Fully Automated Production of Diverse ^{18}F-Labeled PET Tracers on the ELIXYS Multireactor Radiosynthesizer Without Hardware Modification

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Fully automated radiosynthesizers are continuing to be developed to meet the growing need for the reliable production of PET tracers made under current good manufacturing practice guidelines. There is a current trend toward supporting kitlike disposable cassettes that come preconfigured for particular tracers, thus eliminating the need for cleaning protocols between syntheses and enabling quick transitions to synthesizing other tracers. Though ideal for production, these systems are often limited for the development of novel tracers because of pressure, temperature, and chemical compatibility considerations. This study demonstrated the versatile use of the ELIXYS fully automated radiosynthesizer to adapt and produce 8 different ^{18}F-labeled PET tracers of varying complexity. **Methods:** Three-reactor syntheses of 2-deoxy-2-^{18}F-fluoro-β-D-arabinofuranosylcytosine (D-^{18}F-FAC), 2-deoxy-2-^{18}F-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-^{18}F-FMAU), and 2-deoxy-2-^{18}F-fluoro-5-ethyl-β-D-arabinofuranosyluracil (D-^{18}F-FEAU) along with the 1-reactor syntheses of D-^{18}F-FEAU, ^{18}F-FDG, 3-deoxy-3-^{18}F-fluoro-L-thymidine (^{18}F-FLT), ^{18}F-fallypride, 9-(4-^{18}F-fluoro-3-hydroxymethylbutyl)-guanine (^{18}F-FHBG), and *N*-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB), were all produced using ELIXYS without the need for any hardware modifications or reconfiguration. Synthesis protocols were adapted and slightly modified from those in the literature but were not fully optimized. Furthermore, ^{18}F-FLT, ^{18}F-FDG, and ^{18}F-fallypride were produced sequentially on the same day and used for preclinical imaging of A431 tumor–bearing severe combined immunodeficient mice and wild-type BALB/c mice. To assess future translation to the clinical setting, several batches of tracers were subjected to a full set of quality control tests. **Results:** All tracers were produced with radiochemical yields comparable to those in the literature. ^{18}F-FLT, ^{18}F-FDG, and ^{18}F-fallypride were successfully used to image the mice, with results consistent with those reported in the literature. All tracers that were subjected to clinical quality control tests passed. **Conclusion:** The ELIXYS radiosynthesizer facilitates rapid tracer development and is capable of producing multiple ^{18}F-labeled PET tracers suitable for clinical applications using the same hardware setup.

Key Words: automated radiosynthesis; radiochemistry kits; ^{18}F-FAC; ^{18}F-FEAU; ^{18}F-FMAU

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PET is a powerful tool for in vivo imaging, and the diversity of its research and clinical uses has significantly grown in recent years (*1*). Many fully automated radiosynthesizers have been developed to aid radiochemists in routinely producing PET tracers (*2*). For ^{18}F-fluoride radiochemistry (*3,4*), these systems are typically based on either a fixed-tubing design, which requires cleaning after every synthesis run, or a disposable cassette approach (*5,6*). Some fixed-tubing systems can be modified to produce different tracers, though it can be difficult for operators to reconfigure both the tubing layout and the software for new tracers. Furthermore, once a fixed system is configured and optimized for a particular tracer, the radiochemist is often reluctant to modify any aspects of the synthesizer, or use the same system for another tracer, to maintain reproducible production conditions. Disposable cassette-based systems enable the radiochemist to purchase preconfigured cassettes and software programs to avoid the need for operator reconfiguration while enabling the production of several different tracers on the same system. The avoidance of cleaning simplifies operations and expedites compliance with current good manufacturing practice guidelines by eliminating the need for validated cleaning methodologies. However, cassette-based systems are often not amenable to custom modifications or novel tracer development, and thus tracer diversity is limited by the spectrum of kits available from the manufacturer, making them less desirable in research environments.

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Within each of the 2 classes of radiosynthesizers, the features of the system ultimately dictate its capacity for performing complex reactions. Examples of such features include the number of reaction vessels and intermediate purification capabilities, reaction conditions (e.g., temperature and pressure limitations), and reagent compatibility (e.g., volatility and corrosiveness). In some cases, significant modifications to the reaction conditions or even the synthesis pathway have been necessary to adapt optimized manual protocols to the capabilities of existing automated radiosynthesizers, increasing the barriers to synthesis automation.

To address these current challenges in automated radiosynthesis, we combined the benefits of both classes and developed a fully automated 3-reactor radiosynthesizer (ELIXYS; Sofie Biosciences) that is based on a disposable-cassette design but is capable of handling high reaction temperatures and pressures (*7*). The unique reagent delivery and vial-sealing mechanisms allow fluidic connections to be dynamically configured in software, rather than hardware, to accommodate diverse synthesis protocols without the need for any custom plumbing modifications. The versatility of the radiosynthesizer is made possible by mobilization of the reaction vessels, which removes the need for large numbers of valves, including those that isolate the reaction vessel during reaction steps (and that can lead to limitations in reaction temperatures). This allows for a single-disposable-cassette design to be used for developing and reliably producing a wide variety of PET tracers encompassing a broad range of reaction conditions and complexities. The system, therefore, both is suitable for novel tracer development and supports routine production of multiple tracers on a single system (*7*).

