Original Article

# Studying Various Parameters Affecting Labeling Efficiency Of Radiopharmaceuticals In Nuclear Medicine

**Reem H. Elgebaly[1], Monira M. Rageh[1], Mahmoud Adel[2] , Islam Kamal [2].**

*1. Biophysics department Faculty of Science Cairo University*
*2. Nuclear Lab., critical Care department Faculty of Medicine (Kasr El-Ainy) Cairo University.*

*Email: Mahmoud.sci2000@gmail.com*

**DOI:** 10.47750/pnr.2023.14.02.413

## Abstract

The use of various types of radioactive drugs has increased the effectiveness of nuclear medicine. Technetium-99m ($^{99m}$Tc) radiopharmaceuticals are in widespread use owing to the availability and affordability of $^{99}$Mo/$^{99m}$Tc generators and the variety of kits for formulating the desired products. Together, they provide an array of specific tools for diagnosing a large number of diseases affecting the Heart, Kidney and major organs of the body such as the bones, brain, liver, parathyroid, and thyroid. Nuclear medicine requires high quality radiopharmaceuticals and kits that are safe for administration and efficacious for a given application. Since radiopharmaceuticals are intended for human use, it is imperative that they undergo quality control measure.This paper presents various parameters affecting labeling efficiency of radiopharmaceuticals and describes the procedures of preparing from four selected kits. Details of the ingredients are also included. The procedures described here can be used to develop manuals and standard operating procedures in Egypt. This study is expected to serve as a guide to radiopharmaceutical manufacturing centers of such kits. Four kits namely: MIBI, DTPA, MDP, and DMSA are used. Paper Chromatography was involved to disparate the polar and non-polar part of the kit-solution. Quality control testing assures purity, integrity, potency, product identity, biological safety and efficacy of radiopharmaceuticals. The success of diagnostic radiopharmaceutical has been reflected in the quality of images of diseased site.

## Introduction

Nuclear medicine is a medical specialty involving the application of radioactive substances in the diagnosis and treatment of diseases. In nuclear medicine procedures, radionuclides combined with other elements to form chemical compounds, or else combined with existing pharmaceutical compounds to form radiopharmaceuticals. Radiopharmaceuticals have been defined as radioactive drugs that, when used for the purpose of diagnosis or therapy, typically elicit no physiological response from the patient. (**Ruhul Amin M. et al, 2012)**.

Radiopharmaceuticals are made up of a radionuclide (regulates the physical characteristics) and a drug (determines the biological behavior). Radionuclides are unstable nuclides that spontaneously decay to a more stable atom through the emission of particles, electromagnetic radiation, isomeric transition, or capturing electrons. The drug is the organic or inorganic chemical fraction that determines its biodistribution. In other words, it carries the radionuclide to the site of interest within the body. The distribution of the radiopharmaceutical within the body determined by the physiochemical properties of the drug, the stability of the radiolabel, the purity of the radiopharmaceutical preparation, the pathophysiologic state of the patient, and the presence or absence of interfering drugs. **(Juan José Mora-Román et al, 2022), and (Poliane Angelo et al, 2011).**

Nuclear medicine plays a special role in the diagnosis and treatment of the anatomical and biochemical physiology by using the specific characteristics of radioisotopes (RI) and radiopharmaceutical products. A reactor, cyclotron, or generator produces the RI. The radiation dose in nuclear medicine imaging is necessary to improve image resolution. The technetinum-99m ($^{99m}$Tc) produced by the generator is easy to use and easy to obtain by separating the milked $^{99m}$Tc from the mother nuclide molybdenum-99 ($^{99}$Mo). The $^{99m}$Tc, strongly adsorbed on the alumina column in the generator, had eluted. The carrier $^{99}$Tc has little effect on $^{99m}$Tc labeling and radiopharmaceutical products. However, radiopharmaceuticals with monoclonal antibodies tend to have a reduced labeling efficiency because the amount of ligand is very small. In addition, a radiopharmaceutical used as a tracer cannot form a complex with a pharmaceutical ligand during labeling with an RI, so a reducing or stabilizing agent needed. The efficiency of the labeling radioactive isotope $^{99m}$Tc is crucial for the accuracy and reliability of an examination. The accuracy and reliability of efficient $^{99m}$Tc labeling is important for developing

various radiopharmaceuticals and their usefulness in nuclear medicine diagnosis. Quality control (QC) of $^{99m}$Tc and radiopharmaceuticals includes physicochemical purity, that is, radionuclide purity, chemical purity, radiochemical purity. RCP of the resulting radiopharmaceuticals and biological purity, including aseptic and exothermic tests. The radionuclides and their RCP are important factors in nuclear medicine tests because they affect the quality and diagnosis of medical imaging **(Y.H. Jeon et al 2020).**

Since the mid of the 60s of last century technetium-99m radiopharmaceuticals have been and still are the most used tracer agents for planar scintigraphy and single photon emission computed tomography(SPECT). Factors at the basis of this are the favorable radiation and chemical properties of the radionuclide, its continuous availability in hospitals from easy to handle $^{99}$Mo/$^{99m}$Tc generators and the availability of numerous labeling kits that allow simple and efficient in house preparation of numerous $^{99m}$Tc-radiopharmaceuticals shortly before use for diagnosis and follow-up of a variety of diseases. Hundreds of $^{99m}$Tc labeled compounds with diverging biodistribution characteristics have developed in intensive and innovative research and biologically tested during the last 50 years. Today about 30 of these agents are in routine clinical use as licensed diagnostic drugs. Only a few of the $^{99m}$Tc radiopharmaceuticals are inorganic compounds whereas the majority consists of metallo-organic complexes in which the transition metal technetium is present in oxidation states 1, 3, 4 or 5. The $^{99m}$Tc complexing ligands have a widely diverging structure and range from simple organic complexing agents towell-designed Tc-chelating ligands and conjugates in which a bi-functional chelating agent is bound to a receptor targeting chemical, peptide or protein, including antibodies and their fragments. **(JanCleynhens and Alfons Verbruggen, 2022)**

