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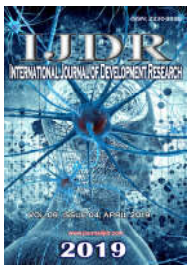

RESEARCH ARTICLE OPEN ACCESS

VALIDATION OF THE ANALYTICAL METHOD OF CHEMICAL PURITY OF 18F RADIOPHARMACEUTICAL FLUDESOXYGLUCOSE (FDG) VIA THIN LAYER CHROMATOGRAPHY

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ABSTRACT

For the release of the 18F-Fludesoxyglucose radiopharmaceutical in humans, one of the tests required in its official monograph present in the American pharmacopeia is the determination of the chemical purity. This test consists of a process of quantification of the analyte Kryptofix 2.2.2, using the realization of a limiting assay, developed in thin layer chromatography. To prove the reliability of the results obtained, the centers producing radiopharmaceuticals should promote the validation of the analytical methods used in quality control by the relevant legislation in the country. Thus, the analytical validation developed in the present work had the objective of verifying the behavior of the analytical method about the parameters: specificity, limit of detection and robustness, based on the criteria established in Anvisa RE 899/2003. Specificity verification assays have demonstrated the ability of the method of detecting the analyte of interest even in the presence of the significant impurities reported for the radiopharmaceutical. For the limit of detection, it is estimated that minimum mass, capable of generating the expected analytical response is equal to 0.03 µg. Finally, the results obtained for the robustness test allow us to state that the method can withstand the modifications proposed for certain stages of the analysis; concluding, therefore, that the technique is duly validated according to the established criteria of RE 899/2003 ANVISA.

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INTRODUCTION

Nuclear medicine is defined as the medical specialty makes use of pharmaceutical preparations called radiopharmaceuticals, for anatomical or physiological assessments, treatments and medical research (ARAÚJO *et al*., 2008). Radiopharmaceuticals are defined as pharmaceutical preparations which have a diagnostic or therapeutic purpose which, when ready for use, contain one or more radionuclides (ANVISA, 2010).

According to the World Health Organization, radiopharmaceuticals can be classified into four categories (WORLD HEALTH ORGANIZATION, 2007):

- Radioactive products ready for use;
- Radionuclide generators;
- Non-radioactive components (lyophilized reagents) for the preparation of compounds labeled with radioactive elements, (generally the eluate of a radionuclide generator);
- Precursors used for marking other substances before administration.

Radiopharmaceuticals intended for therapy, the radionuclide is generally a particle emitting element such as alpha or beta, with energy to promote cell destruction and relatively short range, avoiding the irradiation of healthy tissues located around the target tissue. The radiopharmaceuticals have a short half-life and emit photons or positrons that are detected by equipment such as single-photon emission tomography (SPECT) and positron emission tomography (PET) (ARAÚJO *et al*., 2008). PET becomes increasingly crucial in the evaluation of physiological, biochemical and pharmacological functions at the molecular level, in healthy and pathological conditions. This technique allows the quantitative determination of the distribution of biological components in the human body. For this, the administration of labeled molecules with

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positron emitting radionuclides such as 15O, 13N, 11C or 18F is required, their half-lifes being; 2.037 min, 9.965 min, 20.39 min and 109.8 minutes. The technique requires radiotracers to have high affinity and selectivity for the receptors, low non-specific binding and optimal biological half-life (time taken for an organism to remove half the amount of a given substance by biological elimination). The most commonly used radiopharmaceuticals in the clinics for the evaluation of different processes in the human body, according to Chen; Jacobson (2010), are:

- [18F] - FDG for evaluation of energy and cellular metabolism;
- [13N] - NH3 for perfusion imaging;
- [15O] - H2O for cerebral blood flow;
- [11C] - Raclopride and [18F] - FDOPA for evaluation of the dopaminergic system.

