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Research Article

Rapid preparation of (^{11}C)flumazenil: Captive solvent synthesis combined with purification by analytical sized columns

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Abstract: To produce the radioligand [*N-methyl*-^{11}C]flumazenil at very high specific radioactivity for our small animal imaging studies we have developed procedures for its rapid synthesis, purification and analysis. We have developed 'micro-reactor' apparatus which are assembled from analytical HPLC guard columns packed with stainless steel powder for performing the carbon-11 methylation reactions. These highly efficient reaction columns enable high radiochemical yields to be obtained with very small amounts of precursor (20–40 μg). The very small amount of reactants used enables the use of small analytical-sized HPLC columns for the rapid purification of the radioligand. Combining these techniques has enabled us to consistently prepare [*N-methyl*-^{11}C]flumazenil from [^{11}C]iodomethane with radiochemical yields of 80% (decay corrected). This results in 8–10 GBq of [*N-methyl*-^{11}C]flumazenil at very high specific radioactivities of 520–600 GBq/μmol at the end-of-synthesis. The total preparation time from end-of-bombardment of cyclotron-produced [^{11}C]carbon dioxide to end-of-synthesis is 20 min. A quality control method based on very rapid HPLC analysis (completed within 2 min) on a micro-analytical HPLC column has also being developed to reduce the time from the end-of-synthesis to injection for imaging. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: [*N-methyl*-^{11}C]flumazenil; PET; captive solvent

Introduction

There is an increasing requirement for producing PET radioligands at very high specific radioactivity (SA). A major impetus for these ligands are *in vivo* imaging studies of small animals using dedicated scanners.[1] Successful imaging requires radioligands with low carrier mass for accurate delineation of the binding sites combined with higher radioactivity concentrations in the smaller injection solutions to deliver enough activity for the scanner acquisition protocol. Methods that can help to produce radioligands at high SA are therefore required.

A radiosynthetic method for simplifying and shortening carbon-11 methylation preparations is the captive solvent method. This involves performing reactions on columns packed with support onto which a precursor solution is adsorbed. The methylating agent, which is passed through the support as a gas, is trapped by the precursor solution/support and reacts rapidly. This technique has been successfully used with various supports, including acrylic yarn,[2] silica gel, porapak[3,4] and commercially available C18 disposable cartridges.[5,6] Further simplifications of this technique was introduced by Wilson *et al.*,[7] based on coating a standard HPLC sample loop with the precursor solution. Quantitative loading of the reaction mixture onto the preparative HPLC is then simply obtained by injection.

Here outlined is our method in which by using a captive solvent method we have shortened and simplified our radiosynthesis of [*N-methyl*-^{11}C]flumazenil (Figure 1).[8] Also described are improvements in other

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components of the preparation, the purification and the quality control by HPLC, to produce the radioligand at very high SA at the time of injection and in reduced injection volumes for small animal studies.

Results and discussion

To simplify our standard radiochemical synthesis, performed in 0.5 ml of solvent in a reaction vial, we applied a captive solvent method comparable to the 'loop method' synthesis introduced by Wilson *et al.*,[7] based on the use of a sample loop of a HPLC injection valve. Similarly, this involves one-step trapping of [^{11}C]iodomethane coupled with rapid [^{11}C]methylation followed by quantitative injection of the reaction solution onto a HPLC column for purification of the radioligand. However, to significantly reduce the amount of reagents used but to retain good radiochemical yields (RCY) we have replaced the injection sample loop by a reaction column, which we have termed a 'micro-reactor'. These extremely simple reaction columns, which can be assembled in minutes, are based on HPLC analytical guard columns filled with stainless steel powder. The use of the powder helps to create a large inner surface area that efficiently immobilizes and finely disperses the precursor solution. There are two main parameters that control the efficiency of the 'micro-reactors'. Firstly, the dimension of the reactor which influences trapping efficiency, with larger columns retaining higher amounts of [^{11}C]iodomethane. However, this also results in the use of more precursor and increased helium flow back pressure. The second parameter is the particle size of the stainless steel powder, with smaller particles being more efficient due to increased surface area. However, this can also increase the helium flow back pressure. To obtain the most efficient reaction conditions and also to be compatible with the use of our General Electric MeI module, which produces a maximum pressure of 1 bar upon release of [^{11}C]iodomethane in helium, two 'micro-reactors' columns were selected; Reactor column **1** constructed from a commercially available HPLC column (30 mm × 2.1 mm), while reactor column **2** is constructed from Swagelok fittings (0.40″ × 0.09″). They are both packed with stainless steel powder.

