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# Forensic Characterization Of Dyes From Synthetic Textile Fibers Exposed To Outdoor And Laundering Effects By Ultra Performance Liquid Chromatography And Spectral Analysis

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FORENSIC CHARACTERIZATION OF DYES FROM SYNTHETIC TEXTILE FIBERS  
EXPOSED TO OUTDOOR AND LAUNDERING EFFECTS BY ULTRA-  
PERFORMANCE LIQUID CHROMATOGRAPHY AND SPECTRAL ANALYSIS

by

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Bachelor of Science  
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Submitted in Partial Fulfillment of the Requirements

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## DEDICATION

In memory of Pap, Uncle Joe, and my cousin Scott.

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## ABSTRACT

Textile fibers found in an investigation are trace evidence that can connect a suspect to a victim or crime scene. Examination involves comparison of the color and morphology of a questioned fiber to a known fiber with optical spectroscopy. Fibers are considered class evidence, so evaluating more characteristics increases their significance as evidence if a match cannot be excluded. Acrylic, nylon, and polyester are textile polymers that require different extraction solvents based on the polymer chemistry. Methods have been developed for UPLC analysis of basic dyes on acrylic, acid on nylon, and disperse dyes on polyester. After microextraction from single fibers, a two minute run enables separation and identification of dyes by UV/visible detection with retention time matching and spectral comparison.

However, fibers are rarely found in pristine condition. Over the normal course of the lifetime of a garment, the pattern of dye weathering or photodegradation may even individualize an item of evidence. On the other hand, fibers from a clothed body left in extreme desert conditions might lose dye to photodegradation, lowering their viability as trace evidence. We demonstrate trace analysis of dyestuff residues from single 10 mm fibers of acrylic, nylon, and

polyester samples after exposure to varying humidity and temperature at ASTM testing sites in Phoenix, AZ, and Miami, FL. Despite the loss of dye amounts with increasing environmental exposure, all dyes were detected even in the most weathered fabrics subjected to a year of outdoor exposure. To evaluate the changes in fabrics and dyes after laundering conditions, three brands of detergent (Tide®, Gain®, and Wisk®) were used alone, with bleach, or with Clorox 2® (stain remover and color booster) to wash polyester, acrylic and nylon up to 50 times. Separation and spectral characterization are used to compare spectral differences of dyes extracted from laundered nylon, which are valuable in understanding the forensic relevance of trace fiber evidence.

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## CHAPTER ONE

### FORENSIC EXAMINATION OF TRACE EVIDENCE FIBERS: A REVIEW

Textile fibers are a form of trace evidence that can be used in a forensic investigation to establish associations between individuals involved in a crime as well as the location in which the crime was committed. Fibers are exchanged when an assailant comes into contact with the victim or crime scene because, as stated in Locard's exchange principle, every contact leaves a trace.

"Wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as silent witness against him. Not even his fingerprints or his footprints, but his hair, the fibers from his clothes... — all of these bear mute witness against him. This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are. It is factual evidence. Physical evidence cannot be wrong; it cannot perjure itself; it cannot be wholly absent—only its interpretation can err. Only human failure to find it, study and understand it can diminish its value."

-Dr. Edmund Locard,<sup>1</sup> 1947

Comparisons of the questioned fiber to the known fiber can exclude the possibility that they came from the same source, or reciprocally, suggest an association between the victim and suspect. Fiber examinations involve searching for significant differences between questioned fibers from a crime scene and known fibers originating from a verifiable source with objective of rejecting the null hypothesis that two fibers *could* have come from a single source.

A series of analytical tests are performed with the goal of finding a match exclusion, in which it is confirmed that the two fibers did not come from the same source. In the fiber examination process, the methods are sequenced so that the most exclusionary information is obtained first.<sup>2</sup> Visual comparison is a necessary first step, as differences in color can quickly rule out a match. Optical microscopy (to determine color and morphology), polarized light microscopy (to measure refraction and birefringence indices for generic class), UV/visible microspectrophotometry (to measure the color spectrum of the fiber and dyes), and infrared spectroscopy (to identify polymer type) are essential tools in the discrimination of questioned and known fibers (references). These techniques are rapid and non-destructive, allowing the preservation of evidence, but do not identify dyes.

However, two textile fibers can be visually indistinguishable, regardless of differences in dyes mixtures that the manufacturers have formulated to achieve



the common color. UV/visible spectra of the two dyes might have similar absorptions, with subtle differences in the shapes of the peaks and valleys. The differences in the spectra can be difficult to distinguish even by a trained eye, rendering the judgment of their practical significance to be subjective. This may lead to a standard positive conclusion, in that the data from both the questioned and known fiber are consistent with one another, indicating the possibility of a common material. There is a caveat—it is also possible that the questioned fiber is from an entirely different source, yet physically and optically identical. Fibers are produced in mass quantities, and are therefore a form of class evidence.

Because morphology and color are not unique characteristics, more discriminating factors are necessary to increase the significance of fiber evidence.

The ability to compare individual dyes on a molecular level can provide higher probative value to the results, as it provides information that cannot be measured by spectroscopy alone. Our work has focused on dyes extracted from synthetically manufactured fibers most abundantly found in casework, including acrylic, nylon, and polyester fibers.<sup>3,4</sup> Textiles are colored with specific dye classes according to the polymer type and subsequent dyeing process necessary for the dyes to adhere to the fibers. Dyes are conjugated structures that are often composed of ring systems and unsaturated components that absorb specific wavelengths in response to an excitation source. They are typically classified and

named according to the method by which they are applied and their chemical constitution. Interaction of dyes with a particular fiber is dependent on the chemistry of both the dye class and polymer type, including formation of salt linkages (basic dyes on acrylic, acid dyes on nylon), or dispersion through the fiber (disperse dyes on polyester). Acrylic fiber is a polymer (Figure 1.1) that is formed through free radical polymerization of the acrylonitrile monomer that has been dissolved in a solvent, and is either precipitated in a liquid (wet spinning) or collected after the solvent is evaporated (dry spinning).<sup>5</sup> Dyeability of the pure substance is low, so copolymers are added, not to exceed 15%.<sup>6</sup> In acrylic textile dyeing, cationic sites on basic dye molecules form salt linkages with the negatively anionic copolymer. Polyester fiber (Figure 1.2) is formed from condensation of terephthalic acid and ethylene, and is cooled and solidified by melt spinning.<sup>7</sup> It is hydrophobic, making it inherently resistant to stains while receptive to fire-, soil-, and fire-resistant finishing agents. Polyester fibers are dyed with disperse dyes, which are emulsified in water using surfactants; the dye has a higher substantivity for the nonpolar fiber and adsorbs to it using hydrogen bonds and van der Waals forces. Nylon 6, also prepared by melt spinning, is a polymer condensate (Figure 1.3) of  $\omega$ -aminocarboxylic acid or ring-opening polymerization of lactam. Its elasticity and dyeability are desirable in both industrial and clothing design.<sup>6</sup>

In addition to the multitude of dyes of similar colors, many fibers are often dyed with more than one dye to produce a desired shade. Unusual dye combinations can provide distinctive qualities when compared to fibers colored with seemingly similar shades. The complexity of dye variations establishes a 'fingerprint' of sorts, as an increasing number of components amplifies discriminating characteristics. Textiles used in garments and carpet can be dyed before or after the fibers are spun into yarn, or after it has been woven into fabric.<sup>8</sup> Finishing agents and processes are often applied improve longevity and quality. Based on the subtle differences surrounding its dyeing process, a fiber can be traced back to the product manufacturer, or even the textile or dye facility from which the raw materials originated. Statistical evaluation can be used to determine the probability of finding two identical fibers of separate origin in a criminal investigation.

Following infrared spectroscopy or polarized light microscopy, the polymer type is established, and the dye class can be surmised based on the polymer. Once optical analysis is completed, and the fibers cannot be differentiated, constituent components of the dyes should be analyzed individually. Dyes must be isolated from the fiber, followed by then separation for detection and interpretation. Microextraction enables analysis of the dyes at a molecular level.<sup>9</sup> Because this procedure is destructive, it is used only when other

analyses are inconclusive. Stefan, *et al.* and Dockery, *et al.*<sup>10-13</sup> optimized the extraction of basic dyes on acrylic, acid dyes on nylon and disperse on polyester using experimental design. A literature survey of proposed extraction protocols<sup>14-25</sup> for each dye class were prepared in varying proportions and used to extract respective 10-cm fibers. Absorbances were measured using a plate reader, and modeled to determine the solvent combinations for each fiber type that mostly completely extracted the dyes.

Capillary electrophoresis and liquid chromatography are established separation techniques in many forensic applications, including drug and alcohol testing, DNA analysis, and post-mortem toxicology. CE is excellent for separating ionized dyes with suitable pKa and buffer solution pH, but is unable to facilitate migration of non-ionizable dye classes.<sup>22,23,26,27</sup> Thin layer chromatography is the accepted method of separation of dye components by the Federal Bureau of Investigation,<sup>2</sup> as it has been demonstrated to be complimentary to visible spectroscopy in color comparison.<sup>9,16,19,20,25,28</sup> This method may be impractical on limited sample sizes and pale fibers, requiring higher detection capabilities for forensically relevant samples.<sup>29,30</sup>

Mass-produced textiles that are indiscernible by MSP display inter-batch variation when examined using TLC.<sup>31-33</sup> Dyers are commissioned to formulate dyes to achieve a particular color and in doing so, introduce additives and other

“dyestuffs”. Components may be added or changed to substitute dyes in short supply or alter the final shade. Dyebaths may be adjusted to improve colorfastness. The most important part of a dye run is the end color, not the purity; batches with impurities below 5% are acceptable.<sup>32</sup> Dye mixtures intended to produce the same color can contain different amounts of dyestuffs, possibly individualizing each lot produced.<sup>34</sup> The relative proportions of dyes extracted from fibers may reflect a unique quantitative formulation of a particular product. A questioned fiber that was subjected to environmental conditions may feature dye patterns undetectable by visual comparisons that reveal similarities to the known fiber, requiring more investigation.

UV/visible detection following separation of dyes provides a spectrum of each dye component; if UV/visible microspectrophotometry was performed previously on the same fiber, these results alone may be valuable in assessing the origin of differences found between questioned and known fibers. The sheer volume of textile dyes available makes it impractical to determine the chemical structure of a dye in question, which can often be identified individually by infrared and ultraviolet spectroscopy. Analytical separations thus improve the ability to discriminate fibers by both retention time and spectral comparison, whereas UV/visible microspectrophotometry of fibers will only provide a mixture spectrum due to the combined the absorptions of any and all dyes on

that fiber. Using the known fiber to produce a 'reference' separation and spectrum is necessary to compare and confirm the interpretation of the questioned fiber results.<sup>35</sup> Previous researchers have experienced difficulty with UV/visible detection of dyes extracted from fibers of short length and fibers of lighter shades. Laing, *et al.*<sup>36</sup> used a UV/visible diode array for the detection of acid dyes separated by LC, but did not achieve analysis of fibers of forensically relevant lengths. Some early studies could not discriminate structurally-related dyes from trace fiber extracts by UV/visible detection due to lack of sufficient sensitivity.<sup>27,37-41</sup>

High performance liquid chromatography has been shown suitable for analysis of fibers of 5 to 10 mm lengths with both mass spectrometric and diode array detection.<sup>36,37,42,43</sup> The combination of the stationary phase and mobile phase in liquid chromatography allows tailoring of retention dependent of polarity ranges of dye classes. Target fibers typically found in forensic cases are 2-10 mm in length, and because extraction is destructive to this evidence, it is imperative to preserve as much as possible so that a piece is retained as physical proof. Minimizing the length of sample size necessary for characterization of dyes to fibers of 1 mm length is crucial in transferring the method to a crime laboratory.<sup>39</sup> Additionally, lightly dyed or faded dyes may be difficult to detect after separation by conventional HPLC due to band broadening. An HPLC study by

Wheals, *et al*<sup>44</sup>, found that detection limits of when coupled to UV/visible spectroscopy were 200 ppb with 10 µL injection volumes. Some of the lightly dyed fibers of short lengths did not contain produce sufficient extract for analysis of the major analyte, but could still be discriminated by minor dye components. Textiles are often found after being subjected to degradation and fading due to laundering or prolonged light exposure. Loss of color intensity in a questioned fiber by dye leaching or change in auxochromic configuration complicates comparison to a known fiber if the major dye component cannot be readily detected. The implementation of a method that can detect dyes on fibers that appear colorless, in particular, can increase the probative value of fiber evidence that would normally be classified with indeterminable color spectra by MSP.

HPLC columns used in dye separations are 10-25 cm long columns with internal diameters of 4-5 mm. Trace amounts of dyes are difficult to detect or resolve due to band broadening and require relatively large injection volumes. Ultra-performance liquid chromatography (UPLC) was commercially unveiled in 2004, offering separation with higher sensitivity and speed with higher pressure pumps. UPLC columns have smaller particle sizes that can be uniformly packed to reduce eddy diffusion. Smaller particle sizes also facilitate equilibrium between the stationary and mobile phases, and shorter column lengths decrease longitudinal diffusion. These three factors reduce band broadening to increase

chromatographic efficiency, resulting in higher flow rate capabilities, sharper peaks, and reduced sample volumes. This allows lower limits of detection to be achieved, enabling better discrimination and confidence in the presence or absence of the dyes.

The use of UPLC has steadily increased since its conception, but the majority of articles published pertain to biomedical and environmental applications. Forensics related research is limited to toxicology, illicit drug identification, and occasionally explosives or gunshot residue. The advancement in the development of higher pressure pumps, smaller particle sizes, and shorter columns reduces band broadening, allowing minimization of necessary injection volume. Prepared samples can be concentrated, thus lowering increasing the detector response and lowering detection limits. This, when coupled to UV/visible diode array detection, makes an ideal separation method for dye analysis. Smaller components, including narrower plumbing and reduced flow cell dimension, maximize the performance of the instrumentation. Shorter run times due to faster analyte elution have the potential to increase turnaround volume in forensic analyses and avoid backlogs in crime lab processing. A comparison study<sup>45</sup> of synthetic food dyes using UPLC-DAD and UPLC-MS/MS indicated that diode array detection can offer more sensitivity and lower limits of detection than mass spectrometry for many synthetic food colorants.



Additionally, a study of the same food dyes by Miniotti, *et al.*,<sup>46</sup> using HPLC-DAD with larger injection volumes allows detection of lower concentrations, suggesting that using higher injection volumes with UPLC-DAD can further decrease the quantity of dye necessary to confirm its presence.

The prominence of trace evidence used in court cases has declined in recent years, mostly due to the emphasis and dependence on nuclear DNA, latent print, and mitochondrial DNA evidence.<sup>47</sup> Forensic texts use flippant phrasing and negative implications to describe fiber evidence, such as “hanging by a thread” and referring to it as a pseudoscience, claiming it is unsubstantiated in interpretation.<sup>48,49</sup> As a consequence of being class evidence, fibers cannot definitively be excluded from a match and conversely, determined to be one and the same, despite distinct features.

A 2009 report<sup>50</sup> by the National Academy of Science called for the review and overhaul of forensic science, including trace evidence. The following concerns were raised in the interest of advancing fiber evidence:

- (1) Scientific Working Group for Materials Analysis (SWGMAT) “has produced guidelines, but no set standards, for the number and quality of characteristics that must correspond in order to conclude that two fibers came from the same manufacturing batch. There have been no

studies of fibers (*e. G.*, the variability of their characteristics before and after manufacturing) on which to base such a threshold.”<sup>51</sup>

(2) “Similarly, there have been no studies to inform judgments about whether environmentally related changes discerned in particular fibers are distinctive enough to reliably individualize their source.”

(3) “[T]here have been no studies that characterize either reliability or error rates in their procedures.”

Fiber examination by optical microscopy, polarized light microscopy, UV/visible microspectrophotometry, and infrared spectroscopy are invaluable techniques in initial fiber discrimination efforts. The fast, non-destructive methods allow for preservation of evidence, and in many cases yield enough information (color, morphology, birefringence) to differentiate a questioned fiber from a known fiber. Fibers that are visually and physically indistinguishable can render inconclusive results if UV/visible spectra exhibit seemingly duplicate absorption spectra. The fibers may contain different dyes combinations to impart the same color, generating otherwise identical spectra representing unresolved mixtures of multiple unknown dyes. Analysis of the dyes on both fibers may reveal characteristics to confirm or reject the hypothesis that both fibers came from the same source. Examination of individual dye components strengthens the significance of the results by providing discriminating characteristics that

increase rarity and decrease probability that the fibers came from two separate sources by happenstance. By addressing the questions posed in the NAS report, practical changes can be made to improve the reliability of fiber evidence its legitimacy in legal proceedings. It is essential to investigate and use state-of-the-art technology and procedures until all comparative methods are exhausted, ensuring the most complete inspection for the consideration of potential alternate sources.

The development of methods for separation, detection, and comparison of exclusionary characteristics of trace amounts of dyes using ultra-performance liquid chromatography with diode array detection is the subject of this research presented in this dissertation. The method will be implemented on submillimeter fibers to establish its feasibility in forensic contexts. Dyes extracted from textiles exposed to environmental weathering will be examined to determine if fiber evidence can still be used in investigations involving a body found after prolonged exposure outdoors. The effect of multiple detergents and cleaning additives will be explored to demonstrate the resulting complications that arise from laundering in forensic comparison of dyes extracted from fiber evidence.

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## TABLES

Table 1.1. LOD comparison of food dyes by HPLC-DAD, UPLC-DAD, and UPLC-MS/MS

Food dye	HPLC- DAD LOD <sup>46</sup> (pg) <sup>†</sup>	UPLC- DAD LOD <sup>45</sup> (pg) <sup>‡</sup>	UPLC- MS/MS LOD <sup>45</sup> (pg) <sup>‡</sup>
Tartrazine	<b>37.4</b>	150	2250
Amaranth	<b>204</b>	450	2010
Indigo Carmine	161.8	<b>30</b>	2340
Ponceau 4R	442	<b>120</b>	1200
Sunset Yellow FCF	88.2	<b>30</b>	3300
Allura Red AC	149.2	<b>120</b>	180
Brilliant Blue FCF	<b>54.4</b>	150	60
Azorubine	<b>87</b>	510	240
Patent Blue V	210	390	<b>120</b>
Erythrosine	133.6	1170	<b>90</b>

<sup>†</sup>20 µL injection volume

<sup>‡</sup>3 µL injection volume

FIGURES

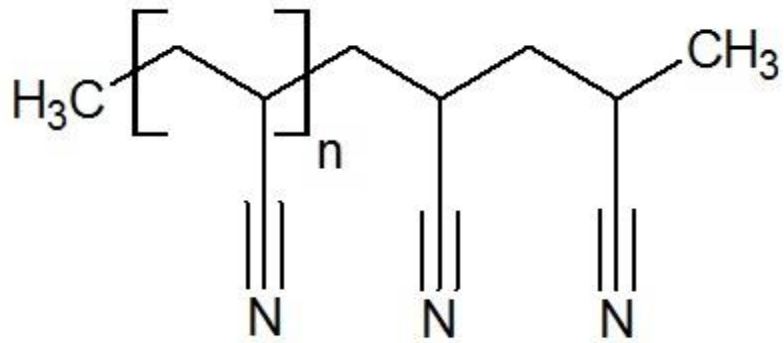


Figure 1.1. Polymeric structure of acrylic fiber.

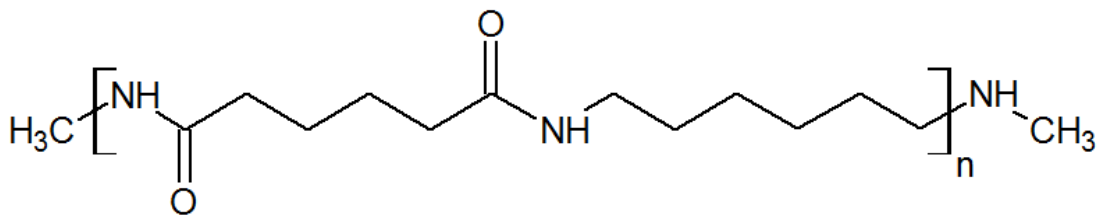


Figure 1.2. Polymeric structure of nylon 6 fiber.

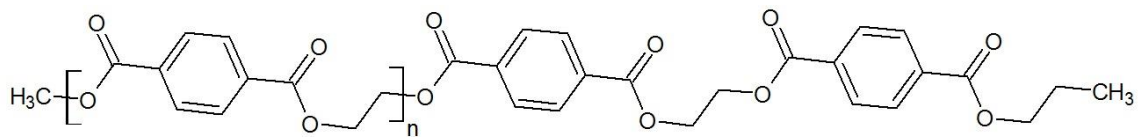


Figure 1.3. Polymeric structure of polyester

## CHAPTER TWO

### DEVELOPMENT OF A LIQUID CHROMATOGRAPHY METHOD AND CALIBRATION OF DYES AT FORENSICALLY RELEVANT LENGTHS

#### ABSTRACT

Development of a method for the separation and detection of disperse, basic, and acid dyes is reported here. A chromatographic method is described for each dye class, allowing separation of analytes in three minutes or less. Limits of detection are determined for semi-quantitative analysis of dyes extracted from polyester, acrylic, and nylon fibers. Although this method is destructive to the fiber, only a small length ( $\leq 1$  mm) is necessary for successful detection by ultra-performance liquid chromatography coupled to diode array detection. With the exception of one acid dye, LODs were found to be less than 4 parts per billion, allowing analysis of dyes on single fibers of forensically relevant lengths.

## INTRODUCTION

Fibers found in criminal investigation are a useful form of trace evidence that can lead investigators to a suspect or piece together the events that took place in an assault. Visually similar fibers can establish an association between two otherwise unrelated subjects. Forensic fiber examinations are centralized around an attempt to find and compare distinct characteristics of a questioned and a known fiber. The objective is to eliminate the possibility that the fibers came from a common source by evaluating individualizing characteristics in the order of maximum discriminating ability.<sup>1</sup> Microscopy techniques and infrared spectroscopy are the first line of inspection, but if these techniques fail to differentiate polymer type, refractive indices, or UV-visible spectra, it is possible that analysis of the dyes may yield information that has higher evidential weight in excluding or confirming a match.

Textiles are often dyed with multiple dyes to achieve a particular color. UV-visible microspectrophotometry is used to measure the color of a fiber, which produces a single absorption spectrum of all of the dyes on the sample. Examining the dyes individually allows a higher degree of discrimination, as more variables can be used to compare the two fibers. The dyes are isolated from the fiber using microextraction, and must be separated prior to spectral analysis.<sup>2</sup> Thin layer chromatography is the separation method used by the Federal Bureau

of Investigation<sup>3</sup>, and capillary electrophoresis and high performance liquid chromatography have been studied for the analysis of dye extracts.<sup>4-11</sup> While CE is able to separate ionized dyes, it performs poorly with non-ionized dyes; HPLC is able to separate both ionized and non-ionized dyes, due to the many stationary phase options and mobile phase that can accommodate the many different dye structures. HPLC coupled to diode-array has shown potential for use in dye comparison, although detection of dyes on fibers of forensically relevant lengths was not achieved. Additionally, HPLC-DAD could not differentiate some structurally similar dyes, although other major dye components could be used to successfully discriminate fibers.<sup>12</sup> Ultra-performance liquid chromatography is an advanced separation system that uses columns with smaller particle sizes, higher pressure pumps, and shorter columns than those used in conventional high performance liquid chromatography. These improvements reduce band broadening, which leads to lower limits of detection, shorter run times and less injection volume required for detection.

The three most abundant synthetically produced fibers are polyester, acrylic, and nylon.<sup>13,14</sup> Polyester fibers are colored using disperse dyes, which are dispersed through the fiber and retained with hydrophobic interactions. Acrylic fibers are dyed with basic dyes; the negatively charged fiber forms salt linkages with the basic (cationic) dyes. Similarly, the positively charged nylon polymer is

dyed with the negatively charged acid dyes by salt linkages. The dyes are removed from the fibers using extraction solvents based on the chemistry of the dye, which involves reducing the substantivity of the dye for the fiber.<sup>2,4,5,15-27</sup> This method is destructive to the fiber, so it should only be implemented once all non-destructive methods have been exhausted without a match exclusion. Fibers found at crime scenes are typically 2-10 mm in length,<sup>28</sup> and in the interest of preserving as much evidence as possible, the method needs to be capable of analyzing fibers  $\leq 1$  millimeter in length, requiring high sensitivity to produce low limits of detection.

## EXPERIMENTAL

Formic acid, HPLC grade water, ammonium hydroxide, HPLC grade ammonium acetate, and HPLC grade acetonitrile were purchased from Fisher Scientific (Pittsburg, PA).

### *Ultra-performance liquid chromatography*

Dyes were separated using a Waters (Milford, MA) Acquity UPLC system. The system was equipped with a room temperature sample manager and a Waters (Milford, MA) Acquity column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID  $\times$  50 mm length) heated to 40 °C. A BEH C18 column was used to separate disperse dyes. The stationary phase of the column used to separate disperse dyes was BEH C18,

and the stationary phase of the column used to separate acid and basic dyes was CSH Phenyl-hexyl. The mobile phase solvent gradient conditions employed for all runs is listed in Table 2.1. The sample injection volumes were 10  $\mu$ L.

#### *UV/Visible diode array detection*

Dyes samples were detected using a UV/visible diode array detector (Waters, Milford, MA) scanning absorbance from 350-675 nm. The peak area on the chromatogram was acquired for each dye using the corresponding maximum wavelength (Tables 2.2, 2.3, 2.4), and was used for comparison to standard dye mixtures to determine the amount of dye on each fiber.

#### *Calibration and limits of detection*

Limits of detection and quantitation were determined from calibration models based on the UPLC-DAD analysis of dye standards at varying concentrations. Basic Dyes were prepared in 25% acetonitrile, acid dyes were prepared in 10% acetonitrile, and disperse dyes were prepared in 70% acetonitrile. Calibration solutions of basic dyes were prepared at concentrations of 10 ppb, 25 ppb, 50 ppb, 75 ppb, 100 ppb. Calibration solutions of acid dyes were prepared at concentrations of 10 ppb, 15 ppb, 20 ppb, 25 ppb, 50 ppb. Another solution of Acid Green 27 was prepared at concentrations 50 ppb, 75 ppb, 100 ppb, 150 ppb, and 200 ppb to encompass the range of the limit of detection estimation. Calibration solutions of disperse dyes were prepared at

concentrations of 2.5 ppb, 5 ppb, 10 ppb, 15 ppb, 20 ppb. Each concentration level was analyzed by UPLC with UV/Visible detection with five replicate 10  $\mu$ L injections.

## RESULTS AND DISCUSSION

### *Chromatographic Analysis of Dyes*

Chromatographic methods were developed to separate dyes in each dye class. In comparison to one comprehensive method for separation of all dye classes, methods tailored to specific dye classes allow simpler gradients and shorter run times. Disperse dyes were prepared in 70% acetonitrile, and injected into a Waters Acquity BEH C18 column and separated using an isocratic gradient of 85% acetonitrile in 15% water. Disperse Red 60, Disperse Yellow 114, and Violet 77 eluted in less than one minute, shown in Figure 2.1.

