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Ion-Pair RP-HPLC Determination of Sugars, Amino Sugars, and Uronic Acids after Derivatization with *p*-Aminobenzoic Acid

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A new, selective, and sensitive ion-pair RP-HPLC method for the simultaneous determination of three classes of natural organic compounds, i.e., carbohydrates, amino sugars, and uronic acids, in environmental samples is presented. *p*-Aminobenzoic acid is used for precolumn derivatization of the analytes, enabling fluorescence (λ_{ex} 313 nm, λ_{em} 358 nm) or photometric detection (303 nm). The dependence of the derivatization yield on the reaction conditions is examined. Derivatives of lactose, galactose, glucose, mannose, xylose, arabinose, galacturonic acid, glucuronic acid, *N*-acetylglucosamine, and glycerinealdehyde were separated on a RP-C_{18} column with hydrophilic end capping within 35 min, applying $TBAHSO_4$ as the ion-pair reagent. The concentration detection limits range between 20 and 30 $\mu g\ L^{-1}$ ($(1-2) \times 10^{-7}$ M) for fluorescence detection and between 30 and 75 $\mu g\ L^{-1}$ for UV detection. A good linearity is achieved in the concentration range from 50 $\mu g\ L^{-1}$ to 100 mg L^{-1} ($r^2 > 0.99$). The described method has been applied for the determination of mono-/disaccharides, uronic acids, and amino sugars in soil solutions and in landfill leachates.

Knowledge of the chemical composition of the dissolved organic carbon (DOC) content of soil solutions is essential for an understanding of biogeochemical soil processes. The low-molecular-weight DOC fraction is composed of many naturally occurring compounds such as sugars, uronic acids, amino acids, carboxylic acids, ketones, aldehydes, and phenols. These substances are ubiquitous in the environment and generated during a multitude of biological processes and biogeochemical reactions.[1−3] Furthermore, the composition and the concentration of the DOC fraction of soil solutions is influenced by environmental factors, such as climate, soil mineralogy, and soil chemistry. It is supposed that anthropogenic pollutants affect the soil microfauna and -flora. As a consequence, the concentration patterns of sugars, amino sugars, and uronic acids change. Possibly the sensitive long-term detection of these biological compounds might provide some information about responses of soil microbial communities to chemical stress.

Due to the lack of suitable analytical tools and methods, earlier studies dealing with the DOC composition of soil solutions were based on the measurement of sum parameters or on the characterization of functional groups and molecular sizes of the prevailing DOC fractions.[4] In recent years, growing interest in the study of environmental processes has led to efforts to develop efficient and sensitive HPLC and GC methods for the determination of naturally occurring hydrophilic compounds. For instance, separation of sugars, amino sugars, and uronic acids can be achieved by anion exchange chromatography (HPAEC). However, to our best knowledge, only one attempt was made to determine simultaneously the three analyte groups of interest by HPAEC.[5] Carbohydrates have neither chromophores nor fluorophores, ruling out direct photometric or fluorometric detection. Refractive index detection is the method of choice, but this technique is not very sensitive and unsuited for gradient elutions. Pulsed amperometric detection (PAD) is a common method for the quantification of saccharides, amino sugars, and uronic acids applying HPAEC with gradient elution. Determination limits are in the range of 100−500 $\mu g\ L^{-1}$. Recently, capillary electrophoresis (CE) in combination with PAD has shown promise as a new tool for this analytical task because of rapid and high resolution of complex matrixes.[6] Since the concentrations of the target substances in environmental samples are assumed to be in the low-ppb range, the PAD sensitivity does not meet the analytical demands. The lowest concentration detection limits reported for the direct determination of monosaccharides (uronic acids and amino sugars not addressed) by CE-PAD were at the 10^{-6} M level, but the separation efficiency decreased with time due to side reactions.[7] Higher detection sensitivities are attainable by labeling of the analytes with fluorometric or chromophoric tags.

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A number of photometric and fluorometric tags have been developed for pre- and postcolumn analysis by HPLC[8−10] and CE.[7,11] The advantages of precolumn derivatization are the modification or removal of the sample matrix and the selective enrichment of the derivatives. Several reagents have been developed for precolumn tagging of carbohydrates, amino sugars, and uronic acids.[12−18] A popular reagent for this purpose is 1-phenyl-3-methyl-5-pyrazolone (PMP).[17] The derivatization procedure comprises several steps including phase-transfer reactions and phase separation and seems therefore to be unsuited for automation.

