Fully automated preparation of $[{^{11}}C]$choline and $[{^{18}}F]$fluoromethylcholine using TracerLab synthesis modules and facilitated quality control using analytical HPLC

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Abstract

Modifications of a GE TracerLab FXC-Pro, which can be implemented for solid-phase $[{^{11}}C]$methylation are described. The simplified procedure for synthesis of $[{^{11}}C]$choline uses a single Sep-Pak CM-Light cation-exchange cartridge for both solid-supported reaction and purification. Compared with the commonly used two Sep-Pak method, the low back-pressure of this Sep-Pak enables efficient and reliable production of $[{^{11}}C]$choline using a TracerLab FXC-Pro without requirement for any gas pressure adjustment. Typical radiochemical yields (RCY) are > 60%, radiochemical purity (RCP) is 99.9% and levels of residual precursor in the final product, which may inhibit the uptake of $[{^{11}}C]$choline, are reduced to 1 μg/mL. Similarly, modification of a GE TracerLab FXC-Pro is reported which enables gas-phase production of $[{^{18}}F]$fluoromethylcholine, suitable for pre-clinical use, (in 4–6% RCY and > 99.7% RCP using a related Sep-Pak method. These modifications can be utilized for solid-phase $[{^{11}}C]$methylation and $[{^{18}}F]$fluoromethylation of other radiotracers, and allow straightforward switching to other module configurations for solution-phase radiochemistry or loop chemistry. In addition, we report a convenient HPLC ion chromatography method, which can monitor residual precursor and the radiochemical purity of product at the same time, providing highly efficient quality control for routine clinical application. The reported HPLC method is appropriate for analysis of doses of both $[{^{11}}C]$choline and $[{^{18}}F]$fluoromethylcholine, and eliminates the need for a GC method to determine residual precursor levels.

1. Introduction

The increasing availability of clinical PET-CT scanners has revolutionized patient diagnosis and therapy follow-up, and has led to a significant increase in the number of positron emission tomography (PET) imaging studies performed in the past few years. To support this increase, highly efficient and reliable methods for preparation and quality control of both carbon-11 and fluorine-18 labeled radiopharmaceuticals are in demand.

$[{^{11}}C]$Choline (Fig. 1) has gained increasing interest as a PET radiopharmaceutical for tumor imaging, where it has been shown to be particularly effective for imaging tumors localized in the brain, lungs, esophagus, rectum, prostate and urinary bladder (see, for example, Hara, 2002). In contrast to $[{^{18}}F]$fluorodeoxyglucose (FDG), the uptake of $[{^{11}}C]$choline in normal brain, heart and urinary tract is negligible, resulting in a higher target to background signal ratio for tumors located near these benign structures (Hara et al., 1997). To support the increasing demand for $[{^{11}}C]$choline, several different synthetic approaches have been described in the literature, including different automated production methods using remote-controlled synthesis modules (Hara and Yuasa, 1999, Pascali et al., 2000; Reischl et al., 2004; Quincoces et al., 2006; Cheung and Ho, 2009). The most popular method uses a C18 Sep-Pak as solid support for methylation and, subsequently, a CM Sep-Pak for purification. We have produced $[{^{11}}C]$choline manually using this method at the University of Michigan. However, when adapting this procedure for automation using a commercially available General Electric (GE) TracerLab FXC-Pro, the back-pressure of two adjacent Sep-Pak cartridges significantly slowed down liquid transfers during the synthesis. This increased synthesis time resulted in lower yields and specific activity of the final dose due to the short half-life of carbon-11 (20 min). To address this problem, herein we report an optimized method for producing $[{^{11}}C]$choline using only one CM Sep-Pak for both reaction and purification. The low back-pressure of this system enables efficient and reliable production of $[{^{11}}C]$choline using a TracerLab FXC-Pro without requirement of any gas pressure adjustment. The modifications to the module are straightforward, and can be utilized for solid-phase $[{^{11}}C]$methylene of other radiotracers whilst also allowing for easy switching between different module configurations for other types of radiochemistry. Furthermore, elimination of the C18 Sep-Pak minimizes residual precursor
(N,N-dimethylaminoethanol, DMAE) in doses of $^{11}$C]choline, which is important as DMAE may compete with the uptake of $^{11}$C]choline into the cell and reduce image quality in the resulting PET scans.

