Neutral pH Formulation for 6- ^{18}F fluoro-L-DOPA With High Radiochemical Stability

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Original research

Keywords: ^{18}F FDOPA, amino acid, stability, enantiomeric purity, positron emission tomography

Posted Date: January 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-142193/v1>

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Abstract

6-[^{18}F]Fluoro-L-DOPA(FDOPA) has always been generally produced under strong acidic conditions, i.e. as an injectable solution of pH 2~3, due to its low stability at a higher or neutral pH. This necessitates the pre-treatment neutralization of this agent with an injectable NaHCO_3 solution. We have developed a neutral pH formulation for [^{18}F]FDOPA using ethanol (EtOH) and phosphate buffer to overcome the radioactive and enantiomeric stability problems at a higher pH. Upon [^{18}F]FDOPA generation by nucleophilic substitution methods, we investigated its radiochemical and enantiomeric purity in accordance with the various pH after 6 hours. After EtOH and three kinds of buffer were added, we further examined this purity at pH 6 ~ 7 after 6 hours. The ascorbic acid did not stabilize the radiochemical purity at the higher pH. A 5% EtOH and PBS buffer matrix produced the best stability for radiochemical and enantiomeric purity at pH 6.5 at the 6 hour time point. This combination maintained a > 95% radiochemical and enantiomeric purity at 6 hours after EOS (end of synthesis). Our new formulation for [^{18}F]FDOPA thus showed a high stability at neutral pH and satisfied QC requirements which was listed in European Pharmacopeia. It has also been approved by the Korean Ministry of Food and Drug Safety.

Background

The radiolabeled aromatic amino acid 3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (6-[^{18}F]fluoro-L-DOPA, [^{18}F]FDOPA) is used for the evaluation of presynaptic dopaminergic function in positron emission tomography (PET) imaging [1, 2], specifically for disorders of the central nervous system such as Parkinson's disease. More recently, 6-[^{18}F]fluoro-L-DOPA has been utilized in a previously unforeseen application, i.e. in the management of neuroendocrine tumors (NETs) [3–5]. Several [^{18}F]FDOPA synthesis reports with nucleophilic substitution methods have been developed to overcome the problems that arose with previous electrophilic substitution methods, such as non-regioselectivity, low radiochemical yields, and low molar activity [6–14]. Lemaire *et al.* reported a general five-step synthesis method using a specific phase transfer catalyst (PTC) that yielded a sufficient degree of enantiomeric purity and radiochemical yield to satisfy the recently adopted European Pharmacopeia (EP) specifications [13, 14].

Regardless of the production method, the final [^{18}F]FDOPA injectable solution has always needed to have a very low pH of between 2.0 and 3.0 because of the instability of this radiochemical at a higher pH. Acidic solutions lead to severe pain however when directly injected into patients. Hence, [^{18}F]FDOPA solutions must be immediately neutralized with an 84 mg/mL NaHCO_3 solution prior to injection. After this neutralization step, the [^{18}F]FDOPA injectable solution must be stored in a refrigerator and used within 3 hours [15]. A previous study has reported that conventional [^{18}F]FDOPA preparations undergo decomposition via oxidative free-radical reactions and that subsequent imaging quality will be reduced by any free [^{18}F]fluoride formation that had not been completely removed during HPLC purification [13].

There have been no prior reports however that have offered solutions to these problems. In our present study, we describe our new formulation for [^{18}F]FDOPA in the mixture of ethanol (EtOH) and buffer

solution at a neutral pH but shows long-term stability without de-[¹⁸F]fluorination.

Materials And Methods

General information

[¹⁸F]Fluoride was produced by a cyclotron (GE healthcare PETtracer, Uppsala, Sweden) using the ¹⁸O(p,n)¹⁸F nuclear reaction with irradiation of an enriched 2 mL of ¹⁸O-H₂O as the target material. All chemicals other than the [¹⁸F]FDOPA preparation cassette and reagent kits were purchased from Sigma-Aldrich (St. Louis, MO)

Production of [¹⁸F]FDOPA under acidic conditions

[¹⁸F]FDOPA was prepared using a nucleophilic method with the Trasis AllinOne Chemistry Module (Liege, Belgium). Disposable cassettes and reagents for [¹⁸F]FDOPA preparation were supplied by Trasis and production procedures were as previously described [13, 14]. We only added 0.1% acetic acid containing 0.2 mg/mL of ascorbic acid to the mobile phase for HPLC purification, and these solutions were freshly prepared prior to the synthesis reactions. The starting radioactivity was 72.3±13.5 GBq. Following the production of [¹⁸F]FDOPA, the radiochemical and enantiomeric purities were checked using reverse phase-high performance liquid chromatography (RP-HPLC) and chiral-HPLC, respectively. The analytical HPLC conditions were as follows: 0.1% acetic acid: methanol = 97:3 v/v, flow rate = 0.8 mL/min, Luna C₁₈ column (250 × 4.6 mm), and monitoring at 280 nm and via a radioactivity detector. Enantiomeric purity was evaluated using an Astec CHIROBIOTIC column (250 × 4.6 mm) and mobile phase system, i.e. 0.01 M NaH₂PO₄:CH₃CN = 20:80 v/v, flow rate = 1.0 mL/min.