In the 1980s, positron emission tomography using [18F] -FDG was introduced as a method of in vivo imaging of the metabolic activity of the human body. This was justified since malignant cells in the great majority have a high glycolytic metabolism concerning normal tissues, which favors the application of the technique and the detection of the disease. The [18F] -FDG assists in the diagnosis of neoplasias, differentiating benign tumors from malignant tumors, staging, evaluation of early and late therapeutic response, assessment of tumor recurrence, and re-staging of cancer patients (SOARES JUNIOR *et al*., 2010). The [18F]-FDG radiopharmaceutical is a glucose analog that has the hydroxyl group of carbon 2 substituted by a fluorine atom, which is a factor that impedes the development of healthy glucose metabolism and what makes this molecule a good indicator of sugar uptake and cell viability. The process of [18F] -FDG production starts with the 18O(p,n)18F nuclear reaction in the cyclotron particle accelerator, by bombarding enriched water (H218O) with protons, a process that causes 18F- (YU, 2006). After the irradiation process, the enriched water is sent to automatic synthesis modules, where the ion of interest will be trapped in the ammonium quaternary ion exchange column (QMA). Fluoride is then eluted from the column with a previously prepared solution by mixing two other solutions, the first of potassium carbonate in water and the second of Kryptofix 2.2.2 in acetonitrile (LEMAIRE *et al*., 2002); thereafter a series of subsequent steps are required to obtain the final product, varying according to the synthesis modulus used and the route of synthesis chosen.

However, the use of Kryptofix 2.2.2, as a phase transfer agent, to facilitate nucleophilic substitution, is a common term among the various models of synthesis modulus and routes employed. Kryptofix 2.2.2 is known to be toxic, presenting an LD50 in rats = 35 mg/kg and may cause apnea and seizure pictures when administered in humans; these facts make the synthesis modules to develop purification steps to minimize the residual concentrations of this molecule in the final product, such as the use of specific columns and filters, which have shown great effectiveness (KILBOURN; SCOTT; 2007; YU; 2006). Thus, in the official monograph of [18F] -FDG one of the tests described for the release of the radiopharmaceutical, is the determination of the chemical purity. This is the quantification of Kryptofix 2.2.2, using a limiting analysis, using the proposed method or another duly validated method (USP, 2015). As the use of the analysis method is not always possible to obtain reliable results due to the difficulty of distinguishing the respective patches from the standard and the sample under analysis (YU, 2006), many producing centers seek to adopt alternatives, either by choosing another technique analytical or stationary phase change, for example (Deng *et al*., 2012). However, for any of the selected alternatives involving the analysis procedures, a validation study should be planned and executed to prove the suitability of the method to the intended purpose (ANVISA, 2003). Based on the information described, the present work had the objective of adjusting and validating the analytical method proposed in the official monograph of radiopharmaceutical [18F] -FDG in the American pharmacopoeia, to determine chemical purity, based on the requirements established in Resolution 899/2003, so that the solution found represents the analytical reliability required for the intended purpose of the analytical method.

MATERIAL AND METHODS

Materiais and Reagents: In the development of the validation study, we used: chromatographic sheets with stationary phase of 20cm x 20cm silica gel (Merck, lot: HX41562953), glass vats; blower; automatic micropipette (0.1 - 10 µL Brand) and suitable tips from the same manufacturer; Kryptofix 2.2.2 standard (Sigma Aldrich, batch: HMBC5690V). The following reagents were used: Methanol (Merck, lot: L1712609), 30% ammonium hydroxide (JT Baker, lot: L01047), Acetaldehyde (Sigma Aldrich, lot: # SHBD9444V), Acetic acid (Modern Chemistry, lot: 00593), Acetonitrile (Merck, lot: L668130), Potassium carbonate (Sigma Aldrich, lot: # SLBJ4496V), Glucose (Sigma Aldrich, lot: # SLBK4621V) and resublimed iodine (Química Nova, lot: 1156).

Analytical Software and Analysis Tool: For the analysis of the results obtained in the robustness tests, we used the Microsoft Excel® 2013 software and data analysis tool ANOVA: single factor.

