



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

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1. Scope

1.1 This guide is intended as an overview of the Thin-Layer Chromatography (TLC) of fiber colorants (or individual dye components) present in dyed fibers. It is intended to be applied within the scope of a broader analytical scheme for the forensic analysis of fiber samples. TLC could provide information that cannot be obtained through other color analyses (such as microspectrophotometry (MSP)) (1).²

1.2 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 (see Practice E3255).

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 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.*

1.5 *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.*

¹ This guide is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.

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² The boldface numbers in parentheses refer to a list of references at the end of this standard.

2. Referenced Documents

2.1 ASTM Standards:³

E620 Practice for Reporting Opinions of Scientific or Technical Experts

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

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

2.2 Other Standards:

ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories⁴

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology E1732.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *adsorbent, n*—the stationary phase for adsorption TLC.

3.2.2 *band, n*—one or more colored areas (circular to elongated shape) on a TLC plate produced by the separation of

³ 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.

⁴ Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, https://www.iso.org.

the dye components for a particular combination of solvent and stationary phase. Bands are created as the solvent (mobile phase) moves past and reacts with the solute, migrating from the origin.

3.2.2.1 *Discussion*—“Spot” may also be used to describe this area.

3.2.3 *chamber, n*—a glass enclosure in which TLC development is carried out.

3.2.4 *chromatogram, thin layer, n*—the series of bands visible on the adsorbent layer after development.

3.2.5 *chromatography, thin layer (TLC), n*—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.

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

3.2.7 *dye extraction, n*—the removal of the dye from a fiber by incubating the fiber(s) in an appropriate solvent.

3.2.8 *eluent, n*—the solvent mixture that acts as the mobile phase in TLC.

3.2.9 *exclusionary difference, n*—a difference in a feature or property between compared items that is substantial enough to determine that they did not originate from the same source.

3.2.10 *mobile phase, n*—the moving liquid phase used for development.

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

3.2.12 *origin, n*—the location of the applied sample or the starting point for the chromatographic development of the applied sample.

3.2.13 *resolution, n*—the ability to visually separate two bands.

3.2.14 *retardation factor (R_f), n*—the ratio of the distance traveled by the solute band’s center divided by the distance traveled by the solvent front, both measured from the origin.

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

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

3.2.17 *spot, n*—a visible concentration of sample applied to the TLC plate; also known as the origin.

3.2.18 *stationary phase, n*—the solid adsorbent coating layer of a TLC plate.

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 the effective application of TLC.

4.2 This 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 TLC is an inexpensive and simple technique that could be used to complement other analytical techniques within a general analytical scheme related to forensic fiber examination.

5.2 Consider the forensic analysis of fiber colorants using TLC for single fiber comparisons only when the sample size is adequate (that is, enough colorant can be extracted for analysis) and it is not possible to discriminate between the fibers of interest using other techniques, such as comparison microscopy and MSP. Larger fibrous units (for example, thread or tuft) can be treated as an individual sample if determined to be homogeneous. Do not treat fibers that cannot be directly related to each other as a collective sample for the purposes of TLC.

5.3 The extraction procedures carried out prior to TLC analysis can provide useful information about dye classification. TLC can provide 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 support the possibility of fibers sharing a common source.

5.4 TLC can be prohibitively difficult or undesirable in some circumstances. Short lengths of fibers or pale-colored fibers can lack adequate amounts of colorant necessary to be examined by TLC. Dye extraction from some fibers can be impossible (2, 3). Some fiber types do not truly extract, but change or lose color. Reactive dyes are covalently bonded to the fiber and typically cannot be removed by conventional extraction methods, but can be released from cotton and wool by disrupting the fiber by enzymatic or chemical digestion, respectively (1). The desire to preserve evidence from deleterious change or for possible analysis by another examiner can preclude removing the color or employing a destructive method for analysis.

6. Sample Handling

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

6.2 Generally, only a single fiber is necessary for extraction (except for cotton and viscose classification).

6.2.1 For very pale fibers, a small tuft is necessary.

6.3 Before working with the unknown, the 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 both known and questioned fibers, using an equivalent amount of material, including similar length and depth of dyeing.

