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Direct Analysis of Silk Dyes from the Murong Zhi Tomb from the Tang Dynasty Using Desorption Electrospray Ionization High-Resolution Mass-Spectrometry Imaging (DESI-MSI)

Qian Yu [1,2,†], Feng Zhang [1,†], Wenchao Lv [1], Yan Wang [3], Lei Zhong [3], Wenting Gu [3], Junmei Liu [2], Xinyan Liu [2], Donghui Xu [2], Guangyang Liu [2,*], Guoke Chen [3,*] and Nasi Ai [4]

[1] Gansu Analysis and Research Center, Lanzhou 730099, China; gfcyq09@163.com (Q.Y.); zfgood_303@163.com (F.Z.); hedwingqian@163.com (W.L.)
[2] State Key Laboratory of Vegetable Biobreeding, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Key Laboratory of Vegetables Quality and Safety Control, Ministry of Agriculture and Rural Affairs of China, Beijing 100081, China; xudonghui@cass.cn (D.X.)
[3] Gansu Province Institute of Cultural Relics and Archaeological, Lanzhou 730015, China
[4] Key Laboratory of Geriatric Nutrition and Health (Beijing Technology and Business University), Ministry of Education, Beijing 100048, China; ainasi@btbu.edu.cn
[*] Correspondence: liuguangyang@caas.cn (G.L.); chenguoke19801228@163.com (G.C.)
[†] These authors contributed equally to this work.

Abstract

The identification of dyes in ancient textiles is crucial for provenance research and scientific conservation. However, the extremely significant value of these cultural relics necessitates the use of non-destructive analytical techniques. To establish a non-destructive, in-situ, accurate, and rapid method for identifying natural dyes in ancient silk fabric samples, we employed desorption electrospray ionization high-resolution mass-spectrometry imaging (DESI-MSI). By optimizing key instrumental parameters—including sample pretreatment method, DESI spray solvent composition, and DESI heated transfer line (HTL) temperature—we determined the optimal mass-spectrometry imaging conditions. The optimal conditions for achieving the highest mass-spectrometry ion peak signal intensity and the best imaging quality were as follows: employing sample pretreatment using double-sided adhesive tape; a spray solvent composed of methanol (100%, v/v) with 0.1% formic acid and 0.1 µg/mL of leucine enkephalin; and an HTL temperature of 400 °C. The characteristic compound in the G42 silk fabric sample was successfully separated. Based on the characteristic mass-to-charge ratio of the major component, the compound was preliminarily identified as berberine. This result was further verified by tandem mass-spectrometry imaging and tandem mass spectra and finally confirmed by comparison with the mass spectrum of a reference standard. Consequently, the source of the dye in the sample was determined to be amur cork tree. The experiments confirmed the applicability and accuracy of the DESI-MSI method for the non-destructive analysis of precious textiles. This work underscores the urgent need to use such non-destructive techniques to provide technical support for the identification of high-value, inaccessible, or fragile silk artifacts and guide the historical tracing and preservation of these cultural relics.

Keywords: DESI-MSI; silk fabric sample; mass-spectrometry imaging and analysis; separation and identification

Academic Editor: Natalia Manousi

Received: 5 April 2026
Revised: 2 May 2026
Accepted: 4 May 2026
Published: 9 May 2026

https://doi.org/10.3390/separations13050145

1. Introduction

The natural dyes found in textiles serve as important carriers of cultural value and technological knowledge [1], making the analysis of dye components highly significant for historical research [2]. Dyeing techniques in ancient textiles were predominantly based on plant dyes, which are rich in variety and have a long history. As dyeing technologies gradually became more systematic and mature, a comprehensive and structured color system emerged, laying an important foundation for textiles in ancient civilizations [3]. Red, blue, and yellow were the three most important colors among ancient plant dyes. However, due to differences in geographical environments and cultural traditions, the types of plant dyes used varied across regions. In Ancient Egypt, Greece, and Medieval Europe, red dyes were mainly derived from Rubia tinctorum [4,5], while cochineal was used in Central and South America [6], and safflower was widely used in China, Japan, and Egypt [7–9]. Additionally, the ancient Mediterranean region utilized dye plants such as dragon's blood and red algae [10]. Among blue dyes, indigo has the longest history of use and was widespread in India, China, and Europe [11]. Its use can be traced back to 3000 BCE, when it was already being employed for textile dyeing, and techniques for its fermentation, oxidation, and reduction have been passed down to the present day [12]. Northern Africa and the Middle East were home to Indigofera tinctoria [13]. Haridra (turmeric, Curcuma longa) served as a major source of yellow dye in Europe, East Asia, South Asia, and beyond [8]. In Central and Western Europe, weld was commonly used as a yellow dye, while in East Asia, pagoda bud and amur cork tree were more frequently utilized [14]. The trade and dissemination of ancient plant dyes primarily occurred through key routes such as the overland Silk Road and the Maritime Silk Road [15]. These routes not only facilitated the exchange of goods but also promoted the transmission of cultures and technologies.

Dye identification holds significant value for cultural relic conservation, historical research, and cultural inheritance. At the level of conservation and restoration, analyzing the sources of dyes, the dyeing process, and the degree of aging can provide a key basis for the formulation of scientific protection plans and the implementation of precise restoration of cultural relics and effectively avoid secondary damage to cultural relics [16]. At the level of historical and cultural research, the types and process characteristics of dyes can directly reflect the technical level, cultural exchanges and trade exchanges of ancient society [17–21] and provide important evidence for age determination and origin traceability regarding textiles [16,18,22], Moreover, dyes also carry symbolic significance and artistic connotation [23]. At the level of technical analysis and process research, the application of modern detection technologies, represented by high-performance liquid chromatography (HPLC), spectroscopy, and mass spectrometry (MS), has deepened our understanding of complex ancient dyeing processes [24]. At the level of cultural inheritance, dye identification is helpful for restoring the lost traditional dyeing techniques and provides a scientific basis for the revival of traditional processes [25]. Furthermore, relevant test data can also be used to build a professional dye database to lay the foundation for subsequent research and protection work [26].

