Application Note

Food and Beverage Testing

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High Throughput LC Method for Soft Drink Additives

Using the Agilent 1260 Infinity III LC system with an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column

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Abstract

Soft drinks represent a major revenue stream for global beverage manufacturers. Consumer loyalty depends on consistent product quality, making the accurate quantitation of key additives essential. This application note presents a fast, robust, and high throughput LC method for the analysis of six common soft-drink additives—acesulfame K, aspartame, saccharin, benzoate, sorbate, and caffeine—using the Agilent 1260 Infinity III LC system, Agilent OpenLab CDS, and an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column. The method delivers excellent chromatographic performance, short runtimes, minimal sample preparation, and straightforward operation for users of varied skill levels. Figures referenced throughout demonstrate the performance of the approach; chromatographic data and calibration results are included.

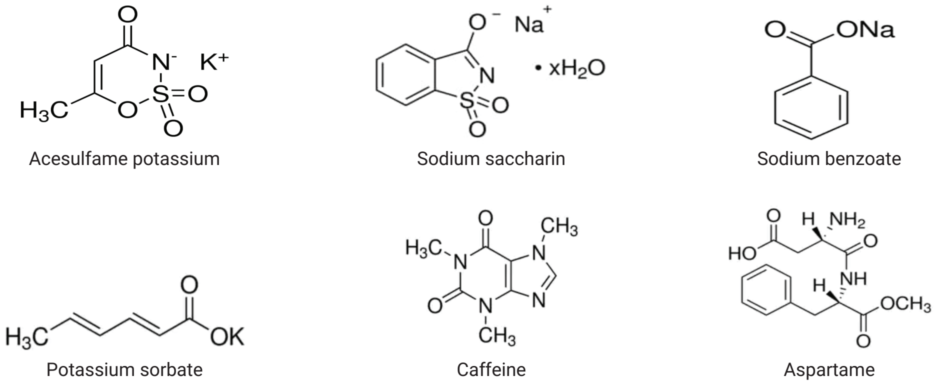
Introduction

Soft-drink products—including carbonated beverages, energy drinks, flavored waters, teas, and diet sodas—represent a major revenue stream for beverage manufacturers and are consumed globally at high volumes. Because these beverages rely on consistent flavor, sweetness, and shelf stability, manufacturers must closely monitor key additives throughout production. These additives typically include caffeine, which functions as a stimulant; benzoate and sorbate, which serve as preservatives; and nonnutritive sweeteners such as acesulfame K, saccharin, and aspartame (Figure 1). Ensuring these components remain within target concentration ranges is essential for maintaining product quality and meeting regulatory and labeling requirements.

In addition to supporting routine quality control, additive analysis plays an important role during product changeovers, reformulation efforts, and verification of ingredient authenticity. For example, caffeine levels may carry regulatory or marketing significance, while sweetener profiles must remain consistent to preserve brand identity. Because soft drinks vary widely in composition—from highly carbonated colas to vitamin-enriched energy drinks—the analytical method must provide reliable quantitation across diverse matrices.

Agilent has published numerous applications describing the analysis of nonnutritive sweeteners and preservatives, using a variety of chromatographic strategies. Many existing methods rely on gradient elution, which can complicate transferability between instruments. Others use traditional C18 columns that may offer limited retention for highly polar compounds, or they require more complex detectors such as evaporative light scattering detectors (ELSD) or mass spectrometry to achieve necessary specificity.[1–7]

The present work focuses instead on a simple isocratic LC method optimized for speed, robustness, and ease of deployment. This approach takes advantage of the phenyl-hexyl stationary phase, which can retain more polar compounds when the organic modifier is minimized or removed.[8] Additionally, the use of diode array detection provides UV-spectral confirmation, enabling more confident identification of analytes. This combination of selectivity, spectral information, and operational simplicity makes the method well suited for high-throughput soft-drink analysis in routine production environments.


Acesulfame potassium

Sodium saccharin

Sodium benzoate

Potassium sorbate

Caffeine

Aspartame

Figure 1. Common soft-drink additives.

Experimental

Instrumentation

All analyses were performed using an Agilent 1260 Infinity III LC system equipped with an InfinityLab Assist Upgrade (G7178A), which includes the Assist Interface (G7179A) and Assist Hub (G7180A). The system also incorporated a 1260 Infinity III quaternary pump (G7111B), a 1260 Infinity III multisampler (G7167A), and a 1260 Infinity III multicolumn thermostat with a standard heat exchanger. Detection was achieved using a 1260 Infinity III diode array detector (G7115A) fitted with a 10 mm standard flow cell. This configuration provided reliable flow delivery, stable retention times, and robust UV detection suitable for routine quality-control workflows.

Column

Chromatographic separation was performed on an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column (3.0 × 50 mm, 2.7 µm; part number 959990-902). The phenyl-hexyl stationary phase offers enhanced selectivity for aromatic and moderately polar analytes while maintaining excellent efficiency. Its ability to operate under 100% aqueous mobile phase conditions without phase collapse is particularly advantageous when analyzing highly polar beverage matrices.[8]

Software

Data acquisition, instrument control, and processing were carried out using Agilent OpenLab CDS (version 2.7 or later). The software enabled consistent method deployment, streamlined data review, and simplified reporting for routine high-throughput environments.

Chemicals and standards

HPLC-grade methanol and ethanol (Honeywell) were used as organic solvents during method development. Analytical standards—including acesulfame potassium, sodium benzoate, sodium saccharin, potassium sorbate, caffeine, and aspartame—were obtained from Sigma-Aldrich. Each standard was dissolved in Milli-Q water at 1,000 µg/mL, stored under refrigeration, and confirmed stable for at least 30 days. The buffer used for the mobile phase was a 20 mM sodium acetate (Sigma-Aldrich) solution adjusted to pH 4.8 with acetic acid (GFS Chemical). A commercially available HPLC-grade buffer (CP Lab Safety, Novato CA; part number CP-SCYSAB-4L) was also evaluated and found to be suitable after filtration. This buffer should be stored in a refrigerator.

Sample preparation

Soft-drink samples were degassed to remove carbonation and then filtered prior to analysis. Most beverages required no dilution before injection. Energy drinks, which typically contain higher caffeine levels and additional polar ingredients, were diluted when necessary to maintain calibration-range compatibility.

Method considerations

A low-pressure mixing pump was chosen to enable accurate preparation of isocratic mobile phases while providing a cost-efficient alternative to high-pressure mixing. This setup ensured consistent solvent delivery throughout the analysis. Temperature control was maintained using the multicolumn thermostat to support retention-time stability and peak-shape optimization.

Results and discussion

Solvent selection: Ethanol versus methanol

Initial method development compared ethanol and methanol as organic modifiers. Ethanol was considered due to its lower toxicity and renewable sourcing; however, its availability in different purity grades (95% and 99.5%) can create ambiguity in laboratories, and its presence in pure form can raise concerns about potential misuse.

