National Center for Natural Products Research[1], Research Institute of Pharmaceutical Sciences, and Department of Pharmacognosy[2], School of Pharmacy, The University of Mississippi, USA

Analysis of sesquiterpenes in *Valeriana officinalis* by capillary electrophoresis

J. R. MIKELL[1], M. GANZERA[1] and I. A. KHAN[1, 2]

A capillary electrophoresis (CE) method permitting the determination of the main sesquiterpenes in *Valeriana officinalis* has been developed. A separation of valerenic acid and its hydroxy and acetoxy derivatives, three compounds characteristic for the species, was achieved using a 40 mM phosphate-borate buffer at pH 8.5, which contained 10% isopropanol as organic modifier. Applied temperature and voltage were 35 °C and 17.5 kV, respectively. This setup allowed a baseline separation of the three compounds within 8 min, with a detection limit of 5.8 μg/ml or less. Out of six market products analyzed, only one contained a detectable amount of the marker compounds, with 0.54% of hydroxyvalerenic acid and 0.13% valerenic acid, respectively. The quantitative results were comparable to those obtained by HPLC.

1. Introduction

Valerian (*Valeriana officinalis,* Valerianaceae) is a perennial herb native to Europe and Asia, whose roots and rhizomes have been used as a sedative for many centuries [1]. According to the German Commission E, Valerian is indicated as an anti-anxiety agent for the treatment of restlessness and sleep disturbances resulting from nervous conditions [2]. The pharmacological properties of Valerian cannot be fully explained yet, but studies indicate that a combination of several compound classes, some yet to be identified, is responsible for its effects. Besides amino acids, activities have been documented for valepotriates (which are bicyclic iridoids) and sesquiterpenes (valerenic acid (**3**), hydroxyvalerenic acid (**2**), and acetoxyvalerenic acid (**1**)) [3, 4]. Valerian sesquiterpenes inhibit the degradation of γ-aminobutyric acid, a CNS neurotransmitter, thus explaining the spasmolytic and muscle-relaxant effects of the plant extract in animal experiments [5].
Valepotriates have been isolated from different *Valeriana* species (e.g. *V. edulis, V. javanica* or *V. wallichii*), whereas the above-mentioned sesquiterpenes are characteristic for the officinally used species [6–8]. Therefore these compounds are used as markers for identification purposes and quality control, and several analytical methods for their determination by TLC, HPLC and GC are reported in literature [9–11]. In continuation of our efforts to introduce CE as an alternative to established techniques in the analysis of natural products, we developed a CE method suitable for the qualitative and quantitative determination of sesquiterpenes in *V. officinalis*.

2. Investigations and results

2.1. Optimization of CE parameters

The electropherogram of a model mixture of **1**–**3** is shown in Fig. 1, and in order to optimize the separation, the influence of several parameters on the resolution (R_s) of adjacent peaks was studied. Most important for a satisfactory result were the pH of the running electrolyte and the addition of a modifier to the buffer. Other parameters (applied voltage, temperature and buffer concentration) had only minor effects on the separation. Preliminary experiments showed that a phosphate-borate buffer is most suitable for a separation of the sesquiterpenes. The effect of different pH-values of the electrolyte (from pH 6.5 to 8.5) on the resolution of **1**–**3** is shown in Fig. 2. Critical was the separation of substance pair **1** and **2**, whereas compounds **2** and **3** were resolved with R_s-values higher than 1.7 at all tested pH-values. As the pH of the buffer was increased, the R_s-values of **1–2** increased as well, with a maximum reached at pH 8.5. Higher pH-values are not possible with this buffer system, and if borate or borate/NaOH buffers were used instead (to obtain a more basic pH), a satisfactory separation was no longer possible. Another important parameter was the right choice of organic modifier (Fig. 3). Because of solubility problems, a separation by just using an aqueous buffer was not possible. The addition of methanol, acetonitrile or isopropanol improved the separation significantly, with 15% of isopropanol in the buffer giving the best results. However, this high concentration of organic modifier de-

