Highly efficient solid phase-supported radiosynthesis of [^{11}C]PiB using tC18 cartridge as a "3-in-1" production entity

Abbreviated title: Solid phase-supported [^{11}C]PiB radiosynthesis

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Abstract.

Pittsburgh compound B ([^{11}C]PiB) is the gold standard PET tracer for the *in vivo* imaging of amyloid plaques. Currently it is synthesized by either solution chemistry or using a "dry loop" approach followed by HPLC purification within 30 minutes starting from [^{11}C]CO_2. Here we report a novel, highly efficient solid phase supported carbon-11 radiolabeling procedure using commercially available disposable tC18 cartridge as a "3-in-1" entity: reactor, purifier and solvent replacement system. [^{11}C]PiB is synthesized by passing gaseous [^{11}C]CH_3OTf through a tC18 cartridge preloaded with a solution of precursor. Successive elution with aqueous ethanol solutions allows for nearly quantitative separation of the reaction mixture to provide chemically and radiochemically pure PET tracer. [^{11}C]PiB suitable for human injection is produced within 10 minutes starting from [^{11}C]CH_3OTf (20 minutes from [^{11}C]CO_2) in 22% isolated yield not corrected for decay and molar activity of 190 GBq/μmol using 0.2 mg of precursor. This technique reduces the amount of precursor and other supplies, avoids use of preparative high performance liquid chromatography and toxic solvents and decreases the time between consecutive production batches. Solid phase supported technique can facilitate [^{11}C]PiB production compliant with Good Manufacturing Practice and improve synthesis reliability.

1. Introduction.

Amyloid beta protein (Aβ) accumulates in the brains of patients with Alzheimer's disease (AD) [1] and the resulting Aβ plaques are currently one of the main targets for AD diagnostic and preventive therapies [2,3,4]. The radiochemical synthesis of carbon-11 labeled thioflavin-T derivatives [5] resulted in development of PET radiotracer Pittsburgh compound B ([^{11}C]PiB) for Aβ imaging in 2003 [6]. The results of the first trials in humans were published shortly after and revolutionized the field of AD diagnostics [7]. For the first time, the Aβ plaques were visualized in the brains of living AD patients, and the correlation between

amyloid deposition and radioactivity accumulation patterns was later validated by the autopsy in post-mortem studies of typical AD brains [8,9]. By highlighting Aβ deposition, [^{11}C]PiB provides earlier and more specific diagnosis in different stages of AD as well as distinguishes AD from other types of non-Aβ forms of dementia such as the frontotemporal lobar degeneration (FTLD) [10]. Amyloid PET for clinical diagnostics has recently been approved by FDA in the US to rule out AD based on the absence of Aβ in the brain, and other countries are expected to follow shortly. Despite recent emergence of various fluorine-18 labeled amyloid tracers, such as florbetapir [11], florbetaben [12], flutemetamol [13], NAV4694 [14] and others, [^{11}C]PiB remains the most studied tracer for Aβ PET imaging due to high affinity for Aβ plaques (K_d = 1.4 nM) [6], fast uptake and low non-specific binding.

The original radiosynthetic procedure of [^{11}C]PiB ([^{11}C]6-OH-BTA-1) involved *N*-methylation of the *O*-methoxymethyl (MOM) protected compound 6-OMOM-BTA-0 using ^{11}C-methyl iodide ([^{11}C]CH_3I) followed by the deprotection of the MOM group with hydrochloric acid (Figure 1, A) [6]. Subsequently, a one-step synthesis from the unprotected compound 6-OH-BTA-0 (0.4 mg) was developed by Wilson [15] using ^{11}C-methyl triflate ([^{11}C]CH_3OTf) either in methylethylketone solution or by elegant captive solvent "loop" method [16,17]. Consequent purification by means of high performance liquid chromatography (HPLC), reformulation and sterile filtration affords [^{11}C]PiB for clinical imaging within 30 minutes after end of bombardment. Currently, most PET centres use between 0.8-1 mg of precursor methods utilizing "loop", solution or on-column [18] approach followed by semi-preparative HPLC purification and reformulation.