Increased concentration of the precursor solution injected onto the reaction columns also results in increased trapping and methylation. However, this increases the reagent load for HPLC purification which we wished to reduce to enable use of analytical sized columns.

Based on these factors optimum conditions for the radiosynthesis of [*N-methyl*-^{11}C]flumazenil using both columns were determined (Table 1).

The [^{11}C]iodomethane trapping efficiency of reactor **1** at 21°C was 90% with reactor **2** having a lower trapping efficiency at 70% due to its smaller surface area. The volume of dimethylformamide solution for both reactors that is needed to trap the [^{11}C]iodomethane is extremely small, 6–12 μl. At room temperature, at the end of the 5 min period required for trapping the [^{11}C]iodomethane on the reactor columns, we obtain a RCY of up to 80% (decay corrected) in its conversion to [*N-methyl*-^{11}C]flumazenil.

A key feature of these reactors is the efficient use of precursor. Only 20–40 μg of precursor is required for the reactions from the initial 180 μg of precursor that is loaded onto the reactors. This is a significant reduction compared to the amount used in the standard solution method (1 mg) or the captive solvent method of Wilson *et al.*,[7] 0.6 mg. This feature would be a significant advantage for precursors that are only obtainable in small quantities and/or are very expensive.

A major advantage of the very small amount of precursor and reagents being used is that this enabled us to use significantly smaller analytical sized HPLC columns instead of semi-preparative sized columns (ID=10 mm) for the purification process. Using a 4.6 mm ID column (Primesphere 5 μ, C18-HC 110 Å, 30 mm × 4.6 mm, Phenomenex) a rapid purification method for [*N-methyl*-^{11}C]flumazenil was developed in which the product elutes within 2.5 min. Shown in Figure 2 are typical radioactivity (Figure 2(A)) and UV (Figure 2(B)) chromatograms of a purification. Often there is residual unreacted [^{11}C]iodomethane (Figure 2(A), R_t=1.0 min) but this is well separated from the [*N-methyl*-^{11}C]flumazenil (Figure 2(A), R_t=2.0 min). The residual amount of [^{11}C]iodomethane

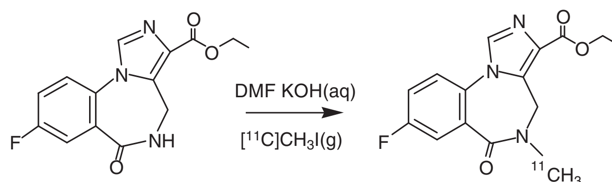

Figure 1 The labelling of [*N-desmethyl*]flumazenil with [^{11}C]CH_3I.

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Table 1 Summary of the results of the synthesis of [*N-methyl*-^{11}C]Flumazenil

Reactor column	DMF (μl)	Precursor (μg)	Time after EOB (min)	Average trapping efficiency of [^{11}C]MeI (%)	[*N-methyl*-^{11}C]flumazenil at EOS (GBq)	SA at EOS (GBq/μmol)
1 ($n = 20$)	12	40	20	90	8–10	525–600
2 ($n = 8$)	6	20	20	70	5.5–10	520–600

