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Production and biological evaluation of [^{18}F]-6-thia-14-fluoro--heptadecanoic acid

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ABSTRACT

BACKGROUND: [^{18}F]-6-thia-14-fluoro-heptadecanoic acid **3b**, a free fatty acid, has been used in myocardial PET imaging. In order to establish an automated synthesis module for routine production in the country, a study was performed for optimization of the production conditions as well as making modifications.

MATERIAL AND METHODS: [^{18}F]Benzyl-14-Fluoro-6-thia-heptadecanoate **2b** was prepared in no-carrier-added (n.c.a) form from Benzyl-14-tosyloxy-6-thia-heptadecanoate **1** in one step at 90°C in Kryptofix2.2.2/[^{18}F] with acetonitrile as the solvent followed by Silica column chromatography. The radiolabelled ester **2** was then hydrolysed to yield [^{18}F]-6-thia-14-fluoro-heptadecanoic **3b**. The final solution was concentrated using the C_{18} SPE system and administered to normal rats for biodistribution and co-incidence imaging studies.

RESULTS: The synthesis took 15 min with overall radiochemical yield of 15–25% (EOS) and chemical-radiochemical purity of more than 90%. Automation was performed using a two-pot synthesis. The best imaging time was shown to be 140–180 minutes post injection.

CONCLUSIONS: Using this procedure a fast, reliable, automated synthesis for the cardial PET tracer, i.e. [^{18}F]FTHA, can be obtained without an HPLC purification step.

Key words: fatty acid, PET, fuorine-18, [^{18}F]FTHA, quality control

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Introduction

Free fatty acids (FFAs) are the main source of energy in well--oxygenated myocardium [1–3] in the fasting state. Myocardial fatty acid utilization rate depends on the availability of exogenous fatty acids and the rate of acetyl-CoA oxidation [4]. [^{18}F]-labelled 14(*R,S*)-fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA) is a false long-chain fatty acid (LCFA) substrate and inhibitor of fatty acid metabolism [5]. After transport into the mitochondria, it undergoes initial steps of β-oxidation, and is thereafter trapped in the cell. [^{18}F]FTHA has been used in the study of the β-oxidation rate of LCFAs [6], fatty acid metabolism in the human heart with positron emission tomography (PET) [7–11] and fasting state [12, 13]. In continuation of our research program on the ^{18}F-labelling of some interesting compounds such as benzodiazepine agonists [14] cholesterol derivatives [15, 16] natural products [17] and [^{18}F]FDG for national use, we became interested in the design and manufacture of an automated synthesis module for the important fatty acid PET tracer, [^{18}F]FTHA, for high scale radiopharmaceutical production followed by preliminary imaging studies in experimental animals.

Material and methods

The chemicals were purchased from Aldrich Chemical Company, Milwaukee, WI. The tosylated precusor was purchased from ABX chemical company, Germany. Thin-layer chromatography (TLC) of non-radioactive products was run on silica gel polymer-backed (F 1500/LS 254, 20 × 20 cm, TLC Ready Foils Schleicher & Schuell) or glass plates (25 × 35 cm, E-Merck). Acetonitrile used for labelling experiments were of "Sure-Seal" grade (Aldrich). Analytical HPLC to determine the specific activity was performed by a Shimadzu LC-10AT, armed with two detector systems: flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu). The specific activity of **3b** was calculated using a standard curve from **3a**. Radiochromatography was performed using a rotary motor equipped with a Canberra germanium detector (model GC1020--7500SL) using polymer-backed silica gel papers. The purification of **3b** was performed by C_{18} Sep-Pak short columns, which were purchased from Waters. Animal studies were performed in accor-

Table I. Retention factors of the chemical species

Chemical species	1	2a	3a
R_f	0.6	0.8	0.35

dance with the United Kingdom Biological Council Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edn.