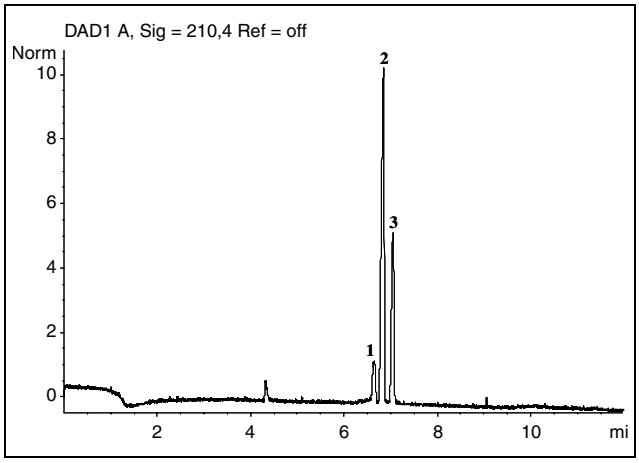
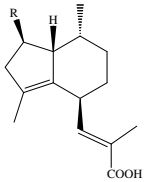


Fig. 1: Electropherogram of a sesquiterpene standard mixture separated under optimized conditions: acetoxyvalerenic acid (**1**), hydroxyvalerenic acid (**2**) and valerenic acid (**3**)



	R
Acetoxyvalerenic acid (**1**)	OAc
Hydroxyvalerenic acid (**2**)	OH
Valeric acid (**3**)	H

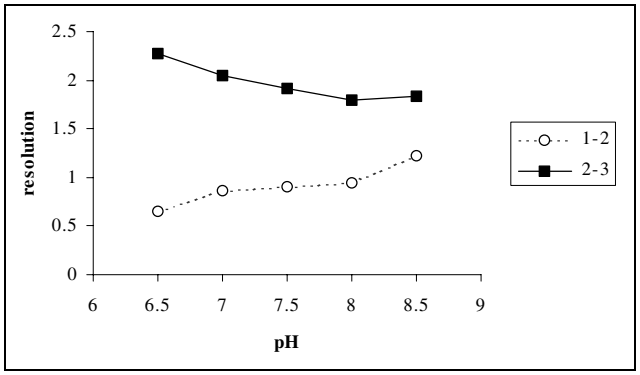


Fig. 2: Effect of the pH-value on the separation of **1–3**; other conditions as for Fig. 1

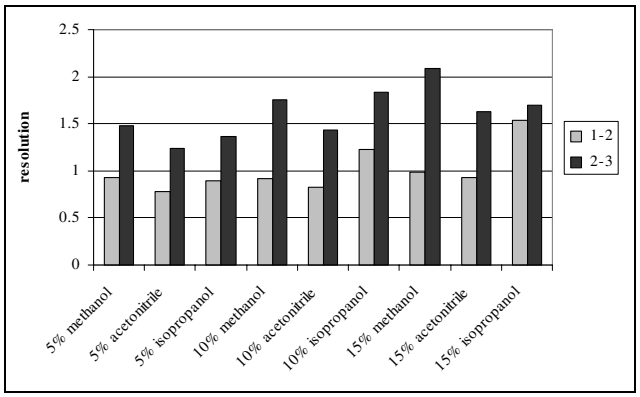


Fig. 3: Effect of organic modifiers on the separation of **1–3**; other conditions as for Fig. 1

creased the over-all reproducibility. Thus the addition of only 10% isopropanol was preferred, still resulting in a sufficient resolution between **1** and **2** ($R_s = 1.2$). The other parameters had only a minor influence on the separation, and because of migration times and peak shape an applied voltage of 17.5 kV, a temperature of 35 °C, and a buffer molarity of 40 mM sodium dihydrogenphosphate/40 mM sodium tetraborate were used.

