# Ultrafast and sensitive analysis of sweeteners, preservatives and flavorants in nonalcoholic beverages using the Agilent 1290 Infinity LC system

## Application Note

Food and Beverages


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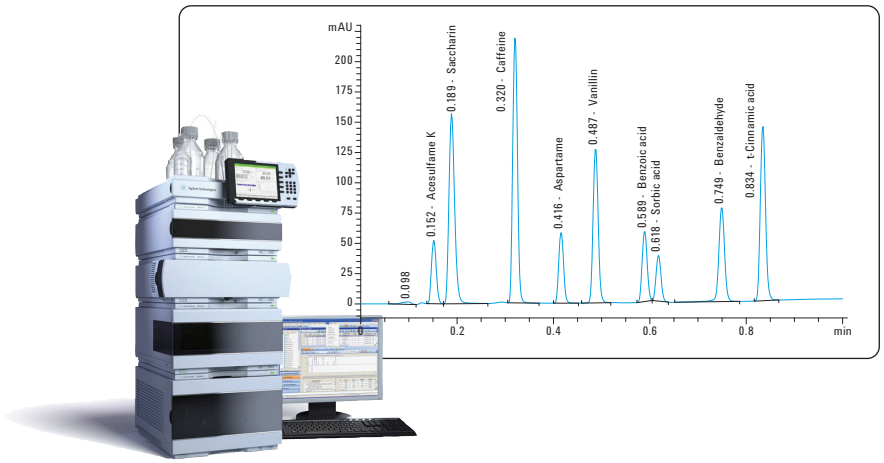



## Abstract

This Application Note describes the separation of nine food additives, acesulfame K, aspartame, caffeine, benzoic acid, sorbic acid, saccharine, trans-cinnamic acid and vanillin using the Agilent 1290 Infinity LC system. The ability of the Agilent 1290 Infinity LC pump to deliver mobile phases at high pressures of up to 1200 bars enables separation of the nine selected additives within a run time of 1 min. The Agilent 1290 Infinity Diode Array Detector (DAD) equipped with Agilent's proprietary Max-Light flow cell provides sufficient sensitivity for reliable analysis of even 1 µL of the sample. The developed method was applied for the screening of additives present in soft drinks and colas.


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## Introduction

Food additives are used for enhancing appearance, flavor, fragrance, and shelf life of processed food and drinks. Stringent regulations mandate careful monitoring of food additive levels to prevent health hazards to the consumers. Simple, sensitive, high-throughput methods that can handle large sample loads while maintaining data quality are required in food and beverage quality control laboratories.

Several analytical techniques including HPLC have been reported for the analysis of sweeteners[1, 2], preservatives[3] and other food additives. An Agilent Application Note[4] describes the separation of seven soft drink components using the Agilent 1200 Series Rapid Resolution LC system. The present Application Note shows the advantages of the Agilent 1290 Infinity LC system for sensitive and rapid analyses of nine additives found in beverages. This ultra high-pressure LC, coupled with the new Rapid Resolution High Definition (RRHD) columns that can withstand up to 1200 bars back pressure can separate the selected additives with a run time of 1 min. UV monitoring at 210 nm provides sufficient sensitivity with an injection volume of only 1 µL of a standard solution containing 50 µg/mL of each of the target analytes in 1:1 methanol/water.

## Experimental Conditions

### System

Agilent 1290 Series Infinity LC consisting of:

| Agilent Part Number | Description |
|---|---|
| G4220A | 1290 Infinity Binary Pump with integrated vacuum degasser and 35 µL Jet Weaver as mixing device |
| G4226A | 1290 Infinity Autosampler |
| G1330B | 1200 Series Thermostat |
| G1316C | 1290 Infinity Thermostatted Column Compartment |
| G4212A | 1290 Infinity Diode Array Detector, with Max-Light 10 mm 1- µL flow cell, |
| Software | ChemStation B.04.02 |

### Columns

Agilent ZORBAX RRHD Eclipse Plus C18 2.1 × 50 mm, 1.8 µm (p/n 959757-902)

Agilent ZORBAX RRHD Eclipse Plus C18 3.0 × 50 mm, 1.8 µm (p/n 959757-302)

### Mobile phase conditions

Organic solvent: acetonitrile (ACN)

Aqueous solvent: 20 mM phosphoric acid in Milli-Q water, pH 3.65 with ammonium hydroxide

### Gradient conditions

See individual chromatograms for flow rate and gradient time.

