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Original scientific paper

Aseptic process validation of [^{18}F]Sodium Fluoride radiopharmaceutical in-house production

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Abstract

Sodium fluoride ([^{18}F]NaF) is a PET radiopharmaceutical for vizualization of the skeletal system and microcalcification. In the originally designed in-house method, [^{18}F]NaF is recovered in aqueous solution after cyclotron irradiation, sterilized by passage through a 0.22 μm sterile filter and dispensed under aseptic conditions. To ensure the microbiological safety of drugs produced under aseptic conditions, validation of aseptic procedures is always recommended. This is essential for radiopharmaceuticals because most of them are released for administration before any sterility test can be completed due to their radioactive nature.

This study reports the validation of the aseptic process applied to the internal production of [^{18}F]NaF carried out in two phases: testing the number of viable microorganisms in radiopharmaceutical product prior to sterilization and process simulation studies (media fill tests). We found that all samples were sterile and the endotoxin concentration was well below the maximum acceptable level reported in the Ph Eur. monograph on [^{18}F]NaF. The results confirmed that the entire production process of [^{18}F]NaF can be carried out under strictly aseptic conditions following the validated procedures preserving the sterility of the final product.

Keywords: [^{18}F]NaF, radiopharmaceutical, validation, aseptic process

Introduction

Radiopharmaceuticals are medicinal products that contain one or more radioactive isotopes (Ph. Eur. 10.0, 07/2016:0125, 2020). Depending on the type of radioisotope's emission, radiopharmaceuticals can be used for diagnostic (gamma and positron emitters) or therapeutic (electron and alpha emitters) purposes. The production of radiopharmaceuticals requires special considerations firstly because of the emission of ionizing radiation, and secondly because most of the radiopharmaceuticals are parenteral drugs. Therefore, the production should be carried out by staff adequately trained both in radiation protection and in aseptic techniques and quality assurance (Fersing et al., 2021; Gillings et al., 2021). For example, to reduce risks of radiation exposure the production of radiopharmaceuticals should be carried out inside hot cells installed in specially designed laboratories kept under negative pressure (International Atomic Energy Agency, 2005). Similarly, to minimize the microbiological and pyrogenic contamination during the production process, all operations should be carried out in environmentally classified areas (EudraLex Annex 1, 2022) using aseptic conditions commonly employed for manufacturing sterile

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medicinal products (EudraLex Annex 3, 2008; PIC/S Guide PE 009-16 Annex 3, 2022). It is helpful to remind here that the goal of aseptic processing is to manufacture a substance that is free from microorganisms and toxic microbial byproducts (bacterial endotoxins) (CDER, 2012). However, a high number of radiopharmaceuticals are released before completion of all necessary quality control tests, including microbiological controls, due to the short decay half-life of associated radionuclides (EudraLex Annex 3, 2008). To overcome these fundamental limitations, the principles of Good Manufacturing Practice (GMP) posit that suitable procedures should be embedded in the design of the production process to ensure the achievement of the requested quality and safety of the final radiopharmaceutical (WHO Technical Report Series 1025 -Annex 2, 2020).

Sodium [^{18}F]fluoride is a radiopharmaceutical for intravenous administration used for skeletal imaging by positron emission tomography (PET). Detection of bone metastases in patients with malignancy is of high clinical importance as it can significantly impact treatment and outcome (Ahuja et al., 2020). This imaging agent has high bone uptake and very rapid blood clearance allowing to attain high bone-to-background ratios within a short time (Blau et al., 1972; Grant et al., 2008). In the last decade, there has been also a sharp increase in the use of [^{18}F]NaF for visualization of calcification activity in the vasculature (Tzolos and Dweck, 2020).

