Optimised quality control method for determination of the radiochemical purity of [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin in a clinical context

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ABSTRACT

Objectives In the context of a supply disruption of mebrofenin (Choledіam)-based kits for radiolabelling with technetium-99m [^{99m}Tc], the medicine agencies allowed the importation of a back-up radiopharmaceutical diagnostic agent, etifenin (Techida), to ensure continuous management of patients with hepatobiliary disorders in nuclear medicine departments. There are still issues regarding the measurement of radiochemical purity (RCP) with these kits based on the European Pharmacopoeia and the Summary of Product Characteristics (SPC). This study aims to identify and to optimise, in a clinical context, the most suitable thin layer chromatography (TLC) method for the determination of the RCP in terms of speed of response and reliability for [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin.

Methods [^{99m}Tc]Tc-etifenin (n=4) and [^{99m}Tc]Tc-mebrofenin (n=5) were individually controlled using six different TLC methods and one high-performance liquid chromatography (HPLC) method for impurity identification ([^{99m}Tc]$(TcO_2)_n$ and Na[^{99m}Tc]TcO_4), RCP (%) and duration of analysis (min). Two TLC methods were selected according to the recommendations of the Pharmacopoeia and SPC, two others were exactly the same but with a heating step, and the other two corresponded to a mix between the methods of the SPC and the Pharmacopoeia that were chosen to optimise RCP determination parameters.

Results Radio-HPLC analysis allowed effective separation of [^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin with a retention time of 8.05±0.02 min and 8.94±0.07 min, respectively, from Na[^{99m}Tc]TcO_4 (retention time 2.76±0.03 min). HPLC showed an absence of Na[^{99m}Tc]TcO_4 for [^{99m}Tc]Tc-mebrofenin and 0.2% for [^{99m}Tc]Tc-etifenin. Among the TLC methods, we identified the most suitable method which ensures the most compliant RCP (98.3±0.9%) in a time of 31.5±1.1 min. Also, it allowed a time saving of 15 min compared with the methods proposed by the Pharmacopoeia and the SPCs.

Conclusion We propose a TLC method that accelerates quality control by an average of 15 min while guaranteeing a reliable RCP.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ [^{99m}Tc]Tc-etifenin is a molecule chemically close to [^{99m}Tc]Tc-mebrofenin which is used to ensure continuous management of patients with hepatobiliary disorders.
⇒ However, the [^{99m}Tc]Tc-etifenin assay is time consuming and not suitable for regular hospital practice.

WHAT THIS STUDY ADDS

⇒ A common quality control method for determining the radiochemical purity is proposed.
⇒ Our proposed TLC-based method ensures radiochemical purity as reliably as radio-HPLC.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This method using heating allows results to be obtained faster than current guidelines, and thus allows for faster administration of the preparations made.

INTRODUCTION

In the context of a supply disruption of mebrofenin (Choledіam)-based kits for radiolabelling with technetium-99m [^{99m}Tc], the medicine agencies allowed the importation of a back-up radiopharmaceutical diagnostic agent, etifenin (Techida), to ensure continuous management of patients in nuclear medicine departments. [^{99m}Tc]Tc-mebrofenin ([^{99m}Tc]Tc-N-(2,4,6-trimethyl 3 bromophenylcarbamoyl) methyl-iminodiacetic acid) is a commonly used radiopharmaceutical for establishing the diagnosis of the hepatobiliary system and the dynamic examination of the hepatobiliary transit.[1 2] [^{99m}Tc]Tc-etifenin ([^{99m}Tc] diethyl-acetanilide-iminodiacetic acid) is a molecule chemically close to [^{99m}Tc]Tc-mebrofenin[3] and has the same indications (figure 1A).[4] The preparation of [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin relies on commercial sterile kits presented in the form of a lyophilised pharmaceutical product.[1 4] Such kits allow [^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin to be easily produced by the addition of sodium pertechnetate (Na[^{99m}Tc]TcO_4), obtained after elution from a molybdenum-99/technetium-99m generator.[1 4]

Radiopharmaceuticals must be controlled according to a validated quality control (QC) method. Radiochemical purity (RCP)—that is, the percentage of ^{99m}Tc that is effectively bound to mebrofenin or etifenin—is determined by identifying radiochemical impurities. If these impurities exceed the defined threshold (>10% for [^{99m}Tc]Tc-etifenin and >5% for [^{99m}Tc]Tc-mebrofenin), they may interfere with the interpretation and eventually lead to repeat examination, causing unnecessary irradiation of the patient.[1 4] Consequently, a straightforward and reliable QC is important before being given to patients and allows the quality of the