6.3.1 If sufficient known sample exists, different fibers may be used for each part of the classification. However, best practice is to use one known fiber cut into smaller pieces to carry out classification.

6.3.1.1 A “blank” can be helpful as a means of easy comparison to see if extraction has occurred. A fiber/piece of fiber is placed in a glass tube with water and heated in the same way as the test piece/fiber.

6.3.2 If the questioned fiber is short or pale in color, there can be insufficient dye for TLC. In such instances, an amount of known sample (equivalent to the amount of questioned sample that is able to be tested) is extracted to determine (a) the efficacy of the extraction solvent and (b) the minimum length or amount of fiber needed to obtain a useful quantity of extracted colorant. Utilize the smallest possible volume of extraction solvent (4).

6.4 Ensure that all pre-treatments (mounting medium, washing solvent, etc.) and sample preparation techniques are identical for all known and questioned fibers being compared on one TLC plate. The following procedure is recommended for removing single fibers from slide preparations.

6.4.1 Clean any traces of marker pen ink from the coverslip using a solvent (for example, acetone).

6.4.2 Remove or crack the coverslip all around the fiber. Apply a solvent that will dissolve or rinse away the mountant, but not affect the fiber or the colorant. Remove the fiber from the mount.

6.4.2.1 *Discussion*—It is recommended that the suitability of the solvent be evaluated with the mounted known fiber before it is applied to the mounted questioned fiber.

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 is determined prior to TLC analysis. See Guides E2224 and E2228.

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 Standard dyes can be used as a control to check eluent performance. Examples for the preparation of standard dye mixtures are given in Appendix X1 and (5).

7.4 Documented extraction schemes (see Appendix X2), based on generic fiber types, can be used to determine the class of dyes present in the fiber. Dye classification is performed on a sample removed from the known item. A new sample can be used for each classification stage.

7.5 *Dye Extraction*—Known and questioned fibers are extracted under the same conditions (time and temperature) using an appropriate solvent ((4), Appendix X2). Fibers can be extracted in a sealable vessel of appropriate size relative to the sample (for example, a short length of a fine capillary tube or a conical vial).

7.5.1 The extractant is introduced ((4), Appendix X2) into the tube in a sufficient volume to immerse the sample. A glass pipette or syringe can be used for this procedure. The vessel is sealed to avoid evaporation and then incubated at a constant

temperature, preferably in an oven. Periodic checks for dye extraction should be made every 15 minutes for up to 1 hour.

7.5.2 Fiber disruption can be used for wool and cotton fibers that are reactively dyed. The colored solutions released are not true dye extracts but are nonetheless amenable to separation and analysis by TLC ((1), pp. 239-240).

7.6 *Non-extractable Dyes*—An extraction with pyridine/water (4:3) at 100 °C for one hour using additional sample should be attempted if the classification indicates that a non-extractable dye other than a reactive dye is present. If both questioned and known fibers “bleed” dye into solution, there may be sufficient dye for analysis. Dyes that typically do not extract are ingrain, sulfur, and vat dyes.

7.7 *Elution*—Silica gel plates, with nominal particle size of 60 microns and incorporating a fluorophore excited at 254 nm (for example, silica gel 60 F₂₅₄, measuring 5 cm by 7.5 cm), are recommended for normal-phase TLC of fiber dyes (6). Plates should be stored in a desiccator; if this is not possible, they should be heat-activated before use.

7.7.1 Both known dyes and questioned dyes to be compared are applied to the same plate. The extracts should be spotted onto the plate about 1 cm from the lower edge. This can be done using a double-drawn capillary tube or other suitable device. Spots should not be too near to the edge of the plate or to each other. Take care to avoid scratching the adsorbent coating layer during spot application.

7.7.1.1 Questioned and known samples are applied to the same plate because the development of each individual TLC plate can show some variability as a result of the coating and conditioning of the plate, solvent condition, and temperature (6).

7.7.2 Include a solvent blank spot (extractant alone) on the same plate as the questioned and known sample(s). A standard dye spot, if used, would also be included on the same plate.

7.7.3 Spots should be dried (for example, using a hair dryer or hot plate) with repeat spot applications made until the spot is strongly colored. The spot size should be uniform and not exceed about 2 mm in size.