Using ethanol, all six analytes demonstrated acceptable peak shapes, with aspartame eluting at 3.1 minutes in 10% ethanol and at 4.2 minutes in 7.5% ethanol. Caffeine and sorbate, however, showed coelution at higher ethanol levels (Figure 2), reducing confidence in quantitation.

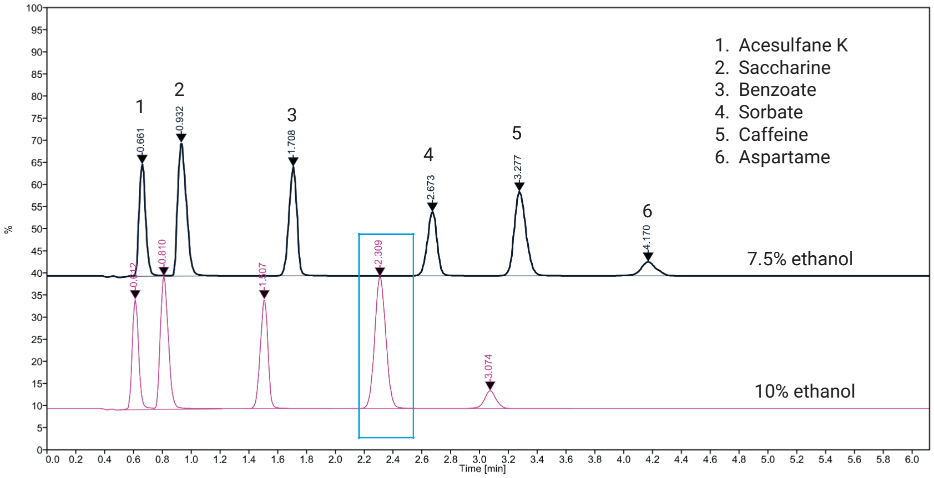


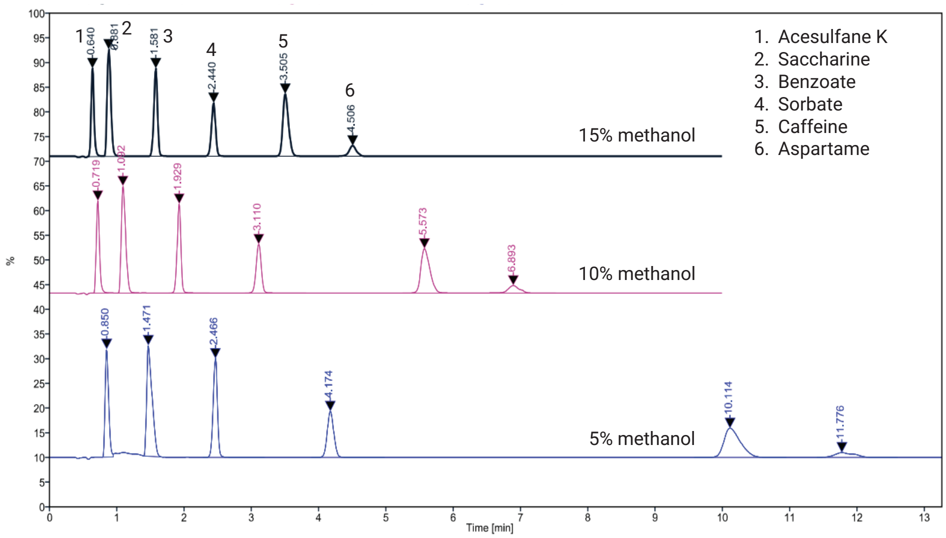
Figure 2. Method optimization using ethanol.

Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
Mobile Phase	(90:10 v/v) 20 mM sodium acetate pH 4.8 (adjusted with acetic acid) and ethanol
Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Flow Rate	0.6 mL/min
Column Temperature	35 °C
Injection Volume	3 µL
Wavelength	218 nm; BW: 4 nm
Reference	360 nm; BW: 100 nm
Peak Width	2.5 Hz
Backpressure	95 bar (7.5% ethanol) 90 bar (10% ethanol)

Methanol was therefore evaluated at 5%, 10%, and 15%. In all cases, analytes displayed strong peak shapes and full resolution, with no change in elution order (Figure 3). This provided a more stable analytical platform, and methanol was selected as the preferred organic modifier.

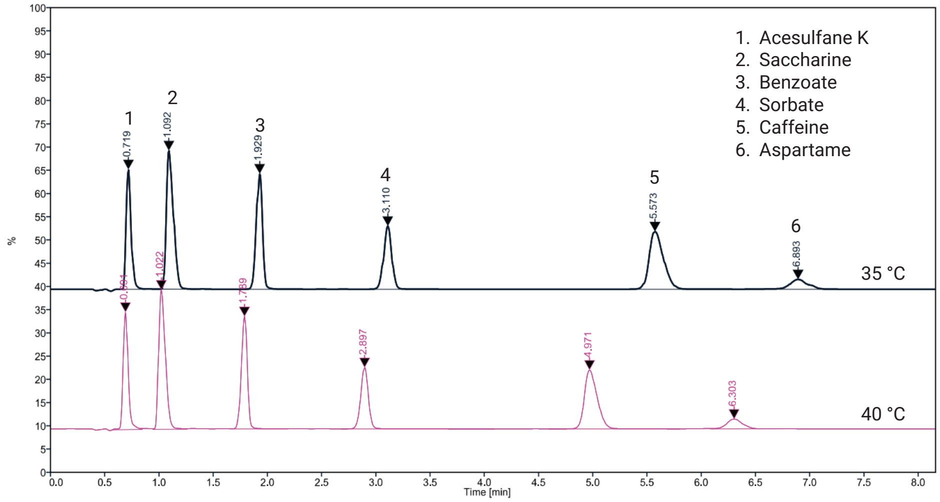
Impact of column temperature

Temperature played a measurable role in chromatographic performance. Although 35 °C is typically recommended as a starting point for method development, increasing the temperature to 40 °C improved aspartame peak shape, decreased overall retention, and maintained adequate separation for early-eluting acesulfame K (Figure 4). This slight temperature elevation also enhanced throughput without compromising resolution.



Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
Mobile Phase	(90:10 v/v) 20 mM sodium acetate pH 4.8 (adjusted with acetic acid) and methanol
Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Flow Rate	0.6 mL/min
Column Temperature	35 °C
Injection Volume	3 µL
Wavelength	218 nm; BW: 4 nm
Reference	360 nm; BW: 100 nm
Peak Width	2.5 Hz
Backpressure	90 bar (15% methanol 92 bar (10% and 5% methanol)

Figure 3. Method optimization using methanol.



Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
Mobile Phase	(90:10 v/v) 20 mM sodium acetate pH 4.8 (adjusted with acetic acid) and methanol
Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Flow Rate	0.6 mL/min
Column Temperature	35 and 40 °C
Injection Volume	3 µL
Wavelength	218 nm; BW: 4 nm
Reference	360 nm; BW: 100 nm
Peak Width	2.5 Hz
Backpressure	92 bar (35 °C) 87 bar (40 °C)

Figure 4. Method optimization using 10% methanol with column temperature at 35 and 40 °C.

