Technical note

In-house development of an optimized synthetic module for routine $^{[11]}$Cacetate production

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$^{[11]}$C-acetate, a radiotracer for PET imaging, is a promising radiopharmaceutical for overcoming the limitation of 2-deoxy-2-$^{[18]}$F-fluoro-D-glucose in a number of cancers. Here, the optimized automatic synthesis of $^{[11]}$C acetate using an in-house-developed module under different conditions has been reported for routine production. $^{[11]}$C 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 $^{[11]}$C acetate. The total reaction time of $^{[11]}$C 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 $^{[11]}$C acetate was achieved by the in-house module. Radiochemical and biological properties of the $^{[11]}$C acetate produced were appropriate for clinical PET study. Nucl Med Commun 36:102–106 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords: automatic synthetic module, $^{[11]}$C acetate, oncology, PET, solid-phase extraction purification

Introduction

$^{[11]}$C 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]. $^{[11]}$C 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-$^{[18]}$F-fluoro-D-glucose [2,3]. Thus, $^{[11]}$C 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 $^{[11]}$C 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 $^{[11]}$C 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 $^{[11]}$C 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.
The PS-OH\textsuperscript{−} 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 \([^{11}\text{C}]\)acetate from the strong anion exchanger to the first product vial.

\([^{11}\text{C}]\)CO\textsubscript{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 \([^{11}\text{C}]\)CO\textsubscript{2} was bubbled through 1.0 ml of 30 \(\mu\text{mol/l}\) 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 \([^{11}\text{C}]\)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 \([^{11}\text{C}]\)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 \([^{11}\text{C}]\)carbonate. Finally, the \([^{11}\text{C}]\)acetate in 9 g/l sodium chloride solution was filtered through a 0.22 \(\mu\text{m}\) sterile filter into a second product vial containing 50 \(\mu\)l 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 \([^{11}\text{C}]\)acetate after synthesis (Supplemental digital content 1, http://links.lww.com/NMC/A34).
Results and discussion

$[^{11}\text{C}]\text{CO}_2$, generated through a cyclotron (20 μA irradiation beam for 10 min), was used to synthesize $[^{11}\text{C}]$ acetate, decaying at 6.01 ± 0.63 GBq (162.33 ± 17.04 mCi), for clinical PET study. $[^{11}\text{C}]\text{CO}_2$ from the cyclotron was trapped in a stainless-steel loop trap cooled in a liquid nitrogen bath. $[^{11}\text{C}]\text{CO}_2$ was released by lifting the trap out of the bath and then transferring it to the reaction vessel. A stream of nitrogen gas containing $[^{11}\text{C}]\text{CO}_2$ 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 $[^{11}\text{C}]$ acetate solution in 9 g/l sodium chloride was filtered out with a 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. $[^{11}\text{C}]$ Acetate was synthesized with 84.33 ± 8.85% (decay corrected) RCY based on $[^{11}\text{C}]\text{CO}_2$. The radiochemical purity was greater than 98%, as determined by analytical HPLC, and none of the previously reported radiochemical impurities, such as $[^{11}\text{C}]$ carbonate, $[^{11}\text{C}]$ acetone, or tert-[^{11}\text{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, $[^{11}\text{C}]$ 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 $[^{11}\text{C}]$ 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 $[^{11}\text{C}]\text{CO}_2$, 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 $[^{11}\text{C}]$ acetate was trapped by a PS-OH$^-$ cartridge than by

| 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 ± SD ($n=6$, each).
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]CO2 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]CO2 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]CO2 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).

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Conflicts of interest
There are no conflicts of interest.
References

