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Research Article

Automated radiosynthesis of [^{18}F]SPA-RQ for imaging human brain NK_1 receptors with PET

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Summary

[^{18}F]SPA-RQ is an effective radioligand for imaging brain neurokinin type-1 (NK_1) receptors in clinical research and drug discovery with positron emission tomography. For the automated regular production of [^{18}F]SPA-RQ for clinical use in the USA under an IND we chose to use a modified commercial synthesis module (TRACERlab $FX_{F\text{-}N}$; GE Medical Systems) with an auxiliary custom-made robotic cooling–heating reactor, after evaluating several alternative radiosynthesis conditions. The automated radiosynthesis and its quality control are described here. [^{18}F]SPA-RQ was regularly obtained within 150 min from the start of radiosynthesis in high radiochemical purity (> 99%) and chemical purity and with an overall decay-corrected radiochemical yield of 15 ± 2% (mean ± S.D.; $n = 10$) from cyclotron-produced [^{18}F]fluoride ion. The specific radioactivity of [^{18}F]SPA-RQ at the end of synthesis ranged from 644 to 2140 mCi/μmol (23.8–79.2 GBq/μmol). Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: [^{18}F]SPA-RQ; Fluorine-18; automation; radioligand; neurokinin type-1; receptor

Introduction

The neurokinin type-1 (NK_1) receptor is acted on by substance P, which has been implicated in several neuropsychiatric disorders such as depression,[1,2] schizophrenia,[3,4] Parkinson's disease[5,6] and Alzheimer's disease.[5,7] Recently, [^{18}F]SPA-RQ (**3**; Figure 1) has been developed for imaging brain NK_1 receptors *in vivo* with positron emission tomography (PET) and validated for imaging in human subjects in Europe.[8–10] However, this radioligand had not been used in human subjects in the USA preceding this work. In 2002 we

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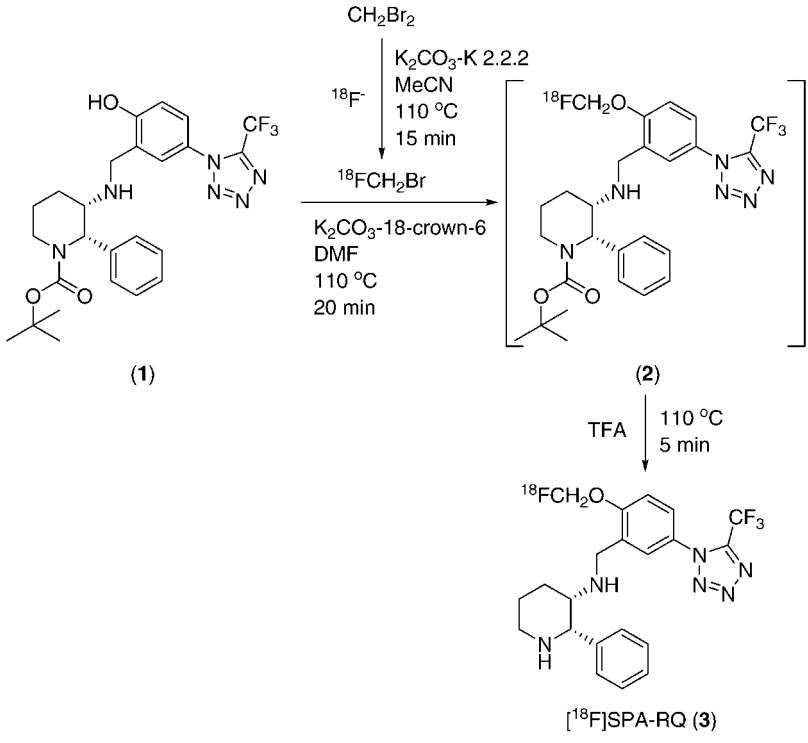

Figure 1. Synthesis of [^{18}F]SPA-RQ

aimed to adapt the then briefly described[11,†] radiosynthesis of [^{18}F]SPA-RQ from cyclotron-produced [^{18}F]fluoride ion (Figure 1) to allow regular production of [^{18}F]SPA-RQ for clinical use under an IND[‡] in the USA. Five main issues needed to be addressed, namely: (i) optimization of the yield of the labeling agent, [^{18}F]fluorobromomethane, from cyclotron-produced [^{18}F]fluoride ion, (ii) optimization of the yield of the subsequent ^{18}F-fluoromethylation of the phenolic precursor (**1**), (iii) optimization of deprotection of the intermediate (**2**), (iv) automation of the radiosynthesis to avoid excessive radiation burden to operating personnel, and (v) efficient formulation of the final dose. Here we describe a satisfactory automated method with respect to radiochemistry, purification, formulation, and quality control for producing [^{18}F]SPA-RQ for human studies under a United States IND.

