Automated Radiosynthesis of Hexadecyl-4-[\(^{18}\text{F}\)]fluorobenzoate ([\(^{18}\text{F}\)]HFB) Using the Explora Radiosynthesizer and a Solid-Phase Extraction / HPLC Procedure

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Direct labeling of stem cells to track their in vivo biodistribution into damaged myocardium has promising therapeutic potential in ischemic heart diseases and dilated cardiomyopathy.

2-[^18F]fluoro-2-deoxy-D-glucose ([^18F]FDG)

- Labeling efficiency of bone marrow mononuclear cells (MNCs) 90.8±1.9% and cell viability > 97% (immediately after radiolabeling)
- However, not sufficient labeling efficiency for many types of stem cells like rat MSCs as significant loss (>90%) of the radiolabel occurs in the washing process.
- In our lab, human circulating progenitor cells (CPCs) displayed labeling efficiency of ~2% (1 million cells) and cell viability >95% (up to five days)
- Low labeling efficiency (10 mCi labeled CPCs, need 500 mCi FDG (2%) or use more stem cells).
Background

\[ N\text{-succimidy}-4-[^{18}\text{F}]\text{fluorobenzoate ([}^{18}\text{F}]SFB) \]

- Labeling efficiency of mouse bone marrow derived dendritic cells > 19 ± 4%.
- The retention of the labeled moiety in the cells was 44 ± 10% at 37°C and 91±3% at 4°C at 4 hours after labeling.
- Poor accumulation of radioactivity in target organ (0.18 ± 0.04%) and unexpected loss of the label from these cells, possibly due to metabolic release of the labeled alkylated moiety.
- Multistep radiochemical synthesis.

\[ [^{64}\text{Cu}]\text{pyruvaldehyde-bis-(n}^4\text{-methyl-thiosemicarbazone)} (\text{[}^{64}\text{Cu}]\text{PTSM) } \]

- Labeling efficiency of glioma cells > 70-85%.
- Significant loss of the radiolabel in the subsequent few hours.
Due to its high lipophilicity, it is quickly absorbed in the cell membrane similar to the fluorescent dyes used for cell labeling.

- labeling efficiency of rat mesenchymal stem cells (MSCs) = 25%
- Greater *in vivo* stability in comparison to the more hydrophilic $^{18}$F-FDG.
- High cell viability (97%).
Objectives and hypotheses

Hypothesis:
- HFB can be radiosynthesized from F-18 and the triflate precursor using an automated radiosynthesizer module in high yield and purity.

Objectives:
- Adapt an appropriate program for producing HFB using the Siemens Explora radiosynthesizer module.
- Assess a solid phase extraction (SPE) conditions for preliminary purification of reaction mixture.
- Determine a suitable HPLC protocol that can be implemented to isolate and identify HFB product.
- Produce HFB in our remote system in high yields and purity.
Synthesis of (\(^{18}\text{F}\))HFB and Cold HFB


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\text{Synthesis of (}\(^{18}\text{F}\))\text{HFB}
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\text{Synthesis of cold HFB as standard}
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The program adapted for the Explora radiosynthesizer module was split into four parts:

- Addition of activated F-18 to the reaction vessel.
- Addition of precursor.
- Synthesis of hexadecyl-4-[\(^{18}\text{F}\)]fluorobenzoate ([\(^{18}\text{F}\)]HFB).
- Delivering the product to the Sep-Pak column.
Radiosynthesis of (\(^{18}\text{F}\))HFB

**Explora Radiosynthesizer Protocol**

1. F-18 is produced from our RDS-111 11 MeV cyclotron via \(^{18}\text{O} (\text{p},\text{n})^{18}\text{F}\) nuclear reaction.
2. F-18 is trapped on the anion exchange cartridge (QMA).
3. F-18 is eluted from the ion exchange resin with kryptofix-222 (96 µmol) and potassium carbonate (48 µmol) solution (2:1) into the reaction vessel where water is then evaporated.
4. Trimethylammonium triflate precursor 7-14 mg (17-34 µmol), dissolved in DMSO (0.5 mL) is added into the reaction vessel and heated 70-100°C for 10-30 min.
5. The reaction was quenched with water and delivered to a pre-activated C-18 reverse phase Sep-Pak column.
Purification of ($^{18}\text{F}$)HFB

**Solid-Phase Sep-Pak purification**
- Column was washed with water (60 mL) to remove unreacted F-18, Kryptofix-222 and salts.
- The organic compounds were eluted with methanol (6 ml) and [${}^{18}\text{F}$]HFB was eluted in last 4 ml of MeOH. All the organic solvents were evaporated.

**Semi-preparative HPLC**
- The product was dissolved in a mixture of DMSO and water and injected to semi-preparative HPLC for further purifications (Prodigy C-8, 10 μm, 250×10 mm, 75/25 acetonitrile/0.1 M ammonium formate, 10 mL/min, retention time (RT) = 21 min).
- The isolated [${}^{18}\text{F}$]HFB was dissolved in 10% DMSO/saline and analyzed by HPLC (Luna C-8, 10 μm, 2 mL/min).
Analytical HPLC profile for the synthesis of $[^{18}\text{F}]$HFB
$^{18}$F]HFB and the co-injection with HFB standard
Results

- Synthesis time ~ 1 hour (From the beginning of the synthesis)
- Radiochemical Yield 20 – 50 %(decay-corrected )
- Radiochemical Purity > 99 %
- Chemical Purity > 90 %

Conclusion

- $[^{18}\text{F}]\text{HFB}$ was produced in acceptable yield, and in high radiochemical and chemical purities.
In progress

- Reaction mixture semi-prep HPLC purification without SPE is currently being tested.
- CPC labeling with HFB are currently in progress.