universität wien

MASTERARBEIT

Titel der Masterarbeit

State of the art techniques in medicinal radiochemistry on the basis of the synthesis of [^{18}F]Altanserin

verfasst von

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And in the end I also want to thank my family, who are always there with a helping hand, and advice or a shoulder to lean on if needed. Thank you all very much!

Die Wissenschaft sucht nach dem Perpetuum mobile -

sie hat es gefunden:

Sie ist es selbst.

Verfasser unbekannt

Abstract English

[^{18}F]Altanserin is an antagonist at the 5-HT_{2A} receptor of the serotonergic system. The serotonergic system plays a role in nearly every known brain function and therefor dysfunctions lead to many different psychiatric disorders.

The goal of the present work was to optimize and establish the synthesis of [^{18}F]Altanserin in the General Hospital of Vienna. Therefore the procedure was parted in different steps, namely the drying procedure of [^{18}F]fluoride, the reaction and the purification and formulation step.

The optimized synthesis was afterwards compared to already published syntheses. All in all [^{18}F]Altanserin was produced via 4 different synthesis methods.
Three methods were done in the vessel based module and one in the microfluidic module.
The first and the second method of synthesis were self-made according to already published syntheses of [^{18}F]Altanserin or the syntheses of other ^{18}F-tracers used in the General Hospital of Vienna.
The third method was gratefully adapted from the Jülich Research Centre and the fourth method, which was done on the microfluidic module, was optimized from an already published description by the working group.

The methods were compared on the basis of the radiochemical yield, the hands-in time, which is important for radiation protection, the duration of the synthesis, the precursor usage, the specific activity, the mean outome with a starting activity of 30GBq, the reliability of the synthesis and if it were be possible to applicate it to a patient by the results of the quality control.

Additionally the reaction was checked for its water-sensibility and furthermore the appearance of a doubled product and precursor peak in three of the four methods was analysed.

Abstract Deutsch

[^{18}F]Altanserin ist ein Antagonist am 5-HT_{2A} Rezeptors des serotonergen Systems.
Das serotonerge System spielt eine wichtige Rolle in fast allen bekannten Hirnfuntionen und eine Funktionsstörung führt daher zu vielen verschiedenen psychiatrischen Störungen.

Das Ziel dieser Arbeit war es die Synthese von [^{18}F]Altanserin zu optimieren und im Allgemeinen Krankenhaus der Stadt Wien zu etablieren. Dafür wurde die Synthese in verschiedene Stufen aufgeteilt, welche der Trocknungsprozess des [^{18}F]Fluorids, die Reaktion und die Aufreinigung und Formulierung des Produkts waren.

Die optimierte Synthese wurde danach anderen, bereits publizierten, Synthesen gegenüber gestellt.

Insgesamt wurde [^{18}F]Altanserin auf vier verschiedene Arten produziert, davon fanden drei Methoden in einem konventionellen (vessel-based) und eine am microfluiden Modul statt.

Die erste und die zweite Methode wurden selbst zusammengestellt in Anlehnung an andere bereits publizierte Methoden der [^{18}F]Altanserin Synthese bzw. anderen ^{18}F-Tracern die im Allgemeinen Krankenhaus der Stadt Wien angewendet werden.
Die dritte Methode wurde dankend vom Forschungszentrum Jülich übernommen und die vierte Methode, welche in einem microfluiden Modul stattfindet, wurde bereits von der Arbeitsgruppe publiziert.

Die Methoden wurden anhand von radiochemischer Ausbeute, der Hands-in Zeit, welche wichtig für den Strahlenschutz ist, der Dauer der Synthese, des Verbrauchs an Ausgangsstoff, der spezifischen Aktivität, der durchschnittlichen Ausbeute bei 30GBq Startaktivität, der Verlässlichkeit der Synthese und ob es, anhand der Richtlinien der Qualitätskontrolle, möglich wäre, das endformulierte Produkt Patienten zu verabreichen, miteinander verglichen.

Außerdem wurde die Reaktion wurde auf ihre Empfindlichkeit gegenüber Wasser untersucht. Ebenfalls wurde das Auftreten eines Doppel-Peaks, bei drei der vier Synthesemethoden in der präparativen sowohl als auch in der analytischen HPLC, untersucht.

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1 Introduction

1.0 A few words…

“What is radioactivity?” is a question I want to start my thesis with. The answer depends on whom you have asked.
If you ask for the public opinion in Austria, radioactivity is one of the biggest evil which has ever been discovered. An opinion based on many bad things, which happened in the nearly 110 years since its discovery.
If you ask a schoolkid, it is the source of super powers from super heroes and makes you glow greenish in the dark.
If you ask our safety representative, it is a lot of work and you need a lot of lead for shielding.
And if you ask me, it is definitely a useful tool for diagnosis and therapy in medicine and as every tool it depends on the user how to handle it.

1.1 Radioactivity

1.1.1 Discovery of radioactivity

The history of radioactivity, as we know it now, started with another important finding, the discovery of the x-rays by Wilhelm Conrad Röntgen in 1895.
Antoine Henry Becquerel, who heard about this, wanted to repeat the experiments of Röntgen with uranium containing minerals. It was the 26.02.1896 when he tried to stimulate potassium uranyl sulfate with sun light, but it was a gray day, so he had to stop his experiments.
Nevertheless, he developed the photographic plate and found that the uranyl mineral had the same effect on the photo plate as Röntgen described. However, it did not need sunlight or anything else to stimulate it. [1]
Two years later, in 1898, Marie Curie found similar properties for thorium. Gerhard Carl

Schmidt made the same discovery in Germany, independently from the work in France. Marie Curie also discovered together with her husband in the following years polonium (1898) and radium (1899).
All in all about 40 kinds of atoms were discovered until 1913 which led to a problem. The periodic table of the elements, set up in 1869 at the same time by L. Meyer and D. Mendeleyev, had only 12 places available on the basis of their chemical properties. This problem was solved by Soddy, who proposed to put several atoms in the same place on the periodic table, calling them isotopes. These isotopes have the same chemical properties but differ by the mass. Some nuclides have a great number of isotopes, tin for example has 10 stable and 18 unstable isotopes. [2, pp. 1-6]

1.1.2 The atom

To explain the difference in mass but not in the chemical characteristics it is the best to leave history and start with the atom itself. In the classic theory by Niels Bohr the atom can be imagined as a ball consisting of an extremely small core, the nucleus, in its center and electron shells.
Is the atom uncharged the number of positively charged protons (Z) in the nucleus is the same as the number of negatively charged electrons in the shells. The binding energy of the electrons is decreasing with the distance to the nucleus. This enables the electrons in the most distant shell to make chemical reactions and gives the atom its chemical properties. [3, p. 29]
Beside protons, there are also neutrons (N) in the nucleus. Both protons and neutrons exist in an unbound form as well, although the neutron is not stable and decays with a half-life of 14.6 min to a proton, an electron, an antineutrino and energy. To explain the decay of a neutron to a proton the quark model is very useful. It states, that a nucleon always consists of three quarks.
A proton consists of two u-quarks and one d-quark, a neutron of one u-quark and two d-quarks. A u-quark has a charge of +2/3 and a d-quark of -1/3, which means a total

charge of +1 for a proton and 0 for a neutron.
During the decay of the free neutron to a proton the d-quark transforms into a u-quark, an electron, an antineutrino and energy is set free.

The mass of an atom is therefore the sum of the mass of electrons, which play a minor part, protons and neutrons minus the energy of the strong interactions in the nucleus. The energy of the interactions in the nucleus is important, because the mass of the free proton or neutron is heavier than the mass of the bound nucleons. Other elementary particles, such as antiparticles form in the moment of decay or during interaction of matter with high-energy particles, which means a transformation of energy to mass. [4, pp. 1,2]

1.1.3 The radioactive decay

Radioactivity is the property of some nuclides to emit spontaneous particle and/or gamma (ɣ) radiation from the nucleus or to emit x-rays after electron capture through the nucleus. It includes all spontaneous appearing nuclear processes, which means both radioactive transformations and isomeric transition.
Out of about 2700 known nuclides only 271 are stable, the rest is unstable and decays with more or less long half-lives until a stable nuclide is reached. [4, p. 33]

The process of the decay can be simplified and described like that:

$$A \rightarrow B + x$$

A = radioactive nuclide
B = stable nuclide
x = emitted particle and/or radiation

The radioactive process depends only on the concentration of A, which means that it follows the reaction rate of the first order.

$$v(A) = -\frac{dc(A)}{dt} = k * c(A)$$

The speed of the decay is the negative change of the concentration of A with the time. It is equal a constant multiplied by the concentration of A.
If this reaction rate is integrated, following equation is obtained:

$$lnc(A) = -k * t + \ln c0(A)$$

ln c0(A) = integration constant
c0(A) = concentration of A at t=0
t = time

If ln c(A) is drawn against the time, a line is obtained with a rise of –k, which meets the axis of the ordinate at ln c0(A).

The equation can be re-written as the well-known “law of decay”:

$$c(A) = c0(A) * e^{-kt}$$

for the concentration or for the number of atoms.

$$N = N0 * e^{-\lambda t}$$

N = number of atoms
N0= number of atoms at t=0
λ = decay constant

The before mentioned half-life (t(½)) is the time, when half of the atoms from t=0 are decayed and it can be calculated the following way:

$$\frac{N0}{2} = N0 * e^{-\lambda t(\frac{1}{2})}$$

with $N = \frac{N0}{2}$ at $t(\frac{1}{2})$
Reformulation leads to:

$$t\left(\frac{1}{2}\right) = \frac{1}{\lambda} * ln2$$

This means that the half-life is independent from the concentration of the decaying atom but depends on λ, which is a nuclide specific constant. [5, pp. 261, 262]

The activity is the number of decaying atoms in a time period and is nowadays given in Becquerel [Bq]. 1Bq is the amount of 1 atom decaying in the time of 1 second. Until 1987 the activity was measured in Curie [Ci]. This unit can sometimes still be found and can be converted like this:

$$1\ Ci = 3{,}7 * 10^{10} Bq \qquad \text{[4, p. 35]}$$

1.1.4 The decay modes

The radioactive decay can be generally described by the following equation:

$$A \rightarrow B + x + \Delta E$$

A = parent nuclide

B = daughter nuclide

x = emitted particle or quant

ΔE = the kinetic energy of the emitted particles or quants

It is enlarged by the term of ΔE in comparison to the equation for the calculation of the kinetic, which was simplified for easier manageability.

The kinetic energy results from the difference in mass before and after the radioactive decay, when all educts and products are in ground state.

On the basis of the emitted particles, the following radioactive decay modes can be distinguished. [4, pp. 44, 45]

Decay mode	Symbol	emitted particle
α decay	α	Helium nuclei
β decay	β^-	Electrons
	β^+	Positrons
Electron capture (EC)	ε	Characteristic x-rays of the daughter nuclide
γ transition	γ	Photons (hv)
Isomeric transition (IT)	I_γ	Photons (hv)
Internal conversion (IC)	e^-	Conversion electrons and characteristic X-rays
Proton decay	p	Protons
Spontaneous fission	sf	Fission products and neutrons

Table 1: The radioactive decay modes [2, p. 48]

1.1.4.1 The α-decay

During the α-decay a He nucleus, the so-called α-particle, is emitted. This leads to a decrease of the atomic number by 2 units and the mass number by 4 units. An α-decays can be observed in nuclides with an atomic number higher than 83. [4, p. 46]
α-particles can be easily absorbed because of the high interactions with matter. A piece of paper, about 0.04mm thick aluminium sheets or a few centimetres of air are enough to absorb them. During their way the α-particles lose their energy by ionisation of other atoms they meet. This ionisation is dependent on their speed and is in air 3000 ion pairs per mm at the beginning to 7000 ion pairs per mm at the end of their journey.

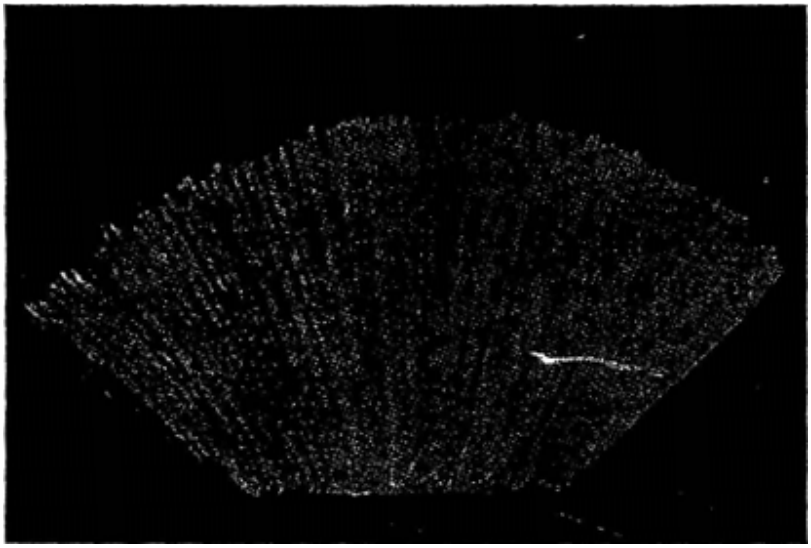


Figure 1: α-rays in a cloud chamber. *[6]*

Figure 1 shows the ionisation of the air in a cloud chamber. In sum, an α-particle produces 10^5 ion pairs until it becomes a neutral He atom. [7, p. 145]
An example for α-decay:

$$^{238}U(\alpha)^{234}Th$$

$t_{½} = 4.5*10^9$ a [4, p. 47]

1.1.4.2 The β-decay

During the β-decay a nucleon of an unstable nucleus transforms into another nucleon. This is only possible if the difference in energy between parent and daughter nuclei is small. With the increasing energy difference the probability for a particle emission as competitive decay increases. [4, p. 50]

The air hardly absorbs β-rays because of their much smaller interactions with matter. They have a range from approximately 10m in it and ionize about 4 ion-pairs per millimetre on their way. On the other hand, the β-rays have a much higher interaction with other electrons and therefore they are much easier kicked off their route. [7, p. 150]

The β-decay can be distinguished into the β^--decay and the β^+-decay.

The β^--decay

A β^--decay can happen spontaneously. This is possible because during the transformation of a neutron into a proton the difference in mass, as the neutron is heavier than the proton, can be compensated by a loss of energy. [4, p. 50] The atomic number increases in this case by one unit, whereas the mass number stays the same. [2, p. 53]

The smallest β^--emitter is the free neutron itself with a half-life time of 14,6min.

$$n \rightarrow p + e^- + \check{\nu} + \Delta E$$

Apart from the energy and the electron, an antineutrino has to be emitted. This particle had been postulated long before it was proofed. On the one hand because a particle with the spin I=-1/2 is needed for the conservation of the spin and on the other hand to satisfy the law of conservation of energy. In contrast to the α-decay, the β-decay has a continuous distribution of energy. The energy of the e^- has a range from 0 to a maximum $E_{\beta(max)}$. $E_{\beta(max)}$ is equal the energy of the decay minus the energy of the recoil. The antineutrino has the rest of the energy. [4, p. 50] Another example for a β-decay is:

$^{14}C(\beta^-)^{14}N$

$t_{1/2}$=5730a [2, p. 48]

The β^+-decay and electron capture

During the β^+-decay a proton in the nucleus transforms into a neutron by emission of a positron and neutrino (see equation below). The atomic number decreases by one unit and the mass number does not change. [2, p. 54] This decay is only possible in nuclei with an excess of protons and if the difference in energy between the educts and the products is higher than 1.022MeV. [4, p. 54]

$$p \rightarrow n + e^+ + v$$

The positron e^+ is an antiparticle of the electron e^- and has the same mass but the opposite charge. [5, p. 645] It is not stable and unites shortly after its formation with an electron. This leads to the electron-positron annihilation, and the whole mass of both particles is transferred into energy. Two ɣ-rays, each with an energy of 511keV, are emitted in opposite directions.

