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研究论文

天冬酰胺合成酶B抑制剂高效液相色谱筛选方法的建立与应用

张继1, 于丹1, 向文胜1, 范志金2, 王相晶1*

1.东北农业大学生命科学学院, 黑龙江 哈尔滨 150030; 2.南开大学元素有机化学研究所, 天津 300071

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摘要 建立了一种快速、高效测定天冬酰胺合成酶B(AS-B)酶活性的反相高效液相色谱法(RP-HPLC)。酶反应体系中的氨基酸经2,4-二硝基氟苯(DNFB)柱前衍生,通过RP-HPLC测定酶反应体系前后底物及产物的变化来分析酶的活性。采用的色谱柱为Agilent C18柱(250 mm×4.6 mm,5 μm),以50 mmol/L醋酸钠缓冲液(pH 6.2)-乙腈(体积比为15:85)为流动相,流速为1.0 mL/min,柱温为30 ℃,检测波长365 nm,于6 min内实现了各组分的基线分离。通过该方法测定反应动力学参数进行AS-B的抑制定量分析。将已知AS-B抑制剂L-谷氨酸-γ-甲酯作用于酶反应体系,测得的抑制剂的抑制常数与文献值相接近,证明该方法可用于AS-B抑制剂的筛选。

关键词 高效液相色谱法 天冬酰胺合成酶B 抑制剂 筛选

Development and application of screening method for asparagine synthetase B inhibitors by high performance liquid chromatography

ZHANG Ji1, YU Dan1, XIANG Wensheng1, FAN Zhijin2, WANG Xiangjing1*

1.Department of Life Science, Northeast Agricultural University, Harbin 150030, China; 2.Research Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China

Abstract

A screening method for asparagine synthetase B (AS-B) inhibitors by reversed-phase high performance liquid chromatography (RP-HPLC) has been established. The contents of asparagines produced in the reaction system can be analyzed by HPLC after the derivatization with 1-fluoro-2,4-dinitrobenzene (DNFB) and used to calculate the total activity of AS-B. The sample was separated on an Agilent C18 column (250 mm×4.6 mm, 5 μm) at the temperature of 30 ℃ with the elution of 50 mmol/L sodium acetate buffer (pH 6.2)-acetonitrile (15:85, v/v) as mobile phase at a flow rate of 1.0 mL/min. The detection wavelength was set at 365 nm. The enzyme reaction system consisted of 100 mmol/L Tris（tris(hydroxymethyl)aminomehane）-HCl buffer (pH 8.0), 100 mmol/L NaCl, 10 mmol/L MgCl2, 5 mmol/L adenosine triphosphate (ATP), 10 mmol/L L-aspartate, 10 mmol/L L-glutamine and 2 μg recombinant soybean AS-B (1 mL of the total volume), then mixed for 1 min and incubated for 15 min at 37 ℃. After quenching with ethanol and centrifugation, the supernatant was derivatized by DNFB and then separated by HPLC. The amino acids in the reaction system were baseline separated within 6 min. The quantitative analysis of AS-B inhibition was performed by determining its dynamic parameters. The inhibitor L-glutamic acid γ-methyl ester was used in the enzyme reaction system to test this method and its inhibition constant obtained was close to the literature value. The established method is fast, accurate, sensitive and suitable for high throughput screening AS-B inhibitors.

Key words high performance liquid chromatography (HPLC) asparagine synthetase inhibitor screening

DOI:

通讯作者 王相晶 wangxiangjing2008@yahoo.com.cn.

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