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Published in:
Journal of Labelled Compounds and Radiopharmaceuticals

DOI:
10.1002/jlcr.3139

Publication date:
2014

Document version:
Submitted manuscript

Citation for published version (APA):

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Download date: 20. Mar. 2024
Good manufacturing practice production of the system A amino acid transport tracer [\textsuperscript{11}C] MeAIB on a commercial synthesis module

Johan Hygum Dam and Kjell Någren* 

In this report, an automated \textsuperscript{11}C-N-methyl-\textalpha-amino-isobutyric acid production on a Tracerlab FXC \textsc{Pro} module is described. Bioburden to the sterile filter was minimized during solid-phase extraction formulation of the HPLC purified product by the use of sterile aqueous solutions. The optimized solid-phase extraction formulation conditions are fast, give a high recovery of the product, and effectively remove organic solvents and adjust the pH. The final product was obtained within 30–35 min, delivering 1.5–5 GBq in 10 mL sterile phosphate buffer. The product fulfills in every respect our stringent batch release specifications including residual solvents, bioburden, and sterility. The robustness of the automated \textsuperscript{11}C-N-methyl-\textalpha-amino-isobutyric acid production has been demonstrated during more than 40 productions for preclinical use without any failures in regard to product amounts or release specifications.

Keywords: \textsuperscript{[11]}CMeAIB; GMP production; automated synthesis; SPE formulation

Introduction

The clinical value of positron emission tomography (PET) using labeled amino acids for oncologic applications is well established. The first successful tracer for this purpose was \textsuperscript{[11]}Cmethionine, which has found wide applications in brain tumor imaging and many other oncologic applications. A number of \textsuperscript{18}F-labeled amino acid analogs such as \textsuperscript{[18]}FET, \textsuperscript{[18]}F6-F-DOPA, and \textsuperscript{[18]}FACBC have also shown their clinical values.

Carbon-11 labeled tracers have several advantages for clinical PET such as a lower radiation dose to the patient and the possibility to perform several PET studies per day using different tracers. Although widely used, \textsuperscript{[11]}Cmethionine imaging is complicated by uptake in non-tumor processes such as inflammation and the fast metabolism of the tracer. Ideally, a tracer should have high specificity for a physiologic process such as an amino acid uptake system and a low rate of metabolism to preclude arterial blood sampling. \textsuperscript{11}C-N-methyl-\textalpha-amino-isobutyric acid (\textsuperscript{[11]}CMeAIB) is a tracer that is very selective for the system A amino acid transport and is metabolically stable in vivo and thus fulfill these two criteria. This tracer has a low radiation burden to the patient and can be used to study system A amino acid transport both in healthy organs such as muscles and in various tumors such as head and neck cancer, chest cancer, and glioblastoma multiforma, and prostate cancer.

The published production of \textsuperscript{[11]}CMeAIB was performed on a home-built remote-controlled synthesis system that included evaporation of the HPLC solvent from the purified product using a rotary evaporator. From a good manufacturing practice (GMP) perspective, these production conditions are today regarded as suboptimal and not suitable for radiopharmaceuticals for clinical use. Most commercial carbon-11 synthesis devices are equipped with storage vessels and valves intended to be used for solid-phase extraction (SPE) formulation purposes. This greatly simplifies the cleaning procedures when compared to a rotary evaporator. The purpose of this work was to establish conditions for automated GMP production of \textsuperscript{[11]}CMeAIB using a commercial synthesis module including solid-phase extraction for the formulation of the purified product in order to fulfill stringent batch release specifications including residual organic solvents and bioburden of the product.

Experimental

General

Production of \textsuperscript{[11]}Cmethyl triflate (\textsuperscript{[11]}CMeOTf) on the GE Tracerlab FXC \textsc{Pro} was performed with manufacturer's specified chemicals. The labeling precursor, \textalpha-amino-isobutyric acid methyl ester hydrochloride, was prepared according to Någren et al. and was crystallized from 100% sterile ethanol. Phosphoric acid (0.2 and 1.0 M), disodium hydrogen phosphate (Na\textsubscript{2}HPO\textsubscript{4}, 2%), and hydrochloric acid (0.1 M) were autoclaved in a Systex DX-23 according to European Pharmacopoeia (Ph. Eur.) before use. Sodium hydroxide (NaOH, 2.0 M) was prepared from Ph. Eur. standard materials. MeOH for HPLC, ≥99.9 %; acetonitrile for HPLC, ≥99.9 %; potassium dihydrogen phosphate, KH\textsubscript{2}PO\textsubscript{4}, anhydrous, for HPLC; pentamethylyperidine, ≥97%; and reference MeAIB, ≥98 % were obtained from Sigma-Aldrich. Strata-X-C 12 mL gigatubes (33 μm, 1 g sorbent) were obtained from Phenomenex. These SPE columns were preconditioned with 10 mL ethanol (96%) and 20 mL phosphoric acid (1 M) before use. Pall Acrodisc HT Tuffryn 0.2 μm sterile filters were obtained from WVR International, Denmark.