Is the energy smaller than 1.022MeV, a transformation of a proton into a neutron can only take place if the nucleus catches an electron from its shells, the so-called electron capture decay (EC). See equation below. [8, p. 7]

$$p + e^- \rightarrow n + v$$

The energy of the transformation emits with the neutrino. The neutrino itself is not easily traceable, therefore an electron capture decay is only detectable by the after-effect, which is caused by the daughter nuclide. It has a missing electron in its closest electron shell. This is filled from the electrons in the shells above and the element's typical x-rays can be measured. [2, pp. 54, 55]

1.1.4.3 The ɣ-decay and other decays

The β-decay seldom leads to the ground state of the daughter nuclide. Most atoms are in an exited state and reach another excited state of lower energy or the ground state by emitting one or more photons, this process is the ɣ-decay. Hence, the β-decay is in most cases followed by the ɣ-decay of the daughter nucleus.
In most cases the lifetime of the exited state is very small and the ɣ-rays are emitted immediately after the decay of the mother nucleus.

In some cases the exited state is metastable and the transition has its own half-life. This transition from the metastable state into the ground state is called isomeric transition (IT). As they are pure ɣ-emitters without α- or β-rays some of these atoms are very important. A famous example is ^{99m}Tc, which has found broad application in nuclear medicine.

Another way of decay from the excited state nucleus into its ground state is the internal conversion (IC). The energy is not emitted as a ɣ-ray but transmitted on an electron, which then is radiated. In contrast to the β^-decay, these electrons are monoenergetic and followed by characteristic x-rays. [2, pp. 61, 62]

The resulting ɣ-rays as well as the annihilation rays are used for scintigraphic measurements. [8, p. 7]

A further decay mode is the proton decay, which occurs when a high excess of protons is reached. The binding energy of the last protons decreases markedly and can even reach zero. This makes proton emission from the ground state energetically possible. This decay was first observed for ^{147}Tm and ^{151}Lu.

The last decay mode I want to describe is spontaneous fission (sf). It is only observed for high mass numbers and its probability increases with an increasing atomic number and a high number of neutrons in the nucleus. The mother nucleus decays into two daughter nuclei, a varying number of neutrons and energy. The resulting nuclei generally have different mass numbers and different atomic numbers. Due to the high

excess of neutrons several of them are emitted during this decay, they are called prompt neutrons. Other neutrons are emitted later on from the fission products and are called delayed neutrons. The energy, which is set free during the decay, can be calculated by a comparison of the masses of the educts and products of the decay. [2, pp. 66-68]

1.2. Flourine-18 radiochemistry

In the routine diagnosis with positron emission tomography (PET) fluorine-18 (^{18}F) is one of the most often used radionuclides. The favorable half-life of 109.7min allows multi-step radiosyntheses as well as PET studies of slower biochemical processes. At the same time, it also guarantees a minor radiation dose to the patient. [9, p. 16]
^{18}F also has a low positron energy of 649keV, which leads to high quality PET images.
In nature, fluorine has only one isomer, the stable ^{19}F, which is rarely present in natural organic compounds and especially rare in biologically active compounds. This fact is very advantageous for the synthesis of ^{18}F-tracers because it is very unlikely to have a high contamination of the product with the stable isotope.
This is important because a tracer is qualified due to its specific activity, which is the activity per mass or mole substance. The less nonradioactive product is obtained, the higher the specific activity. ^{18}F-tracers therefore usually have a high specific activity. [10, pp. 5,9]
Syntheses are evaluated on the radiochemical yield (RCY). It is the activity of the final product in relation to the starting activity. The radiochemical yield can be calculated either decay-corrected or not. [10, p. 14]

1.2.1 Nuclide production

More than 20 nuclear reactions are known for the production of ^{18}F.
The most common and efficient way is $^{18}O(p,n)^{18}F$ on ^{18}O enriched water. High activity of fluorine can be achieved at cyclotrons even with low energy and less than 1h radiation time. For electrophilic labeling reactions $[^{18}F]F_2$ gas is produced. ^{20}Ne and ^{18}O gas targets can be used for this production. [9, pp. 16,17]
The nuclide for radiosynthesis, in this case the ^{18}F, can be diluted with non-radioactive isotopes. There are three ways of this dilution, depending on the amount of non-radioactive, so-called “cold” isotopes.
For the production of $[^{18}F]F_2$ gas, cold F_2 gas is filled into the targets before the

bombardment, to avoid the produced ^{18}F to be trapped on the target walls. The obtained ^{18}F is called carrier-added.
During the production way of ^{18}F with ^{18}O enriched water, no fluorine is added, but also with very careful handling there are still some ^{19}F traces inside, for example as contaminant in the used ^{18}O water. It is called no-carrier-added (nca) ^{18}F.
The third way is called carrier-free and means a pure nuclide without a trace of another isotope.

1.2.2 Labeling with ^{18}F

Most commonly ^{18}F replaces in the labeled molecule one hydrogen atom. The advantage of this way to label is the nearly identical Van der Waals' radius of 1.35Å for fluorine and 1.20Å for hydrogen. The disadvantage is the very different electronic character. Replacing a hydrogen in an aliphatic position by fluorine increases the lipophilicity by a factor of 5, in an aryl group by a factor of 2. [9, p. 17] [9]

The fluorine atom is sterically more similar to oxygen because of the similar bond length to carbon. Therefore the fluorine can also replace a hydroxy group with the advantage of isoelectronicity. [11, p. 1501]

1.2.3 Reactions with ^{18}F

1.2.3.1 *General preparations*

The $^{18}F^-$, which is dissolved in water after its production, has to be transferred into organic solvents, for it is hardly reactive in its high degree and strength of hydration. To remove the water, the fluoride ion is absorbed onto an anion exchange cartridge, for example a PS-HCO_3 cartridge, and eluted with a small amount of aqueous weak base, like potassium carbonate, and a kryptand, such as aminopolyether 2.2.2 also known as K2.2.2. See figure below.

The kryptand captures the potassium cation and because of this separation, the nucleophilicity of the fluoride increases. The same effect can be preserved without a kryptand but with a large cation like Cs^+ or $Et4N^+$. The water is afterwards removed by one or more cycles of azeotropic drying with acetonitrile and the dry fluoride is dissolved in a polar aprotic solvent, such as acetonitrile or dimethyl sulfoxide (DMSO) depending on the subsequent reaction. [12]

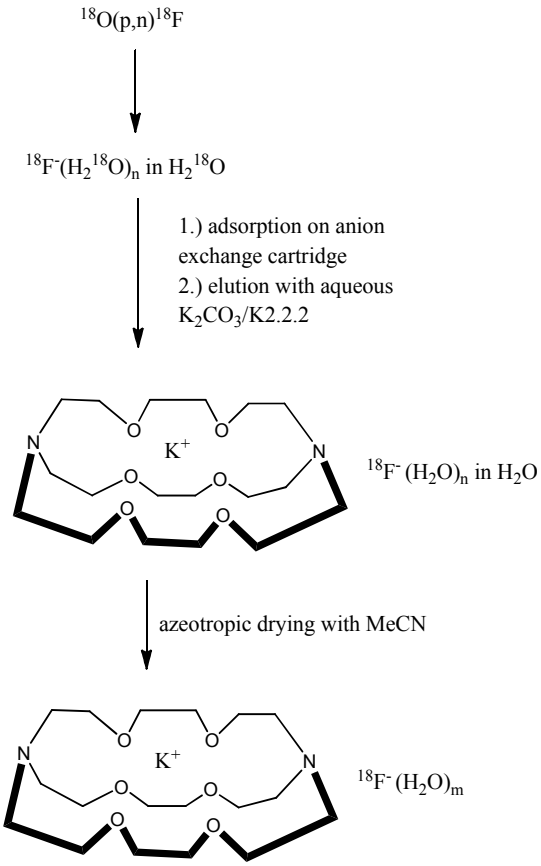


Figure 2: Scheme of the drying process

The degree to which the [^{18}F]fluoride ion is dried or dehydrated in this process is unknown, but it is known that the water-free fluoride is never obtained. The smaller the amount of water in the reaction is, the higher is the nucleophilicity of the fluoride. For difficult reactions, like the synthesis of [^{18}F]Altanserin, which is a nucleophilic

aromatic reaction, it is important to dry it very carefully. For aliphatic nucleophilic substitution reactions the drying process can be shortened.

Not only the presence of water but also the presence of protons can interfere with the reaction. The fluoride ion can be easily protonated, which makes it unavailable for further reactions. Therefore the optimal conditions are weakly basic. [13, p. 2856]

1.2.3.2 Wallach and Balz-Schiemann Reaction

In principal, reactions in the macroscopic organic chemistry can also be adapted into radiochemistry. Since no stoichiometry between reactants in radiochemistry is given, the yields are normally quite different and unpredictable.
The Wallach reaction uses stable diazonium piperidines to introduce fluorine into organic aromatic molecules.
For the Balz-Schiemann reaction solid diazonium tetraflouroborates are used for fluorination. In this case, a maximum radiochemistry yield of only 25% can be reached and isotopic carriers are used. [14, p. 41]
Both reactions are of the S_N1-type, in which a reactive cation interacts with the leaving group and many side products can be expected. [9, pp. 19, 20] The mechanism is shown in Figure 2 below.

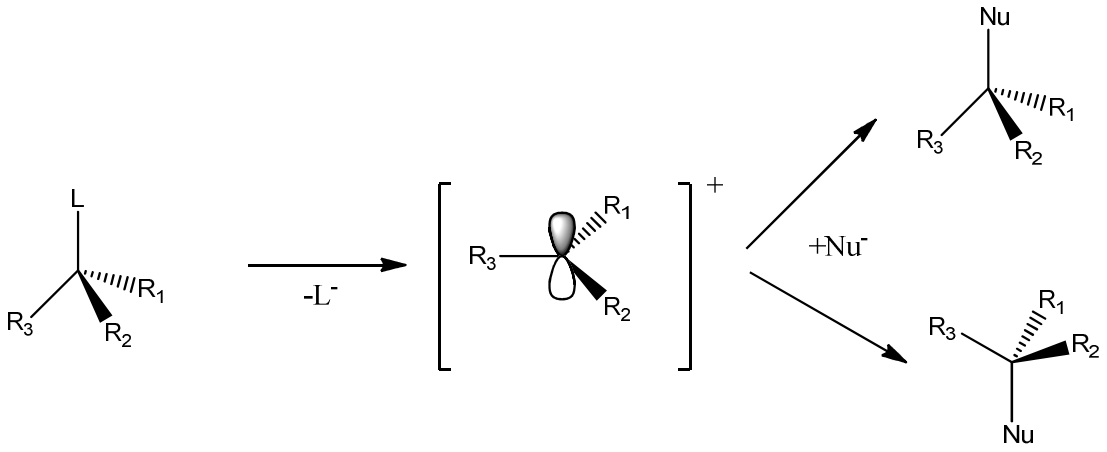


Figure 3: The reaction mechanism of S_N1; with L as leaving group.

Mainly because of their low radiochemical yields, the Balz-Schiemann and the Wallach reactions are nowadays rarely used to prepare ^{18}F-labeld radiotracers. [13, p. 2857] An example for the Balz-Schiemann reaction is the following synthesis of [^{18}F]FDOPA ((2S)-2-amino-3-(2-fluoranyl-4,5-dihydroxyphenyl)propanoic acid), a tracer for the brain dopamine metabolism. [15]

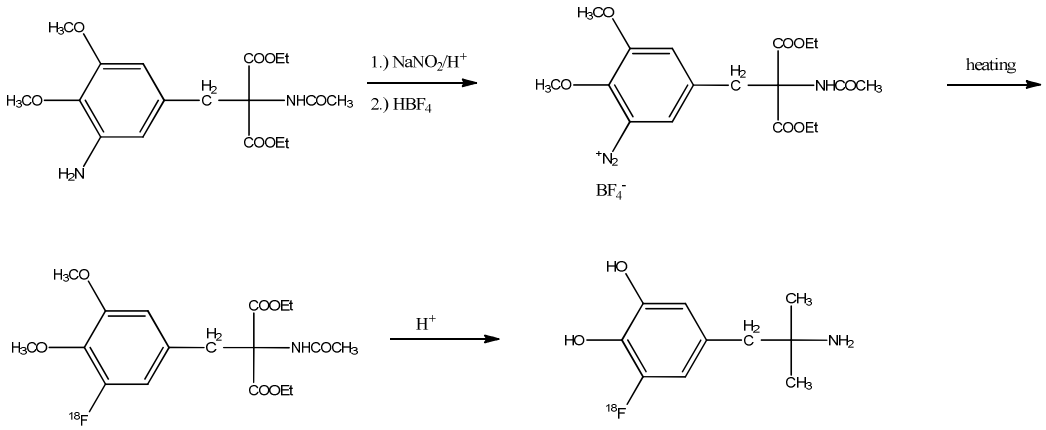


Figure 4: The synthesis of [^{18}F]FDOPA.

The Wallach reaction was used for the synthesis of the dopamine D2-receptor binding radiopharmaceutical 3-(2'-[^{18}F]Fluoroethyl)spiperone. [16]

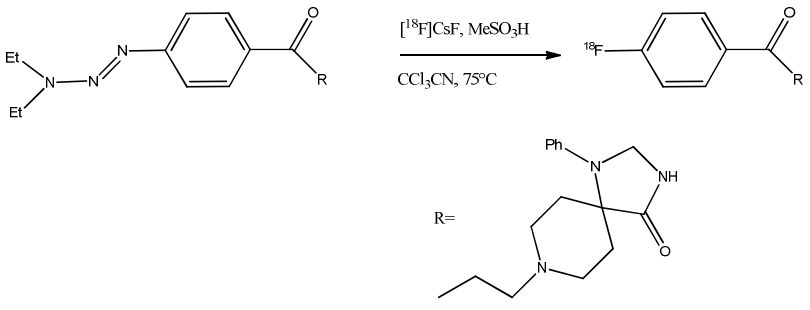


Figure 5: Synthesis of 3-(2'-[18F]Fluoroethyl)spiperone *[17]*

1.2.2.4 Nucleophilic Substitution with the [^{18}F]fluoride ion

The nucleophilic substitution is the most important route for labeling. The dried no-carrier-added [^{18}F]fluoride reacts with a precursor via the mechanism of S_N2 reactions, which is shown in Figure 3 below. [18, p. 240]

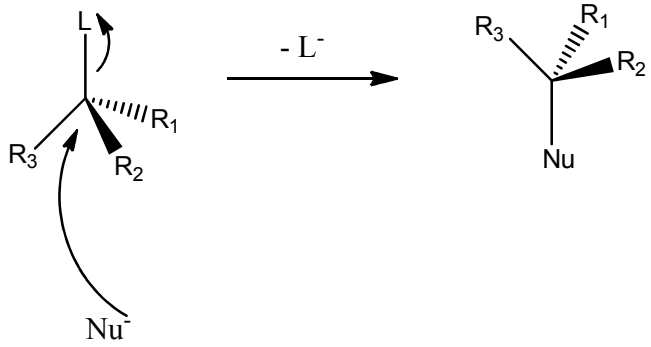


Figure 6: Mechanism of SN2 reaction, with L as leaving group and Nu as nucleophile

Typical leaving groups are halogens or sulphonic acid ester groups like mesylate, tosylate and triflate. The Walden inversion has to be considered for the synthesis of the precursor. A typical example for this reaction type is the synthesis of [^{18}F]FDG ((2R,3S,5S)-3-fluoranyl-6-(hydroxymethyl)oxane-2,4,5-triol or 2-deoxy-2-(^{18}F)fluoroglucose), which represents the most frequently used radiopharmaceutical worldwide in oncology and neurology. [19]

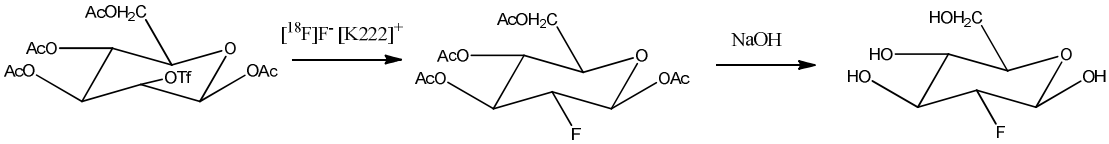


Figure 7: Modern synthesis of [^{18}F]FDG. *[20]*

For the nucleophilic aromatic substitution, activated aromatic molecules are needed. This is the case for aromatic molecules with electron withdrawing substituents in

ortho- or para-position of the leaving group. Typical substituents, with strong electron withdrawing properties, are nitro-, cyano- or carbonyl-groups.
The good metabolic stability of these tracers is a major advantage. [^{18}F]Altanserin follows the SN2 reaction mechanism. [9, pp. 23-26] [21, p. 211]

1.2.2.5 Electrophilic substitution

In contrast to the nucleophilic substitution [^{18}F]F_2 is used for the electrophilic substitution [22]. For some reactions [^{18}F]F_2 is converted into less reactive and more selective reaction agents like acetylhypofluorite ([^{18}F]CH_3COOF) or xenon difluoride ([^{18}F]XeF_2).