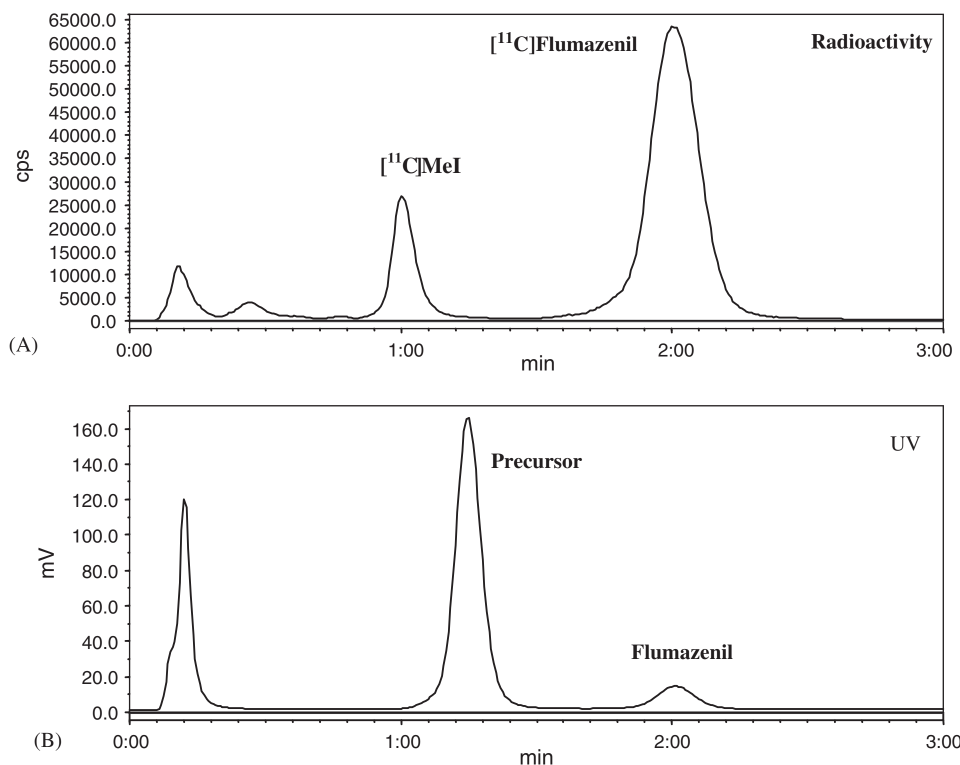

Figure 2 The HPLC radioactivity (A) and UV (B) chromatogram of a [*N-methyl*-^{11}C]flumazenil production with reactor 1.

is usually less than in this example but we chose this chromatogram for its clarity. Figure 2(B) demonstrates that the precursor and flumazenil peaks are well separated.

The use of such smaller columns clearly has the advantage of reduced use of HPLC eluent and reduced volume in the collected product fraction, as well as enabling a more rapid purification process.

Since the HPLC method was based on using ethanol and 'water for injection' as the mobile phase eluent there was no requirement to perform solid phase extraction (SPE) to remove toxic solvents. Instead the [*N-methyl*-^{11}C]flumazenil fraction was diverted directly into the final product vial, containing saline, through a sterile 0.22 μm filter. This resulted in the overall process time from end-of-bombardment, that includes the preparation of [^{11}C]iodomethane (12 min), the radiosynthesis (5 min), purification and formulation (3 min) to be reduced to only 20 min. With decay corrected RCY of up to 80%, based on the conversion from [^{11}C]iodomethane as determined by radio-HPLC, we obtained 8–10 GBq of [*N-methyl*-^{11}C]flumazenil at end-of-synthesis (EOS). The [*N-methyl*-^{11}C]flumazenil fractions were obtained in small volumes (*ca* 2.5 ml) which are compatible for producing small injection volumes for animals (e.g. approximately 160 μl for a rat) with adequate activity for the scanner acquisition protocol.

