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RESEARCH ARTICLE

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Automated GMP production and long-term experience in radiosynthesis of CB_1 tracer [^{18}F]FMPEP-d_2

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Here, we describe the development of an in-house-built device for the fully automated multistep synthesis of the cannabinoid CB_1 receptor imaging tracer (3*R*,5*R*)-5-(3-([^{18}F]fluoromethoxy-d_2)phenyl)-3-(((*R*)-1-phenylethyl)amino)-1-(4-(trifluoromethyl)phenyl)pyrrolidin-2-one ([^{18}F]FMPEP-d_2), following good manufacturing practices. The device is interfaced to a HPLC and a sterile filtration unit in a clean room hot cell. The synthesis involves the nucleophilic ^{18}F-fluorination of an alkylating agent and its GC purification, the subsequent ^{18}F-fluoroalkylation of a precursor molecule, the semipreparative HPLC purification of the ^{18}F-fluoroalkylated product, and its formulation for injection. We have optimized the duration and temperature of the ^{18}F-fluoroalkylation reaction and addressed the radiochemical stability of the formulated product. During the past 5 years (2013–2018), we have performed a total of 149 syntheses for clinical use with a 90% success rate. The activity yield of the formulated product has been 1.0 ± 0.4 GBq starting from 11 ± 2 GBq and the molar activity 600 ± 300 GBq/μmol at the end of synthesis.

KEYWORDS

fluoroalkylation, GMP, nucleophilic ^{18}F-fluorination, positron emission tomography, radiochemistry, radiopharmaceutical

1 | INTRODUCTION

Positron emission tomography (PET) is a medical imaging technique that can be used to study molecular-level function, without interfering with physiological processes in the brain and body. With the application of specific radiotracers, PET can be used to investigate the cannabinoid subtype 1 (CB_1) receptor.[1,2] CB_1 receptors are localized throughout the body, including in the heart, lung, and bone marrow,[3,4] but are primarily found in the brain, including basal ganglia, globus pallidus, entopeduncular nucleus, substantia nigra pars reticulata, cerebellum, and hippocampus.[4–6] CB_1 receptors belong to the G-protein coupled family of receptors and can inhibit the release of various excitatory or inhibitory neurotransmitters.[5] It is believed that abnormalities in CB_1 receptor density or function may contribute to neuropsychiatric and neurodegenerative disorders. To investigate this possibility, CB_1-specific PET radiotracers have been

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developed for preclinical and clinical use. The following radioligands are reportedly suitable for CB_1 receptor imaging with appropriate specific binding: [^{11}C]JHU75528 ([^{11}C]OMAR, 4-cyano-1-(2,4-dichlorophenyl)-5-(4-[^{11}C]methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide),[7,8] [^{18}F]MK-9470 (*N*-{(1*S*,2*S*)-2-(3-cyanophenyl)-3-[4-(2-[^{18}F]fluoroethoxy)phenyl]-1-methylpropyl}-2-methyl-2-[(5-methylpyridin-2-yl)oxy]propanamide),[9] [^{11}C]PipISB and [^{18}F]PipISB (PipISB: *N*-(4-fluorobenzyl)-4-[3-(piperidin-1-yl)indole-1-sulfonyl]benzamide]),[10] [^{11}C]SD5024 ((−)-3-(4-chlorophenyl)-*N*-[(4-[^{11}C]cyanophenyl)sulfonyl]-4-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamidine),[11,12] [^{11}C]MePPEP ((3*R*,5*R*)-5-(3-[^{11}C]methoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)),[13] and [^{18}F]FMPEP-d_2 ((3*R*,5*R*)-5-(3-([^{18}F]fluoromethoxy-d_2)phenyl)-3-(((*R*)-1-phenylethyl)amino)-1-(4-(trifluoromethyl)phenyl)pyrrolidin-2-one).[1]

The synthesis of [^{18}F]FMPEP-d_2 was first reported in 2008 by Donohue et al.,[1] who studied four different radiotracers and found that [^{18}F]FMPEP-d_2 was the most suitable for CB_1 receptor imaging.[1] Moreover, [^{18}F]FMPEP-d_2 is reportedly a superior tracer compared with [^{11}C]MePPEP, due to greater precision and accuracy in detecting significant differences in CB_1 receptor tracer uptake.[2] To date, [^{18}F]FMPEP-d_2 has been used to study conditions associated with abnormal levels of CB_1 receptor binding, including neurological disorders (e.g., schizophrenia)[14] and alcohol abuse.[15,16] In preclinical and clinical studies at Turku PET Centre (TPC), [^{18}F]FMPEP-d_2 has been used to image CB_1 receptor expression in various settings, including in a mouse model of Alzheimer's disease,[17] in human brown adipose tissue,[18] and in psychosis.[19] Additionally, [^{18}F]FMPEP-d_2 has been used to study differences in CB_1 expression between sexes.[20]

Due to radiation safety issues and the high demand for [^{18}F]FMPEP-d_2 at TPC, automation of [^{18}F]FMPEP-d_2 synthesis (Figure 1) was deemed necessary. Herein, we report the construction of a fully automated synthesis device for [^{18}F]FMPEP-d_2 production, as well as 5 years of radiosynthesis experience. All steps in the production and quality control (QC) have been validated according to good manufacturing practices (GMP).[21,22] Here, we focus on the radiolabelling of the particular radiotracer, [^{18}F]FMPEP-d_2, via the [^{18}F]bromofluoromethane-d_2 synthon in order to introduce the prosthetic group [^{18}F]CD_2F- on a heteroatom group attached to the aromatic ring. The practical approaches used in the construction of the synthesis device evolved from our earlier work on similar labelling procedures[23–25] and are generic and applicable for similar types of radiolabelling of various constructs.

