meant to be the first step in the process of a fiber examination. (Refer to the Standard Guide for Forensic Examination of Fibers).

4.2 Microspectrophotometric examinations typically occur in the visible spectral region (~380 to780 nm), where information about the visible color of a sample is found. Some MSP systems are also able to analyze the NIR region (~780 to 1100 nm). For UV-configured systems, analysis in the UV region (~190 to 380 nm) can provide additional information about UV absorbers that may be in or on a fiber. The spectrum of fluorescence emission (UV and visible excitation with UV to NIR emission) can also be captured.

4.3 MSP systems are generally used in fiber analyses because comparisons are typically conducted at the individual fiber level. Additionally, microspectrophotometry is a minimally destructive, highly discriminatory technique.

4.4 Fiber color is usually measured in transmittance, as light is transmitted through an individual fiber. The fraction of light transmitted or absorbed by the fiber at each wavelength is recorded relative to the amount of light transmitted through a control (blank) portion of the preparation. This transmittance spectrum can be plotted as either percent transmittance or absorbance.

5. Significance and Use

5.1 The comparison of color is one of the key steps taken in a fiber comparison, as color is one of the most important discriminating characteristics of fibers. Microspectrophotometers allow for an objective measurement of the color

(based upon selective light absorption) of small samples, which can be complementary to, and more discriminating than, microscopical color comparisons.

5.2 Microspectrophotometric spectral comparison is one part of a multi-analytical comparative approach. It is used in conjunction with techniques that identify the fiber composition, such as polarized light microscopy (PLM) and Fourier transform infrared spectroscopy (FTIR). For the identification of the dye components, other techniques such as thin layer chromatography (TLC), Raman spectroscopy, or liquid chromatography mass spectrometry (LC-MS) can be employed and are complementary to the information provided by microspectrophotometry. For more detailed information regarding PLM, FTIR, and TLC refer to E2228, E2224, and E2227 respectively.

5.3 This guide is designed to assist a FSP in the selection of appropriate sample preparation methods and instrumental parameters for the microspectrophotometric analysis and comparison of fibers. When used for comparison purposes, the goal is to determine whether any exclusionary differences exist between the samples (1-9).

5.4 Limitations to the value of MSP data and comparisons include:

5.4.1 Absorption can be impacted by environmental factors, physical damage, or sample handling. For example, a fiber or textile that has been exposed to environmental factors that irregularly alter the color (e.g., photofading) can exhibit spectral differences to a fiber or textile that has not been similarly exposed.

5.4.2 Very dark or very light fibers may display data of limited value in the visible region.

5.4.3 Certain fiber types naturally absorb in the UV region (e.g., wool, polyester), limiting data collection and interpretation.

5.4.4 MSP is not a practical technique to identify the individual dyes in a fiber.

5.5 Fiber sample spectra are measured using transmittance spectroscopy. The emission of fluorescence by fiber samples is also measurable using an MSP with microspectrofluorimetry capability (9-12).

6. Sample Preparation

6.1 The general collection, handling, and tracking of samples should meet or exceed the requirements of Guide E1459 and Practice E1492.

6.2 The work area and tools used for the preparation of samples are cleaned prior to use.

6.3 Known and questioned samples are mounted and prepared in the same manner. Disperse individual fibers from a sample on the slide in order to minimize interference and the effects of photobleaching (21).

6.4 Transmittance Measurements

6.4.1 The fiber(s) are mounted on a microscope slide under a coverslip in an appropriate medium.

6.4.1.1 A microscope slide with transmittance characteristics appropriate for the region of the spectrum being analyzed is used. Glass slides and coverslips are suitable for measurements in the visible and NIR portions of the spectrum. Typical soda-lime glass slides absorb light in the UV region, therefore mount samples to be analyzed in the UV region of the spectrum between quartz or fused silica slides and coverslips. If performing fluorescence measurements, ensure that the slide and coverslip have low or no inherent fluorescence.

6.4.1.2. A mounting medium that is compatible with the sample (i.e., will not dissolve the fiber or dye) and the spectral range being investigated is selected. Mounting media include, but are not limited to, water, xylene, xylene substitutes, glycerol, and refractive index oils (n = 1.52 or 1.66 are common). When performing fluorescence measurements, a mounting medium with low or no inherent fluorescence is selected. For example, glycerol is a suitable mounting medium when analyzing a sample in the UV, visible, and NIR regions and when performing fluorescence measurements.