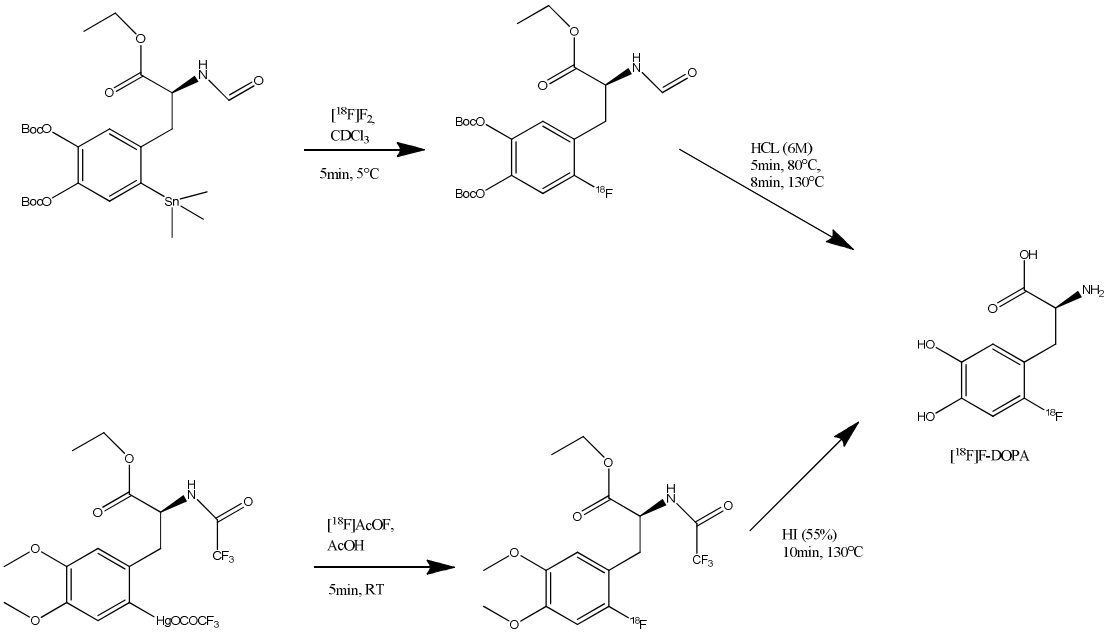


Figure 8: electrophilic synthesis of [^{18}F]F-DOPA via [^{18}F]F_2 *[23]* and [^{18}F]AcOF *[24]*. *[25]*

These methods allow the fluorination of electron-rich compounds, which would not be possible with nucleophilic labeling.
The disadvantage of this method is the high reactivity of fluorine gas, which leads to various side products and even reactions with the solvent can appear. Intensive

cleaning and purification procedures are needed to reach the required purity of pharmaceuticals.

In addition, the specific activity is quiet low due to the added ^{19}F in comparison to the nucleophilic substitutions where no-carrier-added ^{18}F is used. [9, pp. 20-22] [13]

1.3 [^{18}F]Altanserin and the serotonergic system

The name serotonin can be explained by the way of its discovery. In 1947 it was first isolated from blood serum, and it has a vasoconstrictive effect, like a tonic. The combination of the words serum and tonic led to serotonin. [26, p. 44] Its IUPAC name is 3-(2-aminoethyl)-1H-indol-5-ol, another important name is 5-hydroxytryptamine (5-HT). [27]

The serotonin (5-HT) system is the oldest and one of the most complex neurotransmitter systems in the brain and plays a role in nearly every known brain function. [28] [29]

It is involved in many behavioral functions and biological systems, from early developments to neurogenesis and maturation, to apoptosis and neurodegeneration. A dysfunction therefore can lead to many different psychiatric disorders, such as addictions, attention deficit hyperactivity disorder, aggression and violent behavior [30], autism [31], dementia, eating disorders, major depression [32], obsessive-compulsive disorder (OCD), panic and anxiety disorder, personality disorders, schizophrenia and suicidality. [33] [34] [35] Several serotonergic drugs are hallucinogens. [36]

In 1986 a classification on three major types of 5-HT receptors was published, 5-HT_1, 5-HT_2 and 5-HT_3. Several additional receptor classes were detected in the following years. [37] Nowadays at least 14 different receptors, namely 5-HT_{1A-E}, 5-HT_{2A-C}, 5-HT_3, 5-HT_4, 5-HT_{5A-B}, 5-HT_6 and 5-HT_7, and a serotonin transporter are known. All 5-HT receptors, except the 5-HT_3 receptor, which gates a cation-permeable ion channel, are G-protein coupled. [38] The mechanism of the signaling via these receptors is diverse and still not fully understood in real life, but they are able to modulate other systems in the brain such as the glutamatergic and GABA-ergic ones. [39] [40]

The major interest of research in this field was the development of radioligands for the 5-HT_{1A} and the 5-HT_{2A} receptor. The first effective radiopharmaceutical for the serotonergic system has been [^{11}C]WAY-100635, it is used for selective imaging of the

serotonin 1A receptor (5-HT_{1A}). [41] [42]. Subsequently, this compound was radiolabeled in two different positions yielding [*O-methyl*-^{11}C]WAY-100635 and [*carbonyl*-^{11}C]WAY-100635, respectively.

[^{11}C]Ketanserin, [^{18}F]Flouroethylketanserin, [^{11}C]N-methylspiperone, N-([^{11}C]methyl)-2-Br-LSD, [^{18}F]setoperone [31] [43], [^{18}F]MH.MZ, (R)-[^{18}F]MH.MZ, [^{18}F]Altanserin, [^{18}F]deuteroaltanserin, [^{11}C]MDL 10090, [^{18}F]fananserin and [^{11}C]Cimbi-36 are some of the published radio ligands for 5-HT_{2A}. [44] [^{18}F]Altanserin is a preferred radiotracer for labeling of the serotonin 2A receptor (5-HT_{2A}). It binds with high affinity to the receptor as an antagonist and with lower affinity to other serotonin receptor subtypes. [35] Hence, it is an excellent compound to selectively map the distribution of the 5-HT_{2A} receptor using PET. An example is given in Figure 9. [45]

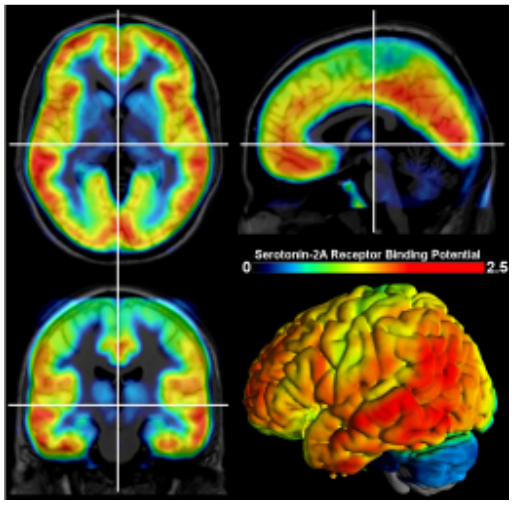


Figure 9: PET scan of the brain using [18F]Altanserin as tracer. Regions with high tracer accumulation are shown in yellow-red. Low uptake is represented by black-to-blue color. *[46]*

[^{18}F]Altanserin crosses the blood-brain barrier (BBB) rapidly with a maximum uptake of 3% of the injected dose in the brain. [35]

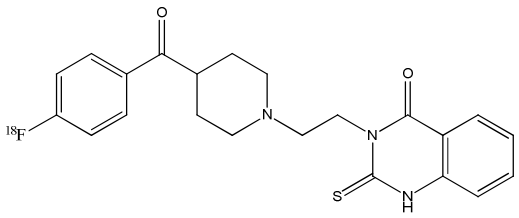


Figure 10: Structure of [^{18}F]Altanserin

In humans, four radiolabeled metabolites of [^{18}F]Altanserin, are known. Two of them, [^{18}F]Altanserinol and [^{18}F]4-(4-fluorobenzoyl)piperidine (FBP), have already been identified. [35] [44]

The synthesis of [^{18}F]Altanserin is a typical S_N2 reaction. The precursor is Nitroaltanserin, with a nitro-group as leaving group onto the aromatic ring and a carbonyl-group as electron-withdrawing group in para-position. [47] Further details to the synthesis are mentioned in the practical part.

1.4 The synthesizer

In the following work two different synthesis modules were used, the vessel-based-batch-mode module and the flow-through-µ-fluidic synthesizer.

1.4.1 The vessel-based-batch mode synthesizer

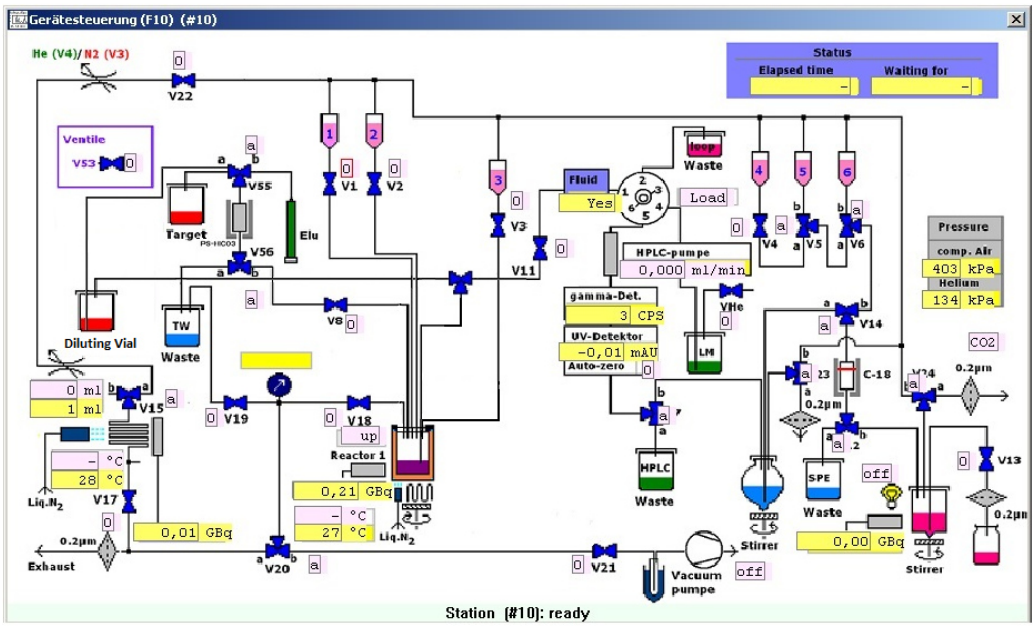


Figure 11: Hydraulic plan of a vessel-based-batch mode synthesizer.

→ Tubing

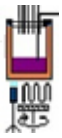

→ Vials 1 to 5

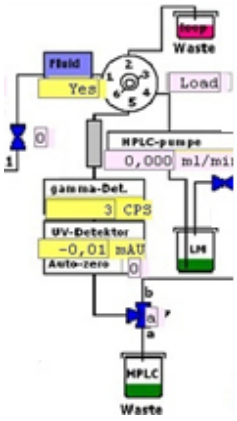

→ Valve; opens automatically during the synthesis and the liquid is flushed along the tubes into another container


→ 3 way valve; either the way to **a** or to **b** can be open

 → Container

 → Bulb; filled with water to dilute the HPLC eluent for removing organic solvents

 → the reactor; the reacting solution can be stirred, heated or cooled



→ Preparative HPLC system with fluid detector, loop, waste bottles, UV- and radio detector

 → cartridges

 → product vial with sterile filtration

 → Product collecting vial

The drying of the Fluoride and the reaction itself takes place in the reactor, which can be stirred, heated or cooled. The whole system could be flushed with helium but the pressure in the system couldn't be regulated precisely. The volumes of the dissolved precursor were in the 100µl to 1ml range.

1.4.2 The flow-through-µ-fluidic synthesizer

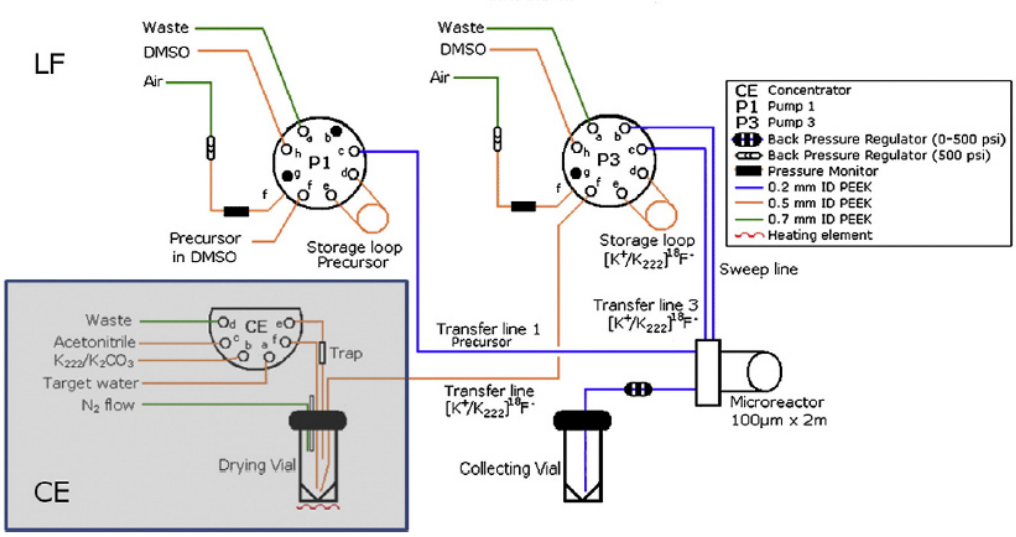
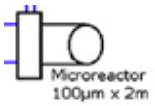


Figure 12: The flow-through-µ fluidic synthesizer

→ Pump 1 or 3

→ Loop to store liquid before pumping it through the reactor

→ Vials



→ Reactor

→ Tubing; as described in Figure 12

→ anion exchange cartridge

The reaction takes place online while the pumps press the dissolved water-free fluoride and the precursor into the microreactor. The reactants were mixed and heated during their way through the reactor. The used reactor itself consisted of a 2m long capillary tube with a diameter of 100µm, which was wound up. The usual diameters of reactors are in the range of 10-300µm. [48] Mixing via diffusion is not efficient in reactors with

dimensions greater than 1mm, but as the diffusion distance is only 100µl the mixing time, in theory, becomes very small. [49] [50]

The main advantages of the microfluidic synthesizer in comparison to the vessel-based-batch mode synthesizer were:

1) smaller amount of solvents and reagents

2) higher concentrations and additional efficiency from rapid heat transfer and mass transport

3) easier modification of the microfluidic reaction chamber

4) easier portability of the device (relative to conventional instruments) [51] [52]

Another benefit is the possibility to do more than one small scale reaction, which makes an optimization of the reaction parameters much faster. [53]

2 Practical part

The synthesis of [^{18}F]Altanserin was optimized. 4 different methods were compared and also smaller changes were implemented to optimize these methods.
For a better comparison of the consequences of different changes, the syntheses were divided in different parts.
The first part is the drying of the fluoride, its quality and efficiency were stated as the turnover of fluoride in the crude product.
The second part is the conversion itself, which was also compared by the turnover rate, measured by HPLC and TLC.
The third part is the cleaning and formulation of the product.

For all purified and formulated products a quality control was done.

