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Automated and efficient radiosynthesis of [^{18}F]FLT using a low amount of precursor

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ABSTRACT

Introduction: Since 1991 until now, many radiosyntheses of [^{18}F]FLT have been published. Most of them suffer from side reactions and/or difficult purification related to the large amount of precursor necessary for the labeling step. A fully automated synthesis using only commercial and unmodified materials with a reduced amount of precursor would be desirable.
Methods: We first explored the possibility to elute efficiently [^{18}F]fluorine from commercial and unmodified cartridges with various amount of base. Based on these results, 10 mg and 5 mg of precursors were used for the fluorination step. The best conditions were transposed in an automated process for a one pot two steps synthesis of labeled FLT.
Results: Using commercial and non-treated carbonate form of QMA cartridges, we were able to elute quantitatively the [^{18}F]fluorine with a very low amount of base (0.59 mg) and, with only 5 mg of precursor, to perform an efficient fluorination reaction with up to 94% incorporation of [^{18}F]fluorine. The synthesis was fully automated and radiochemical yields of 54% (decay corrected) were obtained within a synthesis time of 52 minutes.
Conclusion: We demonstrate that a fully automated and efficient radiosynthesis of [^{18}F]FLT is feasible with only 5 mg of precursor. Compare to the present state of the art, our method provides high yields of pure [^{18}F]FLT and is broadly adaptable to other synthesis automates.

1. Introduction

FLT (3′-deoxy-3′-fluoro-thymidine) was initially developed as a thymidine analogue against HIV virus [1] after the success of 3′-azido-thymidine (AZT). Preliminary tests showed toxicity [2] at high doses (100 mg/day over weeks) but incited its use at a tracer level (several thousand folds less than the therapeutic dose). In 1991 [3] the first radiosynthesis of [^{18}F]FLT and its potential use to monitor drug distribution was described. Later on, Shields et al. [4] reported its use for imaging proliferation in vivo by positron emission tomography.

Mechanisms of [^{18}F]FLT uptake have been extensively studied; as a pyrimidine analogue, [^{18}F]FLT is transported into cells by passive diffusion and active transporters (Na^+ dependent carrier) to participate to the *salvage* pathway in nucleosides synthesis and is further phosphorylated by the thymidine kinase 1 (TK1) leading to its accumulation inside the cell [5,6]. The rationale behind its use to monitor cell proliferation is based on the high TK1 activity in cancer cells; this activity reached its maximum during the late G and S phases of cell duplication. However after phosphorylation it was demonstrated that the radiolabeled [^{18}F]FLT did not significantly incorporate in DNA as it acts as a terminating nucleoside during DNA synthesis [6]. Despite its stability [^{18}F]FLT is eliminated by hepatobiliary pathway after glucuronidation [7].

Even if [^{18}F]FLT exhibits lower uptake than [^{18}F]FDG it has the advantage of being more specific and less sensitive to inflammation process, it is considered as a good indicator of proliferation [8,9] and received FDA agreement in 2009 for proliferation imaging and monitoring of responses to treatments [10–14]. However some authors showed that the uptake did not always correlate with the proliferation index [15,16]. The reasons involved are the difference of mechanisms that can exist among the cancer cell lines (*de novo* versus *salvage* pathway), the endogenous level of thymidine (especially in rodents) and the use of some chemotherapy regimens [17–19]. In spite of these drawbacks [^{18}F]FLT can be used as a staging tool and to monitor therapies if careful validation is used.

The [^{18}F]FLT attractiveness motivated the development of efficient radiosynthesis [20,21] and their automation; Fig. 1 summarizes the main precursors (**I**–**VII**) used for the production of ^{18}F-radiolabeled FLT [22–26,29].

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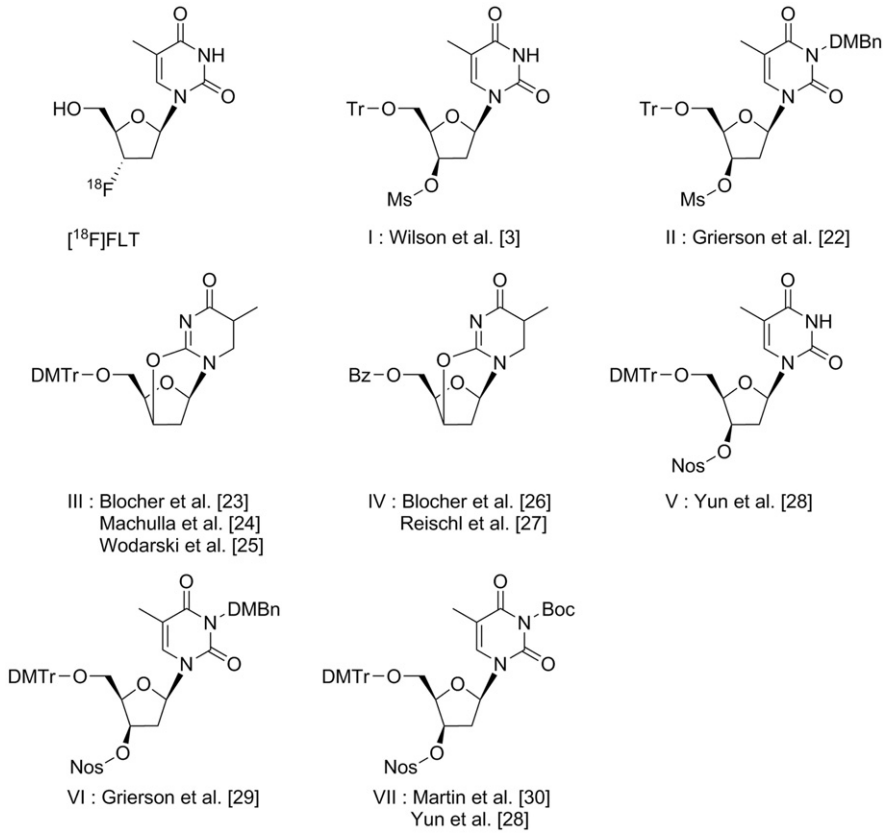

Fig. 1. [^{18}F]FLT and the different precursors used in its radiosynthesis.

