Characterization and Quality Control Analysis of ^{99m}Tc-Bicisate

Chris Tsopelas, PhD; and Douglas Smyth, PhD

RAH Radiopharmacy, Nuclear Medicine Department, Royal Adelaide Hospital, North Terrace, Adelaide, Australia

The aim of this study was to investigate the mini cartridge versus paper chromatography quality control methods for determining the radiochemical purity (RCP) of ^{99m}Tc-bicisate. The 4 methods that were compared with the manufacturer's method included Whatman 17 paper/ethyl acetate solvent, instant thin-layer chromatography (ITLC) silica gel paper/saline solvent, reverse-phase C18 mini cartridge/saline solvent, and strong anion exchange mini cartridge/water solvent. At 30 min after reconstitution, ^{99m}Tc-bicisate was formed at 97%–98% RCP as assayed by the paper and cartridge methods, and the strong anion exchange/water for injection (WFI) system slightly underestimated the percentage at 96%. A significantly lower RCP was obtained for the C18/saline method when a faster flow rate was used. The lipophilic complex moved with ethyl acetate on Whatman 17, was separated from origin impurities on ITLC silica gel/saline, and remained on the column with C18/saline. For strong anion exchange/WFI, components in the radioactive formulation are likely to have influenced the percentage of ^{99m}Tc-bicisate. The time disadvantage for ITLC silica gel/saline analysis made the method less than ideal. The C18 mini cartridge/saline method was found to be the simplest and fastest; a result was obtained in 2 min with use of a safe solvent of elution.

Key Words: ^{99m}Tc-ECD; ^{99m}Tc-bicisate; radiopharmaceutical; quality control; cartridge

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Of the brain perfusion imaging agents developed during the last 20 y, only ^{99m}Tc-exametazime and ^{99m}Tc-bicisate (ethylene cysteine dimer [ECD]) have become routinely adopted into a clinical setting. The former agent should be prepared, evaluated for radiochemical purity (RCP), and then administered to the patient within 30 min of reconstitution or within 4 h when methylene blue is in the formulation. In contrast, the longer shelf life of ^{99m}Tc-ECD allows for quality control analysis and subsequent patient injection beyond 30 min after ^{99m}Tc-pertechnetate addition. The manufacturer's method for assessing the RCP of ^{99m}Tc-ECD requires the use of a Baker-Flex silica gel thin-layer chromatography plate as the stationary phase with an ethyl acetate mobile phase (*1*), and the procedure can be time consuming (*2*). In active nuclear medicine departments, the staff need to determine the RCP of a dose quickly and reliably before administration to patients. Thin-layer chromatography systems using Whatman 3MM paper (*3*) or Whatman 17 paper (*4*) and ethyl acetate solvent were previously validated against the manufacturer's method to assay the lipophilic ^{99m}Tc-ECD. These popular chromatography systems successfully separated ^{99m}Tc-ECD at the solvent front from impurities such as ^{99m}Tc-pertechnetate and $^{99m}TcO_2$ that remained at the origin. Vial A of the 2-vial Neurolite kit (DuPont Merck Pharmaceutical) contains a freeze-dried composition of ECD, stannous chloride, ethylenediaminetetraacetic acid (EDTA), and mannitol, with the latter 2 excipients potentially resulting as ^{99m}Tc-impurities in the reconstituted kit. The Whatman paper/ethyl acetate method can provide a rapid result (*3*), however it is still unclear what effect EDTA and mannitol have on the technique.

Alternative radiopharmaceutical quality control systems have used mini cartridges to assay for ^{99m}Tc-exametazime and ^{99m}Tc-tetrofosmin, comprising reverse-phase C18 column material (*5*), and silica (*2*) or anion exchange resin (*6*) respectively. Other radiopharmaceuticals such as ^{99m}Tc-mertiatide, ^{111}In-pentetriotide, and ^{99m}Tc-sestamibi (*7*) can also be tested in this way. The mini cartridges are simple to use and reduce the analysis time, compared with the paper methods. An aim of this study was to examine the mini cartridge and paper chromatographic methods for quantifying the ^{99m}Tc-species in the reconstituted Neurolite kit. Four quality control systems were investigated to identify which best determined the RCP of ^{99m}Tc-ECD.

