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ORIGINAL ARTICLE

A Practical and Pyrogen-Free Preparation of ^{11}C-L-Methionine in a Good Manufacturing Practice-Compliant Approach

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Aims: ^{11}C-L-methionine, an amino acid tracer used to delineate certain tumor tissues, has proven to be a prevailing nonfluorodeoxyglucose positron emission tomography (PET) radiopharmaceutical. We intended to prepare ^{11}C-L-methionine by following modified synthetic strategies at a rebuilt working area to meet the PET drug current good manufacturing practice (cGMP) and Pharmaceutical Inspection Co-operation Scheme (PIC/S) regulations. Furthermore, we overcame the problem of pyrogen cross-contamination using a cleaner and more efficient program. **Material and Methods:** The task of upgrading air filtration equipment was integrated with the set of Web-Based Building Automation system (WebCTRL®). ^{11}C-L-methionine synthesis was carried out in accordance with redesigned methods to meet the requirements of PET drug cGMP. The product quality was tested by a series of quality control tests and was found to be satisfactory. Depyrogenation was carried out by three different methods with different flow rates and flushing durations. The results were examined through limulus amebocyte lysate clotting test. **Results:** The level of air cleanliness in each section meets the PIC/S GMP standards after the reconstructions. Moreover, after delicate modifications, the radiochemical yield of ^{11}C-L-methionine was 36.20% ± 3.59% (based on ^{11}C-CH_3I, $n = 7$), which is about 10% higher than the average former yield. Besides, the used depyrogenation methods could wipe the bioburden off within 8 h. **Conclusions:** The modifications done not only offer a good production environment but also protect the products from contamination. The modified approaches in both ^{11}C-L-methionine production and depyrogenation resulted in prominent progress in stability and efficiency as well.

Key words: Positron emission tomography, ^{11}C-L-methionine, depyrogenation, good manufacturing practice

INTRODUCTION

^{11}C-L-Methionine is used clinically in positron emission tomography (PET), detection of brain tumors,[1] and imaging several malignancies.[2-5] The default method of ^{11}C-L-methionine synthesis is complicated, and some of the reagents are highly sensitive and easily degraded. Furthermore, some of the steps are not compliant with the quality assurance (QA) requirements of PET drug current good manufacturing practice (cGMP).[6,7] With respect to the aseptic issues, the old program could not guarantee a thoroughly sterile production system. Hence, in this study, we synthesized ^{11}C-L-methionine by a modified protocol designed to overcome these obstacles.

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MATERIALS AND METHODS

Reconstruction of the working area and the cleanliness test

To meet the requirements of Pharmaceutical Inspection Co-operation Scheme (PIC/S) GMP, we redesigned our working area [Figure 1] and divided it into eight isolated sections. Then, we updated the air handling units, components of air circulation, high-efficiency particulate arrestance (HEPA) filters, and the sensors that were controlled by the central control system. The Web-Based Building

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Figure 1: The redesigned clean rooms and the personnel flow. The reconstructions featured clear separation between different work areas and resulted in routes designed according to the principles of good manufacturing practice to keep off the cross-contamination. Furthermore, we set the environmental sensors to monitor the temperature, humidity, and pressure in each section. Information was recorded by Web-Based Building Automation system (WebCTRL®)

Automation system (WebCTRL®) was built by automated logic corporation.

The validation processes

After finishing the rebuilding work, we performed the validation processes including airborne particle count test, airflow test, air pressure differential test, filter leakage test, temperature test, humidity test, and settle plate test. In accordance with the standard operating procedure, we did the annual maintenance tests 1–4 times regularly. In addition, our measurement devices (i.e., the particle counter and air speed meter) were qualified by third party companies or laboratories.