Among these precursors, the 5′-*O*-dimethoxytrityl-3-*N*-Boc precursor **VII** [28,30], is to date the most used and is commercially available at GMP grade.

Different strategies have been published to achieve efficient radiosyntheses of [^{18}F]FLT (Table 1). Most of the reported syntheses are based on the use of a large amount of precursor (up to 40 mg)

Table 1
Summary of [^{18}F]FLT synthesis from 2003 to 2015.

	[Ref.]	Precursor[a] (μmol/mg)	Fluorination conditions	Base	[^{18}F][b]	[^{18}F]FLT[c]
1	[27]	**IV** (30/10)	DMSO, 160 °C, 10′	K_2CO_3 25 μmol, K_{222} 15 mg	46	18
2	[28]	**VII** (40/33)	300 μL CH_3CN, 110 °C, 5′	K_2CO_3 39.8 μmol, K_{222} 28 mg	82	40
		V (41/34)	300 μL CH_3CN, 130 °C, 5′		85	42
3	[31]	**VII** (48/40)	2 mL CH_3CN, 150 °C, 100″	K_2CO_3 50 μmol, K_{222} 22 mg	50	50
4	[32]	**VII** (24/20)	Tertiary alcohols 800 μL, 200 μL CH_3CN, 110 °C, 10′	$TBAHCO_3$ 0.75 μmol	ND	65
5	[33]	**VII** (6/5)	200 μL CH_3CN, 200 μL IL, 120 °C, 15′	$KHCO_3$ 5 μmol	61	30
		VII (12/10)		K_2CO_3 20 μmol	48	25
6	[34]	**VII** (40/33)	CH_3CN, 1 mL, 140 °C, 5′	K_2CO_3 21.7 μmol, K_{222} 10 mg	ND	38
7	[35]	**VII** (24/20)	*t*-BuOH 2 mL, 200 μL CH_3CN, 100–120 °C, 10′	$TBAHCO_3$ 0.75 μmol	85	60
		VII (12/10)			53	ND
		VII (6/5)			32	ND
8	[36]	**VII** (9.6/8)	300 μL CH_3CN, 100 °C, 5′	K_2CO_3 10 μmol, K_{222} 5 mg	64	27
		VII (22/18)			76	ND
		V (11/9)			15	0.6
9	[37]	**VII** (24/20)	2 mL *t*-amylOH, 200 μL CH_3CN, 120 °C, 10′	$TBAHCO_3$ 0.75 μmol	>85	64
10	[38]	**VII** (36/30)	1 mL CH_3CN	K_2CO_3 22 μmol, K_{222} 15 mg	ND	40
		VII (18/15)	1 mL CH_3CN			25
		IV (30/25)	1 mL DMSO			18
11	[39]	**VII** (6/5)	*t*-amylOH 500 μL, 100 μL CH_3CN, 120 °C, 15′	$KHCO_3$ 7.3 μmol	91	ND
12	[40]	**VII** (12/10)	1 mL CH_3CN, 100 °C, 5′	$TBAHCO_3$ 75 μmol	27	ND
		VII (18/15)			36	25
		VII (24/20)			43	ND
		VII (30/25)			58	39
13	[41]	**VII** (30/25)	2 mL CH_3CN, 100 °C, 5′	$TBAHCO_3$ 37.5 μmol	ND	39

t-amylOH = 2-methyl-butan-2-ol, *t*-BuOH = 2-methyl-propan-2-ol, $TBAHCO_3$ = tetrabutylammonium hydrogen carbonate 0.075 M, K_{222} = Kryptofix 2.2.2. ND not determined.
[a] Precursor see Fig. 1 (μmol/mg).
[b] Percentage of incorporation of [^{18}F].
[c] Isolated yield of [^{18}F]FLT (%, decay corrected).

complicating the purification step and generating a large amount of impurities (especially stavudine).

A survey of the literature shows that two main approaches were developed; the first one (entries 4, 5, 7 and 9 in Table 1) consisted in a variation of the solvent with the use of ionic liquids or hindered tertiary alcohols. The second strategy is based on a reduction of the amount of base and the evaluation of the optimal precursor to base ratio (entries 8 and 11 in Table 1) to achieve high yields and to facilitate the purification by reduction of by-products [36,39].

The advantage of using a tertiary alcohol, to preserve the precursor from elimination during the fluorination step, was demonstrated and under optimized conditions [^{18}F]FLT was isolated in 65% yield (decay corrected) on automated synthesizer using 20 mg (24 μmol) of precursor **VII** [32].

When the amount of precursor was reduced to 10 mg (12 μmol) or even 5 mg (6 μmol) a dramatic drop in the fluorination yield was observed. Only Lee et al. [39] (entry 11, Table 1) were successful in the fluorination of 5 mg of precursor **VII** using a low amount of base along with *t*-amyl alcohol. The authors stated that the reduction of the amount of base was only possible by the use of a modified QMA cartridge (preconditioned and eluted with KOMs). Up to 91% incorporation of ^{18}F was observed but the complete synthesis (hydrolysis of the fluorinated intermediate and purification) and its automation were not performed.