$^{99m}$Tc, a metallic radionuclide, is the most widely available isotope in diagnostic nuclear medicine. It is found in oxidation states I to VII, but the technetium (Tc) complexes for medical applications are found mostly in oxidation state V, $^{99m}$Tc, which mainly decays (88%) with a half-life of 6.02 h by gamma emission (E = 140 keV) to the ground state technetium-99 ($^{99}$Tc), is obtained as $^{99m}$TcO4- from a molybdenum-99 ($^{99}$Mo)/$^{99m}$Tc generator commercially available and compatible with the requirements of Good Manufacturing Practices (GMP) **(Frédérique Blanc-Béguin et al,2022)**

Specifically, the radionuclide must have a reasonable half-life, depending on the desired use. In addition, characteristics such as size or charge of the molecule, its specific activity, lipophilicity, stability, and the metabolism of the radiolabeled compounds are directly correlated to the specificity of each biological target. Thus, through quality control tests, aspects concerning the physicochemical, radiochemical, or biological properties are also required. **(Crisan G. et al 2022)**

Radiopharmaceuticals have to be extensively tested in their development before they can be applied on humans. Whereas radiolabeling tests and analytical testing are an essential part to ensure the quality of the radiopharmaceutical, the efficacy in terms of diagnostic performance or therapeutic action as well as the safety is paramount important and has to be characterized before any clinical application can be considered. Traditionally the characterization of pharmacokinetics and targeting of radiopharmaceuticals to provide efficacy data had been performed in animal studies. Today a variety of these aspects can be covered in vitro, thereby reducing efforts and the number of animal studies required.**( Clemens Decristoforo and Joachim Pfister, 2022).**

Nuclear medicine imaging using radiopharmaceuticals is unique, because it is safe, painless, simple, cost effective technique, as same as it provides useful information to doctors about both structure and function of organ to treat disease. **(Shanvi Sachin Gawli and Dipti Hiten Chirmade, 2016).**
Early detection of bone cancer is critical for treating symptoms, minimizing pain, and increasing overall quality of life.**( Meliha Ekinci et al, 2022)**

The altered bio distribution of $^{99m}$Tc radiopharmaceuticals are generally associated with increased amounts of $^{99m}$Tc radiochemical impurities, such as free $^{99m}$TcO4 and particulate impurities, such as $^{99m}$Tc colloids or $^{99m}$Tc-reduced hydrolyzed species ($^{99m}$Tc-RH) these common $^{99m}$Tc impurities, certain radiopharmaceutical preparations result in altered biodistribution. **(Shankar Vallabhajosula et al, 2010).**

Radiopharmaceuticals may also be prepared outside the marketing authorization track or used outside the indications for which they have been registered. Small scale preparations at non-commercial sites thereby represent an important segment.**( Gillings et al, 2021)**

## Materials and Methods

This work of labeling efficiency applied in four NMS that currently are in operation MDP, DTPA, DMSA and MIBI with different parameters of preparing procedure.

**Radiochemical purity assessment**

Radiochemical purity was determined by thin layer chromatography in stationary phase. The chromatography plate was employed silica gel ($^{60}$F) on aluminum foil (thin-layer chromatography silica gel - TLC-SG (Al) - Merck®), whose dimensions are 1x10cm, or (Whatman®) paper, with the same dimensions. Mobile phases were appropriated systems to each radiopharmaceutical, according manufacturer's directions. The stripes of TLC-SG and Whatman paper placed in closed containers ensuring that they do not touch the walls of the same as fig.(1). After the chromatography run, the tapes were cut, as fig.(2) and the activity was determined in Atom lab 500 dose calibrator for each service.

The labeling efficiency was expressed in *% radiochemical purity (%RP):*

$$\%RP = 100 - \frac{(Activity\ of\ 99m\ TcO4\ x\ 100)+(Activity\ of\ Hydrolyzed\ 99m\ Tc\ x\ 100)}{Total\ Activity}$$ **(Gopal Saha, 2010).**

