Evaluation of Physiochemical and Microbiological Stability of 2-[^{18}F] Fluoro-2-Deoxy-D-Glucose, [^{18}F]FDG: Synthesized in the Cyclotron Facility of National Institute of Nuclear Medicine and Allied Sciences

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ABSTRACT

2-[^{18}F] Fluoro-2-Deoxy-D-Glucose ([^{18}F]FDG) is very effective and the most successful radiotracer for the assessment of glucose metabolism in the brain, heart, and lungs. It has also been used for imaging different tumors in oncology. The purpose of this study was to evaluate the stability and quality of [^{18}F]FDG synthesized by the newly installed ^{18}MeV Cyclotron facility at the National Institute of Nuclear Medicine and Allied Sciences (NINMAS). [^{18}F]FDG was synthesized by nucleophilic fluorination of Mannose-triflate followed by basic hydrolysis with an IBA Synthera® auto synthesizer. [^{18}F]FDG solution was collected in a sterilized vial after the filtration by a vented Millex®-GS 0.22µm syringe-driven hydrophilic filter. An aliquot of [^{18}F]FDG solution was taken 0, 2, 4, 6, and 8 hours after the synthesis. All quality control parameters were measured in the first evaluated period, immediately (i.e., 0 hours) after synthesis. Radiochemical purity (RCP) was measured for all [^{18}F]FDG samples and for all time points. The radiochemical and radionuclidic purity was more than 95% and 99.99% respectively. The [^{18}F]FDG was physiochemically & microbiologically stable over 8 hours.

Keywords: 2-[^{18}F]Fluoro-2-Deoxy-D-Glucose ([^{18}F]FDG), quality control, stability, radiopharmaceuticals.

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INTRODUCTION

^{18}F Positron Emission Tomography (PET) AND Computed Tomography (PET-CT) a rapidly developing medical imaging technology, is used to diagnose, stage, and restage a variety of clinical conditions, such as lung cancer, colorectal cancer, lymphoma, melanoma, head and neck cancer, brain cancer, and breast cancer. 2-[^{18}F]Fluoro-2-Deoxy-D-Glucose ([^{18}F]FDG) is very effective and the most successful radiotracer for the assessment of glucose metabolism in the brain, heart, and lungs. The molecular structure and stereo-configuration of [^{18}F]FDG and glucose are identical, with the exception that the hydroxyl group on the 2-carbon of a glucose molecule is replaced with a fluoride. Glucose transporters carry [^{18}F]FDG into cells, where it is phosphorylated by hexokinase to form [^{18}F]FDG-6-phosphate. The transport of [^{18}F]FDG into cancer cells and phosphorylation increase due to overexpression of facilitative glucose transporters and hexokinase. [^{18}F]FDG-6-phosphate does not undergo further metabolization, it is retained in cancer cells and imaged by PET. With a whole-body PET-CT imaging with [^{18}F]FDG assesses glucose metabolism across all organ systems. FDG-PET facilitates the early detection and quantification of metastases.

The first step in the manufacturing of [^{18}F] FDG is the production of [^{18}FF]fluoride in a cyclotron, typically in a target chamber with [^{18}O] water. This is followed by a nucleophilic substitution reaction with 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-D-mannopyranose (mannose triflate) and [^{18}F] fluoride and subsequent removing of protecting groups (acetyl) by acidic or basic hydrolysis, resulting in [^{18}F]FDG formation (Figure 1). The synthesis is carried out in a hot cell using an automated synthesis module following GMP requirements.

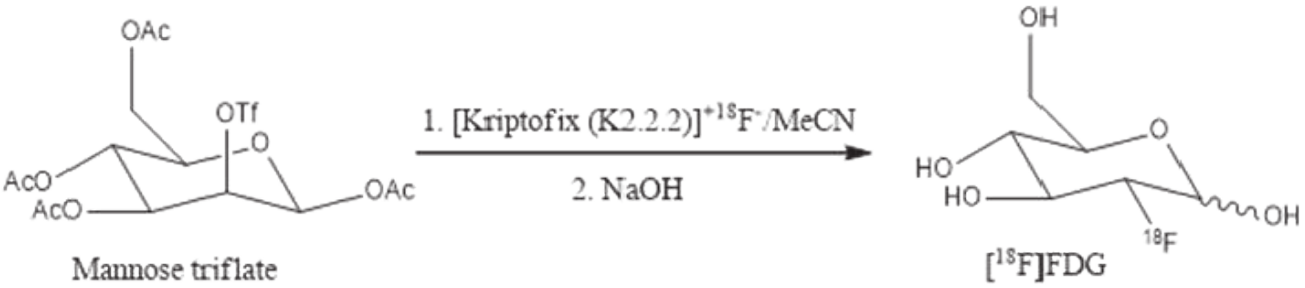

Figure 1: Synthesis of [18F]FDG by Nucleophilic Substitution Reaction

The mannose triflate (precursor) is a sugar molecule that has four acetyl groups protecting the other four possible reaction sites, as well as a well-suited leaving group (trifluoromethanesulfonyl) at carbon-2 for a simple nucleophilic substitution process.