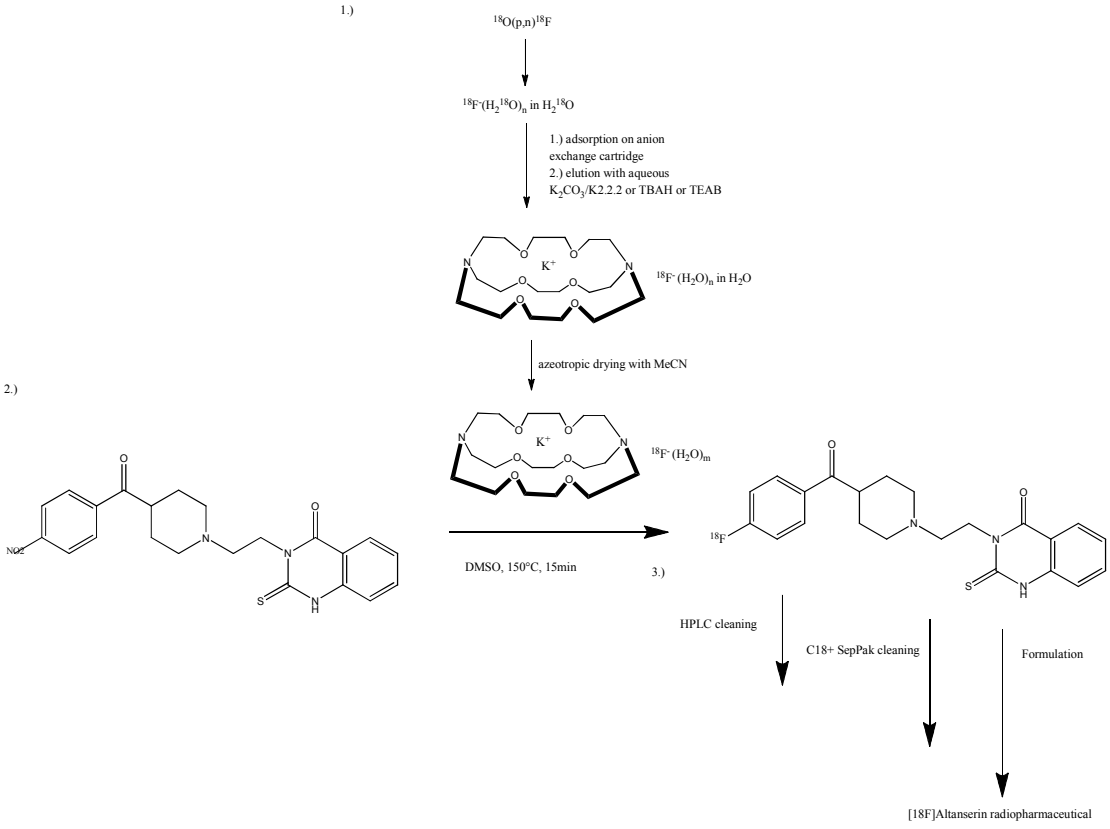


Figure 13: Reaction scheme of [^{18}F]Altanserin production (the steps are marked).

2.1 Cleaning of the equipment

Before a production run could be started, the device was cleaned carefully.

The vial for the precursor was filled with dry DMSO, the vials that contain water or aqueous solutions were filled with water, the vial for ethanol with absolute ethanol and the vial for acetonitrile with dry acetonitrile, the reactor was flushed with acetonitrile, as shown in Figure 14.

1) The elution vial with acetonitrile was opened and emptied into the reactor.

2) The water in the target vial was sucked into the waste with vacuum.

3) The reactor was removed and the vials 1-3 were emptied into a beaker.

4) Vial 6 (filled with water) and vial 5 (containing ethanol) were transferred into the product-collecting vial and washed with water from vial 4.

5) The product-collecting vial was shaken for some time and the ethanol-water mixture was converted through the tubing in direction to the product vial.

6) The tubes were rinsed with water from vial 4.

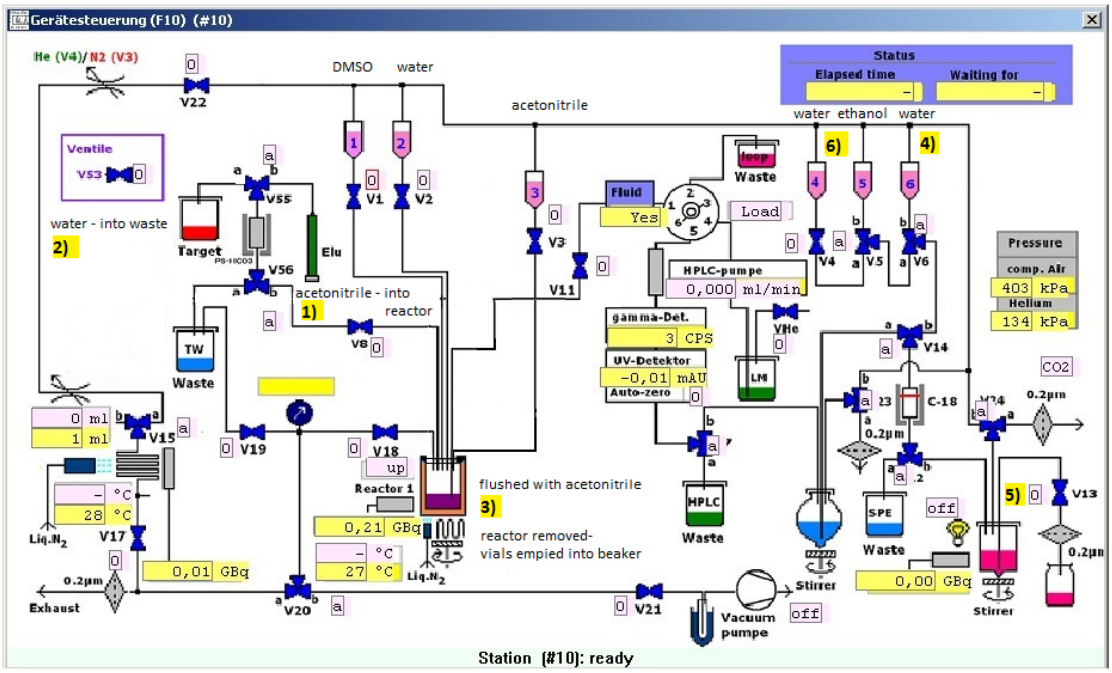


Figure 14: scheme for cleaning the device.

2.2 Production of the [^{18}F]fluoride

^{18}F was produced via the ^{18}O(p,n)^{18}F reaction in a GE PETtrace cyclotron (16.5 MeV protons; GE Medical systems). $H_2{}^{18}O$ was obtained in >98% from Rotem Europe.
The radiation time was approximately 30min to receive 30GBq of ^{18}F for the synthesis. For short reactions, which were aborted after the second step, only 0.5-1.5 GBq Fluoride-18 was used. This was obtained by flushing the cyclotron-target (directly after production of Fluoride-18 for another synthesis) with water.

2.3 The synthesis methods

2.3.1 Method 1)

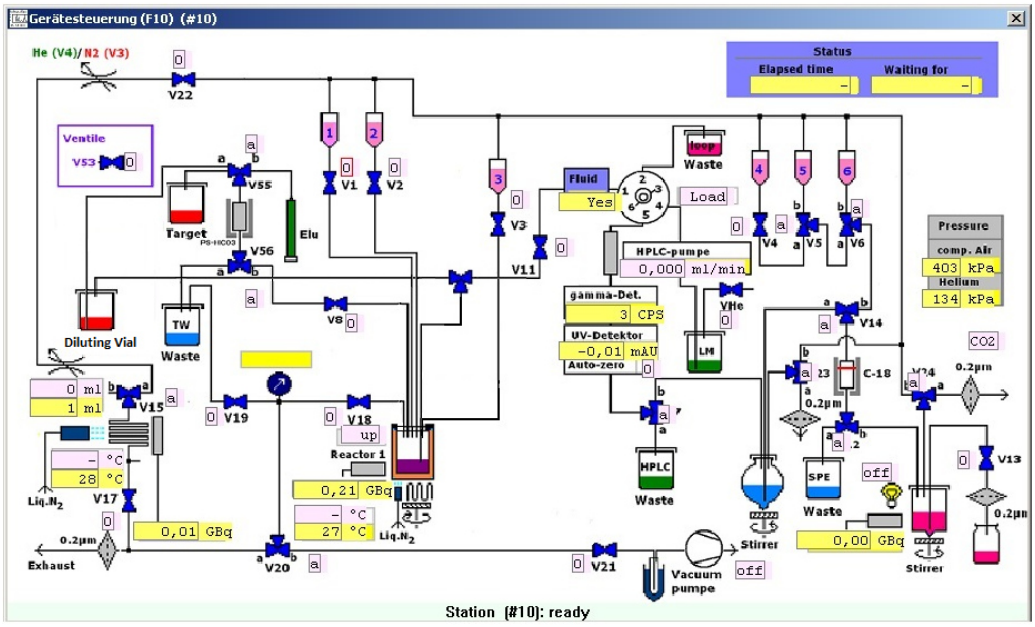


Figure 15: The device for the synthesis method 1

After cleaning, the vials were filled with the following chemicals: (V = Vial)

V1	3mg Nitroaltanserin in 0.5mL DMSO
V2	1.5mL aqueous ammonium acetate solution, 25mM, pH 5.1
V3	0.5mL acetonitrile
V4	5mL 0.9% NaCl solution
V5	1.5mL abs. ethanol
V6	10mL water
Bulb	90 mL water
20mL Diluting vial	10mL 1N, 0.5N or 0.1N HCl or 10mL 0.9% NaCl solution or 10mL water

Product-collection vial	4mL 0,9% NaCl solution;
	1mL 3% NaCl solution;
	1mL phosphate buffer 125mM
Reactor	0.5mL acetonitrile
Elution vial	0.5mL elution solution (22mg K2.2.2, 4.5mg K_2CO_3, 80/20 acetonitrile/water (v/v) in 1mL)
Syringes	1x 0.5mL acetonitrile
	2mL methanol
Product vial (in LAF; hot cell)	5mL 0.9% NaCl solution
between V55 and V56	PS-HCO_3 cartridge
	C-18 plus SepPak (methanol; water) prepared to exchange PS-HCO_3 cartridge after reaction step 2
between V14 and V12	C-18 plus SepPak (ethanol; water)
HPLC column	SymmetryPrep C18 7µm 7.8x150mm
HPLC solvent	67/13/20 = H_2O (25mmol NH_4OAc, pH 5.1)/ THF/ MeOH
Flow	5mL/min
λ	254nm
	Product vial
	Sterilfilter (GV 0.22µm, Millex, yellow)
	Sterilairfilter (0.22µm (Millex white)
	Endotoxin- and Sterility- Vial

When everything was ready, the activity in the target vial was connected to the system. The aqueous solution was sucked out of the target vial through the PS-HCO_3 cartridge into the waste.

The $^{18}F^-$ was trapped on the cartridge. It was eluted with the K2.2.2 solution into the reactor, in which 0.5mL acetonitrile were present. The first azeotropic drying was done;

the reactor was heated to 80°C, 100°C and 120°C for about 2min at every temperature with a constant helium gas-flow of 50ml/min. The acetonitrile in the syringe was transferred into the elution vial and after the reactor was cooled down to 25°C the acetonitrile was added. The second azeotropic drying was similar to the first one. For the 3rd drying another 0.5mL acetonitrile was added through vial 3. Afterwards the precursor was added at 120°C and the reaction mixture was heated to 150°C for 15min. The reactor was cooled down to 25°C and the brown reaction mixture was pumped into the diluting vial. The diluting vial was now connected as target vial and the solution was sucked again through the cartridge, which had been exchanged against the C-18 plus SepPak into the waste. The product was trapped onto the cartridge as brown residue and was eluted with 2mL methanol out of the elution vial.

The methanol was reduced nearly to dryness and the product was again diluted with 1.5mL ammonium acetate solution from vial 2 before it was sent onto the preparative HPLC.

The used column was a SymmetryPrep C18 (7µm 7.8x150mm), the eluent was a mixture of 67/13/20 = 25mmol NH_4OAc (pH 5.1) /THF/MeOH. A flow of 5mL/min was used.

After 8 and 14min the two Altanserin peaks appeared. The peaks were cut into the bulb and stirred for a further minute. The bulb was emptied into the waste via the C-18 plus SepPak between valve 14 and valve 12. The product was fixed on the cartridge, washed with water from vial 6, eluted with ethanol from vial 5 and diluted with 0.9% NaCl solution from vial 4 into the product collection vial.

The final product was transferred via a sterile filter into the product vial.

2.3.2 Method 2)

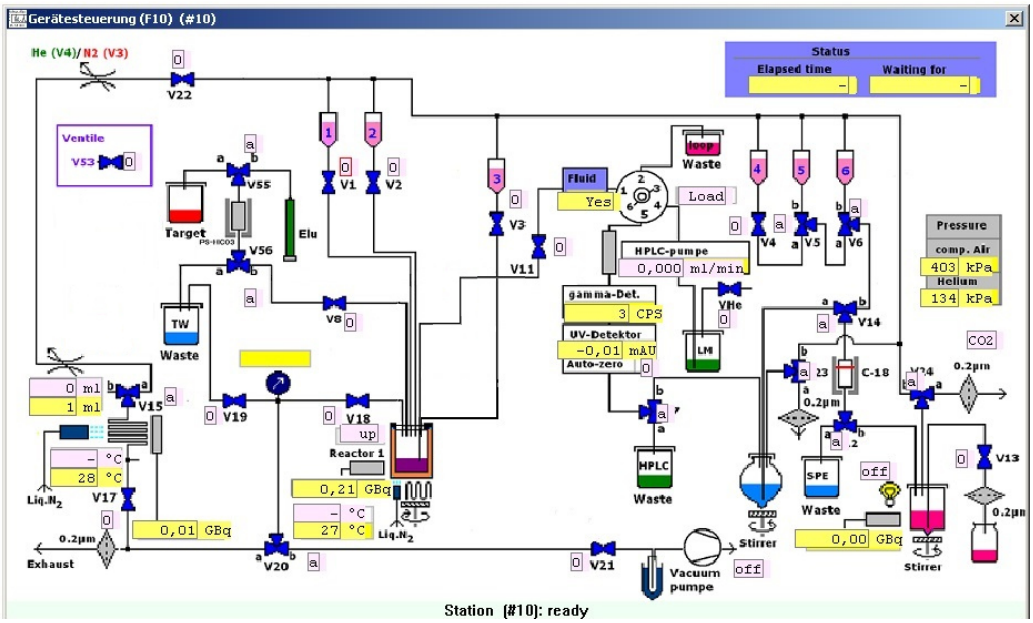


Figure 16: The device for the synthesis method 2, note that, in comparison to method 1, the HCl diluting vial is not used.

All preparation steps were identical to method 1 except V2:

V2	1mL aqueous ammonium acetate solution, 25mM, pH 5.1
	1mL HPLC solvent

This method is very similar to method 1. Until the reaction mixture was cooled down after 15min reaction time, the same steps were used.

The main difference was that after cooling down the reaction mixture, 1mL ammonium acetat solution and 1mL HPLC solvent were added from vial 2. The mixture was stirred for 1min and directly transferred onto the HPLC. No dilution vial and additional setup were necessary. Also the same HPLC column and solvents were used.