A recent trend in PET radiochemistry is the development of cassette-based kits that allow for production of various fluorine-18 labeled tracers with reduced technical effort from the laboratory personnel and minimal maintenance of the equipment between syntheses. The first kit-based synthesis was introduced for the radiolabeling of the most widely used PET

tracer [^{18}F]FDG; lately, similar kits have been developed for other tracers including FMISO, FMC, and others. These kits are commercially available from ABX advanced biochemical compounds [19], Huayi Isotopes [20] and other vendors for most of the existing automated modules. Outside of the physical half-life limitations ($T_{1/2}$ = 20.3 min), one of the major reasons carbon-11 remains a less popular isotope for PET radiochemistry than fluorine-18 is a lack of similar GMP-grade production kits that would allow for fast and reliable radiolabeling. Subsequently, while many ^{18}F-tracers have found application in clinical trials and have been successfully commercialized, ^{11}C-tracers are mostly used in highly specialized research PET centres worldwide. Development of similar kits for ^{11}C-labeled tracers would significantly simplify their production and eliminate the need for cleaning and drying of the module between consecutive batches. Finally, fully disposable kits would prolong the intervals between preventive maintenance of the production modules, improve synthesis reliability and allow avoiding sterilization of the reusable equipment.

Of special interest for the kit development are the reactions that can proceed at room temperature on a disposable cartridge, for example [^{18}F]fluoromethylation of dimethylaminoethanol in production of [^{18}F]fluoromethylcholine, or hydrolysis of [^{18}F]fluorodeoxyglucose tetraacetate in the [^{18}F]FDG synthesis. Although sorbent-supported radiolabeling of several ^{11}C-tracers have been reported, including pioneering work by the University of Michigan PET center [21,22], most of the radiosyntheses rely on time and labor consuming HPLC purification and reformulation of the tracers. As part of our latest endeavours in simplifying the labeling with carbon-11 [23] and fluorine-18 PET isotopes [24], we wish to report a new highly efficient solid phase-supported radiosynthesis of [^{11}C]PiB by [^{11}C]methylation of the 6-OH-BTA-0 on a disposable cartridge used as "3-in-1" entity for reaction, purification and formulation. Briefly, gaseous [^{11}C]CH_3OTf is passed through a disposable cartridge preloaded with 6-OH-BTA-0 precursor and nearly quantitative separation

of synthesized [^{11}C]PiB from starting material and radioactive impurities is achieved using a biocompatible aqueous ethanol as an eluent. Using this very reliable technique, we developed a method for [^{11}C]PiB production using automated module and disposable cassette kits. The resulting tracer meets all specifications to be compliant with requirements of multicenter Dominantly Inherited Alzheimer Network Trials Unit (DIAN-TU) [25] clinical trials: radiochemical and radionuclidic identity; radiochemical purity > 95%; chemical purity – amount of residual precursor or other UV impurities < 1.3 μg; pH 4-8; ethanol content < 10%, sterile and endotoxin free.

2. Experimental.

2.1. Materials and general procedures

All radiochemistry procedures were performed at the PET radiochemistry facility of the Montreal Neurological Institute (MNI) as described before [26]. Briefly, the isotope was obtained in the form of [^{11}C]CO_2 *via* $^{14}N(p,\alpha)^{11}C$ nuclear reaction by irradiation of N_2/O_2 (99.5:0.5) with 18 MeV protons in the gas target of the cyclotron (Cyclone® 18/9 IBA, Louvain-La-Neuve, Belgium). The resulting [^{11}C]CO_2 was converted to [^{11}C]CH_3OTf using a commercially available Synthra module. The [^{11}C]CH_3OTf output line was directly attached to the manifold secured on the Scintomics GRP automated module for ^{11}C-methylation.

