Published in final edited form as:

Appl Radiat Isot. 2017 December ; 130: 230–237. doi:10.1016/j.apradiso.2017.10.002.

Characterization of the radiosynthesis and purification of [¹⁸F]THK-5351, a PET ligand for neurofibrillary tau

Tobey J Betthausen^{*,a,b}, Paul A Ellison^a, Dhanabalan Murali^a, Patrick J Lao^{a,b}, Todd E Barnhart^a, Shozo Furumoto^c, Nobuyuki Okamura^d, Sterling C Johnson^{e,f,g}, Jonathan W Engle^a, Robert J Nickles^a, and Bradley T Christian^{a,b}

^aDepartment of Medical Physics, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA

^bWaisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA

^cDivision of Radiopharmaceutical Neuroimaging, Tohoku University, Sendai, Japan

^dDivision of Pharmacology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, Sendai, Japan

^eDepartment of Medicine, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA

^fGeriatric Research Education and Clinical Center, William S. Middleton Veterans Hospital, Madison, WI, USA

^gWisconsin Alzheimer's Disease Research Center, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA

Abstract

This work characterizes the radiochemical synthesis, purification, and formulation of [¹⁸F]THK-5351, a tau PET radioligand, and develops an automated radiosynthesis routine (ELIXYS, Sofie Biosciences). Nucleophilic radiofluorination reaction was complete by 7 minutes at 110°C with radiochemical yields proportional to precursor mass (0.1–0.5 mg). Optimized HPLC purification produced radiotracer product with no chemical impurities observed on analytical HPLC in formulation. Automated radiosynthesis (ELIXYS), HPLC purification and formulation was completed in 86 minutes producing formulated product suitable for human research use.

*Corresponding author: Tobey J Betthausen, Department of Medical Physics, University of Wisconsin - Madison Waisman Brain Imaging and Behavior, 1500 Highland Avenue, Rm T229, Madison, WI 53705, Tel: +1 (608)-890-2959; Fax: +1 (608)-262-9440, tbetthausen@wisc.edu.

Disclosure

Nobuyuki Okamura and Shozo Furumoto received a grant from GE Healthcare to study tau PET imaging. THK compounds are licensed to GE Healthcare. The remaining authors have no disclosures.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Alzheimer's disease; Positron emission tomography; THK; Radiochemistry; Formulation; Automation; USP; Radiochemical stability

INTRODUCTION

Neurodegenerative diseases, including Alzheimer's disease, have become a focal point of international research due to an increasingly aging population. Many neurodegenerative diseases are characterized by the aggregation of proteins including beta-amyloid plaques and hyperphosphorylated tau tangles (Alzheimer's disease). In relation to Alzheimer's disease, advances in beta-amyloid, and more recently tau positron emission tomography (PET) imaging have stimulated large-scale cross-sectional and longitudinal studies with a focus on *in vivo* characterization of protein aggregates throughout disease progression (Chiotis et al., 2017; Doré et al., 2015; Gordon et al., 2016; Ishiki et al., 2015; Jack et al., 2016; Johnson et al., 2015). [^{18}F](S)-6-(3-Fluoro-2-hydroxypropoxy)-2-(N-methyl-2-aminopyridin-5-yl)quinoline ([^{18}F]THK-5351) is a PET radiotracer developed by Tohoku University to bind to neurofibrillary tau aggregates. While extensive characterization of THK-5351 pharmacokinetics, biodistribution and specific, non-specific and off-target binding in humans remains an active research field (Betthausen et al., 2016; Harada et al., 2016a; Harada et al., 2016b; Ishiki et al., 2016; Ng et al., 2017), there is limited work available on the characterization and optimization of its radiochemical production.

The goal of this work is to characterize the radiosynthesis, optimize the purification and final drug formulation of the tau PET radioligand [^{18}F]THK-5351, and develop an automated synthesis procedure using the ELIXYS radiochemical synthesizer (Sofie Biosciences, Culver City, CA). Chemical synthesis of THK 2-arylquinoline compounds, including [^{18}F]THK-5351, has been reported in detail elsewhere (Harada et al., 2016b; Okamura et al., 2013; Tago et al., 2016). Briefly, [^{18}F]THK-5351 is synthesized through nucleophilic radiofluorination of the 2-tetrahydropyranyl-(OTHP-) protected tosylate precursor (S)-2-(2-methylaminopyrid-5-yl)-6-[[2-(tetrahydro-2H-pyran-2-yloxy)-3-tosyloxy]propoxy]quinoline (THK-5352), followed by acid hydrolysis and neutralization (Figure 1). The crude product is purified using a combination of solid phase extraction(s) (SPE) and high-pressure liquid chromatography (HPLC) prior to final drug formulation. Previously reported methods provide adequate radiochemical yields and molar activity, but require 3 mg of THK-5352 (Harada et al., 2016b; Neelamegam et al., 2017; Okamura et al., 2013). In this report, we show that reasonable radiochemical yields can be achieved with 0.5 mg of precursor. In addition, we describe the optimization of HPLC purification and intermediate SPE methodology leading to dramatic reduction (non-observable) of observable chemical impurities that are otherwise present in final product. Lastly, we demonstrate chemical and radiochemical stability of [^{18}F]THK-5351 formulated in a 10% ethanol/saline solution is dependent on the head gas in the final product vial, which, to our knowledge, has not been reported elsewhere.

