

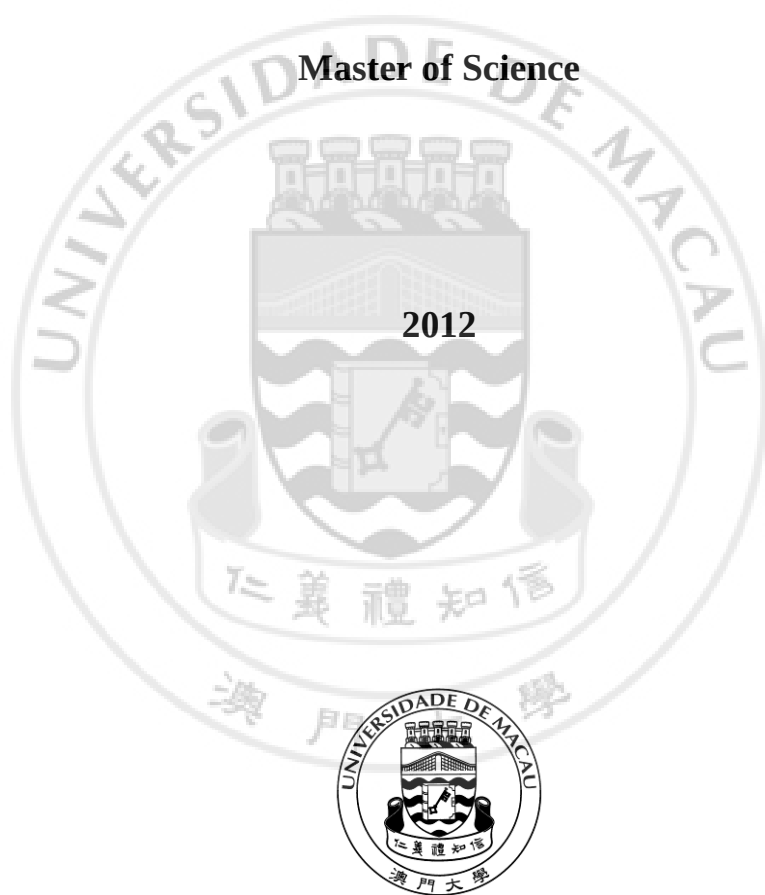
**Qualitative and quantitative analysis of natural and cultured
Cordyceps using high performance thin layer chromatography**

by

Leong Nga I

Master of Science

2012



Institute of Chinese Medical Sciences

University of Macau

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A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science

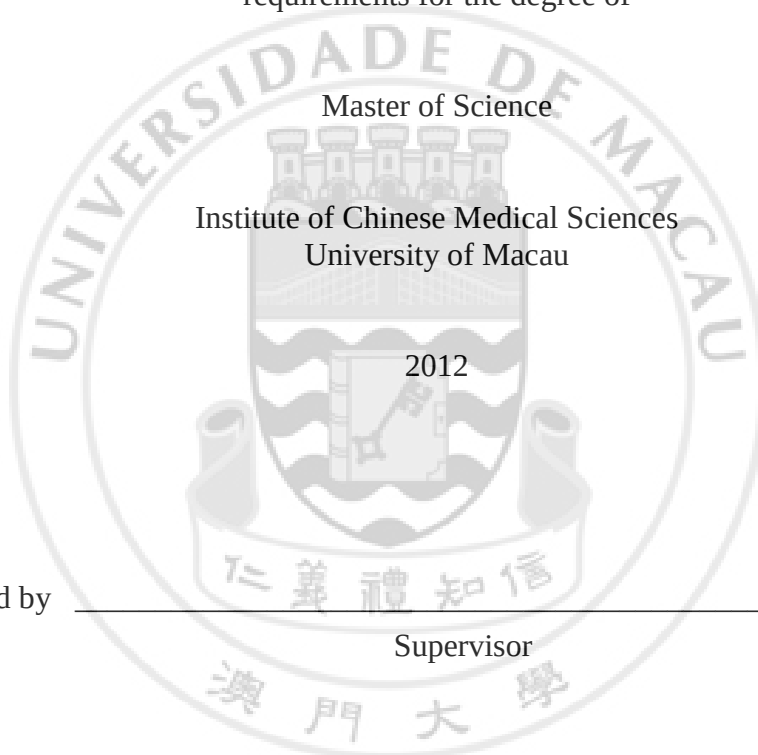
Institute of Chinese Medical Sciences
University of Macau