Labeling by reductive amination using a combination of aromatic amines and reducing reagents is conducted most frequently.[17,19,20] Many reagents classified as monosubstituted aminobenzene derivatives (2-aminobenzamide,[21,22] 4-aminobenzoic acid esters,[23,24] 4-aminobenzonitrile,[25,26] 2-aminobenzoic acid,[27] 3-aminobenzoic acid,[28] 4-aminobenzoic acid[29]) were successfully applied for the formation of derivatives of mono- and oligosaccharides, suited for CE or HPLC analysis. Primarily these derivatization reagents are used in the bioanalytical research for determination of carbohydrates after hydrolyzation of oligo- and polysaccharides and glycoproteins. So far as we know, no attempt has been made to utilize these reagents in environmental analysis.

The newly developed procedure provides for the first time an HPLC method for the simultaneous determination of trace concentrations of carbohydrates, amino sugars, and uronic acids in environmental samples. Additionally, it is the first trace analytical HPLC method using *p*-aminobenzoic acid (*p*-AMBA) for this purpose. In contrast to other analytical procedures, i.e., derivatization with PMP, a complete automation of the analytical process is possible. The performance of the finally elaborated analytical procedure is assessed by applying "real world" samples.

EXPERIMENTAL SECTION

Chemicals and Solvents. The carbohydrates and uronic acids, D(+)-lactose monohydrate (Lac), D(+)-xylose (Xyl), D,L-glycerinaldehyde (GA), *N*-acetyl-D-glucosamine (GlcNAc), D(+)-glucose (Glc), D(+)-galactose (Gal), D-glucuronic acid (GlcUA), D(+)-galacturonic acid (GalUA), D(+)-mannose (Man), D(−)-ribose (Rib), L(+)-arabinose (Ara), and D(+)-maltose monohydrate (Mal) were obtained from Fluka. The amino sugars D(+)-glucosamine (GlcN) and D(+)-galactosamine (GalN) were purchased from Sigma and served together with the carbohydrates and uronic acids as reference compounds. The other chemicals, *p*-aminobenzoic acid (Fluka) and sodium cyanoborohydride (Merck) including the ion-pair reagents tetrabutylammonium hydrogen sulfate ($TBAHSO_4$), tetramethylammonium bromide (TMABr), tetraethylammonium bromide (TEABr), tetrapentylammonium iodide (TPAJ), and hexadecyltrimethylammonium bromide (HTMABr) (all from Fluka), were of analytical grade. Solvents (methanol, acetonitrile) were of HPLC quality. Water was purified by inverse osmosis and then passed through a Millipore Milli-Q unit.

Stock Solutions. Stock solutions of the reference compounds were prepared by weighing of 50 mg of each in a 10-mL volumetric flask followed by dissolution in water. These solutions were stored at −20 °C and used for further dilutions.

Precolumn Derivatization. Reductive amination of the analytes with *p*-aminobenzoic acid (0.35 M) was carried out in a DMSO/acetic acid solution (70:30 v/v). Before usage, the reagent solution was freshly prepared each time. A 200-μL aliquot of the standard solution, 500 μL of the *p*-AMBA solution, and 10 mg of sodium cyanoborohydride were mixed in a 2-mL polyethylene vial. The vials were tightly capped and heated for 15 min at 60 °C. After that the vials were cooled with water and the reaction mixture was dissolved in 6.3 mL of the HPLC eluent.

Apparatus. Chromatographic separations were conducted on a Shimadzu HPLC system consisting of an autosampler SIL 10 A, a controller SCL-10 AVP, a gradient pump LC-10 ADVP, an UV detector SPD-10 AVP (detection wavelength 303 nm), and a fluorescence detector SPD-10 AXL (λ_{ex} 313 nm; λ_{em} 358 nm). Data acquisition and processing were accomplished with the Shimadzu CLASS VP 5.03 software.