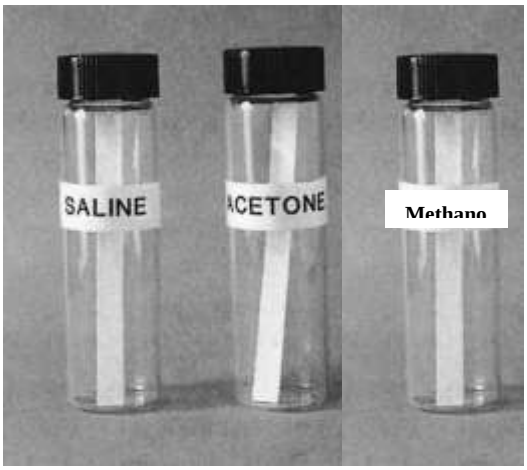


**Fig.(1)**Chromatographic chamber type vials. **(Gopal Saha, 2010)**

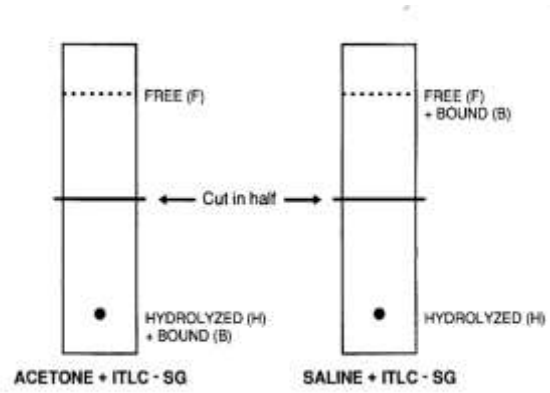


**Fig.(2)**Chromatographic paper cut in half after removing it from chamber **(Gopal Saha, 2010)**

**Statistical analysis**

The statistical method and analysis for evaluation of the results done by calculating arithmetic mean and standard deviation and P values for radiopharmaceuticals labeling efficiency measurements. All these measurements were done for various parameters affecting labeling for all groups; comparison between groups performed using IBM SPSS version 26 program with Paired samples T-Test.

## Results and Discussion

**Radiochemical purity assessment**

Important factors affecting the biodistribution of radiopharmaceuticals can be described, such as factors associated with radiopharmaceutical preparation and formulation. Then several quality control tests need to perform before administration **(S.Vallabhajosula et al, 2010)**.

Radiopharmaceuticals should be prepared according to the instructions of the manufacturer **(European Pharmacopeia, 2011)**, but sometimes the kits were labeling with higher activities than the recommended ones, for example, MIBI in a NMS, it is also hazard to the labeling process, once increasing activity, it increases the carrier Tc-99m that participate in the chelation process; then it causes the decrease at labeling efficiency. **(F. L. N.Marques et al, 2001).**

In terms of stability, the physicochemical parameters, such as temperature, pH, and light must be carefully established for the radiopharmaceutical preparation and storage. With regard to the compound's metabolism, if the radiopharmaceutical compound can metabolically decomposed, thus affected its biodistribution because of the mixture of the intact agents and metabolic fragments from the decomposed radiolabeled molecule.**( Crisan, G. et al 2022).**

Pharmaceuticals intended for human administration, quality control procedures must be in place to ensure the quality of these products. This is also the case for radiopharmaceuticals used for diagnostic imaging by positron emission tomography (PET) and single-photon emission computed tomography (SPECT) and for radiopharmaceuticals for radionuclide therapy. Due to the special characteristics of radiopharmaceuticals, there are a number of specific quality control tests, should performed. Furthermore, for products with short radioactive half-lives, the completion

of some tests may not be feasible before releasing the product and in this regard, a robust quality management system is essential. ( **NicGillings,2022)**

**Statistical analysis**

Paired samples T-Test Showing that the relations between groups of

1. (Normal & Bad storage).
2. (Few amount of Air or Evacuated & Large amount of Air added or non-evacuated).
3. (Large Vol. Activity & Small Vol. Activity).
4. (Conc. Of pharmaceutical added Low Conc. & High Conc.).
5. Fractionation time (Duration After Dilution 0, 2, 5 and 10 Days) for each radiopharmaceutical (**MDP, DTPA, DMSA, and MIBI**)

**Moreover,** for **(MIBI)** the relation between groups of

1. Heating time (5 s - 15 s) with microwave oven.
2. Heating time (5 min - 15 min ref. ) in boiling water bath as shown in the following Tables

**Table (1)** MDP Paired Samples Test statistical analysis for diff. groups of variations.

| | | MDP Paired Samples Test | | | | | t | df | Sig. (2 tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval Of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Normal– Bad Storage | 20.93875 | 2.27408 | 0.80401 | 19.03757 | 22.83993 | 26.04 | 7 | 0.000 |
| Pair 2 | Few Air - Large amount of Air | 3.47500 | 0.88436 | 0.31267 | 2.73566 | 4.21434 | 11.11 | 7 | 0.000 |
| Pair 3 | Activity Large Vol. - Activity Small Vol. | -3.01750 | 1.3271 | 0.46920 | -4.12698 | -1.90802 | -6.431 | 7 | 0.000 |
| Pair 4 | Conc. Of pharmaceutical Added Low Conc. - High Conc. | -1.42125 | 0.36061 | 0.12750 | -1.72273 | -1.11977 | -11.14 | 7 | 0.000 |
| Pair 5 | Duration After Dilution 0 Days - 2 Days | 0.63375 | 0.42541 | 0.15040 | 0.27810 | 0.98940 | 4.214 | 7 | 0.004 |
| Pair 6 | Duration After Dilution 5 and 10 Days | 2.72750 | 0.52931 | 0.18714 | 2.28499 | 3.17001 | 14.57 | 7 | 0.000 |

**Table (2)** DTPA Paired Samples Test statistical analysis for diff. groups of variations.

| | | DTPA Paired Samples Test | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval Of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Normal Storage – Bad Storage | 30.225 | 2.73117 | 0.96561 | 27.94169 | 32.50831 | 31.30 | 7 | 0.000 |
| Pair 2 | Few Air - Large amount of Air | 2.0125 | 1.17527 | 0.41552 | 1.02995 | 2.99505 | 4.843 | 7 | 0.002 |
| Pair 3 | Activity Large Vol. - Activity Small Vol. | -2.9625 | 0.75392 | 0.26655 | -3.59279 | -2.33221 | -11.14 | 7 | 0.000 |
| Pair 4 | Conc. Of pharmaceutical Added Low Conc. - High Conc. | -0.7000 | 0.74833 | 0.26458 | -1.32562 | -0.07438 | -2.646 | 7 | 0.033 |
| Pair 5 | Duration After Dilution 0 Days - 2 Days | 0.4375 | 0.39256 | 0.13879 | 0.10931 | 0.76569 | 3.152 | 7 | 0.016 |
| Pair 6 | Duration After Dilution 5 Days - 10 Days | 5.6750 | 0.73630 | 0.26032 | 5.05943 | 6.29057 | 21.80 | 7 | 0.000 |