For correspondence or reprints contact: Chris Tsopelas, PhD, RAH Radiopharmacy, Nuclear Medicine Department, Royal Adelaide Hospital, North Terrace, Adelaide, Australia.
E-mail: ctsopela@mail.rah.sa.gov.au

MATERIALS AND METHODS

Sodium ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) was obtained from the daily elution of a ^{99}Mo/^{99m}Tc generator (Gentech; Australian Radioisotopes) and used to prepare ^{99m}Tc-ECD according to the manufacturer's instructions, except that the reconstitution volume was ^{99m}Tc-pertechnetate (2–2.5 GBq) in saline (≤1.2 mL). The percentage RCP of the ^{99m}Tc-ECD or $^{99m}TcO_2$ level of kits was determined by column or paper methods simultaneously at 30 min, 2 h and 6 h after reconstitution. ^{99m}Tc-EDTA and $^{99m}TcO_4^-$ were analyzed by column or paper methods at 30 min. The accuracy of each method (testing ^{99m}Tc-ECD kits on multiple occasions) was determined at 30 min after reconstitution and was calculated as the percentage SD of the mean value. The precision of each assay (multiple testing of the same kits) was evaluated ($n = 3$) by 2 operators (excluding strong anion exchange analyses) on separate occasions and was calculated as the percentage SD from the mean value. R_f values were determined as distance migrated by the species divided by distance of the solvent front. Radioactive samples > 3 MBq were counted in a validated counting unit (Atomlab 100$^+$ Dose Calibrator; Biodex Medical Systems), 0.5–3 MBq samples were counted in a large-volume counter (BioSentry; AEI-Ekco) linked to a multichannel analyzer (model 3100; Canberra Industries Inc.), and <0.5 MBq samples were counted in a γ-counter (Auto-Gamma 5650; Hewlett-Packard) over a ^{99m}Tc window (70–210 KeV). All counted samples were background corrected.

Determination of Percentage of ^{99m}Tc-ECD

Paper Methods. ^{99m}Tc-ECD was analyzed according to the manufacturer's method using Baker-Flex silica gel/ethyl acetate, a modified procedure (*8*) in which a Whatman 17 paper strip (1 × 8 cm) was developed in ethyl acetate solvent (cut line R_f, 0.30), and an ITLC silica-impregnated glass fiber strip (1 × 16 cm; Gelman Sciences) developed in saline.

Cartridge Methods. The Amprep C18 cartridge (RPN.1900; Amersham Biosciences) was conditioned by eluting with saline (2 mL) and then air (2 mL). ^{99m}Tc-ECD (4–5 drops) was added by insulin syringe on top of the column bed, and the bolus sample was loaded with minimal air. The column was immediately eluted with saline (2 mL) at either a regular flow rate (estimated as 2–3 drops per second) or a fast flow rate (in a steady stream), and then air (2 mL), into a collection vial. The eluate vial and column were counted separately, and the time of the procedure was recorded. The RCP of ^{99m}Tc-ECD was expressed as the percentage of column counts in the total counts. The procedure was then repeated, using Amprep strong anion exchange (RPN.1918; Amersham Biosciences) in conjunction with water for injection (WFI) as the mobile phase.

Percentage of $^{99m}TcO_2$ in ^{99m}Tc-ECD Kits

The $^{99m}TcO_2$ level was determined by 2 methods using ITLC silica gel strips as the stationary phase. The first method used an alkaline solvent system (*9*) of 1:2:5 concentrated ammonia solution:100% ethanol:WFI (AEW), and the second method used saline (0.9%). The premarked (1 cm) strips were spotted with sample at the origin, developed to 12 cm, and then sectioned and counted. The level of $^{99m}TcO_2$ was calculated as the percentage of origin counts in the total counts.

