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[^{18}F]Fluoromisonidazole synthesis method: development and optimization by cartridge purification

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Abstract: [^{18}F]Fluoromisonidazole ([^{18}F]FMISO) as nitroimidazole derivative with ^{18}F radioisotope is a widely known and studied hypoxia marker for PET imaging. A number of automated synthesis modules and purification strategies for production of [^{18}F]FMISO have been described in recent years. The goal of this work was to develop [^{18}F]FMISO synthesis process with Synthera module with solid phase extraction (SPE) Sep-Pak purification cartridges. To adjust the reaction conditions we synthesized [^{18}F]FMISO under different reaction conditions and using various reversed-phase (RP) purification cartridges (HLB light, HLB plus, tC18, C18 environmental, Chromafix PS-RP). The synthesis was performed by nucleophilic substitution of commercial 1-(2′-nitro-1′-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulfonylpropanediol precursor and subsequent acidic hydrolysis. Further, the product mixture was purified by passing through the SPE cartridge. The produced [^{18}F]FMISO was retained on the cartridge, while the impurities passed through the cartridge into a waste. The retained [^{18}F]FMISO was then eluted with small amounts of ethanol in water and eluates were collected in the final product vial. The product sample was subjected to quality control tests, while for waste sample chemical and radiochemical tests were performed. We have developed an efficient synthesis method of [^{18}F]FMISO with cartridge purification with good radiochemical yield (RCY) and high chemical and radiochemical purity in accordance with the Ph. Eur. Monograph for Fluoromisonidazole (^{18}F) injection.

Keywords: production; purification; purity; Sep-Pak cartridges; [^{18}F]Fluoromisonidazole

1 Introduction

[^{18}F]FMISO is a well-known radiopharmaceutical for hypoxia imaging. It is typically produced through S_N2 nucleophilic fluorination using commercial precursor 1-(2′-nitro-1′-imidazolyl)-2-*O*-tetrahydropyranyl-3-*O*-toluenesulphonylpropanediol (NITTP). Although reversed-phase HPLC is still often used as purification method for [^{18}F]FMISO with high-resolution separation, it requires considerable amount of time which is not favorable for short-lived radiopharmaceuticals with fluorine-18 and it is inconvenient when applied in automatic synthesis [1–4]. To overcome these limitations, in the last decade, the development of solid-phase extraction (SPE) method has been the focus of [^{18}F]FMISO purification.

SPE offers advantages such as simple and faster purification, usage of commercial cartridges, easily adaptable, practical, fast and low cost. In order to achieve adequate radiochemical and chemical purity of the final product, a support mechanism for sorbent type and selectivity optimization plays a significant role [5–7]. Usually more than two cartridges are used or combination of several cartridges with different stationary phases, amounts of the sorbent, particle sizes and shape. SPE is generally rapid but depends on the used synthesis module and the purification procedure development can take considerable time and effort. The choice of SPE cartridge depends on the chemical properties of the final product and impurities generated during synthesis (polarity, molecular weight). On the other hand, the elution solution that can be used with the cartridge also plays an important role.

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Common sorbents for SPE purification of [18F]FMISO can be silica-based chemically bonded phases as C-18 cartridges or hydrophilic lipophilic balanced copolymer as the extraction phase as HLB cartridges, alumina inorganic oxides as alumina cartridges and group-selective or high-specificity sorbents as ion exchange cartridges.

Over the years, there has been ongoing development and optimization of solid-phase extraction (SPE) methods for the purification of [18F]FMISO. These methods are designed to be compatible with different automated synthesis modules and utilize commercial NITTP as the precursor.

Kämäräinen et al. in 2004 (Synthesis module by IBA) [8] and Tang et al. in 2005 (Tracerlab FX F–N module) [9] developed [18F]FMISO synthesis method using SPE purification for the first time, employing C-18, alumina and SCX Sep-Pak cartridges and as precursor they used commercial NITTP Wang et al. in 2009 (FDG4 module, Explora) used also C-18 Sep-Pak column and a neutral alumina Sep-Pak cartridge for the first time [3]. Nandy and Rajan in 2010 (Nuclear Interface Module) used just a single neutral alumina cartridge for purification [10]. Lee et al. (GE TRACERlab MX module) and Blom et al. (IBA Synthera) in 2013 used hydrophilic-lipophilic-balanced (HLB) polymer-based cartridges for the first time, and mixed-mode cation exchange cartridge by Lee or SCX and Alumina B (ALU B) by Blom et al. [11, 12]. Fernandez-Maza et al. and Antuganov et al. in 2018 used the same synthesis module TracerLAB Fx F–N but different solid-phase extraction procedures. Fernandez-Maza et al. produced [18F]FMISO with the shortest radiosynthesis time of 21 min [13] in contrast to the other authors above, using a triple bonding reverse phase tC18 and SCX. Antuganov et al. described several SPE purification procedure based on Chromabond Set, or Alumina N with tC18, HLB and C18 Environmental cartridges [14].

