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Optimization of the Methodology for Determination of Tetrabutylammonium in the radiopharmaceutical 18F-PSMA-1007 by Thin Layer Chromatography

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1. Introduction

Tetrabutylammonium (TBA) is a quaternary ammonium cation often used as a phase-transfer catalyst in radiopharmaceuticals labelling reactions with fluorine-18, like ^{18}F-PSMA-1007. [1] . Elements like kryptofix and TBA are used in automated synthesis, in fluorine-18 elution from anion-exchange cartridge QMA, and are considered impurities on the final product. [2].
Several detection methods for both components can be found on European Pharmacopoeia which, and according to ^{18}F-PSMA-1007 monograph, an amino polyether can be detected by SPOT TEST using a standard solution as a reference, comparing its coloring. The HPLC is an efficient method for detection of TBA, but it requires more time and resources [3]. It is possible to determine TBA through SPOT TEST method, however, the false negative rate of this assay is large, due to the presence of stabilizing agents into samples, that interferes with the test [4].
The thin layer chromatography in silica gel plate (TLC-SG) is a great option to determinate TBA on the ^{18}F-PSMA-1007, being a fast, easy and low-cost method. TLC can promote a semi-quantitative result, separating the product from the impurity to be determined, promoting a suitable result [5]. The present study aimed to evaluate the performance of the TLC-SG method for TBA determination in the ^{18}F-PSMA-1007 radiopharmaceutical. This work investigated critical factors that influence TBA detection on the chromatographic plates.

2. Methodology

^{18}F-PSMA-1007 Synthesis

The synthesis of ^{18}F-PSMA-1007 was fulfilled in an automated radio synthesizer system (TRACERLab MX, GE), using a labelling kit (ABX, Germany). Overall, ^{18}F was produced by irradiation of ^{18}O-enriched water on cyclotron accelerator (Cyclone 18, IBA). QMA cartridge retained the fluorine-18 that was eluted with 750 µL of 0.075 M tetrabutylammonium bicarbonate solution (TBA) into a reactor vessel. The mixture was evaporated to dryness at high temperature under a stream of nitrogen. The precursor PSMA-1007 (1,6 mg) diluted on 2 mL of DMSO was transferred to the reactor vessel, where the radiolabelling occur for 10 minutes at 105 ºC. The product was diluted with ethanol 5,5% and purified by C18 and PSH^+ cartridges, preconditioned with 3 mL of ethanol 30%, followed by 5 mL for product elution. The final product was diluted by PBS with 400 mg of sodium ascorbate on the final vessel.

TLC-SG for TBA determination

The thin layer chromatography in silica gel plate (TLC-SG) was employed to determine TBA as contaminant in ^{18}F-radiopharmaceutical, using methanol:25% ammonia 9:1 (v/v) as solvent [5]. In this chromatographic system, the retention factor (Rf) of TBA was 0,1. After running, the plates were dried and the stain corresponding to the TBA was revealed with iodine vapor.
Some factors that could influence the determination of TBA by TLC-SG method were evaluated, as described below.

Size of the plates

Two sizes of aluminium-base silica gel plates were evaluated: 12.5 X 3.0 cm (two strips of 1.5 cm for ^{18}F-PSMA-1007 sample and TBA standard solution) and 4.0 X 8.0 cm (three strips of 1.3 cm wide for ^{18}F-PSMA-1007 sample, TBA standard solution and negative standard).

Concentration of TBA Standard solution

Three different Standard solution concentrations were studied to appraise the sensibility of the TLC-SG method, which was 0.014 mg/mL, 0.275 mg/mL and 0.1 mg/mL. The choice of these concentrations was based on TBA and/or amino polyether assays described on official monographs and literature. [3] [6] [5].

Solvent for TBA Standard solution

The solvents applied for TBA standard solutions preparation were water and water/ethanol 90:10 (v/v), that were evaluated for each standard concentration [5].

Sample volume

The volume of sample (VS) applied on chromatography plates was 1µL, 2µL and 5µL.

The influence of stabilizer agent

A comparative analysis of chromatographic profile was performed to determine the Rf of final product (^{18}F-PSMA-1007) and sodium ascorbate, which acts as a stabilizing agent for radiopharmaceutical preparations. This determination was important because both are organic species that promote color reaction in the presence of iodine vapor, used for identification of TBA in the TLC-SG system. For this investigation, a solution made of 400 mg of sodium ascorbate was diluted on 0.9% saline solution 20 mL. A parallel analysis was made adding the stabilizer agent in each standard solution on both solvents to determine the separation ability of the chosen method.

