Technical Note

A mild, rapid synthesis of freebase [^{11}C]nicotine from [^{11}C]methyl triflate

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ABSTRACT

A rapid, mild radiosynthesis of freebase [^{11}C]nicotine was developed by the methylation of freebase nornicotine with [^{11}C]methyl triflate in acetone (5 min, 45 °C). A basic (pH 10.5-11.0) HPLC system reproducibly yielded freebase [^{11}C]nicotine as a well-defined single peak. The freebase [^{11}C]nicotine was concentrated by solid phase extraction and formulated in 50 μL ethanol (370 MBq/50 μL) without evaporative loss suitable for a cigarette spiking study. A radiochemical yield of $60.4 \pm 4.7\%$ ($n = 3$), radiochemical purity $\geq 99.9\%$ and specific activity of 648 GBq/ μmol at EOB for 5 min beams were achieved.

1. Introduction

Positron emission tomography (PET) imaging with drugs labeled with carbon-11 is a powerful method for measuring the pharmacokinetics of drugs directly in the human brain (Piel et al, 2014; Volkow et al, 1999; Fowler et al, 1999). Nicotine has been radiolabeled with carbon-11 and its distribution has been measured in healthy subjects and in patients with neurological and psychiatric disorders after intravenous administration (Mazière et al, 1976; Långström et al, 1982; Halldin et al, 1992; Yoko et al, 1993; Muzic et al, 1998). [^{11}C]Nicotine availability has also provided the opportunity to measure its distribution and kinetics in the brain and other organs after different routes of administration. The first such study measured the disposition of nicotine vapor in the respiratory tract administered from a vapor inhaler and from a cigarette, both of which were spiked with [^{11}C]nicotine (Bergström et al, 1995; Lunell et al, 1996). More recently, PET studies showed that peak brain uptake of nicotine occurs within 15 seconds in the human smoker after inhaling a single puff of a cigarette spiked with [^{11}C]nicotine (Berridge et al,

2010). Interestingly, the kinetics of smoked nicotine in the brain and lungs varies with smoking history with brain uptake being lower and slower in heavy dependent smokers than in non-dependent smokers due to higher uptake and slower washout from the dependent smoker's lungs (Rose et al, 2010). The rapid entry of smoked nicotine into the brain is likely to play a role in its rapid behavioral effects, and is more likely to result in addiction similar to other drugs of abuse like intravenous cocaine (Fowler et al, 1989).

In this study we required a method for reliably synthesizing [^{11}C]nicotine in the freebase form and formulating it in a small volume of ethanol which could be spiked into a cigarette or other nicotine delivery device. We evaluated the literature methods for radiolabeling nicotine with carbon-11 including the first radiosynthesis by reductive alkylation of nornicotine with [^{11}C]formaldehyde (Mazière et al, 1976) and several other methods via alkylation with [^{11}C]methyl iodide (Långström et al, 1982; Halldin et al, 1992; Yoko et al, 1993; Apana et al, 2010). Most of these methods used the salt form of nornicotine with the addition of an organic base such as tetramethylpiperidine (TMP) to liberate the freebase with heating at elevated temperatures (85-140 °C). Reverse phase HPLC purification using a pH modifier usually generated [^{11}C]nicotine in the salt form often with very broad tailing or shoulders. In case of normal phase HPLC, methylene chloride (which is highly toxic even in residual amounts) was used with TEA (triethylamine) in order to reduce tailing and produced [^{11}C]nicotine in the salt form (Halldin et al, 1992).

Here we investigated methylation with [^{11}C]methyl triflate (Jewett, 1992), which is generally more reactive than [^{11}C]methyl iodide and gives higher yields with shorter reaction times and lower reaction temperatures for some commonly used radioligands (Någren et al, 1995). We used the freebase form of nornicotine which avoided the need to add (and later remove) an organic base; and we used acetone as the solvent as it can be easily removed prior to HPLC separation. We found that the elution pattern for [^{11}C]nicotine with both the reverse phase and normal phase HPLC systems is highly pH sensitive and variable necessitating the development of HPLC conditions which reproducibly elutes the freebase [^{11}C]nicotine in a single sharp peak even after repeated column use. The volatility of the freebase [^{11}C]nicotine at elevated temperature and under vacuum (Atkins and de Paula, 2001) precluded the use of rotary evaporation to remove the HPLC solvent prior to formulation. For this reason we developed a solid phase extraction method which provided the freebase [^{11}C]nicotine in a small amount of ethanol with minimal loss of radioactivity.

