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A simple and rapid automated radiosynthesis of [^{18}F]fluoroacetate

Ganghua Tang,[a,b]* Xiaolan Tang,[c] Mingfang Wang,[a] Baoyuan Li,[a] Mingquan Liang,[a] and Quanshi Wang[a]

Two fully automated synthetic procedures of [^{18}F]fluoroacetate ([^{18}F]FAC) have been developed using a modified commercial TRACERlab FX$_{FN}$ synthesizer. One was a two-step one-pot procedure, consisting of nucleophilic [^{18}F]fluorination of benzyl-2-bromoacetate as a precursor with no-carrier-added [^{18}F]fluoride, hydrolysis within the same [^{18}F]fluorination reaction vessel, and purification with/without high-performance liquid chromatography (HPLC). The second procedure consisted of nucleophilic [^{18}F]fluorination, hydrolysis on the column, and purification with SEP-PAK cartridges instead of HPLC. The radiochemical purity of [^{18}F]FAC was >95% by the two procedures. The second procedure was a simple, rapid, and fully automated synthesis of [^{18}F]FAC with a high and reproducible radiochemical yield exceeding 60% (decay uncorrected) within the total synthesis time less than 20 min. The new, simple, and rapid on-column hydrolysis procedure should be adaptable to the fully automated synthesis of [^{18}F]FAC at a commercial fluoro-deoxyglucose synthesis module.

Keywords: [^{18}F]fluoroacetate; fully automated synthesis; on-column hydrolysis; solid phase purification

Introduction

[^{11}C]Acetate ([^{11}C]AC) has been used as a tracer for positron emission tomography (PET) to study myocardial metabolism and cerebral oxidative metabolism. PET with [^{11}C]AC also has a high sensitivity for detection of prostate cancer and several other cancers that are poorly detected with [^{18}F]-2-fluoro-deoxyglucose ([^{18}F]FDG).[1] However, the potential to widespread use of [^{11}C]AC is limited by the short radioactive half-life (20.4 min) of ^{11}C, which necessitates production with an in-house cyclotron. [^{18}F]Fluoroacetate ([^{18}F]FAC) is an analog of [^{11}C]AC with a longer radioactive half-life (110 min); therefore, it can be a potential tracer for tumor imaging. Recently, [^{18}F]FAC has been reported to be a useful PET tracer for prostate cancer imaging. Compared with [^{11}C]AC PET, [^{18}F]FAC offers the possibility of delayed imaging, with potential to further increase the tumor-to-background ratios.[2]

In the past few years, production of [^{18}F]FAC was reported by several groups.[3–7] However, most of these syntheses needed a long synthesis time (>60 min) and provided a low radiochemical yield (<34%).[3–6] Padgett et al. [7] reported a chemistry process control unit for the automated production of [^{18}F]FAC using commercial benzyl bromoacetate as a precursor with a high corrected radiochemical yield (>60%). However, this two-step two-pot procedure still needed a long synthesis time (>40 min). More recently, a fully automated synthesis of [^{18}F]FAC via on-column hydrolysis has been reported using ethyl O-mesyl glycolate as a precursor and the high corrected radiochemical yield (about 50%) was achieved within a short synthesis time (32 min) without the need for preparative high-performance liquid chromatography (HPLC) using a commercial [^{18}F]FDG synthesizer, the TRACERlab MX$_{FDG}$.[8] Our group previously carried out a fully automated synthesis of [^{18}F]FAC using commercial benzyl bromoacetate as a precursor via a two-step one-pot procedure with a commercial TRACERlab FX$_{FN}$ synthesizer.[9,10] We obtained an uncorrected radiochemical yield of 45% in about 50 min with preparative HPLC purification [9] and got an uncorrected radiochemical yield of >40% within 40 min with SEP-PAK cartridges instead of preparative HPLC purification.[10] However, all of the previously reported syntheses still needed further optimization to achieve radiochemical yield within a shorter synthesis time.

Here, we report two new automated methods to produce [^{18}F]FAC using commercial benzyl bromoacetate as a precursor with TRACERlab FX$_{FN}$ synthesizer. One is a two-step one-pot procedure with the preparative HPLC or SEP PAK purification, the other is a two-step on-column hydrolysis procedure with SEP-PAK purification. The latter procedure can obtain high radiochemical yield within very short synthesis time.

