

Stability Study and Optimization Conditions of Tc-99m-L,L-Ethylenedicysteine (Tc-99m-L,L-EC)

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ABSTRACT

Technetium - 99m - L, L-EC compound is a renal imaging tracer used especially for renal function study in human. The study shows that radiochemical purity is much higher than 90.00%, pH ~ 10 - 12, shelf life is 240 minutes. Lyophilized kit of L, L-EC can be stored for approximately 6 months. Maximum labeling activity is 400 mCi, in 10 ml volume. Biodistribution studies in mice after 30 mins injection shows that tracer accumulation in kidneys and urine are 8.25 ± 1.32 and $62.56 \pm 2.46\%$ respectively.

INTRODUCTION

Numerous radiolabeled compounds have been proposed and evaluated as potential tracer agents for the radionuclide study of renal function and morphology^(1,2). One of them is radiomercury compounds⁽³⁾ but the major disadvantage is that it gives a significant radiation burden to the patient (Mercury - 203; gamma energy 279.2 keV). Radioiodinated agent⁽⁴⁾ such as I-131 - Hippuran is commonly used as a dynamic qualitative indication of renal function. Recently many Tc-99m labeled tracers have been studied as potential renal tracers. These tracers have the advantages of a low radiation dose to patient and can be specially designed for the study of different specific renal parameters.

One of the Tc-99m labeled tracer for renal imaging is Tc-99m - MAG III (use for renal imaging, relative function and transit times studies and also for determination of the effective renal plasma flow (ERPF).

But it has low stability (shelf life ~ 1 hr.) and after 1 hr. side - products have occurred increasingly with a high hepatobiliary uptake.) Tc-99m - DTPA is use for calculation of glomerular filtration rate. It has high stability (shelf life ~ 5 hrs.). Tc-99m - DMSA has high renal cortex uptake but low urinary excretion rate and low blood clearance. It appropriates for renal parenchyma imaging. Tc-99m-GHA and Iron-ascorbate have poor renal uptake but rapid blood clearance and rapid urinary excretion rate⁽⁵⁾.

Tc-99m - L, - L - EC is one of the Tc-99m labeled renal agent which has better properties than other Tc-99m-labeled renal radiopharmaceuticals because it is rapidly excreted into urine and give better plasma clearance. The distribution in kidneys, liver, intestine is lower compare to Tc-99m - MAG III. The study shows that the properties of Tc-99m-L,-L-EC is similar to that

of I-131 - Hippuran especially in renal function parameter⁽⁶⁾. It is easily labeled at room temperature compare to Tc-99m - MAG III (which has to be labeled in boiling water). The high stability (shelf life ~ 240 mins.) and high radiochemical purity (> 90.00%) are among other advantages.

The stability and appropriate conditions for labeling lyophilized L, L - EC with Tc-99m have to be studied. Paper Chromatography and High Performance Liquid Chromatography (HPLC) are the methods used to determine percent of radiochemical purity (RCP). The results of %RCP at different time intervals have been used to determine the expiration date. For biodistribution, Tc-99m-L,L-EC accumulation in mice have been studied after 10 and 30 mins injection⁽⁷⁾.

This research is covered radiochemical purity, appropriate conditions for labeling (Maximum activity for labeling, shelf life, expiration date, etc.) including the distribution in organs at 10 and 30 mins after injection. The results are compared with other radiopharmaceuticals such as I-131 - Hippuran, Tc-99m - MAG III and Tc-99m - DTPA.

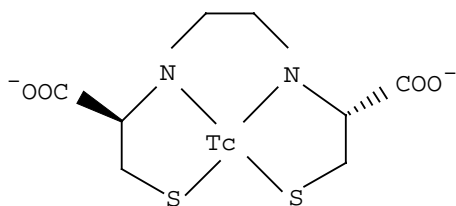


Fig. 1 shows structure of Tc-99m-L,L-EC.

MATERIALS AND METHODS

Materials

1. Sodium Pertechnetate (Tc-99m) from Tc-99m - generator (CIS, France) and lyophilized L, L, EC (contains 1 mg. of N, N - ethylene -

L - dicysteine, 0.1 mg. of SnCl₂ · 2H₂O, 0.5 mg. of Sodium dihydrate and 3.55 mg. of Na₂HPO₄) produced by isotope production division, Office of Atomic Energy for Peace (OAEP).

