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Standard Guide for Forensic Examination of Non-Reactive Dyes in Textile Fibers by Thin-Layer Chromatography¹

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1. Scope

1.1 Metameric coloration of fibers can be detected using UV/visible spectrophotometry. If spectrophotometry is restricted to the visible spectral range only, differences in dye components may remain undetected. One method of detecting additional components is to use thin-layer chromatography (TLC). TLC is an inexpensive, simple, well-documented technique that, under certain conditions, can be used to complement the use of visible spectroscopy in comparisons of fiber colorants. The principle of the method is that the dye components are separated by their differential migration caused by a mobile phase flowing through a porous, adsorptive medium.

2. Referenced Documents

2.1 ASTM Standards:

- E 1188 Practice for Collection and Preservation of Information and Physical Items by a Physical Investigator²
- E 1459 Guide for Physical Evidence Handling and Related Documentation²
- E 1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Laboratory²

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *activation*—the heating of the adsorbent layer on a plate to dry out the moisture and maximize its adsorptive power.

3.1.2 *adsorbent*—the stationary phase for adsorption TLC.

3.1.3 *adsorption*—the attraction between the surface atoms of a solid and an external molecule by intermolecular forces.

3.1.4 *chamber*—a glass chamber in which TLC development is carried out.

3.1.5 *chromatography*—a method of analysis in which substances are separated by their differential migration in a mobile phase flowing through or past a stationary phase.

3.1.6 *development*—the movement of the mobile phase through the adsorbent layer to form a chromatogram.

3.1.7 *dye extraction*—the removal of the dye from a fiber by incubating it in an appropriate solvent.

3.1.8 *eluent*—the solvent mixture that acts as the mobile phase in TLC.

3.1.9 *metameric pair*—two colors that appear the same under one illumination, but different under other illumination.

3.1.10 *mobile phase*—the moving liquid phase used for development.

3.1.11 *normal-phase chromatogram*—adsorption in which the stationary phase is polar in relation to the mobile phase.

3.1.12 *origin*—the location of the applied sample or the starting point for the chromatographic development of the applied sample.

3.1.13 *resolution*—the ability to visually separate two spots.

3.1.14 *retardation factor (RF)*—the ratio of the distance traveled by the solute spot's center divided by the distance traveled by the solvent front, both measured from the origin.

3.1.15 *saturation chamber*—equilibration with mobile phase solvent vapor prior to chromatography.

3.1.16 *solute*—in TLC, a mixture of components to be separated.

3.1.17 *solvent front*—the final point reached by the mobile phase as it flows up or across the TLC plate during development of the chromatogram.

3.1.18 *spot*—a round zone of sample application at the origin, or in a chromatogram, a round zone caused by migration of a separated component of the solute. The sharpness of the spot relates to the efficiency of the chromatographic band.

3.1.19 *spotting*—applying a solute sample at the origin of the TLC plate.

3.1.20 *stationary phase*—the solid adsorbent coating layer of a TLC plate.

3.1.21 *tailing*—a spot distorted during development into an elongated streak.

3.1.22 *thin-layer chromatogram*—the series of spots visible on the adsorbent layer after development.

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² *Annual Book of ASTM Standards*, Vol 14.02.

3.1.23 *thin-layer chromatography (TLC)*—a separation technique in which the flow of solvent causes the components of a mixture to migrate differentially from a narrow initial zone over a planar, thinly-applied porous adsorptive medium.

4. Summary of Guide

4.1 This guide is intended to advise and to assist individuals and laboratories that conduct forensic fiber examinations and comparisons in their effective application of TLC to the analysis of fiber evidence.

4.2 The guide is concerned with the extraction of dyes from single fibers and from bulk material, classification of the dye or colorant, application and development of the extractants on TLC plates using an optimal elution system, and evaluation and interpretation of the resulting chromatograms. The protocols and equipment mentioned in this document are not meant to be totally inclusive or exclusive.

