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Preparation, radiochemical purity control and stability of ^{99m}Tc-mertiatide (Mag-3)

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Background: Scintigraphic image analysis of ^{99m}Tc-mertiatide (Mag-3, mercaptoacetyltriglycine) clearance provides the determination of the blood flow, the tubular transit time and the excretion as well from both kidneys. Radiopharmaceutical routine recommends a radiochemical purity control before administration of the product to a patient. The main objective of this study is to develop a Mag-3 labeling procedure that fits better than the previous one in our daily routine production of radiopharmaceuticals. ***Methods:*** Increasing proportions of ^{99m}Tc-Mag-3 were measured during the heating and cooling steps of the Mag-3 labeling procedure. HPLC analysis was used to confirm the results of a rapid radiochemical quality control assay on standard ITLC-SG paper. ***Results:*** The reconstitution time takes 20–25 minutes from the harvest of pertechnetate to a ready-for-use calibrated patient syringe. The HPLC profile of ^{99m}Tc-Mag-3 including its minor impurities remains unchanged for 24–48 hours after reconstitution. ***Conclusions:*** The application of a programmable Peltier-directed device for heating/cooling provides a better control of the temperature course. The procedure proposed fully meets the labeling criteria recommended by the supplier and can be performed with a minimum of attention within a time-span that we formerly needed for solely the radiochemical purity control assay. Moreover, ^{99m}Tc-Mag-3 prepared in this way seems to be considerably more stable than mentioned in the manufacturer's instructions.

Key words: renal scintigraphy, radiopharmaceutical, technetium-99m-Mag-3 stability, chromatography, radiochemical purity/quality control

INTRODUCTION

EFFICIENT ^{99m}Tc-labeling of Mag-3 requires the incubation in boiling water immediately after addition of pertechnetate to the mertiatide vial followed by cooling down to room temperature in cold water.[1] Heating blocks without cooling facilities may operate inefficiently during heat transfer and unforced cooling-down is time-consuming. After radiolabeling, HPLC analysis is recommended by the manufacturer in order to quantify the radiochemical purity of the preparation before its administration to patients.[1] A simpler alternative is offered by TLC, but this assay requires about 3 hours for development of the reversed phase plates.[1] Also, differential extraction from Sep Pak C18 cartridges[2] is widely applied in daily practice. In fact, the preparation of ^{99m}Tc-Mag-3 is a rather laborious radiopharmaceutical protocol compared to tetrofosmin,[3] oxidronate (HDP) and the albumin-based polymers. Here, we describe the labeling of Mag-3 by putting the vial in a tight-fitting brass pig on top of an

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Abbreviations used: High Performance Liquid Chromatography (HPLC); Thin Layer Chromatography (TLC); Instant Thin Layer Chromatography—Silica Gel (ITLC-SG); (Radiochemical) Quality Control ((R)QC).

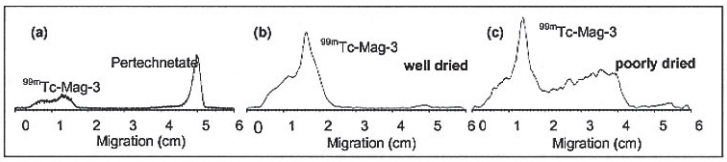

Fig. 1 Radiochemical purity analysis of ^{99m}Tc-Mag-3 on ITLC-SG strips developed in 2-butanone/ethylacetate (40/60% v/v). (a) Profile of ^{99m}Tc-Mag-3 and pertechnetate spotted next to each other on the same strip. (b) Typical profile of a ^{99m}Tc-Mag-3 preparation after being thoroughly dried on the strip before chromatography. (c) The same preparation on a strip poorly dried before chromatography. The shoulder at the left side of the Mag-3 peak is observed in most Mag-3 preparations.

automated, Peltier-directed hotplate. The program of the device directs an unattended transfer from heating to forced cooling down. Measurement of the rates of heat transfer allows a detailed documentation of the temperature course. The time-dependent increase of the Mag-3 labeling efficiency is determined by means of rapid analysis on ITLC-SG paper.[4] As a result, the entire procedure takes as little as 20–25 minutes with an accurate logging of relevant data. Finally, we show by means of HPLC analysis that the labeling properties of ^{99m}Tc-Mag-3 prepared in this way remain unchanged for 24–48 hours after reconstitution.

