

Validation of a Column Method for Technetium-99m Exametazime Quality Control

George Pandos, Stan Penglis and Chris Tsopelas

Queen Elizabeth Hospital Nuclear Medicine Department, Woodville South and Royal Adelaide Hospital, Nuclear Medicine Department, RAH Radiopharmacy, Adelaide, South Australia

Objective: The purpose of this study was to investigate if an octadecyl (^{18}C) minicolumn could be applied successfully for quality control of $^{99\text{m}}\text{Tc}$ exametazime (HMPAO).

Methods: The ^{18}C column system using saline eluent was validated against the Whatman 17 method in calculating the percent radiochemical purity of $^{99\text{m}}\text{Tc}$ -HMPAO. The behavior of $^{99\text{m}}\text{Tc}$ -pertechnetate ($^{99\text{m}}\text{TcO}_4^-$) and hydrolyzed-reduced $^{99\text{m}}\text{Tc}$ ($^{99\text{m}}\text{TcO}_2$) on the column was examined, as well as method reproducibility.

Results: The column method is reproducible and yields results that are highly comparable to the Whatman 17 method. Technetium-99m-pertechnetate is associated with the eluent, and some $^{99\text{m}}\text{TcO}_2$ is retained by the column but this level is insignificant to the final patient dose.

Conclusion: The minicolumn system is safe, simple, rapid and reliable for the quality control analysis of routine $^{99\text{m}}\text{Tc}$ -HMPAO preparations.

Key Words: carbon-18 column; technetium-99m-HMPAO; radiopharmaceutical quality control methods; radiochemical purity analysis methods

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The manufacturer's method for assessing the radiochemical purity (RCP) of $^{99\text{m}}\text{Tc}$ -HMPAO requires the use of 3 solvent types on 2 different stationary phases, and is time consuming (15–20 min) (1). Since the useful life of reconstituted $^{99\text{m}}\text{Tc}$ -HMPAO preparations is only 30 min, rapid quality control (QC) procedures are needed to effectively evaluate % RCP before patient injection. A single-strip, miniaturized chromatography system to quantitate the lipophilic component in $^{99\text{m}}\text{Tc}$ -HMPAO was previously validated against the manufacturer's method (2). This still popular chromatography system consists of Whatman 17 paper (W17) strips using ethyl acetate as the developing solvent, and it successfully separates $^{99\text{m}}\text{Tc}$ -HMPAO at the solvent front from hydrolyzed-reduced $^{99\text{m}}\text{Tc}$

($^{99\text{m}}\text{TcO}_2$), $^{99\text{m}}\text{TcO}_4^-$ or secondary $^{99\text{m}}\text{Tc}$ -HMPAO complex at the origin. QC results are quickly obtained by this procedure (5 min). The strip must be placed in the solvent immediately after spotting, however, since any delay can underestimate the % RCP of $^{99\text{m}}\text{Tc}$ -HMPAO due to oxidation of the primary complex. With the recent availability of ^{18}C minicolumns (Amprep; Amersham UK, Buckinghamshire, England) for sample preparation in Australia, we decided to investigate the application of such products in the QC analysis of $^{99\text{m}}\text{Tc}$ -HMPAO. The W17 paper method was used in our study to validate the Amprep column (AC) system.

MATERIALS AND METHODS

Sodium $^{99\text{m}}\text{Tc}$ -pertechnetate ($^{99\text{m}}\text{TcO}_4^-$) was obtained from the daily milking of a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator and used to prepare $^{99\text{m}}\text{Tc}$ -HMPAO per the manufacturer's instructions. The percent labeling efficiencies were determined by column and paper methods simultaneously on 1 kit within 30 min after reconstitution, then at 1, 1.5, 2, 3, 4 h for $^{99\text{m}}\text{Tc}$ -HMPAO and 20, 60 and 120 min for $^{99\text{m}}\text{TcO}_2$. All QC analyses were performed 3 times or as specified, and a dose calibrator was used for counting all samples. Reproducibility of the column and paper methods was tested on $^{99\text{m}}\text{Tc}$ -HMPAO kits on different days within 30 min after reconstitution. R_f values were determined based on the definition: distanced migrated/distanced migrated by the solvent.

Determining Percent Radiochemical Purity of Technetium-99m-HMPAO

Whatman 17 Method. A previously reported procedure (2) was used where a W17 paper strip (1 × 8 cm) was developed in ethyl acetate solvent. The solvent front contained $^{99\text{m}}\text{Tc}$ -HMPAO (cut line R_f 0.25).

Amprep Column Method. The AC (RPN.1900) was conditioned by eluting with saline (2 mL), then air (2 mL). Technetium-99m-HMPAO (1–3 drops) was added by syringe bearing a needle (22 G × 2 in.) directly onto the sorbent bed. The column was eluted with saline (2 mL), then air (2 mL) into a collection vial. The eluate vial and column each were counted separately. Percent RCP of $^{99\text{m}}\text{Tc}$ -HMPAO was expressed as percent column counts in the total counts of eluate plus column.

