Application Update 202

A Fast Method for Sugar Analysis of Instant Coffee Samples

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Key Words
Dionex CarboPac SA10-4µm Column, Electrochemical Detection, Carbohydrates, HPAE-PAD, AN280, Dionex Integrion HPIC System

Goal
To develop a fast IC method for the determination of sugars in instant coffee using a Thermo Scientific™ Dionex™ CarboPac™ SA10-4µm column with electrolytically generated eluent and an HPIC system with electrochemical detection.

Introduction
Coffee is the most widely consumed hot beverage worldwide. World consumption of coffee has steadily increased in the past 50 years, growing from 57.9 million bags in 1964 to 142 million bags in 2012.[1] The process of bringing the harvested coffee fruits to consumers as a beverage involves a series of steps. Greater control of each step of this process improves the ability to produce a high-quality coffee.

Carbohydrates are an important constituent of the coffee beans. Based on dry weight, the carbohydrates form about 50% of the green coffee bean. These carbohydrates undergo complex changes during the roasting process and can affect the final taste and aroma properties of the coffee. Carbohydrate content is used for detecting coffee adulteration.[2] Because carbohydrates represent a high percentage of the coffee bean's mass, their impact on viscosity plays an important role in soluble coffee processing.[3]

Thermo Scientific Application Note 280[4] (AN280) described a fast method for determining carbohydrates in coffee using the Dionex CarboPac SA10 column with electrolytically generated eluent. The current study updates the fast method described in AN280 with a Dionex CarboPac SA10-4 µm column. Due to the smaller particle size, this column provides more efficient peaks and better resolution, making the sample analysis easier and more reliable. Moreover, the system used for the analysis is updated to the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system. This system combines flexibility and ease-of-use with high sensitivity and selectivity, bringing a new level of convenience and cost effectiveness to simple sugar analysis. The method proposed here separates nine common carbohydrate sugars in less than six minutes, which allows for shorter sample turnaround times and reduced eluent consumption, thereby improving the overall process economics. Using this method, carbohydrates present in soluble and total carbohydrate extracts of instant coffee were quantified in less than six minutes. The results for the method linearity, precisions, and robustness are presented.

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Equipment

- Thermo Scientific Dionex Integrion HPIC system using electrochemical detection includes:
  - Electrochemical Detector (ED)
  - Electrochemical Cell, Reference Electrode and Disposable Working Electrode with Gasket
  - Detector Compartment Temperature Control
  - Tablet Control
  - Vacuum Degas Kit
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control option (P/N 074926) and 1.5/0.3 mL vial tray (P/N 074936)

Reagents and Standards

Reagents

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better

Standards

L(–)-Fucose (Sigma-Aldrich®, P/N F2543)

D-Galactose (Sigma-Aldrich, P/N G0625)

D(+)-Mannose (Sigma-Aldrich P/N M6020)

D-Fructose (Sigma-Aldrich P/N F2543)

D-Xylose (Sigma-Aldrich P/N X1500)

Sucrose (Sigma-Aldrich P/N 84097)

D-Glucose (Avantor®, P/N 1910-01)

D(-)-Arabinose (Sigma-Aldrich, P/N A3131)

Mannitol (Thermo Scientific Acros, P/N AC125340010)

Conditions	
Column:	Dionex CarboPac SA10-4µm Analytical, 4 x 250 (P/N 088233), Dionex CarboPac SA10-4µm Guard 4 x 50 (P/N 088234)
Column Temperature:	40 °C
Compartment Temperature:	30 °C
Flow Rate:	1.5 mL/min
Eluent:	1 mM KOH
Working Electrode:	Gold on PTFE disposable
Electrochemical Cell Gasket:	62 mil
Reference Electrode:	Ag/AgCl
Sampler Tray Temperature:	4 °C
Injection Volume:	0.4 µL (push full mode using a 4-Port Injection Valve pod) *(Note: A High Concentration Carbohydrate Analysis Kit and a 4-port injection valve were installed for this application.)*
Elution Conditions:	Isocratic
Eluent Source:	Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge with Dionex CR ATC-600 Continuously Regenerated Anion Trap Column

Carbohydrate Waveform:

Time (s)	Potential (V)	Integration
0.00	+0.10	
0.20	+0.10	Begin
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

Software

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, CM 7.2 SR4

Consumables

Table 1 lists the consumable products and part numbers.