MATERIALS AND METHODS

Preparation of ^{99m}Tc-Mag-3

Technescan® MAG3 (mertiatide, DRN 4334) was supplied as a ready-for-labeling kit (Tyco Healthcare, Mallinckrodt Medical, Petten, the Netherlands). ^{99m}Tc-pertechnetate was obtained daily from a ^{99}Mo-carrying Utratechnekov® FM generator from the same supplier. Elutions of the generator and amounts added to the reaction vials were done in accordance with the instructions of the manufacturer. Amounts of 1–45000 MBq of ^{99m}Tc were measured in a VDC-405 dose calibrator (Veenstra Instruments, Joure, the Netherlands). A Thermostat*Plus* (Eppendorff) was used for temperature control. A tight-fitting brass pig (thickness 3 mm) provides efficient heating and cooling of the vial as well as an appropriate shielding from radiation. A dual-input thermocouple thermometer with data logging (Fluke 54II) was used to record the temperature course at the inside of a vial.

Radiochemical purity assay

The preparations under investigation were spotted on ITLC-SG strips (1 × 6.5 cm, Gelman, Ann Arbor, USA) at a 1 cm mark. The strips were developed in a mobile phase of 2-butanone/ethylacetate (40/60% v/v)[4] until the solvent front had reached the 5–6 cm marks. After chromatography, the labeling efficiencies were determined by means of a miniGITA scanning device controlled by the GITA v1.64 computer program (Raytest, Hamburg, Germany). Alternatively, the strips were cut and the count rates of the upper and lower parts were determined in a Packard 5650 AutoGamma counter.

HPLC analysis

The HPLC equipment consists of a Gilson 234 autosampler, a binary HPLC pump (Perkin Elmer series 200), a guarded Supelcosil LC18-S column (250 × 4.6 mm, 5 μm; Supelco) and a Radiomatic 525TR detector with a 500 μl cell (Packard). The mobile phases were 0.05 M NaH_2PO_4 pH 4.5 (A) and H_2O/CH_3OH (10/90% v/v, B). A linear gradient (100% A – 100% B) was applied during 20 minutes followed by 100% B for 5 minutes at a flow rate of 1 m*l*/min. The eluant was mixed with an equal volume of Ultima Flo-AP liquid scintillation fluid (Packard) before entering the Radiomatic detector. Injected volumes varied between 10 and 100 μl. Quantifications of the analyses were performed by means of Flow-one for Windows v3.61 (Packard).

RESULTS

Preparation and instant quality control of ^{99m}Tc-Mag-3

The determination of labeling efficiencies during the formation of the ^{99m}Tc-Mag-3 conjugate requires an instant method for the measurement of the radiochemical purity. Instant thin-layer silica gel strips (ITLC-SG) are widely used for these purposes. For the appropriate separation of ^{99m}Tc-Mag-3 from unbound pertechnetate, these strips can be developed in a mobile phase consisting of a mixture of 40% (v/v) 2-butanone (also known as MEK) and 60% (v/v) ethylacetate[4] (see also Fig. 1a). In view of the fair solubility of water in these solvents, the strips should be thoroughly dried in a stream of hot air after spotting and before the chromatography (Fig. 1b and c).

Immediately after addition of pertechnetate to mertiatide, the vial has to be boiled in order to ensure a proper complex formation. The temperature of 100°C indicated by the heating block applied thus far turned out upon measurement within the vial to vary between 110°C and 120°C in practice, being a trigger for a more reliable procedure. Therefore, we tested a "phantom" vial placed in a tight-fitting brass pig on top of a Peltier-directed heating/cooling device. We observed that the brass pig itself reached a temperature of 90°C at 8 minutes after starting the hotplate program (Fig. 2). Preheating the

