Improved RP-HPLC and anion-exchange chromatography methods for the determination of amino acids and carbohydrates in soil solutions

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Abstract

In spite of their low concentrations in soil solutions, low–molecular weight organic substances (LMWOS) such as amino acids, sugars, and uronic acids play a major role in the cycles of C and N in soil. With respect to their low concentrations and to possible matrix interferences, their analysis in soil leachates is a challenging task. We established two HPLC (high-performance liquid chromatography) methods for the parallel determination of amino acids and carbohydrates in soil leachates. The pre-column derivatization of amino acids with an o-phthaldialdehyde (OPA) mercaptoethanol solution yields quantitation limits between 0.03 and 0.44 μmol L^{-1} and S_D values of <8.3% ($n = 9$). High-performance anion-exchange chromatography (HPAEC) on a Dionex CarboPac PA 20 column with a NaOH acetate gradient combined with pulsed amperometric detection (PAD) was used for the determination of carbohydrates. The calibration curves obtained for 11 carbohydrates showed excellent linearity over the concentration range from 0.02 to 50.0 mg L^{-1}. Recovery studies revealed good results for all analytes (89%–108%). Interferences from Hg(II) salts and chloroform used for stabilization of the leachates did not occur with both chromatographic methods. The optimized method was successfully used for quantitative determinations of amino acids and carbohydrates in soil leachates.

Key words: low–molecular weight organic substances / amino acids / soil leachates / HPAEC-PAD / carbohydrates / monosaccharides / amino sugars / uronic acids

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1 Introduction

Usually the main organic constituents of root exudates are amino acids, carbohydrates, and aliphatic carboxylic acids (*Kraffczyk* et al., 1984; *Farrar* et al., 2003). To gain a deeper understanding how root-exudate composition depends on plant-growth conditions, rhizosphere soil solution has to be sampled in such a manner that disturbances of plant–soil interactions, especially of the dynamic of root exudation, are avoided as far as possible. To fulfill these needs, a new soil-leaching technique was developed by *Fischer* et al. (2007) and applied in the present investigation. Since other classes of organisms, *i.e.*, fungi and bacteria, also influence the DOC composition of rhizosphere soil solutions, the analytical determination of the target substance groups in these solutions should provide some hints to their contributions to the chemical composition of soil solutions. For this purpose, the determination of the ratio of hexoses to pentoses (*Oades*, 1984), the ratio of the amino sugars glucosamine to galactosamine (*Glaser* et al., 2006; *Turrion* et al., 2002), and the ratio of the neutral sugars to uronic acids (*Kaiser* et al., 2004) can deliver indicative information.

Derivatization reagents such as 1-fluoro-2,4-dinitrobenzene, ninhydrin, and o-phthaldialdehyde (OPA) are used to determine the total content of amino acids in soil solutions and soil extracts photo- or fluorometrically (*Jones* et al., 2002; *Jones* and *Willett*, 2006). For the simultaneous determination of these derivatization products, RP-HPLC (reversed-phase high-performance liquid chromatography [herein after referred to as HPLC]; *Bachmann* and *Kinzel*, 1992; *Warmann* and *Bishop*, 1985, 1987; *Hertenberger* et al., 2002) is mainly applied. The derivatization step is required for an improved separation and detection of the amino acids which possess neither chromophoric nor fluorophoric functional groups. The pre-column derivatization with o-phthaldialdehyde (OPA) in the presence of 2-mercaptoethanol or other reducing agents is advantageous because the achievable detection sensitivity is higher and in addition, the amino sugars can be determined. Due to the short derivatization time (*Fekkes* et al., 1995; *Fürst* et al., 1990; *Csapo* et al., 2004; *Uhe* et al., 1991), the derivatization procedure can be executed by an autosampler. This procedure has to be tested and optimized with respect to the sample properties, because no sample pretreatment other than preconcentration will be conducted (*Kraffczyk* et al., 1984; *Gransee* and *Wittenmayer*, 2000). In most of the above mentioned analytical studies to determine amino acids in soils, possible matrix effects on the analytical quality in terms of reproducibility, recovery rates, and peak purity were not sufficiently considered.

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Usually sugar concentrations were analyzed in soil extracts or investigated in the context of plant-nutrition studies. For the simultaneous determination of amino sugars together with mono- and disaccharides, the analytes are usually separated by means of high-performance anion-exchange chromatography and subsequently quantified by pulsed amperometric detection (HPAEC-PAD) (*Montero* et al., 2004; *Cheng* and *Kaplan*, 2003; *Kerheve* et al., 2002). For this analytical purpose, the anion-exchange columns CarboPac PA 1 and CarboPac PA 10 (Dionex Corp., Sunnyvale, USA) were often used. Despite of the great number of chromatographic applications elaborated with these columns, some problems regarding insufficient analyte resolution and matrix interferences still remain. For instance, it is disadvantageous that no baseline separation between arabinose and galactosamine and between mannose and xylose can be achieved with the CarboPac PA 1 columns. The co-elution of mannose and xylose on the CarboPac PA 10 (*Jahnel* et al., 1998; *Guignard* et al., 2005; *DeRuiter* et al., 1992; *Borch* and *Kirchman*, 1997) is unfavorable as well. *Currie* and *Perry* (2006) could fully resolve mannose and xylose on a CarboPac PA 10 using a NaOH gradient. However, the gradient program runs 80 min, and for more rapid analysis they used the new CarboPac PA 20. Nine sugars were determined with two different isocratic procedures lasting 20 min each, so that the co-elution of mannose/xylose (9 mM NaOH) and galactose/glucosamine (1.5 mM NaOH) could be eliminated. Compared to the older CarboPac PA 1 and PA 10 columns, the new CarboPac PA 20 has an improved selectivity with respect to the separation of glycoprotein monosaccharides (*Weitzhandler* et al., 2004). In addition, the simultaneous determination of uronic acids is possible by using an appropriate Na-acetate/NaOH gradient (*DeRuiter* et al., 1992; *Currie* and *Perry*, 2006; *Mehrländer* et al., 2002). *Eberendu* et al. (2005) achieved a satisfying separation of neutral sugars and uronic acids in dietary glyconutritional products by means of the CarboPac PA 20, but possible interferences with amino acids were not checked.

