The Hong Kong University of Science and Technology

1. **Authentication of Bulbus Fritillariae Cirrhosae by RAPD-Derived DNA Markers**


*Molecules, 2014, 19, 3450-3459*

**Abstract**

*Bulbus Fritillariae* is the most commonly used antitussive herb in China. Eleven species of *Fritillaria* are recorded as *Bulbus Fritillariae* in the Chinese Pharmacopoeia. *Bulbus Fritillariae Cirrhosae* is a group of six *Fritillaria* species with higher efficiency and lower toxicity derived mainly from wild sources. Because of their higher market price, five other *Fritillaria* species are often sold deceptively as *Bulbus Fritillariae Cirrhosae* in the herbal market. To ensure the efficacy and safety of medicinal herbs, the authentication of botanical resources is the first step in quality control. Here, a DNA based identification method was developed to authenticate the commercial sources of *Bulbus Fritillariae Cirrhosae*. A putative DNA marker (0.65 kb) specific for *Bulbus Fritillariae Cirrhosae* was identified using the Random Amplified Polymorphic DNA (RAPD) technique. A DNA marker representing a Sequence Characterized Amplified Region (SCAR) was developed from a RAPD amplicon. The SCAR marker was successfully applied to differentiate *Bulbus Fritillariae Cirrhosae* from different species of *Fritillaria*. Additionally, the SCAR marker was also useful in identifying the commercial samples of *Bulbus Fritillariae Cirrhosae*. Our results indicated that the RAPD-SCAR method was rapid, accurate and applicable in identifying *Bulbus Fritillariae Cirrhosae* at the DNA level.

2. **Alkaloids of Linderae Radix suppressed the lipopolysaccharide-induced expression of cytokines in cultured macrophage RAW 264.7 cells**


*TANG [humanitas medicine], 2014, 4, e28*

**Abstract**

*Linderae Radix*, the dry roots of *Lindera aggregata* (Sims) Kosterm, has long been used as traditional Chinese medicine for treatment of inflammatory diseases. The total alkaloids are believed to be the active components responsible for anti-inflammatory of *Linderae Radix*. Here, the total alkaloids of *Linderae Radix* were extracted and isolated, including 12 isoquinoline alkaloids and 1 furan sesquiterpene. Within the alkaloids, norisoboldine, boldine, linderaline, isoboldine, reticuline, N-methyllaurotetanine, norjuziphine were found to be the major ingredients. In
lipopolysaccharide-treated macrophage RAW 264.7 cells, application of Linderae Radix extract, or total alkaloids, suppressed the transcription of pro-inflammatory cytokines, interleukin-1β and interleukin-6. Out of the 12 alkaloids, norisoboldine, boldine, and isoboldine were tested in lipopolysaccharide-treated macrophages, and norisoboldine was the strongest alkaloid in suppressing the cytokine expressions. The current studies suggested that the identification of alkaloids from Linderae Radix could provide a plausible explanation for herbal therapeutic functions.

3. Chromatographic fingerprint analysis and simultaneous determination of β–acetoxyisovaleryalkannin in Arnebiae Radix

A. Miernisha, T.X. Dong, J.Y. Guo, H.A. Aisa, K.W.K. Tsim

China Pharmacy, 2014, 3

Abstract

OBJECTIVE: To establish fingerprints to assess the quality of Arnebiae Radix and to determine the contents of β-acetoxyisovaleryalkannin derived from Arnebia euchroma (Royle) Johnst. which is in order to provide the evidence for the quality control of Arnebiae Radix in new version of the Chinese Pharmacopoeia. METHODS: HPLC fingerprinting and content determination methods were applied to evaluate the quality of Arnebiae Radix. Ten batched of samples were detected by an ACE C18 column (4.6 mm x 250 mm, 5 µm) using acetonitrile-0.1 % formic acid with water Isocratic system as mobile phase. The wavelength of detection is 516 nm for fingerprinting and content determination of β-acetoxyisovaleryalkannin in Arnebiae Radix. RESULTS: HPLC fingerprint of Arnebiae Radix was established and could be used for quality assessment of Arnebiae Radix. The results showed the characteristic HPLC fingerprints peaks of these ten batches in Arnebiae Radix. The contents of β-acetoxyisovaleryalkannin showed the differences from different sources of Arnebiae Radix. CONCLUSION: β-acetoxyisovaleryalkannin can be good chemical marker for the quality control of Arnebiae Radix. It has been used in Hong Kong Chinese Materia Medica Standards.

