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Separation of natural pyrethrum extracts using micellar electrokinetic chromatography

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Abstract

The separation of the six pyrethrin esters in a technical pyrethrum extract (Riedel-de-Haën, Cresent Chemical Co. Inc. Hauppauge, NY, USA) by micellar electrokinetic chromatography (MEKC) using both sodium dodecyl sulfate (SDS) and a polymerized surfactant as pseudo-stationary phases has been investigated and optimized. Parameters such as pH, SDS and polymerized sodium *N*-undecyl sulfate (poly-SUS) concentration, type and concentration of background electrolyte and organic modifier, as well as the acetonitrile/water ratio in the sample were studied to optimize the resolution, efficiency, and analysis time. An optimized separation of the six pyrethrin esters was achieved in 25 min with 25 m*M* Tris, buffered at pH 9, containing 30 m*M* SDS, 25% (v/v) acetonitrile, and an equal volume ratio of acetonitrile/water sample matrix at a voltage of 25 kV. The use of 0.5% (w/v) poly-SUS enhanced resolution of the pyrethrin esters and shortened the total analysis time from 25 to 20 min, compared to the SDS mediated separation. The optimized MEKC results are compared to the HPLC separation of these esters and show an improvement in efficiency and total analysis time.

Keywords: Pyrethrin esters

1. Introduction

Pyrethrum is a natural, botanical insecticide extracted in light petroleum from the flower petals of the plant *Chrysanthemum cinerariaefolium* [1]. The active insecticidal ingredients of pyrethrum are the esters pyrethrin I, II; cinerin I, II; and jasmolin I, II. Fig. 1 shows the structural differences between these two ''classes'' of esters (i.e. *Pyrethrin I*=pyrethrin I, cinerin I, and jasmolin I; *Pyrethrin II*=pyrethrin II, cinerin II, and jasmolin II). The three *Pyrethrin I* compounds have a single ester functionality which links the tricyclic and pentyl ring systems together. They differ from each other in the alkene side group attached to the five member ring. The *Pyrethrin II* compounds have an additional ester moiety attached to the three member ring making these compounds more polar than the *Pyrethrin I* molecules.

These esters have high knockdown (*Pyrethrin II*) and lethal (*Pyrethrin I*) activity toward insects and exhibit low mammalian toxicity and short environmental lifetimes [1]. Pyrethrin liquid, dust, and aerosol formulations are applied as household and pet insecticides, in restaurants and food storage

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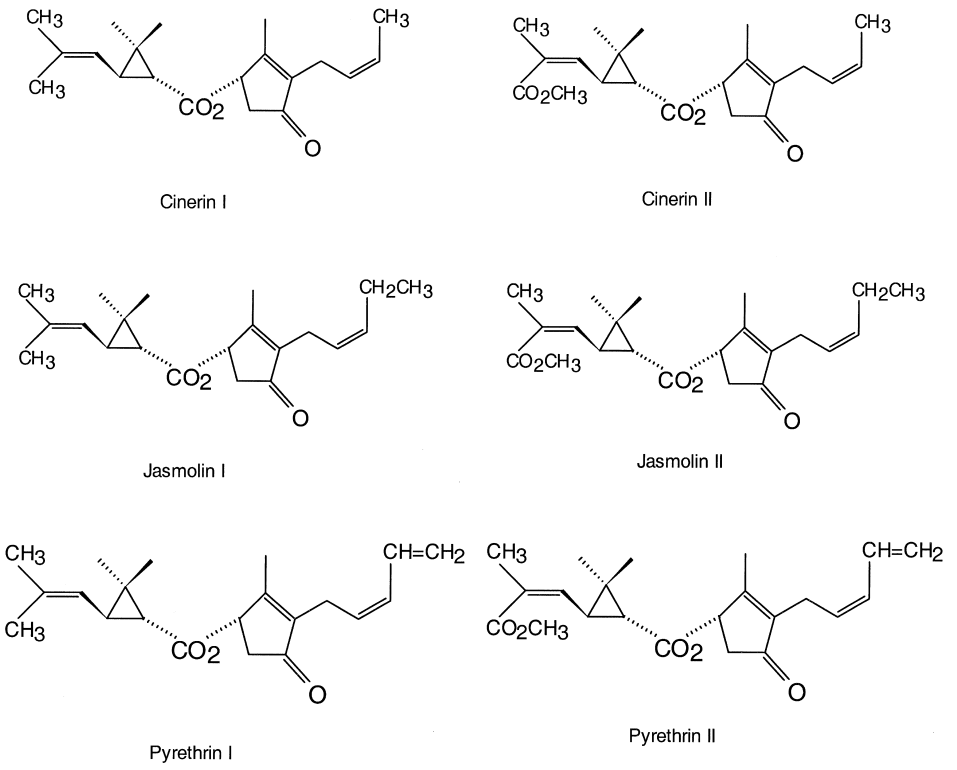

Fig. 1. Structures of the 6 insecticidal esters of natural pyrethrum extracts.

areas, and on crops. In addition, pyrethrin also finds widespread use as an all-natural, organic insecticide for the control of human lice and in mosquito repellent coils [2]. Popular from antiquity to today, pyrethrin is being studied with renewed vigor, specifically due to its natural composition, low mammalian toxicity, and lethal action toward insects.

Separation of the six esters in pyrethrum has been achieved by chemical and thin layer techniques, as well as gas–liquid and liquid chromatographic procedures [2]. Currently, qualitative and quantitative separation of pyrethrum esters is achieved by high-performance liquid chromatography (HPLC). Several HPLC methods exist which can separate the insecticidal esters present in natural pyrethrum samples [3–6]. HPLC is a popular chromatographic technique used in industry due to its capacity for relatively fast, reliable, and reproducible results on a variety of sample matrices. For this reason, pyrethrum producers and chemical manufacturers currently use HPLC as a quality control check.

Another technique that could provide qualitative and quantitative information concerning pyrethrum samples is high-performance capillary electrophoresis (HPCE). HPCE is a powerful separation technique due to its high selectivity, high-resolution, short analysis time, and economy of sample and reagent consumption. To date, there have been no reported attempts to study pyrethrum using capillary electrophoresis (CE), and only a few pyrethroid studies utilizing CE have been reported. These studies have mostly involved capillary isotachophoresis (cITP) which requires cautious control of separation conditions and is otherwise labor intensive [7–9]. Karcher and El Rassi used HPCE to analyze the fluorophore tagged, base hydrolysis products of five popular pyrethroid compounds [10]. Sevcík et al. used micellar electrokinetic chromatography (MEKC) with high SDS and (γ-Cyclodextrin concentrations to achieve partial separation of some pyrethroid stereoisomers [11]. Currently, MEKC is one of the modes of HPCE that effectively separates neutral analytes. The natural pyrethrum esters are non-racemic neutral compounds (see Fig. 1) and

therefore require the MEKC mode of HPCE to achieve separation.