With respect to the recent state of the HPAEC-method elaboration, it was the main objective of the present work to further develop these methods to meet the specific requirements of soil-leachate analysis. The main tasks were to identify possible matrix interferences which could also stem from added chemical stabilizers (Hg[II]salts and chloroform) and to ensure a sufficient quality of the analytical data, *e.g.*, by performing standard addition measurements and by the determination of analyte recovery rates using soil leachates.

2 Materials and methods

2.1 Chemicals and solvents

The carbohydrates, uronic acids, and amino acids D(+)-xylose (Xyl), D(+)-galactose (Gal), D(+)-glucose (Glc), D-glucuronic acid (GlcUA), D(+)-galacturonic acid (GalUA), D(+)-mannose (Man), L(+)arabinose (Ara), and L-asparagine (Asn) monohydrate were obtained from Fluka. L(+)-rhamnose-monohydrate (Rha) was obtained from Merck. The amino sugars D(+)-glucosamine (GlcA) and D(+)-galactosamine (GalA) and the amino acids L-glutamine (Gln) and L-tryptophane (Trp) were purchased from Sigma. D(+)-fucose (Fuc) was obtained by Acros. An amino acid standard H (Pierce Perbio) with the following 17 amino acids L-alanine (Ala), L-arginine (Arg), L-aspartic acid (Asp), L-glutamic acid (Glu), glycine (Gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-methionine (Met), L-phenylalanine (Phe), L-serine (Ser), L-threonine (Thr), L-tyrosine (Tyr), and L-valine (Val) served together with the carbohydrates, amino sugars, and uronic acids as reference materials. The other chemicals, o-phthaldialdehyde (OPA) (Fluka), 2-mercaptoethanol (Fluka), buffer solution pH 10 (Merck), and NaOH (50%) (Baker) including the salts for preparing the eluents and the derivatization solution were of analytical grade. Solvents (methanol, acetonitrile, and tetrahydrofuran [THF]) were of HPLC quality. Water was purified by inverse osmosis and then passed through a MembraPure Astacus Analytical unit.

2.2 Stock solutions

Stock solutions of the reference compounds were prepared by weighing each in a 25 mL volumetric flask followed by dissolution in water (carbohydrates, amino sugars, and uronic acids) or in 0.1 M HCl (amino acids). These solutions were stored at 4°C and used for further dilutions.

2.3 Soil

The soil used in this study was a silty-loamy Haplic Luvisol from Heidfeldhof near Hohenheim University, Stuttgart, SW Germany. An overview of soil properties is given in *Fischer* et al. (2007). One week prior to the beginning of the experiments, the soil was moistened to approx. 75% water-holding capacity (28% [w/w] corresponding to 15 mL) to allow the development of a natural community of microorganisms.

2.4 Experimental set-up

The experimental set-up for the sampling of root exudates is similar to that described by *Fischer* et al. (2007). The peristaltic pump was adjusted to a flow of 15 mL h^{-1}. Samples of leachate were taken at different times between 100 and 1371 min after initiating of the leaching process. The whole experiment was conducted in triplicates.

2.5 Sample preparation

To sterilize the leachates, 5 μL of $CHCl_3$ were added to 10 mL of the sample solution. Then the samples were prefiltered through glass fiber filters (GF/D, Whatman, Brentford, UK) followed by a filtration *via* a polycarbonate filter with a pore size of 0.4 μm (Type 230 Sartorius, Göttingen, Germany) and subsequent 10-fold concentration (from 10 mL to 1 mL) using a speedvac vacuum centrifuge (RVC 2–25, Chris GmbH). Complete drying of the samples was avoided as *Fischer* et al. (2007) found out that some amino acids were not completely redissolved from the formed solids. The leachates were frozen until HPLC analysis.

2.6 Carbohydrate analysis

2.6.1 Apparatus

Chromatographic separations were conducted with a Dionex IC system consisting of an autosampler AS 50, a gradient pump GP 40, and an electrochemical detector ED 40 equipped with a thin-layer-type amperometric cell. The cell comprised a gold working electrode and an Ag/AgCl reference electrode. Data acquisition and processing were accomplished with the Dionex Chromeleon 6.70 software. Columns were CarboPac PA 20 (3 × 150 mm) in conjunction with 3 × 30 mm CarboPac PA 20 guard column and an amino-trap guard column (4 × 50 mm Dionex). The column temperature was kept at 35°C by a Jetstream-Plus column oven (ODS Optilab). The analytes were separated using gradient elution shown in Tab. 1.

Table 1: Gradient profile for carbohydrate separation by HPAEC-PAD on the CarboPac PA20 column.