The [¹⁸F]fluoride content and pH were determined using radio thin layer chromatography (radio-TLC) and a pH meter, respectively. A pH of between 2–3 was used to maintain radiochemical purity after production. 1,517 ~ 3,071 MBq/mL of [¹⁸F]FDOPA injectable solution was applied to the following new formulation development experiments.

Optimized formulation for a stable [¹⁸F]FDOPA preparation at neutral pH

1) Addition of EtOH

After the preparation of the purified [¹⁸F]FDOPA from RP-HPLC, we added ethanol up to a maximum concentration of 30% and evaluated its effect as an additional radiochemical stability agent. We then neutralized the resulting solution with 8.4% NaHCO₃ to obtain a pH of 6, 7, or 8. The radiochemical purity was evaluated by RP-HPLC at 0, 2, 4, and 6 hours after production.

2) Effects of 5% EtOH and temperature

After evaluation of the ethanol concentration effects, we also evaluated temperature effects at a 5% EtOH concentration. These conditions were tested at both pH 2 and pH 7 at 25 °C. We also tested a pH 7

solution containing 5% EtOH at 4 °C to detect any temperature effects after neutralization. The stability of the [^{18}F]FDOPA under these conditions was measured through the formation of free [^{18}F]fluoride detected by radio-TLC [16].

3) Effects of 5% EtOH and different buffer solutions

After evaluating the effects of the ethanol concentration and temperature at pH 7, we further investigated the effects of different buffers on the stability of the [^{18}F]FDOPA compound in 5% EtOH, pH 7 and 25 °C. We tested 5 mg/mL of concentration phosphate-buffered saline (PBS), phosphate buffer, and citrate buffer. PBS was prepared by dilution of a 10 × PBS stock solution. The phosphate buffer was prepared by adding 0.558 g of Na_2HPO_4 and 0.603 g of NaCl to a final volume of 100 mL of deionized water. A 100 mL citrate buffer solution was prepared by the addition of 2.016 g of sodium hydrogen citrate sesquihydrate and 11.512 g of sodium citrate tribasic dihydrate. After mixing the [^{18}F]FDOPA preparation with each buffer solution, we again monitored [^{18}F]fluoride formation by radio-TLC.

4) Evaluation of the final formulation of [^{18}F]FDOPA

After preparation of the purified [^{18}F]FDOPA and formulation with 5% EtOH and PBS buffer, we neutralized the solution with 8.4% NaHCO_3 to obtain a pH of 6.5-7.0. The radiochemical and enantiomeric purities were then checked using RP-HPLC and chiral-HPLC, respectively, for 0, 2, 4, and 6 h. The formation of [^{18}F]fluoride was also monitored by radio-TLC ($n = 3$ for each condition).

High radioactivity production, quality control and stability evaluation of the neutral pH injectable [^{18}F]FDOPA solution

We synthesized 13 batches of [^{18}F]FDOPA with our new formulation such as PBS containing 5% EtOH. The starting radioactivity was 126.1 ± 39.2 GBq. We performed quality control (QC) tests on all solutions including appearance, radionuclide identity and purity, pH, radiochemical identity and purity, radiochemical impurity, enantiomeric purity, molar activity, residual solvent and EtOH, bacterial endotoxin, and sterility. We also evaluated the long time stability of 3 batches up to 6 h after preparation.

Results

Production and quality control of [^{18}F]FDOPA

The radiochemical yield of our [^{18}F]FDOPA preparation was $34.15 \pm 13.38\%$ and the total synthesis time was 82.7 ± 11.0 min. The radiochemical and enantiomeric purities were $99.8 \pm 0.1\%$ and $97.1 \pm 0.37\%$, respectively ($n=19$).

Effects of ethanol addition at various pH levels

Two kinds of radiochemical impurities were detected by RP-HPLC, based on increases in both the pH and retention time (Figure 1). At 2 hours after the addition of the 8.4% NaHCO_3 solution, the radiochemical

purity dramatically decreased at pH 6 without EtOH. Over a pH range of 6–8, solutions containing 1-5% EtOH concentrations showed 10% more stability than those without EtOH up to 6 h. For the 5% EtOH solutions, the radiochemical purity at pH 6 and 7 was above 90% up to 6 h and 4 h, respectively. The decreased radiochemical purity in the 1% EtOH solution was comparable between the pH 7 and 8 solutions. Without EtOH however, the rate of decomposition of [^{18}F]FDOPA was greater at pH 8 compared with pH 7.

