Introduction

- Chinese medicine as the major remedy for preventing and curing disease made great contribution to Chinese nation for its reproduction and prosperity.
- Sheng Nong’s herbal classic (Eastern Han Dynasty, from 1st to 2nd century A.D.) records 365 kinds of Chinese medicines.
- Tang Materia Medica (Tang Dynasty in 659 A.D.) is the first pharmacopeia in China, with 884 Chinese medicines recorded.
- Compendium of Materia Medica (Ming Dynasty in 1578 A.D.), describes 1,892 Chinese medicines.
- The 3rd census of traditional Chinese medicine (TCM) resource) showed existence of 12807 CMM.
- There are about 1200 CMM used commonly.
Introduction

Traits
- Complex origin: multi origins, synonym, or homonym
- Natural resources: easily affected by various factors
- Difficulty in effective control during producing process.
- Comprehensive and chemical constituents and treating functions.

QS
- Quality Uniformity
- Bioequivalence
- Safe Usage

1. Idea of QS Research and Systematic Construction of TCM
1.1. For CMM and its Prepared Slices (PS)

**CMM and PS**

- Chemical research
  - Clarification of chemical constituents
  - Biological or literature research
  - Effective constituents

**TCM QS database**

- Morphological identification
- Microscopic identification of tissues and powders
- Water
- Total ashes
- Acid insoluble ashes
- Heavy metal and deleterious element
- Pesticide residue
- SO2 residue
- Mycotoxins
- Limit examination of toxic and harmful substance

**QS systems of CCM and PS in Chinese Pharmacopoeia**

- **Identification**—Real or Faked—Real Medicine
- **Examination**—Safety—Medicine with Less Toxicity
- **Fingerprint & Determination**—Quality—High-quality Medicine

- **Name**
- **Source**
- **Character**
- **Identification**
  - Microscopic
  - Chromatographic
- **Examination**
  - Water
  - Total ashes
  - Acid insoluble ashes
  - Heavy metal and deleterious element
  - Pesticide residue
  - SO2 residue
  - Mycotoxins
  - Limit examination of toxic and harmful substance
- **Fingerprint/characteristic spectrum**
- **Extracts**
- **Content determination**
- **Processing**
- **Properties, flavors and channels**
- **Functions and indications**
- **Usage and dosage**
- **Notice**
- **Storage**
1.2. For Proprietary Chinese Medicine

CCM

- Fixed origin
- Fixed production places
- Fixed cultivation techniques
- Fixed harvest processing
- Fixed processing technology

Material control

PS

- Extract, solid-liqu separation, purification, concentration, drying, molding

Producing control

Proprietary Chinese medicine

- Morphological identification
- Microscopic identification

Morphological Identification

- Examination
  - Provision and inspection of dosage-form
  - Heavy metal and deleterious element
  - Pesticide residue
  - Endogenous poisonous and harmful ingredients

Microscopic Identification

- Provision and inspection of dosage-form

Effective or major components

Effective or major components

- Literature investigation
- Analysis of chemical components
- Analysis of effective components

QS system of Proprietary Chinese Medicine in Chinese Pharmacopeia

- Name
- Prescription
- Preparation
- Character
- Identification
  - Microscopic identification
  - Chromatographic identification

- Provision and inspection of dosage form
  - Heavy metal and deleterious element
  - Pesticide residue
  - Endogenous poisonous and harmful ingredients

- Fingerprint/characteristic spectrum
- Content determination
- Function and indication
- Usage and dosage
- Attentions
- Specification
- Storage
2 Research of QS System and Enlargement & Amendment of TCM in Chinese Pharmacopoeia 2015 edition

2.1. CCM Identification—Real vs. Faked

- The stigmas of *Crocus sativus* (西红花)
- The stems of *Cistanche tubulosa* (管花肉苁蓉)
- *Cordyceps sinensis* (冬虫夏草)
- *Cordyceps gunnii* (古尼虫草)
- A: *Bombyx Batryticatus*
- B: fakes
- The barks of *Eucommia ulmoides* (杜仲)
- The stems of *Cistanche deserticola* (荒漠肉苁蓉)
- The stems of *Cistanche tubulosa* (管花肉苁蓉)
2.1. CCM Identification—Real vs. Faked

