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Modification of [^{18}F]FMISO Radiosynthesis by Using a Non-Cassette-Based Synthesizer for Supporting Hypoxia Diagnosis in Cancer Patients in Thailand

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Abstract

In cancer cells, hypoxia can lead to resistance to both radiation and chemotherapy. The ability to measure cellular hypoxia is an useful tool in therapeutic planning. [^{18}F]Fluoromisonidazole ([^{18}F]FMISO) is a specific tracer used for the detection of hypoxic tissues by positron emission tomography (PET). In this research, the [^{18}F]FMISO radiosynthesis has been modified to reduce costs to support clinical studies of patients with hypoxia by using an automated module instead of the disposable cassette-based synthesizer. The Synthra RN_{plus} module, a remote-controlled synthesizer, was used for nucleophilic substitution of NITTP (1-(2'-nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonyl-propanediol) with [^{18}F]fluoride anion. Labeling of the 5 mg of NITTP precursor in anhydrous dimethyl sulfoxide (DMSO) was performed at 100 ℃ for 10 min and hydrolyzed with 1 M hydrochloric acid (HCl) at 100 ℃ for 5 min. Finally, the purified product was obtained by using solid phase extraction (SPE) cartridge instead of high-performance liquid chromatography (HPLC). The total [^{18}F]FMISO yield was approximately 29.11 ± 5.00 % (n = 7), the total synthesis time was less than 35 min, and the radiochemical purity was greater than 95 %. These results are very useful for supporting hypoxia diagnosis in cancer patients in Thailand.

Keywords: [^{18}F]FMISO, Synthra RN_{plus}, Radiosynthesis, Radiopharmaceuticals, Hypoxia

Introduction

The incidence of head and neck cancer, one of the top 5 leading cancers in Thailand, is associated with hypoxia leading to therapeutic resistance [1,2]. Hypoxia is a pathological condition in which oxygen is transported to tissues in an insufficient manner. This oxygen-depleted condition may exhibit impaired pathological features such as the inability to control progressive lesions [3,4]. Hypoxia in malignant tumors affects prognosis [5] and increases the likelihood of resistance to all treatment modalities which increases the higher probability of metastasis [6,7]. Therefore, the monitoring of diffuse oxygen concentrations in hypoxia cancer cells is of vital importance to the diagnosis. Over the years, molecular imaging methods, especially for PET/CT diagnostics, have greatly improved the diagnosis of hypoxia [8]. The most widely used radiopharmaceutical for cellular oxygen depletion imaging is [^{18}F]FMISO (half-life = 109.77 min). It is synthesized for the benefit of neuroscience and internal oncology starting from the precursor molecules, a 2-nitroimidazole derivative. Hypoxia imaging with [^{18}F]FMISO takes 20 - 30 min (whole body scan), and the scan is performed 2 - 4 h after intravenous injection. [^{18}F]FMISO is not trapped on areas covered by necrotic cells, instead, it binds living cells with hypoxic pO_2 levels below 10 mmHg [9].

The synthesis of [^{18}F]FMISO was automated using modules such as FXF–N (GE), Explora FDG4 (Siemens), IBA Synthera platform and other general robotic systems [10-16]. In 2005, the first automated synthesis of [^{18}F]FMISO using only SPE purification was reported by Tang *et al.* [13]. The Synthra RN_{plus} is a flexible and completely automated radiosynthesis system for routine production of a wide variety of [^{18}F]fluorine labeled compounds by nucleophilic substitution. It has its own software with manual and automatic working modes [17]. Previously, [^{18}F]FMISO was synthesized on the Synthra RN_{plus} followed by purification using HPLC. However, HPLC purification is time-consuming which should be avoided in daily production.

This report presents the synthesis of [^{18}F]FMISO in a fully automated Synthra RN$_{plus}$ module to support routine clinical use by reducing the cost of radiopharmaceutical production from the disposable cassette-based synthesizer, and reducing time without HPLC for purification.

Materials and methods

[^{18}F]FMISO synthesis

The solvents and chemical reagents were purchased from Sigma-Aldrich (Germany), Merck (Germany) and VWR (Germany) and were used as received. Cartridges were purchased from Waters (Germany). FMISO standard and its precursor 1-(2′-nitro-1′-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonyl-propanediol (NITTP) were purchased from ABX Advanced Biochemical Compounds (Germany).

