Technical Note

Troubleshooting for F-18 Fluorocholine Synthesis: An Institutional Experience

Abstract

Choline is a natural substrate for phospholipid synthesis. F-18 labeled fluorocholine nowadays is routinely used for imaging brain tumors, parathyroid adenoma, and prostate cancer. It is synthesized through nucleophilic substitution reaction using dibromomethane and N, N-dimethylaminoethanol as primary and secondary precursors, respectively. However, sometimes, failures are encountered in F-18 fluorocholine production. Few problems and troubleshooting during synthesis are discussed here.

Keywords: *F-18 fluorocholine, failure, synthesis, troubleshooting*

Rakhee Vatsa, Raman Kumar Joshi[1], Jaya Shukla, Bhagwant Rai Mittal

Department of Nuclear Medicine and PET, Post Graduate Institute of Medical Education and Research, Chandigarh, [1]Department of Neuroimaging and Interventional Radiology, National Institute of Mental Health and Neuroscience, Bengaluru, Karnataka, India

Introduction

Choline is a naturally occurring water-soluble vitamin which comes under the class of quaternary ammonium salts.[1,2] It is used for synthesis of phospholipids in cells. Enhanced cell procreation and increased cell membrane metabolism result in increased choline uptake in tumor cells. C-11 and F-18 labeled choline have been explored for imaging prostate, brain and lung tumors.[3,4] In the present study, F-18 was produced through $^{18}O(p,n)\ ^{18}F$ reaction in an on-site medical cyclotron (PETtrace 4 GE Healthcare). F-18 florocholine (F-18 FCH) was synthesized by nucleophilic substitution reaction as proposed by DeGrado *et al.*,[5] using cassette-based automated synthesis module Neptis virtual synthesizer®, ORA [Figure 1]. Dibromomethane was used as a primary precursor and N, N-dimethylaminoethanol (DMAE) in dimethyl sulfoxide, preloaded on C-18 cartridge was used as a secondary precursor. Postsynthesis purification was accomplished using ready to use cartridges (silica and CM), based on solid-phase extraction chromatography. The final product (F-18 FCH) was eluted in 0.9% NaCl. Although the synthesis is automated, there are still possibilities of mechanical/manual errors leading to production failure. The difficulties faced during F-18 FCH synthesis are herein reported case wise.

Case Reports

Case 1: Reduced F-18 florocholine – Vacuum leak

The usual yield of F-18 FCH was 10%–15%. During one of the syntheses, the obtained yield was less than usual yield (2%). All the reagents released during the synthesis were in order. During weekly self-tests, the vacuum leak was detected as shown in Figure 2. The leak was traced on the waste bottle connection and was repaired.

Case 2: No F-18 florocholine production – Cartridge malfunction

During another F-18 FCH production, no product was obtained after completion of synthesis. F-18 was observed to be normally trapped and released from QMA on real-time synthesis graph and was delivered efficaciously into the reaction vial. While comparing the postsynthesis reports with the previous successful synthesis reports, it was noticed that there was no flow between 27 and 42 min on flow curve as shown in Figure 3. On the following day, radioactivity was also detected in the silica cartridge (top). Further, when checked with N_2 gas, one of the silica cartridges (top) was found to be a defective causing hindrance in the purification of floralbromomethane and subsequently in further reaction with the secondary precursor. The cartridge slot was later on replaced by providers for upcoming synthesis.

How to cite this article: Vatsa R, Joshi RK, Shukla J, Mittal BR. Troubleshooting for F-18 fluorocholine synthesis: An institutional experience. Indian J Nucl Med 2018;33:355-8.

