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Highlighting the versatility of the Tracerlab synthesis modules. Part 2: fully automated production of [^{11}C]-labeled radiopharmaceuticals using a Tracerlab FX$_{C\text{-}Pro}$

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The field of radiochemistry is moving toward exclusive use of automated synthesis modules for production of clinical radiopharmaceutical doses. Such a move not only comes with many advantages but also presents radiochemists with the challenge of re-configuring synthesis modules for production of radiopharmaceuticals that require non-conventional radiochemistry while maintaining full automation. Herein, we continue our series of articles showcasing the versatility of the Tracerlab FX synthesis modules by presenting straightforward, fully automated methods for preparing a range of carbon-11 labeled radiopharmaceuticals using a Tracerlab FX$_{C\text{-}Pro}$. Strategies for production of [^{11}C]acetate, [^{11}C]carfentanil, [^{11}C]choline, [^{11}C]3-amino-4-[2-[(di(methyl)amino)methyl]phenyl]sulfanylbenzonitrile ([^{11}C]DASB), (+)-a-[^{11}C]dihydroterabenazine ([^{11}C]DTBZ), [^{11}C]flumazenil ([^{11}C]FMZ), meta-hydroxyephedrine ([^{11}C]HED), [^{11}C]methionine, [^{11}C]PBR28, [^{11}C]Pittsburgh Compound B ([^{11}C]PiB), 1-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]PMP), and [^{11}C]raclopride are presented.

Keywords: positron emission tomography; automated radiopharmaceutical synthesis; carbon-11; radiochemistry

Introduction

The art and science of radiopharmaceutical production is evolving in a number of ways. First, the environment surrounding production of radiopharmaceuticals is becoming much more regulated, with production of clinical doses according to Current Good Manufacturing Practice (cGMP) becoming standard in most countries. For example, the U.S. FDA approved a set of regulations describing production of positron emission tomography (PET) radiopharmaceuticals according to cGMP, outlined in the Code of Federal Regulations (21CFR212), which are set to become effective in December 2011. Second, in part to address these new regulatory demands, the field of radiochemistry is increasingly migrating toward the use of commercially available automated synthesis modules for production of clinical radiopharmaceutical doses (although manual synthetic techniques can also be conducted in compliance with cGMP). The advantages of using automated synthesis modules over more traditional 'manual' synthetic approaches include (i) automation of radiochemical syntheses providing robust, repeatable processes; (ii) the ability to handle multiple Curies of radioactivity safely enabling PET Centers to produce and distribute hundreds of doses daily; (iii) facilitated regulatory compliance through manufacturer installation qualification/operational qualification/performance qualification and scheduled maintenance protocols performed on synthesis modules by trained service engineers; and (iv) improved radiation safety through reduction (or elimination) of manual operations. However, in addition to these advantages, the move toward exclusive use of automated synthesis modules also has associated challenges. The most significant is the issue of re-configuring synthesis modules for production of radiopharmaceuticals that require non-conventional radiochemistry strategies, while maintaining full automation.

At the University of Michigan, carbon-11 labeled radiopharmaceuticals are prepared using General Electric Medical Systems (GEMS) TracerLab FX$_{C\text{-}Pro}$ automated synthesis modules. As the demand for doses of different carbon-11 labeled radiopharmaceuticals prepared for clinical research, many of which are prepared using complex radiochemical techniques, continues to increase, we[1–4] and others[5–15] have had to address the issue of frequent synthesis module reconfiguration. For example, we recently described preparation of multiple carbon-11 labeled radiopharmaceuticals using a single TracerLab FX$_{C\text{-}Pro}$ that removed the need to open the hot-cell door between syntheses,[1] and in Part 1 of this series of articles, we reported simple modifications to a TracerLab FX$_{FN}$ that enable fully automated production of a range of fluorine-18 labeled radiopharmaceuticals.[16] In Part 2 of this series, we report configurational changes to a TracerLab FX$_{C\text{-}Pro}$ that allow for straightforward switching between production of [^{11}C]-labeled radiopharmaceuticals using routine carbon-11 methylation chemistry, and those prepared using less trivial radiochemical techniques (e.g., alkylation of [^{11}C]CO_2 with Grignard

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reagents). Proof-of-concept is demonstrated through the simple, fully automated production of 12 different [^{11}C]-labeled radiopharmaceuticals (Figures 1 and 2) using a single TracerLab FX$_{C\text{-}Pro}$ (Figure 3).

Automated radiopharmaceutical production using a Tracerlab FX$_{C\text{-}Pro}$

Introduction

General modifications to the Tracerlab FX$_{C\text{-}Pro}$

The Tracerlab FX$_{C\text{-}Pro}$ synthesis module is installed 'out-of-the-box' in its most basic configuration illustrated in Figure 3. This configuration is suitable for straightforward production of radiopharmaceuticals prepared in the reaction vessel, and requiring HPLC purification (e.g., dihydrotetrabenazine (DTBZ), flumazenil (FMZ), meta-hydroxyephedrine (HED), PBR28, and 1-methyl-piperidin-4-yl propionate (PMP), Figures 3 and 4, Section 2.2), but can easily be reconfigured for purification by Sep-Pak cartridge instead (e.g., carfentanil or methionine, Section 2.3).[1] However, with some simple modifications, the TracerLab FX$_{C\text{-}Pro}$ can also be used to produce radiopharmaceuticals using other strategies such as the loop method (e.g., 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB), raclopride, and Pittsburgh Compound B (PiB), Section 2.4),[1] Sep-Pak reaction (e.g., choline, Section 2.5),[3] or alkylation of [^{11}C]CO_2 with Grignard reagents (e.g., acetate, Section 2.6).[4]

The primary modification made to the Tracerlab FX$_{C\text{-}Pro}$ synthesis modules was to remove valve-30 and valve-31 from the HPLC mobile phase set-up (Figure 3) and then use them to control flow of methyl iodide through the silver triflate column (Figures 4, 10, 11, and 16). The benefits of such modifications are numerous. First, control of valve-30 and valve-31 can still be controlled using the same commands in the computer timelists, and so this allows the option for automated bypassing of the triflate column if synthesis with [^{11}C]MeI is being used. Second, it allows for enhanced system performance when conducting loop chemistry (Section 2.4 and Figure 11). In our early work, these valves were not in place, and we found that after conducting loop chemistry, if the synthesis module was left over night before cleaning (not uncommon following an afternoon or evening synthesis using 3 Ci of carbon-11), then residual liquid in the HPLC loop would leach back along the line and poison the silver triflate column. Reconfiguring valves 30 and 31 addresses this issue because they can be closed and can effectively isolate and protect the silver triflate column from any residual liquid in the HPLC loop. One pair of Luer Lock adapters was inserted between valve V7 and the fluid detector. These fittings allow straightforward switching from reactor chemistry to loop chemistry. A second pair of Luer Lock adapters was also inserted between V14 and the round-bottomed flask. This enabled crude reaction mixtures to be injected onto a column for purification using semipreparative HPLC or, alternatively, to be transferred into the round-bottomed flask for purification using solid phase extraction (SPE) techniques without significant module reconfiguration.

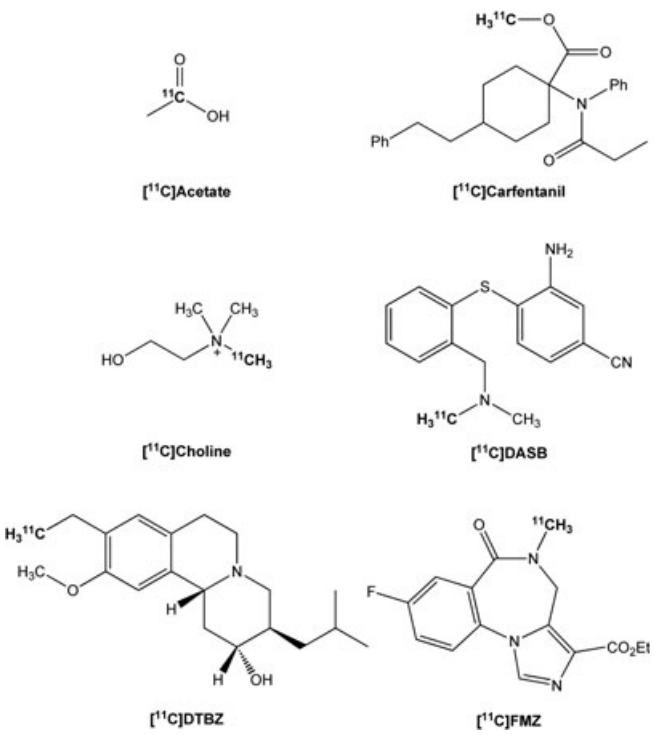

Figure 1. Carbon-11 labeled radiopharmaceuticals.

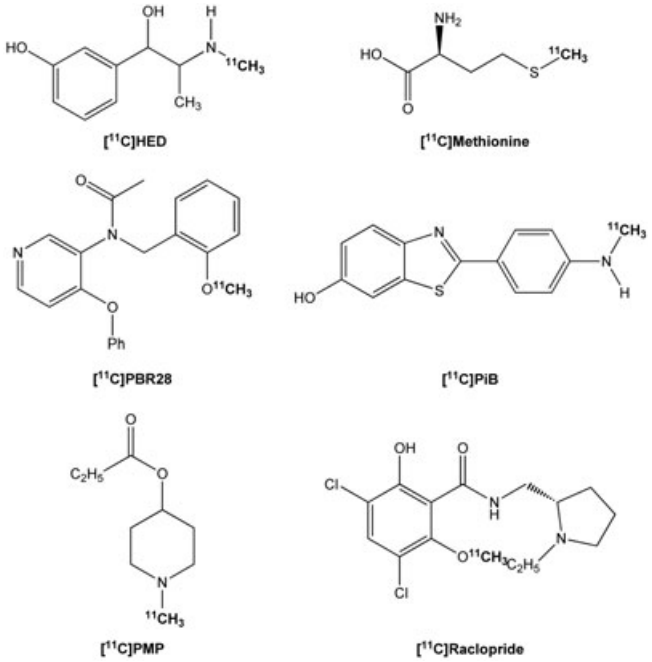

Figure 2. Carbon-11 labeled radiopharmaceuticals (continuation).

General synthesis considerations

Unless otherwise stated, reagents and solvents were commercially available and used without further purification: sodium chloride, 0.9% United States Pharmacopeia (USP), and Sterile Water for Injection, USP, were purchased from Hospira (Lake Foest, IL, USA); ethanol was purchased from American Regent (Shirley, NY, USA); anhydrous acetonitrile, ammonium acetate, hydrochloric acid, acetic acid, dimethyl sulfoxide, Ascarite II, and *N,N*-dimethylformamide (DMF) were purchased from Sigma Aldrich (St. Louis, MO, USA); and HPLC grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). Precursors and standards were commercially available, unless otherwise indicated, and purchased from Aldrich (acetate, choline, carfentanil (contract synthesis)), ABX Advanced Biochemicals (Radeberg, Germany) (FMZ, DASB, HED, PBR28, PIB, Raclopride), Fluka (St.

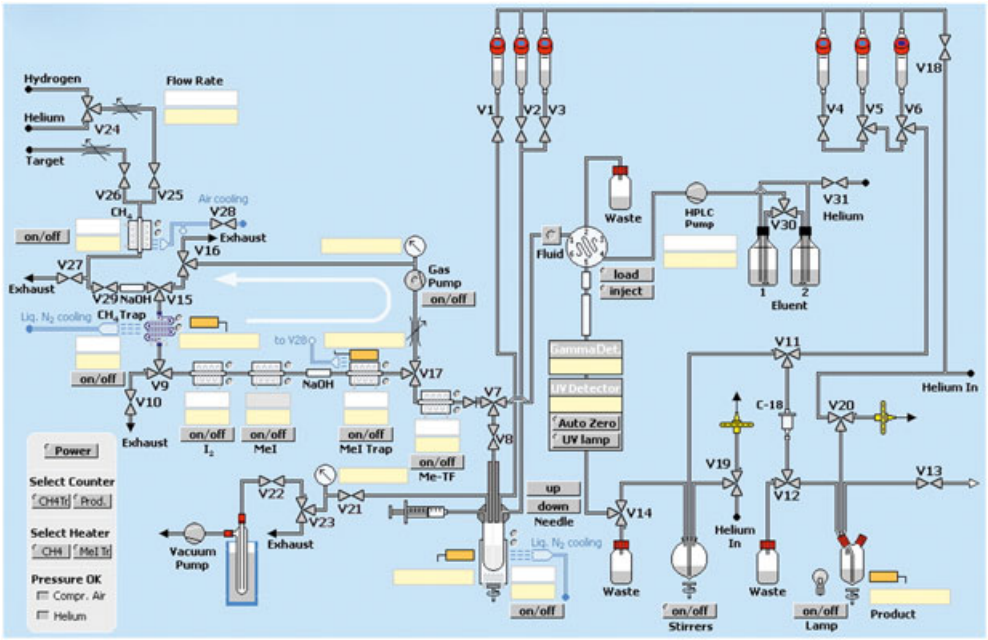

Figure 3. Standard HPLC Tracerlab $FX_{C\text{-}Pro}$ configuration.

