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Original Article

HPLC-free and cassette-based nucleophilic production of [^{18}F]FDOPA for clinical use

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Abstract: Radiotracer 3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (L-6-[^{18}F]fluorodopa or [^{18}F]FDOPA) is widely used for PET imaging of dopamine metabolism in several diseases including Parkinson's Disease, brain tumor, neuroendocrine tumors, and focal hyperinsulinism of infancy. In 2019, [^{18}F]FDOPA was approved by US FDA for detection of dopaminergic nerve terminals in the striatum of adult patients with suspected Parkinsonian Syndromes. A convenient and reliable method is desired for fully automated production of [^{18}F]FDOPA under cGMP compliance to meet the increasing clinical need. In this study, we reported a cassette-based automated production of [^{18}F]FDOPA using a GE Fastlab 2 module and the quality control (QC) under fully cGMP compliant environment. Briefly, automated radiosynthesis of [^{18}F]FDOPA was processed via nucleophilic radio-fluorination using FDOPA Fastlab cassette and solid phase extraction (SPE) purification. The QC tests of [^{18}F]FDOPA, including appearance, pH, half-life, radiochemical purity and identity, enantiomeric purity, chemical impurities, molecular activity, radioactive concentration, filter integrity, endotoxin, and sterility, were conducted at the end of synthesis (EOS) and 8 h after EOS during the validation runs. Three consecutive productions of [^{18}F]FDOPA were reliably achieved with desired radiochemical yield and high radiochemical/enantiomeric purities and molar activity. The uncorrected radiochemical yields of [^{18}F]FDOPA were 9.3-9.8% with a total synthesis time of ~140 min. Both radiochemical and enantiomeric purities of [^{18}F]FDOPA were >99.9% and the molar activities were 2.1-3.9 Ci/μmole at EOS. The full QC results at EOS and 8 h after EOS showed that the produced [^{18}F]FDOPA met all release criteria for clinical use within 8 hours of expiration time. Three consecutive validation runs and QC results demonstrated the efficacy of cassette-based production of [^{18}F]FDOPA for routine clinical use.

Keywords: [^{18}F]FDOPA, radiosynthesis, quality control, PET imaging

Introduction

Positron emission tomography (PET) is a clinical and research molecular imaging technology using positron-emitter labeled bioactive molecules (called radiotracer or radiopharmaceutical) to noninvasively quantify *in vivo* biological process [1]. As an analogue of 3,4-dihydroxy-L-phenylalanine (L-DOPA), 6-[^{18}F]fluoro-L-dopa ([^{18}F]FDOPA) is a well-established F-18 labelled radiopharmaceutical with an ideal half-life of 109.8 min for PET imaging of dopamine metabolism and L-type amino acid transporter in several diseases including Parkinson's Disease (PD), brain tumor, neuroendocrine tumors, and focal hyperinsulinism of infancy [2-9]. Indeed, [^{18}F]FDOPA was originally developed in early 1980s for PET imaging of dopaminergic system in human brain including PD and related disorders [9, 10]. Years later, [^{18}F]FDOPA was successfully explored for PET imaging of brain tumors and a variety of neuroendocrine tumors, as well as congenital hyperinsulinemia of infants [11-13]. In 2019, given its high specificity and sensitivity for PD diagnosis, [^{18}F]FDOPA was approved as a radioactive PET diagnostic agent by United States Food and Drug Administration (FDA or USFDA) for visualizing dopaminergic nerve terminals in the striatum of adult patients with suspected Parkinsonian syndromes [14]. In addition, [^{18}F]FDOPA PET has been used as a biomarker to measure the integrity and function of dopaminergic nerve terminals for patient stratification and therapy response. With the advent of regenerative medicine for PD therapy [15], [^{18}F]FDOPA PET has the potential to serve as an imaging biomarker for monitoring regenerative therapy of PD, as

the mechanism lies in regeneration of dopaminergic pre-synaptic function to take up DOPA and synthesize dopamine in brain [16]. Similarly, [^{18}F]FDOPA PET has been used in monitoring the therapy to distinguish patient's response to the treatment of psychosis [17].

Despite of the advance in diverse PET imaging applications, the utilization of [^{18}F]FDOPA is stagnant partially due to its complicated radiosynthesis. The main challenge is that synthesis of [^{18}F]FDOPA requires radiofluorination on highly electron-rich catechol scaffold. To solve this issue, [^{18}F]FDOPA was initially synthesized using [^{18}F]F_2 or [^{18}F]acetyl hypofluorite desilylation, demercuration or destannylation via electrophilic aromatic substitution [18-21]. And later, more selectively electrophilic fluorodestannylation reaction was commonly applied in clinical research [22-25]. However, such electrophilic radiofluorination requires highly reactive and corrosive electrophilic fluorinating agents and specialized fluorine gas targetry and equipment. Additionally, electrophilic radiofluorination has poor selectivity, and non-radioactive fluorine gas (F_2) is usually used as a carrier gas to flush [^{18}F]F_2 off target body, resulting in low molar activity [^{18}F]FDOPA [26, 27]. Given that radioactive fluoride ([^{18}F]F^-) is readily available in most medical cyclotrons, nucleophilic radiosynthesis of [^{18}F]FDOPA using [^{18}F]F^- is desired to reach large amount of radioactivity with high molar activity. To use [^{18}F]F^- as the nucleophile, the conventional nucleophilic aromatic substitution (SNAr) synthesis of [^{18}F] FDOPA requires the existence of strong electron-withdrawing groups in the ortho- or para-positions of the aromatic structure and an appropriate leaving group [28-31]. Two more efficient approaches for nucleophilic radiosynthesis of [^{18}F]FDOPA were recently reported to use Boron-Pin or stannyl precursor and copper-catalyzed coupling reaction [32-35]. Details in most recent advance [^{18}F]FDOPA synthesis are well documented in systematic reviews [27, 28, 36]. Nevertheless, to overcome the conflict of nucleophilic radiofluorination on electron-rich catechol ring, benzaldehyde precursor with an appropriate leaving group is typically used as the starting step, followed by oxidation of the F-18 labelled aldehyde intermediate and hydrolysis of the resulting ester. Therefore, nucleophilic radiosynthesis of [^{18}F]FDOPA usually requires three steps with either preinstalled or following coupling of the amino acid side chain.