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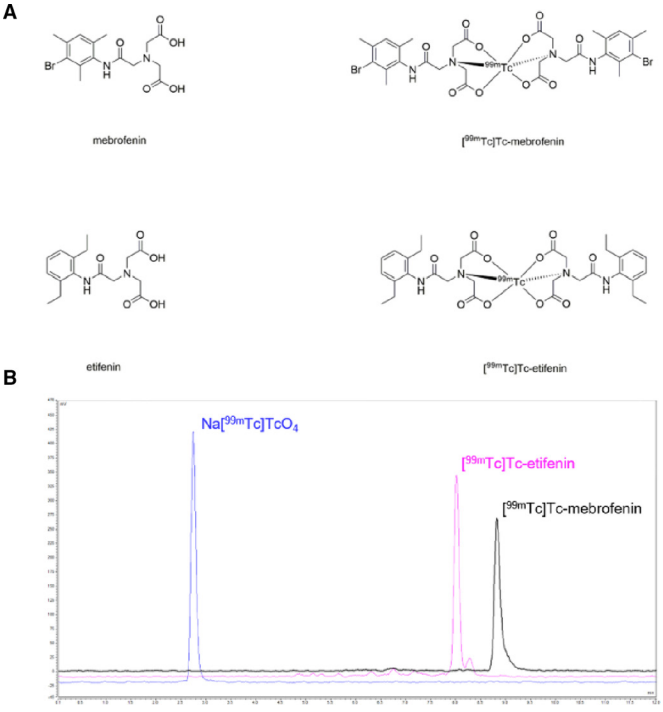

Figure 1 (A) Chemical structure of mebrofenin and [^{99m}Tc]Tc-mebrofenin, and etifenin and [^{99m}Tc]Tc-etifenin. (B) High-performance liquid chromatography (HPLC) radiochromatograms of Na[^{99m}Tc]TcO_4 (blue), [^{99m}Tc]Tc-etifenin (pink) and [^{99m}Tc]Tc-mebrofenin (black) with a retention time of 2.76±0.03 min, 8.05±0.02 min and 8.94±0.07 min, respectively. Absence of Na[^{99m}Tc]TcO_4 impurities was observed on most of the [^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin HPLC radiochromatograms.

examinations to be guaranteed. The RCP of [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin is calculated after quantification of the following radiochemical impurities: reduced technetium [^{99m}Tc]$(TcO_2)_n$ and Na[^{99m}Tc]TcO_4.

Currently, QC methods to measure the RCP of [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin are described in both the Summary of Product Characteristics (SPC)[1 4] and the European Pharmacopoeia.[5 6] The SPC of [^{99m}Tc]Tc-etifenin (IZOTOP) suggests examination by thin-layer chromatography (TLC) using F254 fluorescent silicic acid as the coating substance on a glass plate (Merck 105808)[4] and, for [^{99m}Tc]Tc-mebrofenin, the SPC suggests controlling by TLC with a long drying time.[1] Alternatively, the European Pharmacopoeia recommends for [^{99m}Tc]Tc-mebrofenin using high pressure liquid chromatography combined with a radiodetector (radio-HPLC) to separate and quantify the hydrophilic impurities (Na[^{99m}Tc]TcO_4) and to use TLC with a long drying time (>30 min) to evaluate separately ^{99m}Tc]$(TcO_2)_n$.[5] Finally, for [^{99m}Tc]Tc-etifenin, the European Pharmacopoeia recommends using TLC with a paper for R-chromatography saturated with a sodium bicarbonate solution to assess RCP.[6]

However, there are issues with the RCP measurement proposed by the European Pharmacopoeia and SPC related to nuclear pharmacy practice. On the one hand, radio-HPLC is time consuming and not available in most nuclear medicine departments because of its high cost and its relative complexity. Furthermore, regarding TLC, when it is coupled to fluorescence or when it requires a stage of preliminary saturation of the stationary phase or in the event of drying with free air, these methods are hardly adaptable to the current practice because of a long handling time.

Different teams have published a TLC-based QC method[7 8] but none has reported a suitable method for both [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin in the context of hospital radiopharmacies. Thus, to compensate for these poorly optimised recommendations for routine use, the objective of our study is to identify the most suitable method for determining the RCP in terms of speed of response and reliability for [^{99m}Tc]Tc-mebrofenin, and assessment of its transferability for [^{99m}Tc]Tc-etifenin.

MATERIALS AND METHODS

[^{99m}Tc]Tc-mebrofenin (n=5) and [^{99m}Tc]Tc-etifenin (n=4) were individually controlled by six different TLC methods and a radio-HPLC method, allowing the identification of the following impurities: reduced technetium [^{99m}Tc]$(TcO_2)_n$ and free sodium pertechnetate Na[^{99m}Tc]TcO_4.

