Commentary

Technetium-99m-Exametazime: Pitfalls in Preparation and Quality Control

The Food and Drug Adminstration recently approved the drug exametazime (Ceretec®* or hexamethylenepropyleneamineoxime) for use in preparation of a ^{99m}Tc-chelate suitable for human use. This radiopharmaceutical is indicated for detection of altered regional cerebral perfusion in patients with stroke.

Unlike the majority of radiopharmaceuticals containing stannous ion (Sn^{2+}), which can be prepared by addition of varying volumes and activities of [^{99m}Tc]pertechnetate, successful preparation of ^{99m}Tc-exametazime requires careful control of both volume and activity. According to package insert instructions (*1*), a maximum activity of 30 mCi in a volume of 5.0 ml should be added aseptically to the shielded vial. The necessity for careful control of the final chemical concentration and radioconcentration is based on the minimal amount of Sn^{2+} ion present, typically <4 μg/vial, an amount that is <1% of that found in most commonly used Sn^{2+} ion kits.

Other commonly used ^{99m}Tc radiopharmaceuticals are used with little concern for the stereochemical form in which the complex exists. (Stereoisomerism refers to the arrangement of atoms in a molecule so that a compound with a given formula may have its atoms arranged differently with regard to spatial location around a central atom, usually carbon, producing stereoisomeric versions of the same molecule. These versions often have different properties, especially concerning localization and retention in the body.) For exametazime, the spatial arrangement of atoms in the molecule is critical: in aqueous solution, exametazime changes fairly rapidly from a more lipophilic form commonly referred to as the "secondary complex" (*1*). Since the more lipophilic species is the desirable one (higher brain uptake and significantly longer retention), we must assay the final preparation to insure that the chelate is in the correct stereoisomeric form.

From a chemical standpoint, smaller reconstitution volumes result in higher Sn^{2+} ion concentrations. These higher Sn^{2+} levels tend to prevent radiolabeling of the appropriate exametazime species and affect the delicate kinetics between the various ^{99m}Tc complexes (*2*). While the mechanism for this has not been elucidated, the presumption is that tin, which is considered a heavy metal, is present at levels high enough to accelerate the conversion of the primary complex to the secondary complex.

In addition, kits with a low Sn^{2+} level are prone to oxidation and require special care on the part of the preparer to prevent air from entering the vial and oxidizing the product. The 30-mCi limit has been set due to the low Sn^{2+} levels present and to competition between ^{99m}Tc and its long-lived daughter, ^{99}Tc ($T_{1/2}$ = 213,000 yr). The more ^{99}Tc present, the more likely the competition will favor formation of the ^{99m}Tc complex and less likely the production of the ^{99m}Tc complex.

To counteract this, kits should not be prepared with the first elution of the generator or with an eluate obtained from a generator which has not been eluted within the previous 24 hr. Under these conditions, one can prevent buildup of quantities of ^{99}Tc large enough to result in poor labeling efficiencies. In conjunction with formation of ^{99}Tc, formation of peroxides on generator columns or in eluates due to the intense radiation field increases as a function of time (*3*). Since these compounds are potent oxidizers and can destroy an exametazime kit within minutes post-reconstitution, manufacturers insist that the generator eluate be no more than 2 hr old at time of reconstitution.

The package insert indicates a 30-min shelf-life post-reconstitution. This time interval was established after determining that conversion from the lipophilic to the secondary complex occurs at a rate that permits reliable production of this radiopharmaceutical within this time interval. According to the package insert, the minimum acceptable value for radiochemical purity of the lipophilic form of exametazime is 80%.

PREPARATION OF EXAMETAZIME

Reconstitution Procedure

As a cost-effective measure, insure that the patient is in the nuclear medicine department before reconstituting the vial. This will prevent discard of unnecessarily prepared material. To a shielded vial of exametazime, aseptically add 5.0 ml of sodium pertechnetate solution containing a maximum of 30 mCi. Withdraw air to equalize pressure in the vial, then shake well and permit the vial to stand at room temperature for two minutes. At that time, quality control (QC) procedures are initiated.

