A Better Method of Quality Control for Technetium-99m Sestamibi

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The accepted method of quality control for technetium-99m (^{99m}Tc) sestamibi as published by the manufacturer requires the use of an aluminum oxide-coated plastic thin layer chromatography plate developed with ethanol. This procedure requires meticulous attention to chromatographic technique and requires at least 30 min to complete. We propose a separation of impurities via liquid column chromatography which uses a Sep-Pak alumina N cartridge to provide a faster and simpler method for analysis of the radiochemical purity of ^{99m}Tc sestamibi.

The package insert accompanying the recently approved kit for the production of technetium-99m (^{99m}Tc) sestamibi (Cardiolite) (Du Pont Pharma, N. Billerica, MA) contains a stepwise quality control (QC) procedure using thin layer chromatography (TLC) with an aluminum oxide plate to determine the radiochemical purity (1). The aluminum oxide plates are prone to crumbling when cut, and they must be heat activated and stored in a desiccator. In addition, the spots must be dried for 15 min, the solvent must be pure, (>95% ethanol), and the chamber must be equilibrated with solvent vapor in order to obtain accurate reproducible results. Actual development of the chromatogram requires 10–15 min. The entire process requires 30 min or more. In a busy department, where timely injection of patients is critical, this time commitment can result in delays and increased costs by decreasing the useful shelf-life (6 hr expiration) of this expensive (~$300/vial) radiopharmaceutical, while increasing demands on personnel.

A new QC method which uses a Waters Sep-Pak alumina N cartridge (Millipore Corporation, Milford, MA) eluted with 100% ethanol provides reliable results quickly and easily. In addition, trials were performed with denatured alcohol (95% ethanol, 5% isopropanol) as it is easier to obtain. This paper describes the validation of this method and its possible application.

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MATERIALS AND METHODS

Experiment 1. We determined sodium pertechnetate ($^{99m}TcO_4$) (molybdenum [Mo]/^{99m}Tc generator, Du Pont Pharma, N. Billerica, MA) behavior on Sep-Pak using either normal saline or ethanol as an eluent. The experiment can be replicated by following the protocol below. For NaCl: Equilibrate Sep-Pak by slowly flushing with 5 ml sodium chloride 0.9% solution. Load with 0.1 ml $^{99m}TcO_4$ solution using a 1-cc syringe and a small needle. Load directly onto column through long-neck end of Sep-Pak. Assay loaded Sep-Pak in a dose calibrator. Elute dropwise with 10 ml of 0.9% NaCl solution, and collect 0.5-ml aliquots of the eluate in sequentially numbered test tubes. Push approximately 2 ml of air through the column following elution to ensure removal of all eluate. Assay Sep-Pak and eluate tubes for ^{99m}Tc activity using a dose calibrator. For EtOH: Obtain a fresh Sep-Pak and equilibrate by slowly flushing with 5 ml EtOH and drain. Load Sep-Pak with 0.1 ml $^{99m}TcO_4$ solution using a 1-cc syringe and needle. Load from long-neck end directly onto column. Assay loaded Sep-Pak in a dose calibrator. Elute column with 10 ml of 100% EtOH and collect 0.5 ml-aliquots dropwise in sequentially numbered test tubes. Push air through column to ensure removal of all eluate. Assay Sep-Pak and eluate tubes for ^{99m}Tc activity using a dose calibrator.

Experiment 2. We determined ^{99m}Tc-MIBI behavior on Sep-Pak using normal saline as an eluent. The experiment can be replicated by following the protocol below. Obtain a fresh Sep-Pak and equilibrate with 5 ml 0.9% NaCl solution and drain. Load with 0.1 ml ^{99m}Tc-MIBI solution using a 1-cc syringe and needle. Load directly onto column through long-neck end of Sep-Pak. Assay activity in a dose calibrator. Elute column with 10 ml normal saline dropwise and collect 0.5 ml-aliquots of eluate in sequentially numbered test tubes. Push air through column to ensure removal of all eluate. Assay Sep-Pak and eluate tubes for ^{99m}Tc activity using a dose calibrator.

