

INTRODUCTION OF *HONG KONG CHINESE MATERIA MEDICA STANDARDS (VOLUME 10)* & LATEST DEVELOPMENTS

Chinese Medicine Regulatory Office,
Department of Health of Hong Kong

Hong Kong Chinese Materia Medica Standards
(HKCMMS) Section



Ardisiae Japonicae Herba
矮地茶

溪黃草
Isodonis Herba

Sedi Herba
垂盆草

Dioscoreae Bulbiferae Rhizoma
黃藥子

黑種草子
Nigellae Semen

Dendrobii Caulis
石斛

劉寄奴
Artemisiae Anomalae Herba

Commeliniae Herba
鴨跖草

京大戟
Euphorbiae Pekinensis Radix

苦木
Picrasmae Ramulus et Folium

Ranunculi Ternati Radix
貓爪草

滿山紅
Rhododendri Daurici Folium

HONG KONG CHINESE MATERIA MEDICA STANDARDS (HKCMMS)

Background:

- Launched by the Department of Health in 2001, aiming at establishing reference standards for commonly used Chinese Materia Medica (CMM) in Hong Kong

Aims:

- Promote research of Chinese medicines
- Provide reference standards for CMM trade
- Ensure the safety and quality of CMM
- Promote modernisation and internationalisation of Chinese medicines industry of Hong Kong
- Facilitate trade in Chinese medicines

HKCMMS

Applications:

- Identification of CMM
- Provide references to the trade for selecting/purchasing CMM and formulating quality control standards
- Provide references to the manufacturers of proprietary Chinese medicines (pCm), holders of Certificate for Manufacturer (GMP) of pCm and applicants of registration of pCm for establishing product specifications
- Provide scientific basis for education and research of CMM
- Provide references to laboratories in preparation of seeking ISO/IEC 17025 certification

PRINCIPLES OF SELECTING CMM FOR HKCMMS RESEARCH

Including CMM that are

- Commonly used in Hong Kong
- With relatively high economic values
- Receiving international attention

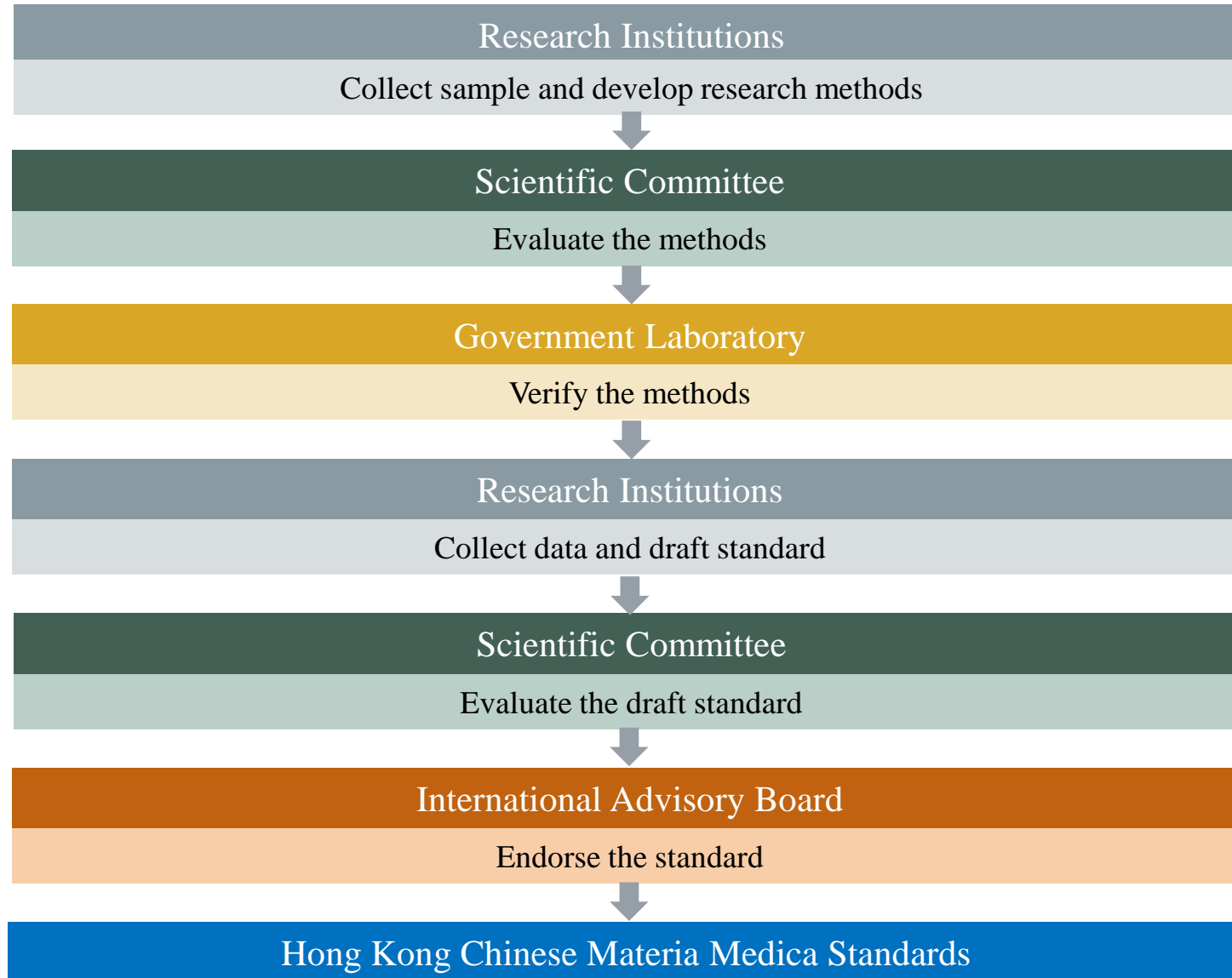
Priority is also given to those listed in the Chinese Medicine Ordinance, Laws of Hong Kong

COLLABORATION PARTNERS

Include

- National Administration of Traditional Chinese Medicine
- National Medical Products Administration
- Research Institutions
- Government Laboratory of Hong Kong

WORKFLOW OF HKCMMS



PROGRESS OF HKCMMS

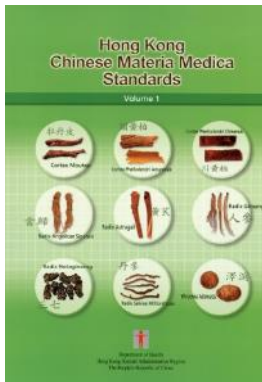
- 10 volumes of HKCMMS were published between 2005-2020
- Total number of CMM standards endorsed: 330



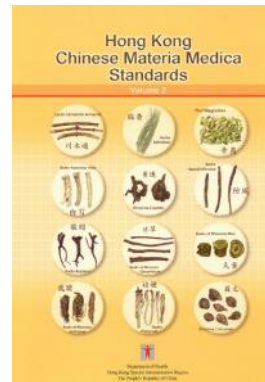
● Lists of Chinese Materia Medica in HKCMMS https://www.cmro.gov.hk/html/eng/useful_information/hkcmms/cmmlist.html

