Box for application of samples to thin-layer chromatograms under nitrogen

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SUMMARY A method is described for the application under nitrogen of samples of phospholipids to thin-layer chromatograms in order to prevent oxidation of the phospholipids and to decrease the influence of atmospheric humidity on R_F values.

KEY WORDS phospholipids . bone matrix . thin-layer chromatography . application . oxidation

WE HAVE BEEN engaged in separating the major phospholipids of bone matrix on thin-layer chromatograms according to the method of Wagner, Hörhammer, and Wolff (1), utilizing a solvent system of chloroform–methanol–water 65:25:4 (v/v). The phosphatides present are lysophosphatidyl choline, phosphatidyl choline, sphingomyelin, and phosphatidyl ethanolamine. The silicic acid bearing each phospholipid was scraped from the plate, the lipid eluted, and quantitative lipid phosphorus determinations were run (2). In order to do this it was necessary to utilize a full 5 cm lane, as otherwise sufficient material could not be applied. The experimental method utilized called for multiple samples, and to conserve time four 5-cm lanes were run simultaneously on a 20 cm plate.

Significant differences were noted between the first lane to which the material had been applied and the last, although nitrogen was used as a drying agent. The time interval between spotting these lanes was approximately 60 min. The R_F of all four phospholipids showed extreme variability between lanes. The most marked difference was found in sphingomyelin, which trailed badly and frequently showed double bands thought to be due to oxidation of the fatty acids by atmospheric oxygen while the mixture was being applied to subsequent lanes. It was also evident that the moisture in the room air was influencing the separations by deactivating the silicic acid.

In order to overcome these difficulties it was decided to carry out the entire procedure under nitrogen, and the device illustrated in Fig. 1 was therefore constructed in the hospital shop. The cover was made of ¼ inch plexiglass with internal dimensions of 21 × 25 × 2.5 cm. An inlet for nitrogen was made in the corner and a saw cut measuring 2 mm was made 2 cm from the opposite edge. Spots were applied by means of a 100 µl Hamilton

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syringe introduced through the saw cut. Since this device has been in use the sphingomyelin has given a single spot without trailing, the separations have been much improved, and the R_F values have been more

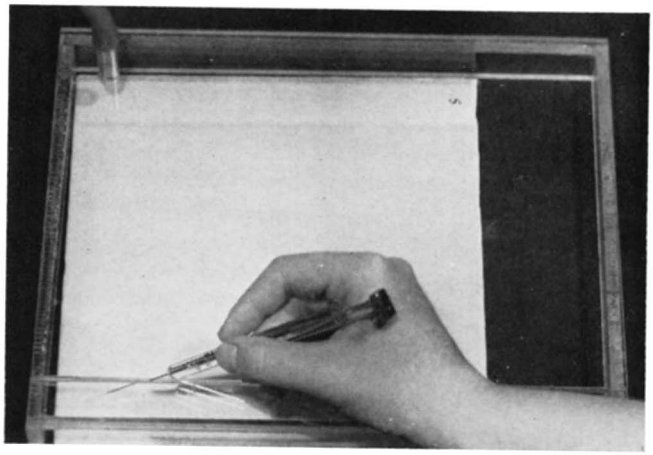

FIG. 1. The box, or cover, through which samples are applied to thin-layer chromatograms. It is made of 0.25 inch plexiglass; internal dimensions are 21 × 25 × 2.5 cm.

consistent. Its simplicity and low cost recommend it. A device similar to the one described is marketed by Brinkmann Instruments Inc., Westbury, New York.

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