

Short communication

Purification of [^{18}F]-fluoro-L-thymidine ([^{18}F]-FLT) for positron emission tomography imaging

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Abstract

3'-Deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]-FLT) has recently been described as a positron emission tomography (PET) radiopharmaceutical for visualizing cellular proliferation *in vivo*. All published radiosyntheses of [^{18}F]-FLT provide crude products that must be purified before injection to human. This study describes a reliable purification procedure for [^{18}F]-FLT. It is based on HPLC. In 17.9 ± 0.5 min, pure [^{18}F]-FLT is obtained that could be injected to human after a passage through a sterile $0.22 \mu\text{m}$ filter.

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1. Introduction

For PET imaging of cell proliferation in neoplastic and metastatic tumors, a new and promising radiopharmaceutical has been synthesized: 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]-FLT) [1,2]. [^{18}F]-FLT is an extemporaneous radiopharmaceutical product and its preparation must be controlled before its injection to human. [^{18}F] fluoride is a short half-life positron emitter (109.6 min). Thus, the radiosynthesis, purification and formulation must be as fast as possible.

Various methods have already been described but analytical information on separation and thus on the purity of the FLT are not defined clearly enough for a clinical trial [3–5].

The aim of this study is the description of the various parameters of the purification of [^{18}F]-FLT from the major synthesis by-product (2',3'-didehydro-3'-deoxy-thymidine) by using solvents compatible with human use in order to save time for an injection to a patient. For radioprotection reasons, this separation was studied first with non-radioactive [^{19}F]-FLT.

2. Materials and methods

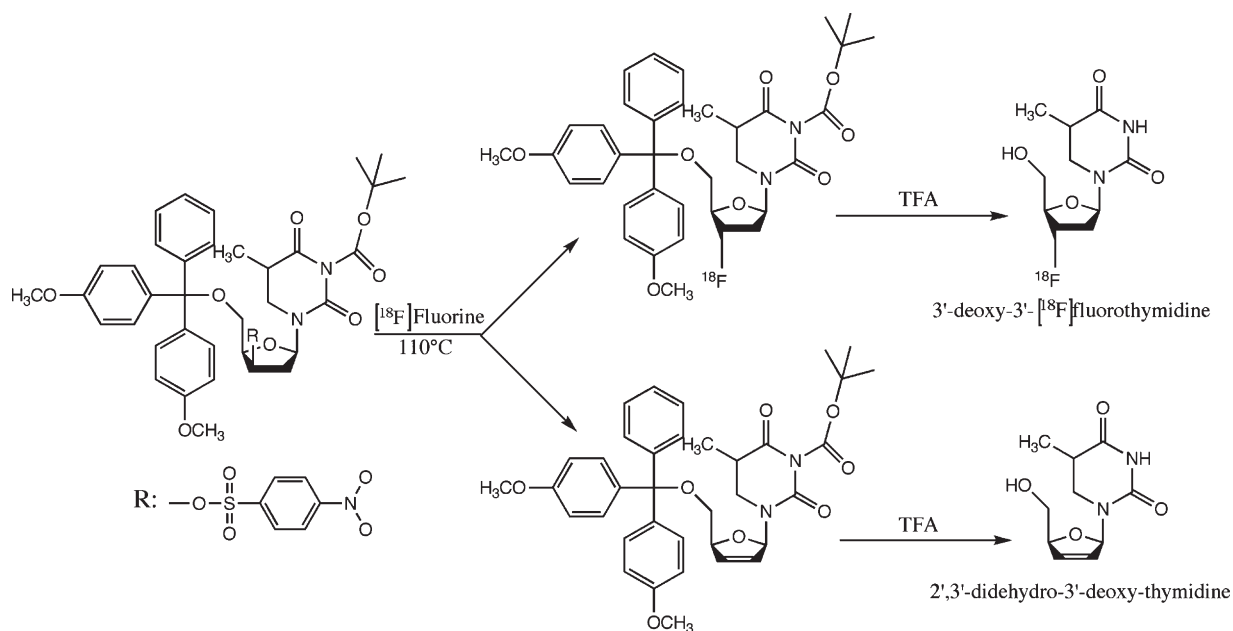
2.1. Radiosynthesis of [^{18}F]-FLT

[^{18}F]-FLT was synthesized from the non-radioactive precursor (5'-*O*-Dimethoxytrityl-2'-deoxy-3'-*O*-nosyl- β -D-threopentofuranosyl-3-*N*-butoxycarbonyl-thymine) in acetonitrile with [^{18}F] fluoride activated by the crown ether 1,10-diaza-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (Kryptofix 2.2.2) at 110°C and deprotection with trifluoroacetic acid. Fig. 1 shows the reaction scheme of [^{18}F]FLT synthesis [6].