Analysis of determination of chemical purity of radiopharmaceutical [^{18}f] –fdg: The analysis of the chemical purity developed in the producer center consists of the development of the thin layer chromatography technique using a solution of methanol: ammonium hydroxide (90:10 v / v) as the mobile phase. The procedure adopted establishes the application of 3 µL of standard solution of Kryptofix 2.2.2 in the concentration of 50 µg/mL in the lower right side, at the point of origin, established 1 cm from the lower edge of the chromatographic sheet previously activated with the aid of hot air blower at 100°C for 5 minutes. The application of 3 µL of a sample of the radiopharmaceutical produced at the point of origin on the lower left side. After use, the chromatographic sheet containing the standard solution and sample of the radiopharmaceutical to be analyzed is subjected to the fixing step, which consists in drying the solutions at 50°C for 1 minute with the aid of a hot air blower. After complete drying, the chromatographic sheet is inserted into a pre-saturated glass vat 10 minutes before starting the analysis with the mobile phase, initiating the chromatographic development, which is interrupted when the mobile phase reaches the end point of the run located 1 cm from the upper border. Immediately after completion, the chromatographic sheet is inserted into another glass vessel containing re-sublimated iodine for performing the development step. Finally, the presence or absence of the Kryptofix 2.2.2 analyte spot in the radiopharmaceutical sample under analysis with the standard solution spot is checked and the retention factor (Rf) obtained is measured. For the release of the radiopharmaceutical for human use, the size and intensity of the spot on the analyzed radiopharmaceutical sample, when present, should be less than the standard solution spot.

Validation Planning: According to Anvisa Resolution no. 999/2003, the determination of the chemical purity of the [18F] -FDG radiopharmaceutical falls within category II, as a limiting test; therefore, it is necessary to elaborate a validation protocol that contemplates the execution of tests that verify the analytical parameters specificity, limit of detection and robustness; in relation to the intended use in order to prove the validation of the analytical method.

The Preparation of Solutions and Chromatographic Sheets: The preparation of the solutions followed the guidelines described in the official monograph of the radiopharmaceutical, present in the American pharmacopeia, and their concentrations were modified according to the proposal and objectives of the specific tests developed in this validation study.

The chromatographic sheets containing the stationary phase of silica gel were prepared and sized as shown below.

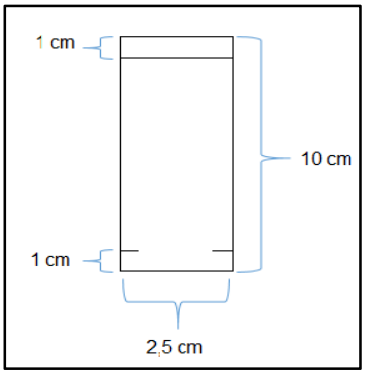

Figure 1. Representation of chromatographic sheet dimensions

Specificity Verification Test: For the evaluation of the specificity, the development of chromatographic runs was performed in triplicate, with two different solutions. The first so-called white solution containing all the major possible chemical impurities in the radiopharmaceutical according to the manufacturer of the reagent cassette used in the synthesis of [18F] -FDG minus the analyte of interest, Kryptofix 2.2.2. The worst-case scenario based on the maximum predicted concentration reported by the manufacturer of the reagent cassette used by the producer of the radiopharmaceutical, To determine the level of each analyte in the white solution, according to the data in Table 1. The second solution, called a robust solution, consisted of the white solution plus the analyte Kryptofix 2.2.2 at 50 µg / mL.

Table 1. List of chemical impurities present in radiopharmaceutical [18F] –FDG

Impurity	Concentration reported	Concentration Solution White
Acetic Acid	900 – 1400 µg / mL	1400 µg / mL
Acetaldehyde	0.1 – 5.4 µg / mL	5.4 µg / mL
Acetonitrile	34 – 134 µg / mL	134 µg / mL
Potassium carbonate	15 – 25 µg / mL	25 µg / mL
Glucose	292 – 402 µg / mL	402 µg / mL

Source: (GE Health Care - Fastlab TM Regulatory Support Information, 2011)

Detection Limit Verification Test: As a means of assessing the limit of detection, a stock solution of 1 mg/ml Kryptofix 2.2.2 was prepared with subsequent dilutions as described in Table 2. For each level of dilution, chromatographic races were run in triplicate. The amount of analyte present in the three µL applied to the chromatographic sheet for the last dilution level of the solution of Kryptofix 2.2.2 was determined as the limit of detection.

