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# A mild anion-exchange HPLC method for analysis of [$^{18}$F]sodium fluoride solution for injection

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**Abstract**
A mild HPLC method for the quality control of [$^{18}$F]sodium fluoride solution for injection was developed. The method used anion exchange column for separation, UV and online radio-detectors for fluoride content and fluorine-18 determination. A mixture of 100 mM sodium acetate and 25 mM sodium chloride was chosen as mobile phase. The method was validated for system suitability, specificity, fluoride concentration and radiochemical purity determination with good linearity, accuracy and precision. The good robustness of the system was also verified, enabling routine analysis of fluoride content and radiochemical purity of the [$^{18}$F]sodium fluoride product.

**Keywords** [$^{18}$F]sodium fluoride · Anion-exchange · HPLC · Mixture mobile phase · Online radio-detection

## Introduction

[$^{18}$F]sodium fluoride ([$^{18}$F]NaF) solution for injection is a sterile radiopharmaceutical drug, which is an excellent bone-seeking positron emission tomography (PET) tracer for detection of osteogenic abnormalities [1–3]. [$^{18}$F]NaF was initially used for bone scintigraphy in early 1960's and approved by the United States Food and Drug Administration (FDA) in 1972 [4]. The early stage clinical use of [$^{18}$F]NaF was limited and the gamma bone scintigraphy tracer, [$^{99m}$Tc]Tc-methylene diphosphonate ([$^{99m}$Tc]Tc-MDP) has been commonly used in nuclear medicine. Recently with the global supply shortage of technetium-99 m sources and clinical advances of PET/CT technique with high resolution, [$^{18}$F]NaF has drawn new interest in nuclear medicine. The [$^{18}$F]NaF PET imaging affords more sensitive and accurate localization and characterization of bone lesions in metabolic bone diseases than gamma planar or SPECT imaging [4–6]. Furthermore, [$^{18}$F]NaF PET imaging can improve the workflow of clinical nuclear medicine and patient convenience.

According to European Pharmacopoeia (EP), the assay of fluoride content of [$^{18}$F]NaF is required to ensure no more than 4.52 mg F-/V, where V is the maximum recommended dose in milliliters at the expiration time [7]. Measurement of the radiochemical purity (RCP) of [$^{18}$F]NaF solution is necessary to meet the quality control requirements. Several analysis methods, including TLC [8, 9] and HPLC were reported to measure RCP of [$^{18}$F]NaF [7, 10–14]. HPLC methods could measure both RCP and fluoride content, and thus is superior to the TLC [7, 10–12]. However, the HPLC analyses using radioactivity and UV detectors recommended by EP [8] and in some studies [13, 14] use strongly basic 100 mM NaOH as mobile phase, which damages the routine HPLC system [11]. The HPLC methods using radioactivity and conductivity detectors for [$^{18}$F]NaF was reported in the US Pharmacopoeia (USP) and recent study [10, 11], which are not popularly settled in radiopharmaceutical laboratories. With this regard, we aim to develop and validate a novel HPLC method using mild mobile phase for determination of fluoride content and RCP of [$^{18}$F]NaF with UV and radioactivity detectors.

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## Experimental

Sterile water for injection and sterile 0.9% saline for injection were purchased from Shijiazhuang No. 4 Pharmaceutical (Hebei, China). $Na_2CO_3$, NaCl and NaOAc of analytical grade and NaF reference standard were obtained from Aladdin Chemical (Shanghai, China). Ultrapure water (resistivity ≥ 18.2 MΩ cm) produced by a ULPHW-ROMB purification system (ULUPURE, Sichuan, China) was used to prepare all aqueous solutions and HPLC mobile phases.

### NaF solution preparation

NaF stock solution of 5 mg/mL in 0.9% NaCl was prepared by dissolving accurately weighed 50 mg of NaF with 0.9% saline for injection and diluting to 10 mL in a volumetric flask, and then diluted serially to make NaF standard solutions of 2.5, 1, 0.5, 0.25 and 0.125 mg/mL. NaF standard solutions in ultrapure water were also prepared in similar method.