Results and discussion

Radiochemistry – preparation of labeling agent

[^{18}F]SPA-RQ is prepared via alkylation of the protected phenolic precursor **1** with [^{18}F]fluorobromomethane.[8,10] In the reported procedure[8] the labeling

[†] A full account of this radiosynthesis was published later (2004) by Solin *et al.*[8]
[‡] Investigational New Drug Application to the Food and Drug Administration.

agent is prepared by treating dibromomethane with cyclotron-produced [^{18}F]fluoride ion in acetonitrile[12,13] and isolated by distillation[11] or gas chromatography[14] for entrapment in a second vessel for reaction with **1** in *N,N*-dimethylformamide (DMF). The decay-corrected radiochemical yield (RCY) of isolated labeling agent is an important factor affecting the overall efficiency with which [^{18}F]SPA-RQ may be produced. The highest RCY reported for this labeling agent is 62% by Coenen *et al.*,[12] who used an extended reaction time (65 min), though Bergman *et al.*[14] have regularly achieved an isolated RCY of $25 \pm 5\%$ ($n = 17$) from an unspecified shorter reaction time. We made various attempts to improve on this yield, using a nitrogen purge to transfer volatile labeling agent into a second vessel containing precursor **1** in DMF. The use of neat dibromomethane, instead of a solution of dibromomethane in acetonitrile, gave [^{18}F]fluorobromomethane in high isolated RCY (37%) (Table 1). However, dibromomethane transferred with the labeling agent and undesirably reacts with the precursor **1** by forming a methylene-bridged dimer. Alternative volatile ^{18}F-fluoromethylating agents were considered, such as [^{18}F]fluoroiodomethane[15–17] or [^{18}F]fluorochloromethane. Attempts to prepare these labeling agents by treating the appropriate dihalomethane precursor with [^{18}F]fluoride ion gave inferior RCYs (Table 1). The RCY of [^{18}F]fluoroiodomethane was especially low (7.8%) in accord with the experience of Bergman *et al.*[14] (RCY: $5.7 \pm 5.5\%$, $n = 30$), although notably Zhang *et al.*[17] (RCY: 14–31%, $n = 13$) and Zheng and Berridge[15,16] (RCY: $40 \pm 8\%$) have reported higher yields. Hence, [^{18}F]fluorobromomethane, prepared from a solution of dibromomethane (10% v/v) in acetonitrile, remained the labeling agent of choice. In order to reduce the transfer of chemical impurities with the labeling agent to the entrapment vessel, a series of four disposable silica gel Sep-Pak Plus cartridges were inserted into the transfer line, as described by Iwata *et al.*[18] The use of Sep-Paks was considered simpler and more convenient than the alternative of gas chromatographic separation described by Solin *et al.*[8] Under

Table 1. Isolated decay-corrected radiochemical yields (RCYs) of ^{18}F-fluoromethylating agents

Labeling agent	Precursor (μl)	Solvent	Isolated RCY[a] (%)
[^{18}F]Fluorobromomethane	CH_2Br_2 (500)	None	37 ($n = 2$)
[^{18}F]Fluorochloromethane	CH_2Cl_2 (100)	MeCN	22 ($n = 1$)
[^{18}F]Fluorobromomethane	CH_2Br_2 (100)	MeCN	27.5 ± 4.5 ($n = 57$)
[^{18}F]Fluoroiodomethane	CH_2I_2 (100)	MeCN	7.8 ($n = 1$)

[a] All reactions were carried out in a sealed reaction vessel in the presence of K 2.2.2 (5 mg; 13.2 μmol) and potassium carbonate (0.5 mg; 3.6 μmol) at 110°C for 10 min. Volatile radioactive product was transferred by nitrogen stream into a second vessel over the course of $\leqslant$30 min until maximal radioactivity had been collected.

these conditions the RCY of isolated labeling agent was 27.5 ± 4.5% (range: 16.5–42.5%; $n = 57$).

Radiochemistry – labeling and deprotection

The RCY of the intermediate **2** from the alkylation of **1** with [^{18}F]fluorobromomethane (Figure 1) and the efficiency of the deprotection of **2** are also major factors in the overall efficiency of [^{18}F]SPA-RQ production. In the reported procedure,[8] cesium carbonate was used as base in DMF as solvent for the alkylation reaction. We explored other bases, (e.g. NaOH, *t*-butylammonium hydroxide (TBAOH) or potassium carbonate-18-crown-6[19]) in attempts to improve the RCY of this reaction. In these experiments the RCY of the intermediate **2** was not measured directly but **2** was deprotected with trifluoroacetic acid (TFA) to give [^{18}F]SPA-RQ (Figure 1) and its overall RCY from [^{18}F]fluoride ion measured usually before or exceptionally after formulation (Table 2). In general, such deprotection reactions are very rapid.[20] Solin *et al.*[8] implemented deprotection of the intermediate **2** with TFA in 2 min

Table 2. RCYs of [^{18}F]SPA-RQ from [^{18}F]fluoride ion under various conditions for alkylation of 1 and deprotection of 2

