U.P.B. Sci. Bull., Series B, Vol. 78, Iss. 1, 2016 ISSN 1454-2331

VALIDATION OF THE HPLC METHOD FOR DETERMINATION OF IDENTITY AND RADIOCHEMICAL PURITY OF [^{18}F]NaF

Mirela MIHON[1], Catalin S. TUTA[2], Carmen MANEA[3], Alina-Catrinel ION[4], Vasile LAVRIC[5]

The development of a high performance liquid chromatography (HPLC) method for the determination of the radiochemical purity evaluation of [^{18}F]NaF was described. HPLC analysis was performed with an Agilent system. Separation was achieved with a Carbopac PA1 column. The method was found to be precise, accurate and specific, assets recommending its use for the determination of radiochemical purity radiopharmaceuticals.

Keywords: validation, identity, radiochemical purity, HPLC, [^{18}F]NaF

1. Introduction

[^{18}F]NaF ([^{18}F]sodium fluoride) is a radiopharmaceutical produced as sterile solution suitable for intravenous administration, which must contain no more than 4.25mg NaF/ V, where V is the maximum administrated total dose at the expiration time [1].

[^{18}F]NaF was first introduced as a bone imaging agent by Blau et al. in 1962 and approved by FDA in 2002 as a radiopharmaceutical for diagnostic agent for positron emission tomography (PET). Fluoride bone uptake mechanism is similar to that of ^{99m}Tc-methylene diphosphonate (^{99m}Tc –MDP). Because of faster blood clearance and higher capillary permeability, fluoride uptake in bone metastases is significantly higher compared with that of normal bone [2,3].

The aim of this work was to validate the methodology for radiochemical purity according to the European Pharmacopoeia (Ph.Eur) and to verify the suitability of the system.

The radiochemical purity (RCP) of a radioactive sample obtained after the labeling reaction was an important parameter to assess the efficiency of a labeling

---

[1] PhD student, Chemical and Biochemical Department , University POLITEHNICA of Bucharest, and National Institute of Physics & Nuclear Engineering Horia Hulubei, Romania, e-mail: mirela.mihon@nipne.ro

[2] PhD, National Institute of Physics & Nuclear Engineering Horia Hulubei, Romania

[3] PhD, National Institute of Physics & Nuclear Engineering Horia Hulubei, Romania

[4] Prof., Department of Analytical Chemistry and Environmental Engineering, University POLITEHNICA of Bucharest, Romania

[5] Prof., Department of Chemical and Biochemical, University POLITEHNICA of Bucharest, Romania

procedure. The RCP should be calculated immediately after synthesis to determine whether any degradation products were generated before, during or after the labeling or purification processes. For high performance liquid chromatography (HPLC) analysis, a comparison of the retention factor (t_R) of the radiolabeled substance to that of the reference can be made to establish the identity of the compound [4].

Only a few reports on this method have been published [5-7]. The radiochemical identity and purity can be confirmed by HPLC equipped with a detector (conductivity or ion chromatography) or by thin layer chromatography (TLC). In this paper the identity and radiochemical purity was determined using HPLC equipped in serial with spectrophotometer and radioactive detectors. Compared with the method described the Ph.Eur a faster method was developed to determine the chemical impurities which may be found in [^{18}F]NaF. The validation of the method follows the International Conference on Harmonization Q2(R1) directives [8-10].

2. Experimental part

Instrumentation and chromatographic condition

The determination of RCP was performed with a high performance liquid chromatograph AgilentBio-inert 1260 with UV equipped with radioactive detector (Gaby star, Raytest), on a CarboPac PA1 column (polystyrene 2% crosslinked with divinylbenzene, agglomerated with 500nm MicroBead quaternary ammonium functionalized latex) 250x 4.5 mm, 1.0 µm internal diameter (Dionex). The HPLC system was controlled using Agilent Chem 32 software, OPEN Lab Chemstation.