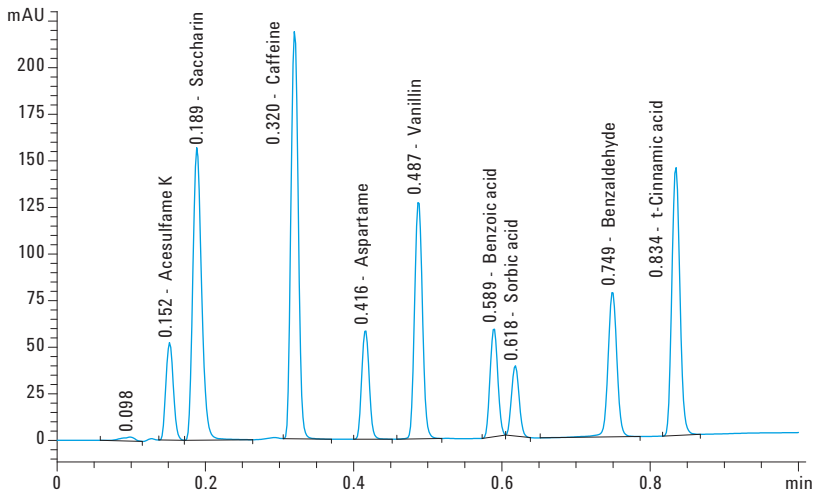
### Samples

1. Standard mixture of acesulfame K, sodium saccharin, caffeine, aspartame, vanillin, benzoic acid, sorbic acid, benzaldehyde, trans-cinnamic acid, all 50 µg/mL in 1:1 methanol/water.
2. Degassed soft drinks.

## Results

An earlier developed application method[4] was adapted for the separation of typical beverage additives using the Agilent 1290 Infinity LC system. The binary pump of the new system can deliver the mobile phase at very high pressures, up to 1200 bars, which are required for rapid analysis. New Rapid Resolution High Definition (RRHD) columns capable of withstanding back pressures of up to 1200 bars were used for analysis in this application work.

Figure 1 shows the analysis of a standard mix using a 3.0 mm × 50 mm, 1.8 µm column. Subsequently a 2.1 mm × 50 mm, 1.8 µm column was also tested for the separation of the analytes present in a mix of standards; the chromatogram obtained with this column is shown in Figure 2. It was possible to separate the components within a run time of 1 min using both the columns. Higher sensitivity obtained with the max light flow cell present in the DAD detector of the Infinity LC made it possible to achieve significant responses even with 1-µL injection volume. Although either of the two columns can be used for sample analysis, the 3.0 mm id column was used in this study as it provided slightly better peak shape and resolution.

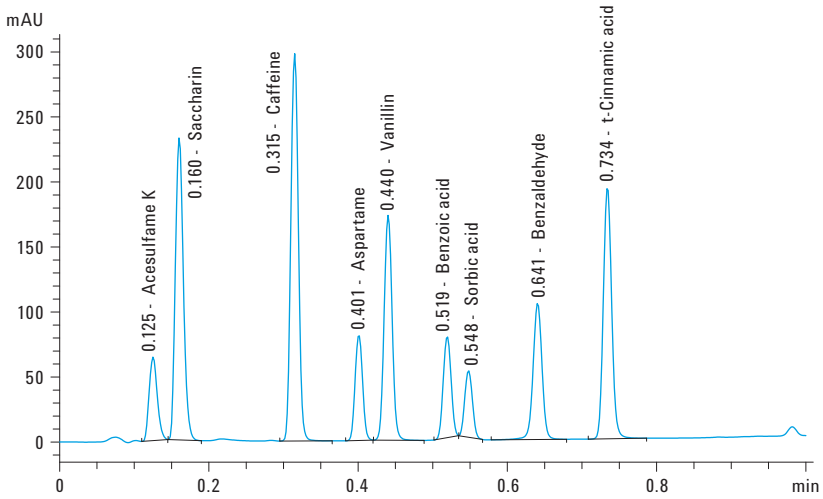



## Conditions

| | | |
|---|---|---|
| Injection volume: | 1 µL | |
| Analysis flow rate: | 2.2 mL/min | |
| Gradient: | 0.0 min | 10% acetonitrile |
| | 1.0 min | 43% acetonitrile |
| | 1.1 min | 10% acetonitrile |
| Post time: | 1 min | |
| Gradient slope: | 3.18% ACN/column volume | |
| Column temperature: | 45 °C | |
| Detection: | UV; 210 nm, 8 nm bandwidth; Reference: 360 nm, 60 nm bandwidth | |

