Original Article

# Establishing the [$^{18}$F]-FDG Production via Two Different Automated Synthesizers for Routine Clinical Studies: Our Institutional Experiences of 4 years

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## Abstract

**Introduction:** [$^{18}$F]-Fluorodeoxyglucose ([$^{18}$F]-FDG) is the most widely used positron-emission tomography tracer used for imaging in clinical studies such as early detection of cancer or its malignancies, quantifications, staging, and restaging of several malignancies. For clinical application, routine production of this tracer is mandatory in compliance to regulatory guidelines. Several dedicated commercial synthesizers are currently used for producing[$^{18}$F]-FDG for clinical usage. Being at hospital radiopharmacy, it is our responsibility and duty to support the clinical service with uninterrupted production and supply of [$^{18}$F]-FDG. This document describes the production of [$^{18}$F]-FDG using two different automated synthesizers in terms of its production yield, time of synthesis, and analyze the quality control (QC) of the produced [$^{18}$F]-FDG. **Materials and Methods:** The precursor, mannose triflate ultra-pure, authentic nonradioactive standard FDG and [$^{18}$O]-water were obtained from ABX, Germany. Solvents and reagents were purchased from Sigma Aldrich India Ltd. and Fisher Scientific India Ltd., (Mumbai, Maharashtra, India). **Results:** The protocol developed for the synthesis with MPS-100 synthesizer yield of [$^{18}$F]-FDG is approximate about 45% End of Bombardment (EOB) with synthesis time of around 35 min, whereas with F300E synthesizer it is around 60% with synthesis time of 25 min. The quality of the tracer produced by both synthesizers is at par with the QC parameter for clinical applications. **Conclusions:** Finally, we have developed the production using two automated synthesis modules which have the capability to produce [$^{18}$F]-FDG, to do the patient studies in good yield and purity. Our protocol is simple, reproducible, and robust.

**Keywords:** *Automation, fluorodeoxyglucose, quality control, synthesizer*

## Introduction

2-[$^{18}$F]-Fluoro-2-deoxy-d-glucose (2 [$^{18}$F] FDG) commonly called fluorodeoxyglucose also abbreviated as 18F FDG or FDG, is one of the most important and Food and Drug Administration (FDA) approved radiopharmaceutical used for the measurement of glucose metabolism using positron emission tomography (PET).[1a-c] It has been used in the diagnosis, staging, and restaging of several clinical conditions such as head and brain cancer, lung cancer, lymphoma, colorectal cancer, melanoma, and neck and breast cancer.[2]

Synthesis of $^{18}$F-FDG is well documented in the literature, but every radiopharmacy has its own challenges to achieve as the cost to be borne by the patients. At hospital pharmacy, the daily challenges to encounter for $^{18}$F-FDG production are: (i) need for rapid and reliable manufacturing of $^{18}$F-FDG, (ii) radiation safety due to the use of multicurie amount of $^{18}$F-produced through proton bombardment of enriched [$^{18}$O]-$H_2O$, (iii) followed current good manufacturing practice (cGMP) as recommended by regulatory bodies (FDA 21CFR212, EU), and (iv) cost-effective to be bear by patients.[3,4]

Currently, the production of $^{18}$F-FDG is exclusively shifted to automated from earlier manual protocols, which compiles the clinical use of it as a generic drug in accordance with 21CFR212 (FDA).[4-6] Various commercial dedicated automated radiosynthesizers such as Tracsis $^{18}$F-FDG QUAD, GE TracerLab MX, GE FASTlab, Bioscan, Inc. "F18-Plus" and IBA Synthera, Sumitomo's F300, etc., are designed to produce $^{18}$F-FDG as per the GMP standards, and it complies the quality controls (QC) tests. These synthesizers are mostly cassette-based modules, run by software template, that are sterile,



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manufactured according to cGMP and are compatible with validated clinical procedures and used for producing large quantity of $^{18}$F-FDG. In addition, the transition to automated systems ensures safety to radiochemists while adhering to as low as reasonably achievable principle during producing excessive larger quantity of production as some center demands due to high patient studies.