2.3.3 Method 3) adapted from the Jülich Research Center

Method on the basis of synthesis instructions of the Jülich research center

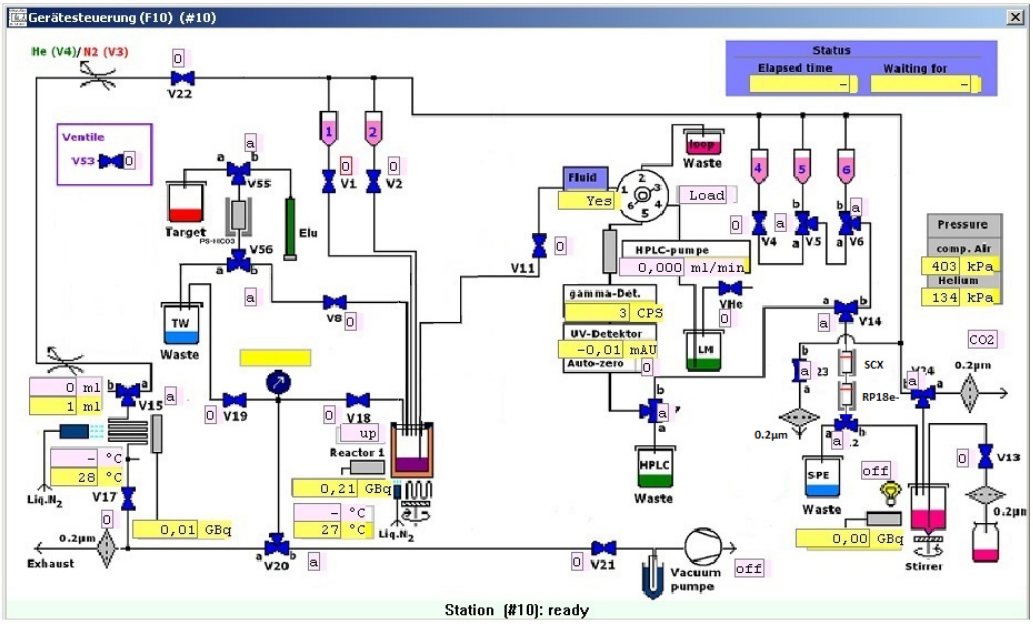


Figure 17: Scheme of method 3. Note the difference in setup as compared to method 1 (Fig.5): the dilution vial and vial 3 were not in use.

After cleaning the equipment, the vials were filled as following:

V1	3mg Nitroaltanserin in 0.5mL DMSO
V2	1ml MeCN, 1ml HPLC eluent
V4	11mL 0.9% NaCl solution with 14mg sodiumascorbat
V5	1mL abs. ethanol
V6	10mL sodiumascorbatsolution (0.79g solved in 10mL water)
Reactor	0.5mL acetonitrile
Elution V	0.9mL elution solution K2.2.2 solution (4.5mg K_2CO_3, 70/20 MeCN/H_2O (v/v), 20mg K2.2.2)
between V55 and V56	PS-HCO_3 cartridge

between V14 and V12	SCX- cartridge 500mg (10mL EtOH, 10mL HCl (1M), water until pH was neutral)
	RP18e-cartridge 200mg (10mL EtOH, water)
HPLC column	Prontosil 120-5C-18 ace-EPS 250x20mm
HPLC solvent	50/50 = H2O (0.1% TEA)/MeCN, pH 6.8
Flow	10mL/min
λ	254nm
	Product vial
	Sterilefilter (GV 0.22µm, Millex, yellow)
	Sterileairfilter (0.22µm (Millex white)
	Endotoxin- and Sterility- Vial

At first the target water vial was emptied into the waste via the PS-HCO_3 cartridge to adsorb the fluoride onto the column. Afterwards it was eluted with the K2.2.2 solution from the elution vial into the reactor. Together with the 0.5mL MeCN, which was submitted to the reactor, the fluoride was dried. At first the reactor was heated to 75°C for 3min, then to 100°C for 4min and to 120°C for another 4min. The whole time the reactor was flushed with a constant helium-flow of 50mL/min.

To the dry fluoride the precursor was added at 120°C, the helium flow was stopped and the reactor was closed. The reaction mixture was heated to 150°C for 15min and thereafter cooled to room temperature. Vial 2, which contained the mixture of HPLC solvent and MeCN, was emptied into the cold crude product and stirred for 1min. To purify the product, the mixture was cleaned via HPLC and the peak was cut onto the SCX cartridge. A prontosil 120-5C-18-ace-EPS column was used with a mobile phase of 50/50= H_2O (0.1% TEA)/MeCN with a flow of 10mL/min. The cartridge was dried for 5min with a helium gas-flow from the bulb to remove the acetonitrile. Vial 6 was emptied into the waste to elute the product from the SCX column to the RP18e column. The product was finally eluted into the product collecting vial with 1mL

ethanol from vial 5 and washed after also in the same vial with 11mL 0.9% NaCl solution with 14mg sodiumascorbat from vial 4. The final product was transferred into the product vial via the sterile-filter.

The difference to the synthesis methods mentioned before is the drying of the fluoride, which was not repeated, and the cleaning and formulation procedure.

2.3.4 Method 4) Altanserin syntheses with the microfluidic module

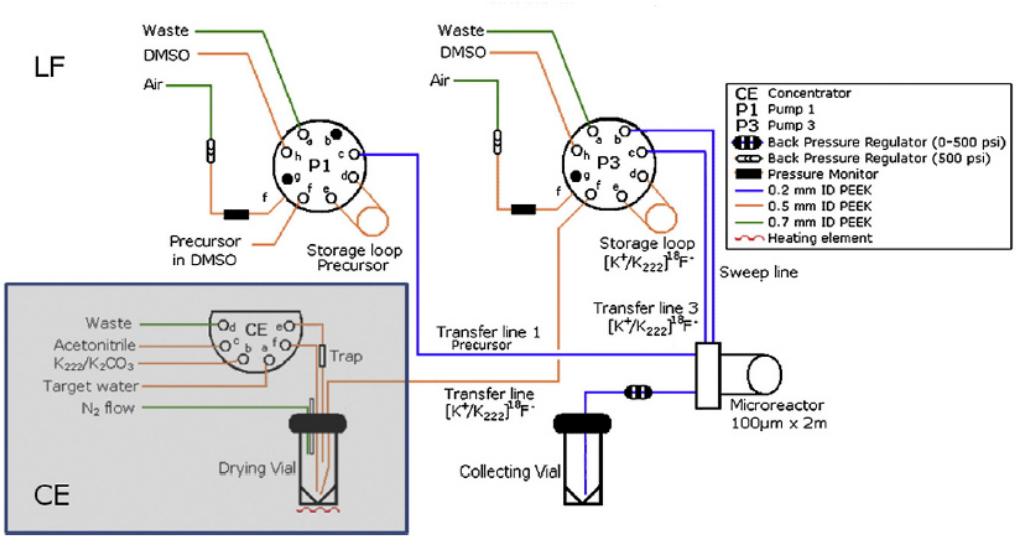


Figure 18: The device for the synthesis method 4.

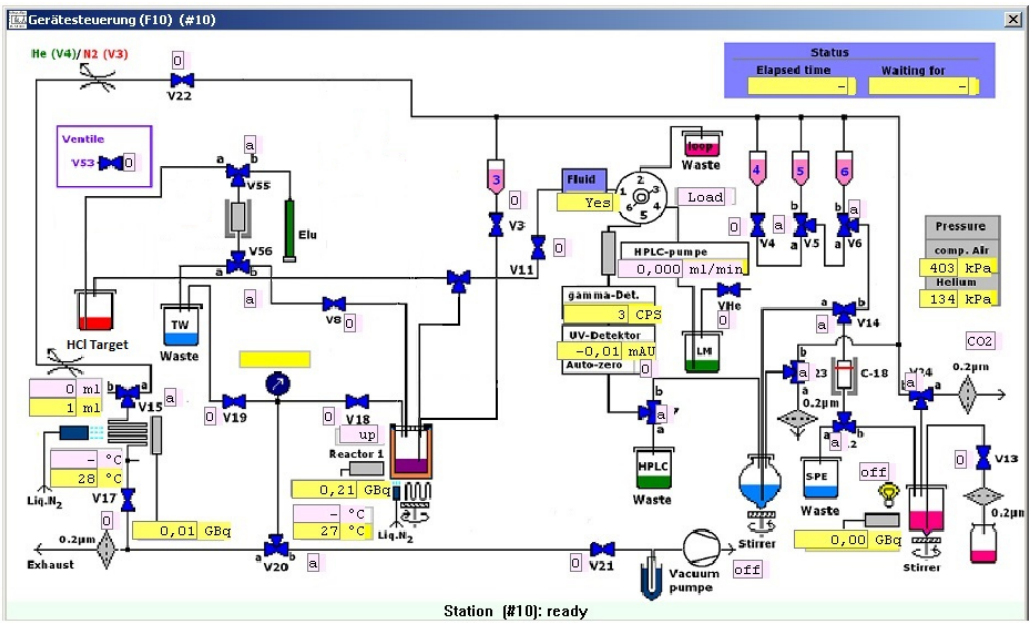


Figure 19: The device for product purification and formulation of synthesis method 4

[28] only vial 3-6 were in use and the product was sucked from the HCl Target.

The vials were filled in the following way:

Eppendorf 1	2mg Nitroaltanserin in 0.5mL DMSO
Eppendorf 2	0.5 mL elution solution (4.5mg K_2CO_3, 22mg K2.2.2, 70/30 MeCN/H_2O (v/v) in 1mL)
V3	1.5mL NH_4OAc (25mM; pH 5.1)
V4	5mL 0.9% NaCl solution
V5	1.5mL abs. ethanol
V6	10mL water
Bulb	90 mL water
20mL Diluting vial	10mL 1N, 0.5N or 0.1N HCl or 10mL 0.9% NaCl solution or 10mL water
Product-collecting vial	4mL 0,9% NaCl solution;
	1mL 3% NaCl solution;
	1mL phosphate buffer 125mM
Product V	5mL 0.9% NaCl solution
between V55 and V56 between V14 and V12	C-18 plus SepPak (methanol; water) prepared to exchange PS-HCO_3 cartridge after reaction C-18 plus SepPak (ethanol; water)
HPLC column	SymmetryPrep C18 7µm 7.8x150mm
HPLC solvent	67/13/20 = H_2O (25mmol NH_4OAc, pH 5.1)/ THF/ MeOH
Flow	5mL/min
λ	254nm
	Product vial
	Sterilefilter (GV 0.22µm, Millex, yellow)
	Sterileairfilter (0.22µm (Millex white)
	Endotoxin- and Sterility- Vial

On the basis of previous work by our working group (Ungersböck et al.) and additional voluble information by Dr. C. Philippe, we set up a microfluidic version of this synthesis. The parameters precursor concentration, reaction temperature, bolus flow rate through the microreactor and the bolus volume were varied.
For a better comparison of this method with the other methods, the parameters with the highest yield were taken.

The syntheses were done in the NanoTek-synthesizer (Advion, Ithaca, USA).
In the beginning the solvents were checked: acetonitrile for the azeotropic drying and DMSO for the reaction. Then the module was cleaned automatically with the standard programs concentrator clean and master clean.
Afterwards the vials were connected as shown in Figure 18 and 19. The target water vial, a 2.5mL glass vial, was connected on CE-a.
An Eppendorf vial with 0.5mL of the elution solvent, containing 10mg Kryptofix 2.2.2, 2.25mg potassium carbonate in 70/30 acetonitrile/water, was connected on CE b.
A PS-HCO_3 cartridge was connected on CE-e between the six-way-valve and the drying vial.
2mg Nitroaltanserin were dissolved in 500µL DMSO in an Eppendorf vial and attached to P1-f.

The target water was sucked through the cartridge and back into the target water vial. Then the [^{18}F]fluoride was eluted into the drying vial, which was standing into a heater. The azeotropic drying was started. There were 3 drying steps at 110°C with twice adding 500µL acetonitrile through the pump of the CE-unit (gray marked in Figure 18). The dried [^{18}F]fluoride was dissolved in DMSO and loaded onto the loop of pump 3. The precursor was loaded onto the loop of pump 1. The reactor was filled with the reactants from the two loops with a flow rate of 10µL/min. The reactor was heated to 220°C and the reaction takes place online. The crude product was afterwards pumped into the HCl-vial and diluted with 10ml 1N HCl.

The product-solution was sucked out of the HCl target vial through the C-18 plus SepPak (methanol; water) into the waste. Afterwards it was eluted into the reactor

with 2mL MeOH from the elution vial. The MeOH was reduced nearly to dryness under a He-flow of 50mL/min. The product was redissolved in the aqueous NH_4OAc solution from vial 3 and loaded onto the HPLC.

The two Altanserin peaks were cut out into the bulb; the solvent between the two peaks was discarded.

After stirring the solution for a short time, the bulb was emptied into the waste through the second C-18 plus SepPak (ethanol; water) and it was washed with water from vial 6.

The product was eluted from the SepPak into the product collecting vial with ethanol from vial 5 and washed with the sodium chloride solution from vial 4. The product was transferred via a sterile filter in the sterile vial. [28]

2.4 Quality control

Since the final aim was the application in human, the final product had to be tested for its radiochemical, chemical and radio nuclear purity as well as for its physical parameters, pH and osmolality, and its microbiological parameters, sterility and endotoxin amount. Most of the quality control was done immediately after the synthesis. For testing the microbiological parameters a sample was taken and the results were received several weeks after the application, which is in accordance with pharmaceutical legislation for short-lived radiopharmaceuticals.

Radiochemical and the chemical purity:
For determination of the radiochemical and the chemical purity a HPLC measurement was performed.

Column -> Gemini 3u C18 110A, 150x4.60mm
solvent -> 60/40 ammoniumacetat buffer (25mmol/l, pH=5.2-5.4)/acetonitrile

