Asian Journal of Chemistry; Vol. 27, No. 1 (2015), 365-367

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18337

Solvent-Dependent Conformational Change in Cephalosporin by HPLC Method

HAILING ZHANG and SHUYU LIU*

College of Chemistry and Chemical Engineering, Shanghai University of Engineering Science, Shanghai 201620, P.R. China

*Corresponding author: E-mail: liushuyu1219@163.com

Received: 17 June 2014; *Accepted*: 17 September 2014; *Published online*: 26 December 2014; AJC-16573

High performance liquid chromatography (HPLC) was generally used to determine drug ingredients. In this article,we used HPLC to detect solvent-dependent conformational change of cephalosporin. Firstly, when the pH value of the mobile phase was constant, it was conducted to research the effect of different solvents on cephalosporin configuration. Then, when solvent was determined, it was carried out to test the influence of pH value of the mobile phase on cephalosporin configuration. It is found that the pH value of mobile phase and different solvents had some effect on cephalosporin configuration by HPLC.

Keywords: HPLC, Cephalosporin, Conformational change.

INTRODUCTION

It is well-known that the molecule structure of the drug has been affected by a number of factors, such as structural flexibility, nature of the solvent, pH of the media, electronic environment, radiation, *etc.*[1]. The molecular structures of drugs have directly effects on their efficacy[2]. Hence, it is necessary to study the behaviour of drug molecules in solution, which is not only help us deeply to understand the mechanism of drugs but also assist us to improve the efficacy by changing its molecular structures[3]. Drug molecules *in vivo* mainly exist in an aqueous solution. Extensive literatures have reported that molecular heterogeneous can be induced by solution. Kanai *et al.*[4] have reported that *N*-phenylbenzohydroxamic acid derivative can change its structure in different solutions. Cephalosporin is a class of compounds containing the same nucleus-7-ACA (Fig. 1)[5]. Cephalosporin nucleus is consisted of an amide bond which can form two configurations in different conditions, *cis* and *trans*[6-9]. Therefore, it is important to research the conformational changes of cephalosporins in different solutions for improving its efficacy.

Currently, the research methods of the molecular conformations are mainly infrared spectrum, ultraviolet spectrum and nuclear magnetic resonance spectroscopy. Gróf *et al.*[10] have reported that NMR spectra were used to study the conformational changes of 3-aminomethylene-2, 4-pentanedione. Zimniak *et al.*[11] have used NMR and IR to investigate the solvent-dependent conformational itions in deacetylephalothin. High performance liquid chromatography (HPLC) is also used to research the conformational changes of molecules. In this article, HPLC was used to study the conformational changes in cephalosporin. Firstly, cephalosporin conformational changes in different solutions were performed by HPLC. Following we conducted the pH value of mobile phase on the influence of molecular conformational changes.

EXPERIMENTAL

The following regents were used in present study *e.g.*, cefoperazone sodium, cefotaxime sodium, ceftazidime, ceftriaxone sodium and cephalothin sodium from Sichuan Industrial of Antibiotic Co., Lid., China; acetic acid from Sinopharm Chemical Reagent Co., Ltd; acetonitrile from Chromatographic Pure, Fisher, USA; watsons pure distilled water from Guangzhou watsons food and beverage Co., Ltd; ammonium acetate from Shanghai Zhanyun Chemical Co., Ltd.

The following instruments were used in our study: Agilent LC-1260 (Agilent USA); Mettler Toledo AB135-S (Mettler Toledo Switzerland).

Cefoperazone sodium,cefotaxime sodium,ceftazidime, ceftriaxone sodium and cephalothin sodium were dissolved in the solutions with different ratios of acetic acid: acetonitrile (1:1,1:2,1:3,1:4,1:5,1;6,1:7,1:8,1:9,1:10), respectively. Different pH values of mobile phase were prepared.

