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STABILITY STUDY OF 2-[^{18}F]FLUORO-2-DEOXY-D-GLUCOSE (^{18}FDG) STORED AT ROOM TEMPERATURE BY PHYSICOCHEMICAL AND MICROBIOLOGICAL ASSAYS

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ABSTRACT

The most widely used radiopharmaceutical in the expanding medical imaging technology of Positron Emission Tomography (PET) is 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}FDG). The increasing demand for ^{18}FDG requires reliable production in large amounts. The synthesis of ^{18}FDG is based on a nucleophilic substitution of the triflate-leaving group from the precursor, mannose triflate, in the presence of Cryptand 2.2.2, as a phase-transfer agent. After labeling, the removal of the acetyl protecting groups from resulting 2-[^{18}F]fluoro-1,3,4,6-tetra-*O*-acetyl-D-glucose is performed by alkaline hydrolysis, followed by purification and final filtration (0.22 μm). It was reported that ^{18}FDG decomposes *in vitro*, resulting in the degradation of the radiochemical purity with time. The aim of this study was to evaluate physicochemical and microbiological stability of ^{18}FDG, stored at room temperature (15-30 °C), at different time intervals. It was investigated how the quality of this radiopharmaceutical varies with time under the influence of environmental factors. ^{18}FDG pH, radionuclidic identity and purity, radiochemical identity and purity, chemical purity, residual solvents, bacterial endotoxins and sterility were evaluated according to the United States Pharmacopeia 31ed. analytical methods and acceptance criteria. The results suggest that ^{18}FDG has physicochemical and microbiological stability up to 10 hours after the end of synthesis, under experimental conditions.

1. INTRODUCTION

The most widely used radiopharmaceutical in the expanding medical imaging technology of Positron Emission Tomography (PET) is the 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}FDG) (Fawdry, 2007) [1]. The synthesis of ^{18}FDG is based on a nucleophilic substitution of the triflate-leaving group from the precursor, 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethane-sulphonyl-β-D-mannopyranose (mannose triflate) in the presence of 4,7,13,16,21,24-hexaoxa-l,10-diazabicyclo(8,8,8)-hexacosane (Cryptand 2.2.2), as a phase-transfer agent (Hammacher *et al.*, 1986) [2]. After labeling, the removal of the acetyl protecting groups from the resulting 2-[^{18}F]fluoro-1,3,4,6-tetra-*O*-acetyl-D-glucose is performed by alkaline hydrolysis, followed by purification and final filtration (0.22 μm).

The increasing demand for ^{18}FDG requires production in large amounts, keeping high quality standard (Mosdzianowski *et al.*, 2002) [3]. In Brazil, the expansion of PET services has not been accompanied by the increase of ^{18}FDG manufacturing centres in the same ratio. As a result, Brazil has currently twenty-three PET centres and only four ^{18}FDG manufacturing centres, which are responsible for supplying ^{18}FDG for the whole country (Personal information).

It was reported that, regardless the radioactive decay, ^{18}FDG decomposes *in vitro*, resulting in the degradation of the radiochemical purity with time (Fawdry, 2007 [1]; Romero *et al.*, 2006 [4]). The purpose of a stability study is to establish, based on testing a minimum of three batches of the drug substance and evaluating the stability information (including, as appropriates, results of the physical, chemical, biological and microbiological tests), a shelf life and label storage instructions applicable to all future batches of the drug product manufactured and packed under similar circumstances (ICH, 2003) [5].

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) issued a series of guidelines on the design and conduct of stability studies of pharmaceuticals and the evaluation of stability data derived from such studies (Lin; Chen, 2003) [6]. However, there are some limitations to adopt these guidelines. As an example, test conditions are based on an analysis of the effects of climatic conditions in European Community, Japan and the United States of America. Besides, the specific tests which should be performed and the testing frequency applicable to radiopharmaceuticals are not mentioned. The Brazilian Regulatory Agency for Medicines (ANVISA) published a guideline for medicine stability study design and execution (RE n° 1/2005) [7]; however, its approach is not appropriate for radiopharmaceuticals.