In general, the radiochemical yield is the most important indicator for the efficiency of the synthesis process. According to recommendation of “The Society of Radiopharmaceutical Sciences (SRS)” the “radiochemical yield” is decay corrected per definition [15].

All those eight research papers, have published an automatic synthesis method with SPE purification methods under similar radiolabeling conditions and produced [18F]FMISO with good decay-corrected radiochemical yield (RCY d.c.) of maximum 55 %, and high radiochemical and chemical purity.

Completely different types of purification cartridges in a Tracerlab FX FDG module improvement in the radiochemical yield was achieved by Cucchi et al. in 2022. The authors introduced polymeric reverse phased sorbent STRATA-X, SPE PS-H+ and QMA for final purification and achieved the highest radiochemical yield of 68 % [16].

Another research group during the last year tried to produce [18F]FMISO with in-house prepared cassettes with the FASTlab system for research and preclinical study with 39 % radiochemical yields and high radiochemical purity using Oasis HLB cartridges and alumina N cartridge as a purification cartridge [17].

In framework of these researches, the findings clarify the roles of SPE purification and the challenges to involve SPE purification instead of classical way of synthesis with high-pressure liquid chromatography (HPLC) purification method. Therefore, the aim of this study was the development and optimization of a method for [18F] Fluoromisonidazole synthesis by cartridge purification because it is characterized by ease of operation, low cost, low solvent consumption and high degree of flexibility. Along with this development the study aimed to adjust synthesis reaction to achieve good radiochemical yield of final product [18F]FMISO with high chemical and radiochemical purity.

2 Materials and methods

2.1 Reagents

Precursor 1-(2′-nitro-1′-imidazolyl)-2-*O*-tetra-hydro-pyranyl-3-*O*-tosyl-propanediol (NITTP), nucleophilic integrated fluidic processor (IFP cassettes), reference standard for [18F]FMISO and reference standard for byproduct of [18F]FMISO, desmethylmisonidazole (DMM) were purchased from ABX (Radeberg, Germany). Enriched [18O]-water was purchased from NUKEM isotopes (Alzenau, Germany); Kryptofix®222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]-hexacosan and potassium carbonate ≥99.0 % ACS were obtained from Sigma-Aldrich (Missouri, United States). Hydrochloric acid solution was prepared by dilution of 36 % HCl Suprapur (Merck, Darmstadt, Germany). Acetonitrile, 99.9+ %, HPLC for gradient analysis, from Thermo Scientific (Geel, Belgium) and ultrapure water (18.2 MΩ cm) for HPLC was prepared by Direct Q3 Millipore (Merck, Darmstadt, Germany).

2.2 SPE cartridges

The following disposable cartridges were used during development: Sep-Pak QMA Light; QMA Carbonate Plus Light Cartridge; Sep-Pak Alumina B; Sep-Pak tC18 Plus Short Cartridge, 400 mg sorbent; Sep-Pak C18 Plus Long Cartridge, 820 mg sorbent (C18 environmental); Oasis HLB Plus Short Cartridge, 225 mg sorbent; Oasis HLB Plus Light Cartridge, 30 mg sorbent were purchased from Waters (Massachusetts, USA); SCX Cartridge from S Pure (Nordcom One, Singapore); Chromafix PS-OH^- and PS-RP cartridges from Macherey-Nagel (Düren, Germany). All cartridges were preconditioned before use according to the manufacturer’s guidelines.

2.3 Production of [^{18}F]F$^-$

PETtrace 16.5 MeV GE Healthcare Cyclotron (Uppsala, Sweden) installed in University Institute of Positron Emission Tomography, Skopje, Republic of North Macedonia was used for the production of radionuclide [^{18}F]F$^-$ with proton irradiation of enriched water ($H_2{}^{18}O$) (NUKEM isotopes, Alzenau, Germany) in the niobium target by the reaction $^{18}O(p,n)^{18}F$.

