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Assessment of Aminopolyether on ^{18}F-FDG Samples

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***Abstract*—**The quality control procedures of a radiopharmaceutical include the assessment of its chemical purity. The method suggested by international pharmacopeias consists of a thin layer chromatographic run. In this paper, the method proposed by the United States Pharmacopeia (USP) is compared to a direct method to determine the final concentration of aminopolyether in Fludeoxyglucose (^{18}F-FDG) preparations. The approach (no chromatographic run) was achieved by placing the thin-layer chromatography (TLC) plate directly on an iodine vapor chamber. Both methods were validated and they showed adequate results to determine the concentration of aminopolyether in ^{18}F-FDG preparations. However, the direct method is more sensitive, faster and simpler when compared to the reference method (with chromatographic run), and it may be chosen for use in routine quality control of ^{18}F-FDG.

***Keywords*—**Chemical purity, Kryptofix 222, thin layer chromatography, validation.

I. Introduction

THE positron emission tomography (PET) technique is very important for the tumoral staging and initial diagnostic. Nowadays the most used PET radiotracer is the 2-[^{18}F] Fluoro-deoxy-D-glucose (^{18}F-FDG) [1], [2]. This molecule is a glucose analogue (in which a hydroxyl group is replaced by a fluorine atom) and it is very useful at clinical imaging applications in neurology, oncology and cardiology [3], [4].

The fluorine-18 is obtained via ^{18}O(p,n)^{18}F nuclear reaction in cyclotrons and the labeling procedure occurs on automatic synthesis modules, where adequate precursors are utilized [3]. The first synthesis procedure lasted up to 2 h (purity over 98% and yield about 8%). In 1986, the use of a catalyst agent (the aminopolyether kryptofix 222) was introduced making the reaction faster (50 min with a yield of over 50%). As the kryptofix 222 could be toxic (causes apnea and convulsion), all the synthesis procedures involves appropriate steps to remove this compound at the final ^{18}F-FDG injection [3].

The quality control procedures performed prior the injection of the drug into the patient should certify that it is safe to human consumption. The methods to access the amount of kryptofix 222 are described in details at USP [5] and European Pharmacopeia (EP) [6]. These compendia describe methodologies based on TLC to perform spotting tests to compare the concentration of kryptofix 222 in test sample and the concentration of the reference standard. The major differences between these compendia are related to the mobile phase (ammonia or 30% ammonium hydroxide, for EP and USP, respectively) and the acceptance limits (the EP states the maximum concentration at 2.2 mg/recommended dose before the dilution with saline while the USP establishes the limit of 50 μg/mL at final solution). In both cases, approximately 20 minutes are required for completion of this test. However, a direct method (without chromatographic run) is cited by Yu [3].

The objective of this study was to suggest a direct method to determine the chemical purity of ^{18}F-FDG, using TLC, and to validate this method in accordance to the parameters recommended by the ANVISA [7]. The chemical purity assay was performed by following two methods: 1) developing the TLC plate in a solution of methanol and ammonia (90:10) and exposing the developed plate to iodine vapor, and 2) placing the TLC plate directly on an iodine chamber.

The validation of an analytical methodology is documented evidence that a method is suitable for its intended purpose, and is considered accurate, specific, reproducible and robust within the analytical conditions [7]-[9]. Several regulatory agencies provide guides for validation of methodologies, such as the ANVISA [7], the Food and Drug Administration (FDA) [8], USP [5] and the International Conference on Harmonisation (ICH) [9], however, there are non-specific for methodologies using ionizing radiation.

II. Experimental

The chemical purity assay was performed by TLC, using silica gel plate as the stationary phase and a methanol:ammonia (90:10) mixture as the mobile phase. Aliquots of standard kryptofix (0.05 mg/mL, diluted in saline) and sample of ^{18}F-FDG are applied on the plate (Fig. 1). In the method 1 (developing the TLC plate), the TLC plate was placed in the beaker containing the developing solution and it was left there until the mobile phase reaches the upper mark (Fig. 1). After drying, the plate was exposed to iodine vapor until two spots are visible, being the test-spot smaller and less intense than standard-spot.

