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Development and Optimization of a Novel Automated Loop Method for Production of [^{11}C]Nicotine

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Abstract

A novel, rapid, and automated loop method for the synthesis of [^{11}C]nicotine was developed and optimized. The method involves, a reaction of the precursor, (+) nornicotine or (–) nornicotine, with a gas-phase produced [^{11}C]CH₃I in an 800 μL loop at 75°C for 5 minutes followed by a semi-preparatory Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) purification. The optimized synthesis and purification process was complete in < 30 minutes and produced [^{11}C]nicotine with >99.9% Radiochemical Purity (RCP), no [^{11}C]CH₃I, no (+) nornicotine, and 105mCi/ μmole specific activity, 7.0 – 7.2 pH, and 16.6% ethanol. The current method can be optimized, to reduce the ethanol content (<10%), and translated to a cGMP production of [^{11}C]nicotine for human clinical trials.

Keywords

Radiosynthesis; radiotracers; gas-phase production of [^{11}C]CH₃I; automated loop method; [^{11}C]nicotine

Introduction

Nicotine is the most abundant alkaloid (98% of the total alkaloids) among more than 20 alkaloids present in cigarette tobacco. It is one of the most commonly abused drugs that affect brain chemistry and physiology which are mediated via nicotinic receptors (Nordberg et al. 1989). Both muscular and neuronal nicotinic acetylcholine receptors (nAChRs), which belong to the superfamily of ligand-gated ion channels, interact with nicotine. It has been reported that density of nicotinic receptors increases in the brains of smokers (Benwell et al. 1988). Additionally, the number of high-density nicotinic receptors are decreased in patients with Alzheimer disease (Nordberg and Winblad 1986; Norberg et al. 1988).

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Competing Interests

The authors have declared that no competing interests exist.

In the recent years, there has been a significant growth in the use of electronic cigarettes (e-cigarettes) among smokers worldwide (Adkison et al. 2013; Berg et al. 2014; Dockrell et al. 2013; King et al. 2013). The health risks of e-cigarettes are unknown, however, they are likely safer than conventional cigarettes and has some success in Nicotine Replacement Therapy as a means to stop smoking. Consequently, it is important to understand the nicotine uptake in the brain, distribution, and pharmacokinetics (PK) of e-cigarette smoking. Recent studies have suggested that e-cigarettes deliver less nicotine per puff than conventional cigarettes and deliver modest nicotine concentration (Scroeder and Hoffman 2014). Walele and coworkers compared plasma nicotine PK profile of unflavored and flavored electronic cigarettes containing 2% nicotine, Nicorette Inhalator, and the conventional cigarette in a randomized crossover study involving 24 human subjects (Walele et al. 2016). The maximum plasma nicotine concentration (C_{max} , ng/mL) and the time to maximum nicotine concentration (t_{max} , min) for the four products were: 3.6, 2.5, 2.5, and 21.2; and 9.0, 10.0, 13.0 and 3.0, respectively.

Radioisotope-based molecular imaging techniques such as Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT) are used to capture brain function and for measurement of pharmacokinetics of drugs directly in the brain (Hargreaves and Rabiner 2014; Piel et al. 2014; Volkow et al. 1999; Fowler et al. 1999). PET is considered superior to SPECT due to the higher sensitivity of the instrumentations and better quantification of regional tissue concentrations of radioisotope-labeled molecular entities. There are numerous published studies related to receptor binding (Sihver et al. 1998) and brain uptake and distribution of nicotine in animals, (Maziere et al. 1976; Saji et al. 1992; Sihver et al. 1999; Nordberg et al. 1989), healthy subjects (Bergstrom et al. 1995; Lunell et al. 1996; Zuo et al. 2017; Apana and Berridge 2010; Berridge et al. 2010; Rose et al. 2010; Halldin et al. 1992; Yokoi et al. 1993; Nyback et al. 1994; Muzic et al. 1994; Garg et al. 2017), and patients with neurological disorders (Nordberg et al. 1990; Kadir et al. 2006; Kadir et al. 2007) after inhalation using a vapor inhaler (Bergstrom et al. 1995; Lunell et al. 1996), or a device (Zuo et al. 2017), or a [^{11}C]nicotine spiked cigarette (Lunell et al. 1996; Apana and Berridge 2010; Berridge et al. 2010; Rose et al. 2010) and intravenous administration (Halldin et al. 1992; Yokoi et al. 1993; Nyback et al. 1994; Muzic et al. 1994; Garg et al. 2017; Nordberg et al. 1990; Kadir et al. 2006; Kadir et al. 2007) of [^{11}C]-labeled nicotine. For nicotine brain uptake and distribution in humans, the positron emission tomography technique was used.