Results

Automated synthesis of [^{18}F]FAC was carried out using the two-step one-pot protocol and the two-step on-column hydrolysis procedure starting from benzyl 2-bromoacetate as a precursor with TRACERlab FX$_{FN}$ synthesizer.

[a]*Nan Fang PET Centre, Department of Nuclear Medicine, Nan Fang Hospital, Southern Medical University, Guangzhou 510515, China*

[b]*Department of Nuclear Medicine, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China*

[c]*Science College, South China Agricultural University, Guangzhou 510642, China*

**Correspondence to: Ganghua Tang, Nanfang PET Centre, Nan Fang Hospital, Southern Medical University, Guangzhou 510515, China.*
E-mail: gtang0224@yahoo.com.cn

The two-step one-pot protocol included nucleophilic fluorination, aqueous basic hydrolysis in the same reaction vessel, and purification with the preparative HPLC or SEP-PAK cartridges in series. The uncorrected radiochemical yield of [^{18}F]FAC by HPLC purification was $>45\%$ ($n=5$) after a synthesis time of about 50 min, while the uncorrected radiochemical yield of [^{18}F]FAC by SEP-PAK purification was 40% ($n=5$) after a synthesis time <30 min. The HPLC purification gave higher uncorrected radiochemical yield than the SEP-PAK purification, but the SEP-PAK purification could reduce the total synthesis time. HPLC purification of crude products showed only two radioactive peaks as free [^{18}F]F$^-$ peak and [^{18}F]FAC peak with a retention time of 8 and 10.2 min, respectively. For HPLC purification, the extent of acetonitrile evaporation also affected the radiochemical yield. Usually, a half of residual acetonitrile (0.5 mL) left after [^{18}F]fluorination would not significantly affect the radiochemical yield. For SEP-PAK purification, complete acetonitrile evaporation after [^{18}F]fluorination would reduce radiochemical yield of about 5–20% compared with the case that left 0.5–1.0 mL of residual acetonitrile after [^{18}F]fluorination.

The on-column hydrolysis procedure included nucleophilic fluorination, basic hydrolysis and purification on four SEP-PAK cartridges in series (an integrated short-column system). A high uncorrected radiochemical yield of $>60\%$ ($n=5$) was obtained within the short total synthesis time of <20 min. Two SEP-PAK C18 cartridges were used to trap and hydrolyze intermediate benzyl 2-[^{18}F]FAC and also used to remove the intermediate and the unreacted precursor. A TSCX cartridge was used to remove K222 and neutralize the basic solution; a SEP-PAK alumina N cartridge was used to remove the unreacted [^{18}F]fluoride.

Purified [^{18}F]FAC solution produced by the two procedures was made to isotonic injection by adding enough saline to vial 14 to achieve a 0.9% concentration. The pH of the final solution resulting from each of the procedures was 6.0–7.0. The identity of the final product was confirmed by comparison of the chromatograms with unlabeled reference materials. The retention time for [^{18}F]FAC, the intermediate benzyl 2-[^{18}F]FAC, and free [^{18}F]fluoride, determined by isocrateic HPLC system with a Hypersil® APS2 column and MeCN/H2O (60/40, v/v) as mobile phase, was 5.0, 2.3, and 8.0 min, respectively. The radiochemical purity of the final product using both one-pot procedure and on-column hydrolysis procedure was over 95%, confirmed by thin layer chromatography (TLC) and HPLC. We did not find any radiochemical or chemical impurities in the final product by analytical HPLC chromatogram and radio-TLC. The average specific activity of the final product ranged from 70 to 140 GBq/μmol. The final product was analyzed with gas chromatography to show that it contained less than 10 ppm acetonitrile. Color spot test for the detection of K222 by TLC also showed no trace of K222 in the final injectable [^{18}F]FAC solution. With high initial radioactivity, [^{18}F]FAC showed good stability, of more than 95% radiochemical purity, at 6 h after synthesis.

Discussion

To perform automated synthesis of [^{18}F]FAC, the most important issues, such as simple and rapid [^{18}F]fluorination, hydrolysis, and purification, must be solved. A comparative study of the two-step one-pot protocol and the on-column hydrolysis protocol was described in this article.