Experiment 3. We determined ^{99m}Tc-MIBI behavior on Sep-Pak using EtOH (95% and 100%) as an eluent. The experiment can be replicated by following the protocol below. Obtain a fresh Sep-Pak and equilibrate with 5 ml (95% or 100%) EtOH solution. Load with 0.1 ml of ^{99m}Tc-MIBI

solution using a 1-cc syringe and needle. Load directly onto column through long-neck end of Sep-Pak. Assay activity in a dose calibrator. Elute column with 10 ml of 100% EtOH. Collect 0.25 ml-aliquots of eluate in sequentially numbered test tubes. Assay Sep-Pak and eluate tubes in a dose calibrator. Repeat the procedure using denatured 95% EtOH. Collect 0.5 ml-aliquots of eluate in sequentially numbered test tubes. Assay Sep-Pak and eluate tubes in a dose calibrator.

Experiment 4. We classified impurities and verified separation. To replicate this experiment, use the following protocol. Obtain a fresh Sep-Pak and equilibrate with 5 ml 0.9% NaCl. Load with 0.1 ml of ^{99m}Tc-MIBI solution using a 1-cc syringe and needle. Load directly onto column from long-neck end. Elute column with 15 ml of 0.9% NaCl dropwise and collect 0.5 ml-aliquots of eluate in sequentially numbered and labeled test tubes. Assay Sep-Pak and eluate tubes in a dose calibrator. Continue eluting column with 10 ml of 100% EtOH. Collect 0.25 ml-aliquots of eluate in sequentially numbered and labeled test tubes. Assay Sep-Pak and eluate tubes in a dose calibrator.

Experiment 5. We validated the EtOH separation method. To replicate this experiment, use the following protocol. Obtain a fresh Sep-Pak and equilibrate with 5 ml EtOH. Load with 0.1 ml of ^{99m}Tc-MIBI solution using a 1-cc syringe and needle. Load directly onto column through long neck-end of Sep-Pak. Elute column with 10 ml of 100% EtOH. Push a small volume of air through the Sep-Pak to ensure removal of all eluate. Place Sep-Pak in a test tube and assay activity of eluate and Sep-Pak in a dose calibrator. Repeat this procedure eleven times using various lots of ^{99m}Tc-MIBI. Repeat the procedure for 10 additional trials, this time using 95% EtOH (denatured with 5% isopropanol) as the eluent. For this procedure, we use Mallinckrodt Reagent Alcohol Absolute Product #7019 (Mallinckrodt Medical, St. Louis, MO). Fifteen different lots were used to perform the above experiments.

Control. This is the package insert TLC method. Using the same ^{99m}Tc-MIBI as above (Experiment 5), perform QC (TLC method) as recommended in the package insert. Count strip portions with a sodium iodide detector.

RESULTS

Experiment 1. When $^{99m}TcO_4$ (5.55 mCi) was loaded onto the column and eluted with 10.2 ml 0.9% NaCl, the resultant activity (decay-corrected) on the column was 6.83 μCi and the assayed eluate contained 5,265.8 μCi (98.87%, peak activity after 2 ml eluted) (Fig. 1). When a fresh Sep-Pak was loaded with 6.24 mCi $^{99m}TcO_4$, the activity (decay-corrected) remaining on the column after elution with 10 ml 100% EtOH remained 6.24 mCi, indicating that no $^{99m}TcO_4$ is removed upon elution with 10 ml ethanol.

Experiment 2. A fresh Sep-Pak loaded with 2,420 μCi ^{99m}Tc-MIBI and eluted with 10 ml 0.9% NaCl had an activity of 2,410.85 μCi (99.62%) when assayed. This indicates that ^{99m}Tc-MIBI is not removed upon elution with normal saline. The remaining 9.15 μCi eluted at a retention volume, which concurred with the behavior of the pertechnetate elution.