The quality control specifications of [^{18}F]FDG are listed in United States Pharmacopeial (USP), European Pharmacopoeia (EP), British Pharmacopoeia (BP), Chemistry Manufacturing and Controls (CMC) section from the United States Food and Drug Administration (US FDA) (1,2)

Meeting the increasing demand for [^{18}F]FDG requires an increase in production without a decrease in quality. It is reported that, besides the radioactive decay, [^{18}F]FDG decomposes in-vitro, resulting in the degradation of the radiochemical purity over time (3,4). Because of the short half-life (109.7 min) of ^{18}F, it is quite challenging to deliver [^{18}F]FDG to PET imaging centers distant from cyclotron and production facilities. This investigation aims to evaluate the physicochemical and microbiological stability and quality of [^{18}F]FDG, such as pH, radionuclidic and radiochemical identity and purity, chemical purity, bacterial endotoxins, and sterility.

METHODS

FDG Synthesis

[^{18}F]FDG was synthesized by standard method from mannose triflate with alkaline hydrolysis. Highly pure (97%) [^{18}O]H_2O was purchased from ABX Advanced Biomedical Compounds (Radeberge, Germany). Cyclotron is the source of anionic fluorine through ^{18}O (p, n) ^{18}F reaction. Produced activity was transferred to the automated synthesizer [Synthera®] where the synthesis and purification were performed within 30 min. In the synthesis path, the aqueous [^{18}F]-fluoride ion was passed through an ion-exchange column to trap the [^{18}F]-fluoride ions. [^{18}F]-fluoride was then eluted to the reaction vessel with a mixture of potassium carbonate and Kryptofix 2.2.2, then water was removed by azeotropic drying with acetonitrile and [^{18}F]-fluoride reacted with mannose triflate. After alkaline hydrolysis, the solution was purified with a sequence of SCX, alumina-B, and C-18 cartridges and eluted with distilled water. The final product was diluted to 15 mL with saline water.

Quality control of [^{18}F]FDG

A) Appearance:

After the synthesis of [^{18}F]FDG, the solution was examined visually with the naked eyes from behind the lead glass.

B) Radionuclide purity:

The radionuclide purity (USP) of the ^{18}F radionuclide was identified by the gamma spectrum and half-life measurement. In our quality control laboratory, a Multi-Channel Analyzer (Elysa Raytest-Mucha, Germany) was used to get the gamma spectrum immediately after synthesis. An aliquot (<1 μL) of the [^{18}F]FDG solution was dropped on a TLC strip and placed on the detector.

C) Half-life test

The half-life of [^{18}F]FDG was measured with ISOMED 2010 Dose Calibrator (Dresden, Germany). An aliquot of [^{18}F]FDG samples was taken in a 1 mL syringe. The initial and final activity of [^{18}F]FDG sample were measured at around 10 min intervals and the observed half-life of ^{18}F was calculated by using the standard formula $t1/2 = (0.693 \times t) \div \ln (A_o/A_t)$, where: t1/2 (half-life), t (time interval in minutes), A_o (initial activity), A_t (activity measured after around 10 min).

D) Radiochemical Purity (RCP)

The radiochemical purity of [^{18}F]FDG was measured by silica gel thin-layer chromatography (radio-TLC). The TLC strip (1.5 x 10 cm) was marked at 1 cm from one end as an origin, and an aliquot (≤ 2µL) of the [^{18}F]FDG solution was spotted onto the origin. The strip was placed in a solvent tank and developed in a 95:5 acetonitrile-water mixture until the solvent mixture reached the top of the strip. RCP was determined by scanning radio-TLC strips using a Radio TLC Scanner (Elysa Raytest- Beta Positron Detector, Germany).

E) Chemical purity

Ethanol, isopropyl alcohol, acetonitrile, and Kryptofix 2.2.2 are the possible chemical contaminants in the synthesized [^{18}F]FDG solution and to ensure the chemical purity of [^{18}F]FDG, the amount of these chemicals was measured.

