papaverine is located at the end of the chromatogram, while with acetone or a mixture of benzene, chloroform and acetone narcotine is found at the top.

Typical chromatograms obtained in this way are shown in Fig. 1, I–IV.

Acknowledgement

The authors wish to express their indebtedness to Dr. S. A. WARSI, Director, for his encouragement and help in preparing this paper.

North Regional Laboratories,
Pakistan Council of Scientific and Industrial Research,
Peshawar (Pakistan)

M. IKRAM
G. A. MIANA
M. ISLAM

[1] A. H. KHAN, *Pakistan J. Sci. Ind. Res.*, 1 (1958) 195.
[2] M. MOTTIER AND M. POTTERAT, *Anal. Chim. Acta*, 13 (1955) 46.
[3] J. DAVÍDEK AND J. BLATTNÁ, *J. Chromatog.*, 7 (1962) 204.

Received September 11th, 1962

J. Chromatog., 11 (1963) 260–263

Use of sequentially applied location reagents and multiple transparent overlays in thin-layer chromatography*

Studies now in progress in this laboratory, utilizing thin-layer chromatography for the separation of tissue lipids[1], have demonstrated a need for a simple, rapid method for permanently recording the positions and total areas of separated lipid classes on the developed chromatoplate prior to quantitation. The separated lipids have differed widely in amount under various experimental conditions, producing a degree of variability as well as interference in the final separations that is not completely standardized by comparison with known lipids. This has necessitated the frequent use of multiple, sequentially applied location reagents. Difficulties have occurred when spots previously demonstrated would be hidden by location reagents applied later in the sequence. It became impossible to relate these sequentially visualized areas unless a permanent record was made after the use of each location reagent. Recent notes by GETZ AND LAWSON[2] and HILTON AND HALL[3] have suggested the feasibility of utilizing standard office photocopiers. The overlay technique described in this communication requires no special equipment and has the added feature that it permits the superpositioning of the series of overlays outlined during sequential spot localization. Direct comparisons can thus be made of the various spots developed with the individual location reagents, positive identifications can be made, and—most important—any incomplete separations can be rapidly determined.

The developed chromatoplate, after being sprayed with the first location reagent, is placed under a plate of either clear glass or plastic. This plate is elevated from the surface of the table enough to allow the chromatoplate to be slipped under-

* This investigation was supported in part by a research contract, Project Number 6X99-26-001-09, from the Medical Research and Development Command, U. S. Army, Washington 25, D. C.

neath without touching the overlying glass or plastic. The spotting guide provided with the Camag* apparatus, inverted, works very well as this stand. A piece of thin, clear, flexible plastic, *e.g.*, 0.005-gauge polyethylene, cut to the size of the glass plates used for the thin-layer chromatography, is then placed on the transparent stand above the developed chromatoplate and the area of each spot outlined. A standard ink glass-marking pen with a fine point works well for this purpose. Notes may be inked in on the overlay plastic, if desired, at this time. Other spots can then be located with other reagents, another overlay being traced each time. The transparent overlays can then be compared by direct apposition. After the spots have been identified, the individual overlays are placed underneath the chromatoplate; with transillumination the total areas containing the particular metabolites desired are easily located.

This method is being applied in this laboratory in a study of the alterations induced by hepatotoxic agents in lipid metabolism of the rat liver. Dichlorofluorescein is first sprayed onto the developed chromatoplate. This visualizes the pH gradient of the plate with different solvent systems[1] and spots the organic acids, all of which are recorded on the first overlay. The chromatoplate is then placed under U.V. light, and the fluorescent areas, which include almost all the lipid fractions in sufficiently high concentration, are outlined on the second overlay. Iodine vapor is then used for detection of unsaturated lipids, a third overlay being made. Finally a fourth overlay is made of the spots located with the Liebermann–Burchard reagent. Direct apposition of these four overlays identifies the various lipids by their relative positions and types of reactions observed with the various location reagents. Incomplete separations are noted. This is particularly important in experiments with shorter chain fatty acids, which usually remain much closer to the origin than the longer chain fatty acids, and are thus incompletely separated from other relatively polar lipids. When ^{14}C-labelled fatty acids are used, radioactivity would be found with these more polar lipids due to the ^{14}C in the incompletely separated fatty acid. In such a case, the separation is repeated, using a different solvent system. The overlays are then placed beneath the glass plate, both chromatoplate and overlay transilluminated, and the silica gel above the desired outline removed for quantitation. Finally, the overlays are stapled together and taped into a loose-leaf notebook with the experimental notes. The data accumulated with the quantitative studies are later summarized on a composite fifth overlay.

Military Environmental Pathology Division,
Armed Forces Institute of Pathology,
Washington, D. C. (U.S.A.)

JOSEPH S. AMENTA

[1] W. C. VOGEL, W. M. DOIZAKI AND L. ZIEVE, *J. Lipid Res.*, 3 (1962) 138.
[2] H. R. GETZ AND D. D. LAWSON, *J. Chromatog.*, 7 (1962) 266.
[3] J. HILTON AND W. B. HALL, *J. Chromatog.*, 7 (1962) 266.

Received October 19th, 1962

* Obtained from Arthur H. Thomas Company, Philadelphia, Pa., U.S.A.