Interestingly, no free [^{18}F]fluoride was detected by RP-HPLC. Additionally, the retention time of free [^{18}F]fluoride was below 3.0 min under our analysis conditions [13]. Hence, we considered the impurities detected by RP-HPLC at the pH range of 6-8 to be unknown radioactive substances. Furthermore, the enantiomeric purity showed no decrease, and the unknown radiochemical impurities detected RP-HPLC were not detected by chiral-HPLC.

Evaluation of [^{18}F]fluoride content by radio-TLC.

We performed radio-TLC analysis because we could not measure the exact amount of free [^{18}F]fluoride by RP-HPLC. [^{18}F]Fluoride formation was monitored by radio-TLC in the [^{18}F]FDOPA solution before and after neutralization according previously described method [13]. Without neutralization, the [^{18}F]fluoride concentration did not increase until 6 hours. However, as soon as the solution was neutralized (pH=6-7), the [^{18}F]fluoride concentration exponentially increased up to 80% at 25°C. The 5% EtOH solution suppressed this rate of de-[^{18}F]fluorination at the earlier time points at 25°C. After 6 hours however, the [^{18}F]fluoride levels were similar to those in the solution without EtOH. Even at 4°C, the [^{18}F]fluoride concentration showed a slow linear increased up to 20% for 6 hours after neutralization (Figure 2).

Under conditions of 5% EtOH and pH 7, the addition of a buffer solution dramatically suppressed the de-[^{18}F]fluorination of [^{18}F]FDOPA (Figure 3). The preparation containing PBS showed no increase in [^{18}F]fluoride concentration up to the 6 hour timepoint (Figure 4). Both the phosphate and citrate buffers also suppressed the formation of [^{18}F]fluoride, but to a lesser degree as the [^{18}F]fluoride levels still continuously increased up to 5% and 10%, respectively.

Stability of the final [^{18}F]FDOPA formulation

The finally formulated [^{18}F]FDOPA (pH= 6.5) in a 5% EtOH/PBS solution showed good stability up to 6 hours. The initial radiochemical and enantiomeric purities were 100% and 97.8±0.3%, respectively. [^{18}F]fluoride was not detected in the [^{18}F]FDOPA (pH=6.5-7.0) solution at EOS. At 6 hours after EOS, the radiochemical purity was slightly decreased to 99.0±0.5% but the enantiomeric purity was maintained as 97.9±0.2%. The formation of [^{18}F]fluoride was slightly increased to 3.0±1.0% (Figure 3) at this time point.

High radioactivity production, quality control and stability of the neutral pH injectable [^{18}F]FDOPA solution

The radiochemical yield of our newly formulated [^{18}F]FDOPA was $30.4 \pm 6.1\%$. All of the QC parameters for this new preparation met the EP (European Pharmacopeia) criteria. The radiochemical and enantiomeric purities were 100% and $95.8 \pm 0.8\%$, respectively, at EOS. The molar activity was $54.7\text{--}15679.7\text{ TBq/mmol}$ at EOS. The ethanol concentration was $4.0 \pm 0.38\%$, and no residual solvents were detected. Although we used EtOH at a 5% concentration in this formulation, it was only detected at $4.0 \pm 0.38\%$ in the GC analysis after sterile filtering and delivery through the instrument tubing. The radiochemical and chiral purities at 6 hours after EOS were $99.0 \pm 0.46\%$ and $98.54 \pm 0.75\%$, respectively. No additional radiochemical impurities formed during the 6 hour period after preparation at pH 6.5–7.0.

Discussion

We have developed and evaluated a new formulation for [^{18}F]FDOPA that achieves a high stability of this compound at a neutral pH (pH 6 ~ 7). We have found that a mixture of EtOH and PBS efficiently suppresses the formation of radiochemical impurities and [^{18}F]fluoride or 6 hours at a neutral pH and 25 °C. These conditions are very amenable for the clinical administration of [^{18}F]FDOPA because no cumbersome neutralization procedures are required prior to injection. In addition, a high degree of radiochemical purity is guaranteed even at a neutral pH during the preparation and transportation of this radioactive compound.