The synthesis process of [^{18}F]FMISO radiopharmaceuticals began with the production of fluorine-18 by the ^{18}O(p,n)^{18}F reaction using 16.5-MeV protons in a cyclotron (model GE PETtrace 840) at the National Cyclotron and PET Center, Chulabhorn Hospital, Chulabhorn Royal Academy, Bangkok, Thailand. The yield of [^{18}F]fluoride was in the range of 29.7 - 44.4 GBq after bombardment for 25 - 30 min. The synthesis of [^{18}F]FMISO was carried out in the Synthra RN$_{plus}$ module (Synthra, Germany), as shown in **Figure 1**. The [^{18}F]FMISO was prepared by nucleophilic substitution of -OTs groups (tosyl or toluenesulfonyl) by [^{18}F]fluoride ion in NITTP, followed by removal of the -OTHP (tetrahydropyran) group with acid hydrolysis (**Figure 2**).

Figure 1 Synthra RN$_{plus}$ module [17].

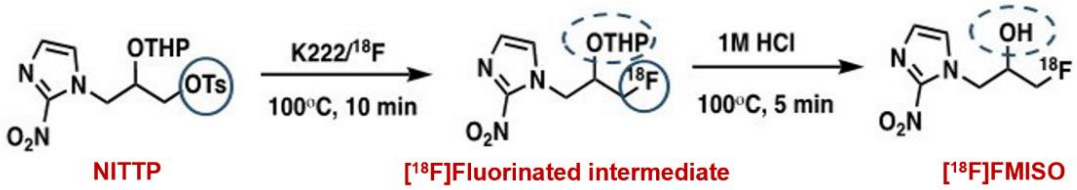

Figure 2 The [^{18}F]FMISO synthesis begins with the labeling of the NITTP precursor by [^{18}F]fluoride (solid circle), followed by an acidic hydrolysis reaction (dash circle).

The radiochemical synthesis and purification of [^{18}F]FMISO using a Synthra RN$_{plus}$ module and Sep-Pak cartridges consists of 3 brief steps below.

Fluorination of the precursor **(Figure 3A)**

First, [^{18}F]fluoride was trapped into the QMA cartridge, then separated from [^{18}O]H_2O by adding 1.5 mL of eluent (15 mg K222, 3 mg K_2CO_3, 1 mL acetonitrile and 0.5 mL H_2O) to elute [^{18}F]fluoride from the QMA cartridge (A1). The [^{18}F]fluoride was then dried with acetonitrile (Azeotropic drying) (A2). Five mg of NITTP precursor dissolved in 0.8 mL DMSO was added (A3) and [^{18}F]fluorination was carried out by replacing the leaving group -OTs with [^{18}F]fluoride at 100 ℃ for 10 min.

Elimination of the protection groups **(Figure 3A)**

Acetonitrile was removed by evaporation and acid hydrolysis was performed by adding 1 mL 1N HCl (A4) at 100 ℃ for 5 min.

Purification with Sep-Pak® cartridge and formulation **(Figures 3A and 3B)**

After hydrolysis, the [^{18}F]FMISO was neutralized with 1.5 mL 0.5N NaOH (A5) before purification using Sep-Pak cartridges (Oasis HLB Plus Extraction Cartridge, Sep-Pak Plus Short tC18 Cartridge and Oasis Wax Plus Short Cartridge) which were preconditioned with 5 mL ethanol, followed by 10 mL water and 30 mL air. The cartridges were rinsed with 12 mL of 3 % ethanol (C3). Finally, the [^{18}F]FMISO was eluted with 2 mL of 50 % ethanol (C2) followed by 7 mL normal saline (C1) into a sterile vial containing 7 mL normal saline and 1 mL citrate buffer. The eluents were passed through Alumina N column for trapping of free ^{18}F, and then passed through a 0.22-micron filter for sterility.

Quality control and identification

The radiochemical purity of the synthesized [^{18}F]FMISO was determined using a thin-layer chromatography (TLC) and HPLC. A TLC was performed using glass-backed silica plates (Merck F254) as stationary phase and acetonitrile:water (95:5) as mobile phase. A radio-HPLC system (Agilent 1200 Infinity Series) with UV-Vis and gamma detector (Gabi Star Raytest, Germany) was used. The typical HPLC analytical columns: Agilent InfinityLab Poroshell 120 EC-C18 column (150×4.6 mm^2, 4 µm) was used as the stationary phase and water:ethanol (95:5 v/v) as a mobile phase with 0.6 mL/min flow rate. Identity of [^{18}F]FMISO was confirmed by comparison of retention time with the certified reference standard (CRS) of main compound [^{18}F]FMISO.