7. Performance Checks

7.1 Prior to use of the instrument, the microscope, illumination sources, and spectrometer are turned on and allowed to stabilize. This is done in accordance with the instrument manufacturers' instructions or laboratory experience, whichever yields consistent results.

7.2 Checking instrument performance verifies that an instrument is operating within required standards. It is essential to demonstrate wavelength and absorbance/photometric accuracy through a performance check, such as that described in Practice E275.

7.2.1 A performance check is conducted each day of use, prior to analysis.

7.2.2 A performance check is conducted after any maintenance or power outages, prior to analysis.

7.2.3 A similar configuration is used each time a performance check is conducted on the system

7.3 Records of all performance checks are maintained. A historical record of this data provides a mechanism for evaluating system performance and allows an operator to monitor system trends and deterioration.

7.4 Performance check parameters:

7.4.1 Wavelength Accuracy – Wavelength accuracy over the measured range is checked with the aid of manufacturer-recommended filters (e.g., holmium-, erbium-, or didymium oxide). The resolution used during the wavelength accuracy checks should be the same as that used in casework and consistent for each wavelength accuracy check. Transmittance is used for these measurements.

7.4.2 Photometric Accuracy -The photometric response of the system is checked to ensure linearity using manufacturer-recommended neutral density filters. A typical set of neutral density calibration filters could include some or all of the following filters: 0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 absorbance units.

7.4.3 Filters are calibrated according to the manufacturer's recommendations.

7.5 Fluorescence Emission - Fluorescence emissions are checked with materials known to fluoresce (e.g., optically-brightened cotton, fluorescent fingerprint powder, SRM 2940) according to the manufacturer's recommendations.

8. Instrument and Scanning Parameters

8.1 MSP instruments can vary and specific details on the operation and system parameters can be found in the manufacturer's manuals and guides.

8.2 Microscope parameters

8.2.1 Illuminator – An illumination source appropriate for the analysis being conducted is selected. The illuminator needs to have sufficient intensity across the entire wavelength range of interest so as to provide a spectrum with an acceptable signal-to-noise ratio according to the manufacturer's recommendation. Tungsten, halogen, and xenon lamps are commonly used for visible and NIR analysis. Xenon lamps are frequently used for UV analysis and mercury lamps are used for fluorescence excitation. While LED illuminators are available over much of the spectrum, they are of little utility for microspectrophotometry due to their lower intensity and limited spectral range (5).

8.2.1.1 Background, system, and reference transmittance spectra can be used to monitor illuminator performance and warn of unsuitable system alignment.

8.2.1.2 Illumination Centration - Slight adjustments to the position of the bulb can serve to increase or decrease the emission over specific regions of the spectrum. For example, it is possible to maximize UV illumination, often at the expense of some light in the visible wavelengths. Generally, the slight loss of intensity in the visible region is not problematic due to the high intensity of modern bulbs.

8.2.1.3 Illumination Intensity - For some illuminators, this can be a fixed parameter. When the voltage of an illuminator is adjustable, it should be held fixed following the photometric intensity performance check (section 7.4.2).

8.2.2 Field Diaphragm - With the specimen in focus, the edges of the field diaphragm are brought into view and then sharply focused by adjusting the substage condenser. The field diaphragm is then opened so that its edges are either just outside the collection aperture or just beyond the field of view to minimize stray light. The focus and size of the field diaphragm is readjusted when the objective is changed.

8.2.3 Substage Aperture (i.e., condenser iris) – The substage aperture is opened until the desired image contrast is obtained. As adjustment of this aperture impacts the amount of light reaching the detector, this aperture setting can

be different from that used to produce an ideal image (9). In some instances, it is desirable to further increase the opening of the substage aperture to allow more light to reach the detector. The aperture is adjusted before collecting the background spectrum and kept in a fixed position between background and sample spectra collection and between samples when they are being compared. The appropriate aperture level is typically that which produces an emission intensity for the most intense emission peak of no more than approximately 80% of the detector saturation value. It is critical that the detector is not saturated anywhere over the region being measured.

8.2.4 Objective – An objective that permits visualization of the fiber to be analyzed is selected. A balance between the objective magnification and size of the measuring aperture is selected by the FSP. Typically, measurements are made using objectives between 10x and 50x. Quartz optics are required for measurements made in the UV region. Once the appropriate objective is selected, all samples being compared are measured at a fixed magnification.