Preparation of [18F]-potassium fluoride

Fluorine-18 anion was prepared by 18 MeV proton bombardment of an enriched $H_2{}^{18}O$ sample (> 95%, Cortec™, France). The sample was held in a gold-coated silver target in a 30 MeV cyclotron at the Nuclear Research Centre for Agriculture and Medicine, Karaj, Iran. After recovery of $H_2{}^{18}O$ over an anion exchange resin (Dowex), the fluorine-18 anion was eluted by a 1% potassium carbonate solution. The eluted solution was directly used in the labelling step.

Preparation of 6-thia-14-fluoro-heptadecanoic acid as cold standard for HPLC studies

Anhydrous acetonitrile (1 ml) was added to a vial containing solid potassium fluoride (2 mg, 34 μmole) and solid Kryptofix 2.2.2 (25 mg), and the mixture was heated to 85–90°C while bubbling using a flow of N_2. The mixture was azeotropically dried 2 more times by the addition of anhydrous acetonitrile portions (1 ml). The vial was cooled and a solution (50 μg/ml, 90 μM) of **1** (40 μl, 2 mg) in anhydrous acetonitrile (3 mL) was added to the dried mixture. The vial was capped and heated to 80°C for 8 min. The TLC control of the reaction mixture using 2 solvent systems (A: diethyl ether; B: ethyl acetate: hexane, 1:3, v/v) demonstrated the completion of the reaction. The heating continued for another 2 minutes to remove acetonitrile residues. After cooling, the mixture was dissolved in diethyl ether (3 mL) and passed through two Si Sep-Pak columns. The organic layer was dried over anhydrous sodium sulphate and purified by preparative TLC silica gel on glass using a mixture of Hexane-EtOAc (3:1, v/v) as the mobile phase. The desired fluoride compound was separated, while the starting material migrated to the higher R_f (not detected by UV). The product was then confirmed to be **2a** using spectroscopic methods.

The ester obtained above (dissolved in 0.2 ml of diethyl ether) was transferred to a conical borosilicate vial containing potassium hydroxide solution (2 ml, 0.2 M) and the reaction mixture was heated to 95°C for 3 minutes, while the temperature was controlled using a cold finger. The reaction mixture was finally cooled to room temperature followed by neutralization using HCl 6M (100 μl). During all the steps the mixture was bubbling using a flow of N_2. The completion of the reaction was checked by TLC using diethyl ether or acetonitrile as the mobile phase. Retention factors of the chemical species are shown in Table I.

Two-step preparation of [18F]-6-thia-14-fluoro-heptadecanoic acid

Compound **3b** was prepared from **1** using the above procedure in small scales. A volume of target solution eluted with 100 μL of a 1% potassium carbonate (1 mg, 7 μmol) solution containing 10–20 mCi of activity, was transferred to a 10 mL conical vessel containing Kryptofix 222 (20 mg, 54 μmol) and anhydrous acetonitrile (0.5 mL). The mixture was evaporated by slight heat and argon flow. Drying was repeated after the addition of two more 0.5 mL portions of anhydrous acetonitrile. A mixture of **1** (0.5 mg, 1 μmol) in anhydrous acetonitrile (0.5 ml) was added to the dried mixture. The reactor 1 vessel was heated to 80°C for 8 min. The heating continued for another 2 minutes to remove acetonitrile. After cooling, diethyl ether (3 mL) was added to the mixture and passed through two Si Sep-Pak columns, while transferring to the second reactor containing potassium hydroxide solution (2 ml, 0.2 M). The reaction mixture was heated to 95°C for 3 minutes, while the temperature was controlled using a cold finger. The reaction mixture was finally cooled to room temperature followed by the addition of HCl 6M (100 μl). During all the steps the mixture was bubbling using a flow of N_2. The mixture was cooled and rapidly passed through a C_{18} Sep-Pak column. The column was washed with diethyl ether (1 mL) and the eluted solution was passed through a short silica column. The active solution was checked for radiochemical purity by developing one drop of the latter solution over a polymer-backed silica gel layer in chloroform as the mobile phase. HPLC and TLC showed a purity higher than 90% in the form of [18F]-compound (Table 1). The final etheric solution was concentrated by a flow of N_2 gas followed by the addition of 5% human serum albumin (1 ml) at 37° with mild stirring.