In this work, we wanted to compare two pseudo-stationary phases: sodium dodecyl sulfate (SDS) and polymeric sodium *N*-undecyl sulfate (poly-SUS). SDS is a well-known anionic surfactant used as a pseudo-stationary phase in MEKC. At concentrations above the critical micelle concentration (CMC), SDS forms micelles which provide a pseudo-stationary phase that can effectively separate neutral compounds based on partitioning between the aqueous mobile phase and the hydrophobic interior of the SDS micelles. Recently, use of micelle polymers in MEKC separations of neutral compounds has gained much interest [12,13]. These covalently stabilized polymeric surfactants share common properties with their unpolymerized counterparts: similar solubilization and conformation in an aqueous environment, as well as surface active properties [12]. However, polymeric surfactants have the added benefit of having a zero CMC which means that a greater range of concentration can be explored compared to the monomer. In addition, organic solvents have little effect on the structure of the polymeric surfactant.

Using SDS and poly-SUS as pseudo-stationary phases, we report the optimum MEKC conditions for the separation of the pyrethrum esters. The MEKC results are compared with traditional HPLC separations of these esters. In reporting this work, we examine the value of HPCE as a separation tool for the analysis of industrially produced chemicals as well as the potential use of achiral polymeric surfactants in the MEKC separation of pesticides.

2. Conditions

2.1. HPLC materials

The natural pyrethrum sample (Riedel-de-Haën–''Pestanal'') used as a standard for initial optimization was purchased from Cresent Chemical Co. Inc. (Hauppauge, NY, USA). This sample was stored in its original tightly capped vials at 4°C in the dark. Sub-samples were obtained by making various mg/ml (w/v) concentrations of pyrethrum samples in HPLC grade dichloromethane (DCM) purchased from Mallinckrodt & Baker Inc. (Paris, KY, USA). The solvent system was composed of equal volumes of water saturated DCM and anhydrous DCM. The water saturated DCM solution was made by mixing 1 part 18 MΩ distilled water and 4 parts anhydrous HPLC grade DCM. The aqueous layer was decanted, and the remaining water saturated DCM was mixed with an equal part of anhydrous DCM.

2.2. HPLC instrumentation

The HPLC separation of pyrethrum samples was performed on a Bio-Rad Model 2700 Solvent Delivery System connected to a Bio-Rad Model AS-100T HRLC Automatic Sampling System, a Bio-Rad Bio-Dimension UV–Vis Monitor detector, and a BioRad Model 2110 Fraction Collector. The system was controlled by a Bio-Rad HRLC System Interface and ValueChrom HPLC System software. Two Waters μPorasil 125Å 10 μm 3.9×300 mm normal-phase HPLC columns were connected in series to afford optimum separation.

2.3. HPLC method

The HPLC separation of pyrethrum samples was obtained following the method of Otieno et al. with slight modifications to the sample composition and injection size [4]. For optimum resolution, 10 μl of 100 mg/ml pyrethrum in DCM sub-samples were auto-injected into the system. For collection of the HPLC fractions of a pyrethrum sample, 50–200 μl of 100 mg/ml pyrethrum in DCM were injected at least ten times and the peak fractions collected. The solvent was evaporated to approximately 1 ml using nitrogen and the fractions were then analyzed by fast atom bombardment mass spectrometry (FAB–MS) for mass identification of the fractions of the pyrethrum samples.

2.4. MEKC materials

The buffers tested as background electrolytes (BGEs) for MEKC were: sodium tetraborate decahydrate ($Na_2B_4O_7 \times 10\ H_2O$), sodium phosphate dibasic (Na_2HPO_4), and (Tris[hydroxymethyl]-aminoethane: ''Tris'') and all were purchased from Sigma (St. Louis, MO, USA). The acetonitrile and methanol were purchased from Burdick & Jackson

(Muskegon, MI, USA), and isopropyl alcohol was purchased from Mallinckrodt & Baker Inc. (Paris, KY, USA). All organic modifiers were HPLC grade. Sodium dodecyl sulfate (SDS) was obtained from Mallinckrodt & Baker Inc. (Paris, KY, USA). All aqueous solutions were made using 18 MΩ distilled water and the pH was adjusted with 0.1 *M* HCl or 0.1 *M* NaOH solutions. The polymerized surfactant poly-sodium *N*-undecyl sulfate (poly-SUS) used in this study was synthesized using a procedure reported earlier [13].

2.5. MEKC instrumentation

All micellar electrokinetic chromatography experiments were performed on a Beckman (Fullerton, CA, USA) P/ACE Model 5510 capillary electrophoresis (CE) instrument. This instrument contains a 0–30 kV high voltage, built-in power supply; wavelength filters for 200, 214, 254, and 280 nm UV detection; and System Gold software for system control and data handling. Separations were performed using bare fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D. and total lengths of 64 cm (60 cm to detector window). The outer polyimide coating was removed from both ends of the capillary in response to the observation that prolonged exposure to acetonitrile damages the coating on the capillary. The capillary was thermostated to room temperature by a fluoroorganic fluid.

2.6. MEKC method

New capillaries were placed into the instrument and flushed for 4 hours with 1 *M* NaOH. At the beginning and end of experiments, the capillary was flushed with 0.1 *M* NaOH for 15 min and 18 MΩ distilled water for 15 min. Before each injection, the capillary was flushed with 0.1 *M* NaOH for 3 min and with the running MEKC buffer for 5 min.

3. Results and discussion

3.1. HPLC separation, and FAB/MS identification of pyrethrum

Fig. 2 shows the HPLC separation of a Riedel-de-Haën pyrethrum sample (approx. 49% w/v total pyrethrins). In the chromatogram, the six major peaks correspond to the six insecticidal esters in the two classes: *Pyrethrin I* and *Pyrethrin II* found in these extracts. The *Pyrethrin II* class contains a second ester functionality and is therefore more polar than the *Pyrethrin I* class esters (see Fig. 1). As expected, the use of a low polarity solvent (DCM) in a normal-phase column resulted in the elution of the least polar compounds first (i.e. *Pyrethrin I* class esters). Each peak was fraction collected, mass analyzed and spiked into a typical pyrethrum sample to determine the identity of each. Table 1 shows the mass spectrometry data used to match peaks with each pyrethrin ester. Because of the trace amounts of Jasmolin I and II in typical pyrethrum samples, FAB/MS was not able to detect these esters. Therefore, the peaks representing Jasmolin I and II were assigned based on polarity considerations and by comparison to chromatograms in the literature [4]. This method yielded excellent separation at low concentrations of pyrethrin esters in under 50 min. The benefits of such a method are obvious: (1) identification of the presence of the pyrethrin esters, (2) collection of individual esters (semi-preparative HPLC), and (3) quantification of the esters in extracts or other matrices.