Basic dyes were prepared in 25% acetonitrile and injected onto the phenyl-hexyl column with an isocratic gradient of 90% ACN and 10% 25mM ammonium acetate in water. It seems that the ionic characteristics of acid and basic dyes require  $\pi$ - $\pi^*$  interactions with the stationary phase for retention and the mobile phase additive for sharper peaks. Figure 2.2 shows the separation of Basic Blue 159, Violet 16, and Yellow 28 in less than one minute.



Acid dyes were prepared in 10% acetonitrile, but Acid Blue 45 was not retained on the C18 column, and eluted at the dead time. This is due to the polar substituents on the ring structure, as opposed to the hydrophobic acid dye, Acid Green 27 (figures shown in Table 2.4). A Waters Acquity CSH Phenyl-hexyl column was employed to take advantage of the  $\pi$ - $\pi^*$  interactions of the dye ring structures with the stationary phase, allowing retention. Although Acid Blue 45 was retained, a dynamic gradient was necessary to elute C. I. Acid Green 27. The initial mobile phase composition of 5% acetonitrile was increased to 50%. The aqueous portion of the mobile phase required a mobile phase additive of 25 mM ammonium acetate to reduce peak tailing. Figure 2.3 shows the separation of Acid Blue 45, Acid Yellow 49, and Acid Green 27. The baseline fluctuation is because of the gradient change, which therefore changes the background absorption. Acid Green 27 consists of two peaks, which is suspected to be due to impurity of the standard. Acid Green 25 is similar in structure (Figure 2.4), with a shorter carbon chain. Both dyes have two absorption maxima, absorbing wavelengths in the orange and blue regions of the visible spectrum, which produce blue and yellow, respectively. This is suspected to be due to two chromophoric groups within the dye molecule. The same anomaly has been observed by Huang, *et al.*<sup>39</sup>, and can be used to differentiate another green dye

with one absorption maximum in the higher visible wavelength range corresponding to an absorption of red light.

#### *Calibration Models of Dye Standards*

Table 2.4 shows UPLC-DAD results for each dye. Figures 2.5-2.13 display calibration plots for the nine dyes investigated. All first order linear calibration models (with intercept and slope parameters) produced coefficients of determination ( $R^2$ ) of 0.9930 or higher. The calibration of Acid Green 27 is fitted with a second order polynomial model, producing a coefficient of determination ( $R^2$ ) of 0.9959. Limits of detection are reported in Tables 2.5-2.7 based on three different estimation approaches. Each method calculates the LOD or LOQ using

$$\text{LOD} = (3.3 \times \sigma_b)/S$$

$$\text{LOQ} = (10 \times \sigma_b)/S,$$

where  $\sigma_b$  is the standard deviation of the blank and  $S$  is the slope of the calibration line. The three methods used differ with how  $\sigma_b$  is estimated.<sup>1,30-33</sup>

LOD1 estimates  $\sigma_b$  using the standard deviation of the integrated blank signals across the width of the actual peak. LOD2 approximates  $\sigma_b$  using the standard deviation of the lowest non-zero concentration calibrator (10 ppb for Acid Blue 45, Acid Yellow 49, and basic dyes; 50 for Acid Green 27; 2.5 ppb for disperse dyes). LOD3 estimates  $\sigma_b$  based on the standard error of the  $y$ -intercept of the calibration model, and are much higher than those of LOD<sub>1</sub> and LOD<sub>2</sub>. The

standard deviations of the residuals are used in calculating the standard error in the  $y$ -intercept, which may be amplified as the standard error of the  $y$ -intercept is calculated from the standard deviation of residuals. The calibrations that exhibit heteroscedasticity (non-constant variability at different concentration levels) indicate a lack of fit of the model, which may have, in turn, inflated LOD<sub>3</sub>. There are multiple estimations of  $\sigma_b$  that can be used in calculating limits of detection, but little discussion in the literature about assumptions made when calculating limits of the detection. The three estimations discussed here demonstrate some of the effects that deviations in calibration models can have on LODs. Most LODs calculated using the standard deviation of the replicate blank samples and lowest concentration calibrator are less than 4.0 ppb, with the exception of Acid Green 27, which has a polynomial calibration model. LOD<sub>3</sub> estimated the most drastic increase from LOD<sub>1</sub> and LOD<sub>2</sub> for the basic dyes, indicating these models have the highest lack of fit. The LOD<sub>3</sub> calculated for the disperse dyes had the smallest increase; it is possible that a lower calibration range for the basic dyes would exhibit lower limits of detection by LOD<sub>3</sub>. This result illustrates an important point: confirming actual detection for a sample concentration at the estimated LOD is required if one plans to operate near the LOD. Conducting the low concentration calibration design achieved this requirement for the present study.

In fiber analysis, it is less important to quantify the dyes on the fiber, and dye amounts can differ along the length of a single fiber. Therefore, the goal of performing calibrations is to determine a level at which it can be confirmed with a degree of certainty that the dye in question is, in fact, on the fiber. When replicate measurements are made, the LOD can be estimated using

$$\text{LOD} = \mu_b + 3.3\sigma_b$$

which gives the fractional risk of a false positive  $\alpha = 0.0005$ . This means that there is a 0.05% chance that the dye will be confirmed to be on the fiber when, in fact, the dye is not present. Figure 2.14 demonstrates this probability, where 99.95% of the measurements confirm that the dye is absent. However, this same estimation gives the fractional risk of a false negative  $\beta = 0.500$ , meaning that 50.00% of replicate measurements will not detect analyte even when it is present. To reduce the false error rate to an acceptably low level of  $\beta = 0.0005$ , the decision limit can be raised to  $3.3\sigma_b$  above the LOD, which can be described as the minimum consistently detectable amount

$$\text{MCDA} = \mu_b + 6.6\sigma_b$$

twice that of the LOD. If a sample containing the MCD amount of analyte is measured repeatedly, 99.95% of the time it will be correctly concluded that the analyte is present (Figure 2.15).

## CONCLUSIONS

Three chromatographic methods have been developed for the analysis of acrylic, polyester and nylon using ultra high performance liquid chromatography, to produce sharp peaks in fewer than 3 minutes each. The chromatography methods for basic dyes and disperse dyes are isocratic; three dyes in each class can be separated in less than a minute. The gradient method for acid dyes allowed for retention and elution of both hydrophilic and hydrophobic compounds. A phenyl hexyl column was employed for both acid and basic dyes, to take advantage of  $\pi$ - $\pi^*$  interactions between the dyes and the stationary phase. Developing a method for disperse dyes on the phenyl hexyl column would allow for faster analysis of multiple dye types, as the column would not have to be changed. Calibrations have been performed to determine detection limits for 8 out of 9 of dyes less than 4.0 ppb. Investigating a lower calibration range for basic dyes could yield lower limits of detection when calculated using the standard deviation of the y-intercept, although LODs calculated using the standard deviation of the blank and lowest non-zero calibrator give sufficiently low, consistent results.

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## TABLES

Table 2.1. Liquid chromatography gradients used for:

(A) the separation of disperse dyes on a Waters BEH C18 column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is water and B is Acetonitrile

Time (min)	% A	% B
0.00	20	80
1.00	20	80

(B) the separation of basic dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	90	10
1.00	90	10

(C) the separation of acid dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	95	5
0.20	95	5
0.50	50	50
1.00	50	50
1.40	95	5
3.00	95	5

Table 2.2. Disperse Dyes

C. I. Name Formula Mol. Wt. (g/mol)	Structure	Absorption Spectrum Maximum Wavelength (nm)
Disperse Red 60 $C_{20}H_{13}NO_4$ 331.32		 514
Disperse Yellow 114 $C_{20}H_{16}N_5O_4$ 424.43		 424
Disperse Violet 77 $C_{21}H_{24}N_6O_5$ 440.45		 547

Table 2.3. Basic Dyes

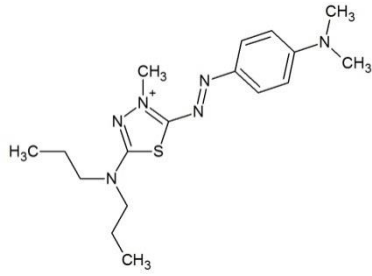
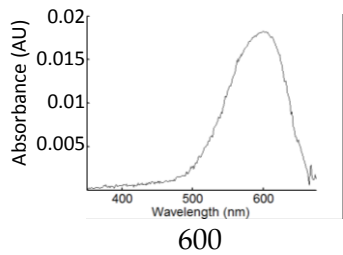
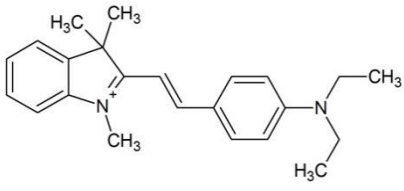
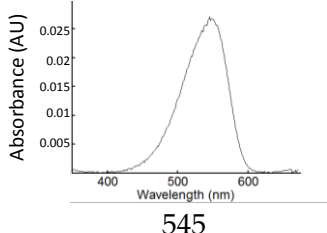
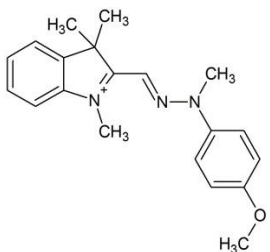
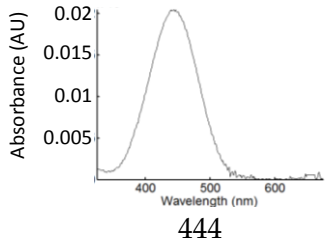
C. I. Name	Structure	Absorption Spectrum Maximum Wavelength (nm)
Basic Blue 159 $C_{17}H_{27}N_6S^+$ 347.50		
Basic Violet 16 $C_{23}H_{29}N_2^+$ 333.49		
Basic Yellow 28 $C_{20}H_{24}N_3O^+$ 322.42		

Table 2.4. Acid Dyes

C. I. Name	Formula	Mol. Wt. (g/mol)	Structure	Absorption Spectrum Maximum Wavelength (nm)
Acid Blue 45	$C_{14}H_{10}N_2O_{10}S_2$	430.37		 614
Acid Yellow 49	$C_{16}H_{13}Cl_2N_5O_3S$	426.28		 420
Acid Green 27	$C_{34}H_{34}N_2O_8S_2$	662.18		 422/616

Table 2.5. Disperse dye limits of detection calculated using  $\sigma_b$  estimated by the standard deviation of: replicate blanks (LOD<sub>1</sub>), lowest non-zero calibrator (LOD<sub>2</sub>), and y-intercept (LOD<sub>3</sub>)

	LOD1	LOD 2	LOD 3
Disperse Red 60	1.65	1.69	0.68
Disperse Violet 77	1.28	0.27	0.31
Disperse Yellow 114	1.59	1.29	0.79

Table 2.6. Basic dye limits of detection calculated using  $\sigma_b$  estimated by the standard deviation of: replicate blanks (LOD<sub>1</sub>), lowest non-zero calibrator (LOD<sub>2</sub>), and y-intercept (LOD<sub>3</sub>)

	LOD 1	LOD 2	LOD 3
Basic Blue 159	1.48	2.40	2.22
Basic Yellow 28	1.09	1.69	1.96
Basic Violet 16	2.33	1.96	2.08

Table 2.7. Acid dye limits of detection calculated using  $\sigma_b$  estimated by the standard deviation of: replicate blanks (LOD<sub>1</sub>), lowest non-zero calibrator (LOD<sub>2</sub>), and y-intercept (LOD<sub>3</sub>)

	LOD 1	LOD 2	LOD 3
Acid Blue 45	3.59	2.18	1.28
Acid Yellow 49	3.62	2.76	1.01
Acid Green 27	25.44	22.79	42.59

FIGURES

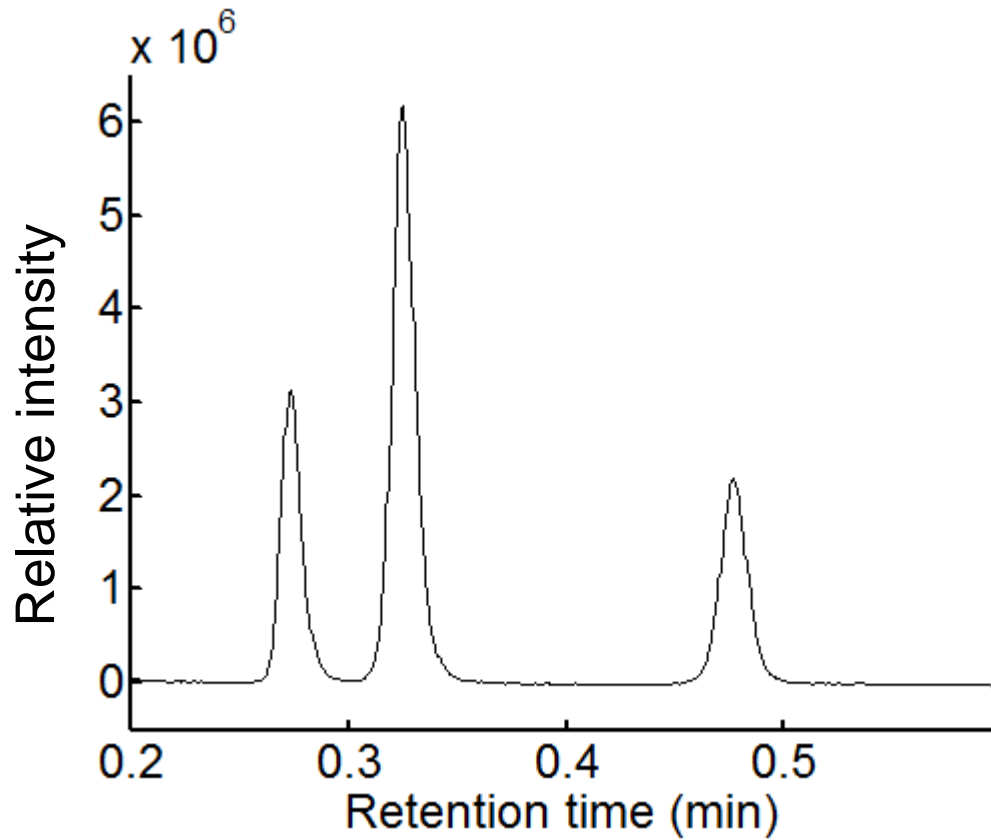


Figure 2.1. Chromatogram of disperse dyes at 25ppb concentration.

Peak Identification: (1) Disperse Yellow 114; (2) Disperse Violet 77; (3) Disperse Red 60.

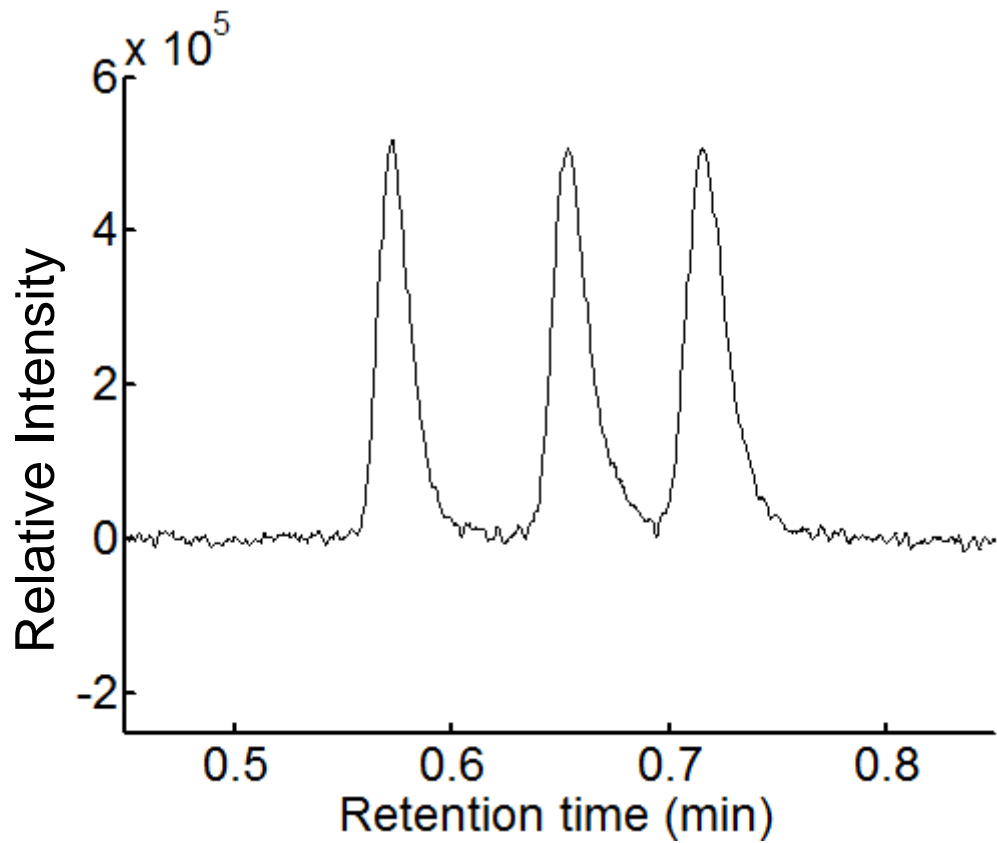


Figure 2.2. Basic dye chromatogram at 150 ppb concentration.  
Peak Identification: (1) Basic Blue 159; (2) Basic Violet 16; (3) Basic Yellow 28.

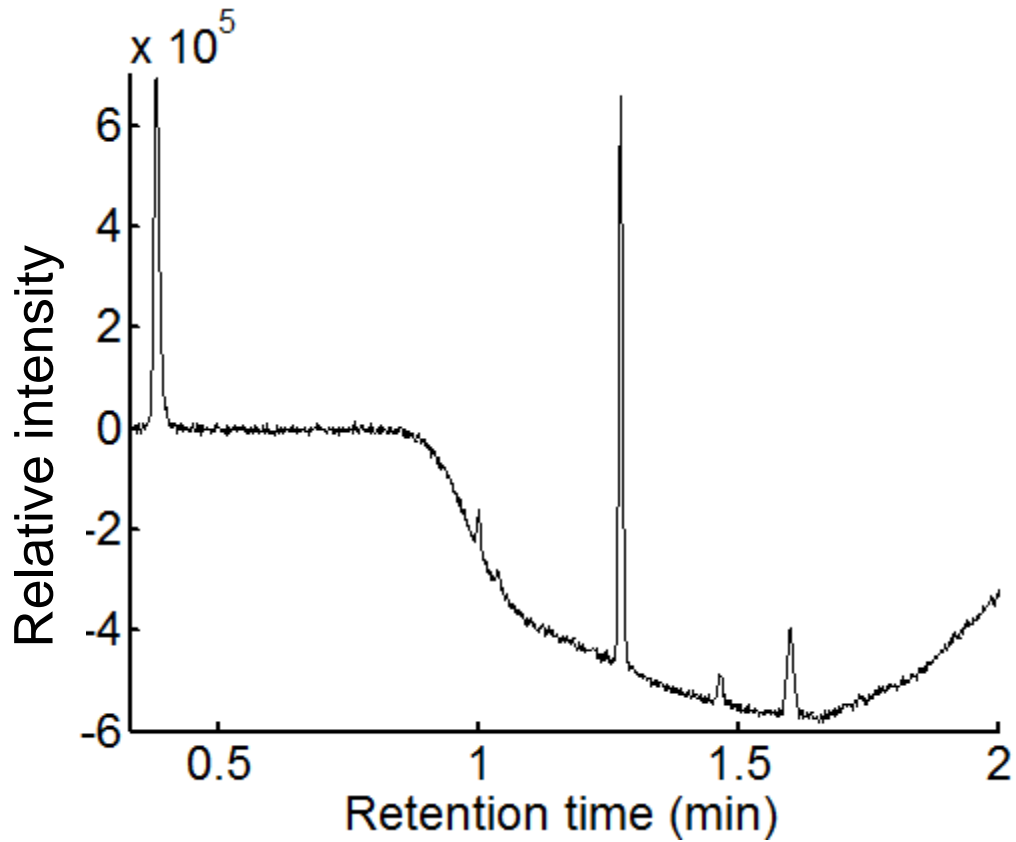


Figure 2.3. Separation of Acid dyes at 250 ppb concentration.

Peak Identification: (1) Acid Blue 281; (2) Acid Yellow 49;

(3) Acid Green 27 (two peaks).

