

## Article

# Determination of Single Sugars, Including Inulin, in Plants and Feed Materials by High-Performance Liquid Chromatography and Refraction Index Detection

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**Abstract:** The exact and reliable detection of sugar monomers and fructans provides important information for the evaluation of carbohydrate metabolism in plants and animals. Using the HPLC method; a large number of samples and single sugars; with both high sensitivity and selectivity; may be analysed. It was shown that the described method—using a Nucleosil column loaded with  $\text{Pb}^{2+}$  ions; a refractive index detector (RID); and HPLC-grade water as the eluent—gives precise and reproducible results regarding the detection of individual sugars in extracts of plants and feed materials. The method can be applied for the detection of sucrose; maltose; lactose; xylose; glucose; galactose; arabinose; fructose; ribose; and mannitol. Furthermore; depending on the plant material; the sugars verbascose; stachyose; and raffinose can be separated. The peaks were well resolved and the reproducibility of the analysis; with 94–108% of recovery (RC) and relative standard deviation (RSD) of up to 5%; was very good. The method was successfully applied to a variety of green forages and samples of sugar beet pulp silages. It is also possible to determine fructan with inulin as a standard; together with the other sugars; or alone by a different protocol and column.

**Keywords:** single sugar; monosaccharides; glucose; fructose; sucrose; fructan; high performance liquid chromatography

## 1. Introduction

Carbohydrates comprise a variety of sugars, mainly lower-molecular carbohydrates which share certain traits regarding chemical structure and reactivity. Carbohydrates form the largest portion of the organic matter in plants and feed materials, so are of great importance as storage compounds, structure elements, and energy sources. They can be assigned to different groups based on certain traits, such as chemical structure (e.g., mono-, di-, oligo-, and polysaccharides, sugar alcohols, sugar phosphates), physico-chemical properties (e.g., redox potential, hydrolytic stability, solubility in different solvents), and function (e.g., storage or matrix/cell wall carbohydrates). Regarding the detection of the carbohydrate fractions in plant and feed materials, it is advisable to divide them into non-structural (NSC) and structural (SC) carbohydrates. The SC comprise hemicelluloses, celluloses, and pectins, as well as plant gums. In contrast to cellulose, hemicelluloses are the polysaccharide part of the plant which can be more easily accessed by chemicals, e.g., by extraction with bases. Mono-, di-, and oligosaccharides, starch, and fructans represent the major compounds of the NSC fraction.

The detection of sugar monomers and fructans provides important information for the evaluation of carbohydrate metabolism in plants and animals. Single sugars can be analysed by enzymatic or chromatographic methods. Although enzymatic assays are characterised by high specificity

and sensitivity, only one sugar species can be detected. Hall [1] reported that reducing sugars and condensation assays are widely used for the analysis of water soluble carbohydrates in feeds. They require the hydrolysis of oligo- and polysaccharides to monomers which will be measured, which means that specific carbohydrates are not detected. In contrast, chromatographic methods (gas-chromatography, GC, and high-performance liquid chromatography, HPLC), offer the advantage of the simultaneous detection of a variety of sugars with rapid, specific, and sensitive measurement. HPLC is mainly used for sugar analysis in foods [2,3]. Furthermore, HPLC is the most common chromatographic method for analysing these compounds in plants and feed materials. An overview, with references, of HPLC methods which are used for sugar analysis in forages is given in Table 1.

For the detection of sugar monomers by HPLC, they must be present in a soluble state. In most cases water or ethanol is used as the extraction solvent.

**Table 1.** Methods to determine individual sugars in plants.

Extraction Solvent	Detector	Column	Sugar Species	Reference
Water	RI	HPX-87P BioRad	Glucose, fructose, sucrose, galactose, stachyose, raffinose, verbascose	[4]
Water, 80% ethanol	HPIC, amperemeter	PA-1, Amino Trap column Dionex	Glucose, fructose, galactose, fucose, arabinose, sucrose, raffinose, stachyose	[5]
Water, 80% ethanol	Electrochemical detector	CarboPac PA200	Glucose, fructose, sucrose, fructane	[6]
Water	Not given	Rezex RPM Phenomex	Sucralose, glucose, fructose, galactose, arabinose, xylose, fructan	[7]
Water	RI	HPX-87C BioRad	Glucose, fructose, sucrose, raffinose, inulin	[8]
Aqueous CaCO <sub>3</sub> —solution 4%	RI	Eurokat Ca 10 µm	Sucrose, glucose, fructose	[9]
18 mM NaOH	Pulsed amperometric detection (PAD)	CarboPac PA 10	Arabinose, galactose, glucose, fructose, fucose, rhamnose, raffinose, salicin, sucrose, inositol, mannitol, sorbitol	[10]

However, which sugar fractions can be separated by chromatography is affected by the solvent used, and its concentration. While aqueous extracts may contain mono-, di-, and oligosaccharides, and fructans [10–12], only a small proportion of lower-molecular fructans dissolve in 80% ethanol [5,13]. The lower the concentration of ethanol in the extracting agent, the more higher-polymeric fructans will be extracted [14].

For the separation of single sugars by HPLC, various columns and detectors can be used. According to Scott [2], columns with polymer-based cations have low detection limits and can be employed in combination with a refraction index (RI) detector. The individual sugars form complexes with cations, e.g., Ca<sup>2+</sup> and Pb<sup>2+</sup>, whose binding strength is affected by the chemical structure of the sugar and the cation species. Deionised water is used as an eluent, and a flow gradient is not needed. In most cases, the column is heated to a temperature of 85 °C. However, Scott [2] reported problems with using this method to separate sucrose from maltose and mentioned, despite reasonable column stability, damage to the column by organic acids, methanol, and salts.

Glucose, fructose, and sucrose belong to the group of the most frequently-occurring lower-molecular carbohydrates in plants. Other monosaccharides, which can be detected after hydrolysis of cell-wall components of plants for food and feed use, are summarised in Table 2.

**Table 2.** Cell wall monosaccharides in plants for feed.

Plant Material	Glu <sup>1</sup>	Fruc <sup>2</sup>	Xyl <sup>3</sup>	Rib <sup>4</sup>	Ara <sup>5</sup>	Gal <sup>6</sup>	Man <sup>7</sup>	Rham <sup>8</sup>	Reference
Grasses/Legumes	X	X	X	X	X	X			[15]
Tropical legumes	X		X		X	X	X	X	[16]
Grasses	X	X	X		X	X	X	X	[17] <sup>11</sup>
Diverse <sup>9,10</sup>	X		X		X	X	X	X	[18,19] <sup>12</sup>
Maize silage, cereal grains	X		X		X	X	X	X	[20,21]
Sorghum/sudan grass	X	X	X						[22]

<sup>1</sup> Glucose; <sup>2</sup> fructose; <sup>3</sup> xylose; <sup>4</sup> ribose; <sup>5</sup> arabinose; <sup>6</sup> galactose; <sup>7</sup> mannose; <sup>8</sup> rhamnose; <sup>9</sup> maize, cereals, rice, different vegetables, flour, bread, and others; <sup>10</sup> wheat straw, wheat bran, sunflower leaves, sugar cane bagasse, soy bean hulls, sugar beet, peanut meal, and citrus trester; <sup>11</sup> additionally 4-o-methyl glucuronic acid; <sup>12</sup> additionally fucose. X: occurrence of substances.

Fructans represent the most prominent storage carbohydrates in temperate and cool zone grasses [23]. Fructans are mainly determined in feeds and forages for horses as they can cause, if overfed, diseases, such as laminitis, in this species [24]. In the literature, five types of fructans in plants are described [25], all with  $\beta$ -linkage: inulin  $\beta$  2-1, levan  $\beta$  2-6, branched  $\beta$  2-1 and 2-6, inulin neoseries  $\beta$  2-1, and levan neoseries  $\beta$  2-6. The HPLC methods mostly use inulin as a standard. According to Apolinário et al. [26], the extraction, isolation, and characterisation of inulin-type fructans have gained attention in recent years due to their wide distribution in nature, and their significant role in industry.