4.3 Not all fiber type/dye class combinations are covered in this guide.

5. Significance and Use

5.1 Forensic analysis of fiber colorants using TLC should be considered for single fiber comparisons only when it is not possible to discriminate between the fibers of interest using other techniques, such as comparison microscopy (brightfield and fluorescence) and microspectrophotometry in the visible range.

5.2 The extraction procedures carried out prior to TLC analysis can provide useful information about dye classification. TLC can provide useful qualitative information about dye components. Similar colors made up of different dye components can be differentiated using this technique. The application of TLC may serve to discriminate between fibers, or it may confirm their similarity.

5.3 TLC may be prohibitively difficult or undesirable in some circumstances. Short lengths of fibers or pale colored fibers may not have an adequate concentration of colorant present to be examined, dye extraction from some fibers may be impossible. The desire to preserve evidence for possible analysis by another examiner may preclude removing the color for analysis.

5.4 Dye from the known material should first be characterized and eluent systems evaluated to achieve optimum separation of the extract. Dye is then extracted from single known and questioned fibers, using an equivalent amount of material.

5.5 The development of each individual TLC plate will show some variability as a result of the coating and conditioning of the plate, solvent condition, and temperature. It is important to evaluate the performance of each TLC plate by spotting known materials along with the questioned samples. See Ref (16).

5.6 Examples for the preparation of Standard dye mixtures are given in Appendix X1.

6. Sample Handling

6.1 The general handling and tracking of the samples should meet or exceed the requirements of Practice E 1492 and Guide E 1459.

6.2 Pre-treatment (mounting medium, washing solvent, etc.) and sample preparation must be identical for all known and questioned fibers being compared on one TLC plate. For removing single fibers from slide preparations the following procedure is recommended.

6.2.1 Any traces of marker pen ink should be cleaned from the coverslip using an appropriate solvent, for example, acetone.

6.2.2 The coverslip should be cracked all around the fiber and an appropriate solvent, that will dissolve the mountant, but not affect the fiber or the colorant, should be used.

6.2.3 The fiber is removed and extracted in an appropriate solvent. Appropriate solvent selection will depend on the mountant and the sample.

7. Analysis

7.1 The ease of dye extraction and the particular extractant required will depend on the generic class of the fiber and the type of dye present. The generic class of the known and questioned fibers must be determined prior to TLC analysis.

7.2 Dye classes are classified into broad groups based on their chemical properties or method of application. The determination of the dye class of the known fibers can be helpful in establishing the best extractant, as well as to assist in the subsequent selection of the most efficient eluent system.

7.3 Documented extraction schemes (see Appendix X2) can be used to determine the dye class of fibers of known generic classes, and thus the optimum extractant. Dye classification is done on single fibers or tufts of fiber removed from the known item. A new fiber or tuft can be used for each classification stage.

7.4 *Dye Extraction*—Known and questioned fibers must be extracted at the same time under the same conditions. Single fibers can be extracted in a short length (about 25 mm) of fine capillary tube (internal diameter of about 1.5 mm), sealed at one end. A fine wire can be useful in pushing the fiber down the tube. The tube must be appropriately labeled.

7.4.1 About 10 µl of the appropriate extractant (as recommended in Appendix X3) should be introduced into the tube to cover the fiber sample. A fine glass pipette or syringe can be used for this procedure. The tube should be heat sealed to avoid evaporation and incubated for a constant time and temperature (as recommended in Appendix X2), preferably in an oven. Periodic checks for dye extraction should be made every 15 min for up to 1 h.

7.5 *Dye Extraction: Bulk Material*—Larger fiber tufts can be extracted in a Durham tube or other suitable small stoppered glass tube, using about 100 µl of solvent in a sand bath or oven heated to 100°C. Periodic checks should be made every 15 min for up to 1 h.

7.6 *Nonextractable Dyes*—If classification indicates that a nonextractable dye or pigment other than a reactive dye is present, then place one known and one questioned fiber in labeled capillary tubes. Add approximately 10 µl pyridine/water (4:3) and attempt to extract at about 100°C for one hour. If neither fiber extracts, a positive association is noted. If the questioned fiber extracts and the known fiber does not, the