Simultaneous Determination of Triptolide, Wilforlide A and Triptonide in Human Plasma by High-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry

Jincheng YAO,* a Luyong ZHANG, a,b Xuyuan ZHAO, b Ling Hu, b and Zhenzhou JIANG a

* National Center of New Drug Screening, China Pharmaceutical University; Nanjing 210038, China; and b Clinical Pharmacy Research Institute, the Second Xiangya Hospital of Central South University; Changsha 410011, China.

Received February 7, 2006; accepted March 22, 2006

A simple and sensitive method for the determination of triptolide, wilforlide A and triptonide in human plasma was developed and validated, using high-performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS). Plasma samples were purified using solid-phase extraction (SPE) columns. The HPLC separation of the analytes was performed on a MACHEREY-NAGEL C18 column (2.0 mm × 125 mm, 3 μm), using 2.7 mM formic acid containing 10 mM ammonium acetate-acetonitrile (55:45, v/v) as mobile phase, with a flow-rate of 0.25 ml/min. The compounds were ionized in the electrospray ionization (ESI) ion source of the mass spectrometer and were detected in the selected ion recording (SIR) mode. The calibration curves were linear in the 0.80—300 ng/ml range for all the three analytes, and the limits of detection were 0.25, 0.40, and 0.35 ng/ml for triptolide, wilforlide A, and triptonide, respectively. The average absolute recoveries for all the three analytes were above 81%. The methodology recoveries were greater than 91% and the relative standard deviations (RSD) of intra-day and inter-day were less than 15%. The developed method was successfully applied to the determination of triptolide, wilforlide A and triptonide concentration in patients’ plasma after taking the medicament containing Tripterygium wilfordii Hook. F.

Key words triptolide; wilforlide A; triptonide; LC-ESI-MS

Tripterygium wilfordii Hook. F (TWHF), which is a perennial twining vine, grows densely on the shaded hill slopes in southern China. The extracts of the roots of the plant have been used in traditional Chinese medicine (named Lei-gong-teng in Chinese) for the treatment of autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, and skin diseases for many years.1,2) A number of diterpenoids and triterpenoids have been isolated from Tripterygium wilfordii,3-8) some of them showed significant biological activities. For example, triptolide, wilforlide A, and triptonide (Fig. 1) have potent antitumor, anti-inflammatory, immunosuppressive, and anti-fertility activities.3) However, most of the terpenoids in Tripterygium wilfordii have a narrow therapeutic index9-8) and some serious side effects.9,10) Therefore, accurate measurement of these terpenoids in blood samples is essential from a safety point of view.

Previous quantitative evaluations of these terpenoids have focused on triptolide in Tripterygium wilfordii and its ethyl acetate extracts. Analytical methods involved UV spectrophotometry,11) thin layer chromatography,12) HPLC with UV detection,13,14) and capillary electrophoresis.15) Most of them, however, were developed for quantitative determination of triptolide in plants or medical preparations. There were fewer methods reported to be used in biological fluids. Yang et al. developed a gas chromatography (GC) method16) for the determination of triptolide in human plasma. Wang et al. developed an HPLC method17) for the determination of triptolide and triptonide in human plasma. Although the published GC method16) was sensitive (LLOQ: 1 ng/ml), it required derivatization for triptolide with trifluoroacetic anhydride and required laborious sample pretreatment, therefore it was not convenient for analysis of large numbers of samples. The HPLC method17) was not sensitive enough (LLOQ: 10 ng/ml) to support the pharmacokinetic and toxicokinetic studies. Furthermore, the two methods needed a tedious long analysis time.16,17) The aim of this study is to develop a simple and selective high-performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) method for the determination of triptolide, wilforlide A and triptonide in human plasma.

MATERIALS AND METHODS

Equipment and Reagents A system of HPLC (Waters 2690, American)-MS with A Micromass QZ mass spectrometer (Wythenshawe, Manchester, U.K.) with mass-selective detector equipped with an electrospray ionization (ESI) ion source, and COMPAQ Deskpro Workstation and MassLynx™ 3.5 software were utilized for determination of the drug concentrations and data analysis.

Triptolide, wilforlide A, and triptonide were purchased from Pharmaceutical Industrial Institute of Fujian province (Fujian, China) and the internal standard (I.S.) diazepam was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Solid-phase extraction columns were 1 cc Oasis™ HLB cartridges (Waters Corporation, Milford, Massachusetts, U.S.A.). HPLC grade reagents (including methanol and acetonitrile) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Fig. 1. The Structures of Triptolide, Wilforlide A, and Triptonide

© 2006 Pharmaceutical Society of Japan
obtained from Tedia Company Inc. (Fairfield, America). Other reagents were of analytical purity grade obtained from Chemical Reagent Factory of Hunan (Changsha, Hunan, China). Deionized water was prepared by Millipore Simplicity water purification system. Control human plasma was obtained from the Blood Center of Shanghai (Shanghai, China).