Reagents and apparatus

Reagents and solvents were purchased from Aldrich and used without further purification. High-performance liquid chromatography (HPLC) analyses (Waters Corporation) were carried out with both ultraviolet and radioactivity detectors. ^{11}C-L-methionine was synthesized with a GE TRACERlab C module, which is an automatic synthesizer used in the production of PET drugs for years. ^{11}C-CO_2 was generated in an IBA cyclone 18/9 cyclotron through the ^{14}N (p, α)^{11}C nuclear reaction. The synthesizer was operated according to the modified sequence based on the wet method.[8-14] The quality control of ^{11}C-L-methionine followed all testing items and procedures listed in the United States Pharmacopeia.[15]

Small commercial package reagents for single use

In the previous protocols, at the reduction reaction step, we had to dilute high concentrations of lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF) solution to meet our requirements. This step was time-consuming, and the quality of the diluted solution was not always consistent. To improve the production stability, the procedure was delicately modified to include adding a small volume (0.5 M, 1 mL) of the commercial package of $LiAlH_4$ from ABX into the reaction vessel before transferring the ^{11}C-CO_2.

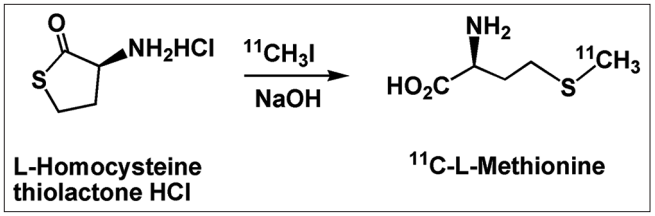
Changes in iodination step

The default configurations of triphenylphosphine diiodide (Ph_3PI_2) and silver trifluoromethanesulfonate (AgOTf) solid-phase extraction (SPE) cartridges were cancelled and 57% hydriodic acid solution was used to accomplish this step instead. After the ^{11}C-CO_2 was transferred to ^{11}C-CH_3OH through the modified reduction reaction procedure, the reaction vessel was heated to 160°C, and 0.5 mL of hydriodic acid was added to form the ^{11}C-methyl iodide (^{11}C-CH_3I). The modified method for the production of ^{11}C-methionine based on GE TRACERlab C module is illustrated in Scheme 1. The reaction steps are described briefly below:

1. After the bombardment, ^{11}C-CO_2 was reduced in the reaction vessel by a solution of $LiAlH_4$ in THF (0.4 mL, 0.1 M)
2. Hydriodic acid (0.5 mL) was added to the reaction vessel to form ^{11}C-CH_3I
3. Five milligrams of L-homocysteine thiolactone. HCl in 0.4 mL of 0.3 M sodium hydroxide reacted with ^{11}C-CH_3I to yield ^{11}C-methionine
4. The crude product was purified by a semi-preparative HPLC system and passed through a 0.22-μm filter for further injection purposes.

Depyrogenation test

HPLC contamination is always a troublesome laboratory issue, and the cleanup procedure is time-consuming. To



Scheme 1: Production of ^{11}C-L-methioine

find an easy and efficient way to wipe off the bioburden, we examined three approaches (methods A, B, and C) with different flow rates and flushing durations, using common reagents (75% ethanol, water, and acetic acid), and checked the results using limulus amebocyte lysate (LAL) clotting test. In these approaches, we used certified standard endotoxin as the positive control (pyrogen-contaminated sample) and LAL reagent water as the negative control.

RESULTS

The improvement of the level of air cleanliness

After reconstructing our working area, it totally had eight chambers: anteroom 1, buffer room 1, buffer zone, anteroom 2, buffer room 2, manufacturing area, aseptic preparation area, and aseptic preparation area in isolator. Each room was equipped with independent HEPA filters and sensors that could send environmental parameters back to the WebCTRL®. In addition, the improved monitoring functions of the new control system provided instant automated warnings if any abnormality occurred [Figure 2]. As shown in Table 1, the data obtained from particle counting demonstrate that the level of air cleanliness in each section meets the PIC/S GMP standards.

The optimization of ^{11}C-L-methionine-automated synthesis

The radiochemical yield of ^{11}C-L-methionine was improved by the modified protocol to 36.20% ±3.59%, which is higher than the former yield by about 10% ($n > 20$). In addition, the radiochemical purity and specific activity reached 99.68% and 1510.85 Ci/mmol (based on $^{11}C\text{-}CH_3I$, $n = 7$), respectively, in a relatively short synthetic time of 20 min from end of bombardment. Therefore, we optimized the automated synthetic procedure and furnished a higher yield and a more stable product through these improvements.

