SYNTHESIS AND EVALUATION OF [^{18}F]LABELLED AMINO ACIDS FOR PET IMAGING.

By

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A thesis submitted to the

Board of Studies in Chemical Sciences

In partial fulfilment of requirements
For the degree of

DOCTOR OF PHILOSOPHY

of

HOMI BHABHA NATIONAL INSTITUTE

August, 2017

Homi Bhabha National Institute[1]

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DECLARATION

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List of Publications arising from the thesis

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a. Publications:

1. Fully Automated Synthesis of O-(2-[^{18}F]Fluroethyl)-L-Tyrosine ([18F]FET) using Solid Phase Extraction Purification with Neutral Alumina. **Lakshminarayanan N**, Amit Kumar, Sushant Roy, YogitaPawar, P. R. Chaudhari, M G R Rajan, Journal of Radioanalytical and Nuclear Chemistry. Journal of Radioanalytical and Nuclear Chemistry, accepted June 2016, DOI 10.1007/s10967-016-4900-8 *Journal of Radioanalytical and Nuclear Chemistry*, December 2016, Volume 310, pages 991–999.

2. Improved method for preparing Ni(II) Complex of (S)-Tyrosine Schiff's Base and its use in the automated synthesis of O-(2'[^{18}F]fluoroethyl)-L-Tyrosine using Solid Phase Extraction purification. **Lakshminarayanan. N**, Amit Kumar, Sushant Roy, YogitaPawar, P. R. Chaudhari, M G R Rajan, DOI: 10.10.16/j.apradiso.2017.05.017 *Applied Radiation and Isotopes*, September 2017, Volume 127, pages 122-129.

3. Synthesis of O-(1'-[^{18}F]Fluoropropan-2'-yl)-L-tyrosine (1-[^{18}F]FPT) via Ni(II) complex of (S) tyrosine Schiff's base precursor.**N Lakshminarayanan**, Sharmila Banerjee, MGR Rajan, DOI: 10.1007/s10967-017-5416-6.*Journal of Radioanalytical and Nuclear Chemistry.*August 2017, Volume 314, pages 483 – 490.

b. Papers presented in Conferences:

1. Preparation of ^{18}F-Fluoroethyl-L-tyrosine ([^{18}F]FET): Preliminary Studies. Lakshminarayanan. N, Arjun G, Rajan M. G. R, Ind. J.Nucl. Med., 26(2011), RP-16.
2. Fully automated One-Pot synthesis of ^{18}F-Fluoroethyl tyrosine using SPE method. N. Lakshminarayanan. N, MGR Rajan, Ind. J. Nucl. Med., 28(2013), RP-21.
3. Synthesis of Ni(II) complex based precursor for ([^{18}F]Fluoroethyltyrosine. N. Lakshminarayanan, MGR. Rajan., Ind. J. Nucl. Med., 28(2013), RP-22.
4. Fully automated synthesis of [^{18}F]Fluoroethyl-L- tyrosine using in-house Ni(II)-complex precursor using SPE method. Lakshminarayanan N, Amit Kumar, M. G. R. Rajan. Conference of Society of Nuclear Medicine SNMICON2014, Kolkata, RPh-O13.
5. Bio-distribution of [^{18}F]Fluoroethyl-L-Tyrosine in B16F10 melanoma bearing C57BL/6 mice. Lakshminarayanan N, Amit Kumar, Sushanta Roy, YogitaPawar, Sheetal Powde, Pradip Chaudhari, M. G. R. Rajan. Conference of Society of Nuclear Medicine SNMICON2014, Kolkata, RPh-P14.
6. Improved and fast method for the synthesis of precursor for [^{18}F]fluoroethyltyrosine based on Ni(II)-(S)BPB-tyrosine complex. Lakshminarayanan N, Amit Kumar, M. G. R. Rajan. National conference on Advancements and Innovations in Chemistal Sciences – Jan 2015, Mumbai University, O-18.
7. Synthesis of [^{18}F]Fluoropropyl-S-tyrosine ([^{18}F]FPT) via Ni(II) Complex of Chiral Schiff's base with tyrosine precursor. Lakshminarayanan. N., M.G.R. Rajan, Sharmila Banerjee. Proceedings of NUCAR-2017, Bhuvaneshwar, F-17, 502.

Dedicated

To my

Parents

Acknowledgements

I wish to record my deep sense of gratitude and sincere thanks to my learned research Co-guide Prof. M. G. R. Rajan, (Guide till June 2016) and guide Prof. Sharmila Banerjee, for their unstinted inspiration, guidance, encouragement, valuable suggestions and good wishes throughout my entire research tenure. Their illuminating guidance and assistance helped me to prepare this thesis.

It is my great privilege to acknowledge Prof. B.S. Tomar,Director, Radiochemistry and Isotope group, BARC who scrutinized my entire research work and offered invaluable scientific suggestions during the course of this research work.

It is my great pleasure to thank Dr. M. R. A. Pillai, Former Head, RPhD, BARC and Dr. N. Ramamoorthy, Former AD, RC&IG and Chief Executive, BRIT, who as Chairman of the Doctoral Committee for their constant encouragement and giving me the opportunity to work on this wonderful field of radio-synthesis of[F-18]Labelled amino acids.

I wish to pay my sincere thanks to Dr. Sunil Ghosh, BOD, BMG, BARC inculcated in me the interest for organic synthesis and for giving me valuable suggestions in the synthesis part throughout my work.

I am very grateful to Associate Prof. Dr. Aruna G. Korde and Prof. Sunil Ghosh of the doctorial committee for the critical reviews and suggestions all through the review of the progress of my work and pre-synopsis viva-voce. I am grateful to them for critically reviewing this thesis.

I shall be ever grateful to Dr. Grace Samuel (now retired), Dr Aruna Korde, IP & AD for providing help for bringing the radio-pharmaceuticals to human use.

I would also like to thank Dr. P. R. Chaudhari, ACTREC, Dr. B. L. Malpani, RMC, Mr. H. Moghe, RMC for providing animal PET/ CT images.

It gives me immense pleasure to acknowledge my friends and PET radiochemistry colleagues in the Medical Cyclotron Facility: Mr. S. Nayak, Mr. Amit Kumar, Dr. S. K. Nandy, Dr.C. Rajesh, Mr. A. Mitra, and Mr. Abhinav Bajpai, for their constant help and cooperation throughout my work.

Finally, I would like to acknowledge the Medical Cyclotron Operation Staffs, Mr. G. Arjun, Mr. B. K. Sharma, Mrs. Kanchan Kushwaha and Mr. Yuvraj Nitin, MCF, RMC, BARC for providing radioactivity as per our requirements.

Last but not the least, I would like to express my deep gratitude to my Wife, Son and Parents, without their constant support, love and encouragement, this thesis would not have been possible.

Feb, 2018 *Lakshminarayanan. N*

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HOMI BHABHANATIONAL INSTITUTE

SYNOPSIS OF Ph. D. THESIS

Name of the Student:	**Lakshminarayanan. N**
Name of the CI:	**Bhabha Atomic Research Centre**
Enrolment No.:	**CHEM01201104027 Dt: 01.09.2011**
Title of the Thesis:	**"Synthesis and Evaluation of [^{18}F] - Labelled amino acids for PET imaging"**
Board of Studies:	**Chemical Sciences**

SYNOPSIS

Nuclear medicine (NM) is a branch of medicine that uses radioactivity for the diagnosis and therapy for a wide range of diseases. To achieve this, nuclear medicine uses radio-isotope-labelled molecules, termed as tracers. These radio-labelled molecules are prepared with adequate purity and uniform composition for administration in patients as radiopharmaceuticals (RPs). RPs are of two types: diagnostic and therapeutic. The former are prepared with radioisotopes that are gamma emitters (γ) or positron emitters (β^+) and the radiation emitted from the RPs is detected by detectors placed outside the body. The radiation data is used to get a tomographic image of the radioactivity distributed in the patient's body. Thus, by choosing specific radiopharmaceuticals that will go to a specific organ or diseased area in the body, a non-invasive and functional image of the organ/disease can be obtained, which allows the clinician in diagnosing and managing the disease. Diagnostic RPs are administered in very small quantities, while therapeutic radiopharmaceuticals, which are largely β^-emitters are administered in much larger quantities to ablate the diseased tissue. The use of α-emitters like ^{223}Ra is finding use in specific diseases because of its very high LET.

Based on the radio-nuclide used, diagnostic nuclear imaging technique can be broadly classified into Single Photon Emission Computed Tomography (SPECT) and Positron

Emission Tomography (PET or PET-CT). The former uses gamma emitting radio-nuclides like ^{131}I, ^{99m}Tc, ^{67}Ga, ^{201}Tl etc, while the latter uses positron emitting radioisotopes like ^{18}F, ^{11}C, ^{68}Ga, ^{13}N etc. While SPECT imaging utilizes the collimated detection of emitted gamma photon, whereas PET imaging makes use of the simultaneously emitted pair of 511Kev gamma, resulting from annihilation of positrons with electrons, and detected by timed coincidence counting (electronic collimation) in order to locate the source of emission. Thus the resolution of a SPECT image is limited by the collimator used, whereas in case of PET, these limitations do not arise and, hence, images with a resolution that is superior to SPECT can be obtained.

Positron emitting radio-nuclides used for PET imaging studies (PET radio nuclides) are generally short lived and are mostly produced in cyclotrons or obtained from generators. The former are the source for^{18}F, ^{11}C, ^{13}N and^{15}O, while ^{68}Ga and ^{82}Rb are generator produced. Since most of these radio-nuclides are isotopes of commonly available stable nuclides (eg. ^{12}C, ^{16}O, ^{14}N, etc), they provide a better scope to study the biomolecules for medical applications, without affecting their metabolic properties[1].

Apart from the above stable nuclides H is the most common element in living systems. Since there is no γ-emitting isotope for ^{1}H, fluorine can be used in its place even though fluorine is not a common element present in biomolecules. ^{18}F can be susbstituted for -H or –OH, in most cases, without causing steric hindrance or affecting biological uptake.^{18}F-RPs are generally metabolically stable compounds. However, substitution of ^{18}F in the biomolecules, may affect its metabolic properties, and they may remain un-metabolized, once transported into the cells. This is actually an advantage, since the accumulation of the RPs inside the cells, known as metabolic trapping, provides better images with high resolution for diagnosis.

[^{18}F]Fluorine is also the PET radio-nuclide of choice because of its relatively higher half-life ($T_{1/2}$ = 109.8 min), and, hence, can be used for a longer period of time, offering logistic advantages of transportation over fairly long distances from its site of production. That is, a medical cyclotron can supply ^{18}F-RPs to several PET-imaging centres. Further, ^{18}F emits positrons with relatively low energy (β_{max}= 630KeV), providing better resolved PET images.[2] Due to its convenient production in cyclotron, by irradiating enriched [^{18}O]H_2O, using the $^{18}O(p,n)^{18}F$ reaction, it is widely used in radio-chemical synthesis.

Among the non-FDG ^{18}F-RPs, [^{18}F]FET is an important one considering its relevance in imaging brain tumours. In this thesis, different aspects of the radio-synthesis of [^{18}F]FET and simple purification procedures using solid phase extraction methods, which can be implemented under GMP compliance production procedures are explored. Results from studies on synthesis of various ^{18}F-labelled amino acids through Ni(II)-(S)-BPB-aminoacid Schiff's base complex precursor have been discussed. The thesis is divided into four different chapters. The contents of each chapter are briefly outlined below.

Chapters 1:

Introduction

This introductory Chapter begins with the historical developments in the use of radio-nuclides in life-science and particularly in nuclear medicine. The differentiation of the non-invasive imaging techniques, depending on the type of radio-nuclide used (SPECT and PET), and the superiority of the latter over the former is discussed in detail. A list of important positron emitting radio-nuclides and their nuclear data is also included. A comparative analyses of the nuclear data of the different positron emitters to show the near-ideal characteristic of ^{18}F as a PET radio-nuclide is discussed. Production pathways of important positron emitting radio-nuclides with a special emphasis on the most four common ones i.e. ^{15}O, ^{13}N, ^{11}C, ^{18}F are described in details. Basic concepts of no-carrier added radio-synthesis, different types of ^{18}F-fluorination techniques with specific examples viz., the production of the most widely used 2-[^{18}F]FDG. The physiological limitationsof 2-[^{18}F]FDG as a PET-tracer and the importance of ^{18}F-labelled amino acids in order to overcome the former's limitations, and commonly used amino acid-PET-RPs are discussed. The chapter describes different synthetic methodologies of ^{18}F- amino acids with specific emphasis to more widely used [^{18}F]FET and [^{18}F]6-fluoro-3,4-dihyroxy-L- phenylalanine([^{18}F]FDOPA) and synthesis of their precursors. The chapter ends with brief introduction to the advantages of using precursors based on novel Ni(II)-(S)BPB-aminoacid Schiff's base complex, for the synthesis of [^{18}F]-labelled fluoro-aminoacids

Chapter 2:

Fully automated one-pot synthesis of [^{18}F]FETand purification using solid phase extraction (SPE) method from commercially available TET precursor

[^{18}F]labelled amino acids have gained more importance as it is convenient for radio-synthesis and clinical studies. In this context, O-(2'-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) has gained high importance due to high uptake in cerebral tumors especially gliomas.[1]

[^{18}F]FET has been synthesized by several methods. Initially the method followed was by indirect labelling, which is a two step synthesis, and later by direct-labelling using a single-pot two-step synthesis method.[2] This method is similar to the 2-[^{18}F]FDG synthesis and, hence, requires single reactor vessel. Moreover, [^{18}F]FET synthesis needs to be enantioselective with >90% *l*-enantiomer in the final formulation, since *d*-enantiomer of [^{18}F]FET is not taken up by cells.[3]

Among the precursors available for the radio-synthesis of [^{18}F]FET, O-(2'-tosyoxyethyl)-N-trityl-L-tyrosine-tert-butyl ester (TET) is reported to provide [^{18}F]FET in its enantiomerically pure form[4] But the authors have performed the final purification using semi-preparative radio-HPLC method.

This Chapter is sub divided into two parts. The first-part concentrates on the process and the methodology utilized for the production of pharmaceutical grade [^{18}F]FET, using different SPE resins for purification. The suitability of producing [^{18}F]FET in radio-chemistry module configured for producing 2-[^{18}F]FDG is also highlighted since this will enable all those producing only 2-[^{18}F]FDG to also consider producing [^{18}F]FET, Step by step standardization of the [^{18}F]FET synthesis process, characterization of various impurities, development of methodologies to identify them thorough radio-TLC are being elaborately discussed. This part briefly describes about the necessity for modifications, and standardization of methods used for the synthesis of [^{18}F]FET in order to simplify the purification step using neutral alumina column and avoiding semi-prep radioHPLC.

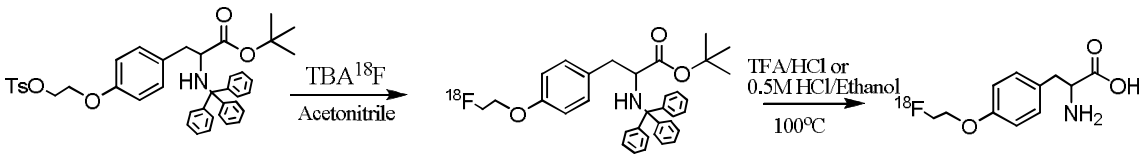

Fig. 1 Synthesis of [^{18}F]FET using TET precursor

The second-part discusses in detail the modifications and developments implemented in the synthesis process of [^{18}F]FET, using in-house prepared neutral alumina column for SPE purification, and also automation of the whole process. This in-house developed, simple and economic procedure for the production of [^{18}F]FET by adapting the [^{18}F]FDG module is discussed in detail along with the quality control tests, which includes radio-chemical, chemical analysis, micro-biological evaluation, etc.

The synthesis of [^{18}F]FET is performed by a single-pot two-step synthesis method. The precursor used was a suitably substituted and protected precursor O-(2'-tosyoxyethyl)-N-trityl-L-tyrosine-tert-butyl ester precursor (TET), which is labelled with [^{18}F]fluorine by nucleophillic substitution in the first step followed by deprotection in the same reactor (Fig. 1). Among the precursors available for the radio-synthesis of [^{18}F]FET, TET precursor is the preferred one as it is reported to provide [^{18}F]FET in its enantiomerically pure form. This method is similar to 2-[^{18}F]FDG synthesis and, hence, can be carried out in a single reactor vessel. Further simplification of the synthesis procedure and fine tuning of the production process are elaborated. It also describes the experiments carried out and results obtained in optimization of the reaction parameters to obtain the maximum radio-chemical yield with the use of minimum amount of precursor to make the developed process highly economical. The improvements in the process to reduce the presence of cold impurities, by varying several parameters, are discussed. Data from bio-distribution studies in tumor bearing mice and PET/CT imaging studies in rabbit are shown and their relevance elaborated. The experimental proofs (Radio-TLC, Radio-HPLC, HPLC by monitoring UV, aluminium ion (Al^{3+}) tests, sterility and bacterial endotoxin tests to ensure microbiological quality control) are given to ensure that the synthesized [^{18}F]FET is of pharmaceutical grade in quality and is free of any radioactive and non-radioactive impurities. Complete evaluation of reliability and reproducibility of the process, formation of SOP, performing trial runs with automated procedure including purification, QC manual and clearance from Radio-pharmaceutical Committee (RPC) clearance are discussed. PET/CT images of normal healthy rabbits and micro-PET/CT with normal and tumor bearing mice models and biodistribution studies at different time points are included. Uptake in tumors and low localization in brain as well as rapid clearance through bladder are shown. Typical PET/CT images of [^{18}F]FET in normal human volunteer studies, have been shown.

In summary, development of an efficient, GMP compliant, fully automated synthesis procedure for [^{18}F]FET using the TET precursor, which can easily be adapted in the simple and more commonly available [^{18}F]FDG module as demonstrated in this chapter.

Chapter 3:

Fully automated synthesis of [^{18}F]FET using Ni(II)-(S)-BPB-alkylated-(S)-tyrosine complex precursor

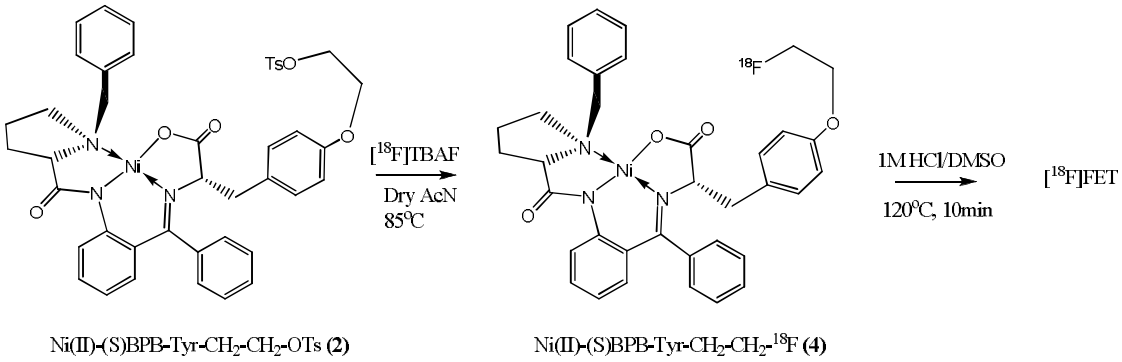

Fig. 2 Synthesis of [^{18}F]FET using Ni(II)-(S)-BPB-(S)-Tyr-CH_2CH_2-OTs precursor

Recently, a novel precursor, based on Ni(II)-(S)BPB-(S)-tyrosine Schiff's base complex was reported to provide [^{18}F]FET with enantiomeric purity of better than 95%[5]. It is a one-pot two-step synthesis similar to 2-[^{18}F]FDG synthesis. The purification procedure, reported by others, followed after radio-synthesis using this precursor is by semi-preparative radio-HPLC. Simplifying the procedure, by replacing cumbersome semi-prep radio-HPLC purification method by SPE by using suitable resins, would reduce the synthesis cost and time. Moreover, the synthesis procedure can easily be adapted in commonly available 2-[^{18}F]FDG synthesis module. Since radio synthesis of [^{18}F]FET based on this Ni(II)-(S)-BPB-(S)-tyrosine Schiff's base complex (Fig. 2), utilizes milder reagents and conditions for the radio-synthesis, it is more attractive as a precursor for regular production. This Chapter describes in detail the optimization of the radio-synthesis procedure, identification of various impurities in the radio-synthesis process, and standardization of purification technique based on in-house prepared economical single neutral alumina cartridge. Minor modifications, and development of fully automated procedure including SPE extraction purification were discussed. Several QC tests of parameters viz., radio-TLC, radio-HPLC, UV absorption-HPLC and chiral-HPLC (for enantiomeric purity), evaluation of Ni(II) ion concentration, aluminium ion tests, sterility and bacterial endotoxin tests are discussed. The latter two are

required to ensure microbiological quality control. All the QC tests are required to ensure that the synthesized [^{18}F]FET is of pharmaceutical grade and suitable for human use. Biodistribution data and micro PET/CT images of [^{18}F]FET produced by this method, in tumor bearing mice models, are shown, in order to evaluate the uptake in tumor.

In summary, the work described in this Chapter demonstrates a GMP compliant and fully automated synthesisprocess for [^{18}F]FET using Ni(II)-(S)-BPB-(S)-tyrosine complex based precursor, which can be easily adapted in any of the commonly available [^{18}F]FDG synthesis modules.

Chapter 4:

Synthesis of Ni(II)-(S)BPB-amino acid alkylated Schiff's base complex precursor for [^{18}F]labelled amino acids

Amino acids are biomolecules which have multiple functional groups. In order to radio-label them with ^{18}F, it is essential to protect these groups, which otherwise will interfere with the ^{18}F-fluorination. Hence, the -NH_2, -COOH and other special functional groups need to be protected in a proper-sequence of steps, and the position in which ^{18}F needs to be radio-labelled should be activated by a suitable leaving group viz. tosylate, mesylate, nosylate, etc. Apart from all these, the synthetic process should not affect the enantiomeric purity of the amino acids significantly. Hence, synthesizing [^{18}F]amino acid precursors are multi-step, tedious and expensive process, which increases the cost of production and consequently the cost of precursor too. This can be circumvented by Ni(II)-(S)-BPB-aminoacid Schiff's base complex precursor, which protects both amino and the acid group by single complex formation [5]. Moreover these precursors, being coloured, can be easily detected during their synthesis and purification steps. These complexes are also found to have a very high stability under basic conditions.

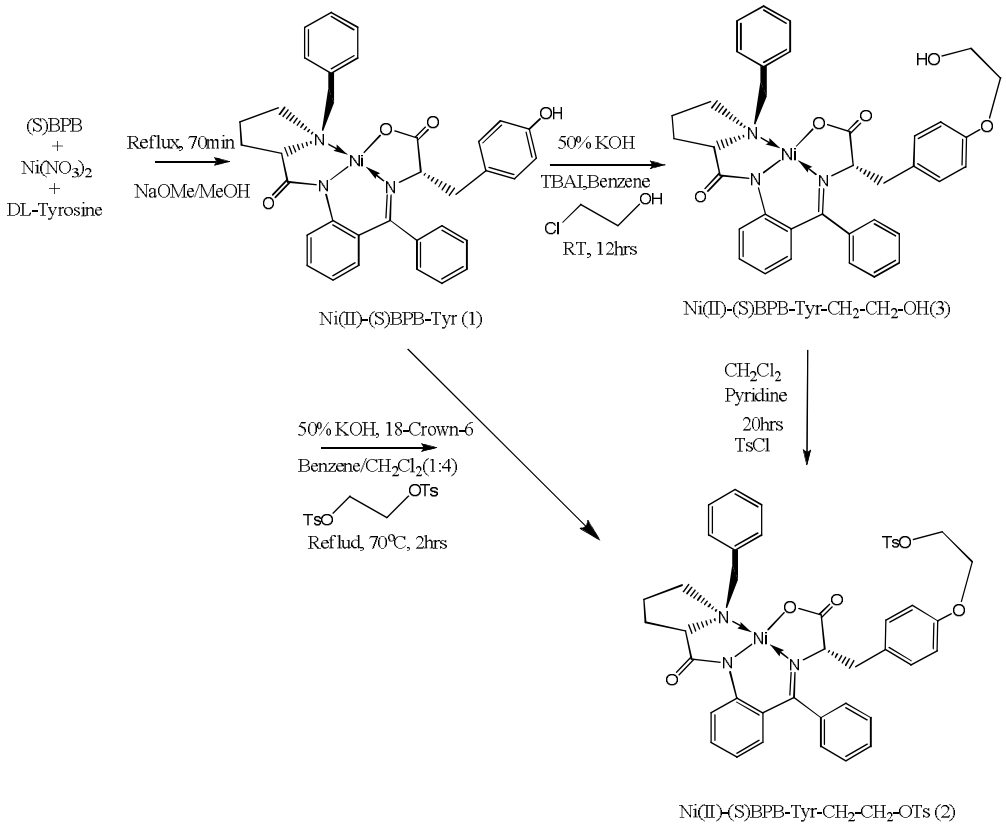

Fig. 3. Synthesis of Ni(II)-SBPB-S-tyr-CH_2-CH_2-OTs precursor for [^{18}F]FET synthesized in two steps in comparison to a reported three step method with reaction time reduced from 33 hrs to 3 hrs.

In this Chapter, synthesis of precursors for various ^{18}F- labelled amino acids via Ni(II)-(S)BPB-aminoacid complex precursor is discussed. (i) Synthesis of amino acid complexes by various routes, starting from glycine complex, for the preparation of [^{18}F]Fluoroethyl and fluoropropyl cysteine. (ii) Synthesis of Ni(II)-(S)BPB-tyrosine complex for the radiosynthesis of [^{18}F]fluoroethyl and [^{18}F]fluoropropyl tyrosine. (iii) Improvement in the synthesis process of Ni(II)-(S)BPB-tyr-CH_2-CH_2-OTs precursor for the synthesis of [^{18}F]FET (radiosynthesis of [^{18}F]FET with this precursor is discussed in Chapter 3). (iv) Fast purification using "Serial Column Flash Chromatography" method for the synthesis of coloured compounds such as Ni(II)-(S)BPB-tyr-CH_2-CH_2-OTs precursor are discussed.

In summary, various approaches for the synthesis of [^{18}F]-labelled fluoro-aminoacids, which may have potential as PET radio-tracers were explored. Improvement in the synthesis and fast purification for the synthesis Ni(II)-(S)BPB-tyr-CH_2-CH_2-OTs precursor for [^{18}F]FET are shown.

Conclusions

The following conclusions can be drawn from the studies outlined in this thesis:

- [^{18}F]FET can be produced by a process, that is as simple as for 2-[^{18}F]FDG, in a fully automated procedure in GMP facilities using neutral alumina column for SPE purification, avoiding toxic solvents and achieving a high order of purity by minimizing chemical impurities.
- Production of [^{18}F]FET using novel Ni(II)-(S)BPB-(S)tyrosine complex is a viable alternative and can provide the [^{18}F]FET with higher yield by neutral alumina SPE purification.
- In the context of the preparation of Ni(II)-(S)BPB-(S)tyrosine Schiff's base precursor, the use of "SerialColumn Flash Chromatography", is a more efficient and economical method for isolating the coloured complex in a pure form.
- The Ni(II)-(S)BPB-aminoacid Schiff's base precursor can be a better method for the synthesis of potentially useful [^{18}F]fluoro-aminoacid PET radio-tracers, due to its ease of production and high chemical stability.
- Ni(II)-(S)BPB-(S)tyrosine Schiff's base precursor is both faster to synthesize and economical to prepare, compared to that of the other precursors available for [^{18}F]FET synthesis.

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LIST OF FIGURES

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Abbreviations

2-D - 2 dimensional

3-D - 3 dimensional

PET – Positron Emission Tomography

CT – Computed tomography

SPECT – Single photon emission computed tomography

MRI – Magnetic Resonance Imaging

RPhs - Radiopharmaceuticals

HCl – Hydrochloric acid

TBA – Tetrabutyl ammonium bicarbonate

FDG – 2-fluoro-2-deoxy-glucose

FET - Fluoroethyltyrosine

HET – Hydroxyethyl tyrosine

FPT – fluoropropyl tyrosine

DOPA – Dihyroxyphenylalanine

DCM - Dichloromethane

DMSO - Dimethylsulphoxide

AcN - Acetonitrile

TFA – Trifluoroacetic acid

EtOH - Ethanol

iPrOH – Isopropyl alcohol

tBu – tertiary-butyl

SBPB – (S)-benzylproly-aminobenzophenone.

Ala - Alanine

Cys - Cystiene

Thr - Threonine

Ser - Serine

Tyr - Tyrosine

Ts – p-toluenesulphonyl.

Trityl- triphenylmethyl

CHAPTER 1
Introduction

1.1. Positron Emission Tomography

The most commonly used tomographic imaging techniques are Magnetic Resonance Imaging (MRI), X-ray Computed Tomography (CT), Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET). MRI and CT are techniques that provides the anatomical information but no significant functional information of the organs being imaged. Over the years, there has been considerable improvement in speed and resolution of imaging, use of contrast agents, but the functional information is still limited. Hence, detection of diseases using these techniques is limited to those, which can cause anatomical changes (structural abnormalities). On the other hand, the latter techniques, (i.e.,) the SPECT and PET, commonly known as emission tomography, can detect the functional abnormality in the diseased organs [1] SPECT-imaging uses gamma-ray (γ) emitting radio-isotopes, while PET-imaging uses positron emitting radio-isotopes. Thus they are non-invasive functional-imaging techniques. Between SPECT and PET, the former has been well explored for the past several decades, whereas the latter emerged in the late 1990's and, hence, is more advanced with better resolution and has a higher level of sensitivity than SPECT. Since 2000, the use of PET and PET-CT imaging has increased by leaps and bounds, worldwide, emerging as an universally accepted technique in clinical research [2-4]. Further, with the development of micro PET-CT, for pre-clinical studies in small lab-animals, the application of PET has become more wider[5]. Due to this, development of PET radiotracers, or PET-radiopharmaceuticals, has gained high importance, with specialized research being done in order to develop PET-tracers for wide varieties of diseases. To

achieve this, it is essential to develop PET radiopharmaceuticals which are readily available and appropriate for a specific clinicalutility. A few PET radiotracers and their important nuclear data are shown in Table 1.1. Positrons are anti-particles of electrons. Hence, they are highly unstable after emission. These positrons (β^+) undergo thermalization, by losing their kinetic energy, by collision with atoms in their path over a short distance. This short distance, known as range, depends on the kinetic energy of the β^+ emitted, which can vary from less than 1mm to several millimeters. After losing its kinetic energy, at rest, these β^+ combines with an electron (β^-) in its vicinity They form an exotic,transient positronium species, similar to a H-atom. They combine together, annihilate and emit γ. Thus, during this process the mass of β^+ and β^- are converted to energy according to the equation $E=mc^2$, where 'm' is the combined mass of positron and electron and 'c' is the velocity of light in space. During annihilation, two γ rays are emitted simultaneously, in opposite directions (~180º angle), for momentum conservation. Each of these γ have energy of 511keV[6]

$$^{18}F \longrightarrow {}^{18}O + \beta^+ + \upsilon$$

Thus the originally emitted β^+ by the radiotracer, converts to γ ray emissions (Fig. 1.1, a). β+ has a range in millimeters, whereas γ rays have high penetrating power. Hence, they travel outside the body, and γ are detected by scintillation detectors. A PET scanner consists of circular array of scintillation detectors. The 511 keV γ emitted by annihilation are detected simultaneously by the detectors. Their incidents are recorded by coincidence logic (Fig. 1-1, b). Thus a single positron emission is registered, only when two detectors detects the annihilation γ rays within a time frame of few nanoseconds. These annihilation events, within the visual field of these detectors are reconstructed as tomographic slices. These slices are further reconstructed into a single 3D image by computer-aided data processing (Fig 1-1). Due to this coincident measurement, the noise created by background radiation and non-

coincident radiations is reduced enormously. Hence, PET provides images of very high resolution as compared to SPECT, and, hence, is a superior imaging technique.

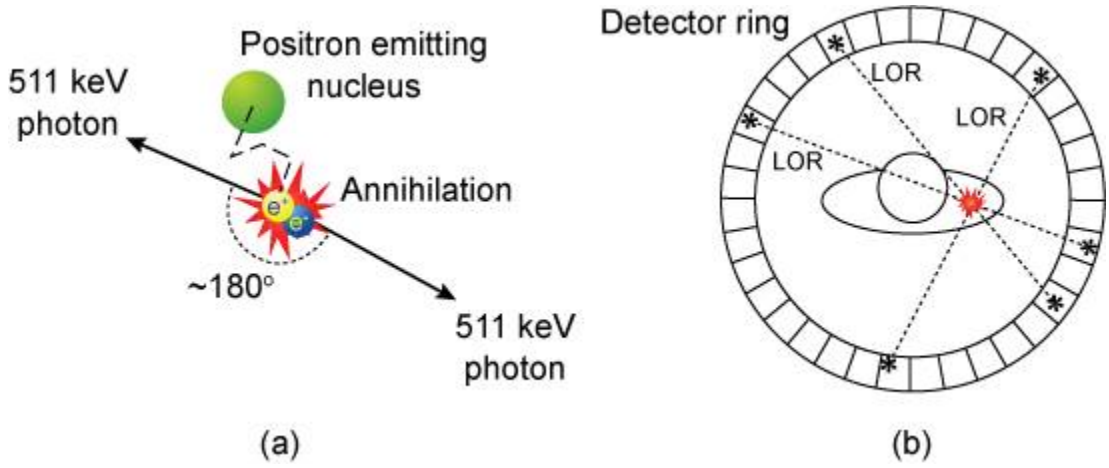


Figure1-1(a) Annihilation of PET radioisotopes in the tissue, (b) Coincidence detection using array of detectors in PET scanner.

Radio nuclide	Decay mode (%)	$E_{\beta+}$ max (KeV)	Half-life
^{18}F	β^+ (97), EC (3)	635	109.6 min
^{11}C	β^+ (99.8), EC (0.2)	960	20.4 min
^{13}N	β^+ (100)	1190	9.96 min
^{15}O	β^+ (99.9), EC (0.1)	1720	2.03 min
^{30}P	β^+ (99.8), EC (0.2)	3250	2.5 min
^{75}Br	β^+ (75.5), EC (24.5)	1740	98 min
^{76}Br	β^+ (57), EC (43)	3900	16.1 h
^{120}I	β^+ (64), EC (36)	4100	1.35 h
^{124}I	β^+ (25), EC (75)	2140	4.18 d
^{38}K	β^+ (100)	2680	7.6 min
^{62}Cu	β^+ (98), EC (2)	2930	9.7 min

^{64}Cu	β^+ (18), β- (37), EC (45)	655	12.7 h
^{68}Ga	β^+ (90), EC (10)	1900	68.3 min
^{82}Rb	β^+ (96), EC (4)	3350	1.3 min
^{86}Y	β^+ (34), EC (66)	1300	14.7 h
^{94m}Tc	β^+ (72), EC (28)	2470	52 min
^{73}Se	β^+ (65), EC (35)	1320	7.1h
^{72}As	β^+ (88), EC (12)	2515	26 h

Table 1-1 Positron emitters used for PET and their important nuclear data[7, 8]

The PET-radiotracers, when injected intravenously in the patient's body, distributes based on their pharmacokinetic behavior. Based on the temporal and local changes of the radio-pharmaceutical, the PET data, combined with a suitable bio-mathematical model, provide quantitative medically relevant images informations are obtained[9] (Fig 1-2).

The most commonly used PET-radiotracers,that are produced using a cyclotron or from generators housing the parent nuclide, are shown in Table 1-1. As it can be seen, they are short lived and some of them are elements that make up bio-molecules (like C, N and O). Hence, true-labelling without disturbing their biological activities are possible. Since the isotopes of these elements are short lived, multiple studies within a short period of time can also be performed.

PET radio-isotopes are generally produced in a compact cyclotron, known as medical cyclotron. The first four PET radioisotopes listed in Table1-1 are produced in a medical cyclotron by nuclear reactions that are shown in Table 1-2.Based on the nature of their production, they form no-carrier-added radio-nuclides, which are high in their specific

activity. This facilitates the study of biological processeswithout disturbing thewm, since the actual quantity of radio-isotope used is very small, and further assures negligible toxicological and immunological effects.

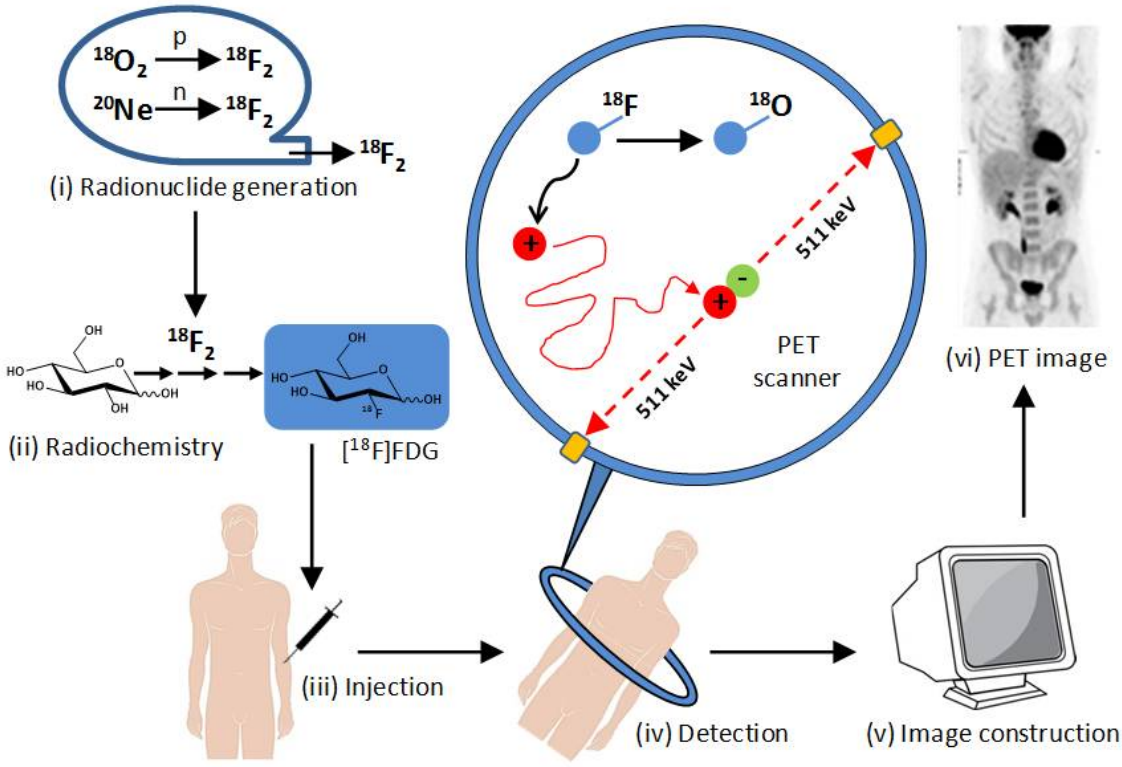


Figure1-2 Process involved in PET imaging using 2-[^{18}F]FDG[10]

Radionuclide	Nucl. Reaction	$T_{1/2}$ (min)	Target	Product	Decay product
^{18}F	^{20}Ne(d,α)^{18}F	110	Ne(+F_2)	[^{18}F]F_2	^{18}O
	^{18}O(p,n)^{18}F		[^{18}O]H_2O	[^{18}F]F^-	
^{13}N	^{16}O(p,α)^{13}N	9.97	[^{18}O]O_2(+F_2) H_2O H_2O + EtOH	[^{18}F]F_2 [^{13}N]NO_x [^{13}N]NH_3	^{13}C
^{11}C	^{14}N(p,α)^{11}C	20.4	N_2(+O_2) N_2(+H_2)	[^{11}C]CO_2 [^{11}C]CH_4	^{11}B
^{15}O	^{15}N(d,n)^{15}O	2.04	N_2(+O_2)	[^{15}O]O_2	^{15}N

Table 1-2 Commonly used radionuclides in PET, their half-lives, production reactions, target materials, products and decay products [11]

As these radio-isotopes are short-lived, it is essential that, the radio-synthesis process to prepare the radiopharmaceuticals has to be fast. The whole process including purification, formulation and quality tests needs to be performed quickly. Moreover, the process used has to be highly efficient and aseptic, since they are intended for human use. The process should not take more than three half-lives in order to obtain sufficient activity for human studies. A very low quantity of tracer - nano to pico grams are utilized for studies.[12] Moreover these radiopharmaceuticals need to be stable, during and after supply to the PET scanner locations, till its clinical use. In order to achieve this, many PET-imaging facilities have cyclotrons (for producing radioisotopes), radio-synthesis unit and PET-scanner facility under a single roof.

The latest development inPET-imaging technique is the co-registration with other tomographic methods like CT and MRI that provides high anatomical information. Due to this, 3D images with exact morphological information, accurate assessment of physiological and biochemical processes are possible.[13] This provides better convenience in clinical management during diagnosis and therapy. Since it is also essential to incorporate an attenuation correction, in order to compensate the variation in attenuation of radiation by tissues as it comes out of the body. This is performed nowadays by the low energy X-rays radiation generated by CT, and suitably extrapolated for high energy of 511keV.

Further, in advanced scanners, co-registration of PET with CT and MRI, not only provides better resolved images, with detailed information, but also reduces the scanning time and the amount of activity required for single scan (Fig 1.3).

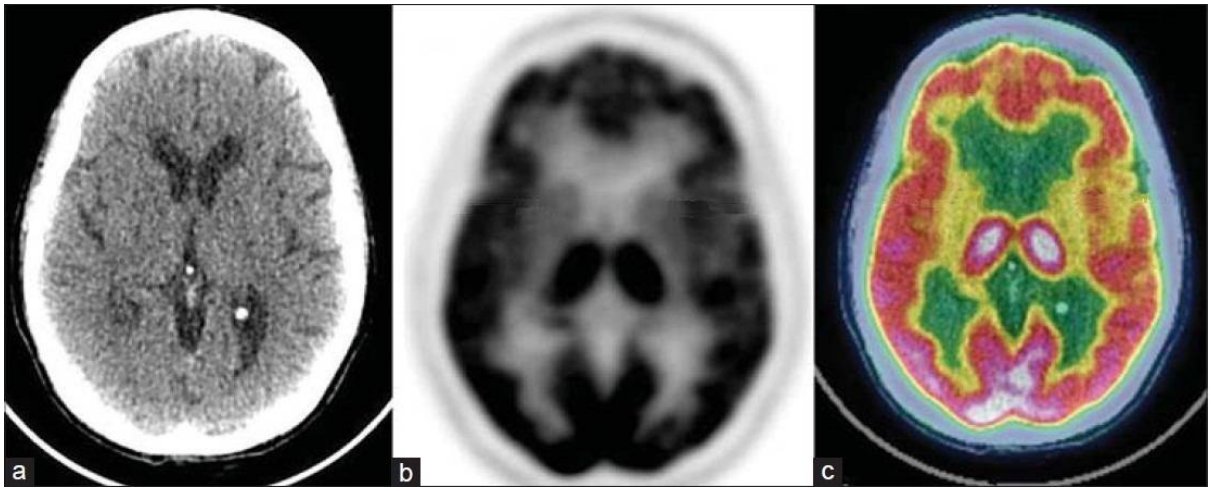


Figure 1-3 Transaxial brain images of 2-[^{18}F]FDG by a. CT, b. PET, c. PET/CT, providing clear Images on fusion of PET and CT.[14]

2-[^{18}F]FDG-PET is the most widely used PET radiopharmaceutical in the field of diagnostic nuclear medicine, especially in oncology[15, 16], as it is an analogue of glucose, which is the primary source of energy for all cells in the body. Hence, organs like brain and heart have high uptake of 2-[^{18}F]FDG, and the latter makes it suitable as a myocardial perfusion imaging agent in the field of cardiology[17-19]. In the field of neurology, it is useful in diagnosing disorders like Alzheimer's, Parkinson's disease, etc.[20-22] Malignant tumours, being the uncontrolled growth of cells, have a very high demand for energy. Thus, 2-[^{18}F]FDG has high uptake in tumors and is very useful in oncology. Apart from this 2-[^{18}F]FDG also has high uptake in infections and inflammations, and serves as an excellent imaging agent for them. 2-[^{18}F]FDG-PET has proven itself a single diagnostic tool in the major fields of nuclear medicine like oncology, neurology and cardiology. Further, PET as a molecular imaging tool, is also expected to contribute to the development of new drugs.[23-25]

1.1.1. Radio-nuclides and tracers for PET registration

PET imaging technique is a highly efficient technique that provides high sensitivity and images of high spatial and temporal resolutions. Hence, radiopharmaceuticals that are highly specific are required for the application and great care in the selection of radiotracers is essential. The PET radio-nuclides are generally divided in three broad categories,viz. organic, analogous and metallic. Organic radionuclides are those, whose stable isotopes are commonly present in the bio-moleculeslike C, N, O, P, etc. The PET isotopes of these elements are ^{11}C ($T_{1/2}$ = 20 min), ^{13}N ($T_{1/2}$ = 10 min), ^{15}O ($T_{1/2}$ = 2 min), ^{30}P ($T_{1/2}$ = 2.5 min), etc. Of them,^{11}C is the one that has a long enough half-life of 20 min, to permit its use in the radio-synthesis of [^{11}C]-labelled compounds purify them and to conduct human studies. However, it has limitations, viz., that preparing [^{11}C]-molecules requiring long synthesis procedures: multistep synthesis process, lengthy purification methods are not possible. Hence, its applications as an organic radiopharmaceutical are restricted. On the other hand, the analogous radiotracers like^{18}F ($T_{1/2}$ = 109.6 min), ^{76}Br($T_{1/2}$ = 16.1 h), ^{124}I($T_{1/2}$ = 4.18 d), etc, have longer half-life, and provide sufficient scope for producing significant amount of radiopharmaceuticals and with feasibility of accomplishing complete studies. The utility of these analogous tracers is generally limited to the similarity in steric and electronic configuration to that of the corresponding bio-molecules. A third category of radio-tracers are radio-metals that are available through generators (eg. ^{68}Ga, ^{82}Rb, ^{62}Cu, etc)[26]. These radio-tracers can also be used by nuclear medicine centres, which do not have an onsite cyclotron. In order to choose a suitable radio-tracer for application, the following criteria are to be considered: 1. Their physical half-life should be optimum in order to synthesize and conduct clinical studies. 2. Their biological half-life should be close to physical half-life in order to perform safe and complete study. 3. The radio-synthesis time should be economic and reliable, 4. The radio-pharmaceutical should be providing relevant information regarding

the disease, 5. The uptake and bio-distribution pattern should be specific for the purpose of its utility.

1.1.2. Fluorine-18 as a nuclide for PET-radiopharmaceuticals

In the current scenario, the most widely used PET radiopharmaceutical worldwide is ^{18}F. Even though ^{18}F is not an element naturally present in common bio-molecules, owing to its similarity to the most commonly present H-atom, it is the element of choice. Fluorine (1.35 Å) has an atomic size close to H-atom (1.2 Å). Moreover, it can be substituted for H and OH without significant change in their steric properties. The C-F bond strength is also strong enough to be stable under biological conditions. Hence, substitution of ^{18}F in the proper position in a molecule results in a biologically stable radiopharmaceutical. However, it may cause some variations in the metabolic pathways, due to its inherent physico-chemical and electronic properties. The most commonly used ^{18}F radiopharmaceutical is 2-[^{18}F]FDG. In this, the OH group at the second position (C-2 position) of glucose molecule is substituted by ^{18}F. Due to the similarity in the uptake pattern of glucose and 2-deoxyglucose, 2-[^{18}F]FDG shows good uptake in cells. After entering the cell, 2-[^{18}F]FDG is converted to glucose-6-phosphate almost instantaneously. The second step of the glycolysis pathway is an isomerization process of glucose-6-phosphate to fructose-6-phosphate. Since the OH in the C-2 is replaced by ^{18}F, the isomerization is not possible in 2-[^{18}F]FDG. Hence, it gets trapped in the cells and accumulates over time. This process is known as metabolic trapping. In addition to this, since tumor cells have very high energy demand resulting in elevated glucose metabolism and enhanced 2-[^{18}F]FDG uptake in tumors [27].

The success rate of cancer therapy can be followed by studying 2-[^{18}F]FDG uptake in the tumor sites. Other than tumors, 2-[^{18}F]FDG is used for diagnosis of various diseases like Parkinson's, Alzheimer's, etc. Other than 2-[^{18}F]FDG, diseases involving receptors like Parkinson's disease can be followed by specialized molecules like [^{18}F]FDOPA. A list of ^{18}F-labelled molecules in clinical use / under development are listed below in Table-3 There is continued research, to develop specialized ^{18}F-moleculesand widen the horizon for the diagnosis of various diseases.

1.1.3. ^{18}F-Radiofluorinated organic molecules

As mentioned earlier, fluorine-18 is the most commonly used PET radiotracer due to its physical and chemical characteristics. It's relatively long half life ($T_{1/2}$ = 109.8 min) makes it suitable for producing a variety of ^{18}F-radiopharmaceuticals and perform clinical studies for a longer time.[28] It has low β^{+}_{max} energy of 650 keV and maximum range of 2.4 mm in water [29]. Hence image resolution is better. Easy labelling sites are available, due to its intermediate atomic size with H and OH, providing less steric effects. Convenient and well established radio-labelling methods for fluorine are developed. The C-F bond formed is strong enough to provide in-vivo stability. Fast incorporation of ^{18}F into the target site can be achieved. Several clinically useful molecules which have been labelled with ^{18}F are shown in table 1-3.

Radio-fluorination into the organic bio-molecules are commonly performed in two ways. Viz. electrophilic and nucleophilic substitution. Since the molar quantities of ^{18}F is extremely low (nano to pico moles range), the fluorination reaction is less dependent on stoichiometry. However, for enzymatic fluorination methods, high specific activity for labelling are required[30].

^{18}F-labelled Compound	Common synthesis route	Clinical application
6-[^{18}F]FluoroDOPA Electrophilic fluorodemetallation;	nucleophilic aromatic substitution	Dopamine metabolism
6-[^{18}F]Fluoro-m-tyrosine	Fluoro-destannylation	Dopamine metabolism
6-[^{18}F]Fluorophenylalanine	Electrophilic fluorination with[^{18}F]AcOF	Neutral amino acid transport
2-[^{18}F]Fluoro-4-boronophenylalanine	Electrophilic fluorination with[^{18}F]AcOF	Neutron capture therapyTitration
[^{18}F]Fluoroethyltyrosine	O-alkylation with [^{18}F]fluoroethyl tosylate	Tumor imaging
[^{18}F]Fluoro-α-methyltyrosine	Electrophilic fluorination with[^{18}F]AcOF	Tumor imaging
6-[^{18}F]Fluorodopamine	Nucleophilic aromatic substitution	Cardiac sympathetic Innervations
(-)-6-[^{18}F]Fluoronorepinephrine	Nucleophilic aromatic substitution	Cardiac sympathetic Innervations
6-[^{18}F]Altanserin	Nucleophilic aromatic substitution	5HT2A receptors
[^{18}F]Fluoroethylspiperone	N-alkylation with [^{18}F]fluoroethyltosylate	D2 receptor
[^{18}F]Fluoromisonidazole	N-alkylation using [^{18}F]epifluorohydrin	Hypoxia imaging
5-[^{18}F]Fluorouracil	Direct electrophilic fluorination with [^{18}F]F_2	Tumor imaging
16α-[^{18}F]Fluoroestradiol	Nucleophilic displacement of aliphatic cyclic sulfone	Estrogen receptor positive Tumors
[^{18}F]Fleroxacin	Nucleophilic displacement of mesylate	Antibiotic pharmacokinetics
[^{18}F]CFT	Fluorodestannylation with high spec. act. [^{18}F]AcOF	Dopamine transport

Table 1-3 Fluorine-18 labelled radiopharmaceuticals in clinical use.[31]

As evident from the literature, the feasibility and versatility of ^{18}F radiochemistry has been sufficiently explored and standardized, in order to perform fast and economic radio-synthesis of small biomolecules and transported to distant locations by road or airfollowing the central unit and satellite concept[32].

1.1.3.1. Production pathway of fluorine-18

PET radio nuclides are neutron deficient isotopes, relative to their stable ones. They are generally produced by nuclear reactions, induced by the bombardment of charged particles. These charged particles are produced in accelerators, commonly in a cyclotron. The chemical form in which these radionuclides are available depends on the nature of irradiation target used and the type of nuclear reaction involved. Hence, the chemical form of ^{18}F for electrophilic and nucleophilic fluorination are different. Table 1-4 shows various production methods of ^{18}F using different target materials. Production of ^{18}F from enriched ^{18}O target is found to be efficient, economic and reliable, and is the most commonly adapted method worldwide through $^{18}O(p,n)^{18}F$ nuclear reaction. Here, enriched ^{18}O-oxygen (>95%) is taken either in the form of O_2 gas or water, depending on the type of radio-chemistry planned. High specific activity of ^{18}F can be obtained with ^{18}O-water target. With proper optimization of the conditions, production of ^{18}F can be quickly and economically performed even with cyclotrons of limited features and low energy. However, for radiolabelling of ^{18}F by electrophilic substitution method, ^{18}F needs to be produced as $[^{18}F]F_2$ gas, by irradiation of $[^{18}O]O_2$ gas target or by irradiating neon gas - $^{20}Ne(d,\alpha)^{18}F$. For efficient trapping of ^{18}F produced, the $[^{18}O]O_2$ gas (or Ne gas) is doped with minute quantities of F_2 gas. The formed $[^{18}F]F_2$ gas is converted to active $^{18}F^+$ ion by converting to suitable reagents (eg. acetylhypofluorite, XeF_2, etc). Since $^{19}F_2$ is used as a carrier gas in these cases, the specific activity of $^{18}F_2$ gas produced is very low.

Reaction	$^{18}O(p,n)^{18}F$	$^{16}O(^{3}He,p)^{18}F$	$^{20}Ne(d,\alpha)^{18}F$	$^{18}O(p,n)^{18}F^{\$}$
Target	$H_2{}^{18}O^{\#}$	H_2O	Ne (0.1-0.2 % F_2)$^{@}$	$^{18}O_2$, Kr (1 % F_2)$^{@}$
Particleenergy [MeV]	16→3	36→0	14→0	10→0
Main product form	^{18}Faq	^{18}Faq	$[^{18}F]F_2$	$[^{18}F]F_2$
Yield [GBq/μAh]	2.22	0.26	0.37-0.44	~0.37
Specific activity [Bq/mmol]	$\leq 3.7\times10^{15}$	$\leq 3.7\times10^{15}$	$\leq 3.7\times10^{10\text{-}11}$	$\leq 3.7\text{-}185\times10^{10}$

Ti-target with Ti-window. @ passivated Ni-target. $ two steps process.

Table 1-4 Most common nuclear reactions for production of fluorine-18.[33-35]

1.1.4. Type of radiosynthesis and specific activity of the radioactive product

In principle, radio-synthesis can be classified as: carrier-free, no-carrier-added (n.c.a.) and carrier-added (c.a.). Ideally, carrier-free systems are only achieved when artificial radio-elements (i.e. astatine) are used and the presence of non-radioactive isotopes of the element is excluded. Nevertheless, in radio-synthesis with cyclotron-produced radionuclides of naturally-occurring elements, traces of stable isotopes of these elements are omnipresent and act as isotopic carriers. Under those conditions radio-synthesis are referred to be performed as no-carrier-added (n.c.a.) processes. In contrast, some circumstances require the addition of weighable quantities of stable isotopes. In the case of $[^{18}F]F_2$ production, fluorine gas is added because n.c.a. $[^{18}F]F_2$ is too reactive to be removed from the walls of targets and tube lines. These methods are termed as carrier-added (c.a.).

The extent to which a radioactive compound labelled with a radionuclide is diluted with the non-radioactive isotopic counterpart is referred to as the specific activity. The specific activity is calculated from the ratio of the amount of radioactive compound (Becquerel, Bq or Curie, Ci) and the molar concentration of the compound (mol), and is usually expressed in GBq/μmol or Ci/mmol[36].The maximum theoretical specific activity of

fluorine-18, with a half-life of 110 min, is 63,000 GBq/μmol (1.7×10^6 Ci/mmol). In practice, much lower specific activities are obtained due to the unavoidable dilution with the common existence of the stable ^{19}F. Since PET investigations using labelled biomolecules are with very low concentrations in the destined tissue (i.e. substrates of enzymes, receptor ligands and reuptake inhibitors), it demand radiotracers of very high specific activities. Hence, it is essential to obtain ^{18}F in its no-carrier-added (n.c.a) form for the radiopharmaceutical synthesis.

1.1.5. Radiochemical synthesis for incorporating ^{18}F into molecules

Fluorination reactions are relatively difficult, due to its highly reactive and corrosive nature, particularly towards glasses. Moreover, fluorination with radioactive ^{18}F adds further constraints to the process. ^{18}F being highly radioactive, emitting pairs of high energetic γ (2 x 511 keV per disintegration), demands handling in safely protected environment from receiving radiation doses, for the chemist. Further, the molar quantity of ^{18}F used being extremely low, characterization without authentic reference materials is difficult. However, identification of radioactive species by radio-TLC and radio-HPLC are efficient. Moreover, the reaction doesn't follow molar stoichiometry due to very low mass of ^{18}F used and the precursor and reagents are taken in very high excess. Thus, these reactions follow a pseudo-first order reaction kinetics. This makes, the ^{18}F radio-fluorination reaction to be extremely efficient, compared to ^{19}F-fluorination carried out under stoichiometric conditions. The incorporation of ^{18}F into organic and bio-molecules can be broadly classified into three categories. Viz (i) electrophilic, (ii) nucleophilic and (iii) indirect labelling methods. Electrophilic labelling methods are found to be efficient and commonly applied in case of aromatic radiofluorintion. Nucleophilic method is applied for aliphatic radiolabelling. The indirect method is used via labelling a prosthetic group and coupling to the target

molecule[3737]. This is adapted when direct labelling in a bio-molecules may demand a lengthy, cumbersome and uneconomical process.

1.1.5.1. Electrophilic substitution

In electrophilic method, the commonly produced radioactive species is $[^{18}F]F_2$ gas[38, 39]. It is further converted to electrophilic and highly reactive reagents that can generate fluoronium ion by converting to $[^{18}F]CH_3OF$[40], $[^{18}F]CH_3COOF$[41], $[^{18}F]FClO_3$[42], $[^{18}F]CsF$ or $[^{18}F]XeF_2$ [43] before further labelling process. Since highly electro negative F bears a partial positive charge (electron deficient condition), the reagents are highly reactive towards electron rich groups like aromatic ring, alkenes, carbanions, etc. Electrophilic fluorination, is a well know radiofluorination method of incorporating radiofluorine in biomolecules,and was the first method in 2-$[^{18}F]$FDG was synthesized, though 2-$[^{18}F]$FDG synthesis was standardized through the well-known nucleophilic method and is the preferred method now. Synthesis of 2-$[^{18}F]$FDG performed by electrophilic method is shown below [Fig 1.4]

Due to inherent nature of production, radiofluorination using $[^{18}F]F_2$ gas is a carrier added process and, hence, provides ^{18}F-labelling with low specific activity. Moreover, theoretically, only 50% of the available fluorine atoms can be utilized in this method, whichresulting in low radio-fluorination efficiency. Further, due to low specific activity, PET-studies that demands high specific activities like enzymatic and receptor based imaging cannot be performed. The physiological toxicity arising due to the stimulation of receptors by cold fluorinated products, produced in the reaction, are also an issue. Hence, electrophilic method is less common and continuous search for equivalent and alternate methods or biomolecular analogues are being explored[44]. In spite of the above facts, electrophilic

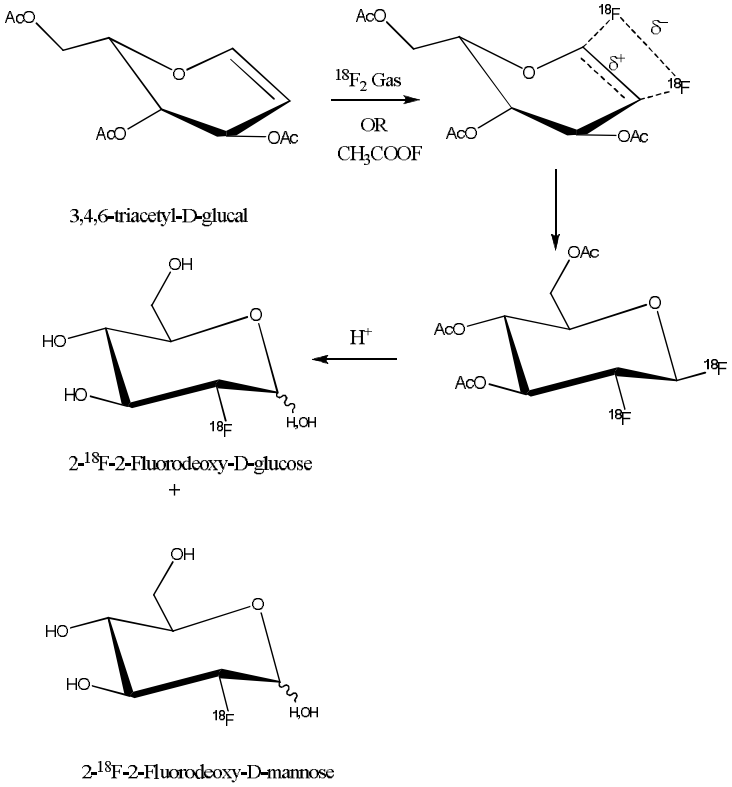


Figure 1-4 2-[^{18}F]-FDG Produced by Electrophilic Fluorination either by using $^{18}F_2$ gas or [^{18}F]acetylhypofluorite.[45]

fluorination has been successfully applied in aromatic moietiesfor wide varieties of molecules using the approach shown in Fig 1-5.

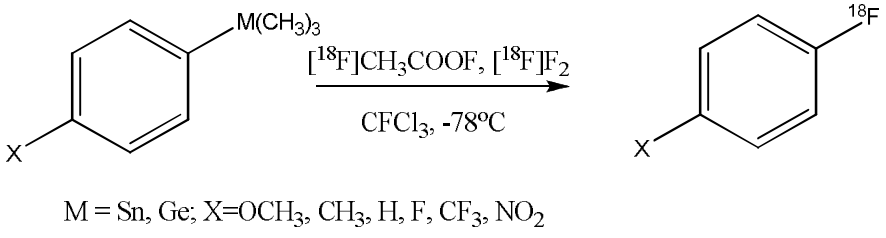


Figure 1-5 Regioselective ^{18}F-labelling via electrophilic fluoro-demetallation reactions. [46]

1.1.5.2. Nucleophilic substitution

In this method, radiofluorination is performed using ^{18}F in the form of $^{18}F^-$ ion. It is directly obtained by irradiation of ^{18}O-water target. Since ^{18}O in a ^{18}O-water target is at a

much higher density compared to gas targets, efficient production of $^{18}F^-$ can be achieved. Moreover, being a liquid, it also retains the advantage, that gas targets provides, such as loading and unloading into and from the target target-cavity through thin, flexible, radiation resistant tubings. This process doesn't require carrier ^{19}F-fluorine for efficient recovery of ^{18}F., though as mentioned earlier some ^{19}F-fluoride would be inevitably present in the ^{18}O-water. Hence, nucleophilic fluorination is the only method in which high specific activity, no-carrier-added ^{18}F-fluorine can be obtained. Presently, nucleophilic fluorination is the most efficient and common method for radio-fluorination. A proof of its suitability is that it is the method used for the production of 2-[^{18}F]FDG worldwide.(Fig 1.6)

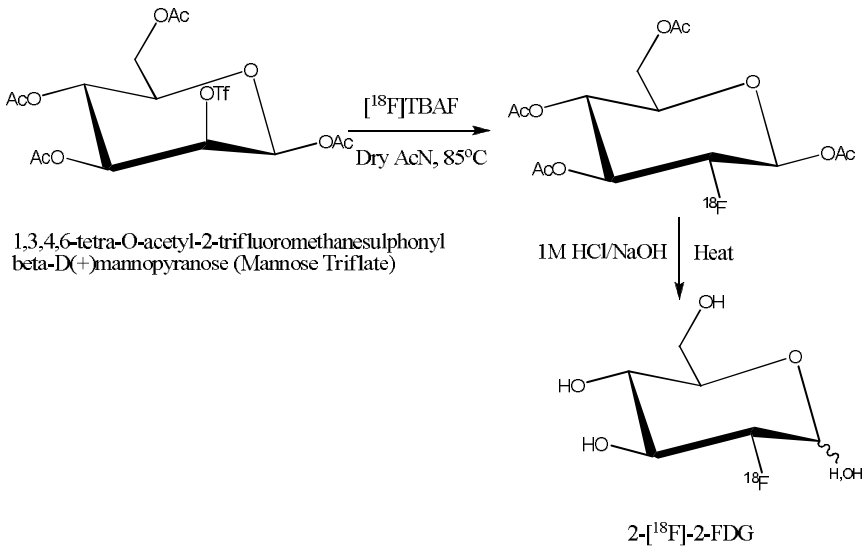


Figure 1-6 2-[^{18}F]-FDG produced by nucleophillic fluorination using [^{18}F]fluoride ion.