Preparation of $^{99m}TcO_2$

A modified procedure (*9*) was used to prepare $^{99m}TcO_2$ dispersions. ^{99m}Tc-pertechnetate (20 MBq/0.5 mL saline) was added to a solution of acidic stannous chloride (pH 4.5; 60 μL; 1.2 mg/mL) in saline (0.5 mL) in a nitrogen-filled vial. The level of $SnCl_2$ (≡72 μg) simulated the mass of stannous chloride in an ECD kit. The $^{99m}TcO_2$ formed was analyzed by the following methods: Baker-Flex silica gel/ethyl acetate, Whatman 17/ethyl acetate, C18/saline, strong anion exchange/WFI, and ITLC silica gel/saline.

Preparation of ^{99m}Tc-EDTA

The contents of the EDTA kit (Royal Adelaide Hospital Radiopharmacy), consisting of ethylenediaminetetraacetic acid (3.7 mg) in WFI (1 mL), were added to an UltraTag RBC Kit (Mallinckrodt) containing lyophilized stannous chloride dihydrate (96 mg), sodium citrate dihydrate (3.7 mg), and dextrose (5.5 mg). This kit was reconstituted with ^{99m}Tc-pertechnetate (400 MBq/1.2 mL saline), mixed with Neurolite buffer (vial B; 0.6 mL), and ^{99m}Tc-EDTA product was analyzed 30 min later by Baker-Flex silica gel/ethyl acetate, Whatman 17/ethyl acetate, C18/saline, strong anion exchange/WFI, and ITLC silica gel/saline. ^{99m}Tc-EDTA was also prepared from a kit containing mannitol (26 mg) and then was analyzed by C18/saline and ITLC silica gel/saline.

Percentage of ^{99m}Tc-EDTA in ^{99m}Tc-ECD Kits

^{99m}Tc-ECD was prepared with ^{99m}Tc-pertechnetate as usual, except that a molar excess of EDTA (3x; 2 mg/mL; 1 mL) was added initially to cold Neurolite vial A. The contents of the kit were analyzed by ITLC silica gel/saline to determine any influence of ^{99m}Tc-EDTA on the migration of ^{99m}Tc-ECD.

Behavior of ^{99m}Tc-Pertechnetate

^{99m}Tc-pertechnetate (20 MBq/10 mL saline) was analyzed by Baker-Flex silica gel/ethyl acetate and strong anion exchange/WFI to verify location on the stationary phase. ITLC silica gel/acetone was performed to determine whether ^{99m}Tc-pertechnetate separated from ^{99m}Tc-EDTA in the ^{99m}Tc-ECD formulation.

Statistical Analyses

Statistical analyses were performed with ANOVA (single factor) or a paired sample *t* test to compare the RCP of ^{99m}Tc-ECD as obtained by the manufacturer's method versus the Whatman 17, C18 cartridge, or ITLC silica gel/saline methods. The paired sample *t* test was used to compare regular versus fast flow rates for the C18 method.

Statistical significance was defined as a P value of less than 0.05. Results are reported as mean ± SE.

RESULTS

Percentage of ^{99m}Tc-ECD

The percentage of ^{99m}Tc-ECD in kits was assayed by different quality control methods at 3 time points up to 6 h after reconstitution (Table 1). As expected, the neutral radioactive complex migrated with the ethyl acetate solvent front (R_f = 0.5–1.0) on both Baker-Flex silica gel and Whatman 17 strips and beyond the origin (R_f = 0.2–0.5) with ITLC silica gel in saline. ^{99m}Tc-ECD was retained by the C18 column of the Amprep cartridge. A comparison of the ^{99m}Tc-ECD RCP results from the manufacturer's method (98%) versus the Whatman 17 or C18 methods (98%), or versus the ITLC silica gel/saline method (97%), indicated no significant differences (Table 2). Similarly, there was no difference in values between the Whatman 17 versus the C18 cartridge methods at 30, 120 and 360 min after reconstitution, and between the Whatman 17 or C18 method versus the ITLC silica gel/saline method at 30 and 120 min after reconstitution. Despite a consistent trend in RCP over time for each method, the small differences between Whatman 17 or C18 and ITLC silica gel/saline were found to be statistically significant. These differences may be attributed to the varying accuracy of the methods and the small sample size. The RCP of ^{99m}Tc-ECD was 98% when excess EDTA was present in the cold formulation. The RCP of ^{99m}Tc-ECD was 98% when the regular elution flow rate was used, versus 94% for the fast flow rate, these values being significantly different at all time points after ^{99m}Tc-pertechnetate reconstitution.