The various national pharmacopeias establish minimum requirements that a drug product must meet at the time of administration to humans (Fawdry, 2007) [1]. Since the Brazilian Pharmacopeia (F. Bras. IV) does not include a monograph for ^{18}FDG, it was adopted the United States Pharmacopeia (USP 31) specifications as acceptance criteria for this study.

The aim of this study was to evaluate physicochemical and microbiological stability of ^{18}FDG, stored at room temperature (15-30 °C), at different time intervals. ^{18}FDG stability is an important issue, especially in Brazil, whose large area imposes distances bigger than one thousand kilometres between some PET centres and the nearest manufacturing place. Additionally, it is worthy to investigate impurities present in ^{18}FDG injections, since they could decrease the resolution power and reliability of PET images.

The influence of radioactive concentration on radiochemical purity of ^{18}FDG was already studied (Fawdry, 2007 [1]; Romero *et al.*, 2006 [4]) and it will not be covered in this paper.

2. EXPERIMENTAL

2.1 ^{18}FDG production

Routine ^{18}FDG synthesis is performed in working days whenever requested by clinical PET scanning centres. Firstly, $^{18}F^-$ is produced by proton bombardment of $H_2{}^{18}O$ (from Cambridge Isotope Laboratories, Inc.) using a GE PETtrace™ 16.5 MeV cyclotron. The synthesis is based in nucleophilic substitution of the triflate-leaving group from mannose triflate (ABX) for $^{18}F^-$ in the presence of Cryptand 2.2.2 (ABX), as a phase-transfer catalyst, followed by alkaline hydrolysis (Fig.1). The synthesis is carried out within a disposable reagent kit from ABX in an automated ^{18}FDG synthesizer (*TRACERlab™ MX$_{FDG}$*, GE). The synthesis lasts, in average, 25 minutes. After synthesis, the ^{18}FDG is purified by passing through a Sep-Pak Plus tC18 and a Sep-Pak Plus Alumina N cartridge (ABX). The final product is filtered in a 0.22 µm membrane (Cathivex®-GS, Millipore) and, then, it is dispensed in glass vials

(Ghipharma) using an automated system for the distribution of the radiopharmaceutical (*Theodorico*®, Comecer).

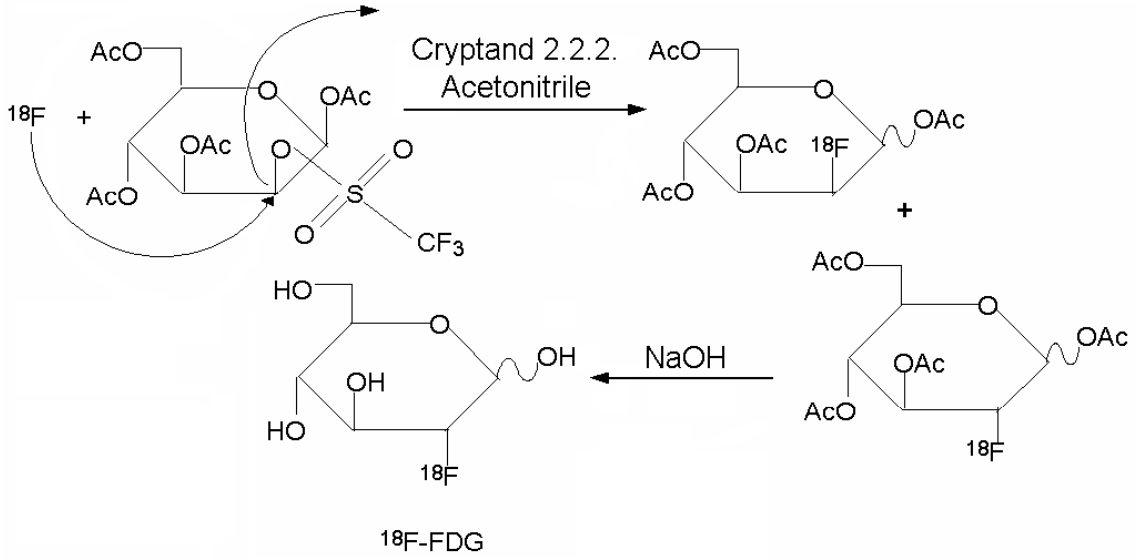

Figure 1. Synthesis of ^{18}FDG by nucleophilic substitution

2.2 ^{18}FDG Quality Control

^{18}FDG quality control is performed according to the USP 31 (2008) [8] analytical methods, which are described in Fludeoxyglucose F 18 Injection monograph. Routine ^{18}FDG quality control assesses physicochemical and microbiological parameters, including: appearance, pH, half-life, radionuclidic identity, radiochemical identity and purity, chemical purity, residual solvents, bacterial endotoxins and sterility.

