Development, Validation and Regulatory Acceptance of Improved Purification and Simplified Quality Control of [^{13}N] Ammonia

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Methodology

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Abstract

Background: [13 N]Ammonia is a widely used cyclotron produced myocardial perfusion imaging agent. With the development of high-yielding [13 N]Ammonia cyclotron targets using a solution of 5mM ethanol in water, there was a need to develop and validate an automated purification and formulation system for [13 N]Ammonia to be in a physiological compatible formulation of 0.9% sodium chloride since there is no widely available commercial system at this time. Due to its short half-life of 10 minutes, FDA and USP regulations allow [13 N]Ammonia to be tested in quality control (QC) sub-batches with limited quality control testing performed on the sub-batches for patient use. The current EP and the original USP method for the determination of the radiochemical purity and identity of [13 N]Ammonia depended on an HPLC method using a conductivity detector and a solvent free of other salts. This HPLC method created issues in a modern cGMP high volume PET manufacturing facility where the HPLC is used with salt containing mobile phase buffers for quality control analysis of other PET radiopharmaceuticals. Flushing of the HPLC system of residual salt buffers which may interfere with the [13 N]Ammonia assay can take several hours of instrument time. Since there are no mass limits on [13 N]Ammonia, a simplified TLC assay to determine radiochemical identity and purity could be developed to simplify and streamline QC.

Results: We have developed and validated a streamlined automated synthesis for [13 N]Ammonia which provides the drug product in 8mL of 0.9% sodium chloride for injection. A novel radio-TLC method was developed and validated to demonstrate feasibility to quantitate [13 N]Ammonia and separate it from all known radiochemical impurities.

Conclusions: The process for automated synthesis of [13 N]Ammonia simplifies and automates the purification and formulation of [13 N]Ammonia in a cGMP compliant manner needed for high-throughput manufacture of [13 N]Ammonia. The novel radio-TLC method has simplified [13 N]Ammonia quality control (QC) and now enables it to be tested using the same QC equipment as F-18 Fludeoxyglucose. Both the streamlined automated synthesis of [13 N]Ammonia and the novel radio-TLC method have been accepted and approved by the US Food and Drug Administration (FDA) for the cGMP manufacture of [13 N]Ammonia.

Background

[^{13}N]Ammonia is a myocardial perfusion imaging agent, which is approved by US Food and Drug Administration (FDA) for diagnostic Positron Emission Tomography (PET) imaging of the myocardium under rest or pharmacologic stress conditions to evaluate myocardial perfusion in patients with suspected or existing coronary artery disease.[1] In the United States, the only other FDA approved alternative PET myocardial perfusion agent is [^{82}Rb]-chloride, which requires a generator system by the patient's side due to the short half-life. With advances in cyclotron capabilities and targetry associated with in-target [^{13}N]Ammonia production, in excess of 37 GBq (1 Ci) of [^{13}N]Ammonia can be produced per batch which makes it feasible with the 10-min half-life to inject and image more than one patient per batch and even transport it short distances from the cyclotron. Due to the expanding infrastructure

globally of cyclotron and PET cameras, there has been rapidly increasing interest in [^{13}N]Ammonia for PET myocardial perfusion imaging outside of the US.[2]

The [^{13}N]Ammonia in-target production method produces [^{13}N]Ammonia in water with trace 5 mM ethanol.[3] This formulation vehicle while acceptable, is less than ideal than a physiological compatible solution like 0.9% sodium chloride. Additionally, the [^{13}N]Ammonia in water may contain trace long lived radionuclidic impurities from the target body and/or target windows depending on the cyclotron target design. Most of the existing methods described for purification and formulation of [^{13}N]Ammonia are either manual loading and elution of solid phase extraction cartridges (SPEs) or complicated dedicated [^{13}N]Ammonia systems, both of which are challenging to validate in a cGMP manufacturing environment.[4,5,6] We set out to design a simple method which could be adapted and validated on several different commercial available platforms, including cassette based systems for easy scale up for high-volume [^{13}N]Ammonia production at a busy PET center.