2.4 Synthesis of [^{18}F]FMISO

IBA Synthera V2 synthesis module (Louvain la Neuve, Belgium) was used for the synthesis of 1H-1-(3-[^{18}F]fluoro-2-hydroxypropyl)-2-nitroimidazole ([^{18}F]FMISO) in consequential steps. The first step after start of synthesis was [^{18}F]F$^-$ trapping on an anion-exchange cartridge and recovery of oxygen-18 enriched water. Trapped [^{18}F]F$^-$ anions were eluted into the reaction vial with a cryptand solution eluent containing 22 mg Kryptofix®222, 4.2 mg K_2CO_3 in water acetonitrile mixture, 1:8. Trapping and elution performances were compared between the three resin types: QMA light, QMA Carbonate Plus Light Cartridge and PS-OH$^-$. The next step was azeotropic distillation under inert gas He at 110 °C. Prepared anhydrous [^{18}F]fluoride/K2.2.2/K_2CO_3 mixture is the main reactant in the next step of radio-fluorination for the production of ^{18}F-fluorinated intermediate via nucleophilic bimolecular S_N2 substitution reaction mechanism. During the development of the synthesis, the radiofluorination was performed for different time frames (3; 5; 7 and 10 min) and at different temperatures (100–140 °C). Also, different concentrations of NITTP precursors (2.5; 5 and 10 mg) were investigated. NITTP dissolved in 2 mL anhydrous CH_3CN was added and the fluorination was performed on heating the reaction mixture. Then, the reaction mixture was cooled and evaporated before next step of hydrolysis. The hydrolysis was carried after HCl solution (0.1 M) was added. After hydrolysis, the mixture was passed through the purification cartridges to waste. The final product was retained, while the impurities were washed out in the waste vial. The last step was elution of [^{18}F]FMISO from the cartridges with 5 % ethanol in water and collection of the eluate in a sterile vial. Activity of produced [^{18}F]FMISO was measured with VIK 202 activity calibrator (Veenstra, Comecer Netherlands). RCY was calculated based on activity of produced [^{18}F] FMISO in GBq expressed as a percentage (%) of related end of bombardment (EOB) activity, decay-corrected (d.c.) at EOB time.

2.5 Quality control tests

The final product [^{18}F]FMISO should meet the quality requirements and acceptance criteria of the EP monograph Fluoromisonidazole (^{18}F) injection 01/2014:2459. Radiochemical purity of the final product and waste sample were tested using HPLC and TLC method.

Agilent liquid chromatography system (Agilent, Santa Clara, USA) comprised of a 1260 Quaternary pump, a 20 µL injection loop, variable wavelength detector and radio-detector (Gabi Raytest Isotopen Messgerate GmbH, Germany) was used with end-capped polar embedded XTerra Shield RP18 Column, 125 Å, 5 µm, 4.6 mm × 250 mm (Waters, United States) and mixture of water and acetonitrile as mobile phase.

For TLC method, Radio-TLC miniGita Star (Raytest, Germany) device was used, silica gel TLC plates (Macherey-Nagel, Düren, Germany), and mixture of water and acetonitrile as a mobile phase. Chemical purity was tested using the same HPLC method as radiochemical purity and by color spot test on TLC plate for residual Kryptofix®222. Approximate pH value of the solution was determined using pH indicator strip with resolution 0.5 pH units (Merck, Darmstadt Germany).

3 Results and Discussion

Radiosynthesis and cartridge purification can be performed by different designed methods depending on the synthesis module and the laboratory resources. Synthesis sequences also should be specially designed for this purpose. In our case the sequence for automated production of [^{18}F]FDG ([^{18}F]Fluoro-2-deoxy-D-glucose) was modified and adapted for automated synthesis of [^{18}F]FMISO with Synthera V2 module. The goal was to develop an efficient synthesis method using cartridge for purification, that could be easily and simply applied for routine use. Even though synthesis with this module has some limitations, such as lack of extra reagent positions beyond four ones, absence of built-in HPLC for separation, we successfully developed method for synthesis with cartridge purification. We can highlight several key factors that are important for an efficient synthesis regardless of the module: types of cartridge for efficient trapping and elution solution for efficient recovery after elution of [^{18}F]F$^-$, effective drying of [^{18}F]F$^-$, precursor concentration, fluorination reaction parameters for effective labeling, hydrolysis reagent and reaction parameters for effective removing of the protective groups and types of SPE purification cartridge for effective purification. Optimization of some of these parameters was performed using multiple runs with starting activity of 20 GBq. Taking into consideration our synthesis experience of [^{18}F]Fluoro-2-deoxy-D-glucose synthesis we were able to choose correct type of strong anion-exchange cartridge and elution solution easily to achieve efficient trapping and elution of [^{18}F]F$^-$ [18]. Retained activity on QMA light, QMA Carbonate and PS-OH$^-$ cartridges arises from non-eluted [^{18}F]F$^-$. The results for retained activity on these cartridges showed high recovery of fluoride ions, more than 98 % for QMA light and QMA Carbonate cartridges and somewhat less, but more than 95 % for PS-OH- cartridge.