Compounds 6-OH-BTA-0 (precursor) and 6-OH-BTA-1 (cold standard) were purchased from ABX advanced biochemical compounds (Cat No 5101 and 5140, respectively). Disposable cartridges Oasis HLB (Cat No. 186000132), Sep-Pak tC18 Plus (Cat No. WAT036810) and Sep-Pak C18 Plus (Cat No. WAT020515) were provided by Waters®. Disposable polycarbonate and solvent-resistant manifolds were purchased from Scintomics (Cat. No. ACC-101 and ACC-201, respectively). HPLC solvents were purchased from Fisher and inorganic salts for buffer preparation from Sigma-Aldrich. Buffer solutions were prepared according to Sigma-Aldrich buffer reference center [27] and their pH verified with pH strips.

Quality control procedures were performed according to the validated SOPs used at the PET radiochemistry facility of the MNI for routine production of radiotracers intended for use in humans. Specifically, Agilent 1200 HPLC instrument equipped with a reversed phase column (MZ Analytical PerfectSil 120 C8 5μm, 100 x 4.0 mm; 40/60 acetonitrile/water at 0.7 mL/min), UV (350 nm) and radioactivity detector (Raytest Gaby) was used to confirm radiochemical identity and purity as well as chemical purity and molar activity. Perkin Elmer Clarus 480 gas chromatograph (GC) equipped with Restek MTX-Wax column (30 m, 0.53 mm) was used for detection and quantification of the residual solvents.

2.2. Separation of 6-OH-BTA-0 and 6-OH-BTA-1.

Our first goal was to find the optimal conditions with regards to solid phase extraction (SPE) sorbent, pH of the aqueous solution and ethanol content for efficient separation of non-radioactive 6-OH-BTA-0 and 6-OH-BTA-1 on a commercially available disposable cartridge. In a typical experiment, premixed aqueous ethanol (ca. 10%) solution containing both compounds was passed through the preconditioned cartridge followed by sequential elution with aqueous solutions of increasing ethanol concentration and all eluate fractions were analyzed by HPLC.

Among three tested cartridges commercially available by Waters®, trifunctional tC18 sorbent provided the best results for separation of 6-OH-BTA-0 and 6-OH-BTA-1 in cold simulation experiment and were later translated to radiolabeling procedure. Other cartridges (Oasis HLB and Sep-Pak C18) performed poorly and were not tested in radiolabeling experiments (data not shown). Excellent separation of the compounds in both "cold" simulations and later in radiosynthesis of [^{11}C]PiB was achieved using 0.2M acetate buffer at pH 3.7, prepared by mixing 0.2M sodium acetate solution (50 mL) with 0.2M acetic acid (450 mL). We rationalized that these aromatic amines have sharper elution profiles and therefore better separation in moderately acidic conditions where they exist predominantly in protonated

forms. Thus, a 20% aqueous ethanol solution in 0.2M acetic buffer at pH 3.7 (80 mL) allowed for almost quantitative washout of the 6-OH-BTA-0 from tC18 cartridge, while the 6-OH-BTA-1 remained trapped. The latter was eluted by increasing the concentration of ethanol to 40% in 20 mL total volume. These conditions were later slightly modified for production of radiochemically and chemically pure [^{11}C]PiB as described below.

2.3. General radiolabelling procedure

In a typical radiolabeling experiment, preconditioned (10 mL water followed by 5 mL acetone) and dried by the stream of nitrogen (50 mL/min, 1 min) tC18 cartridge was slowly loaded with a 2 mg/mL solution of 6-OH-BTA-0 in acetone (50, 100 or 150 μL). [^{11}C]methylation was performed by passing gaseous [^{11}C]CH_3OTf directly through the loaded cartridge secured on a manifold (Fig 2A). Once all the [^{11}C]CH_3OTf was trapped on the cartridge as monitored by the radioactivity detector mounted behind the cartridge holder, it was allowed to react with the precursor for 2-3 min at room temperature and then successively eluted with aqueous (pH 3.7) ethanol solutions of increasing concentrations: first, 12.5% EtOH (92 mL), then 15% EtOH (55 mL) into the waste bottle (Fig 2B, 2C); and finally with 50% EtOH (2.5 mL) followed by sterile phosphate buffer (10 mL) into the final vial (Fig 2D) .