Louis, MO, USA) (methionine), Monomerchem (Research Triangle Park, NC, USA) (DTBZ precursor (contract synthesis)), or SRI (Menlo Park, CA, USA) (DTBZ standard (contract synthesis)). PBR28 and PMP standards and precursors were synthesized in house[17] or can be purchased from ABX Advanced Biochemicals. Shimalite-Nickle was purchased from Shimadzu (Columbia, MD, USA), iodine was purchased from EMD (Rockland, MA, USA), phosphorus pentoxide was purchased from Fluka, and molecular sieves were purchased from Alltech (a division of Grace, Deerfield, IL, USA). Other synthesis components were obtained as follows: sterile filters were obtained from Millipore (Billerica, MA, USA); sterile product vials were purchased from Hollister-Stier (Spokane, WA, USA); C18-light Sep-Paks, CM-light Sep-Paks, and Porapak Q were purchased from Waters Corporation (Milford, MA, USA). C-2 and C-18 Extraction disks were obtained from 3M (St. Paul, MN, USA). Sep-Paks and extraction disks were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use. SAX Sep-Paks were purchased from Grace Inc. and conditioned with 10 mL 70% EtOH, 5 mL NaCl, 0.9% USP, and 10 mL sterile H_2O prior to use.

General procedure for production of [^{11}C]methyl iodide

Carbon-11 at the University of Michigan was produced via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a GEMS PETTrace cyclotron

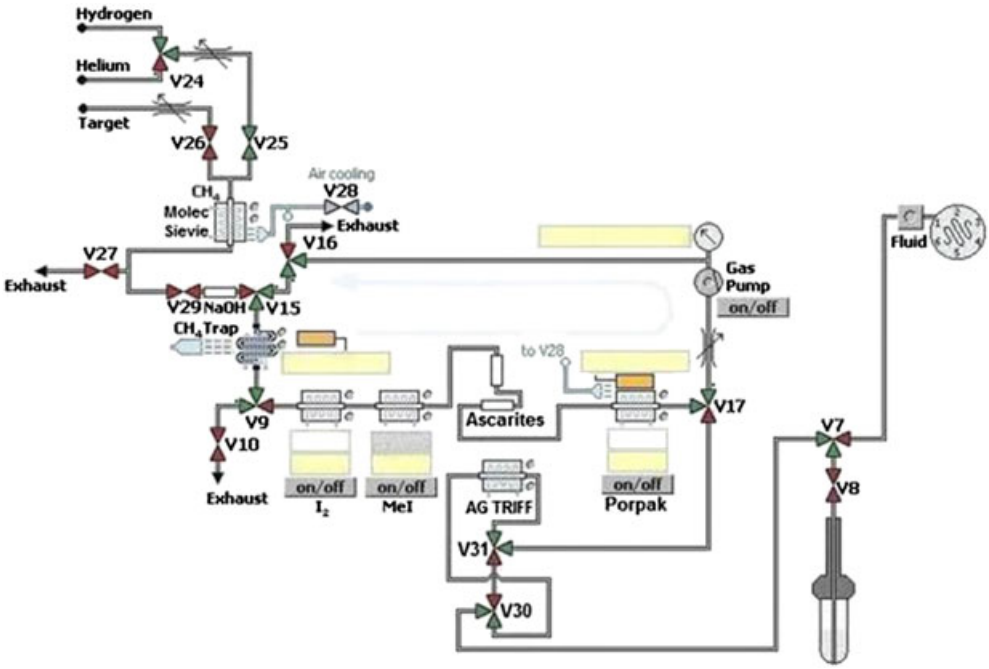

Figure 4. Modified Tracerlab $FX_{C\text{-}Pro}$ configuration for reactor/HPLC.

equipped with a carbon-11 target. Carbon-11 was delivered from the cyclotron target (40 μL, 30 min beam provided ~3 Ci of carbon-11 as [^{11}C]CO_2) *via* a 1/16″ Teflon delivery line by nitrogen pressure directly to a column packed with 0.3 g of molecular sieve and 0.2 g of Shimalite–Nickle where it was trapped at room temperature. The column was then sealed under hydrogen gas and heated to 350°C for 20 s to reduce the [^{11}C]CO_2 to [^{11}C]CH_4. The [^{11}C]CH_4 was passed through a column of phosphorous pentoxide desiccant and trapped on a column of carbosphere cooled to −75°C (with liquid nitrogen). Gaseous [^{11}C]CH_4 was released by heating the carbosphere column to 80°C. Once released, the methane entered a circulation loop, which includes a gas pump, a column of iodine at 100°C, the Tracerlab standard iodine reactor tube at 720°C, two adjacent columns of Ascarite II, and a column of Porapak Q at room temperature. The gaseous mixture was circulated for 5 min, whereas [^{11}C]MeI accumulated on the Porapak column. [^{11}C]MeI (~0.9 Ci) was then released from the Porapak column and either delivered directly to the awaiting reactor (or loop), or passed through a silver triflate column, by heating the Porapak column to 190°C.

General procedure for production of [^{11}C]methyl triflate

[^{11}C]CH_3I was converted to [^{11}C]CH_3OTf by passing it through a silver triflate-Graphpac column pre-heated to 190°C. The contents of the silver triflate-Graphpac column were prepared as follows: silver trifluoromethanesulfonate (5 g) was dissolved in anhydrous acetonitrile (100 mL), and the resulting solution was transferred into a round-bottomed flask containing graphite (10 g). The mixture was stirred, and then the solvent was evaporated to complete dryness *in vacuo*. The resulting [^{11}C]CH_3OTf was delivered to the reactor, loop, or Sep-Pak as outlined in the next paragraphs.

Radiopharmaceutical production via reactor methylation with HPLC purification

Production of [^{11}C]DTBZ

Introduction

Neurodegenerative diseases are frequently associated with characteristic losses of specific neurons in the human brain. For example, progressive losses of dopaminergic neurons are typical in Parkinson's disease, whereas Alzheimer's disease (AD) is associated with decline of the cholinergic neurons. Reflecting this, a number of strategies have been developed for *in vivo* imaging of such neuronal losses using PET. One common approach is the targeting of the vesicular monoamine transporter type 2 (VMAT2) using radioligands such as (+)-α-[^{11}C]dihydrotetrabenazine ([^{11}C]DTBZ: (2*R*,3*R*,11b*R*)-(1,3,4,6,7,11b-hexahydro-9-[^{11}C]methoxy-10-methoxy)-3-(2-methylpropyl)-2-hydroxy-2H-benzo[a]quinolizine).[18,19] The VMAT2 is not specific for a particular monoamine, but rather, it is a common protein capable of transporting dopamine, norepinephrine, serotonin, and histamine.[20] Despite this non-specificity, the utility of VMAT2 imaging in neurodegenerative disease is still possible because of the compartmentalization of neuronal types in the human brain.[18] For example, dopaminergic nerve terminals predominate in the basal ganglia, and so enable specificity for examining losses of such terminals in patients with Parkinson's disease. The VMAT2 is found in presynaptic vesicles, and is responsible for transporting monoamines from the cell cytosol into the storage vesicle, from where they can be released into the synapse.[21]

Synthesis procedures

To prepare DTBZ, the Tracerlab synthesis module was configured as illustrated in Figure 4 and loaded as follows: reaction vessel:

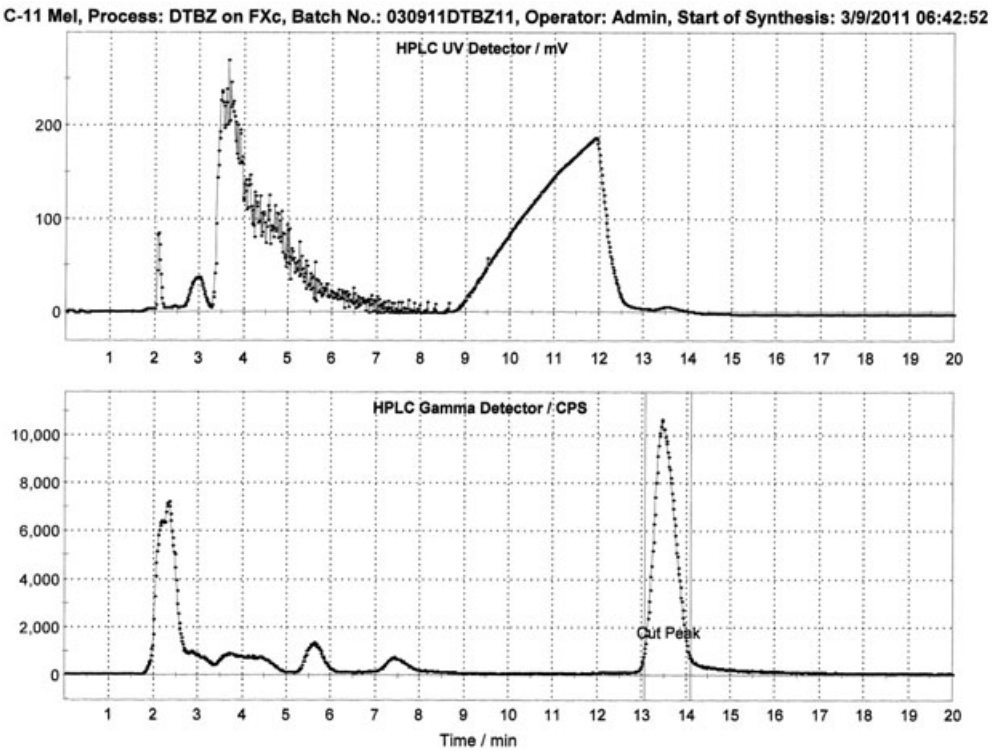

Figure 5. Semipreparative HPLC trace for [^{11}C]DTBZ (CPS = counts per second).

(2*R*,3*R*,11b*R*)-9-O-desmethyldihydrotetrabenazine (DTBZ precursor, 1.0 mg), NaOH (7.5 M, 10 μL); Vial 1: 85/15 20 mM NaH_2PO_4/ethanol (1 mL); Product collection vial: 0.9% NaCl for Injection, USP (7.0 mL). [^{11}C]MeI was prepared according to the general procedure outlined previously and then bubbled into the reaction vessel at a fixed flow rate of 15 mL/min for 3 min. After this time, the flow was stopped, and the solution was stirred for an additional 2 min. The reaction mixture was then diluted with 85/15 20 mM NaH_2PO_4/ethanol (1 mL) and purified using semipreparative HPLC (column: Phenomonex Luna C8(2), 10 × 100 mm; mobile phase: 85/15 20 mM NaH_2PO_4/ethanol; flow rate: 3 mL/min, typical trace: Figure 5). The product fraction (3 mL) was collected (retention time (RT) ~13–14 min) into the product collection vial where it was simultaneously diluted with isotonic saline (7 mL), and the resulting formulation was passed through a 0.22 μm sterilizing filter into a sterile dose vial. The product was then submitted for quality control (QC) testing, and typical yields of [^{11}C]DTBZ prepared using this method were 2.8% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Production of [^{11}C]PMP

Introduction

Acetylcholinesterase (AChE) is the enzyme responsible for the degradation of the neurotransmitter acetylcholine, leading to the termination of cholinergic neurotransmission. AChE deficits in postmortem AD brain samples have been observed, suggesting that cholinergic decline is part of the complex neurodegenerative cascade that occurs in AD.[22,23] Therefore, radiopharmaceuticals suitable for quantifying AChE *in vivo* have potential for tracking the progression of the cholinergic aspect of this cascade in patients with AD. The synthetic AChE substrate, 1-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]PMP),[24] is currently in routine clinical use as a radiopharmaceutical for the study of AChE function in patients with AD at both the University of Michigan and elsewhere,[25–28] and results from such studies have been encouraging. For example, statistically significant decreases in the cortical hydrolysis rate of [^{11}C]PMP in AD brains, versus age-matched controls, have been detected, and correlations identified.[26,28,29]