**Microscopic identification on Basis of Tissue or Powder**

1. resin cana
2. clusters of calcium oxalate
3. cork cell
4. Conduit
5. starch grain

Microscopic traits of Panax ginseng root powder

Microscopic features of transverse section of Panax ginseng root

---

2.1. CCM Identification—Real vs. Faked

**Microscopic identification on Basis of Tissue or Powder**


Microscopic features of transverse section of Coptis rhizoma

Fakes: made of flour

Cordyceps sinensis

Cordyceps gunnii

Cordyceps

The rate of microscopic identification of crude drugs in CHP
2.1. CCM Identification—Real vs. Faked

- TLC identification including
  - Current: reference substance, controlled medicine, and
  - Prospective: TLC identification of CCM compared to reference extract.

Advantages by using the reference extract:
- Multi-component control, a higher specificity
- Large-scale production, higher consistency, uniformity, but lower cost
- Trace, chips, easy to use and carry, but lower cost
- Manual with TLC photo, more accurate and clear

HPTLC images of the roots of *Polygala tenuifolia* (远志)

1~9: *P. tenuifolia* roots; S1: 3,6-disinapoyl sucrose; S2: polygalaxanthone III; RE: reference extract

HPTLC images of the barks of *Magnolia officinalis* (厚朴) (by Z. T. Wang, et al)

A: Visualized by 1% vanillic aldehyde and 10% sulfuric acid ethanol solution; B: By 0.04% DPPH ethanol solution

2.1. CCM Identification—Real vs. Faked


HPTLC images of the rhizomes of *Coptis* (by Z. T. Wang, et al.)

1,2,7: *Coptis chinensis*; 3,4,8: *Coptis deltoidea*; 5,6: *Coptis teeta*
S1: palmatine; S2: berberine; S3: epi-berberine; S4: coptisine; S5: jatrorrhizine; S6: columbamine

HPTLC images of the roots of *Bupleurum* (by P. S. Xie, et al.)

1,2,5: *B. chinensis*; 3,6: *B. scorzonerifolium*; 4,12: *B. falcatum*; 7: *B. longiradiatum*; 8: *B. tricus; 9: *B. polycephorum*; 10: *B. wenchuanense*; 11: *B. marginatum*; 13: *B. yinchowense*; 14: *B. simithii* var. *parvifolia*; 15: *B. tenue*
S1: saikosaponin f
S2: saikosaponin b2
S3: saikosaponin a
S4: saikosaponin d
2.1. CCM Identification—Real vs. Faked

By Characteristic Spectra

- **Advantages**
  - higher separation
  - abundant information
  - higher specificity
  - peak area ratio and semi-quantitative control realized

- **Analytical method**
  - HPLC
  - GC
  - HPCE

- **Evaluation methods**
  - The characteristic peak of retention time and relative retention time
  - The characteristic peak of the peak area ratio
  - Contrast of reference crude drugs and reference extract

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<th>obacunone</th>
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Characteristic chart of the cortexes of *Dictamnus dasycarpus* (白鲜皮)

Case 1: Characteristic Spectrum and QS Establishment of Aquilariae Lignum Resinatum (Agarwood)

- *Aquilaria sinensis* (Lour.) in Thymelaeaceae
- **Valuable medical** with action of moving qi and ceasing pain, stopping vomiting and asthma, used for thoracic abdominal distension stuffy pain, cold stomach vomiting, hiccups, kidney empty reversed flow of qi for urgent.
- **Precious spices and perfumes.**
- **Luxury handicraft raw materials and collections.**
Phytochemical Studies

2-(2-phenylethyl)chromone (PEC, 28)

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Phytochemical Studies

Tetrahydro-2-(2-phenylethyl)chromone (THPEC, 24)

Phytochemical Studies

Sesquiterpenes


Fingerprint Analysis of Agarwood
HPLC Fingerprint of agarwood and Aquilaria wood

HPLC–UV Chromatograms

Agarwood

Aquilaria wood

HPLC-MS TIC Chromatograms

Agarwood

Aquilaria wood

Comparison of HPLC fingerprints of different types of agarwoods

HPLC–UV chromatogram

Highly abundant peaks in artificial sources

Common peaks of natural and artificial sources

7 months after artificial induction
8 months after artificial induction
11 months after artificial induction
Natural sources
Identification of 2-(2-phenylethyl)chromones in agarwood by HPLC-ESI-MS²