Precursor **1** amount (mg; μmol)	Base; amount (μmol)	Alkylation time[a] (min)	Deprotection time[b] (min)	RCY of [^{18}F]SPA-RQ[c] (%)
0.2; 0.4	Cs_2CO_3; 4	15	0.5	3.7
0.2; 0.4	Cs_2CO_3; 4	20	0.5	5.7
0.2; 0.4	Cs_2CO_3; 4	30	0.5	3.6
0.3; 0.6	Cs_2CO_3; 4	30	0.5	5.4
0.3; 0.6	Cs_2CO_3; 31	15	0.5	6.6
0.3; 0.6	Cs_2CO_3; 31	15	1.0	6.1
0.2; 0.4	TBAOH; 4	15	0.5	4.2
0.3; 0.6	NaOH; 10	15	0.5	5.5
0.3; 0.6	K_2CO_3-18-cr.-6[d]; 15-15	15	0.5	3.9
0.3; 0.6	K_2CO_3-18-cr.-6; 15-15	15	5	10.6
0.3; 0.6	K_2CO_3-18-cr.-6; 15-19	20	5	17.0
0.3; 0.6	K_2CO_3-18-cr.-6; 15-19	20	5	8.4 ± 3.6 ($n = 35$)[e,f]
0.3; 0.6	K_2CO_3-18-cr.-6; 15-19	20	5	15 ± 2.0 ($n = 10$)[e,g]

[a] In DMF (0.75 ml) heated at 110°C.
[b] At 110°C.
[c] Overall for HPLC separated product from [^{18}F]fluoride ion, unless otherwise indicated, preceding formulation unless otherwise indicated.
[d] 18-crown-6.
[e] After formulation for intravenous injection.
[f] Semi-automated preparations.
[g] Automated preparations.

at ambient temperature. However, we found that heating of the intermediate **2** with TFA for 5 min at 110°C was necessary to ensure complete deprotection (Table 2). These became the regular deprotection conditions. Among the bases used for the alkylation reaction (Table 2), potassium carbonate (2 mg; 15 μmol)-18-crown-6 (5 mg; 19 μmol) (heated with **1** (0.6 μmol) in DMF (0.70 ml) for 20 min at 110°C) gave consistently superior RCYs of [^{18}F]SPA-RQ and became the base of choice. Shorter reaction time gave lower RCY (Table 2). It should be noted that once the alkylation reaction was complete, it was necessary to remove most of the DMF before the deprotection step. DMF was almost completely removed over a 7–9 min period by heating the vessel while purging it with nitrogen. Evaporation to dryness was routinely avoided since this tended to produce a byproduct that was difficult to separate from [^{18}F]SPA-RQ.

Automation of radiosynthesis

No automated apparatus has been commercially available for the radiosynthesis of the labeling agent, [^{18}F]fluorobromomethane, or for its application in preparing radioligands, such as [^{18}F]SPA-RQ. For the radiosynthesis of [^{18}F]fluorobromomethane, we opted to use the TRACERlab $FX_{F\text{-}N}$ apparatus offered by GE Medical Systems, since this apparatus is specifically designed to perform automated reactions with cyclotron-produced [^{18}F]fluoride ion. The apparatus had to be reconfigured to allow transfer of the labeling agent into a second vessel for the labeling reaction (Figure 2).

Initially, [^{18}F]fluorobromomethane synthesis and entrapment were demonstrated with the TRACERlab $FX_{F\text{-}N}$ module connected to an ice-cooled vial containing the precursor **1** in DMF (RCY: 26 ± 7%; $n = 55$). Deprotection of the generated intermediate **2**, HPLC separation and formulation provided [^{18}F]SPA-RQ ready for administration to human subjects in acceptable but rather variable overall RCY (8 ± 4%; $n = 35$) (Table 2).

In order to achieve consistent production runs with greater radiation safety, a robotic Peltier cooling–heating device (Figure 3) was designed, constructed and implemented as an auxiliary to the TRACERlab $FX_{F\text{-}N}$ module. This device was externally controlled to perform the alkylation reaction in a second reactor (reactor #2). Coupling of this device with the TRACERlab $FX_{F\text{-}N}$ maintained almost the same RCY for isolated [^{18}F]fluorobromomethane (26 ± 5%; $n = 4$). The same reactor was also used for the deprotection reaction and the preparation of the [^{18}F]SPA-RQ for HPLC purification. The use of two reverse phase columns in series was effective in separating [^{18}F]SPA-RQ from a multitude of low level chemical impurities (Figure 4).

Following HPLC, the separated [^{18}F]SPA-RQ was automatically and efficiently formulated by adsorption on a C-18 Sep-Pak, elution with acidified