Whilst $^{11}$C]choline has proven to be an effective PET biomarker in the clinic, as demand for PET tracers at hospitals not possessing a cyclotron continues to grow, there is a concerted effort towards developing the corresponding fluorine-18 labeled analogs of high demand carbon-11 labeled radiopharmaceuticals. The favorable half-life of fluorine-18 (110 min), when compared with that of carbon-11 (20 min), facilitates distribution of radiopharmaceuticals from radiochemistry production facilities to satellite PET centers that do not own a cyclotron. With this issue in mind, DeGrado and co-workers reported a synthesis of $^{18}$F]fluoromethylcholine (2, Fig. 2) from the reactive intermediate, $^{18}$F]fluorobromomethane, in 2001 (DeGrado et al., 2001a,b).

Subsequently, $^{18}$F]fluoromethylcholine has also been prepared from $^{18}$F]fluoromethyl triflate (Iwata et al., 2002), and DeGrado’s method has also been adapted for SPE purification, allowing automated production of $^{18}$F]fluoromethylcholine with a TracerLab MXFDG (Kryza et al., 2008). Since the demand for $^{18}$F]fluoromethylcholine from our PET center has increased heavily of late (at present for pre-clinical evaluation only), we decided to adapt Kryza’s method for use on a TracerLab FXFN, and herein we also present the first results from these research efforts.

Finally, a convenient ion chromatography HPLC method using a conductivity detector has been developed, based upon the literature methods (Mishani et al., 2002), and is now used routinely by our QC department for routine analysis of clinical doses of $^{11}$C]choline. This HPLC method can quantify amounts of residual DMAE precursor, cold mass of unlabeled choline, and the radiochemical purity of product simultaneously, providing highly efficient quality control for routine clinical application. This HPLC method is appropriate for analysis of doses of both $^{11}$C]choline and $^{18}$F]fluoromethylcholine, and eliminates the need for a separate GC method for quantifying DMAE.

2. Materials and methods

2.1. Materials

Chemicals and solvents were purchased from Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Fair Lawn, NJ) and used without further purification. Unlabeled fluoromethylcholine reference standard was purchased from ABX Advanced Biochemicals (Radeberg, Germany). HPLC column: cation-exchange IC-PAK 150 x 3.9 mm (Waters). For preparation of $^{11}$C]choline, Sep-Pak Light Accell Plus CM cation-exchange cartridges were purchased from Waters and conditioned with ethanol (10 mL) and water (10 mL) prior to use. For preparation of $^{18}$F]fluoromethyl choline, Sep-Pak C18 plus cartridges were purchased from Waters and conditioned with ethanol (10 mL) and water (10 mL) prior to use. QMA-light Sep-Pak cartridges were purchased from Waters and conditioned with aq. potassium carbonate (10 mL) and water (10 mL) prior to use. Sep-Pak Light Accell Plus CM cation-exchange cartridges were purchased from Waters and conditioned with 0.5 M HCl (10 mL) and ethanol (10 mL) prior to use. silica Sep-Pak cartridges were purchased from Waters and used, as received, without any pre-conditioning. Note: we believe that using the silica Sep-Pak cartridges without conditioning is important as, in unreported early runs where silica Sep-Paks were conditioned with water, residual moisture appeared to quench the

![Fig. 1. Structure of $^{11}$C]choline and $^{18}$F]fluoromethylcholine.](image1)

![Fig. 2. Modified TracerLab FXC-Pro enabling preparation of $^{11}$C]choline.](image2)
[18F]fluorobromomethane and no [18F]fluoromethylcholine was generated.

2.2. Modification of the TracerLab FXC-Pro synthesis module

The modified configurations for the TracerLab FXC-Pro are shown in Figs. 2 and 3. The three-way valve V30 is borrowed from HPLC unit and inserted between the round-bottomed flask and V11. This valve (V30) is configured normally open to V17, which allows [11C]methyl iodide going to Sep-Pak through V11 for reaction. Activating this valve diverts water from the round-bottomed dilution flask to the Sep-Pak for washing.