At present, the identification of ancient textile dyes has developed into a multidimensional method system combining chromatography, spectroscopy and mass spectrometry. Among these components, high-performance liquid chromatography (HPLC) is often combined with mass spectrometry (MS) due to its high separation efficiency. It is widely used for qualitative and quantitative analysis of complex natural dye components such as Rubia tinctorum [27]. While this method enables the accurate identification of molecular structures, it requires destructive sampling of the artifact in question. For artworks of significant value, preserving their integrity is always a priority. This concern has driven the development of non-invasive analytical techniques, which are increasingly applied in the study of colorants [28]. Spectroscopic techniques are known for their non-destructive testing capabilities: techniques such as Fiber-Optic Reflectance Spectroscopy (FORS) and Fluorescence Spectroscopy (FS) can directly analyze the surface of cultural relics, but they are susceptible to weak signals and fluorescence interference. Surface-enhanced Raman spectroscopy (SERS) offers high sensitivity, but it has difficulty distinguishing mixed dye components [29]. Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) is an ambient, open-air, soft-ionization mass-spectrometry technique. It offers several advantages, including simple sample pretreatment, a simple experimental procedure, fast analysis, and intuitive information. Furthermore, it is characterized by its high specificity, high sensitivity, high throughput, label-free nature, in situ capacity, non-destructiveness, and environmental friendliness [30,31]. At present, this technology has been widely used in fingerprint imaging [32], forensic identification [33,34], poison and drug detection [35,36], cancer diagnosis and treatment, biomedicine, drug analysis, food safety, environmental monitoring, animal and plant physiology, material characterization, and other fields [37–43]. In view of the prominent cultural value of historical relics, research in this vein is often limited by small sample sizes, fragility, and the need to avoid destructive sampling [28,43]. DESI-MS analysis technology shows good application prospects in the study of fragile cultural relics by virtue of its in situ analysis ability. However, reports on the application of this technique for identifying dyes in ancient textiles are still scarce, motivating this study. This paper introduces the application of DESI-MS for characterizing natural dyes in silk fabric unearthed from the tomb of Murong Zhi from the Tang Dynasty in China and presents the preliminary results of imaging experiments.

2. Materials and Methods

2.1. Reagents Solvents and Materials

G42 silk fabric sample (G42) was a small piece (1 cm^2) of brownish-yellow silk fabric that naturally detached from the lining of a robe. It was unearthed from the right side of the body of the occupant of the Tang Dynasty Murong Zhi Tomb (M1) located in Chashan Village, Tianzhu County, Wuwei, Gansu Province, China. Since its discovery, the sample has been preserved without any treatment. It was supplied by Research on the Degradation Mechanism and Protection Technology of Organic Cultural Relics Discovered project in Gansu Province. H_2O, CH_3OH, and CH_3CN (LC-MS grade) were purchased from ThermoFisher Scientific, Waltham, MA, USA. Leucine enkephalin and Berberine were obtained from TargetMol Chemicals Inc., Boston, MA, USA. Double-sided adhesive tape and custom coverslips (76 mm × 26 mm) were bought locally.

2.2. Instrumentation

DESI-MSI was conducted on a platform that included a DESI XS source (Waters, Milford, MA, USA) (Figure 1), a Q-TOF/MS (XevoMRT, Waters), and a solvent delivery system (ACQUITY UPLC M-Class μBSM, Waters).

2.3. DESI-MS Analysis

The distribution of the G42 silk fabric sample was detected using a DESI XS- XevoMRT mass spectrometer (Waters, Milford, MA, USA). G42 silk fabric sample (ca. 1 cm^2) was fixed on a glass slide (76 mm × 26 mm) with double-sided adhesive tape and placed on the stage of the DESI ion source. Data were acquired in positive/negative DESI ionization mode with full-scan MS detection. The working parameters were as follows: The capillary voltage was set to 0.5 kV (negative mode) and 0.7 kV (positive mode); the mass range was m/z 50–1200; the collection angle was 10°; the incident spray angle was 70°; the sprayer-to-surface distance was 1.0 mm; the pixel size was 30 μm × 30 μm; the scan rate was 20 Hz; the N_2 pressure was 0.9 bar; and the source temperature and heated transfer line (HTL) temperature were maintained at 150 °C and 400 °C, respectively. The spray solvent consisted of methanol containing 0.1% formic acid and 100 ppb of leucine enkephalin (an additive in spray solvents used for real-time correction of mass-spectrometry signals in this experiment), delivered at a flow rate of 2 μL/min. To identify the compound, details of each peak were scanned in the MS/MS spectrum. A total of 1000 ions were extracted to generate the MSI (HDI v1.9, Waters). The elemental composition of the ions was determined via MassLynx software (v4.2, Waters).