3. Results and discussion.

The solid-state radiochemical ^{11}C-alkylations in general require lower precursor amount than the comparable reactions in solution as previously reported by Wilson [15-17] and more recently Scott [28]. Our first goal was to investigate if even lower amounts of precursor (0.1, 0.2 and 0.3 mg) would provide high radiochemical yield of the product using on-cartridge technique. The results of the radiochemical yield optimization experiments are summarized in Table 1.

To our delight, as little as 0.2 mg of precursor per synthesis provides excellent radiochemical yields of [^{11}C]PiB in SP-supported procedure (Table 1, entry 2). Notably, reducing the amount even further to 0.1 mg (entry 1) still provides a reasonable quantity of the tracer, although the final amount of [^{11}C]PiB is lower and the yield is less reliable. Increasing the amount to 0.3 mg (entry 3) further improves the yield, while still providing a reasonable chemical purity of the final product despite a higher amount of the precursor in the final product. However, we observed that the acetone used to solubilize the precursor can affect the stability of the polycarbonate manifolds used in the synthesis, which are particularly vulnerable to this solvent. Thus, in two experiments using 150 μL of the precursor solution (0.3 mg), the manifold was destroyed, prompting us to manually intervene in the synthesis to recover the labeled product. This problem can be alleviated by using higher quality solvent-resistant manifolds (See Supporting Information). An optimal amount of precursor will depend on particular needs of the PET center in terms of yield of radiotracer and other considerations. Our attempt to repeat the tC18-suported synthesis using ethanol (Table 1, entry 4) only yielded 3% [^{11}C]PiB (from [^{11}C]CH_3OTf) with less than 70% RCP, deeming this solvent unsuitable for the proposed alkylation technique. Further investigation of ethanol compatibility with solid phase-supported ^{11}C-methylation might be required.

Table 1. Optimization of precursor amount, ^{11}C-methylating agent and solvent.

The most challenging part of the described technique was to find conditions for quantitative separation of the reaction mixture on a relatively short SPE cartridge. After thorough optimization of the ethanol content and volumes of washing solutions, we found the optimal conditions: first 12.5% EtOH (92 mL) is used to elute the radioactive impurities and majority of the 6-OH-BTA-0, then 15% EtOH (55 mL) washes out the residual precursor and a negligible amount of the product (~5%). Finally, the elution of desired [^{11}C]PiB, pure both radiochemically (> 95% RCP) and chemically (<1.3 μg of 6-OH-BTA-0) is achieved at the

50% ethanol concentration. Results of purification optimization can be found in supplementary information. Typical chromatograms and radioactivity distribution between fractions are represented on Figure 3

After the procedure optimisations described above, [^{11}C]PiB is synthesised and prepared for direct injection from a starting activity of [^{11}C]CO_2 ranging from 13 to 37.5 GBq as follows. [^{11}C]CH_3OTf is passed through the preconditioned, dried and loaded with 0.2 mg of 6-OH-BTA-0 tC18 Plus cartridge, allowed to react with precursor for 2-3 min and the reaction mixture is efficiently separated using successive elution with aqueous ethanol solutions of increasing concentrations. From the end of bombardment to the end of the synthesis, the whole procedure is completed within 20 minutes. The final tracer synthesized under optimized conditions meets all the criteria specified in the certificate of analysis (CofA) for use in humans under DIAN-TU clinical trial protocol: radiochemical purity is consistently above 95%, the 6-OH-BTA-0 content is below 1.3 μg per 10 mL dose and the ethanol content is below 10%. [^{11}C]PiB is stable within 1 hour in the final solution, sterile and free from pyrogens and bacterial endotoxins. The results of full batch successful validation runs from [^{11}C]CH_3OTf generated by dry chemistry method are summarized in Table 2.