Synthesis protocols are generated using the accompanying user-friendly software, which allows the user to string together sequences of chemistry-oriented unit operations (*8*). The cassettes were designed to handle any combination of the unit operations (e.g., add reagent, react, evaporate); therefore, different tracers can be synthesized using the same cassette design. For more complex syntheses, up to 3 reactors can be linked with optional intermediate purification (i.e., trapping products on solid-phase extraction cartridges and subsequently eluting them) between each reactor.

Here, we demonstrate the flexibility of the ELIXYS radiosynthesizer through reproducibly synthesizing various ^{18}F-labeled PET tracers with known chemistry: 2-deoxy-2-^{18}F-fluoro-β-D-arabinofuranosylcytosine (D-^{18}F-FAC), 2-deoxy-2-^{18}F-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-^{18}F-FMAU), 2-deoxy-2-^{18}F-fluoro-5-ethyl-β-D-arabinofuranosyluracil (D-^{18}F-FEAU), ^{18}F-FDG, 3-deoxy-3-^{18}F-fluoro-L-thymidine (^{18}F-FLT), (*S*)-N-((1-Allyl-2-pyrrolidinyl)methyl)-5-(3-^{18}F-fluoropropyl)-2,3-dimethoxybenzamide (^{18}F-fallypride), 9-(4-^{18}F-fluoro-3-hydroxymethylbutyl)-guanine (^{18}F-FHBG), and *N*-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB). Batches that were subject to quality control (QC) analysis passed all tests. We further show that the 3 reactors can be leveraged to sequentially synthesize multiple single-reactor tracers (^{18}F-FLT, ^{18}F-FDG, and ^{18}F-fallypride) with minimal manual intervention to enable preclinical imaging with multiple tracers in the same day from a single synthesizer.

MATERIALS AND METHODS

Materials

No-carrier-added ^{18}F-fluoride was produced by the (p,n) reaction of ^{18}O-H_2O (84%, 98% isotopic purity; Medical Isotopes) in an RDS-112 cyclotron (Siemens) using a 1-mL target (11 MeV) or in a PETtrace (GE Healthcare) using a 2.5-mL target (16 MeV). Anhydrous-grade acetonitrile, dimethylsulfoxide, ethyl acetate, toluene, 1,2-dichloroethane, dicholoromethane, methanol, 2,3-dimethyl-2-butanol (thexyl alcohol), hexane, *N,N,N′,N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate, potassium carbonate (K_2CO_3), potassium bicarbonate ($KHCO_3$), potassium phosphate monobasic (KH_2PO_4), ammonium phosphate monobasic ($NH_4H_2PO_4$), ammonium acetate (NH_4OAc), ammonium formate, 0.5 M sodium methoxide (NaOMe) in methanol, 33% hydrobromic acid in acetic acid, 2N sodium hydroxide (NaOH), trifluoroacetic acid, 1 M tetramethylammonium hydroxide in water, 4% tetrabutylammonium hydroxide, triethylamine, 37% hydrochloric acid (HCl, further diluted with water to form a 6N solution), ammonium sulfate ($(NH_4)_2SO_4$), hexamethyldisilazane, trimethylsilyl trifluoromethanesulfonate, and 5-ethyluracil were purchased from Sigma-Aldrich. 1N HCl was purchased from Fisher Scientific. The tC18 (WAT036810) and silica cartridges (WAT020520 and WAT043400) were purchased from Waters. FDG purification cartridges (Chromabond Set V, 730883.1129785) were purchased from MACHEREY-NAGEL GmbH and Co. KG. Regenerated cellulose membrane syringe filters (0.45 μm, AF0-2103-12) were purchased from Phenomenex. ^{18}F Trap and Release Cartridges (MP1) for the 1-reactor D-^{18}F-FEAU synthesis were purchased from ORTG. 2-*O*-(trifluoromethylsulfonyl)-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (D-sugar), 2-*O*-(trifluoromethylsulfonyl)-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (L-sugar), bis(trimethylsilyl)cytosine (FAC precursor), 5-methyl-2,4-bis[(trimethylsilyl)oxy]pyrimidine (FMAU precursor), mannose triflate, FB precursor, 3-*N*-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine (FLT precursor), tosyl-fallypride, tosyl-FHBG, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane (Kryptofix K_{222}; Merck), tetrabutylammonium bicarbonate, preconditioned quarternary methylammonium cartridges, sterile collection vials, and all cold standards were purchased from ABX (Advanced Biochemical Compounds). D-sugar and tetrabutylammonium bicarbonate used for the 1-reactor D-^{18}F-FEAU was prepared as reported by Chin et al. (*9*). Ethanol (EtOH, 200-proof) was purchased from the UCLA Chemistry Department. Any water used was purified to 18 MΩ and 0.1-μm-filtered for all cleaning, preparation, syntheses, and purification. Unless otherwise specified, all reagents were used as received.

Radiosynthesizer Setup

Quarternary methylammonium cartridges were used as received. MP1 cartridges were preconditioned with 1 mL of a 0.84 M $KHCO_3$ solution, rinsed with 1 mL of water, and then dried under helium. FDG purification and tC18 cartridges were preconditioned with 5 mL of EtOH followed by 10 mL of water, and silica cartridges with 10 mL of anhydrous hexane. Unless

otherwise specified, all conditioned cartridges were kept wet before use.

