行政院國家科學委員會補助專題研究計畫成果報告

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毛細管電泳在臨床上的應用 II

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計畫類別：個別型計畫

計畫編號：NSC89－2113－M－040－001

執行期間：88 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：蕭明文

共同主持人：

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執行單位：中山醫學院醫事技術學系

中　華　民　國　89 年 10 月 26 日

行政院國家科學委員會專題研究計畫成果報告

毛細管電泳在臨床上的應用

The Application of Capillary Electrophoresis in the Clinical Settings

計畫編號：NSC 89-2113-M-040-001
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
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一、中文摘要

臨床上經常用來診斷女性病人荷爾蒙的雌激素：雌二醇、雌三醇與雌固酮可以被毛細管電泳予以鑑定分離。此三種荷爾蒙化合物在磷酸鹽溶液 PH7.4, 電壓 10KV 的條件下在 10 分鐘內成功的被分離。

關鍵詞：雌激素、毛細管電泳、

Abstract

The capillary electrophoretic separation of the estrogen compounds (estradiol, estratriol and estrone) typically employed in clinical/medical settings for monitoring the deficency of hormone function. Successful resolution of these compounds was achieved via use of a bile salt micelle system composed of sodium cholate at phosphate buffer (pH 7.4). The elution patterns of these estrogens can be obtained within ten minutes with a voltage of 10 KV. The effect of varying the applied voltage, temperature and the buffer concentrations with bile salt surfactant on the migration behavior is also examined.

Keywords: Estrogen, Capillary electrophoresis

二、Introduction

Sex hormones are responsible for the secondary sex characteristics (1). Testosterone, an androgen, promotes the muscle growth, deepens the voice, monitors the growth of body hair and other male secondary sex characteristics. Testosterone is the precursor of estradiol, an estrogen which regulates the menstrual cycle and its reproductive process. Also, it is obliged for the development of the female secondary sex characteristics.

Presently, radioimmunoassay kits are used most widely for diagnostic examination in the hospital. Such methods are commercially available for the most common estrogen compounds. However, they are expensive and time consuming. Gas chromatography (GC) and high performance liquid chromatography (HPLC), mostly used for endocrine research (2-4), can give a total profile of multiple homones. However, they are still rather time-consuming (30 minutes to over an hour) and need to optimize the analytical conditions for each researcher. The determination of the concentration of free estrogens is variable from one laboratory to another due to the interference from the impurity and running buffers of extraction process(5). Therefore, a convenient and accurate determination of such estrogens in the body fluid is essential for metabolic studies and diagnostic purposes. Since their structures are closely similar, highly hydrophobic and they contain few functional groups, their separation is difficult.

Capillary electrophoresis (CE) was developed to separate the charged molecules in a buffer-filled capillary by the application of a very high voltage (6). Micellar electrokinetic capillary chromatography

(MECC) allows the resolution of uncharged molecules by adding surfactants to modify the conditions to extend the application of CE (7-8). Based on the differential binding to the micellar phase, the partition between the slow moving micelle and the fast moving aqueous phase causes differential resolution of the solutes. Sodium dodecyl sulfate (SDS) has proven very useful for separating water-soluble analytes (e.g. ascorbic acid (9)) and nonionic molecules (10-11). Cole et al. (12) successfully adopted bile salts instead of SDS to optimize the resolution of binaphthyl enatiomer separation. Hsiao et al. (13-14) has successfully separated steroids, nonprotein nitrogen ompounds in phosphate buffer with sodium cholate.

CE methods for urine samples have been developed by Guzman et al. (15) The simultaneous detection of these analytes has not been reported. In this paper, the successful separation of these analytes in phosphate buffer (pH=7.4) containing sodium cholate as well as other effects for such estrogen separation are reported.

三、Result and Discussion

Resolution of these estrogens (estradiol, estratriol and estrone) were not achieved in the buffer range of 0.038 M. They are co-migrated at above concentration. Other buffers are also tried to separate them however they are not successful for the purpose.

MECC with sodium cholate

In CE, the resolution, separation proficiency, selectivity, and elution time of the analytes can be optimized by the capacity factor, k' (e.g. assorted surfactants, surfactant concentrations, organic modifiers, temperature, voltages, etc.). However, separation of these analytes by MECC with addition of SDS in the 0.038 M phosphate or 0.02 to 0.10 M borate buffer solution was not achieved in the range of pH 7-9. Figure 1 shows the resolution of MECC electropherograms of the estrogens in the 0.038 M phosphate buffer of pH=7.4 with 0.04 M sodium cholate at 25^{0}C, 10 KV. The variation of the concentration of phosphate buffer with sodium cholate to adust the capacity factor, K', to separate estrogens was conducted.

Variation of voltage

Variation of voltage can have effects on migration time, resolution, peak sharpness, EOF, and joule heating. The joule heating resulting from an increase in voltage may lead to changes in EOF, ion mobility, analyte diffusion, and band broadening. The migration time decreased when the applied voltage was increased, but the elution sequence did not change with different applied voltages. The joule heating effects (e.g. peak broadening) was not observed.

Temperature effect

Viscosity is a function of temperature. Therefore, as the temperature increased, the viscosity decreased; and then electrophoretic mobility and EOF increase. Some analytes may not be stable at higher temperature, and variation of temperature may lead to conformational change. The migration sequence did not change with the application of different temperatures. An increase in EOF and electrophoretic mobility from increased temperature leads to a shorter analysis time .

Conclusion

In conclusion, these Estrogens are separated by MECC in phosphate buffer (0.38 M) and sodium cholate (0.04 M) at 25°C and 10 KV. This MECC approach can potentially be applied in the clinical settings.

四、Acknowledgement

The authors acknowledge the financial support of a National Science Council grant (#NSC89-2113-M-040-001)

and Chung-Shan Medical & Dental College research fund (CSMC # 82-B032 and 88-OM-A064).

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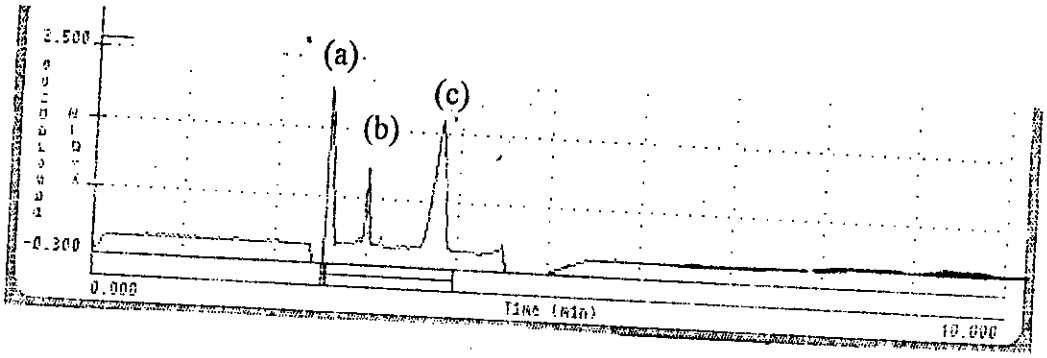

Figure 1. Electropherogram of estrogen using the MECC at phosphate buffer 0.038M, 0.04M sodium cholate and 15% methanol, 10KV and the peaks are (a) Estratriol (b) Estrone (c) Estradiol.