Biodistribution studies

The distribution of [18F]FTHA in tissues was determined in Sprague-Dawdley rats. A volume (0.1 ml) of final [18F]FTHA solution containing 1.48 MBq radioactivity was injected into the dorsal tail vein. The total amount of radioactivity injected into each rat was measured by counting the 1-ml syringe before and after injection in an activity meter with fixed geometry. The animals were sacrificed by ether asphyxiation at selected times (1 and 2 hours, n = 3) after injection, the tissues weighed and their specific activities determined as a percentage of injected dose per gram of tissue, using gamma-ray scintillation.

Imaging studies

0.1 ml volumes of the final [18F]FTHA solution containing 1.85 MBq activity were injected into the dorsal tail vein of healthy rats. The total amount of radioactive material injected into each rat was measured by counting the 1-ml syringe before and after injection in an activity meter with fixed geometry. The animals were relaxed by halothane and fixed in a suitable probe. Images were taken 1, 2 and 3 hours after administration of the radiopharmaceutical in coincidence mode of a Dual-Head SPECT system (SMV, France, Sopha DST-XL). The useful field of view (UFOV) was 540 mm × 400 mm. The spatial resolution in the coincidence mode was 10 mm FWHM at the CFOV, and sensitivity was 20 Kcps/μCi/cc. Sixty-four projections were acquired at 30 seconds per view with a 64 × 64 matrix. Each rat was studied for 3 hours, during which images were taken every 30 minutes.

Results and discussion

Fluorination

Compound **3a** was prepared by direct fluorination of **1** (dissolved in CH_3CN) to an azeotropic dried mixture of 4,7,13,16,21,

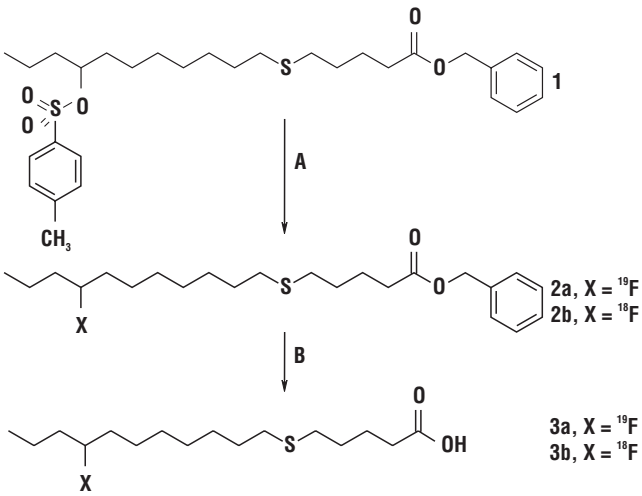

Figure 1. Production steps of [18F]FTHA.

24-hexaoxa-1,10-diazabicyclo [8,8,8]hexacosane (Kryptofix 2.2.2) and potassium fluoride, in order to obtain a standard sample. Separation of the fluorine-19 compound was performed by silica gel chromatography on glass. Infrared, ^{1}H NMR spectroscopy and elemental analysis were used to characterize the product.

The radiolabelled target molecule **3b** was prepared according to the reported method [18] (Figure 1) Compound **2** has two active sites, which are susceptible to nucleophilic attack. Higher temperature and an excess amount of base lead to hydrolysis of the ester functional group. Heating the reaction mixture up to 90°C increased the yield. Further heating of the reaction mixture reduced the yield of synthesis due to decomposition or ester cleavage of precursor and/or product (Figure 1). At the optimum temperature, the reaction yield for **3b** rose to its maximum in 8–9 min and then stayed constant (Figure 2).