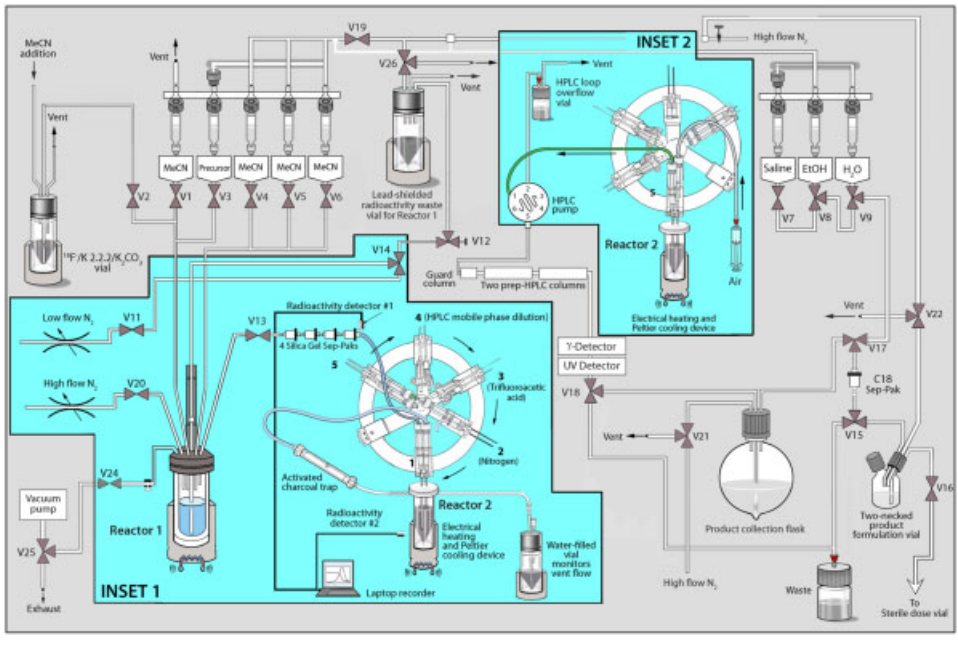

Figure 2. Automated apparatus for entrapment of [^{18}F]fluorobromomethane for the preparation of [^{18}F]SPA-RQ

ethanol, neutralization with sodium bicarbonate in saline and sterile-filtration through a Millex-MP filter. Acidification of the ethanol with acetic acid ensured efficient elution of [^{18}F]SPA-RQ from the Sep-Pak. The Millex-MP filter was superior to alternative filters in that it adsorbed only a very small percentage ($<4\%$) of the [^{18}F]SPA-RQ.

The fully automated process gave an improved and more consistent overall RCY of purified and formulated [^{18}F]SPA-RQ from [^{18}F]fluoride ion ($15 \pm 2\%$; $n = 10$) compared to the partially automated procedure (Table 2). This arrangement also reduced the radiation exposure to the production chemist to an acceptable level of about 70 mrem when using a starting radioactivity of 500 mCi (18.5 GBq). The total time required for the radiosynthesis, purification and formulation was 150 min.

Quality control of final dose

The radiochemical purity of [^{18}F]SPA-RQ preparations exceeded 99% at the end of synthesis and this purity was maintained for at least 6 h (Figure 5). Preparations were also greater than 99% chemically pure, based on the area of the absorbance peak for carrier relative to those of impurities[§] in the HPLC

[§]Carrier SPA-RQ and impurities are assumed to have the same extinction coefficient at $\lambda = 254$ nm.