Depyrogenation method

We examined three different procedures in the depyrogenation step and found that the three procedures worked well as all the final samples passed the LAL clotting test. Surprisingly, we found that method C could efficiently eliminate the bioburden within 8 h. The detailed methods are described in Table 2.

DISCUSSION

Good manufacturing practice-compliant environment

Our facility, built in 1992, has been offering good clinical care to patients for over 10 years. However, the performance of old

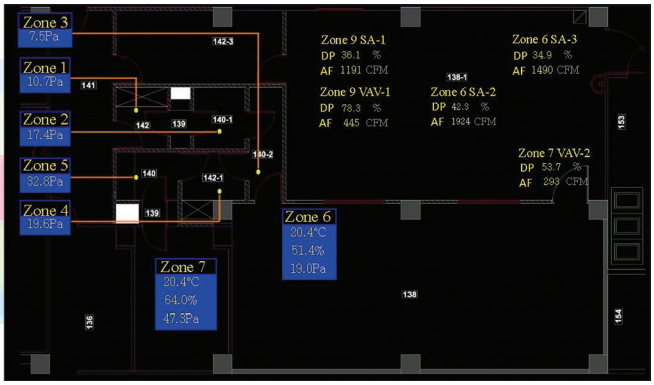


Figure 2: Air filtration and management systems (WebCTRL®) at our facility. Through the monitor, we could have good control of all environmental factors and get alerts instantly if any equipment goes wrong. Besides, all information would be recorded in the database for further analysis

Table 1: Comparison between the level of air cleanliness at our facility and the requirements of Pharmaceutical Inspection Co-operation Scheme good manufacturing practice

Zone	Clean room sections	Tested level (grade)	PIC/S GMP (grade)	Particle number (size ≧0.5 μm)	Particle number (size ≧5.0 μm)
1	Anteroom 1	A	D	159.7	7.3
2	Buffer room 1	A	D	49.3	0.7
3	Buffer zone	A	C	43.7	1.7
4	Anteroom 2	A	C	65.3	2.3
5	Buffer room 2	A	C	34.3	2.3
6	Manufacturing area	A	C	72.2	4.6
7	Aseptic preparation area (background)	A	B	8.5	0.3
8	Aseptic preparation area (isolator)	A	A	0.8	0

According to the results, the cleanliness in all sections met the regulations of the guide to GMP for medicinal products annexes, PIC/S GMP 2015, after the reconstructions. For Grade A/B/C/D (at rest), the maximum permitted number of particles/m^3 is 3520/3520/352,000/3,520,000 for particle size ≧0.5 μm and 20/29/2900/29,000 for particle size ≧5.0 μm, respectively. GMP=Good manufacturing practice; PIC/S=Pharmaceutical Inspection Co-operation Scheme

air filtration equipment diminished gradually despite on-time maintenance. In addition, the flows of material and personnel were not organized. In our new requirements, the materials' flow is allowed only in one direction. The order is Zone 8, Zone 9, passing box, Zone 6, Zone 7, passing box, and finally the injection room. The same order applies for the personnel flow. The paths joining Zone 6 and Zone 7 are unidirectional as well. All staffs need to change their clothes to the first cleanroom suit at Zone 1. Furthermore, the staffs responsible for drug dispensing at Zone 7 need to wear the second cleanroom suit at Zone 4. These rules ensure a high level of air cleanliness and effectively avoid cross-contamination (Zone 7 exhibits the highest level of air cleanliness). Moreover, the set of WebCTRL that integrates all environmental information (temperature, humidity, pressure, and conditions of supplied air) made the management easier to control. All these factors could ensure a high-quality manufacturing environment in our facility.

