

Article

Optimizing HPLC–HRMS Analysis of Indigoid Dyes in Archeological Textiles: Minimizing Extraction-Induced Degradation and Identifying Transformation Markers

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Abstract

The chemical identification of indigoid dyes in archeological textiles remains a significant analytical challenge due to the diversity of indigoid components, their susceptibility to post-deposition degradation, and the risk of further transformation during sample preparation. Seventeen samples of early medieval textiles from the ancient city of Deraheib (Sudan) were studied. Tryptanthrin—an indoloquinazoline alkaloid previously documented only in model solutions—was identified in archeological textiles for the first time, providing a new qualitative marker of indigo-type dyeing that may persist when the original dye has undergone substantial degradation. Two variants of the methanolic hydrochloric acid treatment step—ultrasonication without heating versus boiling in a water bath at 100 °C—and the replacement of dimethylformamide with acetonitrile in the third extraction stage were systematically compared.

Keywords: indigoid dyes; archeological textiles; HPLC–HRMS; Orbitrap; indigotin; tryptanthrin; extraction-induced degradation

1. Introduction

Chemical analysis plays an essential role in modern archeological research [1], enabling, for example, the verification of authenticity in museum textile collections through diverse analytical methods [2]. Despite these capabilities, the identification of dyes in archeological textile samples remains a complex challenge due to the significant diversity of their chemical compositions and the potential presence of decomposition products within the samples [3].

Modern analytical methods, such as high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS), allow for both the reliable determination of known compounds and the identification of unknown ones [4–6]. While low-resolution mass spectrometry (LRMS) using single or triple quadrupole mass analyzers is widely used for analyzing known components in archeological textiles [3], identifying unknown

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compounds is significantly more challenging and typically requires high-resolution mass spectrometry (HRMS) combined with liquid or gas chromatography. Given the complexity and high cost of this specialized equipment, such studies have not yet seen widespread application. Consequently, only a limited number of works have been published to date on the determination of indigo-series dyes using HRMS [2,7,8].

Chromatography coupled with mass spectrometry has been successfully applied to the identification of indigoid dyes in archeological textiles, with established protocols utilizing triple quadrupole [9,10], linear ion trap [11], and their combination [12,13]. Compared to HPLC-LRMS, the use of HPLC-HRMS significantly expands the range of detectable indigoid substances and enhances the reliability of identification [3,14]. Furthermore, recent research has incorporated time-of-flight mass spectrometry for the characterization of blue dye components dyes in archeological textiles [2,7].

For common dyes like indigo, an additional analytical complication arises because the plant-based raw materials initially contain substances that undergo a series of chemical transformations during the dyeing process (Figure 1) [15,16]. These substances may also degrade during the textiles' prolonged exposure to the environment [17]. Furthermore, the very process of extracting dyes from textiles for chemical analysis carries the risk of further transformation and degradation of the substances being analyzed [9,18].

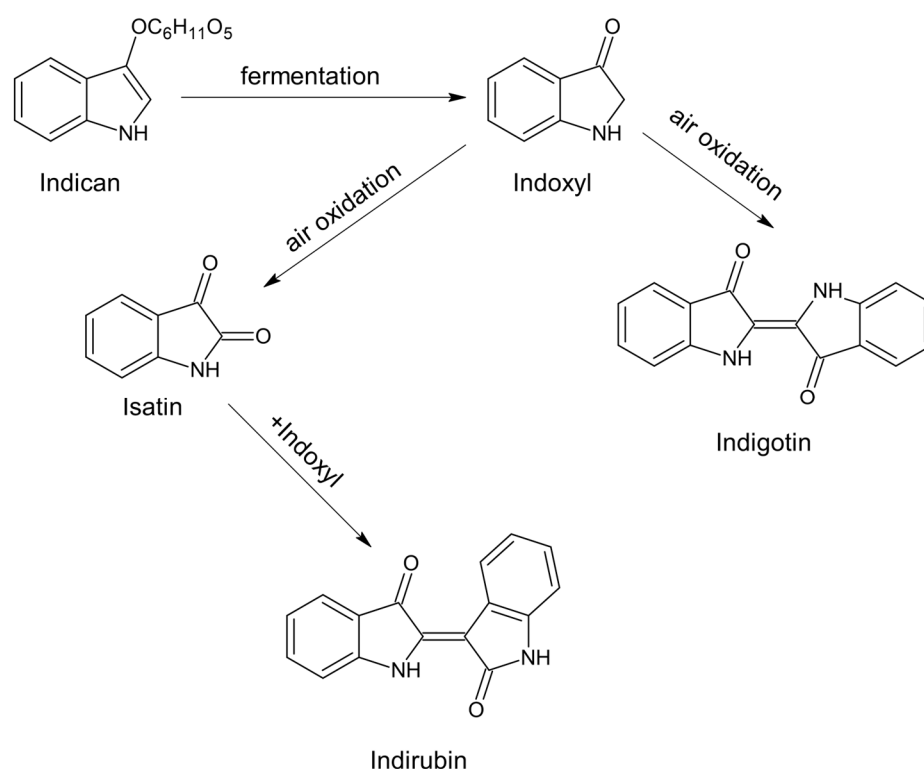


Figure 1. Scheme of the indigo dye production [15].

The first objective of the current publication was to analyze the 17 medieval archeological textile samples supposedly dyed with indigo, identifying known dye markers, which can be accomplished using the HPLC-HRMS method. In order to solve this problem, we had to tackle two other, secondary objectives: the second one was to identify degradation products of dye components in textile samples, enabling the determination of dye types even when the primary substances have significantly decomposed. Finally, the third objective was to minimize the degradation of dye components during sample analysis.

2. Materials and Methods

2.1. Materials

Solvents: methanol (purity $\geq 99.99\%$, Multisolv, S.-Petersburg, Russia), acetonitrile (HPLC grade, Scharlab, Barcelona, Spain), hydrochloric acid (high purity, SigmaTech, Huntsville, AL, USA), formic acid (98–100%, Scharlab, Barcelona, Spain), deionized water from Milli-Q water cleaning system (Millipore, Burlington, MA, USA).