^{18}FDG samples are diluted ten times before radionuclidic identity, half-life, radiochemical identity and purity, and residual solvents testing; and a hundred times before bacterial endotoxin testing. The results are always corrected considering the appropriate dilution factor. Appearance, pH, chemical purity and sterility test are performed without dilution.

For pH assay, an indicator test paper 0-14 (Merck) is used to measure the hydrogen-ion concentration (pH), as an approximate pH value suffices. The pH of both a standard (pH 7,00 - Carlo Erba) and ^{18}FDG sample are measured.

Radionuclidic purity and identity are evaluated by gamma-ray spectrometry (Multichannel Analyzer, Canberra).

Half-life is determined by successive counting of ^{18}FDG activity in a radioisotope dose calibrator (CRC®-25R, Capintec), within the range of 20 minutes, by the equation bellow:

$$t_{½} = (0{,}693)t \div \ln(A_0/A) \quad (1)$$

Where:
$t_{½}$ = half-life;
A_0 = initial activity;
A = activity measured after 20 minutes;
t = time interval (in minutes) between the two measures (t_A-t_{Ao}).

Chemical purity is evaluated by thin layer chromatography, which is performed in silica gel, solvent system: methanol and ammonium hydroxide 30% (9:1). In this test, concentration of aminopolyeter (cryptand 2.2.2) in ^{18}FDG sample is evaluated by comparison with the cryptand 2.2.2 standard (ABX) 50 µg/mL.

Radiochemical identity is also evaluated by thin layer chromatography performed in silica gel, solvent system: acetonitrile and water (95:5). The retection factor of spots of both ^{18}FDG sample and FDG standard (ABX) are compared. The same chromatographic plate is used for radiochemical purity analysis by scanning the chromatogram with a suitable collimated radiation detector (Gina-Star TLC Chromatography Evaluation System®, Raytest).

Bacterial endotoxins are quantified by chromogenic method, using a Portable Test System – PTS® (Endosafe). This device includes a pumping system, a portable spectrophotometer and embedded software to calculate sample data. The test consists in adding product samples inside cartridges (Endosafe), which assures duplicate sample and positive product control testing.

Sterility test is performed within 30 hours after synthesis by direct inoculation of the ^{18}FDG sample in trypticase soy broth (Newprov) and fluid thioglycollate medium (Newprov), which are incubated, respectively, at 20.0 – 25.0 °C and 30.0 – 35.0 °C. The test is performed in duplicate and it assures a negative control testing. The culture mediums are verified daily during a fourteen-day period. The product is considered sterile, when there is no evidence of microbiological growth.

Futher used chemicals and solvents are: silica gel TLC aluminium sheets (Merck), methanol (Merck), ammonium hydroxide solution 33% (Sigma-Aldrich), iodine crystals (Synth), sterile solution of sodium chloride 0.9% (Sanobiol), acetonitrile (J.T.Baker), purified water, sulfuric acid 95.0 – 97.0% (Merck) and apyrogenic water (Endosafe).

2.3 Stability study

It was investigated how the quality of ^{18}FDG varies with time under the influence of environmental factors. The same physicochemical and microbiological parameters evaluated in ^{18}FDG quality control were applied in the stability study. The specifications for the final product were established to meet all the USP 31 (2008) [8] requirements as shown on Table 1. These specifications are used as acceptance criteria for ^{18}FDG approval and they were taken as acceptance criteria for this study.

