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Separation and detection of synthetic sweetners by thin-layer chromatography

A number of chromatographic procedures have been reported for the separation and identification of synthetic sweetners[1–11], but no report on the chromatography of the sweetners on Avicel thin layers* has been given so far as is known. Although both saccharin and dulcin are detectable by their UV absorption (or fluorescence) and other useful methods[1–3,6,8,10] with fairly high sensitivity, sactisfactory methods for the detection of cyclamate are rather poor.

The present paper describes the separation of the three sweetners on Avicel thin layers and their detection by a new sensitive method which consists of spraying with Pinacryptol Yellow reagent and UV irradiation.

Materials

Chromatographic materials. Avicel (or Avirin) is a microcrystalline cellulose manufactured by the American Viscose Division of FMC Co. (Marcus Hook, Pa., U.S.A.). Avicel SF, a finely powdered product of Avicel for use in TLC, was obtained from Funakoshi Pharm. Co. and Asahi Kasei Co. (Tokyo, Japan). Polyamide (Polyamide Woelm TLC), silica gel (Silica Gel G, Merck), alumina (Aluminum Oxide, Woelm neutral), and DEAE-cellulose (Serva DEAE-TLC) were also used for the TLC.

Reagents. The solvents used were purified by conventional methods to meet chromatographic standards. All other reagents were prepared from analytical reagent grade materials.

Preparation of the thin-layer plates

Twenty grams of Avicel SF were mixed in a glass homogenizer with 70 ml of water for *ca.* 60 sec to give a suspension. After deaeration with suction, the suspension was spread on 20 glass plates (10 × 10 cm) or 10 glass plates (10 × 20 cm) with a suitable applicator, pre-set to give 0.25-mm thick layers. The coated plates were kept horizontal and dried in air overnight, and stored in a desiccator containing silica gel.

The layers (0.25 mm thick) coated with the chromato-media other than Avicel SF were prepared by the conventional procedures and stored in the same way as above.

Application of samples and development of chromatoplates

The following sample solutions were used: a 0.06 *M* ethanolic solution of free cyclamate, a 0.02 *M* ethanolic solution of free saccharin, and a 0.02 *M* ethanolic solution of dulcin.

In order to determine the R_F values and examine the color tones, 0.06 μmole of cyclamate, and 0.02 μmole each of saccharin and dulcin were spotted on the starting line 1.5 cm from the edge of the plate (10 × 10 cm). The plate was developed ascendingly at 20–22° in a closed tank until the length of run was 7 cm. When the 20 × 10 cm plate was used, the samples were spotted on a starting line 2.5 cm from the edge of the plate and it was developed until the length of run was 12 cm.

* Avicel is a cellulose product for thin-layer plates and it is known to be superior in many respects to the cellulose products usually used for thin layers[12].

Detection of spots on the chromatoplates

The developed plates were examined by the following methods.

Pinacryptol Yellow–UV (Method I). The plate is sprayed with 0.1% (w/v) Pinacryptol Yellow solution in 95% ethanol and allowed to dry in the dark for 10 min, and then examined in transmitted UV light (3650 Å). Cyclamate appears as an orange fluorescent spot on a light greenish-blue background. Saccharin is a non-fluorescent orange spot and dulcin a dark violet spot.

Bromine–fluorescein–naphthylethylenediamine (Method II)[8]. After exposure to bromine vapor, the plate is sprayed with a 0.1% ethanolic solution of fluorescein, air-dried, and then sprayed with a 2% ethanolic solution of naphthylethylenediamine. Cyclamate appears as a yellow spot, saccharin is yellowish pink, and dulcin is yellowish orange on a dull orange background.

Methyl Red in phosphate buffer solution (Method III). The well dried plate is sprayed with a solution consisting of 1 part of 0.1% (w/v) ethanolic solution of Methyl Red and 2 parts of phosphate buffer solution (pH 7.0). Cyclamate and saccharin give reddish orange spots on a yellow background.

Silver nitrate–pyrogallol (Method IV)[1]. Silver nitrate (0.17 g) is dissolved in 1 ml of water and mixed with 5 ml of 10% NH_3; this solution is diluted with ethanol to 200 ml. The plate is sprayed with the solution prepared as above and then sprayed with a freshly prepared solution of 0.01% (w/v) ethanolic pyrogallol. Cyclamate appears as a transient white spot on a brown background.

UV absorption or fluorescence (Method V). The plate is examined in transmitted UV light (2537 Å), when saccharin appears as a blue fluorescent spot and dulcin as a dark spot.

