

Determination of Monosaccharides in Grain by CE/MS/MS

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Abstract

This Application Note describes a sensitive and reliable method for the determination of monosaccharides in grain. The monosaccharides were extracted from samples by acid hydrolysis using sulfuric acid (H_2SO_4), followed by neutralization with barium hydroxide ($Ba(OH)_2$), which precipitates barium sulfate ($BaSO_4$) and reduces the content of sulfate in the samples. The best separation of fucose, galactose, arabinose, glucose, rhamnose, xylose, mannose, fructose, and ribose, plus inositol as internal standard, was obtained with 0.5 M triethylamine (pH 12.3) as the background electrolyte (BGE) in less than 14 minutes using a fused silica capillary and detection by ESI-MS/MS. The proposed method is simple, fast, and presents linear calibration curves in the range of 0.02 to 2.0 mM greater than 0.997 and relative standard deviation for replicate injections lower than 5 %. The limit of detection (LOD), based on the signal-to-noise ratio (S/N), for all monosaccharides was lower than 3.4 μM . The applicability of this method for the analysis of real samples was demonstrated with soybean, corn, and coffee grain samples.

Introduction

Carbohydrates are one of the most important components in foods, and include a wide range of macromolecules that can be classified by their chemical structure into three groups:

- Mono and disaccharides, with low molecular weight
- Oligosaccharides, with intermediate molecular weight
- Polysaccharides, with high molecular weight

Carbohydrates are the main source of energy for all body functions, particularly brain functions, and they are necessary for the metabolism of other nutrients. Glucose, fructose, and galactose are the primary monosaccharides present in the human diet, while sucrose and some sugar alcohols are often used as food additives. The physicochemical properties of foods such as sweetness, appearance, stability, and texture depend on the type and concentration of all carbohydrates present. Therefore, monitoring of carbohydrates in food samples is essential and important to verify their quality, ripeness, and possible adulterations^{1,2}.

Carbohydrates are nonvolatile compounds, and are therefore suitable for analysis based on high-performance liquid chromatography (HPLC). However, the detection of carbohydrates by ultraviolet visible (UV-Vis) is severely limited because their structures do not contain chromophore groups. The most used analytical techniques for carbohydrates in foods are HPLC with a refractive index detector (RID), HPLC with an evaporate light scattering detector (ELSD), and HPLC with pulsed amperometric detection (PAD). However, every detector has its own limitations, and in all cases the chromatographic methods involve long analysis times, mainly due to lengthy post-run column re-equilibration³⁻⁵.

Capillary electrophoresis (CE) is a powerful separation technique that can provide high resolution efficiency, and is becoming a powerful tool in food analysis⁶. The hydroxyl groups of carbohydrates are weak acids and can be ionized under strong alkaline conditions (pH >12). So, the analysis of underivatized monosaccharides such as fucose, galactose, arabinose, glucose, rhamnose, xylose, mannose, fructose, and ribose was achieved by CE using 0.5 M triethylamine (TEA) (pH 12.3) as the BGE followed by ESI-MS/MS detection, in negative ion mode. Figure 1 shows the molecular structure of the monosaccharides analyzed in this work.

All separations were performed at 25 °C using 0.5 mM TEA, pH 12.3 as the BGE. New fused silica capillaries were preconditioned by flushing 0.1 M NaOH solution (five minutes), deionized water (10 minutes), and BGE (10 minutes). An additional flushing step with BGE for 60 seconds was included between the runs. Samples were introduced hydrodynamically in 30 seconds at 100 mbar and analyzed with an applied voltage of 25 kV. The mass spectrometer was operated in negative ionization mode, using multiple reaction monitoring (MRM) mode for one specific transition. Table 1 lists the migration time (t_M), monitored ions, and other MS/MS acquisition parameters used for the identification and quantification of the targeted monosaccharides in grains.

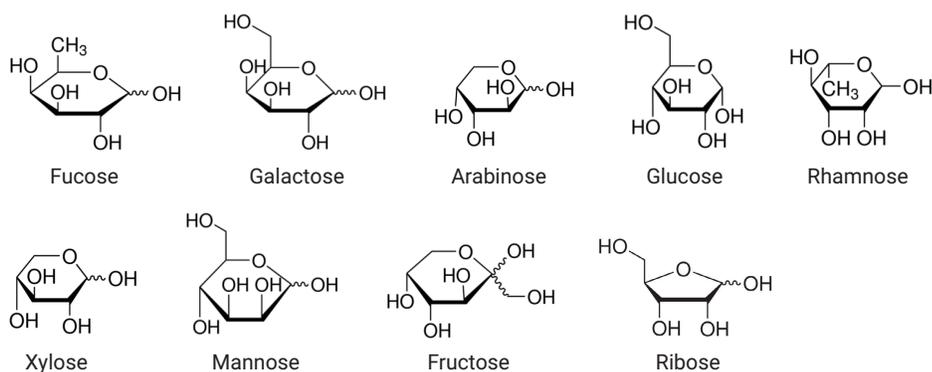


Figure 1. Molecular structure of monosaccharides analyzed.