In the frame of our research project on tumor radiotherapy follow up by [^{18}F]FLT preclinical PET imaging, concentrated formulated solution of [^{18}F]FLT (above 500 MBq/mL) was necessary in order to images 10 to 12 mice a day and ensure a sufficient dilution before *iv* injection to lower the amount of ethanol injected. In a typical batch the amount of ethanol reaches 10%, therefore a 200 μL injection of the formulated solution would result in an ethanol concentration of almost 8 g per liter of blood (for a mouse of 25 g having 2 mL of blood). Moreover HPLC purification would be amenable to be sure to remove completely the stavudine (d4T) formed during the synthesis. For these reasons we reinvestigated the [^{18}F]FLT radiosynthesis on a Raytest automated module to achieve a high yield (and high radioactive concentration) with a low amount of precursor to facilitate the purification step.

Our strategy (Fig. 2) took advantages of the most efficient methods published so far (use of protic solvent and of low amount of base) and involved an automated system with no or minor modifications and with unmodified QMA cartridges along with a low amount of precursor.

2. Materials and methods

2.1. Chemicals, reagents and apparatus

(5′-*O*-DMTr-2′-deoxy-3′-*O*-nosyl-β-D-threo-pentofuranosyl)-3-*N*-BOC-thymine, stavudine (d4T, 2′-3′-didehydro-2′-3′-dideoxythymidine) and 3′-deoxy-3′-fluoro-thymidine (FLT) were purchased from ABX.

K_2CO_3 99.99% and Kryptofix $K_{2.2.2}$ (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-[8.8.8]-hexacosane) were purchased from Aldrich.

Accell plus QMA carbonate light cartridges were obtained from ABX or Waters and used as received.

Alumina N cartridges (Alox N, medium size) were purchased from Macherey-Nagel and washed with 2 × 5 mL of pure water then dried thoroughly before use.

[^{18}O]H_2O ([^{18}O] > 97%) was purchased to Sercon.

Pure H_20 (18.2 MΩ) was produced with a Purelab option Q purification system (Veolia®).

Anhydrous acetonitrile >99.8%, 3-methyl-pentan-3-ol 99%, ethanol (absolute, HPLC grade), methanol (HPLC grade), ethyl acetate, hydrochloric acid, NaOH and sodium acetate were purchased from Aldrich and used as received.

Sodium chloride 0.9% sterile solution was purchased from B BRAUN medical.

Thin layer chromatography was performed on aluminum back coated TLC silica gel $60F_{254}$ plates from Millipore.

HPLC Dionex® U3000 equipped with a DAD detector, a radioactivity detector and a syncronis 250 × 4.6 mm (5 μm) analytical column was used for quality control and specific activity determination.

Radio TLC reader miniGita (Raytest®) was used to determine ^{18}F incorporation in manual fluorination reactions.

Well counter: dose calibrator ISOMED 2010 was purchased from Raytest.

Dry block heater: reacti-therm from Thermo Fisher Scientific® with aluminum block and reacti-vap evaporator connected to nitrogen inlet were used for water evaporation and to carry out manual fluorination reaction.

2.2. Manual synthesis

Manual syntheses were performed in 5 mL V-vials fitted with hole caps and teflon coated septa. A sample of ^{18}F in water (10–50 MBq) was transferred in a 1 mL syringe, counted in well counter and passed through a QMA cartridge. The cartridge was dried with 5 mL of air and

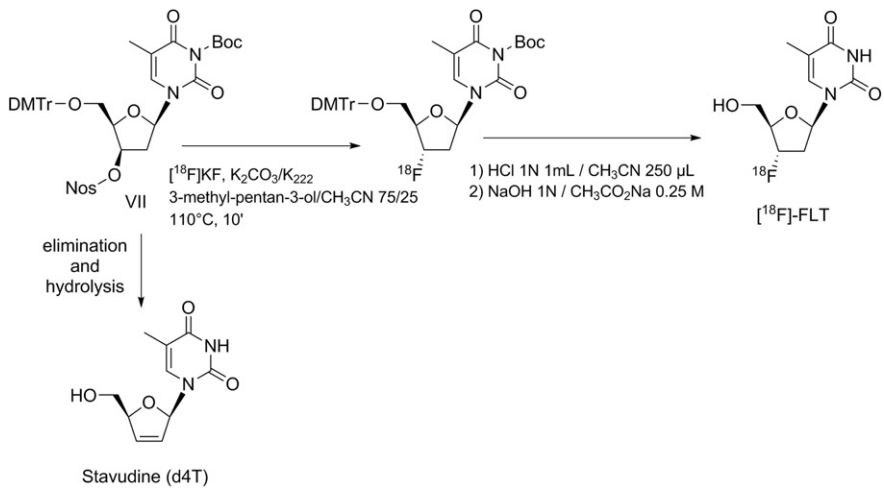

Fig. 2. Radiosynthesis of [^{18}F]FLT and formation of stavudine (d4T).

the radioactivity was eluted with potassium carbonate and K_{222} kryptofix (acetonitrile/water) in a V-vial and counted for determination of eluted activity. The azeotropic solution obtained by iterative addition of acetonitrile (3 × 1 mL) was evaporated in a dry block heater at 100 °C under nitrogen flow. The precursor (5 or 10 mg, solubilized in 750 μL of 3-methyl-pentan-3-ol and 250 μL of acetonitrile) was added and the sealed vial was heated for 15 min at 110 °C. Samples (150 μL) were withdrawn at 5, 10 and 15 min, diluted with methanol and water (50 μL each) and spotted on TLC (eluted with ethyl acetate) for ^{18}F incorporation determination using a minigita radio-TLC reader.