**Table (3)** DMSA Paired Samples Test statistical analysis for diff. groups of variations.

| | | DMSA Paired Samples Test | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval Of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Normal – Bad Storage | 20.9875 | 3.08935 | 1.09225 | 18.40474 | 23.57026 | 19.21 | 7 | 0.000 |
| Pair 2 | Few Air - Large amount of Air | 3.50000 | 0.47509 | 0.16797 | 3.10281 | 3.89719 | 20.83 | 7 | 0.000 |
| Pair 3 | Activity Large Vol. - Activity Small Vol. | -1.52500 | 0.93005 | 0.32882 | -2.30254 | -0.74746 | -4.638 | 7 | 0.002 |
| Pair 4 | Conc. Of pharmaceutical Added Low Conc. - High Conc. | -0.70000 | 0.72506 | 0.25635 | -1.30617 | -0.09383 | -2.731 | 7 | 0.029 |
| Pair 5 | Duration After Dilution 0 Days - 2 Days | 0.52500 | 0.41318 | 0.14608 | 0.17958 | 0.87042 | 3.594 | 7 | 0.009 |
| Pair 6 | Duration After Dilution 5 Days - 10 Days | 19.16250 | 2.13136 | 0.75355 | 17.38064 | 20.94436 | 25.40 | 7 | 0.000 |

**Table (4)** MIBI Paired Samples Test statistical analysis for diff. groups of variations.

| MIBI Paired Samples Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | t | df | Sig. (2-tailed) |
| | | | | | Lower | Upper | | | |
| Pair 1 | Normal Storage – Bad Storage | 20.93875 | 2.27408 | 0.80401 | 19.03757 | 22.83993 | 26.043 | 7 | 0.000 |
| Pair 2 | (Few – Large) Amount Of Air | 3.47500 | 0.88436 | 0.31267 | 2.73566 | 4.21434 | 11.114 | 7 | 0.000 |
| Pair 3 | Activity Large Vol. – Small Vol. | -5.90875 | 0.74769 | 0.26435 | -6.53383 | -5.28367 | -22.352 | 7 | 0.000 |
| Pair 4 | Pharmaceutical Added High Conc. – Low Conc. | 5.90875 | 0.74769 | 0.26435 | 5.28367 | 6.53383 | 22.352 | 7 | 0.000 |
| Pair 5 | Duration After Dilution 0 Days – 2 Days | 0.66250 | 0.76893 | 0.27186 | 0.01966 | 1.30534 | 2.437 | 7 | 0.045 |
| Pair 6 | Duration After Dilution 5Days - 10Days | 21.21250 | 1.58244 | 0.55948 | 19.88955 | 22.53545 | 37.915 | 7 | 0.000 |
| Pair 7 | 5s –15s heating with Microwave | -6.46250 | 0.75674 | 0.26755 | -7.09515 | -5.82985 | -24.155 | 7 | 0.000 |
| Pair 8 | heating with Boiling water bath 5 min -15 min | -0.16875 | 0.16873 | 0.05965 | -0.30981 | -0.02769 | -2.829 | 7 | 0.025 |

It has been found that the relation between the parameters for different groups was significant where P values were ≤ 0.05 for each group as shown in previous Tables.

Considering Storage conditions as recommended by manufactures Normal vs. bad storage parameters such as changing of storage temperature degrees, exposure to light, and using after expiry date as shown in the following figure

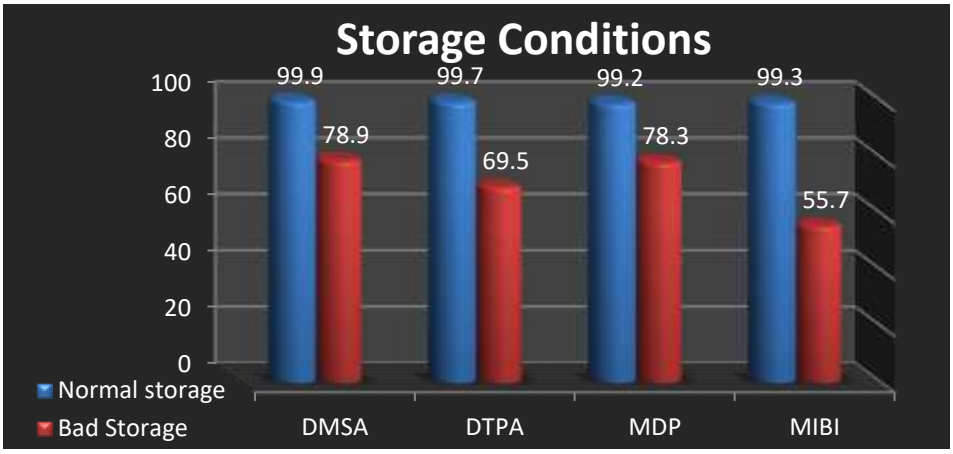


**Fig. (4)** The Effect of storage conditions and the labeling Efficiency percentage of different pharmaceuticals

Thus, we found that
It is necessary to apply manufacture guidelines of storage conditions as recommended by **European Pharmacopeia, 2011**.
Considering adding air: Large amount vs. few as shown in the following figure:

