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Journal of Chromatography B, 800 (2004) 175–179
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Investigation of chromatographic conditions for the separation of cefuroxime axetil and its geometric isomer

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Abstract

The optimal chromatographic conditions for the separation of the syn- and anti-geometric isomers of cefuroxime axetil applying RP-HPLC and micellar liquid chromatography (MLC) methods were investigated. The possibility to separate diastereoisomers of syn- and anti-cefuroxime axetil was observed. Investigations were performed using three columns, two classical silicas and one with hybrid particle technology. Three aqueous-organic and one micellar mobile phases were used. The best results were achieved using micellar mobile phase. Optimization study was performed using different micellar mobile phases. MLC method is sensitive and applicable in purity and stability testing.

Keywords: Optimization; Cefuroxime axetil; Anti-cefuroxime axetil

1. Introduction

Cefuroxime axetil ((1RS)-1-(acetyloxy)ethyl (6R,7R,)-3-[(carbamoyloxy)methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(metoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene 2-carboxylate) is an oral prodrug of the bactericidal cephalosporine antibiotic cefuroxime. Cefuroxime is a broad-spectrum second generation cephalosporin antibiotic active against β lactamase producing strains. It is a semi-synthetic analog of cephalosporin C and the first of a series of α-metoxyiminoacyl-substituted cephalosporins. As acethyloxyethyl ester it is administrated orally, well absorbed and undergoes complete hydrolysis to free cefuroxime during the absorption.

Cefuroxime axetil is a mixture of two diastereoisomers (A and B), syn-geometric isomer has resistance to attack of many β lactamases and the anti-analog is attacked by β lactamases and deactivated that way. Anti-cefuroxime axetil diastereoisomers (A and B) are the process related impurities as well as possible degradation products. Anti-isomers are formed during the manufacturing process or by conversion of cefuroxime axetil under the acidic conditions or on exposure to light.

Cefuroxime was investigated in blood samples by high pressure liquid chromatography (HPLC) [1], by micellar electrokinetic capillary chromatography and HPLC after pretreatment [2], in urine and bile by capillary zone electrophoresis [3] but also in dosage forms using HPLC [4] or spectrophotometric and spectrofluorimetric methods [5,6].

The aim of this study was to develop the optimal chromatographic conditions for the separation of the syn- and anti-geometric isomers of cefuroxime axetil applying RP-HPLC and micellar liquid chromatography (MLC) methods and also to investigate the possibility to separate mentioned diastereoisomers. Separation of geometric isomers in this case is important from the point of purity and stability testing knowing anti-cefuroxime axetil is inactive against β lactamases and can be present in raw material and also in dosage forms, especially liquid. RP-HPLC method was performed using three different columns and four different mobile phases. Considering cefuroxime axetil is a hydrophobic substance, MLC was used as an alternative to classical RP-HPLC. MLC was applied using sodium dodecylsulfate (SDS) to increase aqueous solubility, to form the hydrophilic layer above the surface of the silica and reduce the penetration depth of the components into the bonded phases. The ability of surfactant to self-assemble at its critical micellar concentration and form micellas was used to increase the elution strength of mobile phase and decrease

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doi:10.1016/j.jchromb.2003.08.046

the concentration of organic solvent needed [7]. Influence of co-surfactant 2-propanol and electrolyte sodium chloride in MLC was investigated also.

2. Experimental

2.1. Apparatus

A Hewlett-Packard HP 1100 (Palo, Alto, CA, USA) chromatographic system was used equipped with HP 1100 binary pump and HP 1100 UV-Vis detector. Sample injection was made through Rheodyne injector valve with a 20 μl sample loop and the detection was performed at 280 nm. The mobile phase flow rate was 1.5 ml min^{-1} and the column temperature 50 °C. Columns Alltech C_8 (5 μm particle size, 250 mm × 4.6 mm) (Alltech Associates Inc., Deerfield, Belgium), Alltech C_{18} (5 μm particle size, 250 mm × 4.6 mm) (Alltech Associates Inc., Deerfield, Belgium) and Waters XTerra™ (5 μm particle size, 150 mm × 4.6 mm) (Waters, Milford, Massachusets, USA) were used. Water was deionized using System Simplicity 185 (Milipore, Billerica Massachusetts, USA). The mobile phases and the solution to be injected were degassed and vacuum filtered through 0.45 μm nylon membranes (Alltech Associates Inc., Loceren, Belgium). Data acquisition was made using HP ChemStation software from HP and data treatment was performed with Statistic 6 program.

