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Improved automated synthesis of [^{18}F]fluoroethylcholine and O-(2-[^{18}F]fluoroethyl)-L-tyrosine as a radiotracer for cancer imaging

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To my mother, my family and to my friends

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1- All chemical structures for the compounds and scheme were drawn using ChemDraw software.

2- All data are expressed as mean ± standard deviation (SD).

ABBREVIATIONS

Å	Angstrom (10^{-10} meter)
ACN	Acetonitrile
BETfO	2-bromoethyl triflate
bp	boiling point
β^-	beta particle, electron
β^+	positron
DBE	1,2-dibromoethane
DMAE	N,Ndimethylaminoethanol
DITOS	1,2-bis(tosyloxy)ethane
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
EC	Electron Capture
EOS	End Of Synthesis
eV	electronvolt
[^{18}F]BFE	1-bromo-2-[^{18}F]fluoroethane
[^{18}F]BFM	bromo-[^{18}F]fluoromethane
[^{18}F]FDG	2-deoxy-2-[^{18}F]fluoro-D-glucose
[^{18}F]FECh	[^{18}F]fluoroethylcholine
[^{18}F]FECh	[^{18}F]fluoroethylcholine
[^{18}F]FET	O-(2-[^{18}F]fluoroethyl)-L-tyrosine
[^{18}F]FETos	[^{18}F]fluoroethyltosylate
g	gram
GBq	gigabecquerel
GC	Gas Chromatography
h	hour
HPLC	High-performance liquid chromatography
Kryptofix 2.2.2 / $K_{2.2.2}$	4, 7, 13, 16, 21, 24-hexaoxa-1, 10-diazabicyclo[8.8.8]-Hexacosane
min	minute
mL	millimeter
NMR	Nuclear Magnetic Resonance

***o*-DCB**	1,2-dichlorobenzene
PET	Positron Emission Tomography
RCY	Radiochemical Yield (^{18}F-incorporation)
R_f	Retention factor (TLC)
R_t	Retention time (HPLC)
SPE	Solid Phase Extraction
$t_{½}$	half-life
Ty-Di-Salt	L-tyrosine disodium salt
UV	ultraviolet

1. Introduction

1.1 Nuclear medicine

Nuclear medicine is a branch of medicine and medical imaging that uses radioactive isotopes (radionuclides) to view the physiology and function of organs as diagnostic tool especially early detection disease and to treat diseases.

In nuclear medicine procedures, a radionuclide is combined with other chemical compounds or pharmaceuticals to form a radiopharmaceutical which is then injected into the patient's vein and travels through his body to localize within specific organs or cellular receptors, where it provides energy in the form of gamma rays. This is detected by a special device called a gamma camera. In the case of the use of radionuclides that decay by emission of positrons the gamma ray sent by the annihilation is detected with the positron emission tomography (PET scanner). These devices work together with a computer to measure the amount of radiotracer absorbed by the patient's body and to produce special images. The latter give information about the physiology and the function of the body organ, thus the nuclear medicine physician interprets the image to diagnose or treat the medical problem [1].

Nuclear Medicine imaging is one of the most common nuclear applications. It shows the biological activities in the body organs, rather than just the density of tissues and organs. In addition, a distinctive feature of Nuclear Medicine imaging is that the radiation source is internal, whereas diagnostic radiology passes a beam of radiation from an external source through the patient's body and onto a sensitive surface on the other side [2].

Positron emission tomography/computed tomography (PET) is the most advanced medical imaging technique available today, most PET scans are operated by a combination of PET and CT scanners. The combined PET/CT scanners provide images that pinpoint the exact location of abnormal metabolic activity within the body and detect changes in cell function [3]. This combination allows for earlier and more accurate detection of disease than either CT or PET alone. However, they do not show as much detail as computed tomography (CT) scans or magnetic resonance imaging

(MRI) because the pictures show only the location of the tracer [4]. The PET picture may be matched with those from a CT scan to get more detailed information about where the tracer is located.

In some centres, the PET images can be superimposed with computed tomography (CT) or magnetic resonance imaging (MRI) to produce special views, which are technically known as images fusion or co-registration [5]. These views allow the information from two different studies to be correlated and interpreted on one image, leading to more precise information and accurate diagnoses. In addition, manufacturers are now making single photon emission computed tomography/computed tomography (SPECT/CT) and positron emission tomography/Computed tomography (PET/CT) units that are able to perform both imaging studies at the same time [6]. In this case, CT is used to help us localize anatomical structure whereas PET shows the metabolism of the tracer (physiological procedures).

A PET scan is often used to find cancer; hence it determines the best method for treatment and monitors the patient's progression through the treatment [7].

1.2 Positron emission tomography (PET)

Positron emission tomography (PET) is a nuclear medicine imaging technique which measures the metabolic activity of radiotracers in the body, i.e. glucose metabolism in cancer cells, and produces a three dimensional (3D) image or a map of functional processes in the body. PET is a diagnostic tool that is heavily used in clinical oncology for imaging of tumors and searching for metastases as well as for diagnosis of brain diseases such as dementia. In addition, it is frequently used in patients with brain or heart conditions, as it helps to visualize the biochemical changes taking place in the body, such as the metabolism of the heart muscle. PET is also an important research tool that provides a clear map of human brain, heart functions etc. While other types of nuclear medicine examinations detect the relative amount of a radioactive substance collected in the examined body area or tissues, PET detects quantitatively the radioactivity and allows the measurement of the metabolism within this examined area [8].

PET imaging only used to be available in specialized and specifically equipped centers, thus making it an expensive procedure. However, the advanced new technology incorporated into PET has enabled a faster procedure at a lower cost. Gamma camera systems, a device that tracks the radioactive materials injected within the patient's body, have been adapted to be used as PET scanners.

PET provides a variety of labeled compounds that can be used to characterize tumor biochemistry. Some of these have proved to be of great importance for clinical diagnosis, treatment, and clinical research. [^{18}F]-labeled deoxyglucose ([^{18}F]FDG) was the first and more commonly used PET tracer in oncology and proved to be efficient in the diagnosis of cancer[9]. After injection of [^{18}F]FDG into the patient's body, the positron emitted by ^{18}F loses its kinetic energy within a few millimeters before it combines with an electron (annihilation). The resulting two gamma rays of each 511 keV that spread out with an angle of 180° can be detected by PET. The overall effectiveness of [^{18}F]FDG can be explained in light of fact that cancer cells grow at a very fast rate and that the [^{18}F]FDG molecules are consumed more in fast-growing cancer cells than in normal cells, resulting in concentrations of [^{18}F]FDG and positrons in areas of cancer where they can be easily detected. A PET/CT scanner detects pairs of gamma rays emitted from a patient and provides images that map the locations. The PET [^{18}F]FDG map is combined with the structural detail of the CT image in order to identify the presence of the disease as well as its precise location [10].

1.3 Radionuclides for PET scanning

A PET radionuclide is an atom with an unstable nucleus and decays to stable nuclides mainly by positron emission (also known as β^+ or beta plus decay). The positron, antiparticle to the electron, loses its kinetic energy rapidly and combines with an electron (negative beta particle) before it is annihilated (Figure 1) [11]. As a result, the total mass of the positron and the electron is converted into electromagnetic energy; hence two of 511-keV gamma rays are emitted [12].

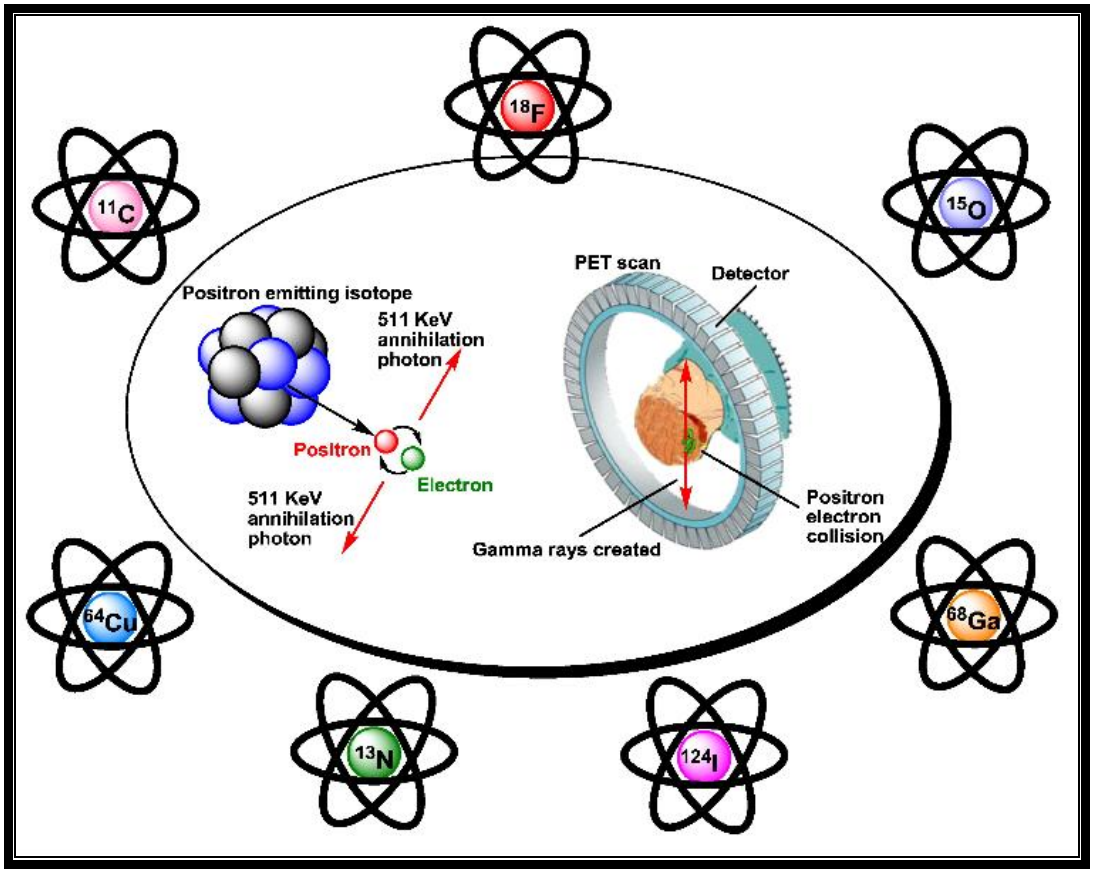


Figure 1. Positron emission and annihilation (From Zibo Li, 2010, image no.1) [13].

Radionuclide which decays to stable nuclides through positron emission can be divided into three main groups:

- Organic radionuclides (^{15}O, ^{30}P, ^{13}N and ^{11}C): the biomolecules consist of carbon, hydrogen, oxygen, nitrogen and phosphorus; wherefore the organic radionuclides allow authentic labelling by exchanging the non radioactive elements with a radioactive isotope of the same element (eg ^{12}C by ^{11}C). In this manner the biochemical and physiological behavior or properties of the labelled molecules do not change. However, their application is of limited value, because the very short half-lives, ranging from two minutes for ^{15}O and 20 minutes for ^{11}C, require the existence of a cyclotron in the vicinity. On the other side they do not allow studies of slow biochemical processes.

- Analogue tracer (radiohalogens like ^{18}F, $^{75,76}Br$, ^{73}Se and $^{120,124}I$): The radiohalogens play a very important role in radiopharmaceuticals used with positron emission tomography. Their longer half-lives, ranging from 1.35 h to 4.15 d allow studies of slower biochemical processes. Structural

analogues are obtained when a halogen atom replaces a hydrogen atom, a methyl or a hydroxyl group, while structural changes are negligible [14].

- Metallic positron-emitters (^{64}Cu, ^{94m}Tc, ^{68}Ga, ^{82}Rb, ^{68}Ga): a few metallic isotopes are produced by generator systems (i.e. ^{82}Rb, ^{62}Cu and ^{68}Ga), thus being readily accessible even in hospitals lacking a cyclotron [15].

Chemistry with short-lived positron emitting radionuclides, principally [^{13}N] nitrogen ($T_{1/2}$ = 10 min), [^{15}O]oxygen ($T_{1/2}$ = 2 min), [^{11}C]carbon ($T_{1/2}$ = 20 min), and [^{18}F]fluorine ($T_{1/2}$ = 109.7 min) (Table 1) [16], has increased over the last decades[17].

Table 1. Physical properties of the four most conventional PET radionuclides [18].

Radionuclide	$T_{1/2}$ min	Decay mode	Production reaction	Max. β^- energy (MeV)	Max. range (mm)
^{15}O	2.04	β^+ (99.9%) e-capture (0.1%)	^{14}N(d, n)^{15}O	1.72	8.2
^{13}N	9.96	β^+ (99.8%) e-capture (0.2%)	^{16}O(p, α)^{13}N	1.19	5.4
^{11}C	20.4	β^+ (99.8%) e-capture (0.2%)	^{14}N(p, α)^{11}C	0.96	4.1
^{18}F	109.7	β^+ (96.9%) e-capture (3.1%)	^{18}O(p, n)^{18}F ^{20}Ne(d, α)^{18}F	0.63	2.4

Synthesis of ^{11}C- and [^{18}F]-labeled compounds is a rapidly growing field with applications in cardiology, neurology, biochemistry and physiology and has been successfully used for the diagnosis of cancer [19]. These radionuclides are incorporated either into compounds that are normally used by the body or into analogues of them [20].

One essential aspect in biological properties of cancer cells as they grow in an uncontrolled manner is that the cancer cells need energy to grow and divide which means a significant increase in glucose uptake. Glucose is also the main source of

energy in the human body [21]. Similarly, it is not surprising that protein helps the growth of cancer cells more quickly. This is usually associated with an elevated uptake of amino acids which are used in build the proteins [22]. Also the growth of cancer cells is usually associated with an increase in the synthesis of phosphor lipid membranes that is associated with choline transporter and choline kinase enzyme overexpression [23,24]. As a result, the considerable high level of intracellular concentration of glucose amino acids and choline (above the normal level) associate with many human cancers[25,26].

Therefore ^{11}C-labeled choline, various amino acids and glucose were developed and have shown their potential in the evaluation of brain tumors, oesophageal carcinoma and prostate cancer [27,28], and have been the most commonly used radionuclides for PET in the last decades. However, due to the short half-life of ^{11}C (20.38min), thus limited usefulness in routine clinical applications, different [^{18}F]-labelled analogues, which have longer half-life (109.7min), were synthesized.

Advantages of ^{18}F

1- A longer half-life of ^{18}F allows relatively complex synthetic manipulations and biological studies.

2- ^{18}F has the lowest positron energy which provides the sharpest imaging with a high-resolution PET.

Disadvantages of ^{18}F

1- Fluorine is a "foreign" element to most biological molecules (non-isotopic labeling).

2- A longer half-life precludes multiple experiments on the same subject in the same day.

Compared to half-life of ^{11}C (20.38 min), the longer half-life of ^{18}F (109.7 min) is more compatible with a clinical routine production of radiopharmaceuticals, including the shipment of the compounds. These factors prompted the production and development of [^{18}F]-synthesis.

Although it is not normally found within human tissues, [^{18}F]fluorine has proved to be an ideal tracer for PET because of its intermediate half-life of 109.7 min and low β^- energy (max. 0.635 MeV).

1.4 [^{18}F]fluorine:

[^{18}F]fluorine is a fluorine radioisotope which decays into the stable isotope [^{18}O]oxygine by sending a positron and a neutrino. The maximum energy of the positrons is E_{max} = 0,635 MeV. It has a mass of 18.0009380 and its half-life is 109.7 min. [^{18}F]fluorineis produced by the reaction of ^{18}O(p,n) ^{18}F.

Decay mode

$$^{18}_{9}\mathrm{F} \rightarrow {}^{18}_{8}\mathrm{O} + {}^{0}_{+1}\mathrm{e} + \nu$$

The positron travels a few millimeters in the tissue before it meets an electron, where both are annihilated producing two gamma ray photons. While the energy of each gamma ray photon is 511 KeV, the energy of the rest mass of the electron plus the positron is lost prior to annihilation. The typical exposure from a PET scan is about 7mSv [29].

Production of [^{18}F]fluorine

There are many methods to produce ^{18}F according to Table 2. The two methods are the most widely used ^{18}O(p,n)^{18}F and ^{20}Ne(d,α)^{18}F since these reactions only requires moderate beam intensities and moderate particle energies [30].

The most common is an irradiation of the stable [^{18}O]oxygen isotope with high energy protons (18 MeV) from a cyclotron [31]**.**

Where [^{18}F]fluoride is prepared by bombarding [^{18}O]oxygen enriched water with protons via nuclear reaction ^{18}O(p,n)^{18}F.

$$^{18}_{8}\mathrm{O} + {}^{1}_{1}\mathrm{P} \rightarrow {}^{18}_{9}\mathrm{F} + {}^{1}_{o}\mathrm{n}$$

[^{18}F]fluoride is then obtained as an aqueous solution of ions [^{18}F]F^- and can be easily separated by ion exchange chromatography. Ionizated ^{18}F can be transferred into the organic solvent and used for the stereospecific nucleophil substitutions. ^{18}F with specific activity 8000 GBq/μmol can be produced after one hour of the irradiation[32].

Table 2. Nuclear reactions for ^{18}F production [33,34,35].

Reaction	Target	E (MeV)	Thick target yield[a]	Main form
$^{18}O(p,n)^{18}F$	$^{18}O_2$	14-0	216 mCi/μA[b,c]	
	$^{18}O_2$	10-00	150 mCi/μA[c]	$[^{18}F]F_2$
	$H_2{}^{18}O$	16-0	110 mCi/μA[c]	$[^{18}F]F^-$
$^{20}Ne(d,\alpha)^{18}F$	^{20}Ne	14	91.9 mCi/μA[b,c]	
	0.1% F_2/Ne	14-2	12.2 mCi/μAh[d]	$[^{18}F]F_2$
	0.18% F_2/Ne	11.2-0	10 mCi/μAh	$[^{18}F]F_2$
	15% H_2/Ne	11.2-0	10 mCi/μAh	$[^{18}F]HF$
	6.7% H_2/Ne	11.2-0	8 mCi/μAh	$[^{18}F]F^-$
$^{20}Ne(d,x)^{18}Ne$[e]	10% H_2/Ne	6.3-0	11 mCi/μA[c]	$[^{18}F]HF$
$^{16}O(\alpha,d)^{18}F$	H_2O	30	1.1 mCi/μAh	$[^{18}F]F^-$
		48	7.0 mCi/μAh	$[^{18}F]F^-$
$^{16}O(\alpha,2n)^{18}Ne$[e]	O_2	40	14 mCi/μAh	$[^{18}F]HF$
$^{16}O(^3He,p)^{18}F$	H_2O	41-14	7 mCi/μAh	$[^{18}F]F^-$
$^{16}O(^3He,n)^{18}Ne$[d]	H_2O	36	7.6 mCi/μAh	$[^{18}F]F^-$
$^{20}Ne(^3He,\alpha n)^{18}Ne$[e]	2% H_2/Ne	27.5 n flux ($cm^{-2}s^{-1}$)	5-7 mCi/μAh Yield	$[^{18}F]HF$
$^{16}O(^3H,n)^{18}F$	Li_2CO_3	$3*10^{13}$	200 mCi/3h	$[^{18}F]F^-$
	$^6LiOH.H_2O$	$3*10^{13}$	250 mCi/3h	$[^{18}F]F^-$

[a] For 1 h experimental irradiation unless otherwise indicated by superscript.
[b] Theoretical yield
[c] Saturation yield
[e] ^{18}Ne decays to fluorine-18 with a half-life of 1.67 s

$[^{18}F]$fluoride can be also made as a radioactive gas via reaction $^{20}Ne(d,\alpha)^{18}F$.

$$^{20}_{10}\mathrm{Ne} + {}^{2}_{1}\mathrm{D} \rightarrow {}^{18}_{9}\mathrm{F} + {}^{4}_{2}\mathrm{He}$$

This method is useful for the electrophil substitutions and requires an addition of the gas $[^{19}F]$ Fluoride to a target as a carrier. Specific activity of a product is lower than 1 GBq/μmol [36].

Currently, the $^{18}O(p,n)^{18}F$ reaction on ^{18}O-enriched water is the most used and effective and reliable for the production of [^{18}F]fluoride ion with high specific activity. Thus, under optimized conditions, high amounts of [^{18}F]fluoride ion can easily be achieved from cyclotrons, even with low-energy machines, within less than one hour of irradiation time [37].

In the case of a neon target, [^{18}F]fluorine can be produced as [^{18}F]acetylhypofluorite from [^{18}F]F_2. In that method, the major problem is the deposition of the produced [^{18}F]fluorine on the target walls. Therefore, the target has to be treated in advance with a 1% fluorine/neon gas mixture in order to coat the target walls with F_2. The fluorine acts as a kind of "carrier gas" by facilitating the transport of [^{18}F]fluorine from the target to the laboratory. The addition of non-radioactive fluorine to the target results in a lower specific activity of the produced [^{18}F]fluorine, so this production method is not applicable for the syntheses of radiotracers that require a high specific activity [38,39].

Half-life

The half-life of [^{18}F]fluorine is 109.7 minutes which means that the radioisotope must be transported and used quickly. The ideal situation is the availability of a cyclotron in a nuclear medicine facility at a hospital. [^{18}F]fluorine is an important isotope in the radiopharmaceutical industry and is primarily synthesized into [^{18}F]fluorodeoxyglucose [^{18}F]FDG for the use in PET [40]. In recent years, the number of newly-developed ^{18}F-labeled pharmaceuticals has increased rapidly, such as [^{18}F]fluoroethyltyrosine and [^{18}F]fluoroethylcholine.

1.5 Labeling of radiotracers with [^{18}F]fluorine

To set up a safe and routine production of a desired radiotracer labeled with [^{18}F]fluorine, [^{18}F]fluoride should be produced first. This can be produced by the $^{18}O(p,n)^{18}F$ reaction with a cyclotron. The several steps depicted in Figure 2 must be completed to produce radiotracers via nucleophilic substitution reaction and which must be chemically and radiochemical pure and also free from pyrogens and sterile.

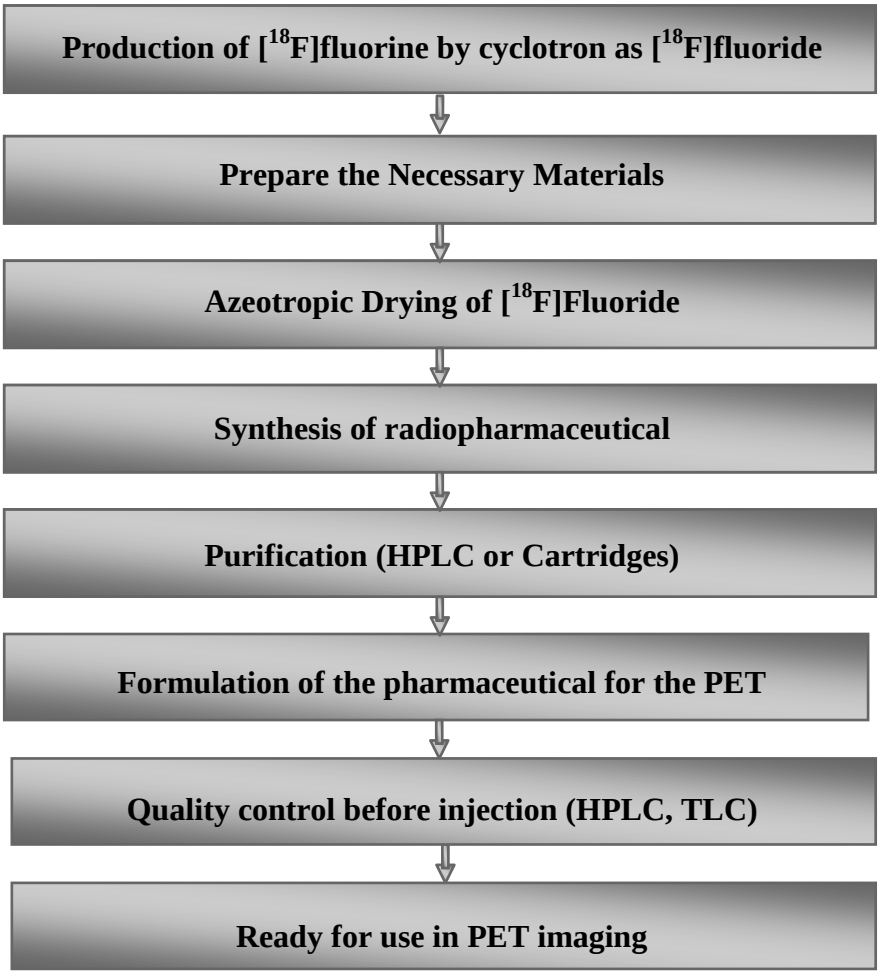


Figure 2. Main steps involved in routine preparation of radiopharmaceuticals.

"From Eeva-Liisa Kämäräinen, 2007, image no.2"[41].

[^{18}F]fluorodeoxyglucose (Figure 3) is the most commonly used radiotracer with PET and PET/CT imaging for the detection of various tumors.

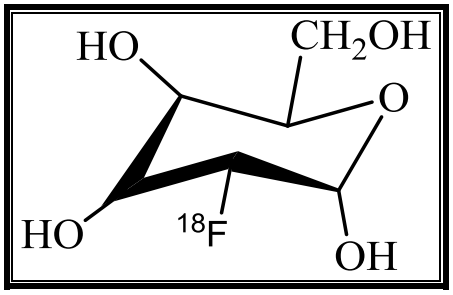


Figure 3. [^{18}F]fluorodeoxyglucose.

Although considerable progress has been made using [^{18}F]FDG in PET, there are some disadvantages which limit its use for the detection of some tumors. It cannot visualize very small tumors and brain tumors due to the high rate of physiological

glucose metabolism in normal brain tissue; the delectability of tumors with modest increases in glucose metabolism is difficult [42,43]. And [^{18}F]FDG also cannot differentiate malignancy and inflammation [44]. This is due to the fact that inflammatory cells, a result of an increased metabolic rate, take up glucose. Thus [^{18}F]FDG accumulates considerably in the same manner as in tumors. This similar accumulation of [^{18}F]FDG may lead to incorrect detection, hence the wrong diagnosis of some types of cancer [45].

Due to this limitation, efforts have been made to develop a new specific ^{18}F-labeled PET tracer. The ^{18}F-labeled amino acids have been suggested because inflammatory cells consume fewer amino acids compared with [^{18}F]FDG and cancer cells [46].

Also [^{18}F]-labeled choline has been suggested because the uncontrolled growth of cancer cells is usually associated with an increased uptake of [^{18}F]choline and phosphorylation of choline to form choline phosphate which is, in turn, used in the synthesis of membrane phospholipids. The inflamed cells consume less choline compared with [^{18}F]FDG and cancer cells. ^{11}C-choline has also been very effective in detecting prostate cancer [47].

1.5.1 Synthesis of ^{18}F-labeled choline

1.5.1.1 Choline

Choline is a special type of phospholipid, also known as 2-Hydroxy-N,N,N-trimethylethanaminum; (beta-hydroxyethyl) trimethylammonium and bilineurine that plays many roles in the body.

The chemical structure of choline is shown in Figure 4.

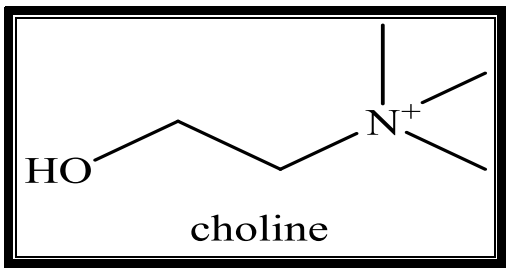


Figure 4. Chemical structure of choline.