Synthesis procedures

PMP precursor was prepared as follows: 4-piperidinyl propionate hydrochloride (0.5 ± 0.1 mg) was dissolved in aqueous saturated sodium bicarbonate (~20 μL) and ethyl ether (2 mL) was added, and the solution was mixed vigorously on a vortex mixture for 1 min. Anhydrous sodium sulfate (0.5 g) was then added to absorb the water, and the dried ether was filtered into DMF (100 μL). The ether was evaporated under nitrogen, leaving the precursor in DMF as freebase. To prepare [^{11}C]PMP, the Tracerlab synthesis module was configured as illustrated in Figure 4 and loaded as follows: reaction vessel: [^{11}C]PMP precursor (0.5 mg in 100 μL DMF prepared as outlined previously); Vial 1: semipreparative HPLC mobile phase (20 mM NH_4OAc in 5% EtOH, 1.0 mL); product collection vial: 0.9% NaCl for Injection, USP (2.0 mL). [^{11}C]methyl triflate was prepared using the general procedure outlined previously and bubbled through the precursor solution at 15 mL/min for 3 min. The reaction mixture was then diluted with 1 mL of HPLC mobile phase and purified using semipreparative HPLC (column: Phenomonex Luna C18, 250 × 10 mm, mobile phase: 20 mM NH_4OAc in 5% EtOH, flow rate: 4 mL/min, typical trace: Figure 6). The product peak was collected (RT ~12–14 min) for 2 min (8 mL), diluted with USP saline (2 mL) to provide a final ethanol concentration <5%, and

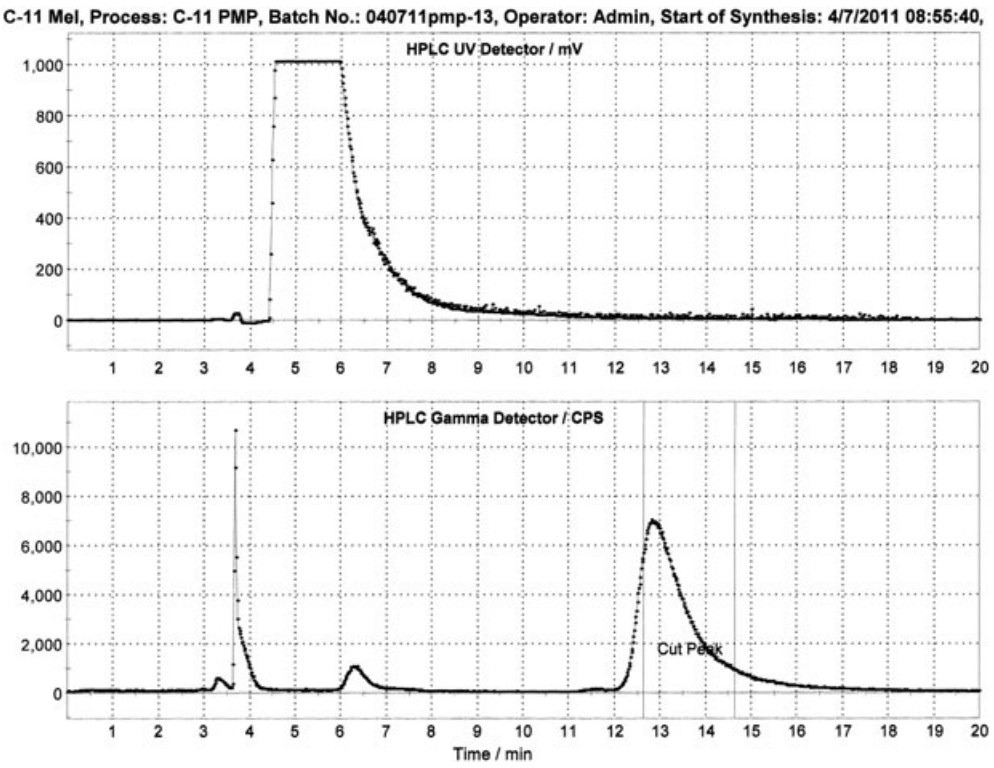

Figure 6. Semipreparative HPLC trace for [^{11}C]PMP.

passed through a 0.22 μm sterilizing filter into a sterile dose vial. The product was then submitted for QC testing, and typical yields of [^{11}C]PMP prepared using this method were 3.0% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Production of [^{11}C]HED

Introduction

Radiolabeled adrenergic neurotransmitters, or closely related analogs that actually behave as false neurotransmitters, are routinely employed for PET imaging in oncology[30] and cardiology.[31] One such example, carbon-11 labeled tracer meta-hydroxyephedrine ([^{11}C]HED or [^{11}C]MHED), is an analog of norepinephrine that was developed to visualize the sympathetic nervous system with PET imaging.[32] [^{11}C]HED accumulates in nerve terminals but does not get metabolized by catechol-*O*-methyl transferase or monoamine oxidase.[33] Because of these favorable characteristics, [^{11}C]HED can be used as a radiopharmaceutical for non-invasive assessment of sympathetic neuronal integrity of the heart using PET imaging.[32,34] Furthermore, early clinical studies showed rapid uptake of [^{11}C]HED in localized tumors of the sympathetic nervous system, and so the [^{11}C]HED is also utilized to image such tumors using PET.[30] Our strategy for producing [^{11}C]HED using a Tracerlab $FX_{C\text{-}Pro}$ is outlined in the next paragraphs. In addition to our efforts, related fully automated production using a Tracerlab $FX_{C\text{-}Pro}$ has also been reported by Lodi and co-workers.[14,33]

Synthesis procedures

To prepare [^{11}C]HED, the Tracerlab synthesis module was configured as illustrated in Figure 4 and loaded as follows: reaction vessel: metaraminol ([^{11}C]HED precursor, 0.5 ± 0.1 mg in DMF (50 μL)); Vial 1: semipreparative HPLC mobile phase (30 mM NH_4OAc in 1% EtOH, 1.0 mL); product collection vial: 0.9% NaCl for Injection, USP (2.0 mL). [^{11}C]Methyl triflate was prepared using the general procedure outlined previously and bubbled through the precursor solution at 15 mL/min for 3 min. The reaction mixture was diluted with 1 mL of HPLC mobile phase and purified using semipreparative HPLC (column: Phenomonex Luna C8, 150×10 mm, mobile phase: 30 mM NH_4OAc in 1% EtOH, flow rate: 4 mL/min, typical trace: Figure 7). The product peak was collected (RT ~12 min) for 2 min (8.0 mL), diluted with USP saline (2.0 mL) and passed through a 0.22-μm sterilizing filter into a sterile dose vial. This product was then submitted for QC testing, and typical yields of [^{11}C]HED prepared using this method were 2.9% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Production of [^{11}C]FMZ

Introduction

Flumazenil (an imidazobenzodiazepine derivative) is a benzodiazepine antagonist that was developed by Hoffmann-La Roche (and marketed as Anexate).[35] Flumazenil is as a competitive inhibitor that acts on the benzodiazepine binding site of the γ–amino butyric acid A receptor and has therefore been used as an antidote for the treatment of overdoses of both benzodiazepines, and non-benzodiazepine sleep enhancers.[36] Subsequently, flumazenil was developed into a radiopharmaceutical for quantifying neuronal density, and literature methods for preparing both carbon-11[37–40] and fluorine-18[41–44] versions of FMZ have been reported. For example, Aigbirhio and co-workers reported an elegant loop method for preparing [^{11}C]FMZ using a GEMS Microlab/Tracerlab FX_C combination.[40] [^{11}C]FMZ is now a well-established radiopharmaceutical used to detect epileptic foci in neocortical epilepsy,[45] explore hippocampus function in patients with temporal lobe epilepsy[46] and as a marker of damage in stroke victims.[43,47]

Synthesis procedures

To prepare [^{11}C]FMZ, the Tracerlab synthesis module was configured as illustrated in Figure 4 and loaded as follows: reaction vessel: desmethylflumazenil (1 mg in DMF (100 μL) and NaOH

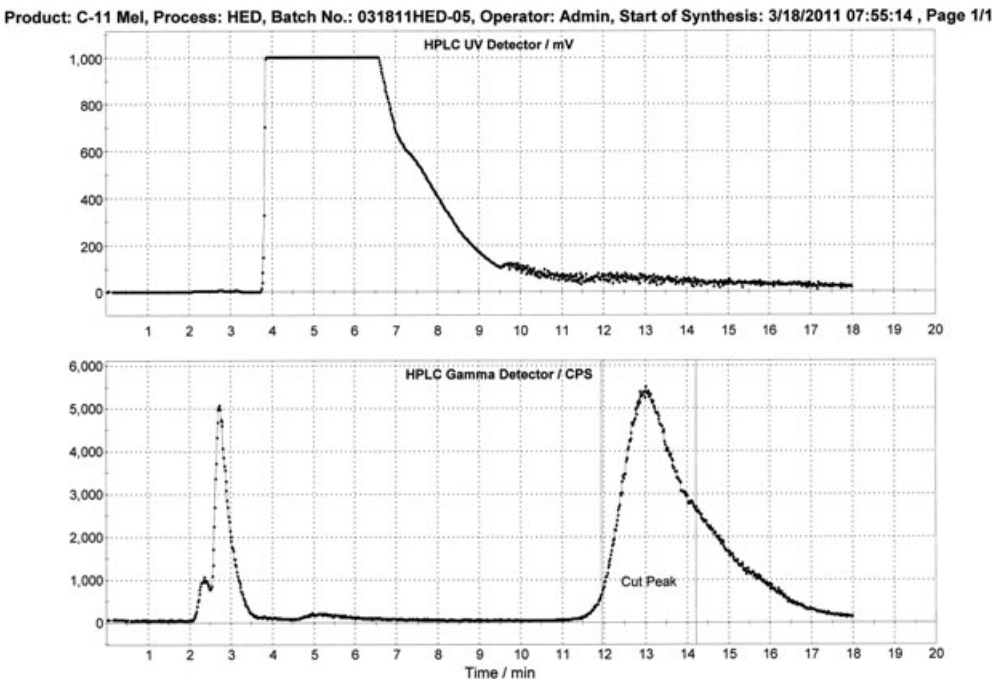

Figure 7. Semipreparative HPLC trace for [^{11}C]HED.

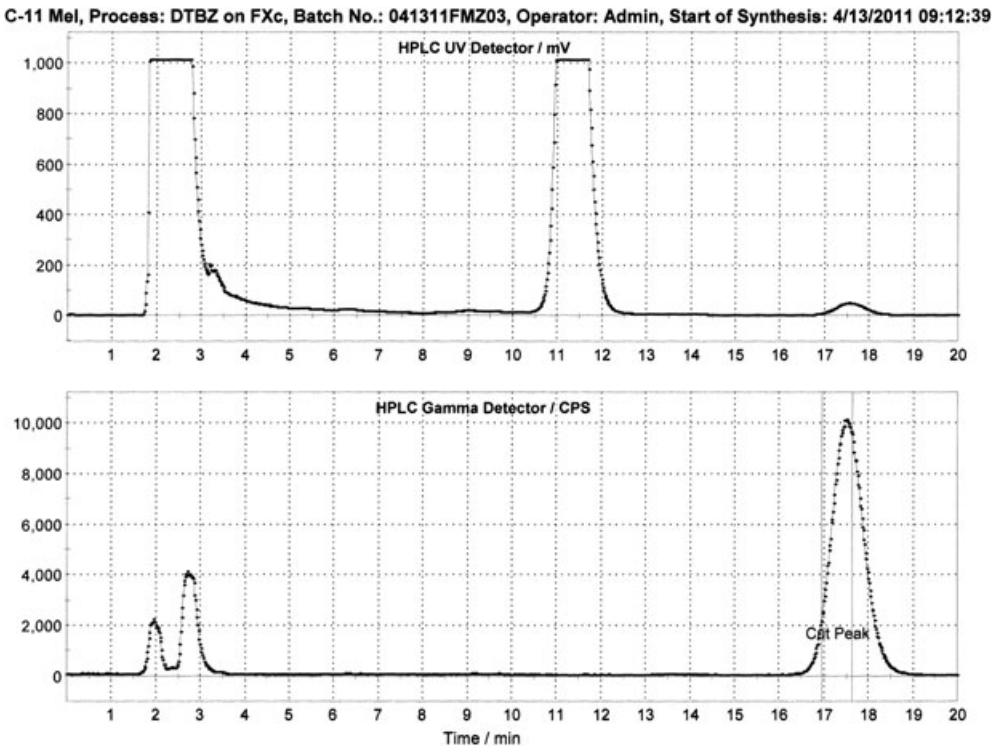

Figure 8. Semipreparative HPLC trace for [^{11}C]FMZ.