The HPLC method development was conducted by applying the following separation columns, packed with C_{18}-RP resins (except d): (a) Nucleosil 100-5C_{18}; 250 × 3.5 mm (Machery & Nagel); (b) HyPurity Elite 5 C_{18}; 150 × 4 mm (Hypersil), (c) AQUA 5 $C_{18;}$ 250 × 3 mm (Phenomex), and (d) anion exchange column AS4A-SC, 250 × 4 mm (Dionex). If not specified otherwise, the column temperature was kept at 22 °C by a Jetstream-Plus column oven (VDS Optilab). Methanol or acetonitrile phosphate buffer mixtures, containing various ion-pair reagents, served as eluents for the RP separations. The pH values of the eluents were adjusted with orthophosphoric acid (85%) and with various phosphate buffers. Separations on the anion exchange column were carried out with various concentration NaOH eluents. All mobile phases were degassed with an on-line degasser GT 104 (Shimadzu) and filtered through a 0.45-μm PTFE filter prior to use. Injection

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volumes varied between 20 and 50 μL, and the flow rates of the eluents ranged from 0.5 to 1.0 mL min^{-1}.

Preparation of Samples. Several soil solutions (seepages from various lysimeters filled with sewage sludge, sand, and gravel) and some leachates from the sanitary landfill of Trier were collected. The samples were filtered through a 0.45-μm PTFE filter, and an aliquot (2 mL) of each sample was derivatized after addition of 5 mL of the derivatization solution and of 60 mg of sodium cyanoborohydride.

RESULTS AND DISCUSSION

Optimization of Precolumn Derivatization. The optimization of the reaction parameters, i.e., time, temperature, *p*-aminobenzoic acid concentration, and pH, was elaborated applying the Nucleosil RP-C_{18} column in combination with a $TBAHSO_4$ containing eluent (phosphate buffered, pH 2.1). The detection mode was UV photometry. The optimization process aimed for a high reaction yield, a short reaction time, and automation of the procedure. The various effects were examined by using selected combinations of analytes for different chromatographic settings because a separation of all derivatized compounds was not possible under initial chromatographic conditions. Some aspects of the derivatization of glucose, fructose, and glucuronic acid were already described.[29] Nevertheless, the practical implications of this investigation are very limited for our analytical task, since the reaction conditions had to be optimized for the common determination of compounds with different structural features.

The effect of the concentration of *p*-aminobenzoic acid on the reaction yield at a temperature of 95 °C is shown in Figure 1A. Generally a high excess of the reagent is needed to accomplish a significant reaction yield, indicated by the areas of the analyte peaks. Except for D(+)-glucosamine and *N*-acetyl-D-glucosamine, maximal detection sensitivities were nearly reached with a *p*-AMBA concentration of 0.1 M. Variation of the concentration of the acetic acid solution, which is applied in a mixture with DMSO to solve the derivatization reagent, did not affect the reaction yield. The substitution of acetic acid by hydrochloric acid or by sulfuric acid had no significant effect on the peak area. For further method development, the concentrations of *p*-aminobenzoic acid and acetic acid were fixed at 0.35 M and 100%, respectively. The amount of sodium cyanoborohydride added was adjusted to 10 mg because that proved to be sufficiently high.

Figure 1B shows the effect of the reaction time on the size of the detection signals, which is related to the degree of completeness of the derivatization reaction. At reaction times of 10 min and shorter, the conversion of galactosamine and *N*-acetylglucosamine was low. The peak areas of the amino sugars increased with increasing time and reached their maximal sizes at ~30 min. On the other hand, the yield of uronic acid derivatives was highest at short reaction times and decreased almost linearly over a period of 45 min. A time span of 10−30 min. proved to be optimal for the conversion of the carbohydrates.

The time dependence of the derivatization was tested at lower reaction temperatures (70, 60, and 30 °C) also. As expected, the time required for the maximal transformation of the analytes increased with decreasing temperature. At 30 °C, the derivatization of the uronic acids proceeded within 100 min.

The impact of the derivatization temperature on the reaction progress at a fixed time of 15 min is illustrated in Figure 1C.

