ANALYSIS OF THE RADIOCHEMICAL PURITY OF ^{99m}Tc-LABELED HUMAN SERUM ALBUMIN BY SIZE-EXCLUSION CHROMATOGRAPHY AND THIN LAYER CHROMATOGRAPHY.

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ABSTRACT

Albumin is a water-soluble polypeptide with a molecular mass of about 68,000 daltons. Human Serum Albumin (HSA) is the major component of plasma protein in healthy adults at concentration 35-50 mg L^{-1}. The function of the albumin is to keep colloid osmotic pressure to the capillary membrane, which prevents plasma loss and maintains the plasma volume. ^{99m}Tc-HSA is an important radiopharmaceutical clinically applied for cardiac function tests or assessment of protein-losing gastroenteropathies. Serum albumin reacts with ^{99m}Tc (in +5 oxidation state) to form ^{99m}Tc-HSA. It is recommended at least 90% radiochemical purity (RCP) of ^{99m}Tc-HSA for clinical use. The present study reports the comparison between two chromatography methods for purity determination of ^{99m}Tc-HSA: size-exclusion chromatography (SEC) and thin layer chromatography (TLC), 30 minutes and 4 hours after reconstitution of the HSA lyophilized reagent with sodium pertechnetate ($Na^{99m}TcO_4$). HSA lyophilized kit and ^{99}Mo/^{99m}Tc generator were from IPEN-CNEN/SP, reagents were from Merck and water was purified in a Milli-RX system from Millipore. A vial containing HSA was reconstituted by the addition of 3 mL eluate containing 166.5 MBq $Na^{99m}TcO_4$. TLC analysis using a silica gel coated glass-fiber sheet (5x20 cm) as adsorbent was performed using 5 µL sample and methanol: water (85:15; v/v) as solvent. The plate was cut in 1 cm strips and the radioactivity measured by a gamma counter with NaI detector. Free pertechnetate has R_f 1.0 and ^{99m}Tc-HSA and TcO_2 remain at the origin. SEC analysis was performed in a HPLC system (LC 20AT Prominence) (Shimadzu) composed by two pumps, auto sampler (SIL 20A), system controller (CBM 20A), a Protein-Pack 300SW column (300 mm x 7.5 mm i.d., 10 µm particle size), diode array (SPD M20A) and a gamma radiation detector (Bioscan). A 200 µL sample volume (^{99m}Tc-HSA and concentrated mobile phase, 1:1, v/v) was injected and the mobile phase disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride and sodium azide, 4:1:1:0.1, m/m) was isocratically eluted, at 0.6 mL min^{-1} flow rate, continuously monitored by the radiation detector. The samples were analyzed in duplicate in the two methods. SEC analyses showed a labeling average efficiency of (97,28 ± 0.09)% and peaks were eluted with retention times 8.71 min, 9.25 min, 11.44 min, 12.58 min, 14.48 min, 20.63 min, corresponding to high molecular mass compound, poly III-albumin, poly II-albumin, poly I-albumin, ^{99m}Tc-HSA and pertecnetate, respectively. Each method gave similar results for radiochemical purity. The difference between TLC and SEC was 2.39%. The identity of some radiochemical impurities of the ^{99m}Tc-HSA was confirmed by 2 different methods. TLC and SEC are important techniques for quality control of ^{99m}Tc-HSA radiopharmaceutical.