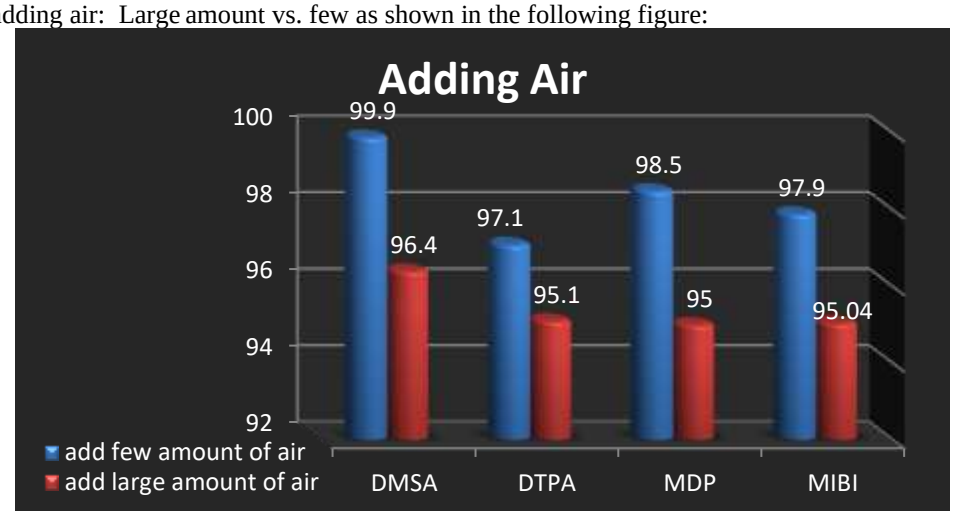


**Fig. (5)** The Effect of adding air during preparation and the labeling Efficiency percentage

Thus we found that:
It is better to evacuate or adding small air rather than large air during preparation as recommended by **manufacturers**.
Considering the volume of certain activity volume added (Large vs. small)
as shown in the following figure

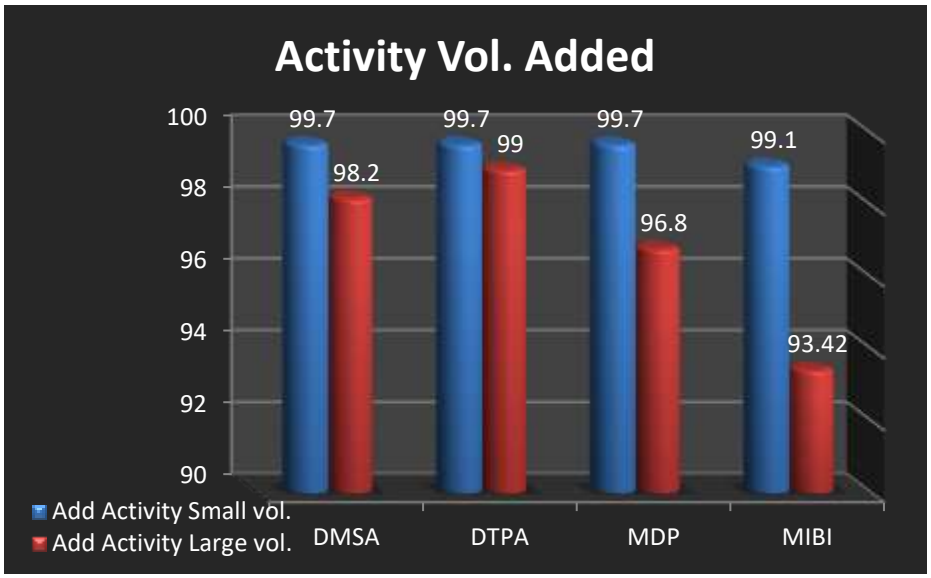


**Fig. (6)** The Effect of change of activity volume with same radioactivity during preparation and the labeling Efficiency percentage

Thus, we found that:
It is better to use small activity volume rather than large volume during preparation as found by **Jeon et al., 2021,** that the labeling efficiency prepared decreased with time but all values were within the standard value.

Considering the *pharmaceutical Conc. Low conc. (Large dilution) vs. high conc. (small dilution) with same radioactivity during preparation and the labeling Efficiency percentage*, as shown in the following figure:

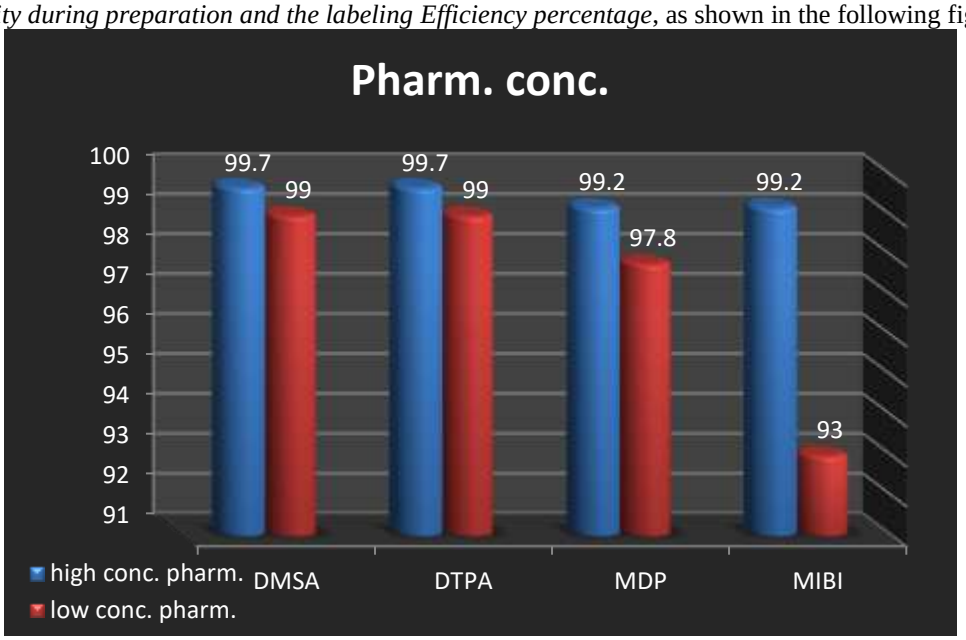


**Fig. (7)** The Effect of change of pharmaceutical conc. with same radioactivity during preparation and the labeling Efficiency percentage

Thus, we found that:
It is better to decrease dilution or increase concentration (high conc.) of pharmaceutical rather than increasing dilution (low conc.) during preparation.

Considering the fractionation time (delay after dilution) from immediately, 2, 5, and 10 days) as shown in the following figures:

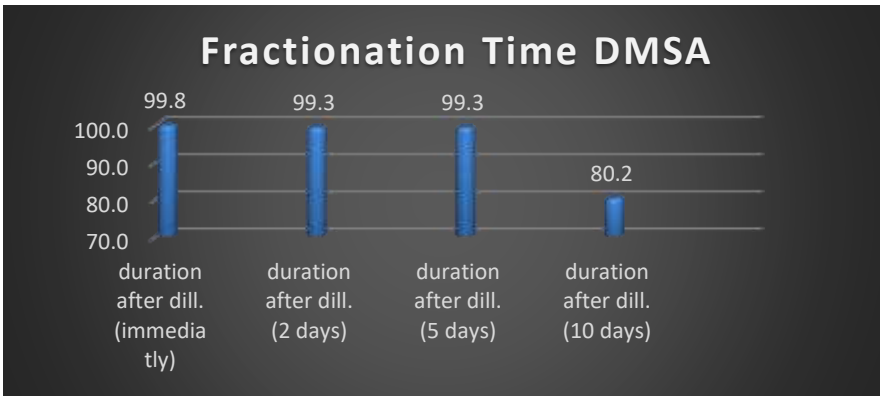


**Fig. (8)** The Effect of Fractionation Time (Time after Dilution) with same radioactivity during preparation and the labeling Efficiency percentage of DMSA

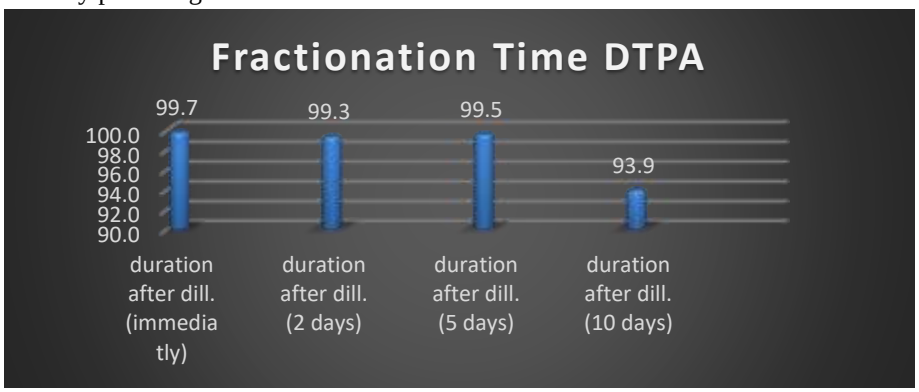


**Fig. (9)** The Effect of Fractionation Time (Time after Dilution) with same radioactivity during preparation and the labeling Efficiency percentage of DTPA

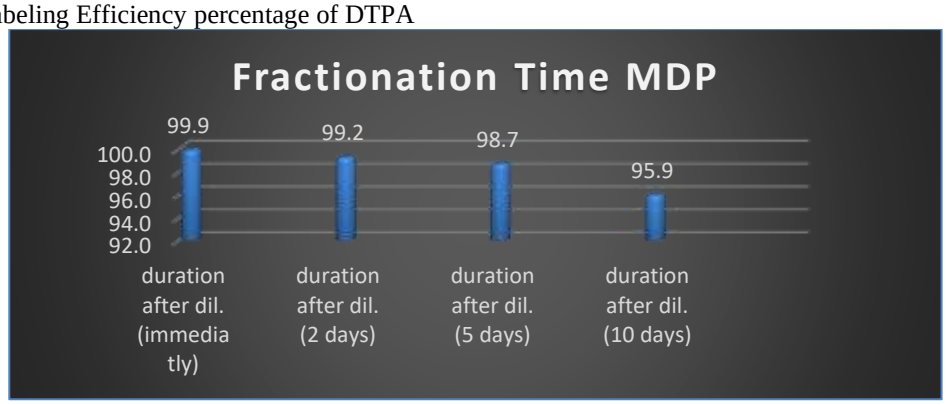


**Fig. (10)** The Effect of fractionation time (Time after Dilution) with same radioactivity during preparation and the labeling Efficiency percentage of MDP