The second method (method 2) for directly developing the kryptofix 222 with iodine vapor (step without chromatographic run) was also performed. The acceptance criterion remains the same.

The methods were validated in accordance to the Publication RDC 166/2017/ANVISA [7] and ICH [9]. The

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parameters studied were specificity, limit of detection and robustness for both methods, as described:

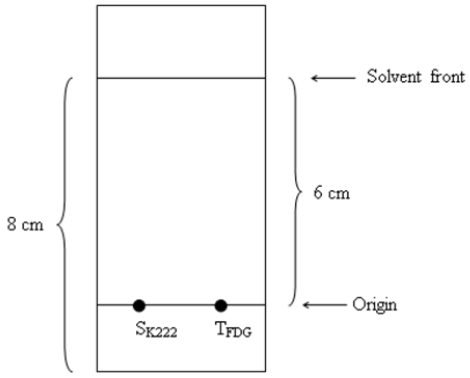


Fig. 1 Schematic preparation of TLC plates

A. Specificity

The standard solutions of ^{18}F-FDG (0.1 and 0.025 mg/mL), fluorodeoxymannose (FDM) (0.1 and 0.25 mg/mL), kryptofix 222 (0.05 mg/mL) and glucose (0.5 mg/mL) were applied in the TLC plate.

B. Limit of Detection

Aliquots with decreasing volumes (5, 4, 3, 2, 1 and 0.5 µl) of kryptofix 222 standard solution (0.05 mg/mL) and aliquots with decreasing concentration (0.05 up to 0.01 µg/mL) of kryptofix 222 were applied the TLC plate simultaneously.

C. Robustness

The influence of the variation of the type of stationary phase (aluminum or glass plate) was evaluated for both methods. Besides, for method 1, the distance traveled by the mobile phase (5.5, 6.0 and 6.5 cm) and the mobile phase concentration (89:11, 90:10 and 91:09 v/v) were also evaluated.

In relation to the method 1, all the plates were subjected to the chromatographic run and exposed to iodine vapor. However, in method 2, the plates were positioned directly on the iodine vapor chamber.

III. Results and Discussion

A. Method 1: With the Chromatographic Run

Specificity: The methods is considered specific because it was only possible to visualize the spot related to the kriptofix 222 (Fig. 2). The spots related to other components (FDG, FDM and glucose) were not visualized on TLC plate after developing procedure.

Limit of detection: The results for the limit of detection are presented on Fig. 3. The minimum concentration of kryptofix 222 detected was 0.014 mg/mL. In relation the variation of the volume of the standard solution applied on the TLC plate, all spots were visualized after developing procedure showing that there is no detection limit on the sample volume (Fig. 4).

Fig. 2 Test for specificity of the method in the presence of possible components of ^{18}F-FDG (FDG: ^{18}F-FDG; K: kryptofix 222; G: glucose; FDM: fluorodeoxymannose)

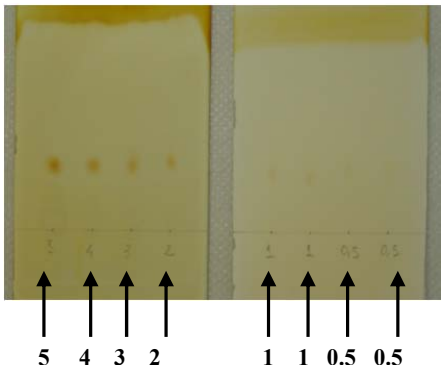


Fig. 3 DL determination for decreasing volumes of the kryptofix 222 standard solution (5 up to 0.5 mg/mL)

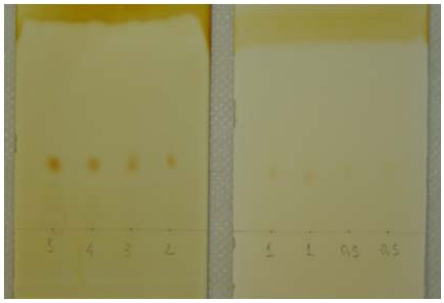


Fig. 4 DL determination for decreasing volumes of the kryptofix 222 standard solution (0.05mg/mL)

Robustness: The results are presented in Table I. The method was not affected by the distance travelled by the mobile phase or by its concentration (in the range studied here), but it was sensitive to the change on type of TLC plate. Moreover, if it is necessary the use of aluminum TLC plates, new validation tests should be performed.

