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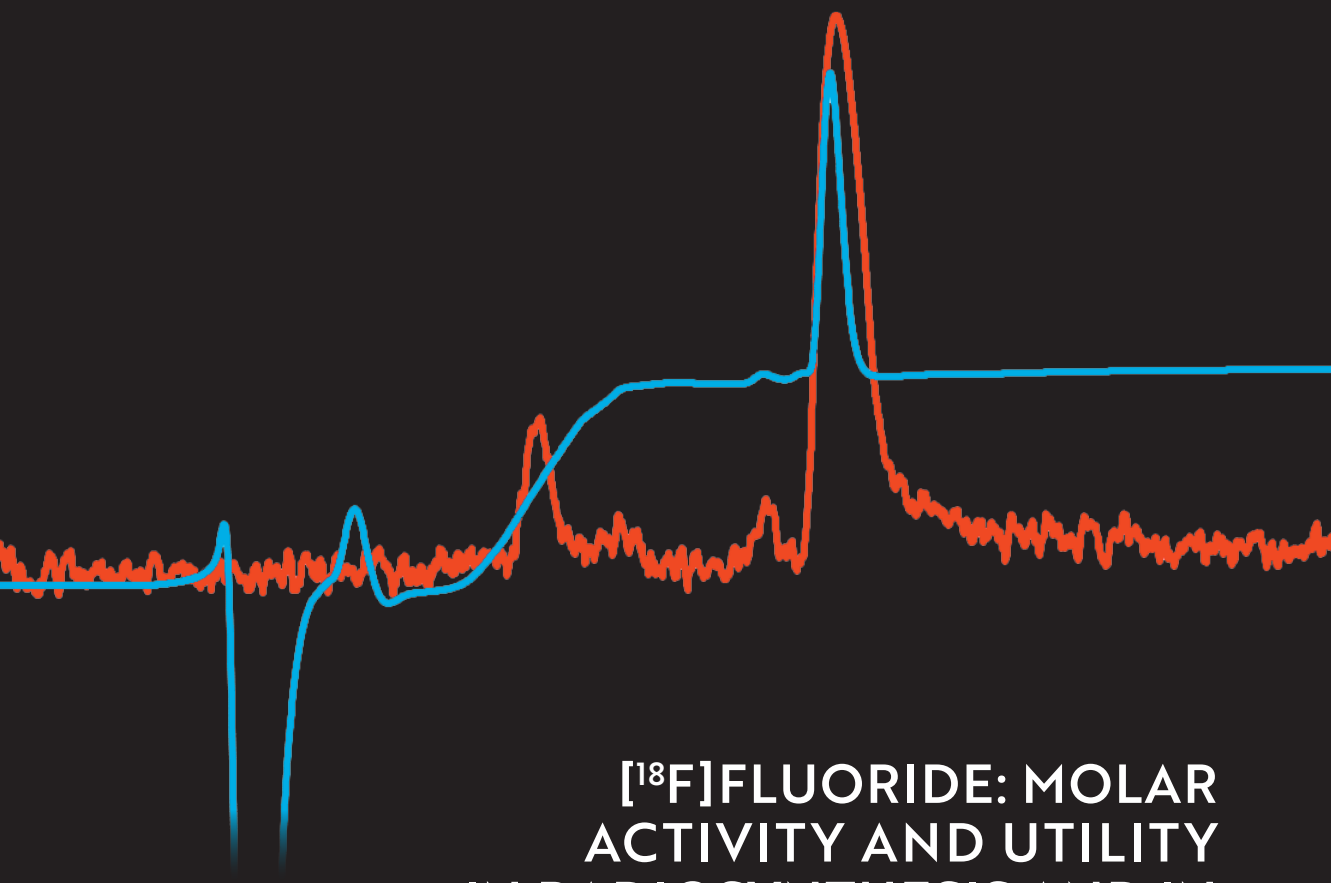

[^{18}F]FLUORIDE: MOLAR ACTIVITY AND UTILITY IN RADIOSYNTHESIS AND IN BIOLOGICAL APPLICATIONS

Nina Sarja

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“You don’t get
what you **wish**
for, you get what
you **work** for."

To Erno, Urho and Aaro

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Kliininen fysiologia ja isotooppilääketiede
Valtakunnallinen PET-keskus
NINA SARJA: [^{18}F]Fluoridi: Molaarinen aktiivisuus, käyttö radiosynteesissä ja biologisissa sovelluksissa
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TIIVISTELMÄ

Radiolääkeaine on radioaktiivisesta isotoopista ja biologisesti mielenkiintoisesta molekyylistä koostuva kokonaisuus, joka kulkeutuu kudoksiin, elimiin tai soluihin elävässä kohteessa. Radiolääkeaineen käyttösovellukset ovat sekä diagnostiikassa että terapiassa. Positroniemissiotomografia (PET) on kajoamaton tutkimusmenetelmä, jossa hyödynnetään lyhytikäisten positronisäteilevien radioisotooppien lähettämää gammasäteilyä. Sen avulla saadaan muodostettua kuva säteilyn jakautumisesta kehossa. [^{18}F]Fluoridi on yksi eniten käytetyistä PET-merkkiaineista.

Molaarinen aktiivisuus (A_m) on oleellinen parametri, joka tulee ottaa huomioon PET-merkkiaineita valmistettaessa. Korkea A_m on haluttu ominaisuus reseptori-kuvantamisessa ja tämän saavuttamiseksi stabiilin nuklidin määrä tulee minimoida. Tässä tutkimuksessa osoitettiin, että lähtöaineena käytettävän [^{18}F]fluoridin kuljetuksessa tuotantokammiosta synteesilaitteelle käytettävä kapillaari saattaa olla stabiilin fluorin lähde. Suositeltavaa on välttää fluorinoituja kapillaarimateriaaleja.

Uuden synteesimetodin kehitys, esim. [^{18}F]FTHA, on hyvin tarkoin säädeltyä GMP-säädöksin. Tähän kuuluu mm. synteesi- ja analyysimetodien validointi.

Molekyylissä kiinni oleva radioaktiivinen leima ([^{18}F]fluoridi) irtoaa jossain vaiheessa metaboliaansa ja kertyy luuhun ja pehmytkudoksiin. Terveillä rotilla tehdyt tutkimukset osoittavat, että tämä kertymä on merkittävästi riippuvainen rotan luun tyypistä ja pehmytkudoksesta.

Yhteenvetona voidaan todeta, että tämä tutkimus kuvastaa PET-radiolääkeaineiden tuotantoon ja käyttöön liittyvän monivaiheisen prosessin eri puolia.

Avainsanat: Radiolääkeaine, stabiilin fluorin lähteet, kuljetuskapillaari, GMP-säädös, validointi, [^{18}F]fluoridin farmakokinetiikka, [^{18}F]NaF, [^{18}F]FTHA

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NINA SARJA: [^{18}F]Fluoride: Molar activity, utility in radiosynthesis and biological applications
Doctoral Dissertation, 129 pp.
Doctoral Program in Clinical Research
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ABSTRACT

A radiopharmaceutical is a radioactive isotope combined with a biologically interesting molecule that targets specific tissues, organs, or cells in the living body. This special class of medicinal product has applications in both diagnostics and therapy. Positron emission tomography (PET) is a powerful non-invasive imaging technique that utilises gamma rays generated by short-lived positron emitting radioisotopes to form an image of the distribution of radioactivity in the subject. [^{18}F]Fluorine is one of the most widely used PET radionuclides.

Molar activity (A_m) is an important parameter that needs to be evaluated when producing PET radiopharmaceuticals. The amount of stable nuclide must be minimized in order to obtain a high A_m, which is an especially desirable property when imaging receptors. This study showed that the tubing used to transport the starting material, [^{18}F]fluoride, from the production chamber to the radiosynthesis device can be a source of stable fluorine; thus, fluorinated tubing material should be avoided.

The development of a new synthesis method, such as [^{18}F]FTHA, is strictly regulated by good manufacturing practices (GMP) guidelines, including validation of the synthesis and analytical methods.

The radioactive label (i.e., [^{18}F]fluoride) becomes detached from the molecule during its metabolism and will accumulate in bone and soft tissues. Studies conducted with healthy rats have demonstrated that this uptake is highly variable in various bone types and soft tissues in rats.

In conclusion, the production and use of PET radiopharmaceuticals involve a multistage process, and the studies presented here reflect the different aspects of this process.

Keywords: Radiopharmaceutical, sources of stable fluoride, transfer tubing, GMP guidelines, validation, pharmacokinetics of [^{18}F]fluoride, [^{18}F]NaF, [^{18}F]FTHA

Table of Contents

List of Original Publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I **Savisto N**, Bergman J, Aromaa J, Forsback S, Eskola O, Viljanen T, Rajander J, Johansson S, Grigg J, Luthra S, Solin O. *Influence of transport line material on the molar activity of cyclotron produced [^{18}F]fluoride.* Nucl. Med. Biol. 2018;64-65:8-15.

II **Savisto N**, Viljanen T, Kokkomäki E, Bergman J, Solin O. *Automated production of [^{18}F]FTHA according to GMP.* J. Labelled Comp. Radiopharm. 2018;61(2):84-93.

III **Savisto N**, Grönroos T, Oikonen V, Rajander J, Löyttyniemi E, Bergman J, Forsback S, Solin O, Haaparanta-Solin M. *^{18}F-fluoride uptake in various bone types and soft tissues in rat.* Submitted

1. Introduction

The isolation and identification of fluoride in 1886 won a Nobel Prize for French chemist Henri Moissan in 1906. However, difficulties in handling this most chemically reactive element, as well as its toxic properties, meant that progress in fluorine chemistry was slow. It was not until World War II that the large-scale industrial exploitation of this element began (e.g., processing nuclear fuel). Today, fluoride is used in the production of fluorocarbons, agrochemicals, and many pharmaceutical products (Strunecká et al., 2004), such as antiviral and antimalarial agents, antibiotics, anti-inflammatory drugs, antidepressants, antipsychotics, general anesthetics, and different biocompatible materials.

After the discovery of natural radioactivity in 1896 by Henry Bequerel and artificial radioactivity in 1934 by Irene and Frederic Joliot-Curie, the exploitation of radioactivity in life sciences has accelerated. Fluorine-18, one of the radioactive isotopes of fluorine, was identified by Arthur Snell in 1936 (Snell, 1937). Three decades later, alongside a few other short-lived positron emitters (^{13}N, ^{15}O, ^{11}C), these isotopes were found to be useful for medical imaging, specifically molecular imaging. In contrast to conventional imaging (e.g., x-ray, computed tomography (CT), and magnetic resonance imaging (MRI)), which provides information on anatomical structures, molecular imaging allows the visualization, characterization, and quantification of functional processes occurring at the cellular and molecular level within the living subject. The imaging modalities involve the use of an image-producing agent, "a probe", that is usually introduced into the body intravenously, and an imaging device that can detect the signals from the probe to produce detailed images.

Positron emission tomography (PET) is a nuclear molecular imaging modality for quantitatively measuring biochemical and physiological processes in vivo using radiopharmaceuticals labelled with positron-emitting radionuclides and by measuring the annihilation radiation using a coincidence technique (Ollinger, 1994, Paans et al., 2002). The advantage of PET imaging is that it can detect diseases at an early stage, often before chemical tests and conventional imaging methods detect any abnormalities. PET can also be combined with CT or MRI, making it possible to obtain a functional and anatomical image during a single imaging session. Today,

the clinical applications of PET are mainly in the fields of oncology, cardiology, and neurology, but it can also be exploited in drug development.

The production of PET radiopharmaceuticals is a multistage process; this is evident in the three studies presented in this thesis, which has been divided into three steps (Fig. 1). The main focus of every study has been on a different step in the production process. In study I, the focus was on the first step, as we studied the molar activity and how it is affected by transport tubing material. Study II focused on the second step; we developed a synthesis method by taking into consideration Good Manufacturing Practise (GMP) regulations. Finally, in study III, the focus was on the last step, as the purpose was to determine the pharmacokinetics of [^{18}F]fluoride uptake in the bone and soft tissues of healthy male and female rats. The focal points in every study block are described in Figure 1.

