Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 66 (2005) 1094–1097
Talanta
www.elsevier.com/locate/talanta

Short communication

Determination of cichoric acid content in dried press juice of purple coneflower (*Echinacea purpurea*) with capillary electrophoresis

Bronja Manček [a,*], Samo Kreft [b]

[a] *Krka, d.d., Novo mesto, Šmarješka cesta 6, 8501 Novo mesto, Slovenia*
[b] *University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia*

Received 31 August 2004; received in revised form 25 November 2004; accepted 14 January 2005
Available online 6 February 2005

Abstract

Purple coneflower (*Echinacea purpurea*) is an immunostimulating drug, containing multiple substances. The most important in activity are polysaccharides, caffeic acid derivatives (cichoric acid), alkamides and glycoproteins. It is not clear yet, which substances are responsible for activity. Cichoric acid is an appropriate marker of the quality of *E. purpurea* containing product, because it has immune stimulatory effects and it is susceptible to degradation.

In this work, an improved capillary electrophoresis method for determining cichoric acid in dried press juice from purple coneflower was developed. The optimal conditions were: electrophoretic buffer—75 mM borate, pH 8.8; injection 20 mbar for 20 s; separation at 20 kV; detection at 350 nm, temperature 35 °C.

Keywords: *Echinacea purpurea*; Purple coneflower; Cichoric acid; Capillary electrophoresis

1. Introduction

Purple coneflower (*Echinacea purpurea* MOENCH), originated in the United States of America, and was brought to Europe in the late 19th century. Per oral use of its aerial parts and juice expressed from the fresh aerial parts is allowed for treating common cold, upper respiratory tract and urinary tract infections, and topical use is allowed for poorly healing wounds [1–3]. It exhibits antioxidative, antibacterial, antiviral and antifungal activities, but most of all it affects the immune system [1–19]. It is one of the few plants that stimulate the non-specific immune system [3,20]. It is used for treating flu and cold, against which symptomatic treatment is an established procedure.

The most important potential active compounds in purple coneflower are polysaccharides, caffeic acid derivatives (especially cichoric acid), alkamides and glycoproteins [4,5]. Despite much research, it is not known which substance or group of substances is effective. Since the active substance is not known, an appropriate marker has to be chosen in order to determine juice or drug quality. Purple coneflower containing products on the European market are standardised on different compounds, also on compounds with no activity (e.g. glycine–betaine [3]). Cichoric acid was chosen, because it has immune stimulatory effects and is most probably absorbed in adequate quantities.

Many articles on LC methods for determination of phenolic acids and/or alkamides in *Echinacea* were published recently [21–26]. Capillary electrophoretic (CE) method for phenolic acids in *Echinacea* [27], and CE method for phenolic acids and alkamides [28,29], have been published. When the method for determination of cichoric acid according to reference [27] was applied on our CE system, retention times and areas under the peaks were found to be irreproducible. In this work, all the critical phases of the method were altered, resulting in an improved CE method for determining cichoric acid content in *E. purpurea* pressed juice. This method is also selective for caffeic acid and its derivatives chlorogenic and caftaric acids. Gentisic acid was used as an internal

* Corresponding author. Tel.: +386 7 331 20 87; fax: +386 7 332 33 86.
E-mail address: bronja.mancek@krka.biz (B. Manček).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.01.028

standard (IS), as it cannot be found in the purple coneflower and it is substantially cheaper than cichoric acid and caftaric acid. In comparison to LC methods [21–26], this CE method has lower operating costs and is more environmentally acceptable.

2. Experimental

2.1. Samples

Several different batches of *E. purpurea* dried press juice were used, standardised to ≥3% cichoric acid. One gram of dried press juice corresponds to 10–15 mL of fresh press juice, with a drug:extract ratio = 8–25:1. Samples were dissolved in water using an ultrasonic bath for approximately 5 min, then filtered. A concentrated solution of internal standard (2 mg/mL) was added to the filtered sample and diluted with water to give a final concentration of 5 mg of sample/mL and 0.4 mg gentisic acid/mL in water.

