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ABSTRACT

The radiopharmaceuticals labelled with Tc-99m are routinely used in Nuclear Medicine Laboratories. A large number of these employ tin(II) reagentes to reduce Tc (pertechnetate-VII) to a lower valence state thereby making it more able to complex forming reactions. The miniaturized chromatography system of Tc-99m labelled compounds using Whatman 3MM (8 x 1cm) or (6 x 1 cm) as a support and 30% NaCl: 0,9% ' NaCl: 85% MeOH and acetone as a solvent permits to assay the radiochemical purity in a few minutes after preparation. In addition this method introduced in routine work not only determines Tc - 99m (pertechnetate) but also determines reduced Tc - 99m unbound to the radiopharmaceuticals (hydrolized reduced Tc-99m). The lyophilized kits for labelling with Tc-99m produzed at IPEN-CNEN/SP are: MDP,DTPA, HSA, GHA, HIDA, Pyro, MAA, MIAA, Sulfur Colloid, Dextran-500, Sn.Cit. and Phytate. Radiochemical quality control of these kits were performed at the first day of preparation and during 12 months for determining' their validity for use. All preparation showed high yield of labelling (95-99%) during this period of time.

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*Paper presented at "5th Congress of the World Federation of Nuclear Medicine and Biology" - August 26-31, 1990 - Montreal, Canada.

CONTROLE DE QUALIDADE RADIOQUÍMICO DOS CONJUNTOS DE REATIVOS MARCADOS COM ^{99m}Tc PRODUZIDOS NO IPEN-CNEN/SP. *

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RESUMO

Os radiofármacos marcados com ^{99m}Tc são rotineiramente usados nos laboratórios de Medicina Nuclear. A maioria destes produtos utilizam Sn^{++} para reduzir o Tc(VII) a um estado de valência menor, facilitando a formação de complexos na reação. O sistema de cromatografia miniaturizada, realizado nos compostos marcados com ^{99m}Tc, usando Whatman 3MM (8x1 cm) ou (6x1 cm) como suporte, e NaCl 0,9% NaCl 30% metanol 85% e acetona como solventes, permite avaliar a pureza radioquímica em poucos minutos após a sua preparação. Este método adaptado nos trabalhos de controle rotineiro determina além do TcO_4^- (pertecnetato) o ^{99m}Tc hidrolizado reduzido. Os conjuntos de reativos liofilizados para marcar com ^{99m}Tc produzidos no IPEN-CNEN/SP, são: MDP, DTPA, HSA, GHA, HIDA, Pyro, MAA, MIAA, S, Dextran-500, Cit. Sn e Fit. Os ensaios de controle radioquímico foram realizados no primeiro dia após a sua preparação e durante 12 meses para determinar a validade de seu uso. Todas as preparações mostraram um alto rendimento (95-99%) de marcação durante esse período de tempo.

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*Trabalho apresentado no 5th Congress of the World Federation os Nuclear Medicine and Biology", de 26 a 31 de agosto de 1990, em Montreal-Canada.

INTRODUCTICN

In the last few years attention was drawn to the need for good routine quality control systems for radiopharmaceuticals particularly those which are prepared "in situ". The rapid growth of the number of Tc-99m - labelled compounds in Nuclear Medicine requires analytical techniques that can accurately identify various components in these products.

Radiochemical purity, defined as a proportion of the total activity present in the stated or desired chemical form in the final product, is an important concept to remember. The nature and potential sources of radiochemical impurities in Tc-99m radiopharmaceuticals must be understood if chromatographic techniques are to be designed and used for their determination.

Method employed for determining the radiochemical purity of labelled compounds are based upon a number of different physical chemical procedures such us: paper chromatography using various developed systems: thin layer chromatography with varying stationary and mobile phase (Billinghurst. 1973):[1] adsorption chromatography (Richards [5] et al 1974): GCS (gel column scanning) method (Persson[4] and Darte. 1974) and eletrophoreses and centrifugation (Patterson[3] and Darte. 1976), each of the above mentioned procedures has its advantages and disvantages, their common drawback being a relativity long time determination.