[^{11}C]Nicotine has been synthesized previously by reductive methylation of nornicotine with [^{11}C]formaldehyde (Maziere et al. 1976) or by *N*-methylation of nornicotine with [^{11}C]CH₃I (Sihver et al. 1998; Saji et al. 1992; Sihver et al. 1999; Nordberg et al. 1989; Apana and Berridge 2010; Berridge et al. 2010; Halldin et al. 1992; Yokoi et al. 1993; Nordberg et al. 1990; Langstrom et al. 1982; Sihver 1977; Nyback et al. 1994; Olson et al. 1992) and more reactive methylation reagent, [^{11}C]methyl triflate (Garg et al. 2017; Xu et al. 2016). In general, a methylation reaction using [^{11}C]methyl triflate gives higher radio-chemical yield (RCY) and specific activity and requires shorter synthesis times and lower reaction temperature than a reaction with [^{11}C]methyl iodide (Nagren et al. 1995; Jewett 1992). The synthesis of [^{11}C]formaldehyde and [^{11}C]CH₃I involves reduction of [^{11}C]CO₂ using lithium aluminum hydride (LiAlH₄) (wet method) and subsequent oxidation or a reaction

with the hydroiodic acid (HI), respectively. Most of these methods involve extensive solution-based chemistry, time-consuming manual synthesis, use of acid/base, and either a liquid/liquid extraction or a normal-phase High-Performance Liquid Chromatography (HPLC) purification method. In addition, most of these methods used ICH (International Conference on Harmonization) Class II solvents e.g. tetrahydrofuran, acetonitrile and methylene chloride during purification, which are not ideal for formulations intended for intravenous (i.v.) administration.

The objectives of the present work were: (1) develop a rapid, efficient and automated loop method for the synthesis of [^{11}C]nicotine, from the reaction of (+) or (–) nornicotine free base and gas-phase produced [^{11}C]CH₃I, (2) avoid using reagents such as LiAlH₄ and organic bases, and (3) minimize amounts of organic solvents in the final [^{11}C]nicotine formulation, for both inhalation and i.v. administrations. While this work was in progress, a similar study was ongoing and published by using gas-phase produced [^{11}C]methyl triflate for the synthesis of [^{11}C]nicotine (Xu et al. 2016). However, there is no information available in the literature related to production of [^{11}C]Nicotine and from reaction of gas-phase produced [^{11}C]methyl iodide and nornicotine. Consequently, in the present work, we have developed and optimized a novel rapid, efficient, and automated loop method (<30 minutes synthesis and purification time) for the synthesis of [^{11}C]nicotine. The method involves a reaction of gas-phase produced [^{11}C]CH₃I with (+) nornicotine or (–) nornicotine free base and avoided any additional base. The product was purified by a semi-preparatory Reversed-Phase HPLC (RP-HPLC) method, using only ethanol and water as a mobile phase, followed by phosphate buffered saline (PBS) dilution making it ready for intravenous (i.v.) administration. The optimized process produces [^{11}C]nicotine with >99.9% radiochemical purity (RCP), no [^{11}C]CH₃I, no (+) nornicotine, 105 mCi/μmole FHigh activity, 7.0 – 7.2 pH, and 16.6% ethanol. The current method can be optimized, to reduce the ethanol content (<10%), and translated to cGMP production of [^{11}C]nicotine for human clinical trials.

Materials and Methods

High purity anhydrous solvents, e.g., dimethylformamide, DMF (99.8%), dimethylsulfoxide, DMSO (>99.8%), acetonitrile, MeCN (99.9%) (all from Sigma Aldrich), and Ethanol, EtOH 100% (USP grade, Fisher) were used without further purification. The pH and ionic strength of the buffers were controlled with sodium monobasic phosphate, sodium dibasic phosphate, ammonium acetate, sodium hydroxide, hydrochloric acid, and sodium chloride. (all from Fisher). A free-base form of the precursor, (+) nornicotine (Alfa Aesar) or (–) nornicotine (Toronto Research Chemicals, Canada), and a nicotine standard (Sigma Aldrich) were used for the radiochemical synthesis and identification of [^{11}C]nicotine, respectively. All other lab supplies, such as 10 & 20 mL sterile vials, sterile needles, and syringes were obtained from either Fisher Scientific or Sigma Aldrich.