Choline is necessary for the structure and function of all cells and is crucial for sustaining life [48]. The three major metabolic functions for choline are as a precursor for the phosphatidylcholine biosynthesis, as a precursor for acetylcholine biosynthesis and as a methyl donor [49]. In addition, choline is the precursor of the phospholipid sphingomyelin. Phosphatidylcholine and sphingomyelin are the structural components of the biological membranes. These phospholipids also serve as precursors for the intracellular messengers ceramide and diacylglycerol. Choline is also the precursor of the signaling lipids, platelet-activating factor (PAF) and sphingosylphosphoryl-choline.

Choline is an essential nutrient that is widely distributed in foods, principally in the form of phosphatidylcholine, but also as free choline. It is also found in foods in the form of the phospholipid sphingomyelin.

Choline is one of the components of phosphatidylcholine, an essential element of phospholipids in the cell membrane. Malignant tumors show a high proliferation and increased metabolism of cell membrane components that will lead to an increased uptake of choline.

Katz-Brull [50] found that choline was incorporated into the tumor cells by a carrier-mediated mechanism and then converted into phosphorylcholine within 1 hour. Haeffner [51] investigated choline transport in Ehrlich ascites tumor cells using 3H-choline and ^{14}C-choline. When incubated with tumor cells at a low concentration, choline was incorporated into the cells by an active-transport mechanism. Then, it was converted into phosphorylcholine within 1 hour and finally integrated into phosphatidylcholine.

Phosphatidylcholine is also known as lecithin. It has the chemical structure shown in Figure 5.

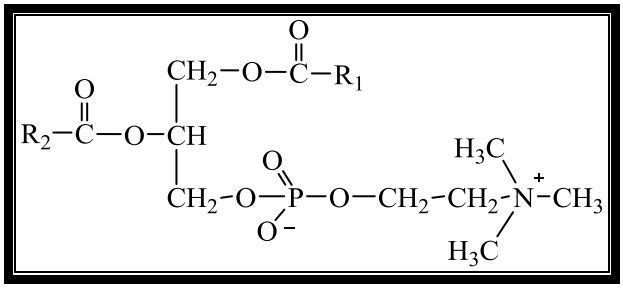


Figure 5. Structure of phosphatidylcholine. R1 and R2 are fatty acids.

Previous studies showed that tumors contain large amounts of membrane phospholipids, especially phosphatidylcholine. Therefore, the visualization of membrane phospholipids can give information about tumor growth. Because of the Previous studies showed that tumors contain large amounts of membrane phospholipids, especially phosphatidylcholine. Therefore, the visualization of membrane phospholipids can give information about tumor growth. Because of the high rate of phospholipid synthesis in tumors, [^{11}C]choline (Figure 6-1) has been used as a radiopharmaceutical for PET. Hara T. et al. Showed [52] that [^{11}C]choline is an effective tracer in imaging. As a result, it has been introduced as a tracer in PET for imaging various human tumors. However, due to the short half-life time of ^{11}C ($T_{1/2}$ = 20 minutes) [^{18}F]-labeled ($T_{1/2}$ = 109.7 minutes), choline analogues have been developed to increase its potential as a routine diagnostic tracer. Figure 6 shows the most prominent choline derivatives.

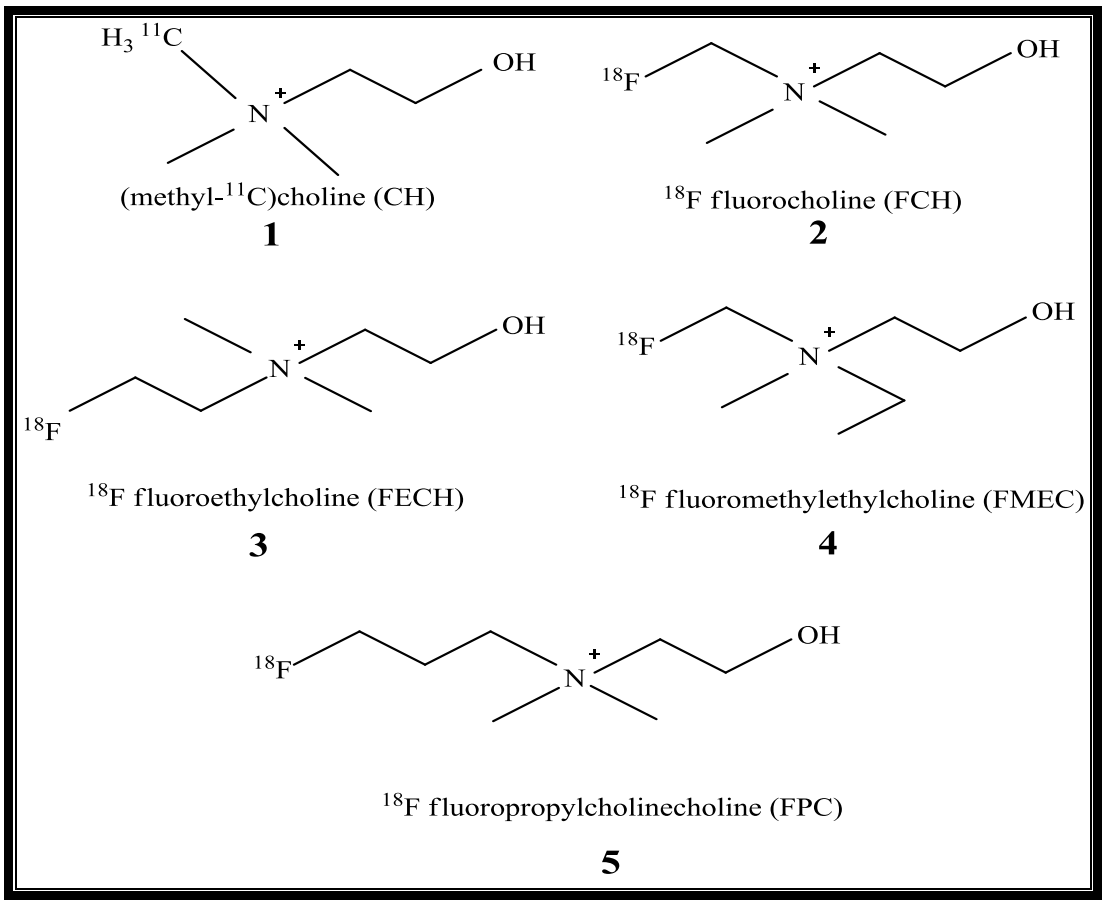


Figure 6. Structure of choline derivatives.

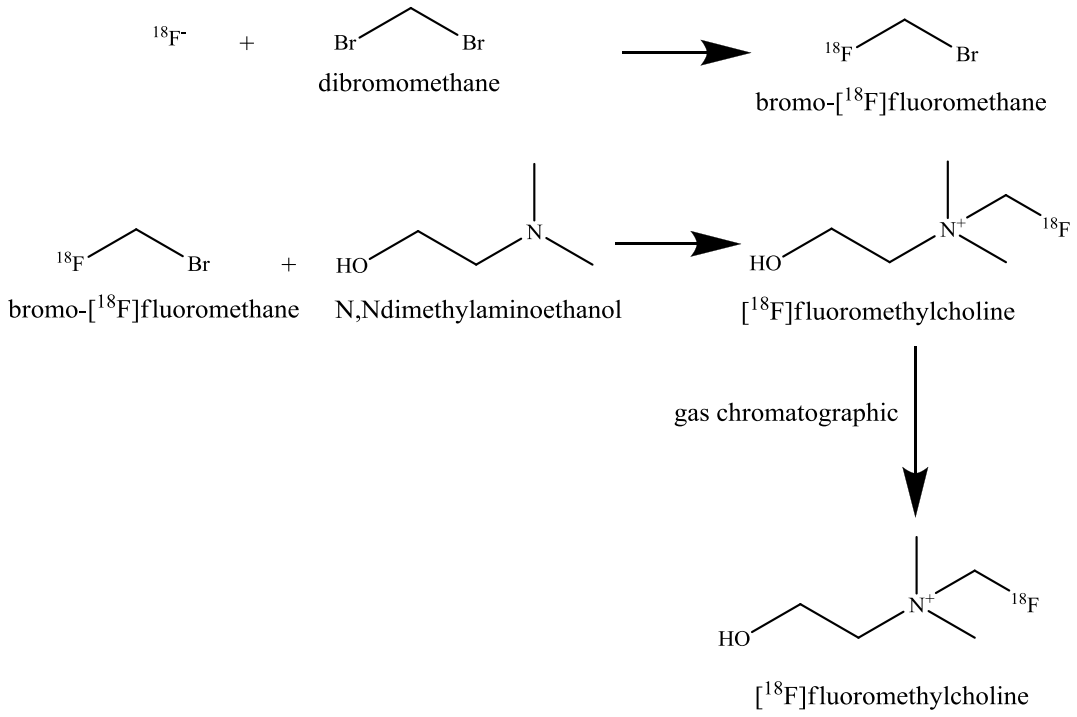
1.5.1.2 The current approaches to automated synthesis of [^{18}F]-labeled cholin

The [^{18}F]fluorocholine ([^{18}F]FC) was synthesized for the first time, by DeGrado et al. [53], by reacting N, N-dimethylaminoethanol (DMAE) with bromo-[^{18}F]fluoromethane ([^{18}F]BFM) (Scheme 1).

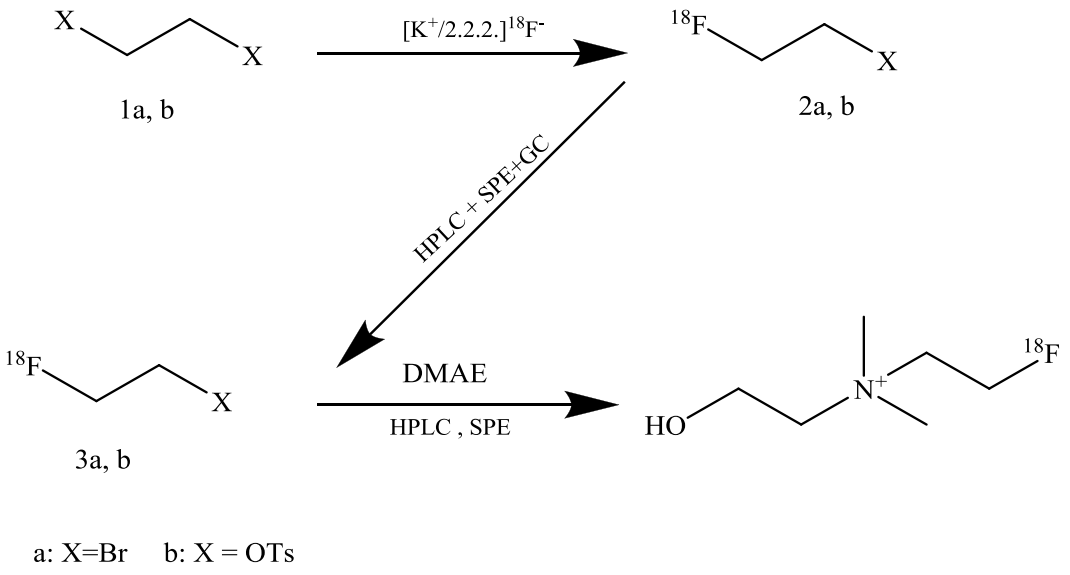
However, because this method is time consuming, for gas chromatographic purification of [^{18}F]BFM during the synthesis [54], a variety of strategies have been introduced to synthesize [^{18}F]-labeled compounds via [^{18}F]fluoroethylation [17,4].

The most successful and commonly used syntheses for [^{18}F]fluoroethylcholine ([^{18}F]FECh) are demonstrated in Scheme 2. Starting from precursor 1,2-dibromoethane (DBE 1a) or 1,2-bis(tosyloxy)ethane (DITOS 1b) the [^{18}F]-labeled ethyl derivatives (prosthetic groups 2a, b) are synthesized in first reaction step by nucleophilic substitution. In case of DBE 1a (bp 131 C), the intermediate 1-bromo-2-[^{18}F]fluoroethane ([^{18}F]BFE 2a) (bp 71.5 C) [55] has to be separated via gas chromatography before the final reaction with DMAE can be performed, leading to [^{18}F]FECh. Even if DITOS 1b is used, it is necessary to introduce an additional purification step, either before starting the second reaction via SPE/HPLC or as a final separation using HPLC [56]. Therefore, both methods require considerable efforts for purification and more importantly consume a significant amount of time. This is why only low yields of [^{18}F]FECh could be achieved until this date. As a result, the yield of a fully automated synthesis of [^{18}F]FECh is limited to only 30% and lasts as long as 50 minutes. For application in clinical services, this means a dose that is of relative high cost, which limits its use.

Consequently, the aim of the present work was to develop a simplified and considerably faster automated synthesis with higher yields, hence producing a more attractive approach for clinical routine use. In this research, the development of a new protocol for the synthesis of [^{18}F]FECh via [^{18}F]fluoroethylation using 2-bromoethyl triflate (BETfO) as the starting material in a fully automated fast synthesis without the need of GC or HPLC purification is described.



Scheme 1. Syntheses of [^{18}F]fluoroethylcholine by DeGrado [53].



a: X=Br b: X = OTs

Scheme 2. Common syntheses of [^{18}F]fluoroethylcholine [55].

1.5.2 Synthesis of [^{18}F]-labeled amino acids

1.5.2.1 Amino acids

Amino acids - as evidenced by the name - are compounds containing an amino group, -NH_2 and a carboxylic acid group, -COOH. They are found in natural proteins (and its number about 22 of type alpha) [57], where the amino acids are classified according to the amine functional group position in carbon chain to four groups (Figure 7).

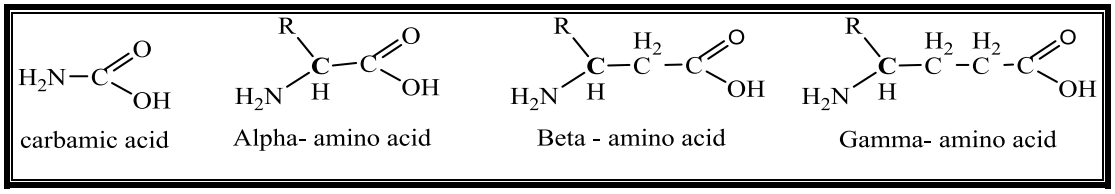


Figure 7. Amino acids classification according to the amine functional group position in carbon chain.

Alpha amino acids are the most common forms found in nature. Apart from glycine, amino acids have the property of optical activity; therefore they can be named by the direction in which they rotate the plane of polarized light. If the light rotates clockwise, the amino acid is labelled (+) and vice versa. The (+) and (−) amino acids have also been termed D- and L- **(**Figure 8).

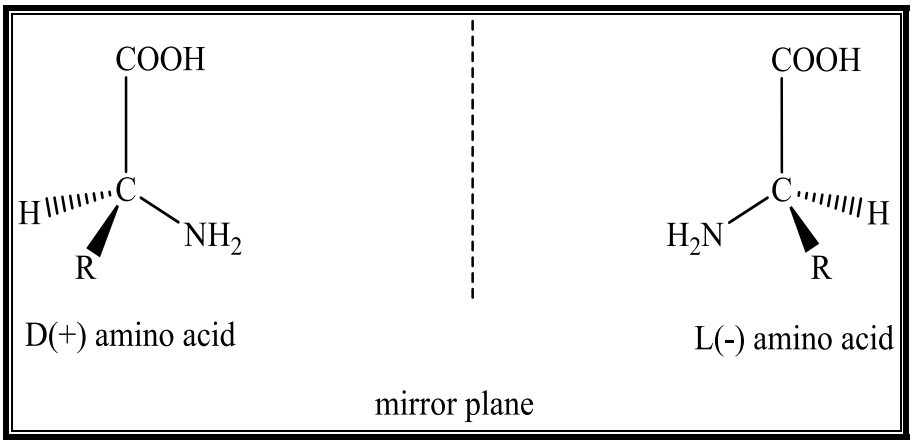


Figure 8. D and L amino acids.

The D/L labelling is unrelated to (+)/(−); it does not indicate. A rule of thumb for determining the D/L isomeric form of an amino acid is the "CORN" rule. The groups are COOH, R, NH2 and H (where R is a variant carbon chain).

If the sequence COOH, R, NH_2 is in a clockwise direction, then it is the L-amino acid. If it is a counter-clockwise, then it is the D-form.

In an amino acid, the amino group will be protonated (positive charges) and the carboxylic acid group will be deprotonated (negative charges) when the number of protonated amino group and deprotonated carboxylic acid group are equal (Figure 9). This is called the zwitterion form.

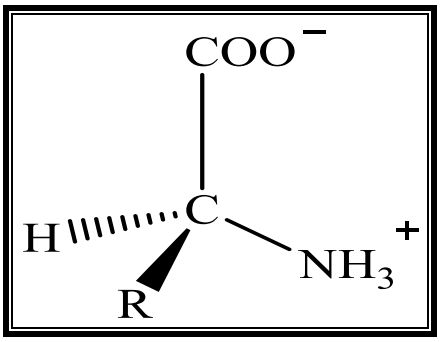


Figure 9. Amino acid is zwitterions.

Amino acids are different only by a variable part of the molecule called the R group, which plays an important role in determining the polarity of the amino acid. Therefore, amino acids are classified, based on polarity, into three groups:

1. **Polar amino acids:** These amino acids do not have any charge on the 'R' group and the R group contains an ALCOHOL, a PHENOL OH group, or N-H bonds which are polar (Figure 10). Amino acids with polar side chain are water soluble because they have extra functional groups on the R (on the side chain) that can form hydrogen bond with water.

2. **NON-POLAR:** They have an equal number of amino and carboxyls groups and are neutral. These amino acids are hydrophobic and have no charge on the 'R' group. The R group contains C-H bonds, non-polar side chain (Figure 12). Amino acids with non-polar side chain are water insoluble because they have no extra functional groups on the R (on the side chain) so, cannot form hydrogen bond with water.

3. **Charged:** the R group contains either a charged carboxyl or amino group Some amino acids have more amino groups as compared to carboxyl groups

making it basic. They have a positive charge on the 'R' group (Figure 11). Other amino acids have more carboxyl groups than amino groups making them acidic. They have a negative charge on the 'R' group (Figure 13).

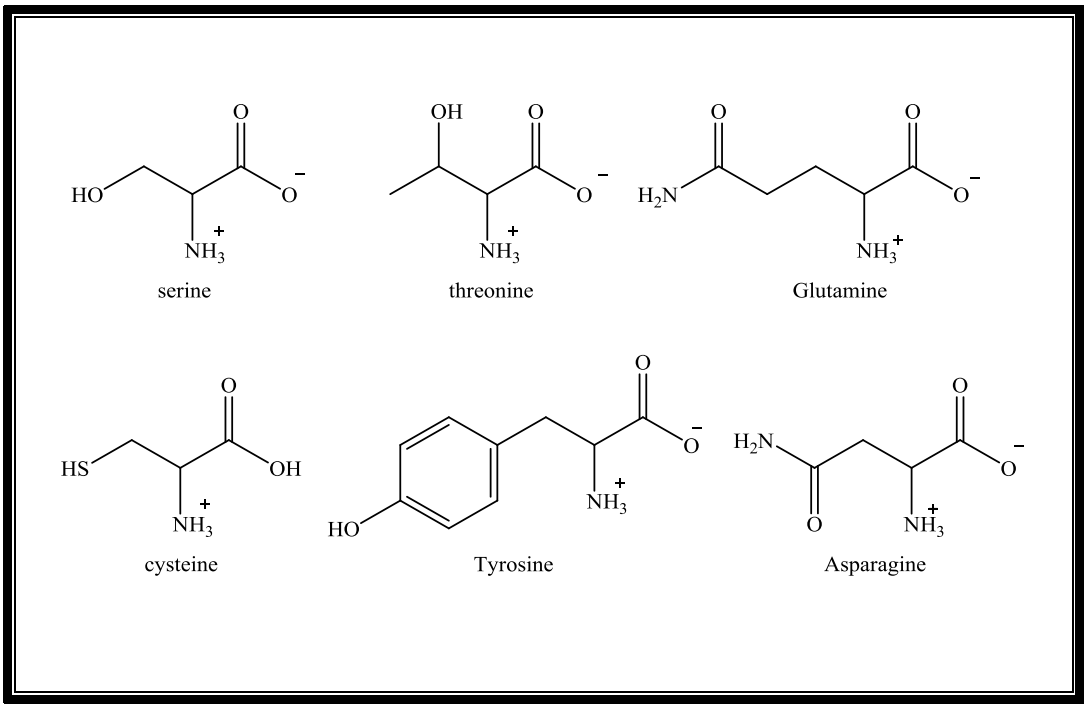


Figure 10. Polar amino acids.

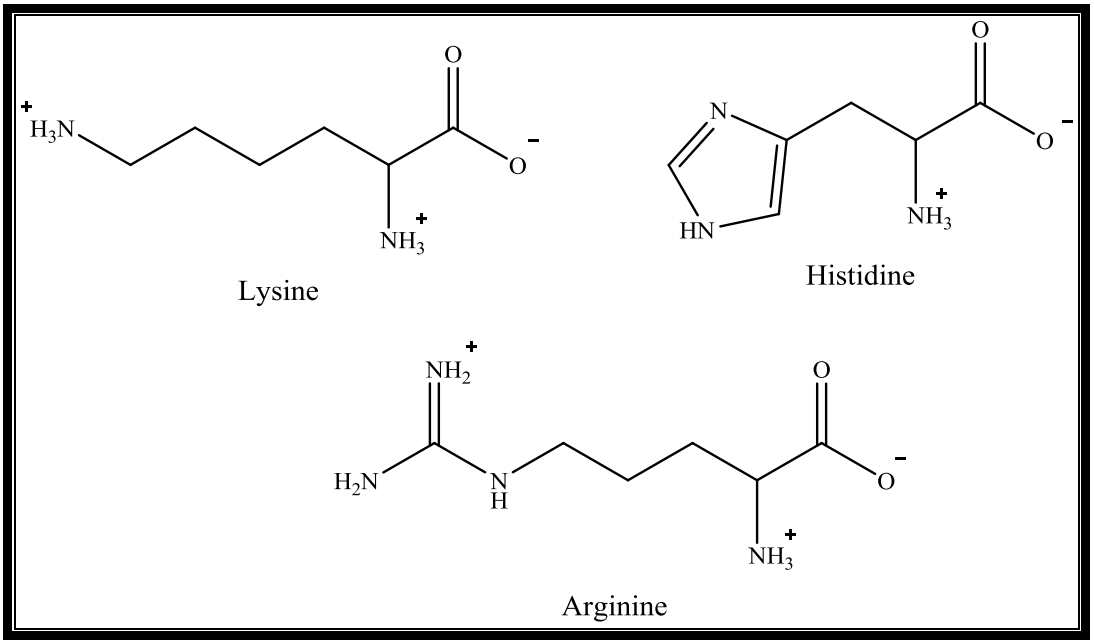


Figure 11. Amino acids have a positive charge on the 'R' group.

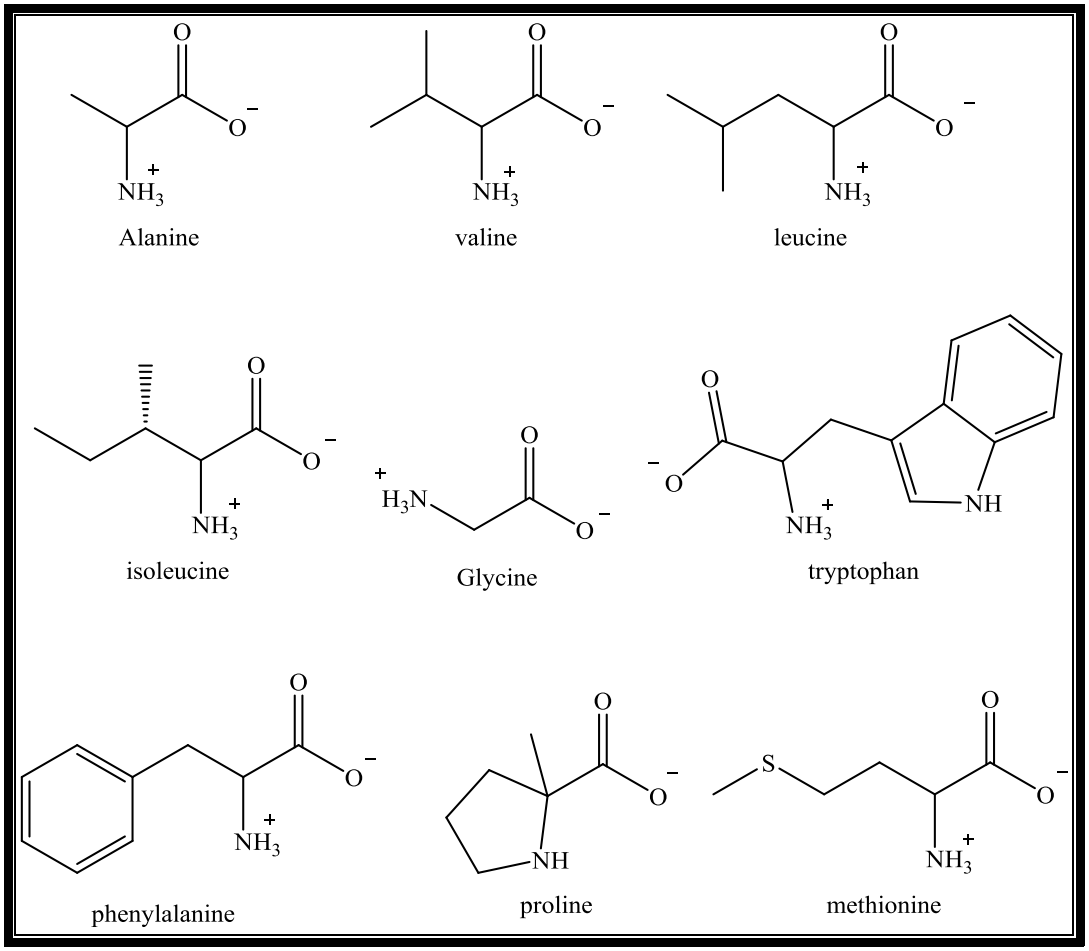


Figure 12. Non-Polar amino acids.

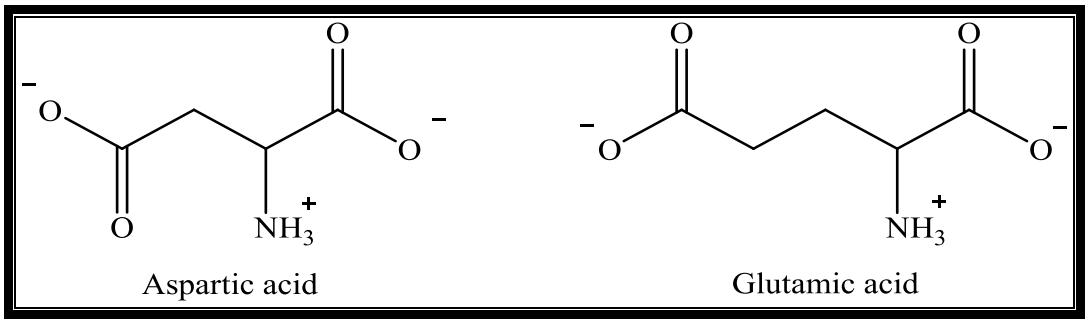


Figure 13. Amino acids have negative charge on the 'R' group.

Amino acids are the monomeric building blocks of proteins and provide essential functions in the cells. They are precursors for other biomolecules such as histamine, thyroxine, adrenaline, melanine and serotonine. In addition, amino acids can be crucial in the metabolic cycles; methionine is a methyl group donor in many biosynthetic pathways [58]. Amino acid contributes to several other processes rather than protein synthesis, namely: transamination and transmethylation. The specific role

of methionine in the initiation of protein synthesis is the use of amino acids as glutamine for energy and as precursors for other biomolecules. On the contrary, the fraction of amino acids entering the protein synthesis may also correlate with proliferation malignancy, due to the fact that tumour cells require nutrients for their growth. Most tumour cells share the ability to concentrate amino acids more efficiently than normal cells. Consequently, amino acid transport is in general increased in malignant transformation. Recently, several amino acids have been labelled with either gamma radiation-emitting isotopes or positron-emitting isotopes, even though PET resolution can be considered better [56].