The original United States Pharmacopeia (USP)[7] and European Pharmacopeia (EP)[8] methods for radiochemical purity and identity determination of [^{13}N]Ammonia currently require the use of an HPLC system configured with a conductivity detector. The current compendial EP and the original USP HPLC conductivity methods are resource intensive, requiring lengthy mobile phase preparation and system suitability determination, while occupying valuable instrument time. Additionally, these detectors are only required for anion or cation chromatography, which is an expensive investment for a PET center to use only for [^{13}N]Ammonia, [^{18}F]Sodium Fluoride and other investigational cation/anion radiopharmaceuticals. Also, HPLC conductivity methods can have a higher incidence of system suitability failures in comparison with other HPLC detection methods, such as UV. System suitability failures can lead to extensive delays in the busy production schedule of PET radiopharmaceuticals.

In this paper, we describe the development, validation and regulatory acceptance by FDA and USP of an ideal alternative method would rapidly and reproducibly determine the radiochemical purity and radiochemical identity of [^{13}N]Ammonia, while requiring less time to complete and fewer resources to maintain than compendial methods. A TLC method for determining radiochemical purity and identity eliminates the need to use HPLC for [^{13}N]Ammonia quality control. This is highly desirable in a PET production facility that produces multiple radiopharmaceuticals per day.

Materials And Methods

Chemicals and Reagents

All chemicals and reagents were obtained from commercial vendors and used without further purification.

Automated Purification and Formulation of [^{13}N]Ammonia

[^{13}N]Ammonia is produced on a modified GE Tracerlab FXFDG synthesis module which was replumbed for the purification and formulation of [^{13}N]Ammonia. The graphic user interface is shown in Figure 1 and the purification and formulation steps are further detailed below.

1. [^{13}N]Ammonia is produced on-site with a GE PETrace cyclotron using the Wieland et al method [1] of irradiation of 5mM ethanol in water in-target synthesis method using a GE niobium body target with HAVAR/Niobium double target window or GE silver body target with HAVAR window (GE Medical Systems, Uppsala, Sweden).
2. [^{13}N]Ammonia is transferred from the cyclotron target via helium overpressure to the automated synthesis unit and through an in-line anion exchange column (Waters, QMA Chloride) to remove any anionic impurities, such as [^{18}F]Fluoride.
3. Using vacuum, the [^{13}N]Ammonia in water is trapped on a cation exchange column (Waters, Accell CM) to quantitatively trap [^{13}N]Ammonia.
4. The [^{13}N]Ammonia is released from the cation exchange column using 8mL of 0.9% Sodium Chloride for Injection, USP.
5. The formulated [^{13}N]Ammonia in 0.9% Sodium Chloride for Injection is then transferred through a 1/16” PFA line via nitrogen overpressure to a ISO Class 5 isolator for sterile filtration through a vented 0.22µ polyethersulfone (PES) membrane filter (B Braun) into a vented 30mL sterile empty vial (ALK OKC Allergy Labs, Hollister Stier or Huayi Isotopes).

The total time of the purification, formulation and sterile filtration of the [^{13}N]Ammonia takes approximately 5 minutes.

Post-formulation in between sub-batches, the fluid pathways in the system unit and the transfer line are washed with sterile for injection, USP and blown to dryness with nitrogen gas.

Quality Control of [^{13}N]Ammonia

The quality control of [^{13}N]Ammonia was performed to ensure the PET drug product met the specifications in Table 1 to satisfy FDA and USP regulatory requirements. Due to the production of [^{13}N]Ammonia via in-target 5mM ethanol solution, the EP/USP test for residual aluminum was not required. The TLC method was validated against the major potential radiochemical impurities, [^{13}N]-NOx and [^{18}F]-Fluoride as detailed in Table 2 and cross-validated against the compendial HPLC method.

A thin layer chromatographic system was developed that uses a Diethylaminoethyl cellulose (DEAE-C) stationary phase (Figure 2). The DEAE-C stationary phase of the chromatography system was chosen due to its ability to attract anionic species. The DEAE-C strip (J.T. Baker) is 1.5 cm × 8 cm and the mobile phase is composed of methanol: water 75:25. The Rf of [^{13}N]Ammonia is 0.7 – 0.9 and the major impurities of [^{13}N]NOx and [^{18}F]Fluorine are retained at the origin (Rf = 0). Originally, the radiochemical identity was confirmed with 100mg/mL Ammonium Chloride USP reference standard which was

visualized with a combination of spray the TLC strip with iodoplatinate reagent, which was allowed to develop for 10 min followed by placement in an iodine chamber to help highlight the ammonium chloride spot. The ammonium chloride spot appears as an orange brown spot against a light maroon background.

