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Note

Analysis of pyrethrins in pyrethrum extracts by high-performance liquid chromatography

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A rapid, reliable method of analysis, which is applicable to products containing pyrethrins, whether these are oleoresins, pale extracts or crude plant extracts, was required for screening pyrethrum lines in an extensive breeding programme.

Methods already described in the literature[1,2] for high-performance liquid chromatographic (HPLC) separation, rely on absorption readings at 254 rather than 229 nm and separation of the components can take up to 60 min[1]. The method presented here takes a step further the method of Ando *et al.*[3] in that it is possible to quantify the amount of pyrethrin present in natural pyrethrum extracts. The spectrophotometric method overestimates total pyrethrins due to the presence of extraneous UV-absorbing material in the pyrethrum extract[3,4]. HPLC techniques offer an opportunity to separate interfering substances from the six esters thus improving the reliability of estimation of pyrethrins.

EXPERIMENTAL

Materials

Pyrethrum extract (premium quality Pyrocide 175) was obtained from McLaughlin, Gormley, King Co., Minneapolis, MN, U.S.A. The *n*-hexane and tetrahydrofuran were Ajax Unichrom and Waters HPLC grade, respectively. The light petroleum (b.p. 40–60°C) was redistilled from bulk supplies.

Equipment

Total pyrethrins from flower samples were determined using a Pye Unicam SP8-200 UV–VIS spectrophotometer. The HPLC analyses were performed using a Waters HPLC system. This included a 6000A solvent delivery system, an U6K injector, an automated gradient controller, a Model 440 absorbance detector with an extended-wavelength module (229 nm) and a Waters RCM 100. Results were collected on a Hewlett-Packard Sigma 10 data station. The HPLC column used was an 8-mm, 10-μm silica Rad-Pak cartridge (Waters) with a 10-μm silica Waters RCSS Guard-Pak pre-column. For large numbers of routine samples a WISP (Waters Intelligent Sample Processor) was used.

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Method

In routine analyses of pyrethrum flowers for pyrethrins content, 0.5 g of dry, finely ground pyrethrum flowers were extracted in 25-ml volumetric flasks with redistilled light petroleum (b.p. 40–60°C) for 2 h[5]. The samples were shaken three times during this period.

Automated analysis of pyrethrins in flowers necessitates that the extract of pyrethrum flowers be suitable for direct injection into the HPLC system. Hence, the dilutions of the standard were chosen to correspond to the range of concentrations usually found in pyrethrum flowers. Care was taken to ensure that no evaporation of solvent occurred from prepared samples.

For the results presented in this paper, the following parameters were used: density of standard, 0.78; density of light petroleum, 0.65; %*P*I in standard (as specified by the manufacturer) 11.77%; %*P*II in standard (as specified by the manufacturer) 8.12%, where %*P*I and %*P*II are the percentages of pyrethrins I (pyrethrin I, jasmolin I and cinerin I) and pyrethrins II (pyrethrin II, jasmolin II and cinerin II), respectively.

A 10-μl volume of each of the dilution series was chromatographed using the following HPLC conditions: flow-rate, 0.7 ml/min for 21 min, then increased to 2.3 ml/min; solvent, *n*-hexane–tetrahydrofuran (96:4). During routine analyses this solvent was recycled with no loss of resolution or accuracy. A chromatogram of a typical HPLC separation is shown in Fig. 1.

RESULTS AND DISCUSSION

Spectrophotometric analyses of the dilution series of the standard indicate that the absorbance was linear within the first half of the concentration range being studied. At concentrations of standard higher than 0.12 ml standard per 25 ml light petroleum, Beer's law did not apply.

In contrast, a linear relationship of total pyrethrins peak area and concentra-

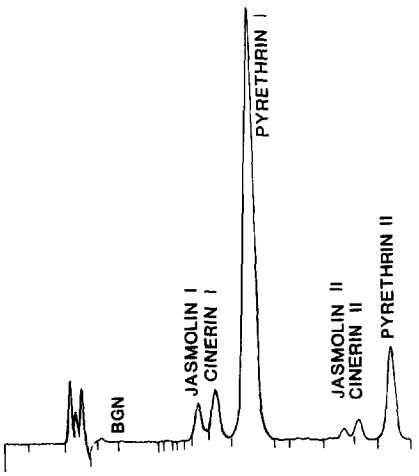

Fig. 1. HPLC separation of a sample of Pyrocide 175.

tion was obtained using HPLC analysis of the same dilution series (correlation coefficient = 0.999).

Pyrethrins I and II are determined independently as it is not yet known if the response factor is identical for the two groups of esters.