This study aims to develop a rapid, sensitive, and reproducible analytical method to determine a variety of sugar monomers and fructans in aqueous extracts of plants and feed materials. For this purpose, an existing HPLC system using an RI detector was enhanced with regard to column type and temperature, eluent, and flow rate. Moreover, the recovery (RC) rate of single sugars and the precision of the method were determined. The scope of the method concerning the detection of fructans with inulin as a standard is discussed.

## 2. Materials and Methods

### 2.1. Chemicals

The reference sugars were: sucrose (purity > 99.5%), D-(+)-glucose (purity > 99.5%), D-(−)-fructose, (purity > 99%), D-(+)-maltose monohydrate (purity > 99%), D-(+)-lactose monohydrate (ACS reagent), L-xylose (purity > 99%), D-(+)-galactose (purity > 99%), L-rhamnose (purity 98%), D-(+)-mannose (purity > 99%), D-(−)-ribose (purity > 99%), isomaltose (purity 98%), mannitol (ACS reagent), verbascose (purity 98%), as well as inulin, which were all obtained from Sigma-Aldrich (now Fa. Merck, Darmstadt, Germany). In addition, stachyose (purity 98%), D-(+)-cellobiose (purity 98%), and D-(+)-raffinose pentahydrate (98%) from Roth GmbH, and D-arabinose (purity 99%) from Alfa Aesar were used. HPLC-grade water and 0.01 N sulfuric acid solution (Fa. Merck, Darmstadt, Germany) were used as mobile phases.

### 2.2. Preparation of Standard Solutions and Mixtures of Standard Solutions

To estimate the retention times of single sugars in the tested chromatographic system, in each case 100 mg of the following single sugars were weighed in a 100 mL calibrated flask: verbascose, stachyose, raffinose, cellobiose, sucrose, isomaltose, maltose, lactose, glucose, xylose, galactose, arabinose, fructose, ribose, mannitol, and inulin.

A six-point calibration curve was developed for each of the following nine single sugars: sucrose, maltose, lactose, glucose, xylose, galactose, arabinose, fructose, and ribose, as well as the sugar alcohol mannitol, and the polysaccharide inulin.

For the quantification of mixtures of single sugars, a stock solution containing either 100 mg (low standard) or 300 mg (high standard) of each sugar species was prepared in a 100 mL calibrated flask. After the transfer of the required sugar quantity, HPLC-grade water was added up to the calibration mark. At first, inulin was weighed in the flask, supplemented with approximately 30 mL

of water, and dissolved in a hot water bath. After complete dissolution and cool-down of the inulin solution, the remaining sugar species were added and, finally, the flask was filled with water up to the calibration mark.

The series of standard solutions for external calibration was prepared by decanting differing volumes of the stock solution into a 25 mL flask, which was subsequently filled up to the calibration mark with HPLC-grade water:

1. Low standard: 1.25, 2.50, 3.75, 5.00, 6.25, 7.50 mL, respectively, in a 25 mL flask, representing concentrations of individual sugars of 5, 10, 15, 20, 25, or 30 mg/100 mL
2. High standard: 1.25, 2.50, 5.00, 12.50, 20.00, 25.00 mL, respectively, in a 25 mL flask, giving final concentrations of individual sugars of 15, 30, 60, 150, 240 and 300 mg/100 mL.

For the quantification of fructan (as inulin) alone, 300 mg of inulin was weighed in a 100 mL calibrated flask and dissolved in water. Next, the flask was shaken in a hot water bath for some minutes. As soon as possible after cooling the serial dilution was prepared: 1.25, 2.50, 5.00, 12.50, 20.00, and 25.00 mL, respectively, in 25 mL flasks, giving final concentrations of inulin of 15, 30, 60, 150, 240, and 300 mg/100 mL.

### 2.3. Optimisation of Chromatographic Analysis for Separation of Carbohydrates

The HPLC system used in this study (LC 20, Shimadzu, Germany) was composed of a pump, an autosampler with a storage temperature of 4 °C, a column oven, and a refraction index detector (RID), and was run isocratically. The following columns were tested: Knauer Eurokat Pb, Nucleosil CHO 620 and Nucleosil CHO 682 (Pb), Machery-Nagel, and BioRad Aminex HPX-87C; all with a length of 300 mm × 7.8 mm particle size, and combined with a pre-column cartridge (21 × 4.6 mm).

The standard solutions (low and high), as well as the single sugar solutions were analysed in the HPLC system with an RID under the following varying analytical conditions: column type, eluent water or 0.01 N sulphuric acid, oven/column temperature (35, 60, 80, 85 °C), injection volume (10, 20, 30, and 50 µL), and flow rate (0.4, 0.5, 0.6, 0.65 mL/min). Those conditions that yielded the best resolution in compound separation were selected for the new sugar HPLC method. After a range of modifications to the original HPLC protocol, the following final analytical conditions for the detection of aqueous solutions of individual sugars, and mixtures thereof were employed: isocratic elution with HPLC-grade water; a flow rate of 0.4 mL/min; an oven temperature of 85 °C; an injection volume of 20 µL; column type Nucleosil CHO 682 (Pb) with pre- and main column (21 mm, ID 4.6 mm; 300 mm, ID 7.8 mm). Data given in Table 3 summarise the retention times ( $r_t$ ) of a variety of mono-, di-, oligo-, and polysaccharides in pure solutions. A single analytical run lasted 50 min. At longer intervals, if the separation deteriorated, the polymer of the column was cleaned with a 50% aqueous solution of acetonitrile at a column temperature of 65 °C, and an elution of 0.1 mL/min overnight. The following day, the acetonitrile solution is replaced by water at 0.1 mL/min until high pressure has subsided.

Fructans with inulin as standard were analysed as a second method, using the column Polypore H (Brownlee, pre-column: 30 mm × 4.6 mm, main column: 220 mm × 4.6 mm ID) and the following HPLC conditions: a column temperature of 80 °C; an eluent of 0.01 H<sub>2</sub>SO<sub>4</sub>; and a flow rate of 0.5 mL/min. The retention time was found to be  $r_t = 2.62$  min.

**Table 3.** Retention times of sugar specie, analysed by HPLC with a Pb<sup>2+</sup> column CHO 682 heated at 85 °C, 0.4 mL/min flow rate, HPLC-grade water as the eluent, with a refraction index (RI) detector.

Groups of Carbohydrates	Sugar	$r_t \pm 0.20$ min
Polysaccharide	Inulin	9.68
Oligosaccharide	Verbascose	13.58
	Stachyose	14.45
	Raffinose	15.70
	Cellobiose	16.68
Disaccharide	Sucrose	16.74
	Isomaltose	17.30
	Maltose	17.64
	Lactose	18.45
Monosaccharide	Glucose	20.06
	Xylose	21.46
	Galactose	23.17
	Rhamnose	23.33
	Arabinose	24.63
	Fructose	25.75
	Mannose	26.17
	Ribose	44.88
Sugar alcohol	Mannitol	39.16

#### 2.4. Precision and Recovery Rate of Chromatographic Analysis

The optimised HPLC method was verified, regarding its precision and recovery rate, to detect nine individual sugar monomers, as well as mannitol and fructan with inulin as the standard in model solutions. Standard deviations (absolute, SD; and relative, RSD) were calculated to estimate the precision.

The recovery (RC) was calculated:  $RC (\%) = \text{Mean of measured value} \times 100\% / \text{Set value}$ .

At first the precision and RC of the method for nine single sugars and mannitol was tested by repeated analysis (10 times during the course of a day) of the same sample. Model solution I contained 60 mg of each sugar species per 100 mL (set value conc. 1), and model solution II contained 300 mg of each sugar species per 100 mL (set value conc. 2).

The next step was to estimate the RC and precision for nine single sugars, mannitol, and inulin after repeated analysis ( $n = 5$ ) on five different days in six different model solutions. The solutions I–VI contained differing concentrations—15, 30, 60, 150, 240, and 300 mg/100 mL—of each sugar species, mannitol, and inulin.

