Study of ^{99m}Tc-DMSA Biodistribution in Experimental Animals

Thais O. M. de Castro[1], Natanael G. da Silva[1], Maria T. Colturato[1], Carlos F Felgueiras[1], Jair Mengatti[1], Neuza T. O Fukumori[1], Margareth M. N. Matsuda[1], Elaine B. de Araújo[1]

[1]Radiopharmacy Center - IPEN/CNEN-SP - São Paulo – SP - Brazil
thais.castrom@gmail.com

ABSTRACT

^{99m}Tc-DMSA, succimer (99m Tc), is a radiopharmaceutical commonly used in nuclear medicine for renal function evaluation by imaging. In order to achieve adequate labeling of the product with good radiochemical yield and standardized biological distribution, the interval of 185 - 3700 MBq should be kept in a maximum volume of 3 mL for product labeling. Moreover, one should avoid exposing the reconstituted solution to oxygen and using the product after four hours post labeling. The aim of the study was to quantify and evaluate the influence of different DMSA complexes on biological distribution of the radiopharmaceutical in experimental animals, taking in account variations in the labeling parameters. Radiochemical purity was determined by paper and thin layer chromatography using both acetone/Whatman 3MM, 0.9% NaCl/TLC-SG and n-propanol/H_2O/acetic acid (4:3:1 V/V/V)/TLC-SG systems respectively for quantification of $^{99m}TcO_4^-$ and $^{99m}TcO_2$ plus some ^{99m}Tc-DMSA complexes. The labeling activity did not significantly affect the extent of the main complex generation. The presence of oxygen and the concentration of ^{99}Tc did not markedly change the percentage of the radiochemical impurities in the preparation. Radiochemical purity tests of the DMSA-^{99m}Tc based on IPEN-CNEN DMSA-TEC reagent and on another producer's reagent showed similar results. Although the routine method used by IPEN-CNEN to determine the radiochemical yield of ^{99m}Tc-DMSA was not able to discriminate among ^{99m}Tc-DMSA complexes, the renal uptake and the kidney to liver plus spleen uptake ratio in rats met the official compendia criteria for the radiopharmaceutical.

1. INTRODUCTION

The meso-2,3-dimercaptosuccinic acid (DMSA) in the solid state is a white crystalline powder with characteristic smell and bitter taste. It was synthetized for the first time by Owen in 1949, later improvements came by several researchers [1]. The molecule has stereoisomers; among them are the meso-isomers, which are used in Nuclear Medicine for evaluation of the kidney function. The spatial distribution of sulfhydryl groups in the meso isomer provides the molecule chelating properties, useful in the treatment of poisoning by heavy metals. The meso isomer is also used in the production of a lyophilized reagent that is labeled with technetium-99m.

~~During~~ In the reaction with ^{99m}Tc, DMSA molecule (Figure 1) is reduced to a few oxidation states that bear distinct complexes (Figures 2 and 3) with varied biodistribution patterns [1].

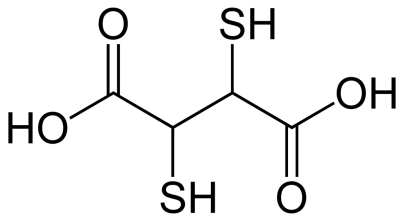

Figure 1: Molecular structure of meso-2,3-dimercaptosuccinic acid (molecular formula: $C_4H_6O_4S_2$; molecular mass: 182.22 g mol^{-1})[2]

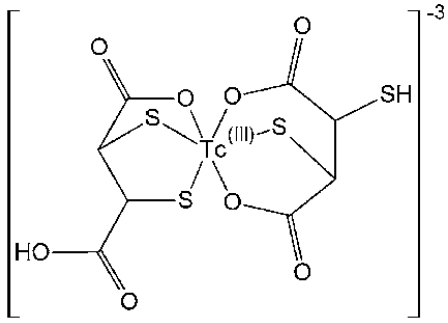

Figure 2: Molecular structure of meso-2,3-dimercaptosuccinic acid 99m technetium-^{99m}Tc(III) (molecular formula: $C_8H_6O_8S_4Tc$; molecular mass: 456.20 g mol^{-1})[3]