Table 1. Specifications for ^{18}FDG (USP 31, 2008)

Appearance	pH	Radionuclidic		Radiochemical	
		Half-life	Identity	Identity	Purity
Clear and free of particulate matter	4.5 – 7.5	$t_{1/2}$ = 105 – 115 min	Main peak energy = 511 keV ± 5%	Sample's R_f similar to Standard's R_f	> 90%
Chemical Purity	**Sterility**	**Bacterial Endotoxins**		**Residual Solvents**	
Cryptand 2.2.2 < 50 µg/mL	Sterile	< 11.6 UE/mL		Ethanol < 0.5% Acetonitrile < 0.04%	

The main goal of this study was to evaluate if ^{18}FDG samples would comply with all these specifications after 10 hours of storage at room temperature (approximately 22.0 °C). This interval was chosen because it is an upper bound for the period between the end of the synthesis and the patient injection time (including transport). The ambient humidity condition was not considered in the analysis, since impermeable containers (glass vials) were used for packaging.

^{18}FDG radioactive concentration was kept between 0.3 - 0.7 GBq/mL, the same level used to supply PET centres requests. As mentioned in early sections, this paper does not intend to discuss the influence of radioactive concentration on radiochemical purity of ^{18}FDG.

2.4 Sample Evaluation

^{18}FDG samples were withdrawn 0, 2, 4, 6, 8 and 10 hours after the final product synthesis. All quality control assays were performed in the first and last evaluated periods, i.e., 0 and 10 hours after synthesis. In the other time intervals, only radionuclidic identity, half-life and radiochemical identity and purity parameters were evaluated. As performed for ^{18}FDG quality control, samples were diluted ten times for radionuclidic identity, half-life, radiochemical identity and purity, chemical purity, residual solvents; and a hundred times for bacterial endotoxin test. The results were corrected considering the appropriate dilution factor. Appearance, pH and sterility test were performed without dilution.

Three batches of purified ^{18}FDG were included in this study. It was not applied a statistical analysis to evaluate the study data, since the study purpose was to evaluate if ^{18}FDG would comply with all the specifications after 10 hours of storage, at room temperature, regarding the phamacopeial acceptance criteria.

3. RESULTS AND DISCUSSION

Results for ^{18}FDG assays in all evaluated time intervals are shown in table 2 as mean ± standard deviation. The results indicated that ^{18}FDG was able to comply with the

specifications (Table 2) up to 10 hours after the end of the synthesis, regarding acceptance limits for each required assay.

Low standard deviations were observed between the measures, what suggests that they are pretty reproducible. Besides, little difference was verified for early, middle time interval and latest results.

Through data analysis it was not possible to find evidence of ^{18}FDG subproducts formation, caused by radiolysis. As it has already been reported, radiolysis increases with the radioactive concentration (Fawdry, 2007) [1]. It is believed that the routine radioactive concentration, which was assayed, was not high enough for observing this effect. However, it is suggested the employment of other methods (HPLC-MS and HPTLC) if the main objective is to perform such a kind of analysis.

Table 2. Results of ^{18}FDG assays at different time intervals

Parameters	Results					
	0 h	**2 h**	**4 h**	**6 h**	**8 h**	**10 h**
Half-life (min)	111.0 ± 1.0	110.3 ± 3.2	110.3 ± 3.2	111.1 ± 1.0	109.5 ± 2.1	110.6 ± 0.5
Radionuclidic identity (keV)	515.6 ± 1.6	511.1 ± 1.0	512.4 ± 1.3	509.8 ± 1.7	514.0 ± 2.0	510.0 ± 2.0
Radiochemical identity (R_f)	0.55 ± 0.04	0.54 ± 0.02	0.54 ± 0.01	0.54 ± 0.02	0.54 ± 0.03	0.54 ± 0.02
Radiochemical purity	95 ± 2%	96 ± 2%	95 ± 1%	96 ± 2%	96 ± 1%	95 ± 2%
pH	6.5 ± 0.5	-	-	-	-	6.5 ± 0.5
Chemical purity	< 50 µg/mL	-	-	-	-	< 50 µg/mL
Residual solvents	Ethanol 0.0381 ± 0.0004%	-	-	-	-	Ethanol 0.0397 ± 0.0007%
	Acetonitrile 0.0056 ± 0.0002%					Acetonitrile 0.0053 ± 0.0003%
Bacterial endotoxins	< 5.0 EU/mL	-	-	-	-	< 5.0 EU/mL
Sterility	Sterile	-	-	-	-	Sterile