With increasing demand for [$^{18}$F]-FDG for human use, production methodologies require short synthesis times with high yield, followed by a fast QC. The first proposed synthesis was documented in 1976,[7] since then, various routes of production and improved yield were well documented.[8-10] Now, the widely used chemistry protocol is the nucleophilic substitution method, followed by an alkaline hydrolysis and subsequent solid phase extraction cartridge purification to afford the [$^{18}$F]-FDG, this all requires about 30 min.[11] All the commercially available synthesis modules work on the above mentioned chemistry mechanism with variation in purification methodology. The purification methods are mostly designed to remove toxic chemical impurities which comprise [$^{18}$F] fluoride, Fluorodeoxy mannose (FDM), partially or fully acetylated glucose molecule ([$^{18}$F] ACY), Kryptofix 2.2.2, and/or acetonitrile (or other residual solvents).

We established the routine production of $^{18}$F-FDG as required by our hospital; for this purpose, we optimized

**Table 1: Quality Control Parameters**

| Quality Parameter | Specification | Methods |
|---|---|---|
| Appearance | Colourless or slightly yellow solution | Visual inspection |
| Identity (radiochemical and radionuclidic) | The radionuclidic and radiochemical identity are combined in the following fashion: Either tests A and C, or B and C may be applied. | Gamma spectrum using gamma spectrometer |
| | A. Gamma ray spectrum exhibits a major peak of 511 keV | Dose calibrator or gamma counter |
| | B. The half-life is between 105-115 min | TLC with radioactivity scanner |
| | C. Distribution of radioactivity on a TLC strip corresponds to FDG | |
| Radionuclidic purity | Not less than 99% of total radioactivity is due to 18F. | Gamma spectrometer |
| Radiochemical purity | Not <95% of total radioactivity in the test chromatogram corresponds to FDG. | TLC with radioactivity scanner |
| pH | pH value, 4.5-8.5 | pH paper (validated with pH meter) |
| Chemical purity: Kryptofix 2.2.2 | Not more than 0.22 mg/mL* | TLC |
| 2-Chloro-2-deoxy-D-glucose | Not more than 1 mg/mL | HPLC |
| Filter Integrity | >50 psig | Bubble point test |
| Bacterial endotoxins | Not more than 17.5/mL | LAL (Limulus amoebocyte lysate) |
| Sterility | Must be sterile | Microbial growth in culture media |

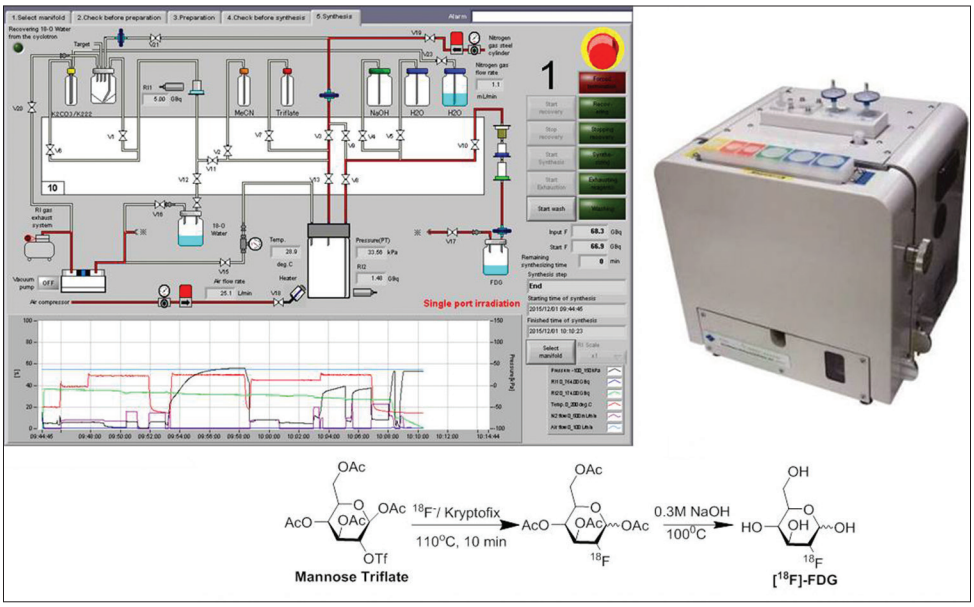


**Figure 1: Software template screenshot and image of the automated synthesis of [18F]-FDG in F300E synthesizer**

the production and quality assurance of $^{18}$F-FDG using two automated synthesizers. We, at our center, have two different synthesizers, manufactures by Sumitomo Heavy Industries Limited, Japan, of which one is a fully dedicated synthetic module for $^{18}$F-FDG production named as F300E module and another one is a multipurpose synthesizer termed as MPS-100 with an option to designed chemistry template for $^{18}$F-chemistry as per user need. In this paper, we document our experience in synthesizing the $^{18}$F-FDG by two automated modules and comparing their results in terms of synthesis time, yield, user friendliness, and QC test of the final product.[12]

