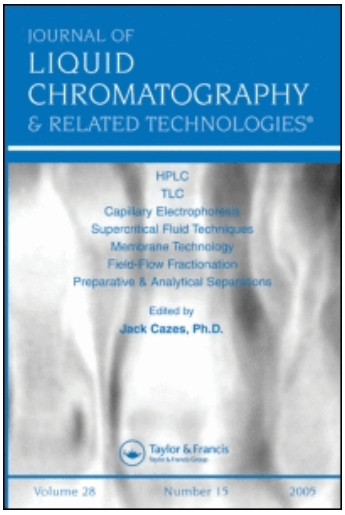

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Zhang, Z. , Chen, X. and Hu, Z.(1997) 'Determination of Three Water-Soluble Active Ingredients in Qiangli Yingqiao Containing Vc Tablets by Capillary Zone Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 20: 19, 3245 — 3255
To link to this Article: DOI: 10.1080/10826079708000487
URL: http://dx.doi.org/10.1080/10826079708000487

J. LIQ. CHROM. & REL. TECHNOL., 20(19), 3245-3255 (1997)

DETERMINATION OF THREE WATER-SOLUBLE ACTIVE INGREDIENTS IN QIANGLI YINGQIAO CONTAINING Vc TABLETS BY CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

A simple and rapid capillary zone electrophoretic (CZE) method was established for separation and determination of chlorphenamin males(CPM), paracetamolum(PAM), and vitamin C(Vc) within 3 min, using borate-phosphate buffer at pH 7.2. The influence of injection time on peak area, peak height and efficiency was studied in detail. The CZE method was successfully applied to quality control of CPM, PAM and Vc in Qiangli Yingqiao containing Vc tablets.

INTRODUCTION

Compared to Western medicine, the pharmacological actions of traditional Chinese medicine are slower, so taking this into consideration and considering their concerted actions, more and more combinations of traditional Chinese medicine and Western medicine have been adopted for pharmaceutical preparations in China.

Qiangli Yingqiao containing Vc tablet is a typical example, consisting of great amounts of Yingqiao extract, which is a kind of water-insoluble traditional Chinese herbal preparation, and small amounts of three water-soluble ingredients--vitamin C(Vc), paracetamolum(PAM) and chlorphenamin males(CPM). Qiangli Yingqiao containing Vc tablet is a very common medicine which can resist influenza virus and treat influenza, cold, fever, cough, sore throat, and headaches due to colds.[1] However, the contents of these three water-soluble ingredients in the tablets aren't clearly indicated; it is, therefore, useful and necessary to develop a rapid and simple method for determination of Vc, PAM, and CPM in order to monitor the quality of the kind of Yinqiao tablet.

CE is being widely established within the pharmaceutical industry for the quality control and determination of drugs, and recently, used for analyses of different traditional Chinese medicines.[2-3] In previous papers,[4] we also developed a CZE method for identification and determination of aesculin and aesculetin in ash bark.

Although many papers have been published for analyses of CPM and PAM,[5-8] as well as Vc[9-10] in drugs, up to date no report appears on simultaneous separation and determination of the three substances. In this paper, we made attempts to enlarge the use of CZE in the pharmaceutical analysis, developing a rapid and simple CZE method for determination of Vc, CPM, and PAM in Qiangli Yinqiao tablet within 3 min. The effects of injection time on peak height, peak area, and peak width at half height were investigated in detail.

EXPERIMENTAL

Instrumentation

The CE system employed was a Waters Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, USA), with a positive power supply. Waters AccuSep fused silica capillary (53cm × 75μm I.D.) were used throughout. A window for on-column detection, by removing the polyimide coating, was created 7.6cm from the end of the capillary (cathode). Direct UV detection was performed with a Hg lamp and a 254-nm optical filter. Samples were introduced from the anodic end of the capillary by hydrodynamic injections, where the sample vial was raised by 10cm. Data acquisition was carried out with a Maxima 820 Chromatography workstation

(Waters) with a system interface module connecting the CE system to the station. Data acquisition rate was 20 points s^{-1}. Collection of electropherographic data was initiated by a signal cable connection between the Quanta 4000 and the system interface module(SIM).

Chemicals

Authentic PAM and CPM were purchased from the National Institute for the Control of Pharmaceutic and Biological Products, P.R. of China. Vc is purchased in Shanghai Chemical plant, China (analytical grade). Two batches of Qiangli Yingqiao containing Vc tablets, from the same pharmaceutical factory, were purchased in a drug store and analyzed. Unless otherwise specified, all chemicals were of analytical reagent grade.

