Journal of Chromatography A 1786 (2026) 467338

Contents lists available at ScienceDirect

Journal of Chromatography A

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

High-throughput HPAEC-PAD for the analysis of neutral sugars and uronic acids in biomass hydrolysates

Christian Marvelous *, Hendrik-Jan Brouwer, Martin Eysberg, Jean-Pierre Chervet, Detlef Jensen

Antec Scientific, Hoorn 131, 2404 HH Alphen a/d Rijn, The Netherlands

ARTICLE INFO

Keywords:
Biomass hydrolysates
Carbohydrate profiling
High performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD)
High-throughput analysis
Method validation

ABSTRACT

Lignocellulosic and marine biomasses are valuable renewable sources of cellulose and alginate polysaccharides. Accurate quantification of the neutral sugars and uronic acids content in complex biomass hydrolysates is essential for effective characterization and use. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) provides sensitive, derivatization-free analysis with good isomer separation. However, traditional methods often involve lengthy gradients and demanding column maintenance, which limit throughput. In this study, we introduce and validate a high-throughput HPAEC–PAD method tailored for quantifying neutral sugars and uronic acids in biomass-derived polysaccharide hydrolysates. Using an efficient anion-exchange stationary phase, optimizing elution conditions, and automating analysis on one column while simultaneously reconditioning the other, a rapid and robust workflow is presented. The presented method separates 12 neutral sugars and 5 uronic acids in a single run, with most analytes baseline resolved ($R_s > 1.5$). The automated approach reduces cycle times from 60 min to 33 min without sacrificing separation quality, quantitation, or sensitivity. This translates to an increased throughput by approximately 82% for combined neutral sugar and uronic acid analysis, and by roughly 160% and 445% when focusing solely on each component class. Calibration was assessed by relative standard error (RSE) in line with the International Union of Pure and Applied Chemistry (IUPAC) guidance. The average RSE on calibration is below 2.3% for all analytes. The detection limits for all analytes range from 0.02 μmol/L to 0.40 μmol/L across all methods and columns. Overall, the results highlight both the efficiency and analytical robustness of this optimized workflow, which supports rapid, high-volume screening of polysaccharides in biomass processing and biopolymer research.

1. Introduction

Lignocellulosic biomass is a crucial renewable resource for producing fuels, chemicals, and high-value products, since its carbohydrate polymers can be converted into fermentable sugars and derivatives. Through processes involving heat, chemicals, enzymatic hydrolysis, and fermentation, cellulose and hemicellulose break down into simple sugars. The resulting hydrolysates contain sample-specific amounts of glucose, xylose, mannose, arabinose, cellobiose, and sugar acids such as galacturonic and glucuronic acids. These uronic acids naturally occur in plant cell walls and can also be formed during processing.

Traditionally, bioactive compounds derived from seaweed have been used in medicine. These compounds are isolated and characterized, offering nutritional, antioxidant, antimicrobial, and antiviral benefits for pharmaceutical and nutraceutical applications. For instance, the gelling properties of alginate enable encapsulation and controlled release, making it useful for oral drug delivery, wound healing, and targeted delivery to tissues such as tumors. Additionally, agar and carrageenan act as emulsifiers and stabilizers in packaging films [1–3]. Biopolymers derived from seaweed and microbes also serve as raw materials for bioplastics and are also studied as soil conditioners to promote sustainable agriculture, improve soil quality, and lessen environmental impact. Seaweed cultivation does not require arable land, as it absorbs CO_2 and nutrients from water, potentially helping to reduce ocean acidification and water pollution. Additionally, it might lower our dependence on traditional plastics [4].

Analyzing carbohydrates in biomass hydrolysates is challenging due to their complex composition, including hexoses, pentoses, oligosaccharides, uronic acids, and co-extracted species such as organic acids and phenolics. Their concentrations can vary, and similar or epimeric

* Corresponding author.
E-mail address: c.marvelous@antecscientific.com (C. Marvelous).

https://doi.org/10.1016/j.chroma.2026.467338
Received 19 June 2026; Received in revised form 4 August 2026; Accepted 5 August 2026
Available online 5 August 2026

structures often require selective chromatographic separation. A variety of chromatographic techniques are employed for their analysis, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), hydrophilic interaction liquid chromatography (HILIC), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [5]. Methods such as HPLC, HILIC, and GC often require derivatization to improve detection. In addition, factors such as pretreatment procedures, ionic strength, and buffers can influence retention times and sensitivity, thereby limiting the effectiveness of these techniques [6].

HPAEC–PAD addresses previous limitations and enables direct analysis of monosaccharides, oligosaccharides, and uronic acids in biomass samples. When the pH exceeds 12, carbohydrates form oxyanions, facilitating their separation on polymer-based anion-exchange columns [7]. Due to different pKa, carbohydrate isomers of monosaccharides and oligosaccharides can be separated using this technique. It can also separate anomeric forms and better retain acidic sugars, such as uronic acids, compared with other methods [7,8]. Pulsed amperometric detection (PAD) offers direct electrochemical detection of carbohydrates with high sensitivity and a broad dynamic range, enabling detection of low-abundance sugars and uronic acids without chemical modification [9–11]. As an example, a mixture of 8 neutral sugars, 2 amino sugars, 2 uronic acids, and 1 alditol standard related to microalgae showed incomplete resolution across 11 different HPLC methods, but improved resolution and accurate quantification using HPAEC-PAD without any derivatization [12]. Several other studies also utilized HPAEC-PAD for plant analysis for complete monosaccharides analysis, using multiple different gradient programs [13,14]. HPAEC-PAD also has been employed for the analysis of neutral sugars, amino sugars, and uronic acids in various marine biomasses such as brown seaweeds, chitins, and alginates [15–17].

As biomass research grows to include larger sample sets for enzyme engineering, pretreatment screening, and process optimization, throughput often becomes a bottleneck. Traditional HPAEC analyses, especially for a variety of saccharides and uronic acids in biomass research, tend to slow down lab productivity due to long gradient runs and the need for column rinsing/reconditioning [18]. To handle the increased sample volume, advanced HPAEC-PAD techniques and hardware setups can be used to boost throughput while maintaining sensitivity and separation quality. This involves employing anion-exchange columns with different selectivity or optimized gradient programs [19,20]. However, such approaches leave the column reconditioning untouched. Given the reliable and consistent results of HPAEC-PAD [21–23], it should be possible to automate the analysis process and manage column reconditioning simultaneously to increase sample throughput.

In this study, we aim to develop and validate an automated dual-pump, dual-column HPAEC-PAD method that removes column reconditioning from the analytical timeline using a column switching valve. To our knowledge, our presented method is the first method that enables the separation of 12 neutral sugars and 5 uronic acids in a single run, with most analytes baseline separated. This enables high-throughput profiling of neutral sugars and uronic acids in biomass hydrolysates. This configuration enables the reconditioning step to be done on one column while the other column performs the separation simultaneously. Unlike traditional methods that involve long, step-by-step rinsing and equilibration, this parallel approach significantly boosts sample throughput—over 80% for simultaneous analysis of sugars and uronic acids, and up to fivefold for targeted profiling—without sacrificing separation quality, sensitivity, or accuracy in complex biomass samples. Additionally, we utilize and show that the use of a newer metric, Relative Standard Error (RSE) [24], to select the most appropriate calibration model, leads to more trustworthy outcomes [25,26]. This method aligns with IUPAC guidelines that recommend against using correlation coefficients in calibration procedures [27]. Overall, the presented method is crucial to overcoming current analytical bottlenecks in biomass polysaccharide processing, enabling faster, large-scale screening for enzyme development, biopolymer research, and process improvement.

