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Comparative Evaluation of Chromatographic Techniques for Radiochemical Control of ^{99m}Tc-MIBI (MIBI = 2-Methoxy-2-isobutyl isonitrile)

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Quality control of radiopharmaceuticals is important, providing products of high standards. The current study refers to comparative evaluation of chromatographic techniques applied to the MIBI (2-methoxy-2-isobutyl isonitrile) after labeling with ^{99m}Tc. Three chromatographic techniques are performed and evaluated taking into account different parameters such as true separation, shape of the chromatographic peaks, time required, ease of handling, cost, *etc*. The scoring system is established according to the relative importance of each factor considered. The results indicated that instant thin layer chromatography is the best quality control method for radiochemical evaluation of ^{99m}Tc-MIBI with application in nuclear medicine. It would appear that PC Whatman 4 and 1Chr can use with success and can replace instant thin layer chromatography method, because of its cost.

Key Words: Radiopharmaceutical, Radiochemical Purity, Labeling Efficiency, Radiochromatographic Techniques.

INTRODUCTION

The need for radiopharmaceutical quality control, as stressed by Saghari *et al.*[1], is emphasized. Because the overwhelming majority of problems occurred in recently developed ^{99m}Tc radiopharmaceuticals (mainly due to their low stannous content), it is likely that this issue is important for some time[2]. The concept of quality control is very important as it provides products of high standards. Quality control of radiopharmaceuticals is a very broad field covering all aspects of the manufacturing process. It must be applied in all steps, *i.e.*, on the radionuclide, on the labeling reaction, on the final product and on radiopharmaceutical aspects of the final product.

^{99m}Tc-MIBI (MIBI = 2-methoxy-2-isobutyl isonitrile) is a positively charged lipophilic complex formed with technetium in a + 1 oxidation state [Tc(I)] as shown by Teran *et al.*[3]. The ligand tetra(2-methoxyisobutyl isocyanide) copper(I) chloride is synthesized by heating 2-methoxyisobutyl isocyanide with anhydrous cuprous chloride in anhydrous ethanol at 90 °C for 1 h. The compound is purified and prepared in a kit form for labeling with $^{99m}TcO_4^-$. After labeling the six monodentate methoxyisobutyl isocyanide (MIBI) ligands are symmetrically attached to the

central ^{99m}Tc atom[4]. There are several recommended methods in the literature for radiochemical purity control of ^{99m}Tc-MIBI radiopharmaceutical. Though, it is unlikely that all methods are equivalent. The present study, comprising part of an extended wok, attempts to address the apparent need of quality control protocols for characterizing ^{99m}Tc-MIBI applied in nuclear medicine.

Finally, it leads to the selection of established analytical techniques, based on simplicity, availability, reliability and information that can be provided. Once a quality control protocol has been established, it can be used to optimize and/or modify the radiolabeling protocol and also monitor the quality of the radiolabeling products prior to being used in nuclear medicine.

Three different chromatographic analytical techniques are originally examined. Different eluents are used creating a lot of chromatographic systems. Some of them do not appear to have found using in nuclear medicine. Some chromatographic of these systems are considered for further investigation. The aim of this study is to compare some of these methods to verify their usefulness and to establish the best way to quantify the radiochemical purity of ^{99m}Tc-MIBI kit.

EXPERIMENTAL

Radiolabeling with ^{99m}Tc: For the preparation of the ^{99m}Tc-labeled MIBI radiopharmaceutical the following procedure is applied[5]: A cold standard MIBI kits (cardiolite) from Du Pont Company and $Na^{99m}TcO_4^-$ (pertechnetate) eluted from a generator Elumatic(III) from CIS Bio. International, after verifying its compliance with the requirements stated in the European Pharmacopoeia are used to prepare ^{99m}Tc-MIBI radiopharmaceutical. It was prepared by adding 1-3 mL of $Na^{99m}TcO_4^-$ containing 5.5-7.4 GBq (150-200 mCi) to the each kit vial. After agitation, the vials are heated in an upright position with appropriate shielding in a boiling water bath to complete the labeling reaction for 10 min. Then the vials are cooled in a cooling water bath for 15 min.

