Journal of Chromatography A 1758 (2025) 466200

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

A method for the simultaneous extraction, concentration, and quantitation of miliacin and 5-*n*-alkylresorcinols from soils and sediments

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ARTICLE INFO

Keywords:
Cereal biomarkers
Miliacin
Alkylresorcinols
GC-MS/MS analysis
Terrestrial archives

ABSTRACT

The analysis of cereal biomarkers provides snippets of human-environmental interactions targeting questions of crop domestication and dietary practices of past societies. Commonly these markers are analysed from ceramics and lacustrine sediments to ensure an optimal preservation. In this paper, we propose a method for the simultaneous extraction, concentration, and quantitation of miliacin and 5-*n*-alkylresorcinols from terrestrial soils and sediments. After Soxhlet extraction, total lipid extracts from a modern and a prehistoric arable soil were purified by solid phase extraction, yielding successively a less polar and a more polar fraction containing miliacin and 5-*n*-alkylresorcinols, respectively. Gas chromatography – tandem mass spectrometry (GC-MS/MS) analysis using multiple reaction monitoring reliably detected miliacin and homologues of 5-*n*-alkylresorcinols from the investigated soils. The method in its current state appears to work better for miliacin than for ARs as evidenced by lower relative standard deviations ($\leq$ 0.07), higher workup efficiencies (95 $\pm$ 15 %, n = 12) and recovery rates (between 74 and 82 %) independent from physiochemical soil characteristics. Future research requires optimisation of AR extraction efficiency from complex soil matrices, especially focussing on the interaction with reactive organic and mineral soil constituents. Nonetheless, this article demonstrates the applicability of the proposed method to simultaneously analyse miliacin and 5-*n*-alkylresorcinols from terrestrial soils and sediments, thus extending the analysis of cereal biomarkers to sample material that it has not or only rarely been applied to so far.

1. Introduction

The analysis of molecular biomarkers has developed as a powerful tool in archaeological contexts to gain insights into past land use and the paleoenvironment [1–3]. Marker molecules are optimally preserved in protected environments (e.g., lacustrine sediments, well-preserved pottery, cave sediments), where weathering processes, microbial degradation, and transformation are reduced [4–6]. But many archaeological settings are not under optimal preservation conditions, posing challenges in data acquisition. Therefore, establishing the analysis of molecular biomarkers from terrestrial soils and sediments is crucial in complementing archaeological datasets as these archives are widespread and can directly be linked to archaeological structures [7,8]. However, analytical challenges like low analyte concentration and interactions with the soil matrix have to be considered when molecular biomarkers are extracted from terrestrial soils and sediments.

In recent decades, molecular biomarkers have been used to decipher past vegetation compositions (e.g., *n*-alkanes, plant-sterols [9,10]), biomass burning (e.g., benzocarboxylic acids, polycyclic aromatic hydrocarbons [8,11]), livestock species, and manuring (e.g., 5*β*-stanols, bile acids [12,13]). The analysis of cereal biomarker from soils and sediments is so far limited to miliacin [14,15] – a molecular proxy for the cultivation of *Panicum miliaceum* (broomcorn millet) – while other cereal biomarker (e.g., 5-*n*-alkylresorcinols, AR) are more common in the field of organic residue analysis of archaeological pottery sherds [5,16,17]. Miliacin (olean-18-en-3*β*-ol methyl ether) belongs to the pentacyclic triterpene methyl ethers, is predominately biosynthesised in seeds of broomcorn millet, and absent from or underrepresented in its hulls, roots, and stems [18]. The production of miliacin has also been shown for a variety of Poaceae worldwide, albeit at much lower concentrations [18–20]. In studies of crop cultivation in prehistory, the analysis of miliacin from ceramics and sediments together with isotopic signatures of bone collagen has recently become popular in the attempt to trace the spread of millet from its domestication origins in north-eastern China 8000 years ago [21–23]. Alkylresorcinols are phenolic lipids consisting of a 1,3-dihydroxybenzene (resorcinol) motif and an alkyl side chain

