

Short communication

Caution to HPLC analysis of tricarbonyl technetium radiopharmaceuticals: An example of changing constitution of complexes in column

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Abstract

Radio-HPLC is a powerful tool for analyzing radioactive species in radiopharmaceutical chemistry. In this paper, we found an example that the commonly used eluting solvent, acetonitrile, could coordinate with the popular radiopharmaceutical nuclides, technetium-99m, during chromatography. $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ showed quite different retention time when they were eluted using acetonitrile/water as mobile phase. However, they almost demonstrated the same retention time when they were eluted using methanol/water as mobile phase. Further analysis showed that both $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ could be changed into $[^{99m}\text{Tc}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{CH}_3\text{CN})_x(\text{H}_2\text{O})_{3-x}]^+$ during the separation, respectively. Some former works mistook the $[^{99m}\text{Tc}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$ for $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ when using acetonitrile and water in analysis. Quality control of the radiopharmaceuticals containing metal complex should be careful since HPLC solvent could replace some liable ligand molecules.

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1. Introduction

Technetium-99m labeled radiopharmaceuticals are currently the main diagnostic agents used in nuclear medicine. A large amount of research on technetium-based radiopharmaceuticals is being carried out all over the world since its ideal characteristics for a nuclear medicine scan. Since the mass amount of technetium in preparation for clinical use is typically in the nanomole range, conventional techniques, such as UV, NMR, IR and elemental analysis, are invalid in analyzing such low concentrated compounds. Radio-HPLC and radio-LCMS developed recently have become powerful techniques for analyzing radio-species and quality control of radiopharmaceuticals [1–3].

Research on technetium-based radiopharmaceuticals has achieved great improvements with the advent of $[^{99m}\text{Tc}(\text{CO})_3]^+$ core and $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ is a frequently used precursor for labeling small organic molecules and biomacromolecules [4–6]. Methanol/water and acetonitrile/water are the most com-

monly used mobile phase for reverse phase HPLC. However, organic solvent could play a role of ligand replacing the water ligand in $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ during the process of separation. In this paper, we explored the possibility of the liable water molecule in $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ being replaced by solvent molecule for HPLC.

2. Experimental

2.1. Materials and reagents

All the reagents used were AR grade and purchased from Aldrich. HPLC solvents were obtained from Fisher Chemical. Potassium boranocarbonate was synthesized according to the literature [7]. $\text{Na}^{99m}\text{TcO}_4$ was obtained from a commercial $^{99}\text{Mo}/^{99m}\text{Tc}$ generator, Beijing Atomic High-tech Co.

2.2. Instrumentation

HPLC analyses were performed on a Shimadzu SCL-10AVP system which consisted of a binary pump with on-line degasser, a model SPD-10 Avp UV detector operating at wave length of

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254 nm and a Packard 500 TR series flow scintillation analyzer. The samples were separated on an RP C-18 Alltech alltima column (5 μ M, 250 mm \times 4.6 mm). Electron spray ionization (ESI) mass spectrums were recorded on Shimadzu LCMS-2010. Infrared spectrums were recorded as KBr disks in the range of 4000–400 cm^{-1} on a Nicolet Avatar 360 IR spectrophotometer. Elemental analyses were performed on an Elementar Vario EI.

2.3. Sample preparation

2.3.1. Preparation of $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor

The $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was prepared by adding 2 ml of $^{99\text{m}}\text{TcO}_4^-$ from a commercial generator (10 mCi) to a 10 ml vial containing potassium boranocarbonate (3 mg), sodium potassium tartrate tetrahydrate (6.7 mg), and potassium tetraborate pentahydrate (5.5 mg). The solution was heated for 15 min in boiling water under N_2 . $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was successfully prepared with high radio-yields (>95%).

2.3.2. Synthesis of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$

Method A. The $\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3\text{Br}$ was synthesized according to the literature [8]. Briefly, 200 mg of $[\text{Re}(\text{CO})_5]\text{Br}$ was refluxed in a 10 ml round-bottom flask for 24 h. Periodic rinsing of the condenser washed unreacted $[\text{Re}(\text{CO})_5]\text{Br}$ into the reaction solution. The crude mixture was cooled and filtered.

