Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1097 (2005) 199–202
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Monitoring of the conversion from triptolide to tripchlorolide in triptergium wilfordii by micellar electrokinetic caillary chromatography

Baozhi Li [a], Jie Bai [c], Gengliang Yang [a,b,*], Zhiwei Li [a], Lijuan Wang [a], Yi Chen [b]

[a] *College of Pharmacy, Hebei University, Baoding 071002, China*
[b] *Center for Molecular Science, Institute of Chemistry, Chinese Academy of Science, Beijing 100080, China*
[c] *Department of Chemistry, Handan University, Handan 056005, China*

Received 27 April 2005; received in revised form 8 June 2005; accepted 14 June 2005

Abstract

A novel concocting method to convert Triptolide (T) into Tripchlorolide (T_4) in the traditional Chinese herb *Tripterygium wilfordii* Hook F. and a micellar electrokinetic capillary chromatographic (MEKC) approach by which the conversion of Triptolide (T) and Tripchlorolide (T_4) was identified and determined had been established. Investigations of the influence of different pH values of boric acid and borax buffer and of sodium dodecyl sulfate (SDS) and organic additive concentrations had been carried out, and the optimum separation for T and T_4 was achieved using boric acid and borax of pH 7.0 with 30 mM SDS and 20% (volume ratio) methanol as the running buffer. It was found that MEKC exhibited good accuracy, precision and repeatability and the content of T_4 was greatly increased in the herb that was treated by the new concocting method.

Keywords: Micellar Electrokinetic capillary chromatography; Triptolide; Tripchlorolide; *Tripterygium wilfordii* Hook F

1. Introduction

Tripterygium wilfordii Hook F. is a traditional Chinese medicine with antiphlogistic, antalgic and antibacterial actions. It is especially effective for the treatment of Rheumatoid arthritis (RA) [1] and has become the most commonly used herbal drug for the disease in China. Triptolide (T) has been demonstrated to be the most important biologically active components in the plant, but it has also been found to be the most toxic component in the herbal drug [2], which is especially harmful to the liver, digest system, nerve system and procreant system and has greatly limited the application of this herb. Tripchlorolide (T_4) has also been separated and identified from the plant and it is approved that T could convert into T_4 after reacting with hydrochloric acid–acetic acid (HCl–HAc) [3] (Fig. 1). Because of the higher performance and lower toxicity of T_4 than T, T_4 is more hopeful for developing new *T wilfordii* drugs. However, the amount of T_4 is so much less than T in the natural plant that it is usually hard to be detected in the natural herbal grass and the raw material source is also a big problem to obtain the pure compound.

Although various TLC [4,5] and HPLC [6–8] methods have been employed for the determination of the diterpenoid triepoxides in *T wilfordii*, no analytical methods for T_4 have been reported. In recent years, capillary electrophoresis has been widely accepted as an attractive method for the identification and determination of Chinese herbal drug [9–12] because of its higher resolving power, shorter analysis time and lower operating cost. The identification and determina-

* Corresponding author. Tel.: +86 312 5079788; fax: +86 312 5971107.
E-mail address: ygl@mail.hbu.edu.cn (G. Yang).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.06.043

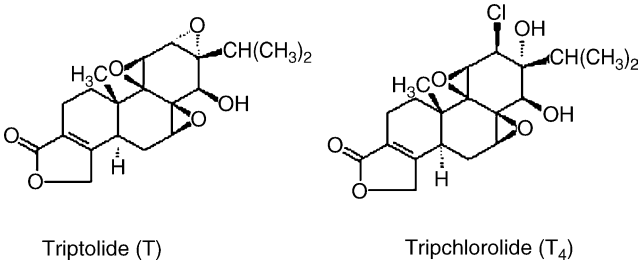

Fig. 1. The structures of Triptolide and Tripchlorolide.

tion of T in *T. wilfordii* by MEKC had also been reported [13,14] and this optimized method was proved to be effective and quick not only for the roots of *T. wilfordii* but also for the multi-glycosides *T. wilfordii* tablet. The purpose of present study is to set up a capillary electrophoresis method for the determination of T and T_4 first, then to use it to monitor the conversion of pure T into T_4 and finally to establish an optimized approach to convert T into T_4 for the herb after it was concocted under the conditions which was set up by the pure T.