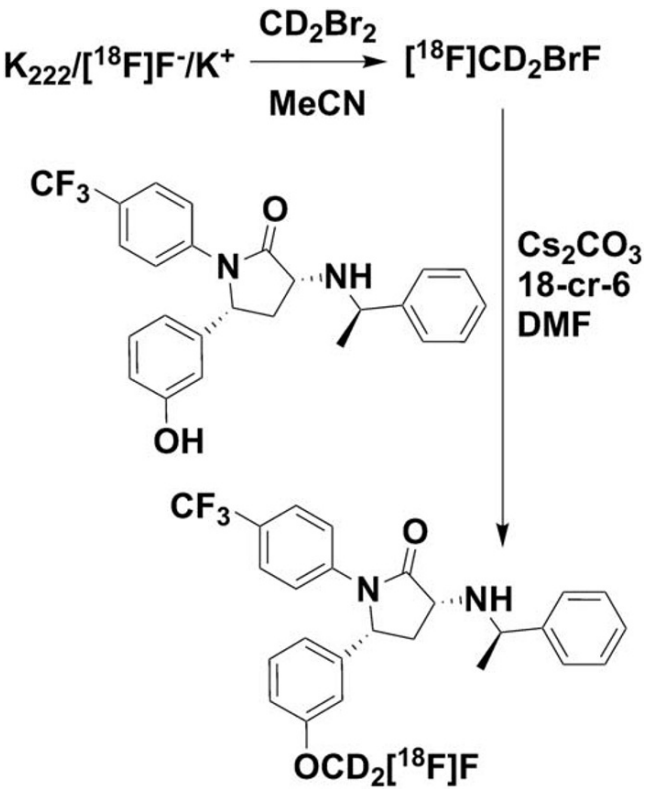

FIGURE 1 Radiosynthesis of [^{18}F]FMPEP-d_2

2 | EXPERIMENTAL SECTION

2.1 | Materials and methods

2.1.1 | Chemicals and materials

The precursor compound (3*R*,5*R*)-5-(3-hydroxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl) pyrrolidine-2-one (GMP grade) and the reference compound (3*R*,5*R*)-5-[3-(fluoromethoxy)phenyl]-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidine-2-one were supplied by PharmaSynth AS (Tartu, Estonia). 1,10-Diaza-4,7,13,16,21,24-hexaoxabicyclo[8,8,8]-hexacosane (Kryptofix 2.2.2, K_{222}), cesium carbonate (Cs_2CO_3; 99.9% trace metals basis), dibromomethane-d_2 (CD_2Br_2; 99 atom% D, contains copper as stabilizer), 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6; ≥99.0%), acetonitrile (anhydrous, 99.8%), *N*,*N*-dimethylformamide (DMF; anhydrous, 99.8%), and trifluoroacetic acid (TFA; ≥99.8%) were obtained from Merck (Dramstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA). Sterile water (aqua sterilisata, i.v.) was obtained from B. Braun Melsungen (Melsungen, Germany) and NaCl solution (9 mg/ml, sterile, i.v.) from Fresenius Kabi (Uppsala, Sweden). Acetonitrile (≥99.9%) was obtained from Honeywell (Muskegon, MI, USA),

ascorbic acid from Sanorell Pharma (Baiersbronn, Germany), ethanol (Ph. Eur.) from Berner (Helsinki, Finland), and helium from AGA (Espoo, Finland). All reagents and solvents were used as received from the commercial suppliers.

Solid-phase extraction (SPE) cartridges, Light C18 Sep-Pak, and Accell Plus QMA Carbonate were obtained from Waters Corp. (Milford, MA, USA). Sterile Millex GP syringe filters with polyethersulfone membrane (0.22 μm, 33 mm) were obtained from Millex, EMD Millipore (Billerica, MA, USA). Sterile Acrodisc® syringe filters with Supor® membrane (hydrophilic polyethersulfone, 0.2 μm, 13 mm) were obtained from Pall Corporation (New York, NY, USA).

2.1.2 | Chromatographic methods

[^{18}F]Bromofluoromethane was purified via semipreparative gas chromatography (GC) using an in-house filled column (8 × 300 mm, Haysep Q, 80/100 mesh or Porapak Q, 80/100 mesh; Supelco Inc, Bellefonte, PA, USA) as previously described.[23,25–27] Helium was used as a carrier gas, and the He flow was controlled outside the hot cell with a pressure reducing valve attached to a central gas helium supply. The column oven temperature was maintained at 90°C during separation. Before every synthesis, the GC column was conditioned at 170°C with He flow for a minimum of 1 h. The GC system is an integral part of the synthesis device.