Address for correspondence:
Dr. Rakhee Vatsa, Department of Nuclear Medicine and PET, Post Graduate Institute of Medical Education and Research, Chandigarh - 160 012, India.
E-mail: rakheevatsa@gmail.com

Access this article online

Website: www.ijnm.in

DOI: 10.4103/ijnm.IJNM_85_18

Quick Response Code:

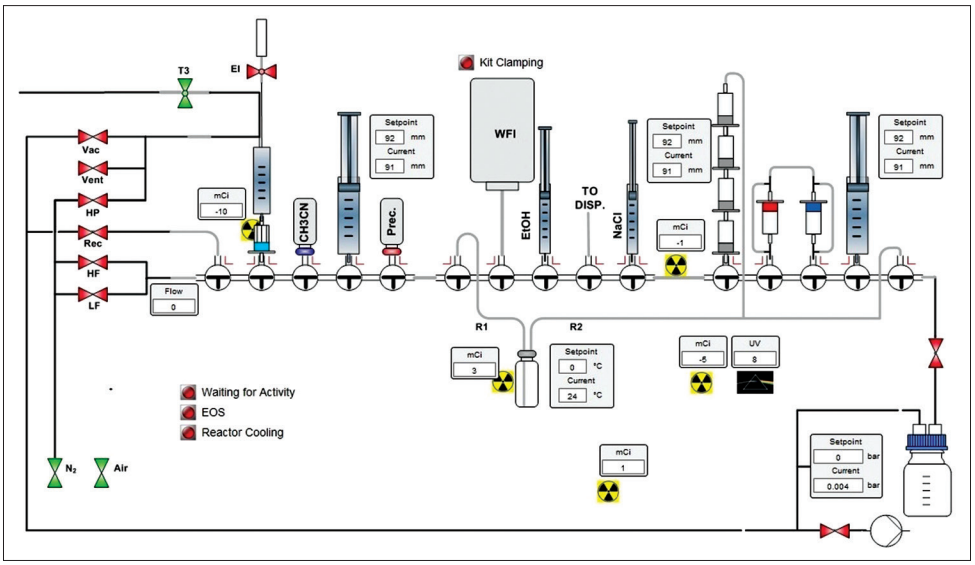

Figure 1: Schematic diagram showing reagents and cartridges used for the synthesis of F-18 florocholine on Neptis virtual synthesizer®

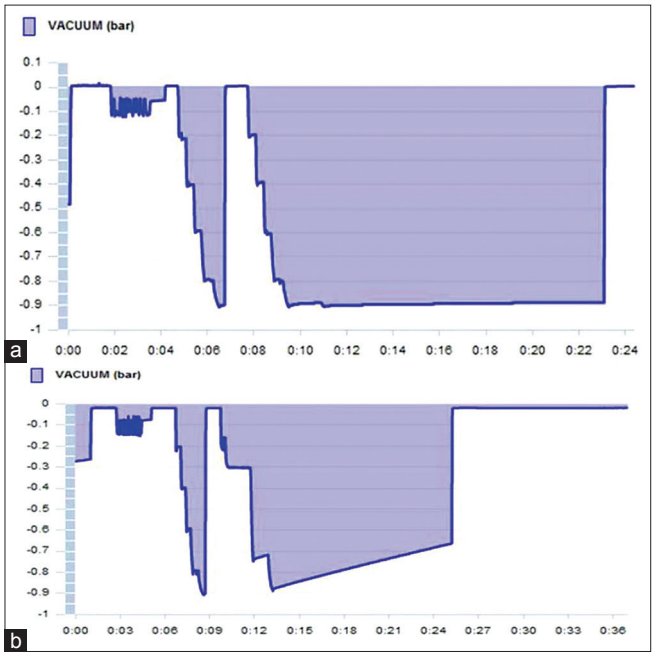

Figure 2: (a) normal vacuum curve and (b) vacuum leak (during 12–24 min) leading to failure of synthesis

Case 3: No production of F-18 florocholine – Reagent malfunction

In another case of failure, error 299 was spotted at 45 min during synthesis. The flow curve showed normal flow during 27–42 min. However, no volume of F-18 FCH was obtained at the end of the synthesis. On observing the cassette on following day, the right syringe in the third manifold of cassette was found to be deformed as shown in Figure 4. The cartridges were checked with N_2 gas flow, before the synthesis. However, postsynthesis CM cartridge was found to be blocked. Rest all other cartridges were working well. It was inferred as there may be problem in reaction with DMAE that led the formation of some intermediates that hindered the flow from CM cartridge during the synthesis.