Preparation and radiolabelling

[^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin were prepared from kits and a ^{99m}Tc saline eluate obtained from a Tekcis molybdenum-99/technetium-99m generator (CIS Bio International) according to the labelling procedure version 2017. For [^{99m}Tc]Tc-etifenin, [^{99m}Tc]TcO_4 was eluted (800–1600 MBq/mL) in no more than 5 mL volume.[4] For [^{99m}Tc]Tc-mebrofenin, [^{99m}Tc]TcO_4 was prepared using the eluate (740–3700 MBq/mL), also in no more than 5 mL volume.[1] After shaking, preparations were incubated at room temperature for 30 min for [^{99m}Tc]Tc-mebrofenin and 15 min for [^{99m}Tc]Tc-etifenin.[1 4]

TLC methods

To perform TLC, 10 µL of the sample was deposited on the specific stationary phase (1.5 cm wide and 8 cm long paper used and marked with a pencil 1.5 cm from the bottom at the sample deposition point and 1.5 cm from the top at the solvent front), and then placed in stoppered development tanks containing mobile phases and left to equilibrate. When the mobile phases reached the solvent front, the strips were removed from the tanks and measured using a radiochromatograph (MiniGita, Elysia Raytest). RCP was calculated as the percentage of [^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin of the total area under the radiochromatograms (Gina Star TLC, Elysia Raytest). Each radiolabelled compound was identified by its retention factor (Rf), defined as the distance at which the compound migrates on the stationary phase divided by the distance travelled by the mobile phase. Chemicals were obtained from Sigma Aldrich unless otherwise specified, and HPLC-grade mobile phases were used.

Two TLC reference methods were selected according to the recommendations of the European Pharmacopoeia without saturation of the stationary phase (Method 1) and according to the recommendations of the SPC (Method 2). The interest in choosing these two methods is to be able to compare them and thus choose which of the two methods gives the best results.

Method 1

According to the European Pharmacopoeia for [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin, the determination of RCP should be performed by a two-strip TLC.[5 6] The recommended mobile phases were water and acetonitrile 40:60% for the detection of [^{99m}Tc]$(TcO_2)_n$ and acetone for the detection of Na[^{99m}Tc]TcO_4. The European Pharmacopoeia mentions as stationary phase a chromatography paper R and a preliminary saturation of the chromatography paper R with a solution of sodium bicarbonate (25 g/L).[5 6] A silica gel instant TLC paper (ITLC-SG) was used without prior saturation, in a way that guarantees reproducibility. We then carried out

drying with free air for 30 min. The different Rf values identified were 0.0–0.2 for [^{99m}Tc]$(TcO_2)_n$ on the first strip and 0.8–1.0 for Na[^{99m}Tc]TcO_4 on the second strip. The overall duration of the QC was 40 min (40 min for the search of Na[^{99m}Tc]TcO_4 and 31 min for the search of [^{99m}Tc]$(TcO_2)_n$).

Method 2

Determination of RCP was performed by two-strip TLC following the recommended mobile and stationary phases: ammonium acetate 10% and methanol 1:1%, according to the SPC of [^{99m}TcTc-mebrofenin, and acetone (in place of methyl ethyl ketone (MEK) indicated in the SPC) with Whatman 3 MM CHR (GE Healthcare). After spotting, the plate was left to air dry for 30 min. The different Rf values identified were 0.0–0.2 for [^{99m}Tc]$(TcO_2)_n$ on the first strip and 0.8–1.0 for Na[^{99m}Tc]TcO_4 on the second strip. The overall duration of the QC was 50 min (50 min for the search of Na[^{99m}Tc]TcO_4 and 33 min for the search of [^{99m}Tc]$(TcO_2)_n$).

In order to optimise the methods recommended by the Pharmacopoeia and SPC, we have proposed two alternative methods (Methods 3 and 4).

Method 3

We chose ITLC-SG chromatography strips as the paper for chromatography R mentioned in the Pharmacopoeia (Agilent Technologies). The mobile phases consisted of a mixture of water and acetonitrile 40:60% and acetone. After spotting, the plate was heated to 120°C for 5 min (heating block, GetHot 100, SB2). The different Rf values identified were 0.0–0.2 for [^{99m}Tc]$(TcO_2)_n$ on the first strip and 0.8–1.0 for Na[^{99m}Tc]TcO_4 on the second strip. The overall duration of the QC was 15 min (15 min for the search of Na[^{99m}Tc]TcO_4 and 7 min for the search of [^{99m}Tc]$(TcO_2)_n$).

Method 4

The stationary phase was Whatman 3MM CHR chromatography paper (GE Healthcare) and the mobile phase consisted of a mixture of acetate ammonium 10% and methanol 1:1% on the one hand and of acetone on the other. After spotting, the plate was heated to 120°C for 5 min. The different Rf values identified were 0.0–0.2 for [^{99m}Tc]$(TcO_2)_n$ on the first strip and 0.8–1.0 for Na[^{99m}Tc]TcO_4 on the second strip. The overall duration of the QC was 25 min (25 min for the search of Na[^{99m}Tc]TcO_4 and 8 min for the search of [^{99m}Tc]$(TcO_2)_n$).