PROGRESS OF HKCMMS

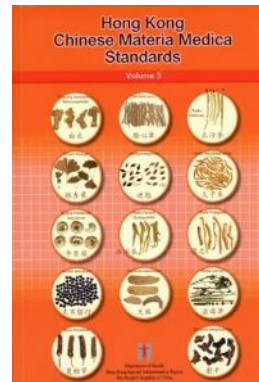
**Volume 1
8 CMM**



**Volume 2
24 CMM**



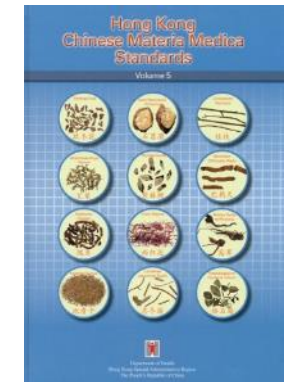
**Volume 3
29 CMM**



**Volume 4
36 CMM**



**Volume 5
42 CMM**



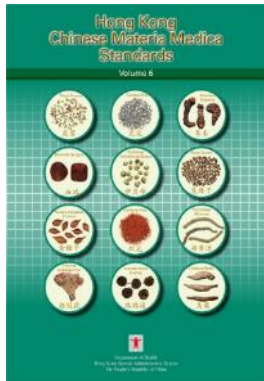
- Developed analytical methods for detecting volatile oil and Aristolochic Acid I in CMM if necessary

- Adopted green chemistry approach
- Employed a new testing technique (LC-MS)
- Included 8 CMM of mineral origin
- Developed X-ray Powder Diffraction as an identification method for CMM of mineral origin

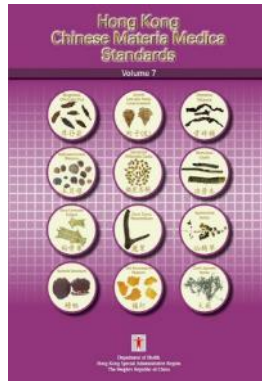
- Included a testing method for acid value in CMM if necessary

PROGRESS OF HKCMMS

**Volume 6
60 CMM**



**Volume 7
36 CMM**



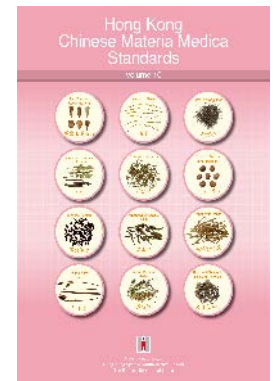
**Volume 8
39 CMM**



**Volume 9
36 CMM**



**Volume 10
31 CMM**



- Included 2 CMM of animal origin
- Employed DNA fingerprinting technology for identification purpose for the first time
- Introduced photochemical derivatization as one of the post-column derivatization method for detecting mycotoxins (aflatoxins)

- Included Reference Assay Chromatograms in electronic edition of monographs

- Included Reference Assay Chromatograms in both print and electronic editions of monographs
- Established testing method and limit of sulphur dioxide residues
- Released DVD compiling all 9 volumes of HKCMMS
- Introduced an online search function that supports different enquiry parameters

- Adopted a new testing method for organochlorine pesticide residues
- Released USB flash drives with all 10 volumes of HKCMMS
- Started to employ UHPLC in fingerprinting and assay

RESEARCH RESULTS

- Research results of HKCMMS are well recognized locally and overseas. Reference standards of HKCMMS are adopted by the Hospital Authority and some manufacturers of pCm with Certificate for Manufacturer (GMP) in the procurement of CMM and quality assurance
- Research work of HKCMMS is of great significance to the standardisation and internationalisation of CMM and can promote Hong Kong to become an international Chinese medicines testing and certification centre

HKCMMS VOLUME 10



The reference standards of volume 10 of HKCMMS were published on **22 December 2020**

CONTENTS OF VOLUME 10 OF HKCMMS

- Reference standards regarding safety and quality aspects of 31 commonly used CMM, including a toxic CMM “Crotonis Fructus (unprocessed)” and an animal origin CMM “Deinagkistrodon (Agkistrodon)”
- Application of DNA fingerprinting technology to identify CMM for the second time
- Introduce new testing technologies, including:
 - Method II was introduced to Appendix VI - Determination of Pesticide Residues. Gas chromatography with tandem mass spectrometry (GC-MS/MS), one of the most internationally advanced testing methods of pesticide residue, was employed
 - Employing Ultra-high Performance Liquid Chromatography (UHPLC) in fingerprinting and assay

VOLUME 10 OF HKCMMS

- As a USB flash drive - compiling 10 volumes of HKCMMS

Convenient to users who choose paperless media to quickly look into monographs of HKCMMS



VOLUME 10 OF HKCMMS

- Introduce an online search function that supports different enquiry conditions

Convenient for users to find needed monograph instantly

The screenshot displays the website for the Chinese Medicine Regulatory Office (CMRO) of the Department of Health, Government of the Hong Kong Special Administrative Region. The page is titled "Hong Kong Chinese Materia Medica Standards (HKCMMS) Section" and "HKCMMS Database". It features a navigation menu on the left with links to Home Page, Important Information, About Us, Government Chinese Medicines Testing Institute, WHO Collaborating Centre, GMP for Proprietary Chinese Medicines Web Resources, Health Information & Activities, Related Links, and Contact Us. The main content area includes a disclaimer stating that the information is for reference only and that the Government is not responsible for any loss or damages. Below the disclaimer are two buttons: "Agree to above disclaimer and continue to the database of HKCMMS" and "Disagree to above disclaimer and back to Main page". The footer contains logos for the Department of Health, Chinese Medicine Council of Hong Kong, and Centre for Health Protection, along with a "Support organ donation Register online!" banner.

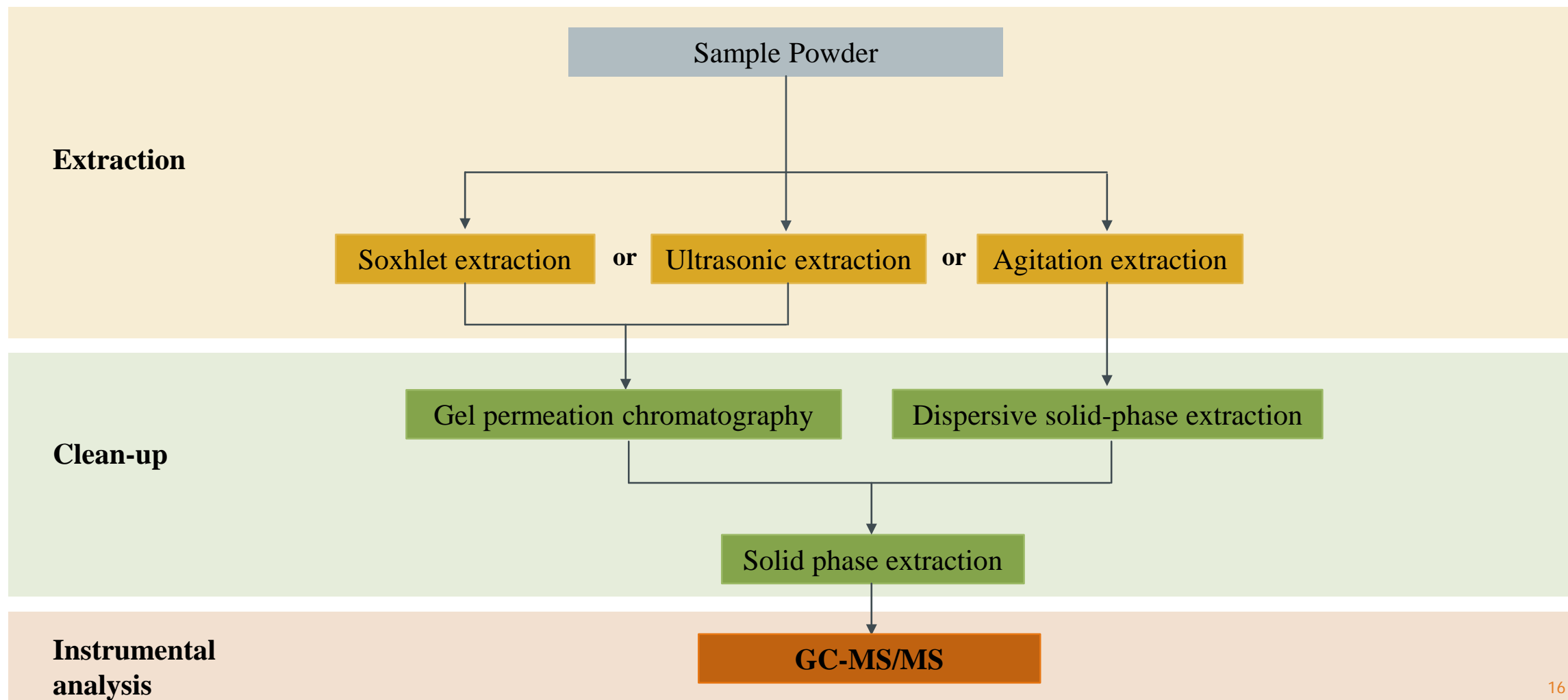
HKCMMS online search function at <https://www.cmro.gov.hk/html/eng/search/disclaimer.php>