Table 1. Consumables for the Dionex Integrion HPIC System.

Product Name	Product Details	Part Number
Thermo Scientific™ Dionex™ IC PEEK Viper™ Fitting Tubing Assembly Kits	Dionex IC PEEK Viper fitting tubing assembly kit for the Dionex Integrion HPIC system: Includes one each of P/Ns: 088805-088811	088798
Dionex IC PEEK Viper Fitting Tubing Assemblies, Included in Kit, P/N 088798	Guard to separator column: 0.007 × 4.0 in (102 mm)	088805
	Valve to guard column: 0.007 × 5.5 in (140 mm)	088806
	EGC Out to CR-TC Eluent In: 0.007 × 6.5 in (165 mm)	088807
	0.007 × 7.0 in (178 mm) –not used in ED	088808
	Separator to ED Cell In: 0.007 × 7.0 in (178 mm), ED	088809
	0.007 × 9.0 in (229 mm) –not used in ED	088810
	CR-TC Out to Degasser In: 0.007 × 9.5 in (241 mm)	088811
Dionex AS-AP Autosampler Vials	Package of 100, polystyrene vials, caps, septa, 0.3 mL	055428
4-Port Injection Valve Pod	Install in place of 6-port valve pod. The 4-port pod has an internal sample loop of 4 µL.	074699
Dionex EGC 500 KOH Eluent Generator Cartridge*	Eluent generator cartridge required when using 4 µm particle columns	075778
Dionex CR-ATC 600 Electrolytic Trap Column*	Continuously regenerated trap column used with Dionex EGC KOH 500 cartridge	088662
Dionex HP EG Degasser*	Degasser module	075522
Electrochemical Detector (ED)	Without cell, with shipping container	22153-62035
Electrochemical Cell	Includes knob and support block	072044
pH-Ag/AgCl Reference Electrode	Reference electrode	061879
Au on PTFE Electrodes	Working electrode, package of six	066480
High Concentration Carbohydrate Analysis Kit	Includes 62 mil gasket and modified spacer block	085324
62 mil Gasket**	If purchased separately, package of two	075499
pH Buffer, pH 7	Reference electrode pH calibration standard	SB107-500***
pH Buffer, pH 10	Reference electrode pH calibration standard	SB115-500***
Thermo Scientific™ Dionex™ OnGuard™ II RP Filter Cartridges	Reversed-phase cartridges for manual sample preparation	082760
Thermo Scientific Dionex OnGuard™ II Ag/H Filter Cartridges	Cation-exchange cartridges for manual sample preparation	082756
Nalgene Syringe Filter	Sterile, PES Membrane, 0.2 µm	725-2520

* High-pressure device recommended for 4 µm particle resin columns.
** 1 mil = 0.001 inch
*** Fisher Scientific P/N

Samples

Two commercially available instant coffee samples, designated as samples A and B, were used.

Two extracts were prepared for both instant coffee samples using the preparation in AOAC Method 995.13,[5] which is described below.

1. Soluble sugars extract in water
2. Total sugars extract in 1M HCl

Methods

Isolation of Carbohydrates

Use soluble coffee without grinding or homogenization. Prepare the free and total carbohydrate extracts using the methods described in AOAC Method 995.13.[5]

Free Carbohydrates

Weigh 300 mg of instant coffee to the nearest 0.1 mg into a 100 mL volumetric flask. Add 70 mL of DI water and shake the flask until dissolution is complete. Dilute the solution to volume with DI water. Prepare a Thermo Scientific Dionex OnGuard II RP cartridge by passing 5 mL methanol and 10 mL DI water. Filter 5–10 mL of solution through the cartridge. Discard the first 3 mL. Pass the filtrate through a 0.2 µm syringe filter prior to injection.

