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Review

Selected Aspects of Forensic Discrimination of Blue and Black/Grey Cotton Fibres Derived from Denim Fabrics

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Abstract: Fragments of single textile fibres are one of the most commonly found microtraces at crime scenes. Among them, the widespread blue and black/grey cotton fibres should be recognized. The analytical methods routinely used in fibre examination mainly focus on color assessment and determination of the fibres' morphological features as well as chemical composition. This publication presents the physicochemical characteristics of blue and black/grey denim fabrics and fibres as well as an overview of the non-destructive and destructive methods used in the discrimination of these fibres. Usually, such fibre microtraces are very difficult to distinguish in forensic examinations due to their widespread abundance, and, thus, their evidential value is not significant. As previous research shows, most denim material samples were colored with indigo dye. However, due to the changing trends in denim production and the fashion market, indigo derivatives may play a more critical role. The literature review shows significant shortcomings in the development of techniques focusing on the analysis of the dyes contained in denim fibres, and this is a research direction worth pursuing.

Keywords: cotton fibres; denim; dye analysis; instrumental analysis; forensic examinations

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1. Introduction

Nowadays, textile fibres accompany humanity in a variety of forms and perform multiple functions. As they have progressed in their production, the possibility of distinguishing between the fibres themselves has also increased. They can be characterized by different chemical compositions, morphological structures, thickness, length, cross-sectional shape, etc. [1]. An additional, but very important feature introducing a significant diversity between the fibres is their color, obtained by the use of different types of dyes or their mixtures and the various dyeing techniques thereof.

One of the sciences dealing with single-fibre examination is forensic science. Due to their high prevalence, fibres are a very important group of forensic traces (mainly microtraces) occurring at the site of an event. This is in line with the principle first formulated by Locard over 100 years ago [2] that material transfer may take place whenever two objects come into contact, and, therefore, also during the perpetration of numerous criminal acts. As a result, the identification and subsequent differentiation of fibres collected at the crime scene and of the evidence secured during its inspection can facilitate the connection of people and places [3]. Direct transfer mainly occurs when the fibres are transferred from the perpetrator's clothing to the victim's clothing or vice versa. Fibre microtraces are revealed not only on garments but depending on the nature of the case, they are sought on the crime items and obstacles overcome by the perpetrator (which are on the route of its movement), car bodies and bumpers or elements inside the vehicle cab. Sometimes fibres are also sought under the fingernails of people involved in the case, on their bodies and hair [4].

Commonly, the vast majority of clothing used today, and thus constituting a major part of the evidence material, is made up, at least to some extent, of cotton [5]. As mentioned above, one of the most important parameters affecting the evidential value of a fibre is its color. According to data collected in the catalogue database (CDB) and international population surveys [6–10], the two most popular groups of colored fibres (in terms of prevalence) are black/grey and blue cotton. Therefore, such fibres have only a limited evidential value, since the potential source of their origin on the evidence examined is difficult to determine [5]. It is undesirable as blue and black/grey cotton fibres are one of the most commonly reported transferred microtraces [10].

Therefore, the aim of this article is to expand the current knowledge on the specifics of denim fabrics and their constituent fibres, as well as the possibility of analyzing this type of blue and black/grey cotton fibres, to show the advantages and disadvantages of the methods used so far and to search for new achievements in this field. The motivation for developing this type of publication is the research currently carried out by the authors and their collaborators on the new possibilities of differentiating real samples of denim fabrics, and their constituent fibres, available on the consumer market.

2. Cotton Fibres Origin, Structure and Usefulness

Cotton is a natural fibre of vegetable origin, in a form of white or white-yellow strands, that surround the seeds of the cotton plants of the genus Gossypium. These fibres are the purest form of cellulose found in nature, its content reaching typically over 90% [11]. In addition, they also contain small amounts of proteins, waxes and minerals [6].

Cotton has a layered structure. Each fibre is created by several dozen (20–30) layers of cellulose coiled and twisted in the shape of a ribbon. The number of turns increases with the degree of cotton ripeness, but when it reaches full maturity, it decreases again and the shape of the fibre is similar to the cylindrical one. The cotton fibre length ranges between 22 and 50 μm, while the average diameter is between 18 and 25 μm. Depending on the fibre type, short, medium- and long-fibre cotton is distinguished [8]. The heterogeneity of the cotton's surface and structure is clearly visible in polarized light microscopy. Dark bands intersecting the fibres at certain intervals indicate points of reversal of the structure, and thus the location of the coils [7].

