ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1241–1244

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Comparison of reversed phase and reversed phase ion pair high performance liquid chromatography for analysis of TcO and TcN complexes of L,L-ethylene dicysteine di-ethylester and its acid analogues[1]

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Received for review 13 September 1995

Abstract

^{99m}Tc(V)-oxo complexes of L,L-ethylene dicysteine (L,L-EC) and its di-ester derivative L,L-ethyl cysteinate dimer (L,L-ECD) are useful tracer agents for evaluation of renal function and cerebral blood flow respectively. Labelling of these molecules with a ^{99m}Tc(V)-nitrido core instead of the ^{99m}Tc(V)-oxo core alters their biological and physiochemical behaviour. In the reversed phase high performance liquid chromatography (HPLC) method presently used to analyse ^{99m}Tc(V)-oxo preparations of L,L-EC and L,L-ECD, the ^{99m}Tc(V)-nitrido–L,L-EC complex and the possible impurities of a ^{99m}Tc(V)-nitrido–L,L-ECD preparation were found to elute with the void volume. In this study, a reversed phase ion pair HPLC method has been developed that is useful for the analysis of both ^{99m}Tc(V)-oxo and ^{99m}Tc(V)-nitrido preparations of L,L-EC and L,L-ECD. Tetrabutylammonium hydroxide is used as a cationic ion pairing agent.

Keywords: L,L-Ethyl cysteinate dimer; L,L-Ethylene dicysteine; Ion pair HPLC; Reversed phase HPLC; Technetium-99m-nitrido; Technetium-99m-oxo

1. Introduction

Tracer agents labelled with an approriate γ-emitting radionuclide may offer the possibility of studying and even quantifying a variety of biological processses, in vivo, depending on their tissue distribution pattern. Technetium-99m (^{99m}Tc) is considered the most ideal radionuclide for such in-vivo studies and is most commonly incorporated in tracer agents in the form of a ^{99m}Tc(V)O core ($[Tc{=}O]^{3+}$). L,L-ethylene dicysteine (L,L-EC) labelled with a ^{99m}TcO core (Fig. 1) has been introduced in nuclear medicine as a tracer agent for evaluation of renal function while the ^{99m}TcO complex of its di-ester derivative L,L-ethyl cysteinate dimer (L,L-ECD) (Fig. 1) is a neutral, lipophilic complex that is in clinical use for the determination of regional cerebral blood flow.

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[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

PII S0731-7085(96)01736-0

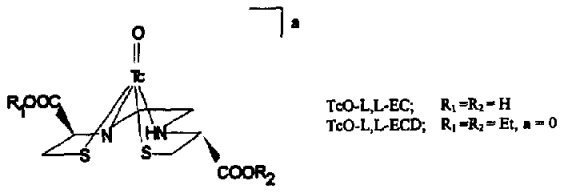

Fig. 1. Structure of TcO complexes of L,L-EC and L,L-ECD.

Replacement of the more common ^{99m}TcO core with a ^{99m}Tc-nitrido core ($[Tc{\equiv}N]^{2+}$) has led to the development of several tracer agents with distinct physicochemical and biological characteristics [1–5] and has also been shown to affect the physicochemical and biological characteristics of the ^{99m}Tc complexes of L,L-ECD and L,L-EC [6].

In this study, we report a high performance liquid chromatography (HPLC) method that has proven to be useful for the routine analysis of the ^{99m}TcO and ^{99m}TcN preparations of L,L-EC and L,L-ECD.

2. Materials and methods

L,L-EC and L,L-ECD were synthesised following reported methods [7]. ^{99m}Tc-pertechnetate solution was eluted from an Ultra-Technekow ^{99}Mo/^{99m}Tc generator (Mallinckrodt Medical, Petten, The Netherlands).

