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The effect of solvent purity on the chromatography of indole-3-acetic acid

In the course of an investigation on the growth substance relationships of diploid and tetraploid races of *Ranunculus ficaria* L. certain anomalies were found with regard to the R_F values quoted for indole-3-acetic acid in various chromatographic solvents.

NITSCH¹ stated that the R_F of indole-3-acetic acid (IAA) using a solvent system of isobutanol, methanol and water (16:1:3, by volume) was 0.24, whilst we have consistently obtained values of 0.80-0.90 using BDH Analar materials. Certain other observations on the effects of solvents on bio-assays prompted us to investigate the possibility of there being present an impurity in one or more of the solvent components causing the differences in R_F quoted. Purification of the isobutanol was achieved by refluxing the alcohol over potassium hydroxide for 2 h, and then fractionating the liquid. The fraction distilling over between 106° and 108° was collected. Methanol was purified by the method of GORDON AND PALEG², whilst the water used was doubly glass distilled and passed through a deionizer.

The R_F values obtained using combinations of repurified and un-repurified components of the solvent mixture are shown in Table I. In all cases purified water was used. Chromatography was carried out using strips of Whatman No. 3 paper, spotted with 2 μ l of a methanolic solution of IAA (10³ p.p.m.). The strips were spotted

TABLE I

THE EFFECT OF DISTILLATION OF ALCOHOL COMPONENTS OF THE SOLVENT SYSTEM ON THE R_F VALUE FOR PURE IAA

Distilled, deionized water used.

Solvent composition	R_F value of IAA
Distilled isobutanol } Distilled methanol }	0.20-0.30
Distilled isobutanol } Undistilled methanol }	0.20-0.30
Undistilled isobutanol } Distilled methanol }	0.80-0.90
Both undistilled	0.80-0.90

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in red light from a photographic Safe-Light, equilibrated over the solvent for 4 h, and run by ascending flow at 25°. The spots were located by their fluorescence under ultra-violet light (Wood's light) and also by Salkowski's reagent.

It is reasonably clear that the R_F value of the IAA depends on the purity of the isobutanol component, and not on that of the methanol. In this connection it is of interest to note that using a solvent of *tert.*-butanol and water (4:1 by volume), GUERN³ quotes an R_F for IAA of 0.27, whilst SEN AND LEOPOLD⁴ using the same solvent system give an R_F value of 0.80. Our own cursory examination of this latter solvent has yielded an R_F of 0.80 for IAA.

It is essential, at least in the conditions obtaining here, to use the purified isobutanol at once after distillation. Table II shows the effect of storage under various conditions of the isobutanol component on the R_F value for pure IAA.

TABLE II

THE EFFECTS OF STORAGE CONDITIONS OF THE ISOBUTANOL COMPONENT ON THE R_F VALUES OF PURE IAA

Conditions of storage of the redistilled isobutanol	Time elapsed after distillation (h)		
	0	24	48
In light, room temperature	0.20-0.30	Streak	0.80-0.90
In dark, room temperature	0.20-0.30	Streak	0.80-0.90
In dark, -10°	0.20-0.30	Streak	0.80-0.90

At present we have no clear indication of the nature of the causes of these effects, but from the results reported here, it seems clear that considerable attention must be paid to the purity of the solvents used in the chromatography of indoles.

Botany Department, Chelsea College of Science and
Technology, London, S.W. 3 (Great Britain)

E. R. TURNER
B. YOUNG*

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* Present address: Department of Botany, University of Alberta, Edmonton, Alberta (Canada).

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