Inside the fibre, there is an empty channel called a lumen, surrounded by a secondary wall and a primary wall. The primary wall is a combination of amorphous cellulose with crosslinked fibrils and other substances such as proteins or ions. Thanks to this specific combination, cotton fibres are flexible, which is particularly important during the growth process [6]. The role of a secondary wall built only of cellulose in crystalline form is to provide strength. The positioning of cellulose fibrils is therefore strictly ordered in it—they are mutually parallel. The outermost layer is the cuticle, a thin film of waxes and fats covering the outer walls of the cells. It has a significant, if not the greatest impact on the properties and thus the use of the cotton [6].

Cotton textiles have been part of the human environment for a long time. The first mentions of the use of cotton in the manufacture of everyday products, such as threads, fabrics or ropes, already appear in the history of ancient civilizations, such as Egypt and India [12,13]. Due to its desirable properties, cotton quickly reached Europe. It was in England in the eighteenth century that the first modern cotton factory was established, which resulted in its rapid popularization, especially in the United States [12]. This situation persists today; for example, about 43% of clothing present on European markets is made of cotton fibres or their blends [14].

Nowadays, cotton is widely used in the textile industry. The fibres are characterized by good strength, absorbency and thermal conductivity. Their application to the production of appropriate materials depends on the quality of the fibres. The basic parameters classifying fibres for a given group are length and color, strength and thickness. The highest quality is associated primarily with the largest length—thanks to this, cotton is elastic and tear-resistant [6]. The use of appropriate chemicals or finishing processes can significantly improve cotton parameters. The refinement treatment consists, among other things, of applying the finishing, smoothing or chemical crosslinking of the fibre-forming polymer. At present, the main trend in the textile industry is the attempt to replace chemical treatment with enzymatic treatment [6].

3. Cotton Dyeing

3.1. General Approach

Dyes are chemical compounds that have the ability to absorb electromagnetic radiation in the visible, near-ultraviolet or near-infrared range. The absorbed radiation is transmitted to the environment in the form of heat, while the reflected radiation creates a color impression perceived by a human being [15]. Among the dyes, synthetic substances as well as those of natural origin can be distinguished. Nowadays, the latter, popular until the 19th century, are practically no longer applicable in mass production. Chemically identical dyes have the same color index name/number [5,16].

There are three main dyeing methods based on different mechanisms of dye and fibre interaction [17]. The first of these involves the adsorption of soluble dye molecules on the surface of the fibre due to the physical forces acting between them. The second method consists of the formation of insoluble dye molecules in the fibres by adsorption of the soluble substance, followed by its oxidation or binding to another component. However, the most common method is to choose a coloring substance, so that its molecules create chemical bonds with the fibre. This method is effective only for fibres containing appropriate functional groups [17].

3.2. Classification of Cotton Dyes

Among the many types of colorants, reactive, direct, azo, sulfur and vat dyes are applied to cotton [18]. Some of them are discussed below, in reverse order to those shown in the previous sentence.

Vat dyes are insoluble in water compounds containing carbonyl groups in their structure. The majority of them originate from anthraquinonoids or indigo chromophores [19]. Therefore, for the coloring of cellulose, their reduced "leuko" forms in the form of soluble salts adsorbed by fibres are used. After the adsorption process, the original insoluble form is reproduced by oxidation [17]. This group includes the natural dye used commonly in denim dyeing: indigo.

Sulfur dyes also occur as pigments insoluble in water. Similar to vat dyes, they need to be reduced to "leuko" form to be used in cellulose coloring. In general, this group of colorants consists of organic compounds containing sulfur atoms, based on amines or phenols. They are commonly used due to their low cost and washing resistance [19].

The direct dyes used in cellulose coloring are primarily synthetic sulphonated azo dyes. They belong to organic compounds formed as a result of coupling reactions of diazonium salts (mainly with phenols and aromatic amines) and contain in their structure an azo group N=N–. The direct dyes group also includes benzidine derivatives. They were replaced with previously used natural dyes due to the direct adsorption of azo dyes by cotton fibres [17].