2. Materials and Methods

Most of the reagents and solvents used for the radio synthesis were purchased from Sigma-Aldrich Chemicals (St. Louis, MO) and used without further purification. Acetone was dried over sufficient amount of Na_2SO_4 for overnight prior use. ($\pm$)-Nor-nicotine was purchased from Asta Tech (Bristol, PA), and used for the method development. Solid phase extraction (SPE) cartridges (SepPak[®] C18 Plus) were obtained from Waters (Milford, MA). The Capintec CRC-712MV and CRC-Ultra Radioisotope Dose Calibrator (Ramsey, NJ) were used for the radioactivity measurement. The Knauer HPLC system with a pump (K-501, Knauer, Germany), a variable wavelength UV detector (model 87, Knauer Germany) and a Geiger-Müller radiodetector were used for the final product purification and quality control. PeakSimple data acquisition system software (version 3.29, SRI Instruments, CA) was used for the HPLC data

collection. The Agilent 7890B GC (Santa Clara, CA) system with integrated software was used for the residual solvent analysis.

2.1 Preparation of [^{11}C]methyl triflate from [^{11}C]methyl iodide

[^{11}C]CO₂ was generated via $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$ nuclear reaction with 18 MeV protons from an EBCO TR-19/9 cyclotron. Following the irradiation, the target gas was released and delivered to an automated gas phase [^{11}C]methyl iodide system (GE Medical Systems, version 1A July 1998, Milwaukee, WI). [^{11}C]MeI was generated and carried by a stream of argon gas through a custom built miniature silver triflate furnace. The [^{11}C]MeOTf/argon gas stream was directly transferred into the freebase nor-nicotine/acetone solution contained in a dry and sealed reaction vessel in an ice bath.

For the methods development runs (Table 1, entries 1-9) we shared fractions of the [^{11}C]CO₂ activity from 40 minute clinical PET beams. For the three repeated production runs of entry 10, a total target bombarded for 5 minute with 33 μA min proton beam was used. Under these conditions, our cyclotron typically produces 17.5 GBq of [^{11}C]CO₂, our [^{11}C]methyl iodide system typically has a 60% conversion of [^{11}C]CO₂ to [^{11}C]CH₃I and the conversion of [^{11}C]CH₃I to [^{11}C]CH₃OTf is about 96-100%.

2.2 Silver triflate preparation and furnace maintenance

Silver triflate impregnated graphitized carbon was prepared according to a literature procedure with minor modification (Jewett, 1992). Briefly, acetonitrile was added to the mixture of silver triflate (1g, Sigma-Aldrich, MO) and graphitized carbon (2 g, GRAPH-PAC-GC, 80-200 mesh, Alltech Inc., IL) to give a slurry. The solvent was removed by rotary evaporation at 100 °C under vacuum. The dried silver triflate was packed into quartz tube (ID x L, 7 x 200 mm) in a furnace and heated at 190-200 °C with argon gas flow for at least 30 minutes or until the tube was visibly dry. Routine daily conditioning by sweeping inert gas (Ar or He) at high temperature (190-200 °C) through the silver triflate furnace, and bi-monthly replacement with fresh AgOTf supported on GraphPAC can significantly increase the reliability of [^{11}C]MeOTf production.

2.3 Freebase [^{11}C]nicotine radiosynthesis and purification

[^{11}C]MeOTf was trapped in the reaction vessel containing nor-nicotine (1.0-1.5 μL , freebase), acetone (200 μL , dried with Na₂SO₄ overnight) in MeCN/dry ice bath (-27 °C), followed by a 5 min heating at 45 °C. Thereafter, the reaction solvent, acetone, was removed with a stream of argon and periodic heating (45 °C). The near dry residue residual crude reaction mixture was diluted with 1.0 mL HPLC solvent containing NaOH (1.2 μL , saturated in MeOH), and purified by HPLC (Gemini C18, 250 x 10 mm, 5 μm , Phenomenex, CA). An isocratic HPLC elution was performed with a pre-mixed HPLC solvent of MeCN:water:trimethylamine (TMA) (20:80:0.1%, v/v/v) at a flow of 5.5 mL/min. UV wavelength of 254 nm. The freebase [^{11}C]nicotine elutes at 12 to 13 min.

