DETERMINATION OF TRIPTONIDE AND TRIPTOLIDE IN MULTI-GLYCOSIDES *TRIPTERYGIUM WILFORDII* TABLETS BY CAPILLARY ELECTROPHORESIS

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A capillary electrophoresis method has been established for the determination of triptonide and triptolide in Multi-glycosides *Tripterygium wilfordii* tablets. Capillary separation was carried out by using 0.01mol L⁻¹ borax + 0.02 mol L⁻¹ boric acid + 0.02 mol L⁻¹ SDS (pH = 8.0) as the buffer, a fused – silica capillary tube column (60.2 cm × 75µm id. 52.6 cm of effective length) as the separation channel, 20 kV applied voltage, 301 K temperature, 5s hydrodynamic sample injection time and 214 nm UV detection wavelength. Baseline separation and good linearity were obtained under the optimum conditions. **Conclusion:** This method was effective and quick.

Key Words: Capillary electrophoresis, Multi-glycosides *Tripterygium wilfordii* tablets, Triptonide, Triptolide.

INTRODUCTION

Multi-glycosides *Tripterygium wilfordii* tablet has antiphlogistic, antalgic and antibacterial actions. It is especially effective for the treatment of Rheumatoid arthritis¹ and has become the most commonly usedmedicine for this disease in China. This medicine is mainly made from *Tripterygium wilfordii* Hook F. Triptolide and triptonide are the important biologically active components and have been found to be novel anti-leukemia diterpenoid triepoxides². However, Multi-glycosides *Tripterygium wilfordii* tablet is regarded as a toxic preparation and triptolide has been found to be the most toxic among the components. Therefore quality control is especially important for this tablet. Generally, the contents of triptonide and triptolide can be considered as a quality index. So it is therefore necessary to develop a simple and rapid method for the identification and determination of triptonide and triptolide in the preparation.

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Figure 1. The structures of triptolide and triptonide

Although there is a variety of analytical methods, such as thin layer chromatography (TLC)⁽³⁾, high performance liquid chromatography (HPLC)⁴⁻⁵, capillary gas chromatography (GC)⁶ and column layer chromatography – ultraviolet spectroscopy⁷, for the determination of triptonide and triptolide in *Tripterygium wilfordii* Hook F, these methods suffer from many limitations, such as more materials and time consuming. For TLC, because the efficiency is low, several prior steps are often required to extract species of interest from the sample and to isolate them from potentially interfering compounds, which make the method time-consuming and the results not accurate. While for HPLC, although it gives more accurate results than TLC, prior steps are also needed to avoid column pollution and it also suffers from the cost for a large amount of solvent. Because of its high resolving power, short analysis time and lower cost, CE has gained more and more attention in the identification and determination of Chinese traditional medicine. In CE, the extract of the herb can be directly injected into the column system without further prior steps. Using different CE modes (CZE, MECC), the neutral and polar components can be easily separated in the run and this is especially suitable for the analysis of plant herbs ⁸⁻¹⁰. The purpose of this paper is to develop a micellar electrokinetic capillary chromatography (MECC) method for the determination of triptonide and triptolide in Multi-glycosides *Tripterygium wilfordii* tablet.

Materials and Methods:

Samples and Reagents

Multi-glycosides *Tripterygium wilfordii* tablets were produced by the Chinese Academy of Medical Sciences (Beijing, China). Triptonide and triptolide were isolated from *Tripterygium wilfordii* Hook F¹¹. Sodium dodecyl sulphate (SDS) was from Beijing Xizhong Chemical Factory (Beijing, China). Di-sodium tetraborate was from Tianjin Chemical Reagent Factory (Tianjin, China). All other reagents were of analytical grade. The buffers were filtered with 0.45 µm film before use.

Sample preparation

A certain amount of Multi-glycosides *Tripterygium wilfordii* tablets (A,B) of two different batch numbers were ground into powder (0.45 mm size) after taking away the outside sugar shell. After 1.25 g (A) and 1.63 g (B) of the

powders were extracted with 100 mL chloroform in a Soxhlet's extractor for four hours, the chloroform extract was removed and the residue refluxed again with 50 mL chloroform. Then the extracts were combined and filtered and the filtrates were evaporated under reduced pressure to dryness. Finally they were dissolved in 10mL ethanol, respectively and stored as stock solutions.

Instruments and Methods

The capillary electrophoresis system was a Waters Quanta 4000 E (Waters Chromatography Division of Millipore, Milford, MA, USA) equipped with an automatic injector, a temperature-controlled equipment and a fused silica capillary of 75 µm ID (total length of the capillary was 60 cm while the effective length was 52.4 cm). A window was created 7.6 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 5s (about 40 nL) where the sample vial was raised by 9.6 cm. Electropherograms were recorded with a Waters Millennium 2010 chromatography system on a Compaq – Prolinea - 4/50 computer. Model pHs-3C acidometer was from Shanghai Leici Instrumental Factory.

