Development of Hong Kong Chinese Materia Medica (HKCMM) standards = 香港中藥材標準的制訂與發展

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Review

Development of Hong Kong Chinese Materia Medica (HKCMM) standards
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Abstract: The development of HKCMM standards is one of the achievements of developing Hong Kong into an international centre for Chinese medicine. In order to enable more people concerned to have an intimate knowledge of this standard, the author was invited to introduce the development of HKCMM standards to readers, including: 1) the history of the project and the basis for legislative supports; 2) the composition and operation of the regulatory division, the research institution and the evaluation committee; 3) the progress of HKCMM standards; 4) the contents and features of HKCMM standards.

Keywords: Hong Kong Chinese Materia Medica standards; HKCMM standards; Quality evaluation

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1. Introduction

Traditional Chinese medicine (TCM) has been serving Chinese people since over 2000 years ago for prevention and treatment of diseases as well as health maintenance. It remains an important part of health care provision in modern China. More and more persons are turning to Chinese herbal remedies with great enthusiasm, attracted by the herbal properties of fewer adverse reactions than chemical drug medicines[1]. Some of the countries which were originally skeptical of TCM began to give serious consideration to it, and the impact of TCM has increased in the international medicinal arena[2].

TCM, although not the mainstream medicine, is nevertheless popular in Hong Kong for it has been used in the community for many years and has made significant contribution to the health of public. A household survey by Census and Statistics Department of Hong Kong showed that about 19.6% of the respondents preferred to consult Chinese medicine practitioners when illness and 60% of the respondents have taken herbal medicine[3]. Since 1998, TCM has been recognized as a strategic development area by
Hong Kong Special Administrative Region (HKSAR). To develop HKSAR into an international centre for Chinese medicine, the HKSAR Government has been continuously laying groundwork for the development of the field since then. The development of HKCMM standards is one of the achievements of these efforts.

2. Background of HKCMM standards

2.1. History of the project and the basis for legislative supports

Starting from the 1980’s, public concern towards Chinese medicine grows and the Hong Kong Government appointed a Working Committee on Chinese Medicine in August 1989. Acting on the Working Committee’s suggestions, the Government appointed a Preparatory Committee on Chinese Medicine in April 1995 to make recommendations to the Government on the promotion, development and regulation of Chinese medicine in Hong Kong[4].

In 1997, the Chief Executive of the HKSAR Government announced the commitment to establish a sound regulatory framework for Chinese medicine[5]. The potential of Chinese medicine was recognized as a possible power for strengthening Hong Kong’s competitiveness. Two years later, in July 1999, the Chinese Medicine Ordinance was enacted while the Chinese Medicine Council of Hong Kong was found in September of the same year to conduct regulatory measures for the practice of TCM practitioners, the manufacture, trading and application of Chinese medicines[6-8]. Following these guidance, the Trade and Industry Department announced a 10-year plan to develop the Chinese medicine industry in Hong Kong[9,10]. The idea of developing Hong Kong into an international centre for Chinese medicine is raised.

To take these roles of Chinese medicine centre and be successful, the quality and safety aspects of Chinese medicine commonly used in Hong Kong[10].

2.2. Composition and operation of the regulatory division, the research institution and the evaluation committee

In 2001, the Department of Health set up the HKCMM standards Office under its Chinese Medicine Division to manage the development of HKCMM standards. In early 2002, an International Advisory Board (IAB), consisting of 12 renowned local, Mainland and overseas (including Australia, Canada, Germany, Japan, Thailand and the United States) experts, was found to give advice on the principles, methodologies, parameters and analytical methods for developing the standards. The IAB is responsible for designing the contents of the HKCMM standards, selecting the Chinese Materia Medica (CMM) and the research institutions, examining as well as endorsing the research results, and promoting recognition by regulatory authorities and organizations worldwide of the future HKCMM standards. In addition, a Scientific Committee, consisting of IAB members, and representatives of the research institutions and Health Department, was set up to supervise the progress of the research work. The Committee is also tasked to resolve various technical problems encountered in the process and check the research results. Local universities were commissioned to develop the standards of the CMM through tender. The Government Laboratory is involved into the project through developing analytical methods for determination of heavy metals, pesticides residues and mycotoxins, and participating in inter-laboratory validation[11,12,13].

