血清試料の直接注入を用いる薬物の高速液体クロマトグラフィー分析のための新除タンパク法

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BUNSEKI KAGAKU, 35, 335-337(1986).

A new deproteinization method for high performance liquid chromatographic analysis of drugs with direct serum sample injection.

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ABSTRACT: Butyl Toyopearl (BT) 650-M(Toyo Soda Co.), a polyvinyl resin with butyl and hydroxyl surface groups, of which the former exhibits hydrophobic interaction with proteins, was used to deproteinize the serum samples for the direct injection HPLC analysis of drugs. It was found that 0.05～0.75% trichloroacetic acid, 0.05～1.0% of trifluoroacetic acid or 0.025～0.5% perchloric acid (PCA) was effective for the adsorption of BSA on BT 650-M as well as conventional 20 ～50% ammonium sulfate solution. In 0.5% PCA solution the quantity of BSA adsorbed was about 40 mg/ml wet gel. For eluting BSA by the reversible desorption, the salting-in (optimum at about 0.2 M phosphate solution, pH 7.0), solubility increase in methanol (tested up to 30%) and hydrogen bond breaking by 5 M urea increased the peak height by 15, 4 and 2.5 times, respectively, than the case of elution by water. It seemed that the hydrogen bonding also functioned for the adsorption. The method was applied to the HPLC determination of 6-mercaptopurine (6-MP), theophylline (TP) and chlorpromazine (CPZ) spiked in rabbit serum at the therapeutic concentration. An excellent reproducibility (R.S.D.:1.8% for 6-MP, 0.78% for TP and 2.1% for CPZ) and good recovery (99.7% for 6-MP, 100.0% for TP and 99.2% for CPZ), regardless of their binding ratio to serum proteins, were ascertained. The use of column switching for troika column system, a BT 650-M precolumn, a minicolumn which trapped analytes and an analytical column, was recommended in order to protect BT 650-M which was fragile at high pressure.

抄録　タンパク質に対して疎水性相互作用を示すブチル基と水酸基を表面グループとするポリビニール樹脂ブチルトヨパール（BT）650-Mを薬物のHPLC分析における血漿試料の直

接注入除蛋白に用いた。0.05–0.75%トリクロロ酢酸，0.05–1.0%トリフルオロ酢酸あるいは0.025–0.5%過塩素酸（PCA）が従来の20–50%飽和硫酸アンモニウムと同様にBSAのBT 650–Mへの吸着に有効であることが見出された。0.5% PCA溶液中で吸着されるBSA量は40mg/mℓ湿潤樹脂であった。可逆的脱着によりBSAを溶離するのに塩溶効果（リン酸塩溶液で最適0.2M)、メタノールへの可溶性（30%まで検討）水素結合切断のための5M尿素で溶離ピークの高さは水溶離に比しそれぞれ15，4，2.5倍となった。本法を血漿中のメルカプトプリン，テオフィリン，クロルプロマジンの除蛋白分析に用い好結果を得た。高圧に弱いBT 650–Mを保護するため分析成分の捕捉のためのミニカウムを用いた，プレカラムーミニカラムー分析カラムの3カラム方式が提案されている。

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