APPENDIX VI - DETERMINATION OF PESTICIDE RESIDUES

	Method I	Method II
Sample preparation	Ultrasonic extraction	Soxhlet extraction, ultrasonic extraction or agitation extraction
Detector	Gas chromatography with electron-capture detector (GC-ECD)	Gas chromatography with tandem mass spectrometer (GC-MS/MS)
	When performing GC-ECD analysis, it needs 2 types of capillary columns with different polarities. If same pesticide is detected on the 2 capillary columns, further confirmation by Gas chromatography-mass spectrometry (GC-MS) is needed	Only 1 capillary column required

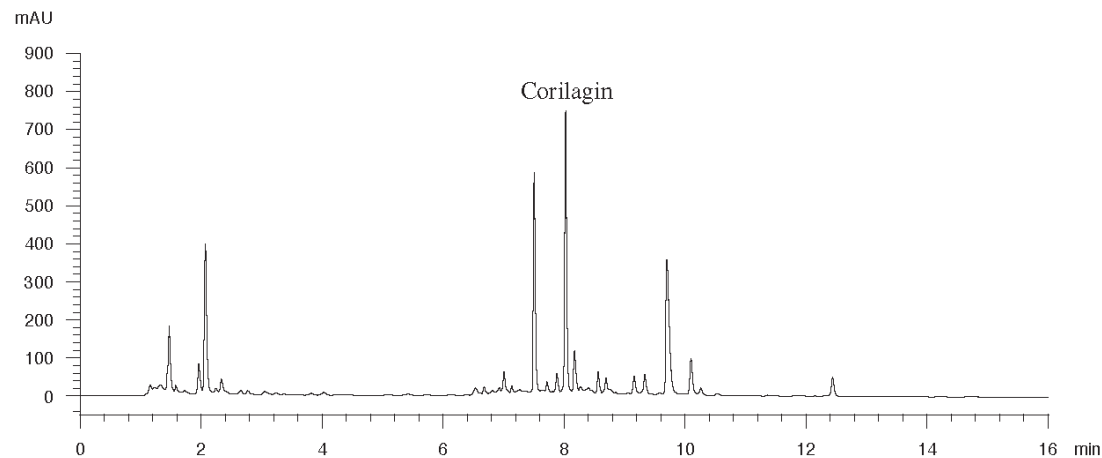
- GC-MS/MS is a method also included for pesticide residues determination in Chinese Pharmacopoeia 2020 Edition

APPENDIX VI - DETERMINATION OF PESTICIDE RESIDUES METHOD II



ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (UHPLC)

- Start to employ UHPLC in fingerprinting and assay
- Used in 7 monographs in Volume 10 of HKCMMS
- Can improve chromatographic separation and separation speed. Since analysis time is shortened, required solvent is also reduced, which is environmental friendly and can lower the cost



UHPLC was used in the assay of
Geranii Caroliniani Herba.
Time required is 16 min

Dioscoreae Bulbiferae Rhizoma



Figure 1: A photograph of *Dioscoreae Bulbiferae Rhizoma* pieces. The pieces are small, irregular, brownish, and fibrous, characteristic of cinnamon bark.

1. NAMES
 Official name: *Dioscoreae Bulbiferae Rhizoma*
 Chinese name: 肉桂
 Chinese name (alternate): 肉桂根
SOURCE
Dioscorea 7, 25. See *Bulbifera* with 2. See also *Dioscoreae Bulbiferae Rhizoma* for the use of *Dioscorea* in the preparation of *Dioscoreae Bulbiferae Rhizoma*.

DESCRIPTION
 The bark is reddish-brown to reddish-orange, 1-3 mm thick, and is fibrous. The bark is broken into small pieces, 1-3 mm long and 1-3 mm wide. The bark is fibrous and has a strong, warm, spicy odor. The bark is bitter and slightly pungent. The bark is used in the preparation of *Dioscoreae Bulbiferae Rhizoma*.

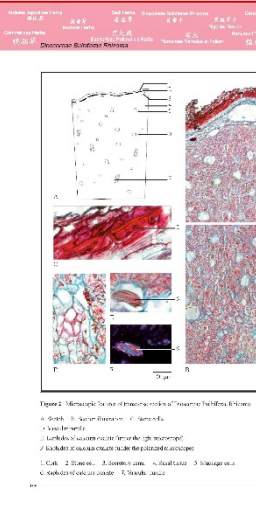


Figure 2: Microscopic image of *Dioscoreae Bulbiferae Rhizoma*. The image shows the cortex and vascular bundles, which are characteristic of cinnamon bark.

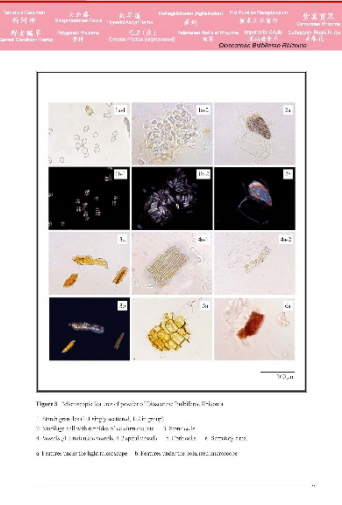


Figure 3: Microscopic image of *Dioscoreae Bulbiferae Rhizoma*. The image shows the cortex and vascular bundles, which are characteristic of cinnamon bark.

Monograph Dioscoreae Bulbiferae Rhizoma

4.2 Thin Layer Chromatography Identification
 Standard solution
 Preparation: Weigh 0.5g of *Dioscoreae Bulbiferae Rhizoma* and extract with 50% ethanol for 30 minutes. Filter and concentrate to dryness. Dissolve in 1 mL of 50% ethanol. This is the standard solution.

