RADIOPHAMATHTICAL, ORIGINAL ARTICLE

^{99m}Tc-HMPAO in Brain SPECT Imaging; Feasibility of Ultra-Utilization of the Cold kit with higher ^{99m}Tc activities - A Chromatographic Study.

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Introduction

Brain SPECT imaging has become one of the most interesting subjects for most specialists in nuclear medicine because of its ability to present clear and detailed data about the brain anatomy and function, allowing the specialist to obtain the greatest sensitivity and specificity for disease detection **(Catafau, 2001).**

Technetium-99m complex of hexamethyl propylene amineoxime (HMPAO) (which is called also exametazime) is widely used as an efficient brain perfusion agent due to the ability of the primary ^{99m}Tc-HMPAO complex to penetrate the intact blood-brain barrier (BBB) which is believed to be attributed to its lipophilic property **(Hung, 1994).**

The primary lipophilic ^{99m}Tc-HMPAO complex is inherently unstable and rapidly converts to a secondary less lipophilic complex. Retention in the brain results from the inability of the secondary complex to cross the blood-brain barrier **(Ponto, 1990)**. Thus, we have here one more impurity ligand in addition to the 2 other persisting impurities which are the free and hydrolyzed-reduced technetium. The radiochemical purity of the ^{99m}Tc-HMPAO preparation could be recognized by using a combination of three thin layer chromatographic (TLC) systems **(Tikofsky, 1993).**

HMPAO commercial kits are one of the most expensive materials in nuclear medicine, in addition to that; the added Tc-99m activities proposed by the commercial venders can be enough to perform only 2 patient studies per vial which is not cost effective especially in developing countries that obstacles depending on brain SPECT as a routine imaging protocol.

To overcome this, there are two postulated solutions; one is to collect a larger number of patients per session which is not practical as it may take several weeks to have a good number of patients especially in small NM facilities, the other solution is to do a cold kit fractionation which is best idea allowing having secured single patient doses at different times; however, this method needs a well trained personnel to perform it.

These two solutions are still not fully applicable because of the limited Tc-99m capacity of the HMPAO vial; this motivated us to study the effect of adding up to 200% of the recommended activity to one of the commercial kits which will allow us to know to which activity limit we can have a safe preparation and if we exceeds these limits what will be the produced impurities out of this, and hence, the preparation is modifiable and recoverable or not.

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Materials & Methods

Labeling ^{99m}Tc-HMPAO preparations were obtained aseptically from a commercial kit *(*DONG-A pharm. Co., ITD, Seoul, Korea**)** containing 0.5mg exametazime ingredient. Pertechnetate in 0.9% aqueous Nacl was eluted from the commercial ^{99}Mo/^{99m}Tc generator (Amertec II, Amersham intl. Laboratories).

In these preparations we used to reconstitute the HMPAO vials with different activities of ^{99m}Tc-pertechnetate which are 20, 30, 40, 50, 60 and 70 mCi. Each preparation with each specified activity had been repeated at least 5 times, an overall number of more than 40 successful preparations have been achieved.

All preparations were obtained while standardizing all other factors which may affect the percentage radiochemical purity (%RCP) and these standards are:

- Age of sodium pertechnetate ≈1 hour
- Order of generator elution = 5th day.
- Specific Concentration = 20 mCi/cm^3.

Chromatography After an incubation time of 5 minutes, %RCP was obtained through a combination of three chromatographic systems that allows a full quantitative assessment of the radiochemical composition of the mixture (**Neirinckx, 1987)**. These systems are:

ITLC-SG / saline (NaCl) system

ITLC-SG / Methyl-ethyl keton

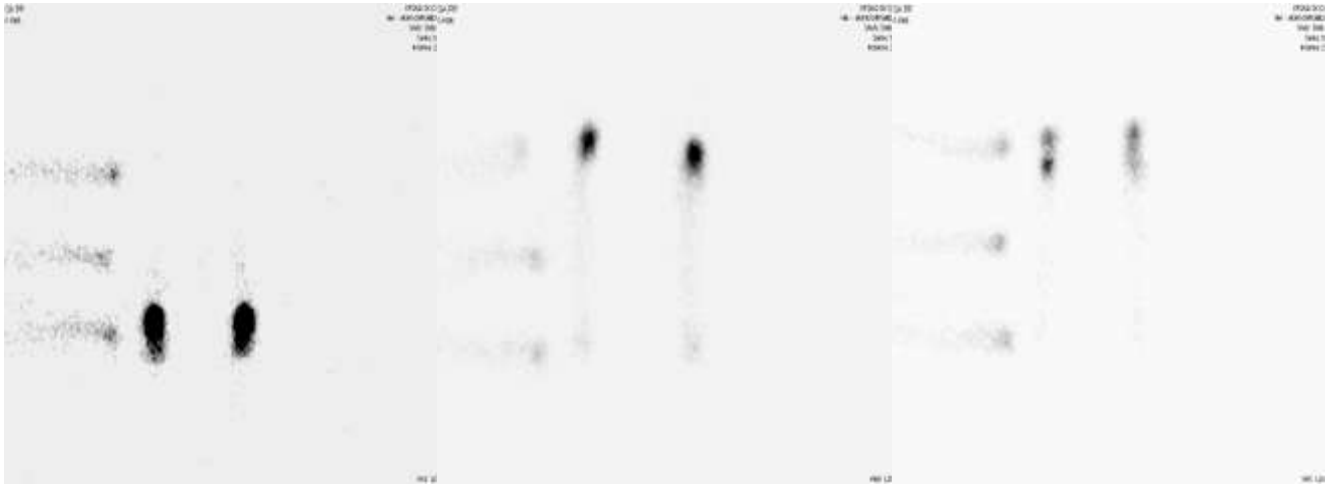
Whatman no.1 / 50% Acetonitril: 50% H_2O

Each of these systems has an origin (R_f = 0) and a solvent front (R_f = 1).

To perform this chromatographic procedure, a small droplet (4 – 8 µl) of the ^{99m}Tc-HMPAO complex was dropped on the chromatography strip and then after the three solvents reaches the solvent front level (R_f= 1) a fast drying method is applied.

Activity distribution along the 3 strips has been determined by calculating the total counts on each side of the strip (starting point and solvent front) that has been detected by drawing a ROI of each side activity in a 2 minutes image of each strip,

Figure(1).



(a) **(b)** **(c)**

Figure (1): Radioactivity distribution along the strips of the three chromatography systems used for radiochemical purity determination of ^{99m}Tc-HMPAO where a) ITLC-SG / saline system, b) ITLC-SG / Methyl-ethyl keton and c) Whatman no.1 / 50% Acetonitril: 50% H_2O. The left-sided three hot spots in the three figure parts showing point source markers at R_f = 0, R_f = 0.5 and R_f = 1.

Using these three chromatographic systems, the chemical composition of the complex was obtained using the following relationships:
% Pertechnetate ($^{99m}TcO_4^-$) = % activity at the solvent front in the ITLC-SG / NaCl system.
% Reduced-hydrolyzed Tc ($^{99m}TcO_2^-$) = % activity remaining at the origin in the Whatman No. 1 / 50% Acetonitril: 50% H_2O system.
% Secondary complex = % activity at $R_f = 0 – 0.3$ in the ITLC/MEK system – % reduced hydrolyzed Tc.
% Primary complex = % activity at $R_f = 0.3 – 1$ in the ITLC/MEK system – % pertechnetate.
The whole chromatography procedure is adjusted to be completed in less than 25 minutes to allow injection of the radiopharmaceutical in a time less than 30 minutes which is the shelf life of the prepared HMPAO mixture.

Results & Discussion

Technetium-99m(^{99m}Tc)-Hexamethyl Propylenamine oxime (HMPAO), was used efficiently for brain SPECT imaging **(Catafau, 2001 and Pi-Lien, 2008)**.
With the addition of oxidant-free ^{99m}Tc pertechnetate, a lipophilic primary complex is formed in the active moiety necessary for optimum brain uptake and crossing blood brain barrier (BBB). With time , the primary lipophilic complex is converted to a secondary hydrophilic form which is unable to effectively cross the blood brain barrier **(Kung, 1990 and Webber, 1992)**.
The two main disadvantages of ^{99m}Tc labeled HMPAO are its instability after labeling with ^{99m}Tc, and the high cost of the kit **(Siddig, 2002)**. The complex stability for many hours had been proven in many research reports **(Solanki, 1994, Hung, 2002 and Siddig, 2002)**.
Before administration of the ^{99m}Tc-HMPAO to the patient, and in compliance with the monographs of the European Pharmacopeia **(Ph.Eur. ,2005)**; a radiochemical purity (RCP) testing must be performed and an RCP value > 80% must be achieved **(Piera, 1990; Piera, 1995*)*** . The rapid decomposition of the primary complex to the secondary form limits the shelf-life of the radiopharmaceutical to 30 minutes after reconstitution **(Ponto, 1990; Tubergen 1991 and Hung, 1994).**
The recommended activity with this particular HMPAO kit is 30 mCi, however, in this study we have reached adding of 70mCi to the vial which is more than double the vender's dose limits. The obtained results of this study have been summarized in table (1) and figure (2) which represents the means value of the %RCP of each species of the ^{99m}Tc-HMPApreparation as a function of the activity added to the kit.