Table 2. Dilution levels for Kryptofix 2.2.2 solutions

Dilution1	Dilution2	Dilution3	Dilution4	Dilution5
50 µg / mL	20 µg / mL	10 µg / mL	5 µg / mL	2,5 µg / mL
0,05 mL "stock solution" + 0,95 mL physiological solution - NaCl 0,9 %	0,020 mL "stock solution" + 0,980 mL physiological solution - NaCl 0,9 %	0,010 mL "stock solution" + 0,990 mL physiologic al solution - NaCl 0,9 %	0,005 mL "stock solution" + 0,995 mL physiologic al solution - NaCl 0,9 %	0,0025 mL "stock solution" + 0,9975 mL physiologic al solution - NaCl 0,9 %

Source: (The Author, 2015)

Robust Verification Tests: To evaluate the robustness of the analytical method, small and deliberate changes in the parameters of some stages of the analysis were established, being:

- Saturation time of the glass vessel with the solvents of the mobile phase;
- Activation time of the chromatographic sheet with the stationary phase;
- The proportion of solvents constituent of the mobile phase;
- Fixation time of the solutions in the chromatographic sheet

For each proposed change the triplicate analyses were performed and at the end of each chromatographic run, the retention factor (RF) and the analysis time were recorded.

Saturation time of the glass vessel with the solvents of the mobile phase: Chromatographic runs were promoted by altering the saturation time of the glass vessel with the mobile phase, so that 5 ml of mobile phase were transferred to the chromatographic vessel at 10, 15 and 20 minutes before the start of the chromatographic analysis and development. Still, between one analysis and another, the residual mobile phase volume was discarded and replaced with a new one, duly transferred from the mother vial storing the mobile phase.

Activation time of the chromatographic sheet with the stationary phase: In each set of analysis, the activation time of the chromatographic sheet necessary for the removal of the moisture contained in the stationary phase, before the development of the analysis runs, was modified with the aid of a hot air blower, set temperature at 100ºC and continuous and repetitive movements developed throughout the leaf extension, varying the time between 01, 03 and 05 minutes.

Proportion of solvents constituent of the mobile phase: The changes in this parameter consisted of varying the proportion of the solvent methanol with the ammonium hydroxide, in the final concentration of the mobile phase. The proportions used were: (80:20 v/v), (85:15 v/v) and (90:10 v/v).

Fixation time of the solutions in the chromatographic sheet: The verification of this parameter occurred by changing the fixation time, under the temperature of 50º C, of the solutions in the chromatographic sheet, changing the time between 01, 03 and 05 minutes. Table 3 summarizes the tests developed in the evaluation of the robustness parameter performed during the validation of the analytical method.

RESULTS AND DISCUSSIONS

Specificity Verification Tests: In the development of the specificity tests, it was not possible to visualize the stain inherent to the Kryptofix 2.2.2 analyte as expected, and when the same solution of the analyte of interest was added to the chromatographic development, (Rf = 0.8125) — thus demonstrating the ability of the method to measure Kryptofix 2.2.2, even in the presence of the main impurities, as shown in Figure 2.

Detection limit Verification Tests: In the development of the assays, the concentration of 10 µg/mL was the lowest to show the Kryptofix 2.2.2 analyte spot with adequate resolution, according to Figure 3. Thus, based on the application volume (3µL) and the concentration of the solution (10 µg/mL); it is estimated that the lowest mass of the Kryptofix 2.2.2 analyte capable of generating a spot with good visibility under the conditions established for the method is equal to 0.03 µg. The Figure 3 show the determination of detection limit:

Robust Verification Tests: The data obtained during the execution of the robustness tests were evaluated using the ANOVA: single factor data analysis tool, to verify if the modifications of the analytical parameters presented significant differences in the obtained results. Forthis, in all the analyzes were established two hypotheses:

(A) $H_0 = \mu1 = \mu2 = \mu3$; where the averages obtained in each set of results for each analysis do not present significant differences;
(B) H_1 = where at least one means obtained presents a significant difference in the other results.

Subsequently, to verify the equality between the means obtained, the results obtained for the execution of Test F were submitted, yet for all conditions analyzed, a level of significance was considered with $\alpha = 0.05$.