Total Carbohydrates

Weigh 300 mg of instant coffee to the nearest 0.1 mg into a 100 mL volumetric flask. Add 50 mL of 1.0 M HCl and swirl the flask. Place the flask in a boiling water bath for 2.5 h. *(Note: Always keep the level of solution in the flask below that of water in the bath.)* Swirl the flask by hand every 30 min, and then cool the flask to room temperature under tap water. Dilute the solution to 100 mL with DI water and filter through folded filter paper. Prepare a Dionex OnGuard II Ag/H cartridge by passing 15 mL of DI water through it. The Dionex OnGuard II Ag/H cartridge is a layered disposable cartridge containing two high-capacity strong acid cation exchange resins: the first loaded with Ag ions to eliminate the chloride anion by precipitation and the second in acid form to trap any Ag that might break through from the first cartridge (thus protecting the column and the working electrode). Pass the solution (10–12 mL) through the Dionex OnGuard II Ag/H cartridge. Discard the first 6 mL. Filter the remaining solution through a 0.2 µm membrane filter prior to IC analysis.

Generation of Potassium Hydroxide Eluent

Generate the potassium hydroxide (KOH) eluent online by pumping high-quality degassed DI water through the Dionex EGC 500 KOH cartridge. The Chromeleon CDS software tracks the amount of KOH used and calculates the remaining lifetime. Although eluents can be prepared manually if needed, we strongly recommend running this application with eluents prepared by an eluent generator and do not recommend using manually prepared eluents. Consistent manual preparation of a 1 mM hydroxide eluent or a 10 mM hydroxide eluent (if proportioning is used) is difficult due to variable carbonate contamination. The impact of carbonate contamination is significant when using low-concentration hydroxide eluents. If eluents must be prepared manually, use NaOH rather than KOH and prepare according to the general instructions for hydroxide eluents in Technical Note 71.[6] For this application, electrolytic eluent generation delivers superior performance and is used for all the data in this study. Performance for this application with manually prepared eluents was not evaluated and could be different than the results reported here.

(Note: Stronger retained matrix components can be accumulated on the column, so that a loss of resolution is observed. Column performance can be restored by washing the column for 3 h with 60 mM KOH.)

Results and Discussion

Separation

Figure 1 shows a standard sample containing nine common sugars separated using a Dionex CarboPac SA10-4µm column. The nine sugars are well resolved within six minutes. As discussed in AN280, two sugar pairs, namely rhamnose/galactose and fructose/ribose, coelute. Rhamnose and ribose were not added to the standard mix based on the knowledge that both are not present in the coffee samples studied. However, for samples that potentially contain coeluting sugars, AOAC Method 995.13 described in AN280[4] should be used.

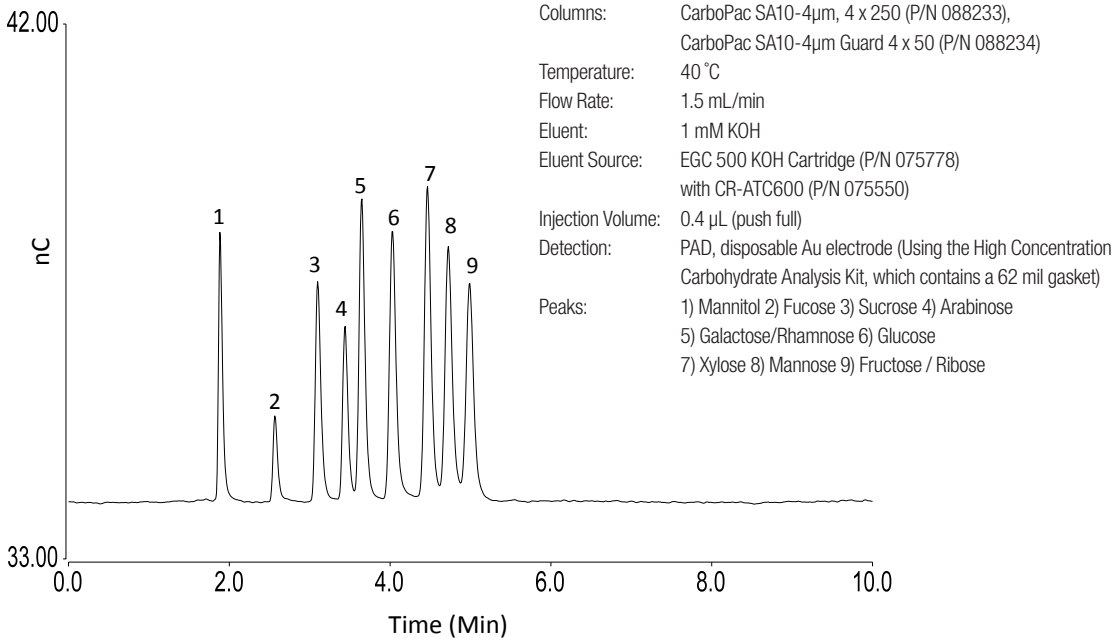

Figure 1. Nine common sugars analyzed using the proposed method.

