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Automated production of [^{18}F]FDDNP using a TRACERlab MX$_{FDG}$

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[^{18}F]FDDNP has been recently described as a potent tracer to image amyloid plaques *in vivo* by positron emission tomography. Such a tool will be advisable to diagnose patient with mild cognitive impairment, to follow the disease progression and to evaluate new therapies. To make this radiopharmacetical affordable for the clinicians, we developed an automated method for [^{18}F]FDDNP radiosynthesis using a commercial [^{18}F]FDG unit. Radiolabeling with fluorine-18 was carried out by a [^{18}F]fluoro-detosylation reaction on the precursor 2-(1-{6-[(2-tosyloxyethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile. The reaction was performed in acetonitrile for 15 min at 90°C, and then the reaction mixture was injected into a semi-preparative high-pressure liquid chromatography. The desire [^{18}F]FDDNP fraction was collected, and an SPE was performed. The [^{18}F]FDDNP was formulated in a sodium chloride/ethanol solution followed by a sterile filtration. Stability of [^{18}F]FDDNP was studied after 4 h and radiochemical purity of [^{18}F]FDDNP remained $>$98%.

The overall decay-corrected radiochemical yield was 15 $\pm$ 3% (n = 8). Radiochemical purity was $>$98% and the specific activity was 164 $\pm$ 25 GBq/μmol at EOS. Pharmaceutical controls, bioburden, sterility, bacterial endotoxin and residual solvent tests were performed. The results were in accordance with the European Pharmacopoeia and demonstrated our ability to produce [^{18}F]FDDNP with a pharmaceutical grade and a high reproducibility.

Keywords: [^{18}F]FDDNP; amyloid plaques; Alzheimer's disease (AD); radiopharmaceutical; TRACERlab MXFDG

Introduction

Alzheimer disease (AD) is a disease that affects several million people around the world. Its prevalence increase due to the fact that age is one of the major risks in the evolution of the disease.[1] Postmortem studies shown that senile plaques and neurofibrillary tangles are abundant in the brain.[2] The major constituent of senile plaques is fibrils formed by β-amyloid (Aβ, 1-40/42),[3] resulting from the amyloid precursor protein (APP), and their presence is correlated with the evolution of AD.[4] More than the monomer β-amyloid, their aggregation in β-plated sheet structure resulting in the formation of deposits in the brain appears to be correlated with the AD progression.[5]

Numerous ligands have been developed to determine the presence of these deposits with the aim to get suitable imaging agents. The first generation of ligands was constituted by compounds with a high ionization potential such as Congo Red or Thioflavin T. These ligands although still used as staining agents are not suitable for *in vivo* imaging. Owing to their ionization potential these molecules cannot cross the blood–brain barrier. Benzothiazole ligands derived from Thioflavin T, and other family compounds were studied such as benzofurane, stillbene, styrylbenzene, naphthalene, have been evaluated with the aim to get a suitable *in vivo* imaging agent for positron emission tomography (PET) or single photon emission computed tomography (SPECT). Among the developed compounds the derivatives presenting the highest potential are the [^{11}C]PIB[6] (benzothiazole), [^{11}C]SB13[7] and [^{18}F] F-BAY94-9172[8] (stillbene) and [^{18}F]FDDNP.[9] These four compounds have been investigated in patients showing their ability to image *in vivo* amyloid plaques. The half-life of fluorine-18 (110 min) allows [^{18}F]fluorinated ligands to be used at distance from the production site and makes [^{18}F]FDDNP[9–11] and [^{18}F] F-BAY94-9172 more attractive imaging agents than ligands labelled with carbon-11 (half-life 20.4 min) such as [^{11}C]PIB[6,7,12,13] and [^{11}C]SB13.

Small and coll.[9] showed that the [^{18}F]FDDNP allows differentiated diagnosis between mild cognitive impairment, AD and healthy patients. As it is radiolabelled with ^{18}F, its production allows multidoses production and multisites distribution for PET examination. [^{18}F]FDDNP appears as a highly potential probe to answer the growing PET examinations request by the geriatric and psychiatric services. We propose and report herein an automated production

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of [^{18}F]FDDNP that can provide doses for several investigations per day using an FDG automate synthesizer.