Glass V-vials (5 mL, W986259NG; Wheaton) with magnetic stir bars (14-513-65; Fisher Scientific) were installed to serve as the reaction vessels for each synthesis. Reagent vials were prepared with the respective reagents and solvents and loaded into disposable cassettes (Supplemental Table 1; supplemental materials are available at http://tech.snmjournals.org), and the cassettes were subsequently installed into the system. For multireactor syntheses, the installation of intermediate-purification cartridges and connections between cassettes were made via Luer fittings preassembled on the cassettes (Fig. 1). When high-performance liquid chromatography (HPLC) purification was needed, the last cassette in the synthesis was connected to the HPLC injector valve, and samples were loaded remotely into the HPLC system.

Chromatography

Semipreparative HPLC was performed with a WellChrom K-501 HPLC pump (Knauer) or Dionex P680 quaternary gradient pump (Fisher Scientific), reversed-phase Gemini-NX column (5 μm, 10 × 250 mm; Phenomenex) or Luna (5 μm, 10 × 250 mm; Phenomenex) columns, ultraviolet detector (254 nm, WellChrom Spectro-Photometer K-2501 or K-2001; Knauer) and γ-radiation detector and counter (B-FC-3300 and B-FC-1000; Bioscan Inc.). Analytic HPLC was performed on a Knauer Smartline HPLC system with a Phenomenex reverse-phase Luna column (5 μm, 4.6 × 250 mm) with an inline Knauer ultraviolet (254 nm) and γ-radiation coincidence detector and counter (B-FC-4100 and B-FC-1000; Bioscan, Inc.) or an Agilent 1200 series (Agilent Technology) with ChemStation software equipped with a quaternary pump, ultraviolet diode-array detector, and model 105S single-channel radiation detector using a Phenomenex Gemini C18 column (5 μm, 250 × 4.6 mm). Unless otherwise specified, chromatograms were collected by a GinaStar (Raytest USA, Inc.) analog-to-digital converter and GinaStar software. HPLC mobile phases, flow rates, and retention times are listed in Supplemental Table 2. Radio–thin-layer chromatography (radio-TLC) was performed on a miniGita Star (Raytest USA, Inc.) using precut silica plates (Baker-flex; J.T. Baker) developed in 95% acetonitrile in water (v/v).

Synthesis of Tracers

For each of the 8 tracers, a synthesis program was created and tested using the ELIXYS software (*8*). After setup and cassette

