New Methods in TCM Research

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Abstract: New methods developed in this laboratory for the analysis and production control of TCM and patent medicines are summarized in this report.

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Introduction

TCM (Traditional Chinese Medicine) has a long history. It can be dated back to the 26th century B.C., to the time of emperor Yan (Shennong), who first tested more than hundred kinds of herbs for use in medical treatment. A temple has been built in Hunan province, which is known to be rich in Chinese herbal medicinal resources, to commemorate his great contribution. Every year, people from all over China come to attend the memorial ceremony held in Yan county.

Recently, state-of-the-art of TCM research has been described, with the emphasis on herbal organic acids and related substances [1]. In this report, new methods developed in this laboratory for the analysis and production control of TCM and patent medicines are summarized.

Establishment of standard methods for TCM production process control by high performance liquid chromatography

Analysis of ginkgolides and bilobalide in Ginkgo biloba L. extract for its producing process control by high-performance liquid chromatography

The dried ripe seed and leaves of Ginkgo biloba L. are two commonly-used traditional Chinese herbal medicines. Ginkgo biloba L. extracts from its leaves are commonly used for the treatment of cerebrovascular and peripheral circulatory problems of the elderly[2]. These extracts contain flavon glycosides, biflavones and terpenes. The pharmacologically active components, the diterpenes ginkgolide A, B and C (GA, GB, GC) and the sesquiterpene bilobalide (BB) are reported to be only present in Ginkgo biloba L. Thus, their contents are often used as the quantitative indices of ginkgo biloba L. extracts (GBE).

We developed a new HPLC method for the analysis of GA, GB, GC and BB. In the proposed HPLC-RF method, ethyl acetate was used to extract the terpenoids from the sample solution, and no peak interference in determination of the terpenoids in real samples was found. GBE was dissolved in methanol in an ultrasonic bath. After mixing with water and ethyl acetate, organic phase evaporated at 40°C. The residue was dissolved in methanol and the sample solution was injected into the HPLC system for analysis.

The above-described method was applied to determine ginkgolides and bilobalide in GBE. Additionally, for manufacturing process control, the analysis was completed on the different manufacturing steps, and the results are shown in Table 1. From this table, it is shown that the quality of GBE as well as the efficiency of extract production in different steps can be monitored by the proposed method.

Table 1. Samples analysis results on different manufacturing steps

<table>
<thead>
<tr>
<th>Step</th>
<th>BB(%)</th>
<th>GC(%)</th>
<th>GA(%)</th>
<th>GB(%)</th>
<th>Total(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.51</td>
<td>0.23</td>
<td>0.72</td>
<td>0.51</td>
<td>1.97</td>
</tr>
<tr>
<td>2</td>
<td>0.72</td>
<td>0.27</td>
<td>0.87</td>
<td>0.56</td>
<td>2.42</td>
</tr>
<tr>
<td>3</td>
<td>0.87</td>
<td>0.54</td>
<td>0.98</td>
<td>0.84</td>
<td>3.23</td>
</tr>
<tr>
<td>4</td>
<td>1.07</td>
<td>0.72</td>
<td>2.37</td>
<td>1.01</td>
<td>5.17</td>
</tr>
<tr>
<td>5</td>
<td>1.12</td>
<td>0.83</td>
<td>2.67</td>
<td>1.43</td>
<td>6.05</td>
</tr>
</tbody>
</table>

Note: The producing tech. is resin tech.

Analysis of Sanguinarine and Chelerythrine in Macleaya cordata (Willd.) R. Br. extract for its producing process control by high-performance liquid chromatography

Macleaya cordata (Willd.) R. Br., a medicinal plant of the Papaveraceae family, has stimulated much interest owing to its chemically and pharmacologically interesting alkaloids. Hu et al. [3] isolated five alkaloids such as sanguinarine, chelerythrine, protopine, α-alloprotopine and β-alloprotopine from the fruits of Macleaya cordata (Willd.) R. Br. Y. Hashimoto et al. analyzed the cell content of Macleaya cordata root [4-5]. Among the alkaloids found in the herb, sanguinarine and chelerythrine are major active components. They have antiseptic, and antitumour activities [6-8].

For the extracts, total content of these two alkaloids and content ratio of sanguinarine vs. chelerythrine are its specification indexes. In general, the total content of these two alkaloids is higher than 50%, and the content ratio of sanguinarine vs. chelerythrine is 1.5:1.0. The content of the alkaloids in different parts such as fruits, stem and leaf of the herb which grew in different areas had been investigated. From the results, the content of the alkaloids in the stem is the lowest among three parts and that of the fruits is highest. And the contents of the alkaloids in the fruits grew in different areas have difference. Increased interest has recently been shown on chelerythrine because of the potential use of the component in antitumour therapy. So, it seems likely that the key problem of the manufacturing technology of Macleaya Cordata (Willd.) R. Br. extracts is to increase the content of chelerythrine in the extracts.