EXPERIMENTAL

Materials

THK-5351 standard and precursor were synthesized and supplied by Tohoku University (Tago et al., 2016). Bulk precursor was dissolved in anhydrous DMSO (Thermo Scientific), aliquoted, and stored at ~5°C. USP grade sterile water for injection (Baxter, SWI), sterile 0.9% sodium chloride for injection (Hospira, saline), and dehydrated ethanol (Decon Labs Inc.) were obtained from the University of Wisconsin (UW) hospital, or through UW purchasing services, and used without further purification. All other chemicals were purchased from Acros Organics, Fisher Scientific, Sigma-Aldrich, and used without further purification. Sep-Pak Accell Plus QMA Plus Light cartridges (Waters Corporation) were preconditioned with 1 mL of 1 M potassium bicarbonate followed by a 10 mL rinse with deionized (DI) water. The tC18 Sep-Pak Plus Light (Waters Corporation, tC18 light) and tC18 Sep-Pak Plus Short (Waters Corporation, tC18 short) cartridges were preconditioned with 5 mL of USP ethanol and rinsed with 10 mL of DI water (intermediate tC18 light) or SWI (formulation tC18 short) prior to use. Semi-preparative and analytical HPLC systems consisted of Waters 515 HPLC pump, Waters 2489 HPLC UV detector set at 360 nm absorbance (Tago et al., 2016), and in-line radioactivity detection (105-S, Carroll & Ramsey Associates). Semi-preparative HPLC purification used either an Inertsil ODS-4 (5µm, 10 × 250 mm, GL Sciences) or a Luna C18(2) (10µm, 10 × 250 mm, 100Å, Phenomenex) column. Analytical HPLC used an Inertsil ODS-4 column (5µm, 4.6 × 150 mm, GL Sciences) with 30:70 acetonitrile:20 mM NaH₂PO₄ mobile phase at a flow rate of 1.5 mL/min.

Radiosynthesis of crude [¹⁸F]THK-5351

Proton irradiation of [¹⁸O]water (Huayi Isotopes, 98% enriched) was performed with the University of Wisconsin PETtrace cyclotron (GE Healthcare). Following irradiation with an integrated current of 4 – 25 µA·hr, 7 – 40 GBq of [¹⁸F]fluoride in water was transferred to an ELIXYS automated radiosynthesizer where it was trapped on a preconditioned QMA cartridge. The [¹⁸F]fluoride was eluted with 700 µL 80/20 anhydrous acetonitrile/water with 5.6 mg 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix® 222, K₂₂₂) and 1.7 mg potassium carbonate, and rinsed with 700 µL of anhydrous acetonitrile. The QMA eluate was azeotropically dried at 110°C under vacuum and argon flow, with two subsequent additions of 1.5 mL anhydrous acetonitrile. Nucleophilic radiofluorination was carried out by adding THK-5352 precursor (0.1–3.0 mg) dissolved in DMSO and heating to 110°C for 10 minutes. After cooling, hydrolysis of the O-THP protecting group was performed (200 µL 2N HCl, 110°C for 3 minutes), followed by cooling and neutralization (200 µL of 2 M potassium acetate (KOAc)).

¹⁸F nucleophilic substitution reaction rate determination

Radiofluorination rates were determined by autoradiography-visualized thin layer chromatography (radioTLC) for varying masses of precursor (0.1 – 0.5 mg). Following azeotropic distillation of [¹⁸F]KF solution, the residue was dissolved in 1 mL of DMSO. The 300 µL volume of [¹⁸F]KF/K₂₂₂/CO₃ DMSO solution (K₂₂₂ and K₂CO₃ masses adjusted to match automated concentrations) was added to each of three vials containing precursor

dissolved in 200 μ L DMSO. Solutions were heated to 110°C for 20 minutes and periodically sampled using capillary tubes. [18 F]KF/K₂₂₂/CO₃ DMSO standard and samples were spotted on silica TLC plates, developed in 80/20 dichloromethane/methanol and imaged by storage phosphor autoradiography (Perkin Elmer). The percentage of reacted [18 F]fluoride was determined at each time point by calculating the percentage of activity within the lane that did not correspond to [18 F]fluoride ($R_f = 0$). As an additional measure of radiochemical yield, each reacted product was hydrolyzed (200 μ L 2 N HCl, 3 minutes at 110°C), neutralized (200 μ L 2M KOAc) and SPE was performed (tC18 light, 6 mL DI water dilution, 6 mL DI water rinse). Decay corrected radiochemical yields were determined by dividing the radioactivity trapped on the tC18 by the total activity in the reaction vessel after subtracting residual radioactivity. Radiochemical yield was also determined post-HPLC purification using the optimized method below.