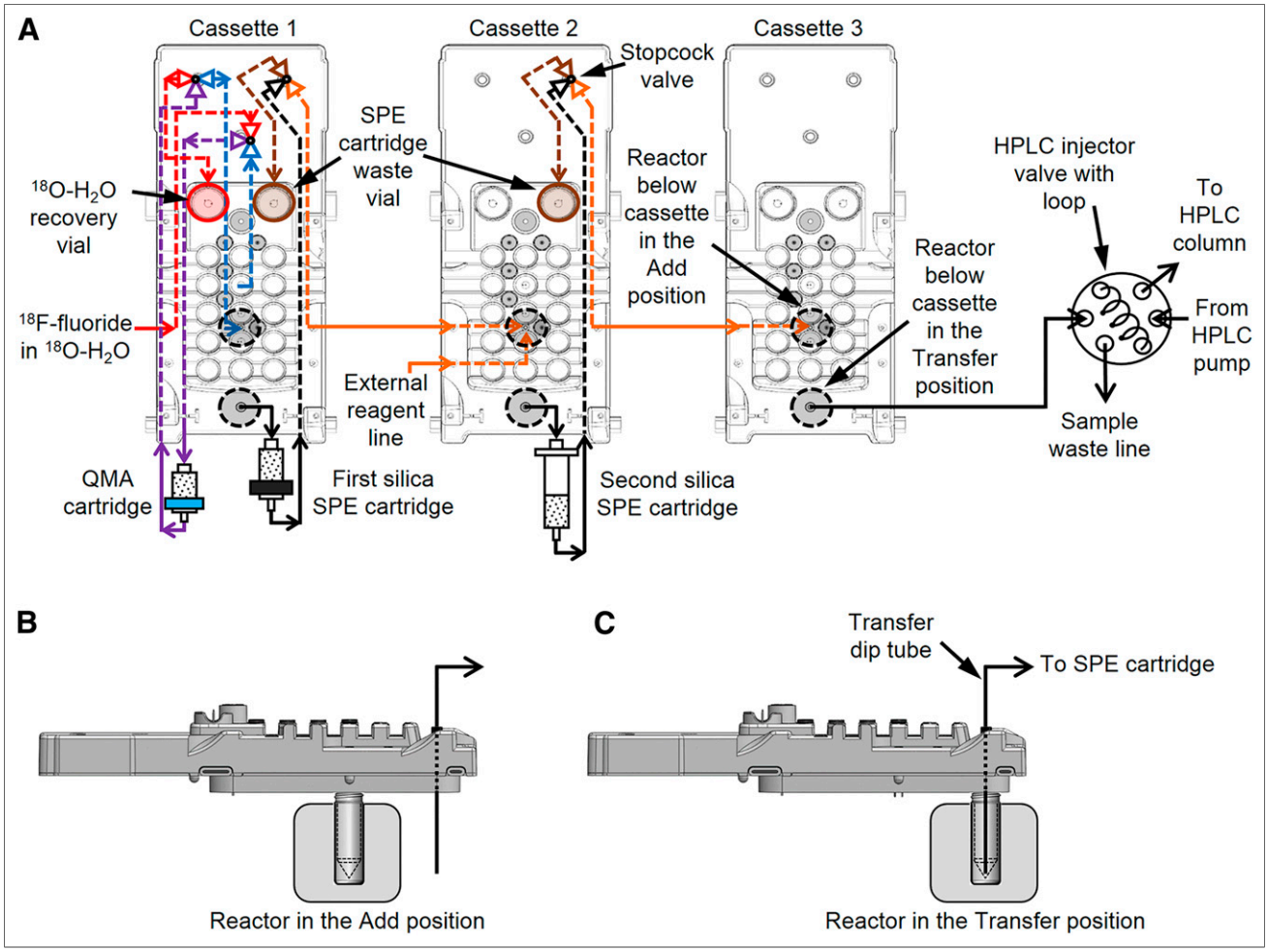

FIGURE 1. Disposable cassette fluidic paths used for 3-reactor syntheses. (A) Top view with fluidic connections inside (dashed) and outside (solid) each cassette, including purification cartridges. Side view of cassettes display reactor in Add position (B), where fluid is delivered into reaction vessel, and Transfer position (C), where fluid is transferred out of reaction vessel. SPE = solid phase extraction; QMA = quarternary methylammonium.

installation, the program was run. After all reaction and evaporation operations, the reactor was cooled to 35°C, with the exception of the ^{18}F-fluoride and base solution drying, for which cooling was applied only after the last azeotropic drying step. All evaporation steps were performed by supplying both vacuum and inert gas to the reaction vessel while heating. Unless otherwise stated, the inert gas supply during evaporation was set to 69 kPag. All reaction steps were performed with stirring. Both the 3-reactor and the 1-reactor D-^{18}F-FEAU syntheses were conducted at the Cyclotron and Radiochemistry Facility in the Stanford University Medical Center Department of Radiology, whereas all other tracers were synthesized at the Crump Radiochemistry and Cyclotron Technology Center at the University of California, Los Angeles (UCLA).

^{18}F-Fluoride Loading

^{18}F-fluoride was prepared in the same manner as for all the tracers listed in this study. A vial of ^{18}F-fluoride in ^{18}O-H_2O was connected via the dedicated transfer line to the first cassette on ELIXYS and transferred via pressure supplied by an inert gas line from the system. Starting radioactivity ranged from 0.3 to 37 GBq (8 mCi–1 Ci) and averaged around 3.7 GBq (100 mCi).

^{18}F-fluoride was then trapped by passing the solution through a preconditioned strong anion-exchanging quarternary methylammonium or MP1 cartridge that allowed the ^{18}O-H_2O to be collected in a recovery vial installed on the cassette. Release of the ^{18}F-fluoride was achieved by passing a base solution (Supplemental Table 1) to release the ^{18}F-fluoride from the cartridge. The ^{18}F-fluoride and base solution eluate was delivered to reaction vessel 1 and subsequently dried at 110°C until almost dry (2.5 min). Anhydrous acetonitrile (1 mL) was then added to reaction vessel 1 to azeotropically remove water from the solution at 110°C until complete dryness (1.5 min). This step was repeated an additional time to ensure activation (i.e., sufficient removal of water).

Three-Reactor Syntheses of D-^{18}F-FAC, L-^{18}F-FMAU, and D-^{18}F-FEAU

Syntheses of these nucleoside tracers were adapted from the literature (*7,10–17*). Programmed unit operations were identical for the synthesis of each tracer and varied slightly from previously published work (*7*). Details are described in the Supplemental "Methods" section.

One-Reactor Synthesis of D-^{18}F-FEAU

One-reactor synthesis of D-^{18}F-FEAU was based on previously reported methods (*9,18*). A D-sugar solution (10 mg in 1 mL of acetonitrile, 17 mM) was added to dried tetrabutylammonium ^{18}F-fluoride in reaction vessel 1 and reacted at 160°C for 15 min. After ^{18}F labeling, the solvent was evaporated at 110°C (2.5 min). A fresh 5-ethyluracil solution was previously prepared by mixing 30 mg of 5-ethyluracil, 4.5 mg of $(NH_4)_2SO_4$, 200 μL of hexamethyldisilazane, and 200 μL of acetonitrile and refluxing the mixture at 95°C under helium for 1 h; then, all liquid was removed by evaporation and the residue was reconstituted with 100 μL of hexamethyldisilazane, 150 μL of trimethylsilyl trifluoromethanesulfonate, and 150 μL of dicholoromethane. The 5-ethyluracil solution was added to the dried fluorinated sugar. The coupling reaction was then performed at 95°C for 30 min, and afterward the solvent was evaporated at 85°C (5 min). NaOMe (2.5 M) in methanol (1.5 mL) was then added and reacted at 105°C for 5 min. HCl (6N, 1 mL) was then added to quench the reaction. The crude product was partially evaporated at 85°C for 1.5 min to remove methanol. Water (1 mL) was added to the reaction vessel, and the mixture was passed through a filter (0.45-μm regenerated cellulose membrane; Phenomenex) before loading onto the semipreparative HPLC.

^{18}F-FDG

Synthesis was adapted from multiple literature reports with slight modifications (*19,20*). Mannose triflate (20 mg in 0.7 mL of acetonitrile, 59 mM) was added to the activated ^{18}F-fluoride residue in reaction vessel 1 and the solution reacted at 130°C for 3 min. After the ^{18}F-fluorination reaction, water (2 mL) was added to reaction vessel 1 and the solution mixed for 15 s. The solution was then passed through a preconditioned tC18 cartridge to trap the fluorinated intermediate, and waste was collected in a vial on the cassette. The tC18 cartridge was subsequently washed with water (2 mL) and sent to waste. For deprotection of the intermediate, 2N NaOH (2 mL) at room temperature was slowly (28 kPag) passed through the tC18 cartridge and then immediately through the FDG purification cartridge. Before entering a vented sterile collection vial, the product was passed through a 0.22-μm sterile filter. Five subsequent rinses with water (2 mL) were performed to remove product from the cartridges. Radiochemical purity was determined by radio-TLC.