[11C]Choline was synthesized by [11C]methylation of N,N-dimethylaminoethanol (DMAE) with [11C]methyl iodide. 40 µL of DMAE was dissolved into 20 µL of ethanol and loaded onto a CM Sep-Pak. The Sep-Pak was then installed on the synthesis module between V11 and V12. [11C]Methyl iodide was produced, based on gas-phase synthetic method, using the normal functions of the GE TracerLab FXC-Pro. Carbon-11 was produced, as [11C]CO2, via the 14N(p,α)11C nuclear reaction using a GEMS PETTrace cyclotron. [11C]CO2 was delivered to the TracerLab FXC-Pro where it was initially reduced (Ni, H2 (g), 350 °C) to [11C]CH4. [11C]CH4 was then reacted with iodine (720 °C furness) to provide [11C]methyl iodide, which was subsequently passed through the CM Sep-Pak at 20 mL/min flow rate for 3 min. The Sep-Pak was washed with 5 mL of ethanol (from reservoir above V4) and 20 mL of water (from round bottom flask). The product was eluted with 0.5 mL of USP saline (from reservoir above V5), followed by 9.5 mL of USP water (from reservoir above V6) into the product vial. The final dose was then transferred into a sterile dose vial through a 0.22 µm sterile filter and submitted for quality control testing.

2.4. Modification of the TracerLab FXCN synthesis module

In order to fully automate Kryza’s synthesis of [18F]fluoromethylcholine in our laboratory (Kryza et al., 2008), simple modifications were made to a GEMS TracerLab FXCN as shown in Fig. 4. Luer lock fittings were incorporated into the line that connects valve-18 to the round-bottomed dilution flask. These fittings offer a straightforward means of connecting the line out from the reactor (through valve-14) directly to the C18 and CM SepPak’s attached to valve-17. Such ease of switching between module configurations is essential as, with the approval of 21 CFR 212 in December 2009 by the U. S. Food and Drug Administration (FDA), the field moves towards a more regulated current good manufacturing practice (cGMP) type of environment.

2.5. Synthesis of [18F]fluoromethylcholine

[18F]fluoromethylcholine was prepared by adapting the procedure reported by Kryza (Kryza et al., 2008) for use with a TracerLab FXCN. The TracerLab was configured as shown in Fig. 4 and the reagent vials were loaded as follows: Vial 1: potassium carbonate (3.0 mg in 0.4 mL water); Vial 2: kryptofix-2.2.2 (15 mg in 1 mL MeCN); Vial 3: dibromomethane (300 µL in 0.7 mL MeCN); Vial 7: sterile water for injection, USP (15 mL); Vial 8: 0.9% sodium chloride for injection, USP (3.0 mL); Vial 9: ethanol (10.0 mL); C18 Sep-Pak (pre-charged with 400 µL of N,N-dimethylamino ethanol) stacked upon a CM cartridge in the module C18 cradle.

Fluoride-18 was produced via the 18O(p,n)18F nuclear reaction using a GEMS PETTrace cyclotron equipped with a high yield fluorine-18 target. Fluoride-18 was delivered from the cyclotron (in a 2 mL bolus of [18O]H2O) and trapped on a QMA-light Sep-Pak to remove [18O]H2O. Fluoride-18 was then eluted into the reaction vessel using aqueous potassium carbonate (3.0 mg in 0.4 mL water). A solution of kryptofix-2.2.2 (15 mg in 1 mL of acetonitrile)
was then added to the reaction vessel and the fluoride-18 was dried by evaporating the water–acetonitrile azeotrope. Evaporation of the azeotrope was achieved by heating the reaction vessel to 80 °C and drawing full vacuum for 4 min. After this time, the reaction vessel was cooled to 60 °C and subjected to both an argon stream and vacuum draw simultaneously for another 4 min.