tC18 Sep-Pak Light ethanol elution profile

The tC18 light ethanol elution profile was determined by stepwise elution of THK-5351 standard and [18 F]THK-5351 in separate experiments. Either 6.5 μ g (20 nmol) of THK-5351 standard (in 200 μ L DMSO) or crude [18 F]THK-5351 reaction product underwent solid phase extraction (tC18 light, 7 mL DI water dilution, 7 mL DI water rinse) with stepwise ethanol elution in 100 μ L fractions. THK-5351 mass was determined for non-radioactive fractions (analytical HPLC) and the percentage of initial THK-5351 was calculated. For [18 F]THK-5351, trapped crude product and eluted fractions were radioassayed using a dose calibrator (CRC-15R, Capintec Inc.) and the percentage of trapped crude product was determined for each fraction after correction for radioactive decay.

Semi-preparative HPLC purification

Six semi-preparative HPLC conditions were investigated for purification of [18 F]THK-5351. One liter batches of mobile phase consisting of 18–25% (v/v) acetonitrile in 20 mM monobasic sodium phosphate buffer were created (18, 19, 20, and 22 % acetonitrile for Luna column, and 22 and 25 % acetonitrile for Inertsil ODS-4). To remove any residual precursor or other late eluting intermediates, HPLC columns were rinsed with 60/40 acetonitrile/DI water and equilibrated with ~100 mL of mobile phase. Prior to HPLC injection, crude product underwent SPE (tC18 Light, 4mL DI water dilution, 6 mL DI water rinse, eluted with 700 μ L ethanol) and eluate was diluted in 1 mL SWI. For each condition tested, 1/6th of the post-SPE product was injected onto the semi-preparative HPLC system. Mass peaks observed near [18 F]THK-5351, and early and late halves of the [18 F]THK-5351 product peak were collected in separate fractions, and were subsequently characterized by analytical HPLC.

Automated Radiosynthesis using the Sofie ELIXYS

Radiosynthesis of 18 F labeled compounds using the ELIXYS and a detailed report of the device features can be found in literature (Lazari et al., 2014; Lazari et al., 2013). The automated sequence described below is available to ELIXYS users on the Sofie Probes Network, a resource hosted by Sofie Biosciences for the dissemination of automated radiosynthesis sequences. Seven automated productions (2 $\times$ 0.2 mg precursor, 3 $\times$ 0.5 mg precursor, 2 $\times$ 3.0 mg precursor) were carried out using the ELIXYS. The automated

synthesis routine developed for the ELIXYS uses two of the three available cassettes and two reactors. Cassette one was loaded according to figure 2, and reactor two was prefilled with 0.5 mL DI water. The following cassette routing was applied: SPE collection output of cassette one was attached to external addition line on cassette two, and the cassette two transfer tube was directly attached to the HPLC injector loading line. In an effort to minimize injecting air using the automated injector, the HPLC loading path, including the transfer tube, was preloaded with mobile phase and a check valve was added to the HPLC loop waste outlet to prevent the preloaded volume from leaking back into the system prior to post-synthesis HPLC loading. Due to residual losses of ~150 µL using automated additions, reagent volumes were increased to account for the loss of volume with the exception of the precursor dilution volume, which was fixed at 500 µL to avoid dilution during reactions. The following steps were carried out using the built-in unit operations:

1. [¹⁸F]Fluoride was transferred from a sealed v-vial, trapped on a QMA cartridge using the positive pressure line on the ELIXYS, and bulk [¹⁸O]water was recovered.
2. The QMA cartridge was eluted into reactor one with K₂₂₂, K₂CO₃ solution (vial 12) and subsequently rinsed with anhydrous acetonitrile (vial 11)
3. Evaporation was carried out under vacuum and argon flush for 4.5 min at 110°C, after which two subsequent evaporations were performed to visual dryness following anhydrous acetonitrile additions (vials 1 and 2, path 1).
4. Precursor was added (vial 3, path 1) to reactor one and the reactor was heated to 110°C for 10 minutes in reaction position 1.
5. 2N HCl was added (vial 4, path 2), and the reactor was heated to 110°C for 3 minutes in reaction position 2.
6. 2M KOAc was added (vial 5, path 2) to reactor one, followed by two additions of DI water (vials 6 and 7, path 2).
7. Diluted crude product was trapped on a tC18 using the SPE path on cassette one using the trap unit operation.
8. DI water (vials 8 and 9, path 2) was added to reactor one, and passed through the tC18 light using the trap unit operation to rinse the cartridge.
9. Ethanol was added to reactor 1 (vial 10, path 2) and used to elute the tC18 light cartridge into reactor two with stirring on.
10. The diluted and mixed eluate was loaded onto the HPLC loop using the automated unit operation and injected onto semi-preparative HPLC.