Case 4: No production – Over tightened check valves connecting cartridge

In another case of failure, no volume of F-18 FCH was observed postsynthesis in the mother vial. All presynthesis tests were passed successfully. All the cartridges were in order as tested on N_2 gas flow test before synthesis. On observing the cassette next day postsynthesis, leakage was detected in the line connecting C-18 cartridge as shown in Figure 5. Later on, it was noticed that the check valves connecting C-18 cartridge were over tightened restricting the free flow of reaction intermediates and leading to failure of synthesis.

Another production failure was noticed due to overtightened valves connecting C-18 cartridge. The flow meter was destroyed due to backflow of reaction mixture in the nitrogen lines as faulty readings were observed on the flow curve as shown in Figure 6. The flow meter was later on replaced with a new one.

Troubleshooting for F-18 fluorocholine synthesis

Gas flow check

Nitrogen

As nitrogen is the carrier gas for the synthesis, it should be very pure (99.9995 ppm or UHP 5.5). Before synthesis nitrogen flow should be checked by opening high flow (HF) valve in the maintenance mode, because a reduced pressure of nitrogen gas may lead to the leakage or poor output of the product. It is wise to have an external calibrated flow meter to check the nitrogen flow in case of production failure and also during planned maintenance. Change the flow meter of the synthesis module if the flow is less than required or if the analog display of the flow meter is not working properly.

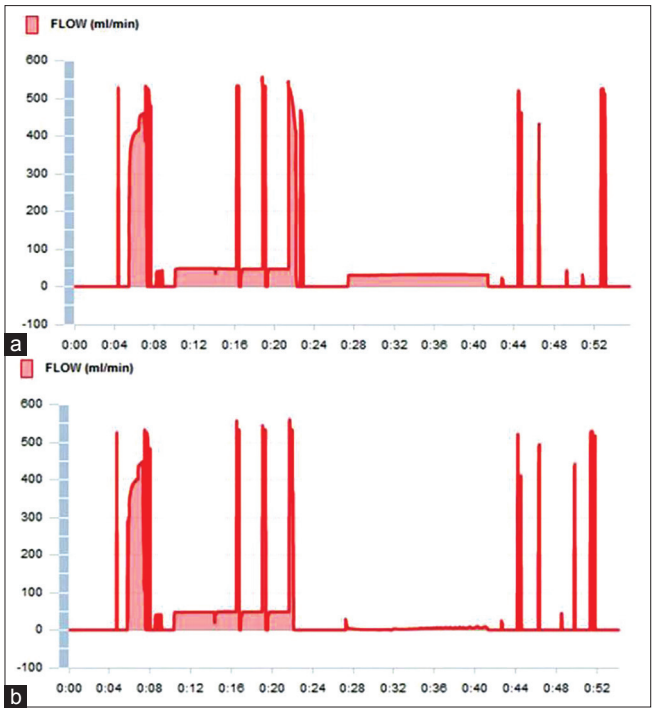

Figure 3: (a) normal flow during a successful run between 27 and 42 min and (b) no flow during failed run