Standard substances of chlorogenic acid (PhytoLab, Labor Addipharma GmbH&Co.); cichoric acid (PhytoLab, Labor Addipharma GmbH&Co.); caftaric acid (ChromaDex Inc.), caffeic acid (Fluka) and gentisic acid (Sigma Chemical Co.) were dissolved in water.

2.2. Capillary electrophoresis

If not otherwise specified, the Hewlett Packard 3D (HP 3D Capillary Electrophoresis System) with a diode array UV–vis detector (DAD), controlled by HP 3D ChemStation 6.03, with a glass capillary (57 cm × 50 μm and bubble detection cell) thermostated at 35 °C was used. The capillary was rinsed for 1 min with methanol and 1 min with buffer prior to each analysis. The electrophoresis buffer was 75 mM borate, pH 8.8. The sample was injected at 20 mbar for 20 s. Separation was performed at 20 kV. Detection was at λ = 350 nm (response time 1 s).

The cichoric acid content was calculated by the formula:

$$\frac{P_{CA} \times C_{GA} \times V \times 0.09365 \times R}{P_{GA} \times m_s} \times 100, \quad (1)$$

where P_{CA} is the area under the cichoric acid peak; P_{GA}, the area under the gentisic acid peak; C_{GA}, the concentration of IS in sample solution (mg/mL); V, the sample volume (mL); 0.09365, the response factor of gentisic acid relative to that of cichoric acid; m_s, the mass of dried press juice to be examined (mg) and R is the dilution factor of sample preparation (usually 4).

The content of caftaric acid and chlorogenic acid (if present) can also be calculated, with use of response factors 0.16369 and 0.29308, respectively. We calculated the response factor as the ratio of slope of the two calibration curves prepared with standard solutions.

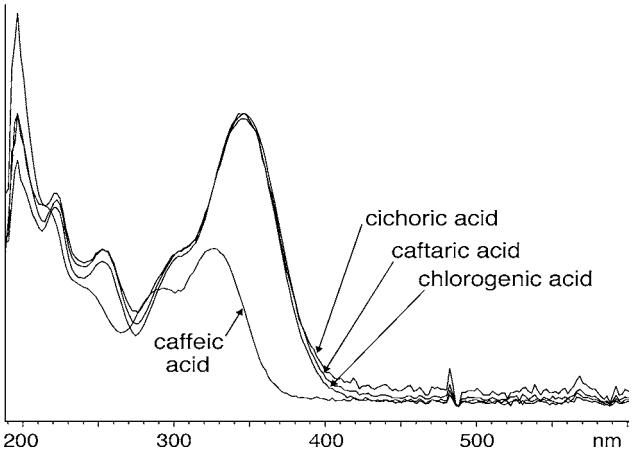

Fig. 1. UV absorbance spectra of cichoric acid, caftaric acid, chlorogenic acid and caffeic acid.

3. Results and discussion

3.1. Detection optimisation

Detection at 300 nm was used by other authors [27]. The spectrum of cichoric acid has a maximum at 350 nm (Fig. 1) with an absorbance twice that at 300 nm. Detection at 350 nm was, therefore, used routinely.

The absorbance is a function of the bandwidth. Bandwidths of 15 nm (342–357 nm), 40 nm (330–370 nm) and 80 nm (310–390 nm) were tested. Narrow range offers the highest signal, but also the highest noise of base line (Table 1) and conversely. The medium range was chosen for the best signal-to-noise ratio.