1. Grassi I, Nanni C, Allegri V, Morigi JJ, Montini GC, Castelluci P, Fanti S. The clinical use of PET with 11C-acetate. Am J Nucl Med Mol Imaging 2012; 2:33–47.
2. Mitterhauser M, Wadsak W, Kralc A, Schmaljohann J, Bartosch E, Eidherr H, et al. New aspects on the preparation of 11C-acetate – a simple and fast approach via distillation. Appl Radiat Isot 2004; 61:1147–1150.
3. Soloviev D, Tamburella C. Captive solvent 11C-acetate synthesis in GMP conditions. Appl Radiat Isot 2008; 64:995–1000.
4. Huo L, Wu Z, Zhuang H, Fu Z, Dang Y. Dual time point C-11 acetate PET imaging can potentially distinguish focal nodular hyperplasia from primary hepatocellular carcinoma. Clin Nucl Med 2009; 34:874–877.
5. Shiomi S, Kawabe J. Clinical applications of positron emission tomography in hepatic tumors. Hepatol Res 2011; 41:611–617.
6. Ho CL, Chen S, Leung YL, Cheng T, Wong KN, Cheung SK, et al. 11C-Acetate PET/CT for metabolic characterization of multiple myeloma: a comparative study with 18F-FDG PET/CT. J Nucl Med 2014; 55:749–752.
7. Nomori H, Shibata H, Uno K, Iyama K, Honda Y, Nakashima R, et al. 11C-Acetate can be used in place of 18F-fluorodeoxyglucose for positron emission tomography imaging of non-small cell lung cancer with higher sensitivity for well-differentiated adenocarcinoma. J Thorac Oncol 2008; 3:1427–1432.
8. Ishiwata K, Ishi SI, Sasaki T, Senda M, Nozaki T. A distillation method of preparing C-11 labeled acetate for routine clinical use. Appl Radiat Isot 1993; 44:761–763.
9. Iwata R, Ido T, Tada M. Column extraction method for rapid preparation of [11C]acetate and [11C]palmitic acids. Appl Radiat Isot 1995; 46:117–121.
10. Krujier PS, Linden TT, Mooij R, Visser FC, Herscheid JDM. A practical method for the preparation of [11C]acetate. Appl Radiat Isot 1995; 46:317–321.
11. Moerlein SM, Gaehle GG, Welch MJ. Robotic preparation of sodium acetate C-11 injection for use in clinical PET. Nuci Med Biol 2002; 29:613–621.
12. Pike VW, Eakins MN, Allan RM, Selwyn AP. Preparation of [1-11C]acetate – an agent for the study of myocardial metabolism by positron emission tomography. Int J Appl Radiat Isot 1982; 33:505–512.
13. Pike VW, Horlock PL, Brown C, Clark JC. The remotely controlled preparation of a carbon-11 labelled radiopharmaceutical [11C]acetate. Int J Appl Radiat Isot 1984; 35:623–627.
14. Roeda D, Dolk F, Crouzel C. An improvement of [11C]acetate synthesis-non-radioactive contaminants by irradiation-induced species emanating from the 11C carbon dioxide production target. Appl Radiat Isot 2002; 57:857–860.
15. Bars DL, Malleva M, Bonnefio F, Tourville C. Simple synthesis of [11C] acetate. J Label Compd Radiopharm 2006; 49:263–267.
16. Cheung MK, Ho CL. A simple, versatile, low-cost and remotely operated apparatus for [11C]acetate, [11C]choline, [11C]methionine and [11C]PIB synthesis. Appl Radiat Isot 2009; 67:581–589.
17. Felicini C, Nägren K, Berton A, Pascali G, Salvadori PA. Development of an automated modular system for the synthesis of [11C]acetate. Nucl Med Commun 2010; 31:1033–1039.
18. Lodi F, Trespidi S, Di Pierro D, Marengo M, Farsad M, Fanti S, et al. A simple Tracerlab module modification for automated on-column [11C]methylamine and [11C]carboxylation. Appl Radiat Isot 2007; 65:691–695.
19. Mock BH, Brown-Proctor C, Green MA, Steele B, Glick-Wilson BE, Zheng QH. An automated SPE-based high-yield synthesis of [11C]acetate and [11C]palmitate: no liquid–liquid extraction, solvent evaporation or distillation required. Nucl Med Biol 2011; 38:1135–1142.
20. Runke AC, Shao X, Tluczek LJ, Henderson BD, Hockley BG, Scott PJ. Automated production of [11C]acetate and [11C]palmitate using a modified GE Tracerlab FXC-Pro. Appl Radiat Isot 2011; 69:691–698.
21. Tang X, Tang G, Nie D. Fully automated synthesis of [11C]acetate as tumor PET tracer by simple modified solid-phase extraction purification. Appl Radiat Isot 2013; 82:81–86.
22. Hur MG, Yang SD, Kim DY, Kim SW. Development of the [11C]acetate synthesis module. J Korean Phys Soc 2013; 63:1390–1394.
23. Davenport RJ, Dowsett K, Pike VW. A simple technique for the automated production of no-carrier-added [11C]acetate. Appl Radiat Isot 1997; 48:1117–1120.
24. Larsson P, Arvidsson D, Bjornstedt M, Isaksson B, Jerusalem I, Motarjemi H, Jacobsson H. Adding 11C-acetate to 18F-FDG at PET examination has an incremental value in the diagnosis of hepatocellular carcinoma. Mol Imaging Radionucl Ther 2012; 21:8–12.