To characterise the precision and RC of the sugar HPLC method in forage sample solutions, aqueous maize and grass silage extracts were used. These extracts were taken at random from silage quality projects. They served to represent the matrix of forage samples. The extracts were filtered at first over a folded paper filter (diameter 125 mm in a funnel), and second through a 0.45 µm minisart filter (Sartorius) into a 1.5 mL HPLC vial, and stored in a freezer at  $-18^\circ\text{C}$  until measurement.

Based on an aqueous maize silage extract that had been fortified (spiking) with six standard solutions at a 1:1 ratio, the measurements were repeated three times per day. The solutions I–VI contained differing concentrations—15, 30, 60, 150, 240, and 300 mg/100 mL—of each sugar species and mannitol. After measurement of the aqueous maize silage extract ( $n = 3$ ), at a ratio of 1:1 with HPLC-water, the set values for each sugar in each model solution were calculated:  $[\text{content in maize silage extract} + \text{set value}] / 2$ . The data concerning precision and recovery rate in spiked grass silage extracts were collected from the measurements of one grass silage sample extract with low and

high standard solutions. The first part covered the low standard concentration of 5–30 mg/100 mL, and the second part covered the high standard concentration of 15–300 mg/100 mL calibration solution. The grass silage extract was combined with HPLC water at a ratio of 1:1, and was measured first. This extract was then spiked with six standard solutions of low, then high, standard, also in a ratio of 1:1, and the set values for each sugar and inulin were calculated: (content in grass silage extract + set value)/2.

For the estimation of the RC and SD of inulin analyses with the Brownlee-H column, two model solutions (9 mg/100 mL and 135 mg/100 mL) were measured 10 times in one day. An external six-point calibration was assumed: 15, 30, 60, 150, 240 and 300 mg/100 mL. Additionally, solutions I–VI were used for the estimation of RC and SD five times on different days.

### 2.5. Comparative Analysis of Sugars in Plant and Feed Materials

This study involved a total of 36 freeze-dried and milled (1 mm sieve) samples (six legumes, five legume-grass mixes, 22 grass mixes, one forage maize, and two whole-crop cereals), and 18 lyophilised and milled samples of sugar beet pulp silages. Of the dried and milled sample, 0.5 g was weighed in a weighing crucible and transferred into a sealable plastic bottle of minimum 120 mL volume, and 100 mL deionised water was added. Subsequently, the bottles were placed in a horizontal shaker, set at 30 revolutions/min for one hour.

After shaking, the contents of the bottle were filtered through a folded paper filter (diameter 125 mm in a funnel) into a 100 mL Erlenmeyer flask. The resulting clear extract was filtered through a 0.45 µm minisart filter (Sartorius) into a 1.5 mL HPLC vial, exactly 1.5 mL. A volume of 20 µL was injected per sample. The compound identification was based on the comparison of retention time with authentic standards. Quantification was performed by the external standard method, and using the high standard.

These plant samples were also analysed by ion chromatography by the University of Kiel, Germany, to compare the accuracy of different methods for glucose and fructose detection. To achieve this, the dried and milled samples were extracted with hot water (five minutes at 100 °C) and centrifuged.

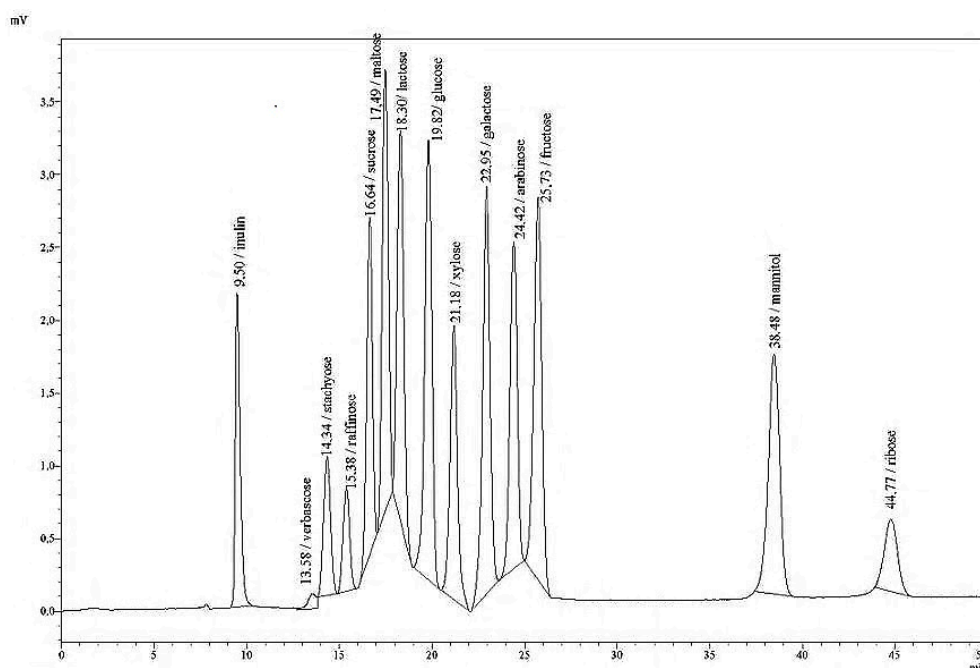
For the analysis, a Carbo Pac PA100 column was used in a Dionex Ionenchromatograph System ICS 2500. The mobile phase consisted of HPLC-water and 200 mM NaOH (pH 10.0–13.5) and the samples were analysed in a gradient mode.

More comparative analyses of sugars have already been conducted and will be published.

## 3. Results and Discussion

### 3.1. HPLC Method for the Detection of Single Sugars

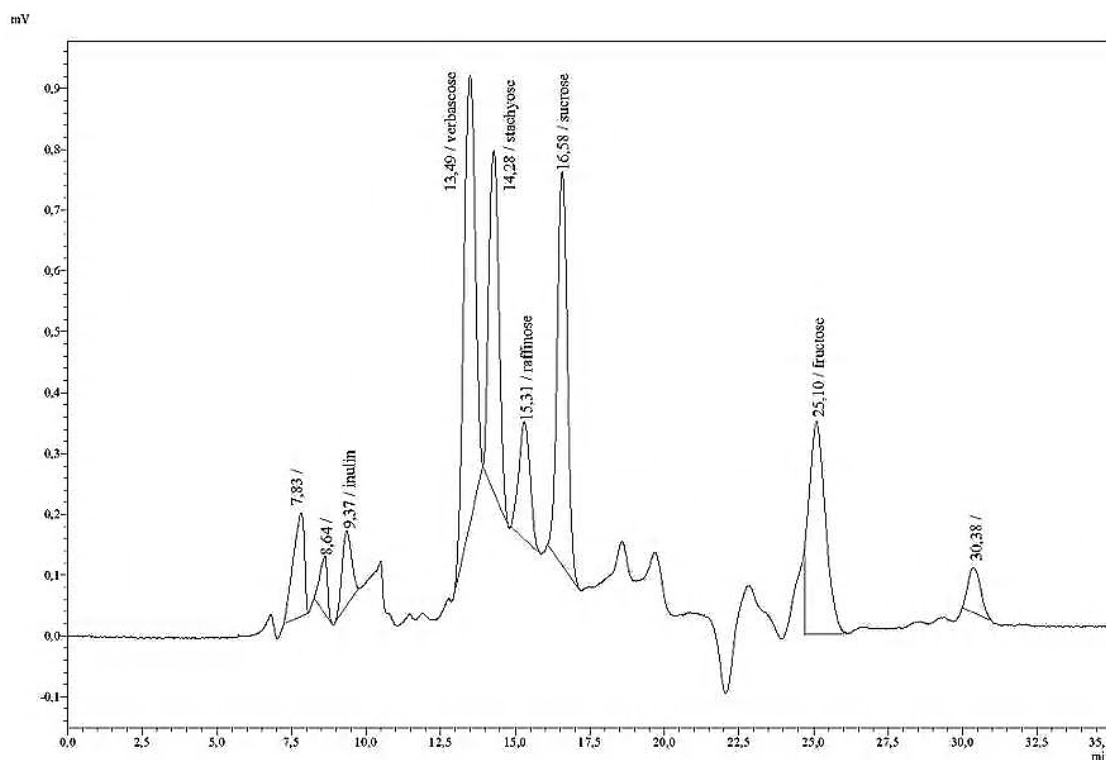
The individual sugars sucrose, maltose, lactose, glucose, xylose, galactose, arabinose, fructose, ribose, fructans (as inulin), and the sugar alcohol mannitol could clearly be separated from one another, thereby enabling exact quantification. The retention times of all compounds varied within the normal range of  $\pm 0.5$  min. The use of a CHO 682 column with Pb configuration at 85 °C, a RID, and HPLC-grade water as the eluent in an isocratic modus gave excellent separation of the most frequent mono- and disaccharides, glucose, fructose, and sucrose, and also showed very good partitioning of additional mono-, di-, oligo-, and polysaccharides (Figure 1).