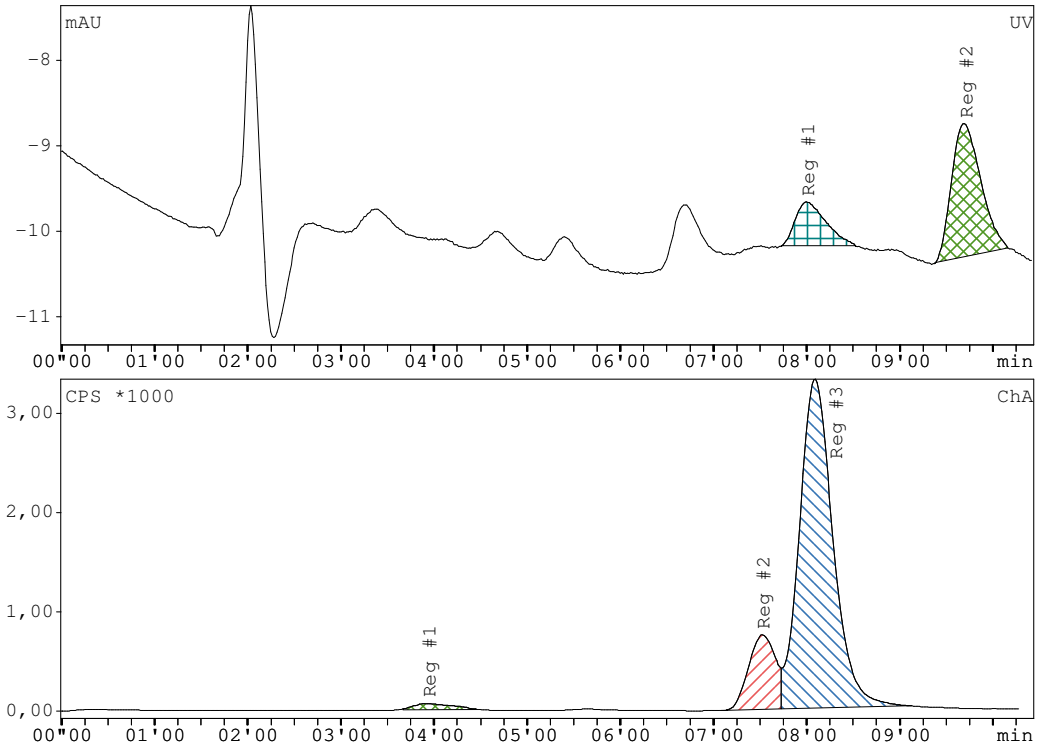
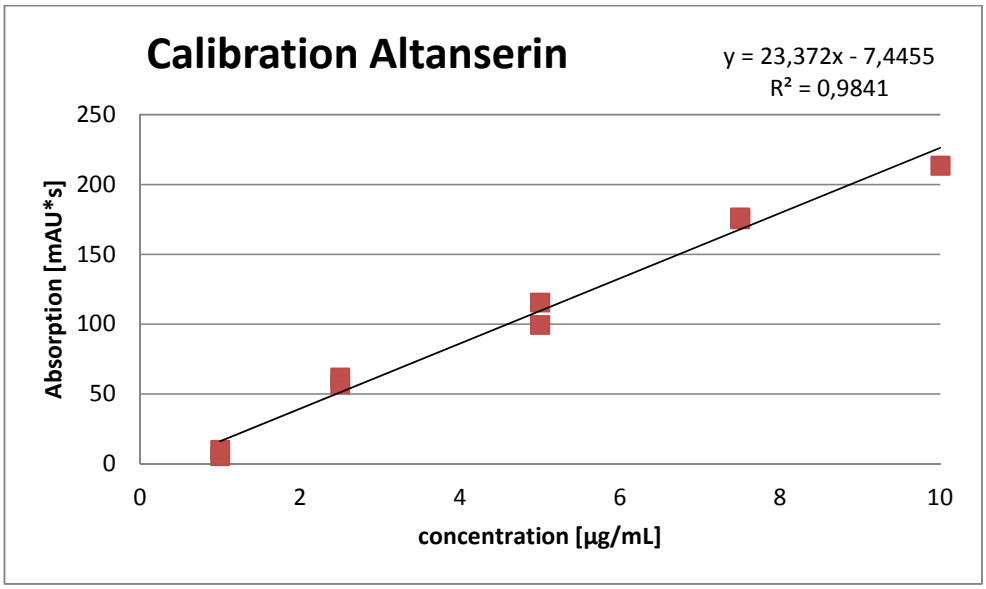
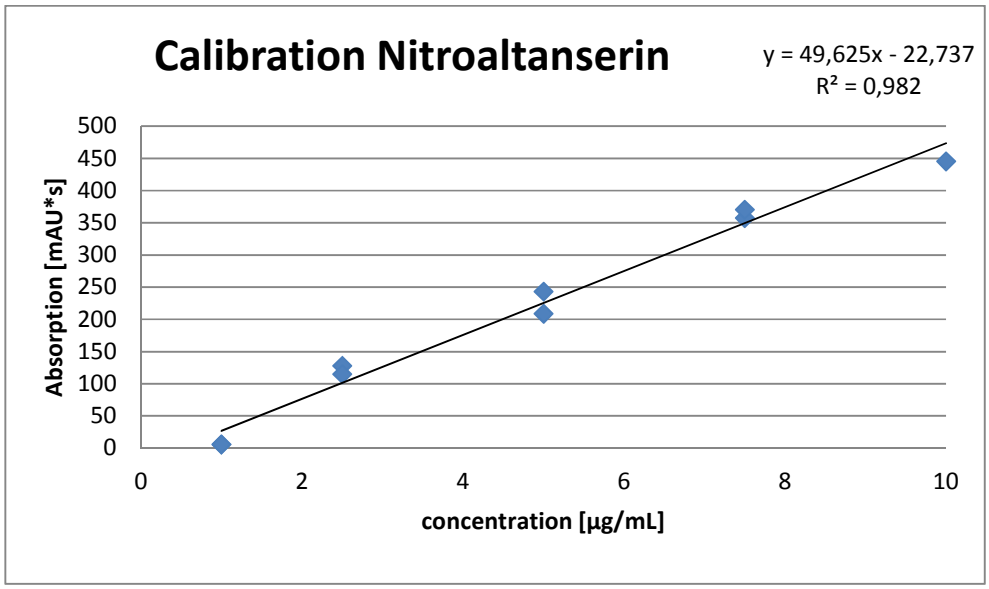


Figure 20: Analytical HPLC of the final product from method 1

Figure 20 shows a typical chromatogram of [^{18}F]Altanserin, produced with method 1, which could be administered to a patient. The upper spectrum shows the UV-channel and the lower the radio-channel. To apply it to the patient there is no official threshold for the amount of precursor or cold Altanserin. Nevertheless the smaller the amount of the cold substances, the better it is. To determine the amounts of the substances calibration curves were measured beforehand. The concentration in µg/mL was plotted against the absorption, as shown in graphs 1 and 2.



Graph 1: Calibration of Altanserin for the analytical HPLC (linear regression)



Graph 2: Calibration of Nitroaltanserin for the analytical HPLC (linear regression)

The specific activity was also calculated on the basis of the following equation:

$$\text{as } [\text{GBq}/(\mu\text{mol})] = \frac{\text{yield } [\text{GBq}]}{\text{conc.}\left[\frac{\mu\text{g}}{\text{ml}}\right] * \frac{\text{Volume}[\text{ml}]}{411.49\left[\frac{\text{g}}{\text{mol}}\right]}}$$

The total volume of the final product was 17.5mL

conc. [µg/ml] = concentration of Altanserin was obtained from the analytical HPLC and the calibration plot.

411.49 g/mol is the molar weight of Altanserin.

In the radio-channel the total amount of the [^{18}F]Altanserin double-peak must be higher than 95%. Two small impurities, like the one at 4min, each smaller than 3% are allowed.

The purity of the radionuclide was determined by measuring a γ-spectrum and the half-life. For this purpose the activity at two different time-points were measured. The lines of the γ-spectrum of the clean product are at 511keV and 1022keV.

The amount of acetonitrile, THF, MeOH, DMSO and ethanol were determined by gas chromatography. The limits of these solvents are the following:

Acetonitrile < 410 ppm
THF < 720 ppm
MeOH < 3000 ppm
DMSO < 5000 ppm
Ethanol < 8.5 %

The pH has to be in a range of 4.5-8.5 and the osmolality between 190-370 mosm/kg.

If all the requirements are met, the product can be released and administered to the patient.

2.5 Materials and Chemicals

2.5.1 Materials

1, 2, 5, 10, 20mL syringes	B. Braun
30-PS-HCO3	CHROMAFIX
Nanotek CE	Adivon
Milli-Q	Merck
TLC Silica gel 60 F254	Merck
Instant Imager	Packard
UV Detector K-2001	Knauer
S1122 Solvent Delivery System	SYKAM
GE PETtrace cyclotron (16.5 MeV protons)	GE Medical systems
Lichrolut RP18e- cartridge	Carl Roth GmbH
NanoTek-synthesizer	Advion
Needles	B. Braun
Prontosil 120-5-C18 ace-EPS 250x20mm	ProntoSIL
SCX-cartridge 500mg	Carl Roth GmbH
Sep-Pak Plus C18	Waters
Gina Star	Raytest
SymmetryPrep C18 7µm 7.8x150mm column	Waters
LaChrom UV Detector L-7400	Merck Hitachi

2.5.2 Chemicals

Acetic acid	Sigma Aldrich
Acetonitrile	Sigma Aldrich
Acetonitrile CHROMASOLV Plus for HPLC, ≥99,5%	Sigma-Aldrich
Acetonitrile hypergrade for LC-MS	Lichrosolv
Altanserin Reference standard for [^{18}F]Altanserin	ABX advanced biochemical compounds
Ammoniumacetat	Sigma-Aldrich
Ascorbic acid	Sigma-Aldrich
Dimethylsulfoxide ≥99,9%	Sigma-Aldrich
$H_2{}^{18}O$ >98%	Rotem Europe
Helium gas	Messer
Kryptofix 222 for synthesis	Merck
Methanol for HPLC ≥99,9%	Sigma-Aldrich
Nitroaltanserin (GMP) precursor for [^{18}F]Altanserin	ABX advanced biochemical compounds
Nitrogen gas	Messer
Sodiumchloride 0.9%	B. Braun
Sodiumchloride 3%	B. Braun
Sodiumhydroxide	Sigma-Aldrich
Steril water	B. Braun
Tetraethylammonium bicarbonate	Aldrich
Tetrahydrofuran Reagent Plus ≥99,0%, contains 250ppm BHT as inibitor	Sigma-Aldrich
Triethylamine ≥99,5%	Sigma-Aldrich

3 Results and Discussion

3.1 Comparison of the radiosynthesis-methods

3.1.1 Optimization steps

3.1.1.1 The drying of the fluoride

To elute the fluoride, which was adsorbed on the cartridge, into the reactor, different elution solvents were tested. The results of conversion to [^{18}F]Altanserin are listed in the table below and calculated from the cleaned and formulated product. The method used for these experiments was method 1).

#	elution solvent	number of experiments	time corrected RCY [%]	standard deviation	Max./Min. yield [%]
1	K2.2.2	6	9.8	4.05	17.6/5.9
2	TEAB	4	12.1	3.99	16.6/6.6
3	TBAH	2	0.05	0.05	0.1/0.0

Table 2: Final yields with different elution solvents

For the first azeotropic drying different amounts of acetonitrile were put in first into the reactor. The results are listed in the table below, for better comparison also method 1) with K2.2.2 as elution solution was used.

amount of submitted acetonitrile [mL]	number of experiments	time corrected RCY [%]	standard deviation	Max./Min. yield [%]
0	3	4.4	0.45	4.9/3.8
0,5	6	9.8	4.05	17.6/5.9

Table 3: Final yields with different amount of submitted acetonitrile

The reactor was heated to 80°C, 100°C and 120°C for about 2min at every temperature.

The number of azeotropic dryings was also tested.

To compare the efficiency of the drying, the syntheses were stopped after the reaction time.

A HPLC and a TLC were done from the crude product and the amount of free [^{18}F]Fluoride was measured.

The table below shows the outcome of these experiments, based on method 1).

number of dryings	number of experiments	% of flouride	standard deviation
1	1	91.67	/
3	2	7.93	0.1

Table 4: Amount of fluoride after different numbers of drying

Between the drying steps the reactor was cooled down to room temperature before the acetonitrile was added.

It was also tested to add the acetonitrile at 80°C, which leads to the very unstable results of 33.4 ± 25% of fluoride (n=3).

To the dry fluoride the precursor in DMSO was added and the reaction mixture was heated to 150°C. It was tested to add the precursor at room temperature or at 120°C.

adding - temperature [°C]	% fluoride	standard deviation
25	89.58	9.31
120	7.93	0.1

Table 5: conversion of fluoride by different adding temperatures

After 15min at 150°C the reaction mixture was cooled down to room temperature.

3.1.1.2 The purification procedure

Method 1)

The crude product was transfered into the dilution vial, which was either filled with 10mL 1N, 0.5N or 0.1N HCl, 10mL 0.9% NaCl solution or 10mL H_2O. The solution was pushed over a C18 plus SepPak into the waste. The product was eluted from the SepPak with methanol into the reactor. Methanol was evaporated; the precipitate dissolved in 1.5mL 25mM ammonium acetate solution and loaded onto the HPLC.
If the solution was diluted with 0.1N or 0.5N HCl, sodium chloride solution or water, an overpressure error occured directly after the injection, since small particles precipitated after addition of ammonium acetate solution.
Therefore it was tried, unsuccessfully, to filter the particles with cotton wool.
For analysis, the particles were dissolved afterwards in acetonitrile and measured onto the analytical HPLC. The precipitate showed to be Altanserin and Nitroaltanserin.
To bring the synthesis to a successful end the HPLC-flow was reduced as far as necessary and the peak was cut out.
The preparative HPLC spectrum of 0.1N HCl shows a very broad fluoride peak and no separation of the remaining [^{18}F]Altanserin and the [^{18}F]fluoride.
When 1N HCl was used no precipitation occurred during the cleaning procedure and therefore also no overpressure.
When another dilution solvent than 1N HCl was used, then generally a higher [^{18}F]fluoride concentration (12.3 ±7.2%) in the final product was obtained. As already mentioned before, the maximum amount of [^{18}F]fluoride for application in the final product was internally set at 3%. Results were mostly within the threshold, when 1N HCl was used.

3.1.2 Method 1) vs. method 2)

Method 2) was much shorter than method 1) and less activity got lost before the crude reaction mixture was loaded onto the HPLC. Nevertheless, the radiochemical yield was smaller than in method 1). The dark crude, which was loaded onto the column, led to an increase in pressure and the flow rate had to be reduced from 5ml/min to 2ml/min. Furthermore, only small parts of the product were separated from the crude. Most of the activity was observed in a broad peak at the beginning of the HPLC run co-eluting with DMSO. The separation, therefore, was incomplete. As there was no HCl in the cleaning steps the spectrum does not show the later described two Altanserin and Nitroaltanserin peaks. (Compare Fig. 21 and Fig. 24)

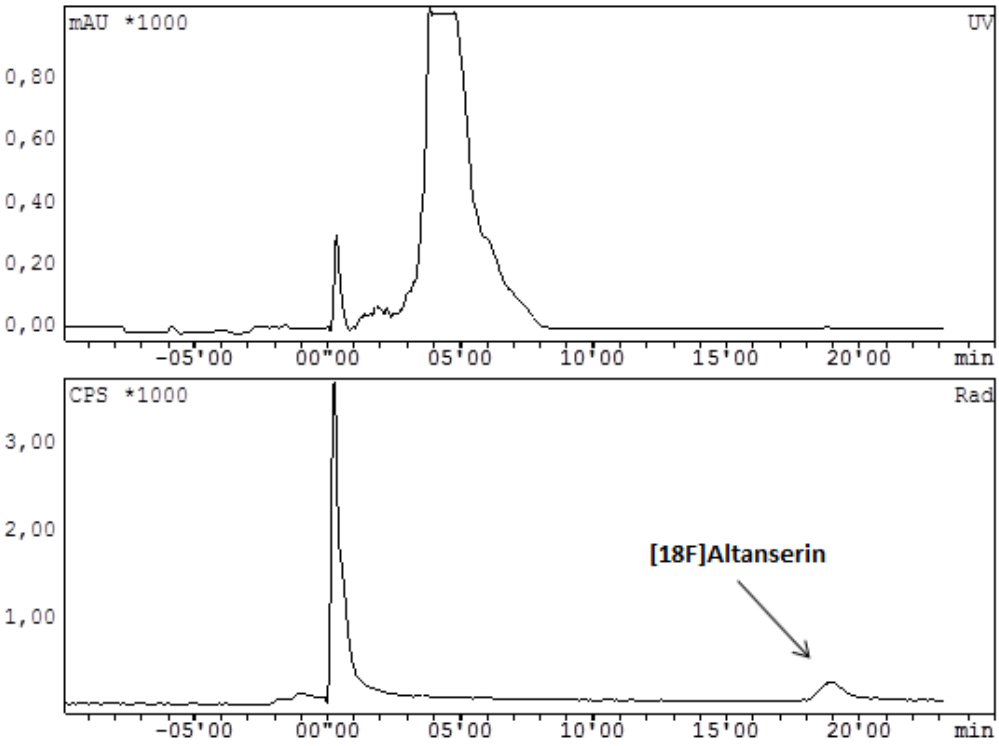


Figure 21: preparative HPLC chromatogram using method 2): [^{18}F]Altanserin visible in the radiochannel (bottom)

3.1.3 Comparison of the 4 methods

To compare the 4 methods reasonably, important key data were considered.
These are: 1) the radiochemical yield; 2) the "hands-in" time, which is important for radiation protection; 3) the duration of the synthesis; 4) the precursor usage; 5) the specific activity; 6) the mean outcome with a starting activity of 30GBq; 7) the reliability of the synthesis; 8) the product's quality live (if would be possible to applicate it to a patient by the results of the quality control).

	Method 1	Method 2	Method 3	Method 4
Synthesis duration (EOB to EOS)	110+/-10'	90+/-8'	67+/- 5'	160+/- 10'
hands-in time [min]	2'12''	1'50''	1'06''	40''
radiochemical yield [%] (corrected from EOB)	9.8±4.05	0.4%	4.2±3%	2.5±0.2
specific activity [GBq/µmol] at EOS	477±203	/	425±332	74±21
Product yield (starting activity ~ 30GBq) [MBq]	1600±650 MBq	0.072 MBq	933±645MBq	770±100MBq
amount of precursor	3 mg	3mg	3mg	2mg
Application possible/ QC passed	yes	no	no	yes
reliability	yes	no	no	yes

Table 6: comparison of the 4 methods (EOB… End of bombardment, EOS… End of synthesis)

This comparison shows that each synthesis method has its advantages and disadvantages.
The final product of methods 2 and 3 could not be administered to the patients. The yield of Method 2 was too small for application. Method 3 was sufficiently pure in terms of radiochemical purity, but showed a huge impurity in the UV-channel, which was even overloading the detector in the analytical HPLC measurement, as shown in Figure 22 below. These two methods were also not very reliable. Method 2 often did

not work at all and the outcome of method 3 fluctuated from almost no conversion to about 2GBq of product.