XIC: m/z 357, 337, 335, 333, 317, 303, 267

XIC: m/z 383, 341, 319, 311, 301, 283, 297

XIC: m/z 367, 365, 349, 347, 343, 331, 327, 313, 281

- 70 of 2-(2-phenylethyl)chromones, including 28 kinds of simple types, 35 species of four hydrogen types, 5 five hydrogen types and 2 six hydrogen types were identified.

HPLC characteristic chromatogram of agarwood included in CP (2015 ed.)

Comparison of characteristic chromatograms of 17 batches

Reference characteristic chromatogram

Agarwood reference
Case 2: Characteristic Spectrum and QS Establishment of Notopterygii Rhizoma et Radix (NR)

- **Origin of Plant:** *Notopterygium incisum* Ting ex H. T. Chang and *N. forbesii* H. de Boiss.
- **Properties:** NR is used to treat a strong cold, headache, rheumatism, paralysis, shoulder pain and other symptoms.
- **Chemical Components:** Coumarins, Phenolic acids, Polyacetylenes, Essential oil, and Terpenoids etc.

Characteristic chromatogram of Chinese agarwoods and fakes
Chemical Constituents from NR

The reference characteristic chromatogram of Notopterygii Rhizoma et Radix

1: Notopterol; 2: Phenethyl ferulate; 3: Isoimperatorin; 4: Falcarindiol

HPLC characteristic chromatogram of NR included in CP (2015 ed.)

The characteristic chromatogram of the roots of N. incisum

The reference characteristic chromatogram of Notopterygii Rhizoma et Radix

The characteristic chromatogram of the roots of N. franchetii

Comparison of the characteristic chromatogram of Notopterygii Rhizoma et Radix(QH) and fakes (WPQH)

1: Notopterol; 2: Phenethyl ferulate; 3: Isoimperatorin; 4: Falcarindiol
Case 3: Characteristic Chromatogram for Identification of Multi-Origin——Clematidis Radix

![Characteristic Chromatogram for Clematidis Radix](image)

Specific LC-MS Identification of Animal Gelatin TCM—Chinese Pharmacopoeia (2015 ed.)

Colla Corii Asini — Donkey skin
Oxhide gelatin — Cowhide
New Colla Corii Asini — Pigskin
Tortoise-shell glue — tortoise-shell
Deerhorn Gelatin — Antlers
2.1 Identification of TCM—Real or Fake

Biological Identification

(1) DNA identification

1. positive CK 2-11. *Zaocys dhumnade*
12. negative CK 13. Blank

(2) DNA barcode

(3) SDS-PAGE: animal drugs

(4) Rapid TCM identification based on monoclonal antibody technique: for TCM with unique components


2.2 Examination—Safety Guaranteed

- Impurities and foreign matter:
- Heavy metals and harmful element: Atomic absorption spectrometry, plasma mass spectrometry
- Pesticide residues: detected by GC, GC-MS, LC-MS
- Mycotoxins: detected by HPLC, LC-MS
- SO₂ Residue: detected by IC, GC
- Endogenous toxic and harmful substances: detected by HPLC, LC-MS, GC, GC-MS

Efficient and sensitive detection methods:
- Monoclonal antibodies
- Chemical reactions
2.2 Examination—Safety Guaranteed

- Heavy metals and harmful elements
  - Pretreatment Method: Microwave Digestion, Wet Digestion, Drying Digestion, High Pressure Digestion.
  - Pb, Cd, As, Hg, Cu: Atomic Absorption Spectrophotometry (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).
  - Speciation and valence determination of As and Hg: HPLC-ICP-MS
  - TCM collected in Chinese Pharmacopoeia (2015 ed.):
    - 17 herbs: Astragali Radix, Lonicerae, Panacis Quinquedolii Radix, Paonieae Radix Alba, Salviae Miltiorrhizae Radix et Rhizoma, Glycyrrhizae Radix et Rhizoma, Lycii Fructus, Crataegi Fructus, Asini Colla, Hirudo, Ostreae Concha, Meretricis Conacha & Cyclinae Concha, Margarita, Laminariae Thallus & Eckloniae Thallus, Sargassum, Sepiae Endoconcha, Propolis;
    - Seven extractions: Total Ginsenoside of Ginseng Stems and Leaves, Notoginseng Total Saponins, Capillary Wormwood Extract, Berviscapine, Cusides, Entella Total Glucosides, Menthol
    - All of the TCM injections.