Calibration linearity and quantitative performance

Five calibration levels (50, 100, 200, 300, and 500 µg/mL) were analyzed to confirm method linearity.

Chromatograms and calibration curves (Figures 5 and 6) demonstrated excellent correlation for all analytes, with R^2 values ≥ 0.999 across the full tested range.

The method showed stable response factors and consistent retention times, supporting high-confidence quantitative analysis in routine environments.

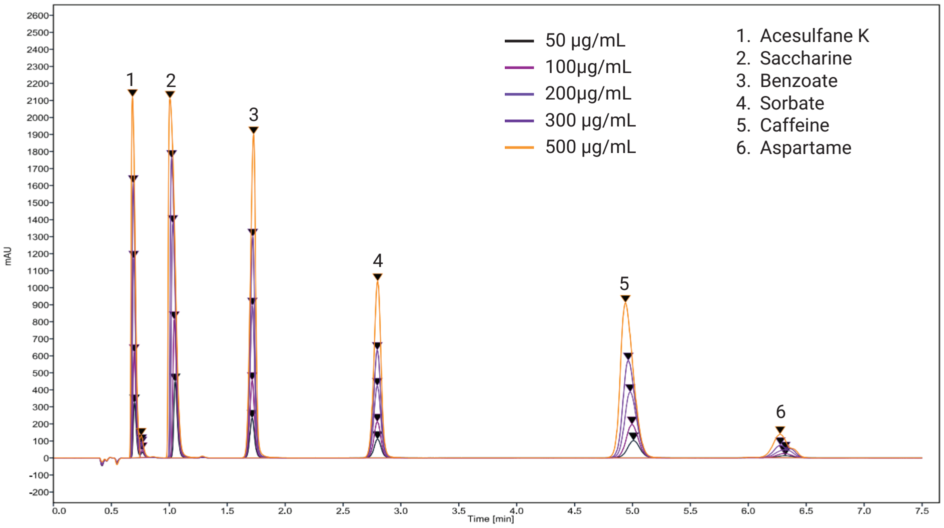
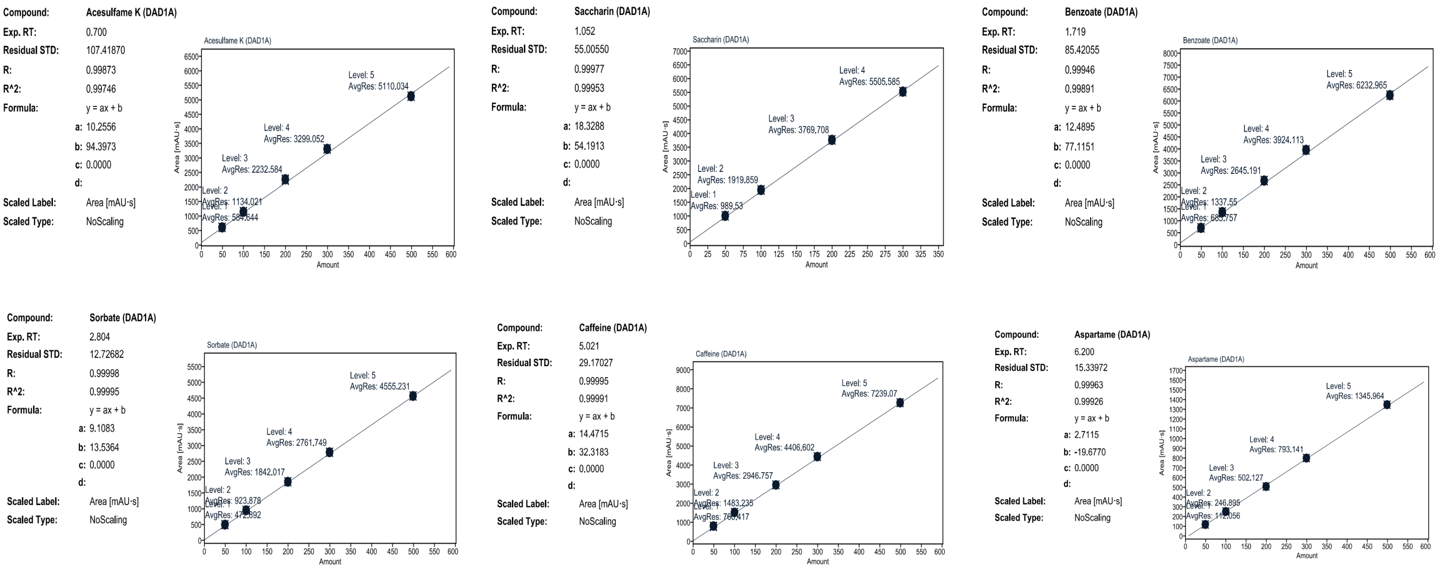


Figure 5. Overlay of calibration chromatograms.

Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
Mobile Phase	(90:10 v/v) 20 mM sodium acetate pH 4.8 (adjusted with acetic acid) and methanol
Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Flow Rate	0.6 mL/min
Column Temperature	40 °C
Injection Volume	3 µL
Wavelength	218 nm; BW: 4 nm
Reference	360 nm; BW: 100 nm
Peak Width	40 Hz
Backpressure	118 bar



Compound: **Acesulfame K (DAD1A)**
Exp. RT: 0.700
Residual STD: 107.41870
R: 0.99873
R^2: 0.99746
Formula: y = ax + b
a: 10.2556
b: 94.3973
c: 0.0000
d:
Scaled Label: Area [mAU·s]
Scaled Type: NoScaling

Compound: **Saccharin (DAD1A)**
Exp. RT: 1.052
Residual STD: 55.00550
R: 0.99977
R^2: 0.99953
Formula: y = ax + b
a: 18.3288
b: 54.1913
c: 0.0000
d:
Scaled Label: Area [mAU·s]
Scaled Type: NoScaling

Compound: **Benzoate (DAD1A)**
Exp. RT: 1.719
Residual STD: 85.42055
R: 0.99946
R^2: 0.99891
Formula: y = ax + b
a: 12.4895
b: 77.1151
c: 0.0000
d:
Scaled Label: Area [mAU·s]
Scaled Type: NoScaling

Compound: **Sorbate (DAD1A)**
Exp. RT: 2.804
Residual STD: 12.72682
R: 0.99998
R^2: 0.99995
Formula: y = ax + b
a: 9.1083
b: 13.5364
c: 0.0000
d:
Scaled Label: Area [mAU·s]
Scaled Type: NoScaling

Compound: **Caffeine (DAD1A)**
Exp. RT: 5.021
Residual STD: 29.17027
R: 0.99995
R^2: 0.99991
Formula: y = ax + b
a: 14.4715
b: 32.3183
c: 0.0000
d:
Scaled Label: Area [mAU·s]
Scaled Type: NoScaling

Compound: **Aspartame (DAD1A)**
Exp. RT: 6.200
Residual STD: 15.33972
R: 0.99963
R^2: 0.99926
Formula: y = ax + b
a: 2.7115
b: -19.6770
c: 0.0000
d:
Scaled Label: Area [mAU·s]
Scaled Type: NoScaling

Figure 6. Calibration curves for common soft-drink components.

