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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 472–477
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Using LC/MS/MS to determine matrine, oxymatrine, ferulic acid, mangiferin, and glycyrrhizin in the Chinese medicinal preparations Shiau-feng-saan and Dang-guei-nian-tong-tang

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Received 27 May 2005; received in revised form 26 July 2005; accepted 29 July 2005
Available online 21 September 2005

Abstract

We have developed a simple, rapid, selective, and reproducible method for the quality control of traditional Chinese medicinal preparations. In this study, we used LC/MS/MS to simultaneously identify and quantify five marker compounds – matrine, oxymatrine, ferulic acid, mangiferin, and glycyrrhizin – in preparations of Shiau-feng-saan and Dang-guei-nian-tong-tang. The calibration curves for the five marker compounds were linear over the concentration range 50–2500 ng/mL ($R^2 > 0.9971$). The matrix effect was minimized and the recoveries of the five marker compounds were >90% at a concentration of 1 μg/mL. Our experimental data reveal that significant differences exist between samples obtained from different sources.

Keywords: Matrine; Oxymatrine; Ferulic acid; Mangiferin; Glycyrrhizin; Shiau-feng-saan; Dang-guei-nian-tong-tang; *Sophorae* Radix; *Angelicae sinensis* Radix; *Anemarrhena* Rhizoma; *Glycyrrhizae* Radix

1. Introduction

1.1. Chinese medicinal preparations

Chinese herbal medicines (CHMs) and Chinese proprietary medicines (CPMs) are used widely throughout the world. In recent years, decoction dosage forms have gradually been replaced by the concentrated dosage forms, which are adapted widely for clinical treatment. Quality control, however, of those concentrated forms is difficult because the materials are derived from many different sources. Hence, to improve the quality of CPMs, specific components have been selected as markers for the analysis of Chinese medicines; this approach is highly promising [1–3].

A number of LC/MS methods have been developed for the determination of one or two constituents in crude drugs [4–6]. There have been few reports, however, on the simultaneous determination of multiple constituents in preparations containing very complicated matrices. The LC/MS technique has attracted a great deal of attention because of the fact that it does not require sample derivatization and because it allows the simultaneous determination of non-volatile and thermally unstable compounds. Consequently, liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) has become the important technique for the analysis of Chinese herbal medicines.

In this study, we examined two concentrated preparation dosages: Shiau-feng-saan and Dang-guei-nian-tong-tang. They both contain the same four herbs – *Sophorae* Radix, *Angelicae sinensis* Radix, *Anemarrhenae* Rhizoma, and *Glycyrrhizae* Radix – and we selected five marker substances for their analysis: matrine, oxymatrine, ferulic acid, mangiferin, and glycyrrhizin. We used liquid chromatogra-

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doi:10.1016/j.jpba.2005.07.036

phy coupled with an ion trap mass spectrometer to verify the presence of these five marker substances. Next, we examined the feasibility of applying this method to determine the five markers in real samples by analyzing commercial Chinese herbal formulations. Our long-term efforts are focused on developing methods for determining every representative marker of each herb in traditional Chinese medicines (TCMs).

1.2. Marker compounds in herbs

Fig. 1 presents the structures of the five marker compounds we chose for the four target herbs. Matrine and oxymatrine are the main active components of *Sophorae* Radix. Matrine displays antibacterial and antitumor activity; oxymatrine has been used as an efficient antitumor treatment [7–9]. These compounds are, however, toxic and may paralyze the respiratory system. The LD_{50} of matrine i.v. to mice is 72.1 mg/kg [10]. Ferulic acid, an aromatic acid, is one of the target compounds of *Angelicae sinensis* Radix. It is the bioactive compound that is used for its anti-inflammatory and anti-asthmatic effects [11]. Mangiferin (MA) is one of the main active compounds in *Anemarrhenae* Rhizoma. It is a natural glucosyl xanthone that is used to treat skin diseases (dermatosis). Recent studies have indicated that MA is an active agent for antitumor, anti-HIV, antiviral, and anticancer treatments [12,13]. It does, however, cause diarrhea when used in overdose amounts. Glycyrrhizin (GL), a triterpene saponin, and a principal active component of *Glycyrrhizae* Radix, has been used to cure Addison's disease, although it may induce heart disease after long-term use. It has also been used as an internal standard in LC/MS for the analysis of soy saponins [14]; other studies of GL through the use of LC/MS are rare. Saikosaponin-a, which we used as the internal standard in this study, is one of the major compounds found in the herbal medicine *Bupleuri* Radix.

