# Constituents and Additives

Food products are analyzed for a variety of reasons, e.g., compliance with legal and labeling requirements, assessment of product quality, determination of nutritive value, and detection of adulteration, etc. According to the Codex Alimentarious Commission – "Food Additive" means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value. The term "Food additive" does not include contaminants or substances added to food for maintaining or improving its nutritive value. "Food additives" do not include use of vitamins, minerals, herbs, salt, spices, yeast, hops, starter cultures, malt extract, etc. "Food additives" are intentionally added to food and must be safe for a lifetime of consumption based on current toxicological evaluation.

"Food additives" are classified on the basis of their functional use and are grouped as:

| | | |
|---|---|---|
| Colors | Preservatives | Acidity Regulators |
| Antioxidants | Anti-caking agents | Antifoaming Agents |
| Artificial sweeteners | Enzymes | Emulsifiers |
| Emulsifying agents | Flavors | Flavor enhancers |
| Modified Starches | Phosphates | Stabilizers |

Thickening and jellying agents.

# Preservatives

A preservative is a naturally occurring or synthetically produced substance that is added to food and beverages to prevent decomposition (either by microbial growth or chemical changes). Preservatives in food can be compounds used alone or combined with other methods of food preservation. Rosemary extract, hops, salt, sugar, vinegar, alcohol, diatomaceous earth and castor oil are used as food preservatives. They are examples of natural food preservatives.

Preservatives can be classified in two groups, Class I and II, where the former are represented by common household products such as vinegar, salt, sugar, honey, and vegetable oil. Class II preservatives refers to those preservatives which are chemically manufactured.

There are antimicrobial preservatives (inhibit growth of bacteria or fungi) such as sorbic acid and its salts, benzoic acid and its salts, calcium propionate, sodium nitrite/sodium nitrate, sulfites (sulfur dioxide, sodium bisulfite, potassium hydrogen sulfite, etc.) and disodium EDTA. There are also antioxidants (inhibit oxidation) such as BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoluene), TBHQ (tert-Butylhydroquinone) and propyl gallate. Other preservatives include ethanol and methylchloroisothiazolinone. Freezing, pickling, smoking and salting techniques can also be used to preserve food.

On the next pages, a method is presented for determination of caffeine in a commercial caffeine containing cola, and caffeine and potassium sorbate in an energy beverage.

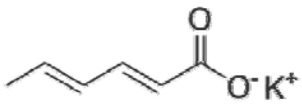
# Caffeine and Potassium Sorbate

**Recommended column:**
Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm (1.52022.0001)

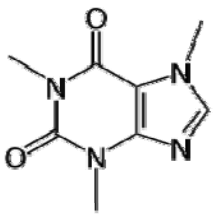
**Recommended solvents and reagents**
Water: Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q® water purification system

Di-ammonium hydrogen phosphate for analysis EMSURE® ACS,ISO,Reag. Ph Eur (1.01207)
ortho-Phosphoric acid 85% for analysis EMSURE® ACS,ISO,Reag. Ph Eur (1.00573)

**Sample Preparation**
Take 5 mL of an commercial caffeine containing cola and sonicate for 5 minutes, thereafter add 0.2 mL of phosphoric acid solution (1M) and make up to a final volume of 10mL by adding water. This gives a dilution factor of 2 for sample.




