Simultaneous Quantitation of Multiple Sugars and Sugar Alcohols on UPLC-ELSD

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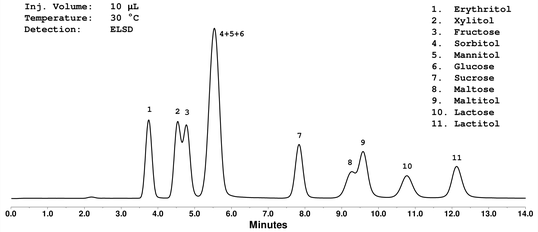
Introduction

Efficient and accurate sugar analysis is very important in order to generate nutrition facts panels. Amine based columns have been widely used for this purpose over recent decades. However, modern food engineering introduced a variety of sugar alcohols as preferred sweeteners, because they provide less calories when digested, and are usually very low in glycemic index. Samples with mixed sugars and sugar alcohols present a significant challenge to traditional sugar analysis by HPLC on amine columns. See chromatogram below.

Column: Aminopropyl silane HPLC, 150 x 4.6 mm
Eluent: 20% Water/80% Acetonitrile
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: ELSD

1. Erythritol
2. Xylitol
3. Fructose
4. Sorbitol
5. Mannitol
6. Glucose
7. Sucrose
8. Maltose
9. Maltitol
10. Lactose
11. Lactitol



An efficient and reliable method has been developed to separate and analyze 5 sugars and 6 sugar alcohols within 20 minutes on a Waters Acquity UPLC (Ultra Performance Liquid Chromatography) system, using a Waters Acquity UPLC BEH Amide column.

Compared to HPLC amine columns, the UPLC Amide column has:

- 1.7 micron particle, leading to shorter run times and higher resolutions between peaks.
- Much more stable amide-based stationary phase, which eliminates Schiff base formation and produces stable retention times over long term.

Evaporative Light-Scattering Detection (ELSD) is preferred over Refractive Index Detection (RID) because:

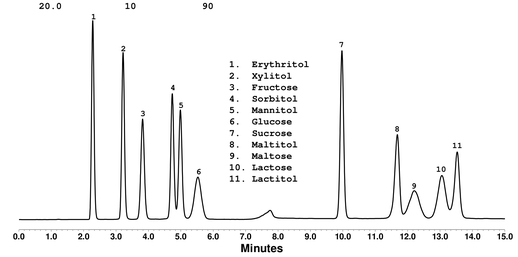
- ELSD is more sensitive than RID.
- ELSD is less affected by change in temperature.
- Unlike RID, ELSD is compatible with gradient elution because of stable baselines.

Chromatographic Conditions

Column: ACQUITY UPLC BEH Amide 1.7 µm, 2.1 x 150 mm
Eluent A: 89.6% Water + 10% CH_3CN + 0.2% Et_3N + 0.2% NH_4OH
Eluent B: 99.5% CH_3CN + 0.1% Isopropanol + 0.2% Et_3N + 0.2% NH_4OH
Temperature: 45 °C
Inj. Volume: 1 µL
Flow Rate: 0.45 mL/min
Run Time: 20 min
STD Concentration: 1 mg/mL each in 25% EtOH and 75% Water

Time (min)	Gradient Profile %A	%B
0.0	10	90
5.0	10	90
5.5	15	85
14.0	15	85
15.0	30	70
16.5	10	90
20.0	10	90

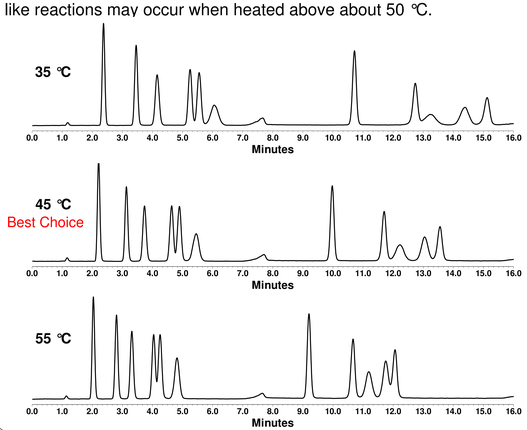
ELSD Settings
Gas Pressure: 40 psi
Nebulizer: Cooling
Drift Tube: 50 °C
Gain: 5
Data Rate: 10 pps
Time Constant: 2 s



The elevated column temperatures and high pH values of the mobile phases (via addition of amine and ammonium hydroxide) help to suppress anomer formation from reducing sugars (glucose, maltose, and lactose), leading to relatively sharp peaks for those analytes.