[^{11}C]Methionine ([^{11}C]MET) is the most common amino acid PET tracer used currently, due to its fast and simple radio synthesis with high radiochemical yield without the need for complex purification steps. The former amino acid analogue MET has been used in many studies [59]. However, due to its short physical half-life of [^{11}C]-label, this tracer remains restricted to a few PET centra with an on-site cyclotron. In order to overcome this, [^{18}F]-fluorinated amino acids, such as *o*-(2-[^{18}F]fluoroethyl)-tyrosine ([^{18}F]FET), have been developed. The first clinical studies with [^{18}F]FET in patients with brain tumours showed similar results in comparison to [^{11}C]MET [60].

1.5.2.2 The current approaches to an automated synthesis of o-(2-[^{18}F]fluoroethyl)-tyrosine

o-(2-[^{18}F]fluoroethyl)-l-tyrosine [^{18}F]FET (Figure 14) is one of the first [^{18}F]-labeled amino acids that can be produced in large amounts for clinical purposes and is applicable for PET studies in a satellite concept similar to the widely used [^{18}F]FDG.

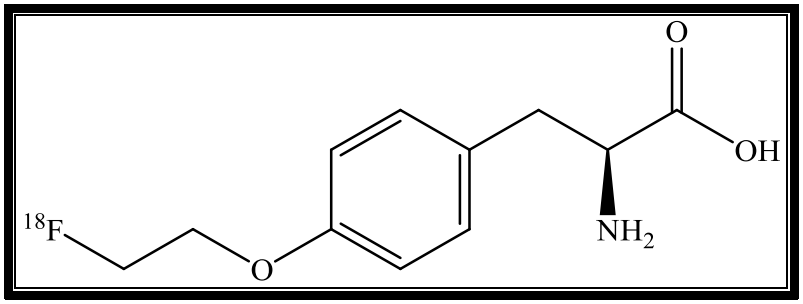


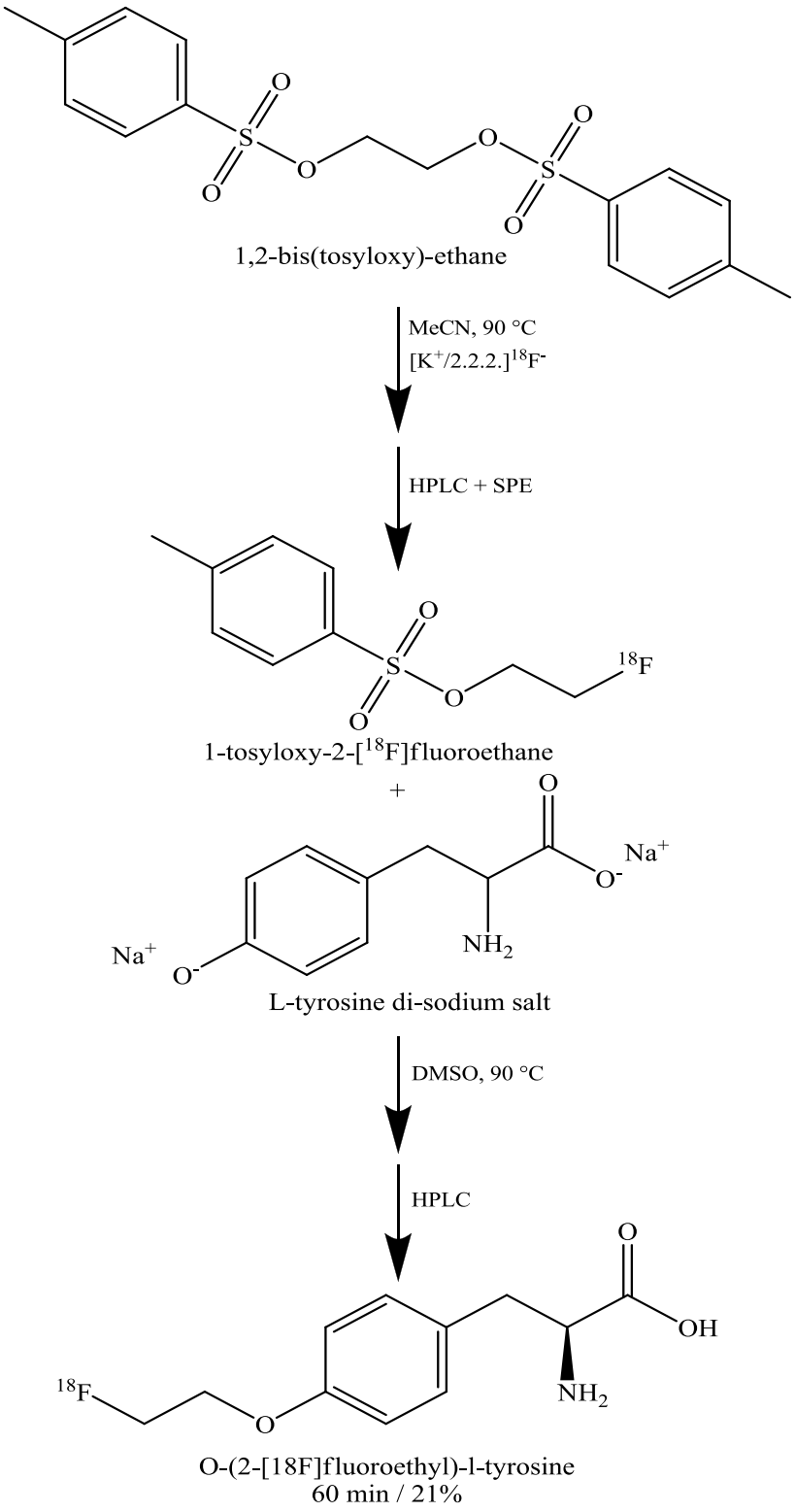
Figure 14. O-(2-[^{18}F]fluoroethyl)-l-tyrosine [^{18}F] FET.

Radio labeled amino acids and their analogues are established tracers in diagnostic oncology. Their use in the single-photon emission tomography (SPECT) and positron emission tomography (PET) is based on their enhanced accumulation into malignant transformed cells by increased expression of amino acid transporters. This reflects an enhanced amino acid metabolism and protein synthesis in growing tumors. O-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) overcomes the disadvantages of [^{18}F]fluorodeoxyglucose, [^{18}F]FDG, and [^{11}C]methionine, [^{11}C]MET. Traditional synthetic methods providing [^{18}F]FET exhibit the disadvantage concerning the necessity of two purification steps during the synthesis including HPLC purification, which causes difficulties in the automation, moderate yields, and long synthesis times >60 minutes. There are two approaches of nucleophilic substitution reaction (Scheme 3 and 4) for the synthesizing of [^{18}F]FET.

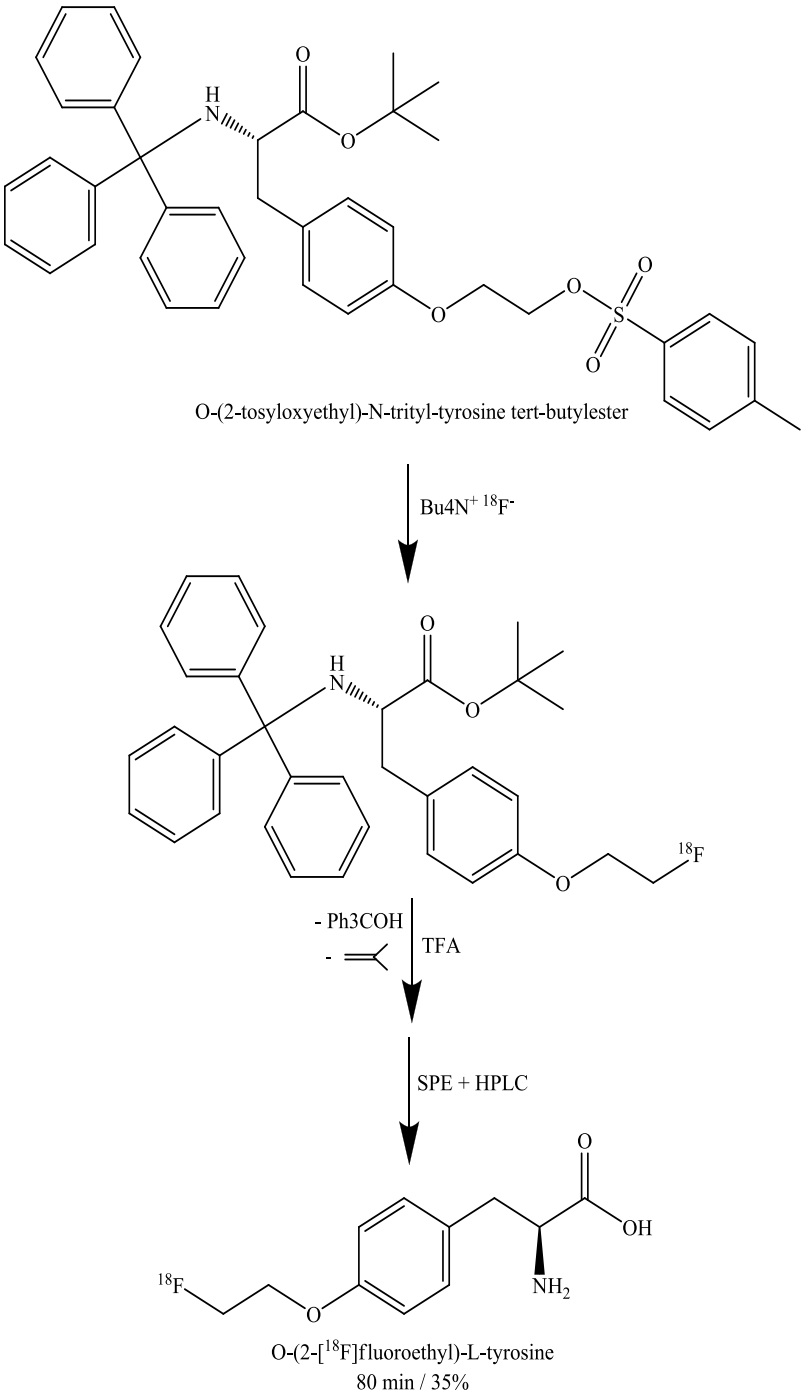
The first one is a two-step reaction starting with the fluorination of 1,2-bis(tosyloxy)-ethane (DITOS) to form 1-tosyloxy-2-[^{18}F]fluoroethane followed by fluoroethylation of unprotected L-tyrosine disodium salt (Ty-Di-Salt) and finally using HPLC for purification to get 21% [^{18}F]FET in 60 minutes [61].

The second is the synthesis of [^{18}F]FET via direct nucleophilic radiofluorination of the protected precursor O-(2-tosyloxyethyl)-N-trityl-tyrosine tert-butylester yielding about 36% [^{18}F]FET in 80 minutes[62]. In both approaches, considerable efforts are required to ensure the purification using HPLC. In addition, they consume a lot of time causing long synthesis times and low yields.

The introduction of a simple solid phase extraction instead of HPLC, to separate the final product after direct nucleophilic radiofluorination of the protected precursor N-Boc-(o-2-tosyloxyethyl)-L-tyrosine methyl ester, led to [^{18}F]FET in only 35% yield after 80 minutes [63]. Thus, the aim of the present study was to develop a simplified and quick automated synthesis with higher yields to pursue the clinical routine use.



Scheme 3. Approach 1 to produce syntheses of [^{18}F]FET [59].



Scheme 4. Approach 2 to produce syntheses of [^{18}F]FET [60].

2. Aims of the study

The general aim of this study was to develop the synthesis of [^{18}F]fluoroethylcholine and synthesis of [^{18}F]fluoroethyltyrosine.

The following objectives were set:

1. To develop an optimized synthesis method for [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine.

2. Production of high radioactivity amounts of [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine.

3. Using 2-bromoethyl triflate (BETfO) as the starting material in a fully automated fast synthesis without the need of GC or HPLC purification.

4. The depended on the distillation of 1-bromo-2-[^{18}F]fluoroethane. Obtain the best radiochemical and chemical purity.

5. Automated synthesis of [^{18}F]fluoroethylcholine and [^{18}F]fluoroethylcholine in the least time possible.

3. Materials and methods

3.1 Materials

Chemicals were commercially obtained and were used without further purification. The Sep-Pak_ Light Accell Plus CM cartridges (Waters) and The Chromafix C18 cartridges (Macherey Nagel) were used. The [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F reaction with a CTI RDS111 cyclotron (Berlin). HPLC was performed with HP 1100 pump with UV and gamma radiation detection (Gabi, Raytest). All demonstrated data are not decay corrected. Gas chromatography was performed with Varian 3350 spectrometer and FID detection.

3.1.1 Sep-Pak ® Light Accell Plus CM cartridge 37-55µm 50/box

Silica-based hydrophilic weak cation-exchanger with large pore-size was used for the isolation of cationic proteins.

Due to the large pore-size, and after pilot procedures, extraction of cationic analytes in aqueous and non-aqueous solutions was optimized for the isolation of cationic proteins.

Pore size (nominal): 300 Å

Particle size: 37-55 µm

Bonding chemistry: Polymeric:

- An acrylic acid/acrylamide copolymer on Diol silica
- Ligand density: 350 µmoles/g
- Approximate protein binding capacity = 175 mg Cytochrome c/gm of packing or 63 mg/ Sep-Pak Accell Plus CM Cartridge.

Surface functionality: $-CO^{2-}$ Na^{+}

pH of a 10% aqueous slurry: 4

Carbon content: 5.5%

Table 3. Properties of the Sep-Pak ® Light Accell Plus CM cartridge**.**

Mode:	Cation Exchange
Format/Type:	Cartridge
Brand:	Sep-Pak®
Chemistry:	Accell CM
Sorbent Substrate:	Silica
Sorbent Weight:	360 mg
Particle Size:	37-55 µm
pH Range:	2 - 8
Pore Size:	300Å
Units in Package:	50/pkg
Ion Exchange Capacity:	370 µeq/mL
% Carbon:	5.5
Water Wettable:	No
Mass Spec Compatibility:	No

The Sep-Pak Accell Plus CM was used. The Sep-Pak Accell Light cartridges (Waters) were activated with EtOH (5 mL) and HCL (10 mL) and water (2, 5, 5 mL) before use.

Figure 15. Sep-Pak ® Light Accell Plus CM cartridge (Waters) [64].

3.1.2 Chromafix 45-PS-HCO_3

UKA [^{18}F]F^- separation cartridge. Anion exchange column, (45 mg, HCO_3^- form). Cartridge filled with strong basic anion exchange based on polystyrene-divinylbenzene in HCO_3^- from. Pore Diameter of resin is 100 Å.

Figure 16. Chromafix 45-PS-HCO_3 (Macherey Nagel) [65].

UKA [^{18}F]F^- separation cartridge was conditioned with 1 mL ethanol, 1 mL water prior to use.

3.1.3 Chromafix C18 cartridges (Macherey Nagel)

C18 Chromafix cartridges (Figure 17) were purchased from macherey-Nagel (Germany). Is octadecyl modified silica phase for SPE, pore size 60Å, particle size 45µm for C18.

Every cartridge was activated with 10 mL of ethanol and washed with 30 mL of NH_4Ac.

Figure 17. Chromafix C18 cartridges (Macherey Nagel) [66].

3.1.4 The synthesis device

Theexplora RN synthesis module was used for synthesis of [^{18}F]fluoroethylcholine or synthesis of O-(2-[^{18}F]fluoroethyl)-L-tyrosine and controlled by the GINA Star SynChrom™ software Version 3.00 release (Raytest GmbH, Straubenhardt, Germany). To carry out the synthesis of [^{18}F]Choline, the valve configuration was modified to the standard assignments. The module was automatically operated during the synthesis. However, manual control of individual components at any time during the process was also available. [^{18}F]fluoroethylcholine synthesis method was written and controlled via the GINA Star SynChrom™ program and a laptop computer in a stepwise time-dependent sequence of events (e.g., valves open and close, vacuum pump on and off, reaction vessel temperature heating and cooling, etc.). The laptop communicates with the GINA Data Acquisition and Control Interface box, which in turn controls the module.

During the synthesis process, different parameters are recorded and became part of the data file, thus documenting each run. These parameters included radioactivity detectors (target, reactor, product), temperature and pressure in the reaction vessel.

A schematic module of the synthesis process of [^{18}F]fluoroethylcholine is shown in Figure 18. And a schematic module of the synthesis process of O-(2-[^{18}F]fluoroethyl)-L-tyrosine is shown in Figure 19.

Reagents and solvents are added from glass vials mounted on their respective valves. The addition and transfer of solvents and reagents was carried out by helium overpressure. The glassy carbon reaction vessel was equipped with a temperature control device, a radioactivity detector, and was connected to a pressure gauge.

The target (radioactive material) was transformed through four additional radioactivity detectors to measure its radioactivity. The reaction vessel has a pneumatically operated PEEK needle for helium overpressure transfer of the reaction mixture to either the C18 Sep-Pak cartridge or to the injection valve loop. Before the synthesis, the module was cleaned by two automated methods. Firstly, an aqueous wash method to remove water-soluble contaminants from the reagent vials and reaction vessel .Secondly, an organic solvent (CH_3CN) wash method to rinse all

valves, glass reagent vials, tubing, reaction vessel. Finally, all module components are dried using helium gas flow under vacuum.

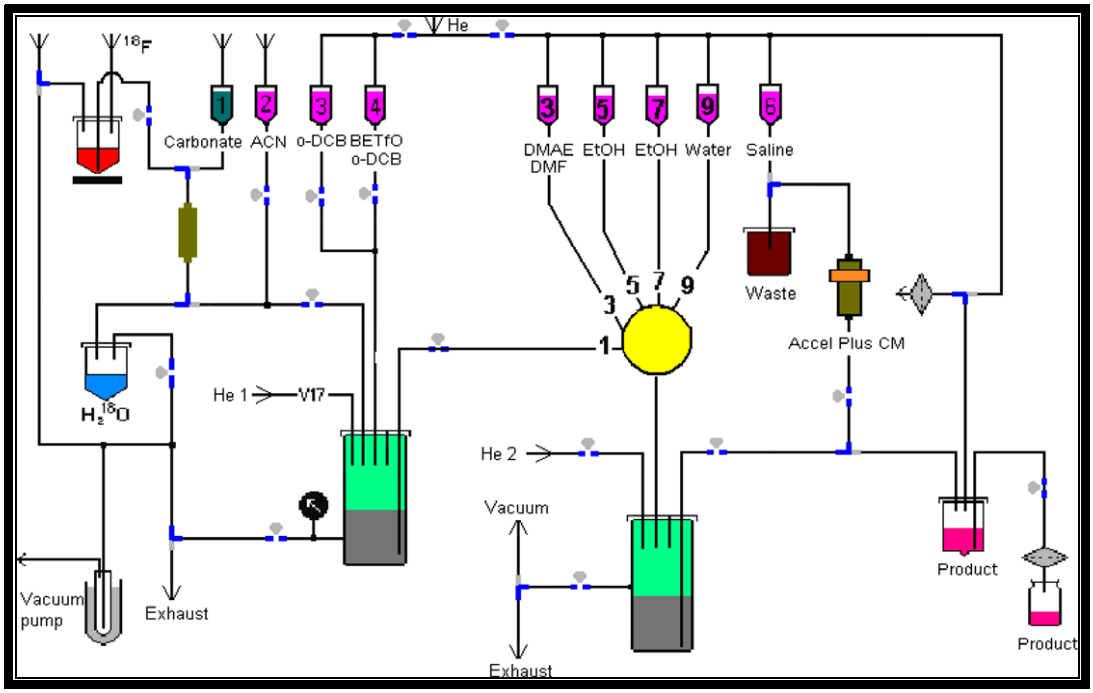


Figure 18. A schematic module of the synthesis process of [^{18}F]fluoroethylcholine. (figure designed in GINA Star SynChrom™)

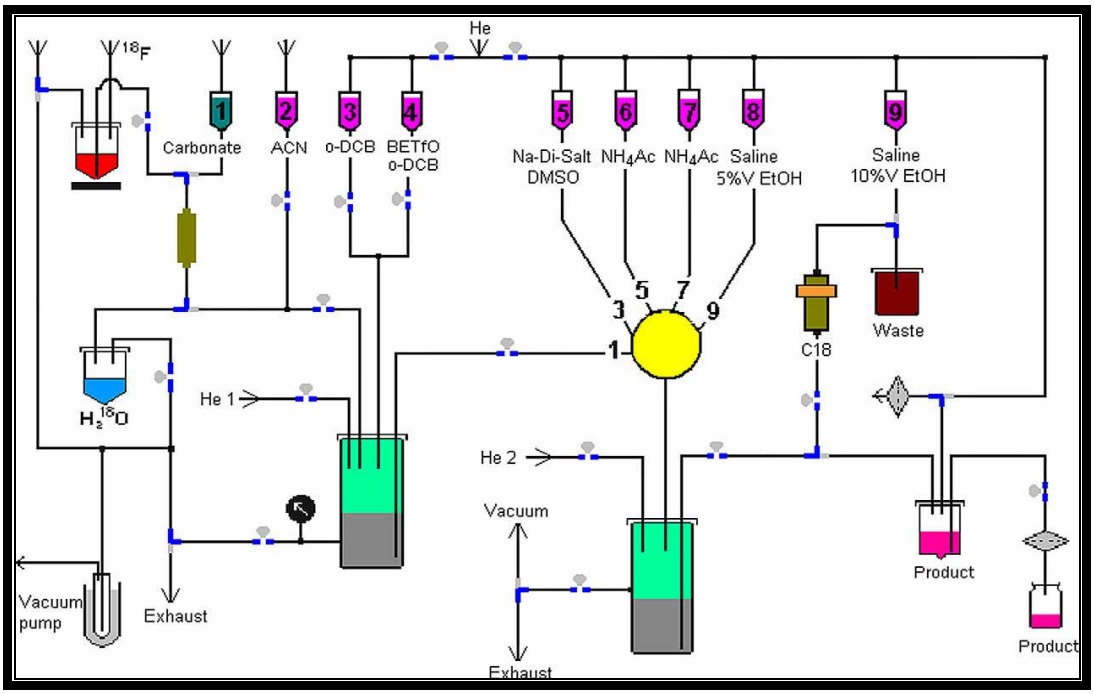


Figure 19. A schematic module of the synthesis process of O-(2[^{18}F]fluoroethyl) L-tyrosine. (figure designed in GINA Star SynChrom™)

3.2 Methods

3.2.1 (2-bromo-1-[((Trifluoromethyl)Sulfonyl)oxy]Ethane)

BETfO [67] was synthesized by dissolving 2-bromoethanol (1 g, 8.0 mmol) in 2.05 mL of 2,6-lutidine (17.6 mmol), diluted with 10 mL of CH_2Cl_2 and cooled to 0 °C. Trifluoromethanesulfonic acid anhydride (2.83 mL, 16.8 mmol) was added dropwise. After being stirred for 30 minutes, the solvent was removed in vacuum and the product was distilled (50 C at 0.5 mm Hg) to give 3.5 g (53%) of BETfO as a colorless liquid: GC chromatogram, single peak t_R = 7.82 minutes (column Rtx® 200, 0.25 mmID, 30m, carrier gas N2, 0.95 mL/min, injector 250 °C, program 50 °C (5 minutes hold, than 10 °C/min).

3.2.2 Radiosynthesis (Automated synthesis and purification of [^{18}F]fluoroethylcholine

Raw materials were tested using validated methods and qualified equipment. The quality control involved measurement of the chemical, the radiochemical purity via HPLC, gas chromatography, the pH and other parameters were tested. [^{18}F]Fluoride ion (2–10 GBq) obtained from the target was trapped in a small anion exchange column (30 mg, HCO_3^- form). The radioactivity was eluted with an aqueous solution of K_2CO_3 (11 mg; 0.08 mmol) in 0.8 mL of H_2O into reactor 1. A solution of K_{222} (30 mg; 0.08 mmol) in 1.5 mL of CH_3CN was added, and the mixture was evaporated at 90 °C under a reduced pressure (mention value) with a helium flow of 100 mL/min for 2 minutes and without helium flow for additional 2 minutes. 0.5 mL of *o*-DCB were then added to the dried [^{18}F]KF–K_{222}. After one minute of stirring at 100 °C, BETfO (25 µL) (dissolved in 0.5 mL of *o*-DCB) was added and the temperature was allowed to increase to 130 °C while [^{18}F]BFE was transferred within 5 minutes into the second reactor containing 0.5 mL of DMAE (5.0 mmol) in 1.7 mL of DMF.

The second reactor was tempered for 15 minutes at 100 °C, then the temperature was decreased to 60 °C. Five milliliters of ethanol were added and the reaction mixture was passed through a Sep-Pak Accell Light cartridge (Waters). The cartridge was then washed with 10 mL of ethanol and 10 mL of H_2O, respectively.

The [^{18}F]FECh was then eluted with 1 mL of 10% NaCl and diluted with 9.0 mL of sterile water. After sterile filtration, 0.94 to 4.7 ± 5% GBq [^{18}F]FECh were obtained. The radiochemical purity was > 99.9% and the specific activity >55 GBq/ µmol.

3.2.3 Radiosynthesis (Automated synthesis and purification of O-(2-[^{18}F]fluoroethyl)-L-tyrosine

The [^{18}F]fluoride (5-10 GBq) was delivered to a synthesis module as a solution in [^{18}O]H_2O (2 mL). This solution was passed through a PS HCO^-_3 cartridge to trap the [^{18}F]fluoride and recycle the [^{18}O]H_2O. The [^{18}F]fluoride was then eluted into the Nuclear Interface glassy-carbon reaction vessel using a solution of aqueous potassium carbonate (11 mg in 0.8 mL H_2O). A solution of kryptofix-[2.2.2] (30 mg in 1,5 mL MeCN) was added and the reaction mixture was azeotropically dried, initially at 90 ºC with both vacuum and helium flow for 2 minutes and subsequently with vacuum for additional 2 minutes.

0.5 mL of *o*-DCB were added to the dried [^{18}F]KF–K_{222}. After one minute of stirring at 100 °C BETfO (15 µL) dissolved in 0.5 mL of *o*-DCB was added while [^{18}F]BFE was transferred within 8 minutes into the second reactor containing 11.5 mg tyrosine (63.4 µmol), 50 µL of 10% aqueous (NaOH) sodium hydroxide, 5 mg NaI (33 µmol), and 800 µL of DMSO.

After [^{18}F]BFE was transferred by distillation into the second reactor the second reactor was tempered for 15 minutes at 110 °C. Afterwards the temperature was decreased to 60 °C, 8 mL of NH_4Ac (0.1 mol/L) were added and the reaction mixture was passed through four C18 Chromafix cartridges (Macherey Nagel). The cartridge was then washed with with 5 mL of NH_4Ac and 5 mL of 10% ethanol, respectively. Then the [^{18}F]FET was eluted with 10 mL of 20% ethanol.

After sterile filtration 2–4.1 ± 5% GBq [^{18}F]FET were obtained (45 ± 2.3%). The radiochemical purity was >99.9% and the specific activity >80 GBq/µmol at the end of synthesis (EOS). Synthesis time was 35 minutes from EOB.

3.2.4 Statistical Analysis

Due to the high level of radioactivity, the equipment used to conduct the experiments could only be used once a day as there had to be about 24-hour interval between experiments due to [^{18}F]fluorine has half-life time (109.7min). The costs of [^{18}F]fluorine also limited the number of the Experiments. Therefore the statistical analysis was conducted with a small number of experiments.