Semipreparative radio-HPLC was performed using a Jasco PU-2089 HPLC pump (Jasco Inc, Easton, Maryland, USA) or a Merck-Hitachi L-6200 HPLC pump (Merck AG) equipped with a miniature Geiger-Müller (GM) tube at the column outlet, and a Luna® C18^2 100 Å column (10 μm, 10 × 250 mm; Phenomenex, Milford, MA, USA). Isocratic elution with 57:43 1% TFA:CH_3CN, with the addition of 500 mg/L ascorbic acid, and a flow rate of 8.0 ml/min was used. Retention time of [^{18}F]FMPEP-d_2 was 13.5 ± 0.7 min.

Analytical radio-HPLC was performed using a Hitachi L-2000 series HPLC pump equipped with a VWR-Hitachi L-2400 UV-absorption detector (λ = 254 nm), a 2 × 2-inch NaI radioactivity detector in series at the column outlet, and a Luna C18^2 column (3 μm, 4.6 × 100 mm; Phenomenex). Isocratic elution with 0.1% TFA:CH_3CN (55:45) and a flow rate of 0.95 ml/min were used. Retention time of [^{18}F]FMPEP-d_2 was 5.5 ± 0.3 min.

2.2 | Automation

2.2.1 | Synthesis device

A fully automated synthesis device for [^{18}F]FMPEP-d_2 production was built in our laboratory (see Figures S1 and S2). This synthesis device was constructed from pneumatic or electric two- and three-port valves and pneumatic six-port distribution valves for liquid and gas path control. Liquid transfer was achieved using either suction or single-use plastic syringes connected to pneumatically operated pistons. Suction was generated using a Teflon membrane pump. Reaction vials 1 and 2 were heated by heaters that utilize compressed air and electrical heating. After several years of use, the heater of reaction vial 2 was changed to an oil bath. The system was interfaced with the GC system, HPLC injector, and semipreparative column. A sterile filtration unit (SFU) was attached to the synthesis device, and this SFU was also used for the sterile filter integrity test (pressure hold test, PHT).

The synthesis device, HPLC injector, semipreparative column, and the SFU were placed inside a hot cell situated in a clean room (EU grade C). These units were controlled from a touch screen. The HPLC pump, ancillary electronics, power supplies, and control systems and the control system for the gas lines were placed in a closed cabinet outside the hot cell.

2.2.2 | Control system

An in-house-built control system for the synthesis device was utilized, as previously reported.[28] Briefly, the control system is based on programmable logic control units (PLC) interfaced with electromagnetic and pneumatic valves for liquid and gas path control. Similarly, transducers for radioactivity, temperature, and position are interfaced with the PLC. A supervisory program, based on one hand on timing sequences and also on responses from the transducers, is in overall control of the automated system. Human control of the system is realized on a touch screen situated outside the hot cell. The human operator can intervene with "pause," "continue," and "skip" commands.

2.3 | Radiochemistry

Absolute radioactivity measurements were performed using a calibrated ionization chamber (VDC-405; Veenstra Instruments, Joure, The Netherlands).

2.3.1 | Preproduction procedures

Before every synthesis, the synthesis device and the SFU were cleaned, and the lines dried following internal device-specific written instructions, and all of the disposable items were changed to new ones. K_{222} (12.0 ± 1.0 mg) was measured into a rounded-bottom glass vial (reaction vial 1) and dissolved in CH_3CN (0.10 ± 0.05 ml). The precursor (0.6 ± 0.2 mg), Cs_2CO_2 (2.0 ± 0.2 mg), and 18-crown-6 ether (5.0 ± 0.5 mg) were weighted into a 2.5-ml glass conical vial (reaction vial 2) and dissolved in DMF (0.8 ± 0.2 ml). EtOH (1.0 ± 0.1 ml) was measured into a 1-ml conical vial. The reaction vials and the EtOH vial were capped with PTFE/silicone septa (Tuf-Bond, Thermo Scientific, Rockwood, TN, USA) and attached to the device (Figure 2). Next, the reagents were loaded into appropriate positions as follows: (1) dibromomethane-d_2 stock solution (0.9 ± 0.2 ml, 90 μl/ml) to reservoir syringe P on valve V4 (Figure 2B); (2) HPLC dilution solution (1.0 ± 0.2 ml, 5 μl/ml ascorbic acid in 1% TFA) to the reservoir syringe on valve V1, port D; (3) the HPLC fraction dilution solution and SPE cartridge washing solution (20 ± 2 ml, 0.5% ascorbic acid in water) to 60-ml plastic syringes S2 and S4 (Figure 2C); (4) sterile filtered air to a 60-ml plastic syringe S3; and (5) the formulation solution (10.0 ± 0.5 ml, 0.9% NaCl solution) to syringe S5 (Figure 2D). The SPE cartridge (Light C18) was preconditioned with ethanol (7.0 ml) and sterile water (10 ml) and connected between valves V11 and V12 (Figure 2C).