The radio-TLC method has since been simplified with the use of resazurin (Millipore Sigma), a visible dye which is used as a marker of system suitability eliminating the need to use ammonium chloride and the complicated development process of iodoplatinate reagent and iodine chamber to visualize the standard. Additionally, the visible dye aids the operator by providing a visual pink-purple streak indicating proper TLC development. The other components of the TLC assay remain the same as described above. The Rf of resazurin is 0.43 – 0.63. Table 2 below contains the validation data of the [^{13}N]Ammonia TLC assay using resazurin as a system suitability marker.

Results

[^{13}N]Ammonia was synthesized as described above. A summary of the 2018-2019 annual validation stability studies is detailed in Table 1 with the FDA approved [^{13}N]Ammonia product specifications and the average results for the five batches. All of the batches met the FDA/USP product specifications. The validation of the radio-TLC method with resazurin as a system suitability indicator for a valid test is detailed in Table 2. We demonstrated that [^{13}N]Ammonia can be adequately separated from the known impurities, [^{18}F]Fluoride and [^{13}N]nitrous oxide (NOx). The two major impurities are retained on the radio-TLC DEAE-C TLC strip at the origin while [^{13}N]Ammonia migrates to the solvent front. Table 2 also contains the Rf data on the resazurin dye to validate it's use as marker for system suitability replacing the ammonium chloride reference standard.

Discussion

In this paper, we described the development of a novel automated purification and formulation system for [^{13}N]Ammonia as well as the development of a novel radio-TLC method for the determination of radiochemical purity of [^{13}N]Ammonia. An automated purification and formulation simplifies the operation of [^{13}N]Ammonia produced via the in-target production method and eliminates the need for operators to turn stopcocks or valves to purify and isolate [^{13}N]Ammonia for formulation. The purification and formulation method described can be adapted with little to no modifications for use on a variety of synthesis platforms, including cassette-based systems such as the Ora Neptis, Trasis All-In-One, and GE FASTlab and MX systems. The method for cassette can be further modified to have a number of purification cartridges on the cassette to one cassette could be used for multiple runs without opening the hot cell.

The radio-TLC method whose development is described in this publication allows for [^{13}N]Ammonia to be simplified and eliminates the need to have ion chromatography HPLC. With the adoption of the novel

TLC method, a PET manufacturing site can perform [^{13}N]Ammonia quality control using the same equipment required for [^{18}F]Fludeoxyglucose. The method has been accepted and adopted by the USP as the new standard for radiochemical identity and purity of [^{13}N]Ammonia with its publication in USP/NF 42-37 in 2019.[9] We describe how the radio-TLC method has been further simplified and improved with the replacement of the ammonium chloride standard with the visible dye resazurin. The use the visible resazurin dye streamlines the radio-TLC test method as it now allows the QC operator to see at time of strip development if the TLC test is valid, saving valuable time with a short-lived isotope. Additionally, it eliminates the need to use iodoplatinate spray reagent and an iodine vapor chamber to visualize the ammonium chloride spot which can be cumbersome for an operator to perform reproducibly.

Conclusion

Both the automated purification and formulation method for [^{13}N]Ammonia produced via the in-target production method and the novel radio-TLC radiochemical purity test method have been accepted by the US FDA. Additionally, the radio-TLC radiochemical purity method has been adopted by the USP and has replaced the radio-HPLC method which was difficult and time consuming to perform. Both methods have been thoroughly validated and are ready to support the wider use of [^{13}N]Ammonia globally for cardiac PET applications.