## Materials and Methods

The precursor, mannose triflate ultra-pure, authentic nonradioactive standard FDG and [$^{18}$O]-water were obtained from ABX, Germany. Solvents and reagents were purchased from Sigma Aldrich India Ltd., and Fisher Scientific India Ltd., (Mumbai, Maharashtra, India). The chemicals were used without any further purification and were commercially available. The United States Pharmacopeia (USP)-grade 0.9% NaCl and sterile water for injection were purchased from B. Braun Melsungen AG, Melsungen, Germany. SEP-PAK cartridges (light QMA cartridges, PS2 cartridges, and Alumina N plus cartridges) were achieved from Waters India Ltd. The Maxi-Clean IC-H cartridges were purchased from Grace Davison Discovery Science, USA. Before they were used, light QMA cartridges were conditioned with 10 ml of 80% ethanol (EtOH), followed by 10 ml of 0.1M $K_2CO_3$ solution, followed by 60 ml of water ($H_2O$), PS2, Alumina N plus, and IC-H cartridges were preconditioned with 10 ml of 80% ethanol (EtOH), followed by 40 ml of $H_2O$. High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1260 HPLC system equipped with a Refractive Index detector (Model 35900E, Agilent, USA) and a radiation detector connected in series. Agilent ZORBAX Eclipse XDB-C18 analytic column (4.6 mm × 150 mm, 5 µm) was eluted with at flow rate of 0.4 ml/min. Radio-thin layer chromatography was carried out using aluminum sheets precoated with silica gel 60 F254 (E. Merck, Germany) (the volume ratio of $CH_3CN$ to $H_2O$ equals 95:5) chromatograms of [$^{18}$F] FDG samples. The apyrogenicity was done using the Charles River EndoSafe Portable Testing System instrument which works on the kinetic chromogenic limulus amebocyte lysate method as specified in the EP/USP.

| Name of synthesizer | F300E | | | MPS-100 | | |
|---|---|---|---|---|---|---|
| Batch Number / Test Performed | FDG-001A | FDG-002A | FDG-003A | FDG-001A | FDG-002A | FDG-003A |
| Particulate Test | clear and colorless | clear and colorless | clear and colorless | clear and colorless | clear and colorless | clear and colorless |
| pH | 7 | 7 | 7 | 7 | 7 | 7 |
| Filter Integrity (psig) | 55 | 60 | 60 | 55 | 58 | 57 |
| Kryptofix [2.2.2] µg/mL | <50 | <50 | <50 | <50 | <50 | <50 |
| RTLC ($R_f$) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Radio Chemical yield | 98.6 | 97.65 | 98.2 | 97 | 98 | 98 |
| Radio Chemical purity (%) | 97.8 | 99.7 | 98.3 | 98 | 98 | 98 |
| Half-life (min) | 110 | 110 | 110 | 110 | 110 | 110 |
| Measured Endotoxin (EU/mL) | <2 | <2 | <2 | <2 | <2 | <2 |

**Figure 2: Quality test of the [18F] FDG synthesized via the automated synthesizer**

## Results

The different quality tests performed for each batch of 18F-FDG produced through both the machines were summarized [Table 1]. All the parameters of the different quality tests comply the standard set by the various regulatory agencies for clinical usage. Both synthesizers produced the final product in more than 95% radiochemical purity. The program template designed for MPS-100 also complies the test parameter set for the QC data to product required yield and purity of the final product.

With the installation of the cyclotron facility as well radiochemistry facility in the year 2015, we served thousands of patients every year with [$^{18}$F]-FDG based PET studies and these production protocols help us to serve the patients uninterrupted. So far, we performed more than 5000 PET scans and more than 700 syntheses using both the synthesizer. The number of PET scans as well as the number of successful synthesis runs self explain the robustness and reliability of the machine as well as chemistry protocol.