Finally, we have identified combinations to search for impurities that guarantee a low handling time and a RCP in accordance with the recommendations (Methods 5 and 6).

Method 5

For the detection of [^{99m}Tc]$(TcO_2)_n$, the mobile phases consisted of a mixture of water and acetonitrile 40:60% with ITLC-SG paper and, for the detection of Na[^{99m}Tc]TcO_4, the mobile phases consisted of acetone with Whatman 3MM CHR chromatography paper (GE Healthcare). After spotting, the two strips were heated to 120°C for 5 min. The overall duration of the QC was 25 min (25 min for the search of Na[^{99m}Tc]TcO_4 and 7 min for the search of [^{99m}Tc]$(TcO_2)_n$).

Method 6

For the detection of [^{99m}Tc]$(TcO_2)_n$, the mobile phases consisted of a mixture of water and acetonitrile 40:60% with ITLC-SG paper. Drying with free air was then recommended, which was carried out for 30 min. For the detection of Na[^{99m}Tc]TcO_4, the mobile phases consisted of acetone with Whatman 3MM CHR chromatography paper (GE Healthcare) heated to 120°C for 5 min. The overall duration of the QC was 31 min (25 min for the search of Na[^{99m}Tc]TcO_4 and 31 min for the search of [^{99m}Tc]$(TcO_2)_n$).

Radio-HPLC method

RCP was first determined using a reverse-phase HPLC (RPHPLC) system (Ultimate 3000; Dionex/Thermo Fischer Scientific, USA) coupled to a multiwavelength detector (DAD-3000; Dionex) set at 254 nm and a dedicated radio flow monitor (HERM LB 500, Berthold, Germany). A Hypersil gold column (100×4.6 mm, 3 μm; Thermo Fischer Scientific) was used. The flow rate was 0.8 mL/min with an isocratic mobile phase including 95% A (0.1% formic acid (v/v) in water) and 5% B (0.1% formic acid (v:v) in acetonitrile) over the first 5 min, followed by the linear gradients: 5–50% B (5–11 min) and 50–95% B (11–12 min). The RCP was calculated as the percentage of [^{99m}Tc]Tc-etifenin or [^{99m}Tc]Tc-mebrofenin of the total area under the radio-detector curve.

Statistical analysis

All data are presented as mean±SD. Statistical significance between experimental groups ($p<0.05$) was assessed using GraphPad Prism 7 software. The RCP measurements were analysed using repeated measures ANOVA and the duration of each QC method was compared using one-way ANOVA analysis followed by the Tukey post-hoc multiple comparisons test. The percentage of Na[^{99m}Tc]TcO_4 was compared between TLC methods versus radio-HPLC as the reference using repeated measures ANOVA followed by Dunnett's post-hoc multiple comparisons test.

RESULTS

Radio-HPLC analysis allowed effective separation of [^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin with a Rt of 8.05±0.02 min and 8.94±0.07 min, respectively, from Na[^{99m}Tc]TcO_4 (Rt = 2.76±0.03 min) (figure 1B).

Quantitative results (%) for the six QC methods were measured and compared in terms of Na[^{99m}Tc]TcO_4 (figure 2A,B), [^{99m}Tc]$(TcO_2)_n$ (figure 2C,D), RCP (%) (figure 3) and overall duration of analysis (min) (figure 4) for [^{99m}Tc]Tc-etifenin and [^{99m}Tc]Tc-mebrofenin. The percentage of radiolabelled impurities during the shelf life of each kit was also measured (see online supplemental figure S1).

For [^{99m}Tc]Tc-mebrofenin, the percentage of Na[^{99m}Tc]TcO_4 was 16.0±6.1 for Method 1, 1.5±2.1 for Method 2, 5.4±2.1 for Method 3, 0.3±0.4 for Method 4 and none for HPLC. There was no significant difference between Method 2 and HPLC on the one hand and Method 4 and HPLC on the other hand, as determined by repeated measures ANOVA ($p>0.05$) (figure 2A). For [^{99m}Tc]Tc-etifenin, the percentage of Na[^{99m}Tc]TcO_4 was 11.2±1.9 for Method 1, 2.3±1.6 for Method 2, 9.2±0.7 for Method 3, 1.3±0.5 for Method 4 and 0.2±0.44 for HPLC. There was no significant difference between Method 4 and HPLC as determined by repeated measures ANOVA ($p>0.05$) (figure 2B).