UV spectral confirmation

Each of the six soft-drink additives provided a distinct UV spectrum (Figure 7), enabling confirmatory identification. Although quantitation can be performed using single-wavelength absorbance, diode-array detection (DAD) offers additional confidence—especially when unknown or unexpected peaks appear, or when new formulations introduce potential coeluting compounds.

Soft-drink sample analysis

The method was applied to commercial beverages, with triplicate injections demonstrating excellent reproducibility (Figures 8 to 14). Across samples:

- Cola beverage contained only caffeine.
- Diet colas contained benzoate, caffeine, and aspartame.
- Lemon-lime soda showed benzoate as the primary additive.
- Orange sodas contained benzoate and consistently produced a small late-eluting peak (~ 8.7 min), identified as a coloring agent. This additional component suggests the need for slightly extended run times for orange beverages to avoid coelution with other components.
- Other soft drinks exhibited varying combinations of benzoate, caffeine, and aspartame, consistent with ingredient labeling.

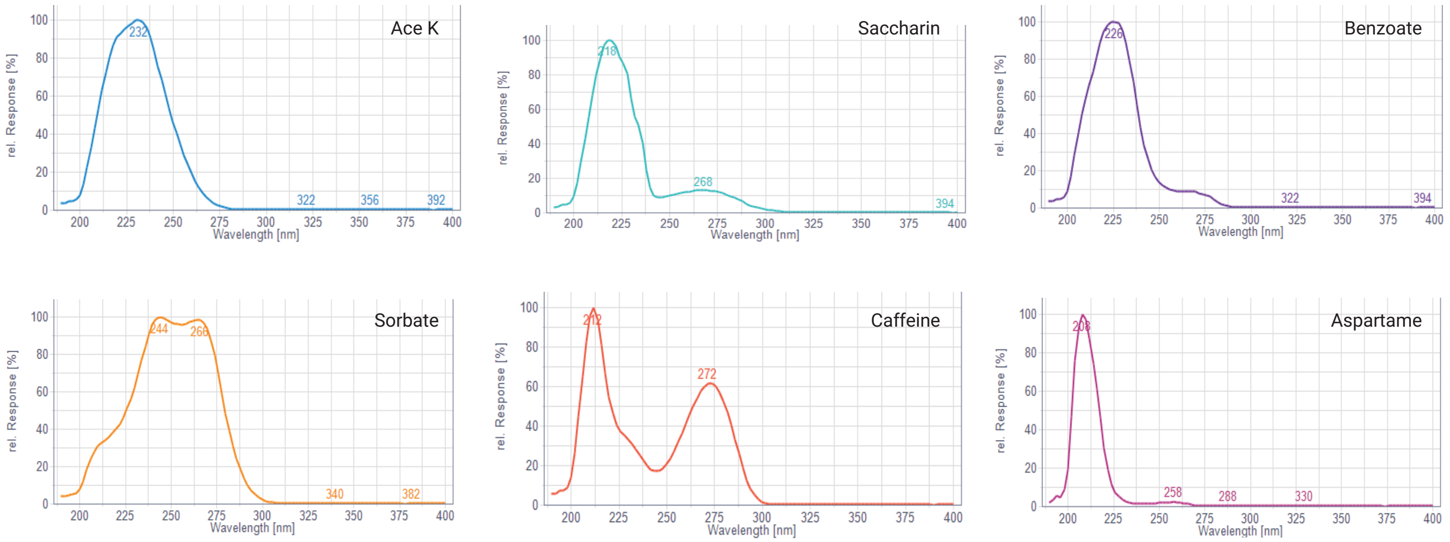


Figure 7. UV spectra of common soft-drink components.

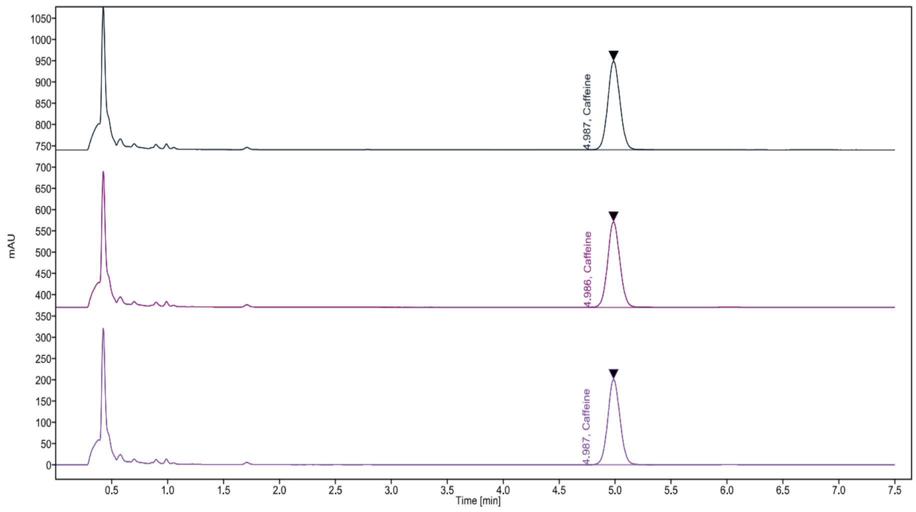


Figure 8. Cola chromatograms.

Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
Mobile Phase	(90:10 v/v) 20 mM sodium acetate pH 4.8 (adjusted with acetic acid) and methanol
Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Flow Rate	0.6 mL/min
Column Temperature	40 °C
Injection Volume	3 µL
Wavelength	218 nm; BW: 4 nm
Reference	360 nm; BW: 100 nm
Peak Width	40 Hz
Backpressure	118 bar
Results	
Caffeine	104.1 µg/mL

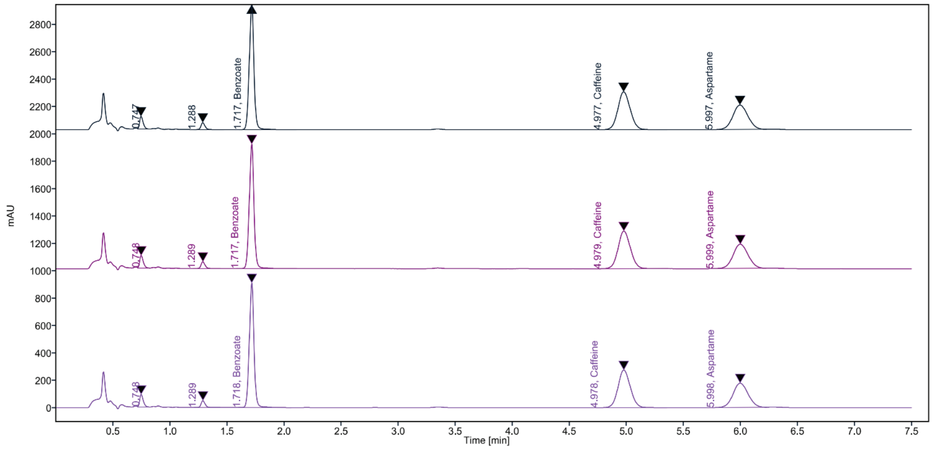


Figure 9. Diet cola chromatograms.