Following drying of the fluoride, dibromomethane was added to the reaction vessel and the reaction was heated to 95 °C for 5 min. After this time, the reactor was cooled (40 °C) and [18F]fluoromethylmethane was distilled over in an argon stream and trapped on the C18 cartridge where it concomitantly reacted with N,N-dimethylamino ethanol to generate [18F]fluoromethylcholine. After 10 mins of distillation/reaction, the C18 Sep-Pak/CM-plus light cartridges were washed with ethanol (10 mL). The C18/CM light cartridges were then washed with sterile water for injection, USP (15 mL) and finally, [18F]fluoromethylcholine was eluted from the CM-Plus Sep-Pak light into the collection flask with sterile saline (3 mL). The dose then passed through a sterile filter into a sterile dose vial and submitted for quality control testing according to Section 2.6.

2.6. Quality control

Quality control of radiopharmaceuticals prepared at the University of Michigan is carried out, in accordance with the U.S. Pharmacopoeia, as described below

2.6.1. Visual inspection ([11C]choline and [18F]fluoromethylcholine)

Doses were examined visually and had to be clear, colorless and free of particulate matter.

2.6.2. Dose pH ([11C]choline and [18F]fluoromethylcholine)

The pH of the doses was analyzed by applying a small amount of the dose to colorPhast® pH 2.0–9.0 non-bleeding pH-indicator strips and determined by visual comparison with the scale provided.

2.6.3. Radionuclidic identity ([11C]choline and [18F]fluoromethylcholine)

Radionuclidic identity was confirmed by determining the half-life of the dose and comparing it with the known half-life of carbon-11 (20 min) or fluorine-18 (110 min). Activities were measured using a Capintec CRC®-15 R Radioisotope Dose Calibrator and half-life was calculated using Eq. (1).

\[ T_{1/2} = -\ln(2) \times \frac{\text{Time Difference}}{\ln(\text{ending activity} / \text{starting activity})} \]

2.6.4. Bacterial endotoxin content ([11C]choline and [18F]fluoromethylcholine)

Endotoxin content in doses was analyzed using a Charles River Laboratories EndoSafe® Portable Testing System and according to the U. S. Pharmacopeia. Bacterial endotoxin limits are ≤175 Endotoxin Units (EU)/dose.

2.6.5. Sterility testing (clinical doses of [11C]choline only)

Culture tubes of fluid thioglycolate media (FTM) and soybean casein digest agar media (SCDM) were inoculated with samples of doses and incubated (along with positive and negative controls) for 14 days. FTM was used to test for anaerobes, aerobes and microaerophiles whilst SCDM was used to test for non-fastidious and fastidious microorganisms. Culture tubes were visually inspected on the 3rd, 8th and 14th days of the test period and compared with the positive and negative standards. Positive standards had to show growth (turbidity) on the plates and dose samples/negative controls had to have no culture growth after 14 days to be indicative of sterility.

2.6.6. Filter integrity test ([11C]choline and [18F]fluoromethylcholine)

The sterile filter from the dose (with needle still attached) was connected to a nitrogen supply via a regulator. The needle was submerged in water and the nitrogen pressure was gradually increased. If the pressure was raised above the filter acceptance pressure (50 psi) without seeing a stream of bubbles, the filter was considered intact. If a stream of bubble occurred < 50 psi, the test failed.
2.6.7. HPLC analysis ([11C]choline and [18F]fluoromethylcholine)

The radiochemical purity (RCP) and concentration of choline (or fluoromethylcholine) and DMAE in each batch were determined using a Shimadzu HPLC system with the following components: SCL-10Avp system controller, DGU-14A in-line degassing unit, LC-10ADvp pump, CDD-10Avp conductivity detector with temperature controlled cell, CTO-20A oven and Bioscan FC3300 flow count radioactivity detector. The system was not equipped with an ion suppressor. Chromatographic separation was performed on a Waters SCX column (IC-Pak™ Cation M/D, 3.9 × 150 mm, pn: WAT036570) with a 5 mM HCl (Fisher Scientific) mobile phase and a flow rate of 1.0 mL/min for [11C]Choline analysis (RT = 7.4 min, RCP = 99.9%) (Mishani et al., 2002) and 1.25 mL/min for [18F]fluoromethylcholine analysis (RT = 6.5 min, RCP ≥ 99.7%).

