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Selective extraction of levoglucosan and its isomers from complex matrices using ligand exchange-solid phase extraction for analysis by liquid chromatography-electrospray ionization-tandem mass spectrometry

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ABSTRACT

The analysis of trace quantities of monosaccharide anhydrides (MAs) in sediments is complicated by the lack of fast and reliable technologies to selectively extract these water-soluble non-ionic compounds from samples of complex composition. Here we describe a solid phase extraction method that takes advantage of the affinity between monosaccharide anhydrides (MAs) and immobilized Na^+ ions related to ligand-exchange processes (LE-SPE). The capacity factor of LE-SPE columns was enhanced by using non-aqueous mobile phases such as DCM/MeOH mixtures. We have used the unique properties of LE-SPE columns to selectively extract MAs from lacustrine, coastal, and deep-sea oceanic sediment samples. The analytical procedure produces extracts with low ion suppression effects (0–20%), resulting in ideal conditions for MAs quantification with LC-ESI-MS/MS systems irrespective of the sedimentary matrix and MAs concentration. The analytical method yields repeatable concentration values (RSD of 9–23% for levoglucosan and 15–34% for mannosan and galactosan) and an IS recovery of 45–70%. The instrumental dynamic range is 10–10000 pg injected, but in practice, the methodological lower limit of quantification is constrained by sample contamination during processing. The combination of LE-SPE and LC-ESI-MS/MS has the potential to produce sensitive and reliable technologies to analyze saccharides and amino acids in environmental and biological samples.

1. Introduction

Levoglucosan (LEV), galactosan (GAL), and mannosan (MAN) are extensively used as selective tracers of fire-derived organic matter to the environment (see Fig. S1 for the chemical structures of LEV, GAL, and MAN) [1–5]. These monosaccharide anhydrides (MAs) are produced by pyrolysis of cellulose during natural and anthropogenic fires and have been detected in polar regions (Greenland [6] and Antarctica [7]), remote continental areas [8–10], and distal oceanic areas [11].

The analysis of MAs in environmental samples has been subject to intense research over the last two decades and many analytical approaches have been published using liquid (LC) or gas chromatography (GC) to separate and detect them at trace levels in aerosol and ice samples (see Table S1 for an overview). Published analytical methods typically involve an extraction step followed by the direct analysis of crude extracts [12]. However, these methods produce unreliable results when applied to samples with complex matrices, such as soils and sediments. The routine analysis of unpurified methanolic sediment extracts results in matrix effects for LC-MS systems [13–15] and irreproducible derivatization of MAs and other low volatile compounds for GC-MS systems [16–19]. However, techniques commonly used to purify and concentrate compounds of interest, such as solid phase extraction and liquid-liquid extraction, fail to selectively extract hydrophilic non-ionic compounds like MAs and other saccharides. Accordingly, new efficient and handy technologies are needed to selectively concentrate trace amounts of saccharides from complex matrices.

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Monosaccharides have long been known to form co-ordination complexes with metal ions in water [20,21] and in MeOH [22,23]. Since 1961, ligand-exchange chromatography (LEC) on cation-exchange resins in the metal form has been extensively used to separate chiral isomers of saccharides [16,21,24]. In LEC, isomers are separated by the differential interaction of 2–3 hydroxyl groups (with adequate spatial geometry) with immobilized metal ions [20,21]. Since LEC is based on the ligand exchange of saccharide and water molecules, compound separation is usually undertaken in pure aqueous media [25–28]. However, the low energy involved in ligand-exchange processes has limited their practical use for sample preparation purposes [27].

Here we describe a method to selectively separate MAs from sediment extracts, based on the interaction between MAs and Na^+ ions immobilized in a strong cation-exchange resin (Dowex 50WX8). We show that the strong affinity between MAs and immobilized ions also occurs in other solvents, such as MeOH and DCM/MeOH, in the absence of water. Since monosaccharides and MeOH also form co-ordination complexes with cations [22,23], we assume that the retention mechanism is also related to ligand-exchange processes. We have optimized the conditions to concentrate quantitatively MAs using a ligand exchange-solid phase extraction column (LE-SPE). This novel technique has enabled us to validate a sensitive and reproducible analytical protocol to quantify MAs in lacustrine and oceanic sediments.

2. Materials and methods

2.1. Standards, solvents, and reagents

Pure standards of mannosan and galactosan were supplied by Cayman Chemical Co. Levoglucosan standard was obtained from Merck, Spain. $^{13}C_6$-Levoglucosan, 98%, was provided by Cambridge Isotope Laboratories Inc.

Methanol (MeOH), dichloromethane (DCM), *n*-hexane, and acetone were of GC grade (SupraSolv®, Supelco). Acetonitrile (ACN) was of LC-MS analysis hypergrade (HiPerSolv Chromanorm® Ultra, Supelco). MilliQ water was produced in house with a Millipore Co. system composed of an Elix prepurification unit and a MilliQ unit.

Silica gel 60 (230–400 mesh), NH_4OH solution (25%), and NaCl (Suprapur grade) were provided by Merck Spain. The strong cationic resin Amberchrom® 50WX8 (hydrogen form, 200–400 mesh, formerly Dowex® 50WX8) and the Mixed Ion Exchange Resin (AmberLite® MB20) were obtained from Merck Spain. The chelating resin Chelex® 100 (200–400 mesh, sodium form) was from Bio-Rad Laboratories Spain.

2.2. Sediment samples

Sample EN651 Mix is a combination of deep-sea sediments from the tropical Atlantic (5–10°N, 21–36°W, 3000–4000 mbsl, meters below sea level), with high $CaCO_3$ (70%) and low TOC (0.6%) content. Sediment MERS ST7 was retrieved in the continental slope off the Ebro River (Spain, 40.07°N, 1.53°E, 1476 mbsl) and has a low TOC (0.8%) and high $CaCO_3$ (45%) content. Sample LUC (42.59°N, 7.11°W) is a TOC-rich lake sediment (TOC = 28%) with low $CaCO_3$ content (<10%).