**Standard Solutions** Triptolide, **wilforlide A**, triptonide, and diazepam (I.S.) were dissolved in methanol at concentrations of 1.0 mg/ml and stored at −20 °C before use. The stock solutions were successively diluted with methanol to

![Chromatograms of Triptolide, Wilforlide A, Triptonide, and I.S.](image)

(A) Control human plasma; (B) standard triptolide, **wilforlide A**, triptonide, and I.S. in control human plasma; (C) patient plasma samples after 1.5 h of oral administration of Lei-gong-teng duo-dai tablets (200 mg each time, three times daily).
prepare series concentrations of working solutions just prior to use. The working solutions were spiked into drug-free human plasma samples to determine the recovery, precision and the limit of detection of LC-ESI-MS method. All these solutions had no change in stability over a period of 1 month tested.

**Chromatographic Conditions** The analytes were separated on a MACHEREY-NAGEL C$_{18}$ column (2.0 mm×125 mm, 3 µm, Germany) with column temperature 45°C. The mobile phase was 2.7 mM formic acid containing 10 mM ammonium acetate–acetonitrile (55:45, v/v) and was filtered through 0.45 µm filters in a solvent filtration apparatus and was never recirculated. The flow-rate was 0.25 ml/min, and the postcolumn splitting ratio was 3 : 1.

**ESI-MS Detection Conditions** Sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The capillary voltage was 3.90 kV. The ion source temperature was 130°C, and the desolvation temperature was 360°C.

**Sample Preparation** Fifty microliters of diazepam (I.S., 200 ng/ml) was added to the sample (0.5 ml). The sample was alkalinized by adding 0.1 ml sodium hydroxide (0.1 M). After vortex-mixed for 1.5 min and centrifuged at 9500 rpm for 5 min, the mixture was immediately loaded onto the Oasis™ HLB extraction cartridge, which had been activated with 1 ml of methanol and balanced with 1 ml of deionized water. The loaded plasma samples were drained away under vacuum (~40 kPa) and the columns were washed with 1 ml of ammonia in water (2%, v/v). The samples were subsequently eluted into glass with 1 ml of methanol containing 2% (v/v) acetic acid. The eluents were dried under nitrogen in a 40°C water bath. The residue was reconstituted in 0.1 ml of mobile phase. A volume of 20 µl sample was injected into the HPLC.

**RESULTS AND DISCUSSION**

**LC-ESI-MS** The LC-ESI-MS in the SIR mode provided a highly selective method for the determination of triptolide, wilforlide A, and triptonide. The retention times of triptolide, wilforlide A, triptonide, and diazepam, respectively. Extractor voltage was 37, 35, 39, and 40 V for triptolide, wilforlide A, triptonide, and I.S., respectively. The capillary voltage was 3.90 kV. The sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The flow-rate was 0.25 ml/min, and the postcolumn splitting ratio was 3 : 1.

**ESI-MS Detection Conditions** Sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The capillary voltage was 3.90 kV. The ion source temperature was 130°C, and the desolvation temperature was 360°C.

**Sample Preparation** Fifty microliters of diazepam (I.S., 200 ng/ml) was added to the sample (0.5 ml). The sample was alkalinized by adding 0.1 ml sodium hydroxide (0.1 M). After vortex-mixed for 1.5 min and centrifuged at 9500 rpm for 5 min, the mixture was immediately loaded onto the Oasis™ HLB extraction cartridge, which had been activated with 1 ml of methanol and balanced with 1 ml of deionized water. The loaded plasma samples were drained away under vacuum (~40 kPa) and the columns were washed with 1 ml of ammonia in water (2%, v/v). The samples were subsequently eluted into glass with 1 ml of methanol containing 2% (v/v) acetic acid. The eluents were dried under nitrogen in a 40°C water bath. The residue was reconstituted in 0.1 ml of mobile phase. A volume of 20 µl sample was injected into the HPLC.

**RESULTS AND DISCUSSION**

**LC-ESI-MS** The LC-ESI-MS in the SIR mode provided a highly selective method for the determination of triptolide, wilforlide A, and triptonide. The retention times of triptolide, wilforlide A, triptonide, and diazepam, respectively. Extractor voltage was 37, 35, 39, and 40 V for triptolide, wilforlide A, triptonide, and I.S., respectively. The capillary voltage was 3.90 kV. The sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The flow-rate was 0.25 ml/min, and the postcolumn splitting ratio was 3 : 1.

**ESI-MS Detection Conditions** Sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The capillary voltage was 3.90 kV. The ion source temperature was 130°C, and the desolvation temperature was 360°C.

