Original Article

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Evaluation of radiochemical purities of some radiopharmaceuticals in Shiraz Namazi teaching hospital

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Abstract

Many radiopharmaceuticals, as a special group of drugs, are eventually prepared at the nuclear medicine departments of the hospitals. Therefore, their quality control procedures such as sterility tests, radionuclide, radiochemical and chemical purity should be carried out in the hospitals. In this study, radiochemical purity for more than 300 preparations of three different radiopharmaceutical formulations from commercial kits were tested using instant thin layer chromatography. The formulations ^{99m}Tc-DTPA, 99mTc-MDP and ^{99m}Tc-MIBI were obtained from Pars Isotope Co. Several paper chromatographic systems including standard and factory recommended thin layer chromatography systems were used in this study. In addition different equipments for detection of radioactivity in paper chromatography like gamma camera and dose calibrator were used. The results showed that the most observed impurities were hydrolyzed reduced technetium (HR-Tc). There were no significant differences between calculated ^{99m}Tc-MIBI radiochemical purities when the radioactive detection device was gamma camera instead of dose calibrator. In case of ^{99m}Tc-DTPA and ^{99m}Tc-MDP, there were significant differences in detection of HR-Tc. On the contrary, no significant differences in free pertechnetate were observed when package insert procedures for quality control were used instead of those recommended in the references. Finally, we observed that the package insert procedures for quality control can offer higher radiochemical purities.

Keywords: Quality control, Instant Thin Layer Chromatography, Radiopharmaceuticals, ^{99m}Technetium, ^{99m}Tc-MIBI, ^{99m}Tc-DTPA, ^{99m}Tc-MDP, HR-^{99m}Tc.

1. Introduction

Nowadays, nuclear medicine obtains important position in diagnostic and therapeutic procedures (1). Nuclear medicine is a functional imaging for the pathological or physiological processes of the organs while other imaging techniques such as CT (Computed Tomography) or MRI (Magnetic Resonance Imaging) are anatomical imaging methods (2). Radiopharmaceuticals are the key elements in nuclear imaging which are formed when radionuclides combine with other chemicals (3). These compounds, once administered to the patient, can localize in specific organs or bind to cellular components (4).

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Based on the used radionuclide, there are two major procedures in nuclear medicine including diagnostic and therapeutic. Diagnostic radionuclides are divided into two major types: Single Photon Emission Computed Tomography (SPECT) radionuclides such as gallium 67, indium 111, thallium 201, technetium 99m (^{99m}Tc) and like this and Positron Emission Tomography (PET) radionuclides including carbon 11, nitrogen 13, oxygen 15, fluorine 18 and so on. On the other hand, the therapeutic radionuclides consist of samarium 153, lutetium 177, rhenium 186 (5).

^{99m}Tc radiopharmaceuticals are the most frequently used radiopharmaceuticals in the nuclear medicine (6). The ^{99m}Tc reveals excellent properties such as proper half-life (6 h), acceptable gamma ray energy (140 Kev), isomeric transition decay as well as good coordination chemistry which make it as the first line

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radionuclide for SPECT imaging (7).

Because of short half-life for ^{99m}Tc, its radiopharmaceuticals should be prepared in the hospital just before administration to the patients. So, instant kits are normally used for preparation of these radiopharmaceuticals. Instates kits are containing all components for the preparation of radiopharmaceuticals except ^{99m}Tc as radionuclide. ^{99m}Tc radionuclide is obtained from Moly generators in the sodium pertechnetate chemical form ($Na^{99m}TcO4$). This chemical form of radionuclide is not able to coordinate with chelating materials which already exist in the instant kits. Reduction of pertechnetate to lower oxidation states improves its coordination properties. Some reducing agents are used for this purpose like stannous chloride, sodium borohydride, dithionite etc. (4, 8, 9). Stannous chloride is the most common reducing agents in cold kits, its salts are nontoxic, stable when lyophilized and can be used in all kind of radiopharmaceutical kits. By adding pertechnetate to the kits, it is immediately reduced by stannous chloride to a lower oxidation state and forms radiopharmaceutical chelates.

