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Using cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography to determine selective serotonin reuptake inhibitors

Hsiu-Li Su, You-Zung Hsieh*

Department of Applied Chemistry, National Chiao Tung University, 1001 Ta Hsueh Road, Hsinchu 300, Taiwan

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ABSTRACT

We have employed a rapid and highly efficient on-line preconcentration method, cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography (CSEI–sweeping-MEKC), for the analysis of selective serotonin reuptake inhibitors (SSRIs) of antidepressant drugs. We monitored the effects of several of the CSEI–sweeping-MEKC parameters – including the pH, the concentrations of high-conductivity buffer (HCB), sodium dodecyl sulfate (SDS), and organic modifier, the injection length of the HCB, and the injection time of the sample – to optimize the separation process. The optimal background electrolyte was 50 mM citric acid/disodium hydrogenphosphate buffer (pH 2.2) containing 100 mM SDS and 22% isopropyl alcohol. The sensitivity enhancements of the SSRIs sertraline, fluoxetine, paroxetine, fluvoxamine, and citalopram ranged from 5.7×10^4 to 1.2×10^5; the coefficients of determination exceeded 0.9938 and the relative standard deviations of the peak heights were less than 3.2%; the detection limits ranged from 0.056 to 0.22 ng/mL. We employed the optimal conditions to analyze these five SSRIs in a plasma sample prepared using solid-phase extraction (SPE) to minimize the influence of the matrix. Although the limits of detection of the SSRIs in human plasma were higher than those in pure water, this present technique is more sensitive than other, more-conventional methods. The recovery of the SPE extraction efficiency was satisfactory (up to 89%). Our findings suggest that, under the optimal conditions, the CSEI–sweeping-MEKC method can be used successfully to determine these five SSRIs in human plasma.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are second-generation antidepressant drugs used for the treatment of depression [1]. They are often employed as first-line therapeutic drugs in place of traditional tricyclic antidepressants. Because of their minimal side effects and low toxicity, SSRIs are generally tolerated well by patients suffering from depression or related conditions. Among the family of SSRIs, fluoxetine (Prozac) is the most widely consumed SSRI drug in the United States, followed by sertraline (Zoloft), paroxetine (Paxile), fluvoxamine (Luvox), and citalopram (Celexa). The mechanism of action of the SSRIs is blockage of serotonin reuptake at the presynaptic nerve terminal. Unfortunately, these drugs have several unwanted side effects, including sexual dysfunction, gastrointestinal effects, and disruption of the central nervous system [2]; when the concentration of SSRIs in the blood is too high, acute symptoms or intoxication might occur. Hence, there is great interest in the development of a simple, rapid method for monitoring the concentrations of these therapeutic drugs in patients.

Several methods have been published for the analysis of SSRIs in plasma and urine, including gas chromatographic separation with mass spectrometric detection (GC/MS) [3] and high-performance liquid chromatographic (HPLC) separation with UV [4,5], fluorescence spectroscopic [6], and mass spectrometric [7] detection. A CE-based technique – employing micellar electrokinetic chromatography (MEKC), UV detection, and a buffer containing sodium dodecyl sulfate (SDS) and an organic modifier – has also been developed for the successful separation of commercial antidepressants [8,9]. Because biological materials can interfere with the detection of the analytes of interest, biological fluids, such as plasma or urine, are usually pretreated through liquid–liquid extraction [8,10] or solid-phase extraction (SPE) [11].

Despite the development of this range of separation techniques, each has unattractive characteristics that hinder its application—e.g., consumption of a large amount of organic solvent (LC), complicated derivation steps (fluorescence detection), poor sensitivity (UV detection), or expensive equipment (mass spectrometric detection). Quirino and Terabe were the first to develop on-line preconcentration techniques for CE analyses; since then,

* Corresponding author. Tel.: +886 3 5731785; fax: +886 3 5723764.
E-mail address: yzhsieh@mail.nctu.edu.tw (Y.-Z. Hsieh).

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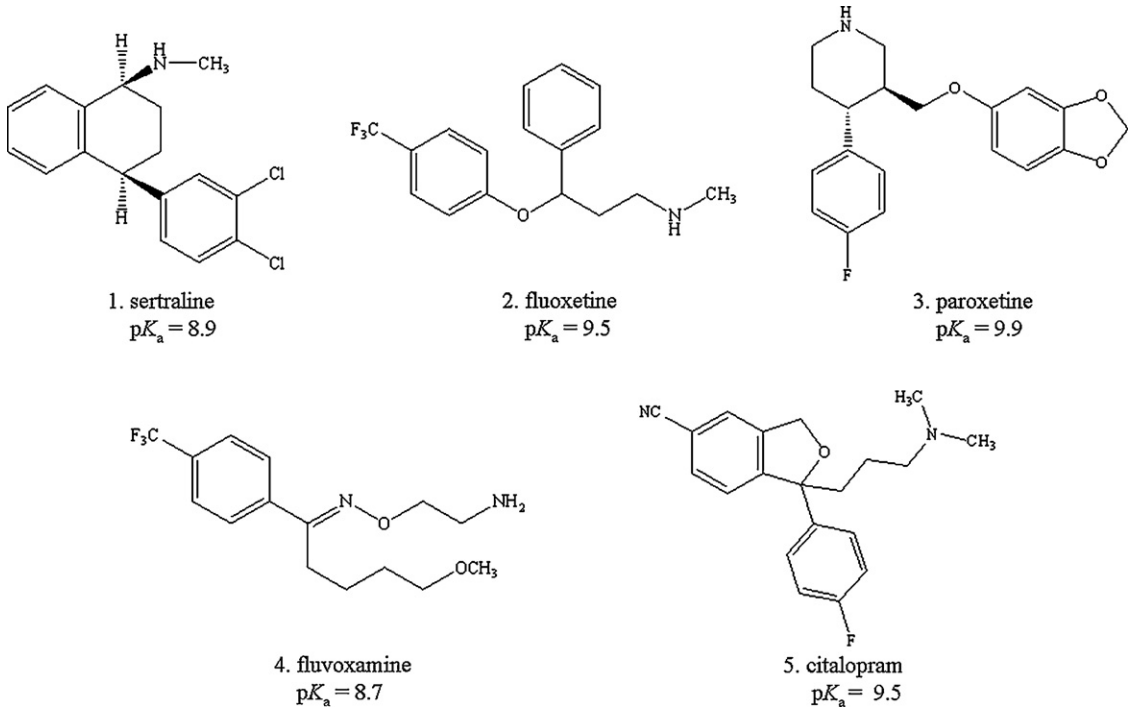