7.7.4 Notes are made of the sample order on the plate itself in pencil in a position that will not interfere with the sample spots (for example, at the top of the plate or well below the origin). Plates shall be thoroughly dried before developing.

7.8 *Development Chambers*—Chromatograms are developed vertically, typically in a glass chamber.

7.8.1 The eluent is added to the tank and allowed to stand in the closed container before development so that the chamber has enough time to become saturated with the eluent vapor (about 30 min). Filter paper can be added to the perimeter of the container to assist in equilibration of the eluent and its vapor.

7.8.2 The level of the eluent in a vertical tank should be at least 0.5 cm below the origin/application spots on the TLC plate.

7.9 Eluent:

7.9.1 Consider the following five parameters when selecting the optimum eluent:

7.9.1.1 Separation of component dyes,

7.9.1.2 Sharpness of bands,

7.9.1.3 Movement of the eluted bands from the origin,

7.9.1.4 Components traveling at or close to solvent front, and

7.9.1.5 Strength of dye extract from questioned fibers.

7.9.2 Two or more eluent systems should be assessed with the known fibers to determine the optimum eluent system. If none of the systems described within this document result in adequate separation, other eluents can be evaluated.

7.9.3 There are numerous published TLC eluent systems that can be applied to the development of particular fiber/dye class combinations (see [Appendix X3](#)).

7.10 The extract from the known material is applied to the TLC plate and developed in the trial eluents as previously described. The plate is eluted until good resolution is achieved (normally 2 cm from the origin), but not so long as to allow the bands to become diffuse, making visualization difficult. The plate is removed from the eluent and the position of the solvent front is marked in pencil. The plate can be evaluated before or after being dried. If the eluents produce poor separation, other eluents appropriate to the dye class should be evaluated.

7.10.1 Different eluent systems or stationary phases can provide additional discriminating power. If the spots do not move from the origin, choose a more polar eluent system. If the bands move with the solvent front, choose a less polar eluent system.

7.10.2 After a suitable eluent system has been found, the comparison of known and questioned fibers can be carried out.

7.11 After drying, the plates are immediately examined in visible and ultraviolet light and band position(s) and color(s) are documented. R_f values can also be determined and recorded.

7.12 The colors, fluorescence, sequence, and position of the bands obtained from the dye of the questioned fibers are compared to those from the corresponding known fibers analyzed.

7.13 The plates are documented by color imaging (for example, photography or scanning) or retained and stored out of direct sunlight in a manner designed to minimize fading, or both.

8. Interpretation

8.1 TLC comparisons are conducted between chromatograms on the same plate.

8.1.1 Compare the chromatograms and make interpretations based on the observation of any differences, or lack thereof, between the sets of TLC data.

8.1.1.1 Visual comparison is an approach for comparing chromatograms where the presence or absence of bands, band shapes, band colors, R_f values, and relative intensities are all considered in the evaluation as to whether exclusionary differences exist between compared samples.

8.1.1.2 When assessing differences between chromatograms, consider sample limitations (for example, small samples, poor dye extraction, pale fibers, dirty samples) and method limitations (for example, uneven silica coating, disruption of chamber, limits of detection). Subtle differences

between known and questioned samples should be carefully considered. Additional sampling of areas of the known textile may be necessary (for example, dye batch variation between back and sleeves of a shirt).

8.1.2 Possible reasons for chromatographic differences include dissimilar sample characteristics (for example, amount of dye, color, dye components, dye class), heterogeneity, contribution from extraneous materials, or origination from different source materials. Additional samples can provide supplemental data to assist in assessing such differences.

8.1.2.1 In regards to UV examination of plates, “extra” bands may result not from dye components, but from fiber finishing agents or contaminants (such as grease or oil). Therefore, use caution when interpreting the significance of a band seen in one sample but not the other.

8.1.3 If suitable chromatographic separation is obtained, comparisons of chromatograms can provide information regarding the potential relationship between the sources of the samples.

8.1.3.1 When exclusionary differences are observed between compared chromatograms, the sources of the samples are considered distinguishable by TLC. Exclusionary differences in TLC chromatographic comparisons occur when differences in the presence and appearance of bands (number, color, size, reaction to UV light): (1) are outside the variability of chromatograms originating from the same source; and (2) cannot be explained by considerations such as sample heterogeneity, contamination, different sample conditions, or different sample histories.