For [^{99m}Tc]Tc-mebrofenin, the percentage of [^{99m}Tc]$(TcO_2)_n$ was 0.4±0.4 for Method 1, 3.2±2.2 for Method 2, 1.6±1.0 for Method 3, and 1.6±1.3 for Method 4. There was a significant difference between Methods 1 and 2 ($p<0.001$) and between Methods 1 and 3 ($p<0.01$) (figure 2C). For [^{99m}Tc]Tc-etifenin, the percentage of [^{99m}Tc]$(TcO_2)_n$ was 0.7±0.3 for Method 1, 3.6±0.9 for Method 2, 3.9±1.5 for Method 3 and 4.7±1.5 for Method

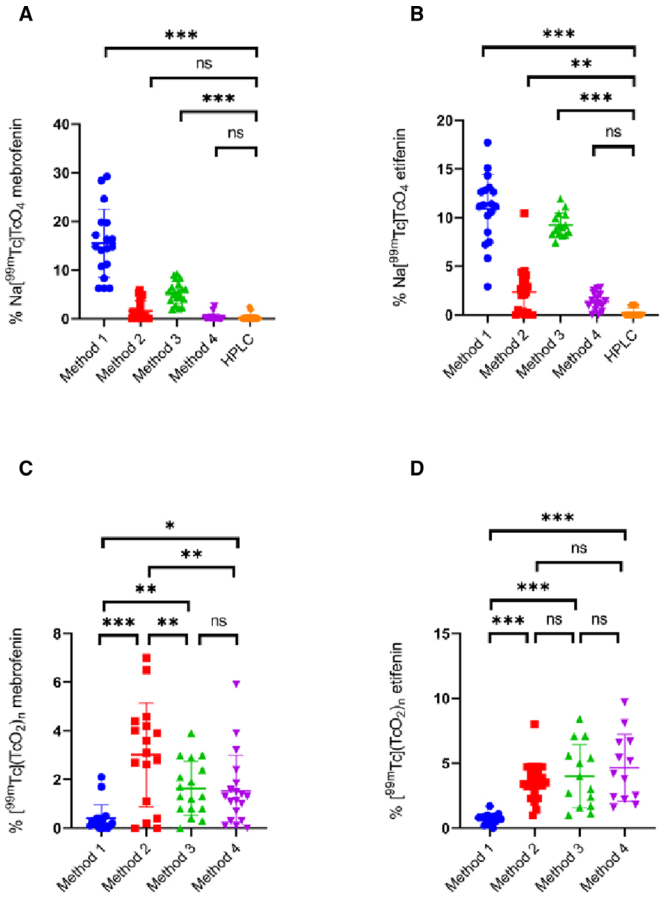

Figure 2 (A) Comparison of the percentage of Na[^{99m}Tc]TcO_4 in [^{99m}Tc]Tc-mebrofenin between Methods 1, 2, 3 and 4 and radio-high-performance liquid chromatography (radio-HPLC). Methods 5 and 6 use the same thin layer chromatography (TLC) characteristics as Method 4 for the search of Na[^{99m}Tc]TcO_4. A statistically significant difference was observed by the multiple comparison test between Method 1 and radio-HPLC ($p<0.001$) and between Method 3 and radio-HPLC ($p<0.001$). (B) Comparison of the percentage of Na[^{99m}Tc]TcO_4 in [^{99m}Tc]Tc-etifenin between Methods 1, 2, 3 and 4 and radio-HPLC. Methods 5 and 6 use the same TLC characteristics as Method 4 for the search of Na[^{99m}Tc]TcO_4. A statistically significant difference was observed by the multiple comparison test between Method 1 and radio-HPLC ($p<0.001$), between Method 2 and radio-HPLC ($p<0.01$) and between Method 3 and radio-HPLC ($p<0.001$). (C) Comparison of the percentage of [^{99m}Tc]$(TcO_2)_n$ in [^{99m}Tc]Tc-mebrofenin between Methods 1, 2, 3 and 4. Method 5 uses the same TLC characteristics as Method 3, and Method 6 uses the same TLC characteristics as Method 1 for the search of [^{99m}Tc]$(TcO_2)_n$. A statistically significant difference was observed by the multiple comparison test between Method 1 and Method 3 ($p<0.01$), and between Method 1 and Method 2 ($p<0.001$). (D) Comparison of the percentage of [^{99m}Tc]$(TcO_2)_n$ in [^{99m}Tc]Tc-etifenin between Methods 1, 2, 3 and 4. Method 5 uses the same TLC characteristics as Method 3, and Method 6 uses the same TLC characteristics as Method 1 for the search for [^{99m}Tc]$(TcO_2)_n$. A statistically significant difference was observed by the multiple comparison test between Methods 1 and 2 ($p<0.001$) and between Methods 1 and 3 ($p<0.001$). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

4. There was a significant difference between Methods 1 and 2 ($p<0.001$) and between Methods 1 and 3 ($p<0.001$) (figure 2D).