Time [min]	**%A** (1 M sodium acetate/ 20 mM NaOH)	**%B** (250 mM NaOH)	**%C** (10 mM NaOH)	**%D** (water)
0	0	0	20	80
16	0	0	20	80
18	5	20	0	75
21	5	20	0	75
35	40	20	0	40
36	0	100	0	0
41	0	100	0	0
45	0	0	20	80

The flow rate was 0.4 mL min^{-1}, and the sample injection volume was 50 μL. The analytes were directly detected by applying a quadrupole-potential waveform on the gold electrode (E_1 = 0.1 V from 0 to 0.4 ms; E_2 = 2.0 V from 0.41 to 0.42 ms; E_3 = 0.6 V from 0.42 to 0.43 ms; E_4 = –0.1 V from 0.4 to 0.5 ms). The analytical data quality was controlled by standard addition methods.

2.7 Amino acid analysis

2.7.1 Apparatus

Chromatographic separations were conducted on a Shimadzu HPLC system consisting of an autosampler SIL 10A, a controller SCL-10AVP, a gradient pump LC-10 ADVP, and a fluorescence detector SPD-10 AXL (λ_{ex} 340 nm; λ_{em} 445 nm). Data acquisition and processing were accomplished with the Shimadzu CLASS VP 6.12 software. The analytical column was a Hypersil ODS (150 × 4 mm; 5 μm) combined with a 4 × 2 mm guard column (Phenomenex). The column temperature was kept at 30°C by a Jetstream-Plus column oven (ODS Optilab). 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.

The eluent system consisted of two components: eluent A was THF/methanol/Na-acetate (0.1 mol L^{-1}; pH 7.2; 5/95/900; v/v/v), and eluent B was methanol. The gradient was formed as follows: 0–20 min: 92.5% A (isocratic); 20–30 min: 92.5% → 60% A; 30–45 min: 60% → 30% A, and 45–50 min: 30% → 0% A. The flow rate was 1 mL min^{-1}.

All glass ware were sterilized by autoclavation.

2.7.2 Derivatization solution

An amount of 68 mg of OPA was dissolved in 1.4 mL of methanol in a 10 mL volumetric flask, and 50 μL of 2-mercaptoethanol were added to the solution. Thereafter, its volume was made up to 10 mL by addition of borate buffer solution (0.4 mol L^{-1} boric acid adjusted to pH 10.4 with NaOH). The reagent mixture was kept in the dark at 4°C and was used after an equilibration period of at least 18 h. Fresh mixtures were prepared every 2 d.

2.7.3 Derivatization procedure

The derivatization was performed automatically by the autosampler. An aliquot of 200 μL of the OPA-reagent solution is pipetted to the sample vial filled with 160 μL of the borate puffer (pH 10) and with 80 μL of sample or standard solution. Afterwards, the liquid components are mixed with the syringe. After a reaction time of 300 s, 50 μL of the derivatized sample were injected into the eluent stream.

3 Results and discussion

3.1 Carbohydrate analysis

3.1.1 Method optimization

The method optimization started with the establishing of the HPAEC method of *Eberendu* et al. (2005). A standard mixture, containing each of the 11 relevant carbohydrates in concentrations of 2 mg L^{-1} but no amino acids, is separated within 24 min, applying the gradient profile listed in Tab. 1. As Fig. 1A elucidates, xylose and mannose are not fully, but sufficiently resolved.

Testing other columns like CarboPac PA 10 or Metroseb Carb 1 (Metrohm) with different concentrations of NaOH in the eluent, with varying column temperatures, and with the addition of borate did not improve the resolution. Higher amino acid contents in the leachates might entail separation problems and symptoms of poisoning of the gold working electrode leading to a high background noise (*Weitzhandler* et al., 1996). For this reason, an amino-trap pre-column was inserted before the CarboPac pre-column leading to a focused retardation of the amino acids. As a consequence, the amino acids eluted within a small time window from 18 to 20.5 min, *i.e.*, after the sugars and before the uronic acids. This effect is exemplified by Fig. 1B which depicts a chromatogram of a mixture containing all of the 11 carbohydrate reference compounds (concentration of each: 0.5 mg L^{-1}). As a result, the gold working electrode was not poisoned. The