2.3. Extraction method

Sediments were freeze-dried and then homogenized with a Mixer Mill MM 400 (Retsch). Selective pressurized solvent extraction was performed using an ASE-350 (Dionex Thermo) equipped with 10 mL extraction cells and 60 mL collection vials. Extraction cells were filled from bottom to top with a glass-fiber filter, 3 g of activated silica, 0.2–3.0 g of sediment, and diatomaceous earth to completely fill the cell. On the top of the cell, a known amount of the internal standard (IS) $^{13}C_6$-Levoglucosan (10 ng, 50 µL of a solution of 200 ng/mL in MeOH) was added for quantification. Sediment samples were previously extracted at 100°C with hexane/acetone 1:1 using the solvent saver mode at 2 mL/min for 10 min to remove lipidic compounds [29]. Sediment samples were then extracted at 100°C with MeOH using the solvent saver mode at 2 mL/min for 19 min to recover MAs. Methanolic extracts were concentrated to dryness at 60°C under a N_2 flow using a TurboVap (Biotage), and redissolved in 1 + 1 mL of MeOH.

2.4. LE-SPE purification method

The Amberchrom® 50WX8 resin was transformed in its sodium form (Dowex-Na) by suspending it in a large excess of 5% NaCl in water. The supernatant was removed, and the resin was resuspended in MilliQ water. The resin was cleaned 2–3 times until discoloration of the suspension liquid. A volume of 5 mL of resin in water (equivalent to 4 g) was loaded into a 10 mL glass syringe. A glass-fiber filter and 0.4 mL of quartz wool were placed below the resin to avoid loss of particles during sample processing. The column was conditioned sequentially with 30 mL of water, 15 mL of MeOH, and 10 mL of DCM/MeOH 9:1 with the occasional assistance of a mild vacuum suction. The replacement of water with MeOH resulted in a volume decrease of the resin from 5 mL to 3.8 mL.

The process of sample loading onto the LE-SPE column involved a sequential addition of 1 mL of DCM, 2 × 1 mL of methanolic sediment extract, and 9 mL of DCM. The final solvent composition (DCM/MeOH 5:1) was optimal to ensure quantitative retention of MAs by the resin. The resulting mixture was percolated at a controlled speed of <5 mL/min to ensure quantitative retention of MAs by the resin. The LE-SPE column was rinsed with 5 mL of DCM/MeOH 9:1, and the resin was dried by applying vacuum and N_2 supply through the column for 3–6 min. The elution of MAs from the LE-SPE column involved a sequential addition of 2 mL of MeOH and 10 mL of MilliQ water. The purified extract was then collected into a polypropylene tube. The LE-SPE column was discarded after use (Text S1).

2.5. Extract desalting and concentration

Purified extracts were desalted using the AmberLite® MB20 mixed-bed ion-exchange resin. Desalting columns were prepared by placing a quartz filter and 5 mL of the MB20 resin (equivalent to 4 g) into 10 mL glass syringes and cleaned with 50 mL of MilliQ water before use. Sample extracts were let to percolate through the column and residual retained MAs were recovered with 30 mL of MilliQ water. Desalted extracts were collected into 50 mL polypropylene tubes and concentrated at 80°C under a N_2 stream. Sample extracts were transferred to polypropylene injection vials and filtered with a 0.45 µm PTFE filter. The final sample volume was 200 µL of MeOH.

2.6. LC-MS/MS analysis

MAs were detected using an LC-MS/MS system composed by a modular HPLC system (Agilent 1290 Infinity LC system with a quaternary pump, an automatic injector and a thermostated column) coupled to a triple quadrupole detector (Agilent 6470A LC/TQ model) using an electrospray ionization (ESI) interface. Both LC-ESI-MS/MS control and data acquisition were performed with the MassHunter Workstation software version 10.0 SR1 (Agilent).

Separation of LEV, MAN, and GAL was achieved with a SeQuant® ZIC-HILIC™ (150 × 2.1 mm, 3.5 µm particle size, Merck) column thermostated at 20°C with a mobile phase flow rate of

0.275 mL/min. The mobile phase was obtained by in-line mixing MilliQ water (Solvent A) and ACN (Solvent B). A linear solvent gradient was programmed from 5% A to 20% A in 8 min [30]. At 8.1 min, the column was cleaned from all injected compounds with 80% A for 2 min. At 10.1 min, the column was reconditioned with only solvent B for 2 min and then under the original conditions (5% A) for 13 min before the following analysis. The injected sample volume was 10 µL and the sample vials were maintained at 16°C.

A post-column addition of 0.1 mL/min of a NH_4OH solution (obtained by adding 1 mL of NH_4OH 25% to 1 L of MilliQ water) was used to enhance the MS/MS signal. Data acquisition was programed between 4 and 8 min. The mobile phase was diverted to waste outside this runtime period. All MAs isomers (LEV, MAN, and GAL) were detected in negative mode using the 161 → 101 *m*/*z* MS/MS transition. The IS ($^{13}C_6$-LEV) was detected using the 167 → 105 *m*/*z* MS/MS transition. The same MS/MS conditions were used for all compounds: dwell time of 100 ms, fragmentor voltage of 110 V, collision energy of 35 V, and a cell acceleration voltage of 0 V. The conditions used at the ion source were the following: gas temperature of 300°C, gas flow of 4 L/min, nebulizer pressure of 60 psi, sheath gas temperature of 380°C, sheath gas flow of 12 L/min, and a capillary voltage of 5000 V.

2.7. Quantification of MAs and estimation of ion suppression

All MAs concentrations were calculated using the peak area ratios between each analyte and the IS ($^{13}C_6$-LEV). The ratios were converted to concentration values using 7 standard solutions of MAs with IS. The calibration curve ranged from 1 to 1000 ng/mL, corresponding to a dynamic range from 10 to 10000 pg injected. The calibration curve was calculated by adjusting a linear equation with the minimum squared method, weighted by the inverse of the squared concentration ($1/x^2$) [31]. The software used for peak integration, run calibration, and quantification was the Quantitative Analysis Module of the MassHunter Workstation version 10.1 (Agilent). To account for changes in the LC-ESI-MS/MS response over an analytical run, several standard solutions were incorporated. Several quality control analyses were distributed within the analytical run to monitor the stability of the LC-ESI-MS/MS detection system over time.