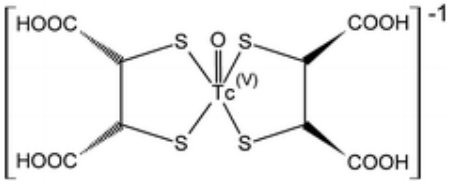

Figure 3: Molecular structure of meso-2,3-dimercaptosuccinic acid 99m technetium-^{99m}Tc(V) (molecular formula: $C_8H_8O_9S_4Tc$; molecular mass: 474.34 g mol^{-1}) [4]

That phenomenon depends on the chemical parameters of the reaction, specially pH and amount of reducing agent [5]. The ^{99m}Tc-DMSA radiopharmaceutical prepared in acid medium bears the ^{99m}Tc-DMSA (III) and is used to renal scintigraphy and renal functions diagnostic [5]. ^{99m}Tc-DMSA complex prepared in medium alkali gives the ^{99m}Tc-DMSA (V) that is used to detect cancer of breast, lung and medullary thyroid carcinoma, tumor of head, neck, brain, metastases of liver and bones.

In order to achieve safe and effective radiopharmaceutical preparations, the product must be tested regularly before clinical use. Routine quality control must evaluate the integrity of labeled compounds, taking in account the efficacy of the labeling procedure, the chemical stability, the radiochemical purity and the biodistribution pattern [6]. The quality control for

^{99m}Tc-DMSA complexes using different chromatographic methods and the organs biodistribution's determines the quality ^{99m}Tc-DMSA complexes [6-7].

According the method used and validated by IPEN-CNEN to ^{99m}Tc-DMSA (III) injectable solution, radiochemical purity should be evaluated by means of chromatographic assay, using paper and thin layer chromatography, with acetone/Whatman 3MM and 0.9% NaCl/TLC-SG systems to quantify $^{99m}TcO_4^-$ and $^{99m}TcO_2$ impurities, respectively. In spite of meeting the criteria of the American Pharmacopoeia [8], the chromatographic systems used by IPEN-CNEN, however, do not separate the different complexed ^{99m}Tc-DMSA species, presumably present in the preparation.

According to the same monograph, biodistribution of the radiopharmaceutical must be evaluated in rats, using a assay that predicts the organs dissection to determine the percentage of administered activity after one hour of intravenous injection of the radiopharmaceutical [8]. Biodistribution could also be qualitatively evaluated by scintigraphy images of animals after radiopharmaceutical administration [9].

Ikeda and colleagues [7] found that technetium could originate four distinct complexes with DMSA: **(I)** Tc(IV)-DMSA, **(II)** Tc(III)-DMSA, **(III)** Tc(V)-DMSA and **(IV)** Tc(VI)-DMSA. The preferential syntheses of a complexes set is driven by pH, pertechnetate concentration, proportion of Sn(II) to Sn(IV) and the amount of oxygen in solution. Complexes **I** and **II** are found in low pH, while high pH brings about complexes **III** and **IV**. Spectrophotometric measurements and stoichiometric titrations of DMSA-pertechnetate with SnCl showed that the complex **I** emerged spontaneously and was later identified as Tc(IV)-DMSA. Further biodistribution studies revealed that bones retain the major part of complex **I**, with low kidney uptake and primary urinary excretion. It was also shown that the complex **I** is converted to complex **II** while in a medium with high Sn(II) concentration; complex **II** is retained mainly in kidneys. Complexes **III** and **IV** rise by titration in alkaline medium of the complexes **I** and **III**, respectively.

Complex **III** is similar to complex **I** regarding bone uptake, and complexes **II** and **IV** concentrate in the kidneys. Complexes I and II are formed from Sn-DMSA kit labeling (such as DMSA-TEC produced at IPEN), due to the low pH. Complexes **I** and **II** should be especially present in the Sn-DMSA kit, due to the characteristic low pH of the medium. The maximal yield of complex **II** is tied to pH, oxygen concentration and reaction time. Renal uptake of the complexes after labeling with ^{99m}Tc doesn't vary when later pH changes [10].

