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Technical note

Radio-UHPLC: A tool for rapidly determining the radiochemical purity of technetium-99m radiopharmaceuticals?

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HIGHLIGHTS

- Radiochemical purity of 3 technetium-99m radiopharmaceuticals was evaluated using radio-UHPLC.
- Results obtained were in agreement with those obtained using the standard method.
- Analysis time was less than 3 min using radio-UHPLC.
- Radio-UHPLC could be proposed as an alternative technique for radiochemical purity determination.

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ABSTRACT

Determining the radiochemical purity (RCP) of technetium-99m (^{99m}Tc) radiopharmaceuticals using the method described in the package insert is a time-consuming process, requiring particular attention in order to achieve accurate RCP results. The purpose of this study was to evaluate whether radio-ultra high performance liquid chromatography (radio-UHPLC) may be an alternative method for RCP testing of ^{99m}Tc-tetrofosmin, ^{99m}Tc-MAG3 and ^{99m}Tc-sestamibi. Results obtained using radio-UHPLC were in excellent agreement with the standard method, with total analysis time being reduced to less than 3 min.

1. Introduction

The radioisotope most widely used in routine nuclear medicine is technetium 99m (^{99m}Tc). This artificially produced radioisotope has almost ideal characteristics for *in vivo* imaging techniques, with a half-life of 6 h, gamma photon energy of 140 keV, and decay by isomeric transition. In addition, it may be routinely obtained from a generator system using molybdenum-99 (^{99}Mo)/^{99m}Tc. ^{99m}Tc-radiopharmaceuticals are usually prepared in a short time by adding saline pertechnetate ($^{99m}TcO_4^-$) to a cold kit containing a chelating agent and a reducing agent. Radiochemical purity (RCP) should be carefully evaluated immediately prior to use, since impurities may arise during the preparation of technetium radiopharmaceuticals and alter the diagnostic imaging accuracy (Vallabhajosula et al., 2010). Numerous methodologies are available to assess the RCP of radiopharmaceuticals (Tonelli et al., 1994; Zolle, 2007), including thin-layer chromatography (TLC), paper chromatography (PC), and high performance liquid chromatography (HPLC). The appropriate method should be sensitive, reproducible, and able to separate all possible components without changing the sample composition, while obtaining the result in the shortest time, as time is a critical parameter in nuclear medicine. In such a way, the chromatographic procedures described in the package inserts for technetium radiopharmaceuticals are slow, requiring particular attention to achieve accurate results and necessitating approximately 25–40 min. HPLC is a useful technique for accessing radiochemical impurities. The recent development of ultra-HPLC (UHPLC) has provided great potential for rapid analysis without sacrificing resolution. The determination of the RCP of ^{99m}Tc radiopharmaceuticals using radio-UHPLC has not yet been reported.

Hence, the main objective of this study was to investigate whether it was possible to use radio-UHPLC as a rapid technique and alternative to the testing recommended by the manufacturer in

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order to determine the RCP of ^{99m}Tc-labeled radiopharmaceuticals. In particular, the RCP of three technetium preparations was evaluated using radio-UHPLC, notably ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3), which is widely used to determine renal function, ^{99m}Tc-tetrofosmin and ^{99m}Tc-sestamibi, which are used for myocardial perfusion imaging.

2. Materials and methods

2.1. Materials

Chemicals and solvents were obtained from Sigma-Aldrich (St Quentin Fallavier, France) and used as indicated. Isotonic saline was acquired from Lavoisier (Paris, France). $Na^{99m}TcO_4$ solution was obtained by the elution of the ^{99}Mo/^{99m}Tc generator (Elumatic, IBA Cis bio, Saclay, France). Radioactivity was measured using a calibration chamber, Medi 404 (Medisystem, Guyancourt, France). The Myoview® labeling kit was purchased from GE (Buckinghamshire, United Kingdom), while Technescan® MAG3 and Technescan® sestamibi were obtained from Covidien Imaging (Elancourt, France).