The neutral ^{99m}Tc-ECD complex was predominantly in the eluent using the strong anion exchange cartridge/WFI. A quality control result was obtained in 2.9 ± 0.1 min (n = 5) from the Whatman 17/ethyl acetate method, 1.9 ± 0.2 min (n = 5) for the regular eluent flow and 1.2 ± 0.1 min (n = 5) for the fast eluent flow with the C18/saline method, and ~10 min for ITLC silica gel/saline. All quality control methods showed good reproducibility in terms of accuracy (≤2.1%) and precision (≤1.3%). The precision values were 0.5%, 1.3%, 0.6%, and 0.5% for the first operator using the C18, strong anion exchange, Whatman 17, and ITLC assays respectively, and 0.4%, 0.3%, and 0.2% for the second operator using C18, Whatman 17, and ITLC assays respectively.

Percentage of $^{99m}TcO_2$

A low level of $^{99m}TcO_2$ was found in ^{99m}Tc-ECD kits according to the ITLC silica gel/saline method (at R_f = 0.0), to the extent of 0.5% ± 0.0% (n = 3), 0.5% ± 0.0% (n = 3), and 1.1% ± 0.2% (n = 3) at 30, 120 and 360 min after reconstitution respectively. These results were confirmed using ITLC silica gel/AEW (0.3% ± 0.1% [n = 3] at 30 min after reconstitution; R_f = 0.0–0.1), for which the alkaline mobile phase moved all other chemical compounds in the kit to the solvent front. When the manufacturer's method was employed, $^{99m}TcO_2$ was identified at the origin (99.7% ± 0.1% [n = 3]; R_f = 0.0–0.1), the same location found by the Whatman 17 paper method (*5*). $^{99m}TcO_2$ remained on the column when the matrix was C18 (*5*) or strong anion exchange (96.4% ± 0.3% [n = 3]).

^{99m}Tc-EDTA

The percentage of ^{99m}Tc-EDTA was assayed using 5 quality control analysis methods at 30 min after reconstitution (Table 3). The Baker-Flex silica gel and Whatman 17 strips that were developed in ethyl acetate resulted in 100% ^{99m}Tc-EDTA at the origin, whereas ITLC silica gel and saline gave the adduct at the solvent front in a sharp band. Of the cartridge methods, no ^{99m}Tc-EDTA was bound to the C18 column matrix and a minor amount (~7%) was adsorbed to the strong anion exchange column. When mannitol was present in the kit, the level of ^{99m}Tc-EDTA in the C18 column eluent was unaffected (99%), however migration of this tracer on ITLC silica gel was retarded in saline, with 100% of ^{99m}Tc-EDTA at R_f = 0.7–1.0 (92% at R_f = 0.9–1.0).

^{99m}Tc-Pertechnetate

^{99m}Tc-pertechnetate was identified at the origin (R_f = 0.0) using either the manufacturer's or the Whatman 17 method, in the saline eluent with the C18 method (*4*), or at the solvent front (R_f = 1.0) with the ITLC silica gel/saline method. The ITLC silica gel/acetone method did not dis-

