Technical note

In-house development of an optimized synthetic module for routine [11C]acetate production

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[11C]Acetate, a radiotracer for PET imaging, is a promising radiopharmaceutical for overcoming the limitation of 2-deoxy-2-[18F]fluoro-D-glucose in a number of cancers. Here, the optimized automatic synthesis of [11C]acetate using an in-house-developed module under different conditions has been reported for routine production. [11C]CO$_2$ was produced in a 16.4 MeV PETtrace cyclotron, and methyl magnesium chloride was used for synthesis. For product purification, ion-exchange solid-phase extraction cartridges were used, connected in series. High-performance liquid chromatography and gas chromatography were used to measure radiochemical and chemical purity. The Limulus amebocyte lysate test and the fluid thioglycollate medium test were performed for quality control of [11C]acetate. The total reaction time of [11C]acetate was within 15 min, and the overall decay-corrected radiochemical yield was 84.33 ± 8.85%. Radiochemical purity was greater than 98% when evaluated on an analytical high-performance liquid chromatography system. No endotoxins or anaerobic bacteria were seen on quality control checks. Optimized production of [11C]acetate was achieved by the in-house module. Radiochemical and biological properties of the [11C]acetate produced were appropriate for clinical PET study. *Nucl Med Commun* 36:102–106 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

[11C]Acetate is quickly metabolized into acetyl-CoA in human cells and can enter the tricarboxylic acid cycle to participate in cell membrane lipid synthesis in tumor cells [1]. [11C]Acetate was initially developed to assess myocardial metabolism in nuclear cardiology; however, it has attracted increasing interest in recent years among oncologists because of its advantages over 2-deoxy-2-[18F]fluoro-D-glucose [2,3]. Thus, [11C]acetate, a radiotracer for PET imaging, is under investigation for use in a number of cancers [1,4–7].

Several methods for radiosynthesis and purification of [11C]acetate have been reported since the 1980s [2,3,8–19]. All reported methods are based on the carboxylation of magnesium halides; however, isolation and purification methods differ. Among these approaches, a Grignard reaction in the reaction vessel or in a loop and solid-phase extraction (SPE) purification using commercially available ion-exchange cartridges have been mainly adopted [3,10,14–18,20]. Especially, purification of [11C]acetate using alumina or AG11A8 cartridges (prepared on site) has been reported in recent years [19,21]. However, most of the reported methods used to develop or modify a synthetic module were difficult and complicated, and synthesis and purification conditions were too varied to be applied clinically. Thus, it is necessary to combine the advantages of each module, such as simple synthesis or purification methods, low concentration of reagents, and high radiochemical yield (RCY), for clinical use. In this study, we modified the prototype of the [11C]acetate synthesis module developed by our group and proved its high reproducibility and simplicity, with high RCY for routine clinical use.

