Poster Note 21431

Determination of Pyrethrins in Pyrethrum Oil Extracts by UHPLC with Charged Aerosol Detection

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Overview

Purpose: Develop a robust method to separate and quantify the major pyrethrins in extracts from pyrethrum oil and commercial products containing pyrethrins. Use complementary detectors to identify and measure minor components and impurities.

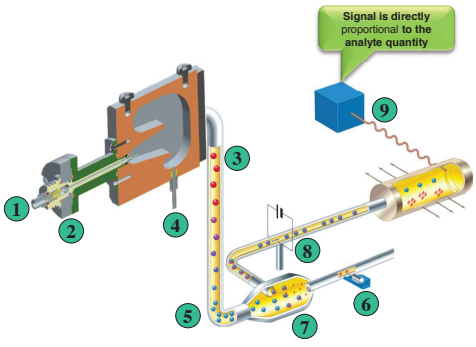
Methods: Gradient UHPLC with photodiode array detection and charged aerosol detection.

Results: Pyrethrum oil was found to be rich in the six major pyrethrins. Numerous minor components were detected at concentrations as low as 0.1% by peak area. The method was sensitive and precise.

Introduction

Pyrethrins are terpenoid esters derived from the flowers of *Chrysanthemum cinerariifolium*. The pyrethrin family includes six similar compounds containing a cyclopropane core, named pyrethrin 1 and 2, cinerin 1 and 2 and jasmolin 1 and 2. Pyrethrins both repel and kill insects by delaying the closure of voltage-gated sodium ion channels in the nerve cells. The insecticidal and insect repellent properties of these compounds have been known for millennia and *Chrysanthemum* species have long been cultivated for this purpose. Interest in using pyrethrin insecticides is growing because of their low toxicity to humans (allowing home use) and favorable, fast biodegradability. On the negative side, pyrethrum oil can trigger allergic reactions in susceptible people. It is also toxic to bees with fatal doses as low as 0.02 micrograms, thus requiring very cautious application. With their increasing use in agricultural and consumer products, there is a need for improved analytical techniques both to assure product quality and to monitor the fate of pyrethrins in the environment. Current reversed phase HPLC methods using UV detection fail to fully resolve the closely eluting components without excessively long run times. In this work, we show that a new UHPLC system comprising a binary parallel pump capable of operating at pressures up to 1500 bar, a high sensitivity diode-array detector with new light-pipe technology and an integrated charged aerosol detector (Figure 1), enables the resolution and detection of more compounds in pyrethrum oil in less time than previously possible.

FIGURE 1. Diagram of the Charged Aerosol Detector



Liquid mobile phase from the LC column enters the detector (1) where it is nebulized by combining with a concentric stream of nitrogen gas or air (2).

The fine droplets are carried by bulk gas flow to the heated evaporation sector (3) where desolvation occurs to form particles. Any larger droplets drain to waste (4).

The dry particles exit from evaporation (5) and combine with another gas stream that first passes over a high voltage Corona charger (6). The charged gas then mixes with the dry particles and transfers charge to the particle's surface (7).

Any high mobility species are removed by an ion trap (8) while the remaining charged particles pass to a collector where the passing particles charges are measured with a very sensitive electrometer (9). The resulting signal is processed by the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System.

Methods

Sample Preparation

Pyrethrum oil (50%) was diluted 10-fold with acetonitrile.

An OTC head lice treatment shampoo was prepared by diluting about 917 mg with 9.17 mL of acetonitrile.

A consumer kitchen aerosol insecticide was prepared by collecting 4800 mg of sprayed product in a 30 mL polypropylene centrifuge tube and adding 4.80 mL of acetonitrile.

Liquid Chromatography

Thermo Scientific™ Vanquish™ UHPLC system with:

- Vanquish Charged Aerosol Detector H:
  - Evaporation Temperature: 35 °C
  - Power function: 1.00
  - Data collection rate: 10 Hz
  - Signal Filter: 2 sec
- Vanquish Diode Array Detector HL:
  - Bandwidth: 4 nm
  - Wavelength: 220, 240 nm; 3D Field 200-400nm
  - Data collection rate: 10 Hz
  - Response Time: 0.4 sec
- Vanquish Binary Pump H
- Vanquish Split Sampler HT
- Vanquish Column Compartment H

Separation:

Column:	Thermo Scientific™ Accucore Vanquish™ C18 1.5 µm, 2.1 × 100 mm
Column Temp:	50 °C
Flow Rate:	0.8 mL/min
Injection Vol.:	0.2 – 0.5 µL
Mobile Phase A:	Deionized water, 18.2 MΩ·cm, < 5 ppm total organic carbon (TOC)
Mobile Phase B:	Acetonitrile, Fisher Optima™ LCMS
Gradient: Time, %B:	0, 45; 7, 80.