Hydrolysis

This step is crucial in the production. In the case of incomplete hydrolysis, an increased amount of un-hydrolysed ester remains in the sample which cannot be removed by solid phase extraction from the final sample, and in the case of injection can cause higher uptake ratios in the liver and finally GI tract. This can not only alter the quality of the image due to lower heart uptake, but also imposes a high absorbed dose to the liver and GI system. At 90–95°C for 2 min 20% of unhydrolysed ester still remains in the reaction vessel. By increasing the time to 5–8 minutes an acceptable product will be obtained (less than 10% unhydrolysed species).

Quality control of [18F]FTHA

Radio thin layer chromatography: 1.5 μl of [18F]FTHA solution was spotted on a polymer backed silica gel paper and developed in solvent system. Figure 3 shows the purity of the pharmaceutical sample before and after passing the sample through C_{18} column.

High performance liquid chromatography

High performance liquid chromatography (HPLC) was used in order to investigate the purity of the final product with higher precision. Since the hydrolysis of the esters was shown to be complete using cold reactions, the only present components were fatty acid components. These species could easily be detected using a reverse phase HPLC column. A C18 Kromasil 100 column (250 × 4.6 mm, particle size 5 μm, Germany, Inchrom) was used as the stationary phase and a mixture of methanol:water:acetic acid (95:4.8:0.2) as the mobile phase (flow rate; 1 ml/min). The fastest component was shown to be fluoride-18 anion (2.23 min) (Figure 4).

The most abundant lipophil compound eluted at 4.75 minutes was shown to be 18F-labelled fatty acid as compared to the cold standard (19F-FTHA) (Figure 5). The best radiochemical purity using the two-step reaction module was shown to be 91% ± 3% according to our experiments.

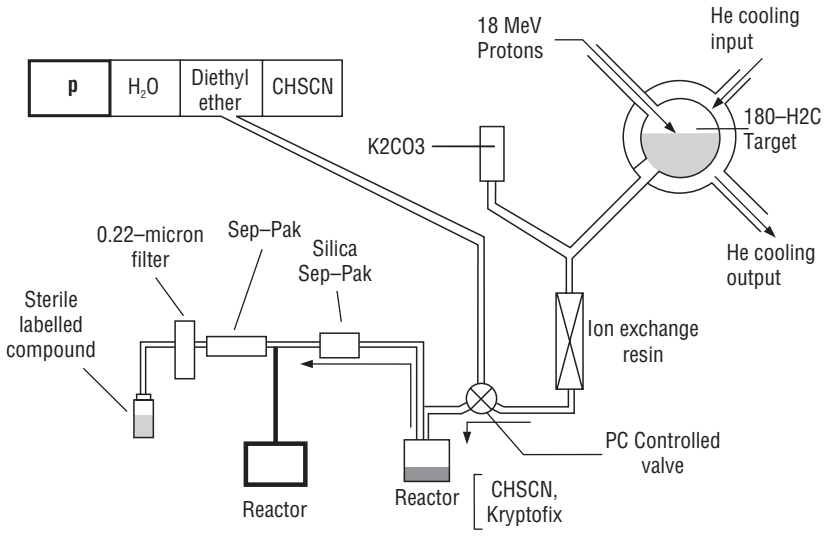

Figure 2. A schematic diagram for two-step reaction module for [18F]FTHA production.