2. Materials and methods

2.1. Chemicals and samples

All chemicals used to prepare the eluents and standards used in this work are listed in the supplementary Table S1. The carbohydrate standards used were prepared as described in the supplement (paragraph S1). Deionized (DI) water was obtained using a laboratory water purification system (YoungIn Chromass Aquapuri Essence+ 393; resistivity >18 MΩ·cm, TOC (Total Organic Carbon) ≤5 µg/L). All mobile phases were manually prepared, sparged, and blanketed with nitrogen (5.0) to minimize carbonate buildup in the eluent, following the procedure described elsewhere [28,29]. The biomass samples were received in a hydrolyzed state, either with sulfuric acid or enzymatically, in accordance with published methods (e.g., [1–4]). Apart from diluting with DI water, no additional sample preparation was applied.

2.2. Instrumentation

All analyses were conducted using the ALEXYS High-Throughput Carbohydrate Analyzer (Antec Scientific), a metal-free HPLC system with PEEK (Polyether Ether Ketone) flow path, equipped with two quaternary gradient pumps, an autosampler, a column thermostat, a ten-port switching valve, an eluent tray, and an electrochemical detector. To remove borate traces from the eluent, a borate ion trap column (2.1 × 50 mm, Antec Scientific) was installed in line, between the pump and injector. For all experiments, two sets of columns were used: a 2.1 × 200 mm analytical column and a 2.1 × 50 mm precolumn. Both columns were packed with a pH-stable polymeric anion-exchange stationary phase (SweetSep AEX20, Antec Scientific). This column was initially designed for the rapid detection of mono- and disaccharides in food and for the analysis of monosaccharides from glycoconjugates. The SenCell electrochemical flow cell (Antec Scientific) was used for detection [30]. This wall-jet flow cell features a 2-mm gold working electrode, a palladium reference electrode (HyREF), and a stainless-steel auxiliary electrode.

2.3. HPAEC-PAD conditions

Neutral sugars were eluted with diluted sodium hydroxide (NaOH) (20 mmol/L). More strongly bound saccharides and uronic acids were eluted with NaOH mixed with sodium acetate, up to 250 mmol/L. The flow rate was set to 0.18 mL/min and the column temperature to 20 °C for all experiments. A 2.5 µL injection volume was used. The adjustable flow cell volume was set to approximately 160 nL (position 2). A four-step potential waveform was applied: +0.10 V for 0.4 s, –2.0 V for 0.02 s, +0.6 V for 0.01 s, and –0.10 V for 0.07 s. The signal cell current is acquired for 200 ms between 0.20 – 0.40 s. The signal output is the average cell current in nA measured during this 200 ms time period with a 10 ms sampling rate. The data rate of the signal output is 2 Hz which corresponds to the 500 ms pulse time duration of the applied 4-step potential waveform. The detection temperature was set to 45 °C.

2.4. Automation

The traditional setup, depicted in Fig. 1(a), consists of an autosampler, a gradient pump, a column set, and a detector. In the high-throughput configuration shown in Fig. 1(b), a second gradient pump and a 10-port, 2-position valve were introduced to enable switching between two column sets with the same selectivity, and preferably from the same batch. The valve switching times were defined in the specific

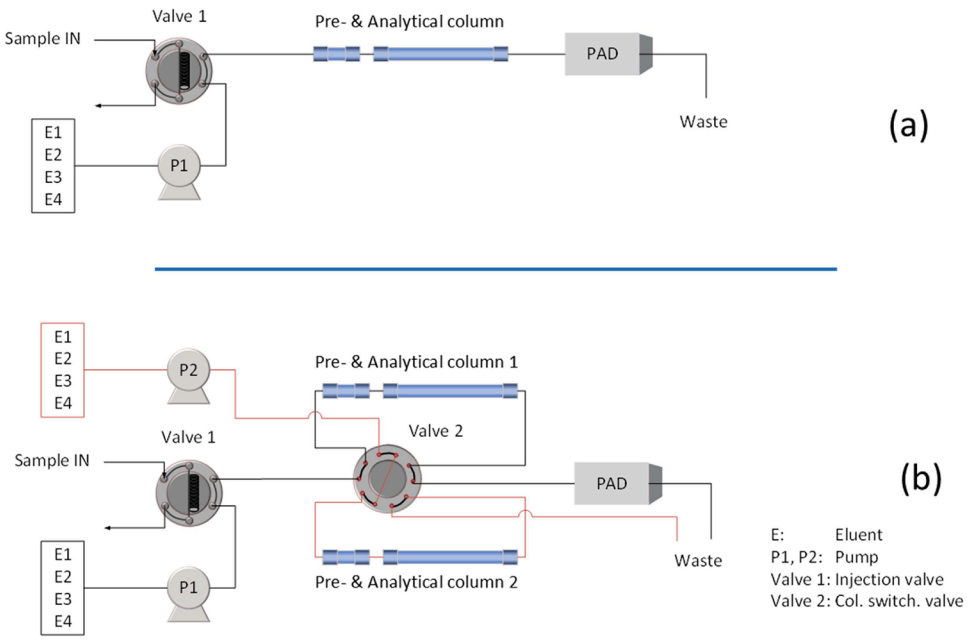

Fig. 1. Schematic setups: (a) conventional HPAEC-PAD instrument and (b) the high-throughput configuration.

methods for each column and could be adjusted if, for example, one of the columns has slightly different retention times due to lot-to-lot variations. The valve flow path configuration allows the columns to be used alternately: one set for separation while the other is reconditioned with the second pump, maintaining the flow direction for both. Consequently, only a single detector cell is needed. This design simplifies calibration and improves both the comparability and repeatability of the analytical results.

For all biomass applications discussed below, except for the conventional single-channel operation (Conventional Analysis of Neutral Sugars and Uronic Acids– reference data), the steps for column rinsing and equilibration (reconditioning) are removed from the main analytical schedule and assigned to a second pump. This change reduces the overall cycle time by reducing the interval between injections, thus increasing sample throughput. Before reintroducing a column into the system's analysis section, both the reconditioned column and the column switching valve must be equilibrated and thoroughly flushed with an eluent matching the starting conditions. This step is essential for consistent, reliable results, preserving the data integrity and separation quality, and achieving repeatability. This setup not only speeds up the simultaneous analysis of neutral sugars and uronic acids but also enables flexible, efficient targeting of either carbohydrate group in high-throughput analyses. Details of the chromatographic conditions are outlined in the following sections.

2.5. Data analysis and method validation

Parametric instrument control, data acquisition, and data processing were performed using Clarity Chromatography Software version 10.1 from DataApex. The method performance was evaluated in terms of repeatability, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and system stability. The same procedures were applied to the reference method and to all high-throughput methods described in this study, on both columns without distinction.

The method for evaluating retention time and peak area repeatability involved 10 injections at each of the three concentration levels (1 μmol/L, 10 μmol/L, and 100 μmol/L) for each carbohydrate. Repeatability was evaluated across all carbohydrates, with no data discrimination at these three levels, and reported as relative standard deviation (RSD).

