DOI: 10.1007/s10967-007-6999-0 *Journal of Radioanalytical and Nuclear Chemistry, Vol. 275, No.1 (2008) 161–164*

Preparation, quality control and stability of ^{99m}Tc-cefuroxime axetil

F. Yurt Lambrecht,* K. Durkan, P. Unak

Department of Nuclear Applications, Institute of Nuclear Sciences, Ege University, 35100 Bornova, Izmir-Turkey

(Received February 23, 2007)

Cefuroxime axetil, a cephalosporin antibiotic used to treat bacterial infections, was investigated to label with ^{99m}Tc. Radiolabeling of cefuroxime axetil was carried out by using stannous chloride method. Effects of pH and stannous chloride amount on the radiolabeling yield were investigated. The radiochemical purity of ^{99m}Tc-cefuroxime axetil was determined by thin layer radio chromatography (TLRC), electrophoresis and high performance liquid chromatography. The maximum radiolabeling yield was 98±1%.

Introduction

Inflammatory diseases, both infections and non-infections are important problems in clinical practice worldwide. The identification of an infection at an early stage of the disease is critical for a favorable outcome. Many current laboratory tests used to guide the diagnostic process rely on factors in the inflammatory response: erythrocyte sedimentation rate, white-blood cell count, acute-phase proteins and cytokines, but the tests are not specific enough to discriminate between infection and inflammation. New techniques, especially within immunology and molecular biology, are yielding new insights into the discrimination of infection and inflammation. Nuclear medical imaging has an important role in discriminating infections from inflammation. Inflammatory processes can be visualized in their early phases, when anatomical changes are not yet apparent since scintigraphic images are based on functional changes of tissues. In this connection, various radiopharmaceuticals, such as radiolabeled antimicrobial peptides, antibiotics, and chemotactic peptides have been developed.[1–3] Radiolabeled antibiotics have been also investigated for infection imaging.[4–6]

Cefuroxime axetil is an oral ester prodrug of the second generation cephalosporin antibiotic cefuroxime (Fig. 1). In humans, gastrointestinal absorption of cefuroxime is negligible, whereas the 1-acety-loxyethyl (axetil) ester of cefuroxime is used to improve its gastrointestinal absorption. After oral administration, cefuroxime axetil is absorbed and rapidly hydrolyzed by esterases in the intestinal mucosa and portal blood to produce cefuroxime. Cefuroxime axetil is used in the treatment of a wide range of infections. Cefuroxime is characterized by a high degree of stability to beta-lactamases and demonstrates favorable in vitro activity against a wide range of beta-lactamase-producing and non-producing gram-positive and gram-negative organisms.[7–10]

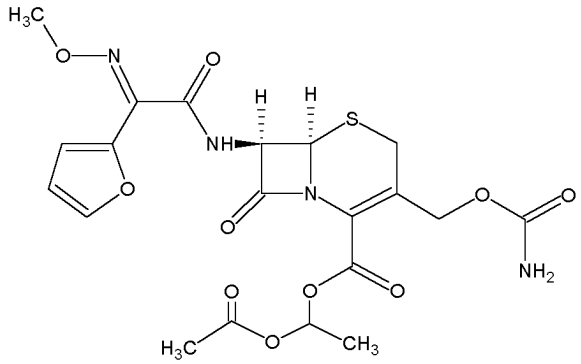

Fig. 1. Structure of cefuroxime axetil

In this paper, we describe the labeling of cefuroxime axetil with the most widely used imaging radionuclide, ^{99m}Tc. The radiolabeling was performed by the stannous chloride method. The quality control procedure using thin layer and radio high performance liquid chromatographies and the stability of labelled compound were investigated.

Experimental

Cefuroxime axetil was a gift from Fako A.S. $Na^{99m}TcO_4$ was supplied by the Department of Nuclear Medicine of Ege University, as ^{99}Mo/^{99m}Tc generator eluent (Monrol, Turkey). Other chemicals were purchased from Merck.

Radiolabeling procedure

In the labeling of ^{99m}Tc-cefuroxime axetil, cefuroxime axetil (0.5 mg in 500 µl 3% acetic acid solution) was added to a vial. To determine the optimal amount of reducing agent, 10–100 µg of $SnCl_2 \cdot 2H_2O$ was used; pH was adjusted by using HCI or NaOH. After addition of all reagents, 37–74 MBq $Na^{99m}TcO_4$ was added into the vial. The vial was incubated at room temperature for 20 minutes.

