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New method for routine production of L-[methyl-^{11}C]methionine: *in loop* synthesis

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A fast, clean and reproducible method for the manufacture of the radiotracer L-[methyl-^{11}C]methionine is reported. The reaction at room temperature of the non-radioactive precursor L-homocysteine (1 mg solution in ethanol/water 50/50) with [^{11}C]CH_3I in an HPLC loop led to the formation of the desired radiotracer with a high radiochemical yield (38.4 ± 4.1% end of synthesis) in a short production time (12 min). Radiochemical purity of the final radiotracer was 99.9 ± 0.05%. Specific activities in the range 11–45 GBq/μmol were obtained. The presence of the undesired enantiomer (D-[methyl-^{11}C]methionine) was not detected in any of the cases.

Keywords: methylation; loop; methionine

Introduction

The majority of positron emission tomography (PET) studies are oncological and use 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG), but the uptake of this radiotracer is not specific to malignant tissue and indeed uptake may depend on factors other than glycolysis alone.[1] Moreover, high FDG accumulation has been observed recently not only in viable cancer cells but also in inflammatory cells and granulation tissue.[2] Because of this lack of specificity of FDG, other metabolic tracers, such as positron emitter-labeled amino acids, are being widely used as indicators of tumor activity. L-[methyl-^{11}C]methionine, one of the most widely used amino acids, is transported by the LAT1 amino acid transporter. It undergoes complex metabolism and is incorporated into proteins; therefore, increased uptake may reflect the metabolic needs of brain tumors.[2]

Since L-[methyl-^{11}C]methionine plays such an important role for PET, an efficient preparation method for this radiotracer allowing frequent syntheses for PET routine is desirable. Because of this, different synthesis methods have been published for the routine production of L-[methyl-^{11}C]methionine starting from [^{11}C]methyl iodide.[3–10] Before Pascali *et al.*[8] introduced the possibility to synthesize L-[methyl-^{11}C]methionine in a C18 solid-phase extraction cartridge, synthesis procedures for the manufacture of this radiotracer usually comprised of trapping [^{11}C]methyl iodide in a solution containing the precursor and an adequate base (usually sodium hydroxide) followed by reaction at high temperature. Semi-preparative HPLC was needed to obtain pure radiotracer. The new approach by Pascali's group was a great advance in L-[methyl-^{11}C]methionine chemistry. More recently, Mitterhauser *et al.*[9] have developed a fully automated system for the production of L-[methyl-^{11}C]methionine (also based on solid-phase extraction cartridge chemistry) without semi-preparative HPLC, obtaining average yields of 21.22 ± 7.9% (not corrected for decay) with respect to [^{11}C]CO_x. Finally, Quincoces *et al.* introduced in 2006 an automated synthesis for simultaneous production of carbon-11-labeled tracers (including L-[methyl-^{11}C]methionine) by solid support methylation.

In the present work, we present a simple, fast and clean procedure for the synthesis of L-[methyl-^{11}C]methionine as an alternative to the SPE cartridge synthesis, by applying the captive solvent method.[11] In this process, without using any additional solid support and without cooling or heating, [^{11}C]methyl iodide is trapped directly into an HPLC loop coated with the precursor solution. After the reaction occurs, the mixture is purified using a C18 SPE cartridge and re-formulated with the help of a self-made automated module.

Results and discussion

Twenty syntheses were performed starting from 38.55 ± 1.11 GBq [^{11}C]CO_2 (end of bombardment, EOB). At the end of synthesis (EOS), 14.8 ± 1.6 GBq of pure L-[methyl-^{11}C]-methionine was obtained, representing a radiochemical yield (non-corrected for decay) of 38.4 ± 4.1%, with average production time of 12 min (corrected yield 57.8 ± 6.2%). According to previous results,[11] [^{11}C]methyl iodide trapping and reaction in an HPLC loop coated with the precursor solution is a suitable procedure for the synthesis of carbon-11-labeled radiotracers by using dimethly formamide and dimethyl sulfoxide. However, it

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has been shown in the present work that solvents with much lower boiling point and that are less able to dissolve methyl iodide (ethanol/water) can also be used successfully. This fact is surprising taking into account that the first step in the synthesis routine is the conditioning of the loop with continuous nitrogen flow (15 mL/min, $t = 10$ s) and that the loop is always open to the atmosphere during [^{11}C]methyl iodide production ($t = 5$ min from EOB). An important advantage of using the 'loop' method is that the implementation of a very simple cleaning procedure in the reaction loop (flushing with water for 150 s at a flow rate of 20 mL/min, flushing with acetone for 150 s at a flow rate of 20 mL/min and drying with nitrogen for 300 s at a flow rate of 20 mL/min) allows multiple consecutive productions with minimum intervention from the operator.

