QUALITY CONTROL AND STABILITY STUDY OF THE SODIUM FLUORIDE INJECTION [18F]NaF

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Abstract

The objective of this study was testing the stability of the radiopharmaceutical [18F]NaF solution, stored at room temperature (15-25°C) at different time intervals. It was investigated the quality of this radiopharmaceutical substance in time under the influence of environmental factors and the influence of material composition of septum from airtight container. The experimental results show that the radiopharmaceutical can be considered stable up to 8 hours after the end of synthesis.

Rezumat

Obiectivul acestui studiu l-a constituit testarea stabilității compusului radiofarmaceutic [18F]NaF sub formă de soluție, ținut la temperatura camerei (15-25°C) la diferite intervale de timp. În acest sens s-a studiat influența factorilor de mediu și compoziția materialului din care este făcut ambalajul asupra calității produsului radiofarmaceutic. Rezultatele experimentale arată că produsul radiofarmaceutic studiat poate fi stabil la 8 ore după sinteză.

Keywords: sodium fluoride (18F) injection, stability, quality control

Introduction

Sodium fluoride (18F) injection as listed in the Ph. Eur (European Pharmacopoeia) is a sterile solution containing fluorine-18 in the form of sodium fluoride ([18F]NaF).

[18F]NaF is a high sensitive bone-seeking positron emission tomography (PET) tracer used for detection of skeletal abnormalities. The uptake mechanism of 18F-fluoride resembles that of 99mTc-MDP (99mTc-methylene diphosphonate) with better pharmacokinetic characteristics including faster blood clearance and two-fold higher uptake in bone. Uptake of [18F]-Fluoride reflects blood flow and bone remodelling [17].

Reasons for an interest in fluorine-18 are related to the physical and nuclear properties of this radioisotope: the half-life 109.8 min, positron decay (97%) and positron weak energy (635 keV maximum, 2.2 mm range in matter) [13, 14].

[18F]NaF was introduced in 1962 and approved by the United States Food and Drug Administration (FDA) in 1972 [5]. [18F]NaF was early limited used due to high energy imaging technical limitations and widespread availability of Tc99m labelled agents. Bone scintigraphy with Tc99m-MDP (methylene diphosphonate) was one of the most common imaging procedures in Nuclear Medicine. Recently advanced in PET/CT technology and the worldwide shortage of technetium-99m led to the new interest in the use of [18F]NaF [7].

PET is a non-invasive, functional and metabolic imaging technique, with good spatial resolution and excellent sensitivity that make it possible to quantify specific biological and pharmacological processes in humans and animals [6].

[18F]NaF is injected intravenously by direct venepuncture or intravenous catheter. The activity for the adult is 185 – 370 MBq (megabecquerel). 18F is normally distributed throughout the entire skeleton. 18F localization in the skeleton is dependent on the regional blood flow, as well as on new bone formation. 18F is substituted for hydroxyl groups in hydroxyapatite and covalently bonds to the surface of new bone [14, 16].

Apart from Pharmacopoeia, several different synthesis and methods of analysis have been described [8-10]. Preparations of the radio-pharmaceutical in greater amounts require the use of a synthesis module (FastLab, TracerLab or Synthera module).

The purpose of a stability study is to provide evidence on how the quality of a drug substance

(minimum 3 batches) varies in time under the influence of a variety of environmental factors and to establish a retesting period for the drug substances or a shelf life and label storage instructions, applicable to all future batches packed under similar conditions. The stability study was conducted on the drug substance packaged in a container closure system that is the same with the packaging proposed for storage and distribution [3, 4]. Stability studies include samples maintained in the inverted position (in contact with the closure) to determine the effects of the closure system on product quality.

Materials and Methods

Production of [^{18}F]NaF in 0.9 % sodium chloride solution
^{18}F-Fluoride was produced at the National Institute for Physics and Nuclear Engineering "Horia Hulubei", Radiopharmaceuticals Research Centre [15], in the Cyclotron TR19 (ACSI). The ^{18}F-fluoride ion was produced in a 19 MeV cyclotron by bombardment of enriched water with accelerated protons (^{18}O (p/n) ^{18}F reaction). The cartridges used in the process were single use, sterile packed. The activity of ^{18}F in target water was transferred to the cartridge. The cartridge was than eluted with 1M solution Na_2CO_3. The preparation was performed in a synthesis module Synthra RN (Germany). The final product was passed through a 0.22 μm filter and then it was dispensed in glass vials using an automated system for the distribution of radio-pharmaceutical – Theodorico (Comecer – Italy).