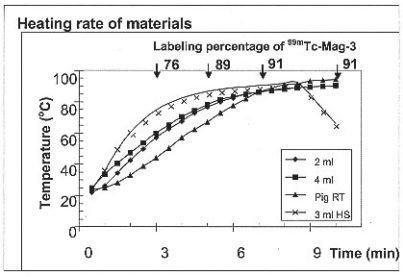

Fig. 2 Rates of heat transfer from hotplate to reaction vial. The lower curve (*triangles*) indicates the increase in temperature of an empty brass pig from room temperature to 90°C. The two intermediate curves (*squares* and *diamonds*) show the temperature course of 2 and a 4 m*l* of water, respectively, placed in the brass pig at a hotplate temperature of 70°C. The upper curve (*crosses*) shows the rate of heat transfer to 3 m*l* of water after preheating the hotplate and brass pig to more than 90°C. At t = 9 min, the test vial was removed from the hotplate. At the four arrows, the radiochemical purity is depicted of a ^{99m}Tc-Mag-3 preparation heated according to the latter curve after reconstitution at $t = 0$.

hotplate to 70°C (and hence the brass pig to 50–60°C) accelerated the heat transfer by 1 minute, approximately, when the vial to be heated is filled with a volume of 2–5 m*l* of water. Larger volumes (6–10 m*l*) only slightly slowed down the rate of heat transfer (not shown) indicating that the brass pig is rate-limiting in the transfer of heat from hotplate to vial. Hence, heat transfer occurred to be fastest when plate and brass pig were preheated to 99°C and 93°C, respectively. Under these conditions, a 2 m*l* ^{99m}Tc-Mag-3 preparation reached the temperature of 90°C within 6 minutes and a labeling percentage of about 90% during the same time course (see also the profile in Fig. 1b).

Routine radiochemical quality control and stability of ^{99m}Tc-Mag-3

The directions for use of ^{99m}Tc-Mag-3 preparations as supplied by the manufacturer[1] mention a storage/stability of 4 hours at room temperature when reconstituted in a final volume of 10 m*l*. More concentrated preparations (4 m*l*) may be used for only 1 hour. For multiple administrations to patients, storage at 4°C is recommended. The procedure proposed here involves reconstitution and heating in a volume of 2 m*l* and withdrawal of a small sample for immediate radiochemical quality control (QC1, Table 1) followed by dilution to 10 m*l* and storage at 4°C. To investigate putative decomposition, we performed a second RQC test (QC2, Table 1) a couple of hours (Δt) after the first one. Due to dilution and radioactive decay, the ITLC strips were not assayed by means of scanning, but instead cut into two pieces and counted in a γ-counter. As shown in Table 1, the values for QC2 significantly exceed those for QC1. During daily routine and not only concerning Mag-3 preparations, we consistently observe a difference of 3–5% in labeling efficiency determined by either scanning or counting in favor of the count rate determination. Differences in geometry and setting may underlie an enhanced background contribution during scanning compared to counting. The results show (Table 1) that significant decomposition of ^{99m}Tc-Mag-3 has not been observed during 5–7 hours after reconstitution, in agreement with previous conclusions based on HPLC analysis.[5]

Table 1 Tenability of ^{99m}Tc-Mag-3 during the day of preparation

	Stability of ^{99m}Tc-Mag-3		
	QC1 (%)	QC2 (%)	Δt (hours)
Mag-3 #1	90.8	94.8	5.0
Mag-3 #2	91.5	97.5	7.5
Mag-3 #3	91.4	94.4	6.5
Mag-3 #4	90.4	94.8	6.5
Mag-3 #5	91.6	96.5	6.5
Mean	91.2	95.5	

A radiochemical purity test was performed by means of ITLC-SG chromatography as described either immediately after the heating procedure (scanning method, QC1) or Δt hours later (counting method, QC2). Note the systematic difference between the rapid scanning method and the time-consuming counting method.

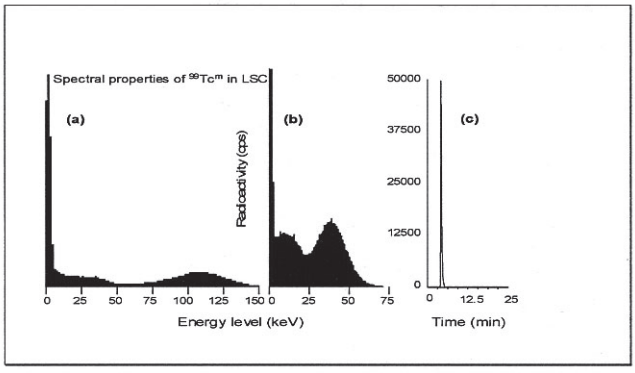

Fig. 3 HPLC and liquid scintillation detection of ^{99m}Tc-pertechnetate. (a) An unquenched spectrum of ^{99m}Tc generated in excess of counting fluid. (b) A quenched spectrum of ^{99m}Tc in a 1/1 mixture of mobile phase and counting fluid. (c) HPLC analysis of ^{99m}Tc-pertechnetate shows elution of radioactivity only at 4.2 minutes after injection.