(0.5 M, 10 μL)); Vial 1: semipreparative HPLC mobile phase (20 mM NH_4OAc in 20% EtOH, 1.0 mL); product collection vial: 0.9% NaCl for Injection, USP (7.5 mL). [^{11}C]methyl iodide was generated using the standard procedure described previously and bubbled through the precursor solution at 15 mL/min for 3 min. The reaction mixture was diluted with 1 mL of HPLC mobile phase and purified using semipreparative HPLC (column: Phenomonex Luna C18, 150 × 10 mm, mobile phase: 20 mM NH_4OAc in 20% EtOH, flow rate: 5 mL/min, typical trace: Figure 8). The product peak around was collected (RT ~17 min) for 30 s (2.5 mL) and diluted with USP saline (7.5 mL). This final formulation was then passed through a 0.22-μm filter into a sterile dose vial and submitted for QC testing. Typical yields of [^{11}C]FMZ produced using this method were 3.0% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 7$).

Production of [^{11}C]PBR28

Introduction

Presently, there is significant interest in imaging the peripheral benzodiazepine receptor (PBR), also known as the translocator protein 18 kDa,[48,49] and a number of PET biomarkers for the PBR have been reported in recent years. The PBR is different to the brain receptors that bind γ–amino butyric acid and synthetic benzodiazepines and is found on the outer membrane of mitochondria in a number of cells, as well as plasma membranes in erythrocytes. Imaging PBR using PET is of interest because PBR has been implicated in cancer, nervous system disorders, and neurodegenerative diseases. Moreover, an increase in PBR levels is considered indicative of inflammation. Reflecting this, a number of PET biomarkers that allow for imaging and evaluation of PBR are known, of which the most common is [^{11}C]PK11195.[50,51] However, [^{11}C]PK11195 has limitations including low brain penetration and high non-specific binding. Therefore, new PET ligands for PBR have been developed, and we selected *N*-(2-[^{11}C]methoxybenzyl)-*N*-(4-phenoxy-3-pyridinyl) acetamide ([^{11}C]PBR28),[52–57] to make available to clinicians. PBR28 was developed by Pike and colleagues and has high affinity for PBR (half maximal inhibitory concentration (IC_{50}) = 0.658 nm) as well as favorable pharmacokinetics and dosimetry.[58]

Synthesis procedures

To prepare [^{11}C]PBR28, the Tracerlab synthesis module was configured as illustrated in Figure 4 and loaded as follows: reaction vessel: des-PBR28 (1 mg) and 60% NaH (in dispersion mineral oil, 1–1.3 mg) in CH_3CN (100 μL); Vial 1: semipreparative HPLC mobile phase (1.0 mL); Vial 4: Sterile Water for Injection, USP (10 mL); Vial 5: Ethanol (1.0 mL); Vial 6: 0.9% NaCl for Injection, USP (9.0 mL); round-bottomed dilution flask: HPLC Grade Water (70 mL). Ten minutes before the end of the bombardment, des-PBR28 (1 mg) and 60% NaH (in dispersion mineral oil, 1–1.3 mg) were dissolved in CH_3CN (100 μL), sonicated for 1 min and transferred into the reactor. [^{11}C]MeOTf was prepared according to the general procedure outlined previously and then bubbled through the reactor via He flow (12 mL/min) until the activity in the reactor reached its maximum (around 3.5 min). The reactor was then sealed and heated for 3 min at 80°C. After cooling the reactor at 40°C, the reaction solution was diluted with HPLC eluent (1 mL) and injected into the HPLC loop for purification using semipreparative HPLC method (column: Phenomenex Prodigy ODS-prep 10 μm 250 × 10 mm, mobile phase: 40% MeCN: 60% water, flow rate: 5 mL/min, typical trace: Figure 9). The radioactive peak was collected (RT ~17 min) into the round-bottomed flask containing HPLC grade water (70 mL), which was then passed through a C18 Empore Sep-Pak. Afterward, the cartridge was rinsed with Sterile

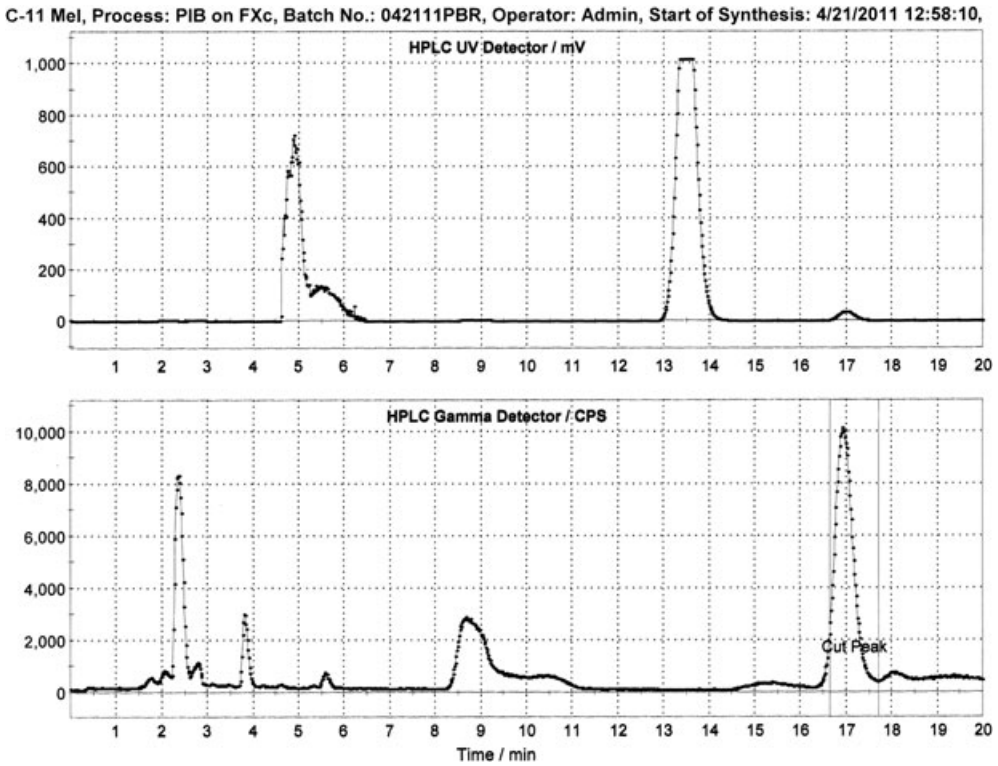

Figure 9. Modified Tracerlab $FX_{C\text{-}Pro}$ configuration for reactor/solid phase extraction.

Water for Injection (10 mL). The labeled compound was then eluted off the cartridge with USP ethanol (1 mL) and diluted with Sterile 0.9% NaCl for Injection, USP (9 mL). This aqueous solution was filtered through a 0.22-μm sterile filter into a sterile dose vial and submitted for QC testing. Typical yields of [^{11}C]PBR28 using this method are 4.2% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 6$).

Radiopharmaceutical production *via* reactor methylation and solid phase extraction purification

Radiolabeling of certain compounds with carbon-11 proceeds cleanly (i.e., no by-products), and the radiopharmaceutical product has sufficiently different polarity to the precursor that

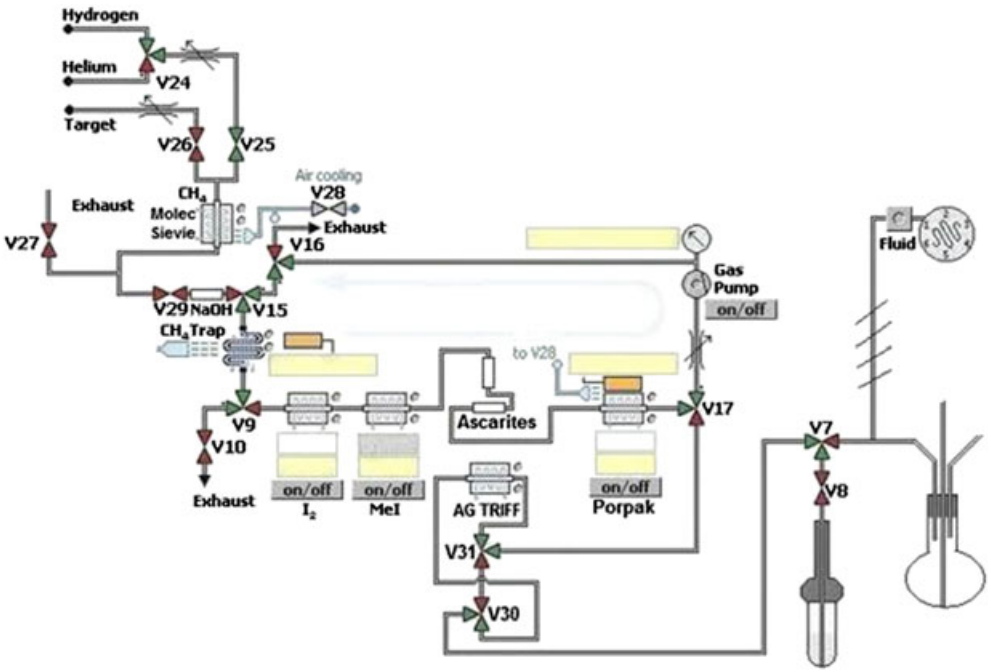

Figure 10. Modified Tracerlab $FX_{C\text{-}Pro}$ configuration for loop/HPLC.

purification can be achieved using SPE or evaporation techniques. Examples of such radiopharmaceuticals include [^{11}C]carfentanil and [^{11}C]methionine, respectively. In order to prepare these radiopharmaceuticals, the TracerLab $FX_{C\text{-}Pro}$ is configured to bypass the HPLC column (Figure 10), and the line out of the reactor (V7) is connected directly to the round-bottomed dilution flask used for reformulation. Reformulation then proceeds as normal (Figure 3).

Production of [^{11}C]carfentanil

Introduction

During the mid-1960s, binding studies using brain homogenates made it apparent that opioid agents probably act through interaction with specific receptors, and the first to be identified were the mu-opioid receptors.[59,60] The mu-opioid receptors are an important class of receptors, with roles in mediating pain and feelings of well-being.[61] Carfentanil, one of the most potent opioids known, has been shown to bind very specifically with mu-opioid receptors as a full agonist. Reflecting this discovery, [^{11}C]carfentanil has found widespread use as a radiopharmaceutical for measuring and mapping the mu-opioid receptors *in vivo*.[62,63] A number of syntheses have been reported,[1,64–68] and [^{11}C]carfentanil has been used to explore many normal and altered neurophysiological and psychic phenomena.

Synthesis procedures

To prepare [^{11}C]carfentanil, the Tracerlab synthesis module was configured as illustrated in Figure 10 and loaded as follows: reaction vessel: desmethyl carfentanil - tetrabutyl ammonium (TBA) salt (0.4 mg in dimethyl sulfoxide (100 μL)); Vial 1: 1% ammonium hydroxide (1 mL); Vial 3: 10% *n*-propanol (3 mL); Vial 4: Milli-Q Water (7 mL); Vial 5: Ethanol (0.5 mL); Vial 6: Sterile Water for Injection (9.5 mL); round-bottomed dilution flask: 1% ammonium hydroxide (5 mL). [^{11}C]methyl triflate was prepared using the general procedure outlined previously and bubbled through the precursor solution at 15 mL/min at room temperature for 3 min. After production, 1% ammonium hydroxide (1 mL) was added to the reaction vessel. The crude reaction mixture was then conveyed via helium gas to the dilution flask containing 5 mL of 1% ammonium hydroxide. This mixture was passed through a 3 M Empore C2 extraction disk where the [^{11}C]carfentanil was trapped. Three milliliters of 10% *n*-propanol followed by 7 mL Milli-Q water were then passed through to remove impurities from the disk. The disk was then dried by passing helium gas through for 1.0 min, and the [^{11}C]carfentanil was eluted off with ethanol (0.5 mL) and diluted with Sterile Water for Injection (9.5 mL). The formulated product was then passed through a 0.22-μm filter into a sterile dose vial, and the product was submitted for QC testing. Typical yields of [^{11}C]carfentanil preparing using this method were 6.3% based upon starting [^{11}C]CO_2 ($n = 10$).