Other identification tests including half-life measurements, pH, chemical purity**,** kryptofix content, bacterial endotoxins, sterile filter integrity, radionuclidic purity and sterility were performed as described for [^{18}F]FDG testing [18].

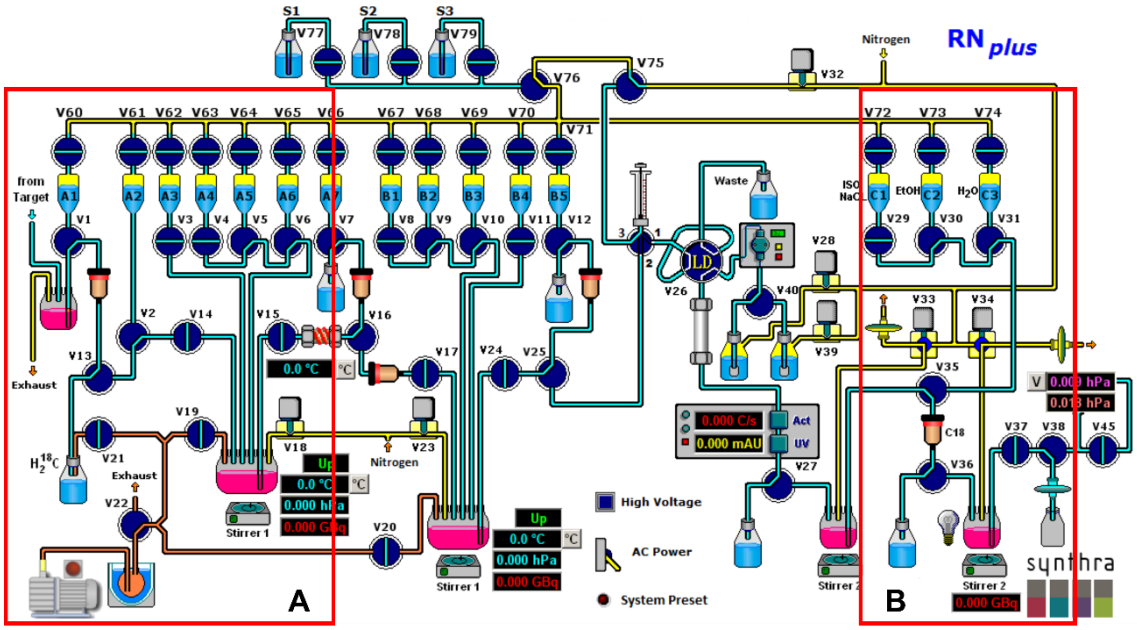

Figure 3 Schematic diagram of Synthra RN_{plus} module [17] used for [^{18}F]FMISO production: A) nucleophilic fluorination of NITTP and B) purification by column chromatography using a Sep-Pak cartridge.

Results and discussion

The [^{18}F]FMISO was successfully synthesized by a Synthra RN_{plus} using the NITTP as a starting material. The synthetic routes consist of 2 main reactions, starting with the substitution of the OTs group with [^{18}F]fluoride followed by the hydrolysis of the intermediate compound by the treatment with hydrochloric acid, as shown in **Figure 2**. Quality control of [^{18}F]FMISO according to the NCI CIP-held IND # 76,042 guidelines [19], including pH, chemical purity, radionuclidic identity, radiochemical purity, sterile pyrogen and others are shown in **Table 1**. A consistently high radiochemical yield and purity of [^{18}F]FMISO were obtained. Final yield was 29.11 ± 5.00 % without decay correction (n = 7). The radiochemical purity determined by a radio-HPLC system was 97.69 ±1.06 %. Total synthesis time was less than 35 min. Our results are in line with previously published works [10,18] but it was found that the modification takes less time to produce radiopharmaceuticals than some previously published [10,11,14].

Figure 4A shows chromatogram obtained from a UV-visible detector in which the signal of reference standard desmethylmisonidazole (by-product of [^{18}F]FMISO synthesis) appeared at 4.990 min and the signal of reference standard FMISO appeared at 8.198 min. **Figure 4B** shows chromatogram obtained from a UV-visible detector in which the signal of citrate buffer, desmethylmisonidazole by-product and [^{18}F]FMISO were recorded at 2.15, 5.044 and 8.291 min, respectively. The peak of [^{18}F]FMISO determined by a radiodetector was found at 8.900 min, as shown in **Figure 4C**.