Hardware and software were developed in our lab and used to perform HPLC data acquisition and fraction collection, post-HPLC purification SPE, and final formulation of [¹⁸F]THK-5351. HPLC purification of crude ELIXYS product was carried out (19/81 acetonitrile/20 mM monobasic sodium phosphate, 10 mL/min) using a new Luna column with a guard column (AJ0-7221 SecurityGuard™, Phenomenex) installed on the inlet. The HPLC fraction corresponding to [¹⁸F]THK-5351 was collected into a pressure bottle

prefilled with 45 mL SWI. SPE was performed using a tC18 short cartridge (15 mL SWI rinse). The tC18 short cartridge was eluted with 1 mL ethanol, USP and flushed with 9 mL saline, USP, which were passed through an in-line sterile filter (SLFG025LS, EMD Millipore Corporation, Darmstadt, Germany) into a 30 mL sterile empty vial (Hospira, Lake Forest, IL) vented with a 20G sterile venting needle (International Medical Industries, Pompano Beach, FL). System losses were characterized for two of the seven productions, and full characterization of the final drug product was performed for four of the seven productions according to USP chapter <823>.

Radiochemical Stability

During the production runs, breakdown of [^{18}F]THK-5351 was observed for activity concentrations 340 MBq/mL beginning around two hours post end-of-synthesis. However, this had not been observed in our previous experience with formulating [^{18}F]THK-5351 manually. After investigating differences in the manual and automated formulation routines, we hypothesized that differences in the formulation vial head gas contributed to the radiochemical stability. To test this, the last two production runs (6 and 7) were formulated using the automated apparatus with USP air in place of ultra high purity argon as a push gas, which had been used in the previous automated runs. Half of the volume (5 mL) was removed from the formulation vial, transferred aseptically into a separate vented 30 mL sterile empty vial, and purged with ultra high purity argon for 1 minute. Sample of 200 μL were taken from both vials hourly and were characterized on analytical HPLC. Samples were also tested for pH at end of synthesis (EOS) and four hours post-EOS and for dissolved oxygen content at four hours post-EOS (CHEMetrics K-7501 and K-7512). Results are reported as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

^{18}F radiolabeling reaction rates

RadioTLC indicated minimal increases (< 3 % higher at 20 minutes) in radiolabeling after seven minutes of reaction for all precursor masses tested (Figure 3). Radiochemical yields measured by radioTLC and SPE were proportional to precursor mass and may also be concentration dependent, although this was not tested. The fixed volume of 500 μL was chosen based on previous experience with synthesizing [^{18}F]THK-5351 in our lab and the ability of automated devices to reliably add small volumes of reagents. [^{18}F]THK-5351 decay-corrected radiochemical yields post-HPLC purification were 23%, 48%, and 55%, using precursor masses of 0.1, 0.2, and 0.5 mg, respectively. These data suggest that adequate [^{18}F]THK-5351 radiochemical yields can be achieved using 6–30 times less precursor mass than previously described (3.0 mg) (Harada et al., 2016a; Neelamegam et al., 2017).

tC18 Sep-Pak Light ethanol elution profile

The THK-5351 ethanol elution profile using the tC18 light cartridge was consistent in both the cold and radiolabeled experiments (Figure 4). Volumes greater than 0.5 mL ethanol only slightly increased (<3%) THK-5351 eluted product. In addition, trapping efficiency of the intermediate crude product in 500 μL DMSO was not compromised with as little as 4 mL

water dilution volume following radiofluorination, hydrolysis and neutralization. In contrast to previous methods, these results allow for a single-step elution and avoid injecting reaction solvents, such as DMSO, onto the purification column, which is typically not recommended by column manufacturers. A disadvantage to using the tC18 light over the tC18 short is the increased backpressure when loading and rinsing the cartridge. As such, this cartridge may be more amenable to small volume dilutions used for intermediate purification and less amenable to SPE steps requiring gross dilution such as those typically performed prior to final formulation.

HPLC purification of [^{18}F]THK-5351

Table 1 indicates the HPLC system backpressure, flow rates and THK-5351 retention times for the tested conditions. Analytical HPLC identified twelve closely eluting chemical impurities, which were numbered 1–12 in order of their retention time on analytical HPLC (table 2). Five of these impurities were observed to co-elute to varying extents with [^{18}F]THK-5351 product in at least one of the semi-preparative HPLC conditions tested. Semi-preparative separations using 20% or 22% acetonitrile with the Luna column and 22% or 25% acetonitrile with the ODS-4 column were unable to separate the identified chemical impurities from [^{18}F]THK-5351 (figure 5). Using 18% or 19% acetonitrile mobile phase with the Luna column allowed for collection of the [^{18}F]THK-5351 peak without any observable chemical impurities. When using 3.0 mg of precursor and 19% acetonitrile with the Luna column, a major chemical impurity (175s analytical retention time) was observed to co-elute on the tail of the [^{18}F]THK-5351 peak, suggesting that the separation is also affected by total mass injected. However, production runs using 0.5 mg of precursor with 19% acetonitrile and the Luna column indicated resolved [^{18}F]THK-5351 devoid of other chemical impurities.