Subsequently, the solvent was removed under vacuum to give the desired product as a light grey-green powder (150 mg, 75%). IR (cm^{-1}): 2018, 1939. Anal. Calcd. for $\text{C}_3\text{H}_6\text{BrO}_6\text{Re}$: C, 8.91; H, 1.50. Found: C, 9.18; H, 1.89. ESI-MS was performed with sample dissolved in methanol: m/z 385.0 $\text{Re}(\text{CO})_3(\text{CH}_3\text{OH})_3 \cdot \text{H}_2\text{O}$ (^{187}Re), 367.1 $\text{Re}(\text{CO})_3(\text{CH}_3\text{OH})_3$ (^{187}Re). Three milligrams of $\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3\text{Br}$ was dissolved in 400 μ l water and 1 equiv. AgNO_3 was added. After the filtration of precipitate, the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ water solution was obtained.

Method B. The $(\text{Et}_4\text{N})_2\text{Re}(\text{CO})_3\text{Br}_3$ was synthesized in a typical reaction [9]. Powered NEt_4Br (170 mg, 0.8 mmol) was slurried in 30 ml of 2,3,5-trioxanone (diglyme) and heated to 80 $^\circ\text{C}$ under dry nitrogen. A suspension of $[\text{Re}(\text{CO})_5]\text{Br}$ (150 mg, 0.37 mmol) in 3 ml of warm diglyme was added. The mixture was heated to 110–120 $^\circ\text{C}$ for 8 h, during which time, some white precipitate was formed. The reaction mixture was filtered while hot and washed with several portions of cold diglyme, diethyl ether and dried in vacuo. The dried light yellow solid was washed with ethanol to remove the unreacted NEt_4Br . Drying in vacuo yielded the product. $(\text{Et}_4\text{N})_2\text{Re}(\text{CO})_3\text{Br}_3$ was yielded as light-yellow solid (200 mg, 70%). IR (cm^{-1}): 1999, 1868. Anal. Calcd. for $\text{C}_{19}\text{H}_{40}\text{Br}_3\text{N}_2\text{O}_3\text{Re}$: C, 29.62; H, 5.23; N, 3.64. Found: C, 29.68; H, 5.41; N, 3.59. Three milligrams of $(\text{Et}_4\text{N})_2\text{Re}(\text{CO})_3\text{Br}_3$ was dissolved in 400 μ l water and 3 equiv. AgNO_3 was added. After

