Waters
Lab Highlights
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ANALYSIS OF SUGARS I
RETENTION TIMES ON SUGAR-PAK™ I

From time to time we receive requests for information regarding retention times of standard sugars on various HPLC separation systems. This highlight presents retention time data collected on a Sugar-PAK™ I Column at four operating temperatures.

The system was a standard Sugar Analyzer I equipped with an M710B WISP™ Injector. The mobile phase consisted of Milli-R/Q™ Water containing 50 mg/l of calcium disodium EDTA, with a flow rate of 0.5 ml/min. The samples (sugars, sugar alcohols, and simple alcohols) were dissolved in water containing 5% acetonitrile, which serves as a fermentation inhibitor. The results are presented in Table 1.

TABLE I

RETENTION TIMES (MINUTES)
SUGAR PAK I

SAMPLE	RETENTION TIME			
	90°	80°	70°	60°
1-Butanol	18.82	19.52	20.07	20.45
2-Deoxyglucose	8.80	8.82	8.82	8.85
2-Propanol	12.95	13.00	13.00	12.95
Acetonitrile	13.45	13.80	14.17	14.65
Adonitol	10.97	11.07	11.22	11.37
Arabinose	10.42	10.55	10.77	11.00
Cellobiose	6.65	6.65	6.65	6.67
Ethanol	12.82	12.85	12.87	12.85
Ethylene Glycol	12.62	12.75	12.90	13.05
Fructose	10.07	10.27	10.52	10.82
Fucose	10.37	10.45	10.57	10.65
Galactose	9.37	9.40	9.47	9.52
Glucose	8.47	8.45	8.45	8.35
Glucose-6-phosphate	4.80	4.82	4.87	4.87
Glycerol	12.02	12.15	12.32	12.52
Lactulose	7.65	7.75	7.87	8.05
Lactose	7.07	7.10	7.12	7.12
Lyxose	10.75	10.90	11.07	11.25
Maltose	6.82	6.85	6.87	6.90
Maltotriose	6.00	6.05	6.10	6.17
Mannitol	12.15	12.47	12.87	13.32
Mannose	9.57	9.60	9.67	9.77
Melibiose	7.00	7.00	7.02	7.05
Methanol	12.52	12.57	12.60	12.62
Panose	5.92	5.90	5.95	5.97
Raffinose	5.97	5.97	6.00	6.02
Rhamnose	9.52	9.50	9.55	9.55
Ribose	14.85	15.75	16.87	18.15
Sorbitol	14.17	14.80	15.57	16.47
Sorbose	9.35	9.32	9.35	9.37
Stachyose	5.42	5.42	5.45	5.50
Sucrose	6.77	6.77	6.77	6.80
Xylitol	14.25	14.80	15.47	16.25
Xylose	9.30	9.30	9.32	9.35
Xylulose	10.55	10.67	10.82	10.95

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At 90° most samples give peaks with a tangential base width of approximately 0.7 min., although there are important exceptions such as ribose, which always gives a broad peak. This means that a one-minute difference in retention time will usually result in a resolution of approximately 1.4 (baseline separation). As temperature is reduced, the peaks of most sugars tend to broaden, as there is partial separation of anomers; thus, a larger difference in retention time will be required to achieve baseline resolution.

It should be remembered that column-to-column variations and differences in pump flow calibration will cause changes in observed retention time. A sample therefore cannot be unambiguously identified by correspondence of its retention time to one of those in Table 1. The best use of the data is in determining retention times relative to common standards such as glucose, fructose, sucrose, glycerol, etc. For example, if sucrose is found to elute (at 90°) in 6.2 min. (vs 6.77 min in Table I) the expected retention time for lactose would be 6.5 min under the assumption that there are no selectivity differences from column to column.