# A combined simple bubbling method with high performance liquid chromatography purification strategy with higher radiochemical yield and purity and faster preparation of carbon-11-raclopride

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**Abstract**

**Objective:** Carbon-11-raclopride ($^{11}$C-R) is a positron-emitting radiotracer successfully used for the study of cognitive control and widely applied in PET imaging. A simple automated preparation of $^{11}$C-R by using the reaction of carbon-11-methyl triflate ($^{11}$C-MeOTF) or $^{11}$C-methyl iodide ($^{11}$C-MeI) with demethylraclopride is described. **Methods:** Specifically we used a simple setup applied an additional "U" reaction vessel for $^{11}$C-MeOTf compared with $^{11}$C-MeI and assessed the influence of several solvents and of the amount of the percussor for $^{11}$C-methylation of demethylraclopride by the bubbling method. The reversal of retention order between product and its precursor has been achieved for $^{11}$C-R, enabling collection of the purified $^{11}$C-R by using the HPLC column after shorter retention time. **Results:** By the improved radiosynthesis and purification strategy, $^{11}$C-R could be prepared with higher radiochemical yield than that of the previous studies. The yield for $^{11}$C-MeOTf was 76% and for $^{11}$C-$CH_3$I >26 % and with better radiochemical purity (>99% based on both $^{11}$C-MeOTf and $^{11}$C-MeI) as compared to the previously obtained purity of $^{11}$C-R using HPLC method with acetonitrile as a part of mobile phase. Furthermore, by using ethanol as the organic modifier, residual solvent analysis prior to human injection could be avoided and $^{11}$C-R could be injected directly following simple dilution and sterile filtration. **Conclusion:** Improved radiosynthesis and HPLC purification in combination with ethanol containing eluent, extremely shortened the time for preparation of $^{11}$C-R, gave a higher radiochemical yield and purity for $^{11}$C-R and can be used for multiple and faster synthesis of $^{11}$C-R and probably for other $^{11}$C-labeled radiopharmaceuticals.

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## Introduction

Carbon-11- raclopride ($^{11}$C-R) is a useful and extensively used positron-emitting radiotracer for the study of cognitive control and it has been widely applied in PET imaging for many years [1-4]. However, many of the difficulties involved a common radiochemistry laboratory, such as the necessity for rapid, reliable, efficient and automated procedures, have limited the preparation of $^{11}$C-R for clinical diagnosis and research. The radiosynthesis procedures have so far been greatly improved by various techniques [5, 6]. Among these, the most common is to simplify and replace parts of the whole procedure and especially the purification procedure. Moreover, radiosynthesis of $^{11}$C-R has been evolved to more efficient $^{11}$C-methylation by substituting $^{11}$C-MeI with $^{11}$C-MeOTf. Since $^{11}$C-MeOTf is 105 times more reactive and less volatile $^{11}$C-methylation agent compared with $^{11}$C-MeI, it provides higher radiochemical yield, less amount of precursor, shorter reaction time and lower reaction temperature, it has recently been used extensively in the synthesis of $^{11}$C-R.

When the synthesis of $^{11}$C-R is completed, the product is generally purified using a conventional (explain the acronym) HPLC purification strategy eventually followed by additional steps, such as evaporation, SEP-PAK C18 purification and formulation to obtain an injectable solution [7, 8]. Many other researchers tried to optimize the total purification time using a semi-preparative column or an analytical column with various fillers such as C18, CN and C16-alkylamine column combined with various mobile phases [9, 10]. The effect of each filler of the HPLC columns on separation is based on differences in lipophilicity, thereby a suitable HPLC column is the one that significantly increases the radiochemical yield of $^{11}$C-R. Unfortunately, attention is often paid to fully optimizing the radiosynthesis procedure or the rest of the procedure (i.e. HPLC separation and formulation), while only a short reaction leading to seperation or formu-

lation can not considerably increase the overall yield.

In order to overcome the current drawbacks in the preparation of $^{11}$C-raclopride, we describe here a very simple modification of Sumitomo Tracerlab which enables performance of both $^{11}$C-methylation using $^{11}$C-MeOTf in the same module and purification using HPLC in combination with ethanol-containing mobile phases. This approach can tactfully combine the conventional bubbling of $^{11}$C-MeOTf into a glass vessel filled with the precursor solution and the HPLC column purification with suitable mobile phases. Furthermore, the effects of precursor amount, solvent, base, methylating agent and temperature were examined to optimize the $^{11}$C-methylation of desmethylraclopride, which provides higher and more reproducible radiochemical yield and purity. In addition, introducing ethanol with concentrations of maximum 10% as an essential part of the mobile phase, the eluent was compatible with a straight forward injection of the solution to the patient.