To further advance the utilization of [^{18}F]FDOPA, a GE Fastlab cassette-based production with incorporation of additional HPLC purification was developed using the well-established three-step nucleophilic method [30]. ABX also developed the cassette-based production of [^{18}F]FDOPA with solid phase extraction (SPE) purification on GE Tracerlab MX, ORA Neptis, Siemens Explora One modules [37], and GE Fastlab module. However, this is no full-text report available for HPLC-free and cassette-based [^{18}F]FDOPA nucleophilic synthesis on GE Fastlab module. To meet the clinical need of [^{18}F]FDOPA PET imaging in our institute, we chose this cassette-based method for the production of [^{18}F]FDOPA using a GE Fastlab 2 module and ABX cassette owing to the wide access to Flastlab module platform. The method has the advantages of convenient cassette set-up, easy switch between different PET tracer production, availability of validated automation sequence, and data integrity and other regulatory compliance. Here we reported this HPLC-free and cassette-based [^{18}F]FDOPA production on GE Fastlab module and quality control tests under fully cGMP compliant environment.

Material and method

Materials and equipment

[^{18}F]FDOPA Fastlab cassette and all related materials, L-6-FDOPA and D/L-6-FDOPA were purchased from ABX advanced biochemical compounds (Radeberg, Germany). The cassette supplies included vials containing tetrabutylammonium bicarbonate (TBA-HCO_3) solution, 30% acetonitrile solution, disodium ethylenediaminetetraacetate dihydrate (Na-EDTA) buffer solution, (S)-3-(5-formyl-4-methoxymethoxy-2-nitro-phenyl)-2-(trityl-amino)-propionic acid tert-butyl ester (precursor of [^{18}F]FDOPA), L-ascorbic acid, dimethyl sulfoxide (DMSO), acetonitrile, meta-Chloroperoxybenzoic acid (mCPBA), ethanol, 30% hydrochloric acid, water for injection, and Light QMA/C18ec/C18/HR-P/WAX/Alumina Light N cartridges. Acetic acid, ethanol, and L-DOPA were purchased from Millipore Sigma (St. Louis, MO, USA). Tetrabutylammonium (TBA) hydroxide, methanol, and acetonitrile were purchased from Fisher Scientific (Waltham, MA, USA).

Ultimate 3000 HPLC (Thermo Scientific, Waltham, MA, USA) equipped with ultra violet

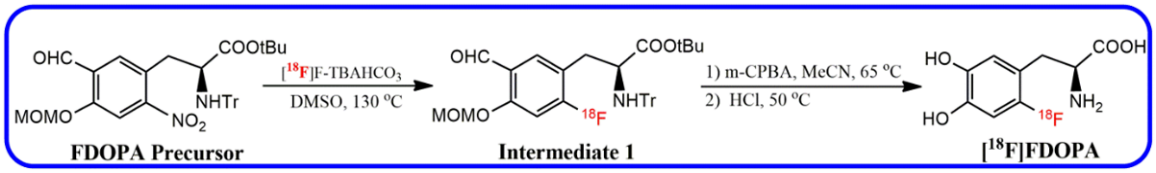

Figure 1. Reaction scheme for preparation of [18F]FDOPA.

