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APPENDIX

High performance liquid chromatography (HPLC)

It is a form of column chromatography used to separate, identify, and quantify sugar compounds based on their polarities and interactions with the column's stationary phase. Different components of the sample are carried forward at different rates by the moving liquid phase, due to their different interactions with the stationary and the mobile phases.

The unknown sample can be identified by comparing retention time of unknown sample with standard sample. The height and area of a peak are proportional to the concentration of the corresponding component. A calibration curve is created using the standard sample. Then, the concentration of the unknown sample can be determined from the peak area of the detected sample using equation obtained from the standard curve.

Glucose concentration (g/l)	Peak area	Retention time (min)
0.8	575840.14	9.030
2.0	1332337.5	9.036
4.0	2006574.25	9.025
6.0	3657789.8	9.032
8.0	4018309.82	9.027
10.0	5882904.13	9.035

Table A Peak areas and retention times of standard glucose

Xylose concentration (g/l)	Peak area	Retention time (min)
0.8	679568.9	9.621
2.0	1536973.35	9.626
4.0	2290611.89	9.614
6.0	4206317.06	9.622
8.0	4549568.17	9.617
10.0	6778413.99	9.625

 Table B
 Peak areas and retention times of standard xylose



Figure A Relationship between peak area and glucose concentration.



Figure B Relationship between peak area and xylose concentration.

Equation of standard glucose:	y = 549711x + 90440
Equation of standard xylose:	y = 628771x + 112549;

y = peak area,

x = sugar concentration

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Presentations:

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