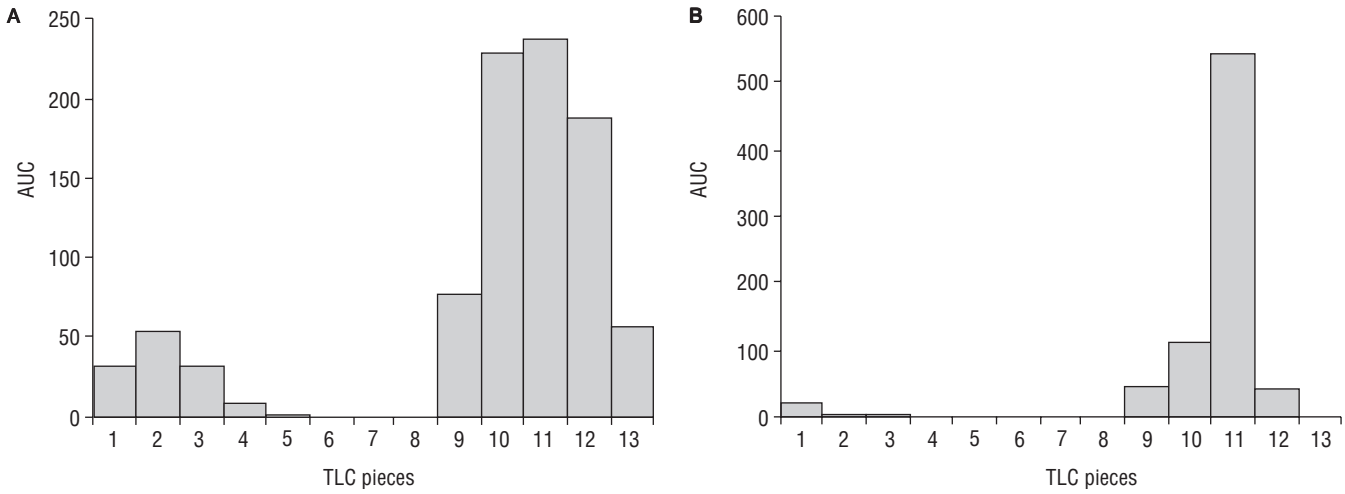

Figure 3.A. Radiochromatogram of the radiopharmaceutical sample before; **B.** C_{18} Sep-Pak passage. AUC — area under curve of 511 keV peak; TLC — thin layer chromatography.

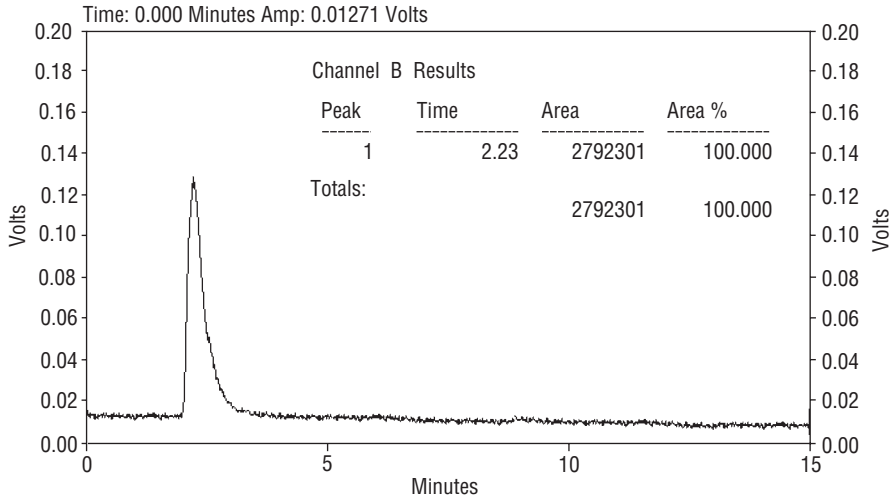

Figure 4. Radiochromatogram of [18F] fluoride using above conditions.