1.1.5.3. Steps involved in nucleophilic fluorination[31]

1.1.5.3.1. Recovery of [^{18}O]water

Since a very small amount of ^{18}O is converted to ^{18}F during proton irradiation, efficient recovery of ^{18}O water would enable its availability for multiple irradiations. In order to recover ^{18}F from the irradiated water, it is passed through anion exchange resin (PS-

HCO_3), to trap $^{18}F^-$., which is then eluted from the column by anionic solution into the reaction vessel. This process further facilitates in concentrating $^{18}F^-$ in a small volume of elution (0.5 mL), compared to the initial concentration in ^{18}O water, after irradiation (1 – 2.5 mL). Since it was well established that CO_3^{2-} and HCO_3^- interferes less during radiofluorination, these solutions are used for elution of $^{18}F^-$ from the resin to the reaction vessel. $^{18}F^-$ is insoluble and non-reactive in anhydrous aprotic medium. Hence Kryptofix(2,2,2) or tetrabutyl ammonium salts are used as phase transfer catalysts (PTC)[47 47]. In many cases, to simplify the PTC addition step, it is together mixed with the bicarbonate solution used for elution, andadded into the radio-chemical reactor. When tetrabutyl ammonium salt is used as PTC, the $^{18}F^-$ elution is carried out by using tetrabutyl ammonium bicarbonate as the eluent.

1.1.5.3.2. Water evaporation

Fluoride being highly electronegative and small in size, it gets easily solvated with protic solvents making it less exposed for nucleophilic substitution. Water being a good nucleophile gets easily substituted into the precursor molecule and interfering in the fluorination. Hence, the fluorination needs to be conducted in polar aprotic solvents like acetonitrile, DMSO, DMF, etc., under anhydrous conditions. Moreover, since the actual ^{18}F content is in nanogram levels, even microgram(microlitre) quantities of water would compete strongly and interfere severely during substitution process. Hence, stringent dryness is required for S_N2 reaction. Direct drying of water by heating would decompose PTC that has temperature sensitive organic moieties like ethyl and butyl groups. Hence acetonitrile is used as a mixture with water in the medium, to dry at lower temperature under azeotropic condition followed by 95^0C drying at vacuum. This can provide sufficient dryness required to carry out the radio-fluorination reaction.

1.1.5.3.3. Radio-fluorination

As mentioned above, in order to perform S_N2 fluorination into organic molecules, a phase transfer catalyst is required, since fluoride is insoluble and hence un-reactive in aprotic solvents. Depending on the conditions required for the substitution reaction, the solvent required for reaction can be chosen. For normal substitution of good leaving groups like tosylates, triflates, etc., in aliphatic and alicyclic chains, acetonitrile is efficient, whose boiling point (82^0C) matches with the reaction temperature (85^0C). For groups which have less leaving abilities like aromatic nucleophilic substitution or poor leaving groups, DMSO or reactions have to be carried out at higher temperatures, DMF is the preferred solvent, however, based on the solubility of organic compounds and the reactivity of fluoride in PTC conditions, acetonitrile is the most commonly used solvent. In recent years, fluorination has also been tried with ionic liquids, which has higher tolerance toward water content during substitution.[48-51]

1.1.5.3.4. Post radio-fluorination steps

Since radio-fluorination of a molecule requires proper protection of interfering functional groups, the precursors are normally molecules, in which the potentially reactive groups are properly protected. Hence, after radio-fluorination, de-protection is done by removing the protecting moieties by hydrolysis. This process is usually done by either acid- or base-hydrolysis. On the other hand, in certain cases, radio-synthesis would be a multistep synthesis, that involves coupling of radio-fluorinated prosthetic groups with the biomolecules. In such cases, these reactions are conducted either as a single-pot or as a two-pot synthesis, post radio-fluorination.

1.1.5.3.5. Purification

Since ^{18}F-radiosynthesis is a pseudo-first order reaction, with excess of other reagents, they are usually associated with large amounts of cold impurities. Moreover, since the radiopharmaceuticals synthesized are intended for human use, the reaction mixture needs to be purified efficiently and sufficiently in order to obtain them in an injectable form, as per the pharmacopoeia specifications. To achieve this, preparative radio-HPLC is often used. Since radio-HPLC is relatively laborious, expensive, demanding high sophistication, replacing it with simple solid phase extraction (SPE) purification is preferred and several groups, worldwide, are working towards developing SPE methods.

1.1.6. Automation technology for PET radio-synthesis

The synthesis of PET radiopharmaceuticals pretty much follows the conventional organic synthesis process. However, due to the constraints in implementing them (radiation safety) and the purpose of application (human clinical use), makes this process much different and unique. The requirement of operator safety from the radioactive compounds and the requirement of production-reliability for regular supply to hospitals, demands the automation or remote-control of the process. As compared to the usual laboratory reaction scale (10 mL – 1000 mL), the quantities used for PET radiochemistry are very small: (10 µL – 2 mL). Hence, modular and compact synthesizers have been designed, which can be fitted into compact, well shielded hot cells. Since PET radiochemistry involves stringent evaporation and high pressure heating, it generates high amount of radioactive gases, that demands closed and air-tight hot cells with controlled exhaust. The air borne activity need to be trapped in a pressurized vessel and stored for sufficient time for delay-decay to permissible level is achieved.

To satisfy all the above requirements, synthesis modules with automated control systems are developed and are commercially available. The automated radiosynthesis system consists of compact modular arrangements of reservoirs and vessels, connected as per the reaction schematics. The electronic controls are connected to a software program in a programmable logiccontroller (PLC) unit, that can operate the time-list, which controls, relays, liquid-flow valves, etc, to perform the general requirements of chemical synthesis, like adding reagents, heating, cooling, evacuation, etc.[52, 53] These components are designed for high-reliability and robust performance for regular production. The concept of automated system for radio-synthesis started as early as 1980's. The technology has evolved through various stages, initially with hard wired modules in the synthesis of ^{11}C-glucose[54].[54] and $^{13}NH_3$ [5555]. Later, the use of microprocessor was explored for automated production of radiopharmaceuticals.[56, 57] The automated radio-synthesis system was recognized as a landmark when it was successfully applied for the regular synthesis of 2-[^{18}F]FDG by a one-pot synthesis.[58]. The technology has undergone enormous improvement with the advent of simplified SPE purification methods, high yield production cyclotron targets, availability of highly enriched [^{18}O]water, and ready-to-use reagent kits.[59, 60]

Further development in the form of compact and easy maintenance synthesis modules using disposable cassette systems for production have increased the number of PET radio-synthesis facilities, since the ease of operation does not require highly qualified chemists to handle the synthesis process.

In the current scenario, the automated production has also been successfully applied to non-FDG PET radiopharmaceuticals like [^{18}F]FDOPA, [^{18}F]FLT, [^{18}F]FMISO, etc.[61, 62] As the importance and reliability of automation have been explored, the technology has also been extended for automated quality control of radiopharmaceuticals[63], computer controlled

infusion systems for the automated injection of radiopharmaceuticals,[64] automated dose dispensing systems[65] and automated delivery of radiopharmaceuticals using pneumatic transport systems.[66] In the current trend with rapid growth of automation, simplified production of various radiopharmaceuticals using solid phase extraction methods,[67, 68] are being explored, that can provide reliable production, with high radiochemical yields, and to the quality required for human clinical studies.

1.2. Amino acids

Amino acids are common biomolecules present in all cells in the body, that serves as building blocks for several proteins, essential for life's various functions. These amino acids link together with amide bonds to form various peptides and proteins. Based on the type of substituted functional groups, they provide various structure and behavior to the peptides and proteins. These interlinks can provide unique structure for the proteins in order to perform specific functions required in a living system.[69]

The proteins present in nature are made up of a total of 20 amino acids. The general structural formula of amino acids can be represented a shown in Fig 1.7. It consists of an amino and a carboxylic acid group. Apart from that, each amino acid is determined by their substituted group present in their α-carbon to the carboxylic acid. The structure and properties of amino acids are shown in table.

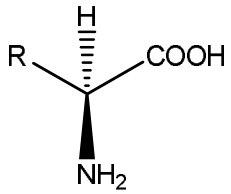


Figure 1-7 General structure of α-L-amino acids

Amino acids can be classified by various methods. Among them, the classification based on their polarity of their substitution are as below.

(1) Non-polar or hydrophobic R group: They have an equal number of amino and carboxyl groups and are neutral. These amino acids are hydrophobic and have no charge on

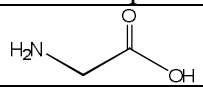
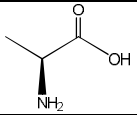
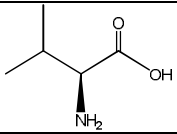
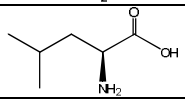
the R group. The amino acids belonging to this group are alanine, valine, leucine, isoleucine, phenylalanine, glycine, tryptophan, methionine and proline.

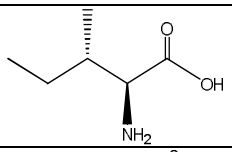
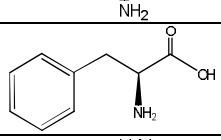
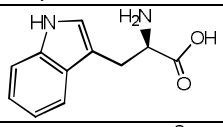
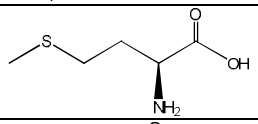
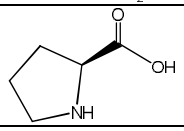
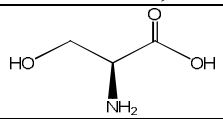
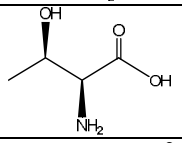
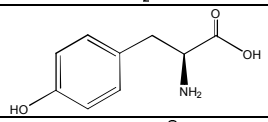
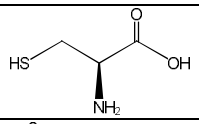
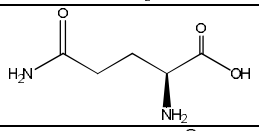
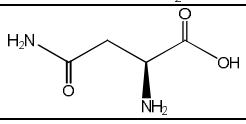
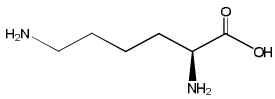
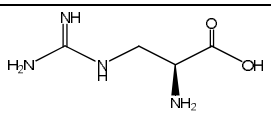
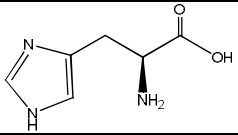
(2) Neutral, uncharged, polar R group: These amino acids also do not have any charge on the R group but participate in hydrogen bonding of the protein structure. The amino acids that belongs to this group are serine, threonine, tyrosine, cysteine, glutamine and aspargine.

(3) Positively charged R group: Polar amino acids with positive charge have more amino groups as compared to carboxyl groups making it basic. These are lysine, arginine and histidine.

(4) Negatively charged R groups: Polar amino acids with negative charge have more carboxyl groups than amino groups making them acidic. They are classified as dicarboxylic mono-amino acids. These are aspartic acid and glutamic acid.

The structure and the physical properties of amino acids are shown in table below (Table 1.5)

S.No	Name and Notation	Structure	pKa_1	pKa_2	PKa_R	pI	Specific Optical Rotation
Non-polar Hydrophobic R groups							
1.	L-Glycine (Gly, G)		2.34	9.6	-	5.97	No chiral center
2.	L-Alanine (Ala, A)		2.34	9.69	-	6.00	$[\alpha]_{20/D}=+14.5^0$ (c=10, 6N HCl)
3.	L-Valine (Val, V)		2.32	9.62	-	5.96	$[\alpha]_{20/D}=+27.5^0$ (c=8, 6N HCl)
4.	L-Leucine (Leu, L)		2.36	9.6	-	5.98	$[\alpha]_{25/D}=-10.8^0$ (c=2.2, 6N

							HCl)
5.	L-Isoleucine (Ile, I)		2.36	9.6	-	6.02	$[\alpha]_{20/D}$=+40.6^0 (c=4.6, 1N HCl)
6.	L-Phenylalanine (Phe, F)		1.83	9.13	-	5.48	$[\alpha]_{20/D}$=-33^0--35.2^o (c=2, water)
7.	L-Tryptophan (Trp, W)		2.83	9.39	-	5.89	$[\alpha]_{20/D}$=-30^0--33^o (c=1, water)
8.	L-Methionine (Met, M)		2.28	9.21	-	5.74	$[\alpha]_{20/D}$=+21.5^0-+25^o (c=2.5, water)
9.	L-Proline (Pro, P)		1.99	10.6	-	6.3	$[\alpha]_{20/D}$=-84^0 (c=4, water)
Neutral, uncharged, polar R groups							
10.	L-Serine (Ser, S)		2.21	9.15	-	5.58	$[\alpha]_{20/D}$=+13.6^0-+16^o (c=2.5, water)
11.	L-Threonine (Thr, T)		2.09	9.1	-	5.6	$[\alpha]_{20/D}$=-26.7^0--29.1^o (c=1, water)
12.	L-Tyrosine (Tyr, Y)		2.2	9.11	10.07	5.66	$[\alpha]_{20/D}$=+19^0-+20.5^o (c=1, water)
13.	L-Cysteine (Cys, C)		1.96	10.13	8.18	5.07	$[\alpha]_{25/D}$=+9.7^0 (c=8, 1N HCl)
14.	L-Glutamine (Gln, Q)		2.17	9.13	-	5.65	$[\alpha]_{20/D}$=+6.3^0-+7.3^o (c=4, water)
15.	L-Asparagine (Asn, N)		2.02	8.8	-	5.41	$[\alpha]_{20/D}$=+33^0-+36^o (c=10, 6N HCl)
Positively charged R groups							
16.	L-Lysine (Lys, K)		2.18	8.95	10.53	9.74	$[\alpha]_{20/D}$=+22.5^0 (c=2%, 6N HCl)
17.	L-Arginine (Arg, R)		2.17	9.04	12.48	10.76	$[\alpha]_{20/D}$=+22^0 (c=12, 10% HCl)
18.	L-Histidine (His, H)		1.82	9.17	6.00	7.59	$[\alpha]_{20/D}$=+10.9^0 (c=0.77, 0.5N NaOH)

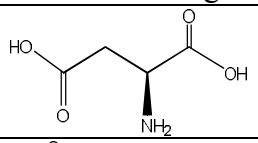
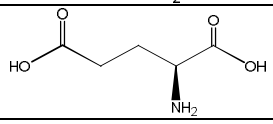
Negatively charged R groups							
19.	L-Aspartic acid (Asp, D)		1.88	9.6	3.65	2.77	$[\alpha]_{20/D}=+25^0$ (c=2, 6N HCl)
20.	L-Glutamic acid (Glu, E)		2.19	9.67	4.25	3.22	$[\alpha]_{20/D}=+31.5^0$-$+32.5^o$ (c=10, 6N HCl)

Table 1-5 List of amino acids, their classifications and their properties.

Note: D and L are absolute configuration of amino acids, with respect to D and L-glyceraldehydes or D and L-lactic acid. R and S notation also represents the absolute configuration as per Corn-Ingold-Prelog rule. Whereas 'd' (dextro rotatory) and 'l' (levo rotatory) are notations with respect to the direction of specific rotation of the plane polarized light by the amino acids.

1.2.1. Amino acid transport across the cell membrane

The transport of amino acids into the cells are mainly through carrier mediated process. In general, all amino acids can however diffuse through into the cells. The capacity of the carrier and their binding affinity towards the amino acids are the factors deciding the activity of the specific carrier. The binding affinity is expressed by the functional carrier density per cell in the extracellular region. During over expression of the amino acid transporter, the increase in amino acid transport is contributed by either of the above factors or both together in combination. These factors and mechanism are described elaborately by Souba et al.[70]

In mammalian cells, three main amino acid transport systems are said to contribute as shown in Fig 1-8. viz., System A, ASC and L. System A is a sodium dependent transport system, by which most neutral amino acids are transported. It is often inhibited by the

intracellular substrates of this system. Contrary to this system, ASC is a transport system, that gets activated by the presence of amino acids interior to the cells. System ASC is also a Na+ dependent and neutral amino acid carrier. As the transporter binds sodium ion in the medium, the affinity of the carrier proteins get initiated. This leads to the formation of an amino acid/sodium/co-transporter complex. Amino acids together with the sodium ion is delivered into the cell by a change in the conformational structure of this complex. The electrical potential present in the membrane which was maintained by the glucose dependent Na/K ATPase transporter, activates the binding of Na^+ ion. The system A and ASC transport process requires energy. Other than the above two, system L is a sodium independent transport process, which is mediated solely by the concentration gradient from inside to outside the cell that determines the direction of flow. Transport of amino acids can also occur against this gradient by a mechanism known as counter-transport. As per this mechanism, the sodium dependent transport systems establishes a known gradient of another amino acid, which activates the efflux of the amino acid through system L. Usually neutral amino acid transport is mediated by system A and ASC, whereas aromatic amino acid transport is mediated by system L.

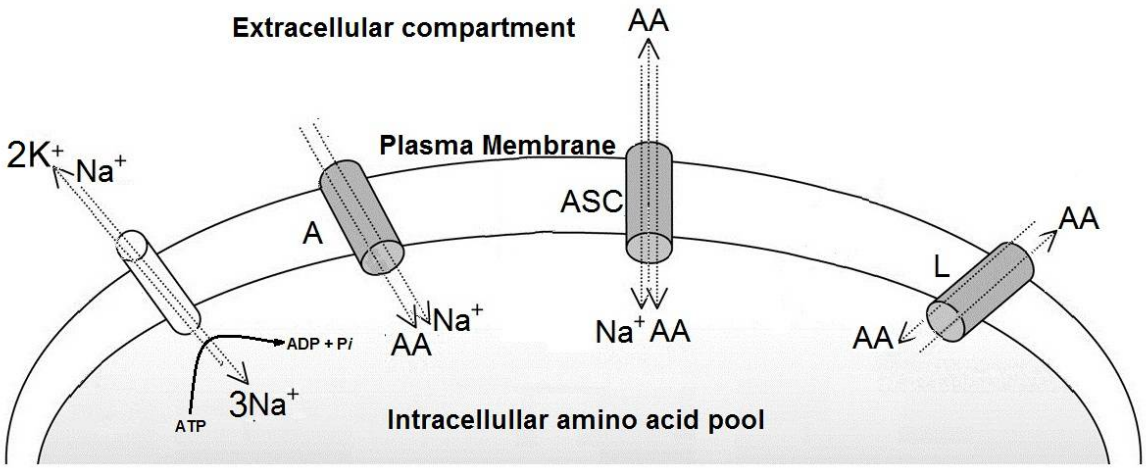


Figure 1-8 Schematic depiction of major amino acid transporter systems.

The system A transporter co-transports one extracellular amino acid with one Na^+ into the cell. The system L transporter exchanges one amino acid from the extracellular compartment with one amino acid from the intracellular compartment and does not require Na^+. The system ASC transporter co-transports one extracellular amino acid with one Na^+ into the cell while transporting one intracellular amino acid out of the cell. The intracellular amino acid pool gradient is maintained by active transport by system A as well as further concentrative amino acid transporters. The sodium ion (Na^+) gradient is maintained by the Na^+, K^+-ATPase[71]

1.2.2. Tumor imaging with radiolabelled amino acids

The uptake of amino acids are said to be elevated in malignant tissues.[72, 73] Amino acids are required by tumor cells in order to synthesize proteins, produce energy and for cell proliferation. Once malignant, the tumor reaches a hypermetabolic state, in which the glucose metabolism, protein synthesis and the amino acid transport are enormously enhanced in comparison to normal tissues.[74] This leads to an excess accumulation of radiolabelled amino acids in the diseased cells during PET imaging studies.

Most commonly useful amino acids for tumor imaging are analogues of phenylalanine and tyrosine.[75] Use of amino acid imaging have been reported in several cases, for studying protein synthesis rate (PSR) or amino acid transport alone, in several cases. It was also reported to have advantage over 2-[^{18}F]FDG for brain imaging. Since the brain metabolizes glucose for its energy requirement, 2-[^{18}F]FDG uptake in normal brainis quite high, because of which high background is observed. Moreover, it also has non-specific uptake in infection and inflammation (due to uptake in macrophages), other than tumors. As far as amino acid analogues are concerned, it has been found that, amino acid transport is the main factor that

decides tumor uptake, rather than incorporation into proteins.[76, 77] The fraction of the amino acid incorporated into the proteins is much smaller in comparison to the quantity injected for PET-studies. Hence, visualization of tumor lesions by PET-amino acid tracers due to the contribution of incorporation of them into proteins is unlikely (Fig 1.9). Hence, analogues of amino acids were developed for quantification of amino acid transport rate.[78-80] The quantification of amino acid transport can be done with the PET-analogue, since, the influx of them in to the blood, back, from the cell after uptake, is relatively very less. Apart from this, the imaging studies can be performed relatively much quicker after injection (~20min) since the transport metabolism of amino acids is pretty fast. On the other hand, for studying PSR, it is essential to perform the studies for extended time after injection. Thus, two types of studies are performed with amino acid analogues: (i) study based solely on amino acid transport (ii) transport and incorporation into protein (otherwise known as PSR studies)[81, 82]

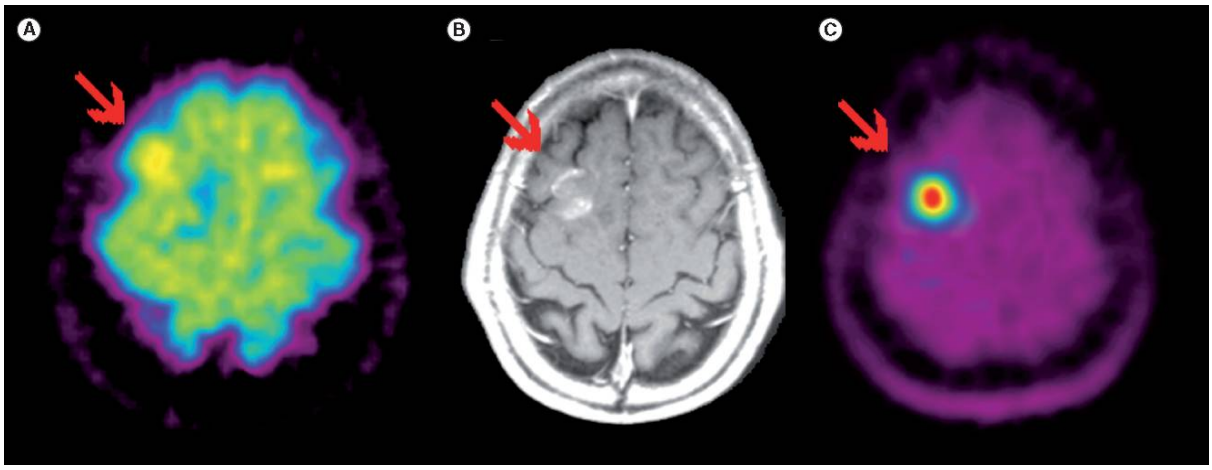


Figure 1-9 Comparison of 2-[^{18}F]FDG and [^{11}C]MET brain images:(A) ^{18}F-FDG; (B) contrast-enhanced MRI; (C) ^{11}C-MET PET. Glioblastoma in the right frontal lobe, which is hard to delineate in the 2-[^{18}F]FDG PET. However, amino-acid PET with ^{11}C-MET clearly shows the lesion with excellent tumor to background contrast[83]

1.2.3. Synthesis of catecholamines from amino acids

Synthesis of catecholamine is another metabolic action in which the amino acids are used in the living organisms. Amino acids, particularly, those that have aromatic substitution are involved in this process. Phenylalanine is the starting material for the synthesis of tyrosine in mammals. Conversion of phenylalanine into tyrosine is catalyzed by the enzyme phenylalanine hydroxylase (PH) which introduces a hydroxyl group in the 4^{th}-position of the aromatic ring of phenylalanine. Tyrosine again acts as precursor in the biosynthesis of catecholamines (CA).

Dihydroxyphenylethylamine (dopamine, DA), norepinephrine (NE), and epinephrine (EP) are synthesized in the adrenal medulla and in adrenergic neurons in the central nervous system. Biosynthesis of CA involves five enzymatic steps as shown in Fig 1.10: phenylalanine is converted by PH in tyrosine; tyrosine hydroxylase (TH) catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA); DOPA is converted to DA by aromatic L-amino acid decarboxylase (AADC); dopamine β-hydroxylase (DBH) catalyzes the enzymatic reaction of DA to NE, and EP is synthesized from NE by phenylethanolamine N-methyltransferase (PNMT).[84]

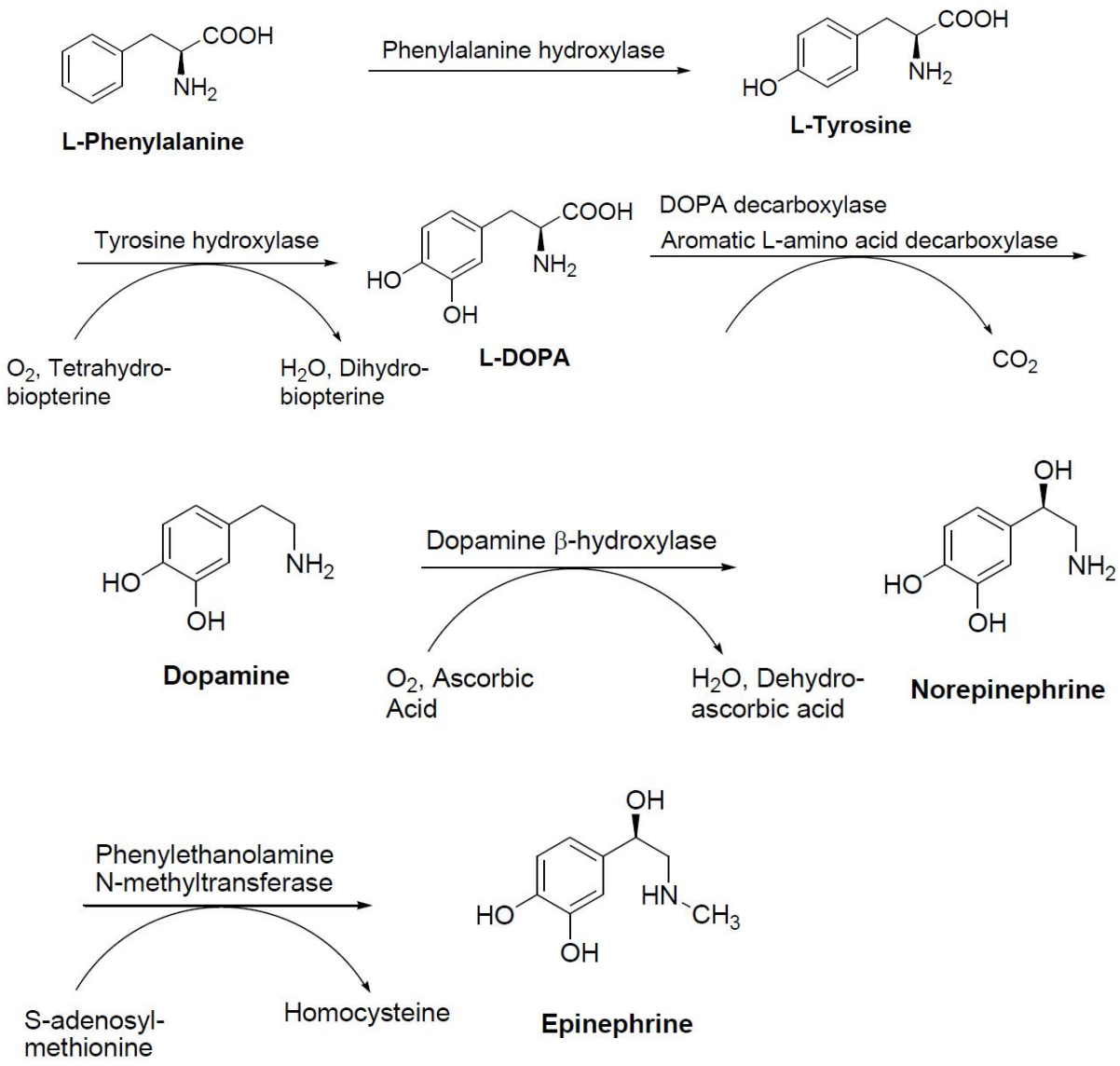


Figure 1-10 Synthesis of catecholamines from phenylalanine.

1.2.4. [^{18}F] Fluoro amino acids

Many amino acids have been radiolabelled to study their potential imaging characteristics. These radiolabelled amino acids differ in ease of synthesis, biodistribution and formation of metabolites in vivo. To date, the most frequently used amino acid tracers for tumor diagnosis are [^{11}C-methyl]-L-methionine (^{11}C-MET) for PET and 3-[^{123}I]iodo-methyl-L-tyrosine (IMT) for single photon emission computed tomography (SPECT).[75] Due to the

short physical half-life of the ^{11}C-label (20.4 min), MET-PET remains restricted to only a few PET centers with a cyclotron on site, and could not be established in routine clinical practice despite of its compatibility with amino acid uptake kinetics and convincing first clinical results. IMT SPECT offers a more widespread application, but the spatial resolution of SPECT is considerably lower than that of PET.[85] Therefore, a number of attempts have been undertaken to label amino acids with fluorine-18.

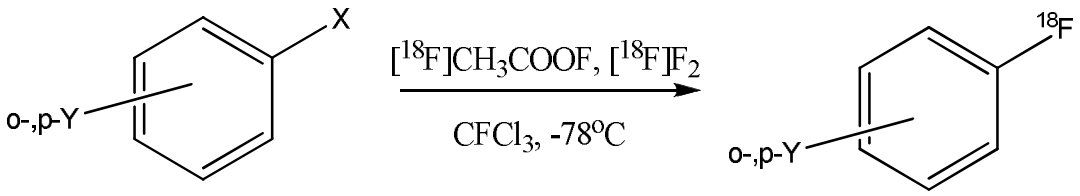
1.2.4.1. 6-[^{18}F]Fluoro-3,4-dihydroxy-L-phenylalanine

3,4-Dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (6-[^{18}F]FDOPA) is a fluorinated analogue of the naturally-occurring L-3,4-dihydroxyphenylalanine (L-DOPA). It has been used extensively for evaluation of the dopaminergic system in the brain, particularly in Parkinson´s disease, as it is a substrate for the enzyme aromatic amino acid decarboxylase (AADC), normally found in high abundance in dopaminergic neurons. For this reason, under normal physiological conditions, there is high uptake and retention of 6-[^{18}F]FDOPA in the substantia nigra and the striatum. Additionally, 6-[^{18}F]FDOPA also shows high uptake in neuroendocrine tumors (NET).[86] and some other tumors, such as cerebral gliomas, which may exhibit an increased accumulation due to amino acid transport.[87] The brain uptake of 6-[^{18}F]FDOPA in animal and humans can be decreased by other system L-substrates such as L-phenylalanine, consistent with a 6-[^{18}F]FDOPA transport via system L.[71] However, accumulation of the radiopharmaceutical in non-NETs, except for cerebral gliomas, is less predictable, and the role of 6-[^{18}F]FDOPA seems to be limited.

1.2.5. Nucleophilic ^{18}F-fluorination in amino acids

Since aromatic amino acids are well established to be useful as PET radio tracers, fluorination in aromatic group was accomplished initially for their synthesis. Since

electrophilic substitution of [^{18}F]fluorine in aromatic group is much straightforward and reliable, it was the first method developed for synthesizing them. Later, due to easy availability of high specific activity of [^{18}F]fluoride by the irradiation of enriched ^{18}O-water target, nucleophilic aromatic ^{18}F-fluorination were explored, in the development of ^{18}F-labelled radiopharmaceuticals. This reaction is activated by electron withdrawing groups ortho and/or para to the leaving group (Fig 1.11). The substitution of [^{18}F]fluorine in the aromatic group, generally show good stability in the metabolic pathways of organisms.



X = NO_2, $N(CH_3)^{3+}$, Br, Cl, I;
Y = NO_2, CN, CHO, COR, COOR, Cl, Br, I;
PTC = K2,2,2/CO_2, R_4N^+, Cs^+, RB^+

Figure 1-11 Nucleophilic ^{18}F-fluorination with activated arenes.[37]

A good leaving group on the benzene ring is needed, and nitro (-NO_2) and trimethylammoniumtriflate (-NMe_3OTf) and diphenyliodonium (Ar-I+-Ar') groups are used in S_NAr reactions.[88] Simple isotopic substitution, ^{18}F for ^{19}F, mostly shows very high radiochemical yield. However, the lower specific activity from these isotopic substitutions makes this process less practical.

As an activating group, functions, such as nitro, cyano and carbonyl groups, are suitable for activating S_NAr reactions. The choice of activating group depends on the structure of the desired final product, or the sequence of synthetic steps to be followed after the introduction of ^{18}F.[89]

Since direct substitution of [^{18}F]fluorine in aromatic is a cumbersome and less effective process, other alternate aliphatic side chains were explored. For this, fluorination of ethyl, propyl or butyl group substituted in the aromatic moiety are explored, in which the [^{18}F]fluorination is performed in the aliphatic moiety by the more efficient nucleophilic substitution reaction. [^{18}F]fluorinated tyrosine analogues are the best examples that demonstrate the above strategies. Substitution of O-fluoroalkyl moiety at the phenolic position in the aromatic ring of tyrosine, are found to have less effect on its uptake, and provides better in-vivo stability. [^{18}F]fluorination was performed by S_N2 method in a single step with a tailored precursor in order to obtain the PET tracer by a single-pot radio-synthesis method. Synthesis of [^{18}F]FET from O-(2'-tosyloxyethyl)-N-trityl-L-tyrosine (TET) precursor is one example that utilized this method.[90]

1.2.6. Indirect ^{18}F-labelling method

The methods mentioned above are direct ^{18}F-labelling methods. In some cases, indirect ^{18}F-labelling methods are applied. These methods mean that a primary ^{18}F-labelled prosthetic group or synthon is prepared first and then coupled with a second molecule to form the product. Important procedures via prosthetic groups are ^{18}F-fluoroalkylation,[91, 92]^{18}F-fluoroacylation[93, 94] and ^{18}F-fluoroamidation.[95](Fig 1.12).

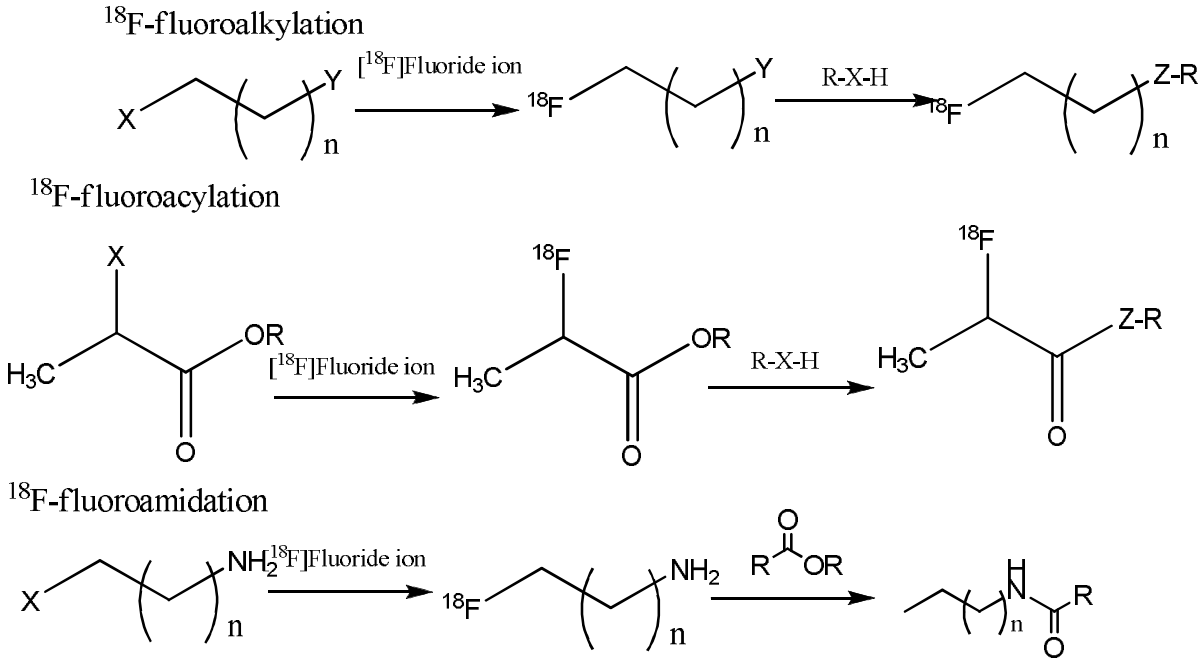


Figure 1-12 Indirect labelling using various synthons.

In addition, the various ^{18}F-synthons used for indirect ^{18}F-labelling methods are 4-[^{18}F]fluorophenyl lithium,[96] 1-[^{18}F]fluoro-4-haloarenes,[97] 4-cyano-1- [^{18}F]fluorobenzene,[98] 4-[^{18}F]fluorophenol[99] and 4-[^{18}F]fluorobenzaldehyde.[100]

The first reported radiosynthesis of 6-[^{18}F]FDOPA is already 30 years old. It was performed through direct electrophilic labelling of 3-methoxy-L-DOPA ethyl ester using [^{18}F]XeF_2 as fluorinating agent and hydrogen fluoride as solvent followed by the cleavage of the methyl group with hydrobromic acid. (Fig 1.13) The radiochemical yield (RCY) achieved by this procedure was 1%.[101] Using the same precursor but [^{18}F]acetyl hypofluorite as fluorinating agent the RCY was improved to 4%.[102]

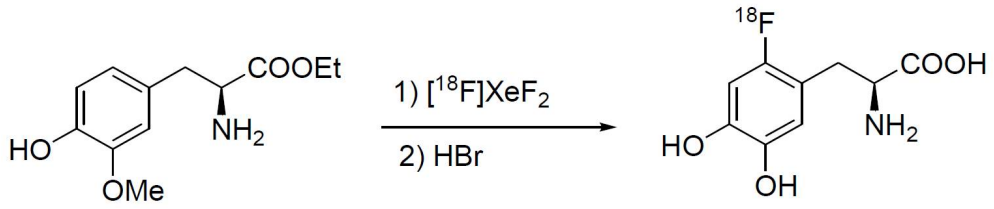


Figure 1-13 First reported electrophilic synthesis of 6-[^{18}F]FDOPA.

Several reports dealing with the direct electrophilic labelling of "naked" L-DOPA in different solvent systems were afterwards published. These experiments provided the desired compound in 3-8% RCY.[103-105] The major drawback of these procedures was the occurrence of all the others probable regioisomers which were not easy to separate from the 6-[^{18}F]FDOPA requiring complicate and time consuming HPLC procedures.Several radio-synthesis procedures for FDOPA using electrophilic fluorination methods have been reported.[106, 107]

Other methods like isotopic exchange methods have also been explored[108].However, radiosynthesis using the most commonly available high specific activity of ^{18}F-fluoride and a nucleophilic synthesis of FDOPA would be more advantageous for common availability of FDOPA. Synthesis of FDOPA using nucleophilic method was explored recently using multistep synthesis process.[109, 110] As a latest development of FDOPA synthesis, by automated synthesis in single-pot using solid phase extraction method in an 2-[^{18}F]FDG module was reported.[111]

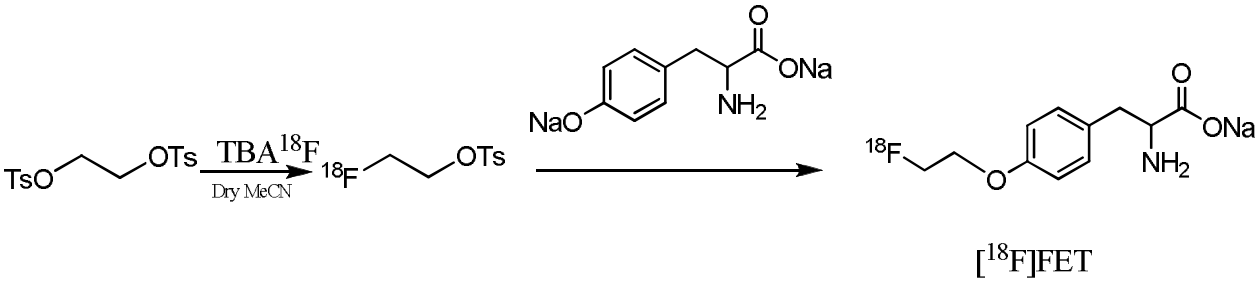
1.2.7. O'-(2-[^{18}F]Fluoroethyl)-L-tyrosine ([^{18}F]FET) as PET imaging agent

Several ^{18}F labelled amino acids have been synthesized ([^{18}F]fluoro-L-tyrosine)[112], [^{18}F]Fluoromethyl tyrosine [113, 114], [^{18}F]fluoroethyl tyrosine[90], [^{18}F]fluoropropyl tyrosine[115], [^{18}F]fluorohydroxy propyl tryptophan[116]. However, out of them, [^{18}F]FET has gained high

importance due to its better labelling efficiency, radiochemical yield and uptake kinetics close to [^{11}C]MET[117, 118].

1.2.7.1. Radio synthesis of [^{18}F]FET by Indirect labelling method

[^{18}F]FET has been synthesized in many ways. Initial synthesis was based on indirect labelling method. Here [^{18}F]FET was synthesized by a two-pot two-step process (Fig 1.14). Initially the purification was performed by semi-preparative radio-HPLC process[80], which was replaced latter by solid phase extraction methods completely[119]. Other than the requirement of two reactors, this method was successful worldwide for producing [^{18}F]FET in sufficient yield and radiochemical purity, with good enantiomeric purity.



[^{18}F]FET

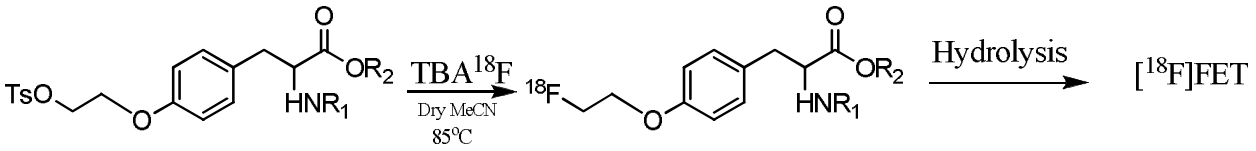
(Ethyleneglycol ditosylate) ([^{18}F]fluoroethyltosylate)

Figure 1-14 Synthesis of [^{18}F]FET by indirect labelling method.

Several modifications in the indirect method has been reported[120].

1.2.7.2. Radio synthesis of [^{18}F]FET by direct labelling method

In direct labelling method a well protected precursor molecule is labelled with [^{18}F]fluoride.[90, 121, 122] The labelled product is further hydrolyzed to form [^{18}F]FET. This is a one-pot two-step method (Fig 1.15).



(Protected precursor molecule)([^{18}F]labelled precursor)

R_1 – boc or trityl group; R_2 – methyl or tert-butyl group.

Figure 1-15 Synthesis of [^{18}F]FET by direct labelling method.

Out of these precursors, the one reported by Krasikova et al[122],[121] is a novel method that utilizes Ni(II)-SBPB-tyrosine complex formation to protect the amino and carboxylic acid groups of amino acids. Preparation of amino acid precursors based on Ni(II)-SBPB-amino acid complex is reported to be stereo selective for both complex formation and further derivatisation. Studies on Ni(II)-SBPB based amino acid complexes are discussed in the following section.

1.3. Ni(II)-SBPB complex based enantioselective synthesis of [^{18}F]labelled amino acids

Synthesis of various amino acids based on (S)-2-N-(N'-benzylprolyl)-aminobenzophenone (BPB) was developed by Y. N. Belekon et al.[123, 124] The method was reported to provide amino acids in high enantiomeric purity. BPB acts as a chiral auxiliary in this process. The method was found to be highly versatile for synthesis of tailor made non-proteinogenic α-amino acids.[125-134] This method was reported to utilize inexpensive reagents and the synthesis method was said to be much simple. The structure of the complex and the reaction mechanism to form the complex was elaborately investigated[132, 135]. Several α-

amino acids were prepared from Ni-BPB-gly complex through aldol reaction of the enolate of glycine moiety with high enantiomeric excess,[136-138].

The Ni(II) complex of the Schiff base of (S)-N-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide and glycine was one of the first glycine synthons used for asymmetric synthesis of carbon-11 and fluorine-18 labelled α-amino acids for positron emission tomography[108, 139-142]. Concerted application of NMR investigation, X-ray structure determinations, mapping of electron density, mass-spectroscopic investigations and MP2 modelling followed by QTAIM analysis of this and similar nickel (II) complexes revealed steric and electron properties of the complexes in solid state, in solutions and in gas phase (Figure 1.16). Results of the physical-chemical investigation resulted in designing modified complexes with much stronger stereo-discriminative power which will allow to shorten the time necessary for the preparation of radiopharmaceuticals for positron emission tomography labelled with short-lived radioisotopes ^{11}C ($T_{1/2}$ - 20 min) and ^{18}F ($T_{1/2}$ - 110min).

Initially the complexes were developed as biomimetic analogues of aminotransferases able to convert racemic α-amino acids to L-α-amino acids. Later, these complexes are used mostly for the preparation of multi-gram and kilogram quantities of non-coded enantiomerically pure α-amino acids for the development of new pharmaceuticals and agrochemicals. Since the procedure is of high commercial value, the synthetic procedures from industrial research are usually not published. The most important procedures briefly described in the Chapter 5 include preparation of α-amino-β-hydroxy acids (substituted serines). Several studies regarding synthesis of β-hydroxy-α-amino acids using Ni-BPB-gly complex were reported in the literature[143-148]. In most of the cases the key compounds used as starting material was the Ni-BPB-glycine complex.

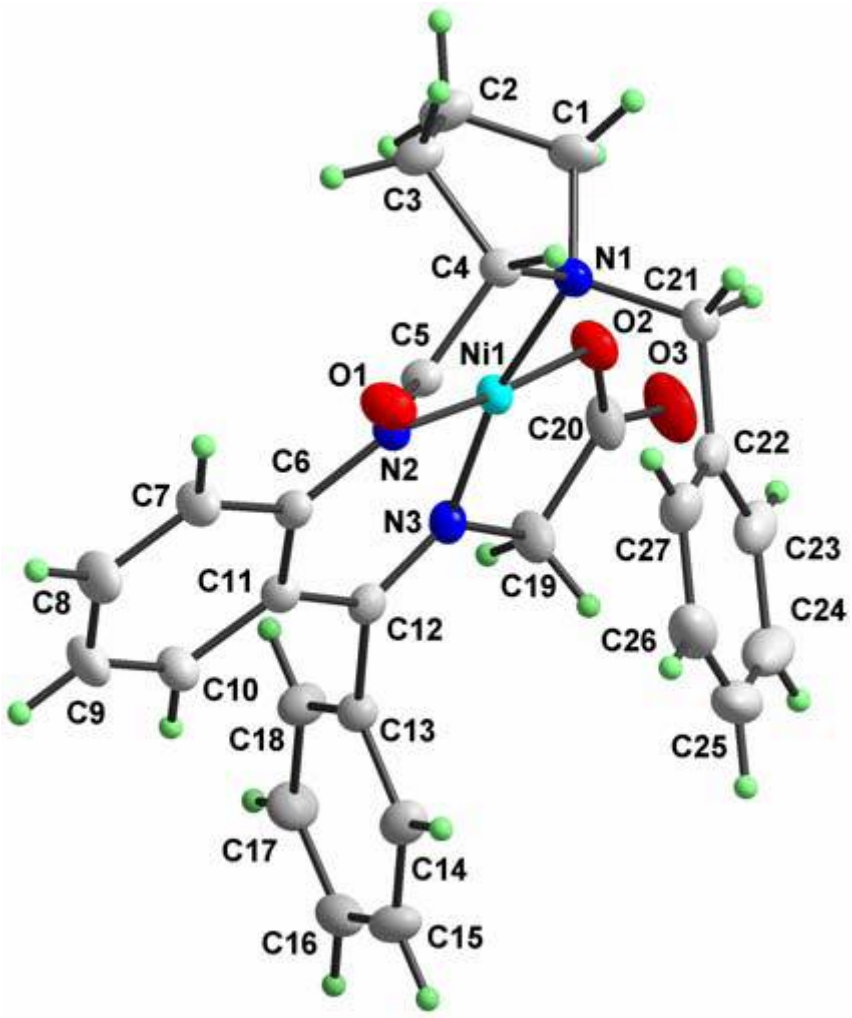


Figure 1-16 X-Ray structure of the nickel(II) complex of the Schiff's bases of BPB and glycine and the numbering of the atoms. [149]

The radio-diagnostic application of this complex was first used as early as in 1990.[150] The chiral glycine complex,[124, 137] was first used for the synthesis of β-hydroxy-amino acids but was later employed with great success in the synthesis of α-amino acids. α-Methyl-α-amino acids were prepared by replacing glycine in the complex with alanine.[146] The Michael type addition of nucleophiles to the α,β-dehydroalanine complex[131] allows the use of various nucleophiles to prepare β-substituted alanines. Reaction of the (S)-BPB-complex with α,β-dehydroaminobutanoic acid generated β-substituted (S)-2-aminobutanoic acids[134]. Since its introduction in 1985[124] the (S)-BPB complex has been employed by several research groups

for the preparation of (S)-α-amino acids[150, 151]. Ni(II)-(S)BPB complex based chiral synthesis was also been applied for the radio-synthesis of [^{18}F]FDOPA[152].

CHAPTER 2

FULLY AUTOMATED ONE-POT SYNTHESIS OF [^{18}F]FET USING TET PRECURSOR AND PURIFICATION USING SOLID PHASE EXTRACTION (SPE) METHOD

2.1. Introduction

In this chapter, O-(2'-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) was synthesized in a modified GE TRACERlab FX-C module, using the O-(2'-tosyloxyethyl)-N-trityl-L-tyrosine-tert-butyl ester (TET) precursor. A fully automated synthesis using solid phase extraction purification method, under aseptic conditions, indented for clinical studies was developed.

Amino acids labelled with positron-emitting radio nuclides are reported to be useful in imaging brain tumours as it provides better diagnostic accuracy than 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG)[75, 153] Due to longer half-life, [^{18}F]fluorine (109.8min) labelled amino acids are more popular over ^{11}C ($T_{1/2}$ = 20 min) based amino acids. Among the former, O-(2'-[^{18}F]Fluoroethyl)-L-tyrosine ([^{18}F]FET) is reported to be the more promising PET radiotracer [154].

O-(2-[^{18}F]Fluoroethyl)-L-tyrosine ([^{18}F]FET) is an analogue of the amino acid L-tyrosine, labelled with Fluorine-18. As compared to the most widely used [^{18}F]FDG, [^{18}F]FET has high uptake in brain tumors and low uptake in normal brain, with a with a potential for grading tumors- particularly gliomas.[80, 155-157]

Several methods of [^{18}F]FET synthesis were reported in the literature. [90, 119, 120, 122, 158, 159]Purification using solid phase extraction for [^{18}F]FET synthesis have also been reported by others[119, 121, 160, 161]. The synthesis procedure by Zuhayra et al[120]used bromoethyl triflate as precursor, with distillation step substituting the HPLC purification. Dirk Mueller et al[119] have synthesized [^{18}F]FET based on a cartridge purification. They prepared [^{18}F]FET by [^{18}F]fluoroethylation of L-tyrosine by a two-step synthesis using a modified [^{11}C]methionine module (Nuclear Interface). The [^{18}F]FET was trapped on an SCX cartridge, eluted with saline solution and trapped again on an HRX cartridge. For a second purification step, the[^{18}F]FET was eluted from the HRX cartridge with ammonium acetate buffer and collected on two SCX cartridges followed by a washing step with water. Out of all these methods, use of O-(2'-tosyloxyethyl)-N-trityl-L-tyrosine-tert-butyl ester (TET) precursor for [^{18}F]FET has gained preference, since it provides better labelling efficiency and high enantiomeric purity. Hamacher and Coenen were the first to report the synthesis of [^{18}F]FET using the TET precursor, in a one-pot two-step synthesis method, followed by semi-preparative HPLC purification.[90] In that procedure, trifluoroacetic acid (TFA) and dichloroethane/ dichloromethane were utilized as hydrolysis solvents. In this chapter, studies were conducted in order to replace the tedious and expensive radio-HPLC purification technique to a simple and economic solid phase extraction purification method.

This section is further subdivided into two parts, viz., Part A and Part B.

Part A: A synthesis method following the procedure reported originally by Hamacher and Coenen[90]was followed. Solid phase extraction purification followed by hydrolysis was developed using reverse phase HR-P and PS-HCO_3 anion exchange resin.

Part B: A fully automated synthesis of [^{18}F]FET was developed with a modified procedure of Part A. Here the purification was carried out by neutral alumina column.

CHAPTER 2 - PART A:AUTOMATED ONE-POT SYNTHESIS OF [^{18}F]FET USING TET PRECURSOR AND PURIFICATION USING REVERSE PHASE SPE RESIN

2.1.1. Introduction

Here, in this chapter [^{18}F]FET is prepared using TET as precursor in a GE TRACERlab FX-C system (configured for producing 2-[^{18}F]FDG). Following [^{18}F]radio-fluorination and hydrolysis, purification was carried out by Solid Phase Extraction, using H-RP & PS-HCO3 resins. The radio-synthesis scheme followed is shown below in Fig 2-1.

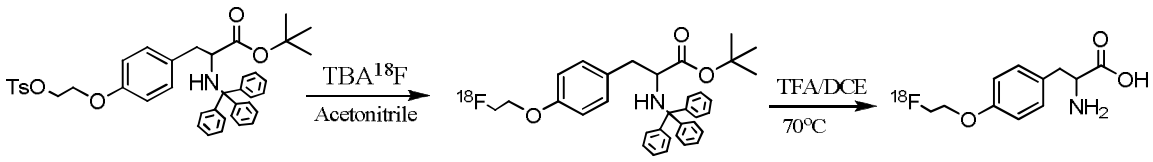


Figure 2-1 Synthesis scheme of [^{18}F]FET using TET precursor and TFA/Dichloromethane as hydrolysis medium

2.1.2. Materials and Methods

TET-Precursor, O-(2'-hydroxyethyl)-L-tyrosine (HET), FET reference standard, resins, Ethanol, WFI, Acetonitrile were obtained from ABX, Germany, Neutral alumina, PS-HCO_3 anion exchange resin and H-RP Reverse Phase resin, from the chromabond® Set IV column procured from ABX was repacked and conditioned with water for injection (WFI). All reagents were prepared in WFI, conductivity less than 0.1µS/cm. TLC plates (1cm x 13cm) of silica gel from Merck, India, were used. Propanol, butanol and acetic acid of AR Grade were from SD Fine Chemicals, Mumbai. Trifluoroacetic acid, DL-tyrosine and Ninhydrin were obtained from Sigma – Aldrich. [^{18}F]Fluoride was produced by $^{18}O(p,n)^{18}F$ reaction in the cyclotron. Radio-HPLC was done with Knauer Smartline HPLC system and

Raytest NaI(Tl) detector, on Nucleosil C-18 column with varying dimensions, flow rate 0.5 - 1mL/min with 5-10% ethanol as the mobile phase, as represented in respective figures. The identity of the labelled compound was confirmed by co-injection with authentic compound on HPLC. Enantiomeric purity was determined by using Daicel Crownpak CR(+) (150X4 mm, 5μm) column, solvent system methanol:water (10:90 v/v), pH-2 using perchloric acid; column temperature = 10°C, UV- 200nm. Radio-TLC was conducted using Raytest GITA radio-TLC Scanner in TLC plates (1cm x 13cm) of silica gel from Merck, India, using acetonitrile: water (8:2) solvent system. Radioactivity was measured using Capintec dose calibrator CRC-15PET. Animals were procured from BARC animal house after obtaining prior permission from BARC animal ethics committee (BAEC) for the studies.

The synthesis was carried out in a modified GE TRACERlab FX-C module (Fig 2)

2.1.2.1. Radiosynthesis of [^{18}F]FET

Table 2-1 shows the reservoirs and chemicals loaded in the module. The schematic representation of the synthesis module is shown in Fig 2-2. The [^{18}F] fluoride was trapped in 45mg PS-HCO_3 anion exchange resin. [^{18}F]Fluoride was eluted with 0.5mL of 75mM Tetrabutylammonium bicarbonate solution to obtain [^{18}F]Fluoride in the form of [^{18}F]TBAF in the reactor vessel. Then to this, 1mL of dry acetonitrile was added and azeotropic distillation was carried out to remove the water. Drying was aided by flushing the reaction vessel with a stream of He-gas. The precursor in acetonitrile (8mg/800μL) was added and heated to 95°C and maintained at this temperature for 5 min. The reaction was then cooled to 40°C with He drying continued inside the reactor. 30% Trifluoroacetic acid (TFA) in 1,2–dichloroethane was added and maintained at room temperature. After 2 min, the temperature was raised to 70°C and maintained for 7min. Purification was carried out by solid phase

extraction. The reaction mixture was loaded onto several SPE resins Viz. Neutral Alumina, anion exchangers and reverse phase H-RP column.

Chemicals	Reservoir No.	Quantity
TBAHCO3	Vial 5	0.6mL, 75mM
Acetonitrile	Vial 1	1mL
TET Precursor	Vial 2	800mg/0.8mL
30%TFA in 1,2-DCE	Vial 3	1mL
WFI	Vial 4	19mL
30% EtOH	Vial 6	15mL

Table 2-1 Reservoirs and Respective chemicals

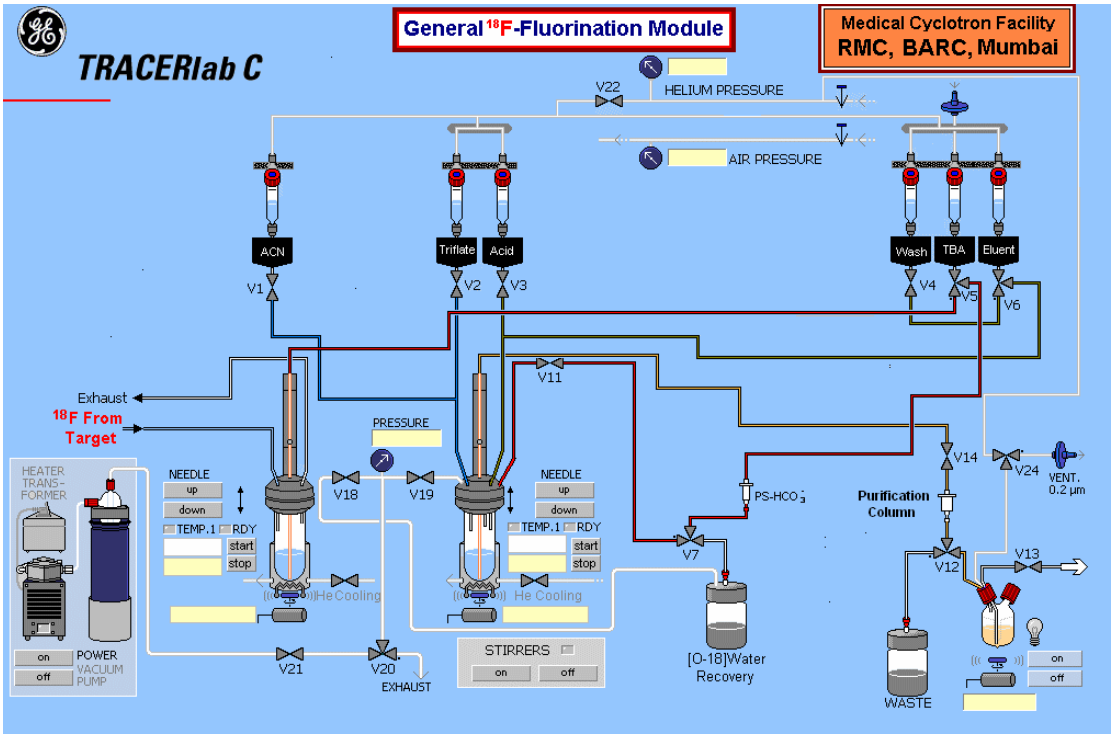


Figure 2-2 Schematic of [^{18}F]FET synthesis module configured for 2-[^{18}F]FDG synthesis

2.1.2.1.1. Preparation of Purification column:

Purification column was prepared by filling HR-P reverse phase resin (machery nagel) in a polypropylene barrel designed for easy and airthight press fit with leurs. The resin layer is packed with having 20µm polyethylene frits in top and bottom. The column is tapped gently for few minutes for close packing and fastened with airtight lid. The column is washed with 20mL of ethanol in order to remove impurities and sanitize the bed. The column is then washed with 100mL of WFI. This column is then stored at 5^{o}C in refrigerator till use (Fig 2-7).

2.1.2.2. Quality Control

2.1.2.2.1. Radio-TLC analysis:

Radio-TLC of the reaction mixture at various stages was developed with various solvent system in a silica TLC plate. The cold FET reference standard was co-spotted and developed in the same solvent in order to match the R_f of cold and HOT peak. The cold spot was developed by spraying 3% ninhydrin solution in acetone and gently heating to develop a purple stain.

2.1.2.2.2. Radio-HPLC analysis:

Radio-HPLC of [^{18}F]FET and non-radioactive reference standard [^{19}F]FET was carried out in isocratic condition (mobile phase:10% ethanol-water; flow rate: 1.0mL/min; UV detector: 254nm; column: C-18 RP 300X4mm, 5µm). Fig 4 shows both the UV and

radio-chromatogram from the HPLC analysis. Radio-HPLC showed single radio-active peak, corresponding to the retention time of [^{19}F]FET (reference standard) from UV chromatogram.

2.1.2.2.3. Gas chromatographic analysis:

The polyethylene glycol immobilized glass capillary column was first conditioned by heating at 180ºC for one hour before analysis with a nitrogen carrier gas flow rate of 2.5mL/min. The column temperature was brought to 50^{o}C and maintained for 30min. 2µL of sample was injected in the column. The column temperature was maintained at 50^{o}C for 5min and increased to 80^{o}C for 5min. Using standard solutions of dichloromethane, dichloroethane, ethanol, acetonitrile and acetone, a calibration graph was generated to estimate the quantities of solvents in the product.

2.1.3. Results and discussions

The reaction was monitored stage by stage and optimized with suitable conditions.

