Simultaneous Analysis of Ingredients in Chinese Medicinal preparations by High Performance Liquid Chromatography

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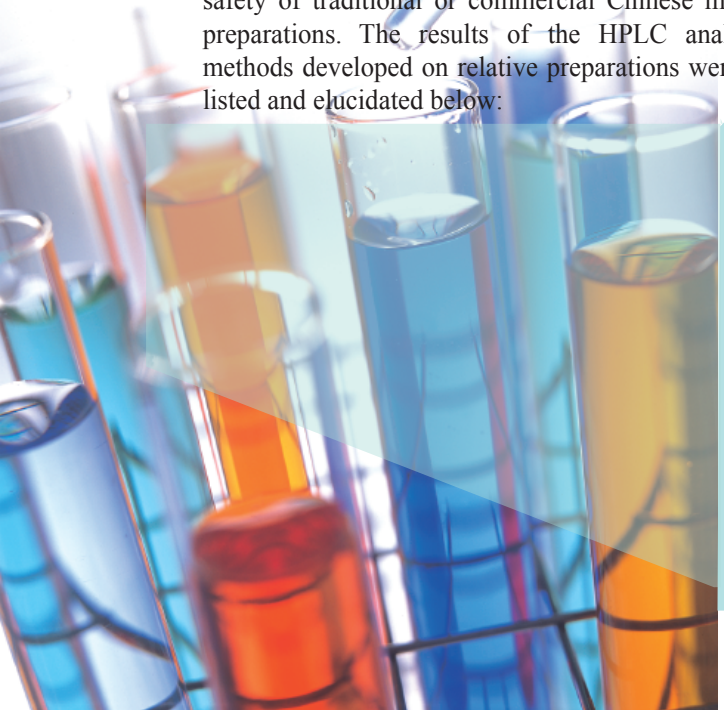
Merits of Chinese medicine products depend on the quality and efficacy of its preparation materials. To extend the development of Chinese medicine, the quality control of Chinese herbal medicines (CHM) must be promoted previously since the pharmacologic efficacy of CHM can be influenced by the uneven quality of CHM. Department of Health, Executive Yuen, announced that certification of validity period extension of new drugs detection registration and drugs permission, no matter indigenously made or imported concentrated preparations, the attached data must follow the act of "Marker components quantification methods and specs notification of concentration preparations of Chinese medicine" and 33 preparations include Ger- Gen-Tang, Siao-Ching-Lung-Tang, Jia-Wei-Siao-Yao-San, Qwei-Je-Tang, Gan-Lu-Yin, Ma-Sing-Gan-Seg-Tang, Bu-Chung-Yi-Chi-Tang, Lieu-Wei-Di-Hung-Wan, Huang-Lian-Jei-Du-Tang, Du-Ho-Gi-Sen-Tang (Jan.1st, 2001), Ze-Bor-Di-Hunag- Wan, Lung-Dan-Sei-Gan-Tang, Sin-Yi-Ching-Fe-Tang, Suei-Fu-Ju-Yu-Tang, Chi- Jui-Di-Hung-Wan, Siao-Fun-San, Ching-Sin-Liang-Zu-Yin, Si-Ni-San, Ding-Chuang- San, Chai-Ger-Jei-Ji-Tang (Feb. 1st, 2003), Che-Gan-Tsau-Tang, Ba-Wei-Di-Hung- Wan, Chuan-Chung-Cha-Tiau-San, Siao-Yao-San, Huo-Siang-Cheng-Chi-San, Siang- Sa-Lieu-Jun-Zu-Tang, Jing-Fang-Bai-Du-San, Su-Jing-Huo-Sei-Tang, Ze-Ker-San, Ji-Seng-Sen-Chi-Wan, Fang-Fung-Tung-Seng-San, Err-Cheng-Tang, Lieu-Jun-Zu- Tang (Aug. 23rd, 2006) were announced. If the data did not follow the rule of the act, new drugs can not be registered and certification of validity period extension will not be allowed. According to the act, every prescription should quantify at least two marker components chosen from different crude materials. However, only 33 preparations including Ger-Gen-Tang were announced that they must do marker component quantification. It will be the trend of the future Chinese herbal medicine development.