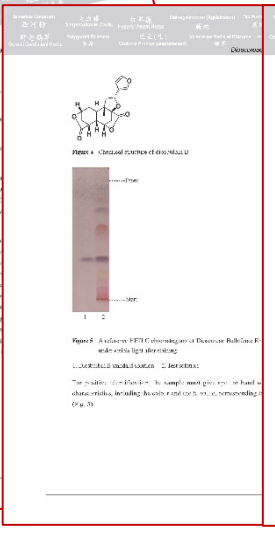


Figure 6: Reference TLC chromatogram of *Dioscoreae Bulbiferae Rhizoma* under ultraviolet light. The plate shows several distinct spots, indicating the presence of various compounds.

4.3 High-Performance Liquid Chromatography Fingerprinting (HPLC)
 Standard solution
 Preparation: Weigh 0.5g of *Dioscoreae Bulbiferae Rhizoma* and extract with 50% ethanol for 30 minutes. Filter and concentrate to dryness. Dissolve in 1 mL of 50% ethanol. This is the standard solution.

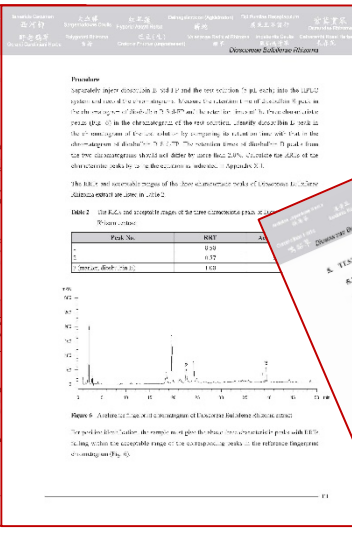


Figure 5: Reference HPLC chromatogram of *Dioscoreae Bulbiferae Rhizoma* showing peaks at retention times 10.2, 11.5, 12.8, and 14.1 minutes.

5. ASSAY
5.1 Assay
 Preparation: Weigh 0.5g of *Dioscoreae Bulbiferae Rhizoma* and extract with 50% ethanol for 30 minutes. Filter and concentrate to dryness. Dissolve in 1 mL of 50% ethanol. This is the standard solution.

Peak No.	RRT	Area
1	10.2	1000
2	11.5	2000
3	12.8	3000
4	14.1	4000

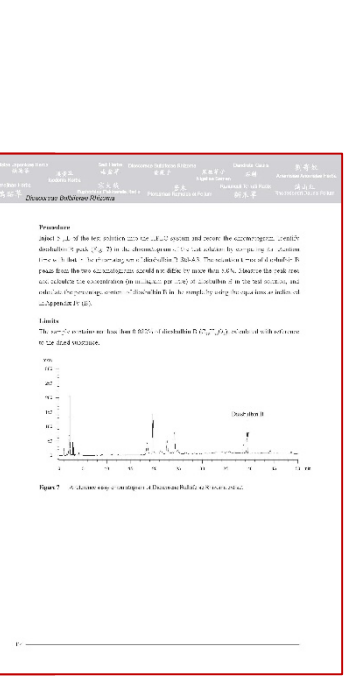


Figure 7: Assay HPLC chromatogram of *Dioscoreae Bulbiferae Rhizoma*. The chromatogram shows several peaks, with the main peak at 12.8 minutes.

1. NAMES

Official name : *Dioscoreae Bulbiferae Rhizoma*

Chinese name : 黃藥子

Chinese phonetic name : Huangyaozi

2. SOURCE

Original plant : *Dioscorea bulbifera* L. (Dioscoreaceae)

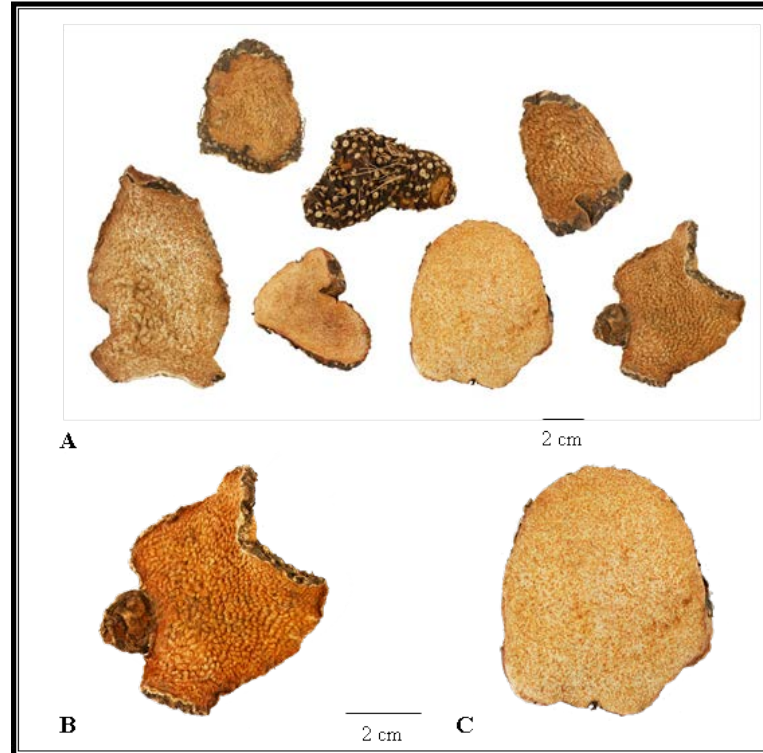
Medicinal part : Tuber

Collection and processing : Collected in autumn, rootlets, residual stems and leaves removed, washed clean, cut into slices when fresh, then dried under the sun

3. DESCRIPTION

- Shape : Mostly transversely cut thick slices, rounded or subrounded, 14-143 mm in diameter, 0.1-1.9 cm in thickness
- Colour and appearance : Cork easily exfoliated, externally brownish-black, shrunken, white punctiform protuberances scars of rootlets numerous, occasionally with remains of curved rootlets. Transversely cut surface yellowish-white to yellowish-brown, flat or bumpy
- Texture : Hard and fragile. Fracture granular, scattered with orange-yellow spots
- Odour and taste : Odour slight; taste bitter

CMM PHOTOGRAPH (DIOSCOREAE BULBIFERAE RHIZOMA)



A photograph of Dioscoreae Bulbiferae Rhizoma

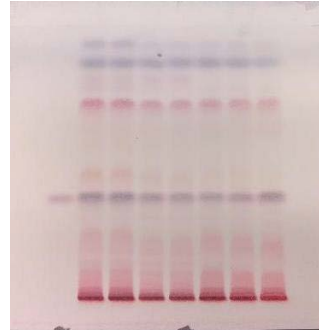
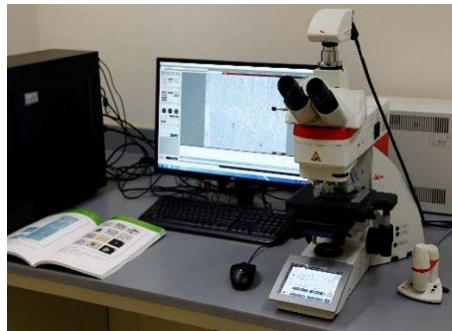
- A.** Dioscoreae Bulbiferae Rhizoma **B.** Magnified image of cut surface (showing granular fracture)
C. Magnified image of cut surface (showing orange-yellow spots)