For [^{99m}Tc]Tc-mebrofenin, the following RCP values (%) were obtained: 83.6±6.2 for Method 1, 95.3±3.3 for Method 2, 93.0±2.7 for Method 3, 98.1±1.6 for Method 4, 98.1±1.3 for Method 5 and 99.3±0.4 for Method 6. A statistically significant

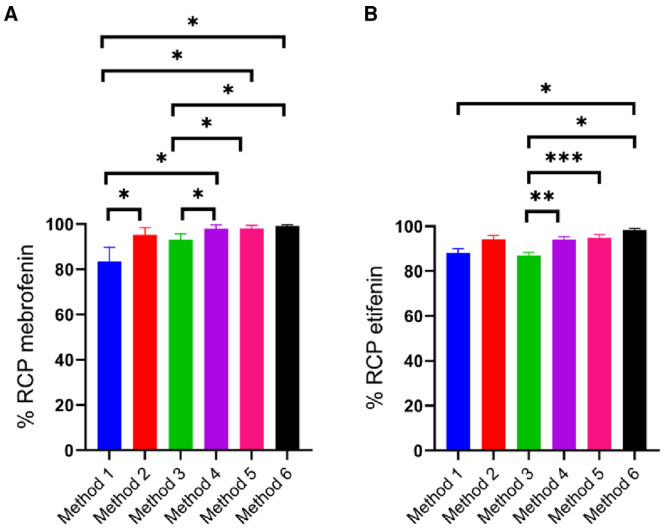

Figure 3 (A) Comparison of the radiochemical purity (RCP, %) of [^{99m}Tc]Tc-mebrofenin between Methods 1, 2, 3, 4, 5 and 6. A statistically significant difference was observed by the multiple comparison test between Method 6 and Method 3 ($p<0.05$) and between Method 6 and Method 1 ($p<0.05$). (B) Comparison of the RCP (%) of [^{99m}Tc]Tc-etifenin between Methods 1, 2, 3, 4, 5 and 6. A statistically significant difference was observed by the multiple comparison test between Method 6 and Method 3 ($p<0.05$) and between Method 6 and Method 1 ($p<0.05$). *$p<0.05$; **$p<0.01$; ***$p<0.001$.

difference was observed by the multiple comparison test between Methods 5 and 3 ($p<0.05$), Methods 5 and 1 ($p<0.05$), Methods 6 and 3 ($p<0.05$) and between Methods 6 and 1 ($p<0.05$) (figure 3A). Regarding [^{99m}Tc]Tc-etifenin, the following RCP values (%) were obtained: 88.1±1.9 for Method 1, 94.2±1.7 for Method 2, 86.9±1.3 for Method 3, 93.9±1.4 for Method 4, 94.7±1.6 for Method 5 and 98.3±0.9 for Method 6. A statistically significant difference was observed by the multiple comparison test between Methods 3 and 4 ($p<0.01$), between Methods 5 and 3 ($p<0.001$), between Methods 6 and 3 ($p<0.05$) and between Methods 6 and 1 ($p<0.05$) (figure 3B).

The overall duration of analysis for [^{99m}Tc]Tc-mebrofenin was as follows: 40.0 ± 0.0 min for Method 1, 52.9±6.9 min for Method 2, 15.0±0.0 min for Method 3, 25.6±2.5 min for Method 4,

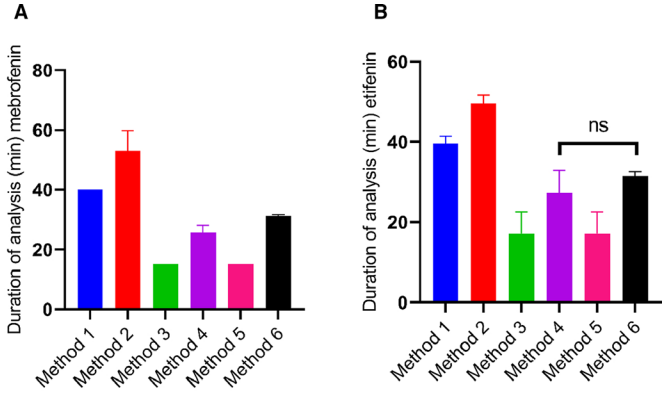

Figure 4 Comparison of the different analysis duration (min) for the quality control methods studied of [^{99m}Tc]Tc-mebrofenin (A) and [^{99m}Tc]Tc-etifenin (B). All methods showed a statistically significant difference in head-to-head comparison except between Method 4 and Method 6 for [^{99m}Tc]Tc-etifenin analysis.