Standard substances: Indican (purity 99.88%, MedChemExpress, Monmouth Junction, NJ, USA), Isatin (purity 99.89%, MedChemExpress), Indigotin (purity $\geq 98.0\%$, MedChemExpress), (-)-epicatechin, (+)-catechin, rutin, vitexin, quercetin, genistin, naringenin, kaempferol, isorhamnetin (all $>98\%$, from Sigma-Aldrich, Burlington, MA, USA).

2.2. Equipment

For preliminary experiments low-resolution mass spectra were obtained using high-performance liquid chromatography–mass spectrometry system Expec L-Chrom MS triple quadrupole (Expec, Hangzhou, China) equipped with an electrospray ionization source.

High-resolution mass spectra were obtained using HPLC–high-resolution mass spectrometry (HRMS) system consisting of Dionex Ultimate 3000 liquid chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) and orbital ion trap mass spectrometer Orbitrap Elite Hybrid (Thermo Fisher Scientific, Waltham, MA, USA), equipped with an electrospray ionization source.

Microbalance AND VM-22 (AND, Tokyo, Japan) was used for textile sample weighting. Genius NM32LA nitrogen generator (Peak Scientific, Inchinnan, Scotland, UK) and nitrogen flow evaporative concentrator NDK200-2N-24 (Miulab, Hangzhou, China) were used for sample evaporation. Ultrasonic bath Sapphire 10456 2.8 L (Sapphire, Moscow, Russia) was used for sample extraction. Mini-centrifuge MiniSpin (Eppendorf, Hamburg, Germany) was used for separation of textile end extract.

2.3. Archeological Site and Textile Samples

The archeological site of Deraheib is located in Sudan's Red Sea Province, at the headwaters of Wadi al-'Allaqi, approximately 400 km from the Nile and 200 km from the Red Sea. Since 2017 it has been investigated by the Nubian Archeological and Anthropological Expedition of the Anuchin Research Institute and Museum of Anthropology, Lomonosov Moscow State University [19,20]. The layers currently under study are dated, on the basis of archeological evidence and written sources, to the 9th–12th centuries.

Deraheib is identified with the town of al-'Allāqī, which stood on the caravan route linking Aswan to the Red Sea port of Aydhab and carried goods from Arabia, Africa, India, and China. It was also the principal center of gold mining in the Nubian Desert [21]. The site comprises the Northern and Southern Fortresses, the town proper, the Southern necropolis, prospectors' settlements, and mines. The Northern Fortress (26.5 × 26.5 m) yielded a rich assemblage of imported and luxury goods, suggesting that it served as the residence of the local ruler rather than as a garrison fort.

Archeological textiles are rare finds in Northern Nubia and the medieval Arab East, and finds from settlement contexts of the Islamic period are especially scarce. At Deraheib about 200 fragments were recovered in 2020 from the fill of Room I of the Northern Fortress, dated by pottery to the 10th–11th centuries [22]. The assemblage is strikingly varied, ranging from coarse goat- and camel-hair fabrics, probably associated with the local Beja nomads, to fine linens, cottons, and patterned silks. Blue-dyed fabrics clearly predominate, making the identification of the blue dye central to the study of the collection as a

whole. Accordingly, seventeen fragments were selected for chemical analysis on the basis of their blue color, and a small portion of each was subjected to HPLC–MS analysis.

All seventeen textiles were recovered from Room 1 of the Northern Fortress at the Deraheib settlement, which, during its period of use, functioned as a refuse dump and latrine. A total of 17 samples (Nos. DB006–022) were examined (see Table S1 in Supplementary Materials for details): 13 are blue (DB006, DB007, DB009, DB010, DB011, DB012, DB013, DB015, DB017, DB018, DB019, DB020, DB022), three are brown or reddish-brown (DB008, DB014, DB021), and one is light-colored (DB016). In terms of fiber composition, the assemblage comprises six wool samples (DB008, DB010, DB011, DB012, DB014, DB021), two flax samples (DB013, DB018), six cotton samples (DB009, DB015, DB016, DB017, DB020, DB022), one silk sample (DB019), and two mixed flax-and-cotton textiles (DB006, DB007). The textiles vary considerably in quality and degree of manufacture: samples DB008, DB015, DB016, DB018, DB019 and DB022 appear to be of particularly fine or high-quality manufacture, while sample DB021 is characterized by a sparse, gauze-like and coarse structure; samples DB011–013 are heavily soiled, substantially limiting their characterization prior to conservation. The remaining samples display intermediate characteristics and differ in thread density, yarn thickness, direction and degree of twist, and uniformity of coloration; these parameters will allow a more precise assessment of textile quality and manufacturing techniques.

2.4. Extraction of Dye Components from Textile Fibers

Extraction of dye components from the textiles was performed with modifications based on the method previously presented in the literature [12]. The textile sample was weighed and transferred to a chromatographic vial, to which 600 μL of an $\text{HCl}:\text{MeOH}:\text{H}_2\text{O}$ mixture (2:1:1, $v/v/v$) was added for acid hydrolysis step. The vial was then placed in an ultrasonic bath for 30 min ($40 \pm 5^\circ\text{C}$). The resulting hydrolysed extract was evaporated under a nitrogen stream (60°C), after which 600 μL of an $\text{MeOH}:\text{H}_2\text{O}$ mixture (2:1, v/v) was added to extract polar fraction. The vial was again placed in the ultrasonic bath ($40 \pm 5^\circ\text{C}$) for 30 min, and an aliquot was removed for analysis of the polar compounds. The remaining solution was evaporated, 600 μL of acetonitrile was added to extract low polar components, and the vial was placed in the ultrasonic bath ($40 \pm 5^\circ\text{C}$) for 30 min. Both extracts were then centrifuged and filtered through a membrane filter before chromatographic separation. Procedure outline is given in Figure 2.