All solutions were prepared using filtered, degassed, and distilled water. Carrier electrolytes were filtered through 0.45μm membrane prior to use.

Sample Preparation

Ten tablets were accurately weighed and ground, an appropriate amount of the resultant powder was accurately weighed and 50mL water was added. After ultra-sonication for 5 min, the mixture was filtered and water was added to make the volume of the filtrate exactly 100mL. For the two batch tablets, the same preparations were used.

Procedure

The capillary was first purged with 0.5mol l^{-1} NaOH for 3 min, 3 min with water and 5 min with running buffer prior to each analysis. Overnight the capillary was stored in 0.5mol l^{-1} NaOH. At the beginning of each day, it was sufficient to rinse the capillary with water for about 10 min. All conditional experiments were completed using the test solution containing 0.22mg mL^{-1} of Vc, 0.27mg mL^{-1} of PAM, and 0.20mg mL^{-1} of CPM.

Experiments were performed to optimize the separation. Unless otherwise specified, the optimum conditions used were: running buffer prepared by mixing 20 mmol l^{-1} sodium tetraborate with 20 mmol l^{-1} sodium dihydrogen phosphate(pH=7.2), applied voltage of 25kV and hydrodynamic injections for 10s. All operations were at room temperature.

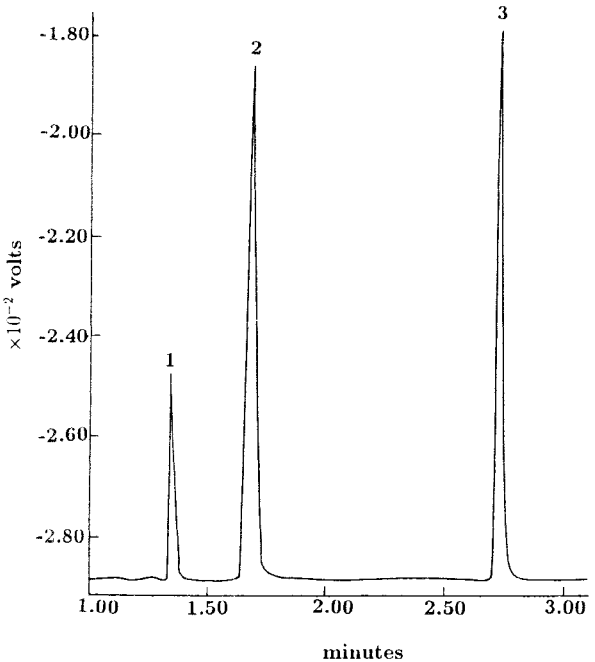

Figure 1. Typical electropherogram of standard mixture of CPM, PAM and Vc. Conditions: 20mmol l^{-1} borate--phosphate buffer at pH=7.2, voltage of 25kV, Hydrodynamic injection time for 10s, other conditions as experimental. Peak identifications are: (**1**) CPM, (**2**) PAM, (**3**) Vc.

RESULTS AND DISCUSSION

Selection of Buffer Solution

According to the complexation mechanism postulated by Lorand and Edwards,[11] borate can form anionic complexes with polyhydroxy compounds (i.e., polyols), thereby facilitating their separation by electrophoresis. The polyol-borate complex formation of catechols[12] and carbohydrates,[13] etc., has been exploited in CE. Here, we make best use of the advantage of the complex of borate with the ortho-dihydroxy in Vc. It is well known that, Vc is not stable and easy to reduce in solution, especially in basic solution, which gives rise to difficulty in determining its content. However, its complexation with borate

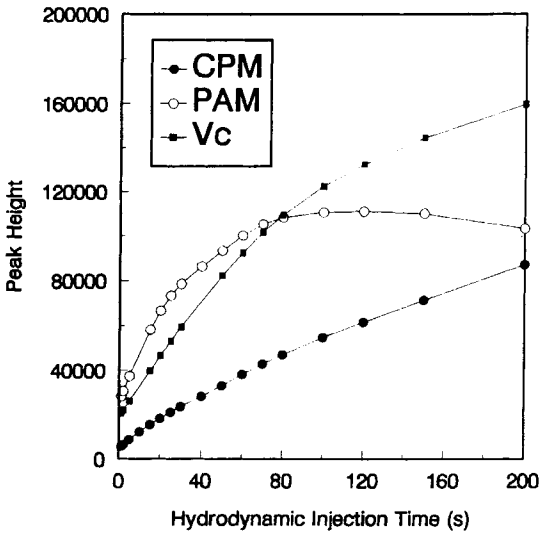

Figure 2. Dependence of peak height on injection time. Conditions as Figure 1 except injection time.