4. IDENTIFICATION

4.1 Microscopic Identification

4.2 Thin-Layer Chromatographic Identification

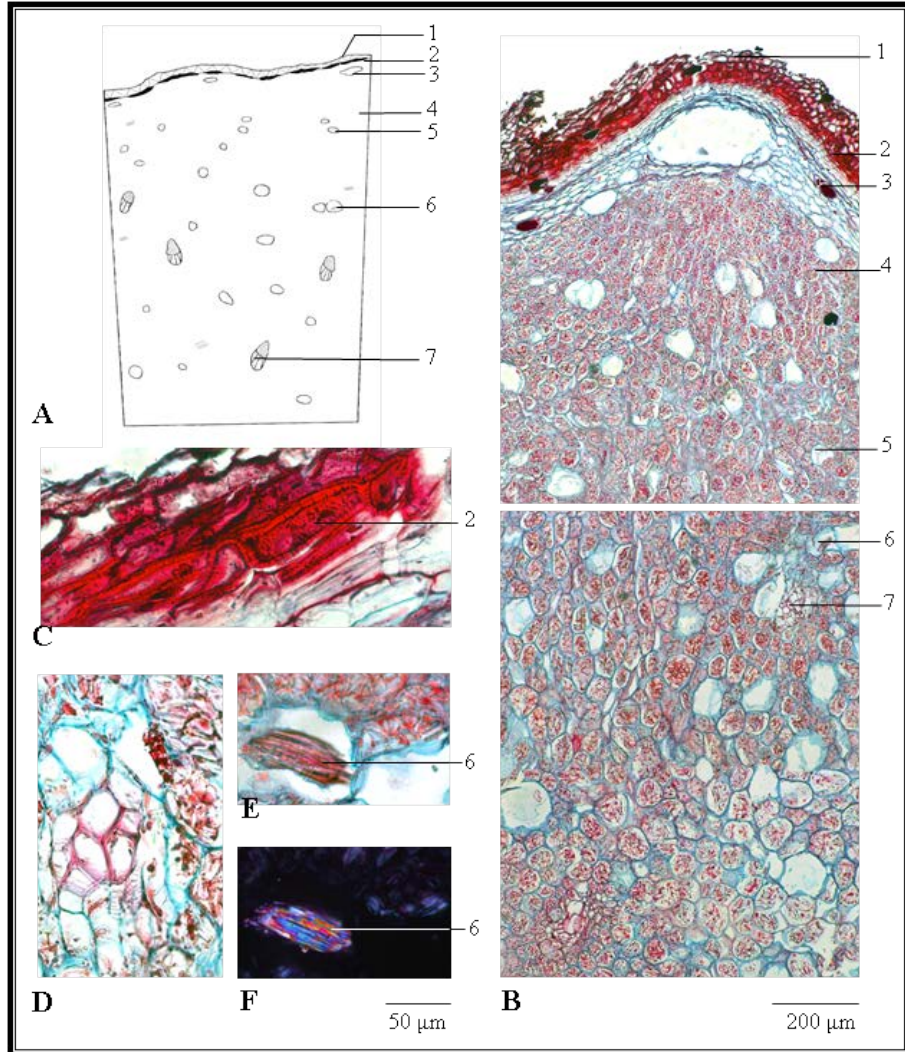
4.3 High-Performance Liquid Chromatographic Fingerprinting



4. IDENTIFICATION

4.1 Microscopic Identification

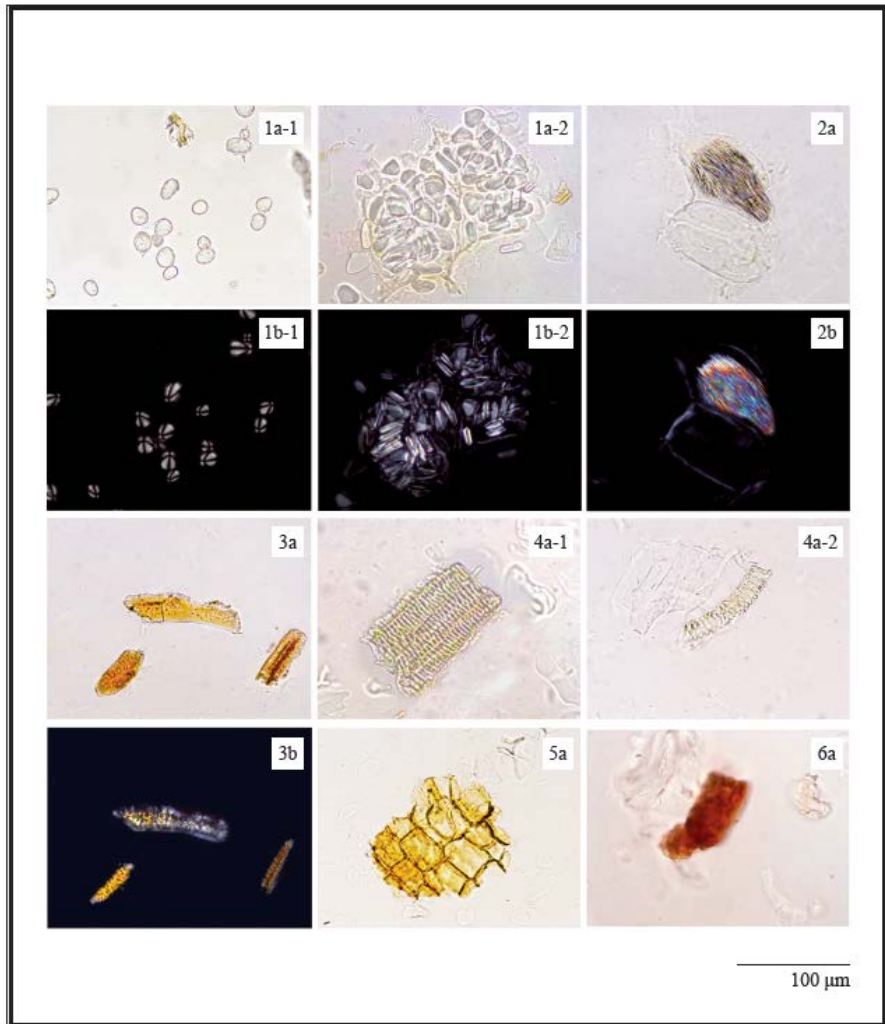
4.1 MICROSCOPIC IDENTIFICATION



A. Sketch B. Section illustration C. Stone cells
D. Vascular bundle
E. Raphides of calcium oxalate (under the light microscope)
F. Raphides of calcium oxalate (under the polarized microscope)

1. Cork 2. Stone cell 3. Secretory canal
4. Basal tissue 5. Mucilage cells 6. Raphides of calcium oxalate 7. Vascular Bundle

