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Separation of the insecticidal pyrethrin esters by capillary electrochromatography

Charles W. Henry III, Matthew E. McCarroll, Isiah M. Warner*

Department of Chemistry, 232 Choppin Hall, Louisiana State University, Baton Rouge, LA 70820, USA

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Abstract

A simple, rapid technique for the direct separation and quantification of the six insecticidally active pyrethrin esters in typical extracts and commercial formulations by capillary electrochromatography (CEC) has been described. The separation of the pyrethrin esters was achieved by optimizing several parameters including the length of stationary phase, the mobile phase composition and column temperature. The mobile phase composition had the most pronounced effect toward resolving these structurally similar compounds. A ternary mobile phase composed of acetonitrile–aqueous buffer–tetrahydrofuran (55:35:10) provided the elutropic solvent strength needed to resolve the six esters from an extract mixture in under 16 min. A 25 cm packed bed of Hypersil 3 μm C_{18} stationary phase was used with the ternary mobile phase at 25°C and 30 kV voltage. These conditions also yielded excellent separation of the pyrethrin esters in two different commercially available insecticidal formulations. In addition, the developed CEC method was shown to be a fast and easy way of quantifying the amount of these esters in typical pyrethrin formulations.

Keywords: Electrochromatography; Mobile phase composition; Pyrethrins; Pesticides

1. Introduction

The six pyrethrin esters are insecticidally active compounds found in the plant *Chrysanthemum cinerariaefolium*. Pyrethrum extract is the insecticidal ingredient in a variety of formulations used for household, agricultural and industrial pest management. Regulatory requirements and quality control considerations have driven the need for better, faster, cheaper ways of determining the presence and quantity of pesticides in different formulations. A variety of methods have been developed to address the determination of pyrethrins in pesticide formulations including spectroscopy, titration, nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC), and high-performance liquid chromatography (HPLC) [1–5]. Among these techniques, GC and HPLC have been the most popular and therefore have found application in the Association of Official Analytical Chemists (AOAC) and the US Environmental Protection Agency (EPA) standard methods texts, respectively [6,7]. Recently, we published a paper highlighting the use of micellar electrokinetic chromatography (MECC) to separate the pyrethrin esters [8]. This technique allowed for the rapid determination of pyrethrins in typical extract samples while utilizing small sample and solvent requirements.

An alternative technique to MECC is capillary

*Corresponding author. Tel.: +1-504-3883-945.

E-mail address: isiah.warner@chem.lsu.edu (I.M. Warner).

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electrochromatography (CEC) utilizing a stationary phase rather than a micellar pseudo-stationary phase. CEC is a hybrid technique that couples the selectivity of HPLC and the separation efficiency of capillary electrophoresis. In CEC, separation of neutral components is dictated by differences in an analytes' distribution ratio between the mobile and stationary phase. Charged compounds have an additional separation component due to their inherent electrophoretic mobility. Therefore, both charged and uncharged compounds can be effectively separated using CEC.

The technique of CEC has gained rapid scientific popularity in a short time for several reasons. It combines the well-characterized retention mechanisms and selectivities of LC with the benefits of electroosmotic flow (EOF). This EOF generates a pluglike velocity profile that yields high efficiency separation in contrast to the parabolic flow profile generated by LC [9,10]. The utilization of EOF in CEC rather than pressure-driven flow makes the use of small particles possible. To date, most studies in CEC have focused on the theory/mechanism [9–13], column packing strategies [14–18], and operational parameters that control CEC performance [12,13,18–21]. Recently, pharmaceutical separations [15,18,22–28], CEC–mass spectrometry (MS) [19,29–31] and CEC repeatability, reproducibility, and quantification [18,24,32,33] have been studied and have yielded encouraging results for this technique.

This paper describes the effects of varying several chromatographic parameters to achieve CEC separation of the pyrethrin esters in high quality standard extracts using CEC with an ODS stationary phase. The optimized method was shown to be effective in separating and quantifying the pyrethrin esters in two commercially available insecticidal products.