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient



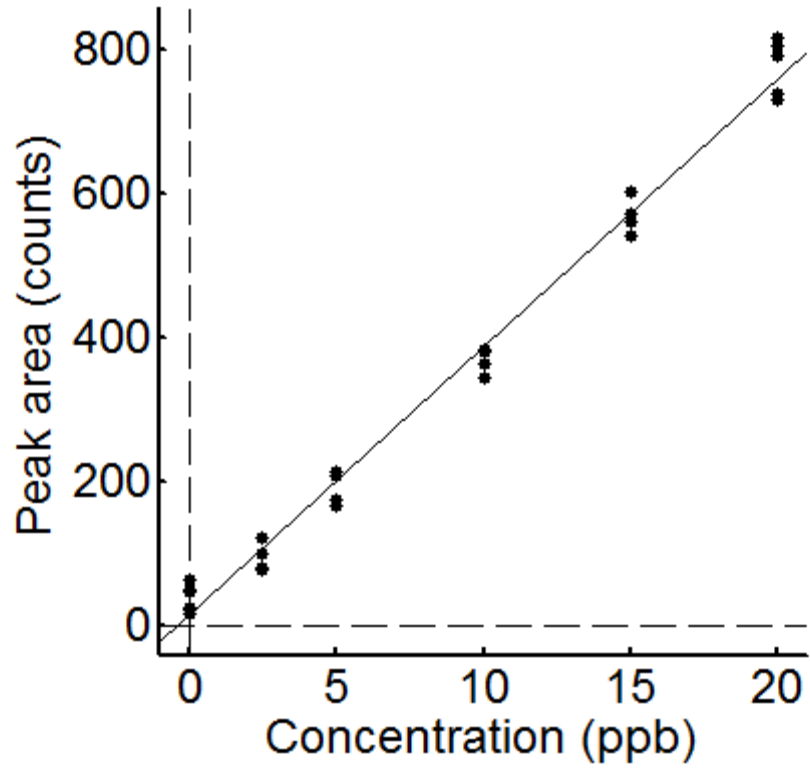


Figure 2.4. UPLC-DAD calibration plot for Disperse Red 60

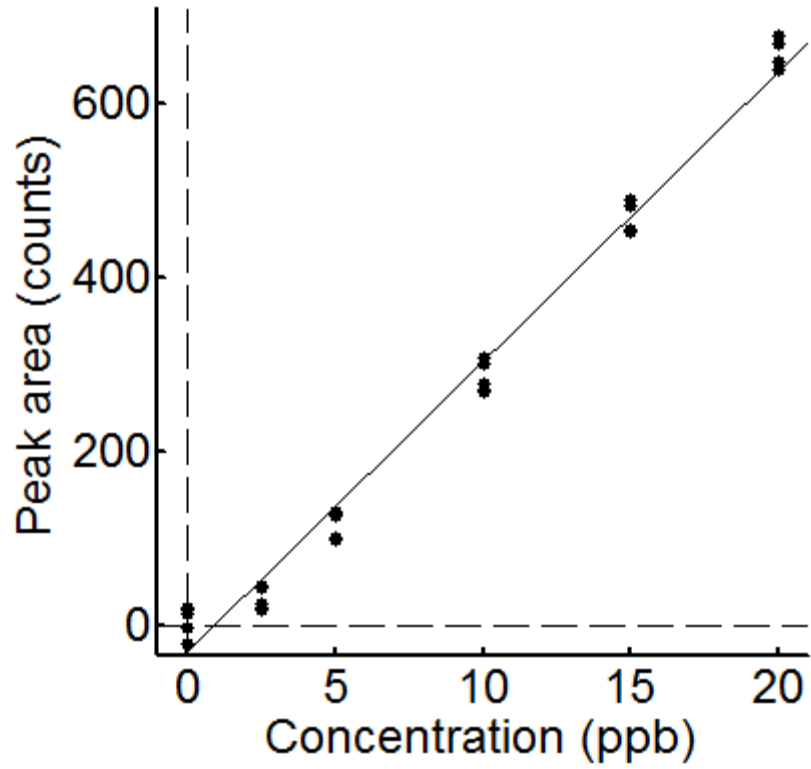


Figure 2.5. UPLC-DAD calibration plot for Disperse Yellow 114

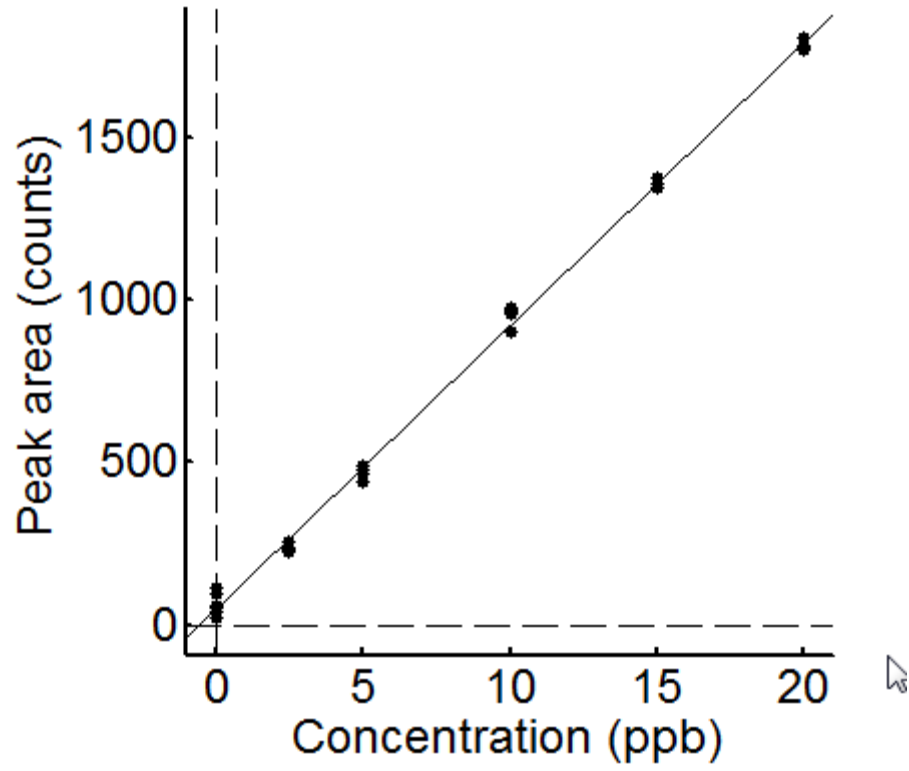


Figure 2.6. UPLC-DAD calibration plot for Disperse Violet 77

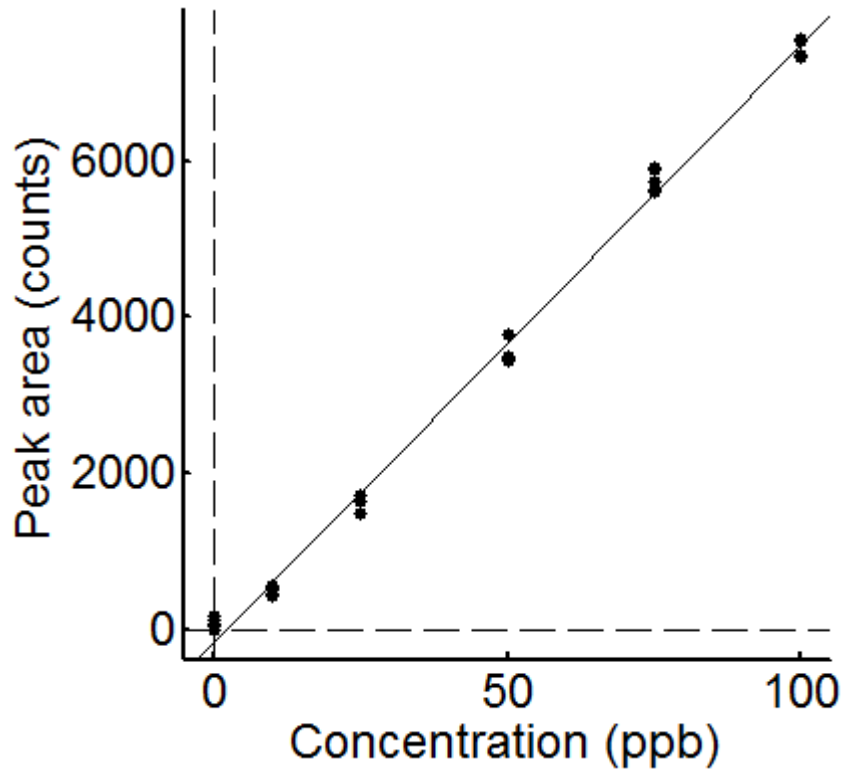


Figure 2.7. UPLC-DAD calibration plot for Basic Blue 159

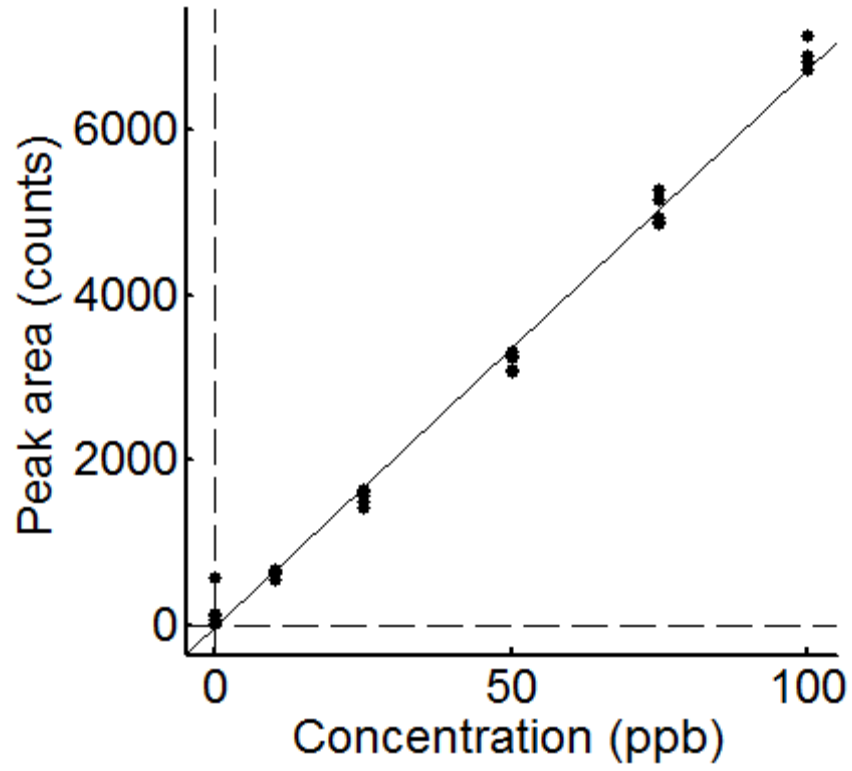


Figure 2.8. UPLC-DAD calibration plot for Basic Violet 16

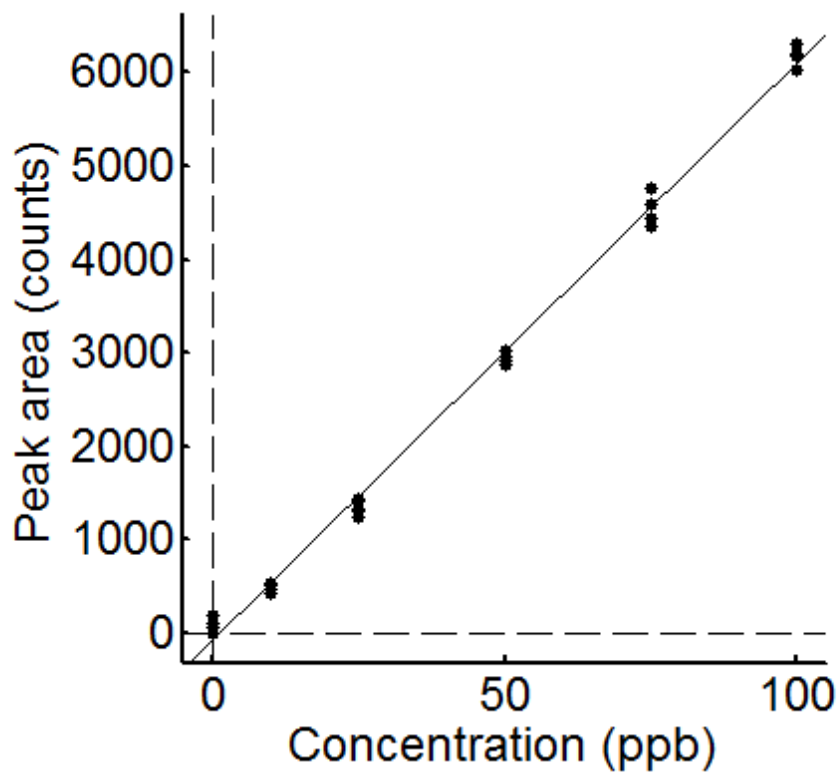


Figure 2.9. UPLC-DAD calibration plot for Basic Yellow 28

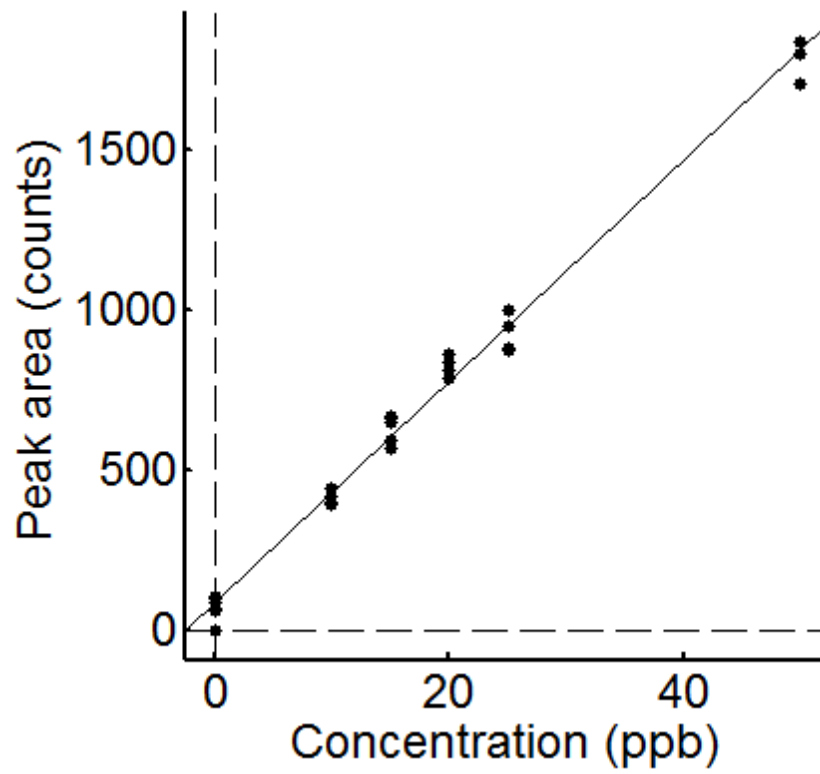


Figure 2.10. UPLC-DAD calibration plot for Acid Blue 45

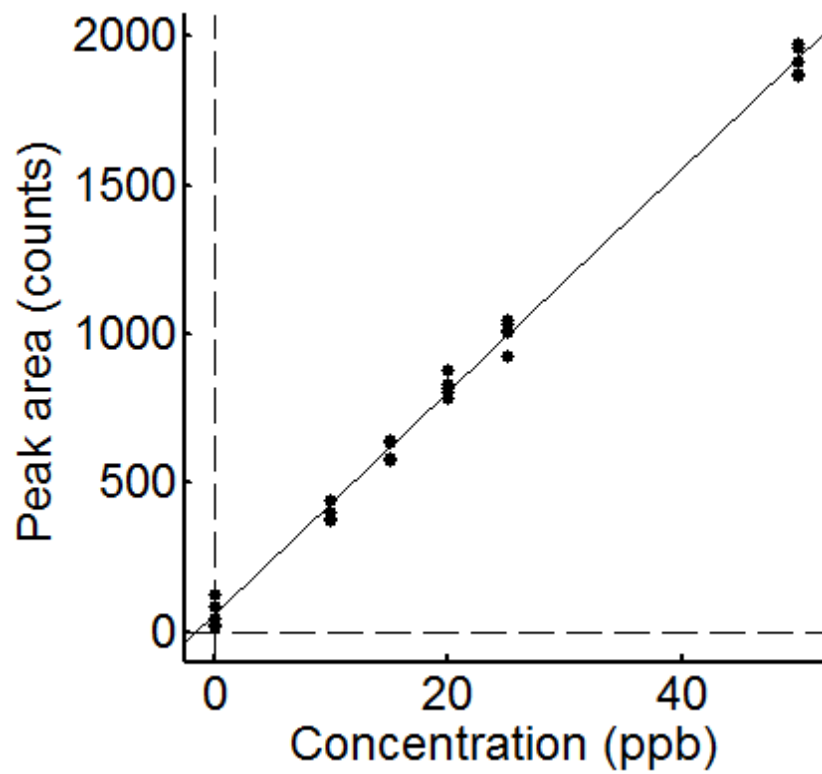


Figure 2.11. UPLC-DAD calibration plot for Acid Yellow 49



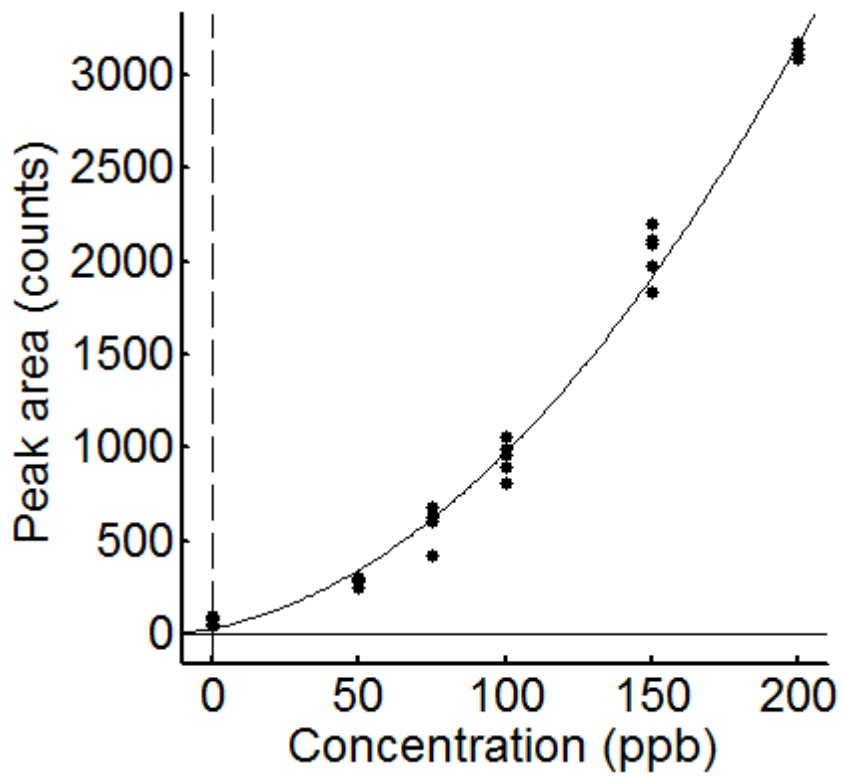


Figure 2.12. UPLC-DAD calibration plot for Acid Green 27

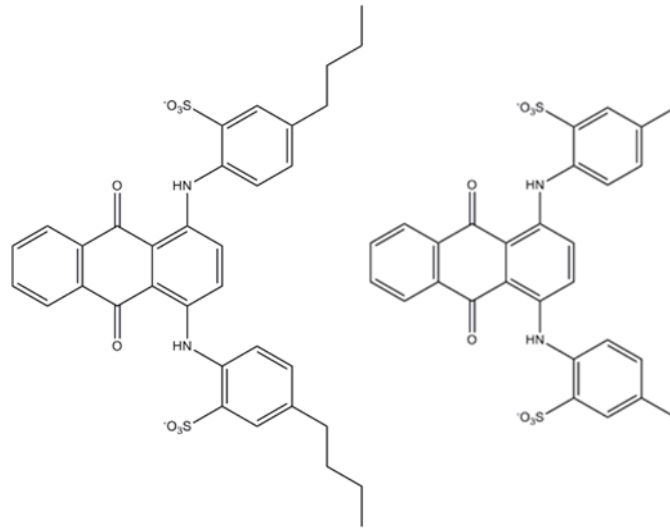


Figure 2.13. Dye structures for (a) Acid Green 27 and (b) Acid Green 25

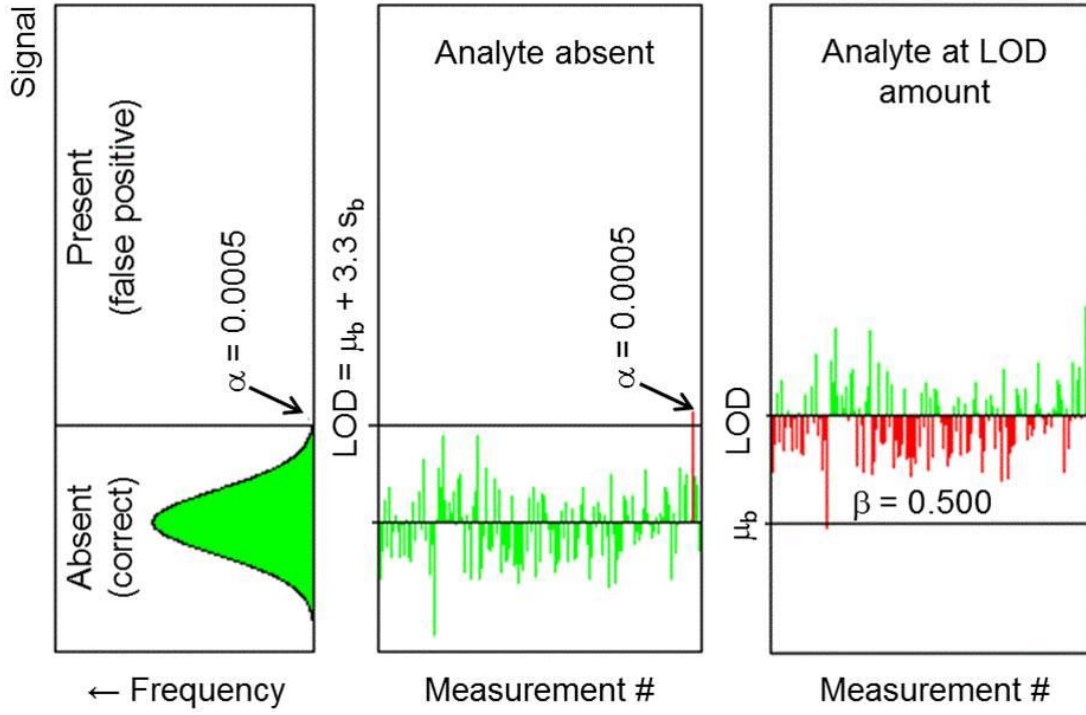


Figure 2.14. Fractional risk of a false negative,  $\beta = 0.500$ , or 50%

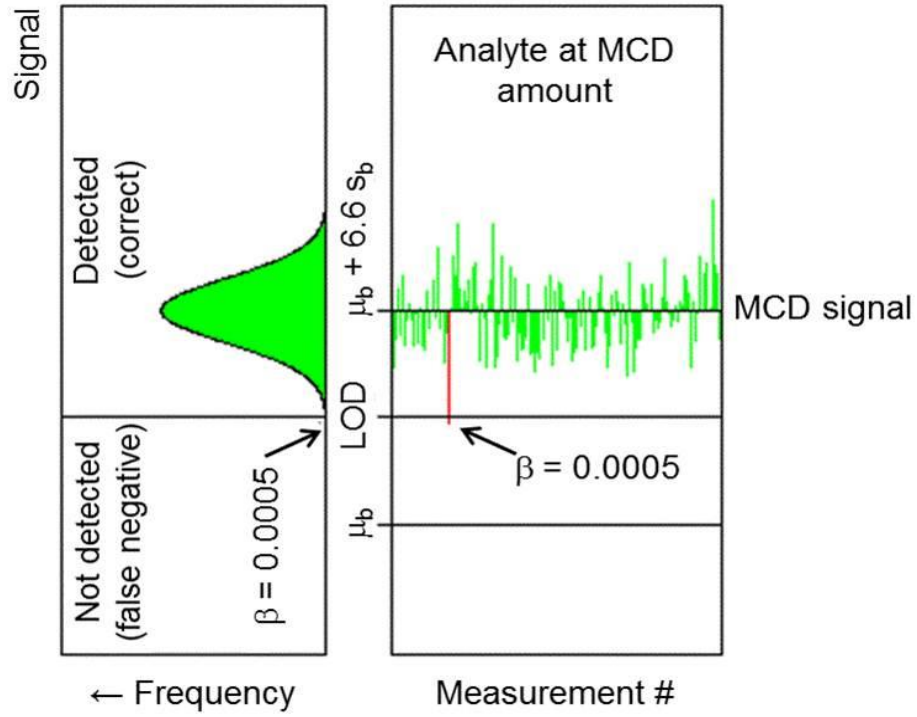


Figure 2.15. Reduce fractional risk of a false negative,  $\beta = 0.0005$  by estimating minimum consistently detectable amount (MCDA)

## CHAPTER THREE

### MICROEXTRACTION OF DISPERSE, BASIC, AND ACID DYES FROM MILLIMETER LENGTH POLYESTER, ACRYLIC, AND NYLON FIBERS

#### ABSTRACT

Microextraction and detection of disperse dyes from polyester, basic dyes from acrylic, and acid dyes from nylon are reported for single fibers of submillimeter lengths. Analysis of individual dye components allows a higher degree of discrimination of fiber evidence in a criminal investigation. Fibers may be dyed with multiple dyes producing similar colors that are difficult to distinguish with microscopic methods. Ultra-performance liquid chromatography coupled with UV/visible detection allows comparison of dyes from fibers that cannot be distinguished using non-destructive methods alone. Dyes are detectable on submillimeter length fibers, requiring only a segment of fibers typically found at crime scene so that evidence is preserved.

## INTRODUCTION

Trace fiber evidence is a tool that can be used to establish contact between an assailant, their victim, and the location at which the crime has taken place. The premise of forensic fiber examination is to compare a questioned and a known fiber with the objective of finding discriminating characteristics that eliminate the possibility of a common origin. Initial investigation includes visual inspection, optical microscopy and UV/visible microspectrophotometry to compare fiber color and morphology. Further testing, such as polarized light microscopy, can be used to determine generic fiber class by refractive index and birefringence measurements; infrared spectroscopy can be used to identify polymer type. However, these methods are incapable of characterizing dyes on a molecular level. Textiles can be colored with multiple dyes to achieve a desired shade, but still produce a spectrum with similar absorption spectra. Therefore, two fibers can be physically and optically indistinguishable, despite being dyed with different dyes. Because fibers are mass produced and subsequently classified as class evidence, it is important to analyze all variables to the maximum capacity. By doing so, the significance of the results increases to strengthen probative value.

Polyester, acrylic, and nylon are the most common synthetic fibers encountered in a forensic setting. The class of dye used to color a fiber is

dependent on fiber polymer chemistries.<sup>1,2</sup> Characterization of the dye molecules requires separation, and thus isolation of the dyes.<sup>3</sup> Microextraction procedures have been investigated for the removal of disperse dyes from polyester, basic dyes from acrylic, and acid dyes from nylon.<sup>4-23</sup> These methods should only be used if the fibers cannot be differentiated using non-destructive optical and spectral techniques. Fibers collected from crime scenes are typically 2-10 mm in length, so to preserve as much of the evidence as possible, a method developed for this analysis should be capable of analyzing fibers down to submillimeter ranges. Achieving the necessary sensitivity requires maximum performance of extraction techniques. Stefan, *et al* and Dockery, *et al* used experimental design to optimize extraction solvents for the most complete removal of dye.<sup>4,5,7</sup> Chlorobenzene is used to reduce the substantivity of disperse dyes for polyester. Anionic sites of acrylic fibers form salt linkages with basic dyes, so dye removal involves the displacement of these dyes using formic acid. Similarly, cationic sites of nylon fibers form salt linkages with acid dyes, which are removed using equal parts pyridine, water, and ammonium hydroxide.

Following dye extraction, dyes must be separated for individual analysis. Thin layer chromatography, capillary electrophoresis, and high-performance liquid chromatography are techniques that have been studied for dye separation, including disperse, basic, and acid dyes.<sup>16-27</sup> Although TLC is an established

method used by the Federal Bureau of Investigation,<sup>22</sup> it may be impractical on lightly colored fibers or small sample sizes. CE is suitable for the separation of acid and basic dyes, but because this method functions on the ability to ionize analytes, it is not adequate for separating non-ionic disperse dyes. HPLC is a technique that has been shown to separate these dye classes extracted from fibers 5-10 mm in length.<sup>24-27,29-31</sup> The variety of stationary phases available in combination with a modifiable mobile phase allows adjustment for analytes with different separation requirements. It has been coupled to UV-visible spectroscopy to characterize the color of a dye using its absorption spectrum, as well as mass spectrometry for dye identification by fragmentation patterns. Prior research revealed difficulty in detection of small samples and poor resolution of structurally similar dyes by HPLC with UV/visible detection.<sup>34</sup> Mass spectrometry allowed discrimination of the dyes, but only down to 5 mm in length.<sup>30-31</sup>

Ultra-performance liquid chromatography improves upon conventional HPLC using higher pressure pumps and smaller particle sizes, allowing shorter, more uniformly packed columns. These advances allow faster flow rates and shorter run times with sharper peaks due to decreased band broadening. As a result, lower limits of detection can be achieved with small injection volumes, affording sensitivity for analysis by UV-visible detection of trace levels of dyes.



Methodology established in a previous study will be implemented to characterize dyes on acrylic, polyester, and nylon fibers. Microextraction procedures will be used on single fibers to isolate dyes in preparation for analysis. Dyes will be separated using ultra-performance liquid chromatography and identified based on their UV-visible absorption spectra. Investigation of detection capabilities will be used to determine the viability of this method for forensic fiber analysis.

## EXPERIMENTAL

Formic acid, HPLC grade water, ammonium hydroxide, HPLC grade ammonium acetate, and HPLC grade acetonitrile were purchased from Fisher Scientific (Pittsburg, PA). Pyridine was purchased from Mallinckrodt Baker (Phillipsburg, NJ) and chlorobenzene was purchased from Acros Organics (Morris, NJ).

Dye standards and finishing agents were donated by dyestuff manufacturers in the southeastern United States. Acid and disperse dyes were solid in phase and basic dyes were liquid in phase. Dyes reported here are named in accordance with the Color Index International database (Society of Dyers and Colourists, Bradford, UK). Dyes were applied to bulk rolls of acrylic, polyester, and nylon fabrics at NC State School of Textiles pilot facility (Raleigh,

NC), at levels consistent with commercial use (2-4% by weight). Two rolls of acrylic, one of which was treated with repellent, were dyed with Basic Blue 159, Basic Yellow 28, and Basic Violet 16. Two rolls of nylon, one of which was treated with antistatic, were dyed with Acid Yellow 49, Acid Blue 45, and Acid Green 27. Two rolls of polyester, one of which was treated with soil release, were dyed with Disperse Yellow 114, Disperse Violet 77, and Disperse Red 60. Dyes standards and fabrics were stored in a dark room to avoid photodegradation.

#### *Extraction*

Single fibers were cut to lengths of 1 mm, and 0.5 mm in replicates of five samples, and placed in 2 mL Big Mouth screw thread autosampler vials with 250  $\mu$ L inserts (Laboratory Supplies Distributor, Millville, NJ). Extraction solvents for respective dye type were dispensed into the inserts in 100  $\mu$ L aliquots, and the vials were sealed and heated to 100°C in an oven. Vials were uncapped and solvents were evaporated at 80°C to dryness, and dye residues were reconstituted with 100  $\mu$ L of appropriate injection solvents. Acrylic fibers were extracted with 88% formic acid and reconstituted with 25% acetonitrile. Nylon fibers were extracted with equal parts pyridine, water, and ammonium hydroxide, and reconstituted with 10% acetonitrile. Polyester fibers were extracted with chlorobenzene and reconstituted with 70% acetonitrile.

### *Ultra-performance liquid chromatography*

Dyes were separated using a Waters (Milford, MA) Acquity UPLC system. The system was equipped with a room temperature sample manager and a Waters (Milford, MA) Acquity column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID  $\times$  50 mm length) heated to 40  $^{\circ}\text{C}$ . A BEH C18 column was used to separate disperse dyes. The stationary phase of the column used to separate disperse dyes was BEH C18, and the stationary phase of the column used to separate acid and basic dyes was CSH Phenyl-hexyl. The mobile phase solvent gradient conditions employed for all runs is listed in Table 3.1. The sample injection volumes were 10  $\mu\text{L}$ .

### *UV/Visible diode array detection*

Dyes samples were detected using a UV/visible diode array detector (Waters, Milford, MA) scanning absorbance from 350-675 nm. The peak area on the chromatogram was acquired for each dye using the corresponding maximum wavelength (Tables 3.2, 3.3, 3.4) and was used for comparison to standard dye mixtures to determine the amount of dye on each fiber.

## RESULTS AND DISCUSSION

### *Polyester (polyethylene terephthalate)*

Figure 3.1 shows the chromatographic separation of three disperse dyes extracted from polyester fibers 1 mm and sub-millimeter lengths. These dye

extracts exhibited the highest S/N ratio, promising detection even at lower analyte concentrations. Disperse dyes Yellow 114, Violet 77, and Red 60 were eluted in less than a minute using an isocratic chromatography method. Due to the hydrophobic characteristics of the fiber, a higher percentage of acetonitrile was required in the injection solvent to maintain solubility and reproducible peak areas. Relative dye amounts extracted from five replicate fibers of both 1 mm and sub-millimeter lengths are compared to the limits of detection calculated in the previous study using the standard deviation of the blank replicates, the lowest non-zero calibrator, and the y-intercept (Table 3.5).

