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Synthesis of ^{11}C-methionine through gas phase iodination using Synthra MeI$_{Plus}$ synthesis module

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Abstract. A method of ^{11}C-methionine synthesis using 'bubbling' method is presented. ^{11}C-methionine was synthesized via ^{11}C methylation from L-cysteine thiolactone (2 mg) in a 300 μL solution of 2:1:1 (v/v) 1 M NaOH, ethanol, and water at ambient temperature (85°C, 5 min). The radiochemical purity of radiotracer was higher than 99% and enantiomeric purity (L-^{11}C-methionine) was 91.6 ± 0.4%. The final product met the requirements of European Pharmacopoeia monograph. The proposed ^{11}C-methionine synthesis is a reliable tool for routine manufacturing in clinical applications and animal studies.

Key words: ^{11}C-methionine • ^{11}C-radiopharmaceuticals • gas phase iodination

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Introduction

Positron emission tomography (PET) is dynamically developing imaging method of nuclear medicine, which allows to diagnose metabolic changes in the human body. Among the β^+ emitting isotopes, produced in medical cyclotrons, carbon-11 is the most convenient radionuclide for labeling of biologically active compounds and tracking their distribution in living organism. Although ^{18}F with $t_{1/2}$ = 109.8 min is routinely applied as a tracer in commercially available radiopharmaceuticals for neuro- and oncological diagnostics, ^{11}C ($t_{1/2}$ = 20.4 min) is often chosen for radiolabeling of the endogenous or investigative compounds – neurotransmitters, amino acids, drugs and drug candidates. Replacement of carbon-12 with carbon-11 does not alter the biochemical properties of a molecule. So a labeled compound is incorporated in metabolic processes and follows the same path as its natural equivalent. Moreover, using the position-specific methylation approach, imaging of metabolites, and metabolic paths of selected functional groups expands the biological information on molecular level.

^{11}C-methionine increased its clinical significance when the methionine uptake was identified as one of the critical factors in tumor growth [1, 2] and started to be a standard method for visualization of gliomas in neurooncology [3–5] as well as a supplementary in adenocarcinoma [6] or radiation therapy planning [7].

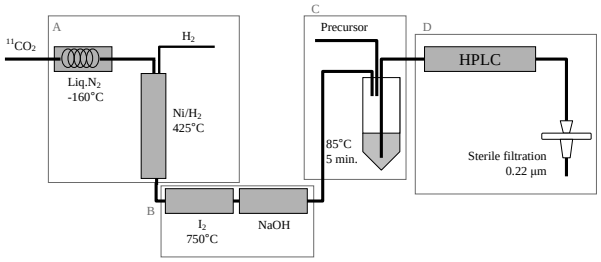

Fig. 1. General layout of Synthra MeI_{Plus} radiosynthesis module. A – liq. N_2 trap and $^{11}CO_2$ to $^{11}CH_4$ conversion unit; B – iodination to $^{11}CH_3I$; C – labeling with ^{11}C methyl iodide; D – HPLC purification and final formulation.

This paper describes a simple and low-cost method for routine production of ^{11}C-methionine. The scope of this study was to synthesize ^{11}C-methionine in a number of consecutive runs and to prove the quality of manufactured radiopharmaceuticals, as a preliminary step in human applications and animal studies.

Materials and methods

^{11}C production

$^{11}CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ reaction with 16.5 MeV proton beam at 20–25 μA in GE Pettrace 840 cyclotron (General Electric, Uppsala, Sweden). The target was gaseous N_2 (6.0), containing 5% O_2 (5.5) in high pressure (170 psi) target body (GE, Uppsala, Sweden). ^{11}C was produced with a total activity of ca. 1000 mCi (37 GBq) after 10–15 min irradiation with 25–30 μA beam current. Then gaseous products were transferred via stainless steel tubing to synthesizer Synthra MeI_{Plus} (Synthra, Germany) (Fig. 1), located in MIP-1100 hotcell (Comecer, Italy), 75 mm shielded, with air compressing system (ACS), collecting radioactive gaseous exhausts from all reaction steps to shield the pressure tanks.