Calibration was performed at nine concentration levels ranging from 1 to 100 μmol/L. The quality of the calibration model was evaluated for each component using the relative standard error (RSE) rather than the correlation coefficient, in accordance with IUPAC guidelines [27]. Using RSE enables an objective comparison among different calibration models. The calculations were performed using a spreadsheet calculator available for download from the NELAC Institute [31]. In our study, the lowest RSE, indicating the best fit, was achieved with a quadratic calibration that excludes the origin and uses a weighting factor $w = 1/\text{amount}^2$.

The limit of detection (LOD) was calculated as the concentration at which the analyte response is 3 × signal-to-noise ratio (S/N) (Eq. 1). The limit of quantification (LOQ) was calculated similarly, using 10 × S/N instead of 3 × (Eq. 2). The analyte responses at 1 μmol/L concentration and the ASTM noise (average peak-to-peak noise of 0.5 min segments) calculated on a blank-region segments (from 50 – 55 min, 11.5 – 13.5 min, 20 – 23 min, and 10 – 11 min, for the single column reference methods, high throughput combined analysis, targeted neutral sugars analysis, and targeted uronic acid analysis, respectively) were used as the S/N.

$$LOD = \frac{3 \times Concentration_{standard}}{S/N} \quad \text{(Eq. 1)}$$

$$LOQ = \frac{10 \times Concentration_{standard}}{S/N} \quad \text{(Eq. 2)}$$

The accuracy of the method was assessed as recovery values. The recovery values were determined by post-spiking the samples before dilution with known amounts of analyte and comparing the amount between the spiked samples and the non-spiked samples. The recovery values were calculated using Eq. 3.

$$Recovery = \frac{Amount_{spiked\ sample} - Amount_{non\ spiked\ sample}}{Known\ amount_{added}} \times 100\% \quad \text{(Eq. 3)}$$

3. Results and discussion

3.1. Conventional analysis of neutral sugars and uronic acids – reference data

The initial approach involved the simultaneous separation of neutral

sugars and acids on a single column, and eluent delivery by a single pump [19,29]. Table 1 presents the gradient conditions employed for this separation, with a total cycle time of 60 min, including rinsing, cleaning, and equilibration steps.

Fig. 2 shows a chromatogram including the analytical separation of the 17 analytes (0 to 24 min) and column reconditioning (24 to 60 min). The latter involves a sodium acetate gradient and a high-concentration sodium hydroxide step to convert the anion exchanger from acetate to hydroxide form before equilibrating to the start conditions. Mono- and disaccharides with pK_a values ≥ 12 [32] elute within 0 to 12 min, with cellobiose appearing around 17 min. Under this condition, baseline separation ($R_s > 1.5$) of all neutral sugars was achieved, except for mannose-xylose pair ($R_s = 1.2$).

Another published HPAEC-PAD method using different conditions listed a faster elution of all neutral sugars of under 10 min. However, some carbohydrates are coeluted (galactose and rhamnose) and not baseline separated (arabinose-galactose/rhamnose pair and xylose-mannose-fructose) even under the optimal condition [33]. Similarly, another published method listed the same cycle time of 60 min as this presented method [18]. However, rhamnose-arabinose and xylose-mannose pair are also not baseline separated. Uronic acids, as anionic carbohydrates ($pK_a \approx 3$–4) [34] elute later between 22 and 26 min and are all baseline separated (Rs > 1.5) (Table 2).

Table 3(a) lists the statistical value of the relative standard deviation for all analytes, with the median values ranging from 0.1% to 0.2%. Table 3(b) summarizes the statistics of the relative standard deviation of the peak areas. The median values for the peak area RSD range from 1.7% at 1 μM to 0.2% at 100 μM, reflecting the heteroscedastic distribution of peak areas with increasing analyte concentration. These repeatability markers are important because they reflect the high chromatographic repeatability through neutral and anionic carbohydrates.

Table 2 shows the RSE values for each analyte, all of which are below 2.3% (full data: Table S2). This is notable because the US EPA (United States' Environmental Protection Agency) recommends acceptable values ranging from 15% to 25% [24]. Although no longer recommended, the table also includes the correlation coefficient and the coefficient of determination for reference.

The LOD and LOQ values are provided in Table 2. The LOD values for the neutral sugars range from 0.03 μmol/L to 0.11 μmol/L. This corresponds to LOD values in 5 μg/L to 25 μg/L concentration range. The uronic acids LODs are slightly higher than those of the neutral sugars, but still under 0.20 μmol/L (corresponding to 37 μg/L). The LOQs for all analytes are below 0.65 μmol/L, indicating a highly sensitive method for quantification of the analytes in the samples.

Under the chromatographic conditions described, eight biomass hydrolysates, originating from two types of seaweed, shrimp shells, two pulp samples, lignin, plant fibers, and wood, were analyzed. The sample solutions provided were diluted and injected for analysis (Figure S1). Fig. 3(a) shows one of the sample chromatograms measured using this conventional method, in which several analytes were identified. Table 4 presents the carbohydrate concentrations in the original solutions, with sample descriptions indicating the sample dilutions. The carbohydrate levels in the samples vary widely depending on the biomass source, ranging from less than 4 μmol/L to about 20,000 μmol/L. It should be noted that acidic hydrolysis can cause unwanted inversion of sucrose; however, in line with the sample owner's preferences, acidic samples were injected without neutralization. Recovery rates for the identified carbohydrates in the samples were determined. Experimentally, sucrose inversion can be identified by glucose and fructose recoveries exceeding 125%. Such values were excluded from the overall summary because they are attributable to sample-preparation and storage-related factors rather than instrument or sample effects. To mitigate the effect, sample neutralization and prompt chromatographic analysis after hydrolysis are recommended. Recovery (Table 5) was achieved by spiking the digested samples, thereby assessing the accuracy of the analytical workflow. With one exception (glucose, 62% recovery in enzymatically hydrolyzed seaweed), recoveries ranged from 88% to 110%, confirming the robustness of the analytical method across a broad range of biomasses and carbohydrates.

3.2. High-throughput analysis of neutral sugars and uronic acids

For all subsequent experiments, the device configuration displayed in Fig. 1(b) was used, effectively separating column reconditioning from the analytical gradient. Table 6 details the task allocation between the two gradient pumps and the valve switching times, specifying when the separation columns switch between the analytical and reconditioning channels. This setup reduces the cycle time from 60 min to 33 min (Fig. 3 (b)), resulting in a nearly 82% increase in throughput (Eq. 4). To assess the practical performance of this high-throughput setup, the same measurement regime, analytical approach, and data-reduction procedures described earlier were applied without distinguishing between the two separation columns (Table 7(a), Figure S2). The high quality of the resulting data is evident from the relative standard deviations of retention time (0.08–1.6%) and peak area (0.05–5.8%), with median values of 0.22% and 0.77%, respectively, across all 17 carbohydrates and calibration levels. Calibration quality is further underscored by an average RSE of 1.8% (median 1.3%), ranging from 0.7 to 7.5% (full data is provided in Table S2). The average RSE is eight to thirteen times lower than the EPA's recommended values [24], highlighting both the efficiency and analytical robustness of this optimized workflow. Additionally, the LOD values on both columns were comparable to the values presented in the previous section (Table S3). These results confirm a significant increase in throughput while maintaining data reliability and the scientific rigor needed for profiling neutral sugars and uronic acids in biomass samples. The recovery of all carbohydrates and sample matrices averaged 99%, ranging from 88% to 113%. This demonstrates high, consistent efficiency in the analytical process, indicating a reliable and robust methodology (Figure S3).

$$\textit{Throughput increase}\ (\%) = \left(\frac{\textit{Reference Cycle Time}\ (60\ \text{min})}{\textit{New Cycle Time}} \times\ 100\right) - 100 \quad \text{(Eq. 4)}$$

3.3. High-throughput and targeted analysis of neutral sugars

In addition to the demonstrated increase in throughput, the method enables targeted analysis of neutral sugars. At the same time, uronic

Table 1
Gradient program for the simultaneous separation of neutral sugars and uronic acids.