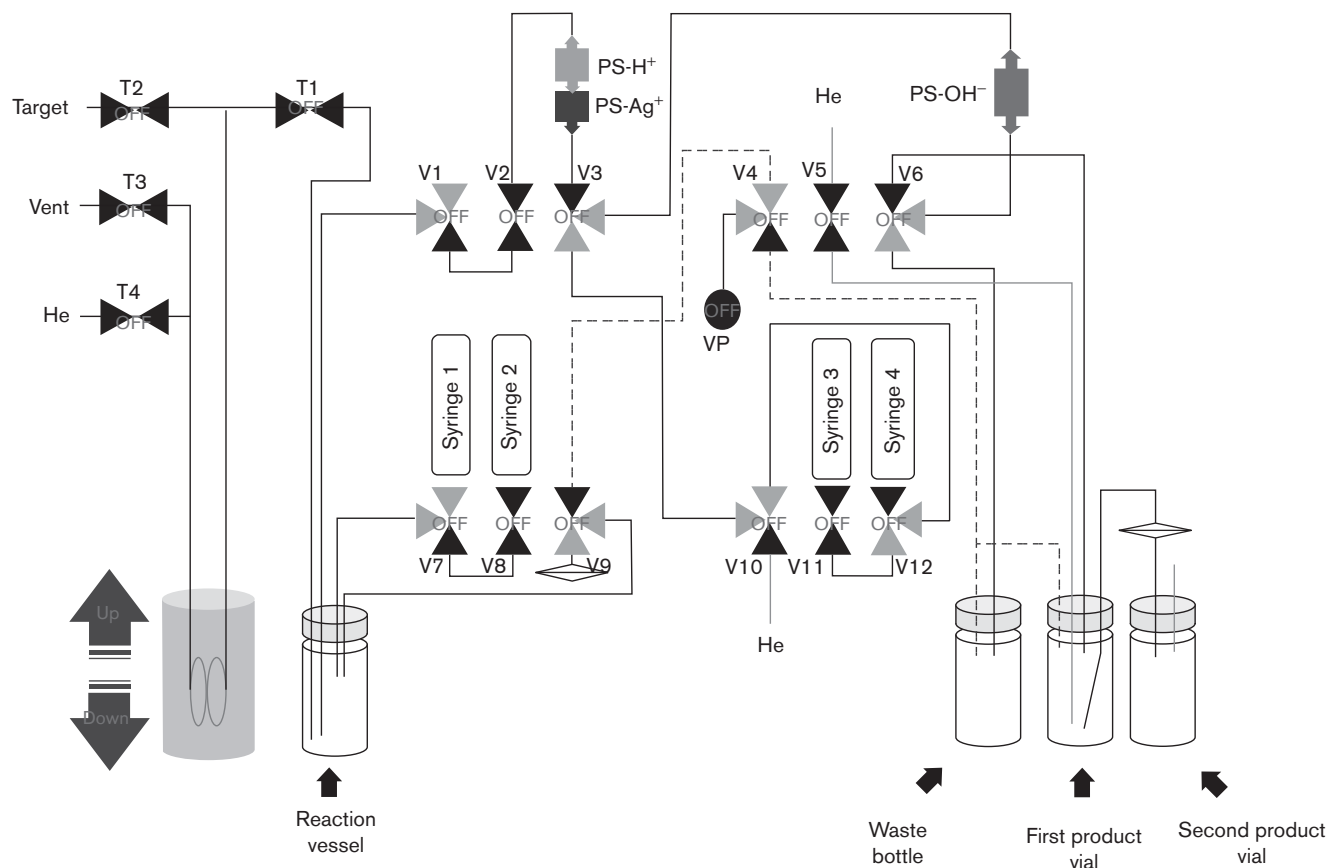## Materials and methods

The synthesis module was configured as shown in Fig. 1. Before synthesis, a 30 μmol/l solution of methyl magnesium chloride in tetrahydrofuran was prepared. All tubes and valves were dried with nitrogen gas. PS-AG$^+$ and PS-H$^+$ cartridges were activated with 10 ml of ethanol, followed by 20 ml of distilled water.

Fig. 1

Synthesis apparatus for [^{11}C]acetate for clinical use. V1–V12 are the solenoid valves of the synthesizer and T1–T4 are the pneumatic valves of the trap system.

The PS-OH$^-$ cartridge was activated with 10 ml of 1.0 mol/l sodium hydroxide solution, followed by 20 ml of distilled water. The Maxi-Clean SAX cartridge was activated with 10 ml of ethanol, then with 5 ml of sterile 9 g/l sodium chloride solution, followed by rinsing with 10 ml of distilled water. Syringes 1 and 2 contained 5 ml of distilled water to move radioactivity from the reaction vessel to the cartridges, and syringe 3 was filled with 30 ml of distilled water to remove impurities of the strong anion exchanger. Syringe 4 contained 10 ml of 9 g/l sodium chloride solution to release [^{11}C] acetate from the strong anion exchanger to the first product vial.

