Journal of Chromatography, 479 (1989) 212–215
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 677

Note

Separation of cationic technetium-99m amine complexes on porous graphitic carbon

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(First received April 17th, 1989; revised manuscript received June 12th, 1989)

Technetium-99m has almost ideal physical properties (half-life, $t_{\frac{1}{2}}$ = 6.1 h, γ energy 140 keV) for diagnostic imaging studies in nuclear medicine using single photon emission computer tomography (SPECT). It is readily available as sodium pertechnetate (TcO_4^-) from commercial generators. Analysis of technetium complexes evaluated as potential radiopharmaceuticals has mainly relied on paper chromatography[1–3] and instant thin-layer chromatography[2–4], although high-performance liquid chromatography (HPLC) is increasingly being employed[5–7].

Cationic technetium complexes of linear and macrocyclic amines have been synthesised as possible imaging agents[8–14], but so far only one HPLC method has been reported for their analysis[15]. This was because of the difficulties in eluting these compounds from silica-based reversed-phase columns[15] and a styrene–divinylbenzene bonded phase (Hamilton PRP-1) was suggested as an alternative. However, although some degree of separation has been achieved with this system using PRP-1, further improvement in resolution is required particularly between pertechnetate (TcO_4^-) and the complexes. This paper describes the complete separation of TcO_4^- and the monocationic amine complexes dioxo(ethylenediamine)-, dioxo(1,5,8,12-tetraazadodecane)- and *trans*-dioxo(1,4,8,11-tetraazacyclotetradecane)-technetium on a porous graphitic carbon (PGC) column with acetonitrile in 1% trifluoroacetic acid as eluent. The system is suitable for monitoring the radiochemical purity of these potential radiopharmaceuticals and for metabolism studies.

EXPERIMENTAL

Materials and reagents

Ethylenediamine (en_2) and stannous tartrate were from Sigma (Poole, U.K.). 1,5,8,12-Tetraazadodecane (ta) was from Aldrich (Gillingham, U.K.). 1,4,8,11-Tetraazacyclotetradecane or cyclam (cyc) was from Lancaster Synthesis (Morecambe, U.K.).

0021-9673/89/$03.50

Sodium pertechnetate (TcO_4^-) was eluted from an Ultratechnekow FM generator (Mallinckrodt Diagnostia, The Netherlands). The eluate was diluted with isotonic saline to give a working solution of 0.2–1 mCi/ml (7.4–37 MBq/ml).

Trifluoroacetic acid (TFA) and hydrochloric acid were AnalaR grade from BDH. Acetonitrile was HPLC grade from Rathburn (Walkerburn, U.K.).

Preparation of technetium amine complexes

Stannous tartrate was used as the reductant. A saturated solution was made by adding the solid to nitrogen purged 0.01 *M* HCl. The solution was filtered through a Millex GS 0.22-μm filter (Millipore, Harrow, U.K.) into a nitrogen-filled vial and constantly purged with nitrogen. The ligand solutions (20–50 m*M*) were made up in water. All reagents were freshly made before used.

The reaction was carried out in a capped vial by mixing 1 ml of ligand solution, 1 ml of sodium pertechnetate working solution and 1 ml of filtered stannous tartrate solution. The mixture was shaken and allowed to stand at room temperature for 15–30 min before injection into the liquid chromatograph. The complexes were stable for at least 2 h.

High-performance liquid chromatography

A Varian Associates (Walnut Creek, CA, U.S.A.) Model 5000 liquid chromatograph was used. The radiomatic detector was home made and consisted of a PTFE tubing wound around the outside of a 1.3-cm diameter plastic tube placed in a thallium-activated sodium iodide crystal well with the photomultiplier tube linked to a scintillation adoptor 288 and ratemeter 248 both from ESI Nuclear (Redhill, U.K.). The detector coil flow volume was 160 μl.

The separation was carried out on a 10 cm × 4 mm PGC (7-μm spherical particles) column (Hypercarb; Shandon Southern Scientific, Runncorn, U.K.). For the separation of mixtures, step-wise elution was used. The column was eluted with acetonitrile–1% TFA (2:98, v/v) for 14 min and then changed to acetonitrile–1% TFA (10:90, v/v) for the elution of the last peak (total time 20 min). For individual technetium amine complexes, isocratic elution with an appropriate mixture of acetonitrile and 1% TFA was sufficient.

RESULTS AND DISCUSSION

PGC is a new packing material for reversed-phase chromatography[16,17]. It is chemically stable to extreme pH and is free of silanol groups which are common to silica-base reversed-phase materials. PGC is much more hydrophobic and retentive than conventional C_8 or C_{18} reversed-phase packings and is therefore ideal for separating compounds which are difficult to retain on silica-based columns. In addition, PGC can interact electronically with solutes having lone pair electrons[18,19] because of the presence of the band of delocalised π-electrons. The above properties suggested the possibility of retaining and separating technetium amine complexes on PGC where silica-base materials have already failed[15].

The separation of a mixture containing $[TcO_2en_2]^+$, $[TcO_2ta]^+$, $[TcO_2cyc]$ and TcO_4^- is shown in Fig. 1. The elution order of the cationic species, $[TcO_2en_2]^+ < [TcO_2ta]^+ < [TcO_2cyc]^+$, is that expected for reversed-phase chromatography since the hydrophobicity of the compounds increased in that order.

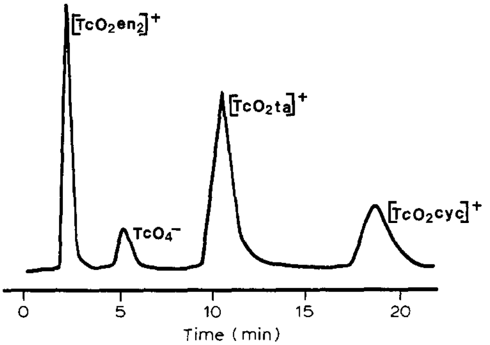

Fig. 1. Separation of technetium-99m amine complexes and pertechnetate on porous graphitic carbon. Mobile phase: acetonitrile–1% TFA (2:98, v/v) for 14 min, then 10:90 (v/v) for 6 min; flow-rate, 1 ml/min. Detection: radiometric.

TFA was chosen as the aqueous component of the mobile phase for two reasons. Firstly, it forms ion pairs with the cationic technetium amine complexes but, unlike long-chain ion-pairing agents, does not confer strong hydrophobicity on the solutes which may lead to excessive retention on the highly hydrophobic PGC. Secondly, TFA is an excellent competing agent for electronic interaction chromatography (EIC) on PGC, thus allowing the retention times of the anionic TcO_4^- to be precisely controlled by simply adjusting the concentrations of TFA in the mobile phase[19]. Retention of inorganic oxo-anions, including TcO_4^-, on PGC has been shown to be exclusively electronic interaction[19]. The PGC column is stable under the strongly acidic condition used and no deterioration of column performance was observed after prolonged usage with 1% TFA as eluent.

The ability of PGC to simultaneously separating cations and anions based on a combination of hydrophobic and electronic interactions is unique. It is anticipated that many novel applications will be developed by exploiting these chromatographic properties. The method described here is well suited for checking the purity of technetium amine complexes and for studying the metabolism of these potential radiopharmaceuticals. It is also well suited for the semi-preparative isolation of individual technetium amine complex with 0.9% NaCl instead of TFA as the major mobile phase component. After removal of acetonitrile by evaporation the preparation in saline can be used directly.

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