4. Study on identification of Cordyceps-A

H. Xu, T.X. Dong, K.J. Zhao, G.K.L. Chan, Y.J. Lin, K.W.K. Tsim

Chinese traditional medicine China Pharmacy Journal, 2014, 49, 120

Abstract

OBJECTIVE: To study on the identification of Cordyceps and establish its traditional pharmacognostic identification and modern identification method based on DNA molecular marker technology. METHODS: The methods of macroscopic, microscopic examination and RAPD-SCAR were employed here to authenticate Cordyceps. RESULTS: Based on the macroscopic identification, the features of transverse section and powder of Cordyceps were described in detail. The digital photographs were presented here in revealing the main macroscopic and microscopic characteristic of Cordyceps. The specific RAPD fragment of Cordyceps was conversed into SCAR
marker, and Cordyceps could be identified by the optimized PCR conditions. CONCLUSIONS: The authenticated method of microscopic and macroscopic identification is intuitive. However, the identification method based on DNA molecular marker is simple, which provides the new scientific evidences for the identification of authenticity of Cordyceps.

5. **Kai-Xin-San**, a Chinese herbal decoction containing Ginseng Radix et Rhizoma, Polygalae Radix, Acori Tatarinowii Rhizoma and Poria, stimulates the expression and secretion of neurotrophic factors in cultured astrocytes


*Evidence-Based Complementary and Alternative Medicine*, 2013: 731385.

Abstract

*Kai-xin-san* (KXS), a Chinese herbal decoction prescribed by Sun Simiao in *Beiji Qianjin Yaofang* about 1400 years ago, contains Ginseng Radix et Rhizoma, Polygalae Radix, Acori Tatarinowii Rhizoma, and Poria. In China, KXS has been used to treat stress-related psychiatric diseases with the symptoms of depression and forgetfulness. Although animal study has supported the antidepressant function of KXS, the mechanism in cellular level is still unknown. Here, a chemically standardized water extract of KXS was applied onto cultured astrocytes in exploring the action mechanisms of KXS treatment, which significantly stimulated the expression and secretion of neurotrophic factors, including NGF, BDNF, and GDNF, in a dose-dependent manner: the stimulation was both in mRNA and protein levels. In addition, the water extracts of four individual herbs did not significantly stimulate the expression of neurotrophic factors, which could explain the optimized effect of KXS in a herbal decoction. The KXS-induced expression of neurotrophic factors did not depend on signaling mediated by estrogen receptor or protein kinase. The results suggested that the antidepressant-like action of KXS might be mediated by an increase of expression of neurotrophic factors in astrocytes, which fully supported the clinical usage of this decoction.

6. **Chemical fingerprinting and quantitative analysis of two common Gleditsia sinensis fruits using HPLC-DAD**


*Acta Pharm*, 2013, 63, 505–515.

Abstract

*Gleditsiae Fructus Abnormalis* and *Gleditsiae Sinensis Fructus* are obtained from different developmental stages of fruits from *Gleditsia sinensis* Lam. (Leguminosae). The possible interchangeable usage of the two fruits, however, has long been very controversial. Here, high
performance liquid chromatography coupled with diode array detection was developed to explore their chemical fingerprinting profiles. Besides, the amounts of aglycones of saponin compounds, echinocystic acid and oleanolic acid in both fruits were quantified. The results indicated that there was no significant difference in the content of aglycones from the two types of fruits. However, their chromatographic fingerprints showed distinct characteristics. Therefore, the interchangeable application of these fruits has to be taken with a specific precaution.

7. Analysis of HPLC fingerprints and determination of isofraxidin and rosmarinic acid of Sarcandreae Herba from Sarcandra glabra (Thunb.) Nakai