8.2.5 Measuring Aperture - If the MSP system is equipped with variable or multiple collection apertures, the same aperture is used for the measurement of all samples being compared. In general, the largest aperture that will remain within the boundaries of the sample area to be measured is selected. Analysis of the edge of a sample should be avoided due to edge effects that could impact the spectrum. An oversized aperture (one that extends beyond the boundary of the sample) is undesirable in transmittance measurements as it decreases the photometric accuracy of the measurement. In fluorescence emission, the spectrum is not the result of a ratio to a reference scan; the strength of the signal is determined by absolute counts. Therefore, an oversized aperture when analyzing fibers for fluorescence emission can be used to increase the signal reaching the detector. As the background is black, there is no significant increase in noise to detract from the quality of the collected data.

8.2.6 Filter Cube - Some systems have a filter cube turret. Filter cubes can include a blank cube for transmission measurements, a mirror for reflection, and combinations of excitation and barrier/emission filters for fluorescence measurements. A filter cube appropriate to the measurement being made is chosen. All samples being compared are measured under a given configuration with the same filter cube in place.

8.2.7 Phototube Diverter - Some systems have an adjustable phototube that permits light to be diverted to the ocular, spectrometer/camera, or split between the ocular and spectrometer/camera. Spectral artifacts (e.g., interference fringes on the baseline) could be visible if the diverter is in the split position. The diverter is set so that all light is directed to the spectrometer during measurements to ensure the highest possible signal-to-noise ratio.

8.3 Spectrometer parameters

8.3.1 Detector - While the detector is not an adjustable variable post-purchase, it plays a critical role during data collection. Two main detector types are available for use with MSP systems.

8.3.1.1 Photomultiplier tube (PMT) detectors consist of a photocathode, held at a positive potential, and a series of dynodes with successively lower potential which amplify the signal and convert photons of light into electrical energy. PMTs are sensitive, provide a high signal-to-noise ratio, and have good spectral resolution. PMTs are typically single channel detectors and are generally used in scanning spectrophotometers in conjunction with a grating. The sample is scanned by stepping through wavelengths to create a spectrum point by point.

8.3.1.2 Semiconductor detectors are composed of a monochromator fitted with a diffraction grating and an array detector (e.g., charge-coupled devices [CCD]) that acts as the photosensitive device. A CCD detector generally has a lower signal-to-noise ratio when compared to a PMT detector, but measurement time is drastically reduced because of simultaneous detection of the full spectral range. For this reason, CCD detectors are far more common than PMT detectors. The CCD detector's resolution will depend on the number of pixels in the array, the dispersion and line spacing of the grating, and the distance between the grating and the array.

8.3.2 Wavelength Range – A wavelength range is selected that is appropriate to the desired range of measurement, instrument capabilities, sample, and sample preparation conditions as discussed in section 6. This range typically falls between 190 and 1100 nm (transmittance UV-Vis-NIR).

8.3.3 Resolution - The resolution is predominantly defined by the grating and slit size. For most commercial MSPs, the grating and slit size are fixed, which results in a fixed maximum resolution. For CCD detectors, pixel size and spacing also affect resolution.

8.3.4 Integration Time - Most MSP systems allow the user to define the integration time. Some software packages have a built-in functionality that automatically adjusts the integration time. When this is not available, the integration time is set such that the detector electronics are not saturated.

8.3.4.1 To manually optimize the integration time, a background spectrum is collected and the highest peak is checked to ensure that it is not saturated. If the detector is saturated (the light intensity is too high), the integration time is reduced. Conversely, if the light intensity is too low, the integration time is increased. In either instance, recollect the dark and background spectra before your sample.

8.3.5 Number of Scans - Most MSP systems allow the user to define the number of scans to average for a single spectrum. Select the number of scans that yields the acceptable signal-to-noise ratio according to the manufacturer's recommendation. Generally, the signal-to-noise ratio is proportional to the square root of the number of scans (e.g., if the number of scans is quadrupled, the signal-to-noise ratio is increased by a factor of 2).