## Materials and Methods

### Materials

Chemicals and solvents were from J & K Scientific (China), except for (S)-O-desmethylraclopride that was purchased from ABX, Radeberg, Germany. All reagents were of great quality and used without further purification. In the Sumitomo Tracerlab, slightly modified, a "U" vessel was inserted between the $^{11}$C-MeI reactor and the methylating reactor bathed in an oil pot. Silver triflate (1.0g) and graphite (1.5g, 80 100mesh) were completely mixed and packed in the"U"vessel.

### Methods: Production of 11C-MeI and 11C-MeOTf

Carbon-11-MeI or $^{11}$C-methyl triflate were prepared starting from $^{11}$C-carbon dioxide. This was produced in a Cypris HM12 cyclotron (Sumitomo Heavy Industries, Inc.) with a starting activity of 26-30GBq and then converted to $^{11}$C-MeI by the catalytic gas-phase iodination reaction (Sumitomo Tracerlab, Japan) [11]. Carbon-11-MeI, swept with a nitrogen flow at 40mL/min, passed through the AgOTf vessel heated at 180 °C, affording $^{11}$C-MeOTf [12].

### Radiosynthesis of [$^{11}$C]raclopride

Carbon-11-MeI or $^{11}$C-MeOTf in carrier nitrogen was bubbled for 3.0min through a glass vial containing a solution of desmethylraclopride, and an equimolar amount of NaOH (1M) at an appropriate temperature. When the radioactivity of the reaction vial reached maximum activity levels, the reaction mixture was diluted with a small volume of HPLC solution and subsequently injected directly onto a reverse-phase HPLC column (Waters™ C18, 5μm, 10×250mm) with a solvent system of 10mM $H_3PO_4$/ethanol 96% (80:20, V/V), flow rate 5.0mL/min and wave length λ=250nm. The product fraction was collected with about 5mL and passed through two consecutive sterile 0.22μm membrane filters into a sterile vial containing 5.0mL 0.1M phosphate buffer pH 7, affording a 10% ethanol solution of 11C-raclopride. After formulation, we measured: the pH using pH strips, the radiochemical purity by thin-layer chromatography (radio-TLC), and sterility. Furthermore, pyrogen tests were performed according to the monographs prescribed in the Chinese Pharmacopoeia [13].

## Results

In this paper we described how $^{11}$C-R was synthesized by $^{11}$C-MeI or by $^{11}$C-MeOTf using automated procedures and a bubbling method with the same setup. The purification using semi-preparative HPLC columns, when combined with an ethanol-containing mobile phase, afforded a straight forward injectable $^{11}$C-R solution. The optimal radiochemical yields of $^{11}$C-R were over 26% (decay corrected, based on $^{11}$C-MeI) and 76% (decay corrected, based on $^{11}$C-MeOTf), respectively. The radiochemical purities of the final products were over 99%. The specific activities of final products were 148-185GBq/μmol. The total time including radiosynthesis and purification of $^{11}$C-R was less than 23min.

The influence of different amounts of the precursor and of the reaction solvents, on the radiochemical yield of $^{11}$C-R was investigated using both methylating agents. There was a significant yield loss when the essential amount of precursors were sharply reduced from 1.0mg to 0.1mg, but the yield reached a plateau after over 1mg of precursors (Figure 1). Acetone was the most suitable solvent for methylation reaction among acetone, acetonitrile and dimethylformamide, and 1mL of acetone was the best solution volume (Figures 2 and 3).