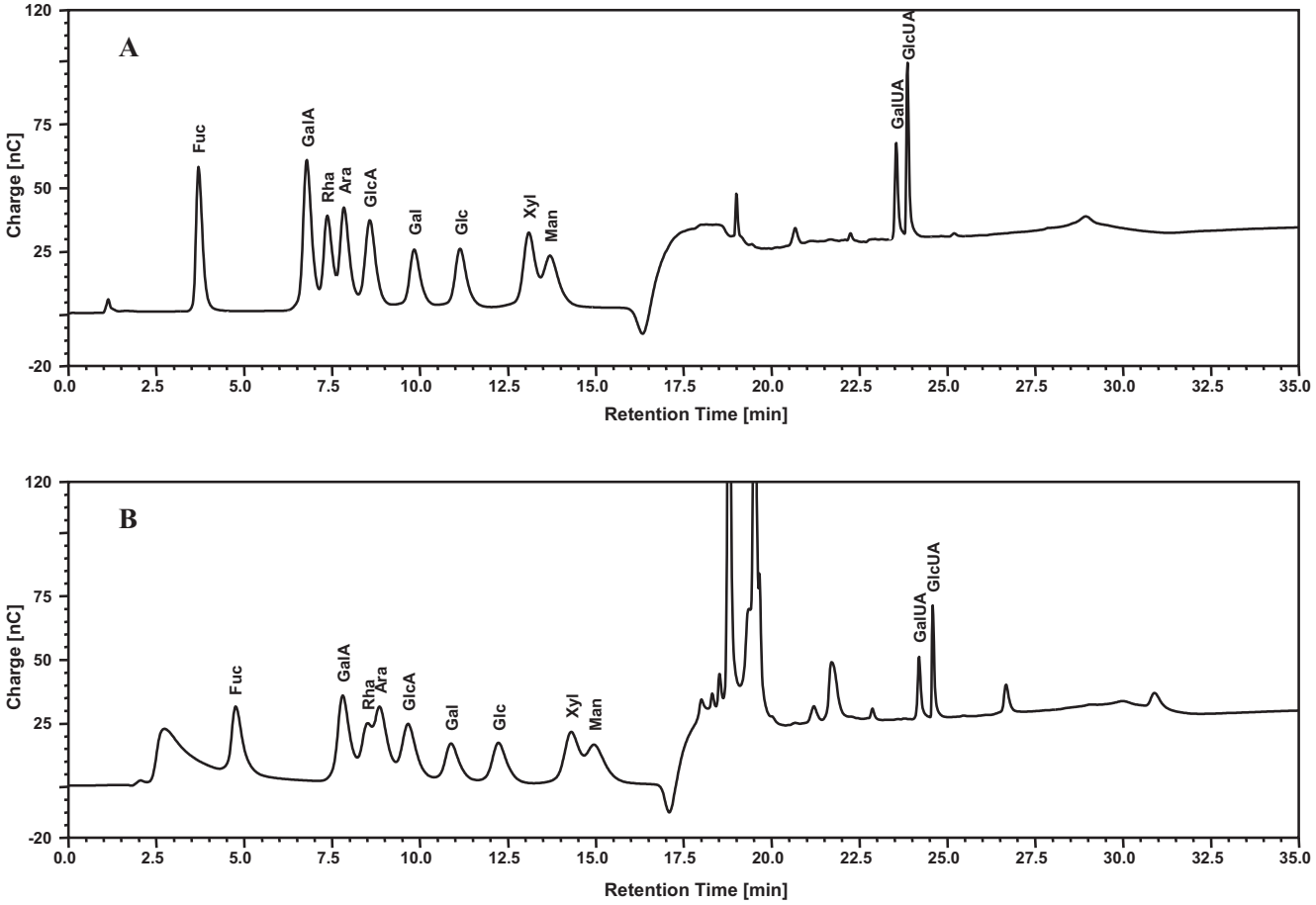

Figure 1: Separation of a carbohydrate standard mixture (concentration of each compound 2 mg L^{-1}) on a CarboPac PA20 anion-exchange column (A) without amino acids and amino-trap pre-column, (B) with an amino acid standard mixture (0.5 mg L^{-1} of each component) and with integrated amino-trap pre-column. Chromatographic conditions: flow: 0.4 mL min^{-1}; column temperature: 35°C; pulsed amperometric detection [PAD]. Elution sequences: A: fucose, galactosamine, rhamnose, arabinose, glucosamine, galactose, glucose, xylose, mannose, galacturonic acid, glucuronic acid; B: as in A; additional signal occurring between 18 and 22 min stems from amino acids.

installation of the amino-trap pre-column only worsened the separation between Rha and Ara (Fig. 1B). In addition to this effect, a slight loss of sensitivity occurred. Trying to optimize the NaOH concentration and the column temperature improved neither the separation nor the detection sensitivity.

Limits of detection (LOD) and limits of quantitation (LOQ) were determined for the carbohydrate reference compounds according to DIN 32645 applying to the same chromatographic conditions as used for the record of the chromatogram shown in Fig. 1B. The LODs spanned from 13 nmol L^{-1} to 54 nmol L^{-1} (Tab. 5). The lowest LOQ was found for xylose (35 nmol L^{-1}), the highest for arabinose (131 nmol L^{-1}). The regression coefficients for linear calibration in the nmol L^{-1} concentration range were 0.9980 or higher.

Eberendu et al. (2005) achieved lower LOQs by using post column addition of NaOH to increase detector response. However, the limits of quantitation mentioned above are sufficient with respect to the expected (and presumably relevant) carbohydrate concentrations. The addition of $CHCl_3$ or Hg(II) salts to the samples neither influenced the detection sensitivity nor the separation efficiency.

3.1.2 Recovery rates

To determine the analytical recovery of carbohydrates solved in soil leachates, eight samples were selected and aliquots of the standard carbohydrate mixture were added to increase the original concentration of each of the relevant carbohydrates by 2.0 mg L^{-1}. Recovery rates between 89% and 106% were found (Tab. 2). Except for glucuronic acid, the standard deviations for the repeatability (n = 3) of the recovery rates were <13%. Taking sample 4 as an example, Fig. 2 illustrates the determination of the recovery rates by means of sample spiking.

It can be inferred from the achieved results that nontarget leachate components do not interfere with the analytical carbohydrate determination and that the amino acid content of the samples is efficiently eluted within an uncritical time window in-between of two of the carbohydrate fractions.

Table 2: Recovery rates [%] determined for the 11 reference carbohydrates by spiking soil leachates numbers 1–8 with a combined carbohydrate standard (concentration increase of each carbohydrate by 2 mg L^{-1}).
Chromatographic conditions: CarboPac PA20 pre- and separation column in conjunction with an amino-trap pre-column; flow: 0.4 mL min^{-1}; column temperature: 35°C; gradient profile as in Tab. 1.