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https://doi.org/10.1016/j.chroma.2025.466200
Received 16 April 2025; Received in revised form 27 June 2025; Accepted 7 July 2025
Available online 8 July 2025

[24]. They are abundant in the intermediate layers (i.e., the inner pericarp) of cereal kernels and serve as potential biomarkers of whole grain intake [25–27]. Various studies reported that the AR homologue composition allows for a discrimination of different *Triticum* species (*aestivum, durum, monococcum, dicoccum*), and in particular the AR-17:0 to AR-21:0 ratio (with numbers before and after the colon indicating the length of the side chain and its degree of unsaturation, respectively) [28, 29]. This implicates an additional relevance of ARs used in archaeological research.

The single and multi-compound analysis of lipid biomarker involves several extraction and clean-up steps before gas-chromatographic and mass-spectrometric analysis [30]. To acquire total lipid extracts (TLE), target compounds are extracted using medium polar solvent systems (e.g., dichloromethane (DCM)/methanol (2:1 *v/v*). Various extraction methods are applied to achieve TLE, of which Soxhlet, ultrasonic, microwave-assisted, and accelerated-solvent extraction are frequently used [31–34]. Soxhlet extraction was used in this method, as recent studies indicate that lipid yields are higher and more reproducible with Soxhlet compared to other extraction systems [31,34,35]. In soils and sediments, where low analyte concentrations and interactions with complex matrices are expected, the long-term 'digestion' of the sample material during Soxhlet extraction may counteract these challenges [31]. Subsequently, the target compounds are concentrated by polarity-based fractionation using chromatography. Solid phase extraction (SPE) is commonly performed – a clean-up step using columns packed with silica gel and solvents of varying polarity – to separate different target compounds [16,32]. To ensure amenability to gas chromatography, polar fractions are derivatized using varying silylating agents. Target compounds are analysed *via* gas chromatography – mass spectrometry (GC–MS) using modes of both non-targeted (e.g., full scan) and targeted identification (e.g., single ion monitoring, SIM or multiple reaction monitoring, MRM). Tandem MS is used in this method to improve the limit of detection and achieve a greater selectivity of target compounds.

In this paper, we present a method for the simultaneous extraction, concentration, and quantitation of miliacin and ARs from soils and sediments to decipher the cultivation of millet and other cereals in geoarchaeological contexts. The method provides three promising aspects: (1) The analysis of cereal biomarkers from terrestrial soils and sediments offers a broader applicability in the field of geoarchaeology, as these archives are ubiquitously distributed within and around archaeological structures. (2) A simultaneous analysis of miliacin and ARs saves laboratory capacity (extraction time, solvent) and counteracts extraction variability. (3) By using tandem MS (i.e., MRM), identification of low concentrated analytes and improved selectivity of target compounds can be achieved.

2. Material and methods

2.1. Sample origin and soil characteristics

Two soil samples from a modern and a prehistoric arable field were analysed in six replicates. The modern arable field is located north of Torino (NW-Italy) in the southern Alpine foreland and has been cultivated with millet in 2024 and with various types of cereals (mainly wheat and maize) in recent years. There, soils mainly developed from alluvial sediments. The prehistoric arable field was documented near Lake Constance in the northern Alpine foreland (SW-Germany) and is covered by > 1.5 m of sediment. The corresponding ploughing horizon can be dated to the late Middle Bronze Age (MBA) period (15^{th} – 16^{th} century BCE) using radiocarbon and optically stimulated luminescence dating [8]. The prehistoric soil developed from Würmian glaciofluvial deposits. The physiochemical characteristics for both soils are given in Table 1.