3.2. Optimization of the MEKC separation of pyrethrum samples

We predict the elution order of the pyrethrin esters using MEKC to be reversed compared to the normal-phase HPLC separation because the aqueous solvent used in MEKC is more polar than the SDS micelles. Based on a review of the literature, the use of approximately 30 m*M* surfactant (CMC of SDS=8 m*M*), a pH of 9.0, and 10 m*M* Borate buffer were reasonable conditions to first apply toward separation of the commercially available pyrethrum sample [14]. However, under these conditions, all of the pyrethrum esters elute simultaneously. Therefore, to optimize the separation of these six esters, a systematic investigation of the organic modifier type and concentration, pH, type and concentration of BGE, as well as SDS concentration was performed. The HPLC fractions (the six pyrethrin esters) were individually spiked into extract samples and sepa-

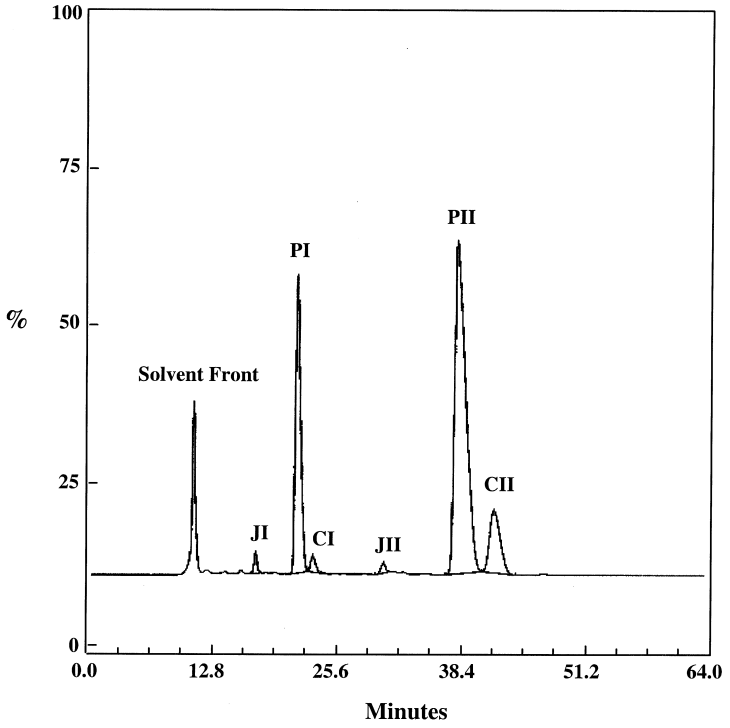

Fig. 2. HPLC chromatogram of the separation of Riedel-de-Haën pyrethrum standard. Separation conditions: 0.6 ml/min flow-rate, dichloromethane solvent system, two Waters μPorasil columns in series, 10 μl injection of 100 mg/ml Riedel-de-Haën pyrethrum standard in DCM, UV detection at 254 nm.

rated by MEKC to identify which peaks represented each of the six pyrethrin esters.

3.2.1. Type and concentration of organic modifier

Since neutral solutes such as the pyrethrin esters are hydrophobic and structurally similar, it is logical to add an appropriate volume fraction of organic solvent to the aqueous buffer system. The use of organic solvent increases the affinity of the pyrethrin esters for the aqueous mobile phase, improves their selectivity, and decreases their partitioning into the pseudo-stationary phase (SDS micelles). From this point of view, three organic modifiers: acetonitrile (ACN), methanol (MeOH), and isopropanol (IPA) were added to the solution containing SDS micelles to alter the solvent strength of the aqueous phase [15].

Table 1
FAB–MS data for the pyrethrins in Riedel-de-Haën pyrethrum extract

Compound	Peak number	Parent ion (m/z)	
		(This work)	(Ref. [13])
Jasmolin I	6	–	330.4
Pyrethrin I	5	328.1	328.4
Cinerin I	4	315.9	316.4
Jasmolin II	3	–	374.4
Pyrethrin II	2	372.2	372.4
Cinerin II	1	360.4	360.4

The electroosmotic mobility (μ_{eo}) inside the fused-silica capillary decreased almost linearly with increasing amount of ACN, MeOH and IPA added to the BGE. Seifar et al. also reported a linear decrease in EOF with increasing ACN and concluded that it was due to the zeta potential (ζ) since the ratio of dielectric constant to solution viscosity (ϵ/η) remains fairly constant in the Cunningham equation, $[\mu_{eo}=(\epsilon\epsilon_o/\eta)\zeta]$, where $\epsilon\epsilon_o$ is the permittivity of free space [15–17]. Note that at equivalent volume fractions of organic solvent added to the BGE, the μ_{eo} increases in the following order: IPA<MeOH< ACN.

When ACN was used, the relative migration times (t_R/t_O) of the pyrethrins first increased to a maximum at 20% ACN and then decreased from 20–40% ACN (Fig. 3a) even though the t_O value continually increased. In all electropherograms, the t_O was determined by a first solvent disturbance (usually a negative peak), indicative of a refractive index change. The initial increase in migration times of the pyrethrum esters observed at low ACN volume fractions could be attributed to a decrease in the zeta potential at the capillary walls [16]. However, the high ACN concentration solvates the hydrophobic