Table 1. Migration time (t_M) and MS/MS acquisition parameters used for the identification and quantification of monosaccharides in grain.

Monosaccharide	t_M (min)	Q1 ^a (m/z)	Q3 ^b (m/z)	CE ^c (V)	FE ^d (V)
Inositol	10.6	179.1	87.1	17	101
Fucose	11.2	163.1	103.0	4	70
Galactose	11.5	179.1	89.0	5	70
Arabinose	11.7	149.0	89.1	9	70
Glucose	11.7	179.1	89.0	5	70
Rhamnose	11.9	163.1	103.0	4	70
Xylose	12.0	149.0	89.1	9	70
Mannose + Fructose	12.1	179.1	89.0	5	70
Ribose	12.2	149.0	89.1	9	70

^a Precursor ion (Q1), ^b Fragment ion (Q3), ^c Collision energy, ^d Fragmentor energy

Sample preparation

Samples of soybean, corn, and coffee were bought in local stores and homogenized using a blender mixer. A 1.0 g aliquot of homogenized material was weighed and transferred to a 50-mL polypropylene tube, followed by the addition of 10 mL of 1.0 M H₂SO₄ to promote the acid hydrolysis. The tube was heated at 90 °C for 150 minutes and manually stirred every 30 minutes. The solution was cooled to room temperature, filtered (Agilent p/n 5190-5264), and the final volume was adjusted to 10 mL with Milli-Q water. The H₂SO₄ hydrolysis increases the conductivity of the sample, making it incompatible with electrophoretic separation. A simple neutralization does not diminish the conductivity to an acceptable level, so the neutralization with Ba(OH)₂ was carried out by mixing 1 mL of 1.0 M Ba(OH)₂ suspension with 1 mL of sample solution and simultaneous precipitation of BaSO₄. After the neutralization and precipitation step, the samples were centrifuged for one minute at 6,000 rpm and filtered through a 0.2 µm PVDF and PP membrane (Agilent Captiva filter cartridges, p/n A5300002), diluted 10 times, then transferred to an Agilent 250-µL polypropylene vial (p/n 5188-2788) for CE/MS/MS analysis.

Results and discussion

The BGE and sheath liquid composition, as well as applied potential and hydrodynamic injection, were optimized for separation efficiency and sensitivity. However, the resolution was not sufficient to separate mannose and fructose. Figure 2 shows a representative electropherogram for the monosaccharide standards and internal standard (IS) at 1 mM each at optimum conditions.

Experimental

CE Conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	0.5 M TEA, pH 12.3
Applied voltage	25 kV
Capillary	Agilent Fused-silica capillary, 20 µm id with 90 cm total length (p/n 160-2660-5, cut down to 90 cm)
Injection	30 seconds at 100 mbar
Temperature	25 °C

MS Conditions

Parameter	Value
Instrument	Agilent 6430 Triple Quadrupole LC/MS
Ion mode	ESI, negative ionization
Sheath liquid	10 mM TEA/methanol (50:50 v:v)
Flow rate	6.0 µL/min
Capillary voltage	4,500 V
Drying gas flow (N ₂)	6 L/min
Drying gas temperature	200 °C
Nebulizer pressure	10 psi