2. Experimental

2.1. Reagents and chemicals

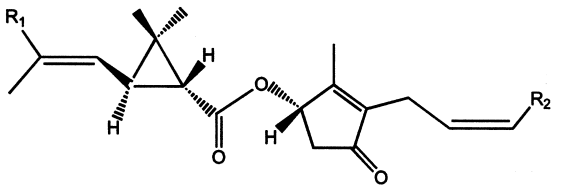
The analytical pyrethrum standard was obtained from Cresent (Riedel-de Haën pyrethrin technical mixture, ''Pestanal''; Hauppauge, NY, USA). Extract samples used for quantification were obtained from Fluka (Ronkonkona, NY, USA) and the Pyrethrum Board of Kenya (WSPE 1992=World Standard Pyrethrum Extract and PBK Pale Extract; Nakuru, Kenya). The Pyrethrin Dip and Flea & Tick Mist (both Adams brand, manufactured for Pet Chemicals, Memphis, TN, USA) were obtained from a local veterinary office. We used 2,2-dimethylpropiophenone purchased from Aldrich (Milwaukee, WI, USA) as an internal standard. HPLC-grade acetonitrile (MeCN) and tetrahydrofuran (THF) were purchased from Mallinckrodt & Baker (Paris, KY, USA) and Fischer Scientific (Pittsburgh, PA, USA), respectively. The buffer Tris (tris[hydroxymethyl]aminomethane) was purchased from (Sigma, St. Louis, MO, USA). The stationary phase, specifically designed for CEC (i.e., no endcapping), was graciously donated by Thermoquest (CEC Hypersil, 3 μm particle diameter, pore diameter=120 Å, pore volume=0.65 ml/g, surface area=175 m^2/g, percentage of carbon=10%; Runcorn, UK). Polyimide coated fused-silica capillary columns of 100 μm I.D.×350 μm O.D. were obtained from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Instrumentation and conditions

A $HP^{3D}CE$ capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detection (DAD) system was used in the CEC mode for all experiments. The dimensions of the capillary were 33 cm×100 μm I.D. (25 cm packed region). The inlet and outlet buffer vials were pressurized at 12 bar using nitrogen to prevent bubble formation (CEC mode). The temperature of the capillary cassette was maintained at 25°C, except for the temperature study. A 30 kV voltage was applied across the capillary to produce EOF. The UV detector was set to 254 nm. All samples were injected electrokinetically (20 kV for 2 s). Data were collected and analyzed using the $HP^{3D}CE$ Chem station software (Hewlett-Packard). The capillary packing pump was a Knauer pneumatic HPLC pump (Berlin, Germany).

2.3. Sample and buffer preparation

The structures of the six pyrethrin esters are shown in Fig. 1. Analytical standard pyrethrum stock



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

Fig. 1. The structures of the six pyrethrin esters.

solutions were made by accurately weighing 20–100 mg of pyrethrum extract in 1 ml MeCN. Pyrethrum extract samples used for quantification were prepared by making various μg/mg (%, w/w) concentrations of pyrethrum extract in MeCN. Before injection, an accurately determined aliquot of the stock was combined with a known amount of the mobile phase to properly match the composition of the sample and mobile phase. Buffer solutions were made by dissolving the appropriate amount of Tris buffer in 10 ml of deionized water and adjusting to pH 9. A volume fraction of the aqueous Tris buffer solution was combined with an appropriate volume fraction of organic modifier depending on the mobile phase being studied.

The Pyrethrin Dip and Flea & Tick Mist were analyzed to show the utility of this method on complex samples. The Pyrethrin Dip was mixed with an equal volume of MeCN to reduce the viscosity of the sample. Because the Flea & Tick Mist contains an order of magnitude less pyrethrins than the Pyrethrin Dip and its viscosity was acceptable for direct injection, the Flea & Tick Mist was analyzed directly from the bottle.