2.3. Automated synthesizer

A Raytest R&D synchrom® dual reactor was used for the radiosynthesis with minor modifications. Only one reactor is used for the synthesis, Al_2O_3 neutral cartridge was inserted between valve C1 and D4, outlet of the pump and vents were connected to gas bags to avoid any radioactive releases in the hot cell ventilation system, 3 additional valves (G3-G5) and a 20 mL vial were added to measure the ^{18}F activity transferred from the target (red rectangle on Fig. 3). The system is equipped with a semi preparative HPLC (Knauer) including an isocratic pump, a 254 nm fixed wavelength UV detector, a radioactivity detector and a 5 mL stainless steel injection loop. Purification was done on a syncronis 250 × 10 mm (5 μm) semi-preparative column at 3 mL/min.

According to the above diagram, the following workflow was used for automated [^{18}F]FLT production using 5 mg or 10 mg of precursor.

Step 1: **Nucleophilic fluorination**

(i) [^{18}F]Fluorine from target vial transferred through a QMA cartridge on the module and water recovered in 10 mL V-vial.
(ii) [^{18}F]Fluorine eluted into reactor 1 with 12–15 mg of K_{222}, 0.59 mg of K_2CO_3 in 800 μL of acetonitrile and 380 μL of water (SC1).
(iii) [^{18}F]KF dried under reduced pressure and Argon flow at 90 °C with successive addition of acetonitrile (SC2, 1.7 mL).
(iv) The precursor 3-*N*-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine (5 mg) dissolved in 250 μL of acetonitrile and 750 μL of 3-methyl-pentan-3-ol (SC3) is added into the reactor.
(v) Fluorination is carried out at 110–112 °C during 15 min.

Step 2: **Acidic hydrolysis**

(i) The reactor is cooled down to 80 °C.
(ii) The solvents are evaporated under reduced pressure and argon flow.
(iii) 250 μL of acetonitrile (SC5) is added to the reactor.
(iv) 1 mL of HCl 1 M (SC4) added to the reactor.
(v) Reactor heated at 90 °C during 10 min. The pale yellow/colorless solution turns to orange progressively (formation of the dimethoxytrityl cation).

Step 3: **Neutralization**

(i) Reactor cooled down to 60 °C.
(ii) Addition of NaOH 1 N/AcONa 0.25 M (1.2 mL) and 2.85 mL of H_2O (SC6).

Step 4: **HPLC purification**

(i) Reaction mixture transferred to the HPLC loop (5 mL) via an Alox neutral cartridge.
(ii) Purification of the crude mixture by HPLC semi-preparative at 3 mL/min. Using 0.9% NaCl/EtOH (92/8 v/v).
(iii) [^{18}F]FLT eluted at R_t = 24 min and collected in a vial or in reactor 2 (average collected volume 7.5 mL).
(iv) HPLC eluent changed (manually) to MeOH/CH_3CN/H_2O 20/20/60 to clean the column.

2.4. Production of [^{18}F]F^-

An ACSI 24 MeV® cyclotron was used for ^{18}F production on a small volume target. Irradiation of [^{18}O]H_2O (1 mL) was performed at 16.5 MeV, 30 μA during 15 min to produce a typical dose of 19 GBq (EOB). After 5 min of cooling the radioactivity is transferred to the hot cell under He pressure and the target is rinsed twice (2 × 1 mL) with pure water. Total transferred activity is counted (well counter) and sent into the Raytest module under He pressure. Residual activity in