2.2 Examination—Safety Guaranteed

- Heavy metals and harmful elements
  - Focus on the element valence state
    - Toxicity of 6 valences of As: Arsenious acid (As$^{3+}$) > Arsenic acid (As$^{5+}$) > MMA > DMA, AsB and AsC are almost innoxious.
    - Toxicity of 4 valences of Hg: Methylmercury > Ethylmercury > Hg$^{2+}$ > Hg$^{+}$, The toxicity of methylmercury and ethylmercury are hundreds-fold higher than inorganic mercury, which might be attributed to the stronger hydrophobicity of heptachlor.

HPLC-ICP-MS Chromatogram

Department of Traditional Chinese Medicine, Shanghai Institute for Food and Drug Control, Shanghai, China
2.2 Examination—Safety Guaranteed

- **Pesticide residues**
  - Chinese Pharmacopoeia (2015 ed.): A total of 24 pesticides including organic chlorine, organic phosphorus and pyrethroids, etc, are required.
  - Chinese Pharmacopoeia (2015 ed.): GC/MS/MS(76); LC/MS/MS (153); 227 kind of pesticide residues in total.
  - TCM formula granule: Cultivated TCM herbs are required to be investigated on pesticide residues, and standards of these carrying servious pesticide residues should be established.

- **Mycotoxins**
  - Methods: HPLC, LC-MS methods.
  - Limits: Aflatoxin B1 ≤ 5 μg/kg; Total aflatoxin G2, aflatoxin G1 and aflatoxin B2 ≤ 10 μg/kg.
2.2 Examination—Safety Guaranteed

☐ **SO₂ Residue**

- **Methods:** Acid-base titration, IC and GC.
- **Limits:**
  - SO₂ residue of 10 Chinese herbal medicine and conventional pieces including Dioscoreae Rhizoma, Asparagi Radix, Trichosanthis Radix, Gastrodiae Rhizom, Achyranthis Bidentatae Radix, Bletilla sfrsia, Atractylodis Macrocephalae Rhizoma, Paonieae Alba Radix, Codonopsis Radix, and Puerariae Thomsonii Radix, which were traditionally deal with sulphur fumigation, should not be more than 400 mg/kg;
  - SO₂ residue of others TCM 150 mg/kg not be more than 400 mg/kg.
- The shapes and properties were revised.

![Color changes of Codonopsis Radix dealed with sulphur fumigation](image)

2.3 Fingerprint Combined Content Determination of Multiple-components

—Quality Evaluation
2.3.1 Establishment of Fingerprint and Determination Standards

- Studies on Establishment of Fingerprint and Determination Standards for Chinese Material Medica (CMM) and Prepared Pieces (PP)
- LC-DAD-MS^n Fingerprint and TCM Chemical Constituents Database

Research of Determination Standards of Fingerprints

- Origin Species
- Medicinal Part
- Locality of Growth
- Cultivation Techniques
- Productive Processing
- Processing procedure

- Preparing method for test solution, control and references
- Methodology: stability, precision and reproducibility.
- Identification of major chromatographic peaks
- Establishment of the control fingerprint
- Determination and evaluation of samples
Research Case: Rhei Radix Et Rhizoma

- **Rhei Radix Et Rhizoma** is one of the most famous TCM herbs, using to treat diarrhea attack plot, clear heat and detoxifying, remove blood stasis, and dredging.
- Its origin resources include *Rheum palmatum* L., *R. tanguticum* Maxim.ex Balf. and *R. officinale* Baill.
- Major components: anthraquinones and its glycosides, tetrahydroxystilbene-glucosides, tannins, and chromones, etc.