2.4. Preparation of packed capillary columns

Capillary columns were packed in our laboratory according to a procedure developed elsewhere [9,10]. Stationary phase was slurried in acetone at a concentration of 0.2 g/ml, and sonicated for 2 min. Then 1 ml of slurry was injected through a Rheodyne injector connected to a stainless steel reservoir. The injector was connected to the pump and the reservoir was connected to a capillary. The other end of the capillary was connected to a union containing a 0.5 μm frit. The pump pressure was set to 400 bar until visible inspection of the capillary showed that it was filled with stationary phase. The pump was then shut off, the pressure was released and the excess slurry was flushed form the reservoir. The capillary was reconnected and the pump was set to 400 bar for 2 h. After the 2 h in which the stationary phase was settled within the capillary, the first frit was made with an electrically heated Nichrome wire. The capillary was removed from the bottom union and the excess stationary phase was expelled using a pump pressure of 200 bar. This pressure was maintained for 2 h and the second frit was then fabricated. Finally, the detector window was made by burning the outer capillary polymer coating 1 cm from the outlet frit. The capillary was then flushed with mobile phase for 1 h and installed in a capillary cartridge. Columns were conditioned with mobile phase by applying 12 bar to the inlet side while ramping the voltage from 5 to 25 kV over a 30-min period. Both the inlet and outlet vials were pressurized and the voltage was set to 30 kV for another 30-min period or until the current response stabilized. This procedure was used whenever a new mobile phase was tested.

3. Results and discussion

3.1. Initial separation of pyrethrins

It is customary to start method development schemes in HPLC by using a binary acetonitrile and water mobile phase. This binary mobile phase has shown utility in CEC method development as well [11,34,35]. Only partial separation of the six pyrethrin esters was achieved using a mobile phase consisting of MeCN–25 m*M* Tris–HCl (80:20, pH 9) as shown in Fig. 2A. The selectivity achieved by this system cannot resolve pyrethrin I (PI) from

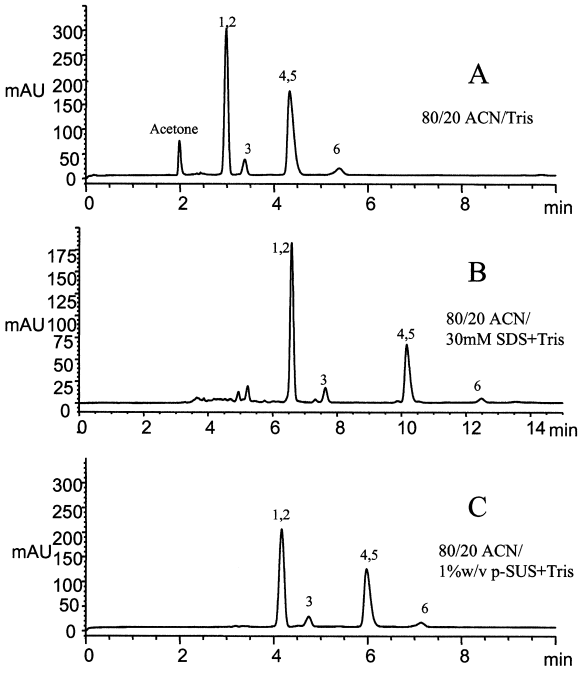


Fig. 2. CEC electrochromatograms of a pyrethrin sample using a mobile phase of MeCN–25 m*M* Tris (80:20, pH 9), and (A) no additive, (B) 30 m*M* SDS, and (C) 1% (w/v) *p*-SUS. 1=Cinerin II, 2=pyrethrin II, 3=jasmolin II, 4=cinerin I, 5=pyrethrin I, 6=jasmolin I.

cinerin I (CI) and pyrethrin II (PII) from cinerin II (CII). Aside from insufficient resolution, these initial results indicate that fast, potentially high efficiency separations were possible using CEC if resolution could be improved. Several factors were studied to achieve complete separation of the six pyrethrin esters including the packed capillary length, column temperature and mobile phase composition. In this work, the pyrethrins were identified using the photodiode array detector and comparing the UV spectra of each peak to the known UV absorption maximums for the esters (see Table 1).