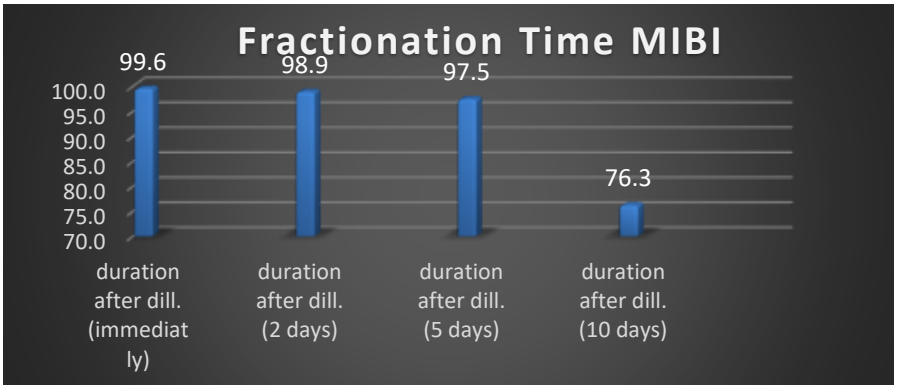


**Fig. (11)** The Effect of fractionation time (Time after Dilution) with same radioactivity during preparation and the labeling Efficiency percentage of MIBI.

Thus, we found that:
It is better to decrease fractionation time less than 5 days especially for DMSA and MIBI because their labeling efficiency percentage are lower than recommended (90 %) after 10 days thus it can't be used thus stability alteration as described by **Crisan, G. et al 2022**.

Considering the heating time of the MIBI vial using microwave oven during preparation as 5, 10, and 15 seconds as shown in the following figure:

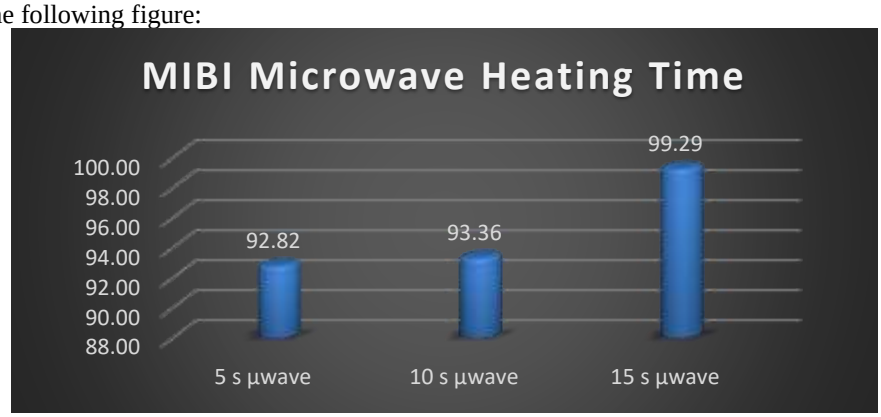


**Fig. (12)** The Effect of change of heating time using Microwave oven with same radioactivity during preparation and the labeling Efficiency percentage of MIBI

Thus, we found that:
Heating 15 second with microwave oven (standard) is better than 10, and 5 seconds because labeling efficiency percentage decreases as **S.H. Hassanpour, et al**, **2021** presented a clinical procedure using ultrasound irradiation technique as an alternative heating process of sesta MIBI with Boiling water bath technique.

Considering the heating time of the MIBI vial using water bath during preparation as 5, 10, and the reference of 15 minutes as shown in the following figure:

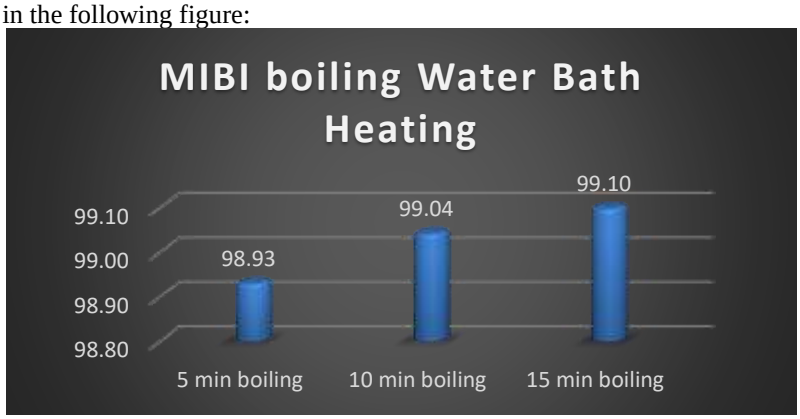


**Fig. (13)** The Effect of change of heating time using Boiling water bath with same radioactivity during preparation and the labeling Efficiency percentage of MIBI

Thus, we found that:
Heating with boiling water bath is better for every time considered of heating and the labeling efficiency slightly increases with time.

## Conclusion

It is possible to obtain labeling of radiopharmaceuticals (RP) with the quality specifications out of manufacturer's standards of the reference values for selected kits (MDP, DTPA, DMSA, and MIBI) through changing various parameters affecting labeling efficiency of radiopharmaceuticals thus, we concluded that:

1. It is necessary to apply manufacture guidelines of storage conditions.
2. It is better to evacuate or adding small air rather than large air during preparation.
3. It is better to use small activity volume rather than large volume during preparation.
4. It is better to decrease dilution or increase concentration (high conc.) of pharmaceutical rather than increasing dilution (low conc.) during preparation.
5. It is better to decrease fractionation time less than 5 days especially for DMSA and MIBI because their labeling efficiency percentage is lower than recommended after 10 days.
6. Heating 15 second with microwave oven (standard) is better than 10, and 5 seconds at same volume because labeling efficiency percentage decreases when time decreases.

7. Heating with boiling water bath is better for every time considered of heating and the labeling efficiency slightly increases with time.

