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Ciprofloxacin radiolabeling with technetium-99m

[Préparation d'une trousse radiopharmaceutique de Ciprofloxacine marquée au Technetium-99m]

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Resumé

En République démocratique du Congo et dans la plupart des pays en voie de développement, l'une des causes de mortalité reste l'infection bactérienne non ou mal diagnostiquée, et la médecine nucléaire joue un rôle important au travers de ses méthodes rapides et précises qui utilisent des trousses radiopharmaceutiques pour diagnostiquer des infections profondes. L'objectif de cette étude était de préparer et d'évaluer la stabilité de la ciprofloxacine marquée au technétium-99m (99mTc) en vue de son utilisation dans le service de médecine nucléaire des Cliniques universitaires de Kinshasa pour le diagnostic des foyers infectieux. Le marquage radiologique de la ciprofloxacine a été réalisé en utilisant en mélangeant 20 mci de 99mTc et un mélange de ciprofloxacine (CPF) de concentration 15 mg/mL, une solution contenant 200 mg d'acide ascorbique et 10 mL (0,2 mg/mL) de chlorure d'étain (SnCl2.2H2O) fraîchement préparé dans une solution d'acide Chlorhydrique (2N) à pH=5,8.
Les tests de pureté et de stabilité radiochimique à température ambiante ont été effectués en utilisant la Chromatographie sur Couche Mince (CCM) avec l'activimètre. La pureté radiochimique du produit était ≥ 98 %, et la stabilité après 4 h était de 95 %. En raison de la facilité de la méthode de préparation de 99mTc-CPF, de l'efficacité élevée du marquage. Il peut représenter un candidat prometteur comme trousse radiopharmaceutique pour l'imagerie des foyers infectieux dans le service de médecine nucléaire des Cliniques universitaires.

Mots-clés: Ciprofloxacine, Technetium-99m, Trousse Radiopharmaceutique.

Abstract

In the Democratic Republic of Congo and many developing nations, undiagnosed or inadequately diagnosed bacterial infections continue to be a significant contributor to mortality rates. Nuclear medicine employs rapid and precise techniques utilizing radiopharmaceutical kits, which are essential for the diagnosis of deep infections. The main goal of this study was to make technetium-99m (99mTc)-labeled ciprofloxacin and test its stability. The department of nuclear medicine at Kinshasa University Clinics planned to use this kit to diagnose infection foci. Ciprofloxacin is radiolabeled by combining 20 mCi of 99mTc with a solution of ciprofloxacin (CPF) at a concentration of 15 mg/mL, 200 mg of ascorbic acid, and 10 mL (0.2 mg/mL) of freshly prepared stannous chloride (SnCl.2HO) in a 2N hydrochloric acid solution at pH 5.8.
Thin layer chromatography (TLC) was employed alongside the radiochromatogram to assess radiochemical purity and stability at room temperature. The radiochemical purity of the product exceeded 98%, and its stability after 4 hours was 95%. The preparation method for 99mTc-CPF is straightforward and demonstrates high labeling efficiency. The 99mTc-CPF preparation method may function as an effective radiopharmaceutical kit for imaging infectious foci.

Keywords: Ciprofloxacin, Technetium-99m, Radiopharmaceutical Kit.

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1. Introduction

Nuclear medicine utilizes radioisotopes characterized by short half-lives, which disintegrate by emitting a substantial quantity of photons (100 to 200 KeV). Medical imaging instruments like single-photon emission tomography (SPECT) and gamma cameras effectively detect these emissions, while positron emission enables quantitative imaging with positron emission tomography (PET) (Lambrecht, 1998). Conventional imaging techniques, including radiology and computed tomography (CT), lack the ability to distinguish between inflammatory and infectious processes. Furthermore, these techniques rely on substantial anatomical changes, which limits their potential for early diagnosis (Simone et al., 2005).