**Figure 1.** Separation of inulin, mono-, di-, oligo- and polysaccharides in a Pb column Nucleosil CHO 682, at 85 °C, with a RID and HPLC-grade water as the eluent (standard solution).

Completeness of separation was checked by recovery studies. The “valley to valley” evaluation method for separated peaks allowed reliable and reproducible identification and quantification. The limit of detection was 1 mg/L. The oligomers verbascose, stachyose, and raffinose, which mainly occur in grain legumes [4], could be separated very well. These oligosaccharides were detected in the forages (legumes, legume-grass mixes, grass mixes, forage maize, and whole-crop cereals) and sugar beet pulp silages, but were only detected at trace concentrations and were, therefore, not added to the mixed stock solutions. Although these oligomer sugars can be detected and quantified with the described method, its precision parameters—RC, SD, and RSD—would still have to be determined. Figure 2 shows a chromatogram for the separation of verbascose, stachyose, and raffinose in freeze-dried peas, recorded with this HPLC protocol after cold water extraction.



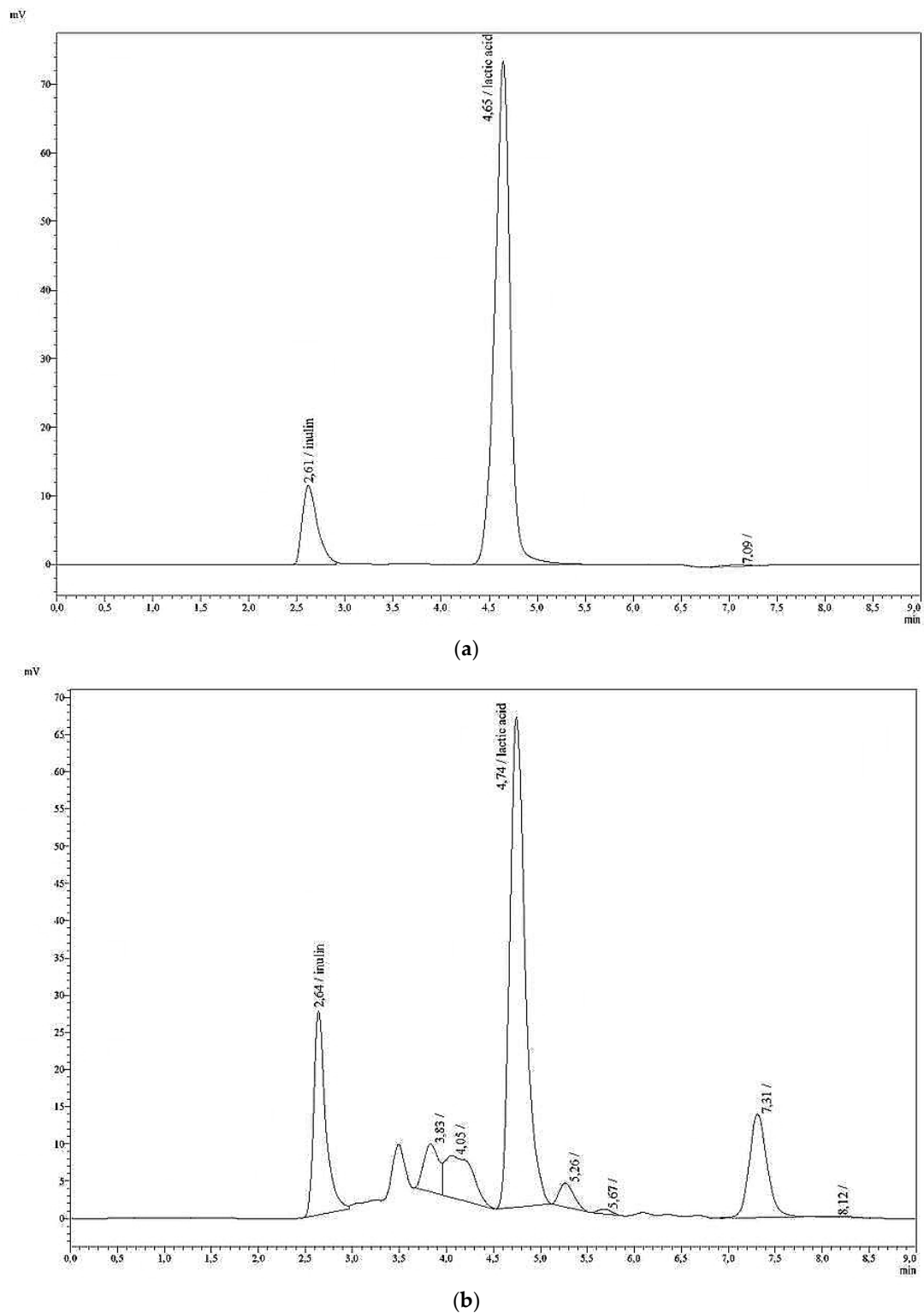


**Figure 2.** Separation of verbascone, stachyose, and raffinose in a Pb column, Nucleosil CHO 682, at 85 °C, with a RID and HPLC-grade water as the eluent in an aqueous extract of peas.

It was demonstrated (see Table 3) that, under the HPLC conditions used, mannose could not be separated from fructose. The poor resolution of fructose and mannose are, according to Agblevor et al. [27], mainly a problem of using a lead carbohydrate column. Ford [28] described the same problems concerning a good separation of fructose and mannose, but with gas-liquid chromatography. Furthermore (Table 3), the rhamnose peak interfered with that of galactose, isomaltose could not be isolated from maltose, and the cellobiose peak overlaid that of sucrose. As rhamnose, mannose, isomaltose, and cellobiose are rarely found in plants and feed materials [20] these substances were not included in the mixed stock solutions and, thus, were not quantitatively analysed. However, the peaks of fructose and galactose in the chromatograms need to be thoroughly checked for tailing and other possible anomalies, because this may indicate the presence of mannose and rhamnose, respectively. The compound identification can be secured by reanalysing after spiking with a standard of fructose or galactose, to further confirm the identity of the compounds.

As the simultaneous detection of fructans, along with other sugars, was desired, water was used as the extraction solvent. Another simple handling HPLC method was developed, in which the same standard substance as that described above is used. Separation and detection is performed using a Polypore H column and a RID. It was shown that inulin-type fructans could also be analysed in acidic silage extracts using this protocol. Along with the detection of lactic acid, as described by Weiß and Kaiser [29], inulin-type fructans were analysed. The chromatogram provided in Figure 3a shows good separation of lactic acid and fructans in a standard solution, and in a grass silage extract (Figure 3b).





**Figure 3.** Separation of inulin in a Polypore H column at 80 °C, with a RID and 0.01 N sulphuric acid as the eluent; (a) standard solution with lactic acid; and (b) aqueous grass silage extract.

For the detection of fructans, inulin was used as a representative compound of this sugar group. Inulin is one of five types of fructan [25], and is a mixture of fructose-derived polysaccharides with 2-1 linkages between fructosyl residues of varying chain length, with one terminal glucose molecule. The use of inulin as standard has been initiated by the food industry, where inulin-type fructans are

most frequently found [26]. Due to the complex structure of fructans, several procedures have been described for the measurement of fructan levels in plants and feedstuffs [30]. Current methods for measuring fructan levels in plant tissues are time-consuming and costly. They often involve multiple or sequential extractions, enzymatic or acid hydrolysis of fructan polymers, and multiple HPLC runs to quantify fructan-derived hexoses [31]. Most chromatography-based techniques present some limitations, but the major problem common to them all is the availability of standards. Therefore, the use of commercially-available inulin as a standard is widespread, but the inulin-based analysed contents of fructans represent only one compound of this polysaccharide group. It is expected that tools and methodologies addressing the difficulties concerning different structure types of fructans and their quantification will become available as fructan research develops.