Time (min)	*%A (water)*	*%B (0.2 mol/L NaOH)*	*%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)*	*Description*
0 - 10	90	10	0	Elution of neutral sugars
10 - 14	20	80	0	Elution of cellobiose
14 - 24	66	6	28	Elution of uronic acids
24 - 28	50	0	50	Column clean-up and equilibration
28 - 38	0	100	0	Removal of acetate ions
38 - 60	90	10	0	Equilibration to starting condition

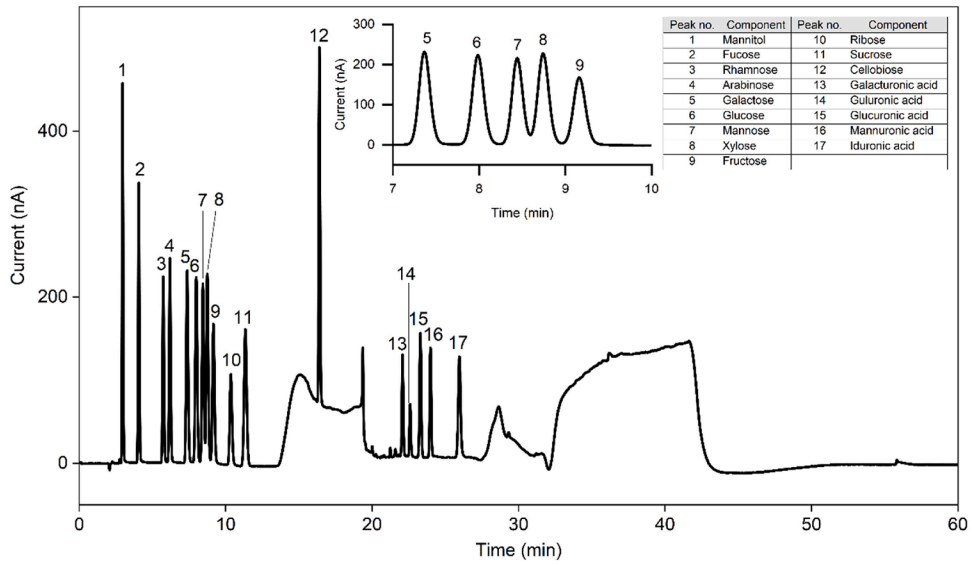

Fig. 2. Chromatogram of 10 μmol/L neutral sugars and uronic acid standards measured using the gradient program in Table 1. Inset shows a zoom-in chromatogram from minute 7 – 10, showing mannose-xylose pair with $R_s = 1.2$.

Table 2
Components investigated and their retention times, limit of detection (LOD) and quantification (LOQ), RSE for evaluation, R and R^2 for reference only.

Component	t_r(min)	*LOD (μmol/L)*	*LOQ (μmol/L)*	*RSE (%)*	*Correl. Coeff. (R)*	*Coeff. Det. (R^2)*
Mannitol	2.96	0.03	0.09	0.63	0.99999	0.99997
Fucose	4.07	0.04	0.12	0.55	0.99999	0.99998
Rhamnose	5.73	0.05	0.18	0.42	0.99999	0.99998
Arabinose	6.18	0.05	0.16	0.46	0.99999	0.99998
Galactose	7.37	0.05	0.18	0.50	0.99999	0.99999
Glucose	7.98	0.05	0.18	0.52	0.99999	0.99998
Mannose	8.44	0.06	0.19	0.39	1.00000	0.99999
Xylose	8.74	0.05	0.18	0.49	0.99999	0.99998
Fructose	9.16	0.07	0.24	0.65	0.99998	0.99996
Ribose	10.4	0.11	0.38	0.50	0.99996	0.99992
Sucrose	11.3	0.07	0.25	0.57	0.99999	0.99998
Cellobiose	16.4	0.03	0.10	0.54	0.99999	0.99998
Galacturonic acid	22.1	0.10	0.32	1.70	1.00000	0.99999
Guluronic acid	22.6	0.19	0.64	1.85	0.99993	0.99984
Glucuronic acid	23.3	0.08	0.26	1.71	0.99998	0.99994
Mannuronic acid	24.0	0.09	0.29	1.85	0.99999	0.99997
Iduronic acid	26.0	0.10	0.34	2.28	0.99983	0.99958

Table 3
Repeatability data of retention times and peak areas at three concentration levels using the chromatographic conditions for the simultaneous elution of neutral sugars and uronic acids.

	%RSD t_R (n = 10)			*%RSD area (n = 10)*		
	1 μmol/L	*10 μmol/L*	*100 μmol/L*	*1 μmol/L*	*10 μmol/L*	*100 μmol/L*
Min	0.1	0.1	0.1	0.1	0.1	0.1
Max	0.2	0.2	0.2	5.2	2.9	0.4
Median	0.2	0.2	0.1	1.6	1.4	0.2
Average	0.2	0.1	0.1	2.2	1.6	0.2

acids and other more strongly retained components are rinsed out during column reconditioning. As shown in Table 8, the analytical and column reconditioning programs have a common cycle time of 23 min (Fig. 3(c)); i.e., after the elution of cellobiose, the column is switched to the reconditioning channel, and the next injection is performed on the second column (Figure S4). Compared to the reference method, sample throughput increases by>160%.

Table 7(b) summarizes the selected performance parameters of the high-throughput method optimized for determining neutral sugars. The same stringent conditions are applied for assessing the relative standard deviation of retention times, peak areas, and RSE (Table S4). Across all components, calibration levels, and both separation columns, the average RSD of retention time was 0.2% (median 0.3%), with a range from 0.1% to 0.3%. Peak area values were similarly within the sub-1% range. The detection limits across both columns were also comparable to the reference data (Table S3). For the recovery assessment, enzyme-treated seaweed, acid-hydrolyzed paper pulp, lignin, and plant fibers were analyzed under the same conditions as described above. On average across all neutral sugars, matrices, and columns, recovery was consistently 100% (median: 99%), with a range of 95% to 118%. This demonstrates the robustness and precision of the method, confirming its suitability for reliable high-throughput analysis of neutral sugars in biomass (Figure S5).