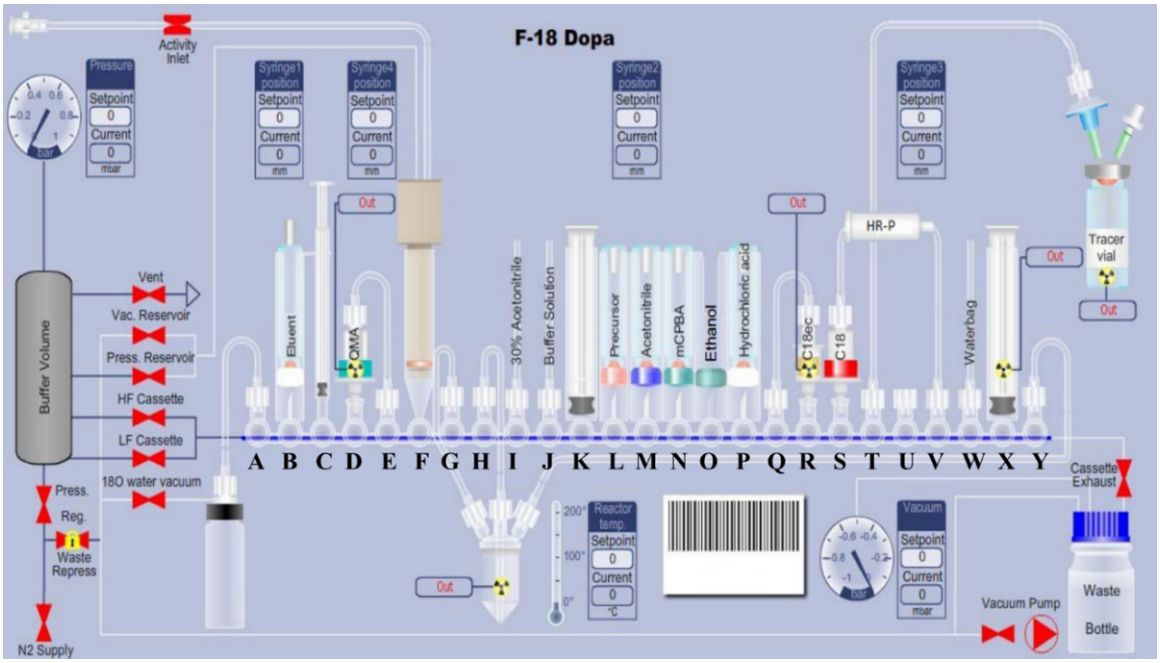

Figure 2. Diagram of Fastlab cassette for [18F]FDOPA production. A: 0-18 water recovery vial; B: 1.05 ml 0.075 M TBA-HCO_3 solution; D: Light QMA cartridge; I: 100 mL 30% Acetonitrile solution; J: 60 mL Buffer solution; L: 30 mg precursor dissolved in 1.3 mL DMSO; M: 3 mL acetonitrile; N: 18 mg mCPBA in 2.3 mL acetonitrile; O: 1.3 mL ethanol; P: 2.1 mL 30% hydrochloric acid; R: C18ec cartridge; S: C18 cartridge and HR-P cartridge; T: WAX/Alumina Light N cartridge (not shown in this figure) and product vial; W: 100 mL water for injection.

(UV) and radioactivity detectors (Eckert & Ziegler, Wilmington, MA, USA) was used to determine radiochemical purity and identity, molar activity and chemical impurities on an analytical reverse-phase column (Phenomenex Luna C18(2) 100 Å, 5 µm, 4.6×250 mm) at the UV wavelength of 282 nm. The injected sample volume was 20 µL and the mobile phase was a mixture of 1% acetonitrile in 0.1% acetic acid aqueous solution (v/v=1/100) with a flow rate of 1 mL/min. The same Ultimate 3000 HPLC was applied for radiochemical enantiomeric purity using a chiral column (Chirobotic T 5 um, chiral chromatography column 4.6 mm×250 mm, Millipore Sigma) and 75% ethanol in water as the eluent with a flow rate of 1 mL/min. Radio-Thin Layer Chromatography (Radio-TLC) system (Bioscan 2000) with silica gel strip (Millipore Sigma, St. Louis, MO, USA) and mobile phase of acetic acid/methanol (v/v=1:9) was used to detect radiochemical purity of L-[18F]FDOPA. Tracer 1310 gas chromatography (Thermo Scientific, Waltham, MA, USA) with a TG-WAXMS column (30 m×0.53 mm×0.50 µm) was used to analyze residual solvents with an injection volume of 0.5 µL sample. Radioactivity was determined with a Capintec® CRC 55tPET dose calibrator (Capintec, Inc., Florham Park, NJ, USA).

Fastlab cassette setup and radiosynthesis of [18F]FDOPA

Using a disposable ABX cassette, the synthesis of [18F]FDOPA was completed on a GE FASTlab 2 synthesizer, as shown in **Figures 1** and **2**.

Table 1. Summary for [^{18}F]FDOPA production

[^{18}F]FDOPA Batch ID	Run 1	Run 2	Run 3
Starting [^{18}F]fluoride Activity	4007 mCi	3625 mCi	4303 mCi
[^{18}F]FDOPA Activity at EOS	389 mCi	354 mCi	400 mCi
[^{18}F]FDOPA Product Volume	27.2 mL	27.3 mL	27.3 mL
Radiochemical Yield (%) (Non-decay corrected)	9.7%	9.8%	9.3%

Briefly, [^{18}F]fluoride anion produced by GE PETtracer 800 cyclotron was trapped on an anion exchange cartridge (QMA), eluted with TBA-HCO_3 solution, and azeotropically dried. By radiofluorination of the precursor at 130°C for 8 minutes, intermediate 1 was formed and trapped on a C18 ec cartridge, and then eluted back into reaction vial for further reaction. Followed by mCPBA oxidation and HCl hydrolysis, the crude product was purified with C18 Sep-Pak and HR-P cartridges, rinsed with water, and eluted with a Na-EDTA/L-ascorbic acid buffer solution via WAX and Light Alumina N cartridges. The final formulated [^{18}F]FDOPA product was delivered into a 30 mL product vial passing through a 0.22 μm sterile filter in an ISO 5 dispensing hot cell.

Quality control (QC)

General quality control of [^{18}F]FDOPA was performed according to USP 823, including appearance, pH, radionuclidic identity and purity, radiochemical identity and purity, enantiomeric purity, chemical purity, TBA residual, solvent residual, bacteria endotoxin, sterility, and stability. The detailed procedure of these QC tests was presented in Supplementary Information.

Results

Radiosynthesis

The radiosynthesis of [^{18}F]FDOPA, which included [^{18}F]fluoride receiving/activation, nucleophilic radiofluorination, oxidation, acid deprotection, multiple SPE purifications, final product delivery and sterile filtration, was automatically processed by FASTlab module. Three consecutive batches of [^{18}F]FDOPA production were successfully completed within defined specifications, and the summary was presented in **Table 1**. Specifically, with a total synthesis time of ~140 min, [^{18}F]FDOPA was obtained in the activities of 354-400 mCi at the end of synthesis (EOS) from starting activities of 3625-4303 mCi. The non-decay corrected radiochemical yields were 9.3-9.8% (n=3). The final products were formulated in buffer with total volumes of 27.2-27.3 mL.