not only helped its separation from the other two analytes, but also prevented its reduction in the buffer solution. It was found out that a buffer solution of 20mmol l^{-1} borate mixing with 20 mmol l^{-1} phosphate in the pH range 7.0-7.5 could obtain better separation for Vc, CPM, and PAM, so pH 7.2 was selected in our experiments. Figure1 shows a typical CZE electropherogram of the standard mixture of Vc, CPM and PAM.

Effect of Injection Time

In the range investigated, it was found out that migration times and mobilities of the samples only change slightly with increasing injection time, so our emphasis was on effects of injection time on peak area, peak height, and the peak width at half-height. In order to observe the effects clearly, it is necessary to point out, emphatically, that for the three analytes, the concentration order is PAM > Vc > CPM and the sensitivity of CPM is also lower than that of PAM and Vc.

For the three analytes, as can be seen from Figure 2, the same tendencies are observed, that is, within the shorter injection time, the peak height increases with direct proportional to increasing injection time, whereas, when

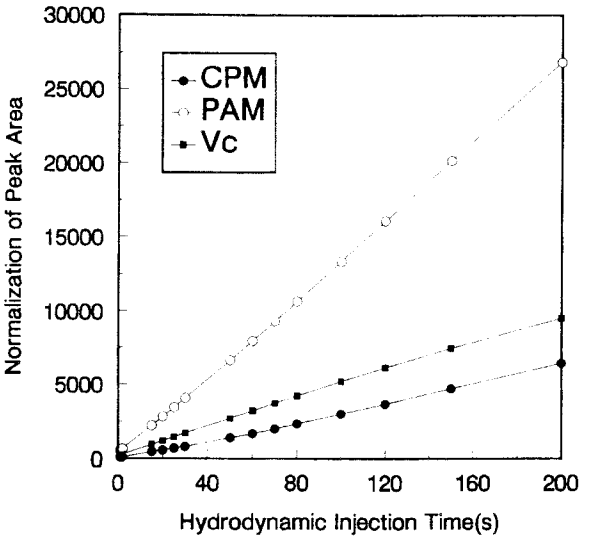

Figure 3. Dependence of peak area on injection time. Conditions as Figure 2.

injection time increases to a degree, the peak height no longer increases linearly with injection time. On the other hand, the greater the concentration, the worse the linear relationship between peak height and injection time. For peak area, however, as indicated in Figure 3, in the whole range studied, peak areas increase linearly with injection time. The reason is that, because the increased injection time results in higher sample concentrations, at higher sample concentration there is peak broadening or distortion which causes changes in peak height but not area. Therefore, for most analyses, peak area is preferred, especially, the normalization of CE peak areas (i.e. division of peak area by migration time) which is necessary to ensure correct quantitation when the solute concentration is high. Peak height can be used for quantitation at the lower concentration, especially, it may be much more desirable for trace analysis where a very small peak is superimposed on a noisy baseline.

As in HPLC, higher detection sensitivity can be obtained by longer injection time, whereas efficiency suffers significantly with injection time increasing, as observed in the experiment (illustrated in Fig 4), due to serious peak broadening, which leads to the reduced efficiency.[14] In other words, the effect of injection time on efficiency also suggested the dependence of efficiency on sample concentration, that is, higher sample concentration will result in a decrease in efficiency. So, a suitable injection time must be considered when selecting optimum separation conditions. Here, 10s injection time was selected.

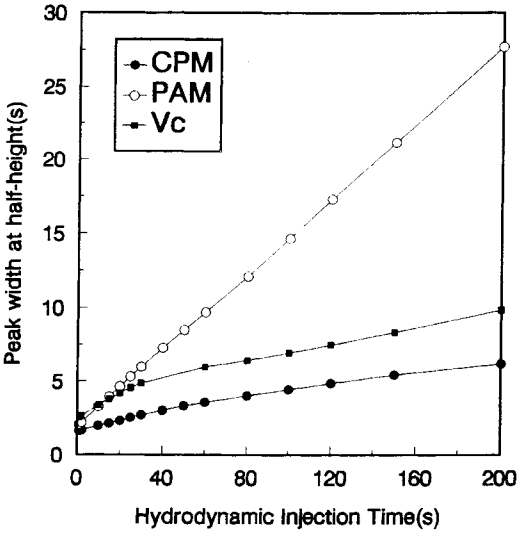

Figure 4. Effect of injection time on peak width at half-height. Conditions as Figure 2.

