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Short communication

Direct determination of pyrethrins in pyrethrum extracts by reversed-phase high-performance liquid chromatography with diode-array detection

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Abstract

A simple, rapid method for the direct determination of six pyrethrin esters in pyrethrum extracts by reversed-phase high-performance liquid chromatography with diode-array detection has been described. The separation of the six esters was based on a binary mobile phase optimization, temperature control and the use of a C_8 octyl column with 5-μm particles. Diode array detection and quantitation were selectively performed at 230 and 240 nm. The method demonstrated acceptable linearity, specificity, limit of sensitivity for the determination of six pyrethrin esters in pyrethrum extracts.

Keywords: Pyrethrins; Pesticides

1. Introduction

Analysis of natural pyrethrins has been of interest in many areas, especially in the insecticidal industry where pyrethrum extracts are used in formulations. Six insecticidally active pyrethrin esters namely, pyrethrin I, jasmolin I, cinerin I, pyrethrin II, jasmolin II and cinerin II, have been isolated from the pyrethrum extracts (Fig. 1) [1]. Other chemical components found in the extracts are sesquiterpenes and sesquiterpenoid lactones, flavonoids (e.g., 7-glucosides of apigenin, luteolin and quercetin), triterpenols and sterols (e.g., β-amyrin and taraxasterol) *n*-alkanes (e.g., *n*-heptacosane, *n*-nonacosane, etc.) and various fatty acids (e.g., lauric, palmitic, linoleic, tricosanoic, etc.) [2,3]. Pyrethrum extracts are normally analyzed by titration, ultraviolet (UV), spectrophotometric, gas–liquid chromatography [4–7] and normal-phase high-performance liquid chromatography methods [8–11]. Due to thermal instability of pyrethrins [12,13], HPLC methods are preferred over GC methods for analyzing pyrethrins and related compounds. But little is mentioned in the literature for the determination of the six pyrethrin esters by reversed-phase HPLC methodology. In a recent paper [14], we presented, for the first time, results of simultaneous determination of the six pyrethrin esters, dipropyl pyridine-2,5-dicarboxylate (MGK 326), *N*-octyl bicycloheptene dicarboximide (MGK 264) and piperonyl butoxide (PBO) in shampoo formulations used on pets (dogs and cats) by reversed-phase HPLC. By using ternary mobile phases comprised of acetonitrile, methanol and water and diode-array detection, all the six pyrethrin esters

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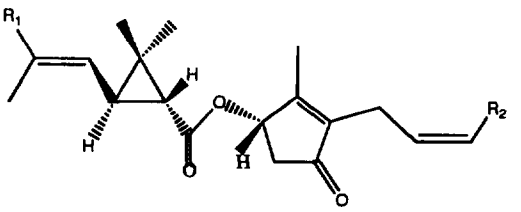

pyrethrin I $R_1 = CH_3$ $R_2 = CH{=}CH_2$ pyrethrin II $R_1 = CH_3O_2C$ $R_2 = CH{=}CH_2$

jasmolin I $R_1 = CH_3$ $R_2 = CH_2CH_3$ jasmolin II $R_1 = CH_3O_2C$ $R_2 = CH_2CH_3$

cinerin I $R_1 = CH_3$ $R_2 = CH_3$ cinerin II $R_1 = CH_3O_2C$ $R_2 = CH_3$

Fig. 1. Structures of the six pyrethrin esters in pyrethrum extract.

along with MGK 264, MGK 326 and PBO, were separated without a sample clean-up procedure. This method could be routinely used for analyzing pyrethrin formulations. In the present communication, we report a simplified method to analyze the pyrethrum extract itself for the six pyrethrin esters by employing a binary mobile phase optimization and no clean-up procedure. In addition, we also report the results of pyrethrum extracts analyzed by the present method and compared them with those obtained by AOAC procedures (Association of Official Analytical Chemists) based on a titration method [15].

2. Experimental

2.1. Materials and reagents

Two different samples of pyrethrum extracts were provided by Testing and Control, Analytical Chemistry, Animal Health, Sandoz Agro, (Dallas, TX, USA). The first sample obtained from Pyrethrum Board of Kenya, Nakuru, Kenya was the analytical standard. The other sample obtained from AgrEvo Environmental Health, New Jersey, USA, was treated as the sample. Acetonitrile, isopropanol and water were all HPLC grade and purchased from Fisher Scientific (Dallas, TX, USA). Volumetric flasks were Pyrex A grade and all samples and solutions were tightly capped and kept in the dark.

