Beitrag zur Verwendung extrem kleinvolumiger Trennkammern in der Dünnschichtchromatographie*

Bei der Entwicklung einer neuen Bestimmungsmethode für Test osteron haben wir für die Abtrennung von Steroidhydrazonen aus Urinextrakten neben der Entwicklungskammer von Desaga (Heidelberg) auch die Sandwich-Kammer (Camag, Muttenz/Schweiz, vgl. Zit. 1) benutzt. Der Lauf in der Sandwich-Kammer erwies sich nach wenigen Zentimetern für unsere Zwecke trotz der Sättigung der Gegenplatte mit dem System Chloroform–Aceton (9:1) als nicht befriedigend. Eine Verbesserung, die darin besteht, dass das nach JÄNCHEN[1] vorbereitete Plattenpaar vor dem Lauf an drei Seiten jeweils 0.5 cm tief in geschmolzenes Wachs getaucht wurde, erwies sich als brauchbar. Wir konnten bei der Isolierung des Testosteronhydrazons durch Einsatz von 20 × 35 cm grossen Platten die bei Verwendung der üblichen Desaga Technik (20 × 20 cm) notwendige zweite Chromatographie weglassen. Wir möchten noch erwähnen, dass die Platten nicht geklammert zu werden brauchen. Sofort nach dem Lauf empfiehlt es sich, zur Erhaltung des scharfen Trenneffektes, die Platten voneinander zu lösen.

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1 D. JÄNCHEN, *J. Chromatog.*, 14 (1964) 261.

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Separation of carbohydrates on borate-impregnated silica gel G plates

Thin-layer chromatography has been used extensively in the separation of saccharides and their derivatives[1–9]. The separation of D-glucose from D-galactose in the presence of other sugars has, nevertheless, remained a problem. According to STAHL AND KALTENBACH[1], many sugars separate better on kieselgur than on silica gel; however, on kieselgur the separation of D-glucose from D-galactose is unsatisfactory.

Silica gel G plates prepared with 0.1 *N* boric acid solution have been used in thin-layer chromatography for separating a limited number of saccharides[2–4]. We have found that a good separation of D-glucose from D-galactose as well as of other carbohydrates can be easily achieved on 0.02 *M* borate buffer (pH 8.0) impregnated silica gel G plates using a mixture of 1-butanol–acetic acid–water (5:4:1) as the developing system.

Experimental

Reagents. Borate buffer, 0.02 *M*, pH 8.0: 100 ml 0.02 *M* boric acid solution (pH 5.9) and 3.0 ml of 0.02 *M* sodium tetraborate solution (pH 9.3). Reagent grade powders from Matheson, Coleman and Bell Chemical Company.

α-Naphthol spray reagent: 10.5 ml α-naphthol (15 % solution in 95 % ethanol w/v), 6.5 ml concentrated sulfuric acid, 40.5 ml 95 % ethanol and 4.0 ml water.

Procedure. Thirty grams of silica gel G (Merck) was manually shaken in a closed jar with 60 ml of borate buffer 60–90 sec. Glass plates (20 × 20 cm) were coated with the slurry to a 0.25–0.275 mm thickness using a Desaga applicator. Good separations were also achieved on plates prepared by the method of LEES AND DEMURIA[10]. The plates were kept at room temperature until they set and were then dried for 30 min at 100°. They were stored at room temperature and activated for 30 min at 100° before use.

A Hamilton microsyringe (Hamilton Company, Inc., Whittier, Calif.) was used to spot the samples, and a warm stream of air from a hair dryer or heat gun was used to dry the spots. The application is carried out by spotting about 0.5 ml, drying, and repeating this procedure until the desired volume is put on the plate. Spots of 1–50 μl were applied.

The plates were allowed to develop by ascending chromatography to a height of 10 cm in closed glass tanks containing 1-butanol–acetic acid–water (5:4:1) as the solvent system. The average development time at 20° was 60–70 min (it was unnecessary to have a saturated atmosphere in the tank). The plates were dried at 100° for 10–15 min, cooled to room temperature, and either returned to the solvent tank for a second run or sprayed with the α-naphthol solution.

The sprayed plate was heated for 3–6 min at 100°. Most of the sugars appeared as blue spots on a light tan background. Deoxyribose gave a gray spot and rhamnose an orange spot. The colors were stable at room temperature for 2–3 days; the only noticeable change was a darkening of the spots on standing.

Results and discussion

Tables I–VI give the R_F values and colors of the separated sugars. The method was effective for saccharide mixtures where the individual sugar concentration varied from 1 to 30 μg.