Quality control performed on the 20 above-mentioned productions showed average radiochemical purity of $99.9 \pm 0.05\%$ (HPLC, method 1). Only one undesired radioactive peak (retention time = 1.2 min) was detected. The presence of L-homocysteine was also determined (HPLC, method 1), obtaining average values of 51 ± 24 μg/mL. As residual solvent, only ethanol was found in average concentrations of 1360 ± 270 mg/L. This amount of ethanol found in the final solution is surprising because when the precursor solution is loaded into the loop (80 μL of 50/50 water/ethanol solution), it contains nearly 32 mg of pure ethanol. At the end of the synthesis, only around 10 mg remains in the solution, and no evaporation step is needed because the limit dose, according to *European Pharmacopoeia* (3rd Edition), is 50 mg per day and person.

Specific activity was calculated from L-methionine concentration obtained by HPLC (method 1). An average value of 28.9 ± 12 GBq/μmol (range = 11–45 GBq/μmol) was obtained at the EOS.

Enantiomeric purity of the final solution was determined by chiral HPLC (method 2). Obtained chromatograms presented very good resolution with relatively short analysis time. In Figure 1, sample chromatograms obtained under method 2 conditions corresponding to (a) L-methionine (reference standard, concentration = 0.1 mg/ml, UV detection, $\lambda = 220$ nm), (b) DL-methionine (reference standard, concentration = 0.1 mg/ml, UV detection, $\lambda = 220$ nm) and (c) sample (final product obtained for the synthesis of L-[methyl-^{11}C]methionine, isotopic detection) are shown. Only the peak corresponding to the L-[methyl-^{11}C]methionine isomer is obtained in the final product. The presence of D-[methyl-^{11}C]methionine was not detected in any of the samples.

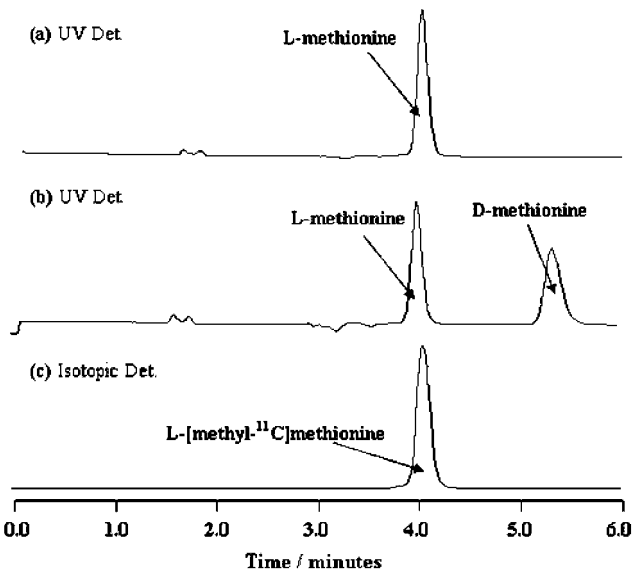

Figure 1. Sample chromatograms obtained under method 2 conditions corresponding to (a) L-methionine (reference standard, concentration = 0.1 mg/ml, UV detection, $\lambda = 220$ nm), (b) DL-methionine (reference standard, concentration = 0.1 mg/ml, UV detection, $\lambda = 220$ nm) and (c) sample (final product obtained for the synthesis of L-[methyl-^{11}C]methionine, isotopic detection).

Thermal stability of the final product at 121°C was explored with the aim of determining the option of autoclaving the sample. The final product was shown to be unstable (radiochemical purity around 85% after 20 min). Thus, the possibility of autoclaving the radiotracer must be discarded and aseptic fractioning with sterilizing filtration must be used before dose release.

Experimental

General

L-homocysteine, lithium aluminum hydride (0.1 M solution in dry THF), hydriodic acid (57% aqueous solution), sodium hydroxide (Ph. Eur. grade), soda lime (ACS reagent, granular, +100 mesh), ethanol (absolute, Ph. Eur. grade), water (sterile, injectable), injectable physiologic solution and sodium phosphate monobasic (anhydrous, USP grade) were commercially available and used as obtained. Solid-phase exchange cartridges (Sep-Pak® Plus Short, Waters, Spain) were conditioned with ethanol (10 mL) and water (10 mL) before use.