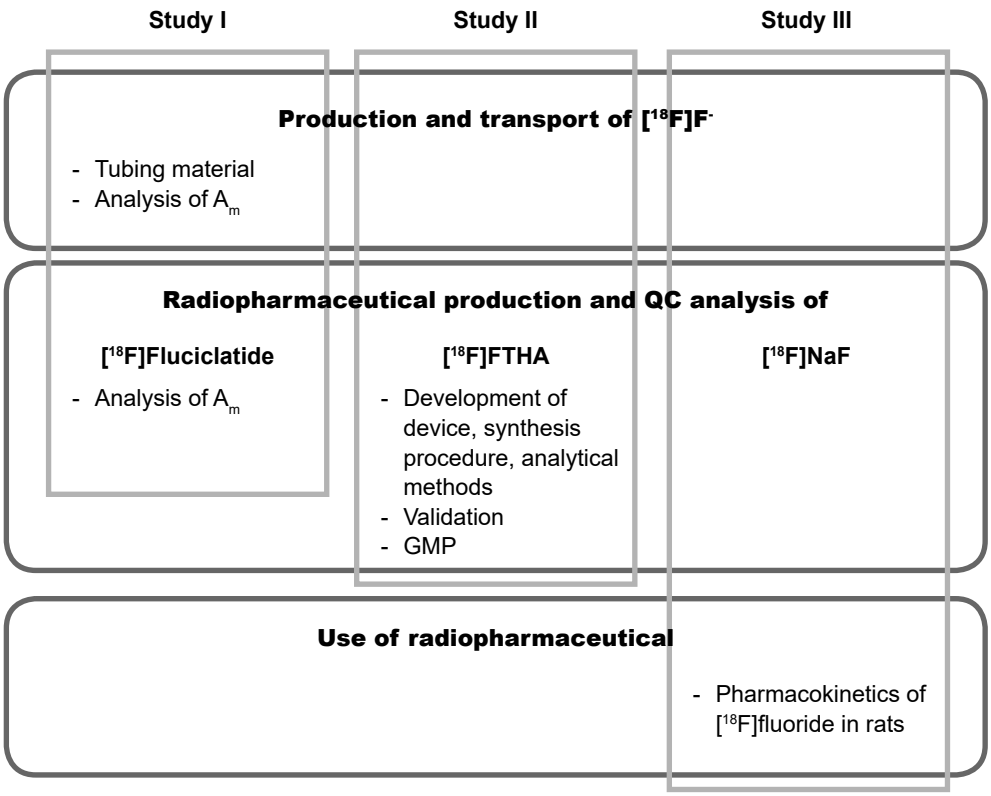


Figure 1. The production process and the use of radiopharmaceuticals divided into three blocks. The relationship between studies I-III.

2. Review of the Literature

2.1. PET radiopharmaceuticals

2.1.1. General aspects of radiopharmaceuticals

Radiopharmaceuticals used in nuclear medicine currently fall into two main categories: those used in diagnostic imaging, called radiotracers and those used for therapeutic purposes. Radiopharmaceuticals and thus radiotracers are defined as radioactive drugs. Radiotracers differ from conventional drugs as well as from therapeutic radiopharmaceuticals because they do not exert pharmacological effects as they are administered in sub-pharmacological doses. This is important when monitoring a particular physiological or pathological process in the body. The second distinctive characteristic of a PET radiotracer as compared to conventional drugs is that production is small scale with the compound being produced just prior to use.

Radiopharmaceuticals consist of two parts as shown in Figure 2 (Wadsak and Mitterhauser, 2010). The molecular structure part acts as a vehicle, transporting the radiopharmaceutical to the target of interest in the body. This determines the biological characteristics as the compound takes part in (bio)chemical interactions within the living system. In most cases, these are small molecules whose molecular weight is less than 2 kDa. In comparison to macromolecules (e.g. peptides, proteins, nanoparticles), small molecules have rather straightforward pharmacokinetics, usually rapidly gaining access to their target (Jeong, 2016). The second part, the radioactive nuclide, works as a signalling agent and it can be detected outside the body. These two parts can be joined together directly (in the case of small molecules) or with a linker (in the case of macromolecules).

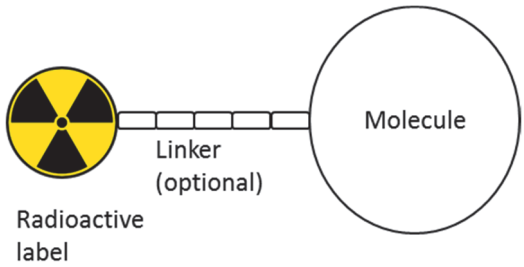


Figure 2. Radiopharmaceutical; radioactive label and the molecule moiety. The presence of a linker between the moieties is not mandatory, in some cases the radioactive label is incorporated into the targeting molecule itself.

In PET radiopharmaceuticals, the radioactive nuclide is a positron (β^+) emitting nuclide that decays to a stable nucleus. The decay process of this neutron deficient nucleus consists of the conversion of a proton into a neutron following the emission of a positron, the antiparticle of an electron and a neutrino. The positron then collides with an electron, forming a positronium (Paans et al., 2002). As the electron and positron are antiparticles, a process called annihilation follows where two photons of 511 keV travelling in opposite directions are produced. These photons can be detected outside the body with a PET camera. Over the years, several thousand radiotracers for PET have been developed. Nonetheless, one PET radiotracer developed at the end of 1970s, 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) (Ido et al., 1978), is still the most widely used radiopharmaceutical in PET today (Vallabhajosula et al., 2011). The success underpinning this glucose analogue lies in its extensive exploitation mainly in the field of oncology and its relative straightforward production with high yields. The production can be conducted with automated commercial synthesis devices.

2.1.2. Production of PET radionuclides

The production of a radionuclide involves a process where a stable atom is transformed to an unstable atom via a nuclear reaction by bombarding the target nuclide with neutrons, protons, deuterons, alpha or other nuclear particles (Srivastava and Mausner, 2013). The source for these nuclear particles can be a nuclear reactor (International Atomic Energy Agency, 2003) or a particle accelerator (International Atomic Energy Agency, 2008). Nuclear reactors used in the isotope production are mainly research reactors (OECD/NEA, 2005). Particle accelerators are either linear accelerators (linacs) or cyclotrons with a circular accelerating path. These two methods, nuclear reactor and the particle accelerator, are complementary because in general, these are used to produce different isotopes.

Often used terms in a radionuclide production are "carrier-free", "non-carried added" and "carried added". Carrier-free radionuclides do not contain any nuclei of the stable element. Non-carrier added production does not involve any intentional addition of the element produced, but unintentional presence of the element is possible. In carried-added production stable nuclei of the produced element are intentionally present.

Reactors are mostly used to produce radionuclides for therapeutic purposes (Volkert et al., 1991, Volkert, Wynn and Hoffman, 1999, Yeong et al., 2014) as these radionuclides are commonly neutron-rich and decay by β^- emission. The production is achieved by exposing an appropriate target material to a neutron flux followed by nuclear reaction where a neutron is captured by the nucleus, followed by emission of γ radiation or nuclear particle(s). The most common nuclear reaction of this type is the (n, γ) reaction, where the nuclide produced is isotopic (equal Z) with the target. The most common radionuclides produced by neutron activation are ^{32}P, ^{90}Y, ^{109}Pb, ^{153}Sm, ^{165}Dy, ^{177}Lu and ^{188}Re (Volkert et al., 1991). The drawback of this method is the low molar activity as the production is not carrier-free (Choppin et al., 2002, International Atomic Energy Agency, 2003). The second production mode involving neutrons is nuclear fission where a heavy nucleus, often ^{235}U, is split into two smaller nuclei. ^{90}Sr, ^{99}Mo, ^{131}I and ^{133}Xe are examples of fission-produced radionuclides (Ruth, 2009b). One disadvantage of reactor production is that radioactive waste from spent nuclear fuel and unintended activation materials is generated during the process. It also should be noted that the use of enriched ^{235}U in the production process is associated with safety concerns as this nuclide is used in nuclear weapons (Ruth, 2009b).

A cyclotron is the most widely used type of particle accelerator due to its circular path that makes it possible to use the same electrode system continually to accelerate particles which makes the device compact (Ruth, 2009a). The radionuclides produced are proton-rich and decay by β^+ emission or by electron capture (EC). These radionuclides are not only used for SPECT and PET imaging but involve also those for therapeutic purposes. The most common radionuclides used in the field of PET are presented in Table 1. The accelerated particles that are most often exploited to produce PET radionuclides are protons and deuterons as can be seen from the production routes described in Table 1. The main advantage of accelerator produced radionuclides is the high molar activity that can be achieved through (p, xn), (p, α) or other charged particle-induced reactions resulting in the production of a different element from the bombarded target Z=Z+1 (Ruth, 2009a). Other factors favouring the cyclotron in production methods are the ease and security of maintenance, the relatively low cost of operation, the stable production of radionuclides (Pashentsev, 2015), the small amount of waste generated during the production and the fact that access to accelerators is easier than to nuclear reactors.

Table 1. Radiochemical properties positron emitting radionuclides (Guillaume and Brihaye, 1986, National Nuclear Data Center, Brookhaven National Laboratory, Schmor, 2010, Vallabhajosula, 2009) with their common production methods. (g = gaseous, aq = aqueous, s = solid)

Nuclide	Half-life	β^+ energy max [MeV]	Branching (β^+) [%]	Target material	Common production method
^{11}C	20.4 min	0.96	99.8	$N_2(g)+O_2(g)$ $N_2(g)+H_2(g)$	$^{14}N(p,\alpha)^{11}C$
^{13}N	9.97 min	1.19	100	$H_2O(aq)$	$^{16}O(p,\alpha)^{13}N$
^{15}O	2.04 min	1.73	99.9	$N_2(g)+O_2(g)$	$^{14}N(d,n)^{15}O$
^{18}F	109.8 min	0.634	96.7	$H_2{}^{18}O(aq)$ $^{20}Ne(g)+F_2(g)$	$^{18}O(p,n)^{18}F$ $^{20}Ne(d,\alpha)^{18}F$
^{64}Cu	12.7 h	0.653	17.6	$^{64}Ni(s)$	$^{64}Ni(p,n)^{64}Cu$
^{68}Ga	67.7 min	1.899	87.7		$^{69}Ga(p,2n)^{68}Ge \rightarrow {}^{68}Ga$ (generator)
^{82}Rb	1.3 min	3.38	81.8		$^{nat}Rb(p,xn)^{82}Sr \rightarrow {}^{82}Rb$ (generator)
^{89}Zr	78.41 h	0.902	22.7	$^{89}Y(s)$	$^{89}Y(p,n)^{89}Zr$ $^{89}Y(d,2n)^{89}Zr$
^{124}I	4.18 d	2.14	10.7	$^{124}Te(s)$ oxide	$^{124}Te(p,n)^{124}I$ $^{124}Te(d,2n)^{124}Te$

The third option available for producing a radiopharmaceutical is a radionuclide generator (Rösch and Knapp, 2011, Saha, 2004) which is actually an application of the two aforementioned methods. The concept of a generator is based on the decay-growth relationship between a parent-daughter nuclide pair where a long-lived radionuclide (the parent) decays into a short-lived radionuclide (the daughter) that can be chemically separated from the parent (Guillaume and Brihaye, 1986). The parent is obtained from uranium fission products, or decay products from ^{233}U or it can be produced directly in an accelerator or a nuclear reactor (Rösch and Knapp, 2011). The most widely used generator is the nuclide pair of $^{99}Mo/^{99m}Tc$ (International Atomic Energy Agency, 2010) where the technetium is a single photon emitter used in SPECT imaging. In PET imaging, generator systems based on the positron emitters $^{68}Ge/^{68}Ga$ and $^{82}Sr/^{82}Rb$ (International Atomic Energy Agency, 2010) are currently most widely used. Table 1 describes the production routes. The major advantage of these kinds of generators is that they can be easily transported, making it possible to provide short-lived radionuclides to other sites e.g. to hospitals where no reactor or accelerator exists, making it a very cost-effective method.

