行政院國家科學委員會專題研究計畫成果報告

毛細管電泳之應用研究

Study of Capillary Electrophoresis

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一、中文摘要

毛細管電泳具有分離效率高，分析時間短，所需樣品體積少，方法開發簡單，多種應用模式，儀器自動化等優點，所以在中藥分析領域中，逐漸成為一重要分析方法。

本計畫建立毛細管電泳方法來分析半夏瀉心湯系列五種以及黃連解毒湯等四種中藥複方湯劑，並討論各分析條件，如 pH **值、緩衝溶液濃度、及加入界面活性劑與環糊精等，對分離效率之影響。並以本研究所建立之毛細管電泳方法，分析經適當前處理的中藥複方湯劑。**

關鍵詞：毛細管電泳、微胞電動層析

Abstract

The techniques of capillary electrophoresis and micellar electrokinetic chromatography for analyzing nine traditional Chinese medicinal preparations were developed under this study. In order to have a better understanding how different experimental setups affect separation performance; various pH, cyclodextrins and buffer concentrations were examined. The analytes were successfully separated at the optimized separation conditions.

Keywords: Capillary electrophoresis, Micellar electrokinetic chromatography

二、Analyses of five traditional Chinese medicinal preparations by cyclodextrin-modified capillary electrophoresis

(一) Introduction

Pan-Hsia-Hsieh-Hsin-Tang, Sheng-Chiang-Hsieh-Hsin-Tang, Kan-Tsao-Hsieh-Hsin-Tang, Huang-Lien-Tang and Ta-Huang-Huang-Lien-Hsieh-Hsin-Tang are common Chinese medicinal preparations. The compositions of crude herbs of these drugs are similar even the same but relative ratio of the herbs are different, thereby making the treatment of these medicine are different. Complex chemical compositions are in Chinese herbs. Berberine (BE) is a compound of alkaloids in Rhei Rhizoma as specific constituent. Three specific constituents including baicalein (BA1), baicalin (BA2) and wogonin (WO) are flavonoid compounds of Scutellariae Radix. Ginsenoside Rg1 (GiR) is the specific constituent in Ginseng Radix. The other specific compounds are homogentizic acid (HA), [6]-gingerol (GI), glycyrrhizic acid (GA), chelidonic acid (ChA), cinnamic acid (CA) and sennoside A (SeA) in this study.

In the last decade, capillary electrophoresis (CE) has become a highly

effective analytical technique in various compounds, such as ionic and neutral compounds. CE poses advantages of a high resolution, high separation efficiency and rapid analysis. Consequently, CE has been employed to analyze Chinese crude herbs and Chinese medicinal preparations. In the present study, CD modified CE method was developed to simultaneously determine selected eleven specific components of the five different commercial concentrated Chinese medicinal preparations. The effects of various separation and buffer conditions on the analytes' migration behavior were also examined. The different extraction methods and the variation of specific component concentrations in two different commercial products of the same Chinese medicinal preparations were discussed.

(二) Results and Discussion

Eleven specific components in five Chinese traditional preparations were selected as analytes in this study. Most of these analytes have dissociated hydroxyl and carboxyl groups. According the differences among those analytes, we choose cyclodextrin-modified CE method to separate these analytes in present study.

Effects of buffer pH value

The migration mobilities of all analytes decreased with increasing buffer pH value. The migration velocity of Be was the highest among those analytes. In contrast, CH has the smallest molecular mass with two carboxyl groups, thus leading to the lowest migration velocity. GiR and GI could be separated by rising pH from 9.3 to 10.0 because of the difference of degree of dissociation was increased. The migration velocity of HO was obviously increased from pH 9.3 to 10.0 results from dissociation of hydroxyl groups was increased even surpassing CA1 and GA. The difference of molecular structure between BA1 and BA2 was that BA2 carry glucuronic acid group causes the larger mobility difference than WO and BA1. Considering both separation time and resolution, pH 10.0 borate buffer was selected for subsequent study.

Effect of buffer concentration

The migration times of all analytes increased with rising buffer concentration. Increasing buffer concentration cause migration velocities decreased of analytes because of EOF decreased. Based on analysis time, efficiency and resolution, 25mM borate buffer concentration was chosen for further investigation.

Effect of cyclodextrins concentration

The migration time of the analytes did not significantly change within altering α-cyclodextrin concentration except for CA1. However, after adding 1mM α-cyclodextrin in the buffer, the adequately separate result could obtain. Although the influence of migration behavior of GA, SeA and BA1 were larger with adding γ-cyclodextrin than α-cyclodextrin, sufficient resolution of all analytes could not observed. Moreover, adding β-cyclodextrin could obtain an unexpected result. According these experimental results, adding 1mM α-cyclodextrin in borate buffer solution could obtain the optimized results.

The optimum separation of eleven analytes was using a 25mM borate buffer solution contain 1mM α-cyclodextrin at pH 9.3. The analysis of these compounds can be complete within 16 min. The R.S.D.s of the migration times were lower than 1.44%. The correlation coefficients of the calibration graphs exceeded 0.9976. The detection limits for those analytes ranged from 2.02 μg/ml to 53.40 μg/ml.

Determination of the analytes in five Chinese medicinal preparations

Five Chinese medicinal preparations were analyzed by the optimized conditions.