TABLE I

SEPARATION OF SACCHARIDES SHOWING R_F VALUES FOR FIRST AND SECOND DEVELOPMENT IN THE SAME SOLVENT

Saccharide	R_F*	R_F**	*Color*
Stachyose	0.05	0.15	Blue
Raffinose	0.10	0.25	Blue
Sucrose	0.30	0.45	Blue
D-Galactose	0.35	0.55	Blue
D-Glucose	0.40	0.65	Blue
D-Xylose	0.50	0.75	Blue
L-Rhamnose	0.55	0.80	Orange
Phenyl-β-D-glucopyranoside	0.65	0.95	Blue

* Developed once in 1-butanol–acetic acid–water (5:4:1).
** Developed twice in the above system with intermittent drying.

TABLE II

SEPARATION OF D-MANNOSE FROM D-XYLOSE IN THE PRESENCE OF OTHER SACCHARIDES

Saccharide	R_F	*Color*
Stachyose	0.05	Blue
Raffinose	0.10	Blue
Sucrose	0.30	Blue
D-Mannose	0.40	Blue
D-Xylose	0.50	Blue
Phenyl-β-D-glucopyranoside	0.65	Blue

TABLE III

SEPARATION OF RIBOSE FROM DEOXYRIBOSE IN THE PRESENCE OF OTHER SACCHARIDES

Saccharide	R_F	*Color*
Stachyose	0.05	Blue
Raffinose	0.10	Blue
Maltose	0.30	Blue
Ribose	0.40	Blue
Deoxyribose	0.50	Gray
Phenyl-β-D-glucopyranoside	0.65	Blue

TABLE IV

SEPARATION OF D-FRUCTOSE FROM D-XYLOSE IN THE PRESENCE OF OTHER SACCHARIDES

Saccharide	R_F	*Color*
Stachyose	0.05	Blue
Raffinose	0.10	Blue
Sucrose	0.30	Blue
D-Fructose	0.40	Blue
D-Xylose	0.50	Blue

TABLE V

SEPARATION OF LACTOSE FROM MALTOSE IN THE PRESENCE OF D-ARABINOSE AND D-XYLOSE

Saccharide	R_F*	*Color*
Lactose	0.35	Blue
Maltose	0.45	Blue
D-Arabinose	0.65	Blue
D-Xylose	0.73	Blue

* Developed twice in 1-butanol–acetic acid–water (5:4:1).

TABLE VI

SEPARATION OF OLIGOSACCHARIDES IN THE PRESENCE OF D-GALACTOSE AND D-MANNOSE

Saccharide	R_F*	*Color*
Maltoheptaose	0.00	Blue
Maltopentaose	0.10	Blue
Maltotetraose	0.15	Blue
Maltotriose	0.25	Blue
Maltose	0.40	Blue
D-Galactose	0.52	Blue
D-Mannose	0.60	Blue

* Developed twice in 1-butanol–acetic acid–water (5:4:1).

The results in Table I show a separation where the concentration of individual sugars in the mixture varied from 2.5 to 12.5 μg. The higher R_F values in this table were obtained when the plate was run twice in the same solvent with intermittent drying.

The separation of the given saccharides was best accomplished on 0.02 *M* borate (pH 8.0) impregnated plates. Silica gel G in water or a mixture of silica gel G and kieselgur (1:1) impregnated with borate buffer (0.02 *M*, pH 8.0) gave poorer separations. The results were also unsatisfactory when pH 8.5 (0.02 *M*) borate buffer or unactivated plates were used.

An optimum separation of D-glucose from D-galactose was achieved by running the plate twice to the 10 cm mark in 1-butanol–acetic acid–water (5:4:1) with intermittent drying. This entire operation takes 2–3 h whereas a separation of D-glucose from D-galactose in a saccharide mixture may take days if it is to be done by paper chromatography.

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1 E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 351.
2 V. PREY, H. BERBALK AND M. KAUSZ, *Mikrochim. Acta*, (1961) 968.
3 G. PASTUSKA, *Z. Anal. Chem.*, 179 (1961) 427.
4 F. GRUNDSCHOBER AND V. PREY, *Monatsh. Chem.*, 92 (1961) 1290.
5 C. WEILL AND P. HANKE, *Anal. Chem.*, 34 (1962) 1736.
6 M. GEE, *J. Chromatog.*, 9 (1962) 278.
7 H. GRASSHOF, *Deut. Apotheker-Ztg.*, 103 (1963) 1396.
8 D. WALDI, in E. STAHL (Editor), *Dünnschicht-Chromatographie*, Springer Verlag, Berlin, Heidelberg, Göttingen, 1962, p. 447.
9 A. SCHWEIGER, *J. Chromatog.*, 9 (1962) 374.
10 T. M. LEES AND P. J. DEMURIA, *J. Chromatog.*, 8 (1962) 108.

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