Recently a miniaturized chromatography procedure has been described for specific Tc-99m - radiopharmaceuticals[2-6]. The procedures are becoming very quick and also relatively easy to perform, reproducible and inexpensive.

Labelled compounds with Tc-99m prepared from non radioactive kits and TcO_4^- generally depend on the efficiency of a reducing agent in the kit to reduce Tc(VII) to Tc(IV) or Tc(V). The reduced form of Tc-99m is labelled to a chelating agent or to other organ specific chemical. When the reduction is incomplete TcO_4^- (VII) may be a radiochemical impurity, unreduced TcO_4^- will not tag. The most common reducing agent is stannous ion.

Three specific forms of radiochemical impurities in stannous reduced Tc-99m radiopharmaceuticals are as follows: TcO_4^- : free Tc-99m that has not been reduced by Sn(II) and/or is present because of reoxidation of reduced technetium, R-Tc: hydrolized reduced technetium includes the insoluble Tc-99m-Tin-colloid and Tc-99m dioxide, Bound-Tc: the desired radiochemical species.

This paper describes the miniaturized chromatography systems used in routine work by Quality Control Division in all lyophilized kits produced at IPEN-CNEN/SP.

MATERIAL AND METHOD

Kits for labelling with Tc-99m are prepared from a solution of the compound to be labelled and an acidic solution of the stannous compound in the appropriate proportions. The pH value of the solution is ajusted to 5-7, and aliquots are dispensed into the vials under nitrogen atmosphere. After lyophilization the vials are capped.

The lyophilized kits prepared by the Radiopharmacy Division at IPEN-CNEN/SP are as follows:

DTPA - Diethylenetriaminepentacetic acid
GHA - Glucoheptonate
DMSA - 2.3 dimercaptosuccinic acid
HSA - Human serum albumin
MAA - Macroagregated albumin
MIAA - Human albumin microagregated
MDP - Methylene diphosphonate
Pyro - Pyrophosphate
SnC - Stannous colloid
Cit Sn - Stannous citrate
Fit - Phytate
Disida - 2.6 diisopropylacetanilideiminodiacetic acid
Dextran - Dextran 500

Technetium-99m is obtained by elution of Mo-99 - Tc-99m generator (IPEN-TEC, from IPEN-CNEN/SP) with saline.

Radiochemical quality control of these kits were performed at the first day of preparation and during 12 months for determining their validity for use and the stability during 6 hours after labelling.

Each vial was labelled with 1 - 5 ml of Tc-99m (3.7 Bq) and the miniaturized chromatography systems used for this determination were the ascending type, in paper Whatman 3MM (8 x1 cm) and (6 x 1 cm) developing in appropriate solvents in glass vials (Fig. 1).

After the solvents move to the top of the strips they are cut in the cut line and the radioactivity of each sections is measured in appropriate gama counter (ANSER-ABBOT). The percentage of TcO_4^- and R-Tc are calculated (Table I-II).

TABLE I

DETERMINATION FREE PERTECHNETATE AND HYDROLIZED REDUCED Tc-99m (R-Tc) IN Tc-99m LABELLED DTPA, GHA, MDP, PYRO, SnC AND DI - SIDA.

1. Place approximately 0.5 mL of acetone (A_2) or 85% MeOH (C_1) into one 10 mL glass vial and 0.5 mL of 0.9 NaCl (A_1) or 30% NaCl (C_2) into an identical another vial.

2. Spot radiopharmaceutical on botton pencil line of Whatman 3 MM paper chromatography strip A (6 x 1cm) or C (8 x 1 cm). (Fig. 1)

3. Develop the strip in vial containing:
   acetone and 0.9% NaCl (DTPA, MDP, PyRO, CitSn and GHA).
   85% MeOH and 30% NaCl (DISIDA).

