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Short communication

On-line concentration and determination of all-*trans*- and 13-*cis*- retinoic acids in rabbit serum by application of sweeping technique in micellar electrokinetic chromatography

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Abstract

A simple and rapid micellar electrokinetic chromatography (MEKC) method with UV detection was developed for the simultaneous separation and determination of all-*trans*- and 13-*cis*-retinoic acids in rabbit serum by on-line sweeping concentration technique. The serum sample was simply deproteinized and centrifuged. Various parameters affecting sample enrichment and separation were systematically investigated. Under optimal conditions, the analytes could be well separated within 17 min, and the relative standard deviations (RSD) of migration times and peak areas were less than 3.4%. Compared with the conventional MEKC injection method, the 18- and 19-fold improvements in sensitivity were achieved, respectively. The proposed method has been successfully applied to the determination of all-*trans*- and 13-*cis*-retinoic acids in serum samples from rabbits and could be feasible for the further pharmacokinetics study of all-*trans*-retinoic acid.

Keywords: All-*trans*-retinoic acid; 13-*cis*-Retinoic acid; Sweeping; Micellar electrokinetic chromatography

1. Introduction

Two of the most clinically useful vitamin A derivatives are all-*trans*-retinoic acid (all-*trans*-RA) and 13-*cis*-retinoic acid (13-*cis*-RA) [1]. All-*trans*-RA can be metabolized through stereoisomerization to 13-*cis*-RA in vivo. Acute promyelocytic leukemia (APL) is the first neoplasm to be successfully treated with all-*trans*-RA, resulting in 80% of patients achieving a complete remission [2]. However, relapse almost widely occurs during continuous treatment with all-*trans*-RA in APL. The study suggests that the clinical relapse and resistance are associated with the progressive reduction of the all-*trans*-RA concentration in plasma [3]. As a less toxic derivative among retinoids, 13-*cis*-RA has been used clinically in the treatment of severe acne [4]. Unfortunately, not only 13-*cis*-RA but also excessive doses of vitamin A are shown to have adverse effects such as hypervitaminosis A and teratogenic potential [4–6]. The toxicity studies of vitamin A have shown that the RA and its metabolites other than retinol may serve as biomarkers for evaluating the risk for vitamin A toxicity [7–9]. Therefore, whether the effectiveness and safety of the clinical application of RA or adequate vitamin A supplementation for patients, pregnant females and healthy people, it is of great importance to establish a rapid and reliable analytical method for determining RA in the biological matrices.

Up to now, several analytical methods for the determination of RA have been reported, including gas chromatography (GC) [10], high-performance liquid chromatography (HPLC) [11] and capillary electrophoresis (CE) [12]. CE is a widely used technique in separation science due to its high separation efficiency, rapid separation, low operational costs and small amounts of reagents or samples required. Nevertheless, the low sensitivity prevents the applicability of CE for determination of low levels

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of RA in biological fluids. Sweeping is a simple and convenient on-line concentration method for either charged or neutral analytes in micellar electrokinetic chromatography (MEKC) [13]. It was first described by Quirino and Terabe [14]. So far, this method has been widely applied in the on-line concentration and determination of metabolites in biological fluids [15] and active components in Chinese herbal medicines [16].

The aim of this work was to develop a simple and sensitive on-line sweeping technique for the determination of all-*trans*-RA and 13-*cis*-RA in the serum sample. To the best of our knowledge, there are no reports published on the simultaneous determination of the RA isomers in the biological fluids using CE.

2. Experimental

2.1. Chemicals and reagents

All-*trans*-RA and 13-*cis*-RA were obtained from Sigma (St. Louis, MO, USA). SDS was purchased from Amresco (Solon OH, USA). Tretinoin Tablets (Shandong Liangfu Pharmaceutical Group Ltd., Shandong, China) were purchased from Shaanxi Provincial People's Hospital. Other chemicals and reagents were analytical or HPLC grade. Deionized water was prepared by a Milli-Q system (Millipore, New Bedford, MA, USA). All solutions and samples were filtered through a 0.22 μm nylon-membrane filter prior to analysis.