Sample injection was performed manually using a bio inert injector with a multiport valve and a 20 µL calibrated loop. The flow rate of the mobile phase was set at 1mL/min, with a total run of 10 min. The column temperature was kept at 20ºC. The detection wavelength for chemical impurities was 220 nm. The column conditioning was performed using sodium hydroxide as mobile phase, with a 1 mL/min flow rate, for 30 minutes.

Sartorius analytical balance with accuracy 0.001mg was used for weighing the reference standards.

Radioisotope dose calibrator (VDC 405, Veenstra) was used to measure the radioactivity.

Reagents

The reference standard of NaF was obtained from Sigma Aldrich.

All aqueous solutions were prepared using ultrapure water from Millipore Milli-Q system (Direct 8/16) with UV lamp and resistivity 18.2MΩ cm (25ºC).

Eluent solution 0.1 M sodium hydroxide (NaOH) from Merck was used.

Stock standard solution: 125 mg NaF solution standard was weighted and diluted with ultrapure water in a 25 mL volumetric flask. Working standard solution was obtained by serial dilution of standard stock solution.

Preparation of sample solution

For the identification and radiochemical purity test we diluted the sample 1:5 with ultrapure water. The injection volume was 20 µL.

Radiosynthesis of [^{18}F]NaF

[^{18}F]NaF was synthesized in our centre using a Synthra RNplus-automated module (11). ^{18}F-fluoride was produced by 15 MeV proton irradiation of ^{18}O-water. The irradiated solution was passed through an anion exchange cartridge and flushed with water for injection and then eluted with sodium chloride solution. Finally the solution was passed through a sterile filter and dispensed in a glass vials using automated system for the distribution (Theodorico, Comecer).

Validation procedure for the HPLC method of analysis

The identification of [^{18}F]NaF is done by the retention time (t_R) value measured on the radiochemical detector -Gaby Star, and the retention time of the NaF reference material measured on the UV detector. The retention times are not identical because the distances from the injector to the radioactive detector and UV detector respectively, are different. Therefore, it is necessary to determine the difference between the detectors expressed in time. After this, the t_R of [^{18}F]NaF peak obtained on the radioactive detector will be corrected.

For this purpose we examine an [^{18}F]NaF batch 5 times. We determine the difference between retention time of [^{18}F]NaF and retention time of NaF expressed in minute and the percentage deviation of the corrected value of [^{18}F]NaF from the reference NaF.

3. Results and discussion

Validation of the chemical purity test of [^{18}F]NaF was carried out according to ICH Q2(R1) guidelines. The parameters of the validation were: linearity, precision (repeatability, reproducibility), limit of quantification, accuracy and range. The required parameters are listed in table 1.

Table 1

Required parameters for validation

Inspected parameter	Requirement
Deviation of retention time of NaF and **[^{18}F]NaF**	± 5%
Linearity	$R^2 > 0.990$
Precision	$RSD_{\%} < 5$ %
Range	LOQ- 5mg/mL
Limit of quantification (LOQ)	0.12 mg/mL

3.1 Validation of the chemical purity test of [^{18}F]NaF

Calibration

Determination of linearity was done on 3 sets of solutions in the concentration range between 0.125-5 mg/mL. From the evaluated chromatograms we determine the peak area and height and plotted graphically the peak area and height values against concentrations and determine the correlation coefficient (R^2). A correlation coefficient value of 0.9991 was achieved (Fig. 1), which is in accordance to acceptance criteria.