* E-mail: fatma.yurt.lambrecht@ege.edu.tr

0236–5731/USD 20.00

Akadémiai Kiadó, Budapest
Springer, Dordrecht

Radiochemical analysis of ^{99m}Tc-cefuroxime axetil

The labeling yield and radiochemical purity were determined by thin layer and radio high performance liquid chromatographies. The reaction product was spotted on silica gel ITLC-SG strips (10×1.5 cm^2 sheets) and developed in acetone and ACD [citrate-dextrose buffer solution (Eczacibasi-Baxter)] solution. After developing, the strips were covered with cello-bands. Then, they were cut into 0.5 cm pieces and counted with a Cd(Te) detector equipped with a RAD-501 single-channel analyzer.

HPLC analysis

HPLC analysis was performed on a Shimadzu HPLC (LC-10 ATvp) equipped with an SPD 10Avp UV detector, quaternary pump, a 1 ml loop, and Cd(Te) detector equipped with a RAD 501 single-channel analyzer. An analytical RP-C18 column (Macherey-Nagel, EC150/4.6 Nucleosil 100-3C-18) was used. The flow rate was adjusted to 1 ml/min with solvents 0.1% TFA (trifluoroacetic acid) in H_2O (solvent A) and 0.1% TFA in CH_3CN (solvent B). The HPLC gradient system started with a solvent composition of 95% A and 5% B and reached 60% A and 40% B in 15 minutes. UV detector was set to 280 nm.[11,12] HPLC analysis of the labeled compound was conducted using a Cd(Te) detector equipped with a RAD 501 single-channel analyzer. Unlabeled cefuroxime axetil was eluted at a retention time of 2.79 minutes, whereas labeled cefuroxime axetil was eluted a retention time of 2.23 minutes. The chromatograms for ^{99m}Tc-cefuroxime axetil and cefuroxime axetil are shown in Fig. 2.

Stability of ^{99m}Tc-cefuroxime axetil in human serum

The stability of ^{99m}Tc-cefuroxime axetil was examined by mixing 0.3 ml of ^{99m}Tc-cefuroxime and 0.6 ml of human serum. The sample was incubated at 37 °C for 24 hours and was tested during the incubation at 15 min, 1 h, 2 hrs, 3 hrs, 4 hrs and 24 hours. The amount of ^{99m}Tc-cefuroxime axetil in the sample was determined by RTLC using serum physiologic and ACD as eluents and the radioactivity was counted using a Cd(Te) detector.

