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SYNTHESIS AND QUALITY CONTROL OF 3-DEOXY-3-[^{18}F] FLUOROTHYMIDINE OR [^{18}F]FLT

Chemistry

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ABSTRACT

OBJECTIVE - In this paper, we discuss in detail the production of 3-Deoxy-3-[^{18}F]-Fluorothymidine or [^{18}F]-FLT using Sumitomo Heavy Industry MPS-100 multipurpose synthesis module and quality control of the produced [^{18}F]-FLT.

SCOPE - [^{18}F]FLT is one of the promising scaffolds for Positron emission tomography (PET) imaging which is the most widely used radiotracer to diagnose the rate of cell proliferation in tumors a marker for proliferation rather than metabolism, it is more specific to tumor tissue than ^{18}F-Fluorodeoxyglucose (^{18}F-FDG). This synthesizer unit produces [^{18}F]FLT by synthesizing ^{18}F-, which is processed by irradiating protons over $H_2{}^{18}O$ from the cyclotron and FLT precursor. After synthesis, separation was done with the HPLC, the liquid was evaporated and the final product [^{18}F]FLT was recovered with the help of saline.

RESULT - The overall synthesis of [^{18}F]FLT has been optimized, the simple procedure we describe will be useful for producing radio-chemically pure doses of [^{18}F]FLT (10 mCi) for PET imaging.

KEYWORDS

3-Deoxy-3-[^{18}F]-Fluorothymidine or [^{18}F]-FLT, ^{18}F-Fluorodeoxyglucose (^{18}F-FDG), Positron emission tomography (PET), radiotracer, irradiating, synthesizer.

INTRODUCTION

Another promising scaffold for PET imaging is 3′-deoxy-3′-fluoro-thymidine [^{18}F]FLT which is one of the mostly widely used radiotracer to diagnose the rate of cell-proliferation in tumors a marker for proliferation rather than metabolism, it is more specific to tumor tissue than 2-deoxy-2-[^{18}F] Fluoro-D-glucose (^{18}F-FDG).[1] After the success of 3′-azido-thymidine (AZT), [^{18}F]FLT was developed as a thymidine analogue which was effective against HIV virus. It was first investigated for this purpose in 1991. [^{18}F]FLT as a PET imaging agent is used to provide a surrogate biomarker for DNA replication. [^{18}F]FLT PET studies have been used in oncology and other highly proliferative tissue. [^{18}F]FLT is monophosphorylated by thymidine kinase-1 (TK1 also known as the rate-limiting enzyme of [^{18}F]FLT metabolism) which is closely connected to cellular proliferation and expressed during the cell cycle phase of DNA synthesis. Phosphorylated [^{18}F]FLT cannot cross the cell membrane and accumulate in cells. In tumor cells, the regulation of cell cycle is aberrant and TK1, the key enzyme, is overexpressed.[2]

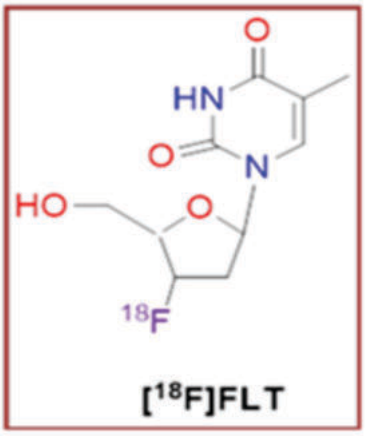


Figure 1. Chemical Structure of [^{18}F] FLT

[^{18}F]FLT the thymidine analogue targets specifically the proliferative activity of malignant lesions. However, the very low radiochemical yield and expensive commercially available synthesizer of [^{18}F]FLT have been major obstacles for routine clinical use of [^{18}F]FLT.[3,4,5,6]

Although an automated synthesis of [^{18}F]FLT by using in TracerLab Mx [^{18}F]FDG synthesis module has recently been reported, the HPLC purification installed in hot cell was necessary in that automated synthesis system.[7]

Classically, production of [^{18}F]FLT suffers from modest yields and time-intensive purification by high-performance liquid chromatography (HPLC).[8] These shortcomings have been partially addressed through development of improved precursors (nosylate, mesylate, tosylate),[9] use of protic solvents,[10] microfluidic modules and double synthesis modules. Further improvements in synthesis time were recently achieved by performing the radiosynthesis in an automated module in conjunction with purification using a single alumina column, giving the shortest reported synthesis time of 68 min and radiochemical purity 495%. Still, the radiochemical yield (decay corrected) remained less than 10%.