Sodium [^{18}F]fluoride for injection is included in the European Pharmacopoeia as a sterile solution containing fluorine-18 in the form of sodium fluoride. The radionuclide fluorine-18 is most produced by proton irradiation of a water target enriched in oxygen-18. Fluorine-18 under the chemical form of monoanionic fluoride-18 is commonly recovered from the water target by adsorption and desorption from anion-exchange resins or by electrochemical deposition and redissolution. Due to the short half-life of the ^{18}F radioisotope, the product is usually released for use before completion of the tests for sterility, bacterial endotoxins, and radionuclidic purity (Ph. Eur. 10, 01/2008:2100, 2020).

Validation of the aseptic process for the production of [18F]NaF was performed following the recommendations described in the publication PIC/S PI 007-6 (PIC/S Guide PI 007-6, 2011). To determine whether the process meet the basic requirements for producing a sterile drug by aseptic processing, media fill (also known as 'process simulation') and bioburden techniques have been employed. Media fill tests were carried out to evaluate the aseptic assembly and operation of the critical (sterile) equipment and qualify the operator's aseptic technique (Todde et al., 2017). Media fill allows the assessment of the performance of an aseptic manufacturing procedure, using a sterile microbiological growth medium in place of the bulk radiopharmaceutical solution, to test whether the aseptic procedures are adequate to prevent contamination during actual drug production (Aerts et al., 2014).

Materials and methods

Materials

Enriched water, [^{18}O] H_2O, was obtained from NUKEM isotopes. Solutions of sodium chloride (NaCl, 0.9% w/w) for injection (1000 mL) and water for injection (10 mL) were purchased from Alkaloid Ad, Skopje. QMA cartridges (WAT023525) were obtained from Waters, sterile filters (0.22 μm) from Merck, Kit Clio from BTC Medical Europe, sterile Y connector from B Barun, sterile vials from Huayi isotopes, kit 10 mL Syringe from BTC Medical Europe and Endosafe®-PTS cartridges from Charles River Laboratories. The growth medium (Trypcase Soy Broth) used for process simulation was purchased from bioMérieux.

Production process

Sodium [^{18}F]Fluoride was delivered by a semi-automated volumetric dispenser for radiopharmaceuticals (Clio, Comecer S.p.A.). This module was originally designed only for dispensing, but at the University PET Centre an in-house method for handling the radiosynthesis of [^{18}F]NaF was developed based on this module. Clio was hosted in Talia hot cell equipped with a Class-B pre-chamber and a Class-A main chamber specially designed for dispensing radiopharmaceuticals. The sterile dispensing kit composed of a sterile Y-shaped connector and QMA cartridge was used for synthesis and dispensing of [^{18}F]NaF as auxiliary equipment. The radioisotope [^{18}F]F^- was produced by a cyclotron (GE PETtrace 16.5MeV) by irradiating the enriched ^{18}O water with protons in a niobium target via the ^{18}O(p,n)^{18}F nuclear reaction. The produced [^{18}F]F^- was transferred from the cyclotron to the Talia hot cell via a delivery line and trapped into a quaternary methyl ammonium (QMA) anion-exchange solid-phase extraction cartridge. All cationic and water-soluble contaminants present in the irradiated enriched water were washed out from the cartridge using a kit of sterile water and collected in a recovery vial. Then a flow of helium was passed through the cartridge. A sterile saline solution was finally used for the elution of [^{18}F]F^- from the QMA cartridge under the chemical form of sodium [^{18}F]fluoride. All operations were performed in a class-A environment using working conditions required for aseptic processing of radiopharmaceuticals (laminar flow, 0.45 m/s; pre-chamber negative pressure, –130 Pa ± 10%; main chamber negative pressure, –100 Pa ± 10%).

Aseptic process validation

Aseptic process validation was carried out by applying the two techniques of bioburden testing and media fill. Three consecutive synthetic batches were utilized, and aseptic dispensing was performed in each stage. The aseptic process validation of [^{18}F]NaF solution for injection was composed by the following steps: (a) transfer of irradiated enriched water ([^{18}O]H_2O) from the cyclotron via

a delivery line, (b) synthesis of [^{18}F]NaF by elution of the cartridge with saline and (c) aseptic dosing through the dispensing module Clio hosted in Talia dispensing hot cell.