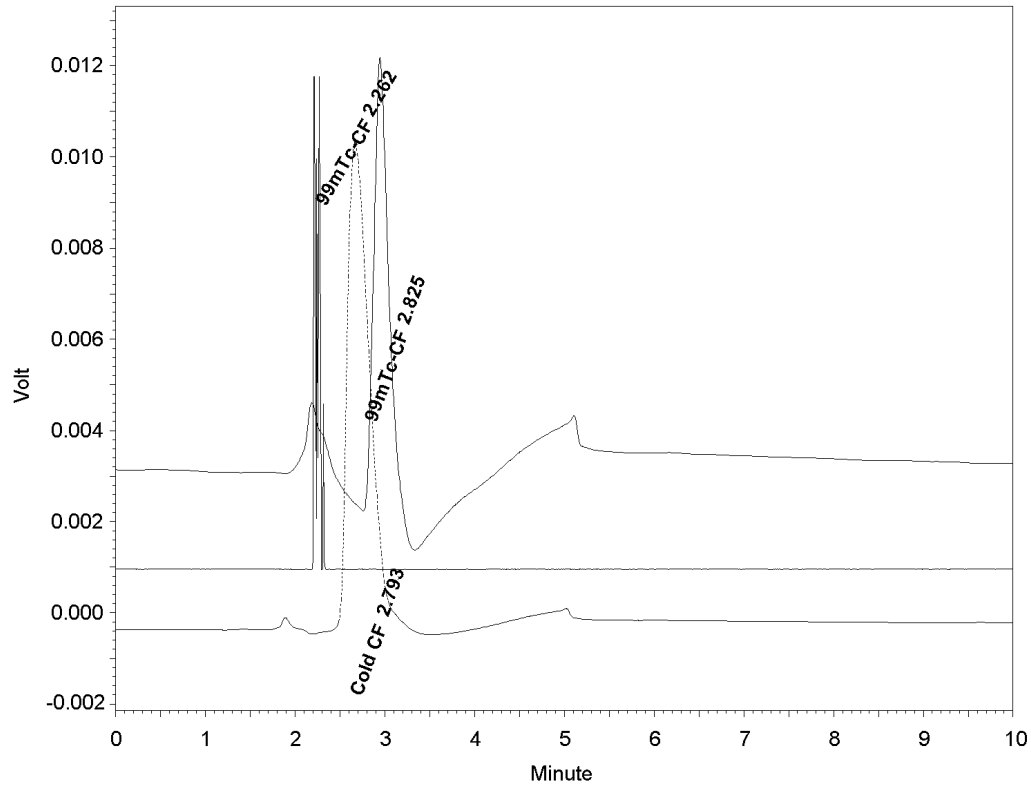

Fig. 2. HPLC chromatogram and RHPLC chromatograms (cefuroxime axetil *rt* = 2.793, and ^{99m}Tc-cefuroxime axetil *rt* = 2.825 at UV detector) (^{99m}Tc-cefuroxime axetil *rt* = 2.262 at radioactivity detector)

Results and discussion

The chemical structure of cefuroxime axetil is presented in Fig. 1. The various complexes of ^{99m}Tc may be formed by interaction between electron donor atoms and reduced technetium. In order to form bonds with technetium, the structure must contain electron donors such as oxygen, nitrogen and sulfur. Although the complex structure is not known, results showed that cefuroxime axetil coordinated with ^{99m}Tc because of its electron donor atoms in the structure. The labeled complex may be formed electron pairs of these atoms with reduced technetium that is +1 or +3 in the reduced states similar to other studies.[6,13]

Labeling efficiency, radiochemical purity and stability were assessed by RTLC and RHPLC chromatographies. In RTLC-SG chromatography using acetone as the solvent, reduced ^{99m}Tc and ^{99m}Tc-cefuroxime axetil remained at the point of spotting, while free $^{99m}TcO_4^-$ moved towards the solvent front. In using ACD solution as solvent, reduced ^{99m}Tc and $^{99m}TcO_4^-$ moved to the front, whereas ^{99m}Tc-cefuroxime axetil remained at the point of spotting.

The effect of pH is shown in Fig. 3. The effect of pH on the radiolabeling yield was examined for pH 2–6. The highest labeling yield is obtained at pH 3. In general, stannous chloride is the most widely used reducing agent. In this state, radiochemical species such as hydrolyzed technetium, ^{99m}Tc-stannous colloids and free pertechnetate are formed especially in alkaline pH medium.[13]

The effects of the amount of stannous chloride are summarized in Fig. 4. The data show that the radiochemical yield was dependent on the amount of $SnCl_2 \cdot 2H_2O$ present in the reaction mixture. The effect of stannous chloride on radiolabeling was studied between 10 μg and 100 μg stannous chloride. The highest labeling efficiency was obtained by using 25 μg of stannous chloride. On the other hand, labeling efficiency decreased at 100 μg stannous chlorides. Therefore radiochemical impurity may be due to formation of tin colloids.[13,14]

The results in human serum showed that the labeling yield of cefuroxime axetil decreased to 92±1.0% and 78±2.0% at 4 and 24 hours, respectively (Fig. 5).

The overall maximum efficiency is achieved at 20 minutes. As a result, cefuroxime axetil was labeled with ^{99m}Tc with high radiochemical yield (98±1.0%).

Conclusions

The radiolabeling yield of ^{99m}Tc-cefuroxime axetil, as measured by chromatographic methods, was 98±1.0%. The labeling of ^{99m}Tc-cefuroxime axetil was optimal in the following reaction mixture: cefuroxime axetil concentration: 0.5 mg/0.5 ml, pH 3, $SnCl_2$ concentration: 25 μg/0.1 ml, reaction time: 20 minutes. The resulting complex of ^{99m}Tc-cefuroxime axetil is quite stable and labelling of 92±1.0% is maintained for up to 4 hours. In conclusion, ^{99m}Tc-cefuroxime axetil may be applied for infection imaging.