Figures 2 and 3 show representative chromatograms for both free and total carbohydrates in two different instant coffee samples, prepared as described by the AOAC method. The free sugar extracts for both coffee samples mainly contained four sugars: arabinose, galactose, mannose, and fructose. The total sugar extracts mainly contain arabinose, galactose, glucose, and mannose. Both types of extracts contain other sugars at lower concentrations.

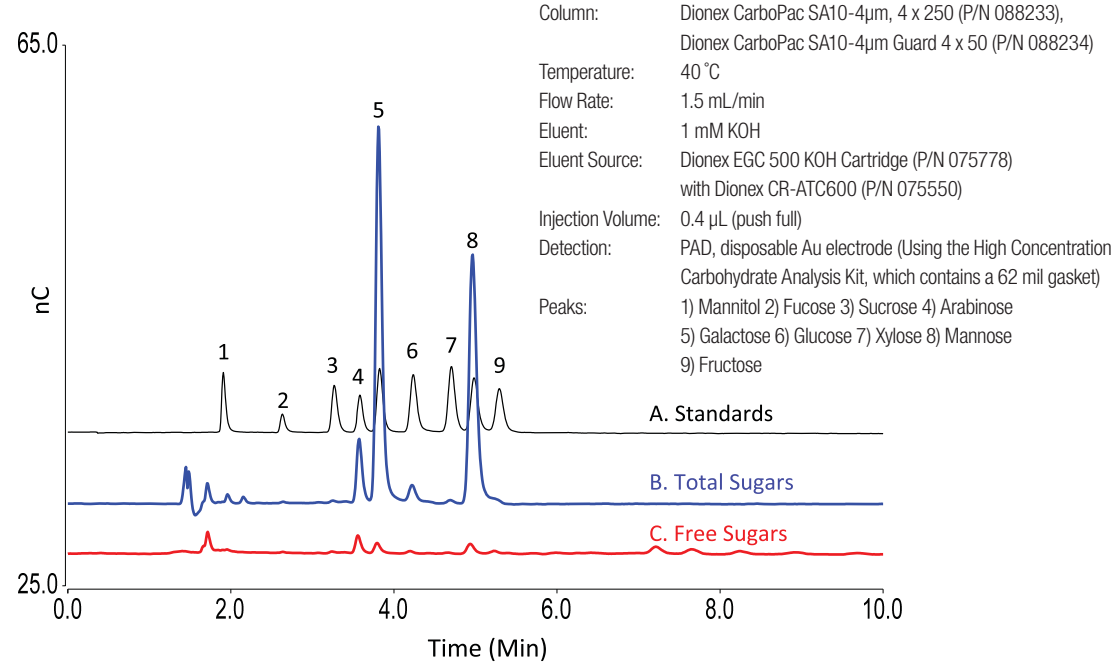

Figure 2. Free and total carbohydrate sugars present in instant coffee sample A.

