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Automated production of [^{18}F]VAT suitable for clinical PET study of vesicular acetylcholine transporter

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Abstract

Automated production of a promising radiopharmaceutical (-)-(1-(8-(2-[^{18}F]fluoroethoxy)-3-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-piperidin-4-yl)(4-fluorophenyl)methanone ([^{18}F]VAT) for vesicular acetylcholine transporter (VACHT) was achieved using a two-step procedure in a current good manufacturing practices fashion. The production of [^{18}F]VAT was accomplished in approximately 140 min, with radiochemical yield of ~15.0% (decay corrected), specific activity > 111 GBq/ μmol , radiochemical purity > 99% and mass of VAT ~3.4 μg /batch ($n > 10$). The radiopharmaceutical product meets all quality control criteria for human use, and is suitable for clinical PET studies of VACHT.

Keywords

Fluorine-18; Vesicular acetylcholine transporter; Automation; Positron emission tomography; Clinical studies

1. Introduction

Loss of brain cholinergic neurons in central nervous system (CNS) disorders such as Alzheimer's disease (AD) represents one of the most typical neuropathological features of dementia (Coyle et al., 1983; Davies and Maloney, 1976; Gidal et al., 1996; Scarpini et al., 2003). The vesicular acetylcholine transporter (VACHT) located in presynaptic cholinergic nerve terminals (Gilmor et al., 1996) is a reliable biomarker for assessing the loss of cholinergic neurons, which correlates to the cognitive dysfunction in dementia (Giboureau et al., 2010; Marien et al., 1987; Roghani et al., 1994). Depletion in VACHT levels has been associated with the onset and severity of dementia progression and VACHT has been a target for quantitative visualization of early neurodegenerative diseases to measure the decline of cholinergic neurons (Efange, 2000; Giboureau et al., 2010; Gilmor et al., 1999).

Positron emission tomography (PET) as a well-established imaging method that offers a unique sensitive and noninvasive way to quantitatively assess neurotransmitter receptors and

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transporters *in vivo* (Meyer et al., 2014). Among the positron emitting radioisotopes, ^{18}F has a favorable half-life (109.8 min) that enables multiple-step radiosynthesis, convenience in clinical imaging procedures, as well as distribution to satellite PET centers.

Since the first structural modification of *trans*-2-(4-phenylpiperidino)cyclohexanol (vesamicol), a lipophilic aminoalcohol that has a unique binding site for VACHT on cholinergic synaptic vesicles, (Rogers et al., 1989), progress has been made in the development of small molecular compounds that bind to this key cholinergic marker with high potency (Kawamura et al., 2006; Kilbourn et al., 2009; Mach et al., 1997; Petrou et al., 2014; Tu et al., 2009). One of the promising fluorinated analogues, (-)-(1-(8-(2- ^{18}F fluoroethoxy)-3-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-piperidin-4-yl)(4-fluorophenyl)methanone (^{18}F VAT), was successfully radiolabeled with ^{18}F by a two-step procedure (Scheme 1). ^{18}F VAT demonstrates favorable tracer kinetics in both rodents and non-human primates (Tu et al., 2015), and radiation dosimetry measurements in primates show that ^{18}F VAT has favorable dosimetry (Karimi et al., 2015). To facilitate clinical measurement of the relationship between cholinergic depletion and the severity of dementia in patients with neurodegenerative diseases, a reliable method for production of ^{18}F VAT that meets regulatory requirements for human administration is needed. We report here the automated production of ^{18}F VAT in a current Good Manufacturing Practice (cGMP) facility that meets all regulatory requirements for clinical use.

2. Materials and methods

2.1 General

Unless otherwise noted, all the chemicals and solvents were purchased from Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Hanover Park, IL) and used as received. The optical pure ^{18}F VAT precursor (1-((-)-3,8-dihydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)(4-fluorophenyl)methanone was made by our lab from the commercially available 1-naphthol (Sigma-Aldrich) and 4-(4-fluorobenzoyl)piperidine (Asta Tech, Inc.) in three steps, then the racemic precursor was resolved by chiral semi-preparative column (Chiralcel OD, 250 $\times$ 10 mm) according to our published protocol (Tu et al., 2015). The nonradiolabeled VAT standard compound was synthesized by the reaction of the optical pure precursor with 2-fluoroethyl 4-methylbenzenesulfonate (Ark Pharm, Inc.) (Tu et al., 2015).