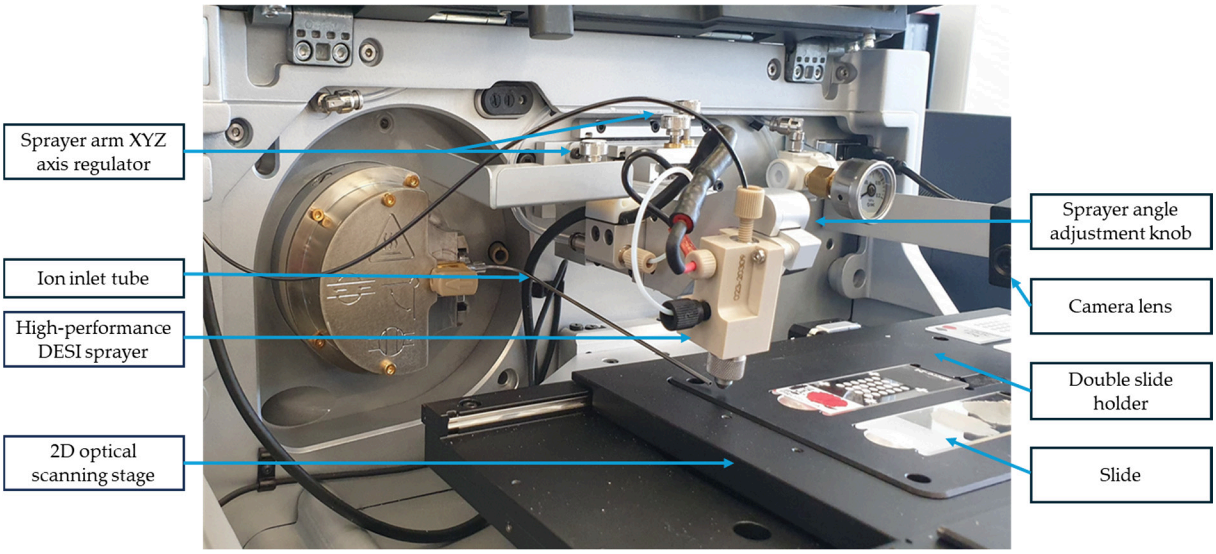

Figure 1. Photograph of DESI XS source.

3. Results

3.1. Preparation of the Silk Fabric Sample

In the preparation of DESI samples, attention should be paid to ensuring the sample is stable so as to ensure repeatability and sensitivity for the target analyte. Fixation is the key step in achieving this goal [41]. Therefore, in order to maintain the original state of the sample to obtain better imaging results, the sample needs to be non-destructively fixed, and the choice of fixation method has an important influence on signal intensity and imaging quality. In this experiment, the effects of two different fixation methods on imaging signals were compared. Two fixation tools, namely, custom coverslips (Figure 2a) and double-sided adhesive tape (Figure 2b), were used in the test, and the fixation tool with greater signal intensity was selected. As shown in Figure 2c,d, the imaging signal intensity of double-sided adhesive fixation was higher than that of the custom coverslips (the brighter the color, the higher the signal intensity). Therefore, using double-sided adhesive was selected as the fixation method in this experiment.

3.2. Optimize DESI-MS Imaging Analysis

DESI is highly dependent on multiple parameters, which are instrumental, geometrical, and chemical in nature, and a small change in one often leads to a necessary change in all the others. Optimization of the spray solvent, heated transfer line (HTL) temperature, EDC mode and other parameters can improve detection sensitivity, reduce background interference, and significantly improve ionization efficiency.

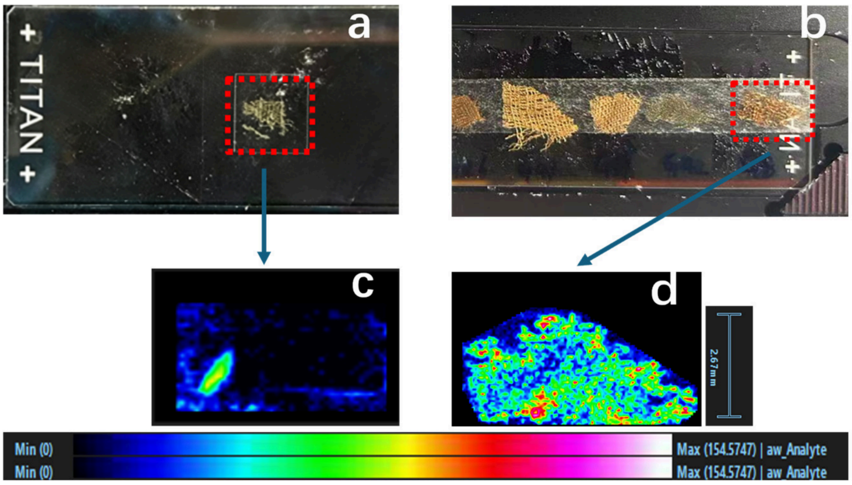

Figure 2. (**a**) The G42 silk fabric sample was placed on a glass slide and held in place with custom coverslips. (**b**) The G42 silk fabric sample was placed on a glass slide and held in place with double-sided adhesive tape. (**c**) The mass-spectrometry imaging map of the G42 silk fabric sample held in place with the custom coverslips. (**d**) The mass-spectrometry imaging map of the G42 silk fabric sample held in place with double-sided adhesive tape.

3.2.1. Optimization of Spray Solvents

The mobile phase is crucial to achieving rapid spatial targeted detection [44]. The properties of the spray solvent affect the ionization efficiency of the analyte. We optimized the type of spray solvent based on the ancient G42 silk fabric sample as the target object. On the basis of existing references [45], we prepared four different types of spray solvents (Table 1 A–D) and applied mass-spectrometry analysis with high spatial resolution to the silk fabric sample. The color differences in the mass-spectrometry imaging map reflect the intensity of the mass spectra of the constituent substances. The brighter the color, the higher the signal intensity. The results show that when using the spray solvent methanol (100%, by volume) with 0.1% formic acid and 0.1 μg/mL of leucine enkephalin added, the mass-spectrometry imaging signal is the strongest (Figure 3a), and the mass peak intensity is the greatest at a mass-to-charge ratio of 336.1239 (Figure 3b). Therefore, this solvent composition was selected as the optimal spray solvent for the sample in this experiment. At the same time, it can be preliminarily inferred that the compound is berberine. These results suggested that mass-spectrometry imaging could be used to determine the distribution and semi-quantifcation of the G42 silk fabric sample.