Radiochemical analysis: Radiochemical purity of the radiolabelled product is defined as the proportion of total radioactivity in a sample associated with the desired radiolabelled species[6]. Radiochemical impurities may arise from the production process as well as during storage. Their physiological behaviour deviates from the desired chemical compound, thus causing difficulties in the application and interpretation of the measurements and leading to the uselessness of the radiopharmaceutical[7]. For determining the radiochemical impurities sensitive methods are used, which make possible the separation of even tiny quantities of the different chemical forms of a radionuclide. Some of them using chromatographic techniques, such as paper chromatography (PC), thin layer chromatography (TLC) and instant thin layer chromatography (ITLC). However, few data are available describing actual results of radiochemical purity determination. The following analytical methods are used comparatively, to characterize the radiolabeled product.

Paper chromatography methods: Paper chromatography Whatmann 4 and Whatmann 1Chr (strips 1 cm × 9 cm) it is used as stationary phase. Ethanol absolute, 0.9 % NaCl, methyl ethyl ketone and acetone are used as mobile phase. About 5 μL of ^{99m}Tc-MIBI radiopharmaceutical is dropped in starting point of chromatography strips. Chromatograms are immediately developed and dried in air at room temperature. At a meantime was checked by the same methods and additionally ITLC-SG elute of $^{99m}TcO_4$ milked from ^{99}Mo-^{99m}Tc generator (CIS). The reason for this measurement is to eliminate any doubt about quality of pertechnetate[8,9] used for labelling.

Thin layer chromatography (TLC) method: TLC in Al_2O_3 and TLC in silica Gel Baker-flex sheets, (TLC-SA) and (TLC-SG), respectively (1 cm × 7 cm strips) are used as stationary phase. All supports are activated in 110 ºC for 0.5 h and storing in desiccators until use. Ethanol (absolute and 96 %), acetone, methyl ethyl ketone, NaCl 0.9 % are used as chromatographic solvents. Glass tubes 2 cm × 20 cm with an appropriate stopper were used as chromatographic chamber. An aliquot of the ^{99m}Tc-MIBI radiopharmaceutical (1-5 μL) was dropped at the origin of the strips, immediately after pre-wetting with the respective solvent. Chromatograms are immediately developed and dried in air at room temperature. The chromatographic patterns are determined by a radiochromato scanner (Tracermaster of Bertold Company) connected to a computer. Using this technique, it is possible not only to determine the R_f of each radioactive compound in the strip, but also other characteristics such as the shape and width of each peak. In some cases the strips, after developing and drying into the air are cut in 1 cm pieces and the activity of each of them is measured in a suitable counter. According to the literature ^{99m}Tc-MIBI complex moves in with the front (R_f = 0.5-1.0) while free and colloid ^{99m}Tc remain in origin (R_f = 0-0.25 and R_f = 0.25-0.55, respectively). The content of ^{99m}Tc-MIBI complexes should be more than 90 %. It is calculated by the following formula:

$$^{99m}\text{Tc-MIBI complex (\%)} = 100 - (^{99m}\text{Tc impurities \%})$$

Instant thin layer chromatography (ITLC) method: Silica gel (ITLC-SG) and polysilicic acid (ITLC-SA) impregnated glass fibre sheets (Gelman Science) for instant thin layer chromatography are used as stationary phase. They are divided in 1 cm × 10 cm and 2 cm × 20 cm strips, respectively and were activated in 110 ºC for 0.5 h storing in desiccators until use. Later 1 cm × 10 cm strips, were used only because it was not observed any difference in behaviour in comparison to the 2 cm × 20 cm strips. Acetone, 0.9 % NaCl and MEK (methyl ethyl ketone) are used as chromatographic solvents. At the 1 cm from the lower end of the strips is dropped 1-5 μL of the ^{99m}Tc-MIBI radiopharmaceutical. The strips are dried in air and developed in chromatographic solvents. They are cut into 1 cm pieces. It is measured activity of each piece in a suitable counter.

