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EFFECT OF THE MOBILE PHASE ON THE RP-HPLC ANALYSIS OF ^{99m}Tc-ECD

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ABSTRACT

Technetium-99m labeled L,L-ethylene cysteine dimer (^{99m}Tc-ECD) is a neutral, lipophilic complex and brain-perfusion imaging agent. The aim of this study was to investigate the effect of the mobile phase on the reversed phase high performance liquid chromatography (RP-HPLC) analysis of ^{99m}Tc-ECD. The HPLC system was LC20AT Prominence model and a Shim-Pack VP-ODS column (250 x 4.6 mm i.d., 5 μm). ^{99m}Tc-ECD was prepared by adding 1 mL of 0.9% NaCl, 1 mL of phosphate buffer (pH 7.5) and 1 mL of $Na^{99m}TcO_4$. The radioactive concentration was 55.5 MBq mL^{-1}. 20 μL sample volume was injected and 1.0 mL min^{-1} flow rate was applied. A linear gradient was performed separately with a mixture of ethanol with three different solvents: 12.5 mmol L^{-1} phosphate buffer (pH 2.5) (solvent A), 0.2 % PIC A (w/v) (pH 6.0) (solvent B) and 0.2 % PIC B5 (w/v) (pH 4.0) (solvent C). ^{99m}Tc-ECD retention times for the mixture of ethanol with solvent A, B and C were 17.38, 17.65 and 17.60 minutes, respectively. These results suggested that the ion pairing reagents (PIC A and PIC B5) did not influence the ^{99m}Tc-ECD analysis, but affected the retention time of impurities as ^{99m}Tc-EC (ethylene dicysteine) and $^{99m}TcO_4^-$. ^{99m}Tc-ECD radiochemical purity determined by RP-HPLC was higher than 96% for the mixture of ethanol with the three solvents. The results indicated that the retention time of ^{99m}Tc-ECD was not influenced by the nature of the mobile phase.

1. INTRODUCTION

Technetium is a group VIIB transition element which has seven electrons in the last layer. It exhibits multiple oxidation state from -1 to +7 and variable cooordination number and geometries. In aqueous solution, the most stable oxidation states are +7 ($^{99m}TcO_4^-$) and +4 ($^{99m}TcO_2$). Technetium-99m (^{99m}Tc) is the most frequently used radionuclide for in vivo imaging studies in Nuclear Medicine. The popularity of the ^{99m}Tc application is due to its physical characteristics (short physical half-life ($t_{1/2}$) of 6,01 h; γ energy of 140 keV; no particulate emission during decay) and chemical properties (technetium easily forms complexes with a large number of ligands) [1].

Technetium-99m labeled L,L-ethylene cysteine dimer (^{99m}Tc-ECD) radiopharmaceutical (Fig. 1) is a neutral, lipophilic complex and brain-perfusion imaging agent, which exhibits selective retention in brain and rapid renal excretion. It is generally prepared by addition of pertechnetate ($Na^{99m}TcO_4$) to the commercially available lyophilized kit containing ECD reagent [2].

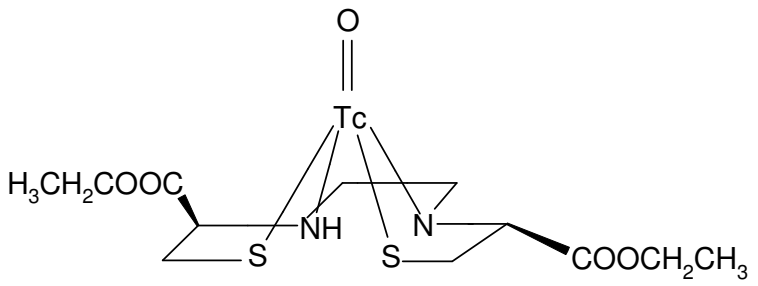

Figure 1. Complex structure of ^{99m}Tc-ECD

In ^{99m}Tc-ECD, an oxotechnetium(V) core is chelated by two amine nitrogen atoms (one of them remains protonated) and two thiol sulphur atoms. The ligand contains two ester functions, which are very important to provide the lipophilic nature to ^{99m}Tc-ECD [2, 3].