Fluorination reaction parameters for effective labeling are key factors for running successful nucleophilic ^{18}F-fluorination and good yield. The influence of fluorination temperature and fluorination time is summarized in Table 1. All experiments were performed with 5 mg NITTP.

Radiochemical yield was calculated based on activity of produced [^{18}F]FMISO in GBq expressed as a percentage (%) of related EOB activity, decay-corrected (d.c.) at EOB time. The influence of temperature and time on the yield

Table 1: Radiochemical yield, decay corrected (d.c. % ± SD) of the product in relation with the fluorination reaction parameters.

Fluorination time (min)	Fluorination temperature (°C)	[18F]FMISO mean RCY[a] (d.c. % ± SD)
3	100	5.32 ± 0.56
5	100	14.45 ± 1.14
7	100	32.33 ± 2.46
10	100	37.61 ± 2.46
10	110	42.79 ± 1.99
7	120	37.93 ± 1.59
10	120	46.77 ± 3.19
10	130	53.18 ± 3.44
7	140	30.07 ± 1.24

[a]All runs (batches) were carried out in triplicate.

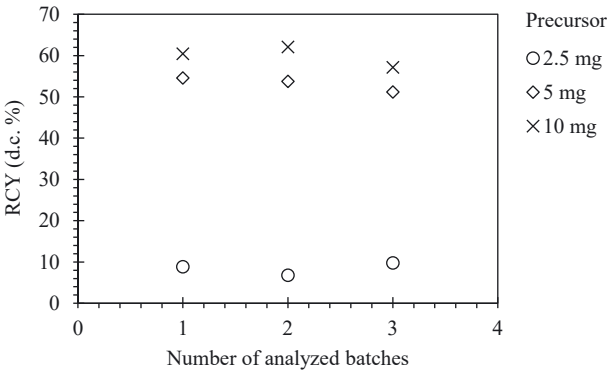

Figure 1: Effect of precursor amount (mg) on the yield of [18F]FMISO (RCY, decay corrected %).

was studied at the optimum heating range and time from 100 to 140 °C during 3–10 min. [18F]FMISO RCY increased with the increasing fluorination time from 3 to 10 min at 100 °C fluorination temperature (from 5.32 ± 0.56 % at 3 min up to 37.61 ± 2.46 % at 10 min). When fluorination time was 10 min, with the increasing temperature from 100 to 110 °C and 120 °C, RCY increased (Table 1). At fluorination temperature of 120 °C, with the decreasing fluorination time from 10 to 7 min the RCY decreased to 37.93 ± 1.59 %. The highest RCY (53.18 ± 3.44 %) was achieved by increasing the temperature to 130 °C during 10 min radio-fluorination. We tried to increase the temperature to 140 °C and decrease the time from 10 to 7 min, but the RCY decrease to 30.07 ± 1.24 % indicating an eventual thermal instability of the 18F-fluorinated intermediate product (Table 1). The findings underline the effect of temperature and time on the RCY. In our case, optimal radiofluorination results at 120 °C and 130 °C during 10 min were selected as the most suitable for this synthesis. Reaction at 130 °C during 10 min was chosen for next experiments. The conditions developed were similar to published radiofluorination data performed with the same commercial precursor but a different synthesizer and reagent kit [10,13,14,19].

Applying the proposed conditions, further optimization was performed. The effect of NITTP precursor amount (mg) on the yield was examined and is shown in Figure 1. It was observed that by increasing the precursor amount the RCY increased. Using 2.5 mg, very low RCY of 8.48 ± 1.26 % was obtained. There was a notable increase in the RCY with 5 mg of precursor (53.18 ± 3.44 %). The highest yield for the [18F]FMISO product at 59.88 ± 2.05 % was obtained with 10 mg of precursor. To choose the optimal precursor amount we were observing the presence of DMM impurity (by-product of [18F]FMISO synthesis) in the runs with 5 and 10 mg precursor amount.