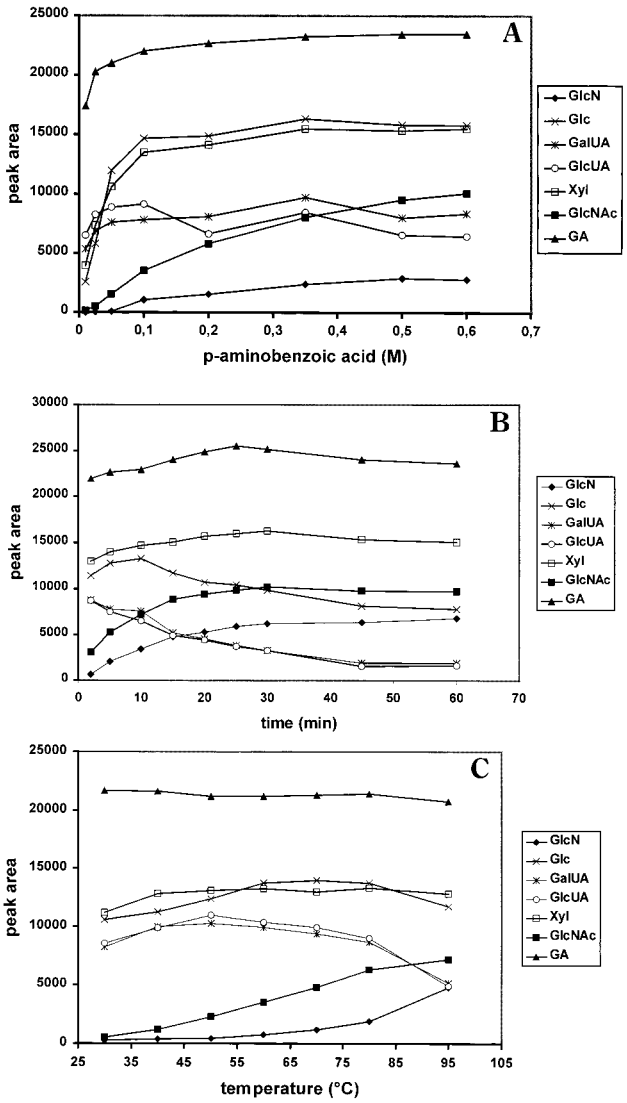

Figure 1. Optimization of reaction conditions for precolumn labeling with *p*-aminobenzoic acid. Chromatographic conditions: Nucleosil 100 $5C_{18}$; 95% $TBAHSO_4$, pH 2.1 (phosphate buffer)/5% methanol; flow, 0.8 mL min^{-1}; UV detection, 303 nm. (A) Effect of *p*-aminobenzoic acid concentration on the peak area (transformation degree) of the derivatives. Reaction conditions: temperature, 95 °C; time, 15 min; carbohydrate concentration 7.5 mg L^{-1} (GlcN, 47.5 mg L^{-1}). (B) Effect of reaction time on the peak area (transformation degree) of the derivatives. Reaction conditions: temperature, 95 °C; *p*-aminobenzoic acid concentration, 0.35 M; carbohydrate concentration, 7.5 mg L^{-1} (GlcN, 47.5 mg L^{-1}). (C) Effect of temperature on the peak area (transformation degree) of the derivatives. Reaction conditions: *p*-aminobenzoic acid concentration, 0.35 M; time 15 min; carbohydrate concentration, 7.5 mg L^{-1} (GlcN, 47.5 mg L^{-1})

Glycerinaldehyde, later used as internal standard, reacted independently from the temperature within the tested range from 30 to 95 °C. The conversion of the amino sugars (GalN not shown) slightly increased with increasing temperature and was highest at 95 °C. The transformation of the other analytes was optimal in the range of 50−80 °C. In principle, shorter reaction times gave similar results, differing in the overall lower degree of reaction completeness.

On the basis of these investigations, a derivatization time of 15 min and a reaction temperature of 60 °C were chosen as standard conditions. All selected parameters are compromises with

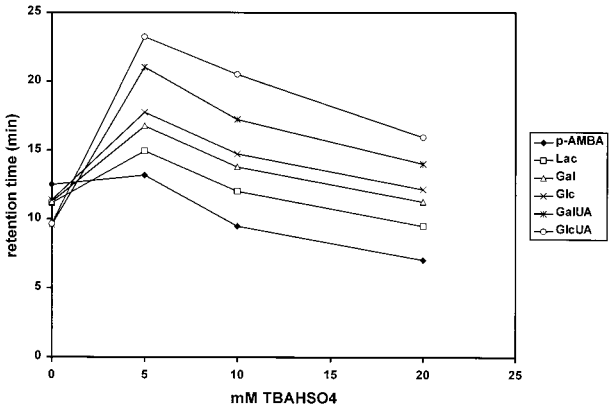

Figure 2. Effect of the eluent component $TBAHSO_4$ on the separation of the derivatized carbohydrates. Derivatization conditions: time, 15 min; temperature, 60 °C; 0.35 M *p*-AMBA; carbohydrate concentrations, 7.5 mg L^{-1}. Chromatographic conditions: see Figure 1.

