Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 831 (2006) 196–204
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Sample stacking for the analysis of penicillins by microemulsion electrokinetic capillary chromatography

Patricia Puig, Francesc Borrull, Carme Aguilar, Marta Calull *

Department of Analytical Chemistry and Organic Chemistry, Rovira i Virgili University, Marcel lí Domingo s/n, E-43007 Tarragona, Spain

Received 3 February 2005; accepted 5 December 2005
Available online 28 December 2005

Abstract

We present a method for determining eight penicillin antibiotics using microemulsion electrokinetic chromatography (MEEKC). We studied how the composition of the microemulsion affected separation by modifying such parameters as the surfactant or the addition of organic solvents. The best microemulsion system consisted of 0.5% ethyl acetate, 1.2% 1-butanol, 2% Brij 35, 10% 2-butanol and 86.3% 10 mM borate buffer at pH 10. We studied the suitability of this microemulsion composition for analyzing a commercial drug. To improve the sensitivity of the method, we used the stacking technique reversed electrode polarity stacking mode (REPSM), which increased the detection limits by about 40-fold.

Keywords: Antibiotics; Capillary electrophoresis; Penicillins; Microemulsion electrokinetic chromatography; Preconcentration

1. Introduction

An antibiotic is any chemical compound that is used to kill or inhibit the growth of infectious organisms, particularly bacteria and fungi. It is generally believed that there is a link between the use of antibiotics in animal fodder, bacterial resistance to these drugs and human diseases [1]. In particular, several penicillin-group antibiotics with various chemical structures are widely used to treat infectious diseases in humans and animals [2]. The presence of these compounds in food chains can lead to the development of allergic reactions and new strains of bacteria that are resistant to antibiotics. These risks led to the legislation of these antibiotics by the Council Regulation of the European Community 2377/90/EC [3]. Annex I of this regulation (updated 22/12/04) establishes the maximum limits of eight penicillins in animal tissues.

Most analytical methods for determining penicillin compounds are based on liquid chromatography [4–10]. Recently, several papers have described how capillary electrophoresis (CE) [1,4,11–19] can be used to analyze these compounds, mostly with micellar electrokinetic chromatography (MEKC) [12–15,17–20] because of its good selectivity and wide applicability.

In the last few years, a technique known as microemulsion electrokinetic chromatography (MEEKC) has been used to insert microemulsions as alternative pseudo-stationary phases in electrokinetic separations [21]. As with MEKC, the technique separates solutes on the basis of their hydrophobicities and electrophoretic mobilities [22] but with different selectivity [21]. Microemulsions are solutions containing dispersed nanometer-sized droplets of an immiscible liquid [23]. Typically, droplets consist of an immiscible oil suspended in water. There is a high surface tension between the layers of immiscible liquids that prevents them from mixing. A surfactant, which is soluble in both layers because it contains both hydrophilic and hydrophobic portions, is added to coat the oil droplets formed. The oil drops are coated in order to reduce the surface tension between the two liquids. The surface tension is further lowered by adding a short-chain alcohol called a co-surfactant, which stabilizes the microemulsion system [22,23]. Therefore, a typical microemulsion used for MEEKC may consist of 0.8% *n*-octane, 3.3% sodium dodecyl sulfate (SDS), 6.6% 1-butanol and 89.3% 10 mM borate buffer at pH 9.2 [24–27]. It should be taken into account that solutes in MEEKC are more able to penetrate the surface of the droplet than the surface of a micelle, which is much more rigid. This means that MEEKC can be applied to a wider

* Corresponding author. Fax: +34 977 558446.
E-mail address: marta.calull@urv.net (M. Calull).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.12.004

range of solutes, including neutral and charged compounds, than MEKC.

One of the advantages of MEEKC is that it takes into account many parameters: the type and concentration of the oil, buffer, surfactant, co-surfactant, counter-ion and the pH all affect the separation performance [26]. As well as the mostly aqueous electrolyte solution and the surfactant responsible for stabilizing the oil droplets, various organic solvents with different properties play an important role in the composition of the microemulsions used in MEEKC [28].

Although MEEKC has been used to separate some penicillins [29], to the best of our knowledge, it has not yet been used to separate the analytes studied in this paper. Altria et al. [29] showed that MEEKC could be used to analyze penicillin compounds. They separated penicillin G and penicillin V from a mixture of several cephalosporins in an analysis time of less than 4 min.