2.1.3. Fluorine-18 as a PET radionuclide

Very few radionuclides are suitable for molecular imaging, especially for PET, even though the number of existing β^+ emitting radionuclides is large (Lambrecht, 1971, Li and Conti, 2010, Qaim, 1986, Ruth et al., 1989). The choice of the most appropriate nuclide needs to take into account many issues e.g. economical aspects, availability,

physical and chemical properties, radiolabelling options and radiopharmacological factors (Wadsak and Mitterhauser, 2010).

The physical aspects needing to be considered (Conti and Eriksson, 2016, Krasikova et al., 2016, Ott et al., 1988, Qaim, 1986, Wadsak and Mitterhauser, 2010) are the half-life and the decay mode of the nuclide as well as the energy of the positron. The half-life of the nuclide should match the half-life of the radiopharmaceutical to enable monitoring of the biological processes of interest. The half-life should preferably be short to minimize the radiation dose to the patient but on the other hand, it should be adequate to accommodate time-consuming synthesis procedures and lengthy imaging protocols and the time span of the biological process imaged. A longer half-life is also beneficial when the radiopharmaceutical must be delivered to hospitals that have no on-site radiopharmaceutical production facilities. The energy of the positron should be as low as possible because high energy will allow the positron to travel further in the tissue before annihilation. This distance travelled by the positron is called the positron range and it is a factor determining the spatial resolution of the PET scanner (Phelps et al., 1975). For example, if the positron range is extended, this will cause blurring of the image. The decay mode of the radionuclide is also important, and it is desirable that the chosen radionuclide is a pure β^+ emitter, meaning that there are no gamma rays other than annihilation radiation present.

^{11}C, ^{13}N, ^{15}O and ^{18}F, which all have a short half-life and a high branching ratio for β^+ decay, are the most widely used non-metal radionuclides in PET radiopharmaceuticals. The advantages of the first three organogenic elements i.e. C, N and O, is explained by the fact that they are isotopes of natural elements. Labelling with these radionuclides does not change the biochemical behaviour or the metabolism of the target molecule, leading to high specificity. The fourth element that can be found in organic molecules is hydrogen but unfortunately, this element does not have a radioactive isotope suitable for PET imaging; instead the isotope of fluorine is exploited. Fluorine incorporation into a target molecule is usually achieved by hydrogen or hydroxyl group substitution in a reaction called bioisosteric replacement (Patani and LaVoie, 1996). Bioisosters are atoms or functional groups which have chemical and physical similarities, making them interchangeable without significantly changing the biological behaviour (Thornber, 1979). In terms of size, fluoride is closer to oxygen than hydrogen as the Van der Waal's radius for fluoride is 1.47 Å, for oxygen, it is 1.52 Å whereas for hydrogen, it is smaller, 1.20 Å (Bondi, 1964). Nonetheless, fluoride seems to be a good hydrogen mimic. Fluoride's electronegativity is the highest of all elements, leading to the creation of a highly polarized C-F bond (Zhou et al., 2016). The role of fluoride in drug design and development has been discussed extensively in scientific articles over the years (Filler and Saha, 2009, Gillis et al., 2015, Hagmann, 2008, Park et al., 2001, Zhou et al., 2016).

In the field of PET, fluoride-18 has become the most important radionuclide due to its unique nuclear-physical properties. Its low positron energy (634 keV) with short tissue range (max 2.4 mm) and with a decay mainly by positron emission (β^+ 97%, EC 3%) all contribute to the production of PET images with excellent spatial resolutions. The half-life of fluoride-18 ($t_{½}$ = 109.8 min) is long enough to allow a multistep synthetic process along with the prolonged imaging procedures necessary for monitoring some moderately slow biological processes. It also permits the delivery of ^{18}F-labelled pharmaceuticals to medical centres further from production sites.

Several nuclear routes to produce fluoride-18 have been reported in the literature using an accelerator or a reactor (Gandarias-Cruz and Okamoto, 1988, Guillaume et al., 1991, Kilbourn, 1990). Many of the routes presented in Table 2 require a cyclotron having a high proton energy or particles that are not readily available in most facilities producing PET pharmaceuticals. Thus, the interest has focused on the two most widely exploited nuclear reactions, ^{18}O(p,n)^{18}F and ^{20}Ne(d,α)^{18}F reactions (highlighted in Table 2), producing either fluoride ion [^{18}F]F^- or molecular fluorine [^{18}F]F_2. The most convenient method to produce [^{18}F]fluoride is the ^{18}O(p,n)^{18}F reaction with highly enriched [^{18}O]H_2O as the target material (Ruth and Wolf, 1979). This non-carrier added (n.c.a.) method (meaning that non-radioactive material with the same chemical identity has not been added intentionally by a person during the preparation of radiopharmaceutical (Paans et al., 2002)) leads to a high molar activity (for definition see chapter 2.3), up to 43 TBq/μmol (Füchtner et al., 2008). It is also advantageous that high activity batches (> 370 GBq) (Jacobson et al., 2015) can be produced using a single irradiation with a proton energy under 20 MeV (Schmor, 2010). Aqueous fluoride can be used either directly in the synthesis or it can separated from the [^{18}O]H_2O by distillation or by passage through a resin column. Highly reactive molecular fluoride [^{18}F]F_2 can be obtained from an irradiation of ^{20}Ne-gas via (d,α)-reaction (Casella et al., 1980) or by ^{18}O(p,n)^{18}F reaction with [^{18}O]O_2 as the target (Nickles et al., 1984). Both reactions are carrier added (c.a.) by F_2-gas in order to extract the product from the target chamber, leading to low molar activity (100-600 MBq/μmol (Coenen, 2007)). In order to minimize the amount of carrier, a post target method to produce [^{18}F]F_2 starting from aqueous [^{18}F]F^- was developed, achieving a molar activity of 55 GBq/μmol (Bergman and Solin, 1997).

Table 2. Nuclear production routes to produce fluorine-18 with small cyclotrons (k<20) (Gandarias-Cruz and Okamoto, 1988, Hellborg et al., 2002, Kilbourn, 1990, Ruth and Wolf, 1979). Part of the reaction routes pass via the β+ decay of short-lived ($t_{1/2}$ = 1.87 s) ^{18}Ne (decay marked with an arrow).

Reaction
$^{16}O(^{3}He,n)^{18}Ne$, $^{18}Ne \rightarrow ^{18}F$
$^{16}O(^{3}He,p)^{18}F$
$^{16}O(\alpha,2n)^{18}Ne$, $^{18}Ne \rightarrow ^{18}F$
$^{16}O(\alpha,d)^{18}F$
$^{18}O(p,n)^{18}F$
$^{19}F(d,t)^{18}F$
$^{20}Ne(^{3}He,\alpha n)^{18}Ne$, $^{18}Ne \rightarrow ^{18}F$
$^{20}Ne(d,\alpha)^{18}F$
$^{20}Ne(p,2pn)^{18}F$
$^{20}Ne(d,p3n)^{18}Ne$, $^{18}Ne \rightarrow ^{18}F$
$^{6}Li(n,\alpha)^{3}H$, $^{16}O(^{3}H, n)^{18}F$

2.1.4. Tracer principle

PET is a molecular imaging technique (as discussed above) that is based on the tracer concept discovered by the Nobel awardee, George de Hevesy, in the early 1900s (Nobel Prize 1943). The concept of the tracer principle is that the radiotracer, the concentration of which can be measured, participates in physiological processes in a similar manner as the non-radiolabelled compound but without disturbing its function (International Atomic Energy Agency, 2008). The method has two important advantages when imaging molecular processes; 1) radiotracers can be used to image molecular processes with great sensitivity and 2) it is a non-invasive method (Wernick and Aarsvold, 2004).

2.1.5. Radiofluorination

Radiolabelling is a chemical reaction where the radioactive label i.e. the radionuclide is incorporated into the target molecule. This should be undertaken as late as possible in the synthesis sequence when short half-life radionuclides are used. For the same reason, fast synthetic strategies are preferable. The rule of thumb is that the total synthesis time including the quality control should not take more than 2-3 half-lives (Gillings, 2013).

Numerous fluorination strategies have been developed over the years. Traditional methods are divided mainly into two categories based on the chemical forms in which the fluoride is used. In nucleophilic labelling, fluoride-18 is introduced into the molecule as a fluoride ion $[^{18}F]F^-$ while in electrophilic radiofluorination, it enters as molecular fluorine $[^{18}F]F_2$. These methods are called direct labelling methods. In addition to these conventional labelling methods, there are indirect labelling methods involving the use of prosthetic groups. Prosthetic

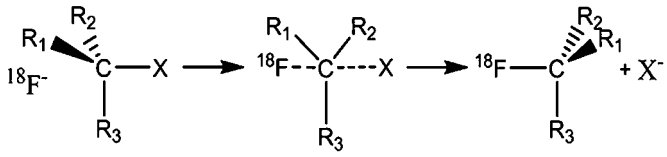
groups are small alkyl or aryl groups labelled with ^{18}F (Schirrmacher et al., 2017). They possess reactive functional groups and they are coupled with more complex biological molecules. Several articles on the labelling methods can be found in the literature (Adam and Wilbur, 2005, Cai et al., 2008, Coenen, 2007, Cole et al., 2014, Gillis et al., 2015, Gu et al., 2011, Jacobson et al., 2015, Miller et al., 2008, Preshlock et al., 2016). This chapter's focus will be on the direct fluorinating methods.

Nucleophilic labelling

Nucleophilic substitution is a process which involves the addition of a nucleophile Nu^-, a highly negatively charged molecule, into a target molecule possessing a leaving group. Currently, it is the preferred method for producing ^{18}F-radiolabeled compounds due to the higher molar activity (A_m), the ratio between amount of radioactivity and mass for a given radiolabelled product. See section 2.3. of the products and the greater selectivity. Nucleophilic labelling methods can be divided into aliphatic nucleophilic substitution and aromatic nucleophilic substitution (Kilbourn, 1990).

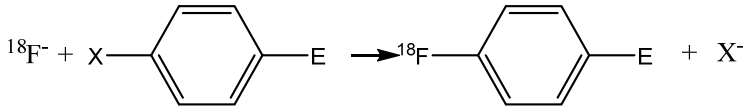
As the [^{18}F]fluoride ([^{18}F]F^-(aq)) is in an aqueous solution after its production, it forms hydrogen bonds with surrounding water molecules, making it inactive and not able to undertake nucleophilic substitution. In order to activate fluoride, the water must be removed, typically by trapping the fluoride in an ion exchange column and eluting it with potassium carbonate in a water/acetonitrile solution and evaporating the water by azeotropic distillation. In addition to potassium, large alkali metal ions e.g. rubidium and cesium (Cacace et al., 1981, Inkster et al., 2012, Kim et al., 2003, Nebel et al., 2017, Shiue et al., 1985) and tetra-alkylammonium salts (Brodack et al., 1986, Jewett et al., 1988, Pascali et al., 2012, Tewson, 1989) have also been used as counterions for fluoride as they have good solubility in organic solvents (Kilbourn, 1990). The solubility of fluoride is increased by adding a phase transfer catalyst such as a crown-ether (Irie et al., 1982) or cryptand (polyaminoethers) (Hamacher et al., 1986) in combination with potassium carbonate. The phase transfer catalyst complexes with the potassium ion thus leaving the fluoride "naked" and highly nucleophilic. Radiolabelling is undertaken in polar aprotic solvents such as acetonitrile, *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) (Vallabhajosula, 2009), all of which improve the nucleophilicity of fluoride as no hydrogen bonding exists. Protic solvents (i.e. water and alcohols) are not suitable for these reactions as they decrease the reactivity of [^{18}F]fluoride by hydrogen bonding as well as interacting with the partial positive charge of these solvents (Miller and Parker, 1961). Nonetheless, some protic tertiary alcohols have been reported to be used successfully as solvents (Kim et al., 2006).

Aliphatic nucleophilic substitution is an S_N2-type reaction (i.e. substitution nucleophilic bimolecular; bond breakage happens simultaneously with bond forming) where [^{18}F]fluoride attacks into the backside of the precursor relative to the leaving group. This results in an inversion of the configuration at the sp^3-center (Scheme 1) (Smith and March, 2007). The choice of leaving group is important and several parameters need to be considered e.g. its reactivity, stability and ease of incorporation into the precursor (Elsinga, 2002). Suitable leaving groups are e.g. halides, iodo-, bromo-, and sulfonic esters, triflate ($CFSO_3^-$), tosylate ($p\text{-}MeC_6H_4SO_3^-$), mesylate ($CH_3SO_3^-$), and nosylate ($m\text{-}NO_2C_6H_4SO_3^-$).