Before the first run of the day, the capillary was rinsed with 0.5 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, double distilled water for 3 min, and finally the separation buffer for 4 min. In the course of experiment, the temperature remained constant at 301 K.

Results and Discussion

As this study focused on the development of a method to analyze the two major components of Multi-glycosides *Tripterygium wilfordii* tablet, the selection of an internal standard and electrophoresis separation optimum conditions were two major tasks to be solved first. From experiments, it was found that acetophenone was found to be a suitable internal standard for the determination of triptonide and triptolide because its peak was close to the two compounds'. Figure 2 illustrates the electropherogram of the extract of Multi-glycosides *Tripterygium wilfordii* tablet with the addition of acetophenone and authentic standards (triptonide and triptolide). It was apparent that the peak of the internal standard did not affect other components. Identification of standard mixture (triptolide and triptonide) was affirmed by comparing the peak area of the sample preparation with the external standard of authentic standards. *Effects of SDS on the Separation*

With other conditions fixed, various concentrations of SDS ranging from 15 mM to 40 mM with steps of 5 mM as a unit were added to the buffer with 20 mM boric acid and 10 mM borax. When SDS concentration was lowered to 15 mM, the peaks of the two active compounds overlapped to a great degree with their related compounds. From figure 3, it can be seen that with the increase of SDS concentration, the separation became better and better. When SDS concentration was 20 mM, triptonide and triptolide were almost baseline separated from other compounds. The

effect of SDS concentration on the number of theoretical plates was also investigated. From the experiments, it was



Figure 2. Electropherogram of the chloroform extract of Multi-glycosides Tripterygium Wilfordii tablets after addition of acetophenone. Conditions: background electrolyte with 20 mM boric acid, 120 mM borax and 20 mM SDS, pH 8.0, the separation was performed at 20 kV applied voltage and 301 K temperture. 1-triptonide, 2=triptolide, 3=acetophenone.



Figure 3. Effect of SDS concentration. Conditions as Figure 2. 1=triptonide, 2=triptolide, C=EOF mark (ethanol)

shown that the numbers of theoretical plates of the two active compounds were lower at 15 mM SDS. When the concentration of SDS increased, the number of theoretical plates of the two active compounds increased quickly. The number of theoretical plates was improved up to the range of $6 \times 10^4 - 3 \times 10^5$ when SDS was 20 mM. So 20 mM SDS was selected for further experiments.



Figure 4. Effect of pH effective mobility. Conditions as Figure 2. 1=triptonide, 2=triptolide, C=EOF mark (ethanol)

Effect of pH

The effects of pH value ranging from 6.0 to 10.0 on the effective mobility of triptonide and triptolide were also investigated in this paper when voltage (20 kV), temperature (301 K) and other conditions were kept constant. From figure 4 it can be seen that the effective mobility of triptonide and triptolide increased generally with the increase of pH from 6.9 to 10.0. When pH was under 6.9, experiment showed that the two active components could not be separated completely. The resolution increased when pH changed from 6.9 to 9.0. Baseline separation was achieved at pH equal to 8.0. But the effective mobility began to decrease when pH was above 9.0. The reason may be that with the increase of buffer pH value, the ionic strength of the buffer increased too and too much Joule heat would be produced at higher pH value. From the above discussion, pH 8.0 was selected as the optimum condition.

Effect of boric acid and borax concentration

From the experiment it can be seen that when boric acid concentration changed from 10 mM to 40 mM and borax concentration from 5 mM to 25 mM with 5 mM as a unit step, the peak areas also increased. This may be due to the formation of borate anions complex with diols, which would increase the UV absorbance. When boric acid concentration was above 20 mM and borax concentration was above 10 mM, the normalized peak area (NA) changed slightly. Based on the experimental results, 20 mM boric acid concentration and 10 mM borax concentration were chosen in the optimum buffer.

Determination of triptonide and triptolide in the sample

The calibration curve was obtained by using the NA of the standards with different concentrations. The calibration curves were Y = 0.02147 + 3.457 X (R = 0.9996) for triptonide in the range of $5.34 \times 10^{-2} - 8.27$ mg mL⁻¹, Y = 0.01793 + 4.888 X (R = 0.9994) for triptolide in the range of $1.86 \times 10^{-2} - 3.46$ mg mL⁻¹, respectively, where X

was the concentration of the standards and *Y* was the NA of the standards, respectively. The detection limits of triptonide and triptolide were 1.26×10^4 mg mL⁻¹, 1.52×10^4 mg mL⁻¹, respectively. In addition, the reproducibility of migration time were 1.06 % and 1.43 % (RSD, n = 5), the reproducibility of normalized peak area were 2.28% and 3.76% (RSD, n = 5), respectively.

As stated above, the NA and migration time showed graphical repeatability, which indicated that a stable and repeatable separation could be obtained under such conditions. The electropherograms of the medicinal extract of chloroform of Multi-glycosides *Tripterygium wilfordii* tablet are shown in figure 2. The contents of triptonide and triptolide are listed in Table 1.