Fig. 2 summarizes how ^{18}FDG radiochemical purity varies with time under experimental conditions up to 10 hours after synthesis. This result is emphasized here because differences in preparation, synthesis, and formulation can have a strong effect on the radiochemical purity of ^{18}FDG (Jacobson et al., 2009) [10]. The graphic indicates that ^{18}FDG samples not only complied with the pharmacopeial specification for radiochemical purity, but also presented precise results (standard deviation smaller than 2%) up to 10 hours after the end of synthesis.

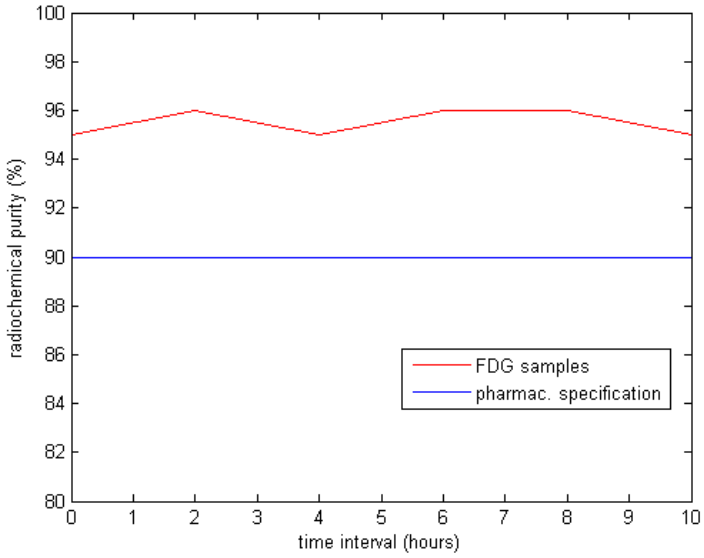

Figure 2. Radiochemical Purity of ^{18}FDG at different time intervals

Fig. 3 and 4 show typical chromatograms of ^{18}FDG samples obtained just after the synthesis and 10 hours later, respectively. The main peak of radioactivity detector corresponds to the ^{18}FDG signal and achieves, respectively, 96.6% and 95.0% of total ^{18}F radioactivity. The spots observed after spraying sulphuric acid solution 2 N in the chromatographic plates corresponds to ^{18}FDG as the retention factors (R_f) were the same obtained for FDG standard solution (approximately 0.5).