[^{11}C]CH_3I production

[^{11}C]CO_2 was produced in an IBA Cyclone 18/9 cyclotron via the ^{14}N(p,α)^{11}C nuclear reaction and was transformed into [^{11}C]CH_3I using the Mel-Plus™ Methyl Iodide Production System (Bioscan Inc., Washington, USA). Briefly, [^{11}C]CO_2 was first trapped in a molecular sieve column at room temperature. After quantitative trapping, [^{11}C]CO_2 was released by heating under continuous nitrogen flow ($T = 250$°C, flow = 15 mL/min, $t = 120$ s) and bubbled into lithium aluminum hydride (0.1 M solution in dry THF, 250 μL). After complete trapping, solvent was evaporated to dryness and hydriodic acid (57% aqueous solution, 250 μL) was added. [^{11}C]CH_3I was distilled with heating under continuous nitrogen flow ($T = 115$°C, flow = 15 mL/min, $t = 120$ s).

L-[methyl-^{11}C]methionine synthesis

A solution of the non-radioactive precursor (1 mg of L-homocysteine dissolved in 80 μL of sodium hydroxide 0.5 M solution in ethanol/water 50/50) was charged in a 2 mL stainless HPLC loop and N_2 was circulated as pre-conditioning (flow = 15 mL/min, $t = 10$ s). After complete trapping of [^{11}C]CH_3I in the loop, the reaction was carried out for 1 min at room temperature. The reaction crude was pushed with the help of an HPLC pump (Agilent 1100 series) with physiologic saline solution (7.5 mL, flow = 5 mL/min, elution time = 1.5 min) into a 10 mL vial (vial 1 in Figure 2).

L-[methyl-^{11}C]methionine formulation

Figure 2 shows a schematic diagram of the automated system for the L-[methyl-^{11}C]methionine formulation. The module consisted of a nitrogen supply, seven 2-way valves (225K012, Neptune Research Inc., USA), two 10-mL sterile-sealed vials (DIN ISO 20 mm H1/10, Comecer, Italy) and one 2-mL sterile-sealed conic vial (Micro Reaction Vessel, Teknokroma, Spain). All valves