For the statistical analysis data obtained from the experiments were put into SPSS version 13 and were analyzed using Student's one-sample t-test.

3.2.4.1 Statistical Analysis for the optimization of reaction parameters

The main aim of this thesis was to obtain a high radiochemical yield of [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine. Therefore the conditions necessary to optimize the radiochemical yield were studied systematically by variation of the reaction parameters (e.g. Drying temperature, helium flow rate, reaction time etc.). The experiments were run five times for each parameter and the means, standard deviation, error standard deviation, coefficient of variation and P-value for the best conditions were determined and expressed in the figures 20-33 as mean ± standard deviation (SD) and P value was less than 0.0001.

3.2.4.2 Statistical Analysis for the optimized synthesis of [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine

After determinating the best conditions, which are necessary to produce [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine with the highest radiochemical yield, the experiment was repeated 10 times using the best conditions. The summarized results are shown in table 4.

Table 4: The Radiochemical yield for [^{18}F]FECh and[^{18}F]FET .

Run	1	2	3	4	5	6	7	8	9	10
Radiochemical yield of [^{18}F]FECh %	44	46	49	48	50	47	43	46	47	50
Radiochemical yield of [^{18}F]FET %	41	43	47	48	47	44	45	43	45	47

The data were put into SPSS version 13 and analyzed using Student's one-sample t-test. The means, standard deviation, coefficient of variation, error standard deviation were calculated (table 5).

Table 5: One-Sample Statistics for [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyl-tyrosine.

Value	N	Mean	Std. Deviation	Std. Error Mean	coefficient of variation
[^{18}F]FECh	10	47.0000	2.35702	0.74536	5
[^{18}F]FET	10	45.0000	2.26078	0.71492	5

P value and statistical significance:

To calculate the P-value our radiochemical yields for [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine were compared with the best radiochemical yield achieved in earlier studies (Piel M et al 30% [56] and Wang H et al 35% [63] (table 6, 7)).

As shown in tables 6 and 7 the two-tailed P value is less than 0.0001. By confidence Interval of 95% , the improvement of the radiochemical yield of 17% and 10% for [^{18}F]fluoroethylcholine and [^{18}F]fluoroethyltyrosine respectievely is considered to be extremely statistically significant.

Table 6: One-Sample Test for [^{18}F]fluoroethylcholine.

Test Value = 30					
				95% Confidence Interval of the Difference	
t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
22.808	9	less than 0.0001	17	15.3	18.6

Table 7: One-Sample Test for [^{18}F]fluoroethyltyrosine.

Test Value = 35					
				95% Confidence Interval of the Difference	
t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
13.988	9	less than 0.0001	10	8.3	11.6

4. Results

[^{18}F]FET or [^{18}F]FECh can be produced in a three -step process:

- The first reaction (formation of [^{18}F]BFE)
- The distillation of [^{18}F]BFE
- The second reaction (formation of [^{18}F]FET or [^{18}F]FECh)

The individual reaction steps were studied systematically to the optimization of the three processes (reaction 1, distillation and reaction 2).

4.1 Reaction 1: Formation of [^{18}F]BFE

The adequate formation of the [^{18}F]KF-K_{222}-complex is essential for the fluorination reaction of BETfO leading to [^{18}F]BFE. Therefore, the effect of the used amounts of the reactants, K_2CO_3/K_{222}, and BETfO, and the effect of the drying temperature as well as the helium flow rate on the formation of [^{18}F]BFE were investigated.

4.1.1 The effect of the quality of K_2CO_3/K_{222}

The [^{18}F]fluoride is usually separated from the target water by an anion exchange column, hence the [^{18}F]fluoride was passed through a small anion exchange column (45 mg, HCO_3^- form) and the [^{18}F]fluoride was fixed by a small anion exchange column.

The main issue is how to extract [^{18}F]fluoride from the anion exchange column and maintain [^{18}F]fluoride. There is no doubt that this step is the most important for the success of the next steps. A counter ion should usually possess sufficient solubility within the reaction media to maintain the solubility of fluoride. Therefore Kryptofix [2.2.2] was used as a complexing agent, improved the K^+ solubility [68].

Therefore, the effect of the quantity of K_2CO_3/K_{222} was investigated. The [^{18}F]fluoride was passed through a PS HCO_3^- cartridge to trap the [^{18}F]fluoride and to recycle the [^{18}O]H_2O. The [^{18}F]fluoride was then eluted into the reaction vessel using a solution of different quantities of potassium carbonate in 0.8 mL H_2O.

A solution of different quantities of kryptofix-[2.2.2] in 1.5 mL MeCN was added and the reaction mixture was dried, initially at 90 °C with both vacuum and argon flow for 2 minutes and subsequently with vacuum for additional 2 minutes.

The results are summarized in Figure 20. This figure clearly shows that when the amount of Kryptofix/Carbonate is increased; the radiochemical yield improves. This improvement continues until a level of 85 µmol has been reached, after which, the radiochemical yield levels off. So, the maximum yield of [^{18}F]BFE (84 ± 1.5%) was achieved when using 85 µmol K_2CO_3/K_{222}.

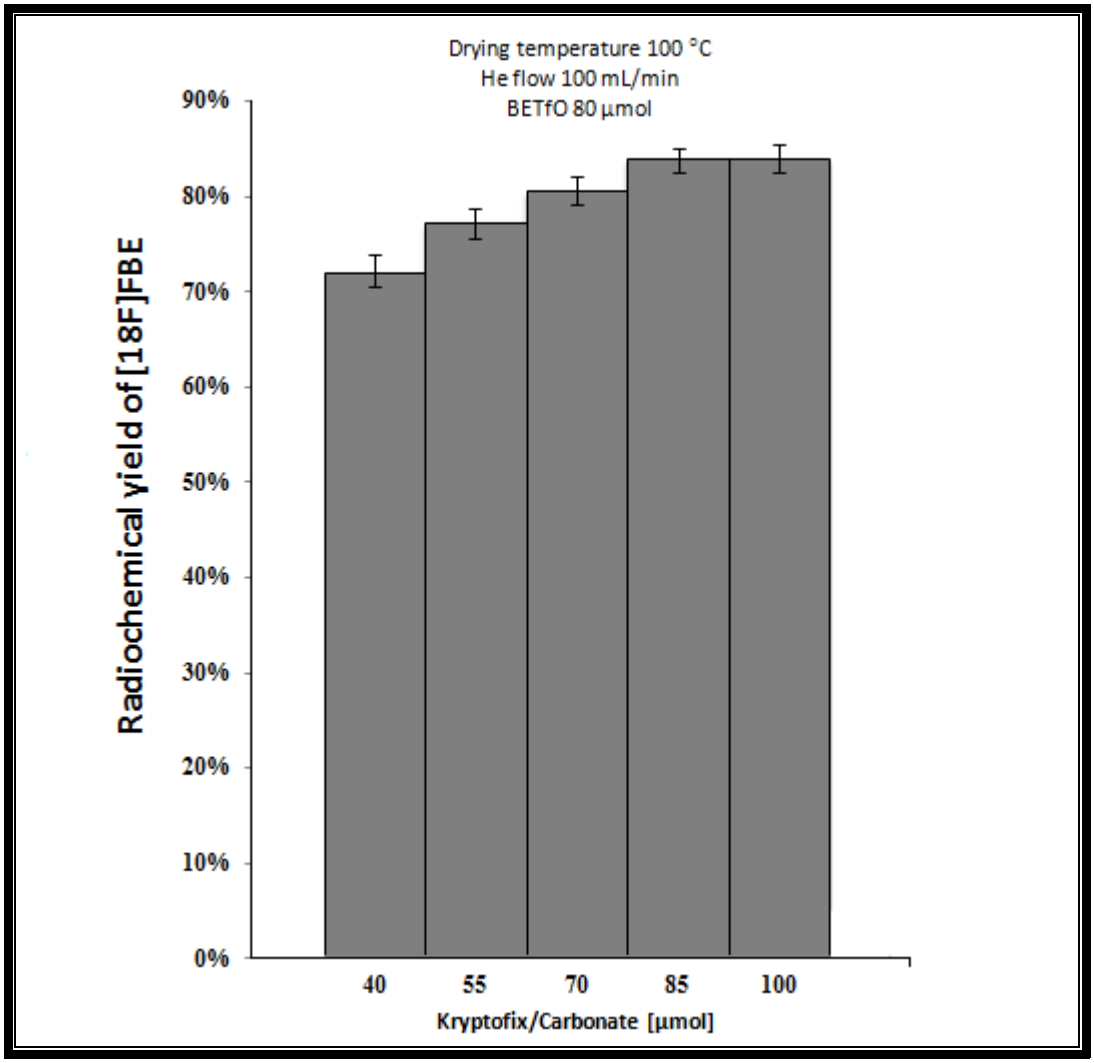


Figure 20. Dependence of the radiochemical yield of [^{18}F]BFE on the amount K_2CO_3/K_{222} . The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.2 The effect of drying temperature

A solution of kryptofix-[2.2.2] (80 µmol in 1,5 mL MeCN) was added and the reaction mixture was dried to exclude MeCN at different temperature degrees. The effect of drying temperature was investigated with both vacuum and helium flow of 100 mL/min for 2 minutes and subsequently with vacuum for additional 2 minutes.

The results are represented in Figure 21. The maximum yield of [^{18}F]BFE (82 ± 1.1%) was achieved when at a drying temperature of 90 °C. At higher temperatures the yield of [^{18}F]BFE became lower due to the decomposition of [^{18}F]KF–K_{222}. At temperatures that are lower than 90 °C, the yield of [^{18}F] BFE became lower because the boiling point of the acetonitrile is about 85 °C.

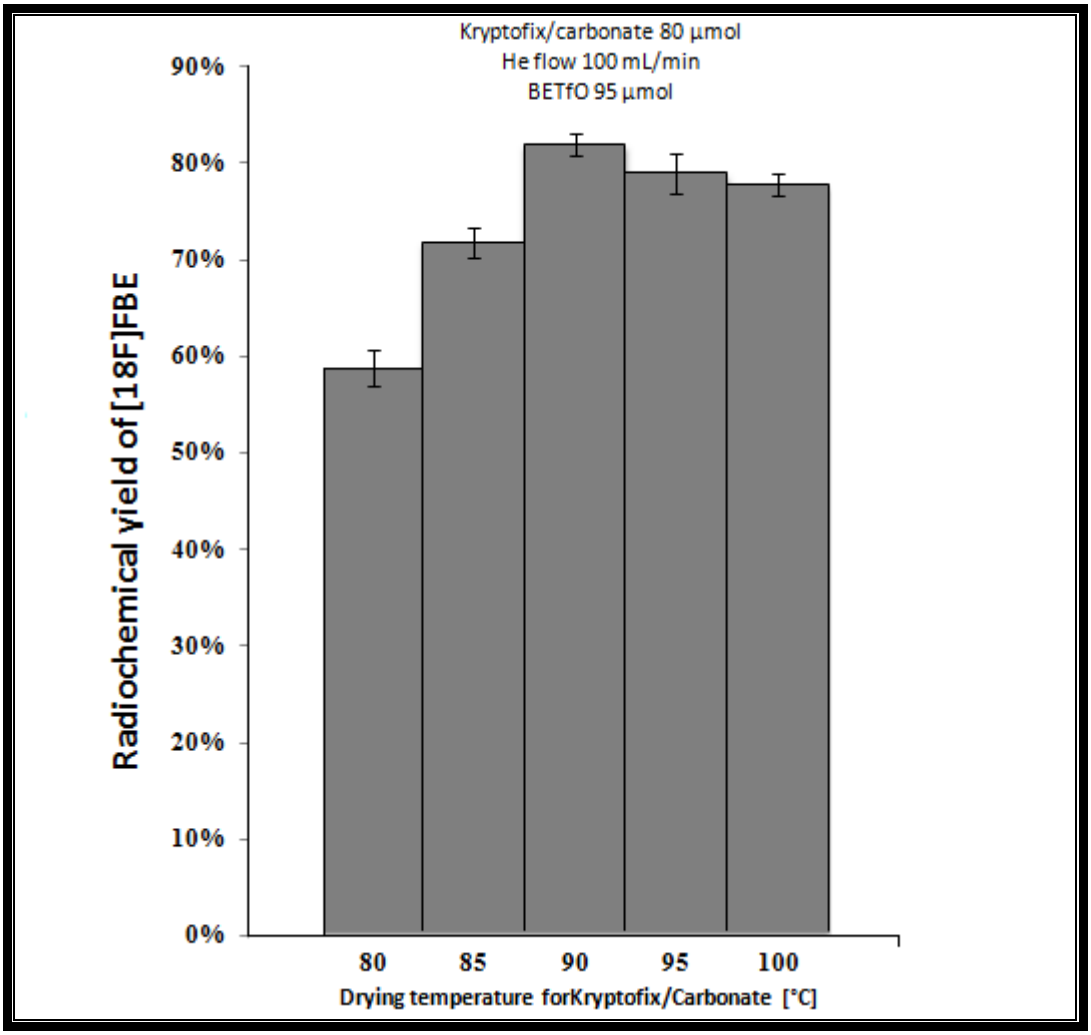


Figure 21. Dependence of the radiochemical yield of [^{18}F]BFE on the amount drying temperature. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.3 The effect of helium flow rate

In our previous study, the maximum yield of [^{18}F]BFE was achieved when using 80 µmol K_2CO_3/K_{222} as well as drying temperature at 90 °C. Therefore a solution of kryptofix-[2.2.2] (80 µmol in 1,5 mL MeCN) was added and the reaction mixture was dried, initially at 90 °C with both vacuum and different rates of helium flow for two minutes and subsequently with vacuum for additional two minutes. The effect of the helium flow rate on the formation of [^{18}F]BFE was examined.

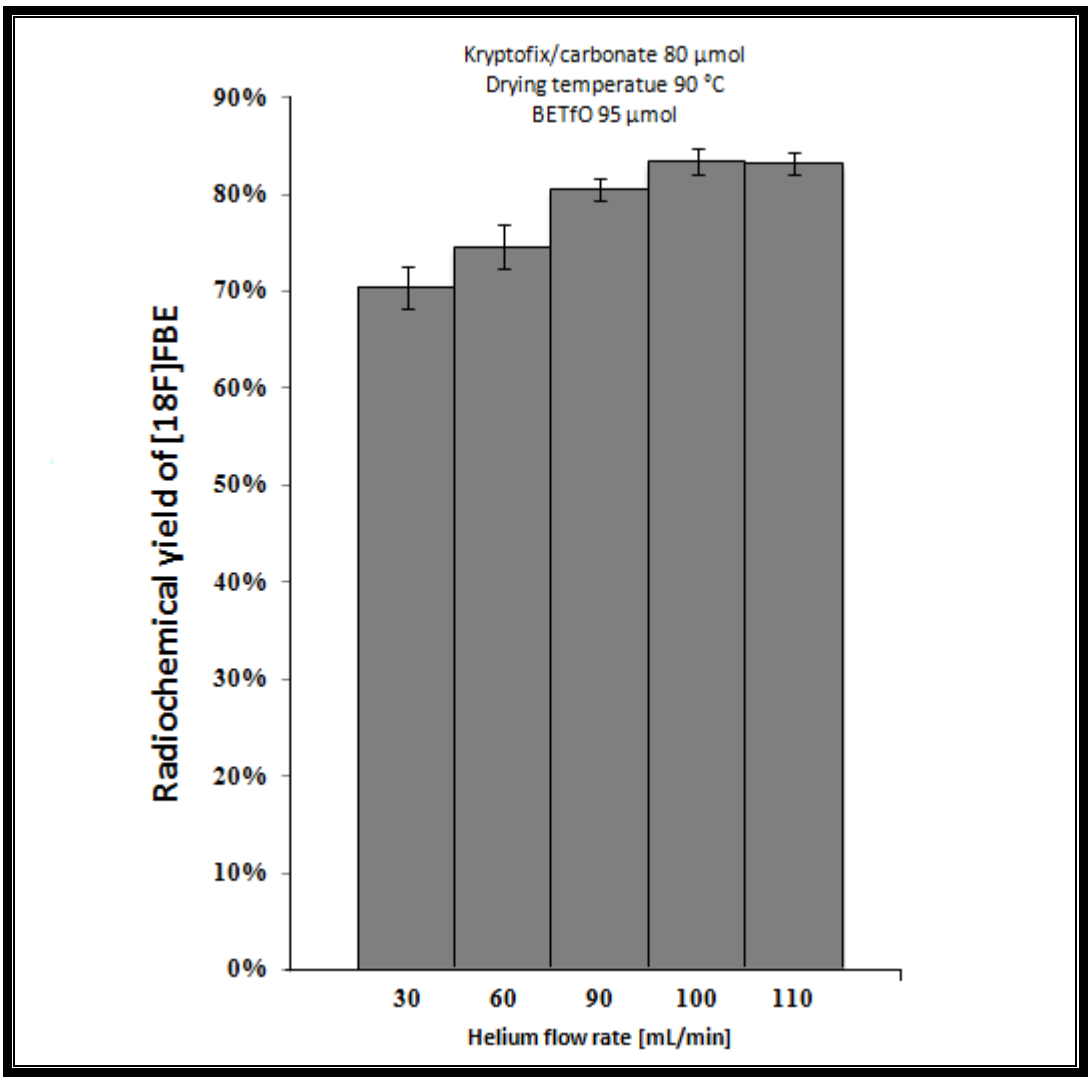


Figure 22. Dependence of the radiochemical yield of [^{18}F]BFE on the amount helium flow. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

The results are summarized in Figure 22. This figure clearly shows that when the helium flow rate is increased; the radiochemical yield improves. This improvement

continues until it reaches a peak at 100 mL/min., after which, the radiochemical yield begins to decrease. The best radiochemical yields of the [^{18}F]BFE (83 ± 1.3%) were obtained when the helium flow rate was 100 mL/min.

4.1.4 The effect of the quantity of triflate (BETfO)

The most successful and frequently used syntheses for [^{18}F]fluoroethylcholine ([^{18}F]FECh) or O-(2-[^{18}F]fluoroethyl)-L-tyrosine are produced Starting from 1,2-dibromoethane or 1,2-bis(tosyloxy)ethane or 2-bromoethyl triflate to produce [^{18}F]FETos or [^{18}F]BFE.

The advantage of BETfO compared to 1,2-dibromoethane (DBE) is not only the higher reactivity of its triflate group compared to the bromine of DBE but especially its higher boiling point (230 °C) that is around 160 °C higher than the boiling point of [^{18}F]BFE (bp 71.5 °C). Thus, in contrast to DBE the use of less volatile BETfO enables a simple distillation of the intermediate [^{18}F]BFE leaving all impurities from the first reaction behind in reactor 1.

BETfO plays an important role in the formation [^{18}F]BFE, hence the effect of the quantity of BETfO was studied. Where 0.5 mL of *o*-DCB were added to the dried [^{18}F]KF–K_{222}. After one minute of stirring at 100 °C, different quantities of BETfO were dissolved in 0.5 mL of *o*-DCB and were added to the dried [^{18}F]KF–K_{222}.

BETfO was subjected to nucleophilic substitution with the [^{18}F]KF-Kryptofix-complex ([^{18}F]KF–K_{222}) in 1,2-dichlorobenzene(*o*-DCB) and [^{18}F]BFE was formatted and distillated at 100 °C.

The results are represented in Figure 23. This figure clearly shows that when the amount of BETfO is increased; the radiochemical yield improves. This improvement continues until it levels off at µmol BETfO. The maximum yield of [^{18}F]BFE (83 ± 1.5 %) was achieved when using 95 µmol and 115 µmol BETfO.

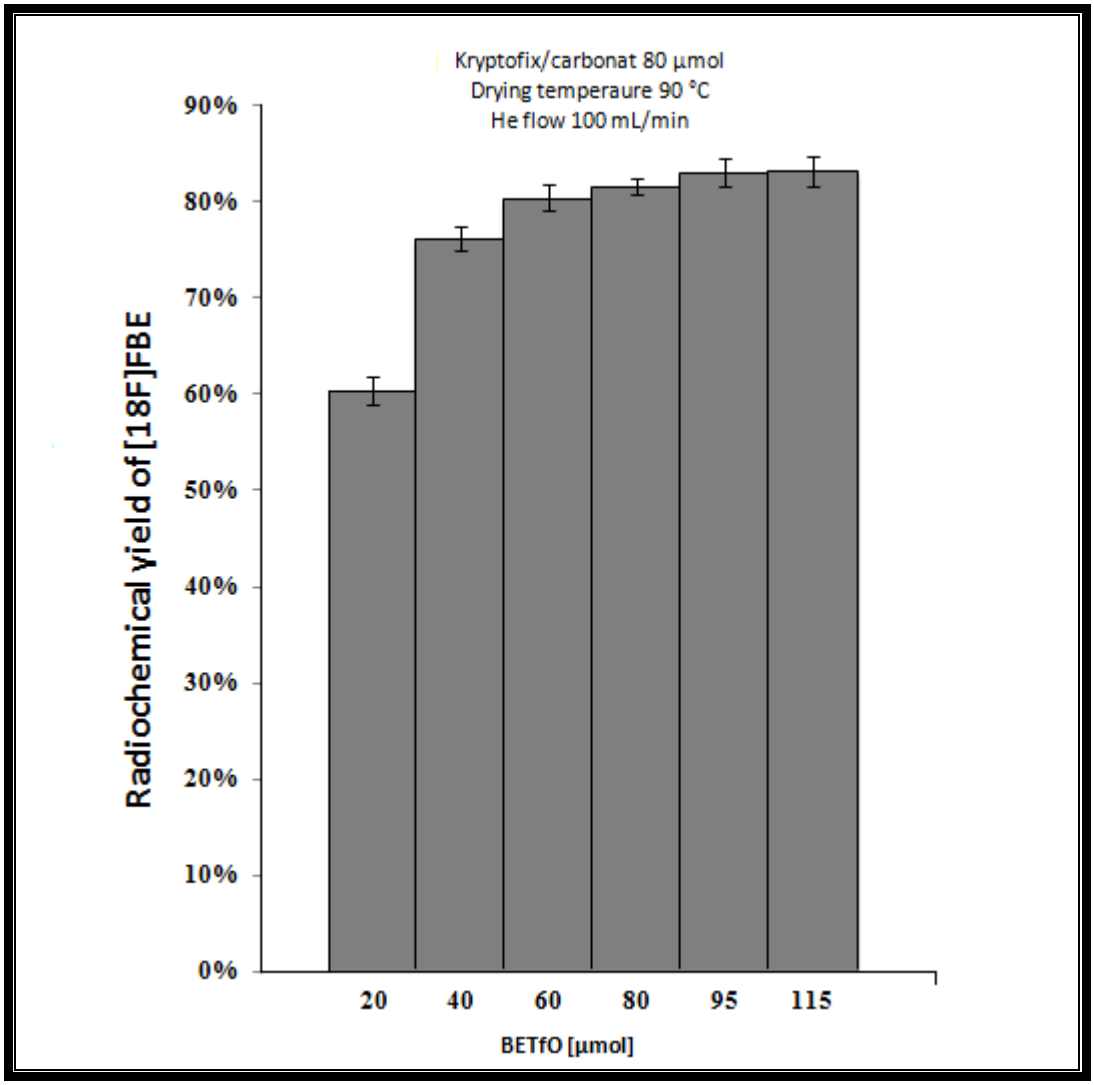


Figure 23. Dependence of the radiochemical yield of [^{18}F]BFE on the amount BETfO. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.5 Effect of tetrabutylammonium hydrogencarbonate (TBA) on formation of [^{18}F] BFE

BETfO was subjected to nucleophilic substitution with [^{18}F]KF– Kryptofix-complex ([^{18}F]KF–K_{222}) as well as with [^{18}F]tetrabutylammonium fluoride ([^{18}F]Bu_4NF) to form the volatile 1-bromo-2- [^{18}F]fluoroethane ([^{18}F]BFE) in 1,2-dichlorobenzene (*o*-DCB) (first reaction).

As described in our previous work, it was found for the case of using of KF–K_{222}-complex that the maximum yield of [^{18}F]BFE was achieved by using 80 µmol K_2CO_3/K_{222} and 95 µmol BETfO as well as helium flow rate and drying temperature

of 100 mL/min and 90 °C, respectively. TBA exhibits lower basic properties compared with Kryptofix. Therefore higher radiochemical yield of [^{18}F]BFE would be expected due to less elimination by-products. But the results in Figure 24 show no significant differences concerning formation of [^{18}F]BFE by using [^{18}F]Bu_4NF instead of [^{18}F]KF–K_{222}-complex.

The data indicate an optimum for the formation of [^{18}F]BFE (84 ± 1.5 %) by using [^{18}F]Bu_4NF at 100 mL/min for the helium flow rate and 110 °C for the drying temperature. These results are summarized in Figure 24. This figure clearly shows that when the drying temperature is increased; the radiochemical yield improves. This improvement continues until it reaches a temperature of 110 °C in case TBA and 90°C in case Kryptofix, after which, the radiochemical yield begins to decrease.

In both cases no transfer of TBA or Kryptofix from rector one into reactor two during the distillation step was detected, due to their high vapor pressures.

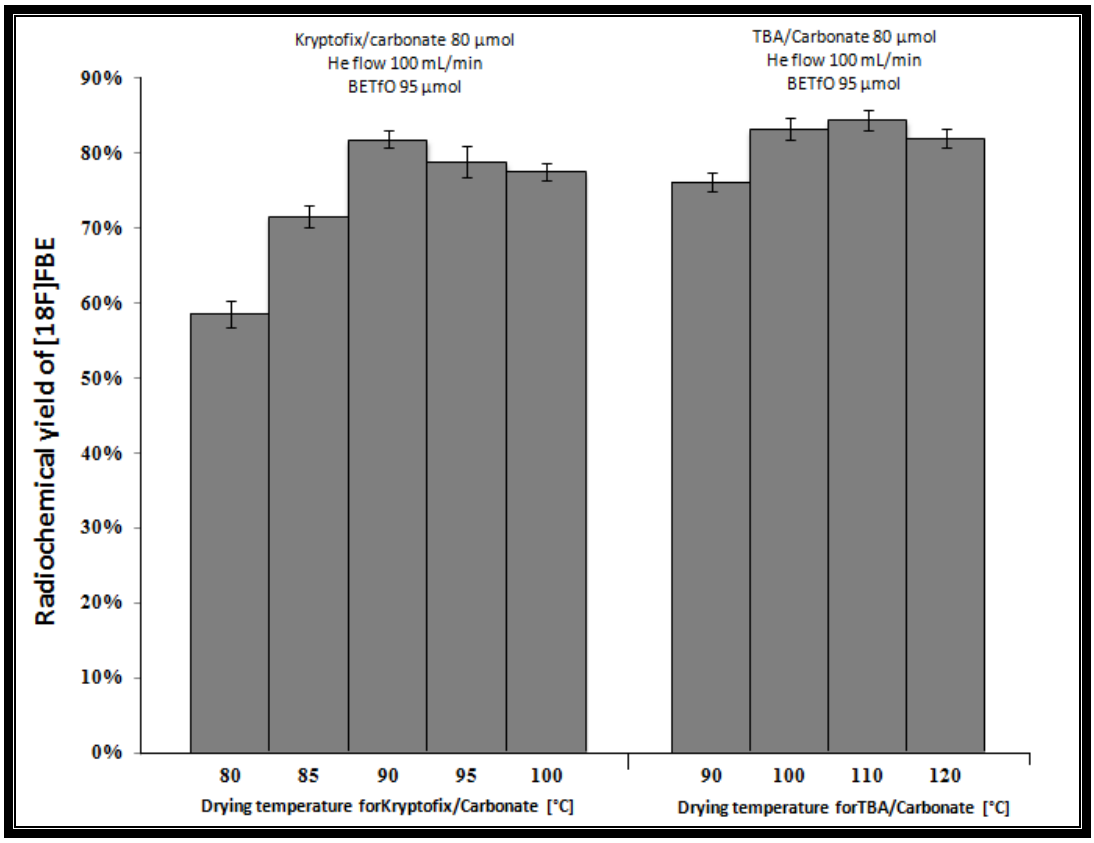


Figure 24. Dependence of the radiochemical yield of [^{18}F]BFE on the amount of reactants, helium flow and drying temperature by using [^{18}F]KF–K_{222}, and [^{18}F]Bu_4NF. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.6 Distillation of [^{18}F]BFE

Distillation is the process of heating a liquid until it boils, capturing and cooling the resultant hot vapors, and collecting the condensed vapors. The distillation of [^{18}F]BFE (bp 71.5 °C) into the second reactor was started directly after the drying step by adding 0.5 mL of *o*-DCB to the dried [^{18}F]KF–K_{222}. After one minute of stirring at 90 °C, BETfO (25 µL) dissolved in 0.5 mL of *o*-DCB the activated [^{18}F]fluoride was added. The temperature was allowed to increase while [^{18}F]BFE was transferred within 8 minutes into the second reactor where a different temperature for the distillation was studied.