2.2. Chemicals

DCM, methanol (both ≥ 99.9 % GC ultra grade), *n*-hexane (≥ 99 % UV/IR grade), toluene (≥ 99.8 % GC ultra grade), and ethyl acetate (≥ 99.5 % HPLC grade) were acquired from Carl-Roth (Karlsruhe, Germany). Pyridine (99 %), the silylating agent consisting of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (99:1, *v/v*), and *n*-octacosane (C_{28} alkane) were from Merck (Darmstadt, Germany). Silica gel (63 – 200 μm, mesh 70 – 230, pore size 100 Å) for SPE was from Macherey-Nagel (Düren, Germany). Miliacin was bought from PhytoLab (Vestenbergsgreuth, Germany). Methyl octadecanoate-d_{35} (FAME d_{35}-18:0; 98 atom% D) was purchased from CDN Isotopes (Pointe—Claire, Canada). AR-18:0 was synthesized in-house.

2.3. Sample preparation, total lipid extraction and purification

All laboratory consumables (glass ware, quartz sand, fibreglass thimbles, silverware, etc.) were prewashed with organic solvents (ethyl acetate, acetone, toluene) and heated at 300 °C for 8 h prior to use. Soil samples were dried at 40 °C for three days, sieved ≤ 2 mm, and finely ground in a ball mill.

For Soxhlet extraction, 5 g of soil were weighed into 23 × 100 mm fibreglass thimbles (Macherey-Nagel, Düren, Germany) and covered by a thin package of quartz sand. Extraction was performed using 210 mL of DCM:methanol (2:1, *v/v*) in 100 mL extractors for > 24 h. FAME d_{35}–18:0 and AR-18:0 (200 μL of ~10 ng μL^{-1}) were added as internal standards ($IS1^a$ and $IS1^b$) to the extraction solution in order to simulate less polar (especially miliacin which is a methyl ether) and more polar compounds (especially ARs which are diols), respectively.

Total lipid extracts were concentrated under reduced pressure (Rotavapor R-100, Büchi, Germany), completely dried under a gentle stream of nitrogen and redissolved in 1 mL of *n*-hexane. Glass SPE-columns (6 mL) were packed with 5 % deactivated silica gel (2.2 cm filling height) and a thin layer of quartz sand. Non-polar contaminants were eluted with 2 × 2 mL of *n*-hexane and discarded. The non-polar fraction containing miliacin and IS^a was eluted with 4 × 2 mL of DCM:*n*-hexane (1:1, *v/v*). Then, the polar fraction containing ARs and $IS1^b$ was eluted with 4 × 2 mL of ethyl acetate:methanol (9:1, *v/v*). To all fractions, *n*-octacosane (200 μL of ~10 ng μL^{-1}) was added as internal standard (IS2) before the solutions were concentrated to dryness under a gentle stream of nitrogen. Both fractions were reconstituted in 200 μL of *n*-hexane and transferred to GC vials for analysis. To ensure amenability to gas chromatography, the polar fractions were additionally silylated using a mixture of BSTFA/TMCS (100 μL) and pyridine (50 μL) (2:1, *v/v*) at 80 °C for 1 h. After silylation, extracts were dried under a stream of nitrogen and reconstituted in 200 μL of *n*-hexane.

Table 1
Physiochemical characteristics of soil samples from a modern and a prehistoric arable soil. WRB: World Reference Base of Soil Resources (2022), C/N: Carbon to nitrogen ratio, TN: Total nitrogen, TOC: Total organic carbon, CEC_{eff}: Effective cation exchange capacity, Ap, 2Apb: Soil horizons according to [36].

Category	Depth [cm]	Horizon (WRB 2022)	C/N	TN [%]	TOC [%]	Sand [%]	Silt [%]	Clay [%]	pH ($CaCl_2$)	pH (H_20)	CEC_{eff} $cmol_{c+}$ kg^{-1}
Modern	0–5	Ap	8.5	0.23	2.0	25	69	6	7.1	7.3	13
Prehistoric	177–182	2Apb	6.9	0.08	0.55	29	40	31	7.4	7.9	26