High purity gases, Helium, 99.9999% (with <0.1 ppm oxygen), Hydrogen, 99.9999% (<0.5 ppm moisture), and Nitrogen target gas (99% nitrogen and 1% mixture) were obtained from Airgas Corp and liquid nitrogen (Praxair), nickel catalyst 120 mesh size, 99.996% (Alfa Aesar), carboxene 60/80 mesh size (Supelco), porapak (Waters), and anhydrous iodine, 10 mesh, 99.999% (Sigma Aldrich) were used during production of [^{11}C]CH₃I and

radiochemical synthesis of [^{11}C]nicotine. Pre-packed sodium hydroxide cartridges were supplied by Machery Nagel. Red PTFE/Silicone, 8 mm size, septa (Supelco) were used in the injection port for the loading of the precursor. Sep-Pak C₁₈ Plus Light cartridges (Waters Corporation) were preconditioned with 5 mL of ethanol followed by 10 mL of water before use. Latex- and silicone-free SUN-SRi syringes were used for handling of all solvents and during experimental work. A Millipore Millex 0.22 μm syringe filter was used for sterile filtration of the final formulation. De-ionized MilliQ water was used for all preparations.

PEttrace 880 (General Electric) cyclotron was used for the production of ^{11}C -labeled carbon dioxide, [^{11}C]CO₂. The [^{11}C]Nicotine production process was developed and optimized using an automated MeI Plus loop system (from Synthra GmbH) equipped with a semi-preparative HPLC system, consisting of a quaternary pump and variable wavelength UV (254nm) and radioisotope detectors. The module was controlled by a Synthraview software and is capable of producing [^{11}C]CH₃I and [^{11}C]methyl triflate. The module was sterilized each time prior to the synthesis with a mixture of 70% ethanol and 30% water. All radioactive work was accomplished in a Comacer BBS2 hot cell. The amounts of radioactivity in the [^{11}C]-labeled materials were measured using a Capintec dose calibrator. The final [^{11}C]nicotine product, along with a nicotine standard and the (+) or (–) nornicotine precursor, was analyzed for radiochemical and chemical purity (RCP) using a model 1260 Agilent HPLC system comprising an infinity quaternary pump system, degasser, temperature controlled column compartment, auto-injector and multi-wavelength UV (254 nm) and a Raytest GABI Star radioisotope detectors. All the components of the system were controlled by Raytest's GINA Star software.

Production of [^{11}C]CO and Preparation of [^{11}C]CH₃I precursor

^{11}C -labeled carbon dioxide, [^{11}C]CO₂, was produced by $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction using a GE PEttrace 880 cyclotron, typically operating at 16.5 MeV, 20 μA , and ~3 minutes proton bombardment of nitrogen gas target, delivering ~200 mCi. This amount is produced and used for radiosynthesis method development and process optimization. Higher amounts of radioactivity have been produced, up to a Curie or more for production, by increasing the bombardment time and energy. The [^{11}C]CO₂ from the cyclotron was transferred to the Synthra module where it was trapped in a cryogenic stainless steel coil at -180°C and converted to ^{11}C -labeled methane, [^{11}C]CH₄, by reduction on a Ni catalyst at 425°C . The [^{11}C]methane was trapped at -120°C on a carboxene column and hydrogen was removed from the system. Subsequently, [^{11}C]CH₄ was released, converted to [^{11}C]-labeled methyl iodide, [^{11}C]CH₃I, in the gas phase by passing through an I₂ quartz oven at 750°C , and trapped on a Porapak Q column. Any unreacted [^{11}C]CO₂ in these steps was retained within NaOH cartridges in the synthesis module.