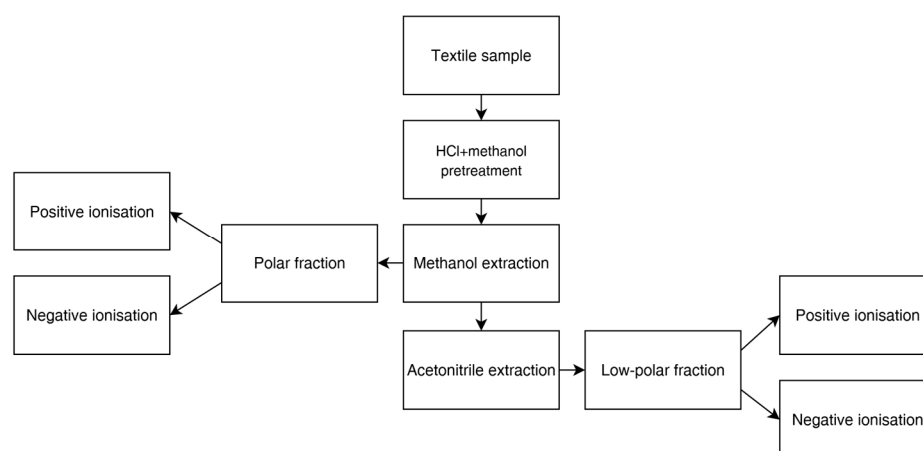


Figure 2. Sample preparation scheme.

2.5. HPLC-HRMS Conditions

Chromatographic separation was carried out on an LC column Acclaim C 18 (120 Å, 2.2 µm, 3 mm i.d., 150 mm length, Thermo Scientific, USA) using 0.1% HCOOH in water (A) and acetonitrile (B) as the mobile phase. Flow rate was 0.4 mL/min. The temperature of the column was 40 °C. Elution gradient program: 0–3 min (5% B), 3–17 min (5 → 95% B), 17–21 min (95% B), 21–22 min (95 → 5% B), 22–25 min (5% B).

Mass spectrometric detection was performed in the data-dependent acquisition (DDA) mode under the following conditions. The nebulizer needle voltage was +3500/−2500 V (for positive and negative ions, respectively). Nebulizer gas (sheath) was 50 Arb, Sweep—1 Arb, Aux—10 Arb. The temperature of the ion-conducting capillary was 325 °C. The temperature of the nebulizer gas was 350 °C. Scanning mode parameters were as follows: m/z range—120–1000, resolution—30000, voltage on the RF lens—70%. Parameters of the information-dependent mode of fragmentation spectra accumulation: dynamic exclusion after 2 spectra within 10 s, exclusion of isotope signals-on, cycle-4 scans. Parameters of fragmentation spectra accumulation: quadrupole isolation window—1 m/z, fragmentation energies 15, 30, 45, 60 V, detector-orbital ion trap, resolution—15000, AGC target—300%, MaxIT—100 ms, spectra format—centroid, mass error—5 ppm.

Identification of compounds for which standard substances were not available was performed using Sirius software [23] (version 6.3.3).

3. Results and Discussion

Indigo dye is a mixture of indigoid compounds, mainly including indigotin and indirubin [9]. Its components can be derived from plants of the genera *Isatis*, *Indigofera*, and, less commonly, *Polygonum*. Because these genera have distinct geographic distributions, the botanical origin of the dye could, in principle, provide information about the geographical origin of archeological textiles [3,24–27].

Regardless of the plant species used, the preparation of indigo dye involves fermentation and oxidation steps, during which the precursor indican is converted into indigotin and various related compounds (see Figure 1). These significant chemical transformations complicate the identification of the specific botanical source of the plant material [28].

3.1. Analysis of Textile Samples from the Ancient City of Deraheib

The 17 archeological textile samples were analyzed via Orbitrap HPLC-HRMS. The obtained MS data were used for the qualitative analysis of all 17 supposedly blue archeological textile samples; the results are presented in Table 1. Selected ion chromatograms for the analyzed compounds are presented in Figure 3.

Table 1. Analysis results of the textile samples from Deraheib.

Sample ID	7-Hydroxyisatin	Isatin	Methyl Indole-1-carboxylate	Indigotin Isomer	Anthranilic Acid, N,N'-Oxalyldi	Tryptanthrin	Indigotin	Indirubin
DB006	+	+	+-	+	+	+	+-	+
DB007	+	+	+	+	+	+	+	+
DB008	+	-	+	-	+-	+-	+-	-
DB009	+	+	+	+	+	+	+	+
DB010	+	+	+	+	+	+	+	+
DB011	+	+	+	+	+	+	+	+
DB012	+	+	+	+	+	+	+	+
DB013	+-	+	-	+-	+	+	+	+
DB014	+	-	-	-	-	+-	+-	-
DB015	+	+	+	+	+	+	+-	+

DB016	+	-	+	+-	+	+-	+-	+-
DB017	+	+	+	+	+	+	+-	+
DB018	+-	+	-	+	+	+	+	+
DB019	+	+	+	+	+	+	+	+
DB020	+	+	+	+	+	+	+	+
DB021	+	-	-	-	-	+-	+-	-
DB022	+	+	-	+	+	+	+	+

Detection criteria were as follows: $S/N > 3$ —compound was detected with confidence (+ in the table), $1 < S/N < 3$ —compound was detected at the background level (+- in the table), $S/N < 1$ —compound was not detected (- in the table).

