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AN INVESTIGATION OF FLAVONES AS FLUORIGENIC SPRAY REAGENTS FOR ORGANIC COMPOUNDS ON A CELLULOSE MATRIX

PART III. QUANTITATION OF THE METHOD

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SUMMARY

A method for the detection and quantitative determination of organic compounds by *in situ* fluorimetry after separation on cellulose layers is discussed. The organothiophosphorus pesticide Proban was used as test sample for quantitative studies. Best results were obtained when the 3-hydroxyflavone robinetin was used as spray reagent. The stability of the fluorescence towards ultraviolet light was investigated. Linear calibration curves between 10 and 0.1 μg were obtained. The method was applied to the determination of Proban in tap water.

INTRODUCTION

New methods for the quantitation of pesticides on thin-layer chromatograms have recently been described. FREI *et al.*[1] have reported on a fluorimetric technique for determining Sevin from water samples. A fluorimetric method has been devised by FREI AND MALLET[2] for the quantitative determination of sub-microgram quantities of organothiophosphorus pesticides. The fluorigenic spray reagent consisted of a mixture of manganese and salicyl-2-aldehyde-2-quinolylhydrazone (SAQH). As low as 0.5 p.p.b. of the insecticide Guthion (azinphosmethyl) was detected from tap water. The procedure has also been applied to the determination of Guthion in blueberries[3].

Recently, it has been found[4,5] that quantities as low as 0.02 μg of some organic pesticides could be detected on cellulose layers sprayed with 3-hydroxyflavones such as flavonol, fisetin, and robinetin. It was also found that organothiophosphorus pesticides could be detected on silicagel layers, after bromination, when sprayed with the same flavones and a quantitation of the method has been described[6].

In this study, the possibility of using flavones as fluorigenic spray reagents for the quantitation of organic compounds on cellulose layers is investigated. The insecticidal organothiophosphate Proban was used as the test sample and should be representative of any organic compound that would normally give fluorescence under the experimental conditions.

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EXPERIMENTAL

For a detailed description, see preceding papers[4,5].

General procedure

A stock solution of the analytical standard Proban (American Cyanamid Company, Princeton, N.J., U.S.A.) was prepared 1000 p.p.m. in methylene chloride, from which dilution series were made. Solutions of the flavones were prepared 0.005 % in isopropanol.

The cellulose powder (MN Cellulosepulver 300 HR, Macherey, Nagel and Co., Düren, G.F.R.) was washed three times with ethyl ether and dried in the oven at 60°. A slurry was prepared by mixing in a Waring Blendor 15 g of the powder with 85 ml of distilled water. For chromatographic separation, the pesticide was spotted 2 cm from the bottom of the plate and eluted 5 cm in a 50 % solution of ethyl ether in *n*-hexane.

After removal from the chromatographic chamber the plate was dried with a stream of warm air and sprayed with the flavone. For best results, the plate was sprayed lightly at first and after observing the fluorescence under UV-light it was sprayed again, if necessary, until maximum fluorescence of the spots was obtained.

In situ quantitative studies

The fluorescence enhancement capability of the different 3-hydroxyflavones was assessed by spraying 2 μg spots of Proban with a solution of each individual flavone and measuring the fluorescence with a Zeiss Chromatogram-Spectrophotometer.

To investigate the stability of the fluorescence towards heat, the plate was spotted with 2 μg of Proban, eluted, sprayed and the fluorescence measured instrumentally (time zero). The plate was then put in an oven at 105° for 5 min and the fluorescence was measured again. The experiment was repeated by heating in the oven for periods of 10, 20, 30, and 60 min, respectively. For measurement of the fading under the UV-lamp of the Zeiss instrument, the spot was positioned visually in the light path and the decrease in the fluorescence was recorded with time. For fading studies under the Hanovia UV-lamp, the disappearance of the fluorescence of a 2 μg spot of the pesticide was monitored instrumentally at different time intervals of exposure.

Nine spots were developed simultaneously on one plate for reproducibility studies and scanned perpendicular to the direction of chromatography. In all studies, the M365 excitation filter was used. The emission monochromator of the Zeiss instrument was set at 528 for flavonol, 538 for fisetin, and 535 for robinetin. Calibration curves were obtained by measuring the fluorescence of a dilution series of the pesticide sprayed with robinetin. The extraction of Proban from tap water was carried out in a manner similar to the extraction of Guthion as described earlier[2]. The concentration of the sample was determined from a calibration curve of concentration *versus* peak area. The results were confirmed by the SAQH-Mn method[2].