According to the Smith *et al.*[10] the content of $^{99m}TcO_4^-$ is calculated from the chromatograms developed in saline (R_f = 0.35 - 1) and the content of $^{99m}TcO_2$ is calculated from the chromatograms developed in acetone (R_f = 0-0.25). Labelling efficiency is calculated as follows:

$$^{99m}\text{Tc-MIBI complex (\%)} = 100 - (^{99m}TcO_4^- + ^{99m}TcO_2\ \%)$$

The content of ^{99m}Tc-MIBI complex should be more than 90 %. This method requires a clear and total separation between the peaks for the correct quantification. The short time needed to complete the test is also important.

RESULTS AND DISCUSSION

Paper chromatography methods: $^{99m}TcO_4^-$ milked from generator allays was pure, do not content any forms of colloids and was suitable to use for labeling. Figs. 1 and 2 present radiochromatograms of ^{99m}Tc-MIBI radiopharmaceutical checked by Whatmann 4 and 1Chr paper, respectively, in methyl ethyl ketone, NaCl, acetone and ethanol eluents.

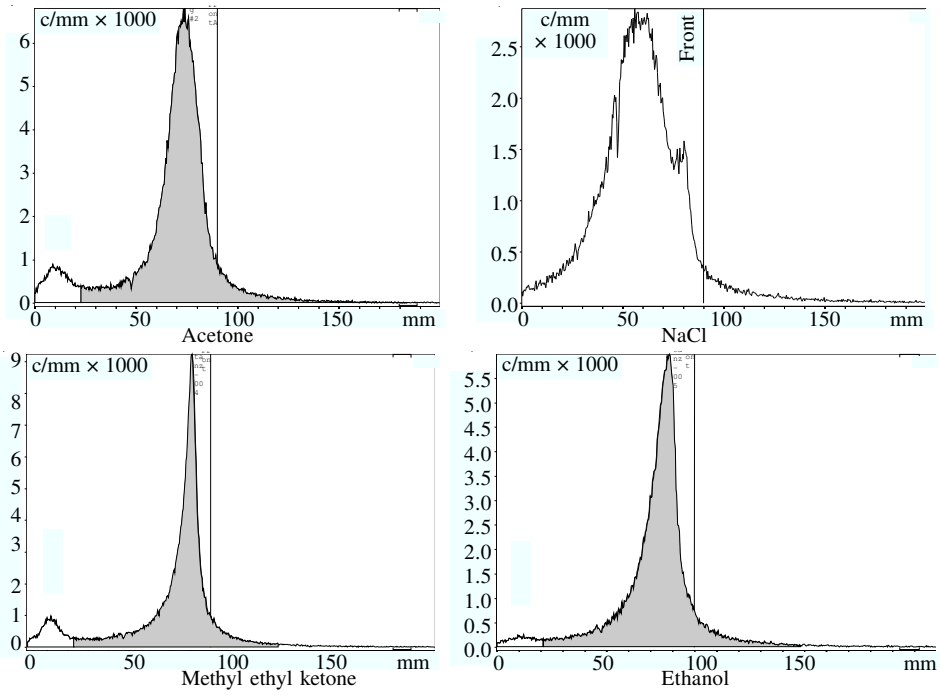

Fig. 1. Chromatograms of ^{99m}Tc-MIBI radiopharmaceutical with PC Whatmann 4

The radiochromatograms obtained using acetone and methyl ethyl ketone as mobile phase, for the both PC (Whatmann 4 and 1Chr), are the similar with radiochromatograms obtained using Baxerflex Al_2O_3 TLC method recommended. Separation of colloidal and complex forms of radiopharmaceutical is very clear defined. In the case of mobile phase NaCl the peaks are not defined and in the case of ethanol absolute content of colloidal form is lower than content obtained by other methods including the Baxerflex Al_2O_3 TLC method.