2.1. Labelling with a ^{99m}Tc-oxo core

To form ^{99m}TcO–L,L-ECD, $^{99m}TcO_4^-$ solution (370 MBq in 2 ml) is added to vial B of an ECD labelling kit. The lyophilisate in vial A is then dissolved in 3 ml of 0.9% NaCl and 1.0 ml of this solution is immediately transferred to vial B which is then incubated at room temperature (RT) for 20 min. Vial A of the ECD kit contains 0.90 mg ECD·2HCl, 72 μg $SnCl_2 \cdot 2H_2O$, 0.36 mg $Na_2EDTA \cdot 2H_2O$ and 24 mg mannitol. Vial B contains 1.0 ml phosphate buffer (pH 7.5; 0.02 M).

^{99m}TcO–L,L-EC is prepared by adding $^{99m}TcO_4^-$ solution (370 MBq in 3 ml) to an EC labelling kit. Maximum ^{99m}TcO–L,L-EC formation is immediate. The EC kit contains, in lyophilised form, 0.5 mg L,L-EC, 10 mg $Na_2HPO_4 \cdot 2H_2O$, 45 mg $Na_3PO_4 \cdot 12H_2O$ and 0.1 mg $SnCl_2 \cdot 2H_2O$.

2.2. Labelling with a ^{99m}Tc-nitrido core

^{99m}TcN complexes of L,L-EC and L,L-ECD are prepared by initially forming an intermediate ^{99m}TcN–succinyl dihydrazide (SDH) complex which then transfers the $[^{99m}Tc{\equiv}N]^{2+}$ core to L,L-EC or L,L-ECD in an exchange reaction. ^{99m}TcN–SDH is prepared by adding $^{99m}TcO_4^-$ solution (3700 MBq in 5 ml) to a SDH labelling kit (CIS Bio International, France) followed by incubation at RT for at least 15 min. The SDH kit contains 10 mg SDH, 5 mg propylenediamine tetraacetic acid, 0.025 mg $SnCl_2 \cdot 2H_2O$ and 1 ml phosphate buffer (pH 7.8; 0.5 M).

^{99m}TcN–L,L-ECD is made by adding 0.5 ml of the ^{99m}TcN–SDH solution to 0.35 mg L,L-EDC·$2H_2O$ in 1 ml 0.9% NaCl and incubating the mixture at RT for 10 min. ^{99m}TcN-L,L-EC is prepared by adding 0.5 ml of the ^{99m}TcN–SDH solution to a mixture of 0.5 mg L,L-EC in 1 ml water and 0.25 ml phosphate buffer solution (pH 12; 0.5 M) followed by incubation at RT for at least 1 h.

2.3. HPLC analysis

The equipment consists of a Merck–Hitachi ternary gradient pump (model L-6200 intelligent pump, Merck, Belgium), a Valco N6 injector (Alltech, Belgium), and a 250 mm × 4.6 mm column filled with Hypersil ODS 5 μm (Shandon Scientific, UK). The column effluent is monitored for level of radioactivity using a 2 in. NaI(Tl) scintillation detector coupled with a single channel analyser and a Rachel analysis program (version 1.40, Lablogic, UK).

In reversed phase HPLC (RP-HPLC), the column is eluted at a flow rate of 1 ml min^{-1} with gradient mixtures of phosphate buffer (pH 2.5; 0.0125 M), 30% v/v ethanol in phosphate buffer (pH 2.5; 0.0125 M), and absolute ethanol. In the case of RP-ion pair(IP)-HPLC, elution is done using gradient mixtures of 10% v/v ethanol in 0.2% w/v tetrabutyl ammonium hydroxide (TBA) (pH 6) and 60% v/v ethanol in 0.2% w/v TBA (pH 6).

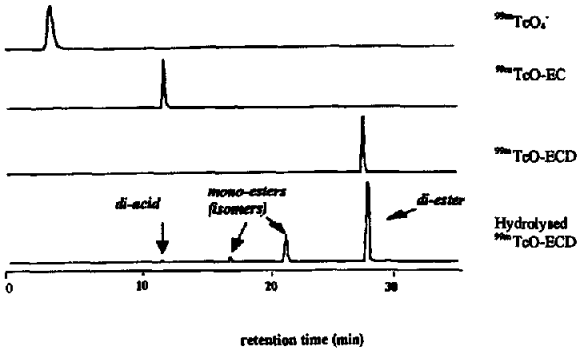

Fig. 2. RP-HPLC chromatograms of ^{99m}TcO preparations of EC, ECD and pertechnetate.