8.1.3.2 When no exclusionary differences are observed between compared chromatographic features, the sources of the samples are considered indistinguishable by TLC. Differences that are not considered exclusionary: (1) are within the variability of chromatograms 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 TLC comparison, samples can be analyzed by other analytical techniques to provide additional information about the potential relationship between the sources of the samples.

8.2 If reporting dye classifications, use language that does not imply that a fiber has been dyed with a particular dye class to the exclusion of all others.

8.3 TLC comparison is one part of a multi-analytical comparative approach. TLC data alone can be used to distinguish the sources of compared samples, but is 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 all of the analytical techniques that were applied.

9. Documentation

9.1 Record the details necessary to support the interpretations made from each comparison (Practice [E620](#)).

9.2 Mark plates for identification (such as case number, sample source, examiner, and date). Case documentation on TLC shall include the source of the samples, method of dye classification, details of extractants/eluent systems tested or used, and the results.

9.3 Case notes should be sufficient to allow an independent analyst to understand and evaluate all the work performed, independently analyze and interpret the data, and draw conclusions.

9.4 Refer to Practices **E1492**, **E620**, and ISO/IEC 17025 for further guidance.

10. Keywords

10.1 fibers; forensic science; thin-layer chromatography

APPENDIXES

(Nonmandatory Information)

X1. SUGGESTED STANDARD DYE MIXTURES

X1.1 The list below suggests some suitable mixtures but is not totally inclusive or exclusive. Any (simple) mixture of dyes separating in these eluents can be used. As many dyes are light-sensitive, the solutions should be stored in sealed amber glass containers.

X1.2 *Recommendations for Preparation of Standard Dye Mixtures*—Approximately 5 mg of each dye component is made up to a final volume of 25 mL with pyridine/water (4:3 v/v). Use until the supply is exhausted.

X1.2.1 *Standard Dye Solution A for Eluents 1, 2, 3, 4, 9, 10, 12:*

X1.2.1.1 Solway green G (CI Acid Green 25), Solway blue RNS (CI Acid Blue 47) and Naphthalene fast orange 2GS (CI Acid Orange 10).

X1.2.2 *Standard Dye Solution B for Eluents 5, 7, 8:*

X1.2.2.1 Supracet fast orange G (CI Disperse Orange 3), Supracet fast violet B (CI Disperse Violet 8) and Supracet scarlet 2G (CI Disperse Orange 1).

X1.2.3 *Standard Dye Solution C for Eluent 6:*

X1.2.3.1 Supracet fast orange G (CI Disperse Orange 3) and Supracet fast violet B (CI Disperse Violet 8).

X1.2.4 *Standard Dye Solution D for Eluent 11:*

X1.2.4.1 Solway green G (CI Acid Green 25), Supracet fast orange G (CI Disperse Orange 3) and Supracet fast violet B (CI Disperse Violet 8).

X1.2.5 *Standard Dye Solution E for Eluents 1, 2, 3, 4, 13, 14, 15:*

X1.2.5.1 Solway green G (CI Acid Green 25), Solway blue RNS (CI Acid Blue 47), and Naphthalene fast orange 2GS (CI Acid Orange 10).

X1.2.5.2 This is recommended as the standard dye mixture for reactive dyes.

X1.3 *Testing of Eluents and Extraction Solutions*—Checks are carried out just prior to use on eluent performance and the extractants are tested to ensure that they have not been contaminated. The standard dye and extraction solution are spotted side-by-side onto a TLC plate approximately 1 cm from the base and then dried to remove any water traces. Drying can be achieved by resting the plate on a hotplate at about 70 °C during spotting, using a heat source such as a blow-dryer, or loading into a 110 °C oven. The plate is eluted as described in Section 7. The chromatogram is compared with historical data to ensure that the separation is acceptable and that there are no visible bands obtained from the extraction solution. If unacceptable, fresh eluent/extractant is made and tested.

X2. EXTRACTION SCHEMES AND CLASSIFICATION OF DYES

NOTE X2.1—Reactive, Sulfur, Vat, Diazo, Ingrain, and pigmented dyes do not extract.