MECHANISM

Fluorothymidine or FLT is an analog of the nucleoside thymidine (deoxythymidine). The 3'- Fluorine atom position on FLT prevents it from performing the thymidine biochemical pathway. It does not freely spread (move) across the cell membrane and by active transport mechanism it is transported from blood into the cells. Phosphorylated FLT is a substrate for thymidine kinase I (TK1) and it is not incorporated into the DNA. The concentration of FLT nucleotides into the cells is proportional to TK1 activity.

One of the advantages of FLT is that it is a specific radiotracer as compared with other fluorinated radiotracers for cellular proliferation. It is only a substrate for TK1 and not for mitochondrial TK2. FLT is not dephosphorylated in vivo but conjugated FLT is cleared via the kidneys and excretion in the urine.[11]

MATERIALS AND METHODS

The precursor, 3-*N*-Boc-5'-*O*-dimethoxytrityl-3'-*O*-nosyl-thymidine ultra-pure, authentic nonradioactive standard [^{19}F]-FLT and ^{18}O Water were obtained from ABX, Germany.

Solvents and reagents were purchased from Sigma (Milwaukee, WI, USA) and Fisher Scientific (Mumbai, India). Sterile vial, USP-grade 0.9% NaCl, and sterile water for injection were purchased from B. Braun Melsungen AG, Melsungen, Germany. The Sep-Pak QMA light cartridge was purchased from Waters Corporation.

Table 1. List of Solutions used in the synthesis.

No.	Name	Method
1	**Potassium carbonate aqueous solution for QMA-conditioning**	16.5 g of K_2CO_3 1.5 H_2O is dissolved in 100 mL of distilled water (For about 10 times).
2	**Potassium carbonate aqueous solution for synthesis**	28 mg of K_2CO_3 1.5 H_2O is dissolved in 2 mL of distilled water (For about 10 times).
3	**Kryptofix 222 acetonitrile solution**	100 mg of Kryptofix 222 is dissolved in 3.5 mL of absolute acetonitrile (For about 5 times).
4	**Phosphoric buffer**	1 pac of Phosphoric buffer into 100 mL water for injection
5	**Ascorbic acid**	0.1 mL of Ascorbic acid into 1.5 mL of water for Injection
6	Eluting solvent for HPLC (15 % Acetonitrile)	150 mL of Acetonitrile for HPLC and 850 mL of water for HPLC

Table 2. List of reagents used in the synthesis.

Vials	Reagent	Amount
Vial 1	Potassium Carbonate Solution (for Synthesis)	0.2 ml
	Kryptofix 222	20 mg
	Absolute Acetonitrile	0.7 ml
Vial 2	Absolute Acetonitrile	1.0 ml
Vial 3	FLT Precursor	20 mg
	Absolute Acetonitrile	1.0 ml
Vial 4	1 mol/L HCL	0.75 ml
Vial 5	1 mol/L NaOH	0.5 ml
	Phosphoric buffer	1.0 ml

Quality control and stability Procedure

Quality Control of [^{18}F] FLT prepared at our laboratory for clinical use is carried out according to the USP recommendations detailed below.

Particulates:- The [^{18}F] FLT product solution was examined visually. The final drug product in the vial should be clear and colorless without any visible particulates.

Filter Integrity Test:- [^{18}F] FLT was collected into the final product vial by passing through a 0.22 □m sterilizing filter. The sterilizing filter was tested for filter integrity by bubble point procedure to indicate product sterility.