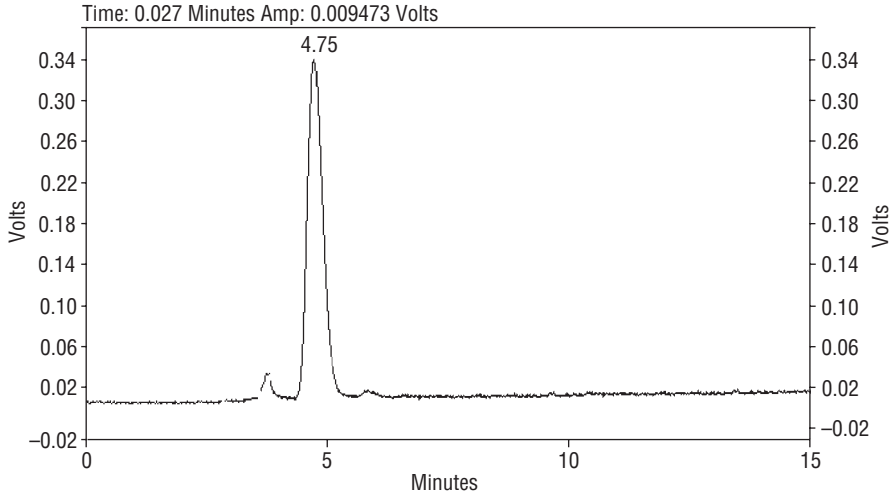

Figure 5. HPLC radiochromatogram of the final [18F]FTHA sample.

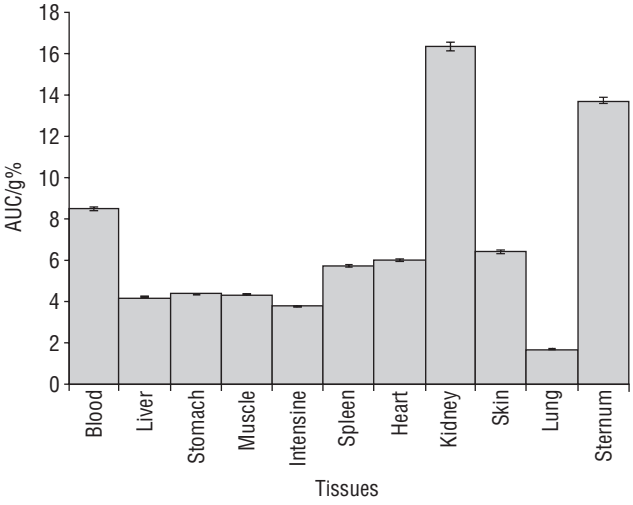

Figure 6. Bio-distribution of ^{18}F-FTHA in normal rats 1 h post-injection.

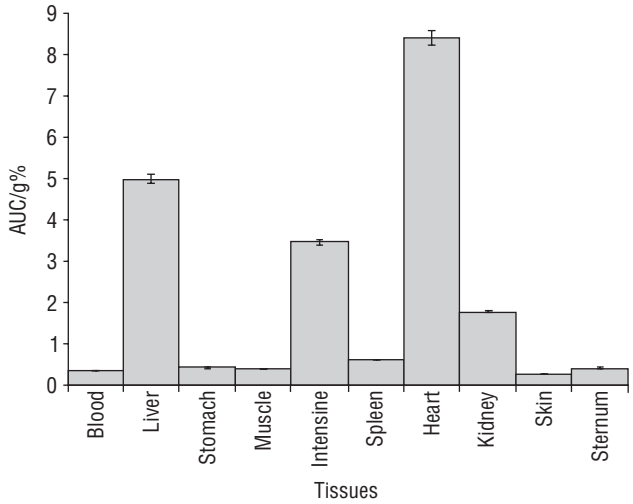

Figure 7. Bio-distribution of [^{18}F]FTHA in normal rats 2 h post-injection.