As the TRACERlab FX_{FN} synthesizer was designed for the automated radiosynthesis of ^{18}F-labeled compounds via a one-pot procedure using HPLC purification, some modifications were required for the production of [^{18}F]FAC. For simplification of the delivery of radioactivity from cyclotron, [^{18}F]fluoride was directly delivered to a SEP-PAK QMA cartridge, where [^{18}F]fluoride was trapped and ^{18}O-water was collected for recycling (Figures 1 and 2). The fully automated synthesis of [^{18}F]FAC via the two-step one-pot protocol using HPLC purification is depicted in Figure 1. For the on-column hydrolysis protocol, TRACERlab FX_{FN} synthesizer was substantially modified and adapted to the production of [^{18}F]FAC as shown in Figure 2. For the two-step one-pot protocol using SEP-PAK purification, some necessary changes of chemical reagents arrangement and SEP-PAK cartridges in Figure 2 were required. In addition, a new program file was written to control the automated synthesis of [^{18}F]FAC via the different procedures.

The automated synthesis of [^{18}F]FAC was modified from the TRACERlab FX_{FN} synthesizer via a one-pot procedure and an on-column hydrolysis procedure that consisted of ^{18}F-fluorination of benzyl 2-bromoacetate as a precursor and subsequent hydrolysis of the benzyl-protected intermediate as shown in Scheme 1. Steps for the preparation of [^{18}F]FAC consisted of three categories: fluorination of the precursor, hydrolysis of the

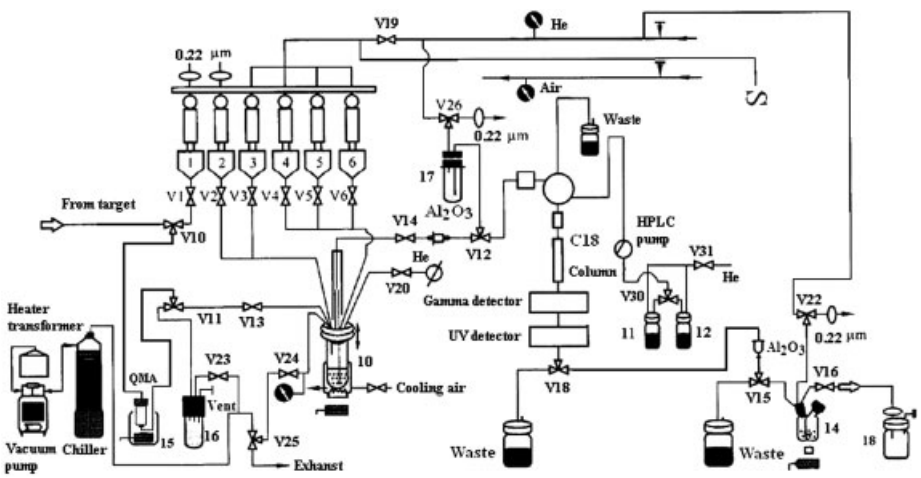


Figure 1. Schematic diagram of the automated synthesis of [^{18}F]FAC using HPLC purification.

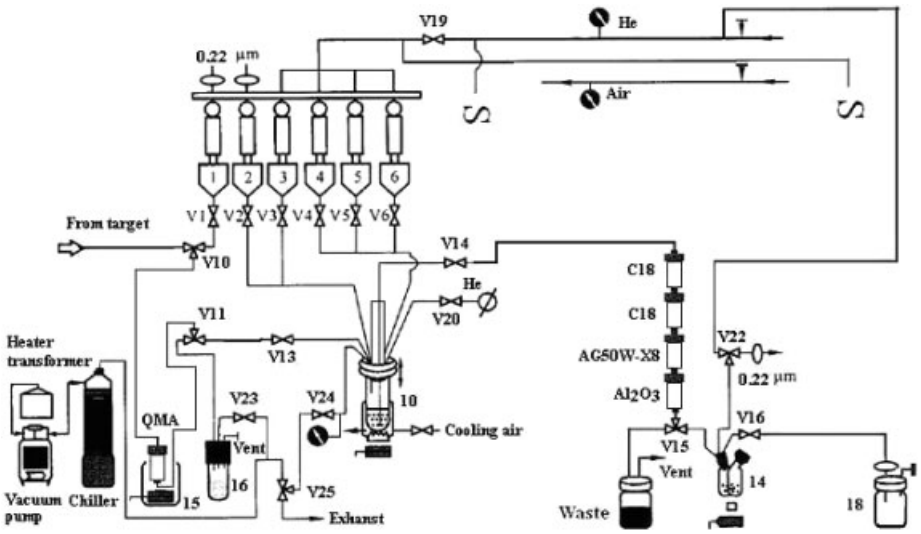
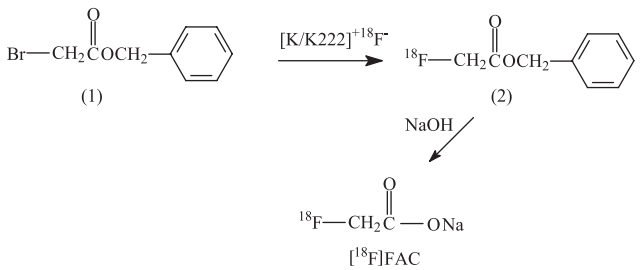