Kryptofix [2.2.2] Test:-. This test was detected by a colour spot test of residual K2.2.2 in the final drug product. As proposed by FDA the maximum permissible level of K2.2.2 in the final product of [^{18}F] FLT is 50 □g/mL.

pH:- pH test was done by spotting on a pH test paper strip and the measured pH must be between pH 6.5 and pH 8 for the [^{18}F]FLT product to be released.

Radionuclidic Identity:- It is measured by the radiopharmaceutical dose's half-life and comparing it to the half-life of fluorine-18 which is 109.77min.

Bacterial Endotoxin:- Bacterial endotoxins analysis was detected by a Charles River Laboratories Endosafe Portable Testing System (PTS) unit. All of the bacterial endotoxin levels were < 175 EU per batch.

Radiochemical purity:- Radiochemical purity was confirmed by High-Performance Liquid Chromatography also known as HPLC on an Agilent HPLC system which was equipped with a UV absorbance detector 254 nm and a radioactive detector (Carroll and Ramsey Associates, CA, USA). The samples were injected onto an analytical C18 column (Agilent, Eclipse Plus C18, 4.6 × 250nm, 110A, 5µm), which was eluted with a mobile phase of 7.5% EtOH and 92.5% Water. The column flow rate was 0.5 ml/min and was kept at temperature 35ºC.

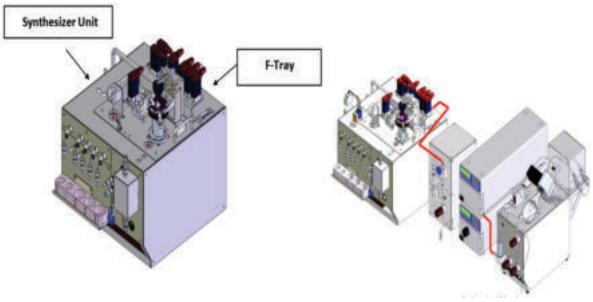


Figure 2. MPS100 synthesizer with ^{18}F standard tray connected with UV detector of HPLC and evaporator.

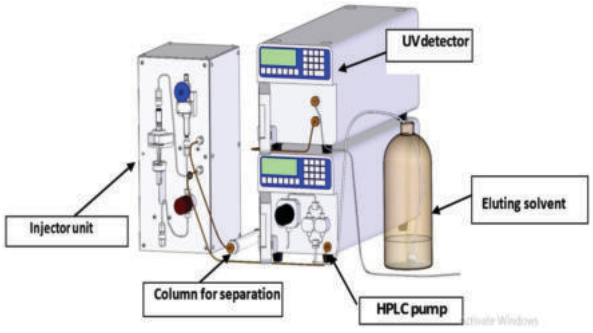


Figure 3. HPLC pump with the injector unit and eluting solvent for HPLC.

RESULT AND DISCUSSION

Radiosynthesis of [^{18}F] FLT

[^{18}F]FLT was synthesized on a fully automated Sumitomo's multipurpose synthesizer or CFN using ^{18}F Tray. [^{18}F]Fluoride was transferred from the cyclotron and was passed through the QMA cartridge which was already preconditioned with a mixture of K_2CO_3 in water & K222 in dry acetonitrile (vial 1) through the cartridge to elute ^{18}F to the reaction vial. The reaction vial was heated at 110^{0}C for 10 minutes than at 80°C for 5 minutes. The solution was azeotropically dried by a stream of dry Nitrogen to remove traces of water and negative pressure to form dried KF^{18} by transferring dry acetonitrile, the drying process was repeated. The fluorination was done by adding 3-*N*-Boc-5'-*O*-dimethoxytrityl-3'-*O*-nosyl-thymidine which was chosen as a precursor for [^{18}F]FLT (20mg in 1ml of dry acetonitrile) to this dried reaction mixture and was heated at 110^{0}C for 15 minutes. The acid-mediated hydrolysis was performed at 120^{0}C for 10 minutes using 0.6 ml of 2.0 N solution of HCl. After hydrolysis, the final reaction mixture was neutralized with 0.5 ml of 1 mol/L aq. sodium hydroxide or NaOH was transferred to the reaction vial. Before transferring the reaction mixture into the HPLC loop the mixture was infused with 0.1 mL of Ascorbic acid (1.5 mL of water for Injection) to maintain the pH of the reaction mixture (as HLC can form salt without pH maintenance). Finally, connect the ^{18}F Tray with the injector unit of HPLC and evaporator. Now the reaction mixture was transferred to the HPLC vial through an activated alumina N Sep-Pak cartridge for purification. Set the UV detector of HPLC at 254nm with a flow rate of 3ml/min with eluting solvent 15% Acetonitrile and 1.0 ml Phosphoric buffer. The purity of the final product was performed with a battery of quality tests.