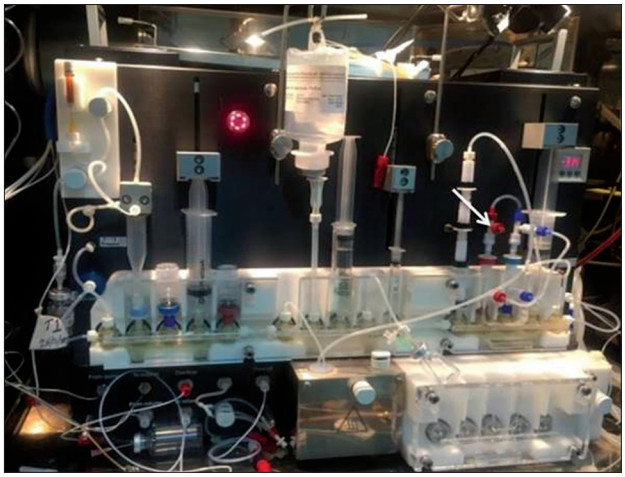

Figure 5: Leakage in line (with arrow) connecting C-18 cartridge due to overtightened check valves into the C-18 cartridge and thus leading to failure of synthesis

Compressed air

Compressed air is used to open the pneumatic valves of the cassette. Always assure that there is required air pressure for the synthesis module. Check all the necessary pneumatic 3-way actuators (valves) on cassette before using the synthesis module for the hot run. Furthermore, ensure the supply of moisture-free filtered air with a flow rate set as per the machine requirement.

Air filter

Notably assure while placing the purification filter for the nitrogen flow for its expiry and nature (hydrophilic/hydrophobic).

Figure 4: Deformed right syringe (with arrow) due to blockage of CM cartridge resulting in failure of synthesis

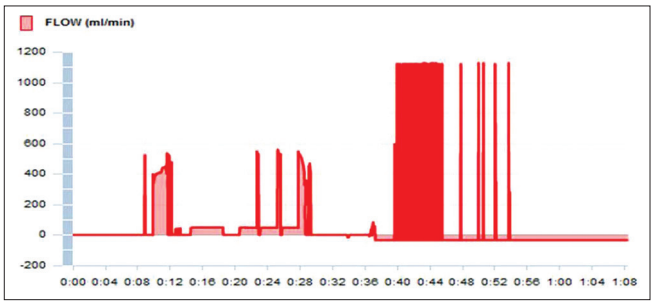

Figure 6: Abnormal flow values after 40 min on flow curve due to the faulty flow meter

This is an important component not only for supplying the sterile nitrogen but also to avoid any backflow of liquid into the flow meter from the faulty distillation accidents.

Size of waste bottle

Always verify that appropriate volume waste bottle is used for collection of waste. A smaller bottle can lead to extra pressure and accidental loosening during the synthesis and vice versa for the larger size bottle (lesser/reduced vacuum throughout the synthesis).

Leak in connector of the waste bottle

Minor leak in the waste bottle may lead to decreased production yield. Always reconfirm that the connectors are well tighten and "O-ring" should always be present inside the lid of the bottle. Leak on the waste bottle can also be present at the back of synthesis module where tubings are connected to the module. To locate the leak, enter a set point of −0.8 bar. Once reached, remove the set point. Pinch the upper waste tubing (which goes to the waste bottle) and check if the vacuum is proper or if the leak is still present. Pinch the lower waste tubing and check if the vacuum is maintained or if the leak is still present.

Silica cartridges block

Due to blockage in silica cartridge, the intermediate product will get struck on the first silica cartridge and it will not

be available for further reaction with DMAE on C-18 cartridge leading to production failure. To locate the silica cartridge blockage, check the flow of individual cartridge before synthesis on HF valve. The normal flow rate values are shown in Figure 7. If one of the cartridges has a flow value too low (10% less than the mentioned values), it should be replaced with a new cartridge for smooth run. Similar test should also be performed before using CM and C-18 cartridges to assure they are also working properly.

Blockage of check valves connecting C-18/CM cartridges

Over tightened check valves (connected on the C18/CM cartridges) into the cartridges may obstruct the flow of gases and the liquid through them. This may lead to faulty distillation or no distillation at all. Hence, check valves should always be tightened carefully.

Mechanical glitches

Physically inspect all the tubing and connections for any blockage. Make sure the cassette syringes are properly clamped. Because this may disable the rinsing of the syringes and there will be no volume in the final product vial at all. While loading the chemicals also cross verify their position in the synthesis module.