**Sample Preparation** Fifty microliters of diazepam (I.S., 200 ng/ml) was added to the sample (0.5 ml). The sample was alkalinized by adding 0.1 ml sodium hydroxide (0.1 M). After vortex-mixed for 1.5 min and centrifuged at 9500 rpm for 5 min, the mixture was immediately loaded onto the Oasis™ HLB extraction cartridge, which had been activated with 1 ml of methanol and balanced with 1 ml of deionized water. The loaded plasma samples were drained away under vacuum (~40 kPa) and the columns were washed with 1 ml of ammonia in water (2%, v/v). The samples were subsequently eluted into glass with 1 ml of methanol containing 2% (v/v) acetic acid. The eluents were dried under nitrogen in a 40°C water bath. The residue was reconstituted in 0.1 ml of mobile phase. A volume of 20 µl sample was injected into the HPLC.

**RESULTS AND DISCUSSION**

**LC-ESI-MS** The LC-ESI-MS in the SIR mode provided a highly selective method for the determination of triptolide, wilforlide A, and triptonide. The retention times of triptolide, wilforlide A, triptonide, and diazepam, respectively. Extractor voltage was 37, 35, 39, and 40 V for triptolide, wilforlide A, triptonide, and I.S., respectively. The capillary voltage was 3.90 kV. The sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The flow-rate was 0.25 ml/min, and the postcolumn splitting ratio was 3 : 1.

**ESI-MS Detection Conditions** Sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The capillary voltage was 3.90 kV. The ion source temperature was 130°C, and the desolvation temperature was 360°C.

**Sample Preparation** Fifty microliters of diazepam (I.S., 200 ng/ml) was added to the sample (0.5 ml). The sample was alkalinized by adding 0.1 ml sodium hydroxide (0.1 M). After vortex-mixed for 1.5 min and centrifuged at 9500 rpm for 5 min, the mixture was immediately loaded onto the Oasis™ HLB extraction cartridge, which had been activated with 1 ml of methanol and balanced with 1 ml of deionized water. The loaded plasma samples were drained away under vacuum (~40 kPa) and the columns were washed with 1 ml of ammonia in water (2%, v/v). The samples were subsequently eluted into glass with 1 ml of methanol containing 2% (v/v) acetic acid. The eluents were dried under nitrogen in a 40°C water bath. The residue was reconstituted in 0.1 ml of mobile phase. A volume of 20 µl sample was injected into the HPLC.

**RESULTS AND DISCUSSION**

**LC-ESI-MS** The LC-ESI-MS in the SIR mode provided a highly selective method for the determination of triptolide, wilforlide A, and triptonide. The retention times of triptolide, wilforlide A, triptonide, and diazepam, respectively. Extractor voltage was 37, 35, 39, and 40 V for triptolide, wilforlide A, triptonide, and I.S., respectively. The capillary voltage was 3.90 kV. The sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The flow-rate was 0.25 ml/min, and the postcolumn splitting ratio was 3 : 1.

**ESI-MS Detection Conditions** Sample cone and desolvation nitrogen were at the rate of 114 and 350 l/h, respectively. The capillary voltage was 3.90 kV. The ion source temperature was 130°C, and the desolvation temperature was 360°C.

**Sample Preparation** Fifty microliters of diazepam (I.S., 200 ng/ml) was added to the sample (0.5 ml). The sample was alkalinized by adding 0.1 ml sodium hydroxide (0.1 M). After vortex-mixed for 1.5 min and centrifuged at 9500 rpm for 5 min, the mixture was immediately loaded onto the Oasis™ HLB extraction cartridge, which had been activated with 1 ml of methanol and balanced with 1 ml of deionized water. The loaded plasma samples were drained away under vacuum (~40 kPa) and the columns were washed with 1 ml of ammonia in water (2%, v/v). The samples were subsequently eluted into glass with 1 ml of methanol containing 2% (v/v) acetic acid. The eluents were dried under nitrogen in a 40°C water bath. The residue was reconstituted in 0.1 ml of mobile phase. A volume of 20 µl sample was injected into the HPLC.
ng/ml). Recovery of I.S. was consistent, precise and reproducible.

**Sensitivity** The limits of quantitation (LOQ) were 0.80 ng/ml for the three analytes (S/N=10). The limits of detection (LOD) were 0.25, 0.40, and 0.35 ng/ml for triptolide, wilforlide A, and triptonide, respectively (S/N=3).

**Analysis of Patient Plasma** Plasma samples were obtained from 5 systemic lupus erythematosus schizophrenia patients (35±4 y, female) after oral administration of Lei-gong-teng duo-dai tablets (200 mg each time, three times daily). The concentrations of triptolide, wilforlide A, and triptonide were determined by the LC-ESI-MS method, and the results are shown in Table 4. Concentrations of triptolide, wilforlide A, and triptonide in these patients’ plasma have obvious individual variation, so the therapeutic drug monitoring (TDM) is necessary to achieve the best therapeutic effects and the least toxicity.

**CONCLUSION**

An LC-ESI-MS method was developed for simultaneous determination of triptolide, wilforlide A, and triptonide in human plasma. The method is simple, accurate and sensitive. Thus, it is suitable for clinical and toxicological studies.

**Acknowledgements** The authors would like to thank Miaoyuan Zhang for clinical sample collection.

**REFERENCES**