Table 1. Spray solvent formulation.

Sample	Spray Solvent Type
A	Methanol:water (95:5, by volume) with 0.1% formic acid and 0.1 μg/mL of leucine enkephalin added
B	Methanol (100%, by volume) with 0.1% formic acid and 0.1 μg/mL of leucine enkephalin added
C	Acetonitrile (100%, by volume) with 0.1% formic acid and 0.1 μg/mL of leucine enkephalin added
D	Acetonitrile:water (95:5, by volume) with 0.1% formic acid and 0.1 μg/mL of leucine enkephalin added

3.2.2. Optimization of the HTL Temperature

In DESI, the temperature of the HTL is a parameter that needs to be optimized based on the specific analyte. By optimizing the HTL temperature, the mass-spectrometric signal intensity of the analyte to be measured can be enhanced. The G42 silk fabric sample was selected as the research object, and berberine was used as the target to optimize the temperature of the HTL. We set five different temperature grades (100 °C\200 °C\300 °C\400 °C\500 °C) and applied mass-spectrometry analysis with high spatial resolution to the silk fabric sample. The results show that when the temperature was 400 °C, the mass-spectrometry imaging signal of red area was the strongest (Figure 4a), and the mass peak intensity was the greatest (Figure 4b). Therefore, we determined that an HTL temperature of 400 °C was the best condition for the analysis of the compounds in the G42 silk fabric sample.

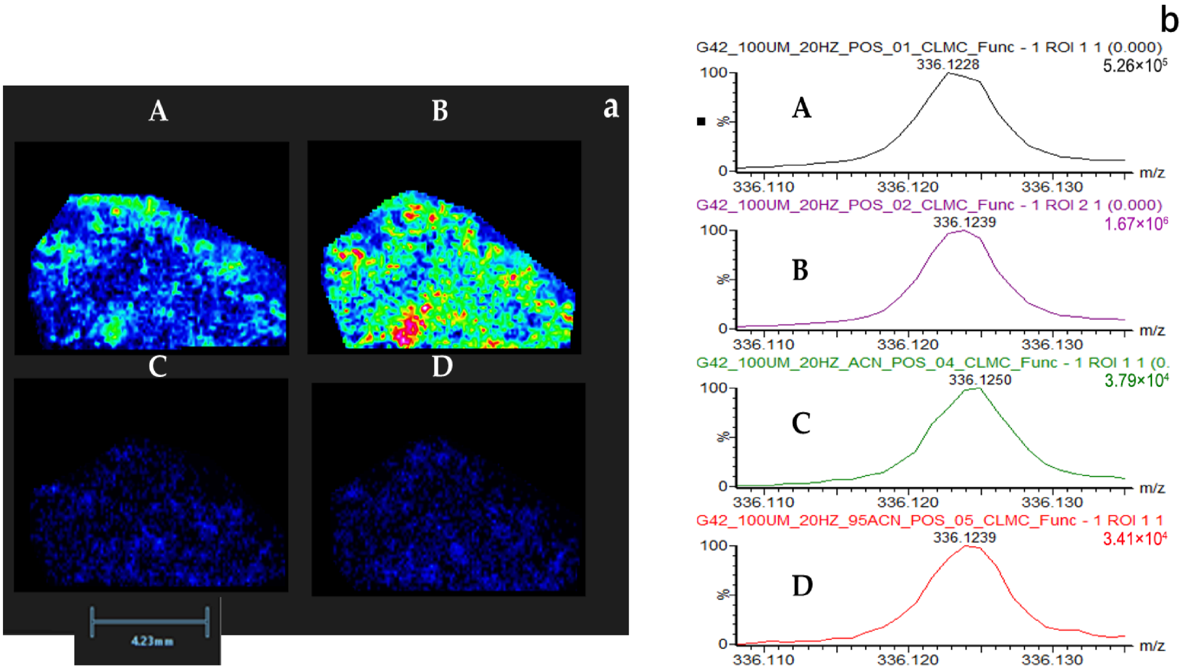

Figure 3. (**a**) The effects of four different spray solvents on mass-spectrometry imaging maps of the G42 silk fabric sample analyzed via DESI-MS. A–D correspond to Table 1. (**b**) The effects of four different spray solvents on the mass-spectrometry peak signals of the G42 silk fabric sample analyzed via DESI-MS. A–D correspond to Table 1.