Table 3. Proposed assays for robustness assessment

Testing of the saturation time of the glass vessel with mobile phase		
Test 1	Test 2	Test 3
10 minutes before the start of the analysis	15 minutes before the start of the analysis	20 minutes before the start of the analysis
Testing of the activation time of the chromatographic sheet with the stationary phase.		
1 minute activation at 100°C	3 minutes activation at 100°C	5 minutes activation at 100°C
Tests to verify the proportion of the solvents constituting the mobile phase		
MeOH:NH_4OH (80:20 v/v)	MeOH:NH_4OH (85:15 v/v)	MeOH:NH_4OH (90:10 v/v)
Testing of solution fixation on the chromatographic sheet		
1 minute fixation under 50 ° C	3 minutes fixation under 50 ° C	5 minutes fixation under 50 ° C

Source: (The Author, 2015)

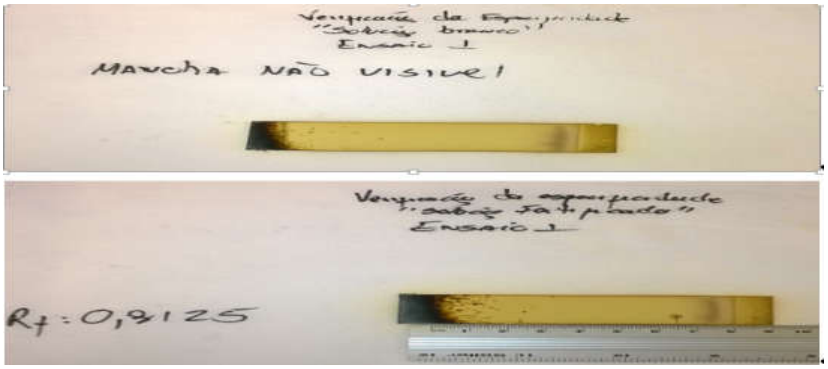

Figure 2. Analysis of white and fortified solutions

Figure 3. Determination of detection limit

General analysis

	Unity	Test 1: 10 min before RF	Test 1: 10 min before Time (min)	Test 2: 15 min before RF	Test 2: 15 min before Time (min)	Test 3: 20 min before RF	Test 3: 20 min before Time (min)
Analysis 1		0,838	19,750	0,788	19,780	0,863	20,560
Analysis 2		0,775	17,750	0,813	21,480	0,775	18,660
Analysis 3		0,800	19,180	0,788	19,460	0,825	18,200
Average		0,804	18,893	0,796	20,240	0,821	19,140
Standard Desviation		0,031	1,030	0,014	1,086	0,044	1,251

Analysis of variance (Anova) - RF

Group	*Counts*	*Sum*	*average*	*Variance*
Test 1: 10 min before	3	2,41250	0,80417	0,00099
Test 2: 15 min before	3	2,38750	0,79583	0,00021
Test 3: 20 min before	3	2,46250	0,82083	0,00193

Source Variation	*SQ*	*gl*	*MQ*	*F*	*F crítico*
Between groups	0,0009722	2	0,000486111	0,4666667	5,1432528
inside of the groups	0,00625	6	0,001041667		
Total	0,0072222	8			

Figure 4. Saturation time of the glass vat with the solvents of the mobile phase. Source :(The Author, 2015)

In this way, it was established that, if the value of F were lower than the value of the critical F, the hypothesis H0 would be considered true and, of course, if the opposite were found, that is, if the value of F were greater than the critical F, the hypothesis H1, would be considered as true. Thus, according to figure 4, the results obtained for the test of evaluation of the saturation time of the glass vat with the solvents of the mobile phase, show F values equal to 0.46 (F = 0.46) and 1.21 (F = 1.21) for the parameters retention factor (Rf) and analysis time respectively, while the critical F for both was 5.14 (critical F = 5.14), making the hypothesis H0 true. Moreover, the relative standard deviation (DPR) analysis, for the three analyzed conditions, shows that the saturation 20 minutes before the beginning

General analysis

	Unity	Test 1: 01 min RF	Test 1: 01 min Time (min)	Test 2: 03 min RF	Test 2: 03 min Time (min)	Test 3: 05 min RF	Test 3: 05 min Time (min)
Analysis 1		0,825	19,650	0,825	21,580	0,813	19,050
Analysis 2		0,813	20,210	0,863	22,900	0,813	20,130
Analysis 3		0,813	19,980	0,800	20,080	0,838	21,650
Average		0,817	19,947	0,829	21,520	0,821	20,277
Standard Desviation		0,007	0,281	0,031	1,411	0,014	1,306