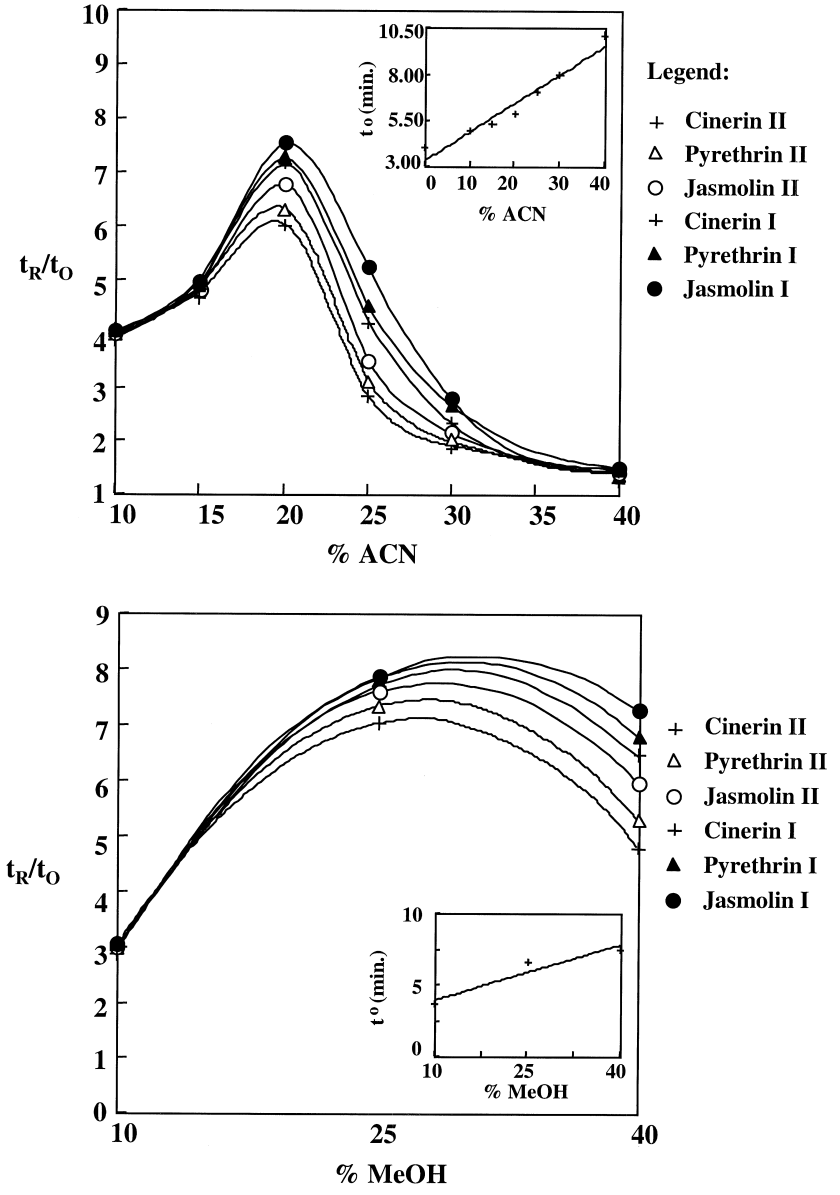

Fig. 3. Relative migration time (t_R/t_O) versus % v/v (volume of organic modifier/total volume) organic modifier for the solvents: (a) ACN, (b) MeOH, (c) IPA. Inset plot is t_O versus % (v/v) organic modifier. Separation conditions: 30 m*M* SDS, 10 m*M* borate, pH 9, 1 s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

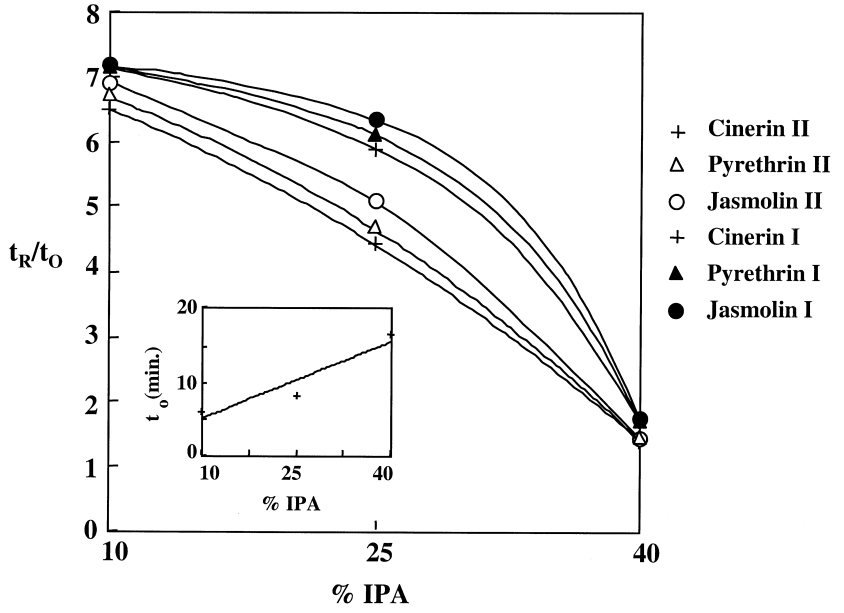

Fig. 3. (*continued*)

pyrethrum esters and reduces their interaction with the micelle. The same trend was observed for MeOH (Fig. 3b) and is consistent with the results of other investigations [18]. Conversely, IPA caused relative migration times of the pyrethrins to decrease with increasing amount of organic modifier (Fig. 3c).

The electropherograms of the pyrethrin separations show that when MeOH was used (Fig. 4b), the more polar *Pyrethrin II* esters exhibited improved peak resolution. Conversely, the *Pyrethrin I* esters were poorly resolved using MeOH compared to that achieved using ACN (Fig. 4a). The same trend was observed when IPA was used (Fig. 4c). These organic modifiers (MeOH and IPA) were less effective (both resulted in longer migration time and lower resolution) than ACN. A comparison of the electropherograms using each of the aforementioned organic modifiers in Fig. 4 shows that ACN is the best choice of organic solvent for separation of the pyrethrin esters.

3.2.2. pH

A 5 m*M* $Na_2B_4O_7$/5 m*M* Na_2HPO_4 buffer was used so that the range of pH between 7–10 could be studied. The running buffer solution contained 30 m*M* SDS and 25% ACN. Generally, at constant ionic strength, increasing the pH results in an increase in EOF (i.e. decrease in t_O) and therefore shorter migration times of the analytes [14]. However, our pH study, which was not conducted at constant ionic strength, showed an increase in t_O and t_R/t_O from pH 7 to 8, a decrease from pH 8 to 9, and an increase again from pH 9 to 10 (Fig. 5). These trends in t_R/t_O are more pronounced for the less polar *Pyrethrin I* esters. Resolution is slightly enhanced at higher pH but at the expense of much higher t_R/t_O values. The best combination of resolution and relative migration time occurs at pH 9.