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Pitfalls During Reconstitution

As described above, it is important to use [^{99m}Tc]pertechnetate solution no older than 2 hr, eluted from a generator which has been eluted within the previous 24 hr, and of an appropriate radioconcentration. Of a more subtle nature, vigorous shaking of the vial can affect labeling efficiency, probably by increasing the amount of dissolved oxygen. In addition, if exametazime vials are routinely stored at 4°C, they should be removed from the refrigerator and permitted to reach room temperature before reconstitution. The labeling reaction will reach equilibrium more quickly, helping to prevent chromatography failures.

Since the Sn^{2+} level in the kit is low, using low dissolved oxygen (LDO) saline to adjust the radioconcentration of the eluate will minimize the amount of dissolved oxygen introduced into the vial during the reconstitution procedure, eliminating one potential source of oxidation. From a practical standpoint, it may not be necessary to go to this extra expense unless QC testing consistently detects the presence of excessive quantities of free ^{99m}Tc. Under no circumstances should air be injected into this or any other product containing Sn^{2+} ion. It is assumed that the expiration date of the cold kit is checked prior to reconstitution. Expired material cannot be used clinically and will not produce the same results as fresh material.

OVERVIEW OF QUALITY CONTROL

Unlike the package insert for other ^{99m}Tc products approved for use in the United States, the exametazime insert states that radiochemical purity (RCP) determination must be performed before administration of the drug to the patient (*1*). At first, radiochromatographic QC of ^{99m}Tc-exametazime appears more complex than that of other ^{99m}Tc products for several reasons: (1) different solvents are used than those for other ^{99m}Tc products; (2) strips are cut at different locations than for these other compounds; in addition, these locations are different for each strip; (3) the chromatography must be performed rapidly and accurately since the time window for use of this product does not permit extensive repetition of the chromatography procedure if unacceptable results are obtained; and (4) there are three (not just two) possible radiochemical impurities. These impurities include the following:

1. *Hydrolyzed reduced technetium* (HR Tc), which is colloidal in nature and thought to be a hydrated technetium oxide. This compound localized almost exclusively in the reticuloendothelial system with a typical distribution of 85% in the liver, 10% in the spleen, and 5% in the bone marrow. It tends to degrade images in certain studies and contributes unnecessarily to the internal radiation dose.
2. *Pertechnetate* (free Tc), which either may have disassociated from the desired compound or never have bound completely to it. Free technetium, which is distributed in the gastric mucosa, choroid plexus, parotid glands, and the thyroid, also tends to degrade images and increases the internal radiation dose.
3. *Secondary exametazime complex*, a stereoisomeric form of the desired technetium chelate with reduced brain uptake and a markedly reduced retention time in the brain. Since the primary form is converted to the secondary complex at a rate such that the product must be used within 30 min of reconstitution or be discarded, it is mandatory to test for its presence. This represents the first ^{99m}Tc radiopharmaceutical approved for human use in the United States for which quantitation of a stereoisomeric form is required.

Experience in our laboratory with preparation and QC of ^{99m}Tc-exametazime has involved many different lots of exametazime. In addition, because of our rotation of duties, several different technologists have been responsible for preparing this radiopharmaceutical. Our overall QC results for 75 separate preparations under these variable conditions are presented in Table 1. We find that a radiochemical purity well above the limit set in the package insert is readily achievable. After having performed the preparation and QC of ^{99m}Tc-exametazime over the past several months, technologists report that the restrictions described above have little impact on their routine operations in the radiopharmacy, and the QC procedure is no more difficult or time-consuming than that of other ^{99m}Tc compounds. In practice, one can routinely obtain radiochemical purity of the lipophilic component in the range of 90%–94%. To do so consistently requires careful adherence to the following QC procedures and awareness of the possible pitfalls.

TABLE 1. Typical Quality Control Data for Preparations of ^{99m}Tc-Exametazime (N = 75)

Component	Radiochemical purity (%)
Lipophilic	91.8 ± 2.1
Free Tc*	0.84 ± 0.53
HR Tc†	2.83 ± 2.31

* Free Tc = unbound [^{99m}Tc]pertechnetate.

† HR Tc = hydrolyzed, reduced ^{99m}Tc (+4).