25.6±2.5 min for Method 5 and 31.2±0.4 min for Method 6. All durations were significantly different in head-to-head comparison (figure 4A). For [^{99m}Tc]Tc-etifenin, the duration of analysis was as follows: 39.6±1.9 min for Method 1, 49.5±2.2 min for Method 2, 17.1±5.4 min for Method 3, 27.1±5.4 min for Method 4, 27.1±5.4 min for Method 5 and 31.5±1.1 min for Method 6. All method durations were significantly different in head-to-head comparison except for Methods 4 and 6 (figure 4B).

DISCUSSION

Determination of the RCP is critical in the context of clinical radiopharmacy and requires simple, quick and reliable techniques of analysis. Issues that limit the determination of [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin RCP such as prior saturation of the stationary phase and long handling time[1 4–6] have fostered the need to optimise the existing QC methods. We previously tested TLC methods immediately after application of the radiopharmaceutical without drying, but the analysis time exceeded 50 min and the results were not reproducible. Our study provided a head-to-head comparison of six different TLC analytical QC procedures suited to hospital radiopharmacies preparing [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin together with radio-HPLC. As [^{99m}Tc]Tc-mebrofenin is chemically close to [^{99m}Tc]Tc-etifenin, it seemed appropriate to develop a common QC method for both radiopharmaceuticals.[2]

Acetone and MEK have very similar characteristics, but we chose to use acetone instead of MEK for several reasons. Acetone is more soluble in water and is cheaper to use than MEK. Furthermore, acetone has a faster evaporation rate, which saves time. All of these reasons allow the dosing method to be optimised.

Radio-HPLC showed an absence or very low amount of Na[^{99m}Tc]TcO$_4$ in both preparations. Thus, Methods 1 and 3 tend to overestimate the true proportion of Na[^{99m}Tc]TcO$_4$ for [^{99m}Tc]Tc-mebrofenin due to the significant difference between these two methods and HPLC ($p<0.001$). Methods 2 and 4 are in agreement with the results obtained by radio-HPLC for [^{99m}Tc]Tc-mebrofenin. As Methods 5 and 6 use the same TLC characteristics as Method 4 for the detection of Na[^{99m}Tc]TcO$_4$, they are also in accordance with the results obtained by radio-HPLC for [^{99m}Tc]Tc-mebrofenin. With regard to [^{99m}Tc]Tc-etifenin, Methods 1, 2 and 3 tend to overestimate the true proportion of Na[^{99m}Tc]TcO$_4$ due to the significant difference between these three methods and HPLC ($p<0.001$ for Methods 1 and 3 and $p<0.01$ for Method 2). Thus, Methods 4, 5 and 6 are in accordance with the results obtained by radio-HPLC for [^{99m}Tc]Tc-etifenin. In addition, Method 4 and, by extension, Methods 5 and 6 use a heating step that saves 25 min compared with Method 2 without heating and with the same TLC characteristics.

Concerning the identification of [^{99m}Tc](TcO$_2$)$_n$, Method 1 had a significantly lower amount of these insoluble impurities compared with the other methods for both [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin. As Method 6 uses the same TLC characteristics as Method 1 for the identification of [^{99m}Tc](TcO$_2$)$_n$, they are the most reliable of the methods studied for determining the percentage of [^{99m}Tc](TcO$_2$)$_n$.

Finally, we found that Method 6 represents the best compromise between the reliable quantification of ^{99m}Tc impurities and the duration of analysis (overall QC duration about 30 min), allowing a saving of about 15 min compared with the methods proposed by the Pharmacopoeia and the SPC (Methods 1 and 2). Moreover, Method 6 had the highest radiochemical purity for both radiopharmaceuticals and was compliant with the SPC characteristics.

The tested TLC methods and the proposed optimised TLC methods presented here were selected because they are derived from regulatory approved documents—namely, the European Pharmacopoeia and SPC. However, the original TLC methods using other stationary and mobile phases may also represent interesting alternatives in a clinical context and warrant further comparative research.[9 10]

CONCLUSIONS

Heating the stationary phase accelerates the quantification of Na[^{99m}Tc]TcO$_4$ and [^{99m}Tc](TcO$_2$)$_n$ by an average of 15 min while guaranteeing a reliable RCP in accordance with the recommendations. We therefore advise the use of Method 6 described in this study for the QC of both [^{99m}Tc]Tc-mebrofenin and [^{99m}Tc]Tc-etifenin.