3.2. Evaluation of micelle polymer mobile phase additives

In a previous study, the micelles of sodium dodecyl sulfate (SDS) and the micelle polymer of sodium *N*-undecyl sulfate (*p*-SUS) were shown to resolve all six of the pyrethrin ester using MECC [8]. In addition, the micelle polymer of sodium *N*-undecanoyl-L-glycinate (*p*-SUG) reduced migration times and increased selectivity in the separation of a complex cholesterol mixture using CEC [36]. Additionally, other researchers have used chiral selector mobile phase additives in combination with ODS stationary phases in CEC to enhance the chiral separations of *N*-derivatized amino acids [37]. Recently, two publications reported the use of SDS below the critical micelle concentration (CMC) to prevent bubble formation in CEC [38,39]. Therefore, in an effort to improve resolution by the addition of another mode of analyte interaction, we added SDS and *p*-SUS to the CEC system, respectively. We believed that the addition of these pseudo-stationary phases would yield the benefits of both MECC and CEC. Fig. 2B and C show the electrochromatograms comparing the separations with the addition of SDS and *p*-SUS. In this work, the concentration of SDS was effectively 30 m*M*, well above the CMC. Interestingly, there was no selectivity gained by the addition of either mobile phase additive. The EOF velocity was approximately the same regardless of the mobile phase additive type and concentration. Resolution (not including the co-eluting pairs CII/PII and CI/PI) and theoretical plates increased when both the micelles and micelle polymer were added yet the selectivity factor remained fairly constant (approx. 1.2). It appears that in this system, the dominant mode of analyte separation is partition into the stationary phase. The added surfactant may simply coat the stationary phase rather than migrate freely in the mobile phase as in MECC.

Table 1
UV absorption data for the isolated pyrethrins [λ_{max} (nm) in solvent described in table]

Pyrethrin	Found (MeCN–Tris, 80:20)	Literature [5] (hexane)
Jasmolin I	226	219
Cinerin I	226	220
Pyrethrin I	226	222.5–223
Jasmolin II	234	229
Cinerin II	234	229
Pyrethrin II	229	227–228

3.3. Evaluation of stationary phase length

Increasing the length of the packed segment of the

capillary from 25 cm to 40 cm resulted in an approximately threefold increase to the migration times and theoretical plates and a twofold increase in resolution. The field strength decreased proportionately with length from 1200 V/cm for a 25 cm column to 750 V/cm for the 40 cm column. The EOF was reduced from 2.1 mm/s to 1.6 mm/s using the longer packed column. There was no increase in selectivity since pyrethrin and cinerin I and II still co-eluted. Only moderate gains in theoretical plates and resolutions were achieved at the cost of longer migration times, the shorter column was therefore used for subsequent experiments.

3.4. Evaluation of the mobile phase composition

The effects of decreasing the proportion of MeCN on selectivity, resolution and retention can be seen in Fig. 3. Other investigators have seen both an increase and decrease in EOF with decreasing proportion of MeCN in the mobile phase [11]. In this study, the EOF decreases with decreasing % MeCN. Conversely, the migration times and selectivity factors increase with decreasing MeCN due to changes in the partitioning of the hydrophobic analytes between the stationary and mobile phase. As the mobile phase becomes more polar (i.e., less organic modifier), the analytes partition into the stationary phase to a greater extent. Enhancing the analyte interaction with the stationary phase causes higher resolution between closely eluting peaks with concomitant longer migration times. In this case, we could alter the selectivity until the co-eluting cinerin and pyrethrin I and II are separated by using 60% MeCN in the mobile phase.

A small fraction of a ternary solvent (THF) was added to the MeCN–Tris (60:40) mobile phase to speed up the migration of analytes while retaining the selectivity achieved by lowering the MeCN concentration. Comparison of Fig. 3C with Fig. 4

