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Automatic Synthesis of [^{18}F]Altanserin, a Radiopharmaceutical for Positron Emission Tomographic Studies of the Serotonergic Type-2 Receptors

Michel Monclus, BS, John Van Naemen, Ir, Eric Mulleneers, Philippe Damhaut, PhD, Andre Luxen, PhD, Serge Goldman, MD

PET/Biomedical Cyclotron Unit, ULB Hôpital Erasme, Brussels, Belgium

Abstract

[^{18}F]Altanserin is routinely used in several centers to study the serotonergic type-2 receptors ($5HT_2$) with positron emission tomography (PET). An automatic production system allowing the preparation of multimillicurie amounts [>1.5 GBq (40 mCi) EOS, mean radiochemical yield 20 ± 6% EOB, specific activity >1 Ci/fimol (mean = 2.8 Ci/ μmol), n = 50] of this radiopharmaceutical within a synthesis time of 90 minutes (quality controls included) is described in this paper. The apparatus includes the recovery of the activity from the target, the preparation of the dried [^{18}F]KF/kryptofix 2.2.2 complex, the labeling reaction using a microwave cavity, the Sep Pak and HPLC purification. A sterile, pyrogen-free and single use unit was also developed for the formulation of the injectable solution. This last part could be used for the formulation of many other radiopharmaceuticals.

Key Words: [^{18}F]altanserin; automatic synthesis; positron emission tomography; $5HT_2$ receptors.

Introduction

Serotonergic mechanisms are implicated in behaviors and psychiatric disorders such as affective disorders, suicide, or obsessive-compulsive disorder. [1] The distribution of the serotonergic type-2 receptors ($5HT_2$) in the human brain has already been studied *in vivo* using several positron emission tomography (PET) radiopharmaceuticals. [2-7] Among these fluorine-18 and carbon-11 labeled compounds, [^{18}F]altanserin presents high specificity and selectivity for $5HT_2$ receptors.[8,9] These properties are now used to investigate the serotonergic system with PET.[10-13]
The first requirement for this radiopharmaceutical to become routinely used is the availability of an efficient, reliable, and safe radiosynthesis unit that allows the production of multimillicurie amounts of labeled compounds within a synthesis time compatible with the half-life of fluorine-18 (109.6 minutes). Several strategies have already been developed to produce large amounts of radiopharmaceutical under safe conditions. [14] Brihaye and coworkers [15] have proposed a robot-assisted synthesis of various [^{18}F]fluorinated compounds. This system using a Zymate II Laboratory Robot allows the production of [^{18}F]altanserin starting from the recovery of fluorine-18 from the target to the formulation of the injectable solution (radiochemical yield = 25% EOB, 120 minutes). The same unit can also be used to prepare other radiopharmaceuticals (4-[^{18}F]fluorotropapride, 6-[^{18}F]fluoro-L-dopa. 2-[^{18}F]fluoro-L-tyrosine) or labeled key intermediates ([^{18}F]fluorinated benzaldehyde or benzyl iodide). The major drawbacks of such a strategy are the high cost of the system and the great shielded space (150 X 175 cm) needed to install the robot. To overcome these constraints, a synthesis box is usually cheaper and more compact. Another strategy has been proposed by Tan et al.[16] who describe a remote-control system including a domestic microwave oven (drying and labeling), a HPLC purification unit, and a formulation apparatus. The radiochemical yield and synthesis time are 23 ± 3.8% and 113 ± 6 minutes, respectively (n = 15). We describe here an automatic radiosynthesis unit dedicated to the production of [^{18}F]altanserin (from [^{18}F]F^- in the target to the injectable solution). The chemical synthesis involves a nucleophilic substitution on the nitroaltanserin precursor in a microwave cavity, followed by a Sep Pak and HPLC purification. A sterile, pyrogen-free and single-use unit for the formulation of the injectable solution is also presented.