Automated production of [^{18}F]THK-5351

Results of the seven ELIXYS production runs are summarized in Table 3. The average run time from QMA loading to end of synthesis was 87 ± 6 minutes with the longer runs being due to pausing the routine to radioassay system components. Syntheses without interruption typically took 80–84 minutes. The greatest radioactivity losses were due to lack of radiolabeling, QMA elution efficiency, and residual losses during automated HPLC injection (Table 4). Compared to the manually performed reaction rate experiment, decay-corrected radiochemical yields (calculated from ^{18}F in [^{18}O]H₂O) were reduced by >50%. This difference can be attributed to QMA radioactivity losses (not accounted for in the reaction rate experiment), HPLC residual losses using the automated routine, and potentially loss of precursor mass and reagent volume due to the automated addition having high residual loss (~30%). These issues could be mitigated to varying extents by further QMA conditioning (data not shown), eluting the intermediate tC18 cartridge into a smaller external vial to avoid eluted product from sticking to the sides of the vessel, and using a manual addition line for precursor to minimize residual losses. Even without these modifications, decay-corrected yields using comparable precursor masses were similar to those previously reported, (Neelamegam et al., 2017) although the total synthesis time was 20 minutes longer in the current work.

Production of [¹⁸F]THK-5351 for Human Use

The final drug product for four of the ELIXYS syntheses was fully characterized according to USP <823> for use in human studies (Table 5). These data indicate that the automated ELIXYS routine combined with our formulation apparatus can reliably produce [¹⁸F]THK-5351 compliant with FDA regulatory requirements for research use of PET radiopharmaceuticals in humans.

Radiochemical Stability

Chemical and radiochemical breakdown were observed in formulated [¹⁸F]THK-5351 stored with argon head gas, but were stable at four hours post-EOS when USP air was used (99.98% ± 0.04%, 99.63% ± 0.00%, 99.52% ± 0.02%, 99.36% ± 0.06%, 99.47% ± 0.11% radiochemical purity at 0, 1, 2, 3 and 4 hours post-EOS, respectively). The formulated tracer stored in argon head gas was stable when tested at EOS (99.89% ± 0.07%) and one hour post-EOS (99.28% ± 0.14%), but deteriorated rapidly from 80.85% ± 6.66% radiochemical purity at two hours post-EOS to 1.59% ± 0.00% radiochemical purity by three hours, and 0.23% ± 0.07% by four hours post-EOS. In addition to radiochemical instability with argon head gas, all chemical products present on analytical HPLC at EOS, including impurities, were no longer observed four hours post-EOS, consistent with radiochemical observations. Dissolved oxygen content measured at four hours post-EOS indicated a significant decrease in the argon-stored sample (0.6 ± 0.2 mg/L) compared to the sample stored in USP air (8 ± 2 mg/L). This result is counterintuitive given proposed mechanisms for radiolysis of formulated radiopharmaceuticals typically requiring the presence of oxygen for the formation of free radicals (Jacobson et al., 2009). While striking, it appears this has been observed before in formulation of ¹⁸F-labeled PET pharmaceuticals in a recent Siemens patent (Kolb, 2016). Further investigation into the interaction of vial head gas, PET radioactive decay products, and ligand stability may provide insight into the mechanisms of radiation induced chemical breakdown.

CONCLUSIONS

This work provides an alternate synthesis method for the radiosynthesis, purification and formulation of [¹⁸F]THK-5351. The automated production described in this work produces adequate yields and product suitable for FDA compliant research use in humans.

Acknowledgments

We would like to acknowledge the Michael Phelps Family Foundation and Sofie Biosciences for their generous contributions to this work.

Funding

Support was provided by National Institutes of Health R01 AG021155, National Institutes of Health R01 AG027161, Alzheimer's Disease Research Center P50 AG033514, National Institute on Child Health and Human Development U54 HD090256, and the National Cancer Institute of the National Institutes of Health under Award Number T32 CA009206.