**Figure 1**
**Gradient separation of soft drink additives on Agilent ZORBAX Eclipse Plus RRHD C18 3.0 mm × 50 mm, 1.8 µm column.**

Six replicate injections of the standard mix were made consecutively on the column to validate the precision of the method. Table 1 shows the retention times, peak areas and peak heights for each of the compounds along with their corresponding RSDs. All RSD values for the three parameters are less than 0.5 indicating excellent precision of the assay.




## Conditions

| | | |
|---|---|---|
| Injection volume: | 1 µL | |
| Analysis flow rate: | 1.6 mL/min | |
| Gradient: | 0.0 min | 8% acetonitrile |
| | 1.0 min | 48% acetonitrile |
| | 1.1 min | 8% acetonitrile |
| Post time: | 1 min | |
| Gradient slope: | 2.6% ACN/column volume | |
| Column temperature: | 45 °C | |
| Detection: | UV; 210 nm, 8 nm bandwidth; Reference: 360 nm, 60 nm bandwidth | |

**Figure 2**
**Gradient separation of soft drink additives on Agilent ZORBAX Eclipse Plus RRHD C18 2.1 mm × 50 mm, 1.8 µm column.**

| Compound | RT (min) | RSD | Peak Area (mAU*s) | RSD | Peak Height (mAU) | RSD |
|---|---|---|---|---|---|---|
| Acesulfame K | 0.15 | 0.31 | 36.43 | 0.19 | 52.23 | 0.15 |
| Saccharin | 0.19 | 0.28 | 128.76 | 0.16 | 156.85 | 0.17 |
| Caffeine | 0.32 | 0.11 | 149.51 | 0.18 | 218.51 | 0.17 |
| Aspartame | 0.42 | 0.10 | 40.80 | 0.36 | 58.57 | 0.33 |
| Vanillin | 0.49 | 0.09 | 93.19 | 0.20 | 127.77 | 0.18 |
| Benzoic acid | 0.59 | 0.07 | 41.70 | 0.30 | 58.13 | 0.21 |
| Sorbic acid | 0.62 | 0.07 | 26.75 | 0.40 | 37.64 | 0.29 |
| Benzaldehyde | 0.75 | 0.05 | 64.58 | 0.36 | 77.98 | 0.19 |
| t-Cinnamic acid | 0.83 | 0.05 | 108.12 | 0.20 | 143.84 | 0.21 |

**Table 1**
**Analysis showing method precision.**

Figures 3A– 3F show the results of the analysis of beverages bought from a local store (Bangalore, India). All the samples were analyzed using 3.0 mm × 50 mm, 1.8 µm column. The experimental conditions were the same as shown in Figure 1.