The results of the chemical purity tests using the HPLC method showed an increase in the DMM by-product in runs with 10 mg of precursor compared to runs with 5 mg of precursor, which confirms that chemical purity was affected by the amount of NITTP precursor. Hence, aiming to achieve on good balance between yield and low amount of the DMM, as well as from economic viewpoint, we selected amount of 5 mg of precursor as optimal condition. As reported in literature, several authors used higher amount of the precursor than 5 mg, but they performed purification of cartridges by washing with water, after transferring of final mixture solution in waste in order to release the trapped polar by-product DMM to achieve high purity of the final product eluate [3,10,14]. IFP cassette in our study has only four positions for vials. Due to this limitation, additional washing with water was impossible. Therefore, as appropriate hydrolyzing reagent volume was chosen 4 mL, 0.1 M HCl solution as suggested concentration and volume by Blom and Antuganov [12,14]. To evaluate the influences of time and temperature with that reagent, we observed the reaction at different temperatures (90–120 °C) and reaction times (2–4 min). Unhydrolyzed [18F]FMISO intermediates were never detected in the final product confirming that complete hydrolysis was achieved in all runs, but at temperature lower than 120 °C more chemical unknown peaks were detected. As optimal hydrolysis conditions for removing the tetrahydropyranyl (THP) protecting group, were chosen the following: 3 min at 120 °C. More attention was given to the purification with SPE cartridges, because the purification principle for [18F]FMISO mentioned above would be more sensitive than the classical way of purification for [18F]FDG.

In our study, the cartridges should retain most of the product, while most of impurities pass through the cartridges and are collected in waste vial. In general, polarity of the impurities and final product, type of

cartridge, particle size and amount of sorbent, could influence the purification efficiency. Six different commercially available reverse phase cartridges (RP cartridges) were examined: 30 mg HLB light; 175 mg PS-RP; 225 mg HLB plus; 820 mg C18 Environmental; 400 mg tC18 and (30 mg HLB light) × 2. In observed runs, as elution solution for final product [18F]FMISO a 5 % ethanol solution was used. To optimize this step, the activity of waste solution, final product and residual activity of RP cartridges was monitored. Additionally, the chromatographic analysis of waste sample was performed by HPLC to determine the content of [18F]FMISO and DMM. Based on this data the percentage fraction of the product (% [18F]FMISO) eluted in the waste in relation to the total activity of the waste was calculated. The results of the main findings in this study along with their advantages and weaknesses are summarized in Table 2. [18F]FMISO was not eluted in waste using 225 mg HLB plus or combination of two 30 mg HLB light. [18F]FMISO ercentage fraction eluted in the waste (un-trapped) was low (1.65 ± 0.48 %) while using 820 mg C18 Environmental cartridges or fraction of final product was 14.44 ± 3.88 % using a 30 mg HLB. [18F]FMISO percentage fraction eluted in the waste was high with PS-RP and 400 mg tC18, 34.42 ± 4.58 % and 20.78 ± 2.31 %, respectively. Besides this factor for trapping performance, important factor is high elution efficacy of the trapped final product from the cartridge expressed through [18F]FMISO retained on RP cartridge in the table. This value represented the residual activity on RP cartridge, measured 5–10 h after the end of synthesis (EOS) and decay-corrected at EOB time, expressed as a percentage (%) of a related EOB activity. The percent of [18F]FMISO retained on the 225 mg HLB plus, 400 mg tC18 or combination of two 30 mg HLB light was high, 29.95 ± 4.67 %, 12.02 ± 1.34 %, and 10.23 ± 2.50 %, respectively. The percent of [18F]FMISO retained on RP cartridge was lower than 2 % on 30 mg HLB light, 175 mg PS-RP, and 820 mg C18 Environmental, indicating high elution efficacy of the trapped final product from these cartridges.

Considering that high chemical purity is the key parameter for effective purification, by-product DMM concentration in waste was compared with DMM concentration in final solution. These results are presented in the table as by-product DMM eluted in waste, and by-product DMM in final product in addition of factors mentioned previously (trapping performance and elution efficacy). As shown in Table 2, it was noted that almost the whole content of DMM by-product passed in the waste using 30 mg HLB, PS-RP and 400 mg tC18.