The estimates of lower limit of quantification (LOQ) were obtained by injecting between between 10 and 10000 pg. The reported LOQ was the lowest concentration that the linear calibration model could reproduce the nominal concentration within a tolerance of ±20%. Sedimentary MAs concentrations were expressed in ng/gdw (grams of dry sediment weight).

Ion suppression was estimated by measuring the peak area increment associated to the standard addition to a sample solution. The signal increment was compared to the peak area measured for the standard without sample matrix. Practical details are provided in Text S2.

2.8. Method validation

To test the analytical robustness, the procedure was validated with the three sediments described in Section 2.2, which have different concentration ranges and matrix composition. Each sediment sample was repeatedly analyzed in three independent batches with all three sediments in triplicate ($n = 9$). Triplicate blanks were included in each batch to determine MAs contamination issues during sample processing. The blanks were prepared similarly to sediment samples, but no sediment was packed in the cell. Each of the three batches was analyzed on different days. In addition to the calibration accuracy and the lack of chromatographic interferences, the following parameters were assessed: 1) background MAs pollution using blank samples, 2) ion suppression effect for MAN, GAL, LEV, and IS (Text S2), 3) total and mass recovery of the IS, and 4) repeatability of the calculated MAs concentrations.

3. Results

Results and processes used to optimize the instrumental and LE-SPE conditions are summarized in Sections 3.1–3.4. Validation of the optimized conditions (as described in Sections 2.3–2.8) is detailed in Section 3.5.

3.1. LC-MS/MS detection setup

Previously published methods used different adducts to detect MAs in both negative and positive mode (e.g., $[M+F]^-$, $[M+Cl]^-$, $[M+CH_3COO]^-$, $[M+HCOO]^-$, $[M+Na]^+$, and $[M+Li]^+$) [30,32–36]. We compared the sensitivity and selectivity of the LC-ESI-MS system upon post-column addition of $HCOONH_4$ [30] and NH_4OH [7] to the mobile phase. The addition of $HCOONH_4$ to the mobile phase produced an intense $[M+HCOO]^-$ signal at *m*/*z* 207 but also provided poor MS/MS spectra, so we monitored the 207 → 207 transition with a low collision energy (10 V). When adding NH_4OH to the eluent, we obtained optimal $[M-H]^-$ responses using the 161 → 101 MS/MS transition. Both $[M+HCOO]^-$ and $[M-H]^-$ adducts yielded a similar sensitivity when analyzing standard mixtures, resulting in lower limits of detection (LOD) of 5–10 pg injected (Fig. 1a and b). However, we also found a poor selectivity and compromised sensitivity for the $[M+HCOO]^-$ adduct when analyzing sediment extracts (Fig. 1c). By contrast, we found a good selectivity for the $[M-H]^-$ adduct when analyzing the same sediment extracts (Fig. 1d). We thus selected the 161 → 101 MS/MS transition to quantify MAs for all subsequent experiments and final method validation.

Quantification of the three MAs isomers requires their complete chromatographic separation because MS and MS/MS detectors are unable to discriminate them. However, MAs show a low affinity with commonly used reverse stationary phases (such as C18) and elute with the solvent front. Hopmans et al. [13] increased the retention of MAs by adding a weak ion-pair reagent to the mobile phase (Et_3N). However, the use of ion-pair in LC-ESI-MS systems causes severe ion suppression issues, resulting in reduced sensitivity. As shown in Table S1, a few published LC methods achieve adequate MAs isomer separation with LEC [28,37], HPAEC [14,35,38,40–43], and HILIC [30]. HPAEC requires the use of specific chromatographs, free of metallic parts, and uses MS-incompatible NaOH-containing mobile phases. Although specific equipment to overcome these issues is commercially available, common LC-MS systems are not compatible with HPAEC and therefore we discarded them for this method development. Since HILIC systems tend to produce optimal results when combined to ESI-MS detectors, we adapted the HILIC method described by Matějíček and Vašíčková [30]. We achieved the same separation with a ZIC-HILIC column (instead of a ZIC-cHILIC column; Fig. 1). However, the resulting method suffered from a cumulative ion suppression effect when analyzing sediment samples. We solved the cumulative ion suppression effect issue by implementing a cleaning step with water/ACN 8:2 after MAs elution.

3.2. Ligand exchange-solid phase extraction (LE-SPE)

Solid phase extraction (SPE) represents a practical approach for sample preparation as it produces fast and reproducible methods to purify sample extracts and make them suitable for instrumental analysis. We performed an initial screening to find the