#### *Acrylic (polyacrylonitrile)*

Figure 3.2 shows the chromatographic separation of three basic dyes extracted from acrylic fibers 1 mm and sub-millimeter lengths. Basic dyes Blue 159 and Violet 16 yield lower responses, indicating that for this fiber in particular, the dye bath used to color this dye contains a higher amount of Basic Yellow 28 than the other two dyes. Relative dye amounts extracted from five replicate 1 mm and sub millimeter fibers are compared to the limits of detection previously calculated based on the standard deviation of the blank replicates, the lowest non-zero calibrator, and the y-intercept (Table 3.6). To obtain sharp peaks, a mobile phase additive of ammonium acetate was necessary to prevent peak tailing and at least 10% acetonitrile required to reproducibly separate the dyes;

use of a completely aqueous injection solvent altered the separation, as portions of some dyes eluted with an adjacent compound, shifting peak area ratios.

*Nylon 6, 6 (polyamide)*

Figure 3.3 shows the chromatographic separation of three acid dyes extracted from nylon fibers 5 mm, 1 mm, and sub-millimeter lengths. Acid Blue 45 eluted at 0.38 minutes using a Waters Acquity CSH phenyl hexyl column. Efforts to develop a method using a Waters Acquity BEH C18 column failed because Acid Blue 45 would not retain if the starting mobile phase was majority aqueous, but Acid Green 27 would not retain if the starting mobile phase was majority organic. This poses a problem because lack of retention can cause separation problems, especially if a questioned fiber extract contains unknown dyes. Acid Blue 45 is an anthraquinone derivative with multiple polar substituents capable of hydrogen bonding. The C18 column was incapable of retaining this dye as the hydrophilic molecule favored interaction with a mostly aqueous mobile phase, causing it to elute at the dead time. Conversely, when gradient started with a larger percentage of organic mobile phase, Acid Green 27 was not retained. A phenyl hexyl column was employed because this stationary phase is capable of pi-pi\* interactions with the ring structures within the dye molecules. Because most, if not all, dyes are conjugated aromatic molecules, they

can be retained regardless of polarity. A gradient method of increasing organic percentage was still necessary to elute dyes Yellow 49 and Acid Green 27.

This separation yielded two peaks for Acid Green 27. This was also observed in the dye standard, leading to the conclusion that this dye has at least two components. Dye manufacturers often add additional compounds to achieve a particular color, and can contain impurities up to 5%. Acid Green 25 is another dye that is structurally similar to Acid Green 27 (Figure 3.4), differing only in the length of two symmetrical hydrocarbon substituents. Huang, *et al.*<sup>32</sup> found that both dyes produce a spectrum with the same absorptions, as well as peaks and valleys, so it is possible that both dyes are present in the dye standard and extract. Acid Green 25 has a lower molecular weight and lacks the elongated carbon chains that potentially interact with the stationary phase. As a result, we hypothesize that the faster eluting peak corresponds to Acid Green 25, and latter to Acid Green 27; this could be tested using tandem mass spectrometry to characterize the fragmentation pattern of each dye.

The UV/visible spectrum of Acid Green 27 is found to absorb in two regions of the visible spectrum correlating to yellow, at 422 nm, and blue, at 616 nm. This indicates that there are two chromophores in the molecule, each absorbing light at different wavelength maxima. When compared to a green dye

with only one maximum wavelength, this would confirm that the fibers are different.

The amounts of Acid Blue 45 and Acid Green 27 extracted from these fibers are difficult to detect in comparison to that of Acid Yellow 49. Therefore, extraction of a 5 mm long fiber (Figure 3.5) was included to show that these dyes are detectable at higher concentrations using this method. Figures 3.6, 3.7 and 3.8 illustrate the respective chromatograms from Figure 3.5 with reduced axis ranges to encompass each dye. The amounts of dyes extracted from five replicate 5 mm, 1 mm, and submillimeter fibers are compared to the limits of detection calculated in the previous study using the standard deviation of the blank replicates, the lowest non-zero calibrator, and the y-intercept (Table 3.7).

## CONCLUSIONS

We have demonstrated the ability to detect and identify dyes extracted from sub-millimeter length fibers, with the exception of Acid Green 27, using UV/visible spectra with retention time matching in ultra-performance liquid chromatograph. Acid Green 27 could be seen on a single 1 mm fiber, which is a reasonable length to be spared for analysis by a destructive method. Additionally, samples are reconstituted using 100  $\mu$ L of the injection solvent; this could potentially be concentrated two-fold, as a 50  $\mu$ L sample can still provide

up to four 10  $\mu$ L injections. The dye amounts extracted from these fibers above the detection limits, proving that this method is feasible for analysis and comparison of fibers in forensic investigations.



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## TABLES

Table 3.1. Liquid chromatography gradients used for:

(A) the separation of disperse dyes on a Waters BEH C18 column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is water and B is Acetonitrile

Time (min)	% A	% B
0.00	20	80
1.00	20	80

(B) the separation of basic dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	90	10
1.00	90	10

(C) the separation of acid dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	95	5
0.20	95	5
0.50	50	50
1.00	50	50
1.40	95	5
3.00	95	5

Table 3.2. Disperse Dyes

C. I. Name Formula Mol. Wt. (g/mol)	Structure	Absorption Spectrum Maximum Wavelength (nm)
Disperse Red 60 $C_{20}H_{13}NO_4$ 331.32		 514
Disperse Yellow 114 $C_{20}H_{16}N_5O_4$ 424.43		 424
Disperse Violet 77 $C_{21}H_{24}N_6O_5$ 440.45		 547

Table 3.3. Basic Dyes

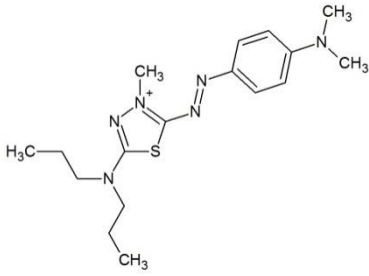
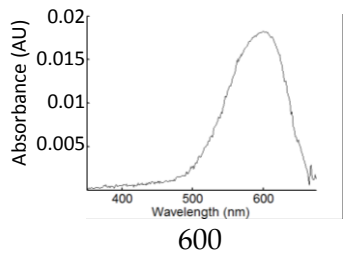
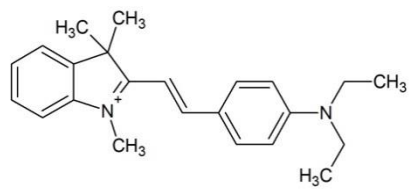
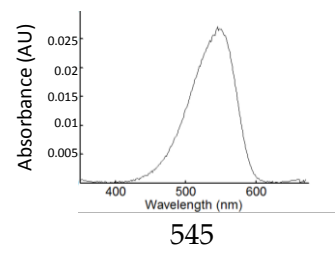
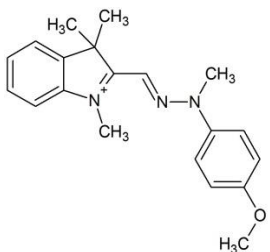
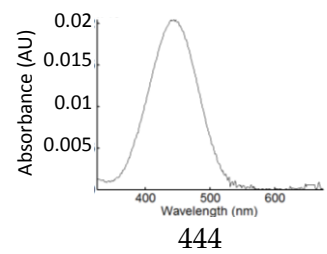
C. I. Name	Structure	Absorption Spectrum Maximum Wavelength (nm)
Formula		
Mol. Wt. (g/mol)		
<p>Basic Blue 159</p> <p><math>C_{17}H_{27}N_6S^+</math></p> <p>347.50</p>		 <p>600</p>
<p>Basic Violet 16</p> <p><math>C_{23}H_{29}N_2^+</math></p> <p>333.49</p>		 <p>545</p>
<p>Basic Yellow 28</p> <p><math>C_{20}H_{24}N_3O^+</math></p> <p>322.42</p>		 <p>444</p>

Table 3.4. Acid Dyes

C. I. Name	Formula	Mol. Wt. (g/mol)	Structure	Absorption Spectrum Maximum Wavelength (nm)
Acid Blue 45	$C_{14}H_9N_2O_{10}S_2$	430.37		 614
Acid Yellow 49	$C_{16}H_{13}Cl_2N_5O_3S$	426.28		 420
Acid Green 27	$C_{34}H_{34}N_2O_8S_2$	662.18		 422/616

Table 3.5. Dye concentrations in parts per billion for polyester 1 mm and sub-millimeter extracts; dye limits of detection calculated using  $\sigma_b$  estimated by the standard deviation of: replicate blanks (LOD<sub>1</sub>), lowest non-zero calibrator (LOD<sub>2</sub>), y-intercept (LOD<sub>3</sub>)

	0.5 mm extract	1 mm extract	LOD1	LOD 2	LOD 3
Disperse Red 60	42.92	150.60	1.65	1.69	0.68
Disperse Violet 77	30.39	106.18	1.28	0.27	0.31
Disperse Yellow 114	58.16	157.09	1.59	1.29	0.79

Table 3.6. Dye concentrations in parts per billion for acrylic 1 mm and sub-millimeter extracts; dye limits of detection calculated using  $\sigma_b$  estimated by the standard deviation of: replicate blanks (LOD<sub>1</sub>), lowest non-zero calibrator (LOD<sub>2</sub>), y-intercept (LOD<sub>3</sub>)

	0.5 mm extract	1 mm extract	LOD 1	LOD 2	LOD 3
Basic Blue 159	7.10	12.87	1.48	2.4	2.22
Basic Yellow 28	7.50	13.76	1.09	1.69	1.96
Basic Violet 16	22.23	53.55	2.33	1.96	2.08

Table 3.7. Dye concentrations in parts per billion for nylon 1 mm and sub-millimeter extracts; dye limits of detection calculated using  $\sigma_b$  estimated by the standard deviation of: replicate blanks (LOD<sub>1</sub>), lowest non-zero calibrator (LOD<sub>2</sub>), y-intercept (LOD<sub>3</sub>)

	0.5 mm extract	1 mm extract	LOD 1	LOD 2	LOD 3
Acid Blue 45	7.81	11.88	3.59	2.18	1.28
Acid Yellow 49	21.47	59.96	3.62	2.76	1.01
Acid Green 27	106.82	275.93	25.44	22.79	42.59



## FIGURES

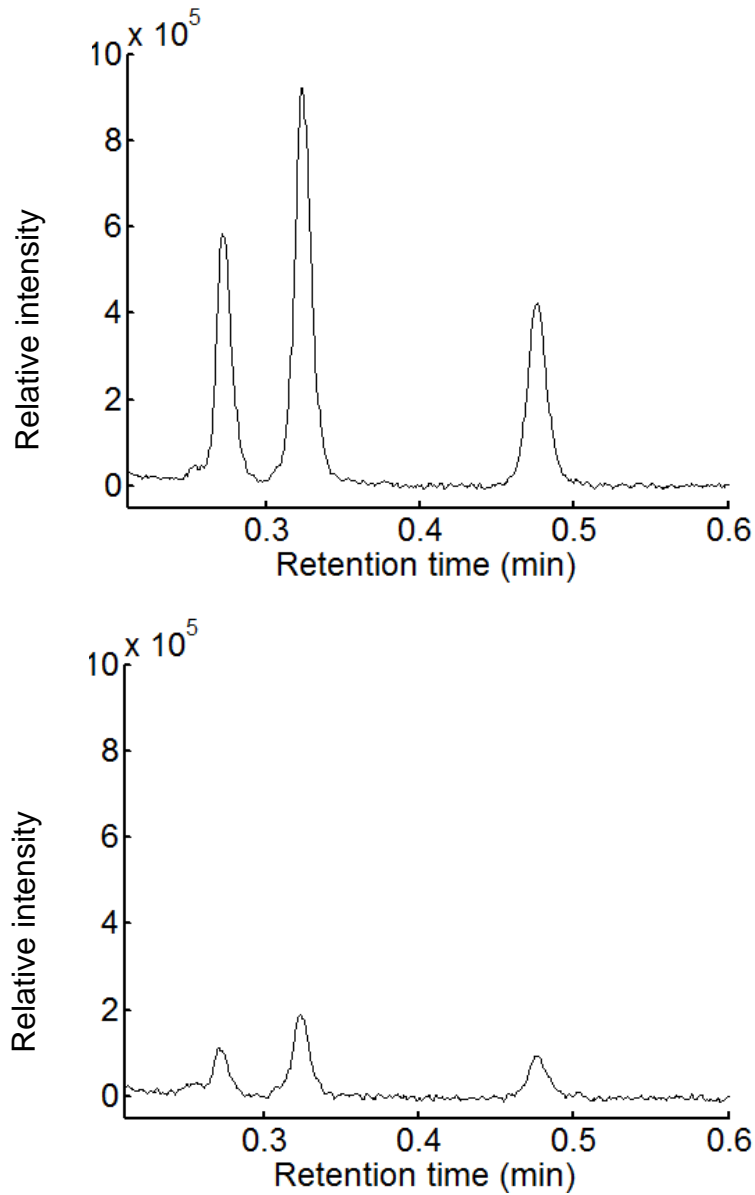


Figure 3.1. Disperse dyes (a) Yellow 114, (b) Violet 77, and (c) Red 60, extracted from unfinished polyester fiber at 1 mm (top) and sub-millimeter (bottom)

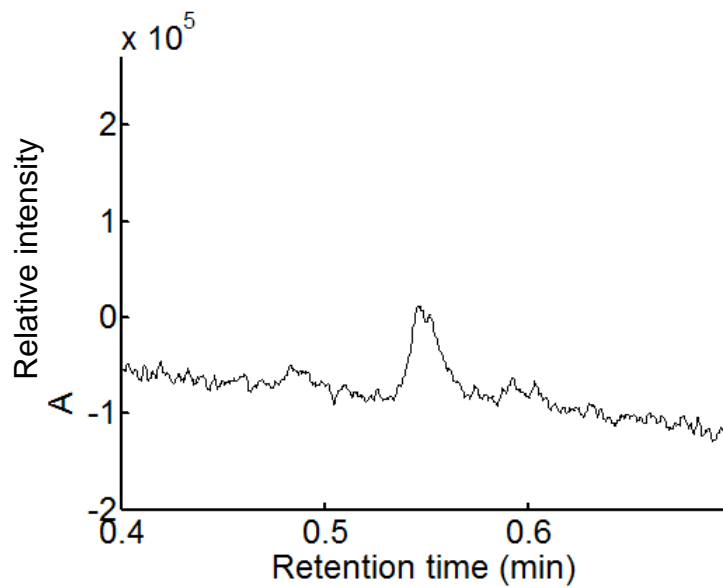
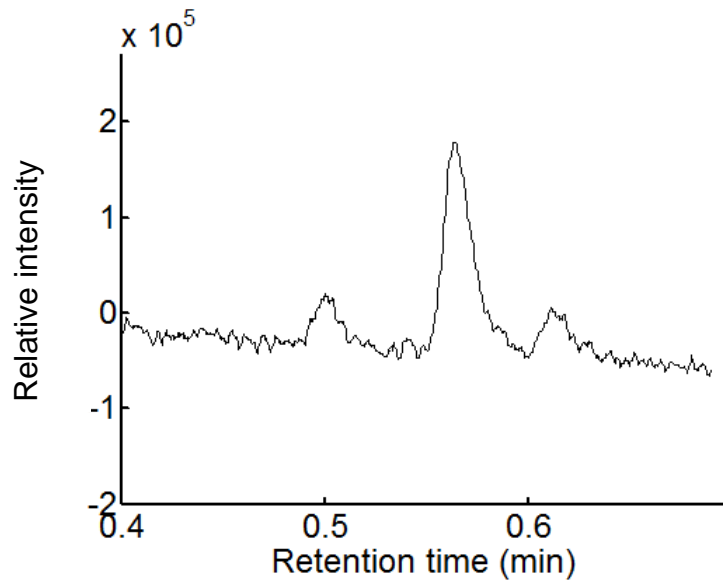


Figure 3.2. Basic dyes (a) Blue 159, (b) Yellow 28, and (c) Violet 16 extracted from unfinished acrylic fiber at 1 mm (top) and sub-millimeter (bottom)

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

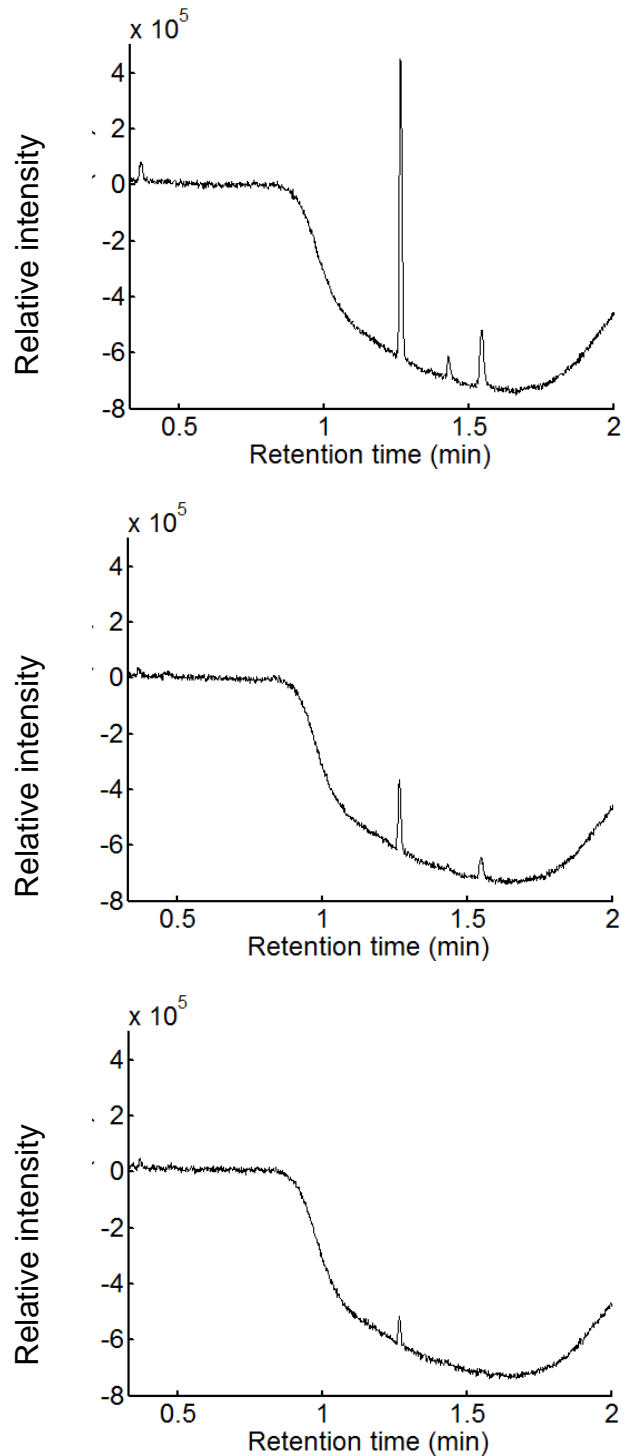


Figure 3.3. Acid dyes (a) Blue 45, (b) Yellow 49, and (c) Green 27 (two peaks), extracted from unfinished nylon fiber at 5 mm (top) and 1 mm (middle), submillimeter (bottom)

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

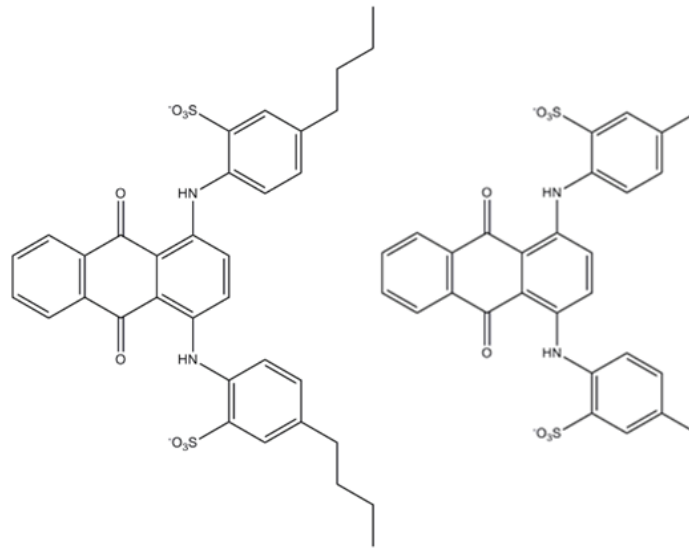


Figure 3.4. Dye structures for (a) Acid Green 27 and (b) Acid Green

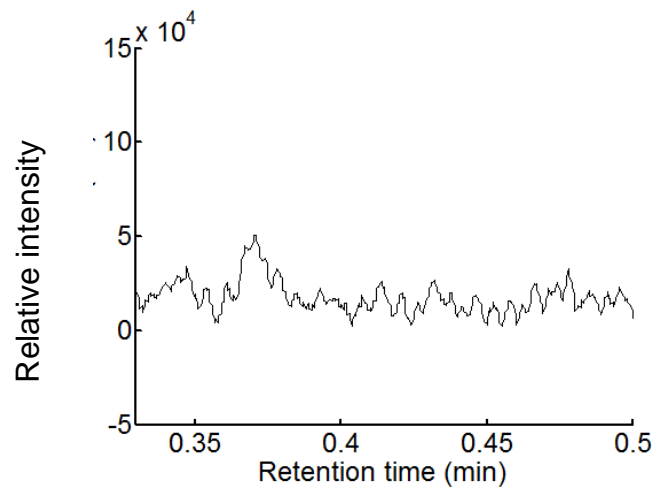
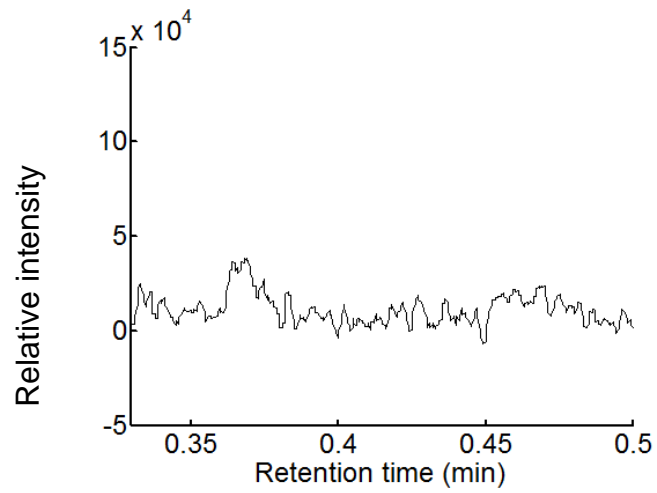
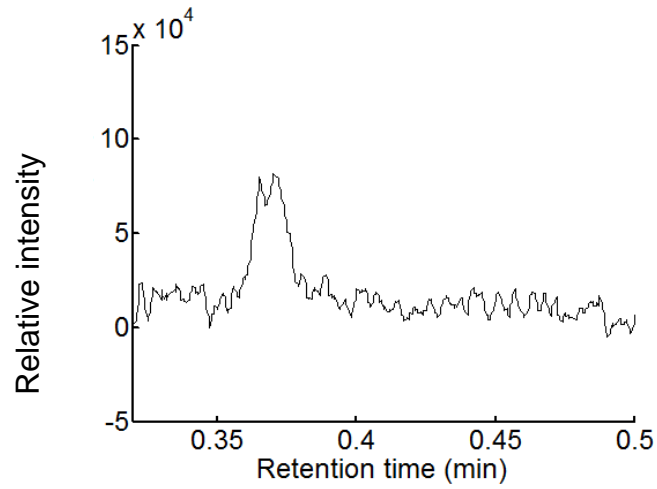


Figure 3.5. Acid Blue 45 extracted from unfinished nylon fiber at 5 mm (top) and 1 mm (middle), submillimeter (bottom)

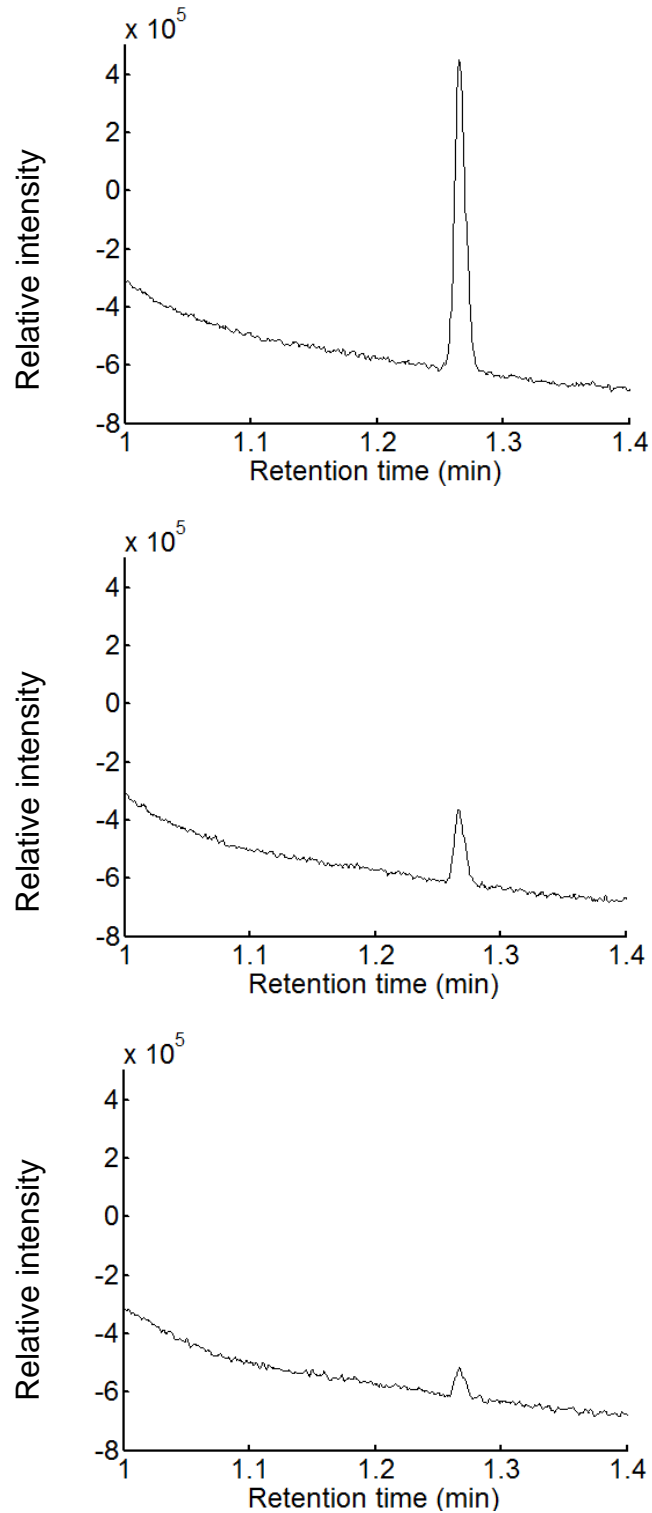


Figure 3.6. Acid Yellow 49 extracted from unfinished nylon fiber at 5 mm (top) and 1 mm (middle), submillimeter (bottom)