The labeling reaction occurs in two consecutive steps: initial fast formation of complex **I**, then slow conversion of complex **I** to complex **II**. Because of that slow step, at least 10 minutes are required for incubation. Complex **II** could be heavily affected by oxygen, as soon as oxidation of the reducing pair Sn(II)/Sn(IV) lowers the system reduction potential and may bring back complex **II** to complex **I**. In order to keep the system reduction potential, nitrogen or argon atmosphere is necessary during complex formation [12-13].

The low kidney uptake is due to fast excretion of complex **I**. Liver uptake raises when 1 mL of air is bubbled into Tc-DMSA solution twenty minutes prior to the injection. Ascorbic acid in the compound formulation retards oxidation [13].

Garnuszek and colleagues [10] studied some chromatographic systems to separate Tc (III) and Tc (V) complexes formed with DMSA. Among the studied systems, which using thin layer chromatography systems with sílica gel (TLC-SG) and n-prophanol/H_2O/acetic acid (4:3:1 V/V/V) mixture as solvent, made it possible to separate all radiochemical species with different Rfs: Rf TcO_4^- = 0.8-0.9; Rf TcO_2 = 0.0; Rf Tc(V) = 0.6 and Rf Tc(III) = 0.0-0.6.

This work used the chromatographic systems described by Garnuszek P. and colleagues [10] to asses the formation of complexed species of DMSA with ^{99m}Tc, from the labeling of DMSA kit produced at IPEN (DMSA-TEC), evaluating the influence of labeling conditions and the use of different labeling agents in the formation of the complexes and the impact on biological distribution of the radiopharmaceuticals in experimental animals.

1. MATERIAL AND METHODS

The DMSA lyophilized reagent (DMSA-TEC®) and the sodium pertechnetate (99m Tc) solution eluted from GERADOR-IPEN-TEC®, both produced at IPEN-CNEN/SP, and the same lyophilized reagent produced by another company, were used in this study.

In the labeling procedure, the producers' instructions were followed - basically, the DMSA reagent was reconstituted with 3 mL of sodium pertechnetate (99m Tc) solution with varied activities, and incubated for 30 minutes at room temperature.

Radiochemical purity was determined by paper and thin layer chromatography, being the chromatographic systems used in this work acetone/Whatman 3MM NaCl 0.9%/TLC-SG (IPEN validated methods) to quantify $^{99m}TcO_4^-$ and $^{99m}TcO_2$ impurities, and n-propanol/H_2O/acetic acid (4:3:1 V/V/V)/TLC-SG to identify several ^{99m}Tc-DMSA complexes formed by labeling DMSA with ^{99m}Tc at low pH, such as I and II complexes previously described by Ikeda et al. [7].

In order to evaluate the labeling parameters influence on both radiochemical purity of the preparation and formation of the different DMSA complexes, the following parameters were studied: (a) labeling activity (185 MBq and 3700 MBq); (b) ^{99m}Tc concentration (using the pertechnetate solution immediately after the elution and 6 hours later); (c) labeled solution oxygenation just after incubation time got finished (oxygen was introduced into the medium by bubbling 3 mL of air two times), radiochemical purity tests were performed 1 and 4 hours after oxygenation.

In addition to the studies carried out with IPEN's DMSA-TEC® reagent, a DMSA kit from another manufacturer was employed to compare the possible rising of distinct radiochemical species due to contrasting kit compositions.

Biodistribution assays of labeled products were performed with different labeling conditions using *Wistar* rats and invasive studies, as described in the United States Pharmacopoeia [13]. About 185 MBq or 3700 MBq (approximately 0.1 mL) of the labeled product were injected in the animals through the tail vein. After 1 hour of injection, the animals were sacrificed and the relevant organs were removed and washed, then the radioactivity of the organs was

determined in a gamma-type well counter (Capintec), and finally the percentage of injected activity per organ (% IA/organ) was calculated.

Alternatively, gamma-scintigraphy images of the animals were acquired in a proper equipment (Mediso-Hungary) several times after the radiopharmaceutical injection, in order to illustrate the radiopharmaceutical distribution pattern.

The results were expressed as mean and standard variation. The data were statistically analyzed by Student's t test (non-parametric and two-tailed) with significance ($P < 0.05$ and confidence interval (CI) equal to 95%. Statistical analyses were performed using GraphPad Prism software (version 7, La Jolla, CA, USA).