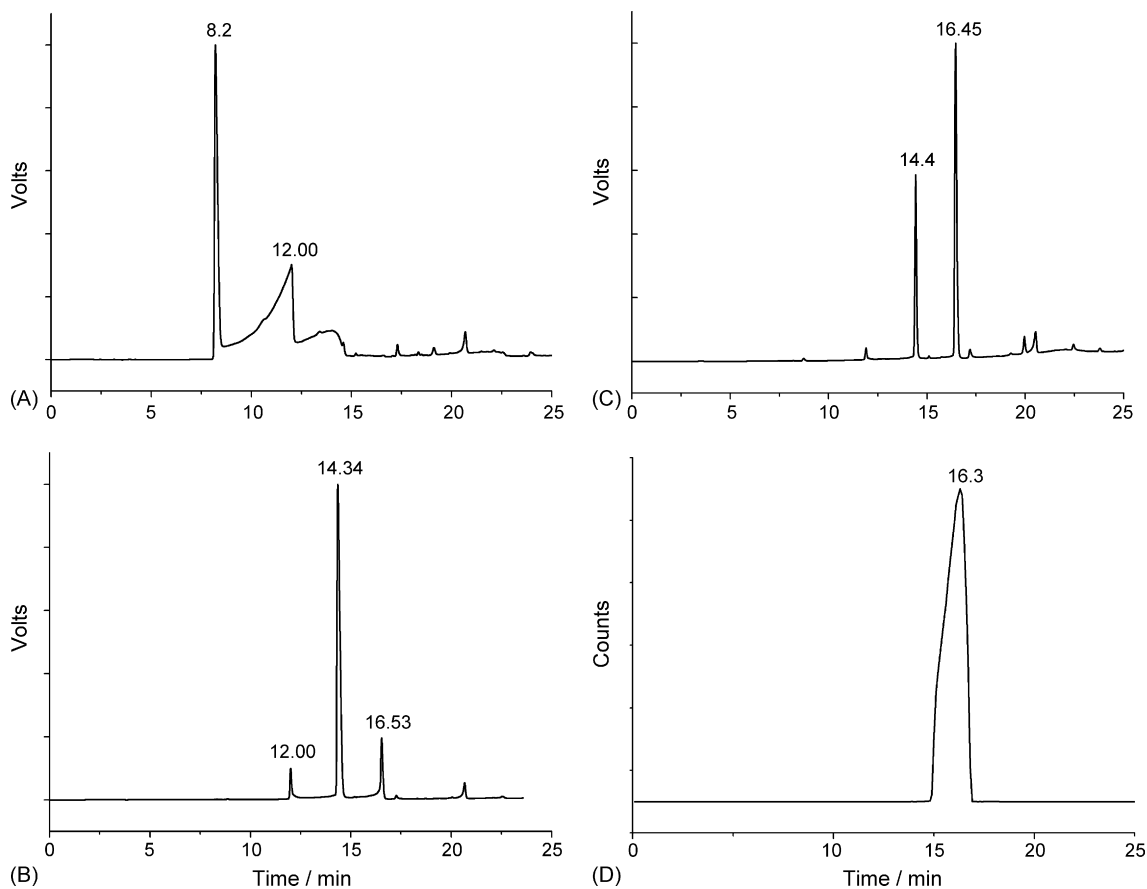


Fig. 1. HPLC chromatogram of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ using system 1: (A) chromatogram of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ in water solution; (B) chromatogram of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ dissolved in 4 $^\circ\text{C}$ acetonitrile/water mixture (v/v = 50:50) solution for 10 min; (C) chromatogram of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ dissolved in acetonitrile/water mixture solution for 60 min; (D) chromatogram of $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ in water solution.

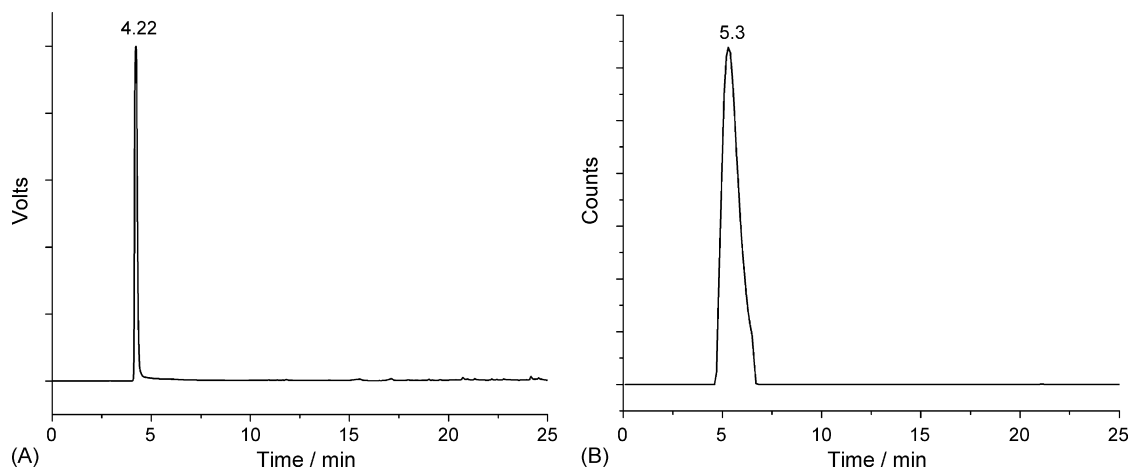


Fig. 2. HPLC chromatogram of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ using system 2: (A) chromatogram of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ in water solution and (B) chromatogram of $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ in water solution.

the filtration of precipitate, the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ water solution was obtained.

2.4. HPLC methods

Two different chromatographic conditions were employed with a flow rate of 1 ml/min. System 1: mobile phase consisted of H_2O containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). The HPLC gradient system started with 10% solvent B with a linear gradient to 90% solvent B from 0 to 28 min. System 2: the mobile phase consisted of aqueous 0.05 M TEAP (triethylammonium phosphate) buffer, pH 2.25 (solvent A) and methanol (solvent B). The HPLC system started with 100% of A from 0 to 3 min. The eluent switched at 6 min to 75% A and 25% B and at 9 min to 66% A and 34% B followed by a linear gradient 66% A/34% B to 100% B from 9 to 20 min. The test solution (2.5 μl) was injected into the column and the elution was monitored by observing the UV profile at 254 nm for rhenium compounds and radio-trace for technetium-99m compound.