[^{18}F]FDG is widely and routinely produced on a TRACER Lab MX_{FDG} automate. Production of radiopharmaceuticals, such as [^{18}F]FMISO[14] [^{18}F]FLT,[15] [^{18}F]Fluoroestradiol[16,17] using a MX_{FDG} automate, has been reported. Using this type of process warrants high reliability and reproducibility and will lower the radiation exposure of the operators. Based on the methods described in the literature for the preparation of [^{18}F]FDDNP,[18,19] we adapted the [^{18}F]FDDNP synthesis on the TRACER Lab MX_{FDG} module with some modifications of the sequence and materials, used for the [^{18}F]FDG preparation, to provide an injectable solutions of [^{18}F]FDDNP.

Results and discussion

We developed an automated synthetic method of [^{18}F]FDDNP, a highly potent radiotracer in Alzheimer diagnosis, using the commercial TRACERlab MX_{FDG} synthesizer. Components of the synthesizer cassette were modified and a specific sequence developed to be able to produce [^{18}F]FDDNP with this unit.

We prepared cold FDDNP and its tosylate precursor with similar yields as those obtained by Liu and coll.[18] Stability of the precursor was checked for several months to assume that no degradation occurred in the produced batch (data not shown).

Low amount of precursor (2–2.5 mg) were used to perform the radiosynthesis of [^{18}F]FDDNP and the nucleophilic substitution with K^{18}F occurred at 95°C during 15 min. In these conditions moderate radiochemical yields were obtained (12 ± 3%, decay corrected). The yields are lower compared with the methods described by Liu[18] and Klok,[19] and the difference can be explained by the fact that both teams used modules able to work with small reactor, using reduced amount of solvent (less than 1 mL of acetonitrile) whereas using an tracer Lab FDG module requires higher volume, 3 mL at least. The use of low amount of precursor allowed us to get high resolution during high-pressure liquid chromatography (HPLC) purification and to have better efficiency. In these conditions, [^{18}F]FDDNP eluted from the column after a retention time of 20 min, retention time was highly reproducible. The moderate yields may also be explained by sticking of the [^{18}F]FDDNP on the filter during sterile filtration and by the presence of numerous radiolabelled by-products. In these conditions, by-products represent around 15% of the radioactivity injected into the HPLC, among these 1-{6-[(2-[^{18}F]Fluoroethyl)(methyl)amino]-2-naphthyl}ethan-1-one or [^{18}F]FENE has been identified as a by-product of the [^{18}F]FDDNP preparation.[20]

The chemical nature of [^{18}F]FDDNP was confirmed by way of HPLC co-injection with its corresponding 19F standard (Figure 3). As it has been described in literature, the stability of [^{18}F]FDDNP remains a problem. In our case, using a sodium chloride and ethanol solution stored at 4°C, we did not observe radiolabelled by-products resulting in degradation of the [^{18}F]FDDNP. Nevertheless, we observed, as reported by Klok and coll,[19] a decomposition of the [^{18}F]FDDNP when left at room temperature to a more polar compound than the parent [^{18}F]FDDNP. With our process and conditions, the chemical purity was >95% and the radiochemical purity of [^{18}F]FDDNP remained >98% up to 4 h after preparation. The specific activity of the preparations was 164 ± 25 GBq/μmol, range of values that is similar to those obtained by Liu and coll.[18]

Bioburden and sterility results showed that no microbiological contamination occurred during the process. Residual solvents analyses, regarding International Conference on Harmonization guidelines, performed by GC, demonstrated that ethanol concentration was below 100 mg/mL of injectable solution, that less than 410 ppm of acetonitrile was found in the injectable doses. The colour spot test excluded the presence of Kryptofix2.2.2 in the final doses. The pH of the injectable dose was in the 6.1–6.4 range.

The total preparation time of [^{18}F]FDDNP was 100 ± 5 min. The preparation time can be splitted into three main parts, the radiolabelling on the synthesizer, 40 min; the purification step, 28 min and the remaining time for the formulation and dispensing process.

Materials and methods

Reagents and equipment

The precursor, 2-(1-{6-[(2-tosyloxyoethyl)(methyl)amino]-2-naphthyl}ethylidene)-malononitrile, and the reference compound, FDDNP, were synthesized in accordance with the literature and with information from the UCLA group.[18] Precursor was stored in dark vials under Argon at 4°C and its stability was checked by HPLC over 1 year. Solvents and reagents were purchased from Sigma-Aldrich and ABX GmbH.

Purification of the crude [^{18}F]FDDNP was performed by HPLC system consisting in a pump (Series 200, Perkin Elmer), a UV detector (Series 200, Perkin Elmer) and a NaI (Tl) radioactive detector (FC-1000F, Bioscan). HPLC quality control was performed on a system equipped with in a pump (P680, Dionex, USA), a UV detector (UV170, Dionex, USA) and a BGO radioactive detector (PET metabolite detector system, Bioscan).