2. Whatman no. 1 (Whatman International Ltd. Maidstone, England), ITLC - SG (Gelman Sciences Inc., U.S.A.), size 1.5 x 10 cm², used as stationary phase of Paper Chromatography.
3. Methyl Ethyl Ketone (MEK) and 0.5 M CH₃COOH used as mobile phase of Paper Chromatography.
4. Column C - 18 m - bondapack, 300 x 3.9 mm. used as stationary phase of HPLC.
5. Phosphate buffer 0.0125 M, pH ~ 2.5 and Ethanol used as mobile phase of HPLC.
6. Radiochromatographic scanner (GITA, Germany).
7. HPLC (Waters, U.S.A.).
8. Mice.
9. Lyophilized kit MAG III, DTPA and I-131 - Hippuran produced by isotope production division, OAEP.

Methods

1. Labeling step

Label lyophilized L, L - EC kit with 10 millicuries of sodium pertechnetate (Tc-99m) in 1.5 millilitre volume. Shake vigorously to produce Tc-99m - L, L - EC.

2. Percent of Radiochemical Purity (% RCP) after labeling.

Two systems of paper chromatography (Whatman no. 1 with MEK and ITLC - SG with 0.5 M CH₃COOH) are performed, as shown in table 1 and figure 2. Percentage RCP can be calculated using equation 1.

Table 1 Two paper chromatography systems

Rf	Whatman no. 1, MEK	ITLC - SG, 0.5 M CH ₃ COOH
0	% H.R. + Tin Colloid + Cpx	% H.R. + Tin Colloid
1	% TcO ₄ ⁻	% TcO ₄ ⁻ + Cpx

Where: Cpx = Tc-99m - L, L - EC
 % H.R. = Hydrolyzed reduced technetium.

$$\% \text{ labelling } (\% \text{RCP}) = 100 - \% \text{TcO}_4^- - (\% \text{H.R.} + \% \text{Tin Colloid}) \dots\dots\dots(1)$$

Radioactivities of each Whatman No. 1 and ITLC - SG region are measured by radiochromatographic scanner linked to NaI (TI) detector (results are shown in Figure 2). Percentage RCP at different time; 5, 60, 120, 180, 240 mins after labeling are studied to determine shelf life of the kit (results are shown in Figure 3). pH of the labeled compound is also determined using pH - paper.

3. Other factors which effect on labeling.

To determine the optimum activity, volume and concentration of sodium pertechnetate for labeling, various activity between 10 - 400 mCi, volume between 1 - 10 ml. and concentration between 10 - 200 mCi / ml. are used for labeling. (results are shown in Figure 4, 5 and 6).

4. Stability of lyophilized L, L-EC kit.

Percent of Radiochemical Purity (% RCP, step 2) determination is repeated every month until the % RCP is less than 90.00% to determine the expiration date of the kit (results are shown in Figure 7).

5. Radiochemical purity of Tc-99m - L, L - EC by HPLC method.

Radiochemical purity of Tc-99m - L, L - EC is determined using HPLC method by injecting 10 ml of Tc-99m - L,L - EC into

HPLC (results are shown in Figure 8). Details of HPLC system is as follow :

Stationary phase :

Column C-18 μ - bondapack, 300x3.9 mm.

Mobile phase :

A ; phosphate buffer 0.0125 M, pH ~ 2.5

B ; Ethanol

min	%A	%B
0	100	0
10	91	9
20	100	0

Flow rate : 1 ml / mins

Detection : UV - VIS absorbance and radiometric

Retention times : 14.2 min

6. Biodistribution studies in mice.

Biodistribution studies in mice is studied by injecting 10 mCi / 0.1 ml of Tc-99m - L, L - EC into 5 mice. Mice are sacrificed 10 and 30 mins after injection. Organs are dissected and organs' radioactivity are measured using NaI (TI) detector linked with SCA analyzer. Percentage organs radioactivity accumulation are calculated by Tiscon Programme (results are shown in table 2). Repeated biodistribution studies, using the same method, are performed with Tc-99m - MAG III, Tc-99m - DTPA and I-131 - Hippuran for comparison. (Table 2 and figure 9)

RESULTS

% H.R. + Tin Colloid + Cpx

% TcO₄⁻ + Cpx

% TcO₄⁻

% H.R. + Tin Colloid

2 a

2 b

Fig. 2 Components of Tc-99m - L,L - EC at 5 mins after labeling (NaTcO₄ ~ 10 mCi / 1.5 ml.) in two paper chromatographic systems.