## Discussion

### Automated synthesis of $^{18}$F-FDG using F300 synthesis module

The synthesis of $^{18}$F-FDG was performed in this dedicated synthesizer. The system was configured with locked synthesis software template, our first priority to establish the synthesis of $^{18}$F-FDG and access its quality for clinical application. We performed the optimization runs using the chemistry protocol as designed by the manufacturer. The synthesis starts with pre-checking of the system internal parameters as such as temperature of reactor, pressure, flow of dry Nitrogen and air, on off of respective valve for flow and leak detection. As the system QC passed, it is ready for the $^{18}$F-FDG synthesis. The system received the required no carrier-added [$^{18}$F]-fluoride in [$^{18}$O] $H_2O$ from an $^{18}$O (p, n) $^{18}$F reaction through in-house cyclotron (Sumitomo's HM-18) connected through internal delivery line. [$^{18}$F] fluoride delivered from a cyclotron was trapped on a preconditioned QMA cartridge and was eluted with $K_2CO_3$: Kryptofix 2.2.2 solution (0.2 ml in $H_2O$: 0.7 ml in dry acetonitrile, phase transfer catalyst), marked as yellow neck vial $K_2CO_3$/K2.2.2 [Figure 1].

The solution was azeotropically dried by stream of dry nitrogen and negative pressure to form dried $KF^{18}$ at 100°C for 8 min by adding dry acetonitrile (0.2 ml); the fluorination was done by adding mannose triflate (20

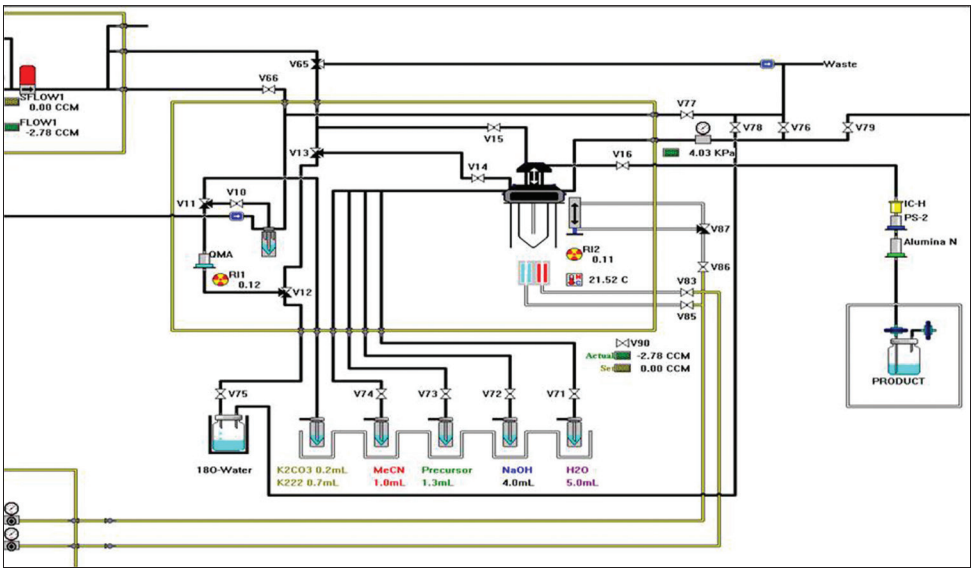


**Figure 3: Software template screenshot for MPS-100 18F-FDG synthesis scheme**

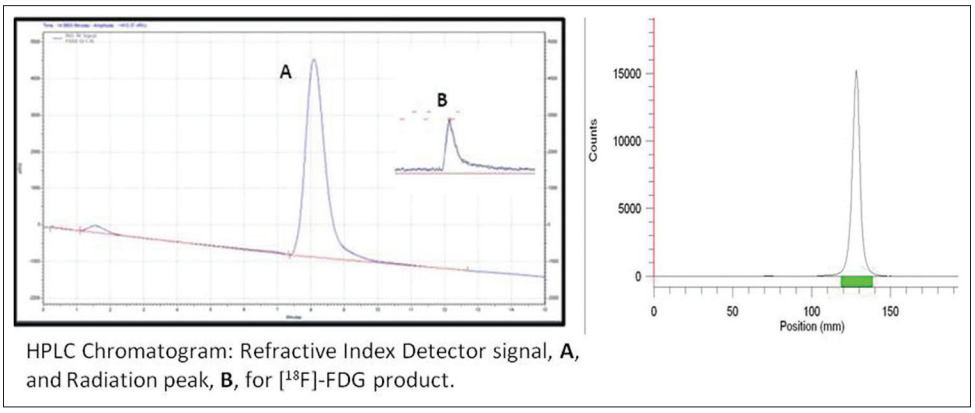


**Figure 4: High-performance liquid chromatography and radioactive thin layer chromatography scan of the [18F]-FDG product**