Reversed phase high performance liquid chromatography (RP-HPLC) constitutes a powerful tool for the separation of impurities in radiopharmaceuticals. The chemical composition of mobile phases plays an important role in the chromatographic separation. In RP-HPLC, an ion pairing reagent can be added, increasing the hydrophobicity of charged molecules. As a consequence, interaction of the molecules with the hydrophobic stationary phase is enhanced occurring, therefore, the separation [4, 5].

The addition of the ion pairing agent to the mobile phase is widely used for the HPLC analysis of proteins and peptides [4, 5], paracetamol and its metabolites in urine [6] and in the separation of catecholamines [7], however it has rarely been used in the analysis of radiopharmaceuticals [8].

The aim of this study was to investigate the effect of the mobile phase on the reversed phase high performance liquid chromatography (RP-HPLC) analysis of ^{99m}Tc-ECD.

2. EXPERIMENTAL

2.1. Materials

ECD lyophilized kit was from IPEN-CNEN/SP (Brazil). Water was purified in a Milli-RX-45 system from Millipore (France). $Na^{99m}TcO_4$ was obtained by elution of a $^{99}Mo/^{99m}Tc$ generator (IPEN-CNEN/SP-Brazil). The ^{99m}Tc activity was measured in a Capintec CRC-35R dose calibrator. Tetrabutylammonium phosphate (PIC A) and pentanesulfonic acid (PIC B5) were from Waters (USA) and other reagents were from Merck (Germany).

2.2. Sample preparation

^{99m}Tc-ECD was prepared by adding 1 mL of 0.9% NaCl, 1 mL of phosphate buffer (pH 7.5) and 1 mL of $Na^{99m}TcO_4$. The radioactive concentration was 55.5 MBq mL^{-1}.

2.3. High Performance Liquid Chromatography

The HPLC system (LC 20AT Prominence) (Shimadzu, Japan) was composed by two pumps, autosampler (SIL 20A), system controller (CBM 20A), diode array (SPD M20A), column oven (CTO 20A), and radiometric detector (Bioscan). The RP-HPLC analysis was performed at room temperature with a Shim-Pack VP-ODS colunm (250 x 4.6 mm, 5 µm). A 20 µL sample volume was injected and 1.0 mL min^{-1} flow rate was applied.

Each sample was analyzed in triplicate. A linear gradient was performed separately with a mixture of ethanol with three different solvents: 12.5 mmol L^{-1} phosphate buffer (Na_2HPO_4) (pH 2.5) (solvent A), 0.2 % PIC A (tetrabutylammonium phosfate) (w/v) (pH 6.0) (solvent B) and 0.2 % PIC B5 (pentanesulfonic acid) (w/v) (pH 4.0) (solvent C). The samples were filtered in a 0.45 µm pore and 25 mm diameter disposable filter unit Millex GV (Millipore).

3. RESULTS AND DISCUSSION

RP-HPLC chromatograms of ^{99m}Tc-ECD and impurities as ^{99m}Tc-EC (ethylene dicysteine) and $^{99m}TcO_4^-$, obtained with a mobile phase composed of a mixture of ethanol and solvent A (phosphate buffer), B (PIC A) and C (PIC B5) are shown in Fig. 2, Fig. 3 and Fig. 4, respectively.