Liquid chromatography: Alltima C_{18} 100 × 4.6 mm chromatographic column; mobile phase, ammonium acetate solution (pH = 3.0, 3.3, 3.5, 3.8, 4.0, 5.0, 0.0166 M, adjusted by acetic acid)-acetonitrile (90:10, v/v); mobile phase, ammonium acetate solution (0.0166 M)-acetonitrile (90:10, v/v);

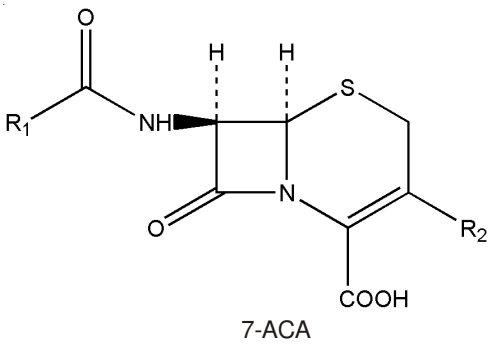

Fig. 1. Cephalosporin nucleus

mobile phase, water-acetonitrile (90:10, v/v); flow rate, 0.3 mL/min; wave length, 254 nm; column temperature, 25 °C; and sample size, 20 µL.

RESULTS AND DISCUSSION

Influence of the solvent: Firstly, cephalosporin were, respectively dissolved the solution of acetonitrile and acetic acid in different proportions. Then HPLC was used to detect the conformational changes of cephalosporin in different solutions. It was found that cephalosporin changed their structures in a mixed solution of acetonitrile and acetic acid by HPLC. Fig. 2 showed that when cephalosporin was dissolved in a solution which the ratio of acetic acid and acetonitrile is 1:1, they only showed one peak. As the amount of acetic acid was decreasing, one peak gradually transformed two peaks. When the amount of acetic acid in a solution was reduced, one peak gradually transformed another peak in chromatogram. It indicated that there were different configurations when cephalosporin was dissolved in solutions with different proportions of acetic acid and acetonitrile. At the same time, the two peaks also produced mutual transformation in solutions. So the amount of acetic acid and acetonitrile in solution has some effect on the configuration of cephalosporin.

Influence of the pH value of the mobile phase: When the solvent is determined, the pH value of the mobile phase on the influence of the molecular structures of cephalosporin was carried out. Firstly, cephalosporin were dissolved the solution that the ratio of acetic acid and acetonitrile was 1:5. Then different pH values of the mobile phase were used to detect the influence on the conformational changes of cephalosporin by HPLC. The result displayed that the pH value of the mobile phase had a certain effect on the molecular structures of cephalosporin. As is shown in Fig. 3, when the pH value of the mobile phase increased, cephalosporin chromatograms gradually changed from two peaks to one peak. Although the solvent is the same, the pH value of the mobile phase is different for different cephalosporin from two peaks to one peak. This indicated that the effect of pH value of the mobile phase on cephalosporin conformational changes is different. The greater pH value of the mobile phase is when it is from two peaks to one peak, the easier the conformational changes of cephalosporin is. When the pH value of the mobile phase was 6.0, cephalothin sodium and ceftazidime still showed two peaks in chromatogram. This manifested that cephalothin sodium and ceftazidime easily happened to conformational changes in solution. The other cephalosporin we studied only showed one peak at last in chromatogram.

Conclusion

The conformation of cephalosporin occurred changes when the solution and the pH value of mobile phase were altered. The changes have been confirmed by HPLC. Cephalosporin showed different conformations in different solutions and