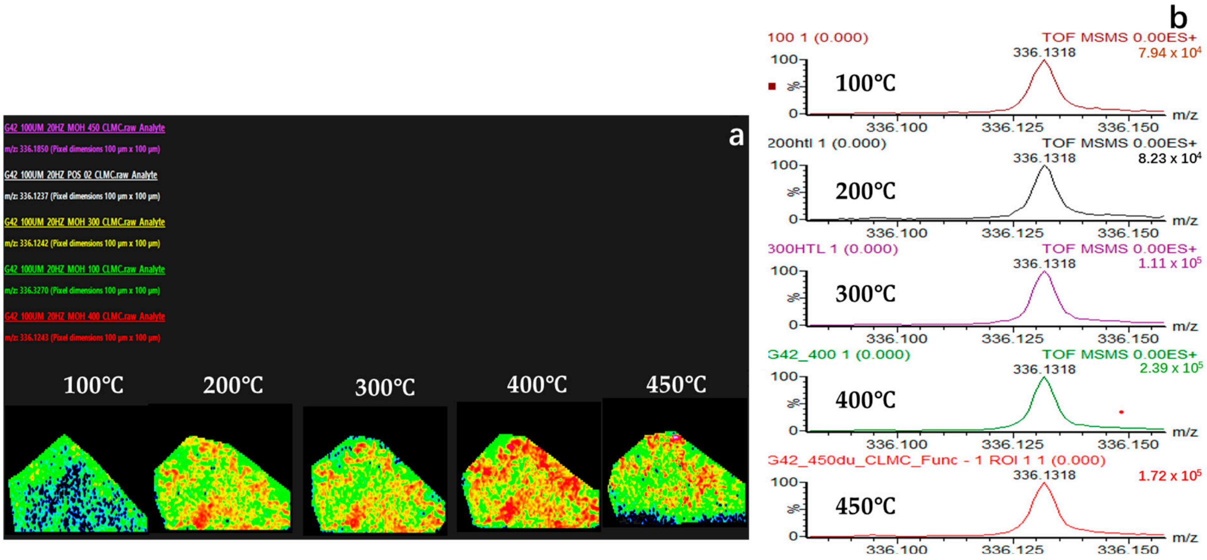

Figure 4. (**a**) The effects of five different HTL temperatures on mass-spectrometry imaging of G42 silk fabric sample analyzed via DESI-MS. (**b**) The effects of five different HTL temperatures on mass-spectrometry peak signals of G42 silk fabric sample analyzed via DESI-MS.

3.3. Verification of Dye Compounds in Silk Fibric Sample Using DESI-MS/MS

Under the optimized experimental conditions, the primary and secondary mass spectra of the characteristic compounds in the G42 silk fabric sample were compared and analyzed, and the data were processed using HDI v1.9 software to obtain the primary mass-spectrum images (Figure 5a), secondary mass spectrum images (Figure 5b–d), and corresponding secondary mass spectrum (Figure 5e) of the compound, thus further verifying that the substance was berberine. At the same time, analysis of the basic information on the seven fragment ions with high signal intensity in the compound (Table 2) showed that the mass accuracy of all fragment ions was within 1 ppm, which fully proves the reliability of this identification approach.

