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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 μm filter.

Keywords: [^{18}F]-fluoro-L-thymidine; FLT; Positron emission tomography; Oncology; Purification

1. Introduction

For PET imaging of cell proliferation in neoplasic 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.

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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-threo-pentofuranosyl-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).

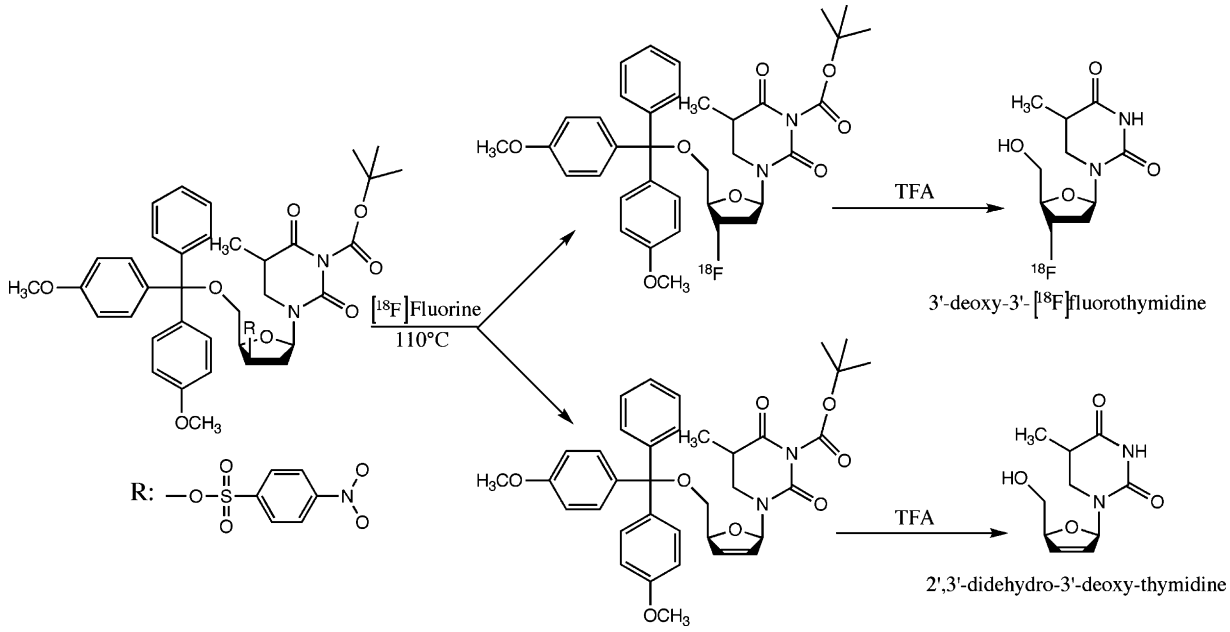

Fig. 1. Radiosynthesis of [^{18}F]-FLT.

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 μbondapak C_{18} Sentry guard column (3.9 mm × 20 mm; 5 μm) was used as guard column. A Waters μbondapak C_{18} (3.9 mm × 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 though 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 × 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).

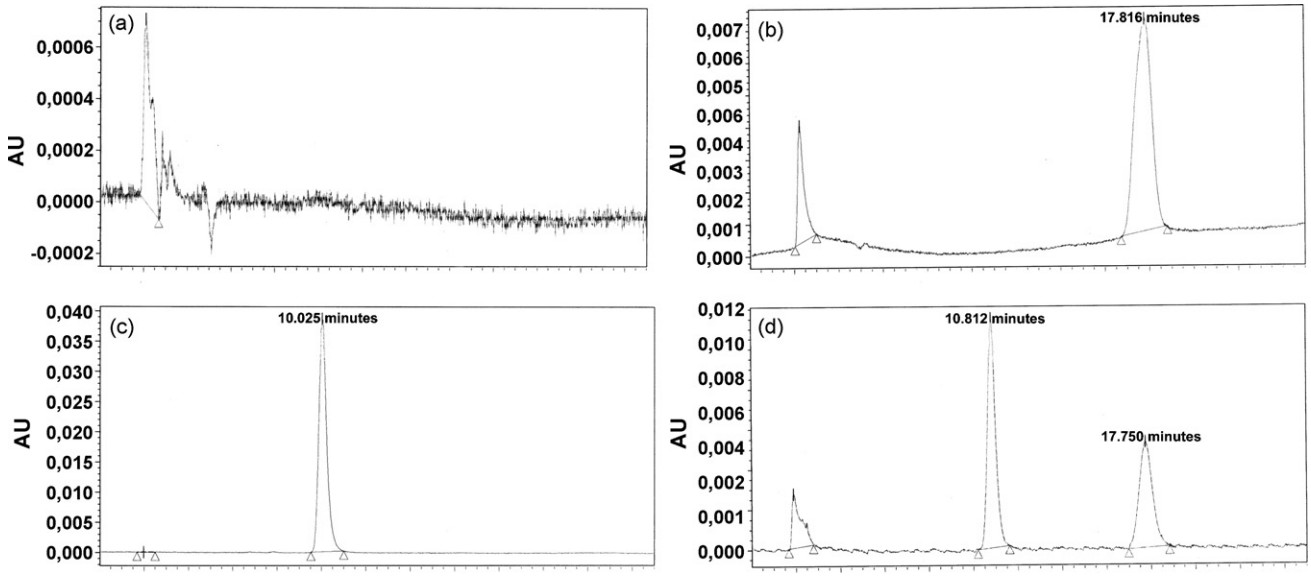

Fig. 2. Typical chromatograms of mixtures of standards on a Waters C_{18} μbondapack (3.9 mm × 300 mm; 5 μm) run in isocratic water–ethanol (9:1, v/v) at 0.65 mL/mn with UV detection at 265 nm. (a) Blank sample. (b) Fluoro-L-thymidine (4.6 μg/mL). (c) Stavudine (12.0 μg/mL). (d) Fluoro-L-thymidine (3.07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