2.2. Chemical

2',3'-Didehydro-3'-deoxy-thymidine (Stavudine[®]), [^{19}F]-3'-fluoro-3'-deoxy-L-thymidine ([^{19}F]-FLT), trifluoroacetic acid and ammonium hydroxide are purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). Sterile water was bought from Fresenius-Kabi (Sevres, France). Absolute ethanol (99.9%) was obtained from Cooper (Melun, France). Methanol is a product of Carlo Erba (Val de Reuil, France).

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Fig. 1. Radiosynthesis of ^{18}F -FLT.

2.3. Instruments and HPLC conditions

HPLC instrumentation consisted of a Waters 600 controller (Saint Quentin en Yvelines, France) and a Waters 486 Tunable Absorbance Detector. Data analysis was carried out using Waters Empower data acquisition and analysis software.

A Waters μ bondapak C_{18} Sentry guard column (3.9 mm \times 20 mm; 5 μm) was used as guard column. A Waters μ bondapak C_{18} (3.9 mm \times 300 mm; 5 μm) was used as the analytical column. The isocratic mobile phase was composed of water–ethanol (90:10, v/v). The mobile phase was filtered through a Whatman (Maidstone, England) nylon membrane

0.45 μm filter. Degassing was performed using a Waters in line degasser AF. Flow rate was set to 0.65 mL/min. The analysis time was 25 min. The UV absorbance was monitored at 265 nm.

A Waters SymmetryPrep C_{18} (7.8 mm \times 300 mm; 7 μm) was used as a preparative column to purify a sample of ^{18}F -3'-fluoro-3'-deoxy-L-thymidine. The isocratic mobile phase was composed of water/ethanol (90:10, v/v) at a flow rate of 3.0 mL/min. In addition, this preparative HPLC equipped with NaI(Tl) detector for γ radiation and a UV detector was used for the identification and purification of ^{18}F fluorine-labeled product. TLC plates (silica gel) are a product of Merck (Darmstadt, Germany).

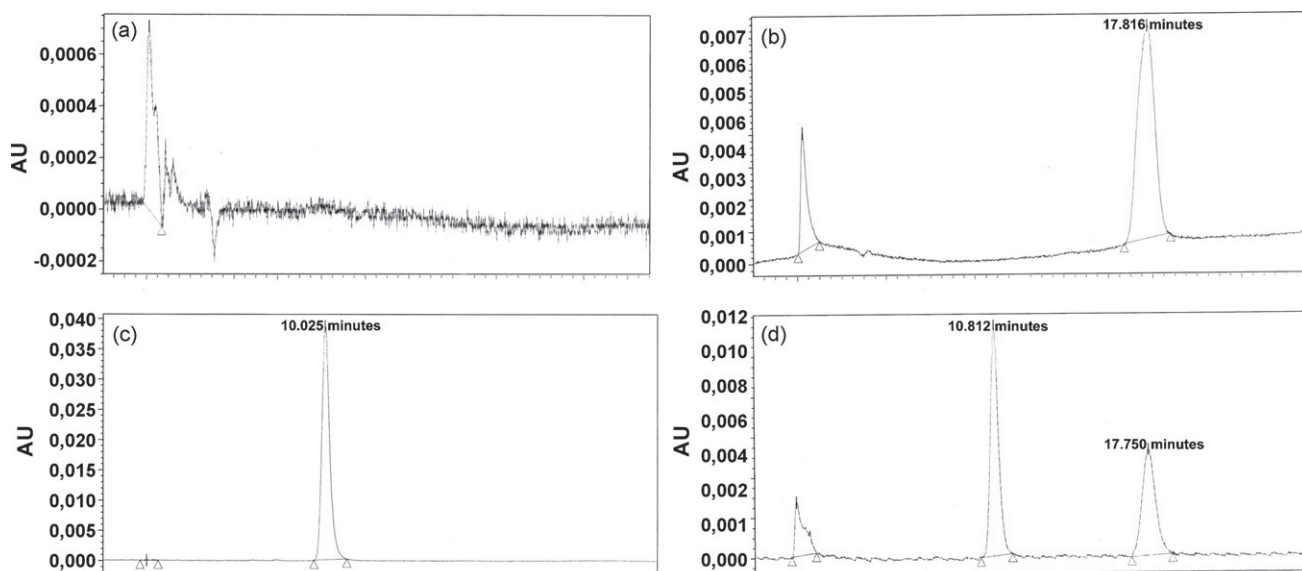


Fig. 2. Typical chromatograms of mixtures of standards on a Waters C_{18} μ bondapak (3.9 mm \times 300 mm; 5 μm) run in isocratic water–ethanol (9:1, v/v) at 0.65 mL/min with UV detection at 265 nm. (a) Blank sample. (b) Fluoro-L-thymidine (4.6 $\mu\text{g/mL}$). (c) Stavudine (12.0 $\mu\text{g/mL}$). (d) Fluoro-L-thymidine (3.07 $\mu\text{g/mL}$) with stavudine (4.0 $\mu\text{g/mL}$).