2. RESULTS AND DISCUSSION

The DMSA-TEC labeling procedures, performed with low and high activity of sodium pertechnetate solution (99mTc), resulted in radiochemical yield exceeding 85%, according to the pharmacopoeial criterion established in the U.S.P monograph (Table 1). The radiochemical purity value of the labeling was obtained by subtracting from 100% the percentages obtained for the radiochemical impurities $^{99m}TcO_2$ and $^{99m}TcO_4^-$. However, the chromatographic systems used in this work to identify these impurities do not allow the separation of the different forms of the previously described ^{99m}Tc-DMSA complex [10]

Thus, Table 1 also presents the percentage of a complexed species of DMSA, possibly the complex I described by Ikeda et al. [7], which is also characterized as a radiochemical impurity of the preparation, since the renal uptake of this species is lower and the biodistribution pattern is different from the ^{99m}Tc-DMSA complex identified such as complex II by the same author, rather the complex of interest in the composition of the radiopharmaceutical for renal study. The labeling activity variation studies presented in Table 1 appear to indicate that this parameter did not influence the percentage of complex I. On the other hand, the complex I percentage increased significantly ($P < 0.05$, CI = 95%) with the labeling time.

Table 1: Labeling of meso-2,3-dimercaptosuccinic acid with high and low ^{99m}Tc activity and quantification of radiochemical impurities (immediately after labeling and four hours later)

Labeling conditions (M)	RADIOCHEMICAL PURITY		
	%TcO_2 (NaCl 0.9%/TLC)	**%TcO_4 Acetone/W3MM (paper)**	**% Complex I (n-propanol:H_2O:acetic acid 4:3:1/TLC)**
M1- Low activity (185 MBq/3mL) immediately after labeling	9.35 ± 0.75	0.28 ± 0.05	12.93 ± 3.05
M1 (after 4 hours)	8.55 ± 2.57	0.25 ± 0.05	18.71 ± 2.33
M2- High activity (3700 MBq/3mL) immediately after labeling	12.65 ± 1.37	0.73 ± 0.29	11.16 ± 0.94
M2 (after 4 hours)	5.42 ± 1.46	0.85 ± 0.07	14.95 ± 0.56

The chromatographic system used, composed of TLC-SG in nPrOH/H_2O/AcOH (4:3:1 V/V/V), allowed the separation of two radiochemical species probably related to the complexes I and II described in the literature [3]. Scintigraphy images of the chromatographic strip obtained in this system were acquired in the gamma camera to verify the spatial distribution, the separation of the radioactive species and the respective Rfs. In addition, a vial of DMSA-TEC was labeled in the presence of sodium bicarbonate solution to generate the pentavalent species ^{99m}Tc-DMSA (V) and to determine the Rf of this specie in the selected chromatographic system.

Figure 4 presents the scintigraphic image of the chromatographic strips used to identify the different ^{99m}Tc-DMSA complexes formed in the labeling of DMSA-TEC with sodium pertechnetate (99m Tc) solution.

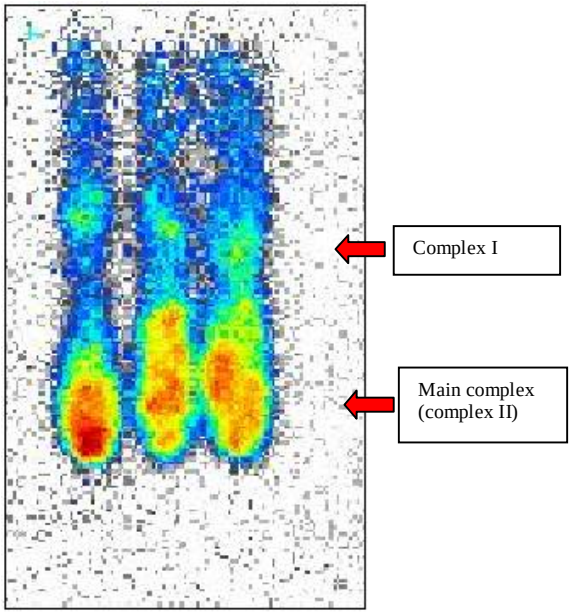

Figure 4: Scintigraphic image of radiochemical purity control chromatographic strips of DMSA labeled with 3700 MBq/3 mL using the TLC-SG and mixture of n-propanol/H_2O/acetic acid 4:3:1. The lower arrow corresponds to the main complex (complex II) and the upper arrow possibly corresponds to the complex I.