Quality control

The QC results showed that the produced [^{18}F]FDOPA met all the release criteria for clinical use. As indicated in **Table 2**, all three batch products were clear, colorless solutions, and free from particulate matter. The pH and half-life value were within the ranges of 4.0-6.0 and 105-115 min. From analytical HPLC and Radio-TLC results, the radiochemical purities (**Figures 3** and S1) and enantiomeric purities (**Figure 4**) were >99%, and the radiochemical identities were within 10% deviation from the retention time of [^{18}F]FDOPA and reference standard. The contents of non-radiochemical FDOPA were 1-2 μg/mL and the total impurities were 37-45 μg in [^{18}F]FDOPA products. The molar activities were 2.1-3.9 Ci/μmol at EOS. The radionuclidic purities were >99.5%, determined by half-life test and no-observance of long-lived isotopes existence in the product after five days decay. The residual TBA concentrations were ≤100 μg/mL. The residual solvents in product were determined to be 2.55-3.68% ethanol, 0.01-0.03% acetonitrile and negligible amount of DMSO on GC. The integrity of the sterile filter was demonstrated by a bubble-point filter test with holding ≥50 psi pressure. The formulated products were sterile and nonpyrogenic from the sterility and endotoxin results. Stability was evaluated by performing a full quality control at EOS and followed by second assessment at the proposed expiration time of 8 hours after EOS. The [^{18}F]FDOPA vial was stored in an inverted position at room temperature during the expiration period. No leaks, sorption or degradation of the container closure were observed. A summary of the stability testing results at 8 h after EOS were given in Table S1. No significant changes were noted in appearance, pH, radiochemical/chemical purity, and endotoxin after

Table 2. Summary of quality control testing results for [18F]FDOPA validation runs

Test	Acceptance Criteria	Run 1	Run 2	Run 3
Appearance	Colorless and free from particulate matter	Pass	Pass	Pass
Radionuclidic Identity	Half-life is 110±5 minutes	109 min	109 min	110 min
Radiochemical Identity	R_t [18F]FDOPA ±10% R_t of pure L-6-FDOPA std	2%	1%	1%
Radiochemical Purity	Activity of [18F]FDOPA ≥95.0% of total radioactivity	100%	100%	100%
Radionuclidic Purity	≥99.5% of the observed peaks should correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of [18F]	Pass	Pass	Pass
Enantiomeric Ratio	L-isomer of [18F]FDOPA >95% of total radioactivity measured	100%	100%	100%
pH	4.0-6.0	4.4	4.4	4.4
Chemical Purity	Residual Solvents			
	Ethanol ≤10.00% (w/v)	3.68%	2.66%	2.55%
	MeCN ≤0.04% (w/v)	0.03%	0.01%	0.02%
	DMSO ≤0.50% (w/v)	0.01%	0.00%	0.01%
	TBA Size/intensity spot test ≤100 μg/mL	Pass	Pass	Pass
	FDOPA ≤20 μg/mL	2 μg/mL	1 μg/mL	1 μg/mL
	Total impurities ≤100 μg/mL	37 μg/mL	45 μg/mL	44 μg/mL
Specific Activity	≥1 Ci/μmol @ EOS	2.1 Ci/μmol	3.2 Ci/μmol	3.9 Ci/μmol
Radioactive Concentration	0.2 to 17.9 mCi/mL @ EOS	14.3 mCi/mL	12.97 mCi/mL	14.65 mCi/mL
Filter Integrity	≥50 psi	Pass	Pass	Pass
Endotoxin	≤175 EU/V*	Pass	Pass	Pass
Sterility	Sterile (No visible growth)	Pass	Pass	Pass

*V means the total volume of final product.

8 hours of room temperature storage. Based on these results, the expiration of [18F]FDOPA was set at 8-hour post EOS.

Discussion

[18F]FDOPA is a radiopharmaceutical for PET imaging of several diseases, including PD, brain tumor, neuroendocrine tumors, and focal hyperinsulinism of infancy. The complicated radiosynthesis has limited its availability for clinical use. It is efficient and convenient to produce [18F]FDOPA by nucleophilic radiosynthesis using commercially available ABX cassette, especially for facilities already equipped with medical cyclotron and Fastlab module for onsite FDG production, which significantly reduced the effort for method development of [18F]FDOPA radiosynthesis. Additionally, this cassette-based [18F]FDOPA production uses SPE purifications, avoiding time-consuming semi-preparative HPLC separation. Furthermore, our results demonstrated that [18F] FDOPA could be achieved with excellent radiochemical/enantiomeric purity and molar activity using this HPLC-free and cassette-based production. In addition to reliable radiochemistry, this method has advantages in module maintenance and fast switch between different cassette-based PET tracer production, which does not require clean procedures with disposable cassettes. Finally, the Fastlab cassette production method enhances data integrity for documentation and FDA CGMP regulatory compliance.

During the method development, FDOPA reference standard and analogs solutions were found stable in the solution of 1% acetic acid for up to six months in refrigerator. As a comparison, the solution of FDOPA in acetonitrile/DMSO/water without acetic acid developed a yellow color in a few days and turned to dark solutions gradually. The improved stability may be obtained from acidification of the catechol and amino acid by acetic acid. Therefore, acetic acid solution was also used in HPLC and radio-TLC analysis [38]. More importantly, the formulation of final product in pH 4-5 is critical to maintain its stability within 8 hours expiration time. In this method, L-ascorbic acid was added in the buffer to stabilize the final product solution, which was shown on HPLC chromatogram of [18F]FDOPA (**Figure 3B**).

