A procedure for the direct reading of fluorescent spots on thin-layer chromatography plates using the Turner fluorometer

Thin-layer chromatography has proved useful for the qualitative identification of several carbohydrates isolated from tobacco products. In particular, rapid methods have been developed for the identification of erythrose, galactose and galacturonic acid. For our needs the thin-layer procedure had certain advantages over conventional paper techniques.

In conjunction with analytical studies, a rather novel use of the Turner Model III Fluorometer* was developed. This involved the direct reading of fluorescent carbohydrate spots on TLC plates of special design. The unique point of this procedure involved the use of a flexible stainless steel plate as a support for the thin-layer medium. Both the steel plate and the thin-layer medium had the ability to bend to the configuration of the rotating drum in the chromatography door of the fluorometer. Fluorescent spots on the plates thus could be read directly; this technique coupled the advantages of separation by thin-layer chromatography with quantitative analyses by an instrument heretofore used almost exclusively with paper chromatograms. The technique should have application to substances other than carbohydrates.

Experimental and results

The chromatography plates were fashioned from 0.005 in. thick stainless steel shim stock, cut in 2 in. $\times$ $8^1/_2$ in. strips and roughened on one surface by rubbing with a coarse grade of emery cloth (Norton Abrasive No. 36). This rough surface facilitated the adhesion of a coating material consisting of a blend of 18 g of Merck (Stahl) Silica gel G with $CaSO_4$, 27 g of Johns-Manville Celite and 125 ml of water. The slurry was applied to the plates in the manner described by LEES AND DEMURIA[1], dried and activated by heating for 1 h at 110° and used the same day. While the silica gel–Celite support was preferred because of its low fluorescent background, some success was obtained with other coating agents namely, Macherey-Nagel and Co. MN Cellulose 300 and finely ground Whatman No. 1 paper (5 μ particle size) with $CaSO_4$ binder. It was necessary to consider the interactions of solvents, binders and supports because of the possible effect in quenching fluorescence or producing extraneous fluorescent products.

Concentrated aqueous carbohydrate solutions were spotted on the plates in 0.1 μl amounts and at levels ranging from 1 to 20 μg for each sugar. After air drying, the plates were placed in a Mitchell chamber[2] for ascending single or double development; usually for periods of 1–2 h at room temperature. The solvent system, *n*-butanol–acetic acid–diethyl ether–water (9:6:3:1) was quite satisfactory for the separation of erythrose. Galacturonic acid, galactose, arabinose and ribose were separated routinely with either an ethyl acetate–pyridine–water (2:1:2) or a *n*-butanol–acetic acid–water (4:1:2) system.

After air drying, the plates were sprayed with an alcoholic solution of *p*-aminohippuric acid and heated for 8 min at 140° (as described by SATTLER AND ZERBAN[3]) to form fluorescent compounds of the carbohydrates. The plates were scanned in the fluorometer usually within 2 h after development.

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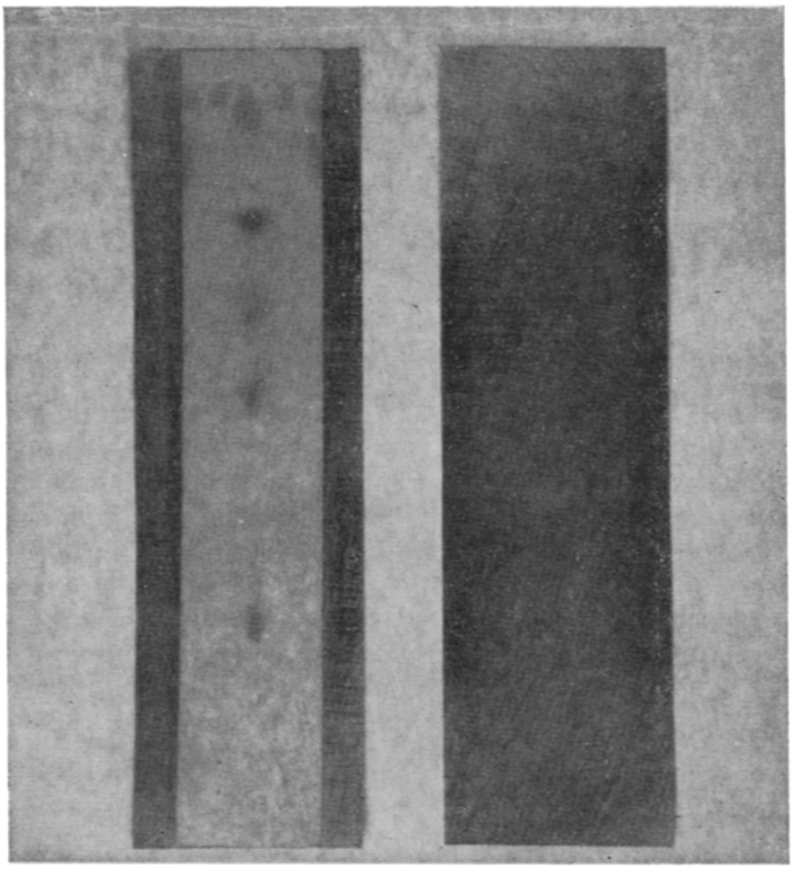

Fig. 1. Flexible stainless steel TLC plates (descriptions in text).