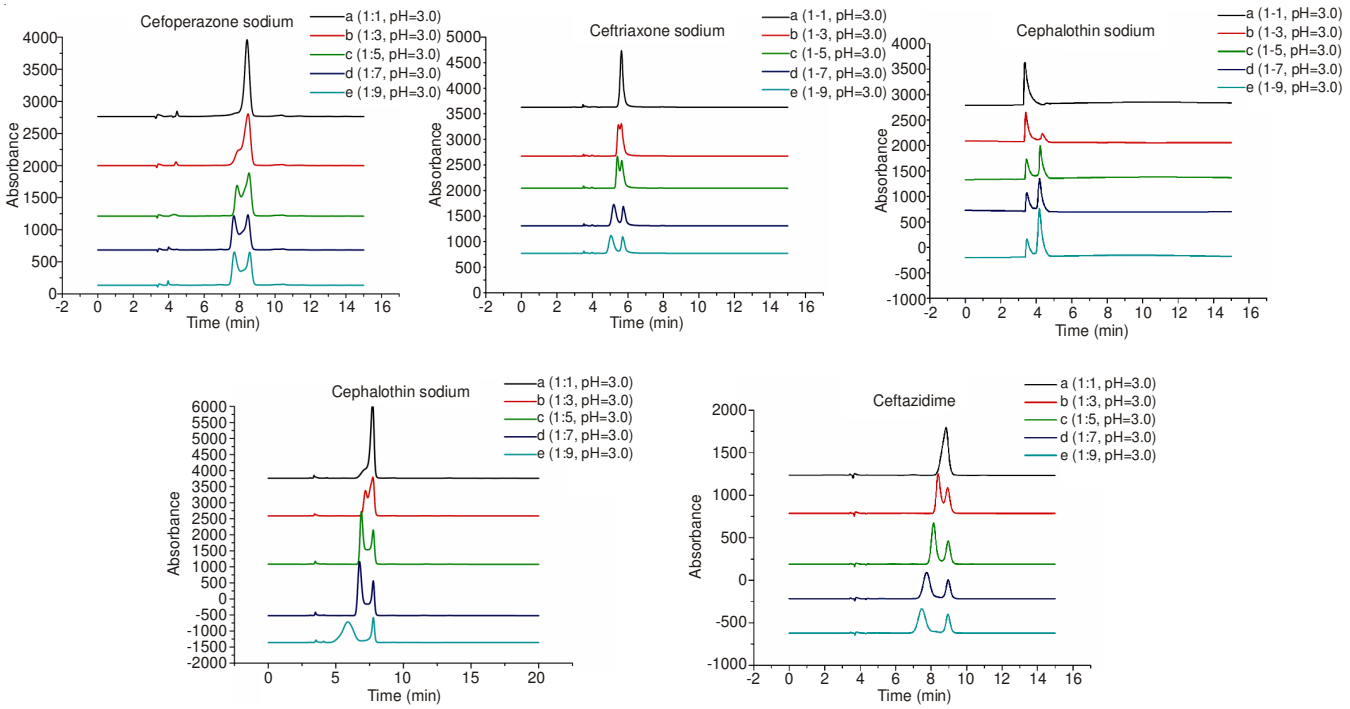

Fig. 2. HPLC chromatogram of cephalosporin in different solvents: a) acetic acid: acetonitrile = 1:1; b) acetic acid: acetonitrile = 1:3; c) acetic acid: acetonitrile = 1:5; d) acetic acid: acetonitrile = 1:7; e) acetic acid: acetonitrile = 1:9. As the content of acetic acid was decreased in the solvent, one peak gradually transformed two peaks