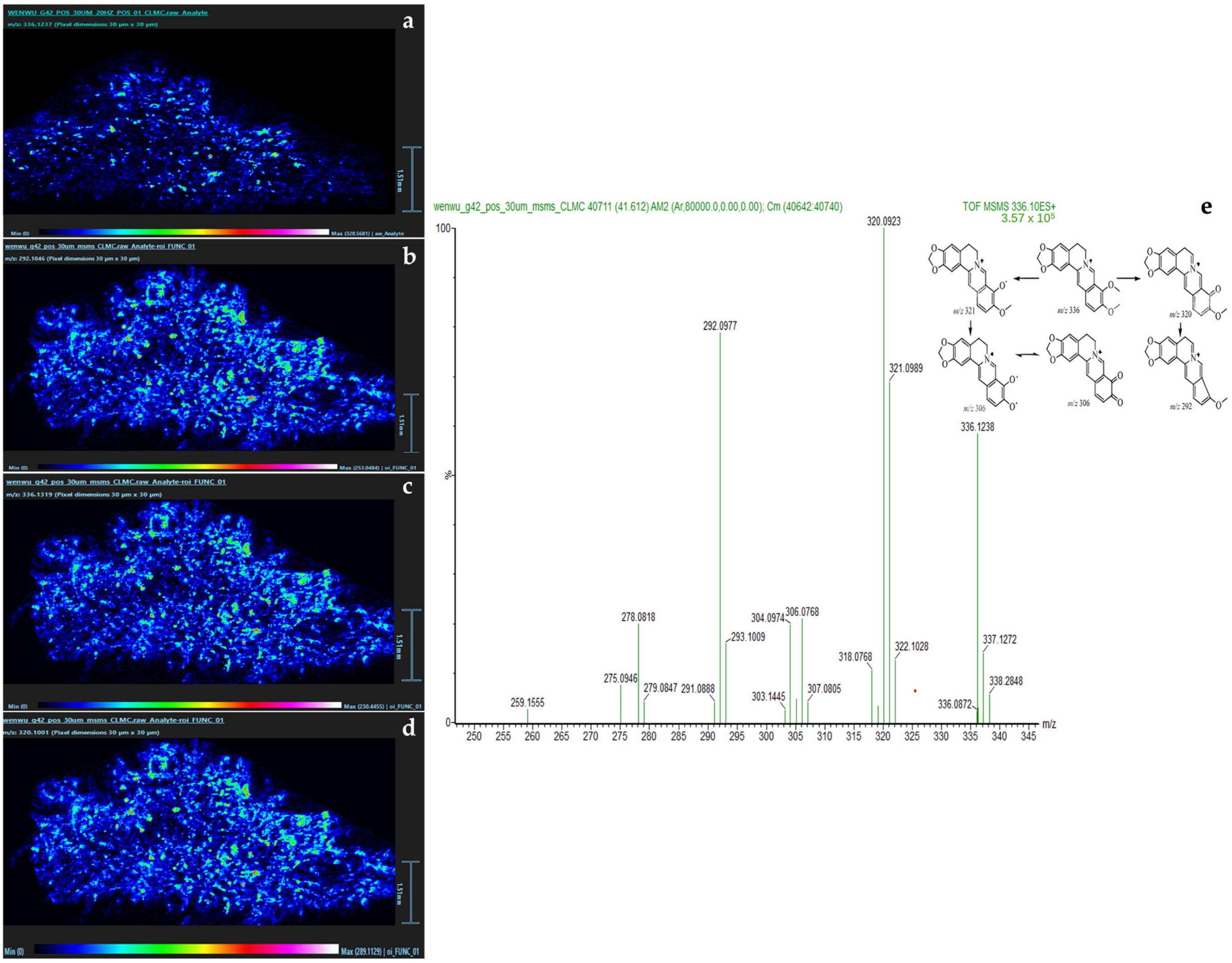

Figure 5. (**a**) Primary mass-spectrometry imaging of berberine, identified as the main dye component in the G42 silk fabric sample via DESI-MS. (**b**–**d**) Secondary mass-spectrum imaging of three daughter ions of berberine, identified as the main dye component in the G42 silk fibric sample via DESI-MS/MS. (**e**) Secondary mass spectra of berberine, identified as the main dye component in the G42 silk fabric sample via DESI-MS/MS.

Selection of DESI-MS/MS Enhanced Duty Cycle (EDC) Mode

DESI-MS/MS Enhanced Duty Cycle (EDC) mode optimizes the timing allocation of ion generation, transmission, and detection, enabling an instrument to acquire more useful signals per unit time. This technique is primarily used to address issues pertaining to weak signals and slow acquisition regarding high-resolution mass spectrometers operating in high-resolution mode. It extends the ion fill time, converting the time originally wasted during the preparatory phase into increased ion counts, thereby enhancing the signal-to-noise ratio. In the experiment, the G42 silk fabric sample was analyzed using DESI-MS/MS EDC in on and off mode for signal intensity comparison. The results show that the MS/MS spectra obtained with EDC mode turned on exhibited an over sixfold increase in signal intensity (Figure 6a,b), and the mass-spectrometry imaging clearly demonstrate higher signal intensity when this mode is on (Figure 6c,d). These findings indicate that turning EDC mode on can aid analysis of silk fabric samples and significantly improve analytical efficiency and data quality.

Table 2. The mass accuracy of seven fragment ions in silk fabric sample G42.

Mass	Calc. Mass	mDa	PPM	DBE	Formula
292.0977	292.0974	0.3	1.0	12.5	$C_{18}H_{14}NO_3$
320.0923	320.0923	0.0	0.0	13.5	$C_{19}H_{14}NO_4$
306.0768	306.0766	0.2	0.7	13.5	$C_{18}H_{12}NO_4$
336.1238	336.1236	0.2	0.6	12.5	$C_{20}H_{18}NO_4$
304.0974	304.0974	0.0	0.0	13.5	$C_{19}H_{14}NO_3$
275.0946	275.0946	0.0	0.0	13.0	$C_{18}H_{13}NO_2$
278.0818	278.0817	0.1	0.4	12.5	$C_{17}H_{12}NO_3$

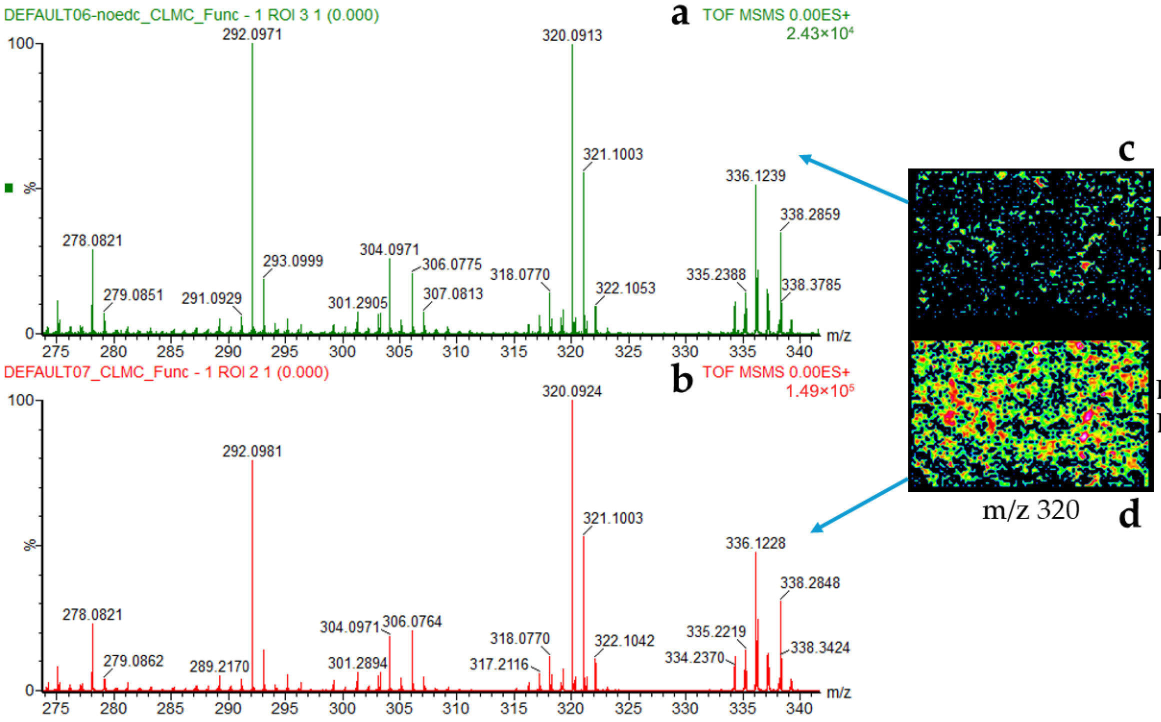

Figure 6. (**a**) Secondary mass-spectrometry signal intensity of berberine with DESI-MS/MS EDC mode off. (**b**) Secondary mass-spectrometry signal intensity of berberine with DESI-MS/MS EDC mode on. (**c**) Secondary mass-spectrometry imaging with DESI-MS/MS EDC mode off. (**d**) Secondary mass-spectrometry imaging map with DESI-MS/MS EDC mode on.