^{11}C-methionine synthesis

^{11}C-methionine was synthesized via ^{11}C methylation from L-cysteine thiolactone (ABX, Radeberg, Germany) in solution using the 'bubbling' method [8]. Target gases were passed through cryogenic trap, where $^{11}CO_2$ was deposited at –165°C. Then $^{11}CO_2$ was converted to methane (H_2/Ni, 400°C) and ^{11}C-methyliodide was synthesized by passing the ^{11}C-CH_4 over iodine in a triplicate loop at an elevated temperature (720°C) and trapping on a Porapak column (dry method). Thermally desorbed $^{11}CH_3I$ was bubbled into the reactor with L-homocysteine thiolactone (2 mg) in a 300 μL solution of 2:1:1 (v/v) 1 M NaOH, ethanol, and water at ambient temperature (85°C, 5 min). The product was then purified by semipreparative HPLC (C18 column, 0.05 M NaH_2PO_4 + 2% EtOH as mobile phase) with a total wet-synthesis time of 20 min (Fig. 2).

The final product was formulated with phosphate buffer, passed through a 0.22 μm filter and dispensed in automatic module DDS-Vials (Tema-Synergie, Italy).

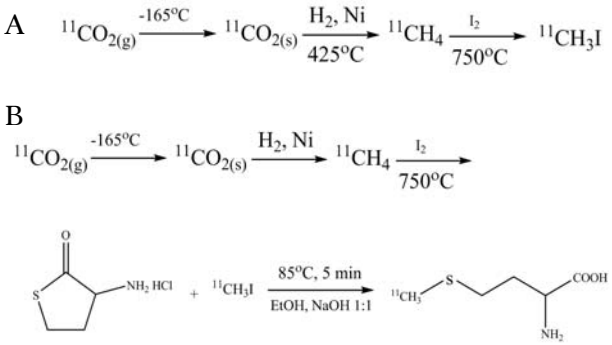

Fig. 2. Synthesis of ^{11}C methionine. (A) Methyl iodide synthesis (dry method). (B) Methylation of L-homocysteine thiolactone hydrochloride.

Identification and quality control

Identification tests were performed as described in [9]. For γ-spectrometry, high-resolution germanium detector GMX-20190-P with digital signal processor (DSPEC, Ortec) and GammaVison software was used. A 2 μL of sample was applied on silica plate, fixed in a holder, and inserted into a 5 cm Pb shielded, low-background housing. Spectra was recorded for 5 min immediately after synthesis.

Half-life was measured with Atomlab300 (Biodex, USA) dose calibrator: 300 μL (0.6–0.8 GBq) sample was crimped in a penicillin vial, fixed in a standard vial holder and measured in triplicate at 5 min intervals.

The identity of the manufactured ^{11}C-methionine was confirmed by comparison of retention time to the certified reference standard (CRS) of the main compound (ABX, Radeberg, Germany).

Radiochemical purity test A was performed with an ion chromatography system Shimadzu AD-20 with a diode array and radiometric (Gabi-Star, Raytest) detectors.

A 10 μL sample was injected via a manual multiport valve. The separation was done on Phenomenex Gemini C18 250 mm × 4.6 mm × 5 μm column with 1.4 g/L solution of potassium dihydrogen phosphate as a mobile phase and 1 mL/min flow rate. Data acquisition and processing were performed with LCsolution® software.

Enantiomeric purity was determined by chiral HPLC, using a Shimadzu AD-20 equipped with a diode array and radiometric detector. A Chirobiotic T column with teicoplanine as chiral selector (150 mm × 4.6 mm × 5 μm, Astec) was used as a stationary phase with acetonitrile:water 70:30 mobile phase at a flow rate 1 ml/min [10].