Considering all these results on trapping and elution behavior of [18F]FMISO and by-product DMM with different RP cartridges, we can point out that high purification efficacy was achieved with 30 mg HLB with 6.26 ± 0.82 mg/mL in final solution, but also showed 14.44 ± 3.88 % passing of [18F]FMISO in waste as not retained product on the cartridge. [18F]FMISO was completely trapped on C18 Environmental cartridge with high purification efficacy (6.70 ± 0.55 mg/mL DMM in final product). Characteristic of this cartridges is its chemical behavior towards DMM, the by-product was retained on the cartridge but was only partially removed in the final product. Subsequently, the presence of DMM was confirmed by HPLC in elution after washing with water. Based on these results we could choose C18 Environmental cartridge for further use, in combination with cation exchange cartridge. On the other hand, 30 mg HLB light cartridge showed good purification performance in context of chemical purity and there was great applicability in this study. According to published data, Antuganov et al. performed successful SPE purification with C18 Environmental cartridge but with washing on sorbent after transferring the final product solution to remove polar impurity. In contrast to those authors, effective purification without water washing was achieved by Blom et al. using 30 mg HLB light cartridge [12,14]. Therefore, we continue to monitor the behavior of these two cartridges in future development to recognize better characteristic in combination with IC-H+ or PS-H^+ cation exchange cartridges instead of typical SCX. Performing the synthesis without or

Table 2: Trapping and elution behavior of [18F]FMISO and by-product DMM with different reversed-phase (RP) cartridges.

RP cartridge[a] (weight of sorbent and type)	[18F]FMISO eluted in waste (mean % d.c. ± SD)	[18F]FMISO retained on RP cartridge (mean % d.c. ± SD)	By-product DMM eluted in waste (mg/mL, mean ± SD)	By-product DMM in final product (mg/mL, mean ± SD)
30 mg HLB light	14.44 ± 3.88	0.48 ± 0.15	40.19 ± 7.83	6.26 ± 0.82
175 mg PS-RP	34.42 ± 4.58	0.89 ± 0.20	37.12 ± 6.66	9.2 ± 0.85
225 mg HLB plus	0	29.95 ± 4.67	3.80 ± 1.33	16.24 ± 4.9
820 mg C18 environmental	1.65 ± 0.48	1.71 ± 0.61	1.60 ± 0.31	6.70 ± 0.55
400 mg tC18	20.78 ± 2.31	12.02 ± 1.34	32.34 ± 5.71	8.27 ± 0.67
(30 mg HLB light) × 2	0	10.23 ± 2.50	17.50 ± 2.23	6.50 ± 0.56

[a]All RP cartridges were tested in triplicate.

minimizing the evaporation time of acetonitrile after the fluorination are also a goal in our future development.

The radiochemical purity (RCP) and chemical purity of the final product [18F]FMISO solution were tested using HPLC and TLC method following the Ph. Eur. monograph procedures. According to this procedure standard solutions for identifying [18F]FMISO and DMM peaks were prepared. Figure 2 shows HPLC UV chromatogram obtained with the concentration 10 mg/mL FMISO and 10 mg/mL DMM. The signal of reference standard DMM appeared at 3.56 min and of FMISO at 6.04 min.

The final [18F]FMISO solution was diluted with saline solution by a factor of two prior to quality control tests. The results of testing chemical purity with HPLC method were in accordance with the acceptance criteria of the Ph. Eur. Monograph for Fluoromisonidazole and related substances. The UV chromatogram of the final product (Figure 3, bottom) indicated presence of five more peaks beside the peak of DMM (R/T 3.52 min) and peak of FMISO (R/T 5.58 min). Areas of FMISO and DMM impurities peaks were not more than the area of corresponding peaks in the chromatogram obtained with reference solutions in Figure 2 (<0.1 mg/V). The area of each of the other impurities was not more than the area of the principal peak in the chromatogram obtained with FMISO reference solution (Figure 2). Total area of all impurities was not more than 5 times the area of the principal peak in the chromatogram obtained with FMISO reference solution (Figure 2). The peak close to DMM is some unidentified labeled polar byproduct, and peak on Reg#5 was probably 1-chloro-3-(2-nitro-imidazol-1-yl)-propan-2-ol (Chloromisonidazole/CIMISO) but we could not confirm this due to the absence of mass spectrometry analysis. Other minor peaks, which may be potentially present impurities, are: 5-hydroxypentanal;