No carrier added ^{18}F fluoride was produced by ^{18}O (p, n) ^{18}F reaction through proton irradiation of enriched ^{18}O water (95%) using a RDS111 cyclotron (Siemens/CTI Molecular Imaging, Knoxville, TN). ^{18}F Fluoride is first trapped on an ion-exchange resin and then is eluted with 0.02 M potassium carbonate (K_2CO_3) solution.

Pre-conditioned Accell plus QMA cartridge, Sep-Pak C18 cartridge, Sep-Pak Alumina N plus light cartridge were procured from Waters. Sep-Pak sodium sulfate cartridge was obtained from Waters. 0.9% sodium chloride was purchased from APP Pharmaceuticals. Sterile Millex-GV filter (0.22 μm $\times$ 13 mm) was purchase from EMD Millipore. USP absolute ethanol was purchased from Pharmaco-AAPER. Sterile 10 mL vial was procured from Hospira. Water was purified by a MilliQ integral water purification system.

Semi-preparative high-pressure liquid chromatography (HPLC, Agilent Zorbax SB-C18 column, 5 μ m, 9.4 $\times$ 250 mm) was conducted by equipment integrated into the Eckert & Ziegler Modular-Lab and TRACERlab™ FX-N Pro. Analytical HPLC were performed using Dionex U3000 system, which includes a Dionex LPG-3400A quaternary gradient pump with degasser, an autosampler, a variable wavelength detector, and a Bioscan radioactivity-HPLC-flow detector. An Agilent Zorbax SB-C18 column (5 μ m, 4.6 $\times$ 250 mm) equipped with a Grace Alltima C18 guard column (5 μ m, 7.5 $\times$ 4.6 mm) was used for quality control. Gas chromatography (GC) was performed using an Agilent 6850 GC system. Analytical HPLC and GC data were acquired and analyzed by a Dionex Chromeleon® software. The identity of labeled compounds was authenticated by injection with the reference standard.

2.2 Hardware and software of the modules

[¹⁸F]VAT was synthesized by a two-step strategy, the first step included the radiosynthesis of 2-[¹⁸F]fluoroethyl tosylate using the Eckert and Ziegler Modular-Lab (Figure 1). The alkylation of [¹⁸F]VAT precursor with 2-[¹⁸F]fluoroethyl tosylate was conducted using a GE TRACERlab FX-N Pro module. Both modules were configured according to [¹⁸F]VAT synthesis sequence program. The Eckert & Ziegler Modular-Lab includes one Peltier reactor module that allows variable temperature control, a magnetic stirrer, internal radioactivity detector, temperature sensors, a pneumatic reactor lift, a reactor camera, two-way and three-way valve modules, one vacuum pump, one HPLC module, one isocratic HPLC pump and UV detector.

The radiosynthesis of 2-[¹⁸F]fluoroethyl tosylate was conducted in a 5 mL V-shaped vessel equipped with a reactor head. A pre-conditioned Accell plus QMA cartridge to trap [¹⁸F]fluoride was installed to the line between the K₂CO₃ vial and the reactor. Activity trapped on the QMA cartridge was transferred to the reaction vessel under vacuum. The reagents loaded in other vials were transferred to the reactor via a positive nitrogen flow. All the transfers were controlled by programmed computer procedures. The aqueous [¹⁸F]fluoride was azeotropically dried by addition of acetonitrile under vacuum with a constant flow of nitrogen monitored by a flow control module. The drying cycle was repeated two times to make sure the [¹⁸F]fluoride was dry enough to subject to the substitution reaction. Upon completion of the reaction, the reaction was quenched with the HPLC mobile phase and loaded to the HPLC loop via an alumina cartridge. The HPLC system includes a semi-preparative Agilent C-18 column, a six-position valve, a sample loop, a fluid sensor, a radioactive detector, a multi-channel wavelength UV detector, an isocratic HPLC pump.

The eluted ethereal solution of 2-[¹⁸F]fluoroethyl tosylate was transferred to a GE TRACERlab FX-N Pro module (GE Healthcare, Waukesha, WI, USA) to complete the second radiolabeling step. The module is equipped a reaction vessel, temperature sensors, a pneumatic reactor lift, a reactor camera, a pump, a UV detector, radioactivity counter, and automated data acquisition. In both automation modules, all processes were remotely controlled by a computer utilizing the dedicated modular-lab software interface. The interface facility flexible programming and GMP-compliant batch records. The systems

were cleaned by a programmed procedure upon completion of each batch synthesis until decay safe (> 10 half-lives of the isotopes).