The linearity, precision, specificity, limits of quantitation and limits of detection of DMAE, choline and fluoromethylcholine for the above HPLC system were determined by serial dilution of concentrated aqueous standard solutions. The resulting detector response was plotted against known concentration and is presented, for DMAE, choline and fluoromethylcholine in the support information. Aqueous solutions of dimethylaminoethanol (Sigma-Aldrich) ranging from 0.2 to 100 mg/mL, choline chloride (Sigma-Aldrich) ranging from 0.2 to 100 µg/mL, choline chloride (Sigma-Aldrich) ranging from 1 to 100 µg/mL and fluoromethylcholine (ABX Chemicals) ranging from 1 to 100 µg/mL were prepared using volumetric glassware and analysed within 24 h.

2.6.8. GC analysis ([11C]choline and [18F]fluoromethylcholine)

Gas chromatography (GC) can also be used to determine the residual concentration of DMAE in doses of [11C]choline and [18F]fluoromethylcholine. The GC system used for comparison herein was a Shimadzu GC-2010 with an AOC-20 autoinjector, split/splitless inlet, a flame ionization detector (FID) and a Stabilwax column (Stabilwax 30 m × 0.25 mm, 0.25 µm G16 stationary phase). Chromatographic conditions are outlined below

<table>
<thead>
<tr>
<th>Injection volume: 0.25 µL</th>
<th>Split ratio: 1:15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injector temp.: 180 °C</td>
<td>Linear velocity: 51.4 cm/sec</td>
</tr>
<tr>
<td>Detector temp.: 250 °C</td>
<td>Oven temp.: 145 °C (isothermal)</td>
</tr>
</tbody>
</table>

2.6.9. Residual kryptofix-2.2.2 (doses of [18F]fluoromethylcholine only)

Residual kryptofix-[2.2.2] levels in [18F]fluoromethylcholine doses were analyzed using the established spot test (Mock et al., 1997; Scott and Kilbourn, 2007). Strips of plastic-backed silica gel TLC plates saturated with iodoplatinate reagent were spotted with water (negative control), 50 µg/mL kryptofix-2.2.2 standard (positive control) and [18F]fluoromethylcholine dose. If kryptofix-2.2.2 is present in a sample, a blue–black spot appears. Spots for the three samples were compared and a visual determination of residual kryptofix-2.2.2 in the dose was made. < 50 µg/mL is acceptable and all doses of fluorocholine prepared in this study were found to contain residual kryptofix-2.2.2 below this level.

3. Results and discussion

3.1. Radiosynthesis of [11C]choline

[11C]Choline has been successfully synthesized in short time, high radiochemical yield and purity, using modified GE TracerLab FXC-Pro. The modification was easy and can be utilized for other solid-phase radiosyntheses such as [11C]methionine. The cleaning and preparation procedures have been fully automated by modifying the time lists of the GE TracerLab FXC-Pro, facilitating reproducible and reliable production of doses for routine clinical application.

A series of experiments were done to optimize the reaction conditions and the results are summarized in Table 1. The flow rate of [18F]methyl iodide passing through Sep-Pak was fixed at 20 mL/min for all the experiments, based on results from previous testing with various flow rates.

Compared with the reported two Sep-Pak (C18/CM) system, the one Sep-Pak (CM only) method outlined herein reduced the total synthesis time by 10 min, which is significant for reactions using carbon-11. The decay corrected yields using the CM only method were slightly lower than their corresponding counterparts. However, the yield of radiotracer is the function of radiochemical yield and time. Thus, the final dose produced using the CM only method was still higher than that produced using the two Sep-Pak system. Furthermore, the high back-pressure of two adjacent Sep-Pak cartridges not only slowed down the washing process, but also increased the chances of leaks, and broken lines and fittings. The one cartridge method has proven more reliable for routine production.