Synthesis and Purification of [^{11}C]-Nicotine

A known amount of precursor, (+) or (–) nornicotine free base was dissolved in 80 μL of 50:50 mixture of DMF and DMSO. In most of the experiments, 2 mg of the precursor was used, except in some process optimization experiments. The 800 μL stainless steel loop was initially coated with 10 μL 50:50 mixture of DMF and DMSO followed by loading of the precursor in solution. The injection port septum was replaced after every two injections to

prevent radioactivity leakage and cross-contamination. Finally, the $[^{11}\text{C}]\text{CH}_3\text{I}$ trapped on a Porapak column was released by heating, up to a temperature of 200°C , into the stainless steel loop. Compressed helium was used as a carrier gas during the entire course of the production. The reaction mixture in the loop was heated by resistive heating to a desired temperature and time in each run for optimization of the reaction conditions. A flow diagram for the synthesis reaction is illustrated in Figure 1.

For purification of the crude product, the reaction mixture from the loop was injected directly onto a Waters Xterra C_{18} semi-prep column (150×10 mm, $5\ \mu\text{m}$ particle size) of the HPLC system. Two purification methods were used for removal of the unreacted precursor, (+) or (–) norm nicotine, and $[^{11}\text{C}]\text{CH}_3\text{I}$. In the first method an isocratic mobile phase containing an 80:20 mixture of 6.5 mM ammonium acetate pH 10 buffer and acetonitrile with 2.5 mL/min flow rate was used. The pH 10 of the mobile phase was necessary to neutralize the protonated forms of the precursor and the final product. A UV detector set at 254 nm and a radioisotope detector were used for monitoring the purification in both methods. Finally, the HPLC purified $[^{11}\text{C}]$ -nicotine was loaded onto a preconditioned Sep-Pak C_{18} Plus Light cartridge. The cartridge was first washed with water to remove any polar impurities and salts followed by elution with ethanol. The final product was collected in a sterile vial fitted with a $0.22\ \mu\text{m}$ sterile filter.

In the second method an isocratic mobile phase containing an 80:20 mixture of water (pH 10, adjusted with NaOH) and ethanol and 5.0 mL/min flow rate were used. No Sep-Pak C_{18} Plus Light purification was needed for samples purified by this method as the HPLC mobile phase did not contain any buffer/salt or acetonitrile. The HPLC purified fraction containing the product was transferred directly into a sterile vial, fitted with a $0.22\ \mu\text{m}$ sterile filter, containing 1.0 mL of 300 mM phosphate buffer and 900 mM NaCl at a predefined pH.

Quality Control and Specific Activity Determination

The $[^{11}\text{C}]$ nicotine stock solution was injected onto a Waters Xterra C_{18} analytical HPLC column (150×4.6 mm, $5\ \mu\text{m}$ maintained at 40°C) for analysis. An isocratic mobile phase containing an 80:20 mixture of ammonium acetate (6.5 mM, pH 10) and acetonitrile with a flow rate of 1.0 mL/min was used. The (+) or (–) nicotine free base was used as a standard for $[^{11}\text{C}]$ nicotine identification (by retention time match) and for determination of the unlabeled nicotine amount. The radiochemical purity of $[^{11}\text{C}]$ nicotine, expressed as a percent, was determined from the peak area of $[^{11}\text{C}]$ nicotine and the total peak areas in the chromatogram as detected by the radioisotope detector.

For specific activity determination, a calibration curve was established between the peak area of nicotine and its mass injected onto the column. From the peak areas of the unlabeled nicotine in the $[^{11}\text{C}]$ nicotine solution and the slope of the calibration curve, the amount of unlabeled nicotine in the sample was calculated. Finally, from the amount of radioactivity (as measured by using a dose calibrator) and calculated mass of unlabeled nicotine the specific activity was determined. In addition, the material was tested for appearance, pH, radionuclidic purity based on the 511 keV PET peak from ^{11}C spectral scans using GABI Star radio detector, and bacterial endotoxins and sterility per USP <85> and <71>, respectively (USP 40 NF 35 2017).