2.3. Criteria of the stated CMMs

The medicines for the development of the standards are selected from the Schedule 1 and Schedule 2 medicines of the Chinese Medicine Ordinance[11]. The selected medicines are commonly used in the local community; of higher economic value; some medicines of international concern in respect to their quality; potent/toxic Chinese herbal medicines. Finally, about 200 medicines are selected for investigation, which made up the majority of herbs being prescribed by local TCM practitioners, and represented the main raw materials in the proprietary Chinese medicines made in Hong Kong[12,13].
2.4. Progress of HKCMM standards

So far, the phase I and phase II of HKCMM standards have been completed on time. Relevant information can be found on the website of the Department of Health (http://www.dh.gov.hk/english/main/main_cm/main_cm_hkcmms.html). Since the announcement, the trade has been encouraged to meet the requirements specified in the HKCMM standards. For phase III, the 6 local universities have already finished the development of standards for 28 CMMs. Inter-laboratory verification studies have also been completed. The results are now compiled as volume 3 of the HKCMM standards, and the publication will be released in 2010. The laboratory work of phase IV is ongoing, and the species selection of phase V consisting of more than 100 herbs is under negotiation. The detailed information is shown in Table 1.[12,13]

3. Characteristic sections of HKCMM standards

With the primary concern of safeguarding the authenticity, safety and quality of CMM, the content of HKCMM standards includes the source, description, identification, test, extractive and assay of the CMM. The characteristic sections of HKCMM standards are described below according to the order of items in CMM monograph.

3.1. Source

On the development of HKCMM standards, the State Food and Drug Administration and the local universities have cooperated in collecting authentic CMM samples[13]. Ten batches of samples were collected for each CMM, including 6 batches obtained from the Chinese mainland and 4 batches obtained from the local market of Hong Kong. The collection of Radix Panacis Quinquefolii is an exception. Forty batches of samples consisting of 10 batches from the Chinese mainland, 10 batches from Wisconsin of USA, 10 batches from British Columbia of Canada and 10 batches from Ontario of Canada were collected for its standard development.

Different from the Chinese Pharmacopoeia (CP), the species of CMM listed are currently available in Hong Kong market. For an instance, three species are listed as the original plants of Rhizoma Coptidis in CP including Coptis chinensis Franch., C. deltoidea C. Y. Cheng et Hsiao, and C. teeta Wall.. However, only the previous two species are stated in HKCMM standards, because the Rhizoma Coptidis obtained from C. teeta Wall. is commercially scarce in Hong Kong area[13]. Similar examples are Herba Ephedrae, Flos Magnoliae, Radix et Rhizoma Glycyrrhizae[13], and so on.

Administration habits are also taken in account by the HKCMM standards, which is another feature of species selection for the development of the standards. For example, Radix Aconiti Praeparata is listed in the HKCMM standards, but its raw material, Radix Aconiti, is not investigated currently because of its limited application in clinics[12].

3.2. Description

For the CMM with multiple sources, the descriptions of the species are consolidated if there is no significant difference among their characteristic features; otherwise, separate descriptions for each species are given in the monograph. For example, a consolidated description of Radix Astagalii with two sources was stated[12], while separate descriptions of Radix Codonopsis[13] with three sources were announced. HKCMM standards listed characteristics based on this method for preliminary authentication of CMM. In the individual monograph, a typical photograph of CMM was taken by modern digital photography technology with accurate hue and strong sense of three-dimensional, which demonstrate the scientific and pragmatic advantages.

3.3. Identification

Correct identification of real medicines from fake medicines, and differentiation of good quality from bad quality are the key elements in the quality control of botanical medicines. It provides a foundation to differentiate different herbal materials with similar morphology, and has been widely adopted in the Pharmacopeias of many countries. In HKCMM standards, the identification includes the following four subsections:

3.3.1. Microscopic identification

In HKCMM standards, both light microscope and polarized microscope have been used to identify CMM
| Phase | Duration | Research institutions | CMM samples |
|-------|----------|-----------------------|-------------|
| I     | 2003–2004 | 1. Hong Kong Baptist University  
2. The Chinese University of Hong Kong | Cortex Moutan (牡丹皮), Cortex Phellodendri Amurensis (黄檗皮), Cortex Phellodendri Chinensis (川黄柏), Radix Angelicae Sinensis (当归), Radix Asaragi (京黄), Radix Ginseng (人参), Radix et Rhizoma Notoginseng (三七), Radix et Rhizoma Salviae Miltiorrhiza (丹参), Rhizoma Alismatis (泽泻) |
| II    | 2005–2006 | 1. Hong Kong Baptist University  
2. The Chinese University of Hong Kong  
3. The Hong Kong University of Science & Technology  
4. The University of Hong Kong | Caulis Clematidis Armandii (川通草), Cortex Magnoliae Officinalis (厚朴枝), Flos Magnoliae (辛夷), Herba Desmodii Symphyoidis (合欢), Herba Ephedrae (麻黄), Radix Aconiti (附子), Radix Aconiti Praeparata (川乌), Radix Angelicae Pubescens (杭白芷), Radix Aucklandiae (款冬), Radix Bupleuri (紫苑), Radix Codonopsis (党参), Radix et Rhizoma Gentianae (龙胆), Radix Glycyrrhizae (甘草), Radix et Rhizoma Rhei (大黄), Radix Paronyae Alba (芦荟), Radix Paeoniae Rubra (红苟), Radix Platycodonis (桔梗), Radix Polygomi Multiflori (韩党), Radix Saposhnikoviae (防风), Rhizoma Chuanxiong (川芎), Rhizoma Cimicifugae (川芎), Rhizoma Copidis (知母), Rhizoma Curcumae (莪术), Rhizoma et Radix Notopterygh (虎杖) |
| III   | 2007–2008 | 1. Hong Kong Baptist University  
2. The Chinese University of Hong Kong  
3. The Hong Kong University of Science & Technology  
4. The University of Hong Kong  
5. The Hong Kong Polytechnic University  
6. City University of Hong Kong | Bulbus Fritillariae Thunbergii (黄花菜), Bulbus Fritillariae Ussuriensis (平贝母), Cortex Eucommiae (杜仲), Cortex Mori (桑白皮), Folium Ginkgo (银杏叶), Fructus Euvodiae (吴茱萸), Fructus Forsythiae (连翘), Fructus Ligustri Lucidi (玄参), Fructus Poriae (茯苓), Herba Andrographis (黄柏), Herba Leonuri (益母草), Herba Taxilli (秦靠丸), Medulla Junci (鸡内金), Radix Glehniae (远志), Radix Pseudostellariae (太子参), Radix Poiretiae (黄芩), Radix Rehmanniae (地黄), Radix Scutellariae (黄芩), Rhizoma Anemarrhenae (附子), Rhizoma Atractylodis Macrocephalae (白术), Rhizoma Belamcandae (射干), Rhizoma Gastrodiae (天麻), Semen Cassiae (决明子), Semen Vaccariae (王不留行), Spica Prunellae (辛秋草) |
| IV    | 2009–2010 | 1. Hong Kong Baptist University  
2. The Chinese University of Hong Kong  
3. The Hong Kong University of Science & Technology  
4. The University of Hong Kong  
5. The Hong Kong Polytechnic University  
6. City University of Hong Kong | Bulbus Fritillariae Hupehensis (湖北贝母), Cortex Dictamni (木通), Folium Isatidis (紫花地), Fructus Arctii (萹蓄), Fructus Aurantri (山楂), Fructus Amomi (豆蔻), Fructus Corni (山茱萸), Fructus Schisandrae (五味子), Herba Artemisiae Annuae (羌青), Herba Ecliptae (萹豆), Herba Houttuyniae (鱼腥草), Herba Gymnosterinae (绞股蓝), Herba Schizonepetae (穿心莲), Herba Scutellariae Barbatae (北柴胡), Herba Cistanches (柴胡), Radix Isatidis (板蓝根), Radix Peucedani (白芷), Radix Scrophulariae (玄参), Radix Sophorae Flavescentis (苦参), Radix Stephaniae Tetrandrae (重楼), Rhizoma Atractylodis (苍术), Rhizoma Corydalis (延胡索), Rhizoma Curculiginis (苍术), Rhizoma Curcumae Longae (姜黄), Rhizoma et Radix Baphicacanthis Cusiae (南板蓝根), Rhizoma Smilacis Glabrae (土黄芩), Rhizoma Polygoni Cuspidati (虎杖), Arsenic Trioxide (砒霜), Arsenolite (砷石), Calomel (硫磺), Cinnabaris (朱砂), Hydragyri Oxidum Rubrum (红药), Mercurius Chloride and Mercuric Chloride (乌鸦丹), Orpiment (雄黄), Realgar (雄黄) |
samples. Besides the corresponding text descriptions, color images of microscopic identification of transverse section and powder for each CMM are taken and listed in the monographs.