Synthesis of [^{18}F]FDDNP using the conventional MX_{FDG} unit

In many centres, TRACERlab MX_{FDG} is daily used for the routine production of injectable solution of [^{18}F]FDG. The unit requires single-use cassette and can be easily manipulated for [^{18}F]FDG preparation without an important background in chemistry or programming. In our case, a TRACERlab MX_{FDG} was modified to allow the preparation of [^{18}F]FDDNP on this automate.

The preparation of [^{18}F]FDDNP includes three main steps: (a) fluorination of the precursor, (b) HPLC purification and (c) formulation (Figure 1). The first step was performed on TRACERlab MX_{FDG}. The diagram of the [^{18}F]FDDNP preparation system is shown in Table 1.

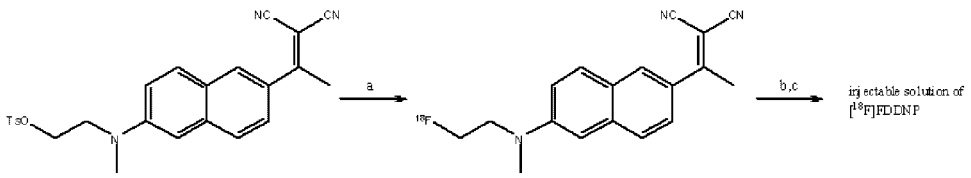

Figure 1. Schematic diagram of preparation of an injectable solution of [^{18}F]FDDNP. (a) radiosynthesis, (b) HPLC purification, (c) formulation, sterile filtration and dispensing.

Preparation of reagents and cassette (Figure 2)

FDG synthesis on the MX_{FDG} proceeds in a sterile single-use cassette provided with an alumina N and two *t*C18 Sep-Pak®. For the [^{18}F]FDDNP radiosynthesis, following modifications on the cassette were performed: removal of the alumina at position 11 on the cartridge and the *t*C18 Sep-Pak® linking connections 12 and 13. The diagram on Figure 3 depicts the cartridge set-up for [^{18}F]FDDNP radiosynthesis. Vial A contains a solution of Kryptofix 2.2.2 (22 mg) and potassium carbonate (7 mg) in 0.6 mL of a mixture acetonitrile-water, 50:50. Vial B is filled with anhydrous acetonitrile (7 mL). Vial C contains the tosyl precursor of FDDNP (2-2.5 mg). The syringe D is filled with 1.5 mL of acetonitrile. Vials A and B with their content were provided by ABX and are identical to those used for FDG synthesis. Connection 12 is used to link the cassette to the HPLC loop.

Table 1. Summary of the main steps to get injectable doses of [^{18}F]FDDNP

A. Transfer of [^{18}F]fluorine to the TRACERlab MX_{FDG}
B. Addition of Carbonate/Kryptofix mixture from vial A to elute [^{18}F]fluoride from QMA cartrdige
C. Drying of [^{18}F]fluoride with acetonitrile
D. Dilution of the precursor with acetonitrile and transfer to the reaction vessel
E. [^{18}F]fluorination at 95°C for 15 min
F. Dilution of the solution in water (syringe 1) and elution through the tC18
G. Rinsing of the reaction vessel with water and elution through the tC18
H. Rinsing of the tC18 with water
I. Elution of [^{18}F]FDDNP with ethanol and transfer of the solution to the HPLC loop
J. HPLC Purification: acetonitrile/ammonium acetate 0.1 M: 55/45, 5 mL/min, 254 nm
K. Collection [^{18}F]FDDNP using THREE-way valve (retention time: 20–22 min.)
L. Dilution with water follow by a Sep Pack
M. Elution with ethanol and dilution with 0.9% NaCl solution
N. Sterile filtration—Filling vials

Synthesis of [^{18}F]FDDNP

The bombardment of 98% ^{18}O-enriched H_2O solution (Rotem) with 14.5 MeV protons (Thales cyclotron, Colombes, France) produced a no-carrier-added [^{18}F]fluoride ion solution (1.85 GBq/μA/h). The [^{18}F] aqueous fluoride solution was transferred to the module and passed through the activated QMA cartridge. After trapping of the [^{18}F]fluoride ion on the QMA, [^{18}F]fluoride was eluted by the potassium carbonate solution from vial A into the reaction vessel. For activation of the [^{18}F]fluoride ion, water was removed by heating at 95°C and azeotropic drying by adding 0.3 mL of acetonitrile from vial B. The precursor in vial C was dissolved in 3 mL of acetonitrile from vial B and the solution was sucked into the reaction vessel under vacuum. The fluorination was carried out at 95°C for 15 min. The solution was then transferred to syringe 1 containing enough water to dilute acetonitrile to 10% of the mixture. The mixture was then eluted through the activated tC18 cartridge. Additional water