TABLE 1
Summary of Synthesis Data

Tracer	Decay-corrected radiochemical yield (%)	Synthesis duration (min)*	Specific activity†	
			GBq/μmol	Ci/μmol
D-^{18}F-FAC	31 ± 4 (n = 11)	163	37–44	1.0–1.2
L-^{18}F-FMAU	49 ± 7 (n = 7)	170	100–170	2.7–4.6
D-^{18}F-FEAU (3-reactor)	39 ± 4 (n = 3)	180	3.8–5.1‡	0.10–0.14
D-^{18}F-FEAU (1-reactor)	28 ± 4 (n = 3)	140	7.4–20‡	0.20–0.53
^{18}F-FDG	70 ± 9 (n = 3)	38	NA	
^{18}F-FLT	69 ± 3 (n = 5)	65	67–481	1.8–13
^{18}F-fallypride	66 ± 8 (n = 6)	56	15–78‡	0.4–2.1
^{18}F-FHBG	11 ± 2 (n = 3)	87	92–189	2.5–5.1
^{18}F-SFB	69 ± 8 (n = 6)	78	63	1.7

*Synthesis duration was defined from start of synthesis to end of synthesis, including purification but not including reformulation.
†Range of specific activities measured at time of injection into analytic HPLC.
‡Starting activity was lower than average and ranged from 1.3 to 1.9 GBq (35–50 mCi).
NA = not applicable.
Radiochemical yields are mean ± SD.

^{18}F-FLT

Synthesis was adapted from multiple literature sources with slight modifications (*21–26*). FLT precursor (30 mg in 0.25 mL of acetonitrile and 0.75 mL of thexyl alcohol, 36 mM) was added to the activated ^{18}F-fluoride residue in reaction vessel 1, and the solution reacted at 120°C for 6 min. The crude fluorinated product solution was evaporated until almost dry at 100°C (3.5 min). HCl (1N, 1 mL) was then added to reaction vessel 1 and the solution reacted at 130°C for 5 min. NaOH (2N, 0.5 mL) was added to reaction vessel 1 to neutralize the HCl. HPLC mobile phase (1 mL, Supplemental Table 2) was added to reaction vessel 1, and the mixture was passed through a filter (0.45-μm regenerated cellulose membrane; Phenomenex) before loading onto the semipreparative HPLC.

^{18}F-Fallypride

Synthesis was adapted from the literature with slight modifications (*27*). Tosyl-fallypride (4 mg in 0.3 mL of acetonitrile and 0.7 mL of thexyl alcohol, 8 mM) was added to the activated ^{18}F-fluoride residue in reaction vessel 1 and the solution reacted at 105°C for 7 min. The crude fluorinated product solution in reaction vessel 1 was evaporated to dryness at 100°C (3 min) and reconstituted in HPLC mobile phase (1 mL, Supplemental Table 2) before injection into semipreparative HPLC for purification.

^{18}F-FHBG

Synthesis was adapted from multiple literature sources with slight modifications (*28–30*). Tosyl-FHBG (4 mg in 1 mL of acetonitrile, 4 mM) was added to the activated ^{18}F-fluoride residue in reaction vessel 1 and the solution reacted at 135°C for 10 min. Solution was evaporated until almost dry at 110°C (2 min). HCl (1N, 1 mL) was then added to reaction vessel 1 and the solution reacted at 105°C for 5 min. NaOH (2N, 0.5 mL) was added to reaction vessel 1 to neutralize the HCl. HPLC mobile phase (1 mL, Supplemental Table 2) was added to reaction vessel 3 before injection into semipreparative HPLC for purification.

^{18}F-SFB

The synthesis was adapted from multiple literature sources with slight modifications (*31–37*). FB precursor (5 mg in 1 mL of dimethylsulfoxide, 14 mM) was added to the activated ^{18}F-fluoride residue in reaction vessel 1 and the solution reacted at 110°C for 15 min. Tetramethylammonium hydroxide (1 M) in water solution (0.025 mL in 1 mL of acetonitrile) was then added to reaction vessel 1, and the solution was evaporated at 110°C for 7 min with a 34 kPag inert gas supply. Immediately after evaporation, anhydrous acetonitrile (2 mL) was added to reaction vessel 1, and the solution was evaporated at 110°C for 3 min with a 34 kPag inert gas supply to further remove traces of water. The *N,N,N′,N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate solution (22.8 mg in 1 mL of acetonitrile, 76 mM) was then added to reaction vessel 1, and the mixture was reacted at 110°C for 5 min. HPLC mobile phase (1 mL, Supplemental Table 2) was added to reaction vessel 3 before injection into semipreparative HPLC for purification.