Thin layer chromatography (TLC) was conducted to determine the radiochemical identity of [^{18}F]FMISO. Tang *et al.* [13] reported the TLC technique performed on aluminum sheet (RP C18 F254s; Merck) using ethyl acetate:ethanol (1:1 v/v) as a mobile phase, and Chang *et al.* [10] reported that the same technique but changing the ethyl acetate:ethanol ratio to 9:1 v/v. In this study, the same mobile phase used for quality control of [^{18}F]FDG, acetonitrile:H_20, 19:1 v/v, was applied and found to be effective. The results showed that the peak sharpness was similar and the Rf values were in the range of 0.843 ± 0.052 (n = 7), while the Rf values of free [^{18}F]fluoride were between 0.1 - 0.2. The radiochemical purity of the final [^{18}F]FMISO was > 95 %. There was no increase in radiochemical or chemical impurities and there were no signs of decomposition on HPLC and TLC signals for up to 6 h (data not shown).

The synthesis of [^{18}F]FMISO using the Synthra RNplus module instead of commercial disposable cassette systems has the advantage of reducing production time and cost. This module is flexible as both automatic and manual modes of operation can be used. Fully automated radiosynthesis system is ideal for routine production. The easy-to-use SynthraView [17] configuration software ensures clean and repeatable conditions for each production. This modification of [^{18}F]FMISO radiosynthesis using a non-cassette-based synthesizer in our laboratory will help to reduce manufacturing and treatment costs, which directly benefits patients in accessing hypoxia imaging.

Table 1 The acceptance criteria for quality control of the [^{18}F]FMISO radiopharmaceutical.

Parameter testing	**Acceptance criteria specification of [^{18}F]FMISO under the NCI CIP-held IND # 76,042 [19]**	**Sample reading (mean ± SD from 7 individual productions)**
Formulation	Solution	Solution
Appearance	Clear and colorless to yellow and free of particles	Clear and colorless
pH	4.5 - 8.0	5.69 ± 0.40
Radionuclidic identity of [^{18}F]fluoride		
Gamma spectrometry	0.511 ± 0.020 MeV	0.511 MeV
Half-life	110 ± 5 min	109.601 ± 0.975 min
Radiochemical identity of [^{18}F]FMISO		
TLC	$0.75 \geq Rf \leq 0.95$	Rf = 0.843 ± 0.052
HPLC	Rt = 7.5 - 9.0 min	Rt = 8.39 ± 0.35 min
Radiochemical purity of [^{18}F]FMISO		
TLC	≥ 95 %	98.43 ± 1.69 %
HPLC	≥ 95 %	97.69 ± 1.06 %
Chemical purity (HPLC)		
[^{18}F]FMISO	≤ 15.0 μg/dose	4.46 ± 3.86 μg/dose
Byproduct and other compounds	≤ 35.0 μg/dose	< 35 μg/dose

Parameter testing	Acceptance criteria specification of [^{18}F]FMISO under the NCI CIP-held IND # 76,042 [19]	Sample reading (mean ± SD from 7 individual productions)
Residual solvent level (GC)		
Ethanol content	≤ 10 %	4.59 ± 1.83 %
Acetonitrile	≤ 410 µg/mL	Not detected
DMSO	≤ 5,000 µg/mL	111.25 ± 36.14 µg/mL
Kryptofix content (TLC)	≤ 50 µg/mL	< 50 µg/mL
Bacterial endotoxins	< 17.5 EU/mL	< 10 EU/mL
Sterile filter integrity	≥ 45 psi	53.14 ± 1.77 psi
Sterility	Sterile	Sterile