2.2. Chromatographic instrument and conditions

A Hewlett-Packard (HP) 1050 high-performance liquid chromatography instrument equipped with a diode-array detector, on-line degasser, heated column compartment and automatic sampler and injector were used. All data acquisition and analyses were made and performed on a Hewlett-Packard 3357 laboratory information management system. Analysis was performed on a Restek Pinnacle C_8 octyl reversed-phase HPLC column (150 mm×4.6 mm I.D., 5 μm). A constant flow-rate of 0.8 ml/min was used. The temperature of the column compartment was maintained at 40°C. Injection volume was 10 μl using a 25-μl sample loop. All analyses were performed after sufficiently equilibrating the column with the mobile phase and allowing the UV lamp to stabilize.

In order to achieve good separation, a two-component mobile phase consisting of acetonitrile and water was used. The volume ratios of the solvents and time table are shown in Table 1.

Table 1
Gradient elution program for pyrethrum extract analysis

Time (min)	% Acetonitrile	% Water
Initial	50	50
10	50	50
15	60	40
25	60	40
30	65	35
36	65	35

Table 2
Absorptivity values at maximum absorbance, retention time, limit of detection and limit of quantification for the six pyrethrin esters in pyrethrum extracts

Compound	λ_{max} (nm)	Retention time (min)	LOD (μg/l)[a]	LOQ (μg/l)[b]
Cinerin II	235	16.7	0.4	1.1
Pyrethrin II	229	17.5	0.1	0.1
Jasmolin II	235	19.9	0.5	1.2
Cinerin I	223	26.3	0.6	1.2
Pyrethrin I	225	27.5	0.2	0.4
Jasmolin I	228	32.2	0.4	0.9

[a] LOD (limit of detection) $= 3s_B/S$ where S is the sensitivity expressed as the slope of the calibration curve (peak area response for 1 μg/L solution) and s_B is the standard deviation of the noise.
[b] LOQ (limit of quantitation) $= 10s_B/S$.

2.3. Sample preparation

Standard and sample solutions were prepared by accurately weighing 0.02–0.05 g of pyrethrum extract in 100 ml of isopropanol in a volumetric flask. A series of dilutions of the standard solution were prepared to study linearity relationships. For the results presented in the paper, the following nomenclature and parameters were used: PY-I was 10.80% and PY-II was 8.62% in the standard pyrethrum extract as specified by the manufacturer (Pyrethrum Board of Kenya). PY-I and PY-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.

3. Results and discussion

To maximize detection and minimize any interferences as far as possible, diode array detection was selectively performed at 230 nm (0–24 min), 240 nm (23–29 min) and 230 nm (>29 min). Detection in the low UV region also gave lower detection and quantitation limits. Table 2 shows the absorptivity values of the six pyrethrin esters at the wavelengths

