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研究论文

对氨基苯甲酸衍生化高效液相色谱法分析多糖中的单糖及糖醛酸组成

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摘要 建立了一种柱前对氨基苯甲酸(p-AMBA)衍生反相离子对色谱法同时分离检测多糖中单糖及糖醛酸组成的方法,筛选出适合于p-AMBA糖衍生物分离的色谱柱,考察了流动相组成对9种单糖和两种糖醛酸的p-AMBA衍生化产物的保留值及分离的影响,优化了反应温度和反应时间等衍生化条件,并应用优化的分析方法测定了螺旋藻中的单糖和糖醛酸的组成。采用紫外检测时,方法的检出限为 (2.55～13.4)×107mol/L;采用荧光检测时,方法的检出限为(3.38～176)×108 mol/L。

关键词

分类号

Analysis of Monosaccharides and Uronic Acids in Polysaccharides by Pre-column Derivatization with p-Aminobenzoic Acid and High Performance Liquid Chromatography

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Abstract

An ion-pair reversed-phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of carbohydrate and uronic acids was developed. p-Aminobenzoic acid (p-AMBA) was used for pre-column derivatization of the analytes, enabling fluorescence (λex=313 nm, λem=358 nm) or ultraviolet (UV at 303 nm) detection. Reaction conditions such as reaction temperature and reaction time were optimized. Atlantis dC18 column with hydrophilic end capping was selected for the separation of derivatives. Effects of mobile phase compositions such as ion pairs and their concentrations and pH on the retention behaviors and separation results of 9 monosaccharides and 2 uronic acids were investigated. Derivatives of fructose, galactose, glucose, mannose, xylose, arabinose, ribose, galacturonic acid, fucose, glucuronic acid and rhamnose were separated within 42 min, applying tetrabutyl ammonium hydrogen bisulfate (TBAHSO4) as the ion pair reagent. The detection limits were between 3.38×108 mol/L and 176×108 mol/L for fluorescence detection and between 2.55×107 mol/L and 13.4×107mol/L for UV detection. Good linearities were obtained with correlation coefficients (r2) above 0.99. The relative standard deviations (RSDs) of the peak area of the derivatives in 1251 h after derivatization were from 2.5% to 3.9%. This method has been applied for the determination of mono-/disaccharides and uronic acids in spirulina polysaccharide after dissolved in trifluoroacetic acid solution (2 mol/L). The results showed this method is suitable for the analysis of monosaccharide compositions in polysaccharides.

Key words polysaccharides, Monosaccharide composition, uronic acid ,pre－column derivatization , high performance liquid chromatography,p-Aminobenzoic acid

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