Postsynthesis Procedures and Measurements

The starting activity was assayed before transfer into the first cassette, and the start of synthesis was marked at the initiation of the automated synthesis program. All activity measurements were collected from a calibrated CRC 25PET dose calibrator (Capintec, Inc.) and decay-corrected to the start of synthesis. For all HPLC-purified samples, the purified fraction from semipreparative HPLC was collected through a selector valve into a collection tube,

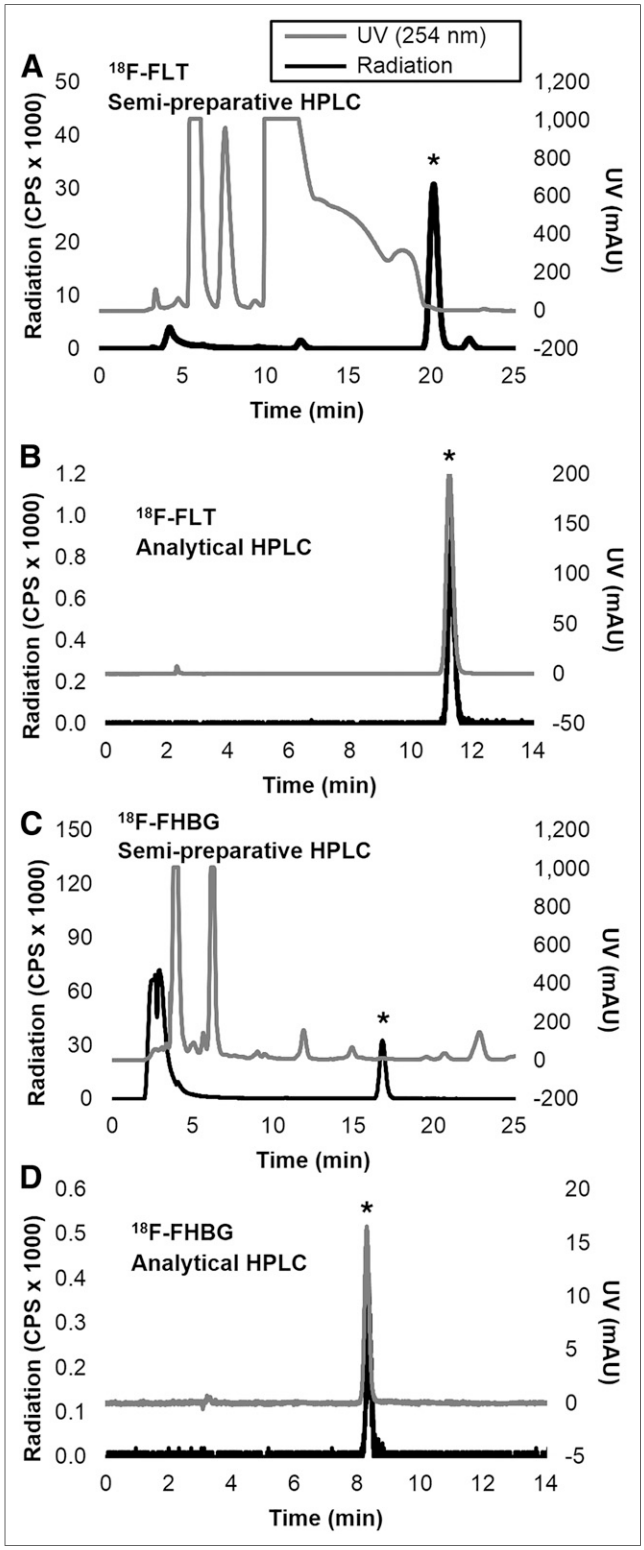

FIGURE 2. Examples of HPLC chromatograms. (A and B) ^{18}F-FLT semipreparative HPLC (A) and analytic HPLC of purified sample with coinjection of standard (B). (C and D) ^{18}F-FHBG semipreparative HPLC (C) and analytic HPLC of purified sample with coinjection of standard (D). Chromatography conditions are found in Supplemental Table 2. *Radioactive peak of interest. AU = absorbance units; CPS = counts per second; UV = ultraviolet.

marking the end of synthesis. The collected purified fraction was assayed, and a sample was taken to analytic HPLC for radiopurity analysis and verification of identity by coinjection with cold standard. Specific activity was determined via analytic HPLC and calculated using the activity of the sample at the time of injection into HPLC. The mass of the sample was determined by a standard curve created for each tracer. For all tracers, radiochemical yield was calculated as the percentage of starting activity that was collected as purified product. For ^{18}F-FDG, radiochemical purity was determined by radio-TLC.

Sequential Syntheses of 3 Tracers with Preclinical Imaging

To demonstrate the high-throughput capabilities of the ELIXYS synthesizer, ^{18}F-FLT, ^{18}F-FDG, and ^{18}F-fallypride were synthesized sequentially in a single day on the same instrument with minimal manual intervention. Each tracer was synthesized in one of the reactors using the above methods—with the exception that a reformulation step was performed—and had its own reagents, cartridges, and starting ^{18}F-fluoride solution. Preparation for all 3 syntheses was completed before radioactivity was introduced into the system. Because ELIXYS currently has only 1 inert gas supply for transferring ^{18}F-fluoride to the cassette, an inert gas stopcock manifold mounted outside the hot cell was used to remotely control the pressurization of each vial containing ^{18}F-fluoride solution. Also, because both ^{18}F-FLT and ^{18}F-fallypride require HPLC purification, 1 quick manual swap of the lines to the HPLC injection valve was required during the ^{18}F-FDG synthesis. These 2 tracers required the same semipreparative HPLC column; therefore, the HPLC system could be cleaned and reconditioned without exposure. All tracers were reformulated for preclinical imaging after purification. All QC testing was performed using the reformulated products. After purification, ^{18}F-FLT and ^{18}F-fallypride were rotary-evaporated, dissolved with pH 7.4 phosphate-buffered saline, and transferred to a sterile vial for injection. For ^{18}F-FDG, the purified product was delivered through a 0.22-μm sterile filter into a sterile vial to which 3 mL of phosphate-buffered saline was previously added, thus making a 15-mL final solution.