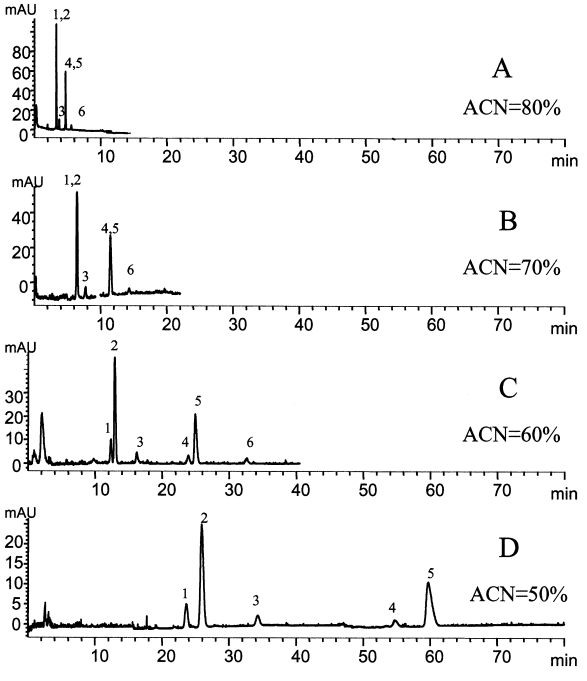


Fig. 3. Effect of percent MeCN on the separation of a pyrethin standard sample. Conditions: acetonitrile–25 m*M* Tris, pH 9, 30 kV voltage, temperature 25°C. 1=Cinerin II, 2=pyrethrin II, 3=jasmolin II, 4=cinerin I, 5=pyrethrin I, 6=jasmolin I.

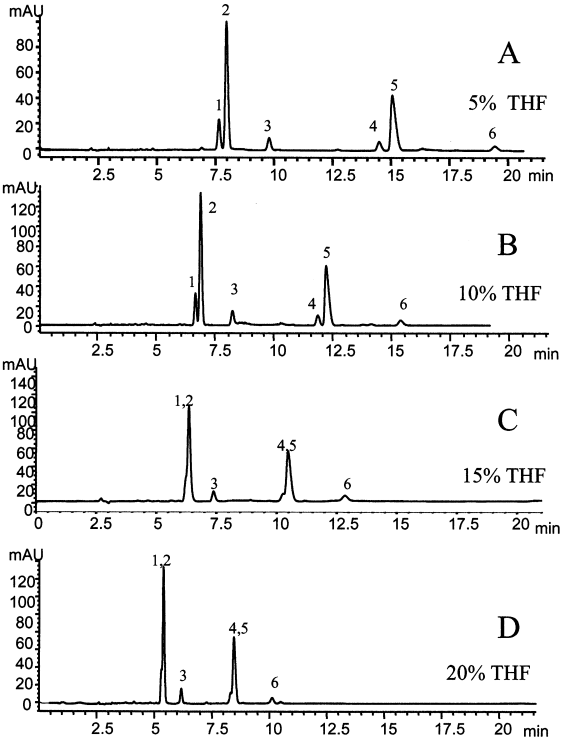


Fig. 4. Effect of percent THF on the separation of a pyrethin standard sample. Conditions: MeCN–25 m*M* Tris (60:40, pH 9), 30 kV voltage, temperature 25°C, UV detection at 254 nm. 1=Cinerin II, 2=pyrethrin II, 3=jasmolin II, 4=cinerin I, 5=pyrethrin I, 6=jasmolin I.

shows the faster migration of analytes upon addition of THF. At THF concentrations higher than 10%, the high elutropic strength of THF destroys the selectivity achieved by the polar mobile phase (i.e., MeCN–Tris, 60:40). The addition of 10% THF to the MeCN–Tris (60:40) mobile phase reduces the total analysis time to below 16 min while maintaining near baseline resolution between cinerin and pyrethrin I and II. Table 2 shows the theoretical plates, resolution and EOF time for each electrochromatogram.

3.5. Evaluation of column temperature

An increase in temperature causes the viscosity of aqueous–organic solvent systems to decrease and therefore increases the EOF [40]. Other researchers have used high temperatures to decrease total analysis time without a sufficient loss to resolution [40]. The pyrethrin analytes are thermally labile and seem to decompose at the studied temperatures giving rise to peak splitting. It is this very property of pyrethrins that makes the GC analysis of these compounds problematic. Therefore, all subsequent separations were performed at 25°C.