Production of [^{11}C]methionine

Introduction

Methionine is a naturally occurring amino acid that is a key component of protein biosynthesis.[69,70] As tumor cells are growing more rapidly than corresponding non-cancerous cells, the formation of new proteins is more rapid in cancerous cells. This increased rate of growth can be detected using PET imaging with [^{11}C]methionine ([^{11}C]MET), and thus, [^{11}C]MET can be considered a biomarker of cell proliferation like, for example, [^{18}F]FDG and [^{18}F]FLT. Although [^{11}C]MET has been used to image a variety of tumors,[71,72] the majority of its clinical use to date has been for imaging brain tumors such as gliomas.[73,74] A number of strategies for preparing [^{11}C]methionine having been reported,[75–79] including recent developments that eliminate the need for HPLC purification.[80–82]

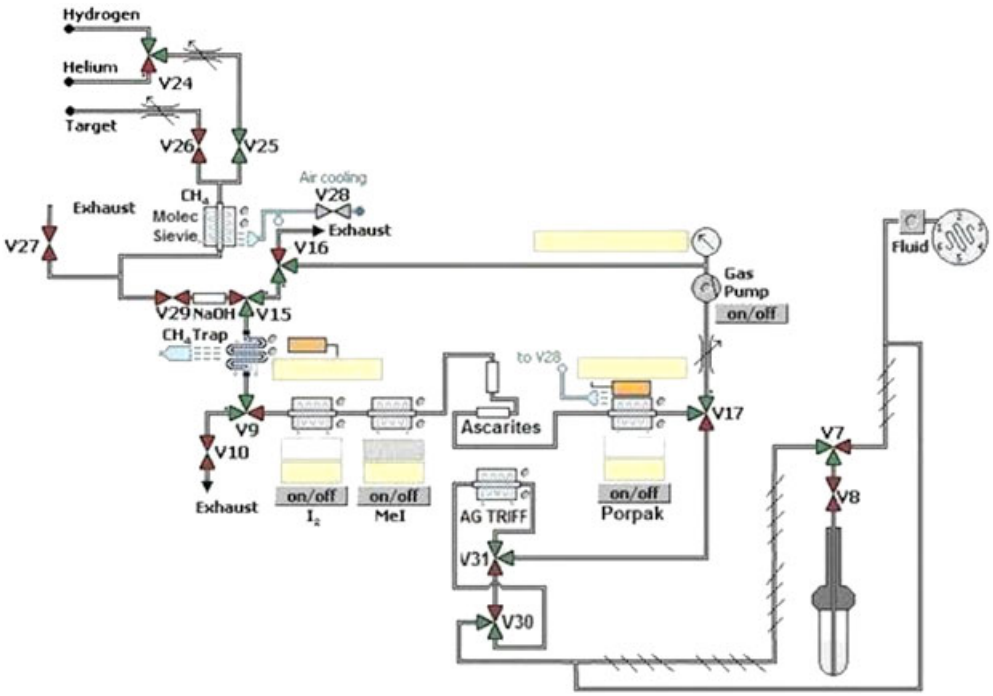

Figure 11. Semipreparative HPLC trace for [^{11}C]DASB.

Synthesis procedures

To prepare [^{11}C]methionine, the Tracerlab synthesis module was configured as illustrated in Figure 10 and loaded as follows: reaction vessel: L-homocysteine thiolactone (2.0 mg) dissolved sodium hydroxide (0.1 M, 0.5 mL) and acetone (0.5 mL); Vial 1: 0.9% NaCl for Injection, USP (1.0 mL); round-bottomed dilution flask: 45 mM Sodium Phosphates for Injection, USP (0.5 mL) + 0.9% NaCl for Injection, USP (8.5 mL). [^{11}C]CH_3I was made according to the general procedure outlined previously and then bubbled through the solution of *L*-homocysteine thiolactone at 15 mL/min for 3 min. After reaction, the acetone and residual [^{11}C]CH_3I were evaporated at 55°C under a helium gas stream for 5 min. The product was then redissolved in 0.9% NaCl for Injection, USP (1.0 mL) and passed into the round-bottomed flask charged with 45 mM sodium phosphates, USP (0.5 mL) and additional 0.9% NaCl for Injection, USP (8.5 mL). This final formulation was then passed through a 0.22-μm filter into a sterile dose vial and submitted for QC testing. Yields of methionine using this method are typically 4.0% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 4$).

Radiopharmaceutical production *via* loop methylation and HPLC purification

Loop chemistry, an early form of microfluidics, has proven a particularly efficient strategy for radiolabeling with carbon-11. A solution of the precursors is deposited as a thin film on the inside of an HPLC loop. The reaction then occurs by blowing [^{11}C]MeI (or [^{11}C]MeOTf) through the HPLC loop, to generate the product. Following reaction, the valve is switched to 'inject', and the contents of the loop are washed by using HPLC mobile phase and injected onto the HPLC column for immediate purification. Examples of such radiopharmaceuticals particularly amenable to loop synthesis include [^{11}C]DASB, [^{11}C]PiB, and [^{11}C]raclopride. In order to prepare these radiopharmaceuticals using a TracerLab $FX_{C\text{-}Pro}$, the synthesis module had to be reconfigured to deliver methyl triflate directly to the HPLC loop, as shown in Figure 11. Following HPLC purification, fraction collection and/or reformulation proceeds as normal (Figure 3).

Production of [^{11}C]DASB

Introduction

Serotonin is one of the monoamine neurotransmitters. Extracellular levels of serotonin in the synaptic cleft are controlled by the serotonin transporter (SERT), which can decrease serotonins level through reuptake.[83] Serotonin is widely known to influence feelings of well-being, whereas, conversely, dysfunction of the SERT (and corresponding disruption of serotonin levels) has been implicated in a range of psychiatric disorders including schizophrenia,[84] depression,[85,86] and anxiety.[86] Reflecting this, SERT is the target for the selective serotonin reuptake inhibitors, a widely used class of antidepressant drugs,[87] and SERT imaging has proven to be an important tool for managing these disorders with, for example, PET imaging. [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile ([^{11}C] DASB) is a compound that binds to the serotonin transporter and is the radioligand of choice for imaging the SERT transporter,[84,88–90] because of a number of characteristics that make it an attractive ligand for clinical imaging. For example, it has high affinity for the SERT (1.1 nM), excellent selectivity over the corresponding norepinephrine and dopamine transporters (NET/SERT = 1230 nM and DAT/SERT = 1300 nM, respectively); high specific binding and, finally, high (but reversible) brain uptake.[91] Syntheses of [^{11}C] DASB have been reported, for example, by Wadsak and colleagues.[88,91]

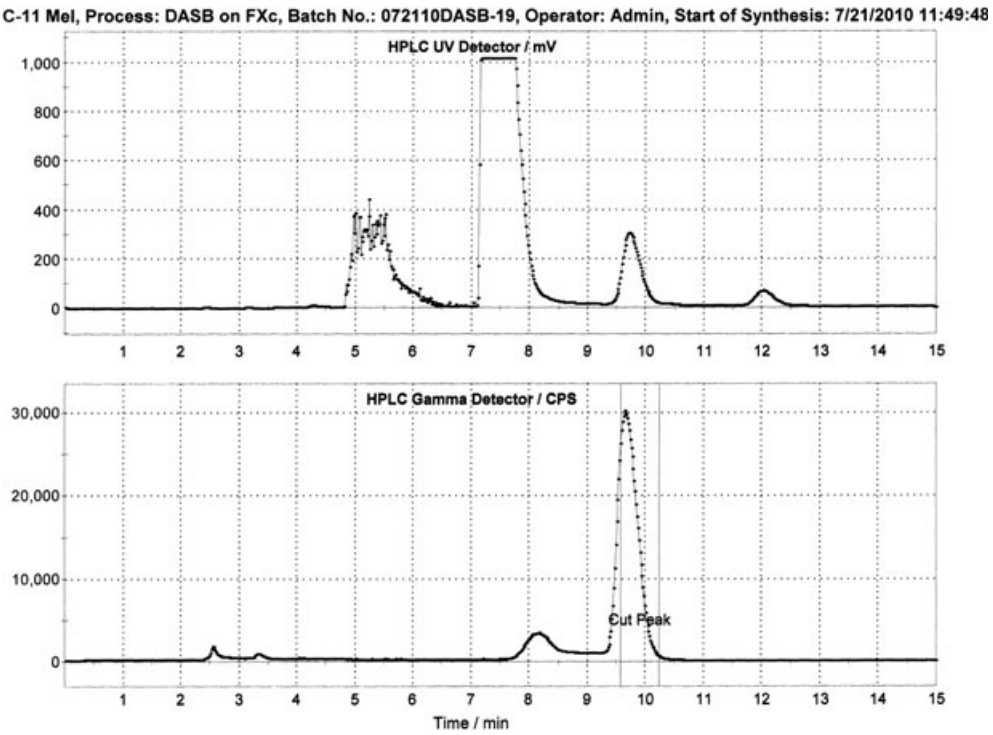

Figure 12. Semipreparative HPLC trace for [^{11}C]PiB.

Synthesis procedures

To prepare [^{11}C]DASB, the Tracerlab synthesis module was configured as illustrated in Figure 11 and loaded as follows: 2 mL steel HPLC loop: *O*-desmethyl DASB (1.0 mg) in 2-butanone (100 μL); Vial 4: Sterile Water for Injection, USP (7 mL); Vial 5: Ethanol (0.5 mL); Vial 6: 0.9% NaCl for Injection, USP (9.5 mL); round-bottomed dilution flask: Milli-Q Water (50 mL). The precursor solution was loaded onto the HPLC loop (2 mL, steel) and conditioned with nitrogen gas for 20 s at 10 mL/min. [^{11}C]MeOTf was prepared according to the general procedure outlined previously and passed through the HPLC loop at 40 mL/min for 3 min. Following reaction, the mixture was purified using semipreparative HPLC (column: Phenomonex Luna C18, 250 × 10 mm, mobile phase: 100 mM NH_4OAc in 30% MeCN, pH = 5.5, flow rate: 4 mL/min, typical trace: Figure 12). The product peak (RT ~10 min) was collected into 50 mL of water. The solution was then passed through a C18 Sep-Pak to remove organic solvent and washed with 7 mL sterile water. The product was then eluted with 0.5 mL of USP ethanol followed by 9.5 mL of USP saline. This final formulation was then passed through a 0.22-μm filter into a sterile dose vial and released for QC testing. Typical yields of [^{11}C]DASB prepared using this method are 4.0% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Production of [^{11}C]Pittsburgh compound B

Introduction

Alzheimer's disease affects 5.3 million Americans, and this number is expected to rise as the baby boom generation comes of age. The cause of AD is presently not entirely understood but what is clear is that it results from a complex neurodegenerative cascade that includes misfolding and aggregation of proteins such as amyloid and tau, with concomitant decline of neurotransmitter systems.[92–94] Although AD can be predicted by clinical diagnosis,[95] differentiating it from other clinically and neuropathologically overlapping dementia diseases can only be achieved definitively by high-risk diagnostic procedures (e.g., brain biopsy) or, more commonly, *post mortem*.[96] This makes it difficult to (a) differentiate AD from other dementias and treat each appropriately before patient death; (b) manage AD early, before cognitive decline; (c) select patients for assisting in AD drug development; and (d) track the impact of new AD therapeutics in clinical trials. Therefore, new non-invasive diagnostic methods for managing AD are in high demand, and PET imaging has enormous potential to impact health care in this regard. Important radioactive drugs that allow diagnosis of AD by visualization of amyloid plaques using PET imaging have been developed (e.g., [^{18}F]Amyvid ([^{18}F]Florbetapir),[97] [^{18}F]Florbetaben,[98] [^{18}F]Flutemetamol,[99] [^{11}C]Pittsburgh Compound B ([^{11}C]PIB)[100]) and are in advanced clinical trials. For example, [^{11}C]PIB is a radiolabeled analog of a dye used to stain amyloid by pathologists (thioflavin-T). [^{11}C]PIB was developed by Mathis and Klunk,[101] although a number of syntheses have since been reported,[15,102,103] and can be used to non-invasively image (and quantify) beta-amyloids plaques *in vivo* using PET imaging.[100]