For correspondence or reprints contact: Chris Tsopelas, PhD, Royal Adelaide Hospital, Nuclear Medicine Dept., RAH Radiopharmacy, North Terrace, Adelaide, South Australia, Australia, 5000.

Percent Hydrolyzed-Reduced Technetium-99m in Technetium-99m-HMPAO Kits (3)

The Whatman 1 paper strip (W1; 1 × 6 cm) was spotted with ^{99m}Tc-HMPAO, developed using freshly prepared water for injection: acetonitrile [50:50] solvent, then cut (*R_f* 0.50) to isolate the origin and solvent front pieces for counting. Technetium-99m-HMPAO, the secondary ^{99m}Tc-complex and ^{99m}TcO₄⁻ migrated with an *R_f* 0.8–1.0 while ^{99m}TcO₂ remained at origin. The percent ^{99m}TcO₂ was calculated as percent origin counts in the total counts of both pieces.

Preparing Hydrolyzed-Reduced Technetium-99m

A modified procedure (4) was used to prepare ^{99m}TcO₂ dispersions. A quantity of 500 MBq/0.5 mL ^{99m}TcO₄⁻ was added to a solution of stannous chloride (SnCl₂; 545.4 μg) in hydrochloric acid (pH 5.0; 1.0 mL) in a nitrogen-filled vial and then diluted [1:306] (≡ 1.78 μg SnCl₂) with water for injection to make Kit A. Kit A was diluted further [1:9.4] with water for injection to make Kit B. Kit B contained a SnCl₂ level (0.19 μg) which simulated the mass of stannous chloride required to produce 2.5% ^{99m}TcO₂. The % RCP of ^{99m}TcO₂ in Kit A was determined using instant thin-layer chromatography paper impregnated with silica gel (ITLC-SG; 1 × 16 cm) and saline (0.9%) as the developing solvent. A cut line at *R_f* 0.1 (1 cm) separated the origin and 9 other pieces (1 cm), and percent ^{99m}TcO₂ was calculated as percent origin counts in the total counts of all pieces.

Behavior of Technetium-99m-Pertechnetate and Hydrolyzed-Reduced Technetium-99m on the Carbon-18 Column

In separate experiments, ^{99m}TcO₄⁻ (5 MBq/mL; 1–3 drops), ^{99m}TcO₂ (1–3 drops) in Kit A and Kit B, were each eluted with saline (2 mL) down the preconditioned column in a procedure, as outlined with ^{99m}Tc-HMPAO above.

RESULTS

Determining Percent Radiochemical Purity of Technetium-99m-HMPAO

Table 1 highlights the change in % RCP of ^{99m}Tc-HMPAO over time according to both methods, as well as the reproducibility

TABLE 1
Percent Radiochemical Purity of Technetium-99m-HMPAO Versus Time as Determined by 2 Quality Control Methods

Time (h)	% RCP ^{99m} Tc-HMPAO		n
	W17 method	AC method	
<0.5	91.1 ± 2.7	92.6 ± 3.2	30
1	78.8 ± 1.5	81.8 ± 0.9	3
1.5	74.1 ± 0.5	75.1 ± 0.3	3
2	61.7 ± 0.2	63.0 ± 0.7	3
3	56.0 ± 1.9	57.2 ± 2.4	3
4	48.0 ± 0.8	48.7 ± 0.6	3

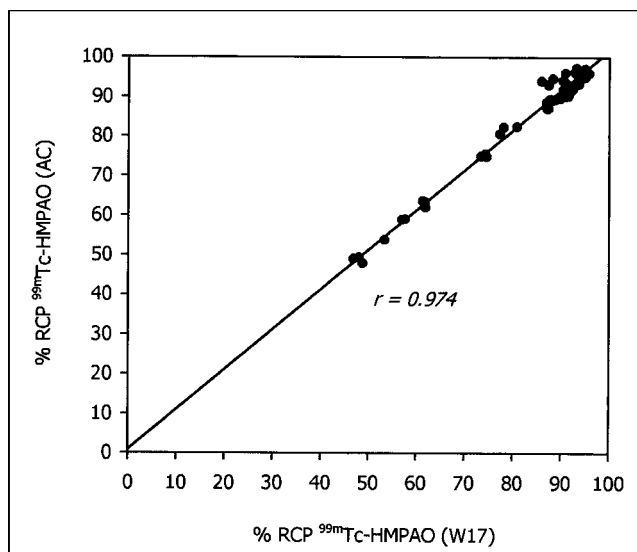


FIGURE 1. Correlation graph of percent radiochemical purity of technetium-99m-HMPAO versus 2 quality control methods.

between different kits (n = 30) over time. Percent RCP calculations were obtained by the AC method in only 3 min, and were higher by 0.7%–3.0% than the W17 method values. Each column was reused at ≥ 2 d after QC analysis to allow for ^{99m}Tc decay. Use of 1 column consecutively on 12 occasions resulted in 2%–3% reduction in % RCP of ^{99m}Tc-HMPAO.