Sample number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean value**	**Std. deviation** (n = 3) [%]
Carbohydrate	recovery rate [%]									
Fuc	75.7	110.3	122.8	103.6	102.8	107.7	98.0	120.9	105.2	13.2
GalA	84.1	109.6	109.3	99.8	95.7	93.0	83.2	98.5	96.7	9.5
Rha	90.2	111.0	107.7	97.4	92.9	96.8	97.2	102.9	99.5	6.9
Ara	81.7	106.8	111.5	100.0	96.7	83.3	91.7	10.1	96.6	9.7
GlcA	81.8	112.8	100.6	105.1	95.6	84.4	89.6	100.7	96.3	11.7
Gal	82.0	111.6	103.3	107.3	101.1	85.9	94.7	110.0	99.5	11.8
Glc	84.8	96.7	98.3	88.9	74.8	89.1	87.1	94.1	89.2	7.9
Xyl	79.8	107.8	115.6	101.0	100.5	94.5	92.2	107.0	99.8	9.7
Man	83.4	111.2	113.7	104.0	87.9	93.0	87.2	89.8	96.3	10.5
GalUA	89.5	120.3	100.2	110.1	116.3	96.5	105.3	109.6	106.0	10.2
GlcUA	82.2	126.5	102.2	102.1	78.4	82.9	79.3	95.9	93.7	18.6

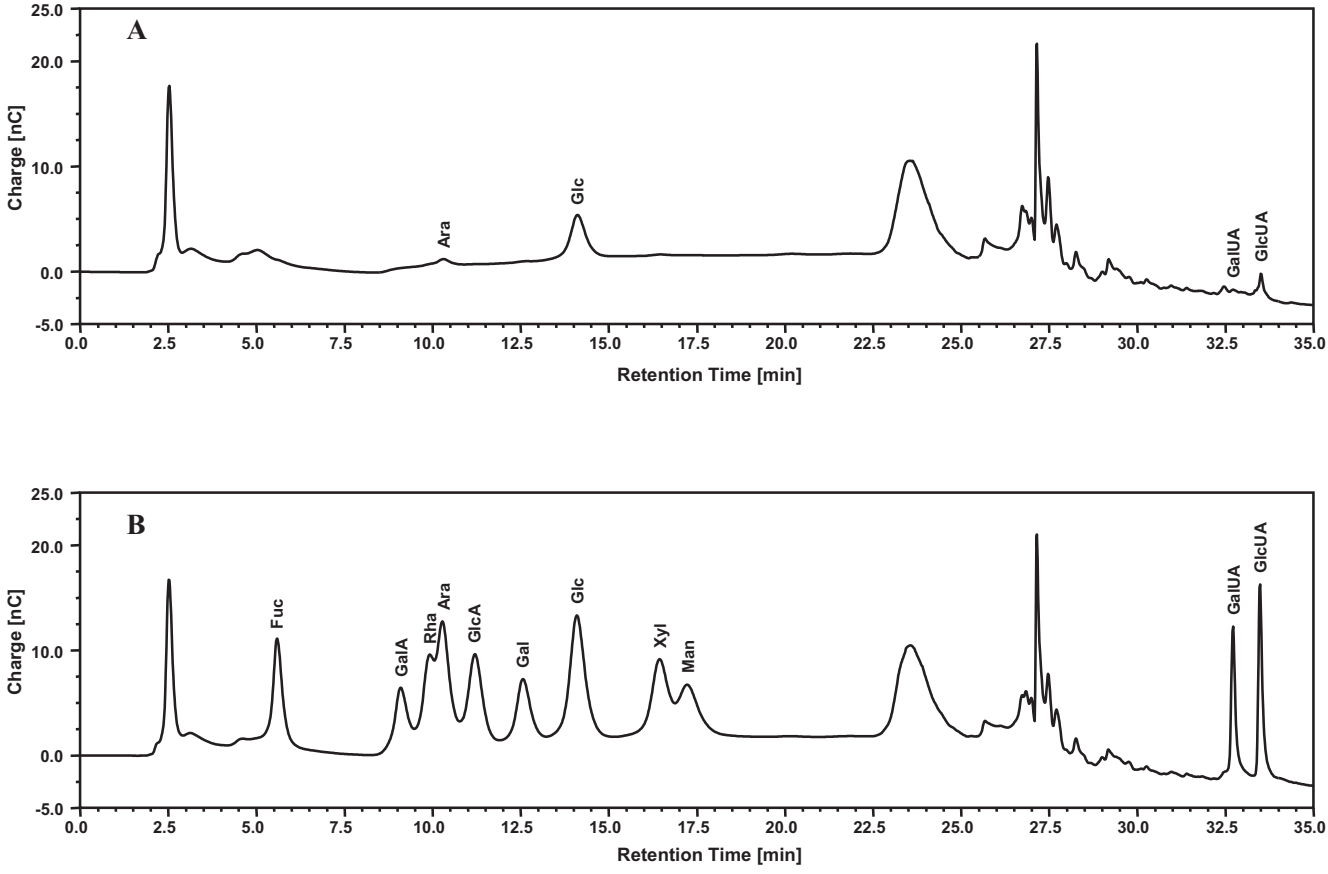

Figure 2: HPAEC-PAD chromatograms of the original soil leachate 2 (A) and the same sample after addition of a standard carbohydrate mixture (B) (concentration of each compound increased by 2 mg L^{-1}). Chromatographic conditions as in Fig. 1. Original carbohydrate contents of leachate 2 (A): Ara 0.20 μmol L^{-1}; Glc: 1.89 μmol L^{-1}; GalUA: < 0.1 μmol L^{-1} (LOQ); GlcUA: 0.41 μmol L^{-1}. Elution sequence of the labeled peaks in (B): fucose, galactosamine, rhamnose, arabinose, glucosamine, galactose, glucose, xylose, mannose, galacturonic acid, glucuronic acid.

