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Note

Quantitative determination of pertechnetate by high-performance liquid chromatography with UV detection

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In the practice of diagnostic nuclear medicine, some chemical form of a gamma-ray emitting isotope is administered to a patient with the goal of having the isotope localize in a specific organ. Subsequent scanning of the organ with a gamma-ray camera provides valuable diagnostic and prognostic data by an essentially noninvasive technique [1,2].

Technetium-99m is the isotope of choice for diagnostic nuclear medicine because of its optimal nuclear properties, its diverse chemistry, and its ready availability by means of the $^{99}Mo/^{99m}Tc$ generator [3—5]. This generator is based upon the nuclear decay scheme

$$^{99}Mo \xrightarrow[66\ h]{\beta^-} {}^{99m}Tc \xrightarrow[6\ h]{\gamma} {}^{99}Tc$$

and the chemical fact that the highest oxidation states of Mo and Tc have different ionic charges (MoO_4^{2-} and TcO_4^-, molybdate and pertechnetate, respectively in neutral, aqueous solution). The manufacturer loads $^{99}MoO_4^{2-}$ onto a shielded alumina column and then ships this generator to the point of use. In the hospital the column is eluted with normal saline (0.15 *M* sodium chloride) once every working day; upon elution, the —1-charged pertechnetate (both $^{99m}TcO_4^-$ and $^{99}TcO_4^-$) is eluted whereas the —2-charged molybdate is retained. This eluate can be used directly to obtain a ^{99m}Tc pertechnetate scan. But more often the eluate is subjected to one of a variety of chemical reactions in which the technetium is reduced to a lower oxidation state and simultaneously chelated by a ligand to generate a reduced ^{99m}Tc radiopharmaceutical with specific biological properties [1—6].

The concentration of ^{99m}Tc in the generator eluate is of prime importance in nuclear medicine applications since it is the gamma-ray emission from this

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isotope which provides the diagnostic image. However, the eluate also contains significant amounts of ^{99}Tc because of the characteristics of the ^{99}Mo—^{99m}Tc—^{99}Tc decay scheme, and this concentration is important for two reasons. First, it is the total chemical concentration of pertechnetate (both $^{99m}TcO_4^-$ and $^{99}TcO_4^-$) in the eluate that determines the chemistry and kinetics of the conversion of pertechnetate into radiopharmaceuticals containing technetium in a reduced oxidation state [3]. Secondly, the amount of ^{99}Tc, which is a long-lived ($t_{½}$ = 2 · 10^5 year) beta-emitter, injected into a patient must be taken into account when calculating the total radiation dose received by the patient.

The total amount of technetium, as both ^{99}Tc and ^{99m}Tc, in generator eluates varies with the age and history of the generator, and, of course, with the time elapsed since the last elution of the generator. The amounts of ^{99m}Tc and ^{99}Tc in any given generator eluate can be calculated from the ^{99}Mo—^{99m}Tc—^{99}Tc decay scheme, given certain assumptions about the history of the generator [7,8]. However, these assumptions are difficult to verify and, to date, the calculations have not been experimentally validated. Determination of ^{99m}Tc in the eluate presents no problems since this isotope can be accurately monitored by means of its gamma-emission. Determination of ^{99}Tc by radiochemical means is very difficult because of its low-energy beta-emission (0.29 MeV), the low concentration of this isotope in the eluate, and the presence of other long-lived radioactive impurities in most generator eluates [9]. We have therefore undertaken this study to determine the total amount of chemical pertechnetate (as both $^{99m}TcO_4^-$ and $^{99}TcO_4^-$) in generator eluates by chemical means. This non-radiochemical procedure employs high-performance liquid chromatography (HPLC) to separate pertechnetate from other generator constituents and impurities, and UV detection to quantitatively monitor the concentration of pertechnetate.

MATERIALS AND METHODS

Chemicals

Technetium in the form of TcO_4^- in 0.9% sodium chloride was obtained from the Radioisotope Laboratory at Cincinnati General Hospital as the first elution of a $^{99}Mo/^{99m}Tc$ generator (7.5 Ci, Union Carbide, Tuxedo, NY, U.S.A.).

