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Radiosynthesis and validation of [*Carboxy-¹¹C]*4-Aminobenzoic acid ([¹¹C]PABA), a PET radiotracer for imaging bacterial infections

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Abstract

In this practitioner protocol, the radiochemical synthesis of [¹¹C] PABA is described in detail, and a quality control summary of three validation productions is presented. The results indicate that the radiotracer product can be produced in good radiochemical yield (14% at end-of-synthesis (EOS)) at high specific activity (molar activity 11 Ci/μmole EOS; 407 GBq/μmole) and high chemical and radiochemical purity as a sterile, pyrogen-free solution suitable for injection conforming to current Good Manufacturing Practice (cGMP) requirements.

Keywords

infection; PABA; QC; synthesis

1 | INTRODUCTION

Given that current imaging techniques remain nonspecific for imaging bacterial infections, there remains a need for developing bacteria-specific radiotracers that identify a wide range of clinically relevant bacteria with high sensitivity.¹

After approximately 1000 small molecules were screened, several promising candidates were identified for radiolabeling and imaging infections, including para-aminobenzoic acid (4-aminobenzoic acid; PABA). In tritiated form, PABA was demonstrated to accumulate in a broad class of bacteria including Gram-positives, Gram-negatives (as well as *Pseudomonas spp.*), and *Mycobacterium tuberculosis*.²

A recent study described a synthesis of [¹¹C] PABA³ with solution radiochemistry and HPLC purification. This paper describes an optimized synthesis of the radiotracer (Figure 1) with full cGMP compliant quality control specifications and results.

2 | EXPERIMENTAL

Reagents and solvents were obtained from the sources indicated in the synthesis description.

Analytical chromatography was performed with an Agilent 1260 Infinity System equipped with a quaternary pump, HiP ALS autosampler, and DAD UV detector with a Max-Light flow cell set to 282 nm as well as a Bioscan Flow-Count interface with a NaI radioactivity detector. The analytical HPLC column was a Phenomenex Synergi 4 μ m Fusion-RP 80 Å 150 $\times$ 4.6 mm column eluted with 5% Acetonitrile: 95% aqueous buffer (57mM TEA adjusted to pH 3.2 with o-phosphoric acid) at a flow rate of 2 mL/min. Chromatographic data were acquired and analyzed with Agilent OpenLAB CDS EZChrom Edition (Rev. A. 04.05).

Radioactivity measurements were made using a Comcer model VDC-606 dose calibrator.

Residual solvent levels were analyzed using an Agilent 7890A gas chromatograph, Agilent OpenLAB CDS EZChrom Edition for data acquisition and analysis, and a WAX (Polyethyleneglycol phase: USP G16, G20) 30 meters, 0.25 mm ID, 0.25 mm film column.

Remote control of the [^{11}C]CO₂ concentration and purification system and the solvent pump and valves was accomplished using National Instruments LabView and cRIO controller (Figure 2).

2.1 | Production of [^{11}C] carbon dioxide

Pressurized ultra-high purity nitrogen gas (99.5%) mixed with 0.5% oxygen (Roberts Oxygen, Baltimore, Maryland) in a standard carbon-11 PETtrace target (General Electric Medical Systems (GEMS), Waukesha, WI) was irradiated with a 16 MeV proton beam of 60 μ A for up to 30 minutes to produce in approximately 1.5 Ci (37 GBq) of [^{11}C] carbon dioxide via the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction.

2.2 | Concentration and purification of [^{11}C] carbon dioxide⁴

Prior to their use, a molecular sieve trap (Alltech, 13X; approximately 0.4 g) and a Carbosphere packed GC column (1/8" x 6' stainless steel column packed with Carbosphere 60/80; Alltech) heated at 325°C and 150°C, respectively, for 5 minutes each while flowing nitrogen gas (UHP; 99.9995%) at approximately 100 mL/min. Following the cyclotron bombardment, the target output of [^{11}C]CO₂ was concentrated on the molecular sieve trap for 120 seconds, while other radioactive gases were diverted to waste. The molecular sieve was heated for 130 seconds from ambient room temperature to a maximum temperature of 325°C, resulting in the transfer of [^{11}C]CO₂ at approximately 85 mL/min in a stream of nitrogen (99.999%; Roberts Oxygen) onto the Carbosphere packed GC column at ambient room temperature. The Carbosphere column was then heated for 195 seconds to a maximum temperature of 150° C while the purified [^{11}C]CO₂ was flushed to the next step of the reaction.

