

Recording short-wave ultraviolet absorption on thin-layer chromatograms by pinhole photography*

It is often necessary to record the locations of compounds on TLC plates under short-wave ultraviolet radiation. When using longer wavelengths, *i.e.* 366 m μ , no problem exists as standard camera optics may be employed¹. Because of the opacity of glass to short wavelengths, quartz optics are indicated but are of prohibitive cost. As will be shown in the present application this difficulty can be overcome by using the classic pinhole diaphragm.

The principle of the pinhole was first described in the sixteenth century² and its value lies in the fact that it will produce an image of any illuminated object placed on either side of it. (The system does not require focussing.) Landscapes, or distant objects, produce acceptably sharp images through a pinhole; however, complications

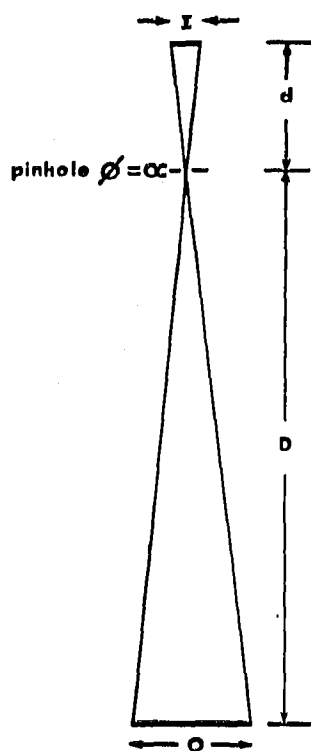
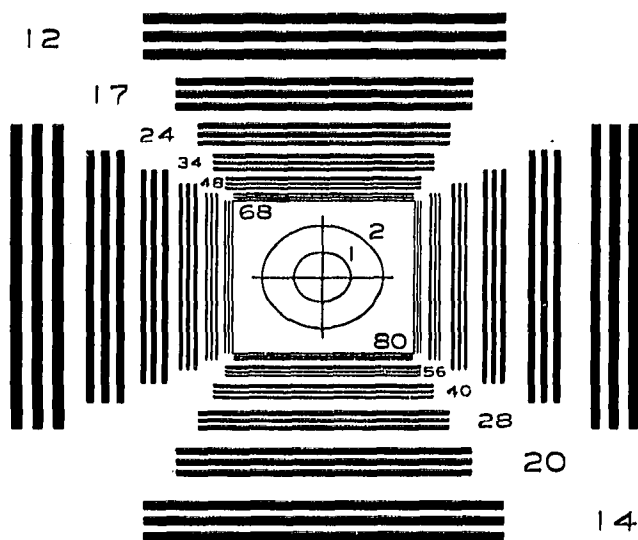


Fig. 1. Geometry of the pinhole diaphragm.

Fig. 2. N.B.S. Resolution Test Chart.



