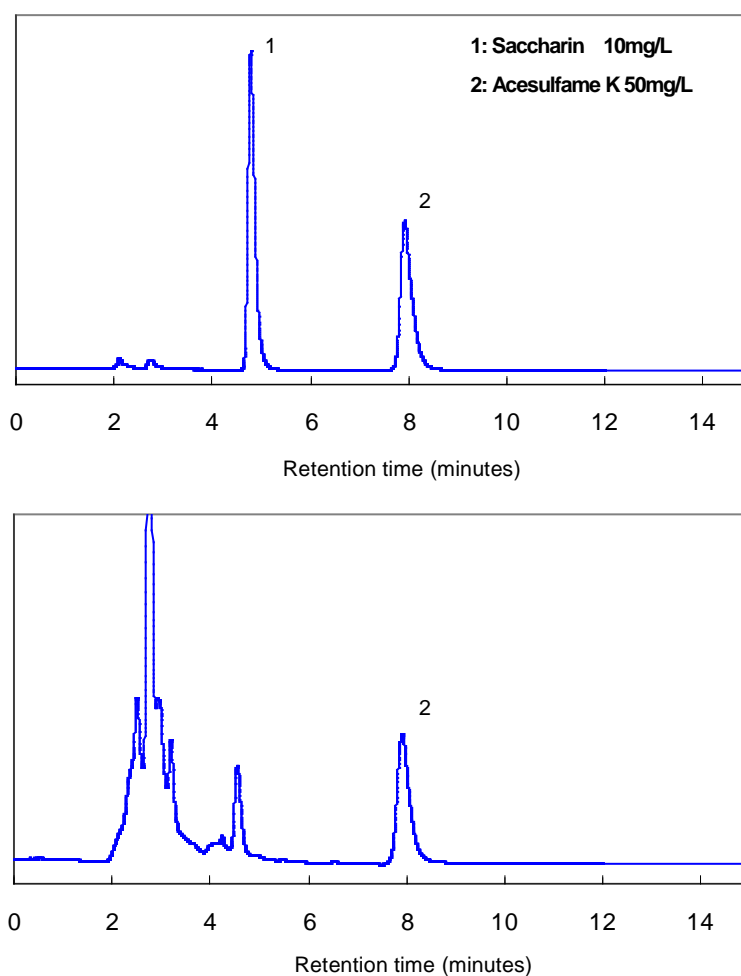


## Analysis of Synthetic Sweeteners in Coffee by HPLC

Synthetic sweeteners are used in many foods because they have fewer calories than sugar. Acesulfame potassium (Acesulfame-K), designated as a food additive in April 2000, has 200 times the sweetness of sugar, and because it has zero calories, is used in sweets and soft drinks as a substitute for sugar. Standards established for use in food include  $\leq 15\text{g/kg}$  as a sugar substitute in foods (for coffee, etc.), and  $\leq 0.5\text{g/kg}$  in soft drinks.

The examples shown here were produced by analyzing Acesulfame-K in coffee using a hydrophilic interaction liquid chromatography (HILIC) column. Under these analytical conditions, saccharine can also be analyzed simultaneously. Samples were pretreated in compliance with the Methods of Analysis in Health Science and Acesulfame-K in canned coffee was analyzed. Quantitation limits ( $S/N=10$ ) in this analytical method were  $0.01\text{mg/L}$  for saccharine and  $0.1\text{mg/L}$  for Acesulfame-K. Also shown for reference purposes is an example of simultaneous analysis of Acesulfame-K, saccharine, and aspartame conducted under analytical conditions that comply with the Methods of Analysis in Health Science (reversed phase chromatography using ion pair reagents).

Figure 1. Chromatograms of standard sample (top)  
Coffee beverage extract (bottom)



**Table 1. Analytical Conditions**

Column:	TSKgel NH <sub>2</sub> -60, 4.6mm ID x 25cm
Mobile phase:	1.0% H <sub>3</sub> PO <sub>4</sub> / acetonitrile = 30/70
Flow rate:	1.0mL/min
Detection:	UV@210nm
Temperature:	40°C
Injection vol.:	5µL

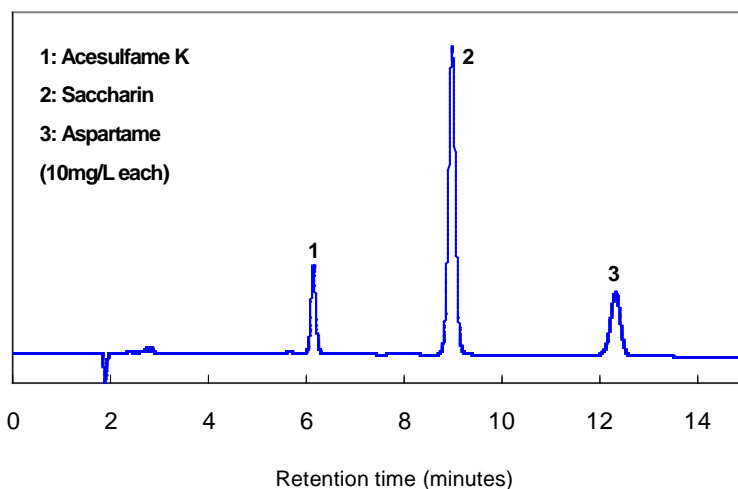
**Figure 2. Procedure for pretreatment of coffee beverage**

1. Inject 20g of coffee beverage into dialysis tube together with 10mL of internal dialysis fluid.
2. Place this dialysis tube into a vessel containing 150mL of external dialysis fluid and add external dialysis fluid to a total volume of 200mL.
3. Leave at room temperature for 24 hours while gently shaking.
4. Mix external dialysis fluid, and dilute with solvent to prepare sample to be analyzed.

Internal dialysis fluid: 1% phosphoric acid + 10 % sodium chloride

External dialysis fluid: 1% phosphoric acid

**Figure 3. Simultaneous analysis by reversed phase chromatography using ion pair reagent (standard substance)**



**Table 2. Analytical Conditions**

Column:	TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
Mobile phase:	10mM tetra-n-propylammonium hydroxide in CH <sub>3</sub> OH/H <sub>2</sub> O = 25/75, pH 4.0 (H <sub>3</sub> PO <sub>4</sub> )
Flow rate:	1.0mL/min
Detection:	UV@210nm
Temperature:	40°C
Injection vol.:	5µL



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