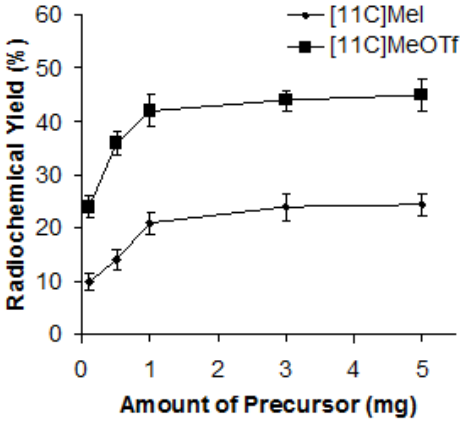


**Figure 1.** Precursor amount vs the $^{11}$C-methylation yield (n ⩾ 3). Solvent: acetone, 0.5mL.

The hydrophobic and polar interactions in reversed-phase HPLC column were taken into consideration when a reaction mixture was injected into the HPLC. We used ethanol as an essential part of the mobile phase in C18 HPLC purification of $^{11}$C-R, because the eluent is compatible with a straight forward produced injectable solution. The mobile phases with ethanol concentrations from 10% to 20% were suita-

ble for the fastest and best separation of $^{11}$C-raclopride from its precursor. Furthermore, the most optimal separation conditions were achieved on a semi-preparative HPLC column combined with 10mM $H_3PO_4$/ethanol: 80/20(V/V) as mobile phase and a flow of 5mL/min (Figure 4). After formulation, the pH, radiochemical purity, sterility and pyrogen tests were analyzed for laboratory, animal, or human clinical testing.

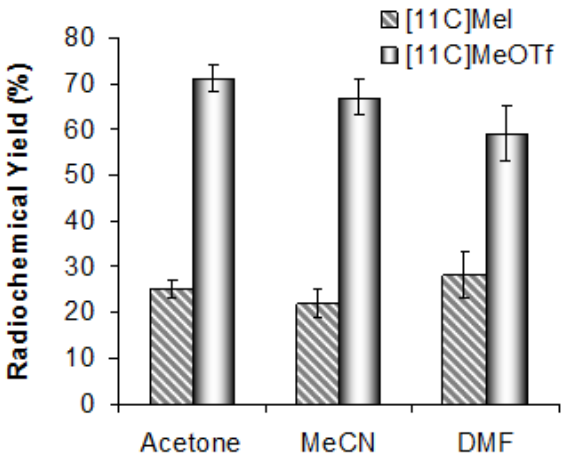


**Figure 2.** Solvent effect on radiochemical yield of $^{11}$C-raclopride (n ⩾ 3). Precursor amount: 1mg.

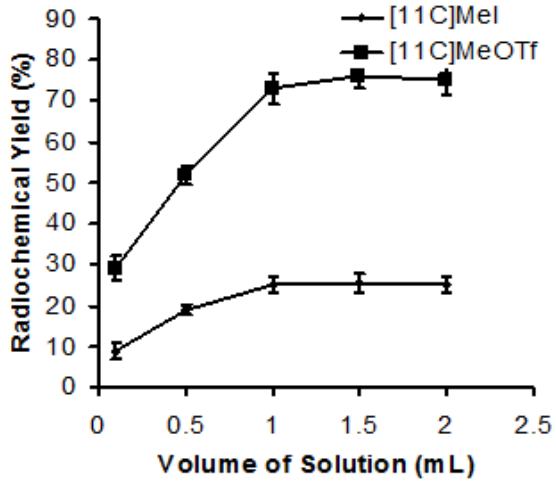


**Figure 3.** Solution volume vs the $^{11}$C-methylation yield (n ⩾ 3). Solvent: acetone.

## Discussion

For many decades, many investigations reported various implementations to optimize the radiosynthesis procedure of $^{11}$C-R or its purification strategy, but only a few considered both its radiosynthesis and purification simultaneously. Comparing the effectiveness of the methylating agent $^{11}$C-MeI or $^{11}$C-MeOTf made it possible to sharply reduce the essential amount of the precursor from 1.0mg to 0.1mg with significant yield loss. The radiochemical yield initially increased rapidly with the amount of the precursor but then after 1mg reached a plateau (Figure 1).