2.3 Radiosynthesis

11–19 GBq [^{18}F]fluoride in 0.2–2.5 mL bolus of [^{18}O]H $_2\text{O}$ was trapped on an Accell plus QMA cartridge (pre-conditioned with 10 mL H $_2\text{O}$). [^{18}F]fluoride was eluted into the reaction vial using aqueous potassium carbonate/kryptofix 2.2.2 (1.2 mg in 0.1 mL water/0.9 mL acetonitrile). The [^{18}F]fluoride was dried by evaporating the water-acetonitrile azeotrope at 105 °C using consistent nitrogen flow applied for 5 min. During this drying step, acetonitrile (3×0.5 mL) were added at intervals. Then the reaction vessel was cooled to 25 °C and subjected to both nitrogen stream and a vacuum draw simultaneously for another 5 min. The [^{18}F]fluoride drying cycle took about 20 min. A solution of ethylene glycol ditosylate (8–10 mg) in acetonitrile (900 μL) was added to dried [^{18}F]fluoride. The reaction was heated to 110 °C for 10 min with stirring. Subsequently the reaction temperature was set to 40 °C and a solution of mobile phase (3 mL of 50% acetonitrile in 50% 0.1 M ammonium formate, v/v, pH 6.5) was transferred to dilute the mixture. The solution was passed through an activated alumina Sep-Pak with the pneumatic reactor lift to the HPLC injection vial. The solution was loaded onto HPLC loop for purification, the loading procedure was monitored by a fluid sensor. The semi-preparative HPLC module includes a semi-preparative column (Agilent Zorbax SB-C18 column, 5 μm , 9.4×250 mm), a UV detector (wavelength at 254 nm), and a radiation detector. The mobile phase (50% acetonitrile in 50% 0.1 M ammonium formate, v/v, pH 6.5) at a flow rate at 5 mL/min was used to purify 2-[^{18}F]fluoroethyl tosylate. The fraction with retention time at 8–10 min was collected into a vented 100 mL vial prefilled with sterile water (50 mL). The diluted fraction was passed through a C-18 Sep-Pak. Upon completion a consistent nitrogen flow was applied to the Sep-Pak for another 5 min to remove residue water. The product trapped on the Sep-Pak was eluted into a sterile vial with 3 mL ether by passing through two stacked sodium sulfate Sep-Pak. After the dose measurement, the activity was transferred to the GE TRACERlab FX-N Pro synthesizer for the second step radiolabeling.

Before starting the automation program, the [^{18}F]VAT precursor (1.0–1.5 mg) was dissolved in DMSO and loaded to vial 3. Cesium carbonate (2 – 3 mg Cs $_2\text{CO}_3$) was added directly to the reaction vessel. Two portions of mobile phase (38% acetonitrile buffered in 0.1 M ammonium formate, pH 6.5) for reaction mixture dilution, each 2 mL was loaded to vial 4 and 5 respectively. Sterile water for washing the C-18 Sep-Pak was loaded to vial 9. EtOH for eluting out the product was load into vial 8 and saline for final formulation was loaded into vial 7. 50 mL sterile water was placed into the dilution flask. A C-18 Sep-Pak was connected between the dilution flask and the product collect vial through two three-way valves. A sterile vial with sterile Millex-GV filter (0.22×13 mm) was connected to the product collect vial for dose delivery and quality control (QC) analysis (Figure 1). Due to two phases was formed in the ether solution of the first step, a needle was placed to the interface of the two phases and made sure only exclusive organic phase was transferred to the reaction vessel. The camera in the hot cell was manually adjusted to acquire better vision. The door was locked and the automation synthesis was controlled by a remote computer. The 2-[^{18}F]fluoroethyl tosylate solution in ether was transferred out by vacuum

into the reaction vessel, subsequently nitrogen flow was applied to the solution to evaporate the ether at room temperature. The evaporation step took about 5 min. [^{18}F]VAT precursor in DMSO was added to the dried 2- ^{18}F fluoroethyl tosylate residue. The mixture was heated at 100 °C for 10 min with stirring. The reaction temperature was cooled down to 40 °C and 2 mL mobile phase was added to dilute the mixture, the resulting solution was transferred out to an injection vial by the pneumatic reactor lift. The process was repeated once with the second part of mobile phase. The combined solution in the injection vial was loaded onto HPLC loop monitored by the liquid sensor. Under the optimized HPLC conditions (Agilent Zorbax SB-C18 column, 5 μm , 9.4 $\times$ 250 mm, isocratic mobile phase 38% acetonitrile in 0.1 M ammonium formate, pH 6.5, flow rate 4.0 mL/min, detection wavelength at 254 nm), the fraction for [^{18}F]VAT with the retention time at 28–31 min was collected into the dilution flask with stirring. The diluted fraction was passed through a C-18 Sep-Pak to trap [^{18}F]VAT. The whole operation was monitored by a camera to ensure complete transfer. The Sep-Pak was rinsed with water (10 mL) loaded in vial 9. [^{18}F]VAT was eluted out to the product collect vial with ethanol (1.0 mL), and formulated with saline (10 mL) loaded in vial 7. The formulation containing 0.9% EtOH was aseptically filtered into a 30 mL sterile glass vial. The dose was ready for delivery and QC analysis.