This simple distillation at 100 °C under helium flow transfers the intermediate [^{18}F]BFE in almost pure form into the second reactor. As shown in the distillation profile presented in Figure 25 the most radioactivity (83%) is already transported after 6 minutes from reactor one into reactor two. About 10% of the start activity remains in reactor one while about 7% of the start activity is lost by the distillation. The dependence of the portion of the intermediate [^{18}F]BFE transferred through the distillation on the temperature and the helium flow rate by using both [^{18}F]KF–K_{222}, and [^{18}F]Bu_4NF is shown in Figure 26. It turned out that the maximum yield of transferred radioactivity was obtained when a flow rate of 100 mL/min and a temperature of 100 °C were applied. Higher temperatures led to less transferred material due to decomposition of reactants. Gas chromatographic analysis of the distilled material showed no presence of BETfO (bp 230 °C) in the second reactor when the temperature was kept ≤130 °C (detection limit 0.0014 mg/mL).

Applying the optimized conditions for the first reaction and for the distillation step has enabled to obtain the intermediate [^{18}F]BFE in a pure form with a RCY of 73% in only 15 minutes without using HPLC or solid phase extraction (SPE). This is a significant improvement against the direct labeling approach using DITOS which provides the corresponding intermediate [^{18}F]fluoroethyltosylate ([^{18}F]FETos) in a RCY of only 65% by using both HPLC-run and SPE for purification.

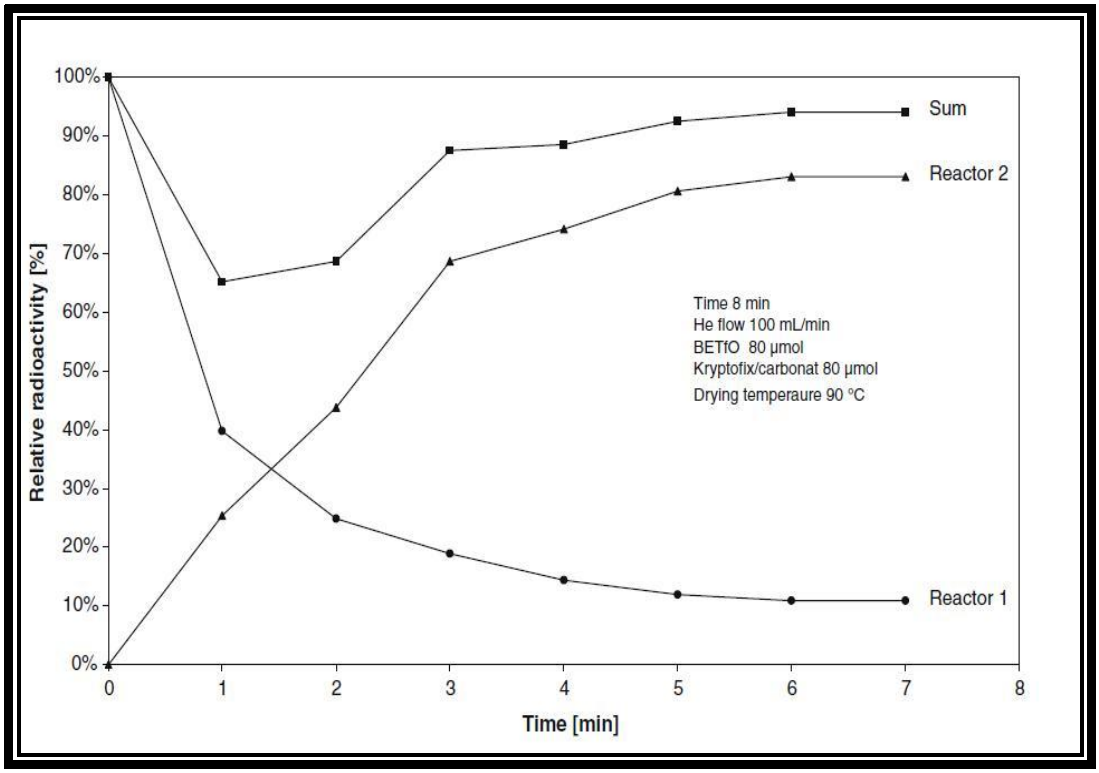


Figure 25. Distillation profile of [^{18}F]BFE at 100 °C.

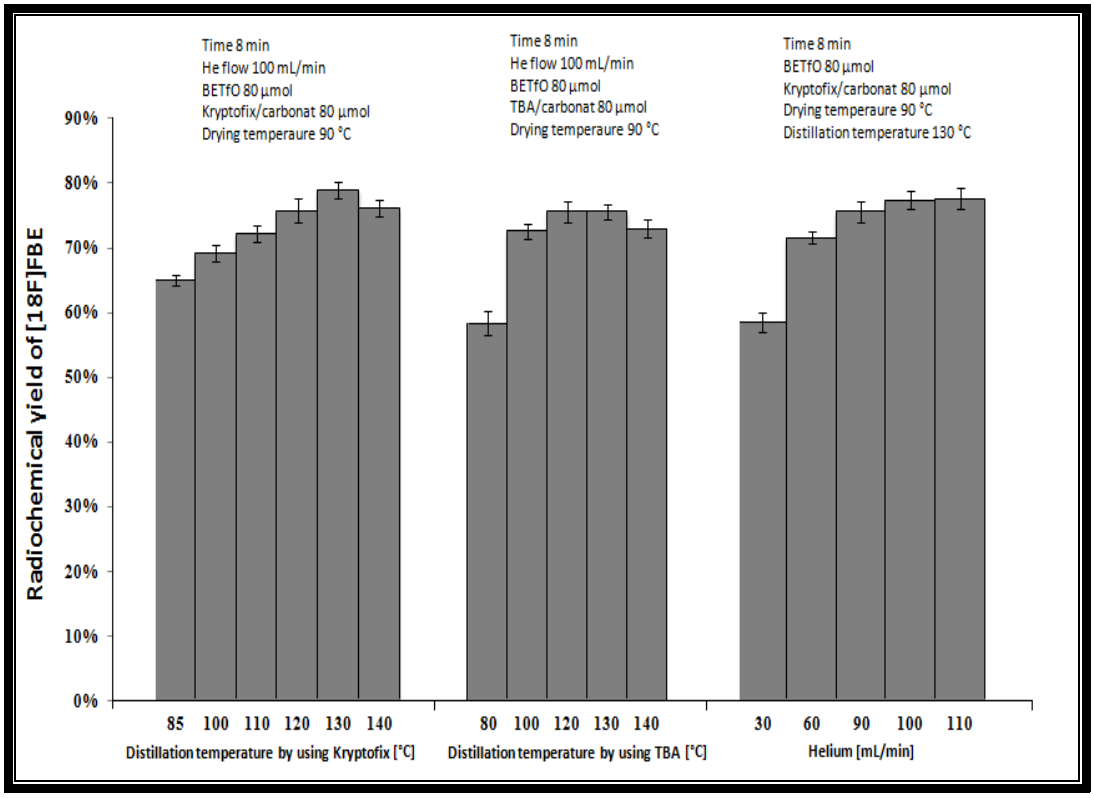


Figure 26. Dependence of the yield of distillation of [^{18}F]BFE on temperature and helium flow. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.7 Reaction 2: Formation of [^{18}F] FECh

After [^{18}F]BFE had been transferred by distillation into the second reactor, the second reaction between [^{18}F]BFE and DMAE was carried out in the second reactor in DMF. In order to optimize the radiochemical yield of [^{18}F]FECh, the effect of reaction time and reaction temperature as well as the effect of the amount of DMAE and DMF were subsequently studied.

4.1.7.1 The effect of the amount of DMAE

DMAE is chemically known as dimethylaminoethanol. It has two methyl groups and is chemically similar to choline, which in turn has three methyl groups. The addition of a methyl group to the molecule of dimethylaminoethanol can lead to the formation of choline. The first method to synthesize ([^{18}F]FC) was reported by DeGrado et al by reacting N,Ndimethylaminoethanol (DMAE) with bromo-[^{18}F]fluoromethane ([^{18}F]BFM).

In the present study, [^{18}F]BFE was transferred within 8 minutes into the second reactor containing a certain quantity of DMAE in 1.7 mL of DMF . The second reactor was then heated for 15 minutes at 100 °C. The temperature was, finally, decreased to 60 °C and the effect of amount the DMAE was studied.

The results are shown in Figure 27. This figure clearly shows that when the amount of DMAE is increased; the radiochemical yield improves. This improvement continues until a level of 0.5 mL of DMAE has been reached, after which, the radiochemical yield levels off. Results showed that 0.5mL of DMAE is the optimum concentration in order to obtain the highest yield of [^{18}F]FECh (65 ± 2%) in reaction.

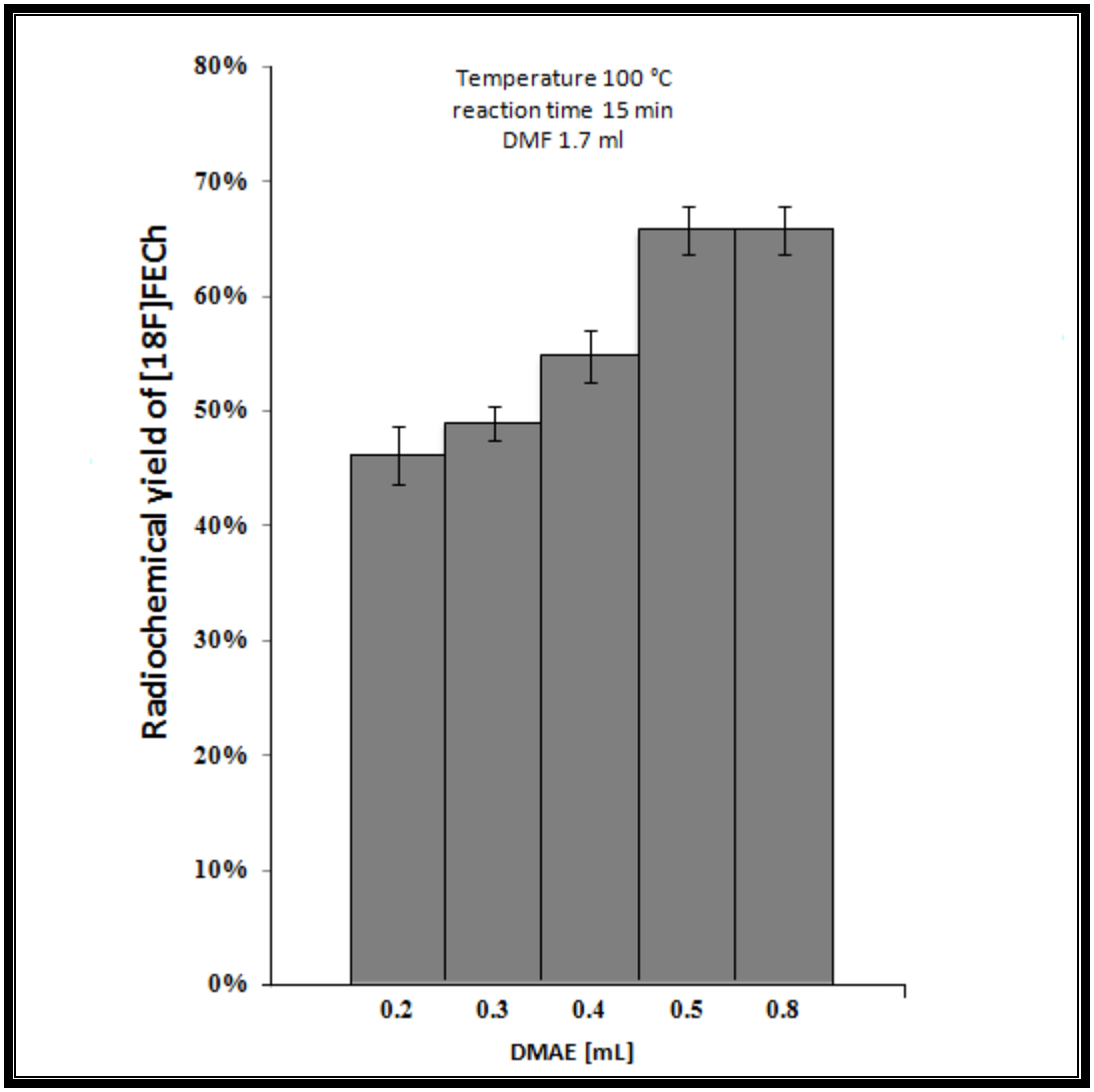


Figure 27. Dependence of the radiochemical yield of [^{18}F] FECh on the quantity of DMAE. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.7.2 The effect of the amount of DMF

0.5mL of *o*-DCB were added to the dried [^{18}F]KF–K_{222}. After one minute of stirring at 100 °C, BETfO (15 µL) dissolved in 0.5 mL of *o*-DCB was added while [^{18}F]BFE was transferred within 5minutes into the second reactor containing 0.5 mL of DMAE in different amount of DMF. The second reactor was then heated for 10 minutes at 100 °C where the second reaction between [^{18}F]BFE and DMAE was carried in DMF. In order to optimize the radiochemical yield of [^{18}F]FECh, the effect of the amount of DMF was subsequently studied.

The results are shown in Figure 28. This figure clearly shows that when the amount of DMF is increased; the radiochemical yield improves. This improvement continues in levels between 1.7 and 2.0 mL of DMF has been reached, after which, the radiochemical yield begins to decrease. Results showed that between 1.7 and 2.0 mL of DMF is the optimum concentration in order to obtain the highest yield of [^{18}F]FECh (69 ± 2%) in reaction.

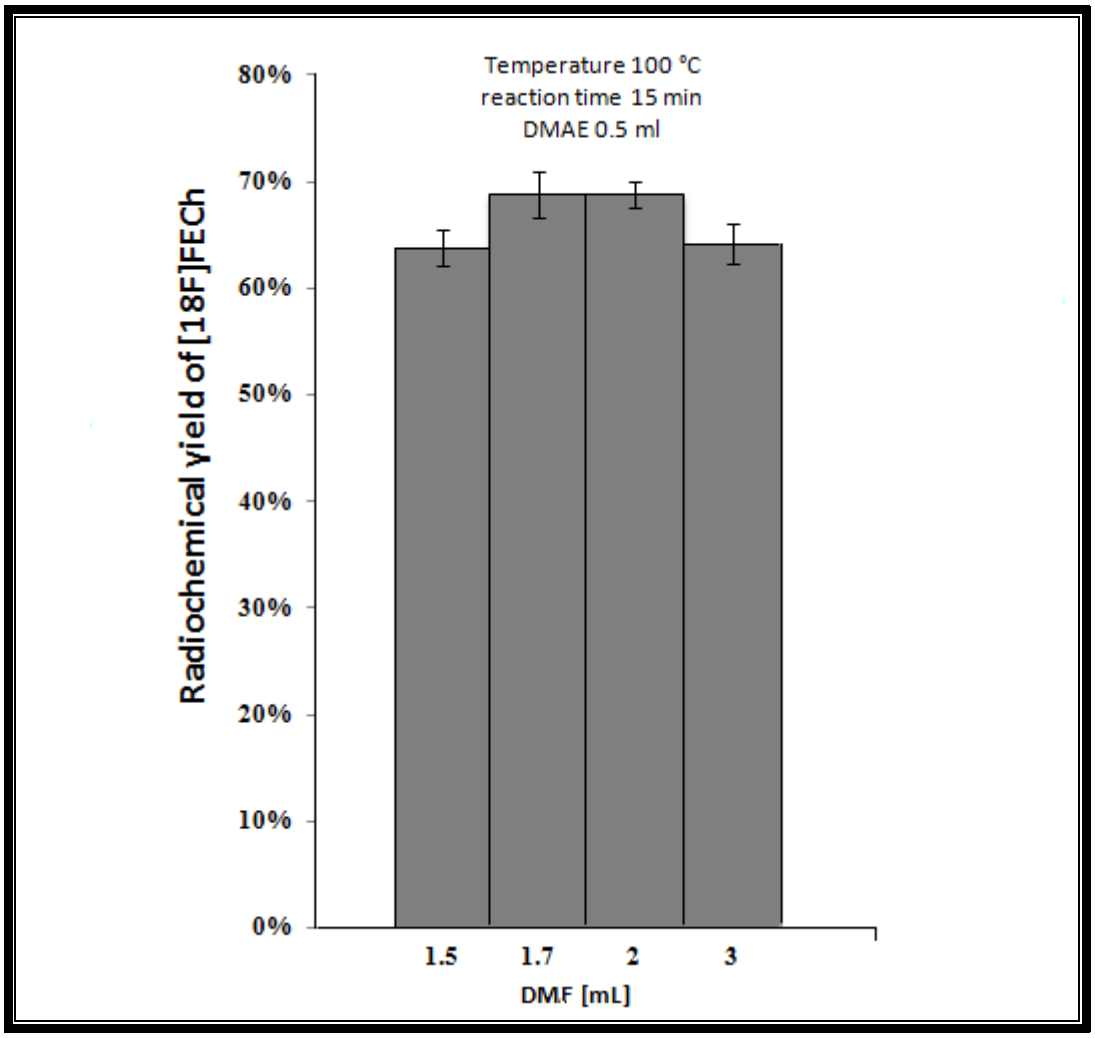


Figure 28. Dependence of the radiochemical yield of [^{18}F]FECh on the quantity of DMF. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.7.3 The effect of the reaction temperature for formation of [^{18}F]FECh

The mixture of 0.5 mL of N,N-dimethylaminoethanol, 1.7ml of DMF and [^{18}F]BFE 2a was heated for 10 minutes at different temperature.

The effects of reaction temperature for mixture of 0.5 mL of N,N-dimethylaminoethanol, 1.7ml of DMF and [^{18}F]BFE 2a were investigated in order to optimize the radiochemical yield of [^{18}F]FECh . Results of this investigation are shown in Figure 21. This figure clearly shows that when the reaction temperature is increased; the radiochemical yield improves. This improvement continues until it reaches a temperature of 100 °C., after which, the radiochemical yield begins to decrease. 0.5 mL of DMAE in 1.7 mL of DMF was found to be the optimum concentration that provides the highest yield in reaction 2 **(**Figure 29**)**. The optimization of the reaction temperature in a maximum yield (84 ± 1.7%) obtained at 100 °C.

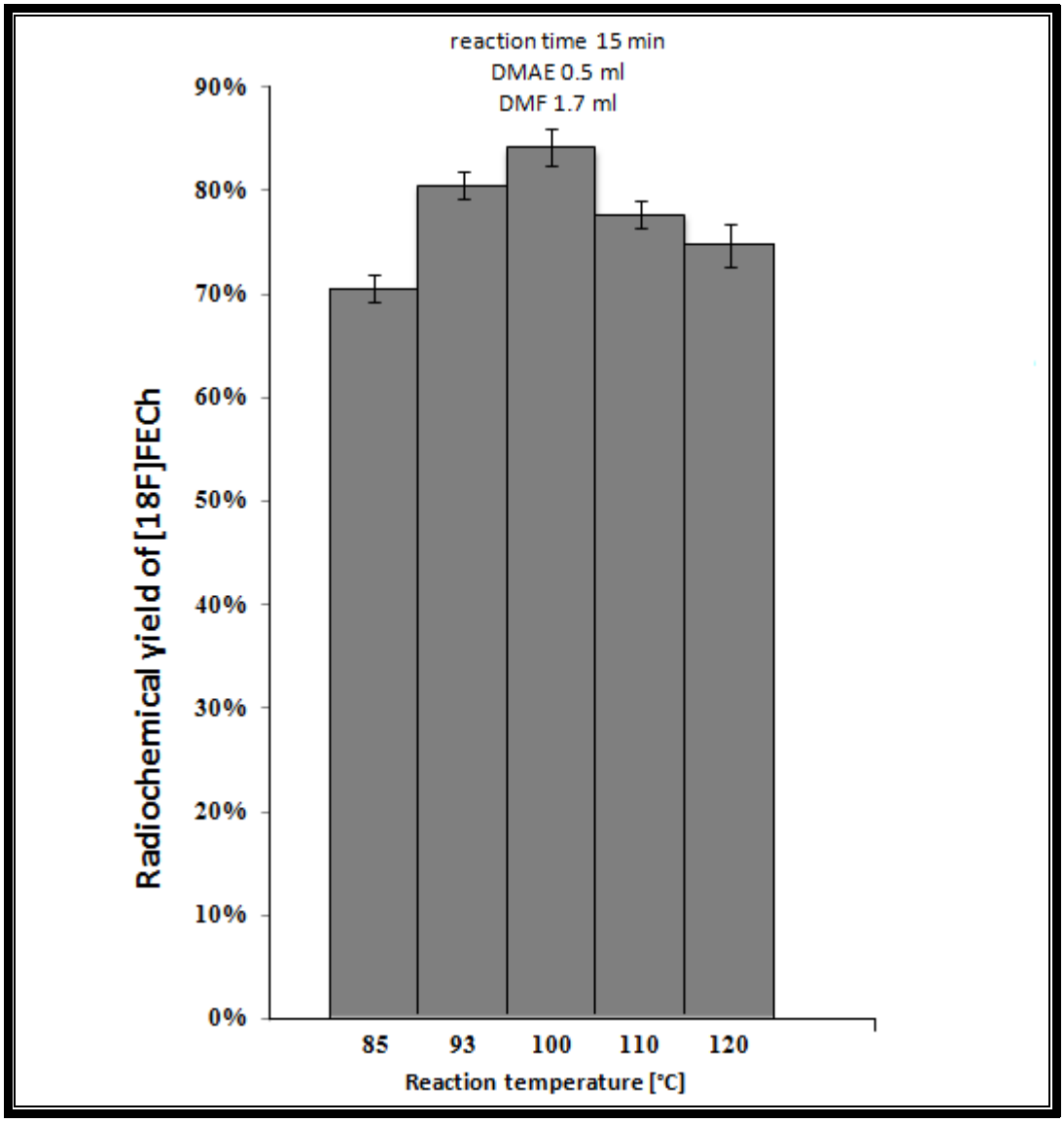


Figure 29. Dependence of the radiochemical yield of [^{18}F]FECh on reaction temperature. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.7.4 The effect of the reaction time for formation of [^{18}F]FECh

In order to optimize the radiochemical yield of [^{18}F]FECh, we investigated the effect of different reaction times (5 to 20 minutes) in the second reactor. The results are shown in Figure 30. Maximum yield (79 ± 2%) obtained in 15 minutes. This figure clearly shows that when the reaction time is increased; the radiochemical yield improves. This improvement continues until a reaction time of 15 minutes is achieved, after which, the radiochemical yield begins to decrease.

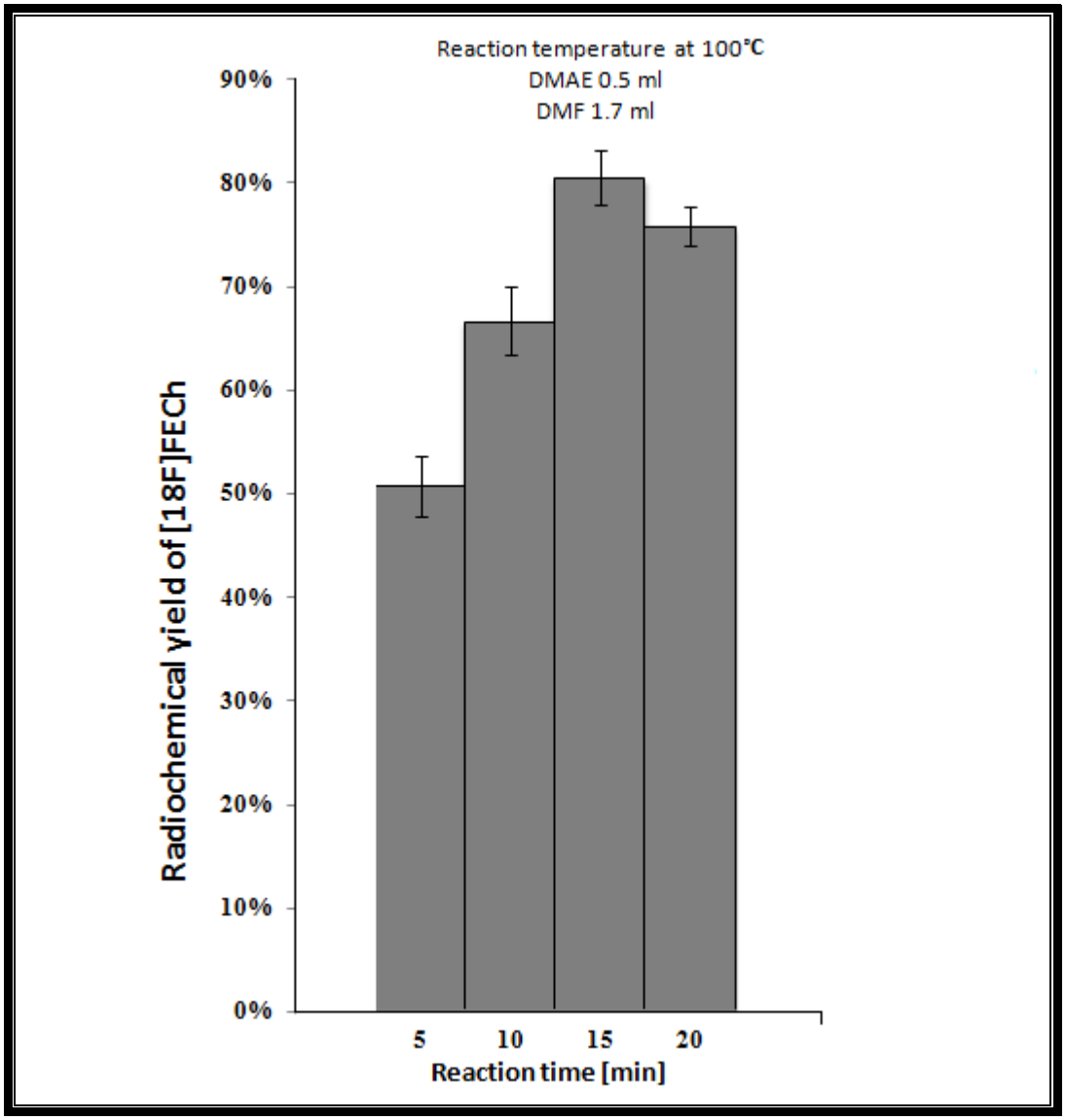


Figure 30. Dependence of the radiochemical yield of [^{18}F]FECh on reaction time. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

Thus 0.5 mL of DMAE in 1.7 mL of DMF proved to be the optimum concentration in order to obtain the highest yield in reaction 2 (Figure 31). The optimization of the

reaction temperature and reaction time resulted in a maximum yield obtained at 100°C and 15 minutes, respectively (Figure 31).