Without use of HPLC purification, minor chemical impurities within acceptance range were found in the final product. Analytical HPLC condition was optimized to quantitate these chemical impurities (**Figure 3**). With 1% acetonitrile in

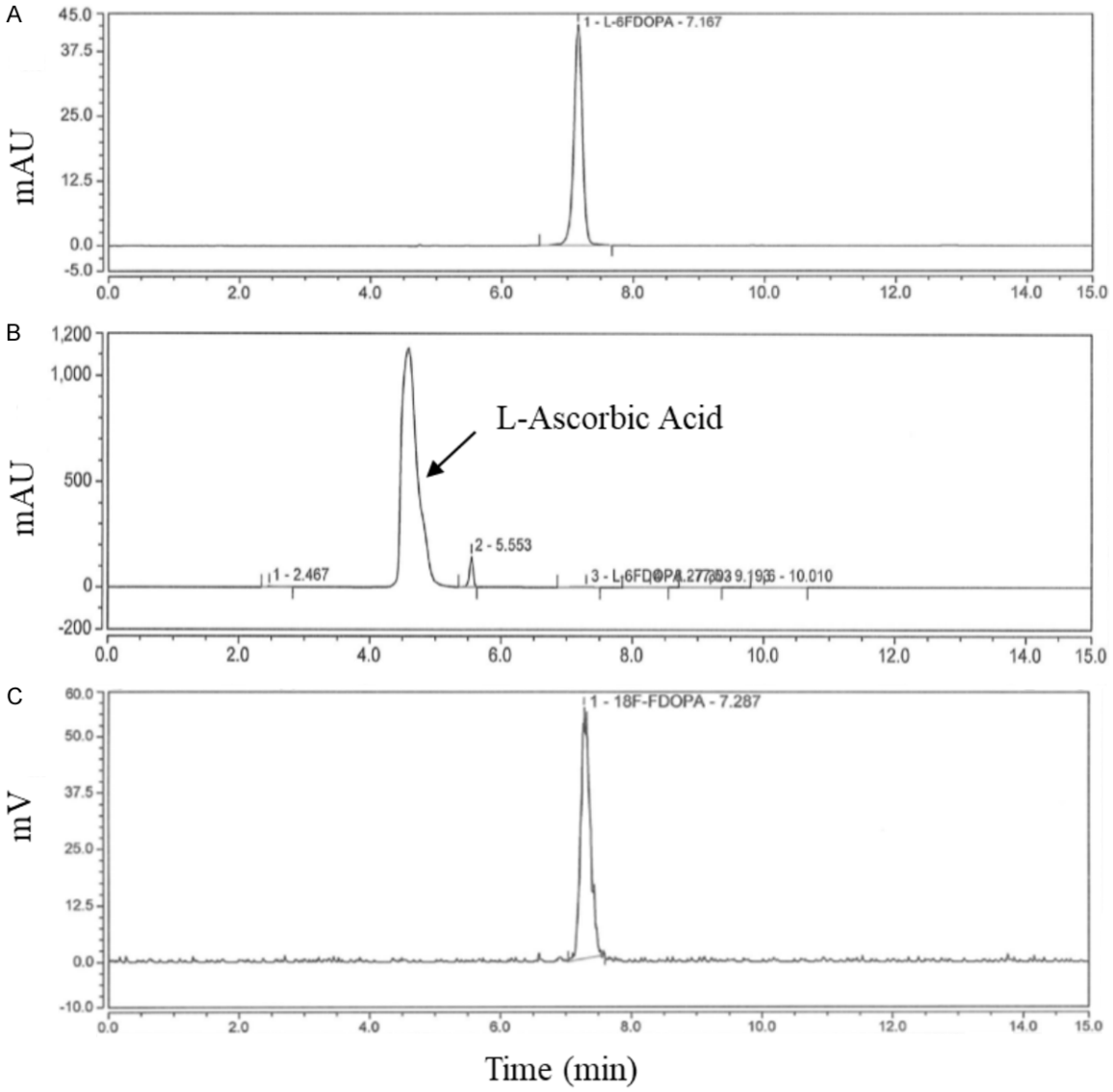

Figure 3. Representative analytical HPLC chromatograms of [18F]FDOPA and its reference standard. (A) HPLC chromatogram for FDOPA reference standard (20 μg/mL); (B and C) HPLC chromatograms for [18F]FDOPA, (B) UV signal; (C) Radioactivity signal.

0.1% acetic acid, the improved analytical HPLC separation was obtained on a reverse-phase Phenomenex Luna C18(2) column. All impurity peaks were integrated and calculated for their quantification. As a stabilizer, L-ascorbic acid was shown as a major peak in the HPLC UV chromatography. Additionally, the quantifiable impurity peak at 2.553 min was likely 6-OH-DOPA, a nucleophilic substitution byproduct. The concentration of nonradioactive FDOPA in the final [18F]FDOPA product was 1-2 μg/mL, and the total mass of impurities (L-ascorbic acid excluded) were 37-45 μg in [18F]FDOPA products, which were under the allowed limits based on European pharmacopeia 9.7-Fluorodopa (18F) Injection [39]. To measure the enantiomeric purity of [18F]FDOPA, chiral chromatography column was used with 75% ethanol in water solution as the eluent. As shown in **Figure 4**, excellent resolutions were obtained between L-6-FDOPA and D-6-FDOPA, with retention times of 5.933 min and 10.297 min, respectively.