3.6. Separation and quantification of pyrethrin extracts and commercial pyrethrin formulations

Because of the unavailability of the individual pyrethin esters, most HPLC quantification methods use a technical pyrethrin sample as a standard. These methods measure the peak height or area of one of the major peaks (i.e., pyrethrin I) to determine the % pyrethrins based on the following formula:

$$\%\ \text{Pyrethrins} = (H/H')(W'/W)(\%\ \text{purity of standard})$$

where H and H' = the average peak heights of sample and standard and W and W' = g of standard and sample [41–43].

In this study we use the sum of the peak areas of pyrethrin I (pyrethrin I, cinerin I, jasmolin I) and pyrethrin II (pyrethrin II, cinerin II, jasmolin II) versus the % (w/w) pyrethrins to generate calibration curves (Fig. 5). The equations of the calibration curves were:

(Pyrethrin I) $y = 259.8x + 105.4$; $r^2 = 0.9970$

(Pyrethrin II) $y = 516.1x + 157.6$; $r^2 = 0.9943$

These working curves allowed for the quantification of different pyrethrum extracts (i.e., Fluka, PBK and WPSE 1992). The composition of the tested pyrethrum extracts and the methods used for their quantification are reported in Table 3. This optimized CEC method yielded excellent quantification data (Table 4). The data in Tables 3 and 4 indicate that the amounts of pyrethrin I, pyrethrin II and the total pyrethrin content determined by CEC compare nicely with those determined by the AOAC, PBK and

Table 2
Theoretical plates (N), resolution (R_s) and dead time marker (t_0) for % THF added to the mobile phase

THF (%)		N	R_s	t_0 (min)
0	CII/PII	16 432	1.47	3.03
0	CI/PI	23 752	1.71	
5	CII/PII	20 833	1.47	2.44
5	CI/PI	23 389	1.54	
10	CII/PII	21 588	1.21	2.35
10	CI/PI	22 448	1.17	
15	CII/PII	13 583	0.64	2.71
15	CI/PI	16 415	0.73	
20	CII/PII	23 352	0.76	2.61
20	CI/PI	25 046	0.73	

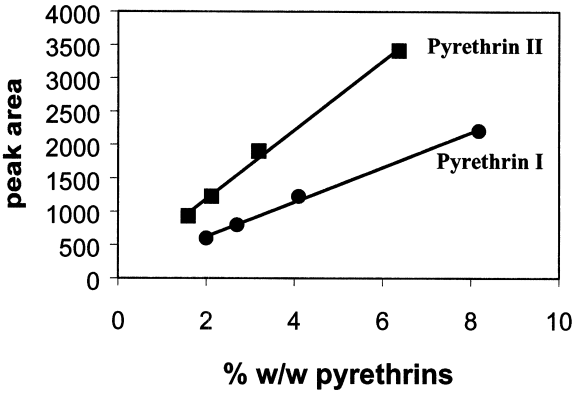


Fig. 5. Calibration curves for the determination of pyrethrin I and II in extract samples. Conditions: MeCN–25 m*M* Tris–THF (55:35:10, pH 9).

Table 3
Composition of the various pyrethrum extracts

Extract (method)[a]	Fluka (HPLC)	W.S.P.E 1992		Pale extract (PBK)
		(PBK)	(AOAC)	
Pyrethrin I (%)	n/a	12.70	10.80	31.42
Pyrethrin II (%)	n/a	8.16	8.62	18.59
Total pyrethrins (%)	Approx. 25%	20.86	19.42	50.01

[a] Method definitions: (1) HPLC = external standard method (assay supplied by manufacturer), (2) PBK: GC quantitation method, (3) AOAC: wet chemical technique.

Table 4
CEC quantification data of the three pyrethrin extracts ($n = 4$)

Extract	Fluka	WSPE 1992	Pale extract
Pyrethrin I	12.6±1.2 (9%)[a]	11.1±1.3 (12%)	24.1±3.9 (16%)
Pyrethrin II	8.6±0.6 (7%)	8.3±1.3 (16%)	19.5±4.3 (22%)
Total pyrethrins	22.9±2.1 (9%)	20.5±2.6 (13%)	45.7±8.1 (18%)

[a] Average±standard deviation (RSD, %).