Synthesis procedures

To prepare [^{11}C]PiB, the Tracerlab synthesis module was configured as illustrated in Figure 11 and loaded as follows: 2 mL steel HPLC loop: 2-(4′-aminophenyl)-6-hydroxybenzothiazole (1.0 mg) in 3-pentanone (100 μL); Vial 4: Sterile Water for Injection, USP (7 mL); Vial 5: Ethanol (0.5 mL); Vial 6: 0.9% NaCl for Injection, USP (9.5 mL); round-bottomed dilution flask: Milli-Q Water (50 mL). The precursor solution was loaded onto the HPLC loop

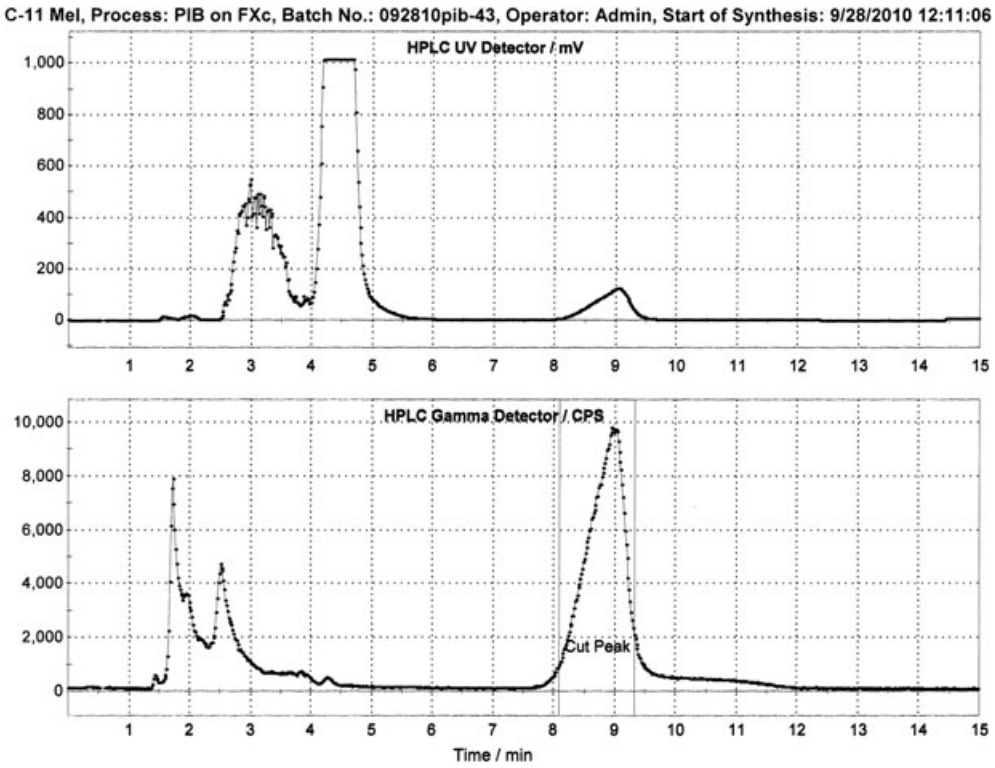

Figure 13. Semipreparative HPLC trace for [^{11}C]raclopride.

(2 mL, steel) and conditioned with nitrogen gas for 20 s at 10 mL/min. The [^{11}C]methyl triflate was passed through the HPLC loop at 15 mL/min for 5 min. The reaction mixture was then purified using semipreparative HPLC (column: Phenomonex Luna C18, 150 × 10 mm, mobile phase: 50 mM NH_4OAc in 40% MeCN, flow rate: 5 mL/min, typical HPLC trace: Figure 13). The product peak (RT ~8 min) was collected into 50 mL of water. The solution was then passed through a C-18 extraction disk to remove organic solvent. The disk was washed with 7 mL sterile water. The product was eluted with 0.5 mL of USP ethanol followed by 9.5 mL of USP saline. This final formulation was then passed through a 0.22-μm filter into a sterile dose vial and released for QC analysis. Typical yields of [^{11}C]PiB prepared using this method are 1.6% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Production of [^{11}C]raclopride

Introduction

[^{11}C]Raclopride, a substituted benzamide, is a radiopharmaceutical used for visualization and quantification of D_2-like dopamine receptors using PET imaging.[104,105] [^{11}C]Raclopride has been used to study neuropsychiatric disorders related to dopamine D_2 receptors for years.[106–108] Several different synthetic approaches have been reported, as well as different automated modules for production of [^{11}C]raclopride. Both [^{11}C]methyl iodide and [^{11}C]methyl triflate have been used for [^{11}C]methylation of *O*-desmethyl raclopride in the presence of base.[109–112] Recently, the loop methylation strategy has also become more popular for preparing [^{11}C]raclopride because of its high efficiency.[11,113–116] With the increasing demand for this radiopharmaceutical, we have recently developed a fully automated and reliable loop method to produce [^{11}C]raclopride for routine clinical application in high radiochemical yield and purity.[1]

Synthesis procedures

To prepare [^{11}C]raclopride, the Tracerlab synthesis module was configured as illustrated in Figure 11 and loaded as follows: 2 mL steel HPLC loop: *O*-desmethyl raclopride tetrabutyl ammonium salt (1.0 mg) in 2-butanone (100 μL); product collection vial: 0.9% NaCl for Injection, USP (5.5 mL). The precursor solution was slowly injected into HPLC loop. [^{11}C]CH_3OTf was prepared according to the general procedure described previously and passed through the loop at a flow rate 40 mL/min. After 3 min, the reaction mixture was injected onto a HPLC column and purified using semipreparative HPLC (column: Phenomonex Luna NH_2, 250 × 10 mm, mobile phase: 20 mM NH_4OAc in 10% ethanol, flow rate: 3 mL/min, typical HPLC trace: Figure 14). The product peak (RT ~10 min) was collected for 90 s (4.5 mL) directly into the product vial containing USP saline (5.5 mL). The final dose was filtered through a 0.22-μm sterile filter into a dose vial and submitted for QC testing. Typical yields of [^{11}C]raclopride prepared using this method are 2.2% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Concomitant production and purification of radiopharmaceuticals using solid phase extraction cartridges

In addition to being used for purification of radiopharmaceuticals, in certain cases, SPE Sep-Pak cartridges can also be used (alone or in series) as the reaction vessel. The reaction then occurs by blowing [^{11}C]MeI (or [^{11}C]MeOTf) over an SPE cartridge, previously charged with precursor, to generate the product. Following reaction, purification can then be achieved by using standard removal of impurities and then elution of the product as previously described. We routinely employ this strategy for preparation of [^{11}C]choline. In order to automate

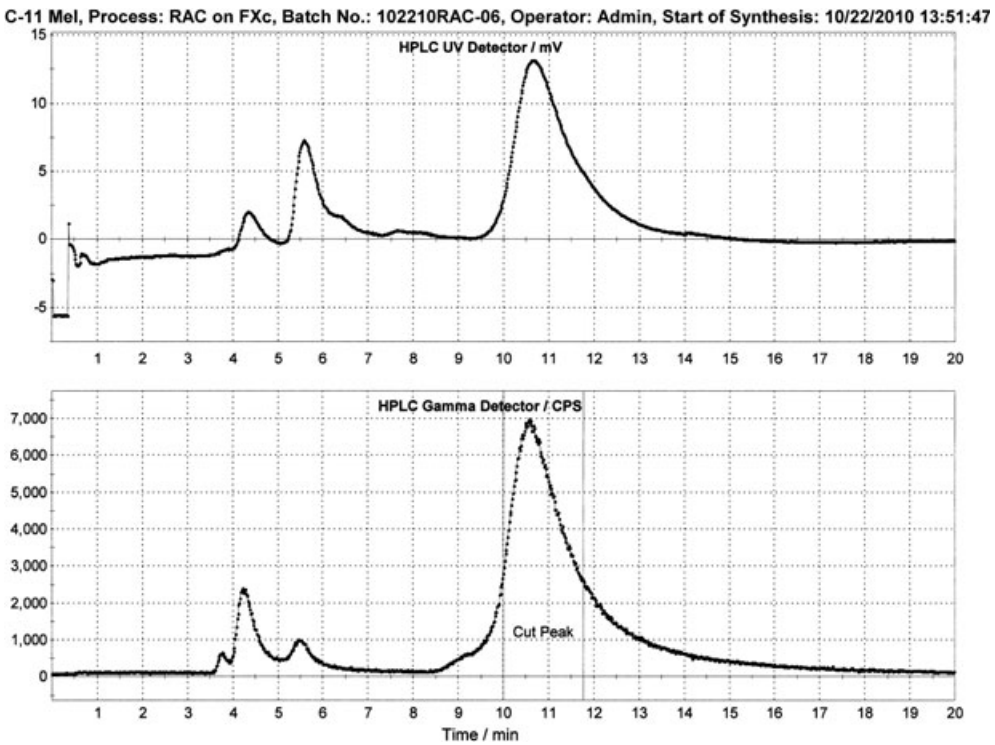

Figure 14. Semipreparative HPLC trace for [^{11}C]PBR-28.

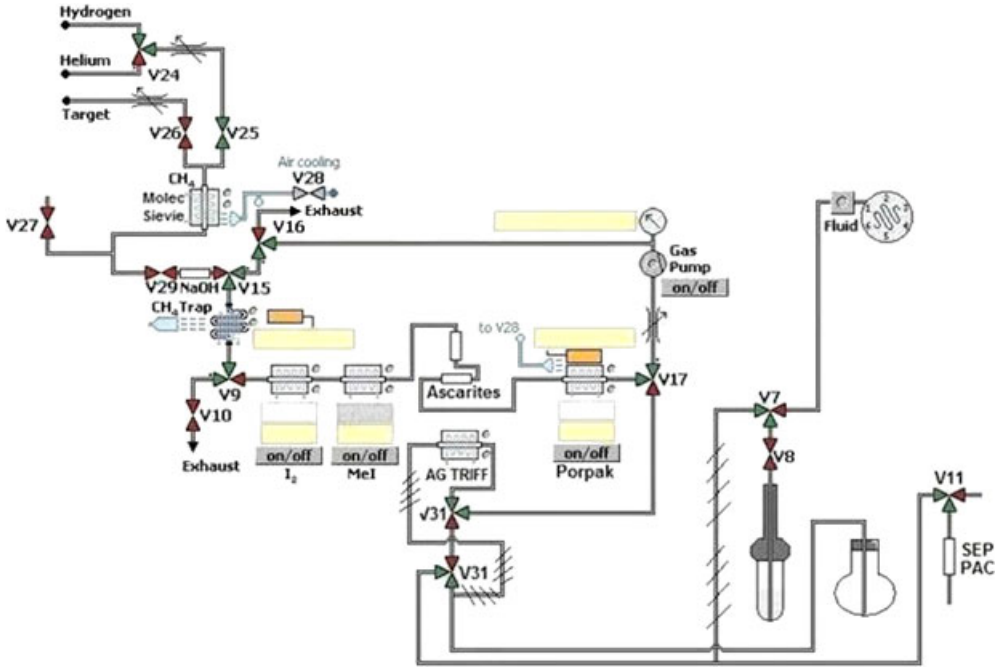

Figure 15. Modified Tracerlab $FX_{C\text{-}Pro}$ configuration for solid phase extraction reaction/purification.

preparation of [^{11}C]choline using a TracerLab $FX_{C\text{-}Pro}$, the synthesis module had to be reconfigured to deliver methyl iodide directly to the SPE cartridge, as shown in Figure 15. Following reaction, purification and reformulation proceeds as normal, utilizing vials 4, 5, and 6.