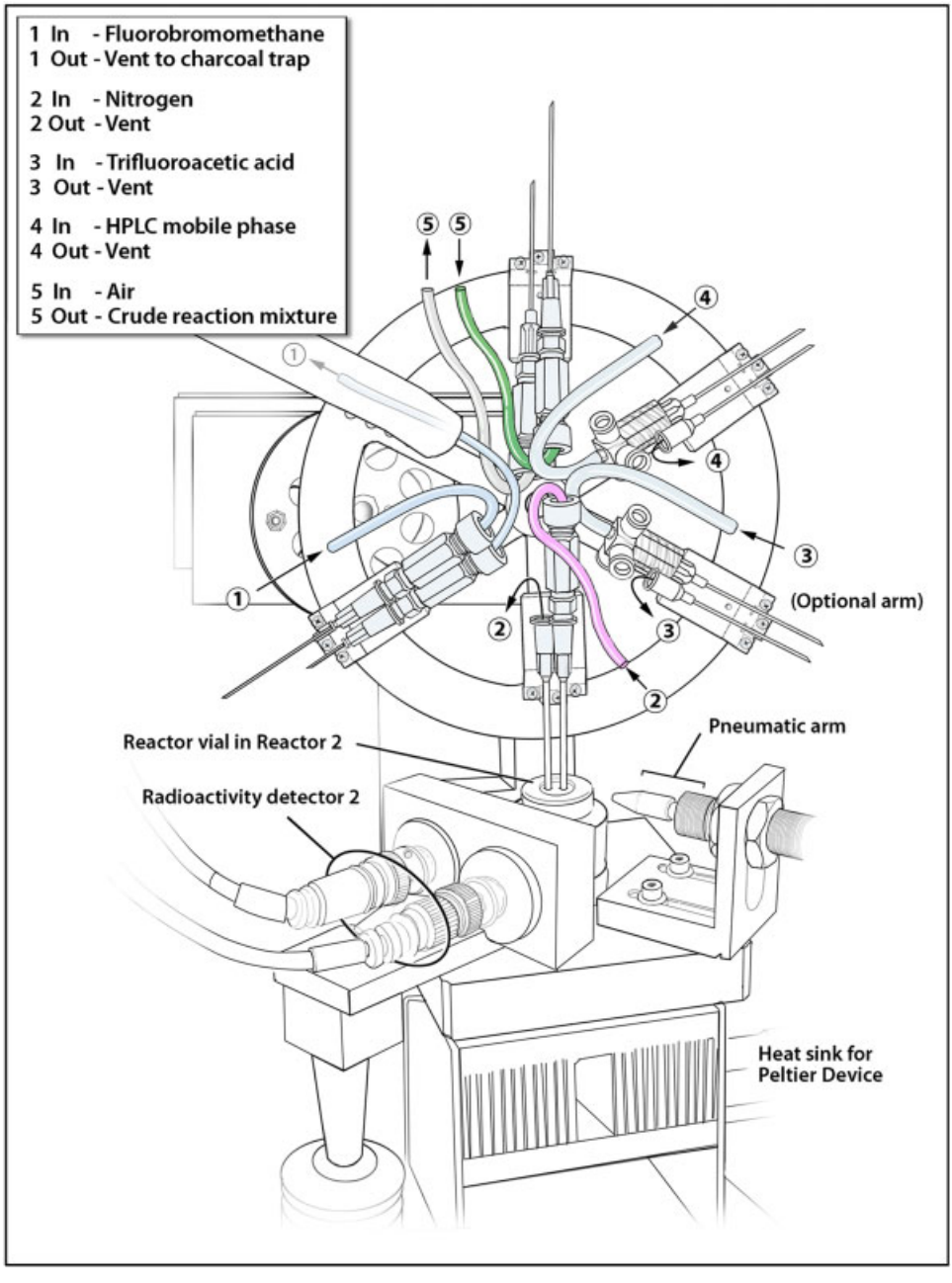

Figure 3. Configuration of TRACERlab FX_{F-N} used to synthesize [^{18}F]fluorobromomethane

analysis when earlier eluting low level compounds introduced with the formulation vehicle are discounted. Specific radioactivities were determined from the HPLC analysis, which was calibrated for mass of SPA-RQ. LC–MS–MS was used to verify carrier identity rapidly (<3 min) preceding

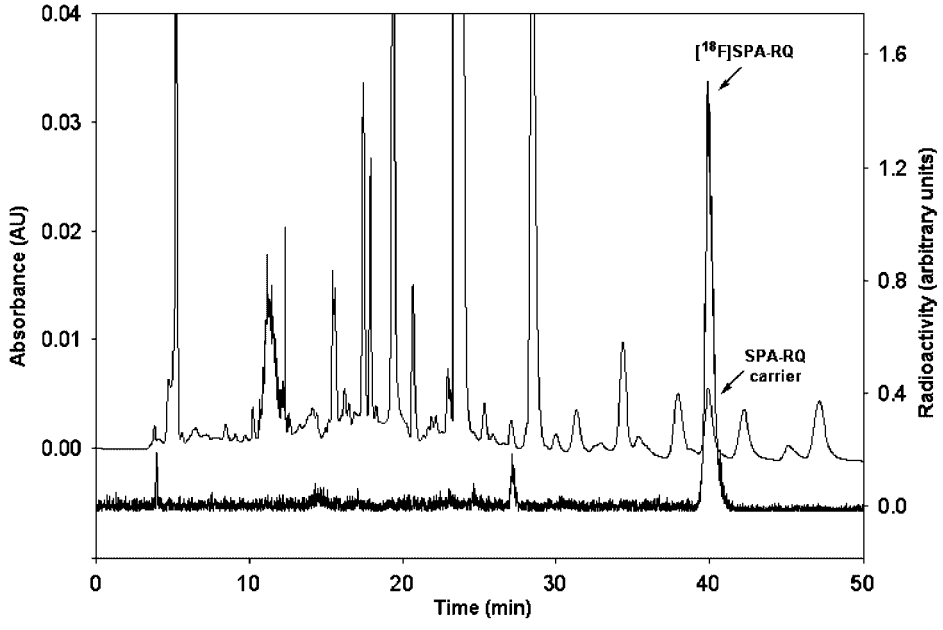

Figure 4. Chromatogram from the HPLC purification of [^{18}F]SPA-RQ

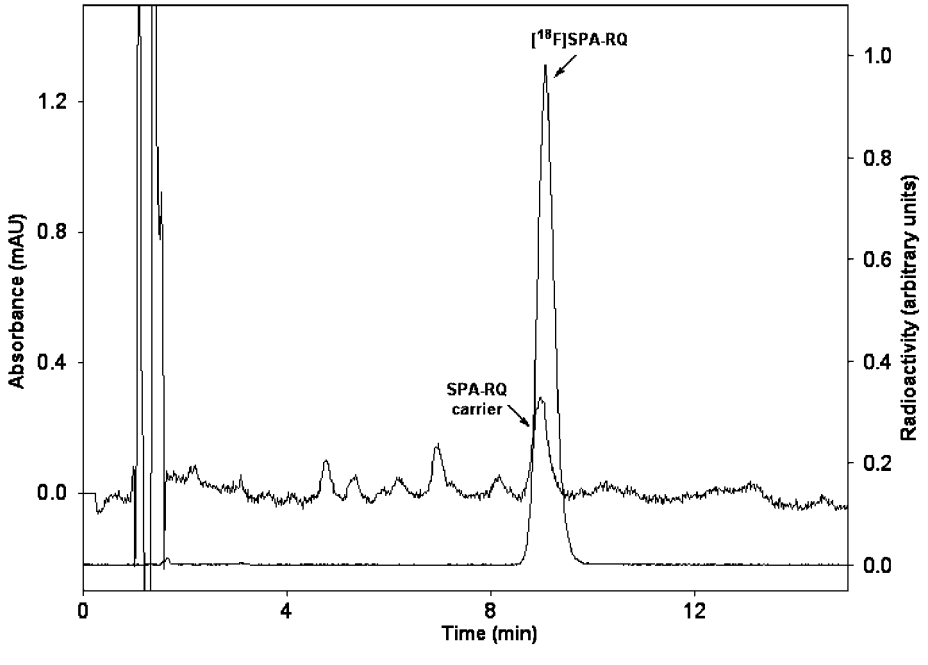

Figure 5. Chromatogram from the HPLC quality control of [^{18}F]SPA-RQ

release of the radioactive product for use. Preparations were free of any significant level of contaminating acetonitrile ($\leqslant$0.04% w/v), had acceptable pH (within range 4.5–7.5) and were sterile and apyrogenic.