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

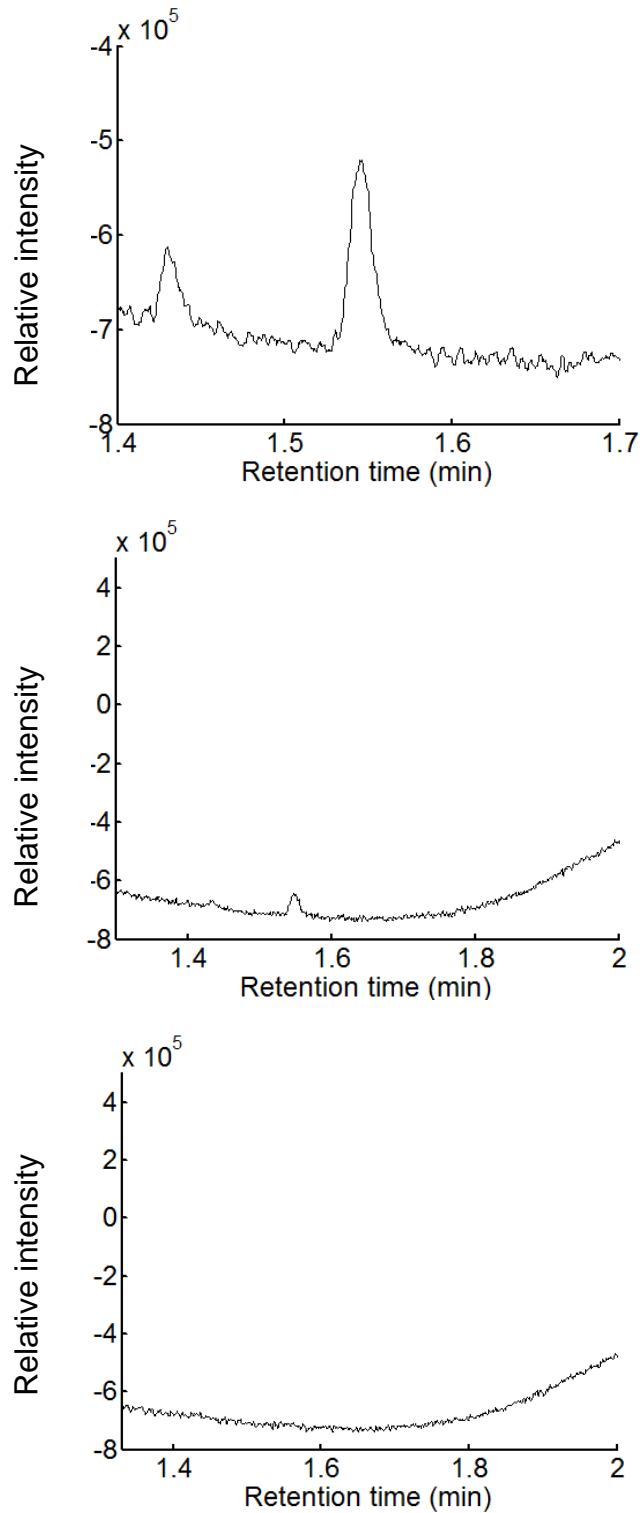


Figure 3.7. Acid Green 27 (two peaks), extracted from unfinished nylon fiber at 5 mm (top) and 1 mm (middle), submillimeter (bottom)

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

## CHAPTER FOUR

### EXTRACTION, SEPARATION, AND DETECTION OF DISPERSE, BASIC, AND ACID DYES FROM POLYESTER, ACRYLIC, AND NYLON FIBERS EXPOSED TO ENVIRONMENTAL WEATHERING

#### ABSTRACT

Fibers found in criminal investigations are rarely found in pristine condition. Loss of dye resulting from environmental exposure can complicate trace fiber examinations, as fibers can appear colorless or different shades when examined by microscopy. Implementation of a microextraction method, followed by ultra-performance liquid chromatography with UV/visible detection, is demonstrated on fibers that have been weathered two hot climates, differing in humidity. Disperse dyes from polyester, basic dyes from acrylic, and acid dyes from nylon are investigated. Dyes can be detected and identified on fibers that have been exposed to photodegradation and leaching for 12 months.



## INTRODUCTION

Examination of fibers collected from a crime scene can place a suspect in the location of the crime scene or establish contact with a victim. Material is exchanged any time two surfaces come into contact with one another, so in an altercation, fibers will be transferred from the suspect to the victim, as well as from the victim to the suspect. Comparison of questioned and known fiber evidence that reveals differences excludes a match between the two fibers, proving that they could not have come from the same source. Forensic fiber investigation is completed by comparison of physical and optical measurements to characterize the fibers with increasing discrimination. Methods for analysis include microscopy and spectroscopy to compare color and morphology, refractive indices, birefringence, and polymer type. When these techniques have been exhausted, and the fibers are indistinguishable, the significance of the fiber is increased with the conclusion that both samples possibly came from the same source.

Determination of polymer type and color alone does not allow for discrimination of dyes on a molecular level; many dyes with different structures emit similar absorptions. Fabrics are often colored dye mixtures, which may be comprised of different combinations of dyes to achieve the same shade.

Characterizing each dye individually could allow differentiation of two

seemingly identical fibers, or further confirm that the fibers are from the same origin. Dye extraction allows separation for analysis of each dye individually,<sup>1</sup> which is usually performed using thin layer chromatography, capillary electrophoresis, and high-performance liquid chromatography.<sup>2-13</sup> These techniques been demonstrated to separate dyes, although limitations include difficulty with detection of limited sample size using TLC,<sup>2-7</sup> ability to separate non-ionized dyes by CE,<sup>8-9</sup> and resolution of dyes due to band broadening and insufficient fiber lengths using HPLC.<sup>10-13</sup>

Textile fibers in crime scene investigation are rarely found in pristine condition. Over the lifetime of a garment or other textile product, dyes are leached or degraded, often resulting in seemingly colorless fibers. Missing person cases may turn into murder investigations if a body is found long after the victim's disappearance. Any evidence collected from a suspected abductor will be sealed, filed, and catalogued for future comparison of fibers found on a body. The effects of saltwater, temperature extremes, and decomposition by composting have been studied to identify changes that may compromise examination of biological polymer types (viscose rayon, azlon, polylactic acid). Optical properties, infrared spectroscopy, and physical properties such as melting points and solubility are used to determine polymer identification. Some

fibers are affected by water submersion, which could explain differences or strengthen the interpretation of fiber evidence.<sup>14</sup>

However, if the body has been exposed to high temperatures, rain, humidity, and photodegradation after prolonged periods outdoors, visual changes and color loss may complicate fiber analysis. These differences between the questioned and the known fiber could result in immediate elimination of a match using visual comparison or microscopy. Methods to analyze fibers with known dye identities that have been purposefully and systematically subjected to weathering conditions can be used to predict the mechanism causing spectral changes and dye loss. The technique used must be sensitive enough to detect low analyte quantities and monitor any spectral changes as a function of time. Fibers found after long periods of exposure are available in larger quantities than the fibers transferred during a crime. Fibers are some of the most fleeting evidence found at a crime scene, and must be collected quickly and carefully to prevent secondary transfer or contamination by fibers shed by the clothing of investigators. As such, fibers from primary transfer would not persist or provide reliable testimony if found long after an investigation; only larger pieces would provide evidential value.

Ultra-performance liquid chromatography allows lower limits of detection than those found using liquid chromatography, because the higher pressure

pumps and smaller particle sizes synergistically reduce band broadening. These sharper peaks can be detected using UV/visible spectroscopy, which can be used to observe changes in the visible spectrum due to photodegradation or disproportionate dye loss on a fabric with multiple dyes. Dye amounts and spectral changes will be compared to dyed textile standards to determine if this method confirm or reject the possibility of a match with <0.05% chance of a false negative.

## EXPERIMENTAL

Chlorobenzene was purchased from Acros Organics (Morris, NJ) and pyridine was purchased from Mallinckrodt Baker (Phillipsburg, NJ). Formic acid, HPLC grade water, ammonium hydroxide, HPLC grade ammonium acetate, and HPLC grade acetonitrile were purchased from Fisher Scientific (Pittsburg, PA).

Dye standards and finishing agents were donated by dyestuff manufacturers in the southeastern United States. Acid and disperse dyes were solid in phase and basic dyes were liquid in phase. Dyes reported here are named in accordance with the Color Index International database (Society of Dyers and Colourists, Bradford, UK). Dyes were applied to bulk rolls of acrylic, polyester, and nylon fabrics at NC State School of Textiles pilot facility (Raleigh, NC), at levels consistent with commercial use (2-4% by weight). Two rolls of

acrylic, one of which was treated with repellent, were dyed with Basic Blue 159, Basic Yellow 28, and Basic Violet 16. Two rolls of nylon, one of which was treated with antistatic, were dyed with Acid Yellow 49, Acid Blue 45, and Acid Green 27. Two rolls of polyester, one of which was treated with soil release, were dyed with Disperse Yellow 114, Disperse Violet 77, and Disperse Red 60. Dyes standards and fabrics were stored in a dark room to avoid photodegradation.

### *Fiber weathering*

Two rolls of polyester, one of which was treated with soil release, were dyed with Disperse Yellow 114, Disperse Violet 77, and Disperse Red 60. Two rolls of acrylic, one of which was treated with repellent, were dyed with Basic Blue 159, Basic Yellow 28, and Basic Violet 16. Two rolls of nylon, one of which was treated with antistatic, were dyed with Acid Yellow 49, Acid Blue 45, and Acid Green 27. These fabrics were dyed at the NC State School of Textiles pilot facility (Raleigh, NC). Rectangular swatches of acrylic, nylon, and polyester fiber samples were prepared and sent to exposure testing sites for natural outdoor weathering in Phoenix, AZ and Miami, FL. Both climates average approximately 22-23 °C throughout the year, but Miami experiences five times more rainfall than the desert. Samples were exposed for up to one year in these conditions, with samples retired at 3 month intervals up to 12 months. The exposure protocol followed ASTM G 147-02(26) and 7-05 (27), with the most exposed

samples (12 months) subjected to a total of 341 MJ/m<sup>2</sup> (Arizona) and 309 MJ/m<sup>2</sup> (Florida) of UV light (295-385 nm). Two different sets of conditions were used: the hot and arid climate of Arizona, and hot and humid climate of Florida. Samples left outside in either Miami or Phoenix after one year of exposure show substantial color loss to the extent that the original color is not easily perceived.

### *Extraction*

Five replicate single fibers were cut to lengths of 1 cm and placed in 2 mL Big Mouth screw thread autosampler vials with 250 µL inserts. Extraction solvents for respective dye type were dispensed into the inserts in 100 µL aliquots, and the vials were sealed and heated to 100°C in an oven. Vials were uncapped and solvents were evaporated at 80°C to dryness, and dye residues were reconstituted with 100 µL of appropriate injection solvents. Acrylic fibers were extracted with 88% formic acid and reconstituted with 25% acetonitrile. Nylon fibers were extracted with equal parts pyridine, water, and ammonium hydroxide, and reconstituted with 10% acetonitrile. Polyester fibers were extracted with chlorobenzene and reconstituted with 70% acetonitrile.

### *Ultra-performance liquid chromatography*

Dyes were separated using a Waters (Milford, MA) Acquity UPLC system. The system was equipped with a room temperature sample manager and a

Waters (Milford, MA) Acquity column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID  $\times$  50 mm length) heated to 40  $^{\circ}\text{C}$ . A BEH C18 column was used to separate disperse dyes. The stationary phase of the column used to separate disperse dyes was BEH C18, and the stationary phase of the column used to separate acid and basic dyes was CSH Phenyl-hexyl. The mobile phase solvent gradient conditions employed for all runs is listed in Table 4.1. The sample injection volumes were 10  $\mu\text{L}$ .

#### *UV/Visible diode array detection*

Dyes samples were detected using a UV/visible diode array detector (Waters, Milford, MA) scanning absorbance from 350-675 nm. The peak area on the chromatogram was acquired for each dye using the corresponding maximum wavelength Table 4.2, and was used for comparison to standard dye mixtures to determine the amount of dye on each fiber.

## RESULTS AND DISCUSSION

### *Polyester*

Polyester fabric has high wash and light fastness because it is non-polar and the dyes are interspersed within the fiber structure, although the exposed fabric showed moderate photofading. A decrease in chromatographic response of disperse dyes Violet 77, Yellow 114, and Red 60 can be seen over 3 month intervals. Figure 4.1 demonstrates the change in amounts of dye on a 1 cm

polyester fiber weathered in Florida for 3, 6, 9 and 12 months. It is apparently that the rate of dye loss is greatest in the period from 0-3 months. Figure 4.2 depicts the weathering rates during the same time period, but in Arizona. There is a large decrease in dye amounts over the 0-3-month time frame, but remains relatively the same from 3-6 months. The period from 6-9 months is also characterized by a large decrease, with a slight decrease from 9-12 months. Over a 12-month period, Disperse Violet 77 and disperse yellow 114 less dye is left on the fabrics in Arizona than in Florida. Disperse Yellow 114 and Violet 77 weather faster in hot, arid conditions, while the amount of Disperse Red 60 was relatively constant. This indicates that dyes do not degrade at the same rate depending on outdoor conditions. Additionally, as they do not degrade at the same rate, the amounts of dyes relative to one another will not necessarily remain constant over time. A factor affecting the rates of degradation could be the differences in climate at particular times of the year combined with the particular fiber chemistry.

### *Acrylic*

The changes in the amounts of basic dyes Blue 159, Violet 16, and Yellow 49 were measured at 3 month intervals in Florida (Figure 4.3) and Arizona (Figure 4.). The decrease is consistent between corresponding dye levels, with a moderately higher level of dye loss in Arizona.



## *Nylon*

Chromatograms demonstrating the dye amounts on nylon fibers over a year at 3 month intervals are shown in Figures 4.5 and 4.6. Successive figures show the same chromatograms, enlarged with smaller axis ranges. Acid dyes Blue 45 (Figures 4.7 and 4.8) and Yellow 49 (Figures 4.9 and 4.10) appear to weather at faster rates in Florida than Arizona over entire exposure period. Acid Green 27 levels seem to remain constant after 3 months in Florida (Figure 4.11), while the decrease in levels of Acid Green 27 weathered in Arizona (Figure 4.12) seems to briefly plateau in the 3-6-month range, and again in the 9-12-month period. This could be due to seasonal climate changes in each location, along with specific dye and polymer chemistries. Acid Blue 45 is an anthraquinone dye with hydroxyl and amino auxophores, which contribute to dye instability, because absorbed light converts the stable keto form to the excited state enol form. The enol form of the dye is vulnerable to attack by the polar nylon polymer, resulting in loss of chromophoric activity. Acid Green 27, conversely, has bulky aromatic substituents that dissipate the energy absorbed by rotation around a single bond. The peak for Acid Yellow 49 decreases rapidly in both Florida and Arizona; this may be attributed to photooxidation of the azo dye.

## CONCLUSIONS

Dye losses are observed in polyester, acrylic, and nylon textiles that are the result of environmental weathering in a hot, humid climate, as well as a hot, arid climate. Although textiles subjected to photodegradation processes are found in larger quantities, the detection of dyes on 1 cm fibers has been demonstrated. Dyes are found to degrade at different rates depending on the polymer, dye class, and environmental conditions. Some nylon dyes were difficult to detect at 9 and 12 months; however, the larger amounts of fiber evidence available after months of exposure would allow more fibers to be analyzed at a time, concentrating the analyte injected for chromatographic separation and UV/visible detection.

## FIGURES

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## TABLES

Table 4.1. Liquid chromatography gradients used for:

(A) the separation of disperse dyes on a Waters BEH C18 column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is water and B is Acetonitrile

Time (min)	% A	% B
0.00	20	80
1.00	20	80

(B) the separation of basic dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	90	10
1.00	90	10

(C) the separation of acid dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	95	5
0.20	95	5
0.50	50	50
1.00	50	50
1.40	95	5
3.00	95	5

Table 4.2. Maximum absorption wavelengths:

Dyes	Maximum $\lambda$ (nm)
Disperse Red 60	514
Disperse Yellow 114	424
Disperse Violet 77	547
Basic Blue 159	600
Basic Violet 16	545
Basic Yellow 28	444
Acid Blue 45	614
Acid Yellow 49	420
Acid Green 27	422/616

FIGURES

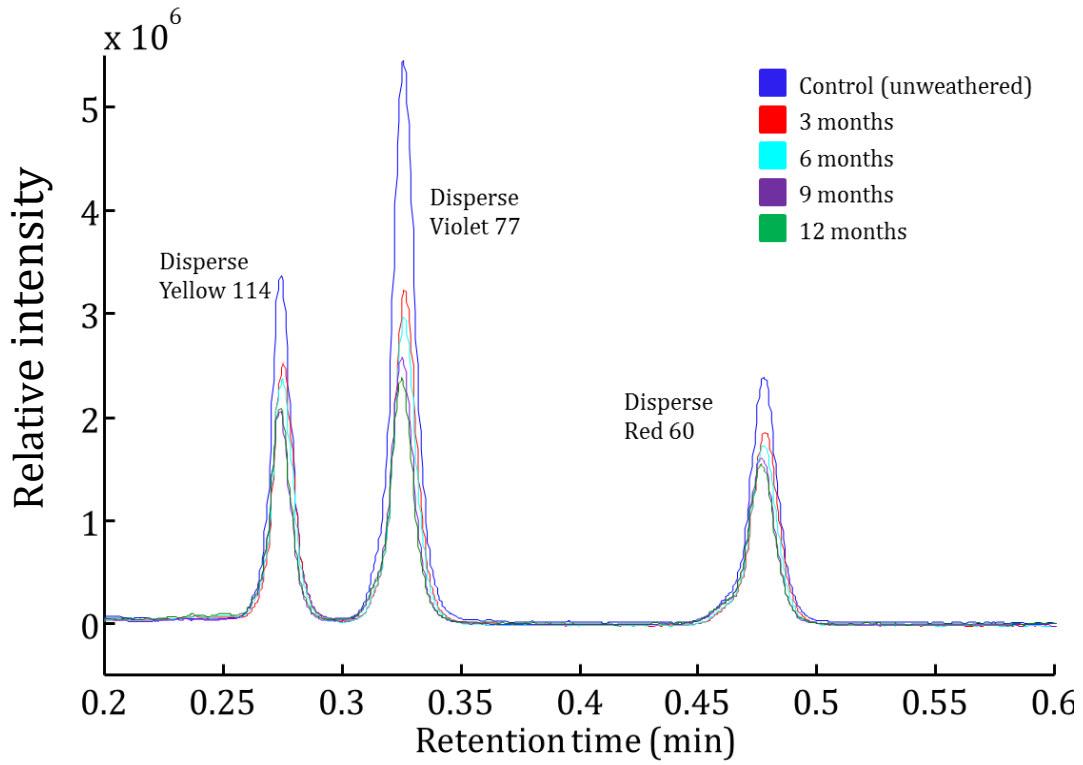


Figure 4.1. Comparison of dyes amounts extracted from polyester fibers weathered in Florida for 0, 3, 6, 9 and 12 months.

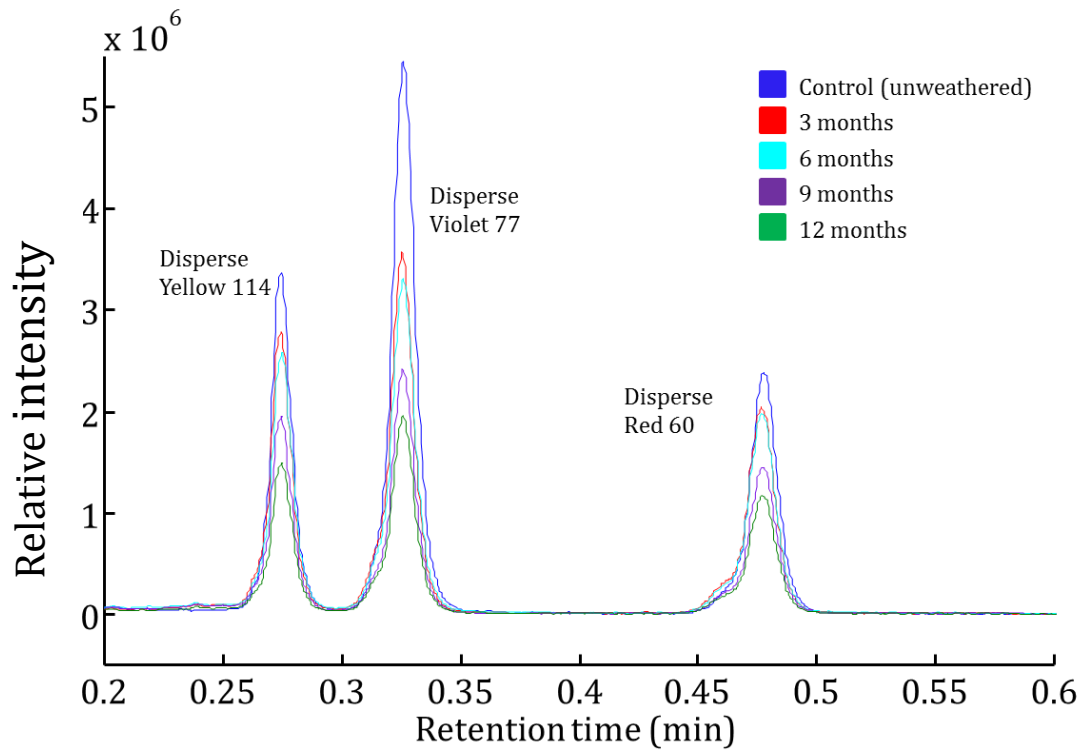


Figure 4.2. Comparison of dyes amounts extracted from polyester fibers weathered in Arizona for 0, 3, 6, 9 and 12 months.



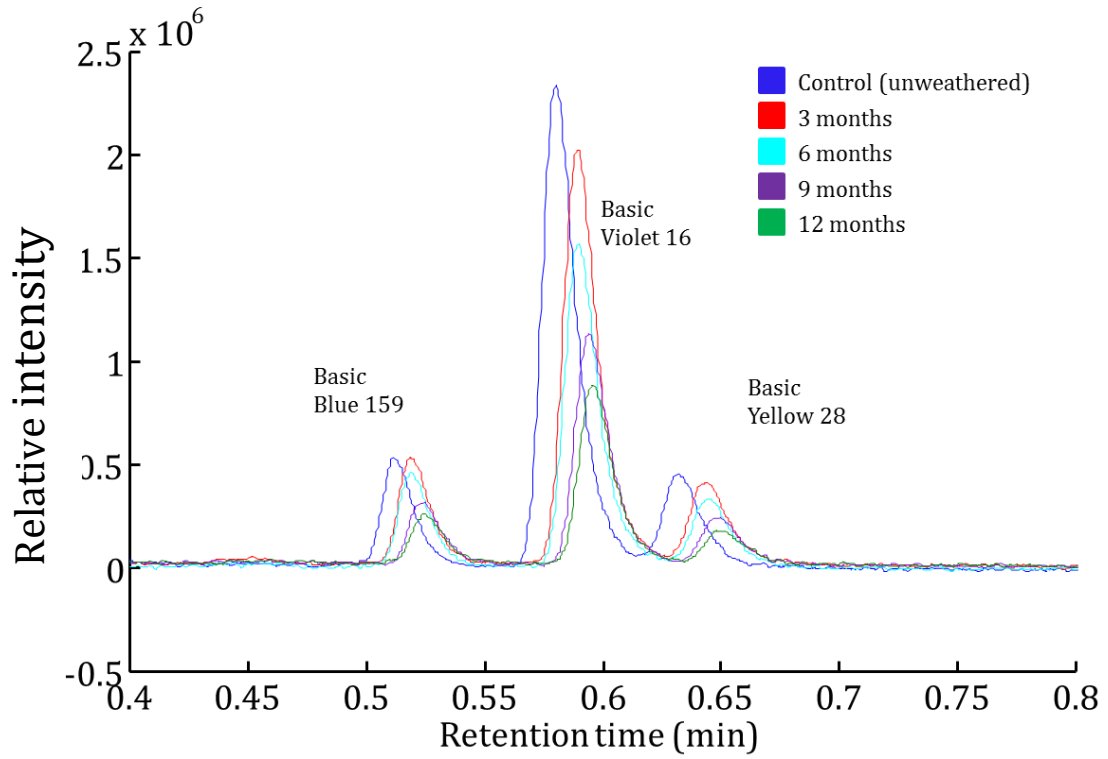


Figure 4.3. Comparison of dyes amounts extracted from acrylic fibers weathered in Florida for 0, 3, 6, 9 and 12 months.

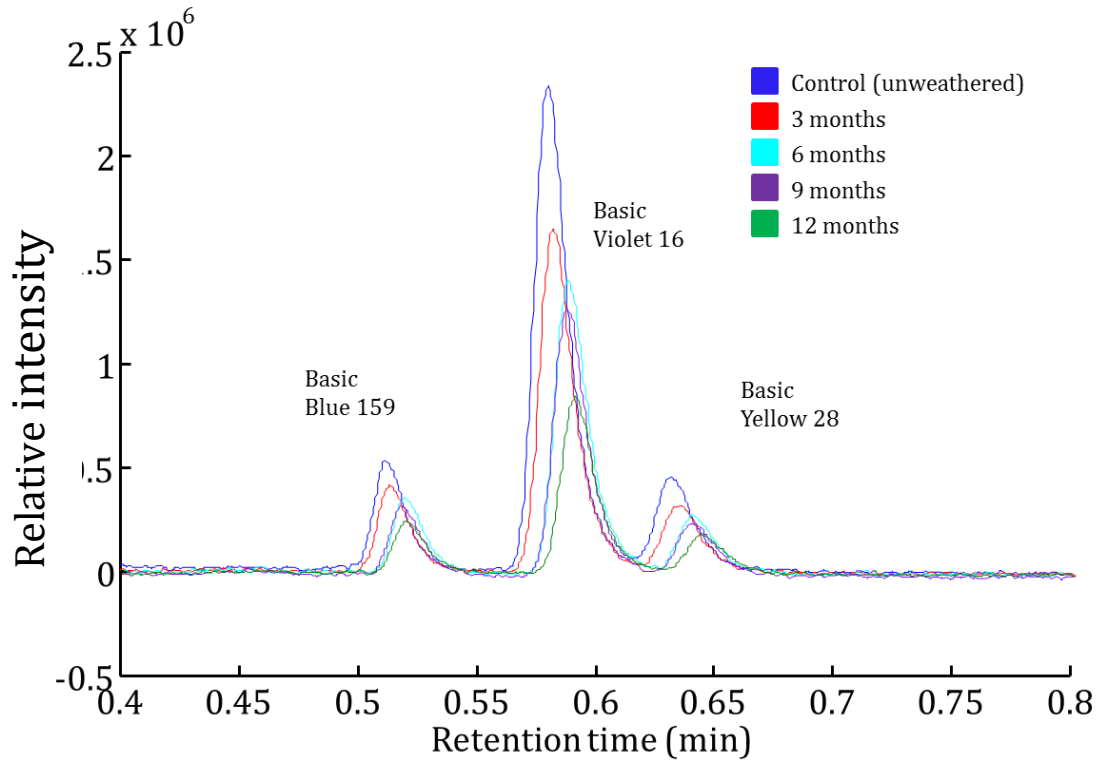


Figure 4.4. Comparison of dyes amounts extracted from acrylic fibers weathered in Arizona for 0, 3, 6, 9 and 12 months.