Production of [^{11}C]choline

Introduction

Choline is a salt that is found in all cells where it is used in the biosynthesis of phospholipids. Such phospholipids are then incorporated into cell membranes, and therefore, radiolabeled analogs of choline can be used to monitor the rate of production of cell membranes *in vivo*, and thus the rate of cell proliferation. [^{11}C]choline, and more recently [^{18}F]fluoromethylcholine, have been employed as biomarkers for imaging human brain, lung, and prostrate cancers.[117] To support the increasing demand for [^{11}C]choline, a number of synthetic approaches have been reported,[15,118–121] the most common of which uses a C18 Sep-Pak as a solid support for methylation and, subsequently, a CM Sep-Pak for purification. We have produced [^{11}C]choline manually using this method, but when adapting this procedure for automation using the TracerLab $FX_{C\text{-}Pro}$, the back pressure

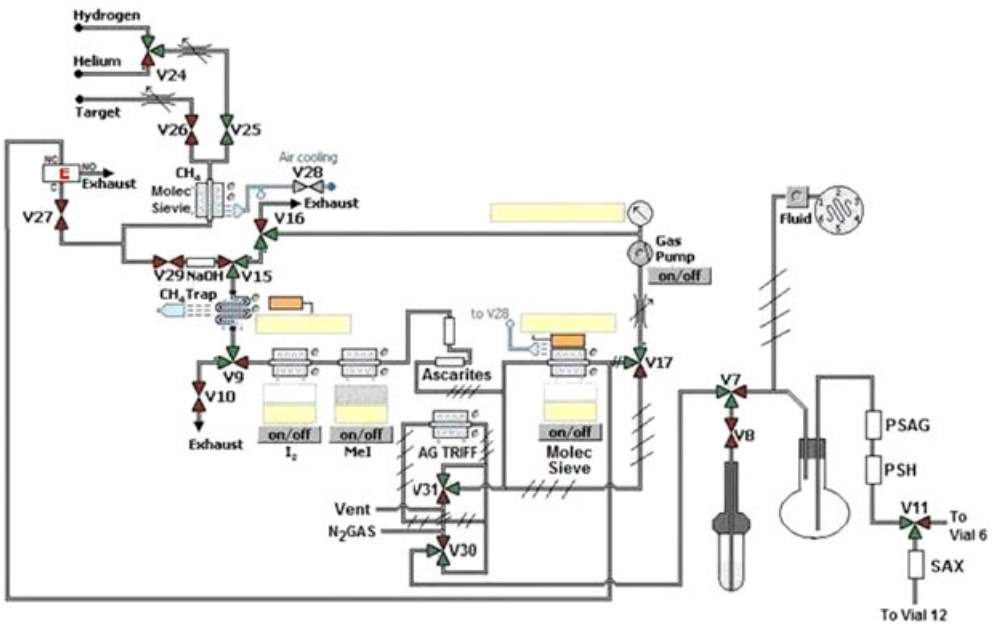

Figure 16. Modified Tracerlab $FX_{C\text{-}Pro}$ configuration for addition of Grignard to [^{11}C]CO_2.

of two adjacent Sep-Pak cartridges significantly slowed down liquid transfers during the synthesis, resulting in lower yields and specific activity of the final dose. To address this problem, we recently reported an optimized method for producing [^{11}C]choline using only one CM Sep-Pak for both reaction and purification.[3,122] The low back pressure of this system enabled efficient and reliable production of [^{11}C]choline using a TracerLab $FX_{C\text{-}Pro}$.

Synthesis procedures

To prepare [^{11}C]choline, the TracerLab $FX_{C\text{-}Pro}$ was configured as shown in Figure 15 and loaded as follows: CM Sep-Pak: 40 μL of *N,N*-dimethylaminoethanol (DMAE) dissolved in 20 μL of ethanol; Vial 4: ethanol (5 mL); Vial 5: 0.9% NaCl for Injection, USP (0.5 mL); Vial 6: Sterile Water for Injection, USP (9.5 mL); round-bottomed dilution flask: Sterile Water for Injection, USP (20 mL). The three-way valve V30 was borrowed from HPLC unit and inserted between the round-bottomed flask and V11. This valve (V30) is configured normally open to V17, which allows [^{11}C]methyl iodide going to Sep-Pak through V11 for reaction. Activating this valve diverts water from the round-bottomed dilution flask to the Sep-Pak for washing. [^{11}C]Choline was synthesized by [^{11}C] methylation of DMAE with [^{11}C]methyl iodide. Forty microliters of DMAE was dissolved into 20 μL of ethanol and loaded onto a CM Sep-Pak. The Sep-Pak was then installed on the synthesis module between V11 and V12. [^{11}C]Methyl iodide was produced according to the general procedure outlined previously and subsequently passed through the CM Sep-Pak at 20 mL/min flow rate for 3 min. After this time, the Sep-Pak was washed with 5 mL of ethanol (from Vial 4) and 20 mL of water (from round-bottomed flask). The product was eluted with 0.5 mL of USP saline (from Vial 5), followed by 9.5 mL of USP water (from Vial 6) into the product vial. The final dose was then transferred into a sterile dose vial through a 0.22-μm sterile filter and submitted for QC testing. Typical yields of [^{11}C]choline produced using this method were 7.3% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 10$).

Radiopharmaceutical production *via* alkylation of [^{11}C]CO_2 with Grignard reagents

Finally, certain radiopharmaceuticals can be prepared directly from [^{11}C]CO_2. In such cases, the synthesis module has to be reconfigured to bypass the methyl iodide and methyl triflate production loop, so that [^{11}C]CO_2 can be bubbled directly through the reaction vessel. For example, we recently reported method for preparing [^{11}C]acetate, in which a Tracerlab $FX_{C\text{-}Pro}$ was reconfigured (as shown in Figure 16) to allow full automation of the synthesis.[4]

Production of [^{11}C]acetate

Introduction

Although the viability of [^{11}C]acetate as a PET radiopharmaceutical was first described almost 30 years ago,[123] its application in myocardial imaging has continued to be of interest since its introduction.[124,125] However, myocardial imaging was quickly discovered to be only a single application of [^{11}C]acetate. By entering the Krebs cycle in the form of the acetyl group in acetyl coenzyme A (acetyl CoA),[126] cells undergoing more or less cellular respiration relative to the surrounding cells are easily identifiable. Thus, recent studies have reported use of [^{11}C] acetate for imaging meningiomas,[127] renal carcinomas,[128] and liver and prostate tumors.[129–132] Strategies for the synthesis of [^{11}C]acetate have been reported by our group,[4] Boschi,[133,134] and others.[15,135–140]

Synthesis procedures

In order to prepare [^{11}C]acetate, the Tracerlab $FX_{C\text{-}Pro}$ was configured as shown in Figure 16 and as previously described.[4] Vials were loaded as follows: reaction vessel: CH_3MgCl in THF (0.5 M, 200 μL); Vial 2: CH_3CO_2H (1 mM, 1 mL); Vial 3: CH_3CO_2H (1 mM, 2 mL); Vial 4: Sterile Water for Injection (10 mL); Vial 6: 0.9% NaCl for Injection, USP (10 mL); round-bottomed dilution flask: CH_3CO_2H (1 mM, 5 mL). The methyl iodide Porapak column was replaced with a second column of molecular sieves, and we installed an electronic valve (Valve E in Figure 16), which directed [^{11}C]CO_2 from the exhaust line of V27 to this second column of molecular sieves. The reaction vessel was pre-charged with 200 μL 0.5 M CH_3MgCl/THF after it had been evacuated of all atmospheric gases *via* nitrogen flow, and the second molecular sieve column was pre-charged with 1 mL of [^{12}C]CO_2 prior to introduction of activity (addition of [^{12}C]CO_2 eliminated over alkylation of [^{11}C]CO_2 by the Grignard). The line out from V7 was connected to the round-bottomed dilution flask. This bypass permitted the transfer of the product from the reaction vessel directly to the dilution flask after quenching. Luer Lock adapters were inserted between the dilution flask and V11 to accommodate PS-H^+ and PS-Ag^+ Sep-Paks. Similarly, the cradle (normally used for the C18 Sep-Pak) was used for the SAX Sep-Pak.

[^{11}C]CO_2 was delivered to the Tracerlab $FX_{C\text{-}Pro}$ module for 2 min and retained on the first molecular sieve column (normally the Shimalite oven). These sieves were then heated to 350°C, at which point the flow rate was decreased to 15 mL/min, to transfer the [^{11}C]CO_2 to the second column of molecular sieves (pre-charged with [^{12}C]CO_2). This second column of sieves was heated to 250°C, V31 and V30 were opened, and the resulting gaseous mixture of [^{11}C]CO_2/[^{12}C]CO_2 was bubbled through the solution of CH_3MgCl in the reactor for 4 min, and stirred for an additional 30 s after bubbling had concluded. The reaction was then quenched with 1 mM CH_3CO_2H (1 mL from Vial 2). The crude reaction mixture was then transferred to the dilution flask where it was further diluted into 5 mL of 1 mM CH_3CO_2H. A final 2 mL of 1 mM CH_3CO_2H was added (from Vial 3) *via* the reactor. The resulting solution was then passed through PS-H^+ and PS-Ag^+ Sep-Paks (to remove excess acid, magnesium, or halide ions, respectively) before passing through a Maxiclean SAX anion exchange column where the [^{11}C]acetate was trapped on the cartridge. The SAX column was rinsed with 10 mL sterile H_2O (from Vial 4), and then [^{11}C]acetate was eluted off the column with 10 mL NaCl, 0.9% USP (from Vial 6), and collected into the product collection flask. Finally, the product was passed through a sterile 0.22-μm sterile filter into a sterile dose vial and submitted for QC testing. Typical yields of [^{11}C]acetate prepared using this method were 8.1% based upon starting [^{11}C]CO_2 (nondecay corrected, $n = 5$).

Synthesis module cleaning

The radiopharmaceuticals described in Sections 2.2–2.6 can be prepared on a single Tracerlab $FX_{C\text{-}Pro}$. Changing between module configurations is that modifications described in Section 2.1 are made to the Tracerlab. In order to clean and dry the synthesis module between syntheses, however, the Tracerlab is

Table 1. Representative quality control data

QC test	Release criteria	Acetate	Carfentanil	Choline	DASB	DTBZ	FMZ
n	N/A	5	10[a]	10[a]	10[a]	10[a]	7
Starting activity (mCi)	N/A	3000	3000	3000	3000	3000	3000
Mean yield (mCi) @ end-of-synthesis (EOS)	N/A	244	188.5	219.0	119.6	82.6	89.3
% Yield (nondecay corrected)	N/A	8.1%	6.3%	7.3%	4.0%	2.8	3.0%
Specific activity (Ci/μmol)	N/A	ND	39 746	ND	5239	6436	11 214
Appearance	Clear, colorless, free of particulates	Pass	Pass	Pass	Pass	Pass	Pass
pH	4.5–8.0	6.0	5.0	5.0	5.8	5.0	5.8
Radiochemical purity	>95% by HPLC	99.3%	97.9%	99.9%	98.0%	99.7	100%
Radiochemical identity	RRT = 0.9–1.1	1.03	1.01	1.09	1.03	1.02	1.01
Radionuclidic identity	$T_{1/2}$ = 18.4–22.4 min	20.2	20.0	20.2	ND	20.2	ND
Residual solvent analysis	Dimethyl sulfoxide (<5000 μg/mL)	N/A	3	N/A	ND	N/A	ND
	n-Propanol (<5000 μg/mL)	N/A	4	N/A		N/A	
	DMF (<880 μg/mL)	N/A	N/A	N/A		81.5	
	Acetone (<5000 μg/mL)	<Limit of Detection (LOD)	142	N/A		26.3	
	THF (<5000 μg/mL)	<LOD	N/A	N/A		N/A	
	DMAE (<20 μg/mL)	N/A	N/A	5.36		N/A	
Filter integrity test	>40 psi	>50 psi	>45 psi	>50 psi	ND	>50 psi	ND
Bacterial endotoxins	<17.5 EU/mL	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile

[a]We have made hundreds of doses of these radiopharmaceuticals using the methods outlined herein. The *n* values included are for a limited number of representative batches.
DASB, [(di(methyl)amino)methyl]phenyl]sulfanylbenzonitrile; DTBZ, dihydrotetrabenazine; FMZ flumazenil, N/A, not applicable; ND, not determined; EU, endotoxin units; QC, quality control; DMF, *N,N*-dimethylformamide; DMAE, *N,N*-dimethylaminoethanol; RRT, relative retention time.

returned to its basic configuration (Figure 4) after completion of a given synthesis, and then daily and weekly clean cycles are performed as outlined in the next paragraphs. Note that the round-bottomed dilution flask and product collection vial are removed, washed, and oven-dried separately between syntheses and after completion of the cleaning cycles. The reaction vessel should be checked afterward to ensure cleanliness and can also be further cleaned and oven-dried manually if required.

