Quality control of instant kit ^{99m}Tc-mercapto acetyl triglycine with inter- and intra-operator measurements

Abstract

This prospective study was *aimed* to assess inter- and intra-operator variability during routine quality control (QC) procedure for technetium-99m mercaptoacetyl triglycine (^{99m}Tc-MAG3) instant kit formulation. *A total of 160 QC analyses* with thin layer chromatography (TLC) for 20 separate MAG3 reconstitutions were performed by 2 radiochemists. The percentage of free and hydrolysed ^{99m}Tc as well as binding efficiency, were calculated according to standard TLC. Each QC analysis was done using silica gel (SG), silica acrylic (SA), Whatman 1 (W1) and Whatman 3 (W3) TLC strips separately at 1h, following labeling MAG3 instant kit with ^{99m}Tc-pertechnetate. To assess the radiochemical stability of ^{99m}Tc-MAG3, the same analysis was performed 4h after kit reconstitution. Visual confirmation for QC with scintigraphy was also performed. At both time points, each radio-chemist repeated all the procedure twice for each of the TLC paper types to analyze the intra-operator reliability. Crombach's Test was used for the reliability analysis. High inter-operator correlation ratios (range: 0.821-0.920) per each TLC strip *were found* where the highest concordance rate was 0.921 for SA. Each TLC strip showed adequate kit reconstitution with acceptable free and hydrolysed ^{99m}Tc percentages both at 1 and 4 h analyses, along with high binding efficiency values of 94.3 ± 2.9 and 92.5 ± 1.9 at 1 and 4 h respectively. Intra-observers reliability showed almost equal high concordance rates (range: 0.888-0.961) for all types of strips. *In conclusion,* all kinds of ITLC/TLC strips were reliable to assess stability of the radiopharmaceutical at 1 and 4 h while analysis with the SA strip had the highest concordence rate. Inter- and intra-operator QC was also reliable.

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Introduction

Radiochemical purity of the radiopharmaceuticals is defined as the proportion of the total radioactivity in the sample associated with desired radiolabeled species. Radiochemical impurities may alter the biodistribution of the radiopharmaceuticals which may result to a distortion in the scintigraphic image leading to major diagnostic and therapeutic failures. For this reason, a radiochemical purity ratio of 95% or above is desired for most of the diagnostic radiopharmaceuticals [1, 2].

In order to assess radiochemical purity, some physicochemical separation methods should be used. Paper chromatography, thin layer chromatography (TLC) and high performance thin layer chromatography are the major types of planar chromatography. All techniques share the same property of having a sample applied to a stationary medium with an appropriate mobile phase. Among many methods that have been used so far, planar chromatography is the most preferred and easily applied method for its advantage of quantification [1-5].

In order to increase the migration speed of the mobile phase, instant TLC (ITLC) materials have been produced and have gained wide acceptance throughout the world. These are composed of glass fibre web impregnated with the modified silica stationary phases, known as silica gel (ITLC-SG) and silicic acid (ITLC-SA). Many different mobile phase such as acetone, 0.9% saline, water and support/stationary phases for both ITLC-SA and ITLC-SG have been recommended for each radiopharmaceutical [2, 3]. Whatman No.1 (W1) and Whatman 3mm (W3) are the most suitable general purpose chromatography papers which are widely used in routine practice.

^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3) is one of the most common renal radiopharmaceuticals which has been in routine nuclear medicine practice since 1986 [6]. ^{99m}Tc-MAG3 provides major advantages over ^{99m}Tc-diethylen triamine pentaacetic acid (^{99m}Tc-DTPA) in renal imaging especially in patients with decreased renal function and with immature glomerular function, such as infants [7]. The correct interpretation of renal function with ^{99m}Tc-MAG3 depends on the stability and ideal biodistribution of the radiopharmaceutical

throughout the body. Following intravenous (i.v.) injection, the blood clearance of MAG3 is quite rapid and biphasic. The half time for the clearance of the first component is 3.18min and that of the second component 16.9min [8]. ^{99m}Tc-MAG3 has a renal extraction fraction of about 54%, and is protein bound in approximately 90%. More than 90% of ^{99m}Tc-MAG3 is secreted by the renal tubules. After i.v. injection ^{99m}Tc-MAG3 is excreted from the kidneys with a ratio of 73% in 30 min and 94.4% in 3h. At 3h postinjection, only 2% of the injected ^{99m}Tc-MAG remains in blood pool and about 2% in the liver, gall bladder and gut [9]. The ideal biodistribution of ^{99m}Tc-MAG3 is crucial for the correct evaluation of renal function and that is why, a proper quality control (QC) procedure for ^{99m}Tc-MAG3 could be undertaken before its administration. Although QC procedures are principally standardized, because of their operator-dependent nature, inter and intraoperator variability is expected to be inevitable.

This study was aimed to assess the inter and intraobserver variability in radiochemical purity analysis of ^{99m}Tc-MAG3 using different chromatography media, which are ITLC-SA, ITLC-SG, W1 and W3.