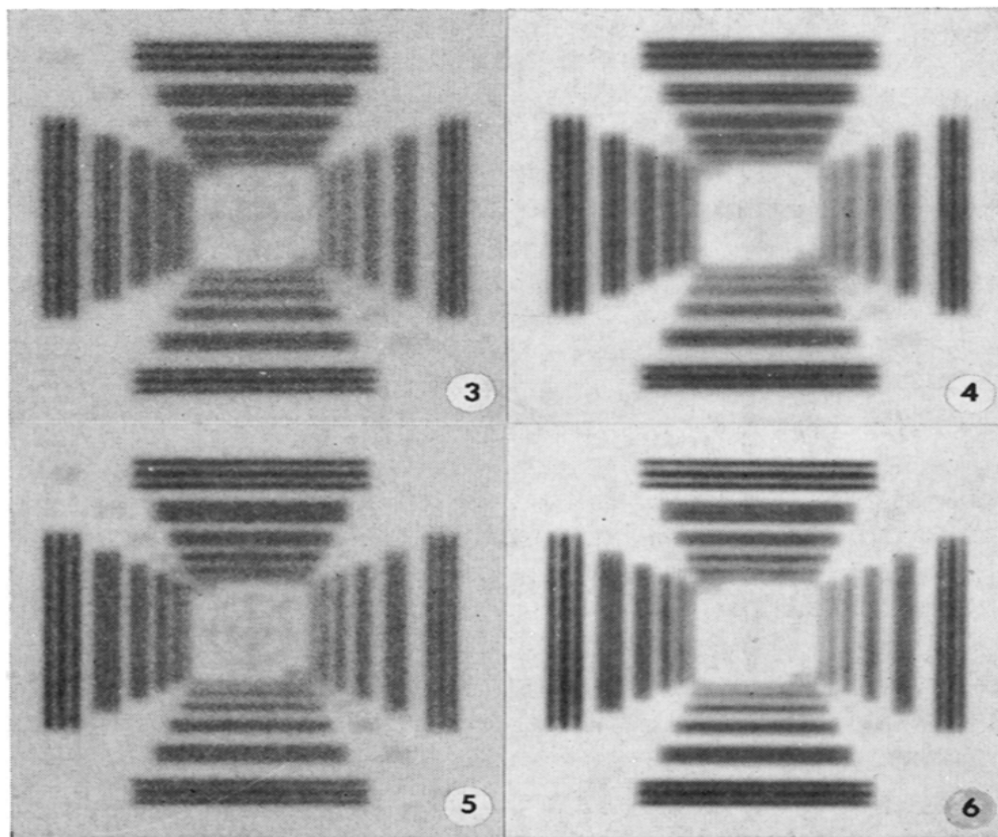
arise when this technique is used for specialized applications. As seen from the geometrical relationship indicated by the diagram in Fig. 1 the size of the image, I , is given by

$$I = Od/D \quad (1)$$

where O is the size of the object and d and D , the distances of pinhole to image and object, respectively. Thus alteration of the d/D ratio simply changes the proportion of I to O .

* Contribution No. 34. Scientific Information Section, Research Branch, Canada Department of Agriculture, Ottawa (Canada).

The quality of the image depends on the size of the pinhole and the smoothness of its edge, down to a diameter at which diffraction effects introduce image deterioration. The definition increases as the diameter, α , decreases; or, as D increases. Aside from the necessity to avoid diffraction effects, limitations in the reduction of pinhole diameter are imposed by constructional difficulties³ and by the necessity to avoid impractically long exposure times. Because of the low level of energy radiated by



Figs. 3 to 6. Comparisons in white light of Kodak Plus-X Pan film (Figs. 3 and 5) and Kodak High Contrast Copy film (Figs. 4 and 6) against pinholes of 0.016 in. diameter (Figs. 3 and 4) and 0.012 in. diameter (Figs. 5 and 6).

U.V. lamps, this factor was of importance in the present application. It is to be noted that the f value of the system is defined by

$$f = d/\alpha \quad (2)$$

Testing the system resolution

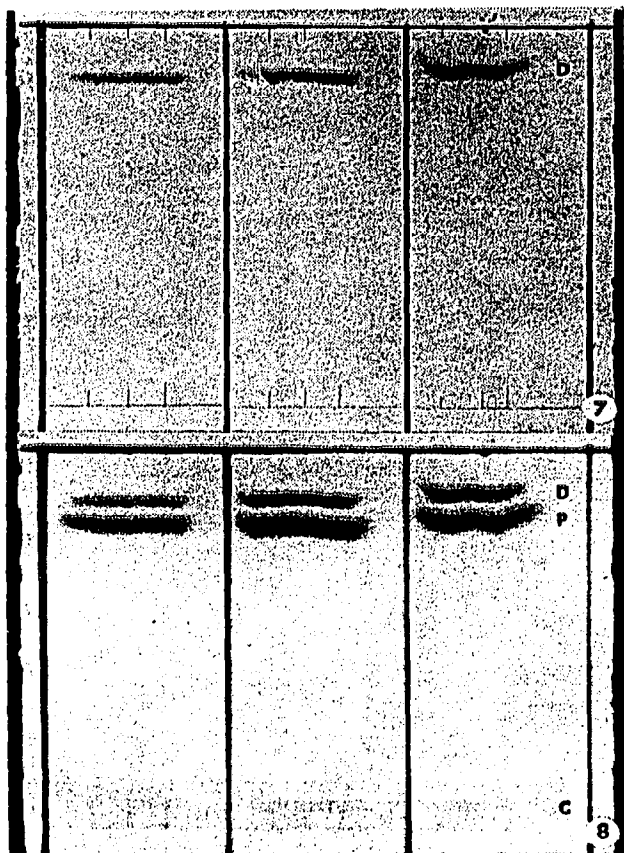
Standard 200 × 200 mm TLC plates were used in the present tests. In order to utilize the full width of 35 mm film a distance $d = 100$ mm of pinhole to film and a distance $D = 950$ mm of pinhole to TLC plate were used. Expression (1) gives $I = 21$ mm in this case; the I/O ratio was 1/9.5.