Some impurities can be formed when sodium pertechnetate is added to cold kits. The two most important impurities during radiopharmaceutical production are hydrolyzed reduced technetium (HR-^{99m}Tc) and free pertechnetate that are determined as radiochemical impurities. The former is prepared when pertechnetate is reduced to lower oxidation state and cannot complex with the chelating agent already present in the kit. The subsequent reaction with water makes colloidal form impurities called HR-^{99m}Tc. The free pertechnetate remains when the reduction process is not efficient enough to make lower technetium oxidation state.

Since ^{99m}Tc radiopharmaceuticals are prepared in-house and immediately administrated to the patient, it is important that they undergo strict quality control procedures. These controls include radionuclide purity, chemical purity and radiochemical purity (RCP) (10). It is very important to evaluate RCP of technetium radiopharmaceuticals immediately after preparation of radiopharmaceuticals because impurities can accumulate during time. There are several methods to evaluate radiochemical purity of radiopharmaceuticals including Solid Phase Extraction (SPE) (11), High Performance Liquid Chromatography (HPLC) (12), extraction(13) and the most important and easy to use method, instant TLC (Thin Layer Chromatography) (14). TLC is an accurate, simple, fast and friendly used method to determine the ^{99m} Tc impurities, including free pertechnetate ($Na^{99m}TcO_4$) and HR-^{99m}Tc. These impurities can rise patient radiation dose and reduce image quality and in some cases alter the normal biodistribution which cause incorrect diagnosis (15) The most frequently used SPECT radiopharmaceuticals in the hospitals are ^{99m}Tc sestamibi (^{99m}Tc -MIBI), ^{99m}Tc-DTPA and ^{99m}Tc-MDP used for myocardial perfusion, kidney and bone scan, respectively. In the present study, we determined the RCP of these radiopharmaceuticals in Namazi teaching hospital using instant TLC to determine the best schedule for nuclear imaging procedures.

2. Material and methods

Acetone, ethanol and phosphoric acid were purchased from Merck and used without further purification. Normal saline for elution and as the mobile phase were provided from Pars Isotope Company and Samen pharmaceutical Co, respectively. Whatmann number 1 paper was provided from whatmann International LTD while silica gel (105554) and alumina (105551) were purchased from Merck. The technetium generators were obtained from Pars Isotope Company. The vial and paper radioactivity was measured by two models of dose calibrators, Isocall II and Capintec 25R. The radioactive counts on paper were measured by planar digital gamma camera (Nucline ™ H.L.). All instant kits were obtained from Pars Isotope Company and kept in 2-8 °C before use.

The significant differences between RCP percentage before and after modification of TLC conditions were examined by Graph Pad Prism 5 software.

2.1. Preparation of ^{99m}Tc-sestamibi (^{99m}Tc-MIBI)

^{99m}Tc-sestamibi was prepared according to its package insert (16). Briefly, up to 150 mCi (5.55 GBq) freshly eluted ^{99m}Tc-pertechnetate (1-3 ml) was added to the corresponding instant kit. The vial was shaked for 10 sec and subsequently placed in the boiling water for 10 min. Finally the vial was allowed to reach to the room temperature for 15 min.

2.2. Preparation of ^{99m}Tc-MDPand 99mTc-DTPA

According to its available package insert (16), the commercial kits were reconstituted with up to 300 mCi (11.1 GBq) ^{99m}Tc-pertechnetate (2-5 ml). The mixtures were shaked gently for 10 sec and kept for 15 min at room temperature to complete the reaction.

2.3. Quality control procedures

The quality control for the radiopharmaceuticals were carried out by instant TLC and paper chromatography using different stationary and mobile phases according to the standards described in references (14) and the package inserts (16).

