EXTRACTION AND ANALYSES OF CHIRAL ISOMERS (EIPGOITRIN/GOITRIN) FROM *ISATIS INDIGOTICA* FORT ROOT EXTRACT USING SFE AND SFC-MS

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Traditional Chinese Medicines (TCMs) have played an important role in Asia as major clinical therapies for thousands of years. In the past decade, the usage of TCMs has expanded globally and gained increasing acceptance as a complement to modern Western medicine. A large number of TCMs are chiral compounds. Since optical isomers could possess considerable different bioactivities, it is therefore important to quantify enantiomers in order to better understand stereo-specific bio-disposition of these enantiomers in TCM.

(R, S)-goitrin are found in *Isatis indigotica* Fort (known as the *ban lan gen* in China), but only the R-goitrin (Epigoitrin) displays the desired antiviral activity. Currently, the quantitative analysis of (R, S)-goitrin often involves a solvent extraction step, which can take several hours to days, followed by a RPLC based quantitation methodology. However, RPLC does not resolve R- and S-goitrin; therefore it can't accurately quantify the bioactive R-goitrin. Recently, Lin et al. demonstrated a normal phase HPLC/UV based chiral separation and quantitation of R- and S-goitrin. The separation time was 50 min. Here, we report a supercritical fluid based workflow for qualitative and quantitative analysis of (R, S)-goitrin: SFE extraction followed by SFC-PDA-MS based separation and quantitation of R- and S-goitrin with significantly reduced overall process time.

We first used the racemate to develop an SFC method for the chiral separation of (R, S)-goitrin. Multiple chiral stationary phases (CSPs) and co-solvents were screened using a generic gradient. Next, we will explore the SFE extraction process. Parameters such as pressure, co-solvent and processing time will be varied to selectively extract (R, S)-goitrin from the *Isatis indigotica* Fort root. The extraction efficiency and specificity will be assessed and compared to the traditional solvent extraction approach. Finally, we will optimize the chromatographic conditions for the extract analysis to reduce the run time. The optimized chromatographic conditions will then be applied to perform a series of quantitative analyses of R- and S-goitrin using SFC-PDA-MS, including LOD, LOQ, linearity, calibration curve, precision, accuracy, reproducibility, stability and recovery. Analyses of several commercially available *ban lan gen* powder will also be presented.