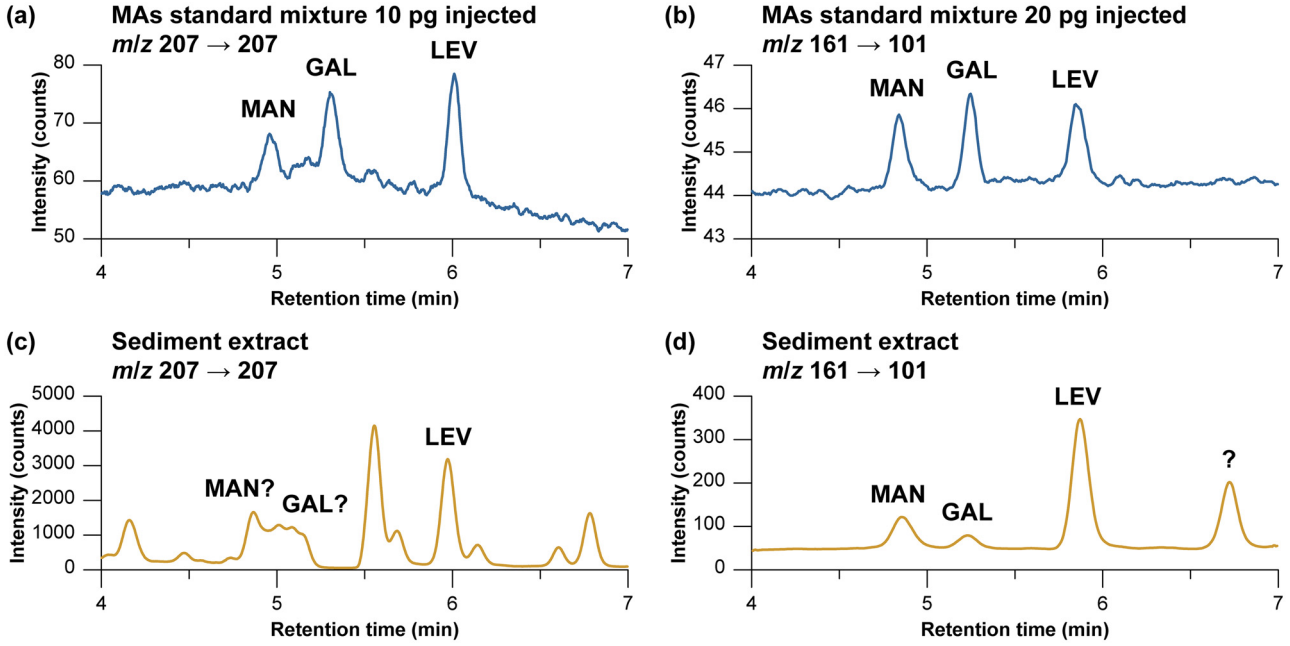

Fig. 1. Comparison of LC-MS/MS sensitivity and selectivity using post-column addition of $HCOONH_4$ (m/z 207 → 207, left) and NH_4OH (m/z 161 → 101, right). (a) and (b) Standard mixtures close to the LOD and (c) and (d) sediment extract. An additional unidentified compound, systematically found in sediment samples, is indicated as "?" in panel 1d.

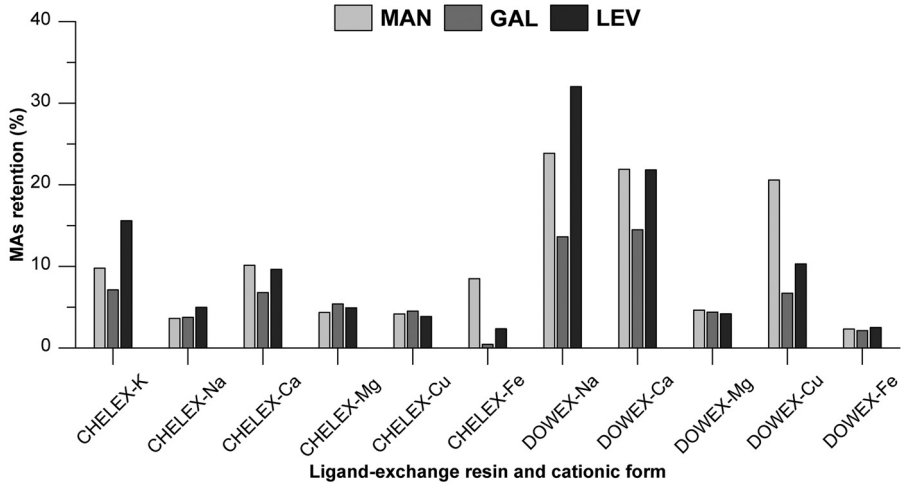

Fig. 2. Percentage of MAs retained on Dowex 50WX8 and Chelex 100 ligand-exchange resins in different cationic forms after a suspension in DCM/MeOH 1:1.

best combination of resin and counter cation capable of extracting MAs from a typical solvent extract in DCM/MeOH. To this end, aliquots of a strong cation-exchange resin (Dowex 50WX8, styrene divinylbenzene polymer with sulfonated groups) and a chelating resin (Chelex^{TM} 100 styrene divinylbenzene polymer with iminodiacetic acid) were transformed to different cationic forms (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{3+}). Each resin (1 g of Dowex or 2 g of Chelex) was suspended in a solution of 1 µg of MAs in 24 mL of DCM/MeOH 1:1 for 16 h. The proportion of MAs adsorbed to the resin in each experiment was quantified by LC-ESI-MS/MS. As shown in Fig. 2, Dowex-Na and Dowex-Ca resins retained a significant fraction of the MAs (15–32%). The Chelex resin retained less than 10% of each MAs in most cases. In addition, transition metals (Fe and Cu) did not perform well in all cases in DCM/MeOH.

To evaluate whether Dowex-Na and Dowex-Ca resins could provide enough retention capacity to quantitatively load/elute MAs in SPE experiments, we loaded 5 mL of each in water into 10 mL glass syringes. Each chromatographic column was conditioned with the appropriate solvent, spiked with 1 µg of MAs, and then eluted with several solvent fractions of 2 mL. The experiment was repeated with three different solvent compositions (water, MeOH, and DCM/MeOH 1:1) to identify the adequate loading and eluting solvents. As shown in Fig. 3, MAs readily eluted from the Dowex-Na resin when using water as the mobile phase. Dowex-Ca resin succeeded in partially retaining LEV and MAN with water. Remarkably, both Dowex-Na and Dowex-Ca resins displayed a far greater capacity to retain MAs when water is replaced with MeOH (Fig. 3c and d). The use of DCM/MeOH 1:1 further improved the retention of MAs by both Dowex-Na and Dowex-Ca resins (Fig. 3e and f). We thus conclude that the interaction of MAs with Ca^{2+} or Na^+ ions immobilized in the ion-exchange resin is strong enough to selectively extract MAs from a DCM/MeOH solution using a SPE column.