HPLC analysis and stability of ^{99m}Tc-Mag-3

From a radiochemical point of view, ^{99m}Tc is a pure γ-emitter with the energy of 140 keV. Since the HPLC system in use has been especially designed for the quantification of weak β-emitters like ^{3}H and ^{14}C, we first assess the behavior of ^{99m}Tc in a liquid scintillation detection system. Under conditions of low-quenching (i.e. excess of counting fluid) the spectrum of ^{99m}Tc nicely

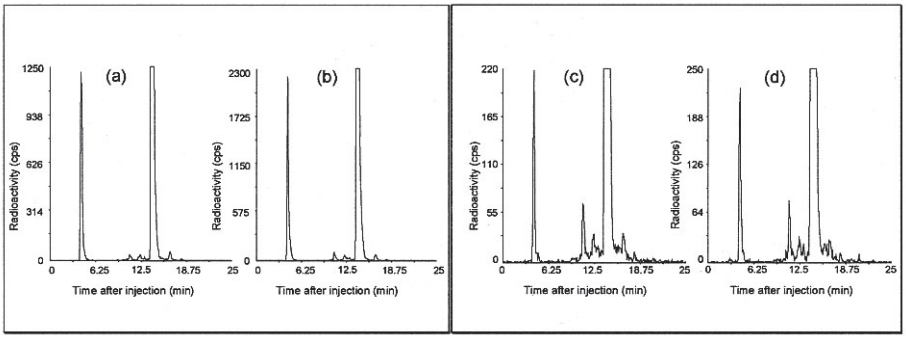

Fig. 4 Stability of ^{99m}Tc-Mag-3 and by-products/impurities. (a) ^{99m}Tc-Mag-3 analyzed by means of HPLC at 3 hours after reconstitution. (b) The same preparation (a) analyzed at 24 hours after reconstitution. (c) Another ^{99m}Tc-Mag-3 (same batch number) preparation analyzed by means of HPLC at 24 hours after reconstitution. (d) The same preparation (c) analyzed at 48 hours after reconstitution. The two major peaks represent free pertechnetate (4.2 min) and ^{99m}Tc-Mag-3 (13.9 min). The ordinates were scaled to display the pertechnetate peaks at full size in order to enhance the visibility of the minor impurities/by-products. Proportions were 9% and 89.5% (4a as well as 4b) and 1.5% and 96.4% (4c as well as 4d) for peaks corresponding to pertechnetate and ^{99m}Tc-Mag-3, respectively.

fits into a ^{14}C window (0–150 keV, Fig. 3a). During our HPLC practice, the mobile phase is mixed with an equal volume of counting fluid before entering the radioactivity detecting compartment and hence, considerable quenching is observed (Fig. 3b). On the basis of these spectral properties, we have set a window of 0 to180 keV for the detection of ^{99m}Tc by means of liquid scintillation. A second window (200–2000 keV) was used to detect $^{99}MoO_4^-$, a potent β-emitter, that in very small amounts may be found in $^{99m}TcO_4^-$ harvests. Pertechnetate is hardly retained and elutes at 4.2 minutes after injection (Fig. 3c). Permolybdate was found to be absent.

Upon injection of ^{99m}Tc-Mag-3, two major peaks emerged in the elution profile (Fig. 4a). The first one elutes at 4.2 minutes after injection and is identical to pertechnetate. Note that the ordinate is scaled to display this peak at full size. The second peak elutes at 13.9 minutes after injection and is most likely ^{99m}Tc-Mag-3. At a closer look, 2–3 minor peaks are observed at the hydrophilic (left) side of ^{99m}Tc-Mag-3 and 1–2 at the hydrophobic (right) side. The presence of 4[6] or 5[5] by-products/impurities has been reported previously. Elution of the hydrophobic peak (also found by Millar et al.[5]) is accelerated in our analyses due to the gradient system applied. In line with these reports, the combined proportion of these by-products/impurities does not exceed the amount of 2% of injected radioactivity.

