Radiosynthesis and Characterization of [^{18}F]FET for Preclinical Imaging of Glioblastoma in Animal Models

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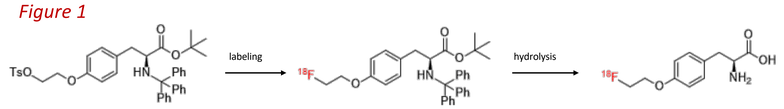
HIPPO
Horizon-Broadening Isotope Production Pipeline Opportunities

INTRODUCTION

In the field of neurooncology, magnetic resonance imaging (MRI) is the standard imaging modality for diagnosis and assessment of gliomas due to its high soft-tissue contrast and spatial resolution. However, accurate tumor delineation and characterization remains a challenge. As such, the use of amino acid PET imaging has emerged as a promising method to overcome these limitations. [1]

[^{18}F]fluoroethyltyrosine ([^{18}F]FET) is one of the amino acid tracers that has gained popularity for glioblastoma (GBM) imaging, as it targets L-type amino acid transporter (LAT) receptors, which are overexpressed in brain tumor cells undergoing rapid proliferation.[2] Studies have shown use of this radiotracer can result in improved tumor delineation, lesion differentiation, and could potentially be used to determine the severity of the tumor.[1]

Figure 1



General radiosynthesis protocols have been established, and can be broadly summarized by Figure 1. However, a standarized methods are still required for cyclotron production, manual radiosynthesis, and compound characterization.

METHODS

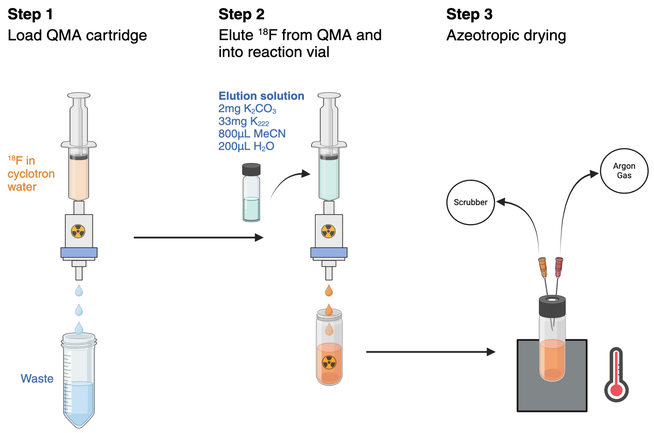
Based on Telix Pharmaceutical's automated synthesis protocol, a manual radiochemistry procedure was established. This procedure can be split into three main parts:

1. Isolation of ^{18}F from target water
2. FET synthesis (labeling and hydrolysis)
3. Product isolation

Isolation of ^{18}F

Fluorine-18 was produced in the University of Wisconsin's GE PETTrace cyclotron via the ^{18}O(p,n)^{18}F reaction. Enriched ^{18}O water was utilized as target medium.

Figure 2



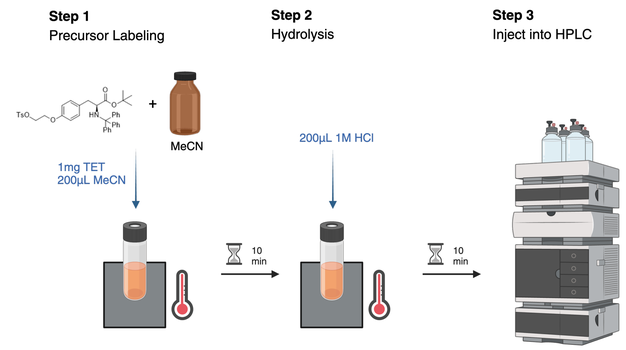
The produced ^{18}F was then isolated from the irradiated water via anion exchange chromatography by use quaternary methyl ammonium (QMA) cartridge. The cartridge was loaded with the ^{18}F/H_2O obtained from the cyclotron into a waste vial, and then eluted with a K_{222} and K_2CO_3 solution. Afterwards, the eluted compound was dried azeotropically with a constant flow of Argon gas. This procedure is summarized in Figure 2.

METHODS

FET Synthesis

(2S)-O-(2-tosyloxyethyl)-N-trityl-tyrosine-tert-butyl ester (TET) was dissolved into MeCN and added to the reaction vial. After 10 minutes, HCl was added to the vial and hydrolysis was allowed to proceed for another 10 minutes. Once reaction was complete, 3.6mL of water were added to the vial, and the solution injected into the HPLC. This procedure is summarized in Figure 3.