3.4. High-throughput and targeted analysis of uronic acids

This approach enables targeted analysis of the stronger retained uronic acids. Due to the higher ionic-strength eluent conditions (Table 9), neutral sugars elute near the void as a sum peak, separated from the target compounds. As a precaution against potential column deterioration caused by stronger retained components, column reconditioning was also performed. As shown in Table 9, the analytical and

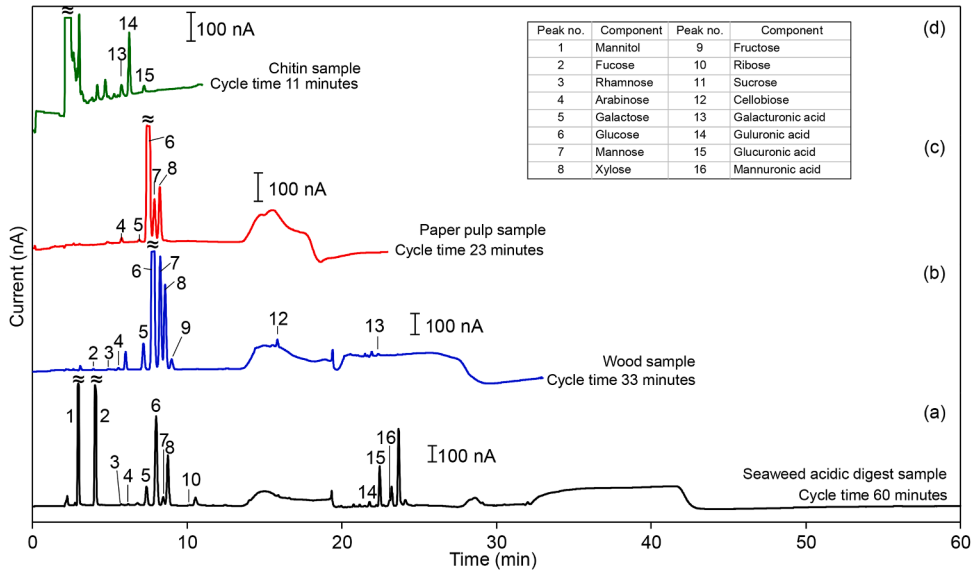

Fig. 3. Overlay of chromatograms analyzing neutral sugars and uronic acids in biomass hydrolysates, (a) conventional analysis with a 60 min cycle time (ct), (b) high throughput analysis (H.T.) of neutral sugars & uronic acids (ct 33 min), (c) H.T. of neutral sugars (ct 23 min), (d) H.T. of uronic acids (ct 11 min).

Table 4
Carbohydrate concentrations in the original solutions, with the sample descriptions indicating the respective dilution factors.

Component	*Actual amount in the sample (µmol/L)*							
	a) Seaweed. enzym. digest. dil 20×	*b) Seaweed. acidic digest. dil 20×*	*c) Chitin. acidic digest. dil 20×*	*d) Pulp. acidic digest. dil 200×*	*e) Paper pulp. acidic digest. dil 200×*	*f) Lignin. acidic digest. dil 20×*	*g) Fiber. acidic digest. dil 100×*	*h) Wood. acidic digest. dil 100×*
Mannitol	27.8	613.2	6.6					
Fucose		908.4				161.6	159	
Rhamnose	6.8	6.2						3.7
Arabinose		6.4	473.4		154	76.4		34.1
Galactose	41.4	94.6	11.4		68	237.4		53.9
Glucose	160.6	475.6	306.6	20292	17460	64.2	6906	1007.4
Mannose		45.4	34.4	428	1480	15.2	486	248.9
Xylose	32.4	255.8		274	1814	153.2	620	176.6
Fructose	34.8		66.2					30.4
Ribose	139.2	6.8						
Sucrose	39.4							
Cellobiose	10.4							9.8
Galacturonic acid								6.2
Guluronic acid	44.6	760.8	204.2					
Glucuronic acid	15.8	29.6						
Mannuronic acid		732						

Table 5
Summary of the recovery data across all carbohydrates detected in the respective sample.

Sample	*Recovery*			
	Min	Max	*Median*	*Average*
a) Seaweed. enzym. digest. dil 20×	62%	106%	98%	95%
b) Seaweed. acidic digest. dil 20×	96%	99%	97%	98%
c) Chitin. acidic digest. dil 20×	92%	99%	97%	96%
d) Pulp. acidic digest. dil 200×	98%	99%	99%	99%
e) Paper pulp. acidic digest. dil 200×	96%	110%	99%	101%
f) Lignin. acidic digest. dil 20×	95%	96%	96%	96%
g) Fiber. acidic digest. dil 100×	97%	106%	98%	100%
h) Wood. acidic digest. dil 100×	88%	97%	95%	95%

column rinsing programs have a cycle time of 11 min. Compared with the reference method, throughput increases by more than 5-fold (>445%; Fig. 3(d), Figure S6).

The data presented in Table 7(c) serve as performance indicators for the high-throughput separation of uronic acids. The method demonstrates excellent repeatability, as reflected in the low RSD values for retention times and peak areas. These values were determined for all investigated uronic acids, galacturonic acid, guluronic acid, glucuronic acid, mannuronic acid, and iduronic acid, as well as for all calibration standards on both separation columns. The RSE, derived from calibration data for both columns, is approximately 2%, with a variability ranging from 0.5% to 5.3% (Table S5). The LODs for all analytes were comparable to those in the reference method (Table S3). The overall recovery rate, accounting for all uronic acids, matrices, and columns, is averaging 97% and has a median of 98%. Recovery was assessed using an enzymatically treated seaweed sample and acid-hydrolyzed shrimp shell, pulp, and wood. In the seaweed sample, additional peaks were observed, which, at the time of manuscript preparation, were attributed to the employed enzyme (Figure S7). This resulted in implausible recoveries for galacturonic and glucuronic acid (ranging from 24% to 90%), which have since been excluded from the report. When such interferences are visible, it is advisable to use acidic hydrolysis or adjust

Table 6
Gradient programs and valve switching times for the high-throughput separation of neutral sugars and uronic acids.

Gradient program (analytical pump)						
Time (min)	Mobile phase	%A (water)	%B (0.2 mol/L NaOH)	%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)	%D (water)	Description
0 – 10	20 mmol/L NaOH	45	10	0	45	Elution of neutral sugars
10 – 14	160 mmol/L NaOH	20	80	0	0	Elution of cellobiose
14 – 24	40 mmol/L NaOH + 140 mmol/L NaOAc	66	6	28	0	Elution of uronic acids
24 – 33	20 mmol/L NaOH	45	10	0	45	Flow path clean-up
32.98						Valve switches positions
Column reconditioning (rinsing pump)						
Time (min)	Mobile phase	%A (water)	%B (0.2 mol/L NaOH)	%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)	%D (water)	Description
0	20 mmol/L NaOH	90	10	0	0	Initial conditions
0.02 – 5	50 mmol/L NaOH + 250 mmol/L NaOAc	50	0	50	0	Column clean-up and equilibration
5 – 18	200 mmol/L NaOH	0	100	0	0	Conversion to hydroxide form
18 – 33	20 mmol/L NaOH	90	10	0	0	Equilibration to starting conditions

Table 7
Summary of analytical data demonstrating the performance of the high-throughput separation of (a) Neutral sugars & uronic acids, (b) Targeted neutral sugars, (c) Targeted uronic acids.

(a)	*RSD t_R*	*RSD Area*	*RSE*	*Recovery*
Min	0.08	0.05	0.7	88%
Max	1.6	5.84	7.5	113%
Median	0.22	0.77	1.3	98%
Average	0.402	1.7	1.8	99%
(b)	*RSD t_R*	*RSD Area*	*RSE*	*Recovery*
Min	0.1	0.1	0.4	95%
Max	0.3	0.6	4.1	118%
Median	0.3	0.2	0.9	99%
Average	0.2	0.2	1.2	100%
(c)	*RSD t_R*	*RSD Area*	*RSE*	*Recovery*
Min	0.1	0.3	0.5	75%
Max	0.2	7.3	5.3	107%
Median	0.2	1.2	1.92	98%
Average	0.2	2.2	2.04	97%

Table 8
Gradient programs and valve switching times for the high-throughput separation of neutral sugars.