The bioburden testing was performed by simulation of the whole production process, but without the presence of radioactive substances and a filter for final sterilization. Each batch included 11 dose simulated samples. The first dose was a 1 mL quality control sample for endotoxins testing. The other 10 doses were filled into 10 mL volume syringes corresponding to the maximum possible dose for a single patient. The enriched water and the sterile water for rinsing were delivered from the cyclotron through the QMA cartridge to the recovery vial. The elution was performed with 0.9% NaCl. After the synthesis of [^{18}F]NaF, dilution and dispensing of single doses were simulated. Eleven doses were filled without passage through a sterilizing filter. The particle counter was sampling the environment during all procedures.

After the results of the first batch of bioburden tests and growth promotion test were obtained, the media fill simulations were performed. Tryptic Soy Broth (TSB) microbial growth medium was used in place of saline both for elution during the synthesis and dilution during the dispensing process. The elution of the QMA cartridge was simulated by media fill and the synthesis product was collected in the dispensing kit bulk vial. The dilution of the product was also simulated by media fill. As for the bioburden testing, eleven doses were filled in each batch.

All samples collected after the synthesis and dispensing from both bioburden testing and media fill, were tested for sterility, and one sample from each batch was tested for the bacterial endotoxins.

Air microbiological sampling was accomplished using settle plates and glove print 5 fingers (Fig. 1) and non-viable monitoring by particle counting in class A. These environmental controls were carried out during the entire aseptic process validation. Recommended frequency of microbiological monitoring with settle plates for class A was set at the end of every working session, and weekly for background environment (class B in the present study). The frequency for glove fingerprint for class A and B was settled on at the end of each working session (PIC/S Guide PE 010-4; Annex 1, 2014). For each batch, settle plates were placed in the pre-chamber, chamber, and the laboratory (class C). The personnel were monitored after completing the aseptic operations in every working session with glove print 5 fingers. Figure 1 illustrates the position of the plates.