2. Experimental

2.1. Reagents and chemicals

Roots of *T. wilfordii* Hook F. were obtained from Anyao Pharmaceutical Group (Hebei; Province, China). T was acquired from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Sodium dodecyl sulfate (SDS) was from Beijing Xizhong Chemical Factory (Beijing, China). Di-sodium tetraborate was from Tianjin Chemical Reagent Factory (Tianjin, China). Methanol of HPLC grade were purchased from Fisher (New Jersey, USA). Unless otherwise specified, all solutions were filtered through a 0.45 μm before use.

2.2. Preparation of T_4

T_4 was prepared by T in our lab [3] and was identified by ESI-MS. The target compound was obtained in 87.0% yield, m.p. 256–259 °C; ESI-MS: 397$[M + 1]^+$, 419$[M + Na]^+$, 421$[M + 2 + Na]^+$, which was consistent with the reported values.

2.3. Instruments

The capillary electrophoresis system was a Waters Quanta 4000 E (Waters, USA) equipped with an automatic injector, a temperature-controlled equipment and a fused silica capillary of 75 μm i.d. (total length of the capillary was 60 cm while the effective length was 52.0 cm). A window was created at 8.0 cm from the end of the capillary (cathode) for on-column detection by removing the polyamide coating. Direct ultraviolet spectroscopy detection was with a 214 nm optical filter. Samples were introduced from the anodic end of the capillary by hydrodynamic injection for 10 s (about 80 nl) where the samples vial was raised by 9.6 cm. Electropherograms were recorded with a CKChrom Chromatography Data System on a Compaq-Prolinea-4/50 computer. Model pHs-3C acidometer was from Shanghai Leici Instrumental Factory. Mass spectra were determined on an APEX11 FT-ICR spectrometer (ESI).

Before the first run of the day, the capillary was rinsed with 1.0 M NaOH for 10min. In order to get better reproducibility, before each run the capillary was purged with 0.1 M NaOH for 3 min, triple distilled water for 3 min, and finally the separation buffer for 5 min. In the course of experiment, the temperature remained constant at room temperature.

2.4. Investigation of the concocting condition of T

To set up the optimum concocting method, stock solution of T was prepared and was treated by HCl with different concentrations at 4 °C, which was more favorable to the unsaturated lactone structure of T under the acidic surrounding than at higher temperature such as room temperature (around 25 °C). Then the stock solution of T was determined and the conversion of T was monitored by MEKC. According to the conversion ratio of T, the most favorable concocting condition of T was set up and this condition would be used for the treatment of the herb later

2.5. Sample pre-treatment

According to the concocting method which had been set up by the above experiment, the roots with outer layer (130 g) of *T. wilfordii* Hook F. were ground to a coarse powder and were treated by 80 ml HCl–HAc before being extracted with 300 ml ethyl acetate. After filtering the ethyl acetate solution, the solvent was washed to neutral and evaporated off under reduced pressure and then 3–4 g Al_2O_3 was added into the residue. After being heated to dryness, the residue was put into an Al_2O_3 column and eluted with ethyl acetate–petroleum (1:9), ethyl acetate–petroleum (1:1) and ethyl acetate–ethanol (24:1), respectively. The eluted solvent of ethyl acetate: petroleum (1:1) was gathered and evaporated to dryness. Finally, the resulting residue was dissolved in 10 ml H_2O and stored as stock solution for MEKC analysis.