3.2. Selection of electrophoresis buffer

A series of borate buffers with different combinations of concentration (25, 50 and 75 mM) and pH 8.3, 8.8, 9.3 and 9.8 were tested. Seventy-five milimolar borate buffer pH 8.8 gave best results. The addition of two surfactants was also tested: sodium dideoxycholate (70 mM), which is suggested in the literature [27], and SDS (70 mM), which is the most frequently used surfactant in capillary electrophoresis. With sodium dideoxycholate, the peak of cichoric acid exhibited a disturbance in shape (Fig. 2) and the retention time was very irreproducible. Reproducibility was improved, when the capillary was rinsed with 0.1 M NaOH for 4 min, followed by water and electrophoresis buffer. Buffer containing SDS resulted in the absence of tailing and good reproducibility,

Table 1
The influence of detection wavelength range on peak height and noise

Range of wavelengths included in detection (nm)	Height of the cichoric acid peak (mAU)	Noise	Signal-to-noise ratio
15	101	0.14	721
40	92	0.07	1314
80	73	0.06	1216

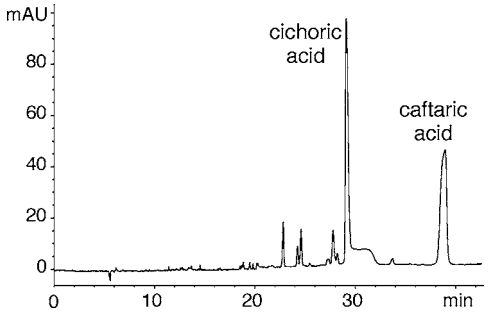

Fig. 2. Electropherogram of *E. purpurea* dried press juice analysed with electrophoresis buffer according to reference [27] (containing sodium dideoxycholate (70 mM)). Cichoric acid peak with a plateau shaped disturbance can be observed.

but the resolution was equal or worse than in absence of any surfactant. Therefore, no surfactant was used in further analyses (Fig. 3).

3.3. Injection optimisation

It is known that large amounts of injected sample produce broader peaks than small amounts. However, the same amount of sample can be injected as a small volume at high concentration or conversely. Experiments showed that dilution of the sample improved the resolution. The peaks of cichoric and caftaric acids were 12% and 13% wider, when a small volume of concentrated sample (10 mg/mL) was injected, than a four-fold higher volume of four-fold diluted sample (Table 2). This is probably due to the sample stacking effect, which is more efficient in diluted samples. During the sample stacking, the molecules of analyte are concentrated at the beginning of the capillary, if the sample solution has much lower ionic strength than that of the electrophoresis buffer. A sample concentration of 2.5 mg/mL with 20 mbar × 20 s injection was used for further analyses.

3.4. Temperature optimisation

Four different capillary temperatures were tested: 20, 25, 35 and 45 °C. The retention times (Rt) were shorter at higher temperatures: at 20 °C Rt for cichoric acid peak was 25–26 min and, at 45 °C, Rt 15–16 min. No influence on separation, except some worsening at 45 °C was observed; thus, 35 °C was chosen for further analyses.

3.5. Stability of sample and standard solutions

The water solution of the sample was stable for 24 h, after which a decrease in cichoric acid concentration and appearance of a white precipitate was observed. There was however, no increase of any peak. Increase of the caffeic acid peak would be expected, if there was degradation due to hydrolysis. Much faster degradation was observed by other authors [30], but caffeic acid did not appear in their experiments either. It is more likely that polyphenol oxidases catalyse oxidation of polyphenols with O_2 [30]. Furthermore, those oxidised products can react with other polyphenols, amino acids or other nucleophiles giving rise to complex polymer structures, which can form a precipitate.

Standard solutions of chlorogenic and gentisic acid in water were stable at least for 30 h, if light protected. Solutions of cichoric acid in water were less stable; in 24 h there was approximately 32% reduction. No decrease was observed in approximately 7 h (the time needed for validation of the method). Caftaric acid was more stable in water than cichoric acid; in 24 h the peak of caftaric acid decreased by approximately 9% (Fig. 3).

3.6. Precision

Reproducibility of the system was established by six successive analyses of actual sample solution with IS at approximately, 0.4 mg gentisic acid concentration. Relative standard deviations of the peak areas were 3.4%, 3.7% and 3.3% for cichoric, caftaric and gentisic acids, respectively. The ratio between peak area and IS was 2.0% and 2.8% for cichoric and caftaric acids, respectively. When the IS was used, the random error was reduced by approximately 1%, which is the error of injection.