3.4. Confirmation of Dye Compounds in the Silk Fibric Sample by Using a Berberine Substance as a Standard

To confirm berberine was a component in the G42 silk fabric sample, a DESI-MS/MS mass-spectrometry image of a berberine standard and that of the berberine in the sample were compared (Figure 7). The results showed that their secondary mass spectra were completely identical. Therefore, it can be confirmed that the component is berberine.

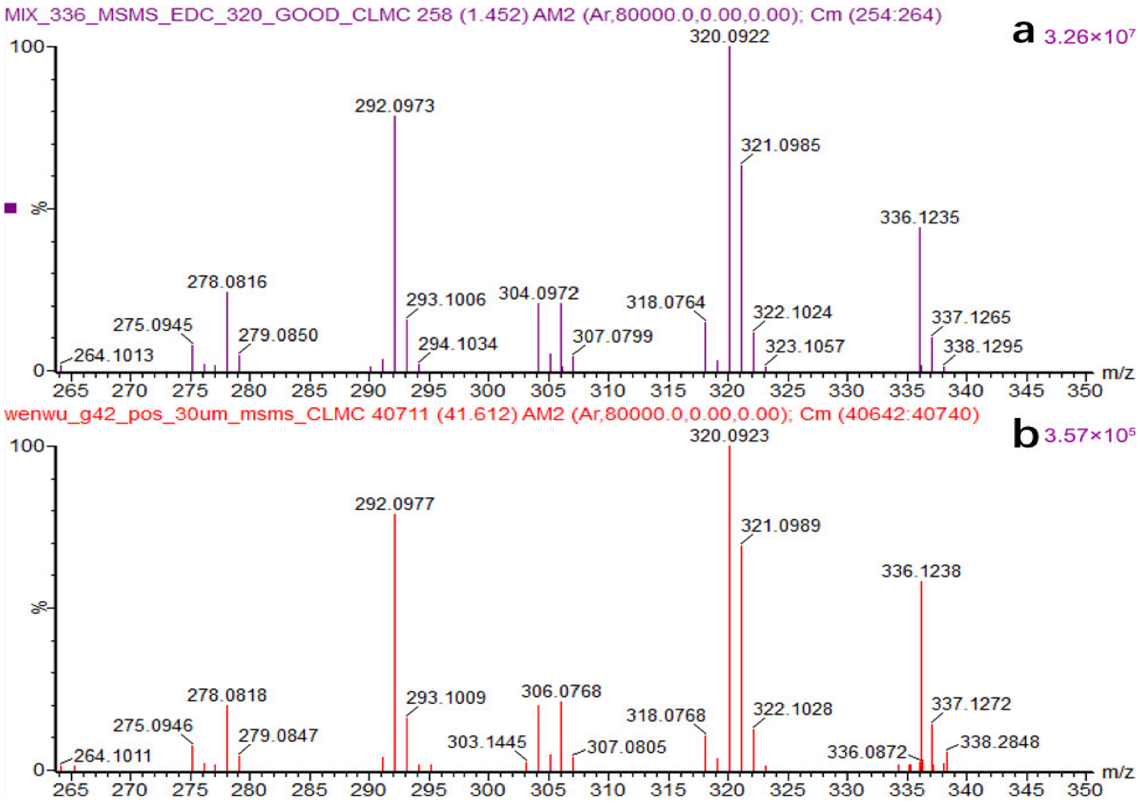

Figure 7. (**a**)The secondary mass spectrum of the standard berberine. (**b**) The secondary mass spectrum of berberine in the G42 silk fabric sample.

Berberine is the main staining component of the amur cork tree, which was commonly used as a natural yellow dye in Ancient China [46]. Berberine is a quaternary ammonium alkaloid with characteristic yellow-green fluorescence. By decocting the amur cork tree, berberine can be extracted. As a cationic dye, it electrostatically interacts with the negatively charged groups on textile fibers such as silk and wool, forming stable bonds and imparting a yellow color to the fibers. This process was an important part of ancient dyeing techniques and is extensively documented in the historical Chinese literature. Therefore, it can be inferred that the dyes of the G42 silk fabric sample unearthed from the Murong Zhi Tomb from the Tang Dynasty originated from the amur cork tree, and this is also consistent with the observed color of the sample.

4. Discussion

The analysis of dyes in ancient silk textiles is a highly complex and multidisciplinary task. Due to the preciousness, fragility, and chemical complexity of ancient fabrics, the analytical process is hindered by a series of unique and significant challenges. The introduction of DESI-MSI technology makes the analysis of dyes in ancient silk more intuitive and efficient. The application of this in situ method for dye analysis is particularly beneficial for the study of silk textiles that have undergone conservation treatments and for which sampling is not feasible. DESI-MSI enables the visualization of the spatial distribution of dyes on textiles, the identification of major dye compounds, and the analysis of patterned textiles, thereby contributing to our understanding of ancient textile dyeing techniques and dye distribution characteristics. DESI-MSI with optimized parameters can significantly enhance mass spectrometry and imaging signal intensities, thereby improving analytical efficiency and accuracy and making the identification results more reliable. In this study, DESI-MSI technology was employed, and through optimization of the testing parameters, the major dye component in the G42 yellow ancient silk fabric sample unearthed from the Murong Zhi Tomb was identified as berberine. This component is consistent with the main constituent of the amur cork tree, a commonly used yellow dye in Ancient China as recorded in the historical literature, and it also matches the color of the sample. We can therefore infer that the dye in the sample originated from the amur cork tree. The experiments confirmed the rationality and reliability of applying DESI-MSI in the identification and analysis of dyes in ancient silk fabrics.