2.4. Preparation of standards

Two stock solutions at different concentrations of [¹⁹F]-FLT were prepared by dissolving 2.3 mg in 500 mL and 2.2 mg in 1 mL of water–ethanol (90:10, v/v), respectively, to yield final concentration of 4.6 and 2200 µg/mL. Working standards of 1100; 550; 275; 137.5; 68.8; 34.4; 17.2; 8.6; 2.3; 1.2; 0.6; 0.3 and 0.14 µg/mL were prepared by two-fold dilution of the stock solution in water–ethanol mixture (90:10, v/v).

Two stocks solutions at different concentrations of 2',3'-didehydro-3'-deoxy-thymidine were prepared by dissolving 3.0 mg in 250 mL and 6.5 mg in 1 mL of water–ethanol (90:10, v/v), respectively, to yield final concentrations of 12.0 and 6500 µg/mL. Working standards of 3250; 1625; 812.5; 406.3; 203.1; 101.6; 50.8; 25.4; 12.7; 6.0; 3.0; 1.5; 0.8; 0.4; 0.2; 0.1 and 0.05 µg/mL were prepared by two-fold serial dilution of the stock solutions in water–ethanol mixture (90:10, v/v).

A solution of trifluoroacetic acid at 0.25 mg/mL in water was also prepared.

2.5. Detection of kryptofix 2.2.2

Because of its lack of UV absorbance, kryptofix was searched in the eluted fraction of FLT using the European pharmacopoeia method for aminopolyethers.

This method consists in a thin layer chromatography on silica gel with a mixture of methanol/ammonia 9:1 as mobile phase. The detection is made by iodine vapor. Under this condition, the R_f of Kryptofix 2.2.2 is 0.1 [8].

2.6. Calibration procedure

The calibration curve was constructed by unweighted linear regression of the peak area against concentration.

2.7. Precision, accuracy and limit of detection

A single set of samples at 4.6 µg/mL for FLT and a sample at 12 µg/mL for stavudine was prepared and analyzed with an independent standard curve each days for 5 days. Inter-day precision was expressed by coefficient of variation (CV).

Five sets of the same sample (same concentration) were prepared and analyzed on the same day, along with an independent standard curve for quantification. CV expressed intra-day precision.

The accuracy was expressed as the percent difference for each sample:

$$\% \text{ difference} = \frac{(\text{determined concentration} - \text{nominal concentration})}{\text{nominal concentration}} \times 100$$

The limit of detection was defined by the concentration with a signal-to-noise ratio of 3.

3. Results

3.1. Chromatographic characteristics

After deprotection with trifluoroacetic acid, the radiosynthesis gives two major products: 3'-deoxy-3' [¹⁸F]fluorothymidine ([¹⁸F]-FLT) and a major non-radioactive by-product, 2',3'-didehydro-3'-deoxy-thymidine (this product was marketed as an anti-HIV drug under the name of Stavudine[®]) [7].

Fig. 2 shows chromatograms of a blank sample, a sample containing 4.6 µg/mL of fluoro-L-thymidine, a sample containing 12 µg/mL of stavudine and a sample containing 3.07 µg/mL of fluoro-L-thymidine with 4.0 µg/mL of stavudine.

The calculated capacity factors (*k'*) for fluoro-L-thymidine and stavudine were 6.98 and 3.84, respectively. Retention times were 17.9 ± 0.5 min for fluoro-L-thymidine (*n* = 5) and 10.0 ± 0.1 min for stavudine (*n* = 5).

Under this chromatographic condition, at a wavelength of 210 nm for detection, trifluoroacetic acid was eluted at 5.7 min and did not interfere with the purification of [¹⁹F]-FLT. Moreover, at the retention time of [¹⁹F]-FLT there is no kryptofix 2.2.2.

3.2. Calibration curve

The analysis of fluoro-L-thymidine and stavudine exhibited excellent linearity (*r*² = 0.9995 and 0.9998, respectively, for fluoro-L-thymidine and Stavudine[®]) over the 2200 µg/mL concentration range for fluoro-L-thymidine and the 1100 µg/mL concentration range for stavudine.

3.3. Precision, accuracy and limit of detection

The results of precision and accuracy are shown in Table 1. The limit of detection was 0.144 µg/mL for [¹⁹F]-FLT and 0.046 µg/mL for Stavudine[®]. At this concentration, the signal-to-noise ratio was 3.