2.2. Reagents

Standards of cefuroxime axetil and anti-cefuroxime axetil were obtained from Ranbaxy, Madhya Pradesh, India. Stock solutions were prepared by dissolving of cefuroxime axetil in acetonitrile to obtain the concentrations of 6 mg/ml (5 mg/ml of cefuroxime) and by dissolving anti-cefuroxime axetil in acetonitrile to obtain the concentrations of 0.6 mg/ml (0.5 mg/ml of anti-cefuroxime). Working solution was prepared in a 50 ml volumetric flask diluting 5 ml of stock solution of cefuroxime axetil and 0.5 ml of stock solution of anti-cefuroxime axetil with acetonitrile up to volume. The final concentrations investigated were 500 μg/ml of cefuroxime and 5 μg/ml of anti-cefuroxime. All reagents were of analytical grade. Glacial acetic acid, potassium chloride, sodium chloride, triethylamine (TEA) and 2-propanol (Merck, Darmstadt, Germany), acetonitrile-gradient grade (Lab Scan, Dublin, Ireland) and 85% ortophosphoric acid (Carlo Erba, Milan, Italy) were used. TEA buffer was prepared by mixing 10 ml of TEA and 990 ml of deionized water. pH of the water phase was adjusted to 4.8 with ortophosporic acid.

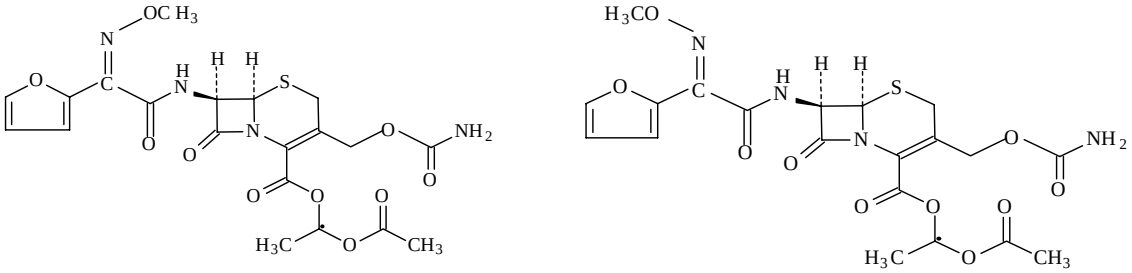
Different micellar mobile phases investigated were prepared with SDS (Merck, Darmstadt, Germany). Aqueous phase of mobile phase was made dissolving SDS in deionized water.

3. Results and discussion

Four mobile phases were studied at every mentioned column, three aqueous-organic and one micellar. Preliminary investigation of aqueous-organic mobile phases were performed with acetonitrile–water at acidic pH adjusted with 85% ortophosporic acid. Because of its low polarity of cefuroxime axetil, retention times were high and peaks were asymmetrical. The effect on separation, retention and peak symmetry was investigated adding glacial acetic acid, potassium chloride (KCl) and tryethylamine (TEA) in aqueous-organic mobile phase. The best results were observed with acetonitrile:water:glacial acetic acid (28:71:1; v/v/v) (A), acetonitrile:0.1 M KCl (28:72; v/v) (B) and acetonitrile:TEA buffer pH 4.8 (28:72; v/v) (C) as aqueous organic mobile phases.

Considering cefuroxime axetil and anti-cefuroxime axetil are mixtures of diastereoisomers, chromatographic conditions were investigated in the course of separating each of them (Fig. 1). In further discussion they will be presented as syn-1 and syn-2 diastereoisomers for peaks corresponding to cefuroxime axetil and anti-1 and anti-2 for peaks of anti-cefuroxime axetil.

