Rapid Screening and Analysis of Components in Nonalcoholic Drinks

Application

Food

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Abstract

Soft drinks, juices, and prepared teas are popular drinks that are carefully formulated to provide unique flavor and reliable product stability. Many end-product facilities have the means to monitor critical ingredients, such as sugars, flavors, colorants, and preservatives, but may find their analyses are not fast enough to keep pace during periods of high seasonal demand. Here we take a classical approach to the analysis of additives and sweeteners and convert to a new method that improves sample throughput and resolution while also reducing solvent consumption.

Introduction

Quality control (QC) laboratories are constantly challenged to meet higher sample loads with fewer instruments, reduced staff, and without sacrificing data quality in the process. While some labs have successfully adopted greater automation in sample preparation and data handling, they still find that opportunities exist for increasing their chromatographic throughput. Methods developed on 5- or 10-µm particle size columns may be candidates for modernization by replacing these columns with smaller-dimension columns packed with smaller particle sizes. The greatest opportunities in beverage QC labs exists with the small molecule separations of additives, preservatives, flavorants, and sweeteners. In methods development and research labs, compatibility with mass spectrometer ionization sources has also been a driving force in reducing column size to support low flow-rate operation.

To reduce analysis time without sacrificing resolution, we can reduce column length and packing particle size simultaneously. The balancing effect is remarkably simple if reasonable optimization of the HPLC system, with respect to gradient delay volume and extracolumn dispersion, is practical. For example, a 250-mm long column with 5-µm particles can be replaced by a 150-mm long column packed with 3-µm particles. If the ratio of length to particle size is equal, in other words, resolution is preserved. Solvent consumption is proportionately reduced, and, if an equal mass of analyte can be successfully injected, the detector response should also increase due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

Liquid chromatography/mass spectrometry (LC/MS) ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal ultra violet UV/VIS optical detection (LC/UV) methods. In MS detection mode, it is

advantageous to reduce the column volume via reduced internal diameter. Lower flow rates will be required, proportional to the cross-sectional area of the inside diameter of the columns. The relationship of flow rate between different column diameters is shown in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

We normally scale the injection mass to the size of the column, and a proportional injection volume would be calculated from the ratio of the void volumes, as shown in Equation 2.

$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj.Vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. There are several other parameters that should be considered. The first of these parameters is the column efficiency relative to flow rate, more correctly efficiency to linear velocity, as commonly defined by van Deemter [1] and others, and the second is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column.

Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, but we are most interested here in his observations that there is an optimum linear velocity for any given particle size, in a well-packed HPLC column, and that the optimum linear velocity increases as the particle size decreases. Graphically, it is typically represented as van Deemter plots [2] of plate height versus linear velocity. Generally speaking, a reduction in particle size leads to higher efficiency, at higher linear velocities, and the optimum range of highest efficiency expands to offer a wide flow range where good performance can be expected.

The second important consideration is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also employed there is a further reduction in the expected peak volume. Care must be taken by the user to minimize the extracolumn volume and to reduce, where practical, the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. It is most useful to express the gradient slope as % change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition, regardless of flow rate or column diameter and length. We can express the gradient as shown in Equation 3.

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End\%} - \text{Start\%})}{\#\text{Column volumes}}\right] \quad \text{(eq. 3)}$$

A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Lower gradient slopes are formed by increasing the gradient time or flow rate, or a combination, within an acceptable range of analysis time and operating pressure.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher capacity value directly increases resolution. The effect is quite dramatic up to a k value of about 5–10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

System

Agilent 1200 Series Rapid Resolution LC, consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C HiP ALS autosampler SL, with thermostatic temperature control
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 50 mm, 1.8 µm

Mobile phase conditions

Organic solvent:	Acetonitrile (ACN) or ACN containing 0.1% formic acid
Aqueous solvent:	20 mm phosphoric acid in Milli-Q water, pH 3.65 with ammonium hydroxide, or Milli-Q water containing 0.1% formic acid

Gradient conditions

Gradient slope:	2.8% per column volume See individual chromatograms for flow rate and gradient time.