QUALITY CONTROL PROCEDURE

Preparation

Before vial reconstitution, have available three 10-ml sterile, empty elution vials, two Gelman ITLC-SG strips†, 6 cm × 0.7 cm (black strips) and one Whatman #31ET paper strip,‡, 6 cm × 0.7 cm (red strips). Prepare the three vials by adding

TABLE 2. Parameters for Thin-Layer Radiochromatography of ^{99m}Tc-Exametazime*

Strip/solvent	Location to cut	Typical elution time
Black strip/MEK	1 cm above origin	45 sec
Black strip/Saline	2.5 cm above origin	45 sec
Red strip/50% ACN	0.5 cm above origin	100 sec

* 0.7 cm × 6 cm strips are used. Black strip = ITLC-SG; red strip = Whatman #31ET.

0.9% saline solution, methylethylketone (MEK), and 50% acetonitrile (ACN), respectively. This last solvent mixture is prepared by mixing equal volumes of HPLC Grade ACN and Water for Injection, USP. Reconstitute the vial of exametazime as previously described.

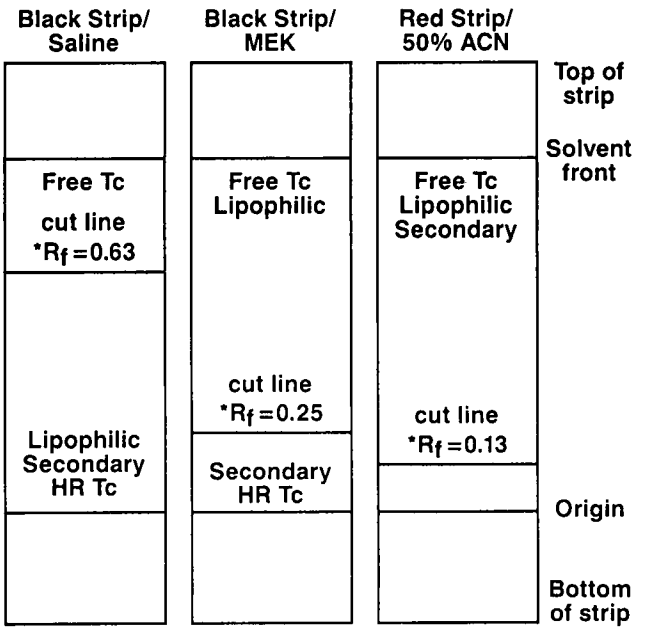
Chromatography

After a 2-min incubation period post-reconstitution, remove a small volume of ^{99m}Tc-exametazime using a Tuberculin syringe equipped with a 25- or 27-g needle and apply 1–2 small drops of the radiopharmaceutical to the origin of each of the three strips. Immediately place one ITLC-SG strip (black strip) in the 0.9% saline solution, the second ITLC-SG strip in MEK, and the paper strip (red strip) in the 50% ACN. Visually monitor each strip. When the solvent front has been reached, remove the strip from the vial using tweezers and immediately cut the strip as outlined in Table 2.

Each strip section should be assayed to determine distribution of activity on the strip. Proper geometry must be maintained to minimize the effects of instrument dead time. Preferred methods of counting include a NaI(Tl) well counter or a gamma camera. Alternate methods include radiochromatogram strip scanners to obtain a histogram of radioactivity distribution or the use of an uptake probe as a radiation detection device. Dose calibrators are not recommended due to the significant fluctuation of readings on the μCi scale and the resultant large inherent error in RCP determination.

Percent-free technetium is represented by the percent of total activity on the top portion of the strip eluted in saline solution. Hydrolyzed reduced technetium (HR Tc) is represented by the percent of total activity on the bottom portion of the strip eluted in 50% ACN. The amount of lipophilic complex present is represented by the percent on the bottom of the saline strip—the percent on the bottom of the MEK strip. The diagrams in Figure 1 indicate the migration patterns for each component and help to explain the calculations described above.

The lipophilic component must represent at least 80% of total activity to be acceptable for injection into patients. Any value lower than 80% requires that the QC procedure be repeated immediately. A second failure indicates a problem with either the product (less likely) or the chromatography procedure (more likely). This failure could result from not only procedural errors or counting errors, but also defective



*R_f refers to the fractional distance traveled between origin and solvent front.