Using mobile phase A on Alltech C_{18} column anti-diastereoisomers were not separated and syn- were asymmetrical. Better results were obtained using Alltech C_8 column due to its higher polarity. Retention times were shorter and symmetry was better. Applying mobile phase A on XTerra™ column gave excellent retention times without



1 2

Fig. 1. Cefuroxime axetil (1) and anti-cefuroxime axetil (2).

tailing. Adding of electrolyte with salting-out effect in mobile phase B, made no big difference in separating isomers on mentioned columns. Low molecular weigh amine, TEA in mobile phase C had influence on separation of syn- and anti-isomers, because of the fronting of the peak of anti-1 diastereoisomer separation factor anti-1/syn-2 was <1, and retention times were prolonged up to 30 min on Alltech C_{18} and Alltech C_8 silicas. In every conditions there was severe fronting and tailing which pointed to great interactions between free silanol groups on Alltech C_{18} and Alltech C_8 column. On XTerra™ the same happened without prolonging of retention times.

In further work, separation of geometric isomers and diastereoisomers of cefuroxime axetil was investigated using micellar mobile phase acetonitrile: sodium dodecyl sulfate (D). Sodium dodecyl sulfate was added to the aqueous phase to resolve the problem of excessive retention [8]. It was used in concentration under and above critical micellar concentration of 8 mM [9] what allowed reducing of the amount of acetonitrile in mobile phase to 7%. On classical silicas Alltech C_8 and Alltech C_{18} mobile phase D did not show any approvement. Investigated substances were not eluted on Alltech C_8 and Alltech C_{18}. Approvement was achieved on XTerra™ column (packing protected with methyl groups), retention times were 9.63 min for syn-1 and 10.79 min for syn-2 diastereoisomers and 19.39 min for anti-1 and 22.66 min for anti-2 diastereoisomers of and peak parameters were acceptable.

In further work, the optimization studies were made on XTerra™ column using mobile phases containing 7% of acetonitrile and changing the concentration of SDS and pH of mobile phase. The best separation of syn- and anti-isomers was obtained using 20 mM of SDS and pH 2.5. The separation factor anti-1/syn-2 was always about 1.8, that pointed good separation of geometric isomers. At the concentration of SDS from 15 to 25 mM syn- and anti-isomers showed good separation and peak fronting was minimized. At the concentration of 5 mM of SDS the best separation and resolution of syn-diastereoisomers was achieved but the retention times were very long. Under those conditions anti-diastereoisomers were not eluted. Changing of pH caused small variation in retention times, retention time depended of the concentration of SDS. Increasing concentration of SDS caused shortening of retention times at every investigated pH. Concentrations of 25 mM and higher caused fronting of syn- and coeluting of anti-diastereoisomers.

As the best separation of syn- and anti-geometric isomers and diastereoisomers was achieved at pH 2.5 and the concentration of SDS at 20 mM, mobile phases were further optimized. At pH 2.5 of mobile phase, investigated percentages of acetonitrile in mobile phase were 6, 7, 8, 10, 11 and 12% with different concentrations of SDS (5, 10, 15, 20, 25 and 30 mM). As the parameter of chromatographic separation selectivity factor anti-1/syn-2 was calculated. On the basis of 36 experiments coefficients of second order polinome

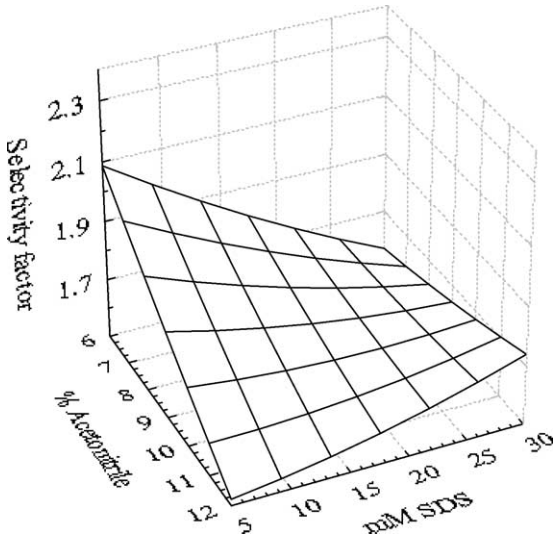


Fig. 2. Three-D diagram selectivity factor anti-1/syn-2 = *f*(% AcN, mM SDS) at pH 2.5. $z = 3.012 - 0.14x - 0.056y + 0.001x^2 + 0.005xy + 0y^2$ (Eq. (1)).