### [$^{18}$F]NaF solution preparation

Firstly, $^{18}F^-$ ion was produced via $^{18}O(p,n)^{18}F$ nuclear reaction by irradiation of [$^{18}$O]$H_2O$ stored in a niobium target with proton beam on a 20 MeV medical cyclotron (HM20S, Sumitomo Heavy Industries, Ltd.). Then the oxygen-18 enriched water containing [$^{18}$F]fluoride ion was transferred to a dedicated automatic synthesis module and passed through a SepPak CM cartridge and a SepPak QMA cartridge that were washed with 10 mL water for injection in advance. The [$^{18}$F]fluoride ion was retained in the QMA cartridge. Following washing the QMA cartridge with 10 mL of water for injection, the [$^{18}$F]fluoride ion was eluted with 10 mL of saline for injection and filtered through a 0.22 μm sterile filter to produce the [$^{18}$F]NaF solution for injection.

### HPLC

An Agilent 1260 Infinity Quaternary HPLC system equipped with degasser, pump, autosampler, variable wavelength UV detector and online radio-detector (Flow-Count B-FC-3500-A diode detector, Eckert&Ziegler, Berlin, Germany) in the study. The Agilent OpenLAB CDS ChemStation Edition software was used for instrument operation and data collection. The anionic exchange HPLC was performed with a Thermo Scientific Dionex CarboPacTM PA1 column (250 mm × 4.5 mm id, 10 μm). The column temperature was held at 25 °C. For HPLC method development, the mobile phases tested included aqueous solution of $Na_2CO_3$, NaOAc, and mixture of NaOAc and NaCl. The injection volume was 20 μl and the elution flow rate was 1 mL/min.

### HPLC analyses using $Na_2CO_3$ as mobile phase

$Na_2CO_3$ was often used as a mobile phase in anionic exchange chromatography analyses. So we tested the HPLC method with $Na_2CO_3$ mobile phase for determination of the [$^{18}$F]NaF solution. The HPLC chromatograms of 1 mg/mL of NaF standard solution were acquired from isocratic elution with different concentrations of $Na_2CO_3$ as mobile phase. Moreover, the HPLC analyses of different concentrations of NaF standard solutions were performed with 100 mM $Na_2CO_3$ mobile phase.

### HPLC analyses using NaOAc as mobile phase

NaOAc solution is nearly neutral providing no corrosion to the HPLC instrument. It has absorption at UV 220 nm which may be useful for fluoride detection. We investigated the HPLC method using NaOAc solution as mobile phase. The HPLC-UV chromatograms of 0.9% NaCl and 1 mg/mL NaF standard solution in 0.9% NaCl were acquired with mobile phases of different concentrations of NaOAc.

### HPLC analyses using NaOAc and NaCl mixture as mobile phase

To improve the elution ability of mobile phase, NaCl was supplied as an additive to the NaOAc solution. The mixtures of NaOAc at different concentrations (15, 30, 50, 75 and 100 mM) and NaCl (25, 50 and 100 mM) were prepared and their potential use as mobile phase for the analysis of [$^{18}$F]NaF solution was investigated and compared.

The most potential method was chosen and validated for system suitability, specificity, linearity and range, accuracy, precision, detection limit (LOD) and quantitation limit (LOQ) and robustness according to the ICH guideline, Q2(R1) [15].

## Results and discussion

### Development of method

Reverse phase (RP) C18 HPLC method has been extensively used for the quality control 0f fluorine-18 radiopharmaceuticals. However, the retention of [$^{18}$F]fluoride on RP C18 columns was seriously effected by pH of mobile phase, tailed fluoride peak and non-quantitative recovery of 18F were often observed with silica based C18 column, whereas polymer based C18 column, such as the Hamilton PRP-1

column showed nearly quantitative recovery of [$^{18}$F]fluoride [16]. The lackage of specific retention meachanism of C18 column for [$^{18}$F]fluoride suggests anion-exchange HPLC is more favorite for the quality control of [$^{18}$F]NaF solution.