Scheme 1. Nucleophilic fluorination, where X is a leaving group (e.g. halide, triflate, tosylate, mesylate) (Vallabhajosula, 2009). $R_{1\text{-}3}$ = alkyl groups

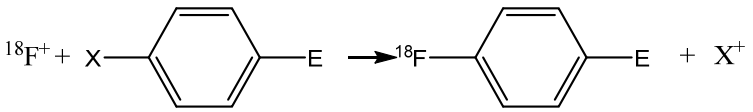
Aromatic nucleophilic substitution (S_NAr) (Scheme 2) with [^{18}F]fluoride is a typical route used to synthesize radiolabelled arenes. Other possible, but rather seldom used, methods, include Balz-Schiemann and the Wallach reactions (Cai et al., 2008). The S_NAr displacement reaction requires the presence of a strong electron-withdrawing group in the ortho- or para-position relative to the leaving group. Nitro (NO_2) and trimethylamine groups as well as halides (F, Cl, Br, I) are good leaving groups (Cai et al., 2008, Kilbourn, 1990). Even though fluoride is a good leaving group, it is rarely used due to the isotopic dilution and the poor molar activity of the final product. Nitro-, trifluoromethyl- and cyano groups are frequently used electron-withdrawing groups (Cai et al., 2008, Kilbourn, 1990).



Scheme 2. Nucleophilic fluorination, where X is a leaving group (e.g. NO_2, R_3N) and E an electron withdrawing group (e.g. NO_2, CN, CHO, COR, COOR) (Vallabhajosula, 2009).

Electrophilic labelling

Electrophilic fluorination (Scheme 3) is a chemical reaction involving the delivery of fluoride to an electron-rich reactant such as an alkene, aromatic ring, or carbanion by an electron-poor fluorinating reagent (Banister et al., 2010). Over the years, electrophilic labelling has enjoyed a pivotal role because many important radiopharmaceuticals, such as [^{18}F]FDG (Ido et al., 1978), have been prepared by electrophilic fluorination before the synthesis was replaced with nucleophilic labelling. At present, [^{18}F]fluoro-L-DOPA and 2-L-[^{18}F]fluorotyrosine are examples of radiopharmaceuticals still prepared by electrophilic labelling. The drawbacks of the electrophilic method are well known. The low molar activity of the end product due to the added carrier when producing [^{18}F]F_2 and the low specificity are factors that have limited the utility of this method. Another factor to be considered is that the radiochemical yield is limited and can be only 50% of the maximum because [^{18}F]F_2 contains both ^{18}F- and stable ^{19}F-isotopes.



Scheme 3. Electrophilic fluorination, where X is a leaving group (e.g. H, SnR_3, HgR) and E an electron donating group (e.g. OH, OCH_3, NH_2) (Vallabhajosula, 2009).

The use of molecular fluorine gas [^{18}F]F_2 is the simplest electrophilic reagent but also the most reactive. The reactivity can be controlled by diluting the fluoride with an inert gas or it can be converted into a milder [^{18}F]F_2-derived secondary reagent. Secondary reagents reported during the years include acetyl hypofluorite (CH_3COO[^{18}F]F) (Fowler et al., 1982), trifluoromethyl[^{18}F]hypofluorite (CF_3O[^{18}F]F), perchloryl[^{18}F]fluoride ([^{18}F]$FClO_3$) (Ehrenkaufer and MacGregor, 1982), xenon di[^{18}F]fluoride (Xe[^{18}F]F_2) (Chirakal et al., 1984), 1-[^{18}F]fluoro-2-pyridone (Oberdorfer et al., 1988b), N-[^{18}F]fluoropyridinium-triflate (Oberdorfer et al., 1988a), N-[^{18}F]fluoro-N-alkylsulphonamides (Satyamurthy et al., 1990), [^{18}F]fluoro-N-fluorobenzenesulphonimide (Teare et al., 2007) and [^{18}F]Selectfluor bis(triflate) (Teare et al., 2010).

2.2. Legislation and good manufacturing practices in the preparation of PET radiopharmaceuticals

2.2.1. Introduction

This section focuses on radiopharmaceuticals; obviously legislation and good manufacturing practises on radiopharmaceuticals are derived from the same for pharmaceuticals. The production of a radiopharmaceutical to be used in human studies is not just a question of choosing an appropriate nuclide or a labelling method. It is a combination of many requirements, for example, the availability of clean room facilities, competent staff, validated production and analytical methods, meaning that the production and the use of pharmaceuticals intended for human use are highly regulated all around the world. The development of these regulations stems from many unfortunate incidents related to use of medicinal products, such as the thalidomide disaster (Kim and Scialli, 2011). These regulations are intended to ensure that medicinal products are both safe and effective for the consumer. As radiopharmaceuticals and thus PET radiopharmaceuticals are defined as medicinal products in the European Union according to Directive 2001/83/EC (European Parliament and Council of the European Union, 2001) and in the USA according to the Federal Food, Drug, and Cosmetic Act (U.S. Food and Drug Administration, 2014), it is evident that they are also subject to many regulations. The production facilities and the production process, use and storage of these products and the training of personnel are subject to many laws, directives, regulations and rules issued by both national and/or regional authorities. In addition, the preparation of PET radiopharmaceuticals is also regulated by radiation safety laws and regulations, making them a very special group of medicinal products. It is occasionally problematic that these can be conflicting, i.e. the regulations governing pharmaceutical preparations may be at odds with those governing radioactive materials. These challenges can usually be resolved with technical applications and in most cases, the requirements for these regulatory documents are congruent in supporting each other. For example, the requirement for air pressure in a clean room area (specification: overpressure compared to surrounding areas) is opposite to the requirements for radiochemistry production facilities (specification: negative pressure compared to surrounding areas). These requirements can be fulfilled by using air-locks with appropriate air pressures.

The preparation of PET radiopharmaceuticals differs considerably from the preparation of conventional medicinal products. Even though the use of PET radiopharmaceuticals has grown in recent years, there are no binding regulations issued by the EU especially concerned with PET radiopharmaceuticals. This is because the EU legislations are focused either on industrial manufacturing of

radiopharmaceuticals or on clinical trial protocols (Decristoforo et al., 2017). The lack of regulations related to "in-house" prepared PET radiopharmaceutical is problematic because the current regulations concerning medicinal products do not take into consideration the specific and unique characteristics of radiopharmaceuticals. In contrast to conventional drugs, the low mass of active component (the tracer principle) means that radiopharmaceuticals should not exert pharmacological effects and the short half-life sets limits on their shelf-life. The parenteral administration of radiopharmaceuticals, which is the principal route of administration, is also a significant factor that has to be taken into consideration in the preparation of radiopharmaceuticals because even more strict rules apply concerning the sterility of these types of products. This is because the injected drug goes directly into the circulatory system, bypassing many human barriers before encountering the immune defences. With conventional medicinal products, the sterility can be tested prior to the release of the product, but for PET radiopharmaceuticals, this is not possible as they have very limited shelf-lives and are administered within a few hours after their preparation. Therefore, to ensure the sterility of PET radiopharmaceuticals prior to patient administration, the production procedures have to be compliant with regulations and the production has to be supervised by qualified personnel. The purpose of all of these regulations is to ensure that radiopharmaceuticals are safe, efficient and fulfil all specifications.

Regulations concerning radiopharmaceuticals, particularly PET radiopharmaceuticals, are a complex web because of the many different authorities and organisations issuing regulations and guidelines. This chapter focuses on the legal documents and guidelines that are related to the PET radiopharmaceuticals used in diagnostic patient studies in the EU, which is essential information for everyone participating in the preparation of PET radiopharmaceuticals. Radiation safety regulations or transport regulations are not addressed in the following chapters.

2.2.2. European regulations of medicinal products

In the member states of the European Union, the European Commission is the regulatory body that is responsible for the legislation that lays down the normative standards on production and use of radiopharmaceuticals. The European Parliament amends and approves these laws. The role of the European Medicines Agency (EMA), an agency of EU, is to evaluate, supervise and monitor the safety of drugs in the EU. In addition to these EU-wide regulations, there is also a national legislation in every member state concerning medicinal products and regulatory guidelines issued by the various regulatory bodies; in Finland, this is Fimea, the Finnish Medicines Agency. In addition to the regulatory bodies, there are many organizations (Table 3) that have issued different guidelines on radiopharmaceutical

practices. These will be introduced in more detail in the following chapters. Good overviews on the European legislation of radiopharmaceuticals can be found in several publications (Decristoforo, 2007, Decristoforo and Schwarz, 2011, Decristoforo et al., 2017, Elsinga et al., 2010, Lange et al., 2015).

Table 3. Regulatory bodies and organisations related to preparation of PET radiopharmaceuticals.

Organisation	Abbr.	Explanation
European Association of Nuclear Medicine	**EANM**	Largest organisation dedicated to Nuclea Medicine in Europe
European Medicines Agency	**EMA**	Agency on EU that evaluates the marketing authorisation applications
European Directorate for the Quality of Medicines and Healthcare	**EDQM**	Body of the Council of Europe that publishes European Pharmacopoeia
Finnish Medicines Agency	**Fimea**	National medicines authority
Food and Drug administration	**FDA**	US government agency
International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human use	**ICH**	International association that aims in focusing global pharmaceutical regulatory harmonization work
Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme	**PIC/S**	International co-operative arrangement between Regulatory Authorities in the field of GMP
World Health Organisation	**WHO**	International organisation of UN that focuses on health issues.

Radiopharmaceutical practices in the member states of the European Union (EU) vary extensively even though one fundamental principle of the EU has been the removal of regulatory barriers to trade throughout Europe. In fact, several regulatory documents concerning the preparation of radiopharmaceuticals have been created to service the industrially manufactured radiopharmaceuticals that are intended to be placed on the market. Nonetheless, the short-lived radiopharmaceuticals including those used in PET studies lie outside of the scope of these regulations. A second explanation for this variability can be found from the directives and from the fact that they have to be translated into national legislation (Table 4). This means that practical implementation of the directives is made by the member states themselves, leaving room for varying interpretations (Decristoforo and Peñuelas, 2009).

Table 4. Definitions of EU legal acts (adopted with modifications from Elsinga et al., 2010).

Legally binding documents
Regulations are mandatory for member states. They are applied without translation* into national legislation.
Directives are rules prepared by the EU Commission and transmitted to the member states. They are mandatory and have to be translated into the national legislation and effectively implemented
Guidance documents
Guidelines are not mandatory. They are recommendations for effective implementation of directives.

*not addressing linguistics

2.2.3. Legal framework of use of PET radiopharmaceuticals

The regulations concerning the preparation of radiopharmaceuticals are dependent on the manufacturer of the radiopharmaceutical and the purpose of its use. When a radiopharmaceutical is produced by a pharmaceutical company and intended for market, then Directive 2001/83/EC (Community code relating to medicinal products for human use) (European Parliament and Council of the European Union, 2001) and Regulation (EC) No 726/2004 (Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency) (European Parliament and Council of the European Union, 2004) are applied. According to Directive 2001/83/EC, a marketing authorisation is needed for the industrially manufactured and commercially distributed radiopharmaceuticals such as radiolabelling kits, radionuclide generators and radionuclide precursors. Marketing authorisation is an official document granted by the national regulatory body (in Finland this is Fimea) or the European Commission, depending on the marketing area (one member state vs. whole EU area) after ensuring that the product is compliant with current requirements of efficacy, safety and quality. However, with respect to the PET radiopharmaceuticals, the marketing authorisation has not been very common. The reasons behind this are economical. The small market is not enough to recoup the investment, and the short half-lives of the products set limits for their delivery (Decristoforo and Peñuelas, 2009). In this sense, [^{18}F]FDG is an exception having a marketing authorisation in many European countries. In addition, some other ^{18}F-labelled PET radiopharmaceuticals do have a marketing authorisation but only in a few European countries; [^{18}F]fluoride, [^{18}F]DOPA, [^{18}F]choline, [^{18}F]FET, [^{18}F]florbetapir, [^{18}F]fluciclovine, [^{18}F]florbetaben and [^{18}F]flutemetamol. In Finland, the marketing authorisation has been granted for [^{18}F]FDG; it is marketed under the product name SteriPET.