(Eq. (1)) were determinated and three-D diagram (Fig. 2) was constructed.

$$z = 3.012 - 0.14x - 0.056y + 0.001x^2 + 0.005xy + 0y^2 \quad (1)$$

The best results were achieved using mobile phase with 8% of acetonitrile, 20 mM of SDS at pH 2.5. The separation factor anti-1/syn-2 was good at every investigated conditions. At 6% of acetonitrile and 5 mM of SDS syn-diastereoisomers were base line separated with acceptable symmetry of peaks but very excessive retention. Retention times were 28.17 min for syn-1 and 32.58 min for syn-2 isomer. Base-line separated anti-1 and anti-2 isomers

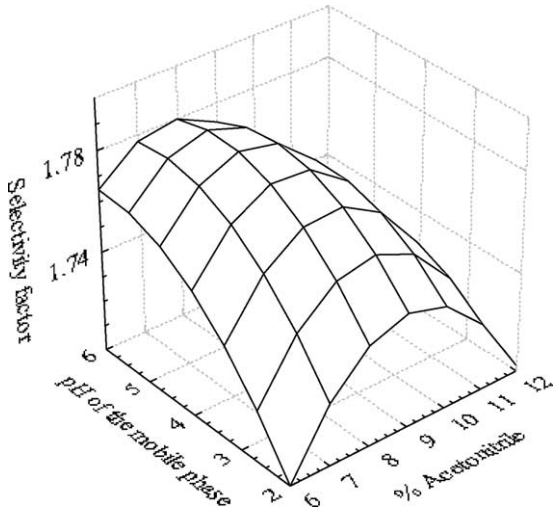


Fig. 3. Three-D diagram selectivity factor anti-1/syn-2 = *f*(% AcN, pH) at 20 mM SDS, $z = 1.218 + 0.096x + 0.071y - 0.005x^2 - 0.00xy - 0.004y^2$ (Eq. (2)).