TABLE 1
Radiochemical Purity of ^{99m}Tc-ECD Stored at Room Temperature

Analysis method	% RCP at time after reconstitution (mean ± SE)			Accuracy of method (%)
	30 min	120 min	360 min	
Baker-Flex SG/EtAc	98.3 ± 0.8 (n=5)	—	—	1.8
W17/EtAc	97.8 ± 0.3 (n=19)	98.8 ± 0.8 (n=3)	98.7 ± 0.3 (n=3)	1.3
C18/saline, regular	98.2 ± 0.2 (n=19)	98.0 ± 0.5 (n=3)	99.0 ± 0.4 (n=3)	1.0
C18/saline, fast	93.8 ± 0.4 (n=19)	93.8 ± 0.5 (n=3)	94.8 ± 0.4 (n=3)	1.8
ITLC SG/saline	97.4 ± 0.1 (n=3)	98.6 ± 0.0 (n=3)	97.0 ± 0.3 (n=3)	0.2
SAX/WFI	96.0 ± 1.2 (n=3)	—	—	2.1

SG = silica gel; EtAc = ethylacetate; W17 = Whatman 17; SAX = strong anion exchange.

TABLE 2
Comparison of Analysis Methods

	P value at time after reconstitution								
	W17/EtAc			C18/saline, regular			ITLC SG/saline		
Analysis method	30 min	120 min	360 min	30 min	120 min	360 min	30 min	120 min	360 min
Baker-Flex SG/EtAc	0.56	—	—	0.71	—	—	0.24	—	—
W17/EtAc	—	—	—	0.80	0.24	0.37	0.73	0.45	0.03
C18/saline, regular	—	—	—	—	—	—	0.51	0.20	0.01
C18/saline, fast	—	—	—	<0.001	<0.001	<0.001	—	—	—

W17 = Whatman 17; EtAc = ethyl acetate; SG = silica gel.

criminate ^{99m}Tc-pertechnetate from ^{99m}Tc-EDTA in the ^{99m}Tc-ECD kit. By the strong anion exchange/WFI method, 100.0% ± 0.0% (n = 4) of ^{99m}Tc-pertechnetate was adsorbed onto the column.

DISCUSSION

The quality control system that the manufacturer recommends for assay of ^{99m}Tc-ECD uses a Baker-Flex silica gel stationary phase with ethyl acetate as the mobile phase and takes approximately 20–25 min to complete. In this radiopharmacy, 98% ^{99m}Tc-ECD was detected in kits, a value greater than the 90% pass criterion for patient injection (*1*). The lipophilic complex migrated with the solvent front in ethyl acetate, leaving behind the hydrophilic compounds at the origin. Of the remaining radioactivity (2%), the potential impurities are $^{99m}TcO_2$, ^{99m}Tc-pertechnetate, or from the list of excipients in the kit formulation, ^{99m}Tc-EDTA. Mannitol also comprises the formulation in an excess molar amount (123:21:1 mannitol:ECD:EDTA) however, this compound is highly unlikely to coordinate ^{99m}Tc (*10*) when the higher-affinity ligands ECD and EDTA are present. Even when more EDTA was added to the contents of Neurolite vial A (1:3 molar ratio of ECD:EDTA), 98% of ^{99m}Tc-ECD was obtained after reconstitution, indicating that ECD is the stronger chelating ligand under these conditions.

EDTA in the kit formulation would be expected to chelate any lower oxidation state ^{99m}Tc that does not bind to ECD, thereby minimizing any $^{99m}TcO_2$ formed by a disproportionate side reaction (*11*). Indeed, the $^{99m}TcO_2$ level was found to be 0.3%–0.5% up to 2 h after reconstitution, increasing to 1% in the kit after 6 h. The remaining hydrophilic activity at the origin of the Baker-Flex paper is accounted for by ^{99m}Tc-pertechnetate and ^{99m}Tc-EDTA, the relative proportions of which could not be discriminated using the quality control systems reported here.

TABLE 3
Radiochemical Purity of ^{99m}Tc-EDTA

Analysis method	% ^{99m}Tc-EDTA*	Location
Baker-Flex SG	99.9 ± 0.0 (n=3)	R_f = 0.0
Whatman 17	99.9 ± 0.0 (n=3)	R_f = 0.0
C18	98.2 ± 0.3 (n=3)	Eluent
ITLC SG	99.9 ± 0.0 (n=3)	R_f = 0.9–1.0
Strong anion exchange	93.3 ± 1.0 (n=3)	Column

*At 30 min after reconstitution (mean ± SE).
SG = silica gel.