Figure 5 presents the profile in the same chromatographic system for the labeling of the DMSA-TEC kit in the presence of sodium bicarbonate (to form the pentavalent species) and without the introduction of sodium bicarbonate (forming the trivalent species at low pH), showing the difference in Rf of the species. The Rf of all the radiochemical species formed in the preparations are shown in Table 2.

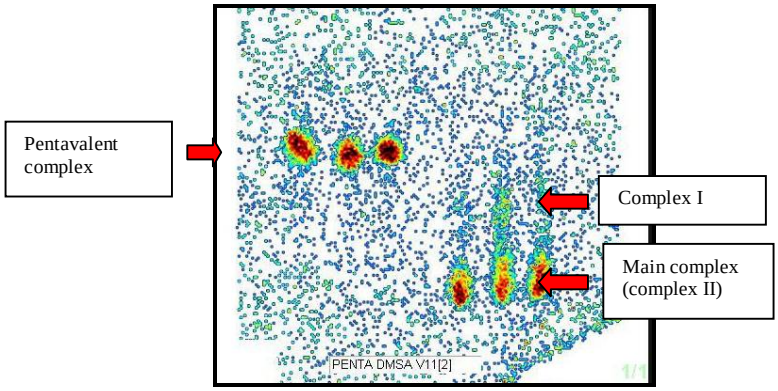

Figure 5: Scintigraphic image of radiochemical purity control chromatographic strips of DMSA labelled with 185 MBq/3 mL in the presence of sodium bicarbonate (triplicate on the left) and in the absence of bicarbonate (triplicate on the right) using the TLC- SG and mixture of n-propanol/H_2O/acetic acid 4:3:1.

The labeling activity variation studies shown in Table 1 seem to indicate that this parameter did not influence the percentage of complex I formation. On the other hand, the percentage of the complex I increased discretely with the labeling time.

Table 2: Rf of radiochemical species identified in the labeling studies of the DMSA-TEC with ^{99m}Tc (with and without sodium bicarbonate)

Radiochemical species	Rf
$^{99m}TcO_4^-$	0.9-1.0
$^{99m}TcO_2$	0.0
^{99m}Tc-DMSA **(III) – complex II**	0.0 – 0.4
^{99m}Tc-DMSA **(III) – complex I**	0.5 – 0.6
^{99m}Tc-DMSA **(V)**	0.7 – 0.8

The results of the studies performed to evaluate the effect of oxygen on the labeling medium as well as the influence of ^{99}Tc concentration (using generator eluate after 6 hours of elution) also did not indicate significant changes ($P < 0.05$; CI = 95%) in the percentage of the complex I or other radiochemical impurities (Table 3).

Table 3: Influence of oxygen and the ^{99}Tc concentration (using the generator eluate with 6 hours) on the radiochemical impurities percentage of the DMSA-TEC labeling with sodium pertechnetate (^{99m}Tc) solution

Labeling conditions (M)	Radiochemical purity control		
	%TcO_2 (0.9 % NaCl/TLC)	**%TcO_4^- (acetone/W3MM paper)**	**% Complex I (n-Propanol:H_2O:AA 4:3:1/TLC)**
M1 (3700 MBq /3mL) immediately after labeling	8.20 ± 1.53	0.19 ± 0.04	13.33 ± 0.84
M1 + O_2 + 1 hour	4.60 ± 1.14	0.08 ± 0.06	19.28 ± 8.78
M1 + O_2 + 4 hours	5.04 ± 1.32	0.27 ± 0.04	15.76 ± 1.39
M2 (3700 MBq /3mL) (Eluted with 6 hours)	9.10 ± 2.41	0.19 ± 0.02	12.40 ± 0.27

In a comparative manner, the radiochemical yield of the labeling of a DMSA reagent from another manufacturer was evaluated. The reagent has different composition of the IPEN DMSA-TEC product and the labeling activity limit is much lower (Table 4).