Using the total peak areas of pyrethrins I (y_1) and pyrethrins II (y_2), the standard curves for each group were found to be linear, having correlation coefficients of 0.9993 and 0.9989, respectively. The regression equations are given by

$$y_1 = 197.073x_1 + 0.677$$

and

$$y_2 = 59.252x_2 + 0.03$$

where x_1 and x_2 are equivalent % of pyrethrins I and II, respectively, per 25 ml light petroleum.

From any pyrethrum sample, the total percentage of pyrethrins can be calculated from $(x_1 + x_2) \cdot$ dilution factor of actual sample.

The standard has been found to remain stable when stored, tightly sealed, at 2°C in the dark.

Identification

A preliminary identification of the six esters was obtained by collecting the six fractions and analysing them on a UV spectrophotometer. Comparison of the results with those presented by Head[6] is given in Table I. The fractions were further subjected to mass spectroscopy and confirmed as being jasmolin I, cinerin I, pyrethrin I, jasmolin II, cinerin II and pyrethrin II (in order of HPLC elution). Mass spectra were obtained from direct insertion probe samples on a VG 70/70F spectrometer at 70 eV, source temperature of 200°C and with a 4 kV accelerating voltage.

It is possible to adapt the method used for calculation of total pyrethrins for calculation of percentages of individual esters. Fig. 2 indicates a linear relationship between peak area and concentration for each ester. It is necessary to assume that the response factor is the same for each ester within either the pyrethrins I or II group. Without authentic samples of the six esters, it is impossible to quantify them

TABLE I

UV-ABSORPTION DATA FOR THE ISOLATED PYRETHRINS

Compound	*Maximum UV-absorption wavelength (nm)*	
	This study	*Ref. 6.*
Jasmolin I (J_I)	220	219
Cinerin I (C_I)	221.8	220
Pyrethrin I (P_I)	224	222.5–223
Jasmolin II (J_{II})	229	229
Cinerin II (C_{II})	229.9	229
Pyrethrin II (P_{II})	225.5	227–228

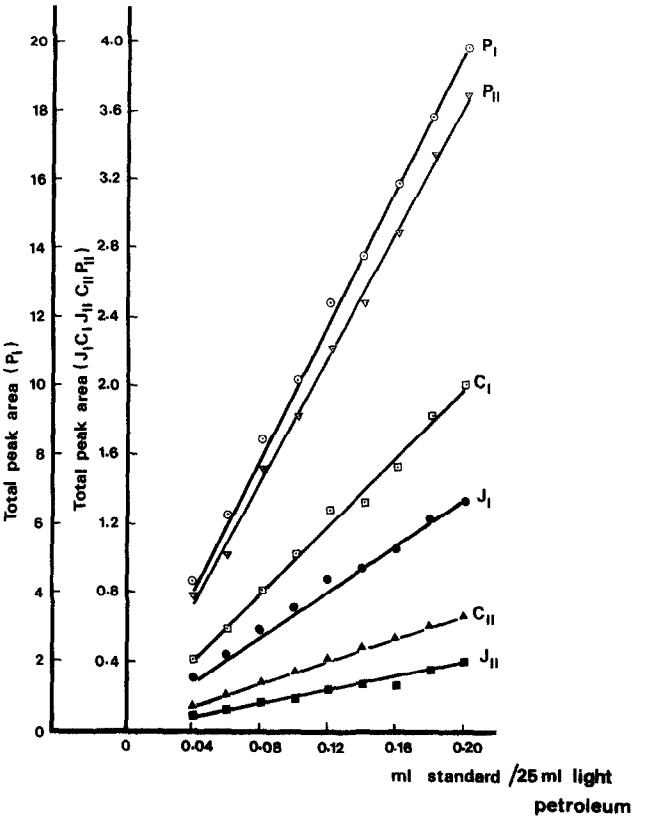

Fig. 2. Area of individual esters (for abbreviations, see Table I) *vs.* concentration.

using regression equations and therefore it is necessary to determine %*P*I or %*P*II as described, then use the peak area of the ester under consideration to calculate the relative percentage of each ester.

An analytical method with the capability for determining relative proportions of the esters and pyrethrins I/pyrethrins II ratios is useful in detecting clones having pyrethrum ratios with specific commercial applications.

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REFERENCES

1 D. A. Otieno, I. J. Jondiko, P. G. McDowell and F. J. Kezdy, *J. Chromatogr. Sci.*, 20 (1982) 566.
2 D. Mourot, J. Boisseau and C. Gayot, *Anal. Chim. Acta*, 97 (1978) 191.
3 T. Ando, Y. Kurotsu and M. Uchiyama, *Agric. Biol. Chem.*, 50 (1986) 491.
4 M. F. Kerslake, personal communication.
5 V. A. Beckley, *Pyrethrum Post*, 2 (1950) 23.
6 S. W. Head, in J. E. Cassida (Editor)), *Pyrethrum*, Academic Press, London, 1973, Ch. 3, p. 25.