J. Malamitsi et al. showed that bone scintigraphy methods that use 99mTc-methylene diphosphonate (MDP) and 67Ga-citrate (67Ga) can't tell the difference between inflammation that isn't caused by bacteria and inflammation that is. To address this issue, we selected ciprofloxacin, a synthetic antibiotic from the fluoroquinolone class, and illustrated its 2D geometry in Figure 1. We acquired the 3D geometry from the PubChem database, identifying it with CID number 2764. It exhibits significant activity against both Gram-positive and Gram-negative bacteria by inhibiting DNA gyrase, thereby preventing DNA synthesis.

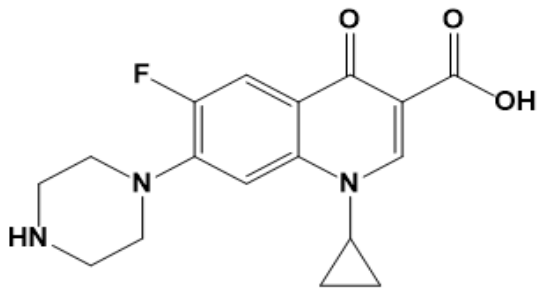

Figure 1. 2D structure of ciprofloxacin (CPF).

The CPF-Tc99m complex is formed with ciprofloxacin serving as a biologically active molecule (vector) for the delivery of diagnostic radiopharmaceutical products to sites of bacterial infection. The CPF-Tc 99m complex has proven effective as a radiotracer for SPECT tomography in diabetic foot syndrome (Koźmiński et al., 2021). The application of radiolabeled antibiotics serves as a valuable diagnostic method for infections, enabling the distinction between septic and aseptic inflammation. Previous studies have noted the application of technetium-99m-ciprofloxacin in diagnosing infections associated with orthopedic devices, joint prosthesis infections, and identifying the etiology of fever of unknown origin (Zhao et al., 2006).

Ciprofloxacin with 99mTc presents itself as a radiopharmaceutical that provides a solution to the issue of differentiating bacterial infections, non-bacterial infections, and even sterile inflammations.

Therefore, the ability to differentiate between infections and other inflammations using a scintigraphy procedure has a major impact on the medical follow-up of patients suspected of having a bacterial infection or not (Zhao et al., 2011).

This work is the result of a collaboration between the University of Kinshasa and the General Commission for Atomic Energy (CGEA) through its Nuclear Medicine Department at the University Clinics of Kinshasa.

The objective of this work is to prepare a 99mTc-Ciprofloxacin kit, with preserved stability and biological activity, capable of identifying a septic focus (*E. coli*) in the experimental infection model.

2. Materials and methods

The Ciprofloxacin used for this study was obtained from Phatkin Laboratory, manufactured by Zhejiang Guobang Pharma/China. All other chemicals were obtained from Sigma-Aldrich and were used without prior purification. The pertechnetate was eluted from an internal alumina-based 99Mo/99mTc generator with saline solution (0.9% NaCl), and its activity was determined in a dose calibrator (Isomed, Germany) according to the British Pharmacopoeia (British Pharmacopoeia, 2009).

The quantitative measurement and quality control of the radioactive activity were performed with the Capintec CRC©-15R Activimeter (figure 2).

Figure 2. Capintec CRC-15R Activimeter

Preparation of 99mTc-CPF

- We prepared a CPF solution (concentration 15 mg/mL) by dissolving CPF in distilled water. From this solution, we added 200 mg of ascorbic acid and 10 mL (0.2 mg/mL) of freshly prepared tin chloride ($SnCl_2 \cdot 2H_2O$) in an HCl (2N) solution. We radiolabel the formulation at room temperature, add 20 mCi of freshly eluted in a maximum of 1 mL of normal saline, and incubate this vial for 10 minutes.

3. Results and discussion

We evaluated the radiochemical purity (RCP) and the stability of the radiopharmaceutical during this work. table I presents the results of the various physicochemical parameters of the ciprofloxacin powder.