Comparisons of MECC with TLC and LC

The TLC- scanner procedure requires considerable expertise for its accurate application and requires visualizing the components with the kedde reagent, which is usually not very stable. Furthermore, TLC has a poor resolution. For qualitative analysis it presents a simple method, but its accuracy is very poor for quantitative determination³. HPLC is an accurate, sensitive and reliable method to separate and determine for triptonide and triptolide in *Tripterygium wilfordii* Hook F, but a number of prior steps are required to extract species of interest from the sample matrix and isolate them from potentially interfering compounds ⁴. Even with prior steps there is still much concern of column pollution in HPLC ⁸⁻¹⁰ because the contents in *Tripterygium wilfordii* Hook F are very complex. But in CE, all of these problems are avoided.

Conclusions

The developed MECC method proved to be a simple and effective method for the analysis of triptonide and triptolide Multi-glycosides *Tripterygium wilfordii* tablets. Under optimized experiment conditions, the specified compounds in *Tripterygium wilfordii* sample were baseline separated within 13 min. The values of relative standard deviations of migration times for triptonide and triptolide were lower than 2.39 (n = 5, %) and the recoveries for triptonide and triptolide were within 94.8 % ~ 98.6 %.

Table 1. Content of	f triptonide ai	nd triptolide in	the sample
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Sample	А		В	
	Triptonide	Triptolide	triptonide	triptolide
Content (mg mL ⁻¹)	0.398	0.033	0.401	0.031
RSD (n=5, %)	1.68	2.39	1.27	2.12
Recovery (%)	101.6	97.2	100.8	98.6

A= Multi-glycosides Tripterygium wilfordii tablets, batch number 990610

B= Multi-glycosides Tripterygium wilfordii tablets, batch number 991119

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REFERENCES

- Liu C, Peng M. Anticancer Chinese Herb Dictionary. *Tripterygium wilfordii* Hook F. Science and Technology Publishing co., Hubei. 382: 1072-6, 1994.
- Kupchan SM, Court WA, Dailay RG. Triptolide and Triptonide Novel Antileukemic Diterpenoid Triepoxides from *Tripterygium wilfordii* Hook. J. Amer. Chem. Soc. 94 (20): 7194-5, 1972.
- Gu WZ, Chen RD, Wein BR. Isolation Purification and Characterization of Immunosuppressive Compounds from *Tripterygium wilfordii* Hook F. Triptolide and Triptonide. Int. J. Immunopharmacol. 17 (5): 351-6, 1995.
- Cai JJ, Tao XL, Lipsky PE. High Performance Liquid Chromatographic Determination of Triptolide and Triptonide in an Ethyl Acetate Extract of *Tripterygium wilfordii* Hook F. J. Liq. Chromatogr. 17 (20): 4479-87, 1994.
- Tao XL, Cai JJ, Lipsky PE. The Identity of Immunosuppressive Components of the Ethyl Acetate Extract and Chloroform Methanol Extract of *Tripterygium wilfordii* Hook F. J. Pharmacology Exp. Ther. 272 (3): 1305-12, 1995.
- Zhang L, Zhang ZH, Sheng LS. Determination of Triptolide in *Tripterygium wilfordii* Hook F from Different Sources by Capillary Gas Chromatography. Journal of Chinese Pharmaceutical University. 23 (3): 158-200, 1992.
- 7. Xia ZL, Chen JY, Xu RQ. Alkaloids in *Tripterygium wilfordii* Hook F by Column Layer Chromatography Ultraviolet Spectroscopy. Chinese Traditional and Herbal Drugs. 23 (11): 579, 1992.
- Zhang HY, Song XR, Shi ZH, Yang GL, Hu ZD. Capillary Zone Electrophoresis for the Determination of Average Hydrodynamic Velocity and Diffusion Coefficient. Fresenius' Journal of Analytical Chemistry, 365(6): 499-503, 1999.
- Wang DX, Yang GL, Engelhardt H, Zhang HY. Micellar Electrokinetic Capillary Chromatography of Psoralen and Isopsoralen. Electrophoresis 20: 1895-1899, 1999.
- Yang GL, Song XR, Zhang HY, Wang DX, Sun HW. Identification and Determination of *Paeonol* and *Paeoniflorin* by Micellar Capillary Electrophoresis. Chinese Journal of Analytical Chemistry, 27 (1): 1-4, 1999.
- Chen JY, Xia ZL and Den FX. Improved Preparation of Triptolide Extract. Journal of Chinese Pharmaceuticals. 20 (5): 195-200,1989.

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毛細管電泳測定雷公藤多 片中 雷公藤內酯酮和雷公藤內酯醇

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建立毛細管電泳測定雷公藤多 片中的有效成分雷公藤內酯酮和雷公藤內酯醇方法。在優 化分離條件下,有效成分可達基線分離,有良好的線性關係。此方法簡便,快速。

關鍵詞:毛細管電泳,雷公藤多 片,雷公藤內酯酮和雷公藤內酯醇。

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