Quality control
The quality control was performed according to the Ph. Eur. analytical methods which are described in "Sodium Fluoride injection" monography: appearance, pH, half-life, radionuclidic identity, radiochemical purity, bacterial endotoxins. [^{18}F]NaF sample was diluted 5 times for radiochemical purity and identity, bacterial endotoxins, residual solvent content and osmolality. Appearance, pH, half-life, radionuclidic identity was performed without dilution.

Each batch was divided into two separate vials. One vial was stored upright and the other one was stored in inverted position for the stability test period. Temperature was maintained between 20 - 25°C. The first sample vial was checked for changes within 8 hours. The inverted vial was checked for changes in appearance, radiochemical purity and pH after the stability period.

Visual inspection for the [^{18}F]NaF was performed behind a PET L-block and must be clear, colourless and free of particulate matter.

pH was determined using pH indicator test paper 2 - 9 (Merck) validated with a ph-Meter (WTW 7111, WTWGmbH, Germany).

Radionuclidic Purity was evaluated by gamma-ray spectrometry (Ortec) just after the synthesis and after 24 hours from irradiation for determination of long living radioactive contaminants.

Half-life was determined by successive counting of activity in a radioisotope dose calibrator (VDC405, VEENSTRA) within the range of 20 min.

Radiochemical Purity and *Identity* were evaluated by an HPLC system (Agilent Bio-inert 1260) with UV and radio-detector (GabiStar, Raytest, Germany) connected in series. The separation was performed on a Thermo Scientific Carbopac PA1 column (250 mm x 4.5 mm id, 10 μm, Dionex) with mobile phase 100 mM sodium hydroxide (NaOH) at 1 mL/min flow rate. UV detection was performed at 220 nm. The reference solution (0.425 mg standard sodium fluoride) was prepared in the same day. 20 μL was injected *via* a manual multiport valve. The HPLC analysis was performed to confirm the retention time and the minimum detectable amount for the sodium fluoride. [^{18}F]NaF was analysed by the same system. The peak response on radioactivity chromatogram was compared to the reference standard to confirm the identity and the radiochemical purity. The UV chromatogram was also obtained to assess the fluoride content, as required by the current edition of the European Pharmacopoeia [1, 2].

Bacterial endotoxins were quantified by chromogenic methods using point-of-use test system (PTS)-Endosafe System. The most likely source of endotoxins in PET radiopharmaceuticals is represented by containers, tubing and non-sterile water and chemicals used in the preparation of the product. The only practical way to avoid endotoxin contamination is to eliminate it at the outset by using endotoxin-free materials and aseptic techniques for all critical steps in the production of a radiopharmaceutical.

Osmolality was determined by cryogenic osmometer (Knauer, Germany) in 150 μL undiluted samples.

For Residual solvents content it was used an Agilent 6890A GC equipped with an flame ionisation detector (FID) and a 7694 HS sampler for the experiments. The headspace gas chromatography (HS/GC) system was controlled using Agilent Chem32 software. The GC column was a J&W DB – 624 (6% cyanopropylphenyl / 94% dimetheylpolysiloxane) fused silica capillary column, 30 m length, 0.53 mm internal diameter, and 3 μm film thickness.

Membrane Filter Integrity Test was performed after the synthesis. The sterile filter was connected to a nitrogen supply. The needle was submerged in water and the nitrogen pressure was gradually

increased. The filter was intact up to acceptance pressure (50 psi).

Chemicals

All solvents were purchased from Merck and used without purification. Ultrapure water was prepared using the Millipore MilliQ Direct 8/16 water purification system. Standards (reference chemicals) were obtained from ABX (Germany).

Methods

For determining the stability of the radio-pharmaceutical we evaluated the quality of [^{18}F]NaF at different periods of time under the influence of environmental factors. The ambient humidity condition was not considered in the analysis because we used sealed glass vials for packaging. But we tested the influence of the septum from the vial. The specification for the final product was established to meet all the Ph. Eur. requirements.

Results and Discussion

The results for [^{18}F]NaF assays in all evaluated time intervals are shown in Table I. The results indicated that [^{18}F]NaF is stable up to 8 hours after the end of the synthesis, regarding acceptance limits for each required assay.