The de-[^{18}F]fluorination rate of previous formulations of [^{18}F]FDOPA was previously reported to be very rapid after neutralization, with [^{18}F]fluoride levels of 10% detectable within 10 min [13]. From our own clinical experience (data not shown), freshly produced [^{18}F]FDOPA in a pH 2 solution does not show any issues when it is injected immediately after neutralization [13]. Notably however, we have occasionally obtained poor quality images after neutralization, as reported also by others. If the patient's schedule is delayed, a neutralized [^{18}F]FDOPA in the NaHCO_3 injectable solution will of course undergo decomposition during the waiting period. Even for neutralized [^{18}F]FDOPA stored at 4 °C, the [^{18}F]fluoride levels will continuously increase and reach 5.2% after one hour. In the aforementioned studies, a 5% [^{18}F]fluoride concentration is reported to affect the imaging quality. Hence, we have in the past strictly controlled the neutralization time for our [^{18}F]FDOPA injections to prevent the uptake of [^{18}F]fluoride into the bone after administration. This process is very cumbersome however and there remains a high possibility that the [^{18}F]FDOPA imaging will be of low quality. Neutralization of low pH [^{18}F]FDOPA preparations has generally involved ascorbic acid or sodium ascorbate as radiolysis reagents, but they cannot prevent the oxidation of [^{18}F]FDOPA.

In our present study, we found that EtOH was sufficient to prevent the formation of radiochemical impurities, as detected by RP-HPLC, but we could not detect free [^{18}F]fluoride using RP-HPLC (Fig. 1E). However, at pH 7 and a temperature of 25 °C (Fig. 2A), a 65% [^{18}F]fluoride concentration was detectable by radio-TLC. A lower temperature more efficiently decreased the formation of [^{18}F]fluoride than EtOH but at 60 min after neutralization, the [^{18}F]fluoride content exceeded 5%. We also could not confirm by radio-

TLC analysis whether the radiochemical impurities were free [^{18}F]fluoride or a mixture of free [^{18}F]fluoride and other [^{18}F]labeled impurities despite performing this modality in accordance with previously described procedures.

EtOH showed good results in terms of radiochemical stability, which was better at 10% EtOH than at 5% EtOH. A 10% concentration is too high for intravenous injection however as it may lead to severe pain [17]. Moreover, storing the solution at 4 °C and at pH 7 showed better results than 5% EtOH alone. Based on these findings, we sought another stabilization method for [^{18}F]FDOPA and decided upon a buffer solution in combination with 5% EtOH. PBS and citrate buffer produced more stability for [^{18}F]FDOPA than phosphate buffers. Moreover, a formulation with two kinds of buffers did not show a linear increase in the concentration of [^{18}F]fluoride with time, which was observed with the phosphate and citrate buffers (R^2 value: 0.99 and 0.90, respectively).

This new formulation with 5% EtOH and PBS showed very stable results for 6 hours at a pH of 6.5-7.0 and all radiochemical purities detected using RP-HPLC, chiral-HPLC, and radio-TLC were at levels under 3%.

Although the mechanism preventing the formation of [^{18}F]fluoride and other radiochemical impurities in a PBS or citrate buffer and 5% EtOH mixture is uncertain, it is likely that PBS and citrate buffer reacts with [^{18}F]FDOPA and causes the formation of a more stable form of this radiochemical compound, even at neutral pH, and that EtOH prevents the formation of free radicals.

Using our newly developed formulation, we can now dispense with the neutralized [^{18}F]FDOPA solutions and prepare multi-dose vials that can be directly injected into patients. This will be particularly important advantage in terms of quality control when supplying other small medical centers with this radiochemical compound because manufacturers or supply centers cannot control the neutralization steps or storage conditions used at external injection sites.

Finally, this new formulation was approved and given marketing authorization in October 2018 from the Korean Ministry of Food and Drug Safety. The product code in Korea is 201907382.

Conclusion

A new formulation for stabilizing [^{18}F]FDOPA has been developed by us and approved for clinical use in Korea. This formulation contains 5% EtOH and a PBS and citrate buffer, which dramatically suppress the decomposition of [^{18}F]FDOPA, including de-[^{18}F]fluorination. The stability results for these preparations show promise that [^{18}F]FDOPA can be dispensed in multi-dose vials and directly administered to the patient for up to 6 hours after production.

Declarations

Acknowledgments

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI18C2383).

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Funding Information: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HI18C2383).The authors have no conflicts of interest to declare.

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Figures

Figure 1

Radiochemical purity evaluation of ^{18}F -FDOPA solutions using RP-HPLC at A) pH 6.0, B) pH 7.0, and C) pH 8.0 under different EtOH concentrations. Chromatograms of $[^{18}\text{F}]$ FDOPA solutions containing 5% EtOH (v/v) after 6 h at D) pH 6.0, E) pH 7.0, and F) pH 8.0.

Figure 2

[18F]fluoride concentration in 18F-FDOPA prepared in A) a stock solution at pH 7.0, and B) three kinds of buffer solutions in 5% EtOH.

Figure 3

Buffer solution effects on [18F]FDOPA solutions at pH 6~7.

Figure 4

QC profile of the new formulation for [18F]FDOPA