Conclusion

An automated method for the regular and consistent production of [^{18}F]SPA-RQ for safe intravenous injection into human subjects was accomplished by coupling a commercial automated apparatus with a custom-made device.

Experimental

Materials

(2*S*,3*S*)-1-*t*-Butoxycarbonyl-2-phenyl-3-[2-hydroxy-5-(5′-trifluoromethyl-tetrazol-1-yl)phenylmethyleneamino]piperidine (**1**) and authentic SPA-RQ ((2-fluoromethoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-benzyl)((2*S*,3*S*)2-phenyl-piperidin-3-yl)-amine) (**3**) were supplied by Merck Research Laboratories and were of >99% chemical purity. The following were obtained commercially, as indicated: sodium chloride injection (0.9% USP; APP Inc.; Schaumburg, IL), sodium bicarbonate injection (8.4% USP; Abbott Laboratories, North Chicago, IL), ethanol (dehydrated USP; Abbott Laboratories), Millex-MP and Millex-GV filters (Millipore; Bedford, MA), silica gel and C-18 Sep-Pak Plus cartridges (Waters Corporation; Milford, MA), Luna C-18 columns (Phenomenex; Torrance, CA), LAL test kit vials and control standard endotoxin (Cape Cod Associates; Falmouth, MA) and ColorpHast® indicator strips (EM Science; Gibbstown, NJ). All other reagents and solvents were obtained from Aldrich (St. Louis, MO).

General methods

No-carrier-added [^{18}F]fluoride ion was prepared by the ^{18}O(p,n)^{18}F nuclear reaction on a PETtrace cyclotron (GE Medical Systems; Milwaukee, WI). ^{18}O-Enriched water (1.8 ml, >95% isotopic enrichment) was added to a titanium target equipped with titanium foils. The [^{18}O]water was bombarded with 18 MeV protons (typically 20 μA for 120 min). Portions of the irradiated water (0.02–0.5 ml), containing up to 500 mCi (18.5 GBq) of [^{18}F]fluoride ion were used for individual experiments.

Radioactivity measurements were carried out with an Atomlab™ 300 dose calibrator (Biodex Medical Systems; Shirley, NY), which was calibrated daily using Cs-137 and Co-57 sources. Each radiochemical yield (RCY) is decay-corrected and reported as a mean ± S.D.

HPLC separations and analysis were performed on Gold HPLC modules (System Gold 126 gradient solvent module coupled with a variable wavelength 166 UV absorbance detector; Beckman Coulter; Fullerton, CA). Radioactivity in HPLC eluates was detected with a pin-diode radioactivity detector (FlowCount; Bioscan; Washington, DC).

Automated radiosynthesis apparatus – description

The automated apparatus for radiosynthesis was housed in a lead-shielded hot-cell. The apparatus comprised a modified commercial device (TRACERlab $FX_{F\text{-}N}$; GE Medical Systems) (Figure 2), which is designed for performing single-step reactions with cyclotron-produced [^{18}F]fluoride ion, linked to a custom-made robotic Peltier cooling–heating device (Figure 3) and to HPLC.

The TRACERlab $FX_{F\text{-}N}$, as supplied, has a glassy carbon reaction vessel (reactor #1; Figure 2) and features automated facilities for drying aqueous [^{18}F]fluoride ion by cycles of addition and evaporation of acetonitrile, for forming a complex of this ion with K^+-Kryptofix® 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-[8,8,8]hexacosane; K 2.2.2) and for conducting single-pot radiochemistry. The apparatus also has facility for heating the glassy carbon vessel up to 135°C for reactions and solvent evaporation. A PC, loaded with TracerLab software, controls and monitors the function of the apparatus. The TRACERlab $FX_{F\text{-}N}$ apparatus was re-configured as shown in Figure 2, mainly by switching valve functions from their original purpose. The major changes and their purposes are further explained in the following section on operation of the apparatus.

The robotic Peltier cooling–heating device, incorporates a second reaction vessel (reactor #2; Figure 3) that may be heated or cooled and penetrated through its top septum with a variety of needles. The device is mainly constructed from stainless steel and aluminum parts on an aluminum platform. Thus, an aluminum wheel (diameter 14 cm), equipped with six aluminum clamps each holding two needles (length: 0.675–2.00 in; diameter 25–18 gauge), was mounted on a stainless steel driveshaft. The driveshaft rides on seal ball bearings under power from an electric-servo motor (HITEC, 6.0 V, 0.19 s/60° speed, 11.5 kg cm torque). The wheel was indexed with a stainless steel 6-position Geneva-gear (WM Berg; East Rockaway, NY). Micro-limit switches were used to control the home position and index location of the wheel. The wheel assembly has a Lexan needle guard and moves up/down vertically on a stainless steel shaft under pneumatic power; micro-limit switches regulate the motion of the pneumatic cylinders. Electrically-activated air solenoids (Norgren; Norgren.com), pneumatic actuators (Clippard Instrument Labs Inc., OH) on aluminum brackets and quick-disconnect air-line fittings (Clippard Instrument Labs Inc.) direct air flow through the pneumatic assembly under remote electronic control. A modified Peltier device (which may cool to −5°C) and dual rod heaters (which may heat to 200°C) regulate the temperature of the Teflon-insulated vial cavity, which is monitored with an attached thermocouple. Two radiation detectors (Bioscan), held by a uni-directional clamping device, externally monitor the radioactivity