Figure 2. Schematic diagram of the automated synthesis of [^{18}F]FAC using on-column hydrolysis and SEP-PAK purification.

protective group, and neutralization/HPLC purification or SEP-PAK purification. For the one-pot procedure, hydrolysis reaction was carried out in the same [^{18}F]fluorination reaction vessel and purification was performed with preparative HPLC or SEP-PAK purification. For the on-column hydrolysis procedure, hydrolysis reaction was carried out in SEP-PAK plus C18 cartridges and purification was performed with SEK-PAK plus C18 cartridges, TSCX cartridge, and SEP-PAK light Alumina cartridge connected in series. After synthesis, cleaning procedures were carried out in between the runs.



Scheme 1. Synthetic scheme for the automatic synthesis of [^{18}F]FAC with benzyl 2-bromoacetate as a precursor.

Several precursors were reported to the automated production of [^{18}F]FAC.[1,3,7,8] Ethyl O-mesyl glycolate [8] (a synthetic precursor) and benzyl bromoacetate [7] (a commercial precursor) were reported to prepare [^{18}F]FAC in good radiochemical yields. In our work, we chose benzyl 2-bromoacetate as the precursor to produce [^{18}F]FAC using the two protocols described above. [^{18}F]Fluorination reaction of benzyl 2-bromoacetate with [^{18}F]fluoride formed benzyl 2-[^{18}F]FAC very easily with a good labeling yield >90%, using more than 3 mg of benzyl bromoacetate dissolved in 1 mL of anhydrous acetonitrile at 80–90°C for more than 5 min. In our present experiments, 10 mg of benzyl 2-bromoacetate, a reaction temperature of 85°C, and a reaction time of 5 min were chosen for [^{18}F]fluorination with a labeling yield of >95% measured by analytic HPLC ([^{18}F]fluoride adsorption on their column was not taken into account).

Most researchers and our previous work reported that hydrolysis reaction usually took place at high temperature.[3,9,10] However, hydrolysis reaction on the column at low temperature was also reported with ethyl O-mesyl glycolate as the precursor in the recent published papers.[1,8] In the present work, we found that the intermediate benzyl 2-[^{18}F]FAC in 2–3 M NaOH solution was also completely hydrolyzed at room temperature within 2 min. In addition, hydrolysis on the short column (SEP-PAK C18 cartridge) with 2–3 M NaOH solution proceeded almost quantitatively at room temperature within 2 min. For the one-pot procedure, complete acetonitrile evaporation before hydrolysis resulted in the loss of much radioactivity and radiochemical yield. For the on-column hydrolysis, the trapping efficiency was about 90% by using one SEP-PAK C18 cartridge. While two SEP-PAK C18 cartridges were used, the trapping efficiency improved to almost 100%. In the present experiments, 2 M NaOH solution, a reaction temperature at room temperature, and a reaction time of 2 min were chosen for the hydrolysis with a yield of almost 100%.

Purification was a key factor for automated radiosynthesis of [^{18}F]FAC. For the one-pot procedure, HPLC purification gave a good radiochemical yield, but there were limitations such as being time consuming and having difficulty in the automated synthesis, while SEP-PAK purification was easy for the automated synthesis of [^{18}F]FAC with a short synthesis time, but complete acetonitrile evaporation before hydrolysis significantly reduced radiochemical yield. For the on-column hydrolysis, basic hydrolysis and purification on an integrated short-column system consisting of four SEP-PAK cartridges in series greatly improved uncorrected radiochemical yield (>60%) and significantly reduced the total synthesis time (<20 min).