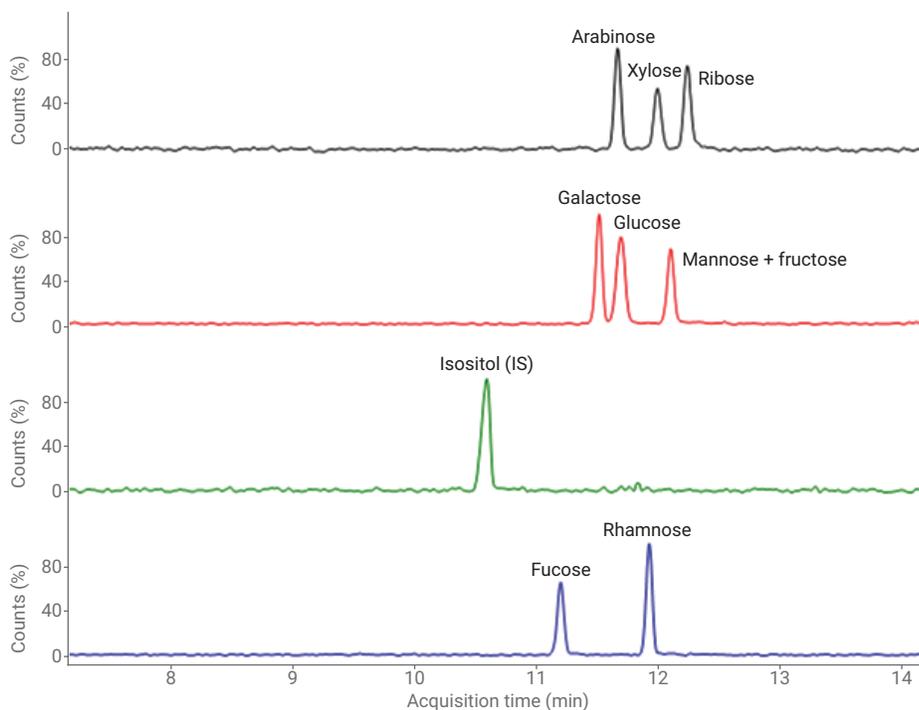


Figure 2. Normalized MRM electropherogram at optimum conditions of the monosaccharides and IS in BGE at 1 mM each.

The linearity of the analytical curve was studied at 11 different concentration levels ranging from 0.02 to 2.0 mM, using inositol at 1.0 mM as the IS. Each concentration level was analyzed in triplicate. Figure 3 shows an example of the response for galactose monosaccharide, using Agilent MassHunter quantitative software (B.08.00).