Chemical Types in Rhei Radix Et Rhizoma

- Anthraquinones
- Anthrone s
- Tetrahydroxystilbene-glucosides
- Acyl glycosides
- Tannins
- Chromones

*Rheum tanguticum* *Rheum palmatum* *Rheum officinale*
HPLC-UV Fingerprint of Rhei Radix Et Rhizoma

A. R. palmatum; B. R. tanguticum; C. R. officiale; D. Comparison of these 3 species.

Evaluation of Fingerprint Similarity
**Evaluation of Fingerprint Similarity of *R. tanguticum***

Fingerprints of 42 batches of *R. tanguticum*

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<td>0.867</td>
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Identification Fingerprint Chromatograms of Rhei Radix Et Rhizoma by LC-DAD-MS

Proposed MS fragmentation for \([M-H]^-\) ions of anthraquinones

Proposed MS fragmentation pathway for the \([M-H]^-\) ions of stilbenes

Proposed MS fragmentation pathway for the \([M-H]^-\) ions of phenylbutanone glucopyranosides

Charaterization of Fingerprint Chromatograms of Rhei Radix Et Rhizoma by LC-DAD-MS

- **TIC chromatograms of Rhei Radix Et Rhizoma**
  A: Anthraquinones; B: Anthrones; C: Tannins; D: Two styrene and butyl benzene ketone; E: Acyl indicant, original ketone and other constituents.

- **271 chemicals Identified**
  Including 34 anthraquinones, 83 anthrones, 46 tannins, 17 stilbenes, 24 benzyl butylketones, 26 acyl indicans, 26 original ketones, and 15 other constituents.
2.3.2 Multicomponent Determination of TCM

- **Methodology**
  - HPLC
  - LC-MS
  - GC
  - GC-MS
  - HPCE
  - SFC
  - NMR

- **Selection of chemical markers**
  - Therapeutic constituents
  - Biological constituents
  - Characteristic constituents

- **Solution to lack references**
  - One marker for multi-components evaluation
  - Multi-components determination using control extract
Multi-components Determination by Markers

**Determination of 14 constituents in Rhei Radix Et Rhizoma**

A. HPLC chromatograms of 14 mixed references; B. HPLC chromatogram of Rhei Radix Et Rhizoma

1. gallic acid; 2. (+)-catechin; 3. (-)-epicatechin-3-O-gallate; 4. isolindleyin; 5. 4-(4'-hydroxyphenyl)-2-butanone; 6. lindleyin; 7. sennoside B; 8. sennoside A; 9. aloe-emodin; 10. 4-(4'-hydroxyphenyl)-2-butanone-4-O-β-D-(2''-O-galloyl-6''-O-cinnamoyl)-glucopyranoside; 11. rhein; 12. emodin; 13. chrysophanol; 14. physcion

Multicomponent Determination by Control Extract

- Simple preparation, low cost
- Quantitative packing, convenient use
- Save reference substance
- Specification with chromatograms, chromatographic peak easily identified
Stem of *Mahonia bealei* (Fort.) Carr. or *M. fortune* (Lindl.) Fedde. has effect of clearing heat, drying wet, purging fire, and detoxification.

**Multi-component determination by control extract**, One marker for multi components evaluation, and qNMR conducted

**Multi-component determination by control extract** was record in Chinese Pharmacopoeia 2015 ed. It is first record in Chinese Pharmacopoeia for this determination method


