The detection of guanidine compounds on paper chromatograms

CONN AND DAVIS[1,2] found that ninhydrin (triketohydrindene hydrate) in alkaline media forms highly fluorescent products with guanidine, and with monosubstituted and N,N-disubstituted guanidines. The above authors developed a method for the fluorimetric estimation of guanidinium compounds.

It has now been found that the above method could be applied to the detection of certain guanidinium compounds on paper chromatograms. The method used was as follows:

The chromatogram was dried and sprayed with a solution of ninhydrin (0.25 % ethanolic solution). After drying the paper was sprayed with ethanolic sodium hydroxide (2 % ethanolic solution). On exposure to ultraviolet light, guanidinium compounds appeared as green fluorescent spots, which faded slowly.

The spray was tested successfully on the following compounds: guanidine, guanidine hydrochloride, guanidine nitrate, creatine, creatinine, glycocyamine, and arginine. Streptidine and diphenyl guanidine could not be detected by this method. Urea also did not give the fluorescent compound.

Although CONN AND DAVIS said that N,N'-disubstituted guanidines did not give the fluorescent compound, it was found that creatinine was detectable on paper chromatograms by this method.

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Colour photography of polycyclic compounds on alumina columns

Most polycyclic compounds are highly fluorescent in ultraviolet light hence their chromatographic development on alumina columns can be readily followed. However, the colour of various compounds and clarity of separation can be permanently recorded by modification of techniques recently developed for medical and biological photographic illustrations[1].

The equipment is illustrated in Fig. 1. A Witrona, 180 Joule Electronic Flash Unit at half power was used as the ultraviolet source. A Woods Glass Filter which

allowed passage of 3650 Å light was cut to the size of the flash holder and attached with a rubber clip ring, using a black felt gasket and opaque masking tape to completely seal off stray light.

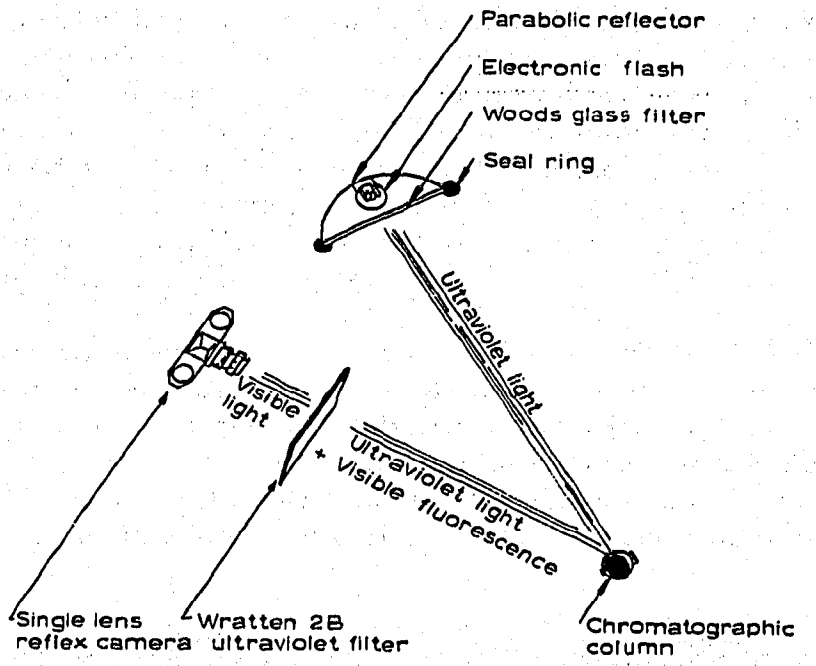

Fig. 1. Photographing chromatographic columns in ultraviolet light.

Ultraviolet energy irradiated the column emitting visible fluorescence plus reflected ultraviolet light. A Kodak Wratten 2B gelatin filter attached to the front of the camera lens absorbed the ultraviolet light and allowed only visible fluorescence to expose the film. Single lens reflex cameras, because of their freedom from parallax and facility for precise focussing proved the most satisfactory for this work. A Topcon 120 R camera with a f 1.8/58 mm lens was used.

The distance from light source to the column was 15 inches and the included angle to camera axis 20°.

Early difficulties with zone clarity and true colour production were overcome by using a fast film, High Speed Ektachrome, ASA 160.

For photographing the full column an exposure of f 5.6 proved most satisfactory while f 8 was used for close photographs of bright individual bands.

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