Table 2. Summary of the [^{11}C]PiB production validation batches under optimized conditions.

In an attempt to develop a fully disposable all-in-one kit for production of [^{11}C]PiB from [^{11}C]CO_2 released from the cyclotron target, we combined this technique with our previously reported "[^{11}C]kits" for cassette-based conversion of [^{11}C]CO_2 into [^{11}C]CH_3I/[^{11}C]CH_3OTf using wet method. Despite a higher radiochemical yield, relatively low molar activity leads to formation of the unidentified radiochemical impurity co-eluting with [^{11}C]PiB, which we attributed to a double methylation product. Although selective elution of radiochemically pure [^{11}C]PiB was achieved (27 mL of 30% aqueous ethanol), low molar activity and high ethanol content would make tracer batches produced by all-in-one kits

unsuitable for clinical applications. We are currently working on improving the molar activity of [^{11}C]CH_3OTf produced with "[^{11}C]kits" by wet method.

4. Conclusion

We have developed a solid phase-supported technique to produce a clinically relevant [^{11}C]PiB tracer from [^{11}C]CH_3OTf in an automated mode and avoiding HPLC purification. Our method takes advantage of the disposable tC18 cartridges as "3-in-1" entity for production, purification and formulation of this tracer. In our work, we optimized the RCY with regards to precursor amount and reaction solvent; and purification with regards to cartridge type, pH of the buffer, ethanol content and volume of the eluent. This synthesis from [^{11}C]CH_3OTf is completed within 10 minutes and affords [^{11}C]PiB in high yield and molar activity as well as excellent radiochemical and chemical purity as an injectable solution for clinical or preclinical imaging. The described methodology allows for reduction of chemicals (including precursor and HPLC solvents) used in the synthesis, shortens the synthesis time and reduces the technical involvement of the personnel. Most importantly, high reliability is achieved by avoiding HPLC-related failures, while use of biocompatible ethanol instead of most commonly used acetonitrile for tracer purification simplifies GMP-compliant quality control procedures. Furthermore, it opens new avenues to develop a fully automated disposable kits for [^{11}C]PiB production. Development of similar highly efficient solid phase-supported syntheses of other clinically relevant tracers produced by ^{11}C-methylation is currently underway in our lab.

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Table 1. Optimization of precursor amount, ^{11}C-methylating agent and solvent.

Entry	Precursor amount	[^{11}C]CH_3X	n	Solvent	RCY*
1	0.1 mg	[^{11}C]CH_3OTf	3	acetone	18.1±3.8%
2	0.2 mg	[^{11}C]CH_3OTf	11	acetone	22.0±3.1%
3	0.3 mg	[^{11}C]CH_3OTf	3	acetone	32.1±3.7%
4	0.2 mg	[^{11}C]CH_3OTf	1	ethanol	<3%

* Not corrected for decay yield starting from [^{11}C]CH_3OTf

Table 2. Summary of the [^{11}C]PiB production validation batches under optimized conditions.

Batch	PiB161025	PiB161027	PiB161110	PiB161118
[^{11}C]MeOTf, GBq	9.21	11.25	7.84	6.44
Amount, GBq	2.26	2.37	2.11	1.41
RCY, %*	24.5	21.1	26.9	21.8
RCP, %	98.0	97.2	97.8	99.2
Molar activity, GBq/μmol	154.6	322.6	121.1	162.1
Residual precursor, μg	0.32	0.55	0.58	0.87
pH	5	5	5	5
EtOH content, %	9.4	8.8	7.7	8.1
Acetone content, ppm	33	38	46	33
BET test	N/A	<10 EU/mL	<10 EU/mL	<10 EU/mL
Sterility test	N/A	No Growth	No Growth	No Growth