4. Cut strip at pencil line into sections 1, 2, 3 and 4 (see Fig. 1 A or C).

5. Count all sections for activity (per unit time) using a gamma counter and substract background.

6. $$\%\ \text{Free-Tc}_{(a)} = \left\{\frac{\text{counts section 4}}{\text{counts section 4 + 3}}\right\} \times 100$$

   $$\%\ \text{R-Tc}_{(b)} = \left\{\frac{\text{counts section 1}}{\text{counts section 1 + 2}}\right\} \times 100$$

   Bound-Tc = 100 - (a + b)

TABLE II

DETERMINATION OF FREE PERTECHNETATE IN Tc-99m LABELLED MAA, MIAA, PHYTATE, SULFUR COLLOID, STANNOUS COLLOID, DEXTRAN AND DMSA. (Fig. 1-3)

1. Place approximately 0,5 mL of acetone or 85% methanol into a 10 mL glass vial.

2. Spot radiopharmaceutical on bottom pencil line of Whatman 3 MM cromatography paper strip (8 x 1 cm).

3. Immediately place strip in vial containing:
   acetone (DMSA, Sulfur colloid, Dextran, Stannous Citrate) and 85% methanol (MAA, MIAA, PHYTATE).
   and develop until solvent front migrates to top pencil line.

4. Cut strip at central pencil line producing 1 and 2 (Fig 1-B).

5. Count each section for activity (per unit time) using a gamma counter, and substract background.

6. $$\% \text{ Free-Tc} = \left\{ \frac{\text{counts section } 2}{\text{counts section } 1 + 2} \right\} \times 100$$