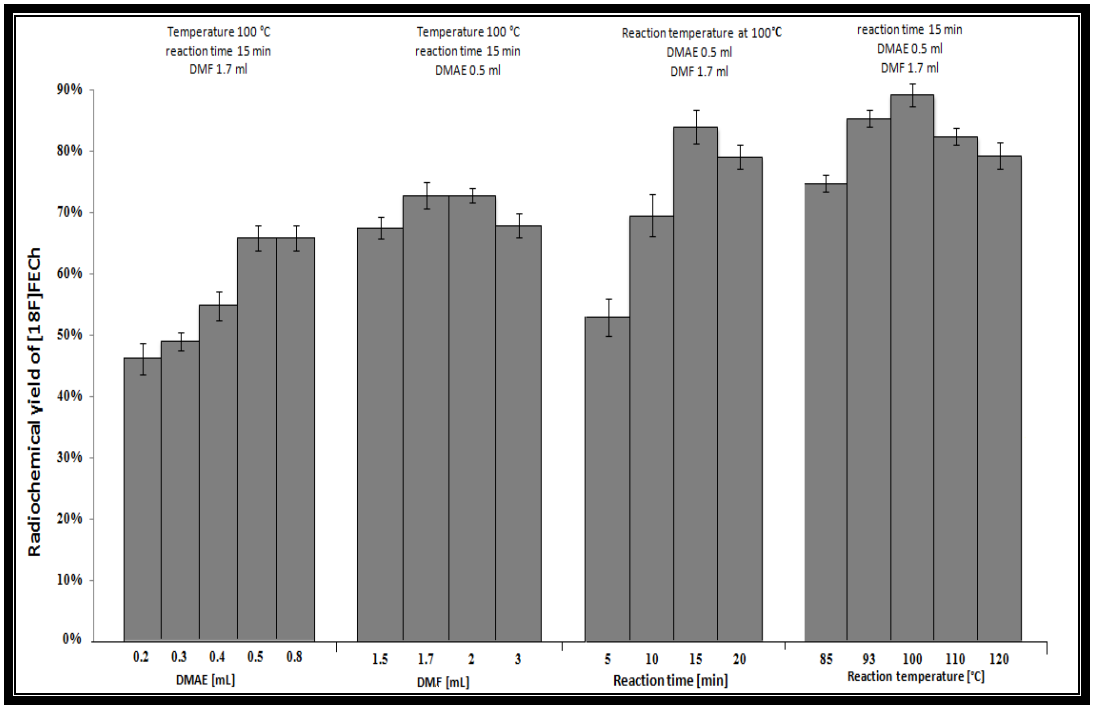


Figure 31. Dependence of the radiochemical yield of [^{18}F]FECh on the quantity of DMAE and DMF as well as on reaction time and temperature. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

4.1.7.5 Efficient alkali iodide for formation of [^{18}F]FECh

Recent reports have shown that the addition of alkali iodides to 1-bromo-2[^{18}F]fluoroethane ([^{18}F]BFE) result in drastically increased radiochemical yields, most probably due to the in-situ formation of 1-iodo-2-[^{18}F]fluoroethane ([^{18}F]IFE). Therefore, the above mentioned new approach was adapted on the fully automated synthesis for the production of [^{18}F]FECh, using the iodide promoted alkylation.

In a recent study, the highest radiochemical yields were obtained via the alkali iodide promoted [^{18}F]fluoroethylation with [^{18}F]FETos by using lithium iodide as a catalyst.

The latter is apparently the most potent of the iodide salts. Due to the toxicity of the lithium, the present study examined the use of sodium iodide as a catalyst which seems more suitable for routine production. A mixture of 0.5 mL of N,N-

dimethylaminoethanol, 1.7 mL of DMF, [^{18}F]BFE and different amounts of NaI, were heated for 10 minutes at 100 °C.

The effect of NaI on mixture of 0.5 mL of N,N-dimethylaminoethanol, 1.7 mL of DMF and [^{18}F]BFE was evaluated. The reaction mixture was passed on HPLC (Figure 32). This figure clearly shows that when the amount of NaI is increased; the radiochemical yield improves. This improvement peaks at 33 µmol of NaI.

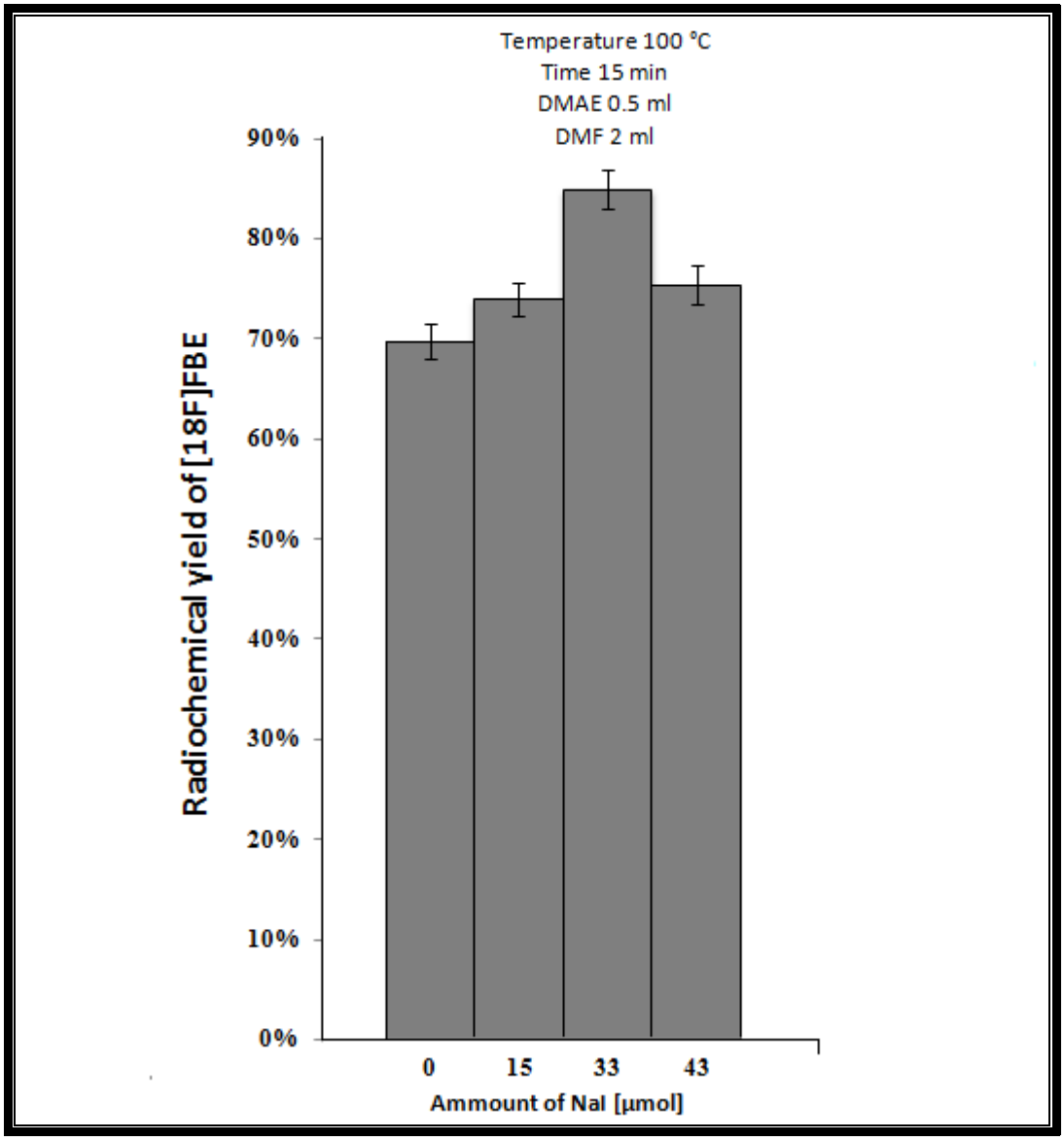


Figure 32. Dependence of the radiochemical yield of [^{18}F]FECh on the quantity of NaI. The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

In a previous related study, we evaluated the effects of NaI for mixture of 0.5 mL of N,N-dimethylaminoethanol, 1.7 mL of DMF and [^{18}F]BFE. The reaction mixture was

passed on the cation-exchanger cartridge. Cation exchanger can attract and hold cations that bypass in a moving solution if these are more attracted to the Na^+ than the $FCH_2CH_2(CH_3)_2N^+CH_2CH_2OH$. It was expected that cation exchanger can attract and hold Na^+ and $FCH_2CH_2(CH_3)_2N^+CH_2CH_2OH$ pass with the moving solution. Thus, the greater amount of NaI resulted in a decrease in [^{18}F]FECh (Table 10).

Table 8. The radiochemical yield of [^{18}F]FECh using different quantity of NaI (n = 5).

Amount of NaI (mg)	0	1,5	5	6	7,5
Rate of [^{18}F] FECh	47 %	25%	10%	6,2%	1,7%

4.1.8 Second reaction: formation of [^{18}F] FET

The second reaction between [^{18}F]BFE and freshly prepared Ty-Di-Salt was carried out after distilling [^{18}F]BFE in a second reactor containing Ty-Di-Salt, NaI and 800 µL DMSO. In order to optimize the radiochemical yield of [^{18}F]FET the effect of reaction time and reaction temperature as well as the effect of the amount of Ty-Di-Salt were subsequently studied. As it was known that NaI increases the yield of fluoroethylation we studied also the effect of NaI on the second reaction. As shown in Figure 33 the optimization of the reaction time and reaction temperature both for the reaction with NaI as well as for the reaction without NaI resulted in a maximum yield obtained at 110 °C and 15 minutes, respectively. By adding of 33 µmol (5.5 mg) NaI the radiochemical yield of [^{18}F]FET could be increased by 30% compared to the reaction without NaI.

The study of the effect of the amount of tyrosine on the radiochemical yield of [^{18}F]FET was started by the use of various quantities of tyrosine which were always dissolved in 50 µL of 10% aqueous sodium hydroxide (NaOH). Figure 33 shows that the radiochemical yield of [^{18}F]FET increases by increasing the amount of tyrosine, but starts to fall after reaching a maximum by 63.4 µmol.

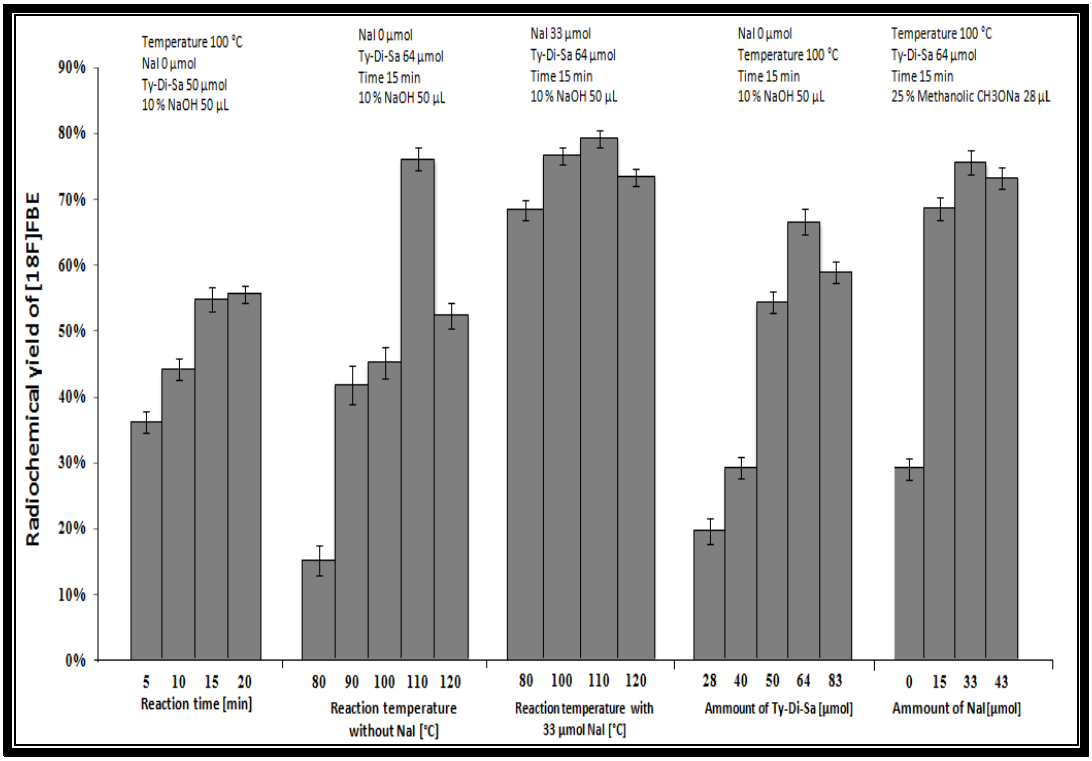


Figure 33. Dependence of the radiochemical yield of [^{18}F]FET on reaction time, reaction temperature as well as on the amount of tyrosine and NaI . The results are shown as means ± SD, ($p < 0.0001$, $n = 5$).

For further investigations, we then varied the amount of the added aqueous sodium hydroxide (NaOH) but kept the quantity of tyrosine constant at 63.4 µmol. We found out that when a molar ratio of NaOH over tyrosine of less than two was used, the radiochemical yield of [^{18}F]FET decreased and the HPLC radio chromatogram of the reaction solution exhibited in addition to [^{18}F]FET a second radioactive substance, compound X, by 13.5 minutes, which is assumed to be the 2-fluoroethyl ester of tyrosine (Figure 34b and c). This assumption corresponds to the fact that a double molar excess of NaOH over tyrosine is necessary for the deprotonation of the phenolic group and building of Ty-Di-Salt. For the unambiguous identification of compound X we performed the cold reaction with [^{19}F]KF with 126.9 µmol tyrosine and 50 µL of 10% aqueous sodium hydroxide (NaOH) in 800 µL DMSO (NaOH molar ratio = 1.0). The HPLC fraction by 13.5 minutes was isolated and investigated with ESI mass spectrometry. The resulting mass spectra of compound X and [^{19}F]fluoroethyltyrosine ([^{19}F]FET) taken with both cone voltages at 20 and 40 V are demonstrated in Figure 35. Both compound X and [^{19}F] FET have the same highest peak at M+1 = 228.1

$(C_{11}H_{15}FNO_3)^+$ (spectra A and C) but they differ in their fragmentation musters at higher cone voltage of 40 V (spectra B and D). With the help of the fragment mass at m/z = 136.0 which belongs to the fragment $(C_8H_{10}NO)^+$ that only appears in the spectrum of compound X at 40 V (spectrum D) we could identify compound X to be 2-fluoroethyl ester of tyrosine because the fragment 10 is built by breaking the C–C bond between the a and b carbon atoms from the tyrosine ester. It is unlikely that the same fragment is derived from [^{19}F]FET since it has to be built by double fragmentation. On the other hand breaking the same C–C bond between the a and b carbon atoms in [^{19}F]FET leads to the fragment $(C_{10}H_{13}FNO)^+$ with m/z = 182.1 which is only present in the spectrum of [^{19}F]FET (spectrum B).

The effect of the amount of NaOH on the radiochemical yield of [^{18}F]FET is dramatic, because of the necessity to deprotonate the phenolic hydroxyl group of tyrosine before it can act as a nucleophilic agent toward [^{18}F] BFE. Due to the lower pKs value of the carboxyl group of tyrosine compared with the pKs value of the phenolic hydroxyl group (pKs_{COOH} = 2.2; pKs_{OH} = 10.07) a molar ratio of NaOH/tyrosine ≥ 2 is necessary to completely deprotonate the phenolic hydroxyl group and avoid the formation of the 2-fluoroethyl ester of tyrosine.

The RCY of the second reaction after optimization of all reaction parameters amounts to 65%. Compared with the second reaction of the indirect labeling approach using 1-tosyloxy-2-[^{18}F]fluoroethane with RCY about 30%, this is an improvement of more than 35%. Also, the total RCY of the here described approach with 45% is about 23% higher than the total RCY of the indirect labeling approach and about 10% more than the total RCY provided by direct labeling methods using protected precursors. In addition, the presented approach allows the synthesis of [^{18}F]FET in significantly higher specific activity without need of purification with the HPLC and reduces the synthesis time by half. Due to the distillation step, which allows to obtain very pure [^{18}F]BFE, we could achieve high specific activity of more than 80 GBq/μmol for [^{18}F]FET. In comparison, the direct labeling approach leads to 18.5 GBq/μmol.

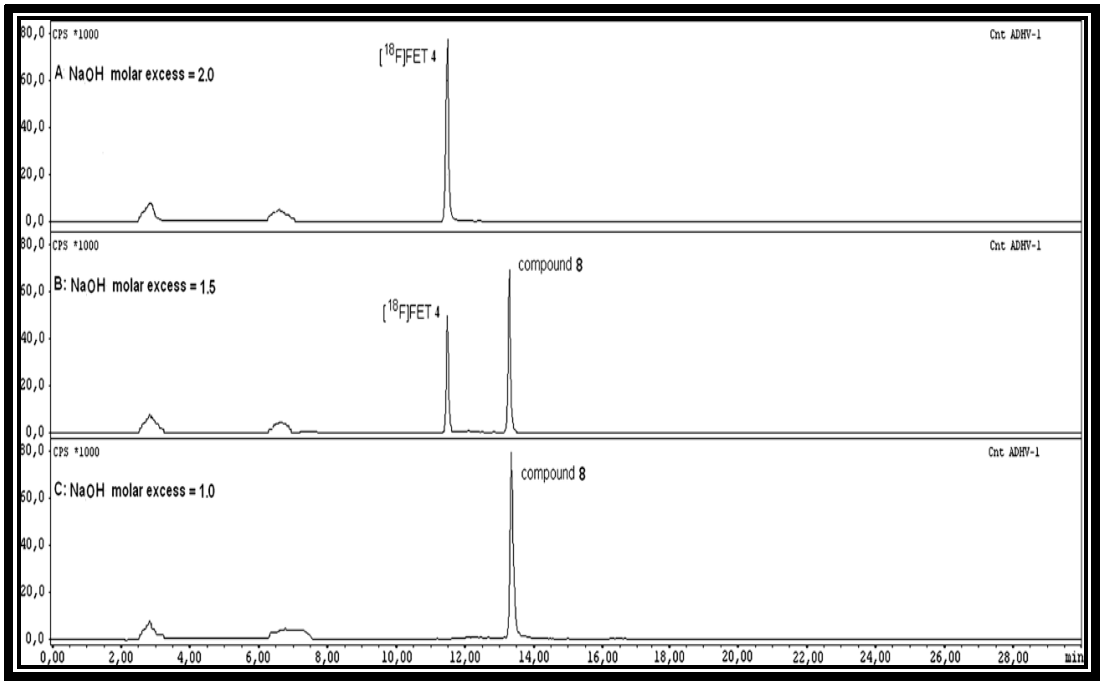


Figure 34. Radio HPLC chromatograms of reaction solutions of reaction two by different molar excess of NaOH over tyrosine.

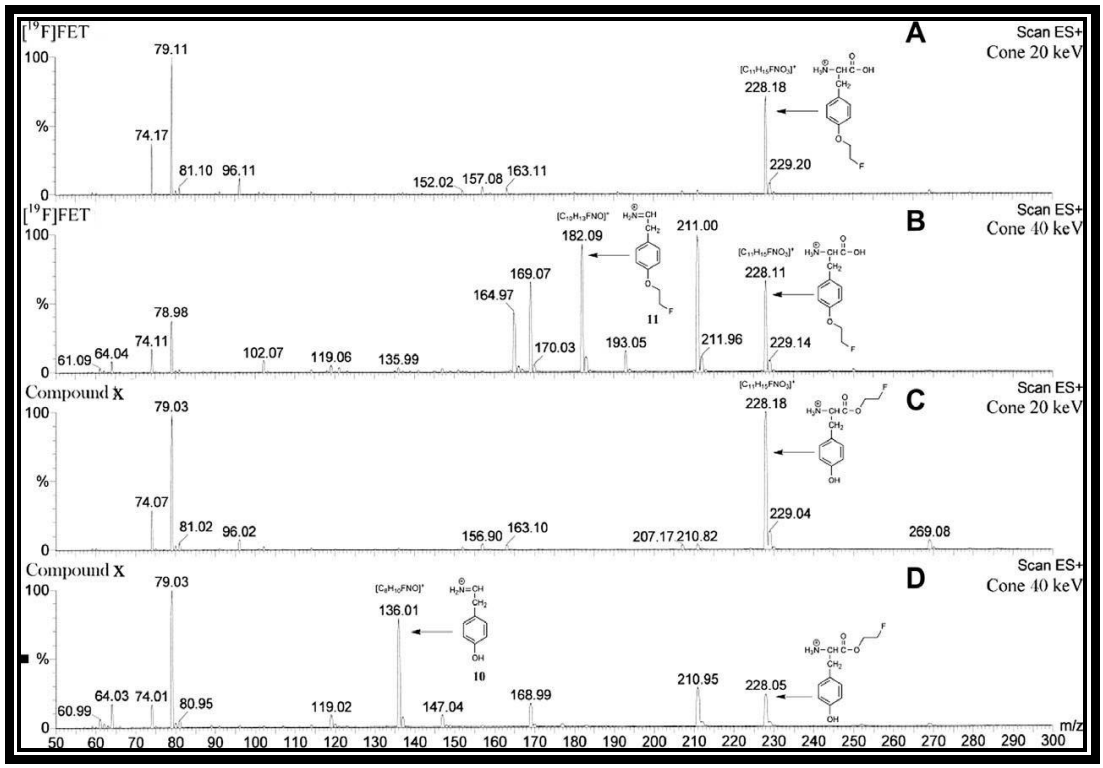


Figure 35. ESI mass spectra of compounds 8 and 9 at cone voltages 20 and 40 V.

4.1.9 Purification of [^{18}F]FECh

After the temperature had been decreased to 60 °C, five milliliters of ethanol were added and the reaction mixture was passed through a Sep-Pak Accell Light cartridge (Waters). The cartridge was then washed with 10mL of ethanol and 10mL of H_2O, respectively. The [^{18}F]FECh was then eluted with 1mL of 10% aqueous NaCl and diluted with 9.0mL of sterile water. After sterile filtration, 0.94 to 4.7 ± 5% GBq [^{18}F]FECh were obtained 47 ± 2.3%, with radiochemical purity of more than 99.9% and the specific activity >55 GBq/μmol.

4.1.9.1 The effect of the ethanol and water on the Sep-Pak Accell Light cartridges (Waters)

In the second reactor, 5 mL of ethanol was added into the reaction mixture which in turn was passed on Two Sep-Pak Accell Light cartridges (Waters). Then ethanol (10 mL) and water (10 mL) were passed on the Sep-Pak Accell Light cartridges, respectively, to wash the cartridges. Ethanol (5 mL), ethanol (10 mL) and water (10 mL) were collected and analyzed by HPLC. [^{18}F]fluoroethylcholine was found in ethanol (5 mL), ethanol (10 mL) and water (10 mL).

Thus, when the mixture passed through two Sep-Pak Accell Light cartridges (Waters), 11% of the radiochemical yield of [^{18}F]FECh (not decay corrected) was passed with waste (Table 9). Then, 0.1M NaCl (10 mL) was passed through the Sep-Pak Accell Light cartridges and [^{18}F]fluoroethylcholine was collected which had yield of 38 % (not decay corrected). Systemization of [^{18}F]FECh resulted in 49% radiochemical yield (not decay corrected) in less than 40 minutes. However, when two Sep-Pak Accell Light cartridges were used, 11 % of the radiochemical yield was lost.

Table 9. Lost radiochemical yield of [^{18}F]FECh with two Sep-Pak Accell Light cartridges

Chemical compound	Ethanol	Ethanol	water
Rate of [^{18}F]FECh	6%	4%	1%

In another related study of mine, in which three Sep-Pak Accell Light cartridges were used, ethanol (5 mL), ethanol (10 mL) and water (10 mL) were collected and analyzed, respectively, by HPLC (Table 10).

Table 10. Lost radiochemical yield of [^{18}F]FECh with three Sep-Pak Accell Light cartridges.

Chemical compound	Ethanol	Ethanol	water
Rate of [^{18}F]FECh	1%	0%	0%

Systemization of [^{18}F]FECh resulted in 48% radiochemical yield (not decay corrected) in less than 40 minutes. However, when three Sep-Pak Accell Light cartridges were used, 1 % of [^{18}F]fluoroethylcholine was lost.

4.1.10 Purification and formulation of [^{18}F]FET

Afterwards the temperature was decreased to 60 °C, 8 mL of NH_4Ac (0.1 mol/L) were added and the reaction mixture was passed through several C18 Chromafix cartridges (Macherey Nagel). The cartridge was then washed with 5 mL of NH_4Ac and 5 mL of 10% ethanol, respectively. Then the [^{18}F]FET was eluted with 10 mL of 20% ethanol. After sterile filtration 2–4.1 ± 5% GBq [^{18}F]FET were obtained (42 ± 5%). The radiochemical purity was >99.9% and the specific activity >80 GBq/µmol at the end of synthesis (EOS). Synthesis time was 35 minutes from EOB.

4.1.10.1 The effect of the ethanol and NH_4Ac on C18 chromafix cartridges (Macherey Nagel)

In the second reactor, 8 mL of NH_4Ac (0.1 mol/L) was added into the reaction mixture which in turn was passed on three C18 Chromafix cartridges (Macherey Nagel). Then 5 mL of NH_4Ac and 5 mL of 10% ethanol were passed on the C18 Chromafix cartridges (Macherey Nagel), respectively, to wash the cartridges. NH_4Ac (8 mL), NH_4Ac (5 mL) and 5 mL of 10% ethanol were collected and analyzed by HPLC. [^{18}F]FET was found in NH_4Ac (8 mL), NH_4Ac (5 mL) and 5 mL of 10% ethanol.

Thus when the mixture passed through three C18 Chromafix cartridges (Macherey Nagel), 25% of the radiochemical yield of [^{18}F]FET (not decay corrected) was passed with waste **(**Table 11**).** Then, 10 mL of 20% ethanol was passed through the three C18 Chromafix cartridges (Macherey Nagel) and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine was collected which had a yield of 19 % (not decay corrected).

Table 11. Lost radiochemical yield of [^{18}F]FET with three C18 Chromafix cartridges (Macherey Nagel).

Chemical compound	NH_4Ac	NH_4Ac	Ethanol
Rate of [^{18}F]FET	5%	14%	6%

Systemization of [^{18}F]FET resulted in 44% radiochemical yield (not decay corrected), in 35 minutes. However, when three C18 Chromafix cartridges (Macherey Nagel) were used, 25 % of the radiochemical yield was lost.

In another related study of mine, in which four C18 Chromafix cartridges (Macherey Nagel) were used, NH_4Ac (8 mL), NH_4Ac (5 mL) and 5 mL of 10% ethanol were collected and analyzed, respectively, by HPLC (Table 12).

Table 12. Lost radiochemical yield of [^{18}F]FET with four C18 Chromafix cartridges (Macherey Nagel)

Chemical compound	NH_4Ac	NH_4Ac	Ethanol
Rate of [^{18}F]FET	0 %	0 %	2%

Systemization of [^{18}F]FET resulted in 42% radiochemical yield (not decay corrected) in 35 minutes. However, when four C18 Chromafix cartridges (Macherey Nagel) were used, 2 % of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine was lost.