* From [^{11}C]MeOTf, not corrected for decay

Figures

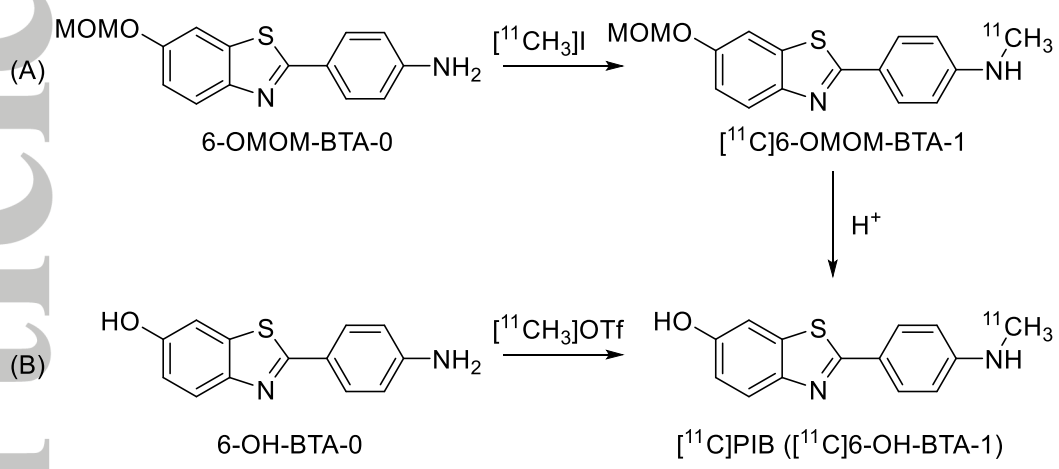


Figure 1. (A) The original radiosynthetic procedure of [^{11}C]PiB ([^{11}C]6-OH-BTA-1) involved *N*-methylation of the 6-OMOM-BTA-0 using [^{11}C]CH_3I and (B) the one-step radiosynthesis of [^{11}C]PiB from the unprotected 6-OH-BTA-0 using [^{11}C]CH_3OTf.

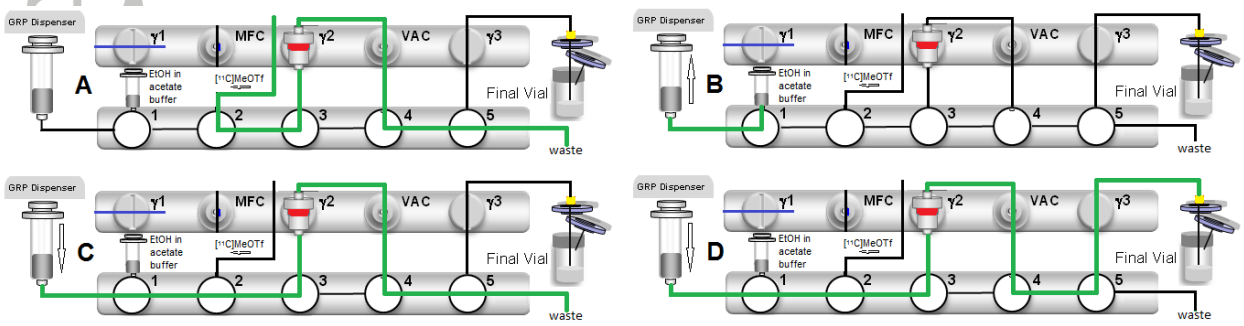
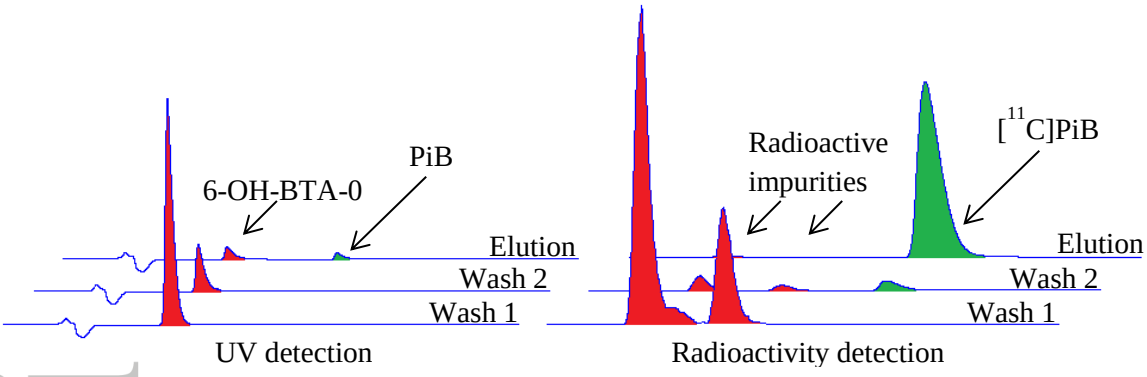