TABLE I
RESULTS OF ROBUSTNESS TEST, ASSESSING THE INFLUENCE OF THE DISTANCE TRAVELLED BY MOBILE PHASE, VARIATIONS IN THE CONCENTRATION OF THE MOBILE PHASE AND THE TYPE OF CHROMATOGRAPHIC PLATE

Distance travelled by mobile phase	Rf[1]
d= 5.5	0.20
d= 6.0	0.20
d= 6.5	0.21
Mean	0.203
Standard Deviation	0.006
Methanol:ammonia proportion	Rf[1]
91:09	0.21
90:10	0.20
89:11	0.20
Mean	**0.203**
Standard Deviation	**0.006**
TLC plate	Rf[1]
Glass	0.20
Aluminium	0.48
Mean	**0.340**

[1]Rf = Distance migrated over the total distance covered by the mobile phase.

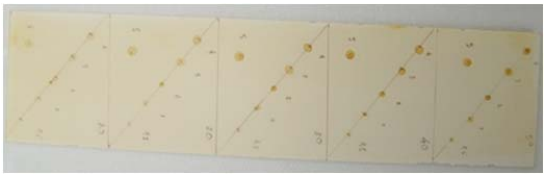
B. Method 2 - Direct Developing

Specificity: The method was considered specific, with the appearance of the spot just at the site of application of kryptofix 222 (Table II).

TABLE II
SPECIFICITY TEST FOR DETECTION OF KRYPTOFIX 222 TO THE DIRECT METHOD

	Spot Intensity
Kryptofix 222(0.05 mg/mL)	+++++
FDG (0.1 mg/mL)	-
FDG (0.025mg/mL)	-
FDM (0.5mg/mL)	-
FDM (0.25mg/mL)	-
Glucose (0.5 mg/mL)	-

(-): Spot not visible; (+++++): Intense spot



(a)

(b)

Fig. 5 DL determination for decreasing concentration of kryptofix 222: (a) 0.05 up to 0.01 mg/mL, and (b) 0.01-0,001 mg/mL, without running the mobile phase

Limit of detection: The aliquots with concentrations of 0.05 to 0.01 mg/mL were detected by spot test (Fig. 5 (a)), therefore, it was necessary to perform more dilutions of standard solution of kryptofix 222 (0.01-0,001 mg/mL) (Fig. 5 (b)). The final concentration of the standard solution that could be detected was 0.003 mg/mL, and this is therefore the DL found to this technique. Then, through this test, it can be concluded that the direct method is more sensitive when compared to with chromatographic run (method 1).

Robustness: The method was not affected by the type of TLC plate utilized once it was possible to visualize the spots corresponding to the standard solution of kryptofix 222 applied on both plates.

The step of chromatographic run aims evidence that the visualized spot corresponds to the kryptofix 222, by analysis of Rf which is a characteristic of each substance. The appearance of a spot in a different Rf means that a nonspecific reaction occurred and the product present does not correspond to kryptofix 222. This method, described by USP Pharmacopeia, however presents disadvantages, such as duration of the test (about 20 minutes, which makes it critical in case of test repetition) and costs (acquisition of mobile phase). The methodology without chromatographic run (direct method) was duly validated, and it can be used safely in the laboratory routine quality control of ^{18}F-FDG. The specificity study ensured that no other substance that may be present in the formulation of the radiopharmaceutical is able to be developed by iodine vapor, not requiring the chromatography running step. In addition, the direct method is much more sensitive (LD = 0.003 mg/ml) and faster.

IV. CONCLUSION

The two methods for detection of kryptofix 222 in ^{18}F-FDG formulations were tested and validated in the present work. The method 1, with chromatographic run, is described at USP Pharmacopeia [5]; the second, the direct method, is cited in former works [3] and was tested at DIPRA/CRCN-NE/CNEN. Both methods have been validated and are suitable for use in the laboratory routine. The direct method (no chromatographic run) was faster, cheaper and more sensitive than method 1.

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