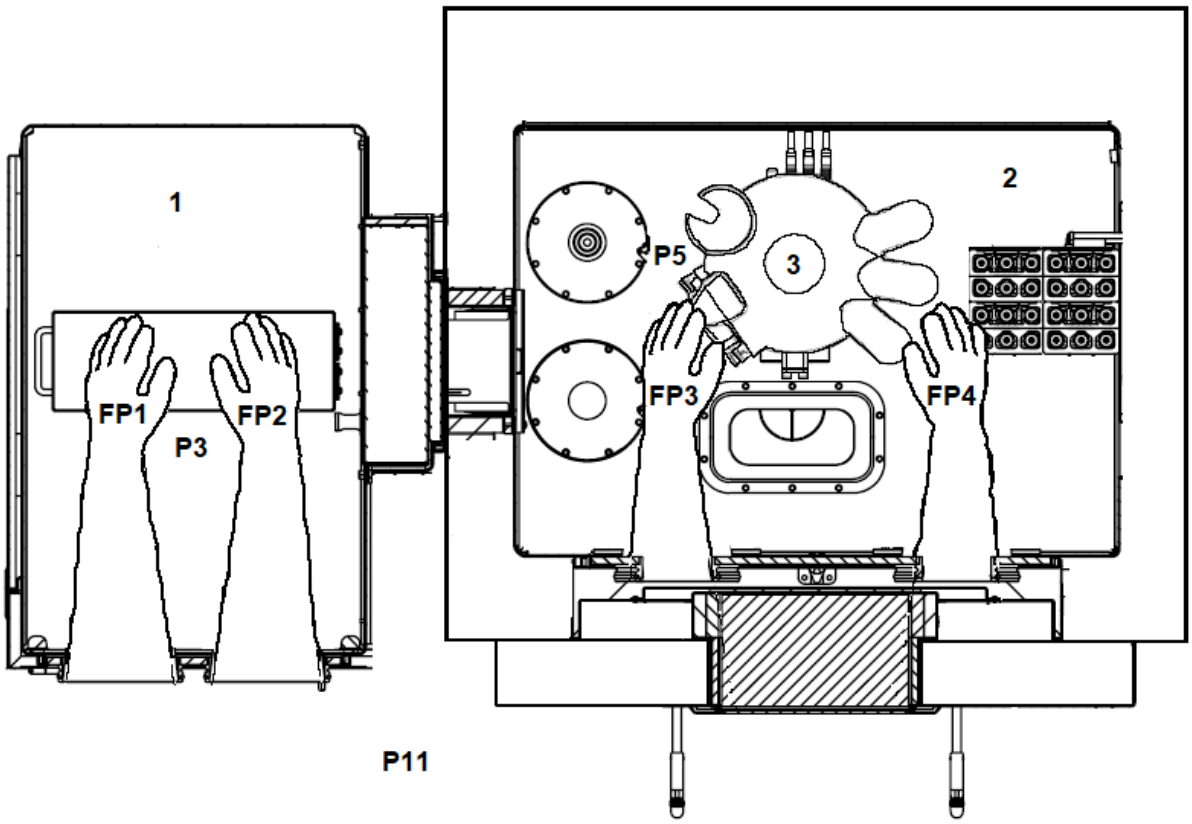

Fig. 1. Selected locations for microbiological controls in the hot cell Talia: (1) Pre-chamber (class B), (2) Main Chamber (class A), (3) Clio dispensing module. FP1 = fingerprint left-hand pre-chamber, FP2 = fingerprint right-hand pre-chamber, FP3 = fingerprint left-hand chamber, FP4 = fingerprint right-hand chamber, P3 = settle plate in the pre-chamber, P5 = settle plate in the main chamber, P11 = settle plate on the floor.

Table 1. Results of aseptic validation

	Bioburden testing			Media fill testing		
	1 batch	2 batch	3 batch	1 batch	2 batch	3 batch
Bacterial Endotoxins EU/mL	<5	<5	<5	6.74	7.21	<5
Sterility samples	All samples were sterile					

Results

The results of the growth promotion test showed that the Tryptic Soy Broth medium (serial number: 1007954450) efficiently supported the growth of all the microorganisms according to the rules dictated by European Pharmacopoeia (Ph. Eur. 10.0, 2.6.1 Sterility, 2020). The results of bioburden and media fill testing are given in Table 1.

The results of microbiological monitoring during aseptic validation are reported in Table 2.

Samples obtained from the three batches of the bioburden testing were found to be fully sterile. Analysis of the sample dispensed for endotoxins testing gave a value < 5 EU/mL which lies within the limits established by acceptance criteria for endotoxins.

Discussion

The results of bioburden testing on three consecutive batches confirmed the effectiveness of the aseptic techniques employed in the validation process. Media fill simulations were carried out on three batches dispensed in different days. No microbiological and endotoxin contaminations were found in the analyzed samples even before sterilization via membrane filtration. One sample from each batch was tested for endotoxins and measured values were below the limit of 17.5 EU/mL established by standard acceptance criteria.

As a worst-case scenario during the dispensing process in the bioburden and media fills testing, the internal door between chamber (class A) and pre-chamber (class B) was open and an additional syringe was added through the pre-chamber. All filled syringes were delivered through the pre-chamber. In a regular dispensing procedure, the filled and measured syringes are delivered through a drawing system. The drawing system allows the transfer of vials or shielded syringes containing radiopharmaceutical from inside the hot cell to the laboratory, while keeping the class A unaltered. Even with open door between class A and B, all results obtained from microbiological monitoring were within the acceptance limits. The acceptance criteria for settle plates prescribed by EudraLex (Volume 4, Annex 1) are no growth of microorganism for class A, less than 5 CFU/4 hour for class B, and less than 50 CFU/4 hour for class C. All settle plates for classes A and B were found without microbiological growth. The results of microbiological monitoring of class C (position P11) showed growth of Staphylococcus coagulase-negative (1 CFU/4 hour). Staphylococcus coagulase-negative is a non-pathogenic bacterium and constitutes a normal component of the microbiota of the skin and mucous membranes of humans and animals (Becker et al., 2014).