The end-product vial set comprised a sterile pyrogen-free glass vial, two sterile filters (Acrodisc® syringe filter for end-product filtration and Millex GP syringe filter for ventilation), and a syringe for QC sampling. This end-product vial set was assembled in an isolator (clean room grade A). The end-product vial was labeled and placed in a lead container on the laminar-air-flow (LAF) box situated inside the hot cell. The tubing from the SFU was attached to the end-product vial via the sterile filter.

2.3.2 | [^{18}F]fluoride production

[^{18}F]Fluoride production and transfer were conducted as previously described.[28] Briefly, oxygen-18 enriched water (98%, Rotem Industries Ltd, Medical Imaging, Dimona, Israel) in an in-house constructed niobium target with a 25-μm-thick stainless steel window (AISI 321, Goodfellow Cambridge Ltd., Huntingdon, England) was irradiated with 17-MeV protons produced with a CC-18/9 cyclotron (Efremov Institute of Electrophysical Apparatuses, St Petersburg, Russia). The beam current was 40 μA. Subsequent to the irradiation, the target water was trapped in a carbonated QMA cartridge which was preconditioned with 10-ml ultrapure (18 MΩ) water. [^{18}F]Fluoride was eluted with 1.5 ml of 3 mg/ml potassium carbonate solution. This solution was transferred to the synthesis device, and the line was flushed with three 1-ml portions of CH_3CN.

2.3.3 | [^{18}F]FMPEP-d_2 production

The automated synthesis (Figure 2) was controlled and monitored from the touch screen and comprised the following automated steps:

1 Transfer of aqueous [^{18}F]fluoride to reaction vial 1. Once the K_2CO_3-containing [^{18}F]fluoride solution and the first CH_3CN batch arrived to the hot cell, the automated synthesis was started upon touching the "start synthesis" button. The [^{18}F]fluoride solution was transferred from the [^{18}F]fluoride reservoir syringe (V1, port A) to syringe S1 via valve V2 and then to reaction vial 1 via valves V3 and V4.
2 Removal of target water part 1. The dry K_{222}/[^{18}F]/K^+-complex was formed by azeotropic distillation under reduced pressure and helium flow. Reaction vial 1 was lowered down to the heater and heated for 6 min at 110°C. Controlled vacuum was applied via valves V5 and V6, and He flow supplied via valves V3 and V4.
3 Removal of target water part 2. Azeotropic distillation was repeated twice, adding one batch of CH_3CN at a time using the same transport system as used for the [^{18}F]fluoride solution (see step 1). Heating occurred for a minimum of 4 min per batch. After the last drying step, reaction vial 1 was removed from the heater and allowed to cool for 1 min under He flow and controlled vacuum.
4 [^{18}F]Bromofluoromethane-d_2 production. Pre-loaded dibromomethane-d_2 was added to reaction vial 1 via valve V4, with suction administered via valves V5 and V6. The reaction solution was mixed for 10 s, with He flow administered via valves V3 and V4 through the solution. The reaction vial was lowered down to the heater and heated for 5 min at 110°C to produce gaseous [^{18}F]bromofluoromethane-d_2. After the reaction, reaction vial 1 was raised from the heater and allowed to cool for 1 min.
5 GC separation. [^{18}F]Bromofluoromethane-d_2 was separated from dibromomethane-d_2 by gas chromatography. He flow was administered through the reaction solution via valves V3 and V4, and to the GC column via valves V5 and M1 (pre-set to chromatographic direction), and out of the column via valve V8.