Results and discussion

Separation and detection of sweetners on Avicel layers. The solvent systems suitable

TABLE I

SOLVENT SYSTEMS FOR CHROMATOGRAPHY OF SYNTHETIC SWEETNERS ON AVICEL LAYERS

Symbol	*Components*	*Ratio (v/v)*
A	AcOEt–conc. NH_3–acetone	1:1:8
B	DMF–EtOH–H_2O	5:4:1
C	Dioxane–pyridine–H_2O	7:2:1
D	Pyridine–EtOH–H_2O	6:3:1
E	Tetrahydrofuran–pyridine–H_2O	6:3:1

for the separation of cyclamate, saccharin and dulcin on the Avicel layers are summarized in Table I. On the small size (10 × 10 cm) plates, these solvents were fairly satisfactory in resolving the sweetners as small dense spots. The R_F values obtained are listed in Table II.

Five detection methods including the Pinacryptol Yellow reagent and other known procedures were examined and the detection limits of the sweetners by these methods were compared with each other. As can be seen in Table III, the sensitivity

TABLE II

R_F VALUES OF SYNTHETIC SWEETNERS ON AVICEL LAYERS

Sample	*Solvent system*				
	A	*B*	*C*	*D*	*E*
Cyclamate	0.15	0.74	0.29	0.47	0.31
Saccharin	0.31	0.83	0.39	0.64	0.41
Dulcin	0.82	0.91	0.80	0.79	0.89

TABLE III

DETECTION LIMITS (μg) OF SYNTHETIC SWEETNERS ON AVICEL LAYERS

Detection: I = Pinacryptol Yellow–UV; II = bromine–fluorescein–naphthylethylenediamine; III = Methyl Red in phosphate buffer; IV = silver nitrate–pyrogallol; V = UV absorption (or fluorescence).

Sample	*Method of detection*				
	I	*II*	*III*	*IV*	*V*
Cyclamate	1	2	4	8	—[a]
Saccharin	0.2	1	1	1	0.1
Dulcin	0.2	1	—	—	0.2

[a] Symbol — means that no spot was detected.

TABLE IV

R_F VALUES OF SYNTHETIC SWEETNERS ON LAYERS OF DIFFERENT CHROMATO-MEDIA

Chromato-media	*Solvent system*	*R_F value*		
		Cyclamate	*Saccharin*	*Dulcin*
Avicel SF	AcOEt–conc. NH_3–acetone (1:1:8)	0.04	0.23	0.88
Polyamide	Benzene–AcOEt–HCOOH[a] (5:10:2)	0.41	0.69	0.81
Silica gel	Acetone–10% NH_3 (9:1)	0.24	0.59	0.84
Alumina	*iso*-PrOH–conc. NH_3 (4:1)	0.14	0.30	0.70
DEAE-cellulose	0.5 *M* $HCOONH_4$ (pH 6.5)	0.74	0.36	0.53

[a] This solvent system was recommended by Dr. R. TAKESHITA.

TABLE V

DETECTION LIMITS (μg) OF SYNTHETIC SWEETNERS BY PINACRYPTOL YELLOW REAGENT ON LAYERS OF DIFFERENT CHROMATO-MEDIA

Sample	*Chromato-media*				
	Avicel SF	*Polyamide*	*Silica gel*	*Alumina*	*DEAE-cellulose*
Cyclamate	1	12	40	1	40
Saccharin	0.2	4	12	0.1	1
Dulcin	0.2	1	1	0.1	0.1

of the new method is comparable to that of the bromine–fluorescein–naphthylethylenediamine method which is the best one for detecting these three sweetners.

Colorations of the synthetic sweetners with Pinacryptol Yellow reagent. When the developed plate is allowed to stand in the dark for 10–20 min after being sprayed with the Pinacryptol Yellow reagent, cyclamate appears as an orange fluorescent spot on a greenish-blue background in long-wave (3650 Å) UV light. The color tone and intensity of the spot are unchanged for a long time, at least eight days, in the dark. The spots of saccharin with this reagent are similar to that of cyclamate in their color tones, but the lack of fluorescence of the saccharin spot distinguishes it from the cyclamate. Dulcin appears as a dark violet spot by this method. Its color tone and intensity are stable for a long time even in the light. The sensitivities of the reagent to both saccharin and dulcin are comparable to those of UV absorption or fluorescence as shown in Table III.

Detection of synthetic sweetners by Pinacryptol Yellow reagent on layers of different chromato-media. In order to examine the colorations given by the Pinacryptol Yellow reagent, cyclamate, saccharin and dulcin were chromatographed on the layers of different chromato-media. The solvent systems used to resolve the sweetners on the layers of each chromato-medium, together with the R_F values, are shown in Table IV. The experimental results shown in Table V indicate that these sweetners are the most sensitive to the reagent on Avicel and alumina layers, and the worst on silica gel layers.

Faculty of Pharmaceutical Sciences,
Kitasato University, Shirokane, Minato-Ku,
Tokyo (Japan)

KINZO NAGASAWA
HISAE YOSHIDOME
KUMIKO ANRYU

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