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Efficient, Baseline Separation of Pyrethrins by Centrifugal Partition Chromatography

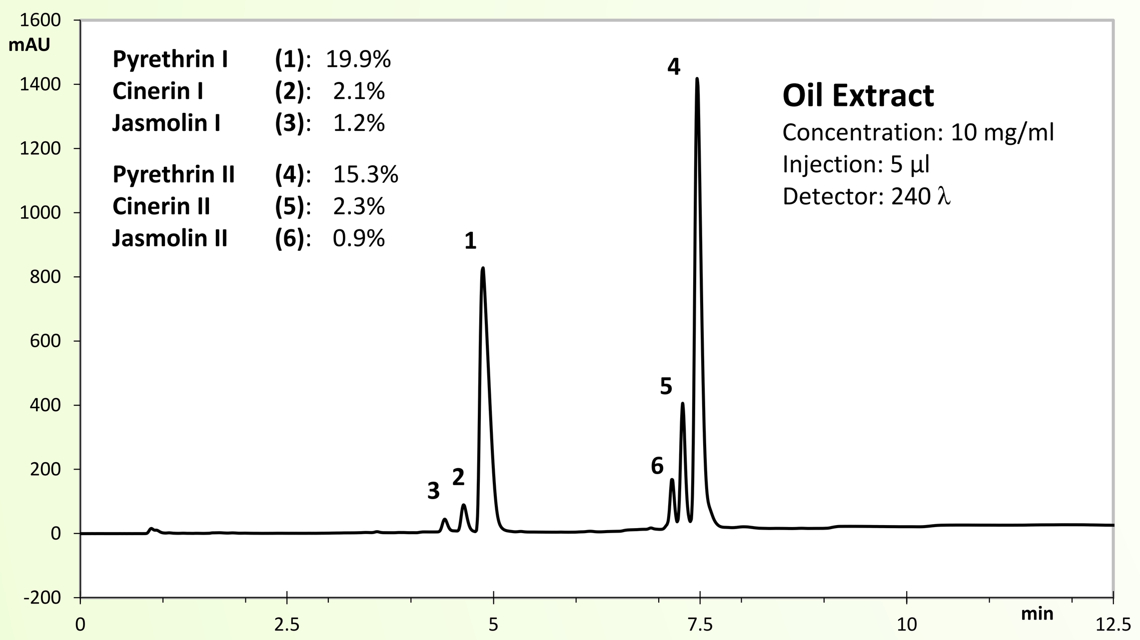
Alan Wong[1], **Vitold Glinski**[1], **Jan A. Glinski**[1]   [1]Planta Analytica LLC, 461 Danbury Rd, New Milford, CT 06776

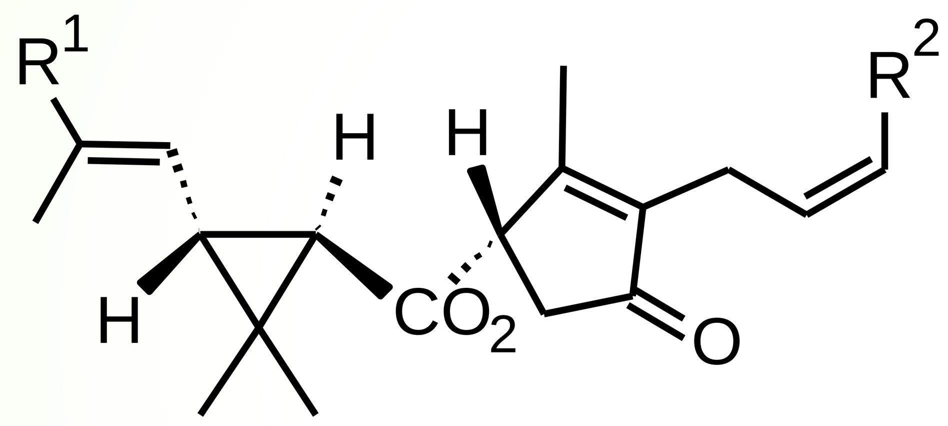
Abstract

Pyrethrins are natural pesticides present in the oil extracted from the flowers of *Chrysanthemum cinerariaefolium*. These six structurally related esters are potent neurotoxins that interfere with sodium channels in neurons of insects. With low toxicity to mammals and an exceptionally safe environmental profile, there is an increasing use of the oil extract as a natural pesticide. The six pyrethrins are either of *trans*-chrysanthemic acid (pyrethrins I group) or pyrethric acid (pyrethrins II group). Both groups comprise pyrethrin, cinerin, and jasmolin, with minute differences in the alkyl chain in the alcohol moiety.