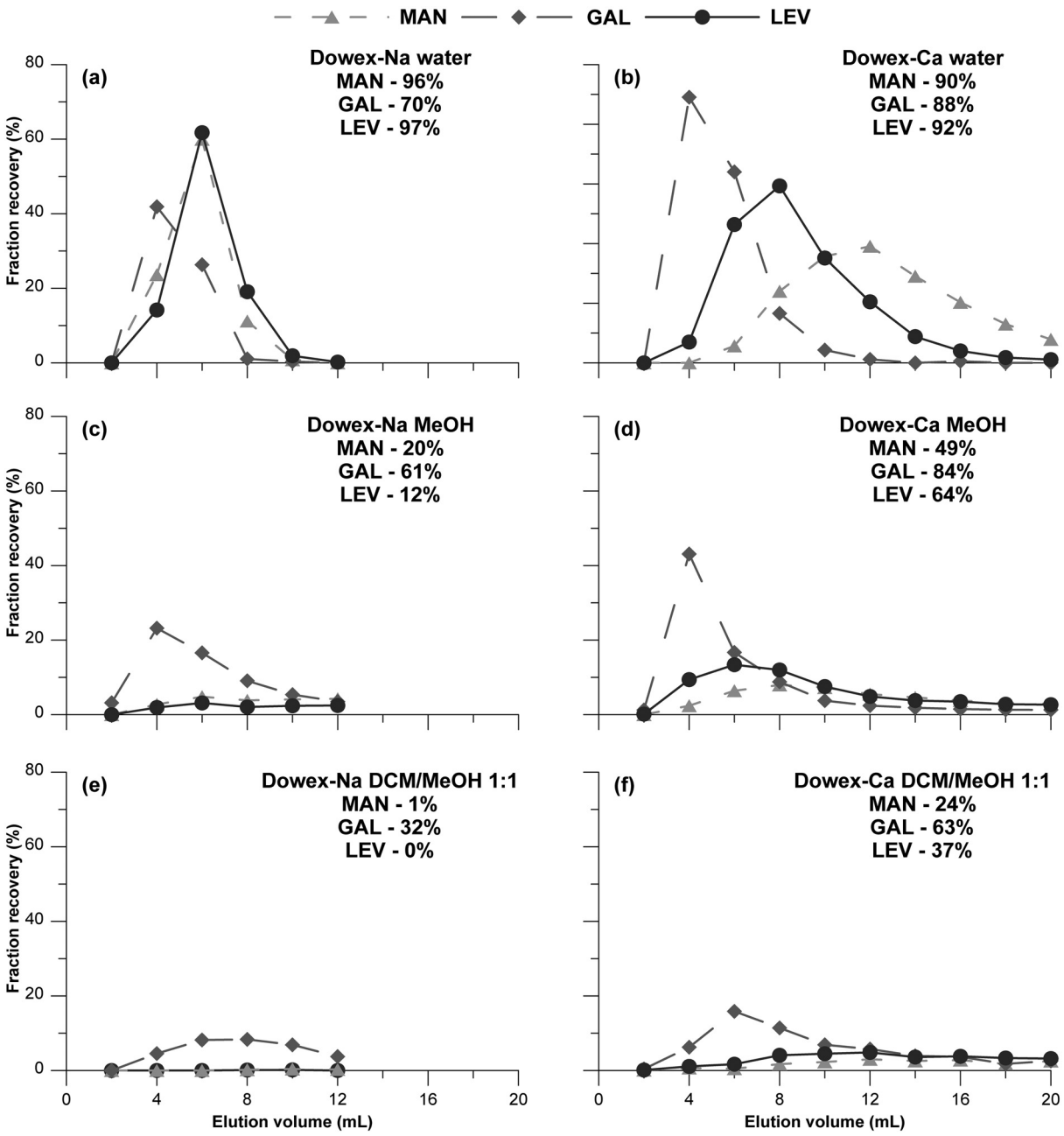

Fig. 3. Elution profiles of MAs in LE-SPE columns containing 5 mL of Dowex-Na (left) and Dowex-Ca (right) resins. Solvents tested were water (a) and (b), MeOH (c) and (d), and DCM/MeOH 1:1 (e) and (f). Values displayed at the top right of each graph indicate the cumulative recovery considering all fractions.

We selected the Dowex-Na resin to further optimize SPE conditions because MAs can be retrieved by eluting with a smaller volume of water (10 mL, Fig. 3a and b).

The retention mechanism of MAs by the Dowex-Na resin is mainly related to complexation of saccharides to immobilized Na^+ (or Ca^{2+}) ions in MeOH, in a similar process to that described for water-based chromatographic systems, such as LEC (ligand exchange chromatography) [20,21,25] and AEC (anion exchange chromatography) [27]. A comparison experiment showed that, under the same experimental conditions, the Dowex-Na resin retained 78–87% of MAs, while the Dowex-H resin only retained 15–18% of MAs. Therefore, only a small proportion of MAs retained by the Dowex-Na (and Dowex-Ca) resins in DCM/MeOH is related to hydrophilic/hydrophobic interactions with the styrene-divinylbenzene copolymer of the resin. The existence of coordination complexes between Na^+/Ca^{2+} ions and hydroxylated organic compounds, such as MeOH and saccharides is well documented [21,22]. Therefore, the exchange of Na^+ ligands (MeOH and MAs) can account for the observed retention/elution of MAs in MeOH-based solvents (Fig. 3). Increasing the hydrophobicity of the loading solvent with DCM further reduces MAs losses by lowering the affinity of the mobile phase to hydrophilic MAs compounds (Fig. 4). After careful optimization, we concluded that the optimal solvents to load and elute MAs from LE-SPE Na^+ columns were DCM/MeOH 5:1 and water, respectively.