Materials and methods

Study design

A total of 160 QC analyses to assess radiochemical purity with TLC for 20 separate ^{99m}Tc-MAG3 reconstitutions were performed by 2 experienced radio-chemists (DK, FM). The percentage of free and of hydrolysed ^{99m}Tc as well as the percentage of bound radiopharmaceutical known as binding efficiency were calculated according to the below-mentioned standard TLC method which has been routinely used in the radio-pharmacy laboratory of our department.

Each QC analysis was performed using SG, SA, W1 and W3 TLC strips separately at 1h following labeling MAG3 instant kit with ^{99m}Tc-pertechnetate eluated from a ^{99}Mo/^{99m}Tc generator.

Visual confirmation of the QC using renal scintigraphy was performed as well. The reconstituted radiopharmaceutical was supposed to keep stability for 4h after labeling according to the product data sheet, published by the manufacturer [10]. Because of this, the same analysis was performed 4h after reconstitution, to assess the radiochemical stability of MAG3. At both time points, each radio-chemist repeated whole the procedure twice for each of the TLC paper types, to test the intra-operators' reliability.

Standard TLC method

Standard 5 cm in length and 1 cm in width, ITLC (SG and SA) and W1 and W3 TLC strips were used for each chromatogram. The strips were marked with pencil 8 mm (origin) and 46 mm (solvent front) from the base and a single spot of ^{99m}Tc-MAG3 was applied with a capillary pipette on the 8mm line. The strip was then placed in a suitable vial fitted with a screw cap. The vial contained a layer of appropriate mobile phase 1-2mm in depth which is 40% methyl ethyl ketone and 60% ethyl acetate for free ^{99m}Tc fraction measurement. Another vial containing 50% acetonitrile was used for hydrolysed ^{99m}Tc fraction. Then the strip was allowed for a few minutes to move forward to the marked solvent front. Afterwards, the strip was removed from the developing vials and dried. The strips were then divided into upper and lower sections by cutting across the premarked line approximately in the mid line. Each section of the strip was rolled and placed in counting tubes. Each tube was counted for 1min in an ionization-chamber gamma counter (Atomlab 950 LPC, Biodex Medical, New York, USA) where measurements were expressed in count-per-minute (cpm) and free pertechnetate, hydrolyzed fraction and bound fraction were calculated according to the below-mentioned formulas:

$$\textit{Free pertechnetate \%} = \frac{\textit{Free counts} \times 100}{\textit{Hydrolysed counts} + \textit{Bound counts} + \textit{Free counts}}$$

$$\textit{Hydrolysed fraction \%} = \frac{\textit{Hydrolysed counts} \times 100}{\textit{Hydrolysed counts} + \textit{Bound counts} + \textit{Free counts}}$$

$$\textit{Bound fraction \% (Binding efficiency)} = 100 - (\textit{Free \%} + \textit{Hydrolysed \%})$$

Statistical analysis

Inter and intra-operator reliability ratios were acquired with Crombach's test and P value below 0.05 was selected as the level of significance. To test the stability, Student's t test was applied for the mean values of binding efficiency, free ^{99m}Tc and hydrolysed ^{99m}Tc at 1 and 4h which were derived from each TLC analysis by using each ITLC and TLC strip type and P value lower than 0.05 was chosen as the level of significance.

Results

Each TLC analysis performed at 1h after kit reconstitution, showed adequate labeling of the radiopharmaceutical (mean binding efficiency: 94.3%±2.9%) with acceptable free (mean: 2.8%±1.7%) and hydrolysed (mean: 1.9%±1.1%) ^{99m}Tc percentages for each of the kit reconstitutions. The repeated TLC analysis for each kit reconstitution at 4h also revealed sufficient labeling efficiency (mean: 92.5%±1.9%), which was consistent with kit stability. The mean values of binding efficiency, free and hydrolysed fraction at 1 and 4h after labelling, were tabulated as in Table 1.

Table 1. *The mean values of free, hydrolysed ^{99m}Tc and binding efficiency, derived from TLC analyses at 1 and 4 h with different ITLC and TLC strips.*

	1 h	4 h	P (Student's t test)
Free ^{99m}Tc %	2.8 ± 1.7	3.6 ± 1.8	0.119
Hydrolysed ^{99m}Tc%	1.9 ± 1.1	2.2 ± 1.3	0.327
Binding efficiency	94.3 ± 2.9	92.5 ± 1.9	0.234

The inter-operator correlation ratios per each strip (range: 0.821-0.920) are given in Table 2. The highest inter-observer concordance was observed with SA, which showed a concordance rate of 0.920.