2.1.3.1. [^{18}F]Radiofluorination

As reported by Hamacher and Coenen[90] the commercially available TET precursor proved to be efficient and reliable for labelling process. In order to optimize the radiolabelling efficiency, and precursor economy, different quantities of precursor with varying concentrations in Acetonitrile were taken, and the labelling studies were carried out. The reaction process was stopped after fluorination and the reaction medium was analyzed through radio-TLC in order to identify the labelling efficiency (Fig 2-3). The fluorination was observed to be reliably 88 – 92%(Table 2-2). In contrast with 10mg of

precursor in 0.5mL acetonitrile, used by Hamacher and Coenen[90], it was observed that a minimum of 8mg/800uL of precursor was sufficient for good labelling efficiency.

S_N2 at 85ºC				S_N2 at 95ºC			S_N2 Rxn Vol. at 95ºC	
1.	6mg/mL	82%		6mg/mL	85%		0.6mL	83%
2.	8mg/mL	88%		8mg/mL	89%		0.8mL	92%
3.	10mg/mL	90%		10mg/mL	93%		1.0mL	93%
4	12mg/mL	91%		12mg/mL	93%		10mg/mL was used	

Table 2-2Optimization of labelling efficiency with respect to the quantity of precursor and S_N2 conditions

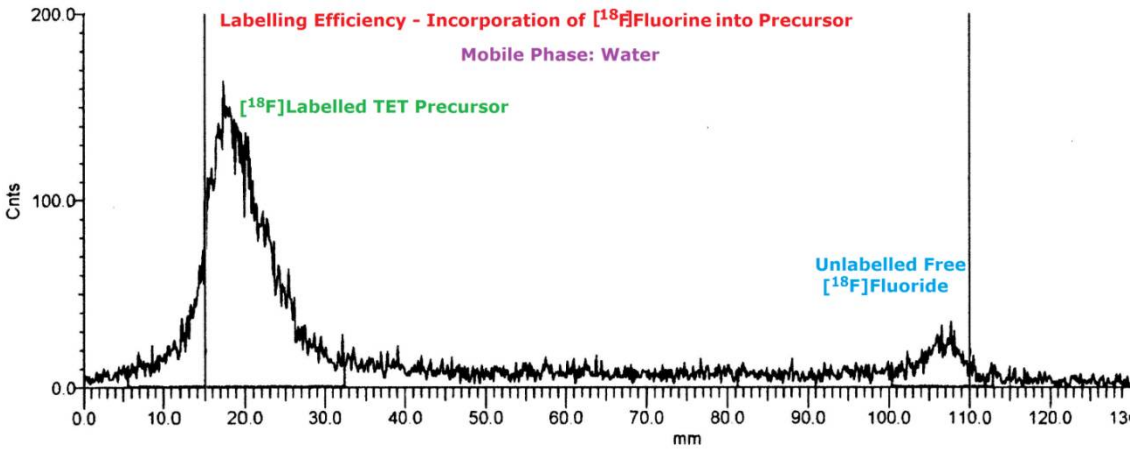


Figure 2-3 Radio-TLC showing labelling efficiency (i.e) Incorporation of ^{18}F

2.1.3.2. Deprotection

A 2 step deprotection was carried out. Deprotection was carried out using 30%TFA in 1,2-dichloroethane. The first step involves deprotection of N-trityl group at room temperature, the next step was carried out at 70ºC for 7 min to remove the tert-butyl group. The reaction mixture was characterized by Radio-TLC to observe the major radio-chemical species present using i-Propanol: Water (70:30) as mobile phase. Four major

radiochemical species – Free Fluoride, Unhydrolised labelled precursor, [^{18}F]FET and presumably a colloidal impurity, were observed in the reaction mixture. On certain occasions it was observed that the colloidal radiochemical impurity was formed in higher quantities, resulting in less [^{18}F]FET yield. This was attributed to degraded TFA on standing, used for deprotection. Fresh TFA was observed to give the best hydrolysis results for [^{18}F]FET synthesis.

The hydrolysis process was smooth with TFA/dichloroethane mixture and was found to be complete. However, dichloroethane is said to be highly toxic considering the intend for human use (<5ppm). But however, other alternates were also reported like dichloromethane or chloroform. Dichloromethane having less toxicity (<600ppm in the final injection) was used as an alternate for dichloroethane for deprotection, and was found to result in completion of hydrolysis.

2.1.3.3. Standardization of Radio-TLC method and identification of several radiochemical species in the reaction mixture

TLC solvent systems generally used for the analysis of aminoacids are butanol:acetic acid:water (4:1:1) or isopropyl alcohol: water (7:3), which are slow moving solvents and hence takes a longer time for their development. Table 2-3 shows the comparison of the time taken for various solvent systems to develop.

Solvent System /Radio-chemical Species	Free Fluoride	Colloidal Impurity	FET	Unhydrolized product	Development time
BuOH:AcOH:H2O (4:1:1)	0	0	0.6	0.8	3h
iPrOH:H2O (7:3)	0.1	0	0.6	0.8	3h
Water	0.9	0	0.7	0	3h
Acetonitrile:H2O(3:7)	0	0	0.5	0.3	40min

Table 2-3 Shows the time taken for development in various solvent systems and R_f of various radioactive species in those solvents.

Considering our applications, in which short lived [^{18}F]fluorine radiolabelled aminoacids to be analyzed, a solvent system that can develop faster with higher moving ability was not yet reported. Hence we have explored a possibility of developing a solvent system, that can develop faster and can also efficiently resolve the radiochemical species that can form in the reaction mixture. The solvent system which commonly used in PET radio-chemistry especially for the most widely used 2-[^{18}F]FDG is acetonitrile:water system. Hence the retention factor (R_f) of various species in the reaction mixture was identified in various commonly used solvent systems whose data are shown in Table 3. Similarly the $R_f$of various species in the solvent system with varying compositions of acetonitrile and water were also studied and the data are shown in Fig 4. Finally the solvent system that suits best for the analysis of [^{18}F]FET, in order to obtain better resolution of all the radiochemical species as well as developing in faster time was found to be Acetonitrile:Water (80:20) (Table 2-4). This solvent system is also convenient to prepare in the PET radiochemistry facility because, of the similarity with the existing solvent used for 2-[^{18}F]FDG i.e Acetonitrile:Water (95:5).

Species acetonitrile: water Composition	**[^{18}F]Free Fluoride**	**[^{18}F]FET**	**Unhydrolized Species 1**	**Unhydrolized Species 2**	**Development Time (min)**	**Remarks**
95:5	0.0-0.01	0.05-0.15	0.85	0.95	15	Quick, Low Resolution
80:20	0.0-0.01	0.35-0.55	0.65-0.8	0.85-0.95	20	Quick, good Resolution
50:50	0.0-0.1	0.4-0.5	0.5-0.6	0.7-0.8	30	Slow, low resolution
30:70	0.0-0.1	0.5-0.6	0.4-0.5	0.2-0.3	40	Slow, best resolution
10:90	0.8-0.9	0.65-0.75	0.1-0.2	0.0-0.1	50	Extremely slow

Table 2-4 Showing R_f of various radioactive species in difference composition of Acetonitrile: Water system.

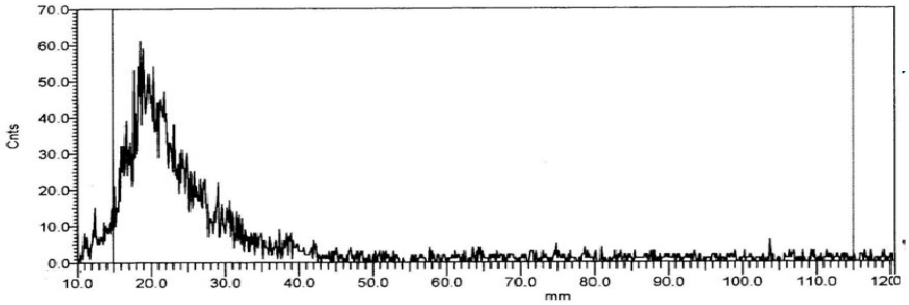
The radioactive species, present in the reaction mixture, showed interesting mobility patterns.

A mixture of partially unhydrolized [^{18}F]FET, unlabelled [^{18}F]free fluoride and [^{18}F]FET revealed following observations:

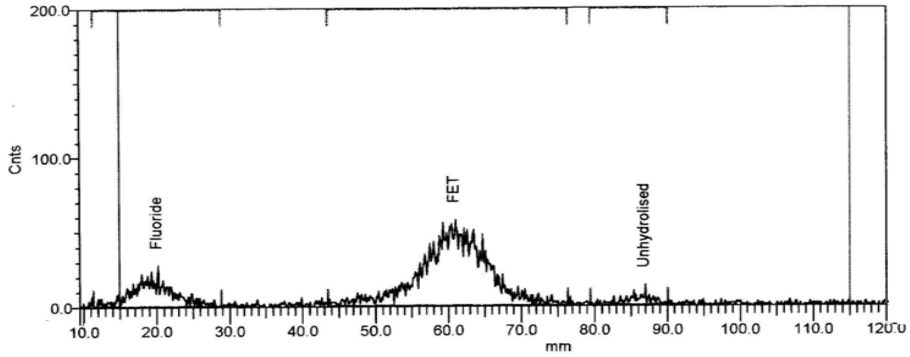
1. Free [^{18}F]fluoride requires atleast 80% water in order to move.
2. A commonly used acetonitrile : water (95:5) is not suitable for separation of the above species. No radio-chemical species were found to move in this system.
3. A minimum of 20% of water is required to move [^{18}F]FET, and a maximum r_f of 0.75 can be obtained with increasing the water content to the maximum.
4. Partially unhydrolized Acetonitrile : Water (80:20) initially had high mobility by increasing the water content till 30%. On further increasing, the mobility started decreasing, and once again came to an R_f of 0 above 80% of water content.
5. The development time was inversely proportional to the acetonitrile content in the mixture.

Based on the above observations, the best separation was obtained when the solvent systems was acetonitrile : water (30:70) (Fig.2-4, TLC 4) with a development time of 30 min. The next best resolution was obtained with a acetonitrile : water (80:20) (Fig 2-4, TLC 5), with a development time of 15 min. However, based on the time of development, Acetonitrile : Water (80:20) is more preferable and chosen for the regular analysis of [^{18}F]FET solutions.

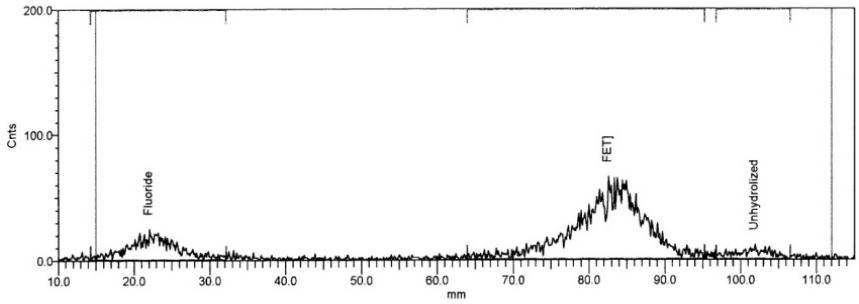
1. Acetonitrile:Water (95:5), development time – 10min



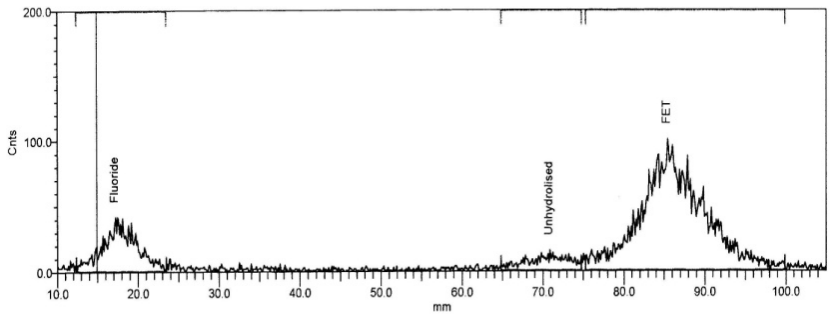
2 Acetonitrile:Water (80:20) - development time – 15min



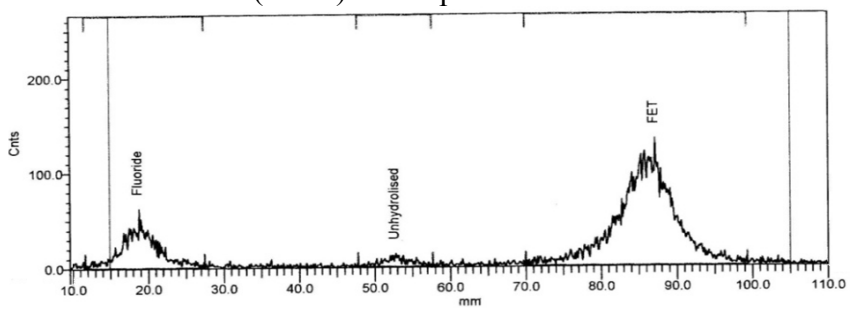
3. Acetonitrile:Water (70:30) - development time – 20min



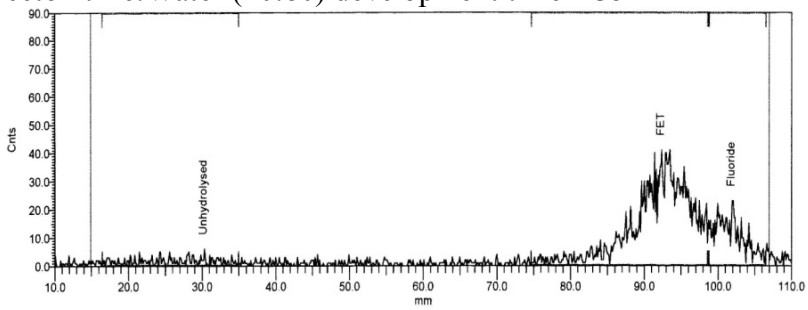
4 Acetonitrile:Water (50:50) - development time – 25 min



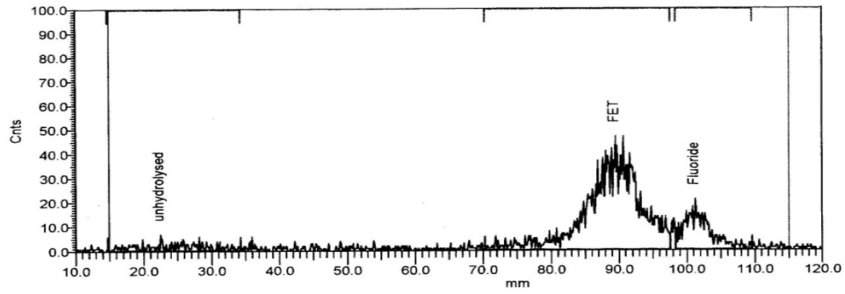
5. Acetonitrile:Water (30:70) development time – 30min



6 Acetonitrile:Water (20:80) development time – 35min



7 Acetonitrile:Water (10:90) development time – 40min



8 Water - development time – 50min

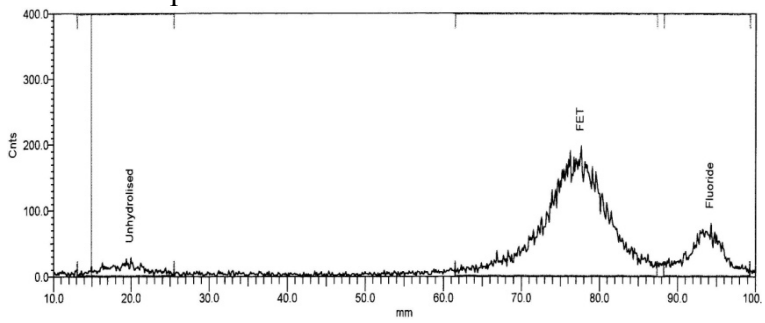


Figure 2-4 Radio-TLC of a mixture of radiochemical species formed in reaction mixture in various acetonitrile:water solvent system.

2.1.3.4. Development of SPE resin for purification of [^{18}F]FET

The SPE resins used for the studies were neutral alumina, PS-HCO3, QMA activated in the bicarbonate form, Dowex - chloride form anion exchanger activated to bicarbonate form and H-RP reverse phase resins were used for this purpose. The reaction mixture was directly loaded onto the various resins. The resin was further eluted using different solvents in order to bring [^{18}F]FET out.

The reaction mixture after deprotection has highly acidic TFA and highly non-polar dichloromethane or dichloroethane in the reaction mixture. It is essential to neutralize the TFA in order to prepare the final formulation. Neutralization of TFA in the reaction mixture, using sodium bicharbonate, has resulted in precipitation of highly non-polar trityl hydroxide, which resulted in the chocking of tubings. Hence further loading of reaction mixture onto the column was not possible. Direct drying of TFA and dichloromethane results in damage of valves involved in evacuation, present in the rear part of the synthesis module. Hence the reaction mixture was directly loaded onto the columns without neutralizing.

Neutral Alumina (4g) being amphoteric, was initially tried out, with various eluents viz. WFI, ethanol and i-Propanol. It was observed that hot 70% i-Propanol could elute the FET activity in volume >10mL. However the solution was found to be highly acidic with a pH of 1 and the colour of the solution was pale yellow, indicating presence of chemical impurities.

As an alternate for neutral alumina, anion exchange resins like PS-HCO_3, QMA and Dowex in bicarbonate form was tried. It was found that [^{18}F]FET gets efficiently trapped in the resins and not getting eluted either by water or highly non-polar solvents like ethanol, isopropyl alcohol, etc.

Non-polar reverse phase HR-P column (1.6g) was then tried out for purification of the reaction mixture. The reaction mixture was found to be efficiently retended in PS-HR-P resin.

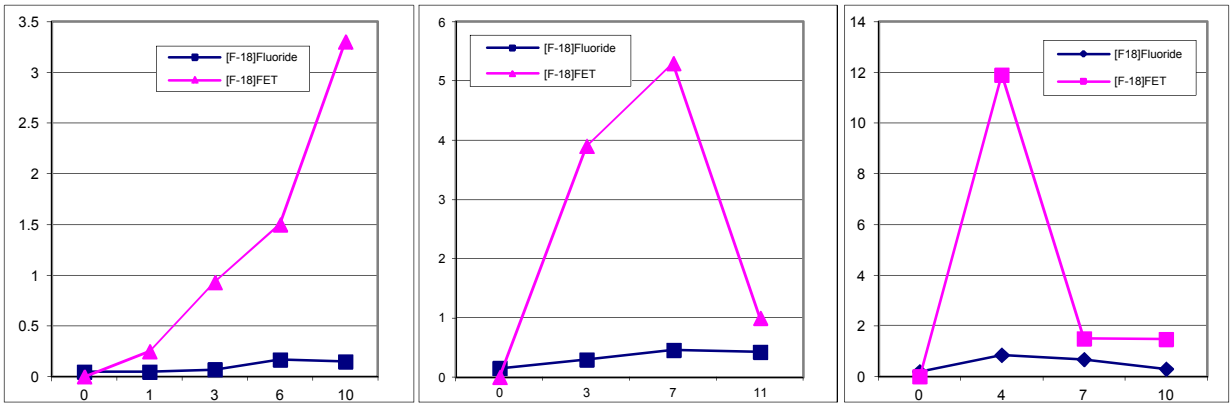
2.1.3.5. Improvement of pH and RCP

Typical pH recommended for a radiopharmaceuticals is of the range 4 to 8. The eluent was also found to be highly acidic in nature due to wash out of TFA. Since [^{18}F]FET was highly retended in the column, and not getting eluted, water wash was continued till the solution becomes neutral. In order to bring the pH to the recommended range the column was washed with excess of WFI to remove TFA present in the column. Due to the limiting capacity of the WFI reservoir, only 19mL can be used for washing. It was observed that pH 6-7 could be achieved by controlling the flow rate of WFI to 2mL/min. The flow rate was maintained by regulating the helium gas flow through valve V1.

Moreover. it was observed that, the unlabelled free fluoride, being highly non-polar separated out by this method. At this stage the [^{18}F]FET was eluted from the resin. Being a non-polar resin, eluents tending to strong non-polar nature was used.

An elution profile with varying composition of ethanolic water was tried as shown in Fig 2-5.

Different compositions of ethanol viz, 10%, 30% and 50% ethanol could elute the [^{18}F]FET out. It was observed that with 50% ethanol, the elution profile was very sharp and in a few milliliter of volume [^{18}F]FET could be collected. However the [^{18}F]FET was accompanied by co-eluted [^{18}F]free fluoride. With 10% ethanol the profile was flatter, requiring higher volume of eluent (>50mL).



Elution profile of [^{18}F]FET and Free [^{18}F]fluoride using 10% ethanol

Elution profile of [^{18}F]FET and Free [^{18}F]fluoride 30% ethanol

Elution profile of [^{18}F]FET and Free [^{18}F]fluoride 50% ethanol

Figure 2-5 Elution profile of [^{18}F]FET and free [^{18}F]fluoride from reverse phase column,in various ethanol-water mixture.

As can be observed 10% ethanol-water solution was not observed to elute the [^{18}F]FET completely. Hence a minimum of 30% ethanol-water mixture was required to elute [^{18}F]FET. 50% ethanol elutes [^{18}F]FET with a less volume, but due to higher ethanolic concentration of the eluent and further loss of [^{18}F]FET when passing further through PS-HCO_3 column, in order to remove minor [^{18}F]fluoride impurities, restricts the use of it.

The study of the fractions eluted from 30% ethanol revealed that the first 3mL predominantly consisted of [^{18}F]Fluoride only and this was discarded to waste. The next 10mL fraction contained [^{18}F]FET with pH in the range 5-6. The purity of [^{18}F]FET obtained on this process was evaluated to be 95%. (Fig 2-6)

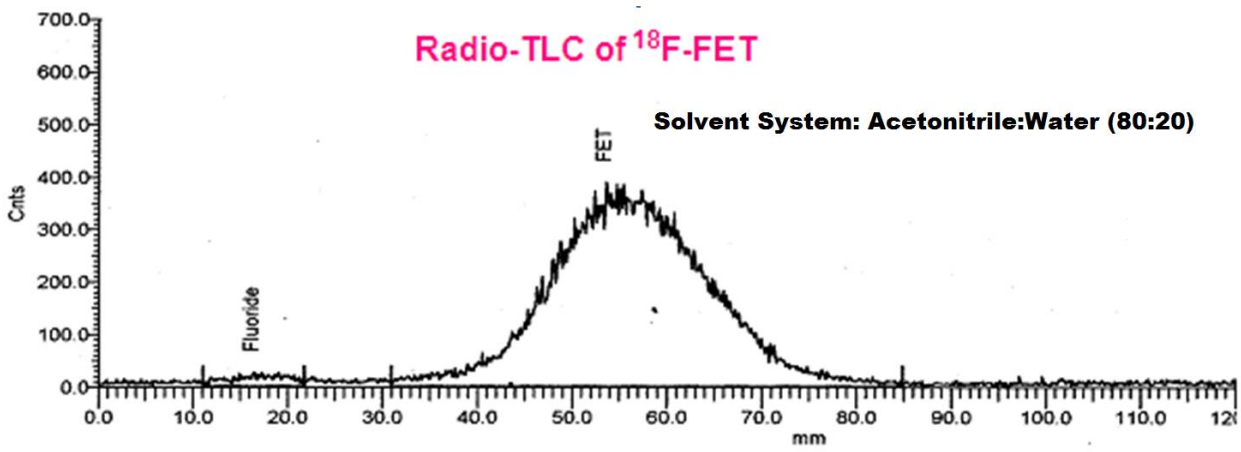


Figure 2-6 Radio-TLC of [^{18}F]FET obtained on elution from the H-RP reverse phase column.

Analysis of the eluted fractions shows that a 5% free [^{18}F]fluoride was present as impurity. In order to remove it, a cartridge containing PS-HCO_3 anion exchange resin was used prior to collection of [^{18}F]FET in the final product. Since, as mentioned above, PS-HCO_3 resin efficiently traps [^{18}F]FET, a bed volume of 200mg was used, which was found to be suitable for trapping free [^{18}F]fluoride, allowing [^{18}F]FET to get collected in the final vessel in 30% ethanolic water(Fig 2-7).

Figure 2-7 QMA anion exchange cartridge in HCO3-form (left); In-house prepared 1.6 g HR-P reverse phase cartridge (middle); 200 mg of PS-HCO3- cartridge for trapping residual fluoride.

Radio-TLC analysis of the final product showed [^{18}F]FET >98% radio-chemically pure. Since the ethanolic content in 30% ethanolic water solution is too high for human injection, the final elution volume is restricted to 8mL and diluted with 8mL of WFI, and finally reconstituted using 1.7mL of 10% sodium chloride solution and 0.7mL of 1M sodium di-hydrogen phosphate buffer solution.

2.1.3.6. Quality control

2.1.3.6.1. Radio-TLCanalysis:

[^{18}F]FET was identified and quantified using Radio-TLC. The TLC was co-spotted with authentic [^{19}F]FET. After development the chromatogram was sprayed with Ninhydrin, followed by gentle heating to determine the R_f of authentic FET in the solvent system. [^{18}F]FET was then identified by the R_f of the cold FET spot. Fig 2-8 shows the radio-chromatogram in solvent system Acetonitrile: Water (8:2).

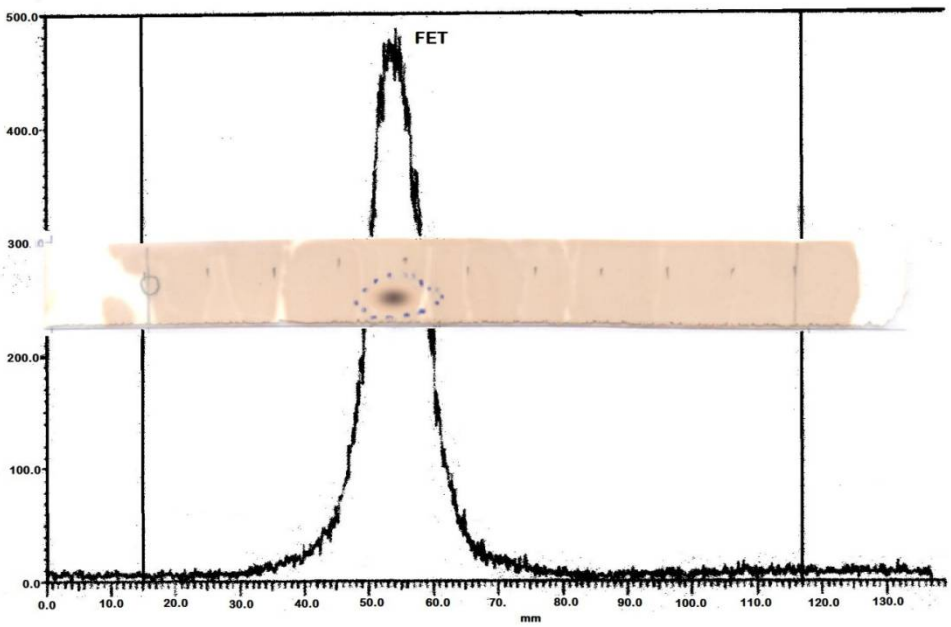


Figure 2-8 Radio-TLC of Purified [^{18}F]FET co-spotted with [^{19}F]FET refrerence standard stained with ninhydrin solution

2.1.3.6.2. Radio-HPLC Analysis:

Radio-HPLC co-injected with authentic FET was carried out along with UV detection at 254nm. Fig 2-9 shows both the UV and radiochromatogram from the HPLC analysis. The peak observed in the UV chromatogram, at a R_t of 14min was found to be present only in the reference standard and not on the [^{18}F]FET sample.

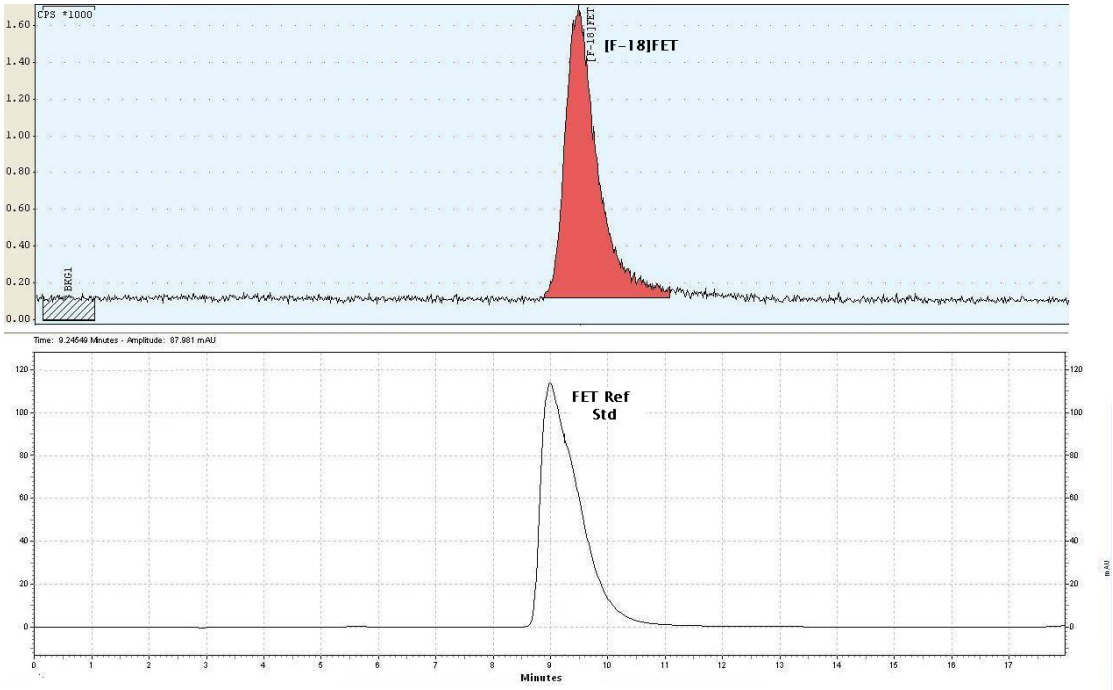


Figure 2-9 Radio-HPLC of [^{18}F]FET and Authentic FET Reference standard. UV-254nmm Flow Rate:1.0mL/min, Rt (UV) of FET = 9.45min, Rt(Radio)of [^{18}F]FET = 9.0 min.

2.1.3.6.3. Gas Chromatographic analysis:

The analysis showed presence of 600ppm of dichloromethane and absence of other solvents (Fig 2-10). This is expected as dichloromethane is used together with TFA as a hydrolyzing agent. However, the toxicity limit of dichloromethane for injections are said to be 600ppm[162], the amount of dichloromethane was found to be in this range and not well below it. This introduces risk for human applications. Due to presence of a high quantity of

30% ethanol in WFI, a saturated peak of ethanol was observed following the dichloromethane peak.

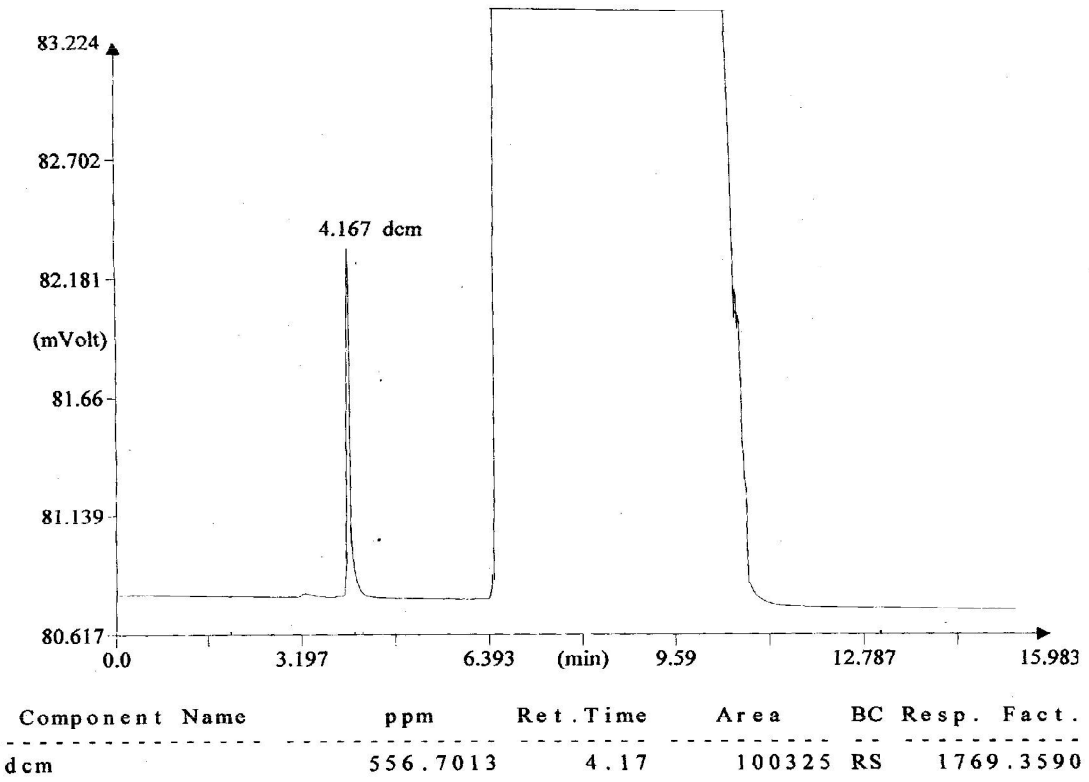


Figure 2-10 Gas chromatogram showing dichloromethane in the final product and high background peak of 30% ethanol.

2.1.3.7. Automation and synthesis yield

The complete synthesis sequence shown in Fig 2-11 was automated. The synthesis time including the slow washing step to improve the pH was one hour. Reliably 36±3.64% decay uncorrected yield was obtained (Table 2-5)

S.No.	Initial Activity (GBq)	Final Acivity (GBq)	Yield
1.	16.65	6.2937	37.8
2.	20.35	6.7562	33.2
3.	21.46	9.05612	42.2
4.	14.06	5.01942	35.7
5.	15.54	5.25252	33.8

Table 2-5 Shows production data of [^{18}F]FET in 15mL of 30% ethanolusing HR-P reverse phase purification.

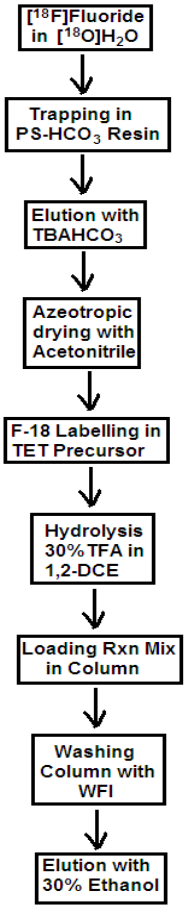


Figure 2-11 Flow diagram showing the schematic sequence of process used in automated production of [^{18}F]FET

2.1.3.8. Conclusions

[^{18}F]FET was synthesized in a GE TRACERlab FX-C module, configured for making 2-[^{18}F]FDG using the TET-precursor. The labelling efficiency, i.e. the incorporation of ^{18}F into the precursor was 90%. 8mg of precursor was sufficient for good labelling efficiency. The various radiochemical species in the reaction mixture were identified. The RCP of [^{18}F]FET was >98% with a radioactive concentration of >10mCi/mL with a radiochemical yield of 36±3.64% (decay uncorrected). The whole synthesis was automated. This simple method, can be easily incorporated in a simplified synthesis module such as the one used for routine [^{18}F]FDG synthesis. HPLC analysis of the final product showed HET to be 35μg/mL, Gas chromatographic analysis showed presence of <600ppm of dichloromethane in the final product, which was found to be safe. However, the range was not found to be well below the stipulated limit. Hence a method involving safer solvents which can avoid toxic solvents like dichloromethane would be recommended for further development.

2.2. CHAPTER 2 - PART B: FULLY AUTOMATED SYNTHESIS OF [^{18}F]FLUOROETHYL-L-TYROSINE USING SINGLE NEUTRAL ALUMINA COLUMN PURIFICATION

2.2.1. Introduction

Radio-synthesis of [^{18}F]FET by One-Pot two-step synthesis procedure using O-(2'-tosyloxyethyl)-N-trityl-L-tyrosine-tert-butyl ester (TET) with semi-preparative HPLC purification was originally reported by Hamacher and Coenen[90]. A one-pot two-step synthesis is similar to 2-[^{18}F]FDG synthesis, which provides a scope for utilizing 2-[^{18}F]FDG module for the synthesis of multiple radio-pharmaceuticals. TET is one precursor which is commonly used for the synthesis of [^{18}F]FET. The TET precursor based synthesis of [^{18}F]FET utilizes highly corrosive Trifluoroacetic acid (TFA) and highly toxic Dichloroethane/ Dichloromethane as hydrolysis solvents. On the other hand Thomas Bourdier et al[159] have carried out a fully automated radio synthesis of [^{18}F]FET using the above TET precursor on GEtracer lab FX_{FN} synthesis module using semi-preparative HPLC purification and 2N Hydrochloric acid as a deprotecting reagent. This procedure adapts a method similar to the most common PET radiopharmaceuticals in use viz. [^{18}F]FDG where the procedures are well standardized, keeping the solvent toxicity into consideration. However the purification was carried out by preparative Radio-HPLC. This lengthens the synthesis time besides requiring a higher degree of sophistication, automation and maintenance. However on both these methods, the TET precursor was reported to provide [^{18}F]FET with good radiochemical and enantiomeric purity in spite of using acids in high concentration. Impressed by this method we have adopted a procedure, which utilizes reagents of low toxicity and corrosiveness

involving acid strength much lesser than the reported ones, utilizing similar to a 2-[^{18}F]FDG reagent kit (Fig 2-12).

The present radio-synthesis procedure is similar to the one reported by Thomas Bourdier et al, with minor modifications, using TET as precursor in a GE TRACERlab *FX-C* module (configured for producing 2-[^{18}F]FDG). Followingradiofluorination and hydrolysis, purification was carried out by Solid Phase Extraction method.

However, several [^{18}F]FDG synthesis modules are available in all PET radiochemistry facilities, synthesis of [^{18}F]FET requires a dedicated synthesis module, demanding more sophistication than FDG synthesis module. This adds restriction for the production of [^{18}F]FET to those centers, availing minimum PET radiochemistry facilities. Synthesis of [^{18}F]FET in [^{18}F]FDG modules, with the reagents used for [^{18}F]FDG synthesis, would facilitate the reach of [^{18}F]FET, even to the primitive PET radiochemistry facilities, availing only [^{18}F]FDG synthesis modules.

Keeping the above factors in consideration, studies have been performed to develop a fully automated synthesis procedure for [^{18}F]FET using SPE method, utilizing reagents which are commonly used for 2-[^{18}F]FDG, the most widely used PET radiopharmaceutical, and sticking towards minimum sophisticated procedure used commonly for the 2-[^{18}F]FDG synthesis module. We have put sufficient efforts in providing [^{18}F]FET in its purest possible form, eliminating all possible chemical and radio-chemical impurities.

Biological evaluation of [^{18}F]FET was studied with C57BL/6 mice bearing B16F10 murine melanoma. We have also conducted Micro-PET imaging studies with the above mice tumor model. Rabbit imaging studies of [^{18}F]FET was also conducted with normal female rabbit using PET/CT imaging technique.

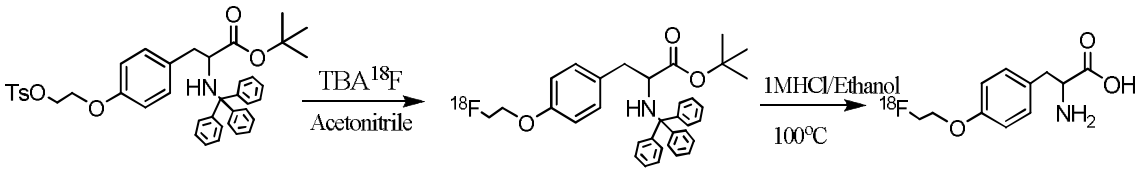


Figure 2-12 Reaction scheme of [^{18}F]FET synthesis from TET precursor using ethanolic HCl mixture for hydrolysis

2.2.2. Materials and Methods

TET-Precursor, FET Reference Standard, Pharmaceutical Grade Ethanol, Water for Injection (WFI) and Acetonitrile were obtained from ABX, Advanced Biochemical Compounds, Germany, QMA cartridge was obtained form Waters, H-RP Reverse Phase resin, from the chromabond® Set IV column procured from ABX was repacked and conditioned with WFI. All reagents were prepared in WFI, conductivity less than 0.1µS/cm. Ninhydrin, microquant Al^{3+} kit was obtained from Sigma – Aldrich. [^{18}F]Fluoride was produced by $^{18}O(p,n)^{18}F$ reaction in GE PEtrace cyclotron. Radio-HPLC was done with Knauer Smartline HPLC system and Raytest NaI(Tl) detector, on Nucleosil C-18 column (300X4 mm, 5µm) at flow rate 0.5mL/min with 5% Ethanol as the mobile phase. Radio-TLC was done using Raytest GITA radio-TLC Scanner in TLC plates (1cm x 13cm) of silica gel from Merck, India, using Acetonitrile: Water (8:2) solvent system. Radioactivity was measured using Capintec dose calibrator CRC-15PET. B16F10 murine melanoma cells were procured from NCCS, pune. C57BL/6 mice were obtained from BARC animal house after taking prior BARC animal ethics committee (BAEC) clearance for the studies.

2.2.2.1. Synthesis Procedure

Synthesis was carried outin a modified GE TRACERlab FX-C module (configured for making FDG) Fig 2-13. shows the schematic of the fluorination module.

The reagents and various chemical loaded in the reservoir are shown in Table 2-6. The [^{18}F]fluoride was trapped in QMA anion exchange resin. [^{18}F]Fluoride was eluted with 0.5mL of 75mM Tetrabutylammonium bicarbonate solution to obtain [^{18}F]Fluoride in the form of [^{18}F]TBAF in the reactor vessel. To this, 1mL of dry acetonitrile was added and azeotropically distilled, to remove the water. Drying was aided by flushing the reaction

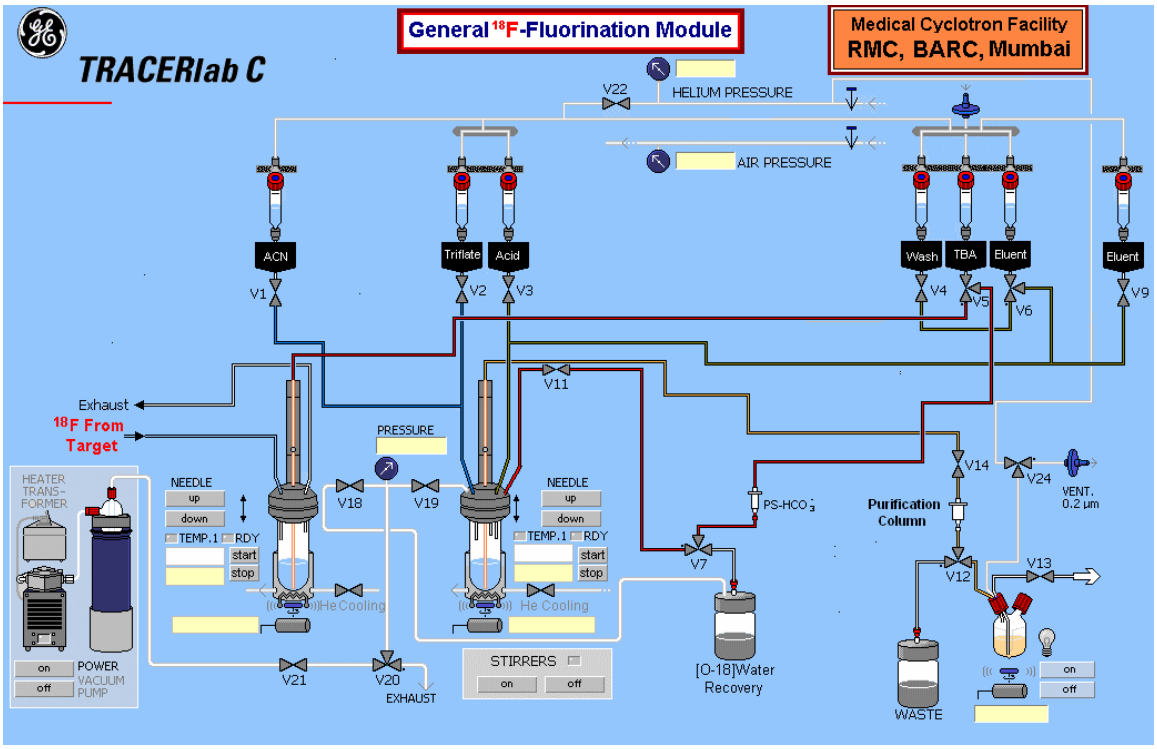


Figure 2-13 Schematic of [^{18}F]FET synthesis module for purification using neutral alumina.

vessel with a stream of He-gas. Fluorination was done by adding precursor in acetonitrile (1-8mg/1mL) and heated to 95°C for 5 min. Acetonitrile was dried with He gas flow at 65ºC for 2 min followed by 95°C for 1min under vacuum. The reaction mixture was then cooled to 40°C with He drying continued inside the reactor. Then a mixture of 0.5mL of 1M Hydrochloric acid and 0.5mL of Ethanol is added for hydrolysis at 40°C. The mixture is stirred for 2min followed by raised the temperature to 100°C and maintained for 10min. The reaction mixture was then loaded on to 12g of Neutral alumina Column. The column was washed with 18mL of 10% Ethanolic Water. The final [^{18}F]FET was collected by washing

the column with 35mL of Water for Injection. The Product is further reconstituted with 10% Sodium Chloride solution and Phosphate Buffer solution and filtered with 0.22µm filter for final injection.

Chemicals	Reservoir No.	Quantity
TBAHCO3	Vial 5	0.6mL, 75mM
Acetonitrile	Vial 1	1mL
TET Precursor	Vial 2	1.5mg/1mL
1M HCl:EtOH (1:1)	Vial 3	1mL
10%EtOH	Vial 4	18mL
Water for Injection	Vial 6	16mL
Water for Injection	Vial 7	16mL

Table 2-6 Reservoirs and Respective chemicals.

2.2.2.2. Quality Control

The purity of the product was evaluated by Radio-TLC using Acetonitrile : Water (8:2) solvent system. Radio-chemical and chemical impurities were evaluated by Radio-HPLC in Smartline Knauer HPLC system with Raytest NaI(Tl) detector using GINA Software. Al^{3+} was tested using Microquant Aluminium testing kit, using the standard protocol provided.

2.2.2.2.1. Radio-HPLC analysis:

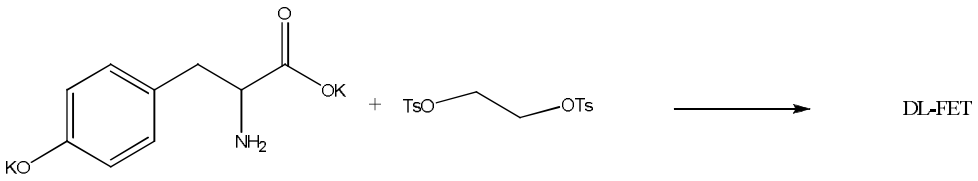
Radio-HPLC of [^{18}F]FET and authentic [^{19}F]FET standard was carried out in isocratic condition (Solvent:7.5% Ethanolic Water; flow rate of 0.5mL/min; UV - 254nm (C-18 RP 250X4mm, 5µm). Fig 4 shows both the UV and radio-chromatogram from the HPLC analysis. Radio-HPLC showed single radio-active peak, corresponding to the retention time of FET cold reference standard from UV chromatogram. An additional UV peak was observed at 11.5min, which was found to be Hydroxyethyl tyrosine (HET) (Fig. 2-18). The quantity of HET in the final product was evaluated from the calibration of cold reference standard of HET.

2.2.2.2.2. Determination of Enantiomeric purity:

Enantiomeric purity of the product was determined using Crownpak CR(+) chiral HPLC column. The solvent system used was water : methanol (90:10, v/v), pH-2 in $HClO_4$, UV-200nm, temperature =10^{o}C, flow rate: 0.8mL/min. The retention time (R_t) of both D and L enantiomer of FET with the R_t of [^{18}F]FET radio peak.

2.2.2.2.3. Synthesis of DL-FET racemic mixture:

DL-FET racemic mixture was prepared by indirect synthesis of FET (Fig 2-14). 1mole of DL-tyrosine was treated with 2moles of KOH in methanol. The methanol layer was evaporated under vacuum. The powder is dissolved in DMSO and added with 2 moles of ethylene glycol ditosylate. The product was neutralized and purified using preparative HPLC. The DL-FET racemic mixture is then used for enantiomeric purity analysis.



(DL-tyrosine di-potassium salt) (Ethylene glycol di-tosylate)

Figure 2-14 Synthesis scheme of DL-FET racemic mixture.

2.2.2.2.4. Gas Chromatographic analysis:

The Polyethylene glycol immobilized glass capillary column was first conditioned by heating at 180°C for one hour before experiment with a nitrogen carrier gas flow rate of 2.5mL/min. The column temperature was brought to 70°C and maintained for 30min. 2µL of sample was injected in the column. Using standard solutions of Ethanol, Acetonitrile and acetone, a calibration graph was first plotted. Based on the calibration curve, the quantities of solvents in the sample were estimated

2.2.2.2.5. Estimation of Aluminium ion (Al^{3+}) Test:

The Al^{3+} ions in the final [^{18}F]FET preparation were estimated using Microquant aluminium test kit, using the standard protocol available. The colour developed in the strip was compared with the standard calibration colours provided in the kit

2.2.2.2.6. Estimation of Tetrabutylammonium ion($[TBA]^+$ion):

Test for TBA^+ ion in the final product was performed by Colour spot test following the procedure reported by Kuntzsch et al[163]. The Pharmacopoeia Limit for TBA^+ ion is <50µg/L. The colour developed with Standard solution was visually compared with the samples. According to the reported procedure 2µL of sample is spotted in TLC Plate and

dried in hot air. 10µL of Ammonium Hydroxide: Methanol (1:9) is added to the dried spot. Plate was placed inside Iodine Chamber immediately for 1min. Intensity of colour developed is directly proportional to quantity.

2.2.3. Results and Discussions

2.2.3.1. Radiosynthesis

The synthesis was carried out in a modified GE TRACERlab FX-C module configured for 2-[^{18}F]FDG synthesis. Table 6 shows the reservoirs and chemicals loaded in the module.

The original method reported by Hamacher et al utilizes corrosive chemicals like Trifluoroacetic acid (TFA) and highly toxic chemicals like Dichloroethane, dichloromethane, chloroform, etc for hydrolysis. Moreover, hexane, diethyl ether and ethyl acetate were also been used. Followed by Radio-HPLC purification, was also reported for purification purpose. On the contrary, the method reported by Thomas Bordier et al, utilizes 2M Hydrochloric acid for the hydrolysis step, followed by Radio-HPLC purification. However, this process was reported to involve precipitation of highly insoluble non polar trityl group in aqueous hydrolyzing medium like hydrochloric acid, which requires prior filteration before purification. This makes automation cumbersome. Impressed by the above method, we have tried with 1M Hydrochloric acid which is used for the synthesis of 2-[^{18}F]FDG.

2.2.3.2. Optimization of hydrolysis using various HCl solvent systems

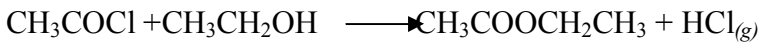
It was observed that, TFA/Dichloromethane or, similar non-polar solvents, with TFA were more suitable for hydrolysis step, leading to the complete hydrolysis of labelled

precursor, under milder conditions. Utilizing aqueous medium, like hydrochloric acid, as a substitute, has not resulted in the complete hydrolysis, leaving around 10% of unhydrolyzed product in the reaction medium. However, considering other factors like corrosiveness of TFA and toxicity of dichloromethane like solvents, hydrochloric acid base method is more reliable for automation and more suitable for human clinical application purposes. Hence we tried hydrolysis using 1M Hydrochloric acid. In order to avoid complications due to precipitation of trityl group during hydrolysis, we used 50% ethanolic hydrochloric acid medium for hydrolysis. We have tried with hydrochloric acid varying in the acidity from 0.5M to 4M. We have not observed any improvement towards completion of hydrolysis by increasing either the molarity of Hydrochloric acid or the reaction time in the process.

Hydrolysis was carried out with various solvent systems in order to obtain complete hydrolysis. Since Bordier et al. have used HCl/Acetonitrile mixture for hydrolysis, a DMSO/HCl mixture was tried instead, since, DMSO is less toxic than acetonitrile. However, it doesn't show improvement in the percentage of hydrolysis. Hence the hydrolysis medium was once again shifted to HCl:ethanol mixture. The reaction temperature was increased from 100^{o}C to 120^{o}C, but still the hydrolysis was not complete. The molarity of HCl was increased from 1M to 4M. But it did not show any improvement, rather higher molarity showed a detrimental result. Reaction time was increased from 10min to 30min, but showed no improvements.

Since one main difference between TFA/dichloromethane mixture and HCl mixture is that, the former is non-aqueous and the later is aqueous. Hence in order to try a non-aqueous hydrolysis mixture based on HCl, a solution of HCl gas dissolved in anhydrous-ethanol was prepared.

The HCl-gas in anhydrous ethanol solution was prepared by adding drop-wise 1mole of acetyl chloride in anhydrous ethanol (in a sealed vial) under ice cooling. The reaction scheme is as shown below (Scheme 2b.1).



$CH_3COCl + CH_3CH_2OH \longrightarrow CH_3COOCH_2CH_3 + HCl_{(g)}$

(acetylchloride) (anh. ethanol) (ethyl acetate) (Hydrogen chloride gas)

Scheme 2b.1: Shows preparation of 1M HCl gas dissolved in anhydrous ethanol.

However use of this mixture has not shown only 10% of hydrolysis.

The results obtained from these studies are shown in following Table 2-7.

S. No.	Solvent	Parameter Varied	% Hydrolysis
1.	1MHCl/DMSO (1:1) @ 100°C for 10min	Solvent	85%
2.	1M HCl gas in Ethanol @ 100°C for 10min	Solvent	10%
3.	1MHCl/ethanol (1:1)@ 100°C for 10min	Solvent	85%
4.	2MHCl/ethanol (1:1) @ 100°C for 10min	Acid strength	85%
5.	3MHCl/ethanol (1:1) @ 100°C for 10min	Acid strength	75%
6.	4M HCl/ethanol(1:1) @ 100°C for 10min	Acid strength	60%
7.	1MHCl/ethanol (1:1) @ 120°C for 10min	Temperature	85%
8.	1M HCl/ethanol(1:1) @ 100°C for 20min	Reaction time	85%
9.	1M HCl/ethanol (1:1)@100°C for 30min	Reaction time	85%

Table 2-7 Standardization of HCl based hydrolysis medium for [^{18}F]FET synthesis.

2.2.3.3. Purification of [^{18}F]FET using neutral alumina column

The reaction mixture, post hydrolysis shows presence of about 10% of un-hydrolyzed product and about 5% of unlabelled free [^{18}F]fluoride. [^{18}F]fluoride has very high affinity towards neutral alumina resin. Hence, passing the reaction mixture directly through the column

removes [^{18}F]fluoride. The un-hydrolyzed products are highly non-polar in nature. Hence washing with sufficiently non-polar solvent system like ethanol-water mixture would elute out un-hydrolyzed product initially followed by [^{18}F]FET. It is essential that the non-polar radio-active impurities are removed completely during purification in order to obtain [^{18}F]FET in its highest possible radio-chemical purity. For this various proportions of ethanol-water mixture was tried. With a 10% ethanol water mixture, un-hydrolyzed product was found to get eluted out completely, without much loss of [^{18}F]FET.

Various bed volume of neutral alumina was tried for purification starting from 4g till 12g. It was observed that a total bed volume of 12g is required in order to separate un-hydrolyzed radio-labelled product completely from the reaction mixture (Fig 2-15).

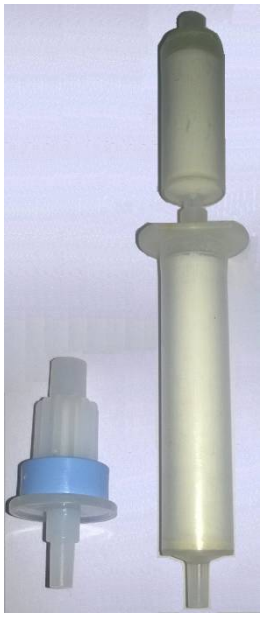


Figure 2-15 Anion exchanger cartridge for trapping [^{18}F]Fluoride from [^{18}O]Water (Left),In-house prepared 8g neutral alumina purification cartridge.(Right).

2.2.3.4. Washing out impurities from neutral alumina column and purification of [^{18}F]FET

The radio-synthesis of [^{18}F]FET originally reported by Hamacher and Coenen[90], utilized mixture of TFA and dichloroethane as hydrolysis reagents. Later many have reported use of dichloromethane or chloroform to give better results instead of dichloroethane. However, these reagents are extremely corrosive and highly toxic. This can have detrimental effect on the components like valves and rubber septas of the synthesis module, requiring to change them frequently. However, these reagentsare found to be extremely efficient even at lower temperatures like 70°C resulting in complete hydrolysis of the labelled precursor. Utilizing HCl:ethanol mixture undergoes hydrolysis leaving [^{18}F]FET as the major component, but however leaving some amount of unhydrolized component in the mixture. The comparison of radio-TLC of the reaction mixture post hydrolysis using TFA/dichloromethane and HCl/ethanol mixture is shown in Fig. 2-16.

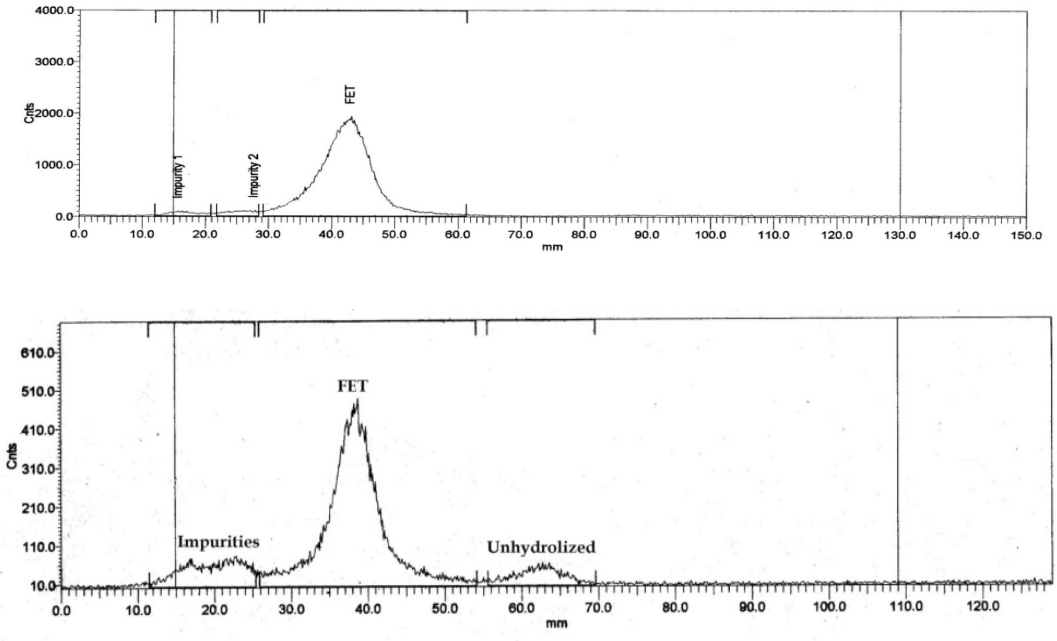


Figure 2-16 Radio-TLC of [^{18}F]FET using TFA/dichloromethane as hydrolysis medium (Top) and 1M HCl/Ethanol as hydrolysis medium (Bottom).

Washing the neutral alumina column with 10% ethanol is found to remove the non-polar unhydrolyzed radio-active impurities at first, followed by [^{18}F]FET. In order to obtain complete separation of [^{18}F]FET from unhydrolyzed impurities, 12g of neutral alumina was found to be sufficient. Neutral alumina column has been reported as a purification resin in several reports. Moreover, neutral alumina is always a part of SPE technique reported by most of the studies in the literature. We have tried with several resins, including reverse phase and anion exchange resins in combination with neutral alumina. But the results were sufficiently satisfactory with single neutral alumina bed. However, we also consider further scope of development using Sep-pak columns for purification, but have not succeeded. Under this condition the purification cartridge was washed with 18mL of 10% ethanol, which was found to remove the unhydrolized radio-active impurities completely. Elution of the neutral alumina column further with de-ionized water for injection was found to provide [^{18}F]FET. A total volume of 32mL is sufficient to recover [^{18}F]FET to a maximum extent. This was found to be 19.07±3.69%(n=9) yield (non-decay corrected, Table 2-18). Radio-HPLC and Radio-TLC showed >99% of radiochemical pure[^{18}F]FET (Fig 2-17&Fig 2-20). However UV-HPLC chromatogram showed presence of two cold peaks, R_t time 7.8min and 9.1min. The peak at 7.8 min is identified to be O-(2'-hydroxyethyl)-L-tyrosine, obtained from unfluorinated precursor, and the peak at 9.1min is an unknown species (Fig 2-17).

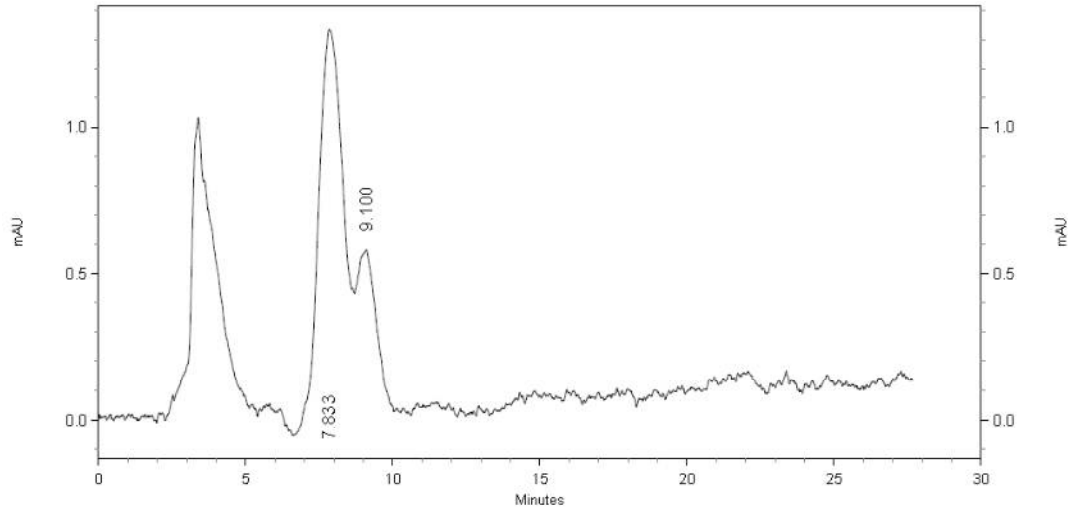


Figure 2-17 UV-HPLC Chromatogram of [^{18}F]FET: C-18 RP column; Flow Rate: 0.8mL/min, UV-254nm; R_t of HET – 7.833 min, R_t of unknown species – 9.1 min.

Restricting the elution volume to 16mL, avoided the 9.1min peak with a reduced radiochemical yield of [^{18}F]FET. Whereas the HET peak at 7.8min was unavoidable. It was quantified using HET reference standard to be 18µg/mL. Since the toxicological data for HET is not available in the literature, we were skeptical to ignore its presence in the injectables. In order to reduce the HET further, the only way we could do was by reducing the precursor concentration used for radiolabelling. The precursor quantity was reduced from 8mg/mL to 2mg/mL, which was found to reduce the radiochemical yield of [^{18}F]FET to a noticeable level (Table 2-8). Reliably [^{18}F]FET yield of 13.44 ± 1.20 % and 7.03 ± 0.79% (n=9) in 35mL and 17 mL of buffered saline were obtained, when 2mg of TET precursor was used (Table 12). The UV-HPLC chromatogram showed HET <2µg/mL(Fig 2-19).

