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A New Method of Separation of Four Benzodiazepines by RP-CEC

Jin Lan ZHANG*, Tong Hui ZHOU

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050

Abstract: A new method to separate diazepam, nitrazepam, estazolam, alprazolam was established on both C_{18} and C_8 CEC columns. The influence of separation voltage, Tris concentration, column temperature and the percentage of acetonitrile on the resolution and retention behavior of four benzodiazepines was investigated. The results showed that the percentage of acetonitrile had the largest effect on the resolution and retention behavior of the four benzodiazepines. Other separation conditions had also effects on the resolution and retention behavior, but smaller than the concentration of acetonitrile. Optimum separation conditions were obtained to separate four benzodiazepines on C18 and C8 CEC columns.

Keywords : Capillary electrochromatography, diazepam, nitrazepam, estazolam, alprazolam.

Capillary electrochromatography (CEC) is a new kind of separation and analytical technique. It combines the advantages of high performance liquid chromatography (HPLC) and capillary electrophoresis with high resolution, high efficiency and better selectivity. It has been used in pharmacy, food and environment analysis. In this paper a new method to separate four benzodiazepines was established by RP-CEC, and the effect of different separation conditions on the resolution and retention behavior of the four benzodiazepines was investigated.

Experimental

Instrument and materials

A Beckman P/ACE System 5500 was used for all analysis. Data acquisition was accomplished with Beckman Gold Software and an IBM personal computer. The vacuum pump used to pack capillary column was from The Great Wall Technology and Trade Company (Henan, China). Fused silica capillary column with internal diameter of 100μm was pur-chased from Yongnian Optical Fiber Factory (Hebei, China). ODS of 3μm size was obtained from Jinouya Technology and Development Co. Ltd.. 5μm ODS crossed with SE54 was provided by Pharmacy School of Health Science Center of Peking University.

* E-mail: zhjl2002by@sohu.com

Sodium dihydrogen phosphate, thiourea, benzene, toluene and naphthalene (AR) were purchased from Beijing Chemical Reagent Factory. Trihydroxymethylaminomethane (Tris) (AR) was obtained from Chengdu Chemical Reagent Factory. Acetonitrile was of HPLC grade from Fisher Chem. Co. . Water was deionized. Nitrazepam, estazolam, alprazolam and diazepam were purchased from National Institute for The Control of Pharmaceutical and Biological Products. C8 (3μm) column packed by electrokinetic packing method[1] with internal diameter of 75μm was provided by the Mongolia Unicom Technology Co..

Separation conditions

The total length of 3μm C18 CEC column with internal diameter of 100 μm was 27 cm and the effective length was 16.8 cm. The total length of 3μm C8 CEC column with internal diameter of 75μm was 27 cm and the effective length was 19.8 cm. The mobile phase was consisted of acetonitrile and 1mmol/L Tris (65% : 35%). The separation voltage was 13kV. The detection wavelength was 214 nm. The column temperature was 20℃. The samples were loaded by electrokinetic injection for 5 s under 4 kV.

Column preparation

C18 (3μm) CEC column with internal diameter of 100μm was successfully prepared by combination of vacuum filling and pressure compressing . The detailed description has been published elsewhere in 2002 [2]. The efficiency of column was up to 100,000 plates per meter. The reproducibility of migration time of benzene, toluene and naphthalene was better than 2.2%.