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) 7.2

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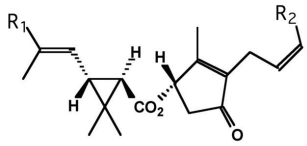
FIGURE 2. Structures of pyrethrins used in this study



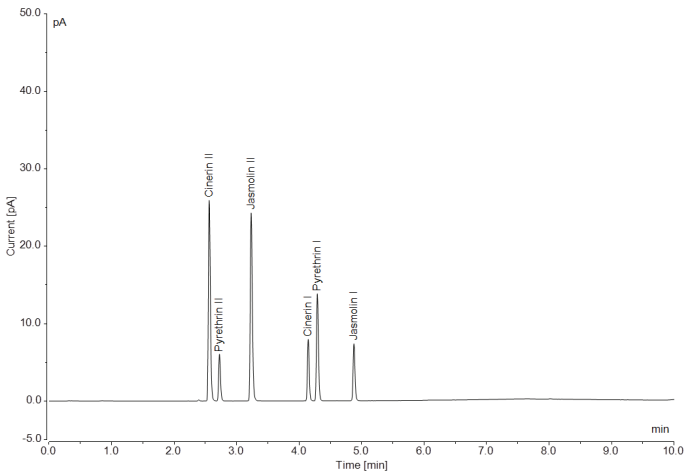
	R1	R2
Cinerin I	CH_3	CH_3
Cinerin II	CO_2CH_3	CH_3
Pyrethrin I	CH_3	$CH{=}CH_2$
Pyrethrin II	CO_2CH_3	$CH{=}CH_2$
Jasmolin I	CH_3	CH_2CH_3
Jasmolin II	CO_2CH_3	CH_2CH_3

Results and Discussion

Chromatography

Because the pyrethrins are thermally labile, liquid chromatography is preferred over gas chromatography for their analysis. Recent HPLC methods have included HPLC with UV detection[1,2] and LCMS[3]. To update the analytical method, we performed the analysis on the state-of-the-art Vanquish UHPLC system with an Accucore Vanquish C18 column that features 1.5 μm solid core particles. This system and column can operate at pressures up to 1500 bar to provide increased speed and resolution. We determined that a simple water:acetonitrile gradient provided better separation than when either methanol or formic acid were included in the mobile phase. A mixed pyrethrin standard analyzed by the final UHPLC-CAD method is shown in Figure 3. Under these conditions the six major pyrethrins are baseline resolved in under five minutes. For the analysis of pyrethrum oil and consumer product samples, the run time was extended to elute several highly retained hydrophobic compounds observed in those samples.

FIGURE 3. Vanquish UHPLC platform speeds analysis while maintaining resolution. UHPLC-CAD chromatogram of a mixed pyrethrin standard



UHPLC-UV-CAD Performance

The Vanquish UHPLC system uses two detectors that work in tandem with complementary strengths. The diode array detector with LightPipe™ is superbly sensitive for measuring species that possess a chromophore. The photodiode array detector responds nonspecifically at low UV wavelengths to most organic analytes and matrix components. UV achieves some specificity for particular functional groups by monitoring specific wavelengths. The charged aerosol detector is complementary to the diode array detector because it's response to all non-volatile analytes is typically uniform for a given mass of analyte. This uniform response is especially useful when one needs to estimate the quantity of unknown minor components or impurities as for which one does not have a calibration standard. Many components in addition to the six Type I and II pyrethrins are seen in the HPLC-CAD chromatograms shown in Figure 4.

When combined with a robust UHPLC method using state-of-the-art instrumentation, the method demonstrated here is both sensitive and precise. The limits of detection range from about 8 to 80 ng on-column by CAD and 29 – 66 ng by UV (data not shown). A typical response range of several orders of magnitude allows detection of impurities at below 0.1% of total peak area. Precision and calibration data for the charged aerosol detector are summarized in Table 1.

Table 1. UHPLC-CAD method, precision and calibration performance data

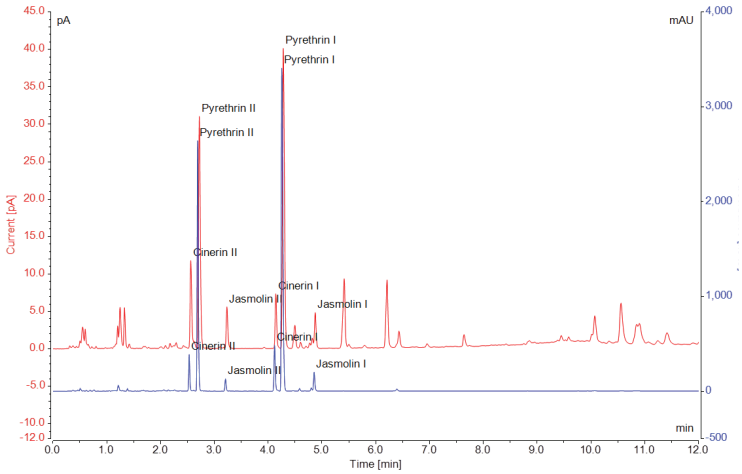
Analyte	RT (min)	Precision, % RSD[1]		Linearity		
		RT	Peak Area	Range (ng/μL)	LOD[2] (ng/μL)	R^{2**}
Cinerin II	2.51	0.09	1.68	4.2 - 2163	101	0.99894
Pyrethrin II	2.68	0.09	1.81	3.8 – 1950	15.0	0.99997
Jasmolin II	3.18	0.08	1.55	3.9 – 2013	82.0	0.99917
Cinerin I	4.09	0.07	2.32	3.7 - 1873	164	0.99849
Pyrethrin I	4.24	0.08	1.72	3.3 – 1712	43.9	0.99975
Jasmolin I	4.83	0.07	1.91	3.4 - 1720	120	0.99879

