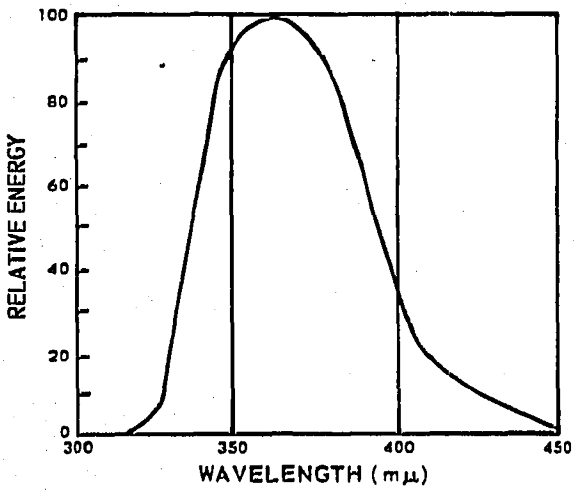
## Color recording of ultraviolet fluorescence in thin-layer chromatograms

In thin-layer chromatographic techniques it has been found that a visual evaluation of the separations (in white light) is not always accurate. In some cases, locations may be determined by well-defined color spots, while in others, the color may be too subtle to detect. As certain compounds either absorb ultraviolet or fluoresce under its influence, unstained spot locations may be determined by this response. Chromatographic studies were conducted at the Plant Research Institute, Canada Department of Agriculture, Ottawa, to determine differences in species of *Trifolium*. These required permanent recording of the spot locations which fluoresced under U.V. excitation. Because the fluorescing spots had extremely subtle color variations, color emulsions were indicated in preference to black-and-white records.

Several positive color emulsions were tested and Kodachrome II Daylight was found to give the best reproduction. Color films balanced for either daylight or artificial light may be used, depending on the colors to be emphasized. Emulsions balanced for daylight accentuate yellows and reds, while those balanced for artificial light render the blues more brilliantly. The respective ASA speeds of the films are not too important as the exposures are relatively long and do not differ greatly between one emulsion and another.

Exposures by transmitted radiation were made using a Blak-Ray fluorescent lamp* containing two self-filtering General Electric 15-W Blacklight F15T8-BLB tubes. The maximum emission of these tubes occurs at 366 mμ, Fig. 1 (the curve is plotted to peak at 100 on a relative scale). As the plates are usually 20 cm square, they may



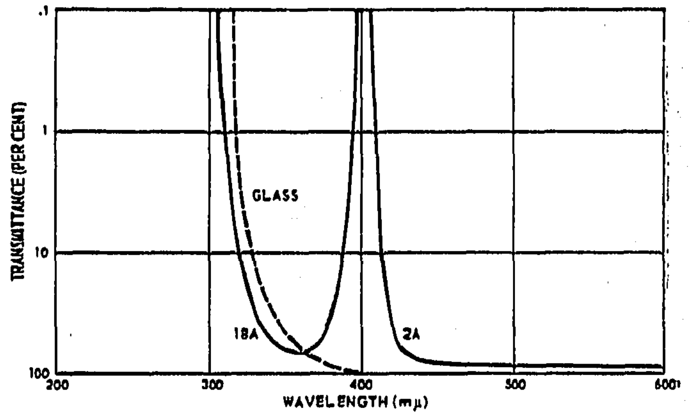




Fig. 1. Emission curve of G.E. F15T8-BLB tubes.

Fig. 2. Spectrophotometric absorption curves.

be placed directly on the edges of the reflector without sacrificing any of the information area. This leaves about 1 inch between the tubes and the underside of the plate. The exposed portions of the tubes should be masked off. The efficient design of the reflector permits an even exposure over the whole plate without light "bars" being detectable. Optimum exposure is a matter of trial and error, being dependent upon

* Ultraviolet Prods., San Gabriel, Calif., U.S.A.

the intensity of the fluorescence and the degree of background density desired. Final exposures for these particular studies varied between 15 and 20 sec with the lens at full aperture (f/2.8) and an image reduction to fill the 35 mm format.