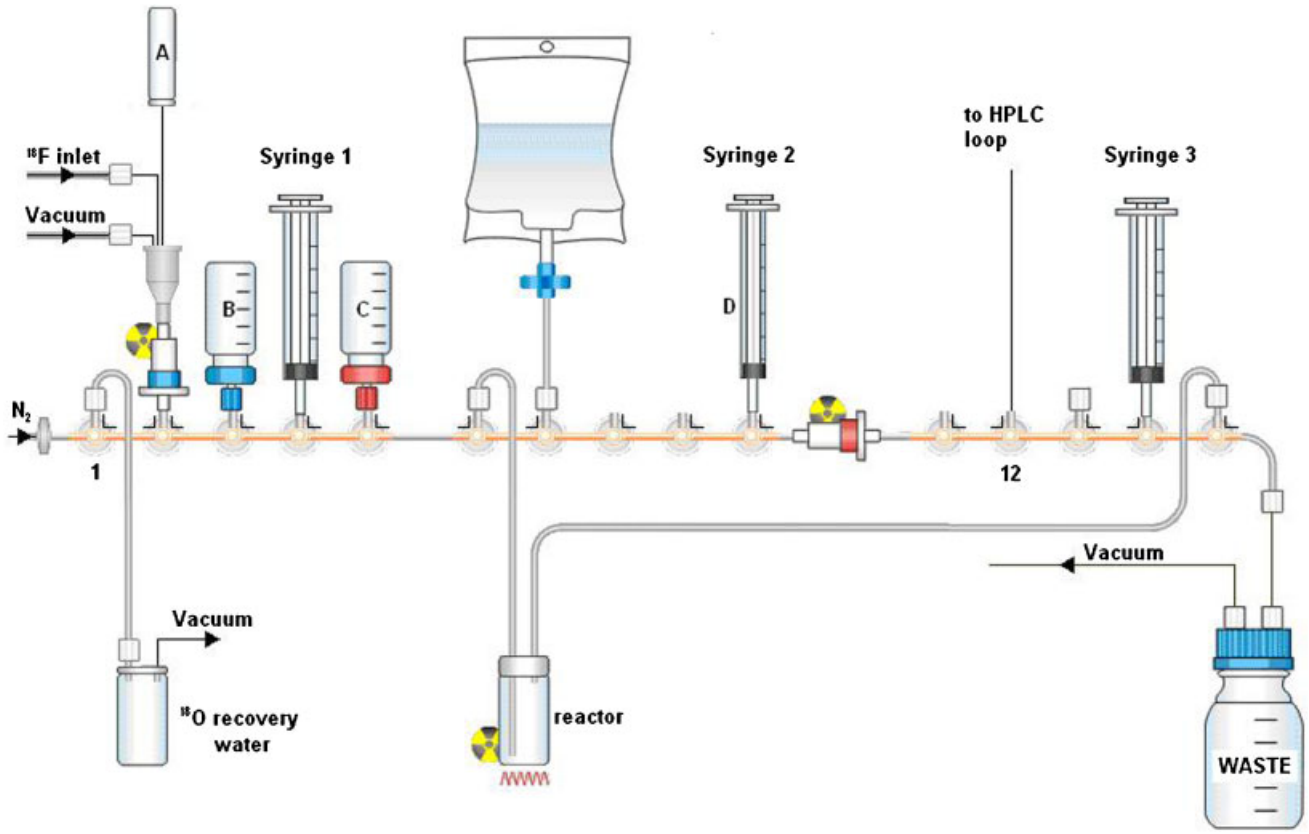

Figure 2. Diagram of a disposable cassette for the preparation of the automated production of [^{18}F]FDDNP. A: potassium carbonate/Kryptofix solution; B: acetonitrile; C: precursor; D: acetonitrile.