3.2.3. Type and concentration of background electrolyte

Borate, borate/phosphate, and Tris buffer were compared as BGEs under similar conditions to determine which resulted in the best separation of the six neutral esters. First, 100 m*M* Tris was compared to the 10 m*M* borate and 5 m*M* borate/5 m*M* phosphate buffers. Fig. 6 shows the separation of a Riedel-de-Haën pyrethrum sample using these three buffer systems. The borate/phosphate buffer yielded excellent separation and resolution of the first three peaks (i.e. *Pyrethrin II*), but did not separate *Pyre-*

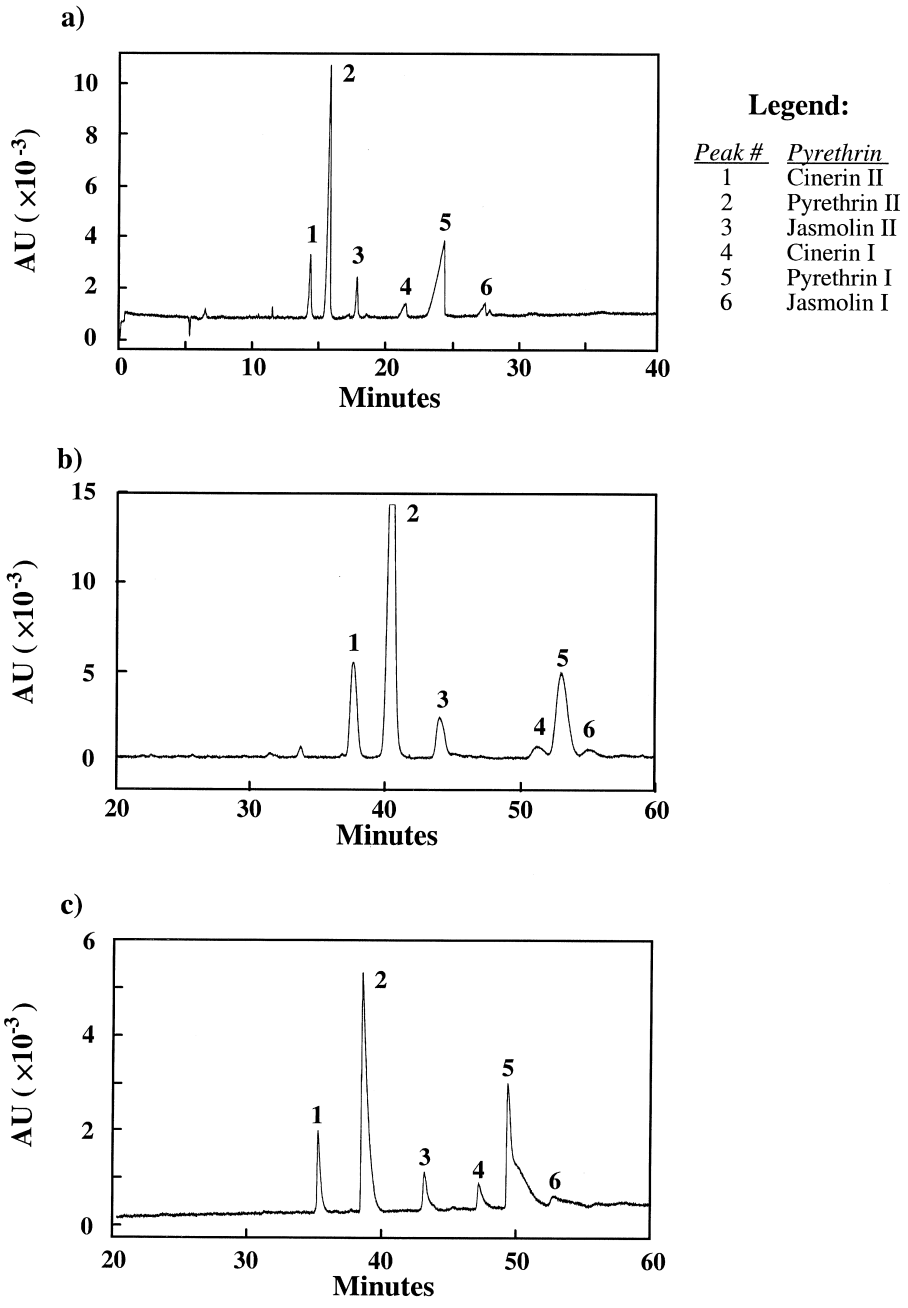

Fig. 4. Separation of Riedel-de-Haën pyrethrum standard using the optimized fraction % (v/v) of organic solvents: (a) 25% ACN, (b) 30% MeOH, (c) 10% IPA. Separation conditions: 30 m*M* SDS, 10 m*M* borate, pH 9, 1 s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

thrin I well. Comparison of the efficiencies of each buffer system indicated that Tris had the highest overall efficiencies (Table 2). The borate/phosphate buffer yielded a higher efficiency for Cinerin I and the borate buffer had a higher efficiency for Jasmolin II. Because of Tris buffer's low conductivity, all of

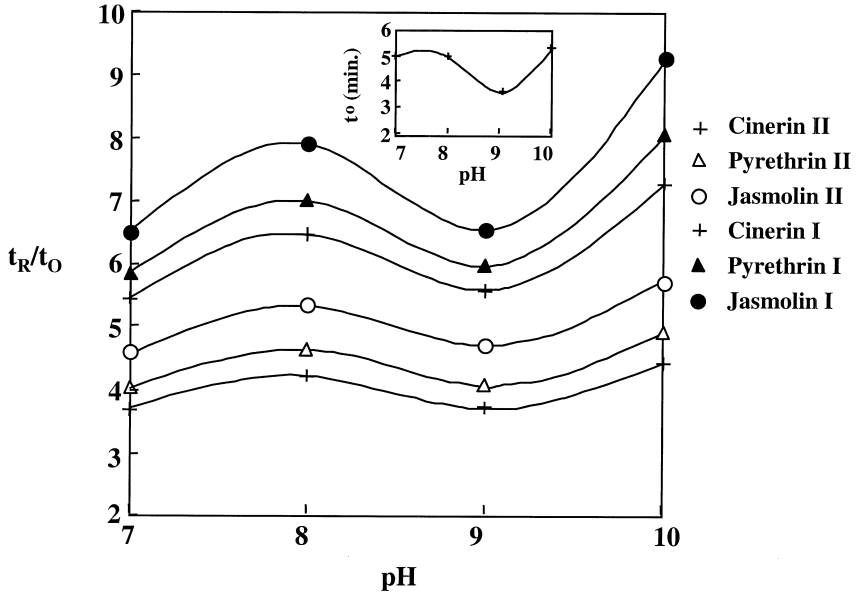

Fig. 5. Relative migration time versus pH. Separation conditions: 5 m*M* borate/5 m*M* phosphate, 30 m*M* SDS, 25% (v/v) ACN, 1 s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

the pyrethrin esters were separated in the shortest time with high resolution.

Resolution was also greatly enhanced by optimizing the concentration of Tris buffer. Fig. 7 shows the t_R/t_O values of the pyrethrin esters as a function of Tris concentration. The increased resolution of the esters at high concentrations of Tris (above 100 m*M*) buffer was obtained at the expense of very long migration times (ca. 72 min for Jasmolin I). Note that high concentrations (up to 100 m*M*) of Tris can be conveniently used because its low conductivity does not contribute to joule heating. Moreover, the high Tris concentration likely improved resolution by minimizing interactions between the pyrethrin esters and the capillary walls, thus suppressing band-broadening [14]. The best compromise between resolution and migration time occurred at 25 m*M* Tris.