Figure 2. Step-by-step synthesis and purification of [^{11}C]PiIB on tC18 cartridge: A) gaseous [^{11}C]CH_3OTf is passed through the cartridge loaded with 6-OH-BTA-0; B) aqueous ethanol (18.5 mL) is withdrawn into the 20 mL dispenser syringe (100 mL/min); C) the impurities are washed out into a waste bottle (50 mL/min); D) [^{11}C]PiB is eluted into the final vial through a sterile filter.



Solution	V, mL	[11C]CH3X*		[11C]PiB		6-OH-BTA-0	
		%**	MBq***	%**	MBq***	%****	µg
Wash 1	92.0	62.3	4070	<0.01	<4	~90	~180*****
Wash 2	55.0	0.2	15	2.4	163	~10	~20******
Elution	12.5	0.3	18	34.8	2276	0.3	0.58

*[11C]CH3I, [11C]CH3OTf and [11C]CH3OH; **Quantity expressed as a percentage of the total activity corrected for decay; ***Decay-corrected values; ****Percentage of the total mass of 6-OH-BTA-0 eluted from the cartridge; *****Absolute values outside of the calibration curve.

Figure 3. Elution profile with aqueous (pH 3.7) ethanol solution of increasing concentrations. **Wash 1** (92 mL of 12.5% EtOH), **Wash 2** (55 mL of 15% EtOH) and **Elution** (2.5 mL of 50% EtOH) were sequentially passed through the cartridge to elute the radioactive impurities, the residual 6-OH-BTA-0 and finally the [11C]PiB, respectively.

References

[1] Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron 1991;6:487-498.

[2] Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol. 2013;12:357-67.

[3] Carrillo MC, Brashear HR, Logovinsky V, Ryan JM, Feldman HH, Siemers ER, et al. Can we prevent Alzheimer's disease? Secondary "prevention" trials in Alzheimer's disease. Alzheimers Dement. 2013;9:123-131.

[4] Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367:795-804.

[5] Mathis CA, Bacskai BJ, Kajdasz ST, McLellan ME, Frosch MP, Hyman BT, et al. A lipophilic thioflavin-T derivative for positron emission tomography (PET) imaging of amyloid in brain. Bioorg Med Chem Lett. 2002;12:295-298.

[6] Mathis CA, Wang Y, Holt DP, Huang GF, Debnath ML, Klunk WE. Synthesis and evaluation of ^{11}C-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. J. Med. Chem. 2003;46:2740-2754.

[7] Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. Ann. Neurol. 2004;55:306–319.

[8] Ikonomovic MD, Klunk WE, Abrahamson EE, Mathis CA, Price JC, Tsopelas ND, et al. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. Brain 2008;131:1630–45.

[9] Villeneuve S, Rabinovici GD, Cohn-Sheehy BI, Madison C, Ayakta N, Ghosh PM, et al. Existing Pittsburgh Compound-B positron emission tomography thresholds are too high: statistical and pathological evaluation. Brain 2015;138:2020-33.

[10] Nordberg A. PET imaging of amyloid in Alzheimer's disease. 2004;3:519-527.

[11] Wong DF, Rosenberg PB, Zhou Y, Kumar A, Raymont V, Ravert HT, et al. In vivo imaging of amyloid deposition in Alzheimer's disease using the novel radioligand [^{18}F]AV-45 (Florbetapir F 18). J Nucl Med 2010;51:913–20.