To approach a facile anion-exchange HPLC method for the quality control of fluoride content and RCP of [$^{18}$F]NaF solution for injection, different mobile phases, including aqueous $Na_2CO_3$, NaOAc, and NaOAc and NaCl mixture were tested.

## HPLC analyses using aqueous $Na_2CO_3$ as mobile phase

The HPLC-UV chromatograms of 1 mg/mL of NaF standard solution in 0.9% NaCl under different concentrations of $Na_2CO_3$ mobile phase displayed a systemic solvent peak, fluoride peak and chloride peak with increasing retention time (Fig. 1). The retention times of fluoride and chloride reduced with increasing $Na_2CO_3$ mobile phase concentration. The areas of negative fluoride and chloride peaks also decreased with increasing $Na_2CO_3$ concentration, however, the skewed peak shape of fluoride turned symmetric. The systemic solvent peak at retention time of 1.27 min changed from positive peak to negative peak with increasing $Na_2CO_3$ concentration.

As 100 mM $Na_2CO_3$ of mobile phase resulted in symmetric fluoride peak of the HPLC-UV chromatogram, it was further tested for the analysis of NaF standard solutions. The chromatograms of different NaF standard solutions (Fig. S1) showed the retention time of fluoride were not constant,

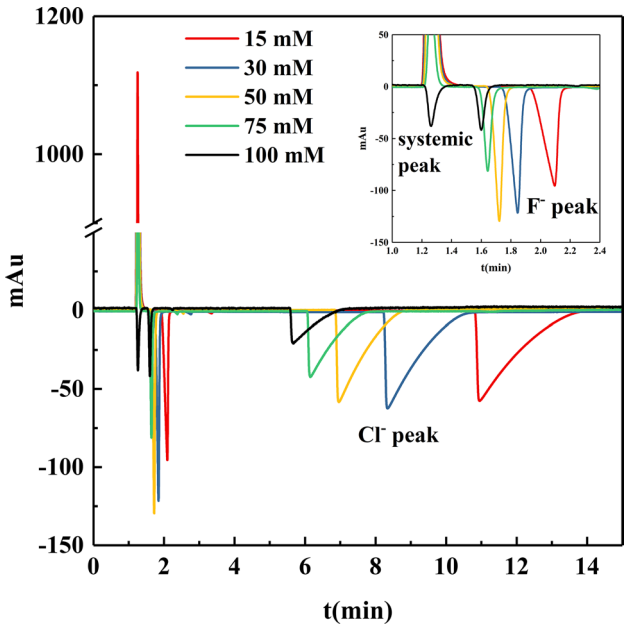


**Fig. 1** HPLC-UV chromatograms of 1 mg/mL of NaF in 0.9% NaCl under different concentrations of $Na_2CO_3$ mobile phase

but increased a little bit with increasing NaF concentration. For both this reason and the short retention time of fluoride adjacent to the systemic solvent peak, $Na_2CO_3$ was not recognized as an appropriate mobile phase for the HPLC assay of [$^{18}$F]NaF.

## HPLC analyses using NaOAc as mobile phase

When 15 mM NaOAc was used as mobile phase, 1 mg/mL of NaF standard sample had a tailed fluoride peak at retention time of 11.88 min, and the chloride ion could not be eluted by the 15 min HPLC run (Fig. S2). The retention times of fluoride and chloride ions reduced with increasing NaOAc concentration up to 100 mM (Fig. 2). The peak symmetry of fluoride was improved. However, the chloride ion from 0.9% NaCl caused not only a severely tailed broad peak with longer retention time than that of fluoride but also a broad systemic peak at RT 2.30 min just before fluoride. These results indicated the inefficient elution ability of NaOAc towards chloride in the [18F]NaF solution for injection.