Crystalline $NH_4{}^{99}TcO_4$ (Oak Ridge National Laboratories, Oak Ridge, TN, U.S.A.) was converted to $K^{99}TcO_4$ by metathesis with potassium hydroxide. This material was recrystallized and dried before being used for the preparation of standard solutions.

Standard solutions

Stock solutions of $6.00 \cdot 10^{-4}$ *M* and $4.35 \cdot 10^{-4}$ *M* $K^{99}TcO_4$ were prepared by weight in 0.10 *M* and 0.03 *M* acetate, pH 4.5, respectively. Appropriate dilutions of these with the corresponding elution buffers yielded a series of standard solutions.

Reagents

Elution buffers, 0.10 *M* and 0.03 *M* in total acetate concentration, pH 4.5, were freshly prepared from glacial acetic acid, A.R., sodium hydroxide, A.R., and triply distilled, charcoal filtered water. Prior to use, these were filtered through 0.22-μm GS membranes (Millipore, Befored, MA, U.S.A.) and de-aerated by sonication under vacuum.

Chromatographic apparatus

The chromatographic equipment included a Waters Assoc. Model M45 solvent delivery system, a Rheodyne Model 7125 injection valve fitted with a 100-μl sample loop and a PD-2 12-in. pulse dampener, all obtained from Bioanalytical Systems (West Lafayette, IN, U.S.A.).

A LiChrosorb 10-μm amino bonded phase 5-cm cartridge guard column (Brownlee Labs., Santa Clara, CA, U.S.A.) was installed between the injection valve and the analytical column.

The analytical column was a 250 mm × 4.6 mm stainless-steel Knauer column (Unimetrics, Anaheim, CA, U.S.A.) slurry packed with Spherisorb amino bonded phase, 5 μm (Phase Separations, Hauppauge, NY, U.S.A.). Isocratic elution of these columns with the aqueous acetate buffers at 1.5 ml/min resulted in typical operating pressures around 12.4 MPa (1800 p.s.i.) at ambient temperature.

UV detection

The absorbance of the eluent was monitored at 254 nm with a Beckman Model 153 UV detector equipped with an 8-μl flow-cell of 1 cm pathlength. The detector output was recorded with an Omniscribe B-5000 dual-channel strip chart recorder (Houston Instruments, Austin, TX, U.S.A.).

Radiometric detection

Chromatographic detection of the gamma activity of eluting $^{99m}TcO_4^-$ was accomplished with a scintillation spectrometry system (Harshaw Chemical Co., Solon, OH, U.S.A.). This consisted of a NA-23 stabilized amplifier/single channel analyzer, a NR-22 linear ratemeter, and a NV-32A high-voltage power supply all mounted in an AP-2H nuclear instrument module (Berkeley Nucleonics, Berkeley, CA, U.S.A.). The detector was a shielded 5.1 × 5.1 cm cylindrical NaI(Tl) crystal with a 1.4 cm I.D. hole optically coupled to a 5.1-cm Amperex PM 2202 photomultiplier tube. The ratemeter signal was fed to the second channel of the recorder permitting simultaneous monitoring of gamma activity and optical absorbance. Finally, absolute amounts of ^{99m}Tc activity in collected fractions were determined with a CRC-6A Radioisotope Calibrator (E.R. Squibb and Sons, Princeton, NJ, U.S.A.).

Quantitative analysis

A standard curve (Fig. 1A) under conditions of minimum retention and maximum detectability was generated by sampling eighteen different concentrations of $KTcO_4$, ranging from $6.00 \cdot 10^{-8}$ *M* to $6.00 \cdot 10^{-4}$ *M* in 0.10 *M* acetate buffer, pH 4.5. A second standard curve (Fig. 1B) under conditions more suited to the analysis of $^{99}Mo/^{99m}Tc$ generators was obtained by sampling