## References


1. **Clemens Decristoforo and Joachim Pfister**, "In vitro studies with radiopharmaceuticals", Nuclear Medicine and Molecular Imaging, Elsevier, Volume 1, **(2022)**, Pages 631-639,
2. **European Pharmacopeia 6**, http://www.uspbpep.com/ **(2011).**
3. **F. L. N.Marques, M. R. Y. Okamoto,A. A.Buchepiguel**, **"***Alguns Aspecto sobre Geradores e Radiofármacos de Tecnécio-99m e seus controles de qualidade Radiologia Brasileira*", *Rev. Brasileira de Radiologia*, **Vol. 34**, n.4, p.233-239**,(2001).**
4. **Frédérique Blanc-Béguin, Simon Hennebicq, Philippe Robin, Raphaël Tripier , Pierre-Yves Salaün and Pierre-Yves Le Roux,** "Radiopharmaceutical Labelling for Lung Ventilation/Perfusion PET/CT Imaging: A Review of Production and Optimization Processes for Clinical Use**"**, Pharmaceuticals (**2022**), 15,518.
5. **George Crisan, Nastasia Sanda Moldovean-Cioroianu, Diana-Gabriela Timaru, Gabriel Andries ,Calin Cainap and Vasile Chis, "** Radiopharmaceuticals for PET and SPECT Imaging: A Literature Review over the Last Decade**"** International Journal of Molecular Sciences**,** (**2022), 23, 5023.**
6. **Gopal Saha, "*Fundamental of Nuclear Pharmacy*"**, Springer, 6ª Ed., New York, **(2010).**
7. **Jan Cleynhens and Alfons Verbruggen,** "Technetium-99m radiopharmaceuticals—Radiochemistry and radiolabeling", Nuclear Medicine and Molecular Imaging, Elsevier, Volume 1, **(2022)**, Pages 79-94.
8. **Juan José Mora-Román, Alejandro Esquivel-Álvarez, Elías Quesada-Morales, María Paz Villegas-Calero, Marianela Chavarría-Rojas, María Fernanda Rojas-Salas and German Madrigal-Redondo,** "Radiopharmaceuticals: A Mini Review Of Applications And Innovations On Nuclear Medicine", World Journal of Pharmaceutical Research, Volume 10, Issue 13, 64-79., ISSN 2277– 7105, DOI: 10.20959/wjpr202113-22132, (**2021).**
9. **Meliha Ekinci, Derya İlem Özdemir, Emre Özgenç, Evren Gündoğdu, Makbule Aşıkoğlu Department of Radiopharmacy, Faculty of Pharmacy, Ege University, Turkey**," Radiolabeling, Quality Control and Cell Binding Studies of a New 99mTc-labeled Bisphosphonates: 99mTc-Ibandronate Sodium**"** DOI: 10.4274/tjps.galenos.2022.01346, Turkish Journal Of Pharmaceutical Sciences (**2022**).
10. **Nic Gillings, Olaug Hjelstuen, Jim Ballinger, Martin Behe, Clemens Decristoforo, Philip Elsinga,Valentina Ferrari, Petra Kolenc Peitl, Jacek Koziorowski, Peter Laverman, Thomas L. Mindt, Oliver Neels, Meltem Ocak, Marianne Patt and Sergio Todde, "**Guideline on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals", EJNMMI Radiopharmacy and Chemistry, https://doi.org/10.1186/s41181-021-00123-2 **(2021)**.
11. **NicGillings, "**Quality control of radiopharmaceuticals: Basics and instrumentation**",** Nuclear Medicine and Molecular Imaging, Volume 1, Elsevier, (**2022)**, Pages 250-259.
12. **Poliane Angelo de Lucena Santos, Wellington Gomes de Andrade, Fernando Roberto de Andrade Lima and Fabiana Farias de Lima**, "quality evaluation of radiopharmaceuticals in nuclear medicine services in the states of Alagoas", international nuclear Atlantic conference – INAC, **(2011)**, ISBN: 978-85-99141-04-5.
13. **Ruhul Amin M., Azizul Haque, Avishek Biswas and Taufiq Hassan Mozumder**, Preparation and labeling of technitium-99m kit in pharmaceutical grade clean room, Journal of Chemical Engineering, IEB Vol. ChE 27,No. 2, December, **(2012).**
14. **S.H. Hassanpour, H. Vafapour, S.Z. Karami,** "The presentation of clinical results of radiolabelling of 99mTc-MIBI complex using ultrasound technique for myocardial perfusion SPECT scanning"**,** Nuclear Medicine 45 271–278, Elsevier, **(2021)**
15. **Shankar Vallabhajosula, PhD, Ronan P. Killeen, MD, and Joseph R. Osborne, MD, PhD, "**Altered Biodistribution of Radiopharmaceuticals:Role of Radiochemical/Pharmaceutical Purity**,** Physiological, and Pharmacologic Factors"**,** Semin Nucl Med 40:220-241 © Elsevier Inc., **(2010)**.
16. **Shanvi Sachin Gawli, Dipti Hiten Chirmade,** a review on radiopharmaceuticals and radiochemical method in analysis, Indo American Journal of Pharmaceutical Research, ISSN NO: 2231-6876**, (2016)**
17. **Y.H. Jeon, Y.H. Cho, J.H. Jung, J.H. Choi**,"Quality control of radiochemical purity and safety of $^{99m}$Tc-HDP and $^{99m}$Tc-phytate radiopharmaceuticals labeling"**,** International Journal of Radiation Research, Volume 18, No 1, **(2020).**