Samples

1. Standard mixture of sodium saccharin, caffeine, aspartame, vanillin, benzoic acid, sorbic acid, benzaldehyde, all 50 µg/mL in 1/1 methanol/water
2. Various soft drinks, decarbonated where applicable

Results

The separation in Figure 1 was performed on a 4.6 × 250 mm, 5-µm ZORBAX SB-C18, column thermostatted to 30 °C using buffered phosphate conditions. At this pH, benzoate and sorbate were adequately resolved on the SB-C18 material with ACN organic modifier. With the formic acid modifier, nominal pH 2.5, poor resolution of these two components was observed, though selectivity and peak shape of other components was comparable. Additionally, on-column degradation of benzaldehyde was observed, presumably to benzoate, at the lower pH. This has been observed in other experiments (data not shown) and is particularly evident as column temperature is increased. Preparing ammonium formate buffer at about the same pH as the phosphate would likely solve both problems and would be MS compatible.

The method was then scaled in flow and time for exact translation to 3.0 × 100 mm, 3.5-µm and 1.8-µm columns (data not shown). The gradient was recalculated for 3.0 × 50 mm, 1.8-µm material (Figure 2). Subsequent gradient calculations yielded higher speed examples (Figures 3 and 4) without resolution loss.

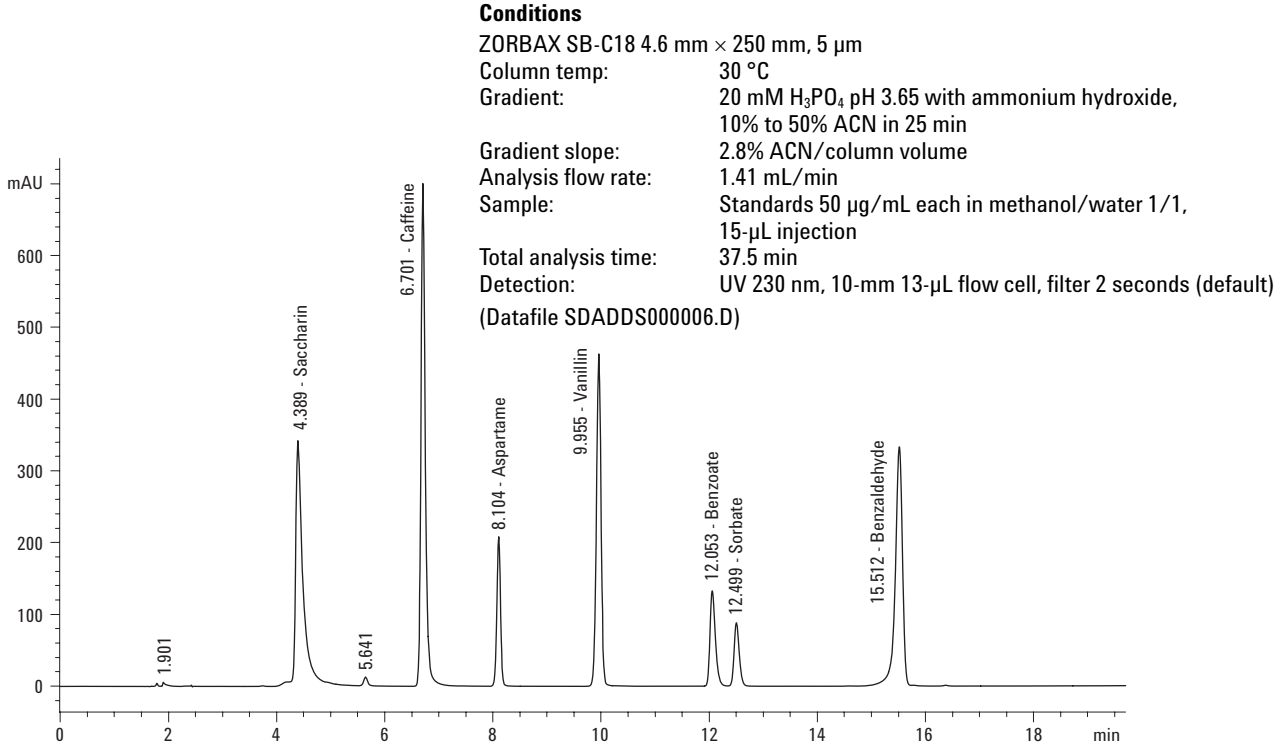
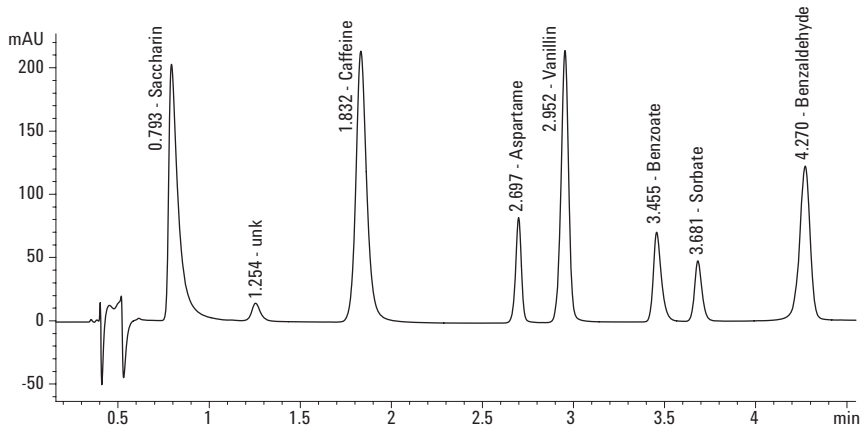


