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论文

用高效液相电化学检测直接测定羟自由基

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摘要:

应用DMPO捕捉羟自由基(-OH)生成DMPO—羟游离基(DMPO-OH),经高效液相电化学检测羟自由基。选用ODS反相柱(10 μm)及柠檬酸30 mmol/L—乙酸钠50 mmol/L—3%乙腈为流动相,流速1-2 ml/min,检测电压0.6 V。采用EDTA-H₂O₂-Fe²⁺(FeSO₄ 300 nmol/L, EDTA 300 μmol/L, H₂O₂ 180 μmol/L及DMPO 2 mmol/L和H₂O₂光照(H₂O₂ 18 mmol/L, DMPO 2 mmol/L光照6 min)两种产生-OH的体系作为药物筛选及作用机制探讨。其RSD分别为6.1和8.0%。检测灵敏度和ESR相似,本文介绍了O₂⁻的检测方法。

关键词: 羟自由基 高效液相电化学检测器 DMPO Penton's反应 过氧化氢 超氧阴离子

DIRECT MEASUREMENT OF OXY-RADICALS USING HPLC-ECD

Y Liu and JT Zhang

Abstract:

A direct, sensitive, simple and specific high-pressure liquid chromatographic (HPLC) method was used for the quantitation of hydroxyl radicals by means of oxy-radical trapping of DMPO to form DMPO-OH adducts. The DMPO-OH adduct peak was separated successfully and identified by HPLC-ECD with a Waters ODS reversedphase 10 μm column. The mobile phase composed of citric acid/sodium acetate (citric acid—30 mmol/L—sodium acetate 50 mmol/L—3% acetonitrile, pH 5. 1), at a flow rate of 1.2 ml/min and detection potential of 0.6 V with Ag/AgCl as reference electrode. Both EDTA—Fe²⁺—H₂O₂ (FeSO₄ 300 μmol/L, EDTA 300 μmol/L, H₂O₂ 180 μmol/L and DMPO 2 mmol/L) and H₂O₂ photolysis (H₂O₂ 18 mmol/L and DMPO 2 mmol/L photolysis for 6 min) systems were taken to produce hydroxyl free radicals for screening new drugs and studyins the mechanism of action. The relative standard deviations were 6.1 and 8.0% respectively. The sensitivity of the method was shown to be similar to that of ESR. The method for detection of superoxide anions with HPLC-ECD was also described.

Keywords: Hydroxyl radical 5,5- dimethylpyrroline-1-oxide (DMPO) EDTAH₂O₂-Fe²⁺ H₂O₂-UV O₂⁻ HPLC- ECD

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