R.W.L. Tang, Y.Y. Ng, D. Bi, R. Duan, K.W.K. Tsim, T.X. Dong

China Pharmacy, 2013, 47

Abstract

OBJECTIVES: To determine the contents of isofraxidin and rosmarinic acid, and establish fingerprints to assess the quality of Sarcandreae Herba from Sarcandra glabra (Thunb.) Nakai, which is in order to establish the quality evaluation of Sarcandreae Herba. Methods: HPLC fingerprint and content determination were applied to evaluate ten batches of Sarcandreae Herba from Sarcandra glabra (Thunb.) Nakai. For the determination of isofraxidin and rosmarinic acid, the samples were separated by Inertsil ODS-4 column (250 mm × 4.6 mm id. 5 μm) using acetonitrile and 0.1% phosphoric acid gradient system as mobile phase. The flow rate was 1.0 ml.min⁻¹, and the detection wavelength was at 342 nm. Results: HPLC fingerprint of Sarcandreae Herba was established with good separation and repeatability, which could be used for quality assessment of Sarcandreae Herba. The results showed that the HPLC fingerprints are similar among the ten batches. The contents of rosmarinic acid from ten batches were obviously different from each other. Conclusion: The method is sensitive, repeatable and accurate, it can be used as quality control for Sarcandreae Herba.

8. Study on Quality Standard of Andrographis Herba

L.J. Liang, K.J. Zhao, T.X. Dong, K.W.K. Tsim

Chinese Journal of Information on Traditional Chinese Medicine, 2013, 9, 63-65

Abstract

Objective: To improve the quality standard of Andrographis Herba through determination of effective components, moisture, total ashes, acid insoluble ashes, extracts and heavy metals. Methods: TLC and HPLC were used for qualitative and quantitative identification of andrographolide and dehydroandrographolide in Andrographis Herba. Routine examinations were based on the procedures recorded in the Appendix IX A, IX H, IX K, IX A and IX E of Chinese Pharmacopoeia(2010), for foreign matter, moisture, ashes, extracts determination and heavy metal test respectively. Results: Total content of andrographolide and dehydroandrographolide, extractives(70% ethanol) all complied with Chinese Pharmacopoeia. Conclusion: The established method was simple, accurate and can be used
as the quality standard for the quality control of Andrographis Herba.

9. **HPLC determination of 5-heneicosylresorcinol and linoleic acid in Fructus Tritici Levis**
M.J. Gao, D. Bi, W.L. Tang, C. Xu, T.X. Dong, K.W.K. Tsim

*China Pharmacy, 2013, 3*

**Abstract**

To develop an HPLC method for determination of 5-heneicosylresorcinol and linoleic acid in Fructus Tritici Levis

**Methods:** The separation was carried out on Phenomenex Luna C18 (250 mm x4.6, 5 μm) column. The mobile phase was acetonitrile and phosphoric acid in aqueous solution (0.1%) with gradient elution. The flow rate of mobile phase was maintained at 1.0 mL·min-1. The detection wavelength was set at 210 nm.

**Results:** There were good linear relationships between the concentrations and peak areas of 5-heneicosylresorcinol and linoleic acid in the ranges of 2.83 – 113.2 μg/mL (r=0.9998) · 17.55 – 702.0 μg/mL (r=0.9997), respectively. The average recoveries (n=6) were 97.0% and 98.2%, and RSDs were 1.8% and 2.5%, respectively.

**Conclusion:** This method is simple, precise, reliable and can be used for the quality control of Fructus Tritici Levis.

10. **Pharmacognostical identification of Allii Tuberosi Semen**

*China Pharmacy, 2013, 3*

**Abstract**

To establish the pharmacognostic identification and authentication method of Allii Tuberosi Semen.

**METHODS:** The methods of macroscopic, microscopic examination and thin layer chromatography (TLC) were employed here to authenticate Allii Tuberosi Semen.

**RESULTS:** Based on the macroscopic identification, the features of transverse section and powder of Allii Tuberosi Semen were described in detail. The digital photographs were presented here in revealing the main macroscopic and microscopic characteristic of Allii Tuberosi Semen. Thin layer chromatography method was established to identify the crude drugs of Allii Tuberosi Semen.

**CONCLUSIONS:** The authenticated method is sample and intuitive, and which provides the basis for further establishment of quality control of Allii Tuberosi Semen.
11. Determination of Galactitol in Cistanche deserticola and Cistanche tubulosa by HPLC-ELSD
K.J. Zhao, L.J. Liang, D. Bi, T.X. Dong, K.W.K. Tsim