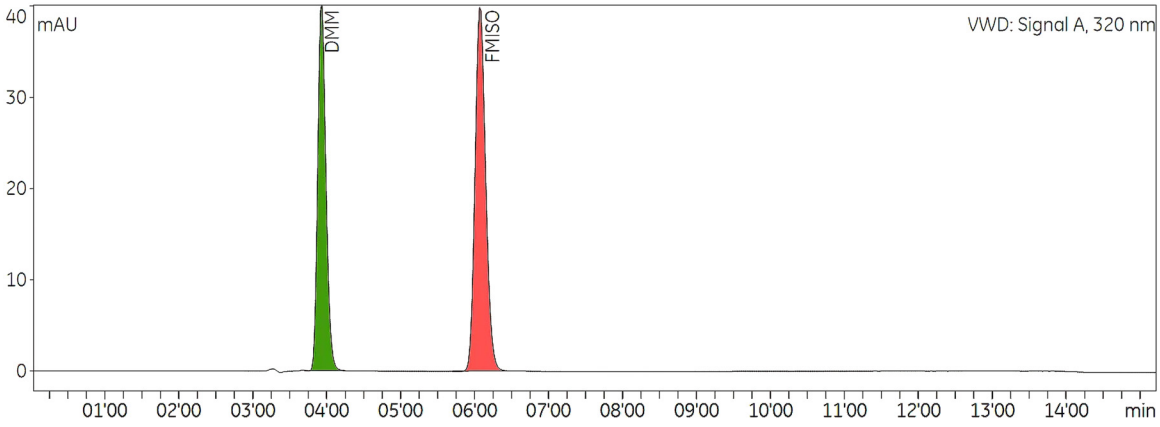

Figure 2: HPLC chromatogram of DMM and FMISO reference solutions (10 mg/mL each).

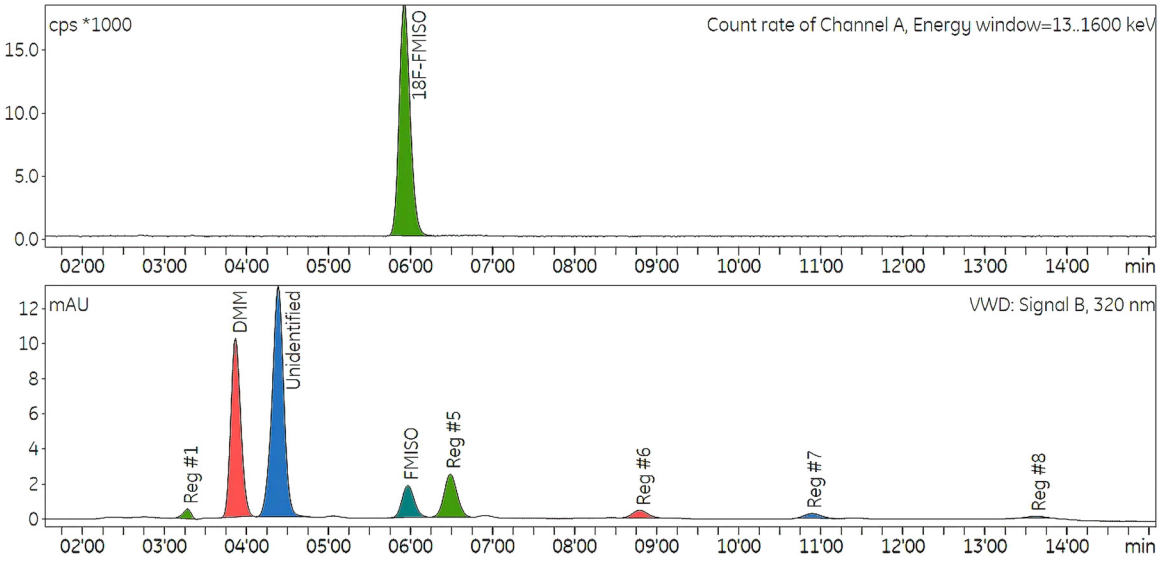

Figure 3: HPLC chromatograms of the purified [18F]FMISO. Radio chromatogram (top): peak of [18F]FMISO; and UV chromatogram (bottom): peaks of chemical impurities.