Temperature vs. Retention Times

At 35 ℃, reducing sugar peaks, especially that of maltose, are too wide. Column temperatures higher than 45 ℃ yields much sharper peaks, but however at the cost of peak resolution. Additionally, apparent Maillard-like reactions may occur when heated above about 50 ℃.

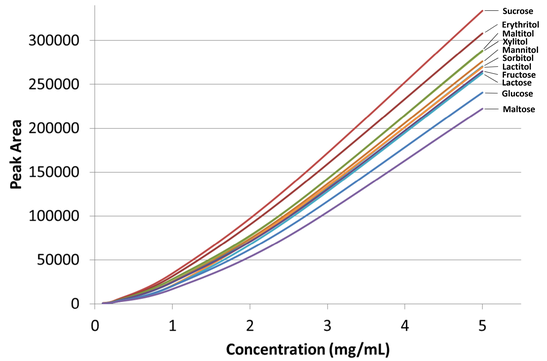


Non-Linear Response on ELSD

Analyte concentration affects the particle size after solvent is removed in the detector's drift tube. Therefore, ELSD responses are generally not linear. The relationship between peak area and the concentration of analyte can be expressed as:

$$\textbf{Peak Area} = \mathbf{a} \times \textbf{Concentration}^{\mathbf{b}},$$

which is observed:



Log-Log Linear Curves

Double logarithmic transformation allows linear curves:

$$\textbf{Log(Peak Area)} = \mathbf{b} \times \textbf{Log(Concentration)} + \textbf{Log(a)}$$

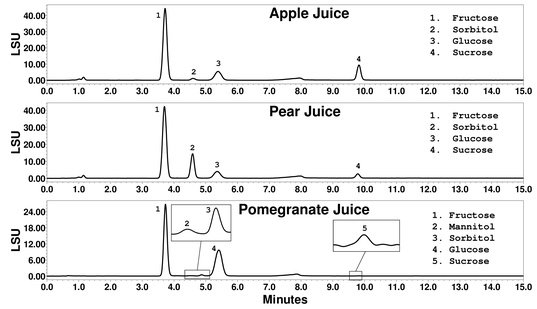
All 11 analytes were found to have excellent linearity over a 50-fold concentration range, as well as good repeatability.

Analyte	Retention Time (min)	b	Log(a)	Correlation Coefficient (R)	Lowest Conc. (mg/mL)	Highest Conc. (mg/mL)	%RSD of Peak Area*
Erythritol	2.168	1.567	3.970	0.9993	0.1	5	3.47
Xylitol	3.056	1.538	3.951	0.9997	0.1	5	2.85
Fructose	3.634	1.561	3.898	0.9998	0.1	5	1.25
Sorbitol	4.499	1.580	3.887	0.9997	0.1	5	2.29
Mannitol	4.746	1.546	3.920	0.9998	0.1	5	2.55
Glucose	5.295	1.564	3.779	0.9994	0.1	5	1.50
Sucrose	9.759	1.489	4.059	0.9996	0.1	5	1.78
Maltitol	11.421	1.529	3.943	0.9999	0.1	5	2.36
Maltose	11.940	1.505	3.879	0.9991	0.1	5	1.26
Lactose	12.726	1.522	3.914	0.9990	0.1	5	1.13
Lactitol	13.185	1.599	3.879	0.9995	0.1	5	1.81

* Analysis of ten replicate injections of a 1 mg/mL standard.

Examples

Three commercially available fruit juices are diluted 5x with 25% ethanol then filtered through 0.2 µm filter into LC vials before analysis.



Conclusions

A UPLC method has been successfully developed to separate and quantitate up to 11 mixed sugars and sugar alcohols. UPLC amide column provides superior pH and temperature stability in addition to excellent peak resolution. Mobile phase ingredients were carefully chosen in consideration of peak shape (NH_4OH and Et_3N in both eluents), resolution (0.1% IPA in CH_3CN in Eluent B), and inhibition of microbial growth (10% CH_3CN in Water in Eluent A).

Both repeatability and linearity (Log-Log) were found to be satisfactory. This method is applicable to a variety of sample types, especially juices where both sugars and sugar alcohols are found. Typical analysis time per injection is 20 minutes.

However, it is suspected that during UPLC separation, Maillard-type reactions occur within samples containing both proteins and carbohydrates, causing sharply increased back pressures or even total blockage. Therefore, it is not recommended that such sample be analyzed with this UPLC method.

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