3.3. Optimization of the selective pressurized liquid extraction

A number of studies have used pressurized liquid extraction using MeOH [10,13–15,40,44–46] or DCM/MeOH mixtures [9,11,47–51] to obtain crude MAs-containing sedimentary extracts. However, the use of DCM/MeOH mixtures yields increased matrix

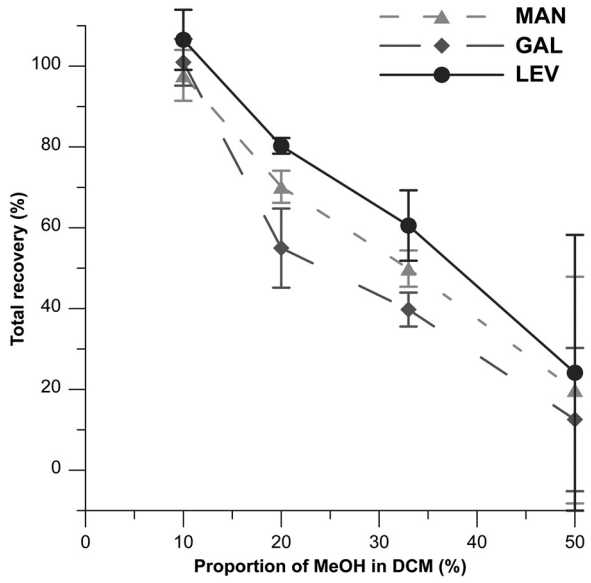

Fig 4. Relationship between loading solvent composition and total MAs recovery using a LE-SPE column containing 5 mL of Dowex-Na. Error bars represent standard deviations of duplicates.

effects for LC-ESI-MS systems [13] and poor recoveries [46] compared with methanolic sediment extracts. We further investigated these issues by testing hexane/acetone 1:1 and DCM/MeOH from 9:1 to 0:10 for selective pressurized liquid extraction (SPLE) from spiked blanks and sediment samples from a TOC-rich lake (TOC = 28 %). The other SPLE conditions are identical to those described in Section 2.3. Spiked blanks proved that the activated silica gel quantitatively retains MAs and IS when using hexane/acetone 1:1, which enables a pre-purification step by SPLE. Adequate MAs recoveries from the silica gel required highly hydrophilic extraction solvents (20–100% MeOH, Fig. 5a). Similarly, the total IS recovery from the TOC-rich sediment maximized with DCM/MeOH mixtures containing more than 40% MeOH (Fig. 5b).

Total IS recoveries for $CaCO_3$-rich sediments remained consistently low (10–30%, Fig. 5b) when using DCM/MeOH 6:4. Further experiments involving sequential extraction with DCM/MeOH 6:4, MeOH, and water confirmed that MAs extraction with DCM/MeOH 6:4 was not complete for $CaCO_3$-rich sediments. The reduced recovery when extracting $CaCO_3$-rich sediments is likely related to strong interactions between MAs and cations present in the inorganic matrix (i.e., Ca^{2+} ions). In situations where IS and MAs are strongly retained by the stationary phase, adding the IS on top of the sediment can lead to an underestimation of the IS recovery. These low recoveries have been reported to produce significant overestimation of concentration values in SPLE methods [52]. Nevertheless, our $CaCO_3$-rich sediment extracts in DCM/MeOH 6:4 and MeOH yielded consistent differences in total IS recoveries in favor of the methanolic extracts, so we selected MeOH as the SPLE extraction solvent for MAs.

3.4. Extract desalting

During method development, we found that some extracts produced reduced MAs signals due to ion suppression of the ESI-MS/MS signal by interfering compounds. Signal losses occurred randomly with both blank and sediment extracts. We attributed this issue to accidental leaching of Na^+ (and other ions) from the Dowex-Na resin during LE-SPE treatment. These cations form extremely stable adducts at the ESI interface even at trace levels [30,36,43,53], resulting in severe signal suppression problems. Residual interfering ions in the sample extract thus compromised the sensitivity of the analysis and substantially biased the quantification of MAs. To address this issue, we implemented a sample desalting step with the Amberlite MB20 mixed-bed ion-exchange resin. This sample desalting step also eliminated interferences in LC-ESI-MS/MS chromatograms (Fig. S2) when analyzing $CaCO_3$-rich sediments.

3.5. Method validation

3.5.1. Linearity and sensitivity

The LC-ESI-MS/MS method provided linear responses in the range from 10 to 10000 pg injected (Table 1 and Fig. S3) with a LOD of approximately 5 pg injected. This instrumental sensitivity is comparable to the ones reported by Matějíček and Vašíčková [30] (LOD of 4–9 pg injected and LOQ of 10–30 pg injected) and Sanz Rodriguez et al. [35] (LOD of 5–25 pg injected and LOQ of 15–76 pg injected), which are among the best published instrumental sensitivities in terms of injected MAs amounts (Table S1). Only Gambaro et al. [7], You et al. [39], and Yao et al. [36] achieved better instrumental sensitivities by two orders of magnitude (LODs of 0.30, 0.55, 0.20 pg injected, respectively), though at the expense of LEV separation from MAN and GAL.

3.5.2. Repeatability

The analytical method provided repeatable MAs concentration results, irrespective of the sedimentary $CaCO_3$ and TOC content, over a wide MAs concentration range (Table 2). The LUC sediment yielded the highest MAs concentrations (LEV/MAN/GAL 717/162/34.3 ng/gdw), the EN651 Mix sediment yielded the lowest MAs concentrations (LEV/MAN/GAL 6.6/0.7/0.3 ng/gdw), and the MERS ST7 sediment yielded MAs concentrations twice as high as those of the EN651 Mix sediment (LEV/MAN/GAL 13.6/1.4/0.7 ng/gdw). Global RSDs for LEV ranged between 9% and 23%, with the poorest repeatability for the deep-sea sediment (EN651 Mix). Global RSDs for MAN and GAL were consistently poorer compared to LEV (15–34%), likely related to the absence of isotopically labeled IS for these MAs isomers.

