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研究简报

无胶筛分毛细管电泳法检测微量蛋白质骨桥蛋白

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摘要 采用非涂层毛细管,以150 mmol/L硼酸盐缓冲液为电泳缓冲液,30 g/L聚乙二醇(PEG)20000为筛分介质,经对分离条件进行优化,成功地建立了用无胶筛分毛细管电泳检测微量蛋白质的方法。用所建立的方法测定骨桥蛋白,其批内、批间迁移时间的相对标准偏差均小于5%,回收率大于95%,被检测样品中骨桥蛋白的含量与其峰面积呈良好的线性关系(相关系数为0.996),最低检测限为0.079 g/L。考察了无血清饥饿培养对血管平滑肌细胞分泌骨桥蛋白的影响,结果表明无血清饥饿培养24 h骨桥蛋白的合成与分泌达到高峰,此后随时间延长含量随之降低,此结果与采用Western blot方法检测的结果一致。该方法具有进样量小(nL级)、检测速度快、可自动化等优点,是一种简便、快捷的检测微量蛋白质的好方法。

关键词 毛细管电泳 聚乙二醇20000 无胶筛分 骨桥蛋白 血管平滑肌细胞

分类号

Determination of Osteopontin at Trace Levels by Non-Gel Sieving Capillary Electrophoresis

Abstract

A method of non-gel sieving capillary electrophoresis (NGSCE) was established to determine osteopontin at trace levels. The capillary used was uncoated fused silica with a size of 57 cm×75 μm i.d. and an effective length of 50 cm. The electrode buffer was a 150 mmol/L boric acid-borate buffer containing 30 g/L polyethylene glycol 20000 (pH 10.0). Other conditions were as follows: separation voltage 23 kV; detection wavelength 214 nm; pressure of injecting sample 3.4 kPa (0.5 psi)×5 s; and column temperature 25 ℃. The NGSCE method had excellent linearity with correlation coefficient of 0.996, and reproducibility with the relative standard deviation of migration time of osteopontin less than 5%. The recovery was 95% and better, the sensitivity was 0.079 g/L. Osteopontin secreted by vascular smooth muscle cells was determined by the NGSCE method at different times after serum withdrawal, and the results were in agreement with those of Western blot method. The results indicate that NGSCE is a simple and rapid method of determining osteopontin at trace levels. This method only needs a micro-amount of sample and is easily automated.

Key words capillary electrophoresis polyethylene glycol 20000 non-gel sieving osteopontin vascular smooth muscle cell

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