3.2.4. SDS concentration

As the micelle concentration was increased, the probability of the analyte partitioning into the micelle increased, causing both longer analyte migration time and increased resolution. Therefore, the SDS concentration was varied between 10–40 m*M* and plotted against t_R/t_O values (Fig. 8). Between 10–25 m*M* SDS, t_O remained constant within experimental error. The sharp increase of t_O from 25–35 m*M* SDS is most likely due to viscosity changes at these higher levels of SDS. Fig. 8 illustrates the enhanced resolution at higher SDS concentrations of 35 and 40 m*M*; however, the latter peaks (*Pyrethrin I*) did not elute within a reasonable time period (200 min). The best compromise between resolution and analysis time was achieved at the originally chosen SDS concentration of 30 m*M*.

3.2.5. Voltage

Varying the applied voltage from 5–25 kV showed that the best separation occurred at the highest attainable applied voltage. The electroosmotic flow and electrophoretic velocity are both directly proportional to the field strength causing the shortest analysis time at the highest applied voltage [19]. The resulting current, due to the applied voltage, is linear throughout the range (5–25 kV) tested. Therefore, there is no voltage-induced joule heating or temperature gradient in this range.

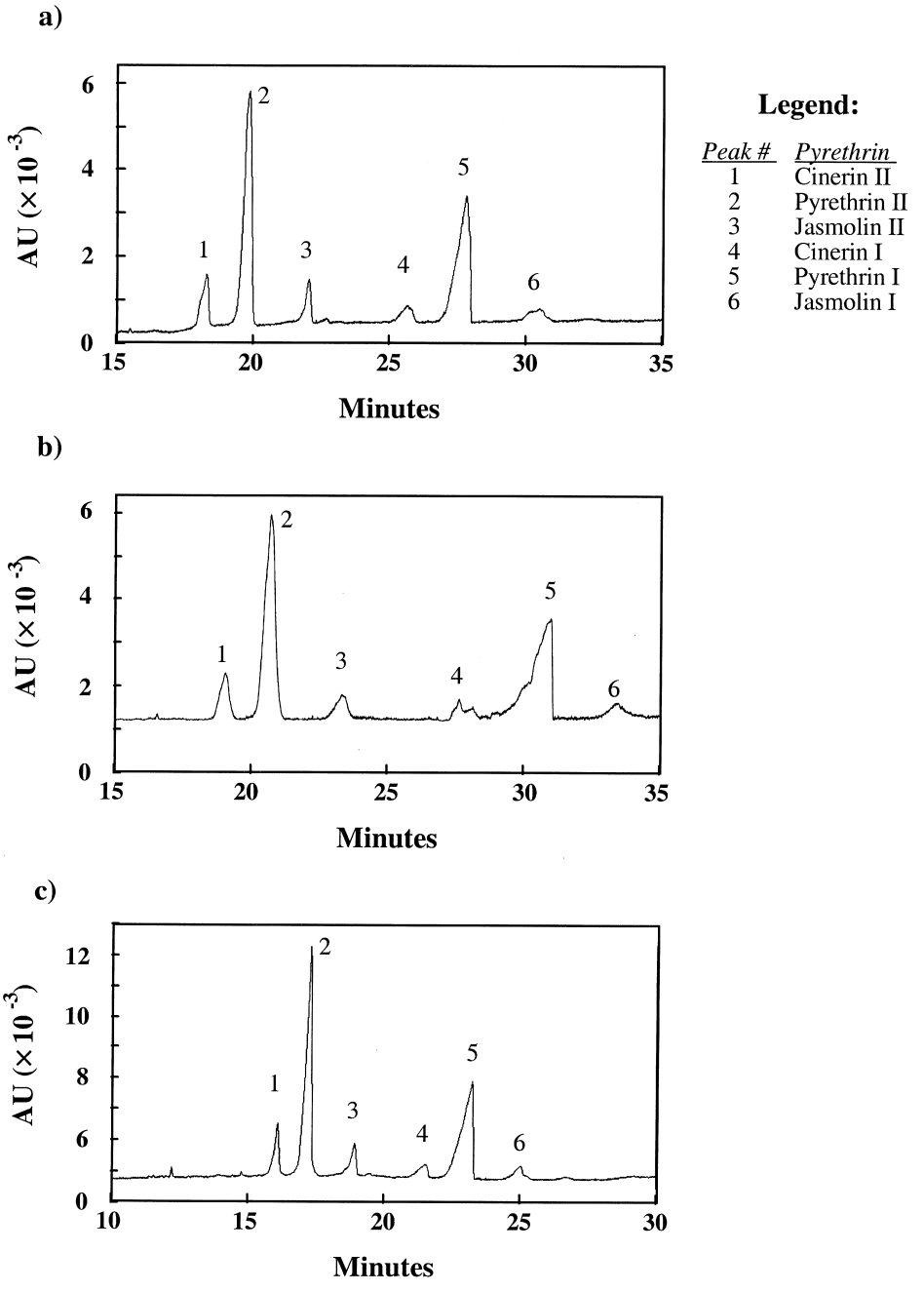

Fig. 6. Optimum electropherograms of the separation of Riedel-de-Haën pyrethrum standard using three buffered systems: (a) 5 m*M* Borate/5 m*M* phosphate, (b) 10 m*M* BORATE, (c) 100 m*M* Tris. Separation conditions: 30 m*M* SDS, pH 9, 25% (v/v) ACN, 1 s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

Table 2
Efficiencies of the pyrethrins in Riedel-de-Haën pyrethrum extract using three different buffer systems

Compound	Peak number	*N* Tris	*N* Borate/phosphate	*N* Borate
Jasmolin I	6	28 000	21 000	12 000
Pyrethrin I	5	27 000	19 000	12 000
Cinerin I	4	27 000	37 000	13 000
Jasmolin II	3	15 000	14 000	18 000
Pyrethrin II	2	14 000	16 000	8300
Cinerin II	1	19 000	11 000	6400

3.3. MEKC separation of pyrethrum sample using poly-SUS

Polymerized sodium *N*-undecyl sulfate (poly-SUS) was used in place of SDS micelles to compare the separation of pyrethrum esters. The optimized separation conditions were used to determine if the use of poly-SUS could achieve similar or enhanced separation of the pyrethrins. Fig. 9 shows the series of MEKC electropherograms for the separation of the Riedel-de-Haën pyrethrum sample using various percent (w/v) of poly-SUS. At 0.25% (w/v) poly-SUS, all the esters were sufficiently separated in under 20 min compared to the approximately 20–35 min needed to achieve comparable separation efficiency and resolution using SDS. Peak splitting was observed in all pyrethrin separations using the polymerized surfactant. The observed peak splitting could have been caused by a mismatch between the injected sample (i.e. 100% ACN) and the running buffer (25% ACN/75% aqueous buffer). This prompted us to perform a sample composition study. Fig. 10 shows that an equal volume ACN/water sample caused peak splitting to disappear. The buffer