Other common possible causes of failure

No or partial transfer of F-18 to module can lead to either synthesis failure or less yield. Transfer lines may get clogged after using for long time or leak may also be present in the lines. Before F-18 production, it is always recommended to fill the target with $H_2{}^{16}O$ followed by drying of transfer line with helium. The collected volume must be measured to rule out any possibility of obstruction/leakage. This will also help in checking the delivery path to correct hot cell.

No trapping of F-18 in the quaternary methyl ammonium (QMA) anion exchange column is one of the root causes for failed synthesis. Always ensure that the QMA is properly conditioned and dried before installation in the cassette.

No release of F-18 from QMA is another source for the failure of synthesis. As eluent position is slightly out of the way, each time confirm that the eluent is placed at respective position and is pricked properly in the eluent needle. The eluent line should also be pinched during synthesis. Before each run line must be washed with water and dried to rule out any obstruction.

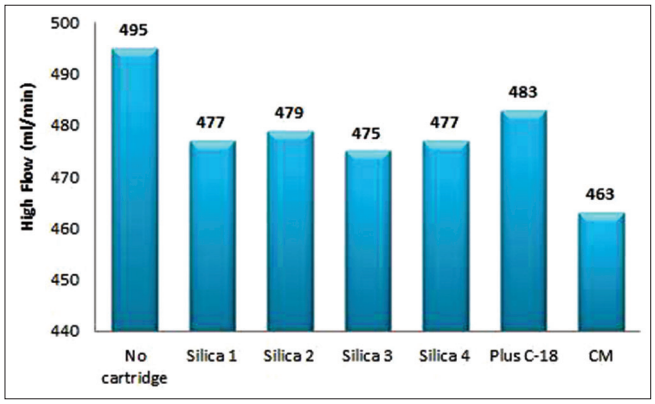

Figure 7: Normal nitrogen flow values of various cartridges before synthesis

Always observed for the color change of the QMA column resin and recovered O-18 water in the recovery bottle next day of synthesis. There will be a color change from pale yellow to brown if the irradiated O-18 water has added impurities of the target body. This will lead to poor retention/release of the F-18 ions by the QMA column.

Software problem

Sometimes, it is witnessed that even after passing all the tests, the synthesis is failed. Later on, it is observed that some valves did not open during the hot run due to which the reagents are not released at required steps. This may occur due to the problem in software. It is always recommended to perform the self-test for the machine at least once in a month to reassure that all the valves, syringe actuators, vacuum pump, and reactor heater are working well.

In addition to these, always ensure the expiry and storage condition requirement of the chemicals and precursor. Keep a track on the graph values for the pressure, temperature, and radioactivity. This will aid in the easy identification of the possible cause in case of failure/reduced yield.

Acknowledgments

The author gratefully acknowledges the support given by Guillaume Villeret for solving the synthesis issues as and when needed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Choline. Human Metabolome Database. The Metabolomics Innovation Centre, University of Alberta, Edmonton, Canada; 17 August, 2016. Available from: http://www.hmdb.ca/metabolites/HMDB0000097. [Last retrieved on 2016 Sep 13].
2. Institute of Medicine: Food and Nutrition Board. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantotheni Acid, Biotin and Choline. Washington, DC: National Academy Press; 1998.
3. Kryza D, Tadino V, Filannino MA, Villeret G, Lemoucheux L. Fully automated [18F]fluorocholine synthesis in the tracerLab MX FDG coincidence synthesizer. Nucl Med Biol 2008;35:255-60.
4. Vali R, Loidl W, Pirich C, Langesteger W, Beheshti M. Imaging of prostate cancer with PET/CT using (18)F-fluorocholine. Am J Nucl Med Mol Imaging 2015;5:96-108.
5. DeGrado TR, Baldwin SW, Wang S, Orr MD, Liao RP, Friedman HS, *et al.* Synthesis and evaluation of (18)F-labeled choline analogs as oncologic PET tracers. J Nucl Med 2001;42:1805-14.