2.3 | Reaction with Grignard solution

Prior to its use, a 2-mL reaction loop of 1/8" ID fluorinated ethylene propylene tubing was sequentially washed with 5 mL aliquots of hydrochloric acid (2 M), HPLC water, and acetone, after which the loop was flushed with nitrogen gas (UHP, 99.9995%) for at least 15 minutes (100 mL/min). Purified [^{11}C]CO₂ from the previous step was flushed through the loop that was prefilled with 4-[bis (trimethylsilyl)amino] phenylmagnesium bromide in tetrahydrofuran (THF) (100 μL , 0.5 M; Sigma-Aldrich, St. Louis, MO) mixed with THF (100 μL , Sigma-Aldrich). Trapping of the [^{11}C]CO₂ in the Grignard loop took approximately 40 seconds.

2.4 | Purification of the radiotracer product

Using a remotely controlled, multiport Cavo pump (Tecan, Morrisville, North Carolina), a mixture (8 mL) of 1:99 Dehydrated Alcohol USP:0.75 M phosphate buffer pH 2.2 solution (Dehydrated Alcohol USP, Pharmco-Aaper, Shelbyville, Kentucky, and sodium dihydrogen phosphate monohydrate and phosphoric acid for the buffer solution, Sigma-Aldrich) was passed through the reaction loop, a syringe filter (to trap particulates; Millipore-Sigma, Millex 0.45 μm LCR filter, 25 mm), and an Oasis HLB Sep-Pak Plus (Waters Corp, Milford, Massachusetts) to waste (note: Flow path dead volume through the loop and syringe filter intentionally results in 4 mL of solution from passing through the Sep-Pak). Next, the pump washed an additional 7 mL of 1:99 Dehydrated Alcohol USP: 0.75 M phosphate buffer pH 2.2 solution through just the Oasis HLB Sep-Pak Plus alone to waste. Then, the pump passed 7 mL of Sterile Water for Injection (Hospira, Lake Forest, New Jersey) through the Oasis HLB Sep-Pak Plus to waste, followed by 5 mL of filtered lab air. The [^{11}C] PABA radiotracer product was eluted from the Oasis HLB Sep-Pak Plus with 10 mL of 10:10:80 Dehydrated Alcohol USP: 8.4% Sodium Bicarbonate Inj: Sodium Chloride, 0.9% Injection (sodium bicarbonate and sodium chloride obtained from Hospira) through the Millipore FG sterile filter assembly (Millipore-Sigma) into a sterile, pyrogen-free vial (NUCMECOR, San Francisco, California) prefilled with 4 mL of sodium chloride, 0.9% Injection. The final product vial was immediately transferred from the production area to the quality control (QC) laboratory for analysis.

2.5 | Quality control procedures

QC procedures for [^{11}C] PABA were performed based upon current requirements for PET radiotracers for human use set forth in 21 CFR Part 212 "Current Good Manufacturing Practice for Positron Emission Tomography Drugs"⁵ and the US Pharmacopeia Chapter 823 "Radiopharmaceuticals for Positron Emission Tomography—Compounding."⁶

Our recent practitioner report⁷ on [^{18}F] FNDP described in detail the standard QC procedures for cGMP compliance. Therefore, those test procedures were not repeated here. Only two minor changes are noted. For [^{11}C] PABA, no Kryptofix 2.2.2 was used; thus, the QC test for residual Kryptofix was unnecessary. For residual solvent analysis, a standard GC solution containing a known amount of THF was used for calculating residual THF in the final product solution. The previously described specification for chemical purity⁸ notes removal of 99.5% of starting precursor (in this case, the Grignard reagent) and setting the all

other UV absorbing peaks in the QC HPLC to be less than 0.5% total (0.33 $\mu\text{g/mL}$) as the standard used.