in the reaction vial when it resides in the vial cavity. A stainless steel pneumatic arm with a nylon tip enables the reaction vial to be held in place during removal of wheel assembly needles from the vial via the septum. Nitrogen flow to the secondary reactor was controlled by a flow controller connected to the Local Set Point Module using a Top Connector Socket (Alicat Scientific Inc.; Tucson, AZ).

Automated radiosynthesis apparatus – general features of operation

General features of operation of the automated radiosynthesis apparatus (Figures 2 and 3) are as follows. Valve 2 (Figure 2) allows [^{18}F]fluoride ion, K 2.2.2 and potassium carbonate in [^{18}O]water, followed by acetonitrile, to be introduced into the glassy carbon reaction vessel (reactor #1). Then azeotropic drying is performed through two subsequent cycles of acetonitrile addition and evaporation. The PEEK tee between the outlet lines of valves 1 and 2 allows acetonitrile from the first azeotropic drying step to be transferred through valve 1, under reduced pressure, to rinse out any residual activity from the preceding loading of activity into the reaction vessel. Acetonitrile is added through valve 5 for the second azeotropic drying step. After the [^{18}F]fluoride complex is dry, precursor (dihalomethane) in acetonitrile is added through valve 3 for the synthesis of the radioactive labeling agent. After this synthesis, the glassy carbon reaction vessel is washed twice with acetonitrile introduced via valves 4 and 6. A conical vial (10 ml) is present between valves 12 and 26 and used to store residual radioactivity in a shielded environment after the synthesis.

A Teflon tee introduced before valve 20 provides for a new nitrogen source which is regulated with an adjustable flow meter (150 mm Cole-Palmer) and switched on or off by valve 11. Valve 11 is connected to the 'normally closed' side of three-way valve 14. Labeling agent is transferred out of the glassy carbon reaction vessel in a nitrogen gas stream (flow rate: 30–35 ml/min), released by valve 11, and allowed to bubble through the reaction mixture and out through valve 13. Labeling agent is purified by passage through a series of four silica gel Sep-Paks and trapped in a cooled secondary reaction vessel (reactor #2; Figure 3) containing *des*-fluoromethyl precursor (**1**), potassium carbonate and 18-crown-6 in DMF with the Peltier device in position #1 (Inset 1; Figure 2). Radioactivity transfer was monitored with detectors (Bioscan) at the terminus of the silica gel cartridges and at the secondary reactor (Figure 3). The rotating wheel assembly was put in position #5 (Inset 2, Figure 2), to reconnect the secondary reactor to the TRACERlab $FX_{F\text{-}N}$ synthesis module at the HPLC loop. The crude reaction mixture was loaded onto the loop by pressurizing the secondary reactor with an external syringe (20 ml).

Synthesis of [^{18}F]fluorohalomethanes – standard automated procedure

Cyclotron-produced [^{18}F]fluoride ion in [^{18}O]water was delivered into a vial containing K 2.2.2 (5 mg; 13.2 μmol) and potassium carbonate (0.5 mg; 3.6 μmol) in acetonitrile–water (9:1 v/v; 100 μl). The [^{18}F]fluoride ion mixture was transferred to reactor #1 of the automated radiosynthesis module, chased by acetonitrile (1 ml). A further amount of acetonitrile was added (1 ml) and the mixture taken to dryness at 90°C under reduced pressure with nitrogen flow. A further cycle of acetonitrile (2 ml) addition and evaporation was performed. Dihalomethane (CH_2Cl_2, CH_2Br_2 or CH_2I_2; 100 μl) in anhydrous acetonitrile (1.0 ml) was added to the dry [^{18}F]fluoride ion-K 2.2.2-potassium carbonate complex which was then heated to 110°C for 15 min. The reaction vessel was then cooled to 35°C. Nitrogen gas was used to transfer the volatile [^{18}F]fluorohalomethane through a series of four silica gel cartridges (Sep-Pak Plus) and into a pre-cooled vessel, a V-vial (volume 1 ml) with a crimp-seal silicone-Teflon septum cap.