	2a	2b
Stationary phase :	Whatman no. 1	ITLC - SG
Mobile phase :	MEK	0.5 M CH ₃ COOH

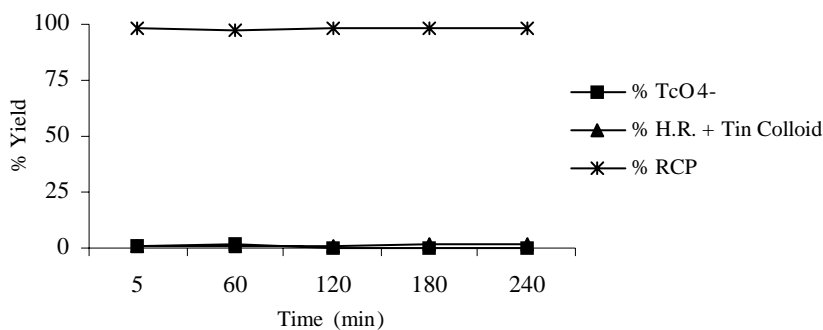


Fig. 3 Percent Radiochemical Purity and % Impurity (TcO₄⁻, H.R. + Tin Colloid) of Tc-99m - L, L - EC at 5, 60, 120, 180 and 240 mins after labeling (NaTcO₄ ~ 10 mCi / 1.5 ml.).

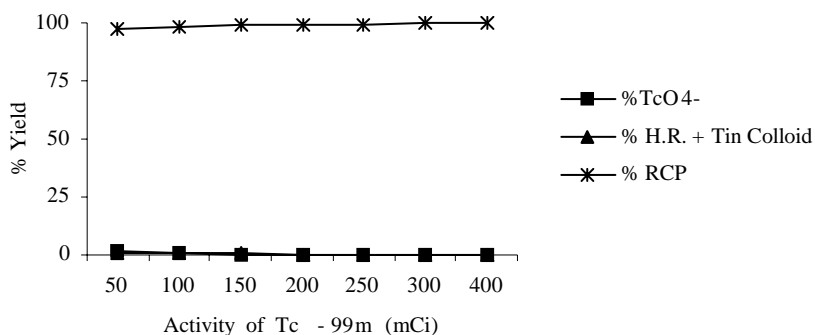


Fig. 4 Percent Radiochemical Purity of Tc-99m - L, L - EC at 5 mins after labeling with various activity of Tc-99m (from 50 to 400 mCi).

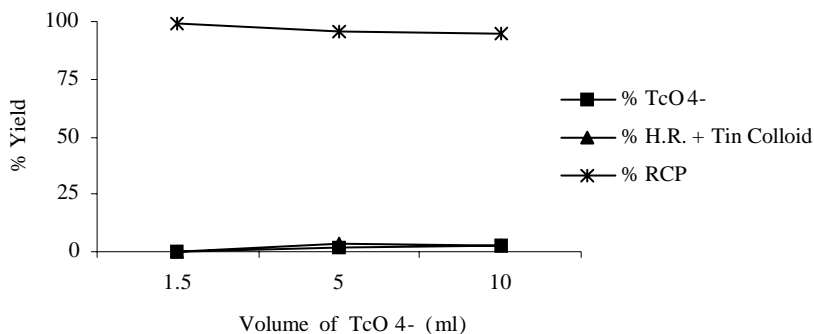


Fig. 5 Percent Radiochemical Purity of Tc-99m - L, L - EC at 5 mins after labeling with various volume of Tc-99mO₄⁻ (from 1.5 to 10 ml.).