FIG. 1. Radiochromatographic migration patterns of the radiochemical species present in ^{99m}Tc-exametazime.

TABLE 3. Common Pitfalls in Quality Control Procedures for ^{99m}Tc-exametazime

Description of Problem	Result
Strip spotted with drop larger in diameter than one-half the width of the strip or with drop too close to edge of strip	Inaccurate measurement of RCP. Spot new strip.
Spot permitted to dry before elution of strip	Oxidation of product yielding artificially low RCP. Discard strip; spot fresh one.
Age of solvent, *especially MEK*. Buy fresh at least annually; use only HPLC Grade	Inconsistent, inaccurate RCP results.
Age/condition of chromatography strips	Strips tend to absorb moisture; this affects the migration patterns, especially on silica gel, and can lead to inconsistent, inaccurate results.
Handling strips with fingers	Fingerprints can affect migration patterns, leading to inaccurate or inconsistent results.
Strip is eluted past marked solvent front line	If cut line is not changed to maintain same R_f value, erroneous results may be obtained.
Strips are counted *in* NaI(Tl) well counter instead of *above* it in a fixed geometry	Dead time of crystal may be excessive since 20–30 μCi are typically spotted; if so, "hot" strips will appear to have an artificially lower count rate than actually present on strip, in effect reducing the RCP and causing test failure.
Strips are counted in dose calibrator	Readings tend to fluctuate in the μCi range; variations of only 1–5 μCi can lead to large errors in determining RCP.
Red and black strips reversed or placed in incorrect solvents	Results will be inaccurate; repeat entire QC procedure.
After strip is spotted and placed in solvent, spot is submerged or strip becomes wet and sticks to wall of vial	Results will be inaccurate; spot another strip.

strips or solvents tabulated in Table 3 are common sources of error observed in performing radiochemical QC procedures for exametazime as well as other radiopharmaceuticals and the effect of each error on the radiochemical purity of the final product.

DISCUSSION AND CONCLUSIONS

Introduction of ^{99m}Tc-exametazime has resulted in the first required radiochromatographic QC procedures for a ^{99m}Tc radiopharmaceutical. This determination of radiochemical purity is warranted due to the delicate kinetics established between stereoisomeric forms of the ^{99m}Tc complex. The fairly rapid formation of the secondary complex from the primary (lipophilic) complex necessitates testing to insure that only minimal quantities of this secondary complex are present prior to patient administration.

Although the chromatographic systems are different than those routinely used for other ^{99m}Tc complexes, the spotting, elution, cutting, and counting procedures are essentially identical. Preparing special pre-marked strips prevents errors associated with cutting strips in the incorrect location. Experience in our laboratory indicates that technologists familiar with radiochromatography readily adapt to these procedures and have no unusual difficulties in performing them.

From a practical standpoint, the restrictions associated with preparation and use of ^{99m}Tc-exametazime essentially have posed no problems. By ensuring that the patient is in the department prior to reconstitution of the vial, discard of unused, expired material can be essentially eliminated. The chromatography rarely must be repeated, so that the 30-min time requirement has not been a problem. Departments dependent upon a centralized radiopharmacy for their supply of [^{99m}Tc]pertechnetate should specify when ^{99m}Tc-exametazime is scheduled to be prepared, so only the freshest eluate will be shipped to the department.

It is theoretically possible that eluate collected in a "wet column" generator might produce poorer labeling efficiencies with this product thant those obtained using eluate from a "dry column" generator. If this proves to be the case, these decreased labeling efficiencies are probably attributable to either peroxide or free-radical formation on the column.

In summary, by carefully following the manufacturer's recommendations described in the package insert and avoiding the pitfalls outlined above, successful preparation and QC of exemetazime can be as routine as that for any other ^{99m}Tc radiopharmaceutical.

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NOTES

*Ceretec ®, Amersham Corp., Arlington Heights, IL
†Gelman ITLC-SG strips, Atomic Products Corp., Shirley, NY
‡Whatman paper strip, Atomic Products Corp., Shirley, NY

REFERENCES

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