Daily clean cycle

The module is reset to its normal configuration illustrated in Figure 4. Between daily runs, we wash and dry the vials, reaction vessel, and HPLC loop. The vials are loaded as follows: Vial 1: water (1 mL); Vial 2: acetone (1 mL); Vial 3: acetone (3 mL); Vial 4: ethanol (10 mL); Vial 5: ethanol (10 mL); Vial 6: 70% ethanol (10 mL). The water from Vial 1 is initially passed through the reactor and the HPLC loop to waste, followed by the acetone. Helium is then blown through for 5 min to dry the HPLC loop. The reaction vessel is then heated to 80°C and placed under vacuum for 5 min to dry it. The reformulation components are also then cleaned with ethanol and dried by using helium flow. The daily clean cycle takes approximately 20 min.

Weekly clean cycles

The module is reset to its normal configuration illustrated in Figure 4. Once per week, we wash and dry the vials, reaction vessel, and HPLC loop. The first 'clean' cycle washes everything with water (Vials 1–6 are filled with water). This is followed by a 'clean/disinfect' cycle (Vials 1–6 are filled with 70% ethanol), and finally, a 'dry' cycle (Vials 1–3 are filled with acetone and vials 4–6 are filled with ethanol). During the 'clean' and 'clean/disinfect' programs, water and 70% ethanol are washed through the reactor and the HPLC loop to waste, respectively. The dry cycle washes acetone through the reaction vessel and HPLC loop to waste, before heating the reaction vessel to 80°C and placing it under vacuum for 5 min. During the 'dry' cycle, the reformulation components are cleaned with ethanol and dried by using helium flow.

Quality control procedures

Quality control of radiopharmaceuticals prepared for clinical use at the University of Michigan PET Center is carried out using guidelines outlined in Chapter 823 of USP and as detailed in the next paragraphs.[141] After successfully meeting release

Table 2. Representative quality control data (continuation)

QC test	Release criteria	HED	Methionine	PBR28	PMP	PiB	Raclopride
n	N/A	10[a]	4	6	10[a]	12[a]	10[a]
Starting activity (mCi)	N/A	3000	1500	3000	3000	3000	3000
Mean Yield (mCi) @ EOS	N/A	87.7	120.5	119	90.2	46.9	64.4
% Yield (nondecay corrected)	N/A	2.9%	8.0%	4.0%	3.0%	1.6%	2.2%
Specific activity (Ci/μmol)	N/A	1884	<LOD	12 000	<LOD	7177	8325
Appearance	Clear, colorless, free of particulates	Pass	Pass	Pass	Pass	Pass	Pass
pH	4.5–8.0	6.0	6.3	5.0	6.3	5.0	5.6
Radiochemical purity	>95% by HPLC	100%	96.7%	100%	98.0%	98.8%	98.2%
Radiochemical identity	RRT = 0.9–1.1	1.03	<LOD	1.01	<LOD	1.01	1.01
Radionuclidic identity	$T_{1/2}$ = 18.4–22.4 min	20.2	20.3	20.2	20.2	20.2	20.0
Residual solvent analysis	MeCN (<410 μg/mL)	ND	N/A	25	ND	3.6	N/A
	Butanone (<5000 μg/mL)		N/A	N/A		N/A	<LOD
	Acetone (<5000 μg/mL)		1201	145		139.4	9.3
	Ether (<5000 μg/mL)		N/A	N/A		N/A	N/A
	3-Pentanone (400 μg/mL)		N/A	N/A		3.9	N/A
Filter integrity test	>40 psi	ND	>50 psi	>40 psi	ND	>50 psi	>50 psi
Bacterial endotoxins	<17.5 EU/mL	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile

[a]We have made hundreds of doses of these radiopharmaceuticals using the methods outlined herein. The n values included are for a limited number of representative batches.
HED, meta-hydroxyephedrine; PMP, 1-methylpiperidin-4-yl propionate; PiB, Pittsburgh Compound B; N/A, not applicable; ND, not determined; EU, endotoxin units; QC, quality control; RRT, relative retention time.

criteria (Tables 1 and 2), doses are released to physicians for clinical use.

Visual inspection

Doses are visually examined and must be clear, colorless, and free of particulate matter.

Dose pH

The pH of the doses is analyzed by applying a small amount of the dose to colorpHast® pH 2.0–9.0 non-bleeding pH indicator strips and determined by visual comparison to the scale provided.

Chemical purity and radiochemical purity/identity

Chemical and radiochemical purity/identity are analyzed using a Shimadzu VP-Series HPLC equipped with a Bioscan FC3300 radioactivity detector and either a conductivity detector ([^{11}C]choline) or a UV detector ([^{11}C]acetate, [^{11}C]carfentanil, [^{11}C]DASB, [^{11}C]DTBZ, [^{11}C]FMZ, [^{11}C]HED, [^{11}C]methionine, [^{11}C]PBR28, [^{11}C]PiB, [^{11}C]PMP, and [^{11}C]raclopride) using conditions described in the next paragraphs. Although radiochemical purity for doses typically must be >95%, there are currently no chemical purity requirements for release of radiopharmaceuticals in clinical research. Radiochemical identity is confirmed and quantified by calculating the relative retention time (=[retention time of radiochemical peak]/[retention time of unlabeled reference standard peak]).

[^{11}C]Acetate

Column: Phenomonex Luna SCX, 150 × 4.6 mm; 0.5 N (0.25 M) aqueous sulfuric acid; flow rate: 1.0 mL/min; UV: 220 nm; RT = 7.0 min. [^{12}C]Sodium acetate was purchased from Sigma Aldrich and used as the non-radioactive reference standard.

[^{11}C]Carfentanil

Column: Phenomenex Luna C8(2) 5 μ, 100 × 2.0 mm; mobile phase: 35% MeOH: 65% 20 mM NH_4OAc; pH 4.5; flow rate: 0.8 mL/min; oven: 40°C; UV: 218 nm; RT = 4.5 min. Non-radioactive [^{12}C]carfentanil was used as the reference standard.

[^{11}C]Choline

Column: Waters IC-Pak Cation M/D Column, 150 × 3.9 mm; mobile phase: 5 mM aqueous hydrochloric acid, flow rate: 1.0 mL/min; conductivity detector: negative polarity; RT = 7.0–7.5 min. [^{12}C]Choline non-radioactive reference standard was purchased from Sigma Aldrich.

[^{11}C]DASB

Column: Phenomonex Luna C18 5 μ, 100 × 2.0 mm; mobile phase: 35% MeOH: 65% 20 mM NH_4OAc; pH: 4.5; flow rate: 0.3 mL/min; oven: 30°C; UV: 254 nm; RT = 4.5 min. [^{12}C]DASB non-radioactive fluoride-19 reference standard was purchased from ABX Advanced Biochemicals.

[^{11}C]DTBZ

Column: Phenomonex Luna C8(2) 5 μ, 100 × 2.0 mm; mobile phase: 25% MeOH: 75% 20 mM aqueous NH_4OAc; pH 4.5; flow rate: 0.7 mL/min, oven: 40°C; UV = 240 nm; RT = 1.8 min. [^{12}C]DTBZ reference standard was purchased from SRI (contract synthesis).

[^{11}C]FMZ

Column: Phenomonex Luna C8(2) 5 μ, 100 × 2.0 mm; mobile phase: 25% MeOH: 75% 20 mM NH_4OAc; pH: 4.5; flow rate: 0.8 mL/min; oven: 40°C; UV: 240 nm; RT = 6.0 min. [^{12}C]FMZ non-radioactive reference standard was purchased from ABX Advanced Biochemicals.

[^{11}C]HED

Column: Phenomonex Luna C18(2) 5 μ, 150 × 2.0 mm; mobile phase: 5% MeOH: 95% 20 mM NH_4OAc; pH: 4.5; flow rate: 0.25 mL/min; oven: 30°C; UV: 280 nm; RT = 8.1 min. [^{12}C]HED non-radioactive reference standard was purchased from ABX Advanced Biochemicals.

[^{11}C]Methionine

Column: Phenomonex Luna C18(2) 5 μ, 150 × 4.6 mm; mobile phase: 8 mM $Cu(OAc)_2$: 17 mM L-proline: 30 mM NaOAc; flow rate: 1.5 mL/min; oven: 40°C; UV: 304 nm; RT = 10.7 min. [^{12}C]Methionine non-radioactive reference standard was purchased from Fluka.

[^{11}C]PBR28

Column: Phenomonex Luna C18(2) 5 μ, 150 × 4.6 mm; mobile phase: 45% MeCN: 10 mM Ammonium Formate; flow rate: 3.0 mL/min; oven: 40°C; UV: 220 nm; RT = 2.4–2.5 min. [^{12}C]PBR28 non-radioactive reference standard was prepared in house[17] (although it is also now commercially available from ABX Advanced Biochemicals).

[^{11}C]PiB

Column: Phenomenex Luna C8(2) 5 μ, 100 × 2.0 mm; mobile phase: 45% MeOH: 55% 20 mM NH_4OAc; pH 4.5; flow rate: 0.6 mL/min; oven: 40°C; UV: 350 nm; RT = 4.0 min. [^{12}C]PiB non-radioactive reference standard was purchased from ABX Advanced Biochemicals.

[^{11}C]PMP

Column: Phenomonex Luna C18(2) 5 μ, 150 × 2.0 mm; mobile phase: 5% MeOH: 95% 20 mM NH_4OAc; pH 4.5; flow rate: 0.35 mL/min; UV: 220 nm; RT = 4.5 min. [^{12}C]PMP non-radioactive reference standard was purchased from ABX Advanced Biochemicals.

[^{11}C]Raclopride

Column: Phenomenex Luna C8(2) 5 μ, 100 × 2.0 mm; mobile phase: 35% MeOH: 65% 20 mM NH_4OAc; pH 4.5; flow rate: 0.8 mL/min; oven: 40°C; UV: 218 nm; RT = 3.5 min. [^{12}C]Raclopride non-radioactive reference standard was purchased from ABX Advanced Biochemicals.

Residual solvent analysis

Levels of residual solvents in doses were analyzed using a Shimadzu GC-2010 with an AOC-20 autoinjector, split/splitless inlet, a flame ionization detector, and a Restek column (Stabilwax 30 m × 0.25 mm, 0.25 m G16 stationary phase). Limits of residual solvents are based upon the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines.

Radionuclidic identity

Radionuclidic identity is confirmed by measuring the half-life of radiopharmaceutical doses and comparing it with the known half-life of carbon-11 (20.8 min). Activities are measured using a Capintec CRC®-15R Radioisotope Dose Calibrator, and half-life is calculated using equation [1]. Calculated half-life must be 18.4–22.4 min.

$$T_{1/2} = -\ln 2(\text{TimeDifference}/(\ln(\text{endingactivity}/\text{startingactivity}))) \quad (1)$$

Sterile filter integrity (Bubble Point) test

Sterile filters from doses (with needle still attached) are connected to a nitrogen supply *via* a regulator. The needle is then submerged in water, and the nitrogen pressure is gradually increased. If the pressure is raised above the filter acceptance pressure (typically 40 psi) without seeing a stream of bubbles, the filter is considered intact.

Bacterial endotoxins

Endotoxin content in radiopharmaceutical doses is analyzed using a Charles River Laboratories EndoSafe® Portable Testing System and according to USP. Doses must contain <175 endotoxin units.

Sterility

Culture tubes of fluid thioglycolate media (FTM) and tryptic soy broth (TSB) are inoculated with samples of [^{11}C]-labeled radiopharmaceutical doses and incubated (along with positive and negative controls) for 14 days. FTM is used to test for anaerobes, aerobes, and microaerophiles, whereas TSB is used to test for non-fastidious and fastidious microorganisms. Culture tubes are visually inspected on the 3rd, 7th, and 14th days of the test period and compared with the positive and negative standards. Positive standards must show growth (turbidity) on the plates and dose/negative controls must have no culture growth after 14 days to be indicative of sterility.

Conclusions

In conclusion, the Tracerlab $FX_{C\text{-}Pro}$ has proven to be an efficient automated synthesis module for preparation of many carbon-11 labeled radiopharmaceuticals in our laboratory. The results disclosed herein demonstrate that through simple modification of the 'standard' synthesis module configuration, the system can be configured (and re-configured) in a straightforward manner to allow production of clinical doses of many different radiopharmaceuticals. The production of the radiopharmaceuticals discussed herein demonstrates that numerous different

carbon-11 radiolabeling strategies can be adapted for use with the Tracerlab $FX_{C\text{-}Pro}$. Each synthesis has been fully automated, and all radiopharmaceutical doses for clinical use meet or exceed established QC criteria.

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