Analysis of variance (Anova) - RF

Group	*Counts*	*Sum*	*average*	*Variance*
Test 1 - 01 min	3	2,45	0,8167	5,208E-05
Test 2 - 03 min	3	2,49	0,8292	0,0009896
Test 3 - 05 min	3	2,46	0,8208	0,0002083

Source Variation	*SQ*	*gl*	*MQ*	*F*	*F critico*
Between groups	0,0002431	2	0,000121528	0,2916667	5,1432528
inside of the groups	0,0025	6	0,000416667		
Total	0,0027431	8			

Figure 5. Activation time of the chromatographic sheet with the stationary phase Source: (The Author, 2015)

General analysis

	Unity	Test 1 MeOH : NH4OH (80:20 v/v) RF	Test 1 MeOH : NH4OH (80:20 v/v) Time (min)	Test 2 MeOH : NH4OH (85:15 v/v) RF	Test 2 MeOH : NH4OH (85:15 v/v) Time (min)	Test 3 MeOH : NH4OH (90:10 v/v) RF	Test 3 MeOH : NH4OH (90:10 v/v) Time (min)
Analysis 1		0,85	19,95	0,79	19,08	0,79	19,03
Analysis 2		0,86	22,13	0,83	20,66	0,85	22,80
Analysis 3		0,84	22,08	0,85	22,71	0,79	20,11
Average		0,85	21,39	0,82	20,82	0,81	20,65
Standard Desviation		0,01	1,24	0,03	1,82	0,04	1,94

Analysis of variance (Anova) - RF

Group	*Counts*	*Sum*	*average*	*Variance*
Test 1 - MeOH:NH4OH(80:20 v/v)	3	2,5500	0,8500	0,0002
Test 2 - MeOH:NH4OH(85:15 v/v)	3	2,4625	0,8208	0,0010
Test 3 - MeOH:NH4OH(90:10 v/v)	3	2,4250	0,8083	0,0013

Source Variation	*SQ*	*gl*	*MQ*	*F*	*F critico*
Between groups	0,002743	2	0,00137	1,68085	5,14325
inside of the groups	0,004896	6	0,00082		
Total	0,007639	8			

Figure 6. Proportion of the solvents constituting the mobile phase Source :(The Author, 2015)

General analysis

	Unity	Test 1 01 min of fixation RF	Test 1 01 min of fixation Time (min)	Test 2 03 min of fixation RF	Test 2 03 min of fixation Time (min)	Test 3 05 min of fixation RF	Test 3 05 min of fixation Time (min)
Analysis 1		0,78	19,38	0,81	21,90	0,81	21,46
Analysis 2		0,81	20,68	0,78	21,16	0,81	20,88
Analysis 3		0,83	20,81	0,81	22,06	0,81	21,00
Average		0,80	20,29	0,80	21,71	0,81	21,11
Standard Desviation		0,03	0,79	0,02	0,48	0,00	0,31

Analysis of variance (Anova) - RF

Group	*Counts*	*Sum*	*average*	*Variance*
Test 1 - 01 min of activation	3	2,4125	0,8042	0,0007
Test 2 - 03 min of activation	3	2,4000	0,8000	0,0005
Test 3 - 05 min of activation	3	2,4375	0,8125	0,0000

Source Variation	*SQ*	*gl*	*MQ*	*F*	*F critical*
Between groups	0,000243	2	0,0001215	0,318182	5,14325285
inside of the groups	0,002292	6	0,0003819		
Total	0,002535	8			

Analysis of variance (Anova) - Time

Group	*Counts*	*Sum*	*average*	*Variance*
Test 1 - 01 min of activation	3	60,870	20,290	0,625
Test 2 - 03 min of activation	3	65,120	21,707	0,231
Test 3 - 05 min of activation	3	63,340	21,113	0,094

Source Variation	*SQ*	*gl*	*MQ*	*F*	*F critical*
Between groups	3,036867	2	1,5184333	4,797241	5,14325285
inside of the groups	1,899133	6	0,3165222		
Total	4,936	8			