Table I

Quality control data for [^{18}F]NaF at different interval times

QC test	Release criteria [Eur. Ph.]	End of synthesis	2H	3H	4H	6H	7H	8H	8 H inverted vial
Appearance	Clear, colourless, free of particulate	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
pH	4.5 - 8.5	8	8	8	8	8	8	8	8
Radiochemical Purity	> 95% by HPLC	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5
Radiochemical Identity	Retention Time = 4.2 - 4.65	4.611	4.55	4.591	4.585	4.59	4.6	4.585	4.594
Radionuclidic Identity	$T_{1/2}$ = 105 - 115 min	111	109.8	-	-	-	-	109.2	108.6
Bacterial Endotoxins	< 17.5 Eu/mL	< 5 Eu/mL	< 5 Eu/mL	-	-	-	-	< 5 Eu/mL	< 5Eu/mL

[^{18}F] was identified by recording the principal gamma peak at 511 keV (Ph. Eur.) and determination of half-life (105-115 min). The gamma spectrum was used for determination of radionuclidic purity (Figure 1).
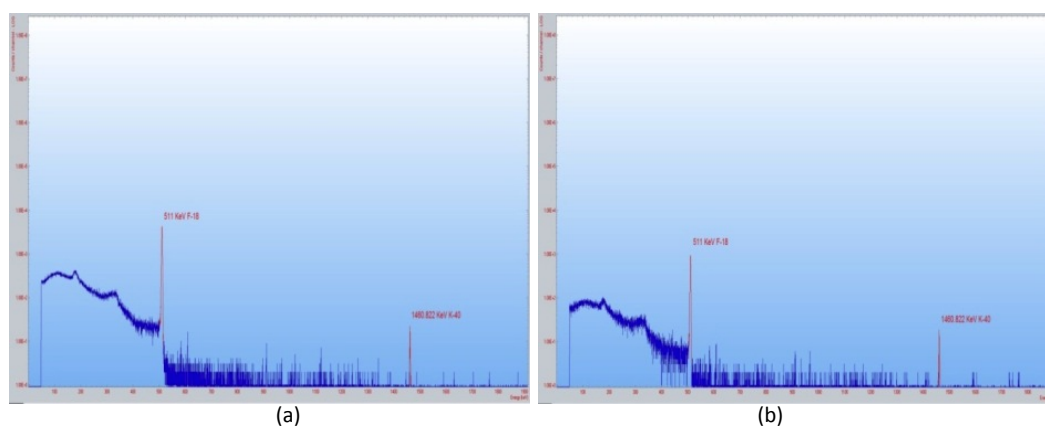

Figure 1.

Gamma spectrum with [^{18}F] principal peak: (a) Immediately after synthesis; (b) 24 h after synthesis

Was checked the presence of any peaks with an energy different from 511 keV. We found only signals coming from Pb-X-rays (range 70 - 80 keV). [^{18}F]NaF activity was determined by the dose calibrator type VDC-405. This is a dose calibrator digitized, composed of ionization chamber, power supply for ionization chamber and soft. The calibrator provides fast and accurate measurements of radioactivity in the samples with known radionuclides. The ionization chamber has measuring range up to 740 GBq (20 Ci) of F-18 and 0.001MBq resolution. The calibration of the device was made with a source of Cs-137. ^{18}F was identified by determination of half-life (105 - 115 min.) in the dose calibrator (Figure 2).
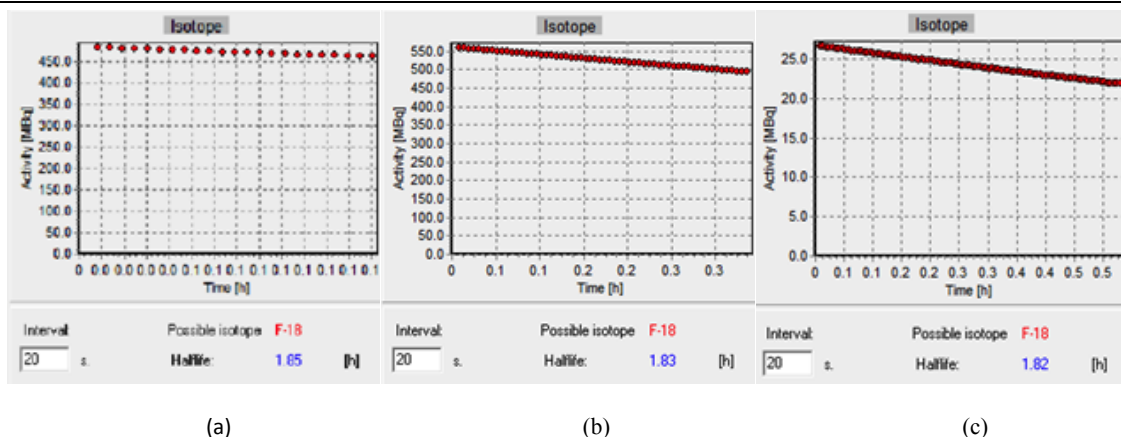
(a)　　　　　　　　　　(b)　　　　　　　　　　(c)