NOTE X2.2—All solvent ratios are volume:volume.

TABLE X2.1 Classification of Dyes from Wool Fibers (7)

Stage 1	Pyridine/water (4:3), 100 °C, 10 min Good extraction = ACID DYE Little or no extraction—Go to Stage 2
Stage 2	2 % aqueous oxalic acid, 100 °C, 20 min then pyridine/water (4:3), 100 °C, 10 min Improved extraction = METALLIZED DYE Little or no extraction = REACTIVE DYE

TABLE X2.2 Classification of Dyes from Cotton and Rayon Fibers (8)

Stage 1	Glacial acetic acid, 100 °C, 20 min Good extraction = AZOIC DYE Little or no extraction—Go to Stage 2
Stage 2	Pyridine/water (4:3), 100 °C, 20 min Good extraction = DIRECT DYE Little or no extraction—Go to Stage 3
Stage 3	Dithionite/polyvinylpyrrolidone solution ^A , 100 °C, 20 min Apply extract to TLC plate; observe color of fiber and dried spot. Spot colorless / not original fiber color Fiber color changes = REACTIVE DYE Fiber color unchanged = INGRAIN DYE Spot original fiber color Fiber color changed—Go to Stage 4
Stage 4	With a new fiber, 10 % to 14 % Sodium hypochlorite, 100 °C, 10 min Fiber color changed = SULFUR DYE Fiber color unchanged = VAT DYE

^A 80 mg sodium dithionite, 30 mg polyvinylpyrrolidone, 450 µL 10 % sodium hydroxide, 9 mL water, use immediately and discard any excess.

TABLE X2.3 Classification of Dyes from Acrylic/Modacrylic Fibers (9)

Stage 1	Formic acid/water (1:1), 100° C, 20 min Good extraction—Go to Stage 2
Stage 2	TLC procedure—methyl acetate eluent Movement = DISPERSE DYE No movement—Go to Stage 3
Stage 3	TLC procedure—methanol eluent Sharp line at solvent front = ACID DYE Little or no movement or smearing = BASIC DYE

TABLE X2.4 Classification of Dyes from Nylon Fibers (10)

Stage 1	Chlorobenzene, 150 °C, 15 min Good extraction = DISPERSE DYE Little or no extraction—Go to Stage 2
Stage 2	Pyridine/water (4:3), 100 °C, 20 min Good extraction—Go to Stage 3 Little or no extraction = REACTIVE OR DIAZO DYE
Stage 3	TLC procedure—methanol eluent Sharp line at solvent front = ACID DYE Little or no movement or smearing = BASIC DYE

TABLE X2.5 Classification of Dyes from Polyester Fibers (10)

Stage 1	Chlorobenzene, 130 °C, 10 min Good extraction = DISPERSE DYE Little or no extraction—Go to Stage 2
Stage 2	Dimethyl formamide/formic acid (1:1), 100 °C, 20 min Good extraction = BASIC DYE

TABLE X2.6 Classification of Dyes from Polypropylene Fibers (11)

Stage 1	Methyl acetate/water/acetic acid (5:5:1), 100 °C, 20 min Good extraction = DISPERSE DYE Little or no extraction—Go to Stage 2
Stage 2	Pyridine/water (4:3), 100 °C, 20 min Some extraction—Go to Stage 3 No extraction = PIGMENT
Stage 3	2 % aqueous oxalic acid, 100 °C, 20 min then pyridine/water (4:3), 100 °C, 20 min Improved extraction = METALLIZED DYE No improvement = ACID DYE

TABLE X2.7 Classification of Dyes from Acetate / Triacetate Fibers (12)

Stage 1	Acetate - pyridine/water (4:3), room temp., 15 min Triacetate - pyridine/water (4:3), 100 °C, 20 min Little or no extraction = DIAZO DYE ^A Good extraction = DISPERSE DYE
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^A Diazo dyes are typically only used for black and navy blue coloration. Little/no extraction may also be indicative of a rarely used dye class.

[ASTM E2227-23e1](https://standards.iteh.ai/catalog/standards/sist/f91f7a03-4b5e-45df-bf70-27713789993e/astm-e2227-23e1)

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