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论文

高效液相色谱法与荧光光度法检测中药材中黄曲霉毒素的比较

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摘要：

目的比较研究了免疫亲合柱（IAC）净化-HPLC柱后溴化衍生荧光法检测黄曲霉毒素B_1，B_2，G_1和G_2的含量与溴化荧光光度法（SFB法）检测黄曲霉毒素总量的方法。方法在IAC-HPLC柱后溴衍生荧光法中，药材经甲醇-水（70∶30）溶剂系统提取后，采用免疫亲合柱净化、富集黄曲霉毒素，净化后的样品溶液通过高效液相色谱柱分离后，进入柱后衍生管中与过溴化溴化吡啶溶液发生反应，最后进入荧光检测器分别检测黄曲霉毒素B_1，B_2，G_1和G_2含量。在SFB法中，样品经甲醇-水（70∶30）提取后，再经免疫亲合柱净化、富集，在处理后的样品溶液中添加一定量一定浓度的溴水溶液后，迅速置于荧光光度计中读数。结果IAC-HPLC柱后溴衍生荧光法中，考察了3种不同药材中添加2个浓度水平的混合对照品的回收率实验，回收率平均值在93%-97%之间。黄曲霉毒素B_2和G_2的最低检出限为0.06 μg·kg^{-1}，黄曲霉毒素B_1和G_1的最低检出限为0.20 μg·kg^{-1}。精密度实验的RSD值在0.8%-1.4%之间。运用此高效液相色谱法检测了39种中药中黄曲霉毒素的含量。同时运用SFB法检测了上述39种中药中黄曲霉毒素的总量。结论SFB法不适合用来检测中药中黄曲霉毒素的总量。

关键词： 黄曲霉毒素 高效液相色谱法 荧光光度法 免疫亲合柱

Comparison between the post-column derivatization with bromine by HPLC and the fluorometric analysis for determination of aflatoxins in medicinal herbs and plant extracts

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Abstract:

AimTo compare the post-column derivatization technique (IAC-PCD-HPLC) for the determination of aflatoxins B_1, B_2, G_1 and G_2 and the rapid procedure with fluorometric analysis (SFB) for the determination of total aflatoxins. MethodsThe method of post-column derivatization with bromine by HPLC consisted of extraction of the sample with MeOH-H_2O (70∶30) followed by clean-up of the extracts with immunoaffinity columns and finally, HPLC determination with fluorescence detection. Aflatoxins B_1 and G_1 were determined as their bromine derivatives, produced in an on-line post-column derivatization system. In SFB method, samples were ground and extracted with methanol-water (70∶30). A portion of the extract was cleaned up by passage through a immunoaffinity column, One mL of purified extract was derivatized with a bromine reagent, and fluorescence of the solution was immediately quantified with a calibrated fluorometer containing a broad wavelength pulsed xenon light source. ResultsIn IAC-HPLC method, the overall average recoveries for three different medicinal herbs spiked at levels of 1.3 and 2.6 ng·g^{-1} of total aflatoxins ranged from 93% to 97%. The detection limit was 0.06 μg·kg^{-1} for both G_2 and B_2 and 0.20 μg·kg^{-1} for both G_1 and B_1, based on a signal/noise 3∶1 and the precision (within-laboratory relative standard deviation) ranged from 0.8% to 1.4%. Each of aflatoxins B_1, B_2, G_1 and G_2 in 39 kind medicinal materials were determined by IAC-PCD-HPLC, and the total aflatoxins were determined by SFB. ConclusionThe SFB method is not the suitable method for the determination of total aflatoxins in medicinal herbs and plant extracts.

Keywords: HPLC fluorometry immunoaffinity column aflatoxins

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