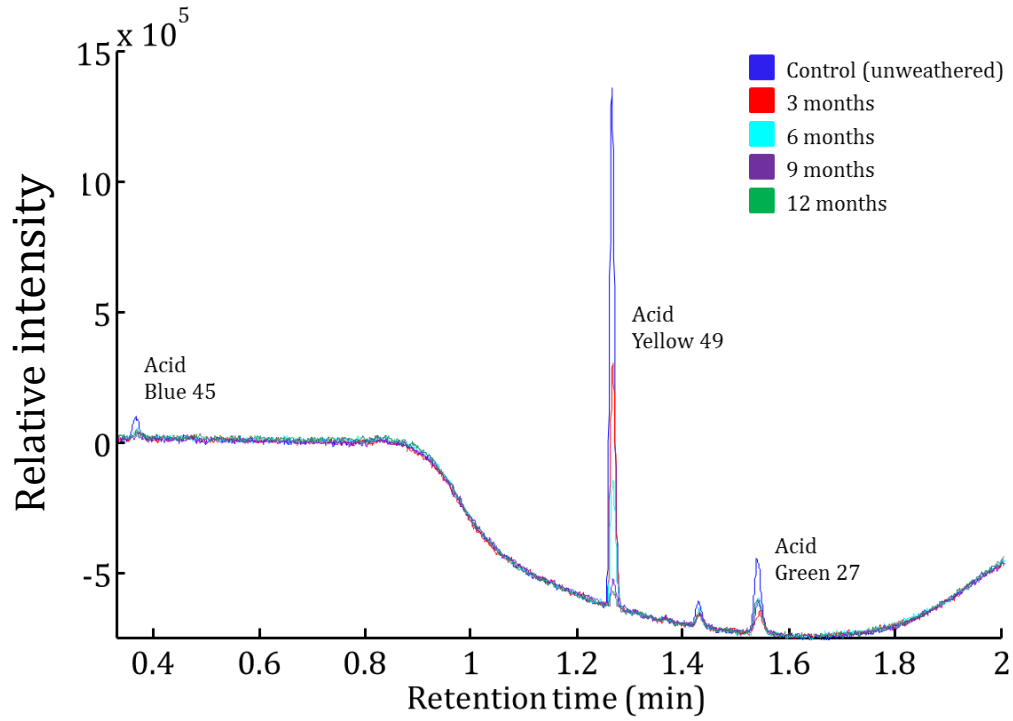


Figure 4.5. Comparison of dyes amounts extracted from nylon fibers weathered in Florida for 0, 3, 6, 9 and 12 months.

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

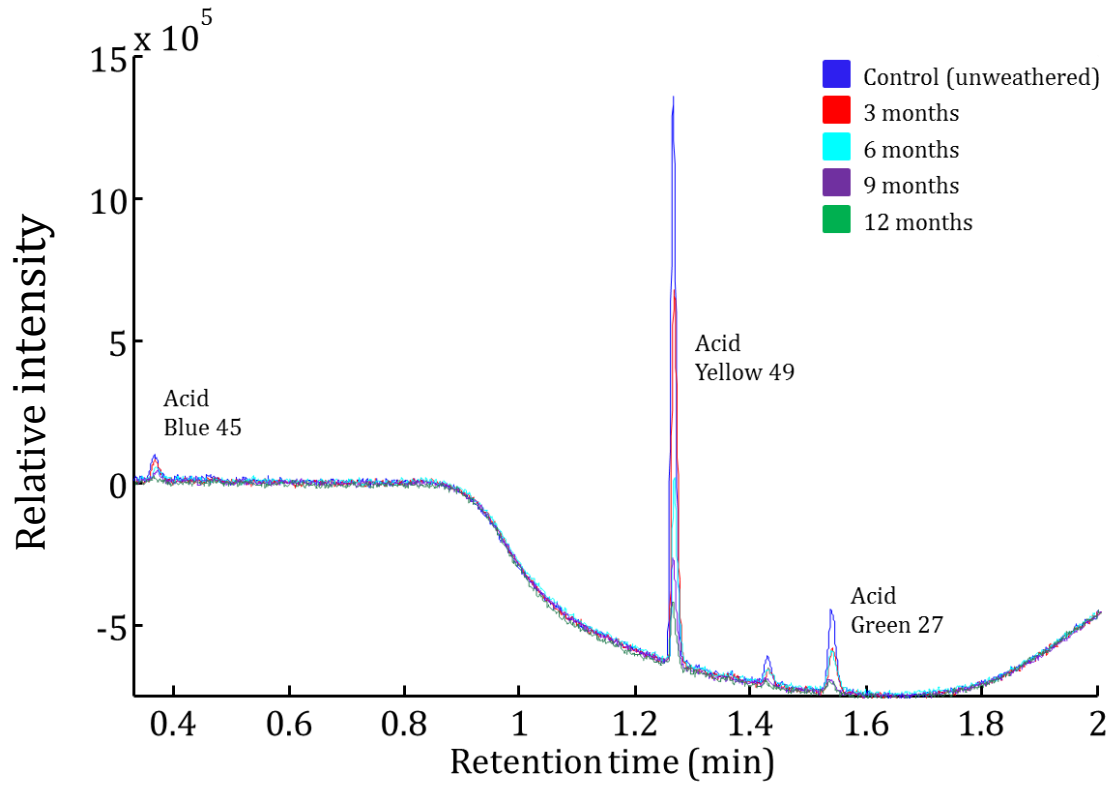


Figure 4.6. Comparison of dyes amounts extracted from nylon fibers weathered in Arizona for 0, 3, 6, 9 and 12 months.

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

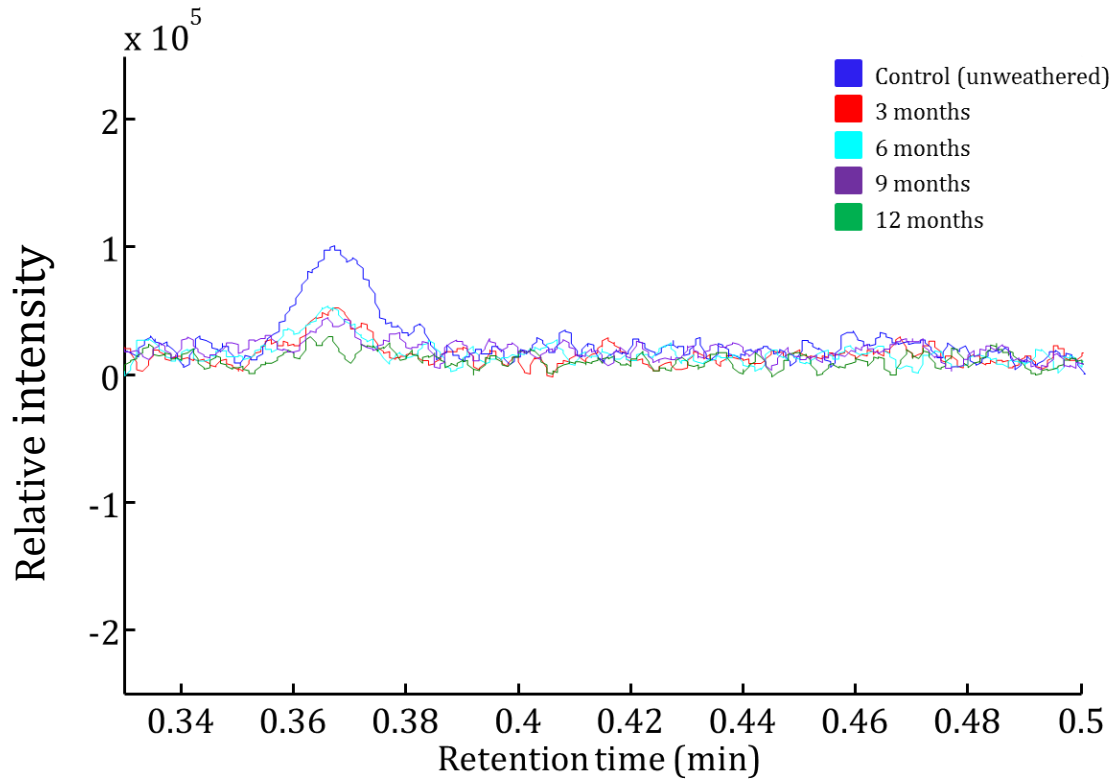


Figure 4.7. Comparison of amounts of Acid Blue 45 from nylon fibers weathered in Florida for 0, 3, 6, 9 and 12 months

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

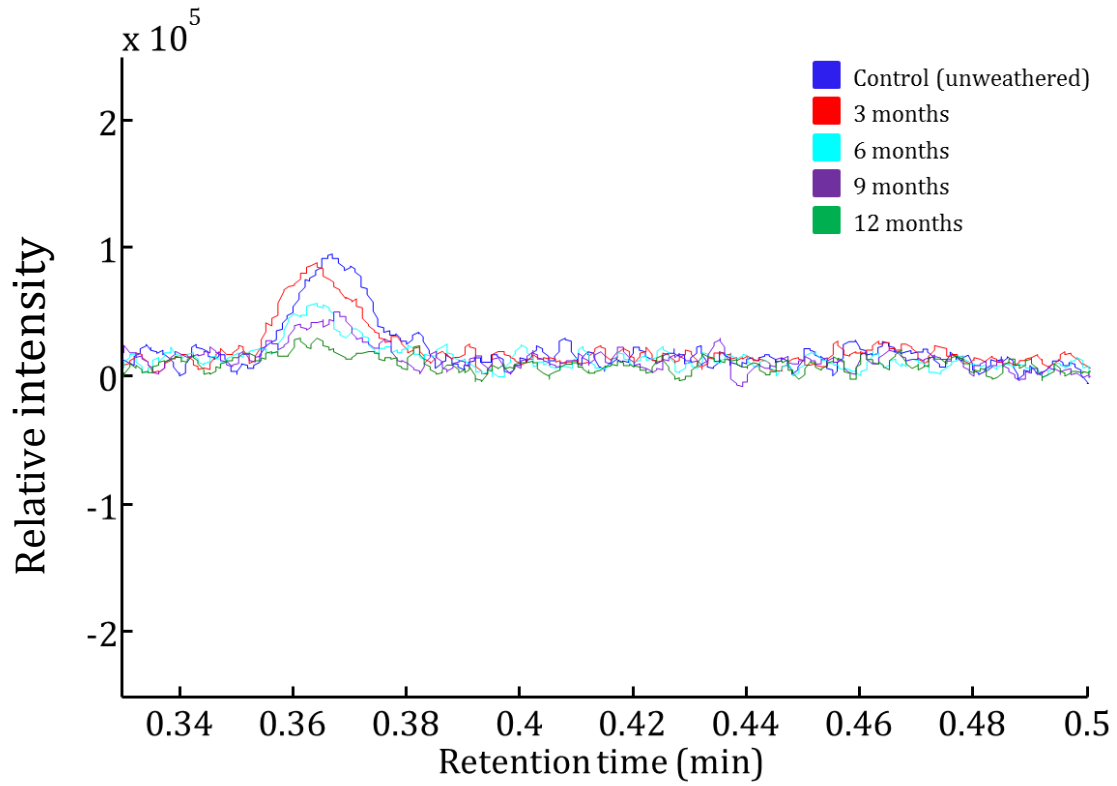


Figure 4.8. Comparison of amounts of Acid Blue 45 from nylon fibers weathered in Arizona for 0, 3, 6, 9 and 12 months

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

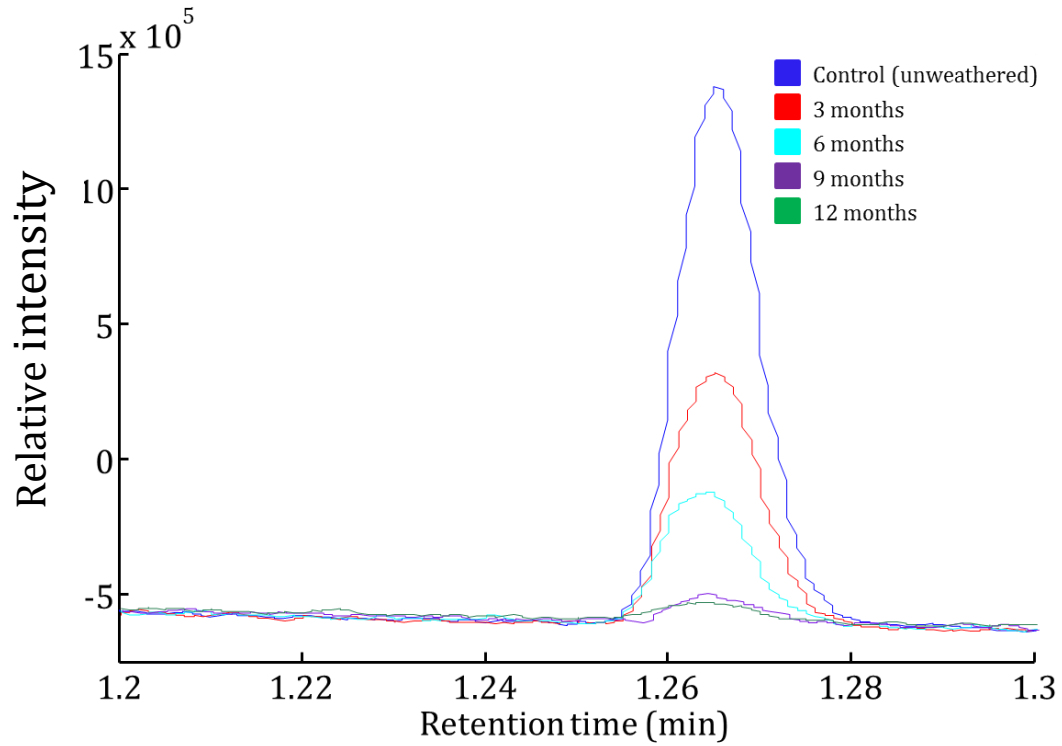


Figure 4.9. Comparison of amounts of Acid Yellow 49 from nylon fibers weathered in Florida for 0, 3, 6, 9 and 12 months.

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

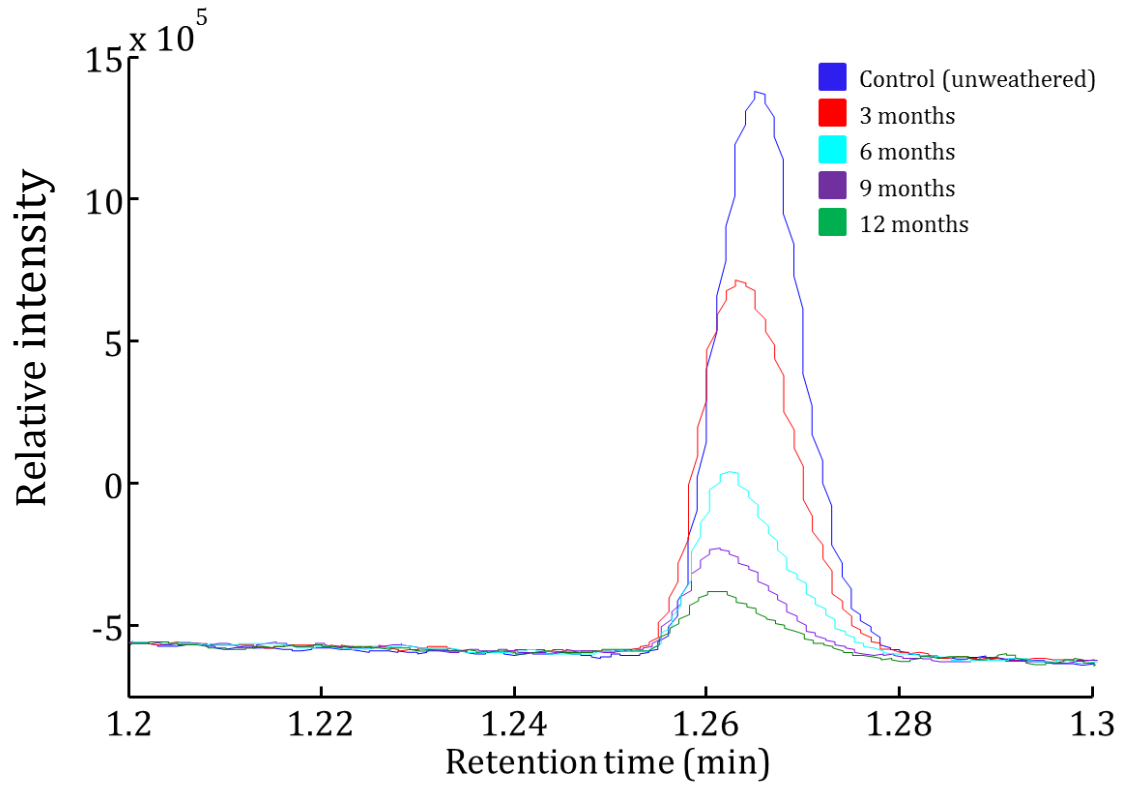


Figure 4.10. Comparison of amounts of Acid Yellow 49 from nylon fibers weathered in Arizona for 0, 3, 6, 9 and 12 months.

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient



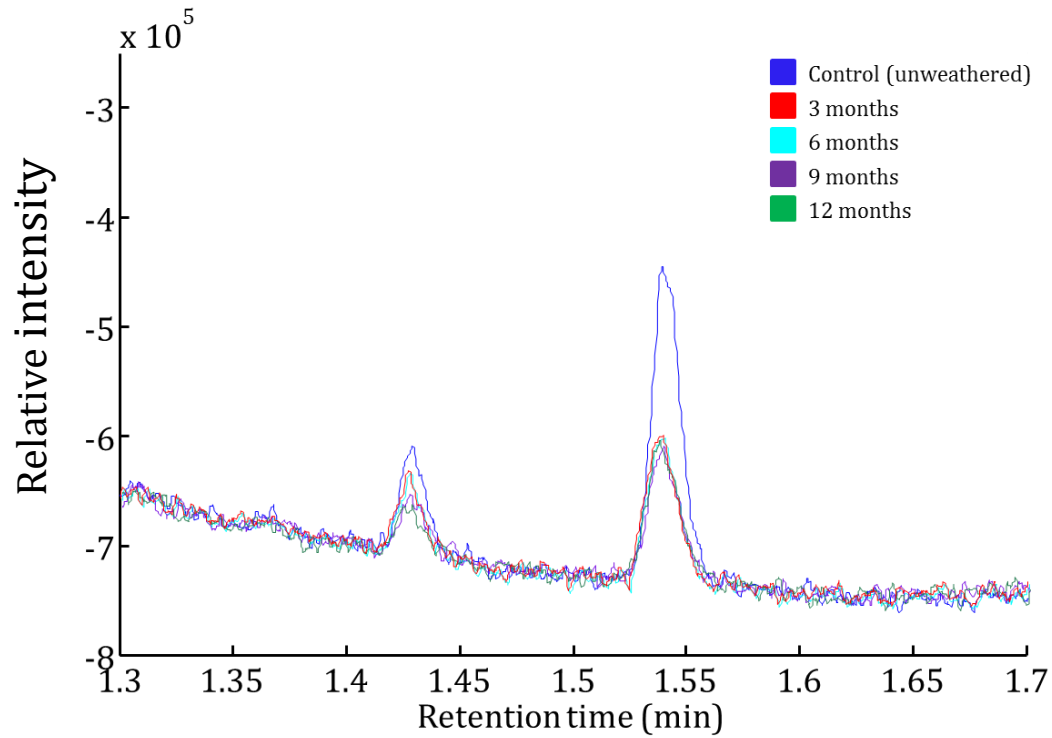


Figure 4.11. Comparison of amounts of Acid Green 27 from nylon fibers weathered in Florida for 0, 3, 6, 9 and 12 months.

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

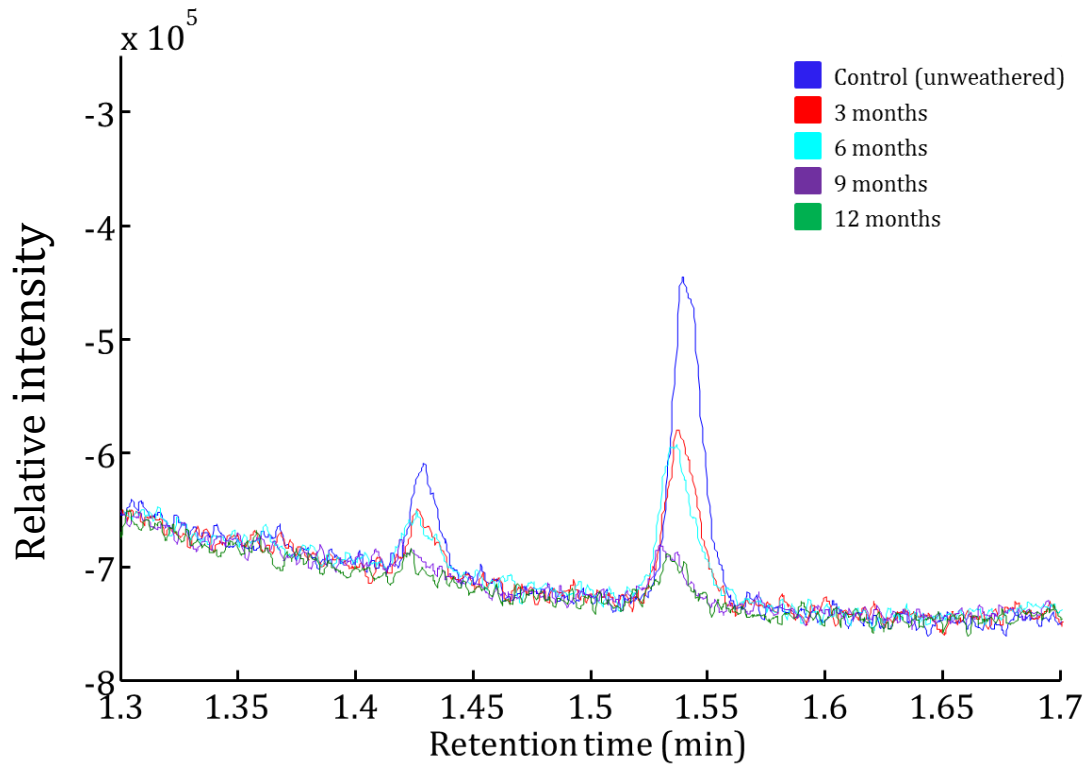


Figure 4.12. Comparison of amounts of Acid Green 27 from nylon fibers weathered in Arizona for 0, 3, 6, 9 and 12 months.

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

## CHAPTER FIVE

### DETERGENTS, BLEACH, AND STAIN REMOVERS: EFFECTS ON FORENSIC FIBER INVESTIGATIONS

#### ABSTRACT

Forensic trace fiber evidence is often subjected to laundering before it is found in a criminal investigation. Dyes are extracted from synthetic fibers that have been washed using detergents with bleach and Clorox 2<sup>®</sup>. Acrylic and polyesters show little change in dye intensity using any of the cleaning agents. Nylon fibers show substantial color loss as a result of laundering, with the greatest loss observed in fabrics washed with detergent and bleach. Ultra-performance liquid chromatography and UV/visible diode array detection are employed to evaluate the degree of weathering caused by repeated laundering cycles, and determine if the dyes are present on fibers that appear colorless.

## INTRODUCTION

Textile fibers collected after an altercation has taken place can provide indirect evidence leading to an association between suspect and the victim. Comparison of a questioned fiber and a known fiber can reveal if contact occurred, which would result in an exchange of material. Techniques are used to evaluate discriminating properties of the fibers, including polarized light microscopy, UV/visible microspectrophotometry, and infrared spectroscopy. Differences in color, morphology, birefringence, refractive indices, and polymer type allow a fiber examiner to successfully exclude a match. If the fibers cannot be distinguished by any of these methods, it is determined that they could have the same origin. It is impossible to determine this with complete certainty because textiles are mass produced, they could come from separate, yet identical sources.

When all microscopic techniques and spectral measurements have been completed, and the results between the questioned and known fibers are found to be consistent, the evidence is found to be significant, as a match cannot be excluded. However, multiple combinations of dyes can be used to produce the same color, so differences in the visible spectra may be difficult to recognize, with subtle differences in the rise and fall of the maximum wavelength absorptions.<sup>1</sup> A method capable of analyzing each dye component individually

could characterize any dissimilarities or further confirm the possibility of a match on a molecular level.

Polyester, acrylic and nylon fibers are the most frequently encountered synthetic textiles in forensic investigations.<sup>2,3</sup> The dye components from each fiber must be separated prior to individual analysis, which is completed using microextraction protocol specific to polymer and dye class chemistries that have been proposed in the literature.<sup>4</sup> TLC is a currently used separation technique, which is implemented with UV/visible spectroscopy for color comparison, but it is invariable for application to limited samples sizes or lightly colored fibers.<sup>5-10</sup> Capillary electrophoresis has been shown to effectively separate acid and basic dyes using their ionic character, but disperse dyes cannot be separated due to their inability to ionize.<sup>11,12</sup> High performance liquid chromatography has been paired with UV/visible spectroscopy or mass spectrometry, but is often unable to detect low amounts of dye or distinguish between structurally similar dyes, due to band broadening.<sup>13-16</sup>

Fiber evidence often contains degraded polymers and dyes, or dye loss in general, due to weathering conditions encountered over the lifetime of a garment. Each time a fabric is laundered, there is a potential for dye leaching as a result of less than optimal color fastness. Integrity of the dye structure may be compromised by exposure to detergent constituents, including surfactants and

fluorescent brighteners, bleach, or stain removers. Mujumdar, *et al.*<sup>17</sup> used fluorescence microscopy of single acrylic, cotton, and nylon fibers to distinguish washed fibers from unwashed fibers. Cotton and nylon textiles washed with detergents containing whitening agents could always be distinguished from unwashed textiles; little detergent was characterized with acrylic fibers, and subsequently not recommended for classifying washed from unwashed textiles. In addition to color loss, there is potential for changes in the UV/visible spectra resulting from interaction of detergent chemicals. Characterization of the spectral changes of dyes extracted from fiber evidence could provide explanation for complications in fiber analysis due to exposure to detergents, dyes, and stain removers after multiple laundering cycles.