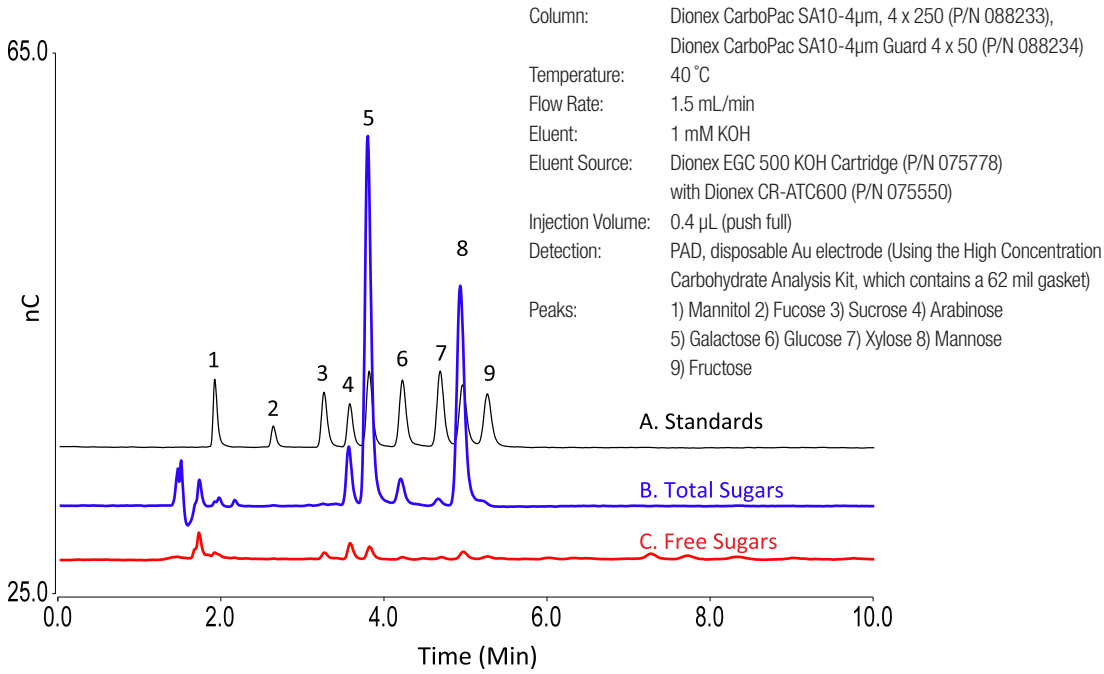

Figure 3. Free and Total carbohydrate sugars present in instant coffee sample B.

Linearity and Precision

The linearity of the method was studied using nine concentration levels of the nine sugars used in this study. The calibration data contained in Table 1 shows that the responses are linear for all concentration levels studied with coefficients of determination ≥ 0.997. To determine method precision, seven replicate injections of a standard sample containing 100 mg/L sugars were used (except fucose and arabinose, which were at 50 and 25 mg/L, respectively). The RSD values for retention time as well as peak area were < 2%.

Accuracy

Recovery studies were performed to assess method accuracy. Both free and total carbohydrate extracts were prepared in triplicate, and recovery studies were performed on each extract separately. The main four sugars in each extract were evaluated. Each sample was spiked with 50–100% of the original amount present. Sugar recoveries from the spiked samples were calculated using seven replicate injections of the same sample. As shown in Tables 2–5, recoveries for all sugars in free as well as total carbohydrate extracts are well within the 70–130% requirement specified by the United States Food and Drug Administration in the Office of Regulatory Affairs (ORA) laboratory manual.[7]

Conclusion

A method for the determination of sugars present in instant coffee was developed. The smaller particle size of the Dionex CarboPac SA10-4µm column allows for higher resolution separation of nine dominant sugars within 6 min. The method demonstrated excellent precision and accuracy, making it an ideal candidate for the fast analysis of coffee extracts to determine both the amount of free carbohydrates and total amount carbohydrates. The method proposed here offers increased reliability and sensitivity due to electrolytic eluent generation as well as all the ease-of-use features of the Dionex Integrion HPIC system.

Table 1. Calibration and precision data for carbohydrate standards used in this study (N=7).

Carbohydrate	Range (mg/L)	Retention Time (min)	Coeff. of Determination	Average RT (min)	RT RSD (%)	Average Peak Area (N=7)	Peak Area RSD (%) (N=7)
Mannitol	3.125–800	1.90	0.9996	1.90	0.16	0.30	1.86
Fucose	0.7–200	2.61	0.9997	2.61	0.18	0.11	1.49
Sucrose	3.125–800	3.23	0.9993	3.23	0.09	0.34	0.60
Arabinose	1.56–400	3.54	0.9997	3.55	0.09	0.25	0.58
Galactose	3.125–800	3.78	0.9997	3.78	0.00	0.47	0.84
Glucose	3.125–800	4.19	0.9994	4.19	0.00	0.51	1.28
Xylose	3.125–800	4.65	0.9978	4.65	0.07	0.60	1.18
Mannose	3.125–800	4.93	0.9987	4.93	0.06	0.52	0.83
Fructose	3.125–800	5.21	0.9999	5.22	0.06	0.42	2.05

Table 2. Sugars present in the free carbohydrate analysis of instant coffee sample A (N=7).