Materials and Methods

General

Kryptofix 2.2.2 [(4,7,13,16,21,24)hexaoxa-1,10-diaza-bicyclo(8.8.8)hexacosan] was purchased from Merck (Darmstaadt, Germany). The gold-label dimethyl sulfoxide (DMSO) and acetonitrile were purchased from

Aldrich (Arlington Heights, IL). Nitroaltanserin was prepared according to the literature. [9,17,18] Altanserin was a gift of Janssen Pharmaceutica (Beerse, Belgium).
The microwave heating system (Pr. Luypaert, KUL, Leuven, Belgium) consisted of a microwave cavity (diameter = 10 cm) connected to the magnetron (2.45 GHz, 0-500 W variable intensity) with a coaxial cable.[19] C18 Sep Pak cartridges (Waters, Milford, MA) were activated with 5 mL of ethanol and 10 mL of sterile water. HPLC purification was carried out using a Lichrosorb RP Select-B column (7 μm, 250 X 25.7 mm) eluted with MeOH/THF/water pH 5 (100 mM AcOH, 100 mM NH_4Ac): 13/32/55 at a constant flow rate of 10 mL/min. The chemical and radiochemical purities and the specific activity of the final product were checked on a Gilson (Middleton, WI) HPLC system using a reverse-phase ODS C18 column (5 μm, 250 X 4.6 mm) eluted with H_2O (28 mM NH_4Ac, 290 mM AcOH)/CH_3CN: 60/40 (pH = 5.37) at a flow rate of 1.5 mL/min. The effluent was monitored with a UV detector (Gilson, 254 nm) and with a NaI scintillation crystal with associated electronics (Canberra, Schwardorf, Austria) for radioactive detection.
Teflon tubing, luer adapters, and flange free tools were purchased from Alltech Associates (Deerfield, IL). The single use, sterile and pyrogen-free three-way valves (valves 14–20) and connecting tubing were available from Baxter (Santa Ana, CA) and B. Braun (Melsugen, Germany), respectively. Telfon valves (valves 1-13, 21-22) were purchased from Resco Trade (Kortrijk, Belgium). Valve 23 (pinch valve) was home made. All valves (except 23) were pneumatically switched (actuator SMC, Antwerpen, Belgium). The liquid transfers were made by use of argon pressure (0.1 bar) or suction (— 880 mbars, ventury, SMC, Antwerpen, Belgium). The various parts of the system were attached to an aluminum support (50 cm wide and 40 cm high).
The small magnetic stirrers (minimum size: 12 X 12 X 5 mm) were build by Variomag and commercially available from Resco Trade.
All valves and HPLC system (including radioactivity detector) were controlled by a Simatic 135U (Siemens, Müchen, Germany).

Production of [^{18}F]Fluoride

The no-carrier-added aqueous [^{18}F] fluoride solution was produced by the $^{18}O(p,n)^{18}F$ reaction in a silver target equipped with a havar window (25 μm). The target material (250 ML of 95% ^{18}O-enriched water) and the activity were delivered directly to the synthesis unit through a 10-m PEEK tubing (od: 1.6 mm, id: 0.8 mm).

Synthesis of [^{18}F]Altanserin

The synthesis of [^{18}F]altanserin were conducted in the unit depicted in Figure 1 and is described step by step below.

[K/222]$^+$ $^{18}F^-$ complex. The activity ([^{18}F]F^-) was recovered from the target in a vial (**G**) containing a mixture of acetonitrile (665 μL) and water (35 μL) with kryptofix 2.2.2 (13 mg) and potassium carbonate (2.3 mg) placed in the microwave cavity. The solvent was evaporated by heating (400 W, 3 minutes) under a helium stream and the complex was dried by addition and evaporation (400 W, 5 minutes) of 1 mL of acetonitrile (**A**).

Labeling reaction. Nitroaltanserin (**B**, 3.6-4.5 mg in 0.9 mL of DMSO) was added and the solution was heated in the microwave cavity (450 W) for 210 seconds.

Sep Pak purification. The reaction mixture was diluted in 30 mL of water (**F**) and passed through a C18 Sep Pak (1 g, Waters). The [^{18}F]altanserin was then eluted in vial **H** (with stirring) with 3 mL of ethanol (**C**) and the cartridge was rinsed with 1.5 mL of water (**D**).

HPLC purification. The HPLC 5 mL loop was filled with the activity. When the volume of the loop was reached (detection switch, Figure 1), the HPLC valve was pneumatically rotated to the INJECT position. The HPLC effluent was controlled by a UV and a radioactivity detector. Information coming from this last detector was analyzed by the Simatic automate, which, after a defined time (20 minutes) and when a defined radioactivity threshold was reached, allowed collection of the effluent in a reserve vial (beginning of the [^{18}F]altanserin peak, valve 12) and then in water (300 mL, vial **I**, valves 13 and 14). Only the second fraction was used.