2.4 Freebase [^{11}C]nicotine formulation into small volume

The [^{11}C]nicotine fraction from semi-prep HPLC purification was collected into 50 mL of water containing 0.1% TMA (v/v), mixed well and passed through a SepPak C18 Plus SPE cartridge. The majority of the residual water inside the cartridge was displaced by pushing through 20 mL of air through a syringe. The freebase [^{11}C]nicotine was eluted with diethyl ether (1.5 mL) and passed through a Na_2SO_4 drying column (20 x10 mm) into a conical vial (3-mL) containing 50 μL ethanol (200 Proof). The ether was removed in about 30 sec by a stream of argon gas and intermittent heating (45 $^\circ\text{C}$). The [^{11}C]nicotine remained in the droplets of ethanol which were collected at the bottom of vial by a quick centrifuge spin, and ready to be spiked onto cigarette for the cigarette smoking PET studies.

Radiochemical yield is reported as the [^{11}C]nicotine collected from the HPLC relative to the total C-11 collected in the reaction vessel expressed as a percent. All radioactivities are decay corrected back to the end of cyclotron bombardment (EOB).

2.5 Quality Control and Specific Activity Determination

An aliquot (2 μL) of the final product was diluted to 200 μL with HPLC solvent, and 60 μL of this solution was injected onto an analytical HPLC column (Gemini-NX C18, 150 x 4.6 mm, 5 μm , Phenomenex; mobile phase: MeCN :0.04% TMA(aq) (30:70, v/v); UV: 254 nm; flow rate: 0.6 mL/min). The freebase [^{11}C]nicotine eluted at 6.5 minutes.

The radiochemical purity is determined by comparing the radioactivity in the [^{11}C]nicotine peak relative to the total C-11 injected onto the HPLC column expressed as a percent. The chemical and radiochemical identity of the product was determined by HPLC analysis of a 60 μL aliquot of the final product (as described above) co-injected with a freebase nicotine standard. The specific activity (GBq/ μmol at the End of Bombardment) were calculated as the ratio of analytical HPLC net injected [^{11}C]nicotine radioactivity (GBq) and its corresponding mass (μmol).

Residual solvent analysis for ether was performed on 1 μL of the final formulated product diluted 50-fold with ethanol (see Supplemental **S5** for GC conditions).

3. Results and Discussion

Table 1. Freebase [^{11}C]nicotine radiosynthesis using [^{11}C]MeOTf and freebase nornicotine. Conditions for entries 1-9 were tested at least 2 times and conditions for entry 10 were tested 3 times for reproducibility of optimized conditions.

Entry	(+)-Nornicotine (free-base)	[¹¹ C]Methylation Reaction Conditions	Radiochemical Yield (%)
1	1.0 μ L (0.023 μ M)	THF (300 μ L), 30 $^{\circ}$ C, 3 min	1.4
2	1.0 μ L (0.023 μ M)	Butanone (300 μ L), 45 $^{\circ}$ C, 5 min	11.8
3	1.0 μ L (0.023 μ M)	Acetone (300 μ L), NaOH (6N, 1.2 μ L), 30 $^{\circ}$ C, 3 min	31.5
4	1.0 μ L (0.023 μ M)	Acetone (300 μ L), NaOH (6N, 1.2 μ L), 30 $^{\circ}$ C, 5 min	39.8
5	1.0 μ L (0.017 μ M)	Acetone (400 μ L), 30 $^{\circ}$ C, 5 min	27.2
6	1.0 μ L (0.017 μ M)	Acetone (400 μ L), 30 $^{\circ}$ C, 10 min	48.4
7	2.0 μ L (0.046 μ M)	Acetone (300 μ L), 45 $^{\circ}$ C, 3 min	44.8
8	1.0 μ L (0.023 μ M)	Acetone (300 μ L), 45 $^{\circ}$ C, 5 min	31.8
9	1.5 μ L (0.034 μ M)	Acetone (300 μ L), 45 $^{\circ}$ C, 5 min	46.7
10	1.5 μ L (0.051 μ M)	Acetone (200 μ L), 45 $^{\circ}$ C, 5 min	60.4 $\pm$ 4.7 (n = 3)