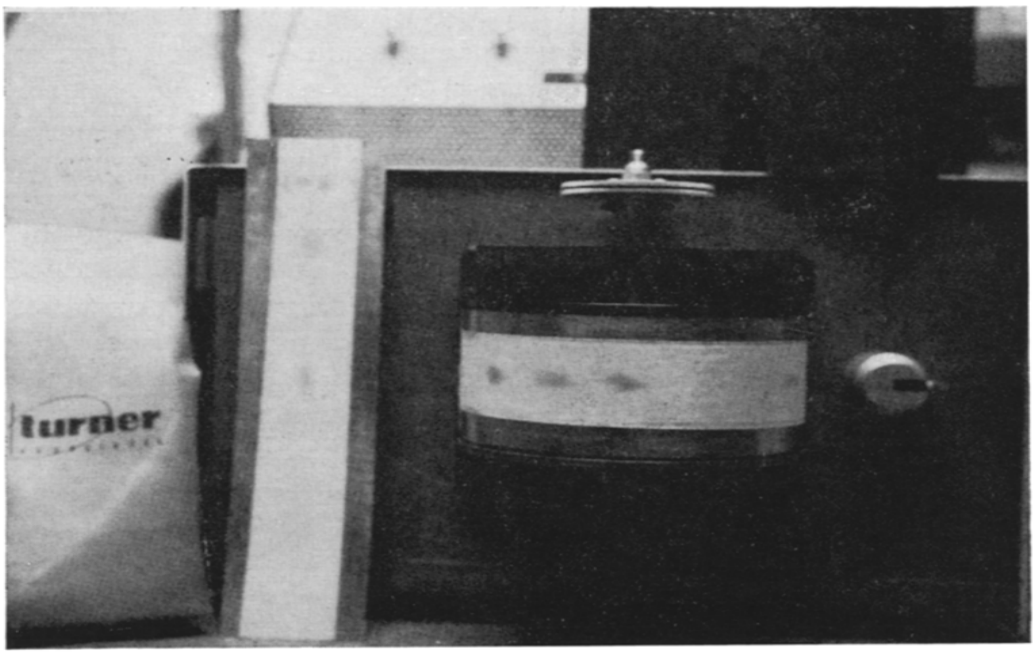

Fig. 2. Flexible TLC plate attached to rotating drum of fluorometer. At the left another chromatogram awaits reading.

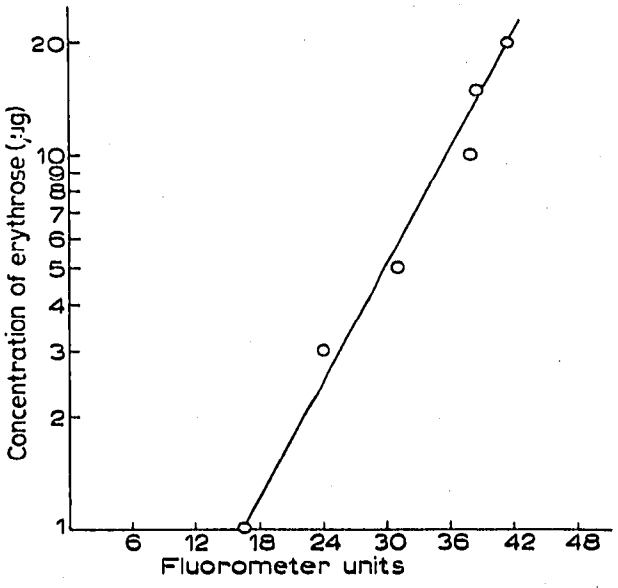

Fig. 3. Calibration curve for erythrose.

The plates were secured to the rotating drum of the chromatography door by the use of small pieces of masking or adhesive tape. An oblong mask was selected which outlined a reading area of 3 × 25 mm on the plates. The scale of the instrument was zeroed at a blank portion of the chromatogram, and the fluorescent intensity of the carbohydrate zones was measured by scanning at 0.5 cm intervals along the entire length of the chromatogram. It was found that a light intensity opening of 30 × with a 7–37 primary filter and a 2A–12 secondary filter with a 2 ND neutral wedge sufficed for most of the tests. On a weight basis, erythrose was the most intensely fluorescing carbohydrate studied under these conditions, and a sizeable range of the fluorometer scale was utilized for concentrations between 1 and 20 μg. Standard calibration curves were prepared each time a sugar was tested.

In Fig. 1 appear, from left to right, a completed chromatogram and an uncoated stainless steel plate. The chromatogram illustrates in ascending order: galacturonic acid, galactose, arabinose and ribose. The topmost spots are impurities which migrate with the solvent system.

Fig. 2 illustrates the Turner fluorometer with a flexible chromatogram affixed to the rotating drum of the chromatography door. To the left, leaning on the instrument, is another chromatogram, shown only to demonstrate the relative rigidity of the stainless steel plates. A calibration curve for erythrose is shown in Fig. 3. The log of the carbohydrate concentration is plotted *versus* fluorometer units. A fairly linear plot is obtained over a range of from 1 to 20 μg.

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Received April 13th, 1964