Obviously, larger volume of precursor would lead to higher radiochemical yields, but also higher residue of DMAE. As DMAE may inhibit the incorporation of [11C]choline into the cell membranes, it is important to minimize the DMAE residue in the final product. According to HPLC and GC analysis results shown in Table 1, 40 µL of DMAE was found to be optimal, since it allowed us to achieve high yield and still keep residue of precursor relatively low. Practically, DMAE was diluted with 20 µL of ethanol to have a reasonable total volume for easily loading on the Sep-Pak.

It should be noted that elimination of the C18 Sep-Pak significantly minimized the residual precursor in doses of [11C]choline. Using the CM only method, the final product contained much lower amounts (less than half) of DMAE based upon HPLC and GC analysis. Even with 60 µL of precursor, the residual DMAE content in the final product was only 7.62 µg/mL, lower than 9.18 µg/mL from 20 µL precursor and the two Sep-Pak method. Different kinds of C18 Sep-Pak including tC18 and C18 packed with different amount of material were also investigated (data not reported).

### Table 1

<table>
<thead>
<tr>
<th>Sep-Pak</th>
<th>DMAE used (µL)</th>
<th>Synthesis time (min)</th>
<th>DMAE residue (µg/mL)</th>
<th>CHL (µg/mL)</th>
<th>RCP (%)</th>
<th>n⁴</th>
<th>RCP (%)</th>
</tr>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18+CM</td>
<td>20</td>
<td>30</td>
<td>4.89</td>
<td>9.18</td>
<td>&lt;1</td>
<td>21.2</td>
<td>99.9</td>
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<td></td>
<td>40</td>
<td>30</td>
<td>16.2</td>
<td>13.6</td>
<td>&lt;1</td>
<td>70</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>30</td>
<td>10.9</td>
<td>17.3</td>
<td>&lt;1</td>
<td>90.0</td>
<td>99.9</td>
</tr>
<tr>
<td>CM only</td>
<td>20</td>
<td>20</td>
<td>2.75</td>
<td>5.1</td>
<td>&lt;1</td>
<td>20.7</td>
<td>&gt;10</td>
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<td></td>
<td>40</td>
<td>20</td>
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<td>5.2</td>
<td>&lt;1</td>
<td>63.1</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20</td>
<td>2.76</td>
<td>7.62</td>
<td>&lt;1</td>
<td>77.4</td>
<td>99.9</td>
</tr>
</tbody>
</table>

* Values reported in Table 1 are mean values for all runs.
All two Sep-Pak systems retained more precursor and resulted in higher residual DMAE in the final products. The CM only method proved to be preferable for routine clinical production of \(^{11}C\)choline.

### 3.2. Radiosynthesis of \(^{18}F\)fluoromethylcholine

The TracerLab FXFN was configured as illustrated in Fig. 4 and described in Section 2.4. The front end of the TracerLab FXFN was configured as for a typical radiofluorination reaction, and the line from the reactor needle out through valve-14 (normally connected to an alumina cartridge) was connected to 3 \(\times\) silica Sep-Pak cartridges (connected in series). The back silica cartridge was then connected (by luer fitting) to V17. DMAE was loaded onto a Sep-Pak (CM-plus only or a combination of C18 and CM-plus) and then this was placed in the TracerLab FXFN C18 cradle. A range of experiments were carried out to optimize production of \(^{18}F\)fluoromethylcholine using a TracerLab FXFN and are summarized below and in Table 2. Clearly the reduced reactivity of \(^{18}F\)fluorobromomethane compared with \(^{11}C\)methyl iodide is reflected through the lower radiochemical yields obtained in the fluorine-18 labeling reactions.