2.4 Quality control

Quality control of [^{18}F]VAT produced in three consecutive production batches was performed to assure that the product met acceptance specifications set for clinical use of the radiopharmaceutical. Conventional methodology as described in the United States Pharmacopoeia was employed, and included visual inspection, testing for filter integrity, pH, radionuclidic identity, apyrogenicity and sterility. HPLC methods were utilized to determine radiochemical purity, chemical purity and radiochemical identity, and gas chromatographic methods were employed for analysis of residual solvents. Residual Krytox 2.2.2 was determined using a color spot test (Mock et al., 1997).

3. Results and Discussion

3.1 Radiosynthesis and formulation

A two-step automatic procedure was used for producing [^{18}F]VAT in our GMP facility for clinical investigation (Scheme 1). The first step was the radiosynthesis 2- ^{18}F fluoroethyl tosylate using the Eckert and Ziegler Modular-Lab. [^{18}F]fluoride was produced in the cyclotron facility of Washington University in St. Louis by an $^{18}\text{O}(\text{p}, \text{n})^{18}\text{F}$ reaction. The fluoride was delivered in a [^{18}O]H₂O bolus of the synthesis module and trapped on a Accell plus QMA Sep-Pak to remove most [^{18}O]H₂O. The trapped [^{18}F]fluoride was then eluted into the reaction vessel by using aqueous potassium carbonate (1.2 mg in 0.1 mL of water/0.9 mL acetonitrile). The trapping and releasing procedures are very typical in automatic radiosynthesis and usually result in minor [^{18}F]fluoride loss although may decrease a little bit of specific activity (Gao et al., 2010). The [^{18}F]fluoride was azeotropically dried by applying heat at 105 °C and vacuum at the same time, which greatly shortened the drying step. A second portion of acetonitrile was added to the vial and the drying step was repeated, which would guarantee the [^{18}F]fluoride free of water to subject next substitution reaction. The drying step was monitored by a camera equipped inside the

reaction vessel and remotely controlled by a computer. The drying step typically took 20 min. Subsequently a solution of ethylene glycol ditosylate (8–10 mg) in acetonitrile (900 μ L) was added to dried [18 F]fluoride. The reaction was heated at 110 $^{\circ}$ C for 10 min in the sealed reaction vessel. Upon completion of the substitution step, reaction was cooled to 40 $^{\circ}$ C and 2 mL of mobile phase was added to dilute the mixture. The solution was passed through an alumina Sep-Pak, which was connected between the reaction vessel and injection vial to retain major free [18 F]fluoride. The solution was transferred into an injection vial by a pneumatic reactor lift prior to loading it to the HPLC column for purification. The reaction vessel was rinsed with a second portion of mobile phase and transferred to the HPLC injection loop. The product was chromatographically purified using a stationary phase consisting of an Agilent Zorbax SB-C18 column (5 μ m; 9.4 $\times$ 250 mm) and a mobile phase of 50% acetonitrile in 0.1 M ammonium formate (pH 6.5) v/v, flow rate 5.0 mL/min. The retention time for 2-[18 F]fluoroethyl tosylate was 8–10 min while the precursor was 18–21 min (Figure 2).

The desired fraction was diluted with 50 mL sterile water and passed through a C-18 Sep-Pak with pressurized nitrogen. The activity trapped on the Sep-Pak was eluted off with ether through two stacked sodium sulfate Sep-Pak cartridges to remove residual water. The yield of 2-[18 F]fluoroethyl tosylate was measured, and the labeling intermediate transferred to the GE TRACERlab FX-N Pro module for the next step in the reaction sequence. The first step was highly reproducible, and took 50 min including the [18 F]fluoride drying step to give a radiochemical yield was $80 \pm 5\%$ (decayed-corrected). In this step, it was observed that trace amount of water in 2-[18 F]fluoroethyl tosylate production did have a significant impact on the yield of [18 F]VAT. However, using two stacked Sep-Pak drying system is more reliable for efficiently drying the ether phase and the yield of [18 F]VAT is more consistent. Compared to acetonitrile or ethanol, using ether to elute the product of 2-[18 F]fluoroethyl tosylate has two advantages: (1) the lower boiling point of ether makes it easy to be removed from the collection vial; (2) ether does not like acetonitrile/ethanol that is a water-miscible solvent, water is able to be removed easily from ether than either acetonitrile or ethanol.