## HPLC analyses using NaOAc and NaCl mixture as mobile phase

With the addition of NaCl to 15 mM or 30 mM NaOAc mobile phase, the retention time of chloride was significantly reduced, whereas the fluoride peak was still skewed or not resolved well from chloride peak (Fig. 3a). The HPLC chromatograms showed the symmetry of fluoride peak was otherwise improved with increasing NaOAc concentration from 15 mM to 100 mM, while 25 mM NaCl coexisted in the mobile phase (Fig. 3b). As appropriate retention of fluoride and good separation of fluoride from chloride were reached, we investigated in detail the HPLC method with the 100 mM NaOAc and 25 mM NaCl mixture mobile phase.

## System suitability

The system suitability was evaluating by five replicate assays of the 1 mg/mL NaF standard solution. The relative standard deviation (RSD) for the retention time and peak area of fluoride ion were 0.05% and 0.62%, respectively, indicating the good suitability of the system. The good column efficiency was otherwise verified by the tailing factor.

## Specificity

The specificity of the HPLC assay was evaluated by the retention times of anions from NaF standard solution, saline for injection and [$^{18}$F]NaF solution. The retention times of fluoride and chloride in UV chromatograms were 2.0 and 3.5 min, respectively. The radioactive chromatogram of [$^{18}$F]NaF solution showed a peak at retention time of 2.11 min