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REFERENCES

1 Summary of Product Characteristics for Choledium Kit for Radiopharmaceutical Preparation, 2017. Available: http://agence-prd.ansm.sante.fr/php/ecodex/rcp/R0302099.htm
2 Kula M, Karacavus S, Baskol M, *et al*. Hepatobiliary function assessed by 99mTc-mebrofenin cholescintigraphy in the evaluation of fibrosis in chronic hepatitis: histopathological correlation. *Nucl Med Commun* 2010;31:280–5.
3 van Wyk AJ, Fourie PJ, van Zyl WH, *et al*. Synthesis of five new 99mTc-HIDA isomers and comparison with 99mTc-HIDA. *Eur J Nucl Med* 1979;4:445–8.
4 Summary of Product Characteristics for Techida Kit for Radiopharmaceutical Preparation, 2017. Available: http://www.izotop.hu/pdf/spc/techida_a_20170712.pdf
5 European Pharmacopoeia 2.2.27. Technetium (99mTc) mebrofenin injection monograph, P 4345, 2019
6 European Pharmacopoeia 2.2.27. Technetium (99mtc) etifenin injection monograph, P. 1283. 2008.
7 Jovanović V, Konstantinovska D, Memedović T. Determination of radiochemical purity and stability of 99mTc-diethyl HIDA. *Eur J Nucl Med* 1981;6:375–8.
8 Billinghurst MW, Eckert K, Mang'era K. Alternative quality control for technetium-99m IDA complexes. *Appl Radiat Isot* 2004;61:1151–5.
9 Ghibellini G, Leslie EM, Pollack GM, *et al*. Use of Tc-99m mebrofenin as a clinical probe to assess altered hepatobiliary transport: integration of in vitro, pharmacokinetic modeling, and simulation studies. *Pharm Res* 2008;25:1851–60.
10 Kowalsky R, Falen S. *Radiopharmaceuticals in nuclear pharmacy and nuclear medicine*. American Pharmacists Association, 2004.

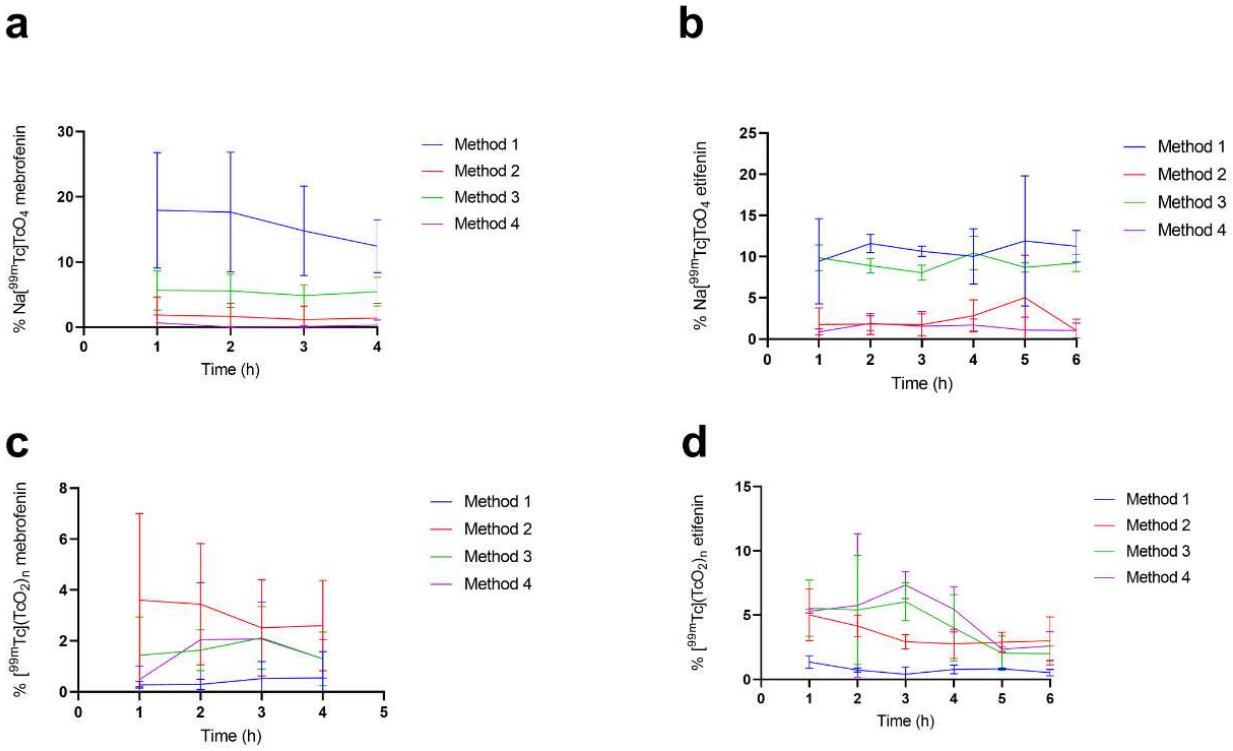

Fig. S1. Evolution of the percentage of Na[^{99m}Tc]TcO_4 and [^{99m}Tc]$(TcO_2)_n$ as a function of time following the radiolabeling of [^{99m}Tc]Tc-mebrofenin (**a** and **c**) and [^{99m}Tc]Tc-etifenin (**b** and **d**), according to methods 1 to 4.