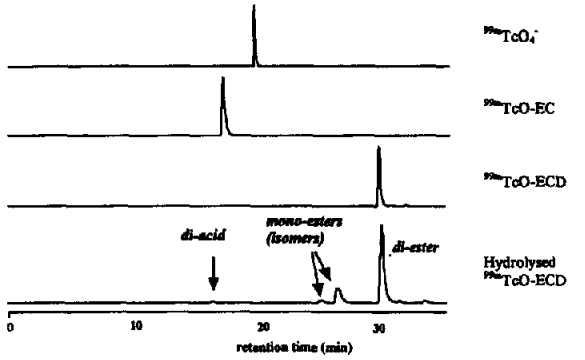

Fig. 4. RP-IP-HPLC chromatograms of ^{99m}TcO preparations of EC, ECD and pertechnetate.

The pH of the TBA solutions is adjusted with phosphoric acid solution (0.5 M).

3. Results and discussion

RP-HPLC chromatograms of ^{99m}TcO preparations of L,L-EC and L,L-ECD are given in Fig. 2. Similarly, chromatograms of corresponding ^{99m}TcN preparations are given in Fig. 3. Chromatograms of ^{99m}Tc-pertechnetate and of ^{99m}TcN–SDH, which are potential impurities in ^{99m}TcO–L,L-EC and ^{99m}TcN–L,L-EC preparations respectively, are also shown. ^{99m}Tc complexes of the isomeric monoester monoacids ECM1 and ECM2 (Fig. 1, either R_1 or R_2 = Et, the other = H) and of the diacid (EC) may be additional impurities in ^{99m}TcO– or ^{99m}TcN–L,L-ECD preparations.

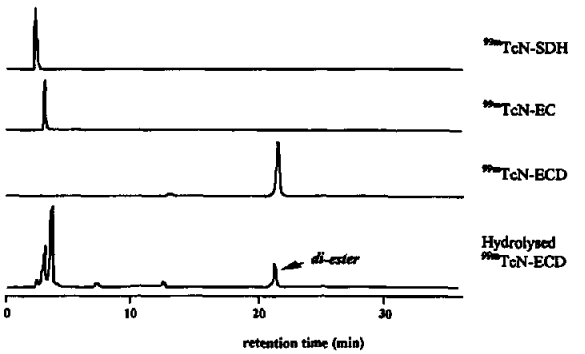

Fig. 3. RP-HPLC chromatograms of ^{99m}TcN preparations of EC, ECD and some potential impurities.

In RP-HPLC, ^{99m}TcO–ECD elutes with a retention time (R_t) of 28 min and is well separated from its possible impurities ^{99m}TcO–EC (R_t = 11 min) and pertechnetate (elutes with the void volume). A ^{99m}TcO–ECD preparation incubated with 0.2 ml 0.5 M NaOH for a few minutes gives a peak consistent with ^{99m}TcO–EC, a peak due to the residual ^{99m}TcO–ECD complex, and two peaks at retention times of 17 and 21 min (Fig. 2). These peaks at intermediate retention times are probably due to ^{99m}TcO–ECM1 and ^{99m}TcO–ECM2, as these two complexes are expected to be less polar than ^{99m}TcO–EC and more polar than ^{99m}TcO–ECD. In contrast, for ^{99m}TcN-labelled preparations only ^{99m}TcN-ECD elutes with an adequate retention time (21 min) in the RP-HPLC system. ^{99m}TcN–EC, pertechnetate and the intermediate ^{99m}TcN–SDH complex all elute with the void volume. In the alkaline-hydrolysed ^{99m}TcN–ECD preparation, a residual ^{99m}TcN–ECD peak is seen but the hydrolytic products ^{99m}TcN-ECM1, ^{99m}TcN–ECM2 and ^{99m}TcN–EC all elute with retention times less than 5 min.