4.2 Quality control procedures

4.2.1 Quality control of [^{18}F]FECh

Chemical and radiochemical purity are important for radiopharmaceuticals. In this study, the chemical and radiochemical purity of [^{18}F]fluoroethylcholine in a

radiochemical yield was determined using gas chromatography (GC), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

4.2.1.1 Gas chromatography:

While all non-volatile reactants and products of reaction 1, K_{222}, K_2CO_3 and [^{18}F]KF–K_{222} remained in reactor 1, only [^{18}F]BFE is transferred to reactor 2 for distillation at 130 °C. Thus, they do not interfere with the second reaction. The only compounds that might be also transferred are *o*-DCB (bp 180 °C) and BETfO (bp 230 °C) due to their considerable vapour-pressure. Analysis of the residual solvent levels, acetone (from drying procedure), DMF, DMAE, *o*-DCB, ethanol and acetonitrile, in [^{18}F]FECh doses was carried using a Varian 3500GC Gas Chromatography. The latter method used the column (Rtx® 200) is 0.25 mmID and 30m long) carrier gas N_2, 0.95 mL/min, injector 250 °C, program 50 °C (5 min hold, than 10 °C/min).

As demonstrated in Figure 36 and Table 15, the investigation of the final sterile saline solution of [^{18}F]FECh via gas chromatography showed the presence of only minimal amounts of acetone resulting from the cleaning procedure (0.24 ng/mL) and ethanol (11.5 ng/mL) (Table 13).

Table 13. The mean values of the chemical purity of the [^{18}F]FECh-saline solution for human use when detected by the gas chromatography.

Solvent	**Acetone**	**Ethanol**	***o*-DCB**	**DMF**	**DMAE**
(ng/mL)	0.24	11.5	-	-	-
Det. Limit (µL/mL)	0.007	0.007	0.002	0.007	0.025

GC measurements of the distil, however, clearly showed the presence of a trace amount of *o*-DCB (5 µL/mL) in the distillate, whilst BETfO was not detected (detection limit <0.01 ng/mL). This is a significant finding because BETfO could had also reacted with DMAE to give, for example, 2-bromoethylcholine which in turn would have caused problems in the next step, separation of [^{18}F]FECh via the cationic exchange cartridges.

The contamination with *o*-DCB, however, was easily removed together with DMF and DMAE by diluting the reaction mixture with ethanol, loading it onto the Sep-Pak cartridges, and subsequent cleaning it with ethanol and water.

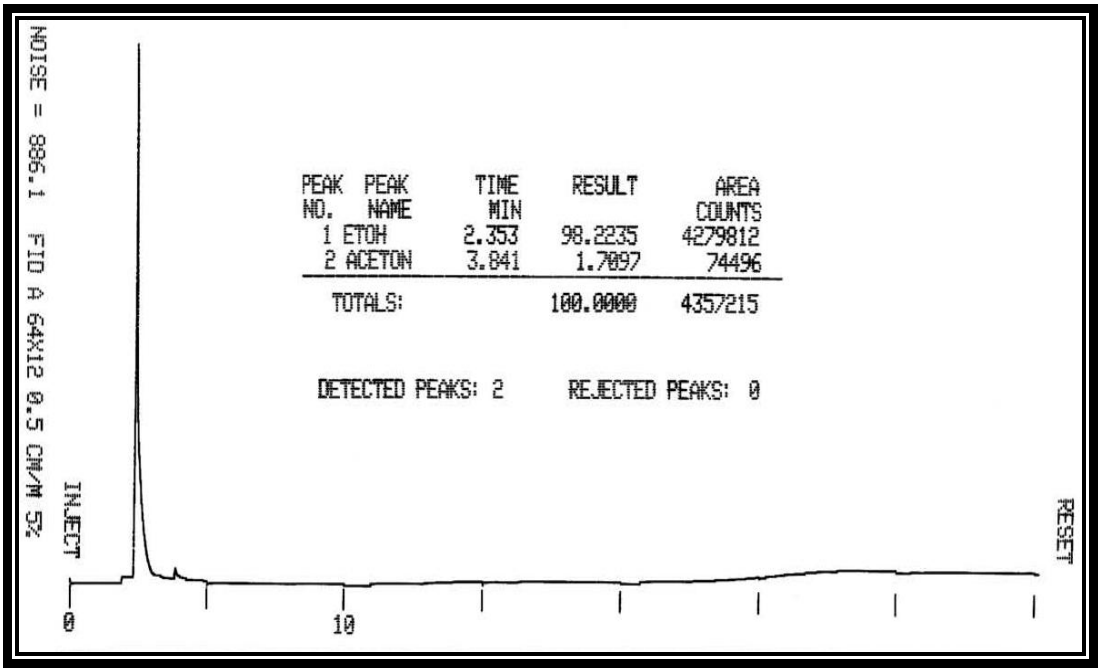


Figure 36. Chemical and radiochemical purity detection with gas chromatography.

4.2.1.2 Thin-layer chromatography (TLC)

TLC is a simple, quick, and inexpensive procedure to determine how many components are in a mixture. It is also used to support the identity of a compound in a mixture when the retention factor (R_f) of the compound is compared with that of a known compound (preferably both are run on the same TLC plate). The R_f is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent}}$$

The R_f for a compound is constant from one experiment to the next one only if the chromatography conditions below are also constant:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- temperature

The radiochemical purity of [^{18}F]fluoroethylcholine was determined by a thin-layer of chromatography (TLC) with 0.2mm silica gel and flourescent indicator $CH_2Cl_2/CH_3OH/CH_3OOH/H_2O$ (55/35/5/5 volume ratio) as a developing agent. After drying, the developed plates were exposed to iodine vapour for 3 minutes.

The R_f for [^{18}F]fluoroethylcholine was 0.34 (Figure 37). Since these factors are difficult to keep constant from experiment to another, relative R_f values are generally considered. "Relative R_f" means that the R_f values are compared with those of the compounds which run on the same plate at the same time (Figure 37).

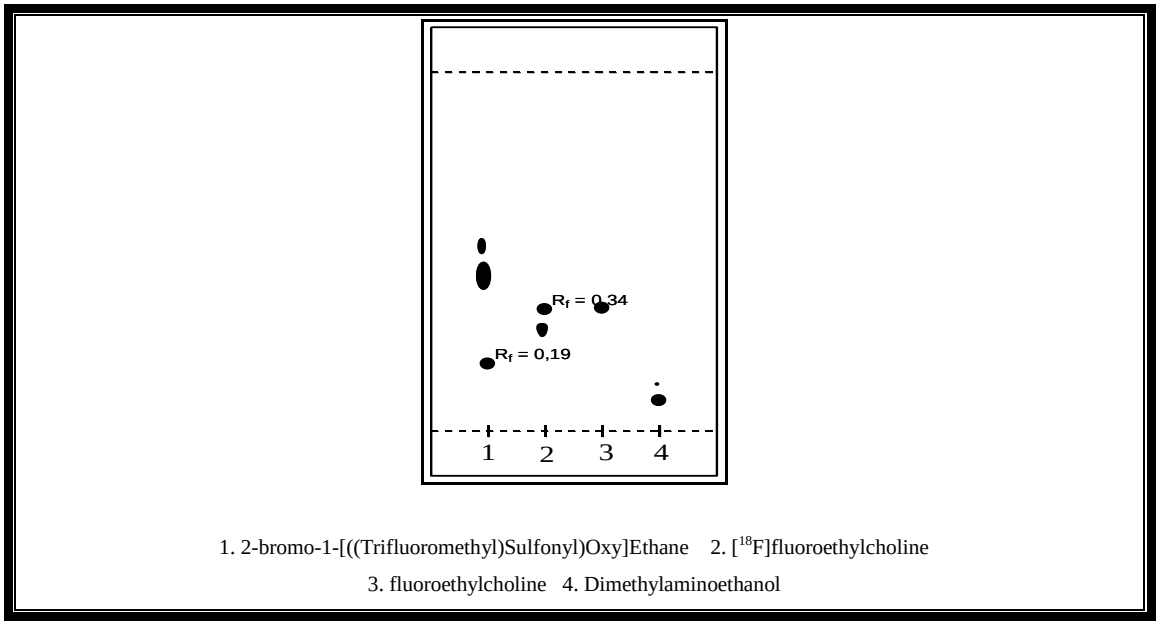


Figure 37. thin-layer chromatography.

4.2.1.3 High-performance liquid chromatography (HPLC)

The [^{18}F]FECh was analysed using Nucleosil® C18 column (100-5, 250×4 mm).

Run: flow rate 1ml/min

Buffer A: 0.1% TFA(Trifluoroacetic Acid) in acetonitrile

Buffer B: 0.1% TFA (Trifluoroacetic Acid) in H_2O

Gradient: 0 to 5 minutes 100% Buffer A decreased to 50%

5 to 15 minutes 50% Buffer A

The [^{18}F]FECh was analysed in accordance to the above mentioned conditions with UV-detection at 260nm. The purity and identity were checked by ion exchange chromatography (Partisil 10 SCX 250 4.6mm, 0.25M H3PO4/KCl, 10%V acetonitrile,

flow rate: 1.0 mL/min). The radiochemical yield was 47 ± 2.4% with a radiochemical purity of >99.9% and a specific activity >55 GBq/µmol. Typical chromatograms of the quality control are shown in Figure 38.

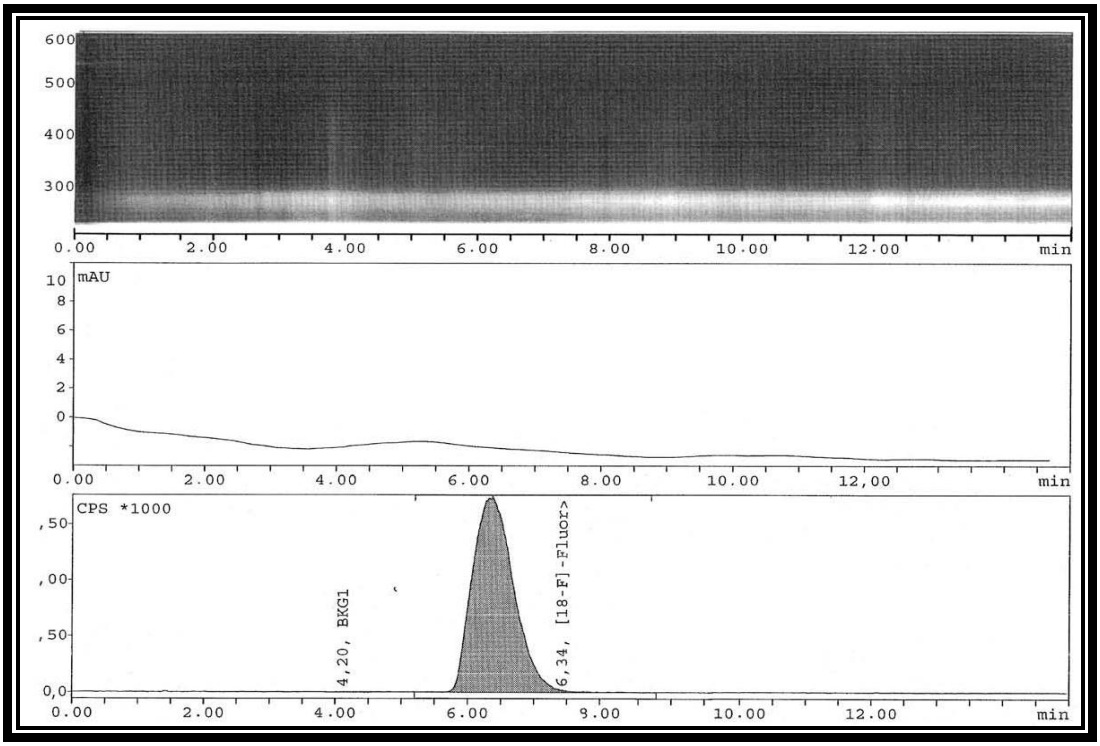


Figure 38. Chemical and radiochemical purity detection with radio HPLC with diode array detection.

4.2.2 Quality control of [^{18}F]FET

The Chemical and Radiochemical purity of [^{18}F]FET in a radiochemical yield was determined using gas chromatography (GC), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) .

4.2.2.1 Gas chromatography (GC)

The [^{18}F]FET was produced by the use of non-volatile 2-bromoethyl triflate as the starting material and the introduction of a distillation step for the prostetic group [^{18}F]BFE instead of the HPLC purification.

All non-volatile reactants and products of the first reaction, K_{222}, K_2CO_3, [^{18}F]KF-K_{222} as well as TBA and [^{18}F]Bu_4NF stay behind in reactor one and only [^{18}F]BFE is transferred into reactor 2 upon distillation at 100 °C. Thus, they do not interfere with

the second reaction. The only compounds that might possibly be also transferred into reactor 2 would be ***o***-DCB (bp 180 °C) and BETfO (bp 230 °C) due to their considerable vapour pressure.

GC measurements of the distillate, however, clearly revealed that only a trace amount of ***o***-DCB (5 µL/mL) is actually present in the distillate whereas BETfO was not detected at all (detection limit 0.002 µL/mL).

Analysis of residual solvent levels (acetone (from drying procedure), DMSO, *o*-DCB, ethanol and acetonitrile) in [^{18}F]FET doses was conducted using a Varian 3500 GC Gas Chromatograph. Column: Rtx® 200, 0.25 mmID, 30m, carrier gas N_2, 0.95 mL/min, injector 250 °C, program 50 °C (5 minutes hold, than 10 °C/min).

As shown in Figure 39, the investigation of final sterile solution of [^{18}F]FET via gas chromatography showed only the presence of minimal amounts of acetone from the cleaning procedure (0.24 ng/mL) and ethanol (11.5 ng/mL). No other solvents could be detected (Table 14).

Table 14. Chemical purity of the final ready for human use solution of [^{18}F]FET in saline detected with the gas chromatography.

Solvent	**Acetone**	**Ethanol**	***o*-DCB**	**DMSO**	**MeCN**
(µL/mL)	1.29	200	-	-	-
Det. Limit (µL/mL)	0.005	0.006	0.002	0.007	0.020

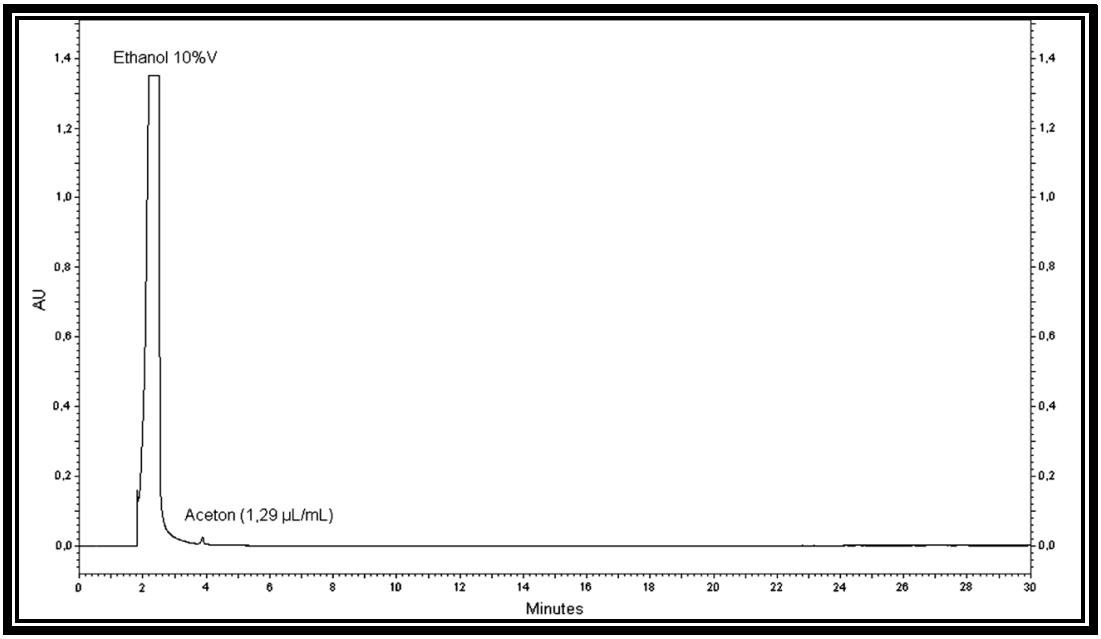


Figure 39. Chemical and radiochemical purity detection with (A) gas chromatography.

4.2.2.2 Thin-layer chromatography (TLC)

The analytical TLC plates used were active silica (SiO_2) gel on plastic plates. Spots were detected by ultraviolet (UV) illumination then TLC plates were cut into many equal sections, which were analyzed for radioactivity with a γ counter. Radiochemical purity was >97.7 %.

4.2.2.3 High-performance liquid chromatography (HPLC)

HPLC analysis of radiochemical identity was conducted using an Agilent 1100 LC-Liquid Chromatography fitted with diode array detector and Gina γ-detector (column: Nucleosil 100-5 HD 4·250 mm, Solvents: A: NH_4Ac (0.1 M); B: Acetonitrile; Gradient: 0-5 minutes 100%A, 5-30 minutes 0-100% B; flow rate: 1.0 mL/min). The retention time of [^{18}F]FET is compared to that of the [^{19}F]FET reference standard and must be ±10% (relative retention time (RRT) must be 0.9 – 1.1). Radiochemical purity must be >95%. Representative analytical HPLC traces are displayed in Figure 40.

4.2.2.4 Residual kryptofix-[2.2.2] analysis

Residual kryptofix-[2.2.2] levels in [^{18}F]FET doses were analyzed using the established spot test. Strips of plastic-backed silica gel TLC plates saturated with

iodoplatinate spray reagent were spotted with water (negative control), 50 µg/mL kryptofix-[2.2.2] standard (positive control) and [^{18}F]FET dose. If kryptofix-[2.2.2] is present in a sample, a blue-black spot appears. Spots for the three samples were compared and a visual determination of residual kryptofix-[2.2.2] in the [^{18}F]FET dose was made. <50 µg/mL is acceptable.

4.2.2.5 Sterile filter integrity test

The sterile filter from the [^{18}F]FET (with needle still attached) was connected to a nitrogen supply via a regulator. The needle was submerged in water and the nitrogen pressure was gradually increased. If the pressure was raised above the filter acceptance pressure (50 psi) without seeing a stream of bubbles, the filter was considered intact. If a stream of bubble occurs <50psi, the test fails.

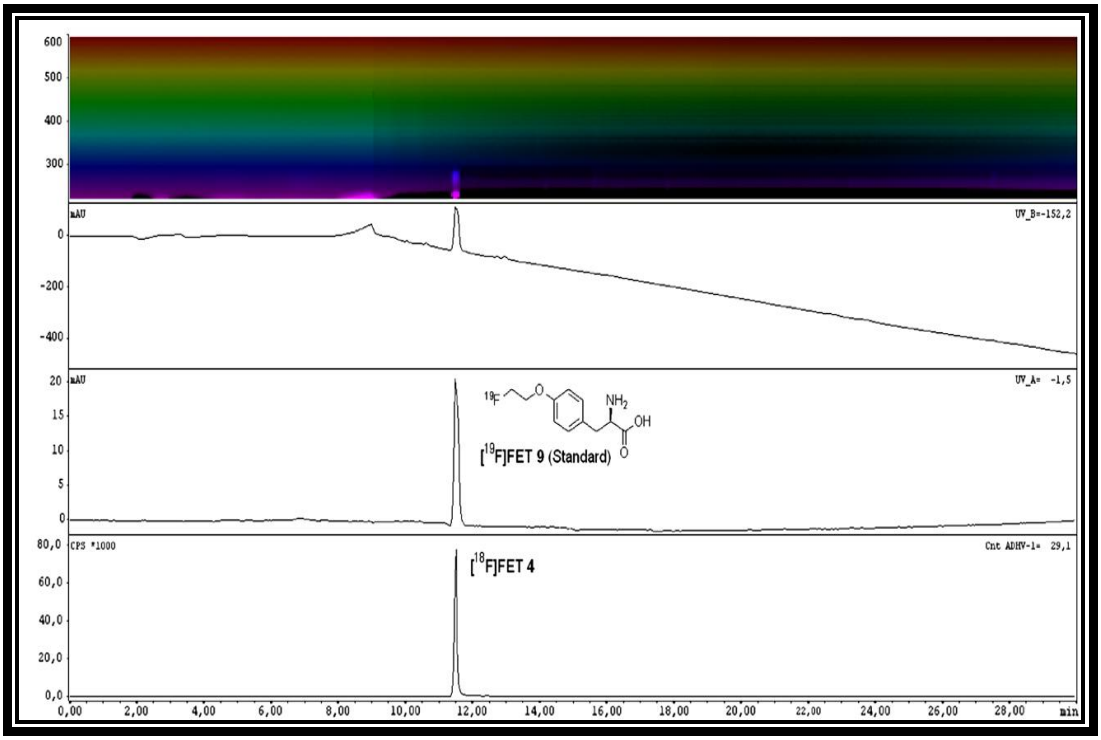


Figure 40. Chemical and radiochemical purity detection with radio HPLC with diode array detection.

4.2.2.6 Radionuclidic identity

Activities were measured using a MED®-Radioisotope Dose Calibrator and half-life was calculated using equation (1). Calculated half-life must be 105 – 115 minutes.

$$T_{1/2} = \frac{\ln(\text{Time Difference})}{\ln\left[\frac{\text{ending activity}}{\text{starting activity}}\right]} \quad (1)$$

4.2.2.7 Endotoxin analysis

Endotoxin content in doses of [^{18}F]FET was analyzed by a laboratory of the university hospital testing system and according to the US Pharmacopeia. Doses must contain ≤17.5 Endotoxin Units (EU) / mL.

4.2.2.8 Sterility testing

Samples of [^{18}F]FET were placed onto fluid thioglycolate media (FTM) plates and soybean casein digest agar media (SCDM) plates. FTM plates are used to test for anaerobes, aerobes and microaerophiles whilst SCDM plates are used to test for nonfastidious and fastidious microorganisms. [^{18}F]FET plates were incubated along with positive and negative controls for 14 days. FTM plates were incubated at 32 °C and SCDM plates were incubated at 22 °C according to current USP guidelines. Plates were visually inspected on the 3rd, 8th and 14th days of the test period and compared to the positive and negative standards. Positive standards must show growth (turbidity) on the plates and [^{18}F]FET / negative controls must have no culture growth after 14 days to be indicative of sterility.

5. Discussion

One essential aspect in biological properties of cancer cells is their uncontrolled growth. Therefore they need a lot of energy and protein to grow. This leads to an increased uptake in glucose which is the main source of energy for the human body. Similarly, the uptake of amino acids, which are used to build proteins, is usually higher in these cells than in normal cells. Abnormal growth of cancer cells also leads to a significant increase in the synthesis of phosphor lipid membranes that are associated with a choline transporter and choline kinase enzyme overexpression. As a result, the considerably high level of intracellular concentration of glucose amino acids and choline (above the normal level) are associated with many human cancers. Therefore [^{18}F]-labeled choline; various amino acids and glucose were developed and have shown their clinical potential in the evaluation of various human tumors using PET. But The most famous and most widely-used PET of these radiolabeled tracers is 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) which has been proven to be useful as a positron emission tomography (PET) probe for many kinds of cancers due to the higher demand of glycolysis in the tumor than that of normal counterparts. However, [^{18}F]FDG PET for the brain lesion imaging is limited for several reasons: firstly, the glucose metabolism in the normal brain tissue is usually higher and is difficult to be differentiated from that of the tumor; secondly, [^{18}F]FDG also cannot differentiate malignancy and chronic inflammation. This is due to the fact that inflammatory cells, a result of an increased metabolic rate, take up glucose. Thus [^{18}F]FDG accumulates considerably in the same manner as in tumors. This similar accumulation of [^{18}F]FDG may lead to incorrect detection, hence the wrong diagnosis of some types of cancer.

For these reasons [^{18}F]-labeled amino acids were developed particularly for clinical routine. Compared with [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) radiolabeled amino acids are more specific tracers for the differentiation between tumor and inflammation since the expression of transporters for amino acids is not stimulated by cytokines. And in a recent study [^{11}C]-labeled choline has been shown to be a valuable tracer for brain tumors, prostate carcinoma and bladder cancer where [^{18}F]FDG shows inadequate sensitivity. Because of the short half-life of ^{11}C ($T_{1/2}$ =20.38 minutes) is short, that leads to a limited usefulness for clinical routine. To overcome this problem [^{18}F]-labeled choline was developed.

In recent methods in the automated synthesis of [^{18}F]fluoroethylcholine and O-(2-[^{18}F]fluoroethyl)-L-tyrosine, all methods have required considerable efforts concerning purification as well as needing HPLC and gas chromatographic. Because of this, it takes a lot of time and yields are low. For an application in clinical services this means a relatively high dose cost rate, which certainly limits a widespread use of [^{18}F]FECh or [^{18}F]FET. Thus, our aim was to develop a simplified and considerably faster automated synthesis with higher yields making this approach much more attractive for clinical routine use.

In this study, we developed new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET via [^{18}F]fluoroethylation using 2-bromoethyl triflate (BETfO) as the starting material in a fully automated fast synthesis without the need of GC or HPLC purification. The new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET depends on the use of non-volatile 2-bromoethyl triflate as the starting material and the introduction of a distillation step instead of the HPLC purification.

In the new protocol for the synthesis of [^{18}F]fluoroethylcholine, [^{18}F]FECh is produced in a three-step process which consists of the formation of the [^{18}F]fluoroethylating agent, followed by the reaction with DMAE (Scheme 5).

Step one:

1-bromo-2-[^{18}F]fluoroethane synthons is produced by 2-bromo-1-[((Trifluoromethyl)Sulfonyl)Oxy]Ethane.

Step two:

1-bromo-2-[^{18}F]fluoroethane is distilled and it alone is transferred to second reaction.

Step three:

[^{18}F]fluoroethylcholine ([^{18}F]FECh) is synthesized by reacting 1-bromo-2-[^{18}F]fluoroethane ([^{18}F]FBE) with N,N-dimethylaminoethanol (DMAE) which has a similar structure to choline. This labeling synthon has to be cleaned up by Sep-Pak ® Light Accell Plus CM cartridge purification, hence purification of HPLC was not required.

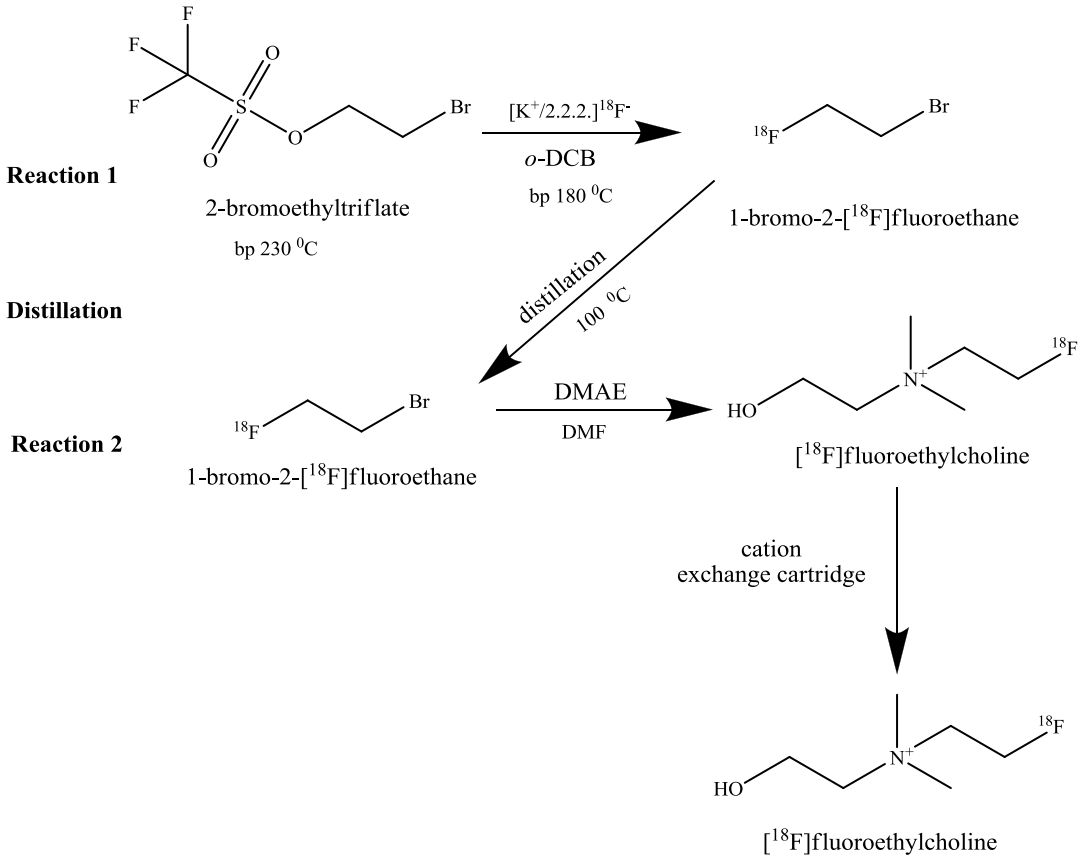
The most successfully used common syntheses for [^{18}F]fluoroethylcholine ([^{18}F]FECh) are produced starting from precursor 1.2-dibromoethane(DBE). But the new protocol for [^{18}F]fluoroethylcholine ([^{18}F]FECh) is produced starting from a precursor 2-bromoethyl triflate (BETfO). BETfO subjected to nucleophilic substitution with the [^{18}F]KF-Kryptofix-complex ([^{18}F]KF–K_{222}) in 1.2-dichlorobenzene (*o*-DCB) (Scheme 5). The advantage of BETfO compared to DBE is not only the higher reactivity of its triflate group compared to the bromine of DBE but especially its higher boiling point (230 °C) that is around 160 °C higher than the boiling point of [^{18}F]BFE (bp 71.5 °C).