4.1 MICROSCOPIC IDENTIFICATION



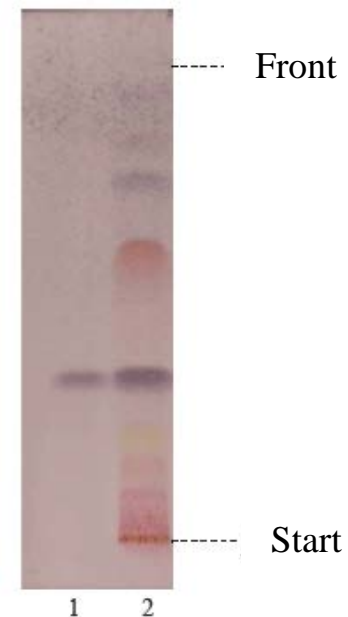
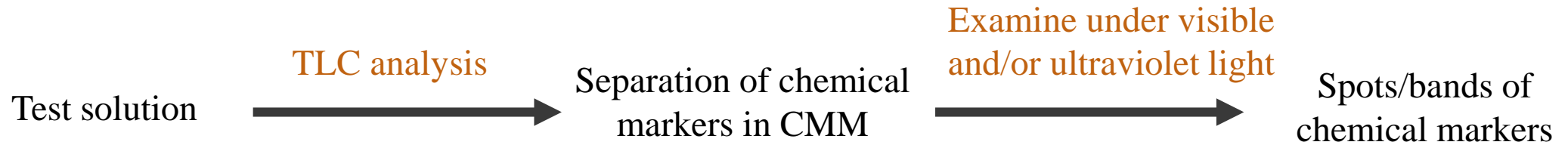
1. Starch granules (1-1 singly scattered, 1-2 in group)
2. Mucilage cell with raphides of calcium oxalate
3. Stone cells
4. Vessels (4-1 reticulate vessels, 4-2 spiral vessel)
5. Cork cells
6. Secretory canal

- a. Features under the light microscope
- b. Features under the polarized light

4. IDENTIFICATION

4.2 Thin-Layer Chromatographic Identification

4.2 THIN-LAYER CHROMATOGRAPHIC (TLC) IDENTIFICATION



4.2 THIN-LAYER CHROMATOGRAPHIC (TLC)

IDENTIFICATION



1. Diosbulbin B standard solution
2. Test solution

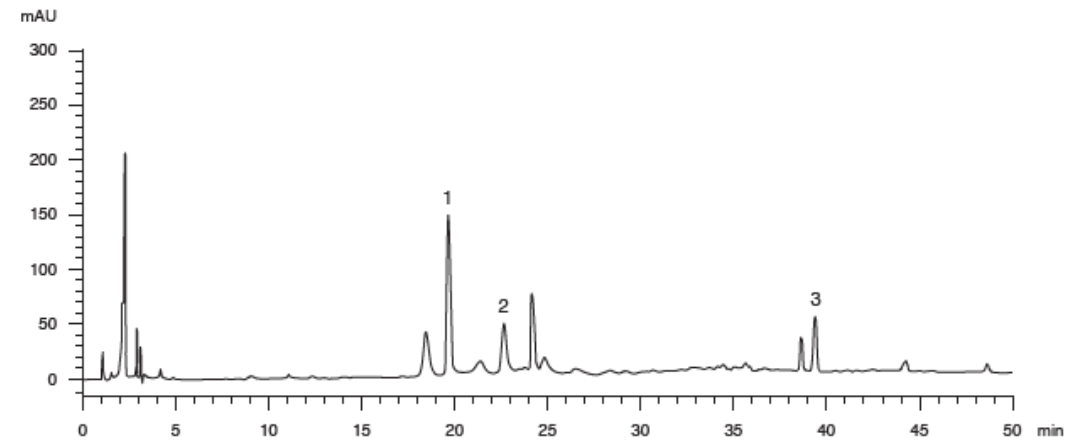
Chemical marker of *Dioscoreae Bulbiferae Rhizoma*
– Diosbulbin B

A reference HPTLC chromatogram of *Dioscoreae Bulbiferae Rhizoma* extract observed under visible light after staining

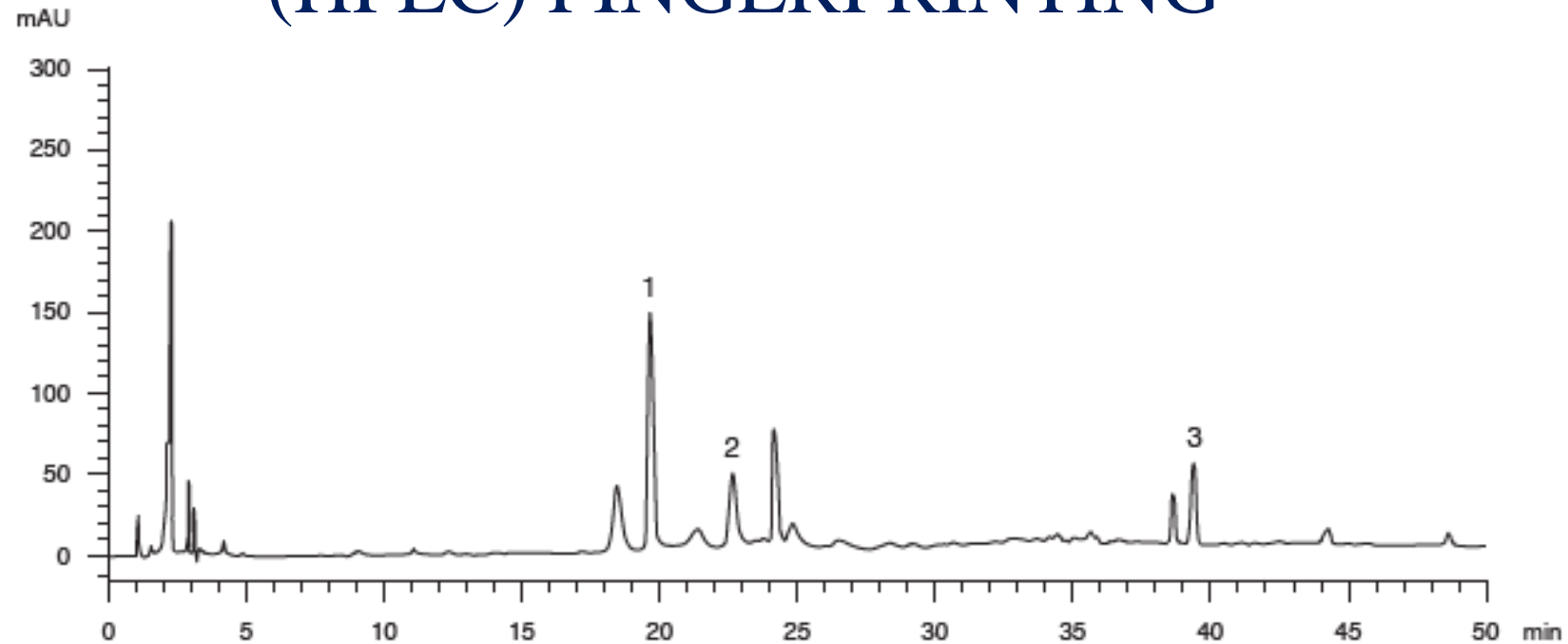
4. IDENTIFICATION

4.3 High-Performance Liquid Chromatographic Fingerprinting

4.3 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) FINGERPRINTING



4.3 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) FINGERPRINTING



A reference fingerprint chromatogram of Dioscoreae Bulbiferae Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.50	± 0.03
2	0.57	± 0.03
3 (marker, diosbulbin B)	1.00	-

5. TESTS

- 5.1 Heavy Metals
- 5.2 Pesticide Residues
- 5.3 Mycotoxins (Aflatoxins)
- 5.4 Sulphur Dioxide Residues
- 5.5 Foreign Matter
- 5.6 Ash
- 5.7 Water Content

5. TESTS

Safety Tests

- 5.1 Heavy Metals
- 5.2 Pesticide Residues
- 5.3 Mycotoxins (Aflatoxins)
- 5.4 Sulphur Dioxide Residues