Ultra-performance liquid chromatography coupled to UV/visible spectroscopy enables comparison of textiles with low levels of dyes resulting from repeated laundering. Spectral changes will be monitored for each dye after exposure to 5x, 25x, and 50x wash cycles using Tide<sup>®</sup>, Gain<sup>®</sup>, or Wisk<sup>®</sup>, as well as each detergent with bleach or Clorox 2<sup>®</sup>. Tide<sup>®</sup>, Gain<sup>®</sup>, and Wisk<sup>®</sup> are detergents that can be used alone or in conjunction with bleach or Clorox 2<sup>®</sup>. Bleach is chemical agent that oxidizes dyes by breaking the bonds of the chromophore, as well as stains. Clorox 2<sup>®</sup> is a stain remover and color booster that is recommended for laundering colored garments, as the 'color-friendly' additive

does is not as harsh as bleach. The effects of these laundering chemicals that complicate fiber examination on each dye will be demonstrated by spectral comparison. Dyes will be extracted from fibers washed and dried for 5x, 25x, and 50x cycles with commercial detergents with or without stain-fighting laundering additives. The goal of this study is to determine if dyes can be detected after repeated washes, as well as to determine if fibers can be characterized despite dye loss and possible structural changes that result from laundering. Dyes will be extracted from fibers exposed to these conditions and analyzed by UPLC to compare any trends in dye leaching that can be attributed to a specific detergent.

## EXPERIMENTAL

Formic acid, HPLC grade water, ammonium hydroxide, HPLC grade ammonium acetate, and HPLC grade acetonitrile were purchased from Fisher Scientific (Pittsburg, PA). Chlorobenzene was purchased from Acros Organics (Morris, NJ) and pyridine was purchased from Mallinckrodt Baker (Phillipsburg, NJ).

Dye standards were donated by dyestuff manufacturers in the southeastern United States. Acid and disperse dyes were solid in phase and basic dyes were liquid in phase. Dyes reported here are named in accordance with the Color Index International database (Society of Dyers and Colourists, Bradford,

UK). Bulk rolls of the most common classes of synthetic fibers were dyed at levels consistent with commercial use (2-4% by weight) at the NC State School of Textiles pilot facility (Raleigh, NC). The acrylic textile was dyed with Basic Blue 159, Basic Yellow 28, and Basic Violet 16. The nylon textile was dyed with Acid Yellow 49, Acid Blue 45, and Acid Green 27. The polyester textile was dyed with Disperse Yellow 114, Disperse Violet 77, and Disperse Red 60. Dyes standards and fabrics were stored in a dark room to avoid photodegradation.

#### *Fiber weathering*

Textile samples were weathered in accordance with the AATCC Test Method 124-2001 to simulate the “Permanent Press” cycle of a washer. Each fabric was washed and dried for 5, 25, or 50 cycles. Detergents were added to each cycle in equal volumes as recommended by the manufacturer. Tide® and Gain® (Proctor and Gamble, Cincinnati, OH) were added in 187 mL volumes, and Wisk® (The Sun Products Corporation, Trumbull, CT) was added in 115 mL volumes. Clorox® concentrated bleach was added in 115 mL volumes, and Clorox 2® (The Clorox Company, Oakland, CA) was added in 177 mL volumes.

#### *Extraction*

Three replicate threads were cut to lengths of 1 cm and placed in 2 mL Big Mouth screw thread autosampler vials. Extraction solvents for respective dye type were dispensed into the inserts in 0.5 mL aliquots, and the vials were sealed



and heated to 100°C in an oven. Vials were uncapped and solvents were evaporated at 80°C to dryness, and dye residues were reconstituted with 1 mL of appropriate injection solvents. Polyester fibers were extracted with chlorobenzene and reconstituted with 70% acetonitrile. Acrylic fibers were extracted with 88% formic acid and reconstituted with 25% acetonitrile. Nylon fibers were extracted with equal parts pyridine, water, and ammonium hydroxide, and reconstituted with 10% acetonitrile.

#### *Ultra-performance liquid chromatography*

Dyes were separated using a Waters (Milford, MA) Acquity UPLC system. The system was equipped with a room temperature sample manager and a Waters (Milford, MA) Acquity column (1.7 µm particle size, 2.1 mm ID × 50 mm length) heated to 40 °C. A BEH C18 column was used to separate disperse dyes. The stationary phase of the column used to separate disperse dyes was BEH C18, and the stationary phase of the column used to separate acid and basic dyes was CSH Phenyl-hexyl. The mobile phase solvent gradient conditions employed for all runs is listed in Table 5.1. The sample injection volumes were 10 µL.

#### *UV/Visible diode array detection*

Dyes samples were detected using a UV/visible diode array detector (Waters, Milford, MA) scanning absorbance from 350-675 nm. The peak area on the chromatogram was acquired for each dye using the corresponding maximum

wavelength Table 5.2, and was used for comparison to standard dye mixtures to determine the amount of dye on each fiber.

## RESULTS AND DISCUSSION

### *Polyester*

Laundering of polyester was not found to reveal substantial dye loss after being subjected to any of the combinations of Tide<sup>®</sup>, Gain<sup>®</sup>, Wisk<sup>®</sup>, Clorox<sup>®</sup> bleach, or Clorox 2<sup>®</sup>. This polymer is resilient to commercial stain removers, and is subsequently desired to avoid dye fading while still allowing the removal of stains Figure 5.1 shows the polyester fabrics laundered for 50 cycles.

### *Acrylic*

Acrylic showed no decrease in the amount of dye after laundering with Tide<sup>®</sup>, Gain<sup>®</sup>, Wisk<sup>®</sup>, bleach, or Clorox 2<sup>®</sup>. The colorfast quality of the polymer dyed with basic dyes, as well as its light weight, make it a cheap, efficient alternative to natural fibers like wool. Figure 5.2 shows acrylic fabrics laundered for 50 cycles.

### *Nylon*

Figure 5.3 shows the color change comparison of nylon fabrics resulting from 5, 25, and 50 cycles of Tide<sup>®</sup>, Gain<sup>®</sup> and Wisk<sup>®</sup>, as well as the detergents used with bleach or Clorox 2<sup>®</sup>.

Figures 5.4 demonstrates the change in dye amounts as a result of repeated washing with Tide<sup>®</sup>, Gain<sup>®</sup>, and Wisk<sup>®</sup>, respectively. Changes in

retention time can be attributed to an ingress of CO<sub>2</sub> into the mobile phase over the course of the injection batch, which decreases the pH, resulting in shorter retention times for basic molecules like the deprotonated form of the acid dyes in solution. The subsequent figures are the same chromatograms, enlarged simply for comparison for interpretation. Acid Blue 45 shows similar rates of dye loss (Figure 5.5) with each detergent unaided by stain removers. Acid Yellow 49 (Figure 5.6) appears to weather most rapidly when laundered using Wisk<sup>®</sup>, followed by Gain<sup>®</sup>, then Tide<sup>®</sup>; the same trend is seen with respect to Acid Green 27 in Figure 5.7.

The chromatograms in Figure 5.8 show the comparison of detergents used with bleach. Acid Blue 45 could not be detected after 25 washes with Tide<sup>®</sup> or Gain<sup>®</sup>, and none could be detected on when Wisk<sup>®</sup> was used with bleach (Figure 5.9). Similarly, when Acid Yellow 49 was laundered with both detergent and bleach (Figure 5.10) it could not be detected at after 25 or more cycles for any of the detergents. Tide<sup>®</sup> proved to be the most destructive on Acid Green 27 when used with bleach (Figure 5.11), and no dye was detected following 50 cycles of Gain<sup>®</sup> with bleach, or after 25 cycles of Wisk<sup>®</sup> with bleach.

Figure 5.12 characterizes dye loss when fabrics were washed with detergent and Clorox 2<sup>®</sup>. When washed with Clorox 2<sup>®</sup>, Acid Blue 45 could be detected after 25 cycles of washing with any of the detergents, but not after 50

washes (Figure 5.13). This was also observed for levels of Acid Yellow 49 when washed using Clorox 2® (Figure 5.14) with Tide® or Gain®; some dye was still detected after 50 cycles when used in combination with Wisk®. Gain® with Clorox 2® proved to cause the most decrease in the levels of Acid Green 27 (Figure 15), followed by Tide® with Clorox 2®. Wisk® used with Clorox 2® did not decrease the amount of Acid Green 27; fronting of peaks associated with laundering is responsible for discrepancies in peak height.

## CONCLUSIONS

Extraction of polyester, acrylic, and nylon were performed to assess the influence of repeated washings on colorfastness using Tide®, Gain®, and Wisk® detergents, as well as bleach or Clorox 2®. Minimal color loss was seen on weathered acrylic and polyester fibers as high as 50 wash cycles of detergent alone, or in combination with bleach or Clorox 2®. Nylon demonstrated substantial color loss that could be seen after 5 laundering cycles. When Tide® or Wisk® was used with bleach, no dye was present after 25 washes. Although there was significant color loss after 25 cycles with Gain® and bleach, dyes could still be seen. Clorox 2® demonstrated its color boosting claims, as higher amounts of dye were when used with all of the detergents, than with the detergent alone. Wisk® appears to have the harshest effect of the three detergents used.

Detection of dyes has been demonstrated in all combinations of laundering agents investigated at 5 cycles. Some difficulty was seen when bleach was combined with detergent, but when washed with detergent alone or with Clorox 2<sup>®</sup>, all dyes could be detected even after 50 laundering cycles.

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## TABLES

Table 5.1. Liquid chromatography gradients used for:

(A) the separation of disperse dyes on a Waters BEH C18 column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is water and B is Acetonitrile

Time (min)	% A	% B
0.00	20	80
1.00	20	80

(B) the separation of basic dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	90	10
1.00	90	10

(C) the separation of acid dyes on a Waters CSH Phenyl hexyl column (1.7  $\mu\text{m}$  particle size, 2.1 mm ID, 50 mm length), where A is 25 mM ammonium acetate in water and B is Acetonitrile

Time (min)	% A	% B
0.00	95	5
0.20	95	5
0.50	50	50
1.00	50	50
1.40	95	5
3.00	95	5



Table 5.2. Maximum absorption wavelengths for all dyes.

Dyes	Maximum $\lambda$ (nm)
Disperse Red 60	514
Disperse Yellow 114	424
Disperse Violet 77	547
Basic Blue 159	600
Basic Violet 16	545
Basic Yellow 28	444
Acid Blue 45	614
Acid Yellow 49	420
Acid Green 27	422/616

FIGURES

Tide 50x	Tide + Bleach 50x	Tide + Clorox 2 50x
Gain 50x	Gain + Bleach 50x	Gain + Clorox 2 50x
Wisk 50x	Wisk + Bleach 50x	Wisk + Clorox 2 50x
Control		

Figure 5.1. Polyester laundering comparison; control vs. 50x cycles. Tide®, Tide® w/bleach, Tide® w/ Clorox 2®; Gain®, Gain® w/bleach, Gain® w/ Clorox 2®; Wisk®, Wisk® w/bleach, Wisk® w/ Clorox 2®.

Tide 50x	Tide + Bleach 50x	Tide + Clorox 2 50x
Gain 50x	Gain + Bleach 50x	Gain + Clorox 2 50x
Wisk 50x	Wisk + Bleach 50x	Wisk + Clorox 2 50x
Control		

Figure 5.2. Acrylic laundering comparison; control vs. 50x cycles. Tide®, Tide® w/bleach, Tide® w/ Clorox 2®; Gain®, Gain® w/bleach, Gain® w/ Clorox 2®; Wisk®, Wisk® w/bleach, Wisk® w/ Clorox 2®.

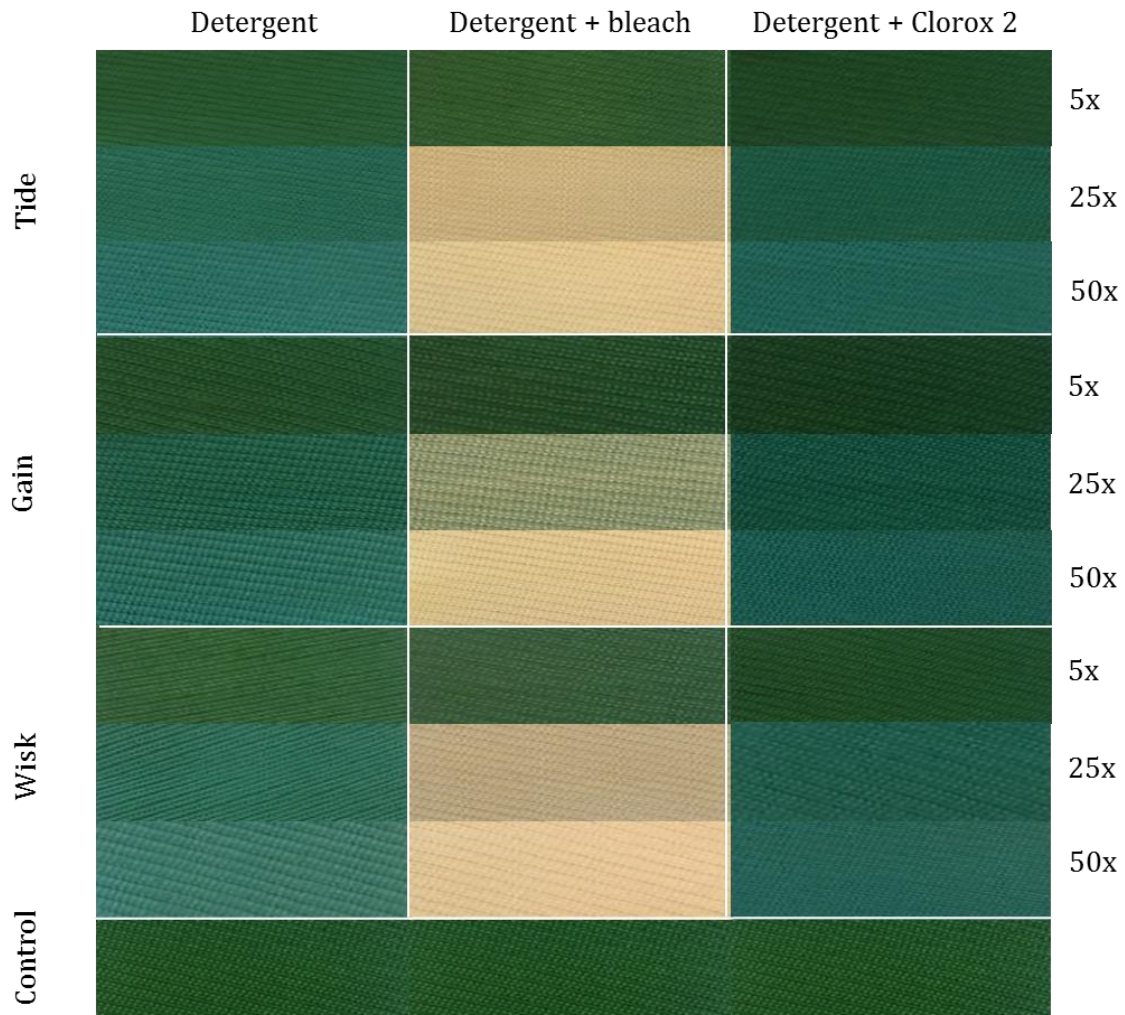


Figure 5.3. Nylon laundering comparison; control vs. 5, 25, and 50x cycles. Tide®, Tide® w/bleach, Tide® w/ Clorox 2®; Gain®, Gain® w/bleach, Gain® w/ Clorox 2®; Wisk®, Wisk® w/bleach, Wisk® w/ Clorox 2®

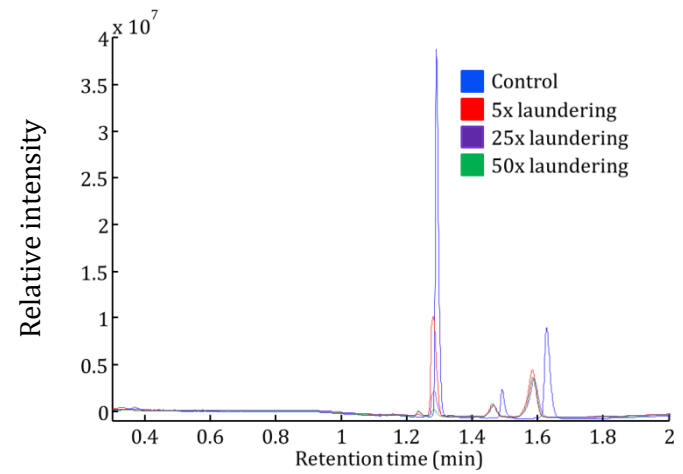
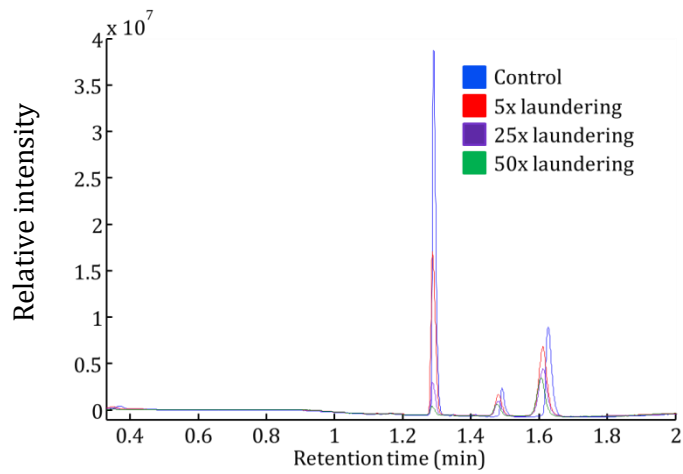
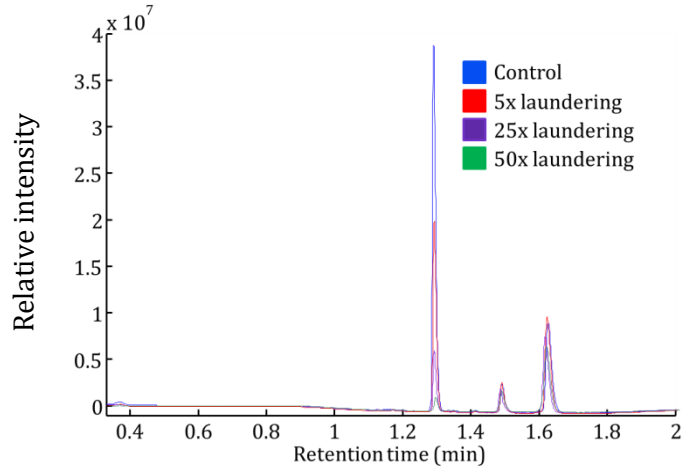


Figure 5.4. Separation of dyes from 1 cm nylon thread; (A) Tide®, (B) Gain®, (C) Wisk®

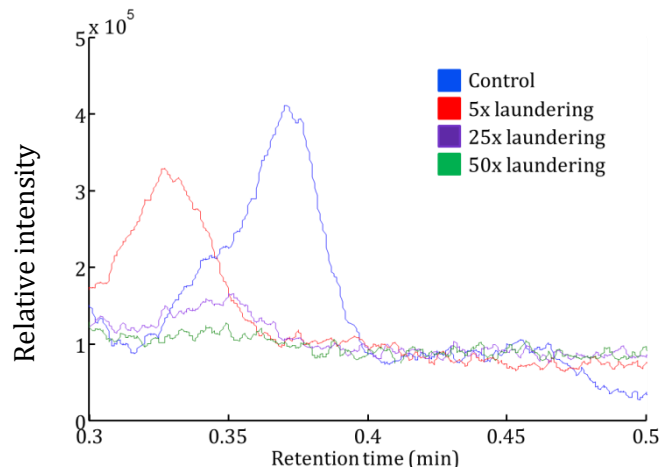
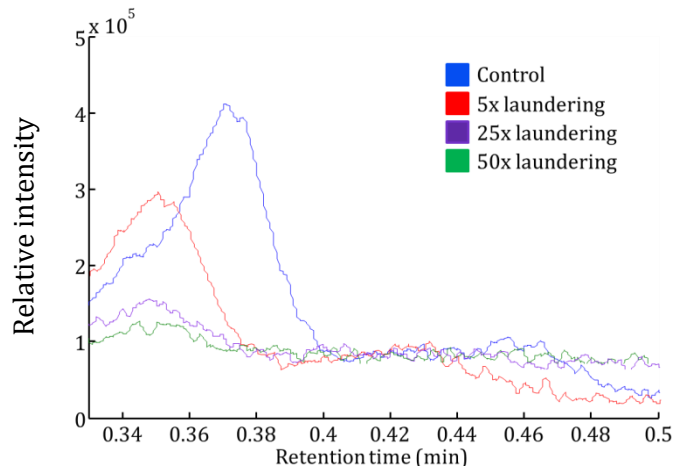
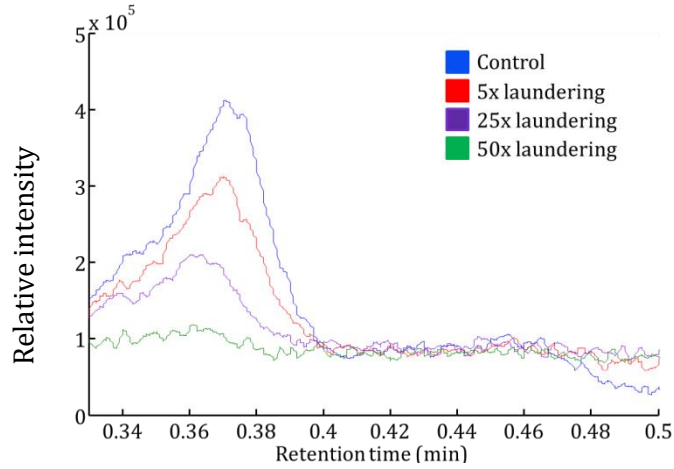


Figure 5.5. Acid Blue 45 1 cm nylon thread comparison; (A) Tide®, (B) Gain®, (C) Wisk®



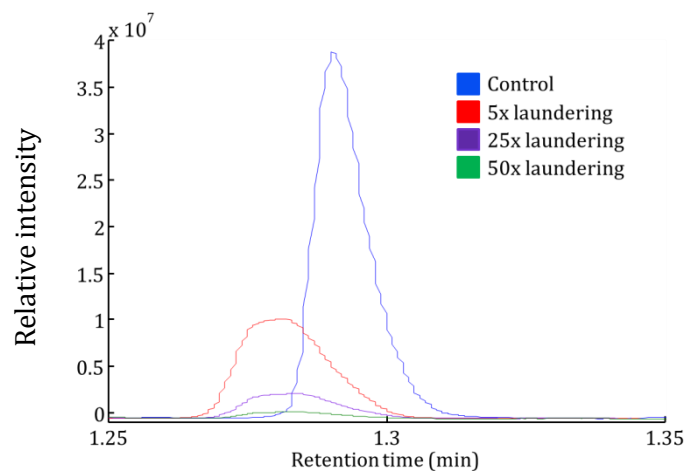
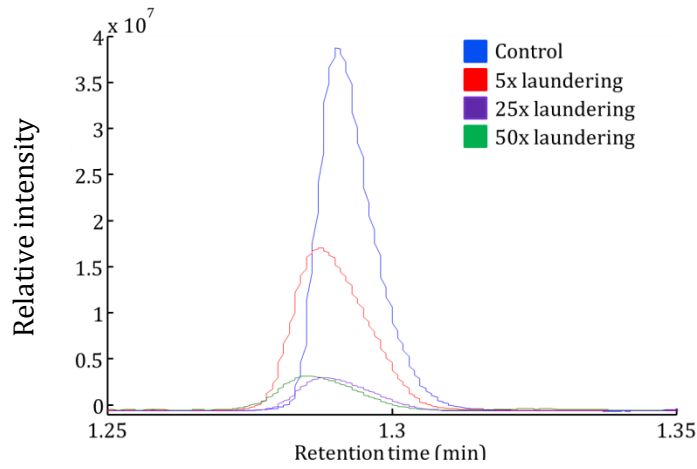
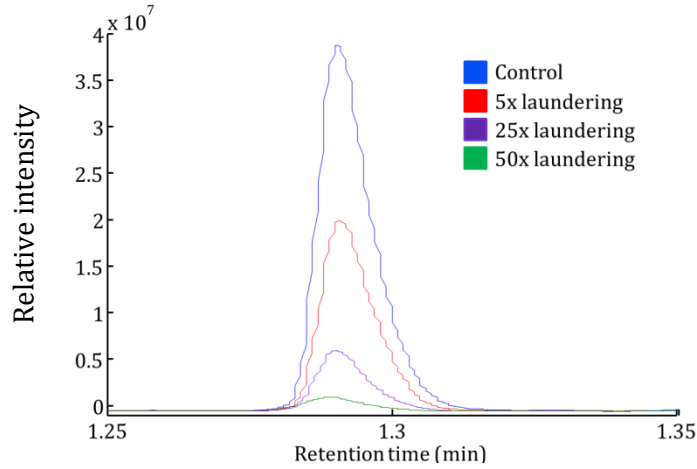


Figure 5.6. Acid Yellow 49 1 cm nylon thread comparison;  
(A) Tide®, (B) Gain®, (C) Wisk®

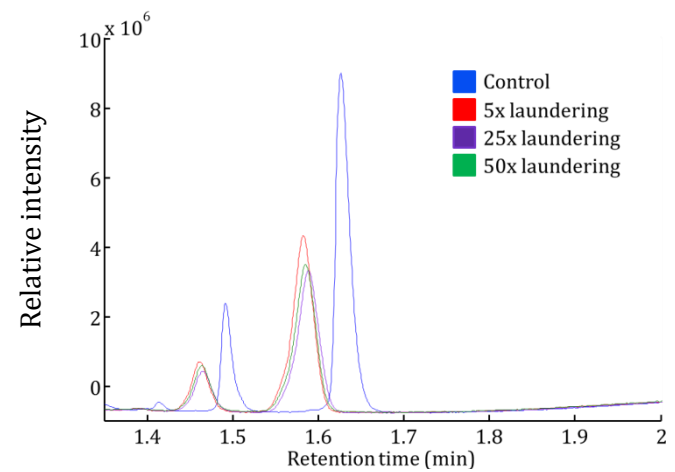
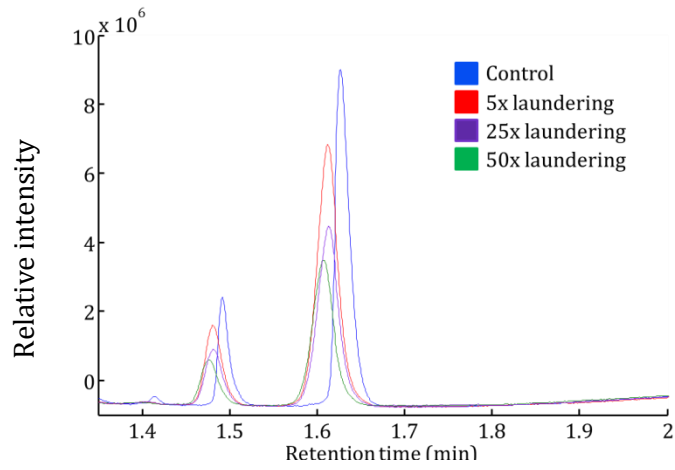
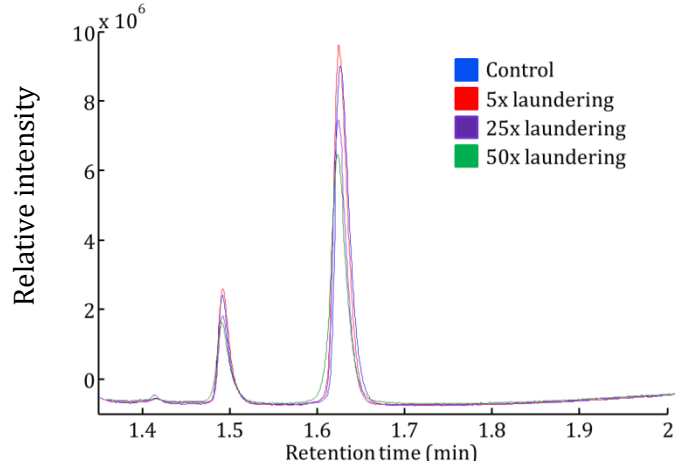