Potassium Sorbate



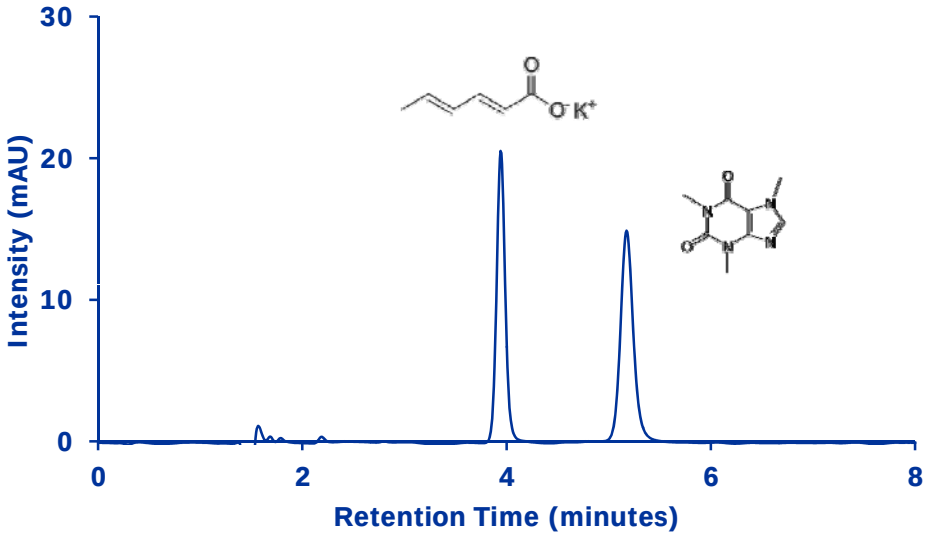

Caffeine

# Caffeine and Potassium Sorbate - Standards

## Chromolith® HighResolution RP-18 endcapped

### Chromatographic Conditions

| | | |
|---|---|---|
| Column: | Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm | (1.52022.0001) |
| Injection: | 10 µL | |
| Detection: | UV, 220 nm | |
| Cell: | 1 µL/10 mm | |
| Flow Rate: | 1.0 mL/min | |
| Mobile Phase: | A: 15 mM ammonium acetate and 50 mM potassium di-hydrogen phosphate 40:60 v/v<br>B: Acetonitrile<br>C: Methanol<br>Composition: A:B:C= 85:3:12 (v/v) | |
| Temperature: | 30 °C | |
| Diluent | water | |
| Sample: | 5µg/mL of potassium sorbate and caffeine diluted in water | |
| Pressure Drop: | 47 Bar (681psi) | |




### Chromatographic Data

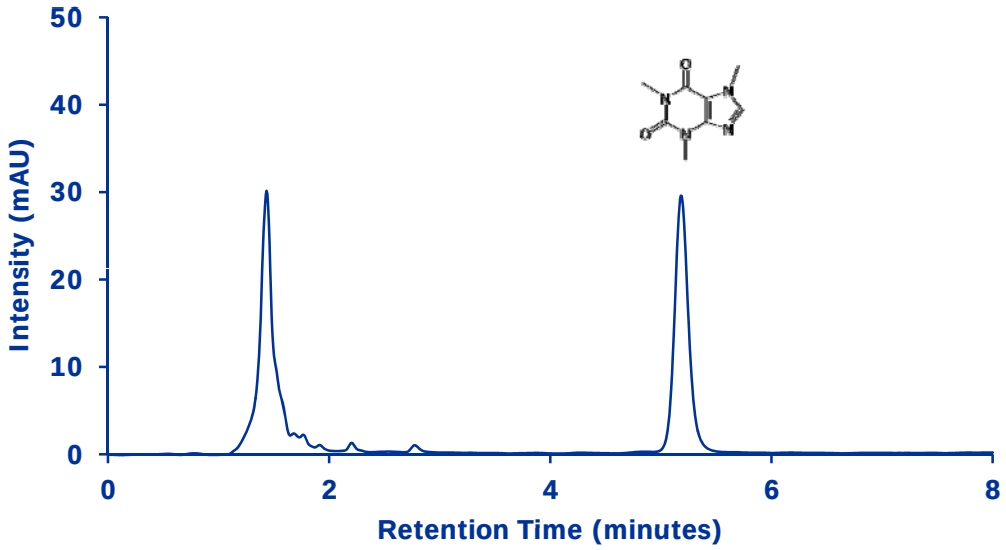
| No. | Compound | Retention Time (min) | Theoretical plate | Asymmetry |
|---|---|---|---|---|
| 1 | Potassium sorbate | 3.9 | 10886 | 1.1 |
| 2 | Caffeine | 5.2 | 8600 | 1.1 |

# Caffeine in Beverage

## Chromolith® HighResolution RP-18 endcapped

### Chromatographic Conditions

| | | |
|---|---|---|
| Column: | Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm | (1.52022.0001) |
| Injection: | 10 µL | |
| Detection: | UV, 220 nm | |
| Cell: | 1 µL/10 mm | |
| Flow Rate: | 1.0 mL/min | |
| Mobile Phase: | A:15 mM ammonium acetate and 50 mM potassium di-hydrogen phosphate 40:60 v/v<br>B: Acetonitrile<br>C: Methanol<br>Composition: A:B:C= 85:3:12 (v/v) | |
| Temperature: | 30 °C | |
| Diluent | water | |
| Sample: | 10mL of a commercial caffeine containing cola was sonicated for 5 minutes.<br>1ml of the degassed cola was thereafter diluted to 10mL by water. (sample is diluted 10 times) | |
| Pressure Drop: | 47 Bar (681psi) | |




### Chromatographic Data

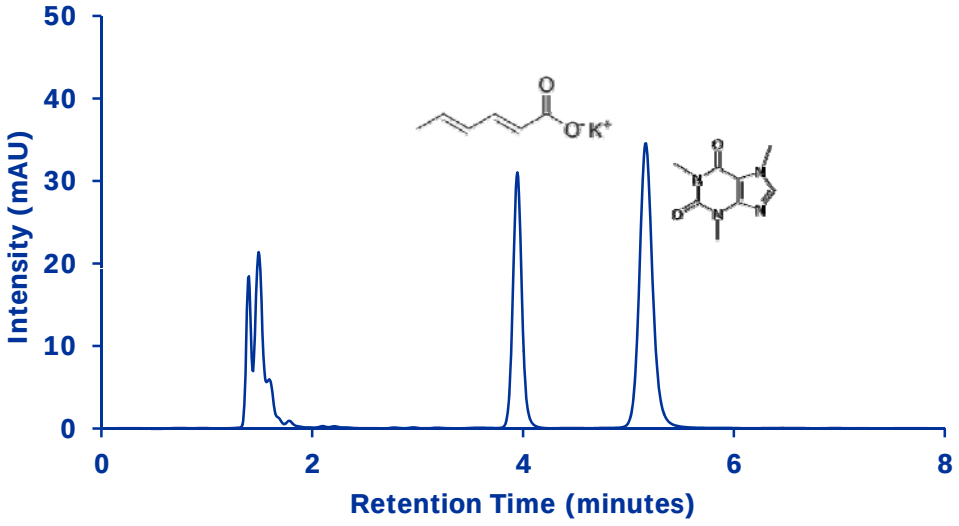
| No. | Compound | Retention Time (min) | Theoretical plate | Asymmetry |
|---|---|---|---|---|
| 1 | Caffeine | 5.2 | 8600 | 1.1 |