Figure 7. Fixation time of the solutions in the chromatographic sheet. Source :(The Author, 2015)

of the analysis was the condition that presented the worst results, both for the factor retention factor and for the time of analyzing. The condition 10 minutes before, presented the lowest variability for the parameter time of analysis and the situation 15 minutes earlier, the best results for the retention factor (Rf). Even so, all conditions presented values below 2% relative standard deviation (DPR) in both parameters, indicating good repeatability for the method. The figure 4. The analysis of the values of the relative standard deviation (RPD), for the time required for the development of the analysis and retention factor (Rf), in the 3 conditions analyzed, was used to evaluate the activation time of the chromatographic sheet with the stationary phase, did not show significant differences, presenting very close values for both parameters. Also, the value of F obtained for the parameter retention factor (Rf) was 0.29 (F = 0.29) and for the parameter time of analysis the value of F was equal to 1.64 (F = 1, 64), making the hypothesis H_0 valid and accepted as true, according to the data available and shown in Figure 5. In the assay to evaluate the proportion of the solvents constituting the mobile phase, as described in figure 6, the result of the F value for the retention factor parameter (Rf) was 1.68 (F = 1.68). For the time parameter, we obtained a result equal to 0.15 (F = 0.15), demonstrating that the data obtained for the means of results of each condition tested did not present significant differences between them. The proportion of solvents that presented the lowest values for the relative standard deviation (DPR) results of each parameter was test 1, with the concentration of 80 parts of methanol and 20 parts of ammonium hydroxide, however, the increase in the values for the parameters of analysis time and retention factor (Rf).The figure 6 show the results: Finally, in the assay to evaluate the fixation time of the solutions in the chromatographic sheet, test 3 was presented as the best condition, due to the low value of the relative standard deviation (DPR) for the analyzed parameters, being those less than 0, 5% indicating good repeatability.

The condition studied in the tests programmed for test 2, evidenced an average time to carry out the analysis greater than the other experiments, which was equal to 21.71 minutes. The results obtained for the F values were 0.31 (F = 0.31) for the factor retention factor (Rf) and 4.79 (F = 4.79) for the analysis time making the hypothesis H0 valid and accepted as true, thus evidencing the absence of significant differences between the averages obtained, as reported and demonstrated in Figure 7: Thus, in general, the data obtained in each analysis, demonstrate that the changes developed in the analytical method, in order to verify the robustness of the same does not present significant differences between the means of results obtained, thus making the hypothesis H0 true , for all the conditions tested, evidencing the robustness of the method against the proposed modifications. Considering also that the objective of a study on validation of analytical methodologies is to demonstrate that the technique is appropriate for the intended purpose, thus demonstrating its suitability for qualitative, semi-quantitative or quantitative determination of drugs and other substances, in pharmaceutical products, by proving the specific analytical parameters. In this way, validation aims to obtain reliable analytical data, capable of leading to assertive decisions, reducing costs and avoiding irreparable financial losses (RIBANI *et al*., 2004). In particular, in the case of radiopharmaceuticals, the pharmacist should be consulted in the decision-making on the release of the medicinal product in humans, stating that, based on the results obtained, the analysis of the analytical parameters defined by Resolution 899/2003 , that the proposed analytical method for determining the chemical purity of the [18F] - FDG radiopharmaceutical has the required reliability required for the technique, since these demonstrated satisfactory values consistent with the purpose of the method. Thus, given the arguments presented, it is considered that the execution of the analytical method according to the procedures described above besides being suitable for the intended purpose represents. The Technique is a crucial alternative process to the pharmacopeia method described in the official monograph of the radiopharmaceutical [18F] - FDG.

The technique to prover and save of the financial resources necessary to carry out the analysis, since, according to data available in the literature, it is not always possible to carry out the analysis with the officially described method due to the difficulty of visualization and distinction of the inherent stains in the standard solution and sample under analysis. This leads the producer centers to look for new alternatives, ranging from the acquisition of more sophisticated equipment or exchange of the stationary phase, resulting in an increase in the costs of the analysis and consequently in the final price of the radiopharmaceutical.

Conclusion

The activities inherent to quality control for the release to the use of short half-life radiopharmaceuticals, such as 18F-Fludeoxyglucose, should be established and adequate for the intended purpose to provide reliable results. In this way, a validation study to prove such parameters must be performed. Thus, for the analysis of the chemical purity of the [18F] - FDG radiopharmaceutical, the adjustments made in the analytical method proved to be efficient, so that the analysis can be developed in accordance with the parameters established in its official monograph, mainly the analytical process, which, with the results obtained, can be considered validated, being in good agreement with the national regulations in force.

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