Before the ether solution was transferred to the reaction vial in the GE synthesizer, the needle used for the transfer was inserted into the interface of the solution of two phases formed in the first ether elution step. The ether was blown off with gentle nitrogen flow at room temperature. Heat or vigorous nitrogen flow applied to the ether solution will result significant loss of 2-[18 F]fluoroethyl tosylate due to its volatility. The reaction of VAT precursor with 2-[18 F]fluoroethyl tosylate was conducted in DMSO at 100 $^{\circ}$ C. Upon completion of the reaction, two portions of mobile phase (each 2 mL) were added to the reaction vial and moved to the transfer vial.

The combined fractions were loaded onto an HPLC loop (5 mL) for HPLC separation. To our delight, when a pH 6.5 solution (38% acetonitrile in 0.1 M ammonium formate, v/v) was employed, peak shape was improved significantly and the collected volume was greatly decreased. With a stationary phase consisting of an Agilent Zorbax SB-C18 column and mobile phase 38% acetonitrile / 62% 0.1 M ammonium formate (pH 6.5) (v/v) at 4 mL/min, [18 F]VAT eluted at 28 - 31 min, cleanly resolved from a side product eluting at 26 -27 min (Figure 3).

The [^{18}F]VAT fraction from the HPLC was collected to a dilution flask filled with 50 mL sterile water and the activity was trapped on a C-18 Sep-Pak. The Sep-Pak was rinsed with 10 mL of water, and [^{18}F]VAT was eluted with 1.0 mL USP ethanol into a product vial, and reformulated with 10 mL of 0.9% Sodium Chloride Injection, USP. The radiopharmaceutical product vial was sterilely terminally filtered (sterile Millex-GV filter, $0.22\ \mu\text{m} \times 13\ \text{mm}$) into a sterile empty vial. The total synthesis time for the two-step radiolabeling sequence was $140 \pm 10\ \text{min}$, with an average radiochemical yield of $\sim 15.0\%$ and specific activity $111\ \text{GBq}/\mu\text{mol}$ (decay-corrected to end of synthesis). It is no doubt that the procedure of producing [^{18}F]VAT could be further optimized if needed. In the process of establishing the protocol, we had tried one step radiosynthesis of [^{18}F]VAT via corresponding tosylate/fluoride exchange reaction. However, the reaction yield was not as good as the currently reported procedure, particularly when starting tosylate precursor that was not fresh made. It may result from the competition between the tosylate/fluoride exchange and the decomposition of the tosylate precursor under the reaction conditions. Similarly, lower radiochemistry yield was observed in our lab for producing the F-18 radiopharmaceuticals using some tosylate precursors that were not fresh made. Considering the reproducibility of producing [^{18}F]VAT and the reliability of this procedure, we decide to use this two-step procedure to deliver the dose of [^{18}F]VAT for human studies at currently e-IND clinical phase 0 investigation. So far, our GMP facility staffs are able to deliver the dose for clinical investigations without failure.

3.2 QC of the radiopharmaceutical

For IND application, three consecutive validation syntheses were completed to qualify our synthetic method for clinical production of [^{18}F]VAT. As shown in Table 1, the formulated [^{18}F]VAT met all quality specifications set for clinical release of the radiopharmaceutical. All batches for three consecutive validation runs were clear, particle-free and colorless. The filter membrane integrity test met the manufacturer's pressure rating. The radionuclidic identity as assayed by the half-life measurement was $109.0 \pm 2.0\ \text{min}$, which was within the release criteria (105–115 min). The product pH determined via pH test strips was 5.5, and the residual level of kryptofix 2.2.2 determined by a color spot test was lower than $50\ \mu\text{g}/\text{mL}$. Residual solvents for DMSO, acetonitrile, ethanol, ether were determined by GC using standard calibration curves, and all were below the limit stated in the USP recommendations.