Reactive dyes are considered to be one of the most important classes of cotton dyes [5]. They have the ability to permanently chemically bind to colored fibres due to the presence of reactive groups in their molecules [19]. Contrary to the earlier belief that cellulose is chemically inactive towards them, these dyes are currently one of the most commonly used in the dyeing process of cotton [17]. In many cases, bi- or trifunctional reactive dyes containing two or three reactive groups are used. The dyeing efficiency of such dyes increases as all groups can effectively bind to the cellulose chain [18].

3.3. Use of Indigo and Its Derivatives

As previous research shows [20,21], the majority of the denim material samples were colored with indigo dye (Figure 1), regardless of the type of clothing, the place of origin, or the country of production. In most cases, such fibres were not taken into account in forensic examination due to their widespread abundance. Denim fibres displaying other physicochemical properties were considered to be rare and their evidential value was significant [21]. However, due to the changing trends in denim production and the fashion market, it is possible that indigo derivatives play a more important role (Figure 2). This is a direction of research worth pursuing.

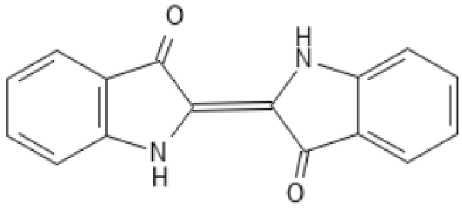

Figure 1. Chemical structure of Vat Blue 1 (indigo).

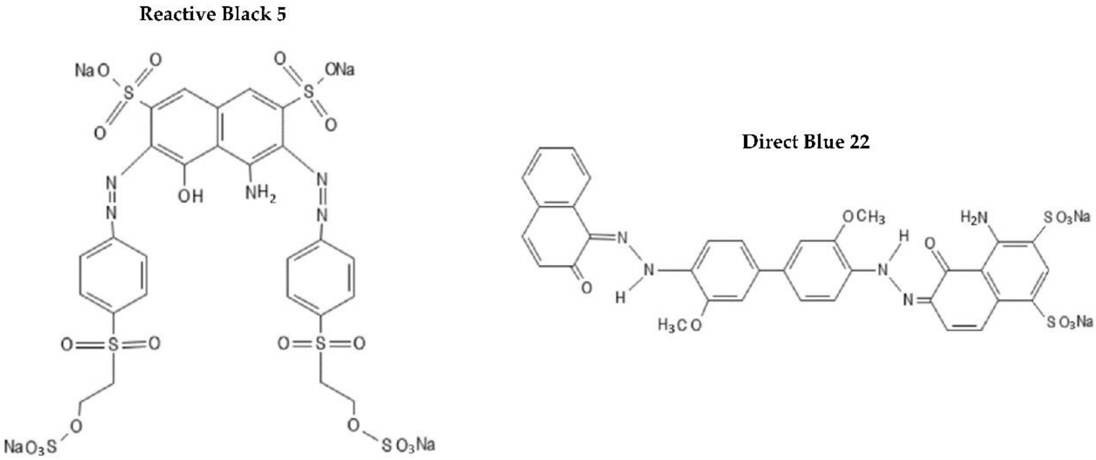

Figure 2. Chemical structure of Reactive Black 5 and Direct Blue 22.

4. Denim Fabric

Levi Strauss is considered to be the popularizer of wearing jeans as an element of everyday clothes. The trousers that he produced were widely used by gold miners, mainly because of their durability. Moreover, a tight weave provided good protection against moisture [22]. It is believed that denim materials are mainly dyed with indigo because stains and dirt are not so visible on the blue color.

Nowadays, over 50% of denim is manufactured in Asia. In the European Union, Italy is the leader in jeans manufacturing. Among other countries with a significant jeans industry, Romania, Spain and Portugal should be mentioned [23]. Due to their prevalence, blue and black/grey cotton fibres are not only one of the most common forensic microtraces, but also pose a serious ecological threat. Recent studies show that they are accumulating even in regions as remote as the Arctic, and the authors made an important statement that blue jeans are an “indicator of the widespread burden of anthropogenic pollution” [24].