2.5. GC–MS/MS analysis

Fractions were analysed by GC-MS/MS using a 7890B-Triple Quad 7000D equipped with a 30 *m* × 0.25 mm i.d. × 0.25 μm film thickness DB-5 Ultra Inert column (Agilent Technologies, Santa Clara, CA, USA). Samples (1 μL) were injected into a split/splitless inlet operated continuously at 300 °C in pulsed splitless mode (30 psi for 1 min) and fitted with a 5183–4711 liner (single taper, split, deactivated glass wool, Agilent Technologies). This was followed by depressurization back to operating column flow rate (helium as carrier gas at a flow rate of 1.5 mL/min) for 0.25 min and a split vent purge at a flow rate of 100 mL/min for 0.25 min. The oven was kept at 80 °C during this time (1.5 min in total), then increased to 300 °C at a rate of 10 °C/min. After an isothermal plateau of 8 min, the temperature was ramped up to 325 °C at 25 °C/min and maintained for 5 min. Analytes were ionized *via* electron ionization at 70 eV. Transfer line, ion source, and quadrupole temperatures were set to 250 °C, 230 °C, and 150 °C, respectively. Targeted mass spectrometry data was collected in multiple reaction monitoring (MRM) mode, and additional analysis in scan mode (m/z 50 - 650 at a scan time of 250 ms) was performed to track other soil markers and laboratory contamination. For MRM, nitrogen was used as collision gas and helium as quench gas at flow rates of 1.5 mL/min and 2.25 mL/min, respectively. Ion transitions were selected for the highest possible specificity at the lowest possible interference as confirmed by previously performed measurements in product ion mode (Tab. S1, Fig. S2).

For quantitation, a serial standard series in pure solvent (0.00125 – 10.22 ng μL^{-1} for miliacin and 0.00065 – 10.62 ng μL^{-1} for AR 18:0; 200 ng μL^{-1} of IS2 in each dilution step) was used for external calibration, where AR-18:0 was representative of all ARs. The quantifier transition peak areas of miliacin and AR-18:0 were corrected against IS2. The resulting values were used to create regression curves, and sample analytes were quantitated accordingly (Tab. S2). Noise levels for the calculation of signal-to-noise ratios (SNR) were achieved by the peak-to-peak algorithm of the Qualitative Navigator B.08.00 software (Mass Hunter, Agilent Technologies).

3. Results and discussion

3.1. Identification of target compounds and quality assurance

The proposed method enabled a simultaneous extraction, concentration, and discrimination of miliacin (Fig. 1A, B) and ARs (Fig. 1C, D). The identification of target compounds was achieved by using retention times and molecular-specific ion transitions as well as by comparing chromatograms and mass spectra with those of pre-analysed cereal grain extracts. The most distinct ion transition peaks were used for quantitation (*), while each target compound was additionally verified by two qualifier ion transition peaks (**) (Tab. S1). Miliacin concentration > 0.0200 ng μL^{-1} and AR concentration > 0.0026 ng μL^{-1} could be reliably quantified based on signal-to-noise ratios (limit of quantitation (LoQ), SNR > 10 for quantifier ion transition peaks and SNR > 3 for qualifier ion transition peaks, Tab. S3). Limits of detection (LoD) were reached at 0.0100 ng μL^{-1} and 0.0012 ng μL^{-1} for miliacin and AR, respectively (SNR < 3 for at least one ion transition). Consequently, miliacin concentrations could be unexceptionally quantitated, while 8 % of identified ARs did not match the LoQ and were excluded from further calculations. In most studies on lipid biomarker analysis, information on detection and quantitation limits are omitted. Birk et al. [32]. analysed fecal lipid biomarker (Δ^5-sterols, stanones, stanols and bile acids) from soils and sediments and showed LoQs between 1.3 and 10.0 ng g^{-1}_{soil}. The tandem MS approach used in this paper enabled a reliable quantitation of very low analyte concentrations (< 1 ng $g^{-1}_{sediment}$).