To verify if MEEKC has any advantages over MEKC in terms of resolution and analysis time, this paper studies the potential of MEEKC to separate and determine the eight legislated penicillins used as veterinary drugs. The microemulsion parameters (pH, the nature of the surfactant, the temperature of the capillary, the nature and concentration of the buffer and the addition of organic solvents) were optimized. We evaluated the usefulness of the method by analyzing a commercial drug sample.

Because CE capillaries are small, only very small sample volumes can be loaded into the column. CE is therefore not a very sensitive technique. To preconcentrate samples and increase the amount of sample that can be loaded into the column without degrading the separation, several techniques have been developed in the various electrophoretic modes [19,30,31]. One of them is the reversed electrode polarity stacking mode (REPSM), which has been used as a stacking technique in MEKC [19,31]. REPSM introduces the sample into the capillary hydrodynamically. A stacking voltage is then applied at negative polarity to preconcentrate analytes at the interface between the sample zone and the background electrolyte, and the sample matrix is pumped out from the capillary by EOF. This technique was first coupled to MEEKC to analyze some NSAIDs by Macià et al. [32]. In the present paper, we study how the on-line coupling of REPSM-MEEKC can be used to analyze penicillins at trace levels for the first time.

2. Experimental

2.1. Chemicals

Penicillin V potassium salt was purchased from Riedel-de-Häen (Seelze, Germany). Sodium dodecyl sulfate (SDS), amoxicillin, dicloxacillin sodium salt, nafcillin and sodium cholate were obtained from Sigma (Saint Louis, USA). Penicillin G potassium salt, oxacillin sodium salt, cloxacillin sodium salt, ampicillin sodium salt, sodium tetraborate and polyethylene glycol dodecyl ether (Brij 35) were obtained from Fluka (Buch, Switzerland). Sodium hydroxide and hydrochloride acid 35% were obtained from Prolabo (Bois, France). Ethyl acetate and 1-butanol were obtained from Merck (Darmstadt, Germany), octane and methanol from SDS (Peypin, France), Tris(hydroxymethyl) aminomethane (Tris) buffer and dibutyl tartrate from Aldrich (Steinheim, Germany) and *n*-heptane from Probus (Badalona, Spain). Water was obtained from a Millipore Milli-Q system (Millipore, Bedford, USA).

2.2. Equipment

MEEKC analyses were performed using a Hewlett Packard ^{3D}CE Capillary Electrophoresis System (Hewlett-Packard, Palo Alto, CA, USA) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. A HP ChemStation (Agilent) version A:04.01 was used for instrument control, data acquisition and data analyses.

2.3. MEEKC conditions

Separations were performed on 45-cm long (detection window at 36.5 cm), 75-μm internal diameter, uncoated fused-silica capillaries (Supelco, Bellefonte, USA). Unless otherwise specified, the capillary was thermostated at 25 °C, the voltage was kept constant at 10 kV during analysis and the detection wavelength was 210 nm. Normal sample injection was carried out using the pressure mode for 5 s at 50 mbar.

New capillaries were conditioned with 1 M sodium hydroxide for 5 min at 60 °C, water for 10 min and electrolyte for 30 min at 25 °C. At the beginning of each day, the capillaries were rinsed successively with 0.1 M sodium hydroxide, then with water for 8 min each and finally with microemulsion solution for 10 min. The capillaries were rinsed between injections with the microemulsion solution for 2 min. When not in use, the capillaries were washed with 0.1 M sodium hydroxide, with water for 8 min, and then dry stored.

2.4. Buffers and standards

Microemulsions were prepared by weighing the appropriate ratio of components to obtain different compositions. The order of addition was the same in all cases: initially the oil was mixed with the co-surfactant, and then the surfactant and the buffer were added. When organic solvents were used, they were added before the surfactant. The mixture was sonicated for 30 min to aid complete dissolution and an optically transparent microemulsion was formed. The pH was adjusted using 1 M NaOH or HCl 35%. Before use, the microemulsion solutions were filtered through a 0.22 μm microfilter. The solution remained transparent and stable for several weeks.

Stock standard solutions of penicillins were prepared by dissolving each compound in Milli-Q water to obtain a concentration of 1000 mg L^{-1}. For MEEKC experiments, a standard mixture of 100 mg L^{-1} was prepared and working solutions were made by dissolving a volume of this solution in water to obtain the final concentrations.

A commercial pharmaceutical preparation containing amoxicillin was used to test the suitability of the method for industrial samples. The individual solution was prepared by dissolving a quantity of the preparation in water to give an amoxicillin concentration of 100 mg L^{-1}. This was then diluted to obtain a final concentration of 10 mg L^{-1}.