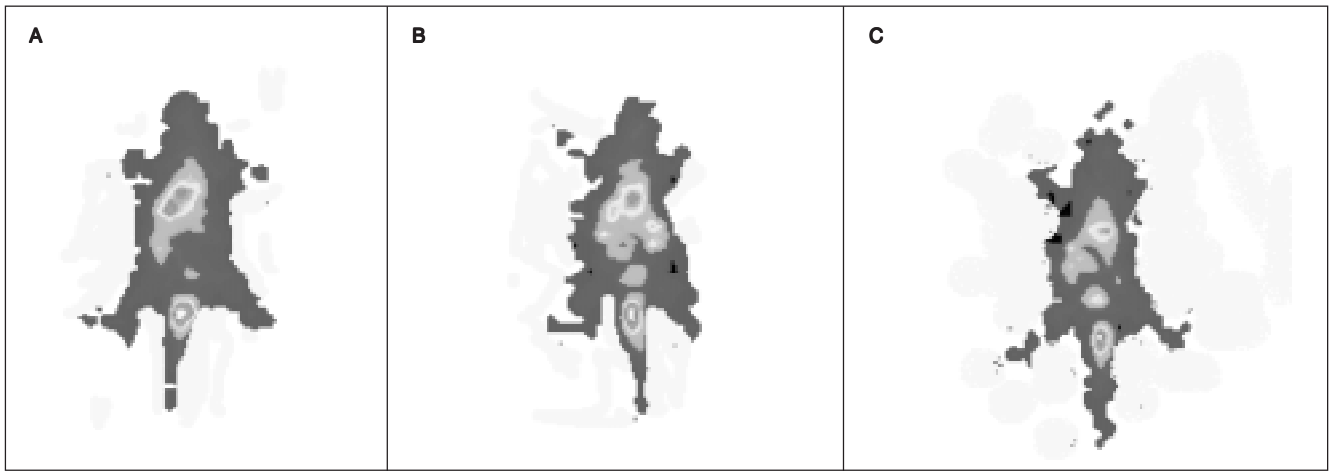

Figure 8A. Coincidence scans of [^{18}F]FTHA (50 μCi) dissolved in 5% serum albumin 1 hour; **B.** Two hours post injection; **C.** Three hours post injection.

Biodistribution of [^{18}F]FTHA in normal rats

To determine its biodistribution, [^{18}F]FTHA was administered to normal rats. A volume (25–50 μl) of final [^{18}F]FTHA solution containing 1.5 MBq radioactivity was injected intravenously to rats through their tail vein. The animals were sacrificed at exact time intervals (1 and 2 h), and the specific activity of different organs was calculated as a percentage of injected dose per gram of tissue using gamma-ray scintillation by means of an HPGe detector equipped with a sample holder device (Figures 6, 7).

In the first hour a fraction of activity was found in the kidney and sternum, possibly due to the presence of free fluoride in the solution and/or production of some radiolabelled metabolites.

Imaging of [^{18}F]-FTHA in normal rats

The best time period for scanning was shown to be 2 to 3 hours post injection; at less than 2 hours post injection a rather high accumulation is observed in the liver and GI system of the rats. After 3 hours the animals show reduced activity in the above tissues while the most of the accumulation takes place in the heart (Figure 8). Thus, 140–180 min was confirmed to be the best acquisition time post [^{18}F]FTHA injection. Interestingly, this data is comparable to other reported methods in humans (140 min post injection). Since the radiotracer is diluted in the formulation step by the addition of 5% serum albumin, the final sample is slightly viscous, and due to the fragility of rat tail veins, infiltration of the tracer in the injection area is observed in the scans.

Conclusion

A high specific activity was achieved via displacement of tosylate by the [^{18}F]-fluoride ion as previously reported. We did not observe unlabelled products upon TLC or HPLC analysis of the final products. The fluorine-18 compound prepared from **1** was

examined repeatedly by different chromatographic methods and showed a consistent final specific activity in excess of 74 GBq/ /mmol (limit of detection). The value was consistent with the use of high specific activity, n.c.a. [18F]-fluoride. Furthermore, there was little (if any) dilution of the specific activity by [19F]-fluoride ion in the reagents or precursors, nor an exchange of fluorines between the [18F]-fluoride ion and the trifluoromethyl group of the triflate counter ion. The reaction conditions (temperature, time, solvent and Kryptofix/base ratio) were optimized. [18F]-FTHA can be produced in less than 20 minutes using a two-pot module at acceptable pharmaceutical purity.

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