Solvents residues

The amount of residual solvent was measured by Gas Chromatography, (GC-2010 plus Shimadzu, Germany). A very small amount (0.5µl) of [^{18}F]FDG standard (ABX, Germany) and synthesized [^{18}F]FDG solution were injected separately into a hot injector port which was heated at 250^{o}C. The separation of the organic compounds took place between the carrier gas Helium (pressure: 29.4 kPa, flow rate: 30 mL/min) and the high boiling liquid (stationary phase) within the GC capillary column (Shimadzu-624, dimension 30m x 0.32mm x 1.8µm, USA). Table 1 shows the set oven temperature.

Table 1: Gas Chromatography (GC) oven temperature gradient

Oven ramp	Rate (°C/min)	Temperature (°C)	Hold time (min)
Initial	0	40	2
1	10	80	1
2	40	160	1
3	60	200	5

The flame-ionization detector (FID) was heated at 280^{o}C by Hydrogen gas (flow rate: 40mL/min) /Air (flow rate: 400mL/min) flame. The mixture of solvents reached the detector at varying times to oxidize and produce electrically charged particles (ions). The ions were collected and produced an electrical signal. The signal was sent by this detector to the recorder through an amplifier. The results with all peaks were sent to the system computer which stored, displayed, and analyzed the data.

Kryptofix 2.2.2

Kryptofix 2.2.2 (K 2.2.2) has toxicity, that's why USP and FDA recommend maximum contents of K2.2.2 equivalent to 0.22µg/mL in the injectable solution. As its toxicity is more than acetonitrile, according to Good Laboratory Practice (GLP), it is better to keep K 2.2.2 concentration in an [^{18}F]FDG injectable solution as low as possible (5-7). 5µL each of [^{18}F]FDG test samples and the reference standard of K 2.2.2 (0.22µg/mL) was spotted side by side on a silica gel plate. The spots were then air-dried without the application of heat. The plate was then developed with the mobile phase composed of methanol: ammonia (30%) (9:1, v/v). The developed plate, after drying, was exposed to iodine vapor in a closed container to visualize the spots and then the spot's intensity of [^{18}F]FDG test sample and reference standard of K 2.2.2 were compared.

F) pH measurement

A properly calibrated pH meter (Knick, pH meter 765 Calimatic, Made in Germany) and mColorpHast pH indicator strips, (Millipore Sigma) were used to check the pH of [^{18}F]FDG. According to US pharmacopeia, the physiological range of pH for [^{18}F]FDG is 4.5-8.5 and it was checked from batch to batch.

G) Sterility and bacterial endotoxins (LAL) test

Bacterial endotoxin (BET) and sterility tests were evaluated for the confirmation of the microbiological purity of the final [^{18}F]FDG product. A sterility test involves incubation of a test sample for the cultivation of microorganisms with two different growth media, Tryptic Soy Broth (TSB) and Fluid Thioglycollate Medium (FTM) (100 mL fill, Merck, Millipore, France) at different temperatures 25^{o}C and 35^{o}C respectably for 14 days. One tube was taken for each growth media. [^{18}F]FDG sample was added to two tubes (0.1mL sample to each tube), incubated, and monitored for up to 14 days.

A portable testing system (PTS) [Endosafe®-PTS, Charles River Laboratory] was used to quantify bacterial

endotoxins. Ten times diluted 25µL [18F]FDG sample was added into each sample reservoir of Limulus amebocyte lysate (LAL) reagent containing cartridge (Endosafe®-PTS, Charleston, SC) without creating bubbles

H) Sample Evaluation and Stability study

The main goal of this study was to evaluate if [18F]FDG samples would comply with all the specifications of USP (5) during 8 hours of storage at room temperature. This interval was chosen because it's an upper bound for the period between the end of the synthesis (EOS) and the patient injection time (including transport) in the perspective of our country, Bangladesh. The radiochemical purity at different time intervals was evaluated in [18F]FDG quality control and applied in the stability study. A small amount of [18F]FDG samples were taken at various time points 0, 2, 4, 6, and 8 hours after the final product's synthesis, to measure the RCP by the TLC method. However, all other quality parameters, including appearance, pH, radionuclidic identity and purity, radiochemical identity and purity, residual solvents, bacterial endotoxin, and sterility, were only assessed immediately after the synthesis of [18F]FDG. Five batches of purified [18F]FDG were evaluated in this study.