The degree of resolution obtainable with two pinhole sizes, *i.e.* 0.012 and 0.016 in., was determined with each of two emulsion types. The tests were conducted in white light using the NBS high resolution Test Chart⁴ (Fig. 2) and the I/O ratio 1/9.5. The results, Figs. 3 to 6, were examined visually, no attempt being made to assign

numerical values for the resolution obtained. It was estimated that the definition and contrast achieved with the 0.012 in. pinhole in combination with High Contrast Copy Film (Fig. 6) were adequate to the present application.

Testing the system with short-wave U.V. light

A TLC plate was used in this test. Illumination was provided by two Mineralight lamps (Ultraviolet Products Inc., San Gabriel, Calif.), directed at 45° on each side and



Figs. 7 and 8. TLC plate in white light, Fig. 7, showing only dye band D. The same plate under shortwave U.V., Fig. 8, showing absorption in bands D, P and C.

at 10 in. distance from the center of the plate. The f value with the 0.012 in. hole and $d = 100$ mm was 330. It was found that about an 8-min exposure produced negatives of sufficient density for subsequent reproduction; direct visual evaluation from negatives was possible with 3-min exposures.

The plate, prepared and developed by applying known techniques⁵, had been spotted⁶ with mixtures of steroids. By ordinary light (Fig. 7) only the dye band D, used as a reference for the determination of R_b values, was visible (R_b value = 1.00). In the same plate, under U.V. illumination, absorption by this band and that corresponding to progesterone, P, produced clear zones on the negative translated as dark bands on the print (Fig. 8). Weaker absorption by a third compound produced band C.

Fluorescence induced by U.V. radiation will appear as light bands on the print, provided the energy thus emitted is higher than that reflected by the background.

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Received January 10th, 1967

J. Chromatog., 29 (1967) 252-255

Thin-layer chromatographic separation of primary and secondary amines as 4-(phenylazo)benzenesulfonamides

The direct chromatography on thin layers of primary and secondary amines in most cases causes difficulties due to the strong adsorption of the NH-group to the adsorbents generally used. Several methods have, nevertheless, been developed for work with the free amines, but the use of derivatives is often to be preferred. Thus derivatization has been performed with reagents such as 2,4-dinitrochlorobenzene^{1,2}, 3,5-dinitrobenzoyl chloride^{3,4}, 1-dimethylaminonaphthalene-5-sulfonyl chloride⁵, 4-toluenesulfonyl chloride^{4,6} or benzoyl chloride⁴.

In the present investigation, 4-(phenylazo)benzenesulfonyl chloride has been used for preparing derivatives of the amines. These derivatives have previously been described, and fair separations were achieved by column chromatography⁷. The 4-(phenylazo)benzenesulfonamides formed have several advantages over the free amines as well as over derivatives used before. They are thus well suited for thin-layer chromatography, *e.g.* on alumina plates, the compounds are easy to detect on the plates, as they are intensely colored, and the preparation of the derivatives can be carried out very simply even from aqueous solutions of the amines or their salts. Due to these facts the method is also very suitable for separation and characterization of volatile amines arising from the cleavage of more complicated molecules, *e.g.* of biological origin. The procedure described here was originally developed for the lower aliphatic amines, as only very few methods are available for the identification of these compounds, but results are also given for some amines of pharmacological interest. The method discussed in this paper has been included in the course in organic identification at the Technical University of Denmark.

Preparation of the derivatives

The reagent, 4-(phenylazo)benzenesulfonyl chloride, was prepared from azobenzene (Fluka, puriss.) and chlorosulfonic acid⁸. The derivatives were prepared according to the following procedure:

J. Chromatog., 29 (1967) 255-258