Measurements of pH were conducted with MulitiSeven pH-meter (Mettler-Toledo, Germany) with microelectrode vs. 4.01, 7.01, and 9.21 buffers (Hamilton, UK).

Head-space gas chromatography was performed on 7890A Agilent system, equipped with a J&W HP-5 column (30 m × 0.32 mm × 0.25 μm) and a flame ionization detector (FID). GC system was supplied with an H_2 (30 mL/min) from CFH200 generator (Peak Scientific, UK), ensuring 99.9995%

of hydrogen purity, zero-air (400 mL/min) from a Jun-Air 0F301-4B generator (Jun-Air, UK), and He (6.0, Air Products, flow rate 25 mL/min). Gas chromatographic system was operated on the following conditions: oven temperature 40°C for 1 min, inlet temperature 150°C, detector temperature 180°C. The samples were injected via 7694E Agilent head-space system. For a comfortable sample application, 5-μL single-use capillaries (Drummond Scientific, USA) were used for transfer of the radioactive sample to 10 mL penicillin vials with aluminum caps and PTFE/Si septa (Agilent). Head-space injector was set to 80°C for 2 min, and then the sample was equilibrated for 0.2 min and transferred to the GC system. The loop and transfer line were heated to 105°C and 110°C, respectively. Chemstation software was used for operation of chromatograph, acquisition, and processing of data.

To ensure the quality of measurements, methods were developed and validated with certified reference standards (CRS) of main compound and impurities.

Results and discussion

Synthesis

^{11}C-methionine was synthesized by conventional 'bubbling' method [8]. $^{11}CH_3I$ was passed through the reactor, containing precursor, L-homocysteine thiolactone in the presence of ethanol and sodium hydroxide. The reaction was followed with preparative HPLC, ensuring good separation of final product from impurities. Radiochemical yield was 21.3 ± 4.6% (not corrected), total synthesis time, including activity transfer, gas phase iodination of CH_4, labeling, purification, and final formulation was 27–32 min. The produced activity was sufficient for the injection of two patients in a standard imaging mode. Recently, synthesis on solid supports has gained some more attention due to simplicity (room temperature, immobilized precursor) and shorter reaction time (elimination of time-consuming preparative HPLC purification). The described methods using on-column precursor deposition [11, 12] or on-loop synthesis [13] showed excellent potential (radiochemical yield >60%, reaction time 11–13 min, radiochemical purity >99%) but relatively short half-life still limited application to two to three patients from a single run.

Identification

^{11}C was identified by recording the principal γ-peak at 511.5 ± 0.3 keV (Fig. 3) and determination of half-life (20.5 ± 0.3 min). ^{11}C-methionine was confirmed, comparing the retention times of standards, observed in the reference chromatogram with retention time of the principal signal in the radiochromatogram (Fig. 4). Recorded retention times were 3.05 min for the standard and 3.07 min for the principal peak in radiochromatogram.

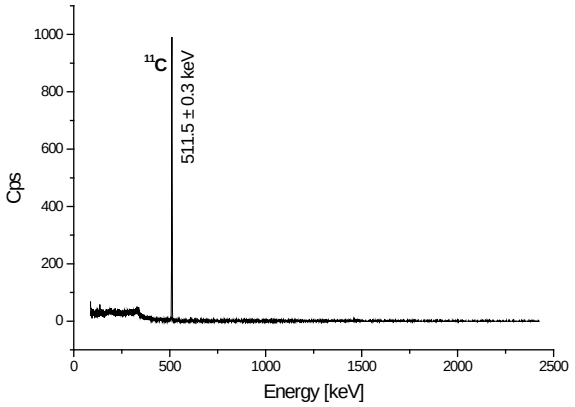

Fig. 3. Identification of ^{11}C. γ Spectra with principal peak at 511 keV.