Thin layer chromatography (TLC) method: Chromatograms developed in absolute and 96 % ethanol, while TLC in Al_2O_3 is used as stationary phase, are

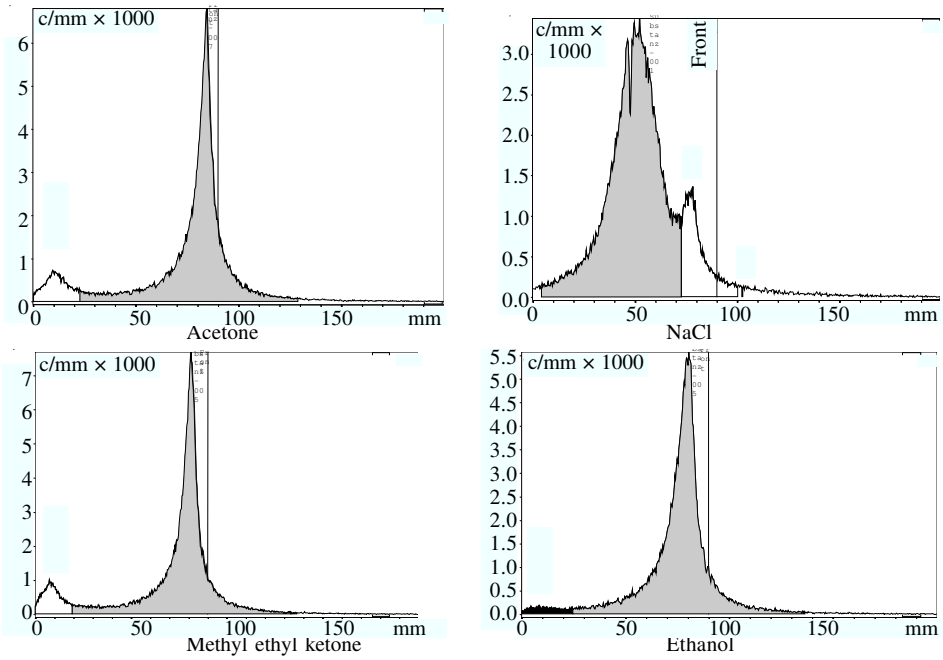

Fig. 2. Chromatograms of ^{99m}Tc-MIBI radiopharmaceutical with PC Whatman 1Chr

presented in Fig. 3. It clearly shows a good separation between two peaks, impurities and ^{99m}Tc-MIBI complex, but we can also envisage a small difference between the two typical chromatograms. Chromatogram developed in absolute ethanol shows a good separation between two impurities (free and colloid ^{99m}Tc), too. This is not completed when 96 % ethanol is used as mobile phase.

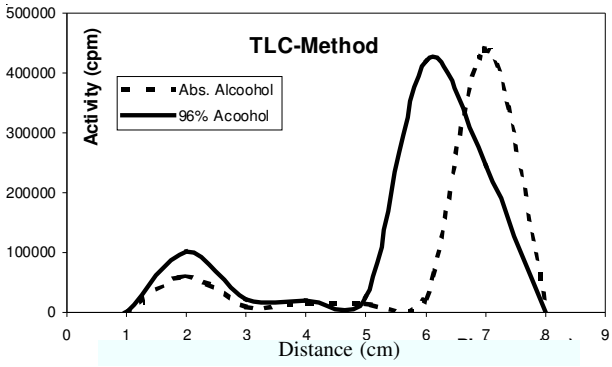

Fig. 3. Typical curve of TLC-Al_2O_3 method

A number of experiments, not recommended in the literature, are also performed using TLC in silica gel (TLC-SG) as stationary phase and absolute alcohol, acetone, NaCl and methyl ethyl ketone as mobile phases in order to find another proper

chromatographic system to determine the content of radioactive ^{99m}Tc-forms in ^{99m}Tc-MIBI radiopharmaceutical. The typical chromatograms are given in Figs. 4-7, respectively.