Isolation of multi-gram quantities of all six pyrethrins in high purity is a prerequisite to synthesizing derivatives for studying toxicity and soil degradation. High hydrophobicity and close structural similarity between the individual pyrethrins within their groups make this task challenging. As a result, large quantities of these esters have not been commercially available. To facilitate the study, we have developed a two-step purification process, in which the two groups were first separated by normal phase column chromatography on silica gel. For the separation of pyrethrin, cinerin, and jasmolin within each group we applied Centrifugal Partition Chromatography (CPC). We explored and successfully optimized solvent systems that were subsequently applied to achieve multi-gram scale separation. In both cases, gram quantities of individual pyrethrins in purity exceeding 99% were produced.

Normal phase HPLC separation





Pyrethrin I (1): $R^1 = CH_3$ $R^2 = CH{:}CH_2$
Cinerin I (2): $R^1 = CH_3$ $R^2 = CH_3$
Jasmolin I (3): $R^1 = CH_3$ $R^2 = CH_2CH_3$

Pyrethrin II (4): $R^1 = CO_2CH_3$ $R^2 = CH{:}CH_2$
Cinerin II (5): $R^1 = CO_2CH_3$ $R^2 = CH_3$
Jasmolin II (6): $R^1 = CO_2CH_3$ $R^2 = CH_2CH_3$

Experimental

Centrifugal Partition Chromatography:

Kromaton FCPC A (Rousselet Robatel, Annonay, France) equipped with 1 L rotor
Waters HPLC pump model 590 for solvent delivery; 33 ml/min
Dynamax pump model SDS-200 for sample injection; 20 ml/min
Varian ProStar 701 fraction collector
Operated in ascending mode at 1500 rpm

Pyrethrins I Group:

Solvent system: SS-02 heptane-methanol-acetonitrile (6:1:2, v/v)
SS-03 heptane-MTBE-acetonitrile (8:1:5, v/v)
Sample: 8 g load of est. 65% pyrethrins I group per run; 8 runs
Result: 28.3 g pyrethrin I **(1)**, 5.4 g cinerin I **(2)**, 4.6 g jasmolin I **(3)**

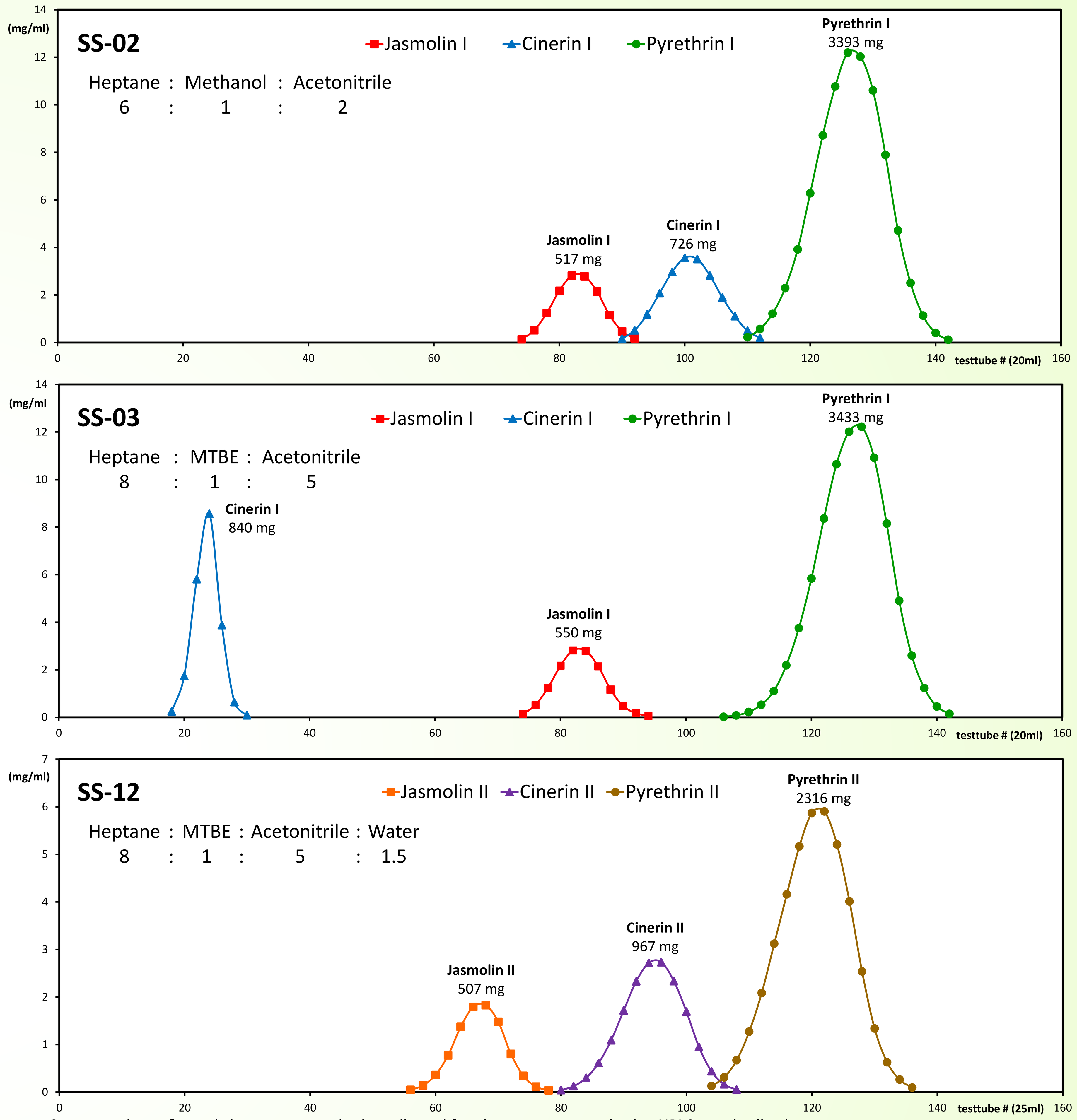
Pyrethrins II Group:

Solvent system: SS-12 heptane-MTBE-acetonitrile-water (8:1:5:1.5, v/v)
Sample: 8 g load of est. 60% pyrethrins II group per run; 9 runs
Result: 24.3 g pyrethrin II **(4)**, 4.8 g cinerin II **(5)**, 3.2 g jasmolin II **(6)**

Normal Phase HPLC:

System: Agilent HPLC model 1100; YMC 4.6 x 100 mm Cyano column, S-3 micron, 120 Å
Gradient: A: hexane; B: 10% THF in hexane;
2.0 ml/min; step gradient 0% B to 12% B at 5.0min
Result: jasmolin I **(3)** 4.42 min, cinerin I **(2)** 4.63 min, pyrethrin I **(1)** 4.89 min, jasmolin II **(6)** 7.17 min, cinerin II **(5)** 7.30 min, pyrethrin II **(4)** 7.48 min

CPC elution pattern of pyrethrins I group in (A) SS-02, and (B) SS-03, and of pyrethrins II group in (C) SS-12 determined by HPLC.



Concentrations of pyrethrin components in the collected fractions are measured using HPLC standardization.

Solvent systems for pyrethrins I group

Solvent System	Hep	MTBE	MeOH	MeCN	H_2O	Cpd	K_{asc} ascending	K_n/K_{pyr} ratio
						1	0.43	1.00
SS-01	1.5	--	1	--	0.03	2	0.38	0.88
						3	0.30	0.70
						1	2.63	1.00
SS-02	6	--	1	2	--	2	2.08	0.79
						3	1.61	0.61
						1	2.64	1.00
SS-03	8	1	--	5	--	2	0.68	0.26
						3	1.64	0.62

Partition coefficients (*K*) measured for the pyrethrins I group (*left*) and pyrethrins II group (*right*) in selected solvent systems. Comparison of the *K* values of cinerin and jasmolin with the K values of pyrethrin (K_n/K_{pyr}) provides information about the selectivity of the solvent system with the pyrethrin group.

Solvent systems for pyrethrins II group

Solvent System	Hep	MTBE	MeOH	MeCN	H_2O	Cpd	K_{asc} ascending	K_n/K_{pyr} ratio
						4	6.25	1.00
SS-04	5	--	2.5	0.5	--	5	5.26	0.84
						6	4.76	0.76
						4	12.53	1.00
SS-05	5	--	1.5	1.5	--	5	9.53	0.76
						6	7.16	0.57
						4	8.82	1.00
SS-06	5	--	1	2	--	5	6.63	0.75
						6	4.85	0.55
						4	3.41	1.00
SS-07	6	1	2	1	--	5	3.10	0.91
						6	2.98	0.87
						4	2.94	1.00
SS-08	6	0.5	2	1	0.5	5	2.44	0.83
						6	1.79	0.61
						4	13.42	1.00
SS-09	8	1	--	5	--	5	9.87	0.74
						6	7.33	0.55
						4	7.84	1.00
SS-10	8	2	--	4	--	5	6.18	0.79
						6	4.90	0.63
						4	9.36	1.00
SS-11	7	2	--	5	--	5	4.17	0.76
						6	5.58	0.59
						4	4.54	1.00
SS-12	8	1	--	5	1.5	5	3.53	0.78
						6	2.26	0.50

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