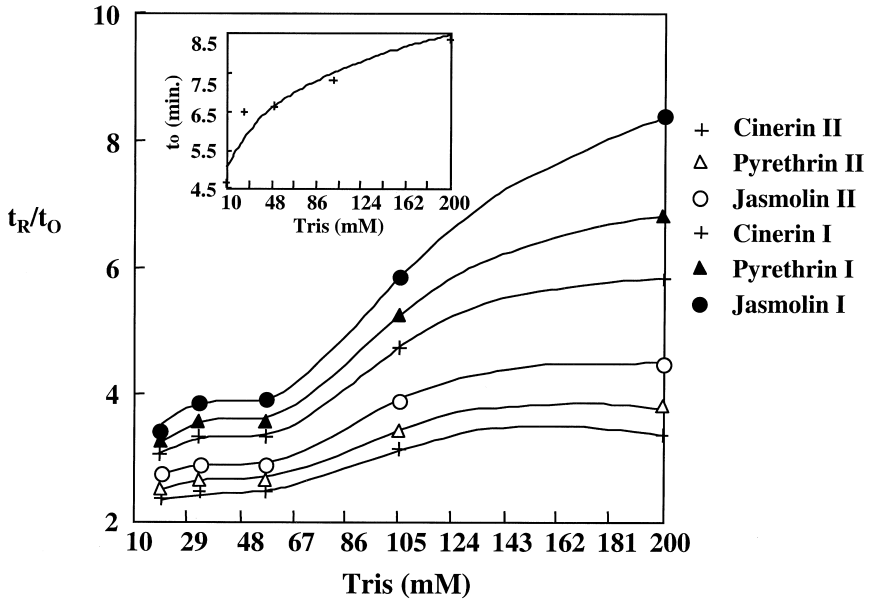

Fig. 7. Relative migration time versus Tris buffer concentration. Separation conditions: 30 m*M* SDS, pH 9, 25% (v/v) ACN, 1s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

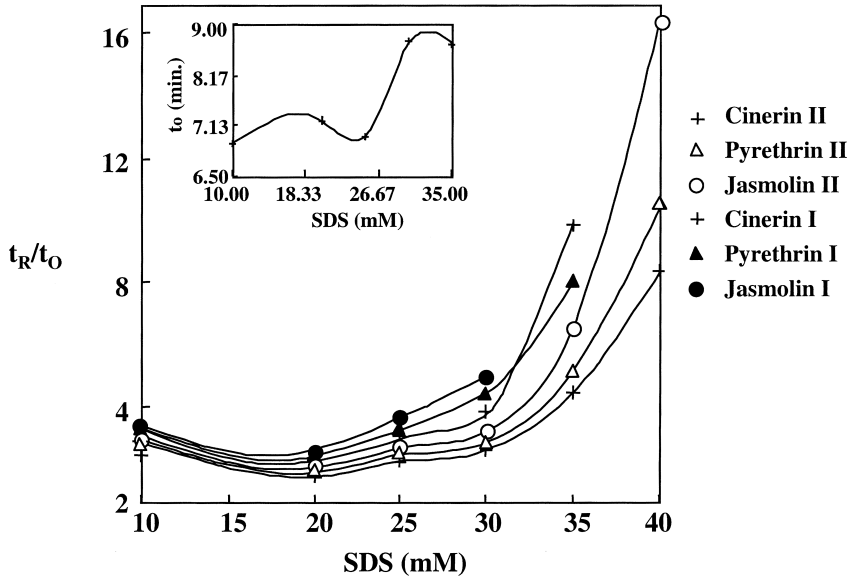

Fig. 8. Relative migration time versus SDS concentration. Separation conditions: 25 m*M* Tris, pH 9, 25% (v/v) ACN, 1s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

type and concentration, as well as pH, were varied in conjunction with 0.25% (w/v) polymerized SUS but yielded no better separation than the optimized conditions (25 m*M* Tris, pH=9).

3.4. Reproducibility and detection limits

In our study, the reproducibility of peak areas and retention times were determined experimentally by comparison to an internal standard (Table 3). Low relative standard deviations (RSD) of the peak areas between 0.9%–3.5% were obtained, indicating excellent run to run reproducibility. Likewise the retention time reproducibility (RSD) of 0.9%–2.4% is excellent and therefore can be used for identification of each pyrethrin on a run to run basis. The detection limits of the six esters ranged from 1 to 14 ppm and, although higher than some HPLC techniques, provide reasonably low detection limits for routine industrial analysis of concentrated extracts.

4. Concluding remarks

The results of our studies show that MEKC is a viable technique for the separation of the pyrethin esters of typical pyrethrum extracts. Our systematic optimization revealed a distinct set of conditions (30

Table 3
Reproducibility of peak areas and retention times and the detection limits of the pyrethrins in Riedel-de-Haën pyrethrum extract

Compound	Peak number	Peak area % RSD	Retention time % RSD	Detection limit ppm
Jasmolin I	6	1.8	0.9	1.4
Pyrethrin I	5	3.1	1.0	14.1
Cinerin I	4	1.4	1.4	2.3
Jasmolin II	3	0.9	1.9	1.1
Pyrethrin II	2	2.9	2.2	10.6
Cinerin II	1	3.5	2.4	2.4