3.3.2. Physicochemical identification

Physicochemical identification was adopted in phase 1 and phase 2 of the HKCMM standards to test the representative constituents in CMM, while it was unemployed in phase 3 because of its weak selectivity in the complicated botanical matrix. In phase 4, this identification method could play a key role in the identification of 8 mineral medicines.

3.3.3. Thin-layer chromatographic (TLC) identification

In CP, both chemical markers and authentic Materia Medica are used as the reference substances for TLC identification; nevertheless, only chemical markers are adopted as the reference substances in HKCMM standards. Chemical structures of the markers are shown in this section.

3.3.4. High-performance liquid chromatographic (HPLC) fingerprinting

With the merits of high selectivity, high sensitivity, high separation rate and requiring a small amount of sample, chromatographic fingerprinting identification becomes the most significant advantage of the HKCMM standards. In general, one or more chemical markers and characteristic peaks can be identified in the chromatographic fingerprinting. Internal standards are used, if there is no peaks can be identified in the CMM, such as Caulis Clematidis Armandii[13].

The fingerprinting method is validated by using 10 batches of samples from different origins and by spiking sample of CMM with markers. System suitability parameters, including the reproducibility of peak area, the retention time, the column efficiency and the resolution between the marker peak and the adjacent peak, should match the requirement stated in the individual monograph. Identification of marker compound is based on comparison of its retention time in the standard solution with that in the sample extract under the same experimental conditions. For positive identification, the sample must give the characteristic peaks with relative retention times falling within the acceptable range of the corresponding peaks in the listed reference fingerprint chromatogram.

3.4. Tests

With increasing industrialization, urbanization and modernization in China, pollution is invading the far distant growing districts. Many Chinese medicine herbs and raw materials now contain certain levels of noxious materials.

In the HKCMM standards, each CMM must meet the requirements of test item, including the limits of heavy metals, pesticide residues, mycotoxins (aflatoxins), foreign matter, ash, water content and other chemical components which should be monitored[12,13].

3.4.1. Heavy metals

Arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are those heavy metals with relatively high toxicity to human beings. To assure the safety of CMM in use, the heavy metals are of specific concern in the HKCMM standards. Microwave assisted acid digestion is applicable for the preparation of test sample, and quantitative detection of As, Cd, Pb and Hg by using inductively coupled plasma-mass spectrometry (ICP-MS) with indium as an internal standard is the officially affirmative procedure. The consistent requirements are enacted for each CMM, and the recommended limits of heavy metals are shown in Table 2.

As a comparison, only six CMMs are required to meet the limits of heavy metals specified in CP, including Radix Paeoniae Alba, Radix Astagali, Radix et Rhizoma Salviae Miltiorrhizae, Radix Panacis Quinquefolii, Radix Glycyrrhizae and Flos Lonicerae[14].

3.4.2. Pesticide residues

Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other animals. For the safety of CMM, pesticide residues are another issue worthy of concern. In the HKCMM standards, gel permeation chromatography and solid phase extraction is employed for clean-up of the test sample extract. Gas chromatography (GC) using 2,4,5,6-tetrachloro-m-xylene as an internal standard is used for quantitative and qualitative analysis. The uniform requirements are enacted for each CMM, and the recommended limits of pesticide residues are shown in Table 3.

As a comparison, only two CMMs are required to meet the limits of pesticide residues specified in CP, including Radix Astagali and Radix Glycyrrhizae[14].
3.4.3. Mycotoxins

Mycotoxins, including aflatoxins, are naturally occurring toxins that are produced by many species of *Aspergillus*. Since aflatoxins are toxic and among the most carcinogenic substances known, a reliable method is developed to study the contents of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in CMM to protect human health.

In the HKCMM standards, an immunoaffinity column containing antibodies specific for aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> is used for clean-up of the test solution, and an HPLC system coupled with a post-column reactor and a fluorescence detector are used for quantitative analysis. The uniform requirements are enacted for each CMM, and the recommended limits of aflatoxins are shown in Table 4.

3.5. Assay

Most of the assay in the HKCMM standards is based on analysis of chemical markers by HPLC method; the determination of polysaccharides and total alkaline by colourimetric method are also listed.