---

**A Research Case: Mahonia Stems**

<table>
<thead>
<tr>
<th>Material</th>
<th>Ethanol extract</th>
<th>50% Ethanol eluent</th>
<th>Control extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>70% Ethanol reflux, 3 times 1h each</td>
<td>70% Ethanol reflux, 3 times 1h each</td>
<td>70% Ethanol reflux, 3 times 1h each</td>
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<tr>
<td>Dry to the concentration of 1.1g/ml</td>
<td>Dry to the concentration of 1.1g/ml</td>
<td>Dry to the concentration of 1.1g/ml</td>
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<tr>
<td>D101 macroporous resin CC; 2BV water elution, abandon; 2BV 10% ethanol elution, abandon; 2BV 50% ethanol elution, collected</td>
<td>D101 macroporous resin CC; 2BV water elution, abandon; 2BV 10% ethanol elution, abandon; 2BV 50% ethanol elution, collected</td>
<td>D101 macroporous resin CC; 2BV water elution, abandon; 2BV 10% ethanol elution, abandon; 2BV 50% ethanol elution, collected</td>
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<tr>
<td>concentration</td>
<td>concentration</td>
<td>concentration</td>
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<tr>
<td>freeze dryer</td>
<td>freeze dryer</td>
<td>freeze dryer</td>
<td></td>
</tr>
</tbody>
</table>

**Preparation Protocol**

**HPLC chromatograms**

A. Hybrid reference substance; B. Standard extract; C. Mahonia: C. Columbamine; J. jateorhizine; P. palmatine; B. berberine

---

**Control extract**

<table>
<thead>
<tr>
<th>reference extract batch</th>
<th>Crude medicine</th>
<th>inventory</th>
<th>yield</th>
<th>productivity</th>
<th>The transfer rate of total alkaloids</th>
<th>content of total alkaloids</th>
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<tr>
<td>022101</td>
<td>贵州</td>
<td>200g</td>
<td>4.69g</td>
<td>2.34%</td>
<td>51.4%</td>
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<tr>
<td>031101</td>
<td>贵州</td>
<td>5kg</td>
<td>132g</td>
<td>2.64%</td>
<td>50.3%</td>
<td>40.82%</td>
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<tr>
<td>031102</td>
<td>贵州</td>
<td>5kg</td>
<td>137g</td>
<td>2.74%</td>
<td>50.1%</td>
<td>40.32%</td>
</tr>
</tbody>
</table>
**Quality Standard of *Mahonia* Stems by control extract**

**Source:** Mahonia is the stem of *Mahonia* bealei (Fort.) Carr. or *M.* fortune (Lindl.) Fedde. belonging to berberidaceae, produced for standard extraction.

**Preparation**

**Character** Yellowish-brown powder, slightly acid odour, taste bitter

**Solubleness** Soluble in methanol, ethanol and water, insoluble in chloroform and ethyl acetate

**Identify**

**Examination**

**Fingerprint**

**Assay** Calculated by dry goods, including columbamine, jateorhizine, palmatine, berberine, should more than 35.0%.

**Package and storage**

**Application** For Mahonia stems analysis use.

---

**Determination data *Mahonia* Stems by control extraction**

<table>
<thead>
<tr>
<th>No</th>
<th>Place</th>
<th>columbamine (mg/g)</th>
<th>jateorhizine (mg/g)</th>
<th>palmatine (mg/g)</th>
<th>berberine (mg/g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CE</td>
<td>RSD (%)</td>
<td>CE</td>
<td>RSD (%)</td>
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<tr>
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<td>1.05</td>
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<td>1.69</td>
<td>6.19</td>
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<tr>
<td>No</td>
<td>Place</td>
<td>columbamine</td>
<td>jateorhizine</td>
<td>palmatine</td>
<td>berberine</td>
</tr>
<tr>
<td>----</td>
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<td>-------------</td>
<td>--------------</td>
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<td>-----------</td>
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<tr>
<td></td>
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<td>CE Meth. (%)</td>
<td>CE Meth. (%)</td>
<td>CE Meth. (%)</td>
<td>CE Meth. (%)</td>
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</table>

Determination data Mahonia Stems by control extraction

3 High Efficiency Analysis Technology of Complex TCM System

- Sample preparation
  - PWWE
  - online SPE

- LC Separation
  - 2D LC system
  - column switching LC

- MS Detection
  - IT-TOF-MS: MDFIF, etc.
  - Qtrap-MS: stepped MRM, sMRM, stepped MIM, etc.
  - Qtof-MS: UNIFTM, etc.
  - Multi-platform combined, overall strategy
Direct Analysis of Constituents from TCM Based on Online Pressurized Solvent Extraction Technique

Traditional extraction method of TCM
- Solvent, dosage of herb use much
- Sample preparation time is long
- May cause degradation compounds
- Unable realize the direct analysis of the sample