3.5.3. Blank contributions

Atmospheric MAs in the laboratory environment is a source of contamination during sample processing [7,8,39,54–56]. We found a systematic concentration background in the final extract in the range of 5–30 ng/mL for LEV and 0.2–10 ng/mL for MAN and GAL. Therefore, blank contributions should be considered for reliable MAN, GAL, and LEV quantifications (Table 2). During this validation exercise, we found that contamination during sample processing could contribute significantly (up to 29%) to calculated MAs concentrations. Accordingly, these blank issues impose a practical lower limit of quantification of the analytical procedure (about 5–10 ng/gdw in sediment), which is significantly higher than the instrumental LOQ (0.1 ng/gdw assuming 60% recovery and 3 g of sediment). Therefore, achieving extremely low analytical LOQ requires processing samples in a clean room [7,39] or under a laminar flow bench [7,8,54–56].

3.5.4. Total and mass IS recovery and ion suppression effect

Total recoveries (TR) were estimated by comparing the IS signal obtained for processed samples with a standard solution. The average TR values obtained during the validation exercise ranged between 39% and 61% (Table 3). Remarkably, blank and sediment samples provided similar TR, indicating that the relatively low TR is independent of the sample matrix (i.e., $CaCO_3$ and TOC content).

The TR is a combination of the mass recovery (MR, the proportion of MAs recovered after sample processing) and the matrix effect (mainly related to ion suppression at the ESI interface;

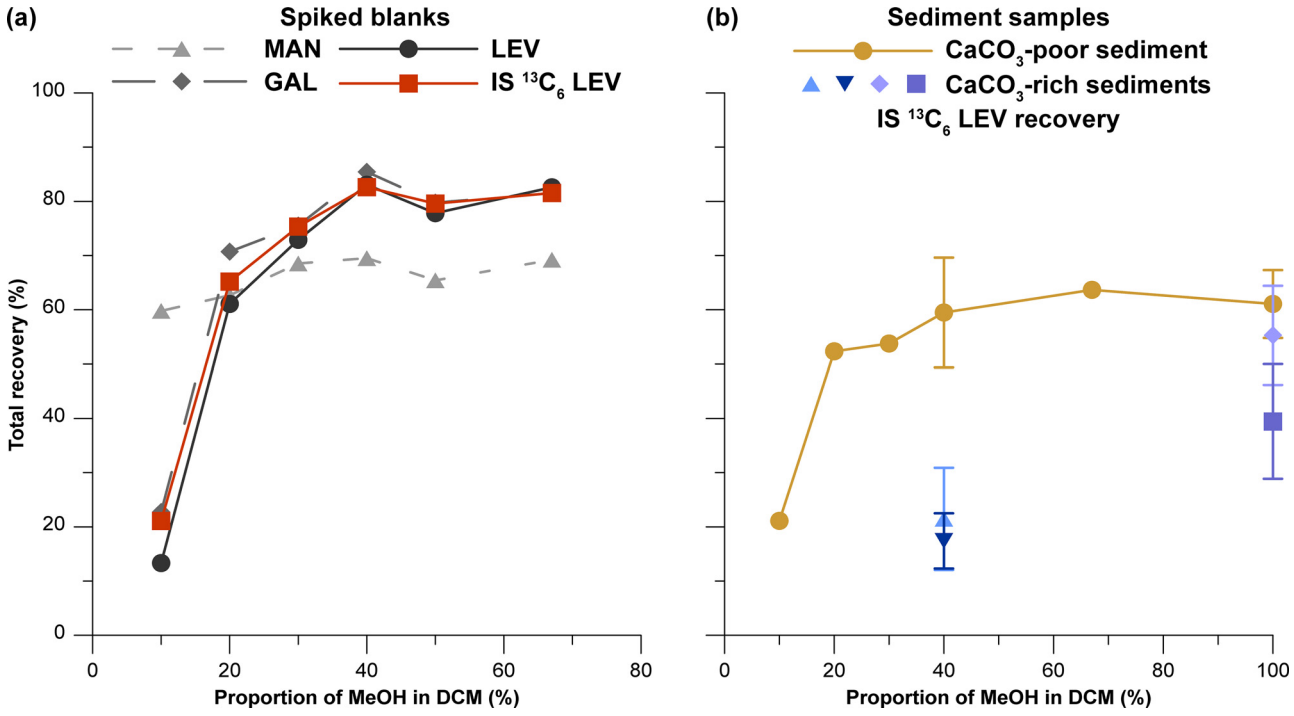

Fig. 5. Solvent composition effect on total MAs and IS recovery with selective pressurized extraction. (a) Total MAs and IS recovery from spiked blanks and (b) total IS recovery from sediment samples. Error bars represent standard deviations (n = 4–9).

Table 1
Linearity and accuracy of the method.

	MAN	GAL	LEV
Calibration standard mixtures			
Correlation coefficient (R^2)[a]	0.995–0.999	0.990–1.000	0.991–0.999
Accuracy (%)	89–111	94–118	91–113
Quality control standard mixture			
Batch 1 accuracy (mean ± SD, %)[b]	103 ± 12	95 ± 14	93 ± 3
Batch 2 accuracy (mean ± SD, %)[b]	106 ± 7	96 ± 8	101 ± 4
Batch 3 accuracy (mean ± SD, %)[b]	104 ± 6	99 ± 6	106 ± 6
Global accuracy (mean ± SD, %)[c]	105 ± 9	97 ± 10	100 ± 7

[a] Linear regression adjusted with the minimum squared method, weighted by the inverse of the squared concentration ($1/x^2$).
[b] Calculated from 7 injections of 200 pg per independent batch.
[c] Calculated from 21 injections of 200 pg over 3 independent batches.

Table 2
Repeatability of calculated MAN, GAL, and LEV concentrations and blank contributions.

Sample	MAs concentration [mean, ng/gdw (%RSD)][a]			Blank contribution (%)[a]		
	MAN	GAL	LEV	MAN	GAL	LEV
LUC (lacustrine sediment)	162.2 (15%)	34.3 (16%)	717.4 (9%)	1	3	2
MERS ST7 (coastal sediment)	1.4 (26%)	0.7 (30%)	13.6 (14%)	11	10	8
EN651 Mix (deep-sea sediment)	0.7 (34%)	0.3 (30%)	6.6 (23%)	29	23	18

[a] Calculated from 9 replicates over 3 independent batches.