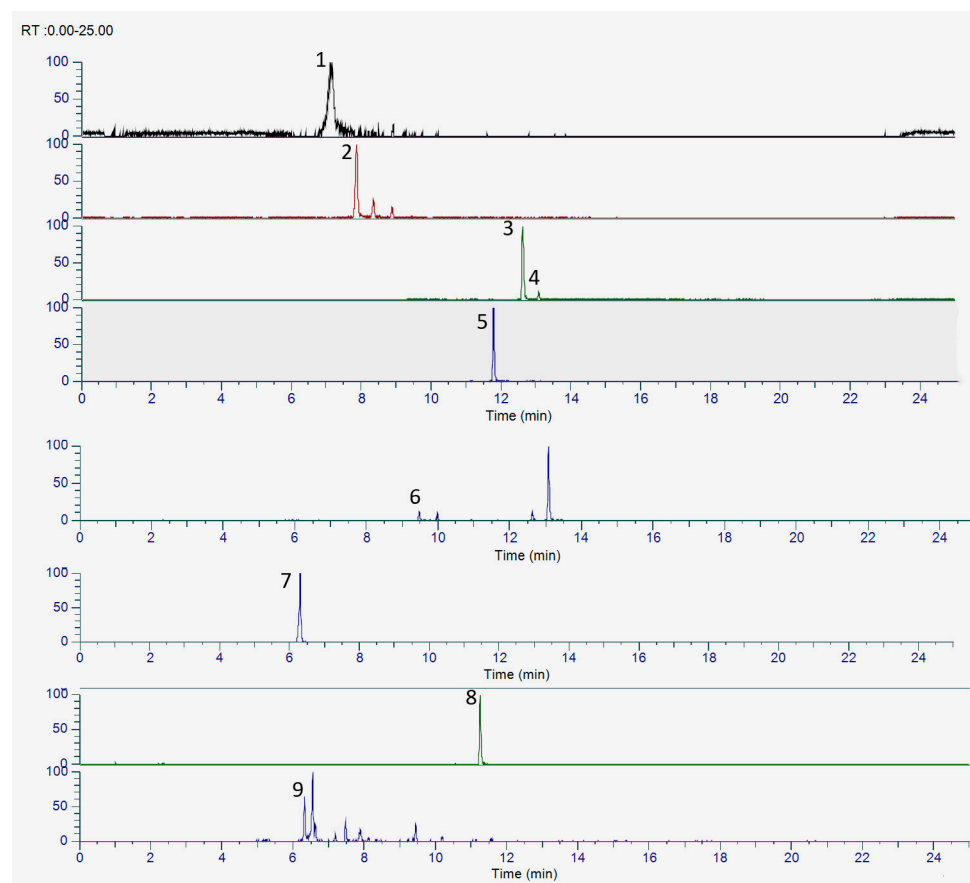


Figure 3. Selected ion chromatograms for the analyzed compounds. 1—*isatin*, 2—*methyl indole-1-carboxylate*, 3—*indigotin*, 4—*indirubin*, 5—*tryptanthrin*, 6—*indigotin isomer*, 7—*indican*, 8—*N,N'-oxalyldianthranilic acid*, 9—*7-hydroxyisatin*.

Indigotin and *indirubin* were detected simultaneously in 10 samples (DB007, DB009, DB010, DB011, DB012, DB013, DB018, DB019, DB020, and DB022), serving as the primary markers of the blue dye indigo [29]. In samples DB006, DB015, and DB017, the analytical signal for *indigotin* was at the background level, whereas *isatin*, *N,N'-oxalyldianthranilic acid*, *indirubin*, and *tryptanthrin* were identified, which may indicate the transformation of indigoid components in these samples [18]. In samples DB008, DB014, DB016, and DB021, *indigotin* was identified only at the background level, suggesting either the absence of blue dye or its destruction resulting from sample aging [30]. For instance, in sample DB016, where *indigotin* and *indirubin* were detected at the background level, the identification of *N,N'-oxalyldianthranilic acid* may indirectly confirm the use of indigo for dyeing [9]. Samples DB008, DB014, and DB021 were likely not blue, and the 7-hydroxyisatin and methyl indole-1-carboxylate found in them are not specific markers of indigoid

components, unlike isatin; the latter was found in all samples (except DB008, DB014, DB016, and DB021) and is a confirmed degradation product of the indigotin molecule [18].

Among the 17 analyzed samples, indican could not be reliably identified, a result of its transformation into indoxyl and subsequent conversion into indigotin during the fermentation and oxidation processes in the vat [28,31].

When considered alongside the technological characteristics of the fabrics (see Table S1), the HPLC–HRMS results indicate that indigo was used across a range of textile structures and fiber types, including wool, flax, cotton and silk, and in both monochrome and striped fabrics. In samples DB007, DB009–013, DB018–020 and DB022, the simultaneous detection of indigotin and indirubin provides the clearest evidence for indigo dyeing, while the presence of degraded indigoid markers in DB006, DB015 and DB017 is consistent with substantial transformation of the original dye. These results are particularly significant when correlated with the textile structure: in samples such as DB007, DB017, DB018 and DB020, dyed and undyed yarns were deliberately combined to produce striped effects, while the microscopic characteristics of several samples suggest that dyeing may have taken place at the fiber stage before spinning.

Fragmentation mass spectra were subsequently acquired in data-dependent mode for several components, including isatin, 7-hydroxyisatin, methyl indole-1-carboxylate, indigotin isomer, indigotin and indirubin (Figure 4).

The mass spectrum of indigotin displays an intense signal for the molecular ion $[M+H]^+$ at m/z 263.0814, as well as fragment ions at m/z 235.0867 and m/z 219.0919 (Figure 4), which correlates with reported low-resolution mass spectral data [13]. The product ion at m/z 235.0867 is attributed to the loss of a CO-group, while the ion at m/z 219.0919 results from the subsequent loss of an NH_2 -group [5]. For indigotin, the fragment ion at m/z 235.087 is more intense than the fragment at m/z 219.092; in contrast, indirubin displays a higher intensity for the fragment at m/z 219.092. Since indirubin is a structural isomer of indigotin, the use of fragmentation spectra significantly improved the selectivity of their identification when both were present in the sample extract.

The acquisition of fragmentation spectra increases sensitivity and selectivity compared to single-step MS, particularly for samples with complex matrices [13,32]. The indirubin (Figure 4, major protonated molecular ion $[M+H]^+$ at m/z 263.0815) was identified in the analyzed samples, with a fragmentation spectrum exhibiting product ions at m/z 235.0865 and m/z 219.0916, which correlates with literature data [13]. The product ion at m/z 235.0865 corresponds to the loss of a CO group from the molecular ion, while the ion at m/z 219.0916 results from the subsequent loss of an NH_2 group [5]. Additionally, indigotin isomer (m/z 263.0814) was detected. In its MS/MS spectra, a fragment ion at m/z 235.087 formed upon the elimination of a CO group—a feature common to indigotin and indirubin—while a fragment ion at m/z 245.071 formed upon the elimination of an H_2O molecule from the molecular ion [33,34].