2012

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碩士學位論文

天然和人工蟲草高效薄層色譜分析

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日期：2012 年 7 月



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Abstract

Qualitative and quantitative analysis of natural and cultured *Cordyceps* using high performance thin layer chromatography

Leong Nga I

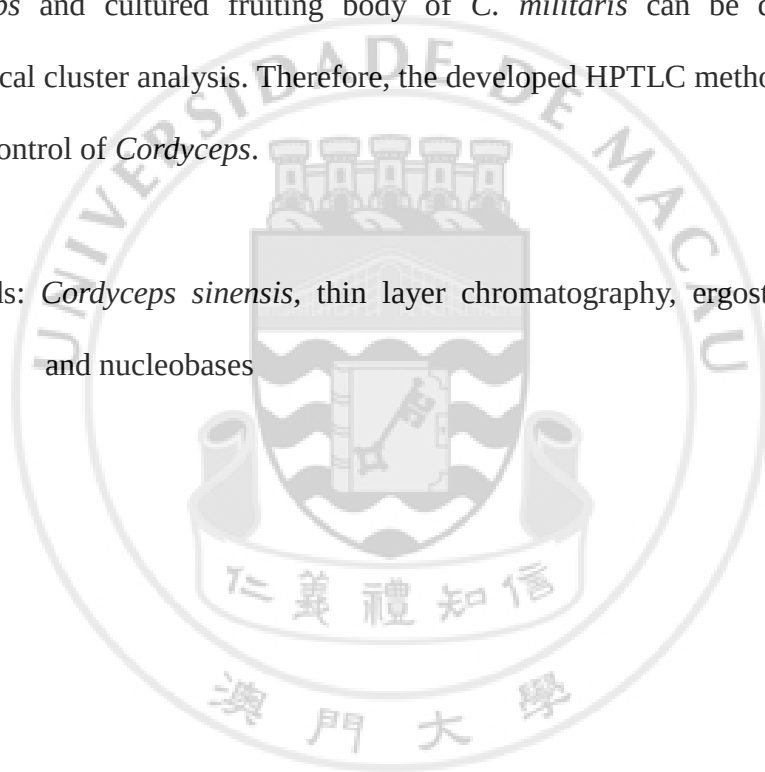
Master of Science

Supervisor: Prof. Shao-ping Li

Cordyceps sinensis is a well-known traditional Chinese medicine. Because of its rarity and outstanding therapeutic effects, nowadays, there are lots of substituted products of wild *Cordyceps* including other *Cordyceps* species and artificial cultivation are commonly sold as health food products in the market. Obviously, quality control of *C. sinensis* and its related market products is very important in order to ensure the safety and efficacy. The aim of this research was to establish a simple and rapid assay for authentication and quality control of *Cordyceps*. In this study, eight kinds of nucleosides and nucleobases, as well as ergosterol have been selected as markers and high performance thin layer chromatography (HPTLC) was established for qualitative and quantitative discrimination of different samples. The chromatography was performed on silica gel 60F₂₅₄ plate with hexane-ethyl acetate-formic acid (0.5:6:0.2, v/v/v) and methanol-chloroform-isopropanol-ammonia solution (0.5:5:5:5:1, v/v/v/v) as mobile phases for hexane extracts and methanol extracts, respectively. After the development, the plates were imaged under UV light

at 254 and 365 nm and then stained with vanillin-H₂SO₄ solution. All the samples were discriminated obviously based on their characteristic of the TLC profiles. Furthermore, the simultaneous quantification of eight nucleosides and nucleobases including inosine, guanosine, uridine, hypoxanthine, adenosine, uracil, adenine and cordycepin were carried out by densitometer scanned at λ scan = 251 nm and with λ ref = 360 nm. The calibration curve of each reference standards had good linearity ($r^2 > 0.9924$) within test ranges. In addition, natural *Cordyceps*, cultured mycelia of *Cordyceps* and cultured fruiting body of *C. militaris* can be divided by using hierarchical cluster analysis. Therefore, the developed HPTLC method can be used for quality control of *Cordyceps*.