2.2. Apparatus

All experiments were carried out on a Hewlett-Packard^{3D} capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detection (DAD) system. The pH of solution was measured by an Orion 250A pH meter (Orion research, Beverly, USA). Conductivities were measured using a sensION378 conductivity meter (HACH, Loveland, CO, USA). Centrifugation was performed on a Sigma 3K30 centrifuge (Sigma, Osterode, Germany).

2.3. Animals and blood sampling

In order to prevent photoisomerization and degradation of RA, all sample manipulations involving RA were performed in dark rooms under dim yellow light.

Three healthy New Zealand white rabbits, weighing about 2.0–2.5 kg, were obtained from the Laboratorial Animal Center of Xi'an Jiaotong University. All animal manipulations were strictly in accordance with *the Guide for the Care and Use of Laboratory Animals* (National Research Council of USA, 1996). Before drug administration, rabbits were fasted for at least 12 h and given free access to water. Tretinoin Tablets were suspended in 0.5% (w/v) carboxymethyl cellulose sodium salt (CMC-Na) aqueous solution and administered a dose of 15 mg/kg body weight by intragastric gavage. Blood samples (1.0 mL) were withdrawn from the marginal ear vein at 2.0 h after administration of Tretinoin Tablets, and then immediately centrifuged at 3000 rpm for 15 min at 4 °C. The serum layer was collected and stored in the amber Eppendorf tubes at −20 °C until analyzed.

2.4. Sample preparation

Stock standard solutions of all-*trans*-RA (220 mg/L) and 13-*cis*-RA (250 mg/L) were prepared in methanol. The working standard solutions were prepared daily by diluting the stock standard solutions to obtain appropriate concentrations, and finally contained 20 mM borate buffer (pH 8.5), 15 mM NaCl and 15% (v/v) methanol. All solutions were protected from light exposure and stored at −20 °C before analysis.

The serum samples were 6:4 (v/v) diluted with methanol, and then mixed for 1 min using a vortex mixer, finally centrifuged for 15 min at 10,000 rpm. Subsequently, a 150 μL aliquot of supernatants was transferred into amber Eppendorf tubes and diluted with water and borate buffer to obtain 600 μL of sample solution comprising 20 mM borate buffer and 15% (v/v) methanol with pH 8.5.

2.5. Electrophoresis procedure

All the separations were performed on a fused-silica capillary of 48.5 cm (40 cm effective length) × 75 μm I.D. (Yongnian Optical Conductive Fiber Plant, Hebei, China) at 20 °C with a voltage of +15 kV. Detection wavelength was set at 350 nm. The running buffer (conductivity, 1.2 mS/cm) consisted of an aqueous solution with 30 mM borate buffer (pH 8.5), 30 mM SDS and 15% methanol (v/v). The sample matrix (conductivity, 0.25 mS/cm) contained 20 mM borate buffer (pH 8.5), 15 mM NaCl and 15% (v/v) methanol. Sample was introduced by pressure (50 mbar, 1 mbar = 100 Pa) injection. New capillaries were conditioned by rinsing with methanol (5 min), followed by 1.0 M NaOH (30 min), 0.1 M NaOH (15 min), purified water (20 min) and finally by the running buffer (20 min). To ensure repeatability between consecutive analyses, the capillary was preconditioned for 1 min by flushing with methanol, followed by 1 min with 0.1 M NaOH and 2 min with purified water, finally by rinsing with running buffer for 5 min. The running buffer was renewed after every three runs.

3. Results and discussion

As a simple and versatile method, sweeping can occur when the sample has a conductance that is lower, similar or higher than the background solution (BGS) and no pseudostationary phase (PS) exists [17]. A unique focusing effect is caused by partitioning or interaction between analytes and PS when charged PS penetrates the sample zone during the application of voltage. The injected length of an analyte zone is theoretically narrowed by a factor equal to $1/(1+k)$ (k, retention factor) and the concentration can be increased approximately by a factor, $1+k$ [18]. The greater the value of k, the greater the concentrating effect will be. In practical applications, several experimental parameters could also interfere with the concentrating effect and their influences are systematically investigated and optimized in the following section.