2.3.1. Quality control of ^{99m}Tc-sestamibi

The radiochemical purity tests were carried out using instant TLC. An aliquot (5 µl) of the prepared ^{99m}Tc-sestamibi was spotted on an ethanol pre-wetted stationary alumina phase (1×7 cm). Then the strip was placed in a chamber and developed by ethanol 99%

Table 1. Radiochemical purity of radiopharmaceuticals.

	% Preparations with % CP < 90%		% RCP mean±SE		% Free pertechnetate±SE		% HR-99mTc±SE	
	B*	A**	B	A	B	A	B	A
^{99m}Tc-MIBI	7.70%	3.30%	96.60±4.44 %	97.46±2.64 %	ND***	ND	ND	ND
^{99m}Tc-DTPA	11.90%	2.80%	94.88±4.10%	98.21±2.20 %	0.49±0.45%	0.26±0.31%	4.62±4.16%	1.50±2.18%
^{99m}Tc-MDP	37.70%	10.00%	90.20±5.87%	95.9 ±3.87%	0.54±0.71%	1.00±1.35%	8.72 ± 5.84%	3.08±3.60%

*B is denouncing before modification; **A is denouncing after modification; ***ND means not determined

as mobile phase. In this chromatography system, all radiochemical impurities including free pertechnetate and HR-^{99m}Tc remained in the origin (R_f=0.0-0.1) whereas the desired radiopharmaceutical complex migrated to the solvent front (R_f=0.9-1). Cutting the strip from the middle line resulted two pieces. Their activity was thereafter measured by dose calibrator or gamma camera. The RCP percentage was calculated using equation No 1.

$$\%\text{Radiochemical purity} = \frac{\text{net radioactivity on the upper piece}}{\text{net total activity on the whole stript}} \times 100 \quad [1]$$

The net activity was, calculated by extracting background activity from total activity.

2.3.2. Quality control of ^{99m}Tc- MDP, ^{99m}Tc- DTPA according to standard procedures

Unlike MIBI the quality control tests for ^{99m}Tc- MDP and ^{99m}Tc- DTPA pharmaceuticals were carried out using two different instant and paper chromatography systems. Each system could indicate one of the possible radiochemical impurities. At first, a paper chromatography system with whatman 1 as stationary and acetone as mobile phase (system 1) was utilized. In this system, the free pertechnetate migrated to the solvent front (R_f=0.9-1) while the desired radiopharmaceutical and HR-^{99m}Tc remained in the origin (R_f= 0.0-0.1). On the other hand, the HR-^{99m}Tc technetium impurity was determined using an instant TLC system composed of silica gel and normal saline as stationary and mobile phase, respectively (system 2). In this system, the free pertechnetate and radiopharmaceutical migrated to the solvent front and the HR-^{99m}Tc remained in the origin. Finally, each strip was cut from the middle line and the radioactivity on each piece was measured by dose calibrator or gamma camera according to the bellow (equations 2 and 3).

$$\%\text{pertechnetate impurity} = \frac{\text{net radioactivity on the upper piece in system 1}}{\text{net total activity on the whole stript in system 1}} \times 100 \quad [2]$$

$$\%\text{HR 99mTc impurity} = \frac{\text{net radioactivity on the lower peice in system 2}}{\text{net total activity on the whole stript in system 2}} \times 100 \quad [3]$$

As described above the net activity was calculated by extracting background activity from total activity.

