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Determination of free ^{99m}Tc in Labeled Compounds by means of Thin Layer Chromatography*

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Introduction

The widespread use of radioisotope generators is associated with an urgent need for fast, simple and reliable methods for determining the radiochemical purity of the compounds labeled with short-lived radionuclides. Chromatography is one of the best and most widely used methods for this purpose. Several papers regarding ^{99m}Tc-labeled compounds have been published[2–5] but the procedures are rather time consuming and it is not so easy to complete the control before administering the dose to the patient.

In a previous paper,[6] we described thin layer chromatography as a simple and rapid method for the determination of inorganic iodide in radiodinated compounds. In this communication we present our results with thin layer chromatography for the control of the most common ^{99m}Tc-labeled radiopharmaceuticals.

Material and Methods

The following compounds, prepared according to the published methods, were assayed by thin layer chromatography: ^{99m}Tc-sulphur colloid[7] antimony sulphide colloid,[8] stannous hidroxide colloid,[4] ^{99m}Tc-albumin,[5] ^{99m}Tc–Fe complex,[3] and ^{99m}Tc-DTPA.[5] The purity of all samples was checked by either or both, paper chromatography and paper electrophoresis.

Each sample was chromatographed on silica gel *G* (Merck) plates of 250 μ thickness, prepared in the usual manner[9] and on three types of commercially available plates:

K—100 μ silica gel with polyvinyl alcohol as a binder (Eastman Chromagram Sheet Type K 301R2 or 6061).
S—Glass microfiber (10) with potassium silicate (Gelman I.T.L.C. Type S).
SG—Glass microfiber with silica gel (Gelman I.T.L.C. Type SG).

Nondiluted samples (5–20 μl) were applied with micropipettes and dried with cool air. Sandwich-type chambers (Eastman Chromagram Developing Apparatus) were used for developing plates *K*, *S* and *SG* and an ordinary chromatography jar for plates *G*. A distance of 7 cm from the origin to the front was enough for a good separation.

Radiometric evaluation of chromatograms was done by each or both of the following techniques:

(a) By means of a radiochromatography scanner (Actigraph III Nuclear Chicago or Packard 7200) and plannimetry.

(b) By means of a scintillation camera (Pho/Gamma III Nuclear Chicago) provided with a 1000 hole collimator and with the controls set for divided field operation. The chromatograms were placed across the dividing line, so origin and front fell in different sections, providing readings corresponding to the radioactivity of each part of the plate. At the same time, it was possible to obtain one "instant autoradiogram" on Polaroid film, using the oscilloscope display of the camera.

In all cases of doubtful results, the labeled compounds were chromatographed again with the addition of a known amount of $^{99m}TcO_4^-$, followed by a quantitative evaluation to confirm the complete separation of the components of the mixture.

Results

The results concerning developing time and R_f values for different compounds, plates and solvents, are given in Table 1. A good correlation between scanning and evaluation with the scintillation camera was always obtained for the same plate.

* The preparation of the technetium labeled compounds and the studies with the scintillation camera were done at the Hospital "20 de Noviembre" del I.S.S.S.T.E., Mexico City.

TABLE 1

Compound	Plate	Solvent	Time	R_f Compound	R_f Free ^{99m}Tc
^{99m}Tc–S colloid	S or SG	MEK	3	0·0	1·0
	K		8		
	G	NaCl 0·9%	15	0·0	0·85
	SG		3		
	K		25		
^{99m}Tc–Sb_2S_3 colloid	SG	MEK	3	0·0	1·0
	G	NaCl 0·9%	15		0·85
	K		25		
^{99m}Tc–$Sn(OH)_2$ colloid	G	NaCl 0·9%	15	0·0	1·0
	K		25		0·85
^{99m}Tc–Albumin	S or SG	MEK	3	0·0	1·0
	G or K		8		
	G	NaCl 0·9%	15	0·0	1·0
	K		25		0·85
^{99m}Tc–DTPA	SG	MEK	3	0·0	1·0
	G or K		8		
Tc–Fe complex	SG	MEK	3	0·0	1·0
	G or K		8		

Discussion

Developing time in either thin layer or paper chromatography depends strongly upon the solvent in use. However, shorter times for longer distances are always achieved with the same solvent and thin layer chromatography. Furthermore, with the use of glass microfiber plates ("instant thin layer chromatography plates"), the time is even more considerably reduced. The separation produced by thin layer chromatography is always sharper at a shorter distance.

Out of the four kinds of plates we tried, the *S* and *SG* had the distinct advantage of a considerably faster separation, but in the case of Tc–Sn $(OH)_2$ colloid, a higher amount of free technetium was found when compared with the *G* and *K* types.

Mixtures of organic solvents with water (e.g. 85 per cent methanol or butanol–ethanol–water, 2:2:1), often used for chromatography of Tc-labeled compounds, provide good separations in general, but have the disadvantage of a rather slow flow-rate. It was found that 0·9 per cent NaCl, which is a very mild agent, can be used for almost all the compounds assayed. With the same solvent and using *G*, *K* of *SG* plates, it was also possible to separate ionic indium (R_f 0·0) from ^{113m}In-EDTA or ^{113}In-DTPA (R_f 0·8); 5 per cent glucose or 0·1*N* NH_4OH were also found to be good solvents for this purpose.

Since Tc-DTPA and TcO_4^- have about the same R_f in 0·9 per cent NaCl, we recommend the use of methyl–ethyl–ketone (MEK) for their separation.

Tc–Fe complex behaves like a single compound in two of the tested systems (MEK and BuOH–EtOH–H_2O), but it can be resolved into two components (R_f 0·0 and R_f 0·8) when chromatographed in 0·9 per cent NaCl. This unexpected finding strongly suggests that Tc–Fe complex is actually a mixture of two compounds, since no decomposition is thought to be produced in this solvent.

Summary

A simple and rapid method, using thin layer chromatography for determining the radiochemical purity of the most widely used ^{99m}Tc-labeled radiopharmaceuticals is described. The use of a scintillation camera is proposed for a faster radiometric evaluation of chromatograms. Through these procedures, radiochemical purity can be checked within 5–30 min of the preparation of the labeled compound.

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