Keywords: *Cordyceps sinensis*, thin layer chromatography, ergosterol, nucleosides and nucleobases



摘要

冬蟲夏草是傳統名貴中藥材，它是麥角菌科真菌冬蟲夏草菌 *Cordyceps sinensis* (BerK.) Sacc. 寄生在蝙蝠蛾科昆蟲的幼蟲上的子座及幼蟲屍體的複合體。夏初子座出土、孢子未發散時挖取，曬乾至六七成，除去似纖維狀的附著物及雜質，曬乾或低溫乾燥。

現代藥理及化學研究證實冬蟲夏草含有多種藥理活性成分，包括核苷類、甾醇類、多糖類物質、甘露醇和氨基酸是冬蟲夏草具有重要保健和藥理作用的物質基礎。冬蟲夏草具有免疫調節、抗癌、調節內分泌、抗菌、促進造血、抗病毒、護肝和鎮靜作用；此外還可用於心血管系統、呼吸系統和腎臟等疾病的調節和治療。冬蟲夏草因其重要的藥用價值，引起人們廣泛的注意。由於野生冬蟲夏草數量稀少，市場上已經出現了天然的同屬替代品和大量的人工發酵活性產物包括人工菌絲體，它們都是功能性健康食品的重要資源。因此，為了確保冬蟲夏草及其替代品的安全和功效，對它們進行質量控制是很重要的。

實驗目的是通過超聲波提取，用高效薄層色譜(HPTLC)建立一個分離速度快、樣品處理較簡單的定性和定量質量控制方法，此實驗採用矽膠 60F₂₅₄ 薄層板，以正己烷-乙酸乙酯-甲酸 (0.5:6:0.2 v/v/v)和甲醇-氯仿-異丙醇-氨水 (0.5:5:5:5:1 v/v/v/v)分別作為正己烷和甲醇提取的展開劑。展開後再以香草醛-濃硫酸為顯色劑。對於核苷類成份的定量分析，顯色前先進行掃描，測定波長為 251 nm，參比波長為 360 nm。各斑點包括肌苷、鳥苷、尿苷、次黃嘌呤、腺苷、尿嘧啶、腺嘌呤和蟲草素在範圍內呈良好線性關係 ($r^2 > 0.9924$)。樣品可以以薄層圖進行定性析，同時天然蟲草、人工菌絲體和人工蛹蟲草子實體可用階層式集群分析分辨。本方法簡便、準確、重現性好，可用於冬蟲夏草及其相關產品的質量控制。

關鍵詞：冬蟲夏草，薄層色譜，麥角甾醇，核苷

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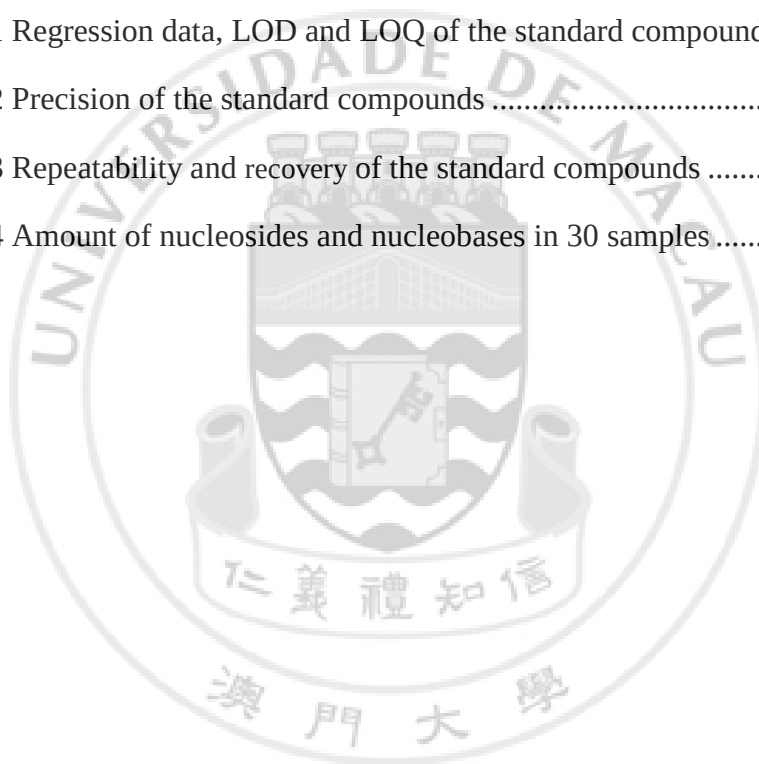
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List of Abbreviations

CE	capillary electrophoresis
DAD	diode array detection
ELSD	evaporative light scattering detectors
GC	gas chromatography
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
IP-RP-LC-MS	ion-pairing reversed-phase liquid chromatography-mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry
PLE	pressurized liquid extraction
RSD	relative standard deviation
TLC	thin layer chromatography
UPLC	ultra-performance liquid chromatography
UV	Ultraviolet
USE	Ultrasonic extraction