When it comes to the structure of the material, denim material is a strong cotton twill (weave) with a 2/1 or a 3/1 diagonal rib. This diagonal ribbing distinguishes denim from other cotton textiles. Usually, only one of the threads used for weaving is colored. As a result, blue is predominant on the outer side of the denim fabric and white is predominant on the inner side (Figure 3).

Figure 3. Structure of denim fabric on the outer side (**left image**) on the inner side (**right image**).

One of the most characteristic features of denim yarn is ring dyeing; fibres are woven into thread and then dyed. It results in such an effect, that only an outer layer of thread consists of colored fibres and the core of the thread remains white. Due to this particular technological process, the denim fibres are dyed unevenly and exhibit high intra-subject and inter-object variability, as shown in Figure 4.

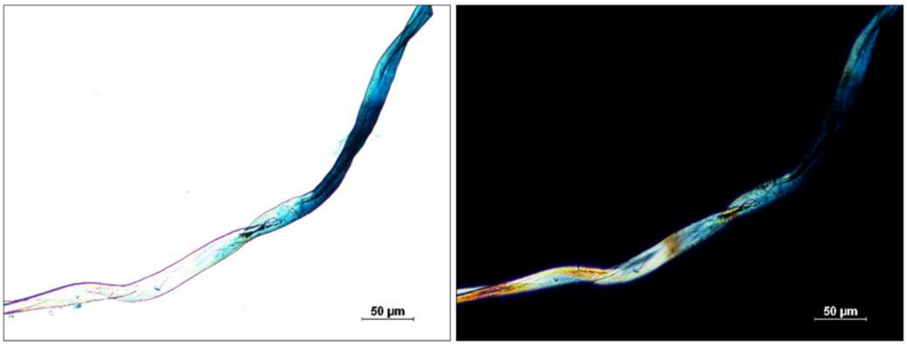

Figure 4. Typical uneven dyeing of cotton fibre retrieved from blue denim fabric in transmitted white (**left image**) and polarized (**right image**) light.

Currently, many admixtures are added to denim textiles, such as polyester, elastane, and recently also viscose and lyocell. The addition of these types of fibres to the fabric improves its utility values, including better strength and stretchability of the material, and reduces production costs.

5. Analysis of Dyed Cotton Fibres

Forensic examination of fibres relies on the determination of their physicochemical properties and subsequent identification and differentiation [4]. In contrast to biological or fingerprint marks, fibres are not specific to one person or one object [3]. The analysis scheme and the order of methods used to identify and differentiate fibres in the context of forensic science are essential, as the number and nature of the comparisons carried out have a significant impact on the evidential value of the results obtained. Currently, there are many methods used to identify and discriminate fibres. These techniques are usually non-destructive because the fibres tested are often treated as evidence in the case [16]. They are used in the following order [4]:

1. High-power microscopy in transmitted white light (HPM), polarized light microscopy (PM) and fluorescence microscopy (FM);
2. Microspectrophotometry in the UV–Vis range (UV–Vis MSP);
3. Microspectrometry in the IR range (micro-FTIR);
4. Raman microspectrometry (micro-RS).

Sometimes, the information obtained using the techniques mentioned above is not sufficient. In such cases, with the consent of the court and with the appropriate amount of material, destructive techniques can be used. These are mainly chromatographic methods: liquid chromatography (HPLC), thin-layer chromatography (TLC) and capillary electrophoresis (CE), primarily.

5.1. Optical Microscopy Techniques

HPM is a simple and useful way to identify and compare fibres, with a combination of various illumination sources, filters, and attachments. At each stage of the examination, known and recovered fibres are compared to determine if they exhibit the same microscopic characteristics and optical properties [11]. Microscopic examination of textile fibres provides the quickest and least destructive means of determining their morphological features. The presumptive polymer type can be determined by PLM. Additionally, a side-by-side microscopic comparison provides a fundamental discriminating method for determining if two or more fibres are consistent with originating from the same source. With the use of HPM, most natural fibres can be easily and quickly identified. In difficult situations, differences can be observed using PM [16,25]. The fibre feature evaluated in PM is its birefringence. The anisotropic behavior of fibres used in this technique results from differences in the refractive index between parallel and perpendicular fibre axes. The interference colors visible on the recorded image correspond to the values of these differences [16] and provide, among others, the degree of crystallinity of fibres [1]. This technique is used to determine the general class of fibre [16]. Moreover, PM allows for the detection of dichroism—the dependence of the observed color on the direction of the plane of polarized light [25]. Another microscopic method, FM, distinguishes dyed fibres based on the different fluorescence of fluorophores contained in dyes, or optical brighteners used during production or during the washing of textile products [25]. These techniques are used routinely with every fibre comparison test.