### 3.2. Precision and Recovery Rate of Single Sugar Detection

The recovery rate and precision of the method were affected by the different sugar species and their concentration in the model solution. In Table 4, the results concerning the RC and precision of all nine single sugars, mannitol, and inulin is given for 10 repeated measurements on one day, in two model solutions. The mean RC ranged between 102.63% (model solution 60 mg/100 mL) and 99.97% (model solution 300 mg/100 mL). The relative standard deviation (RSD) varied from 1.79% to 0.96%.

In Table 5, the results concerning the RC and precision of all nine single sugars, mannitol, and inulin is given for five repeated measurements on one day, in six model solutions. The mean RC, depending on concentration, ranged from 98.74% to 101.37%, and had an RSD of 0.86% to 2.10% (Table 5). Depending on the sugar species, the recovery rate lay between 95.71% and 102.32%, with an RSD of 0.32% to 3.93%.

The data summarised in Table 6 characterise the precision and RC in aqueous maize silage extracts. Recovery rates varied between 98.20% and 102.82% and the RSD ranged between 0.45% and 4.15%, depending on concentration level. The variation in recovery rate (94.31–107.89%) and RSD (0.32–3.92%) was affected by the sugar species. Figure 4a shows an example of a chromatogram of this separation in spiked aqueous maize silage extracts. Results on the recovery rate of individual sugars in aqueous grass silage extracts (chromatogram example shown in Figure 4b) differing in sugar concentration after spiking with low and high standard solutions are given in Table 7.

**Table 4.** Recovery rate (RC, %), absolute (SD) and relative (RSD, %) standard deviation of two model solutions (conc. 1: 60 mg/100 mL and conc. 2: 300 mg/100 mL), measurements performed on one day ( $n = 10$ ).

Description	Set	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Ribose	Mannitol	Fructan	Mean
	value	mg/ 100 mL											
Conc. 1	60												
Real value		61.20	60.48	66.21	60.42	61.24	60.92	60.56	61.75	60.02	63.56	60.99	61.58
RC		102.00	100.81	110.36	100.70	102.07	101.53	100.93	102.91	100.03	105.93	101.64	102.63
SD		1.24	0.80	3.53	0.36	0.85	0.78	0.85	1.73	0.88	1.18	0.14	1.12
RSD		2.02	1.32	5.33	0.59	1.39	1.28	1.40	2.80	1.47	1.85	0.22	1.79
Conc. 2	300												
Real value		301.92	298.94	291.16	300.28	307.95	301.59	297.56	295.89	301.07	301.98	300.71	299.91
RC		100.64	99.65	97.05	100.09	102.65	100.53	99.19	98.63	100.36	100.66	100.24	99.97
SD		1.04	0.99	14.60	1.32	1.98	2.83	1.71	4.97	0.81	0.55	0.22	2.82
RSD		0.34	0.33	5.01	0.44	0.64	0.94	0.57	1.68	0.27	0.18	0.11	0.96
Mean													
RC		101.32	100.23	103.70	100.40	102.36	101.03	100.06	100.77	100.20	103.30	100.94	
RSD		1.18	0.83	5.17	0.52	1.02	1.11	0.99	2.24	0.87	1.02	0.17	

**Table 5.** Recovery rate (RC,%), absolute (SD) and relative (RSD,%) standard deviation of six model solutions with varying sugar concentrations from 15 to 300 mg/100 mL, measurements performed on five different days ( $n = 5$ ).

Description	Set	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Ribose	Inulin	Mannitol	Mean
	value	mg/ 100 mL											
	15												
Real Value		14.71	14.75	15.30	14.14	12.23	15.62	13.91	16.55	16.39	14.39	15.33	14.85
RC		98.07	98.31	101.99	94.25	81.53	104.15	92.73	110.33	109.29	95.93	102.20	98.98
SD		0.14	0.05	0.10	0.22	0.02	0.64	0.17	1.31	0.51	0.27	0.18	0.33
RSD		0.95	0.35	0.68	1.56	0.19	4.09	1.26	7.94	3.10	1.85	1.15	2.10
	30												
Real Value		29.63	30.86	31.23	30.17	29.51	29.70	30.07	32.05	30.62	29.77	30.92	30.41
RC		98.77	102.88	104.10	100.57	98.38	98.99	100.24	106.83	102.06	99.22	103.06	101.37
SD		1.48	0.11	0.13	0.22	0.51	1.39	0.26	0.36	0.76	0.22	0.19	0.51
RSD		4.99	0.34	0.42	0.74	1.73	4.68	0.86	1.12	2.47	0.72	0.63	1.70
	60												
Real Value		61.49	60.68	60.76	60.11	60.08	56.43	60.48	60.42	57.30	60.54	60.54	59.89
RC		102.48	101.14	101.27	100.19	100.14	94.06	100.79	100.70	95.50	100.90	100.90	99.82
SD		0.59	0.11	0.29	0.26	0.22	2.38	0.31	1.31	1.11	0.28	0.30	0.65
RSD		0.96	0.18	0.47	0.44	0.37	4.22	0.51	2.16	1.94	0.46	0.50	1.11

Table 5. Cont.

Description	Set	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Ribose	Inulin	Mannitol	Mean
	150												
Real Value		150.79	149.75	149.30	149.45	153.72	137.74	148.44	147.84	142.01	150.59	149.59	148.11
RC		100.53	99.83	99.53	99.64	102.48	91.83	98.96	98.56	94.68	100.39	99.73	98.74
SD		0.44	0.49	0.62	0.16	0.36	5.26	0.91	1.92	2.44	0.29	0.72	1.24
RSD		0.29	0.32	0.42	0.11	0.24	3.82	0.61	1.30	1.72	0.19	0.48	0.86
	240												
Real Value		241.25	239.81	238.46	241.39	247.24	220.76	236.31	236.29	232.63	239.05	239.46	237.51
RC		100.52	99.92	99.36	100.58	103.02	91.98	98.46	98.45	96.93	99.60	99.78	98.96
SD		0.74	0.90	0.96	0.63	0.27	7.40	1.32	2.75	4.34	1.02	1.40	1.98
RSD		0.31	0.37	0.40	0.26	0.11	3.35	0.56	1.16	1.87	0.43	0.59	0.86
	300												
Real value		305.19	301.50	301.03	305.01	311.89	279.71	297.18	297.20	294.18	300.24	301.47	299.51
RC		101.73	100.50	100.34	101.67	103.96	93.24	99.06	99.07	98.06	100.08	100.49	99.84
SD		2.81	1.02	1.69	3.66	2.68	9.60	1.18	3.24	4.41	1.25	0.38	2.90
RSD		0.92	0.34	0.56	1.20	0.86	3.43	0.40	1.09	1.50	0.42	0.12	0.99
Mean RSD		1.40	0.32	0.49	0.72	0.58	3.93	0.70	2.46	2.10	0.68	0.58	
Mean RC		100.35	100.43	101.10	99.48	98.25	95.71	98.37	102.32	99.42	99.35	101.03	

Table 6. Recovery rate (RC, %), absolute (SD) and relative (RSD, %) standard deviation in an aqueous maize silage extract ( $n = 3$ ) spiking with authentic standards.