The volatility of freebase [¹¹C]nicotine stimulated the development of a low temperature synthesis method to avoid evaporative product losses. The key was to find a solvent which is capable of facilitating rapid methylation reaction without the base catalyst and which also has a moderately low boiling point for easy removal prior to HPLC purification. We evaluated three low boiling point polar aprotic solvents, THF (B.P. 66 $^{\circ}$ C), butanone (B.P. 80 $^{\circ}$ C) and acetone (B.P. 56 $^{\circ}$ C) (Supplement **S4**). THF is commonly used in C-11 methylation at higher temperature (85 $^{\circ}$ C) with [¹¹C]MeI. A radiochemical yield (RCY) of 35%-45% was reported for [¹¹C]nicotine salt synthesis when [¹¹C]MeI and freebase nornicotine were used in the presence of sodium hydroxide at 85 $^{\circ}$ C for 10 min (Apana et al, 2010). With a 10 min heating at 85 $^{\circ}$ C, we achieved an average RCY 22.7% (n = 5) using THF as the methylation solvent, the [¹¹C]MeI and the nornicotine biscamsylate salt as the precursors, in the presence of sodium hydroxide. However, at 30 $^{\circ}$ C, even though the more reactive precursors, [¹¹C]MeOTf and freebase nornicotine were used, the low yield (RCY = 1.4%) (**Table 1**, Entry 1) discouraged us to investigate THF as the reaction solvent further for this reaction. We also briefly investigated butanone as another potential reaction medium, and obtained a radiochemical yield of only 11.8% after a 5-min heating at 45 $^{\circ}$ C (**Table 1**, Entry 2). Its higher boiling point than the nicotine's critical evaporation point of 61 $^{\circ}$ C (Atkins and de Paula, 2001) also discouraged its use. Acetone (B.P. 56.3 $^{\circ}$ C) was selected as the best polar aprotic solvent for freebase [¹¹C]nicotine synthesis when [¹¹C]MeOTf was used as the radioactive precursor (**Table 1**, Entry 10). With a mild 5 minutes heating (45 $^{\circ}$ C), a radiochemical yield of 60.4 $\pm$ 4.7 % was achieved in 3 repeated production syntheses. Acetone, along with unreacted [¹¹C]MeOTf and a trace of unreacted [¹¹C]MeI, were also readily removed prior to HPLC in about 30 seconds by a stream of argon gas with intermittent 45 $^{\circ}$ C heating in an oil bath.

Although the mass of no-carrier-added [¹¹C]MeOTf is relatively fixed, the concentration of freebase nornicotine can be increased to increase the radiochemical yield. As expected, the radiochemical yields were significantly increased as the concentration of freebase nornicotine increased (**Table 1**, Entry 7, 8, 9 and 10). This gave us the opportunity to reduce the reaction

time by increasing the concentration of nornicotine (Entry **7** vs **8** and **9**). The precursor concentration was increased further by reducing the reaction solvent volume without increasing precursor loading (**Table 1**, Entry **3** vs **4**, **5** and **6** vs **10**).

Using the freebase nornicotine, we briefly evaluated the effect of NaOH on [^{11}C]nicotine yield. Although added NaOH base somewhat improved the yield of the reaction (**Table 1**, Entry **4** vs **8**), a satisfactory radiochemical yield ($60.4\pm 4.7\%$) was achieved (**Table 1**, Entry **10**) in the absence of base. Mild heating to 45 °C was used to compensate for reaction cooling due to the high air flow in our hot cell.