[^{11}C]CO$_2$ was released by lifting the trap out of the liquid nitrogen bath and then transferring it to the reaction vessel with a gentle flow of nitrogen from a gas cylinder. The stream of nitrogen gas with [^{11}C]CO$_2$ was bubbled through 1.0 ml of 30 μmol/l methyl magnesium chloride solution for 4 min in a 10 ml sealed reaction vessel. Distilled water (5.0 ml) from syringe 1 was added to the reaction vessel using a vacuum pump (VP) and the mixture was aspirated with the VP through valves 1, 2, 3, and 6 through the cation exchanger and anion exchanger cartridges into the waste bottle. The reaction vessel was rinsed once more with 5.0 ml of distilled water from syringe 2. The anion exchanger with trapped [^{11}C]acetate was washed with 30 ml of distilled water from syringe 3 and the washings were aspirated through valves 11, 12, 10, 3, and 6 into the waste bottle using the VP. The [^{11}C] acetate was flushed out with 10 ml of 9 g/l sodium chloride solution from syringe 4 into a first product vial containing 0.2 ml of 0.1 mol/l hydrochloric acid. Gaseous nitrogen was then bubbled vigorously through the solution for 3 min to eliminate [^{11}C]carbonate. Finally, the [^{11}C]acetate in 9 g/l sodium chloride solution was filtered through a 0.22 μm sterile filter into a second product vial containing 50 μl of saturated sodium hydrogen carbonate for neutralization. Quality control [thin-layer chromatography, high-performance liquid chromatography (HPLC), gas chromatography, pH, endotoxin test, fluid thioglycollate medium (FTM) test] was performed to validate [^{11}C]acetate after synthesis (Supplemental digital content 1, *http://links.lww.com/NMC/A34*).

Table 1 Self-developed module operation for the preparation of [^{11}C]acetate injection

Step	Operation	Explanation	Time (s)
1	T2, T3 on	[^{11}C]CO_2 delivery and trapped	120
2	T2, T3 off, T1 on	Liquid N_2 trap down for increasing temperature	60
3	T4 on	[^{11}C]CO_2 transferred to reaction vessel	60
4	T1 off, T3 on, V9 on, V7 off, VP on, and VP off	Syringe 1 down	20
5	V4 on, V3 on, V2 on, V9 off, VP on, and VP off	Reaction mixture transferred to cartridges (first time)	80
6	V3 off, V7 on, V8 on, V9 on, V4 off, VP on, and VP off	Syringe 2 down	20
7	V4 on, V3 on, V9 off, VP on, and VP off	Reaction mixture transferred to cartridges (second time)	80
8	V3 off, V11 on, VP on, and VP off	PS-OH cartridge washing	90
9	V6 on, V12 on, and VP on	[^{11}C]acetate flushing to first product vial	60
10	V5 on	Bubbling	180
11	VP off, V4 off, and V10 on	[^{11}C]acetate transferred to second product vial	130

In-house-developed module operation for the preparation of [^{11}C]acetate injection.
VP, vacuum pump.

Results and discussion

[^{11}C]CO_2, generated through a cyclotron (20 μA irradiation beam for 10 min), was used to synthesize [^{11}C] acetate, decaying at 6.01 ± 0.63 GBq (162.33 ± 17.04 mCi), for clinical PET study. [^{11}C]CO_2 from the cyclotron was trapped in a stainless-steel loop trap cooled in a liquid nitrogen bath. [^{11}C]CO_2 was released by lifting the trap out of the bath and then transferring it to the reaction vessel. A stream of nitrogen gas containing [^{11}C]CO_2 was bubbled through methyl magnesium chloride solution. The reaction was quenched with water and the solution was aspirated using a VP, through cation and anion exchangers, into a waste bottle. The anion exchanger was washed with distilled water and the washings were flushed out with 9 g/l sodium chloride solution into the first product vial, giving an acidic solution. After nitrogen gas was bubbled through the first product vial, the [^{11}C] acetate solution in 9 g/l sodium chloride was filtered through a 0.22 μm sterile filter into the second product vial, giving a basic solution neutralized using 50 μl of saturated sodium hydrogen carbonate solution. The total synthesis time was less than 20 min. The time sequence is listed in Table 1. [^{11}C]Acetate was synthesized with $84.33 \pm 8.85\%$ (decay corrected) RCY based on [^{11}C]CO_2. The radiochemical purity was greater than 98%, as determined by analytical HPLC, and none of the previously reported radiochemical impurities, such as [^{11}C]carbonate, [^{11}C]acetone, or tert-[^{11}C]butanol, were observed [10]. Gas chromatography revealed a very low concentration of tetrahydrofuran (31.42 ± 0.45 ppm,

$n = 6$), which is acceptable in clinical use. An endotoxin test was performed using a portable detector system, and an FTM test was used to check sterility. The endotoxin value was less than 1.0 EU/ml. No bacteria, yeasts, or fungi were observed in the FTM after 15 days, and the pH value was 6.0–7.0.