Gradient program (analytical pump)					
Time (min)	*Mobile phase*	*%A (water)*	*%B (0.2 mol/L NaOH)*	*%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)*	*Description*
0 – 10	20 mmol/L NaOH	90	10	0	Elution of neutral sugars
10 – 14	160 mmol/L NaOH	20	80	0	Elution of cellobiose
14 – 23	20 mmol/L NaOH	90	10	0	Flow path clean-up
22.98					Valve switches position
Column reconditioning (rinsing pump)					
Time (min)	*Mobile phase*	*%A (water)*	*%B (0.2 mol/L NaOH)*	*%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)*	*Description*
0	20 mmol/L NaOH	90	10	0	Initial conditions
0.02 – 5	50 mmol/L NaOH + 250 mmol/L NaOAc	50	0	50	Column clean-up and equilibration
5 – 10	200 mmol/L NaOH	0	100	0	Conversion to hydroxide form
10 – 23	20 mmol/L NaOH	90	10	0	Equilibration to starting conditions

the chromatographic conditions. Specifically, decreasing the ionic strength of the eluent and increasing the cycle time can help improve chromatographic resolution. These aspects are included in our facility's future development plans. If no components elute after the uronic acids, rinsing is unnecessary. This allows for isocratic elution of the uronic acids, as described in Table 9. The cycle time could be as short as before, yet the instrument configuration given in Fig. 1(a) would suffice.

3.5. Evaluation of detection stability over all experiments

Table 10 displays calibration curve slope values, as an indicator of detector sensitivity [35,36], for all analytes and all the experiments mentioned above. For each analyte, observed slopes are summarized with Max, Min, Average, RSD, and Median. Sensitivity varies among analytes: disaccharides such as cellobiose and certain neutral sugars exhibit the highest slopes, indicating strong responses, whereas uronic acids tend to be less sensitive.

In a uronic acid, the terminal –CH_2OH group of the aldose was oxidized to a carboxyl group, leaving only two of the three more acidic hydroxyl groups of the neutral sugar. Under high-pH conditions, only the remaining two hydroxyl groups are hypothetically deprotonatable, compared with three in neutral sugars [32]. Since only alcoholates are oxidized at the working electrode, this indicates reduced sensitivity toward uronic acids [37]. The high correlation of mean and median values supports the robustness of the slope measurements, indicating minimal influence from outliers. Most analytes have slope RSDs between 1.6% and 3.7%, with ribose showing the highest at approximately 6.3%. The low analyte-specific slope RSDs signify consistent method repeatability across columns and setups.

Table 9
Gradient programs and valve switching times for the high-throughput separation of uronic acids (cycle time = 11 min).

Gradient program (analytical pump)					
Time (min)	*Mobile phase*	*%A (water)*	*%B (0.2 mol/L NaOH)*	*%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)*	*Description*
0 – 11	40 mmol/L NaOH + 140 mmol/L NaOAc	66	6	28	Elution of uronic acids
10.98					Valve switches position
Column reconditioning (rinsing pump)					
Time (min)	*Mobile phase*	*%A (water)*	*%B (0.2 mol/L NaOH)*	*%C (0.5 mol/L NaOAc + 0.1 mol/L NaOH)*	*Description*
0	40 mmol/L NaOH + 140 mmol/L NaOAc	66	6	28	Initial conditions
0.02 – 3	50 mmol/L NaOH + 250 mmol/L NaOAc	50	0	50	Column clean-up and equilibration
3 – 11	40 mmol/L NaOH + 140 mmol/L NaOAc	66	6	28	Equilibration to starting conditions

Overall, the dataset demonstrates consistent, reliable detector responses across the experiments discussed and over the full range of neutral sugars and uronic acids. Ribose and, to a lesser extent, some uronic acids, warrant further optimization to improve consistency or sensitivity based on routine analytical goals. These results suggest that calibration can be transferred from one experiment to another when necessary, requiring only minor adjustments to the method calibration.

4. Discussion

The high-throughput HPAEC–PAD workflow provides a reliable, efficient analytical platform that significantly improves the profiling of neutral sugars and uronic acids in complex biomass hydrolysates. Building on the improvements described earlier, the method consistently demonstrates high chromatographic performance across all modes: neutral sugars and uronic acids in one run, neutral-sugar-targeted, and uronic-acid-targeted. In simultaneous analysis, the dual-pump, dual-column setup cuts the cycle time from 60 to 33 min, resulting in an 82% increase in throughput while maintaining excellent retention-time stability, peak-area repeatability, and calibration quality. The latter is reflected in average RSD below 0.4% for retention times and below 2.2% for peak areas, and low relative standard errors of calibration, confirming the reliability of the quadratic, weighted calibration method.

Mannose and xylose under the separation condition in this study were not fully resolved, with R_s=1.2. The mannose-xylose resolution could be improved further, for example by decreasing hydroxide concentration during the elution [13,38], or decreasing the separation temperature. Uronic acid and cellobiose will not be affected by these changes because they are strongly retained in HPAEC columns and can only be eluted by significantly increased hydroxide/acetate concentration. However, care should be exercised because decreasing hydroxide concentration can result in (1) coelution of other sugar pairs such as arabinose-rhamnose, (2) a decrease of PAD response, and (3) difficulties in reproducing the results due to the precision in manually preparing a low-concentration NaOH mobile phase. Decreasing the separation temperature is directly correlated with an increase in column backpressure. Despite not being fully resolved, quantification of mannose and xylose can be done accurately due to the low RSE values (0.39% for mannose, and 0.49% for xylose, Table 2).

Targeted workflows also highlight the method's flexibility and accuracy. In neutral-sugar mode, switching columns after eluting cellobiose reduces the cycle time to 23 min, nearly tripling throughput without losing analytical reliability. Recovery rates of enzymatically or acid-digested biomass samples, including seaweed, lignin, pulp, and plant fibers, remain close to 100%, highlighting the method's robustness even with challenging samples. Likewise, the uronic-acid method, despite the strong retention of acidic sugars, shortens the cycle time to 11 min, more than five times faster. It also keeps excellent repeatability and calibration accuracy, with average recoveries of 97% and RSE values around 1%. Additionally, the analyte-specific detection sensitivity remains largely unaffected by the degree of automation, the chosen analytical approach, and the samples.

Table 10
Comparison of calibration slopes to assess detector sensitivity across all experiments.