Table (1*):* mean value of %RCP of the 4 components of the HMPAO complex as a function of the activity added to the kit

	20	**30**	**40**	**50**	**60**	**70**
Primary HMPAO	**92.30**	**91.60**	**92.00**	**92.10**	**87.50**	**78.90**
Secondary HMPAO	**4.60**	**4.40**	**4.70**	**4.70**	**3.10**	**3.50**
TcO_4	**1.40**	**2.00**	**1.10**	**1.10**	**2.30**	**6.60**
TcO_2	**2.80**	**3.40**	**1.90**	**2.90**	**7.10**	**10.20**

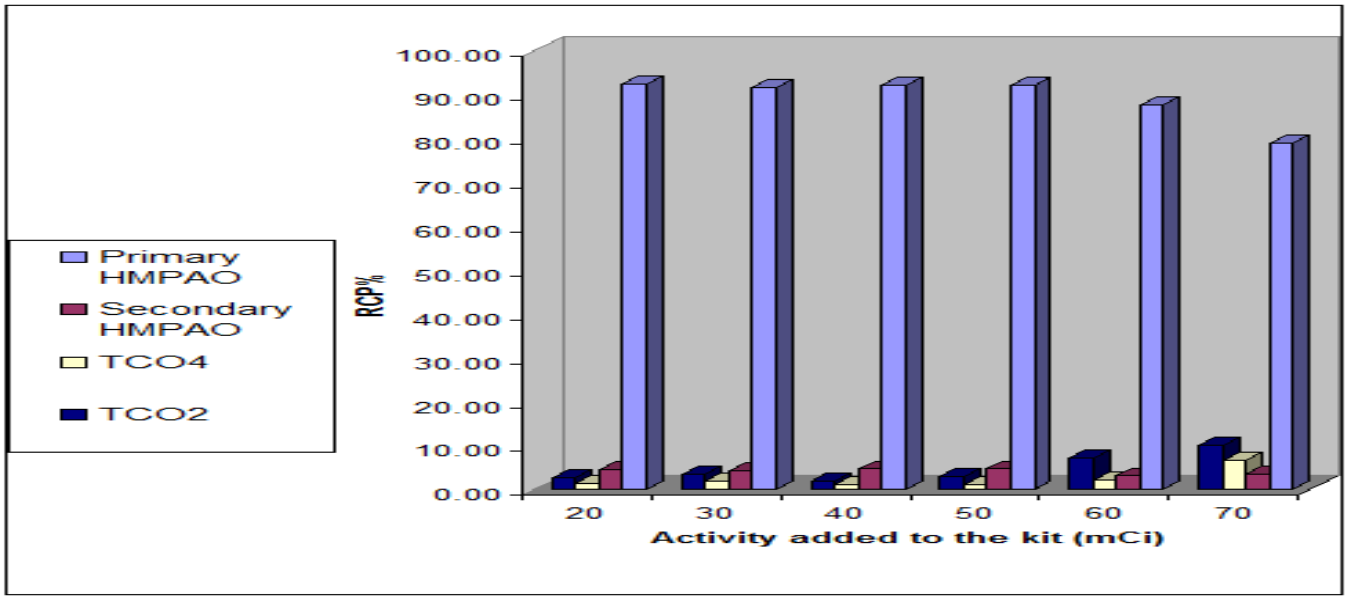


Figure (2)*:* Effect of the activity added to the kit on the %RCP of the 4 components of the HMPAO complex

Regarding Primary HMPAO; our results revealed that, there were no obvious variations in (% RCP) when adding an activity of 20 up to 50 mCi to the kit. Increasing the activity above this level led to a well differentiated loss in the (% RCP) of the primary HMPAO complex aggressively and progressing with time ($\rho \approx 0.001$). However, we have to state here that, even with this degradation of the %RCP of the primary complex, it is still higher than the accepted %RCP stated by the European pharmacopeia (>80%) with activities up to 60 mCi. With activity of 70 mCi, the mean %RCP is just lower than the accepted limit (78.9%) which is still close to the safe limit, however, we have to take into our consideration that, this is a mean value which means that we had multiple preparation with this activity are considered failed ones since they have a %RCP of less than 75%.