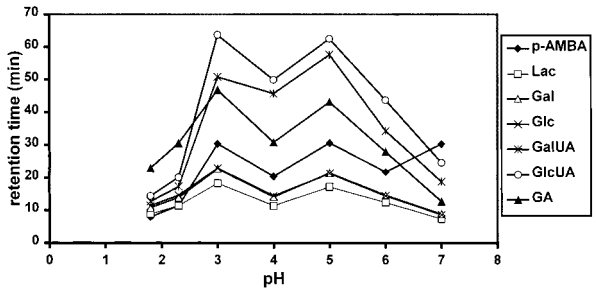

Figure 3. Influence of the eluent pH on retention of the derivatized reference compounds. Derivatization and chromatographic conditions: see Figure 2 (except for pH).

regard to the simultaneous determination of analytes belonging to different structural types. Due to the low detection sensitivity and to poor separation from the reagent peak, glucosamine was excluded from the subsequent method development.

A sensitive determination of fructose was not possible under the described conditions. The detection limit was ~1000-fold higher than the other analytes.

In contrast to other derivatization reagents, e.g., PMP, benzoylhydrazine, 2,4-dinitrophenylhydrazine, etc., the application of *p*-AMBA is marked by (i) an uncomplicated and relatively fast single-step derivatization procedure, (ii) a low impact of reagent excess on subsequent chromatographic separation under developed separation conditions, and (iii) the possibility to integrate the derivatization reaction in a sample pretreatment routine of an autosampler.

Separation of *p*-Aminobenzoic Acid Derivatives. Because of the coelution of some carbohydrates and of both uronic acids, an isocratic separation of the combined analytes with various methanol/water eluents was not possible on the Nucleosil column. Variations of the eluent pH between 2.0 and 7.0 and changes of the composition and concentration of the buffer added did not improve the resolution.

Depending on the eluent pH, the carboxylic group of the derivatized analytes is more or less dissociated and even in strong alkaline medium the carbohydrate unit of the derivatives is negatively charged so that anion exchange chromatography may be feasible under certain conditions. Various solvent mixtures and gradient profiles were applied to obtain a sufficient resolution on the AS4A-SC column. A separation of the sugars and of the uronic acids into two coarse fractions was possible. The amino sugars coeluted with the derivatization reagent. Efforts to resolve the two coarse fractions failed.

As shown in Figure 2, the application of the ion-pair reagent $TBAHSO_4$ in combination with the Nucleosil RP-C_{18} column and the methanol/water (phosphate-buffered) eluent marks a significant improvement of the separation conditions. The other ion-pair reagents TMABr, TEABr, TPAJ, and HTMABr influenced the retention of the analytes but they did not favor their separation. The retention time of the *p*-aminobenzoic acid peak did not change considerably after addition of $TBAHSO_4$. This ion-pair reagent affected an retention time increase of the derivatized compounds, in particular of the uronic acid derivatives. Applying $TBAHSO_4$ concentrations higher than 5.0 mM, the retention of all compounds slightly decreased and the resolution was enhanced.