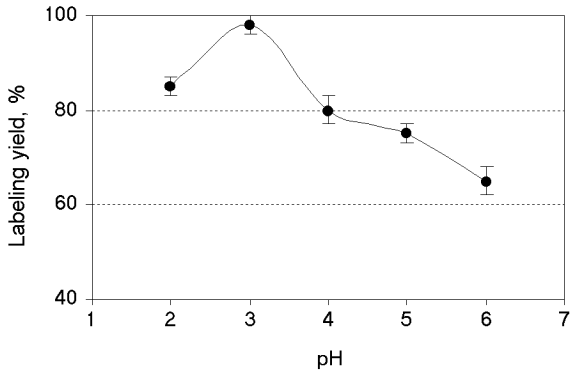

Fig. 3. Effect of pH on the labeling efficiency of ^{99m}Tc-cefuroxime axetil

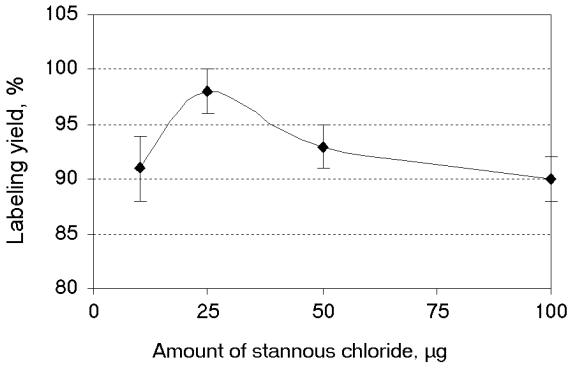

Fig. 4. Effect of stannous chloride amount on the labeling efficiency of ^{99m}Tc-cefuroxime axetil

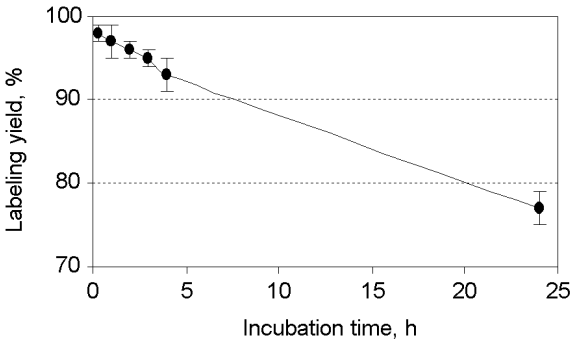

Fig. 5. In-vitro stability of ^{99m}Tc-cefuroxime axetil in human serum

*

Part of this study was presented at the 7th International Symposium on Technetium in Chemistry and Nuclear Medicine, 329-330, Bressanone (BZ), Italy, September 6–9, 2006. The authors gratefully acknowledge the financial support received from the Ege University Department of Scientific Projects.

References

1. H. J. J. M. RENNEN, O. C. BOERMAN, W. J. G. OYEN, F. H. M. CORSTENS, Medicinal Chem. Rev., 1 (2004) 27.
2. S. S. DAS, A. V. HALL, D. D. W. WAREHAM, K. E. BRITTON, Brazil. Arch. Biol. Technol., 45 (2002) 25.
3. A. LUPETTI, M. M. WELLING, E. K. J. PAULWELS, P. H. NIBBERING, Lancet Infection Diseases, 3 (2003) 223.
4. K. ALEXANDER, WM. T. DROST, J. S. MATTOON, J. J. KOWALSKI, J. A. FUNK, A. C. CRABTEE, Can. J. Veter. Res., 69 (2005) 272.
5. R. H. SIAENS, H. J. RENNEN, O. C. BOERMAN, R. DIERCKX, G. SLEGERS, J. Nucl. Med., 45 (2004) 2088.
6. S. ROOHI, A. MUSHTAQ, M. JEHANGIR, S. A. MALIK, J. Radioanal. Nucl. Chem., 267 (2006) 561.
7. P. RUIZ-CARRETERO, A. NACHER, M. MERINO-SANJUAN, V.G. CASABO, Intern. J. Pharm., 202 (2000) 89.
8. V. F. PATEL, N. M. PATEL, AAPS Pharm. Sci.Technol., 7 (2006) 1.
9. P. RUIZ-CARRETERO, M. MERINO-SANJUAN, V. G. CASABO, Eur. J. Pharm. Sci., 21 (2004) 217.
10. P. RUIZ-CARRETERO, A. NACHER, V. G. CASABO, M. MERINO-SANJUAN, Intern. J. Pharm., 234 (2002) 101.
11. I. IVANA, Z. LJILJANA, Z. MIRA, J. Chromat., A1119 (2006) 209.
12. A. JELINSKA, I. DUDZINSKA, M. ZAJAC, I. OSZCZPOWICZ, J. Pharm. Biomed. Anal., 41 (2006) 1075.
13. E. A. EL-GHANY, A. M. AMINE, A. S. EL-SAYED, M. T. EL-KOLALY, F. ABDEL-GELIL, J. Radioanal. Nucl. Chem., 266 (2005) 125.
14. E. A. EL-GHANY, M. T. EL-KOLALY, A. M. AMINE, A. S. EL-SAYED, F. ABDEL-GELIL, J. Radioanal. Nucl. Chem., 266 (2005) 131.