<table>
<tr><th colspan="2">Chromatographic Conditions</th></tr>
<tr><th colspan="2">Agilent 1260 Infinity III Quaternary LC</th></tr>
<tr><td colspan="2">All chromatographic conditions are the same as Figure 8</td></tr>
<tr><th colspan="2">Results</th></tr>
<tr><td>Benzoate</td><td>204.1 µg/mL</td></tr>
<tr><td>Caffeine</td><td>140.8 µg/mL</td></tr>
<tr><td>Aspartame</td><td>608.4 µg/mL</td></tr>
</table>

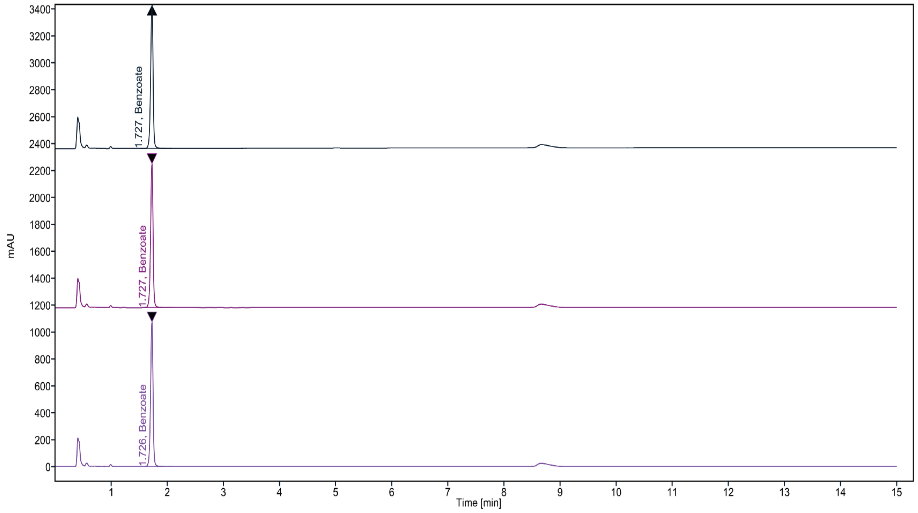


Figure 10. Lemon-lime soda chromatograms.

<table>
<tr><th colspan="2">Chromatographic Conditions</th></tr>
<tr><th colspan="2">Agilent 1260 Infinity III Quaternary LC</th></tr>
<tr><td colspan="2">All chromatographic conditions are the same as Figure 8</td></tr>
<tr><th colspan="2">Results</th></tr>
<tr><td>Benzoate</td><td>196.8 µg/mL</td></tr>
</table>

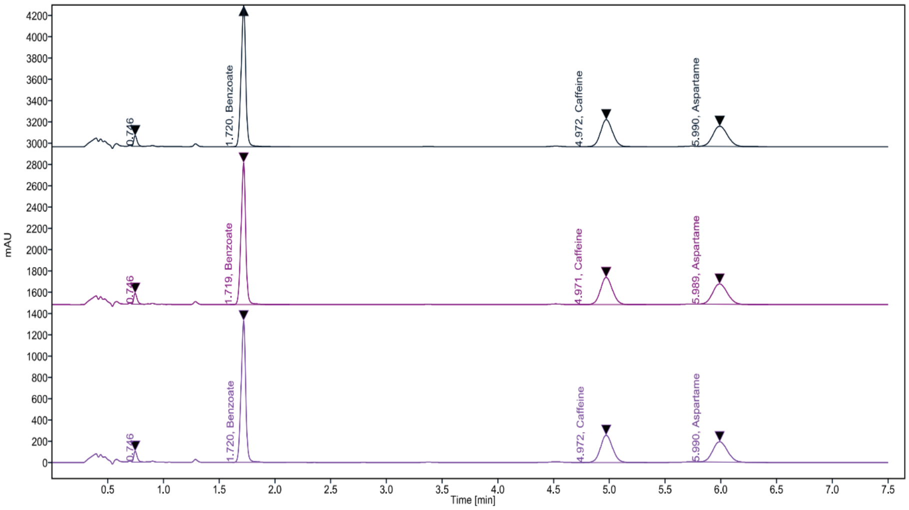
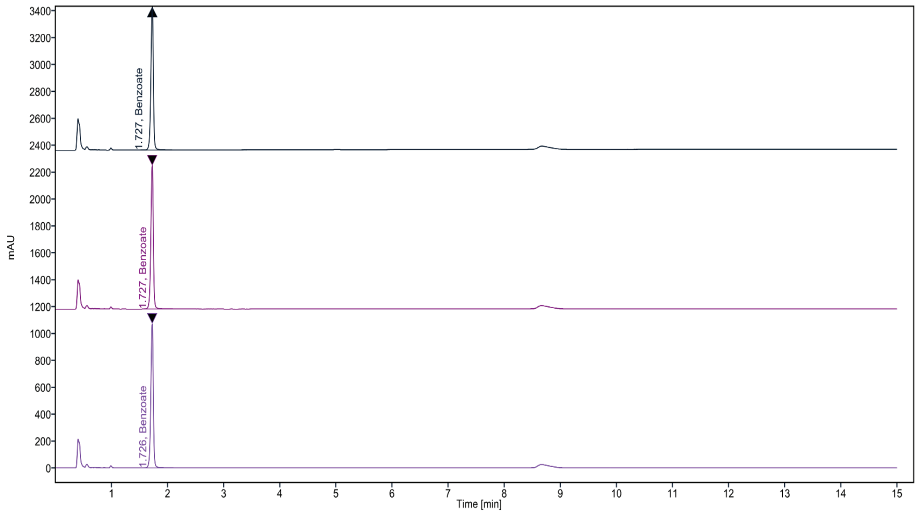


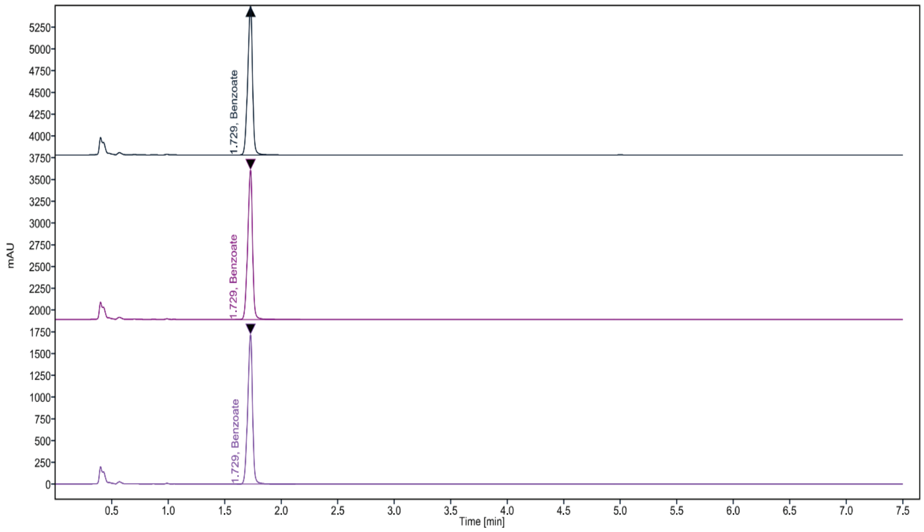
Figure 11. Diet pepper soda chromatograms.