List Of Abbreviations

cGMP – current good manufacturing practices; DEAE-C - diethylaminoethyl cellulose; EP- European Pharmacopeia; FDA – United States Food and Drug Administration; FDG – F-18 Fludeoxyglucose; HAVAR - UNS R30005, alloy of cobalt; NOx – nitrous oxide; PES – polyethersulfone; PET – Position Emission Tomography; PFA – perfluoroalkoxy fluoropolymer plastic; QC – quality control; Rf – retention factor; TLC – thin layer chromatography; USP – United States Pharmacopeia

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: All data generated or analyzed during this study are included in this published article

Competing interests: Authors DY, PR and Massachusetts General Hospital are patent holders of the purification method described in this publication for [^{13}N]Ammonia.

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Authors' contributions: DY is responsible for the overall design of the experiments, analysis and preparation of the manuscript. PR was responsible for experiment execution, co-developer of purification method and initial radio-TLC method development with DY, as well as for experimental data review. RN was responsible for the development of the improved radio-TLC method as well as experimental execution. GEF was responsible for overall conduct of the experiments as well review and editing of the manuscript.

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Tables

Table 1: Ammonia N 13 Injection 2018-2019 Annual Stability Testing Summary Table		
Quality Control Specification	**Acceptance Criteria**	**Average Result***
Activity at End of Synthesis	1.11 – 76.96 GBq @ EOS 30 to 2080 mCi @EOS	10.18 ± 0.22 GBq 572 ± 60 mCi
Product Volume	8mL ± 20%	7.61 ± 0.2mL
pH Determination	4.5 – 7.5	5
Visual Inspection	Clear, colorless solution. Absent of foreign matter. Product vial is intact.	Pass
Radionuclidic Identity	Principal photopeaks are found at 0.511 MeV, 1.02 MeV and Compton scatter	Pass
Half-life Determination (minutes)	The measured half-life is between 9.5 – 10.5 minutes	9.99 ± 0.1 minutes
Radiochemical Identity	Rf of resazurin = 0.43 – 0.63	0.5 ± 0.05
Radiochemical Purity	NLT 95.0% Ammonia N 13 via TLC	98.61 ± 0.4 %
Residual Solvent Assay	Ethanol NMT 3.1 mg/mL	< 3.1mg/mL
Sterile Filter Integrity	≥ manufacturer specification of 46 psi	>46 psi
Bacterial Endotoxin Testing (EU/mL)	NMT 10.9 EU/mL	<5 EU/mL
Sterility	Sterile	Sterile
Long Lived Radionuclidic Purity	<0.5% at time of expiry	<0.001% (less than lower limit of detection)

*Average of 5 validation stability runs

Table 2 – [^{13}N]Ammonia TLC Method Validation Results						
Ammonia Standard Plus Reszaurin	NH_3 Peak Start (mm)	NH_3 Peak End (mm)	NH_3 Rf Value	Resazurin Peak Start (mm)	Resazurin Peak End (mm)	Resazurin Rf Value
NH_3Cl Standard + Resazurin Mean of 3 TLC Strips	45	57	0.81	25	47	0.67
Ammonia N 13	% N 13 NH3 integrated	Peak Start (mm)	Peak End (mm)	Peak centroid (mm)	Rf Value	Resazurin Standard Range (mm)
Ammonia N 13 – TLC Strip #1	99	20.3	81.8	50.8	0.846	0 - 40
Ammonia N 13 – TLC Strip #2	98.96	17.7	79.2	51.7	0.862	0 - 40
Ammonia N 13 – TLC Strip #3	99.06	17.7	80.1	51.2	0.853	0 - 45
Mean	***99.01%***				***0.854***	
Fluoride F 18	% F 18 fluoride integrated	Peak Start (mm)	Peak End (mm)	Peak centroid (mm)	Rf Value	Resazurin Standard Range (mm)
Fluoride F 18 – TLC Strip #1	99.5	-17.3	30.5	7.1	0.119	0 – 41
Fluoride F 18 – TLC Strip #2	99.78	-16.5	25.4	4.3	0.072	0 - 44
Fluoride F 18 – TLC Strip #3	99.77	-15.6	30.5	5.7	0.095	0 - 44
Mean	***99.68***				***0.095***	
NOx N 13	% N 13 NOx integrated	Peak Start (mm)	Peak End (mm)	Peak centroid (mm)	Rf Value	Resazurin Range (mm)
NOx N 13 – TLC Strip #1	98.45	-15.6	30.5	4.1	0.068	0 - 34
Mean	***98.45***				***0.068***	