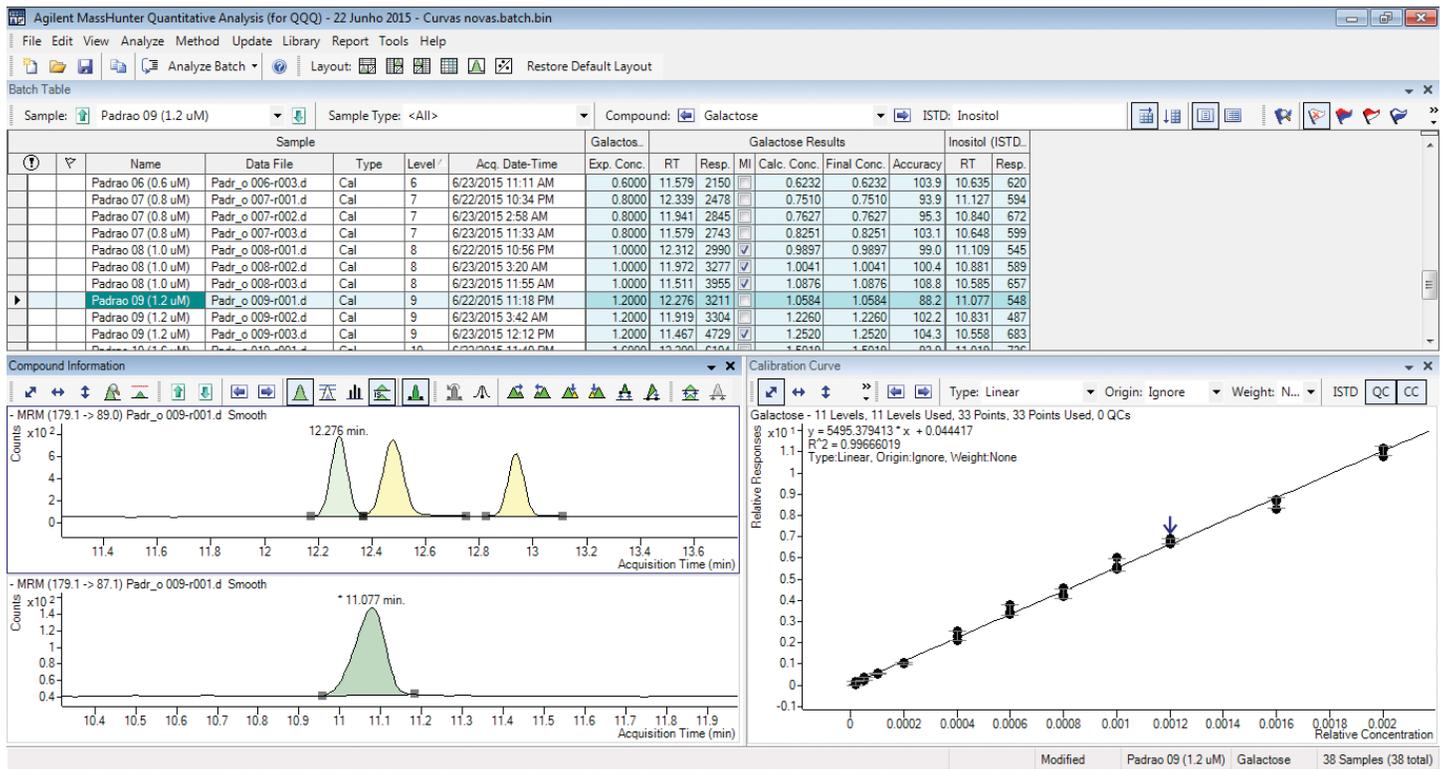


Figure 3. Calibration curve of galactose using MassHunter quantitative software (B.08.00).

The response function was found to be linear, with coefficient of determination (R^2) values higher than 0.997 for all calibration curves. The LODs and limits of quantification (LOQs) were determined considering the corresponding concentration to produce a signal 3 and 10 times the baseline noise, in a close region to the migration time of each monosaccharide, respectively. The proposed method enabled us to detect monosaccharides at levels between 1.2 to 3.4 μM . Table 2 shows the regression equations and other characteristic parameters for the developed method.

Monosaccharides were determined by external calibration in commercial samples of soybean, corn, and roasted coffee. The results and standard deviations expressed in percentages of monosaccharides are presented in Table 3.

Conclusion

We have shown that CE/MS/MS is well suited for the analysis of monosaccharides in grain. The proposed method presented a linear response in the concentration range of 0.02 to 2.0 mM, with excellent precision data for replicate injections and LOD lower than 3.4 μM . In addition, the method is simple and fast, spending less than 14 minutes per sample and uses a small amount of sample with low reagent consumption. The combination of the hydrolysis and neutralization step using $\text{Ba}(\text{OH})_2$ simplifies this procedure, rendering it suitable for use in other types of grain.

Table 2. Linear range, regression equation, determination coefficient, standard deviation of residuals, limits of detection (LOD) and quantitation (LOQ) of the proposed method for monosaccharides in grain.

Monosaccharide	$Y = a + bx$	R^2	$S_{y/x}$	LOD (μM)	LOQ (μM)
Fucose	$y = 0.044 + 1.336 x$	0.998	0.043	2.4	8.1
Galactose	$y = 0.097 + 0.285 x$	0.999	0.010	1.4	4.6
Arabinose	$y = -0.103 + 2.355 x$	0.998	0.079	1.9	6.2
Glucose	$y = 0.246 + 0.441 x$	0.997	0.015	1.9	6.2
Rhamnose	$y = -0.106 + 2.018 x$	0.998	0.059	3.4	11.3
Xylose	$y = -0.065 + 3.395 x$	0.998	0.112	2.8	9.3
Mannose + Fructose	$y = -0.064 \pm 2.279 x$	0.998	0.076	1.5	5.0
Ribose	$y = -0.187 + 2.825 x$	0.998	0.099	1.2	3.8

^a Intercept, ^b Slope, R^2 = Determination coefficient, $S_{y/x}$ = Standard deviation of residuals

Table 3. Levels of monosaccharides (% w/w) obtained by CE/MS/MS ($n = 3$) in samples of coffee, soybean, and corn.

Monosaccharide	Coffee	Soybean	Corn
Fucose	ND	0.28 ± 0.01	ND
Galactose	8.78 ± 0.30	4.53 ± 0.20	ND
Arabinose	2.45 ± 0.02	2.93 ± 0.02	0.59 ± 0.03
Glucose	0.29 ± 0.01	4.26 ± 0.19	58.60 ± 3.22
Rhamnose	0.32 ± 0.02	0.37 ± 0.02	0.02 ± 0.00
Xylose	9.84 ± 0.47	2.27 ± 0.07	ND
Mannose + Fructose	0.14 ± 0.01	0.64 ± 0.02	1.16 ± 0.03
Ribose	0.05 ± 0.00	0.27 ± 0.01	0.03 ± 0.00

^a The level is expressed as percentage of the monosaccharide after hydrolysis over the total dry mass of the sample. ND = not detected.

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