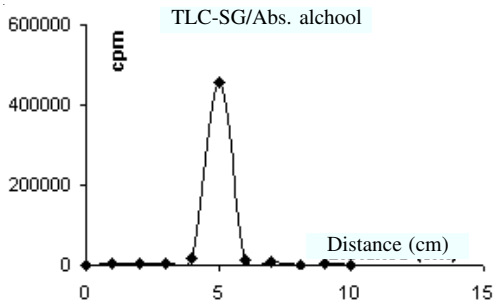

Fig. 4. Typical curve of TLC-SG/abs. alc. system

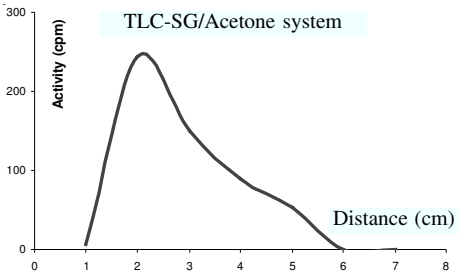

Fig. 5. Typical curve of TLC-SG/acetone system

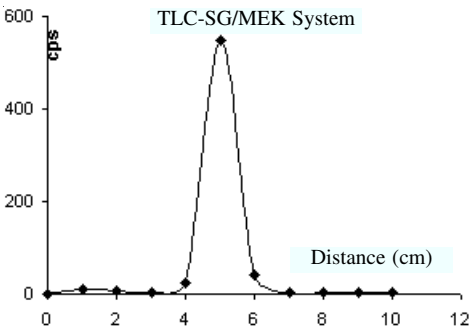

Fig. 6. Typical curve of TLC-SG/NaCl system

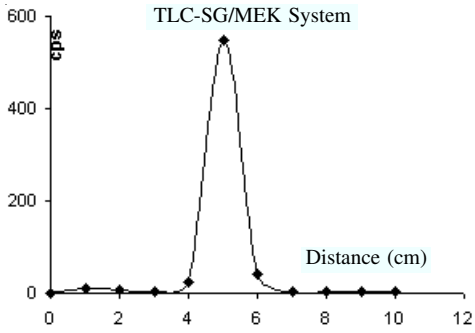

Fig. 7. Typical curve of TLC-SG/MEK system

These figures TLC-SG/absolute alcohol and TLC-SG/NaCl systems perform a good separation of ^{99m}Tc-forms, while TLC-SG/MEK system can not perform a good separation. The results from this system are not in accordance with the reported literature. TLC-SG/Acetone system cannot perform separation of ^{99m}Tc-forms in ^{99m}Tc-MIBI radiopharmaceutical.

This method is expensive and takes a long time during the developing of the chromatograms. It is needed to scan the chromatograms because it is difficult to cut the TLC strips and to measure each cm peaces. The other problem resulting from this method is that the contents of free and colloid technetium forms could not be established apart.

Instant thin layer chromatography (ITLC) method: The results taken after calculations of ^{99m}Tc-MIBI complex, free and colloid technetium forms contents by given formula, when ITLC-SG sheets are used as stationary phase and acetone and saline as mobile phases show that in all of them the content of ^{99m}Tc-MIBI complex is more than 98 %.

Figs. 8 and 9 presented typical curves taken after measuring of the activity of each 1 cm peaces of the chromatograms developed in acetone and NaCl, respectively, using ITLC-SG sheets as stationary phase.