8.4 Determine polarization in the system

8.4.1 The set-up of some MSP microscopes result in polarization of the transmitted light, therefore each system should be assessed to determine the effects of possible polarization. Use a moderately to highly dichroic fiber at different orientations (e.g., 0, 45, and 90 degrees) and compare the resultant spectra. If the orientation is found to affect spectral results, casework samples should be oriented in the same direction during analyses to minimize the effects of the possible polarization or spectra should be collected from a wide range of fiber orientations.(9, 21)

9. Sample Analysis

9.1 Prior to sample analysis, performance checks are done in accordance with Section 7.

9.2 The illumination field, measuring aperture, and magnification for the sample under investigation are optimized. Experimental conditions and instrument settings (e.g., objective, aperture size, illumination intensity, number of scans, resolution) should be identical for compared samples.

9.2.1. In order to reduce sample degradation (i.e., photo-degradation), the amount of time the sample is illuminated when it is not being actively analyzed is minimized (9, 20, 21). The potential for sample degradation can be assessed on known samples prior to analyzing questioned fibers. Photo-degradation during sample analysis is typically due to the UV wavelengths from the MSP light source.

9.2.1.1 A UV blocking filter can be placed between the condenser and the light source while spectra are not being collected.

9.2.2 Consistent sample conditions are especially critical when collecting fluorescence data because the amplitude of the resulting spectrum is directly correlated with the collection conditions (i.e., there is no reference scan to ratio against).

9.2.3 Consistent or similar sample orientations are preferred when comparing samples. This can be accomplished most easily via a rotating stage but can also be done by ensuring fibers are mounted in the same general orientation.

9.2.3.1 Dichroic fibers in different orientations can yield spectra with significant differences when using polarized light. Collecting spectra from a dichroic fiber in both the parallel and perpendicular orientations provides more data for comparison.

9.3 Collection of Reference Spectra

9.3.1 Dark Scans

9.3.1.1. A dark scan is a reference spectrum collected when the light from the microscope is blocked from the detector and is a measurement of instrument noise.

9.3.1.2 A dark scan is collected prior to the analysis of each new microscope slide or sample preparation.

9.3.1.3. A dark scan is required for transmittance, reflectance, and fluorescence emission spectra.

9.3.2. Background Scans

9.3.2.1 A background scan is a spectrum that measures the light transmitting/absorbing effect of all the system components (i.e., light source, optics, microscope slide, cover slip, and mounting medium) without the sample of interest in the light path. The background scan is also called the "reference scan."

9.3.2.2. A background scan is required for transmittance and reflectance measurements but is not relevant to fluorescence emission spectra.

9.3.2.3. For transmittance measurements, a new background scan is collected for each new microscope slide and coverslip preparation, aperture size, or instrument configuration. In general, a background scan collected before every sample scan will best compensate for the effects of adjusting the fine focus on the microscope and of heterogeneities in the mounting medium.

9.4. Collection of Sample Spectra

9.4.1 For fibers with differences in thickness due to cross-sectional shape (e.g., trilobal, triangular, flattened), sampling areas are chosen in the compared fibers with similar thicknesses and orientations (e.g., through a single lobe of a trilobal fiber or the flat area of a flattened/ribbon fiber).

9.4.2 Multiple spectra should be collected from each uniformly-colored sample, each representing different sample areas. For fibers that are not uniformly-colored (e.g., cotton and other natural fibers), a larger number of spectra is recommended. The number of replicate analyses can be adjusted in an effort to capture the variation present within the sample. Small sample size or poor sample conditions could limit the acquisition of multiple spectra.

9.4.3 Known sample(s) representative of the variation in color within the textile are selected. Differences could arise in measurements of fiber samples from the same garment or textile because of differences in inherent variation in color between fibers, weathering (e.g., sunlight exposure), spot staining/bleaching, or repaired areas (e.g., use of a fabric marker to cover a discolored area, application of a patch).

10. Spectral Comparison and Interpretation

10.1 Spectral comparisons should be conducted between spectra collected using the same sample preparation methods, similar sample characteristics (e.g., color intensity, thickness, orientation), and the same instrumental parameters.

10.2 The spectra are compared based on the observation of any spectral differences, or lack thereof, between the sets of microspectrophotometric data.

10.2.1 The sample comparison begins with the examination of the whole spectrum, followed by critical examination of each specific peak. The comparison includes examination of peak shape, minima, maxima, inflection points, troughs, shoulders, relative peak intensities, and the curves or slopes between peaks.

10.3 Spectral overlay is a method for comparing data where the presence or absence of peaks, peak shapes, and relative intensities are all considered in the evaluation as to whether exclusionary differences exist between compared samples.