The lower overall yields obtained with reactor **2** is due to the poorer [^{11}C]iodomethane trapping efficiency. However, excellent SA of 520–600 GBq/μmol at EOS can be achieved with both reactors. This is primarily a

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result of the very high SA of the [^{11}C]iodomethane used (up to 800 GBq/μmol at EOS) however the reduction in the preparation time with these reactors enables the SA of the [*N-methyl-*^{11}C]flumazenil at EOS to be higher than obtained with standard solution radiosynthesis.

The quality control (QC) method based on HPLC analysis was also improved in order to obtain the chromatograms rapidly and therefore shorten the time from EOS to injection for scanning. Therefore we developed a very rapid HPLC QC method involving the elution of the [*N-methyl-*^{11}C]flumazenil radiochemical peak within 70 s and the completion of the analysis within 2 min. Figures 3(A) and 3(B) shows the radioactivity and UV chromatograms from the analysis of the [*N-methyl-*^{11}C]flumazenil fraction obtained from the purification process outlined in Figure 2. Figure 3(B) shows there is a small amount of precursor present (R_t=32 s) in the final product. This was determined to be between 0.1 and 0.4 μg in the total production samples which also contains approximately 5 μg cold flumazenil. This results in 2–8 ng of precursor and 0.1 μg of cold flumazenil to be present in a standard injection dose for a rat. The final product was shown to have a radiochemical purity exceeding 99% with only trace levels (< 1%) of [^{11}C]iodomethane and [^{11}C]methanol (Figure 3(A), R_t=29 and 10 s, respectively).

Conclusion

In summary, to enhance the final specifications of the carbon-11 radioligand [*N-methyl-*^{11}C]flumazenil for our small animals imaging programme we have developed methods to improve key components of the radiosynthetic process. Starting with [^{11}C]iodomethane of high SA, we have developed highly efficient 'microreactors' for the one-step trapping and reaction of [^{11}C]iodomethane by the precursor solution. These simple reaction columns, which can be assembled in minutes, can be reused without further modifications for numerous reactions. Reactor column **1** has been used routinely for more than 100 preparations without loss of efficiency. Reactor column **1** was considered to be the most suitable for regular and reliable use due to; (i) been constructed from a commercially available HPLC column which is appropriate for high pressure applications, and (ii) a higher trapping efficiency of [^{11}C]iodomethane.

The high efficiency of these reactors which use very small amounts of precursor and reagents has enabled us to perform very rapid HPLC methods on small analytical sized columns for the purification process. Coupled with a rapid QC HPLC method our process time from EOB of [^{11}C]carbon dioxide to injection of the