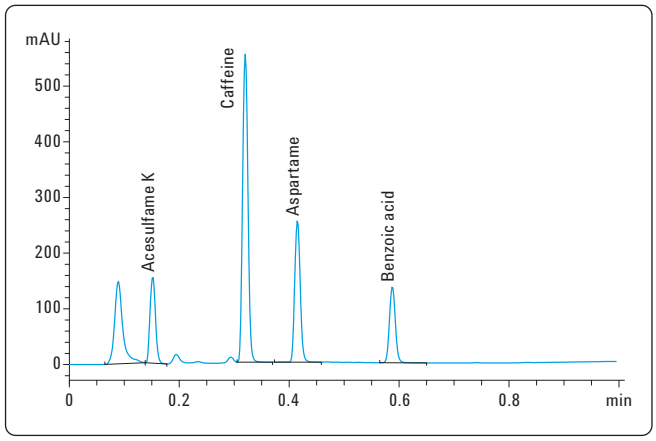



**Figure 3A**
**Cola A.**

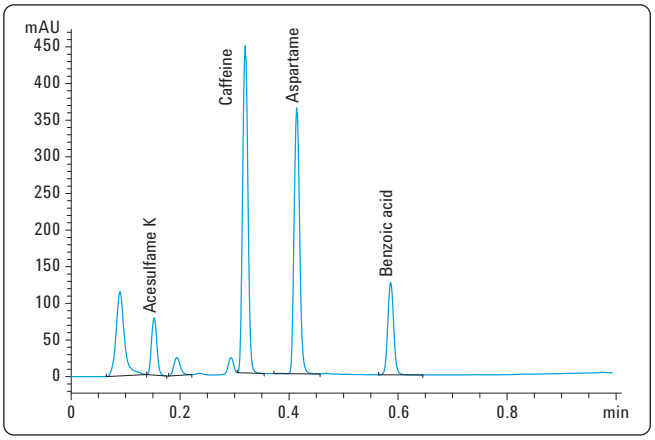



**Figure 3B**
**Cola B.**

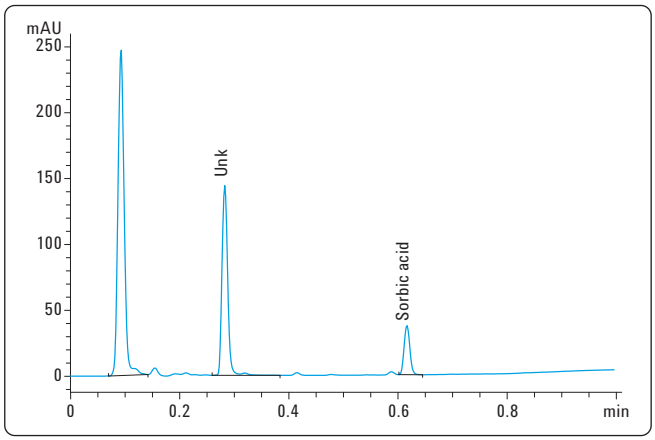



**Figure 3C**
**Orange flavored soft drink.**

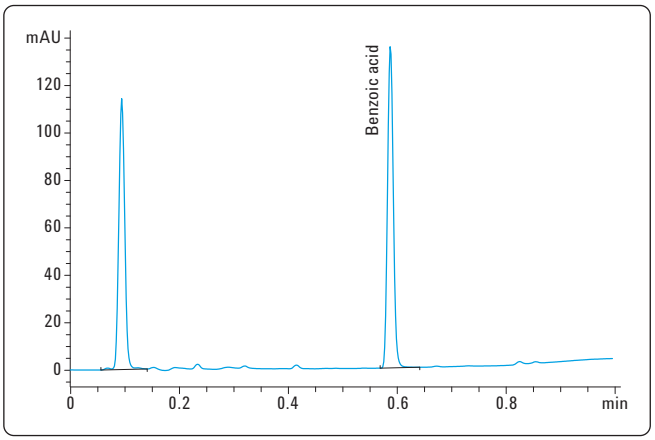



**Figure 3D**
**Lime flavored soft drink.**

A visual inspection of the chromatogram of the beverage containing <1% sugars (Figure 3e) shows two peaks at the retention times of benzoic and sorbic acids. Since the drink contains low concentrations of the sugars, it could be expected to have preservatives; yet the product label does not indicate the presence of either benzoic or sorbic acids. To establish the presence of these compounds in this soft drink, a second sample from a different batch was analyzed. Once again two peaks were seen at the retention times of benzoic and sorbic acids.