Table I. Results of physicochemical analyses of Ciprofloxacin powder

Physical appearance :	White powder		
Physicochemical Tests			
Parameters	Requirements	Obtained results	Testing methods
Solubility: water		Satisfying	ph Eur 6 : 2007
Solubilité : Méthanol		Satisfying	ISO 4159 :1978
pH (2,5%)	3.5-4.5 pH Eur 6:2007	4,05	ISO 4159 :1978
Dessiccation tolerance	Max 6,7% pH Eur 6:2007	4,23%	ISO 672-1978
Identification	Requirements	Obtained results	Testing methods
Ciprofloxacin HCL	Positive	Positive	ph Eur 6 : 2007
Dosage	Requirements	Obtained results	Testing methods
Ciprofloxacin HCL	98,0 – 102,0 %	100,68 %	ph Eur 6 : 2007

The results of table I show that the ciprofloxacin powder is pure and meets the requirements of the cited standards.

Labeling of ciprofloxacin

We characterized the 99mTc-CPF in a 0.9% (w/v) NaCl solution and in terms of radiochemical purity using instant thin-layer chromatography (ITLC). We developed the ITLC plates (silica gel) in acetone. In the same solvent, 99mTc-CPF remains at the application point (Rf ≈ 0.0–0.1) while free $99mTcO^{4-}$ is carried with the solvent (Rf ≈ 1). Once the development process was complete, we dried the two radiochromatograms, cut them into 0.5 cm pieces, and separately analyzed them using the activimeter (Capintec CRC-15R) to determine the PCR of the 99mTc-CPF complex and the free $99mTcO^{4-}$, as presented in table II.

Table II. Quality Control of Tc-99m Labeled Ciprofloxacin

Method	**mobile phase**	**Spotted Volume**	**Migration time**	**Relative migration**	**R_f**
ITLC-SG	Acetone	1 to 5 µL	About 3 minutes	^{99m}Tc-CPF	0,0 - 0,1
				$^{99m}TcO_4^-$	0,9 – 1,0
		Activity (mCi)	Volume (mL)	Bruit de fond (mCi)	Incubation time (min)
E. 01	Acetone	18,59	3	0,01	3
E. 02	Acetone	19,33	3	0,01	3
E. 03	Acetone	20,01	3	0,01	3

The parameters monitored during quality control (table III) for 99mTc-CPF preparations showed acceptable radiochemical purity according to the specifications of the European Pharmacopoeia (Seyedeh, 2012).

Table III. Parameters monitored during quality control

Complex	Appearance	pH	Radiochemical purity*	Humidity level	Sterility
^{99m}Tc-CPF	Clear solution	5.88 (±0.13)	99.33 % (±0.86)	<0,0001%	Sterile

The labeling of CPF with technetium-99m was carried out at room temperature and at different time intervals. The labeling reaction was completed after 10 minutes with a radiochemical yield of 100% (with the radioactivity meter). The formed complex remained stable for up to 4 hours, then the yield decreased again to reach 43.3% after 6 hours (figure III).

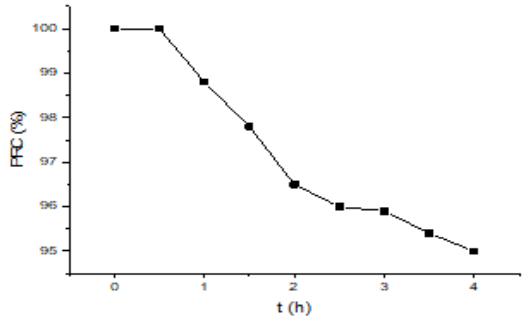

Figure III. Radiochemical purity in terms of time

4. Conclusion

Many radiotracers have been developed to image infectious foci, but few have succeeded in distinguishing an infection from inflammation.

During this work, Ciprofloxacin was prepared and labeled with technetium using a simple method. Based on the physicochemical and biological properties of CPF-based radiopreparations, we can consider that CPF is a good vector for radiopreparations aimed at exploring sites of bacterial infection using SPECT or PET imaging.

The radiotracer was obtained with good radiochemical purity; the labeling stability lasted for several hours and is compatible with potential routine use.

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