2.3.3. Quality control of ^{99m}Tc- MDP, ^{99m}Tc- DTPA according to package inserts procedures

The recommended standard TLC and paper chromatography systems were replaced by those available in package inserts. Accordingly, the TLC conditions for ^{99m}Tc-MDP were whatmann 1 as stationary and the mixture of methanol and acetone (1:1) as the mobile phase for detecting free pertechnetate. The other system to detect the HR-^{99m}Tc was whatmann no. 1 and phosphoric acid 15%. In case of ^{99m}Tc –DTPA, the TLC systems were changed as bellow: the package

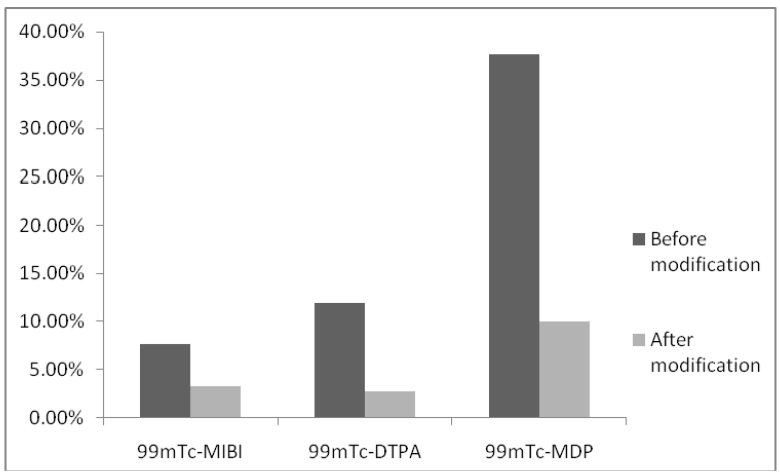

Chart 1. Percentage of preparations with RCP less than 90%.

insert enforces that to detect free pertechnetate whatmann 1 and the mixture of methanol and acetone (1:1) should be used as stationary and the mobile phase, respectively. The TLC system for HR-^{99m}Tc was whatmann no. 1 and normal saline. As described in the previous section (2.3.2) the radiochemical purity was determined by new TLC systems.

In order to compare the modifications for different groups of radiopharmaceuticals, t-test studies were performed.

3. Results

The radiochemical purity of the desired radiopharmaceuticals was controlled and the results were summarized in Table 1 and depicted in Figures 1 and 2. As it is indicated in Table 1 in this study 107 ^{99m}Tc-MIBI formulations were tested for their RCP percentages. As described in section 2.3.1, the chromatography system for ^{99m}Tc-MIBI was not changed and only radioactive detection system was changed. The results showed that using more sensitive detection system (gamma camera instead of dose calibrator) leads to increasing the percentage of passed formulations with RCP more than 90%. However, the RCP percentages for ^{99m}Tc-MIBI didn't have significant differences between these two situations. In case of ^{99m}Tc-MDP 93, formulations were tested. At first 62 formulations were evaluated using recommended quality control procedures and the rest were evaluated using package insert procedures as well as using gamma camera as detection device. The results showed that the percentage of formulations with RCP less than 90% decreased dramatically (37.70% vs. 10.00%). From two possible impurities only % HR- ^{99m} Tc was decreased significantly. Similar results were obtained for ^{99m}Tc-DTPA. Briefly, from 103 preparations, 67 cases were tested using recommended quality control procedures and radiochemical purities for 36 formulations were obtained using package insert procedures and gamma camera as radioactive detection equipment. The percentage of formulation with RCP less than 90% decreased (11.90 vs. 2.80). Here, again the RCP percentages significantly increased and only HR-^{99m}Tc impurities decreased and the free pertechnetate didn't decreased, significantly.