References

- Betthausen T, Lao PJ, Murali D, Barnhart TE, Furumoto S, Okamura N, Stone CK, Johnson SC, Christian BT. In vivo comparison of tau radioligands ^{18}F -THK-5351 and ^{18}F -THK-5317. *J Nucl Med*. 2016
- Chiotis K, Saint-Aubert L, Rodriguez-Vieitez E, Leuzy A, Almkvist O, Savitcheva I, Jonasson M, Lubberink M, Wall A, Antoni G, Nordberg A. Longitudinal changes of tau PET imaging in relation to hypometabolism in prodromal and Alzheimer's disease dementia. *Mol Psychiatry*. 2017
- Doré V, Bourgeat P, Fripp J, Macaulay L, Ames D, Ellis KA, Martins RN, Masters CL, Rowe CC, Salvado O. Interaction between 18 F-THK5317, 18 F-flutemetamol SUVR, and cortical thickness. *Alzheimers Dement*. 2015; 11:P313.
- Gordon BA, Friedrichsen K, Brier M, Blazey T, Su Y, Christensen J, Aldea P, McConathy J, Holtzman DM, Cairns NJ. The relationship between cerebrospinal fluid markers of Alzheimer pathology and positron emission tomography tau imaging. *Brain*. 2016:aww139.
- Harada R, Furumoto S, Tago T, Katsutoshi F, Ishiki A, Tomita N, Iwata R, Tashiro M, Arai H, Yanai K. Characterization of the radiolabeled metabolite of tau PET tracer 18F-THK5351. *Eur J Nucl Med*. 2016a:1–8.
- Harada R, Okamura N, Furumoto S, Furukawa K, Ishiki A, Tomita N, Tago T, Hiraoka K, Watanuki S, Shidahara M, Miyake M, Ishikawa Y, Matsuda R, Inami A, Yoshikawa T, Funaki Y, Iwata R, Tashiro M, Yanai K, Arai H, Kudo Y. 18F-THK5351: A novel PET radiotracer for imaging neurofibrillary pathology in Alzheimer disease. *J Nucl Med*. 2016b; 57:208–214. [PubMed: 26541774]
- Ishiki A, Harada R, Okamura N, Tomita N, Rowe CC, Villemagne VL, Yanai K, Kudo Y, Arai H, Furumoto S, Tashiro M, Furukawa K. Tau imaging with [18 F]THK-5351 in progressive supranuclear palsy. *Eur J Neurol*. 2016
- Ishiki A, Okamura N, Furukawa K, Furumoto S, Harada R, Tomita N, Hiraoka K, Watanuki S, Ishikawa Y, Tago T, Funaki Y, Iwata R, Tashiro M, Yanai K, Kudo Y, Arai H. Longitudinal assessment of tau pathology in patients with Alzheimer's disease using [18F]THK-5117 positron emission tomography. *PLoS One*. 2015; 10:e0140311. [PubMed: 26461913]
- Jack CR, Wiste HJ, Weigand SD, Therneau TM, Lowe VJ, Knopman DS, Gunter JL, Senjem ML, Jones DT, Kantarci K, Machulda MM, Mielke MM, Roberts RO, Vemuri P, Reyes D, Petersen RC. Defining imaging biomarker cut points for brain aging and Alzheimer's disease. *Alzheimers Dement*. 2016
- Jacobson MS, Dankwart HR, Mahoney DW. Radiolysis of 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG) and the role of ethanol and radioactive concentration. *Appl Radiat Isot*. 2009; 67:990–995. [PubMed: 19231219]
- Johnson KA, Schultz A, Betensky RA, Becker JA, Sepulcre J, Rentz D, Mormino E, Chhatwal J, Amariglio R, Papp K. Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Ann Neurol*. 2015; 79:110–119. [PubMed: 26505746]
- Kolb, HCW., Joseph, Mocharla, Vani, Mu, Fanrong, Gangadharmath, Umesh. Office, U.S.P. Formulation, apparatus and method for stabilizing radiopharmaceuticals. Siemens Medical Solutions USA, Inc; (Malvern, PA) United States: 2016.
- Lazari M, Collins J, Shen B, Farhoud M, Yeh D, Maraglia B, Chin FT, Nathanson DA, Moore M, van Dam RM. Fully automated production of diverse 18F-labeled PET tracers on the ELIXYS multireactor radiosynthesizer without hardware modification. *J Nucl Med Technol*. 2014; 42:203–210. [PubMed: 25033883]
- Lazari M, Quinn KM, Claggett SB, Collins J, Shah GJ, Herman HE, Maraglia B, Phelps ME, Moore MD, van Dam RM. ELIXYS - a fully automated, three-reactor high-pressure radiosynthesizer for development and routine production of diverse PET tracers. *EJNMMI Res*. 2013; 3:52. [PubMed: 23849185]
- Neelamegam R, Yokell DL, Rice PA, Furumoto S, Kudo Y, Okamura N, El Fakhri G. A report of the automated radiosynthesis of the tau positron emission tomography radiopharmaceutical, [18 F]-THK-5351. *J Labelled Comp Radiopharm*. 2017; 60:140–146. [PubMed: 27859483]

