CHROM. 4222

An "opposite sense" developing system in thin-layer chromatography

ZAPPI has previously proposed a methanol extraction followed by monodimensional, ascending TLC resolution of the product as a method for the identification of circulating thyroid hormones and related iodophenols[1-3].

The methanol extraction is highly effective for the preparation of such substances but gives rise—like all other alcoholic extractions—to lipid impurities which cannot be totally eliminated afterwards despite treatment of the extract with different organic solvents. When the extract is spotted on a chromatographic thin layer (cellulose powder) the lipids arrange themselves as a peripheral crust which is almost impermeable to the solvent mixture used in this particular case: acetone–0.1 *N* acetic acid (2:8). Serious distortion of the expected chromatographic patterns occurs as reported by BARKER in similar circumstances[4].

In order to overcome this difficulty, an ascending chloroform run prior to the resolutive one and in a transverse sense to this was tried. This permitted the displacement of the impurities, which form an irregular, tail-shaped spot starting from the origin, while the thyroid hormones, which are insoluble in chloroform[5] remain unaffected by the action of the solvent (Figs. 1a and b). However, these impurities block the flow of the acetone–acetic acid mixture through the cellulose layer during the second run and the iodoamino acids are pushed sideways along their ascending displacement line, as seen in Fig. 1c. For this reason, an "opposite sense" running system was devised, as outlined in Figs. 2 a and b. The extract and the reference substances are spotted equidistant from the sides of the plate and 4 cm from one edge. An ascending chloroform run is started on the opposite edge from this. Since the progress of the solvent decreases exponentially during the run, the front reaches the spots slowly, thus permitting an effective removal of the lipids, which are displaced to the upper

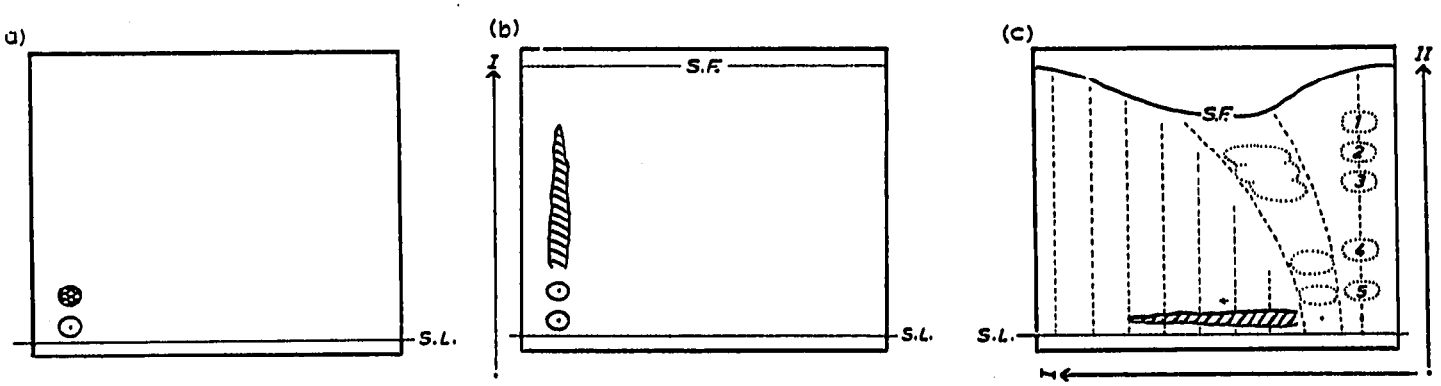

Fig. 1. S.L. = solvent level in the chromatographic jar. S.F. = final solvent front. The arrows indicate the direction of runs I and II. (a) The dark and the white circles represent the extract and the reference standard substances, respectively on the thin-layer plate. (b) The irregular, streaky mark shows the position of the displaced impurities. (c) The spots in the column on the right are dispersed according to the R_F values of the reference substances in the above mentioned TLC system. 1, 2, 3, 4, 5, are monoiodo- and diiodo-tyrosine, diiodo- triiodo- and tetraiodo-thyronine, respectively. The spots without a label show the approximate positions on the plate of the substances arising from the extract after chromatography. The direction of the solvent flow is arbitrarily represented with dashed lines.

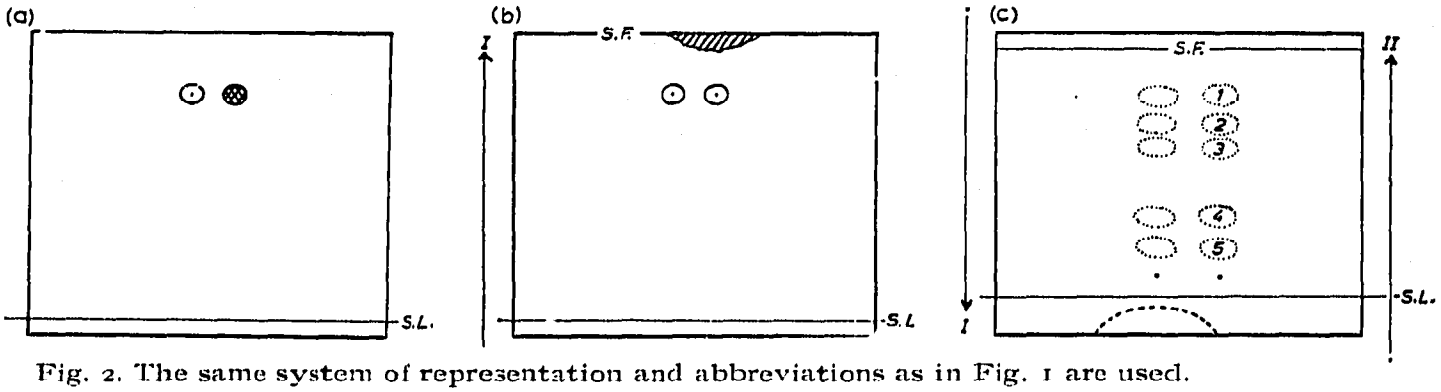

Fig. 2. The same system of representation and abbreviations as in Fig. 1 are used.

edge of the plate. The iodoamino acids, on the contrary, remain in their original position. After finishing this first run the part of the layer containing the impurities is scraped off and the plate is turned through 180° with respect to the first position. The ascending, resolutive run in acetone–acetic acid is carried out. The development of the chromatogram shows close agreement between the spots arising from the extract and those of the corresponding reference substances (Fig. 2c).

This "opposite sense" technique can also be used when the extract is applied in the form of a line instead of as a round spot[6]. It can be adapted, too, for removal of impurities from spots before bidimensional runs, provided that solvent mixtures with the necessary characteristics can be used. It could eventually be used as a preparative procedure for the separation of one particular fraction in an extract, which can then be eluted from the support medium and further analysed.

*1. Med. Abteilung** des städtlichen Auguste-Viktoria Krankenhaus, Berlin-Schöneberg (G.F.R.)*

EDUARDO ZAPPI*
MICHAEL SCHMIDT
FRED PRANGE

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Received June 11th, 1969

* Present address and address for reprint requests: Department of Microbiology, New York Medical College, 5th Av. at 106th St., New York City, N.Y. 10029, U.S.A.
** Chefarzt Prof. Dr. K. H. PFEFFER.