<table>
<tr><th colspan="2">Chromatographic Conditions</th></tr>
<tr><th colspan="2">Agilent 1260 Infinity III Quaternary LC</th></tr>
<tr><td colspan="2">All chromatographic conditions are the same as Figure 8</td></tr>
<tr><th colspan="2">Results</th></tr>
<tr><td>Benzoate</td><td>304.6 µg/mL</td></tr>
<tr><td>Caffeine</td><td>130.3 µg/mL</td></tr>
<tr><td>Aspartame</td><td>657 µg/mL</td></tr>
</table>



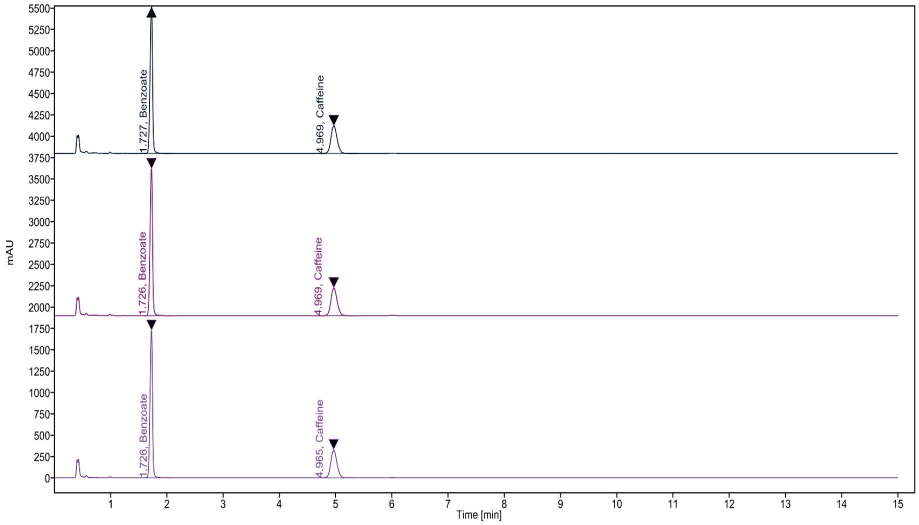
Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
All chromatographic conditions are the same as Figure 8	
Results	
Benzoate	246 µg/mL

Figure 12. Orange soda chromatograms.



Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
All chromatographic conditions are the same as Figure 8	
Results	
Benzoate	402.7 µg/mL

Figure 13. Ginger ale chromatograms.



Chromatographic Conditions	
Agilent 1260 Infinity III Quaternary LC	
All chromatographic conditions are the same as Figure 8	
Results	
Benzoate	407.0 µg/mL
Caffeine	168.8 µg/mL

Figure 14. Citrus-flavored soft-drink chromatograms.

Energy-drink chromatography and aqueous compatibility

Energy drinks present unique challenges due to high caffeine content and complex matrices rich in vitamins and botanicals. These highly polar compounds can interfere with early-eluting analytes, particularly acesulfame K.

A major advantage of the Poroshell 120 Phenyl-Hexyl column is its ability to operate under 100% aqueous mobile-phase conditions without phase collapse.[8] Chromatograms (Figure 15) demonstrated that decreasing methanol content reduced interference between acesulfame K and matrix components in energy drinks. At 0% methanol, baseline resolution was achieved. However, reduced organic content increased total runtime for later-eluting compounds.