However, the analysis of natural dyes in textiles using DESI-MSI is still hindered by several challenges. For instance, in the sample fixation step, the commonly used double-sided adhesive tape method for textiles may introduce polymer background signals and volatilization interference. To mitigate these risks, it is necessary to select double-sided tape with low volatility and minimal polymer content and conduct blank background testing to evaluate signal interference. Alternatively, developing new fixation equipment specifically designed for textiles could be considered. Regarding the use of mordants, which can lead to reduced ionization efficiency, and sample degradation causing chemical property changes, as well as the poor water solubility of natural dyes resulting in low desorption efficiency, it is essential to assess the compatibility between different spray solvent systems and the DESI detection of natural dyes in silk textiles. Other viable approaches include exploring unconventional solvents to enhance ionization or optimizing key instrument parameters to improve the accuracy and applicability of the analysis. These methods can significantly enhance signal intensity and detection sensitivity, ultimately improving the accuracy, comprehensiveness, and applicability of the analysis.

Future research will focus on applying DESI-MSI to more complex systems and unknown analytes as well as strengthening interdisciplinary collaboration by integrating knowledge from multiple disciplines such as history, chemistry, and materials science. This will expand the application of this method in the analysis of complex dyes, enhance its comprehensive identification capability, and promote further development in the study of ancient silk dyes.

5. Conclusions

In this study, we developed an optimized desorption electrospray ionization mass-spectrometry imaging (DESI-MSI) method and established a corresponding visual analytical workflow for the non-destructive detection of natural dyes in ancient silk fabrics. The identification of natural dyes in ancient silk fabrics provides important evidence for archaeological dating and provenance tracing as well as scientific guidance for the conservation and restoration of cultural relics. By determining key experimental parameters—using double-sided adhesive tape for sample fixation; using the optimal DESI spray solvent, i.e., methanol (100%, by volume) with 0.1% formic acid and 0.1 μg/mL leucine enkephalin added; and setting the heated transfer line (HTL) temperature to 400—we significantly enhanced the signal intensities in both mass-spectrometry imaging and mass spectra. Following these optimizations, the compound was effectively separated. Based on the characteristic mass-to-charge ratio of the major component in the silk sample, the compound was preliminarily identified as berberine, and this was further verified by tandem mass-spectrometry imaging. Moreover, the use of DESI-MS/MS EDC mode increased the intensity of the mass-spectrometry signal sixfold, markedly improving the reliability of identification. Finally, the compound was confirmed to be berberine through comparison with the tandem mass spectrum of a reference standard. This compound is consistent with the main component of the amur cork tree, a commonly used yellow dye in Ancient China as recorded in the historical literature, and matched the color of the sample. Therefore, it can be inferred that the dye in the sample originated from the amur cork tree. The optimized DESI-MSI method was successfully applied to the analysis of natural dye samples in ancient silk fabrics, demonstrating its applicability and accuracy for the non-destructive analysis of precious textiles. This study underscores the urgent need for such non-destructive techniques. Throughout the entire analysis in this experiment, the sample was only gently fixed with double-sided adhesive tape and could be easily removed after the analysis was complete, after which it could be directly returned to storage or used for other supplementary analyses. This technique can support the identification and guide the conservation of precious, inaccessible, or fragile silk artifacts.

Author Contributions: Conceptualization, Q.Y., F.Z. and W.L.; methodology, W.L., Y.W., L.Z. and X.L.; software, W.L., W.G. and X.L.; validation, Y.W., W.G., X.L. and N.A.; formal analysis, Q.Y., F.Z., G.L. and G.C.; investigation, F.Z., Y.W., L.Z. and J.L.; resources, D.X., G.L. and G.C.; data curation, W.G., J.L. and G.L.; writing—original draft preparation, Q.Y. and F.Z.; writing—review and editing, D.X., G.C. and N.A.; visualization, Q.Y., L.Z. and J.L.; supervision, F.Z. and N.A.; project administration, D.X.; funding acquisition, Q.Y. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Gansu Provincial Science and Technology Major Project [25ZDFA014], the Gansu Longyuan Young Talent Program, the Special Project for Enhancing the Scientific Research Innovation Capacity of Provincial Research Institutes [25CXTA001], the Basic Research Center, the Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-BRC-202612), the Beijing Natural Science Foundation (6242028), the National Key Research and Development Program of China (2022YFF0606800), the Special Fund for the Industrial System Construction of Modern Agriculture of China (CARS-23-E03), the Cross-Innovation Open Project of Food Flavor and Health, and the Beijing Technology and Business University.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Acknowledgments: We thank Xiaofei Jia from Waters Technology (Shanghai, China) Co., Ltd., for providing technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DESI-MSI	Desorption Electrospray Ionization High-Resolution Mass-Spectrometry Imaging
DESI-MS	Desorption Electrospray Ionization Mass Spectrometry (MS scan mode)
DESI-MS/MS	Desorption Electrospray Ionization Tandem Mass Spectrometry (MS/MS Scan Mode)
HTL	Heated Transfer Line
EDC	Enhanced Duty Cycle
HPLC	High-Performance Liquid Chromatography
MS	Mass Spectrometry
FORS	Fiber-Optic Reflectance Spectroscopy
FS	Fluorescence Spectroscopy
SERS	Surface-Enhanced Raman Spectroscopy
LC-MS	Liquid Chromatography Mass Spectrometry

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