RESULTS & DISCUSSION

The appearance of the [18F]FDG solution was clear, colorless and non-particulate solution. Radionuclide purity, radiochemical purity, chemical purity, microbiological purity, pH and stability of the [18F]FDG had been evaluated at the cyclotron and radiochemistry laboratory of NINMAS.

3.1 Radionuclide Purity

a) Gamma-ray spectrometry

Radionuclidic purity was confirmed by taking the gamma spectrum of [18F]FDG samples. There was no other peak recorded except the peak with energy 511 keV as shown in Figure 2 for all the five batches of [18F]FDG production which confirmed radionuclidic purity of more than 99.9%.

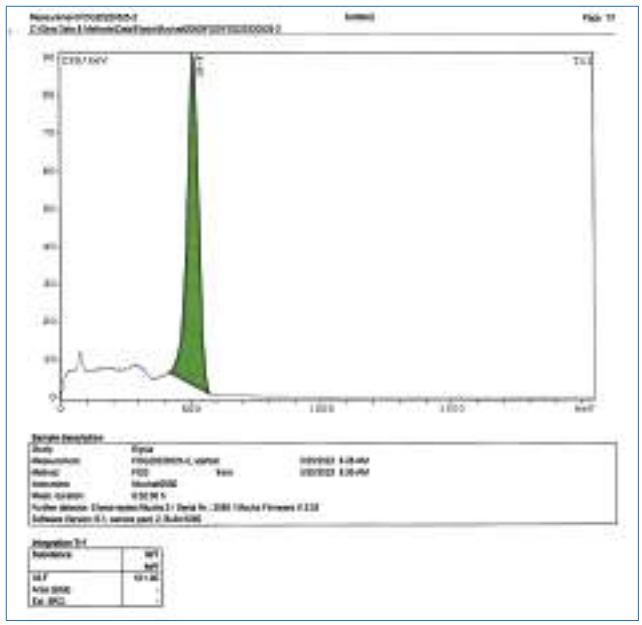

Figure 2: Gamma ray spectrum of ^{18}F with 511 keV peak

b) Half-life test

The average half-life (109.05 ± 0.85) of [18F]FDG (Table 2) was almost the same as the 18F half-life (109.7 min). A typical report of half-life of 18F is shown in Figure 3.

Table 2: Measured radio-activity and average half-life of the synthesized [18F]FDG

No.	Time	Initial activity (mCi)	Final activity (mCi)	Half-life ($t_{1/2}$) in minute	Average Half-life (Mean ± SD)
1	09:51-10:01	13.6	11.10	109.9	109.05 ± 0.85
2	10:01-10:11	8.5	7.15	108.2	