3.2 Amino acid analysis

3.2.1 Optimization of the method

Starting from the automatic determination of amino acids with OPA and 2-mercaptoethanol (*Fekkes* et al., 1995), the pre-column derivatization procedure was carried out by the HPLC autosampler. In contrast to the original method, the reagent solution was pipetted into a sample vial which already contained the sample. Then the joint sample and reagent solutions were mixed by drawing up and ejecting the syringe needle several times. Immediately afterwards, 50 μL of the mixture were injected. The separation of the 17 amino acids on the Hypersil column was optimized concerning the column temperature and the composition of the gradient profile. Nearly all peaks of the amino acid derivates were baseline separated; merely the resolution between Met and Val was not complete (Fig. 3). OPA does not react with secondary amines. Therefore, proline was not measurable principally. The fluorescence intensity of the lysine-OPA derivate is not high enough for enabling its sensitive detection (*Godel* et al., 1984). Since the yield of the reaction of cysteine with OPA amounts to approx. 5% only (*Fisher* et al., 2001), this amino acid was generally not detected.

The automatic pre-column derivatization of the amino acids was optimized with respect to the following factors:

– the volume ratio between the sample and the derivatization solution;

– the pH value of the buffer solution added to the sample;

– the reaction time and the mixing cycles of the sample with the syringe.

Optimal derivatization conditions were obtained when the samples were mixed twice after the addition of the reagent (reaction time of 300 s). For most analytes, the largest peak areas yielded at a reaction pH of 10 and at a mixing ratio of 20:16:8 (v/v/v: OPA solution to buffer to sample). The background impurity mentioned by *Warman* and *Bishop* (1987) could be minimized when the OPA solution was allowed to equilibrate over night, when all storage flasks for the used liquids were sterilized in an autoclave, and when the separation column was cleaned with an aqueous methanol (50:50 v/v) solution including sodium azide (1 g L^{-1}) every 5 d of continuous operation. The background contents of Asp, Glu, Asn, Ser, Gly, Ala, Tyr, Leu varied from day to day and had to be determined daily. As a consequence, the determination of the LOD and LOQ values as well as the generation of the calibration functions had to be repeated at considerable changes of the amino acid background contents. Therefore, the amino acid LOD and LOQ values listed in Tab. 5 are typical but not fixed values.

In spite of the amino acid background content of the deionized water, the reproducibility of the determination of a 2 μmol L^{-1} amino acid standard prepared in this solvent was good except for Gly and Asn. The standard deviations of nine subsequent injections were <8.3% for each of the other amino acids (Tab. 3).

Table 3: Standard deviations calculated for nine subsequent injections of the amino acid reference standard (nominal concentration of each amino acid: 2 μmol L^{-1}), prepared in deionized water. Analytical conditions as in Fig. 3.

Amino acid	**Std. deviation** [%]
Asp	7.6
Glu	6.4
Asn	13.6
Ser	5.5
His	5.8
Gly	10.5
Thr	2.9
Arg	8.3
Ala	6.3
Tyr	2.3
Met	3.3
Val	2.9
Trp	3.7
Phe	4.8
Ile	3.0
Leu	3.2

3.2.2 Recovery rates

Recovery rates for the 17 amino acids of the combined reference standard were determined after spiking of 16 soil leachate samples. The spike concentration of each amino acid was 2.5 μmol L^{-1}. Using eight randomly selected samples as examples, Tab. 4 shows that the recovery rates for each of the 17 amino acids, given as means of triplicates, ranged from 95% to 100%. Even if related to the whole analytical process, including the derivatization step, the chromatographic separation, and the background signal of the blank value, the standard deviations of these recovery rates were <15%. Recovery rates for the other eight samples were in this range as well. As a consequence, the method presented here proved to be suitable for the determination of amino acids in these soil leachates.

Figure 3C illustrates the determination of the recovery rates for amino acids by spiking of the amino acid reference standard (concentration of each amino acid: 2.5 μmol L^{-1}).

3.2.3 Interferences

The examination of a possible interference of the amino acid determination by amino sugars showed that in accordance to *Georgi* et al. (1993), the amino sugars were well-separated from the relevant amino acid derivates. Neither higher concentrations of chloroform nor of Hg^{2+} ions in the processed soil leachates impaired the determination of the amino acids.

Table 4: Recovery rates of the amino acids determined for samples 1–8. Spike concentration of each amino acid: 2.5 μmol L^{-1}. Analytical conditions as in Fig. 3.

Sample number	1	2	3	4	5	6	7	8	Mean value	Std. deviation [%]
Amino acid	recovery rate [%]									
Asp	85.2	106.3	100.4	95.4	105.9	113.5	89.8	96.3	99.1	9.4
Glu	91.1	104.4	100.0	94.1	104.5	115.3	73.7	89.5	96.6	13.0
Asn	92.2	107.7	102.3	96.1	106.1	113.7	74.5	90.8	97.9	12.6
Ser	92.7	106.4	102.3	101.5	115.0	108.4	76.2	90.1	99.1	12.4
Gln	91.7	105.4	101.0	93.7	105.7	116.9	74.3	91.9	97.6	13.1
His	109.1	104.5	99.6	94.8	107.5	117.5	71.6	90.1	99.3	14.2
Gly	88.8	102.4	106.0	100.7	120.0	107.8	87.9	75.0	98.6	14.3
Thr	85.9	106.5	96.5	93.8	106.9	116.0	74.2	92.2	96.5	13.7
Arg	84.4	105.2	97.1	92.3	107.0	117.3	74.1	98.2	97.0	14.0
Ala	87.0	106.0	98.9	93.6	110.1	113.7	73.9	97.0	97.5	13.3
Tyr	89.4	105.2	98.1	94.2	107.3	109.3	74.3	91.6	96.2	12.0
Met	89.4	104.1	97.9	92.5	102.7	113.2	73.7	89.9	95.4	12.5
Val	89.5	105.6	98.6	93.1	103.9	113.8	74.7	90.3	96.2	12.5
Trp	90.2	105.8	99.1	93.5	104.6	115.3	73.8	89.7	96.5	13.1
Phe	89.8	105.5	99.3	93.3	104.1	115.9	83.8	89.8	97.7	10.8
Ile	89.2	103.8	99.7	93.4	103.9	114.3	86.3	91.4	97.8	9.6
Leu	96.4	96.2	99.4	87.8	103.1	115.5	74.6	89.4	95.3	12.6