In the case of secondary HMPAO; the differences of (% RCP) could not considered of great value. However, it was sometimes clinically significant (ρ-value < 0.05). This phenomenon is related also to the elevation of added activity over (50) mCi, whereas it was not seen in levels of 20 to 50 mCi.

For $^{99m}TcO^-_4$; consideration of the statistical parameters revealed tendency of (% RCP) towards a great increase when exceeding the limit of 50 mCi activity. This effect was not seen with using 20 – 50 mCi of radio technetium.

The (% RCP) of $^{99m}TcO^-_2$; was considered fluctuating all over the test, so we could not say that it was standard when using 20 – 50 mCi. But, its concentration has markedly increased when the activity increased over fifty millicuries.

We could explain the variation of the (% RCP) of the primary HMPAO complex by the increased concentration of free technetium and hydrolyzed-reduced technetium. The elevated concentration of the free Tc ($^{99m}TcO^-_4$) is due to the absence of enough amounts of stannous ions that are responsible for reduction of the Tc-99m ions from the oxidation state 7 to 3 or 4 to be ready for labeling. Unlike most of the technetium based cold kits, HMPAO contains a very limited amount of stannous ions and this due to the well defined relation between the increased stannous concentration and the transformation of the primary HMPAO molecules to the secondary form which is has a very limited capability of crossing the Blood Brain Barrier.

This elevated concentration of $^{99m}TcO_4^-$ can be avoided pre-labeling by adding an adjusted amount of stannous ions in the form of stannous chloride, bromide, fluoride or hydrate in a process called Stannous Augmentation. Post-labeling, if the primary complex is >80% and the preparation s suffering from the high concentration of $^{99m}TcO_4^-$, safe chemical compounds can be administered to the patient before HMPAO injection to block thyroid and choroid plexus preventing accumulation of the free Tc-99m atoms in these sites which deteriorate the brain imaging process.
The increased concentration of the hydrolyzed-reduced Technetium (TcO_4) can be attributed to the following:

a) Since there is a limited number of HMPAOmolecules, increasing the activity to this limit means more than double Tc-99m molecules have been introduced to the preparation with no equal amount of HMPAO molecules which leading to a competition of Tc-99m atoms for the labeling procedure leaving the unlabeled ones in their reduced state.

b) Radiolysis, as the main reason of decomposition of the labeled complexes increases with higher activities and specific concentration leading to a release of the Tc-species after the complete breaking of the ^{99m}Tc-HMPAO complexes that appeared obviously beyond 50mCi Tc-99m activities.

The decrease in the (% RCP) of the secondary HMPAO could be attributed only to the decrease in (% RCP) of primary complex as it's a bi-product of the primary complex.

The results of **Neirinckx et al., (1987)** concluded that, no more than 30 mCi should be added to the kit to prevent the degradation of the (% RCP) of the primary HMPAO complex. But, **Ballinger et al., (1990)** stated that no (% RCP) variations could be detected in the range of (35–65) mCi. Accordingly, the results of the present work do not fully agree with the results reported by these two groups of research work.

Conclusion

In this work which have aimed to study the effect of adding up to 70mCi of ^{99m}Tc-Pertechnetate to the HMPAO commercial kit on the %RCP of the labeled complex, it have proved that adding of activities between 20mCi to 50mCi will not have any effect on the purity of the labeled mixture. With increasing the activity to 70mCi, the %RCP has been degraded significantly; whoever, this degradation cannot obstacle using of the labeled radiopharmaceutical since the obtained %RCP is still in the safe limits recommended by the European Pharmacopeia (>80%). When adding an activity of 60-70mCi, an accurate QC procedures are mandatory since we had a marked number of failed preparation with this activity range.

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