Figures

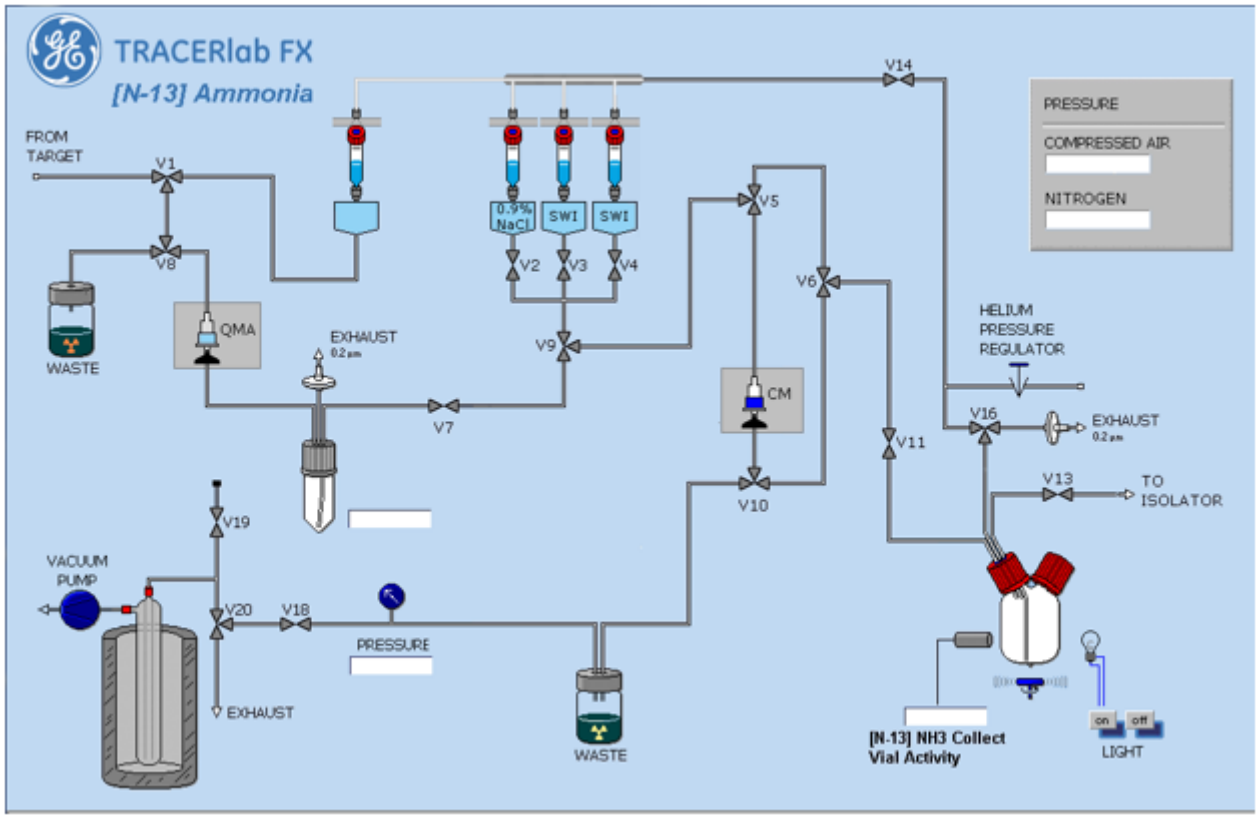

Figure 1

N-13 Ammonia Purification and Formulation Module Graphic User Interface

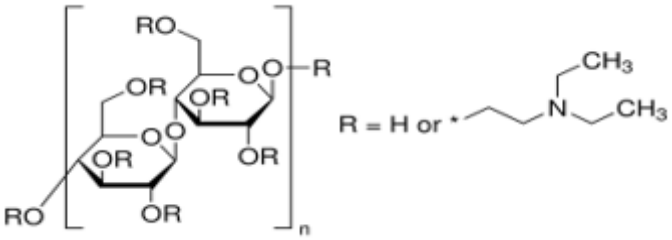

Figure 2

Diethylaminoethyl cellulose (DEAE-C)