In our previous work, the fully automated synthesis of [^{18}F]FDG using the on-column hydrolysis procedure in TRACERlab FX_{FN} synthesizer was carried out in a high uncorrected radiochemical yield (>60%) within a short total synthesis time (<20 min),[11] which should be adaptable to the fully automated synthesis of [^{18}F]FAC at a commercial FDG synthesis module, such as TRACERlab MX_{FDG} synthesizer, because the synthetic steps to produce [^{18}F]FAC were similar to those used to prepare

Table 1. Automated synthesis of [^{18}F]FAC via different approaches

Method	Synthesis time (min)	Radiochemical yield (%)	Radiochemical purity (%)	Reference
One-pot—HPLC purification	50	>45 (Uncorrected)	>99	This work and Tang *et al.*[9]
One-pot procedure—SEP-PAK purification	<30	40 (Uncorrected)	>95	This work and Tang *et al.*[10]
Two-pot procedure	42	66 (Corrected)	>99	Padgett *et al.*[7]
On-column hydrolysis	<20	>60 (Uncorrected)	>95	This work
On-column hydrolysis	32	50 (Corrected)	>99	Sun *et al.*[8]

[^{18}F]FDG. For automated synthesis of [^{18}F]FAC, our on-column hydrolysis procedure had some advantages over the hydrolysis method on four Oasis HLB plus cartridges reported by Sun et al.[8] For example, we could obtain a higher radiochemical yield within a shorter total synthesis time; Toxic matter K222 could be completely removed by using TSCX cartridge. In addition, hydrolysis and neutralization were carried out on an integrated short-column system, which could further simplify the automated procedure of [^{18}F]FAC using TRACERlab MX_{FDG} synthesizer. A comparison of automated synthesis of [^{18}F]FAC using several methods is shown in Table 1.

Experimental

Reagents and apparatus

Benzyl 2-bromoacetate and 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222, K222) were obtained from Sigma-Aldrich. SEP-PAK light QMA cartridge, SEP-PAK plus C18 cartridge, and SEP-PAK light Alumina cartridge were obtained from Waters (Milford, MA). TSCX cartridge was prepared by opening SEP-PAK C18 cartridge (Waters) and filling them with AG 50W-X8 (H^+ form, Bio-Rad). The cartridges were washed with 10 mL of sterile water before use. All reagents were used without further purification. Radioactivity was determined using a calibrated ion chamber (Capintec CRC-15R). TRACERlab FX_{FN} synthesizer was purchased from GE Medical System (GEMS). HPLC for [^{18}F]FAC purification was carried out in the TRACERlab FX_{FN} synthesizer built-in HPLC system with a semi-preparative reverse-phase C18 column (10 mm × 250 mm, Waters) and C18 precolumn equipped with a UV detector and a radioactivity detector. For the quality control, HPLC analysis was carried out on a modular HPLC system with a Hypersil® APS2 column (4.6 mm × 250 mm, ThermoQuest Company, UK), consisting of two LC-10ATvp pumps (Shimadzu Corporation of Japan) and a variable wavelength SPD-10ATvp UV detector (Shimadzu Corporation of Japan), a LB 508 Radioflow detector with a two-channel analyzer (EG &G, Germany) and a computer (Japan). The UV signal was monitored by a UV Lambda Max detector at 220 nm.