A second group are the radiopharmaceuticals used in clinical trials, having at the same time marketing authorisation as an objective. These are studies performed to

investigate the safety or efficacy of a medicine (pharmacodynamics) in human volunteers, or the absorption, distribution, metabolism and excretion (pharmacokinetics) of the drug in the human body, or both at the same time. The medicinal product being tested or used as a reference in the clinical study is called an "investigational medicinal product" (IMP). PET radiopharmaceutical used in a clinical trial can be the object of the investigation i.e. the IMP itself but it can also be a non-investigational medicinal product (NIMP) and simply be used as diagnostic tool e.g. to study the effects of some drug (Verbruggen et al., 2008). Before the trial can start, an ethical and scientific review of the study has to be conducted and clinical trial authorisation (CTA) has to be sought according to Regulation (EU) No 536/2014 (Clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC) (European Parliament and Council of the European Union, 2014).

Nonetheless, PET radiopharmaceuticals produced for diagnostic purposes mainly in hospital pharmacies, academic research centres and PET centres are left outside of these above mentioned indications. Their production is often denoted as "small-scale" preparation. The term small-scale is not related to the size of the facility but to the batch size which is one dose or a few doses at a time, differing greatly from the industrial, large-scale, production where drugs can be made in batches of hundreds of kilos and intended to treat thousands of patients. In the case of radiopharmaceuticals, production is called extemporaneous preparation and a marketing authorisation is not needed based on an exemption of Directive 2001/83/EC. In that case, the preparation is undertaken in a pharmacy either in accordance with a medical prescription (magistral formula) or according to a pharmacopoeia monograph (officinal formula). For example, in Turku PET Centre, the PET radiopharmaceuticals are prepared under the licence of the hospital pharmacy with a pharmacist being responsible for the production and release. This is not the case in many other EU member states where the preparation may be undertaken according to specific local regulations or under the responsibility of a medical doctor (Decristoforo, 2007).

2.2.4. Standards of preparation – good manufacturing practice

Good manufacturing practise abbreviated to GMP is a set of rules; their principle is to ensure that radiopharmaceuticals or medicinal products in general are consistently produced and controlled according to quality standards (European Parliament and Council of the European Union, 2003). GMP-rules describe the minimum standard that must be met in the production process when preparing radiopharmaceuticals. These rules refer to the whole production process; facilities, starting materials, equipment, record keeping, personnel qualification and

validation. Thus, an appreciation of these regulations is vital for the whole personnel participating in the production process. It is essential to have written, detailed instructions (SOP: Standard Operating Procedure, MET: Method Description) for each process that can have an effect on the quality of the end product. The whole production process has to be documented to show that correct procedures have been followed every time that the product is made. With regard to PET radiopharmaceuticals, the compliance with GMP is absolutely essential because certain quality control tests (e.g. sterility tests) cannot be done before the release of the product. In this case, the quality has to be "build into the process" so to speak.

In the EU, the principles and the legal basis of GMP are laid down in Directive 2003/94/EC (European Parliament and Council of the European Union, 2003) adopted by the European Commission. More specific rules on GMP are published in Eudralex Volume 4 (European Commission, 2010). Eudralex is the collection of European pharmaceutical legislation housed in the internet and consisting of 10 volumes. Two of these, Volume 1 (EU pharmaceutical legislation for medicinal products for human use) and Volume 5 (EU pharmaceutical legislation for medicinal products for veterinary use), comprise official legislation while the 8 other volumes, including Volume 4 (Guidelines for good manufacturing practices for medicinal products for human and veterinary use) are supporting guidelines. Volume 4 consists of three parts and eighteen annexes (Table 5). It is noteworthy that the former Annex 18 is now published as Part II "Basic requirements for Active Substances used as Starting Materials". GMP related documents that explain the regulatory expectations are described in part III.

With respect to PET radiopharmaceuticals the scope of Directive 2003/94/EC, the legal base for GMP, refers to medicinal products with marketing authorisation or investigational medicinal products with an authorisation to manufacture or import the products. Thus, the PET radiopharmaceuticals prepared extemporaneously are left outside of the scope of this legal requirement. However, the Introduction to GMP guidelines published in Volume 4 (European Commission, 2010) states that "They (GMP regulations) are also relevant for pharmaceutical manufacturing processes, such as that undertaken in hospitals" meaning that they apply to the preparation of PET radiopharmaceuticals. Nonetheless, it should be noted that these are simply guidelines meaning that they are not legally enforced. They only describe the acceptable practices and principles allowing the possibility for implementation of alternative methods. This can be problematic, and harmonization of practices would be beneficial (Decristoforo and Peñuelas, 2009).

Table 5. Contents of Eudralex Volume 4: Guidelines for good manufacturing practices for medicinal products for human and veterinary use (European Commission, 2010).

Part I: Basic requirements for Medicinal Products		**Annexes**	
1	Pharmaceutical Quality System	1	Manufacture of Sterile Medicinal Products
2	Personnel	2	Manufacture of Biological active substances and Medicinal Products for Human Use
3	Premise and Equipment		
4	Documentation	3	Manufacture of Radiopharmaceuticals
5	Production	4	Manufacture of Veterinary Medicinal Products other than Immunological Veterinary Medicinal Products
6	Quality control		
7	Outsourced activities	5	Manufacture of Immunological Veterinary Medicinal Products
8	Complaints and Product Recall		
9	Self Inspection	6	Manufacture of Medicinal Gases
Part II: Basic requirements for Active Substances used as Starting Materials		7	Manufacture of Herbal Medicinal Products
		8	Sampling of Starting and Packaging Materials
Part III: GMP related documents (e.g. Site Master File)		9	Manufacture of Liquids, Creams and Ointments
		10	Manufacture of Pressurised Metered Dose Aerosol Preparations for Inhalation
		11	Computerised Systems
		12	Use of Ionising Radiation in the Manufacture of Medicinal Products
		13	Manufacture of Investigational Medicinal Products
		14	Manufacture of Products derived from Human Blood or Human Plasma
		15	Qualification and validation
		16	Certification by a Qualified Person and Batch Release
		17	Parametric Release
		19	Reference and Retention Samples

Annex 1 "Manufacture of Sterile Medicinal Products", Annex 3 "Manufacture of Radiopharmaceuticals", Annex 11 "Computerised Systems", Annex 15 "Qualification and validation" and Annex 16 "Certification by a Qualified Person and Batch Release" apply when preparing PET radiopharmaceuticals for diagnostic use. These rules do not distinguish between the in-house preparation and the industrial preparation, nor have the regulatory authorities a different set of rules when inspecting manufacturers. This can be challenging especially for those manufacturing PET radiopharmaceuticals extemporaneously as they may struggle to comply with the increasingly stringent demands (Schmidt et al., 2017).

In addition to the GMP rules set by EU/EMA, there are other international guidelines published by different parties. The radiopharmacy committee of the European Association of Nuclear Medicine (EANM) has issued "Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals" (Elsinga et al., 2010) and "Guidance on current good radiopharmacy practice for the small-scale preparation of radiopharmaceuticals

using automated modules: a European perspective" (Aerts et al., 2014). The first guidance document addresses in a comprehensive manner all the integral parts of the preparation of radiopharmaceuticals e.g. personnel and resources, facilities and equipment, documentation, preparation and process controls (Elsinga et al., 2010). The second document is a complement to the first one, focusing on aspects relating to the use of automated synthesis modules in the production process. Even though these two documents can be viewed as useful references in the preparation of radiopharmaceuticals, they are not binding nor do the inspection authorities consider them as reference materials. In reality, the inspection authorities inspect the small non-commercial manufacturers based on the same requirements that concern commercial large-scale medicinal products preparation.

The World Health Organisation (WHO), an agency of the United Nations, has published its own GMP guidance document regarding radiopharmaceuticals. The scope of the annex 3 "Guidelines on Good Manufacturing Practices for radiopharmaceutical products" (World Health Organization, 2002) is the preparation of radiopharmaceuticals in hospital radiopharmacies and PET centres. The WHO GMP guide differs in detail from EU GMP guidance, but the main principles are the same.

The Pharmaceutical Inspection Convention and the Pharmaceutical Inspection Co-operation Scheme (jointly know as PIC/S) is a non-binding co-operative arrangement between pharmaceutical inspection authorities in the field of GMP. The aim of the 52 participating authorities is to harmonise inspection procedures globally by developing common GMP standards. The main instrument for harmonisation is the PIC/S GMP Guide (PIC/S, 2017), which is equivalent to the EU GMP guidelines with regards to GMP requirements. Annex 3 "Manufacture of radiopharmaceuticals" in PIC/S GMP Guide also takes into account PET radiopharmaceuticals.

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is an international association that gathers together the regulatory authorities of Europe, Japan and the United States and pharmaceutical industry to discuss scientific and technical aspects of drug registration. The aim is to increase the harmonization of technical requirements worldwide to ensure that safe, effective and high-quality medicines are developed and registered in a cost-effective manner. In order to achieve this goal, ICH has developed guidelines on quality, safety and efficacy and also technical requirements for medicine development and approval.

2.2.5. European Pharmacopoeia

Pharmacopoeia is an official book devoted to medicinal products containing quality requirements for medicinal substances, excipients, and medicinal products. There are several pharmacopoeias in use today such as the International Pharmacopoeia, the United States Pharmacopoeia, the Japanese Pharmacopoeia and the European Pharmacopoeia.

The European Pharmacopoeia (Ph. Eur.) issued by the European Directorate for the Quality of Medicines & Healthcare (EDQM) consists of a collection of monographs that describe individual and general quality standards of substances and medicines, general methods of analysis, and some general requirements for dosage forms (e.g. capsules, injections, tablets). It has a legal status in the European Union and in most non-EU countries in Europe. A total of 38 European countries have signed the Pharmacopoeia convention to accept Ph. Eur. as a legally binding document.

The present officially binding version of Ph. Eur (Council of Europe, 2017) contains 2376 monographs from which only a small part refer to ^{18}F-labelled radiopharmaceuticals. The small number is explained by the fact that monographs are drafted for those radiopharmaceuticals that have a marketing authorisation or are used widely (Decristoforo et al., 2017). For these radiopharmaceuticals, the regulatory acceptance is usually more simplified. In addition to these individual radiopharmaceutical monographs, Ph. Eur. has recently published a general chapter on the extemporaneous preparation of radiopharmaceuticals (Council of Europe, 2016). This is especially targeted to the small-scale preparation of radiopharmaceuticals, e.g. giving minimal requirements for PET radiopharmaceuticals. As this is a general chapter, it should be noted that is not legally binding unless cited in a monograph (Decristoforo et al., 2017).

2.3. Concept of molar activity

According to a recently updated terminology issued by the Working Group on "Nomenclature in Radiopharmaceutical Chemistry and related areas", initiated by Drug Development Committee by the European Association of Nuclear Medicine, the term **molar activity (A_m)** is recommended to be used instead of **specific activity (A_s)**, if the molar amount of radioactive compound is expressed (Coenen, et al., 2017). The publication by the Working Group defines the A_m as measured activity per mole of compound; measured in Bq/mol (GBq/µmol). In contrast, the definition for A_s is the measured activity per gram of compound; measured in Bq/g (GBq/µg). It should be noted that the time point of measurement must be stated because the A_m decreases over time as the radioactive nuclide decays. In addition, other terms e.g. **apparent molar activity** and **apparent specific activity** and also **effective molar**

activity and **effective specific activity** are used. The first two terms take into account the amounts of the labelled and non-radiolabelled impurities present (using moles, or weight, respectively) but are not able to distinguish between them analytically (Coenen et al., 2017). The last two terms address the chemically, biologically, or pharmacologically 'active' fraction of radioactive and non-radioactive materials present in a sample, competing with the labelled product in its chemical or biological reactions. In this case, the "effectivity" must be determined by an additional analytical process; e.g. receptor or enzyme binding assay, side-product analysis, etc." (Coenen et al., 2017).