- Ng KP, Pascoal TA, Mathotaarachchi S, Therriault J, Kang MS, Shin M, Guiot MC, Guo Q, Harada R, Comley RA, Massarweh G, Soucy JP, Okamura N, Gauthier S, Rosa-Neto P. Monoamine oxidase B inhibitor, selegiline, reduces 18F-THK5351 uptake in the human brain. *Alzheimers Res Ther.* 2017; 9:25. [PubMed: 28359327]
- Okamura N, Furumoto S, Harada R, Tago T, Yoshikawa T, Fodero-Tavoletti M, Mulligan RS, Villemagne VL, Akatsu H, Yamamoto T. Novel 18F-labeled arylquinoline derivatives for noninvasive imaging of tau pathology in Alzheimer disease. *J Nucl Med.* 2013; 54:1420–1427. [PubMed: 23857514]
- Tago T, Furumoto S, Okamura N, Harada R, Adachi H, Ishikawa Y, Yanai K, Iwata R, Kudo Y. Structure-activity relationship of 2-arylquinolines as PET imaging tracers for tau pathology in Alzheimer disease. *J Nucl Med.* 2016; 57:608–614. [PubMed: 26697966]

Highlights

- Characterization of [^{18}F]THK-5351 radiosynthesis with varying precursor mass
- Optimization of pre-HPLC and HPLC purification
- Automated radiosynthesis of [^{18}F]THK-5351 using the Sofie ELIXYS
- Formulation instability of [^{18}F]THK-5351 under inert head gas

FIGURE 1. Radiosynthesis of [¹⁸F]THK-5351
Schematic of the radiosynthesis of [¹⁸F]THK-5351.

FIGURE 2. ELIXYS Cassette Setup

Cassette load out for the ELIXYS automated radiosynthesis routine of [^{18}F]THK-5351. Volumes stated are the actual vial fill volumes and do not reflect the $\sim 150\ \mu\text{L}$ dead volume after the contents are added during the synthesis.

FIGURE 3. Nucleophilic Reaction Rates

Reaction rates for nucleophilic radiofluorination of [¹⁸F]THK-5352 using 0.1, 0.2 and 0.5 mg of precursor in 500 μ L DMSO determined by radio-TLC. Filled points represent decay-corrected radiochemical yields after acid hydrolysis determined by solid phase extraction (tC18 Light Sep-Pak).

FIGURE 4. tC18 EtOH Elution Profile

THK-5351 (blue) and [^{18}F]THK-5351 (red) elution profile using a Waters tC18 Light Sep-Pak with an ethanol (EtOH) eluent. 0.5 mL ethanol recovered greater than 90% of product for both experiments.

FIGURE 5. Semi-preparative HPLC Purification

Semi-preparative chromatograms with 360 nm absorbance (black) and radioactivity (red) peaks for various concentrations of acetonitrile (CH₃CN) in 20 mM NaH₂PO₄, with Phenomenex Luna or Inertsil Prodigy ODS-4 column. Impurity peaks were identified based on their retention times on analytical HPLC (see Table 2). Both 18% and 19% CH₃CN resulted in [¹⁸F]THK-5351 product with no UV impurities present on analytical HPLC. The ODS-4 conditions were not able to completely separate [¹⁸F]THK-5351 from impurities. Lower acetonitrile concentrations were not tested with the ODS-4 column due to high backpressure at flow rates above 7 mL/min. Shifts in chemical impurity retention times relative to THK-5351 were observed for small changes in acetonitrile concentrations (1%).

FIGURE 6. Analytical HPLC Results

Analytical HPLC chromatograms [^{18}F]THK-5351 early (left column) and late (right column) product fractions from preparative HPLC using the optimized method (top) (19/81 acetonitrile/20 mM NaH_2PO_4 , Luna), compared to the previously reported method (bottom) (25/75 acetonitrile/20 mM NaH_2PO_4 , ODS-4). Early and late fractions represent the first and second half of the semi-preparative radiopeak corresponding to [^{18}F]THK-5351. Note the radioactive peaks are relative to the time the samples were assayed and do not reflect differences in molar activity.

TABLE 1
Tested Semi-preparative HPLC Conditions

Semi-preparative HPLC conditions tested in this study along with retention times of THK-5351 and column backpressure for each condition.

Column	Acetonitrile Concentration [%]	Flow Rate [mL/min]	Backpressure [kpsi]	THK-5351 Retention Time [min]
<i>Luna</i>	22	5	1.8	20.08
<i>Luna</i>	20	7	2.8	20.90
<i>Luna</i>	19	9	3.3	21.02
<i>Luna</i>	18	9	3.4	26.13
<i>ODS-4</i>	25	5	2.2	18.93
<i>ODS-4</i>	22	7	3.2	21.13
<i>Luna (New with Guard)</i>	19	10	1.9	18.21

TABLE 2
Analytical HPLC Observed Chemical Impurities

Impurity number assignment based on retention time on analytical HPLC. The retention time of THK-5351 was 257 s.

