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An American National Standard

Standard Guide for Forensic Examination of Dyes in Textile Fibers by Thin-Layer Chromatography[1]

This standard is issued under the fixed designation E2227; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Editorially updated 6.2.1 in June 2023.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E620 Practice for Reporting Opinions of Scientific or Technical Experts

E1459 Practice 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 Laboratories[4]

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

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

[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.