At least one specific component of all crude herbs could be detected except for two specific constituents CH and HO of crude herb Sheng jiang and Ban xia respectively. Altering extraction solvent from Ethanol-water (7/3, v/v) to water could determine CH in Pan-Hsia-Hsieh-Hsin-Tang and Huang-Lien-Tang. Chinese medicinal preparation Huang-Lien-Tang including two ingredients could be detected two specific components BE and SeA. The R.S.D.s of the quantities of the eleven analytes in total seven actual samples were less than 7.06%. The differences of the crude herb sources or ratios and pretreatment processes by the manufacturer may cause the differences.

(三) Conclusions

In this study, we have simultaneously separated the eleven specific components of five traditional Chinese medicinal preparations. Those analytes were separated within 16 min using a 25 mM borate buffer solution contain 1mM α-cyclodextrin at pH 9.3. Moreover, the difference of two different commercial Chinese medicinal preparations of same medicine could be effectively determined by the cyclodextrin modified CE method. The extraction method for actual samples was relatively simple and efficient. Consequently, the cyclodextrin modified CE technique potentially offers a rapid, simultaneous and efficient separation method for analyzing specific constituents in Chinese medicinal preparation analysis.

三、 Determination of seven constituents of four traditional Chinese medicinal preparation by micellar electrokinetic chromatography

(一) Introduction

In this study, the high-performance micellar electrokinetic chromatography method was developed for the simultaneous determination seven specific components of four traditional Chinese medicinal preparations. These preparations include Huang-Lien-Chieh-Tu-Tang, Tang-Kuei-Liu-Huang-Tang, Szu-Wu-Tang and Wen-Ching-Yin. The effects of pH, buffer and surfactant concentration of the buffer solution on the migration and separation of the analytes were also studied. The optimized separation buffer was a pH 10, 30 mM borax/NaOH buffer containing 100 mM SDS. The whole separation time was less than 14 minutes. The relative standard deviations of migration times are less than 1.62% under the optimized separation condition. The correlation coefficients of the analytes' linear calibration graphs exceeded 0.997. Moreover, the amounts of the seven constituents in four different traditional Chinese medicine samples are also determined by the MEKC method with a relatively simple extraction procedure.

(二) Results and discussion

These analytes have quite different molecular structures and polarity. The molecules carry glucosyl moiety is more hydrophilic such as PA and GE. BE has a quaternary nitrogen atom on its molecule and carry positive charge without adding any modifier in running buffer. The different partition coefficients between the aqueous solution phase and the micellar pseudostationary among those analytes could distinguish their respective migration velocities. Based on UV absorbance spectra of those analytes, 200 and 226nm were selected herein for detection.

Effect of buffer pH value

Since there is no any dissociated group on PA, GE and TMP molecular structures, migration velocities are identical with the EOF. In order to study the buffer pH value effect, 50mM SDS was added to the buffer solution. The migration mobility of FA was heavily influenced from pH 8.0 to 10.0, increased with rising buffer pH value.

However, the migration behaviors were unaffected for the other analytes within the pH range. Thus, the pH 10.0 borax-NaOH buffer solution was selected in the subsequent study.

Effect of SDS and buffer concentration

With an increasing in the SDS concentration, the analytes' velocities decreased especially for BE, TMP, GE and PA, even surpassing CA1 and BA2 of TMP. The migration time of BE was the longest than the other six analytes. In contrast, more hydrophilic molecules like CA1, BA2 and FA, the migration times did not significantly change. The resolutions among of PA, GE and EOF were enhanced with increased SDS concentration. Considering the resolutions and analysis time, 100 mM SDS was the preferred choice to separate the analytes.

The migration times of the analytes increased as the buffer concentration were increased. At lower buffer concentrations, the resolution was inadequate even though SDS was maintained at 100mM. Although higher buffer concentration implies a more satisfactory resolution, the peak resolution and the total analysis time are necessary to compromise. Therefore, 30-mM buffer was the most effective choice.

The optimized separation condition was 30-mM borax-NaOH buffer at pH 10 containing 100 mM SDS. The analysis of these compounds can be completed within 14 min. The R.S.D.s of the migration times were lower than 1.62%. The correlation coefficients of the calibration graphs exceeded 0.9970. The detection limits for those analytes ranged from 2.95 μg/ml to 51.60 μg/ml.

Determination of the analytes in four Chinese medicinal preparations

The specific constituents of four Chinese medicinal preparations were determined by the optimized analysis method. Three specific constituents of the medicine are detected in Huang-Lien-Chieh-Tu-Tang. The specific components in Wen-Ching-Yin were all detected except for TMA. The ingredients of the traditional Chinese medicinal preparations are quite complex and some components may alter during the manufacturing process. In addition, the relative ratio of the amounts for the seven analytes varied among those actual samples, likely owing to different sources of crude herb or different manufacturing processes. Some specific compounds could not detected in the medicines may due to the reasons mentioned above.

(三) Conclusions

In present study, analyzing seven specific constituents in four commercial Chinese medicinal preparations were completely separated within 14 min using a 30 mM borax-NaOH (pH 10.0) containing 100 mM SDS buffer. Experimental results indicates BE was interfered from other components in the medicine, further investigations on extraction solvents are necessary. The MEKC method poses a rapid, simultaneously, and efficiently analyzing for specific compounds in complex Chinese medicinal preparations. Combining MEKC method with a simple extraction process could effectively analyze other traditional Chinese medicinal preparations.