Results and Discussion

Synthesis and Purification of [^{11}C]Nicotine from the Reaction of (+)Nornicotine and Gas-Phase Produced [^{11}C]CH₃I

In addition to other methods, cyclotron produced [^{11}C]CO₂ is, traditionally, trapped in a solution of LiAlH₄ in tetrahydrofuran (THF). After evaporation of THF, hydroiodic acid is added. The [^{11}C]CH₃I produced is trapped in a suitable solvent under a stream of nitrogen followed by the reaction with a precursor at the desired temperature. In the present work, the production of [^{11}C]CH₃I involved reduction of [^{11}C]CO to [^{11}C]CH₄ on a Ni catalyst and finally conversion of [^{11}C]CH₄ to [^{11}C]CH₃I in gas phase over iodine (details are given above). Initial trials using this procedure were either unsuccessful or gave poor yields for production of [^{11}C]CH₃I. Various investigations and optimization experiments were conducted and led to the conclusion that high purity helium and hydrogen gases were critical for the successful production of [^{11}C]CH₃I. For example, helium gas with low amounts of carbon dioxide, carbon monoxide, and oxygen, and CH₄ (all < 0.1 ppm) and hydrogen with low moisture (0.5 ppm) and carbon dioxide and carbon monoxide (both < 0.1 ppm) gave acceptable results. Under these conditions typical conversion yield of [^{11}C]CO₂ to [^{11}C]CH₃I was as high as 65%.

Several solvents have been used in the past for the synthesis of [^{11}C]nicotine from the reaction of LiAlH₄ produced [^{11}C]CH₃I and nornicotine salt or base form of nornicotine. These include acetonitrile, DMF, a mixture of DMF and DMSO, and tetrahydrofuran. In some cases, bases like 2,2,6,6, -tetramethylpiperidine and sodium hydroxide were used. The reaction temperatures and times varied between 85°C and 130°C and 5 to 10 minutes, respectively. In a recent study (Xu et al. 2016), several solvents, tetrahydrofuran (BP 66°C), butanone (BP 80°C), and acetone (BP 56°C) were evaluated for the *N*-methylation of nornicotine by [^{11}C]methyl triflate. The objective of the study was to find a suitable solvent that is capable of facilitating rapid methylation reaction without a base and which is easy to remove prior to HPLC purification. Acetone was selected as a best polar aprotic solvent, with mild reaction conditions (i.e., heating 45°C for 5 minutes without addition of a base) for the synthesis of [^{11}C]nicotine from the reaction of a free-base form of nornicotine and [^{11}C]methyl triflate.

In the present work, tetrahydrofuran, acetonitrile, DMF, DMSO and combinations of DMF and DMSO solvents were initially used to dissolve the precursor for the synthesis of [^{11}C]nicotine. Using these solvents, there was either no reaction or the yield of the experiments were low and unacceptable. However, a 1:1 mixture of DMF and DMSO was subsequently found to be the best choice for the radiochemical synthesis. In a typical reaction, 2 mg (+) nornicotine (dissolved in 80 μL of 1:1 mixture of DMSO and DMF) with gas phase produced [^{11}C]CH₃I for 5 minutes at 100°C loop temperature gave [^{11}C]nicotine with 13.4 % decay corrected (EOB, end of bombardment) radiochemical yield. Replacing (+) nornicotine with just the S or (–) isomer did not show any significant improvement in the yield. Hence, the racemic mixture was used for all subsequent trials.

A semi-preparatory RP-HPLC method for purification (less than 10 minutes run time) of the crude product was developed which was suitable for removal of unreacted (+) nornicotine

and [^{11}C]CH₃I. A HPLC chromatograms were monitored in real time to detect and collect the desired peak corresponding to [^{11}C]nicotine. Using the first HPLC purification method, the [^{11}C]nicotine eluted at ~8 min while the retention times for [^{11}C]CH₃I and (+)nornicotine precursor were ~2.8 and ~3.2 min, respectively. A second purification step using a pre-conditioned Sep-Pak C₁₈ Plus Light cartridge was then implemented for the removal of ammonium acetate and acetonitrile from the HPLC mobile phases. After loading the [^{11}C]nicotine from the HPLC column onto the Sep-Pak C₁₈ Plus Light cartridge, the latter was first washed with 5 mL water to remove any polar impurities and salts. The final product was then eluted with 2 mL ethanol. The total time for the synthesis of [^{11}C]nicotine including purification time was ~37 minutes. Using Sep-Pak CM cartridges to trap [^{11}C]Nicotine after loop followed by 0.9% sodium chloride elution was also considered, however, it is impossible to remove the precursor from the final product by this method.