Modified method of ^{11}C-L-methionine synthesis

According to the original instructions given by the provider, some reagents used in ^{11}C-L-methionine preparation should be treated before being loaded in the synthesizer, such as Ph_3PI_2 on Al_2O_3 and AgOTf cartridges that were used to produce ^{11}C-Met and ^{11}C-MeOTf, followed by coupling with the precursor to afford the crude ^{11}C-L-methionine. Nevertheless, those two materials were extremely sensitive and highly susceptible to degradation while preparing the SPE cartridges (weighing it, filling it in the cartridge, and sealing the cartridge). Thus, the qualities of those materials were not consistent among the steps of the synthesis and completely depended on the handling skills of the personnel. This does not agree with the principles of QA. To overcome this problem, we developed and reschemed the protocols, using 57% hydriodic acid as the iodination reagent to convert ^{11}C-CH_3OH to ^{11}C-CH_3I (commercialized package, directly added in reaction vessel without further process). The commercially available hydriodic acid has packed in a light-protective bottle and stored at 2–8°C. Both hydriodic acid and $LiAlH_4$ (0.5 M in THF, 1 mL) were purchased from ABX for single use to assure their quality. More importantly, $LiAlH_4$/THF must be loaded to the reaction vessel only after purging the vessel with helium gas. Eventually, the yield of the final product increased by 10%, and the average standard deviation decreased by 6.5% in comparison with the previous results. In addition, the stability of the products was improved. The comparison between the default and selected reaction reagents is shown in Table 3.

New depyrogenation method

Pyrogen contamination is a tedious problem in radiopharmaceutical production. Although there are several methods proposed to address this problem,[16] it is still difficult to be solved in a quick and effective way. Our experience showed that 0.1–1.0 M sodium hydroxide solution could be used to avoid pyrogen contamination. Nonetheless, this method was not suitable due to the low tolerance to high pH in the reverse phase column (VP 250/16 NUCLEOSIL 100-7 C18). Several trials were done using 1% acetic acid solution to cope with this issue. Finally, method C was found to be the best procedure to accomplish the preparation of ^{11}C-L-methionine without pyrogen contamination. Not only did it solve the pyrogen contamination problem but also it completed the whole depyrogenation procedure within 8 h, which demonstrates how this approach is timesaving.

Table 2: Methods for cleaning up pyrogen contamination

Solvents	Flow rate/time		
	Method A	Method B	Method C
75% ethanol	2.5 ml/min for 2 h	2.5 ml/min for 2 h	2.5 ml/min for 2 h
Water	4 ml/min for 2 h	4 ml/min for 2 h	4 ml/min for 1.5 h
Acetic acid	1 ml/min, overnight	1 ml/min for 6 h	2.5 ml/min for 2 h
Water	4 ml/min for 2 h	4 ml/min for 2 h	4 ml/min for 1.5 h
Pyrogen test	Negative	Negative	Negative

We developed three methods of different flow rates and flushing durations to decontaminate the HPLC column from pyrogen burden. The results showed that all methods were effective in decontaminating the HPLC column, but method C was the most efficient approach. HPLC=High-performance liquid chromatography

Table 3: Comparison between the synthetic agents utilized in the default and modified methods of ^{11}C-methionine production

Method type	Conventional	Modified
Reduction agent	$LiAlH_4$	$LiAlH_4$
Iodination agent	Ph_3PI_2 on Al_2O_3	Hydriodic acid
Other agent	Silver triflate	-
Reaction phase	Solid	Aqueous

In our modified method, aqueous hydriodic acid was used to replace Ph_3PI_2 and silver triflate filled in the cartridges for better stability and yield. Ph_3PI_2=Triphenylphosphine diiodide; $LiAlH_4$=Lithium aluminum hydride; Al_2O_3=Aluminum oxide

CONCLUSIONS

The modifications in production environment not only provide a GMP compliant manufacturing areas to produce the guaranteed clinical radiopharmaceuticals but also improve the level of air cleanliness. The modified approaches guarantee a stable production of PET drugs and offer an efficient way to prominently remove the possible pyrogen contaminations.

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Conflicts of interest

There are no conflicts of interest.

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