Figure 5.7. Acid Green 27 1 cm nylon thread comparison;  
(A) Tide®, (B) Gain®, (C) Wisk®

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient



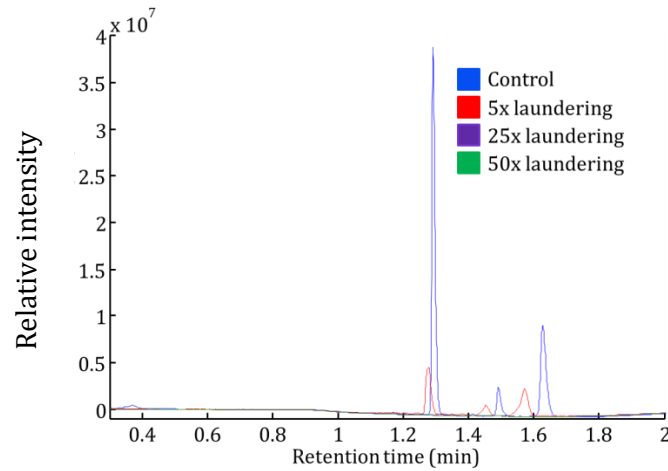
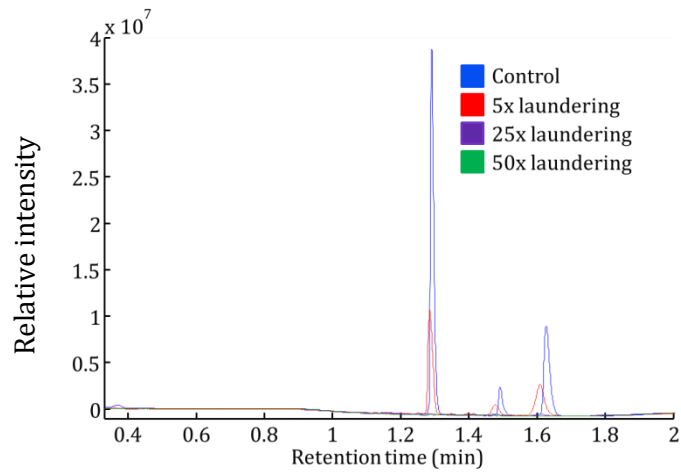
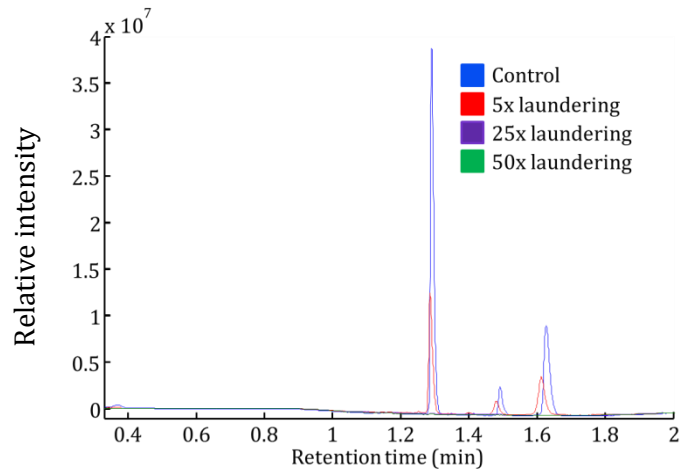


Figure 5.8. Separation of dyes from 1 cm nylon thread;  
 (A) Tide® with bleach, (B) Gain® with bleach, (C) Wisk® with bleach

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

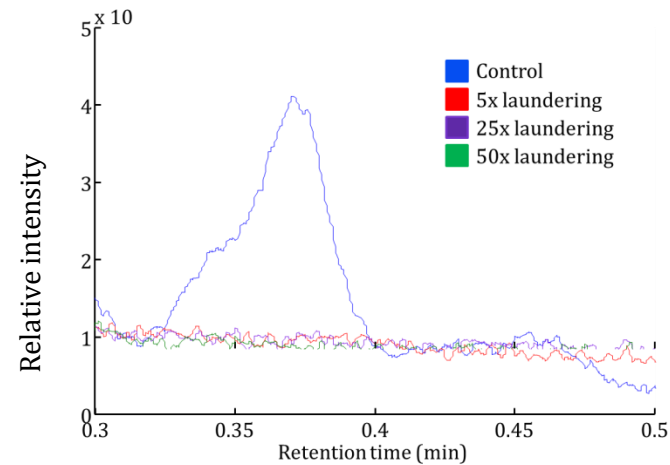
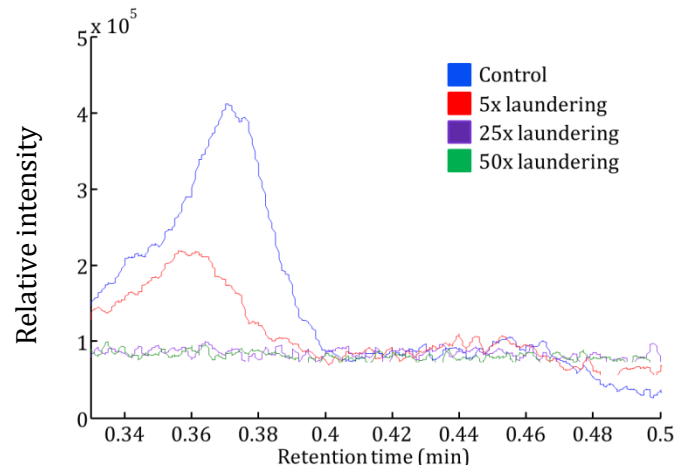
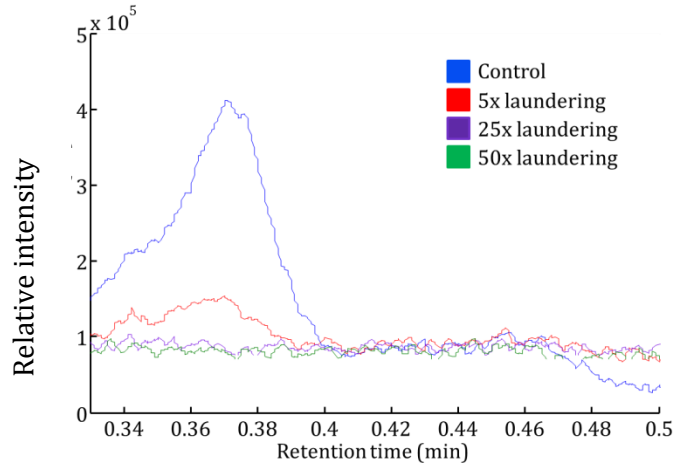


Figure 5.9. Acid Blue 45 1 cm nylon thread comparison;  
 (A) Tide® with bleach, (B) Gain® with bleach, (C) Wisk® with bleach

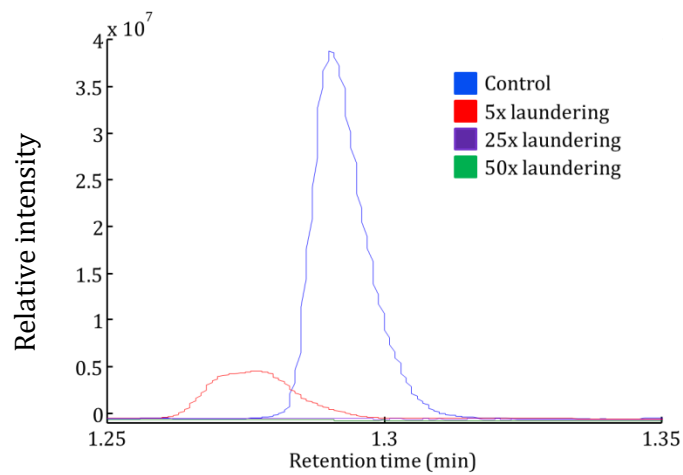
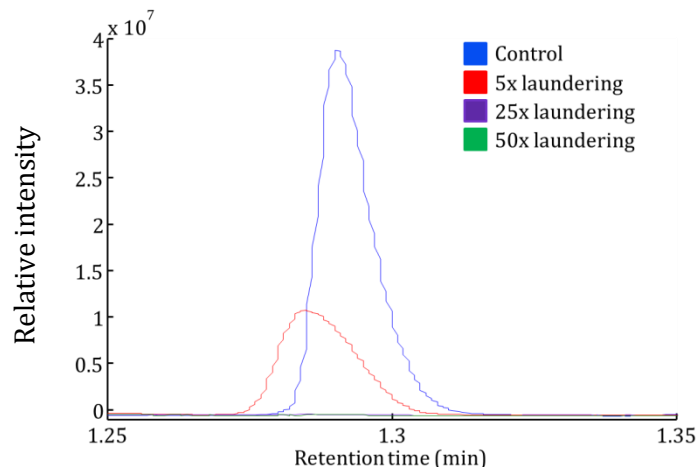
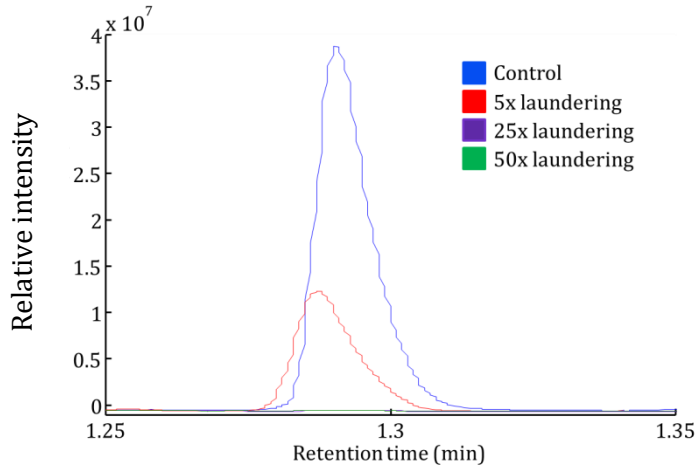


Figure 5.10. Acid Yellow 49 1 cm nylon thread comparison;  
 (A) Tide® with bleach, (B) Gain® with bleach, (C) Wisk® with bleach

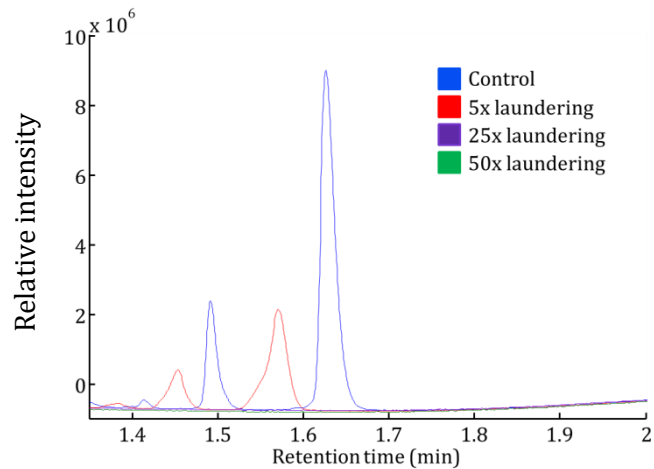
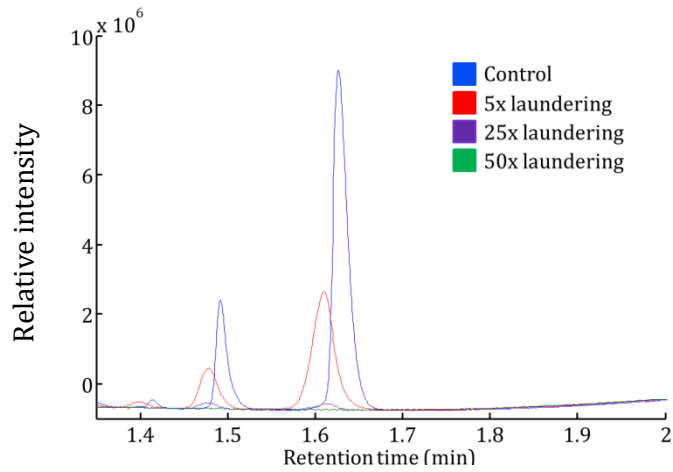
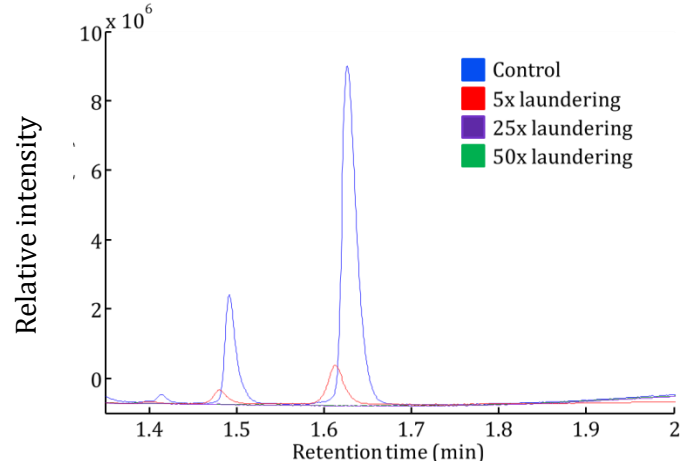


Figure 5.11. Acid Green 27 1 cm nylon thread comparison;  
 (A) Tide® with bleach, (B) Gain® with bleach, (C) Wisk® with bleach

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

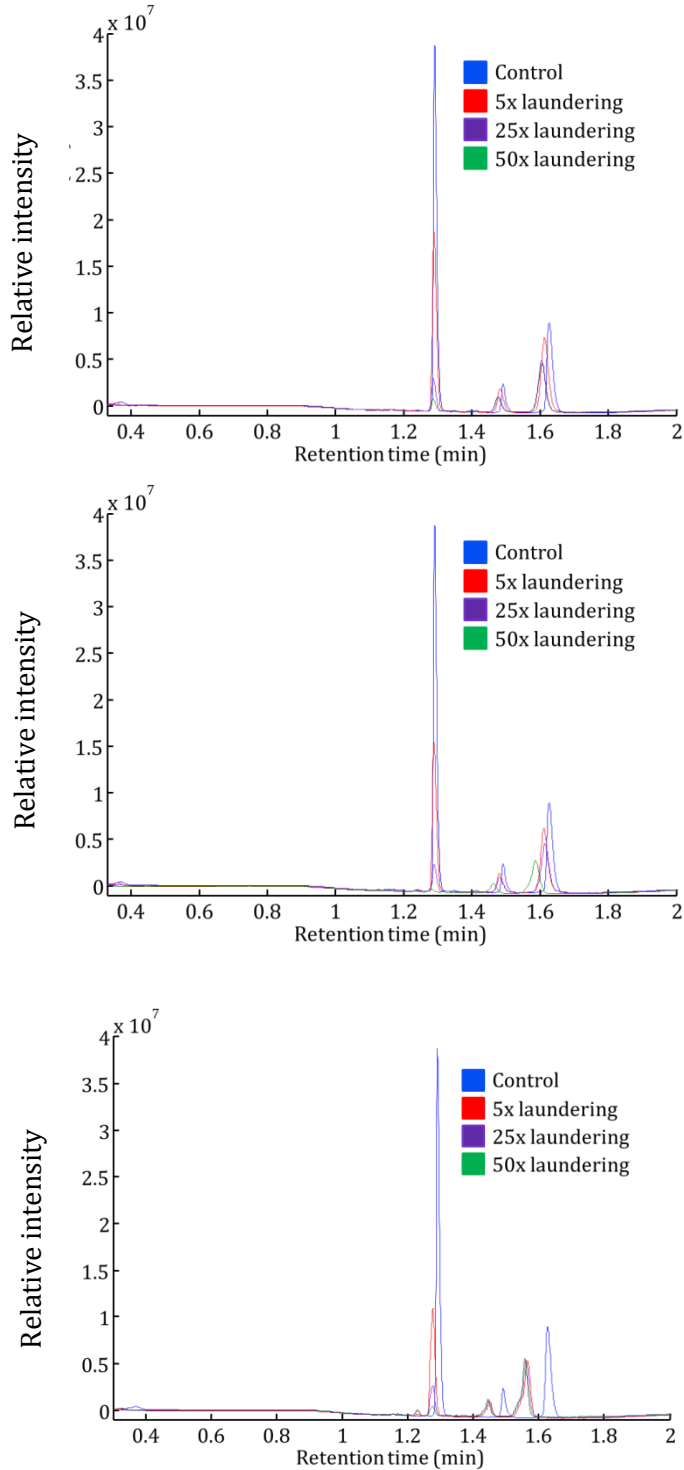


Figure 5.12. Separation of dyes from 1 cm nylon thread;  
 (A) Tide® with Clorox 2®, (B) Gain® with Clorox 2®, (C) Wisk® with Clorox 2®

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

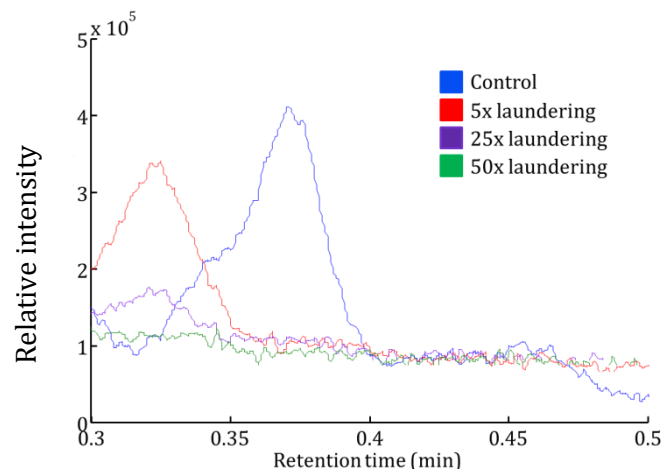
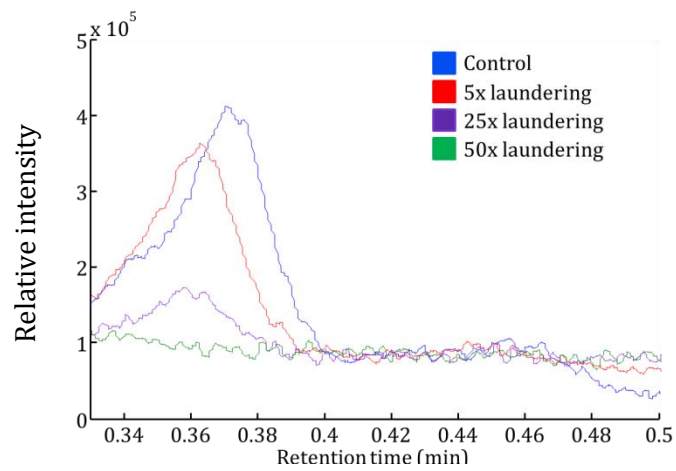
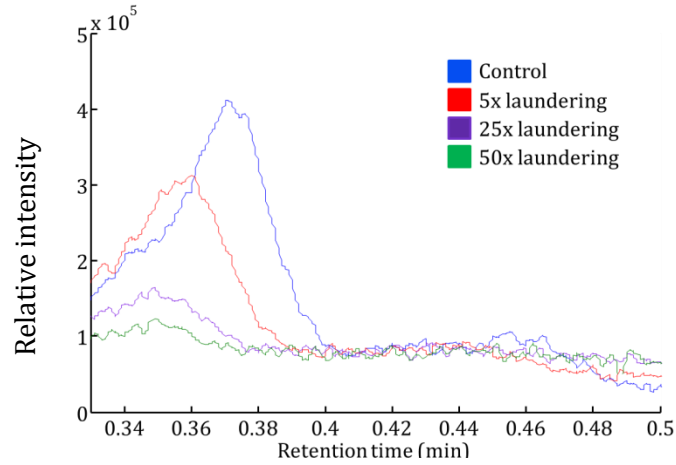


Figure 5.13. Acid Blue 45 1 cm nylon thread comparison;  
 (A) Tide® with Clorox 2®, (B) Gain® with Clorox 2®, (C) Wisk® with Clorox 2®

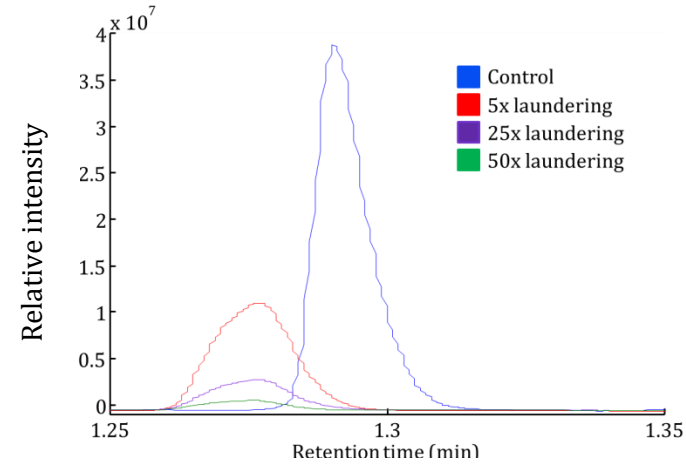
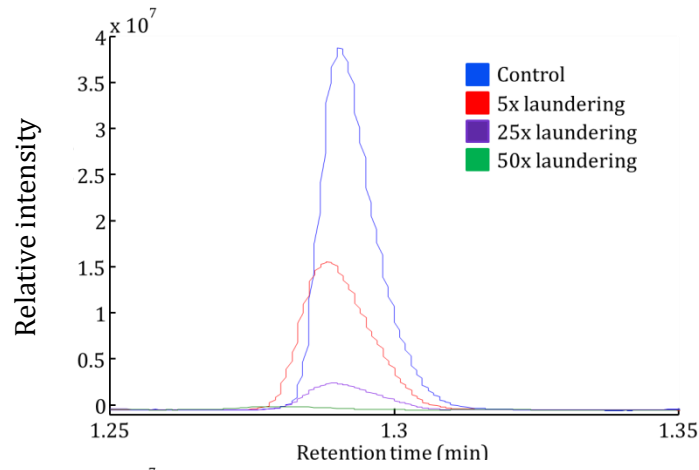
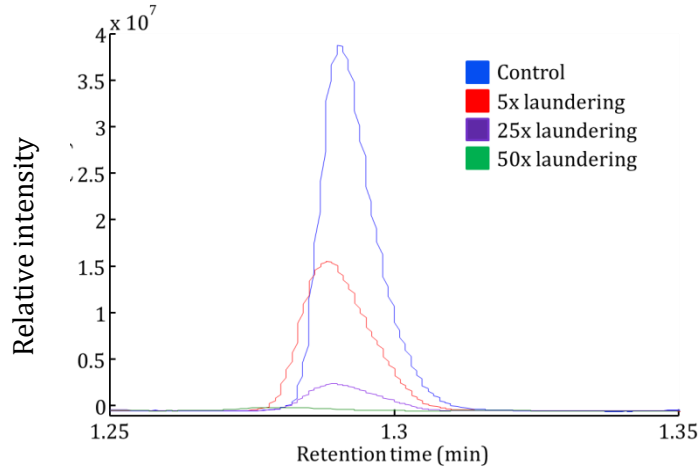


Figure 5.14. Acid Yellow 49 1 cm nylon thread comparison;  
 (A) Tide® with Clorox 2®, (B) Gain® with Clorox 2®, (C) Wisk® with Clorox 2®

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient

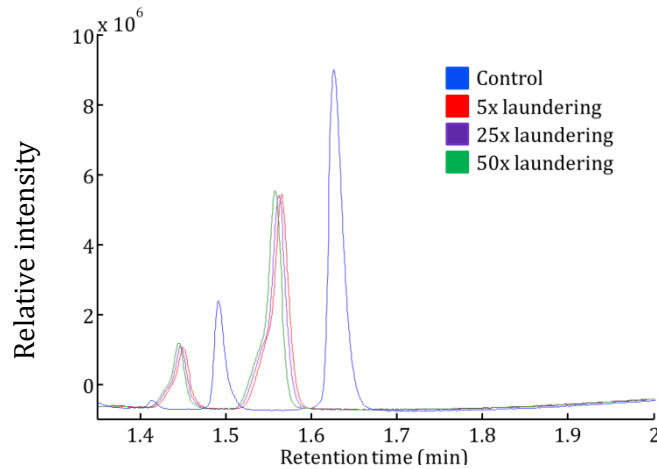
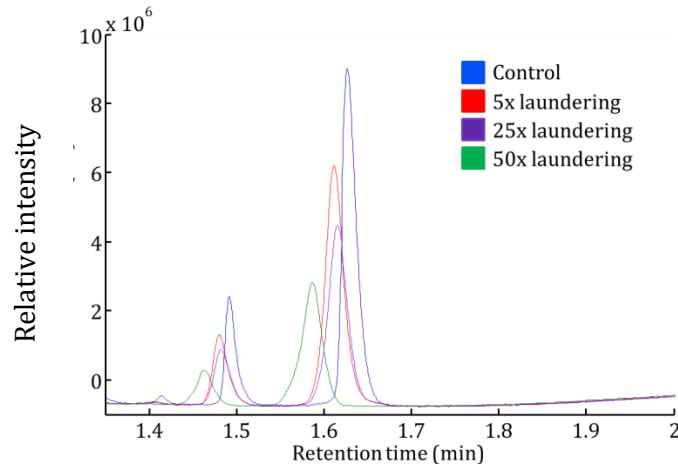
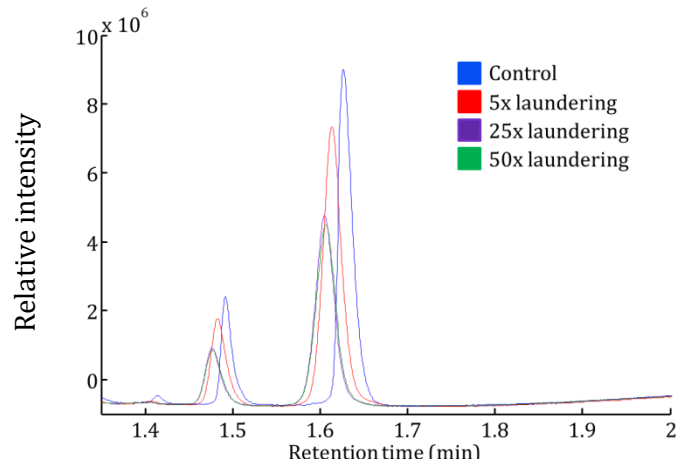


Figure 5.15. Acid Green 27 1 cm nylon thread comparison;  
 (A) Tide® with Clorox 2®, (B) Gain® with Clorox 2®, (C) Wisk® with Clorox 2®

\*Negative y-axis values are due to changes in refractive index of mobile phase gradient