# Potassium Sorbate and Caffeine in Beverages

## Chromolith® HighResolution RP-18 endcapped

### Chromatographic Conditions

| | | |
|---|---|---|
| Column: | Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm | (1.52022.0001) |
| Injection: | 10 µL | |
| Detection: | UV, 220 nm | |
| Cell: | 1 µL/10 mm | |
| Flow Rate: | 1.0 mL/min | |
| Mobile Phase: | A: 15 mM ammonium acetate and 50 mM potassium di-hydrogen phosphate 40:60 v/v<br>B: Acetonitrile<br>C: Methanol<br>Composition: A:B:C= 85:3:12 (v/v) | |
| Temperature: | 30 °C | |
| Diluent | water | |
| Sample: | 10mL of the beverage was sonicated for 5minutes.<br>1ml of the degassed beverage was diluted to 25 mL by water (sample is diluted 25 times). | |
| Pressure Drop: | 47 Bar (681psi) | |



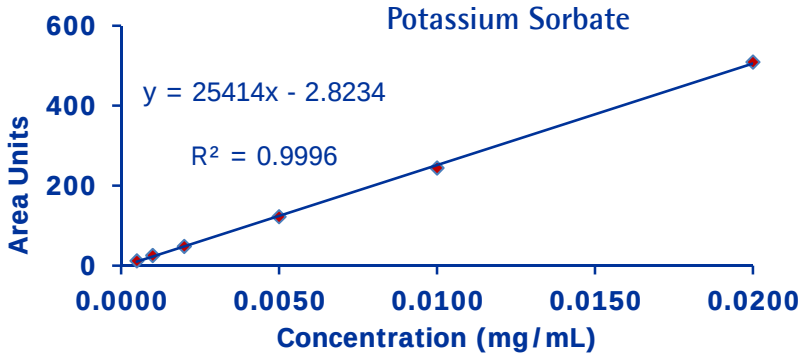

### Chromatographic Data

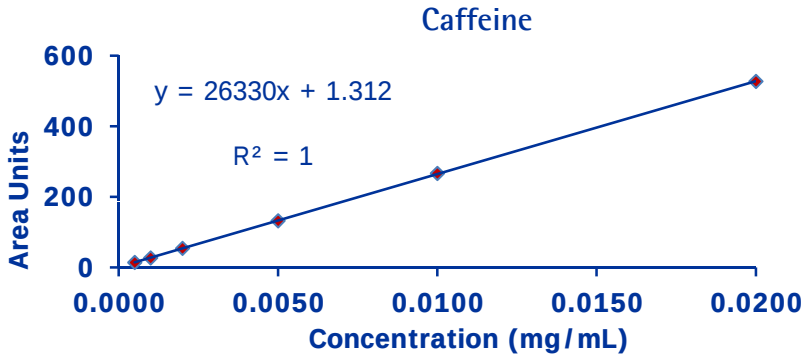
| No. | Compound | Retention Time (min) | Theoretical plate | Asymmetry |
|---|---|---|---|---|
| 1 | Potassium sorbate | 3.9 | 10700 | 1.1 |
| 2 | Caffeine | 5.2 | 8600 | 1.1 |

# Potassium Sorbate and Caffeine in Beverages

## Chromolith® HighResolution RP-18 endcapped

Calibration curves were constructed in the range 5-20 ppb (µg/mL) for potassium sorbate and caffeine. Five (n=5) replicate injections of standard solution were analyzed at the five different concentration levels to determine the method linearity. The relative standard deviation for replicate injections at all concentration levels was better or equal to 1.5% for both compounds.




The detection limits (LOD's) and the quantitation limits (LOQ's) were 0.54/1.63 ppb for potassium sorbate and 0.14/0.42 ppb for caffeine, respectively. The energy drink contained 0.18 mg/mL of potassium sorbate and 0.29 mg/mL caffeine.

In total 1000 injections of standards and diluted energy drink samples were analysed and on the following page sample injection 1, 500 and 1000 are shown. The method is robust and can be used for quantitation of potassium sorbate and caffeine in beverages.

# Potassium Sorbate and Caffeine in Beverages

## Chromolith® HighResolution RP-18 endcapped