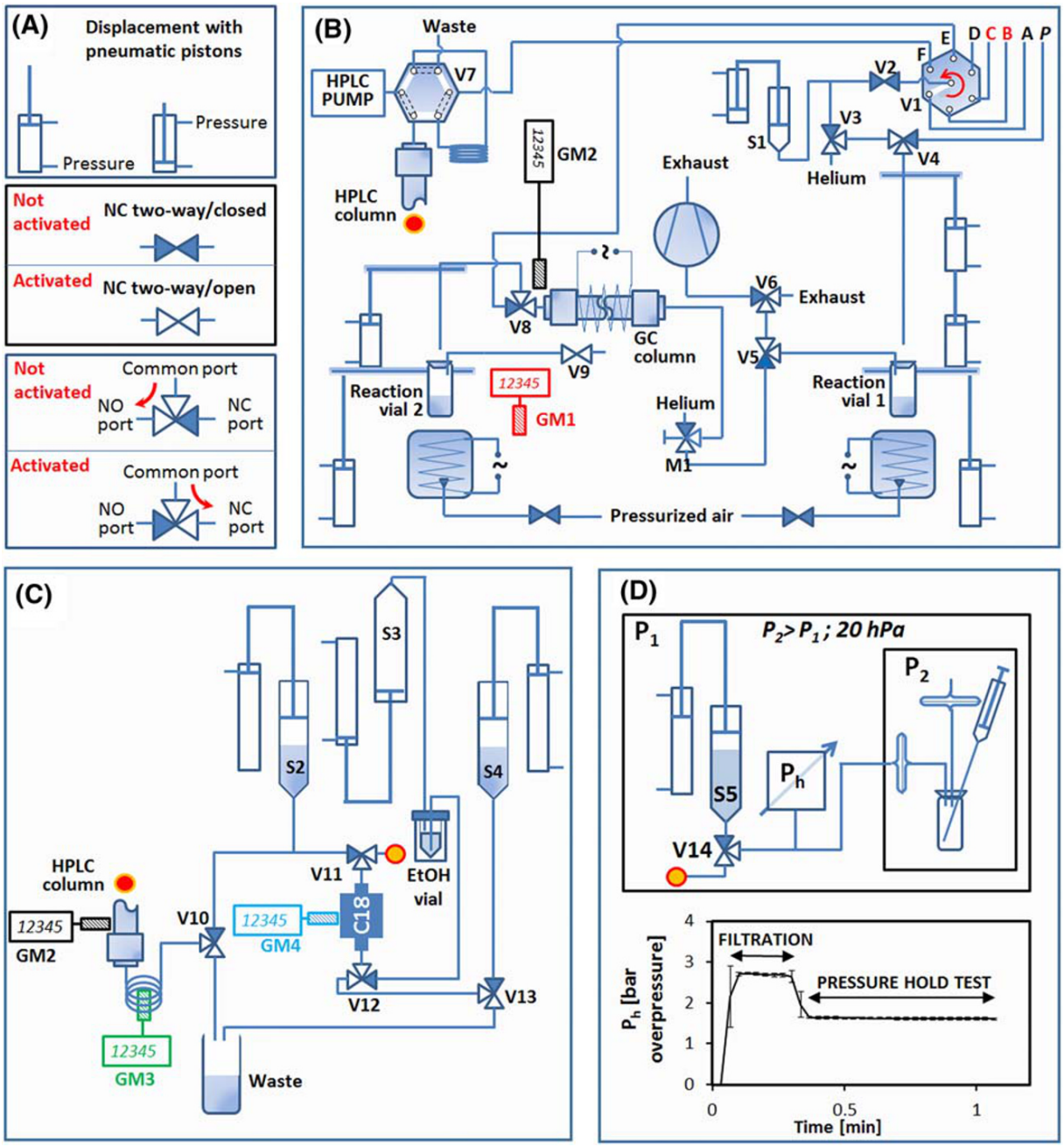

FIGURE 2 Schematic diagrams of the automated system used for [^{18}F]FMPEP-d_2 synthesis. (A) Explanations for the valves and pistons are presented (NO, normally open; NC, normally closed). (B) The reaction units, the GC separation unit for [^{18}F]FBrCD$_2$ separation and the HPLC purification unit of [^{18}F]FMPEP-d_2. (C) Collection of the HPLC fraction and the solvent exchange unit with a solid-phase extraction (SPE) cartridge. (D) The sterile filtration unit (SFU), which is also used for the pressure hold test for sterile filter integrity. The vial containing the end product is situated in a separate LAF box inside the hot cell. The pressure difference between the hot cell and the LAF box inside the hot cell is 20 mbar

Separation was monitored by a GM tube situated at the outlet of the GC column (GM2) (Figure 3).

6 Collection of [^{18}F]bromofluoromethane-d_2. When the count rate of the GM2 exceeded a pre-set level, [^{18}F] bromofluoromethane-d_2 collection was automatically initiated by setting the needle to the bottom of the reaction vial 2 containing the precursor solution. When the GM2 indicated that all of the [^{18}F] bromofluoromethane-d_2 had passed through the column, the operator pressed the "Skip" button on the touch screen to terminate collection, and the next step immediately started.

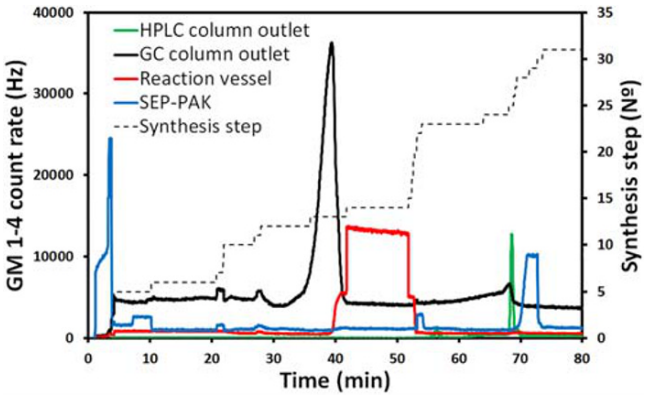

FIGURE 3 The data collected from the four GM tubes interfaced to the synthesis device. Colors of the traces are according to the color coding of GM tubes in Figure 2