[1] for n = 30 replicates;
[2] Hubaux-Vos method
**quadratic fit with offset

Analysis of Pyrethrum Oil

The analysis of a concentrated pyrethrum oil illustrates the high resolving power of this method and the complementary nature of the UV/Vis absorbance and charged aerosol detectors. While the UV detector with Lightpipe is highly sensitive for detection of the six major Type I and II pyrethrins, the charged aerosol detector is clearly superior for the highly hydrophobic components eluting after Jasmolin I at 5 min in Figure 4.

FIGURE 4. complementary Detectors: VCAD and UV/Vis. Analysis of pyrethrum oil by UHPLC-CAD



Analysis of Commercial Products

Pyrethrum oil is used as a botanical insecticide in a variety of commercial products, including treatments for lice, bed bugs, and other insects. After a simple dilution with acetonitrile, the UHPLC-UV-CAD method was used to analyze some of these products, with two examples shown below in Figure 5. The unknown peak eluting immediately before Jasmolin II at about 3 min may be piperonyl butoxide, which is listed as an adjuvant in both of the products shown. The pyrethrin content of the samples is summarized in Table 2.

FIGURE 5. UHPLC-UV chromatograms of two commercial products containing pyrethrins.

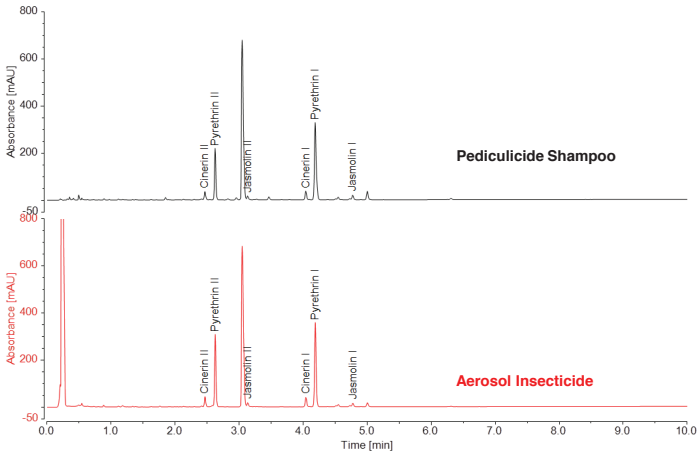


Table 2. Measured[1] pyrethrin content of selected samples determined by UHPLC-CAD

Analyte	Pyrethrum Oil	Pediculicide Shampoo	Aerosol Insecticide
Cinerin II	1,760	16.3	37.4
Pyrethrin II	21,900	692	973
Jasmolin II	700	38.8	26.6
Cinerin I	3,440	--	82.4
Pyrethrin I	11,400	443	272
Jasmolin I	2,780	47.4	58.2

[1] Amounts in ng/µL in samples as prepared

Conclusions

- The UHPLC-UV-CAD method resolves and quantifies all of the Type I and II pyrethrins found in extracts from flowers of the chrysanthemum daisy.
- The method is simple, requiring only dilution, filtering and injection.
- This quantitative method is precise (peak area RSD below 2 %) and sensitive (LODs 29 – 66 ng by UV absorbance and 8 – 80 ng on column by charged aerosol detection.)
- Charged aerosol detection is sensitive to non-chromophoric analytes and is compatible with UHPLC methods that elute the major pyrethrins in < 5 min.
- The UV./is and charged aerosol detectors furnish complementary information and together detect more peaks than either one separately.

References

1. Loper B., Anderson K., Determination of pyrethrin and pyrethroid pesticides in urine and water matrixes by liquid chromatography with diode array detection, *Journal of AOAC International*, Vol.86, No 6 (**2003**) 1236 - 1240.
2. Essig, K., Zhao, Z., *J. Chromatographic Science*, Vol. 39, **2001**
3. Achille Cappiello, Bruno Tirillini, Giorgio Famiglini, Helga Trufelli, Veronica Termopoli and Cornelia Flende. Determination of Natural Pyrethrins by Liquid Chromatography-Electron Ionisation-Mass Spectrometry. *Phytochemical Analysis*, **2012**, 23,191-196.

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