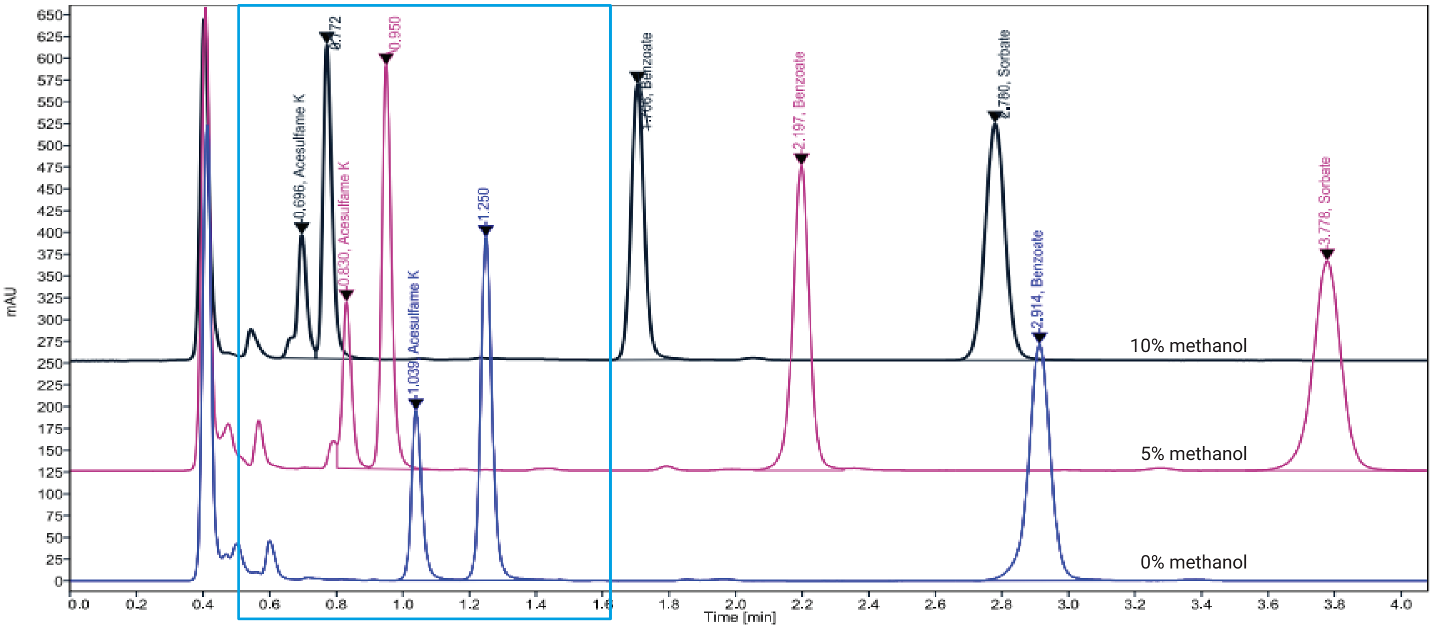
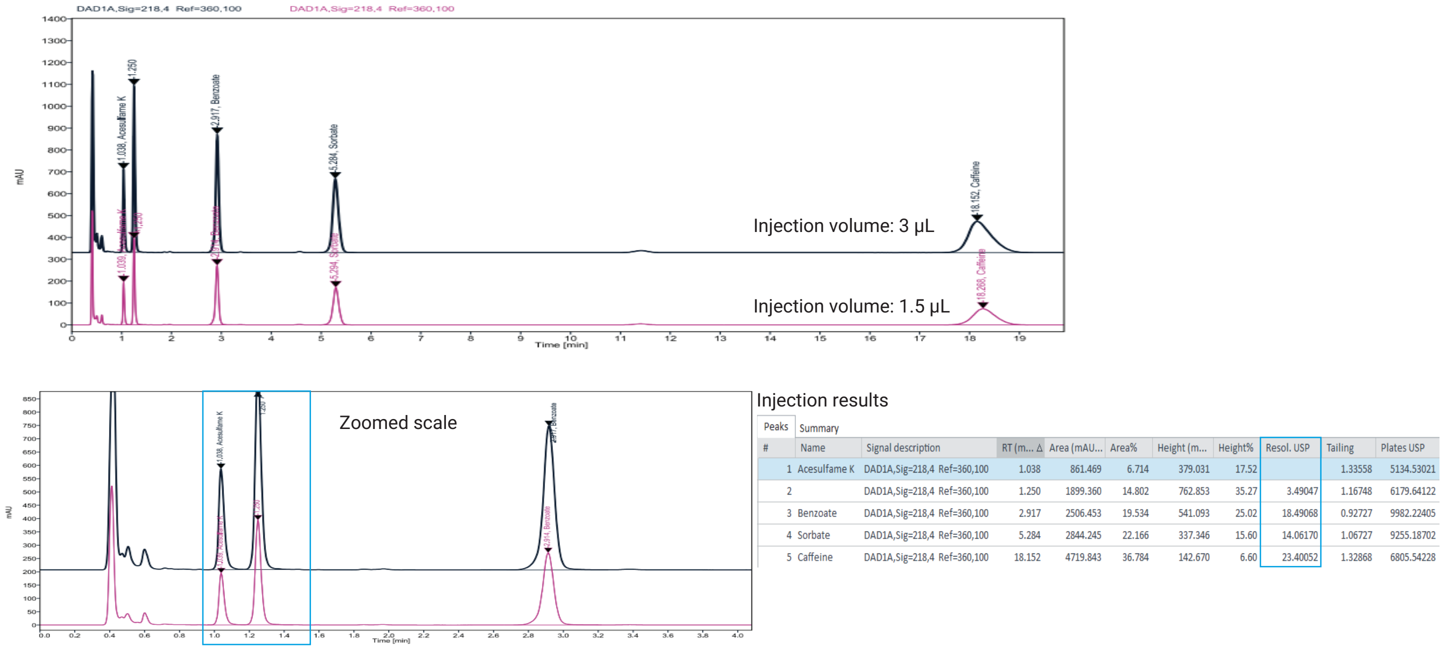


Figure 15. Popular energy drink chromatograms at varied methanol concentrations: 10, 5, and 0%.

Several energy drinks were evaluated, and none were found to contain aspartame, the latest eluting compound in the standards. Results for these beverages are shown in Figures 16, 17, and 18. Although caffeine eluted at approximately 18 minutes, the method provided excellent resolution for acesulfame K and several unidentified polar components eluting immediately afterward. This demonstrates the suitability of the phenyl-hexyl chemistry for resolving highly polar additives in complex, layered matrices such as energy drinks.

To ensure long term column performance, care should be taken to avoid storing the column in buffer. Based on recommendations from multiple column manufacturers, the buffer should be displaced with filtered water, followed by a rinse with 50:50 water:acetonitrile. Columns should then be stored in 100% aprotic solvent—typically acetonitrile—to prevent microbial growth, buffer precipitation, or deterioration of chromatographic performance over time.

Chromatographic Conditions (Figures 16 to 18)	
Agilent 1260 Infinity III Quaternary LC	
Mobile Phase	(100%) 20 mM sodium acetate pH 4.8 (adjusted with acetic acid)
Column	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Flow Rate	0.6 mL/min
Column Temperature	40 °C
Injection Volume	1.5 and 3 µL
Wavelength	218 nm; BW: 4 nm
Reference	360 nm; BW: 100 nm
Peak Width	40 Hz