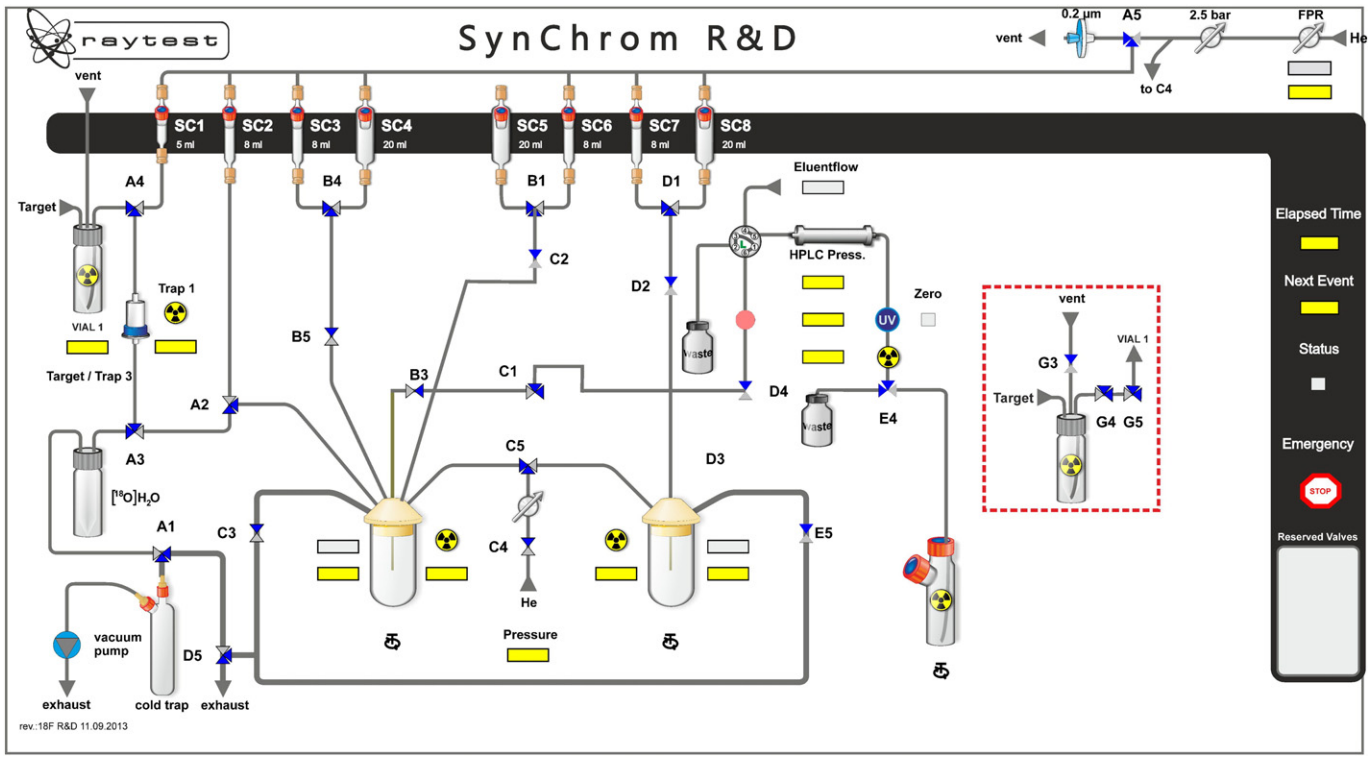

Fig. 3. Synchrom R&D layout for [^{18}F]FLT production.

the intermediate vial after transfer is counted. Starting from 19 GBq (EOB), 16 GBq was transferred to the synthesizer for [^{18}F]FLT synthesis.

2.5. Quality control and specific activity determination for preclinical use

Purified [^{18}F]FLT was injected on analytical HPLC (25 μL) for determination of chemical and radiochemical purities. A second injection with the non-radioactive authentic reference FLT (3′-deoxy-3′-fluorothymidine) was done to assay the identity of the radioactive compound. The column was eluted with a gradient of acetonitrile in water (H_2O/CH_3CN 90/10 to 80/20 over 10 min then 80/20 to 70/30 over 15 min.) at 0.7 mL/min and UV detection was performed at 265 nm.

The specific activity was determined by injection on analytical HPLC of a known radioactive dose of [^{18}F]FLT (0.7 to 1.5 MBq) and quantification of the amount of FLT by reporting the UV signal area on a calibration curve ($R^2 = 0.999$).

The pH of the solution was determined by applying a drop of the purified solution onto a pH indicator strip and comparing the result with the provided scale.

The volumic activity (MBq/mL) at the end of synthesis was determined by measuring the activity of a known volume of purified [^{18}F]FLT (25 to 50 μL).

3. Results and discussion

3.1. Manual synthesis, QMA elution

During the synthesis of [^{18}F]FLT, one main problem encountered is the base mediated elimination of the nosylate, leading to the formation of the stavudine and consuming the precursor. Suehiro et al. [36] investigated carefully the influence of the base amount and highlighted the importance of the precursor to base ratio on the ^{18}F fluorine incorporation during the labeling step. Our first approach to minimize the formation of stavudine was to reduce the amount of base used during the elution of the QMA cartridge. Using commercially available and untreated (used as received) QMA cartridges under carbonate form we were able to elute more than 95% of the radioactivity with low amount of K_2CO_3. Typically, 10 to 50 MBq of [^{18}F]fluorine was adsorbed on QMA cartridges and eluted with a mixture of CH_3CN (800 μL) and water (270 μL) containing 12–15 mg of K_{222} and various amount of K_2CO_3. From 1 mg down to 0.63 mg (7.2 to 4.6 μmol) of K_2CO_3 in 270 μL of water the radioactivity was almost quantitatively eluted (more than 95% recovery of activity); with lower amount (0.59 mg, 4.3 μmol) only 80% of radioactivity was recovered in these conditions. However using a larger amount of water (380 to 400 μL) the QMA was fully eluted (>95%) with as low as 4.3 μmol (0.59 mg) of K_2CO_3 in presence of K_{222} (12–14 mg in 800 μL of CH_3CN).

3.2. Manual synthesis, determination of ^{18}F incorporation

The following step of our evaluation was to perform the radiofluorination of precursor **VII** under various conditions (amount of precursor and base). The solutions eluted from QMA cartridges (see 3.1) were evaporated under N_2 flow at 100 °C with iterative addition of CH_3CN (3 × 1 mL). After drying, the precursor 5 or 10 mg in 1 mL of solvent (3-methyl-pentan-3-ol/CH_3CN 75/25) was added and the vial was heated for 15 min at 110 °C. Samples were withdrawn at 5, 10, and 15 min, diluted and analyzed by radio-TLC (eluted with 100% ethyl acetate, Fig. 4). The precursor to base ratio (P/B in Fig. 5) was critical to achieve high incorporation of the ^{18}F fluorine (Fig. 5). No significant difference in conversion was observed between 10 and 15 min. Better results were obtained when the precursor to base ratio was superior to 1.3; using such conditions 97% and 93% conversions were observed using 10 mg and 5 mg of precursor respectively. Although these results represent the higher incorporation percentages reported so far, it is worth to note that they reflect only the composition of the liquid phase, the radioactivity in solution usually represents 90–95% of the initial amount introduced in the vial (part of it get stuck on the glass wall of the V-vial). We were not able to identify a parameter that could influence positively or negatively this amount of lost radioactivity. One reason might come from the manual drying step that led to some fluctuating binding of ^{18}F fluorine on the glassware.