For the determination of trace concentrations of the analytes, a significant difference in the retention of the derivatization agent, which remains in high excess after the reaction is finished, and of the analytes is necessary. With regard to this aspect and to an efficient resolution of all peaks, the pH of the mobile phase, containing 20 mmol L^{-1} $TBAHSO_4$, was varied between 1.8 and 7.0. As Figure 3 reveals, conditions facilitating an appropriate separation of the derivatization agent from the analytes could not be found. Presumably due to the pH-dependent formation of cationic, neutral (zwitterionic), and anionic analyte species, the retention times changed irregularly with pH. For a limited pH range, a certain correlation between the isoelectric points of the analytes and their retention times seem to exist. The pk_a values of *p*-AMBA are 2.41 and 4.85.[30] The pk_{a2} values of the N-alkylated derivatives (secondary amines), e.g., *N*-methyl-*p*-AMBA (pk_{a2}, 5.05) are about 0.2−0.3 unit higher than that of the primary amine *p*-AMBA. As a consequence, the isoelectric points of *p*-AMBA and of its derivatives are in the pH range between 3.65 and 3.80, corresponding to the noticed retention time minimum of the ion pairs at about pH 4.0. The decrease of the retention times at pH 6.0 and higher may be traced back to the fact that the eluent contained a higher amount of phosphate buffer. Furthermore, at near-neutral pH, the concentration of the $[HPO_4^{2-}]$ species sharply increases. This species competes for association with the ion-pair reagent.

Minimal resolution of most of the analytes, except glucose and galactose, was obtained at the lowest pH value tested. Nevertheless, only under this condition did *p*-aminobenzoic acid elute before the analytes. Furthermore, the separation of the derivatives of glucose and galactose under isocratic conditions required a pH below 3.0.

Reflecting these results, a linear methanol A/water (b, including 20 mmol L^{-1} $TBAHSO_4$, pH 2.0) gradient was applied starting with 0% A and ending up with 50% A after 60 min (reequilibrium time included).

Despite this new approach, the separation was not satisfying. Coelution of lactose with *p*-aminobenzoic acid and of *N*-acetylglucosamine with galacturonic acid occurred and the resolution of most of the other analytes was incomplete (Figure 4A).

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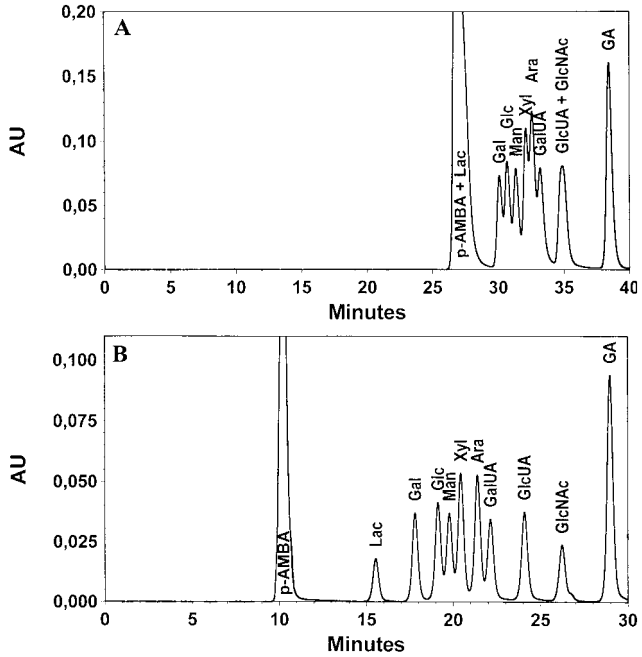

Figure 4. Separation of a standard of reference compounds (5 mg L^{-1} each) on the Nucleosil (A) and the AQUA column (B). Chromatographic conditions: gradient profile, see text; flow rate, 0.8 (A) and 0.5 mL min^{-1} (B); fluorescence detection.

Table 1. Retention Times and Resolution of the Reference Compounds on Different HPLC Columns[a]

	resolution			retention time (min)		
column	Nucleosil	HyPurity	AQUA	Nucleosil	HyPurity	AQUA
Lac		1.73	7.47	26.5	12.0	14.5
Gal	2.50	3.16	3.6	29.1	14.7	16.8
Glc	0.68	1.32	2.07	29.7	15.8	18.1
Man	0.74	1.27	0.98	30.3	16.8	18.8
Xyl	0.79		0.97	31.1	16.8	19.4
Ara	0.45	1.34	1.47	31.6	17.8	20.4
GalUA	0.55	1.43	1.10	32.2	18.9	21.1
GlcUA	1.54	1.49	2.85	33.8	20.3	23.1
GlcNAc			3.18	33.9	20.4	25.2
GA	3.77	2.29	4.12	37.4	23.0	28.0

[a]Conditions: gradient elution; pH 2.0; 20 mm $TBAHSO_4$; flow rate, 0.5 (AQUA) and 0.8 mL min^{-1} (Nucleosil and HyPurity).