3.3 Application of the chromatographic methods to soil leachates

The optimized chromatographic methods were used to analyze amino acids, sugars, and uronic acids in several soil leachates (Tab. 5). From the carbohydrates, only glucose was found in all leachates (mean concentration: 1.67 μmol L^{-1}). Glucosamine was detectable in one sample only.

A number of 4 out of 17 amino acids was present in all leachates. From these four compounds, leucine occurred in highest concentrations (mean: 1.04 μmol L^{-1}), followed by alanine (0.87 μmol L^{-1}), glycine (0.77 μmol L^{-1}), and asparagine (0.39 μmol L^{-1}). Glutamine and histidine could be quantified in two leachates only. Almost equally high overall concentrations of amino acids were measured in leachates 2 and 4 (approx. 12.0 μmol L^{-1}), whereas the overall concentration of amino acids in sample 7 amounted to 1.5 μmol L^{-1} only. The glucose concentration was highest in leachate 4.

In order to compare these results with those of other authors, the measured sample concentrations must be divided by a factor of 10 (concentration factor), multiplied by the eluate volume, summed-up over all the leaching cycles, and related to the original water content. The result will be the soil-solution concentration at the beginning of the leaching period. Soil-solution concentrations calculated in this manner were 8.18 μmol L^{-1} for total free amino acids and 2.41 μmol L^{-1} for carbohydrates (sugars and uronic acids).

These data are in the range of those given by *Christou* et al. (2006) who reported amino acid concentrations in a vineyard soil leachate in the range of 16 μmol L^{-1}. As mentioned above, no data of sugars in soil solutions are available.

4 Conclusions

Soil leachates were sampled with a modified technique which was developed to reduce artificial impacts on the soil–soil solution distribution equilibrium of low–molecular weight organic substances (LMWOS). After adequate sample preparation performed to reduce the sample volume, amino acids and carbohydrates were determined. Out of the 17 amino acids and 11 carbohydrates, used for calibration of the chromatographic methods, 16 amino acids and 7 carbohydrates were detected in at least one of the sampled soil leachates. Due to the 10-fold enrichment of the sample analyte concentrations as low as 0.1 μmol L^{-1} and even lower could be determined. Therefore, changes of the composition of these leachates caused by root exudation or by the activity of microorganisms can be examined in detail.

The determination of amino acids *via* pre-column derivatization with OPA was automated, and the separation of 17 amino acids on the applied chromatographic column was possible without matrix interferences.

In order to simultaneously determine sugars, amino sugars, and uronic acids *via* anion chromatography, these com-

