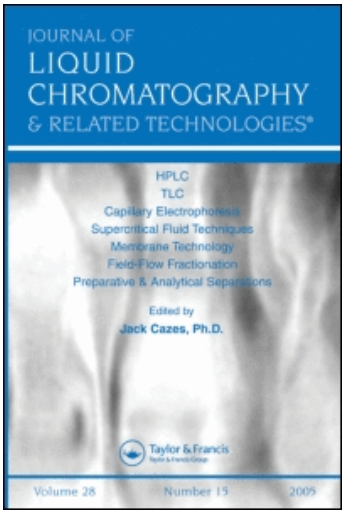

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A Rapid Method for Enkephalin Analysis in Tissues by Capillary Electrophoresis

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A RAPID METHOD FOR ENKEPHALIN ANALYSIS IN TISSUES BY CAPILLARY ELECTROPHORESIS

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Abstract

A rapid method for the determination of the pentapeptide, met enkephalin , in samples of rat retina is described. The method uses ultrafiltration as a sample preparation followed by free solution capillary electrophoresis with detection at 210 nm. The method is rapid with an analysis time of less than 10 minutes.

Introduction

There continues to be intense interest in methods for the determination of neurochemicals especially the pentapeptides met and leu enkephalin. Additionally, there is growing interest in the use of capillary electrophoresis to perform this assay. A number of researchers have reported the use of capillary electrophoresis (CE) for the determination of these compounds (1-4). Some of these reports focus on the development of the

appropriate conditions for the separation of the individual compounds while others report the analysis of complex samples. In those cases where complex samples were analyzed, the solid phase technique pioneered by Desiderio and others (5-7) was used to prepare samples for subsequent analysis . In this communication, we report the use of ultrafiltration (UF) coupled with CE using UV detection at 210 nm for the qualitative determination of met enkephalin in samples of retina obtained from a laboratory rat. This sample preparation is rapid and entails the use of no additional chemicals when compared to the solid phase extraction (SPE) protocol.

Materials and Methods

Sample Preparation

Retinas were obtained from 1 day and adult Sprague-Dawley rats (Charles River; Wilmington, MA). The material was homogenized in 50 mM Tris buffer containing protease inhibitors in a 1:10 ratio (v/v) using a Polytron tissue homogenizer (8) . After homogenization samples were centrifuged through a Centricon filter unit with a 10,000 Mw cutoff membrane (Amicon; Danvers, MA). Samples were stored at -70 ° C prior to analysis.

Equipment and Analytical Conditions

A SpectraPhoresis 1000 Capillary Electrophoresis Unit (Thermo Separation Products; Fremont, CA) operating under Version 1.04 of the SpectroPhoresis software was used in this study. A 44 cm x 75 um UVT Capillary (Polymicro; Phoenix, AZ) with a buffer system of 40 mM phosphate at pH 9.0 was used (9). The resulting electropherogram was monitored at 210 nm. The run time was 15 min. at a temperature of 30 °C with 15 kV applied voltage.
Samples of met and leu enkephalin (Sigma , St. Louis, MO) were dissolved in distilled water at concentrations of approximately 100 ug/ml. Samples and standards were injected using an electrokinetic injection of 10 kV for 1 s. The sample extract was compared with the standard to verify migration times.

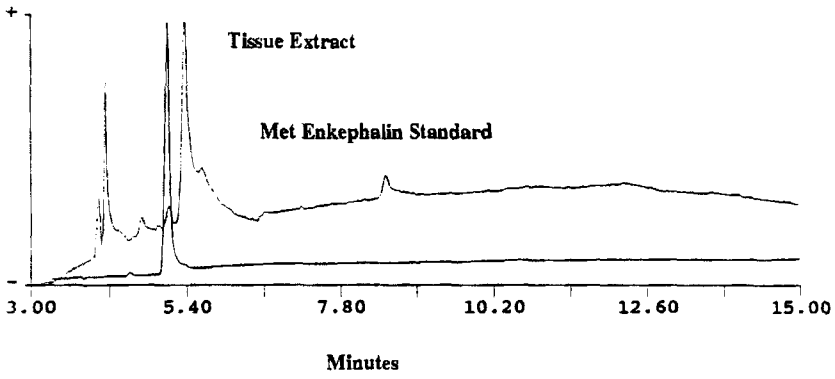

Figure 1
Overlay of Electropherogram of Met Enkephalin Standard and Tissue Extract
Conditions of Analysis: 44 cm x 75 um UVT Capillary with a 40 mM Phosphate Buffer @ pH 9.0 Detection @ 210 nm

Results and Discussion

Injections of met and leu enkephalin using the conditions described indicated that the two compounds migrated at differing rates as has been previously reported by others (1-3). A sample of retina from a developing rat was injected under the same conditions and a peak was seen at the same migration time as met enkephalin. To verify sample clean-up, samples of authentic standard were prepared using the same conditions as the sample and 100% recovery was seen by comparing peak areas of filtered and unfiltered met enkephalin standard. Additionally, the sample was spiked with met enkephalin standard to verify the migration time of the peak . The detector wavelength of 210 nm was chosen to monitor the peptide bond.Figure 1 illustrates this separation showing a comparison of the standard with the retina extract.

No quantitative data were developed in this experiment since the purpose was to illustrate the utility of ultrafiltration as a method of sample preparation for these compounds. UF has been widely used as a method of sample preparation for other biologically related compounds (10 -12) in similar systems so there is no reason not to expect success in these preliminary studies. Additionally, this sample preparation has only been applied initially to this one matrix but the results are encouraging enough to report on these preliminary studies.

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