Figure 1 Gradient separation of soft drink additives on 4.6 × 250 mm, 5-µm ZORBAX SB-C18.



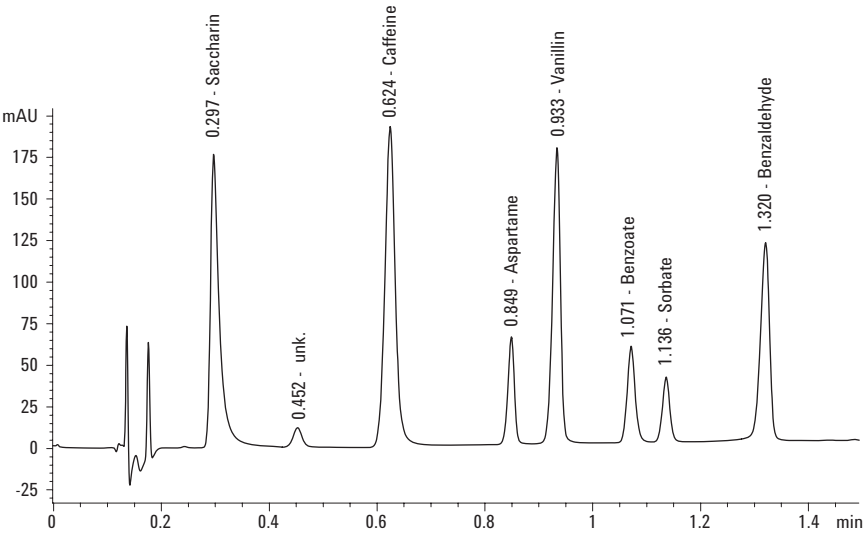
Conditions

ZORBAX SB-C18 3.0 mm × 50 mm, 1.8 µm

Column temp:	45 °C
Gradient:	20 mM H_3PO_4 pH 3.65 with ammonium hydroxide, 10% to 50% ACN in 5 min
Gradient slope:	2.8% ACN/column volume
Analysis flow rate:	0.6 mL/min
Sample:	Standards 50 µg/mL each in methanol/water 1/1, 2.5-µL injection
Total analysis time:	9 min
Detection:	UV 210 nm, 6-mm 5-µL flow cell with 0.12-mm id inlet heat exchanger, filter 0.2 seconds

(Datafile SDADDS_3MM21004.D)

Figure 2. Gradient separation of soft drink additives on 3.0 × 50 mm, 1.8-µm ZORBAX SB-C18.