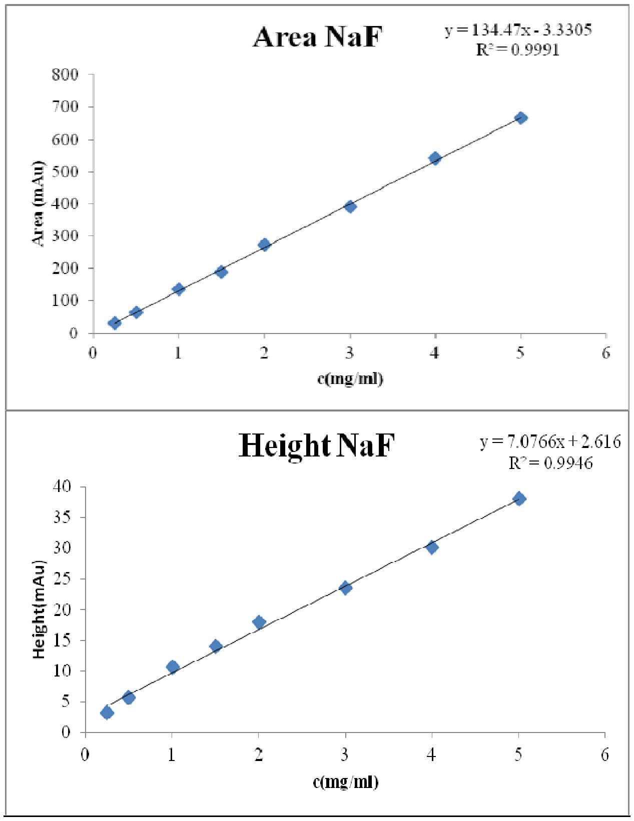

Fig. 1. The calibration curve of standard NaF obtained with the HPLC method

Precision

To determine the precision of the chromatographic method we injected the standard NaF with concentration of 2.1 mg/mL five times. With the evaluation of the obtained chromatograms, determine the peak area and retention times then calculate the standard deviation (SD) of the obtained values. The relative standard deviation (RSD) was found out to be less than 5%. All values are listed in table 2.

Table 2

Precision of the HPLC method

Number	t_R(min)	Area (mAU min)
1 injection	4.09	44.3
2 injection	4.08	44.9
3 injection	4.08	44.7
4 injection	4.09	46
5 injection	4.09	44.8
Average	4.09	44.94
Standard deviation	0.006	0.64
RSD	**0.14**	**1.41**

To perform the repeatability were carried out 3 measurements by 3 values of the range of linearity. Standard solutions were selected and injected for 3 times. Intermediate precision was made the other day by preparing fresh solutions

The limit of detection (LOD) was calculated with formula (1)

$$LOD = (s \times 3)/ Slope \qquad (1)$$

where s is the standard deviation of the response. The slope was calculated from three calibrations curves in the range 0.1-5 mg/mL.

The limit of quantification (LOQ) was calculated with formula:

$$LOQ = (s \times 10)/ Slope \qquad (2)$$

Limit of quantification for NaF was 0.12 mg/mL

Robustness

The aims were to demonstrate that variations of operational parameters of the method do not significantly affect the results. Robustness was set by analyzing the sample in six replicates with the optimized method and by changing the operational parameters. Operational parameters investigated are: mobile phase composition, temperature influence column and mobile phase flow. The method was robust with regard to small variation in the method parameters.

Accuracy

The method's accuracy was validated through recovery experiments by spiking with known amount of impurity. They were taken into working samples of standard NaF so as to obtain concentrations at 80%, 100% and 120% of the maximum admissible concentration in radiopharmaceutical. Sample concentrations were prepared by diluting the stock standard solution with ultrapure water. Each concentration was prepared in triplicate and percent recovery was calculated. All data of recovery were found within the range 90%-110%. The coefficient of correlation was greater than 0.99 and the RSD of the recovery values was less than 2%. In this range of concentrations to average accuracy of the studied method has value 99.78%.

3.2 Validation of the identity and radiochemical purity test of $[^{18}F]NaF$

The results of the validation inspection of the HPLC method for determination of identity are shown in table 3. The retention time of $[^{18}F]NaF$ should be the same (± 10%) as the retention time observed for the reference standard. The deviation of retention time of NaF and $[^{18}F]NaF$ was in the range 0-0.55%.