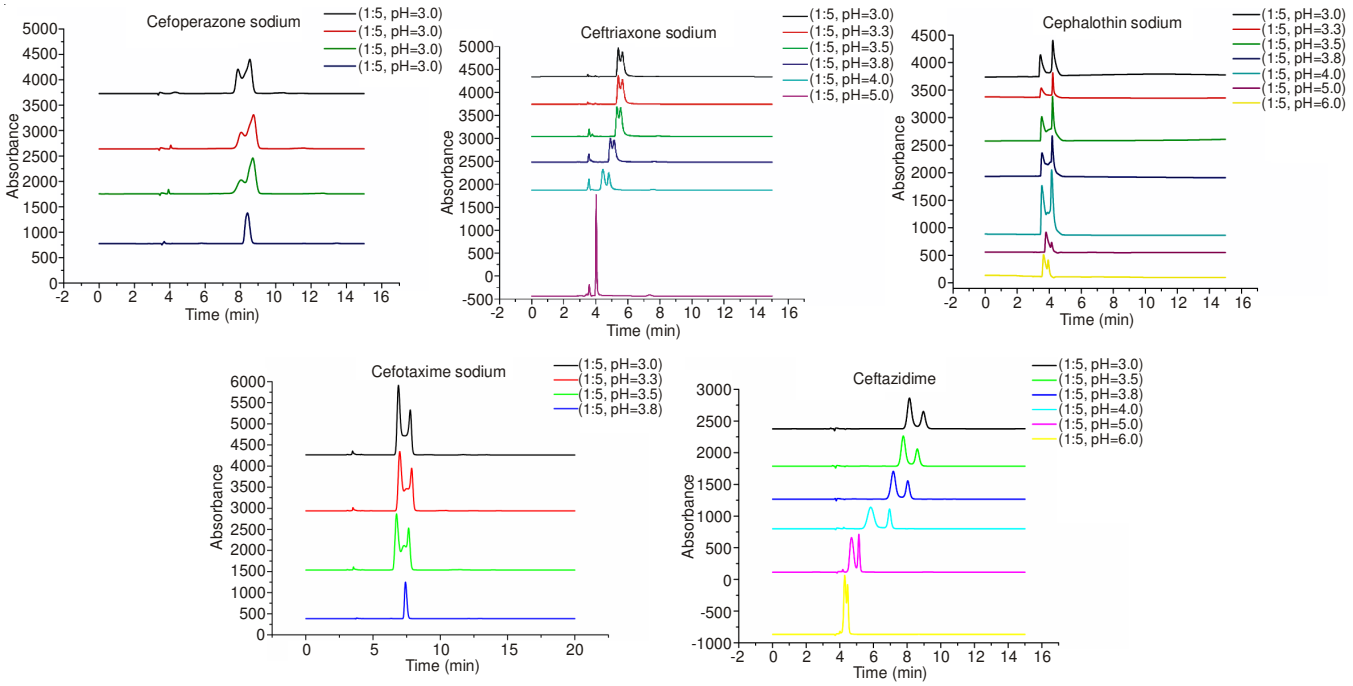

Fig. 3. HPLC chromatogram of cephalosporin in different pH value of mobile phase

different pH value of mobile phase. The different conformations of cephalosporin were mutual transformation in different solutions. This indicated that we can obtain a particular drug molecule by changing the solvent during drug production.

REFERENCES

1. N.K. Jena and N.A. Murugan, *J. Phys. Chem. C*, **117**, 25059 (2013).
2. K.N. Nwogu, *Engl. Specif. Purposes*, **16**, 119 (1997).
3. J. Mehta, *Leuk. Res.*, **21**, 183 (1997).
4. M. Kanai, T. Hirano, I. Azumaya, I. Okamoto, H. Kagechika and A. Tanatani, *Tetrahedron*, **68**, 2778 (2012).
5. C.H. O'Callaghan, S.M. Kirby, A. Morris, R.E. Waller and R.E. Duncombe, *J. Bacteriol.*, **110**, 988 (1972).
6. R. Yamasaki, A. Tanatani, I. Azumaya, S. Saito, K. Yamaguchi and H. Kagechika, *Org. Lett.*, **5**, 1265 (2003).
7. J. Leis, K.D. Klika and M. Karelson, *Tetrahedron*, **54**, 7497 (1998).
8. R. Crespo-Otero, A. Mardyukov, E. Sanchez-Garcia, M. Barbatti and W. Sander, *ChemPhysChem*, **14**, 827 (2013).
9. I. Okamoto, M. Nabeta, M. Yamamoto, M. Mikami, T. Takeya and O. Tamura, *Tetrahedron Lett.*, **47**, 7143 (2006).
10. M. Gróf, A. Gatial, V. Milata, N. Prónayová, L. Sümmchen and R. Salzer, *J. Mol. Struct.*, **843**, 1 (2007).
11. A. Zimniak, I. Oszczapowicz, A. Sikora and I. Wawer, *J. Mol. Struct.*, **443**, 115 (1998).