Impurity Number	Analytical Retention Time [s]
1	110
2	120
3	147
4	175
5	190
6	212
7	220
8	226
9	241
10	249
11	276
12	322

TABLE 3

Automated ELIXYS Outcomes

Descriptive statistics for the seven automated productions using the Sofie ELIXYS. Percentage yields were determined from the amount of [^{18}F]fluoride in [^{18}O]H $_2$ O at the time of automated synthesis start. Runs 1, 6, and 7 were longer due to pausing the sequence to assay system components. Detailed analysis of the ELIXYS system losses is provided for runs 6 and 7 in Table 5 (NDC: non-decay corrected, DC: decay corrected)

Run	Precursor Mass [mg]	Run Time [min]	NDC Yield [MBq]	NDC Yield [%]	DC Yield [%]	Molar Activity [MBq/nmol]
1	0.2	100	1051	9.7	18.5	97
2	0.2	84	977	5.1	9.3	144
3	0.5	80	3437	13.5	22.7	317
4	0.5	83	2320	10.5	18.1	210
5	0.5	83	4740	12.3	21.5	322
6	3.0	88	5061	16.0	28.2	289
7	3.0	89	5099	15.6	27.8	635
Mean	1.1	87	3200	11.8	20.9	290
Std	1.2	6	1700	3.5	6.0	160

TABLE 4

Automated Routine Loss Characterization

Characterization of automated ELIXYS system losses determined by radioassay. Run numbers correspond to those in Table 3 (3mg precursor). Columns 2–7 describe the decay corrected radioactivity of the system components radiosynthesis using the automated ELIXYS routine. Column eight is the residual loss from the HPLC injection using the automated injector on the ELIXYS. Column nine is the amount of radioactivity that was recovered from the residual corrected HPLC injection after HPLC purification, solid phase extraction and sterilization and formulation of final product.

Run	[¹⁸ F] vial residual [%]	QMA Residual [%]	tC18 eluate [%]	tC18 cartridge residual [%]	tC18 waste [%]	ELIXYS unaccounted for [%]	HPLC Injection Residual [% of tC18 eluate]	HPLC, SPE and Formulation Recovery [% of injected eluate]
6	0.4	17.6	37.3	1.5	34.7	8.5	13.5	87.6
7	0.5	15.6	37.4	1.6	36.9	8	15	87.4
<i>Mean</i>	0.5	16.6	37.4	1.6	35.8	8.3	14.3	87.5
<i>Std</i>	0.1	1.0	0.1	0.1	1.1	0.3	0.8	0.1

TABLE 5

USP <823> Tested Batch Results

Results from four ELXIYS production runs tested according to United States Pharmacopeial Convention, Chapter 823.

Test	Specification	Run #2	Run #3	Run #4	Run #5	Mean ± SD
Radiochemical Yield	EOS Yield	0.98 GBq (26.4 mCi)	3.44 GBq (92.9 mCi)	2.32 GBq (62.7 mCi)	4.74 GBq (128.1 mCi)	2.87 ± 1.39 GBq (77.5 ± 37.5 mCi)
Strength	Radioactivity concentration at EOS	97 MBq/mL	343 MBq/mL	232 MBq/mL	474 MBq/mL	287 ± 139 MBq/mL (7.8 ± 3.8 mCi/mL)
Visual Solution Inspection	Clear, colorless, and free of particulate matter	Pass	Pass	Pass	Pass	Pass
Vial Integrity	Intact	Pass	Pass	Pass	Pass	Pass
Membrane Filer Integrity	Bubble point > 13 psi	21	21	20	20	20.5 ± 0.5 psi
pH	4–8	7.2	7.2	7	7	7.1
Radiochemical Identity	Retention time within ±10 % of THK-5351 standard	1.5 %	1.9 %	0.8 %	1.1 %	1.3 ± 0.4 %
Radiochemical Purity	[¹⁸ F]THK-5351 peak > 90% of the sum of all radioactivity peaks	100 %	99.9 %	100 %	99.8 %	99.9 ± 0.1 %
Chemical Purity	Non-THK-5351 mass peaks < 3.7 µg in 10 mL (360nm abs)	< 0.2 µg (none detected)	< 0.2 µg (none detected)	< 0.2 µg (none detected)	< 0.2 µg (none detected)	< 0.2 µg (none detected)
Molar Activity	> 18.5 GBq/µmol	144 GBq/µmol	317 GBq/µmol	210 GBq/µmol	322 GBq/µmol	248 ± 75 GBq/µmol
Residual Solvents	Acetonitrile <0.41 mg/mL	Pass	Pass	Pass	Pass	Pass
Radionuclidic Identity	Half-life 105–115 minutes	108.6 min	109.0	108.4	109.5	108.9 ± 0.4 min
Radionuclidic Purity	>99 % of total counts at 511 keV, 1022 keV or Compton scatter	100 %	100 %	100 %	100 %	100 ± 0 %
Bacterial Endotoxin Testing	<17.5 EU/mL	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL	< 2 EU/mL
Sterility	No growth at 14 days post- inoculation	No Growth	No Growth	No Growth	No Growth	No Growth
Residual Kryptofix 2.2.2	< 50 µg/mL (spot visibility less than standard)	No Spot, Pass	No Spot, Pass	No Spot, Pass	No Spot, Pass	No Spot, Pass