タンパク質固定ODSカラムの特性と血漿試料の直接注入による薬物定量への応用

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Some Characteristics of Protein-coated ODS Column and Its Use for the Determination of Drugs by the Direct Injection Analysis of Plasma Samples

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ABSTRACT It was found that ODS column of small pore (~15nm) which was coated with denatured plasma proteins (protein-coated ODS) no longer adsorbed plasma proteins from an aqueous solution, but still held the characteristics of native ODS for small hydrophobic molecules. Elemental analysis and nitrogen desorption (BET) analysis showed that the protein-coated ODS contained ca. 25mg proteins/g dry packing and that the pore diameter or pore volume was similar to that of native ODS. Capacity factors for small molecules were also similar to that of native ODS, but theoretical plate numbers were reduced by 20–60%. The coated denatured proteins, which were seemed to be adsorbed on the outside surface of the porous packings were not eluted with usual reverse phase elution condition. As either an analytical column or a pre-column, this (protein-coated ODS) column was used to analyze spiked-drugs in plasma. Plasma sample was directly injected onto the column and the plasma proteins and hydrophilic components were eluted out with aqueous solution near the void volume, then, the drugs were eluted by reverse phase mode. The spiked-drugs were procainamide, propranolol, doxorubicin, methotrexate etc. The recovery of all the tested drugs was almost quantitative (98–102%) with good reproducibilities (c.v., less than 2%). The present method was useful for the determination of total (=free + bound to plasma proteins) hydrophobic drugs in view of accuracy and simplicity.

抄録　小孔径ODSカラムを変性血漿タンパク質でコートすると，タンパク質の吸着能は無くなるが脂溶性低分子に対するODSの特性は依然として保持されることを見出した。元素分析とBET分析の結果，タンパク質固定ODSは約25 mgタンパク質／g乾燥量を含み，孔径分布は元のODSカラムと余り変らなかった。低分子物質に対するk'値も元と変らず，ただ理論段数が20～60

%減少した。タンパク質はODS樹脂の外表面に吸着すると考えられ，通常の逆相系溶離条件では溶出しない。これを分析カラムあるいはプレカラムとして血漿中に添加した薬物の定量に適用した。血漿試料を直接注入すると血漿タンパクと親水性成分はほぼ排除体積の水溶液として溶出し，次いで薬物が逆相モードで溶離した。用いた薬物はプロカインアミド，プロプラノロール，ドキソルビシン，メトトレキセートなどであった。回収率は定量的（98～102%）であり，再現性は2%以下と良好であった。本法は薬物の遊離型とタンパク結合型両者の合計量の定量に有用である。

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