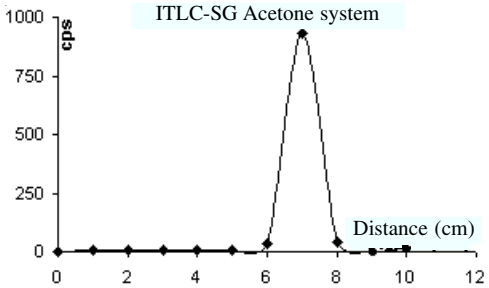

Fig. 8. Typical curve of ITLC-SG/acetone system

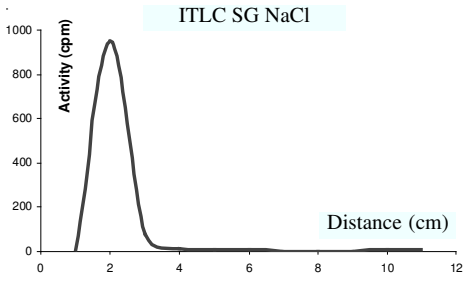

Fig. 9. Typical curve of ITLC-SG/NaCl system

A good separation of three radioactive technetium forms in ^{99m}Tc-MIBI radiopharmaceutical. Chromatographic peaks are clearly defined.

In Figs. 10 and 11 are presented typical curves using ITLC-SA sheets as stationary phase and acetone and NaCl, respectively, as mobile phases. These systems are not recommended before in the literature.

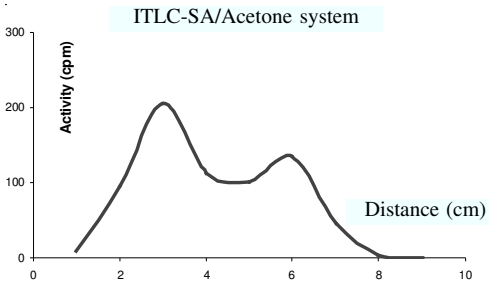

Fig. 10. Typical curve of ITLC-SA/acetone system

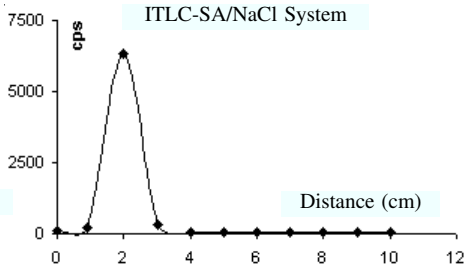

Fig. 11. Typical curve of ITLC-SA/NaCl system

It is clearly shown that in this case the peaks are clearly defined while saline is used as chromatographic solvent. The shape of chromatographic peaks is not clear in case of acetone is used as chromatographic solvent. Therefore, it needs further effort to clarify this situation. We used 2 cm × 10 cm ITLC-SA strips and replaced acetone with methyl ethyl ketone (Fig. 12).

ITLC SA/MEK system can not perform good separation between ^{99m}Tc-forms. This method can be completed in few minutes. It is relatively easy to handle but expensive.

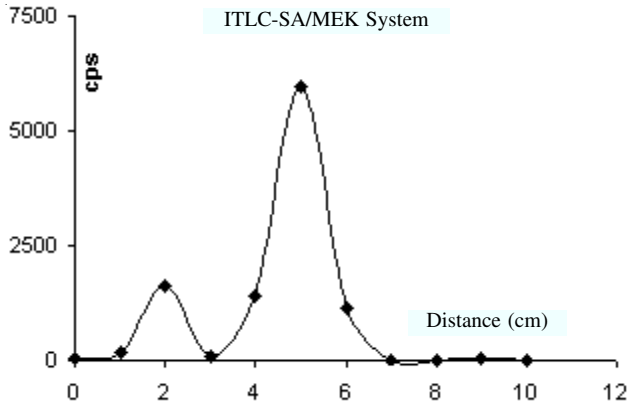

Fig. 12. Typical curve taken from ITLC-SA/methyl ethyl ketone system

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