[12] Zhang W, Oya S, Kung MP, Hou C, Maier DL, Kung HF. F-18 Polyethyleneglycol stilbenes as PET imaging agents targeting Aβ aggregates in the brain. Nucl Med Biol. 2005;32:799-809.

[13] Vandenberghe R, Van Laere K, Ivanoiu A, Salmon E, Bastin C, Triau E, et al. ^{18}F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial. Ann Neurol 2010;68:319-329.

[14] Cselényi Z, Jönhagen ME, Forsberg A, Halldin C, Julin P, Schou M, et al. Clinical validation of ^{18}F-AZD4694, an amyloid-β-specific PET radioligand. J Nucl Med. 2012;53:415-424.

[15] Wilson AA, Armando Garcia A, Chestakova A, Kung H, Houle S. A rapid one-step radiosynthesis of the β-amyloid imaging radiotracer *N*-methyl-[^{11}C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]-6-OH-BTA-1). J. Labelled Comp. Radiopharm. 2004;47:679–682.

16 Wilson AA, Garcia A, Houle S, Vasdev N. Utility of commercial radiosynthetic modules in captive solvent [^{11}C]-methylation reactions. J. Labelled Comp. Radiopharm. 2009;52:490-492.
17 Wilson AA, Garcia A, Jin L, Houle S. Radiotracer synthesis from [^{11}C]-iodomethane: a remarkably simple captive solvent method. Nucl Med Biol. 2000;27:529.
18 Yamada A, Baba S, Isoda T, Maruoka Y, Kitamura Y, Kimura S, Honda H. Effective synthesis of Pittsburg Compound B (^{11}C-PIB) for clinical application by on-column ^{11}C-methylation. J Nucl Med. 2015;56(S3):1004.
19 http://abx.de/kits.html.
20 http://hic.cc/index.php?m=Product&a=index&id=33.
21 Watkins GL, Jewett DM, Mulholland GK, Kilbourn MR, Toorongian SA. A captive solvent method for rapid *N*-[^{11}C]methylation of secondary amides: application to the benzodiazepine, 4′-chlorodiazepam (RO5-4864). Appl Radiat Isot. 1988;39:441-444.
22 Jewett DM, Ehrenkaufer RL, Ram S. A captive solvent method for rapid radiosynthesis: Application to the synthesis of [1-^{11}C]palmitic acid. Appl Radiat Isot. 1985;36:672-674.
23 Jolly D, Hopewell R, Kovacevic MM, Li QY, Soucy JP, Kostikov A. Development of "[^{11}C]kits" for a fast, efficient and reliable production of carbon-11 labeled radiopharmaceuticals for Positron Emission Tomography. Appl Radiat Isot. 2017;121:76-81.
24 Kostikov AP, Chin J, Orchowski K, Niedermoser S, Kovacevic MM, Aliaga A, et al. Oxalic acid supported Si-^{18}F-radiofluorination: One-step radiosynthesis of succinimidyl 3-(di-tert-butyl[^{18}F]fluorosilyl)benzoate ([^{18}F]FSiB) for protein labeling. Bioconjugate Chem. 2012;23:106-14.
25 https://dian-tu.wustl.edu/en/home/.
26 Gibon J, Kang MS, Aliaga A, Sharif B, Rosa-Neto P, Séguéla P, et al. Towards the PET radiotracer for p75 neurotrophin receptor: [^{11}C]LM11A-24 shows biological activity *in vitro*, but unfavorable *ex vivo* and *in vivo* profile. Bioorg Med Chem. 2016;24:4759–4765
27http://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html.
28 Shao X, Hoareau R, Runkle AC, Tluczek LJM, Hockley B, Henderson BD, Scott PJH. Highlighting the versatility of the Tracerlab synthesis modules. Part 2: fully automated production of [^{11}C]-labeled radiopharmaceuticals using a Tracerlab FXC-Pro. J. Labelled Comp. Radiopharm. 2011;54:819– 838