Figure 2.
Determine isotope: (a) Immediately after synthesis; (b) 2 hours after synthesis; (c) 8 hours after synthesis

Identification of [18F]NaF was performed by determining the retention time measured with a radiometrical detector and then this value was corrected with the value determined for the retention time measured on an UV detector. The radioactive compound has the same standard deviation as the reference, if retention time measured value is below 5%. The main peak on the radiochromatogram obtained using HPLC for the [18F]NaF test solution had the same retention time, about 4.5 min, as the peak for the same retention time with standard solution (Figure 3). The response for the fluoride ion is a negative peak at 4.1 min.
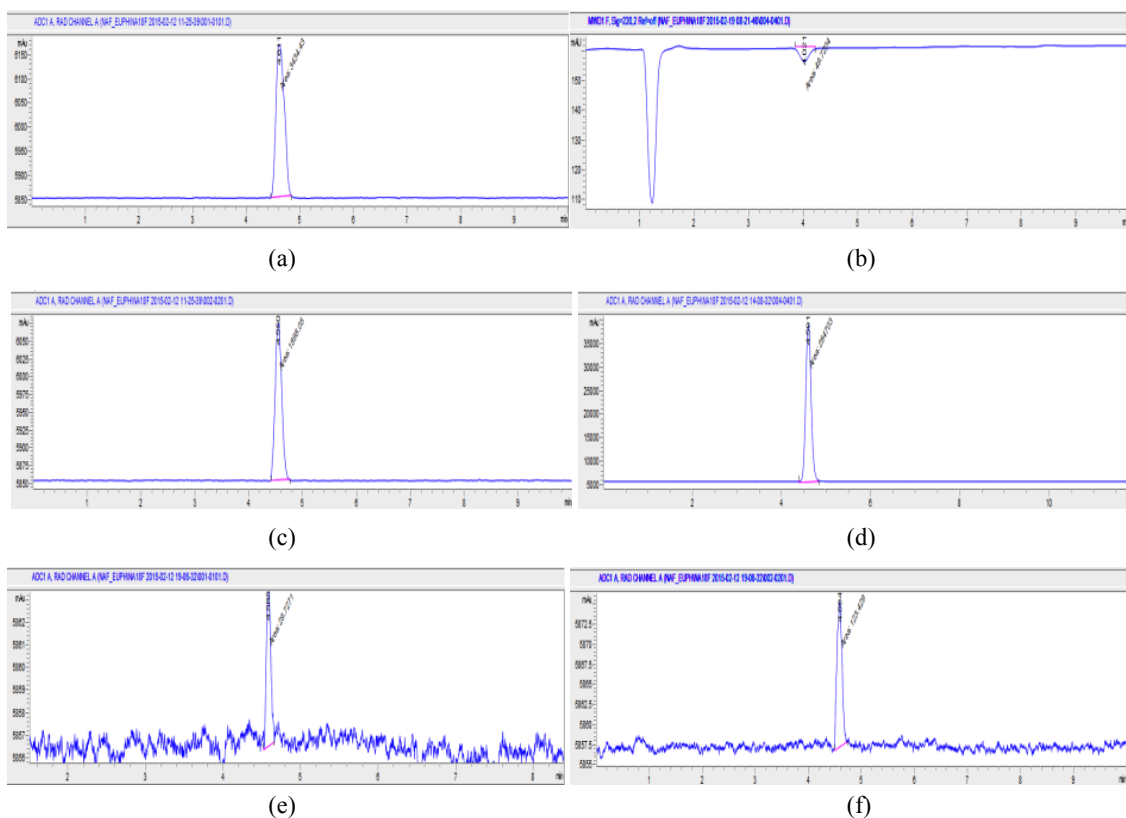

(a)　　　　　　　　　　　　　　　　(b)

(c)　　　　　　　　　　　　　　　　(d)

(e)　　　　　　　　　　　　　　　　(f)

Figure 3.
(a) HPLC chromatogram after synthesis; (b) Chromatogram of standard solution;
(c) HPLC chromatogram 1 h after synthesis; (d) HPLC chromatogram 2 h after synthesis;
(e) HPLC chromatogram 8 h after synthesis; (f) HPLC chromatogram 8 h after synthesis (inverted position)

We used ethanol for the purification of Sep-Pak cartridges (ABX, Germany) resulting in some ethanol in the final product. We checked the presence of ethanol in the final product with our

developed method for the determination of the residual solvent. The results of the test performed on the HS-GC were in compliance with the requirements established by the Ph. Eur., showing that the sample contained less than 5000 ppm ethanol.