   % Bound-Tc = 100-A

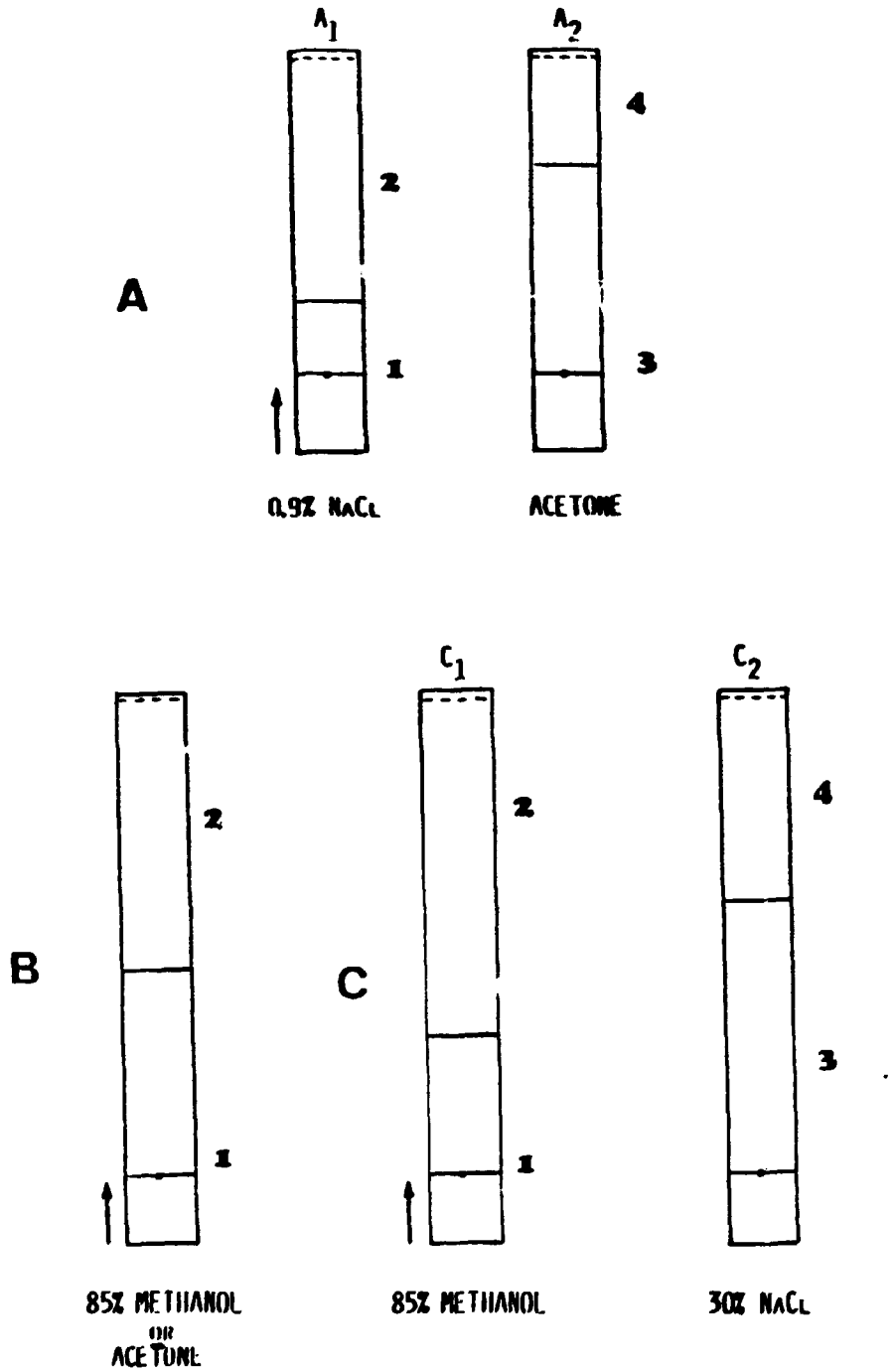

FIGURE 1 - CHROMATOGRAPHY STRIP USED FOR RADIOCHEMICAL QUALITY CONTROL OF Tc-99m LABELLED KITS; **A** - WHATMAN 3MM CHROMATOGRAPHY PAPER (6x1cm); **B** - WHATMAN 3MM CHROMATOGRAPHY PAPER (8x1cm); **C** - WHATMAN 3MM CHROMATOGRAPHY PAPER (8x1cm).

RESULTS AND CONCLUSION

All preparations showed high yield labelling (95-99%) during 12 months after preparation and during 6 hours after labelling with Tc-99m. (Table III, IV, V, VI).

The Rf values of the compounds studied in the various solvents and chromatographic media are presented in Figure 2. (A, B, C).

The miniaturized chromatography procedure is extremely fast, inexpensive, easy to be performed and can be used for practically all Tc-99m radiopharmaceuticals produced at IPEN/SP. Labelling or tagging efficiences can usually be determined within 3 - 25 minutes after spotting. The method is suitable for determinating the free pertechnetate content in strong and weak chelate compounds and the hydrolyzed form of reduced Tc that is present most frequently as radiochemical impurities.

It is important to study the radiochemical purity of a radiopharmaceutical product, in order to assure its use in the specified chemical form. Radiochemical impurities modify useful information in Nuclear Medicine Studies.

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TABLE III

Radiochemical purity of GHA labelled with Tc-99m during a period of time: Month after its preparation and minute after labelling.

Time (Month) / Time (min.)	1	2	4	6	8	10	12
15	98.3*	96.9	97.7	95.9	95.8	95.7	94.8
	1.4	2.8	1.5	2.9	2.7	2.9	3.7
	0.3	0.3	0.8	1.2	1.5	1.4	1.5
30	97.5	96.7	96.5	95.4	95.6	95.6	94.9
	1.8	2.9	2.7	2.7	2.5	3.1	3.8
	0.7	0.4	0.8	1.9	1.9	1.9	1.3
60	97.4	96.5	96.3	95.7	95.5	95.7	94.6
	2.5	2.8	2.9	3.1	2.7	2.5	3.7
	0.1	0.7	0.8	1.2	1.8	1.8	1.7
120	97.6	96.6	97.7	95.4	95.6	95.5	94.5
	1.8	2.7	1.8	2.7	2.8	3.1	3.7
	0.6	0.7	0.5	1.9	1.6	1.4	1.8
180	97.5	96.3	97.2	95.3	95.4	95.2	93.9
	1.6	2.8	1.7	3.7	2.9	3.9	4.2
	0.9	1.9	1.1	1.0	1.7	0.9	1.9
240	97.7	96.4	96.4	95.4	95.3	95.1	94.3
	1.6	2.8	2.7	3.1	3.2	3.7	3.8
	0.7	0.8	0.9	1.5	1.5	1.2	1.9
300	97.6	96.2	96.3	95.2	95.4	95.2	95.3
	1.7	2.9	2.8	3.7	3.9	3.8	2.9
	0.7	0.9	0.9	1.1	0.7	1.0	1.8
360	97.2	96.3	96.2	95.4	94.9	95.2	95.1
	1.7	2.8	3.1	2.7	3.2	2.9	2.7
	0.9	0.9	0.7	1.9	1.9	1.9	2.2