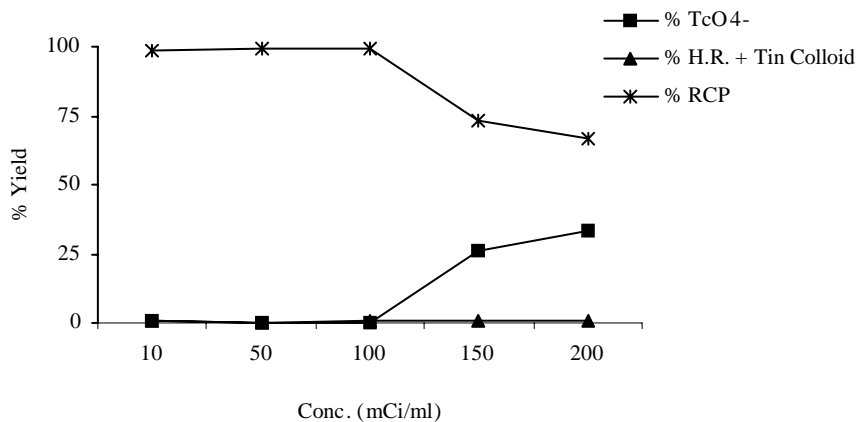


Fig. 6 Percent Radiochemical Purity of Tc-99m - L, L - EC at 5 mins after labeling with various concentration of Tc-99mO₄⁻ (from 10 to 200 mCi / ml).

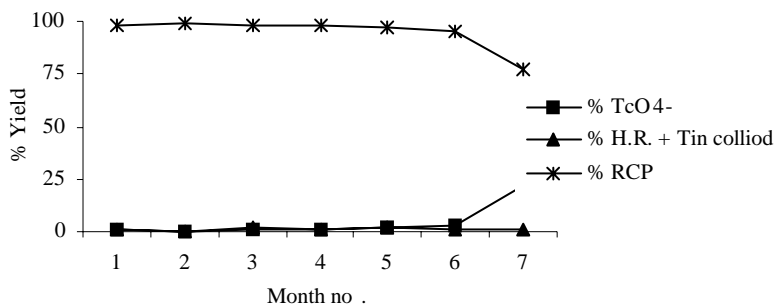


Fig. 7 Percent Radiochemical Purity of Tc-99m - L, L - EC at 5 mins after labeling using lyophilized L, L - EC which are stored from 1-7 month (NaTcO₄ ~ 10 mCi / 1.5 ml.).

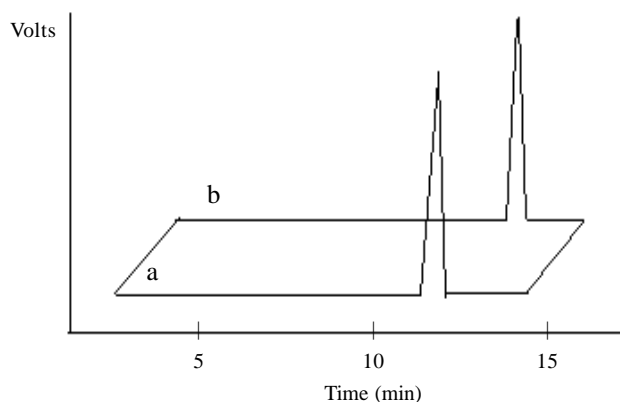


Fig. 8 Radio - HPLC Chromatograms result of Tc-99m - L,L - EC :
 a : labeled at pH ~ 12
 b : labeled at pH ~ 12 and adjusted to pH ~ 7.0 with 0.5 NaH₂PO₄ 0.4 ml.

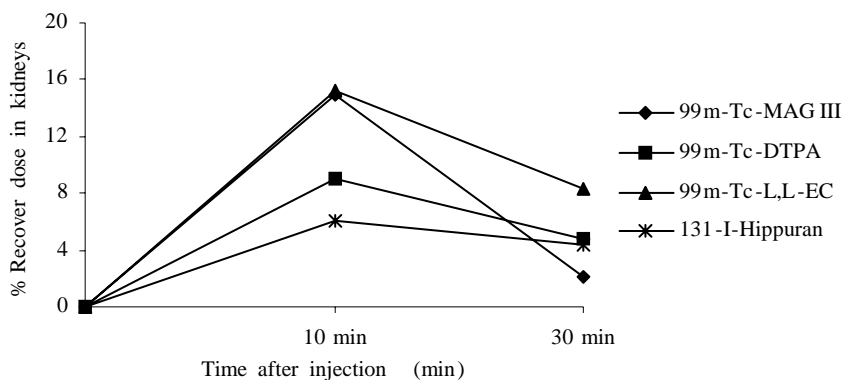


Fig. 9 Percent recover dose in kidneys of Tc-99m - L, L - EC, Tc-99m - MAG III, Tc-99m - DTPA and I-131 - Hippuran in mice (n = 5).