The remaining two methods, method 1 and 4, were demonstrated to be well-balanced. Method 1 was 50min shorter; method 4 used only 2/3 of the precursor amount of method 1. The hands-in time of method was also shorter, because of less manual interventions. The specific activity of method 1 and the final yields were higher than in method 4 and would therefore, sufficient for more than one patient, in contrast to method 4.

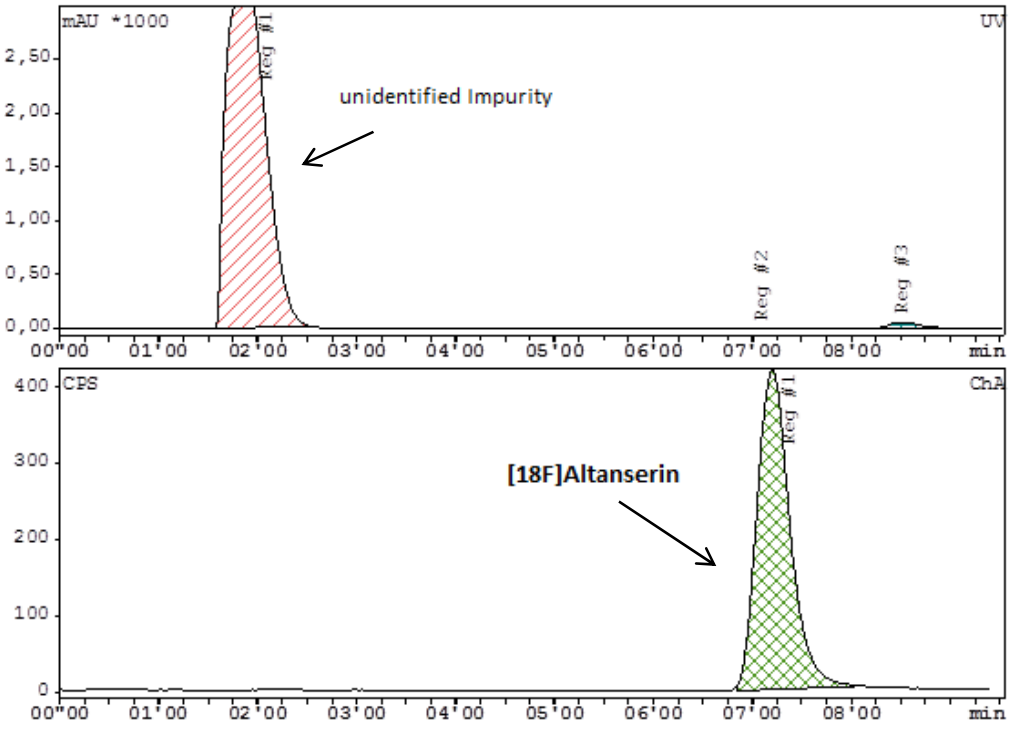


Figure 22: Analytical HPLC measurement of method 3

3.2 Comparison of the HPLC methods

Different HPLC methods were tested, as shown in Table 7 below.

Synthesis Method	1 + 4	2	3
column	SymmetryPrep C18 7µm 7.8x150mm column		Prontosil 120-5-C18 ace-EPS 250x20mm
solvent	67/13/20 = H_2O (25mmol NH4OAc, pH 5,1) /THF/MeOH		50/50= MeCN/H_2O (0,1% TEA), pH 6,8
solvent of the crude reaction mixture	25mmol/l NH_4OAc in H2O	1/2/2=DMSO/NH_4OAc/eluent	1/2/2=DMSO/NH_4OAc/eluent
retention time and capacity factor of [^{18}F]Altanserin	2 peaks at 10min and 18min; K'= 0.7 and 1.5		10min; K'= 0.7
flow rate	5ml/min		20ml/min

Table 7: The different HPLC methods ($K' = (t_R - t_0)/t_0$; t_R…retention time of the peak; t_0…. time a molecule with no interaction with the column needs from injection to detection)

As already mentioned the separation in method 2) did not work properly, and a big amount of [^{18}F]Altanserin got lost. Also the dilution solvent played a major role in method 1 and 4, to avoid a blockage of the HPLC system.
This means that the first HPLC method the crude had to be prepared. The DMSO had to be eliminated and also the MeOH had to be evaporated before it could be loaded onto the HPLC (for the preparative HPLC spectrum see chapter 3.4).
In contrast, the crude in method 3 was loaded onto the HPLC column directly after it was cooled to room temperature without any further ado. On the HPLC, the elution of the product needed approximately 8min less than in the other HPLC method, which is shown in Figure 23.

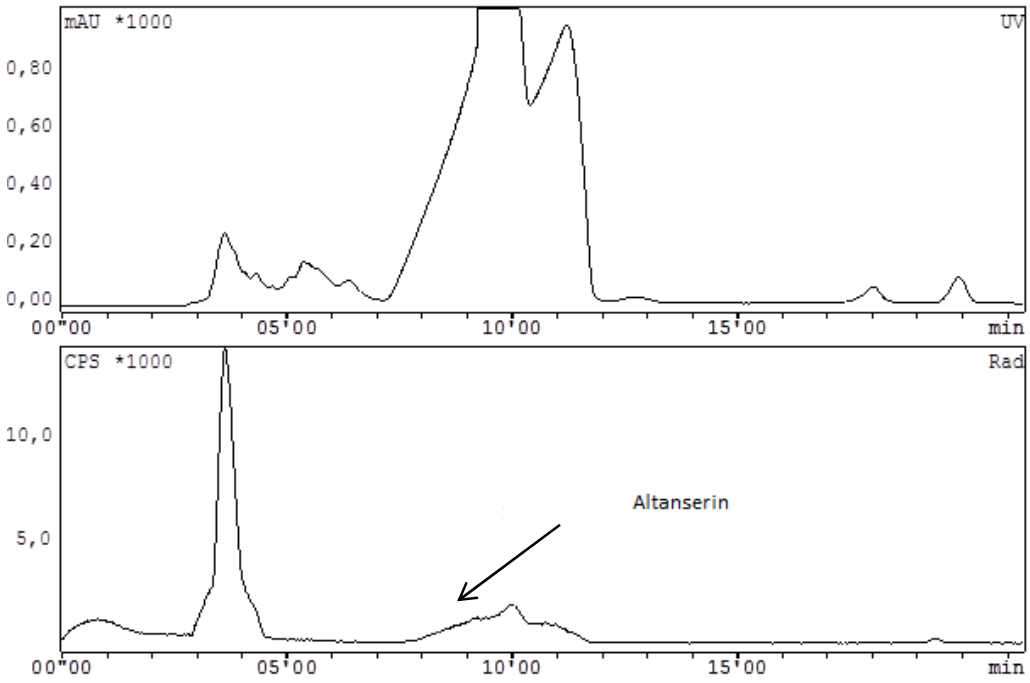


Figure 23: HPLC spectrum from method 3, [^{18}F]Altanserin is visible at 10min.

Method 3 therefore is favorable because there is no preparation before the HPLC cleaning and the whole purification time is shorter. However, it was not able to eliminate a nonradioactive impurity from the product fraction. This leads to the conclusion, that the final product cannot be administered to a patient.
Other disadvantages of method 3 are the much higher amount of solvent that is need and also the high flow rate and consequently the higher pressure, which can be a problem for the pump, tubings and connections.

3.3 Loss of Activity

During the elution of the [^{18}F]fluoride from the cartridge half of the activity was lost. It could neither be measured in the waste nor on the cartridge.
This loss of activity during the elution from the cartridge may be explained by a volumetric effect. The higher the volume is in the reactor, the smaller the measured activity. During the drying steps the activity increases as the volume decreases. Furthermore, only half of the starting activity can be obtained after diluting the crude product in the dilution vial, sending it through the C18+ SepPak and eluting it with methanol. Again, the activity cannot be found in the vials or on the cartridge. In this case the volume is doubled in comparison to the first dilution, but also after evaporation of the solvent it does not increase significantly. It may stick in the tubing, but as they cannot be easily to removed and measured and also to avoid a long exposition to high amounts of radiation this could not be proofed.

It should be noted that the amount of eluted [^{18}F]fluoride slightly decreases with an increasing number of [^{18}F]Altanserin syntheses. This may be explained by the used acid, which could interact with the tubing materials and might activate their surface for higher adsorption of fluoride.

3.4 The “double-peak” phenomenon

The recorded HPLC spectrum of method 1) seemed to show a doubled measurement. The [^{18}F]Altanserin peak in the radio-channel as well as the Nitroaltanserin peak in the UV-channel seemed to appear twice, as shown in Figure 24 below.

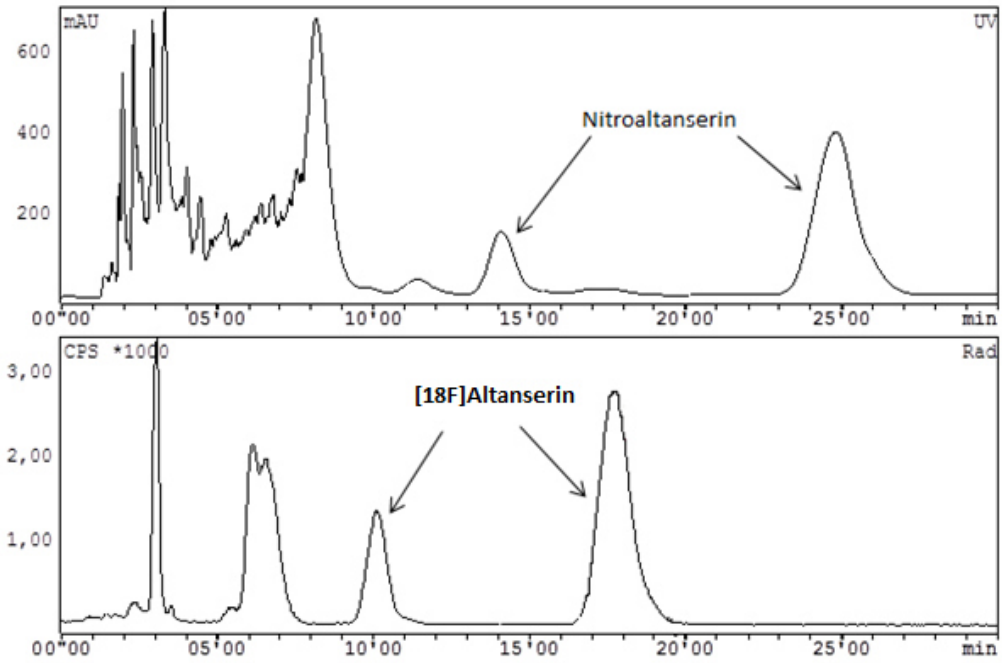


Figure 24: Preparative HPLC spectrum of method 1 and 4

Both [^{18}F]Altanserin-fractions were first collected in separate vials and measured with the analytical HPLC to check if they are both the product. The following spectrum was measured in the analytical HPLC with the pooled, formulated fractions (Figure 25).

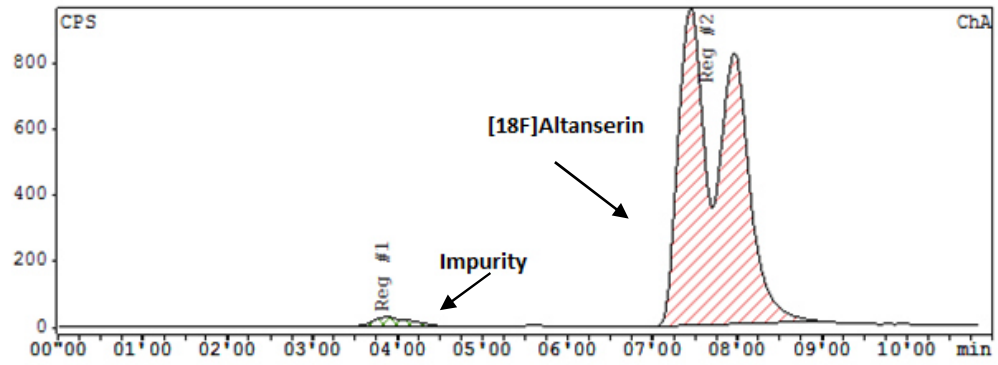


Figure 25: Analytical HPLC spectrum of the pooled [^{18}F]Altanserin peaks

Reg #1 at 03’51 had 2.29% of the sum of the peak areas and Reg#2 at 07’25’’ had 97.71%.

The spectrum showed that the two peaks were not exactly the same.

For comparison, Figure 26 shows the analytical spectrum directly after the reaction (from the crude product without cartridge and HPLC purification).

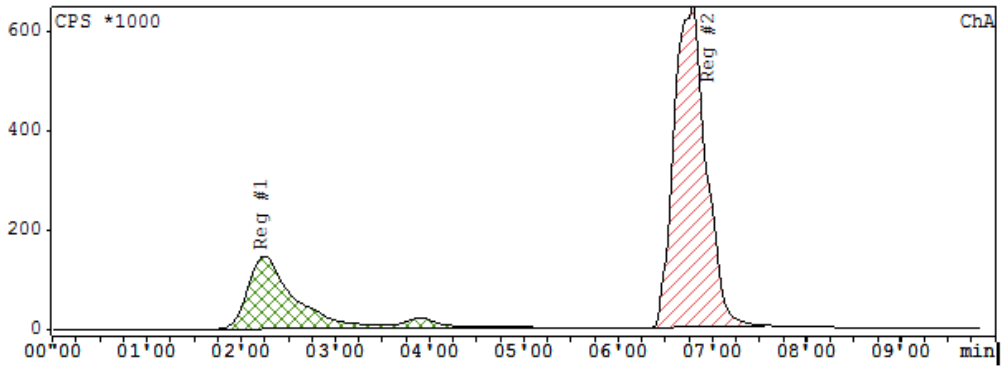


Figure 26: Analytical HPLC of the crude product

The spectrum showed a [^{18}F]fluoride peak 2’15’’ and also a impurity at 03’51 is visible, but only one [^{18}F]Altanserin peak 6’55’’.

To find out what caused the two peaks at ~7min, 1mg/ml Altanserin standard was dissolved in DMSO and diluted to 10µg/ml with the preparative HPLC solvent and stored at room temperature for 30min before measuring. The analytical HPLC showed

no changes.

Then the Altanserin was diluted to 10µg/ml with 1N HCl which was used in the syntheses in the SPE procedure. The reaction mixture was diluted with 10ml HCl and pushed over a C18 plus cartridge, as described in the practical part (method 1) and 4)). In the analytical HPLC a peak was visible a little bit earlier than the usual Altanserin Peak. By overlapping this spectrum with the Altanserin standard in DMSO, the before observed double-peak could be recognized.

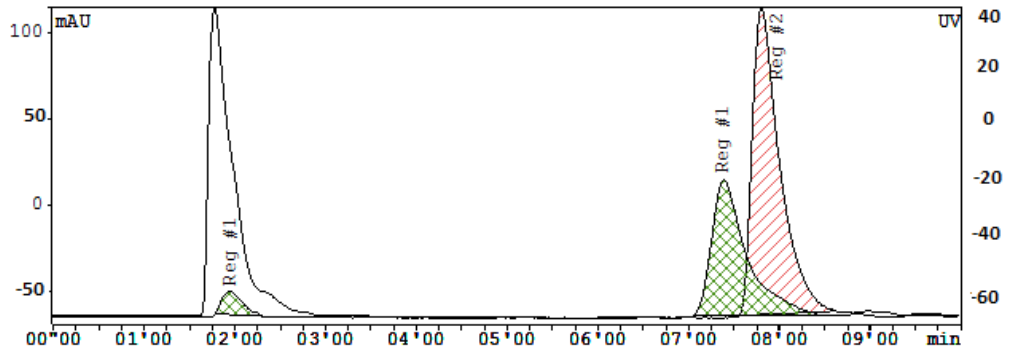


Figure 27: Altanserin diluted in HCl, green peak and left scale, and in HPLC solvent, red peak and right scale

This effect can be explained by protonation of Altanserin, for example on one of the oxygens.

