A simple method for photographing ultra-violet absorbing spots on paper chromatograms

Recent studies in this laboratory have necessitated the purification of folic acid derivatives. These compounds may be observed when run on paper chromatograms by virtue of their ultra-violet absorption properties. In order to have a permanent and readily observable record of these chromatograms the following procedure for photographing was devised.

The chromatogram to be photographed was placed on a piece of Agfa "Copyrapid negativ" paper, and the two kept in contact by placing a piece of sheet glass on top of them. The chromatogram was exposed, through the glass, to a U.V. source by scanning with a U.V. lamp (in this case a Mineralight, model SL 2537) at a height of about 12 inches. The correct exposure time depended necessarily on the thickness of the chromatogram and the sheet of glass. It was in the order of 5–20 seconds. The exposed negative was developed in a "Copease Book Copier" machine using Agfa "Copyrapid developer" and Agfa "Copyrapid positiv" paper. The whole procedure of exposing and developing was carried out in a semi-darkened room.

The final positive consisted of a completely white background with the absorbing areas black. With short exposures any fluorescent spots present will appear as grey areas, with longer exposures they will be absent.

As many laboratories possess some model of a photocopying machine, and hence the necessary positive and negative material, developer and means of developing, the method described provides a very simple and convenient way of photographing U.V. absorbing areas on paper chromatograms.

Department of Pharmacology,
Stanford University School of Medicine,
Palo Alto, Calif. (U.S.A.)

A. S. MILTON

Received December 15th, 1961

A simple chromatographic technique for the removal of dinitrophenyl-amino acids from excess dinitrophenyl-artifacts

In the dinitrophenylation (DNP) method[1] of N-terminal analysis of a protein, excess fluoro-dinitrobenzene (FDNB) is generally removed from the DNP-reaction mixture by washing the acidified solution with organic solvents prior to the acid hydrolysis of the DNP-protein. Small amounts of bound FDNB produce dinitrophenol (DNP-OH) on acid hydrolysis and this is normally removed from the ether-soluble DNP-amino acids by either cold finger sublimation[2] or column chromatography[3,4] before the DNP-amino acids can be estimated by paper chromatography[5]. The method of LI AND ASH[3] employs a silicic acid column containing water as the stationary phase and chloroform equilibrated with water as the mobile phase. The most soluble