Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 623–628

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Analysis of cephalosporins by hydrophilic interaction chromatography

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ARTICLE INFO

Article history:
Received 19 July 2010
Received in revised form
21 September 2010
Accepted 30 September 2010
Available online 8 October 2010

Keywords:
Cephalosporins
Hydrophilic interaction chromatography
The Click β-CD column
Reversed-phase high performance liquid chromatography

ABSTRACT

A simple hydrophilic interaction chromatography (HILIC) method was developed to analyze seven cephalosporins. These seven cephalosporins could be separated well on the Click β-CD column and Atlantis HILIC Silica column. The effects of buffer concentration and pH on the retention under HILIC mode were studied. Except cefepime hydrochloride (**4**), the retention of other six cephalosporins increased with increasing buffer concentration, while decreased with increasing pH. Different separation selectivities could be observed on the Click β-CD column and Atlantis HILIC Silica column, and changing pH also resulted in the changing of separation selectivity. The separations of cephalosporins by HILIC and reversed-phase high performance liquid chromatography (RP-HPLC) were compared, and the two separation modes had good orthogonality. In addition, cefotaxime sodium (**1**) and its degradation were separated well on the Click β-CD column, which indicated that the Click β-CD column by HILIC can be used for studying the stability of cephalosporins.

1. Introduction

Cephalosporins can treat infections caused by Gram positive and Gram negative bacteria by interfering the formation of bacterial cell wall. They are the most frequently used antibiotics due to their broad antibacterial spectrum. More than 60 cephalosporins in four generations have already been available in the market, so it is important to develop analysis methods for their quality control. The analysis of cephalosporins is not only limited in pharmaceutical analysis, but also extent to food safety and environmental protection, such as monitoring antibiotic residues in milk, edible tissues of animals [1] and wastewater from butcheries or hospitals [2]. All these analytical tasks need high performance separation.

Cephalosporins are semi-synthetic antibiotics derived from 7-aminocephalosporanic acid including a dihydrothiazine ring and a β-lactam ring. There is at least one carboxyl group in the structure, and some cephalosporins possess zwitterionic structure. In the last few decades, RP-HPLC has been the most widely used method for the analysis of cephalosporins [3]. With this method, the buffer, acid and ion-pair are often used as additives [4–9]. Ion-pair, such as, tetrabutylammonium hydroxide can partially neutralize the charged analytes, so that both the retention and peak shape of these analytes can be improved [8]. In addition, multidimensional HPLC system has been developed to analyze cephalosporins. Nishino et al. used two dimensional HPLC system with coupled ion-exchange and RP columns to separate cefmatilen hydrochloride hydrate and its metabolites in plasma and urine [10]. Capillary HPLC is also used to analyze cephalosporins at lower concentration [11,12], which is especially suitable for the trace determination of cephalosporins in environmental and food samples. Besides the HPLC method, cephalosporins can also be analyzed by capillary electrophoresis [13] and ion-exchange planar electrochromatography [14].

In 1990, A. J. Alpert firstly proposed HILIC which is particularly promising for the separation of polar compounds [15]. With the development of HILIC, it has been widely used in many fields, such as metabonomic study [16], analysis of pharmaceutical and their impurities [17] and two dimensional (2D)-LC analysis [18]. It is reported that HILIC was also used to analyze cephalosporin C [19,20]. The feasible mobile phase of HILIC and its compatibility with MS open a new door for the analysis of cephalosporins. In this paper, HILIC was developed to separate seven commonly used cephalosporins. Column, buffer concentration and pH were investigated to illustrate their effects on the retention and separation selectivity of cephalosporins. The orthogonality between HILIC mode and RP-LC mode for cephalosporins was also investigated. Furthermore, a successful HILIC method was developed to analyze cefotaxime sodium (**1**) and its degradation products.