A Wratten 2A filter must be used over the lens of the camera to exclude all ultraviolet radiation and allow the emulsion to record only the fluorescing spots which are in the visible portion of the spectrum; *i.e.* the 2A transmits only wavelengths beyond about 410 m$\mu$ (Fig. 2). If this filter is not used, the excessive U.V. will overpower the subtle spots and they will not be photographed at all. Exposures of this type should of course be made in a darkened room where existing light will not record an image on the film. If an exposure is required using only reflected ultraviolet to show general outlines of the separation of the compounds (with the effect of fluorescence subdued) then a Wratten 18A filter must be used over the lens to exclude *all* visible light and record only the ultraviolet[1]. These exposures can be made in normal room light as the 18A transmits only from around 310 m$\mu$ to 400 m$\mu$. As the wavelengths used here are of the "long" variety, *i.e.* 366 m$\mu$, the consideration of the glass plate acting as a filter is not important. The dash line in Fig. 2 approximates the transmission curve for standard glass, which filters everything below 320 m$\mu$. (Glass quality should be "selected window", free from bubbles, scratches and waves.) On the other hand, "short" wave U.V. would be filtered out by the glass, that is in lamps having filtered tubes which peak around 250 m$\mu$.

Because of the reciprocity characteristics of Kodachrome II Daylight (or any

TABLE I

COMMONLY USED COLOR EMULSIONS AND THEIR COMPENSATIONS

(Reproduced with permission of Canadian Kodak Sales Co. Ltd.)

| KODAK FILM AND TYPE | EXPOSURE* AND FILTER COMPENSATIONS FOR RECIPROCITY CHARACTERISTICS | | |
|---|---|---|---|
| | EXPOSURE TIME IN SECONDS | | |
| | 1 | 10 | 100 |
| Ektacolor Prof. S | ** | ** | ** |
| Ektacolor L | *** | *** | *** |
| Kodacolor | None – No Filter | ** | ** |
| Kodacolor-X | None – No Filter | +1 Stop – No Filter | +2 Stops – No Filter |
| Ektachrome Day (E-3) | +1 Stop – CC30 B | +2 Stops – CC40 B + CC10 M | +3 1/3 Stops – CC50 B + CC10 M |
| Ektachrome B (E-3) | None – No Filter | + 1/3 Stop – No filter | +1 Stop – No Filter |
| " (High speed) Day | +1 Stop – CC10 B | +1 2/3 Stops – CC05 G | +2 2/3 Stops – CC05 M |
| " (High speed) B | + 2/3 Stop – CC05 G | +1 1/3 Stops – CC10 G | +2 Stops – CC05 Y |
| Ektachrome-X | + 2/3 Stop – CC05 Y | +1 1/3 Stops – CC20 Y | + 2 2/3 Stops – CC40 Y |
| Kodachrome II Day | + 2/3 Stop – CC10 R | +1 1/3 Stops – CC20 R | +2 1/3 Stops – CC25 R |
| Kodachrome II Prof. A | + 2/3 Stop – CC10 R | +1 1/3 Stops – CC20 R | +2 Stops – CC25 R |
| Kodachrome-X | + 1/3 Stop – CC05 M | + 2/3 Stop – CC05 M | +1 2/3 Stops – CC10 R |

*The exposure increase, given in lens stops, includes the adjustment required by any filter or filters suggested.

** Not recommended

*** No filter from 1/5 to 60 sec.

color film, for that matter) filter compensations should be made for the increased exposure times being used. In this case, a CC20R filter will be required. Naturally, other emulsions would require different filtrations for their particular color shifts. Color compensating filters usually require a slight increase in exposure because of their density, but with this type of work, the small factor involved is lost in the lengthy overall exposure required for the fluorescence. Table I lists some commonly used color emulsions and their compensations.

Fig. 3 is a three-color reproduction of a typical thin-layer plate (silica gel G) showing the color variations required for these particular interpretations.

If a photograph of the plate is required in visible light, Kodachrome II Type A with 3200°K lamps is recommended. Although this particular emulsion is balanced for exposure by standard photofloods, *i.e.* 3400°K, it has been found that the drop of 200°K in the temperature of the source is advantageous in overcoming the subtle bluish cast which sometimes occurs in the background of these plates. Ordinarily, in using this light source and film, an 82A filter would be required to bring the combination back into balance.

