

# Sulfonamide Artificial Sweeteners in Food Products

## Analysis of Acesulfame, Saccharin and Cyclamate by HPLC with Post-Column Derivatization



Nonnutritive sweeteners are widely used in foods and beverages. Since some studies raised questions about long-term safety of these compounds, their concentration in food products is regulated. Among the artificial sweeteners used worldwide Saccharin and Cyclamate cause most controversy and their use is restricted in many countries, including the US. Liquid chromatography is a method of choice for artificial sweeteners but Cyclamate analysis is complicated by the fact that this compound does not exhibit noticeable UV absorbance.

This method allows for simultaneous analysis of Acesulfame, Saccharin and Cyclamate by LC with post-column derivatization followed by fluorescence detection. Since the sweeteners are commonly used in combination, a single method of analysis is preferred.

### Method

#### Analytical Conditions

**Analytical Column:** Reversed-phase column, C<sub>18</sub>, 2x100 mm

**Temperature:** 50 °C

**Flow Rate:** 0.2 mL/min

#### Mobile Phase:

0.02 M Potassium Phosphate Monobasic in water. Isocratic for 8 min, followed by washing the column with 70 % ACN – 30 % 0.02 M KH<sub>2</sub>PO<sub>4</sub>

**Injection Volume:** 10-50 uL

#### Post-Column Conditions

**Post-Column System:** Onyx PCX, Pinnacle PCX or Vector PCX

**Reagent:** Hexadecyltrimethyl ammonium bromide (10<sup>-3</sup> M),  
1,6 Diphenyl-1,3,5-hexatriene (4x10<sup>-6</sup> M)

#### Reagent Preparation:

To 250 mL of DI water add 0.09 g of Hexadecyltrimethylammonium bromide and sonicate until fully dissolved. Add 300 uL of 1,6-diphenyl-1,3,5-hexatriene (DPH) stock solution (i), mix well. Protect the reagent from light.

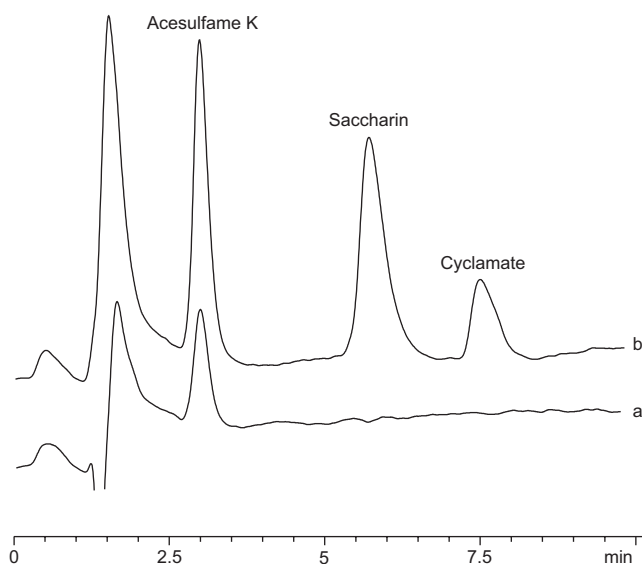
- (i) Dissolve 0.007 g of 1,6-diphenyl-1,3,5-hexatriene (DPH) in 10 mL of dry THF. Store the stock solution in refrigerator, protected from light.

**Reactor Volume:** 0.15 mL

**Reactor Temperature:** 40 °C

**Flow Rate:** 0.3 mL/min

**Detection:** FLD Detector,  
 $\lambda_{ex}$  : 365 nm,  $\lambda_{em}$  : 460 nm



Chromatograms of a) Diet cola containing Acesulfame K; b) Soft drink spiked with 60 ppm of artificial sweeteners.

### References

James F. Lawrence, Analyst, vol. 112, No 6 (1987), 879-881

James F. Lawrence, Claudette F. Charbonneau, J. Assoc. Anal. Chem., vol. 71, No 5 (1988), 934-937