In our laboratory committed developing HPLC analysis methods for different Chinese medicine preparations and dosage formulations. The analysis techniques will be established by first using column chromatography of various packing materials (including silica gel, sephadex LH-20), or preparative thin layer chromatography to purify and to identify marker components. Meanwhile, HPLC analysis methods of marker components were developed and were tested by inter-day, intra-day, and recovery validation. The development of high stability, high reproducibility, and trusty technology of simultaneous multi marker components analysis may reduce the analysis period of materials and preparations, enhance efficacy of quantification, and setup standard operation procedure and criteria of detection to reach the goal of quality control and assure the efficacy and safety of traditional or commercial Chinese medicine preparations. The results of the HPLC analysis methods developed on relative preparations were listed and elucidated below:



1 Simultaneous Analysis of Eight Components in "Pin-Wei-San" by High Performance Liquid Chromatography

Simultaneous determination of eight marker substances was established for the quality control of Chinese medicinal preparation "Ping-Wei-San" by HPLC. These substances were glycyrrhizin in Glycyrrhizae Radix, hesperidin, nobiletin, 3',4',3,5,6,7,8-heptamethoxy flavone, tangeretin, 5-hydroxy-3',4',6,7,8- pentamethoxyflavone in Citri Leiocarpae Exocarpium, honokiol, magnonol in Magnoliae Coatex. Extracted sample were run through the HPLC column (Inertsil ODS-80A 5μm, 4.6 mmφ×250 mm) at 30℃ and the column was developed with a mixture of 20% acetonitrile (pH 2.5) and 70% acetonitrile (pH 2.5) aqueous solution employing linear gradient elution method at a flow-rate of 1.0 ml/min. The detection wavelength varied with time. It was 275 nm during 0~19 min, 250 nm during 19~80 min.

2 Simultaneous Analysis of Six Components in "Tzyy-Yun-Gau" by High Performance Liquid Chromatography

Simultaneous determination of five marker substances was established for the quality control in traditional Chinese medicine ointment preparation "Tzyy-Yun-Gau". These substances included shikonin, deoxyshikonin, β,β-dimethylacryl shikonin, and acetylshikonin in Macrotomiae Radix; ferulic acid in Angelicae Sinensis Radix. Tzyy-Yun-Gau was partitioned at the mixture of n-hexane and methanol, sample from take out the methanol layer to analyze. The samples was run through the HPLC column (Inertsil ODS-2, 4.6 mm I.D.×250 mm) at 30℃ with a mobile phase, a mixture of methanol, acetonitrile and 2% acetic acid aqueous solution, by linear gradient elution metuanol in a flow-rate of 1.0 mL/min. The detection wavelength varied with time, which was 325 nm during 0~25 min, 525 nm during 25~58.5 min, 440 nm during 58.5~62 min, and lastly 525 nm during 62~80 min.

3 Studies on the component analysis and quality control in tonic wine preparation of King-Mon-Long-Fong-Jyo

Simultaneous determination of seven marker substances was established for the quality control in tonic wine preparation of "King-Mon-Long-Fong-Jyo". These marker substances were gomisin A and schizandrin from Schizandrae Fructus, loganin from Corni Fructus, cinnamic acid and cinnamaldehyde from Cinnamomi Cortex, and scopoletin and ferulic acid from Angelicae Radix. Different rice wine extraction volume and extraction temperature conditions were performed to evaluate quality of King-Mon-Long-Fong-Jyo. Extracted samples were run through the HPLC column (Inertsil 5 ODS-2, 4.6 I.D. × 250mm.) at 30℃ and the column was developed with a mixture of 20% acetonitrile and 70% acetonitrile aqueous solution and then employed linear gradient elution method at a flow-rate of 1.0 mL/min. An UV 250 nm was used for the detection of the marker substances.

4 Simultaneous Analysis of Nine Components in "Byi-Liang-Tang" Preparation by High Performance Liquid Chromatography

Simultaneous determination of nine marker substances was established for the quality control of "Byi-Liang-Tang" by HPLC. These marker substances include ferulic acid from Cnidii Rhizoma, paeoniflorin from Paeoniae Radix, glycyrrhizin from Glycyrrhizae Radix, cinnamic acid and cinnamaldehyde from Cinnamomi Cortex, puerarin and daidzin from Puerariae Radix, and baicalin and baicalein from Scutellariae Radix. Extracted samples were analyzed with reverse-phase column (Inertsil 5 ODS-2, 4.6 I.D. × 250 mm.) at 30℃ and eluted with a mixture of 20%, 50% and 90% acetonitrile aqueous solution in gradients at a flow-rate of 1.0 mL/min, and detected at 230 nm.