Examples of dichroism proving to be useful in the examination of black/grey and blue cotton fibres can be found in some published articles [5,26]. It was noted that this phenomenon mostly occurs with direct, reactive and vat dyes. Moreover, dyes used commonly in dyeing denim, such as Vat Blue 1, Reactive Black 5 and Direct Blue 22, were examined. All of them proved to show a dichroic effect; however, the most popular of them (Vat Blue 1) was characterized only by a weak dichroic ratio [26]. Fluorescence emission microscopy studies combined with appropriate chemometric analysis allow the differentiation of visually indistinguishable fibres [27]. So far, however, there are no reports of the effective application of these techniques in the discrimination of blue and black/grey cotton fibres.

5.2. Microspectrophotometry in the UV–Vis Range

UV–Vis MSP is an important, highly developed technique in the study of colored fibres, comparably to microscopic methods, as the visual determination of the color of the fibre can be subjective. It enables the measurement and objective comparison of the color of single fibres as a beam of light can be focused on a very small area of the sample. The absorption and luminescence spectra obtained with this technique are associated with electron and vibrational passages occurring between individual energy states of the dye molecule [25,28]. These transitions are caused by the absorption of a certain amount of light by a sample [25,29].

It has been proven that UV–Vis MSP is applicable in distinguishing cotton fibres characterized by a similar shade of color [30,31], particularly in the case of blue-colored fibres [32,33]. Examples of the use of UV–Vis MSP or only visible microspectrophotometry to examine blue and black/grey cotton fibres are widely described in the scientific literature [21,26,31,34–36]. This technique is particularly useful when the observation is also carried out in the ultraviolet region, as it provides structural information and enables the discrimination of samples, having only single fibres available [21]. In the case of black/grey cotton, considered to be very difficult to distinguish by this technique, conducted studies show that discriminating fibres stained with dyes other than vat can be feasible [5], which increases their evidential value. In the preliminary examinations conducted by authors (Figure 5), it has been proven that the analysis of the UV–Vis spectra, particularly in the form of its first derivative, can lead to denim fibre discrimination within the blue and black/grey color groups, especially those with a light color.

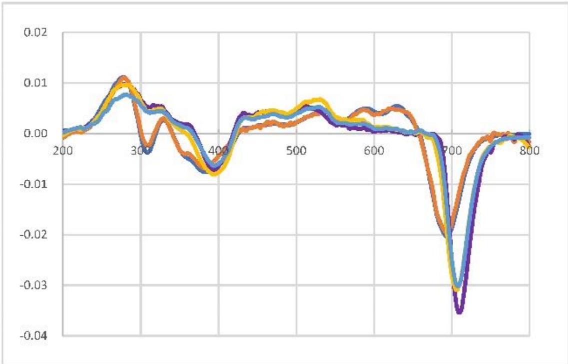

Figure 5. The comparison of the first derivative of MSP absorbance spectra obtained for blue denim fibres from the five experimental fabrics. The results obtained for the fibres constituting the individual fabrics are presented in different colors.

5.3. Fourier Transform Infrared Spectrometry

Infrared spectrometry is one of the most important analytical techniques, based on the vibrations of the atoms of the molecule. An IR spectrum is obtained by passing the radiation from this range through the sample and determining which part of the incident radiation with a certain energy is absorbed. Energy, at which any maximum of the absorption spectrum appears, corresponds to the vibration frequency of a part of the sample [25,37,38]. Spectrum features (such as the number of bands present, their shape and intensity) in the infrared range are directly related to the structure of the compound [28].

Micro-FTIR spectrometry has proved to be useful in the identification and discriminative analysis of fibres as it provides information concerning the chemical structure of a fibre-forming polymer and thus makes it possible to find a difference between fibres that look very similar [3]. In addition, it is characterized by many advantages, such as minimal sample preparation, favorable signal-to-noise ratio, high precision, and the ability to use a small amount of sample [16]. Examples of its use can be found in the literature, especially with regard to chemometric analysis [39].

