Simultaneous Determination of Paeoniflorin, *Trans*-cinnamic Acid, Schisandrin and Glycyrrhizin in So-Cheong-Ryong-Tang by HPLC-DAD and HPLC-ESI-MS

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Abstract – High performance liquid chromatographic method with diode-array detection (HPLC-DAD) has been performed for the simultaneous determination of four marker constituents, paeoniflorin, *trans*-cinnamic acid, schisandrin and glycyrrhizin in traditional herbal medicinal preparation, So-Cheong-Ryong-Tang (SCRT). The presence of paeoniflorin, *trans*-cinnamic acid, schisandrin and glycyrrhizin in this decoction was ascertained by retention time, spiking with each authentic standard, UV spectrum and ESI mass spectrum. All four compounds showed good linearity (r² > 0.998) in a relatively wide concentration ranges. The RSD for intra-day and inter-day precision was less than 3% and the limits of detection (LOD) were less than 30 ng. The mean recovery of each compound was 94.1 – 113.0% with RSD values less than 3.0%. These results suggest that the developed HPLC method is simple, effective and could be readily utilized as a quality control method for commercial SCRT products.

Keywords – So-Cheong-Ryong-Tang (SCRT), HPLC-DAD, HPLC-ESI-MS, validation

Introduction

Traditional herbal medicinal preparations are mostly used in combination of many herbs. Multiple constituents from each herb are known to be responsible for their therapeutic effects (Xue and Roy, 2003), however, the quality of each herb has been affected by many factors such as cultivation environment and manufacturing process (Wang et al., 2002; Antonnen et al., 2006). In addition, even though each herb has been mixed in the same ratio, different preparation procedure such as cutting size of herbs, temperature, time, pressure for extraction may affect the amounts of various constituents in the decoction. In other words, all these factors can affect the therapeutic effects and/or safety of traditional medicinal preparation. Therefore, the need for quality assessment of major active components in traditional herbal medicinal preparation has been increased. As such, numerous studies related to quality control have been carried out, mainly by the determination of major and/or active constituents (Zang and Cheng, 2006; Sheng et al., 2005; Li et al., 2006).

So-Cheong-Ryong-Tang (SCRT) is combinational herbal decoction that consists of *Ephedra sinica*, *Paeonia lactiflora*, *Asarum heterotropoides var. mandshuricum*, *Zingiber officinale*, *Glycyrrhiza glabra*, *Cinnamomum cassia*, *Pinellia ternata* and *Schizandra chinensis*. SCRT has been used as herbal medicines for allergic diseases, such as allergic rhinitis and asthma, for hundreds of years in Asian countries (Amagaya et al., 2001; Kao et al., 2001; Ko et al., 2004). Untill now, several analytical methods using HPLC have been reported for each herbal component of SCRT (Jung et al., 1998; Okamura et al., 1999a; Okamura et al., 1999b; Wang and Yang, 2007; He et al., 2005; Halstead et al., 2007; Dong et al., 2007). However, there have been no reports about the simultaneous quantitative determination of their major constituents of SCRT. Thus, to ensure the efficacy and safety, a suitable assay method for quality control has been required.

In the present study, paeoniflorin (1), *trans*-cinnamic
acid (2), schisandrin (3) and glycyrrhizin (4), the four marker constituents of SCR T that KFDA has designated, were screened and identified by HPLC-ESI-MS technique. In addition, a HPLC/DAD method was developed and validated.

Experimental

Materials — All four compounds, paeoniflorin (1), trans-cinnamic acid (2), schisandrin (3) and glycyrrhizin (4), were purchased from Wako (Osaka, Japan). All of the plants were purchased from Kyungdong traditional herbal market (Seoul, Korea) and were authenticated by Prof. Jong Hee Park in the College of Pharmacy, Pusan National University. The commercial SCR T products from medicinal companies were purchased from local providers. HPLC grade solvents (acetonitrile, water and methanol) and reagents were obtained from BDH chemicals (Poole, UK). Phosphoric acid (analytical grade) was purchased from Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

Instrumentation and chromatographic conditions — The HPLC system was consisted of a chromatographic pump (P680, Dionex, Germany), an injector (7725i, Rheodyne, USA) equipped with Photo Diode Array (UVD 340U, Dionex, Germany). The output signal of the detector was recorded using a Dionex Chromelon™ Chromatography Data System. Chromatographic separation was achieved on a Waters XTerra™ RP18 (5 μm, 4.6 mm I.D. × 150 mm). A linear gradient elution of A (0.03% phosphoric acid) and B (100% acetonitrile) was used (0 min, 10% B; 18 min, 10% B; 40 min 30% B; 55 min, 47% B; 58 min, 10% B; 60 min, 10% B; v/v) at a flow rate of 1.0 ml/min. The diode-array UV/vis detector (DAD) was used for the detection and the wavelength for quantification was set at 250 nm.

HPLC-ESI-MS system consisted of Finnigan Surveyor HPLC system with a pump, an autosampler, a PDA plus detector, and Finnigan LCQ advantage MAX (ion trap mass spectrometer) with Xcalibur software. Separation was achieved on a Waters XTerra™ RP18 (5 μm, 4.6 mm I.D. × 150 mm). A linear gradient elution of A (0.03% formic acid) and B (100% acetonitrile) was used (0 min, 10% B; 30 min, 40% B; 55 min 55% B; 56 min, 10% B; 60 min, 10% B; v/v) at a flow rate of 0.3 ml/min. The flow rate was 0.3 ml min⁻¹, and the injection volume 5 μL. The conditions were as follows: capillary temperature 270°C, i spray voltage 5 kV, sheath gas flow rate 22 arbitrary units, aux/sweep gas flow rate 0 arbitrary units.