Generally, [^{11}C]acetate was synthesized by the carboxylation of magnesium halides, and there were many types of purification methods, including liquid–liquid extraction, HPLC purification, the distillation approach, and SPE [21]. Among them, SPE revealed a high RCY and short purification time [17,19–21]. In addition, this method is very simple and is an easy-to-establish automatic system. These factors are very important for clinical use of radiopharmaceuticals.

In this study, the previously reported prototype of the [^{11}C]acetate module, developed by our group for clinical use, has been improved [22]. A Grignard reaction in the vessel and SPE for purification were adopted in the previous module. However, the RCY of the former prototype module was around 30% (decay corrected) and was insufficient. Thus, optimization was needed for synthesis and purification. The liquid nitrogen trap has been modified to increase the RCY by changing the trap volume, the flow of [^{11}C]CO_2, and the lifting speed of the liquid nitrogen trap. In addition, the anion exchangers, hydrolysis, and flushing solution have been changed to increase the RCY. Results showed that significantly more [^{11}C]acetate was trapped by a PS-OH$^-$ cartridge than by

Table 2 RCY of [^{11}C]acetate according to different synthesis conditions

Anion exchanger	Quenching and transfer solution	Recovery solution	Radiochemical yield
Maxi-Clean SAX	Distilled water	Sodium chloride solution (9 g/l)	68.40 ± 4.42^a
Maxi-Clean SAX	Distilled water	Citrate buffer (pH 4.7)	21.82 ± 5.50
Maxi-Clean SAX	Acetic acid (1 mmol/l)	Sodium chloride solution (9 g/l)	35.32 ± 3.75
Maxi-Clean SAX	Acetic acid (1 mmol/l)	Citrate buffer (pH 4.7)	5.02 ± 0.79
PS-OH$^-$	Distilled water	Sodium chloride solution (9 g/l)	84.33 ± 8.85
PS-OH$^-$	Distilled water	Citrate buffer (pH 4.7)	26.84 ± 5.41
PS-OH$^-$	Acetic acid (1 mmol/l)	Sodium chloride solution (9 g/l)	55.93 ± 5.84
PS-OH$^-$	Acetic acid (1 mmol/l)	Citrate buffer (pH 4.7)	34.20 ± 3.01

RCY, radiochemical yield.
[a]RCYs are expressed as mean$\pm$SD ($n = 6$, each).