2. Experimental

2.1. Materials and reagents

Matrine and oxymatrine were obtained from Chuang Song Zong Pharmaceutical Company (Pingtung, Taiwan). Mangiferin was purchased from Sigma (St. Louis, MO, USA). Ferulic acid was obtained from Aldrich (USA). Glycyrrhizin was purchased from Nacalai Tesque (Japan).

Shiau-feng-saan and Dang-guei-nian-tong-tang were purchased from the Chuang Song Zong Pharmaceutical Company (Ligang, Pingtung; samples S-01 and D-01), Sun Ten Pharmaceutical Company (Taichung; S-02 and D-02), and Sheng Foong Pharmaceutical Company (Taipei; S-03 and D-03) of Taiwan.

Shiau-feng-saan is used to cure eczema, urticaria, rubeola, and medica mentosa dermatitis. Shiau-feng-saan contains 13 herbs, including *Sophorae* Radix, *Angelicae sinensis* Radix, *Anemarrhena* Rhizoma, *Glycyrrhizae* Radix, *Soposhinkoviae* Radix, *Atractylodis* Rhizoma, *Akebiae* Caulis, *Gypsum* Fibrosum, *Arctii* Fructus, *Rehmanniae* Radixet Rhizom, *Cicadae* Periostracum, and *Schizonepetae* Herba.

Dang-guei-nian-tong-tang is used as a remedy for waist soft-tissue strain, rheumatic arthritis, sciatic neuralgia, and rheumatoid arthritis. Dang-guei-nian-tong-tang contains 15 herbs, including *Sophorae* Radix, *Angelicae sinensis* Radix, *Anemarrhena* Rhizoma, *Glycyrrhizae* Radix, *Artemisiae capillaris* Herb, *Notopterygii* Rhizoma, *Cimicifugae* Rhizoma, *Puerariae* Radix, *Atractylodis* Rhizoma, *Scutellariae* Radix, *Ginseng* Radix, *Polyporus*, *Alismatis* Rhizoma, and *Atractylodis* Rhizoma.

2.2. Preparation of standard solutions

Matrine, oxymatrine, ferulic acid, mangiferin, and glycyrrhizin were dissolved in 70% methanol and in matrix solution (in the extract of Dang-guei-nian-tong-tang) to provide a series of concentrations in the range 50–2500 ng/mL, respectively. The internal standard, saikosaponin-a, was added to each vial at a concentration of 500 ng/mL. Calibration curves were plotted after linear regression of the ratios of the peak areas of the analytes to those of the internal standard.

2.3. Extraction and preparation of samples

The powder form of the crude drug was accurately weighed (0.5 g), washed with *n*-hexane, and then a 10-fold mass of a methanol–water mixture (7:3, v/v) was added. The solutions were extracted for 30 min in an ultrasonic bath and then were centrifuged for 10 min at 5000 rpm. The supernatant was collected and 70% methanol was added up to a total of 10 mL to provide the real sample stock solution. This solution was diluted 20-fold. An appropriate amount of the internal standard was spiked. The sample solution for analysis was obtained after filtering through a 0.22-μm membrane filter.

2.4. Liquid chromatography conditions

LC analyses were performed using a Surveyor liquid chromatography system (Thermo Finnigan, San Jose, CA, USA). The five marker substances and the internal standard were separated on a Phenomenex 5-μm Luna C18 column (150 × 2.0 mm ID) using an injection volume of 5 μL. Gradient elution was achieved using two solvents – (A) 0.005% (v/v) trifluoroacetic acid buffer (pH 3) and (B) acetonitrile – at a flow rate of 200 μL/min. The linear gradient program that gave the optimal sensitivity was the following: a linear increase from 5% solution B to 10% over 1.5 min, increased to 40% solution B over 8.5 min, maintained isocratically for 5 min, increased to 50% solution B over 7 min, increased to 90% solution B over 5 min, increased to 100% B over 3 min, and then maintained at that level for a further 8 min.