Formulation of the injectable solution. In order to remove the HPLC solvent, the diluted peak (vial **I**) was passed through a conditioned C18 Sep Pak (100 mg, Waters) both by pressure and suction. The cartridge was rinsed with 10 mL of water (**N**), [^{18}F]altanserin was eluted in vial **K** with 0.7 mL of ethanol (**M**) and diluted through the Sep Pak with 10 mL of 9% NaCl (**L**).

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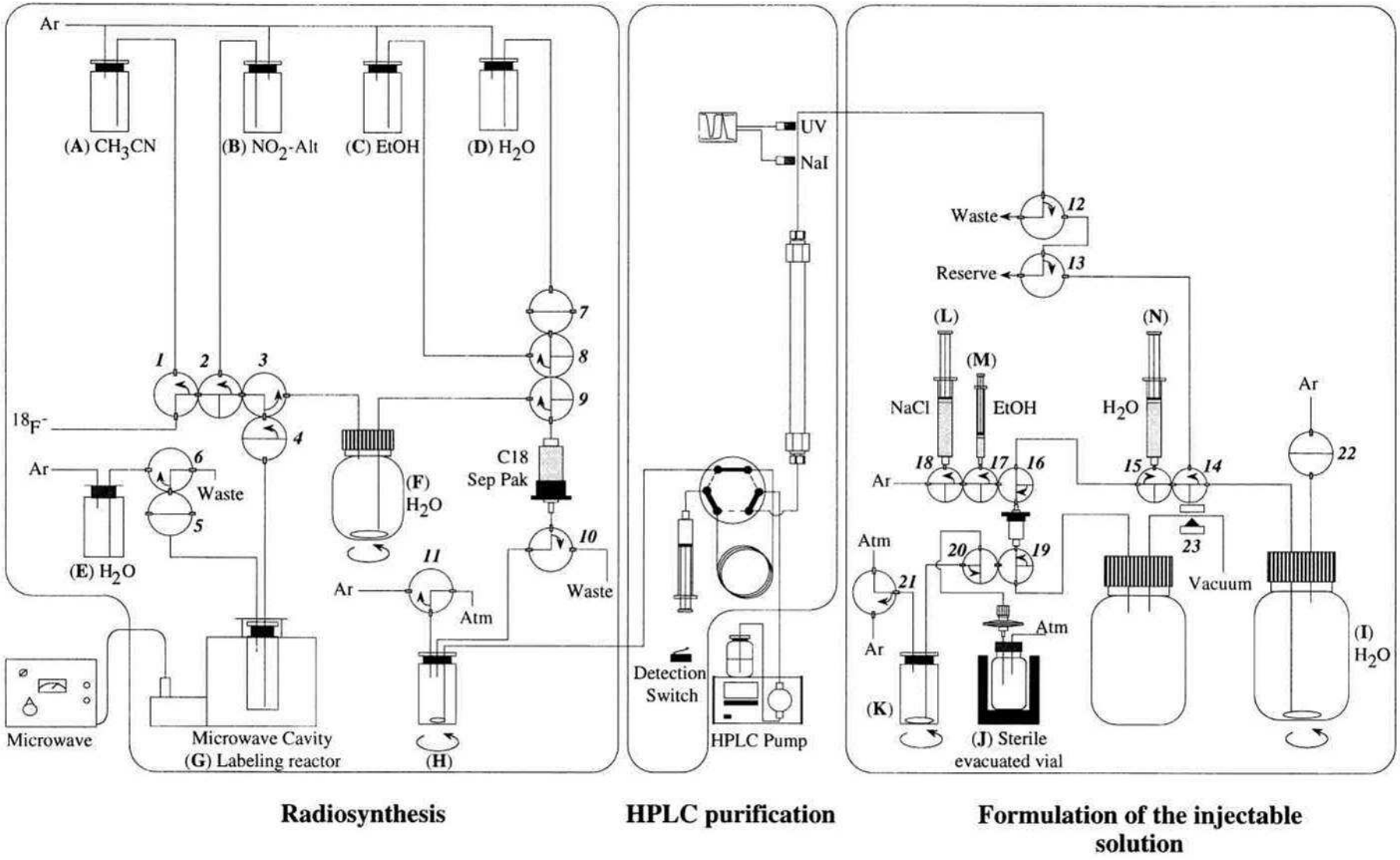

Figure 1. Automatic system for the preparation of an injectable solution of [^{18}F]altanserin (NO_2-Alt = nitroaltanserin).

Addition of these solvent contained in the various syringes was realized using a pneumatic system. The whole mixture was then passed through a 0.22 µm filter (Millex GV, Millipore, Bedford, MA) and collected in a sterile evacuated vial (**J**, final volume = 10.7 mL).