Chinese Journal of Information on Traditional Chinese Medicine; 2012, 8, 52-54

Abstract

Objective: To establish a method for the determination of galactitol in Cistanche deserticola and Cistanche tubulosa, and to provide evidence for the quality control of Cistanches Herba. Methods: The contents of galactitol in 20 batches samples of Cistanche deserticola and Cistanche tubulosa were determined by HPLC-ELSD. The samples were separated by Alltech Pevail Carbohydrate ES column (4.6 mm×250 mm, 5 μm) with acetonitrile-water (80:20) as mobile phase. The flow rate was 1.0 mL/min and the injection volume was 10 μL. Shift tube temperature of ELSD was set at 80 ℃ and the gas flow rate was 3.0 L/min. Results: The linear range of galactitol was 89.6-1 120 mg/L (r=0.999 0), method detection limit was 15.998 mg/L, limit of quantitation was 79.903 mg/L. The average recovery rate of galactitol in Cistanche deserticola and Cistanche tubulosa was 100.34% and 101.11% respectively with RSD of 1.72% and 1.33 % (n=5). Conclusion: The method is sensitive, repeatable and accurate, which can be used for the quality control of Cistanches Herba.

12. Analysis of HPLC fingerprints of Fructus Aurantii from different habitats and contents of naringin, neohesperidin and synephrine
K.J. Zhao, Y.Z. Zheng, T.T.X. Dong, K.W.K. Tsim

Chinese Pharmaceutical Journal, 2011, 46, 955-959

Abstract

OBJECTIVE To establish the HPLC fingerprints for assessing the quality of Fructus Aurantii from different habitats, and to determine the contents of naringin, neohesperidin and synephrine.

METHODS HPLC fingerprinting and content determination methods were applied to evaluate 10 batches of Fructus Aurantii from different habitats. The samples were separated by Alltima C18 column (4.6 mm x 250 mm, 5 μm) using acetonitrile 0.1% phosphoric acid and 0.1% SDS water solution as mobile phase with gradient elution. The flow rate was 1.0 mL · min-1. The detection wavelengths were 224 nm for fingerprinting, 283 nm for content determination of naringin and neohesperidin, and 224 nm for content determination of synephrine, respectively.

RESULTS HPLC fingerprint of Fructus Aurantii was established with good separation and repeatability, which could be used for quality assessment of Fructus Aurantii. Six common peaks were defined in characteristic fingerprints, and similarity evaluation system was applied to evaluate the fingerprints of the 10 batches of Fructus Aurantii. The contents of naringin, neohesperidin and synephrine in Fructus Aurantii from different habitats were obviously different from each other.

CONCLUSION The method is sensitive, repeatable and accurate, which can be used for the quality control of Fructus Aurantii. The contents of naringin, neohesperidin and synephrine vary significantly
Acknowledged to the support of Department of Health

13. Study on Determination of Indigo and Indirubin Contents and HPLC Fingerprints of Isatidis Folium from Various Sources


*Modern Chinese Medicine*, 2011, 13, 15-18

**Abstract**

Objective: To determine the contents of indigo and indirubin and establish fingerprints of *Isatidis Folium* from various sources in order to control their qualities. Methods: We selected 17 batches of *Isatidis Folium* from various sources. The contents of indigo and indirubin were determined and the fingerprints were established by high performance liquid chromatography (HPLC) method. The fingerprints were compared using similarity evaluation software published by the Committee of China Codex. Results: The indigo content in *Polygonum Folium* was higher than that in *Isatis Folium* while its indirubin content was lower than that in *Folium Isatis*. Indigo and indirubin were not detected in the leaves of *Baphicacanthus cusia* and *Clerodendron cyrtophyllum*. The contents of indigo and indirubin in *Isatidis Folium* from different sources are varying largely. Conclusion: The above methods have desirable precision, reproducibility, stability, providing an experimental basis for quality control of *Folium Isatidis*.

Acknowledged to the support of Department of Health

14. Quality evaluation of Radix Glehniae (Glehnia littoralis) by HPLC-DAD chromatographic fingerprinting and quantitative analysis of the herbs from different regions of China


*Asian Journal of Traditional Medicines*, 2010, 5, 40-48

**Abstract**

High performance liquid chromatography coupled with diode array detection (HPLC-DAD) was developed to evaluate the quality of *Radix Glehniae*. Chromatographic fingerprints and the amounts of coumarins and polycyclics were determined in *Radix Glehniae* collected from different regions of China. The analysis was carried out on a Prevail C18 analytical column (250 mm ×4.6 mm id. 5 μm) using linear gradient elution of acetonitrile-water. The correlation coefficients of similarity were determined and twelve common peaks were defined from the HPLC fingerprints: five of the peaks were identified as psoralen, imperatorin, isoimperatorin, falcarindiol and falcarinol. The contents of these five identified compounds were subsequently determined by the validated HPLC-DAD method.
with high sensitivity, precision, repeatability and accuracy. This newly developed method involving a combination of chromatographic fingerprinting and quantitative analysis is suitable for the quality of Radix Glehniae.