3.4. Preparative HPLC of [¹⁸F]-FLT

Fig. 3 shows a chromatogram of [¹⁸F]-FLT. Under this chromatographic condition, only one radioactive product, [¹⁸F]-fluoro-L-thymidine, was detected with a retention time of 11.06 min. UV detection showed a major by-product: 2',3'-didehydro-3'-deoxy-thymidine with a retention time of 6.9 min.

4. Discussion and conclusion

A pre-purification on Sep-Pak column would eliminate trifluoroacetic acid and kryptofix 2.2.2, but this technique does not allow us to separate stavudine from [¹⁸F]-FLT. A rapid and reproducible method has been developed here for the preparation of [¹⁹F]-FLT. This method is applicable to [¹⁸F]-FLT.

During the radiosynthesis, a small quantity of [¹⁹F]-fluoro-L-thymidine may be formed. This is due to the presence of [¹⁹F] fluoride traces in the [¹⁸H]H₂O target. The concentration in

Table 1
Intra- and inter-day precision and accuracy of [^{19}F]-FLT and stavudine

	Theoretical concentration ($\mu\text{g/mL}$)	Measured concentration ($\mu\text{g/mL}$)	SD ($\mu\text{g/mL}$)	CV (%)	Accuracy (%)	<i>n</i>
Intra day						
FLT	4.60	4.64	0.08	1.53	0.87	5
Stavudine	12.00	12.03	0.07	0.48	0.25	5
Inter day						
FLT	4.60	4.61	0.08	1.43	0.22	5
Stavudine	12.00	11.90	0.26	1.91	0.83	5

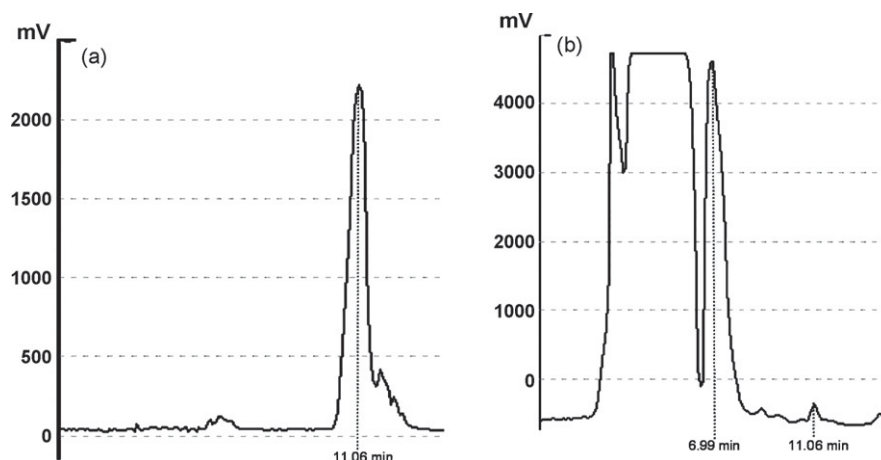


Fig. 3. Preparative chromatograms obtained with Waters SymmetryPrep C_{18} (7.8 mm \times 300 mm; 7 μm). (a) Detection with a NaI(Tl) detector. (b) Detection with a UV detector at 265 nm.

the sample may be quantified by UV detection using a calibration curve. If the total quantity in the injected dose is less than 10 μg , the regulatory authorities only require an acute animal toxicity study. If the injected dose is more than 10 μg , it is necessary to perform a toxicity study for each process of chemical manufacturing. It is thus very important to choose the best quality of [^{18}H] H_2O to prepare [^{18}F]-FLT. Under the radiolabeling condition, UV quantification of [^{18}F]-fluoro-L-thymidine is impossible because the quantity synthesized is lower than the limit of detection (Fig. 3). In routine, a scintigraphic detector calibrated to the 511 keV annihilation photons of [^{18}F] fluorine and a UV detector for non-radioactive product (2',3'-dideoxy-3'-deoxy-thymidine and [^{19}F]-FLT), should be used.

The methodology described in this publication affords [^{18}F]-FLT without the major impurity obtained during the radiolabeling, 2',3'-dideoxy-3'-deoxy-thymidine. In analytical conditions, the retention time of the [^{18}F]-FLT is the same as that of [^{19}F]-FLT, i.e. 17.9 ± 0.5 min and the retention time is 10.0 ± 0.1 min for stavudine. For preparative chromatography, pure [^{18}F]-fluoro-L-thymidine can be obtained at 11.1 min.

After a passage through a sterile 0.22 μm filter, the purified [^{18}F]-FLT fraction collected in a vial may be injected to human because the mobile phase of HPLC (water/ethanol (90:10, v/v)

mixture) may be injected to human patients for PET imaging if solvents are of pharmaceutical grade.

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