$[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ were examined using both system 1 (Fig. 1) and system 2 (Fig. 2). In system 1, only one radioactive peak at 16.3 min could be observed as shown in Fig. 1D when $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was injected. $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ showed retention time of 8.20 min, and two leading peaks could be seen at time of 12.00 and 14.0 min respectively as shown in Fig. 1A. When $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was dissolved in 4 °C acetonitrile/water mixture (v/v = 50:50) solution for 10 min before injection, the peak at 8.20 min disappeared and the two leading peaks became sharp peaks (Fig. 1B). The compound corresponding to 14.3–14.4 min became the primary component rather than $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ after acetonitrile was introduced into the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ for only 10 min. If $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was mixed with acetonitrile/water mixture for 60 min before injection, the complex became the final product with retention time of 16.4–16.5 min, as showed in Fig. 1C. In system 2, only one UV peak was found for $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ at 4.22 min and the retention time for $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was 5.3 min as shown in Fig. 2.

3. Results and discussion

Identity confirmation across $^{99\text{m}}\text{Tc}$ - and ^{99}Tc - or Re-complexes could be performed by co-injection on HPLC and comparison of UV and radiometric detector signal. $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ showed equivalent retention time when they were analyzed using methanol/water as mobile phase. However, $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ showed different retention time when they were analyzed using acetonitrile/water as mobile phase. Only after the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was mixed with acetonitrile, the final complex showed the same retention time as $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ did. The $^{99\text{m}}\text{Tc}$ -complex with a retention time of 16.3 min should have a similar structure with the Re-complex with a retention time of 16.4–16.5 min. Mass spectrum analysis demonstrated that the molecular weight of the compound with retention time at 16.4–16.5 min was 394.1 (Fig. 3), which is corresponding to $[\text{Re}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$. So, the $^{99\text{m}}\text{Tc}$ -complex eluted out should be $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$ rather than $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$. The three water molecules for $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ could be fully replaced by acetonitrile during chromatography. This can also explain why the hydrophilic $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ demonstrated such a long retention in the column.

Some former works [10,11] using system 1 to characterize the $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ showed the retention time of 13.6–13.7 min for radioactivity. According to our experimental results, the eluted complex should be $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$ rather than $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$.

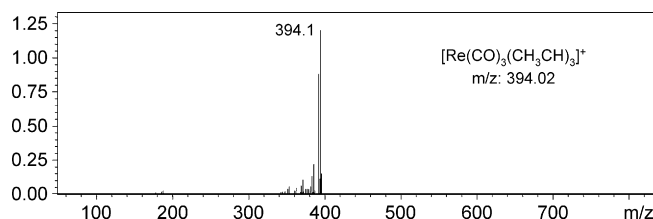


Fig. 3. ESI-MS spectrum of the compound $[\text{Re}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$.

The mass amount of $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was in range of 10^{-10} to 10^{-8} mole, so its three water molecules could be fully replaced resulting in $[^{99m}\text{Tc}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$ during the separation. Whereas, the quantity of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was in range of 10^{-6} to 10^{-3} mole, and only one or two water molecules of partial $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ could be replaced by acetonitrile in the HPLC column. The primary eluted component for $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was still unchanged $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ (retention time of 8.2 min) with partial $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_2(\text{CH}_3\text{CN})]^+$ (retention time of 12.0 min) and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})(\text{CH}_3\text{CN})_2]^+$ (retention time of 14.4 min).

$[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ has been and will be studied extensively because of its stability and kinetic inertness. Radio-HPLC is a very helpful tool to analyze the product of radio-synthesis and acetonitrile/water was commonly used as the mobile phase for reverse phase HPLC. Though acetonitrile can not replace any strong ligand, it probably can replace the “1” weak ligand molecule in complex with a “2 + 1” coordination pattern. This will cause a change in constitution of complex after purification with HPLC, and then some important characteristics in vitro and in vivo.

4. Conclusions

In the paper, we found that $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ could be changed into $[^{99m}\text{Tc}(\text{CO})_3(\text{CH}_3\text{CN})_3]^+$ and $[\text{Re}(\text{CO})_3(\text{CH}_3\text{CN})_x(\text{H}_2\text{O})_{3-x}]^+$, respectively, during the process of separation when they were eluted using acetonitrile/water mixture in HPLC. So, HPLC characterization should be carried out carefully when $[\text{M}(\text{CO})_3(\text{H}_2\text{O})_x]^+$ was eluted by acetonitrile/water. Methanol/water can be used as mobile phase since no replacement has been observed during the chromatography.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2006.11.031.

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