When it comes to comparing the dyes contained in fibres, this technique is of little use. This is due to the fact that the dye constitutes a minor part of the sample (about 5%). However, there are examples of such applications of the IR method in the writings. Currently, studies focus on the application of the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) in combination with chemometric methods for determining the type of dye, as the reflectance measurements are far more responsive. As described in the literature, it enabled the non-destructive discrimination of cotton fibres, blue among others, colored with various reactive dyes [40,41].

5.4. Raman Spectrometry

Raman spectrometry provides information about the structure of the dye at the molecular level. The Raman effect is the result of the scattering of light. In the molecule, virtual states of energy, considered a short-term distortion of the electron cloud, are induced through a high-energy monochromatic laser beam [25,42]. As a result of a return to the basic energy state, the particle emits light with a wavelength corresponding to the wavelength of the excitation light. The Raman spectrum consists of both types of bands, symmetrically distributed, but with different intensities, and corresponding Raman shifts [25,28,42]. Like IR spectrum features, Raman spectrum characteristics are directly related to the molecular structure of the compound. On the other hand, the condition of the molecule's activity is a change in the polarization of electron clouds during interaction with incident radiation [28].

Micro-RS finds its application in forensic fibre research [43,44], as it does not require a large amount of sample and its preparation is nondestructive. Unfortunately, in most cases, the analysis of colored fibres has encountered a fluorescence problem. The solution is occasionally the use of an excitation laser with a longer wavelength (FT-Raman, λ = 1064 nm). In the study of blue and black/grey colored fibres, RS is broadly described in the literature [34,44–51]. Raman resonant spectrometry [34] and surface-enhanced Raman spectroscopy (SERS) [49], characterized by increased sensitivity and selectivity, are also used, which are of particular importance when analyzing dyes and their mixtures in blue and black/grey cotton fibres.

5.5. High-Performance Liquid Chromatography

Chromatography is one of the most developed and most commonly used separation techniques. The separation process taking place in the chromatographic column occurs since there is phase equilibrium between the components of the analytical sample and the chromatographic system consisting of the stationary and mobile phases that pass through it. In liquid chromatography, the mobile phase consists of different solvents and by alternating the composition of their mixture, changes in the retention times of the ingredients and in the selectivity of the method are obtained. The correct choice of the two phases is a significant step to reach the preferred rates of migration of the components and, thus, desired separation [25]. High-performance liquid chromatography (HPLC) provides the highest efficiency in the separation process. It is therefore applicable for the analysis of compounds such as pigments and dyes [52].

Chromatographic techniques seem to be effective in the analysis of colored fibres. In the first stage, the dye is isolated from the collected fibre. In such a situation, the results are not altered by signals generated by the background which in this case is the fibre polymer. The most commonly used for this purpose is HPLC combined with DAD and MS detectors in various configurations [11,53–58]. As a mobile phase, a mixture of methanol with water and acetonitrile and water with the addition of various salts was usually used to separate the extracts. The stationary phases of the columns were based on silica modified with octadecyl groups. A few researchers described the use of HPLC in the analysis of blue cotton fibres. However, it is worth emphasizing that this is a unitary work, therefore the area of application of chromatographic techniques for the analysis of blue and black/grey cotton dyes is poorly recognized.

5.6. Thin Layer Chromatography

Thin-layer chromatography is the simplest method of all commonly used chromatographic methods. Thanks to the optimization of techniques and materials as well as the use of available instruments, efficient separation and accurate and precise determination of the components of the analyzed samples can be achieved [59]. The basis of TLC is a two-phase system: stationary and mobile. The most commonly used stationary phase is silica gel and the mobile phase is a mixture of solvents with a polarity opposite the polarity of the stationary phase [18,25]. If both phases are properly selected, the components of the mixture applied on the plate, due to the action of capillary forces, migrate at a different rate [59].