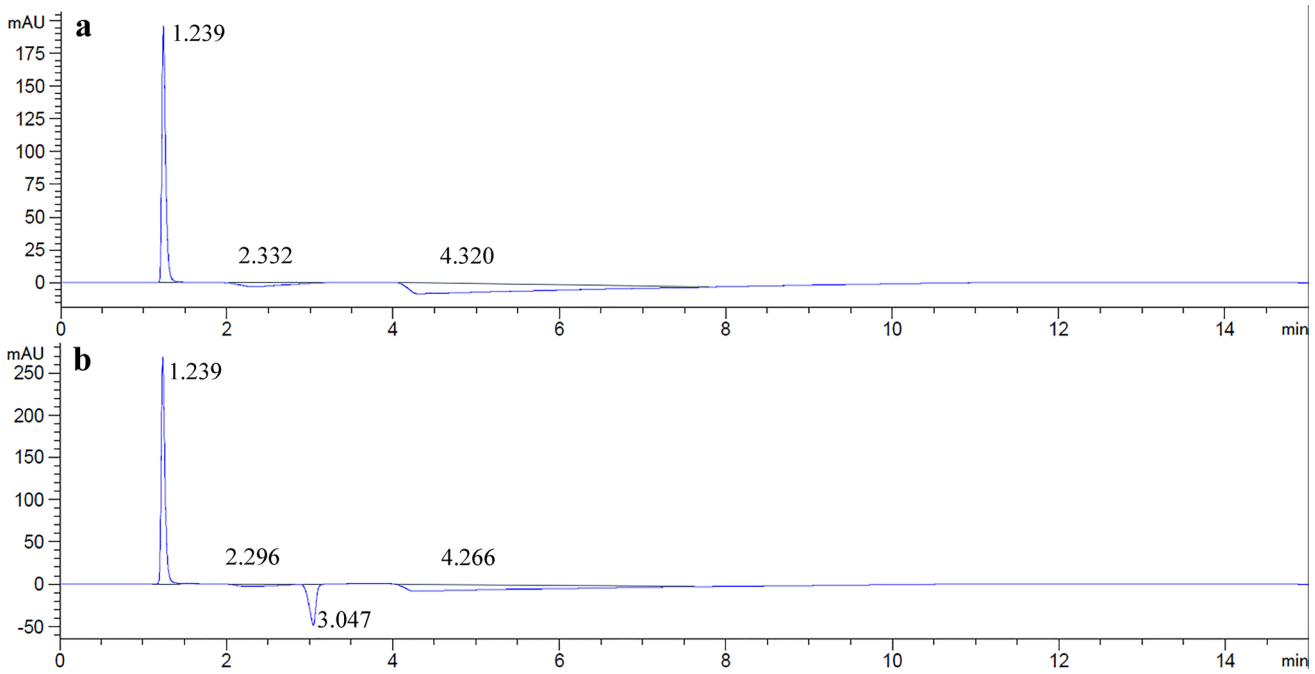


**Fig. 2** HPLC-UV chromatograms of 0.9% NaCl (**a**) and 1 mg/mL NaF solution in 0.9% NaCl (**b**) with 100 mM NaOAc mobile phase

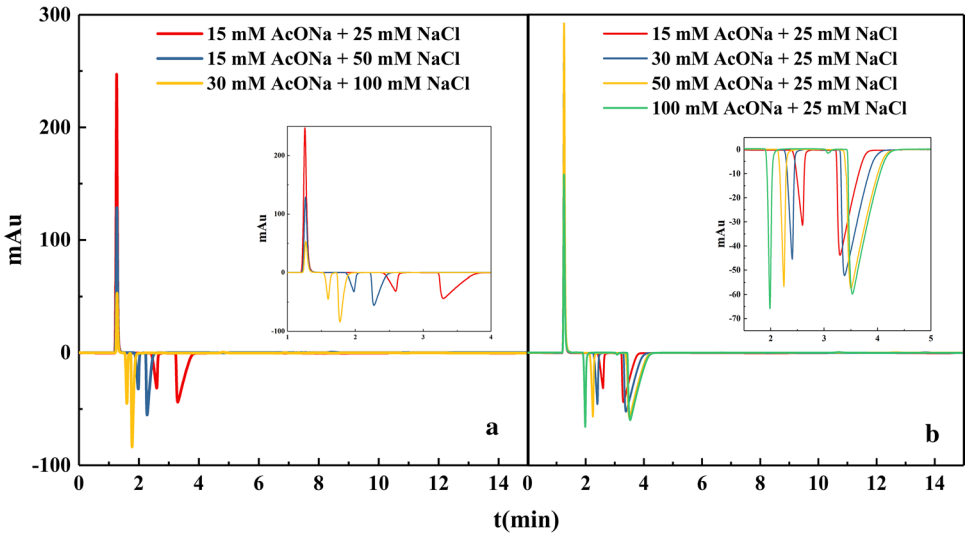


**Fig. 3** HPLC-UV chromatograms of 1 mg/mL NaF in 0.9% NaCl using different concentrations of NaOAc and NaCl mixture as mobile phase

(Fig. 4), same as that of fluoride ion of NaF standard solution after adjustment of the 0.2 min delay caused by the tubing between the UV detector and the radioactivity detector.

## Linearity and range

Six NaF standard solutions at concentrations of 0.05–2 mg/mL and blank solution were measured for validation of linearity. The standard solution of each concentration was measured triplicate. The fitting by least-square regression method showed good linear relationship between UV 220 nm peak area and fluoride concentration over the investigated range (Fig. 5a). Correlation coefficients ($R^2$) of the obtained calibration curves were consistently equal to 0.9999.

The prepared [$^{18}$F]NaF solution was diluted serially with 0.9% NaCl and measured by HPLC. The radioactive chromatogram showed the radioactive peak area of $^{18}F^-$ at retention time of 2.18 min had good linear response to the injected 18F-radioactivity concentration within a range of 3.7–714.1 MBq/mL (Fig. 5b).