Radionuclidic purity

Gamma spectrum recorded for identification was used for the determination of radionuclidic purity. The presence of any peaks with an energy different from 511 keV was checked and, except signals coming from Pb-X-rays (range 70–80 keV), none was found. That confirmed a better than 99.9% radionuclidic purity. Then ^{11}C-methionine sample was left for 2 h to decay the carbon and again impurities were tested with no significant peaks, except residual activity of ^{11}C. The abovementioned results complied with radionuclidic purity tests A and B.

Purity

Chemical purity was determined in all produced ^{11}C-methionine samples. L-homocysteine thiolactone hydrochloride (impurity A), ^{11}C-methionine, and homocysteine (impurity B) were identified by a qualitative comparison with the reference solution, containing maximum available concentration of respective CRS as described in [9]. The retention times were 2.51 min for impurity B, 3.05 min for methionine, and 3.72 min for impurity A, which corresponded to resolution factors 1.02 and 1.28 vs. metionine. In all ^{11}C-methionie samples, the area of the corresponding peaks were smaller than obtained

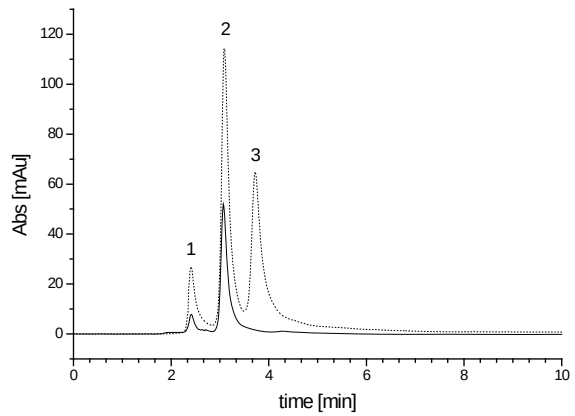

Fig. 4. UV-Chromatogram of reference standards (dot line) and ^{11}C-methionine sample (solid). 1-L-homocysteine (t_r = 2.51 min), 2-methionine (t_r = 3.05 min), 3-L-homocysteine thiolactone hydrochloride (t_r = 3.72 min).

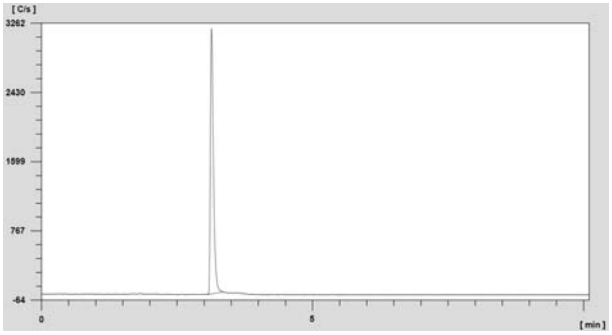

Fig. 5. Radiochromatogram of ^{11}C-methionine (t_r = 3.07 min).

with reference solution and signal from precursor (L-homocysteine thiolactone hydrochloride) was negligible or not even observed (Fig. 4).

Radiochemical purity was determined by high-performance liquid chromatography (HPLC) with radiometric detection, where the peak of ^{11}C-methionine was observed at 3.07 min, with no other signals recorded (Fig. 5). The average content of ^{11}C-methionine was higher than 99%. Six consecutive runs were analyzed in triplicate each.

The synthesis on solid support with SPE separation showed the presence of precursor in the final formulation [14, 15], lower radiochemical purity (98% [15], 97–99% [14]) and additional impurities (iodide, homocystine [16]) but still at the acceptable level. It could be explained with more efficient preparative HPLC purification than SPE, resulting in the separation of reaction mixture to its individual components and controlled collection of ^{11}C-methionine fraction.