5 Simultaneous Analysis of Ten Components in patch preparation of Wan-Yin-Gau by High Performance Liquid Chromatography

Simultaneous determination of seven marker substances was established for the quality control in patch formula preparation of Wan-Yin-Gau by HPLC. These marker substances included cinnamic acid, cinnamaldehyde (Cinnamomi Cortex), isoimperatorin (Angelicae Dahuricae Radix and Notopterygii Rhizoma), ferulic acid (Angelicae Sinensis Radix), paeoniflorin (Paeoniae Radix), glycyrrhizin (Glycyrrhizae Radix), harpagoside (Scrophulariae Radix), emodin, sennoside A, and sennoside B (Rhei Rhizoma). The ingredients in the formula for water-base and oil-base patches from different manufactures were also analyzed for quality evaluation. Extracted samples were analyzed with reversed-phase column (Inertsil 5 ODS-2, 4.6 i.d. × 250 mm.) at 30℃ and eluted with a mixture of 20% acetonitrile and 70% acetonitrile aqueous solution in gradient manner at a flow-rate of 1.0 mL/min, and detected at 250 nm.

6 Simultaneous Analysis of Nine Components in Patch Preparation of Ru-Yi-Jin- Huang-San by High Performance Liquid Chromatography

Simultaneous determination of seven marker substances was established for the quality control in patch formula preparation of Ru-Yi-Jin-Huang-San by HPLC. These marker substances included berberine (Phellodendri Cortex), curcumin (Curcumae Rhizoma), imperatorin (Angelicae Dahuricae Radix), magnolol (Magnoliae Cortex), hesperidin (Citri Leiocarpae Exocarpium), glycyrrhizin (Glycyrrhizae Radix), and emodin, sennoside A, sennoside B (Rhei Rhizoma). The ingredients in the water-based and oil-based patches of the formula from different manufactures were also analyzed for quality evaluation. Extracted samples were analyzed with reversed-phase column (Inertsil 5 ODS-2, 4.6 I.D. × 250 mm) at 30℃ and eluted with a mixture of 20% and 70% acetonitrile aqueous solution in gradient manner at a flow-rate of 1.0 ml/min. The detection wavelength varied with time, which was 275 nm during 0~72 min, 250 nm during 72~105 min, and lastly 220 nm during 105~145 min.

REFERENCES

1. Horng-Liang Lay, Chien-Chih Chen. 2000. Simultaneous Analysis of Eight Components in"Pin-Wei-San" by High Performance Liquid Chromatography. Journal of Liquid Chromatography & Related Technologies 23: 1439-1450.
2. Horng-Liang Lay, I-Jen Shih, Chih-Ho Yeh, Chwan-Fwu Lin and J-Wen Liang. 2000. Simultaneous Determination of Five Constituents in "Tzyy-Yun-Gau" Medicine by High Performance Liquid Chromatography. Journal of Food and Drug Analysis 8: 304-308.
3. Horng-Liang Lay, Shiow-Chyn Huang, Chia-Chi Chen and Tian-Shung Wu. 2002. Studies on the component analysis and quality control in tonic wine preparation of King-Mon-Long-Fong-Jyo. Journal of Food and Drug Analysis 11: 201-208.
4. Horng-Liang Lay, Chia-Chi Chen and Shu-Tuan Chiang. 2004. Simultaneous Analysis of Nine Components in "Byi-Liang-Tang" Preparation by High Performance Liquid Chromatography. Journal of Food and Drug Analysis 12: 115-119.
5. Horng-Liang Lay, Chia-Chi Chen, Shiow-Chyn Huang, Thau-Ming Cham, and Tian-Shung Wu. 2005. Simultaneous Analysis of Ten Components in patch preparation of Wan-Yin-Gau by High Performance Liquid Chromatography. Journal of Food and Drug Analysis 13:118-124.
6. Horng-Liang Lay, Chia-Chi Chen, Shiow-Chyn Huang, Thau-Ming Cham, Tian-Shung Wu, I-Hsin Lin. 2010. Simultaneous Analysis of Nine Components in Patch Preparation of Ru-Yi-Jin-Huang-San by High Performance Liquid Chromatography. Journal of Natural Medicines 64: 194-202.