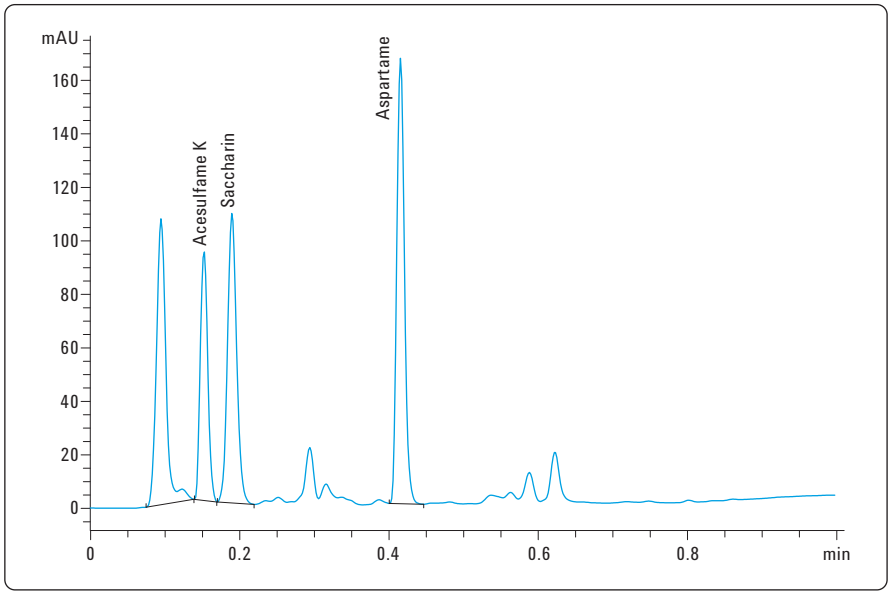



**Figure 3E**
**Juice containing natural fruit extracts, < 1% sugars.**

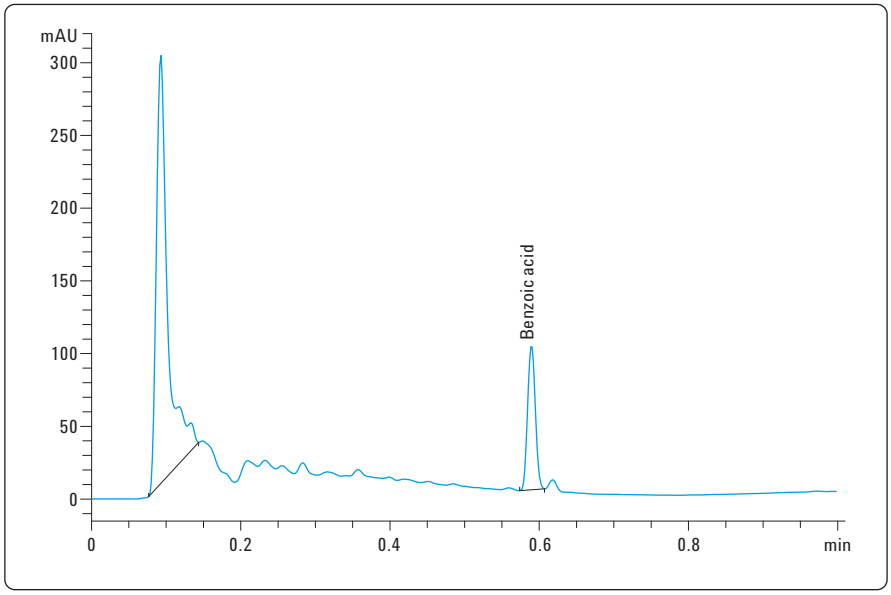



**Figure 3F**
**Sparkling grape drink.**

To determine the identity of these peaks, the UV spectra of the peaks were compared with the UV spectra of the standards using the spectral matching feature of ChemStation. This required reintegration of the chromatogram with modified integration parameters. Figure 4 shows the reintegrated chromatogram of the second sample with the two peaks now labeled as benzoic and sorbic acids.

Spectral matching is a powerful tool that can help confirm the presence of a compound in the sample matrix. Figure 5 shows the overlay of the spectrum, of the benzoic acid peak from the sample, and the spectrum of the standard. The excellent correlation confirms the identity of the peak and the presence of benzoic acid in the sample. This technique also helped prove that the peak labeled sorbic acid is not sorbic acid. The spectral matching, shown in Figure 6 suggests that the UV spectrum of the compound corresponding to this peak does not match the sorbic acid spectrum. This helps eliminate the presence of sorbic acid in the sample.