Fig. 2
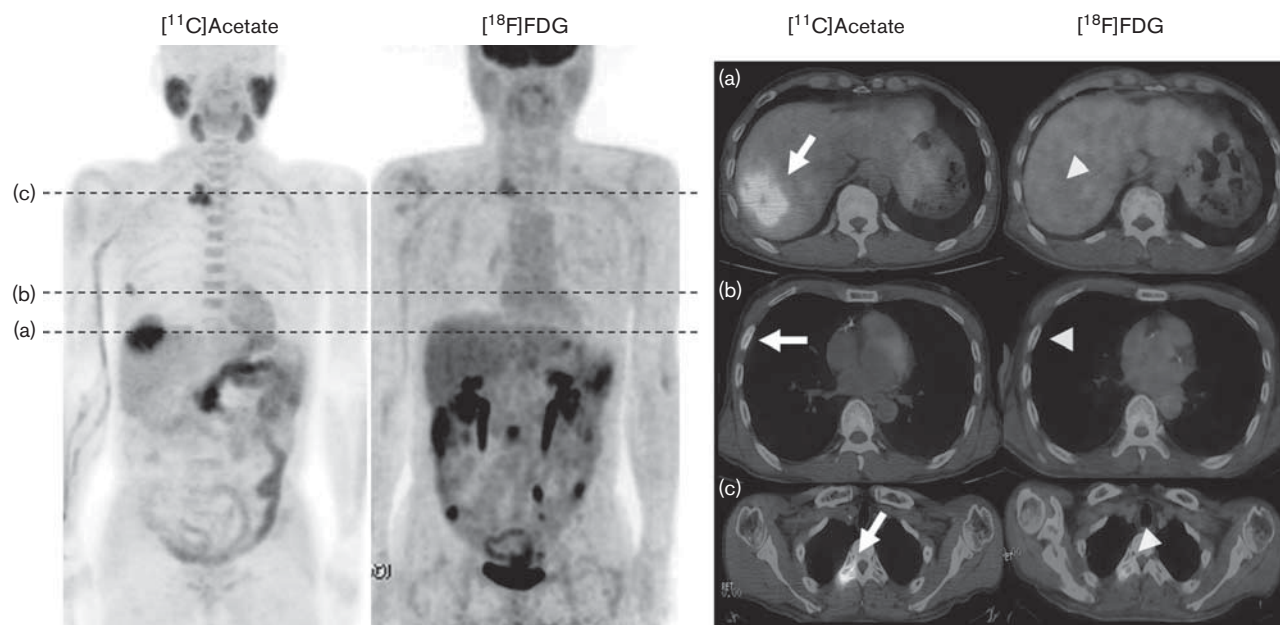
[¹¹C]Acetate PET-CT images were compared with [¹⁸F]FDG PET-CT images of HCC patients. (a) [¹¹C]Acetate PET-CT showed substantially increased uptake in the right hepatic lobe (SUV$_{max}$: 9.0, arrow). [¹⁸F]FDG PET-CT showed isometabolism not separated from the surrounding hepatic tissue (SUV$_{max}$: 2.9, arrow head). (b, c) [¹¹C]acetate PET-CT showed substantial hypermetabolism in metastatic lesions of the right fifth rib [SUV$_{max}$: 3.3, arrow in (b)] and the T3 vertebra [SUV$_{max}$: 6.1, arrow 9 (c)]; [¹⁸F]FDG PET-CT showed mild or slightly high metabolic activities [SUV$_{max}$: 1.5 in the right fifth rib, arrow head in (b), and 3.2 in the T3 vertebra, arrow head in (c)]. CT, computed tomography; [¹⁸F]FDG,2-deoxy-2-[¹⁸F]fluoro-D-glucose; SUV$_{max}$, maximum standardized uptake value.

the Maxi-Clean SAX cartridge. Distilled water was better than aqueous acetic acid (1 mmol/l) for hydrolysis and quenching. Sodium chloride solution (9 g/l) was superior to citrate buffer (pH 4.7) for flushing out [¹¹C]acetate from the anion cartridge into the product vial (Table 2).

Furthermore, to minimize the risk of contamination by inorganic impurities, the concentration of Grignard reagent was reduced to 30 µmol/l, which is substantially less than that used in the prototype module (200 µmol/l). This concentration can be considered safe because magnesium and bromide ions normally present in human blood are at concentrations higher than 30 µmol [23]. Another reason for the use of a low concentration of Grignard reagent was the reduction of the failure rate of [¹¹C]acetate synthesis. Previously, a white precipitate, formed when the reaction mixture was quenched with acetic acid at a quantity depending on the concentration of Grignard reagent, often obstructed the lines and valves of the module. Further, a single product vial in the prototype module often led to retention of unreacted [¹¹C]CO$_2$ and [¹¹C]carbonate, which were also trapped by the anion exchange cartridge and flushed out with the [¹¹C]acetate to contaminate the product vial. To effectively remove [¹¹C]CO$_2$ and [¹¹C]carbonate, a first vial containing 0.2 ml of 0.1 mol/l hydrochloric acid was added, a 9 g/l sodium chloride solution was used to flush out the anion exchange cartridge, and nitrogen gas was bubbled

vigorously. Both [¹¹C]CO$_2$ and [¹¹C]carbonate had to be removed with nitrogen flow under slightly acidic solutions. Thereafter, [¹¹C]acetate in the acidic 9 g/l sodium chloride solution was transferred to a second product vial for neutralization as previously mentioned.

[¹¹C]Acetate was synthesized simply and efficiently under optimized conditions using an in-house-developed module based on [¹¹C]carboxylation. The radiochemical and biological properties of the [¹¹C]acetate was appropriate for clinical PET study. [¹¹C]Acetate produced through the current in-house module was used to visualize hepatocellular carcinoma in patients that was not detected by 2-deoxy-2-[¹⁸F]fluoro-D-glucose PET-computed tomography, which is in line with previous studies [24]. Image quality was also acceptable for diagnostic purposes (Fig. 2).

Conflicts of interest
There are no conflicts of interest.

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