Component	Reference* Neutral sugars & uronic acids	H.T.** Neutral sugars & uronic acids		H.T.** Targeted neutral sugars		H.T.** Targeted uronic acids		Evaluation across all experiments and columns				
	Column 1	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2	Max	Min	Average	RSD	Median
Mannitol	232	227	220	219	214			232	214	222	3%	220
Fucose	209	200	200	196	195			209	195	200	2%	200
Rhamnose	159	146	146	142	143			159	142	147	4%	146
Arabinose	186	178	178	172	172			186	172	177	3%	178
Galactose	223	213	215	204	203			223	203	212	3%	213
Glucose	221	215	218	208	205			221	205	213	3%	215
Mannose	201	192	194	192	194			201	192	195	2%	194
Xylose	219	212	214	205	199			219	199	210	3%	212
Fructose	176	172	176	169	165			176	165	171	3%	172
Ribose	126	123	124	110	109			126	109	118	6%	123
Sucrose	216	204	209	196	197			216	196	204	4%	204
Cellobiose	283	273	272	273	270			283	270	274	2%	273
Galacturonic acid	84	80	79			81	76	84	76	80	3%	80
Guluronic acid	40	41	35			41	38	41	35	39	5%	40
Glucuronic acid	124	113	117			117	106	124	106	115	5%	117
Mannuronic acid	97	93	90			98	85	98	85	93	5%	93
Iduronic acid	136	141	134			146	134	146	134	138	4%	136

*) Setup Fig. 1A; **) Setup Fig. 1B; H.T.: High Throughput.

As mentioned in the method section above, adaptation of this method preferably requires two HPAEC columns with similar selectivity and the same production batch. If, for instance, the two columns have different selectivity, individual method for each column could be applied. If this is the case, the valve switching and total cycle time are determined by the longest cycle time out of the two columns.

Finally, these results show that the combined chromatographic and hardware approach accelerates analysis while ensuring data reliability and operational efficiency. The method works well for a wide range of targets without needing major changes, making it a useful tool for high-volume biomass screening, biopolymer research, and process development. Its consistent results across different sample types and analytical modes demonstrate the method's suitability for adoption in laboratories that require both high throughput and data accuracy.

5. Conclusion

A high-throughput workflow with a dual-pump, dual-column HPAEC–PAD setup has been developed for precise analysis of neutral sugars and uronic acids in complex biomass hydrolysate samples. Parallel analysis and column reconditioning steps shortened the cycle time from 60 min to 33 min for the combined separation of neutral sugars and uronic acids, and to 23 and 11 min for the targeted analysis of neutral sugars and uronic acids, respectively. This corresponds to the throughput gains of 82%, 160%, and 445% relative to the single-column reference method. The cycle time reduction did not compromise the analytical performance. Instead, the method maintains the high resolution separation of 12 neutral sugars and 5 uronic acids, with stable retention times and consistent peak areas. The calibration models were evaluated in accordance with IUPAC guidelines, giving average RSE values below 2.3% for all analytes. The sensitivity of the method is evident from the detection limits between 0.02 to 0.40 μmol/L.

The method presented in this study solves key issues of traditional workflows, especially the long reconditioning times that limit lab productivity. The successful recoveries (averaging close to 100%) across various biomass hydrolysates confirm the method's suitability for analyzing complex samples and underscore its potential in biopolymer research, biomass process improvement, and large-scale screening required for advancements in enzyme engineering, biopolymer research, and process optimization. These findings show that this high-throughput HPAEC–PAD method is an efficient, reliable, and versatile method for analyzing biomass-derived hydrolysate samples.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit organizations. Antec Scientific is the manufacturer of the electrochemical detectors and columns used in this research.

CRediT authorship contribution statement

Christian Marvelous: Writing – review & editing, Visualization, Validation, Investigation, Formal analysis. **Hendrik-Jan Brouwer:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Martin Eysberg:** Funding acquisition. **Jean-Pierre Chervet:** Writing – review & editing, Funding acquisition. **Detlef Jensen:** Writing – original draft, Validation, Methodology, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationship which may be considered as potential competing interests: All authors are employees of Antec Scientific. M.E. and J.P.C. are board members of Antec Scientific.

Acknowledgement

The authors thank Mr. Younes Tazini for providing the columns used for this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2026.467338.

Data availability

Data will be made available on request.

References

[1] W.A. Akbar, H.U. Rahim, F.A. Rutigliano, Microbial- and seaweed-based biopolymers: sources, extractions and implications for soil quality improvement and environmental sustainability - a review, J. Environ. Manag. 359 (2024) 120964, https://doi.org/10.1016/j.jenvman.2024.120964.
[2] R.S. Baghel, P. Suthar, T.K. Gajaria, S. Bhattacharya, A. Anil, C.R.K. Reddy, Seaweed biorefinery: a sustainable process for valorising the biomass of brown seaweed, J. Clean. Prod. 263 (2020) 121359, https://doi.org/10.1016/j.jclepro.2020.121359.
[3] S. Lomartire, A.M.M. Gonçalves, Novel technologies for seaweed polysaccharides extraction and their use in food with therapeutically applications—A review, Foods 11 (2022) 2654, https://doi.org/10.3390/foods11172654.
[4] I. Santana, M. Felix, C. Bengoechea, Seaweed as basis of Eco-sustainable Plastic materials: focus on Alginate, Polymers 16 (2024) 1662, https://doi.org/10.3390/polym16121662.
[5] M. Herrero, A. Cifuentes, E. Ibáñez, M.D. del Castillo, Advanced analysis of carbohydrates in foods. Methods of Analysis of Food Components and Additives, CRC Press, 2012. https://digital.csic.es/handle/10261/109484. accessed November 22, 2023.
[6] Z. Zhang, N.M. Khan, K.M. Nunez, E.K. Chess, C.M. Szabo, Complete monosaccharide analysis by high-performance anion-exchange chromatography with pulsed amperometric detection, Anal. Chem. 84 (2012) 4104–4110, https://doi.org/10.1021/ac300176z.
[7] R.D. Rocklin, C.A. Pohl, Determination of carbohydrates by anion exchange chromatography with pulsed amperometric detection, J. Liq. Chromatogr. 6 (1983) 1577–1590, https://doi.org/10.1080/01483918308064876.
[8] L. Bhattacharya, J.S. Rohrer, Glycan analysis by high performance anion exchange chromatography with pulsed amperometric detection. Applications of Ion Chromatography For Pharmaceutical and Biological Products, 1 edition, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012, pp. 323–338, https://doi.org/10.1002/9781118147009.
[9] Y.C. Lee, High-performance anion-exchange chromatography for carbohydrate analysis, Anal. Biochem. 189 (1990) 151–162, https://doi.org/10.1016/0003-2697(90)90099-u.
[10] C. Marvelous, D. Vetter, H.-J. Brouwer, M. Eysberg, N.J. Reinhoud, J.-P. Chervet, Separation of all classes of carbohydrates by HPAEC-PAD, LCGC Int. 1 (2024), https://doi.org/10.56530/lcgc.int.es8884l6.
[11] C. Corradini, A. Cavazza, C. Bignardi, High-performance anion-exchange chromatography coupled with pulsed electrochemical detection as a powerful tool to evaluate carbohydrates of food interest: principles and applications, Int. J. Carbohydr. Chem. 2012 (2012) 1–13, https://doi.org/10.1155/2012/487564.
[12] D.W. Templeton, M. Quinn, S. Van Wychen, D. Hyman, L.M.L. Laurens, Separation and quantification of microalgal carbohydrates, J. Chromatogr. A 1270 (2012) 225–234, https://doi.org/10.1016/j.chroma.2012.10.034.
[13] A. Nagel, S. Sirisakulwat, R. Carle, S. Neidhart, An acetate–Hydroxide gradient for the quantitation of the neutral sugar and uronic acid profile of pectins by HPAEC-PAD without postcolumn pH adjustment, J. Agric. Food Chem. 62 (2014) 2037–2048, https://doi.org/10.1021/jf404626d.
[14] Z. Zhang, N.M. Khan, K.M. Nunez, E.K. Chess, C.M. Szabo, Complete monosaccharide analysis by high-performance anion-exchange chromatography with pulsed amperometric detection, Anal. Chem. 84 (2012) 4104–4110, https://doi.org/10.1021/ac300176z.
[15] L. Cao, J. Wu, X. Li, L. Zheng, M. Wu, P. Liu, Q. Huang, Validated HPAEC-PAD method for the determination of fully deacetylated chitooligosaccharides, IJMS 17 (2016) 1699, https://doi.org/10.3390/ijms17101699.
[16] J. Lu, H. Yang, J. Hao, C. Wu, L. Liu, N. Xu, R.J. Linhardt, Z. Zhang, Impact of hydrolysis conditions on the detection of mannuronic to guluronic acid ratio in alginate and its derivatives, Carbohydr. Polym. 122 (2015) 180–188, https://doi.org/10.1016/j.carbpol.2015.01.008.
[17] D. Manns, A.L. Deutschle, B. Saake, A.S. Meyer, Methodology for quantitative determination of the carbohydrate composition of brown seaweeds (Laminariaceae), RSC. Adv. 4 (2014) 25736–25746, https://doi.org/10.1039/C4RA03537B.
[18] L. Basumallick, Determination of Uronic Acids and Wood Sugars in Wood-Based Hydrolysates, Thermo Fisher Scientific Application Note, 2015, pp. 1–6.