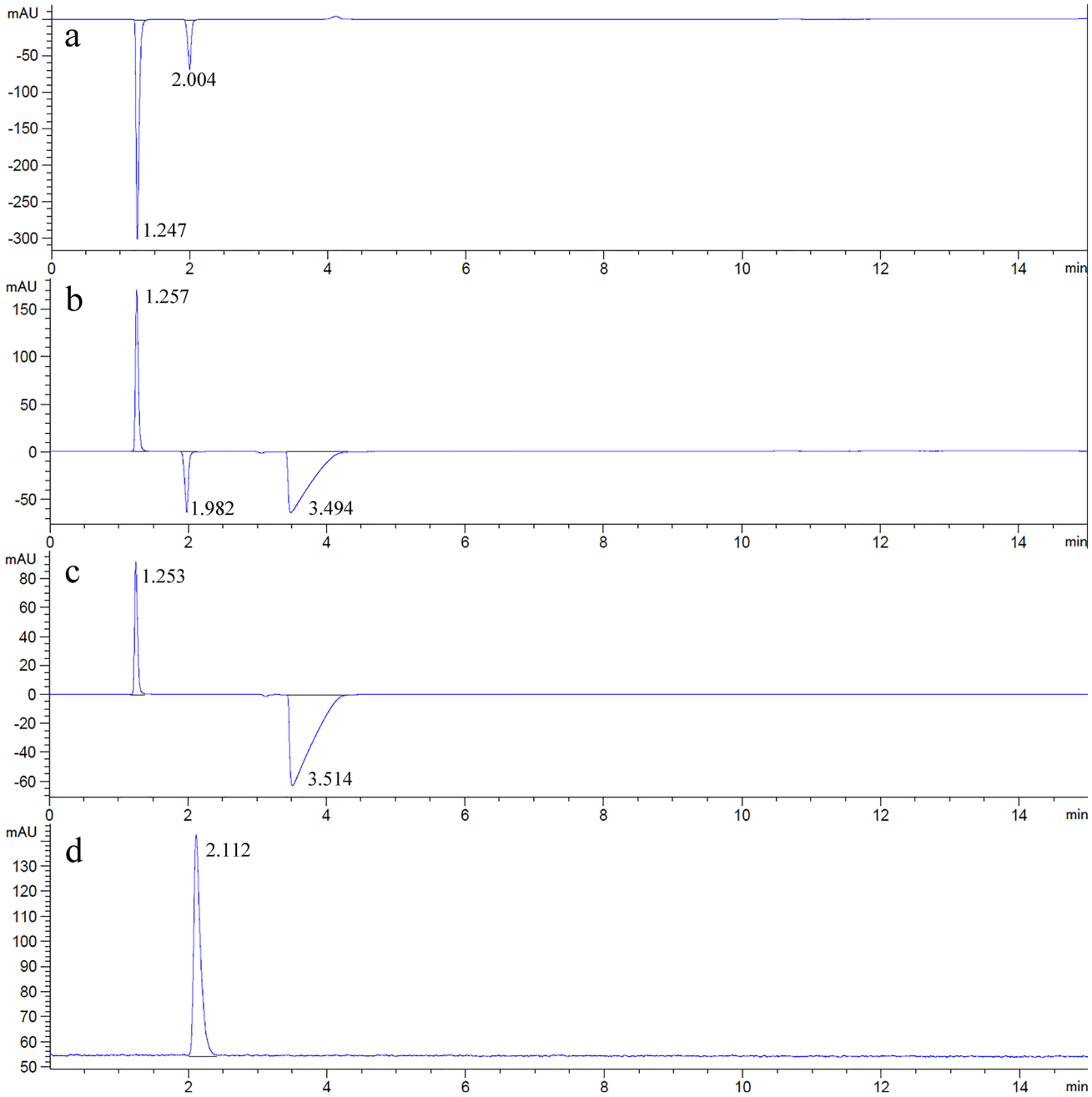


**Fig. 4** HPLC chromatograms of NaF aqueous solution (**a** UV absorbance at 220 nm), NaF in 0.9% NaCl (**b** UV absorbance at 220 nm), and [$^{18}$F] NaF in 0.9% NaCl (**c** UV absorbance at 220 nm, **d** radioactivity) using 100 mM NaOAc and 25 mM NaCl mixture mobile phase

## Accuracy

The accuracy was evaluated by the recoveries of NaF solutions of different concentrations, which were calculated by comparing the theoretical concentrations from the calibration curve with the nominal concentrations. All the data of recovery were within the range of 98–102%, indicating the acceptable accuracy of the HPLC method (Table 1).