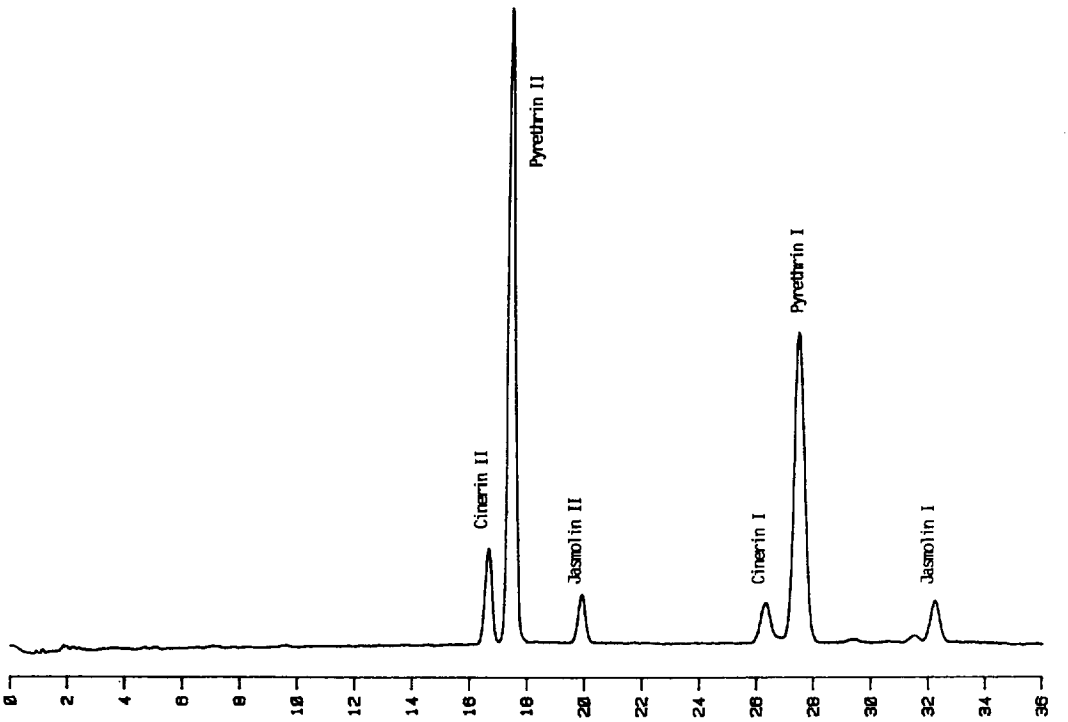

Fig. 2. Reversed-phase HPLC chromatogram of pyrethum extract.

of maximum absorbance, their respective retention times and limits of detection and quantitation.

The six active ingredients were analyzed individually and the results were used to identify the relative positions of the pyrethrin esters in pyrethrum extracts. The chromatogram of the standard pyrethrum extract (obtained from Kenya) is shown in Fig. 2. Under the described conditions of binary mobile phase, column temperature and selective detection, the six pyrethrin esters were well resolved. The advantage of reversed-phase HPLC over normal-phase HPLC methods reported earlier, was the very low level of interferences in the chromatography (Fig. 2). The power of diode array detection was put to use in the quantitation and separation of cinerin I and pyrethrin I. Cinerin I and pyrethrin I were monitored at 240 nm that resulted in well resolved peaks as shown in Fig. 2. If the monitoring UV wavelength was kept at 230 nm, cinerin I peak had a shoulder (probably from an interference peak) that affected its peak purity as well as an integrable area. Also the resolution between cinerin I and pyrethrin I was affected.

Based on an approach previously described by McEldowney and Menary [8], a linear relationship of PY-I and PY-II peak areas versus concentration was obtained by preparing a series of pyrethrum extract (obtained from Kenya) standard solutions over a wide range of concentrations. Using the total peak areas of PY-I (y_1) and PY-II (y_2), the standard curves for each group were found to be linear with correlation coefficients of 0.9999 and 0.9999, respectively. The regression equations are given by

$$y_1 = 1668\,x_1 + 7860$$

and

$$y_2 = 1272\,x_2 + 4479$$

where x_1 and x_2 are concentrations of PY-I and PY-II, respectively. In the absence of an authentic sample for each ester, it was not possible to quantify for each one of them in the PY-I and PY-II groups. Fig. 3 indicates a linear relationship between peak area and concentration for the six different esters. Since the slope for jasmolin (I and II) and cinerin (I and II) were fairly close, the main plot is shown in an expanded form for the four esters. The plot for pyrethrin I and II is shown as an inset of Fig. 3.

The new reversed-phase HPLC method was tested on a sample obtained from AgrEvo. The results were compared with analytical data of the sample using an established AOAC procedures [15] that is based on a titation method. Results by the AOAC method was provided by the manufacturer. The sample was tested against the standard obtained from Kenya. Table 3 shows the results of analyses by the two methods.

It is clear that the new reversed-phase HPLC method is as accurate as the AOAC titration method and this level of accuracy underlines the suitability of the method for the analysis of pyrethins in pyrethrum extract, residues in soil and other matrices. The agreement between the two sets of data in Table 3 is similar to that reported for normal-phase HPLC and AOAC procedures of pyrethrum extracts [11].

Selectivity, as a measure of the method's sensitivity to potential sample-related interferences, was evaluated by analyzing peak purities for all the peaks. The peak purities were all good indicating no interference from other constituents of the extract at the retention times of the six pyrethrin esters.