[19] H.J. Brouwer, J. van Schaik, C. Marvelous, J.P. Chervet, D. Vetter, M. Eysberg, Novel Stationary Phase for High-Resolution Separation Of Carbohydrates in Biomass Hydrolyzates, 2025. https://antecscientific.com/wp-content/mu-plugins/antec-downloads/files/posters/food/221_276_01%20-%20Novel%20stationary%20phase%20for%20high-resolution%20separation%20of%20carbohydrates%20in%20biomass%20hydrolysates%20-%20EPNOE%202025.pdf. accessed December 11, 2025.
[20] C. Marvelous, T. Mulder, J.-P. Chervet, N.J. Reinhoud, H.-J. Brouwer, Evaluation of a novel stationary phase for the separation of lactose and isomers in lactose-free products using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), J. Chromatogr. A 1716 (2024) 464661, https://doi.org/10.1016/j.chroma.2024.464661.
[21] W.R. LaCourse, Pulsed Electrochemical Detection in High Performance Liquid Chromatography, Wiley, New York, 1997. https://www.wiley.com/en-gb/Pulsed+Electrochemical+Detection+in+High-Performance+Liquid+Chromatography+-p-9780471119142.
[22] S.M. Haslam, D.I. Freedberg, B. Mulloy, A. Dell, P. Stanley, J.H. Prestegard, A. Varki, R.D. Cummings, J.D. Esko, P. Stanley, G.W. Hart, M. Aebi, D. Mohnen, T. Kinoshita, N.H. Packer, R.L. Schnaar, P.H. Seeberger, Structural analysis of glycans. Essentials of Glycobiology, 4th Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), 2022, https://doi.org/10.1101/glycobiology.4e.50.
[23] S. Ricci, B. Fabbri, S. Giannini, S. Bernardini, M. Morelli, S. D'Ascenzi, High performance anion-Exchange Chromatography with pulsed amperometric detection (HPAEC-PAD) analysis of GBS glycoconjugate vaccines: from fermentation to final product. Applications of Ion Chromatography for Pharmaceutical and Biological Products, 1 Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012, https://doi.org/10.1002/9781118147009.ch23.
[24] U.S. EPA, 40 CFR 136 - Guidelines establishing Test Procedures for the Analysis of Pollutants, 2023. https://www.govinfo.gov/content/pkg/CFR-2023-title40-vol25/pdf/CFR-2023-title40-vol25-part136.pdf. accessed August 5, 2024.
[25] R. Burrows, J. Parr, Evaluating the goodness of instrument calibration for chromatography procedures, LCGC Supplements 38 (2020) 38. –38.
[26] D.A. Edgerley, Techniques for Improving the Accuracy Of Calibration in the Environmental Laboratory, in: n.d.: pp. 181–187. https://clu-in.org/download/char/dataquality/aedgerley.pdf.
[27] K. Danzer, L.A. Currie, Guidelines for calibration in analytical chemistry. Part I. Fundamentals and single component calibration (IUPAC Recommendations 1998), Pure & Appl. Chem. 70 (1998) 993–1014, https://doi.org/10.1351/pac199870040993.
[28] Antec Scientific, Technical Note: Introduction to HPAEC-PAD – Eluent Preparation, 2025. https://antecscientific.com/wp-content/mu-plugins/antec-downloads/files/apps/tech/220_045_01%20-%20Eluent%20preparation%20in%20HPAEC-PAD.pdf. accessed March 5, 2026.
[29] Antec Scientific, Application Note: Uronic acids & Neutral Sugars in Biomass Hydrolyzates, 2025. https://antecscientific.com/wp-content/mu-plugins/antec-downloads/files/apps/carb/plants/220_037_05%20-%20Neutral%20sugars%20&%20uronic%20acids%20in%20biomass%20hydrolyzates.pdf. accessed May 14, 2026.
[30] H.R. Louw, H.-J. Brouwer, N.J. Reinhoud, Electrochemical Flow cell, US9310330B2, 2016. https://patents.google.com/patent/US9310330B2/en.
[31] The Nelac Institute, RSE Calculation Spreadsheet, 2017. https://nelac-institute.org/docs/comm/emmec/Basic%20RSE%20calculatorv4.xlsx. accessed May 14, 2026.
[32] J.A. Rendleman, Ionization of carbohydrates in the presence of metal hydroxides and oxides, in: carbohydrates in solution, Adv. Chem. (1973) 51–69.
[33] L. Basumallick, J. Rohrer, Determination of Carbohydrates in Acid Hydrolysates of Wood, Thermo Fisher Scientific Application Note, 2014, pp. 1–7.
[34] Ed. G. Jander, K.F. Jahr, G. Schulze, J. Simon, in: J. Simon, Berlin, Walter de Gruyter (Eds.), Theorie Und Praxis der Titrationen mit Chemischen Und Physikalischen Indikationen, 16 ed, 16th Edition, Walter de Gruyter GmbH, Berlin, 2003, p. 2003. Ed.
[35] L.S. Ettre, Nomenclature for chromatography (IUPAC Recommendation 1993), Pure Appl. Chem. 65 (1993) 819–872.
[36] A.D. McNaught, A. Wilkinson, IUPAC, Compendium of Chemical Terminology (the "Gold Book"), 2nd Edition, Blackwell Scientific Publications, Oxford, 1997.
[37] W.R. LaCourse, Pulsed electrochemical detection in ion chromatography. Applications of Ion Chromatography for Pharmaceutical and Biological Products, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012, pp. 49–90.
[38] T. Yeats, T. Vellosillo, N. Sorek, A. Ibáñez, S. Bauer, Rapid determination of cellulose, neutral sugars, and uronic acids from plant cell walls by one-step two-step hydrolysis and HPAEC-PAD, Bio Protoc. 6 (2016), https://doi.org/10.21769/BioProtoc.1978.