Workup efficiencies were calculated from the internal standards ($IS1^a$, $IS1^b$) to estimate loss of target compounds during extraction and purification steps. FAME d_{35}-18:0 was well recovered in all samples (95 ± 15 %, n = 12) compared to AR-18:0 (56 ± 32 %, n = 9) (Tab. S4). Similar observations were obtained from a spiking experiment to test the extraction efficiency of miliacin and ARs from the analysed soils (details on the experimental setup are given in the supplementary information). The recovery rate of miliacin and its observed isomers (δ- and β-amyrin methyl ethers) was between 74 and 82 %, while between 5.4 and 41 % of AR-18:0 were recovered. Extraction efficiency was comparatively equal for miliacin and its isomers among the analysed soils, but distinctly different for AR-18:0. The soil of the prehistoric field, which is fine-grained (> 30 % of clay), retained ~ 95 %, while the soil of the modern arable field (6 % clay, 69 % silt) retained ~ 60 % of AR-18:0. These differences indicate strong interactions between the soil matrix (reactive mineral surfaces) and the target compounds. Especially clay minerals have a high specific surface area and therefore high exchange capacities at the intermediate layers and boundaries of their tetrahedric-octahedric crystalline structures. Consequently, polar lipids, such as ARs with their phenolic hydroxyl groups, may bond more effectively to clay minerals compared to coarser grained soil material [37,38]. Acid pretreatment of soil samples is reported to lower the effect of mineral protection of lipids and improve extractability of target compounds [39]. This gives one way to possibly increase extraction efficiency of ARs from clay-rich soil matrices in future studies. A wide range of recovery rates (0 to > 100 %) of different lipid groups extracted from partially fine-grained lake sediments were reported by Dieferndorf [35], whereby these differences are also attributed to different extraction systems and solvents used. In general, studies on the extraction efficiency and recovery of lipid biomarker from soils and sediments are rare but necessary to understand occurring effects of the soil matrix on target compounds [40].

Miliacin was detected in all samples, showing comparable concentrations (mean ± standard deviation in ng $g^{-1}_{sediment}$) for the modern (48.2 ± 3.4) and prehistoric arable soil (48.5 ± 3.1). ARs were also identified among all analysed soil samples, but at distinctly lower concentration (∑ARs) than miliacin and with differences between the soils of the modern (16.8 ± 6.4) and prehistoric arable field (1.3 ± 0.4). (Fig. 2). Relative standard deviations (RSD) were ≤ 0.07 and ≤ 0.45 for miliacin and ∑ARs, respectively. Homologues (17:0 –27:0) of ARs were identified in all replicates of the modern soil with a predominance of AR-21:0 and AR-23:0. For the prehistoric arable soil, AR-25:0 and AR-27:0 were below LoD and the homologue composition was more balanced and shifted towards shorter-chain ARs (Table 2). Analysis of reference subsoils that were largely unaffected by the input of cereal grains showed background levels of ARs up to 0.80 ng $g^{-1}_{sediment}$.

The significantly lower concentrations of ARs compared to miliacin in the analysed soils may have several reasons. Firstly, the natural level of ARs in cereal grains is generally low, resulting in lower concentrations of ARs that can be transferred to soils and sediments. ARs are found only in the testa and inner pericarp of cereal kernels [26,29], whereas miliacin is enriched in embryonic cell compartments, including the albumen [18]. Secondly, ARs are more prone to microbial degradation in oxic environments as shown from an incubation experiment with dosed sherd material [16]. And thirdly, ARs likely adsorb stronger to mineral surfaces in soils, which reduces extraction efficiency. Despite these challenges, the proposed method enabled a reliable identification and quantitation of ARs from soils of modern and prehistoric contexts. However, AR concentrations < 1 ng $g^{-1}_{sediment}$ should be used with