In our initial efforts, fluoride-18 was delivered from the cyclotron and then trapped on a QMA-light Sep-Pak to remove \(^{18}O\)H\(_2\)O. Fluoride-18 was then eluted into the reaction vessel using q. potassium carbonate. A solution of kryptofix-2.2.2 in acetonitrile was also added to the reactor and the fluoride-18 was dried by evaporating the water-acetonitrile azeotrope. Following drying, dibromomethane precursor was added to the reactor and the reaction was heated to 95 °C for 5 min. After this time, the reactor was cooled (40 °C), and the \(^{18}F\)fluorobromomethane was distilled through 3 \(\times\) silica Sep-Paks, C18 Sep-Pak and, finally, the CM Sep-Pak to waste. The argon supply to the TracerLab FXFN synthesis module is fixed at 40 psi and this was used to transfer the crude reaction mixture. 3 \(\times\) silica Sep-Paks removed any unreacted dibromomethane (b.p. = 97 °C) and acetonitrile (b.p. = 82 °C) that, despite their higher boiling points, may otherwise have distilled over. Non-volatile components, including kryptofix-2.2.2 and potassium carbonate, remained in the reaction vessel. In contrast, the volatility of \(^{18}F\)fluorobromomethane (b.p. = 9 °C) allowed pseudo-distillation over to valve-17 where it was trapped on the C18 cartridge and concomitantly reacted with DMAE (400 μL) to generate \(^{18}F\)fluoromethylcholine. After 10 min of distillation, the C18 Sep-Pak cartridge was washed with ethanol to elute the \(^{18}F\)fluoromethylcholine as well as unreacted \(^{18}F\)fluorobromomethane and DMAE. As \(^{18}F\)fluoromethylcholine is positively charged, it was trapped on the CM-Plus cation-exchange resin, whilst the uncharged precursor species were washed to waste. The CM was then washed with Sterile Water for Injection, USP to remove any residual solvents and by-products. Finally, \(^{18}F\)fluoromethylcholine was eluted from the CM-Plus Sep-Pak into the collection flask with sterile saline (3 mL). The dose was passed through a sterile filter into a sterile dose vial and released for quality control testing (see Table 2 and Section 2.6). Typical decay corrected yields of \(^{18}F\)fluoromethylcholine using this optimized production method were 4–6%, similar to yields obtained using a TracerLab MXFDG (Kryza et al., 2008), pH = 5.5 and radiochemical purity was > 99.7% \((n = 7)\). Following production, doses underwent quality control testing as outlined in Section 2.6. Doses were free of residual solvents, kryptofix-2.2.2 and dibromomethane, but were found to contain residual DMAE.

Whilst the radiochemical yields were lower than hoped for using this synthesis method, doses produced were acceptable to meet our present pre-clinical demand (\(\sim 60–90\) mCi of \(^{18}F\)fluoromethylcholine is obtained from a 1500 mCi target dump). Nevertheless, we explored the synthesis further to try and elucidate the causes of inefficient labeling and DMAE contamination (Table 2). Analyzing Sep-Paks after the synthesis revealed that significant radioactivity (presumably corresponding to \(^{18}F\)fluorobromomethane) remained trapped on the silica Sep-Paks. Therefore we repeated the synthesis using only 1 silica Sep-Pak (Table 2, Entry 2), but this did not improve the yield of \(^{18}F\)fluoromethylcholine. Moreover, the dose contained higher levels of impurities (e.g. dibromomethane) when the number of silica Sep-Paks was reduced. Therefore, 3 silica Sep-Paks appear optimum for preparing a pure product and 4–6% is the maximum radiochemical yield obtained to date using the Sep-Pak method. It was considered that \(^{18}F\)fluorobromomethane may not be reactive enough to do the chemistry on Sep-Pak. However, attempts to bubble \(^{18}F\)fluorobromomethane through a solution of DMAE (400 μL of DMAE in 700 μL MeCN) in a vial (Table 2, Entry 5) proved less efficient and provided \(^{18}F\)fluoromethylcholine in less than 0.5% radiochemical yield. Therefore it was decided to remain with the Sep-Pak method and investigate residual DMAE levels.