In Figs. 4 and 5, RP-IP-HPLC chromatograms of ^{99m}TcO preparations and ^{99m}TcN preparations of L,L-EC and L,L-ECD respectively are given. Chromatograms of possible impurities are also shown. Alkaline-hydrolysed ^{99m}TcO–ECD and ^{99m}TcN–ECD give a peak consistent with the corresponding ^{99m}Tc complex of EC, a residual peak due to the ^{99m}Tc complex of ECD and two peaks attributable to ^{99m}TcO or ^{99m}TcN complexes of ECM1 and ECM2. It is thus possible to

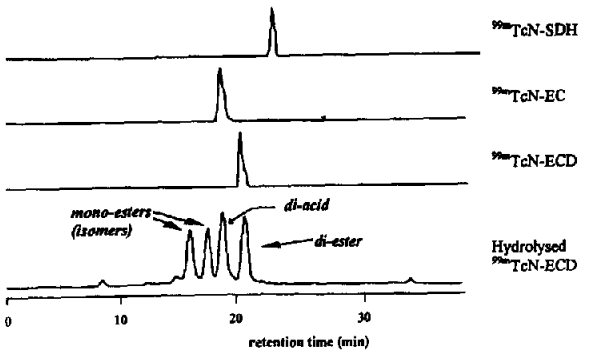

Fig. 5. RP-IP-HPLC chromatograms of ^{99m}TcN preparations of EC, ECD and some potential impurities.

separate ^{99m}TcO or ^{99m}TcN complexes of EC or ECD from their possible impurities with this RP-IP-HPLC method. However, it is evident from Fig. 5 that baseline separation of ^{99m}TcN–ECD from ^{99m}TcN–ECM1 and ^{99m}TcN–ECM2 is not achieved. The large amounts of ^{99m}TcN–ECM1 and ^{99m}TcN–ECM2 in the alkaline-hydrolysed ^{99m}TcN–ECD preparation contribute significantly to this poor separation. It is expected that in routine ^{99m}TcN–ECD analysis, small amounts of ^{99m}TcN–ECM1 and ^{99m}TcN–ECM2 are present and baseline separation is therefore achievable.

In RP-IP-HPLC, ^{99m}TcN–L,L-EC elutes later than the ^{99m}TcN complexes of ECM1 and ECM2, contrary to the situation observed with the corresponding ^{99m}TcO complexes. A possible explanation may be a higher negative charge at pH 6 on ^{99m}TcN–L,L-EC compared to ^{99m}TcO–L,L-EC [6]. The higher negative charge may lead to one molecule of ^{99m}TcN–L,L-EC associating with more molecules of the ion pairing agent (TBA) than a molecule of ^{99m}TcO–L,L-EC. The effect of the ion pairing agent on the elution of ^{99m}TcN–L,L-EC would therefore be greater than on ^{99m}TcO–L,L-EC and may sufficiently prolong column retention of ^{99m}TcN–L,L-EC to cause the complex to elute after the ^{99m}TcN–ECM1 and ^{99m}TcN–ECM2 peaks.

The results obtained show that the described RP-HPLC method is efficient for separation of the ^{99m}TcO complexes of EC and ECD from their possible impurities but that the system is not appropriate for analysis of the corresponding ^{99m}TcN preparations, as ^{99m}TcN–EC, ^{99m}TcN–ECM1, ^{99m}TcN–ECM2, ^{99m}TcN–SDH and pertechnetate all elute with retention times less than 5 min. However, RP-IP-HPLC visualises the ^{99m}TcO and ^{99m}TcN complexes of L,L-ECD and L,L-EC and all their mentioned impurities and is therefore applicable for analysis of both the ^{99m}TcO and ^{99m}TcN preparations of L,L-EC and L,L-ECD.

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