Dioscoreae Bulbiferae Rhizoma –
should meet the requirements

5. TESTS

5.1 Heavy Metals

- Extraneous pollutants
- Absorbed and accumulated in CMM
- Arsenic, cadmium, lead and mercury
- Quantitative analysis –
Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

5. TESTS

5.1 Heavy Metals

References:

- Chinese Pharmacopoeia
- Green Standards of Medicinal Plants and Preparations for Foreign Trade and Economy (2005)
- World Health Organization
 - Quality control methods for medicinal plant materials (1998)
- United States – National Sanitation Foundation
 - Dietary Supplement – NSF International Standard (NSF/ANSI 173-2006), National Sanitation Foundation International (2006)

5. TESTS

5.1 Heavy Metals

Maximum permitted limits of heavy metals in CMM samples (Appendix V)

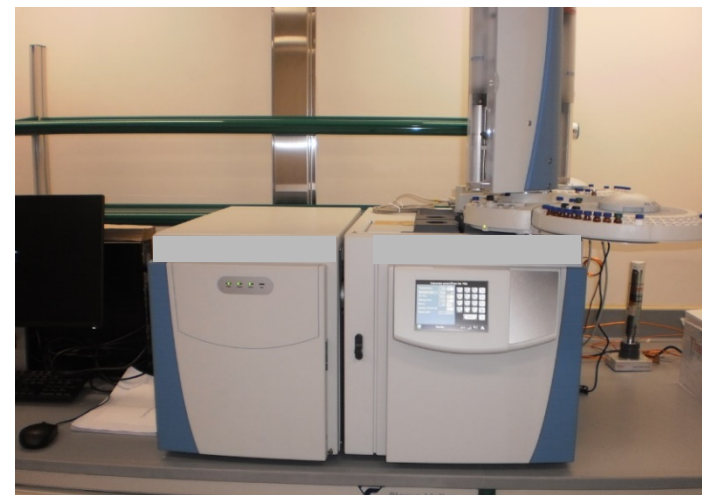
Heavy Metal	Limit (Not more than)
Arsenic	2.0 mg/kg
Cadmium	1.0 mg/kg
Lead	5.0 mg/kg
Mercury	0.2 mg/kg

Dioscoreae Bulbiferae Rhizoma –
should meet the requirements

5. TESTS

5.2 Pesticide Residues

- Synthetic compounds, a natural or biological substance or mixtures of the above
- Prevent pests or regulate their growth
- Organochlorine pesticides
- Quantitative and qualitative analysis – Gas chromatography with electron-capture detector (GC-ECD), Gas chromatography with tandem mass spectrometry (GC-MS/MS)



Gas chromatography with electron-capture detector (GC-ECD)

5. TESTS

5.2 Pesticide residues

References:

- Chinese Pharmacopoeia
- United States Pharmacopoeia
- British Pharmacopoeia
- European Pharmacopoeia

5. TESTS

5.2 Pesticide residues

Maximum permitted limits of pesticide residues in CMM samples (Appendix VI)

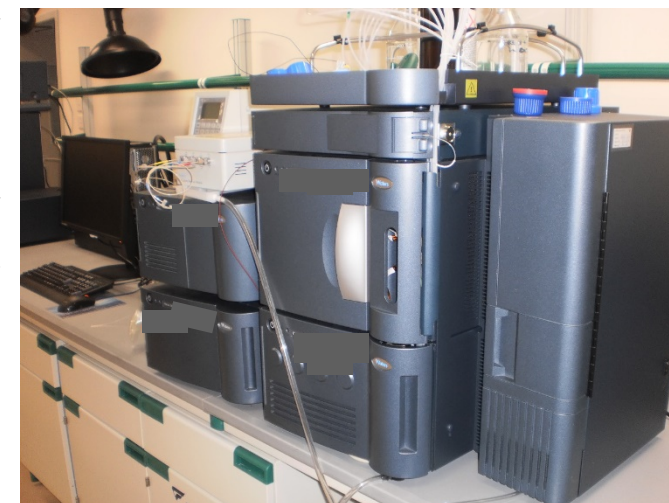
Pesticide	Limit (Not more than)
Aldrin and Dieldrin (sum of)	0.05 mg/kg
Chlordane (sum of <i>cis</i> -, <i>trans</i> - and oxychlordane)	0.05 mg/kg
DDT (sum of <i>p,p'</i> -DDT, <i>o,p'</i> -DDT, <i>p,p'</i> -DDE and <i>p,p'</i> -TDE)	1.0 mg/kg
Endrin	0.05 mg/kg
Heptachlor (sum of heptachlor and heptachlor epoxide)	0.05 mg/kg
Hexachlorobenzene	0.1 mg/kg
Hexachlorocyclohexane isomers (α -, β - and δ - hexachlorocyclohexane)	0.3 mg/kg
Lindane (γ -hexachlorocyclohexane)	0.6 mg/kg
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0 mg/kg

Dioscoreae Bulbiferae Rhizoma—
should meet the requirements

5. TESTS

5.3 Mycotoxins (Aflatoxins)

- Toxic metabolites produced by molds and/or fungi
- CMM may be contaminated with aflatoxins
- Aflatoxins B₁, B₂, G₁ and G₂
- Quantitative analysis – High Performance Liquid Chromatography (HPLC) with fluorescence detector
- Either iodine derivatization or photochemical derivatization can be selected as the post-column derivatization method for aflatoxin detection



High Performance Liquid Chromatography with fluorescence detector (HPLC-FLD)

5. TESTS

5.3 Mycotoxins (Aflatoxins)

Post-column derivatization for aflatoxins detection

(1) Iodine derivatization

reaction temperature set at 70°C and 0.5 mM iodine solution as post-column derivatization reagent. Flow rate of the reagent set as 0.3 mL/min

(2) Photochemical derivatization

a post-column reactor system with a 254 nm UV lamp and reaction coil for derivatization

5. TESTS

5.3 Mycotoxins (Aflatoxins)

References:

- Chinese Pharmacopoeia
- United States Pharmacopoeia
- British Pharmacopoeia
- European Pharmacopoeia
- Green Standards of Medicinal Plants and Preparations for Foreign Trade and Economy

5. TESTS

5.3 Mycotoxins (Aflatoxins)

Maximum permitted limits of aflatoxins in CMM samples (Appendix VII)

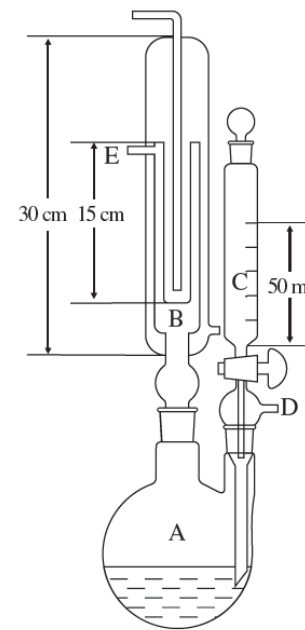
Aflatoxin	Limit (Not more than)
Aflatoxin B ₁	5 µg/kg
Aflatoxins (sum of B ₁ , B ₂ , G ₁ and G ₂)	10 µg/kg

Dioscoreae Bulbiferae Rhizoma –
should meet the requirements

5. TESTS

5.4 Sulphur Dioxide Residues

- CMM may be fumigated with sulphur in order to prevent spoilage
- Sulphur dioxide residues in CMM can cause allergic reactions in some people
- Quantitative analysis – Acid-base titration



Apparatus for the determination of sulphur dioxide residues

5. TESTS

5.4 Sulphur dioxide residues

References:

- Chinese Pharmacopoeia
- United States Pharmacopoeia
- British Pharmacopoeia
- European Pharmacopoeia

5. TESTS

5.4 Sulphur dioxide residues

Limit of sulphur dioxide residues in CMM samples (Appendix XVI)

CMM	Limit of Sulphur Dioxide Residues (Not more than)
All CMM, unless in the case of a CMM of mineral origin or as otherwise specified	150 mg/kg

Dioscoreae Bulbiferae Rhizoma –
should meet the requirements

5. TESTS

5.5 Foreign Matter

- Appendix VIII: Determination of Foreign Matter
- Definition of Foreign Matter
 - Same biological origin specified in monograph but appearance or botanical part is different
 - Different biological origin as specified in monograph
 - Foreign mineral matters such as stones, sand and lumps of soil

Dioscoreae Bulbiferae Rhizoma –
Foreign Matter not more than 1.0%

5. TESTS

5.6 Ash

- Appendix IX: Determination of Ash
- Ash of CMM includes: (i) amount of non-volatile inorganic materials (physiological ash) remaining after ignition of the plant materials and (ii) amount of ash (non-physiological ash) derived from extraneous matter such as sand and soil
 - Total Ash
 - Acid-insoluble ash

Dioscoreae Bulbiferae Rhizoma –
Total ash – not more than 4.5%
Acid-insoluble ash – not more than 0.5%

5. TESTS

5.7 Water Content

- Appendix X: Determination of Water Content
 - Determine the water content of CMM samples by Oven dried method
 - CMM may be prone to mold infestation and deterioration if water content is too high

Dioscoreae Bulbiferae Rhizoma –
Water content not more than 15.0%

6. EXTRACTIVES

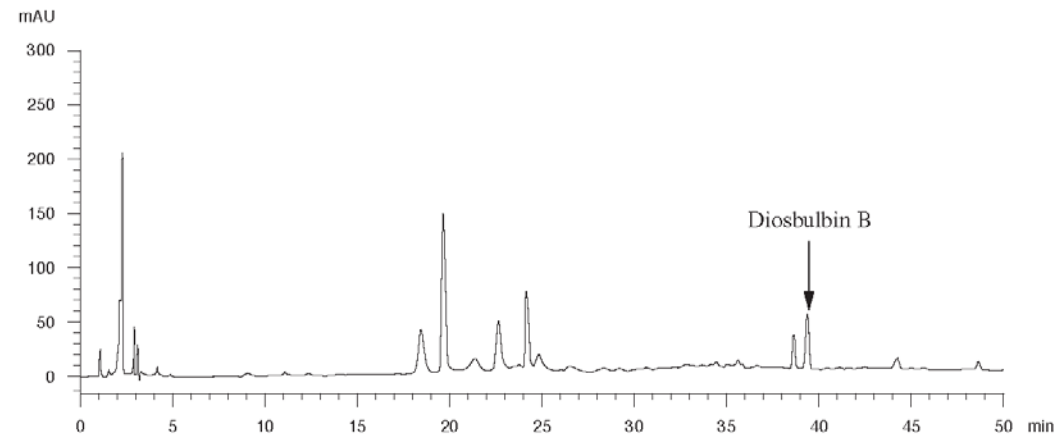
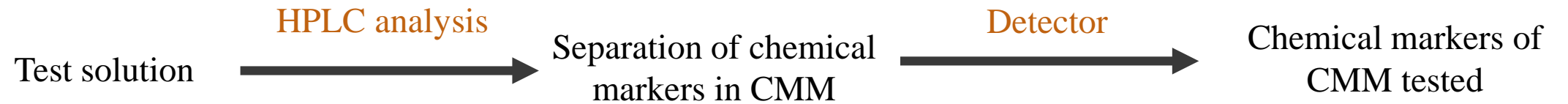
- Appendix XI: Determination of Extractives
 - Ethanol-soluble extractives
 - Water-soluble extractives

Dioscoreae Bulbiferae Rhizoma

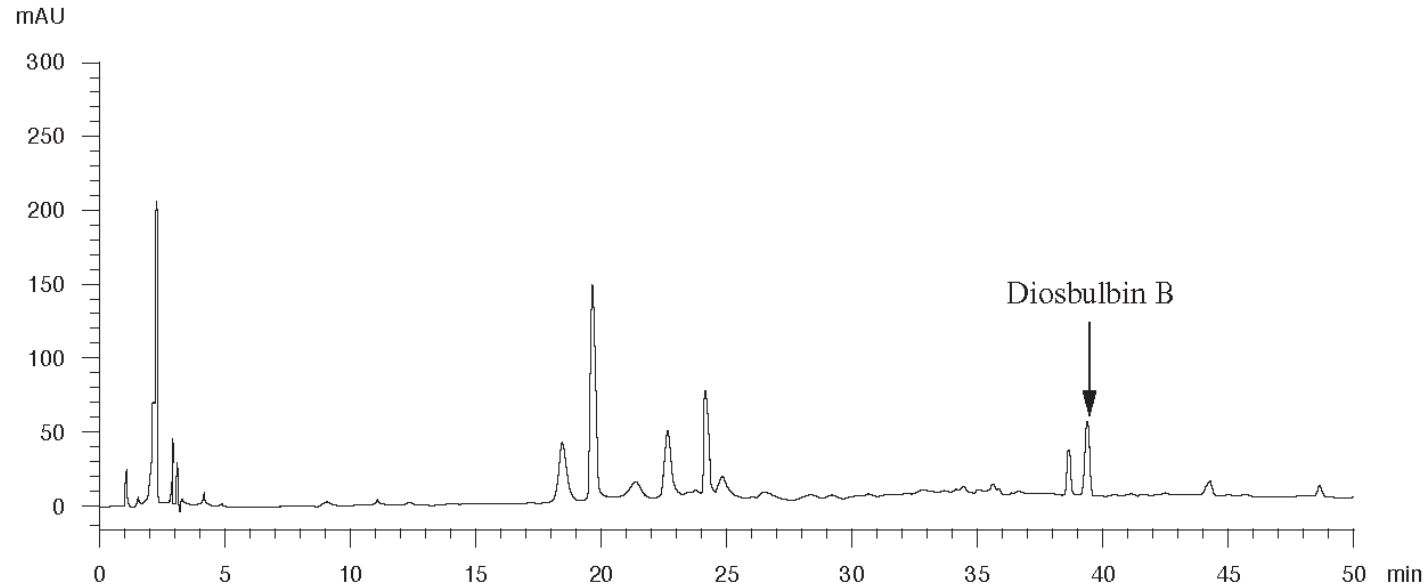
Water-soluble extractives (cold extraction method) : not less than 8.0%

Ethanol-soluble extractives (cold extraction method) : not less than 5.0%

7. ASSAY



7. ASSAY



A reference assay chromatogram of Dioscoreae Bulbiferae Rhizoma extract

The sample contains not less than 0.082% of diosbulbin B ($C_{19}H_{20}O_6$) calculated with reference to the dried substance

COMMENT AND ENQUIRY

- E-mail : hkcmmso@dh.gov.hk
- Fax : (852) 2788 2962
- Website : www.cmro.gov.hk
- Address : 2/F, Public Health Laboratory Centre
382 Nam Cheong Street, Kowloon
Hong Kong Chinese Materia Medica Standards Section of
Department of Health
The Government of HKSAR

THANK YOU