Conclusions

The quality of the [^{18}F]NaF meet the criteria specified in the European Pharmacopeia analytical methods for [^{18}F]NaF up to 8 hours after the end of synthesis. These suggest that all physicochemical and microbiological stability characteristics are kept up to 8 hours in the radioactive concentration tested. The material made of rubber cup did not interfere with radioactive substance.

An extension of this work is the evaluation of other factors which might affect the radiopharmaceutical stability, different packaging materials and longer storage periods.

Acknowledgements

References

1. ***, EDQM, European Pharmacopoeia. 8.0, Radiopharmaceutical preparation: Sodium fluoride (18F) injection, European Directorate for the Quality of the Medicines; Strasbourg, 2014; 1079-1080.
2. ***, EDQM, European Pharmacopoeia. 8.0, Radiopharmaceutical preparation, European Directorate for the Quality of the Medicines; Strasbourg, 2014; 759-763.
3. ***, ICH, Stability testing of a new drug substances and products, Q1A(R2), 2003.
4. ***, ICH, Stability testing of biotechnological/biological products, Q5C, 2003.
5. Blau M., Nagler W., Bender M.A., Fluorine-18: a new isotope for bone scanning. *J. Nuclear Med.*, 1962; 3: 332-334.
6. Gambhir S.S, Molecular imaging of cancer with positron emission tomography. *Nat. Rev. Cancer*, 2002; 2: 683-693.
7. Jadvar Hossein, Radionuclide Imaging of cancer Therapy, in Cancer Theranostic, Chapter 4, Eds. X., Chen and S. Wong, 2014.
8. Hockley B.J., Scott P.J.H., An automated method for preparation of [18F] sodium fluoride for injection, USP to address the technetium-99m isotope shortage. *Applied Radiation and Isotopes*, 2010; 68: 117-119.
9. Kumar R., Sonkawade R.G., Tripathi M., Sharma P., Gupta P., Kumar P., Pandey A.K., Bal C., Damle N.A., Bandopadhayaya G., Production of the PET bone agent 18F-fluoride ion, simultaneously with 18F-FDG by a single run of the medical cyclotron with minimal radiation exposure-A novel technique. *Hellenic Journal of Nuclear Medicine*, 2014; 17(2): 106-110.
10. Martinez T., Cordero B., Medín S., Sánchez Salmón A., Adaptation of the 18FDG Module for the Preparation of Sodium Fluoride [18F] injection Solution in Agreement with the United States (USP32) and European Pharmacopoeia (PhEur 6). *Rev. Esp. Med. Nucl.*, 2011; 30: 351-353.
11. Moisei A., Gligor F., Bojiță M., Chiş A., Totan M., Vonica-Gligor L.A., Ciurba A., Compatibility and stability studies of antihypertensive/excipients by thermal methods, used in the preformulation phase. *Farmacia*, 2014; 62(6): 1239-1248.
12. Oprea E., Pintilie V., Bufnea V., Aprotosoaie A.C., Cioancă O., Trifan A., Hăncianu M., Radionuclides content in some medicinal plants commonly used in Romania. *Farmacia*, 2014; 62(4): 658-663.
13. Saha G.B., Synthesis of PET radiopharmaceuticals, In: Basics of PET Imaging Physics, Chemistry, and Regulations, Springer, New York, 2004, 1-18; 111-124.
14. Segal G., Delbeke D., Stabin M.G., Even-Sapir E., Fair J., Sajdak R., Smith G.T., Sodium 18F–Fluoride PET/CT Bone Scans. *Journal of Nuclear Medicine*, 2010; 51: 1813-1820.
15. Ursu I., Craciun L., Niculae D., Zamfir N.V., The Radiopharmaceuticals Research Centre of IFIN-HH at Start. *Romanian Journal of Physics*, 2013; 58: 1327-1336.
16. Vallabhajosula S., 18F-labeled positron Emission tomographic radiopharmaceuticals in oncology: an overview of radiochemistry and mechanism of tumor localization. *Semin. Nucl. Med.*, 2007; 37: 400-419.
17. Wong Ka Kit, Morand Piert, Dynamic Bone Imaging with 99mTc-Labeled Diphosphonates and18F-NaF: Mechanisms and Applications. *Journal of Nuclear Medicine*, 2013; 54: 590-599.