2.2. Instruments

2.2.1. Liquid chromatography

The radio-liquid chromatography (LC) system consisted of a Dionex rapid separation liquid chromatography (RSLC) Pump (Dionex, Voisin le Bretonneux, France), containing the conventional LC and UHPLC in a single system, equipped with an autoinjector, UV detector, and gamma flow count detector (Bioscan, Washington DC, USA). Analysis was carried out using a RP C18 column (XTerra™ 5 μm × 4.6 × 250 mm, Waters) for conventional LC and a Waters ACQUITY UPLC® BEH column (1.7 μm × 2.1 × 50 mm). Data collection was performed using the Chromeleon chromatography software package. Table 1 portrays the radio-UHPLC analysis conditions. For conventional LC analysis of ^{99m}Tc-MAG3, the column was eluted at 1 mL min^{-1} with 100%A 0–10 min (A: ethanol/10 mmol L^{-1} phosphate buffer pH 6(5:95); B: methanol/water (90:10)), followed by 0% A for 7 min and finally 100%A for 4 min at 2 mL min^{-1}. For UHPLC analysis of ^{99m}Tc-MAG3, the column was eluted with ethanol/10 mmol L^{-1} phosphate buffer pH 6(5:95) for 1.5 min at 0.5 mL min^{-1} and then methanol/water (90:10) for 1.5 min at 0.5 mL min^{-1}. For the analysis of ^{99m}Tc-tetrofosmin, the ACQUITY UPLC® BEH column was eluted at 0.5 mL min^{-1} with a linear gradient from 100% 10 mmol L^{-1} phosphate buffer (pH=7.5) to 100% tetrahydrofuran. For UHPLC analysis of ^{99m}Tc-sestamibi, the ACQUITY UPLC® BEH column was eluted with a flow rate of 0.8 mL min^{-1} using a gradient system: in 1 min from 50 mmol L^{-1} Ammonium formate: methanol (20:80) to (95:5) and constant composition (95:5) up to 3 min.

For analysis, 2 μL of diluted preparation was applied on the radio-UHPLC system.

Radiochemical purity was determined by expressing the counts in the ^{99m}Tc-radiopharmaceutical peak as a percentage of the total counts in the chromatogram.

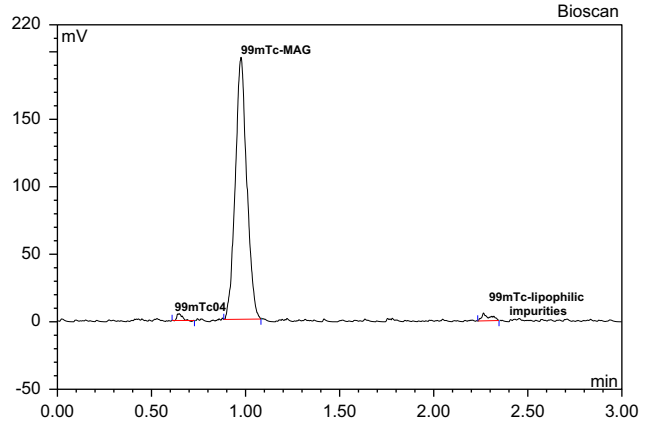

Fig. 1. Radio-ultra high performance liquid chromatography of ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3).

2.2.2. Thin-layer chromatography

The RCP of ^{99m}Tc-tetrofosmin was assayed with a gamma isotope TLC analyzer (Raytest, Courbevoie, France) using silica gel strips (ITLC-SA) developed in two solvent systems (acetone/dichloromethane, [35:65]). Free ^{99m}Tc-pertechnetate moved with the solvent front, while ^{99m}Tc-tetrofosmin complex migrated toward the center of the strip. Hydrolyzed-reduced ^{99m}Tc and any hydrophilic impurities remained at the origin. The RCP of ^{99m}Tc-sestamibi was performed using alumina TLC plates and absolute ethanol as solvent. Hydrolyzed-reduced ^{99m}Tc and free ^{99m}Tc-pertechnetate remain at the origin while ^{99m}Tc-sestamibi is measured at an Rf of 0.9.

2.3. Radiolabeling

2.3.1. Preparation of ^{99m}Tc-MAG3

^{99m}Tc-MAG3 was prepared according to the labeling procedures provided by Covidien by adding generator eluate containing 1100 MBq ^{99m}Tc-pertechnetate to the kit vial and diluting it in 10 mL saline. The labeling mixture was placed in a boiling water bath for 10 min and then allowed to cool.

2.3.2. Preparation of ^{99m}Tc-tetrofosmin

An available commercial kit was reconstituted with 12 GBq $^{99m}TcO_4^-$ in 8 mL saline. The labeling mixture was then shaken, with a complete reaction achieved after a 15 min period at room temperature.

2.3.3. Preparation of ^{99m}Tc-sestamibi

^{99m}Tc-sestamibi was prepared by adding generator eluate (3 mL) containing 11 GBq ^{99m}Tc-pertechnetate to the kit vial. The labeling mixture was placed in a boiling water bath for 10 min and then allowed to cool for 15 min.

2.4. Statistical analysis

The RCP of the 20 preparations for each radiopharmaceutical kit was analyzed in duplicate by two chromatographic techniques, namely radio-UHPLC and the technique recommended by the manufacturer. The results obtained were then compared, with statistical analysis being performed using Student's t-test. Differences with a value of $p < 0.05$ were considered significant.

3. Results and discussion

The determination of RCP in technetium radiopharmaceuticals is relevant, as radiochemical impurities in a radiopharmaceutical preparation may degrade the image quality, resulting in major diagnostic failure. In general, the RCP of ^{99m}Tc-radiopharmaceuticals are often determined using TLC techniques. Nevertheless, such techniques require long preparation times. For this reason, the RCP measurement of three radiopharmaceuticals has been investigated by means of two methods: radio-UHPLC and the technique recommended by the manufacturer.