Several indole derivatives were preliminarily identified in the analyzed samples, namely isatin, 7-hydroxyisatin, and methyl indole-1-carboxylate (Figure 4). Their molecular ions were recorded at m/z 148.0393, m/z 162.0197, and m/z 176.0705, respectively, and fragmentation spectra for these compounds were subsequently obtained. Methyl indole-1-carboxylate is characterized by fragment ions at m/z 148.0756, m/z 118.0650, and m/z 91.0541, which are formed by the sequential elimination of CO, CH_2O , and CHN fragments, whereas 7-hydroxyisatin exhibits a product ion at m/z 134.0248 due to the loss of a CO_2 fragment. The identification of methyl indole-1-carboxylate and 7-hydroxyisatin was performed using the Sirius software. Similarly, the fragmentation spectrum of isatin yields product ions at m/z 130.0284 and m/z 120.0443 (Figure 4), attributed to the presumed loss of H_2O - and CO-fragments by the protonated molecular ion [13]. Finally, the fragmentation spectrum of indican contains signals at m/z 132.0455, m/z 131.0377, m/z

113.0244, 101.0244 and m/z 71.0138, corresponding to the loss of the glycoside residue and the formation of indoxyl, followed by its subsequent fragmentation [5,13].

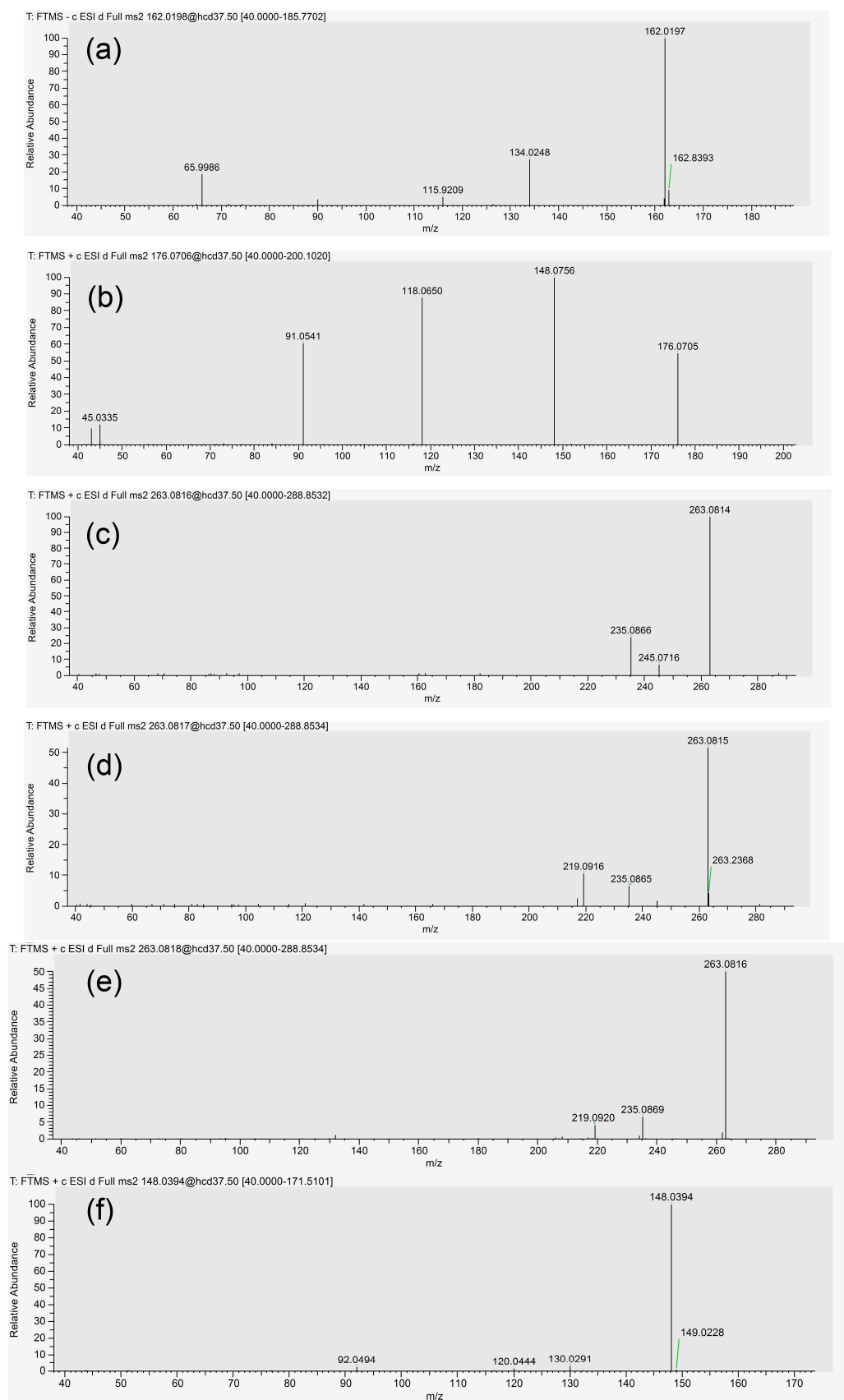


Figure 4. Mass spectra of compounds identified in textile samples from Deraheib: 7-Hydroxyisatin (a), Methyl indole-1-carboxylate (b), Indigotin isomer (c), Indirubin (d), Isatin (e) and Indigotin (f).

Taking into account that the existing literature lacks second-generation mass spectra for isatin and indigotin obtained via orbital ion trap mass spectrometry, one of the objectives of this study was to acquire these spectra under various analytical conditions. In this study, fragmentation spectra were acquired for standard substances of indigotin, isatin, and indican (Figure S1), with the resulting spectra in MSP format provided in the Supplementary Materials.

It is important to note that no other known dye markers were found in the studied textile fragments; notably flavonoids (luteolin, etc.) and tannins (gallic acid, etc.) were absent.

For a number of flavonoids, certified samples of pure substances were available (see Section 2.1), enabling the determination of analyte retention times under our chromatographic experimental conditions, as well as the acquisition of experimental mass spectra.