Table 3

Validation of the $[^{18}F]NaF$ identification test

$t_R[^{18}F]NaF$, min	$t_{R\ NaF}$, (min)	t_R deviation, min	t_R average deviation, min	t_R corrected with average deviation, min	Deviation, %
4.31	4.11	0.20	0.20	4.11	0.00
4.31	4.09	0.22		4.11	0.48
4.27	4.09	0.18		4.07	0.24
4.28	4.13	0.15		4.08	0.55
4.31	4.09	0.22		4.11	0.48
4.31	4.09	0.24		4.11	0.48

The determination of the linearity of the radioactive detector tells us how the concentration range of the $[^{18}F]NaF$ changes in direct proportion with the detector signal. For the examination of the linearity, from the radioactive concentration value (related to the reference time of the sample), the radioactive concentrations of $[^{18}F]NaF$ related to the given time were calculated. In the range, the standards with different concentrations are determined not by measurement, but with the determination of the radiochemical decay, respectively with the calculation of concentration related to the time of injection (table 4).

Table 4

Linearity of the radiochemical purity test

Time (hh:mm)	Area [18F]NaF (mAU min)	[18F]NaF radioactive concentration (MBq/mL)
09:40	279889.6	1385
10:10	259786.1	1153
10:50	216167.2	954.2
11:50	161151.1	653.3
12:30	117876.3	476.1
13:40	85848	306
14:30	75469.5	223.3

The radioactivity concentration related to the peak areas and the times of injection were plotted graphically, and correlation coefficient (R^2) was determined (Fig. 2).

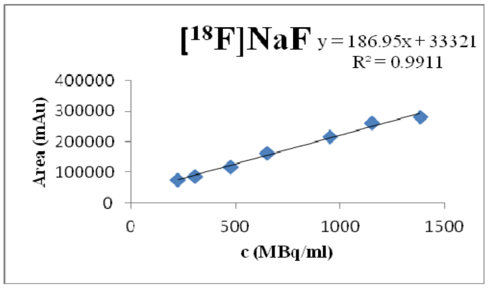

Fig. 2. Calibration curve for the radiochemical purity test

The precision of the chromatographic method was determined and a [18F]NaF sample was injected five times. With the evaluation of the obtained chromatograms, the radioactive purity of [^{18}F]NaF was determined (radiochemical purity 99.5%) and then the standard deviation of the obtained values was calculated ($RSD_{\%}$- 0.36%). Identifying peaks was done by comparing UV absorption spectra and retention time corresponding to peaks in the chromatograms of the sample and standard solutions of NaF. Recorded retention time were 4.13 min for the standard and 4.33 min for the radiopharmaceutical [^{18}F]NaF (Fig. 3).

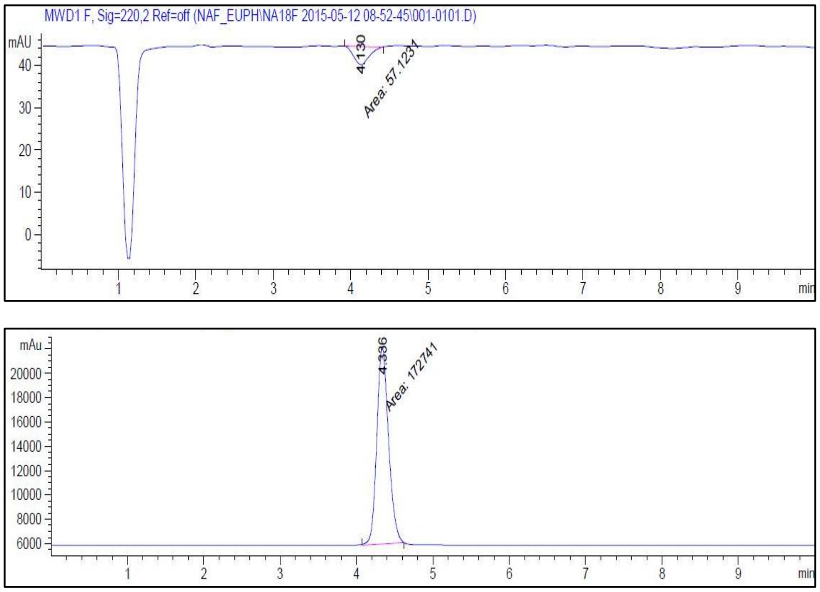

Fig.3. Chromatograms of the standard solution UV detector (left) and [^{18}F]NaF-radioactive detector (right)