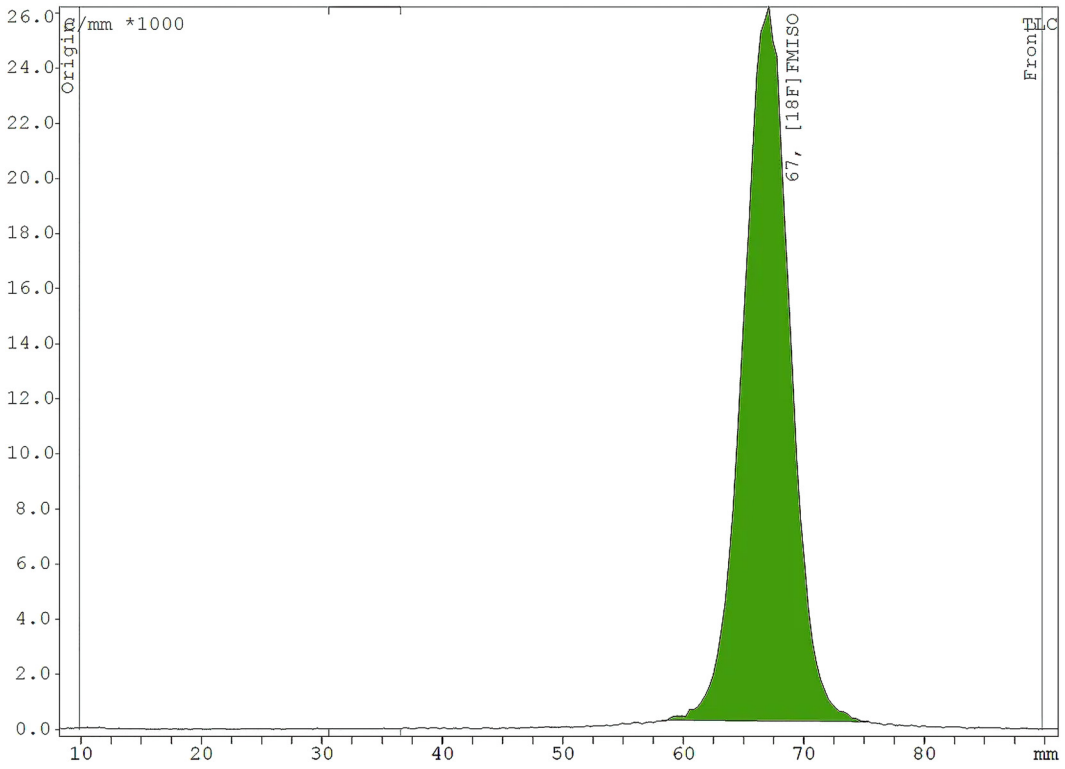

Figure 4: Radio-TLC of the final [18F]FMISO solution.

4-methylbenzenesulfonate, 2-nitro-1-phenylpropane-1,3-diol or 2-(5-nitroimidazol-1-yl)ethanol.

As part of testing chemical purity, residual Kryptofix®222 was checked by color spot test on TLC plate. The results in all tested samples showed no presence of K2.2.2.

The results of testing radiochemical purity with HPLC and TLC method were also in accordance with the acceptance criteria of the Ph. Eur. Monograph, minimum 95 % [18F]FMISO of the total radioactivity due to fluorine-18. All HPLC radiochromatograms and TLC chromatograms showed radiochemical purity more than 99 % (Figure 3, top and Figure 4 respectively).

Although the neutralization was not performed after the hydrolysis, the pH value was 6.5–8.0, tested by pH test strip.

4 Conclusions

In this study, the objective was to develop suitable synthesis reaction conditions for the production of [18F]FMISO using the Synthera V2 module, along with optimizing the purification process using Sep-Pak purification cartridges and the NITTP commercial precursor. Since multiple parameters can influence the quality and yield of the product, the researchers were tasked with examining these parameters.

The [18F]FMISO product was successfully produced with good RCY (53.18 ± 3.44 %) compared with recent published articles. Quality control analysis of the final product solution showed no radiochemical impurities and very low levels of chemical impurities, i.e. results of testing radiochemical and chemical purity were in accordance with acceptance criteria stated in the Ph. Eur. Monograph for Fluoromisonidazole (18F) injection. This developed method might be promising and an easily applicable synthesis process for [18F]FMISO production. Furthermore, based on this study, we continue to investigate in more details, in order to understand the challenge of high recovery yields and purity.

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