

研究论文

二氢叶酸还原酶抑制剂的高效毛细管电泳法筛选模型的建立与应用

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摘要 建立了一种采用毛细管电泳法(CE)测定二氢叶酸还原酶(DHFR)反应动力学参数的新方法。以含0.002% Brij-35的50 mmol/L的硼砂缓冲溶液(pH 9.18)作为电泳介质,检测波长280 nm,于19 min内实现了体系中各组分的基线分离。根据酶反应过程中反应物和反应产物的浓度变化计算有关反应动力学参数。将已知的氨甲喋呤、甲氧苄啶和叶酸3种抑制剂作用于所建立的二氢叶酸还原酶体系,测得抑制剂的半数抑制浓度与文献值相接近,证明本体系可用于二氢叶酸还原酶抑制剂(DHFRi)的筛选。

关键词 [高效毛细管电泳法](#) [二氢叶酸还原酶](#) [抑制剂](#)

分类号

Development and Application of Screening Method for Steroid Dihydrofolate Reductase Inhibitors by High Performance Capillary Electrophoresis

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Abstract

A novel screening method for dihydrofolate reductase inhibitors (DHFRi) using high performance capillary electrophoresis (HPCE) was developed. The separation was performed in a fused silica capillary column using disodium tetraborate (50 mmol/L, pH 9.18) buffer solution with 0.002% Brij-35. The separation temperature was controlled at 25 °C and a voltage of 25 kV was applied. The detection wavelength was 280 nm. Five µL of various concentrations of inhibitor was added to the enzyme reaction system consisting of 25 µL of 50 mmol/L pH 6.0 potassium phosphate buffer, 10 µL of 0.025 mg/mL dihydrofolate (FH2), 10 µL of dihydrofolate reductase (DHFR) (0.25 unit) and 5 µL of 0.5 mg/mL NADPH in a 300-µL sample tube, then mixed for 1 min and incubated for 30 min at room temperature. The reaction mixture injections were performed by pressure at 3.45 kPa (0.5 psi) for 10 s. With the developed CE method the substrate and product were baseline resolved within 19 min. The time course of the reaction was studied to show excellent correlation. Quantitative analysis of DHFR inhibition was performed by determining its dynamic parameters. The difference of peak areas between FH2 and FH4 was used to calculate the inhibitory rate. Three inhibitors, amethopterin, trimethoprim and folic acid, were used in the enzyme reaction system to test this method and their IC50 values determined were close to the literature values. It was demonstrated that the developed method is suitable for screening DHFRi.

Key words [high performance capillary electrophoresis](#) [dihydrofolate reductase inhibitor](#)

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