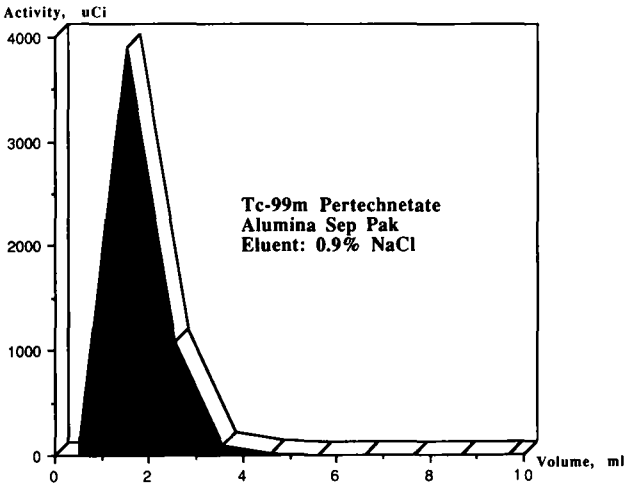

Fig. 1. Elution of alumina Sep-Pak containing ^{99m}Tc pertechnetate with 10 ml of 0.9% saline. Eluted samples were collected as 0.5-ml aliquots in test tubes, and the activity in each tube (vertical axis) is plotted against the accumulated elution volume (horizontal axis). Note that the majority of activity if contained in the first 2 ml of eluate.

Therefore, the activity contained in the eluate can be considered to be pertechnetate, an impurity.

Experiment 3. A second Sep-Pak, when loaded with 3.11 mCi ^{99m}Tc-MIBI and eluted with 10 ml 100% EtOH, yielded a column activity (decay-corrected,) of 61.19 μCi (2.13%) (Fig. 2). The majority of activity was contained in the first ml of eluate. An additional column loaded with 2,896 μCi of ^{99m}Tc-MIBI and eluted with 95% EtOH using the same procedure yielded activity of 2,821 μCi, (97.4%,) in the eluate and 74.8 μCi (2.6%,) on the Sep-Pak (Fig. 3). Again, the majority of activity was assayed in the first ml with some tailing evident.

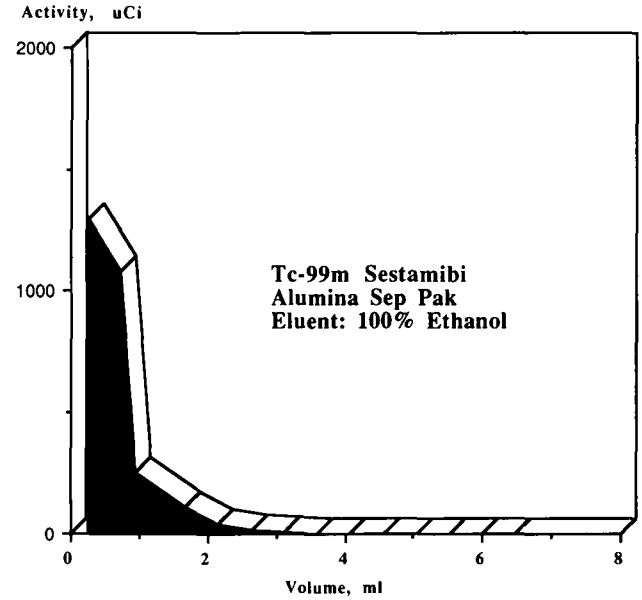

Fig. 2. Elution of alumina Sep-Pak containing ^{99m}Tc sestamibi with 10 ml of 100% ethanol. Eluted samples were collected as 0.25-ml aliquots in test tubes and the activity in each tube (vertical axis) is plotted against the accumulated eluted volume (horizontal axis). Note that the majority of activity is eluted in the first ml.

