Radiopharmaceutical Details: $^{13}$N-NH$_3$

1. Name of Radiopharmaceutical (active ingredient)

$^{13}$N-Ammonia

2. Indicate the chemical structure for low molecular weight drug molecules
   (not required for antibodies, proteins, or polymeric agents)

$\text{NH}_3$

4. State specific details of techniques to analyze and quantify the compound
   (ex: spectrophotometer – make, settings, sample dilutions, etc.)

$^{13}$N-Ammonia is produced from $^{13}$N-nitrates using the GE PETtrace system. As only trace amounts of ammonia are produced, and it is a naturally occurring blood component no routine quantification is performed. Radiochemical purity, radiochemical identity, pH, radionuclidic identity, visual inspection, filter membrane integrity testing and pyrogen testing will be performed on each batch of $^{13}$N-ammonia prior to release. Chromatography (HPLC) will be performed using a Luna 5 SCX ion exchange column (Phenomenex, Torrance, CA; 4.6 x 50 mm) and elution with 40 mM potassium nitrate, pH 3.1 at 0.5 mL/min. The production process includes the use of Devarda’s alloy which contains aluminum. The aluminum content of representative batches of $^{13}$N-Ammonia was determined by the independent testing laboratory EMSL Analytical Inc. using inductively coupled plasma mass spectrometry. These batches were found to comply with the limits of aluminium set forth by the USP monograph for $^{13}$N-Ammonia. Representative batches of $^{13}$N-Ammonia will be submitted to EMSL for aluminum testing at six-month intervals.

4b. Indicate the minimum detectable mass of the drug by HPLC analysis.

The mass of ammonia in the dose is not routinely determined.

5. Radioisotopes

Nitrogen-13

6. Method of assaying radioisotope activity prior to administration
   (ex: Capintec ion chamber, gamma counter, or liquid scintillation counter; include details of make, setting, type of standard, etc.)

The amount of radioactivity in each unit dose is assayed prior to administration using a calibrated Capintec 712M dose calibrator dialed into a setting of 457. A daily constancy test is performed using an NIST traceable Cs-137 source. Accuracy, linearity and geometry testing is performed as required by the NRC or equipment manufacturer.
7. Radionuclidic Purity
(in %)

>98%

8. Significant radionuclidic impurities and means of assay

Nitrogen-13 is produced by the $^{16}\text{O}(p,\alpha)^{13}\text{N}$ nuclear reaction from $^{16}\text{O}$-water. The bombardment produces N-13 as the major radionuclide, but also oxygen-15 by the $^{16}\text{O}(p,pn)^{15}\text{O}$ reaction. The N-13 ammonia is distilled from the reaction vessel to the formulation vessel during the production process. This distillation effectively separates most of the $^{13}\text{N}$-Ammonia from $^{15}\text{O}$-water. This is verified by radiometric HPLC mentioned in section 4. Trace amounts of $^{18}\text{F}$fluoride ion are also produced from the 0.2% naturally occurring $^{16}\text{O}$-water by the $^{18}\text{O}(p,n)^{18}\text{F}$ nuclear reaction. These traces of fluoride remain in the reaction vessel after the distillation of $^{13}\text{N}$-Ammonia. A half-life determination will be performed for each batch of Ammonia and shall be between 9.5-10.5min.

9. Radiochemical Purity
(in %)

>95%

10. Significant radiochemical impurities and means of assay
(ex: Chromatographic techniques and procedure for analyzing radiochromatogram).

$^{15}\text{O}$-water is the only significant potential radiochemical impurity. Chromatography (HPLC) using a Luna 5 SCX ion exchange column (Phenomenex, Torrance, CA; 4.6 x 50 mm) and elution with 40 mM potassium nitrate, pH 3.1 at 0.5 mL/min. This system separates the impurities mentioned in section 8 from the desired $^{13}\text{N}$-ammonia which are detected using a Bioscan flow-through NaI radioactivity detector. $^{15}\text{O}$-water is typically <2% of the total radiochemical purity of the final product.

11. Provide evidence that the tracer will be stable over period of storage prior to administration.
(Give details of storage conditions and on-going quality assurance procedures for sterility, pyrogenicity, and radiochemical purity.)

The 10 minute physical half-life of N-13 requires $^{13}\text{N}$-ammonia to be administered within 20 minutes (maximum) of synthesis. The tracer will be stored at room temperature prior to patient administration. Routine quality control testing has verified that the product remains radiochemically stable for 30min post synthesis. Formal stability data has not been collected.
14. Provide detailed information on how the pharmaceutical quality of the radioactive drug will be assured at the time of administration.

Include the following:

- **pH**
- **Sterility**
- **Apyrogenicity**
- **Identity (chemical and radiochemical purity)**
- **Concentration**

a. **pH**

A small amount of each batch of the final drug products will be spotted on pH paper (range 2-9). The pH will be in the physiological range (4.5-7.5) and will be consistent from batch to batch.

b. **Sterility**

The product is delivered in a sterile multi-dose vial. No addition of liquid or aliquotting to another storage container is permitted. Individual doses are removed from this vial using aseptic techniques and only by trained staff or nuclear medicine technicians.

During preclinical studies the final drug product was produced utilizing established synthesis procedures [See appended Master Formula Card]. When tested as described below the prepared batches were sterile. During clinical studies the radioactive drug product will be produced utilizing these established procedures and on a synthesis apparatus which will be appropriately maintained. Sterility tests will be routinely performed on batches of the drug product in an ex post facto manner utilizing residual samples.

An aliquot of the final drug products will be inoculated into each of the appropriate sterility test media and incubated according to USP recommendations:

i. Fluid Thioglycollate Media (BBL, Division of Becton-Dickinson Co., Cockeysville, MD): 14 days at 30-35° C.

ii. Soybean Casein Broth (BBL, Division of Becton-Dickinson Co., Cockeysville, MD): 14 days at 20-25° C.

Positive growth is indicated by cloudiness in the culture media. Results will be compared to positive and negative controls. The efficacy of a 0.22 µm membrane filter for terminal sterilization [See appended Master Formula Card] warrants release of the drug products for patient administration prior to results of sterility testing as stipulated by current USP.

c. **Apyrogenicity**

An aliquot of the final drug product will be tested for the presence of bacterial endotoxin utilizing a Limulus Amebocyte Lysate (LAL) Test. The test is performed using a Charles River Endosafe-PTS Portable Testing System which is based on a kinetic chromogenic BET. The established USP endotoxin limit is 175 EU per dose for radiopharmaceuticals and is not exceeded.
d. Identity (chemical and radiochemical purity)

Radiochemical purity will be determined by HPLC analysis as described above. The chemical purity of the dose will not be determined routinely.

e. Concentration

i. Mass Concentration (mass/volume)

Not applicable.

ii. Activity Concentration (activity/volume)

5-25 mCi/mL

iii. Specific Activity (activity per mass of drug)

Not applicable.

For questions or concerns, contact:

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