Therefore, two C_{18} columns (HyPurity Elite and AQUA), recommended for an improved separation of hydrophilic, polar compounds, were included in the method development. These columns are characterized by a reduced silanophilic activity, very low metal contamination, resulting from the manufacturing of a specially purified silica gel (HyPurity Elite), and a hydrophilic end capping (AQUA). The latter property offers the choice of mobile phases consisting of high portions of water or exclusively of water.

A comparison of the resolution and the retention of the analytes and of the internal standard GA on three RP columns is presented in Table 1. Figure 4B displays the separation of the reference compounds on the AQUA column with a gradient optimized with the DryLab software. The AQUA column facilitates an early elution of *p*-AMBA, a high resolution of *p*-AMBA and lactose, and a complete separation of glucuronic acid and *N*-acetylglucosamine. Compared with a conventional RP-C_{18} column (Nucleosil), the separation on AQUA is faster.

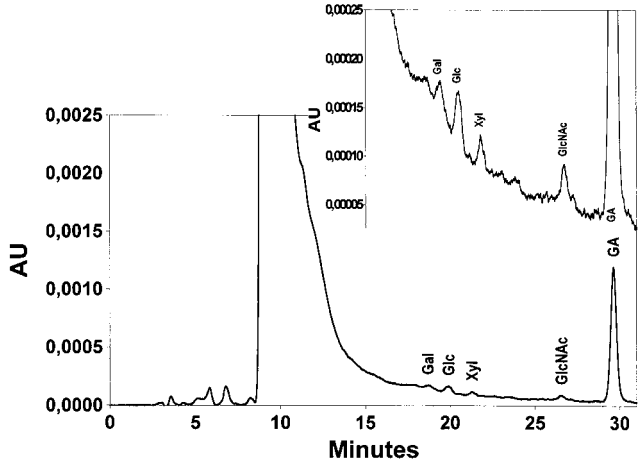

Figure 5. Chromatographic determination of carbohydrates in a soil solution after derivatization with *p*-aminobenzoic acid. Derivatization and chromatographic conditions: see Figure 4B.

As Figure 4B and Table 1 reveal, half of the components are not baseline separated. A further improvement of the resolution is possible but time-consuming, since a reduction of the eluent flow is required for the adjustment of a less steep gradient profile. As Figure 5 illustrates, the achieved resolution is adequate for the chosen practical application.

The temperature dependence of the retention of the reference compounds on the AQUA column was also tested. When the column temperature was elevated, the retention of the analytes decreased more than the retention of the derivatization reagent, resulting in a loss of resolution. At 70 °C, the lactose peak eluted before the *p*-aminobenzoic acid signal. Because of pressure limitations, the column temperature could not be reduced below 22 °C at a given flow rate.

Linearity, Precision, and Detection Limits. The calibration of peak areas against concentrations generated linear functions (coefficient of determination ≥0.995) for all analytes within a range from 50 μg L^{-1} to 100 mg L^{-1}. The standard deviations of the retention times ($n = 9$) were less than 0.5%. At a signal-to-noise ratio of 3, the detection limits were 20−30 μg L^{-1} ((1−2) × 10^{-7} M) using fluorescence detection and 30−75 μg L^{-1} applying UV detection.

The precision of the analytical process (including derivatization) and of the determination method (excluding derivatization) was examined separately by the calculation of coefficients of variation (CVs) for the repeated injection of solutions containing the complete set of standard compounds. To evaluate the repeatability of the analytical process, the derivatization reaction was conducted in triplicate, and each generated reaction solution was injected three times (Table 2).

Examining the precision of the determination method for low-level injection (50 μg L^{-1}), the determined CVs were less than 10% for most analytes except Lac and Gal. At the high concentration level (500 μg L^{-1}), the variation coefficients spanned from 1 to 6%. Except for Lac and Gal, the repeatability of the analytical process matched the determination method at high analyte concentrations, but the dispersion of the analytical results was significantly higher (CV, 13−31%) at the low concentration level.