Table 3
Total and mass IS recoveries and ion suppression effect on MAN, GAL, LEV, and IS signals.

Sample	IS recovery (mean ± SD, %)[a]		Ion suppression effect (mean ± SD, %)[a]			
	Total recovery	Mass recovery	MAN	GAL	LEV	IS
Blank	42 ± 10	45 ± 10	3 ± 5	2 ± 5	12 ± 7	5 ± 5
LUC (lacustrine sediment)	61 ± 7	70 ± 12	2 ± 8	1 ± 10	4 ± 10	11 ± 7
MERS ST7 (coastal sediment)	55 ± 9	62 ± 7	12 ± 7	18 ± 7	7 ± 9	11 ± 9
EN651 Mix (deep-sea sediment)	39 ± 11	47 ± 11	15 ± 7	19 ± 10	12 ± 8	16 ± 10

[a] Calculated from 9 replicates over 3 independent batches.

Text S2). Estimates of the ion suppression, obtained by measuring the signal increment caused by the addition of a known amounts of MAs (and IS), remained at acceptable levels for all MAs in all samples considered (0–20%, Table 3). Low ion suppression values show that LE-SPE provides MAs extracts free of interferents for LC-ESI-MS/MS. Therefore, the relatively low TR estimates are caused mainly by MAs losses during sample processing.

The MR of the IS, as estimated by removing the ion suppression effect from the TR, ranged between 45% and 70% (Table 3), with the lowest values obtained for the blank samples. Further tests directed at improving the MR showed that the final MR was the result of small losses that occurred at every step of the analytical setup and no significant improvement could be achieved.

4. Discussion

Trace amounts of ions in the mobile phase can produce drastic changes in mass spectra of MAs in LC-ESI-MS systems. Although this problem can be partially solved by promoting specific adduct forms, such as the $[M\text{-}H]^-$ adduct by adding NH_4OH to the mobile phase in this study, the instrumental response factor in multiple reaction monitoring or selected ion monitoring mode can still be severely affected during the analysis of samples with high ionic compound concentrations. Analytical protocols based on LC-ESI-MS require proper evaluation of the ion suppression by the sedimentary matrix, including the sensitivity, accuracy, and precision in real samples [57]. In principle, accuracy can be partially ensured by using an isotopically labelled IS that compensates for changes in the instrumental response factor. In the case of crude sediments extracts, the analytical method suffered from major ion suppression issues that hampered the detection of MAs. Therefore, the analysis of MAs by LC-ESI-MS required exhaustive elimination of salts and other potential interferents from the sediment extract.

Previous studies implemented crude desalting methods, such as sample percolation through cotton wool [46], Na_2SO_4 columns [13,46], or with ACN [13] or DCM/MeOH 9:1 [46]. Another existing MAs purification protocol employed centrifugation and C18 columns [10]. However, we have shown that the use of apolar solvents and Na_2SO_4 columns may lead to significant MAs losses and introduce interfering ions. The efficiency of these purification methods cannot be evaluated because matrix effect and ion suppression were not reported.

The hydrophilic character of saccharides makes impractical the use of established methods to purify and concentrate sample extracts. For example, liquid-liquid extraction fails to separate MAs from salts and other water-soluble interferents. Also, the low affinity between saccharides and commercially available chromatographic stationary phases limits the applicability of SPE protocols. On the other hand, ligand-exchange chromatography is commonly applied to separate and identify isomeric forms of saccharides and amino acids, but these systems typically have low retention capacities that have hampered their applicability in sample purification processes [27]. We have demonstrated that the retention capacity of ligand-exchange systems is enhanced in a methanolic context, compared to traditionally used water-based LC systems (Figure 3). In this context, the elution capacity of the mobile phase can be further reduced by adding hydrophobic organic solvents to the mobile phase, such as DCM. Ligand-exchange interactions between MAs and immobilized Na^+ ions occurred even in highly hydrophobic solvents, such as DCM/MeOH 9:1 (Figure 4). Since ligand-exchange interaction is highly specific to polyhydroxylated compounds such as saccharides, purified extracts were free of interfering compounds commonly found in sediments and soils, such as fulvic acids, lipids, and salts. In this study, we have demonstrated that LE-SPE can be applied to selectively extract trace amounts of MAs in sediment extracts with a reasonable efficiency (mass recoveries of 45–70%, Table 3). However, we believe that other ligand-exchange-based approaches can be developed to purify and concentrate other saccharides and amino acids in complex matrices, such as biological and environmental samples.

5. Conclusions

We describe a solid phase extraction method that takes advantage of the affinity between MAs and immobilized Na^+ ions related to ligand-exchange processes in non-aqueous, methanolic solvents. The capacity of LE-SPE columns to retain MAs can be enhanced by increasing the hydrophobicity of the mobile phase with DCM. We have applied the unique properties of LE-SPE columns to selectively extract MAs from samples of complex composition such as sediment extracts. The analytical procedure produces extracts with low matrix effect and is suitable for reliable MAs quantification in sediments with LC-ESI-MS/MS systems. The resulting analytical method provides robust and repeatable concentration values for LEV, MAN, and GAL irrespective of the sample matrix and is applicable to a wide concentration range. This analytical method represents a proof of concept, and other ligand-exchange-based SPE protocols may be developed to quantify trace amounts of other saccharides and amino acids in biological and environmental samples.

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.

CRediT authorship contribution statement

Nina Davtian: Writing – original draft, Investigation, Validation, Formal analysis, Visualization. **Nuria Penalva:** Validation. **Antoni Rosell-Melé:** Funding acquisition. **Joan Villanueva:** Writing – original draft, Writing – review & editing, Visualization, Methodology, Supervision.

Data availability

All data generated from this study are presented in Figs. 1–5, S2, and S3 and Tables 1–3. Detailed method validation results are available in Tables S2–S5.

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Supplementary materials

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