For other known plant dye markers, we generated extracted-ion chromatograms based on the monoisotopic masses of protonated and deprotonated molecules (in positive and negative ionization modes, respectively). The absence of peaks with a signal-to-noise ratio greater than 3 in these chromatograms indicates that significant quantities of flavonoids and tannins are not present in these samples.

3.2. Degradation Products of Indigoid Dyes in Textile Samples

Indigotin, the main component of indigo dye, is an unstable compound that undergoes degradation upon exposure to light, atmospheric oxygen, and elevated temperatures, forming various degradation products. Literature on experiments with model solutions of indigo dyes has identified isatin, isatoic anhydride, tryptanthrin, and others among these products; see Figure 5 [18,30,35]. Data on indigotin degradation products in the context of archeological textile analysis is limited. However, substances such as anthranilic acid, methylisatin, and other indole derivatives—presumed to be indigotin degradation products—have been identified in ancient textiles [17].

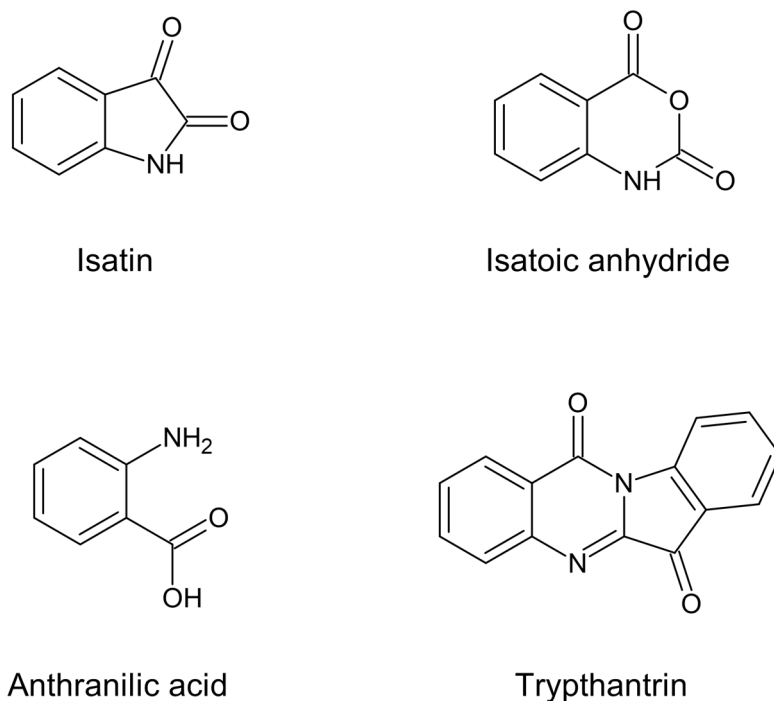


Figure 5. Some indigotin degradation products according to literature data [18].

One of the degradation products of indigotin is tryptanthrin, an indoloquinazoline alkaloid. While it has been identified in model solutions during the study of indigo transformation processes [18], we have found no literature documenting its presence in archeological textiles.

In this study, the analysis of 17 archeological textile samples enabled the preliminary identification of three substances as putative indigotin degradation products: isatin, tryptanthrin, and *N,N'*-oxalyldianthranilic acid (see spectra in Figures 4 and 6). The MS2 spectrum of tryptanthrin exhibits fragment ions at m/z 221.0710 and m/z 130.0288, consistent with the potential loss of CO^- - and $\text{C}_6\text{H}_5\text{N}^-$ - fragments from the positively charged molecular ion, as noted in the previous literature [30]. Notably, our study marks the first identification of tryptanthrin as a component within the qualitative composition of archeological samples.