The % RCP of ^{99m}Tc-HMPAO values obtained by both methods (n = 45) are depicted as a correlation graph in Figure 1. Note that the regression line is slightly staggered up the y axis.

Percent Hydrolyzed-Reduced Technetium-99m in Technetium-99m-HMPAO Kits

Hydrolyzed-reduced ^{99m}Tc was found in ^{99m}Tc-HMPAO kits as a minor impurity (Table 2), and observed to vary insignificantly with time.

Behavior of Technetium-99m-Pertechnetate and Hydrolyzed-Reduced Technetium-99m on the Carbon-18 Column

Technetium-99m-pertechnetate was not retained by the column matrix, but appeared in the eluent exclusively (99.9% ± 0.0%). Hydrolyzed-reduced technetium-99m remained at the origin of the ITLC-SG strip quantitatively (96.8% ± 1.3%). This species was better retained by the column with Kit B (Table 3).

TABLE 2
Percent Hydrolyzed-Reduced Technetium-99m in Technetium-99m-HMPAO Kits Over Time

Time (min)	% ^{99m} TcO ₂	n
20	2.1 ± 0.5	6
60	2.5 ± 0.5	4
120	2.3 ± 0.4	5

TABLE 3
Percent Hydrolyzed-Reduced Technetium-99m
Retained by the Column Matrix

^{99m} TcO ₂ kit	SnCl ₂ (μg)	% Radioactive counts	
		Saline eluent	Column
A	1.78	50.4 ± 1.0	49.6 ± 1.0
B	0.19	11.2 ± 0.2	88.8 ± 0.2

DISCUSSION

Use of an aqueous mobile phase in the AC procedure was a major advantage over the W17 method because it eliminates the need for hazardous organic solvents. Reproducibility of both QC methods was very good, giving percent radiochemical purity of ^{99m}Tc-HMPAO as 91.1% ± 2.7% (+2.1% ± 0.5% for ^{99m}TcO₂) by the Whatman methods and 92.6% ± 3.2% by the AC method. The correlation coefficient shows that the AC method can determine % RCP of ^{99m}Tc-HMPAO equally effectively as the W17 method (Fig. 1).

Technetium-99m-pertechnetate was not retained by the column and thus did not influence the final % RCP of ^{99m}Tc-HMPAO result. However, ^{99m}TcO₂ was retained by the cartridge to the extent of 49.6% ± 1.0% with Kit A and 88.8% ± 0.2% with Kit B. These results show that the ¹⁸C column binds a higher proportion of ^{99m}TcO₂ impurity when it is present at lower levels, as normally found in ^{99m}Tc-HMPAO kits (2.1%). Thus, for a ^{99m}Tc-HMPAO kit containing 2.1% ^{99m}TcO₂, the cartridge will remove 1.9% by column adsorption and allow 0.2% to pass into the eluent.

This ^{99m}TcO₂ level of column adsorption is in agreement with the range of difference of % RCP values (0.7%–3.0%) for both methods (Table 1). The staggered regression line in Figure 1 can be explained by the low level of column bound ^{99m}TcO₂. Thus, ^{99m}TcO₂ accounts for the higher % RCP values by the AC method (Table 1), and fortunately exists in ^{99m}Tc-HMPAO kits as a minor impurity (Table 2). Furthermore, we found this amount varies only slightly with time, as previously observed (5).

Although the % RCP of ^{99m}Tc-HMPAO is slightly overestimated by the AC method, the impurity level is insignificant because all values within 30 min greatly exceed the manufacturer's permissible % RCP limit (80%) for patient injection.

The secondary ^{99m}Tc-HMPAO complex is hydrophilic (6), remaining at the origin with W17 method and, in comparison to the charged ^{99m}Tc-pertechnetate ion, it was not expected to be retained by the ¹⁸C column after saline elution. The secondary ^{99m}Tc-HMPAO complex is a degradation product of ^{99m}Tc-HMPAO, an impurity that significantly increases with time beyond 30 min. From Table 1, all the % RCP of ^{99m}Tc-HMPAO values decrease at the same rate with time, suggestive of poor retention of the secondary complex by the column.

CONCLUSION

Percent RCP results for different ^{99m}Tc-HMPAO kits were highly comparable between the W17 method and the AC analysis system. The AC system employs an inexpensive, reusable ¹⁸C column in conjunction with a safe and readily available mobile phase. This method was found to be both simple and rapid for the QC analysis of routine ^{99m}Tc-HMPAO preparations.

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