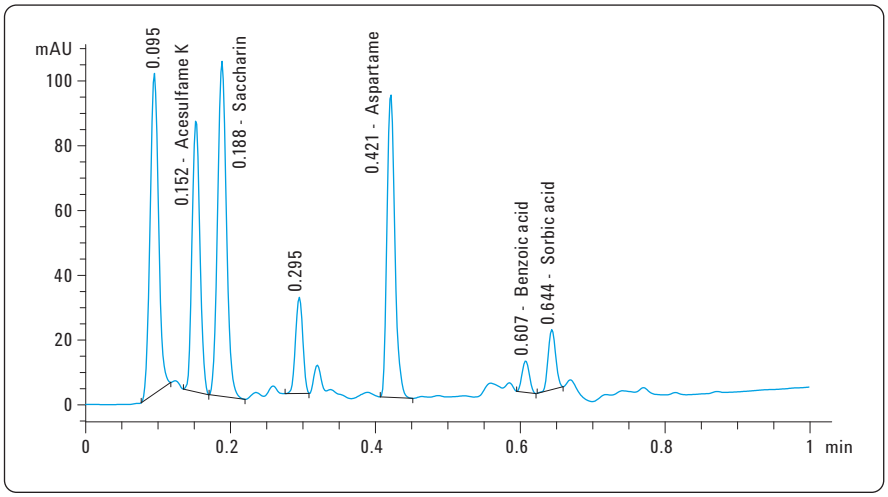



**Figure 4**
**Reintegrated chromatogram of the juice containing natural fruit extracts, < 1% sugars.**

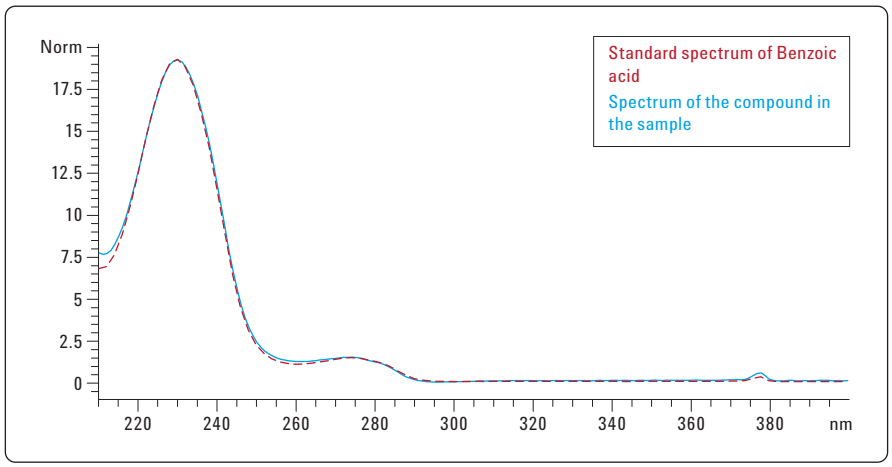



**Figure 5**
**Confirmation of the benzoic acid peak by spectral matching.**

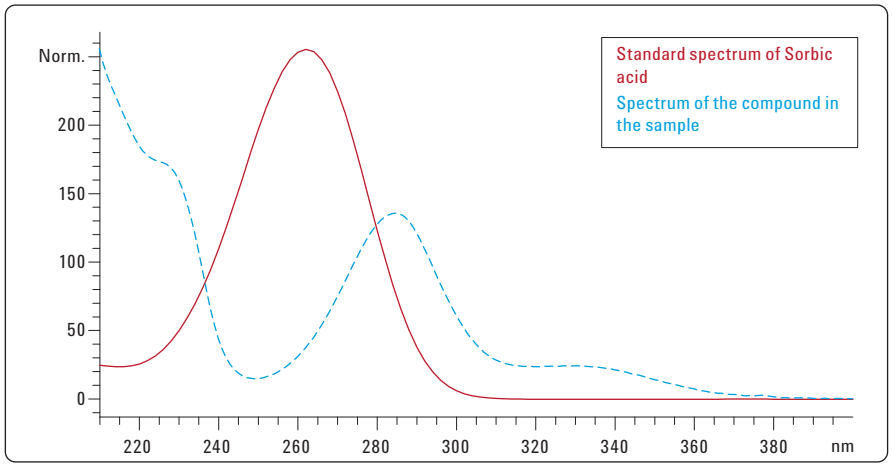



**Figure 6**
**Elimination of the sorbic acid peak by spectral matching.**

## Conclusion

In this Application Note, we have demonstrated that the Agilent 1290 Infinity pump can deliver mobile phases at high pressures of up to 1200 bars. When this pump is used with the new Rapid Resolution High Definition (RRHD) columns that can withstand high back pressures, separation of the nine selected additives can be achieved within a run time of 1 min. The Max-Light flow cell of the detector provides sufficient sensitivity for reliable analysis of even 1 µL of the sample. The developed method was applied for the screening of additives present in soft drinks and colas.

## References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www.agilent.com/chem/1290



April 15, 2010
Publication Number 5990-5590EN


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