Table 2
Cichoric and caftaric acid peak width on the electropherograms as a function of sample concentration and injection volume

Sample concentration (mg/mL)	Peak	Injection			
		5 mbar × 10 s	10 mbar × 10 s	20 mbar × 10 s	20 mbar × 20 s
10	Cichoric acid	**0.2928**	0.3213	0.3508	0.4461
5		0.2819	**0.2715**	0.2887	0.3461
2.5		0.2684	0.2561	**0.2612**	0.2811
1.25		0.2663	0.2425	0.2522	**0.2614**
10	Caftaric acid	**0.3966**	0.4768	0.5714	0.8514
5		0.3543	**0.3764**	0.4470	0.7040
2.5		0.3093	0.2946	**0.3499**	0.5101
1.25		0.2869	0.2813	0.3001	**0.3652**

Since the retention times were not influenced by injection procedure and sample concentration, wider peaks are related to smaller resolution. Figures in bold represents the analyses where the same amount of analytes were injected.

Table 3
Statistical parameters defined according to ICH guidelines [31,32]

Standard	Tested range (mg/mL)	Determination coefficient	Slope (mAU/(mg mL))	Intercept	Bias (%)
Gentisic acid	0.1000–0.8000	>0.995	1031 ± 38	−18 ± 19	100–6.5
Cichoric acid	0.0741–0.5928	>0.973	11006 ± 1048	−256 ± 392	100–12.2
Caftaric acid	0.0440–0.3522	>0.980	6297 ± 513	−121 ± 114	100–18.5
Chlorogenic acid	0.0399–0.2794	>0.992	3517 ± 174	−9 ± 31	100–2.4

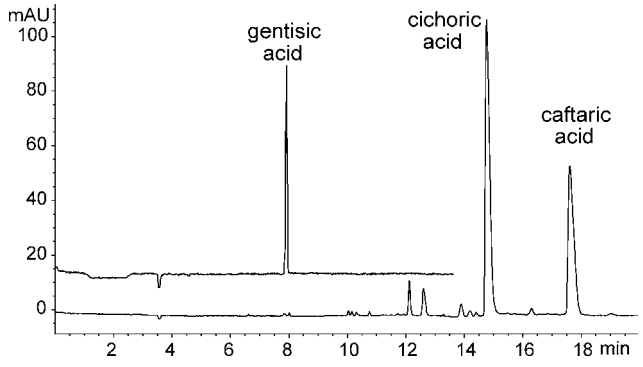

Fig. 3. Electropherogram of solution of the actual sample of *E. purpurea* dried press juice (containing approximately 0.2 mg cichoric acid/mL) and of gentisic acid standard (approximately 0.4 mg gentisic acid/mL).

3.7. Linearity

Statistical parameters describing linearity of peak area as function of concentration are presented in Table 3.

3.8. Limits of detection and quantitation

The approach based on signal-to-noise ratio was chosen, which can be applied to analytical procedures, which exhibit baseline noise. The limit of detection for cichoric acid was 1.1×10^{-3} mg/mL (signal-to-noise ratio of 3:1) and quantitation limit 3.5×10^{-3} mg/mL (signal-to-noise ratio of 10:1).

4. Conclusion

An improved CE method was developed. Since cichoric and caftaric acids standards are extremely expensive, for routine analyses a cheaper standard is preferred. The CE method described uses gentisic acid as IS and response factors content of cichoric, chlorogenic and caftaric acids are calculated in one run.