Additional experiments were conducted to optimize the purification method. This included varying the HPLC flow-rate, the ratio of ammonium acetate and acetonitrile in the HPLC mobile phase and the amount of ethanol (1, 2, and 3 mL) for elution of [^{11}C]nicotine from the Sep-Pak C₁₈ Plus Light cartridge. Purification experiments performed on the radiotracer, produced under the following conditions: 2 mg precursor, 5 minutes reaction times, and 100°C loop temperature, suggested that (1) a ratio of 80:20 ammonium acetate (6.5 mM, pH 10): acetonitrile at a flow rate of 2.5 mL/min for the HPLC purification step, and (2) a two mL ethanol volume followed by a 1 mL water for the Sep-Pak C₁₈ Plus Light Cartridge purification step gave the best results, i.e., no [^{11}C]CH₃I and (+)nornicotine and a 19% EOB radio-chemical yield. However, the total ethanol content in the final sample was ~66%. This amount of ethanol is acceptable for administration by inhalation, however, is not suitable for i.v. administration.

To achieve lower ethanol content in the final [^{11}C]nicotine sample, the purification procedure was modified as mentioned in the experimental section above (second method). In the modified method 6.5 mM ammonium acetate pH 10 buffer and acetonitrile were substituted by water (pH 10 adjusted with NaOH) and ethanol, respectively, however, the ratio of water and ethanol was kept 80:20. To compensate for the polarity difference between acetonitrile and ethanol and keeping the purification run time within 10 minutes, the flow rate was increased to 5 mL/min. Under these conditions, the retention times for [^{11}C]CH₃I, nornicotine, and [^{11}C]nicotine were 3.1, 3.8, and 8.2 min, respectively. The 5.0 mL HPLC purified fraction containing the product was transferred directly into a sterile vial, fitted with a 0.22 μm sterile filter, containing 1.0 mL of 300 mM phosphate buffer and 900 mM NaCl at a predefined pH. The final [^{11}C]nicotine sample had 50 mM PBS concentration, 16.6% ethanol and pH between ~7.0-7.2. Using the modified mobile phase and eliminating the Sep-Pak C₁₈ Plus Light purification step, the total time for the synthesis and purification of [^{11}C]nicotine was reduced to <30 minutes. This is the shortest synthesis time reported so far.^{14,23, 35} The amount of ethanol in the final formulation can be further reduced by decreasing the amount of ethanol in the mobile phase and increasing the flow rate during purification. For example, by using the 90:10 (water:ethanol) composition of the mobile phase (at pH 10), a flow rate of 10 mL/minute for HPLC purification, and by diluting 5 mL purified fraction with 1 mL 300 mM phosphate buffer containing 900 mM NaCl would give a 8.3% ethanol concentration in the final formulation.

Involvement of mg quantities of (+) or (–) nornicotine, a requirement of HPLC purification, and volatility of [^{11}C]nicotine free base stimulated optimization of various process parameters, i.e., the mass of the precursor, the loop temperature, and the reaction time. Uncorrected (EOS, end of synthesis) and EOB radiochemical yields (RCY) of the synthesis under various conditions are summarized in Table 1.

Initially, 2 mg precursor was reacted with [^{11}C]CH₃I in the loop at 25°C and 125°C for 1, 3, and 5 minutes. The EOB radiochemical yields of the synthesis were between 10 to 17%. 0.5, 1, and 2 mg precursor loaded in the loop were reacted with [^{11}C]CH₃I at 75°C for 5 minutes. Data given in Table 1 and Figure 2 demonstrated a linear increase in the radiochemical yield with the increased amount of the precursor. A comparison of the percent EOB radiochemical yield of the synthesis of [^{11}C]nicotine at various temperatures can be made as, 12.3, 19.0, 13.4, and 18.9 at 25, 75, 100, and 125°C, respectively. It appears that raising the radiochemical synthesis temperature above 75°C does not improve the radiochemical yield, leading to the conclusion that the best radiochemical yield can be achieved by the reaction of 2 mg (+) nornicotine with gas-phase produced [^{11}C]CH₃I at 75°C for 5 minutes (i.e., 19%). These conditions gave ~ 50-70 mCi of the radiolabeled product on a routine basis. Additional experiments conducted by using similar synthesis conditions but using method 2 for purification gave significantly higher yields, i.e., up to 35% (EOB). Similar or higher radiochemical yields have been achieved in the past by using wet method and using [^{11}C]-methyl triflate method (Maziere et al. 1976; Halldin et al. 1992; Sihver et al. 1998, 1999; Langstrom et al. 1982; Xu et al. 2016). Lower yield by method 1 may be either due to variation in the radiochemical yield or loss in the purification method.