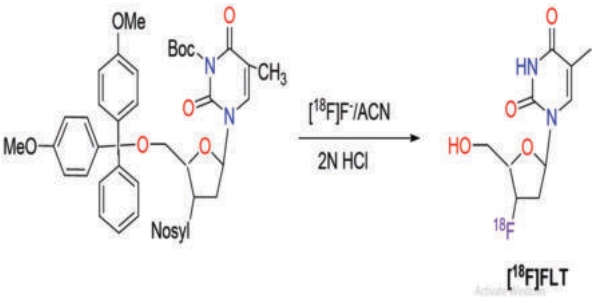


Figure 4. Reaction scheme for the automatic synthesis of [^{18}F]FLT.

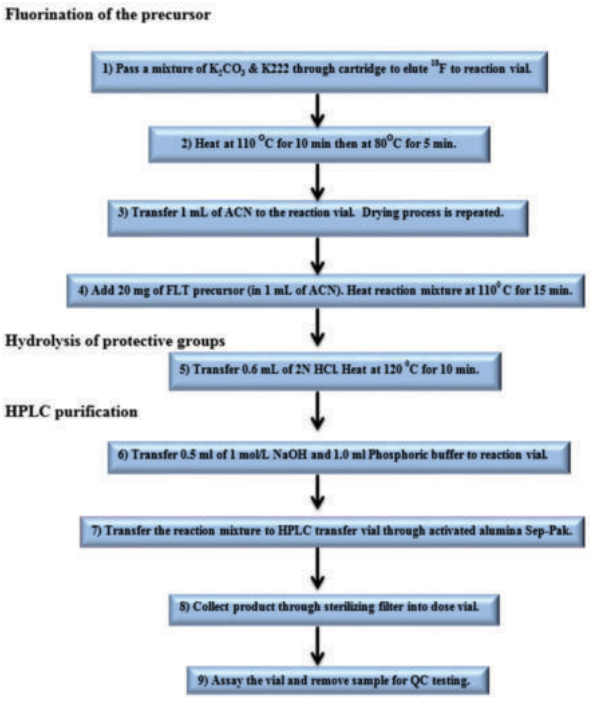


Figure 5. Summary of all the Operational Steps involved during au tomated radiosynthesis of [^{18}F]FLT.

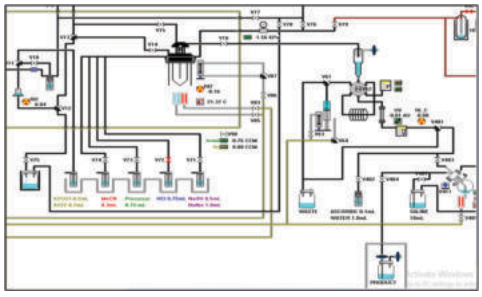
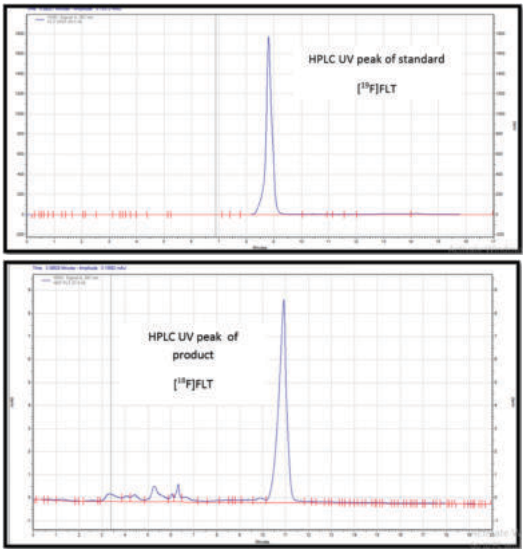


Figure 6. Automated production of [^{18}F]FLT.