S. No	Quantity of Precursor (mg) in 1mL Dry Acetonitrile	% Yield (non-decay corrected , n=3(in 35mL buffered saline	% Yield (non-decay corrected , n=3(in 17mL buffered saline	Quantity of HET Concentration of HET (µg/mL , n=3(
1.	10	23.6±1.61	12.6±1.01	34.7±3.1
2.	8	19.8±1.25	10.2±1.21	18.0±2.1

3.	6	18.1±0.77	9.6±0.79	12.8±1.2
4.	4	16.3±0.81	8.4±0.86	7.7±0.7
5.	2	14.1±0.62	7.2±0.94	1.8±0.4
6.	1	8.9±0.57	4.3±1.19	<0.5

Table 2-8 Quantities of precursor used, overall yield of [^{18}F]FET and quantity of HET as an impurity in the first 16mL of elution

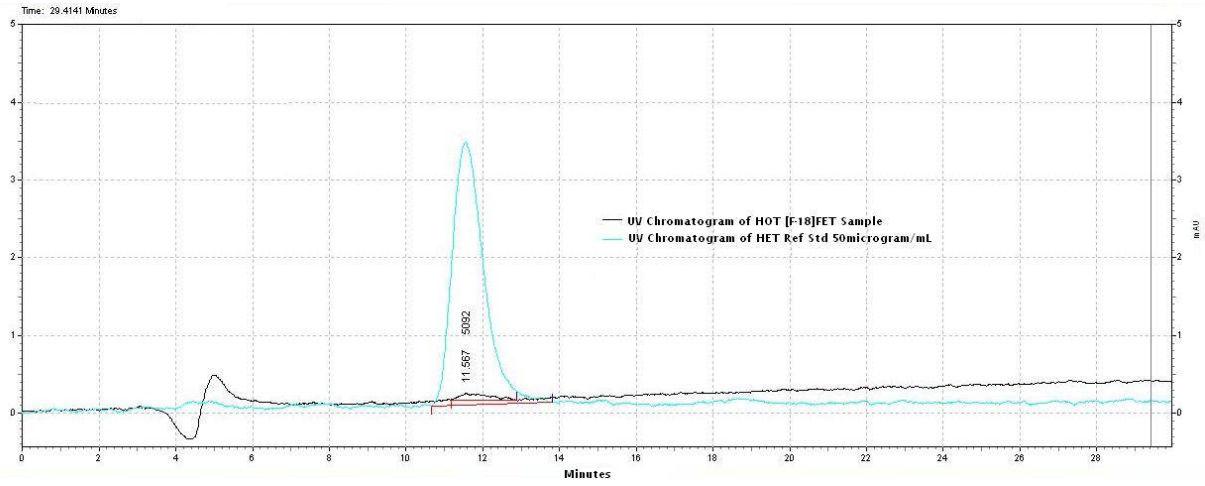


Figure 2-18 HPLC of [^{18}F]FET showing UV Chromatogram of [^{18}F]FET (Black) overlaid with reference Standard of O-(2'-hydroxyethyl)-L-tyrosine [HET] (Blue). From the Peak area integration, HET content in the final product is estimated to be 1.3µg/mL. UV-254nmm FR:0.5mL/min, R_t of HET = 11.6min.

2.2.3.5. Simplification of [^{18}F]FET synthesis by using common reagents available from 2-[^{18}F]FDG reagent kit

[^{18}F]FDG synthesis and its reagents are well established to be safer and reliable for automation. Utilizing these reagents for synthesis of [^{18}F]FET provides safety and reliability for its production. Moreover, 2-[^{18}F]FDG is the most commonly and the only widely used PET radio-pharmaceutical world wide. Hence reagents and module for producing 2-[^{18}F]FDG would be readily available in all PET centers. Hence, all these PET radio tracer synthesis units can easily produce [^{18}F]FET, by adapting this method. Table. 2-9 presents reagents used for 2-[^{18}F]FDG and [^{18}F]FET by reported procedure and by our modified

procedure in order to compare the similarity in the reagents of the procedure that we have developed with that of reagents used for 2-[^{18}F]FDG synthesis.

S. No.	2-[^{18}F]FDG reagents	[^{18}F]FET reagents (Bourdier et al)	Modified [^{18}F]FET procedure
1.	$TBAHCO_3$	$TBAHCO_3$	$TBAHCO_3$
2.	Acetonitrile	Acetonitrile	Acetonitrile
3.	Mannose triflate	TET Precursor	TET Precursor
4.	1M HCl	2M HCl/Acetonitrile	1M HCl/EtOH
5.	WFI – 2mL	Radio-HPLC	10% Ethanol-water – 19mL
6.	WFI-Elusion	-	WFI-Elusion.

Table 2-9 Comparison of reagents used for synthesis of [^{18}F]FET and 2-[^{18}F]FDG.

By adapting this procedure, the GMP compliant reagents used for 2-[^{18}F]FDG can be directly used for [^{18}F]FET. This also ensure the easy and reliable availability of reagents for the regular preparation of [^{18}F]FET.

2.2.3.6. Quality Control

2.2.3.6.1. Radio-TLC analysis:

Radio-TLC analysis of the final product was performed in acetonitrile:water (80:20) as mobile phase. Radio-TLC chromatogram shows single peak of >99% of [^{18}F]FET in the final product mixture (Fig 2-20).

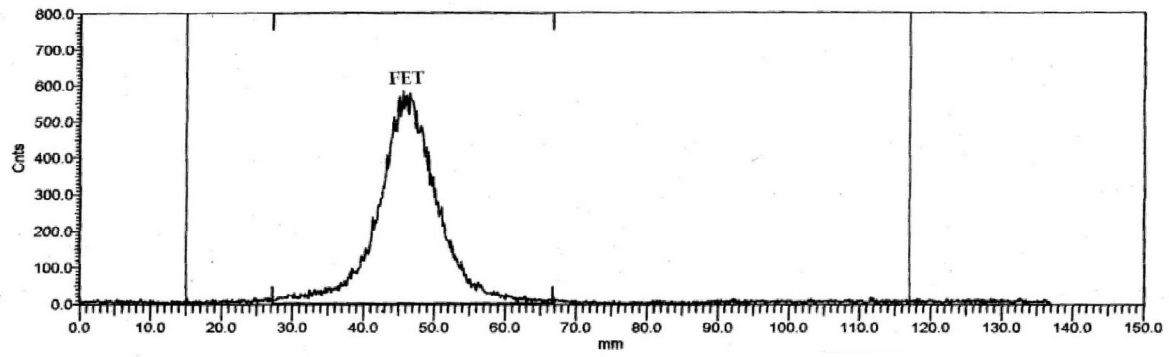


Figure 2-19 Radio-TLC of Purified [^{18}F]FET. Solvent System: Acetonitrile: Water (8:2)

2.2.3.6.2. Radio-HPLC analysis:

The comparison of [^{18}F]FET obtained by, reducing the precursor to 2mg, and quantification of HET in the final product together with the purity of [^{18}F]FET is shown in Fig 2-21 as a single chromatogram.

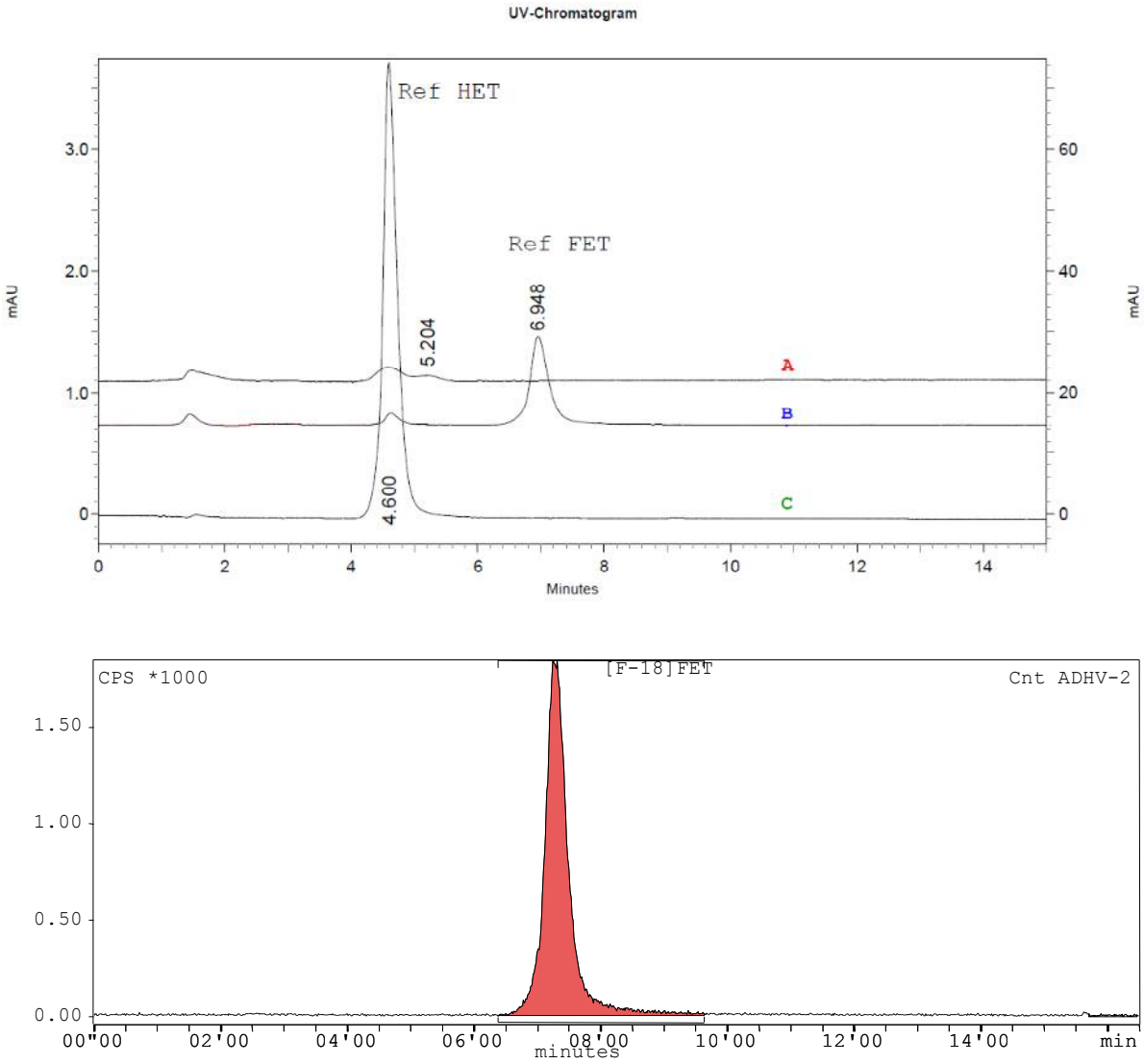
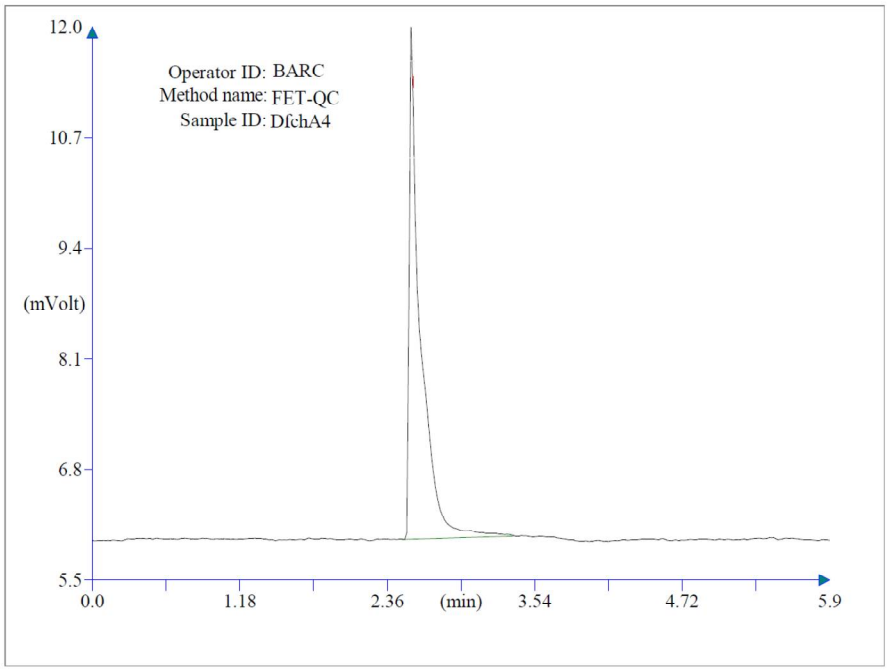


Figure 2-20 Radio-HPLCColumn Nucleosil C-18 column 300X4 mm, 5µm, UV-254nmm Flow Rate:1.0mL/min: ***A.*** *HPLC [^{18}F]FET Sample showing 1.3µg/mL (detection limit 0.2µg/mL) in restricted elusion volume of 16mL and unknown peak at 5.2 min in full elusion of 35mL.* ***B.*** *Radio-HPLC of [^{18}F]FET co-injected with [^{19}F]FET Reference standard. R_t (UV) of [^{19}F]FET = 6.95min, R_t of [^{18}F]FET = 7.25 min.* ***C.*** *Ref std of HET (50µg/mL). R_t of HET – 4.6 min.*

It is worth mentioning that, the precursor quantity used by others were in the range of 6mg to 12mg. The fluorination of the TET precursor at low concentration was found to be sufficiently high even at low concentration of 2mg/mL. This high radiolabelling, even at low concentration, was attributed, probably due to an additional [^{18}F]Fluorination, occurring while the removal of acetonitrile, post S_N2 step, as the concentration of reaction mixture increases during evaporation, while all reagents required for S_N2 substitution of ^{18}F is available in the reaction medium.

2.2.3.6.3. Gas Chromatographic analysis:

The analysis showed absence of solvents other than 2% of Ethanol (Fig 2-22). This is expected as the cartridge is washed with 10% Ethanol prior to final elution of [^{18}F]FET with Water for Injection. No other solvent impurities were observed in the final product.



Component Name	ppm	Ret.Time	Area	BC	Resp. Fact.
EtOH	20691.5300	2.28	801955	RS	87.0866

Figure 2-21 Gas chromatogram shows single peak corresponding to ethanol quantified as 2.07ppm.

2.2.3.6.4. Estimation of Tetrabutylammonium ion (TBA^+ ion):

Test for TBA^+ ion was performed by colour spot test following the procedure reported by Kuntzsch et.al.,[163] and compared with samples from the final product. The pharmacopoeia limit for TBA^+ ion is <50µg/L. According to the reported procedure, 2µL of sample is spotted in TLC Plate and dried in hot air. 10µL of Ammonium Hydroxide: Methanol (1:9) is added to the dried spot and the TLC plate was immediately placed inside a chamber saturated with iodine vapour for 1min. Intensity of colour developed is directly proportional to quantity of TBA^+ ion in the final product. The quantity of TBA^+ ion in the final product was found to be well below 50µg/mL (Fig 2-23).

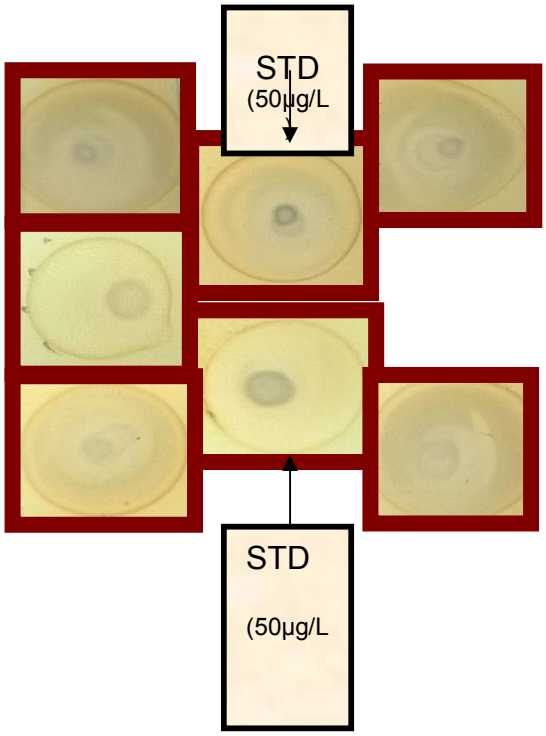


Figure 2-22 Colour spot test for TBA+ ion in the final product comparison to 50µg std.

2.2.3.6.5. Estimation of Aluminium ion (Al^{3+}ion):

Aluminium ion test performed using Microquant aluminium testing kit, using the protocol provided with the product, showed <5µg/mL of Al^{3+} ion in the final product (Fig 2-24).

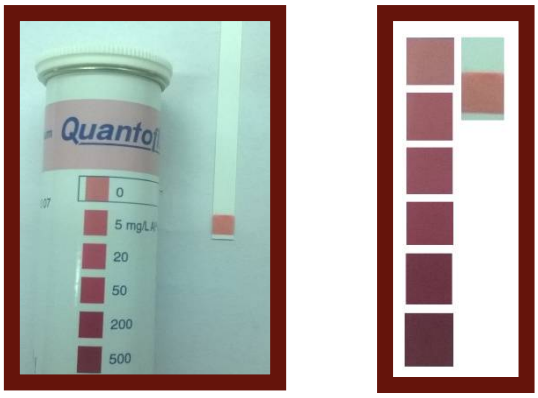


Figure 2-23 Shows colour developed in microquant aluminium test kit.

2.2.3.6.6. Determination of Enantiomeric purity of [^{18}F]FET produced:

Enantiomeric purity analysis was performed in Crwonpak CR(+) chiral HPLC column (Fig 2-25). The product shows presence of single L-enantiomer which was compared with racemic mixture of [^{18}F]FET. The Enantiomeric purity was found to be >99%, which is in agreement with the results reported by others[90, 159].

2.2.3.7. Tumor Uptake studies

Tumor uptake studies of [^{18}F]FET were conducted with B16F10 melanoma tumor bearing C57BL/6 mice based on the studies reported by Wang Ming et al.[158] Tumor was developed in C57BL/6, by injecting B16F10 murine melanoma cell lines (10^5 Numbers) on shoulder muscles. The tumor was allowed to develop to a palpable size for 15 days. The animals are injected with 200µL of [^{18}F]FET (4mCi/mL).

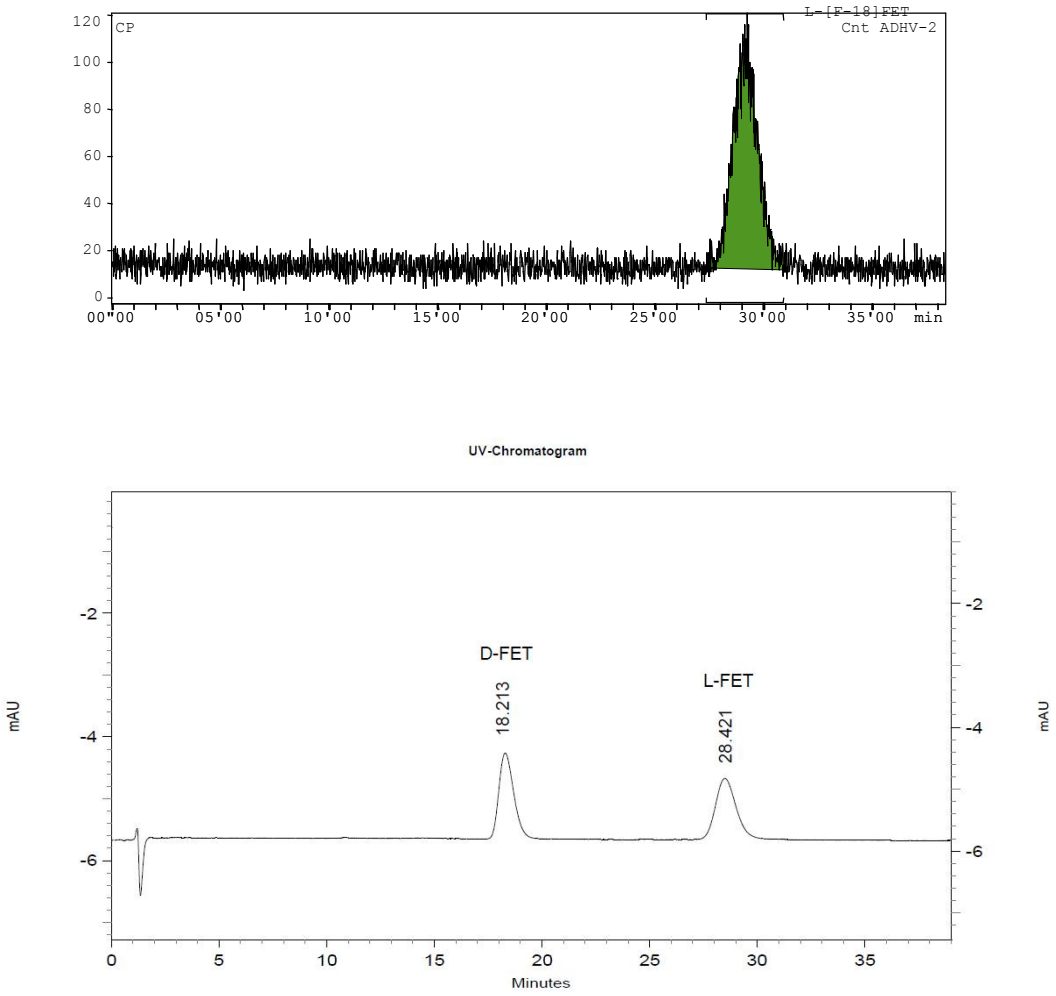


Figure 2-24 Chiral HPLC using Crownpak CR(+) column. Flow Rate:0.8mL/min; Solvent: MeOH: Water (10:90,V/V); pH = 2 in HClO4; UV – 200nm; Column temperature = 10□C. Bottom:Rt (D-FET) UV- 18.2min; Rt (L-FET) UV - 28.4 min; Top: Rt(L-[^{18}F]FET) Radio - 29.1min (Radioactive peak follows the UV peak with a delay due to placement of detectors in sequence)

2.2.3.7.1. Biodistribution studies:

Bio-distribution data of [^{18}F]FET in C57BL/6 mice bearing B16F10 murine melanoma. The animals were sacrificed in different time points (15min, 60min and 120min). Three animals were sacrificed in each time point. The radioactivities in different organs were counted and the percentage Injected dose per gram were measured (%ID/g).Tumor uptake was found to be 5.54%ID/g and tumor/brain ratio is found to be 2.8 (Table 2-10).

%ID/g(Normal)				%ID/g (Tumor)		
Organ/ Dissection Time Pt	**15min**	**60min**	**120min**	**15min**	**60min**	**120min**
Blood	3.11±0.23	2.38±0.46	1.54±0.26	3.27±0.58	2.41±0.36	1.46±0.29
Bone	2.01±0.31	3.07±0.45	1.81±0.47	2.04±0.47	2.89±0.55	1.40±0.56
Muscle	2.58±0.12	3.47±0.81	1.92±0.51	2.23±0.89	2.98±0.64	1.65±0.28
Liver	2.33±0.86	2.66±0.27	1.46±0.40	2.55±0.61	3.05±0.62	1.54±0.29
Stomach	3.16±1.02	4.15±1.16	1.61±0.13	3.11±0.86	4.41±1.06	1.23±0.57
Int+GB	4.05±0.26	4.85±1.25	2.12±0.33	2.77±0.80	3.15±0.54	1.80±0.41
Kid+Blder	3.55±0.95	5.24±1.53	4.45±1.02	3.64±1.23	5.90±2.70	3.20±1.43
Heart	3.11±0.63	3.76±0.56	1.11±0.31	2.75±0.38	3.28±0.54	1.62±0.30
Lungs	3.23±0.37	4.05±1.25	1.81±0.78	3.15±1.50	3.96±1.14	1.63±0.35
Spleen	3.66±0.42	3.87±0.96	2.24±0.81	3.64±1.23	4.04±0.75	1.92±0.36
Brain	1.72±0.33	2.51±0.67	1.51±0.31	1.45±0.76	1.98±0.35	1.20±0.22
Pancreas	22.31±5.13	27.45±6.76	11.18±5.73	21.89±5.33	26.46±9.53	8.43±2.65
Skin	3.12±0.91	2.62±0.53	1.23±0.29	2.83±0.90	2.51±0.34	1.95±0.75
Tumor				2.78±0.23	5.54±1.31	3.30±0.84

Table 2-10Bio-distribution data of [^{18}F]FET in C57BL/6 mice bearing B16F10 murine melanoma. Tumor uptake was found to be 5.54%ID/g and tumor/brain ratio is found to be 2.8.

2.2.3.7.2. Micro-PET imaging studies:

A Micro-PET imaging studies were also conducted for normal and tumor bearing C57BL/6 mice, which showed significant [^{18}F]FET uptake in tumor 60min post injection. Tumor/brain ratio was found to be 3.3 which is in good agreement with the bio-distribution data. (Fig 2-26)

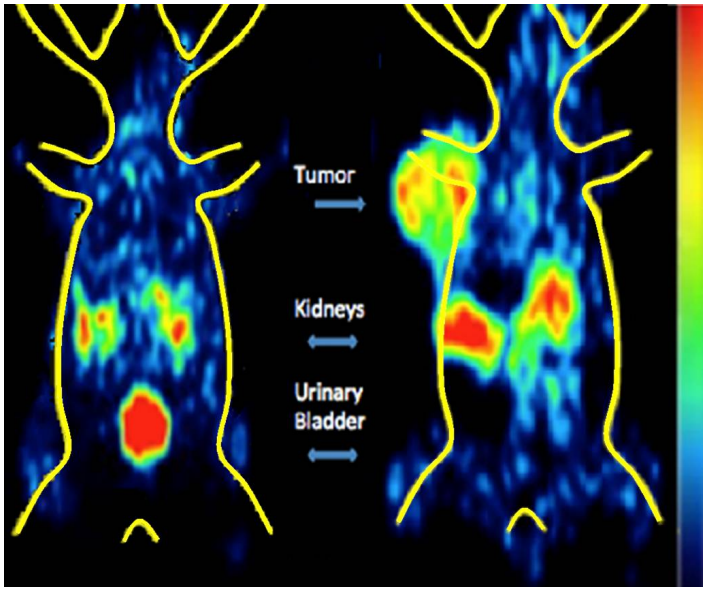


Figure 2-25 Micro PET imaging of [^{18}F]FET in C57BL/6 normal mouse (left) and tumour bearing mouse (B16F10 melanoma) (right)

2.2.3.8. PET/CT imaging in Normal Rabbit

PET/CT image was performed in normal healthy female rabbit (2.62 Kg) after fasting for six hours. 370MBq of [^{18}F]FET in 1mL was injected through ear vein. The rabbit was anaesthetized by injecting ketamine and the images ware recorded after 1 hour post injection. Rabbit image shows (Fig 2-27), no significant uptake in normal brain and other organs with good clearance in bladder.

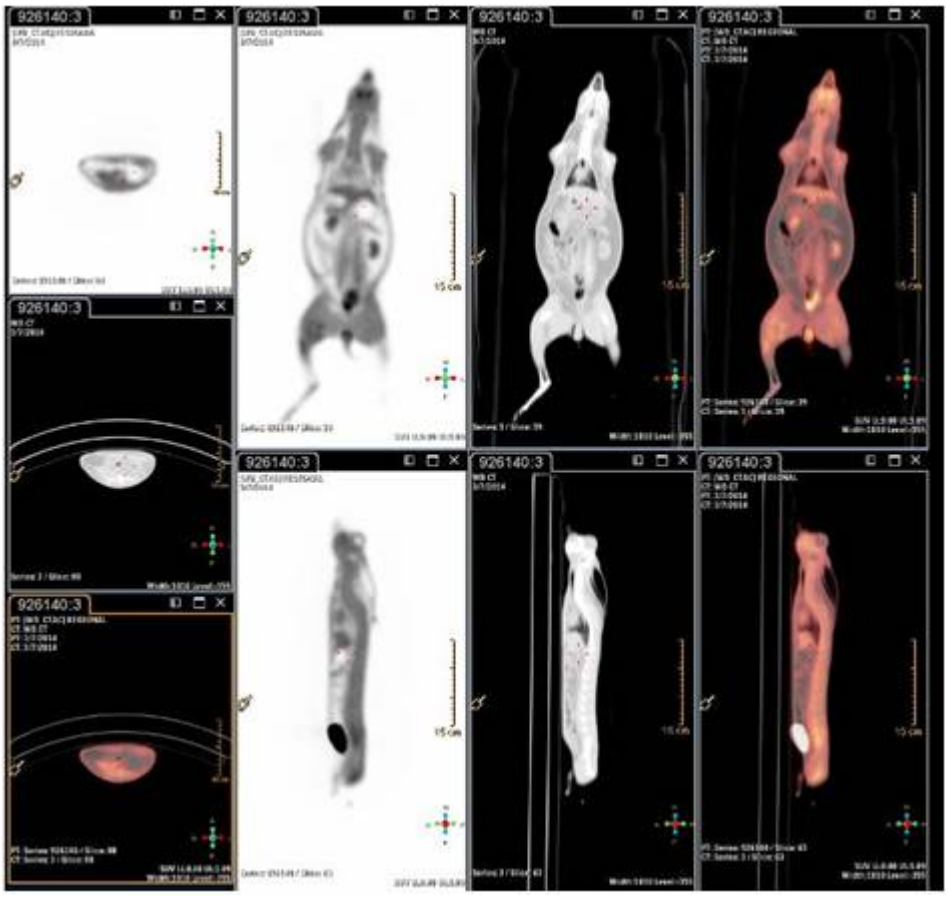


Figure 2-26 Normal Rabbit PET/CT of [^{18}F]FET 1hr post injection.

2.2.3.9. Automation

The whole synthesis process including purification has been automated by program time list. The schematic flow diagram of process involved is shown in Fig 2-28.

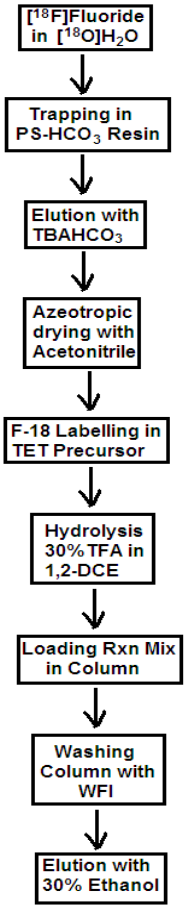


Figure 2-27 The Flow diagram shows the schematic sequence of process used in automated production of [^{18}F]FET using neutral alumina column purification.

The synthesis was conducted several times with varying amount of activities(3.7GBq to 26.6GBq). The synthesis time including the purification was 55±3 min. Reliably 19.07 ± 3.69 % (n=9) decay uncorrected yield was obtained in 35mL of buffered saline with 8mg of precursor whose data is shown below in Table 2-11. The precursor quantity was reduced further to 2mg in order to reduce the HET quantity in the final product. The production data with 2mg of precursor is shown in Table. 2-12 below.

S.No	MBq	16mL	%Yield	35mL	%Yield	Reaction Time (min)
1	3700	333	9.00	777	21.00	57
2	5550	463	8.33	1184	21.33	53
3	9250	870	9.40	1739	18.80	55
4	12950	1110	8.57	2294	17.71	55
5	16650	1554	9.33	3034	18.22	54
6	20350	1665	8.18	3811	18.73	56
7	14060	1184	8.42	2486	17.68	55
8	11840	1006	8.50	2294	19.38	58
9	26640	2442	9.17	4995	18.75	54

Table 2-11 Production Data of [^{18}F]FET using 8mg of precursor in 1mL of dry acetonitrile

S.No	Initial Activity (MBq)	Activity of [^{18}F]FET in 16mL Elution (MBq)	%Yield	Activity of [^{18}F]FET in 35mL Elution	%Yield	Reaction Time (min)
1	16650	1348.65	8.1	2414.25	14.5	58
2	18870	1264.29	6.7	2509.71	13.3	62
3	26640	1571.76	5.9	3036.96	11.4	55
4	24050	1875.9	7.8	3487.25	14.5	55
5	14060	1012.32	7.2	1996.52	14.2	58
6	14800	1021.2	6.9	1894.4	12.8	53
7	24050	1394.9	5.8	2837.9	11.8	55
8	19980	1478.52	7.4	2897.1	14.5	60
9	9250	693.75	7.5	1295	14	57

Table 2-12 Production Data using fully automated procedure using 2mg precursor in 1mL dry acetonitrile

2.2.3.10. Human PET/CT Studies

The production and bio-distribution data obtained in this study was submitted to radiopharmaceutical committee (RPC) in order to undergo further human studies. After clearance from RPC, human studies were conducted.

[^{18}F]FET was synthesized through automated procedure. After quality control tests the radiopharmaceutical was injected in human clinical studies.

5mCi of activity was injected. PET/CT scan was performed after 30min PI. As expected, no uptake in normal brain was observed, and fast clearance through kidneys and bladder are visible (Fig 2-28 and Fig 2-29).

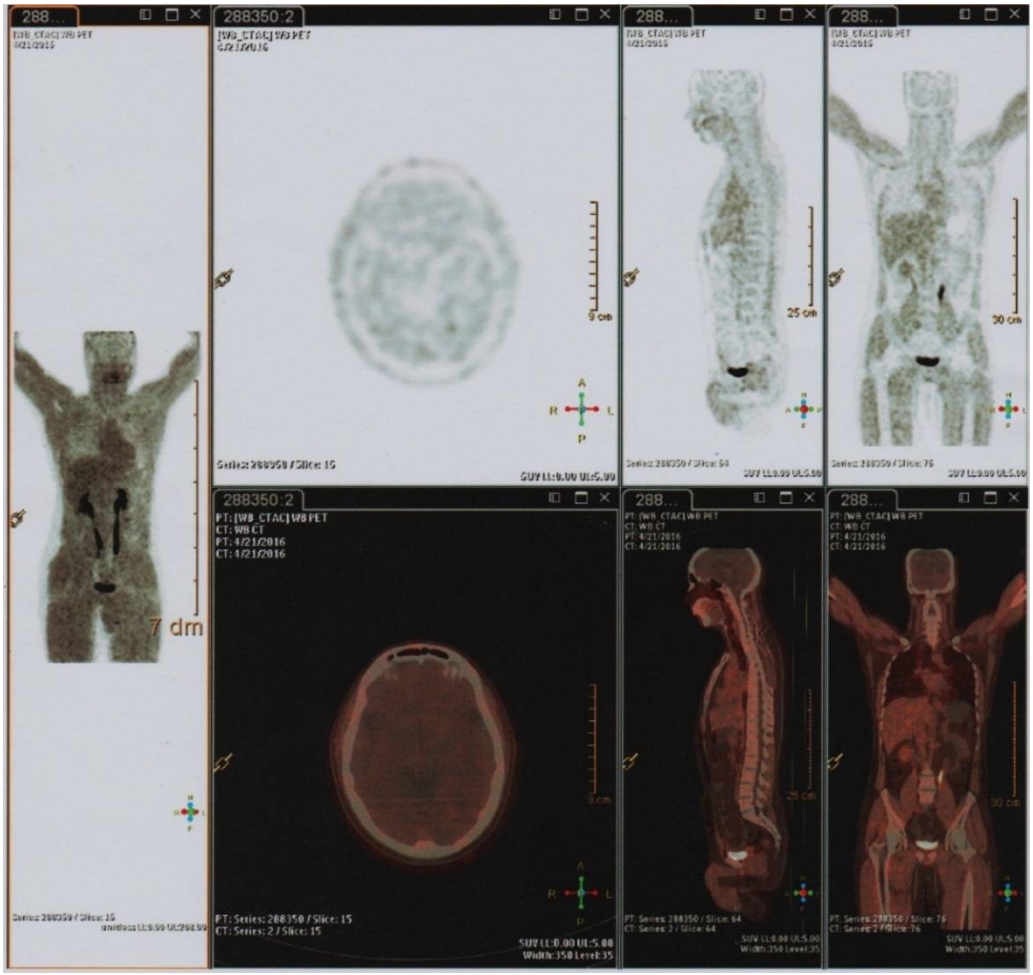


Figure 2-28 Shows whole body uptake of [^{18}F]FET in normal human.

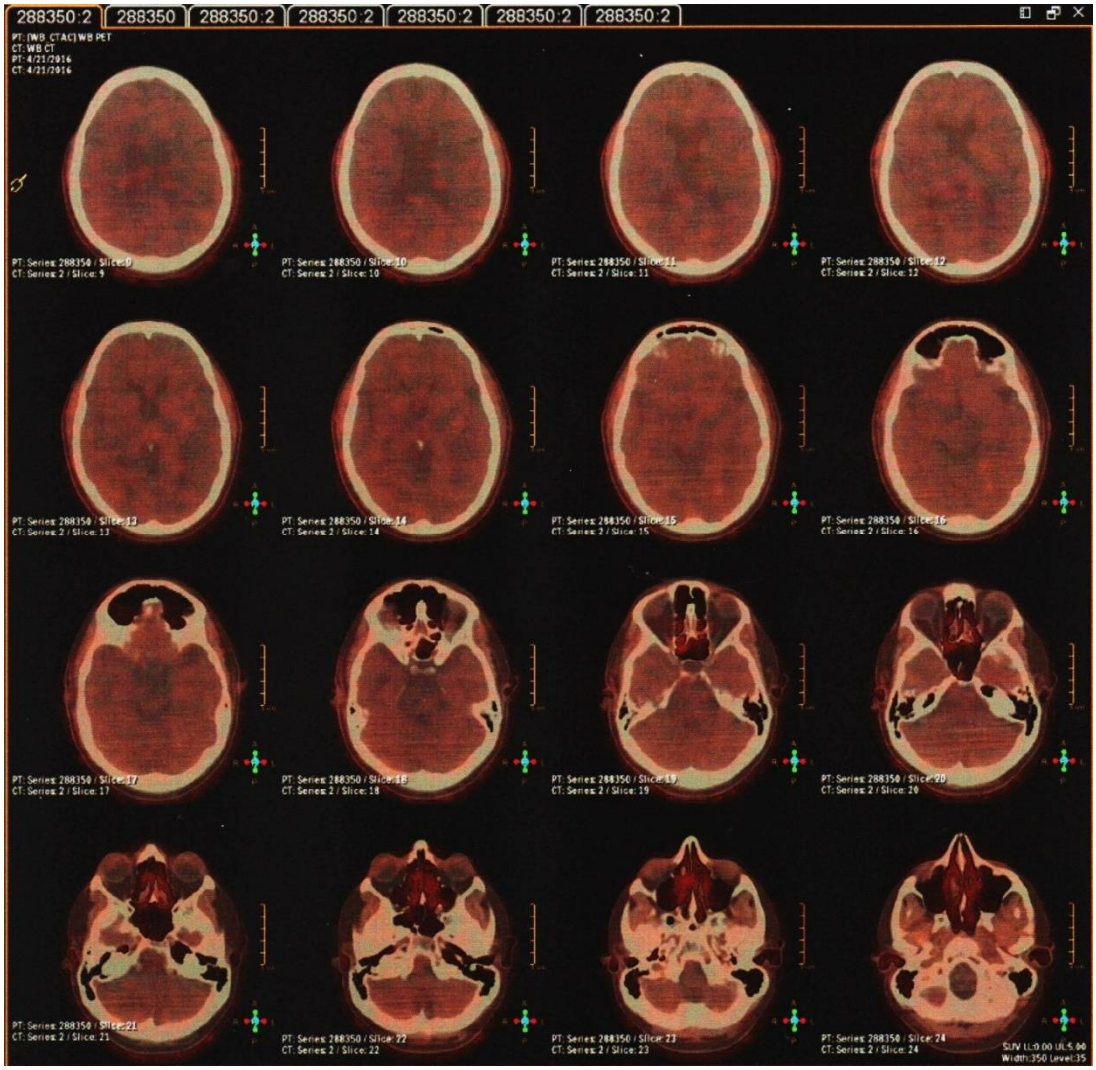
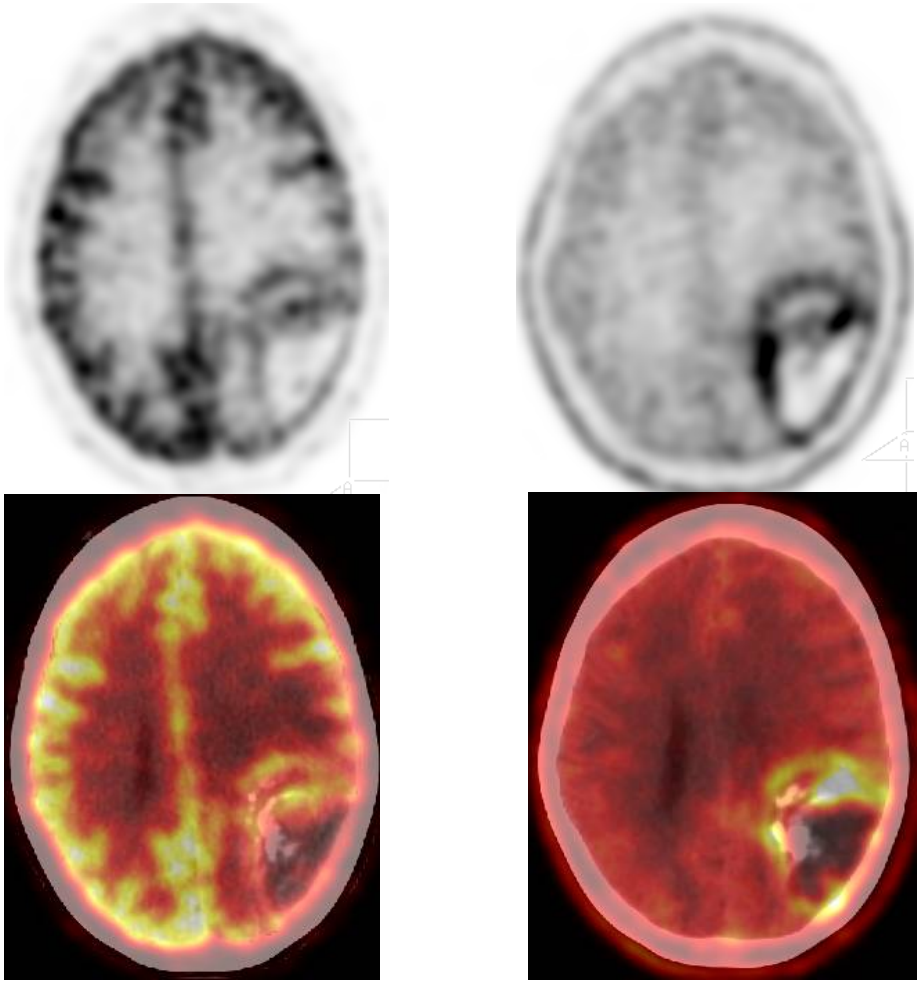


Figure 2-29 Normal brain image in human showing no significant uptake of [^{18}F]FET.

2.2.3.11. Comparison of 2-[^{18}F]FDG-PET/CT and [^{18}F]FET-PET/CT:

[^{18}F]FET shows better tumor to background contrast in brain tumors in comparison to [^{18}F]FDG. (Fig 2-30).



2-[^{18}F]FDG PET/CT **[^{18}F]FET – PET/CT**

Figure 2-30 Brain PET/CT image of glioblastoma patient, showing better tumor to background radio with [^{18}F]FET in comparison to 2-[^{18}F]FDG. (Courtesy: Department of Nuclear Medicine & Molecular Imaging, TATA Memorial Hospital, Mumbai, India)

2.2.3.12. Comparison of purification methods of [^{18}F]FET using reverse phase resin and neutral alumina column

The comparison of [^{18}F]FET production by both the methods adapted in Part A and Part B is shown in Table 2-13. It can be observed that, there are certain advantages by using the reverse phase column method described in Part A, such as high radiochemical yield and high radioactive concentration. But however, due to utilization of highly corrosive and toxic chemicals involved in the reaction and high content of ethanol in the final [^{18}F]FET preparation, the method is not used for human clinical studies.

S. No.	Purification with H-RP Column	Purification with Neutral Alumina Column
1.	High yield (36±3.64%)	Low Yield (19.07±1.3%)
2.	Low total volume of elution (15mL)	High total volume of elution (32mL)
3.	High ethanol content – 20-30%	Low ethanol content – 2-3%
4.	Involves corrosive acids (TFA)	Involves mild acid 1M HCl
5.	Utilizes toxic chemicals like dichloromethane, dichloroethane or chloroform	Utilizes ethanol and not toxic chemicals.
6.	Complete hydrolysis after radiolabelling (100%)	Partial hydrolysis after radiolabelling (85%)
7.	High RAC - >11mCi/mL starting with 500mCi of [^{18}F]Fluoride.	Low RAC – 2-3mCi/mL starting with 500mCi of [^{18}F]Fluoride.
8.	Has less similarity towards commonly used 2-[^{18}F]FDG chemicals.	Can be produced from commonly used 2-[^{18}F]FDG chemicals, excluding precursor.

Table 2-13 Comparison of [^{18}F]FET production using HR-P reverse phase resin and neutral alumina column.

2.2.4. Conclusions

[^{18}F]FET was synthesized in a GE TRACERlab FX-C (configured for making [^{18}F]FDG) using the TET-precursor. Decay uncorrected synthesis yield (n=9) was found to be 19.07 ± 1.3 % and 8.76 ± 0.46% in 35mL and 17 mL of buffered saline when 8mg of TET precursor was used, and 13.44 ± 1.20 % and 7.03 ± 0.79% in 35mL and 17 mL of buffered saline when 2mg of TET precursor was used respectively. The RCP of [^{18}F]FET was >99%. A unreported acetonitrile:water 80:20 mobile phase was found to be suitable for the rapid radio-TLC analysis of [^{18}F]FET. Radiochemical and enantiomeric purity were identified by

Radio-HPLC and found to be >99%. By reducing the precursor quantity to 2mg in 1mL of dry acetonitrile the quantity of HET was reduced to <2µg/mL. Bio-distribution studies in C57BL/6 mice bearing B16F10 murine melanoma showed tumor/brain ratio of about 3 confirmed by micro-PET imaging. PET imaging in rabbit did not show any significant bone or brain uptake.

CHAPTER 3

FULLY AUTOMATED SYNTHESIS OF [^{18}F]FLUOROETHYL-L-TYROSINE USING NOVEL NI(II) COMPLEX OF TYROSINE SCHIFF'S BASE

3.1. Introduction

Several methods for preparation of [^{18}F]FET are available in the the literature[90, 119, 120, 122, 158, 159]. Out of all the one-pot two-step synthesis method which involves tailored precursors, on which, the radio-labelling and deprotection can be performed in a single reactor vessel are of high importance. These methods utilizes a single pot module, like 2-[^{18}F]FDG synthesis module which is more economic and commonly available in almost all PET centers. Moreover, it also provides potential possibility of utilizing 2-[^{18}F]FDG module for the regular production of [^{18}F]FET, thus providing easy availability of the radiotracer to all PET centers. Since the radio-isotope used, [^{18}F]fluorine, is a fast decaying positron emitter, the precursors used for [^{18}F] PET radiochemistry needs to have high radiolabelling efficiency and radiochemical yield, so that transporting of the radiotracer to remote PET facilities by satellite concept, after production, are possible. Moreover, amino acids are chiral molecules, with L-enantiomer being biologically active. Hence radiosynthetic method needs to provide high enantiomeric purity of the radiotracer. Ni(II)-(S)BPB-alkylated tyrosine Schiff's base complex precursor is one such type of precursor, which have been reported recently by recently byKrasikova et al[122]. The precursor was said to have high radiolabelling efficiency, and provides 95% enantiomerical purity of [^{18}F]FET, with high radiochemical yield (35%). The enantioselectivity of the complex is due to the sterric hinderance exerted between the

benzyl moiety of (S)BPB and the phenolic group of tyrosine in the complex, thus resulting in the significant entantiomeric excess of (S)tyrosine Schiff's base complex(fig 3-1).

The precursor utilizes HCl as hydrolyzing reagent that is safe and more commonly used in PET-radiochemistry modules. Moreover, the reaction conditions are also said to be milder. The purification of the [^{18}F]FET radio-synthesized by this method was originally reported to be conducted by semi-prep radio-HPLC. Later,a SPE method was reported[160].

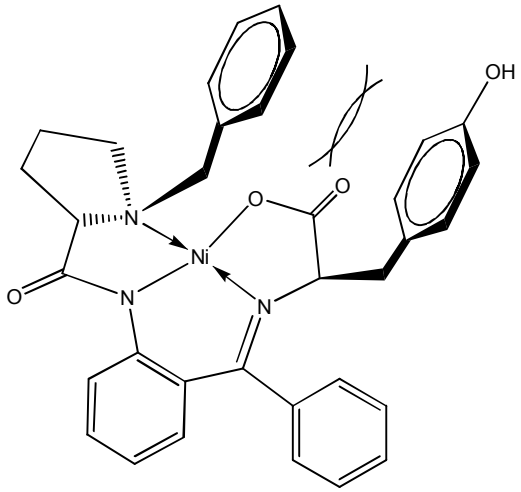


Figure 3-1 Ni(II)-(S)BPB-(R)tyrosine complex showing sterric hindrance for its formation.

However, the process involves acetonitrile during acid hydrolysis. Moreover, complication arising due to precipitation of reaction mixture before purification was reported, which was said to be managed by filtration using 0.22μm filter before purification. A method is explored which can avoid both these issues in this study.

The precursor is not available commercially from any source. However, it can be produced by a three step process reported[122]. In this study, the precursor, Ni(II)-L-Tyr-O-CH_2-CH_2-OTs (fig 3-2) was synthesized by a reported three step process shown in schematic (fig 3-3) and a two by a improved two step process discussed in chapter 4 (fig 4-3). The precursor was used for production of [^{18}F]FET, followed by a SPE purification technique in GE-TRACERlab

FX-C module (configured for 2-[^{18}F]FDG synthesis). A fully automated procedure was developed including purification. The final purity of the product including enantiomeric purity was evaluated. The final product was studied for tumor uptake in C57BL/6 mice bearing B16F10 murine melanoma bearing tumor models.

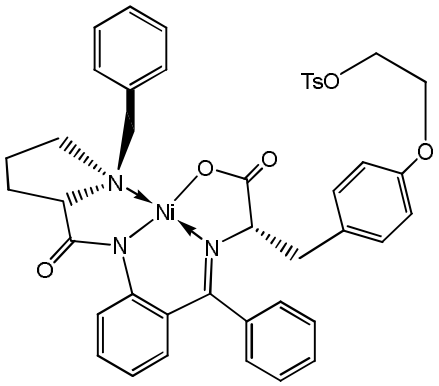


Figure 3-2 Structure of [^{18}F]FET precursor based on Ni(II) Complex.

3.2. Materials and methods

(S)BPB was procured from Merck, Germany, all other chemicals were procured from sigma-aldrich. HPLC analysis using a C-18 reverse phase analytical column (LIChroCARTs 250X4 5µm HPLC cartridge LIChrosphers 100 RP-18 5µm) was done on Knauer smartline HPLC systems. Raytest radioactive detector used for Radio-HPLC. Enantiomeric purity was tested by Chiral column Crownpak CR(+) (150X4mm, 5µm). O-(2'-hydroxyethyl)-L-tyrosine reference standard was obtained from ABX, Germany. ICP-MS was performed in Element XR, Thermo Fisher Scientific, Germany attached to Magnetic Sector (High Resolution) Inductively Coupled Plasma Mass Spectrometer.

[^{18}F]Fluorine in fluoride form was produced in GE PETtrace Medical Cyclotron by irradiation of enriched [^{18}O]water target by the nuclear reaction $^{18}O(p,n)^{18}F$. Radio-synthesis of [^{18}F]FET was carried out in a GE-TRACERlab FX-C configured for 2-[^{18}F]FDG radio-synthesis. Radio-TLC was carried out on Silica Gel 60, 20X20 (Merck, Germany) and

scanned using a Raytest TLC scanner with a BGO scintillation detector and chromatogram analyzed with GITA software.

3.2.1. Precursor synthesis:

Precursor was synthesized by a three step reaction as shown below (fig 3-3)

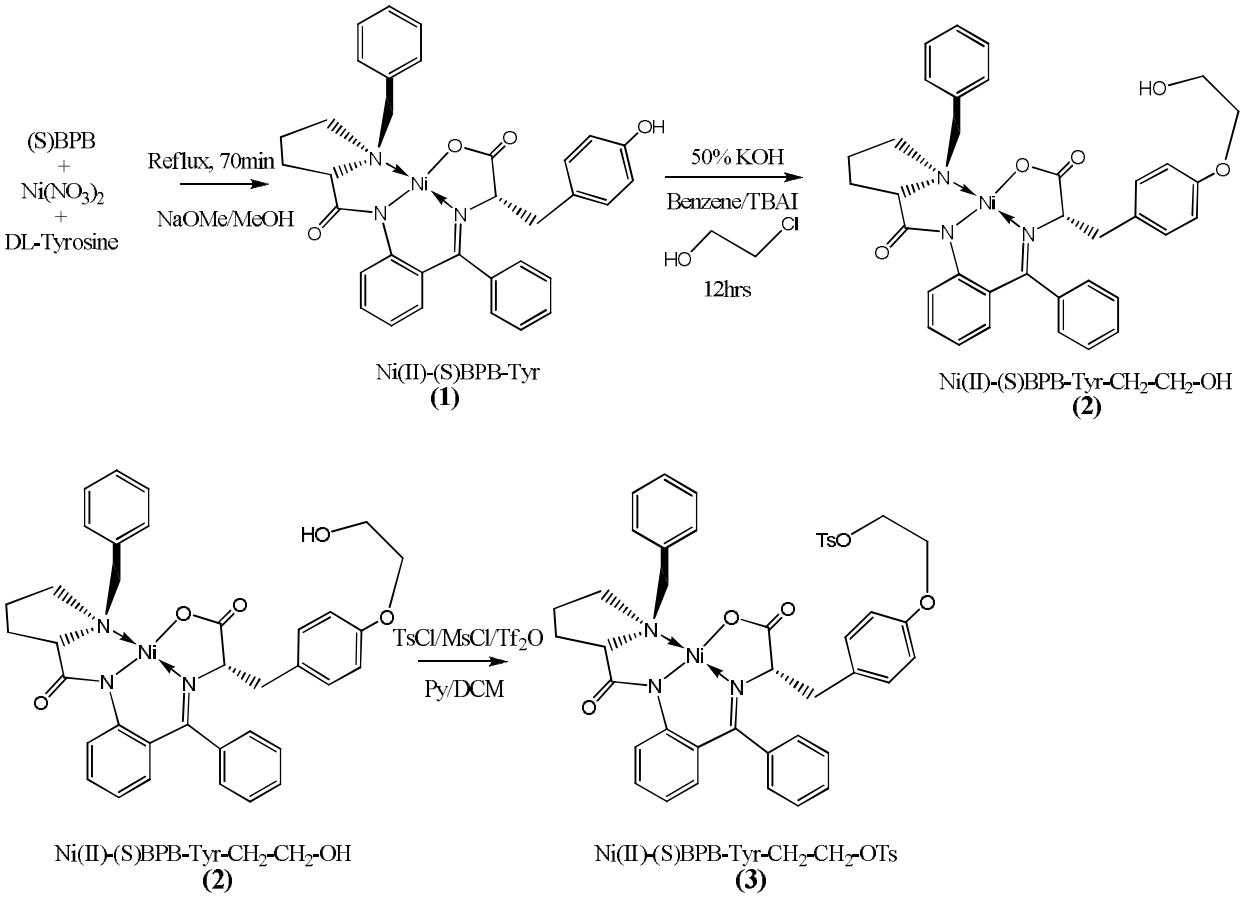


Figure 3-3 Reaction schematic of synthesis of Ni(II)-(S)BPB-(S)-tyrosine-CH_2-CH_2-OTs complex precursor.

3.2.1.1. Synthesis of Ni(II)-(S)BPB-(S)-tyrosine complex (1)

To a three necked round bottomed flask, crushed and thoroughly mixed mixture of DL-tyrosine (1.0 g, 0.55 $X10^{-3}$ mol), (S)BPB (0.446 g, 1.11 $X10^{-3}$ mol) and $Ni(NO_3)_2 \cdot 6H_2O$ (0.65 g, $2.3X10^{-3}$ mol), are taken and dissolved in 10mL of dry methanol with a magnetic stirrer. The mixture was heated upto 45°C. To this MeONa (1.4g, $2.6X10^{-2}$mol) was quickly in one portion. The mixture was allowed to reflux in an thermostat at 90°C for 70min. The

reaction mixture was analyzed by TLC (mobile phase: Chloroform : acetone (7:1). Once the parent (S)BPB spot disappears, the reaction was stopped. The reaction mixture was added to crushed ice in a beaker. To this glacial acetic acid was added drop wise to neutralize the mixture by monitoring the pH in litmus paper. The mixture was extracted with chloroform and allowed the mixture to settle for 1h. The red organic layer was collected, dried with anhydrous sodium sulphate, and evaporated to dryness. This mixture was triturated with hexane to obtain reddish orange precipitate of complex. The precipitate was further dried, to get the amorphous powder of the complex. This complex was further purified by flash chromatography with hexane : ethyl acetate mobile phase. (Yield = 0.652g, 93% with respect to (S)BPB)

3.2.1.2. Synthesis of Ni(II)-(S)BPB-(S)-tyrosine-CH_2-CH_2-OH complex (2)

To a flat bottomed flask with magnetic stirrer, Ni-(S)-BPB-(S)-Tyr complex (1) (300 mg, 0.49 X 10^{-3} mol), 2-chloroethanol (1.3 mL, 1.5 X 10^{-2} mol) and TBAI (0.2g, 0.5X10^{-4} mol)were added and dissolved in 5mL of benzene. To this mixture 4mL of 50% aqueous potassium hydroxide was added in 1mL/hr portions and allowed to stir vigorously for overnight. The reaction mixture was then tested for completion of the reaction by TLC (SiO_2, mobile phase: 100% ethyl acetate). Once the reaction was complete, the mixture was extracted with dichloromethane, washed with water till the pH of aqueous layer comes to neutral. The chloroform layer was then dried with anhydrous Na_2SO_4 and concentrated. The crude mixture is then purified with chromatography using hexane : ethylacetate mobile phase. This mixture was triturated with hexane to obtain reddish orange precipitate of complex. The precipitate was further dried, to get the amorphous powder of the complex (218mg, Yield = 0.68%).

3.2.1.3. Synthesis of Ni(II)-(S)BPB-(S)-tyrosine-CH_2-CH_2-OTs complex (3)

In a round bottomed flask with magnetic stirrer, Ni(II)-(S)BPB-(S)-tyrosine-CH_2-CH_2-OH complex (2) (0.1 g, 1.54 X 10^{-4} mol) and 700µL of triethyl amine was added and dissolved in 2 mL of dry CH_2Cl_2. The solution was cooled to 0ºC, and to this p-toluenesulphonyl chloride(0.09 g, 4.72 X 10^{-4} mol) was added in three portions within 3 hrs. The solution was allowed to stir at room temperature for 20h. The reaction mixture was analyzed by SiO_2 TLC (mobile phase: 100% ethyl acetate). Once the reaction is complete, the reaction mixture was washed with cold water. The aqueous layer was neutralized with 0.5M HCl while extraction in order to remove triethylamine from the organic layer. After neutralization, the organic layer was collected, washed with water, dried with anhydrous Na_2SO_4, and concentrated. The final concentrate was then purified by flash column chromatography using hexane:ethyl acetate mobile phase. Yield 65% (80 mg).

3.2.2. Radio-synthesis of [^{18}F]FET

The chemicals loaded in the respective reservoirs are shown in the table 3-1.

The radiosynthesis was carried out in GE TRACERlab FX-C module configured for FDG shown in fig 2.2 of chapter 2. The radio-synthesis procedure are as follows (fig 3-4).

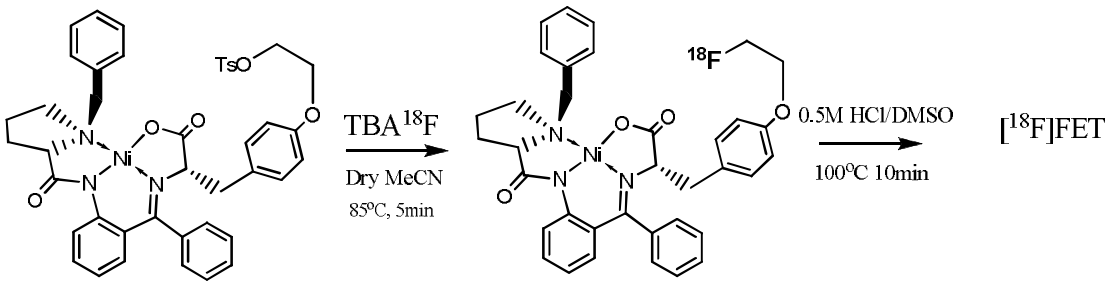


Figure 3-4 Radiosynthesis schematic of [^{18}F]FET using Ni(II)-(S)BPB-(S)-tyrosine-CH_2-CH_2-OTs complex precursor.

The [^{18}F] fluoride was trapped in 45mg PS-HCO_3 anion exchange resin. [^{18}F]Fluoride was eluted with 0.5mL of 75mM Tetrabutylammonium bicarbonate solution to obtain [^{18}F]Fluoride in the form of [^{18}F]TBAF in the reactor vessel. Then to this, 1mL of dry acetonitrile was added and azeotropic distillation was carried out to remove the water. Drying was aided by flushing the reaction vessel with a stream of He-gas. The precursor in acetonitrile (5mg/1mL) was added and heated to 95°C and maintained at this temperature for 5 min. The reaction was then cooled to 50°C with He drying continued inside the reactor. To this, 1M HCl, mixed with a suitable organic solvent was added, and the temperature was raised to 120°C and maintained for 10min. Purification was carried out by solid phase extraction. The reaction mixture was loaded onto neutral alumina column. The column is washed with WFI and eluted with WFI.

Chemicals	Reservoir No.	Quantity
TBAHCO3	Vial 5	0.6mL, 75mM
Acetonitrile	Vial 1	1mL
TET Precursor	Vial 2	800mg/0.8mL
1M HCl/solvent mixture	Vial 3	1mL
WFI	Vial 4	10mL
WFI	Vial 6	15mL

Table 3-1 Reservoirs and Respective chemicals.

3.2.2.1. Preparation of Purification column

Purification column was prepared by filling neutral alumina (active, Brockmann-I Grade) in a polypropylene barrel designed for easy and airthight press fit with leurs. The alumina layer is packed with having 20µm polyethylene frits in top and bottom. The column is tapped gently for few minutes for close packing and fastened with airtight lid. The column

is washed with 30mL of ethanol in order to remove impurities and sanitize the bed. The column is then washed with 100mL of WFI. This column is then stored at 5°C in refrigerator till use (fig 3-13).

3.2.3. 3.2.3 Quality Control

3.2.3.1. Radio-TLC analysis

The radio-TLC was performed in different stages of reaction in order to identify the radio-chemical species formed in those steps. The purity of the product was evaluated by SiO_2-TLC plates using Acetonitrile : Water (8:2) as mobile phase.

3.2.3.2. Radio-HPLC analysis

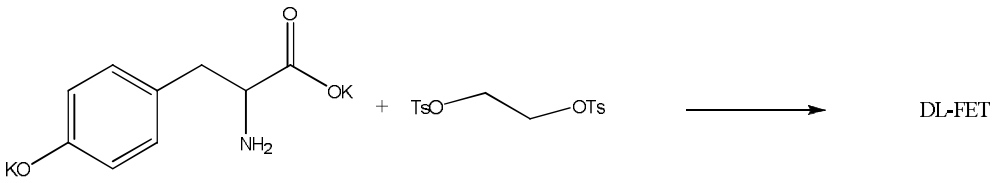
Radio-HPLC of [^{18}F]FET and authentic [^{19}F]FET standard was carried out in isocratic condition (Solvent:7.5% Ethanolic Water; flow rate of 0.5mL/min; UV - 254nm (C-18 RP 300X4mm, 5µm).

