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In-capillary derivatization and analysis of ephedrine and pseudoephedrine by micellar electrokinetic chromatography with laser-induced fluorescence detection

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ABSTRACT

This paper describes an automatic rapid approach for in-capillary derivatization of ephedrine (E) and pseudoephedrine (PE) and subsequent sensitive determination of the derivatives by micellar electrokinetic chromatography (MEKC) with laser-induced fluorescence (LIF) detection using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) as fluorescent reagent. The unique feature of this method is the capillary being used as a small reaction chamber, in which the sample, derivatization buffer and reagent solutions were injected directly into the capillary by tandem mode, followed by an electrokinetic step (5 kV, 15 s) to enhance the mixing efficiency of analytes and reagent plugs. Standing a specified time of 1 min for reaction, the derivatives were then immediately separated and determined. Several parameters for in-capillary derivatization and subsequent MEKC separation were systematically investigated. Under these optimized conditions, a baseline separation of the two analytes was achieved within 10 min and the derivatization concentration limits of detection were found to be 4.8 ng mL^{-1} for E and 1.6 ng mL^{-1} for PE, respectively. The method was validated in terms of precision, linearity, accuracy and successfully applied for the determination of the two alkaloids in ephedra herb and its preparations.

1. Introduction

Capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection has documented a powerful tool for the determination of a variety of compounds [1–4], as it possesses the advantageous characteristics of high separation efficiency, high detection sensitivity and short analysis time. However, a derivatization procedure with a suitable fluorogenic or fluorescent reagent to produce a fluorescent adduct is necessary for the development of a CE–LIF method [5,6], because few analytes themselves can emit strong fluorescence. Also, analysis of native fluorescent compounds is limited by their differing excitation wavelengths, and their compatibility with available laser sources. Generally, derivatization procedures can be performed before (pre-capillary), during (in-capillary) or after (post-capillary) the electrophoretic separation [7–10]. The most suitable approach depends on the reason why a derivatization procedure is introduced, the number of samples that have to be analyzed, the physicochemical properties of the analyte and reagent, etc. Recently, in-capillary derivatization has received attractive attention because this approach exhibits some remarkable advantages over conventional pre- and post-capillary derivatization, such as low consumption of reagents and sample, short reaction time, and the possibility of automation without extra equipment. In this set-up, the front-end of the capillary is used as the reaction chamber, which means that sample dilution can be reduced to a minimum. As a result, this approach is especially suitable for the determination of analytes present in extremely small sample volumes such as single cell [11] or vesicle [12] analysis.

One of the most important features of in-capillary derivatization is to select a proper fluorogenic or fluorescent reagent. An excellent reagent for in-capillary derivatization should have the advantages of rapid reaction rate, high derivatization efficiency and less fluorescent side products. Up to now, only a few reagents, such as *o*-phthalaldehyde (OPA) [13–21], naphthalene-2,3-dicarboxaldehyde (NDA) [11,12,22,23], 1,2-naphthoquinone-4-sulfonate (NQS) [24,25], 3-(2-furoyl)-quinoline-2-carboxaldehyde (FQ) [26–28], and 5-(4,6-dichloro-*s*-triazin-2-ylamino)fluorescein (DTAF) [29], have already been employed for in-capillary derivatization. Although these reagents have their own merits, it should be emphasized that they require either the use of seriously toxic chemicals such as sodium cyanide (OPA, NDA and FQ) or traditional UV detection (OPA, NQS), or more complex background signals would

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be observed (DTAF). 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), introduced by Watanabe and Imai [30], has been identified to be a useful and sensitive fluorescent agent for pre- or post-capillary derivatization due to its several remarkable advantages such as good reactivity with both primary and secondary amines, high reaction rate, few fluorescent side products and well compatibility with commercial argon ion laser. However, no study has been reported on the use of NBD-F as in-capillary derivatization reagent for capillary electrophoresis until recently, the potential of NBD-F as in-capillary derivatization reagent was exploited by Zhang et al. [31] and our group [32], respectively, using different test analytes. However, the related study is still very scarce. Further study is significant to understand the mechanisms and extend the application of in-capillary derivatization.