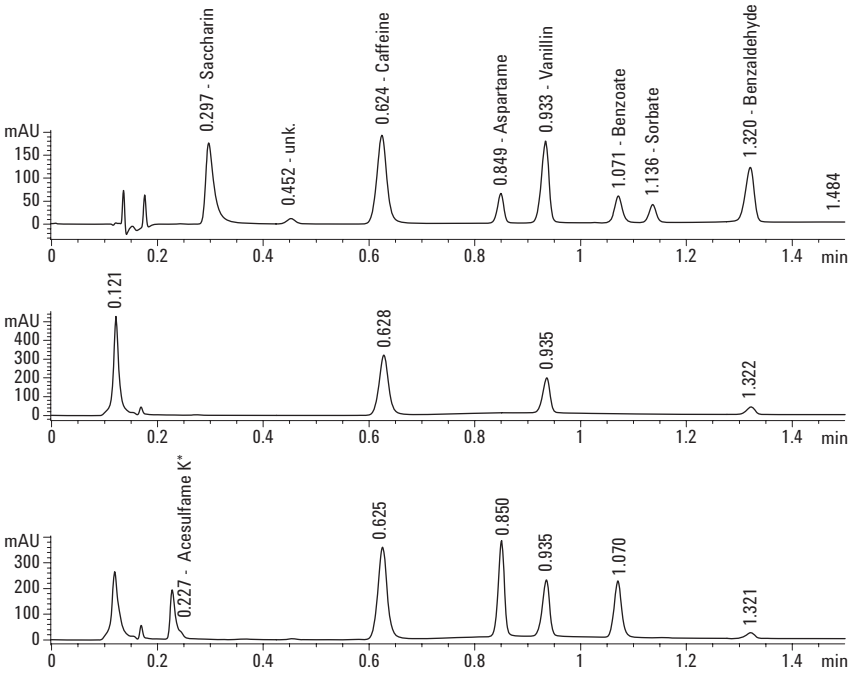
Conditions

ZORBAX SB-C18, 3.0 mm × 50 mm, 1.8 µm

Column temp:	45 °C
Gradient:	20 mM H_3PO_4 pH 3.65 with ammonium hydroxide, 10% to 50% ACN in 1.5 min
Gradient slope:	2.8% ACN/column volume
Analysis flow rate:	2.0 mL/min
Sample:	Standards 50 µg/mL each in methanol/water 1/1, 2.5-µL injection
Total analysis time:	3 min
Detection:	UV 210 nm, 6-mm 5-µL flow cell with 0.12-mm id inlet heat exchanger, filter 0.2 seconds

(Datafile SDADDS_3MM31029.D)

Figure 3. High-speed gradient separation of soft drink additives on 3.0 × 50 mm, 1.8-µm ZORBAX SB-C18.



Conditions

See Figure 3 for chromatographic conditions

*Acesulfame K tentative identification by reference to other samples under similar conditions and product label claim

Sample: Upper panel: mixed standard
Center panel: black cherry vanilla cola, 20-oz polymeric bottle, decarbonated, 2.5-µL injection
Lower panel: diet black cherry vanilla cola, 20-oz polymeric bottle, decarbonated, 2.5-µL injection

(Datafiles SDADDS_3MM31029.D, SDADDS_3MM31043.D, SDADDS_3MM31044.D)

Figure 4. High-speed gradient separation of soft drink additives on 3.0 × 50 mm, 1.8-µm ZORBAX SB-C18.

The conditions in Figures 3 and 4, using a 2.8% slope at increased linear velocity on 3.0 × 50 mm, 1.8-µm material, yield a separation with better resolution than the original 4.6 × 250 mm method. Because the absolute plate count is lower for the 1.8-µm column (12,000 vs. 24,000 predicted for the 250-mm 5-µm column, based on typical calculations), the resolution increase is presumably related to the increased operating temperature, which lowers both solvent viscosity and nonspecific column-analyte interactions, and an improvement in solvent linear velocity relative to the optimum for 1.8-µm materials. With only a 3-minute total analysis time, this is an excellent procedure for high-throughput screening and quantitation of a large number of samples.

Conclusion

Careful translation of gradient slope and the use of optimum linear velocity with sub-2-micron particles can enable users to take advantage of small format columns that yield fast analysis times without compromising resolution. Optimization of extracolumn volume helps minimize resolution losses that unduly degrade column performance, assuring the maximum resolution possible while improving analysis time substantially. We have demonstrated this potential with a complex mixture of typical beverage additives and encourage users to contact us for guidance on this application and when other method translation opportunities are identified.

References

1. J. J. van Deemter, F. J. Zuiderweg, A. Klinkenberg, *A. Chemical Engineering Science* 1956, **5**, 271–289

2. The Influence of Sub-Two Micron Particles on HPLC Performance, Agilent Technologies, application note 5989-9251EN, May 2003

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