### Table 2. Recommended limits of heavy metals in CMM samples

| Heavy metal   | Limit (Not more than) |
|---------------|-----------------------|
| Arsenic (As)  | 2.0 mg/kg             |
| Cadmium (Cd)  | 0.3 mg/kg             |
| Lead (Pb)     | 5.0 mg/kg             |
| Mercury (Hg)  | 0.2 mg/kg             |

### Table 3. Recommended limits of pesticide residues in CMM samples

| Pesticide                                              | Limit (Not more than) |
|--------------------------------------------------------|-----------------------|
| Aldrin and dieldrin (sum of)                           | 0.05 mg/kg            |
| Chlorodane (sum of cis-, trans- and oxychlorodane)     | 0.05 mg/kg            |
| DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-TDE) | 1.00 mg/kg            |
| Endrin                                                 | 0.05 mg/kg            |
| Heptachlor (sum of heptachlor and heptachlor epoxide)  | 0.05 mg/kg            |
| Hexachlorobenzene                                     | 0.10 mg/kg            |
| Hexachlorocyclohexane isomers (α-, β- and δ- hexachlorocyclohexane) | 0.30 mg/kg |
| Lindane (γ-hexachlorocyclohexane)                      | 0.60 mg/kg            |
| Quinotriene (sum of quinotriene, pentachloroaniline and methyl pentachlorophenyl sulphide) | 1.00 mg/kg |

### Table 4. Recommended limits of aflatoxins in CMM samples

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

Chemical markers specified in the HKCMM standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), Sigma and other chemical companies. A small number of chemical markers, which are commercially unavailable or unstable in store, are isolated from the CMM in laboratory, such as Δ<sup>7</sup> stigmastenol-3-O-β-D-glucoside from Radix Pseudostellariae and Z-ligustilide from Radix Angelica Sinensis[13]. Moreover, it is widely accepted that multiple constituents might be involved in any herb's therapeutic functions, and that the content of a single or a few marker compounds cannot accurately reflect the quality of herbal products[15]. Therefore, in order to upgrade the scientific levels of the HKCMM standards, multiple constituents are usually chosen as markers for the quality evaluation of CMMs in the HKCMM standard.

Sometimes, the choice of markers for a part of CMM is not an easy task. For an instance, the assay of Caulis Clematidis is absent because of the unavailability of chemical markers. Moreover, the newly isolated compound also cannot be used as a marker, if its content is less than 100 mg/kg in the raw CMM. For example, there are not suitable markers for the quantitative analysis of Radix Pseudostellariae based on the literature. The newly isolated compound (Δ<sup>7</sup> stigmastenol-3-O-β-D-glucoside) from Radix Pseudostellariae is low abundance; hence, this compound is suitable as the marker for fingerprinting identification, but not suitable for assay. In this case, the quality evaluation based on the contents of a type of chemicals is employed. Finally, the determination of polysaccharides was endorsed for the assay of Radix Pseudostellariae. In the monograph of Radix Pseudostellariae, polysaccharides are extracted by hot water, precipitated by ethanol and re-dissolved in water. Then polysaccharides are hydrolyzed and polymerized to form a coloured complex in the presence of sulfuric acid and anthrone, and the coloured complex is measured quantitatively by ultraviolet/visible spectrophotometer. Anhydrous glucose is used as the reference substance.

The development of limit of assay is the core of the HKCMM standards. The possible contributing factors such as mean content of ten batches of CMM samples, outlier of the data points, measurement uncertainty for the protocol, and reference value obtained from other pharmacopoeia are carefully considered and statistically calculated.
4. Summary

The increase in the use of herbal medicines, including CMM, is a global trend. Hong Kong has made positive progress on a number of CM fronts since the beginning of the new century. The development of the HKCMM standards is a good example in setting an internationally acceptable standard for most CMM. The announcement of the HKCMM standards plays a pivotal role in galvanizing the components, such as trading, R&D, and education into a vibrant economic sector to serve HK's healthcare system.

The HKCMM standards provides recommendations and references regarding the safety and quality standards for a number of Chinese medicines commonly used in Hong Kong. From the results of the HKCMM standards, evaluation standards of CMM concerned have been established and improved to varying degrees. The experiences and advantages raised from the HKCMM standards deserve to be referred for the revision of pharmacopoeia of other countries including Chinese mainland.

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