Tabela 4: IPEN DMSA-TEC Kit comparative composition and the kit evaluated from another commercial source

KIT COMPOSITION / LABELING PARAMETERS	DMSA-TEC IPEN	DMSA Another Manufacturer
DMSA (mg)	1.0	1.0
$SnCl_2.2H_2O$ (mg)	0.44	0.40
Ascorbic acid (mg)	0.70	-
Inositol (mg)	50	-
Labeling Volume (mL)	2-3	1-3
Maximum labeling activity (MBq)	3700	555
Stability (hours)	4	Not informed

In spite of differences in the formulation, when labeling the DMSA reagent from other manufacturer, at the maximum limit suggested by the manufacturer (555 MBq) and also using high activity (3700 MBq), the radiochemical yield was similar, when compared with those obtained with IPEN DMSA-TEC reagent (Table 5). However, while at IPEN kit the complex I percentage appears not to drop from the labeling activity, but increases with the labeling time, in the case of the other manufacturer's kit, the complex I percentage increases with the labeling activity, but does not increase with the labeling time. Perhaps for this reason, the labeling activity kit of the other manufacturer is limited to 555 MBq. Differences in the kits formulation, basically the ascorbic acid antioxidant presents on IPEN kit formulation may influence the observed differences.

Table 5: Percentage of radiochemical impurities present in the labeling of DMSA kit from the other manufacturer, with low and high activity (555 MBq and 3700 MBq) of sodium pertechnetate solution (^{99m} Tc) immediately after labeling, and 4 hours after the labeling.

Labeling conditions (M)	Radiochemical purity control		
	$\%TcO_2$ (0.9% NaCl/TLC)	**$\%TcO_4^-$ (acetone/W3MM paper)**	**% Complex I (n-propanol:H_2O:AA 4:3:1/TLC)**
M1 (555 MBq /3mL) immediately after labeling	12.73 ± 3.42	1.23 ± 0.42	10.40 ± 2.60
M1de (555 MBq /3mL) After 4 hours of labeling	9.18 ± 1.89	0.91 ± 0.16	10.77 ± 1.31
M2 (3700 MBq /3mL) immediately after labeling	11.30 ± 5.91	0.90 ± 0.17	15.41 ± 0.74
M2 (3700 MBq /3mL) After 4 hours of labeling	13.66 ± 1.37	1.45 ± 0.07	12.55 ± 1.25

Legend - AA: acetic acid; TLC: thin-layer chromatography; W3MM: Whatman 3MM chromatographic paper.

The ^{99m}Tc-DMSA preparations obtained with the DMSA-TEC IPEN reagent and the reagent from another manufacturer were evaluated in animal biodistribution studies for both the low activity and high labeling activities.

Table 6: Biological distribution comparison in *Wistar* rats of DMSA produced at IPEN and another producer labeled with high and low activity of sodium pertechnetate (99m Tc) solution.

ORGAN (USP CRITERIA)	% IA/organ for ^{99m}Tc-DMSA from IPEN		% IA/organ for ^{99m}Tc-DMSA from another producer	
	Low activity 185 MBq/3mL	High activity 3700 MBq/3mL	Low activity 185 MBq/3mL	High activity 3700 MBq/3mL
Kidneys (≥ 40 % IA)	55.10 ± 1.36	44.87 ± 1.62	43.15 ± 1.62	41.25 ± 0.61
Liver (no criterion)	2.82 ± 0.53	3.86 ± 0.31	6.58 ± 0.49	6.01 ± 0.81
Spleen (no criterion)	0.17 ± 0.10	0.15 ± 0.02	0.40 ± 0.04	0.28 ± 0.05
Carcass (no criterion)	22.79 ± 4.51	27.29 ± 4.80	33.78 ± 1.01	31.68 ± 1.61
Kidneys/(Liver+Spleen)(≥ 6)	18.84 ± 3.58	11.22 ± 0.51	6.19 ± 0.20	6.64 ± 0.90

Legend – % IA: percentage of the injected activity.