4. Conclusions

High performance liquid chromatography of the reference standard (0.425 mg/mL) was performed and validate and the system showed good signal to noise ratio and chromatographic method complies with all requirements (table 1). The advantage of this method over other described previously is the short analyze time (10 minutes), compared with the analyze time of the method described by Li et al. (20 minutes) [7]. This method is useful for synthesis process control and determination of impurity NaF in radiopharmaceutical. Deviation of retention times of NaF and [^{18}F]NaF is in the range of 0-0,55%. The calibration curve was linear with a good coefficient (R^2) of 0.9911 for the radiochemical detector.

Acknowledgements

Mirela Mihon acknowledges the support of the Sectorial Operational Program Human Resources Development (SOP HRD), financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390/.

REFERENCES

[1]. ***Sodium (^{18}F) injection. The European Pharmacopoeia. 8th ed. Strasbourg, France: European Directorate for the Quality of the Medicines, 2014, pp.1052 – 1054

[2]. *Shankar Vallabjosula*, ^{18}F-labeled positron Emission tomographic radiopharmaceuticals in oncology: an overview of radiochemistry and mechanism of tumor localization, Seminars in Nuclear Medicine, 37, pp.400-419

[3]. *K.K. Wong and M. Piert*, Dynamic Bone Imaging with 99mTc-Labeled Diphosphonates and18F-NaF: Mechanisms and Applications, Journal of Nuclear Medicine, 54, 2013, pp. 590-599

[4]. A. *Chopra, L. Shan, W.-C. Eckelman, K. Leung,* Important parameters to consider for the characterization of PET and SPECT imaging probes, Nuclear Medicine and Biology, 38, 2011, pp. 1079-1084

[5]. *G.B. Saha*, Synthesis of PET radiopharmaceuticals, In: Basics of PET Imaging Physics, Chemistry, and Regulations, Springer, New York, 1-18; 2004, pp. 111–124

[6]. *M.N. Leonardi, G.-A. Casale, J. Nicolini, P.-D. Zubata, M.-J. Salgueiro, M.-B. Zubillaga*, Validation of a paper chromatographic methodology as an alternative for determination of the radiochemical purity of Na^{18}F, Journal of Nuclear Medicine Technology, 40, 2012, pp. 271-274

[7]. *C.-C. Li,S.-S. Farn, Y.-H. Yeh, W.-J. Lin, L.-H. Shen,* Development and validation of an anion-exchange HPLC method for the determination of fluoride content and radiochemical purity in [^{18}F]NaF, Nuclear Medicine and Biology, 38, 2011, pp. 605-612

[8]. ***Validation of Analytical Procedures: Text and Methodology, Q2(R1), ICH Harmonised tripartite guideline, 2005

[9]. *I. Ion, D. Bogdan, A.-C. Ion,* Improvement in the determination of traces of nitrate and nitrite in natural mineral waters by ion chromatography, U.P.B. Sci. Bull., 76, 2014, pp.112-122

[10]. *C.M.Riley, T.W.Rosanske,* Development and Validation of Analytical Methods, Elsevier Science Ltd, 1996

[11]. I. *Ursu, L. Craciun, D. Niculae, N. -V. Zamfir*, The Radiopharmaceuticals Research Centre of IFIN-HH at Start, *Romanian Journal of Physics*, 58, 2013, pp 1327–1336, 2013