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一次性膜分离灵芝多糖肽和灵芝酸对照品方法的研究

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Abstract：目的 灵芝化学成分中多糖(肽)类和三萜类是重要生物活性物质，也是灵芝及其产品质量控制的两项指标。作者针对灵芝化学成分复杂体系，采用膜超滤法从灵芝提取液中一次性分离灵芝多糖肽和灵芝酸对照品。方法 采用切向流超滤膜分离 10KD 截留液和透过液，1)10KD 截留液经浓缩、醇沉，再经高效凝胶制备色谱纯化得到灵芝多糖肽对照品。色谱柱为 TSK G4000PW，流动相为水，检测波长 280nm。2)10KD 透过液经浓缩得析出物，采用全息快速纯化色谱仪纯化制备得到灵芝酸纯品。色谱柱为 XB-C_{18}柱，流动相为 0.05%磷酸：乙腈(65∶35)，检测波长 254nm。结果 1)灵芝提取液 10KD 截留液纯化制备得到的灵芝多糖肽对照品纯度 97%以上，得率为原料 0.49%，其峰位分子量(Mp)5.0×10^4Da。GL-$PPSQ_2$结构重复单元为主链由→3)-β-D-Glc *p*-(1→构成，每 4 个→3)-β-D-Glc *p*-(1→在 *O*-6 位连接一个长支链，该支链由α-D-Glc *p*-(1→、→4,6)-β-D-Glc *p*-(1→、→4)-β-D-Glc *p*-(1→和→6)-β-D-Glc *p*-(1→依次相连构成。2)10KD 透过液浓缩析出物经纯化制备得到 3 个化合物纯度均达 97%以上，总得率为浓缩物的 4.5～5.5%。经中国医学科学院北京协和医学院药物研究所鉴定，3 个化合物分别为灵芝酸 A、B 和 C_2。结论　采用膜分离技术，一次性分离灵芝多糖肽和灵芝酸对照品，可应用于药理研究和检验用的对照品。与传统分离、提取相比，膜分离快速，简化了醇提取工艺步骤，既保护生态环境，又提高了经济效益。

Study on Method of Rapid Membrane Separation *Ganoderma lucidum* Polysaccharide Peptide and Ganoderic Acids Reference Substance

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Abstract: Objective The polysaccharides (peptides) and triterpenoids are important bioactive substances in the chemical constituents of *Ganoderma lucidum* (*G. lucidum*), and are also two indicators for the quality control of *G. lucidum* and its products. The authors separated *G. lucidum* polysaccharide peptide (GL-$PPSQ_2$) and the Ganoderic acid reference substance from *G. lucidum* extract by membrane ultrafiltration. Methods 10KD retentate and permeate were separated by tangential flow ultrafiltration membrane. 1) 10KD retentate was concentrated, alcohol precipitated, and purified by high performance gel chromatography to obtain GL-$PPSQ_2$. The Chromatographic column was TSK G4000PW with water as mobile phase, and the detection wavelength was 280 nm. 2) The 10KD permeate was concentrated to obtain a precipitate, and purified by a holographic rapid purification chromatograph to obtain pure ganoderic acids. The chromatographic column was XB-C18 column with 0.05% phosphoric acid acetonitrile (65:35) as mobile phase, and the detection wavelength was 254 nm. Results 1) The purity of GL-$PPSQ_2$ from the extract of *G.*

lucidum was over 97%, the yield was 0.49% of the drug, and its peak molecular weight (Mp) was 5.0×10^4 Da. The GL-PPSQ$_2$ structural repeat unit was composed of →3)-β-D-Glc *p*-(1→, every four→3)-β-D-Glc *p*-(1→connected at *O*-6 long-chain branch, which was composed of α-D-Glc *p*-(1→,→4,6)-β-D-Glc *p*-(1→,→4)-β-D-Glc *p*-(1→ And →6)-β-D-Glc *p*-(1→ successively connected. 2) 10KD permeate concentrated precipitates were purified to obtain three compounds with a purity of over 97%, and the total yield was 4.5～5.5%. The three compounds were identified as ganoderic acid A, B and C_2 by the Institute of Materia Medica, Beijing Union Medical College, Chinese Academy of Medical Sciences. Conclusion Membrane separation technique is used to separate *G. lucidum* polysaccharide peptide and Ganoderic acid at one time, which can be applied to offer the reference substance for pharmacological research and testing. Compared with the traditional separation and extraction, the membrane separation is fast, and the alcohol extraction process step is simplified, thereby protecting the ecological environment and improving the economic benefit.