Molar activity is one of the most important parameters for PET radiopharmaceuticals. It indicates the extent to which the ^{18}F-labelled compound is contaminated with the non-radioactive isotopic compound (Preshlock et al., 2016). High A_m means a low mass of the radiotracer which is desirable in many cases, especially when imaging various receptor systems. The importance of a high A_m value can be seen in Figure 3 as the limited number of target sites do not differentiate between the ^{18}F- or ^{19}F-labelled molecules. Due to a low mass of radioligand (usually less than 1-10 nmol), the receptor binding sites are not saturated with non-radioactive ligand and possible pharmacological and toxic effects of the tracer molecule are minor (Cai et al., 2008, Füchtner et al., 2008, Horti and Villemagne, 2006, Jacobson and Chen, 2010). An extensive review on the significance of A_m has been published a few years ago (Lapi and Welch, 2013).

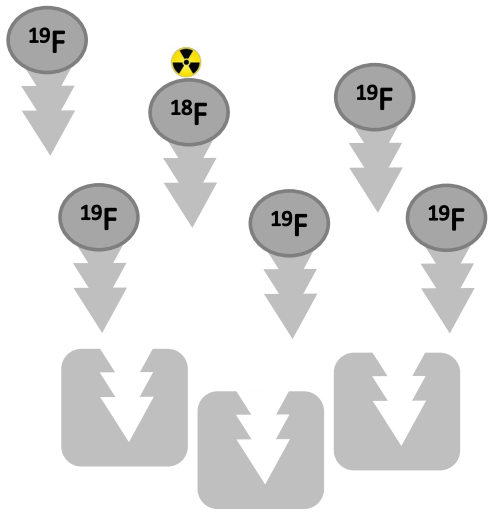


Figure 3. Relationship between ^{18}F- and ^{19}F-labelled molecules. Only a small part of the labelled molecules have a radioactive label. The ^{18}F and ^{19}F marked constructs depict radiolabelled and non-labelled molecules. The receiving sites depict e.g. receptors that are targeted.

A_m of fluoride-18 can be determined by measuring the absolute amount of a sample radioactivity and by measuring either fluoride ion concentrations by e.g. ion chromatography or other methods. Alternatively the fluoride can be incorporated into a molecule, and the activity and mass of the particular molecule is determined by any practical means- in practise very often by UV-absorption chromatography.

The maximum theoretical A_m for a specific radionuclide is defined by equation 1, where $t_{1/2}$ is the half-life of the nuclide (s) and the N_{av} is the Avogadro constant ($6.022 \cdot 10^{23}$ 1/mol).

$$A_m(\max) = \frac{\ln 2}{t_{1/2}} N_{av} \quad (1)$$

A_m(max) for PET isotopes are high, because of their short half-lives. The value for fluorine-18 is 63 TBq/µmol, when no stable fluoride is present. Molar activities reported in the literature for fluorine-18 at the end of bombardment (EOB) in irradiated oxygen-18 water are significantly lower, varying from 22 to 9900 GBq/µmol. (Bergman et al., 2001, Berridge et al., 2009, Dence et al., 1995, Kilbourn et al., 1984, Kilbourn et al., 1985, Shiue et al., 1985, Solin et al., 1988). One notable exception from this was reported by Füchtner et al. (Füchtner et al., 2008), 43 TBq/µmol.

It is not easy to achieve a high A_m but the value can be improved with an increase in the starting activity or by removing the carrier fluorine-19 as extensively as possible. The contamination with stable fluorine-19 can happen in the radionuclide production in a cyclotron from ^{18}O-enriched water or during the preparation of the radiotracer. It is important to appreciate that even very small amounts of fluorine-19 will decrease A_m considerably as the molar amount of [^{18}F]fluoride is only 0.58 nmol when the starting activity is 37 GBq (typical activity for many fluoride synthesis) and no stable fluoride is present i.e. A_m is 63 TBq/µmol. There are many different sources of fluorine-19 encountered during the production process and they are often difficult to control. Even though this has raised some interest among many research groups and generated quite a few diverse studies on this subject, many have been anecdotal and only a few have investigated this topic in-depth. This is somewhat surprising as resolving this problem would benefit many research groups. Various suggestions about the possible sources of carrier fluorine have been proposed such as oxygen-18 water, chemicals and solvents being used in the synthesis, transfer tubing and target chamber material, length of irradiation and radiolytic degradation of fluorinated materials (Berridge et al., 2009, Füchtner et al., 2008, Hersh et al., 2009, Link et al., 2012, Nishijima et al., 2002, Schlyer et al., 1993, Shiue et al., 1985, Tewson and Welch, 1980, Vaidyanathan et al., 2009). It seems probable that no single source is the culprit, instead it will be a combination of many different factors (Bergman et al., 2001).

2.3.1. Target water and synthesis reagents as sources of carrier fluorine

^{18}O-enriched target water has most often been proposed to be the source of carrier fluorine. However, when virgin target water has been analysed, only small traces (e.g. 0.30 ppm, also under detection limit in some cases) of fluorine-19 were found to be present and thus it has been concluded that water is not the most crucial source of the carrier (Berridge et al., 2009, Füchtner et al., 2004, Füchtner et al., 2008, Link et al., 2012, Schlyer et al., 1993). Two other studies seem to support this view even though they did not focus on determining the sources of fluoride (Nishijima et al., 2002, Shiue et al., 1985). The analysis of pre-irradiation target water samples revealed low fluoride concentrations (≤0.002 ppm also under detection limit in some case) whereas in post-irradiation samples significant amounts of fluoride (≤0.58 ppm), were present.

A few of the reagents used in the synthetic procedures such as cesium hydroxide, cesium carbonate, potassium carbonate, kryptofix, acetone, triflate precursor (Berridge et al., 2009, Shiue et al., 1985, Tewson and Welch, 1980, Vaidyanathan et al., 2009) have been shown to contain fluorine-19 as impurities. Thus, chemicals and solvents of high quality should be used in the synthesis of ^{18}F-labelled radiopharmaceuticals demanding high molar activity.

2.3.2. Transfer lines as a source of carrier fluorine

Irradiated aqueous [^{18}F]fluoride is transferred from the target chamber to the synthesis apparatus via the transfer tubing. The length of the tubing can be several meters as the cyclotron vault is usually situated some distance from the radiochemistry laboratory. During this transfer, the target water comes into contact with valves, fittings and transfer tubing made of various materials. There are several commonly used types of transfer tubing e.g. polyether-ether-ketone (PEEK), polyethylene (PE), polypropylene (PP), stainless steel or different fluoropolymers such as polytetrafluoroethylene (PTFE), ethylene chlorotrifluoroethylene (Halar), ethylene tetrafluoroethylene (ETFE), fluorinated ethylene propylene (FEP) or perfluoroalkoxy (PFA) (Berridge et al., 2009, Brodack et al., 1986, Füchtner et al., 2008, Link et al., 2012, Schlyer et al., 1993). Fluoropolymers are materials that contain fluorine atoms in their chemical structures. The fluorine can totally or partly replace the hydrogen atoms in the polymer's structure (Teng, 2012).

The transfer process as a possible source of carrier fluoride has been mentioned briefly in some research papers over the years (Brodack et al., 1986, Horti and Villemagne, 2006, Kiesewetter et al., 1984, Schlyer et al., 1993). However, it is only recently that these suggestions were confirmed in controlled studies (Berridge et al., 2009, Füchtner et al., 2008, Link et al., 2012). The focus has mostly been on studying

different tubing materials in the transfer lines with only the briefest of mentions about the fittings and valves used in the transport system. As stated above, the variety of transfer line material is extensive. The fluorine containing tubing material used to transport irradiated ^{18}O-water e.g. PTFE (Füchtner et al., 2004), FEP, PFA, Halar (Link et al., 2012) has been shown to contribute significantly to the fluorine-19 amount. Radiolysis is the mechanism by which the carrier fluoride originates in the transfer process i.e. "radiation-induced release of fluoride" from plastics (Allayarov et al., 1999). The amount of carrier fluoride released seems to be dependent on the amount radioactivity (Berridge et al., 2009). It was shown that there is a threshold effect between these two parameters. When the produced [^{18}F]fluoride exceeded 15 GBq, a clear elevation was detected in the amount of carrier fluoride.

It has recommended that the tubing material should be either stainless steel (Link et al., 2012) or PP (Füchtner et al., 2004, Füchtner et al., 2008), i.e. a non-fluorinated material. It should be noted that in the study where PP tubing was found to be a better choice, this type of tubing was used in the transfer of non-irradiated ^{18}O-water whereas PTFE-tubing was used to transport irradiated ^{18}O-water. The molar activities in this case (using PTEF-tubing) varied from 43000 to 3794 GBq/μmol (Füchtner et al., 2008). This remarkable value of 43 000 GBq/μmol was achieved by rinsing the target system and cleaning the transfer lines with water after irradiation (Füchtner et al., 2008).

2.4. Pharmacokinetics of fluorine-18

Already in the 60s and 70s Blau and co-workers reported that ^{18}F-labelled sodium fluoride ([^{18}F]NaF) could be used for imaging of skeletal malignancies (Blau et al., 1962, Blau et al., 1972). The building blocks of bone are mainly type 1 collagen, bone minerals (mainly hydroxyapatite) and inorganic salts (McCann, 1953, Neuman and Neuman, 1958). Fluoride binds to hydroxyapatite where new bone is formed and is hence a good marker for bone blood flow and osteoblastic activity (Blake et al., 2001, Piert et al., 2001, Reeve et al., 1988).

The pharmacokinetics of [^{18}F]fluoride in bones and soft tissues of healthy rats have not been well documented in a comprehensive manner. Most of the previously published fluoride-18 distribution studies have been only partial or focused on the long-term exposure to fluoride (Wallace-Durbin et al., 1954, Bonner et al., 1956, Whitford 1996). However, it is important to understand the pharmacokinetics of [^{18}F]fluoride in various bones and soft tissues in a comprehensive manner when evaluating ^{18}F-labelled radiotracers which release [^{18}F]fluoride in their metabolic pathways. This also applies when [^{18}F]NaF is used in human bone or calcification studies, or in monitoring therapeutic interventions targeting metabolic, traumatic or neoplastic bone diseases in appropriate animal models.

3. Aims of the Study

The aim of this thesis was to study the factors affecting molar activity in the production of [^{18}F]fluoride and an ^{18}F-labelled compound. ^{18}F-labelling synthesis of [^{18}F]FTHA was developed according to GMP and the biodistribution of fluoride-18 in the body of rat was evaluated.

The following objectives were set for the studies included in this thesis:

1. To study the sources of stable fluoride encountered in the production of fluoride-18 and to determine the molar activity of the produced fluoride. According to the working hypothesis, the tubing used in the transfer of the aqueous [^{18}F]fluoride from the cyclotron to the synthesis device is a significant contributor of stable fluoride.

2. To develop an automated GMP-level synthesis device for nucleophilic ^{18}F-labelling. An automated device was developed that can be used for the production of [^{18}F]FTHA while fulfilling the requirements of good laboratory manufacturing practices.

3. To investigate the pharmacokinetics of [^{18}F]fluoride uptake in the compact (cortical) and cancellous (trabecular) bones and soft tissues of healthy male and female Sprague-Dawley rats using in vivo PET imaging and ex vivo organ measurements.

4. Materials and Methods

4.1. General

The radiotracers used in the studies were all prepared in the Turku PET Centre Radiopharmaceutical chemistry laboratory. [^{18}F]Fluciclatide was used a model compound in study I because both the synthesis and the quality control involving determination of the A_m are reliable and reproducible. Study II presents the process of taking a new synthesis method into use, the synthesis of [^{18}F]FTHA is used as an example. Setting up a novel synthesis procedure in Turku PET Centre sometimes involves the building of a new synthesis device instead of utilizing a commercial device. The in-house choice also involves the requirement to design a computer program that will control the synthesis device. An in-house designed synthesis device can be seen in many cases as an advantage as the device can be made to be versatile so that it is better suited for different syntheses by allowing modification to the control system, if needed. However the inconvenience in this option as compared to a commercially bought synthesis device is that the qualification has to be also performed on-site, requiring highly qualified personnel and many work-hours.