Automated synthesis of [^{18}F]FAC via a one-pot procedure

[^{18}F]fluoride was obtained through the nuclear reaction ^{18}O(p, n)^{18}F by irradiation of a 95% ^{18}O-enriched water target with a 16.5 MeV proton beam at the PETtrace cyclotron (GEMS). Before delivery of [^{18}F]fluoride to the synthesizer, vial 1 was filled with a mixture of 15 mg of K222, 3 mg of K2CO3, 1 mL of acetonitrile, and 0.5 mL of water, vial 3 was filled with benzyl 2-bromoacetate dissolved in 1 mL of acetonitrile, vial 4 was filled with 0.8 mL of 3 M HCl solution or >10 mL of water, vial 5 was filled with 1 mL of 3 M NaOH solution, and vial 6 was filled with 1 mL of HPLC eluent (5% EtOH) or 10 mL of water. For isotonic adjustment, a calculated amount of NaCl was added to vial 14. The radioactivity was collected on a SEP-PAK QMA cartridge, where [^{18}F]fluoride was trapped and ^{18}O-water was collected for recycling. A volume of 1.5 mL of a solution of K_2CO_3 and K222 from vial 1 was eluted through the SEP-PAK QMA cartridge, in which the trapped $^{18}F^-$ was eluted into the reaction vessel. The solvent was evaporated under a stream of helium at 85°C. After complete removal of the solvent, the precursor in vial 3 was added to the reaction vessel containing the dried [K/K222] $^{+18}F^-$ complex and the vessel was heated for 5 min at 85°C. Then, the reaction mixture was cooled, concentrated, and the resulting reaction mixture was added with 3 M NaOH from vial 5. The mixture was hydrolyzed to remove the benzyl-protected group at room temperature for 2 min. After cooling the mixture was neutralized with 3 M HCl from vial 4 and passed through a SEP-PAK alumina cartridge. The eluate was collected in a glass vial. Before HPLC purification, 1 mL of HPLC eluent from vial 6 was added to the reaction vessel and the solution was then passed through the same SEP-PAK alumina cartridge. The eluate was collected in the same glass vial. Finally, [^{18}F]FAC was purified by HPLC system consisting of a pump, an automatic sample injector, a reverse-phase C18 column (250 × 10 mm), a UV absorption detector (220 nm) and radiodetector. The mobile phase used was H_2O/C_2H_5OH (95/5, v/v) at a flow rate of 8 mL/min. The peak corresponding to [^{18}F]FAC was collected and passed through 0.22 μm sterile filter into a sterile vial to obtain the final formulation. The radiochemical yield was expressed as the amount of radioactivity in the [^{18}F]FAC fraction divided by the total ^{18}F-radioactivity.

For SEP-PAK purification, the fluorination reaction mixture was evaporated to dryness and cooled. Then removal of the protective group was achieved by hydrolysis with 1 mL of 3 M NaOH solution (vial 5) and kept at room temperature for 2 min. The mixture was neutralized with 0.8 mL of 1 M HCl (vial 4) and passed through a SEP-PAK plus C18 cartridge, a TSCX cartridge, and a SEP-PAK light alumina N cartridge in series. The SEP-PAK cartridges were washed with 10 mL of sterile water (vial 6). The eluates were combined and further passed through 0.22 μm sterile filter into a sterile vial to obtain the final [^{18}F]FAC.

Automated synthesis of [^{18}F]FAC via on-column hydrolysis procedure

The [^{18}F]fluorination reaction steps were carried out according to the one-pot procedure described above. After fluorination,

the reaction mixture was concentrated, cooled, diluted with >10 mL of water (vial 4), and passed through two SEP-PAK plus C18 cartridges, a TSCX cartridge, and a SEP-PAK light alumina N cartridge in series. The SEP-PAK cartridges were dried under a flow of helium and the eluate was collected in a waste vial. Removal of the protective group trapped in the first two Sep-Pak plus C18 cartridges was achieved by hydrolysis on the cartridges with 0.9 mL 2 M NaOH (vial 5) at room temperature for 2 min. The SEP-PAK cartridges were washed with 10 mL of sterile water (vial 6) and the eluate was collected in sterile vessel 14. Finally, the solution in vessel 14 containing solid NaCl was further passed through 0.22 μm sterile filter into a sterile vial 18 to obtain the final [^{18}F]FAC (pH ~ 7.0) under a flow of helium.

Analysis of [^{18}F]FAC

The aforementioned analytical HPLC system with a Hypersil® APS2 column (4.6 mm × 250 mm, ThermoQuest Company, UK) was used for the checking of radiochemical purity and specific activity eluted with MeCN/H_2O (60/40, v/v) at a flow rate of 1 mL/min, and a radio-TLC developed with 90% MeCN as the solvent system was also used for the checking of radiochemical purity. K222 detection test was performed on the silica gel 60 coated plate developed with methanol/ammonium hydroxide (90/10, v/v) as the solvent system, and iodine vapor was used for staining the spots to render them visible.[12]

Conclusion

The automated synthesis of [^{18}F]FAC has been successfully accomplished via the two-step one-pot procedure and the on-column hydrolysis procedure. The on-column hydrolysis procedure is a very simple and rapid automated radiosynthesis of [^{18}F]FAC. Using the on-column hydrolysis procedure in TRACERlab FX_{FN} synthesizer, the final product can be obtained in <20 min after the end of bombardment with a radiochemical yield of >60% (decay uncorrected). Radiochemical purity of [^{18}F]FAC is >95%. The on-column hydrolysis procedure can be adaptable to the fully automated synthesis of [^{18}F]FAC in a commercial FDG synthesis module, such as TRACERlab MX_{FDG} synthesizer.

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