Description	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Ribose	Mannitol	Mean
	mg/ 100 mL										
Sample + water	2.13	1.59	0.00	7.42	26.71	27.46	12.30	0.00	0.00	127.91	
SD	1.86	1.39	0.00	1.04	0.36	2.59	0.32	0.00	0.00	1.06	
RSD	87.28	87.22	0.00	14.05	1.35	9.45	2.60	0.00	0.00	0.83	
Sample + 7.5 mg/100 mL	9.72	11.66	7.72	11.78	34.68	32.70	19.54	6.88	7.81	140.50	
SD	0.14	0.27	0.37	1.15	0.62	1.32	0.88	0.48	0.45	0.19	0.59
RSD	1.43	2.32	4.80	9.79	1.79	4.05	4.48	7.03	5.71	0.13	4.15
Set value	9.63	9.09	7.50	14.92	34.21	34.96	19.80	7.50	7.50	135.41	28.05
RC	100.97	128.27	102.98	78.94	101.36	93.54	98.68	91.78	104.07	103.76	100.43
Sample + 15 mg/100 mL	16.09	19.37	18.41	20.79	44.83	41.84	27.86	13.71	14.91	147.40	
SD	0.30	0.16	0.32	0.41	0.38	0.55	0.51	0.08	0.95	0.52	0.42
RSD	1.87	0.80	1.75	1.99	0.84	1.31	1.84	0.56	6.37	0.35	1.77
Set value	17.13	16.59	15.00	22.42	41.71	42.46	27.30	15.00	15.00	142.91	35.55
RC	93.93	116.78	122.76	92.72	107.48	98.54	102.06	91.38	99.43	103.14	102.82
Sample + 30 mg/100 mL	31.68	33.48	29.75	35.62	60.37	56.22	42.21	29.54	33.65	159.28	
SD	0.52	0.45	0.39	0.30	0.26	0.33	0.08	0.46	2.34	0.50	0.56

Table 6. Cont.

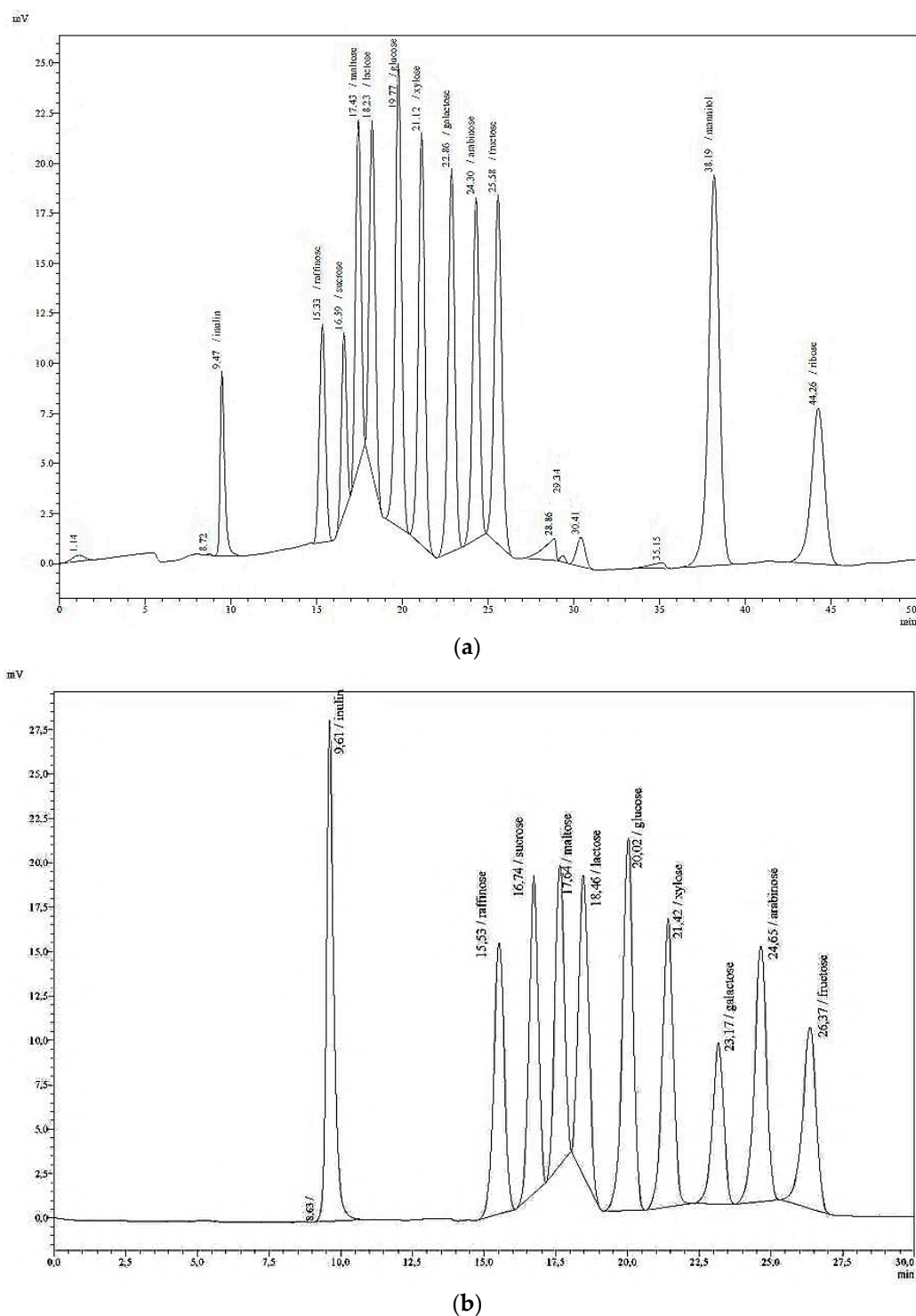
Description	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Ribose	Mannitol	Mean
RSD	1.64	1.34	1.30	0.85	0.43	0.59	0.19	1.55	6.96	0.31	1.52
Set value	32.13	31.59	30.00	37.42	56.71	57.46	42.30	30.00	30.00	157.91	50.55
RC	98.61	105.97	99.17	95.18	106.45	97.85	99.78	98.48	112.18	100.87	101.45
Sample + 75 mg/100 mL	74.14	76.44	74.37	81.66	105.17	99.08	88.62	69.96	72.11	203.42	
SD	1.13	1.42	1.22	1.13	1.62	1.08	0.74	0.41	0.47	1.46	1.07
RSD	1.52	1.86	1.64	1.38	1.54	1.09	0.84	0.59	0.65	0.72	1.18
Set value	77.13	76.59	75.00	82.42	101.71	102.46	87.30	75.00	75.00	202.91	95.55
RC	96.13	99.80	99.16	99.08	103.40	96.70	101.51	93.28	96.14	100.25	98.54
Sample + 120 mg/100 mL	114.72	117.59	117.55	125.65	146.43	142.97	131.77	121.51	116.31	248.59	
SD	0.44	0.10	0.97	0.24	0.30	0.78	0.20	2.00	0.35	0.46	0.58
RSD	0.38	0.09	0.82	0.19	0.20	0.54	0.15	1.65	0.30	0.19	0.45
Set value	122.13	121.59	120.00	127.42	146.71	147.46	132.30	120.00	120.00	247.91	140.55
RC	93.93	96.71	97.96	98.61	99.81	96.96	99.60	101.26	96.93	100.27	98.20
Sample + 150 mg/100 mL	146.05	151.27	149.35	159.54	179.09	177.42	164.93	151.87	150.65	279.62	
SD	0.47	6.52	0.89	0.33	0.16	0.27	0.23	0.62	5.34	0.54	1.54
RSD	0.32	4.31	0.59	0.21	0.09	0.15	0.14	0.41	3.54	0.19	1.00
Set value	152.13	151.59	150.00	157.42	176.71	177.46	162.30	150.00	150.00	277.91	170.55
RC	96.01	99.79	99.57	101.34	101.34	99.98	101.62	101.25	100.43	100.62	100.19
Mean RSD	1.20	1.79	1.82	2.40	0.82	1.29	1.27	1.96	3.92	0.32	
Mean RC	96.60	107.89	103.60	94.31	103.31	97.26	100.54	96.24	101.53	101.48	

**Table 7.** Recovery (RC,%) in an aqueous grass silage extract supplemented with calibrated model solution ( $n = 1$ ) at low standard level (5 to 30 mg/100 mL) and high standard level (15 to 300 mg/100 mL).