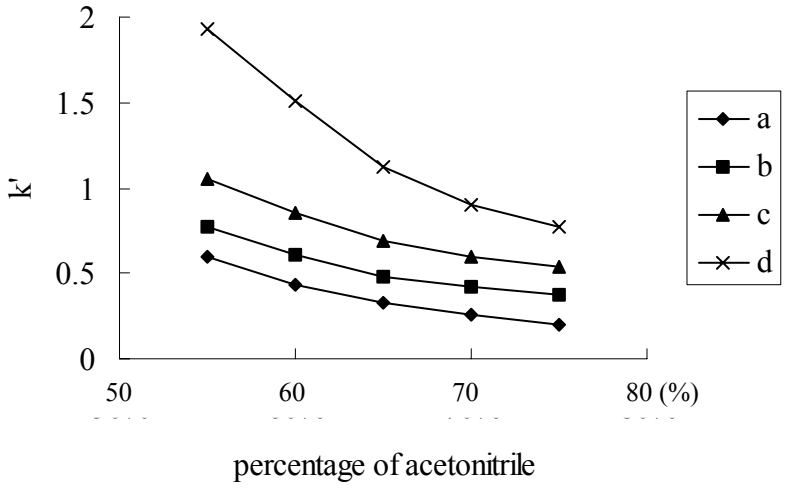
Results and Discussion

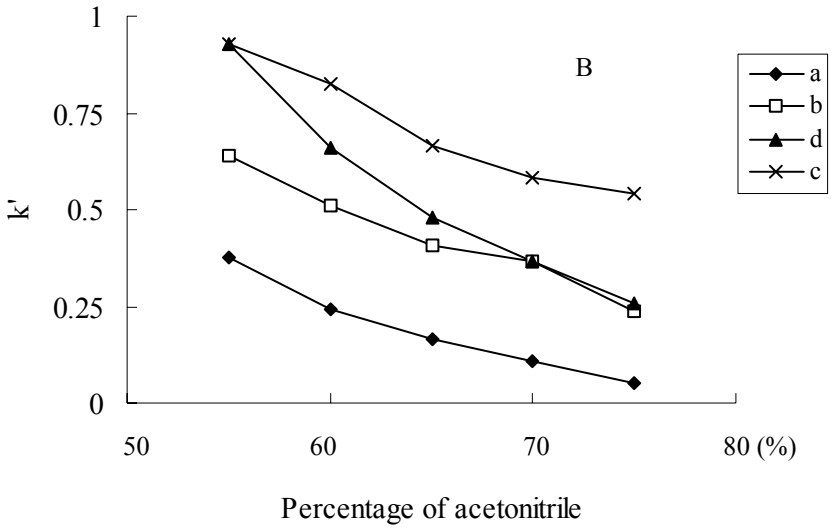
Four benzodiazepines (nitrazepam, estazolam, alprazolam and diazepam) were separated on both C18 and C8 CEC columns. The optimum separation conditions, such as separation voltage, Tris concentration, the percentage of acetonitrile and column temperature were investigated. The results showed that the percentage of acetonitrile in the mobile phase had the largest effect on capacity factors and resolution of the four benzodiazepines. Other separation conditions had smaller effect. High percentage of acetonitrile reduced retention while low percentage of acetonitrile was good to resolution (**Figures 1**). **Figure 2** shows the CEC electrochromatograms of the four benzodiazepines on C18 CEC column with thiourea added as a reference. Satisfactory separation condition was obtained with a mobile phase of 65% acetonitrile content and separation voltage at 13 kV. Under these circumstances the reproducibility of the retention time and area (n=4) was good with RSD≤ 1% and 4% respectively. The elution order of the four benzodiazepines on C18 CEC column was nitrazepam, estazolam, alprazolam and diazepam. But there was some difference on C8 CEC column, diazepam was eluted faster than alprazolam.

Conclusion

This work has shown the usefulness of CEC in the rapid analysis of four benzodiazepines, thus enlarging the scope of the application of the newly emerging microseparation technique.

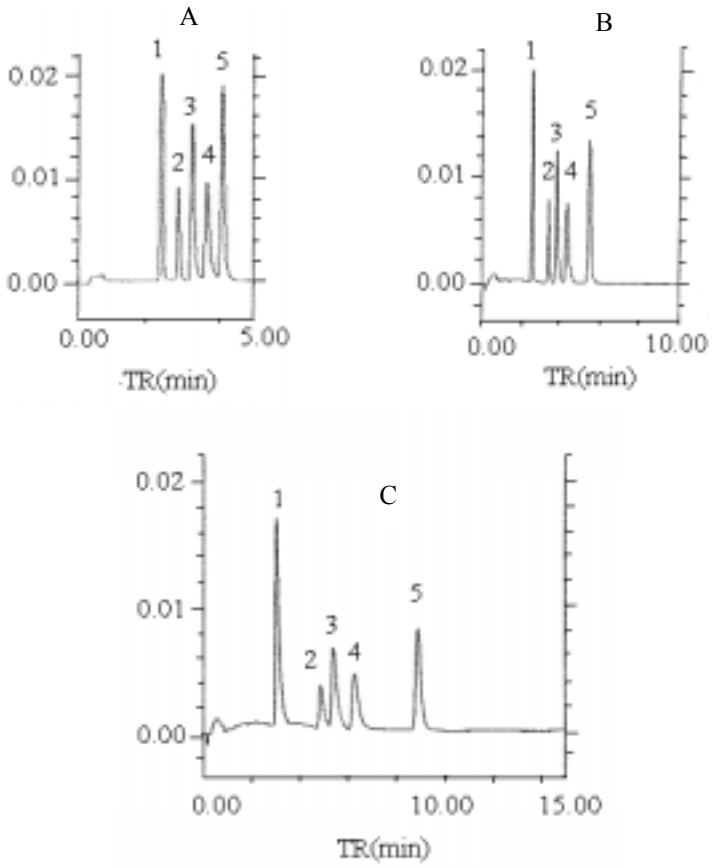
Figure 1 Effect of the percentage of acetonitrile on capacity factor of four benzodiazepines on C18 (A) and C8 columns(B)





a.nitrazepam b. estazolam c. alprazolam d. diazepam

Figure 2 Effect of percentage of acetonitrile on retention behavior of four benzodiazepines on C18 column



Percentage of acetonitrile : A. 75% B. 65% C. 55%
1. thiourea 2. nitrazepam 3. estazolam 4. alprazolam 5. diazepam

References

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