## Precision

The precision was evaluated by the repeatability from triplicate determinations of three NaF preparations and the intermediate precision from inter-day determinations of the NaF preparations. All the values of RSD were less than 1% (Table 1), indicating the good repeatability of the HPLC method.

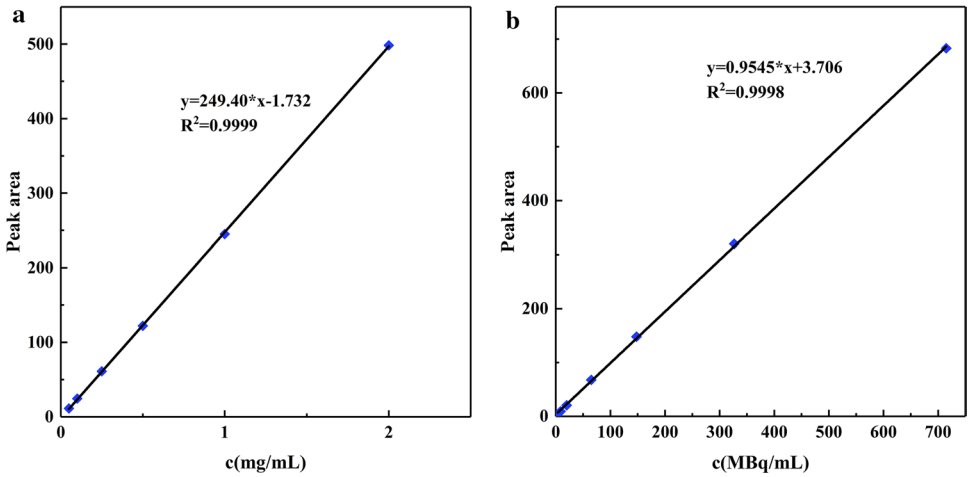


**Fig. 5** The linear relationship of UV absorbance peak area with $F^-$ concentration (**a**) and $^{18}F^-$ radioactive peak area with $^{18}F^-$ radioactivity adjusted to injection time (**b**)

**Table 1** Validation of HPLC anlysis of NaF solution

| Analyst | NaF(mg/mL) | Peak area mean ± SD ($n=3$) | RSD (%) | Rocovery (%) |
|---|---|---|---|---|
| 1 | 0.1 | 24.0 ± 0.10 | 0.417 | 100.27 |
| | 0.5 | 122.1 ± 0.99 | 0.811 | 99.91 |
| | 1 | 245.0 ± 0.82 | 0.335 | 100.02 |
| 2 | 0.1 | 24.2 ± 0.058 | 0.24 | 101.53 |
| | 0.5 | 121.3 ± 0.20 | 0.165 | 99.46 |
| | 1 | 244.9 ± 0.71 | 0.29 | 100.12 |
| 3 | 0.1 | 24.3 ± 0.10 | 0.412 | 101.29 |
| | 0.5 | 120.6 ± 0.76 | 0.63 | 99.54 |
| | 1 | 242.8 ± 0.35 | 0.144 | 100.10 |

## LOD and LOQ

The LOD and LOQ of fluoride ion were estimated based on the standard deviation of the UV 220 nm response of blank samples (regressed y-intercept) and the slope of the calibration curve of NaF solutions, which were represented by α and S, respectively. The LOD and LOQ were 4.4 μg/mL and 13.4 μg/mL, respectively, as calculated according to the equations LOD = 3.3 α/S and LOQ = 10 α/S. These data indicated that the HPLC method is appropriate to test the quality of [18F]NaF solution about the fluoride content.