7 ^{18}F-Fluoroalkylation reaction. Reaction vial 2 was lowered to the heating unit and heated for 5 min at 80°C or 10 min at 110°C. After the reaction, reaction vial 2 was removed from the heating unit and allowed to cool for 1 min.
8 Dilution of reaction solution. The pre-loaded HPLC dilution solution was first transferred from the reservoir syringe on valve V1 (port D) to syringe S1 via valve V2. It was then transferred to the six-port valve V1 (port E) via valve V2 and finally to reaction vial 2 via valve V8.
9 Injection on the semipreparative HPLC column. The diluted reaction solution was transferred back to syringe S1 via the same route as in step 8. From syringe S1, the diluted reaction solution was loaded to an injector loop (V7) via valve V2 and the six-port valve V1 (port F) and then injected to the semipreparative HPLC column. The HPLC flow was manually controlled from outside the hot cell.
10 HPLC separation. Separation was monitored by a GM tube (GM1) situated on the outlet of the HPLC column. The [^{18}F]FMPEP-d_2 retention time varied between 13.0 and 14.5 min.
11 Collection of [^{18}F]FMPEP-d_2. When the count rate of the GM1 exceeded a pre-set level, collection was automatically initiated by directing the HPLC flow via valve V10 to syringe S2, which was prefilled with the dilution solution. Collection lasted for 60 s, after which the flow was again directed to the HPLC waste via valve V10.
12 Solid-phase extraction and formulation of [^{18}F]FMPEP-d_2. The diluted HPLC fraction containing the end product was trapped on a pre-activated SPE cartridge by emptying syringe S2 to the waste container via valves V11, V12, and V13. The trapping of [^{18}F]FMPEP-d_2 was monitored using the GM4 situated next to the SPE cartridge. The SPE cartridge was washed by emptying the prefilled syringe S4 to syringe S2 via valves V13, V12, and V11. [^{18}F]FMPEP-d_2 was eluted from the SPE cartridge using ethanol by pushing sterile filtered air from syringe S3 through the ethanol vial, via valves V12, V11, and V14, into the end-product vial, through the sterile filter. Immediately after the final product in EtOH, the prefilled formulation solution from syringe S5 was pushed into the end-product vial via valve V14 and through the sterile filter. After addition of the formulation solution, PHT was performed to test the sterile filter integrity.
13 Sample (0.5 ml) for QC. The end-product vial was shaken, and a QC sample of the formulated end product was withdrawn into a pre-set 1-ml syringe. After obtaining the QC sample, all filters and needles were removed from the end-product vial and the radioactivity was measured.

2.4 | Specifications and quality control (QC) tests

QC tests on the QC sample were performed following the EudraLex, Volume 4, GMP guidelines.[22,29] Table 1 presents the specifications and analysis methods for [^{18}F]FMPEP-d_2.

2.5 | Qualification and validation

2.5.1 | Qualification of the synthesis device

Both the synthesis device and the sterile filtration unit were qualified (Installation Qualification/Operational Qualification) according to the TPC Validation Master Plan, which is in accordance with the EudraLex, Volume 4, GMP guidelines, Annex 15.[29] [^{18}F]FMPEP-d_2 was produced according to the GMP guidelines set by European Union (EU GMP Annex 3).[22]

2.5.2 | Validation of the synthesis procedure

Process validation was performed to confirm that the [^{18}F]FMPEP-d_2 production process reliably and reproductively produced a radiopharmaceutical that fulfilled the

TABLE 1 Specifications and quality control (QC) test methods

Test	Acceptance criteria	Method
Appearance[a]	A clear and colorless solution, free of particles	Visual inspection
Radiochemical identity[a]	R_t([^{18}F]FMPEP-d_2) = R_t (FMPEP) ± 0.5 min	LC
Radioactivity[a]	The injection contains ≥90.0% and ≤ 110.0% of the declared fluorine-18 radioactivity at the date and time stated on the delivery sheet	Measured in dose calibrator
Radiochemical purity[a] (RCP)	The fluorine-18 radioactivity in the form [^{18}F]FMPEP-d_2 is ≥95.0%	LC
Radionuclidic identity[b]	Half-life of 105–115 min	Measured in dose calibrator
Radionuclidic purity[c]	≥99.9% of the radioactivity corresponds to fluorine-18	HPGe detector
Chemical purity[a] (CP)	The combined mass of FMPEP-d_2 and any relevant organic (UV-absorbing) impurities are ≤10.0 μg/max injected dose (max V_{INJ})	LC
Residual solvents[b]	The end product contains CH_3CN ≤ 410 ppm DMF ≤ 880 ppm	GC
Content of ethanol[b]	The end product contains ≤10%	GC
pH[a]	4.0–7.5	pH indicator strip
Bacterial endotoxins[b,d]	Endotoxin limit is <17.5 IU/ml	Ph. Eur.
Sterility[b,d]	Sterile according to Ph. Eur.	Ph. Eur.
Shelf-life[b]	To be used within 2 h of the date and time of manufacture	QC analysis of CP, RCP, and pH
Sterile filter integrity[a]	Relative pressure decrease (RPD) <10%	Pressure hold test

[a]Performed on each batch (prior administration).
[b]Performed on the process validation batches.
[c]Analyzed on a quarterly basis from [^{18}F]fluoride from irradiated target.
[d]5% of the production runs are tested.

product specifications until the end of its shelf-life. Process validation was executed according to the TPC Validation Master Plan in accordance with EudraLex, GMP guidelines.[22,29]

For process validation, we produced three consecutive batches of [^{18}F]FMPEP-d_2 following the production method described in Sections 2.3.1–2.3.3. The QC tests were performed on the end product at the end of synthesis (EOS), as described in Table 1. Chemical purity (CP), radiochemical purity (RCP), and pH were also examined at EOS + 60 min and EOS + 180 min for shelf-life determination. An additional batch of [^{18}F]FMPEP-d_2 was produced for microbiological verification of bioburden prior to sterile filtration. From the above-mentioned validation syntheses, the end-product vials were sent to a contract research organization (CRO) for validated sterility and endotoxin testing. Sterility and bacterial endotoxins were determined using European Pharmacopeia (Ph. Eur.) procedures. After process validation, changes to the process were executed and documented according to GMP.