The analysis of fibre dyes with the use of TLC should be used for single fibres only after testing with all available non-destructive techniques [60]. Despite the low popularity of TLC in the analysis of dyes used in the textile industry, the literature describes the essential procedures and examples of its application [35,60,61]. The use of this method in the analysis of blue and black/grey cotton is mentioned in research conducted by the team of De Wael et al. [35], in which red, black/grey and navy blue textiles were analyzed. It was proved that for reactive dyes, greater discrimination was achieved with the use of UV–Vis microspectrophotometry than with the high-performance thin-layer chromatography (HPTLC). There seems to be no piece of work concerning the application of TLC in the examination of blue and black/grey denim fibres. However, in the examinations conducted by authors, it has been proven that the use of TLC coupled with the video spectral comparator (VSC) and observation of the TLC plates under diverse light spectra [61] can lead to denim fibre discrimination within the blue and black/grey color groups.

5.7. Capillary Electrophoresis

Capillary electrophoresis is a modern technique used in the analysis of colored fibres [62]. The separation of the components is possible by the difference in their electrophoretic mobility, and hence the speed at which molecules and ions migrate along the capillary. After the application of the electric field, the charged particles suspended in the separation buffer (characterized by the appropriate pH, concentration and ionic strength) move toward the cathode or anode, in accordance with the possessed charge [25]. This technique is suitable for a great number of diverse compounds, as numerous variations of the technique have been developed, enabling the choice of the most appropriate option for the analyzed sample. Additional advantages of this method include high efficiency obtained even with the administration of a small amount of sample and high resolution, which are important when examining trace evidence [62].

The possibility of using capillary electrophoresis to analyze the most popular classes of dyes isolated from fibres was described by Morgan and his colleagues [63]. It was noted that, while using a DAD detector, the identification of dyes contained in a 10 mm fibre is probable. More promising was the MS detector, as its application allowed shortening the length of tested fibre up to 2 mm. Currently, there is a very limited number of literature sources describing the use of CE in the study of blue and black/grey cotton fibres. One of them mentions the promising application of sample-induced isotachophoresis-micellar electrokinetic capillary chromatography (ITP-MECC) [62]. However, the area of application of capillary electrophoresis for the analysis of blue and black/grey cotton dyes is, as yet, poorly recognized. Scientific publications on the analysis of textile dyes using non-aqueous capillary electrophoresis (NACE) can also be found [64,65]. They concern various groups of dyes, but exclude vat dyes commonly used in the dyeing of denim fibres.

In the preliminary experiments conducted by authors and their collaborators (Figure 6), it was proven that the use of CE could lead to denim fibre discrimination within the blue and black/grey color groups.

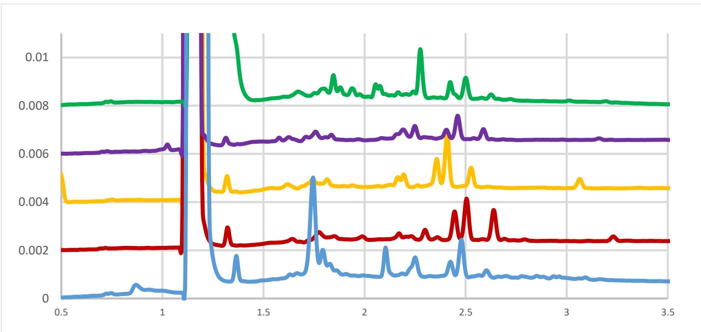

Figure 6. The comparison of electropherograms (PDA at 220 nm) obtained for blue denim fibres from five experimental fabrics. The results obtained for the fibres constituting the individual fabrics are presented in different colors.

6. Conclusions

Blue and black/grey cotton fibres, derived from denim textile products, are some of the most frequently collected microtraces at crime scenes. Their identification is not an analytical problem, but it appears during the comparative studies of fibres, e.g., secured at the scene of a crime and making up the denim fabric of a suspect’s trousers. However, a significant amount of data can be obtained by examining the dyes used to color such textile fibres. For this reason, the development of analytical techniques used in the discrimination of colorants is extremely important.

As the review of the available subject literature has shown, studies on the dyes contained in blue and black/grey denim fibres are scarce and insufficient. The analysis of such fibres presents many challenges due to their worldwide distribution to consumers, as an environmental pollutant, heterogeneous distribution of dye, a too dark or too light color of the fibres (impeding the full use of microscopic and spectroscopic techniques), and the problematic process of extracting dyes from the fibres for further analysis. Nevertheless, the development of dye analysis methodologies, especially if combined with chemometrics, may significantly increase the evidential value of such fibres, and thus help investigators solve cases in which denim fibres are collected as evidence.

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