3. Results and discussions

In order to monitor the conversion of T into T_4 in the traditional Chinese herb *T. wilfordii,* a micellar electrokinetic capillary chromatographic (MEKC) approach for the identification and determination of the diterpenoid triepoxides had to be established. So the optimum electrophoresis separation condition of T and T_4 was the first task to be solved. Investi-

gations of the influence of different pH values of the buffer, SDS and organic additive concentrations had been carried out. The identification of T and T_4 was affirmed by adding standard T and T_4 into the sample.

3.1. Effects of SDS and pH on the separation

The effects of SDS on the separation were investigated with SDS concentration ranging from 0 to 40 mM with steps of 5 mM as an unit when the buffer contained 98 mM boric acid and 0.6 mM borax. Experiments showed that the resolution of T and T_4 was satisfactory and good separation was achieved when the concentration of SDS was 20 mM. So 20 mM SDS was selected for further experiment.

When the running voltage (20 kV) and other conditions were kept constant, the effects of pH values of the buffer ranging from 6.2 to 8.1 on the separation of T and T_4 showed that when pH was over 8.1, T and T_4 overlapped together. Baseline separation of T and T_4 was achieved at pH 7.5 and the resolution increased when pH value was lower than it. Optimum separation was obtained at pH 7.0. Although better separation could also be obtained at lower pH value than 7.0, the migration time of T and T_4 was much longer. So pH 7.0 was selected as the optimum value.

3.2. Effects of the organic additives on the separation

Although baseline separation of T and T_4 could be obtained, the peaks of T_4 and other related compounds in the sample were somehow overlapped at above conditions, which would affect the content determination of T_4 in the concocted herb. So organic additives were used for further investigation.

When methanol was added in the buffer solution as the organic additives, the resolution between T_4 and its neighbor compound at the right side of the electropherogram was improved greatly. Although the resolution increased with increase of methanol in the buffer solution, no obvious change happened when methanol concentration was over 20%. So 20% methanol was chosen.

3.3. Calibration

With the developed MEKC method above, calibration curve was obtained by using the peak area of the standards with different concentrations. The linear equations were $Y = 3.969 \times 104X\text{-}305.2$ ($R = 0.9984$) for T_4 in the range of 0.002–0.24 mg ml^{-1}, and $Y = 3.971 \times 104X\text{-}374.8$ ($R = 0.9972$) for T in the range of 0.002–0.17 mg ml^{-1}, where X was the concentration and Y was the peak area of the standard. In addition, the repeatability of migration time of T and T_4 were 1.16 and 1.52% (RSD, $n = 5$), the repeatability of peak area of T and T_4 were 3.38 and 4.07% (RSD, $n = 5$), respectively.

3.4. Establishment of optimized concocting conditions from T to T_4

Because of the complexity of the components in the herbal drug, T was chosen as the mark to explore the optimum concocting condition. It could be seen that both the conversion ratio of T and the quantity of T_4 grew apparently during the first 10 h, but no more change would happen after 36 h and the result was shown in Fig. 2A. So the optimum concocting time for T was chosen as 36 h. It was also be found that the higher the concentration of HCl system was, the higher the conversion ratio of T was. Because the stronger acidic circumstance was unfavorable to the unsaturated lactone structure of T and T_4 [3], it was found that many other unknown compounds would appear at stronger acidic circumstance, which would reduce the conversion ratio of T. After testing HCl–HAc system at different concentrations, it was showed that 0.4 M HCl–HAc could give satisfactory results and the effects of pH value on the conversion ratio was given in Fig. 2B. So