3.2.3.3. Determination of Enantiomeric purity

Enantiomeric purity of the product was determined using Crownpak CR(+) chiral HPLC column. The solvent system used was water : methanol (90:10, v/v), pH-2 in $HClO_4$, UV-200nm, temperature =10°C, flow rate: 0.8mL/min. The retention time (R_t) of both D and L enantiomer of FET with the R_t of [^{18}F]FET radio peak.

3.2.3.3.1. Synthesis of DL-FET racemic mixture:

The synthesis of racemic mixture of DL-FET was performed from the procedure provided in chapter 2. as per the procedure shown below in the synthesis sequence.



(DL-tyrosine di-potassium salt) (Ethylene glycol di-tosylate)

3.2.3.4. Gas Chromatographic analysis

The Polyethylene glycol immobilized glass capillary column was first conditioned by heating at 180ºC for one hour before experiment with a nitrogen carrier gas flow rate of 2.5mL/min. The column temperature was brought to 70ºC and maintained for 30min. 2µL of sample was injected in the column. The column temperature was kept at 70ºC for 4min and heated to 180ºC over a period of 7min. Using standard solutions of Ethanol, Acetonitrile, acetone and DMSO, a calibration graph was first plotted. Based on the calibration curve, the quantities of solvents in the sample were estimated from the area under the peak.

3.2.3.5. Estimation of Aluminium ion (Al^{3+})

The Al^{3+} ions in the final [^{18}F]FET preparation were estimated using Microquant aluminium test kit, using the standard protocol provided with it. The colour developed in the strip was compared with the standard calibration colours provided in the kit

3.2.3.6. Estimation of Tetrabutylammonium ion ($[TBA]^+$ion)

Test for TBA^+ ion in the final product was performed by Colour spot test following the procedure reported by Kuntzsch, M. et al[163]. The Pharmacopoeia Limit for TBA^+ ion is <50µg/L. The colour developed with Standard solution was visually compared with the samples. According to the reported procedure 2µL of sample is spotted in TLC Plate and dried in hot air. 10µL of Ammonium Hydroxide: Methanol (1:9) is added to the dried spot.

Plate was placed inside Iodine Chamber immediately for 1min. Intensity of colour developed is directly proportional to quantity.

3.2.3.7. Estimation of Ni(II) ion concentration

Ni(II) ion concentration was estimated by ICP-MS.

3.2.3.8. Tests for sterility and bacterial endotoxins

Sterility tests were performed according to the Indian Pharmacopoeia (IP 1996 and IP addendum 2005) protocol. In this test, 1mL of the [^{18}F]FET sample after radioactive decay, was inoculated in fluid thioglycollate medium and incubated at 37^{o}C for 14 days to observe the growth of aerobic and anaerobic bacteria. Similarly, 1mL of the decayed [^{18}F]F2ET sample was also inoculated in soyabean casein digest medium and maintained at 22–25^{o}C for 14 days to detect fungal growth. The bacterial endotoxin test was performed in accordance with USP XXV. The test was based on the formation of gel clot in the sample by Limulus Amoebocyte Lysate (sensitivity: 0.125EU/mL) reagent.

3.2.4. 3.2.4 Bio-evaluation Studies

3.2.4.1. Bio-distribution in tumor model mice

B16F10 murine melanoma cells were procured from NCCS, Pune, Maharashtra, India. C57BL/6 mice were obtained from our institutional animal house. Tumor was developed in C57BL/6 mice, by injecting B16F10 cells (10^5 cells) into the shoulder muscle based on the studies reported by Wang Ming et al[158]. The tumor were allowed to develop for 15days to a palpable size of about 0.5cm. The animals are injected with 74MBq of [^{18}F]FET in 200µL intravenously and were sacrificed at different time points (15min, 60min and 120min). Three animals were sacrificed in each time point. The radioactivity in different organs were counted and the percentage injected dose per gram were measured (%ID/g).

3.2.4.2. Micro-PET imaging studies in tumor model mice

A Micro-PET imaging studies were also conducted for normal and tumor bearing C57BL/6 mice, which showed brain to tumor ratio of 2.8 at 60min post injection.

3.2.4.3. PET/CT imaging in Normal Rabbit

PET/CT image was taken in normal healthy female rabbit (2.45 Kg) after fasting for six hours. 37MBq of [^{18}F]FET in 0.5mL was injected through ear vein. The rabbit was anaesthetized by injecting ketamine and the images were recorded after 1 hour post injection.

3.3. Results and discussions

The radio-synthesis reaction of [^{18}F]FET was followed by analyzing the reaction mixture at various stages in order to standardize the various steps of synthesis.

3.3.1.1. Radiolabelling

The reaction mixture was removed after S_N2 substitution of [^{18}F]fluorine. Under this conditions the radio-TLC analysis showed that the radiolabelling efficiency was observed to be around 70% with 5mg of precursor in 1mL of acetonitrile. But however, the labelling efficiency was found to increase to 80% as reaction mixture was dried prior to hydrolysis (fig 3-5). The acetonitrile used in the S_N2 was evaporated, and after complete evaporation step, the reaction mixture was once again redissolved in acetonitrile and further analyzed by radio-TLC. This additional increase in the radio-fluorination of 10% is attributed to the increase in the concentration of the reactant during evaporation of acetonitrile.

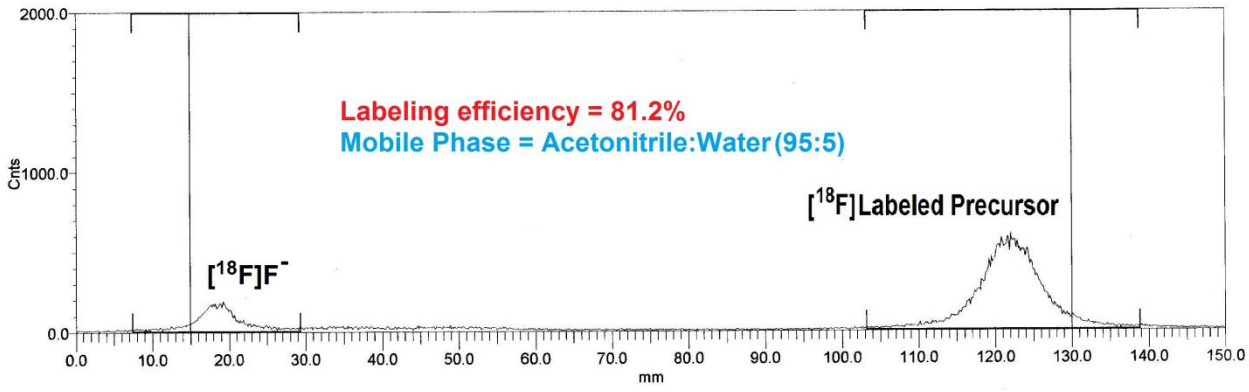


Figure 3-5 Radio-TLC of reaction mixture before hydrolysis. mobile phase: acetonitrile:water (95:5).

3.3.1.2. Standardization of hydrolysis process

Hydrolysis of the [^{18}F]labelled precursor was reported to be carried out by 0.5M aqueous HCl. Even though the 0.5M HCl is a mild acid, it was reported to be efficient. But however, it requires a polar organic solvent. Hence the acetonitrile used for S_N2 step was not evaportated before hydrolysis. This was reported to increase the solubility of reaction mixture with the aqueous acid. Since acetonitrile is toxic in nature, we tried it replacing with other relatively less toxic polar organic solvents like DMSO and ethanol. When both these solvents were used instead of acetonitrile the hydrolysis was found to be incomplete leaving 10% of unhydrolized product (fig 3-6). This results in multiple radiochemical species present in the reaction mixture.

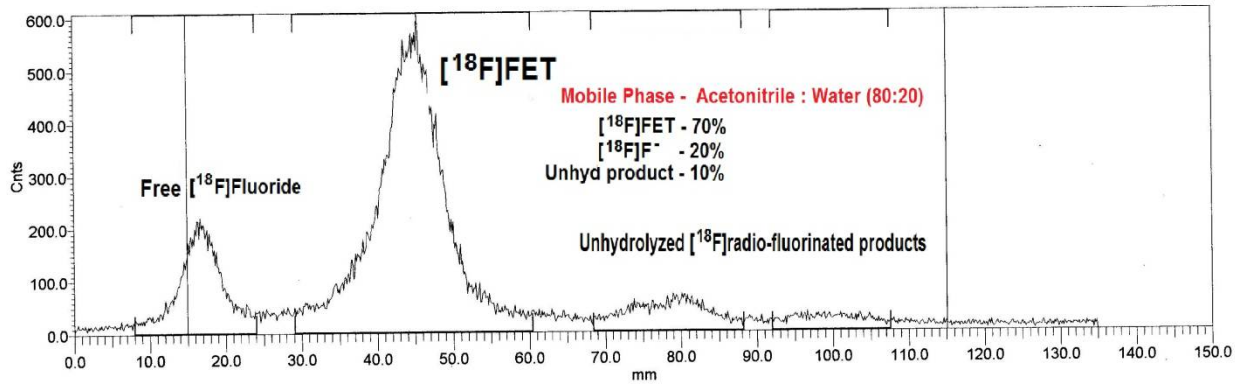


Figure 3-6 Radio-TLC analysis of reaction mixture after hydrolysis with 0.5M HCl.

It is essential to obtain complete hydrolysis, since it will leave only two radiochemical species in the reaction mixture viz. free [^{18}F]fluoride and [^{18}F]FET. In order to achieve complete hydrolysis various reaction parameters were varied (table 3-2).

On increasing the acid strength from 0.5M to 3M, the hydrolysis did not show any improvement. Instead of 0.5M HCl, a mixture of HCl/ethanol was used. Radio-TLC showed an improvement in the hydrolysis of one product. But, however, it showed presence of a unhydrolyzed species in the mixture(fig 3-7).

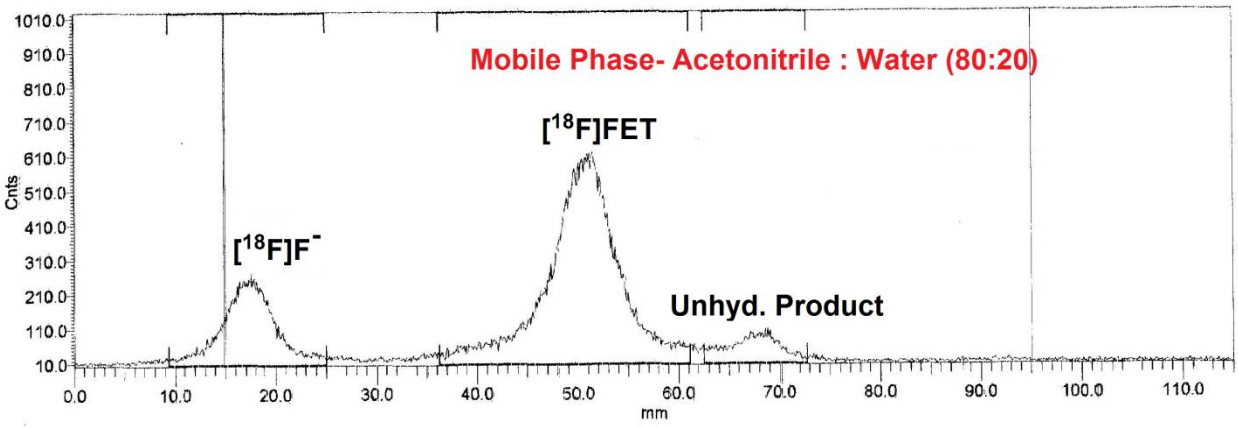


Figure 3-7 Radio-TLC post hydrolysis with HCl/ethanol mixture.

The reaction temperature was varied from 100ºC to 130ºC. The solvent system was varied with acetonitrile, DMSO and ethanol. Similarly, the reaction time was varied from 10 min to 30min. Heating with low boiling solvent like acetonitrile upto 130ºC may exert high pressure in the reaction vessel. Moreover, the search was to substitute an alternate solvent for acetonitrile, preferable ethanol. Heating at 130ºC was conducted only with DMSO.

The results obtained from these studies are shown in following table 3-2.

S. No.	Solvent	Parameters Varied	Hydrolysis %
1.	1MHCl/acetonitrile (1:1) @ 100ºC for 10min	Temperature	75%
2.	1MHCl/acetonitrile (1:1) @ 110ºC for 10min	Temperature	80%
3.	1MHCl/acetonitrile (1:1) @ 120ºC for 10min	Temperature	90%
4.	1MHCl/DMSO (1:1) @ 120ºC for 10min	Solvent	85%
5.	1MHCl/DMSO (1:1) @ 130ºC for 10min	Temp &Solvent	85%
6.	1MHCl/ethanol (1:1)@ 120ºC for 10min	Solvent	75%
7.	2MHCl/DMSO (1:1) @ 120ºC for 10min	Acid strength	80%
8.	3MHCl/DMSO (1:1) @ 120ºC for 10min	Acid strength	60%
9.	1M HCl/DMSO(1:1) @ 120ºC for 20min	Reaction time	90%
10.	1M HCl/DMSO (1:1)@120ºC for 30min	Reaction time	45%
11.	1MHCl/ethanol (1:1) @ 120ºC for 20min	Temp & Solvent	85%
12.	1MHCl/DMSO (1:1) @ 120ºC for 10min followed by stirring at 55ºC for 10 min	Solvent	95%
13.	1MHCl/DMSO (1:1) @ 120ºC for 10min followed by stirring at 55ºC for 20 min	Solvent	97%
14.	1MHCl/DMSO (1:1) @ 120ºC for 10min followed by stirring at 55ºC for 30 min	Solvent	100%

Table 3-2 Standardization of HCl based hydrolysis medium for [^{18}F]FET synthesis.

The results show that DMSO is more suitable substitute for acetonitrile than ethanol, since, complete hydrolysis was not able to achieve by ethanol, moreover, the degree of hydrolysis at any given conditions were higher in DMSO compared to ethanol. At last the hydrolysis reaction was found to be complete when the reaction mixture was allowed to stir at 55^{o}C for 30min in DMSO/HCl medium (fig 3-8). On the other hand, when ethanol/HCl mixture was used as the hydrolysis reagent, completion of reaction was not observed even allowing the reaction mixture to stay for 60min.

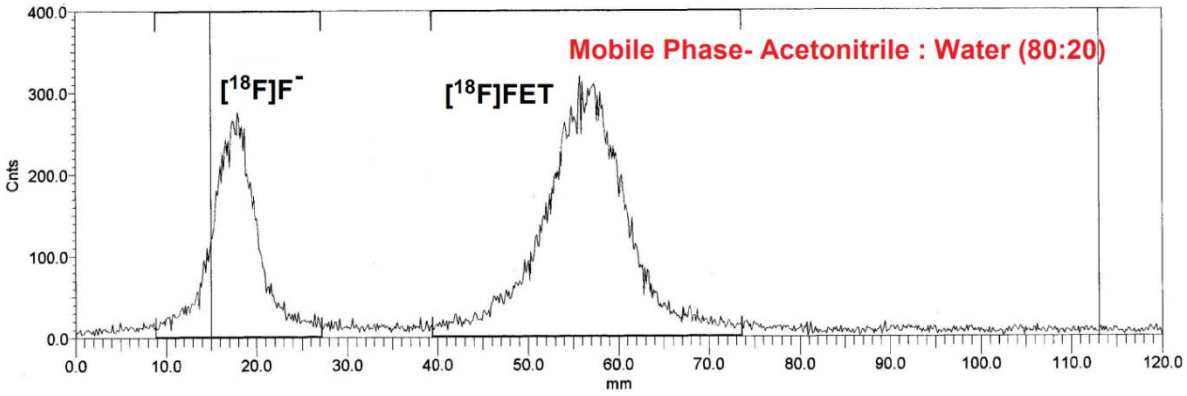


Figure 3-8 Radio-TLC showing complete hydrolysis of labelled precursor in HCl/DMSO mixture.

3.3.2. Purification of [^{18}F]FET after hydrolysis

Since the reaction mixture contains only two radio-chemical species, after achieving complete hydrolysis, radio-chemical purification basically involves removal unlabelled [^{18}F]fluoride. Moreover, the reaction mixture is aqueous in nature, and all compounds except (S)BPB is polar, and water soluble. Hence, purification using neutral alumina resin should retend free [^{18}F]fluoride strongly and other polar chemical species. Various bed volumes of neutral alumina varying from 4g to 12g was tried. The reaction mixture was loaded onto the purification column directly after hydrolysis.

After loading the reaction mixture, the column was washed with several compositions of ethanol-water solvents of varying polarity. For this a solution of 0% to 30% variation was used. It was observed that, ethanol concentration more than 5% was found to elute [^{18}F]FET faster without resolving from its unhydrolyzed impurities. However, the reaction mixture does not contain any unhydrolyzed radioactive impurities, a negligible quantity of it can arise when high amount of radio activity was taken for radiosynthesis (>37GBq). Hence the ethanol content was kept to a minimum as possible. i.e. 0-5%. The concentration of ethanol in the wash volume depends on the bed volume of alumina used for purification. Hence the neutral alumina bed volume was first standardized.

3.3.2.1. Standardization of bed volume of neutral alumina

As compared to the TET precursor, the amount of unhydrolyzed product with Ni(II)-(S)BPB-(S)-tyrosine complex precursor is very less. Moreover, the reaction mixture itself contains DMSO as a non-polar medium, which assists in the removal of non-polar and unhydrolyzed species from the purification column. Hence, the ethanol content required for elution of [^{18}F]FET to the final product vial is very less. Even though the hydrolysis is complete, there can be occasions where a minor quantity of unhydrolyzed product my result in the reaction mixture. Hence the elution profile for purification was evaluated. In order to purify the reaction mixture efficiently, it is essential to resolve the radio-active species present in the reaction mixture. For this the bed volume of neutral alumina required need to be standardized.

3.3.2.1.1. Purification with 4g bed volume of neutral alumina:

A bed volume of 4g neutral alumina was found to be insufficient to resolve the radiochemical impurities. The radio-TLC analysis showed a mixture of unlabelled [^{18}F]fluoride and [^{18}F]FET eluting together with unhydrolyzed product (fig 3-9). This concludes that the bed volume of 4g is insufficient for efficient purification of the reaction mixture.

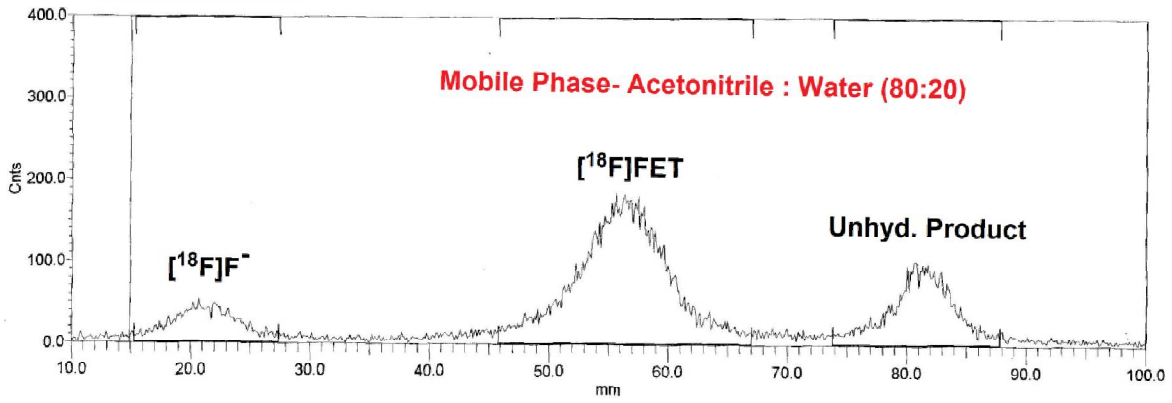


Figure 3-9 RadioTLC of wash after loading reaction mixture in 4g neutral alumina column.

3.3.2.1.2. Purification with 12g bed volume of neutral alumina:

When 12g bed volume of neutral alumina was used, the resolution was found to be better. But however, retention of [^{18}F]FET in the purification column was found to be very high. In order to recover the [^{18}F]FET to the maximum extent from the column, a large volume of water was required to wash the column. Thus the radioactive concentration of [^{18}F]FET in the final product was reduced significantly.

3.3.2.1.3. Purification with 8g bed volume of neutral alumina:

Since complete hydrolysis of the reaction mixture can be achieved by choosing proper hydrolyzing mixture and proper condition, the amount of unhydrolyzed product in the reaction mixture is going to be relatively negligible. Hence purification of [^{18}F]FET can be efficient by using less than 12g bed volume of neutral alumina. This reduces the elution volume with sufficient resolution of radioactive species, and consequently increases the radioactive concentration of [^{18}F]FET, thus resulting in its good recovery in the final product vial.

Since DMSO is present in the reaction mixture, by washing the column with water, the unhydrolyzed product was found to get eluted in the first 3mL (Fig 3-10).

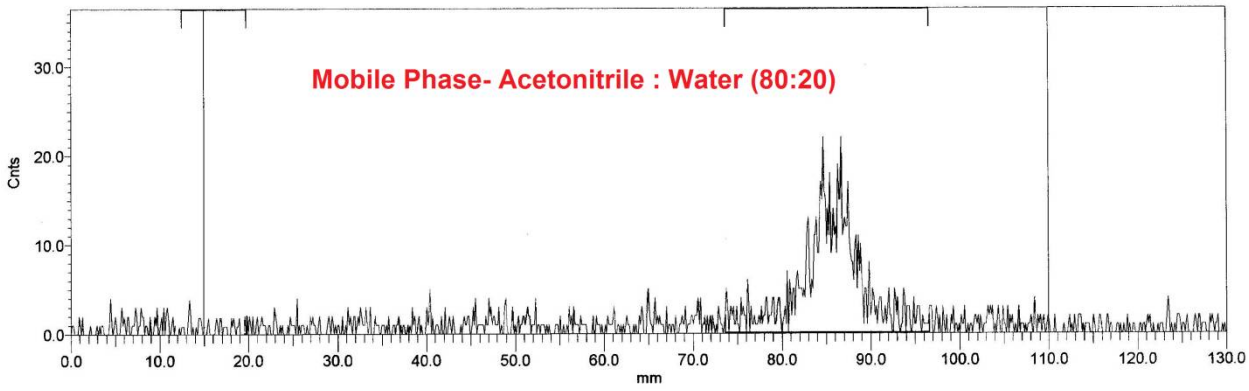


Figure 3-10 Radio-TLC of first 3mL wash of the purification column, where unhydrolyzed product comes out.

Second elution of product with 3mL of WFI showed further removal of unhydrolyzed product with minor quantities of [^{18}F]FET (fig 3-11).

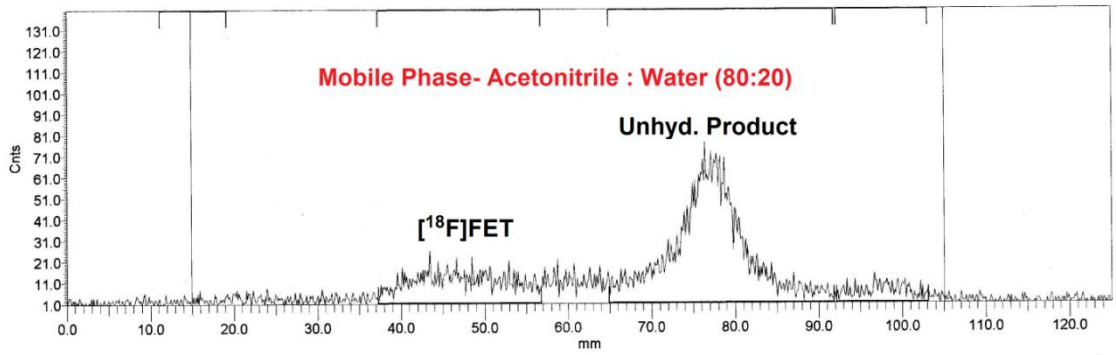


Figure 3-11 Shows radio-TLC of second 3mL elution of purification column

Third wash of column with 4mL of WFI showed complete removal of uhydrolized product with some loss of [^{18}F]FET (fig 3-12).

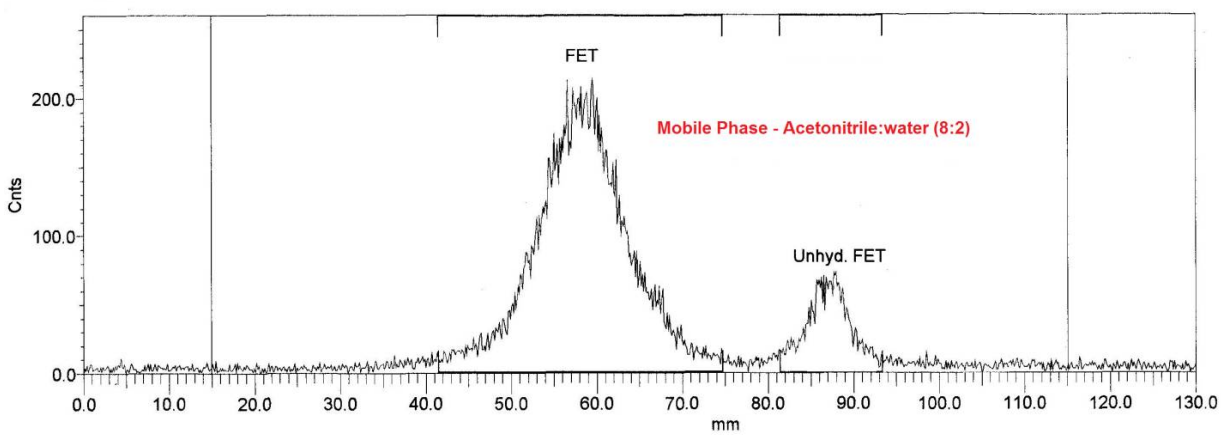


Figure 3-12 Shows the radio-TLC analysis of 4mL third wash of purification column with WFI.

3.3.2.2. Recovery of [^{18}F]FET from the column

The reaction mixture was finally loaded in 8g of neutral alumina column (fig 3-13). The column was washed with 10mL of WFI, which was found to wash out the unhydrolysed products completely. [^{18}F]FET was finally eluted from the column, by using 15mL of water for injection after washing the column with 10mL of WFI. Thus [^{18}F]FET obtained from the column was found to be reliably pure. The column was further eluted with 16mL of WFI to obtain [^{18}F]FET. The product vial was filled with 1M NaH_2PO_4 buffer and 10% NaCl solution. The final product was sterilized by filtering with 0.22µm filter.

Figure 3-13 Anion exchanger cartridge for trapping [^{18}F]Fluoride from [^{18}O]Water (Left),In-house prepared 8g neutral alumina purification cartridge.(Right)

3.3.3. Quality control

Since neutral alumina is amphoteric in nature, 0.5M HCl used for hydrolysis, was found to get neutralized on loading the reaction mixture onto the purification column. The [^{18}F]FET obtained after purification was analyzed with Radio-TLC, Radio-HPLC, GC, Chiral-HPLC for Enantiomeric purity and for sterility and BET. Apart from this tetrabutyl ammonium ion (TBA^+), aluminium ion (Al^{3+}) and Nickel(II) ion concentration in the final product mixture were analyzed.

3.3.3.1. Radio-TLC analysis

Radio-TLC analysis was performed using mobile phase acetonitrile : Water (8:2). The purity of the final product was found to be >98%.(Fig 3-14) Radioactive sample was co-spotted with reference standard [^{19}F]FET. The TLC plate was stained by spraying with 3% ninhydrin in acetone, and gently heated in order to get the purple spot.

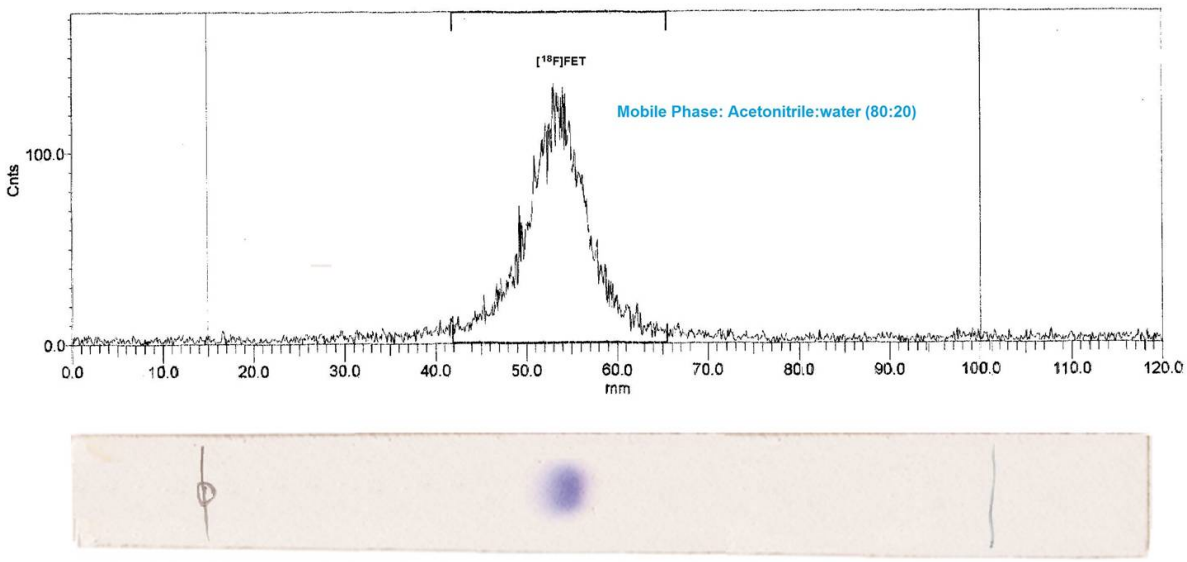


Figure 3-14 Radio-TLC of [^{18}F]FET co-spotted with FET reference standard after purification through 8g of neutral alumina column.

3.3.3.2. Radio-HPLC analysis

Radio-HPLC was performed in Knauer Smartline Radio-HPLC System. Column (C18 RP 300X4mm, 5µm); UV: 254nm; Flow rate: 0.5 mL/min. Hot [^{18}F]FET sample was mixed with [^{18}F]FET reference standard and co-injected. The retention time of FET in cold and hot chromatogram are matched. Fig 16 shows both the UV and radio-chromatogram from the HPLC analysis. Radio-HPLC showed single radio-active peak, corresponding to the retention time matched with [^{19}F]FET cold reference standard from UV chromatogram. An additional UV peak was observed at 11.5min, which was found to be O-(2'-hydroxyethyl)-L-tyrosine (HET) arising due to the hydrolysis of unfluorinated precursor (Chromatogram B in fig. 3-15). The quantity of HET in the final product was evaluated from the calibration of cold reference standard of HET (Chromatogram A in fig.3-15). This was quantified from the reference HET to be 8µg/mL.

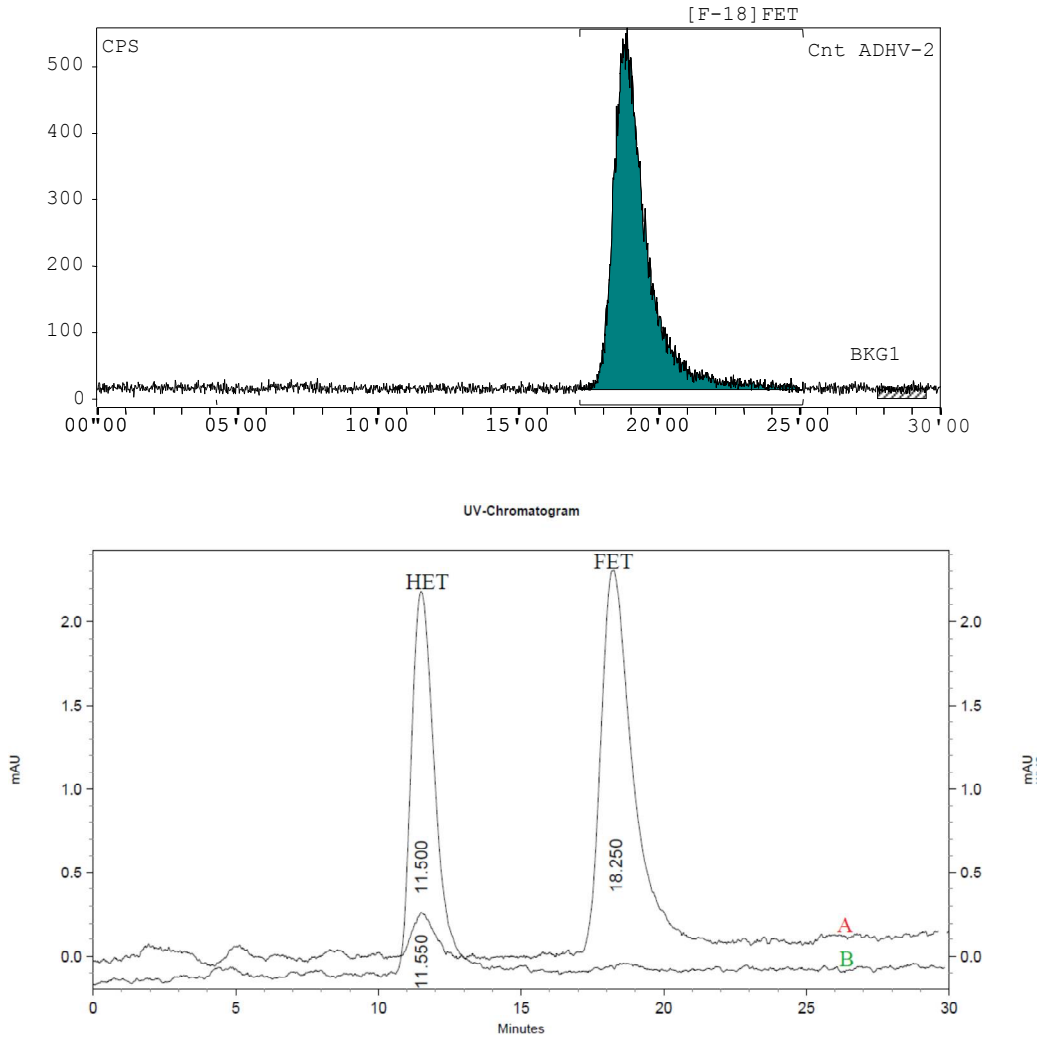


*Figure 3-15 Radio-HPLC and UV-HPLC:Column Nucleosil C-18 column 300X4 mm, 5µm, UV-254nmm Flow Rate:0.5mL/min: (Top) Hot [^{18}F]FET sample Radio-HPLC, showing peak of [^{18}F]FET at Rt– 18.48min. (Bottom) A. HPLC [^{18}F]FET Sample showing 8µg/mL of HET(detection limit 0.2µg/mL) in elusion volume of 16mL and reference standard [^{19}F]FET at R_t - 18.25min mixed and co-injected with hot sample **B.** UV-HPLC HET Reference standard (50µg/mL) R_t HET = 11.55min. Delay in the R_t of the peak between UV and Radio active chromatogram is due to sequential placement of detectors.*

3.3.3.3. Determination of Enantiomeric purity of [^{18}F]FET produced

Enantiomeric purity of the product was determined using Crownpak CR(+) chiral HPLC column. Solvent system: Water:Methanol(90:10, v/v), pH-2 in HClO4, UV-200nm, temperature-10ºC, flow rate: 0.8mL/min. (Fig 3-16)

The product shows presence of major peak of L-enantiomer which was compared with racemic mixture of [^{18}F]FET. The Enantiomeric purity was found to be 94%, which is in agreement with the results reported by others[108, 160].

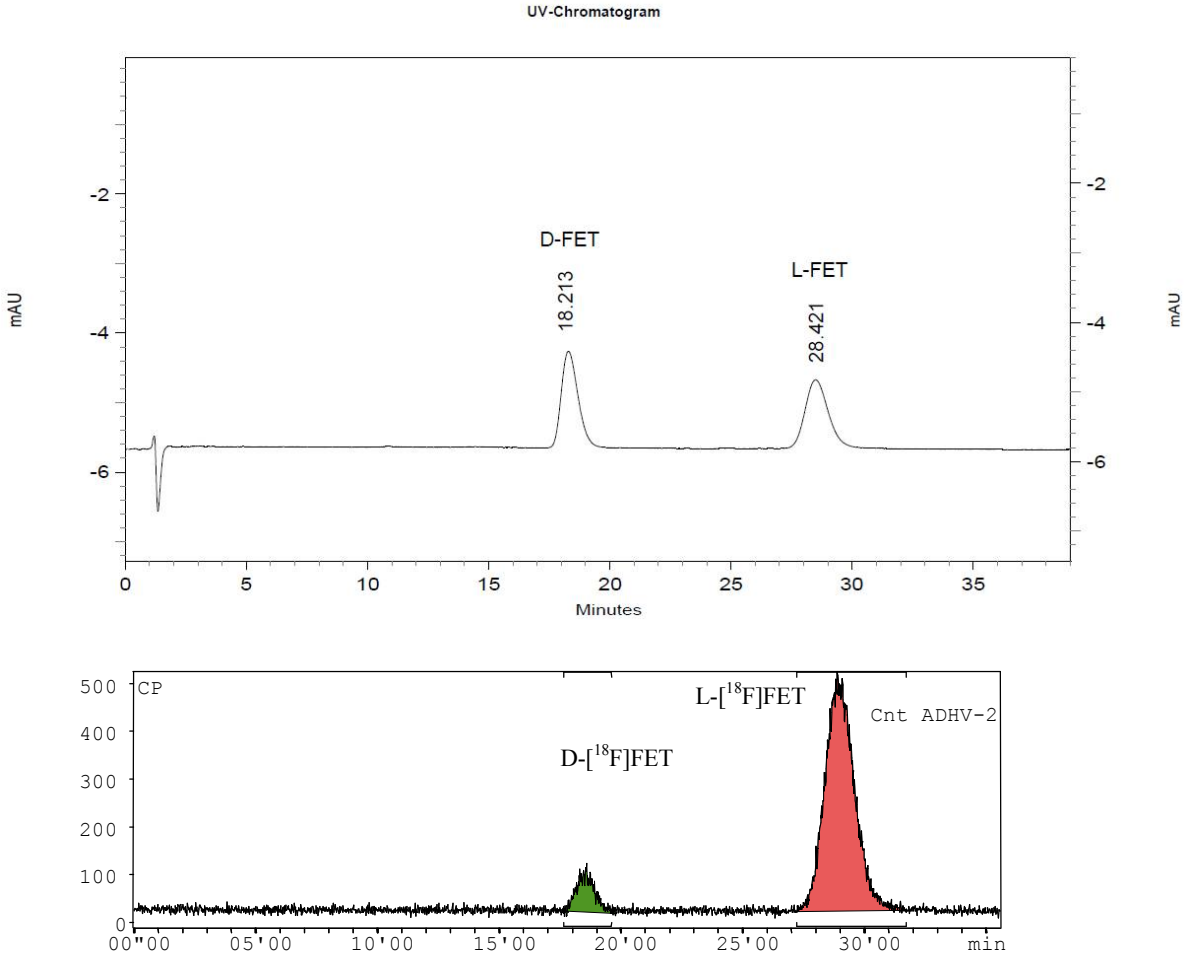
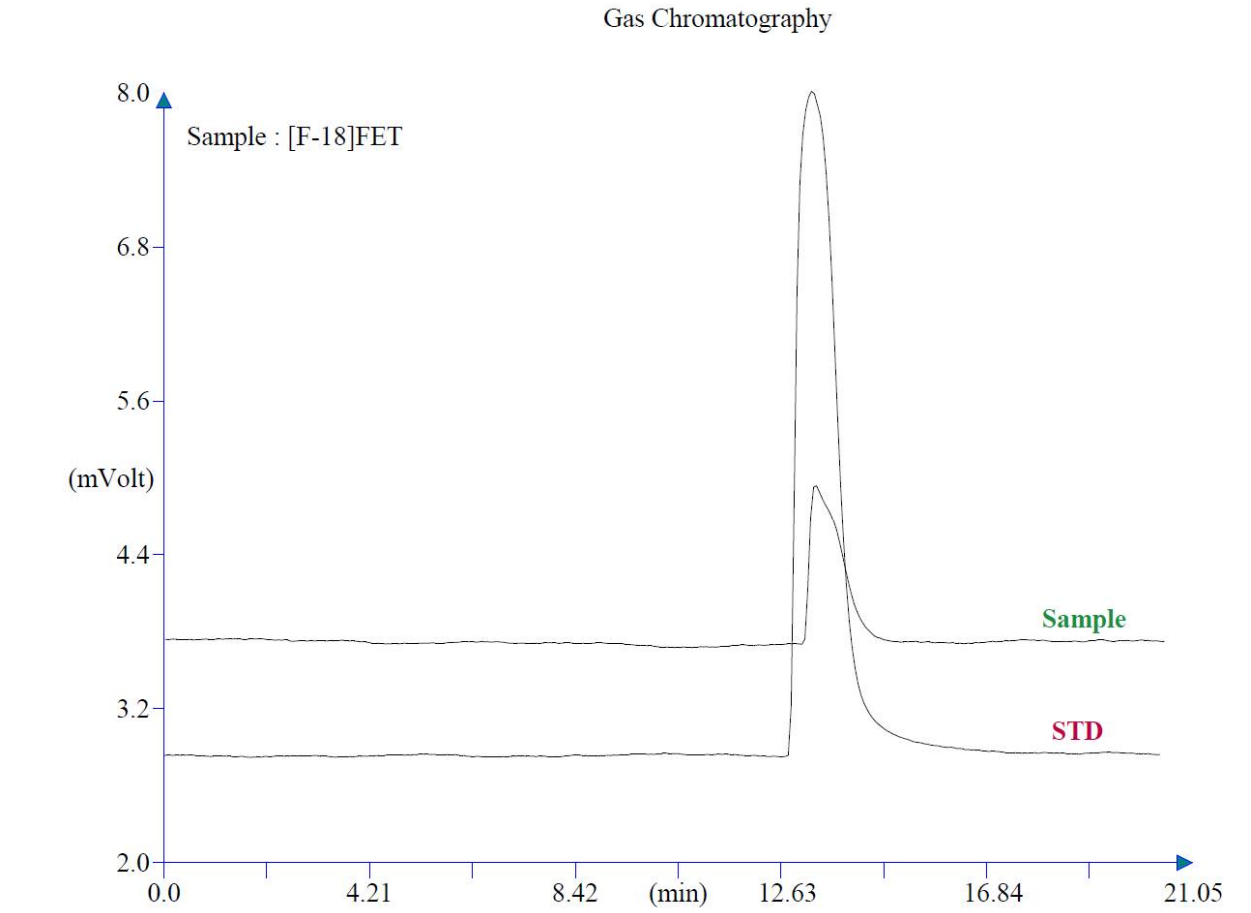


Figure 3-16 Chiral UV and radio-HPLC using Crownpak CR(+) column. Flow Rate:0.8mL/min; Solvent: MeOH: Water (10:90,V/V); pH = 2 in HClO4; UV – 200nm; Column temperature = 10°C. Top:Rt (D-FET) UV- 18.2min; Rt (L-FET) UV - 28.4 min; Bottom: Rt(L-[^{18}F]FET) Radio -29.1min and R_t (D-FET) 18.6min (Radioactive peak follows the UV peak with a delay due to placement of detectors in sequence)

3.3.4.4. Gas chromatographic analysis:

GC showed presence of 1000ppm of DMSO, and absence of any other solvents (fig 3-17). This is expected since, DMSO is used as a solvent for hydrolysis, which is not removed

from the system. Since DMSO is 1000 times less toxic than acetonitrile, DMSO is replaced for acetonitrile, which is safer for human clinical studies.



Component Name	ppm	Ret.Time	Area	BC	Resp. Fact.
DMSO	987.59	3.02	165047	mi	13531.4000

Figure 3-17 GC of decayed [^{18}F]FET sample compared with the 5000ppm standard of DMSO.

Gas chromatographic analysis of the product showed that solvents like acetone, ethanol and acetonitrile are well below the permissible limits. Presence of DMSO was observed in the final product and was quantified to be around 1000 ppm in the final [^{18}F]FET solution.

3.3.3.4. Quantification of Tetrabutyl ammonium ion [TBA^+]

Test for TBA^+ ion was performed by colour spot test following the procedure reported by Kuntzsch et.al.,[163] and compared with samples from the final product. The pharmacopoeia limit for TBA^+ ion is <50µg/L. According to the reported procedure, 2µL of sample is spotted in TLC Plate and dried in hot air. 10µL of Ammonium Hydroxide: Methanol (1:9) is added to the dried spot and the TLC plate was immediately placed inside a chamber saturated with iodine vapour for 1min. Intensity of colour developed is directly proportional to quantity of TBA^+ ion in the final product. The quantity of TBA^+ ion in the final product was found to be well below 50µg/mL (fig 3-18).

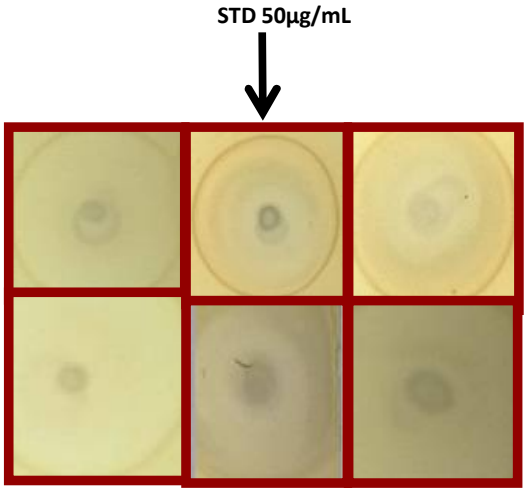


Figure 3-18 Spot test for TBA^+ ion estimation. Colour developed compared to the standard (50µg/mL)

3.3.3.5. Quantification of Aluminium (III) ion (Al^{3+})

Aluminium ion test performed using Microquant aluminium testing kit, using the protocol provided with the product, showed <5µg/mL of Al^{3+} ion in the final product (fig 3-19).

Figure 3-19 Colour developed in microquant aluminium test kit.

3.3.3.6. Determination of Ni(II) ion concentration

Ni(II) ion concentration was estimated by ICP-MS. It was estimated to be 52.41±6.34ppb i.e. 52.41µg/L (n=5). Since Ni(II) ion, present as $NiCl_2$, in the reaction mixture, after hydrolysis of the complex, is a highly polar species, and hence is expected to get highly retended in the neutral alumina column. The toxicity of $NiCl_2$for human intravenous administration is however not estimated clearly anywhere in the literature, in rats and mice was estimated to be >5mg/Kg which may be extrapolated to a crude estimate for human as 12.5 mg for infants to 250mg for average adults. Comparing to these values, the Ni(II) ion concentration in the samples being around 50µg/L may be persumed to be negligible for human injection. Moreover, Ni is an essential trace element and reported to be involved in improvement of growth and fertility, whose deficiency was observed to result in suppressed growth and reproductivity related symptoms.[164]

3.3.3.7. Sterility and Bacterial endotoxins

The product was found to be sterile and bacterial endotoxins below the permissible limit (<25EU/mL).

3.3.4. Bio-distribution studies

Bio-distribution data of [^{18}F]FET in C57BL/6 mice bearing B16F10 murine melanoma. The animals were sacrificed in different time points (15min, 60min and 120min). Three animals were sacrificed in each time point. The radioactivities in different organs were counted and the percentage Injected dose per gram were measured (%ID/g).Tumor uptake was found to be 6.2%ID/g and tumor/brain ratio is found to be 2.74 at 60min PI (Table 3-3).

%ID/g(Normal)				**%ID/g (Tumor)**		
Organ/ Dissection Time Points	**15min**	**60min**	**120min**	**15min**	**60min**	**120min**
Blood	3.57±1.46	2.91±1.54	1.85±0.67	3.62±1.66	2.62±0.65	1.31±0.64
Bone	2.61±0.89	3.42±1.23	2.35±0.85	2.45±1.41	3.22±1.16	1.82±0.47
Muscle	2.97±1.12	3.02±1.42	2.21±0.94	2.46±0.76	2.59±1.03	1.90±0.46
Liver	2.56±0.99	2.84±0.76	1.61±0.56	2.75±1.13	3.25±0.89	1.69±0.38
Stomach	3.27±1.47	4.54±1.48	2.05±0.77	3.58±1.41	4.21±1.71	1.76±0.69
Int+GB	4.84±1.02	5.63±2.01	2.53±0.69	3.06±1.58	3.66±1.38	2.15±0.80
Kid+Blder	4.11±1.34	5.95±2.41	5.15±1.72	4.07±1.11	6.70±3.34	3.70±1.21
Heart	2.54±0.47	4.44±1.16	0.91±0.41	3.04±1.12	3.87±1.55	1.32±0.66
Lungs	3.67±1.21	4.71±1.54	2.06±0.38	3.53±0.97	4.61±1.78	1.85±0.46
Spleen	4.03±1.22	4.30±2.09	2.46±0.84	3.97±1.47	4.49±1.46	2.11±0.15
Brain	2.14±0.81	2.87±1.03	1.73±0.53	1.36±0.66	2.26±0.67	1.38±0.36
Pancreas	20.08±5.65	25.23±6.48	10.06±3.96	20.80±7.07	24.32±10.16	7.59±1.97
Skin	2.86±0.66	2.88±1.25	1.13±0.28	2.80±0.71	2.76±1.12	1.79±0.45
Tumor				3.06±0.84	6.20±2.17	3.60±1.21

Table 3-3 Bio-distribution data of [^{18}F]FET with C57BL/6 mice bearing B16F10 melanoma.

3.3.5. Micro PET/CT imaging studies

A Micro-PET imaging studies were also conducted for normal and tumor bearing C57BL/6 mice, which showed significant [^{18}F]FET uptake in tumor 60min post injection. Tumor/brain ratio was found to be 3.3 which is in good agreement with the bio-distribution data. (Fig 3-20)

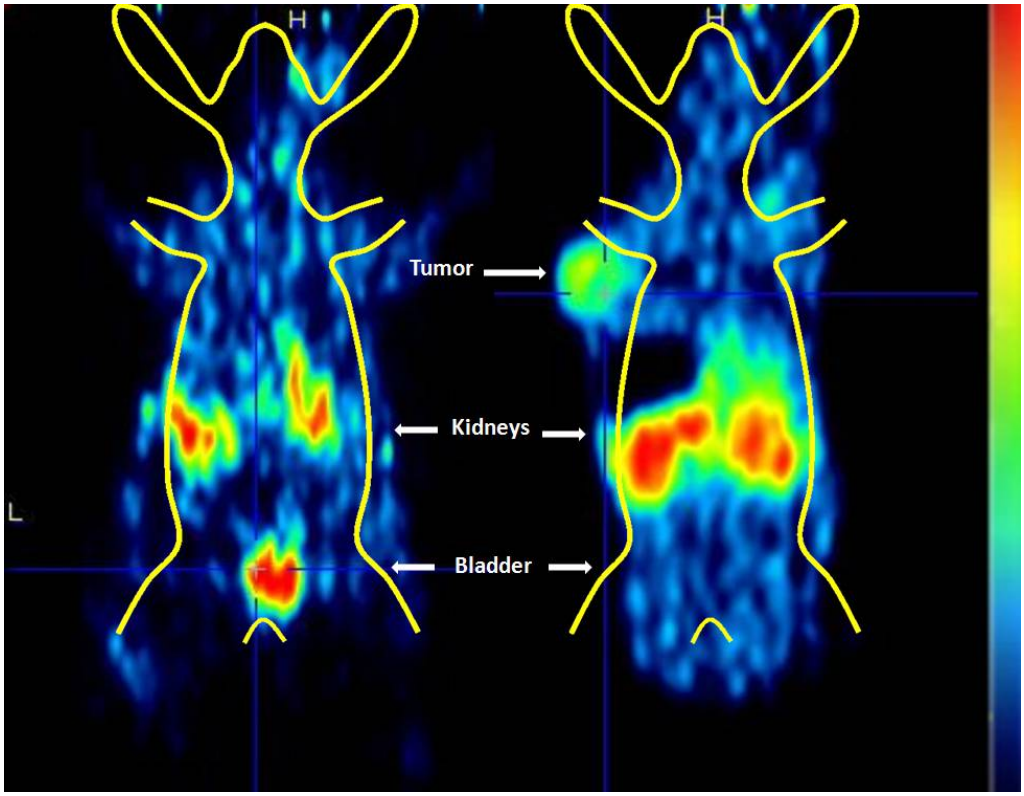


Figure 3-20 MicroPET image of C57BL/6 mice bearing B16F10 melanoma.

3.3.6. Rabbit PET/CT imaging studies

PET/CT image was performed in normal healthy female rabbit (2.45 Kg) after fasting for six hours. 37MBq of [^{18}F]FET in 0.5mL was injected through ear vein. The rabbit was anaesthetized by injecting ketamine and the images ware recorded after 1 hour post injection. Rabbit image shows (fig 3-21), no significant uptake in normal brain and other organs with good clearance in bladder.

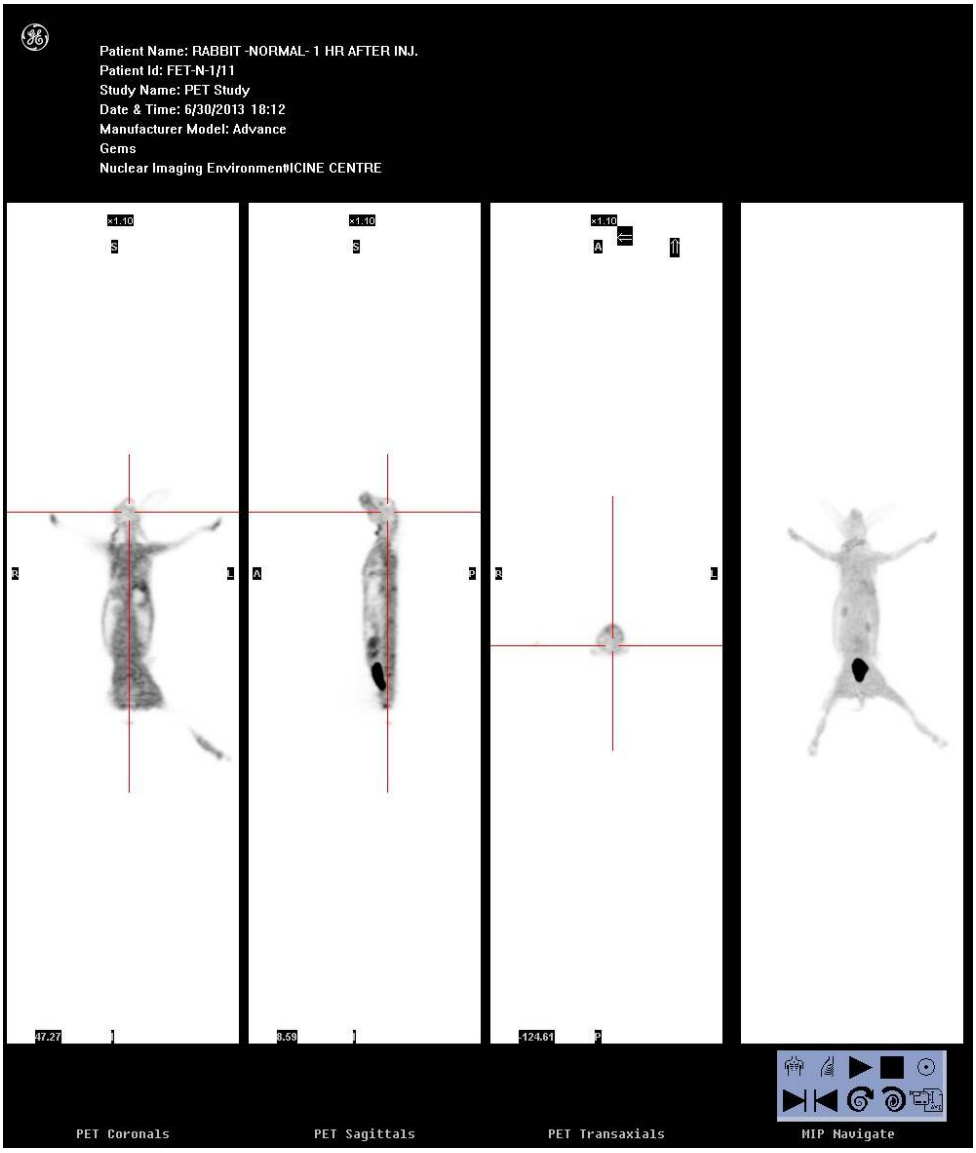


Figure 3-21 Shows normal rabbit PET/CT image of [^{18}F]FET 60min PI.

3.3.7. Automation of radio-synthesis program

The complete synthesis procedure was automated by programmed time lists. The schematic flow diagram of process involved is shown in fig 3-22

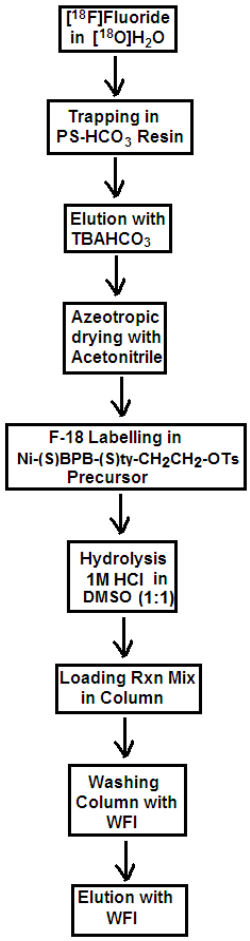


Figure 3-22 The Flow diagram shows the schematic sequence of process used in automated production of [^{18}F]FET using Ni(II)-(S)BPB-(S)Ty-CH_2CH_2-OTs) precursor and neutral alumina column purification.

The synthesis was conducted several times with varying amount of activities (5.55GBq to 25.9GBq). The synthesis time including the purification was around 70 min. Reliably 19.78 ± 1.08 % (n=5) decay uncorrected yield was obtained in 18mL of buffered saline with 5mg of precursor in 1mL of dry acetonitrile, whose data is shown below in table 3-4.

S.No	Initial Activity (GBq)	Activity of [^{18}F]FET in 16mL Elution (GBq)	%Yield	Reaction Time (min)
1	9.25	1.887	20.40	69
2	5.55	1.036	18.67	71
3	11.1	2.368	21.33	71
4	25.9	4.921	19.00	70
5	24.05	4.699	19.54	72

Table 3-4 Production Data using fully automated procedure.

3.3.8. Comparison of [^{18}F]FET synthesized from TET precursor and Ni-(S)BPB-alkylated (S)tyrosine Schiff's base complex precursor

However alternate precursor and methods for one-pot two-step synthesis of [^{18}F]FET is available viz. TET[90], both these methods show several advantages and disadvantages over the other. The pros and cons of [^{18}F]FET by using Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor as compared to the previously developed procedure using TET precursor demonstrated in Chapter 2, are shown in table 3.5.

S. No.	**Synthesis of [^{18}F]FET using TET precursor**	**Synthesis of [^{18}F]FET using Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor**
Advantages of TET precursor over Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor		
1.	Provides better Enantiomeric purity	Provides 94-95% enantiomeric purity
2.	High radio-labelling efficiency. (95% with 8mg of precursor)	Relatively low radio-labelling efficiency (80% with 5mg of precursor)
3.	Ethanol present as the only chemical in final injection.	DMSO present as the only chemical in the final injection.
4.	Commercially available	Not commercially available and hence has to be synthesized.
5.	Contains less HET impurity (2µg/mL with 2mg precursor used for synthesis)	Contains high HET impurity (4µg/mL with 5mg of precursor used for synthesis)

Advantages of Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor over TET precursor		
1.	Provides less radioactive concentration (2-3mCi/mL) starting with 500mCi of initial activity.	Provides high radioactive concentration (5-6mCi/mL) starting with 500mCi of initial activity.
2.	Contains high quantity of ethanol in the final product (2%).	Contains no ethanol, but low quantity of DMSO as the only content (1000ppm)
3.	TET precursor is less stable (i. e) highly sensitive for decomposition by light and temperature.	Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor is found to have high stability over exposure to light, temperature and basic conditions.
4.	Requires highly corrosive and toxic chemicals like TFA, dichloroethane or dichloromethane for efficient synthesis.	Requires mild reagents like 0.5M HCl for complete hydrolysis, which is well established to be safe for PET radiochemistry.

Table 3-5 List of pros and cons of producing [^{18}F]FET using TET and Ni(II)-(S)BPB-(S)tyr-CH_2-CH_2-OTs precursor.

However the above pros and cons are observed for these precursors, used for [^{18}F]FET synthesis, the procedure that was developed by these studies, for the radio-synthesis of [^{18}F]FET, using both these precursors, infer several advantages, which are outlined in below.

- Both these methods use simple purification procedure like neutral alumina which is simple and economical to prepare.
- Solid Phase extraction (SPE) purification was adapted in both these methods, thus reducing the complexity and sophistication required for synthesis of [^{18}F]FET.
- Using less toxic and mild reagents, like HCl, for radio-synthesis was adapted in both these methods.
- Using commonly available chemicals with 2-[^{18}F]FDG synthesis kit, except precursors, can be utilized for the synthesis of [^{18}F]FET in both these methods. This

provides GMP compliance, and except precursors, easy availability of chemicals for all PET centers, for its regular production and supply.

- Both these methods can be easily adapted in 2-[^{18}F]FDG synthesis module. This increases the adaptability of [^{18}F]FET in almost all PET radiochemistry facilities, since 2-[^{18}F]FDG, is by and large, the most commonly produced PET radio-pharmaceutical in all PET centres, its synthesis module will be commonly available.

3.4. Conclusions

[^{18}F]FET was synthesized in a GE TRACERlab FX-C module (configured for making [^{18}F]FDG) using the Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor. Decay uncorrected synthesis yield (n=5) was found to be 19.78 ± 1.08 % by using 5mg of precursor in 1mL of dry acetonitrile in synthesis time of 70min. The RCP of [^{18}F]FET was >98%. Radiochemical purity analysis using radio-HPLC showed the [^{18}F]FET purity to be >98%. UV-HPLC showed HET as the only chemical impurity, and was quantified to be 8µg/mL by using 5mg of precursor. Enantiomeric purity was identified by Chiral-HPLC and was found to be >94%. GC showed presence of around 1000ppm of DMSO. Bio-distribution studies in C57BL/6 mice bearing B16F10 murine melanoma showed tumor/brain ratio of about 3 confirmed by micro-PET imaging. PET imaging in rabbit did not show any significant bone or brain uptake.

The reaction utilizes milder reagents like 1M HCl which are commonly available together with 2-[^{18}F]FDG kit. Radioactive concentrations of about 10mCi/mL can be obtained starting with 1Ci of activity. This method to prepare [^{18}F]FET using the Ni(II)-(S)BPB-(S)tyrosine-CH_2-CH_2-OTs precursor and SPE purification with just neutral alumina can be easily incorporated in any synthesis module such as the one that are routinely used for 2-[^{18}F]FDG synthesis.

CHAPTER 4

SYNTHESIS OF NI(II)-(S)-BPB-AMINO ACID SCHIFF'S BASE PRECURSORS FOR [^{18}F]LABELLED AMINO ACIDS

4.1. Introduction

PET radiochemistry involves, fast synthesis process, since radio-tracers involved are of fast decaying in nature. Hence these precursors involve highly reactive compounds, having low thermal stability. These precursors are also required in the high order of purity, since their radio-labelled products are intended for human use. Moreover most of the procedures for the synthesis of these precursors are multi-step synthesis process involving lower yields and giving other undesirable side products. Separating them becomes a laborious task, thus adding to its preparation cost. However, these precursors are commercially available; this remains a challenge in the precursor synthesis of PET radiopharmaceuticals.

Amino acids are bifunctional biomolecules that are commonly present as building blocks of living organisms. There are 20 common amino acids that are present in nature. As the name suggests, all amino acids have an amino and an acid group in them. Several amino acids have an extra functional group other than these two. Thus they are multifunctional compounds. Radio-labelling [^{18}F]fluorine in amino acid is a challenging process due to its multifunctional nature. These functional groups need to be protected sequentially, before it could be labelled. After protecting them, the labelling site are activated by suitable leaving

groups like tosyl, triflyl, mesyl, , etc[165]. Moreover, the synthesis needs to be conducted carefully, such that, the already protected groups shouldn't get deprotected, during the successive processes, thus leading to additional loss of synthesized compounds. Thus preparing a [^{18}F]labelling precursor for amino acids are multistep reactions requires high level of optimization.