10.3.1 Spectral comparisons can be conducted with the spectra displayed in percent reflectance, percent transmittance, or absorbance formats. Certain information, however, is observed more readily in one format or the other. Absorbance is better for observing differences in darkly colored fibers. Transmittance is better for observing differences in lightly colored fibers. At a minimum, spectra are presented on the same x-axis scale when providing overlays or performing comparisons.

10.3.1.1 Mean value spectra (i.e., averaged) can be generated from replicate scans of each sample. Spectra are typically averaged and then compared to the averaged spectra of another item. Mean spectra should be calculated from absorbance data.

10.3.1.2 Plots of standard deviation spectra (calculated from multiple spectra collected from a given sample) can also provide a useful point of comparison. Standard deviation curves can be useful for estimating the known sample variation range; however, comparisons based upon standard deviation spectra use intensity as a criterion for comparison. Standard deviations should be calculated from absorbance spectra.

10.3.1.3 First and second derivative functions of the spectra can also assist in identifying inflection points and aid in the discrimination of samples. Effective use of derivative functions requires that spectra have high signal-to-noise ratios. Conduct spectral derivative calculations on absorbance data.

10.4 Consider sample limitations (e.g., small samples, dirty samples, color intensity variations) and instrumental limitations (e.g., sampling size) when assessing differences between spectra.

10.4.1 Possible reasons for spectral differences include dissimilar sample characteristics, heterogeneity, contribution from extraneous materials, or origination from different source materials. Collecting spectra from additional samples can provide supplemental data to assist in assessing such differences. Slight differences in peak heights can indicate differences in dye concentrations, light exposure (fading), or dye uptake (1, 5, 9).

10.4.2 Some spectral differences are subtle and visually difficult to discern. In these instances, chemometric analysis could help assess compared samples. It is noted that consensus has not been reached in the relevant scientific literature on the most appropriate chemometrics for application to microspectrophotometric data (22, 23).

10.5 If suitable spectra are produced, comparisons can provide information regarding the potential relationship between the sources of the samples.

10.5.1 Distinguishable sources: when exclusionary differences are observed between compared spectral features, the sources of the samples are considered distinguished by microspectrophotometry. Exclusionary differences in microspectrophotometric spectral comparisons: 1) are outside the variability of spectra originating from the same source; and 2) cannot be explained by considerations such as sample heterogeneity, contamination, different sample conditions, or different sample histories.

10.5.2 Indistinguishable sources: when no exclusionary differences are observed between compared spectral features, the sources of the samples are considered indistinguishable by microspectrophotometry. Differences that are not considered exclusionary: 1) are within the variability of spectra originating from the same source; or 2) can be explained by considerations such as sample heterogeneity, contamination, different sample conditions, or different sample histories. If no exclusionary differences are observed in a microspectrophotometric spectral comparison, samples can be analyzed by other analytical techniques to provide additional information about the potential relationship between the sources of the samples.

10.6 Microspectrophotometric spectral comparison is one part of a multi-analytical comparative approach. Microspectrophotometric data alone can be used to distinguish the sources of compared samples, but they are not used independent of data obtained from other analytical techniques to reach an overall opinion regarding the potential relationship between the sources of the samples. An overall opinion that sources are indistinguishable is only reported when no exclusionary differences are observed in any of the analytical techniques that were applied.

11. Examination Documentation

11.1 The details necessary to support the interpretations made from each comparison are recorded (E620).

11.2 A description of the evidence analyzed by MSP, the method of sample preparation (including any mounting medium used), the analytical instrumentation used, mode of operation (transmission, fluorescence, etc.), and its optimized operating parameters (e.g., aperture size, objective, scan/spectrum averaging, spectral resolution, fluorescence filter cube) is included in the case notes, case record, or otherwise recorded in accordance with laboratory procedures.

11.3 Instrumental data used for interpretations are included in the case notes. Notes should be sufficient to allow an independent FSP to understand and evaluate all the work performed and independently analyze and interpret the data.

11.4 Spectra are provided either in color or in a format such that spectra from various samples plotted together can be attributed to a legend when viewed in grayscale.

11.5 If chemometric methods are applied, the data analysis method(s) and all information (e.g., software name and version, confidence intervals) necessary to review the result are recorded.

11.6 Refer to E1492, E620, ISO 17025, and WK78747 for further guidance.

12. Keywords

12.1 forensic fiber analysis; microspectrophotometry; MSP; microspectrophotometer

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