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研究论文

基于适配体亲和毛细管电泳-激光诱导荧光检测的凝血酶分析

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摘要 以一种高亲和力适配体作为亲和荧光探针, 以自建的毛细管电泳-激光诱导荧光(CE-LIF)检测装置为基础, 建立了一种高灵敏、快速测定人凝血酶的方法。荧光标记的凝血酶适配体特异性地与凝血酶结合并形成稳定的凝血酶-适配体复合物, 采用CE-LIF对复合物进行分离检测, 从而测定凝血酶浓度。探讨了盐离子种类及浓度对适配体与凝血酶结合的影响, 并在选定的电泳条件下对凝血酶检测的线性范围、检出限和重现性进行了测定。结果表明, 盐离子存在的条件下适配体与凝血酶的亲和力降低, 不利于两者的结合; 人血清溶液中, 凝血酶浓度在0.25～10 nmol/L范围内与复合物峰面积具有良好的线性相关性(r20.991), 检出限(S/N3)为55.6 pmol/L; 精密度和回收率测定结果均能满足分析的要求。

关键词 亲和毛细管电泳 激光诱导荧光检测 凝血酶 适配体 血清

Determination of human thrombin by an aptamer based on affinity capillary electrophoresis-laser induced fluorescence detection

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Abstract

The method for the determination of human thrombin by an aptamer was developed based on capillary electrophoresis (CE) with laser induced fluorescence (LIF) detection. The concentration of thrombin was calculated through the peak area of the thrombin-aptamer complex, which was separated and detected by CE-LIF. Because of the binding favorable G-quartet conformation potentially involved in the specific aptamer, it was assumed that monovalent and bivalent cations promoting the formation of a stable G quadruplex conformation in the aptamer may enhance the binding of the aptamer and thrombin. Therefore, the effects of various metal cations on the binding of human thrombin and the aptamer were investigated. The results showed that the cations like K+ and Mg2+ could not stabilize the affinity complex. The linear range, detection limit and reproducibility were measured. The linear range was 0.25~10 nmol/L (r20.991), and the detection limit of thrombin was 55.6 pmol/L. Regarding the advantages of high efficiency and rapid separation, low sample consumption, and high sensitivity, CE-LIF is a potential and powerful alternative to conventional immunoaffinity assays in clinical diagnostics.

Key words affinity capillary electrophoresis laser induced fluorescence (LIF) detection thrombin aptamer serum

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