

Abstract

Radix Notoginseng (Sanqi) is a valuable Chinese Medicine (CM) and widely used in clinical. Some previous studies showed that saponins are the main active ingredients in this CM. Xuesaitong injection, a sterilized injection, was prepared by extracting the total saponins from the crude materials of Radix Notoginseng. Previous pharmacological studies indicated that this CM has many cardiovascular activities such as anti-platelet aggregation, anti-thrombosis and anti-arrhythmias. According to the standard set by the Ministry of Health P. R. China(The pharmaceutics of Chinese medicine prescription, volume 19) , a double wave scan of thin layer chromatography (TLC) was recommended for the detection of the content in Xuesaitong injection. However, factors such as structural diversity, large molecule structure, lack of chromophores of different saponins make analysis of individual saponin very difficult. In addition, TLC is an opening system which means the results produced by this method are highly affected by the environmental conditions and therefore show poor reproducibility. In some previous studies, a quantitative method was developed to analyze the Sanqi saponins by using HPLC coupled with UV or diode array detector (DAD). The main problem for this method is due to the lack of ultraviolet (UV) absorption in most of the saponins. Although evaporating light scattering detector (ELSD) can solve the problem of no UV absorption, it has a drawback of not able to detect most trace components within the detectable limits. Therefore, in this study, HPLC-MS-MS method was employed as a key technique for the qualitative and quantitative analysis of the injection.

In the present studies, Agilent 1100 Series LC/MSD VL Trap System and LC/MSD Trap Software Version 4.2 were used for data analysis. A gradient elution system consisted of water and acetonitrile was used for mobile phase. Additionally, 8 mM ammonium acetate was added in the water phase as electrolyte solution. The flow rate of the mobile phase was set at 0.4 ml/min and the total analytical time was 40 min. At the same time, an electro-spray ionization (ESI) source and negative ion mode were used in the mass spectrometer and the scan range was from 200 to 1400u. Full scan mode (auto MS/MS) and selected reaction monitoring (SRM) were used for the qualitative and quantitative analysis. In the test results of limit of detection (LOD) and limit of quantification (LOQ),

the detection limit was found to reach pg level for most components in the injection. The result showed a high sensitivity for this detection method. In the qualitative analysis, 27 peaks could be identified with the help of 9 reference standards and the fragmentation patterns in the mass spectra. Comparing 10 different batches and/or sources of Xuesaitong injection and their corresponding crude material (Sanqi), the results showed that all samples were with very similar saponin components. In the quantitative analysis, notoginsenoside R_1 , ginsenoside R_{b1} , R_{b2} , R_{b3} , R_c , R_d , R_e , R_f and R_{g1} were quantified. The results obtained from the 10 batches of Xuesaitong injection showed that the notoginsenoside and ginsenoside content varied a lot from batch to batch, even for those injections manufactured by the same factory. The content of the components was found to vary as follows: notoginsenoside R_1 from 6.63% to 10.50%, ginsenoside R_{g1} from 21.30% to 39.00%, R_e from 1.99 to 4.89%, R_f from 0.04 to 0.26%, R_{b1} from 37.49 to 50.37%, R_c from 0.03 to 0.04%, $R_{b2} + R_{b3}$ from 0.52 to 0.93% and R_d from 8.61 to 16.62 %. From these findings, it is better to ensure the quality of Xuesaitong injections by multi-ingredient evaluation or fingerprinting method. In conclusion, HPLC/MS/MS provides a quick, sensitive and highly effective method for the qualitative and quantitative analysis of those components without UV absorption. Moreover, it may also be used as a potential method for the quality control of crude CM material and its related products.

Key words: HPLC-MS, *panax notoginseng*, Xuesaitong (Sanqi) injection, notoginsenoside, ginsenoside, quality control