3.3. Automated synthesis

The above mentioned results prompted us to adapt the manual synthesis on our Raytest automate. We first adapted the conditions using 10 mg of precursor along with 1 mg of K_2CO_3. As the reactor of the synthesizer is suitable for reaction volumes of 1 mL no dilution was necessary and the concentration of the precursor could be kept the same as in manual synthesis. Classical automated sequence was used to transfer the radioactivity into the reactor and to dry the [^{18}F]-KF. The

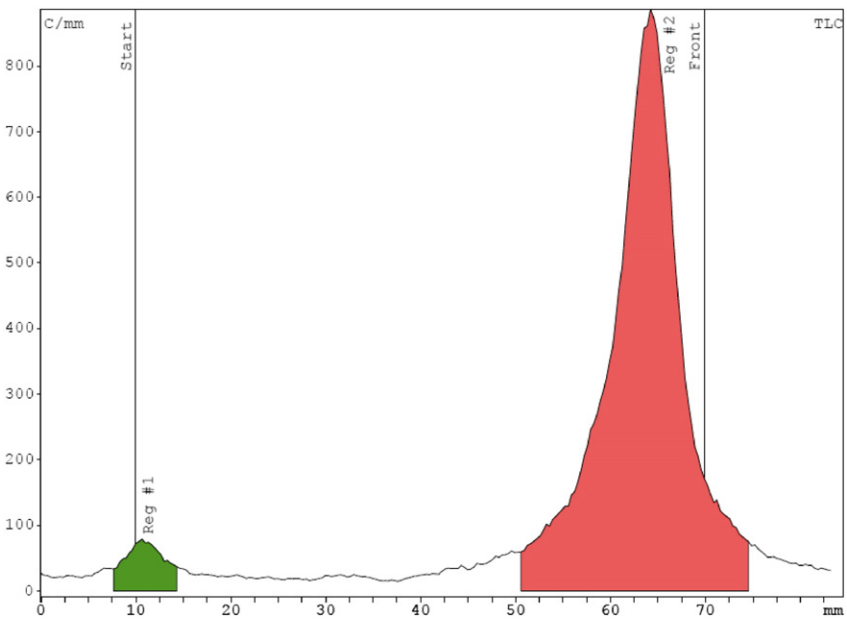

Fig. 4. Example of radio TLC obtained with 10 mg of precursor, 1 mg of K_2CO_3 and 13 mg of K_{222} at 110 °C after 10 min.

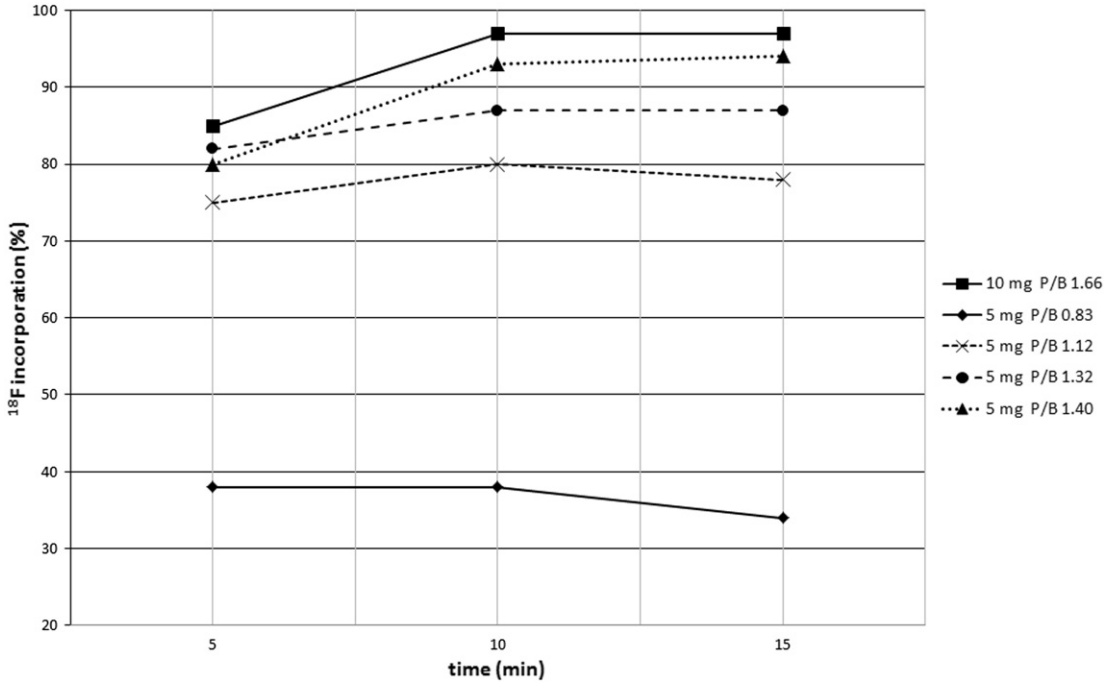

Fig. 5. ^{18}F incorporation over time using various amounts of base and precursor. Reactions carried out at 110 °C in 1 mL of solvent (3-methyl-pentan-3-ol/CH_3CN 75/25). P/B: Precursor (μmol)/Base (μmol).

temperature of the reactor was raised to 110 °C before addition of the precursor dissolved in acetonitrile and 3-metyl-pentan-3-ol. Hindered alcohols are now often used in FLT synthesis to reduce the formation of by-products especially when they are generated by the competitive elimination reaction [32]. Among the potential hindered alcohols we choose 3-methyl-pentan-3-ol for its 123 °C boiling point; in such conditions the reaction can be performed at high temperature without generating an excessive pressure in the reactor. During our synthesis the maximum pressure observed never exceeded 230 KPa (2.3 bars). The other advantage is the easy elimination of the alcohol under reduced pressure, this evaporation step is mandatory as otherwise the deprotection step using 1 M aqueous HCl fails due to the non-miscibility of the reagents (deprotection using other acid, ie CF_3CO_2H was not attempted).