2.2. Quantitative analysis of Valerian samples

The practical applicability of the CE method was verified by analyzing different Valerian samples (authenticated plant material and six market products). Calibration data for **1**–**3** is combined in Table 1, and indicates the linearity of the detector signal in the concentration range tested (12.5 to 100.0 μg/ml), as well as a detection limit of 5.8 μg/ml (**1**) or less. The CE separation of a *Valeriana officinalis* root extract under optimized conditions is shown in Fig. 4. Compounds **1** and **3** were assigned by comparison of the migration times and the appropriate UV spectra, as well as by spiking the sample with the standard compounds. Marker compound **2** was not detectable in the sample. Finally, different market products purchased in the USA were analyzed (see Table 2 for results). Out of 6 commercial products (tablets, capsules and teabags) analyzed, sesquiterpenes were detectable only in one (sample NPC-VO-2), with 0.54% of **1** and 0.13% of **3**, respectively. In a previous study the same products were analyzed by HPLC for their content of valerenic acid (**3**). The results were comparable, as sample NPC-VO-2 was found to contain 0.11% of **3** in the HPLC study.

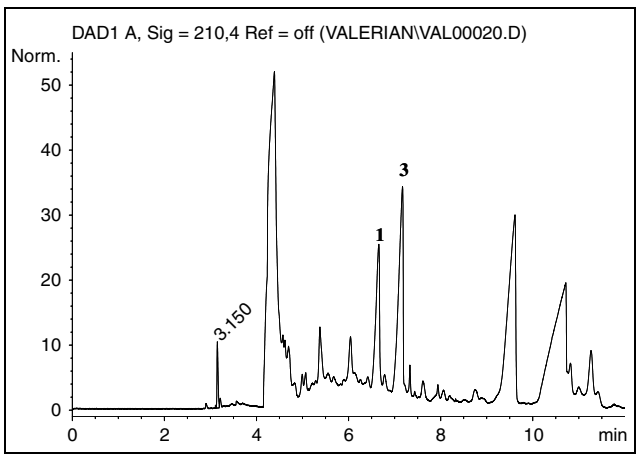


Fig. 4: CE separation of a *Valeriana officinalis* extract; conditions and peak assignment as for Fig. 1

Table 1: Calibration data for compounds 1–3

Compd.	Regression equation	R^2	LOD (μg/ml)
1	$y = 11.38\ x - 111.54$	0.997	5.8
2	$y = 39.60\ x - 3801.89$	0.996	3.4
3	$y = 48.41\ x - 1325.28$	0.997	2.2

Regression equation (y represents the peak area; x the amount in μg/ml), correlation coefficient (R^2) and limit of detection (LOD)

Table 2: Percentage (g/100 g) of sesquiterpenoids 1–3 found in different valerian market products, determined by CE and compared to values for 3 obtained by HPLC

Sample	Type	CE			HPLC
		1	**2**	**3**	**3**
NPC-VO-1	Capsule	–	–	–	–
NPC-VO-2	Softgel	0.54 (4.32)	–	0.13 (0.46)	0.11 (2.16)
NPC-VO-3	Teabag	–	–	–	–
NPC-VO-4	Capsule	–	–	–	–
NPC-VO-5	Capsule	–	–	–	–
NPC-VO-6	Tablet	–	–	–	–

Data represents the means of three replicates, relative standard deviations are given in parentheses

3. Discussion

Many dietary supplements, including *Valeriana officinalis* have no regulatory status in the USA, analytical methods for their standardization are therefore in demand. Capillary electrophoresis is an interesting approach, as it combines high separation efficiency with precision, sensitive detection, automated instrumentation and low analytical cost. The results of our study of the sesquiterpenoids in Valerian showed that CE can be seen as an equivalent alternative to well established techniques such as HPLC. With CE a sensitive detection of the compounds of interest was possible and the results were reproducible as well as comparable to those obtained by HPLC. Of the 6 market products analyzed, 4 claimed the content of *V. officinalis* whereas the others did not indicate the exact species used. Out of these six products just one would have passed the identity test for *V. officinalis* according to the European Pharmacopoeia. Only NPC-VO-2 contained the marker

compound valerenic acid. Results like these clearly indicate the need for an improved quality control in order to assure safety and efficacy of dietary supplements.