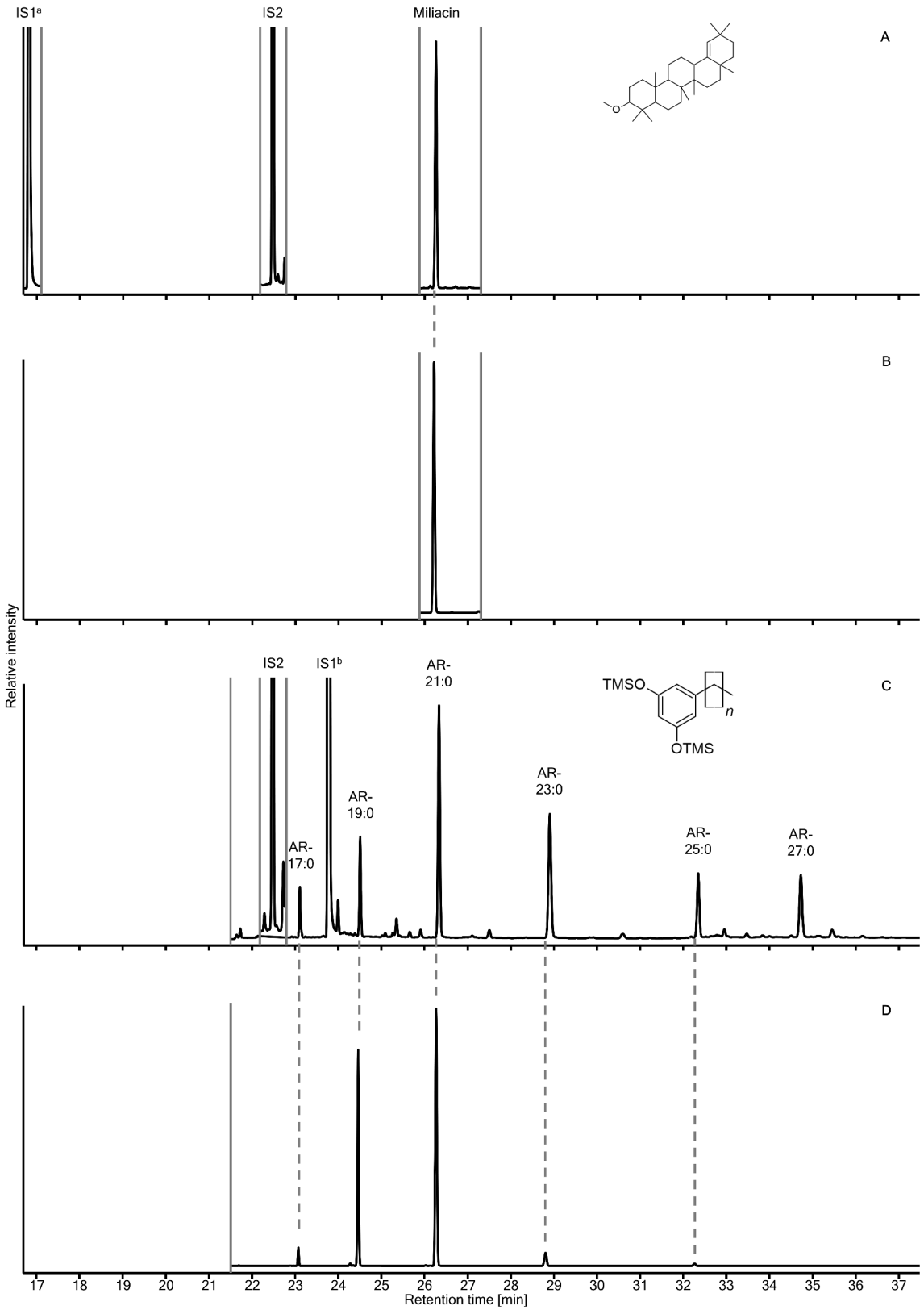

Fig. 1. Extracted transition chromatograms of soil and grain samples recorded in multiple reaction monitoring mode; A: Zoomed-in overlayed transitions of miliacin (*m/z* 204.2 → 189.2), IS1[a] (*m/z* 91.1 → 58.1), and IS2 (*m/z* 99.1 → 57.1) of soil extracts from the prehistoric arable field; B: Transition of miliacin (*m/z* 204.2 → 189.2) in an extract of broomcorn millet; C: Zoomed-in overlayed transitions of ARs (*m/z* 268.2 → 73.1) and IS2 (*m/z* 99.1 → 57.1) of soil extracts from the modern arable field; D: Transition of ARs (*m/z* 268.2 → 73.1) in an extract of spelt; solid grey lines denote the limits of recording windows; AR = 5-*n*-alkylresorcinol; IS1[a] = d_{35}-octadecanoic acid methyl ester, IS1[b] = AR-18:0; IS2 = *n*-octacosane; *n* = 16, 18, 20, 22, 24, 26; AR chain length = *n* + 1.

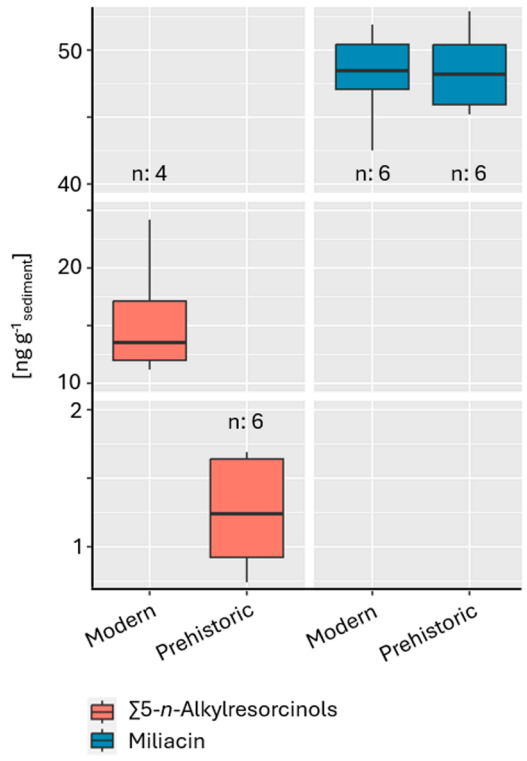

Fig. 2. Mean concentrations [ng $g^{-1}_{sediment}$] of ∑5-*n*-alkylresorcinols and miliacin in soil samples from a modern and a prehistoric arable field, n: number of replicates, black line in boxplots: median, scale breaks are between 2, 10 and 25, 40 ng $g^{-1}_{sediment}$.

caution, as they may be influenced by natural background levels of ARs in soils.

In summary, the method in its current state performs better for miliacin than for ARs, as evidenced by lower RSDs, higher workup efficiencies, and recovery rates independent from physiochemical soil characteristics. The higher polarity of ARs that likely results in stronger interactions with reactive organic and mineral soil surfaces and their overall lower abundance in soils and sediments make them more complex lipid biomarker candidates that requires further optimisation efforts.

3.2. Archaeological significance of cereal marker analysis from soils and sediments

The levels of miliacin in the analysed soils likely indicate the cultivation of *Panicum miliaceum*. While the modern arable soil was cultivated with millet in 2024, the sufficient preservation of miliacin in the prehistoric soil can be associated with a rapid sedimentation of the former surface by erosion [8]. A high resistance of miliacin against biodegradation due to anti-microbial properties [14] may explain comparable results between the modern and prehistoric arable soil. A study of Ukrainian paleosols in the Donets floodplain revealed miliacin concentrations between 3 and 15 ng $g^{-1}_{sediment}$ [15]. Together with charred millet grains from the infilling of a stratigraphically related soil pit, an *in-situ* cultivation of broomcorn millet during the Early Iron Age is assumed. Higher concentrations of miliacin (up to 350 ng $g^{-1}_{sediment}$) were found in fillings of cereal pits in NE France [14]. These pits are interpreted as storage facilities since the Bronze Age. Jacob et al. [41]. found elevated levels of miliacin (up to 450 ng $g^{-1}_{sediment}$) in lacustrine sediments in the French Alps, proving millet cultivation around Lake Le Bourget in the Bronze Age, Roman and Medieval periods.