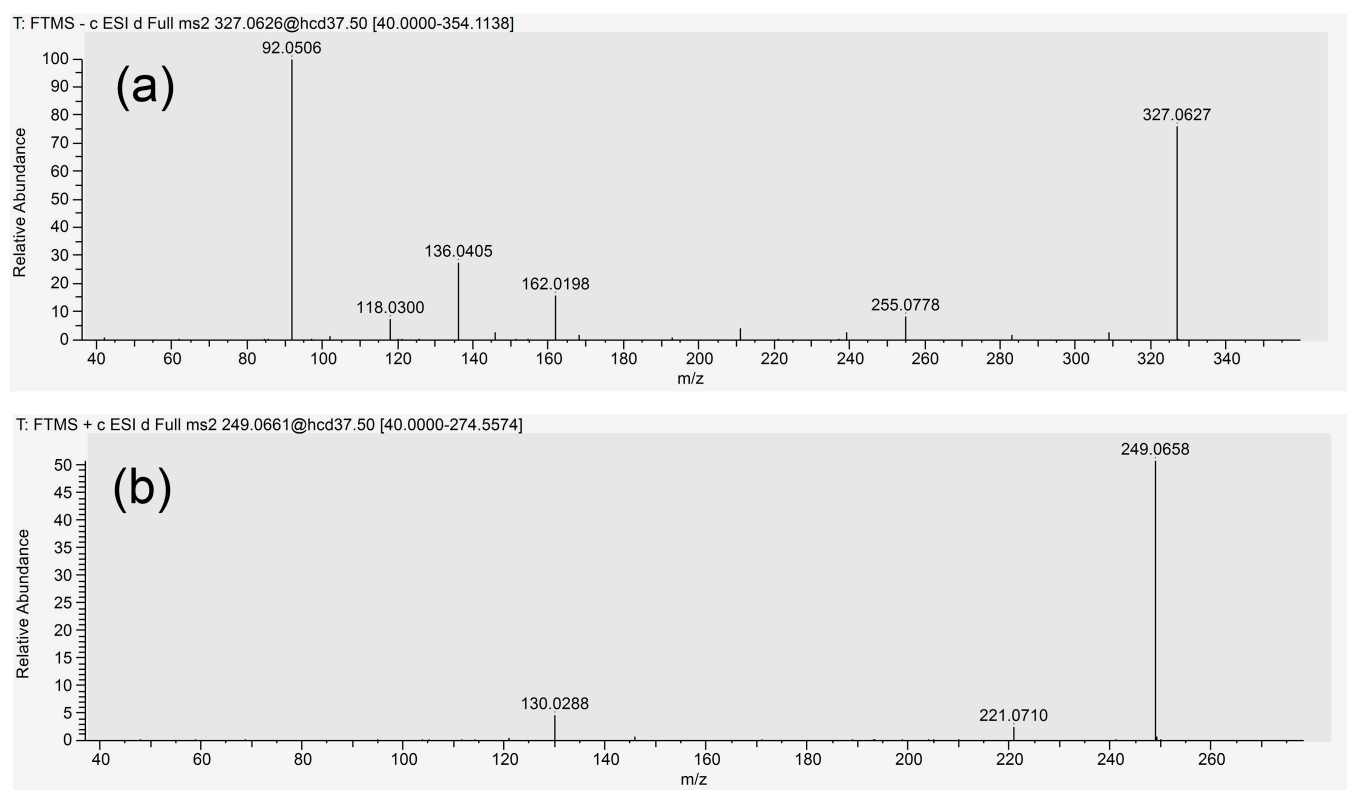


Figure 6. Mass spectra of substances preliminarily identified as degradation products of indigotin—*N,N'*-oxalyldianthranilic acid (a) and tryptanthrin (b).

Since second-generation Orbitrap mass spectra for the indigotin degradation product *N,N'*-oxalyldianthranilic acid is absent from public databases such as MoNA (MassBank of North America), we interpreted our fragmentation spectra using Sirius software. The proposed fragmentation pathways for *N,N'*-oxalyldianthranilic acid involve the sequential or parallel elimination of CO^- , CO_2^- , $\text{C}_6\text{H}_5\text{N}^-$, $\text{C}_7\text{H}_5\text{N}^-$ and H_2O^- groups from the molecular ion at m/z 327.0627 [9], findings which are supported by our experimental data. The product ion at m/z 255.0778 corresponds to the loss of CO^- and CO_2^- groups from the molecular ion simultaneously. The product ions m/z 162.0198 and m/z 136.0405 indicate the formation of deprotonated ions of isatoic anhydride and anthranilic acid, which the following transformation produce m/z 118.0300 by the loss of CO_2^- and H_2O^- groups respectively. The transformation of deprotonated ion of anthranilic acid by losing CO_2^- group provides m/z 92.0506.

3.3. The Influence of Extraction Conditions of Analytes from Textile Fibers on Their Degradation

Analyte degradation can occur not only during the archeological sample's environmental exposure but also during the analysis itself, particularly at the extraction stage. This complicates the extraction and subsequent determination of indigotin, especially when component ratios are used to identify the dye's origin [8].

Natural textile dyes are typically classified into vat, mordant, and direct categories based on the technology utilized for dye application and fixation [15]. Many natural dyes function as mordant dyes, which are fixed to fibers through the application of transition metal salts. Consequently, preparing textile samples for analysis often involves acid treatment to break down the resulting complexes formed between mordant dyes and transition metal ions [3]. However, this common use of acid for extraction can negatively affect vat dyes, particularly indigo [36], which are fixed to textile fibers via weak hydrogen or van der Waals bonds [37]. Indigoid dyes, therefore, are typically extracted directly using solvents such as dimethyl sulfoxide, (DMSO) [38], dimethylformamide (DMF) or pyridine [3].

Consequently, a three-stage extraction scheme is typically employed to recover unknown dye components from textile fibers. In the initial stage, fabric fibers are treated with a mixture of hydrochloric acid and a water–methanol solution to decompose potential metal-dye complexes [3,12]. This is followed by sequential extraction: first using a methanol solution to isolate more polar components, and then dimethyl sulfoxide or dimethylformamide to extract weakly polar components [5,33].

An extraction protocol involving boiling in a water bath with methanolic hydrochloric acid is commonly used to identify dyes in archeological textiles [12,33]. Notably, this specific method was employed in a study analyzing textiles from medieval Sudan using HPLC-MS [12]. However, several studies have noted the risk of dye degradation under such harsh extraction conditions [9,30]. To address this issue, a number of milder procedures have been proposed, differing in both the extraction agents and the extraction conditions used [2,39,40].

In our study, we aimed to compare the acid extraction method (using a water bath) for indigotin and its derivatives with a milder approach involving ultrasonic bath treatment at a lower temperature. This comparison provided data on the relative changes in the concentrations of indigotin, isatin, and tryptanthrin within the resulting extracts (see Figure 7). In this study, we focused primarily on indigo-based blue dyes; the sample selected for comparing extraction methods contained a sufficiently high concentration of indigotin specifically. No dyes of other colors were present in the sample, which means the results obtained cannot yet be generalized to a broader range of dyes.

The presented diagrams indicate that exposure to heat leads to a decrease in indigotin concentration, accompanied by a corresponding increase in the levels of its degradation products, isatin and tryptanthrin [18,36], see reaction scheme in Figure 7. The differences for indigotin, isatin and tryptanthrin are significant according to two-sided t-test (see Supplementary Materials Table S2).

Consequently, employing ultrasonication instead of boiling is the preferred method for extracting indigoid components from fabric fibers with solvents of varying polarity. This approach avoids heating during the extraction process, thereby minimizing the degradation of sensitive compounds.

Using DMF or DMSO to extract low-polarity textile dye components presents significant challenges, including solvent toxicity and ion suppression effects within the mass spectrometer [41]. Consequently, an experiment was conducted to evaluate the replacement of DMF with acetonitrile during the third stage of the extraction process. We evaluated two parameters: the change in the extraction of indigotin and the change in the extraction of two of its degradation products— isatin and tryptanthrin.