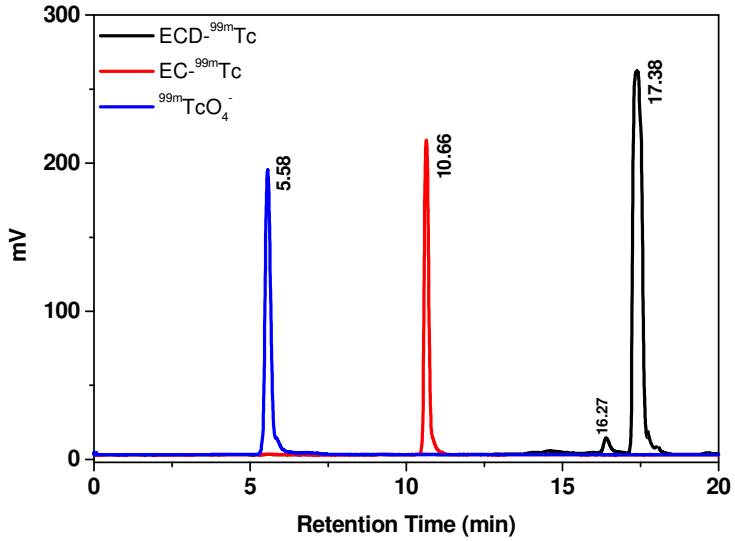

Figure 2. Chromatograms of ^{99m}Tc-ECD, ^{99m}Tc-EC and $^{99m}TcO_4^-$. Radioactive concentration: 55.5 MBq mL^{-1}. Conditions: Shim-Pack VP-ODS column (250 x 4.6 mm; 5 µm); 20 minutes linear gradient of 0 to 100% ethanol- phosphate buffer (pH 2.5); 1.0 mL min^{-1} flow rate; room temperature; 20 µL volume injection.

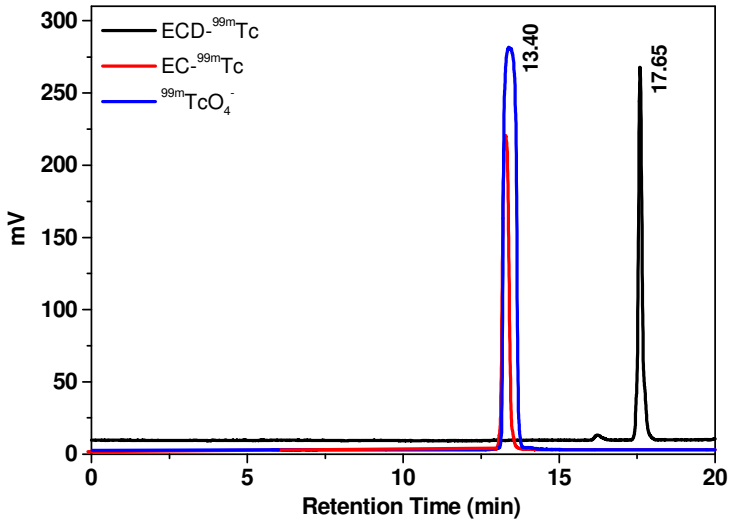

Figure 3. Chromatograms of ^{99m}Tc-ECD, ^{99m}Tc-EC and $^{99m}TcO_4^-$. Radioactive concentration: 55.5 MBq mL^{-1}. Conditions: Shim-Pack VP-ODS column (250 x 4.6 mm; 5 µm); 20 minutes linear gradient of 0 to 100% ethanol- 0.2% PIC A (pH 6.0); 1.0 mL min^{-1} flow rate; room temperature; 20 µL volume injection.