The development of HPLC purification conditions which reproducibly eluted freebase [^{11}C]nicotine in a single well-defined peak presented a challenge. In preliminary studies evaluating literature methods, we identified the following factors affecting the elution pattern of [^{11}C]nicotine: the presence of residual polar reaction solvent and organic base (TMP) and the pH of HPLC eluent. Polar reaction solvents, such as DMF, DMSO, butanone, and acetone all contributed to variability in the elution pattern of freebase [^{11}C]nicotine. More often, when residual reaction solvent was not removed prior to HPLC, the nicotine HPLC profile shown in Supplement **S2** changes from batch to batch and nicotine eluted as multiple or poorly shaped peaks. This is not suitable for tracer GMP production for human use. Fortunately, acetone which is the reaction solvent of choice, was easily removed prior to HPLC purification. In addition, our reaction conditions do not require an organic base. The pH of the HPLC eluent is also a crucial factor. For example, we obtained five well separated peaks from a pure nicotine standard (pH adjusted to simulate a physiologic pH 7) injection on the analytical HPLC system (Supplement **S3,A**). In contrast, a single peak was obtained when the freebase standard of nicotine was injected at high pH (Supplement **S3,B**). Thus a variety of the nicotine neutral form, mono- and diprotonated solution species were formed while equilibrations were established at pH 7 while only the freebase form dominates the species population at the high pH. We suggest that the presence of residual reaction solvent and organic base interferes with the interactions between the analytes and the stationary phase.

Based on the above analyses, we developed a HPLC purification procedure which would tolerate basic conditions (pH: 10.5-11.0). We first diluted the crude reaction mixture (after the acetone evaporation) with 1 mL of HPLC solvent which was made basic (pH: 10.5-11.0) by the addition of a saturated solution of NaOH in methanol so that the freebase form [^{11}C]nicotine became the dominant species before the HPLC purification. The high pH tolerance of the Gemini C18 column (working pH range 1-12) allowed the use of a mobile phase containing an organic base (trimethylamine, pKa 9.81, b.p. 4 °C) and provided the opportunity to separate the [^{11}C]nicotine from the nornicotine and other chemical contaminants, in a single, sharp and well-defined peak eluting at 12-13 min (Figure 1A). There was no obvious degradation of column performance through 57 injections.

Figure 1. (A) Typical semi-preparative HPLC plot showing the UV absorbance (top) and radioactivity profiles with unlabeled nicotine mass and [^{11}C]nicotine indicated; ((B) typical analytical HPLC profile of an aliquot of the final purified freebase [^{11}C]nicotine showing a process blank, UV absorbance and radioactivity profiles. For HPLC conditions see **Materials and Methods**.

The next step was to formulate the collected freebase [^{11}C]nicotine into clinically useful medium. The freebase form [^{11}C]nicotine was preserved through the SPE formulation process. The low temperature application for ether evaporation and ethanol extraction avoided product loss through evaporation. The freebase [^{11}C]nicotine which was extracted into 50 μL of ethanol was suitable for use for the cigarette spiking or other applications where freebase [^{11}C]nicotine is needed. Radioanalytical HPLC showed a radiochemical purity of 99.9% (Figure 1B). No radiolysis was observed at 36 min after the end of synthesis for this highly concentrated product (370 MBq/50 μL) in the presence of ethanol. GC analysis showed no residual ether in the final product (Supplement S5).

5. Conclusion

A new radiosynthesis method for freebase [^{11}C]nicotine was developed. It used [^{11}C]MeOTf as radioactive precursor and freebase nornicotine as the non-radioactive substrate. The methylation of freebase nornicotine with [^{11}C]MeOTf was carried out in acetone under mild condition (45 $^{\circ}\text{C}$) and short reaction time (5 min) without the addition of organic or inorganic base. The total synthesis time was 32- 36 minutes. An overall radiochemical yield of 60.4 ± 4.7 % ($n = 3$) was achieved with high radiochemical purity (99.9 %), high radioactivity concentration (370 ± 185 MBq/50 μL) and high specific activity (648 GBq/ μmol at EOB for a 5 min cyclotron irradiation). The final product formulated in 50 μL of ethanol was ready to be used for future nicotine brain uptake study via cigarette smoking or other routes of administration. In addition, a robust and reproducible basic HPLC purification method was developed for the freebase nicotine purification.