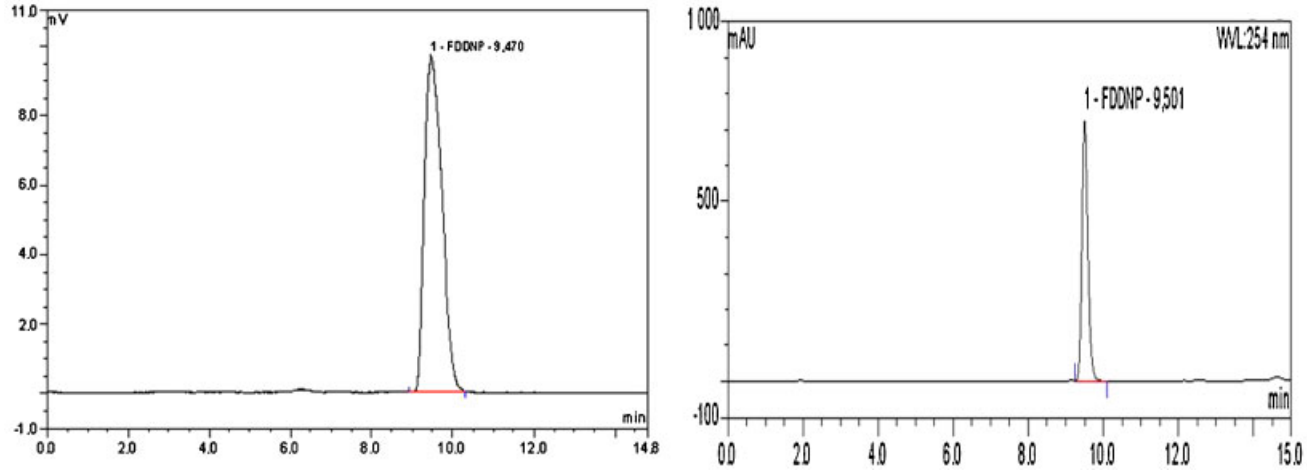

Figure 3. QC HPLC analysis of the HPLC formulated [^{18}F]FDDNP solution. Left: radioactivity trace, Right: UV(254 nm) signal for a co-injection of cold FDDNP solution (10 L of 2 mg/10 mL and 10 L of the formulated [^{18}F]FDDNP preparation). The retention time of [^{18}F]FDDNP was 9.5 min.

was used to rinse the reaction vessel and the tC18 cartridge. Crude [^{18}F]FDDNP was eluted from the cartridge using acetonitrile in syringe at the D position and loaded into HPLC injector loop using the 12 position connector on the cassette.

The HPLC purification and the formulation steps

The HPLC purification was performed using a C18 Alltima column (Alltech, 5 μm, 250 × 10 mm), eluted with acetonitrile/ammonium acetate 0.1 M:55/45 at a flow rate of 5 mL/min. The radioactive fraction of [^{18}F]FDDNP was collected (≈5 mL) in flask containing 25 mL of water (Rt ≈ 20 min). The solution was passed through an activated tC18 cartridge, which was rinsed with water (10 mL). [^{18}F]FDDNP was eluted from the cartridge with 2 mL of injectable ethanol, diluted with 20 mL of a 0.9% NaCl solution and the solution was passed through a sterile 0.22 μm filter (Millex-GS, Millipore, MA) to a pyrogen-free vial to get the final injectable doses.

Quality control and stability

The chemical and radiochemical purity of final product for clinical use were determined by HPLC analysis using a Luna Phenyl-Hexyl column (4.6 mm × 250 mm, Phenomenex, USA) eluted with acetonitrile/ammonium acetate 0.1 M: 65/35 at a flow rate of 1.0 mL/min.

Pyrogen test was carried out using a LAL Cambrex method (ACM Pharma, Orléans, France). The sterility and Bioburden tests were performed (ACM Pharma, Orléans, France). K_{222} detection test was performed using the method described by Mock. The residual organic solvent test in the final product was using a gas chromatography method following the European Pharmacopoeia (01/2005: no. 50400, pp. 541–549). The system is a Varian CP3800 equipped with a Varian column (CP select 624 CB, 30 m, 0.32 mm ID, 1.8 μm film) and a Chromeleon (Dionex) software. The injection was performed at 180° (split 1/20) and a FID detector (250°, range 12, attn 1) was used.

The K_{222} and residual solvents tests were performed by Cyclopharma (Saint Beauzire, France). The K_{222} test was performed following the European Pharmacopeia guidelines for injectable solution of (^{18}F) Fluorodesoxyglucose ([^{18}F]FDG) (01/2005: no. 1325, pp. 822–825).

The stability of the injectable solution was checked by HPLC up to 4 h after end of preparation (t_0) storage at 4°C.

Conclusion

We developed an automated method for production of injectable doses of [^{18}F]FDDNP. This method was carried out on a commercial FDG synthesizer, TRACERlab MX_{FDG}, with some modifications of the cassette and operation program of the controller unit. This automated process warrants safety of the operators and provides pharmaceutical grade [^{18}F]FDDNP for diagnosis of AD patients.

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