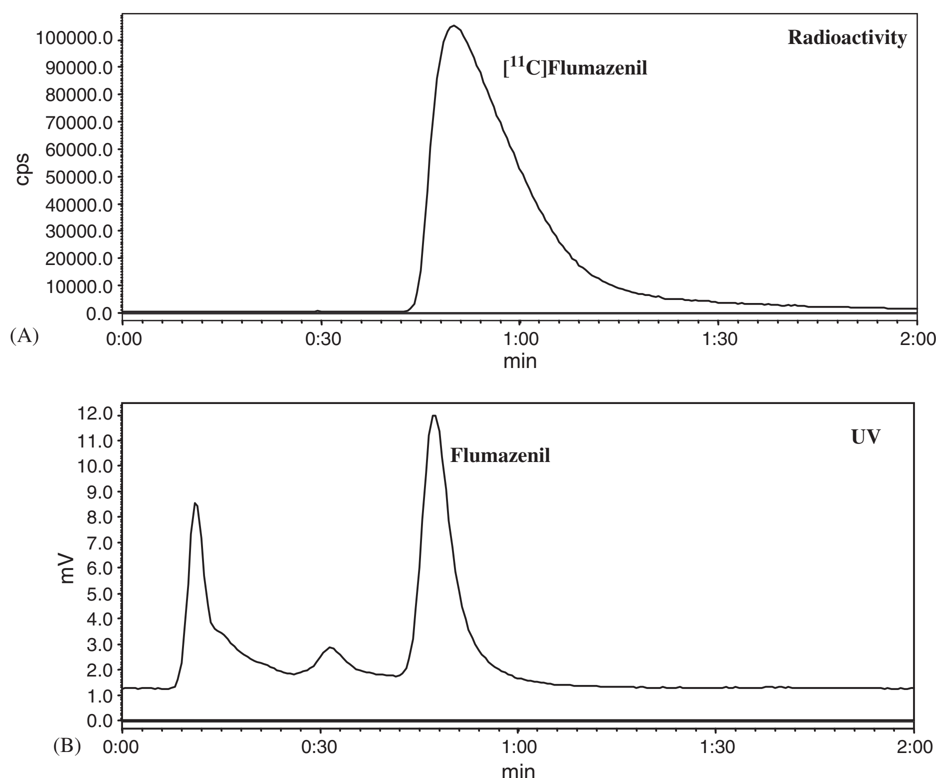

Figure 3 Quality control HPLC analysis: radioactivity (A) and UV (B) chromatograms.

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radiotracer has been significantly reduced to produce [*N-methyl-*^{11}C]flumazenil at very high SA and in small injection volumes suitable for small animal imaging studies.

The components outlined in this preparation, in particular the 'micro-reactor' columns, could be applicable for preparing other radioligands synthesised by carbon-11 methylations to improve their SA and reduce the final product volume. Key items that would need to be addressed to obtain successful reactions would be the amount of base and the concentration of the precursor solution. We are now developing this method for preparing other radioligands, e.g. [^{11}C]raclopride and [^{11}C]methyl-spiperone, that are been used for our small animal imaging programme.

Materials and methods

The *N*-desmethyl-flumazenil precursor and flumazenil standard were kindly donated by F.Hoffman-La Roche Ltd. (Basel). Other reagents and solvents at analytical grades were obtained from commercial sources and used without any further purifications. [^{11}C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ reaction with a GE Medical Systems PETtrace cyclotron using 16.4 MeV proton irradiation (20 min, 40 μA) of nitrogen gas with 0.5% oxygen. [^{11}C]Iodomethane was synthesised from [^{11}C]carbon dioxide utilising a GE Medical Systems MeI Microlab®, with the system optimised for very high SA preparations (up to 800 GBq/μmol at EOS). Radiochemical preparations were performed on a Nuclear Interface Methylation Synthesizer, which contained a two position 6-port stainless steel HPLC injection valve (Valco Instruments Co. Inc, Part Number: AC6W). The preparative HPLC column was a Primesphere 5 μ, C18-HC 110 Å, 30 mm × 4.6 mm (Phenomenex). Analytical HPLC procedures were performed using a Gilson 104 pump with Gilson 118 UV absorbance detector (245 nm) in series with a Bioscan Flow-Count radio detector. Data was acquired and analysed using a Laura radio-chromatography system (Lablogic).

Experimental

Reactions columns ('micro-reactors')

Reaction Column 1: This was constructed from a commercially available PEEK lined stainless steel HPLC column (Alltech) 30 × 2.1 mm (Part Number: 66183).

Reaction Column 2: This was made from a stainless steel Swagelok® union (internal dimensions: 0.4″ × 0.09″, Part Number: SS-200-6) equipped with stainless steel frits.

Both reactors are filled with stainless steel powder; Goodfellow AISI 304, maximal particle size 45 μm. The reaction columns are then placed in the loop position of the preparative HPLC injection valve using low volume peak tubing (0.007″ ID).