## Robustness

The robustness of the HPLC method was investigated by analyzing an 1 mg/mL NaF solution in 0.9% NaCl and evaluating the performance parameters after individually varying composition concentration of mixture mobile phase (NaOAc: ± 5 mM/NaCl: ±2 mM), HPLC pump flow rate (± 5%) and column compartment temperature (± 5 °C).

The mobile phase was a mixture of NaOAc and NaCl. The variation of concentration of each component in the mixture mobile phase in the investigated range had a slight reverse effect on the retention time of fluoride. However, the effect of varying concentration of NaOAc and NaCl on the peak area of fluoride was complicated. The peak area of fluoride decreased with increasing NaOAc concentration, but slightly increased with increasing NaCl concentration. The performance parameters of resolution for fluoride and chloride, tailing factor and number of theoretical plates were not significantly changed by the varying concentration of mobile phase (Table 2).

The column temperature was set up at 25 °C. The increase of column temperature in range of 20–30 °C caused the retention time decreased and the peak area increased. The resolution, tailing factor and number of theoretical plates were not significantly affected by the change of temperature (Table 2).

The effect of flow rate on the chromatograms of sodium fluoride was measured at flow rate of 5% variation. Higher flow rate resulted in shorter retention time and reduced peak area of fluoride (Table 2). The retention time and peak area of fluoride were consistent, respectively at each flow rate with values of RSD smaller than 1%.

The above tests verified the robustness of the HPLC method for measurement of the radiochemical purity and fluoride content of the [18F]NaF solution.

**Table 2** Robustness of HPLC analysis of NaF solution under variable conditions

| | Retention time (min) mean ± SD ($n$ = 3) | RSD (%) | Area mean ± SD ($n$ = 3) | RSD (%) |
|---|---|---|---|---|
| NaOAc/NaCl concentrations | | | | |
| 105 mM/25 mM | 1.96 ± 0.0005 | 0.024 | 238.2 ± 1.07 | 0.449 |
| 95 mM/25 mM | 2.00 ± 0.0005 | 0.024 | 249.4 ± 1.37 | 0.549 |
| 100 mM/25 mM | 1.98 ± 0.0010 | 0.041 | 245.0 ± 0.67 | 0.273 |
| 100 mM/23 mM | 2.01 ± 0.0005 | 0.024 | 242.3 ± 0.25 | 0.103 |
| 100 mM/27 mM | 1.95 ± 0.0016 | 0.084 | 249.2 ± 0.98 | 0.393 |
| Column temperature | | | | |
| 20 ℃ | 1.96 ± 0.0008 | 0.042 | 239.1 ± 0.64 | 0.268 |
| 25 ℃ | 1.98 ± 0.0010 | 0.041 | 245.0 ± 0.67 | 0.273 |
| 30 ℃ | 1.99 ± 0.0017 | 0.085 | 247.4 ± 1.18 | 0.477 |
| Flow rate of mobile phase | | | | |
| 0.95 mL/min | 2.09 ± 0.0005 | 0.023 | 254.7 ± 0.76 | 0.298 |
| 1.00 mL/min | 1.98 ± 0.0010 | 0.041 | 245.0 ± 0.67 | 0.273 |
| 1.05 mL/min | 1.89 ± 0.0005 | 0.025 | 240.5 ± 1.23 | 0.511 |

## Conclusions

A facile anion exchange HPLC method has been developed for the analyses of fluoride content and radiochemical purity of [$^{18}$F]NaF solution. The use of mixture of 100 mM NaOAc and 25 mM NaCl as mobile phase produced good resolution and sensitive UV detection of fluoride ion. The characteristics including system suitability, specificity, linearity, accuracy and precision were validated according to relevant ICH guidelines. The method was also proved to be robust, providing a convenient assay for routine QC analysis of [$^{18}$F] NaF solution in PET radiopharmaceuticals.

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