2.5.3 | Validation of analytical methods

The HPLC method (described in Section 2.1.2) used for analyzing RCP and CP, and for determination of the radiochemical identity of the end product, and the gas chromatography method for analyzing residual solvents were validated following the guidelines of the TPC and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH; Q2(R1)).[30] The validated parameters for RCP included specificity and limit of detection (LOD), and those for CP were specificity, linearity, repeatability, accuracy, range, and vulnerability. Precision was determined by using the FMPEP reference standard. The validated parameters for GC were specificity and LOD.

3 | RESULTS AND DISCUSSION

3.1 | Optimization and validation of [^{18}F]FMPEP-d_2 synthesis

The increased clinical need for [^{18}F]FMPEP-d_2 at TPC prompted us to design an automated device for [^{18}F]FMPEP-d_2 synthesis. We followed the [^{18}F]FMPEP-d_2 synthesis method of Pike[1] with slight modifications (Figure 1), including adjustments of the reaction

temperatures and times, and improved purification of [^{18}F]bromofluoromethane-d_2[23] and [^{18}F]FMPEP-d_2. For [^{18}F]bromofluoromethane-d_2 synthesis, we found that 5 min at 90°C constituted sufficient reaction conditions for successful radiolabeling. However, in routine production at TPC, we use a 5-min reaction at 110°C so that we do not have to change the heater temperature after [^{18}F] fluoride activation via azeotropic distillation. [^{18}F] bromofluoromethane-d_2 was successfully purified with GC at 90°C over 15 min. We found that reconditioning the GC column at 170°C before each synthesis was crucial for the repeatability of the purification process, as observed in our previous studies.[23,25–27] For the ^{18}F-fluoroalkylation reaction, 5 min at 80°C was satisfactory. The semipreparative purification conditions of [^{18}F] FMPEP-d_2 were optimized, ending with a 15-min purification. In general, our synthesis procedure follows that of Donohue et al. 2008.[1] However, for separation of [^{18}F] bromofluoromethane-d_2 from dibromomethane, organic solvents as well as radiolabeled impurities we used a simple semipreparative GC separation. We feel that this approach is more robust and reproducible than the method described by Donohue et al. 2008.[1]

In the clinical production of [^{18}F]FMPEP-d_2, we encountered problems with radiolysis and the lipophilicity of the [^{18}F]FMPEP-d_2. Radiolysis becomes an issue when the formulated [^{18}F]FMPEP-d_2 has a high radioactivity concentration (RAC)—that is, in the order of 50 MBq/1 ml and higher. In [^{18}F] bromofluoromethane-d_2 production, deuterated dibromomethane is used to reduce defluorination of the final product.[1,31] Despite this, the final product was highly prone to undergo defluorination due to radiolysis. To decrease radiolysis of the product, ascorbic acid was added to the reaction dilution solution, the semipreparative HPLC eluent, and the HPLC fraction dilution solutions. Additionally, the high lipophilicity of [^{18}F] FMPEP-d_2 causes it to easily become attached to single-use sterile materials, such as plastic syringes and sterile filters. We tested several kinds of plastic syringes and sterile filters to minimize the adherence. For the end-product sterile filtration, the sterile filters with hydrophilic polyethersulfone membrane diminished the adherence of the tracer to the filter. For radiopharmaceutical administration, we found that two-component syringes made of polypropylene and polyethylene minimized the adherence of the product to the syringe materials, presumably due to the lack of lubricants, such as silicon oil.

The synthesis device and the synthesis process were successfully qualified and validated, and the analytical HPLC and GC methods were successfully validated. Results of the process validation batches are presented in Table S1. The process was considered to be appropriate for [^{18}F]FMPEP-d_2 production.

3.2 | Long-term radiosynthesis experience at TPC

[^{18}F]FMPEP-d_2 produced at TPC using our in-house-built synthesis device has been in continuous clinical use since 2013. Up to the end of 2018, we have performed 223 syntheses, from which 149 batches have been for clinical use and 20 for quality control, with a 90% success rate. During the past 5 years, 54 test syntheses have been performed for development of the synthesis process or for preclinical studies only. Table 2 presents the synthesis results. The total synthesis time was 83 ± 7 min—including GC purification, semipreparative HPLC purification, and formulation for injection. The [^{18}F]FMPEP-d_2 shelf-life at room temperature was determined to be 120 min. The radioactivity of the formulated [^{18}F] FMPEP-d_2 was 1060 ± 400 MBq at EOS (n.d.c) when the starting activity was 11 ± 2 GBq at EOB. The radiochemical yield (RCY) was 16 ± 6% decay corrected to the end of bombardment (EOB). The radiochemical purity of [^{18}F]FMPEP-d_2 exceeded 95.0% in all syntheses released for clinical use. The molar activity (A_m) of [^{18}F]FMPEP-d_2 was 600 ± 300 GBq/μmol at EOS. The radioactivity distribution within the synthesis apparatus has been monitored throughout the years. The relatively low RCY is due to radioactivity losses in incomplete radiochemical reactions, as well as to a lesser extent to side reactions. There is room for further optimization; however, we are able to produce sufficient amounts of the tracer for our imaging needs.