4. Experimental

4.1. Materials

Authenticated *V. officinalis* root extract was obtained from Nutra Source (San Carlos, CA, USA). Standard compounds **1** and **2** were purchased from Apin Chemical Ltd. (Oxon, UK); **3** was bought from Indofine (Belle Mead, NJ, USA). Valerian market products were purchased from various drug stores in Oxford/Mississippi; voucher specimens of all samples are deposited at the NCNPR. Water, methanol, acetonitrile and isopropanol were of HPLC grade and obtained from Fisher (Fair Lawn, NJ, USA). Sodium dihydrogenphosphate and sodium tetraborate were purchased from Sigma (St. Louis, MO, USA).

4.2. Preparation of samples and standards

1.000 g of the sample (capsules or tablets containing Valerian root powder or extract, teabags) was extracted 3-times with 3 ml of methanol by sonication for 10 min. After centrifugation the supernatant was combined and the solvent evaporated under reduced pressure. The resulting extract was dissolved in 10.00 ml isopropanol.

Standards were prepared by dissolving 1.00 mg of each reference compound in 1.00 ml isopropanol (stock solution); further calibration levels were prepared by diluting the stock solution with isopropanol (see Table 1 for calibration data).

Prior to injection all solutions (standards and samples) were diluted 1 : 10 with the running electrolyte, filtered through a 0.45 μm membrane filter (Gelman, Ann Arbor MI, USA) and centrifuged for 5 min at 9000 rpm. Samples were analyzed in triplicate and the standard deviation was below 5.0% in all experiments.

4.3. CE-conditions

All CE experiments were performed on a Biofocus 3000 capillary electrophoresis system (Bio-Rad, Hercules, CA, USA), equipped with multiple wavelength UV/VIS detector, automatic injector, water cooled column cartridge with a fused silica capillary (60 cm × 50 mm ID; Polymicro Technologies, Phoenix, AZ, USA) and autosampler. The running electrolyte was prepared by mixing aqueous solutions of 40 mM sodium dihydrogenphosphate and 40 mM sodium tetraborate, to adjust a pH of 8.5. Separation temperature, voltage and detection wavelength were kept constant at 35 °C, 17.5 kV and 210 nm, respectively. After each injection the capillary was washed with H_2O (1 min), 0.01N NaOH (2 min), and again with H_2O (1 min), followed by an equilibration period with buffer for 3 min.

Acknowledgements: This work was funded in part by the United States Department of Agriculture, ARS Specific Cooperative Agreement No. 58-6408-7-012.

References

1 Tyler, V. E.: Herbs of choice, p. 117, Pharmaceutical Products Press, New York 1994
2 Bisset, N. G. (Ed.): Herbal drugs and phytopharmaceuticals, p. 513, CRC Press, Boca Raton 1994
3 Ferreia, F.; Santos, M. S.; Faro, C.; Pires, E.; Carvalho, A. P.; Cunha, A. P.; Macedo, T.: Rev. Port. Farm. **46**, 74 (1996)
4 Hendriks, H.; Bos, R.; Woerdenbag, H. J.; Koster, A. S.: Planta Med. **49**, 28 (1985)
5 Riedl, E.; Hänsel, R.; Ehrke, G.: Planta Med. **46**, 219 (1982)
6 Bos, R.; Woedenbag, H. J.; Hendriks, H.; Wikstroem, H. V.; Scheffer, J. C.: Flavour Fragance J. **11**, 321 (1996)
7 Pande, A.; Uniyal, C. G.; Shukla, Y. N.: Indian J. Pharm. Sci. **56**, 56 (1994)
8 Hänsel, R.; Sticher, O.; Steinegger, E.: Pharmakognosie – Phytopharmazie, p. 460, Springer Verlag, Berlin – Heidelberg 1999
9 Moravi, M.; Molnar-Perl, I.: Chromatographia **25**, 37 (1988)
10 Bockstaller, S.; Schmidt, P. C.: Pharmazie **52**, 552 (1997)
11 Schimmer, O.; Roeder, A.: PZ Wiss. **5./137** (1992)

Received June 8, 2001
Accepted July 4, 2001

Ikhlas A. Khan
National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
USA
rikhan@olemiss.edu