Description	Fructan	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Mean
mg/ 100 mL										
Low standard										
Sample + water	7.60	6.00	0.00	0.00	0.00	8.00	0.00	0.00	8.13	
Sample + 2.5 mg/100 mL	10.40	8.69	1.99	2.30	2.95	10.97	2.74	2.06	11.10	
Set value	10.10	8.50	2.50	2.50	2.50	10.50	2.50	2.50	10.63	
RC	102.99	102.31	79.52	91.80	117.80	104.55	109.48	82.40	104.44	96.48
Sample + 5 mg/100 mL	13.32	11.98	4.86	5.28	6.34	13.98	5.46	4.92	13.92	
Set value	12.60	11.00	5.00	5.00	5.00	13.00	5.00	5.00	13.13	
RC	105.71	108.96	97.16	105.52	126.76	107.56	109.14	98.36	105.96	107.24
Sample + 7.5 mg/100 mL	15.94	14.62	7.55	7.96	8.94	16.82	8.15	7.60	16.86	

Table 7. Cont.

Description	Fructan	Sucrose	Maltose	Lactose	Xylose	Glucose	Galactose	Arabinose	Fructose	Mean
Set value	15.10	13.50	7.50	7.50	7.50	15.50	7.50	7.50	15.63	
RC	105.58	108.28	100.65	106.19	119.17	108.53	108.72	101.28	107.87	107.36
Sample + 10 mg/100 mL	16.57	14.92	8.15	8.56	9.52	17.43	8.83	7.12	17.79	
Set value	17.60	16.00	10.00	10.00	10.00	18.00	10.00	10.00	18.13	
RC	94.13	93.24	81.48	85.60	95.20	96.83	88.30	71.21	98.13	89.34
Sample + 12.5 mg/100 mL	18.645	17.233	10.25	10.71	11.68	19.66	10.51	10.25	19.87	
Set value	20.10	18.50	12.50	12.50	12.50	20.50	12.50	12.50	20.63	
RC	92.77	93.17	82.02	85.69	93.47	95.92	84.08	81.97	96.32	89.49
Sample + 15 mg /100 mL	24.02	22.13	15.79	16.26	17.33	25.53	16.05	15.62	25.47	
Set value	22.60	21.00	15.00	15.00	15.00	23.00	15.00	15.00	23.13	
RC	106.30	105.40	105.29	108.41	115.52	111.00	106.97	104.15	110.10	108.13
Mean RC	101.25	101.89	91.02	97.20	111.32	104.07	101.12	89.90	103.80	
High standard										
Sample + water	8.35	6.98	0.00	0.00	1.26	8.38	0.00	0.00	9.43	
Sample + 7.5 mg/100 mL	16.01	12.99	8.45	8.90	9.54	16.69	8.93	8.16	17.32	
Set value	15.85	14.48	7.50	7.50	8.76	15.88	7.50	7.50	16.93	
RC	101.04	89.67	112.72	118.71	108.95	105.07	119.11	108.75	102.30	107.36
Sample + 15 mg/100 mL	22.48	19.37	15.21	15.58	16.59	23.51	16.20	15.13	24.60	
Set value	23.35	21.98	15.00	15.00	16.26	23.38	15.00	15.00	24.43	
RC	96.27	88.10	101.40	103.86	102.00	100.56	108.02	100.87	100.69	100.20
Sample + 30 mg/100 mL	38.05	34.50	31.09	31.43	32.36	38.66	31.22	30.99	39.76	
Set value	38.35	36.98	30.00	30.00	31.26	38.38	30.00	30.00	39.43	
RC	99.23	93.28	103.63	104.76	103.51	100.72	104.07	103.29	100.82	101.48
Sample + 75 mg/100 mL	81.36	77.94	74.98	75.07	75.34	83.45	75.18	74.61	84.10	
Set value	83.35	81.98	75.00	75.00	76.26	83.38	75.00	75.00	84.43	
RC	97.61	95.07	99.97	100.10	98.79	100.08	100.24	99.49	99.61	99.00
Sample + 120 mg/100 mL	128.73	124.65	122.21	122.12	121.76	129.46	120.78	120.82	130.38	
Set value	128.35	126.98	120.00	120.00	121.26	128.38	120.00	120.00	129.43	
RC	100.30	98.16	101.84	101.76	100.41	100.84	100.65	100.68	100.73	100.60
Sample + 150 mg/100 mL	161.81	158.10	155.91	155.68	155.28	163.64	155.96	155.16	163.67	
Set value	158.35	156.98	150.00	150.00	151.26	158.38	150.00	150.00	159.43	
RC	102.19	100.71	103.94	103.79	102.66	103.32	103.97	103.44	102.66	
Mean RC	99.44	94.17	103.92	105.50	102.72	101.77	106.01	102.75	101.14	



**Figure 4.** Separation of inulin, mono-, di-, oligo-, and polysaccharides in a Pb column, Nucleosil CHO 682, at 85 °C, with a RID and HPLC-grade water as the eluent; (a): aqueous maize silage extract, spiked with standard solution (300 mg/100 mL per sugar); (b): aqueous grass silage extract, spiked with standard solution (300 mg/100 mL per sugar).

For inulin-type fructans, sucrose, glucose, and fructose, recovery rates of 101.25% to 104.07% in the low standard concentration range were determined, which are in line with those observed for the aqueous maize silage extract (Table 6). Regarding all other individual sugars, the deviation in recovery rates was markedly larger. The values of the low standard measurement series have not been



used in the summarising data for RC and RSD. Recovery rates using the high standard concentration were from 94.17% to 106.01% for all single sugars and, therefore, better than using a low standard concentration, and similar to those for the maize silage extract.

The individual detection of inulin in standard solutions with the Brownlee-H column ( $n = 10$ ) showed recovery rates of 123.73% and 99.30%, for the tested concentrations of 9 and 135 mg/100 mL, respectively. The RSD ranged from 0.04% to 7.52%. RC depends on the concentration of inulin in the model solutions (Table 8). In the range between 15 and 300 mg/100 mL the RC is very accurate at concentrations from 60 mg to 100 mL.

**Table 8.** Recovery rate (RC,%), absolute (SD) and relative (RSD,%) standard deviation of six model solutions varying in inulin concentrations from 15 to 300 mg/100 mL, measurements performed on five different days ( $n = 5$ ), column Polypore H (Brownlee).

Description	15	30	60	150	240	300	Mean
	mg/ 100 mL						
Real Value	15.88	28.25	60.54	150.25	238.94	299.01	
RC	105.86	94.15	100.90	100.16	99.56	99.67	100.05
SD	0.03	0.04	0.094	0.17	0.12	0.42	0.14
RSD	0.17	0.13	0.156	0.11	0.05	0.14	0.13

For both stock solutions and samples supplemented with known amounts of individual sugars, the method showed good recovery rates, ranging between 94.0% and 108.0%. Additionally, the mean RSDs of 0.7–5.2% confirmed the high accuracy of the method. In a study by Hall [1], in which the reduction of sugar assays was used for the estimation of glucose, fructose, galactose, sucrose, maltose, lactose, raffinose, and inulin in feedstuffs, recoveries in the range of 92.0–108.0% were assessed as acceptable. The standard deviation was between 0.1% and 4.05%, but the RSD for unhydrolysed substrates are between 0.1% and 6.25% (61.1% for sucrose, 65.0% for raffinose) and, therefore, less precise than the method described in this study. In a study of the simultaneous quantification of stachyose, raffinose, and galactose with HPLC and RI-detection [32] the authors calculated recoveries of 97.71–102.61% with an RSD of 0.92–2.41%. This method was found to be useful for the quantification of these three sugars in the enzymatic degradation of stachyose. Duarte-Delgado et al. [33] published a method concerning the development and validation of a HPLC method with RI detection to quantify sucrose, glucose, and fructose in tubers of solanum, and reported an acceptable range of RSD from 5.3% to 7.3% and the limit of detection of 3.0 mg/L for the three sugars. Both parameters are less precise compared with the method presented here. Agblevor et al. [27] developed an HPLC method for biomass sugars (pre-treated corn stover feedstock) with an evaporative light-scattering detector (ELSD) and an RID. They analysed arabinose, xylose, fructose, glucose, and sucrose up to a maximum of 6.67% RSD, but higher for mannose (22.07%) and galactose (23.29%). Longland et al. [8] described a HPLC method for the detection of fructan (inulin) in pasture grasses. The RSD showed a wide spread, between 0.67% and 14.82%, calculated based on the published data.