Table 2. *Inter-operator concordance for quality control of ^{99m}Tc-MAG3 for different ITLC/TLC strips at 1h after kit reconstitution.*

ITLC/TLC strip	Inter-operator concordance	P correlation coefficient
SA	0.920	0.000
SG	0.821	0.000
W1	0.869	0.000
W3	0.869	0.000

After each successful kit reconstitution, the radiopharmaceutical was given to a patient and visual assessment of biodistribution of ^{99m}Tc-MAG3 was used as a feed-back to quantitative radiochemical purity analyses. Each kit reconstitution yielded normal bio-distribution of ^{99m}Tc-MAG3, no sign of radiochemical impurity of ^{99m}Tc-MAG3 such as increased hepatic uptake, was observed.

Intra-operator reliability for DK and FM (range: 0.888-0.961), is given in Table 3 showing high concordance rates for all strip types.

Table 3. *Intraoperator concordance for 2 radiochemists (DK and FM) for different ITLC strips at 1h after kit reconstitution.*

	DK	FM	P correl. coef.*
SA	0.961	0.889	0.000
SG	0.920	0.929	0.000
W1	0.888	0.927	0.000
W3	0.913	0.940	0.000

* P value represents the intraoperator correlation between the repeated analyses which were done separetly by 2 radiochemists DK and FM.

Discussion

^{99m}Tc-MAG3 is a widely used dynamic renal imaging radiopharmaceutical in nuclear medicine practice [6]. As a tubular renal agent, it can display renal blood flow, extraction and excretion function of kidneys, which enables its use in many different indications such as renal failure, hydronephrosis, obstructive renal disease and renovascular hypertension. In order to achieve a sufficient scintigraphic interpretation and a reasonable clinical impact, the radiopharmaceutical must keep its integrity as a stable chemical compound beginning from the moment it has been labeled until a certain time point, which is supposed to be 4h for ^{99m}Tc-MAG3 instant kit formulation as indicated by the manufacturer on the product data sheet [10]. Therefore, adequate QC should detect radiochemical purity as well as stability of the bound compound. The practically quantifiable radiochemical impurities of ^{99m}Tc-MAG3 kit reconstitutions are mainly the free and hydrolysed ^{99m}Tc fractions, as in many other radiopharmaceuticals labeled with ^{99m}Tc pertechnetate. By using chromatographic methods, both free and hydrolysed ^{99m}Tc fractions can be calculated, which enables us to calculate the bound fraction better known as the binding efficiency of instant kit reconstitution [1-3].

Among many methods that have been used so far, planar chromatography is the most preferred and easily applied. The major advantage of planar chromatography over column and elution methods is that the total amount of applied radioactivity remains on the chromatoplate which enables quantification of various segments or strips under standard conditions. All major kinds of planar chromatography namely paper chromatography, TLC and high performance thin layer chromatography share the common property of having a sample applied to a stationary medium with an appropriate mobile phase, although they differ from each other in many ways [1-5].

W1 and W3 are the most suitable general purpose chromatography papers, widely used in routine practice [1-3]. W3 paper has some technical advantages compared to W1 paper as having more mechanical strength which avoids tearing in aqueous solvents. It can also absorb a spot of a sample with less spreading [1-3]. In order to increase the migration speed of the mobile phase, ITLC materials have been produced and have gained acceptance. These chromatography materials are composed of glass fibre web impregnated with the modified silica stationary phases, composed of either silica gel (ITLC-SG) or silicic acid (ITLC-SA). Many different mobile phases such as acetone, 0.9% saline, water and support/stationary phases of either ITLC-SA or ITLC-SG, have been recommended for each radiopharmaceutical, which for ^{99m}Tc-MAG3 were acetone and water [10, 11].

ITLC and TLC both serve as advantageous chromatographic techniques, which can be performed routinely in most of the radiopharmacy units in a short period of time. However, they may have some drawbacks: The potential of low reproducibility and possibly high operator dependancy. This prospective study was aimed primarily to answer the question of how reproducible and reliable TLC/ITLC for ^{99m}Tc-MAG3 is and to answer this question, inter and intraobserver concordance rates of 2 experienced radiochemists who routinely deal with QC procedures for routine ^{99m}Tc radiopharmaceuticals, were assessed. To the best of our knowledge, this is the first paper on inter and intraoperator variability in QC for radiochemical purity of ^{99m}Tc-MAG3, although there are some studies in the literature which compare the use of different quality control methods for radiochemical purity [5].

Stability tests of the content of the ^{99m}Tc-MAG3 kit both at 1h and at 4h ITLC/TLC analyses, have high binding efficiency values as suggested by us and indicated by the manufacturer [10].

Visual assessment of ^{99m}Tc-MAG3 biodistribution in patients, was performed because increased hepatic uptake of ^{99m}Tc-MAG3 is considered as indicating radiochemical impurities [7, 12]. Abnormal biodistribution pattern was not observed.

In conclusion, all kinds of ITLC/TLC strips were reliable to assess stability of the radiopharmaceutical at 1 and 4h while analysis with the SA strip had the highest concordence rate. Inter- and intraoperator QC was also reliable.

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