There are a few noticeable limitations to be addressed in further method development of

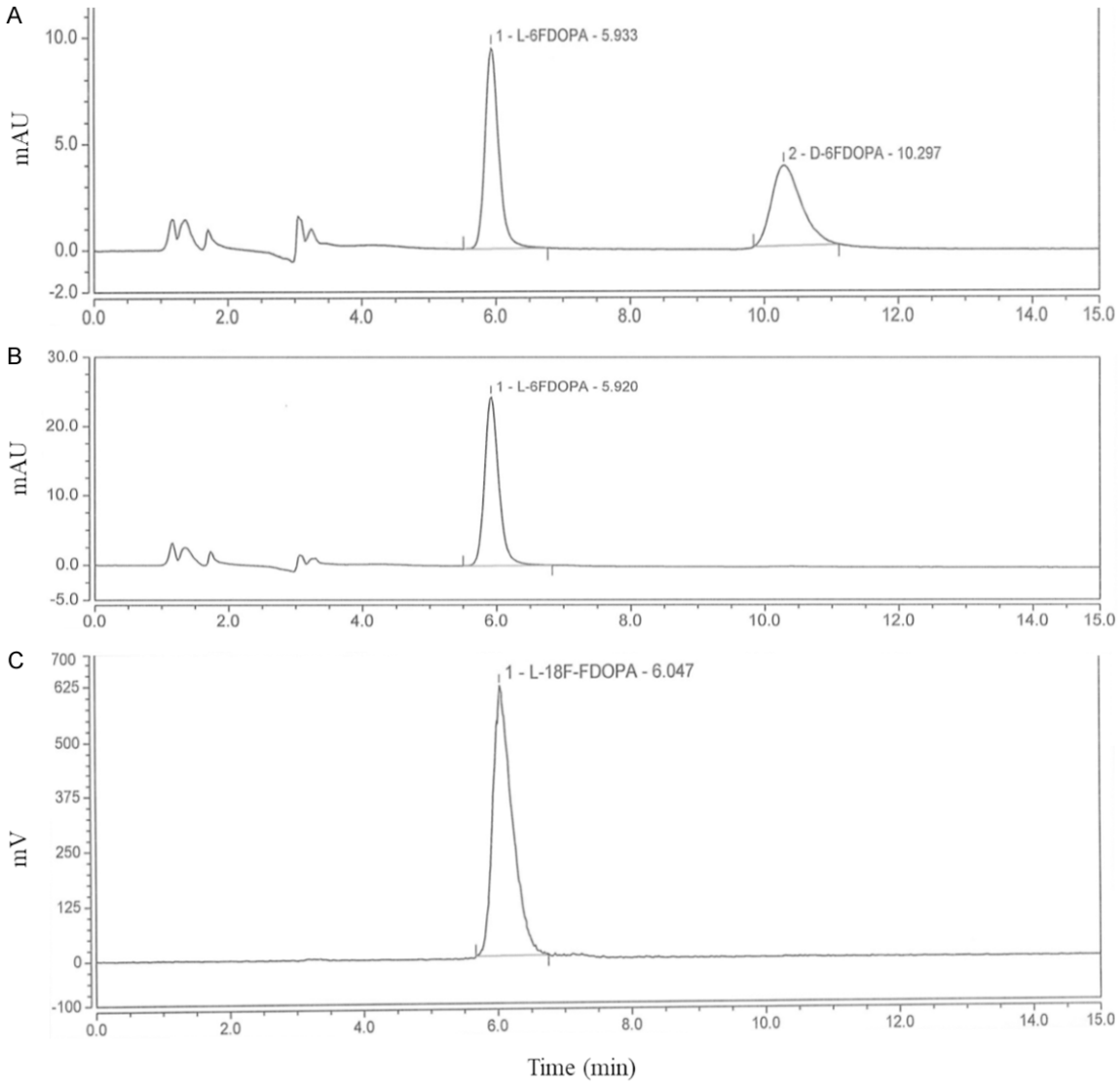

Figure 4. Representative chiral analytical HPLC chromatograms of [18F]FDOPA and D/L-6-FDOPA. A: Chiral HPLC chromatogram for D/L-6-FDOPA (20 μg/mL); B: Chiral HPLC chromatogram for L-6-FDOPA (20 μg/mL); C: Chiral HPLC chromatogram for [18F]FDOPA.

Fastlab-cassette based [18F]FDOPA production. Firstly, although [18F]FDOPA was obtained successfully in our laboratory, residual solvent acetonitrile contents in final product were variable from 0.01 to 0.03%, shown in **Table 2**. It may reach to 0.04% in rare case for large scale routine production, resulting in out of specification for acetonitrile residual. Therefore, further optimization in the purification steps may be required to reduce acetonitrile residual, e.g. extra flush or more water rinse at the cartridge purification steps. Secondly, during the product delivery, unexpected leakage was observed at the joint of alumina N and WAX cartridges due to the back pressure built from the long-distance transferring tubing between synthesizer and final product vial. A home-designed and 3-D printed cartridge holder was used to lock alumina N and WAX cartridges to avoid potential leakage, which is strongly suggested for more than 30 feet tubing delivery. Thirdly, unlike FDG, current clinical usage of [18F]FDOPA are limited to the small number of patients in a few academic medical centers, the cost for each batch production using FDOPA Fastlab cassette is more expensive in comparison to conventional research production method. Finally, with continuous improvements and

progress in nucleophilic radiosynthesis of [18F]FDOPA, more simple and convenient methods are reported, such as one-pot two-step radiosynthesis of high-molar-activity [18F]FDOPA by Cu-mediated fluorination of a pinacol boronate (BPin) [32, 33], or by direct arene radiofluorination via photoredox-mediated halide catalysis [40, 41]. These most recent advance may be applicable to next generation cassette-based radiosynthesis of [18F]FDOPA. Nevertheless, current cassette-based nucleophilic radiosynthesis of [18F]FDOPA allows for obtaining hundreds of millicuries product with high radiochemical purity and molar activity for clinical use.

Conclusion

Using nucleophilic radiofluorination and SPE purification, HPLC-free and cassette-based production of [18F]FDOPA on GE Fastlab module was efficiently and reproducibly achieved in large-scale production with desired radiochemical yield. The produced [18F]FDOPA has excellent radiochemical/enantiomeric purities, molar activity, and stability, and met all release specifications for clinical use.