4. Discussion

The results showed that the radiochemical purity for MIBI is higher than two other radiopharmaceuticals. From the two possible impurities, the reduced hydrolyzed technetium (colloidal form) was shown to be more than the free pertechnetate. This impurity is able to localize in liver and other reticuloendothelial system (RES) and increase radiation dose for these sites (17). The data in Table 1 show that the average amount of HR-^{99m}Tc in ^{99m}Tc-MDP is about twice more than that of DTPA. As the image qualities didn't show high absorption in the RES sites, which is associated to this kind of impurity, it was proposed that the real amounts of HR-^{99m}Tc impurity were less than those calculated by standard procedure, so, a more powerful technique using accurate equipment and package insert TLC conditions was utilized. Based on the reports (14), there are several factors affecting the accuracy and precision of TLC results in quality control of radiopharmaceuticals. The size of drop and its activity can alter the migration pattern of radioactive species. The bigger and more active spot makes more tailing in the TLC results leading to lower radiochemical purity report. The commercial dose calibrators have 1µCi detection limit. Therefore, it is highly recommended to keep the spot activity more than 100 µCi in order to decrease the errors. Moreover, for decreasing the spot activity and size, more sensitive equipment such as gamma camera could be utilized. Gamma camera may be used as a counter instrument and it was therefore used in this study due to its high sensitivity and accuracy. At least in one reference (18), using gamma camera led to better visualization for the migration of radioactivity which resulted in a better judgment about RCP. The dose calibrator was accordingly replaced

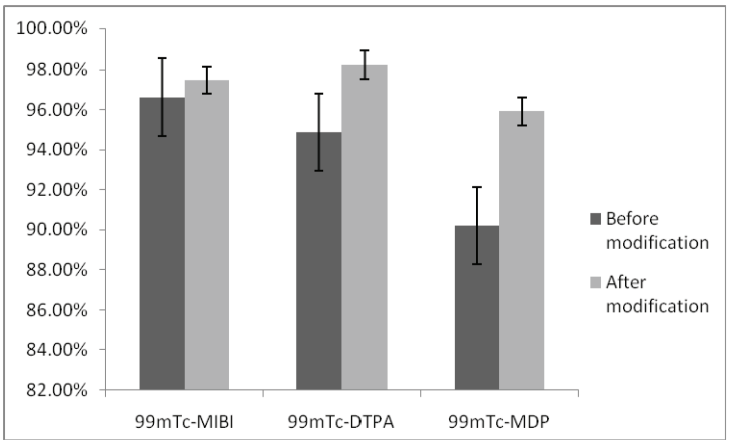

Chart 2. Means of radiochemical purity for tested radiopharmaceuticals.

by digital gamma camera (Nucline ™ T.H.) and due to more sensitivity of gamma camera, the activity on the strips decreased dramatically. The quality control results after this modification are shown in Table 1, Figures 1 and 2.

Based on the obtained t-test p values, there was no significant difference between RCP percentages for ^{99m}Tc-MIBI before and after using gamma camera (p value=0.3231). On the contrary, in case of ^{99m}Tc-MDP and ^{99m}Tc-DTPA significant values of RCP percentages were observed (p<0.0001). These data showed that using a suitable method for chromatography is more important than using more accurate devices. As described before, there are two kinds of impurities in the samples (HR-^{99m}Tc and free pertechnetate). Based on the data displayed in Table 1, the impurities in ^{99m}Tc-DTPA and ^{99m}Tc-MDP have significantly decreased after utilizing proper chromatography systems. It might be due to the presence of some ingredients which could alter the retention factor and migration pattern of radiopharmaceutical complex and impurities. This study supports the idea that each radiopharmaceutical should be controlled by the method described in the package insert. Every nuclear medicine center should, therefore, observe the quality control procedures developed by the factory rather prior to those principles presented by other references. It can be concluded that the ingredients in the radiopharmaceutical kits can alter the retention factor (Rf) of the radiochemical species and consequently alter the final decision about the radiochemical purity.

Since the ^{99m}Tc-MIBI showed the least impurities in all procedures and was independent of further modifications, the nuclear medicine center decided to change the imaging schedule. The generators are handed on Sundays and the better radiochemical purities will be achieved when fresh generators are used. Therefore, the bone scans with ^{99m}Tc-MDP was carried out on Sundays and Mondays and the myocardial scans was carried out since Tuesdays. Finally it should be emphasized that it was the first attempt in the Namazi Teaching Hospital to control the quality of these in-house radiopharmaceuticals.

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Conflict of Interest

None declared.

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