

一种基于二喹啉甲酸- Cu⁺显色反应的毛细管电泳检测蛋白质的新方法

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A novel capillary electrophoretic method for protein determination using bicinchoninic acid-Cu⁺ colorimetric reaction

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摘要

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摘要 采用毛细管电泳法和蛋白质显色反应-二喹啉甲酸(BCA)法,结合微波辅助反应,在60 mmol/L硼酸盐缓冲液(pH 9.5)中,实现了快速毛细管电泳分析检测。同时以 β -环糊精为包合添加剂,实现了BCA-Cu⁺复合物和游离BCA的分离,从而在波长200 nm处以测定特征吸收峰,通过BCA-Cu⁺复合物来间接检测蛋白质,其峰强度比直接检测蛋白质自身吸收的峰强度提高了2个数量级。对于转铁蛋白、蓖麻毒素,其线性范围为2~200 mg/L和2~100 mg/L,检出限分别为0.33和0.37 mg/L。将该方法成功地应用于第一届蓖麻毒素国际实验室间比对测试,含量测定结果满意。

关键词: 毛细管电泳 二喹啉甲酸 亚铜离子 显色反应 β -环糊精 蛋白质

Abstract: A new rapid, sensitive method for protein determination using capillary electrophoresis and specific colorimetric reaction of bicinchoninic acid (BCA) was established, assisted by microwave incubation. With 60 mmol/L borate buffer (pH 9.5) and inclusion additive of β -cyclodextrin, the complex of BCA-Cu⁺ and free BCA molecules were efficiently separated. The peak intensity of BCA-Cu⁺ was higher than those of native proteins about two order magnitude at a low wavelength of 200 nm. The linear ranges of this method were from 2 to 200 mg/L for transferrin and 2 to 100 mg/L for ricin. The limits of detection for transferrin and ricin were 0.33 and 0.37 mg/L, respectively. This method was also successfully applied in the determination of some ricin samples in the First International Proficiency Test. The results of content determination were satisfactory.

Keywords: capillary electrophoresis (CE) bicinchoninic acid (BCA) Cu⁺ colorimetric reaction β -cyclodextrin

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