For the preclinical imaging of ^{18}F-FLT, six 6- to 8-wk-old female severe combined immunodeficient mice were injected subcutaneously with 1.0×10^6 A431 cells in a 1:1 mixture of DMEM: Matrigel (BD Biosciences). For ^{18}F-FDG and ^{18}F-fallypride imaging, 2 and 4 non–tumor-bearing 9- to 10-wk-old BALB/c mice were used, respectively. Small-animal PET was performed as previously described (*7*). Briefly, conscious tumor-bearing severe combined immunodeficient or non–tumor-bearing BALB/c mice were injected with 740 kBq (20 μCi) of ^{18}F-FLT, ^{18}F-FDG, or ^{18}F-fallypride followed by a 60-min uptake period. Before being scanned, the mice were anesthetized with 2% isoflurane and placed in a dedicated imaging chamber (Sofie Biosciences, Inc.). Whole-body PET images were acquired using a GENISYS^4 (Sofie Biosciences, Inc.), and images were acquired for 10 min and analyzed using OsiriX Imaging software (Pixmeo). All animal experiments were approved by the UCLA Animal Research Committee and were performed according to the guidelines of the Division of Laboratory Animal Medicine at UCLA.

QC Analysis

Multiple batches of D-^{18}F-FAC and ^{18}F-FDG were subjected to QC testing for clinical use. The D-^{18}F-FAC samples were reformulated by rotary-evaporating the HPLC-purified fractions, dissolving with phosphate-buffered saline, and transferring the product through a sterile filter to a sterile vial before testing. The ^{18}F-FDG batches did not require further reformulation after purification. These tracers were chosen as models for multireactor HPLC-purified and single-reactor solid-phase extraction cartridge–purified methods of production. The tests included optical clarity via visual inspection, filter integrity, pH, radionuclide identity and purity via half-life and energy, radiochemical identity and purity via analytic HPLC, residual solvent analysis via gas chromatography, Kryptofix K_{222} spot, pyrogenicity, and sterility. Detailed methods for these tests can be found in our previous work (*17*).

RESULTS

A summary of decay-corrected radiochemical yield, duration of synthesis (including purification, not including reformulation), and specific activity at time of injection into analytic HPLC is listed in Table 1 for the individual syntheses. All tracers had greater than 99% radiochemical purity. Examples of the semipreparative and coinjected analytic HPLC chromatograms can be found in Figure 2.

^{18}F-FLT required an additional 20 min for reformulation (total synthesis duration of 85 min), and ^{18}F-fallypride

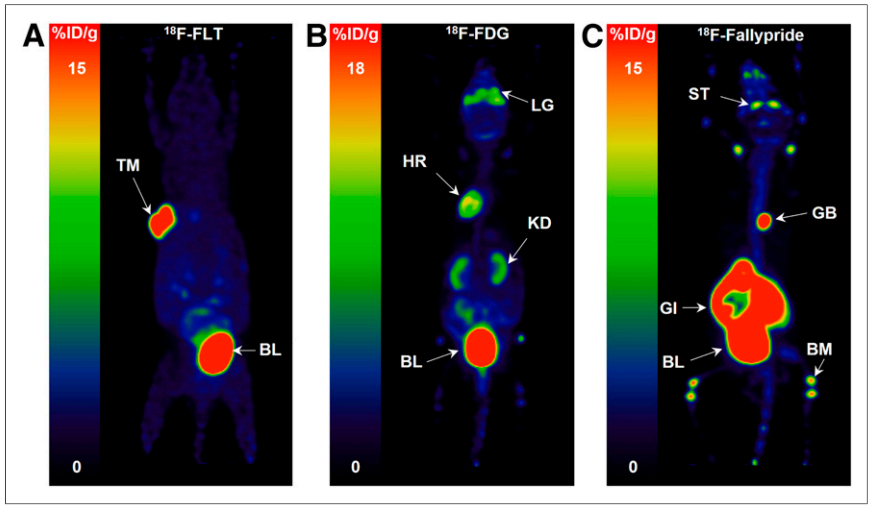

FIGURE 3. Small-animal PET imaging using same-day sequentially synthesized PET tracers on single automated radiosynthesizer. Sample images are shown for ^{18}F-FLT (A), ^{18}F-FDG (B), and ^{18}F-fallypride (C). Scale for percentage injected dose per gram (%ID/g) is listed for each image. BL = bladder; BM = bone marrow; GB = gallbladder; GI = gastrointestinal tract; HR = heart; KD = kidneys; LG = lacrimal glands; ST = striatum; TM = A431 implanted tumor.

reformulation required 10 min (total synthesis duration of 66 min). Analysis of the reformulated products showed no distinguishable variation in purity to their respective HPLC-purified samples collected from the individual syntheses. Yields, synthesis durations, and specific activities of the 3 tracers likewise were equivalent to having been synthesized individually. Additionally, the pH (7–7.5) and the radiochemical purity were acceptable for both preclinical and clinical applications.