Amino acids are also chiral molecules. The L-amino acids are the one that are biologically active. D-amino acids are found to have negligible uptake in cells. Hence the precursor synthesis should either incorporate the desired chiral centre, or shouldn't degrade the chiral purity of the stereo centers, during the synthesis process. Due to this, the chemistry with amino acids are highly demanding, thus leads to high preparation cost of their [^{18}F]fluorine labelling precursors.

[^{18}F]labelled amino acids can also be prepared by an indirect two step labelling method, which requires two pot radiosynthesis module. However, which could avoid the requirement of a precursor, several precursors have been synthesized and used for [^{18}F] radio-labelling of amino acids, in spite of the above challenges. This is due to the advantage that, a readymade precursor would facilitate the economic way of radiosynthesis, with the commonly available synthesis modules. Synthesis of [^{18}F]fluoro amino acids using readymade tailored precursors would provide an opportunity to radio-synthesize them in a single reactor. Thus a more simple module like a 2-[^{18}F]FDG synthesis module, that are commonly available in all PET facilities, can be utilized for radio-synthesis of [^{18}F] amino acids using readymade precursors, thus providing economic and easy availability of them.

Even though, the above conveniences are achievable by using a readymade precursors from commercial sources, the synthesis and availability of these precursors are still a challenge. This is due to the above mentioned laborious process involved in its synthesis. By overcoming all these challenges, a simplified alternate method for the synthesis of

[^{18}F]labelled amino acid precursor for [^{18}F]FET was reported by Krasikova et al[122]. This novel methodology, has incorporated a new strategy for the [^{18}F]amino acid precursor synthesis by several advantages. In this method, a Ni(II) complex, formed with a Schiff's base of (S)BPB and amino acids, which acts as chiral auxiliary, is used for the synthesis of the precursor. This method shows high scope due to its convenience in synthesis, simple and safe radio-synthesis procedures and high enantioselectivity.

The enantioselective synthesis of Ni(II)-(S)-BPB-amino acid complexes are well established since 1985[124, 166]. However, their application towards PET radio-chemistry have been recently introduced[122]. Moreover the scope of these transition metal complexes for the synthesis of [^{18}F]labelled amino acids are not yet well explored and documented. The enantioselective nature of the complex is attributed to the sterric effect provided by the benzyl group of the chiral auxiliary (S)-BPB to the α-substituted groups of the amino acids.(fig 4-1)

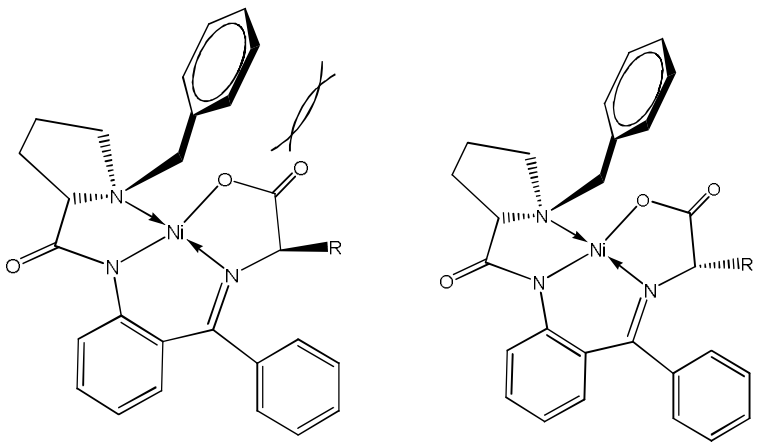


Figure 4-1 Shows the sterric interaction for the formation of (R)-amino acid complex, (B) shows the absence of sterric effect in the (S)-amino acid complex.

Laboratory synthesis of amino acids always ends in racemic mixture. In order to prepare an enantiomerically pure amino acid, these racemic mixtures need to be chirally

separated in large quantities. Thus, it becomes an expensive process. On the contrary, all amino acids available naturally are L-enantiomers. Thus nature adapts an efficient enantioselective approach, with its complex biomolecules, for synthesis of amino acids and proteins. This natural enantioselective property is utilized smartly when amino acid analogues are synthesized, through these complexes, for an economical advantage. The (S)-BPB, which acts as a chiral auxiliary, is synthesized by the reaction of (S)-proline, benzyl chloride and 2-amino-benzophenone[124]. Since (S)-proline is a naturally available amino acid, is readily and cheaply available. This makes synthesis of (S)-BPB, an economical process. Thus the chiral selectivity provided by the (S)-BPB, turns to be an economical way of achieving the enantioselectivity of amino acids through formation of Ni(II)-(S)BPB-complex with aminoacid. Moreover, these complexes are coloured and have extraordinary stability under basic conditions. Hence synthesis and purification are more convenient.

([^{18}F]FET) is one of the PET radio-pharmaceuticals which had proved to be more useful in the imaging of Brain tumors. [18F]FET can be synthesized by a one-pot two-step method through Nickel (II) complex of (S)BPB precursor[122]. This precursor was synthesized by a three step method.

Inspired with the above attractive properties of this method, in this study, the scope of synthesizing several [^{18}F]fluoro amino acids based on Ni(II)-(S)BPB-amino acid Schiff's base complex is explored. Several [^{18}F]labelling precursors for several amino acids were synthesized, characterized and radio-labelled in this study.

A simplified method for the synthesis of the [^{18}F]FET precursor based on Ni(II)-(S)-BPB-Ty complex was developed, which is an improvement of the procedure reported earlier [122], reducing both the number of reaction steps and reaction time significantly. A new purification technique, which provides better suitability for the purification of coloured complexes were introduced, which was coined the name "Serial

Column Flash Chromatographic Technique" (SCFC). This method is more specific for compounds of the kind used in this study, providing fast and economic means of purifying coloured mixtures. The development and performance of this new technique, applied for the purification of the products obtained in various steps in the synthesis of this precursor are also discussed in details.

4.2. Materials and methods

(S)BPB was procured from Merck, Germany, $Ni(NO_3)_2.2H_2O$, (DL)tyrosine and flash chromatography silica gel (mesh 200-400) were procured from sigma-aldrich. Polypropylene barrels and poplyethylene frits used for purification are prepared by emptying the used and decayed resins of Chromabond Column, that came together with 2-[^{18}F]FDG kit from ABX, Germany. HPLC analysis using a C-18 reverse phase analytical column (LIChroCARTs 250X4, 5µm HPLC cartridge LIChrosphers 100 RP-18 5µm) was done on Knauer smartline HPLC systems and Spectra Physics equipment. Raytest radioactive detector used for Radio-HPLC. [^{18}F]Fluorine in fluoride form was produced in GE PETtrace Medical Cyclotron by irradiation of enriched [^{18}O]water target by the nuclear reaction $^{18}O(p,n)^{18}F$. Radio-synthesis of [^{18}F]FET was carried out in a GE-TRACERlab FX-C configured for 2-[^{18}F]FDG radio-synthesis. Radio-TLC was carried out on Silica Gel 60, 20X20 (Merck, Germany) and scanned using a Raytest TLC scanner with a BGO scintillation detector and chromatogram analyzed with GITA software.

4.2.1. Precursor synthesis

The precursor was synthesized by a two step process as shown in the reaction schematic below (fig 4-2). The details of synthesis are given in the experimental section (page 174)

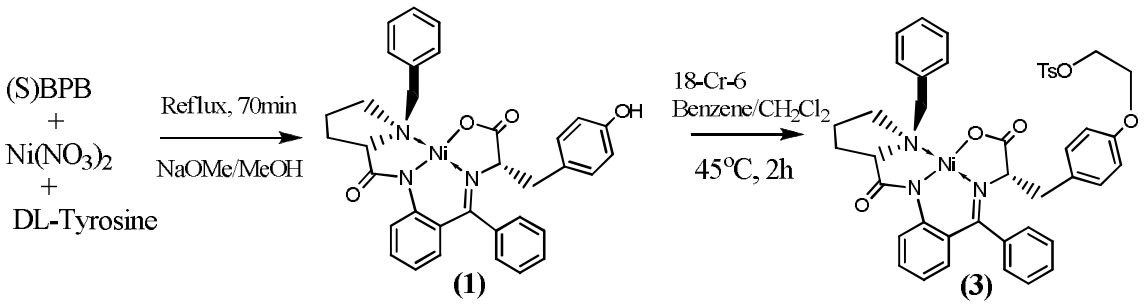


Figure 4-2 Reaction schematic of synthesis of Ni(II)-(S)BPB-(S)-tyrosine-CH_2-CH_2-OTs complex precursor.

4.2.2. Purification of compounds

Generally, purification of compounds is performed by various column chromatographic techniques. Akin to that, in this study, we have developed modified version of flash chromatographic technique which was coined the name "Serial Column Flash Chromatography" technique (SCFC). The purification of compounds, obtained in various steps, is performed by SCFC technique. This is an indigenously developed technique, suits perfectly for the compounds, of the type, prepared in this study. Due to this novel technique, the recovery of the compounds can be faster, efficient and economic. The process and development of the SCFC technique are discussed in detail in Annexure I of this thesis.

4.2.3. Synthesis of Ni(II)-(S)-BPB-amino acid precursors

The synthesis of various amino acid precursors are discussed in the experimental section on the last part of this chapter.

4.2.4. [^{18}F]Radiofluorination of precursors

The radiosynthesis was carried out in GE TRACERlab FX-C module configured for 2-[^{18}F]FDG shown in fig 2-2 of chapter 2.

The [^{18}F] fluoride was trapped in 45mg PS-HCO_3 anion exchange resin. [^{18}F]Fluoride was eluted with 0.5mL of 75mM Tetrabutylammonium bicarbonate solution to obtain [^{18}F]Fluoride in the form of [^{18}F]TBAF in the reactor vessel. Then to this, 1mL of dry acetonitrile was added and azeotropic distillation was carried out to remove the water. Drying was aided by flushing the reaction vessel with a stream of He-gas. The precursor in acetonitrile (5mg/1mL) was added and heated to 95°C and maintained at this temperature for 5 min. The reaction mixture was then cooled to 50°C. To this, HCl was added for hydrolysis, and the temperature was raised to 120°C and maintained for 10min. The reaction mixture was then cooled to 50ºC. The final reaction mixture was analyzed with Radio-TLC (SiO_2, Acetonitrile:Water (8:2)) to identify the radiochemicals.

4.3. Results and discussions

The synthesis procedure for Ni(II)-(S)BPB complex based [^{18}F]FET precursor reported by Krasikova et al is a three step process as shown in figure 2. Since it was explored that, the tosylate was the most efficient precursor over triflate and mesylate, for radio-fluorination, in this study, the tosylate was prepared directly from the Ni-(S)BPB-(S)-tyrosine complex to the tosyloxy alkylated precursor complex, thus bypassing the hydroxyethylated complex. Thus the number of steps have reduced to two from one.

The main modification of the precursor synthesis is in the second step of the precursor synthesis, i.e, tosyloxy ethylation step. This step is followed close to the procedure reported[122]. The solvent used was benzene. Here when we have tried benzene as the solvent, it was observed that, the Ni-(S)BPB-(S)-Ty complex gets separated out from the mixture on addition of ethylene glycol ditosylate. Hence in order to improve the solubility, few volumes of dichloromethane was added. We have used dichloromethane, because, it is a low boiling solvent, and has less probability of forming reactive carbene species like dichlorocarbene,

(whose probability is more in chloroform). Moreover, it provides milder conditions for the reaction while refluxing, and can be removed faster, at low temperature, from the reaction mixture after workup, as the precursor complex is a highly sensitive compound. We have tried other solvents like carbon tetrachloride. It was observed that, the tosyloxyethylation was sluggish and the hydroxyl complex was formed to a larger extent. Hence we finalized with a mixture of dichlormethane:benzene (1:3) as the reaction medium.

In the reported procedure, TBAI was used as a phase transfer catalyst (PTC). In our study, it was observed that, use of TBAI, resulted in significant amount of iodination of the tosylate formed. This results in the loss of precursor and additional impurity, that would be difficult to remove. Moreover, it also adds up the iodine content in the final product. Hence, reaction without phase transfer catalyst (PTC) was tried. Since the reaction didn't proceed, an alternate PTC was tried. Since KOH is used as the base, 18-Crown-6, which is more specific PTC for K^+ ion was tried. Under the presence of PTC, the reaction went smoothly, as expected.

The precursors used in PET radiochemistry involves highly reactive and sensitive compounds. Ni-(S)-BPB-(S)-tyr-CH_2-CH_2-OTs precursor is not also an exception of it. Hence the post synthesis process, like extraction, purification, recrystalization, etc. needs to be faster. The loss of the synthesized precursors, due to the purification processes involved in the post synthesis steps, is directly proportional to the number of stages in the purification process and the time taken for it. Hence efforts for reducing the time of preparation in all stages are taken into consideration. The reaction time reported for the synthesis of the precursor altogether was over 35 hrs. Apart from that, the purification in each step may lead to a much longer time. On the contrary, a procedure was developed in this study in which, the total synthesis time was significantly reduced, to 4 hours, excluding purification time.

The reaction also uses commonly available chemicals which are economic and easy to procure. Since the complexes involved have a bright orange-red colour, it is easy to follow the progress of the reaction by TLC analysis during reaction and while purification. Since faster purification of these highly reactive precursors, would save their loss during their preparation, purification by flash chromatography reduces the time of production. Further using the newly explored SCFC purification method (Annexure I), the purification time further reduces. Moreover, the longer the steps involved in purification, the volume of chemicals used are also large. Since SCFC purification method inherently has a technical property of reducing the purification process by shortening the column used, the wastage of solvents is further saved, making the process more economic and time saving as well. The precursor synthesis procedure reported by us is a simplified and fast method with a good overall yield (75%). The method reported earlier involves three steps.This was reduced by both the number of reaction steps, and the reaction time significantly as shown in fig 4-3

The synthesized precursor was characterized with ^{1}HNMR &^{13}CNMR. The total time taken for the precursor synthesis, was found to complete within 4-5 hours excluding column purification steps. The final product mixture was analyzed by HPLC and observed that the reaction was complete to around 90%. The reaction mixture showed an extra spot other than reactant and the product. This was characterized by ^{1}H-NMR, to be hydroxylated product Ni(II)-(S)-BPB-(S)-tyr-CH_2-CH_2-OH complex, arising due to hydroxylation of the formed precursor (fig.4-4), whose ^{1}HNMR data are shown below

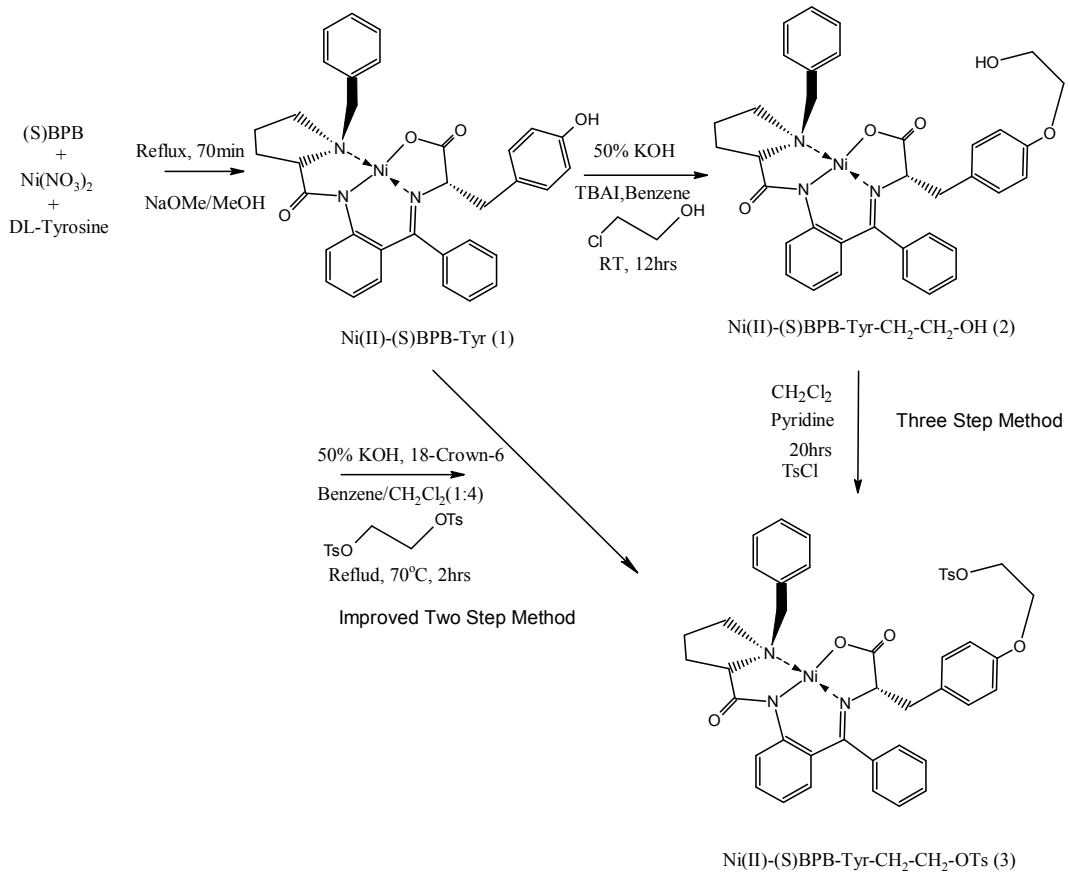


Figure 4-3 Three step reaction sequence originally reported

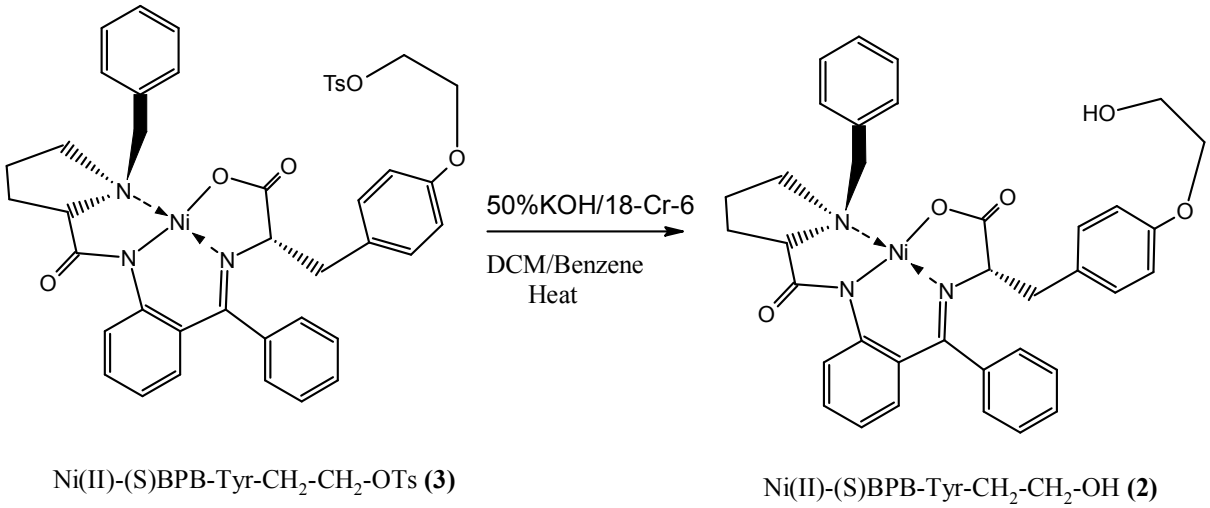


Figure 4-4 Formation of Ni(II)-Tyr-CH_2-CH_2-OH complex in the reaction mixture

^{1}H-NMR of Ni(II)-(S)-BPB-(S)-Ty-CH_2CH_2OH(3) (fig. 4-33): 1.66 (m, 1H, Pro), 1.96 (m, 1H, Pro), 2.34 (m, 3H, Pro), 2.78 and 3.01 (AB part of ABX, J_{AB} = 14 Hz, J_{AX} = 5.7 Hz, J_{BX} = 4.3 Hz, 2H, –CH_2–Ty), 3.09 (m, 1H, δ-Pro), 3.29 (m, 1H, α-Ty), 3.41 (s, 1H, OH),

4.21 (m, 1H, α-Pro), 3.42 and 4.25 (AB, J_{AB} = 12.5 Hz, 2H, –CH2–Bn), 3.94 (m, 2H, OCH_2CH_2OH), 4.07 (m, 2H, OCH_2CH_2OH), 6.64 (d, 2H, Ar), 6.86 (d, 1H, Ar), 6.93 and 7.07 (AB, JAB = 8.4 Hz, 4H, Ty), 7.13 (m, 2H, Ar), 7.28 (m, 3H, Ar), 7.42 (m, 1H, Ar), 7.51 (m, 2H, Ar), 8.00 (d, 2H, Ar), 8.19 (d, 1H, Ar).

The reaction mixture thus had around 5% of reactant and the hydrolyzed product Ni(II)-(S)-BPB-(S)-Ty-CH_2CH_2OH each as shown in the TLC (fig 4-5-2h).In order to perform the reaction at milder condition, to avoid post hydroxylation, tosyloxyethylation reaction was carried out at room temperature. It takes overnight (about 14hrs) for completion and resulted in large quantities of hydrolyzed product (about 50%), which was evaluated by TLC analysis (mobile phase: ethylacetate). It was observed that, as the tosyloxyethylation progresses, the hydroxylation of the formed tosylate was a sequencial reaction, since a strong solution of 50% KOH is already present in the reaction mixture, which was unavoidable. The reaction pattern was followed by TLC, and the reaction time was optimized based on the conditions at which the desired precursor was formed as the major product. The reaction pattern is shown in fig 4-5.

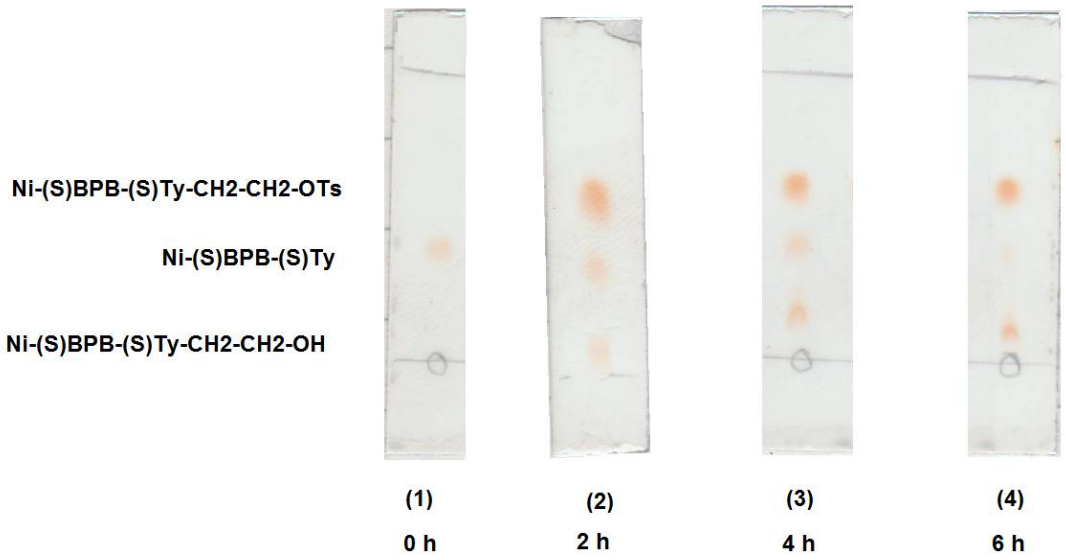


Figure 4-5 The TLC pattern in which the reaction was found to progress.

As visible from the TLC pattern, TLC (1) shows the reactant at the start of the reaction. As the reaction progresses, the major product that formed within 2 h was the precursor, with a minor quantity (~5%) of hydroxylated product (TLC 2). As the reaction was allowed to progress further for 4h, the hydroxylated product increased, which can be clearly observed from the relative intensities of the spots (TLC 3). When the reaction was allowed to complete either by a overnight vigorous stirring at room temperature or refluxing for 6h, it was observed that the reaction mixture contained two major spots, corresponding to the precursor and the hydroxylated compounds. The hydroxylated product was found to be of higher quantity (~30%), as observed from the colour intensity of the spot (TLC 4). Thus the reaction time was optimized to 2hrs in which a minor quantity of reactant and hydroxylated product was formed.

The hydrolyzed product was recovered separately and characterized by ^{1}H-NMR. This can also be tosylated separately to finally get the precursor Ni(II)-(S)-BPB-(S)-Ty-CH_2CH_2OTs (fig 4-6). Thus this procedure doesn't leave any undesired product for disposal.

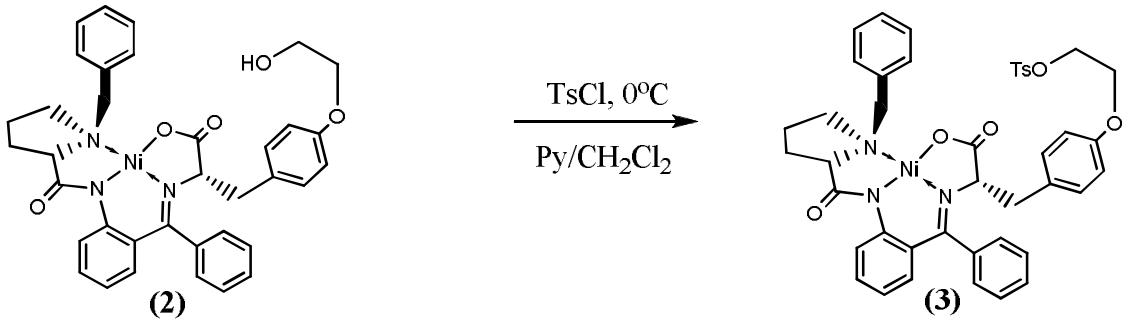


Figure 4-6 Synthesis scheme of tosylation of hydroxyl complex formed as side product.

Further, it was also observed, an extra hydroxylation happens when the column purification was performed, which was reduced by performing the purificationquickly by using SCFC method.

4.3.1. Standardization of TLC solvent for following the reactions of precursorsynthesis

The originally reported TLC solvent by Krasikova et al was Chloroform: Acetone based solvents. Two different compositions of solvents were used for various steps of the reactions. For complex formation, chloroform : acetone (3:1) was used as mobile phase. For hydroxyethylation and tosylation chloroform:acetone (7:1) was used. To begin with, we have used the above solvents. It was observed that, the hydroxyethylated complex and the Ni-(S)BPB-L-tyrosine complex both had the R_f, that are so close, that they were not well resolved in both these mobile phases of chloroform:acetone system.

Hence with these solvent system, however the tosylation pattern was observed, the hydroxylation progress cannot be followed. Since hydroxylation happens as a side reaction, by the consumption of formed precursor, in the reaction mixture, it is essential to follow the hydroxylation, in order to stop reaction, and recover the precursor formed. Thus the yield of the precursor is highly dependent on this stage. Hence an alternate solvent system was searched for this purpose. It was observed that, a 100% ethyl acetate system can be applied for the identification of all the three complexes formed in the synthesis of the precursor.

The TLC pattern of the mixtures in both these solvent systems are shown in fig. 4-7. As the TLC pattern shows, it is visible that the separation of all the three complexes involved in the synthesis are well resolved when 100% ethyl acetate was used as the mobile phase. On the other hand the chloroform: acetone system could resolve the tosylate complex from the other two, the reactant complex and the hydroxylated complex are not well resolved.

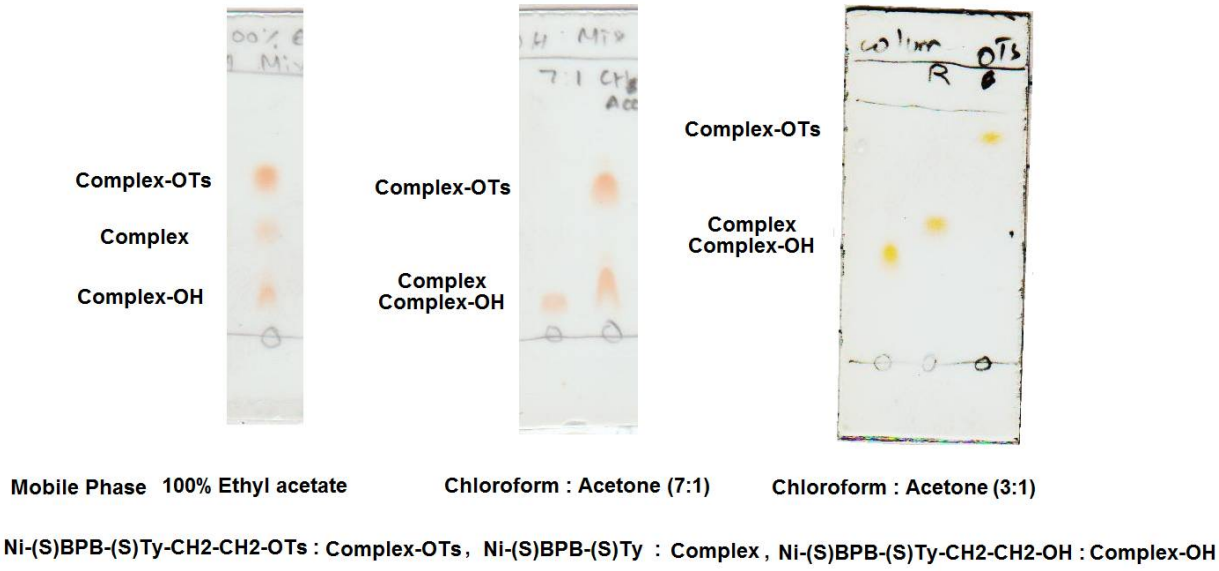


Figure 4-7 Shows TLC pattern of the mixture of complexes, being well resolved in 100% ethyl acetate.

4.3.2. HPLC analysis of the precursor

After synthesis the crude mixture was purified using Serial Column Flash Chromatography. The crude product and the purified precursor were analyzed in Spectra Physics HPLC system; Column:C18 RP Nucleosil (250X4mm, 5µm); mobile phase: acetonitrile:water (6:4); UV – 284nm; flow rate : 0.5 mL/min. A typical HPLC of the crude reaction mixture which was stopped at a time when no hydroxylated product was not visible, and extracted from the mixture is shown in fig. 4-8. The reagents were removed through a flash chromatography to recover the crude complex mixture, before analyzing the mixture. HPLC showed 75% of the precursor in the crude mixture. A diastereomeric peak of 5% is visible in all the three complexes.viz. Ni(II)-(S)BPB-Ty-CH_2CH_2-OTs precursor, Ni(II)-(S)BPB-Ty complex and Ni(II)-(S)BPB-Ty-CH_2CH_2-OH complex.

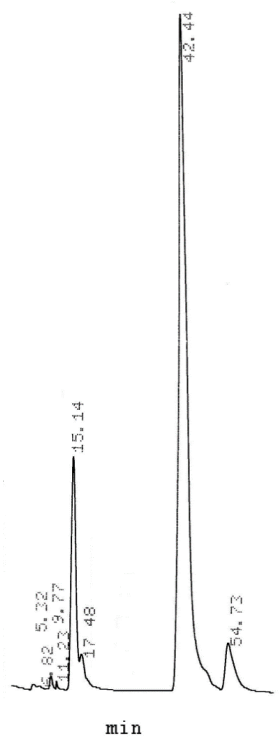


Figure 4-8 HPLC of crude precursor mixture, showing 75% Ni(II)-(S)BPB-Ty-CH_2CH_2-OTs precursor (Rt – 42.44 min), 24% Ni(II)-(S)BPB-Ty complex (Rt – 15.14 min) and 1% Ni(II)-(S)BPB-Ty-CH_2CH_2-OH complex (Rt –6.82 min). 5% of Enantiomeric complex Ni(II)-(S)-BPB-(R)-Ty-CH_2CH_2-OTs is present in R_t – 54.73min.

The precursor was synthesized in bulk quantity (600mg scale). The mixture was purified using the serial column flash chromatography. The purity of the product was shown in fig. 4-9. The HPLC of the purified product shows 98.5% pure precursor, with diasteriomers Ni(II)-(S)-BPB-(S)-Ty-CH_2CH_2-OTs – 95% and Ni(II)-(S)-BPB-(R)-Ty-CH_2CH_2-OTs – 5%.

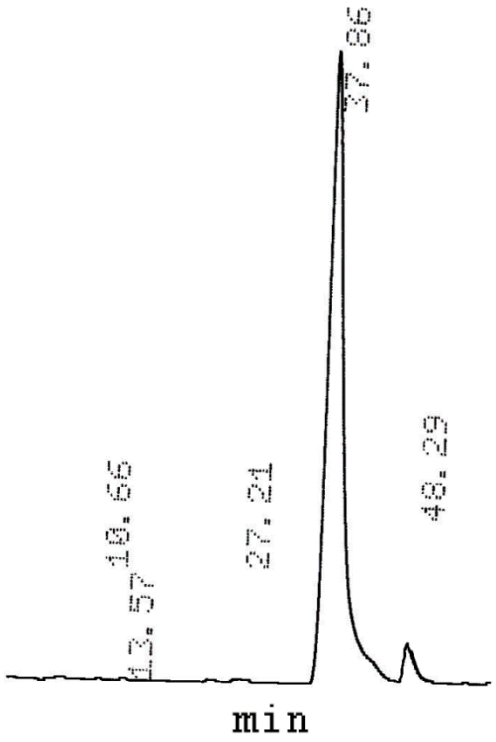


Figure 4-9 HPLC of crude precursor mixture, showing 98.5% Ni(II)-(S)BPB-Ty-CH_2CH_2-OTs precursor, Flow Rate: 0.6 mL/min Mobile Phase: Acetonitrile:Water (6:4) Ni(II)-(S)-BPB-(S)-Ty-CH_2CH_2-OTs; Rt – 37.86 min; 95% Ni(II)-(S)-BPB-(R)-Ty-CH_2CH_2-OTs; Rt – 48.29 min; 5%

4.3.3 Radio-synthesis of [^{18}F]FET using the In-house synthesized precursor:

[^{18}F]FET was synthesized using the above precursor and the final product mixture was characterized by Radio-TLC (mobile phase: acetonitrile: water(80:20)). The labelling efficiency was found to be 60% at 80°C with 5mg/mL of precursor in dry acetonitrile. The radio-labelled precursor was also hydrolyzed with 0.5M HCl at 120°C. The hydrolysis was found to be 85% efficient leaving 15% of unhydrolyzed product (fig. 4-10)

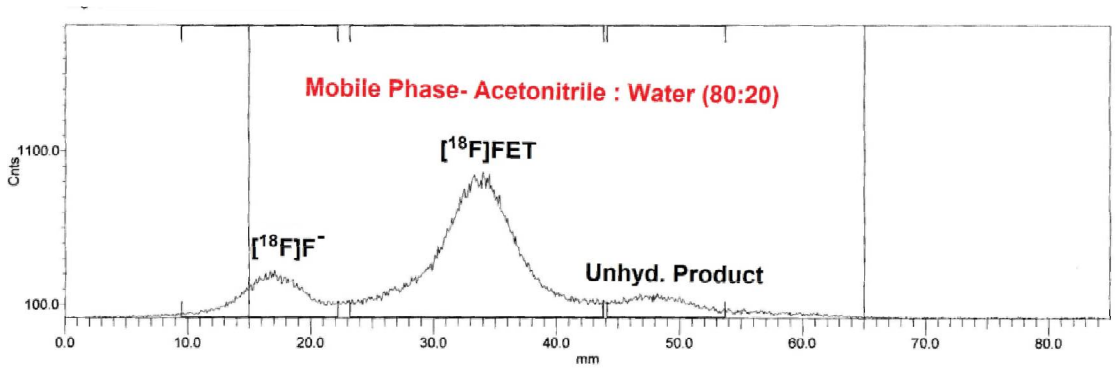


Figure 4-10 Radio-TLC of the reaction mixture post hydrolysis.

The radio-labelling efficiency and the spectroscopic data of the final precursor are in good agreement with the reported one [122]. It was observed that, the raidolabelling efficiency increases to 80% when the S_N2 reaction temperature was increased to 95˚C.

4.4. Synthesis of other [^{18}F]Fluoroaminoacids through Ni(II)-(S)BPB complex

As a first strategy to synthesize[^{18}F] fluoro amino acid precursor, was planned by choosing amino acids that have a hydroxyl group as their functionality. By forming the Ni(II)-(S)-BPB-amino acid Schiff's base complex, it can be further tosylated to form the precursor, that can be further fluorinated.(fig 4-11)

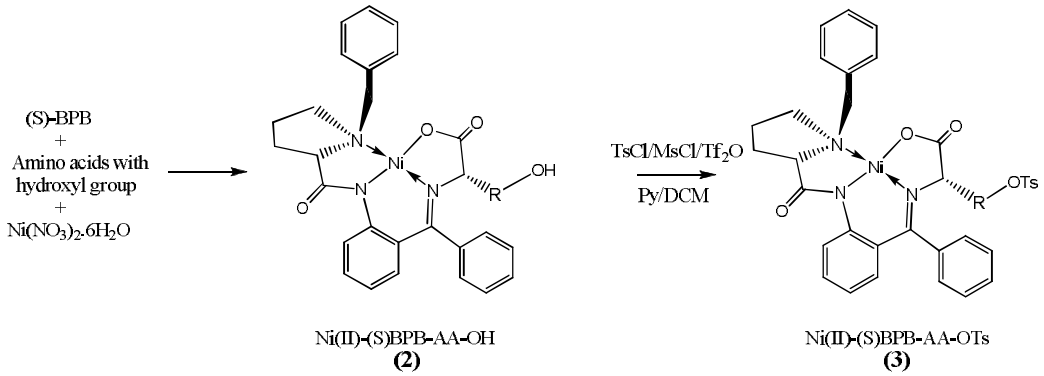


Figure 4-11 General strategy of synthesizing a tosylate complex as a [^{18}F]fluoro amino acid precursor.

4.4.1. Synthesis of serine and threonine- Ni(II)-(S)-BPB complexes

For this threonine and serine were chosen and the Ni(II)-(S)-BPB-AA complex synthesis was attempted which didn't succeed. Hence it was realized that, these amino acids were not suitable candidates for forming the complex.

Formation of Ni(II)-(S)-BPB-glycine complex was reported to be highly efficient [167]. An alternate procedure of preparing serine complex from glycine complex was reported by Smith et al.[168].Hence serine complex from the glycine complex was synthesized.(fig 4-14)

The serine complex was tosylated to form the precursor for synthesizing [^{18}F]fluoroalanine. [^{18}F]radiolabelling using Ni(II)-(S)-BPB-serine-OTs complex was carried out. Radio-TLC analysis of the reaction mixture prior to hydrolysis showed 80% radiolabelling efficiency.(fig 4-12) On further hydrolysis of the [^{18}F]labelled serine complex using HCl, it was observed to undergo defluorination, to form persumably an eliminated product together with the hydrolysis of the complex.(fig 4-13).

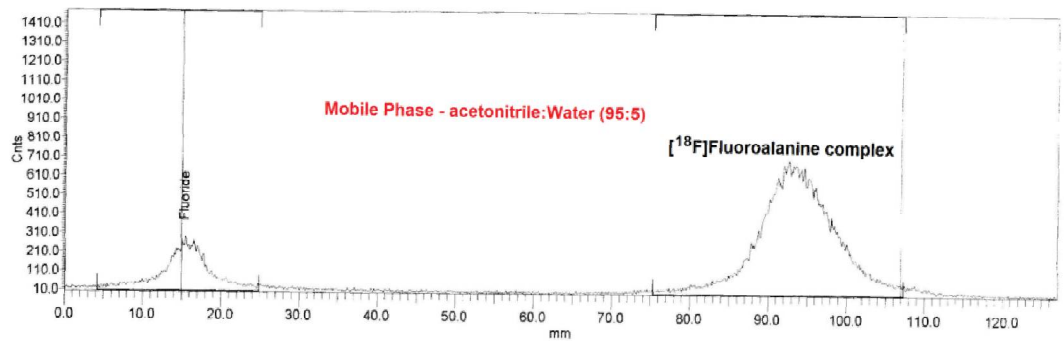


Figure 4-12 Radio-TLC analysis of Ni(II)-(S)-BPB-Ser-OTs complex showing 80% radiofluorination

Moreover, the synthesis of Ni(II)-(S)-BPB-serine-OTs complexprecursor, itself showed partial elimination of the product, as a side reaction to form the serine elimination complex (12) (fig 4-11).

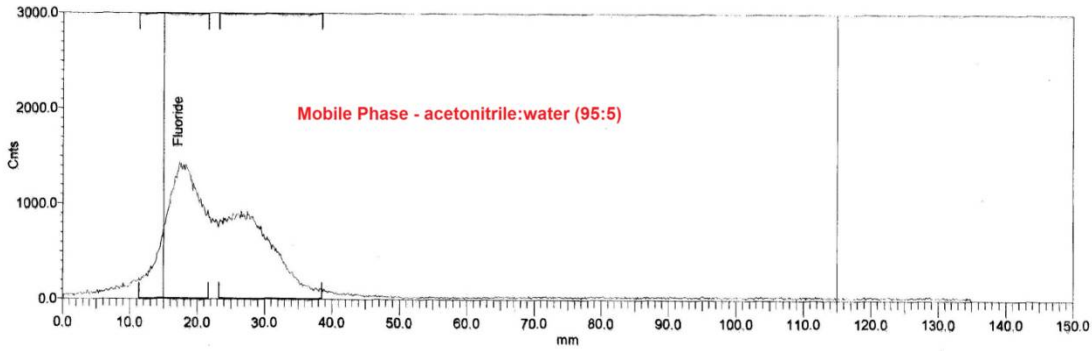


Figure 4-13 Radio-TLC analysis showing elemination of HF after fluorination of serine-OTs complex.

This is expected due to the product stability, because of extended conjugation, provided by the adjacent phenyl and the carbonyl groups.(compound 4 in fig 4-14)

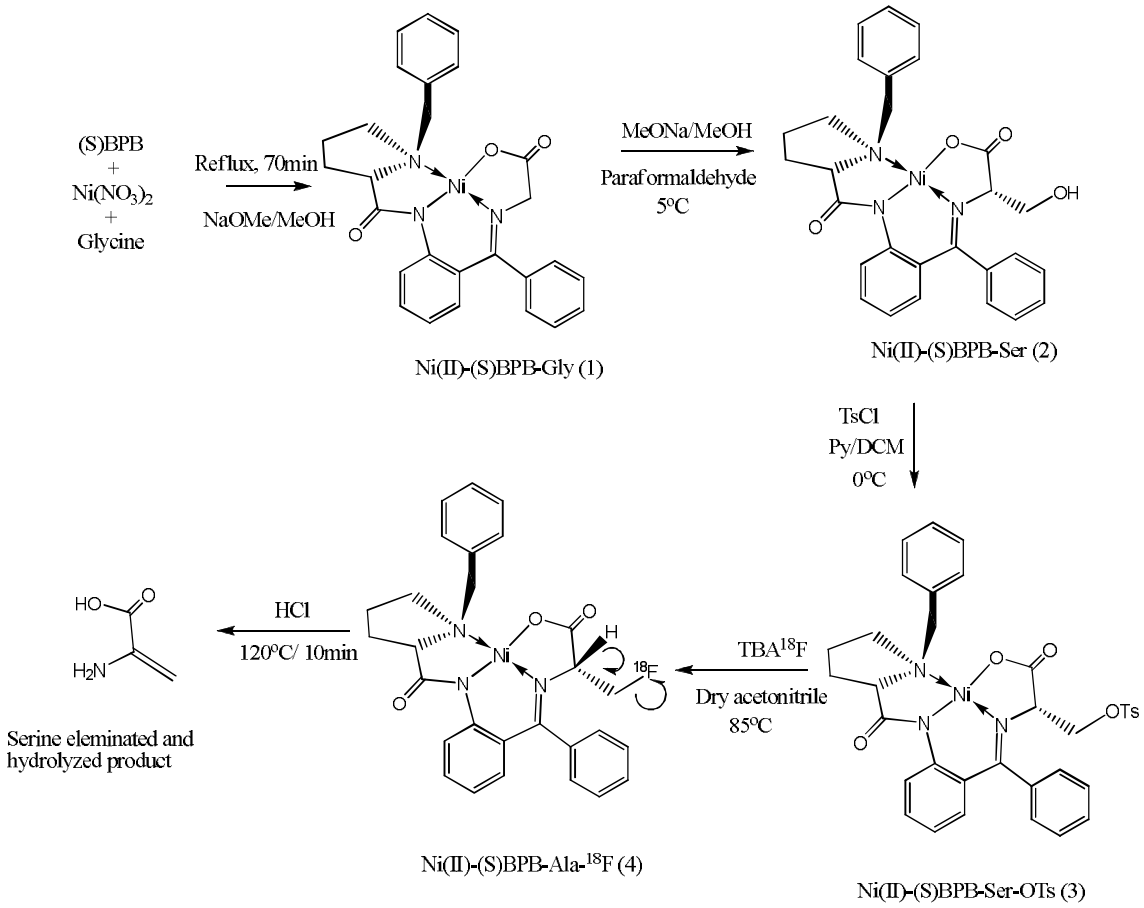


Figure 4-14 Schematic of fluoroalanine synthesis and elimination of HF on hydrolysis.

4.4.2. Synthesis of [^{18}F]fluoroethylcysteine and [^{18}F]fluoropropylcysteine from Ni(II)-(S)-BPB complexes

The serine elimination complex (12) was coupled with the most commonly available mercaptoethanol to form hydroxyethyl cysteine complex fig 4-15).

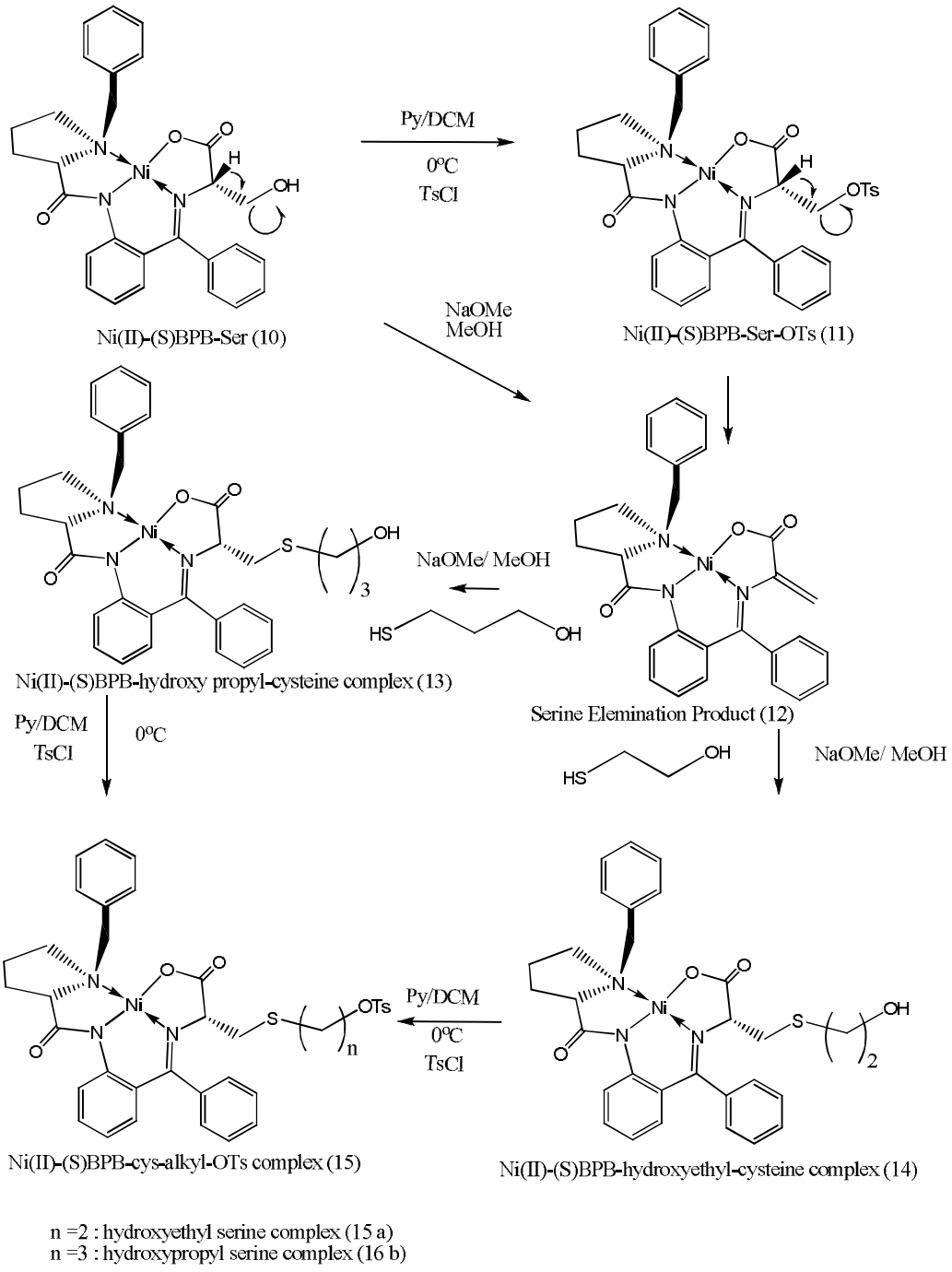


Figure 4-15 Synthesis schematic of fluoroethyl and fluoropropyl cysteine complex precursors

The Ni(II)-(S)-BPB-hydroxyethyl cysteine complex was further tosylated and fluorinated. This precursor showed a fluorination efficiency of 30% (fig 4-16). On hydrolysis, with HCl it was observed to undergo defluorination. This is presumably due to

the presence of sulphur atom in the β-position, which can stabilize α-carbanion, making it acidic, resulting in elimination of HF (fig 4-17).

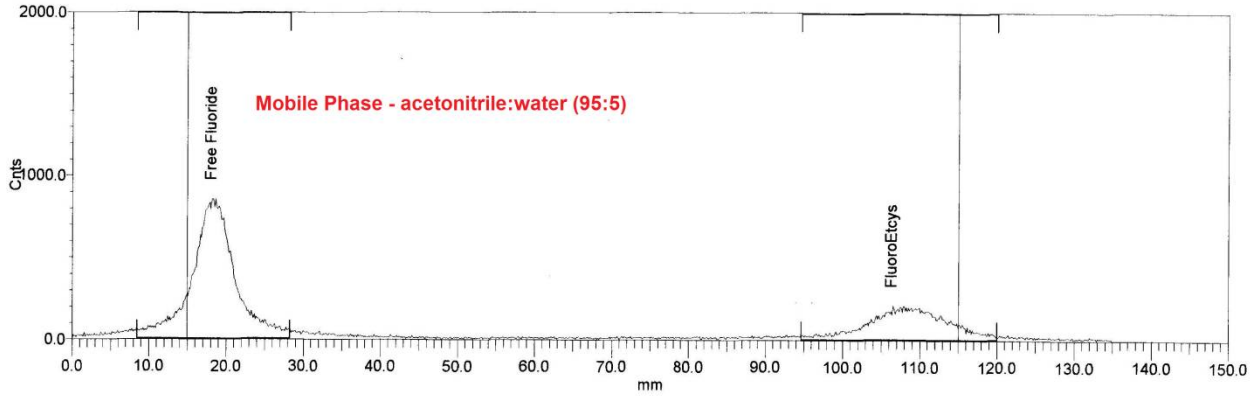


Figure 4-16 Radio-TLC analysis of fluoroethyl cycteine precursor complex showing 30% labelling.

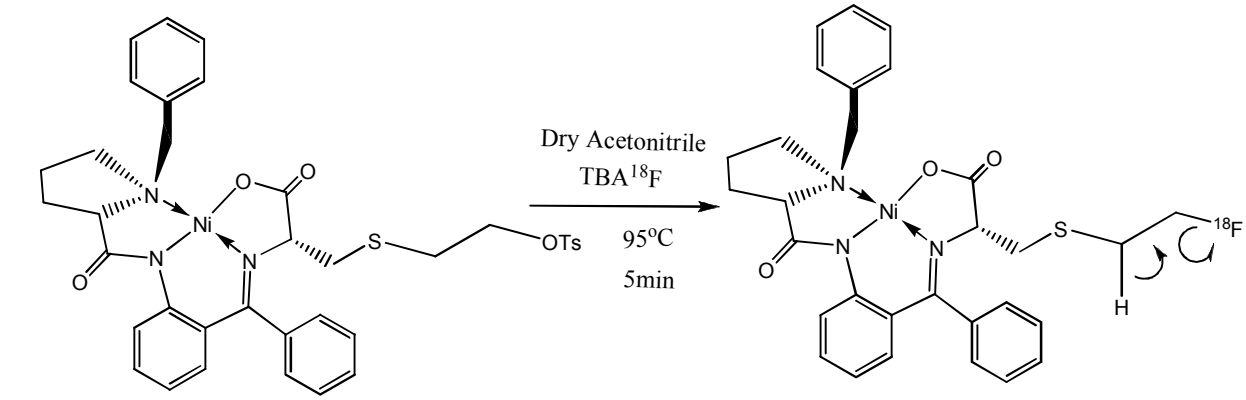


Figure 4-17 Elemination of HF with fluoroethylcysteine complex.

Hence the above serine elimination precursor complex (12) was coupled with the mercaptopropanol to form hydroxypropyl cysteine complex. The Ni(II)-(S)-BPB-hydroxypropyl cysteine complex was further tosylated and fluorinated. This precursor showed a poor fluorination efficiency of 7%. Further attempts for hydrolysis of the complex

using 3M HCl showed [^{18}F]Fluoropropylcycsteine ([^{18}F]FPC) with 2.7% of unhydrolyzed product (fig 4-18).

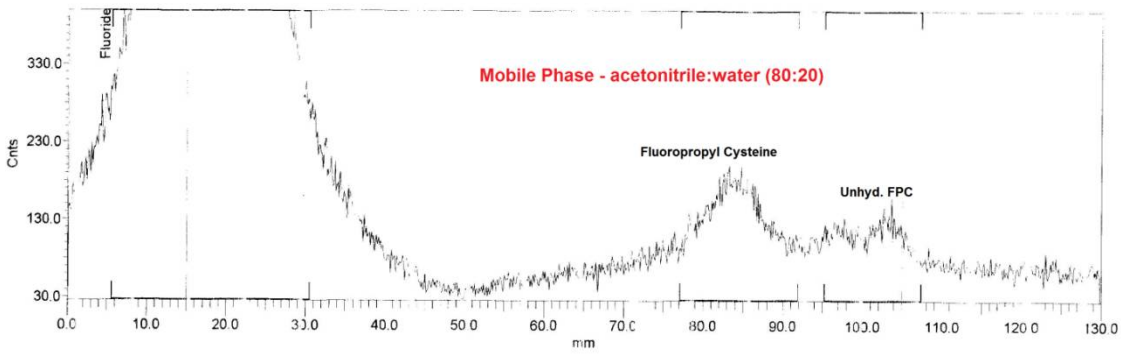


Figure 4-18 Radio-TLC showing [^{18}F]FPC with 4.5% in the reaction mixture.

The reaction mixture was purified with neutral alumina. Radio-TLC analysis showed single major spot of the [^{18}F]FPC with minor unhydrolyzed compound. The radio-chemically pure product was analyzed with radio-HPLC. The retention in the peak was matched with the cold compound prepared by cold fluorination of the precursor (fig 4-19).

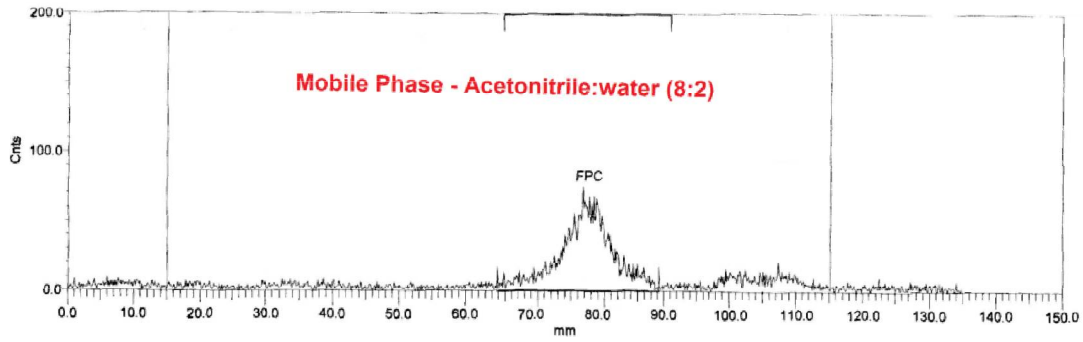


Figure 4-19 Radio-TLC of purified [^{18}F]FPC.

The [^{18}F]FPC precursor complex was analyzed by ESIMS (fig 4-36). The relative isotopic abundance peak of Ni and S are matched with the theoretical values and found to be in good agreement. (fig 4-20)

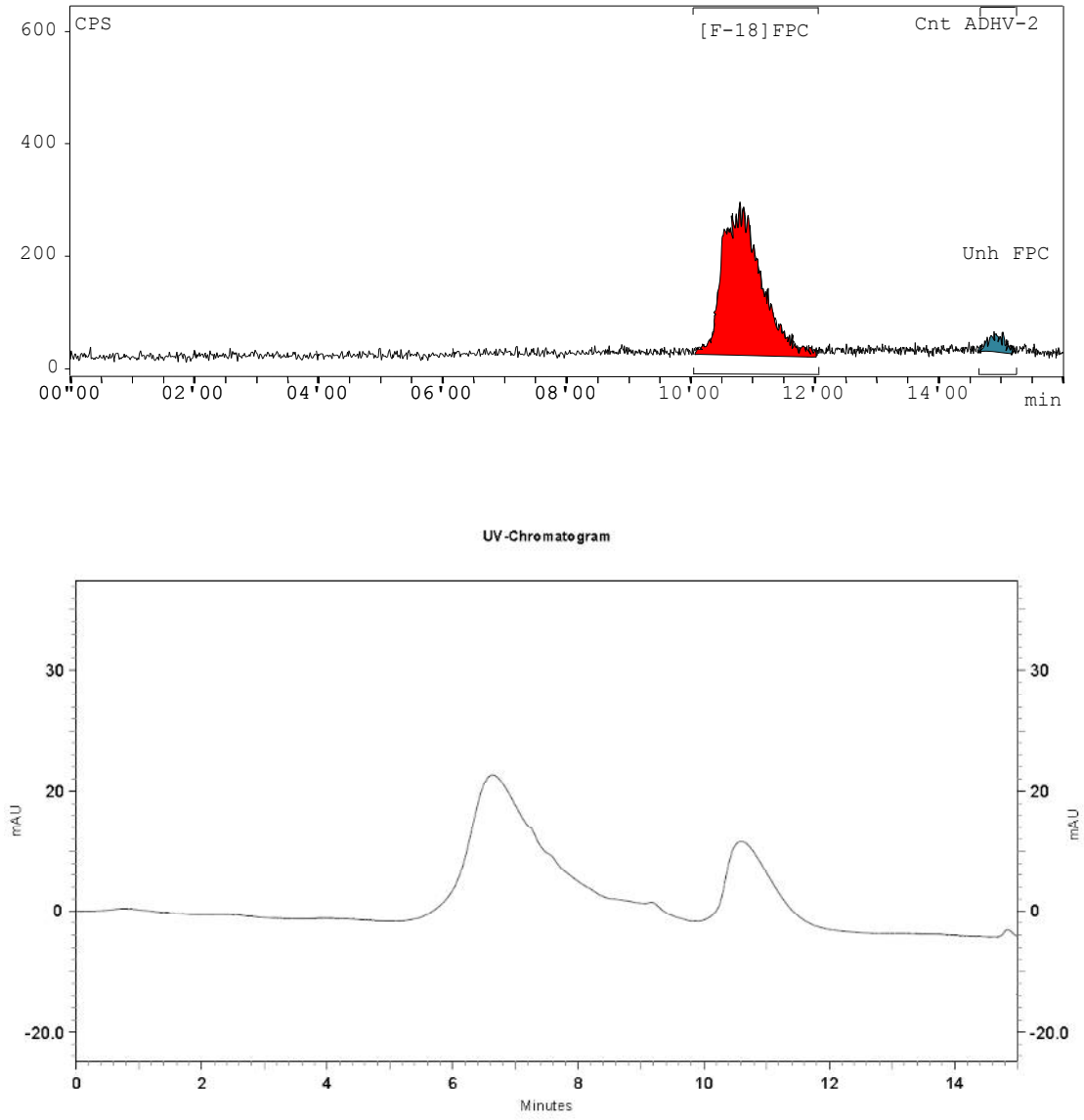


Figure 4-20 Radio-HPLC and UV-HPLC of [^{18}F]FPC co injected with cold [^{19}F]FPC.:Column Nucleosil C-18 column 250X4 mm, 5µm, UV-254nmm Flow Rate:1mL/min; mobile phase: 10% ethanol. (Top) Hot [^{18}F]FPT sample Radio-HPLC, showing peak of [^{18}F]FPCat Rt– 10.81 min. ***(Bottom)*** *[^{19}F]FPC at R_t - 10.59 min mixed synthesized by cold fluorination. Delay in the R_t of the peak between UV and Radio active chromatogram is due to sequential placement of detectors.*

4.4.3. Synthesis of O-(1'-[^{18}F]Fluoropropan-2'-yl)-L-tyrosine (1-[^{18}F]FPT) via Ni(II)-(S)-BPB-complex

In search of a new [^{18}F]labelled amino acid precursor using Ni(II)-(S)BPB-aminoacid complex strategy, 3-[^{18}F]fluoropropyl-(L)-tyrosine ([^{18}F]FPT) precursor was synthesized. Other than L-FET, L-Fluoropropyl tyrosine (L-FPT) was reported to be more promising radio tracer, showing more tumor specific than L-FET.[169] Several methods for the synthesis of 3-[^{18}F]fluoropropyl-(L)-tyrosine ([^{18}F]FPT) has been reported in the literature. [115, 170-172] Here in this study we have synthesized (1-[^{18}F]FPT) via Ni(II)-SBPB complex precursor, viz. Ni(II)-(S)-BPB-Tyr-(CH_2)-$CH(CH_3)$-OTs complex precursor. 1-[^{18}F]FPT precursor was synthesized starting from Ni(II)-(S)-BPB-(S)-Tyr complex as shown below (fig 4-12). The precursor was synthesized by preparing the ditosylate of more commonly available propylene glycol. .