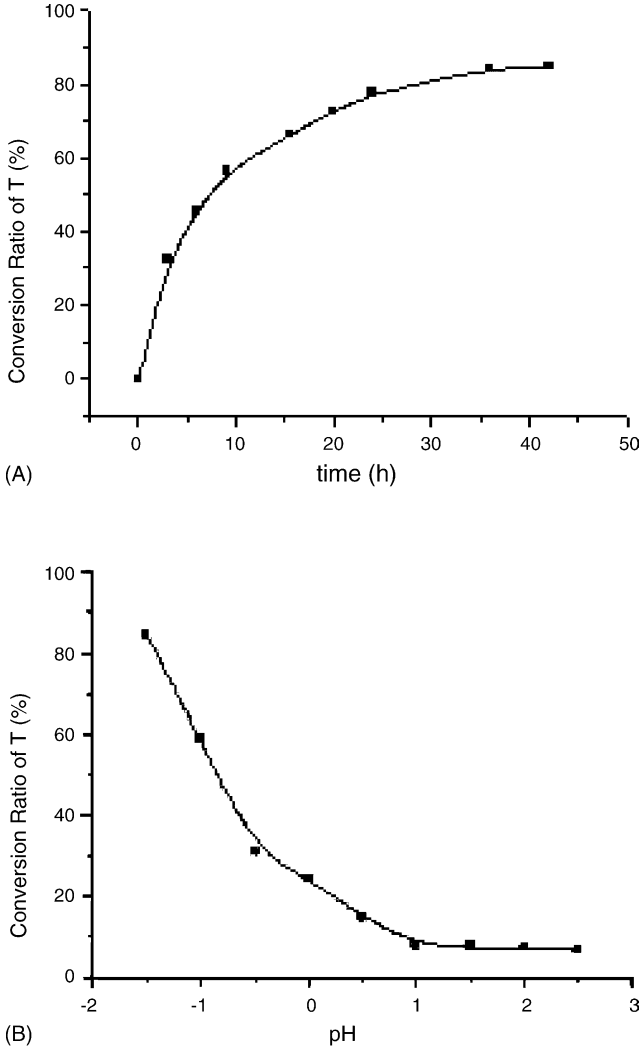

Fig. 2. Effect of: (A) reaction time on the conversion ratio of T and (B) pH on the conversion ratio of T.

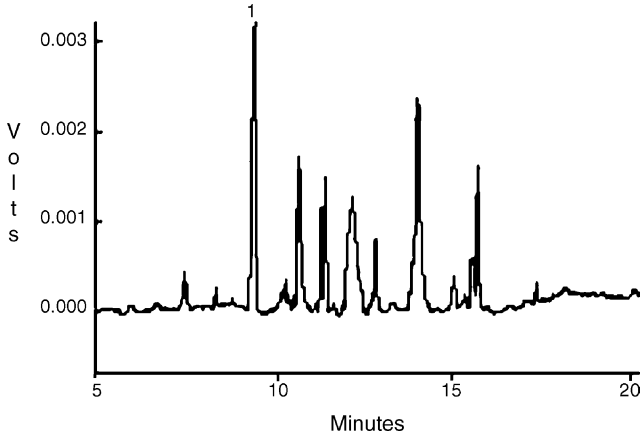

Fig. 3. Electropherogram of the ethyl acetate extract of *Tripterygium wilfordii*. Condition: background electrolyte with 98 mM boric acid, 0.6 mM borax, pH 7.0, and 20% methanol and 30 mM SDS were added, the separation was performed at 20 kV applied voltage, room temperture and 214 nm UV wavelenth. 1: Triptolide.

the final optimized conditions was 0.4 M HCl–HAc system at 4 °C for 36 h.

3.5. Concocting of the herb

With the above concocting condition for T, the herb was treated with the same method. Fig. 3 was the electropherogram of the ethyl acetate extract of the drug without being treated by HCl–HAc, Fig. 4 was the electropherograms of the ethyl acetate extract of *T. wilfordii* after it was dealt under the final concocting condition. Compared Fig. 3 with 4, it could be seen that before the herb was concocted, almost no T_4 could be found in the herb. After being concocted, the content of T changed from 0.10 mg ml^{-1} in the ethyl acetate extract of the natural herb to 0.015 mg ml^{-1} in the concocted herb and the content of T_4 was 0.06 mg ml^{-1} in the treated herb. By this way, most T in the natural herb can be converted into T_4.

