Qualitative and Quantitative Analysis of Volatile Components in *Curcuma longa* Based on Virtual (Digital) Chemical Standards

By

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Supervisor

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碩士學位論文

基於數字化參照品的薑黃揮發性成份定性定量分析

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Abstract

Qualitative and quantitative analysis of volatile components in *curcuma longa* based on virtual (digital) chemical standards

by

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Thesis Supervisor: Professor Li Shao-Ping

Major: Chinese Medicinal Sciences

Curcuma longa L. belongs to the family Zingiberaceae, is one of the most valued medicinal herbs in traditional Chinese medicine (TCM), essential oil and curcuminoids are considered as main fractions of *Curcuma longa.* Recent research showed that: *Curcuma longa* volatile components have effects on anti-oxidant, anti-tumor, anti-inflammation and anti-atherosclerosis.

As recorded in Chinese pharmacopoeia 2010, Curcuminoids is used as the marker for quality control of *Curcuma longa*, and total volatile oil should not be less than 7%. Because the chemical components especially the volatile compounds from TCMs are not only difficult to obtain, but also hard to be preserved due to their instability. Therefore, absence of chemical standards has become a bottleneck for the internationalization of Chinese medicines. *Curcuma longa* oil has no quantitative items for its quality control. Therefore, comparison of chemical volatile components in oil is very important for quality control of *Curcuma longa*. The thesis contained four chapters:

Chapter 1 is a review of volatile compounds in *Curcuma longa*, including the research of chemical components, pharmacological activity and its quality control.

Chapter 2 is focused on the isolation and purification of components in oil of *Curcuma longa*. Four pure compounds ar-turmerone, β-turmerone, α-turmeron and α-atlantone were obtained, which were confirmed by UV, MS. The purities of them are 98%, 97%, 97%, 99%.

Their quality control method of volatile oil in *Curcuma longa* was developed in

Chapter 3. Optimized GC-MS condition: The column temperature was at 80 °C for injection, then programmed at 20 °C /min to 150 °C and held for 10 min, then at ,40 °C /min to 280 °C . Split injection (2 μL) was conducted with a split ratio of 10:1 and high purity helium was used as carrier gas of 1.0 mL/min flow rate. Five main compounds were identified by the method of digital qualitative and quantitative analysis. Through methodology validation, this method show high stability, veracity, reliability. Application of digital chemical standards method in chapter 4 proved its possibility. The results also showed that powder sample, which are extracted by PLE has low contents of ar-curcumene and zingiberene.

5.1 To sum up, five n-alkanes have been selected as markers for analysis of *curcuma longa* oil. And a rapid qualitative and quantitative analysis GC-MS method was established for volatile components in *Curcuma longa* with good selectivity of detection and high repeatability. Therefore, this study established a virtual (digital) chemical reference substance, and provided new method to qualitatively and quantitatively analyses the volatile components of TCMs, in the absence of physical chemical reference substance. This method may solve the shortage of the status quo of traditional Chinese medicine physical chemical markers in same ways.

Key words: *Curcuma longa*; Standards; Volatile components; Digital; GC-MS; PLE

摘　要

基於數字化參照品的薑黃揮發性成份定性定量分析

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薑黃是我國名貴的傳統中藥材，其主要成份為揮髮油和薑黃素類。現代研究表明薑黃的揮發性成份具有抗氧化、抗炎和保肝等作用。據 2010 版中國藥典記載，薑黃以其薑黃素類成份含量為質量控制標準，同時規定其全揮髮油含量不得少於 7%。由於中藥揮發性成份的不穩定性，其有效成份不僅難獲得且難以保存，缺乏對照品。現階段缺少化學成份標準品成為了中藥材國際化道路上的瓶頸。薑黃揮髮油揮發性成份的質量控制方面缺少定性，定量分析。因此，中藥中揮發性化學對照品庫的建立對中藥材的質量控制有至關重要的意義。本研究旨在本實驗室前期研究基礎上，建立薑黃油的數字化對照品庫。本論文包含以下章節：

第一章：全面綜述了薑黃揮發性成份，包括其化學組成，藥理活性研究，多種提取方法的比較，現代分離、製備揮發性單體技術的總結，中藥揮發性成份分析方法以及中藥標準品研究現狀等。

第二章：重點講述了通過分子蒸餾儀富集薑黃揮髮油中含氧倍半萜類化合物。分子蒸餾餾出物經高速逆流色譜處理后實現了很好的分離及純化，獲得了四種純的化學成份：芳薑黃酮、β 薑黃酮、α 薑黃酮和大西洋酮，并經過 UV 掃描和 MS 分析確認，其純度分別為 98%、97%、97%、99%以上。

第三章：建立了薑黃揮髮油成份質量控制新方法，並在本章中詳細陳述。主要分為三個部份：第一節.GC-MS 方法的優化最終條件為程序升溫梯度：初始柱溫為 80℃,以 20℃/分鐘到 150℃，保持 10℃，以 40℃ /分鐘升到 280℃停止。分流模式進樣，進樣量為 2 μL，分流比為 10：1，以高純氮氣為載氣，流速 1mL/min。

第二節在優化過的 GC-MS 分析條件下確定定性參數的虛擬化。第三節主要為揮發性成份定量參數的虛擬化及該方法的方法學驗證。

第四章：數字化對照品在薑黃藥材 5 種揮發性成份定性定量分析中應用。結果顯示，以正構烷烴做內標對薑黃藥材中揮發性成份定性分析準確度高。薑黃藥材中揮發性成份在一定濃度範圍內以虛擬對照品進行定量的結果與個對照品的實際濃度基本一致，說明該方法在沒有對照品的情況下對薑黃揮發性成份定性定量分析是可行的。

總結：本研究建立了以系列正構烷烴作為分析薑黃揮髮油的替代對照品，快速對薑黃油揮發性成份進行定性，定量分析的 GC－MS 方法。本實驗旨在建立虛擬對照品方法對薑黃揮發性成份進行定性,定量分析，以期能夠解決傳統化學對照品難以分離和保存等問題，為中藥薑黃揮發性成份的質量控制提供了一個新方法。

關鍵字：薑黃；揮髮油；標準品；虛擬數字化；GC-MS；PLE

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List of Abbreviation

Abbreviations	Full Name
AMDIS	Automatic Mass Spectral De convolution and Identification System
CAS	Chemical Abstracts Service
DAD	Diode Array Detector
ELSD	Evaporative Light Scattering Detector
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HSCCC	High Speed Counter-Current Chromatography
LOQ	Limit of quantitation
LOD	Limit of detection
MD	Molecular Distillation
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NIST	National Institute of Standards and Technology
PLE	Pressurized Liquid Extraction
RSD	Relative standard deviation
SD	Standard deviation
SFE	Supercritical Fluid Extraction
RSD	Relative standard deviation
TNF	Tumor Necrosis Factor