The analytical HPLC injection volume was 10 μL . A standard curve relating mass to UV absorbance consisting of seven mass points between approximately 3 and 200 pmoles with six replicate injections per mass was established prior to the validation runs ($r^2 = 0.999986$). The limit of detection was 0.3 pmoles and the limit of quantitation was 2.56 moles.⁹

3 | RESULTS AND DISCUSSION

This synthesis used a dual solid-phase reversible trapping of [^{11}C] CO_2 (molecular sieves as well as Carbosphere) to limit the effect of unwanted miscellaneous chemical species known to be produced by high energy proton irradiation of nitrogen/oxygen target gas mixtures (in particular, oxides of nitrogen (NO_x)) that can potentially interact with Grignard solutions and interfere with subsequent radiolabeling chemistry.⁴

For Grignard radiolabeling, we adapted the loop technique described previously for the synthesis of [$1\text{-}^{11}\text{C}$] acetate.¹⁰ This is in contrast to the solution chemistry previously reported for this radiotracer.³

For purification, we developed a simple cartridge (Sep-Pak) purification procedure that eliminated the need for semi-preparative HPLC purification that has been previously reported.³

Figure 3A,B,C shows sample quality control chromatograms (reference standard, final product QC, and “spiked” final product containing added reference standard for radiochemical identity confirmation).

Table 1 presents a summary of 3 validation productions of [^{11}C]PABA.

4 | CONCLUSIONS

A cGMP-compliant synthesis of [^{11}C] PABA has been described. The overall synthesis of the radiotracer product required approximately 15 minutes from end-of-bombardment. Subsequent QC testing adds another 15 minutes to the overall process. [^{11}C] PABA was obtained in good radiochemical yield (approximately 14% calculated at EOS), high specific activity (molar activity), high chemical and radiochemical purity. [^{11}C] PABA was shown to be sterile and free of pyrogens and be stable for at least 60 minutes. The final radiotracer product is suitable for future human PET studies.

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FIGURE 1.
Production of $[^{11}\text{C}]\text{PABA}$

FIGURE 2.
Schematic drawing of synthesis

FIGURE 3.
Sample QC chromatograms for [^{11}C]PABA

Release and stability test data for three qualification batches of [¹¹C] PABA injection manufactured at the JHUPET radiotracer center^a

TABLE 1

[¹¹ C] PABA Validation Productions				
Test	Specification	PABA-Validation 1	PABA-Validation 2	PABA-Validation 3
Initial appearance	Clear, colorless solution, no visible particulate matter	Conforms	Conforms	Conforms
Appearance 60 minutes after EOS	Clear, colorless solution, no visible particulate matter	Conforms	Conforms	Conforms
Initial radiochemical purity, % (t = 0 minutes)	95%	100%	100%	100%
Expiry radiochemical purity, % (t = 60 minutes)	95%	100%	100%	98.8%
pH, initial	7–9	8.5	8.5	8.0
pH, expiry	7–9	8.5	8.5	8.0
Chemical purity	All others 0.33 µg/mL	0.06 µg/mL	0.07 µg/mL	0.09 µg/mL
Yield	30 mCi (1.1 GBq) [¹¹ C] PABA (referenced to assay recorded at end-of-filtration)	292.2 mCi (10.8 GBq)	221.1 mCi (8.2 GBq)	129.5 mCi (4.8 GBq)
Specific activity (also called molar activity)	500 mCi/µmole (18.5 GBq/µmole) of [¹¹ C] PABA (referenced to end of filtration)	11726 mCi/µmole (433.8 GBq/µmole)	13043 mCi/µmole (482.6 GBq/µmole)	9189 mCi/µmole (340.0 GBq/µmole)
Radionuclidic identity	t _{1/2} = 19.4–21.4 min	20.21 min	19.85 min	19.74 min
Identity (HPLC)	HPLC retention time with 10% of reference standard	0.55%	0.41%	0.48%
Bubble point	13 psi	16 psi	16 psi	16 psi
Endotoxin	12 EU/mL	<5 EU/mL	<5 EU/mL	<5 EU/mL
Residual solvent	Ethanol 10% THF 514 ppm	EtOH: 7.0% THF: 93.9 ppm	EtOH: 6.8% THF: 94.1 ppm	EtOH: 6.6% THF: 90.9 ppm
Sterility	No growth observed over 14-day period	Conforms	Conforms	Conforms

^aYield and specific activity (molaractivity) measurements were reported at end-of-synthesis and are therefore not corrected for decay