Interferences. The occurrence of interferences with other sample constituents was tested with amino acids especially, since

Table 2. Precision of the Analytical Process (Including Derivatization) and of the Determination Method (Excluding Derivatization), Expressed by the Coefficients of Variation of Peak Areas (Fluorescence Detection)

concn (μg L^{-1})	Lac	Gal	Glc	Xyl	GalUA	GlcUA	GlcNAc	GA
Relative Deviation (%) of the Determination Method ($N = 5$)								
50	13	13	5	6	7	2	6	5
500	5	6	2	1	3	2	3	2
Relative Deviation (%) of the Analytical Process ($N = 9$)[a]								
75	31	28	16	17	19	13	16	13
500	13	10	4	4	5	2	5	4

[a] Derivatization reaction conducted in triplicate, three subsequent injections of each reaction solution.

Table 3. Determination of Carbohydrates, Amino Sugars, and Uronic Acids in Various Soil Solutions (1−3) and Landfill Leachates (4−6) ($N = 6$)

sample	concentration (μg L^{-1})						
	Lac	Gal	Glc	Xyl	GalUA	GlcUA	GlcNAc
1	nd[a]	nd	nd	36 ± 4	nd	nd	44 ± 7
2	nd	nd	51 ± 6	54 ± 7	nd	nd	36 ± 6
3	nd	nd	nd	77 ± 10	117 ± 35	38 ± 11	42 ± 7
4	nd	98 ± 22	nd	100 ± 11	nd	201 ± 55	nd
5	63 ± 11	30 ± 7	nd	42 ± 5	67 ± 20	47 ± 13	nd
6	97 ± 17	45 ± 10	nd	57 ± 7	73 ± 22	51 ± 14	nd

[a] nd, not detectable.

it is known that amino acids interfere with the determination of carbohydrates, amino sugars, and uronic acids by means of HPAEC-PAD.[31] The addition of a 100-fold excess of amino acids (lysine, cysteine, glycine, alanine, threonine) to standard solutions of the reference compounds prior to the derivatization procedure had no effect on separation and on UV or fluorescence detection.

Environmental Analysis. To assess the applicability of the developed method in the environmental analysis of the target compounds, several soil solutions and leachates from a sanitary landfill were investigated. The chromatographic separations were performed on an AQUA column.

A typical chromatogram obtained from the analysis of a soil solution is shown in Figure 5. The peaks were identified by comparison with the retention times of the reference compounds, determined by the injection of standard solutions and by standard addition of the analytes. The concentrations of the carbohydrates and uronic acids found in these samples are summarized in Table 3. The concentrations of mono- and disaccharides are very low compared with data reported for various soil hydrolysates.[32−34] It can be assumed that a high portion of these components is generated by the hydrolytic breakdown of polysaccharides.

Generally, the number and the concentrations of compounds are higher in the landfill leachates than in the soil solutions. Xylose is detectable in all samples (mean, 61 μg L^{-1}). Lactose and galactose are present in the landfill leachates only, whereas the detection of *N*-acetylglucosamine is restricted to soil solutions.

The applicability of the analytical method to samples as different as soil solutions and landfill leachates elucidates its versatility. Due to the selectivity of the derivatization reaction and of the fluorescence detection, complex and highly loaded matrixes such as waste seepage can be investigated with little sample pretreatment, i.e., membrane filtration.

CONCLUSION

The first HPLC method designed for environmental analysis of mono- and disaccharides, uronic acids, and amino sugars by applying *p*-AMBA as a precolumn derivatization reagent is described. The main features of the method are the simultaneous determination of the different groups of analytes with high ($(1-2) \times 10^{-7}$ M) and approximately uniform sensitivity, the relatively fast and uncomplicated single-step derivatization procedure suited for automation, and the robustness against matrix interferences. The poor detection sensitivities reached for glucosamine and for the nonreducing sugar fructose demonstrate the limitations of the method.

Due to the hydrophilic and polar character of the derivatives, chromatographic separations with the aid of the ion-pair reagent $TBAHSO_4$ gave the best results if performed on a RP-C_{18} phase with hydrophilic end capping. Under these conditions, the differences in the retention of the derivatives and of the remaining derivatization agent are remarkable, facilitating the detection of trace concentrations of analytes in the presence of a great excess of *p*-AMBA. Presumably caused by almost identical pK_a values, charge numbers, and polarizabilities of the derivatives, attempts to separate the compounds on an anion exchange column failed.

The determination of the target compounds in soil solutions and landfill leachates supports the conclusion that the developed method is tailored to environmental analytical issues.

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