Quality Control Methods and Specific Activity of [^{11}C]Nicotine

Normal-phase and reversed-phase analytical HPLC methods (with 15 to 30 minutes run times) (Saji et al. 1992; Halldin et al. 1992; Xu et al. 2016) were used in the past for the analysis and quality control of [^{11}C]nicotine samples. Given the short half-life of ^{11}C (20 minutes), it was essential to develop an RP-HPLC method with a shorter run time than reported previously. An analytical RP-HPLC method with short run time, <10 minutes (using Water Xterra C₁₈ column, 150 × 4.6 mm, 5 μm, 80% 6.5 mM ammonium acetate at pH 10 and 20 % acetonitrile mobile phase, flow rate of 1 mL/min) was found suitable for the determination of radiochemical purity of [^{11}C]nicotine and the amount of unlabeled nicotine in the samples. Figure 3 shows the analytical HPLC chromatograms for a [^{11}C]nicotine sample, the nicotine standard, the (+) nornicotine precursor, and a [^{11}C]CH₃I sample, with excellent separation (retention times in minutes in the parenthesis) between [^{11}C]CH₃I (2.4), (±) nornicotine (3.1), and [^{11}C]Nicotine/nicotine (5.5/6.0). The method was validated (Ghosh et al. 2017) for specificity, linearity, range, accuracy, precision, and the LOD, and the LOQ per the ICH guidelines (ICH Q2 (R1) 2005) and used to analyze [^{11}C]nicotine samples. Typically, ^{11}C -nicotine was produced with >99.9% radiochemical purity (RCP), no [^{11}C]CH₃I, no (+) nornicotine was observed.

Specific activity for a radiotracer is a ratio between the amount of radioactivity (i.e., specific radioactive molecule) and the number of total molecules (i.e., radioactive and stable molecules expressed as mass or molar quantity). A linear correlation, i.e., a calibration curve,

between UV peak areas and the amount of nicotine injected onto an HPLC column (slope = 97404 Peak Area/mg of nicotine, $r^2 = 0.9999$) is shown in Figure 4. From the UV peak area of a [^{11}C]nicotine sample and the slope of the curve the amount of nicotine followed by the specific activity was calculated as 105 mCi/ μmole . Higher specific activity have been achieved in the past by using a wet method or using [^{11}C]methyl triflate for *N*-methylation (Halldin et al. 1992; Sihver et al. 1998, 1999; Garg et al. 2017; Xu et al. 2016).

[^{11}C]Nicotine PET imaging of nicotinic acetylcholine receptors (nAChRs) is a target-binding based technique and high specific activity of the tracer is important. Additional experiments are planned in the future, in our laboratories, to improve the specific activity.

Synthesis of [^{11}C]-labeled radiotracers in high radiochemical yields with high specific activity is always challenging. Theoretical specific activity for ^{11}C is very high (e.g. 341.1 TBq/ μmol or 9219 Ci/ μmol), however, the specific activity of synthesized ^{11}C -labeled radiotracers, in general, is by far lower than the expected value. The actual radiochemical yield and specific activity achieved depend on the radionuclide production methods and radiochemical synthesis line procedures. The main source of stable carbon, responsible for the reduction in specific activity, could be from the target, the target gas (a mixture of 99% nitrogen and 1% oxygen), helium, and hydrogen. High-purity gases were used in the present work to minimize the amount of carbon (e.g. < 0.1 ppm CO_2 and < 0.1 ppm CO in each helium). Additional optimization experiments are being conducted for improvement of the specific activity.

Historically, [^{11}C]CH $_3$ I is produced by LiAlH $_4$ reduction of [^{11}C]CO $_2$ followed by hydroiodic acid reaction. It has been suspected that the LiAlH $_4$ can absorb CO $_2$ from the atmosphere or carrier carbon dioxide most likely can be originated in the methanol formed from traces of tetrahydrofuran remaining in LiAlH $_4$ after the evaporation step. The gas-phase production of [^{11}C]CH $_3$ I, eliminates all these challenges and appears to be superior to the LiAlH $_4$ method.