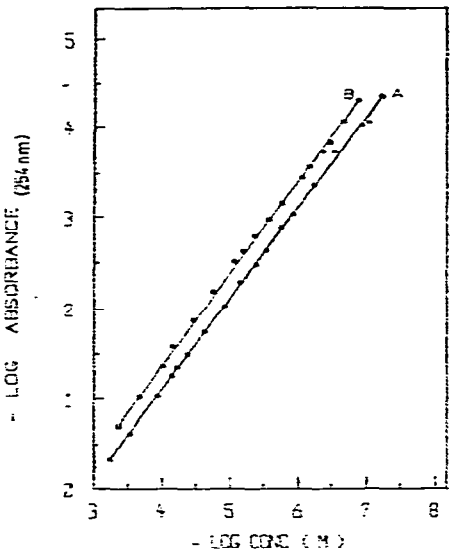

Fig. 1. Calibration graphs of $K^{99}TcO_4$ with mobile phases of (A) 0.10 *M* acetate, pH 4.5 (t_R = 4.3 min) and (B) 0.03 *M* acetate, pH 4.5 (t_R = 7.3 min). Conditions: flow-rate 1.5 ml/min; detection 254 nm; temperature ambient.

seventeen different concentrations of $KTcO_4$, ranging from $1.30 \cdot 10^{-7}$ *M* to $4.35 \cdot 10^{-4}$ *M* in 0.03 *M* acetate buffer, pH 4.5. For each concentration of $K^{99}TcO_4$, six replicate chromatograms were obtained; the peak heights were corrected for noise and measured manually. The six peak heights were then averaged, and the standard deviation of the mean (σ_m) was calculated. Logarithmic calibration curves were constructed by plotting average peak height [in absorbance units, weighted as $1/(\sigma_m)^2$] vs. $K^{99}TcO_4$ concentration, and were then analyzed by a linear least squares treatment (Table I).

RESULTS AND DISCUSSION

Initial experiments designed to optimize conditions for pertechnetate analysis resulted in a detection limit of $6.00 \cdot 10^{-8}$ *M* TcO_4^- in 0.10 *M* acetate, pH 4.5, the retention time of pertechnetate being 4.3 min. However, it was found that analysis of pertechnetate eluted from a $^{99}Mo/^{99m}Tc$ generator was not possible under these conditions due to refractive index changes and other unknown chromatographic interferences occurring at or near this retention time. The mobile phase was therefore adjusted to increase the retention time of pertechnetate. With 0.03 *M* acetate, pH 4.5, the retention time of pertechnetate is 7.3 min and the detection limit for $K^{99}TcO_4$ is $1.30 \cdot 10^{-7}$ *M*. A typical chromatogram obtained under these conditions is shown in Fig. 2A.

Fig. 1 shows that the calibration curves under both sets of conditions are linear over four orders of magnitude of pertechnetate concentration. This linearity extends at least two orders of magnitude beyond the pertechnetate concentrations expected in $^{99}Mo/^{99m}Tc$ generator eluates. Statistical data describing each calibration curve are summarized in Table I.

Analysis of the first eluate from 7.5 Ci Union Carbide $^{99}Mo/^{99m}Tc$ generator

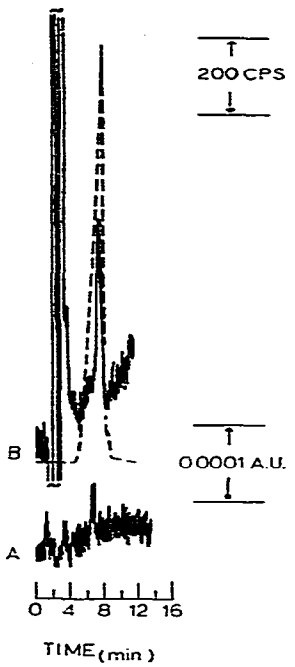

Fig. 2. Typical chromatograms of (A) $1.30 \cdot 10^{-7}$ *M* $K^{99}TcO_4$ and (B) $^{99}Mo/^{99m}Tc$ generator first eluent (37 μCi/ml). Conditions: eluent 0.03 *M* acetate, pH 4.5; flow-rate 1.5 ml/min; detection 254 nm (——), γ (— — —); temperature ambient.