The fluorinations was carried out at 110–112 °C during 15 min and after evaporation 250 μL of acetonitrile was added to the reactor to facilitate the solubilization of the intermediate during the deprotection step. In our classical set-up, CH_3CN and HCl are located in separated solvent containers (SC4 and SC5) but a mixture of CH_3CN and aqueous HCl can also be used in a single solvent container. Addition of a larger volume of CH_3CN considerably impaired the final HPLC purification.

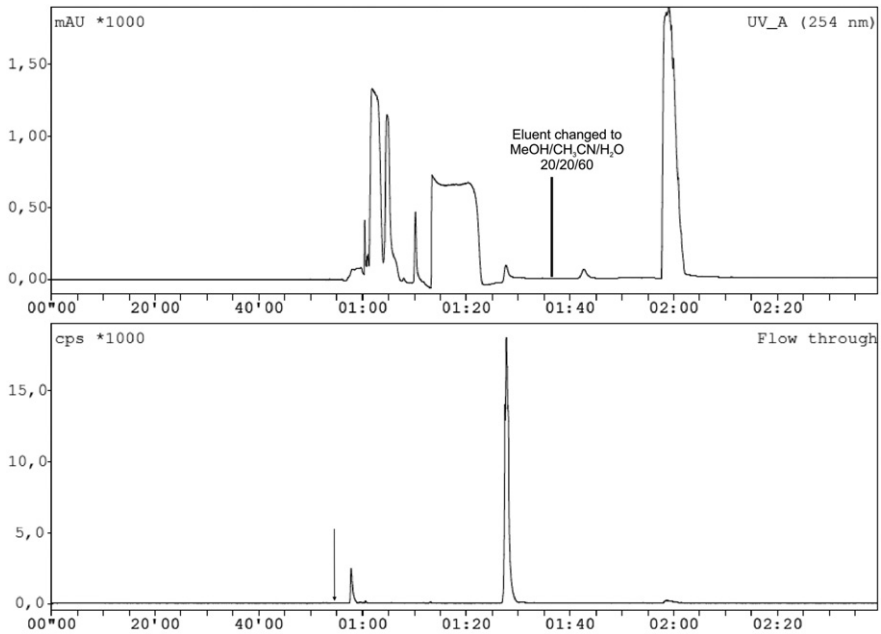

Fig. 6. HPLC chromatogram of crude [^{18}F]FLT during purification step on synthesizer. Top: UV detector. Bottom: radioactive detector. X axis represents time from start of the module at the beginning of the synthesis, black arrow on radioactive channel denotes the injection time on semi-preparative HPLC.

Table 2
Comparison of decay corrected yields of [^{18}F]FLT.

Ref.	Precursor[a]	Precursor mg	Fluorination %	[^{18}F]FLT % (DC)
[33]	12 μmol	10 mg	48	25
[35]	12 μmol	10 mg	53	ND
[40]	12 μmol	10 mg	27	ND
Our Work	**12 μmol**	**10 mg**	**97**	**56**
[36]	11 μmol	9.2 mg	15	0.6
[36]	9.6 μmol	8 mg	64	27
[33]	6 μmol	5 mg	61	30
[35]	6 μmol	5 mg	32	ND
[39]	6 μmol	5 mg	91	ND
Our Work	**6 μmol**	**5 mg**	**93**	**54**

[a] All entries refer to the same precursor (**VII**, 5′-*O*-DMTr-2′-deoxy-3′-*O*-nosyl-β-D-threo-pentofuranosyl-3-*N*-BOC-thymine) except entry 5, Ref. [36] using 11 μmol (9.2 mg) of precursor **V**, 5′-*O*-DMTr-2′-deoxy-3′-*O*-nosyl-β-D-threo-pentofuranosyl-thymine.

Deprotection was carried out at 90 °C during 10 min, the reactor was cooled down and the reaction was neutralized using NaOH 1 N with 0.25 M CH_3CO_2Na. The crude product was then passed through an alumina neutral cartridge and directly sent into the HPLC loop for semi-preparative purification on a syncronis column. Pure [^{18}F]FLT was collected at 24 min (Fig. 6, chromatogram of semi-preparative HPLC) after injection and ready to use (already formulated in NaCl 0.9% with 8% ethanol), the average yield (decay corrected) was 56% (±5%, n = 5). Starting from 16 GBq we obtained a ready to inject solution of 7.5 mL with a [^{18}F]FLT concentration greater than 500 MBq/mL. The synthesis time was 52 min without purification and 81 min from start of synthesis to the final collect of the purified product (HPLC included).

With 5 mg of precursor the automated synthesis required only minor modifications; the QMA cartridge is eluted with a solution containing 12–14 mg of K_{222}, 0.59 mg of K_2CO_3 in 800 μL of CH_3CN and 380 μL of H_2O. As the amount of water eluted from QMA was more important, 1 min of drying was added to the sequence. The remaining of the synthesis remained unchanged. Using such a low amount of precursor, [^{18}F]FLT was obtained after purification in 54% (n = 4, min. 45% max. 60%) decay corrected yield.