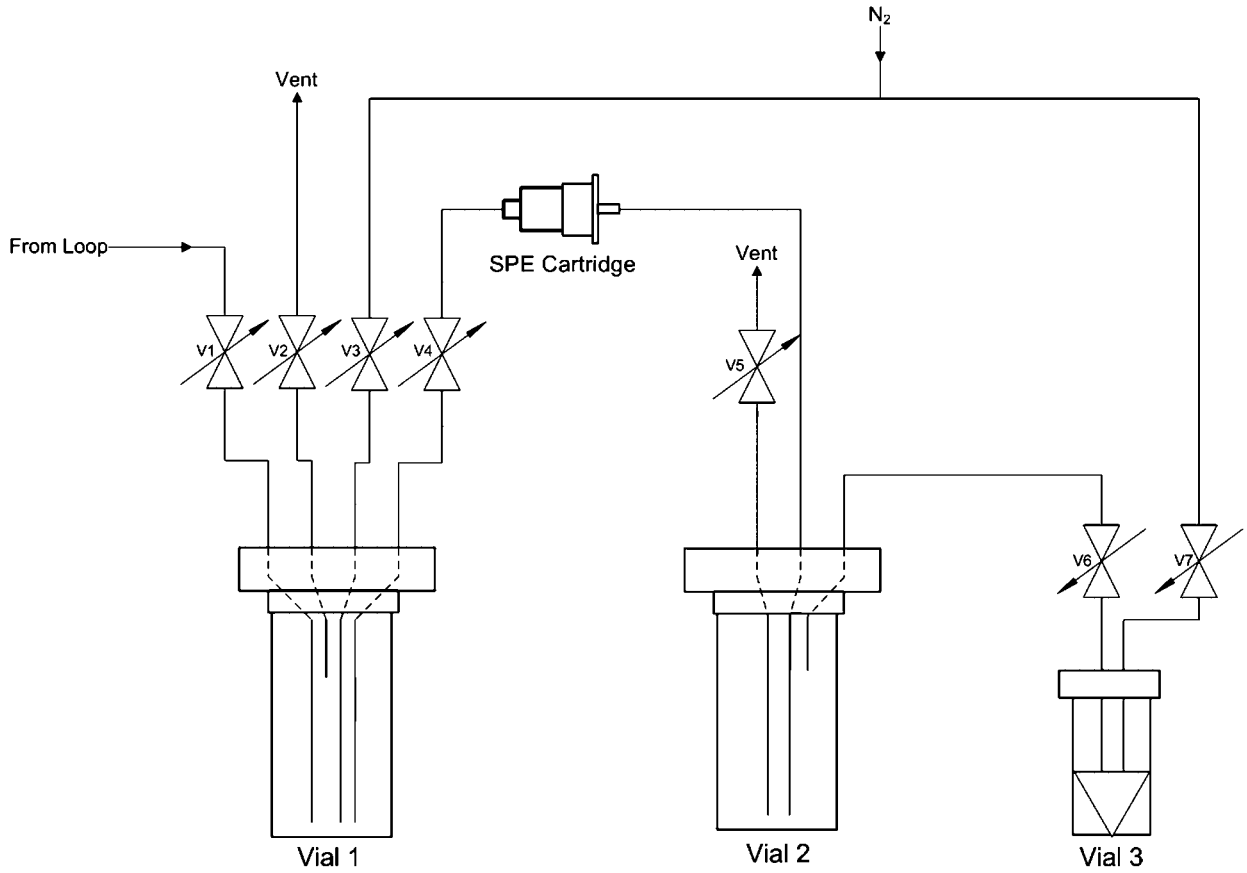

Figure 2. Schematic diagram of the automated system for the L-[methyl-^{11}C]methionine formulation.

were controlled remotely through a Programmable Logic Controller (PLC, DL-06-050D, Automation Direct) equipped with one analogical input module and one Ethernet module. A laptop (IBM ThinkPad R40) was used as an interface between the system and the operator (software Indusoft, WebStudio v6.0 SP4). Connections were realized via luer-locks and 1/16 in teflon tube. Before synthesis started, vial 3 was filled with 300 μL of monobasic sodium phosphate aqueous solution (0.2 g/mL). Vials 1 and 2 were empty.

During transfer of the reaction crude from the loop, V1 and V2 were open. After collecting the reaction crude with physiologic saline solution in vial 1 (final volume = 7.5 mL), V3 was opened and V1 was closed. Nitrogen was bubbled for 15 s to homogenize the solution and then V2 was closed and V4 and V5 were opened to allow the transfer of the product solution to vial 2 through the C18 SPE cartridge. At this step, [^{11}C]CH_3I was trapped in C18 cartridge and L-[methyl-^{11}C]methionine was collected in vial 2. After complete transfer, V3 and V4 were closed and V6 and V7 were opened to transfer the sodium phosphate solution into the collecting vial to neutralize sodium hydroxide. This final solution was submitted to quality control tests after automatic aseptic fractioning into a laminar flow cabinet (THEO, Comecer, Italy).

Quality control

Radiochemical purity was determined by radio HPLC using method 1. An Agilent 1100 series HPLC equipped with a quaternary pump, a multiple wavelength detector (λ = 220 nm) and an isotopic detector (Gabi, Raytest) was used. A Zorbax Eclipse SB-Aq C18 column (4.6 × 250 mm, 5 μm, Teknokroma, Spain) was used as stationary phase, and potassium dihydrogen phosphate aqueous solution (1.4 g/L, Panreac, Spain) was used as mobile phase at a flow rate of 1 mL/min. Chromatograms were run for 13 min (retention time for L-[methyl-^{11}C]methionine = 4.12 min, retention time for [^{11}C]CH_3I = 11.3 min).

The quantification of L-homocysteine and L-methionine (the last one to calculate specific activity) was also carried out by HPLC under experimental conditions of method 1. The areas obtained for L-homocysteine (retention time = 2.88 min) and L-methionine (retention time = 4.12 min) were compared with the areas obtained after the injection of a standard solution of L-homocysteine and L-methionine (0.01 mg/mL each). The limit of detection for this method was 0.22 μg/mL for L-methionine and 0.47 μg/mL for L-homocysteine. The analytical method was shown to be linear in the range 0.002–0.04 mg/mL for both compounds.

Enantiomeric purity was determined by means of HPLC (method 2), using an Agilent 1100 series HPLC equipped with a quaternary pump, a multiple wavelength detector (λ = 220 nm) and an isotopic detector (Gabi, Raytest). A Chirobiotic T column (4.6 × 250 mm, 5 μm, Astec) was used as stationary phase, and water/methanol 50/50 was used as mobile phase at a flow rate of 1 mL/min.

A residual solvent quantification was carried out for all solvents used during synthesis and system cleaning (ethanol, diethyl ether, acetone and THF). The quantification was carried out by means of gas chromatography using an Agilent 6890N Gas Chromatograph equipped with flame ionization detector; a TRB-1, 60 m × 0.32 mm, 0.5 μm column was used as stationary phase and ethyl acetate as internal standard.

Conclusion

The reaction of L-homocysteine with [^{11}C]CH_3I in the presence of sodium hydroxide in an HPLC loop led to the formation of L-[methyl-^{11}C]methionine with high radiochemical yield (38.4 ± 4.1% EOS) and moderate specific activity (28.9 ± 12 GBq/μmol) in short synthesis time (12 min). Radiochemical purity of the final radiotracer was 99.9 ± 0.05%.

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