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Disclosure of conflict of interest

None.

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Supplementary Information

Procedure for [^{18}F]FDOPA quality control

Appearance

Three validation batches of [^{18}F]FDOPA product vial was observed visually through radiation shielding glass for any color or particulate matter. Final product was a clear, colorless, and particle-free solution without presence of particulate matter.

Ph

The pH of [^{18}F]FDOPA was measured using pH test strips (0.3 units) and was visually confirmed by referencing the pH chart provided by the vendor. Each batch of [^{18}F]FDOPA was tested to assure that the pH valve of final product was within the acceptable ranges of 4.0-6.0.

Radionuclidic identity

Radionuclidic identity was determined by half-life ($T_{½}$). By placing a sample of drug product into a properly calibrated dose calibrator, the activity was recorded at two timepoints with at least 10 minutes apart. The $T_{½}$ was calculated by linear regression to assure that the $T_{½}$ was within 105-115 min ranges.

Radiochemical identity

A 20 μL sample of drug product was injected into an HPLC system with a UV setting at 282 nm in line with a radioactivity detector. Briefly, reverse phase column (Luna 5 μm C18 (2) 100 A, 250×4.6 mm, Phenomenex) was used with 1% acetonitrile in 0.1% Acetic acid aqueous solution as the eluent at a flow rate of 1 mL/min. The retention time of radioactive [^{18}F]FDOPA was recorded and compared to that of non-radioactive L-FDOPA reference standard. The acceptable deviation in retention times of the [^{18}F]FDOPA and the L-FDOPA reference standard was within ±10%.

Radiochemical purity

The same 20 μL drug product injection into the HPLC system for radiochemical identity was also used to measure radiochemical purity. All peaks that appeared in the radioactive chromatography were integrated and the peak areas were recorded. Acceptable radiochemical purity from radio-HPLC analysis was ≥90%. To avoid the potential free [^{18}F]fluoride ion in the final product which may not be detected by radio-HPLC system, radio-Thin Layer Chromatography (Radio-TLC, Bioscan 2000) with mobile phase of acetic acid/methanol (V/V=1:9) was introduced to confirm radiochemical purity of L-[^{18}F]FDOPA during validation process. Acceptable radiochemical purity from Radio-TLC analysis was ≥90%. The radiochemical purity determined by radio-TLC will be performed once per year as part of an annual validation after three validation runs.

Molar activity

Three validation batches of [^{18}F]FDOPA were analyzed for molar activity at the end of synthesis (EOS). Molar activity was determined by dividing activity of final product by the mass of nonradioactive FDOPA. Recommended molar activity was ≥1000 mCi/μmole at EOS.

Radiochemical enantiomeric purity

The radiochemical enantiomeric purity of [^{18}F]FDOPA was determined using the same HPLC system with chiral column (Chirobotic T 5 um, 4.6 mm×250 mm, Millipore Sigma) and 75% ethanol in water as the

eluent at a flow rate of 1 mL/min. The retention times of the radioactive L-[^{18}F]FDOPA and D-[^{18}F]FDOPA were recorded and compared to non-radioactive L-FDOPA and D/L-FDOPA reference standards. There was no D-[^{18}F]FDOPA detected in three validation batches of [^{18}F]FDOPA, and the deviation in retention times of the L-[^{18}F]FDOPA and the L-FDOPA standard was within ±10%. The enantiomeric radiochemical purity of [^{18}F]FDOPA product determined by chiral radio-HPLC will be performed once per year as part of an annual validation after three validation runs.

Chemical purity

Chemical purity test includes residual solvent analysis, tetrabutylammonium hydroxide (TBAOH) residual test, and nonradioactive mass of FDOPA and related impurities analysis.

The amounts of residual solvents present in [^{18}F]FDOPA product were measured using GC. A 0.5 μL [^{18}F]FDOPA was injected into the GC and evaluated for the presence of ethanol, acetonitrile, and DMSO. Acceptable residual solvent content for ethanol, acetonitrile, and DMSO were ≤10.00% (w/v), ≤0.04% (w/v), and ≤0.50% (w/v), respectively.

The amount of residual TBA present in the drug product was measured with TLC staining technique by comparing the intensity of [^{18}F]FDOPA with that of 100 μL/mL TBAOH standard solution. The volume of 2 μL [^{18}F]FDOPA and TBAOH standard were spotted on the same TLC strip separately, and followed by addition of 5 μL methanol/ammonia (v/v=9/1) solution on top of each spot. The stained spots were observed after placing TLC strip into an iodine chamber for 1 min. The [^{18}F]FDOPA spot must be less intense than that of the 100 μg/mL TBAOH reference sample.

Mass of nonradioactive FDOPA and related impurities in [^{18}F]FDOPA were determined by HPLC analysis. The amount of [^{18}F]FDOPA (retention time was about 6.9 min) and other impurities present in the final product were quantitated by comparing to a standard solution of FDOPA (20 μg/mL). The mass of total nonradioactive unidentified chemical impurities (except solvent front of the mobile phase and L-ascorbic acid) in final product was determined to be <100 μg/mL, assuming they have the same molecular weight of FDOPA, which was under allowed limit based on European pharmacopeia 9.7-Fluorodopa (^{18}F) (prepared by nucleophilic substitution) Injection. Typically, the amount of FDOPA in the final preparation was 1-2 μg/mL.

Radionuclidic purity

Radionuclidic purity testing of [^{18}F]FDOPA was performed by gamma spectroscopy of a decayed product sample using a germanium detector. This test was completed as part of an annual validation. Samples of [^{18}F]FDOPA product were allowed to decay for no longer than one week for analysis. The total radioactivity of any long-lived radionuclides was measured via gamma analysis.