Although the radiochemical purity results of the DMSA reagent produced at IPEN and from another manufacturer are similar, biodistribution sudy in rats demonstrated that in IPEN product the percentage of the radiopharmaceutical in the liver is lower and, consequently, the ratio kidney/liver and spleen is much higher than the another manufacturer, although the acceptance criteria to the biodistribution study are met for both producers. For both producers, organs uptake values do not appear to vary significantly with respect to labeling activity.

Through the comparison between high and low DMSA IPEN activity, it was observed that in the kidneys the uptake was higher for low activity, in the liver the uptake was higher for high activity and the kidney/(liver + spleen) ratio was higher for low activity.

In the low activity comparison between DMSA IPEN and DMSA from another manufacturer showed greater uptake in the kidneys to DMSA IPEN, liver and spleen uptake was higher for the another manufacturer, the ratio kidney/(liver + spleen) was higher for DMSA IPEN and the uptake in the carcass was greater for the other manufacturer.

With the high activity comparison between DMSA IPEN and another manufacturer, it was observed that in the kidneys the uptake was greater for DMSA IPEN, in the liver and spleen the uptake was higher for another manufacturer and the ratio kidney/(liver + spleen) was higher for DMSA IPEN.

To complement the biodistribution studies, scintigraphic images were obtained in rats administered with ^{99m}Tc-DMSA, from DMSA-TEC IPEN (Figure 7). Sequential images show radiopharmaceuticals acumulation in the renal tissue and in later times accumulation in the bladder is already observed. In one of the animals, there is a slight uptake in the liver, which decreases as a function of time.

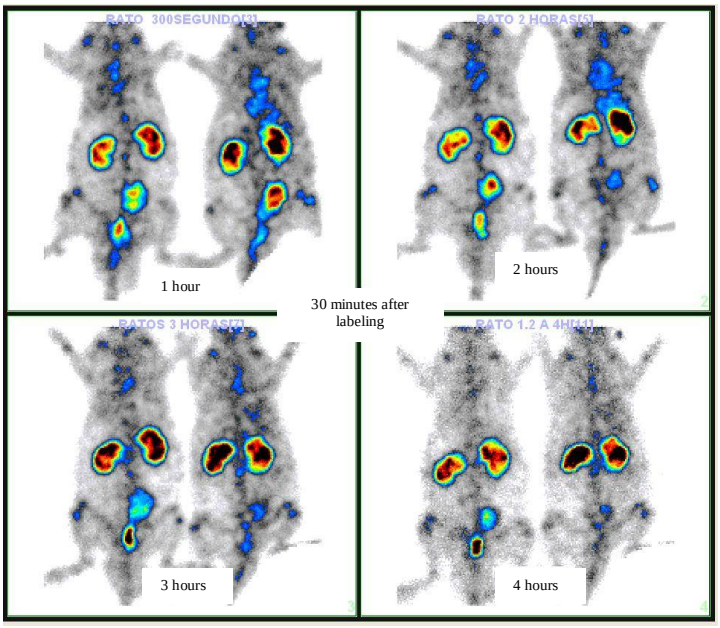

Figure 7: Gamma-scintigraphy images (Nuclide TH22, Mediso) of ^{99m}Tc-DMSA biodistribution in 2 *Wistar* rats, administered immediately after labeling DMSA-TEC IPEN. The images were acquired 1, 2, 3 and 4 hours after administration of the radiopharmaceutical with the animal in ventral decubitus position.

The same biodistribution pattern was observed in the scintigraphy image obtained with DMSA reagent from another manufacturer (Figure 8).

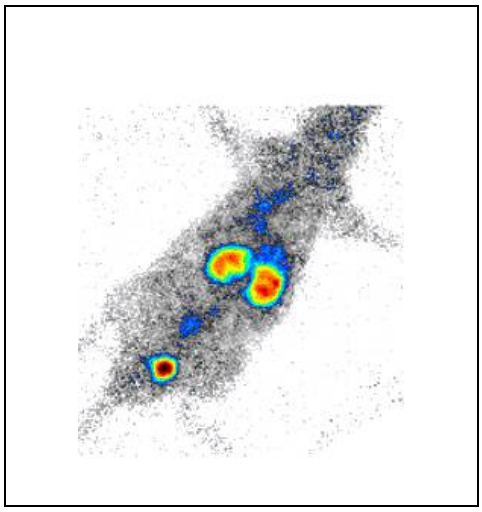

Figure 8: Biodistribution gamma-scintigraphy imaging (Nuclide TH22, Mediso) of ^{99m}Tc-DMSA in 1 *Wistar* rat, administered immediately after ^{99m}Tc-DMSA labeling, obtained with reagent from another manufacturer. The image was acquired 1 hour after the radiopharmaceutical administration with the animal in a ventral decubitus position.