* GHA - ^{99m}Tc (%)
R - Tc (%)
TcO_4^- (%)

TABLE IV

Radiochemical purity of methylene diphosphonate (MDP) labelled with Tc-99m, during a period of time: Month after its preparation and minute after labelling (3.7 Bq)

Time (Month) / Time (min.)	1	2	4	6	8	10	12
15	99.26*	99.96	98.86	99.88	99.71	98.74	98.38
38	99.68	99.94	98.69	99.36	99.88	98.76	98.57
68	99.47	99.72	98.59	99.88	98.63	98.74	98.19
128	99.57	99.13	98.57	99.16	98.33	98.14	97.53
188	99.18	99.88	98.38	98.42	98.76	98.28	97.38
248	99.63	99.43	98.39	99.88	99.86	98.48	97.48
300	99.52	99.33	98.78	99.28	99.98	98.22	97.43
368	98.78	99.12	98.75	98.83	98.58	98.14	97.83

* MDP - ^{99m}Tc (%)

n = 3

TABLE V

Radiochemical purity of phytate (FIT) labelled with: Tc-99m, during a period time: Month after its preparation and minute after labelling.

Time (min.) \ Time (Month)	1	2	4	6	8	10	12
15	99.88*	99.96	99.73	99.96	99.83	99.94	99.98
	0.12	0.04	0.27	0.04	0.17	0.06	0.02
30	99.77	99.94	99.95	99.97	99.87	99.98	99.99
	0.23	0.06	0.05	0.03	0.13	0.02	0.01
60	99.89	99.96	99.85	99.98	99.94	99.99	99.99
	0.11	0.04	0.15	0.12	0.06	0.01	0.01
120	99.84	99.96	99.48	99.99	99.89	99.58	99.43
	0.16	0.04	0.52	0.01	0.11	0.42	0.57
180	99.84	99.94	99.70	99.98	99.97	99.79	99.84
	0.16	0.06	0.30	0.02	0.03	0.21	0.16
240	99.91	99.70	99.95	99.93	99.83	99.80	99.73
	0.09	0.38	0.05	0.17	0.17	0.20	0.27
300	99.89	99.87	99.10	99.98	99.99	99.88	99.90
	0.11	0.13	0.90	0.02	0.01	0.12	0.10
360	99.87	99.12	99.27	99.79	99.79	99.69	99.79
	0.13	0.88	0.73	0.21	0.21	0.31	0.21

*Fit-^{99m}Tc (%)
$^{99m}TcO_4^-$ (%)

(n = 3)

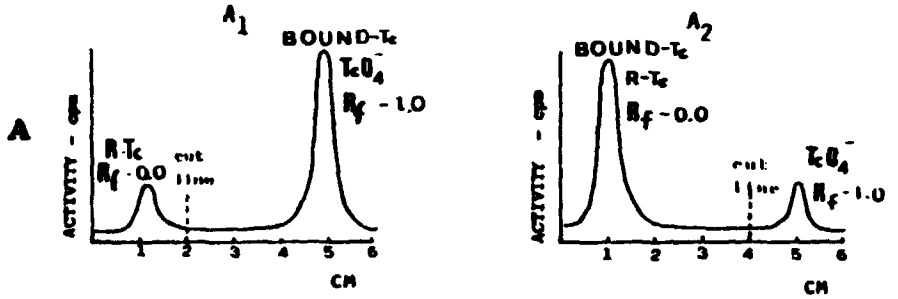
TABLE VI

Radiochemical purity of DMSA labelled with Tc-99m during a period of time: Month after its preparation and minute after labelling.

