

CHROM 3962

The separation and identification of four synthetic sweeteners by thin-layer chromatography

Several paper and thin-layer chromatographic methods¹⁻⁶ have been reported for the separation of combinations of saccharin with sodium or calcium cyclamate, or of saccharin, cyclamates, and dulcin. Some of these methods are rather complex and none has included P4000 (5-nitro-2-*n*-propoxyaniline). A method is here presented for a rapid separation and identification of all four sweeteners by thin-layer chromatography.

Experimental

A 0.25 mm layer of silica gel (Adsorbosil-1, Applied Science Laboratories, State College, Pa.) is prepared and allowed to dry at room temperature. This must not be oven-dried or stored over desiccant. The plate is spotted with dulcin and P4000, both in 95% ethanol, and sodium saccharin and calcium cyclamate, both in a mixture of equal volumes of 95% ethanol and water. The developing solvent is a mixture of butanol-ethanol (95%)—ammonia (28%)—water (40:4:1:9). The plates are developed 10 cm from the spotting line in a standard tank saturated with developing solvent vapor.

The developed plates are promptly examined in short-wave (254 m μ) U.V. radiation, in which saccharin is a blue fluorescent spot and P4000 a dark spot. A solution of 1% chloranil in benzene is immediately sprayed heavily on the plate, which is then heated in a 100° oven about 5 min. Saccharin and cyclamates appear as white spots on a lavender background. A light spraying with 1% *p*-dimethylamino-benzaldehyde in 10% (w/v) HCl then reveals dulcin as a bright yellow spot on a white background, while the P4000 spot remains lavender. It should be noted that each sweetener is detected differently, as well as having different R_F values.

Alternatively, the developed plate is sprayed with 1% α -naphthylamine in acetic acid and viewed in long-wave (360 m μ) U.V. radiation. Cyclamates and dulcin appear light, saccharin and P4000 dark. This spray reagent is not recommended due to the health hazard involved in its use.

R_F values obtained with this system are: calcium cyclamate, 0.30; sodium saccharin, 0.41; dulcin, 0.75; P4000, 0.84. Values are based on the average of ten determinations. The sensitivity of the first detection method is 5 μ g of calcium cyclamate, 2 μ g of sodium saccharin, and 1 μ g each of dulcin and P4000. The α -naphthylamine method detects 40 μ g calcium cyclamate, 20 μ g sodium saccharin, 20 μ g dulcin, and 2 μ g P4000.

St. Joseph's College,
Philadelphia, Pa. (U.S.A.)

TREAN KORBELAK*
JOSEPH N. BARTLETT

- 1 A. CASTIGLIONI, *Z. Anal. Chem.*, 145 (1955) 188.
- 2 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 38 (1955) 943.
- 3 IN SUK KO, IN SOO CHUNG AND YOUNG HOO PARK, *Rept. Nat. Chem. Lab. (Korea)*, 3 (1959) 72; *C.A.*, 54 (1960) 10181d.
- 4 T. SALO, E. AIRO AND K. SALMINEN, *Z. Lebensm.-Untersuch.-Forsch.*, 124 (1964) 20.
- 5 G. J. DICKES, *J. Assoc. Publ. Analysts*, 3 (1965) 118.
- 6 W. KAMP, *Pharm. Weekblad*, 101 (1966) 57.

First received October 3rd, 1968; revised manuscript received January 23rd, 1969

* Present address: U.S. Food & Drug Administration, Philadelphia, Pa. 19106 (U.S.A.).