Sugar	Extract Number	RT (min)	Average Amount Present (mg/L)	Average Amount Recovered (mg/L)	Theoretical Spiked Amount (mg/L)	Average Spike Recovery (%)	Recovery RSD (%)
Arabinose	1	3.6	23.8	25.0	30.0	83.2	2.6
	2	3.6	23.4	25.8	30.0	86.1	4.0
	3	3.2	23.0	25.2	30.0	83.9	0.9
Galactose	1	3.8	12.7	13.5	12.0	112	8.4
	2	3.8	12.4	11.2	12.0	93.6	3.8
	3	3.8	11.9	12.5	12.0	104	6.2
Mannose	1	5.0	12.1	17.8	15.0	123	2.8
	2	4.9	12.6	18.0	15.0	128	6.5
	3	4.9	12.2	18.5	15.0	125	2.7
Fructose	1	5.3	1.4	10.7	10.0	106	6.5
	2	5.2	1.8	10.5	10.0	104	6.2
	3	5.2	1.6	10.8	10.0	108	8.2

Table 3. Sugars present in the free carbohydrate analysis of instant coffee sample B (N=7).

Sugar	Extract Number	RT (min)	Average Amount Present (mg/L)	Average Amount Recovered (mg/L)	Theoretical Spiked Amount (mg/L)	Average Spike Recovery (%)	Recovery RSD (%)
Arabinose	1	3.6	15.2	7.9	10.0	79.4	2.0
	2	3.6	15.5	8.0	10.0	80.3	4.5
	3	3.6	15.6	8.5	10.0	85.3	4.8
Galactose	1	3.8	15.0	11.0	12.0	92.0	5.7
	2	3.8	14.9	11.4	12.0	95.0	4.5
	3	3.8	14.8	12.9	12.0	106	2.8
Mannose	1	5.0	4.6	4.8	4.0	120	10.3
	2	5.0	4.6	4.9	4.0	122	8.3
	3	5.0	4.5	5.5	4.0	138	10.2
Fructose	1	5.3	2.7	4.8	4.0	112	11.3
	2	5.3	3.0	4.2	4.0	104	12.3
	3	5.3	2.9	4.8	4.0	115	4.8

Table 4. Sugars present in the total carbohydrate analysis of instant coffee sample A (N=7).

Sugar	Extract Number	RT (min)	Average Amount Present (mg/L)	Average Amount Recovered (mg/L)	Theoretical Spiked Amount (mg/L)	Average Spike Recovery (%)	Recovery RSD (%)
Arabinose	1	3.5	87.8	78.6	100.0	78.6	0.9
	2	3.5	86.1	83.1	100.0	83.1	0.4
	3	3.2	85.9	80.5	100.0	80.3	1.2
Galactose	1	3.8	632	650	650.0	100	0.3
	2	3.7	626	658	650.0	101	0.2
	3	3.7	640	643	650.0	99.2	0.4
Glucose	1	4.2	21.9	18.1	20.0	90.3	1.5
	2	4.1	21.4	17.1	20.0	85.4	1.8
	3	4.1	22.0	16.8	20.0	83.9	2.2
Mannose	1	5.0	613	762	600.0	128	0.6
	2	4.9	604	646	600.0	107	0.4
	3	4.8	618	686	600.0	113	0.8

Table 5. Sugars present in the total carbohydrate analysis of instant coffee sample B (N=7).

Sugar	Extract Number	RT (min)	Average Amount Present (mg/L)	Average Amount Recovered (mg/L)	Theoretical Spiked Amount (mg/L)	Average Spike Recovery (%)	Recovery RSD (%)
Arabinose	1	3.6	65.0	28.1	40.0	70.3	1.5
	2	3.5	63.4	27.4	40.0	68.6	2.0
	3	3.2	62.1	27.2	40.0	67.7	2.5
Galactose	1	3.8	528	547.2	500.0	109	0.3
	2	3.7	524	588.1	500.0	117	0.1
	3	3.7	525	592.0	500.0	118	0.3
Glucose	1	4.2	35.9	44.9	40.0	107	10.7
	2	4.1	35.2	43.3	40.0	109	1.6
	3	4.0	35.3	44.8	40.0	112	1.2
Mannose	1	4.9	483	516.6	500.0	103	0.8
	2	4.8	480	516.9	500.0	103	0.6
	3	4.9	410	578.5	500.0	115	1.7

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