Radiosynthesis of (*N*-methyl-^{11}C)flumazenil

From a stock solution of *N*-desmethyl-flumazenil (3.3 mg) in dimethylformamide (1 ml), 60 μl is transferred into a small vial. Subsequently, 3 μl of 12 M potassium hydroxide (aq) solution is added immediately after EOB of [^{11}C]carbon dioxide. This mixture is then injected onto the 'micro-reactor' with the HPLC valve in the 'load' position (Diagram 1). Subsequently the reaction column is conditioned by passing a stream of helium (10 ml/min). This conditioning process is continued until a bolus of the precursor solution is eluted (approximately 1 min). [^{11}C]Iodomethane is then slowly passed through the reaction column in a stream of helium (10 ml/min). With the aid of a radio-detector positioned next to the reactor, the amount of [^{11}C]iodomethane trapped is monitored with optimal trapping occurring after 4–6 min (15–17 min after EOB). The HPLC valve is then switched to the 'inject' position for the quantitative injection of the reactants onto the HPLC column. To prevent excess back pressure occurring due to the HPLC eluent flow through the reaction column, the flow rate is initially set at 2 ml/min for 20 s. The HPLC valve is then switched back to the 'load' position and the flow rate is increased to 4 ml/min (ethanol/water=15/85, v/v). With UV and radioactivity analysis (Figure 2) the required [*N-methyl-*^{11}C]flumazenil fraction is then collected (2.5 ml) into a product vial via a sterile 0.22 μm filter. Saline (5 ml) is then added to the give the final product solution containing 5% v/v ethanol.

Cleaning the reaction column

1 ml of acetone is flushed through the reaction column with the HPLC valve in the "load" position. The system is then dried by gently passing a stream of helium through the reaction columns for 5 min.

HPLC methods for quality control of (*N*-methyl-^{11}C)-flumazenil (Figure 3)

Mercury MS Luna C18(2), 3 μ, 20 × 2.0 mm (Phenomenex). Flow rate at 1.3 ml/min. Eluent: ethanol/water=15/85 (v/v). UV detection at λ=245 nm. Analysis injection volume, 20 μl of the final product solution.

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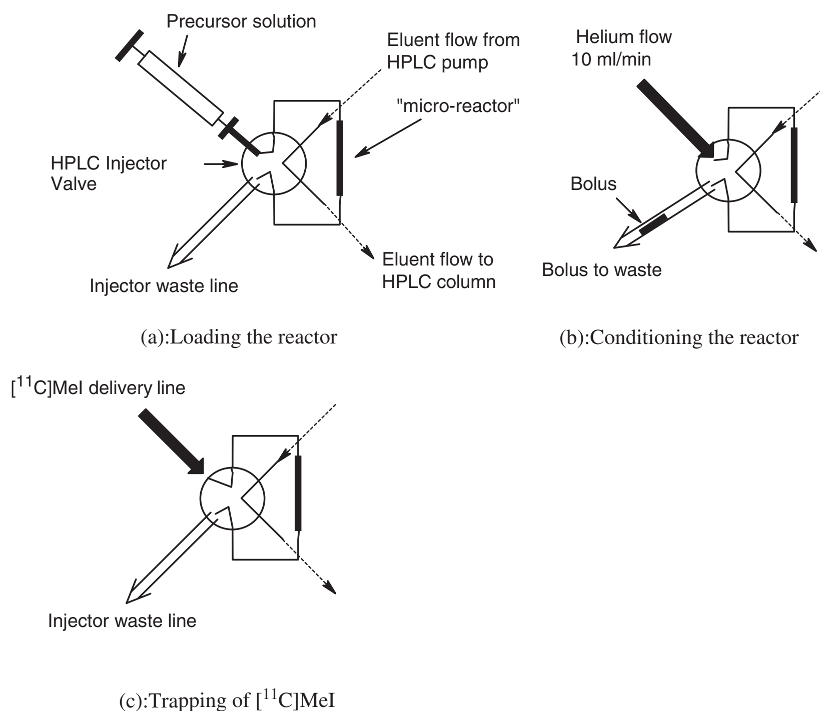

(a):Loading the reactor

(b):Conditioning the reactor

(c):Trapping of [^{11}C]MeI

Diagram 1 Preparation of the reactor columns for labelling.

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