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1 *Infrared and Ultraviolet Photography*, 7th Ed., Eastman Kodak Co., Rochester, N.Y., 1961.

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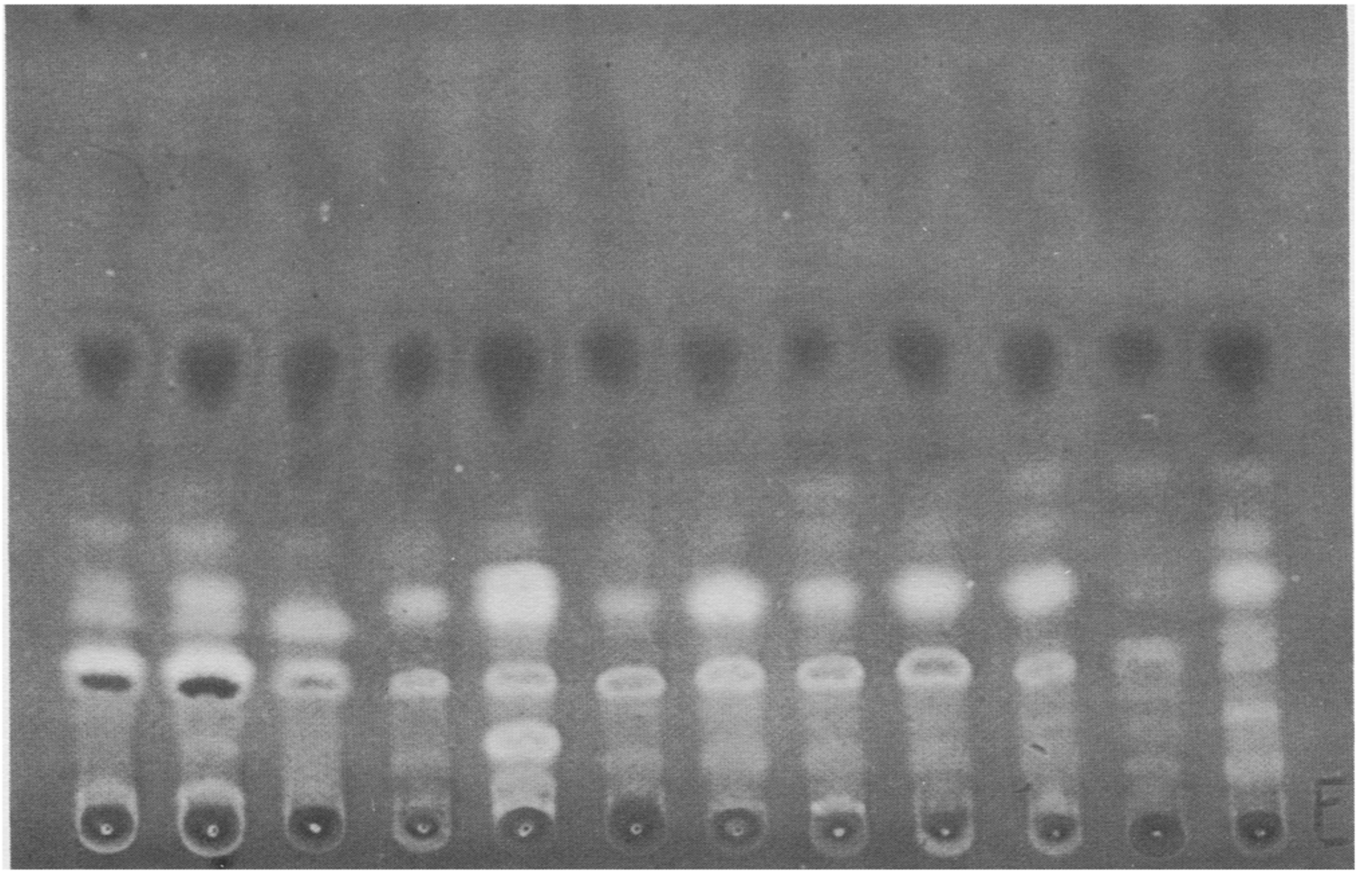


Fig. 3. Three-color reproduction of fluorescing TLC silica gel G plate (U.V. 366 m$\mu$).