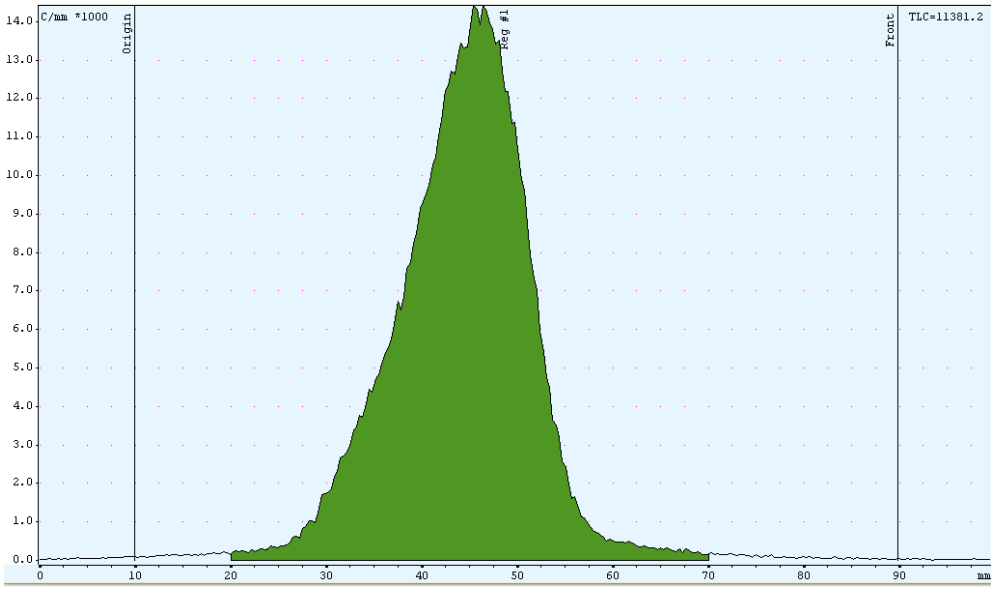

Figure 3. Chromatogram of ^{18}FDG just after synthesis

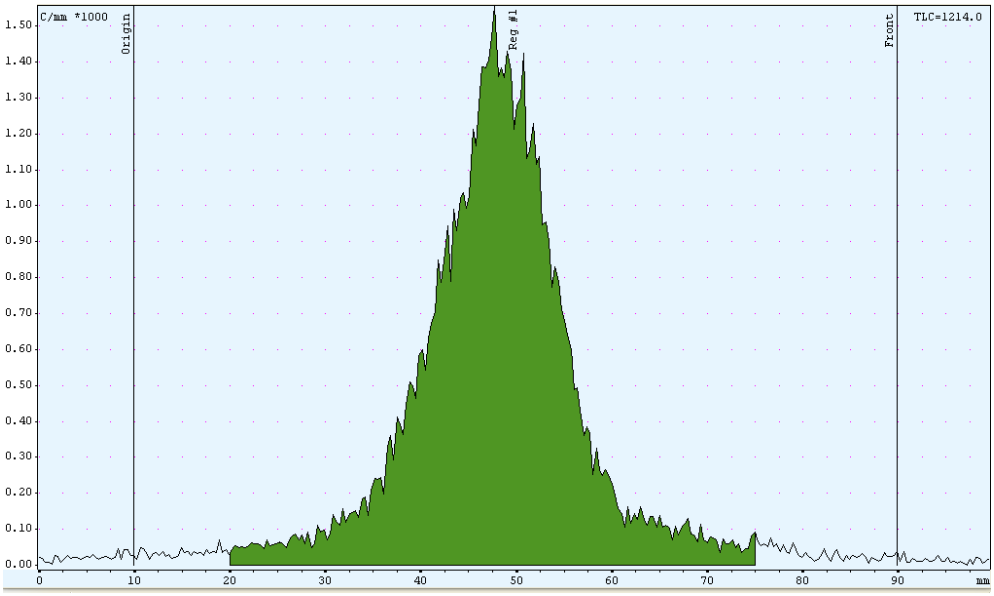

Figure 4. Chromatogram of ^{18}FDG 10 hours after synthesis

^{18}FDG epimerizes in alkaline conditions forming the epimer ^{18}Fluorodeoxymannose (^{18}FDM) and free ^{18}Fluoride ($^{18}F^-$) (Varelis and Barnes, 1996 [11]; Meyer et al., 1999 [12]). Free $^{18}F^-$ is the main active product of radiolysis and it is considered the main decomposition product (Meyer et al., 1999) [13]. The radiochemical purity analysis makes possible the evaluation of the percentage quantity of free fluoride ($^{18}F^-$), which can appear as a secondary peak, since its R_f (0.0) is lower than ^{18}FDG's one (approximately 0.4) (Hung, 2002) [13]. However, in test conditions, there is no evidence of $^{18}F^-$ formation in significant quantity, as just a major peak appears in all chromatograms obtained for radiochemical purity. The major peak was identified as ^{18}FDG by R_f value and comparison with the standard.

4. CONCLUSION

The samples of ^{18}FDG which were assayed using the USP 31 (2008) [8] analytical methods for Fludeoxyglucose F 18 injection complied with all the specifications up to 10 hours after the end of the synthesis. These findings suggest that ^{18}FDG has physicochemical and microbiological stability up to 10 hours, if stored at room temperature, in the radioactive concentration tested.

A possible extension of this work is the evaluation of other factors which might affect radiopharmaceutical stability, such as different packaging material, longer storage period and higher temperatures. It is expected that some complementary results will be able to provide enough background to estimate the expiration period of ^{18}FDG with high accuracy and reduced bias.

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