Filter membrane integrity (bubble point test)

The filter membrane integrity was checked using the bubble test at the end of synthesis to assure filter integrity, as recommended by the manufacturer of the filter. The pressure was recorded with the reading on a calibrated pressure gauge. No breakthrough bubbling at 50 psi was considered as accepted filter membrane integrity.

Bacterial endotoxin

The bacterial endotoxin (BET) test was performed using the Endosafe Nexgen-MCS cartridge technology provided by Charles River Laboratories. [^{18}F]FDOPA product was diluted with LAL water by the factor of 1:100 and 25 μL diluted samples were pipetted into a sterile, disposable LAL test cartridge with a sensitivity of 0.05 EU/mL that provides quantitative LAL results within 15-20 minutes. Less than 175 EU/V (V= maximum dose administered in mL) was considered acceptable.

Sterility

Sterility testing of [^{18}F]FDOPA was completed retrospectively, and initiated after the release of the drug product for patient use. Within 30 hours of end of synthesis, a 0.2-1.0 mL sample of the undiluted drug product was inoculated in both tryptic soy broth (TSB) and fluid thioglycollate (FTM) media and incubated for 2 weeks alongside negative control samples. The TSB and FTM tubes were incubated at 20-25°C and 30-35°C, respectively. The sample was considered sterile with no visually detectable growth in either media 14 days later.

Radioactive concentration (strength)

The final drug product activity at end of synthesis (EOS) was measured using a properly calibrated dose calibrator. The weights of final [^{18}F]FDOPA product vial and empty vial were used to determine product volume assuming the density was 1 g/mL. The activity was divided by the total volume of drug product to calculate radioactive concentration. [^{18}F]FDOPA strength was determined and recommended to be 0.2-17.9 mCi/mL at EOS.

Stability testing

Stability was evaluated by performing a full quality control at the end of synthesis (EOS), followed by an assessment of appearance, pH, radiochemical identity/purity, chemical purity, and bacterial endotoxin at the proposed expiration time of 8 hours after EOS. The [^{18}F]FDOPA product vial was stored in an inverted position at room temperature for the duration of the expiration period. No leaks, sorption or degradation of the container closure were observed.

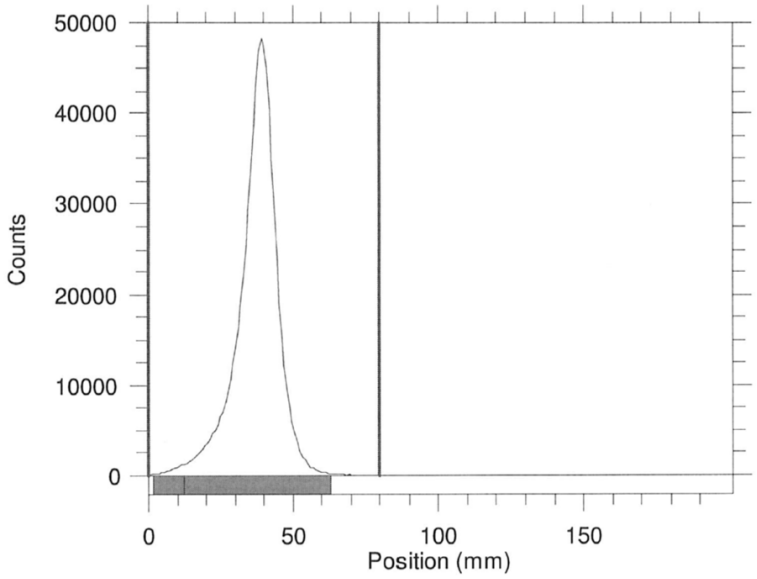

Figure S1. Representative radio-TLC chromatograms for [^{18}F] FDOPA.

Table S1. Summary of stability testing results of [^{18}F]FDOPA

Test	Acceptance Criteria	Run 1		Run 2		Run 3	
		EOS	8 hours	EOS	8 hours	EOS	8 hours
Appearance	Colorless and free from particulate matter	Pass	Pass	Pass	Pass	Pass	Pass
Radionuclidic Identity	Half-life is 110±5 minutes	109 min	112 min	109 min	108 min	110 min	112 min
Radiochemical Identity	R_t [^{18}F]FDOPA ±10% R_t of pure L-6-FDOPA std	2%	3%	1%	1%	1%	2%
Radiochemical Purity	Purity of [^{18}F]FDOPA ≥95.0% of total radioactivity	100%	100%	100%	100%	100%	100%
Enantiomeric Ratio	L-isomer of [^{18}F]FDOPA >95% of total radioactivity measured	100%	100%	100%	100%	100%	100%
pH	4.0-6.0	4.4	4.4	4.4	4.4	4.4	4.4
Chemical Purity	Residual Solvents						
	Ethanol ≤10.00% (w/v)	3.68%	3.71%	2.66%	2.65%	2.55%	2.46%
	MeCN ≤0.04% (w/v)	0.03%	0.03%	0.01%	0.01%	0.02%	0.01%
	DMSO ≤0.50% (w/v)	0.01%	0.01%	0.00%	0.00%	0.01%	0.00%
	TBA Size/intensity spot test ≤100 μg/mL	Pass	Pass	Pass	Pass	Pass	Pass
	FDOPA ≤20 μg/mL (0.002%)	2 μg/mL	2 μg/mL	1 μg/mL	1 μg/mL	1 μg/mL	1 μg/mL
	Total impurities ≤100 μg/mL (0.01%)	37 μg/mL	35 μg/mL	45 μg/mL	41 μg/mL	44 μg/mL	40 μg/mL
Endotoxin	≤175 EU/V*	Pass	Pass	Pass	Pass	Pass	Pass

*V means the total volume of final product.