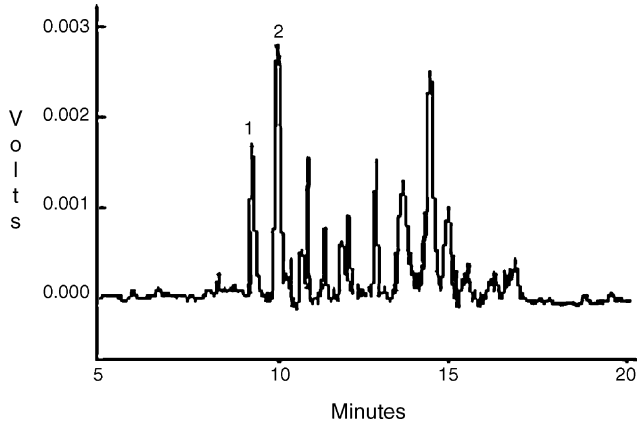

Fig. 4. Electropherogram of the ethyl acetate extract of the concocted *Tripterygium wilfordii*. Conditions as Fig. 3. 1: Triptolide, 2: Tripchlorolide.

4. Conclusion

A novel concocting method to convert Triptolide into Tripchlorolide in the traditional Chinese herb *T. wilfordii* Hook F. had been established. The method established in this paper cannot only be used for the conversion of pure T into T_4, it can also be utilized for the treatment of the natural herb. In addition, the developed MEKC method was proved to be a simple and effective method to monitor the conversion of T. Under the optimized MEKC conditions, the specified compounds in the plant sample were baseline separated within 13 min. This work supplied not only a quick and effective method but also an academic base for developing new *T. wilfordii* drugs with lower toxicity.

Acknowledgements

One of the authors, Gengliang Yang, gratefully acknowledges the financial assistance provided by National Natural Science Foundation of China (Grant No. 20375010), “Bairen” Project of Chinese Academy of Sicences, Natural Science Foundation of Hebei Province (Grant No. 202096), Screening platform of the active components in natural drugs of Hebei Province (Grant No. 04276402D) and Excellent Youth Program and Doctorate Station Foundation of Chinese Education Ministry.

References

[1] C.N. Liu, M. Peng, Anticancer Chinese Herb Dictionary, Science and Technology Publishing Co., Hubei, 1994, p. 1072.
[2] X.Z. Guo, Toxic Traditional Chinese Herb Dictionary, Science and Technology, Tianjin, 1994, p. 580.
[3] X.Y. Lu, P.C. Ma, Y. Chen, C.P. Zhang, Acta Acad. Med. Sinicae 12 (1990) 157.
[4] L.R. Yu, J.L. Li, J. Tradit. Chin. Med. 19 (1988) 18.
[5] K. Nie, W.J. An, G.C. Xia, J. Tradit. Chin. Med. 25 (1994) 539.
[6] J. Cai, X.L. Tao, P.E. Lipsky, J. Liquid Chromatogr. 17 (1994) 4479.
[7] W.Z. Gu, R.D. Chen, B. Wein, Int. J. Immunopharmac. 17 (1995) 351.
[8] Y.P. Mao, J. Cai, X.L. Tao, L. Ma, P.E. Lipsky, J. Liquid Chromatogr. 21 (1998) 2699.
[9] X.R. Song, G.L. Yang, H.Y. Zhang, D.X. Wang, J. Tradit. Chin. Med. 30 (1999) 85.
[10] X.R. Song, G.L. Yang, H.W. Sun, J. Li, Chin. Tradit. Patent Med. 22 (2000) 158.
[11] G.L. Yang, X.R. Song, H.Y. Zhang, D.X. Wang, Chin. J. Anal. Chem. 27 (1999) 1.
[12] H.Y. Zhang, X.R. Song, Z.H. Shi, G.L. Yang, Fresenius J. Anal. Chem. 365 (1999) 499.
[13] X.R. Song, G.L. Yang, X.J. Zhao, H.W. Sun, J Chin. Med. 12 (2001) 91.
[14] X.R. Song, G.L. Yang, X.J. Zhao, Y. Chen, Phytochem. Anal. 14 (2003) 132.