Acknowledges to the support of Department of Health

15. Quality evaluation of Rhizoma Belamcandae (Belamcanda chinensis (L.) DC.) by using high-performance liquid chromatography coupled with diode array detector and mass spectrometry


Journal of Chromatography A, 2009, 1216, 2071-2078

Abstract

A high-performance liquid chromatography coupled with diode array detector and mass spectrometry (HPLC-DAD-MS) method was developed to evaluate the quality of Rhizoma Belamcandae (Belamcanda chinensis (L.) DC.) through establishing chromatographic fingerprint and simultaneous determination of seven phenolic compounds. The analysis was achieved on an Alltima C(18) analytical column (250 mm x 4.6 mm i.d. 5 microm) using linear gradient elution of acetonitrile-0.1% trifluoroacetic acid. The correlation coefficients of similarity were determined from the HPLC fingerprints, and they shared a close similarity. By using an online APCI-MS/MS, twenty phenols were identified. In addition, seven of these phenols including mangiferin, 7-O-methylmangiferin, tectoridin, resveratrol, tectorigenin, irigenin and irisflorentin were quantified by the validated HPLC-DAD method. These phenols are considered to be major constituents in Rhizoma Belamcandae, and are generally regarded as the index for quality assessment of this herb. This developed method by having a combination of chromatographic fingerprint and quantification analysis could be applied to the quality control of Rhizoma Belamcandae.

Acknowledges to the support of Department of Health

16. Simultaneous determination of phenols in Radix Polygalae by high performance liquid chromatography: quality assurance of herbs from different regions and seasons.


Journal of separation science, 2007, 30, 2583-2589

Abstract

Radix Polygalae, roots of Polygala tenuifolia or of Polygala sibirica, is a Chinese herbal medicine commonly used to prevent dementia. Reliable chemical markers for quality assurance of this herb
are missing. Here, a high performance liquid chromatography method coupled with diode array detection was developed to simultaneously determine nine different phenols in Radix Polygalae, including sibiricose A(5), sibiricose A(6), glomeratose A, tenuifoliside A, glomeratose D, 3',6-di-O-sinapoyl sucrose ester, mangiferin, polygalaxanthone III, and polygalaxanthone XI. By using two different detection wavelengths in the HPLC analysis, the developed method was able to determine the phenols with excellent resolution, precision, and recovery. This established method was therefore applied to determine the amounts of phenols in thirty-two samples from different cultivation regions and harvest seasons in China, and significant variations were revealed. The amounts of phenols in the roots of P. tenuifolia collected in Shanxi and Shannxi Provinces were markedly higher than in roots collected from other Provinces. Moreover, the samples harvested in the spring contained higher contents of phenols than those collected in other seasons.

Acknowledged to the support of Department of Health


Q. Xia, K.J. Zhao, Z.G. Huang, P. Zhang, T.T.X. Dong, S.P. Li, K.W.K. Tsim

Journal of agricultural and food chemistry, 2005, 53, 6019-6026

Abstract

Rhizoma Curcumae (Ezhu) is a traditional Chinese medicine that has been used in removing blood stasis and alleviating pain for over a thousand years. Three species of Curcuma rhizomes are being used, which include Curcuma wenyujin, Curcuma phaeocaulis, and Curcuma kwangsiensis. In China, the production of Rhizoma Curcumae largely depends on agricultural farming. The essential oils are considered as active constituents in Rhizoma Curcumae, which include curdione, curcumol, and germacrone. On the basis of the yield of curdione, curcumol, and germacrone in an orthogonal array design, the optimized extraction condition was developed. The amounts of these compounds within essential oils in Rhizoma Curcumae varied according to different species and their regions of cultivation. Chemical fingerprints were generated from different species of Curcuma, which therefore could serve as identification markers. In molecular genetic identification of Rhizoma Curcumae, the 5S-rRNA spacer domains of 5 Curcuma species, including the common adulterants of this herb, were amplified, and their nucleotide sequences were determined. Diversity in DNA sequences among various species was found in their 5S-rRNA spacer domains. Thus, the chemical fingerprint together with the genetic distinction could serve as markers for quality control of Curcuma species.

Acknowledged to the support of Department of Health