Assessment of retention factors in the TLC-SG system

TLC was performed using Polygram SIL (Merck, Germany) 4.0 × 8.0 cm by applying three separate spots on the respective lines containing the reference standard, test solution ^{18}F-PSMA-1007 and negative standard each 10 mm from the bottom. The TLC plate was properly dried at ambient temperature and then developed in the TLC chamber pre-conditioned. After the run, the TLC plate was properly dried at the heater at 60 ºC and then placed in a second chamber filled with a few crystals of iodine for 1 min. This iodine chamber was gently heated from outside at 50–80 ºC, which results in the formation of iodine vapors. Thereafter, the dark brownish spot was identified and registered. This revealing process was performed for each run of 12.5 X 3.0cm plate and with sodium ascorbate samples.

3. Results and Discussion

Throughout the chosen method, was executed a comparison between de ^{18}F-PSMA-1007 product retention factor and the TBA standard solutions of reference. The visualization of Rf occurred by coloring the TBA by iodine vapors, showing a characteristic dark brownish spot. The acceptance criterion was the degree of intensity of the TBA standard spot, where the corresponding ^{18}F-PSMA-1007 spot samples should not be more intense than that of the TBA reference standard [5].

As expected, the standard spot of TBA showed red-brownish color in all concentrations, while the spots of the product showed orange-brownish color. According to Giesel et. al., the reference standard should show a dark brownish spot approximately at Rf 0.2–0.3 whereas the ^{18}F-PSMA-1007 spot, if it shows some spot at the same Rf, should be in lower intensity. [5]. The results found were similar, with the standard spots at Rf 0.1, while no other spot was found on the same Rf with the ^{18}F-PSMA-1007 samples. This indicates that, the TBA concentration on the final product was below the limit established by standard solutions (0.014 mg/mL, 0.1 mg/mL and 0.275 mg/mL).

Regarding concentrations used, the one that offered better visualization of the TBA spot was the 0.275 mg/mL (Figure 1, B), the concentration used as a TBA reference by HPLC on European Pharmacopoeia [3]. In comparison with the other concentrations studied, this one has the highest amount of TBA, and resulted in the most intense spot. Even with its higher acceptance limit and not being so strict, it remains acceptable in the toxicity standards with an LD_{50} value of 10 mg/Kg (i.v.), almost 4 times lower than kryptofix (LD_{50} (i.v.) between 32 - 35 mg/Kg) [7] [2].

The influence on the solvent used for standard dilution is less visible but comparable. Considering that the TLC-SG plate is strongly polar and the presence of ethanol in the mobile phase affects the retention factor more than the development itself, the intensity of the spots should not change. Nevertheless, the spots identified with the water/ethanol solution were slightly less intense than the spots seen with the standard diluted in only water (Figure 1, C and D) [8]. In Figure 1, it is possible to see that the standard retention factor for both solvents remains the same, proving that the water/ethanol solution used by Giesel and collaborators can also be used for TBA identification [5].