Synthesis of [^{18}F]SPA-RQ – standard automated procedure

The glass reaction vessel for reactor #2 was charged with **1** (0.3 mg; 0.6 μmol), potassium carbonate (2 mg; 15 μmol) and 18-crown-6 (5 mg; 19 μmol) in DMF (0.8 ml) and cooled to −5°C. [^{18}F]Fluorobromomethane was transferred to this solution under computer control from the TRACERlab $FX_{F\text{-}N}$ module. Radioactivity transfer was monitored by two external Bioscan detectors (Figure 2) with Hotcell software and was stopped when radioactivity in the vessel reached a maximum. The vessel was then heated at 110°C for 20 min and the DMF removed almost to dryness with a nitrogen stream. Trifluoroacetic acid (TFA; 0.1 ml) was added to the dry radioactive residue and heated at 110°C for 5 min. The acidic mixture was diluted with aqueous mobile phase A (water–MeCN–TFA, 95:5:0.1 by vol.; 0.9 ml) and injected remotely onto HPLC for purification on two Luna C-18 columns (10 × 250 mm; 10 μL) connected in series and eluted over 40 min at 9 ml/min starting with mobile phase A changing linearly to A–MeCN (78:22 v/v) over 20 min. Eluate was monitored for absorbance at 254 nm and radioactivity (Figure 4). Purified [^{18}F]SPA-RQ was collected and diluted with sterile water (85–100 ml).

Formulation of [^{18}F]SPA-RQ – standard procedure

A Sep-Pak Plus C-18 cartridge was eluted at about 10 ml/min with ethanol (10 ml) and then water (10 ml). Under TRACERlab $FX_{F\text{-}N}$ module control, the aqueous solution of purified [^{18}F]SPA-RQ was passed through the prepared cartridge. The loaded cartridge was then washed with sterile water (5 ml). Finally, [^{18}F]SPA-RQ was eluted from the cartridge with ethanol (USP; 0.95 ml) containing glacial acetic acid (2 μl). 'Sodium chloride injection' (0.9%

USP; 9 ml) with 'sodium bicarbonate injection' (8.4% USP; 40 μl) were pushed through the Sep-Pak C-18 cartridge and combined with the ethanolic solution of [^{18}F]SPA-RQ. This solution was thoroughly mixed and pushed through a sterile Millex-MP filter (25 mm) into a sterile dose vial (10 or 30 ml).

QC analysis of [^{18}F]SPA-RQ

An aliquot (1 ml) of the formulated [^{18}F]SPA-RQ was removed from the dose vial with a pre-attached sterile syringe (1 ml size). A portion (0.8 ml) was then transferred to a sterile vial and the remainder placed in a pyrogen-free tube for an apyrogenicity test. Further portions were taken from the sample in the sterile vial for HPLC (2×100 μl), LC–MS–MS (50 μl), GC (50 μl), sterility testing (100 μl added to a sterile 10 ml saline vial) and pH test (50 μl).

[^{18}F]SPA-RQ was analyzed for radiochemical and chemical purity by reverse phase HPLC on a Luna C-18 column (250×4.6 mm; 10 μm) eluted with a mixture of acetonitrile (21%) and water–acetonitrile–TFA (95:5:0.1 by vol.) (79%) at 2.5 ml/min. Eluate was monitored for radioactivity and absorbance at 254 nm. Absorbance response was calibrated for mass of SPA-RQ to permit the amount of carrier in the preparation to be determined and the specific radioactivity to be calculated.

Carrier in [^{18}F]SPA-RQ preparations was routinely identified by mixing analyte (50 μl) with acetic acid (0.5%; 50 μl) in an autosampler vial and injecting a sample of this mixture (10 μl) into an LC–MS (LCQ Deca instrument; Thermo Electron Corporation; San Jose, CA). The mobile phases were water–methanol–acetic acid (90:10:0.5 by vol.) (mobile phase A) and methanol–acetic acid (100:0.5 v/v) (mobile phase B). Chromatography was performed on a reverse-phase column (Luna C18; 50×2 (i.d.) mm; 3 μm) eluted with an equal mixture (v/v) of A and B at 150 μl/min, with eluate introduced into the MS probe for electrospray ionization. The electrospray source voltage was 4.5 kV and the capillary voltage 16 V. The capillary temperature was 260°C. For nebulization, the sheath gas (nitrogen) flow rate was set at 44 units. MS–MS analysis of SPA-RQ was achieved by isolation (width $m/z = 1.5$) of the parent ion ($m/z = 451$) and its collision-induced dissociation using a collision energy level of 24%. The resulting parent–daughter ion spectrum contained intact $m/z = 451$ ion as well as major fragment ions ($m/z = 279, 247$ and 160) suitable for definitive identification of SPA-RQ.

A reported GC procedure[21] was modified for the measurement of residual acetonitrile in preparations of [^{18}F]SPA-RQ. Samples were analyzed on an instrument (Agilent 6850; Agilent Technologies Inc.; Palo Alto, CA) equipped with a flame ionization detector and a DBWax column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness; J&W). Analyte (50 μl) was mixed

with aq. propionitrile (internal standard; 386 ppm; 50 μl) and a sample (1 μl) of this mixture injected into GC. The initial column temperature was 50°C and after 1 min was increased to 150°C at 20°C/min. This temperature was held for 0.5 min and then increased to 220°C at 50°C/min. After 3 min the column temperature was returned to 50°C. The concentration of residual solvent was calculated from a previously acquired calibration curve.

Apyrogenicity (limulus amebocyte lysate; Pyrotell®; Cape Cod Inc.; Falmouth, MA) tests were performed in house to ensure that doses of [^{18}F]SPA-RQ contained <1.25 endotoxin units per ml. Sterility tests were performed by the National Institutes of Health Microbiology Service.

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