The results showed a chemical and radiochemical purity of greater than 95%. The radiochemical purity of the final solution of [^{18}F]FLT was confirmed by the analytical HPLC and the retention time for the desired compound is at 10.9 minutes which was confirmed by co-injection of the standard injection of [^{19}F]FLT. The pH of the solution ranged from 6 to 7 and the volume activity was superior to 500 MBq/mL for each batch (n = 9) with a specific activity of 35 to 70 GBq/μmol.



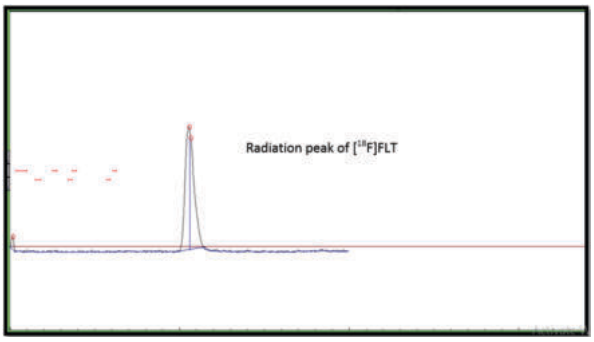


Figure 7. [^{19}F]FLT HPLC UV peak of standard, [^{18}F]FLT HPLC Product peak, Radiation peak of [^{18}F]FLT.

Table 3. Quality Control Tests for the [^{18}F]FLT

Particulate Test	clear & colourless	clear & colourless	clear & colourless
pH	7	7	7
Half-life (min)	110	110	110
Filter Integrity (psig)	55	60	60
Kryptofix [2.2.2] μg/mL	<50	<50	<50
Radio Chemical yield	98.6	97.65	98.2
Radio Chemical purity (%)	95	95	95
RTLC (R_f)	0.6	0.6	0.6
Measured Endotoxin (EU/mL)	<2	<2	<2

Our protocol for synthesizing the [^{18}]FLT using automated synthesizer MPS-100 is straightforward with the capability to produce the desired PET tracer in good reproducible yield. With the use of the low amount of precursor (3-4 mg per run), the ^{18}F ions were incorporated into the 3'-position of the thymidine scaffold via nucleophilic substitution of the nosylate which serves as leaving group. After the initial incorporation of fluoride ions, the hydrolysis of protecting groups (Butyl carbonate and dimethoxy trityl) was performed using the 2N HCl solution. The reaction mixture was neutralized and transferred in a line-connected semi-preparative HPLC system for purification and isolation of the desired product. The mobile phase for isolating the desired peak (add mobile phase and column details) and the peak was collected and collected to an in-built rotary evaporator for concentrating the solution and making it physiologically compatible. Our protocol is very simple to adopt and reproduced in any kind of automated synthesizer. The total reaction time minutes and yield in the range of GBq. After performing synthesis the small fraction of the isolated pure product was subject to a battery of quality control tests such as radiochemical purity, radionuclide purity, pH, endo toxicity, and sterility.

CONCLUSION

Our aim to optimize the synthesis of [^{18}F]FLT has been documented, our methodology is very simple and robust and producing radio-chemically pure doses of [^{18}F]FLT (10 mCi) for PET imaging studies. We present our synthesis in its simplest format, with the aim that it will be easy to identify how this synthesis can be adapted to dedicated systems that produce other ^{18}F-labeled radiopharmaceuticals.

Consent for publication: No data, figures, tables were taken from any author in this manuscript.

Availability of data and material: The data are mentioned in this manuscript, No data is available as supplementary data.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent: This article does not contain any studies with human and animal subjects performed by any of the authors.

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