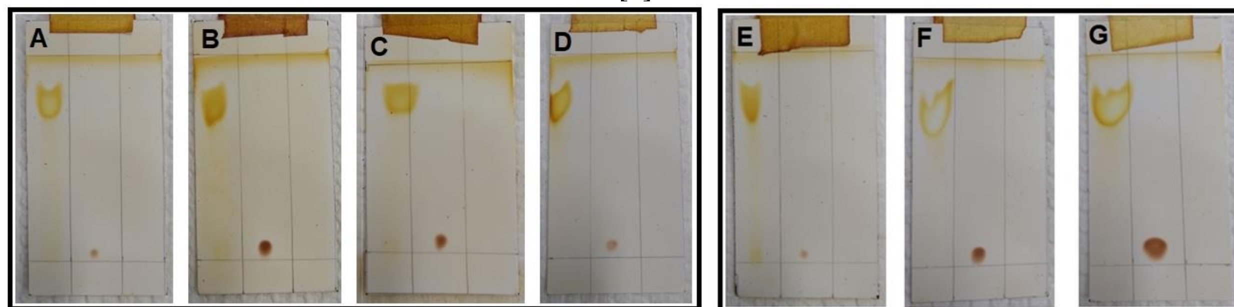

Figure 1: Comparison between the solutions used on references standards of TBA and ^{18}-PSMA-1007 product on 4 x 8 cm TLC plates. From left to right: ^{18}F-PSMA-1007; reference standard; negative standard. A) Concentration of 0.014 mg/mL diluted in water, AV of 2 µL; B) concentration of 0.275 mg/mL diluted in water, AV of 2 µL; C) concentration of 0.1 mg/mL diluted in water, AV of 2 µL; D) concentration of 0.1 mg/mL diluted in water/ethanol, AV of 2 µL. E) AV of 1 µL, concentration of 0.014 mg/mL diluted in water; F) AV of 2 µL, concentration of 0.275 mg/mL diluted in water; G) AV of 5 µL, concentration of 0.275 mg/mL diluted in water/ethanol.

In contrast to solvent, the VS applied for the TBA detection directly influenced the visualization of the spot. Among the three volumes evaluated, the volume of 2µL showed the best performance, especially in the spot samples visualization (Figure 1, F). The runs with 1µL showed very intense color of the radiolabelled product, but concerning the reference standard, the spot was very small, almost imperceptible (Figure 1, E). However, the spots of 5µL were too large, interfering with Rf identification and exceeding the limits for each strand (Figure 1, G). This volume is normally used for SPOT TEST, not recommended for TLC-SG as it uses unnecessary extra volume.
Considering the size of the TLC plates, both have a good performance on separating TBA from the final product ^{18}F-PSMA-1007, in the identification and quantification. The method proved to be appropriated for this assay, with acceptable results within a relatively short time limit. For the 4.0 x 8.0 cm plates, the running time was 15 minutes, whereas the 12.5 x 3.0 cm was 45 minutes. Between these options, the minor size was the most indicated for fast and efficient analysis, an even smaller TLC plate size can be used, considering that the TBA has a practical Rf of 0.1 and the product runs through the tape, staying close to the front of the solvent.

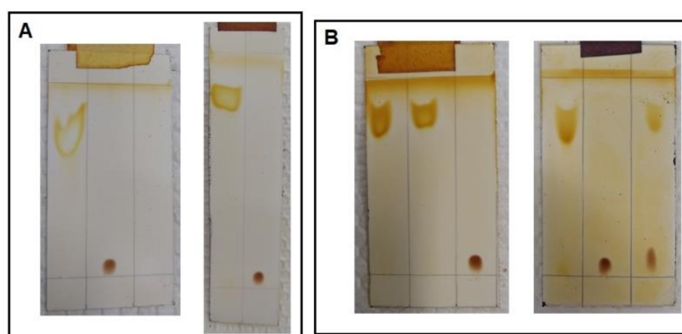

Figure 2: A) Comparison between the 4cm x 8cm (left) and 12.5cm x 3cm (right) TLC plate with AV of 2µL, standard solution of 0.275 mg/mL diluted in water; B) 4 cm x 8cm TLC plate with AV of 2µL, standard solution of 0.275 mg/mL diluted in water. Left: Comparison between the Rf of sodium ascorbate, ^{18}F-PSMA-1007 and the TBA standard (from left to right). Right: Presents the separating ability of the

method when combined sodium ascorbate with the TBA reference standard, not interfering with each Rf. (from left to right: ascorbate solution, TBA standard and the both solutions).

Another point that can be visualized is the color of the product obtained from the iodine vapor. In all TLC plates, the final product showed a very intense brownish, in some other cases stronger than the TBA standard spot. This occurs due to the presence of sodium ascorbate, which stains by iodine vapors. This fact was verified with runs performed with ascorbate only, which had the same Rf and the same intensity of color (Figure 2, B). Thereby, the SPOT TEST is not indicated for the determination of TBA in formulations where there is the presence of stabilizers. False positive and negative are common on this test, confirming the need for a reliable and low-cost method, like TLC. The TLC can determine the presence of TBA and perform a semi-quantitative analysis of it, isolating it in preparations with large amounts of TBA. [9].

4. Conclusions

The method used to determine the TBA proved to be efficient for the ^{18}F-PSMA-1007 product. The concentration of 0.275 mg/mL found for HPLC in the European pharmacopoeia is the best option for the detection of TBA, even for TLC-SG. The indicated solvent for its preparation is water, with a volume of 2µL for application of the samples, providing good visualization of the stain revealed with iodine vapor. The 4.0 x 8.0 cm plate was ideal for separating the TBA from the product, with a revealing time between 15 to 30 seconds, since the brown spot appears at the moment the steam contact the plate

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