Similar to the synthesis of [^{18}F]FET precursor. Ni(II)-(S)-BPB-(S)-tyr complex was coupled with propylene glycol ditosylate to form Ni(II)-(S)-BPB-(S)-tyr-(CH_2)-$CH(CH_3)$-OTs complex. On propyloxy-tosylation of the Ni(II)-(S)-BPB-(S)-Tyr, the coupling would provide two positional isomers, as expected. Moreover, the chiral center present in the propylene glycol, further provides multiple stereoisomers

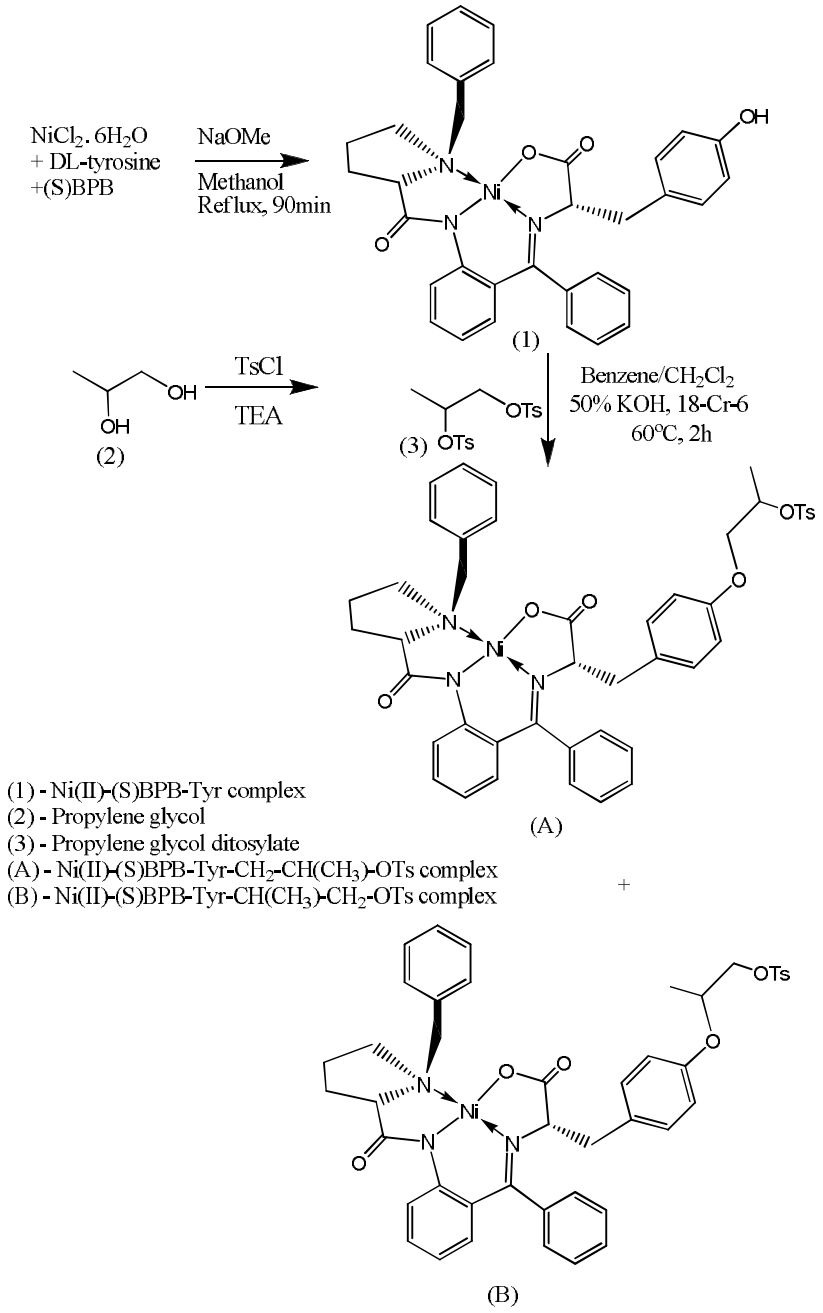


Figure 4-21 Synthesis scheme of Ni-(S)BPB-Tyr-CH(CH_3)-CH_2-OTs precursor.

The HPLC analysis of the purified precursor showed two discrete peaks, corresponding to R_t of 25.5 min and 28.9 min. The peak at R_t – 28.9 min showed a single peak, whereas the peak at R_t 25.5 min was found to have shoulder indicating the presence of multiple compounds. ^{1}H-NMR of these compounds after further separation showed, presence

of two isomers compound A and compound B (fig 1), which are precursors for compound (a) and Compound (b) in fig.4-22

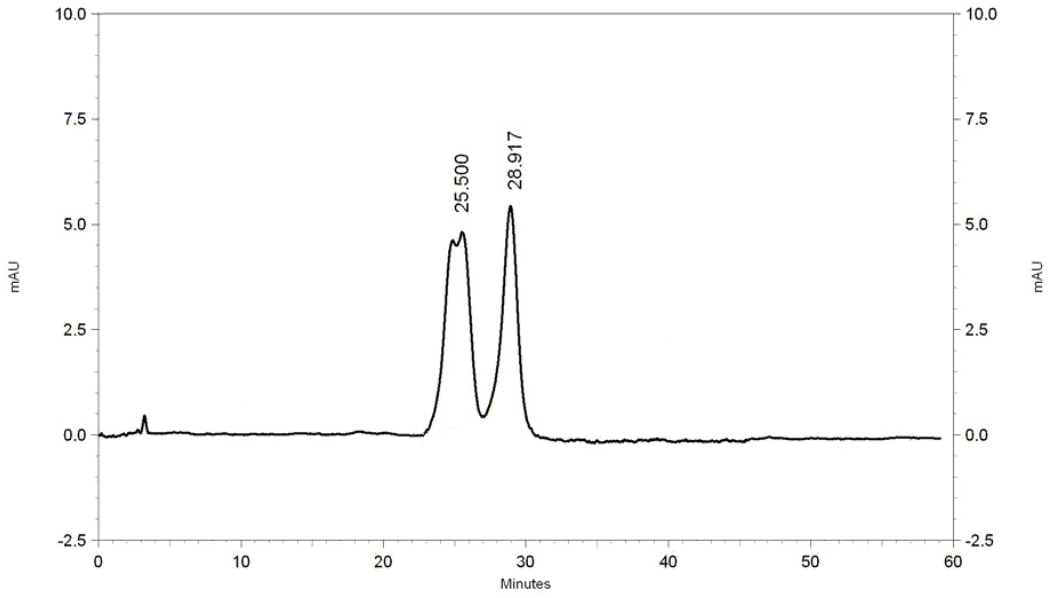


Figure 4-22 HPLC of Ni(II)-(S)BPB-Tyr-Propylene-OTs based precursor for [^{18}F]FPT: HPLCColumn Nucleosil C-18 column 300X4 mm, 5µm, UV-254nmm Flow Rate:1.0mL/min; Inj Vol:10µL Comp (A) = 28.917 min;Comp B Rt= 25.50 min. (Confirmed by ^{1}H-NMR)

Radiofluorination was performed as shown in reaction scheme below (Fig. 4-23) Radiosynthesis of [^{18}F]FPT using compound A provided final compound showed single peak by radio-TLC analysis which was found to be highly non-polar with a R_f of 0.9 (Mobile Phase: acetonitrile: Water (80:20)) corresponding to compound (a) in fig 4-24. The radio-HPLC analysis of this compound further showed multiple radiochemical peaks, in association with unhydrolized compounds making separation and purification much difficult.

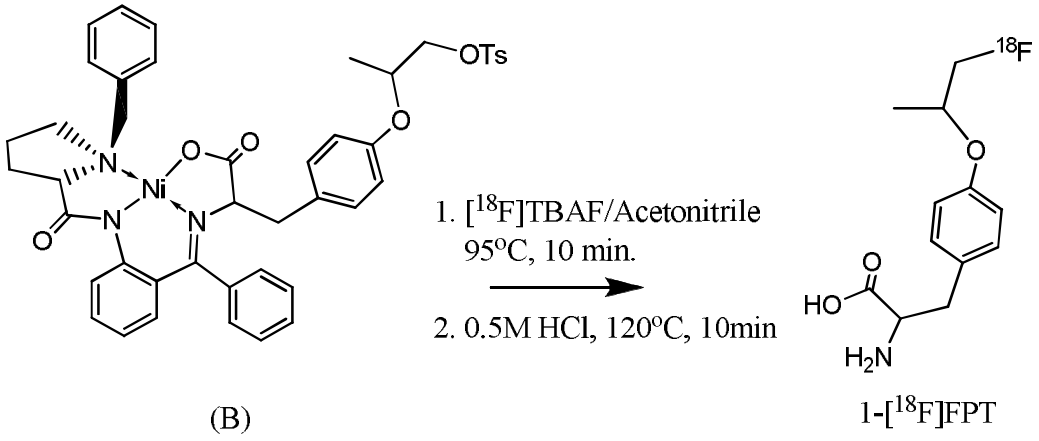


Figure 4-23 Showing Radio-synthesis scheme of 1-[^{18}F]FPT

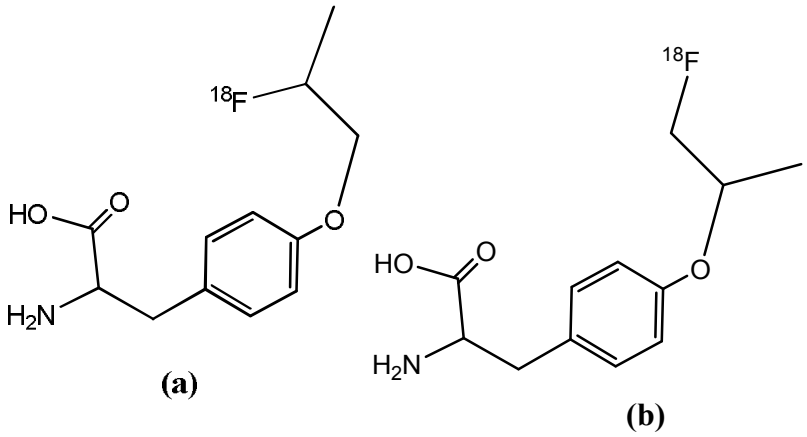


Figure 4-24 Radio-chemicals formed after radiosynthesis of [^{18}F]FPT.

Radiosynthesis of [^{18}F]FPT using compound B provided final compound showed high polarity with single peak by radio-TLC analysis(fig. 4-25), at R_f–0.5 (Mobile Phase: acetonitrile: Water (80:20)) corresponding to the compound (b) in fig 4-24. The radio-HPLC analysis of this compound further showed two peaks (R_t – 15.8 and R_t- 19.45 min) corresponding to the diastereomers of compound (b)(fig 4-24). Enantiomeric purity of compound (b) showed 4 radio-chemical species (fig 4-25) with R_t – 52'56, 1:04, 1:19 and

1:31 min respectively, corresponding to stereo isomeric compounds b1, b2, b3 and b4 (table.4-1)

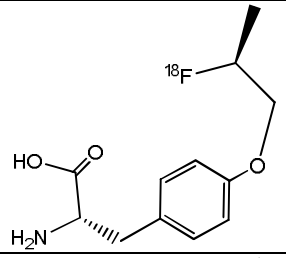
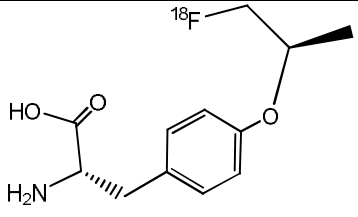
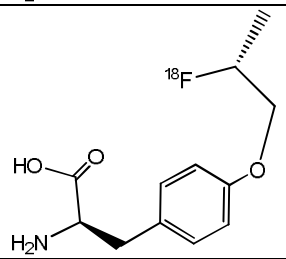
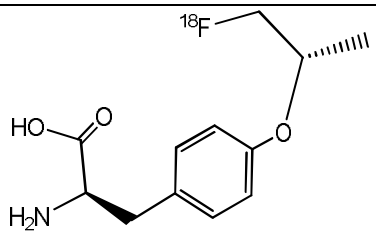
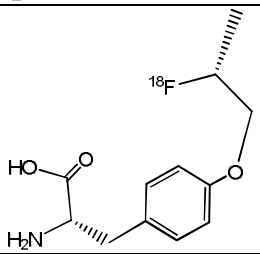
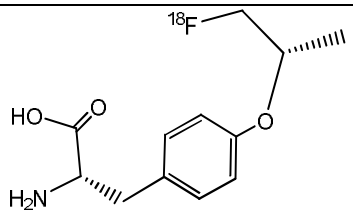
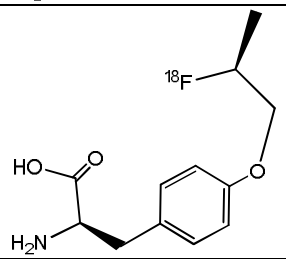
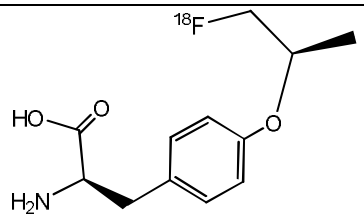
a	Structure of Stereoisomers	b	Structure of Stereoisomers
a1		**b1**	
a2		**b2**	
a3		**b3**	
a4		**b4**	

Table 4-1 Radio-chemicals formed after radiosynthesis of [^{18}F]FPT.

Out of all these stereo isomeric compounds, the L stereoisomer was found to be >93% in the mixture. Compound (b) which was found to be highly polar was found to be separated well in comparison to Compound (a). Hence compound (b) was purified efficiently using 12g of neutral alumina.

The final product after purification was analyzed with radio-TLC and radio-HPLC. Radio-TLC showed 98% purity of the 1-[^{18}F]FPT after purification.

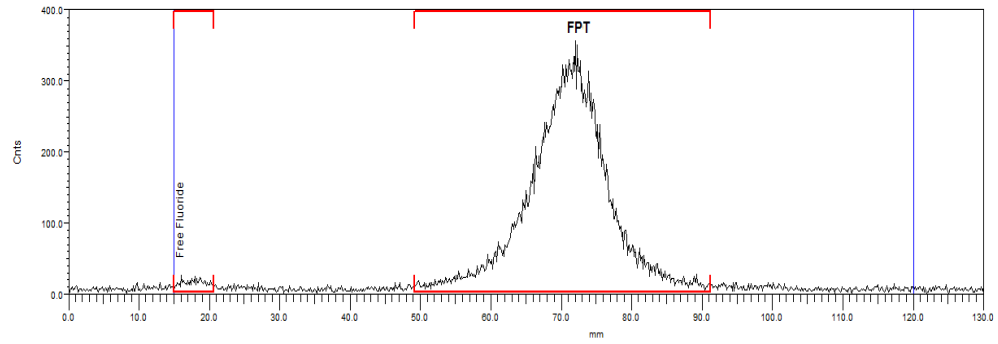


Figure 4-25Radio-TLC of the 1-[^{18}F]FPT, after purification, showing >98% radiochemical purity of the product. Mobile Phase: Acetonitrile:Water (8:2)

Radio-HPLC showed a mixture of stereoisomers of 1-[^{18}F]FPT viz. compounds b1, b3 and b2, b4 shown in table 4-1, which are diastereomers (fig 4-26). The final product showed no radiochemical impurities.

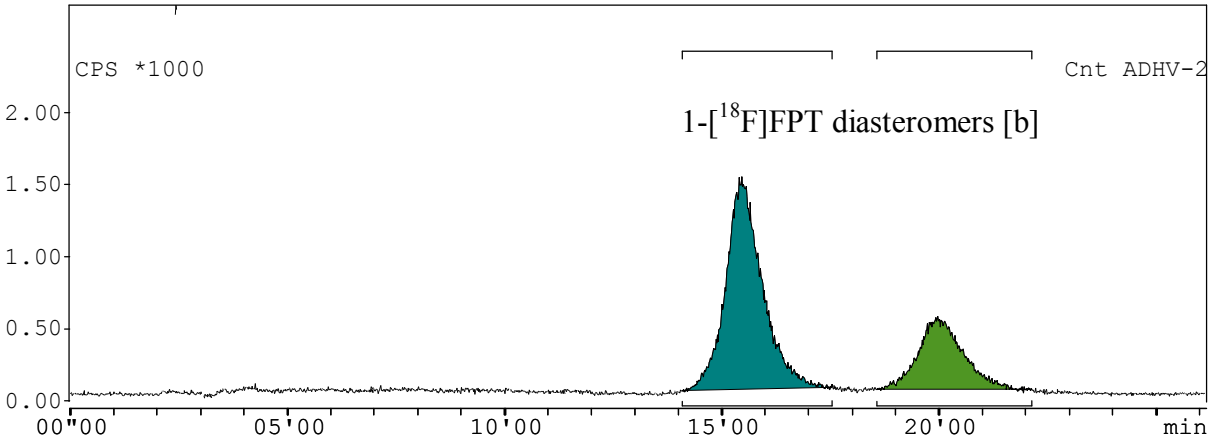


Figure 4-26 Radio-HPLC of [^{18}F]FPT: Radio-HPLCColumn Nucleosil C-18 column 300X4 mm, 5µm, UV-254nmm Flow Rate:1.0mL/min; Inj Vol:10µL.Radio-HPLC chromatogram with 100µCi/mL of activity, showing diasteriomers of [^{18}F]FPT (Comp B) peak at Rt= 15.18 min and 19.45 min

Enantiomeric purity of 1-[^{18}F]FPT was found to be 93% (Fig 4-27). Al^{3+} ion test in the final 1-[^{18}F]FPT injection showed <5µg/mL of Al^{3+} ion. Concentration of Ni(II) ion in the final 1-[^{18}F]FPT preparation, estimated by ICP-MS, was found to be 51.6±4.2 ppb (n=3). TBA^{+} ion test with samples showed concentration of <50µg/mL.

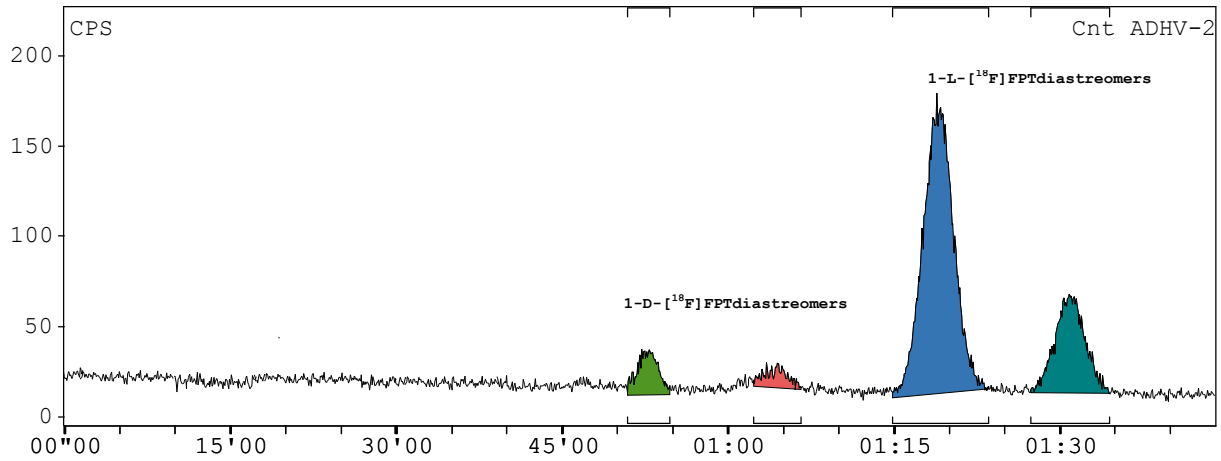


Figure 4-27 Chiral HPLC using Crownpak CR(+) column. Flow Rate:0.8mL/min; Solvent: MeOH: Water (10:90,V/V); pH = 3 in $HClO_4$; Column temperature = 10□C; Inj Vol: 10µL; radioactivity injected: 0.1µCi/mL. R_t(D-1-[^{18}F]FPT) Radio - 52'56min&1:04min;R_t (L-1-[^{18}F]FPT) – 1:19 min & 1:31 min.

4.4.3.1. Micro-PET imaging studies in tumor model mice

Micro-PET imaging studies were conducted with normal and B16F10 melanoma bearing C57BL/6 mice. Significant uptake of [^{18}F]FPT was observed in tumor (fig. 4-28).

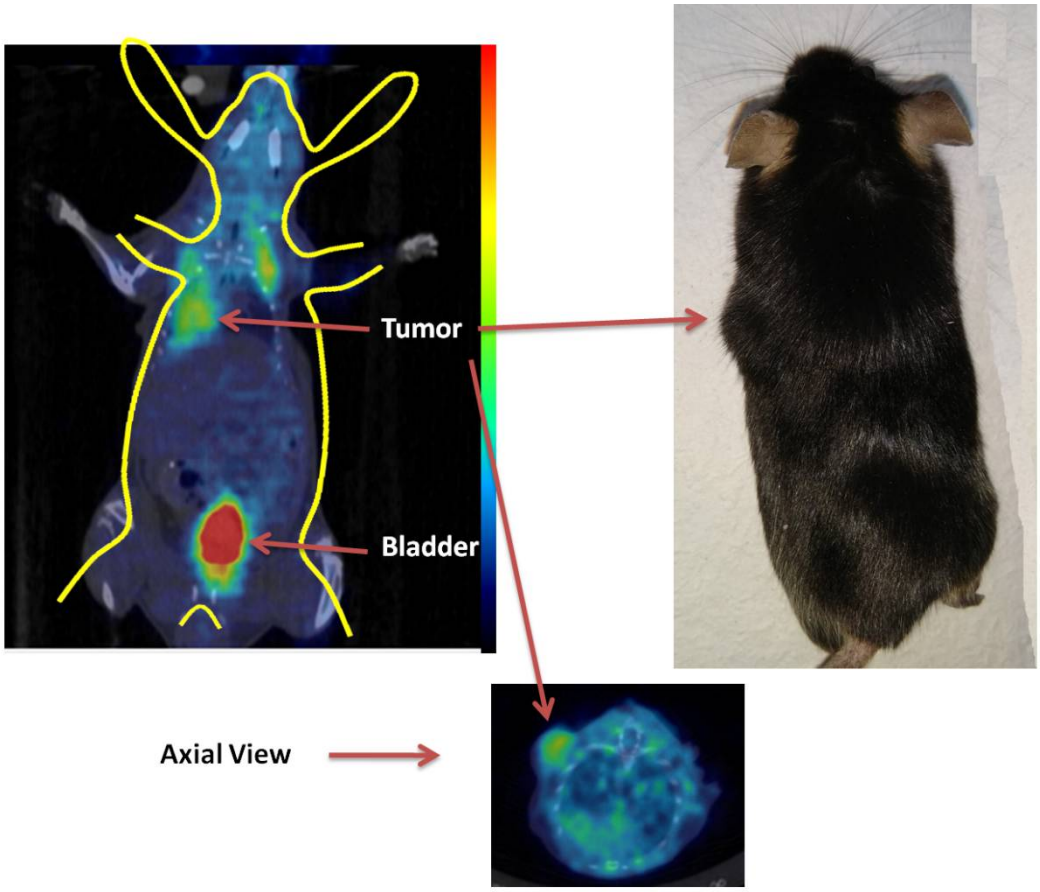


Figure 4-28 MicroPET/CT image of [^{18}F]FPT in C57BL/6 mice bearing B16F10 melanoma.

4.5. Experimental

4.5.1. Synthesis of [^{18}F]FET Precursor

4.5.1.1. Synthesis of Ni-(S)BPB-Ty (1)

The complex was prepared by the procedure reported elsewhere[122] with minor modifications. (DL)tyrosine (1.8 g), (S)-BPB · HCl (0.84 g), $Ni(NO_3)_2 \cdot 6H_2O$ (1.16 g), and 10 mL of MeOH were taken. The mixture was heated up to, then 6.5mL of 4 N KOH was quickly added under stirring at 45˚C. The mixture was refluxed with stirring for 70 min. Then the reaction mixture was neutralized with Acetic acid and diluted with 25mL of water and the precipitate was filtered off. The precipitate was re-dissolved in $CHCl_3$, and the residual tyrosine was removed by filtration. The solution was evaporated to give 1.12 g (92%) of the target product Ni-(S)-BPB-(S)-Ty. A part of the final product was purified by serial column flash chromatography using hexane:ethylacetate (70:30) for acquiring analytical data.

^{1}H NMR (fig 4-29):1.65 (m, 1H, Pro), 1.94 (m, 1H, Pro), 2.34 (m, 3H, Pro), 2.72 and 2.91 (AB part of ABX J_{AB} = 13.5 Hz, J_{AX} = 4.8 Hz, J_{BX} = 2.2 Hz, 2H,–CH_2-Ty), 3.07 (m, 1H, δ-Pro), 3.33 (m, 1H, α-Ty), 4.24 (m, 1H, α-Pro), 3.32 and 4.19 (AB, J_{AB} = 12.4Hz, 2H, –CH2-Bn), 6.65 (m, 2H, Ar), 6.84 (d, 1H, Ar), 6.99 and 7.02 (AB, J_{AB} = 8.4 Hz, 4H, Ty), 7.13 (m, 2H, Ar), 7.26 (m, 3H, Ar), 7.42 (m, 1H, Ar), 7.52 (m,2H, Ar), 8.04 (d, 2H, Ar), 8.12 (d, 1H, Ar)

^{13}C NMR (fig 4-30): 23.08 γ-C [Pro], 30.91 β-C [Pro], 38.51 β-C[Ty], 57.79 δ-C [Pro], 63.77 CH_2 [Bn], 70.75 α-C[Ty], 71.76 α-C [Pro], 115.97 m-Ar [Ty], 121.09 3-Ar[2-ABPh], 123.59 5-Ar [2-ABPh], 126.17 *i*-Ar [Ty],126.45 1-Ar [2-ABPh], 127.19 4-Ph [Bn], 127.83 6-Ar[2-ABPh], 128.89 3,3'-Ph [Bn], 128.93 4-Ph [2-ABPh],129.19 2-Ar [Ty], 129.93 4-Ar [2-

ABPh], 131.49 3.3'-Ph [2-ABPh], 131.62 2,2'-Ph [Bn], 132.39 2-Ph [2-ABPh],133.51 *i*-Ph [Bn], 133.59 2'-Ph [2-ABPh], 134.14 *i*-Ph [2-ABPh], 142.24 2-Ar [2-ABPh], 157.08 4-Ar [Ty], 171.22 –CONH–, 179.34 >C=N–, 180.75 –COO–.

4.5.1.2. Synthesis of Ni-(S)-BPB-Ty-CH_2-CH_2-OTs (2)

Ni-(S)-BPB-Ty (1) (500mg), Ethylene glycol ditosylate (1.0g), 18-crown-6 (catalytic quantity) was dissolved in 1.5mL of dichloromethane. To this 5mL of benzene, 1.5mL of 50% KOH was added and stirred vigorously at 45˚C for 2h. The organic layer was separated, extracted with water, and neutralized with Acetic acid. The final organic mixture was concentrated and purified by serial column flash chromatography using Hexane:Ethylacetate (8:2) mixture. The final product was obtained by crystallizing in Hexane (411mg, 82.2%).

^{1}H NMR (fig 4-31):1.67 (m, 1H, Pro), 1.95 (m, 1H, Pro), 2.30 (m, 3H, Pro), 2.45 (s, 3H, $CH_3C_6H_4SO_3$–), 2.75 and 2.97 (AB part of ABX, J_{AX} = 5.5 Hz, J_{BX} = 4.45 Hz, 2H, –CH_2-Ty), 3.07 (m, 1H, δ-Pro), 3.31 (m, 1H, α-Ty), 4.20 (m,1H, α-Pro), 3.43 and 4.24 (AB, J_{AB} = 12.46 Hz, 2H, –CH_2-Bn), 4.03 (m, 2H, OCH_2CH_2OTs), 4.35 (m, 2H,OCH_2CH_2OTs), 6.65 (d, 2H, Ar), 6.85 (d, 1H, Ar), 6.84 and 7.05 (AB, J_{AB} = 8.8 Hz, 4H, Ty), 7.12 (m, 2H, Ar), 7.26 (m, 3H, Ar), 7.43 (m, 1H, Ar), 7.35 and 7.82 (AB, J_{AB} = 8.06 Hz, 4H, Ts), 7.52 (m, 2H, Ar), 8.02 (d, 2H,Ar), 8.19 (d, 1H, Ar).

^{13}C NMR (fig 4-32): 21.75 CH_3-[Ts], 23.13 γ-C [Pro], 30.73 β-C[Pro], 38.92 β-C [Ty], 57.33 δ-C [Pro], 63.43 CH_2[Bn], 65.59 –OCH_2CH_2OTs, 68.15 α-C [Ty], 70.36 α-C[Pro], 71.62 –OCH_2CH_2O-, 114.79 m-Ar [Ty], 120.68-Ar [2-ABPh], 123.44 5-Ar [2-ABPh], 126.20 1-Ar [2-ABPh], 127.25 4-Ph [Bn], 127.87 6-Ar [2-ABPh],128.10 2,2'-Ar [Ts], 128.64 *i*-Ar [Ty], 128.85 3,3'-Ph[Bn], 128.96 2-Ar [Ty], 129.17 4-Ph [2-ABPh], 129.86 4-Ar [2-ABPh], 129.98 3'-Ar [Ts], 131.58 3,3'-Ph [2-ABPh], 131.67 2,2'-Ph[Bn], 132.43 2-Ph [2-ABPh],

133.29 *i*-Ph [Bn], 133.58 2'-Ph [2-ABPh], 134.23 *i*-Ph [2-ABPh], 142.86 2-Ar [2-ABPh], 145.10 *i*-Ar [Ts],157.72 4-Ar [Ty], 171.14 –CONH–, 178.70 >C=N–, 180.42 –COO–.

4.5.2. Synthesis of Ni(II)-(S)-BPB-glycine and Ni(II)-(S)-BPB-alanine complex

Ni(II)-(S)-BPB-glycine complex was synthesized by the procedure reported elsewhere[124].

A mixture of (S)-BPB (1g, 2.6 mmol), $Ni(NO_3)_2.6H_2O$ (1.5 g, 5 mmol) and amino acid (either glycine (1g, 13 mmol) or alanine (1.1 g, 13 mmol)) were taken in dry methanol (10mL) in a three necked round bottomed flask (250 mL). The solution was heated at 40-45ºC and stirred till a homogenous solution was obtained. To this KOH (1 g, 17.8 mmol) in dry methanol (4mL) was added in a single portion. The solution was refluxed for 1h in case of glycine and 2h in case of alanine. The reddish brown solution of complex in then added to crushed ice (20 g) in a beaker (250 mL), glacial acetic acid (0.3mL) was added to neutralize the medium. The reddish orange precipitate of complex was re-dissolved in dichloromethane, extracted with water, dried with anhydrous Na_2SO_4 and concentrated in rotary evaporator. The concentrated solution (1mL) was triturated with hexane to obtain the complex. This complex was further purified using flash column chromatography in order to remove excess (S)-BPB. Complex yield Glycine - 1.1 g (83%), Alanine yield (1.1 g, 82%).

Glycine Complex:^{1}H NMR (fig 4-34):2.04 (m, 2H, Pro), 2.38 (m, 1H, Pro), 2.42 (m, 1H, Pro), 2.34 and 2.37 (2H, α–Gly), 3.8 (m, 1H, δ-Pro), 4.24 (m, 1H, α-Pro), 3.65 and 4.47 (AB, J_{AB} = 12.8 Hz, 2H, $–CH_2$-Bn), 6.67 (m, 2H, Ar), 6.78 (d, 1H, Ar), 7.00 (m, 2H, Ar), 7.31 (m, 3H, Ar), 7.46 (m, 1H, Ar), 7.48 (m,2H, Ar), 8.06 (d, 2H, Ar), 8.27 (d, 1H, Ar).

^{13}C NMR (fig 4-35): 23.77 γ-C [Pro], 30.79 β-C [Pro], 57.54 δ-C [Pro], 61.37 α-C [Gly], 63.18 CH_2 [Bn], 69.93 α-C [Pro], 120.92 3-Ar[2-ABPh], 124.33 5-Ar [2-ABPh], 125.73 1-Ar [2-ABPh], 126.32 4-Ph [Bn], 128.99 6-Ar[2-ABPh], 129.19 3,3'-Ph [Bn], 129.41 4-Ph [2-ABPh], 129.67 4-Ar [2-ABPh], 129.81 3.3'-Ph [2-ABPh], 131.80 2,2'-Ph [Bn], 132.29 2-Ph [2-ABPh],133.25 *i*-Ph [Bn], 133.36 2'-Ph [2-ABPh], 134.68*i*-Ph [2-ABPh], 142.59 2-Ar [2-ABPh], 171.70 –CONH–, 177.37>C=N–, 181.44 –COO–.

4.5.3. Synthesis of Ni(II)-(S)-BPB-serine complex from glycine complex

In a round bottomed flask (10 mL), glycine complex (250 mg, 0.5 mmol) was dissolved in dry methanol (3 mL) and stirred well. The solution was cooled to 15ºC. To this paraformaldehyde (30 mg, 1mmol) and sodium methoxide (38 mg, 0.7 mmol) were added in one portion and the reaction mixture was allowed to stir for 1min. The progress of the reaction was confirmed by TLC (mobile phase: 100% ethyl acetate). Once the reaction was complete, the mixture was then poured in crushed ice (1g) in a beaker (50 mL). The mixture was then neutralized with glacial acetic acid (10 µL). The solution was extracted with dichloromethane. The organic layer was washed with deionized water, dried with anhydrous Na_2SO_4 and concentrated in rotary evaporator. The crude product was then further purified by flash chromatography. The concentrated solution (<0.3mL) was triturated with hexane and dried to obtain reddish orange powder (Yield – 220 mg, 83%). m.p. 159–161ºC.

^{1}H NMR (fig 4-36): 1.98 (m, 1H, Pro), 2.06 (m, 1H, Pro), 2.46 (m, 1H, Pro), 3.77 (m, 3H, Ser), 2.74 (m, 1H, δ-Pro), 4.00 (m, 1H, α-Pro), 3.54 and 4.34 (AB, J_{AB} = 12.4Hz, 2H, –CH2-Bn), 6.64 (m, 2H, Ar), 7.98 (d, 1H, Ar), 7.13 (m, 2H, Ar), 7.21 (m, 3H, Ar), 7.34 (m, 1H, Ar), 7.45 (m,2H, Ar), 8.06 (d, 2H, Ar), 8.14 (d, 1H, Ar).

4.5.3.1. Synthesis of Ni(II)-(S)-BPB-Serine –OTs Precursor

In a round bottomed flask with magnetic stirrer, Ni(II)-(S)BPB-(S)-serine complex (2) (0.100mg, 0.189 mmol) and triethyl amine (50 µL, 0.48 mmole)were added and dissolved in 2 mL of dry CH_2Cl_2. The solution was cooled to 0ºC, and to this p-toluenesulphonyl chloride(0.09 g, 4.72 X 10^{-4} mol) was added in three portions within 3 hrs. The solution was allowed to stir at room temperature for 20h. The reaction mixture was analyzed by SiO_2 TLC (mobile phase: 100% ethyl acetate). Once the reaction was complete, the reaction mixture was washed with cold water. The aqueous layer was neutralized with 0.5M HCl (10 µL) while extraction in order to remove triethylamine from the organic layer. After neutralization, the organic layer was collected, washed with cold deionized water, dried with anhydrous Na_2SO_4, and concentrated. The final concentrate was then purified by serial column flash chromatography using hexane:ethyl acetate mobile phase. Yield 61.9% (80 mg).

^{1}H NMR (fig 4-37):2.01 (m, 2H, Pro), 2.20 (m, 1H, Pro), 2.27 (s, 3H, $CH_3C_6H_4SO_3$–),2.50 (m, 1H, Pro), 3.74 and 4.12 (AB part of ABX, J_{AX} = 3.3 Hz, J_{BX} = 2.2 Hz, 2H, –CH_2- OTs), 2.86 (m, 1H, δ-Pro), 4.00 (m,1H, α-Pro), 3.56 and 4.34 (AB, J_{AB} = 12.8 Hz, 2H, –CH_2-Bn),6.28 (d, 2H, Ar), 6.57 (d, 1H, Ar), 7.12 (m, 2H, Ar), 7.20 (m, 3H, Ar), 7.23 (m, 1H, Ar), 7.26 (m, 2H, Ar), 7.33 and 7.77 (AB, J_{AB} = 8.06 Hz, 4H, Ts), 8.04 (d, 2H,Ar), 8.29 (d, 1H, Ar).

4.5.3.2. Synthesis of Ni(II)-(S)-BPB-hydroxy ethyl cysteine complex

In a round bottomed flash (10 mL), serine elimination complex complex (12) (100 mg, 0.196 mmol) was dissolved in dry methanol (3 mL) and stirred well at room temperature. To this sodium methoxide (38 mg, 0.7 mmol) was added. To this stirring mixture, mercaptoethanol (0.5 mL, 7mmol) was added and the reaction mixture was allowed

to stir for 5 min. The mixture was then poured in crushed ice (1g) in a beaker (50 mL). The mixture was then neutralized with glacial acetic acid (10 µL). The solution was extracted with dichloromethane. The organic layer was washed with deionized water, dried with anhydrous Na_2SO_4 and concentrated in rotary evaporator. The crude product was purified by flash column chromatography. The concentrated solution (<0.3mL) was triturated with hexane and dried to obtain reddish orange powder (Yield – 95 mg, 82.4%).

4.5.3.3. Synthesis of Ni(II)-(S)-BPB-hydroxyethyl cysteine tosylate complex

In a round bottomed flask with magnetic stirrer, Ni(II)-(S)BPB-(S)-hydroxyethyl cysteine complex (100 mg, 0.169mmol) and triethyl amine (50 µL, 0.48 mmole)were added and dissolved in 2 mL of dry CH_2Cl_2. The solution was cooled to 0ºC, and to this p-toluenesulphonyl chloride(90mg, 0.4mmol) was added in three portions within 3 hrs. The solution was allowed to stay for 36h at 5ºC. The reaction mixture was analyzed by SiO_2 TLC (mobile phase: 100% ethyl acetate). Once the reaction was complete, the reaction mixture was washed with cold water. The aqueous layer was neutralized with 0.5M HCl (10 µL) while extraction in order to remove triethylamine from the organic layer. After neutralization, the organic layer was collected, washed with cold deionized water, dried with anhydrous Na_2SO_4, and concentrated. The final concentrate was then purified by serial column flash chromatography using hexane:ethyl acetate mobile phase. Yield – 83 mg (65.8%).

4.5.4. Synthesis of Ni(II)-(S)-BPB-hydroxypropylcysteine complex

In a round bottomed flash (10 mL), glycine elemination complex (100 mg, 0.196 mmol) was dissolved in dry methanol (3 mL) and stirred well at room temperature. To this sodium methoxide (38 mg, 0.7 mmol) was added and stirring continued. To this

mercaptopropanol (20 µL, 0.217 mmol) was added and the reaction mixture was allowed to stir for 1min. The mixture was then poured in crushed ice (1g) in a beaker (50 mL). The mixture was then neutralized with glacial acetic acid (10 µL). The solution was extracted with dichloromethane. The organic layer was washed with deionized water, dried with anhydrous Na_2SO_4 and concentrated in rotary evaporator. The crude mixture was purified by flash column chromatography. The concentrated solution semi solid mixture was triturated with hexane and dried to obtain reddish orange powder (Yield – 92 mg, 78%).

4.5.4.1. Synthesis of Ni(II)-(S)-BPB-hydroxypropylcysteine-OTs complex

In a round bottomed flask with magnetic stirrer, Ni(II)-(S)BPB-(S)-hydroxypropyl cysteine complex(100mg, 0.166mmol) and triethyl amine (50 µL, 0.48 mmole)were added and dissolved in 2 mL of dry CH_2Cl_2. The solution was cooled to 0ºC, and to this p-toluenesulphonyl chloride(90mg, 0.47mmol) was added in three portions within 3 hrs. The solution was allowed to stay for 36h at 5ºC. The reaction mixture was analyzed by SiO_2 TLC (mobile phase: 100% ethyl acetate). Once the reaction was complete, the reaction mixture was washed with cold water. The aqueous layer was neutralized with 0.5M HCl (10 µL) while extraction in order to remove triethylamine from the organic layer. After neutralization, the organic layer was collected, washed with cold deionized water, dried with anhydrous Na_2SO_4, and concentrated. The final concentrate was then purified by serial column flash chromatography using hexane:ethyl acetate mobile phase. Yield 65% (82 mg).

4.5.4.2. Synthesis of propylene glycol ditosylate

In a round bottomed flask (100 mL) with a magnetic stirrer propylene glycol (2 mL, 26.2 mmole) and triethylamine (6.8 mL, 65.5 mmole) was added and stirred. To this p-toluenesulphonyl chloride (12.5 g, 65.4 mmole) was added in three portions within 15 min.

The solution was allowed to stir for half an hour. The white mass was allowed to dissolve in dichloromethane (5mL) while stirring. The solution was further stirred for 1 hr. The mixture was then poured to crushed ice (3 g) in a beaker 250mL. The solution was extracted and the mixture was neutralized by 0.5 M HCl to remove triethyl amine. The organic layer was washed with deionized water, dried with anhydrous Na_2SO_4,concentrated with rotary evaporator. The mixture was further purified by flash column chromatography using hexane : ethyl acetate mobile phase. The purified compound was concentrated and kept under vacuum desiccator overnight. The white solid obtained (7.8g, yield – 77%) was further used for reactions. The structure of compound was confirmed from ESIMS analysis shown in **fig 4-36.**

4.5.4.3. Synthesis of Ni(II)-(S)BPB-Tyr--$CH(CH_3)$-CH_2-OTs (2)

The precursor was synthesized by a two step process as shown in fig 1. First Ni(II)-(S)BPB-Tyr complex (1) was prepared by the reported procedure [24].Complex (1) (500mg, 8.27X10-4 moles), and 18-crown-6 (catalytic quantity) was dissolved in 1mL of dichloromethane. To this 4mL of benzene, 1.5mL of 50% KOH was added. 1,2-ditosyloxypropane (1.27g, 6.35X10-4 moles) was added to this mixture and refluxed at 70˚C for 1h. The mixture is further added with a portion of 1,2-ditosyloxypropane (1.27g, 6.35X10-4 moles) and the reflux continued for further 1h. The organic layer was separated, extracted in water, and neutralized with acetic acid, washed with deionized water and dried with anhydrous sodium sulphate. The final organic mixture was concentrated and purified by flash column chromatography using hexane:ethylacetate mixture. The final product was obtained by crystallizing in Hexane (544 mg, 82.2%).

The purity of the final product was evaluated by HPLC. HPLC showed multiple compounds with two discrete peaks that are well resolved. This was expected due to the various

possibilities arising while coupling with propylene glycol ditosylate (fig 2). These compounds were separated and characterized by 1H-NMR.

^{1}H NMR of Compound (A) (DMSO-D_6) (fig 4-39): 1.04 (d, 3H, CH-CH_3), 1.67 (m, 1H, Pro), 2.01 (m, 1H, Pro), 2.38 (m, 3H, Pro), 2.39 (s, 3H, $CH_3C_6H_4SO_3$–), 2.64 and 2.70 (AB part of ABX, J_{AB} = 13.7 Hz, J_{AX} = 5.66 Hz, J_{BX} = 4.2 Hz, 2H, –CH_2- Tyr), 2.86 (m, 1H, δ-Pro), 3.39 (m, 1H, α-Tyr), 3.90 (m,1H, α-Pro), 3.37 and 3.88 (AB, J_{AB} = 12.2 Hz, 2H, –CH_2-Bn), 3.55 and 4.08 (AB, J_{AB} = 12.7 Hz, 2H, -CH_2-$CH(CH_3)$-), 5.24 (m, 1H, -$CH(CH_3)$-OTs), 6.54 (d, 1H, Ar), 6.64 (d, 1H, Ar), 6.82and 6.94 (AB, J_{AB} = 9 Hz, 4H, Tyr), 7.07 (m, 2H, Ar), 7.21(d, 1H, Ar), 7.30 (m, 2H, Ar), 7.52 (m, 1H, Ar), 7.61 (m, 2H, Ar), 7.45 and 7.75 (AB, J_{AB} = 8.1 Hz, 4H, Ts), 8.01 (d, 1H,Ar), 8.28 (d, 2H, Ar).

^{1}H NMR of Compound (B) (DMSO-D_6) (fig 4-40): 1.02 (d, 3H, CH-CH_3), 1.67 (m, 1H, Pro), 2.01 (m, 1H, Pro), 2.36 (m, 3H, Pro), 2.48 (s, 3H, $CH_3C_6H_4SO_3$–), 2.66 and 2.70 (AB part of ABX, J_{AB} = 13.7 Hz, J_{AX} = 5.66 Hz, J_{BX} = 4.2 Hz, 2H, –CH_2- Tyr), 2.85 (m, 1H, δ-Pro), 3.35 (m, 1H, α-Tyr), 3.91 (m,1H, α-Pro), 3.37 and 3.41 (AB, J_{AB} = 12.2 Hz, 2H, –CH_2-Bn), 4.49 and 5.02 (dd, 2H, –CH_2-OTs), 6.54 (d, 1H, Ar), 6.64 (d, 1H, Ar), 6.82 and 6.94 (AB, J_{AB} = 9 Hz, 4H, Tyr), 7.06 (m, 2H, Ar), 7.21 (d, 1H, Ar), 7.30 (m, 2H, Ar), 7.52 (m, 1H, Ar), 7.61 (m, 2H, Ar), 7.45 and 7.75 (AB, J_{AB} = 8.1 Hz, 4H, Ts), 8.01 (d, 1H,Ar), 8.28 (d, 2H, Ar).

4.5.4.4. Synthesis of [^{19}F]fluoroaminoacids as reference compounds

The cold fluorinated amino acid compounds were prepared by taking [^{18}F]tetrabutyl ammonium fluoride([^{18}F]TBAF) mixed [^{19}F]TBAF for the reaction. For this the irradiated water containing [^{18}F]fluoride was trapped in anion exchanger (45 mg, PS-HCO_3 cartridge). It was then eluted with 0.5 mL of [^{18}F]TBAF. To this 1mL of [^{19}F]TBAF (10 mg, 37.5 µmole) dissolved in acetonitrile was added. The solution was azeotropically distilled. To

this dried mixture, precursor (10 mg) in 1 mL dry acetonitrile was added and heated at 95ºC for 5 - 10 min. The resultant solution was hydrolyzed with 1M HCl at 120ºC for 10 min. The reaction mixture was loaded onto alumina column. Purified to separate the radiochemical species and analyzed with SiO_2 radio-TLC (mobile phase: acetonitrile : water (80:20)). The compounds were then analyzed with radio-HPLC.

4.5.5. Advantages of Ni(II)-(S)-BPB-(S)-Ty-CH_2-CH_2-OTs precursor over other precursors for [^{18}F]FET radiosynthesis

From the studies conducted with the synthesis and radio-labelling of Ni(II)-(S)-BPB-(S)-Ty-CH_2-CH_2-OTs precursor for [^{18}F]FET, the following advantages were realized as outlined below.

- The Ni(II)-(S)-BPB-(S)-Ty-CH_2-CH_2-OTs precursor is a one-pot two-step synthesis precursor for [^{18}F]FET, that can be synthesized with 2-[^{18}F]FDG module and common chemicals of its kits.

- The method protects the acid and the amino group of tyrosine, by forming a single Schiff's base complex. Thus, it provides a single step protection and deprotection method.

- The Ni(II)-(S)-BPB-(S)-Ty complex and its other compounds including precursor are coloured compounds. Hence can be easily visualized during synthesis and purification.

- By the improved synthesis method developed in this chapter, the precursor can be synthesized by a two step process, with a total synthesis time of 12h, including processing and purification.

- With the advantage of the coloured complexes involved, the newly developed SCFC technique can be applied, which reduces the production time and cost of the precursor.

- This precursor also provides a better enantiomeric purity of 95%. Since the remaining 5% is D-[^{18}F]FET, that doesn't have uptake in any specific organ, and will be directly cleared by bladder, it is highly acceptable considering the above advantages of this precursor.

4.6. Conclusions

Several Ni(II)-(S)-BPB-amino acid complexes were synthesized starting from glycine complex. We have successfully synthesized Ni(II)-(S)-BPB-Ty-CH_2-CH_2-OTs precursor of ^{18}F-FET. The procedure used is an improved procedure which involves lesser reaction sequence and lower reaction time. The synthesis time is further reduced by introduction of a novel "Serial Column Flash Chromatography" technique. Synthesis of [^{18}F]fluoroalanine was attempted through Ni(II)-(S)-BPB-serine-OTs complex precursor. [^{18}F]fluoroalanine and [^{18}F]fluoroethyl cysteine resulted in elimination of [^{18}F]fluoride. However, [^{18}F]fluoropropyl cysteine and [^{18}F]fluoropropyl tyrosine were successful. These compounds were identified by their cold compounds through HPLC. The precursor complexes were characterized by ^{1}H-NMR. The compounds after radiosynthesis were purified using neutral alumina column.

The above study shows that Ni-(S)-BPB-amino acid based synthesis of [^{18}F]labelled amino acids have high scope for further exploration of other [^{18}F]-amino acids. Further potential of [^{18}F]fluoropropyl cysteine and [^{18}F]fluoropropyl tyrosine as a PET radiotracer and further possibilities to form other analogues of tyrosine and cysteine can be explore in future.

Figure 4-29 *^{1}H-NMR of Ni(II)-(S)BPB-(S)-Ty complex(1)*

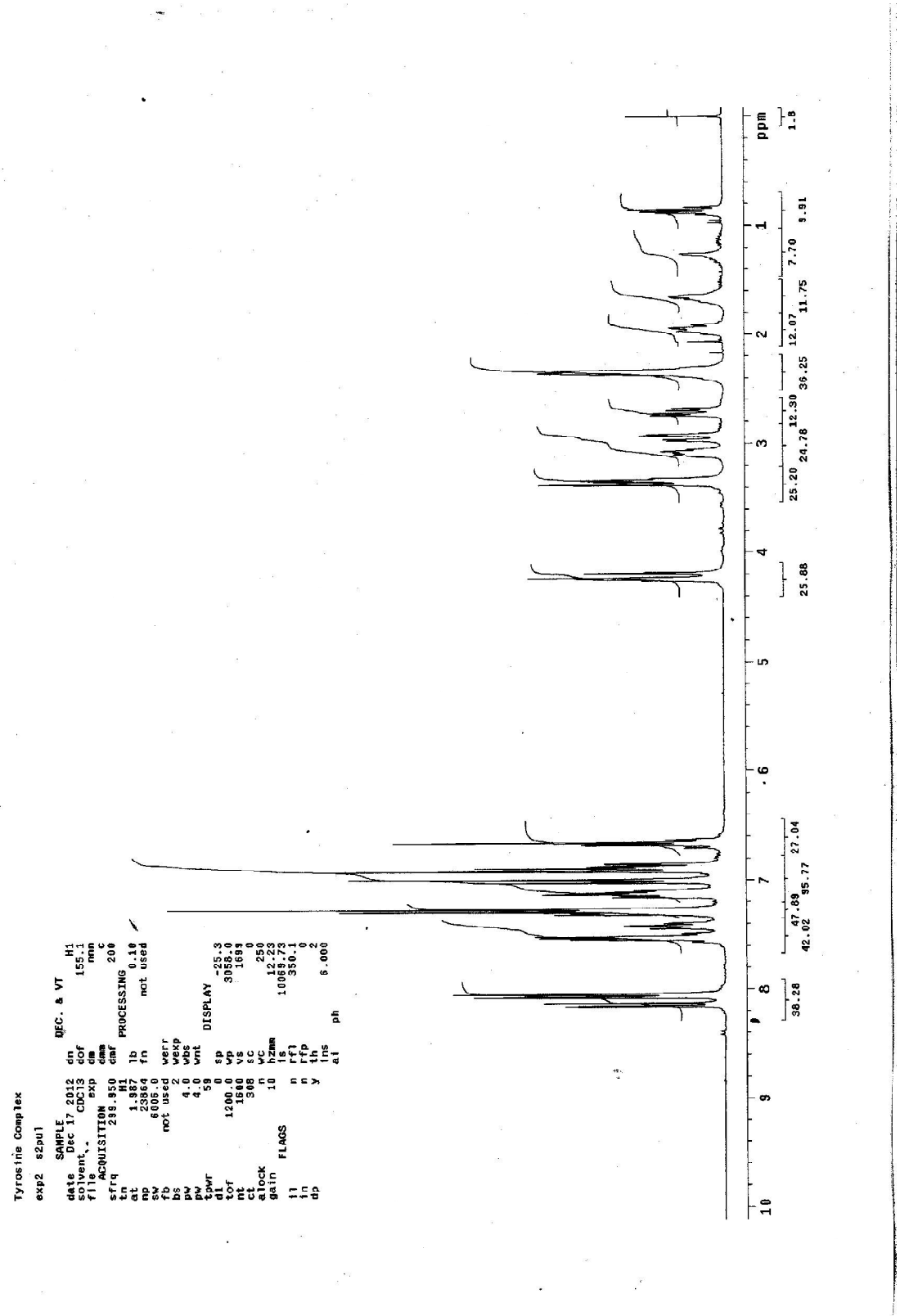


Figure 4-30 *^{13}C-NMR of Ni(II)-(S)BPB-(S)-Ty complex (1)*

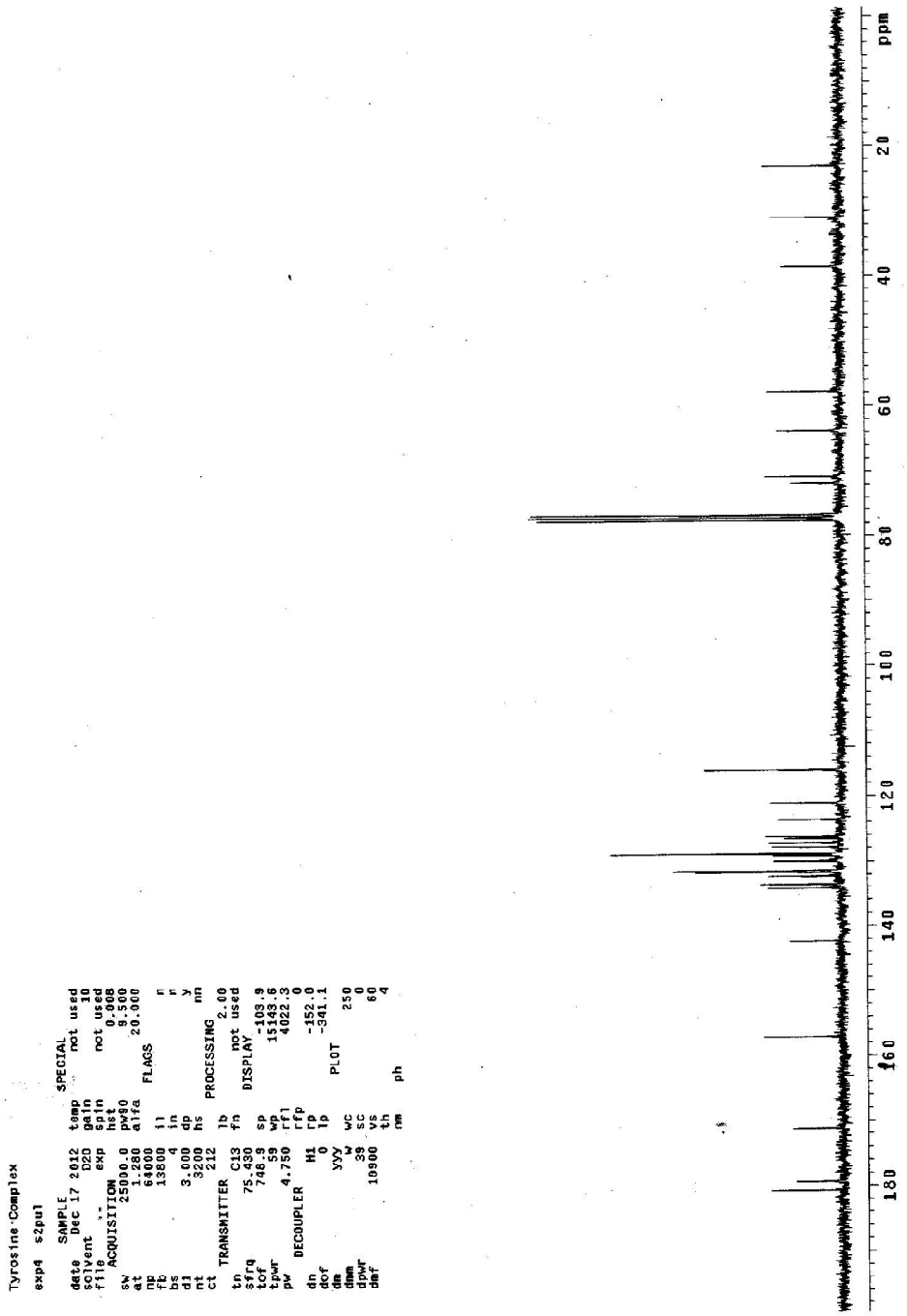


Figure 4-31 *. ^{1}H-NMR of Ni(II)-(S)BPB-(S)-Ty-CH_2-CH_2-OTs complex (3).*

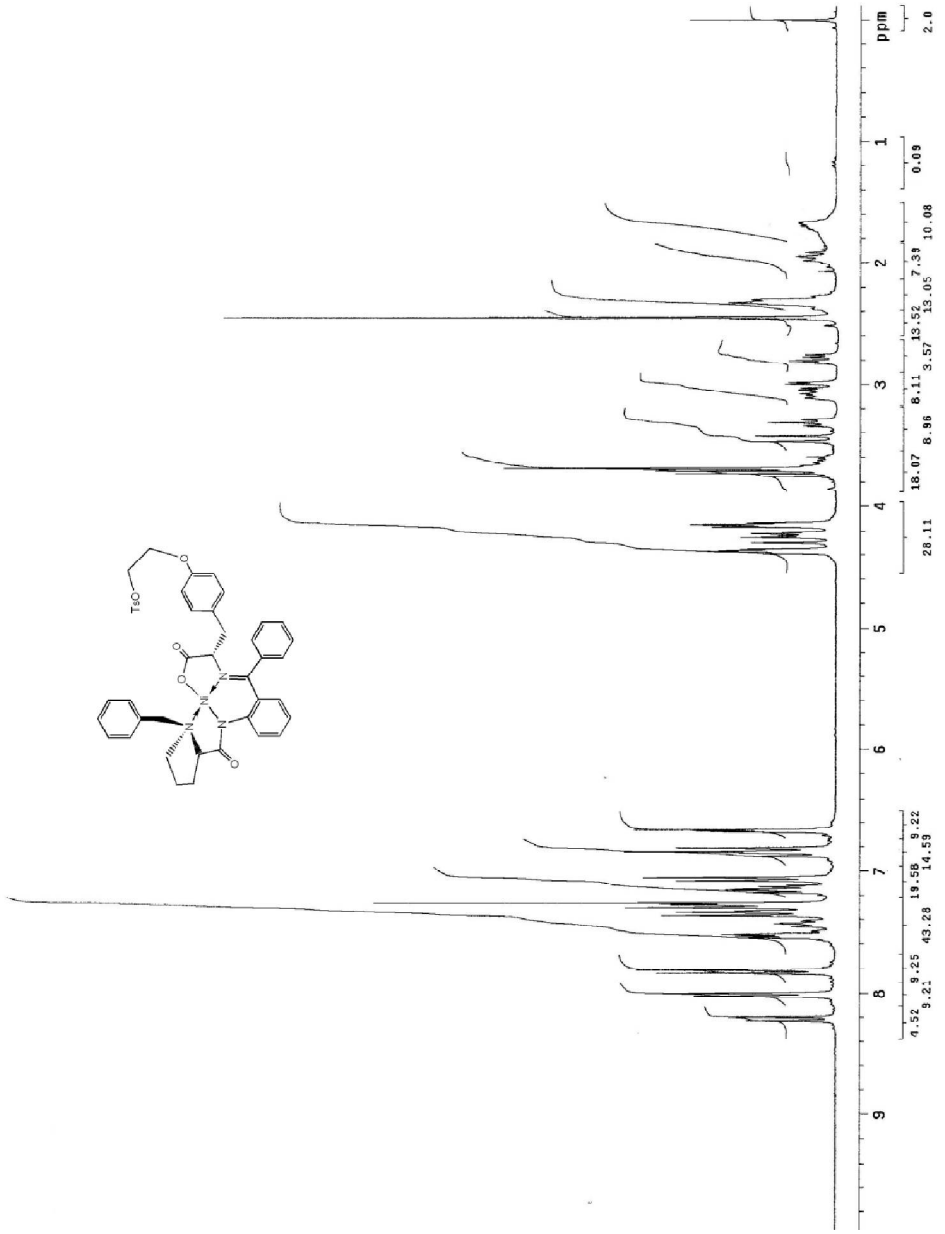


Figure 4-32. *^{13}C-NMR of Ni(II)-(S)BPB-(S)-Ty-CH_2-CH_2-OTs complex(3)*

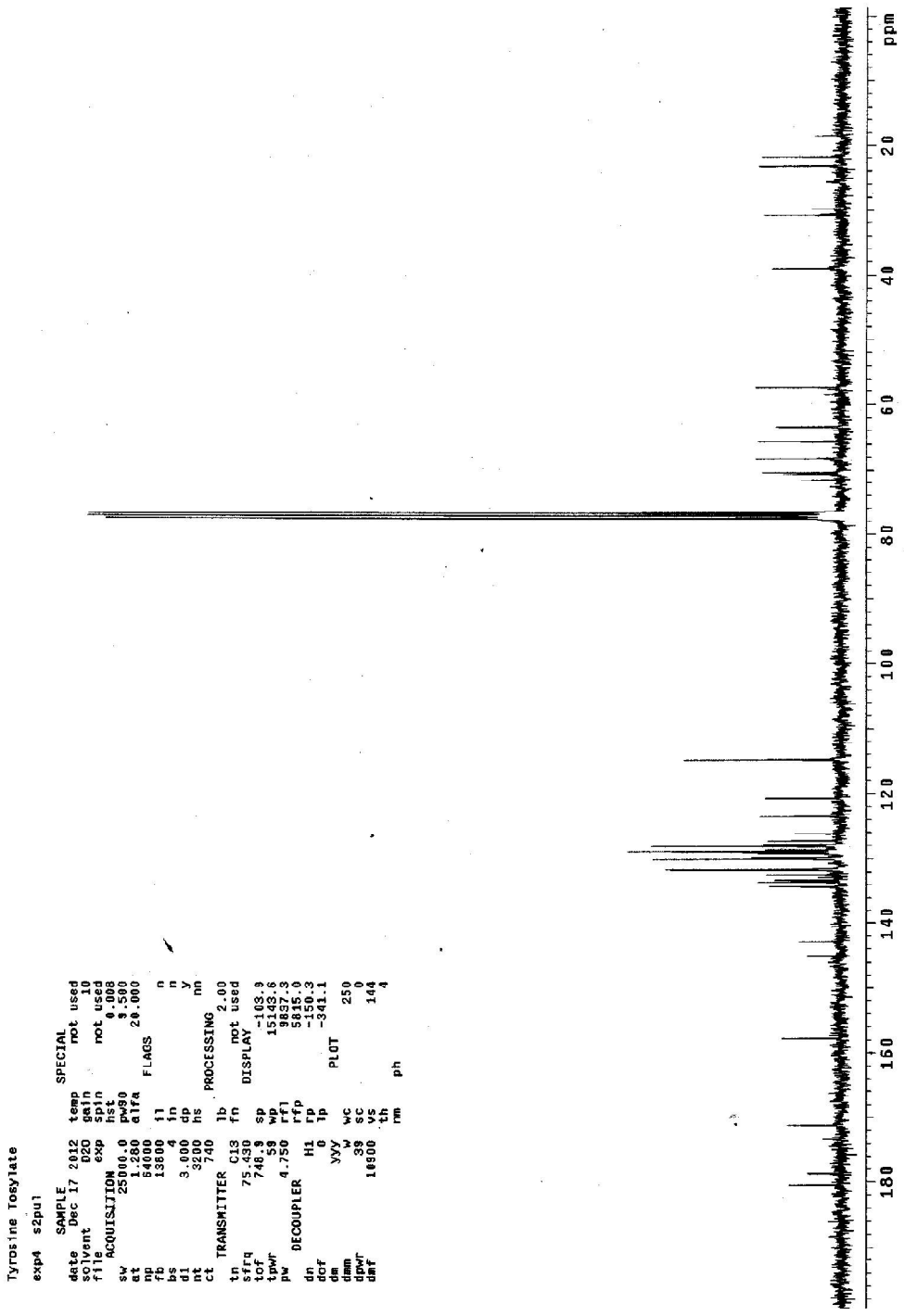


Figure 4-33 *. ^{1}H-NMR of Ni(II)-(S)BPB-(S)-Ty-CH_2-CH_2-OH complex (2)*

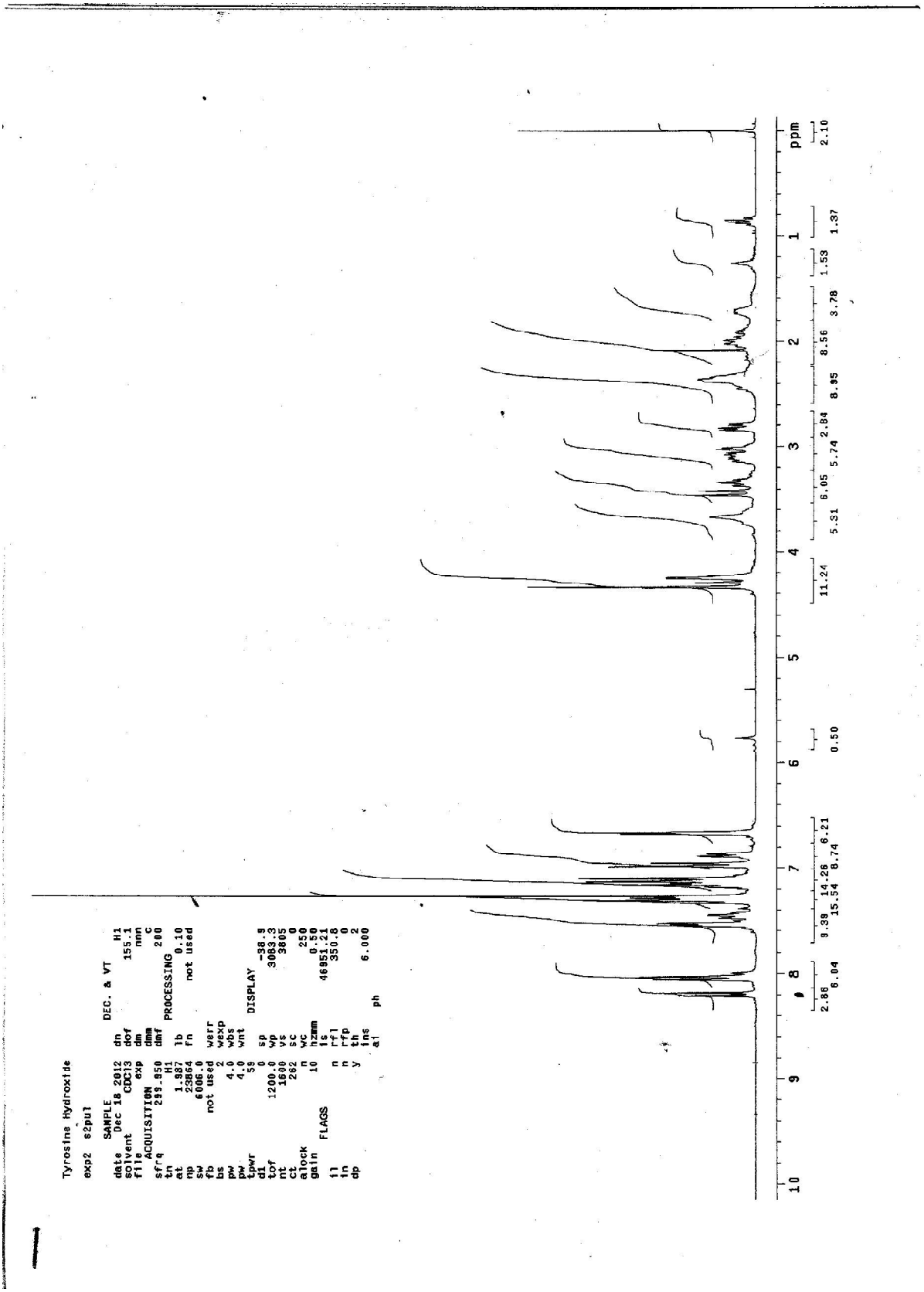


Figure 4-34 ^{1}H NMR of Ni(II)-(S)-BPB-Gly complex

:

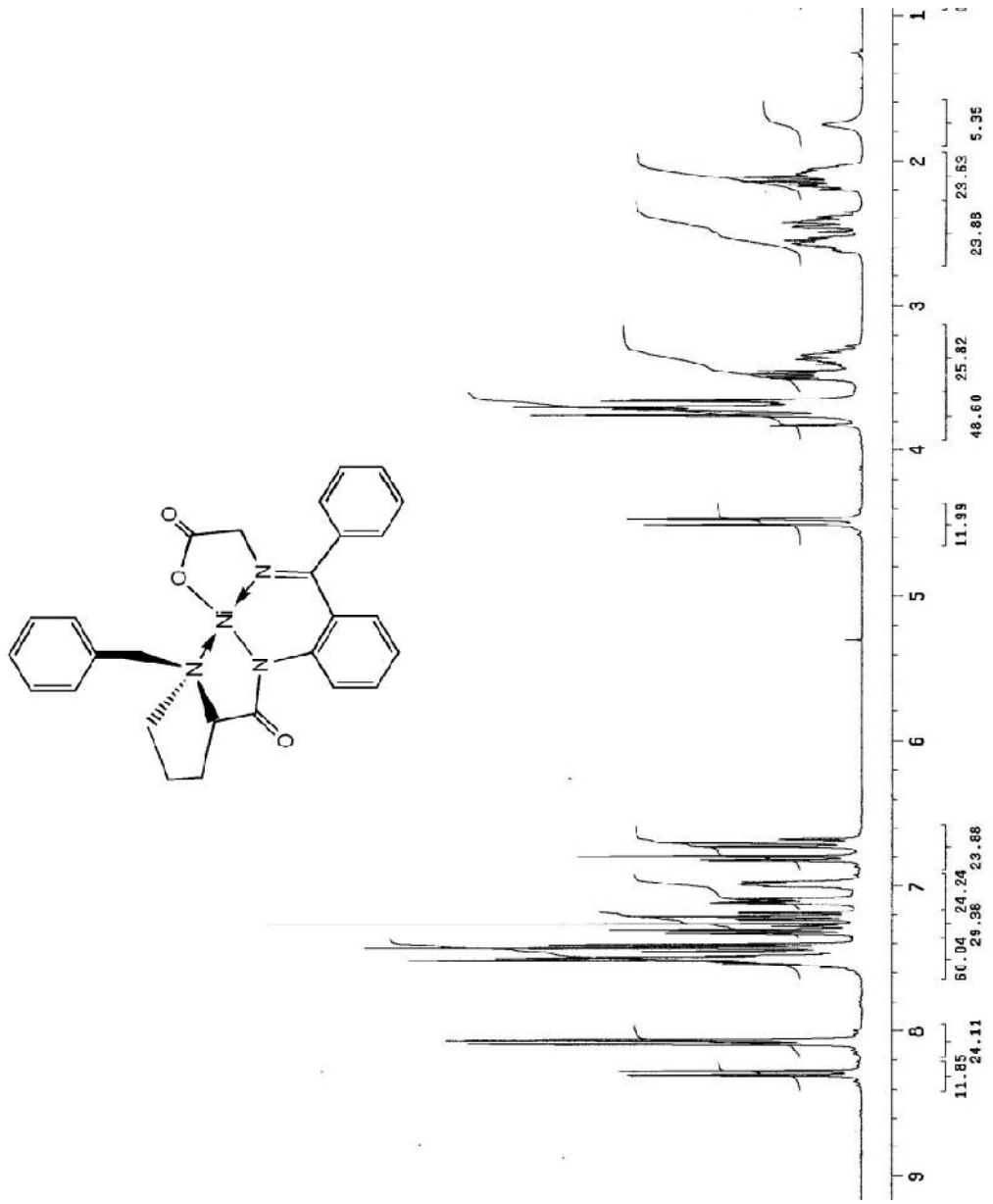


Figure 4-35 ^{13}C NMR of Ni(II)-(S)-BPB-Gly complex

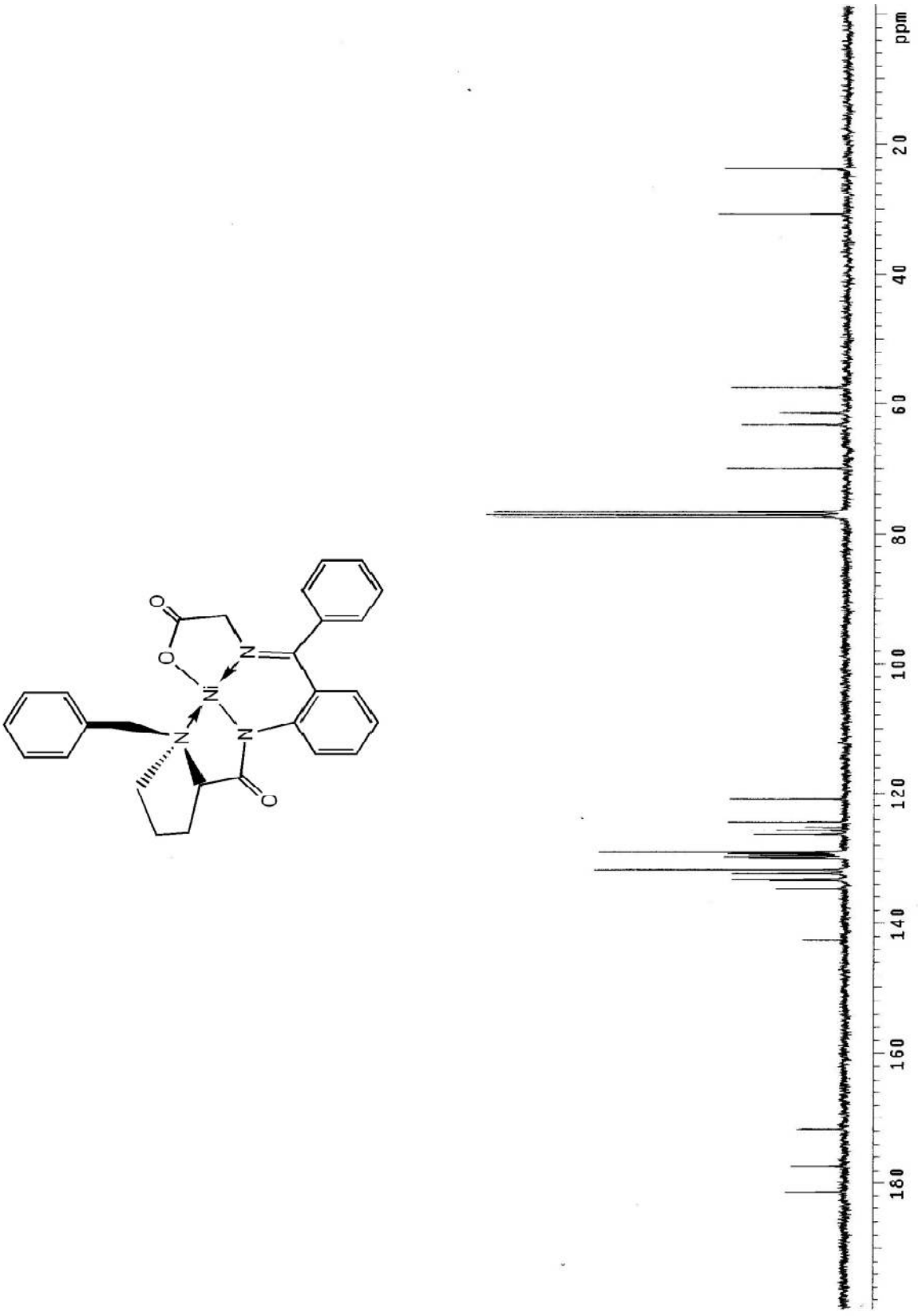


Figure 4-36 ^{1}H NMR of Ni(II)-(S)-BPB-Ser complex

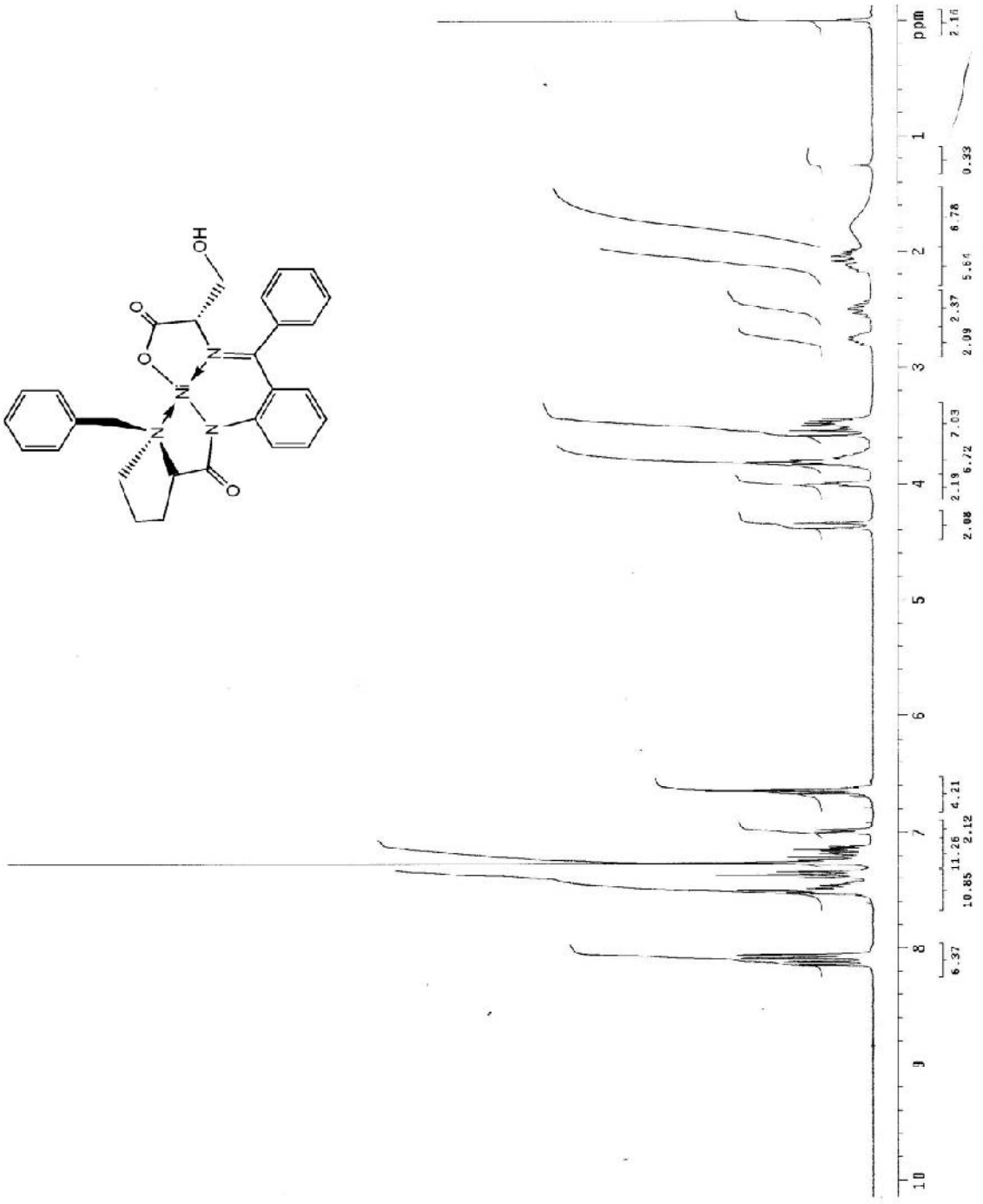


Figure 4-37 ^{1}H NMR of Ni(II)-(S)-BPB-Ser-OTs complex

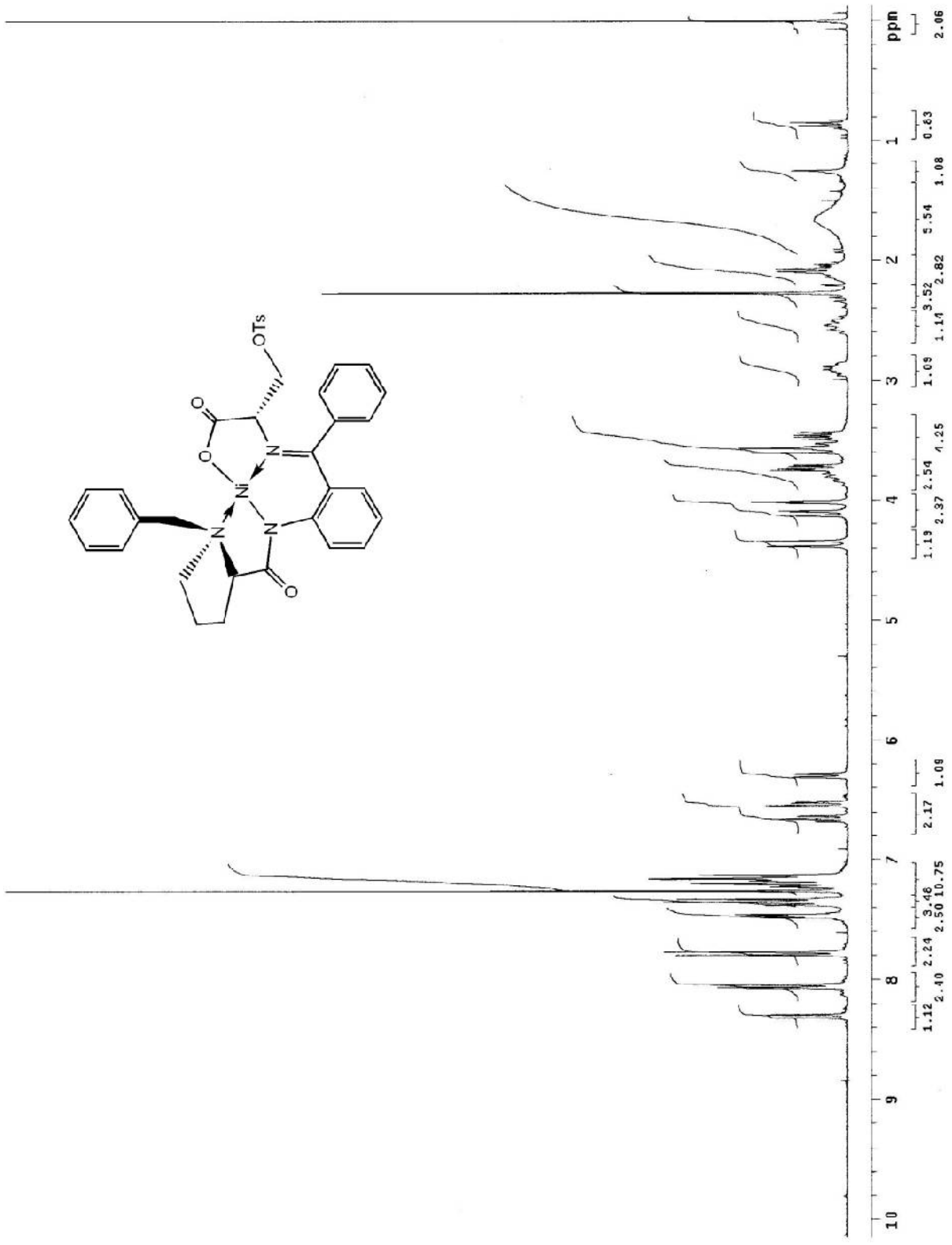
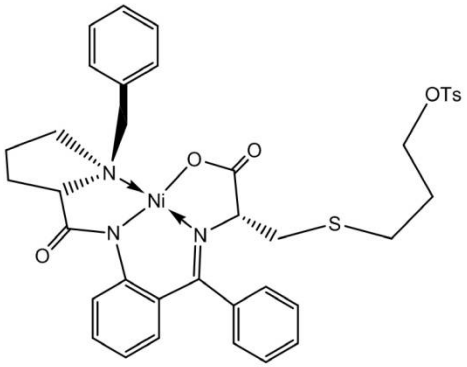
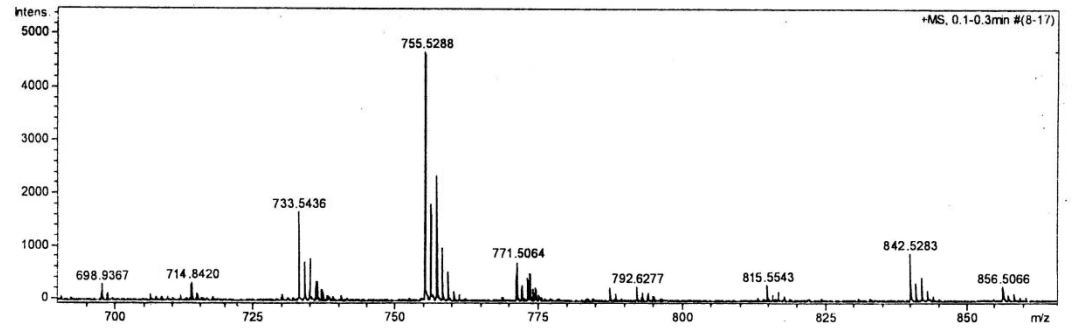

OTs
O
O
N
Ni
N
N
O
ppm
10
9
8
7
6
5
4
3
2
1
1.12
2.40
2.24
3.46
2.50
10.75
2.17
1.09
1.19
2.37
2.54
1.25
1.09
1.14
3.52
2.82
5.54
1.08
0.63
2.06

Figure 4-38ESIMS of Ni(II)-(S)-BPB-propylcysteine-OTs complex: Showing realative isotopic abundance peak of Ni(II) and sulphur corresponding to two sulphur atom in the compound



Relative Isotopic Abundance of Peaks		
m/Z	**Theoretical**	**Observed**
755.5288	**100%**	**100%**
756.5287	**44.5%**	**44.29%**
757.5290	**58.4%**	**58.39%**
758.5289	**24.7%**	**24.71%**
759.5288	**15.1%**	**14.88%**
760.5290	**4.0%**	**4.01%**
761.5288	**2.4%**	**2.45%**



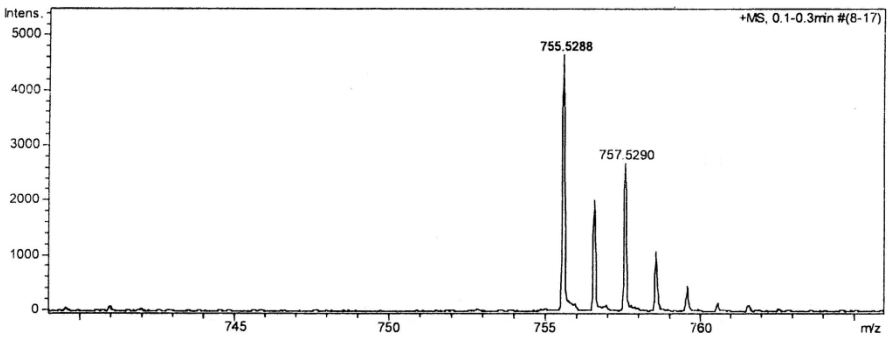


Figure 4-39 ^{1}H NMR of Ni(II)-(S)-BPB-Tyr-CH(CH_3)-CH_2-OTs complex

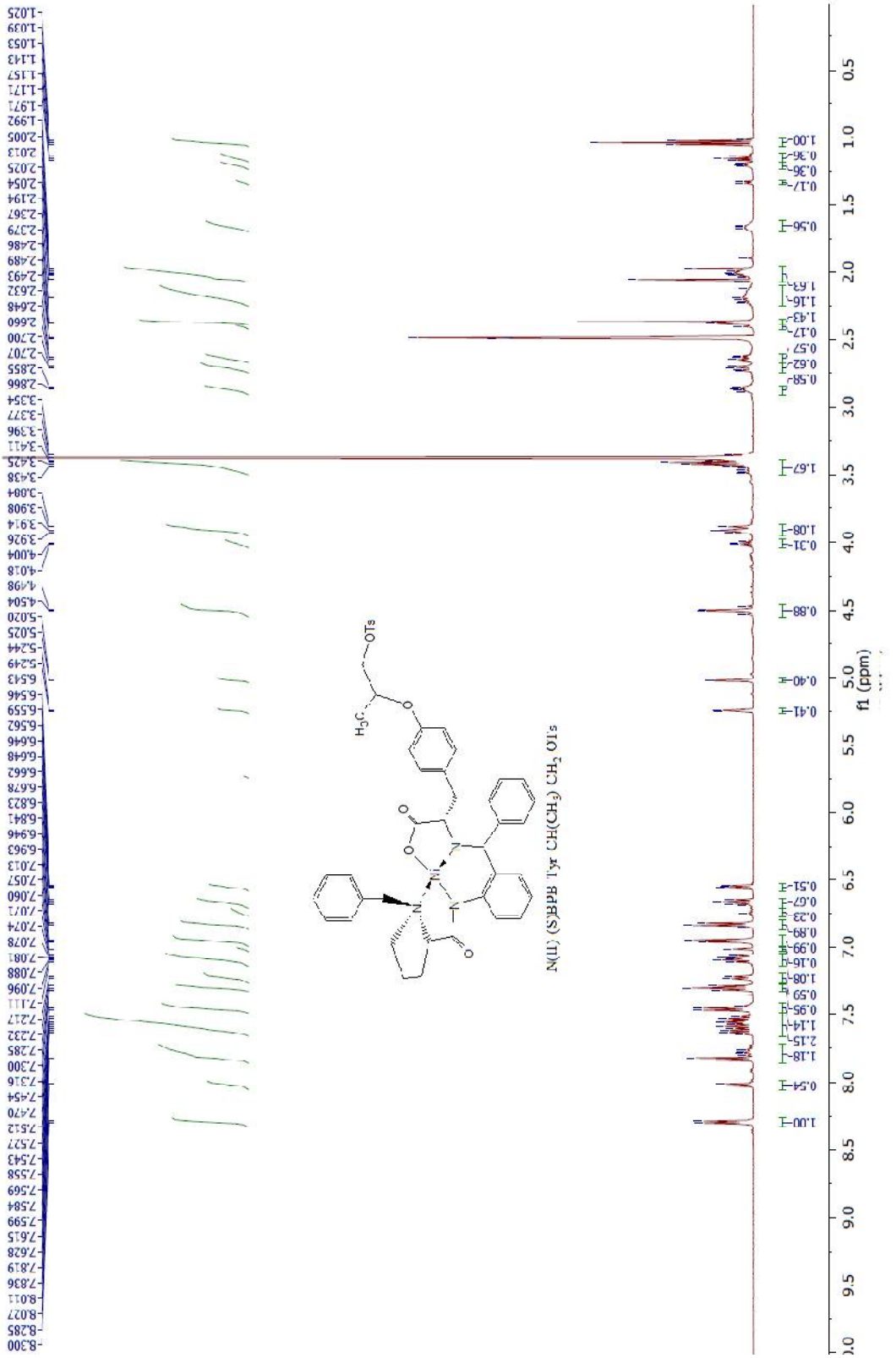
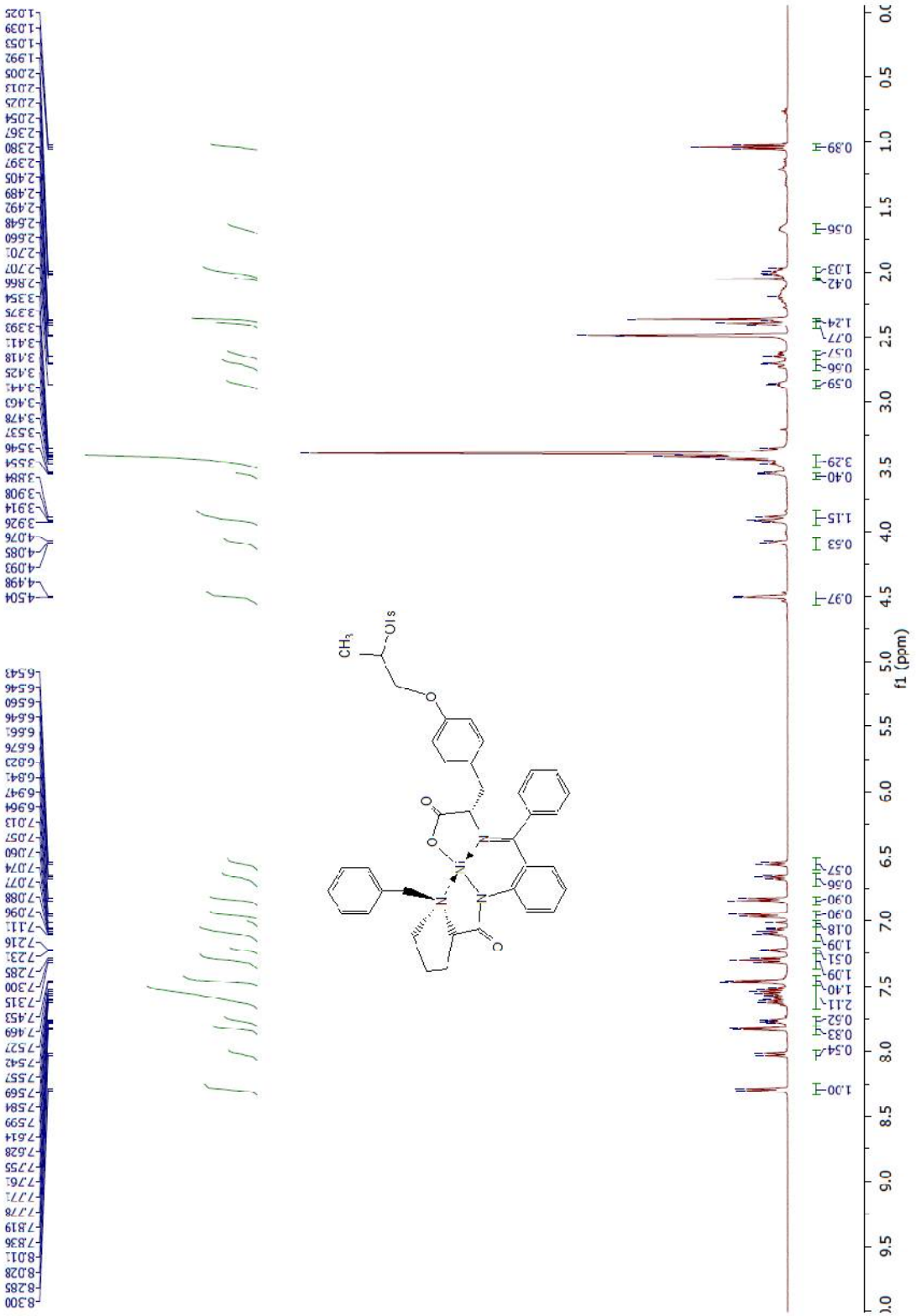


Figure 4-40 ^{1}H NMR of Ni(II)-(S)-BPB-Tyr)-CH_2-$CH(CH_3)$-OTs complex



CHAPTER 5

Summary

5.1. General discussion

[^{18}F]Fluoroethyl tyrosine ([^{18}F]FET) is a PET-radiopharmaceutical, (PET-RP) that is of great significance in nuclear medicine imaging. Its usefulness as a brain imaging agent has been well established over the past several years. Since PET-radiopharmacyuses fast decaying positron emitters, hence, the procedures developed to prepare the RPs, from the time the radionuclide is produced in the cyclotron and till it is ready for injection into patients, needs to be rapid and reliable. [^{18}F]FET,being an amino acid analogue, its enantiomeric purity in the final product is of high importance, in order to obtain satisfactory PET-images for the clinical use. Synthesis of the labelling precursor for ^{18}F-amino acid is a laborious multistep synthesis process that normally gives low yield. However, synthesis of labelling precursors using the Ni(II)-SBPB-amino acid complex based process, provides a quicker method to synthesize the precursors with higher yields. Moreover, it shows good chemical and thermal stability with good enantiomeric purity. The aim of the work, carried out in this thesis, is to explore the possibilities of developing a few ^{18}F- labelled amino acids for use as PET-RPs for imaging brain tumors. Procedures were developed, for routine use,for a pure and aseptic preparation, considering that these RPs will finally be used in humans for a clinical diagnosis.

Several ^{18}F-PET-RPshave been successfully produced and used in patients, as can be seen from the numerous publications. Radiosynthesis of 2-[^{18}F]FDG, which started the era of modern PET-imaging, is now a well standardized process, that can be easily and reliably carried out, and is produced daily in more than 600 medical cyclotron facilities worldwide.

India has 17 such facilities, with the first one installed at RMC in 2002. The production of 2-[^{18}F]FDG has evolved, from the early 1980s to a virtually fail-proof method using a relatively simple andcompact lead-shielded radiosynthesis module, that has single reactor vessel, a solid phase extraction (SPE) purification procedure, and the whole process is controlled by programmable logic controller. SPE purification does away the need for an expensive semi-preparative radio-HPLC process. The reagents used are readily available from many sources, and with a reasonably long shelf-life. Moreover, the automated radiosynthesis process, which takes between 30 – 40 minutes, is compatible with the short half-life of ^{18}F ($T_{1,2}$ – 110 min), and the decay-corrected yield at the end of synthesis is 60 – 70%, which is sufficiently high to cater the needs of several PET imaging centers from one medical cyclotron facility. Thus production of 2-[^{18}F]FDG has been established to be a very cost-effective process for the preparation of PET-RPs. Using this as model, scientists are developing radiosynthesis methods for other useful ^{18}F-RPs that are simple, rapid, reliable and user friendly. In this work, a fully automated procedure for the synthesis of [^{18}F]FET from commercially available TET precursor was described in Chapter 2. Using this method [^{18}F]FET can be prepared using the standard and readily available radiosynthesis module used for the synthesis of 2-[^{18}F]FDG.

[^{18}F]FET radiopharmaceutical has been prepared by several methods, as can be seen in literature, using both direct and indirect methods. Synthesis from the TET-precursor was reported to as a direct labelling method that requires a single reactor. Moreover, it was also reported to provide enantiomerically pure [^{18}F]FET. However,the radiosynthesis of [^{18}F]FET through TET precursor that were initially reported followed a radiosynthesis process that uses aggressive reagents like trifluoroacetic acid and toxic chemicals like dichloroethane. The purification was also reported to be accomplished by semi-preparative radio-HPLC method. In this work, described in **Chapter 2 Part - A**, we have developed a radiosynthesis method of

[^{18}F]FET, closely following the published procedure but attempted to do the purification by SPE, avoiding semi-preparative radio-HPLC purification. However, due to the reagents used, the process was found to be corrosive to the miniature valves, tubing and other components in the synthesis module. Moreover, it was difficult to avoid toxic chemicals like dichloroethane of dichloromethane in the final product, though they were found to be within permissible limits. In order to develop a safer alternate method, we have tried to radio-synthesize [^{18}F]FET using reagents that are also used for 2-[^{18}F]FDG, which is described in **Chapter 2Part – B**. Due to high insolubility of trityl hydroxide formed in aqueous solutions like HCl, during hydrolysis, automated loading of the reaction mixture on the SPE column was found to be difficult as it was not quantitatively transferred. Moreover, it was also observed that, the radio-synthesis process was more efficient with aggressive reagents like TFA/dichloroethane mixture, even at milder conditions as compared to aqueous reagents like HCl. Thus, with TFA/dichloroethane mixture, the reaction was found to be complete leading to 95% of [^{18}F]FET formation in the reaction mixture, providing minimum amount of radiochemicals impurities. Hence, in order to develop a less corrosive radiosynthesis process, albeitless efficient, usingrelatively safer reagentslike aqueous HCl, that are compatible with the radio-synthesis module, we could finally develop a purification method using neutral alumina, with modifications and optimization of reaction parameters. Further, in order to reduce the cold impurities in the final product, we have reduced the initial precursor quantity to an optimum level, which was as low as 2 mg of TET precursor for one [^{18}F]FET production batch. This also saves on the cost of production, since precursors of PET radiochemistry are very expensive. The quality of the product was evaluated by radio-HPLC, Chiral HPLC and GC and was found to be satisfactory. The bio-quality control of the product obtained by final purification was evaluated and found to be satisfactory. The [^{18}F]FET preparation was evaluated by tumor studies in C57BL/6 mice bearing B16F10

melanoma, and found to have a better uptake compared to [^{18}F]FDG. The production procedure for [^{18}F]FET using the above said TET precursor, was found to be efficient, GMP compliant, and the steps can be fully automated, which can easily be adapted in the simple and more commonly available [^{18}F]FDG synthesis modules.

The product was used for human studies, after clearance from the DAE-RadiopharmaceuticalsCommittee, and clinical studies in humans were performed. The [^{18}F]FET is now routinely produced, once a week in the RMC medical cyclotron facility.

Amino acids being multifunctional compounds, the synthesis labelling-precursors of the L-enantiomers, which are specifically taken up the amino acid symporters, is a multistep and laborious process, since the multiple functional groups have to be protected, prior to activation of the labelling site of the molecule. This is absolutely essential for the enantiomeric purity of the product**.** A novel Ni(II) complex based precursor, that uses SBPB as chiral auxiliary for the synthesis of enantiomerically pure amino acids has been described in literature and successfully used. By forming a single Ni(II)-SBPB-aminoacid complex, both the amino and the acid groups were found to be simultaneously protected, along with the advantage of obtaining an enantiomeric excess of L-optical isomer. Radiosynthesis of [^{18}F]FET was achieved by this precursor, with 95% enantiomerically pure product starting with racemic mixture of tyrosine. Moreover, this process was also reported to follow a direct labelling procedure, using single reactor, akin to 2-[^{18}F]FDG procedure. This precursor is not commercially available, but can be synthesized from the reported procedure.Since the precursor has attractive properties in order to adapt in a simple 2-[^{18}F]FDG module, the precursor was synthesized in-house, and evaluated for the preparation of [^{18}F]FET. Moreover, it also provides an in-house source of labelling precursor for [^{18}F]FET preparation, that makes the process more reliable, avoiding degradation of precursors during transport,

when purchased from commercial sources. Based on this, a synthesis procedure for [^{18}F]FET was developed**,** using solid phase extraction procedure,by a one-pot two-step procedure using Ni(II)-(S)-BPB-(S)-tyrosine complex based precursor, which is described in **Chapter 3.** The synthesis procedure was modified considering the radio-chemical yield and the chemical purity required for final use for human studies. The purification step, based on simple neutral alumina cartridge was developed. Quality control of the final [^{18}F]FET preparation showed radio-chemical and chemical impurities well below the prescribed limits. Enantiomeric purity of the final product was evaluated to be 94%, which was in agreement with the reported value of 95%. Tests for trace level impurities like TBA^{+}, Al^{3+} and Ni^{2+} were also found to be below pharmacopoeia limits. The product was found to pass bio-QC evaluations like sterility and BET. Animal studies with tumor models (C57BL/6 mice bearing B16F10 melanoma) showed tumor/brain ratio of ~3, which is in good agreement with that expected for brain tumor imaging agents reported in literature. The radiosynthesis procedure was fully tested with varying amounts of radio-activity, to start with, and was found to be consistent and reproducible. The method developed for the synthesis of [^{18}F]FET by the novel Ni-(S)BPB-Tyr based precursor was found to be efficient and can easily be adapted in commonly available 2-[^{18}F]FDG radio-synthesis modules.

We have used the synthesis procedure for the Ni(II)-(S)BPB-tyr precursor for [^{18}F]FETis reported in the literature, but found that the procedure takes a long time and requires stringent purification process prior to labelling. In effort to reduce the time and steps involved, a simplified route for the synthesis of this precursor is described **in Chapter 4.** The number of steps for the precursor synthesis was reduced from three to two, and the time of synthesis was reduced from 33 h to 3 h excluding purification. Since these compounds are coloured, a simplified purification procedure was developed, which is a modified version of flash chromatography purification method. The method termed as “Serial Column Flash

Chromatographic method"**(SCFC)** method, can be applied for purification of all coloured compounds, and is a fast, efficient and economic purification technique for coloured compounds. Thus applying SCFC purification method to the improved synthesis procedure for Ni(II)-(S)BPB-tyr compounds, makes the precursor synthesis very fast and efficient. Since synthesis of Ni(II)-(S)BPB-tyr complex precursor for [^{18}F]FET looks to be more simple and economic, after applying the above improvement, we tried to apply the above method of precursor synthesis for various various ^{18}F-labelled fluoro-amino acids. [^{18}F]Fluoro propyl tyrosine ([^{18}F]FPT) was synthesized based on the above procedure, by coupling the Ni(II)-(S)BPB-tyr complex with propylene glycol ditosylate. After fluorination, various products formed were analyzed and found to have >90% enantiomerically pure. Various precursors for ^{18}F-labelling were synthesized starting from glycine complex.Several complexes for other amino acids,that were synthesized, were characterized using ^{1}H-NMR, ^{13}C-NMR and HPLC methods. This procedure can save time and solvents, and is a versatile one for better separation of various compound mixtures obtained during synthesis. Synthesis of this precursor and the [^{18}F]FET radio-synthesis using this precursor has several advantages, as it is economical and reliable for routine production, as compared to the most commonly used, and expensive TET precursor.

5.2. Conclusion

In conclusion**,**the following can be summarized from the studies carried out in this thesis:

- [^{18}F]FET can be produced from commercially available TET precursor, as easily as 2-[^{18}F]FDG, using a similar, fully automated procedure, in GMP facilities using neutral alumina column as SPE purification, avoiding toxic solvents and achieving high order of purity by minimizing chemical impurities.

- Production of [^{18}F]FET using novel Ni(II)-(S)BPB-(S)tyrosine complex is a viable alternative and can provide the [^{18}F]FET with higher yield, and high order of purity by neutral alumina SPE purification.
- Synthesis of Ni(II)-(S)BPB-(S)tyrosine Schiff's base precursor is both faster and economical compared to that of the other precursors available for [^{18}F]FET synthesis.
- In the context of the preparation of Ni(II)-(S)BPB-(S)tyrosine Schiff's base precursor, the use of segmented column flash chromatography, is a more efficient and economical method for the purification of the coloured complex.
- The Ni(II)-(S)BPB-amino acid Schiff's base precursor can be a better method for the synthesis of potentially useful [^{18}F]fluoro-amino acid PET radio-tracers, due to its ease of production and high chemical stability.

5.3. Future outlook

The Ni(II)-(S)BPB-amino acid based complexes provides a better alternative for synthesizing labelling precursors for [^{18}F]-fluoro-aminoacids. Ni(II)-(S)BPB-amino acid complex based compounds shows good chemical and thermal stability. Hence, it is more convenient to synthesize them. The method provides higher yields of stereo-specific fluoro-aminoacids, and provides the convenience to do the radiosynthesis in the readily available 2-[^{18}F]FDG synthesis module. The modified synthesis procedure adapted in Chapter 4, coupled with SCFC purification method reported in Annexure I, for the Ni(II)-(S)BPB-amino acid precursor, provides better alternatives for developing new [^{18}F]fluoro-aminoacids with better enantiomeric purity and has scope for further exploration. This gives a wide opportunity for developing non-natural [^{18}F] fluoro-amino acid analogues, with higher stereo-specific products that can mimic natural amino acids.

CHAPTER 6

Appendix

Description of Serial Column Flash Chromatography (SCFC) purification method used in Chapters 3 and 4.

6.1. Purification using "Serial Column Flash Chromatography" – SCFC method

Ni(II)-(S)-BPB-(S)-Ty-CH_2-CH_2-OTs is a very highly reactive precursor, which is susceptible for hydrolysis or decomposition on exposure to even mild reaction conditions. Hence, it gets hydrolyzed even during purification, due to contact with the silica adsorbent for longer time. Hence a conventional chromatographic separation would not be helpful in efficient recovery of the synthesized precursor. In these cases, flash chromatographic separation technique is used, which is faster and efficient for separation and purification. The compounds involved in this study are transition metal complexes, that are having intense colours. Ni(II)-(S)BPB-tyrosine complexes are bright orange red in colour.

The Ni(II) salt viz. $Ni(NO_3)_2$ used as the starting material is green in colour. As the complex is formed, the reaction mixture turns into red colour, indicating the formation of the Ni(II)-(S)BPB-tyrosine complex. The progress of this reaction can be visualized by naked eye due to its colour change. On the other hand, the various derivatives of the Ni(II)-(S)BPB-tyrosine complexes prepared are of same colours. Hence these derivatives cannot be directly distinguished by mere observation through naked eye. But however, these products can be easily visualized by TLC analysis or during chromatographic separation (Figure 10). In case of usual organic compound mixtures, that does not show intense colour or large variations in colours, (the situation in most of the cases) have to be analyzed by TLC and processed in

order to visualize the composition in the mixtures. On the contrary, the easy visualization of various derivatives while separation of these coloured complexes, provides an extra advantage for the easy recovery of compounds while purification.

Utilizing the above characteristics, of easy visibility of the complexes, while analyzing the reaction mixture by TLC, SCFC is a new method, indigenously explored. The typical flash chromatography used in our facility is shown in Figure.6-1

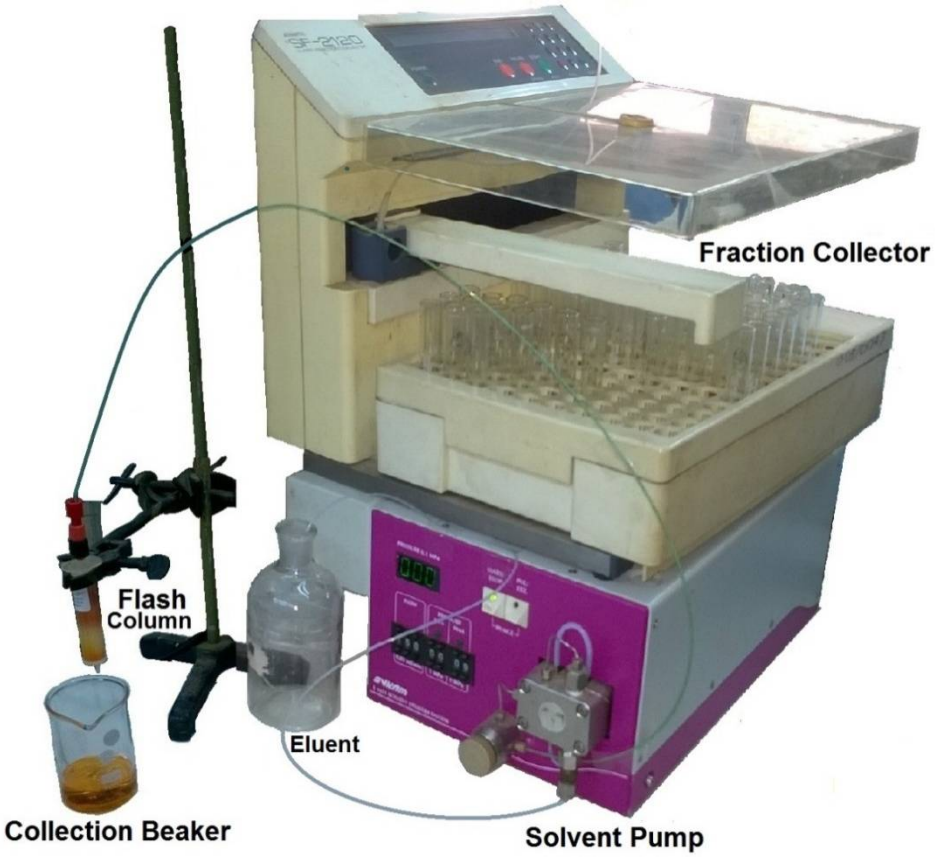


Figure 6-1 Flash column chromatographic system in our facility.

In conventional chromatographic separation, the crude mixture obtained after reaction are loaded on to the top of the column, and they are eluted with suitable mobile phase. The compounds are recovered, from the bottom, after separation through the column, in a sequence based on their separation pattern. An experienced organic chemist, has a rough

estimate of when, and in which sequence, to expect these compounds to get eluted during separation. However, in order to confirm the actual point of elution, of those compounds, a TLC needs to be developed frequently, for efficient recovery and less wastage of the desired products. For a beginner, however, this process of confirming the beginning and end point of the compounds, during column purification, becomes even tedious task. Adding to these, every compound needs to be collected from the bottom of the chromatographic column, as and when they elute out. Certain compounds, which may have high retention and low mobility in the solvent system used, would take a longer time for a separation chemists to realize and respond, in order to replace a stronger eluent for further recovery. This requires lot of practice and imagination, in order to realize the behavior of these compounds in the column. Added to these, a TLC analysis is required, even to find the nature and concentration of the collected fractions after their separation.

Contrary to these facts, coloured complexes are visible during their separation, and the intensity shows the concentration of these compounds present in their fractions. Since these compounds are visible as and when they get separated in the column itself, a time comes, when the separation gets sufficiently adequate, that the compounds in the mixture are well resolved from each other (Figure6-2).

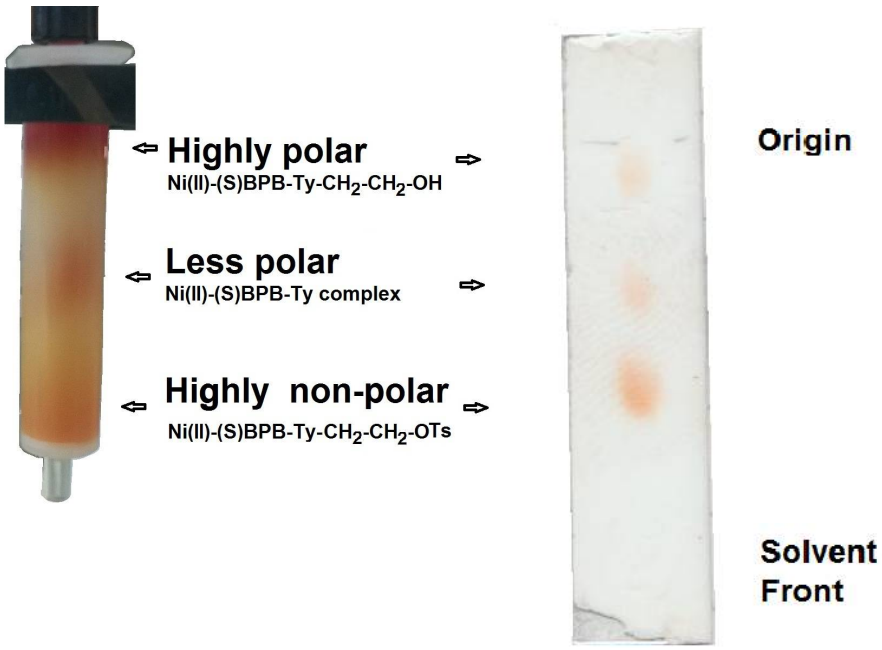


Figure 6-2a. Flash column showing separation of three compounds; b. TLC showing separation of three compounds – mobile phase – 100% ethyl acetate.

If the compounds can directly be recovered by break opening the column and collecting the respective adsorbent layers independently would simplify the recovery. Later, these adsorbents can be washed with suitable solvent, to recover the compounds, directly, in its pure form. This avoids the chemists to wait till all the desired compounds get eluted one by one from the bottom. This moreover saves the time and solvent used for further elution.

However, this is not possible in normal case, since, the column has its own integrity. Removing the adsorbent layer by layer after break opening the column, is once again a messy and tedious process.

In order to make this process simpler, the column is used in the form of segments, whose bed volume can be decided by the separation chemists. These serial columns can be

connected in sequence by a simple and efficient press fit, in order to form long column of desired bed volume (Figure6-3).

Figure 6-3 Small segments, that can be connected to form a long column

The columns are made of polypropylene barrels, and polyethylene frits. The barrels and poplyethylene frits used for the preparation of purification column was obtained by emptying the used and decayed resins of Chromabond Column, that came together with 2-[^{18}F]FDG kit, ABX, Germany. The empty barrel, after removing the used resins, is repacking with Silica gel used for flash column chromatography (mesh 200-400).

A Typical schematic representation of the technique is shown in (Figure 12). The column is packed tightly with SiO_2 gel (mesh 200-400). The column is thoroughly washed with hexane. The compound mixture is then loaded onto the column, and eluted with suitable solvent gradients. On elution, the mixture gets separated, as they move. In a particular point, the compound's position falls in separate columns. At this time, the column may be disconnected and compounds can be recovered by independently passing suitable strong solvents (Figure6-4). The bed volume of the column segments and the number of segments, can be varied and designed as per the requirements.

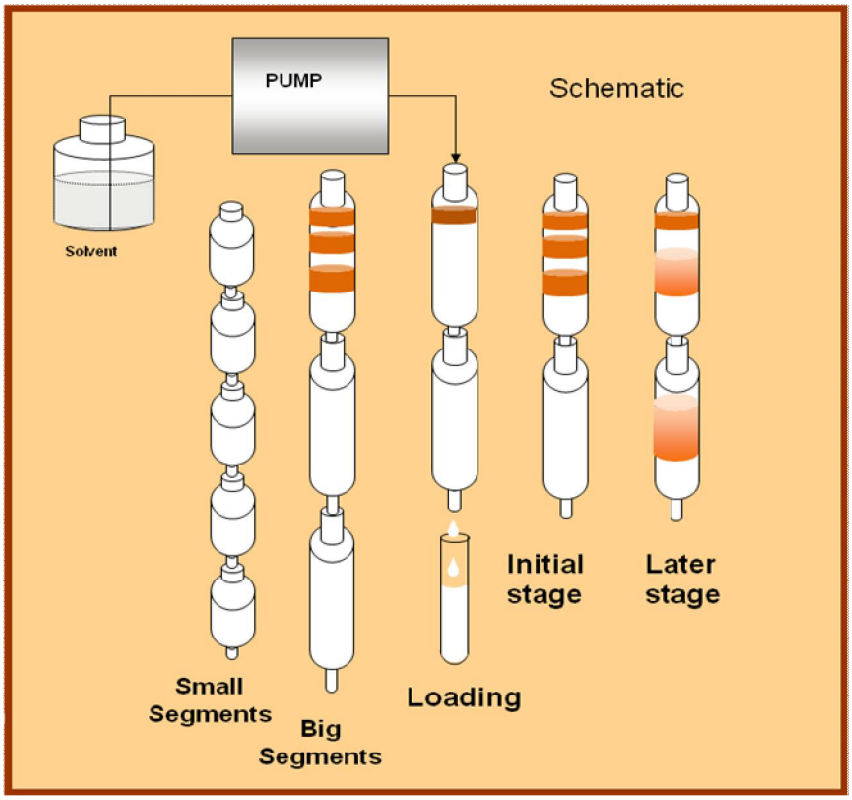


Figure 6-4 Schematic representation of "Serial Column Flash Chromatography"

6.1.1. Preparation of column

The column was prepared by filling the silica gel (mesh 200-400) in a polypropylene barrel, after placing a polyethylene frit in the bottom. The column was tapped gently in order to make the packing close and tight. After packing, a polyethylene frit was place above the layer for protection and the column was closed tightly (Figure6-5 and 6-6). In order to make column segments of different sizes, the standard size barrel was cut into smaller size, as per requirement and fastened by lid, after placing a polyethylene frit over it.

Figure 6-5 Packing of polypropylene barrel with SiO_2 gel (mesh 200-400)

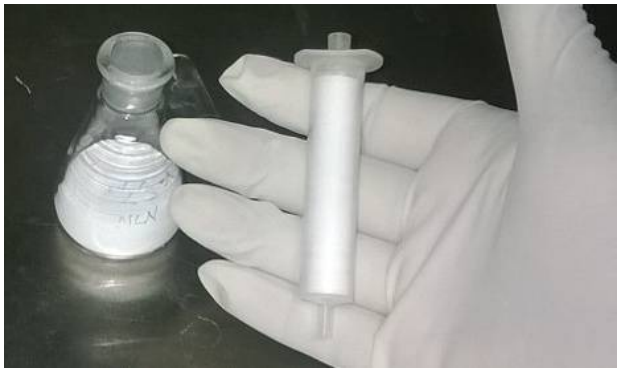
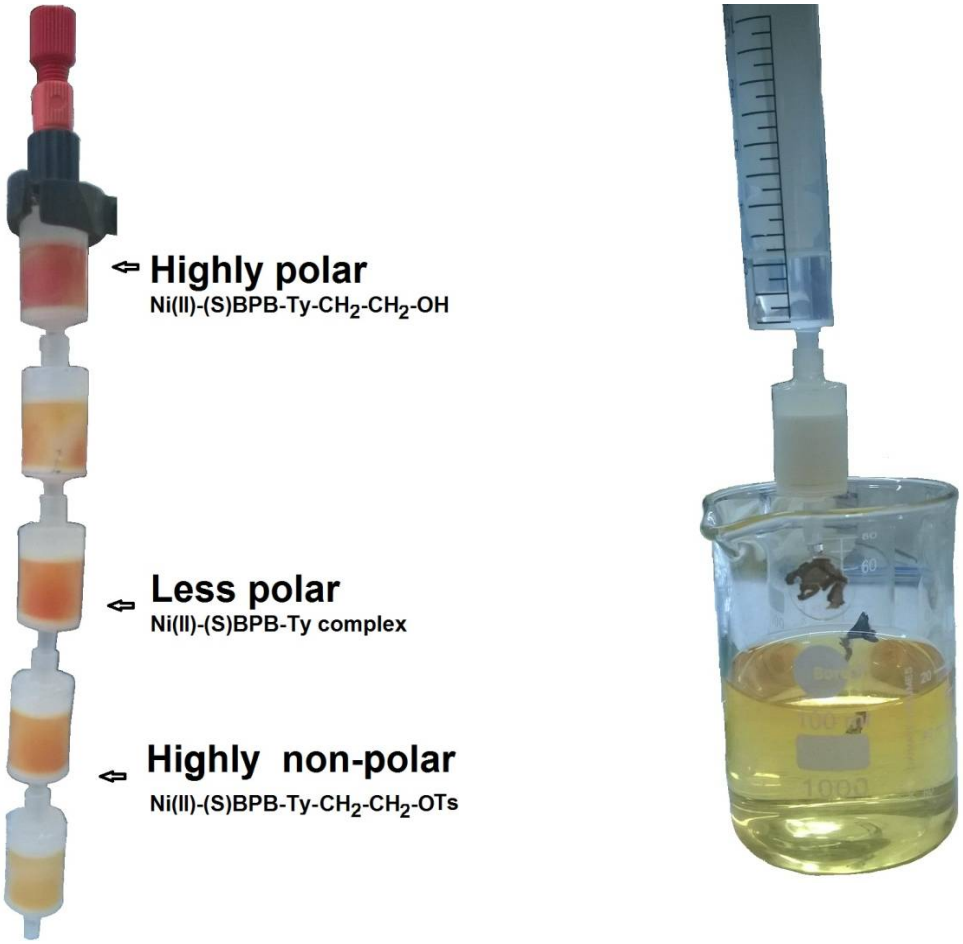


Figure 6-6 Packed Serial column.

6.1.2. Purification of precursor using serial column flash chromatography

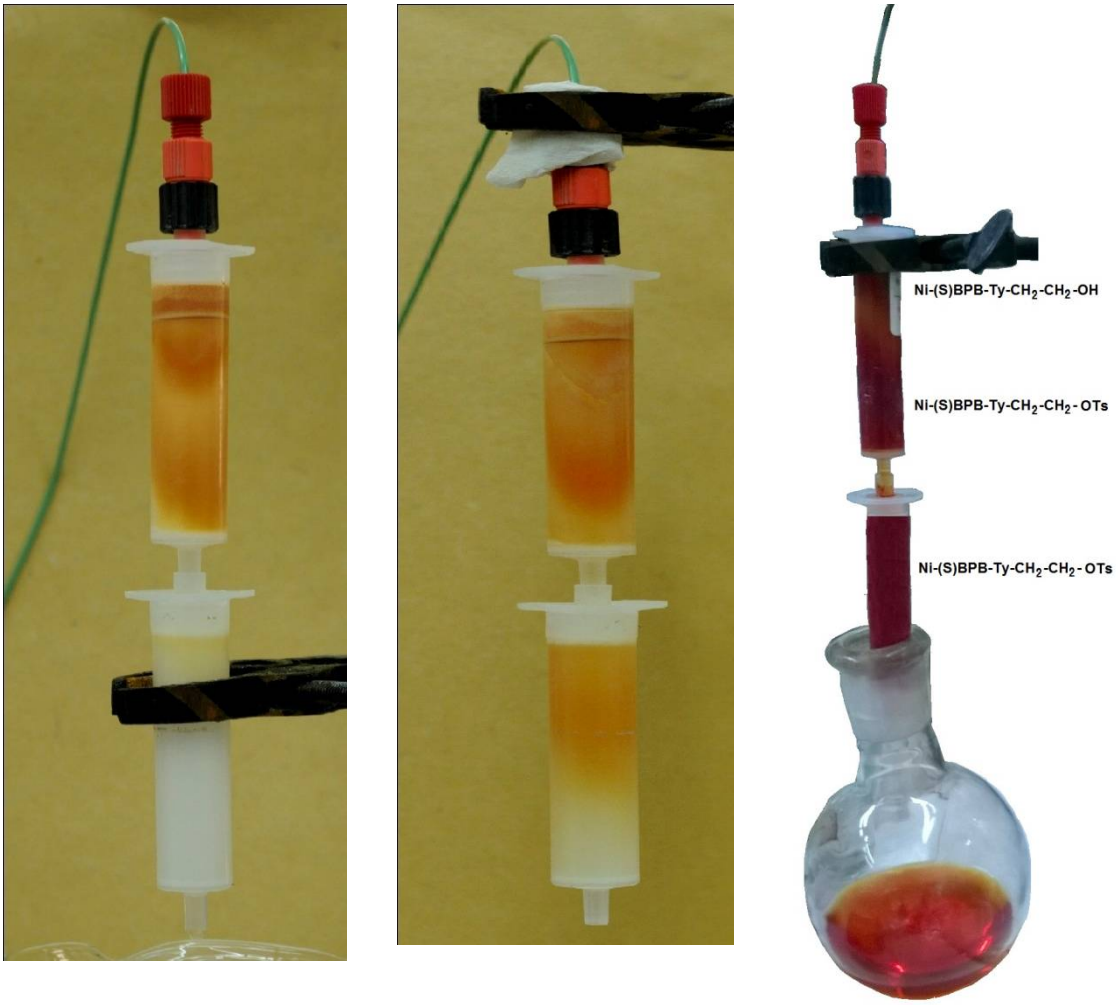
The separation of the Ni(II)-(S)-BPB-(S)-Ty-CH2-CH2-OTs precursor, Ni(II)-(S)-BPB-(S)-Ty-CH2-CH2-OH complex, obtained as a by-product, and Ni(II)-(S)-BPB-(S)-Ty complex using serial column flash chromatography is shown in (Figure6-7).



A **B**

*Figure 6-7 **A**. Using serial column Flash chromatography for separating the precursor from reactants.The column fragments are disconnected and independently eluted with strong solvents to recover the compounds of interest. **B**. The column fragments are disconnected and independently eluted with strong solvents to recover the compounds of interest*

The purification was applied for Small quantities as well as larger quantities upto 500mg scale for bulk preparation and separation of complex, which is shown in (Figure6-8)



A. **B.** **C.**

*Figure 6-8 **A**. Highly non-polar tosylate getting separated fast.**B**. The Highly non-polar tosylate collected in below segment.**C.**Bulk quantity of precursor purification (500mg scale)*

6.1.3. Other advantages of the SCFC technique

6.1.3.1. Easy termination or extension of separation process

Since the columns are in segments, the experiment can be terminated by disconnecting the segment of interest or may be extended further by attaching new segments.

6.1.3.2. Replacement by fresh columns for further separations

Since different compounds have different separation factors, some compounds in the mixture may fall in close proximity with each other, for which a long column may be required by conventional methods. Under such cases, the segments which has these compounds can be directly disconnected and extended with fresh column segments in the bottom and the separation can be achieved.

6.1.3.3. Future Prospects of the SCFC method

This technique, in this study, is applied to coloured compounds. However, it has high scope that it may also be extended to other compounds by development of a visualization means, for example, like fluorescence coated adsorbents, that can show compounds by UV irradiation, etc.

At present scenario, the column segments, of required size, are manually made. This may be a labour consuming process. But however, based on the practical utility of the technique and commercial availability of either empty barrels, or packed readymade flash column segments, may simplify the "serial column flash chromatographic" technique and may be applied to a wide varieties of organic compounds.

Development of more robust barrels, which can provide an easy means of packing and can withstand high pressures, can further make this method useful for separations of more complex mixtures.

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