TABLE I

WEIGHTED LINEAR LEAST SQUARES ANALYSIS FOR $K^{99}TcO_4$ STANDARD CURVES (LOG VS. LOG)

	t_R (A) (4.3 min)	t_R (B) (7.3 min)
Slope	1.011 ± 0.004	1.043 ± 0.006
Y-Intercept	−2.926 ± 0.015	−2.80 ± 0.03
Correlation coefficient	0.9998	0.9997

(detection at 254 nm) yields the chromatogram shown in Fig. 2B (solid line), with a single component eluting at a retention time of 7.3 min. Gamma detection (Fig. 2B, broken line) also shows a single major component with a retention time of 7.3 min, confirming that the component is indeed pertechnetate. From the slope and intercept parameters for standard curve B (Table I) the concentration of pertechnetate in the $^{99}Mo/^{99m}Tc$ generator eluate is calculated to be $(8.0 \pm 1.6) \cdot 10^{-7}$ *M*, five times greater than the detection limit. In this experiment, 100 ± 7% of the initially injected gamma activity is recovered from the HPLC column within the peak at a retention time of 7.3 min. Therefore, this particular generator eluate does not contain significant amounts of reduced forms of ^{99m}Tc, and the HPLC procedure itself does not induce significant reduction of pertechnetate. Upon increasing the gamma sensitivity, two additional radioactive components eluting prior to TcO_4^- are observed

(Fig. 3). These extremely minor components each comprise approximately 0.01% of the total activity applied and have been verified by multichannel pulse height analysis to contain only ^{99m}Tc (possibly as reduced hydrolyzed species).

Fig. 3. Chromatogram of $^{99}Mo/^{99m}Tc$ generator eluent (≈60 mCi/ml) with gamma-detection. Conditions: eluent 0.1 *M* acetate, pH 4.5; flow-rate 1.5 ml/min; temperature ambient.

Attempts at detecting MoO_4^{2-} (a breakthrough product of $^{99}Mo/^{99m}Tc$ generators) were performed by chromatographing standard Na_2MoO_4 solutions under similar conditions of generator analysis. Since no peaks were observed after 5.5 h of elution (1.5 ml/min), it is concluded that MoO_4^{2-} is either totally retained on the column or has been sufficiently diluted so as to be undetectable, and it thus poses no serious interference in the analysis of TcO_4^-.

From these results it is clear that HPLC with UV detection has the sensitivity and selectivity necessary to monitor total chemical pertechnetate in $^{99}Mo/^{99m}Tc$ generator eluates. The applicability of this technique has been substantiated by our current investigation involving the measurement of total TcO_4^- in the eluates of several $^{99}Mo/^{99m}Tc$ generators over their entire useful clinical lifetime [10]. We have noticed that the absolute sensitivity varies slightly, however, due to both the age of the column and variability in column-to-column efficiencies.

Although some column deterioration (loss of retention) was observed over the course of the study, this analysis should be of considerable utility in monitoring the function of clinical generators, as well as in the routine analysis of reduced ^{99m}Tc radiopharmaceuticals for the presence of undesired pertechnetate.

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REFERENCES

1 B.A. Rhodes and B.Y. Croft, Basics of Radiopharmacy, C.V. Mosby, St. Louis, MO, 1978, pp. 33—51.
2 G. Subramanian, B.A. Rhodes, J.F. Cooper and V.J. Sodd (Editors), Radiopharmaceuticals, Society of Nuclear Medicine, New York, 1975.
3 E. Deutsch, Radiopharmaceuticals II, Society of Nuclear Medicine, New York, 1979, p. 129.
4 N.D. Heindel, H.D. Burns, T. Honda and L.W. Brady (Editors), The Chemistry of Radiopharmaceuticals, Masson, New York, 1978.
5 W.C. Eckelman and S.M. Levenson, Int. J. Appl. Radiat. Isot., 28 (1977) 67.
6 J.W. Keyes, Jr., CRC Manual of Nuclear Medicine Procedures, 3rd ed., CRC Press, Cleveland, OH, 1978.
7 M.L. Lamson, A.S. Kirschner, C.E. Hotte, E.L. Lipsitz and R.D. Ice, J. Nucl. Med., 16 (1975) 639.
8 V. Hušák and J. Vlček, Int. J. Appl. Radiat. Isot., 30 (1979) 165.
9 L. Johansson and S. Mattsson, J. Nucl. Med., 21 (1980) 1091.
10 E. Deutsch, W.R. Heineman, J.P. Zodda, T.W. Gilbert and C.C. Williams, Int. J. Appl. Radiat. Isot., in press.