4. Conclusions

The new reversed-phase HPLC method using diode-array detection is suitable for the analysis of pyrethrum extracts and is simple, rapid and precise considering the complex nature of the extract. The method achieved good separation of the six pyrethrin esters and could be used for routine analysis of pyrethrum extracts. Detection in the low UV region resulted in lower detection limits and wider linearity. Finally the reversed-phase HPLC method was as good as the AOAC titration method.

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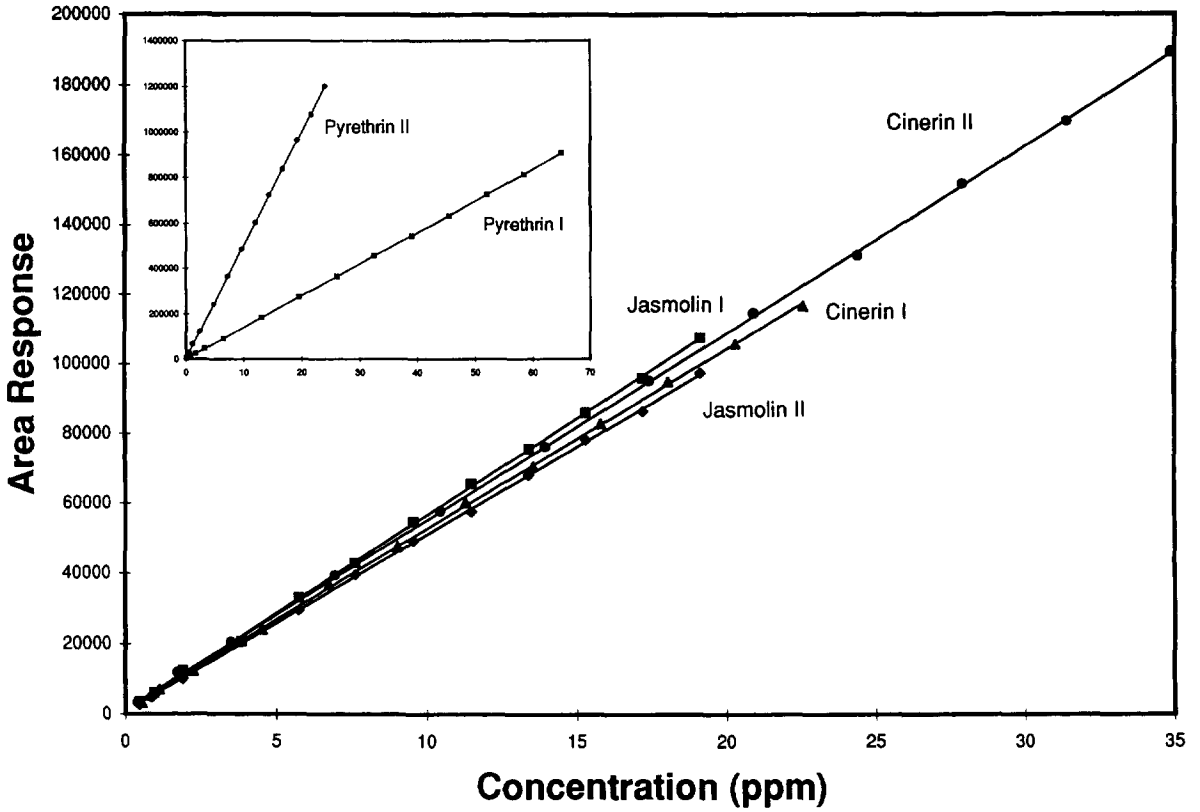

Fig. 3. Peak area response versus concentration for cinerin I and II and jasmolin I and II in pyrethum extract. Inset shows plot of pyrethin I and II in pyrethum extract.

Table 3
Comparison between results of the sample from AgrEvo obtained by reversed-phase HPLC and AOAC methods

Compound	RP–HPLC (%)[a]	AOAC (%)[b]
PY-I	28.13±0.02	28.14
PY-II	22.81±0.03	22.48
Total (PY-I + PY-II)	50.94±0.05	50.62

[a] Mean±standard deviation for $n=3$.
[b] Values provided by AgrEvo Environmental Health.

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