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The semi-preparative HPLC system consisted of a SYKAM S1021 HPLC pump, a six-way Valco Instruments Co. Inc. injector (C6W) with a 2 mL loop, a Phenomenex Luna NH₂ 250 × 10 mm, 5 μm HPLC column, a Knauer K-2001 UV detector, and a Geiger-Müller tube radioactivity detector. The analytical Hitachi LaChrome Elite HPLC system consisted of an L-2130 HPLC pump, an L-2200 autosampler, an L-2455 diode array detector, and a Bioscan FC-4000 with FC-4100 dual Bismuth Germanium Oxide radiodetector. A Varian 3900 gas chromatograph equipped with a Varian VF-200ms, 30 m × 0.32 mm inner diameter capillary column and a flame ionization detector was used to quantify residual solvents.

Figure 1. Synthesis of [11C]MeAIB.

Figure 2. Semi-preparative HPLC chromatogram from the purification of [11C]MeAIB. Upper: UV detection at 200 nm, lower: radiodetector. (1) [11C]MeAIB and (2) unidentified by-product.

Figure 3. Analytical HPLC chromatograms of the final product with co-injected reference MeAIB. Upper: UV detection at 210 nm, lower: radiodetector. (1) [11C]MeAIB and (1'): MeAIB.
Endotoxin levels were measured with an Endosafe®-portable test system from Charles River. Bioburden and sterility tests were performed by Statens Serum Institut, Copenhagen, Denmark. The pH was measured with a SevenMulti system from Mettler Toledo. Radioactivity was measured using a Capintec CRC®-15 PET dose calibrator.

**Labeling procedure**

The labeling procedure is shown in Figure 1. \(^{[11C]}\)MeOTf was prepared on a Tracerlab FXC Pro module (GE Healthcare) by standard in-line gas-phase conversions from \(^{[11C]}\)CO\(_2\) and was trapped in the reaction mixture cooled to 5°C. This mixture consisted of 1 mg precursor in 300 μL pentamethylpiperidine. After delivery of \(^{[11C]}\) MeOTf, the vial was heated to 60°C for 1 min, at which point, 0.1 mL NaOH (2M) was added. The heating was continued for 3 min, and the reaction mixture was diluted with 0.3 mL HPLC eluent.

**Preparative HPLC**

Following dilution, the reaction mixture was automatically injected onto the semi-preparative HPLC column through the 2 mL loop. The mobile phase consisted of 3:1 acetonitrile:7.5 mM KH\(_2\)PO\(_4\), pH 4.3, and the purification was performed at a flow of 9.5 mL/min and monitored by UV detection at 200 nm and by radiodetection. The purified \(^{[11C]}\)MeAIB fraction was collected using manual computer control by comparison with a master chromatogram.

**Formulation**

The HPLC purified \(^{[11C]}\)MeAIB was subjected to solvent exchange by SPE. The solution was diluted with 20 mL sterile phosphoric acid (0.2 M) and passed through a Strata-X-C column. Following loading of \(^{[11C]}\)MeAIB on the SPE column, it was washed with 15 mL sterile hydrochloric acid (0.1 M) and 3 mL isotonic sterile saline. The \(^{[11C]}\)MeAIB was then eluted from the column with sterile 10 mL Na\(_2\)HPO\(_4\) (2%) through a 0.2 μm sterile filter into a sterile product vial.

**Analytical methods**

Radiochemical purity was analyzed by HPLC on a Phenomenex Bondclone NH\(_2\) (300 × 3.9 mm, 10 μm) HPLC column, at a flow of 2 mL/min and at a UV detection at 210 nm. The elution was performed as a gradient following: A, 7.5 mM KH\(_2\)PO\(_4\) (pH 4.3) and B, CH\(_3\)CN:H\(_2\)O (500:70); 95 to 50% B, 0–8 min. Identification was performed by co-injection with reference MeAIB.

The amount of residual solvents was determined by gas chromatography using an injector temperature of 220°C, a detector temperature of 270°C, and a column temperature of 40°C for 5 min, followed by a 50°C/min increase up to 240°C. Helium gas flow was 2 mL/min. The concentrations of CH\(_3\)CN and ethanol in the product solution were measured by comparison with a reference solution containing 350 ppm CH\(_3\)CN and 1% (w/v) ethanol.