In addition to the radiochemical purity and specific activity, other test methods were developed and established for quality control of [^{11}C]nicotine. These include: testing for radionuclidic purity, pH, bacterial endotoxin (USP <85>) and sterility (USP <71>). A summary of these testing results with specifications is given in Table 2.

Conclusion

A rapid, efficient, and automated loop method (<30 minutes synthesis time) for the radiochemical synthesis of [^{11}C]nicotine, from the reaction of (+) or (–) nornicotine free base and gas-phase produced [^{11}C]CH $_3$ I, was developed and optimized. This method produces [^{11}C]nicotine in 19 to 35% radiochemical yield (EOB) by using method 1 and 2 for purification, respectively, high radiochemical purity (>99.9%), and specific activity, (105 mCi/ μmole). Lower yield by method 1 may be either due to variation in the radiochemical yield or loss in the purification method. The process is being used routinely in our laboratories for the production of [^{11}C]nicotine for pre-clinical testing in animal models. Experiments are in progress for improving the specific activity of the final product. An analytical HPLC method with a short run time was developed and validated for quality

control of the material produced. The material produced pass all tests required for imaging pharmaceuticals. The automated loop method for synthesis can be translated for GMP production of [^{11}C]nicotine with lower ethanol content (<10%) for human clinical trials.

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highlights

- A novel, rapid, and automated loop method for synthesis of [^{11}C]nicotine is reported.
- The reaction involves gas-phase produced $^{11}\text{CH}_3\text{I}$ and base form of (+) nornicotine or (–) nornicotine.
- The optimized process produced [^{11}C]nicotine with >99.9% Radio Chemical Purity (RCP), no [^{11}C]CH $_3\text{I}$, no (+) nornicotine.
- An analytical HPLC method with a short run time was developed.
- The automated loop method for synthesis can be translated for GMP production of [^{11}C]nicotine with lower ethanol content (<10%) for human clinical trials.

Figure 1.
Flow diagram of the Automated Synthra MeI Plus Loop System (courtesy Synthra GmbH).

Figure 2.
Correlation Between Corrected (EOB) Radiochemical Yield With the Amount of the Precursor, (+)Nornicotine Base, Used.

Figure 3.
Analytical RP-HPLC chromatograms of (a) ^{11}C -Nicotine (b) Nicotine standard (c) (+) Nornicotine Precursor and (d) $^{11}\text{CH}_3\text{I}$.

Figure 4.
A Linear Correlation Between Peak Areas and the Amount of Nicotine.

EOS and EOB Radiochemical Yields for the Synthesis of [^{11}C]Nicotine Under Various Experimental Conditions**Table 1**

Run	Precursor, mg	Reaction time, min	Temperature, °C	EOS RCY [*] , %	EOB RCY [*] , %
1	2.0	1	25	0.00	0.00
2	2.0	3	25	4.3	17.0
3	2.0	5	25	3.6	12.3
4	0.5	5	75	2.4	8.7
5	1.0	5	75	3.5	13.0
6	2.0	5	75	4.6	19.0
7	2.0	5	100	3.5	13.4
8	2.0	1	125	3.5	12.1
9	2.0	3	125	2.9	10.0
10	2.0	5	125	4.1	18.9

* RCY = Radiochemical Yield

Table 2 Summary of Analytical Testing Results of [^{11}C]Nicotine

Test	Method	Specification	Result
Appearance	Visual	Clear and Colorless Solution	Clear and colorless solution
Identification	HPLC/ID	Retention times of product and standard match + 0.5 minute	Retention times of product and standard were within + 0.5 minute
pH	pH	pH between 6.8 and 7.4	pH between 7.0 and 7.2
Radiochemical and Chemical Purity	HPLC	Purity >95%	Purity > 99.9%
Radionuclidic Purity	HPLC Spectral Scan	Peak at 511 keV characteristic of C-11 PET	Confirmed 511 keV peak
Specific Activity	HPLC	>100 mCi/ μmole	105 mCi/ μmole
BET	USP <85>	175/mL	Passed <1.00 EU/mL
Sterility	USP <71>	Meets the USP requirements for sterility	Sterile - No growth observed in 14 days