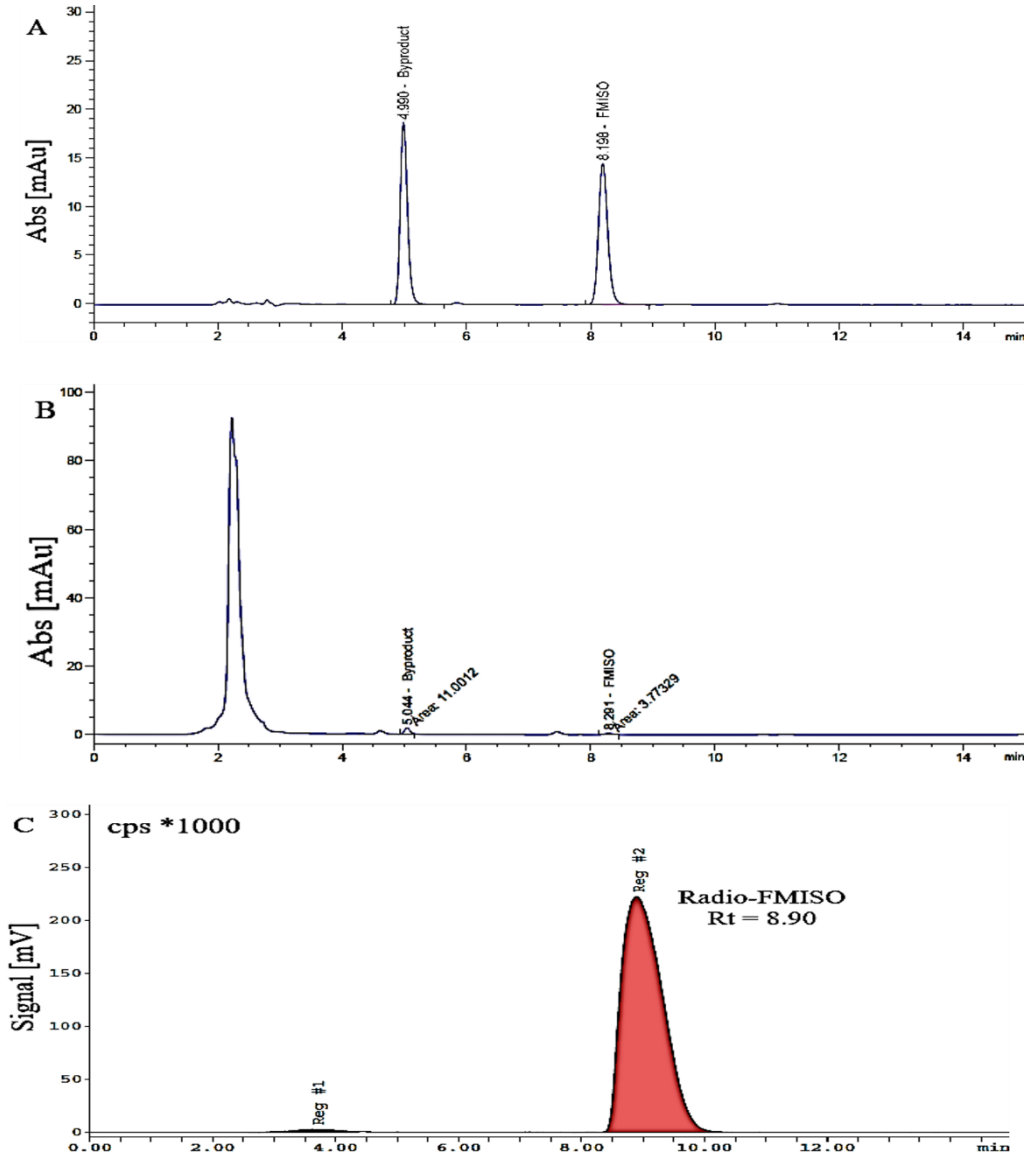

Figure 4 HPLC chromatogram of the purified [^{18}F]FMISO. A) UV chromatogram at 254 nm of reference standard desmethylmisonidazole by-product and reference standard FMISO appeared at 4.990 and 8.198 min, respectively. B) UV chromatogram of the synthesized [^{18}F]FMISO showing peaks of citrate buffer, desmethylmisonidazole by-product and [^{18}F]FMISO at 2.150, 5.044 and 8.291 min, respectively. C) Radio-HPLC chromatogram of the final [^{18}F]FMISO appeared at 8.90 min.

Conclusions

The [^{18}F]FMISO radiopharmaceutical was successfully synthesized using the Synthra RN_{plus} module instead of the disposable cassette-based synthesizer. The Sep-Pak cartridges were used for purification instead of HPLC. The quality of the synthesized [^{18}F]FMISO meets the acceptance criteria according to the NCI CIP-held IND #76,042 guidelines. The overall [^{18}F]FMISO yield is approximately 29.11 ± 5.00 % (n = 7), the total synthesis time is less than 35 min, and the radiochemical purity is greater than 95 %. These results are very useful for hypoxia PET imaging in diagnostic nuclear medicine in Thailand.

Acknowledgements

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