HPLC methods. The manufacturer reported data concerning WSPE 1992 shows that there is variability of about 8% between the AOAC and PBK methods for determining the amount of pyrethrins. Likewise, the relative standard deviation (RSD) for the CEC determination of the pyrethrins is between 7 and 22%. The accuracy of the CEC determinations is high compared to the other methods used for quantification. The percent difference between the CEC determined values and the manufacturer's reported values for the various extracts are 2–9%.

Both the Pyrethrin Dip and Flea & Tick Mist were chosen as representative commercial samples since each contained different % of pyrethrins and other complex matrix components (see Table 5). Electrochromatograms of the Dip and Mist are shown in Fig. 6. The presence of the pyrethrin ester in these samples was confirmed by matching retention times to the standard extract separation, UV spectra of the peaks, and by spiking pyrethrin standard mix to the samples.

4. Conclusions

Our goal in this work was to show the benefits of using CEC to quickly separate and identify the pyrethrin esters in formulations that contain various concentrations of these insecticidal esters. Using the optimized conditions determined in these experiments, one can completely separate the six pyrethrin esters in under 16 min. Currently, most HPLC methods require gradient elution to achieve pyrethrin separation in about 20 min [44]. Due to the limited

Table 5
Composition of the Pyrethrin Dip and Flea & Tick Mist

Ingredient	Pyrethrin Dip	Flea & Tick Mist
Pyrethrins	0.97%	0.15%
Piperonyl butoxide	3.74%	1.50%
N-Octylbicycloheptene dicarboximide	5.70%	0.50%
Di-*N*-propyl isocinchomeronate	1.94%	N/A
Nylar 2-[1-methyl-2(4-phenoxyphenoxy)ethoxy]pyridine	N/A	0.15%
Inert ingredients	87.65%	97.70%

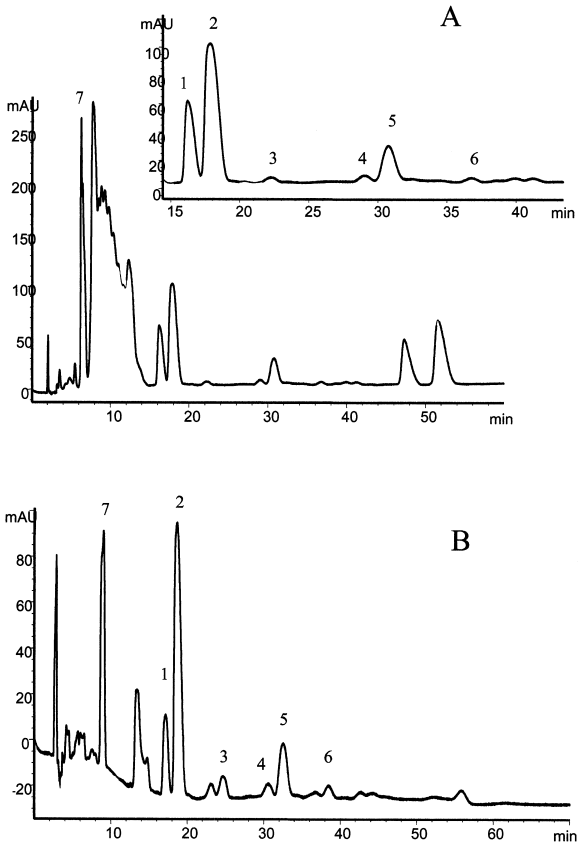


Fig. 6. CEC of (A) Pyrethrin Dip and (B) Flea & Tick Mist. Conditions: MeCN–25 m*M* Tris–THF (55:35:10, pH 9). 1= Cinerin II, 2=pyrethrin II, 3=jasmolin II, 4=cinerin I, 5= pyrethrin I, 6=jasmolin I, 7=piperonyl butoxide.

concentration sensitivity of CEC, samples with low % pyrethrins must first be extracted and possibly concentrated. Depending on the sample matrix and pyrethrin content in these formulations, simple extraction procedures can be used that make the complete determination fast and sensitive.

Acknowledgements

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