We have analyzed two different ^{99m}Tc-Mag-3 preparations of the same batch, which differ by age from 3 (Fig. 4a) to 24 (Fig. 4c) hours after reconstitution. The main difference between the two preparations resides in the 9% (4a) and 1.5% (4c) proportion, respectively, of the peaks corresponding to free pertechnetate. After storage at 4°C for 24 hours, both preparations again were analyzed by means of HPLC, and the profiles (Figs. 4b and 4d) as well as the proportions of the peaks corresponding to ^{99m}Tc-Mag-3 and to unincorporated ^{99m}Tc were amazingly similar to the previous ones (Figs. 4a and 4c). These data illustrate that ^{99m}Tc-Mag-3 preparations that were made according to the above protocol maintain their labeling properties for at least 24 hours.

DISCUSSION

The attainment of a temperature of 100°C is not a prerequisite for a successful preparation of ^{99m}Tc-Mag-3. Hence, the process of heat transfer may overlap with the labeling itself. Preheating of materials accelerates the initial rate of labeling and does not affect the final labeling efficiency, provided that heat transfer starts immediately after reconstitution. The use of a programmable heating/cooling device allows an unattended change from heating into cooling. Alternatively, cooling may quickly be introduced by dilution from 2 to 10 m*l* after release of overpressure by means of a simple needle. Throughout the entire procedure, temperatures are well documented in conjunction with the increase in labeling efficiency. These data and tools may contribute to a further development of evidence-based protocols. In addition, there is no need to manipulate the preparation outside of the laminar flow cabinet.

^{99m}Tc is readily detected in a ^{14}C window during liquid scintillation counting as shown by the above HPLC profiles. The two ^{99m}Tc-Mag-3 preparations analyzed above display proportions of free pertechnetate that are at opposite sides of admissibility (9% and 1.5%, respectively). Nevertheless, these proportions appear unchanged after 24 hours of storage, illustrating the stability of ^{99m}Tc-Mag-3 prepared in this way. Minor impurities or by-products are consistently present in amounts less than 2% of total radioactivity. ITLC-SG analysis is not able to discriminate between major and minor products in a ^{99m}Tc-Mag-

3 preparation. As mentioned above we found a substantial difference in labeling efficiency after either scanning or counting an ITLC-SG chromatogram. With respect to the scanning method, it should be kept in mind that in contrast to the counting method, the relatively poor shielding from background radiation (probably caused by the strip itself) slightly enhances the count rate at the solvent front part of the strip, where free pertechnetate would have migrated if present. However, the scanning of a ^{99m}Tc-Mag-3 ITLC-SG chromatogram as described above is a very rapid procedure, generates an easy accessible profile and hence provides sufficient information for a fair estimate of the labeling efficiency of the preparation under investigation. Having passed this simple test to assess its radiochemical purity, a preparation of ^{99m}Tc-Mag-3 maintains its labeling properties for at least 24 hours. Finally, a summary of the procedure is provided:

- Preheat materials
- Take 1110 MBq of ^{99m}Tc-pertechnetate in a volume of 2 m*l*
- Avoid contamination of the inner side of the septum as follows
- Make sure that the needle is filled with air
- Push the needle through the septum as far as possible
- Slowly deliver the pertechnetate straight onto the Mag-3 powder
- Carefully mix the content of the vial in an upright position
- Start the heating/cooling program
- Mix the entire content of the vial after the appearance of condensation at the septum
- After 10 minutes, release overpressure and take a sample for radiochemical purity control
- Dilute to a final volume of 10 m*l* and store at 4°C.

As a result, this procedure saves considerable time, fits quite well in our daily routine production and meets the labeling criteria formulated by the manufacturer. At the time of resubmission of this paper, we have approved the radiochemical purity of more than 250 ^{99m}Tc-Mag-3 preparations made according to this protocol. Scintigraphic abnormalities possibly related to a putatively deviant property of any of these Mag-3 preparations have not been observed.

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