Abstract

Since the events of aristolochic acid poisoning were reported widely around the world, the safety of Traditional Chinese Medicine (TCM) was greatly concerned. The thesis optimized the supercritical fluid extraction (SFE) conditions of aristolochic acids I & II (AAI & II) from Aristolochia plants---*Fructus Aristolochiae* and *Caulis Aristolochiae manshuriensis*. The influences of related factors on the extraction efficiency and possible reasons were discussed. Selectivity of the optimized SFE method was investigated for the potential application of removing aristolochic acids in the future.

Extraction pressure (p), temperature (T) and mole fraction (x) of the entrainer---absolute ethanol were mainly optimized, which had great interactions. Based on the CO_2-ethanol P-T-x phase diagram, a special univariate design was introduced that was able to assist with fast analysis of results. Extraction time and mode were also investigated. In the expèriments, the sample powder was extracted by supercritical fluid and the residue was then analyzed on the extraction rates of AAI & II by pressurized liquid extraction (PLE) and high performance liquid extraction (HPLC), which were all optimized.

After optimization, the final SFE conditions were the extraction pressure of 194 bar, extraction temperature of 50℃, mole faction of the entrainer---absolute ethanol of 0.20, extraction time of 4 hours including 3-hour dynamic extraction. Under this method, the extraction rates of AAI & II for *Fructus Aristolochiae* and *Caulis Aristolochiae manshuriensis* were 65.23%, 59.07% and 81.25%, 81.19%, respectively. Mole fraction of the entrainer was dominant factors, while the extraction pressure and temperature had little influences. Besides, extraction mode had great effect. Dynamic mode was much better than the static one. Selectivity, depicted by the change of peak area of individual peak or peak combination, was investigated and the optimized SFE method did show its selectivity. In a word, the optimized SFE method was able to extract AAs from *Fructus Aristolochiae* and *Caulis Aristolochiae manshuriensis*. Mole fraction of the entrainer and extraction mode were the determinants. Selectivity of this method offered opportunity for the future application of SFE method in the removal of AAs from *Aristolochia* plants.

The optimized HPLC condition was able to separate and detect the analytes

satisfactorily. PLE method, mainly influenced by the extraction solvent, particle size of the sample powder, extraction temperature and pressure, exhibited high efficiency and good applicability in the pretreatment of *Aristolochia* samples and was much better than conventional methods.

Five chapters are included in this thesis. Chapter 1 is the introduction to the background, objective and the experimental design of this research and related information on *Aristolochia* plants and aristolochic acids. Chapter 2 focuses on the optimization of the HPLC method and discusses the generations of errors. Chapter 3 gives the overview of the optimization of PLE method by the univariate way and recovery, applicable and comparison experiments were also presented. Chapter 4 is the key content of this thesis. SFE optimization is discussed including several factors such as p-T-x. as well as other factors based the phase diagram. Selectivity of SFE method is also investigated. Chapter 5 is the conclusion of this study.

Key words: Supercritical fluid extraction; *Fructus Aristolochiae*; *Caulis Aristolochiae manshuriensis*; Aristolochic acid; HPLC; PLE; Univariate Design