Only few percent of yield difference were observed during our synthesis when 5 mg was used instead of 10 mg. When compared to recently published radiosynthesis using 10 mg or even 5 mg of precursor (Table 2) our automated synthesis achieved high yields of pure [^{18}F]FLT.

3.4. Quality control and specific activity.

A quality control for pre-clinical use was performed. The results demonstrated a chemical and radiochemical purity above 95% (Fig. 7) and co-injection of authentic FLT proved the identity of the produced [^{18}F]FLT (R_t = 12.4 min).

The pH of the solution ranged from 6 to 7 and the volumic activity was superior to 500 MBq/mL for each batch (n = 9) with a specific activity of 35 to 72 GBq/μmol.

No stavudine could be detected by HPLC in the final product (R_t = 9.1 min for the stavudine under same analytical conditions).

4. Conclusion

Combining a low amount of base and hindered alcohol as solvent, we synthesized [^{18}F]FLT in high yield (up to 60%) on an automated module. The one pot, two steps, radiosynthesis can be carried out on any single reactor synthesizer without any modification and using only commercially and unmodified materials. Under such conditions, as low as 5 mg of precursor can be used facilitating the purification step. The synthesis took 52 min and after semi-preparative HPLC purification [^{18}F]FLT is obtained in a ready to inject solution for preclinical

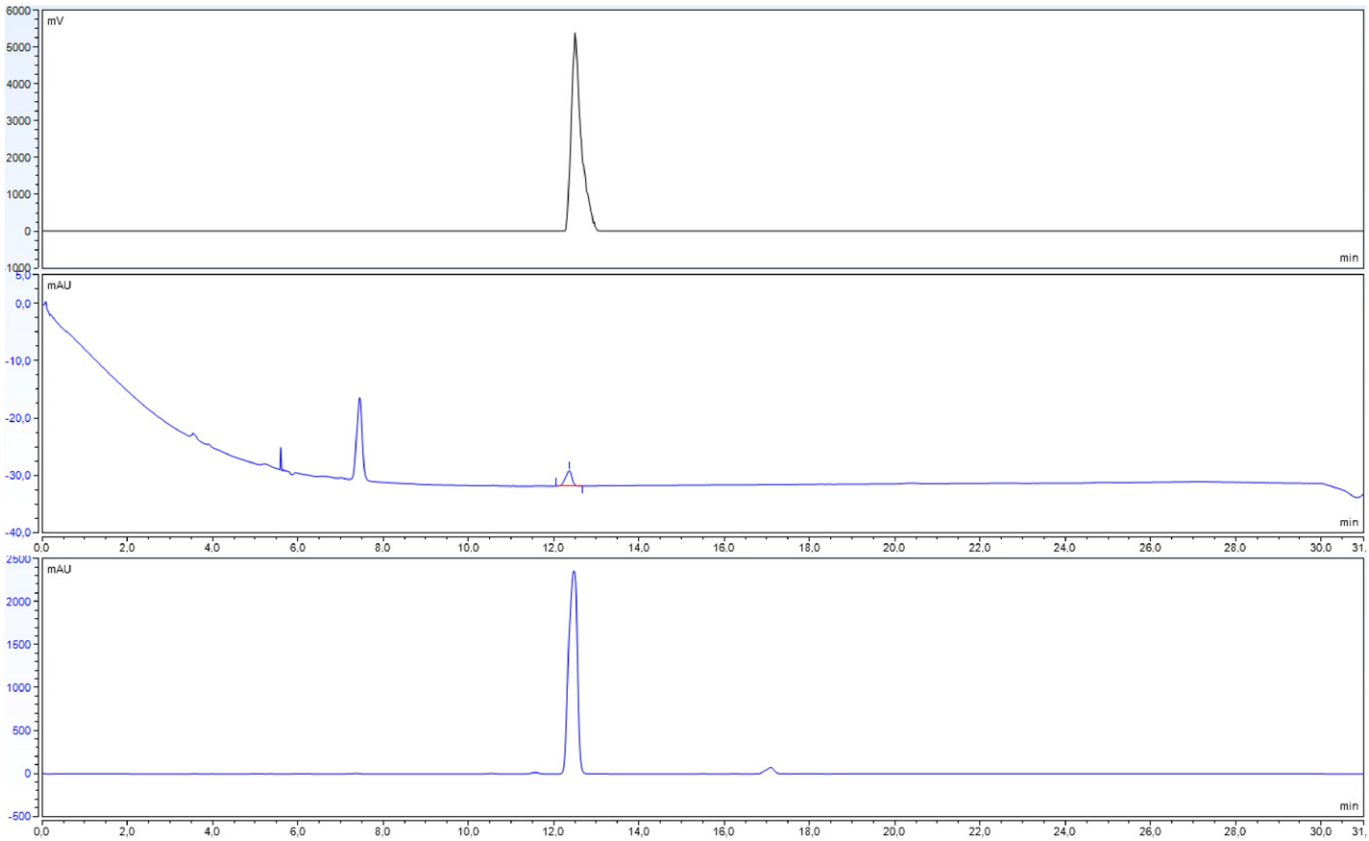

Fig. 7. HPLC chromatograms of purified [^{18}F]FLT. Top: radioactive channel. Middle: UV channel 265 nm. Bottom UV channel 265 nm co-injected with authentic FLT reference. In the same condition Stavudine (d4T) has a retention time of 9.1 min.

imaging. The volumic activity was greater than 500 MBq/mL for every batch enabling the use of the solution during 4 h for preclinical imaging and ensuring a low amount of ethanol injected. The method seems highly suitable for other base sensitive compounds prone to fast elimination reaction.

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