Replacement of the stationary phase with Whatman 17 paper gave the same RCP for ^{99m}Tc-ECD at 3 different testing occasions (Table 1). In comparison to the manufacturer's method, the Whatman 17/ethyl acetate system has the advantage of a shorter analysis time, taking less than 3 min for completion. Whatman 3MM paper and ethyl acetate have been reported to determine the RCP of ^{99m}Tc-ECD in less than 5 min (*3*). Therefore, using the faster-flowing chromatography paper Whatman 17 provided a time advantage of approximately 2 min.

Like $^{99m}TcO_2$, ^{99m}Tc-EDTA remained at the origin when ethyl acetate was used in conjunction with either of the paper methods however ^{99m}Tc-EDTA did migrate with the solvent front on ITLC/saline. With mannitol present in the formulation, ^{99m}Tc-EDTA was slightly less resolved but the percentage of ^{99m}Tc-ECD by ITLC silica gel/saline was not affected. While ^{99m}Tc-ECD migrated away from the origin in saline to give a distinct separation, a drawback of this method was the longer time required to obtain the final result.

The Amprep strong anion exchange cartridge/WFI system gave 96% ^{99m}Tc-ECD in the eluent, where $^{99m}TcO_2$ (>96%), ^{99m}Tc-pertechnetate and ^{99m}Tc-EDTA (>93%) were each adsorbed onto the column. Initial experiments with this cartridge plus saline resulted in poor retention (~70%) of ^{99m}Tc-pertechnetate by the ion exchange column matrix because of a surplus of competing solvent chloride ions. Although it is not clear why this method slightly underestimates the percentage of ^{99m}Tc-ECD, one possibility is that some of the product may be adsorbed onto the column. Also, a variable level of sodium chloride is added to the kit during reconstitution, and it is uncertain how these chloride ions might alter binding of ^{99m}Tc-EDTA and ^{99m}Tc-pertechnetate to the column matrix during the quality control procedure.

The Amprep C18 cartridge/saline system retained ^{99m}Tc-ECD on the column to the extent of 98%, a value not significantly different to those derived from the manufac-

turer's method, the Whatman 17 method, or the ITLC silica gel/saline method for 2 h after reconstitution. $^{99m}TcO_2$ was adsorbed onto the column, whereas ^{99m}Tc-pertechnetate and ^{99m}Tc-EDTA (>98%) passed into the eluent cleanly. The level of $^{99m}TcO_2$ on the column was found to be low, adding a minor contribution to the final ^{99m}Tc-ECD percentage. The solvent elution rate strongly influenced the final RCP value, where the regular flow rate (~2–3 drops per second) was superior to the fast flow of saline in a steady stream. This result indicates that the physical adsorption of ^{99m}Tc-ECD on the short C18 column material can be desorbed by a fast flow rate (and pressure) provided by the eluting solvent. Even at the regular flow rate, this entire procedure took approximately 2 min to produce a final quality control result, which is particularly advantageous in a busy radiopharmacy. Another benefit of this simple technique is that it incorporates the common, inexpensive hospital consumable 0.9% saline as the solvent, and avoids the special safety conditions required for use of ethyl acetate and other flammable organic solvents.

CONCLUSION

The RCP of ^{99m}Tc-ECD was found to be 98% by the manufacturer-recommended Baker-Flex silica gel/ethyl acetate method, the same as values derived by other analysis methods such as Whatman 17/ethyl acetate, Amprep C18/saline, and ITLC silica gel/saline. The lengthy analysis time of ITLC silica gel/saline was inconvenient, and the strong anion exchange/WFI method underestimated the percentage of ^{99m}Tc-ECD. The simpler, faster and safer Amprep C18/saline system, eluted at a regular flow rate is recommended as the method of choice for routine quality control analyses of ^{99m}Tc-ECD.

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