Effect of Applied Voltage

The influence of applied voltage was investigated. As observed from Fig. 5, increased voltage results in decrease in peak width at half-height, indicating that higher efficiency is obtained with increasing voltage. In addition, shorter analysis time is also observed, so 25 kV was selected.

Quantitative Analysis

Under the optimized conditions, the method showed good linear correlations based on the linear relationship between the normalization of CE peak area for PAM and Vc and peak height for CPM and sample concentration as shown in Table 1, where A is the normalization of CE peak area for Vc and PAM and the peak height for CPM, and C is concentration in $\mu g\ mL^{-1}$. Approximate detection limits were calculated at a signal-to-noise ratio of 2. The relative standard deviations (N=5) at 80 $\mu g\ ml^{-1}$ for all three analytes are 1.83%, 0.71%, and 2.10% for CPM, PAM, and Vc, respectively.

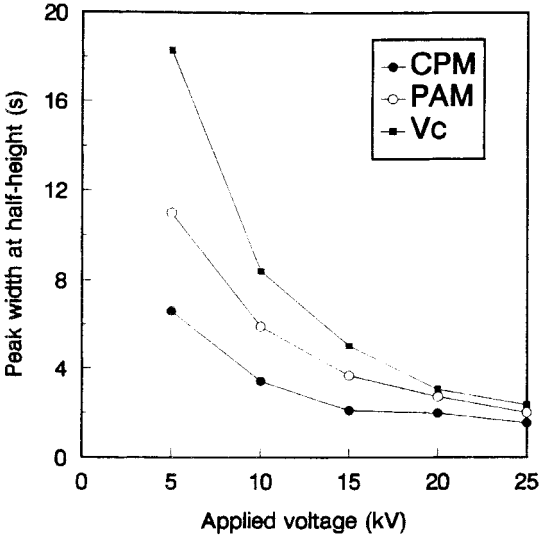

Figure 5. Effect of applied voltage on peak width at half-height. Conditions as Figure 1 except voltage.

Table 1

Linear Regression Analysis

Species	Linearity ($\mu g\ mL^{-1}$)	Detection Limit ($\mu g\ mL^{-1}$)	Linear Regression Equation	Correlation Coefficient
CPM	16.0 - 600.0	7.0	A = 0.97 + 0.27C	0.9998
PAM	6.0 - 2000.0	3.0	A = 7.82 + 1.61C	0.99993
Vc	3.0 - 1000.0	2.0	A = 3.2 + 1.42C	0.99991

Application

The CZE method established has been successfully applied to the separation and determination of three water-soluble ingredients in Qiangli Yinqiao containing Vc tablets. Table 2 gives the results of standard addition of recovery for these three ingredients in two batch tablets. Due to the content of CPM being very lower, it must be determined at the excess of PAM and Vc, consequently, two typical electropherograms for batch II are shown in Fig. 6.