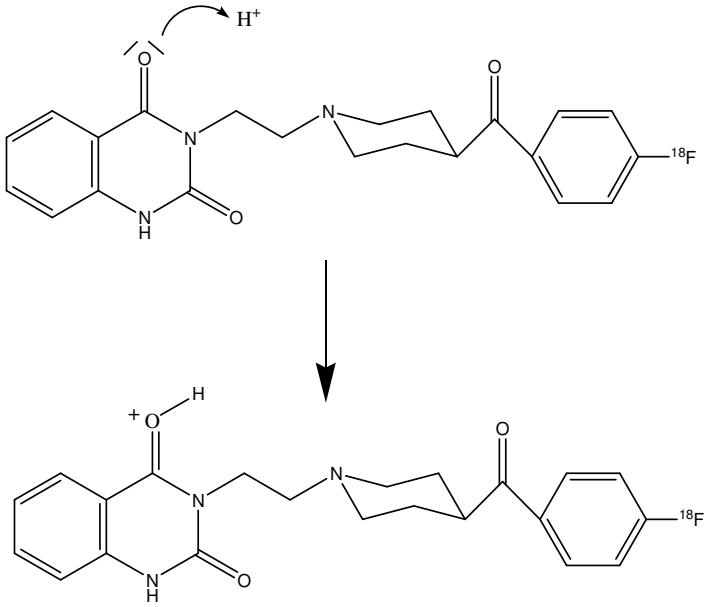


Figure 28: Suggested protonation of [^{18}F]Altanserin

From this, we realized that the product even with the double-[^{18}F]Altanserin peaks was very pure (as far as it could be determined in the analytical HPLC). Deprotonation could be explained by the character of THF, which is part of the preparative HPLC solvent. Tetrahydrofuran has the ability to react as Lewis base in the presence of a Lewis acid like HCl.

To prove this theory right, the product with the two peaks was titrated with NaOH. The following spectrum showed, that at pH14 only one broad [^{18}F]Altanserin peak was visible.

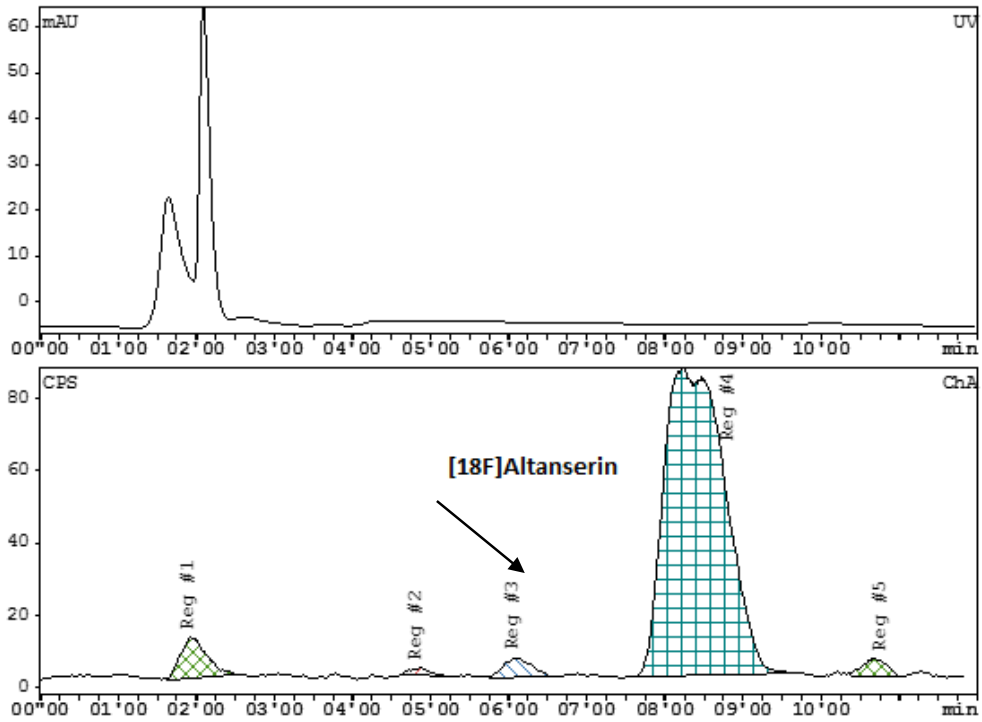


Figure 29: [^{18}F]Altanserin titrated with NaOH at pH 14

Another strong indication that our theory of the protonation of [^{18}F]Altanserin was correct, was found using 0.9% NaCl solution or water instead of HCl. Then only one [^{18}F]Altanserin peak occurred in the preparative HPLC. also in the analytical HPLC spectrum only one peak was visible as shown in the spectrums below. (Fig. 20 and 21)

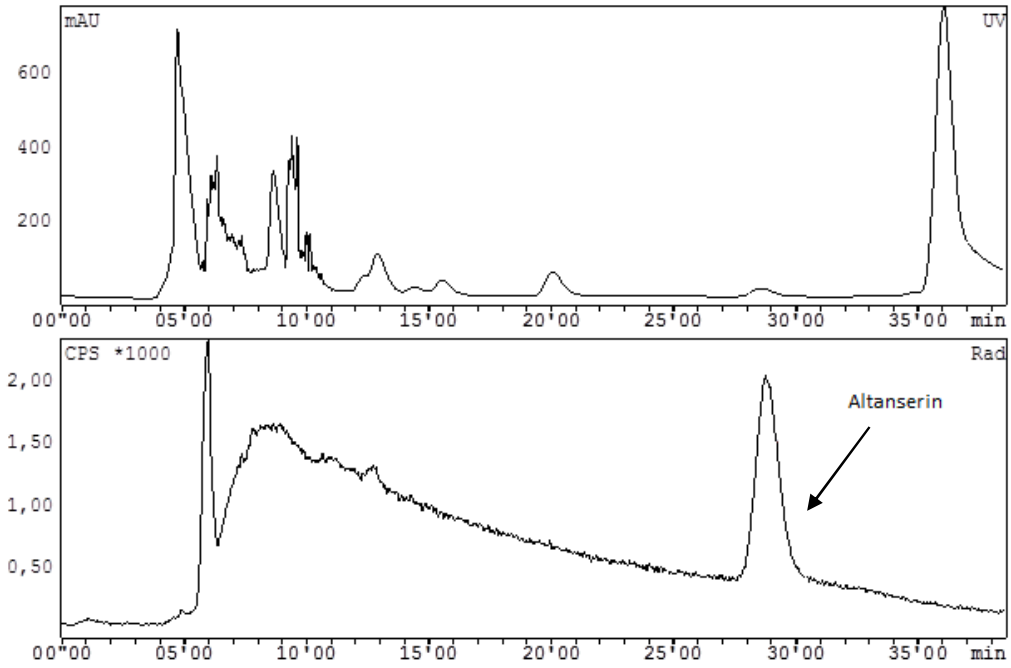


Figure 30: Preparative HPLC spectrum of method 1 with 0.9% NaCl solution (no acid)

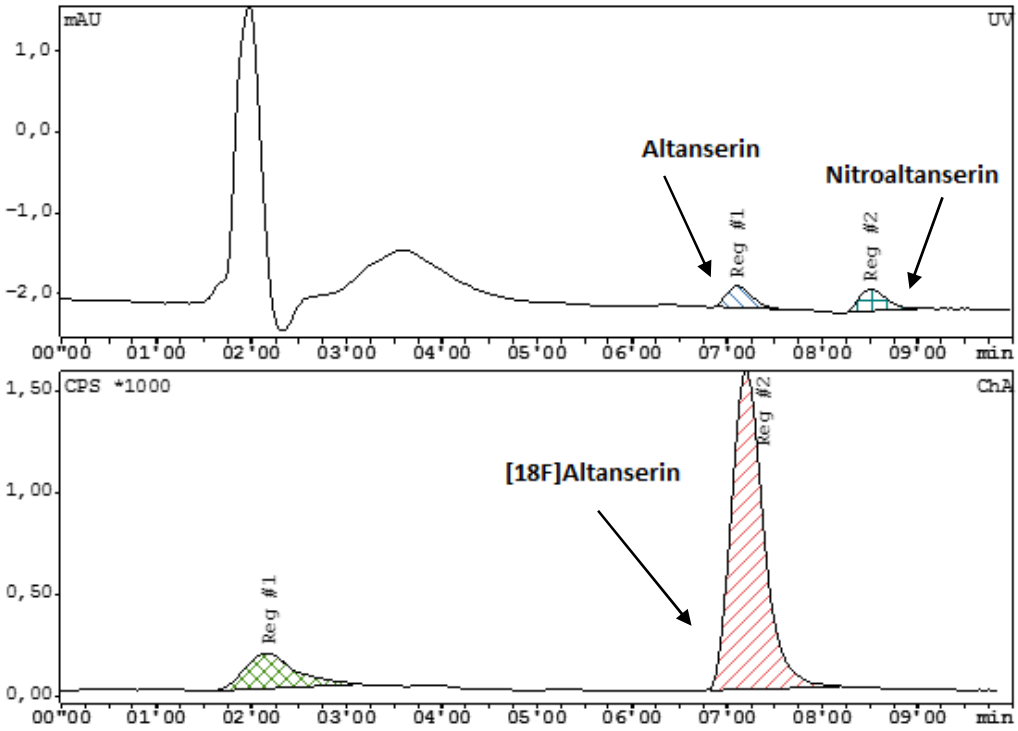


Figure 31: Analytical HPLC measurement of method 1 with 0.9% NaCl solution (no acid)

The two [^{18}F]Altanserin-peaks showed a difference in the elution time of approximately 5min on the preparative HPLC-system at a flow rate of 5ml/min. Therefore 25mL HPLC solvent between the peaks without product but with Nitroaltanserin and maybe other impurities were cut into the bulb. To prevent this, in the further peak cutting was interrupted by manually switching the three-way-valve from the bulb-position back into the HPLC waste position after the first peak and again to the bulb position when the second peak started. After the second peak was collected quantitatively the peak cut mode was stopped.

3.5 Water sensibility

No conversion was observed when the [^{18}F]fluoride was eluted with the base Tetrabutylammoniumhydroxide 30 hydrate (TBAH).
Therefore it was tested whether the water content duw to the 30x H_2O were too much for this water-sensible reaction.

The elution solution contained 20mg TBAH.
The molar mass of TBAH is 799,5g/mol. Hence 25nmol of the compound were used.
Consequently 30 hydrates are to equal 0,75mmol water.
Therefore the total amount of water, which was brought into the reaction mixture by TBAH, is 13.5mg or 13.5µl. This corresponds to 2.6% water in 500µl DMSO.

To check the water sensitivity of the syntheses, small amounts of water were deliberately added into the precursor. The fluoride was dried three times and the precursor in DMSO with 1, 2 or 3% water was added. The results are listed below.

percent of water in DMSO [%]	Mean percent of fluoride in the crude product [%]
1	27,69
2	94,12
3	100

Table 8: water-sensitivity experiments

It shows that there is a small conversion with 2% water in the presursor, but none at all with 3%. 2.6% therefore are definitly too to expect significant conversion the precursor with [^{18}F]fluoride to gain sufficient amount of product.
(For this calculation it was supposed, that after aceotropic drying the fluoride no water was in the reaction mixture.)

4 Conclusion

All four methods definitely had their advantages and disadvantages.
Method 1 (three times azeotropic drying, reaction conditions of 3mg precursor in 500µl DMSO, heated for 15min at 150°C, cleaned by HPLC with a complex preparing procedure prior; including dilution with 1N HCl, cartridge cleaning, methanol elution evaporation and dilution with 25mmol/l NH_4OAc solution, and formulated with ethanol, NaCl solution and phosphate buffer) was reliable and had the highest radiochemical and product yield, but also the longest "hands-in" time.
Method 2 (which differs from method 1 in the cleaning procedure; the step prior HPLC cleaning was skipped) was shorter in time but has a poor radiochemical and product yield and also not reliable. This made it useless for patient synthesis.
Method 3 (only one azeotropic drying step, reaction conditions similar to method 1 and 2, HPLC cleaning, with different HPLC assay, and formulation with ethanol and sodiumascorbat solution) was the shortest synthesis, the radiochemical and product yield was fair but the final product was neither reliable nor pure.
Method 4 (azeotropic drying and the reaction took place in the flow-through-µ-fluidic synthesizer, the cleaning and formulation procedure was the same as for method 1) had the shortest "hands-in" time, needed less precursor than the other synthesis and the radiochemical and product yields were in the center span, this synthesis took the longest time.
The same can be said about the different modules. As for the vessel-based-batch mode synthesizer only the synthesizer itself was needed. It was directly connected to the HPLC and also further purification could be done directly on the module.
The flow-through-µ-fluidic synthesizer had the advantage of less "hands-in" time and less precursor required but for cleaning and formulation of the product, the vessel-based-batch mode synthesizer was also needed.

The examined data are advising the use of method 1 with 1N HCl as favorable method for the synthesis of [^{18}F]Altanserin. It leads to a final product, which can be

administered to more than one patient and it is also more reliably in outcome, than the other synthesis methods.

A disadvantage of all methods which use HCl in their cleaning procedure was the double peak in the analytical HPLC, but as it was explained and the explanation proofed in many different ways, this could easily be tolerated.
The trial to prepare the crude reaction mixture with sodiumchloride solution or water instead of the acid, to avoid the double-peak, led to a precipitation of the product and the precursor. This decreased the final yield and led to a blockage of the HPLC system.

For the elution solvent TEAB as well as K2.2.2 could be used. The advantage of TEAB is the slightly higher conversion, whereas the disadvantage is that the elution solvent had to be prepared freshly before every synthesis. This extended the preparation time prior the synthesis.
TBAH did not lead to an adequate conversion. It could be showed, that the water content caused by the 30 hydrates in the solid TBAH (Tetrabutylammonium hydroxide 30 hydrate) was enough to interfere with the reaction. The conversion decreased with the amount of water. A total amount of 3% water, which is 15µl water in 500µl DMSO, inhibited the reaction completely.

It was also advisable to clean the parts of the module, which were in contact with the acid, directly after each synthesis. A longer interaction of the acid with the tubing led to a higher loss of activity while sending it through them during the following synthesis. It seemed, that the acid activated the surface of the tubings for a higher adsorption of fluoride. As the cleaning could be done automatically, after connecting the target water tube with a vial filled with water, it was not a big temporal expenditure.

5 Outlook

As the synthesis of [^{18}F]Altanserin is now well-established, further experiments and studies with it can be done.

At the moment a study with 12 patients and 12 healthy volunteers is already planned. These patients have undergone a surgery to remove the thyroid and after 4 to 6 weeks a hypofunction of the thyroid hormones showed up.

Shortly before these patients start the radioiodine therapy and take synthetically produced hormones, they are measured the first time with [^{18}F]Altanserin in PET or PET-CT.

This short time-window is used to examine possible psychic mood swings during the hypofunction.

The patients are measured a second time after their radioiodine therapy. The hormones, which are given to the patients during the therapy, lead to a hyperfunction. The goal of the second measurement is to explore the possible psychic emotional changes (as reported before) during the hyperfunction on the basis of the density of the 5-HT_{2A} receptor. [29]

6 Register

6.1 Register of Figures

6.2 Register of tables

6.3 Register of literature

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6.4 Register of acronyms

5-HT	5-Hydroxytryptamine
BBB	Blood-brain barrier
Bq	Becquerel
Ci	Curie
DMSO	Dimethylsulfoxide
EC	electron capture
EtOH	Ethanol
EOB	End of bombardment
EOS	End of synthesis
FBP	4-(4-fluorobenzoyl)piperidine
He	Helium
HPLC	high performance liquid chromatography
IC	internal conversion
IT	internal transition
IUPAC	International Union of pure and applied Chemistry
K2.2.2	Kryptofix 2.2.2
MeCN	Acetonitrile
n	neutron
nca	no-carrier added
OCD	obsessive-compulsive disorder
p	Proton
PET	positron emission tomography
RCY	Radiochemical yield
SN	nucleophilic substitution
TBAH	Tetabutylammoniumhydroxide
TEAB	Tetraethylammonium bromide
V	Vial

Chemical formulas and common units are not mentioned.

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