Fig. 1. Molecular structures of the five SSRIs.

the application of sweeping-MEKC [12,13], anion-selective exhaustive injection (ASEI)–sweeping-MEKC [14,15], and cation-selective exhaustive injection (CSEI)–sweeping-MEKC [16,17] has expanded into several fields. Because these methods are simple to use, provide high sensitivity, and do not require additional equipment, they have found widespread use. An appropriate preconcentration technique can be selected based on the analyte's properties. If sweeping-MEKC concentration is employed, the detection limit can be improved by ca. 10–1000-fold [18]; if, however, sweeping-MEKC is coupled with selective electrokinetic injection, the sensitivity can be enhanced by up to a million-fold [19]. Among the many on-line preconcentration methods, one of the best preconcentration efficiencies is provided by the CSEI–sweeping-MEKC technique—used for the detection of cationic analytes—performed in conjunction with selective electrokinetic injection, followed by the addition of surfactants to effect the sweeping process. The CSEI–sweeping-MEKC technique has been applied, for example, to the analysis of lysergic acid diethylamide (LSD) in mouse blood [20], ephedra alkaloids in dilute herb extracts and mouse sera [21], and methamphetamine, ketamine, morphine, and codeine in human urine [22]. Those studies have indicated the practicality of the method in terms of the linear relationship of the quantitative data, the reproducibility of the measurements, and the application to real samples.

In this study, we used CSEI–sweeping-MEKC to improve the detection limit and enhance the sensitivity of the determination of five SSRI antidepressant drugs: fluoxetine, sertraline, paroxetine, fluvoxamine, and citalopram (Fig. 1). We optimized several separation and sensitivity enhancement parameters, including the pH, the concentrations of surfactant, buffer, and organic modifier, the injection length of the high-conductivity buffer (HCB), and the injection time of the sample. We also compared the sensitivity enhancements using CSEI–sweeping-MEKC and sweeping techniques. In addition, we applied the developed method to analyze the five SSRIs in human plasma samples. Using SPE, a simple technique for sample preparation, we decreased both the degree of interference and the matrix effect.

2. Experimental

2.1. Chemicals

All of the reagents and chemicals were of analytical grade. Sertraline, fluoxetine, paroxetine, fluvoxamine, citalopram, and SDS were obtained from Sigma (St. Louis, MO, USA). Disodium hydrogenphosphate (Na_2HPO_4) and sodium hydroxide (NaOH) were purchased from Fluka (Buchs, Switzerland). Citric acid and acetonitrile were obtained from Merck (Darmstadt, Germany). Methanol, isopropyl alcohol (IPA), acetic acid, and ammonium hydroxide were obtained from Aldrich (St. Louis, MO, USA). Water was purified through a Milli-Q water system (Millipore, Milford, MA, USA). The blank human plasma samples were obtained through donation.

2.2. Apparatus

A Beckman P/ACE MDQ CE system (Fullerton, CA, USA) was used to effect the separations. A diode-array detector was employed for detection. Separations were performed in a 60 cm (50 cm effective length) × 50 μm i.d. fused-silica capillary tube (Polymicro Technologies, Phoenix, AZ, USA). The capillary tube was assembled in the cartridge format. A personal computer using 32 Karat software controlled the P/ACE instrument and allowed data analysis. The conductivity of the samples was measured using a cond 340i conductivity meter (WTW, Weilheim, Germany). The human blood samples were centrifuged using a Hettich EBA21 apparatus (Tuttlingen, Germany). Prior to use, the separation capillary was preconditioned sequentially with methanol (10 min), 1 M HCl (10 min), deionized water (2 min), 1 M NaOH (10 min), and deionized water (2 min). Stock solutions of 0.2 M citric acid and 0.4 M disodium hydrogenphosphate were first prepared. Different buffer solutions were prepared by mixing suitable amounts of the stock solutions with water to obtain a specified concentration and pH. Under typical conditions, the non-micellar background electrolyte (BGE) consisted of 100 mM citric acid/disodium hydrogenphos-