When compared to other studies, it can be concluded that the precision and accuracy of the new HPLC method is very high. The recovery rates were improved by applying the high standard solutions of up to 300 mg/mL, as opposed to the least concentrated solutions (max. 30 mg/100 mL). Lower sugar concentrations in calibration solutions, e.g., 150 or 200 mg/mL, could also be used. In any case, the sugar concentration of the calibration solutions should be adjusted to the expected content of individual sugars in the analysed samples. A summary of values for recovery rate (RC, range) and relative standard deviation (RSD, maximum value) concerning the precision of the HPLC method for single sugar analysis, using a Pb column, and high standard calibration is given in Table 9.

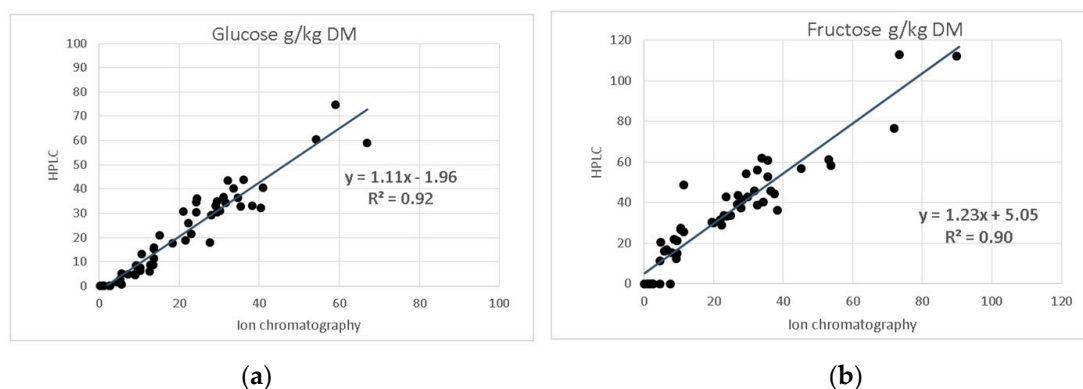
**Table 9.** Single sugars with recovery rate (RC, %) and relative standard deviation (RSD, %).

Sugar Species	RC (%)	RSD (%)
Sucrose	94.2–101.3	1.4
Maltose	100.2–107.9	1.8
Lactose	101.1–105.5	5.2
Xylose	94.3–102.7	2.4
Glucose	98.3–103.3	1.0
Galactose	95.7–106.0	3.9
Arabinose	98.4–102.8	1.3
Fructose	96.2–102.3	2.5
Ribose	99.4–101.5	3.9
Mannitol	101.0–103.3	1.0
Fructan (as Inulin)	99.4–100.9	0.7

#### 4. Comparative Analyses

The freeze-dried samples from forages ( $n = 36$ ) and sugar beet pulp silages ( $n = 18$ ) were subjected to cold-water extraction and filtration, and subsequently analysed with the HPLC method described herein. The results from the sugar species glucose and fructose were compared with those obtained by the laboratory of the University of Kiel, which uses an ion-chromatographic method after hot-water extraction. By HPLC with RID, glucose was detected at up to 74.86 g/kg DM, whereas fructose was found at up to 123.90 g/kg DM. Moreover, very low levels of sucrose ranging between 1.22 and 9.35 g/kg DM were detected with the HPLC method in eight samples. In addition, three samples with concentrations between 16.85 and 21.73 g/kg DM were found using the HPLC method. These results were not confirmed by ion-chromatography. In sugar beet pulp silage samples sucrose was also not detectable using the HPLC method.

As shown in Figure 5, a very close correlation exists between HPLC and ion-chromatography data for glucose ( $R^2 = 0.92$ ) and fructose ( $R^2 = 0.90$ ), regardless of the different conditions of extraction and detection. In addition, xylose was found in 20 samples at 2.99 to 30.15 g/kg DM and lactose in 11 samples at 28.11 to 38.88 g/kg DM using the HPLC method. For these and other individual sugar species, and fructan, comparative results are not available.



**Figure 5.** Correlation between the concentrations of glucose (a) and fructose (b), analysed by HPLC-RID and ion chromatography ( $n = 54$ ).

The comparison of the determined HPLC-RID values on glucose and fructose with those analysed by ion chromatography revealed a high degree of correlation, although the fructose contents detected were consistently higher with ion-chromatography.

To the authors' knowledge, an official reference method for the detection of a variety of sugar species in plants and feed materials does not exist. Sugar analyses by other laboratories also only

cover the most frequently occurring sugars—glucose, fructose, and sucrose—and are unpublished in-house methods.

Further studies comparing different methods, e.g., colorimetry, and separation conditions are underway, and are currently being prepared for publication.

## 5. Effect of Sample Preparation and Storage Temperature on Sugar Concentration Analyses

It is well-known that changes can occur in the composition of water-soluble carbohydrates (WSC) in plant materials between sampling and analysis. For conservation and sample storage, freeze-drying of the sample is superior to oven-drying because microbial activity in the early stages of the oven-drying process cannot be ruled out. According to Smith [34], drying at temperatures below 50 °C allows time for DM losses by respiration and enzymatic conversions. Heat-drying at temperatures above 80 °C can cause thermo-chemical degradation. McGrath [35] published a study concerning water-soluble carbohydrates in ryegrass, and found that heating at temperatures over 100 °C resulted in a loss of WSC, since values were lower than those obtained after freeze-drying. In general, lower sugar concentrations can be expected in oven-dried samples when compared with a lyophilised specimen. Furthermore, Smith [34] concluded that changes occur in non-structural carbohydrate concentrations during the storage of both freeze- and heat-dried tissues, and that the analyses should be conducted as soon as possible after sampling. It should be stressed that, from extraction to analysis, the degradation of sugars by microorganisms must be prevented. Extraction should also be carried out as quickly as possible, and the samples measured immediately, if possible. Samples should be stored at 4 °C in the HPLC sampler until injection, as significant sugar losses may otherwise occur. In particular, it was found that the concentrations of glucose and fructose were markedly lower in the samples that had not been stored in the fridge after extraction. This is likely to have been caused by microbial sugar degradation. According to Smith [34] this probably reflects the resistance of enzymes to the temperature effects before and during the drying process. Attempts to stabilise the extracts with solutions of either silver chloride or sodium azide (0.01%) failed due to interference regarding sugar separation, and resulted in extensive/laborious regeneration processes of the  $\text{Pb}^{2+}$ -column.

Comparative measurements of fructan with a Brownlee-H column in 36 aqueous silage extracts either immediately after extraction, or after storage in the freezer showed a very high degree of correlation ( $R^2 = 0.92$ ) with low RSD, varying between 0% and 2.18%. Preliminary evidence suggests that fructans can also be detected without loss in silage extracts which had been stored in the freezer. In most cases, the silage extracts are weakly acidic (pH under 5 or 4) and conserved with toluol, which is used as an inhibitor for microbial activity.

## 6. Conclusions

The HPLC method using a Nucleosil column loaded with  $\text{Pb}^{2+}$  ions, a RI detector, and HPLC-grade water as an eluent gives precise and reproducible results regarding the detection of individual sugars in extracts of plants and feed materials. The method can be used for the detection of sucrose, maltose, lactose, xylose, glucose, galactose, arabinose, fructose, ribose, and mannitol. Furthermore, depending on the plant material, the sugars verbascose, stachyose, and raffinose can be separated. The method was extended to the analysis of fructan with inulin as standard. The peaks were well resolved and the reproducibility of the analysis with 94–108% recovery (RC) and, a relative standard deviation (RSD) of up to 5%, was very good.

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**Author Contributions:** Manuela Alt performed the experiments, analysed the data; Kirsten Weiß conceived and designed the experiments, wrote the paper.

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