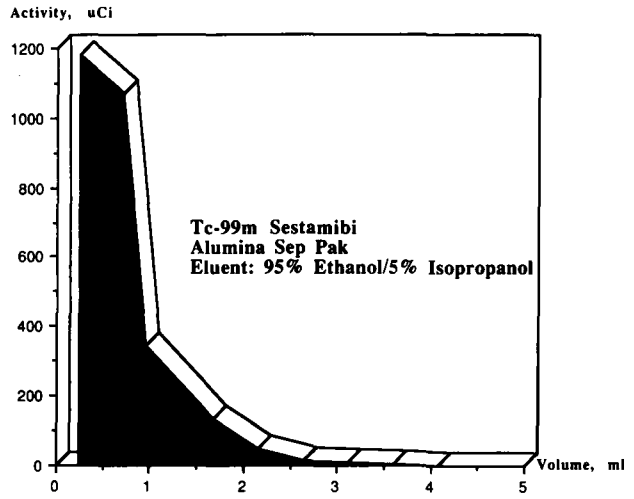

Fig. 3. Elution of alumina Sep-Pak containing ^{99m}Tc sestamibi with 10 ml of 95% ethanol. Eluted samples were collected as 0.5-ml aliquots in test tubes, and the activity of each tube (vertical axis) is plotted against the accumulated eluted volume. Note that tailing occurs due to the nature of the solvent.

Experiment 4. A fresh Sep-Pak loaded with 2,295 μCi ^{99m}Tc-MIBI and eluted with 15 ml 0.9% NaCl yielded 9.153 μCi (0.399%) in the eluate. Further elution of the same column with 100% EtOH yielded 2,249.6 μCi (98.015%) in the eluate and left 36.39 μCi, (1.586%) on the Sep-Pak (Fig. 4).

Experiment 5. The results of 21 elutions using either 95% or 100% EtOH are shown in Table I. Statistical analysis of the results showed a mean of 97.424% tagged material with a s.d. of 0.513.

Control. The results obtained when ^{99m}Tc-MIBI was analyzed with TLC as recommended by the manufacturer (Table I, column 2) showed a mean of 97.8% tagged material with a s.d. of 0.549.

Four of the above elutions were performed on the same lot to affirm the reproducibility of the procedure. Statistical analysis of these four trials showed a mean of 97.425% tagged material with a s.d. of 0.69. In comparison, four trials of TLC analysis on the same lot yielded a mean of 97.9% tagged material with a s.d. of 0.432.

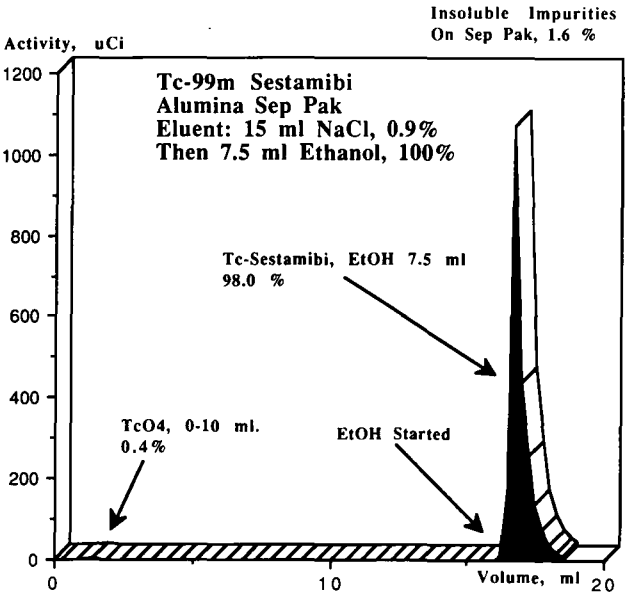

Fig. 4. Elution of alumina Sep-Pak containing ^{99m}Tc sestamibi with 15 ml of 0.9% NaCl followed by 7.5 ml of 100% EtOH. Eluted samples were collected in 0.5-ml aliquots (NaCl) and 0.25-ml aliquots (EtOH) in test tubes, and the activity in each test tube (vertical axis) is plotted against the accumulated eluted volume.