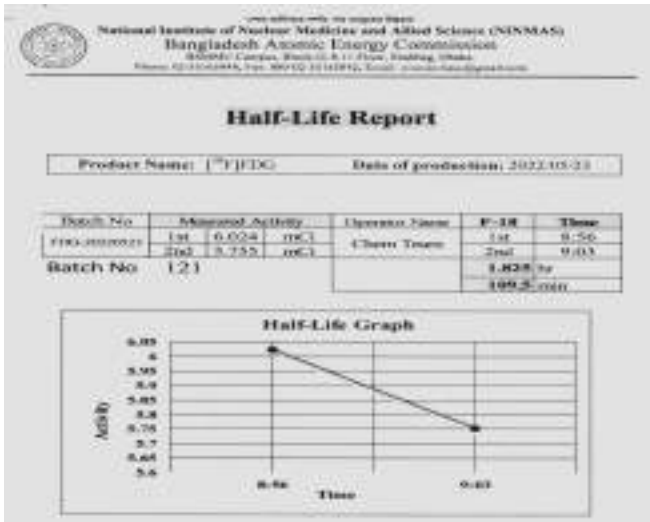

Figure 3: Half-life report [18F]FDG

[18F]FDG Quality Control Report				
Results Summary				
Before release [18F]FDG				
Test Items		Specification	Results	Comment
1. Appearance		Colorless/Clear/ No particle	☑ Colorless ☑ Clear ☑ No Particle	☑ Pass ☐ Non-Pass
2. Identification				
	Gamma-ray energy	511/1022 KeV	511KeV	☑ Pass ☐ Non-Pass
	Half-life	105 - 115 min	109.05 ± 0.85 min	☑ Pass ☐ Non-Pass
	Radiochemical identity	R_f = 0.4 - 0.6	0.44 ± 0.035	☑ Pass ☐ Non-Pass
3. PH		4.5 – 8.5	6.5-7.0	☑ Pass ☐ Non-Pass
4. Purity				
	Radiochemical purity	> 95%	95.88 ± 0.34%	☑ Pass ☐ Non-Pass
	Radionuclidic purity	511/1022 KeV	511 KeV	☑ Pass ☐ Non-Pass
	Chemical purity Kryptofix2.2.2 test	0.22mg/mL	< 0.22 mg/mL	☑ Pass ☐ Non-Pass
5. Endotoxin test		< 17.5 EU/mL	0.2-0.8 EU/mL	☑ Pass ☐ Non-Pass
After release [18F]FDG				
6. Chemical Purity				
	Residual Acetonitrile	< 410 ppm	< 10 ppm	☑ Pass ☐ Non-Pass
	Residual Ethanol	< 5000ppm	< 400 ppm	☑ Pass ☐ Non-Pass
7. Sterility test				
	FTM	No microbial growth	No turbidity	☑ Pass ☐ Non-Pass
	TSB	No microbial growth	No turbidity	☑ Pass ☐ Non-Pass

Table 3: Tests Performed, acceptance criteria and results.

3.2 Radiochemical Purity (RCP)

Five consecutive batches were analyzed with Thin Layer Chromatography (TLC) to check the RCP of the synthesized [18F]FDG. A typical thin-layer radio chromatography is shown in Figure 4. RCP of all samples of [18F]FDG was 95.88 ± 0.34% (mean ± SD) which was in the expected ranges (>95%) mentioned in Pharmacopeia (8).

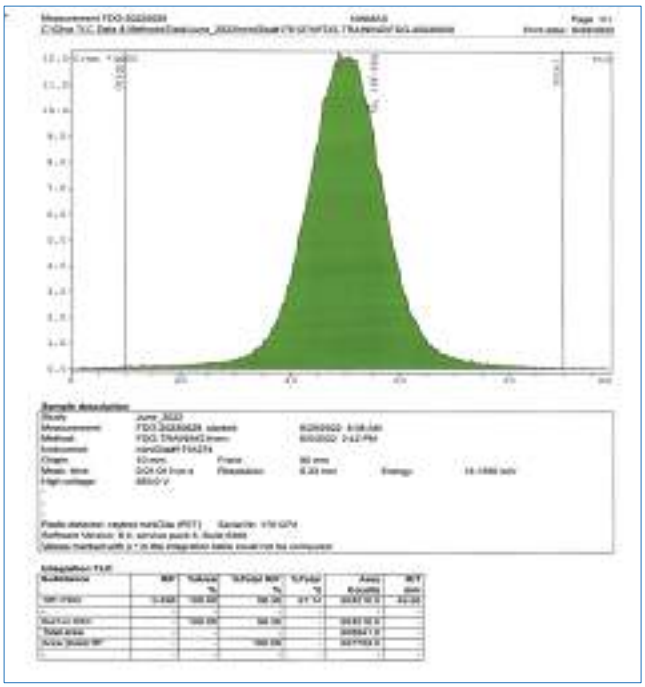

Figure 4: Radio-chromatogram of 18F-FDG.

3.3 Chemical purity

Kryptofix and Residual solvent test

The phase transfer catalyst kryptofix 2.2.2 was measured by the TLC spot method. The amount of residual kryptofix 2.2.2 in the 18F-FDG solution was negligible. The amount of residual acetonitrile and ethanol was also determined quantitatively by GC equipped with an FID detector. It took a total of 15 min to analyze the residual solvent in [18F]FDG solution. The separation of ethanol, isopropyl alcohol, and acetonitrile was completed in 7.5 min (Figure 5a and 5b). The average amount of ethanol in all samples of [18F]FDG was less than 500 ppm, and isopropyl alcohol and acetonitrile (CH_3CN) were less than 8 ppm. The observed amount of residual solvents in the 18F-FDG solution was much lower than their acceptance limit mentioned in Pharmacopeia (6,8).