Typical doses of \(^{18}F\)fluoromethylcholine prepared using the method outlined above (Table 2, Entry 1) were found to contain 100–1000 μg/mL of DMAE. This result was high, although not all together unexpected given similar results reported by others (e.g. Kryza et al., 2008). Moreover, whilst DMAE could also interfere with \(^{18}F\)fluoromethylcholine in vivo, presently there is no specific activity release criteria defined (particularly for pre-clinical studies). However, a number of attempts were made to try and reduce residual DMAE levels. A trial run was conducted using a Waters C18-light Sep-Pak in place of a regular C18 Sep-Pak (Table 2, Entry 3) but, somewhat surprisingly, this had a negative impact on the synthesis and provided < 0.5% radiochemical yield of \(^{18}F\)fluoromethylcholine. Similarly, when we eliminated the C18 Sep-Pak from the \(^{18}F\)fluoromethylcholine synthesis altogether (Table 2, Entry 4), and attempted to replicate the highly efficient CM only conditions outlined above for production of \(^{11}C\)choline, a similar marked decline in radiochemical yield was observed. At this stage those conditions outlined in Table 2, Entry 1, represent our most efficient conditions for preparing \(^{18}F\)fluoromethylcholine using a TracerLab FXFN.

### 3.3. Quality control

Detector Linearity, precision and limits of detection (LOD) were established for choline, dimethylaminooethanol and fluoromethylcholine. Limits of detection were defined as the lowest concentrations that resulted in signal-to-noise ratios of not less than 2:1. Detector linearity and precision were verified using the data generated when determining the LOD. Precision at a given concentration level was deemed suitable if the relative standard deviation of at least three replicate injections at that concentration was less than 15%. The detector linearity was deemed suitable over a given concentration range if the trendline resulting from a linear regression analysis had a coefficient of determination \( (R^2) \) value > 0.99 (see supporting information).

It was determined herein that the limits of detection for choline, DMAE and fluoromethylcholine are 1, 0.2 and 1 μg/mL, respectively. The precision of the method was found to be suitable over a

<table>
<thead>
<tr>
<th>Sep-Pak</th>
<th>DMAE used (μL)</th>
<th>Si Cartridges</th>
<th>RCY (%), EOS</th>
<th>n</th>
<th>RCP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 C18+CM</td>
<td>400</td>
<td>x 3</td>
<td>4–6</td>
<td>7</td>
<td>&gt; 99.7%</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>x 1</td>
<td>4%</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>3 C18-light+CM</td>
<td>400</td>
<td>x 3</td>
<td>&lt; 0.5%</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>4 CM only</td>
<td>40</td>
<td>x 3</td>
<td>&lt; 0.5%</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>5 Vial</td>
<td>400</td>
<td>x 3</td>
<td>&lt; 0.5%</td>
<td>1</td>
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</tr>
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range of 1–100 μg/mL for dimethylaminoethanol, 2–100 μg/mL for choline and 2–100 μg/mL for fluoromethylcholine. The detector had a linear response to choline, dimethylaminoethanol and fluoromethylcholine over ranges of 1–100 μg/mL, 0.2–100 μg/mL and 1–100 μg/mL, respectively.

The results obtained from the IC HPLC system (see supporting information) were comparable with those obtained by GC analysis, and actually more reliable at lower concentrations. This was due to interference, from ethanol and saline, that caused variation when samples of lower concentration were run on the GC. This indicated that analysis by GC can be replaced with the multifunctional IC HPLC analysis disclosed herein. The IC HPLC analysis has been demonstrated to be suitably linear, repeatable and sensitive for the analysis of \[^{11}\text{C}\]choline, and \[^{18}\text{F}\]fluoromethylcholine within functional ranges that are routinely encountered in the preparation of radiopharmaceuticals.

4. Conclusion

Simple modifications to a GE TracerLab FXC-Pro enabled fast, automated and reliable production of \[^{11}\text{C}\]choline suitable for clinical use. The optimized method using only one CM Sep-Pak with 40 μL of precursor produced high yields of product with very low residual DMAE. Similarly, modifications to a GE TracerLab FXC allows automated production of \[^{18}\text{F}\]fluoromethylcholine suitable for a range of pre-clinical applications. Moreover, quality control analysis of both \[^{11}\text{C}\]choline and \[^{18}\text{F}\]fluoromethylcholine can be streamlined by utilizing a single ion chromatography HPLC system, with electrical conductivity detection, for the analysis and quantitation of all critical components of the formulated dose. This HPLC method eliminates the need to purchase and maintain expensive IC instrumentation and also eliminates the need for analysis of the same dose using multiple analytical systems (GC and HPLC).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.apradiso.2010.09.022.

References