Quality Controls

The chemical and radiochemical (UV and gamma detectors) purities of [^{18}F]altanserin were checked on an analytical reverse-phase HPLC (retention time = 5.56 minutes). The area of the UV absorbency peak of altanserin (254 nm, automatic integrator, Rainin, Woburn, MA) was compared with a calibration curve realized with an authentic sample of altanserin. The value obtained was combined with the total activity produced to obtain the specific activity (Ci/µmol).
The radionuclidic purity was checked by determination of the decay over a 1-hour period.
The chemical, radiochemical, and radionuclidic purities as well as the pH were controlled at each production.
The sterility, the pyrogenicity (LAL), the isotonicity (cryoosmometry), and the presence of residual solvent (methanol and tetrahydrofuran, by gas chromatography using a HayeSep P column, 60/80 mesh, Alltech) were checked periodically.

Washing of the Synthesis Box

After each production, the vials **A, B, C, D, E, F,** and **G** were replaced by new ones containing ether. All the multiple-use parts (valves 1-11 and related tubes) were rinsed using an automatic procedure. This step was reproduced a second time with ethanol and the system was finally dried by flushing with argon. Clean vials were used at each production.

Synoptic of the Automatic Production of [^{18}F]Altanserin

Valves are depicted on Figure 1 in CLOSED position. A typical automatic synthesis is detailed step by step on Table 1.

Results and Discussion

The general chemical pathway applied to the preparation of [^{18}F]altanserin includes a nucleophilic substitution on nitroaltanserin with the kryptofix/K_2CO_3 activated [^{18}F]fluoride anion in DMSO (Figure 2). [9]
Compared to domestic microwave ovens used in systems previously described, [15,16] the major advantages of the microwave cavity described above lies in the low level of by-products appearance during the labeling reaction, which results in a considerably easier HPLC purification. Furthermore, this system can be easily integrated in an automatic synthetic unit. This system provides several advantages in terms of radiochemical yield, synthesis time, and control of the reaction parameters. [20]
The second part of the synthetic box includes a C18 Sep Pak [21] and an HPLC purification. The HPLC effluents are controlled by a UV and a radioactivity detector. Information coming from this last detector are analyzed by the Simatic automate, which, after a defined time and when a defined radioactivity threshold is reached, allows collection of the peak.
The formulation of the injectable solution is realized in a sterile, pyrogen-free and single use unit. The diluted activity is passed through a C18 Sep Pak and eluted with ethanol. Aqueous sodium chloride (0.9%) is added before passing through the 0.22 µm filter in order to avoid any solubilization of the membrane by pure ethanol. Quality controls including chemical, radiochemical (>99%), and radionuclidic purities, specific activity (>1 Ci/µmol, mean = 2.8 Ci/µmol), and pH (ranging from 5.0 to 7.0) demonstrated the efficacy of the system. Sterility, pyrogenicity, and isotonicity of the final solution were good in all batches tested. The concentration of tetrahydrofuran was lower than 5×10^{-5} g/mL. The peak of methanol was under the detection threshold of the system (concentration < 5×10^{-5} g/mL).
[^{18}F]Altanserin is obtained after a synthesis time of 90 minutes (quality controls included, Table 2) with a radiochemical yield of 20 ± 6% (EOB, n = 50) and a specific activity higher than 1 Ci/µmol (EOS). At this level of specific activity, no pharmacological effect of the radiotracer has ever occurred during the numerous PET studies performed and quantification of the binding may be conducted.[10] The preparation and the automatic washing of the box take 60 and 30 minutes, respectively. After washing, the apparatus is ready to perform another synthesis.
This automatic production box provides several advantages in terms of shielding, space requirement (50 X 40 X 50 cm including the microwave cavity and the gamma and UV detectors; magnetron and HPLC pump are outside this area), and therefore safety and cost. The radiochemical yield and synthesis time are in the same

range as those reported with other synthesis systems. [15,16] Even if this synthesis box is dedicated to the production of only one radiopharmaceutical, the various units developed (microwave heating, sep pak and HPLC purification, formulation of the injectable solution) can be easily adapted to the preparation of many other labeled compounds.

Conclusion

The system described in this paper allows the preparation of [^{18}F]altanserin starting from aqueous [^{18}F]fluoride ions recovered from the target to the injectable solution.