3. CONCLUSION

This study presented important results about the influence of labeling conditions on the formation of different DMSA complexed species with technetium-99m. Using an alternative chromatographic system to that normally employed in routine quality control for determination of TcO_4^- and TcO_2, it was possible to separate different complexes formed during the labeling. Further studies should be carried out in order to investigate in a larger number of animals the influence of the labeling conditions of the complexed species on the biodistribution pattern of ^{99m}Tc-DMSA.

ACKNOWLEDGMENTS

The authors are grateful for the funding provided by IPEN-CNEN / SP for this study and CNPQ (PCI grant).

REFERENCES

1. S.Roman, J. Svetlík, I. Benkovsky, "DMSA and its complexes with radioisotopes: review", *J.Radioanal. Nucl. Chem*., vol. **293**, pp. 545-554, 2012.
2. "Succimer for Merck Index Online", <4https://www.rsc.org/Merck-Index/monograph/m10265/succimer?q=unauthorized> (2017)
3. J. L. MORETTI, J. R. RAPIN, J. C. SACCAVIN, LAGERON, A. PONCIN, M. L. A. BARDY, "2,3-Dimercaptosuccinic acid chelates: structure and pharmacokinetic studies"**,Int.** *J. Nucl. Med. Biol***.**, **vol. 11**, n. 314, pp. 270-274, 1984.
4. P. J. BLOWER, J. SINGH, S. E. M. CLARKE, "The chemical identity of pentavalent technetium-99m-dimercaptosuccinic acid", *J. NucI.Med***.**, **vol. 32**, pp. 845-849, 1991.
5. R. Stanik, I. Benkovsky, "99mTc-DMSA complex preparation: the effect of pH and amount of reducing agent", *Acta Facult. Pharm. Univ. comenianae Tomus*, **vol. 5,** pp. 90-95, 2010.
6. H. M. A. Al-azzawi, S. M. D. Al-Nuzal, J. M. Bandi, "Comparative biological parameters for the evaluation of common renal pharmaceutical preparations: 99mTc-DTPA, 99mTc-GH, and 99mTc-DMSA", *J. Radioanal. Nucl. Chem*., **vol. 307**, pp. 123-130, 2016.
7. Ikeda, O. Inoue, K. Kurata, "Preparation of various Tc-99m dimercaptosuccinate complexes and their evaluation as radiotracers", *J. Nucl. Med*.,**vol. 18**, pp. 1222-9,1997.
8. TECHNETIUM Tc 99m SUCCIMER INJECTION, *The United States Pharmacopeia*, 40th Ed
9. M. Z. Sadiq, M. B. Imran, S. J. Khurshid, "Radiobiokinetic Evaluation of Domestic Cold Kits", *J. Radioanal. Nucl. Chem*., **vol. 199 (2)**, pp. 79-88, 1995.
10. P. Garnuszek, D. Pawlak, M. Maurin, D. Jankovic, U. Karczmarczyk, R. Mikołajczak, "Comparison of chromatographic methods for quality control of DMSA complexes with 99mTc and 188Re at (III) and (V) oxidation states", *Nucl. Med. Rev*.,**vol. 15 (2)**, pp. 95-100, 2012.

11. Taylor,. Optimal handling of dimercaptosuccinic acid for quantitative renal scanning, *J. Nucl. Med*., **vol. 21**, pp. 1190-1193, 1980.
12. C. D. Russell, P. G. Bischoff, K. L. Rowell, "Quality control pf 99mTc-DTPA for measurement of glomerular filtration", *J Nucl. Med.*, **vol. 14,** pp. 722, 1983.
13. U. Jáuregui, A. Blanco, D. Hernández, Z. Rodríguez, "Actualidad y retos de la química de los complejos 99mTc, 186/188Re-DMSA", *Alasb. J.*, 25 de agosto de 2016.