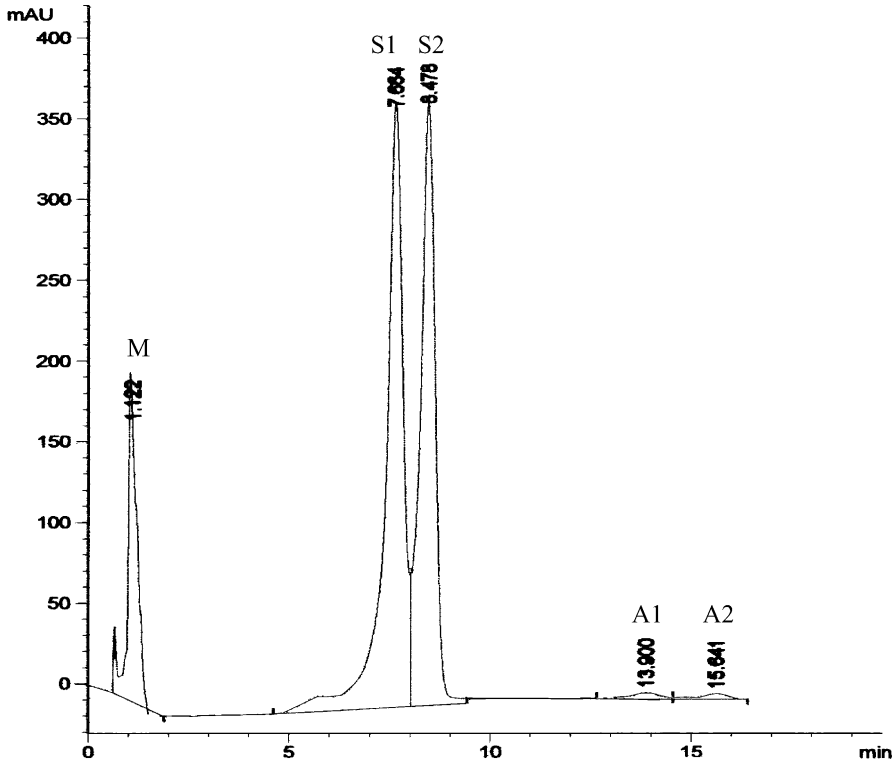


Fig. 4. Chromatogram of working solution of cefuroxime axetil (S1 and S2) and anti-cefuroxime axetil (A1 and A2) (M: peak coresponding to the mobile phase) (mobile phase: acetonitrile: 20 mM SDS (8:92; v/v), pH adjusted to 2.5 with ortophosporic acid, flow rate 1.5 ml min^{-1}, column temperature 50 °C and detection at 280 nm).

were eluted at 68.11 and 80.65 min, respectively. Mobile phases with 10–12% of acetonitrile and low concentration of SDS coeluted syn-diastereoisomers.

In further work, the mobile phase with 20 mM of SDS was optimized changing the percentage of acetonitrile and pH of mobile phase. As the parameter of chromatographic separation selectivity factor for anti-1/syn-2 was calculated. 36 experiments were carried out using mobile phases containing 20 mM of SDS and different percentages of acetonitrile (6, 7, 8, 10, 11 and 12%) at the pH values 2.0, 2.5, 3.0, 4.0, 5.0 and 6.0. Coefficients of second order polinom (Eq. (2)) were determinated and three-D diagram (Fig. 3) was constructed.

$$z = 1.218 + 0.096x + 0.071y - 0.005x^2 - 0.00xy - 0.004y^2 \quad (2)$$

Considering the great influence of pH on forming of micelles and retention [10], it had no influence on separation of geometric isomers under the constant concentration of 20 mM of SDS.

The best separation and acceptable retention of geometric and diastereoisomers were achieved using mobile phase acetonitrile:20 mM SDS (8:92; v/v) (E), pH adjusted to 2.5 with ortophosporic acid. Retention times were 7.66 min for syn-1 and 8.48 min for syn-2 diastereoisomer and 13.90 min for anti-1 and 15.64 min for anti-2 diastereoisomer. Under these conditions the influence of temperature was investigated. The temperature range 25–50 °C was investigated. The best retention time and symmetry were achieved at 50 °C. Representative chromatogram is shown in Fig. 4.

In the following investigations, the influence of co-surfactant 2-propanol and electrolyte sodium chloride in micellar mobile phase E on the retention was investigated also. 2-Propanol was added in different concentrations to increase elution strength [11]. Concentrations up to 1% of 2-propanol in mobile phase gave shorter retention times, especially for anti-isomers. The separation of syn-diastereoisomers was better without co-surfactant. Adding of sodium chloride in concentrations up to 100 mM had no big influence on retention of syn-isomers. It made the elution of anti-isomer faster for approximately 2 min which is not of great importance because resolution of diastereoisomers lowers with addition of sodium chloride.

4. Conclusion

A new micellar liquid chromatographic method for the separation of the syn- and anti-geometric isomers of cefuroxime axetil was developed. The best results were achieved on XTerra^{TM} column on 50 °C using acetonitrile: 20 mM SDS (8:92; v/v) as mobile phase, pH 2.5 (adjusted with

ortophosporic acid), at flow rate 1.5 ml min^{-1} with UV detection.

The optimization of mobile phase was done changing the percentage of acetonitrile, concentration of SDS and pH of mobile phase. Optimization of micellar mobile phase showed good separation of geometric isomers. There was no big influence of acetonitrile in concentration range from 6 to 12% and pH range from 2.0 to 6.0 on retention times. Retention was significantly a function of concentration of SDS. Investigated parameters had great influence on peak parameters and separation of diastereoisomers. The method is sensitive what gives the opportunity to use this method for the determination of geometric isomers of cefuroxime axetil in standard substances as a purity testing and in dosage forms for stability testing.

Acknowledgements

These results are the part of project “Molecular structures, chemical transformations, physicochemical characterization, pharmaceutical purity and analysis of pharmacologically active compounds” (No. 1458), which are partly financial supported by Serbian Ministry of Science and Technologies.

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