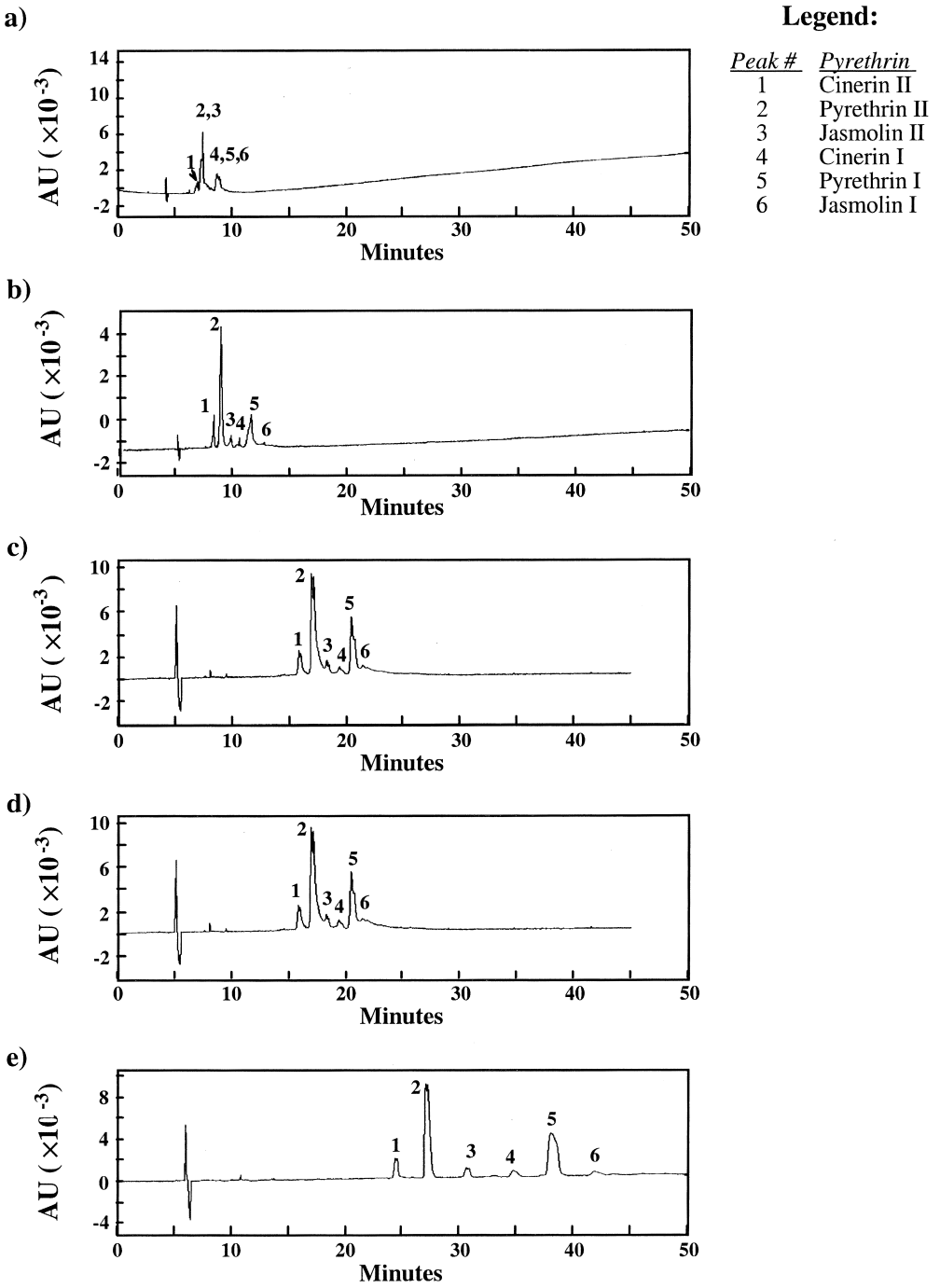

Fig. 9. Electropherograms of Riedel-de-Haën pyrethrum standard at various % (w/v) of poly-SUS: (a) 0.10%, (b) 0.25%, (c) 0.50%, (d) 0.80%, (e) 1.00%. Separation conditions: 25 m*M* Tris, pH 9, 25% (v/v) ACN, 1 s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

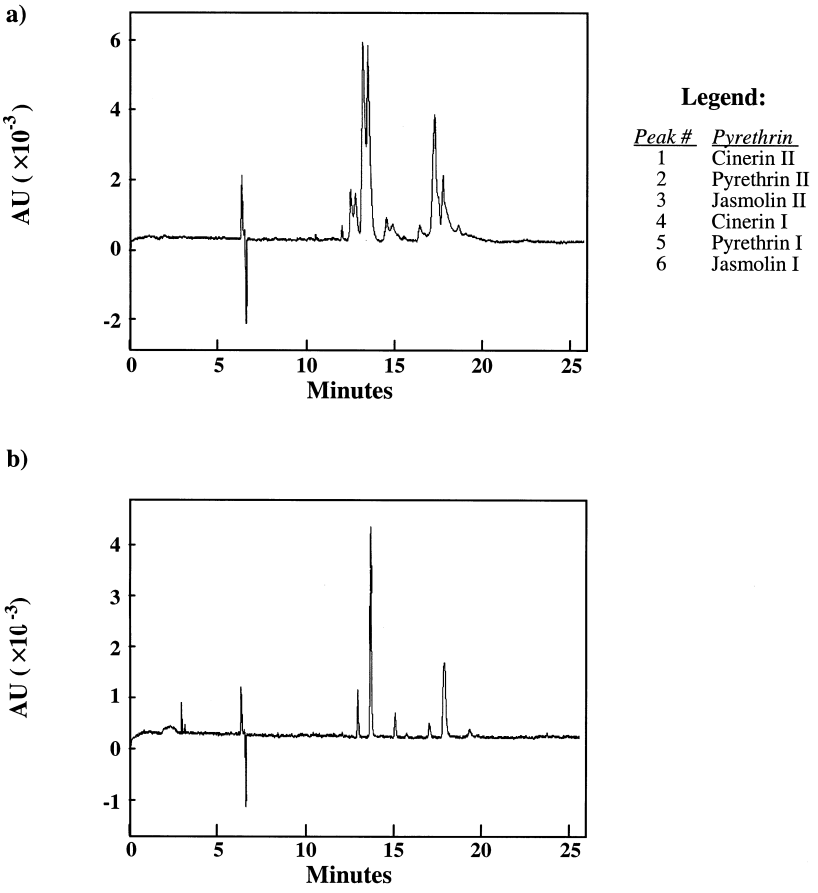

Fig. 10. Electropherograms of Riedel-de-Haën pyrethrum standard separation using different sample composition: (a) 100% (v/v) ACN, (b) 50% (v/v) ACN/(v/v) 50% water. Separation conditions: 2.0% (w/v) poly-SUS, 25 m*M* Tris, pH 9, 25% ACN, 1 s pressure injection, 25 kV applied for separation, UV detection at 254 nm.

m*M* SDS, pH 9, 25% (v/v) ACN, 25 m*M* Tris, 25 kV voltage and an equal volume ACN/water sample) that yielded excellent separation of these esters in a shorter time (ca. 25 min) than that achieved by HPLC (ca. 50 min). The analysis time of the pyrethrins was further reduced using 0.25% (w/v) poly-SUS (ca. 20 min). The ease of method development in MEKC combined with the inherent advantages of CE (small sample and solvent requirements, excellent peak area and retention time reproducibility, and modestly low detection limits) make it a potentially excellent quality and quantity control method for industrial application.

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