mg in 1.8 ml of dry acetonitrile) to this dried reaction mixture. The nucleophilic substitution reaction was performed for 6 min at 100°C by replacing triflate ions by fluoride ion to produce acetylated fluorinated glucose (2-fluoro-1,3,4,6-tetra-O-acetyl-D-glucose). The system performed the one more drying cycle at 90°C for 5 min, in order to remove the volatile impurities, especially acetonitrile. The base-mediated hydrolysis was performed at 100°C for 5 min using 0.3M solution of NaOH. The reaction mixture was cooled down and loaded on purifying cartridges assembly. The preconditioned cartridges are used to remove the impurities such as to trap Kryptofix 2.2.2., $^{18}F^-$ 1, 3, 4, 6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl -β-D-mannopyranose (TATM), and fluorinated TATM, and the activity was transferred to the product vial. Finally, the product was eluted with 5 ml of the sterile water which passes through the reaction vial to refining columns, in order to wash out any activity left to reaction vial, into the sterile product vial having a 0.22 μM sterile membrane filter. The total synthesis takes about 26 min and yield is about 65%. The [$^{18}$F]-FDG solution is clear, colorless, neutral, and isotonic. It is subjected to pass the quality tests before being used for clinical studies. After successfully performing ten hot runs and comparing their QC data, we moved for support the clinical studies [Figure 2].

## Automated synthesis of $^{18}$F-FDG using MPS-100 multipurpose synthesis module

After successful optimization of the production of $^{18}$F-FDG through the F300 module, we move forward to work with the multipurpose synthesis module. This module has capabilities to perform the $^{11}$C, $^{13}$N, $^{15}$O, and$^{18}$F chemistries as desired by the user. The module has open software operation with programming capabilities to modify it as per chemistry. For doing $^{18}$F-chemistry, it has individually operated five vial valves and one closed reactor. These valves and reactor can be programmed as per need and operated with a designed software template. After completion of the reaction, the crude can be either loaded to appropriate solid Sep-Pak cartridge system for

purification or loaded into an integrated semi-preparative HPLC system for purification; the purified product will be transferred to a housed rotatory vacuum evaporator to reduce the HPLC solvent. The final purified product will then diluted to physiological solution to finally transfer through a sterile filter for clinical studies after performing the quality assurance tests.

In brief, the bombarded [$^{18}$O]-$H_2O$ was transfer to the collection vial cyclotron line and then valve V66, V10, V11, and V75 open to trap the $^{18}$F ions to preconditioned QMA cartridge, and remnant [$^{18}$O]-$H_2O$ was collected to vial marked as $^{18}$O-water. The trapped activity was eluted with mixture of $K_2CO_3$ and K2.2.2 (0.2 ml/0.7 ml) and transferred to the reaction vessel through the opening of valve V11, V12, V13, and V14. The azeotropically drying of the reaction mixture was performed at 100°C for 8 min, followed by the second cycle of drying through the addition of acetonitrile (0.2 ml) via opening of valve V74 for 5 min at 90°C. After cooling down the reactor, the precursor mannose triflate was added via the opening of valve V73. The fluorination was performed at 100°C for 10 min, followed by drying of excess of acetonitrile [Figure 3].

The hydrolysis accomplished at 100°C for 3 min, by the addition of 0.3M NaOH to the reaction vessel. The reactor was cooled down and crude was loaded to set of purification cartridges. To accomplish the complete transfer of crude mixture, the reaction vessel was washed with 5 ml of water of injection, and this water is passed through the purification cartridge; the order of IC-H, PS2, and Alumina N from the upstream are connected to each other to trap Kryptofix222, K+, Na+, $CO_3^{2-}$, free fluoride ions, fluorinated TATM, and TATM, to elute out the purified [$^{18}$F]-FDG, whereas all the impurities were trapped within these cartridges. The final$^{18}$F-FDG was collected in sterile product vial through sterile filter and is ready for QC and clinical studies. The total synthesis time was about 35 min and yield is approximately 45% [Figure 4].

## Conclusions

We developed two different protocols over these machines in order to serve the patients who are coming to our PET center for their studies. The development of the $^{18}$F-FDG synthesis software template over the MPS-100 synthesizer facilitates our center to serve the patients when our fully dedicated synthesizer failed the production. The failure of a dedicated synthesizer may account either to some technical issues or QC test failure. The protocol developed with MPS 100 synthesizer for production of [$^{18}$F] FDG, yielded approximate 45% (EOB), with synthesis time of around 35 min. Finally, our centers have two automated synthesis modules which have the capability to produce [$^{18}$F]-FDG in good yield and purity, to do the patient studies. Our protocol is simple, reproducible, and robust to work over it.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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