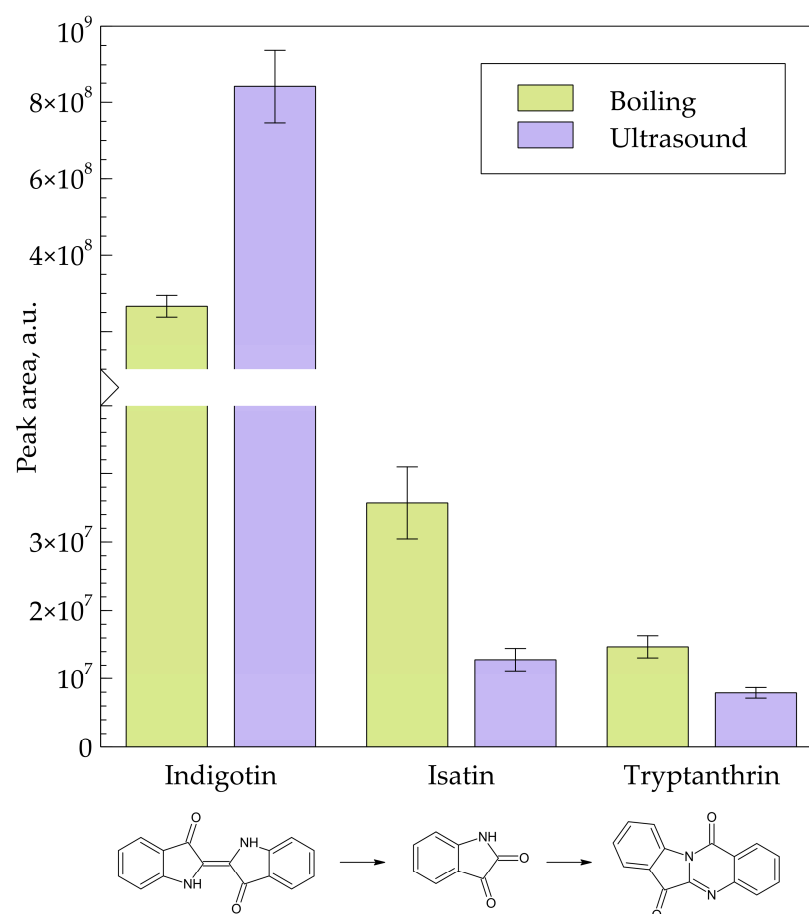


Figure 7. Extraction results of indigotin and its degradation products from textile fibers under various conditions ($S_r = 11\text{--}14\%$; $n = 3$). The masses of textile samples were identical ($m = 0.0020 \pm 0.0001$ g) in each experiment.

Indigotin extraction using DMF was slightly higher than with ACN (Figure 8), but we found no statistically significant difference between the two values based on the t-test (Table S3). Consequently, it can be concluded that the use of ACN does not result in a substantial reduction in the extraction of the primary analyte compared to DMF.

At the same time, the statistically significant increase in isatin and tryptanthrin signals observed with DMF could result either from their more complete extraction or from the partial degradation of indigotin during the experiment.

Based on the optimization results, the following procedure was adopted to extract dye components from textile fibers. Initially, the textile sample was treated with a hydrochloric acid and water–methanol solution using ultrasonication. Subsequently, two extraction stages were performed—first with methanol and then with acetonitrile, both under ultrasonic conditions—to isolate the polar and weakly polar fractions, respectively. The resulting extracts were then analyzed via HPLC-HRMS in both positive and negative ion detection modes (Table S4, Supplementary Materials).

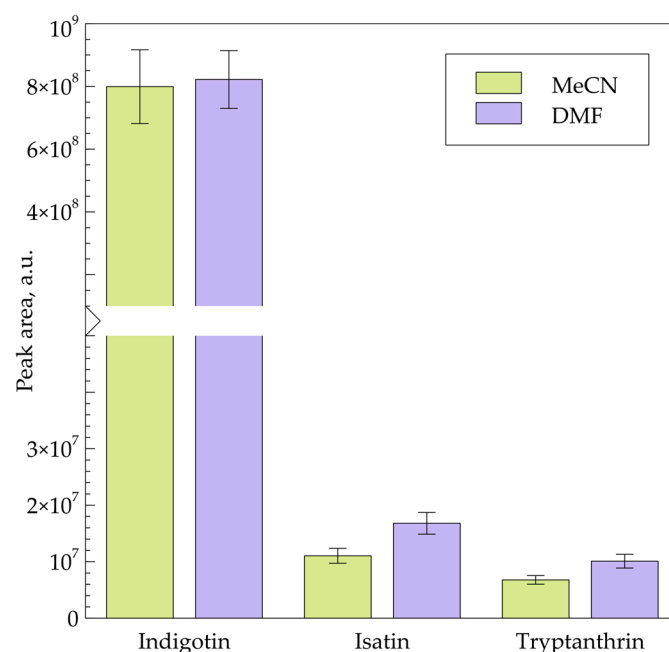


Figure 8. Extraction results of indigotin and its degradation products from textile fibers using dimethylformamide (DMF) or acetonitrile (MeCN) ($S_r = 11\text{--}15\%$; $n = 3$). The masses of textile samples were identical ($m = 0.0020 \pm 0.0001$ g) in each experiment.

4. Conclusions

Seventeen samples of early medieval textile from the ancient city of Deraheib (Sudan) were studied. The chemical and technological data together point to the use of indigo within several distinct manufacturing and dyeing practices rather than a single standardized process. First- and second-generation high-resolution mass spectra of isatin and indigotin standards were obtained for the first time using an orbital ion trap mass spectrometer. Tryptanthrin, a degradation product of indigotin, was identified in archeological textile samples for the first time. Regarding the extraction of indigoid dyes, it was demonstrated that ultrasonication without heating is preferable to the conventional boiling procedure with methanolic hydrochloric acid.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/analytica7040075/s1>, Table S1. Information on textile samples. Table S2. Extraction results of indigotin and its degradation products from textile fibers under various conditions. Table S3. Extraction results of indigotin and its degradation products from textile fibers using dimethylformamide or acetonitrile. Table S4. List of analytes. Figure S1. Mass spectra of indican (a), isatin (b), indigotin (c) standards. Experimental mass spectra in MSP format.

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Abbreviations

The following abbreviations are used in this manuscript:

DDA	data-dependent acquisition
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
LC	liquid chromatography
LRMS	low-resolution mass spectrometry
MeCN	acetonitrile
MS	mass spectrometry

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