Enantiomeric purity was assessed by chiral HPLC. L- and D-isomers were identified by HPLC with UV and radiometric detectors (Fig. 6). The percentage of L-^{11}C-methionine was 91.6 ± 0.4%. Other recently published papers reported values from 89% [14] to pure L-isomer [13]. The commonly used method for enantiomers determination

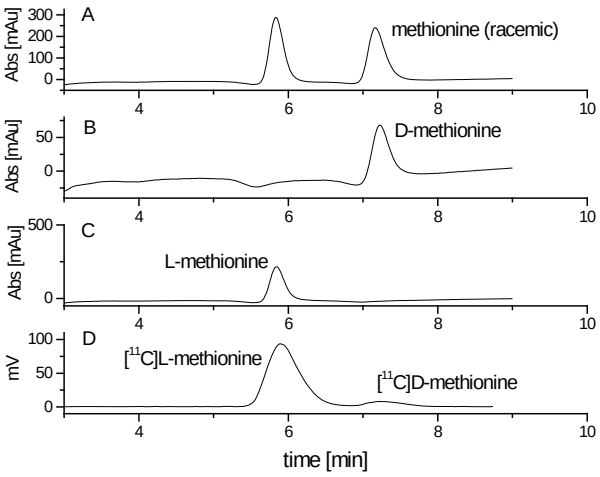

Fig. 6. Standard and sample chromatograms for enantiomeric purity evaluation. A – UV spectra of racemic methionine CRS; B – UV spectra of D-methionine CRS; C – UV spectra of L-methionine CRS; D – radiochromatogram of ^{11}C-methionine sample.

is chiral HPLC, instead of the time-consuming pharmacopoeial TLC method. Two approaches are applied: first with chiral column [13] and the other with C8-18 reverse phase chromatography with L-proline in mobile phase as a chiral selector [14, 17]. Nevertheless, the D-form does not influence tumor uptake [18].

Residual organic solvents

The analysis of methionine samples needs to consider a certain percentage of ethanol. ^{11}C-methione is autodegraded by its own radiation [19] and an important issue is to ensure the stability in routine production due to radiolysis. Thus up to several percent of ethanol may be added as a scavenger and a stabilizer [20]. The produced ^{11}C-methionine formulations were tested and the mean value of the ethanol concentration was 33 ± 2 g/L. No traces of

Table 1. Determined QC parameters in ^{11}C-methionine. Acceptance criteria according to [9]. (IU/V – Endotoxins unit per recommended dose in mL; mg/V – milligrams per recommended dose in mL; * – indicative value)

Determined values in n = 6 consecutive runs		
Parameter	Acceptance criteria	Value
Identification	Principal peak 511 keV	511.5 ± 0.3 keV
	Half-life 19.9–20.9 min	20.5 ± 0.3 min
	CRS methionine, t_r = 3.05 min	^{11}C-methionine, t_r = 3.07 min
pH	4.5–8.5	5.8–6.0
Radiochemical purity	^{11}C-methionine >95%	>99%
	D^{11}C-methionine <10%	8.4 ± 0.4%
	L-homocysteine thiolactone <0.6 mg/V	Pass
	L-homocysteine <2.0 mg/V	Pass
	Methionine <2.0 mg/V	Pass
Residual solvents	Ethanol <5000 mg/kg*	33 000 ± 2200 mg/kg
	Acetone <50 mg/V	<0.1 mg/V
Sterility	Sterile	Pass
Bacterial endotoxin test	<175/V IU/mL	Pass
Radionuclidic purity test A	511 keV >99.9%	Pass

acetone or acetonitrile were detected. Some cited works report the ethanol content on similar levels, but others do not determine it quantitatively. Thus addition of ethanol, recommended concentrations, and control of its level should be officially regulated.

Other tests

pH, sterility, and endotoxin test, as generally known procedures, are summarized in Table 1. All parameters were according to the quality criteria for ^{11}C-methionine.

Conclusions

In this work, the 'bubbling' method of ^{11}C-metionine synthesis as an alternative way to SPE synthesis was presented. The final product met the requirements of European Pharmacopoeia monograph. All the impurities were efficiently determined and then eliminated in the purification process. The final product was free from main radionuclidic and chemical impurities. The proposed ^{11}C-methionine synthesis is a reliable tool for routine manufacturing in clinical applications and animal studies.

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