A fully automated synthesis device is very advantageous, especially in the routine production of PET radiopharmaceuticals in terms of radiation protection and reliability compared to a semiautomatic or remote-controlled device. Automated synthesis procedures are preferred in laboratories following GMP guidelines and having GMP compliant hot cells. In addition, the intervention of the operator is minimised with automated synthesis procedures, thus reducing human errors and the risk of bacterial contamination of human origin (Decristoforo, 2007).

The radioactivity of [^{18}F]fluoride and the radiotracers was measured with VDC-405 ionization chamber (Veenstra Instruments, Joure, the Netherlands). All the materials and instrumentation used in the production of radiopharmaceuticals and specific details of the transportation are described in the original papers I, II and III.

4.2. Production and molar activity of [^{18}F]fluoride

4.2.1. Production of [^{18}F]fluoride (I-III)

No-carrier added aqueous [^{18}F]fluoride for all the studies was produced by irradiating 2.2 mL of ^{18}O-enriched water (GMP grade, 98%, Rotem Industries Ltd., Medical Imaging, Dimona, Israel) in a niobium target with 17 MeV protons from a CC-18/9 cyclotron (Efremov Institute of Electrophysical Apparatuses, St. Petersburg, Russia). The beam current was 20–40 μA.

In the preparation of [^{18}F]FTHA, the produced [^{18}F]fluoride was trapped into an SPE cartridge (Sep-Pak Light cartridge, Accell Plus QMA Carbonate, Waters Corp.). The [^{18}F]fluoride was removed from the resin by elution with aqueous potassium carbonate and transferred to the synthesis device.

4.2.2. Transportation of [^{18}F]fluoride to the synthesis device (I-III)

The transportation of the [^{18}F]fluoride used as a starting material for ^{18}F-labelling synthesis was conducted via the transfer tubing connecting the cyclotron target and the synthesis device situated in the hot cell. In studies II and III, the transportation of irradiated water was undertaken using polyether ether ketone (PEEK) tubing as this has been the preferred material in Turku PET Centre.

In study I, in order to evaluate the significance of the transfer tubing material as a source of stable fluoride and therefore exerting an influence on the A_m of [^{18}F]fluoride, four different tubing materials – PEEK, polypropylene (PP), polytetrafluoroethylene (PTFE), ethylene tetrafluoroethylene (ETFE) were chosen. The lengths of the tubings were ~40 m, bore 0.75 or 1 mm. The first two of these tubing materials represented non-fluorinated polymers while the other two were fluorinated polymers. For this project, new tubing was installed between the cyclotron and a hot cell. Every tubing was rinsed before taken into use with 100 mL of ultra-pure water (UPW) and then again with 5 mL of UPW prior to each use. Collected samples, denoted as “total water boluses” (TWB), consisted of irradiated or non-irradiated oxygen-18 water batches. These batches were transported with tubing (route 1 and 3) or without polymer tubing (route 2) to the synthesis laboratory. A detailed description of the transportation routes is presented in Figure 4.

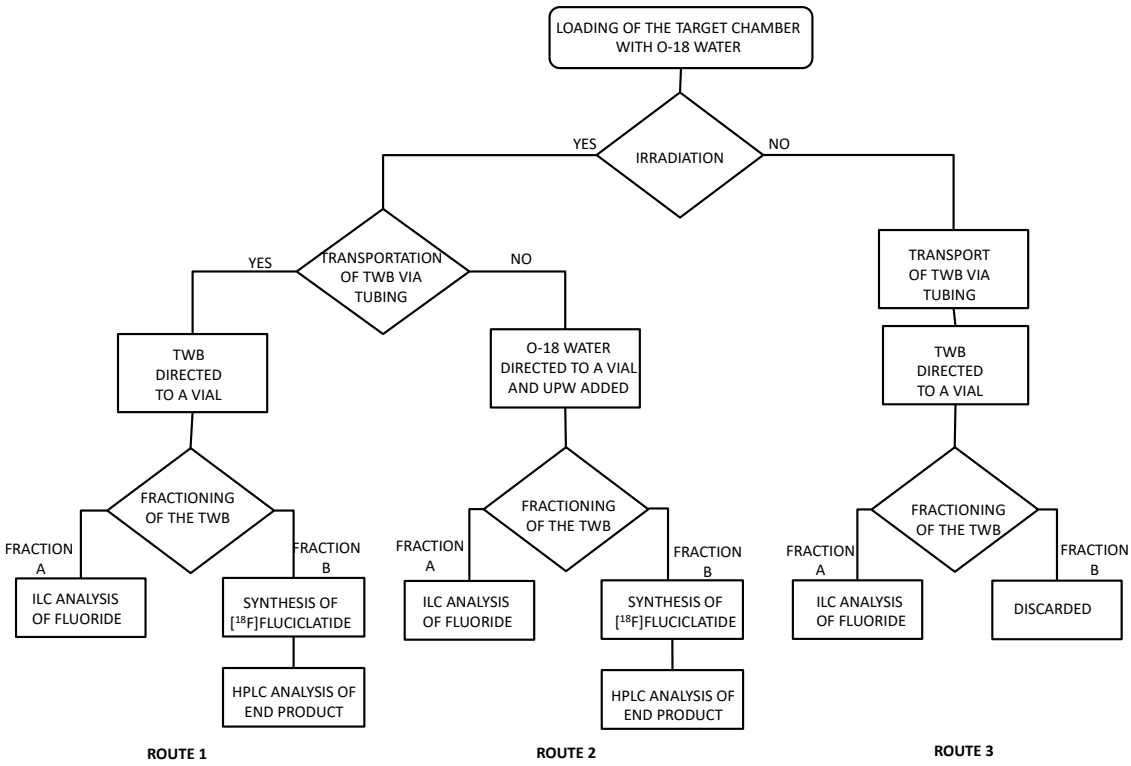


Figure 4. Flow chart of collection and processing of different fractions of target water in study I (TWB = total water bolus, UPW = ultra pure water, ILC = ion liquid chromatography).

4.2.3. Determination of [^{18}F]fluoride molar activity (I)

In study I, 45 TWBs were collected to prepared in high-density polyethylene (HDPE) vials; 19 irradiated (routes 1 and 2) and 26 non-irradiated (route 3), and divided into two fractions A and B. The equipment and methods were chosen as such to minimize the risk of fluoride contamination.

Samples that were denoted as fractions A, were measured for fluoride concentration with a suppressed anion exchange chromatography system (Ion Liquid Chromatography (ILC); Merck-Hitachi L-6000 HPLC pump, Merck-Hitachi, Darmstadt, Germany; Waters 432 conductivity detector, Waters Corporation, Milford, MA, USA), fitted with a Dionex AMMS III 4-mm Micromembrane Suppressor (Dionex Corporation, Sunnyvale, CA, USA) combined with a 2×2 inches NaI-crystal for radioactivity detection. In the anion separation, a Waters IC-PAK Anion HR (4.6×75 mm) column was used. (More detailed information on ILC analysis, see publication of study I.) These fluoride concentration results were combined with radioactivity measurement to calculate A_ms. Fractions B collected via

route 1 and 2 (non-radioactive fraction B collected from route 3 was discarded) were used to synthesize [^{18}F]fluciclatide. [^{18}F]fluciclatide was synthesized in order to verify the A_m results. The concentration of fluciclatide in the final product was determined using high-performance liquid chromatography (HPLC) for mass determination.

4.3. Synthesis of radiotracers

4.3.1. Synthesis of [^{18}F]fluciclatide (I)

[^{18}F]Fluciclatide (Fig. 5) was synthesized from fractions B collected via route 1 (n = 15) and route 2 (n = 3) with a FASTLab® synthesizer using single-use cassettes and a synthesis sequence designed for the production of this tracer (Engell et al., 2011, Engell et al., 2013). Three syntheses per tubing and no tubing, were performed. The end product contained [^{18}F]fluciclatide in phosphate-buffered saline containing ethanol (3%) and sodium-4-aminobenzoate (2 mg/mL).

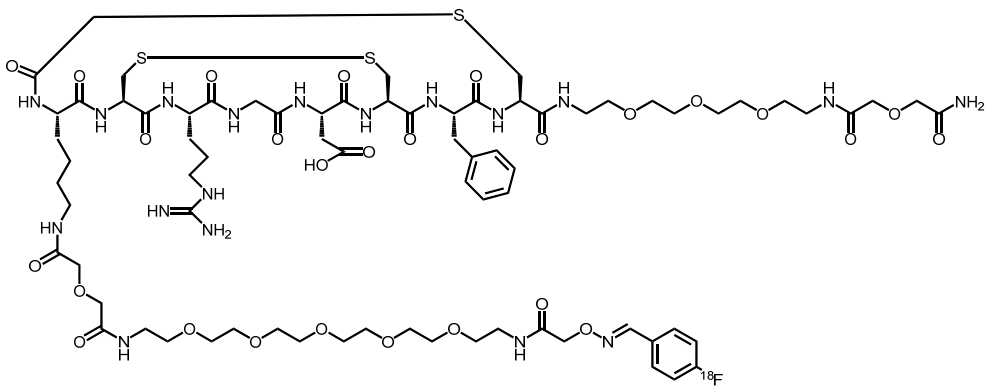
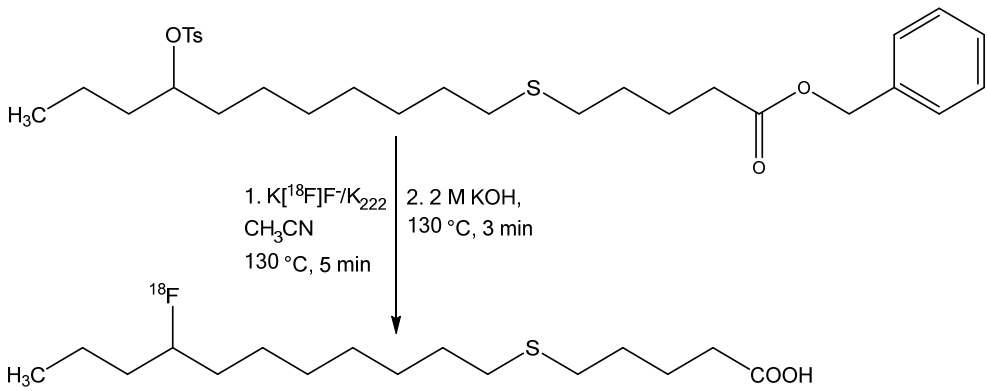


Figure 5. Molecular structure of [^{18}F]fluciclatide

4.3.2. Synthesis of [^{18}F]FTHA (II)

Synthesis of [^{18}F]FTHA was conducted with an in-house built automated synthesis device suited for nucleophilic ^{18}F-fluorination purposes. A sterile filtration unit (SFU), which was also used for the sterile integrity test, was combined into the synthesis device. The synthesis device and the SFU were placed in a hot cell situated in a grade C clean room. Both devices were controlled from a touch screen and cleaned before synthesis using in-house validated methods.

[^{18}F]FTHA was produced according to GMP guidelines set by the European Union (European Commission, 2008). Synthesis was performed by using a slightly modified method reported by DeGrado (DeGrado, 1991) (Scheme 4). The step-by-step procedure of the automated synthesis can be found in paper II. Since its publication, there have been some modifications to the synthesis procedure: the air bath of the synthesis device has been converted back to an oil bath to ensure a more stable heating process and the temperature of the heating unit has been unified throughout the synthesis and is currently set at 110 °C. In view of these modifications, the current synthesis procedure is as follows; K_2CO_3 (aq) solution containing [^{18}F]fluoride is transported to the reaction vessel containing Kryptofix 2.2.2 in CH_3CN. Kryptofix 2.2.2/[^{18}F]/K^+-complex which is heated to 110 °C using an air bath for 4 min to evaporate the water under He flow. The azeotropic distillation is repeated by adding a batch of CH_3CN and continuing heating for 7 min. Benzyl-14-(*R,S*)-tosyloxy-6-thia-heptadecanoate, precursor for [^{18}F]FTHA-synthesis, is dissolved in CH_3CN and added to the dried cryptate. The labelling reaction is conducted by heating the solution for 5 min at 110 °C to produce a fluorinated intermediate. The mixture is cooled to room temperature and CH_3CN is evaporated under He flow at room temperature. KOH is added to the residue. Hydrolysis is carried out in 110 °C for 3 min to remove the protection group of the fluorinated intermediate. Before neutralization with a mixture of CH_3COOH and HPLC mobile phase, the reaction mixture is cooled at room temperature. Hydrolysate is then injected into a semi-preparative column (Phenomenex Gemini 10µ C18 110A, 250 x 10.00 mm, Phenomenex, Torrance, CA, USA) HPLC purification (HPLC pump: Jasco PU-2089, Jasco Inc., Easton, Maryland, USA). The automatically collected end product is monitored at the outflow of the column using a GM tube. The end product is trapped in an SPE cartridge, washed with phosphate buffer-ascorbic acid mixture end eluted with ethanol followed by a phosphate buffer-ascorbic acid mixture.