By using the proposed method, satisfactory separation of sanguinarine and chelerythrine was achieved, and interference caused by other components in Macleaya cordata (Willd.) R. Br. was eliminated. Acetonitrile concentration in the mobile affected the retention and separation characteristics of sanguinarine and chelerythrine. When the concentration of acetonitrile in the mobile increased, the retention time and the resolution of the two alkaloids decreased. The ratio (V:N) of acetonitrile vs water in the mobile was optimized.

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Analysis of ginsenosides Rgl, Re, Rd, Re, Rb1 and Rb2 for quality control of ginseng products by high-performance liquid chromatography

There are seven species and three varieties of the genus Panax grown in China. Thus, China has the most abundant gene resources of Panax. Ginsenosides are main active constituents of ginseng. 28 ginsenosides were found in the roots, root-stocks, stems, leaves, flowers and flower-buds of ginseng plant. According to their chemical structure, ginsenosides can be divided into three types: oleanolic acid, panaxadiol and panaxatriol types. Rgl, Re, Rd, Re, Rb1 and Rb2 are main components. The total content of these six constituents is always used as quality index of ginseng materials and products. The HPLC techniques developed in this lab can be used to standardize the effective constituents in commercial products. A rapid, effective reversed-phase high performance liquid chromatography procedure has been developed to evaluate the ginsenoside content of commercially available products and cultivated ginseng samples.

Development of new detection methods

The quartz crystal (QC) device has played an important role in probing interfacial processes [9]. The high sensitivity, low cost and conceptual simplicity of this method portend its development in a variety of commercial and research application. Several type of piezoelectric quartz crystal (PQC) detection devices have been developed in this laboratory, including series piezoelectric quartz crystal (SPQC), electrode-separated piezoelectric crystal (ESPC) and double cell quartz crystal (DCQC). These novel detectors have been used successfully in liquid chromatography. Compared with conventional detector, the quartz crystal detector is considered advantageous in sensitivity and simplicity of construction. In this section, we describe the quartz crystal detector and its application for the determination of some active constituents in traditional Chinese herbal and patent medicines with HPLC.

Piezoelectric quartz crystal sensor as a new detector for the detection of active components in TCM with HPLC

SPQC detector for HPLC or ion chromatography (IC) is constructed as follows: two platinum wires are used as conductivity electrodes and inserted oppositely in the detection cell. The distance between the two electrodes is 0.5 mm. The body of the detector is made of two Plexiglas plates with a thickness of 5 mm and a cylindrical trough with a diameter of 2 mm. A length of 8 mm within these Plexiglas plates is used as the detection cell. Two stainless steel tubes (0.5 mm) are used as the inlet and outlet of the mobile phase into the detection cell. The distance between the electrode and the inlet is 5 mm. The electrodes of SPQC detector is connected with an AT-cut 9 MHz piezoelectric quartz crystal. The cell constant of the electrodes is 3.5 cm. With the help of a time-division multiplex (TDM) frequency-voltage conversion circuit, the data of the PQC detector is directly transferred to a chromatographic workstation in real-time.

Using SPQC detector coupling with HPLC, ion chromatography (IC) or ion exclusion chromatography (IEC), we determined vitamin C in apple, lactic acid, pyruvic acid, Zn²⁺, Ca²⁺, Mg²⁺ and Cu²⁺ etc successfully.

Bulk acoustic wave (BAW) sensor as a new detector for the detection of organic acids in TCM with HPLC

Double cell quartz crystal (DCQC) detector was made in this laboratory. Figure 1 shows the schematic diagram of the DCQC detector. The quartz crystal was mounted on the top of a Teflon column with one side facing liquid. The electrode on this side was removed, therefore the two opposite electrodes inducing an alternating electrical field across the crystal were separated by two flow-through conductivity cells, cell 1 and cell 2. Cell 1 was adjustment cell and one side of the crystal was in contact with liquid in this cell. Cell 2 was sample cell and mobile from the chromatographic column flows through the cell. The cell constant of cell 1 can be adjusted by changing the position of PTFE column with the crystal and the cell constant of cell 2 is 0.85 cm. The piezoelectric crystal used was a 9 MHz and AT-Cut quartz. A frequency-to-voltage converter (made in this laboratory) was used to transform the frequency signal of the DCQC detector to a chromatographic workstation, which was used to record chromatograms in real-time and to integrate peak areas.

Figure 1 schematic diagram of the DCQC detector

For the DCQC detector, the ΔF vs ΔA which relationship (ΔF/ΔA) is linear when all other parameters are kept unchanged. And we found that the response sensitivity of the DCQC is independent of the background conductivity of solution in cell 2 (G2) when conductivity of solution in cell 1 (G1) is ca.500μS. Cell constants can affect the detector performance. In this work, the cell constants were optimised k1=1.0 cm, k2=0.85 cm.

We determined fumaric acid, succinic acid and tartaric acid in herbal drugs such as Schefflera arboricola Hayata, Tamarindus indica L., Angelica sinensis (Oliv) Diels, Vitis vinifera L. and some patent drugs by using the proposed HPLC-DCQC system. The patent drugs were made in this laboratory according to ancient formulae and traditional manufacture technology.

Acknowledgements

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References

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