DISCUSSION

The alumina Sep-Pak is essentially the same column as is found in the Mo/Tc generator. It is well established that hydrolyzed-reduced technetium (Tc_{HR}) has a high affinity for alumina and stays bound to the column even with very polar solvents such as 0.9% NaCl (2) and that TcO_4 is readily elutable with 0.9% NaCl. Our results concur. We found a small, unelutable fraction, Tc_{HR} and TcO_4 eluted in the first 1–2 ml with 0.9% NaCl. This is a somewhat smaller retention volume than is seen with generators and is due to the smaller column height of the Sep-Pak.

Our results show that no TcO_4 is eluted with 10 ml or less of ethanol but ^{99m}Tc-MIBI is eluted totally with the same volume. There is a bit of tailing evident with denatured ethanol and hence 100% ethanol is preferable, if available. The denatured solvent is acceptable and is easier to obtain if the facility doesn't have authorization for tax-free ethanol. Our comparison of 21 analyses of 15 lots by both TLC and our method shows remarkably similar results (Table I). The average difference between techniques is smaller than the s.d. of replicate determinations using the same technique. Hence, the Sep-Pak method is shown to be equivalent.

TABLE 1. Percent of Tagged Material Assayed: Sep-Pak Versus TLC Method

Sample No.	% Tagged Sep-Pak	% Tagged TLC	EtOH (Eluent) Purity
1	97.4	98.0	100%
2	98.0	98.0	100%
3	97.9	98.0	100%
4	97.7	97.3	100%
5	97.7	97.9	100%
6	97.4	97.1	95%
7	97.3	98.1	95%
8	97.9	98.4	95%
9	98.1	97.8	95%
10	96.5	97.5	95%
11	96.6	97.5	95%
12	97.2	98.1	95%
13	97.1	97.2	95%
14	96.4	98.1	100%
15	97.9	98.3	100%
16	97.4	96.0	95%
17	98.0	98.0	95%
18	97.8	98.1	100%
19	96.9	98.2	100%
20	97.1	98.2	100%
21	97.6	98.0	100%

Our method is as follows.

1. Slowly push 5 cc of ethanol through a fresh Sep-Pak alumina cartridge.
2. Load 0.05–0.1 ml of ^{99m}Tc-MIBI on the long-neck side of the column, making sure it gets on the column and not in the tube neck.
3. Push 10 cc of ethanol through the column slowly, drop by drop, using a 10-cc syringe and collecting the eluate in a test tube. Follow with a few ml of air to collect all of the ethanol.
4. Place the Sep-Pak in a second test tube.
5. Assay each tube in a dose calibrator.
6. Calculate the % Labeled:

$$\% \text{ Labeled} = \frac{\text{Ethanol Activity}}{\text{Total Activity in Both Tubes}}$$

This method can be completed in less than five min. It gives better counting statistics with the dose calibrator and does not have the stringent requirements of the TLC method. After using it in both a centralized nuclear pharmacy and a large hospital nuclear pharmacy, we find it much more convenient and efficient than the insert method.

In order to quantify both the TcO_4 and the Tc_{HR} separately, first elute the column with 10 cc of 0.9% NaCl and then elute with 10 cc of ethanol. In this case, pre-elute the column with 5 cc of 0.9% NaCl. The TcO_4 is in the 0.9% NaCl eluate, the MIBI is in the ethanol eluate, and the Tc_{HR} is on the column. This would not need to be done routinely but would give useful information when investigating a poor tag or altered biodistribution in a patient.

REFERENCES

1. Package insert for Cardiolite kit for the production of ^{99m}Tc sestamibi. Du Pont Radiopharmaceuticals, N. Billerica, MA, 1991.
2. Boyd RE. Recent developments in generators of ^{99m}Tc. In *Radiopharmaceuticals and labeled compounds*. Vol I. Vienna: IAEA; 1973:3–26.