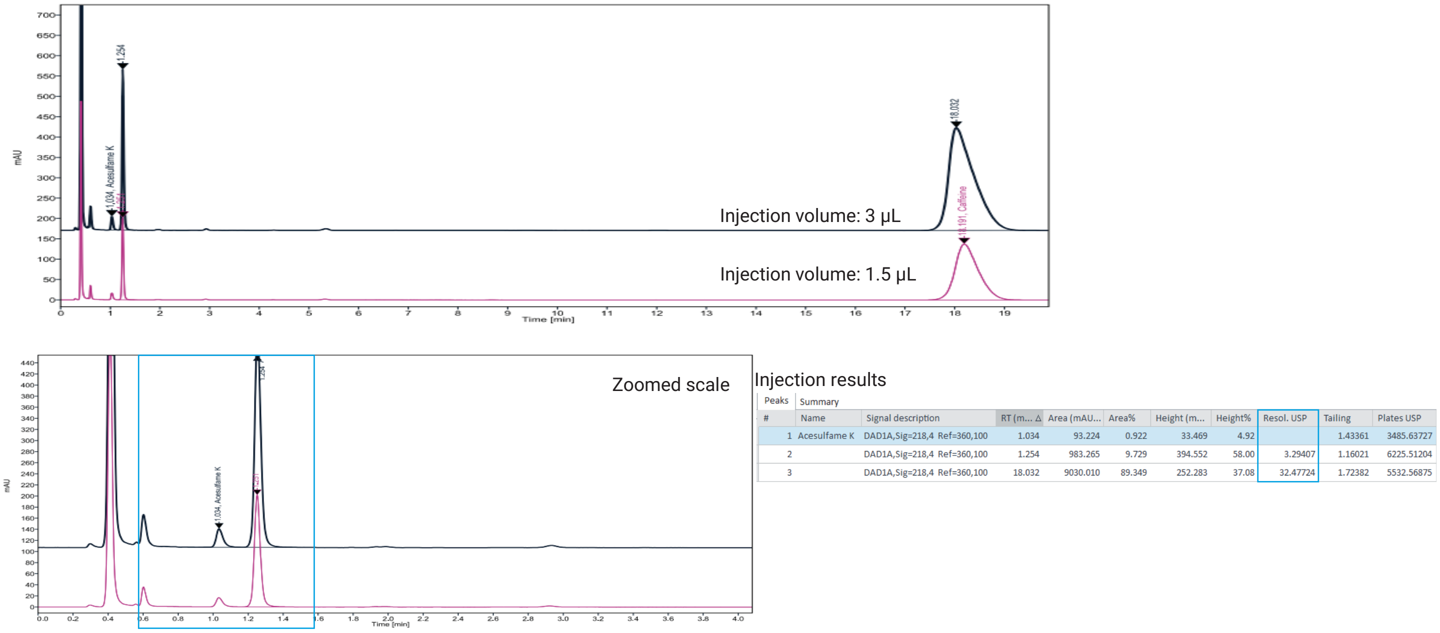


Injection results

Peaks | Summary

#	Name	Signal description	RT (m... Δ	Area (mAU...	Area%	Height (m...	Height%	Resol. USP	Tailing	Plates USP
1	Acesulfame K	DAD1A,Sig=218,4 Ref=360,100	1.038	861.469	6.714	379.031	17.52		1.33558	5134.53021
2		DAD1A,Sig=218,4 Ref=360,100	1.250	1899.360	14.802	762.853	35.27	3.49047	1.16748	6179.64122
3	Benzoate	DAD1A,Sig=218,4 Ref=360,100	2.917	2506.453	19.534	541.093	25.02	18.49068	0.92727	9982.22405
4	Sorbate	DAD1A,Sig=218,4 Ref=360,100	5.284	2844.245	22.166	337.346	15.60	14.06170	1.06727	9255.18702
5	Caffeine	DAD1A,Sig=218,4 Ref=360,100	18.152	4719.843	36.784	142.670	6.60	23.40052	1.32868	6805.54228

Figure 16. Energy drink 1 chromatograms at 0% methanol.

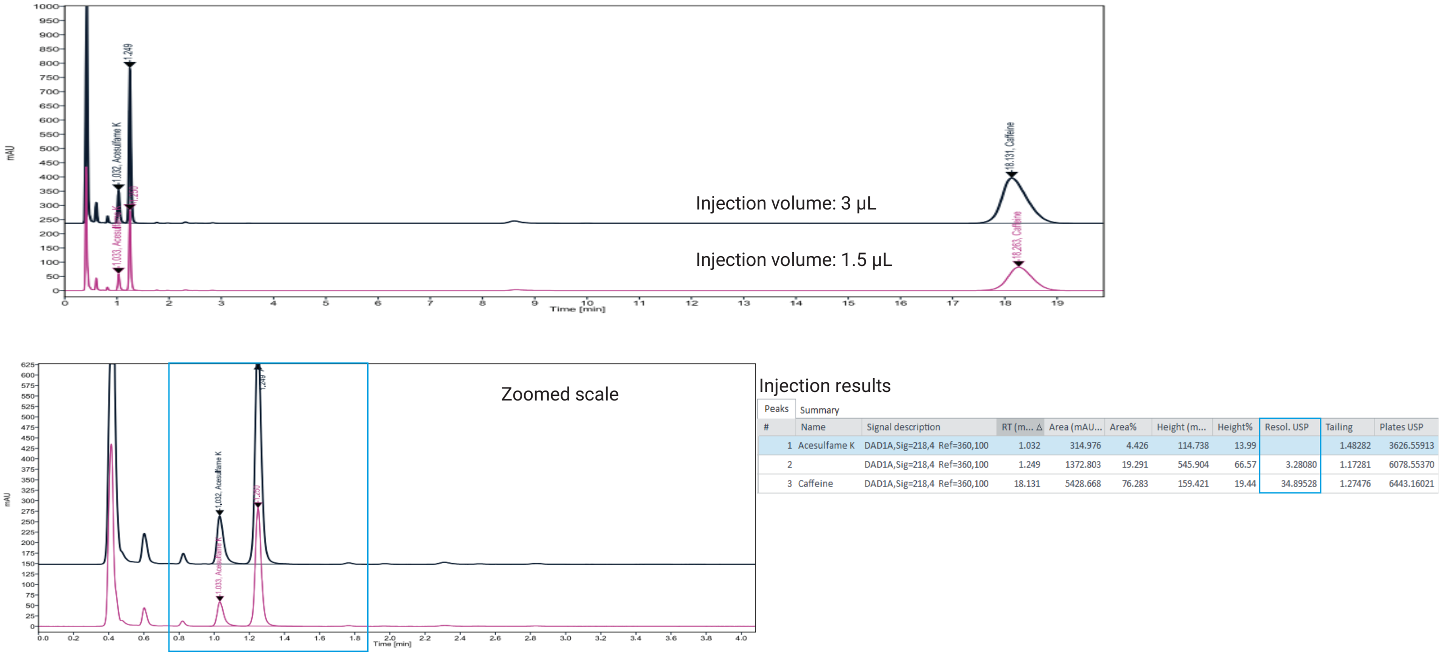


Injection results

Peaks Summary

#	Name	Signal description	RT (m... Δ	Area (mAU...	Area%	Height (m...	Height%	Resol. USP	Tailing	Plates USP
1	Acesulfame K	DAD1A,Sig=218,4 Ref=360,100	1.034	93.224	0.922	33.469	4.92		1.43361	3485.63727
2		DAD1A,Sig=218,4 Ref=360,100	1.254	983.265	9.729	394.552	58.00	3.29407	1.16021	6225.51204
3		DAD1A,Sig=218,4 Ref=360,100	18.032	9030.010	89.349	252.283	37.08	32.47724	1.72382	5532.56875

Figure 17. Energy drink 2 chromatograms at 0% methanol.



Injection results

Peaks Summary

#	Name	Signal description	RT (m... Δ	Area (mAU...	Area%	Height (m...	Height%	Resol. USP	Tailing	Plates USP
1	Acesulfame K	DAD1A,Sig=218,4 Ref=360,100	1.032	314.976	4.426	114.738	13.99		1.48282	3626.55913
2		DAD1A,Sig=218,4 Ref=360,100	1.249	1372.803	19.291	545.904	66.57	3.28080	1.17281	6078.55370
3	Caffeine	DAD1A,Sig=218,4 Ref=360,100	18.131	5428.668	76.283	159.421	19.44	34.89528	1.27476	6443.16021

Figure 18. Energy drink 3 chromatograms at 0% methanol.

Conclusion

This work demonstrates a fast, reliable, and high throughput LC method for quantifying six key additives commonly found in soft drinks using the Agilent 1260 Infinity III LC, Agilent OpenLab CDS, and an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column. The method provides strong chromatographic performance, excellent calibration linearity, and minimal sample preparation, making it well suited for routine quality-control environments. Its compatibility with highly aqueous mobile phases extends its applicability to complex matrices such as energy drinks, while diode array detection offers additional assurance in peak identification and helps distinguish coeluting components when formulations change.

Beyond finished-product testing, the method can be easily adapted for syrup and concentrate analysis through simple dilution with mobile phase. This flexibility enables earlier-stage quality checks during beverage production, helping bottling plants identify off-spec batches before filling operations begin. Catching these issues upstream can prevent significant material loss and operational downtime, saving manufacturers substantial cost. Together, these capabilities highlight the method's value as a robust, high throughput solution suitable for a wide range of soft-drink quality-control applications.

References

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