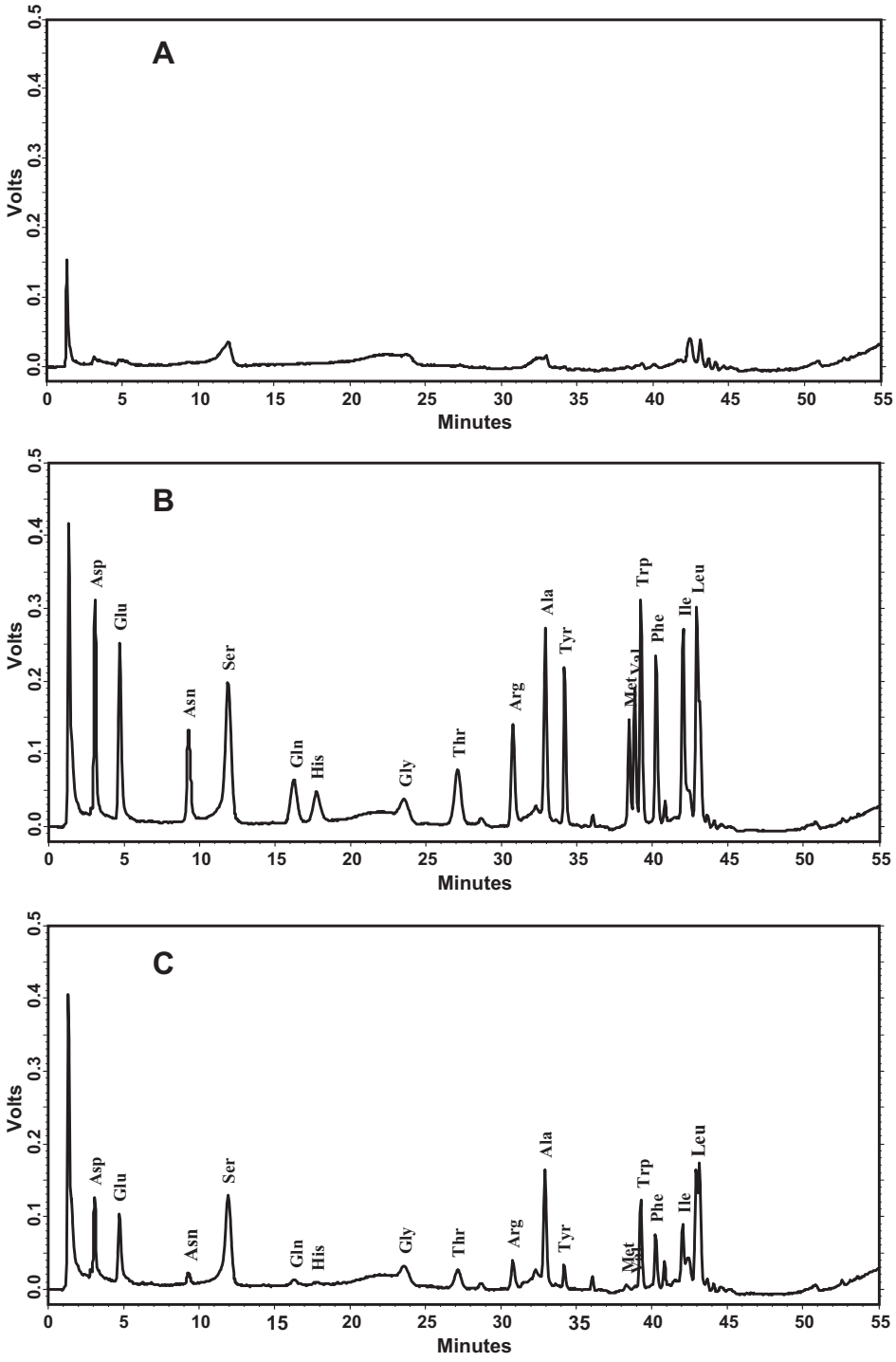

Figure 3: Determination of the recovery rates of amino acids in the sample 9: (A) blank (distilled water used for the preparation of the amino acid standard mixture), (B) sample 9, (C) sample 9 after standard addition (concentration increase of each amino acid by 2.5 μmol L^{-1}). Analytical conditions: OPA derivatization; gradient profile as described in section 2; flow: 1 mL min^{-1}; fluorescence detection.

pounds were separated with a Dionex CarboPac PA 20 column. Interferences by amino acids were eliminated by installing an amino trap pre-column. Thus, an extensive sample preparation was not necessary.

The good reproducibility and the acceptable recovery rates achieved by both analytical methods document the suitability of the methods for the described analytical task.

Table 5: Carbohydrates and amino acids in various soil leachates, analyzed in triplicates. Data of LOD and LOQ calculated according to DIN 32645.

Sample number	1	2	3	4	5	6	7	8	LOD*	LOQ*
Carbohydrates						[μmol L^{-1}]				
Rha	n.d.	n.d.	0.43	0.37	0.49	0.24	n.d.	0.18	0.051	0.122
Ara	0.13	0.20	0.27	0.13	n.d.	n.d.	n.d.	n.d.	0.054	0.131
GlcA	n.d.	n.d.	n.d.	n.d.	n.d.	0.22	n.d.	n.d.	0.026	0.064
Glc	0.72	1.89	1.22	2.44	2.00	1.50	1.50	2.05	0.017	0.039
Xyl	n.d.	0.07	0.07	0.07	n.d.	n.d.	n.d.	n.d.	0.013	0.035
GalUA	d.	d.	d.	0.10	n.d.	n.d.	0.10	n.d.	0.045	0.103
GlcUA	0.36	0.41	0.67	0.67	0.62	n.d.	0.10	n.d.	0.037	0.087
Amino acids										
Asp+	0.52	0.73	0.50	0.82	0.05	0.37	0.07	0.06	0.018	0.048
Glu+	0.68	1.03	0.67	1.01	n.d.	n.d.	0.09	n.d.	0.024	0.083
Asn	0.11	0.14	0.11	0.14	n.d.	n.d.	0.03	n.d.	0.014	0.044
Ser+	0.50	1.16	0.75	1.09	n.d.	n.d.	n.d.	n.d.	0.176	0.435
Gln	n.d.	0.18	n.d.	0.13	n.d.	n.d.	n.d.	n.d.	0.013	0.036
His+	n.d.	0.16	n.d.	0.13	n.d.	n.d.	n.d.	n.d.	0.019	0.033
Gly	0.24	1.63	0.98	1.45	0.45	0.64	0.38	0.35	0.087	0.215
Thr+	0.69	0.69	0.40	0.67	n.d.	0.11	n.d.	n.d.	0.043	0.113
Arg	0.78	0.34	0.30	0.39	n.d.	0.21	n.d.	n.d.	0.075	0.189
Ala+	1.39	1.77	1.34	1.82	0.17	0.15	0.17	0.16	0.034	0.121
Tyr	0.16	0.29	0.24	0.29	n.d.	n.d.	n.d.	n.d.	0.009	0.060
Met	n.d.	0.09	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	0.013	0.051
Trp+	0.63	0.92	0.63	0.95	n.d.	n.d.	n.d.	n.d.	0.094	0.210
Phe+	0.32	0.55	0.28	0.50	n.d.	n.d.	n.d.	n.d.	0.098	0.245
Ile+	0.38	0.78	0.38	0.63	n.d.	n.d.	n.d.	n.d.	0.121	0.331
Leu	0.55	2.06	0.83	1.70	0.45	0.65	0.64	1.40	0.049	0.287

* Amino acid LOD and LOQ values are typical but varied with varying amino acid background concentrations (system blank).
+ blank from the system
d.: detectable, concentration < LOQ
n.d.: not detectable
Fuc, GalA, Gal, Man, and Val could not be detected in any of the samples.

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