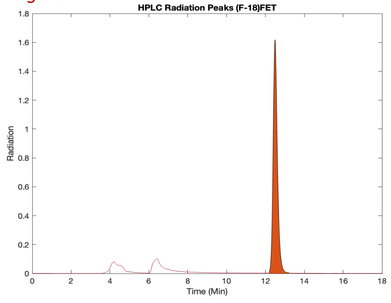
Figure 3



[^{18}F]FET Isolation

Using the HPLC Chromatograph, the product was isolated by manually moving the output line into a sterile vial once the desired radiopeak appeared. (Highlighted in Figure 4) Once standard conditions had been established, the peak was collected in 500µL intervals, and the overall concentration and composition of the peak analyzed through various methods

Figure 4



RESULTS

Tested synthesis conditions included heating at both 120° and 130° C, leaving needles in during reaction, having a completely sealed vial, and breaking down labeling into two separate steps. RCC and ndcRCY were calculated for more accurate comparison.

$$RCC = \frac{Decay\ Corrected\ Activity\ of\ Fraction}{Total\ Activity\ Collected\ from\ HPLC} \qquad ndcRCY = \frac{Activity\ at\ End\ of\ Synthesis}{Starting\ Activity}$$

Overall, it was found that higher yields were obtained when reaction occurred at 120° C and needles were pulled out after dry-down. Some of these results are summarized in Table 1.

Temp	Needles	RCC	ndcRCY
120°	Taken Out	32%	15%
130°	Left In	19%	5%
130°	Taken Out	21%	10%
120°	Taken Out	65%	40%

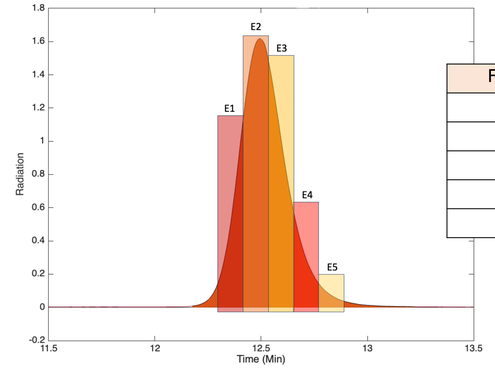
Table 1: RCC and ndcRCY for various conditions

Further testing was done at these optimized conditions in order to analyze reproducibility. For these replicates, the RCC was found to have a value of **54% ± 19%**, while the ndcRCY was found to be **23% ± 14%.** Values show clear improvement over other conditions tested, although there still remains considerable variation between replicates.

RESULTS

Similarly, a fractionated analysis of the product peak (as shown in Fig. 4) showed higher concentration (in terms of µCi/µL) on the fraction corresponding to the peak. In Figure 5, a general guideline for the 500µL fractionation is shown, although it is important to note that neither the volume nor the timing is exact due fraction collection being done manually.

Figure 5



Fraction	% of Peak Act.
E1	20% ± 10%
E2	51% ± 11%
E3	22% ± 8%
E4	6% ± 4%
E5	2% ± 1%

CONCLUSIONS & FUTURE WORK

- Optimized reaction conditions (120° heat with fully sealed vial) show high RCC and ndcRCY values
- Fractionation of the FET peak shows highest concentration corresponding to fraction containing the peak (E2 in our diagram)
  - From our activity measurements, we also noticed fractions contain enough activity for animal imaging (based on 400µCi in 500µL being used in each scan)
- More work is needed to determine other factors affecting variation levels in replicate synthesis.

Future work includes translating the methods utilized for this manual chemistry into automated procedures for a chemistry box, as well as increasing replicability of product yield. Similarly, more work is needed in order to standardize the procedures utilized for quality control and product characterization.

Overall, the methods described show consistent success at synthesizing [^{18}F] FET, and can reliably be used for production of the amino acid tracer in future pre-clinical imaging studies.

REFERENCES

[1] Galldiks, Norbert, et al. "Amino acid PET in neurooncology." Journal of Nuclear Medicine 64.5 (2023): 693-700.

[2] Jackson, Luke R., et al. "Use of multimodality imaging, histology, and treatment feasibility to characterize a transgenic Rag2-null rat model of glioblastoma." Frontiers in Oncology 12 (2022): 939260.

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