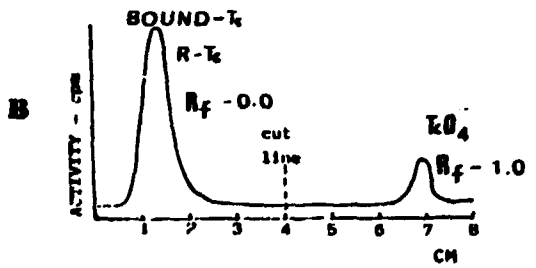
Time (Month) / Time (min.)	1	2	4	6	8	10	12
15	99.9*	99.7	99.7	99.6	99.8	99.9	99.8
	0.1	0.3	0.3	0.4	0.2	0.1	0.2
30	99.9	99.9	99.7	99.8	99.1	99.8	99.8
	0.1	0.1	0.3	0.2	0.9	0.2	0.2
60	99.9	99.8	99.8	99.1	99.8	99.8	99.1
	0.1	0.2	0.2	0.9	0.2	0.2	0.9
120	99.9	99.6	99.7	99.2	99.4	99.7	99.7
	0.1	0.4	0.3	0.8	0.6	0.3	0.3
180	99.9	99.5	99.4	99.4	99.2	99.3	99.5
	0.1	0.5	0.6	0.6	0.8	0.7	0.5
240	99.9	99.7	99.7	99.3	99.1	99.7	99.6
	0.1	0.3	0.3	0.7	0.9	0.3	0.4
300	99.9	99.7	99.5	99.3	99.4	99.4	99.5
	0.1	0.3	0.5	0.7	0.6	0.6	0.5
360	99.9	99.8	99.6	99.5	99.3	99.4	99.4
	0.1	0.2	0.4	0.5	0.3	0.6	0.1

* DMSA - ^{99m}Tc (%)
$^{99m}TcO_4^-$ (%)

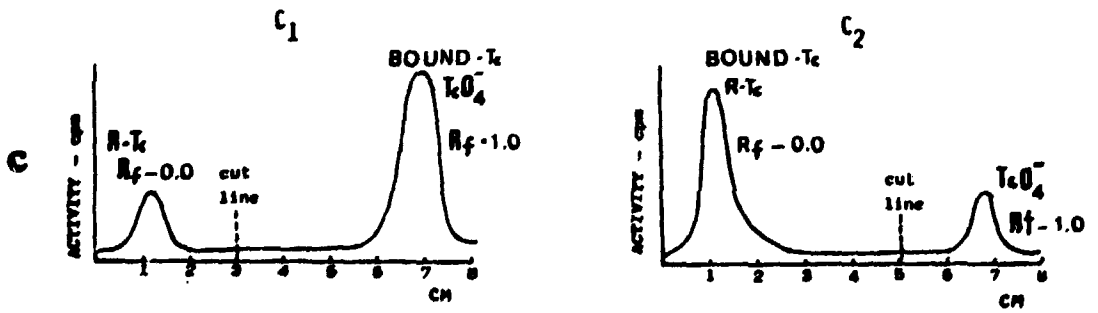
(n=3)



SCAN OF IMPURE DTPA, MDP, PYRO, GHA AND CIT.Sn LABELLED WITH Tc-99m.



SCAN OF IMPURE MIAA, MAA.SnC.DMSA AND HSA LABELLED WITH Tc-99m.



SCAN OF IMPURE Tc-99m-DISIDA

FIGURE 2 – R_F VALUES OF THE IMPURITIES IN KITS LABELLED WITH Tc-99m IN DIFFERENT CHROMATOGRAPHIC SYSTEMS.

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