Table 2 Biodistribution in Mice (n = 5) of Tc-99m - L,L - EC, Tc-99m -MAG III, Tc-99m - DTPA and I-131 - Hippuran at 10 and 30 mins after injection.

	Time (min. p.i.)	Blood	Liver	Intestines	Kidneys	Urine	Stomach
		%Recover dose ± s.d.					
Tc-99m-L,L-EC	10	5.31±0.37	9.63±1.30	7.21±0.83	15.26±2.85	38.83±5.57	0.34±0.08
	30	2.38±0.16	5.11±0.55	11.95±1.15	8.25±1.32	62.56±2.46	0.19±0.05
Tc-99m-MAG III	10	4.82±1.01	5.09±1.55	2.77±0.39	14.95±7.07	41.26±14.68	0.47±0.10
	30	1.41±0.20	1.41±0.39	4.01±0.64	2.09±0.82	75.64±2.67	0.31±0.20
Tc-99m-DTPA	10	8.79±1.94	2.39±0.22	2.47±0.45	8.97±1.44	32.62±6.06	0.46±0.05
	30	3.31±0.73	1.63±0.61	1.92±0.76	4.77±0.69	67.32±5.27	0.28±0.17
I-131-Hippuran	10	3.41±1.37	2.48±0.76	1.65±0.31	6.01±2.19	57.93±5.66	0.31±0.05
	30	0.42±0.14	0.68±0.19	3.28±0.44	4.40±5.22	71.08±8.50	0.53±0.07

DISCUSSION

Percent Radiochemical Purity (RCP) of Tc-99m - L, L - EC by Paper Chromatography (Stationary phase ; Whatman No. 1, ITLC - SG and mobile phase ; Methyl Ethyl Ketone (MEK), 0.5 M CH₃COOH is much higher than 90.00% (Figure 2). The impurities such as TcO₄⁻, H.R., Tin Colloid have been separated clearly from the complex.

The shelf life of Tc-99m - L, L - EC is 240 mins after labeling. (Figure 3). The activity of Tc-99m can be used up to as high as 400 mCi. The volume for labeling is up to 10 ml. and the maximum concentration is 100 mCi / ml. (Figure 4, 5, 6).

Figure 7 shows that %RCP are much higher than 90.00% up till 6 month after reconstitution and slightly decreased in 7th month. It means that the expiration date of Lyophilized L, L - EC kit is 6 months after manufacturing date.

Figure 8 shows the HPLC chromatograms of a reaction mixture (a) immediate after labeling with pH ~ 12 and (b) after neutralize to pH ~ 7. Both studies have Radiochromatogram at retention time of 14.2 mins.

The results of tracer of Tc-99m - L, L - EC in kidneys is higher at 10 mins. than at 30 mins. (15.26 ± 2.25 , 8.25 ± 1.32 respectively) and accumulation in urine is less

at 10 mins. than at 30 mins. (38.83 ± 0.57 , 62.56 ± 2.46 respectively). This means that excretion of Tc-99m - L, L - EC into urine is rapid and high with minimal retention time in kidneys.(Table 2)

Compare to Tc-99m - MAG III, Tc-99m - DTPA and I-131 - Hippuran (Figure 9), Tc-99m - L, L - EC approaches Tc-99m - MAG III more closely than Tc-99m - DTPA and I-131 - Hippuran at 10 mins. But at 30 mins the accumulation of Tc-99m - L, L - EC in kidneys is higher than Tc-99m - MAG III. (8.25 ± 1.32 , 2.09 ± 0.82 respectively) It means that excretion of Tc-99m - MAG III into urine is rapid and high with minimal retention time in kidneys. (Table 2 and Figure 9)

CONCLUSION

On the basis of our experiment with Tc-99m - L, L - EC, it can be concluded that this agent has some very attractive properties that can make it more appropriate for renal function studies than Tc-99m - MAG III. It can be labeled very easily and efficiently at room temperature starting from a labeling kit with long shelf life (240 mins.) resulting in a preparation with excellent radiochemical purity and stability (% RCP > 90.00%, expiration date = 6 months.). It would thus constitute a very practical and reliable radiopharmaceutical that can be available in a few minutes without a boiling step.

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