药学学报 2007, 42(5) 529-533 DOI: ISSN: CN:

论文

HPLC-ESI-ITMS在药品质量控制中确证胰岛素和胰岛素B链C端氨基酸序列的应用研究

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摘要：

在药品质量控制中应用HPLC-ESI-IT (Ion Trap) MS分别确证胰岛素和胰岛素B链的C端氨基酸序列。取胰岛素标准品溶液或者胰岛素B链溶液适量，加入羧肽酶P及羧肽酶Y溶液适量降解胰岛素或者胰岛素B链C端，在预定的时间点取出酶降解反应液适量，加入等体积1%甲酸停止反应，混旋均匀后制成供试液，供HPLC-ESI-IT MS分析测定。采用Zorbax Prosphere C_{18}柱(2.1 mm×150 mm，300A，5 μm)分离，以流动相进行梯度洗脱［A相：水-乙腈-三氟乙酸(98∶2∶0.02)，B相：乙腈-水-三氟乙酸(98∶2∶0.02)］。柱后修饰“TFAfix”溶液为丙酸-异丙醇(2∶8)的混合溶液。ESI-ITMS测定供试液中梯度多肽系列(彼此相差1个C端氨基酸残基)的分子质量，计算出分子质量最相近的相邻两肽段的系列分子质量差值，即可得到待测样品C端系列氨基酸残基质量的实验值。胰岛素的B链C端的第1个氨基酸残基丙氨酸残基可以被准确地确证；同样还原修饰的胰岛素B链C端的第1个氨基酸残基丙氨酸残基也能被准确地确证。本研究在nmol样品水平准确地确证胰岛素和胰岛素B链各一个C端氨基酸，符合重组DNA制品中试产品的质量控制要求。本实验方法不必将胰岛素经还原修饰将A链从B链裂解，直接确证胰岛素的C端氨基酸残基，避免还原修饰和纯化的烦琐操作，方法简便。

关键词： HPLC-ESI-ITMS 蛋白质C端测序 羧肽酶P和羧肽酶Y 胰岛素

Application of HPLC-ESI-ITMS in the quality control of carboxyterminal sequence confirmation for insulin and insulin chain B

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Abstract:

Application of HPLC-ESI-ITMS in the quality control of carboxyterminal sequence confirmation for insulin and insulin chain B was studied. The solution of intact insulin or insulin chain B was added to the solution of carboxypeptidase P (CPP) and carboxypeptidase Y (CPY). Fractions of appropriate volume were removed at some appointed time points, acidified with the same amount of 1% formic acid to stop the digestion, and then briefly vortexed for HPLC-ESI-ITMS analysis. Mobile phase A consisted of 0.02% TFA in 98% ultra-pure water and 2% acetonitrile. Mobile phase B consisted of 0.02% TFA in 98% acetonitrile and 2% ultra-pure water. The solution used for post-column fix consisted of propionic acid and isopropyl alcohol (20∶80, *v*/*v*). Chromatographic separation was carried out on a reversed-phase column (Zorbax Prosphere C_{18}, 300A, 5 μm, 2.1 mm ID×150 mm length). The molecular weights of the multiply charged ions representing consecutive truncated losses of carboxyterminal amino acids were determined by the use of HPLC-ESI-ITMS. The differences between the consecutive truncated peptides are the experimental weights of the carboxyterminal amino acid residues. The carboxyterminal amino acid residue Ala, which released from chain B of intact insulin, was confirmed in the nanomolar concentration range by analyzing the molecular weight of the truncated peptides. Another one carboxyterminal amino acid Ala was confirmed in the nanomolar concentration range of insulin chain B. In the quality control for recombinant DNA product or natural protein, the confirmation of 1-3 carboxyterminal amino acid residues is regarded to be up to standard. One amino acid residue of insulin or insulin chain B could be confirmed accurately in the nanomolar concentration range. The results showed that intact insulin could be directly sequenced in the quality control without separating chain B from chain A. There would be no need to separate chain A from chain B to identify carboxyterminal of intact insulin. Furthermore, the method saved us a lot of trouble from the preparation and purification of insulin chain A and chain B.

Keywords: carboxyterminal sequence confirmation CPP and CPY insulin HPLC-ESI-ITMS

收稿日期 2006-11-14 修回日期 网络版发布日期

DOI:

基金项目:

通讯作者: 吴梧桐

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参考文献：

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