Table 2. Results of microbiological monitoring during aseptic validation

Microbiological monitoring during aseptic validation								
		Settle plates (CFU/4 hours)			Glove print -5 fingers (CFU/glove)			
	Positions	P3	P5	P11	FP1	FP2	FP3	FP4
Batch No.	1 Bioburden	0	0	1	0	0	0	0
	2 Bioburden	0	0	0	0	0	0	0
	3 Bioburden	0	0	0	0	0	0	0
	1 Media fill	0	0	0	0	0	0	0
	2 Media fill	0	0	1	0	0	0	0
	3 Media fill	0	0	0	0	0	0	0

Airborne particle monitoring was conducted during all aseptic processes. The acceptance criteria for class A are 3520 counts/m^3 for 0.5-μm particles and 20 counts/m^3 for 5-μm particles. The results of the six batches were well within the limits of acceptance criteria. The maximum counts for 5-μm particles were 4 counts/m^3 and for 0.5-μm particles were 1688 counts/m^3, but still within the limits of acceptance criteria.

Conclusion

The aseptic process validation of [^{18}F]NaF radiopharmaceutical production has been completed successfully. It demonstrated that the microbiological safety of the radiopharmaceutical can be obtained following the procedure and working conditions for routine production of [^{18}F]NaF. The results of the analyzed bioburden and media fill samples, including the results of microbiological and physical monitoring, performed during the validation, confirmed the effectiveness of the aseptic process carried out for [^{18}F]NaF production. It has been demonstrated that even in the worst-case scenario, no microbiological growth occurs during the processes of synthesis and dispensing performed under aseptic conditions in a class-A chamber equipped with a class-B loading area. Aseptic process validation showed that the originally designed, in-house procedure for the production of the radiopharmaceutical [^{18}F]NaF ensures the absence of microbial and pyrogen contamination in the final product and its safety for intravenous administration.

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Резиме

Валидација на асептичен процес на производство на [^{18}F]NaF радиофармацевтик

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Клучни зборови: [^{18}F]NaF, радиофармацевтик, валидација, асептичен процес

[^{18}F]Натриум флуорид ([^{18}F]NaF) е радиофармацевтик за парентерална интравенска администрација кој се користи во нуклеарната медицина за визуелизација на скелетен систем и микрокалцификати, со позитронско емисиона томографија (ПЕТ). [^{18}F]Натриум флуорид раствор за инјектирање се произведува во асептични услови со стерилизација на радиофармацевтикот со 0,22 μm филтер. Изведувањето на валидација пред имплементирање на нов метод за производство на ПЕТ радиофармацевтик е клучен фактор за докажување на ефикасноста на асептичното работење.

Оваа студија ја прикажува постапката на валидација на асептичен процес на производство на [^{18}F]Натриум флуорид раствор за инјектирање, спроведена врз основа на препораките дадени во водичите за валидација на асептичен процес. Валидацијата беше спроведена во две фази: тестирање на биолошко оптеретување на производот и тест на симулација на процесот со подлога која што поддржува раст на микроорганизми. Испитувања на стерилност и бактериски ендотоксини беа спроведени на примероците произведени при валидација на асептичниот процес. Сите примероци беа стерилни, а концентрацијата на ендотоксини беше под лимитот на прифатливост, согласно монографијата на [^{18}F]NaF од Ph.Eur 01/2008:2100. Резултатите од анализираните примероци вклучително и резултатите од микробиолошки и физички мониторинг, спроведен за време на изведбата на валидацијата, го потврдија асептичниот процес на производство на [^{18}F]NaF радиофармацевтик.