Sequentially synthesized ^{18}F-FLT, ^{18}F-FDG, and ^{18}F-fallypride were evaluated in vivo on the same day by small-animal PET. High ^{18}F-FLT tumor uptake in A431 tumor–bearing mice was observed (Fig. 3A), consistent with previous results (*38*). In non–tumor-bearing mice, ^{18}F-FDG demonstrated the anticipated uptake in brain and heart (Fig. 3B), whereas the striatum could be clearly identified with ^{18}F-fallypride (Fig. 3C) (*39*).

The reformulated batches of D-^{18}F-FAC and ^{18}F-FDG passed full clinical QC testing, and their respective chromatograms can be found in Figure 4. The specific activity and pH of all reformulated products were within the acceptable range for both clinical and preclinical production.

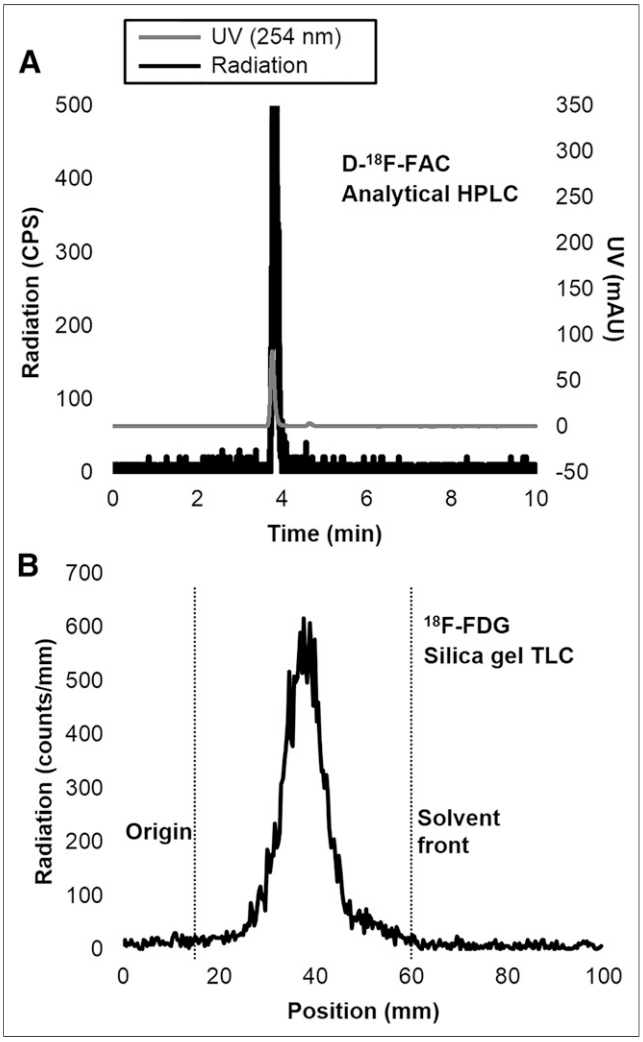

FIGURE 4. Chromatograms of purified and reformulated products. (A) D-^{18}F-FAC analytic HPLC with coinjection of standard. (B) ^{18}F-FDG radio-TLC. Chromatography conditions are found in Supplemental Table 2. AU = absorbance units; CPS = counts per second; UV = ultraviolet.

DISCUSSION

The various radiotracer syntheses were performed on the ELIXYS radiosynthesizer without hardware modifications regardless of the complexity of the synthesis or the reagents used. The only changes needed were in the programmed sequences, the reagents and purification cartridges installed in the cassettes, and the methods of HPLC purification. This ability of the ELIXYS radiosynthesizer contrasts with other fully automated cassette-based radiosynthesizers currently available (e.g., IBA Synthera, GE FASTlab and TRACERlab MX, Eckert and Ziegler PharmTracer, TRASIS AllinOne, and Siemens Explora One models), which tend to be limited in the number of reactors, reagents, purification capabilities, or reaction temperatures and pressures. In some reports, synthesizer limitations have been overcome by custom hardware modifications, but these often make it difficult to accommodate the syntheses of various tracers, particularly novel tracer development, using only one of these systems. Universal cassettes and software-controlled fluid paths used by ELIXYS may obviate such custom modifications.

Syntheses were not extensively optimized, as this study was intended as a proof-of-concept demonstration. However, yields and synthesis durations were comparable to those reported in the literature (*7,9–37*), and there may be room for improvement with further optimization. The tracers described here included some purified by cartridge and some by semipreparative HPLC. For multiple sequential syntheses requiring HPLC, it was necessary to clean the HPLC system between syntheses. For several tracers of wide interest (e.g., ^{18}F-FLT (*40*)), there have been efforts toward developing cartridge purification methods. These methods could be adapted to ELIXYS, thus simplifying the procedure for multitracer synthesis.

Three tracers were successfully produced on the same day using a single system and used for preclinical imaging. Sample tracers also passed full clinical QC testing. These qualities combined with a wide range of synthesizable ^{18}F-labeled tracers demonstrate the effective use of the ELIXYS radiosynthesizer to adapt previously developed synthesis protocols for immediate transition to production.

CONCLUSION

We have demonstrated the syntheses of 8 well-known ^{18}F-labeled PET tracers on the ELIXYS radiosynthesizer without the need for any hardware reconfiguration. The software enabled automated synthesis programs to be quickly created from published protocols. All tracers were produced with radiochemical yields and synthesis durations comparable to those reported in the literature. Several batches were reformulated and passed clinical-level QC testing. In addition to supporting diverse synthesis protocols, the 3 reactors of ELIXYS could be leveraged to accomplish multiple single-reactor syntheses sequentially with minimal manual intervention.

DISCLOSURE

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