The analysis of ARs from soils and sediments is rarely performed [5,42], but commonly applied in the field of organic residue analysis of archaeological pottery sherds [5,16,43,44]. Under optimal (anoxic) preservation conditions, AR concentrations < 50 ng $g^{-1}_{pottery}$ were determined from ceramic fragments of Neolithic Scottish crannog sites [5]. Numerous studies reported that the AR homologue composition enables a differentiation of *Triticum* species by using the AR-17:0 to AR-21:0 ratio [25,28,29]. Together with AR-17:0 to AR-21:0 ratios between 0.1 and 0.2, an increased input of common wheat (*Triticum aestivum*) grains into the soil of the modern arable field is indicated [28,29,44]. The soil of the prehistoric arable field showed AR-17:0 to AR-21:0 ratios > 0.9 (Table 2), pointing to an increased input of rye or millet grains into the soils [45–47]. The cultivation of rye is known to be relevant only since the Medieval periods [48]. Earlier studies reported a predominance of shorter-chain ARs (especially AR-17:0, 19:0, 21:0) in grains of *Panicum miliaceum* and *Setaria italica* [46,47]. However, recent studies [25,49] and our own measurements (Fig. S3) have not confirmed the presence of ARs in extracts of broomcorn millet grains. It is likely that the relative AR homologue composition is shifted because AR-25:0 and AR-27:0 were < LoD as well as due to possible degradation and adsorption processes of ARs in these soils. The alteration of lipid biomarker signals in the presence of reactive mineral surfaces was also reported by Li et al. [39]. More reference sample material needs to be analysed in future studies, as suggested by Mallol et al. [6], to verify possible shifts of AR homologue composition against the local background signal of lipid biomarkers in the analysed soils.

4. Conclusion

In this paper, a method for the simultaneous extraction, concentration, and quantitation of miliacin and ARs from soils and sediments is presented. After acquisition of TLE by Soxhlet extraction, the purification includes a SPE in which two phases containing miliacin and ARs, respectively, are sequentially separated. The subsequent GC–MS/MS analysis using MRM reliably detected miliacin and homologues of ARs from modern and prehistoric arable soils. Therefore, the proposed method is applicable to studying past and present human-environment interactions targeting questions of crop domestication and dietary practices using terrestrial soils and sediments. In its current state, the method performs better for the analysis of miliacin than ARs, as evidenced by several quality parameters. Future optimisation efforts including the improvement of extraction efficiency by acid pretreatment of soil samples as well as the integration of more and local reference sample material, possibly increase the performance and accuracy of AR analysis from complex soil matrices. The simultaneous extraction, concentration, and quantitation of miliacin and ARs from terrestrial archives offers an extension of cereal marker analysis to sample materials that it has not or only rarely applied to so far.

Table 2
Mean distribution of homologues of 5-*n*-alkylresorcinols (AR) in soil samples from a modern and a prehistoric arable field, SD: standard deviation, LoD: limit of detection.

Soil samples	AR homologue content [%] (SD)						AR ratio
	17:0	19:0	21:0	23:0	25:0	27:0	17:0/21:0
Modern	5.3 (0.9)	9.2 (1.1)	30.0 (3.2)	24.1 (1.7)	11.2 (1.1)	12.9 (2.1)	0.18 (0.02)
Prehistoric	17.0 (5.2)	18.4 (4.0)	10.7 (1.5)	12.5 (2.0)	< LoD	< LoD	1.55 (0.38)

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Sascha Scherer: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **George Janzen:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Moritz Leistner:** Investigation, Formal analysis, Data curation. **Sabine Fiedler:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Dr. Klaus Kaiser (Martin-Luther Universität Halle-Wittenberg) for putting us in touch with Dr. Daniel Said Pullicino (University of Torino), who kindly collected the samples from the field where the millet was first grown.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2025.466200.

Data availability

Data will be made available on request.

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