RESULTS AND DISCUSSION

The qualitative aspect of the use of flavones as fluorigenic spray reagents has

already been discussed[4,5]. Only flavonol, fisetin and robinetin were found to be suitable for quantitative evaluation. A relative comparison of the fluorescence enhancement properties of these three flavones showed that robinetin gives the highest fluorescence intensity (Table I) and consequently further studies were carried out with these flavones.

Fig. 1 illustrates the stability of the fluorescence of robinetin towards UV-light. A drastic decrease in fluorescence can be observed, which is even greater when the UV-lamp of the Zeiss instrument is used for irradiation. The effect is, however, stronger on the background than on the fluorescent spot (compare curves 1 and 2). Under the less intense UV irradiation of the Hanovia lamp, the decrease in fluorescence is not as remarkable. In order to determine the feasibility of carrying out quantitative work with the Zeiss instrument, the effect of repetitive scanning on the fading of fluorescence was studied and it was found that around 23 % of the fluorescence is lost after ten successive scans of the spot; *i.e.*, an average of 2.3 % per scan, which is acceptable. Visible light has no apparent effect on the fading of fluorescence and in the dark the fluorescent spot can be kept for months without change.

The effect of heat on the fluorescence has also been investigated. An increase in fluorescence was noted for the first 20 min, dropping down again upon further heating. This was probably due to the drying process, as observed before[2]. A similar increase in the background fluorescence was observed, resulting in increased noise and hence little gain in sensitivity.

TABLE I

RELATIVE COMPARISON OF FLUORESCENCE ENHANCEMENT OF 3-HYDROXYFLAVONES

Flavone	*Excitation (nm)*	*Emission (nm)*	*Relative fluorescence*
Flavonol	360	528	1
Fisetin	370	533	12
Robinetin	375	535	19

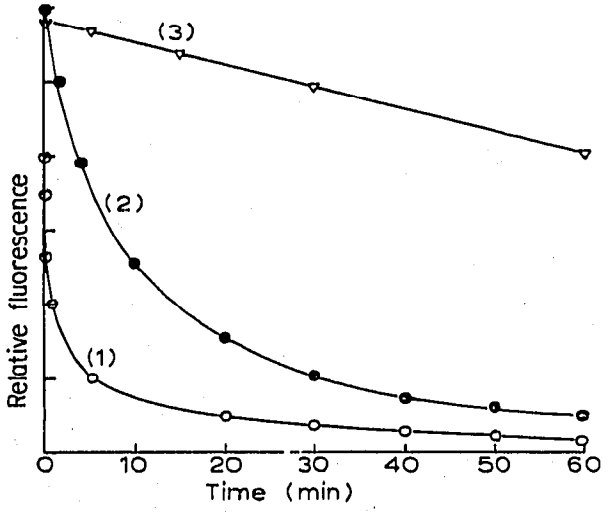

Fig. 1. Influence of UV light on the fluorescence of robinetin sprayed on silica gel layers. (1) Background, Zeiss Hg lamp; (2) spot, Zeiss Hg lamp; (3) spot, Hanovia Hg lamp.

Concentration *versus* fluorescence relationships have been studied with a dilution series of the pesticide Proban. A weakly bent curve was obtained between 10–1 μg while a straight line passing through the origin was observed below 1.0 μg. Although spots of concentrations lower than 0.1 μg are clearly visible under UV light, they can hardly be recorded because the background fluorescence interferes too much at high sensitivities. Standard addition methods are recommended in this concentration range.

A study of the reproducibility of the method after elution is presented in Table II. The values obtained for the relative standard deviations at the various concentrations compare favorably with those obtained in previous studies on *in situ* fluorimetric methods[2,6].

TABLE II

REPRODUCIBILITY AS A FUNCTION OF CONCENTRATION OF PROBAN

Concentration (μg)	*Average* ($n = 4$) *of rel. S.D.* (%)
10	5.7
5	3.3
1	3.1
0.5	5.5
0.1	12.2

Proban in tap water was determined at the 1 p.p.b. level to test the utility of the method. The results were double-checked by the SAQH-Mn method described earlier[2]. An average recovery of 83 % was obtained with the robinetin spray as compared with 85 % with the SAQH-Mn reagent. Thus the results were in good agreement.

CONCLUSIONS

The method discussed is suitable for the quantitation of organic compounds on cellulose layers. The reproducibility and sensitivity are similar to those of other *in situ* fluorimetric techniques on TLC[2,6]. Bromination is not required as compared with the previous techniques. Because of the non-specificity of the spray reagent, however, analysis of complex samples may be difficult. Nevertheless, a certain degree of selectivity exists for organic materials because many compounds are capable of quenching the fluorescence of the spray reagent[5]. Otherwise, the method is simple and the spray reagents are readily available. The technique should be useful when dealing with water samples or for checking out formulations.

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