Analytical HPLC confirmed the chemical and radiochemical purity (60% acetonitrile in 0.1 M ammonium formate, v/v, pH 6.5, 1.5 mL/min). The radioactive product was authenticated by injection of the nonradioactive VAT standard as shown in Figure 4, and the retention time difference was less than 10%. Radiochemical purity was over 99% for all batches. No change in radiochemical or chemical purity was observed for up to 4 hours at controlled room temperature. The average mass in the final [^{18}F]VAT product was $\sim 3.4\ \mu\text{g}$ ($n > 10$).

Biological testing demonstrated that the bacterial endotoxins measured by USP chromogenic methods are all below the regulatory maximum of (175 endotoxin units). Testing of the three batches of [^{18}F]VAT using USP sterility testing methods demonstrated that the radiopharmaceutical product prepared using our methodology is sterile.

4. Conclusions

We report a robust, reliable automated production method for production of the promising VACHT tracer [^{18}F]VAT. A two-step synthetic strategy was adapted for use in our cGMP facilities. Validation studies of [^{18}F]VAT prepared in this manner were completed with high radiochemical yield, good reproducibility and high product quality to qualify the methodology for clinical production of the radiopharmaceutical. [^{18}F]VAT was achieved in approximate 140 min with an average yield of ~15% (decay corrected, radiochemical purity >99%, chemical purity >90%, specific activity > 111 GBq/ μmol , and average VAT mass ~3.4 μg /batch. We have instituted this automated production system for the preparation of [^{18}F]VAT for ongoing clinical PET investigation cerebral cholinergic activity in healthy and diseased human subjects.

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HIGHLIGHTS

- Automated production of a novel PET tracer [^{18}F]VAT for VACHT was established using a two-step procedure.
- [^{18}F]VAT were completed with high radiochemical yield, good reproducibility and high product quality.
- The radiopharmaceutical product of [^{18}F]VAT is suitable for clinical PET studies.

Figure 1.
 Schematic diagrams of the Eckert&Ziegler Modular-Lab (top panel) and GE TRACERlab FX-N Pro Module (bottom panel) for [^{18}F]VAT.

Figure 2.

Chromatograms of representative semi-preparative HPLC purification of 2-[^{18}F]fluoroethyl tosylate. Agilent Zorbax SB-C18 column, 50% acetonitrile in 50% 0.1 M ammonium formate, v/v, pH 6.5, 5 mL/min. Panel A: radiochemical trace and panel B: UV trace at 254 nm.

Figure 3. Chromatograms of representative semi-preparative HPLC purification of [^{18}F]VAT. Agilent Zorbax SB-C18 column, 38% acetonitrile in 62% 0.1 M ammonium formate, v/v, pH 6.5, 4 mL/min. Panel A: radiochemical trace and panel B: UV trace at 254 nm.

Figure 4.

Representative analytical HPLC trace of VAT reference and formulated $[^{18}\text{F}]\text{VAT}$. Panel A: VAT standard reference; Panel B shows UV trace for control run using 10% EtOH in saline; panel C: $[^{18}\text{F}]\text{VAT}$ UV trace at 254 nm; panel D: radiochemical trace.

Scheme 1.

Radiosynthesis of $[^{18}\text{F}]\text{VAT}$ using a two-step procedure.

Table 1

Product release specifications and validation test results (n = 3).

Quality control test	Description	Release criteria	Results
Particulates and color	Visual inspection for color and particulates	Clear, particle-free and colorless	Pass
Filter integrity	Bubble point test	Meet manufacturer's requirement	Pass
pH	Using pH strip test	4.5 – 7.5	5.5
Residue kryptofix 2.2.2 test	Color spot test	< 50 µg/mL	Pass
Radionuclidic identity	Half-life determination	105 – 115 min	109.0 min
Radiochemical purity	Determined by HPLC	90%	> 99%
Radiochemical identity	Determined by HPLC	Retention time of [¹⁸ F]VAT and injected VAT must be within ± 10%	Pass
Residual solvent: DMSO	Determined by GC	5 mg/mL (50 mg/day max)	< 0.34 g/mL
Residual solvent: acetonitrile	Determined by GC	0.41 mg/mL (4.1 mg/day max)	0.05 mg/mL
Residual solvent: ethanol	Determined by GC	100 mg/mL	64 mg/mL
Residual solvent: ether	Determined by GC	5 mg/mL (50 mg/day max)	< 0.11 mg/mL
Bacterial endotoxin	Lysate by gel clot	175 EU/dose	< 53.5 EU
Sterility test*	USP sterility test	No visible microorganism growth	Sterile

* Not a release criteria.