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References

- Apana, S.M., Berridge, M.S. 2010. Smoking produces rapid rise of [^{11}C]nicotine in human brain." *J. Label. Compd. Radiopharm* 53: 6-10.
- Atkins, P., de Paula, J. 2001. Water nicotine azeotropic mixture. Chap. 8 in *Physical Chemistry*, by Julio de Paula and Peter Atkins. New York: W.H. Freeman and Company.
- Bergström, M., Nordberg, A., Lunell, E., Antoni, G., Långström, B. 1995. Regional deposition of inhaled ^{11}C - nicotine vapor in the human airway as visualized by positron emission tomography. *Clin. Pharmacol. Ther.* 58: 309-317.
- Berridge, M.S., Apana, S.M., Nagano, K.K., Berridge, C.E., Leisure, G.P., Boswell, M.V. 2010. Smoking produces rapid rise of [^{11}C]nicotine in human brain. *Psychopharmacology* 209: 383-394.
- Fowler, J.S., Volkow, N.D., Wang G.-J., Ding Y.-S., Dewey S.L. 1999. PET and drug research and development. *J. Nucl. Med.* 40: 1154-1163.
- Fowler, J.S., Volkow, N.D., Wolf, A.P., Dewey, S.L., Schlyer, D.J., MacGregor, R.R., Hitzemann, R., Logan, J., Bendriem, B., Gatley, S.J. 1989. Mapping cocaine binding sites in human and baboon brain in vivo. *Synapse* 4: 371-377.
- Halldin, C., Någren, K., Swahn, C.-G., Långström, B., Nybäck, H. 1992. "(S)- and (R)- [^{11}C]nicotine and the metabolite (R/S)-[^{11}C]cotinine. Preparation, metabolite studies and in vivo distribution in the human brain using PET. *Int. J. Rad. Appl. Instrum. B Nucl. Med. Biol.* 19: 871-880.
- Jewett, D.M. 1992. A simple synthesis of [^{11}C]methyl triflate. *Int. J. Rad. Appl. Instrum. A Appl. Rad. Iso.* 43: 1383-1385.
- Långström, B., Antoni, G., Halldin, C., Svärd, H., Bergson, G. 1982. Synthesis of some C-11-labeled alkaloids. *Chemica Scripta* 20: 46-48.
- Lunell, E., Bergström, M., Antoni, G., Långström, B., Nordberg, A. 1996. Nicotine deposition and body distribution from a nicotine inhaler and a cigarette studied with positron emission tomography. *Clin. Pharmacol. Ther.* 59: 593-594.
- Mazière, M., Comar, D., Marazano, C., Berger, G. 1976. Nicotine- ^{11}C : Synthesis and distribution kinetics in animals. *Eur. J. Nucl. Med.* 1: 255-258.
- Muzic Jr, R.F., Berridge, M.S., Friedland, R.P., Zhu, N., Nelson, A.D. 1998. PET quantification of specific binding of carbon-11-nicotine in human brain. *J. Nucl. Med.* 39: 2048-2054.

Någren, K., Müller, L., Halldin, C., Swahn, C.-G., Lehtikainen, P. 1995. Improved synthesis of some commonly used PET radioligands by the use of [^{11}C]methyl triflate. *Nucl. Med. Biol.* 22: 235-239.

Piel, M., Vernaleken, I., Rösch, F. 2014. Positron emission tomography in CNS drug discovery and drug monitoring. *J. Med. Chem.* 57:9232-9258.

Rose, J.E., Mukhin, A.G., Lokitz, S.J., Turkington, T.G., Herskovic, J., Behm, F.M., Garg, S., Garg, P.K. 2010. Kinetics of brain nicotine accumulation in dependent and nondependent smokers assessed with PET and cigarettes containing ^{11}C -nicotine. *Proceedings of the National Academy of Sciences U.S.A.* (107): 5190-5195.

Volkow, N.D., Fowler, J.S., Ding Y.-S., Wang G.-J., Gatley S.J. 1999. Imaging the neurochemistry of nicotine actions: studies with positron emission tomography. *Nicotine Tob. Res.* 1 Supp (2): S127-32.

Yoko, F., Komiyama, T., Ito, T., Hayashi, T., Iio, M., Hara, T. 1993. Application of carbon-11 labelled nicotine in the measurement of human cerebral blood flow and other physiological parameters. *Eur. J. Nucl. Med.* 20: 46-52.