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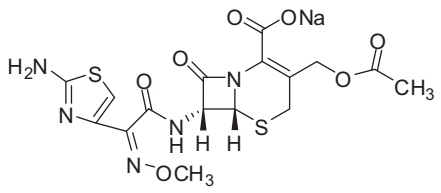
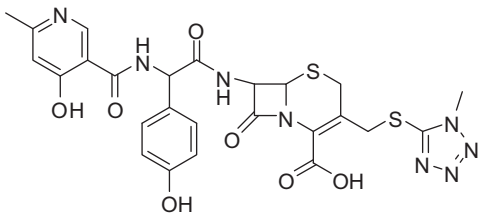
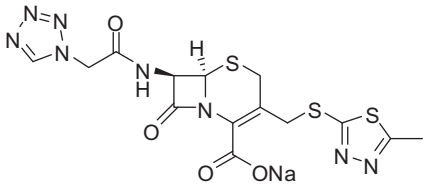
0731-7085/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.jpba.2010.09.040



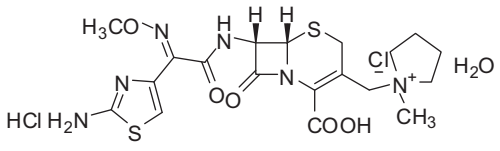
cefotaxime sodium (1)



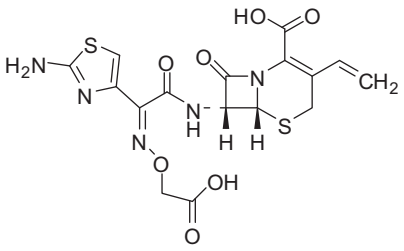
cefpiramide (2)



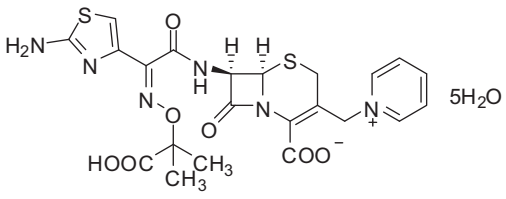
cefazolin sodium (3)



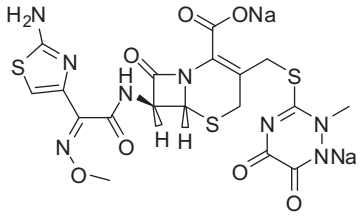
cefepime hydrochloride (4)



cefixime (5)



ceftazidime (6)



ceftriaxone sodium (7)

Fig. 1. Chemical structures of seven cephalosporins.

2. Experimental

2.1. Reagents and materials

The raw materials of seven cephalosporins were gifts from northeast general pharmaceutical factory. And the structures of them are shown in Fig. 1. Formic acid (98% pure) was purchased from Acros (USA), and ammonium formate (analytical-reagent grade) was purchased from Aladdin (China). HPLC grade acetonitrile was purchased from TEDIA (USA). The water used in this study was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instruments

All the chromatographic separations were performed on an Agilent 1200 HPLC system (Agilent, USA), which comprised G1312B binary pump, G1379B degasser, G1367C autosampler, G1316B thermostatic column compartment and G1315C diode array detector (DAD).

The mass spectrometry (MS) determination was performed on Waters ACQUITY UPLC™ system with a Quattro Micro MS (triple quadrupole) operating in ESI^+ mode (Waters, USA).

2.3. Chromatographic and MS conditions

The columns used under HILIC mode were the Click β-CD column [21] (150 mm × 2.1 mm i.d., 5 μm, 10 nm pore size, home made) and Atlantis HILIC Silica column (100 mm × 2.1 mm i.d., 5 μm, 10 nm pore size, Waters, USA). An XTerra MS C18 column (150 mm × 2.1 mm i.d., 5 μm, 12.5 nm pore size, Waters, USA) was used under RP-HPLC mode. All HPLC experiments were done under the following conditions: Flow rate was 0.2 mL/min. Column temperature was controlled at 30 °C. 254 nm was chosen as the detection wavelength. The mobile phase was filtrated with microp-