References

[1] M. Blumenthal, The Complete German Commission E Monographs, American Botanical Council, 1999 (CD-ROM).
[2] S. Percival, Biochem. Pharmacol. 2 (60) (2000) 155.
[3] R. Bauer, Zeitschrift für Phytotherapie 18 (1997) 270.
[4] R. Bauer, H. Wagner, Echinacea, Handbuch fur Ärzte, Apotheker und andere Naturwissenschaftler, Wissenschaftliche Verlagsgesellschaft GmbH Stuttgart 1990, 182 pp.
[5] M.L. Chavez, P.I. Chavez, Hosp. Pharm. 33 (1998) 180.
[6] World Health Organisation, WHO Monographs On Selected Medicinal Plants, vol. 1, Geneva, 1999, p. 136.
[7] European Scientific Cooperative on Phytotherapy (ESCOP), Monographs on the Medicinal Uses of Plant Drugs, Fascicule 6, *Echinaceae purpureae* herba (purple coneflower herb), October 1999, 10 pp.
[8] R. Bauer, Phytotherapie 2 (1994) 12.
[9] S.E. Binns, J. Hudson, S. Merali, et al., Planta Med. 68 (9) (2002) 780.
[10] R. Maffei Facino, M. Carini, G. Aldini, et al., Planta Med. 61 (6) (1995) 510.
[11] M. Nardini, M. D'Aquino, G. Tomassi, et al., Free Radic. Biol. Med. 5 (19) (1995) 541.
[12] B.D. Sloley, L.J. Urichuk, C. Tywin, et al., J. Pharm. Pharmacol. 53 (2001) 849.
[13] M. Nardini, F. Natella, V. Gentili, et al., Arch. Biochem. Biophys. 342 (1) (1997) 157.
[14] J. Roesler, A. Emmendörffer, C. Steinmüller, et al., Int. J. Immunopharmacol. 13 (7) (1991) 931.
[15] H. Wagner, H. Stuppner, W. Schäfer, et al., Phytocemistry 27 (1) (1988) 119.
[16] E. Speroni, P. Govoni, S. Guizzardi, et al., J. Ethnopharmacol. 2 (79) (2001) 265.
[17] A. Wacker, W. Hilbig, Planta Med. 33 (1978) 89.
[18] G. Mazza, T. Cottrell, J. Agric. Food Chem. 47 (1999) 3081.
[19] A. Cheminat, R. Zawatzky, H. Becker, et al., Phytochemistry 9 (27) (1988) 2787.
[20] V. Schulz, R. Hänsel, V.E. Tyler, Rational Phytotherapy: A Physicians Guide to Herbal Medicine, third ed., Springer-Verlag, Berlin, Heidelberg, New York, 1998, pp. 273–278.
[21] P. Molgaard, S. Johnsen, P. Christensen, C. Cornett, J. Agric. Food Chem. 51 (24) (2003) 6922.
[22] X.B. Luo, B. Chen, S.Z. Yao, J.G. Zeng, J. Chromatogr., A 986 (2003) 73.
[23] S. Gocan, L. Radu, M. Hadaruga, Chromatographia 57 (9–10) (2003) 677.
[24] M. Laasonen, T. Wennberg, T. Harmia-Pulkkinen, H. Vuorela, Planta Med. 68 (6) (2002) 572.
[25] G.W. Schieffer, M. Kohn, J. Liq. Chromatogr. Relat. Technol. 25 (2) (2002) 263.
[26] G. Giancaspro, Pharmacopeial Forum 30 (2004) 557.
[27] R. Pomponio, R. Gotti, M. Hudaib, et al., J. Chromatogr., A 945 (2002) 239.
[28] R. Gotti, R. Pomponio, C. Bertucci, V. Cavrini, J. Sep. Sci. 25 (2002) 1079.
[29] P. Pietta, P. Mauri, R. Bauer, MEKC analysis of different *Echinacea* species, Planta Med. 64 (1998) 649.
[30] B. Nüsslein, M. Kurzmann, R. Bauer, et al., J. Nat. Prod. 12 (63) (2000) 1615.
[31] International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline: Text on Validation of Analytical Procedures Q2A, on http://www.ich.org/pdfICH/Q2A.pdf, accessed 10.12.2002.
[32] International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Methodology Q2B, on http://www.ich.org/pdfICH/Q2B.pdf, accessed 10.12.2002.