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研究论文

3-氨基-9-乙基咔唑衍生化寡糖混合物的高效液相色谱分离及激光解吸电离飞行时间质谱分析

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摘要 建立了以3-氨基-9-乙基咔唑(AEC)为衍生化试剂对寡糖的标记方法。寡糖的还原端与AEC的伯氨基反应生成烯胺,再被NaBH3CN还原为二级胺,使得寡糖被AEC标记。衍生物通过反相高效液相色谱分离纯化,采用的色谱柱为Waters Symmetry C18柱(3.9 mm×150 mm,5 μm),乙腈和乙酸铵水溶液(pH 4.5)为流动相,梯度洗脱,在254 nm波长处检测,并以基质辅助激光解吸电离飞行时间质谱进行分析。在此衍生化条件和色谱条件下,葡寡糖衍生物分离良好,并且AEC衍生可显著提高葡寡糖的质谱检测灵敏度。该方法适用于寡糖的分离纯化和结构分析,并与生物质谱具有良好的兼容性,表明该方法在微量寡糖链分析方面有广阔的应用前景。

关键词 高效液相色谱(HPLC) 基质辅助激光解吸电离飞行时间质谱 3-氨基-9-乙基咔唑; 寡糖

Separation and identification of oligosaccharides labeled with 3-amino-9-ethylcarbazole using high performance liquid chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract

A pre-column derivatization method for the determination of oligosaccharides based on a labeling reagent 3-amino-9-ethylcarbazole (AEC) was proposed. The enamines were generated by the reaction of the reducing ends of oligosaccharides and the primary amines of AEC, and then reduced to secondary amines by NaBH3CN, making oligosaccharides labeled by AEC. The derivatives were separated by reversed-phase high performance liquid chromatography （RP-HPLC）, and then directly analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The HPLC separation was carried out on a Waters Symmetry C18 column (3.9 mm×150 mm, 5 μm) with a gradient elution (acetonitrile and ammonium acetate as mobile phases at a flow rate of 1 mL/min) and ultraviolet detection at 254 nm. Under the optimized derivatization and HPLC conditions, the derivatized oligosaccharides were separated, and the derivatization with AEC increased the sensitivity of MS detection. The developed method for the analysis of oligosaccharides is satisfactory.

Key words high performance liquid chromatography (HPLC) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry （MALDI-TOF-MS） 3-amino-9-ethylcarbazole oligosaccharides

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