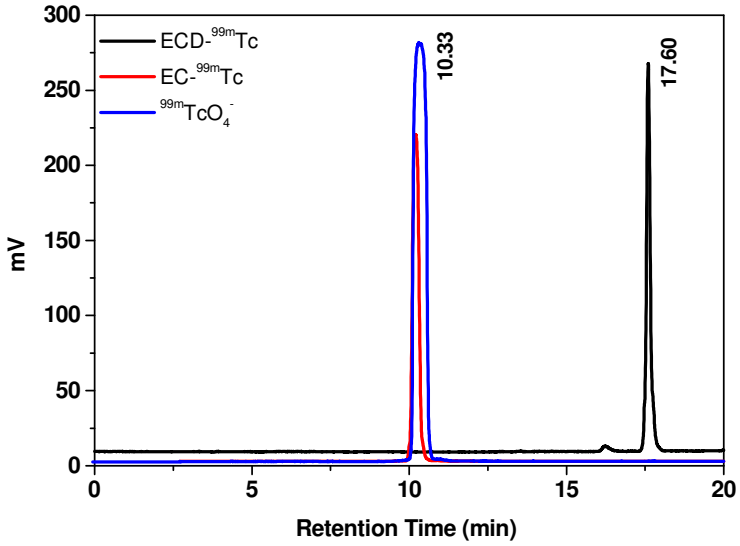

Figure 4. Chromatograms of ^{99m}Tc-ECD, ^{99m}Tc-EC and $^{99m}TcO_4^-$. Radioactive concentration: 55.5 MBq mL^{-1}. Conditions: Shim-Pack VP-ODS column (250 x 4.6 mm; 5 µm); 20 minutes linear gradient of 0 to 100% ethanol- 0.2% PIC B5 (pH 4.0); 1.0 mL min^{-1} flow rate; room temperature; 20 µL volume injection.

All chromatograms showed symmetrical peaks and stable baseline. ^{99m}Tc- ECD retention time (Rt) in mobile phase composed of the mixture of ethanol with solvent A (phosphate buffer) (Fig. 2) was 17.36 minutes. The product peak (^{99m}Tc- ECD) was separated from the impurities as ^{99m}Tc-EC (Rt = 10.36 min) and $^{99m}TcO_4^-$ (Rt = 5.54 min). On the other hand, ^{99m}Tc-EC and $^{99m}TcO_4^-$ coeluted (Rt = 13.40 min and Rt = 10.33 min, for solvent B and solvent C, respectively), in the mixture of ethanol with solvent B (PIC A) (Fig. 3) and solvent C (PIC B5) (Fig. 4).

The results of retention time suggested that the ion pairing reagents (PIC A and PIC B5) did not influence the ^{99m}Tc-ECD retention, most probably due to its neutral characteristics, so retention time was similar in all solvents [6].

The addition of ion pairing reagent (PIC A) to the mobile phase affected the retention time of negatively charged impurities as ^{99m}Tc-EC and $^{99m}TcO_4^-$ (Fig. 3). The alkyl group of the PIC A (positively charged) interacts with the stationary phase of the column while the polar group interacts with the sample ion. ^{99m}Tc-EC and $^{99m}TcO_4^-$ can form an ion pair, resulting in increasing of the retention time of the charged sample [9].

Mobile phase containing PIC B5 (negatively charged) could not interact with ion pairing reagent because sample and ions presented the same charge. Thereby, ^{99m}Tc-EC and $^{99m}TcO_4^-$ are rapidly eluted (Fig. 4).

The differences in retention behavior (Rt = 13.40 min and Rt = 10.33 min, for PIC A and PIC B5, respectively) might be due not only by electrostatic repulsion but also to other forces acting inside the system [8, 9]. The effect of the mobile phase on the RP-HPLC in the retention time of ^{99m}Tc-EC and $^{99m}TcO_4^-$ was more prominent than ^{99m}Tc-ECD.

^{99m}Tc-ECD radiochemical purity determined by RP-HPLC was higher than 96% for the mixture of ethanol with the three solvents. From a clinical standpoint, it is important to note that the radiochemical purity was not below the recommended level of 90% [10].

4. CONCLUSIONS

Three mobile phases were evaluated in the separation of ^{99m}Tc-ECD by RP-HPLC. The results indicated that the retention time of ^{99m}Tc-ECD was not influenced by the presence of ion pairing agent in the mobile phase. However, only in a linear gradient of phosphate buffer-ethanol, the separation of ^{99m}Tc-EC and $^{99m}TcO_4^-$ impurities were unfeasible.

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