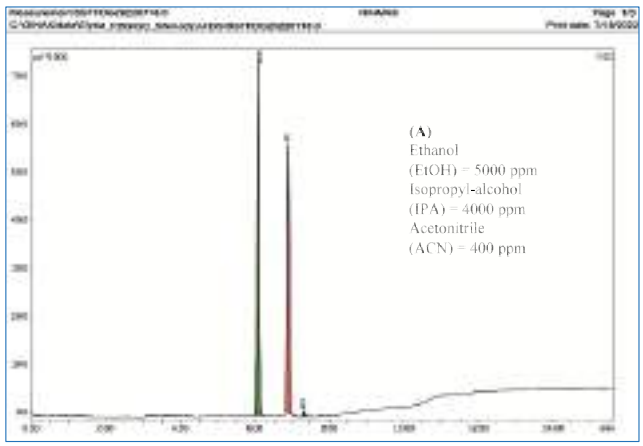

Figure 5a: GC-chromatogram of residual solvents in [18F]FDG-standard solution, Elution order: Ethanol, Isopropyl-alcohol, Acetonitrile.

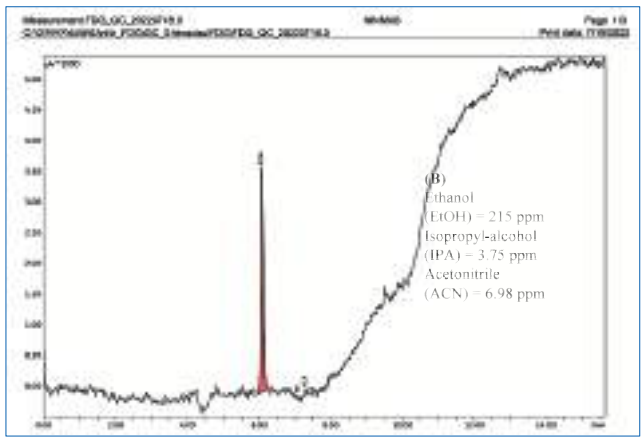

Figure 5b: GC-chromatogram of residual solvents in synthesized [18F]FDG sample, Elution order: Ethanol, Isopropyl-alcohol, Acetonitrile.

3.4 Sterility and bacterial endotoxins test

The test tubes containing [18F]FDG test samples in TSB and FTM Medium were examined visually. The TSB and FTM medium were found clear during 14 days of incubation period Figure 6. All the [18F]FDG samples passed this test successfully.

Figure 6: Sterility test of the final [18F]FDG product, (A) TSB media incubated at 22.5°C (B) FTM media incubated at 32.5°C for 14 days.

3.5 Sample evaluation and Stability study

A drug's stability is determined by how well it retains its qualities during storage and use, as well as how quickly these properties change (6). To measure how drug quality changes over time, the radiochemical purity (RCP) of [18F]FDG was measured at different time points 0, 2, 4, 6, and 8 hours after the end of synthesis (EOS). We observed that RCPs were more than 95% for each time point (Figure 7).

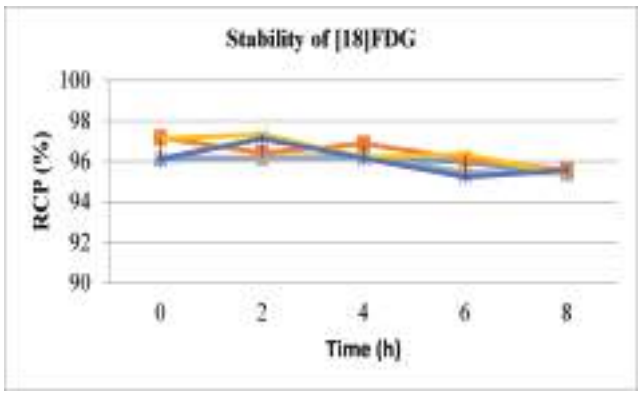

Figure 7: Radiochemical purity (RCP) of [18F]FDG at different time intervals.

The evaluation indicated that the [18F]FDG was stable up to 8 hours after the end of the synthesis. We did not consider the time point longer than 8 hours because this period is sufficient to use [18F]FDG.

Specifications and results for [18F]FDG assays for all evaluated samples in five batches at room temperature are shown in Table 3. The results showed, [18F]FDG was able to meet the specifications regarding acceptance limits for each required assay.

4.0 CONCLUSIONS

The [18F]FDG samples evaluated in this study met all the specifications set by EP & USP and the radiopharmaceutical was found radio chemically pure and stable. These findings demonstrated that the [18F]FDG synthesized in the cyclotron and radiochemistry facility of National Institute of Nuclear Medicine and Allied Sciences (NINMAS) has the suitable physicochemical and microbiological stability up to 8 hours after the end of the synthesis if stored at room temperature, that is highly recommended for the safe and effective [18F]FDG PET-CT study.

5.0 ACKNOWLEDGEMENTS

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