**Results and discussion**

Generally, 10–15 GBq of \(^{[11C]}\)MeOTf was produced on the Tracerlab FXC Pro module. We confirmed the reported high synthesis yield of \(^{[11C]}\)MeAIB from \(^{[11C]}\)MeOTf, which was in the order of 70% decay corrected radiochemical yield. Total synthesis time from end of bombardment to the crude product was 20–21 min.

The Phenomenex Luna NH\(_2\) column proved to be a proper substitute for the Phenomenex Selectosil SAX column, which is no longer commercially available. This robust column has to be thoroughly washed before first use and then withstands the basic injected solution well. \(^{[11C]}\)MeAIB fraction eluted at 5.5–6.0 min and a minor radiochemical impurity, which eluted at 4.5 min, was well separated from the main product (Figure 2). This impurity increased with decreasing specific radioactivity.

More than 99.7% of \(^{[11C]}\)MeAIB was retained by the Strata-X-C column. A wash with 15 mL 0.1M HCl followed by a wash with 3 mL saline resulted in a neutral pH of the product, and an acceptable acetonitrile content. Using the optimized SPE procedure, more than 99% of the applied \(^{[11C]}\)MeAIB was recovered in the eluted product fraction within 4–7 min.

**Table 1. Quality control of \(^{[11C]}\)MeAIB productions**

<table>
<thead>
<tr>
<th>Item</th>
<th>Methodology</th>
<th>Acceptance criteria</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity</td>
<td>Dose calibrator</td>
<td>Clear and colorless</td>
<td>1500–5000 MBq</td>
</tr>
<tr>
<td>Appearance</td>
<td>Visual inspection</td>
<td>≥95%</td>
<td>Clear and colorless ≥99.9%</td>
</tr>
<tr>
<td>Radiochemical purity</td>
<td>HPLC</td>
<td>4.5–8.5</td>
<td>6.5–6.7</td>
</tr>
<tr>
<td>pH</td>
<td>pH meter</td>
<td>19.9–20.9 min</td>
<td>20.1–20.6 min</td>
</tr>
<tr>
<td>γ-ray spectra</td>
<td>γ-spectrometer</td>
<td>511 ± 25 KeV</td>
<td>511–520 KeV</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Gas chromatograph</td>
<td>≤0.7%</td>
<td>0.1–0.5%</td>
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<tr>
<td>Acetonitrile</td>
<td>Gas chromatograph</td>
<td>≤350 ppm</td>
<td>135–347 ppm</td>
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<td>Sterile filter</td>
<td>Bubble point</td>
<td>≥3.1 bar</td>
<td>≥3.1 bar</td>
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<tr>
<td>Endotoxins</td>
<td>LAL</td>
<td>≤17.5 EU/mL</td>
<td>&lt;1.0 EU/mL</td>
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<tr>
<td>Sterility</td>
<td>Culture media (Ph. Eur.)</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Bioburden*</td>
<td>Culture media (Ph. Eur.)</td>
<td>&lt;1 colony forming unit/4 mL</td>
<td>&lt;1 colony forming unit/4.5 mL</td>
</tr>
<tr>
<td>Shelf life</td>
<td>HPLC and visual inspection at 30–60 min</td>
<td>≥95% and clear and colorless</td>
<td>100% and clear and colorless</td>
</tr>
</tbody>
</table>

Tests of sterility, bioburden, and shelf life were performed for three productions, and the other quality control parameters were determined for six productions.

*The productions for test of bioburden were performed without sterile filtration of the product solutions.
The optimized procedure for production of $[^{11}C]$MeAIB gives up to 5 GBq of a clear and colorless solution within 30–35 min from end of bombardment. The specific radioactivity, determined for other $^{11}$C-radiopharmaceuticals produced on our Tracerlab FXC Pro module, has been in the order of 20–70 GBq/μmol. Radiochemical purity of the final product, determined by HPLC, has been higher than 99% (Figure 3), and the radiochemical purity was maintained for 60 min in batches up to 5 GBq. The result of our validation of the $[^{11}C]$MeAIB production is shown in Table 1.

**Conclusions**

The automated $[^{11}C]$MeAIB production has shown to be very reliable. The optimized conditions for SPE formulation of the HPLC purified product are fast, give a high recovery of the product, and effectively remove organic solvents and adjust the pH. More than 40 productions have been performed for in vivo imaging of human glioblastoma in nude rats. There have been no failures in these productions. The production and quality control conditions that comply with cGMP requirements have been validated and accepted by the Danish Health and Medicines Authority. The product fulfills in every respect our stringent batch release specifications including residual solvents, bioburden, and sterility.

**Conflict of Interest**

The authors did not report any conflict of interest.

**References**