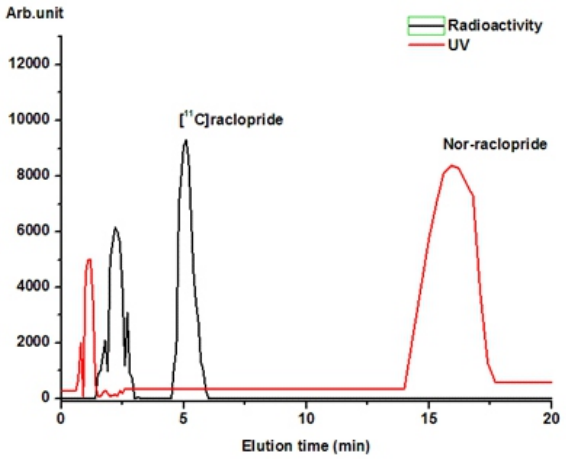


**Figure 4.** Semi-preparative HPLC separation of $^{11}$C-raclopride.

The influence of three reaction solvents acetone, acetonitrile (MeCN) and dimethylformamide, (DMF), on the radiochemical yields of $^{11}$C-raclopride was studied for both methylating agents (Figure 2). Dimethyl Sulphoxide (DMSO) as a polar aprotic solvent was the first solvent to be considered in the radiosynthesis of $^{11}$C-raclopride from $^{11}$C-MeI. However, it gave too low yield to perform further studies [5]. Acetone, MeCN and DMF were commonly used for $^{11}$C-methylations with $^{11}$C-MeOTf or $^{11}$C-MeI. By using DMF instead of acetone and MeCN we had a lower radiochemical yield for $^{11}$C-MeOTf but a higher yield for $^{11}$C-MeI (Figure 2). Acetone having higher polarity and volatility can sustain the most nucleophilic reaction between the precursor and $^{11}$C-MeOTf but not between precusor and $^{11}$C-MeI, since $^{11}$C-MeOTf is more active in the bimolecular nucleophilic substitution reaction (SN2) than $^{11}$C-MeI. On the other hand, low radiochemical yields of the methylation reaction when using $^{11}$C-MeI in acetone explained the above results (Figure 3). So, acetone as a solvent was more suitable for $^{11}$C-methylations using $^{11}$C-MeOTf, and DMF could act as the reaction solvent for $^{11}$C-MeI. The radiochemical yield of $^{11}$C-R based on $^{11}$C-MeOTf and acetone solvent is higher than that using simple-loop method [5, 9], and far more than that of $^{11}$C-R based on $^{11}$C-MeI and DMF solvent. The radiochemical yield is shown in Figure 3.

After the methylation reaction, the product is more lipophilic than the precursor and minimizes the opportunities to form hydrogen bonds with the C18 column stationary phase. Then a reversal of the retention order between product and its precursor may result. The optimal separation of $^{11}$C-raclopride from the precursor was achieved on a semi-preparative HPLC column in combination with an ethanol-containing mobile phase and a flow of 5mL/min. Optimization of radiosynthesis and of purification, not only increased the radiochemical yield by reducing the whole radiosynthesis time and the elution time of $^{11}$C-R to only about 5min, (Figure 4), but also improved the radiochemical purity because there was no tailing on the HPLC column after cutting down the amount of the precursor. $^{11}$C-R could be faster prepared and with higher radiochemical purity (>99% based on both

$^{11}$C-MeOTf and $^{11}$C-MeI) as compared to the previously obtained purity of $^{11}$C-R using HPLC method with acetonitrile as a part of mobile phase [6]. In addition, as the product fraction was collected within about 1min and transferred through a sterile 0.22μm membrane filter into a sterile vial containing 5.0mL of 0.1M phosphate buffer pH 7, the introduction of the semi-preparative HPLC column made it possible to greatly reduce the final formulation volume to a 10mL solution. After optimization of radiosynthesis and purification, it was possible to achieve high radiochemical yields and significantly reduce purification time to a total of 7-8 min. The total time including radiosynthesis and purification of $^{11}$C-R was less than 23min, which saved 8-10 min from $^{11}CO_2$ to $^{11}$C-R as compared to the other preparation method [5, 6, 9].

*In conclusion,* by a simple setup and an additional method for $^{11}$C-MeOTf compared with $^{11}$C-MeI, the injectable solution of $^{11}$C-raclopride was prepared with higher radiochemical yield (76% and 26% based on $^{11}$C-MeOTf and $^{11}$C-MeI, respectively) and better radiochemical purity (>99% based on both $^{11}$C-MeOTf and $^{11}$C-MeI). With proper chromatographic conditions, $^{11}$C-raclopride was eluted from the HPLC column prior to its less lipophilic precursor. Furthermore, by using ethanol and aqueous buffers in the eluent systems, $^{11}$C-raclopride could be formulated by a simple dilution of the collected HPLC fraction, without lengthy evaporation or solid phase extraction procedures for the removal of the organic solvents. The preparation method of $^{11}$C-raclopride could also be used for a multiple faster and reliable synthesis of other $^{11}$C-labeled radiopharmaceuticals in a faster and reliable manner.

*The authors declare that they have no conflicts of interest*

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