Online pressurized solvent extraction
- Solvent, dosage of medicine use little
- Water or low proportion of organic solvent extraction, analysis environmental
- Automatic extraction and analysis online switch, high flux
- Avoid light, extraction time is short, avoid degradation compounds
- LC - MS online connection, analysis directly

- online PWWE-TFC-LC-DAD apply on C. deserticola constituent analysis
- online PWWE-TFC-LC-MS/MS apply on Polygala constituent analysis

Mixed standards

C. deserticola

Polygalae Radix

PWWE-TFC-LC-MS/MS

Chemical analysis
Chemical analysis of Complex System Based on multi-column Chromatography

- Large polarity diversity
- Separate of HILIC or RPLC cannot achieve full retention
- Several compounds outflow cannot be accurate quantitative
- difficult to achieve HILIC and RPLC connect directly
- conventional capture column caused serious peak broadening

- HILIC-TFC-LC-MS/MS method established
- Comprehensive analysis for plasma samples chemical composition after Baoyuan decocction administration

Research Case 1: Ginseng saponin by LC-Qtrap-MS

- ginseng saponins are main active ingredient in ginseng.
- It produces [M+HCOO]⁻ and [M-H]⁻ ion;
- [M+HCOO]⁻ broken into [M-H]⁻ most appropriate collision to -32eV;
- Establish a step-wise MRM-IDA-EPI method, achieve comprehensive chemical constituent analysis 221ginseng saponins
- UHPLC-sMRM method established, and achieved all peaked saponins quantitative analysis
The primary metabolites, phenylethanol, iridoid glycosides and lignin glycosides show different behavior in MS.

Through PI, NL and predefined MRM realized the detection of all ingredients, and through the EPI, identification of each structure.

Introduction of sMRM method for all 513 constituents relative quantitative analysis.

Using multivariate statistical analysis to distinguish two original plants in quantitative chemical groups.

Betaine and tubuloside B are the biomarkers.

Current Difficulties

- TCM reference substance is difficult to get comprehensively
- Difficult to achieve compound detection
- MS data matrix containing adduct fragment ions, redundant information
- Isomers are difficult to distinguish
- MS quantitative parameter difficult to optimize
- Peak intensity how to translate into content
- Large scale chemical constituent range
- Difficult to achieve large compound quantitative all

"Three Steps"
Rapid Characterization of *Carthamus tinctorius* L. Extract Metabolites by UFLC/IT-TOF-MS and UPLC/Qtrap-MS

- *Carthamus tinctorius* L., also known as ‘Hong hua’ in TCM, belongs to Asteraceae
- Total flavonoids from *C. tinctorius* L. have anti-myocardial ischemia effect;
- 137 metabolites and 19 prototypes were identified using diagnostic ion strategy, including 63 in plasma; 73 in urine; 50 in bile; 17 in faeces;
- UPLC/Qtrap-MS used to quantitatively and semi-quantitatively analyze 156 in vivo components

Typical metabolic pathways

![Typical metabolic pathways](image)
Establishment of Fingerprints and in vitro Metabolic profiles of Bao-Yuan-Tang based on LC-MS Global Strategy

The method UPLC/Q TOF-MS, HPLC/Qtrap-MS and UNIFI software was adopted to construct the LC-MS overall strategy in order to establish a fast and comprehensive identification of the fingerprint of Bao-yuan-Tang.

A total of 236 compounds including 139 saponins, 83 flavonoids, 6 procyanidins, 4 lignans, and 4 diterpenoids were defined from Bao-yuan-Tang.

On the basis of fingerprints of Bao-yuan-tang, HPLC/IT-TOF-MS and UPLC/Qtrap-MS were used to analyze the metabolic fingerprints of it.

A total of 286 metabolites were detected and identified, among which 77 were plasma, 117 in bile, 139 in urine, and 4 in faeces, including 178 flavonoids, 105 saponins and 3 small molecular phenolic compounds.

Metabolic profiles analysis of Bao-yuan-tang

Proposed metabolic pathways of liquiritigenin-4-O-glucoside in rats

Proposed metabolic pathways of Licorice-saponin G2 in rats
THANKYOU !

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