Thus, in contrast to DBE the use of less volatile BETfO enables a simple distillation of the intermediate [^{18}F]BFE leaving all impurities from the first reaction behind in reactor 1. In this way, the second reaction of [^{18}F]BFE with DMAE can be easily performed under pure conditions resulting in higher specific activity of [^{18}F]FECh. We then turned our attention to the individual optimization of the three processes (reaction 1, distillation and reaction 2) outlined in Scheme 5.

In reaction 1, the adequate formation of the activated [^{18}F]fluoride either as [^{18}F]KF–K_{222}-complex by using K_2CO_3/Kryptofix is essential for the subsequent fluorination reaction of BETfO leading to the volatile intermediate [^{18}F]BFE. As described in the previous chapters, it was found for the case of using of KF–K_{222}-complex that the maximum yield of [^{18}F]BFE was achieved by using 80 µmol K_2CO_3/K_{222} and 95 µmol BETfO as well as a helium flow rate and drying temperature of 100 mL/min and 90 °C, respectively. At higher temperatures the yield of [^{18}F]BFE became lower due to decomposition of [^{18}F]KF–K_{222}. The distillation of [^{18}F]BFE (bp 71.5 °C) into the second reactor was started directly after the drying step by adding 95 µmol BETfO that was dissolved in 0.5 mL *o*-DCB to the activated [^{18}F]fluoride. This simple distillation at 130 °C under helium flow transfers the intermediate [^{18}F]BFE in almost pure form into the second reactor containing DMAE in DMF. The maximum yield was obtained with a flow rate of 100 mL/min and a temperature of 130 °C. Higher temperatures led to less transferred material because of partial decomposition of BETfO. Gas chromatographic analysis of the distilled material showed no presence of BETfO (bp 230 °C) in the second reactor when the temperature was ≤130 °C. The second reaction between [^{18}F]BFE and DMAE was carried out in the second reactor in

DMF. 0.5 mL of DMAE in 1.7 mL of DMF was found to be the optimum concentration in order to obtain the highest yield in reaction 2. The optimization of the reaction temperature and reaction time resulted in a maximum yield obtained at 100 °C and 15 minutes respectively.

Thus and after optimization of the synthesis parameters during the three processes (reaction 1, distillation and reaction 2) and selected the appropriate conditions to produce [^{18}F]fluoroethylcholine ([^{18}F]FECh) and due to the use of non-volatile 2-bromoethyl triflate as a starting material and the introduction of a distillation step instead of an HPLC purification, a significant simplification, and improvement of the synthesis of [^{18}F]fluoroethylcholine in a short time was achieved. At the same time there was a considerable increase in the radiochemical yield and the chemical purity. With such significant findings, the 47 ± 2.4% radiochemical yield of [^{18}F]fluoroethylcholine is, to a great extent, comparable with that of [^{18}F]FDG. Thefore, our optimized and easy manipulation synthesis provides access to this important compound for routine clinical use as a radio diagnostic tool.



Scheme 5. New approach to produce syntheses of [^{18}F]FECh.

As mentioned above we have a new protocol for fast and high yielding fully automated synthesis of [^{18}F]fluoroethylcholine via [^{18}F]fluoroethylation using 2-bromoethyl triflate (BETfO). Now BETfO will also be used as starting material for synthesizing [^{18}F]FET. BETfO was subjected to nucleophilic substitution with [^{18}F]KF–Kryptofix-complex ([^{18}F]KF–K_{222}) as well as with [^{18}F]tetrabutylammonium fluoride ([^{18}F]Bu_4NF) to form the volatile 1-bromo-2-[^{18}F]fluoroethane ([^{18}F]BFE) in 1.2-dichlorobenzene (*o*-DCB) (first reaction in Scheme 5). Because of the significantly lower boiling point of [^{18}F]BFE (bp 71.5 °C) compared with the precursor BETfO6 (bp 230 °C) and the solvent, *o*-DCB: (bp 179 °C), the intermediate [^{18}F]BFE could be elegantly distilled in a simple way into a second reactor within 8 min at 100 °C leaving all impurities of the first reaction behind in the first reactor. In this way, the second reaction of [^{18}F]BFE with Ty-Di-Salt can be easily performed under pure conditions in dimethylsulfoxide (DMSO) resulting in higher specific activity for [^{18}F]FET. The individual reaction steps are shown in Scheme 6.

[^{18}F]FET also is produced in three steps (Scheme 6) as in [^{18}F]FECh:

Step one:

1-bromo-2-[^{18}F]fluoroethane synthons is produced by 2-bromo-1-[((Trifluoromethyl)Sulfonyl) Oxy]Ethane.

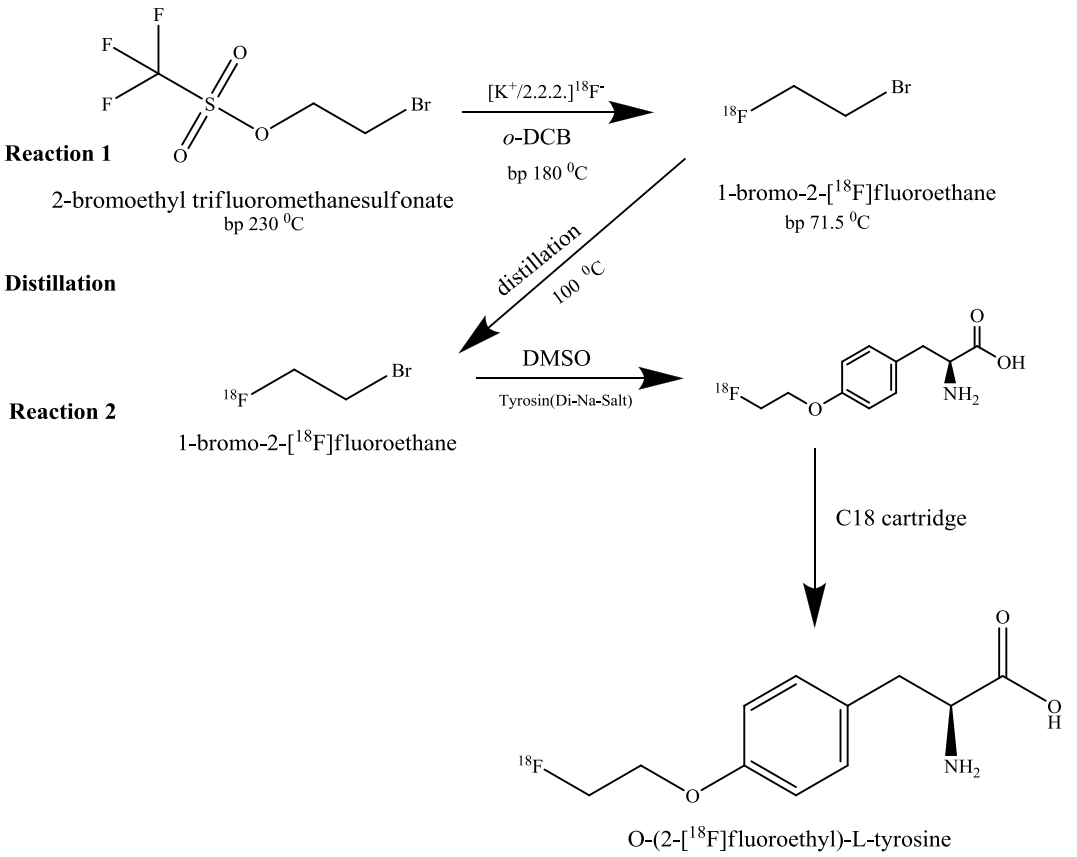
Step two:

1- bromo-2-[^{18}F]fluoroethane is distilled and it alone is transferred to second reaction

Step three:

[^{18}F]BFE reacts with freshly prepared L-tyrosine di-sodium salt to create [^{18}F]FET. This labeling synthon has to be cleaned up by C18 Chromafix cartridges purification, hence purification of HPLC was not required.

We then turned our attention to study the appropriate conditions which are necessary to produce [^{18}F]BFE during the three processes (reaction 1, distillation and reaction 2) to get the best result.



Scheme 6. New approach to produce syntheses of [^{18}F]FET.

In the first step, the adequate formation of the activated [^{18}F]fluoride either as [^{18}F]KF–K_{222}-complex or as [^{18}F]Bu_4NF by using K_2CO_3/Kryptofix or tetrabutylammonium hydrogencarbonate (TBA) is essential for the subsequent fluorination reaction of BETfO leading to the volatile intermediate [^{18}F]BFE. TBA exhibits lower basic properties compared with Kryptofix. Therefore higher radiochemical yield of [^{18}F]BFE would be expected due to less elimination by-products. But the results show no significant differences concerning formation of [^{18}F]BFE by using [^{18}F]Bu_4NF instead of [^{18}F]KF–K_{222}-complex. The data indicate an optimum for the formation of [^{18}F]BFE by using [^{18}F]Bu_4NF at 100 mL/min for the helium flow rate and 110 °C for the drying temperature. In both cases no transfer of TBA or Kryptofix from reactor one into reactor two during the distillation step was detected due to their high vapor pressures. The distillation of [^{18}F]BFE (bp 71.5 °C) into the second reactor was started directly after the drying step by adding 95 µmol BETfO that was dissolved in 0.5 mL *o*-DCB to the activated [^{18}F]fluoride. This simple distillation at 100 °C under helium flow transfers the intermediate [^{18}F]BFE in almost pure form into the second reactor containing Ty-Di-Salt and sodium iodide

(NaI) in DMSO. The most radioactivity (83%) is already transported after 6 minutes from reactor 1 into reactor 2. About 10% of the start activity remains in reactor 1 while about 7% of the start activity is lost during the distillation process. The second reaction between [^{18}F]BFE and freshly prepared Ty-Di-Salt was carried out directly after distilling [^{18}F]BFE in a second reactor containing Ty-Di-Salt, NaI and DMSO. As it was known that NaI increases the yield of fluoroethylation thus by adding of 33 µmol (5.5 mg) NaI the radiochemical yield of [^{18}F]FET could be increased by 30% compared to the reaction without NaI.

While in case [^{18}F]FECh the reaction mixture was passed onto the cation-exchanger cartridge. Cation exchanger can attract and hold cations that by pass in a moving solution if these are more attracted to the Na^+ than the $FCH_2CH_2(CH_3)_2N^+CH_2CH_2OH$. Therefore, the greater amount of NaI resulted in a decrease in [^{18}F]FECh.

So during the production of [^{18}F]FET, the second reactor contains 11.5 mg tyrosine (63.4 µmol), 28 µL of 10% aqueous sodium hydroxide (NaOH), 5 mg NaI (33 µmol), and 800 µL of DMSO. But when the use of various quantities of tyrosine which were always dissolved in 28 µL of 10% aqueous sodium hydroxide (NaOH), the radiochemical yield of [^{18}F]FET increases by increasing the amount of tyrosine, but starts to fall after reaching a maximum of 63.4 µmol. For further investigation, we then varied the amount of the added aqueous (NaOH) sodium hydroxide but kept the quantity of tyrosine constant at 63.4 µmol. We found out that when a molar ratio of aqueous (NaOH) over tyrosine of less than two was used, the radiochemical yield of [^{18}F]FET decreased and a new compound will form, which is assumed to be the 2-fluoroethyl ester of tyrosine. This assumption corresponds to the fact that a double molar excess of aqueous (NaOH) over tyrosine is necessary for the deprotonation of the phenolic group and building of Ty-Di-Salt. Therefore, the effect of the amount of NaOH on the radiochemical yield of [^{18}F]FET is dramatic because of the necessity to deprotonate the phenolic hydroxyl group of tyrosine before it can act as a nucleophilic agent toward [^{18}F]BFE. Due to the lower pKs value of the carboxyl group of tyrosine compared with the pKs value of the phenolic hydroxyl group (pKsCOOH = 2.2; pKsOH = 10.07) a molar ratio of NaOH/tyrosine $\geq$ 2 is necessary to completely

deprotonate the phenolic hydroxyl group and avoid the formation of the 2-fluoroethyl ester of tyrosine.

Thus and after optimization of the synthesis parameters including the distillation step of [^{18}F]FEBr combined with the final purification of [^{18}F]FET using a simple solid phase extraction instead of an HPLC run, the synthesis [^{18}F]FET could be significantly simplified, shortened, and improved. The radiochemical yield (RCY) was about 45% (not decay corrected). Synthesis time was only 35 minutes from the end of bombardment (EOB) and the radiochemical purity was >99% at the end of synthesis (EOS). Thus, this simplified synthesis for [^{18}F]FET offers a very good option for routine clinical use.

As mentioned above, the new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET is higher than all other approaches. Where the radiochemical yield (RCY) for the other approaches was about 25% (not decay corrected), but the radiochemical yield (RCY) for the new approach is about 47% (not decay corrected). This is because the other approaches need HPLC before the second reaction and during the purification, but the new approach to produce [^{18}F]FECh and/or [^{18}F]FET does not need HPLC before the second reaction simply due to the distillation step. This leads to a reaction 2 being easier and done under pure conditions. And [^{18}F]FECh and [^{18}F]FET also could be obtained ready for human use as physiological solution after fixation on the appropriate cartridges and formulation with saline without the need of GC or HPLC purification.

The new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET does not only give a higher yield, it is also less expensive. This is because the other approach needed 4 GBq of [^{18}F]floride ([^{18}F]F^- activity that was delivered from the cyclotron) per patient. This is because all other methods require considerable efforts concerning the purification and even more important consume a lot of precious time. That is why only low yields of [^{18}F]FECh and [^{18}F]FET could be achieved in all other methods. This means a relative high dose cost rate, which certainly limits a widespread use of [^{18}F]FECh and [^{18}F]FET. But the new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET needs less than 2 GBq of [^{18}F]floride. This yields a savings 50% per patient.

All other methods also consumed a lot of precious time. They took about 60 minutes. This is because all other methods require considerable efforts concerning the purification and used HPLC more than once during the production of [^{18}F]FECh and [^{18}F]FET. Whereas, the new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET takes about 35 minutes simply due to the distillation step, which allows us to obtain very pure [^{18}F]BFE, and also the final purification of [^{18}F]FET or [^{18}F]FET using the appropriate cartridges instead of HPLC. Thus, the new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET eliminates the use of HPLC completely.

The new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET is not only the fastest, it is also the most secure to preventing the risk of ionizing radiation because exposure time to ionizing radiation and radioactivity of [^{18}F]fluoride is less than other approaches and this will lead to a decrease in exposure rate for radiation to chemist and workers. This is because the ionizing radiation may cause damage in living tissue due to the ionizing radiation passing through living tissues induces damage, this damage is caused directly or indirectly with producing free radicals and toxic agents. Interaction of free radicals with critical macromolecules such as DNA is leading to genotoxicity and then mutation and carcinogenesis. In nuclear medicine practice, personnel and patients are exposed to ionizing radiation through radiopharmaceutical preparation and administration. Although, gamma irradiation produced by radiopharmaceuticals is not high level, it can induce genotoxicity.

Thus [^{18}F]FET or [^{18}F]FECh was produced by the use of non-volatile 2-bromoethyl triflate as the starting material and the introduction of a distillation step. And it could be obtained ready for human use as physiological solution after fixation on the appropriate cartridges and formulation with saline without the need of GC or HPLC purification. Radiochemical purity was >99.9% and no contamination of the sterile solution with chemicals used during the synthesis was detected. Thus, the new protocol for the synthesis of [^{18}F]FECh and [^{18}F]FET has the best radiochemical purity due to all non-volatile reactants and products of the first reaction, K_{222}, K_2CO_3, [^{18}F]KF–K_{222} as well as TBA and ([^{18}F]Bu_4NF) stay behind in reactor one and only [^{18}F]BFE is transferred into reactor two upon distillation at 130°C. Thus, they do not interfere with the second reaction. This leads to a reaction that is easier and done under pure condition.

The synthesis of [^{18}F]FECh and [^{18}F]FET could be significantly simplified, shortened, and improved. The use of the non-volatile 2-bromoethyl triflate as the starting material and the introduction of a distillation step substitute the HPLC purification steps in common synthesis procedures making the synthesis simple and short. At the same time both the radiochemical yield and the chemical purity could be increased dramatically. Thus, the routine synthesis of [^{18}F]FECh and [^{18}F]FET became more practicable and economically profitable.

The radiochemical yield (RCY) was about 45% (not decay corrected). Synthesis time was only 35 minutes from the end of bombardment (EOB) and the radiochemical purity was >99% at the end of synthesis (EOS). Thus, this simplified synthesis for [^{18}F]FET offers a very good option for routine clinical use.

With such significant findings, the 47 ± 2.4% radiochemical yield of [^{18}F]fluoroethylcholine is, to a great extent, comparable with that of [^{18}F]FDG. Therefore, our optimized and easy manipulation synthesis provides access to this important compound for routine clinical use as a radio diagnostic tool.

O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) is widely used as a positron emission tomography tracer for brain tumors.

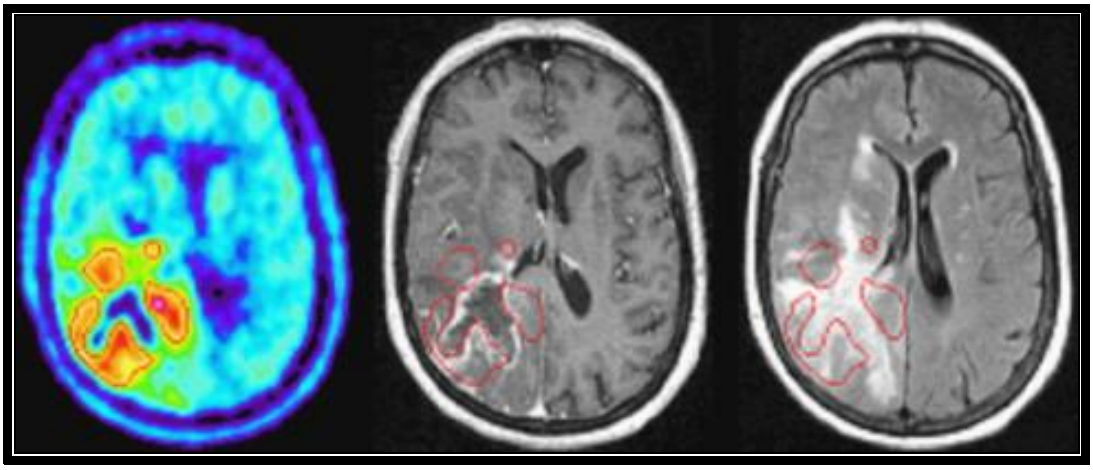


Figure 41. ***O***-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) used with PET for brain tumors (From Karl-Josef Langen, 2006, image no.1).

[^{18}F]fluoroethylcholine is used to detect and to determine the stage of cancers. PET is a unique and effective diagnostic tool in noninvasive assessment of metabolic and

physiological rates through tracer techniques. Differentiation of malignant cancer tissue from neighboring benign tissues may be accomplished by exploiting changes in choline processing that may occur in response to metabolic, genetic, or micro structural changes in malignant cells.

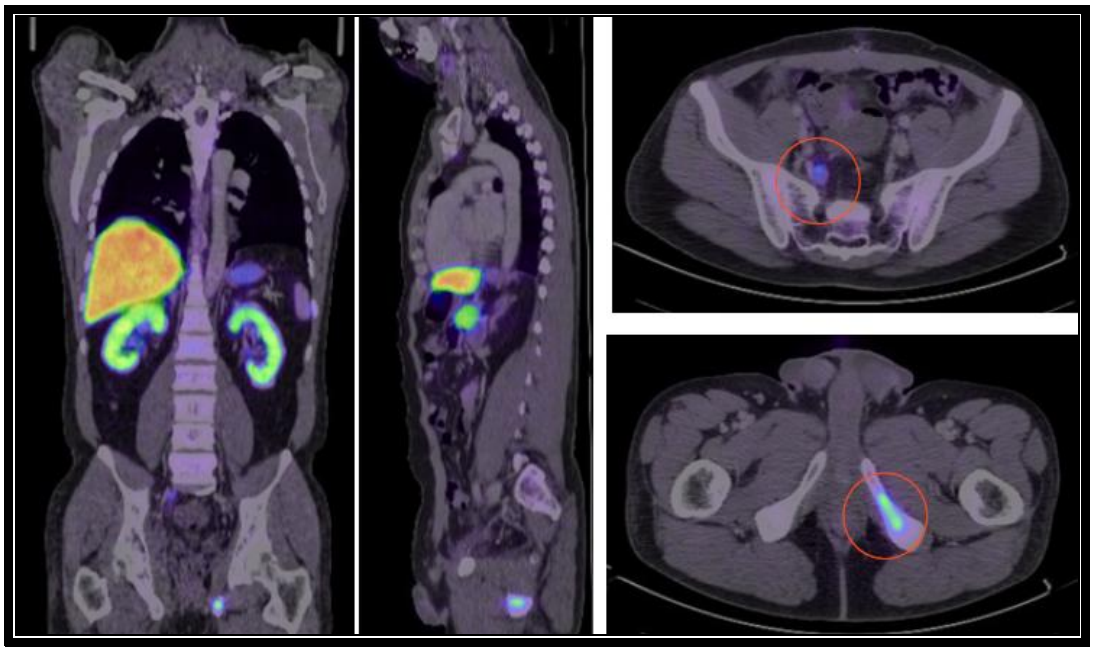


Figure 42. [^{18}F]fluoroethylcholine is used with PET to detect and to determine the stage of cancers (From PD Dr. Dietmar Dinter, 2011).

6. Summary

[^{18}F]fluoroethylcholine and O-(2-[^{18}F]fluoroethyl)-L-tyrosine are used as a radiotracer for cancer imaging and have shown good results with PET.

[^{11}C]- and [^{18}F]-labeled choline analogues are successful tracers for prostate cancer imaging when used with positron emission tomography (PET). Due to the practical advantages of the longer-living radioisotope [^{18}F] ($t_{1/2}$ = 110 minutes) instead of ^{11}C ($t_{1/2}$= 20 minutes), [^{18}F]fluoroethylcholine has been introduced to increase the opportunity of widespread clinical application. Nevertheless, the previous synthetic methods provided [^{18}F]fluoroethylcholine for human use only in moderate overall yields of up to 25% .

O-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) is one of the first [^{18}F]-labeled amino acids for imaging amino acid metabolism in tumors. This tracer overcomes the disadvantages of [^{18}F]fluorodeoxyglucose, [^{18}F]FDG, and [^{11}C]methionine, [^{11}C]MET. Nevertheless, the various synthetic methods providing [^{18}F]FET exhibit a large disadvantage because they need two purification steps during the synthesis. This includes HPLC purification, which causes difficulties in automation, moderate yields, and long synthesis times >60 minutes.

To overcome these problems, a new approach has been developed for the synthesis of [^{18}F]FET and [^{18}F]FECh starting from 2-bromoethyl triflate as precursor.

This approach also depends on the introduction of a distillation step of [^{18}F]FCH_2CH_2Br. [^{18}F]FET and [^{18}F]FECh could be obtained as physiological solution ready for human use after fixation on the appropriate cartridges and formulation with saline without the need of GC or HPLC purification.

After optimization of the synthesis parameters, which include the distillation step of [^{18}F]FCH_2CH_2Br in combination with the final purification of [^{18}F]FECh or [^{18}F]FET, the synthesis of [^{18}F]FECh or [^{18}F]FET could be significantly simplified, shortened, and improved. The radiochemical yield (RCY) was about 45% (not decay corrected and calculated relative to [^{18}F]F^- activity that was delivered from the cyclotron). Synthesis time was only 35 minutes from the end of bombardment (EOB) and the

radiochemical purity was about 99% at the end of the synthesis (EOS). Thus, this simplified synthesis for [^{18}F]FET offers a very good option for routine clinical use.

The synthesis of [^{18}F]FECh and [^{18}F]FET could be significantly simplified, shortened, and improved. The use of the non-volatile 2-bromoethyl triflate as the starting material and the introduction of a distillation step substitute the HPLC purification steps in common synthesis procedures making the synthesis short and simple. At the same time, both the radiochemical yield and the chemical purity could be increased dramatically. Thus, the routine synthesis of [^{18}F]FECh or [^{18}F]FET became more practical and economically profitable.

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9. Curriculum Vitae

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Education and working experience

- 1998-2002: Bachelor of Chemical & Physical Sciences - University of Aleppo.
- 2002-2003: Graduate student, Department of Analytical Chemistry - University of Aleppo, Syria.
- 2003- Diplom degree in Analytical Chemistry - University of Aleppo, Syria.
- 2007-2012: PhD student, Clinic of Nuclear Medicine - University of Kiel & Luebeck, Germany.

Publications

1. Simplified fast and high yielding automated synthesis of [^{18}F]fluoroethylcholine for prostate cancer imaging.
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Presentations

1. Optimierung der Cholinsynthese ([^{18}F]Cholin) Neue Aspekte im Strahlenschutz und Strahlenschutzunterweisung
   A. Alfteimi und Dr. rer. nat. M. Zuhayra, Klinik für Nuklearmedizin, UKSH- 2007.

2. Radiochemische Markierung: Verbesserte, neue Synthese für [^{18}F]Fluoroethyltyrosin [^{18}F]FET
   A. Alfteimi, Klinik für Nuklearmedizin, UKSH- 2008.

3. Entwicklung und Optimierung der radiochemischen Synthesen von [^{18}F] Fluorethylcholin und [^{18}F]Fluorethyltyrosin
   A. Alfteimi, Klinik für Nuklearmedizin, UK S-H -2010.

Poster presentations

1. Improved automated synthesis of [^{18}F]fluoroethylcholine as a radiotracer for prostate cancer imaging - Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin vom 23.-26. April 2008 in Leipzig.

2. Improved automated synthesis of O-(2-[^{18}F]fluoroethyl)-L-tyrosine as a radio-tracer for cancer imaging - München 2008.

3. Improved automated synthesis of [^{18}F]fluoroethyltyrosine as a radiotracer.
   47. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin vom 22. bis 25. April 2009 in Leipzig.

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Deutsch: Elementary