Table 1. Synoptic of the automatic production of [^{18}F]altanserin

No	Step	Time (min)	Description
1	Recovery of [^{18}F]F$^-$	5	Valves 4 and 5 OPEN Target valves (not shown) OPEN
2	Drying	3	Microwave oven ON (400 W)
3	Addition of CH_3CN (**A**)	5	Valve 1 OPEN
4	Addition of nitroaltanserin (**B**)	0.25	Valve 2 OPEN Valve 1 CLOSED
5	Labeling reaction vial (**G**)	3.50	Valves 2, 4, and 5 CLOSED Microwave oven: 450 W
6	Dilution (**F**)	1	Microwave oven OFF Valves 3, 4, 5, and 6 OPEN
7	Transfer on the C18 Sep Pak	4	Valves 9 and 10 OPEN
8	Elution (**H**)	0.58	Valve 8 OPEN Valves 9 and 10 CLOSED
9	Washing of the C18 Sep Pak	0.50	Valve 7 OPEN Valve 8 CLOSED
10	Filling of the HPLC loop		Valves 10 and 11 OPEN
11	Injection		The Rheodyne valve turns automatically when the desired volume is loaded (detection switch)
12	HPLC purification Beginning of the peak Collected activity (**I**)	30	 Valve 12 OPEN Valves 13 and 14 OPEN
13	Transfer on the C18 Sep Pak	±20	Valve 14 CLOSED Valves 22 and 23 OPEN
14	Washing of the Sep Pak	0.75	Valve 22 CLOSED Valve 15 OPEN
15	Elution (**K**)	0.75	Valves 15 and 23 CLOSED Valves 16, 17, 19, and 20 OPEN
16	Dilution with 0.9% NaCl (**K**)	1.25	Valve 17 CLOSED Valve 18 OPEN
17	Transfer into the sterile vial (**J**)	1.50	Valve 20 CLOSED Valve 21 OPEN
End of the synthesis			

A sterile, pyrogen-free and single-use unit for the formulation of the injectable solution has been developed. This automatic unit routinely furnishes the radiopharmaceutical with a radiochemical yield of 20 ± 6% (EOB) within a synthesis time of 90 minutes and with a specific activity higher than 1 Ci/μmol (EOS, n = 50).

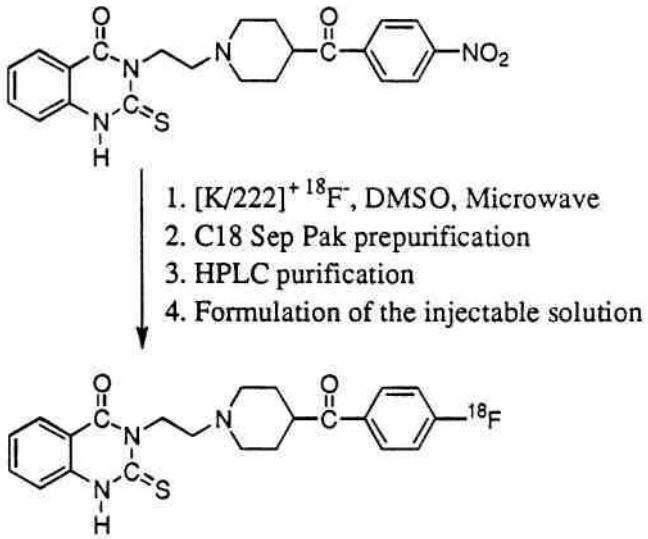

Figure 2. General synthetic pathway used to prepare [^{18}F]altanserin.

The quality controls of the final solution are in accordance with the general quality criterions for a short-lived radiopharmaceutical. [22]
The major advantages of this fully automated synthesis box, which does not require any contact between operator and radioactivity, are its low cost, the small shielded space required, and the good radiochemical yield. [^{18}F]Altanserin prepared by the described method has already been used for numerous human PET studies.

Table 2. Synthesis time for a typical production of [^{18}F]altanserin

Step	**Time for each step (min)**
Recovery of [^{18}F]F$^-$ from the target	5
Radiosynthesis and prepurification	20
HPLC purification	30
Formulation of the injectable solution	25
Quality controls	10
Total	90

In particular, this tracer has led us to demonstrate higher $5HT_2$ binding capacity in men than in women, particularly in the frontal and cingulate cortices. [11] Clinical applications of this tracer may soon appear since its binding in orbitofrontal and insular cortices is reduced in patients with unipolar depression. [12] Applications of this tracer in other neuropsychiatric diseases are still under investigation in different centers.
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