The rapid and sensitive analysis of ephedrine alkaloids and analogue is of continual interest in the fields of foods, pharmaceuticals and forensic application. So far, many analytical methods have been developed for qualitative or quantitative analysis of these analytes using gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis. Different CE–LIF approaches have also been established for sensitive determination of ephedrine alkaloids after pre-capillary derivatization [33–37]. However, these methods, in many cases, are laborious and require large sample volumes. In addition, the reaction times are usually long and the derivatization of very diluted samples is problematic. Based on these, a new MEKC–LIF method utilizing in-capillary derivatization with NBD-F as fluorescent reagent was developed in this study for sensitive determination of E and PE. Experimental conditions for in-capillary derivatization and subsequent MEKC separation were systematically investigated. This proposed method is proved a fast and easy-to-use approach for the fully automated analysis of E and PE in ephedra herb and its preparations.

2. Experimental

2.1. Reagents and solutions

Standard E and PE (chemical structures shown in Fig. 1) were obtained from National Institute for Control of Pharmaceutical and Bio-products (Beijing, China). NBD-F was the product of Tokyo Kasei Kogyo (Tokyo, Japan). Samples of ephedra herb (the aerial parts), Keke capsule (Guizhou Ebay Pharmaceutical), and White & Black tablets (Qidong Gaitianli Medicines) were purchased from a local pharmaceutical store. All other reagents used were of analytical reagent grade and used without further purification. Distilled water was used throughout.

Stock standard solutions of E and PE with the concentration of 200 μg mL^{-1} were prepared by dissolving the appropriate amount in distilled water and standard mixtures were prepared by dilution of the corresponding stock solutions with distilled water as required. All standard solutions were stored at 4 °C. 10 mM NBD-F stock solution was prepared in acetonitrile (ACN), stored at −18 °C, and diluted to the desired concentration with ACN before use.

2.2. Preparation of the electrolytes

The running buffers employed for separation were prepared from stock solution of 100 mM sodium tetraborate and 200 mM sodium dodecyl sulfate (SDS). An electrolyte solution of 20 mM tetraborate was used for derivatization. Before use, all buffers were adjusted accurately to the desired pH value with 1.0 and 0.1 M HCl or 1.0 and 0.1 M NaOH, and filtered through a 0.45 μm pore size cellulose acetate membrane.

2.3. Sample preparation

2.3.1. Ephedra herb

The crude drug of ephedra herb was finely powdered, and then 0.50 g powder was extracted with 10 mL distilled water in an ultrasonic bath for 1 h. After centrifugation for 10 min and filtration through a 0.45 μm filter membrane, the extract was diluted by 400 times with distilled water for direct injection.

2.3.2. Keke capsule

One of the capsules was opened and the powder was extracted with 10 mL distilled water in an ultrasonic bath for 1 h. After centrifugation for 10 min and filtration through a 0.45 μm filter membrane, the extract was diluted by 80 times with distilled water for direct injection.

2.3.3. White and black tablets

A couple of tablets were weighed and finely powdered, and then half of powder (0.2703 g for white tablet and 0.2637 g for black tablet) was extracted with 25 mL distilled water in an ultrasonic bath for 1 h. After centrifugation for 10 min and filtration through a 0.45 μm filter membrane, the extract was diluted by 800 times with distilled water for direct injection, respectively.

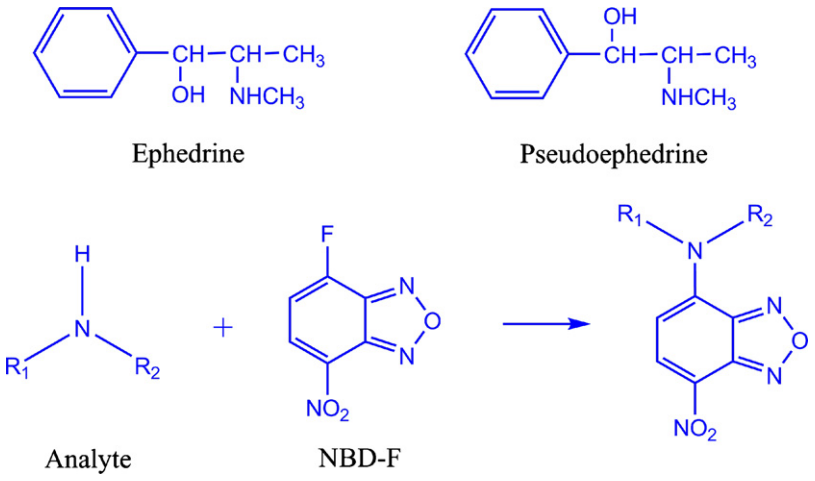

Fig. 1. Chemical structures of E and PE, and the scheme of the derivatization reaction with NBD-F.