Clinical batches have been rejected due to leakages in the synthesis device that result in low RCY or no product, blockage of the sterile filter, or low RCP. After each rejected batch, the cause of the rejection has been determined and addressed. Due to deviations in [^{18}F]FMPEP-d_2 production, we have made three major controlled changes to the synthesis process. First, the shelf-life of the final product was shortened from the original 180 to 120 min to ensure product quality during the whole shelf-life, even with high RAC. Second, the GC column stationary phase was changed from Porapak Q to Haysep Q, due to a change in the commercial supply; the change did not affect the product quality. Third, the heating unit used in the ^{18}F-fluoroalkylation reaction was changed from a heater to an oil bath due to suspected inaccuracy and non-repeatability of the heater's temperature. Simultaneously, the ^{18}F-fluoroalkylation reaction time was increased from 5 to 10 min, and the temperature raised from 80°C to 110°C.

TABLE 2 Synthesis results during the years 2013–2018

Year	Total	For preclinical use or test only/QC/for clinical use	Rejected[a]	RCP (%)	Radioactivity at EOS (GBq)	A_m at EOS (GBq/μmol)
2013	46	39[b]/1/6	0	99.1 ± 0.7 (97.8–99.7)	0.8 ± 0.4 (0.5–1.3)	430 ± 260 (150–870)
2014	38	8/3/27	5[c]	98.5 ± 0.8 (96.6–99.5)	0.8 ± 0.4 (0.3–1.7)	410 ± 250 (70–1030)
2015	35	2/5[d]/28	3[c]	98.2 ± 1.0 (95.5–100.0)	1.1 ± 0.4 (0.2–1.7)	730 ± 290 (110–1200)
2016	37	0/3/34	2	97.9 ± 0.8 (96.2–99.3)	1.2 ± 0.3 (0.7–2.0)	790 ± 220 (470–1560)
2017	27	4/2/21	6[e]	98.1 ± 1.0 (96.4–100.0)	0.9 ± 0.3 (0.2–1.4)	540 ± 190 (270–1000)
2018	40	1/6[d]/33	3[c]	98.5 ± 0.8 (95.9–99.6)	1.1 ± 0.3 (0.4–1.6)	660 ± 350 (200–1550)
Total	223	54/20/149	19			

Note. Values are presented as mean ± SD (range).
Abbreviations: EOS, end of synthesis; RCP, radiochemical purity.
[a]For clinical use and QC syntheses; results from rejected syntheses are not included in the RCP, radioactivity, and molar activity calculations.
[b]The syntheses for analytical method validation and process validation.
[c]Two of the rejected batches were of acceptable quality but the amount of radioactivity achieved was too low for human injection.
[d]Operator qualification syntheses.
[e]Three of the rejected batches were of acceptable quality but the amount of radioactivity achieved was too low for human injection.

These changes were executed and documented according to GMP.

In 2017, we encountered several difficulties in [^{18}F]FMPEP-d_2 production, which resulted in rejections and low A_m (Table 2). Originally, we thought that the difficulties in [^{18}F]FMPEP-d_2 synthesis were caused by variation in the ^{18}F-fluoroalkylation reaction temperature; thus, the heater was changed to an oil bath, the reaction temperature was increased, and the reaction time was prolonged, as described above. However, these changes did not improve the synthesis as expected and further studies and careful analysis of our documentation during 2018 raised questions regarding the validity of the DMF used as a reaction solvent. Specifically, when the used DMF bottle had been open for over 1 year, the RCY of [^{18}F]FMPEP-d_2 drastically decreased. Additionally, the dibromomethane-d_2 stock solution is only usable for a relatively short time. The expiration of the dibromomethane-d_2 stock solution can be visually observed, as the solution eventually turns bright yellow over time, and in addition, the activity yield of the radiosynthesis starts to slowly decrease. In our experience, the dibromomethane-d_2 stock solution is valid for 4 months. After the controlled changes to the synthesis process and changes in monitoring the validity of reagents, especially DMF, the synthesis has become more reliable and reproducible. In addition, as the real cause of the production failures was not in the heater, the 5-min reaction at 80°C is suitable conditions for [^{18}F]FMPEP-d_2 production.

4 | CONCLUSION

Here, we describe the development of an automated GMP-compliant synthesis device for the clinical production of [^{18}F]FMPEP-d_2. The separation of dibromomethane-d_2 from [^{18}F]bromofluoromethane-d_2 by GC is simple, effective, and reproducible. The entire process is straightforward, and our success rate has been over 90% during 2013–2018. This user-friendly device and process provides adequate quantities of [^{18}F]FMPEP-d_2 with moderate RCY and high A_m for multiple clinical studies per batch.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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