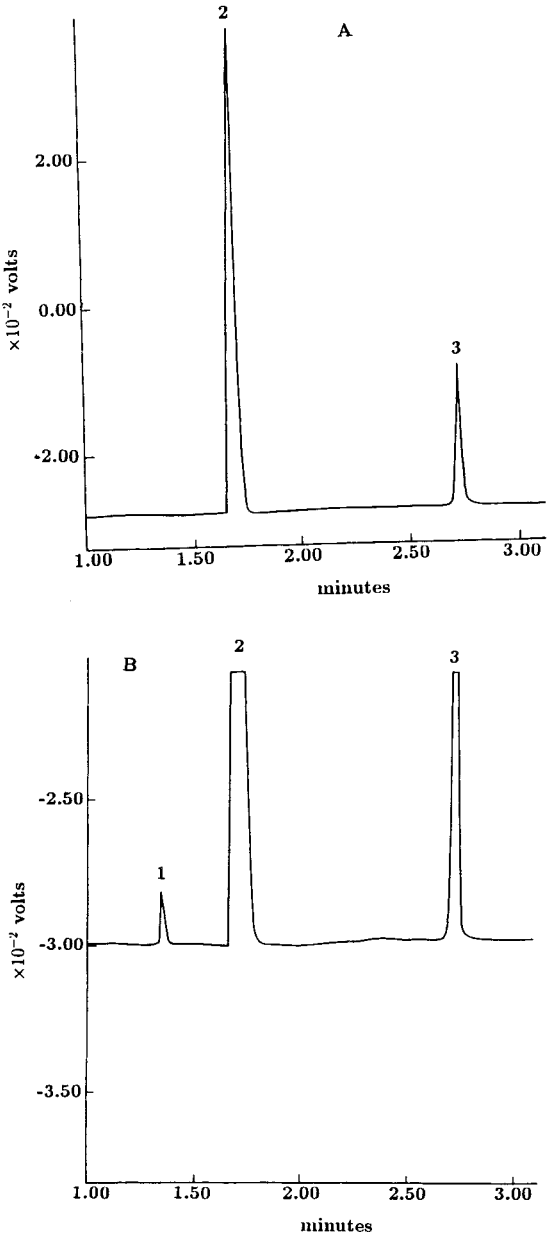

Figure 6. Electropherograms of CPM, PAM and Vc in Qiangli Yingqiao containing Vc tablet from Batch II. (A): for determination of PAM and Vc, (B): for CPM. Conditions as Fig. 1.

Table 2

Quantitative Analysis

	Species	Determined (mg tablet^{-1})	RSD (n= 9) (%)	Added (μg)	Recovery (%)	RSD (n=3) (%)
	CPM	0.203	3.17	120	98.6	0.65
				80	101.8	1.36
Batch I	PAM	60.57	1.53	120	99.0	0.23
				80	97.2	0.89
	Vc	12.15	2.78	50	96.5	2.6
				80	99.6	1.37
	CPM	0.214	2.84	80	102.2	1.16
Batch II	PAM	66.54	1.39	80	98.4	0.76
	Vc	15.42	2.47	80	98.7	1.43

CONCLUSION

The proposed CZE method was successfully used for separation and determination of CPM, PAM, and Vc in Qiangli Yinqiao containing Vc tablets within 3 min, indicating that the method can be promising in the quality control of such kinds of medicine containing these three ingredients. The relationships between injection time and peak area and peak height and peak width at half-height, suggest that normalization of CE peak area can ensure the correct quantitation either at lower or higher concentration, and that selection of injection time is important to CE separation.

The normalization of CE peak areas for quantitative analysis of Vc and PAM and peak height for CPM confirms that normalization of CE peak areas is preferred to peak height in quantitative analysis.

ACKNOWLEDGMENTS

This project is financially supported by the National Natural Science Foundation, Natural Science Foundation of Gansu Province, and the Doctoral Point Foundation of the State Education Commission of P. R. of China.

REFERENCES

1. **New Edited Handbook of Chinese Medicine**, National Medicine and Science & Technology Press, Beijing. p.658(1988).

2. L. M. Liu, S. J. Sheu., J. Chromatogr., **639**, 323-328 (1993).

3. C. T. Chen, S. J. Sheu, J. Chromatogr., **710**, 323-329 (1995).

4. Z. Zhang, Z. Hu, G. Yang, Chromatographia, in press.

5. H. Nishi, T. Fukuyama, M. Matsuo, S. Terabe, J. Pharm. Sci., **79(6)**, 519-523 (1990).

6. H. Nishi, T. Fukuyama, M. Matsuo, S. Terabe, J. Chromatogr., **498**, 313-323 (1990).

7. M. E. Swartz, J. Liq. Chromatogr., **14(5)**, 923-938 (1991).

8. S. Fujiwera, S. Honda, Anal. Chem., **59**, 2773-2776 (1987).

9. S. Fujiwara, S. Iwase, S. Honda, J. Chromatogr., **447**, 133-140 (1988).

10. C. P. Ong, C. L. Ng, H. K. Lee, S. F. Y. Li, J. Chromatogr., **547**, 419-428 (1991).

11. J. P. Lorand, J. O. Edwards, J. Org. Chem., **24**, 69-774 (1959).

12. J. P. Landers, R. P. Oda, M. D. Schuchard, Anal. Chem., **64**, 2846-2851 (1992).

13. J. Lui, O. Shirota, M. Novotny, Anal. Chem., **63**, 413-417 (1991).

14. J. W. Jorgensen, K. D. Lukas, Anal. Chem., **53**, 1298-1302 (1981).

Received December 20, 1996
Accepted January 30, 1997
Manuscript 4342