Scheme 4. Labelling synthesis route of [^{18}F]FTHA

4.3.3. Synthesis of [^{18}F]NaF (III)

The [^{18}F]NaF was produced using the in-house built synthesis device. The irradiated [^{18}F]fluoride (aq) was transported to the synthesis device and collected into a syringe. The solution was passed through an anion-exchange resin (Sep-Pak Accell Light QMA cartridge, Waters Corporation, Milford, MA, USA). Before trapping, the SPE cartridge was conditioned by washing with a 700 µL NaCl (0.9 mg/mL) / 9.3 mL water solution. The trapped [^{18}F]fluoride was rinsed with 10 mL sterile water to remove contaminants and traces of irradiated water. [^{18}F]fluoride was then eluted from the SepPak with 10.0 mL NaCl-solution (9 mg/mL). This solution was formulated for injection by filtering it through a sterile filter (Millex GP 0.22 µm, EDM Millipore Billerica, MA, USA) into a sterile, pyrogen-free vial. A sterile filtration unit (SFU) combined with the synthesis device was used for this step.

4.3.4. Quality control of [^{18}F]fluciclatide, [^{18}F]FTHA and [^{18}F]NaF

The quality control (QC) tests of [^{18}F]fluciclatide was performed according to the method provided by GE Healthcare. [^{18}F]NaF and [^{18}F]FTHA were analysed by the methods in use at Turku PET Centre. The analysis of all radiotracers was conducted in an analytical HPLC connected to UV and radioactivity detectors.

In the QC of [^{18}F]fluciclatide, an Agilent Infinity 1200 series analytical HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with UV (215 nm) and radioactivity detectors was used. Phenomenex Luna C5 (5 µm, 150×2.0 mm) in series with a Guard column C5 (Phenomenex, Torrance, CA, USA) were used as the column. The column was eluted with aqueous $CH_3CO_2NH_4$ (0.05 M) and CH_3CN (75:25, v/v) at 0.2 mL/min. A_m, radiochemical purity (RCP) of [^{18}F]fluciclatide (produced from fraction B) and the concentration of fluciclatide were determined. The activity concentration of [^{18}F]fluciclatide was calculated on the basis of end product activity and volume.

In the QC of [^{18}F]FTHA, a Hitachi L-2000 series HPLC (Hitachi Ltd., Tokyo, Japan) equipped with radioactivity and UV detectors (230 nm) was used. A Phenomenex Gemini C18 110A column (250 × 4.6 mm, 10 µm) was used as the column. The QC tests consisted of identity, assay of chemical impurities, pH and RCP. In addition, the content of FTHA was determined.

In the QC of [^{18}F]NaF, a Hitachi L-2000 series HPLC equipped with UV (220 nm) and radioactivity detectors was used. A Waters IC-Pak Anion HR (4.6 × 75 mm) was used as the column. The column was eluted with $NaHCO_3$ (1.6 mM) / Na_2CO_3 (1.4 mM) at 1mL/min. The QC test consisted of checking the identity, RCP and chemical purity.

4.4. Qualification and validation

The validations in studies I and II were made according to ICH Q2(R1)-guideline (International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use, 2005) and EudraLex volume 4 - GMP guidelines, Annex 15 (European Commission, 2015).

4.4.1. Validation of the ILC analysis method for fluoride (I)

The calibration curve for the analysis of fluoride with ILC was made by diluting fluoride stock solution (1 mg/mL, prepared from solid NaF dissolved in UPW) with ILC eluent ($NaHCO_3$ (1.6 mM)/Na_2CO_3 (1.4 mM)) to an appropriate concentration. The reference standards were dissolved in the ILC eluent to minimize the void volume signal. The linearity was verified by the analysis of five concentrations (25, 50, 75, 100, and 200 ng/mL) of fluoride, a range chosen so that the highest concentration represented the expected maximal amount of fluoride which would be present in the collected fractions A. The lowest concentration represented the lower limit of the linearity range of the method.

A linear calibration curve was determined from the peak area of the fluoride reference standards as a function of concentration. The linearity of the calibration curve was determined via regression analysis and evaluated by calculating the correlation coefficient.

Repeatability and accuracy were evaluated using five concentrations of fluoride and three replicate injections. To determine the method repeatability the relative standard deviation (RSD) of the peak area was calculated. The accuracy was determined as the recovery calculated from the calibration curve compared to the known amount of fluoride.

To determine specificity the chromatograms of virgin ^{18}O-water and the ILC mobile phase were compared to the chromatograms of the irradiated fractions A. The resolution factor (R_s) between fluoride and carbonate was calculated.

4.4.2. Qualification of the synthesis device and the cleaning procedure (II)

The synthesis device built for nucleophilic ^{18}F-fluorination synthesis ([^{18}F]FTHA) and the SFU utilizing the synthesis device were qualified (Instalation Qualification IQ/Operational Qualification OQ/Performance Qualification PQ) according to the Turku PET Centre Validation Master Plan, which follows the EudraLex volume 4 - GMP guidelines, Annex 15 (European Commission, 2015). Because the synthesis device was intended to be used in the synthesis of various tracers, the cleaning method to be used between every synthesis was validated. The purpose of this

procedure was to show that the cleaning method would be efficient enough to remove all residues from previous syntheses that could affect the synthesised end product. The validation was performed by taking into consideration the concentration of the most biologically active precursor used in the device. In the validation, a cold synthesis was performed until the step involving preparative HPLC purification, after which the synthesis device was left uncleaned for 2 days. The device was then cleaned and dried according to the standard procedure using a CH_3CN/H_2O solution. The device was flushed with CH_3CN and the flush analysed by HPLC. The validation criteria were that the concentrations of residuals from precursor + end product would be lower than the limit of detection (LOD). This was then repeated two times.

4.4.3. Validation of synthesis procedure for [^{18}F]FTHA (II)

To validate that the synthesis procedure could produce [^{18}F]FTHA reliably and reproducibly, three consecutive batches of [^{18}F]FTHA were prepared according to the approved synthesis method and the QC tests. The following time points were applied for testing end product stability; end of synthesis (EOS), EOS + 120 min, and EOS + 240 min. Residual solvents, pH, and Kryptofix 2.2.2 were analysed only at EOS. To test the bioburden, one batch of [^{18}F]FTHA was produced without sterile filtration of the end product. Validated sterility and endotoxin testing were conducted by a contract research organization for all process validation batches.

4.4.4. Validation of the analytical method of [^{18}F]FTHA (II)

Validation of the HPLC method used for analysing the end product (radiochemical purity, chemical purity as a limit test, determination of radiochemical identity) was undertaken according to the guidelines of Turku PET Centre and ICH Q2(R1) (International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use, 2005). Procedures were validated for specificity, LOD and repeatability.

4.5. Preclinical studies with [^{18}F]NaF (III)

4.5.1. Experimental animals

Male (n = 23) and female (n = 18) Harlan Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were bred and housed in the Central Animal Laboratory, University of Turku, Turku, Finland. The study was approved by the

Animal Experiment Board of the Province of Southern Finland for animal experiments.

4.5.2. In vivo PET/CT imaging

Male rats (n = 5, 272 ± 26 g) were imaged in an Inveon multimodality PET/CT scanner (Siemens Medical Solutions, Knoxville, TN, USA) after being anesthetized with an isoflurane/oxygen gas mixture. A 10 min CT image was taken as an anatomical reference of the animals and for attenuation correction. Subsequently, (18.7 ± 2.4 MBq) of [^{18}F]NaF was administered intravenously following a 60 min dynamic PET scan. Volumes of interest (VOIs) were drawn over compact (cortical) bone and cancellous (trabecular) bones. Compact bones were from the tibia and radius and from the surface of flat bones. Cancellous bone samples were from tibia head and radial head. VOIs were also drawn over whole brain, heart (left ventricle), liver, kidneys, bone marrow, and bladder using Inveon Research Workplace Image Analysis software (Siemens Medical Solutions). From the VOIs, time–activity curves (TACs) were obtained and the uptake of [^{18}F]fluoride was expressed as a percentage of injected dose per millilitre of tissue (%inj.dose/mL).

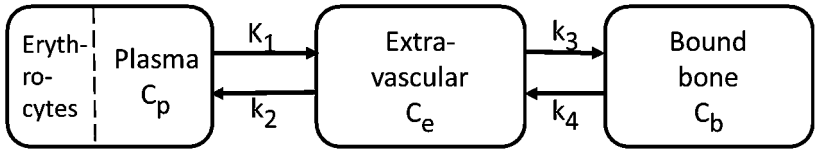


Figure 6. Three-compartment model of [^{18}F]fluoride ion kinetics in bone. C_p, C_e and C_b refer to the plasma, extravascular and bound bone compartments. K_1, k_2, k_3 and k_4 are rate constants describing the exchanges between compartments (Hawkins et al., 1992).

The skeletal [^{18}F]fluoride ion kinetics (bone perfusion (K_1), the net plasma clearance of [^{18}F]fluoride to bone (K_i) reflecting regional bone turnover, the rate constants k_3, and the ratio between K_i/K_1 describing the unidirectional extraction efficiency to bone minerals was calculated using a three-compartment model and the plasma input function was derived from the left ventricle cavity of the heart (Fig. 6).

4.5.3. Ex vivo biodistribution studies

[^{18}F]NaF was injected via a tail vein into male (n = 18, 32.3 ± 6.4 MBq) and female rats (n = 18, 32.2 ± 4.5 MBq) under a brief isoflurane/oxygen anesthesia. There was an age difference between male (55 ± 16 d) and female (112 ± 6 d) rats but their body

weights (male; 286 ± 8 g, and female; 260 ± 25 g) were nearly the same. The animals (n = 3/time point) were sacrificed at 6 different time points (15, 30, 60, 120, 240 or 360 min) after injection. Blood, urine, and organs of interest were immediately removed, weighed, and measured for ^{18}F-radioactivity in a NaI(Tl) well counter (3 × 3-inch, Bicron, Newbury, USA). The uptake of ^{18}F-radioactivity was expressed as a percentage of the injected dose per gram of tissue (%inj.dose/g).

4.6. Statistical analyses

All values were calculated as an average and uncertainties expressed as standard deviation (SD) when $n \geq 3$.

In study I, statistical analyses were performed with GraphPad Prism 6.0 (San Diego, CA, USA). Changes in the fluciclatide concentration between non-fluorinated and no tubing were compared using one-way ANOVA. Differences between PTFE and ETFE, non-fluorinated vs. fluorinated tubing materials, and non-fluorinated vs. no-tubing were compared using unpaired t-tests.

In study III, the analyses were made with SAS software, version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). The area under curve (AUC) and maximum values for bone time-activity curves, and the K_1, K_i, K_1/K_i values were compared to other bone in the same rat, therefore the analysis was done using repeated measures techniques, where bone is a repeated factor (hierarchical linear mixed model). While overall differences were detected between the bones, pairwise comparisons were made between the bones. Urinary excretion of radioactivity in females and males was analysed using two way analysis of variance (ANOVA), using time and gender as explanatory variables (at each time point different rat was measured). A P-value less than 0.05 (two-tailed) was considered as significant.

5. Results

5.1. Validation

5.1.1. Validation of the ILC method (I)

The ILC method used to analyse fluoride was validated with the following results recorded:

- Specificity: the method distinguished the fluoride from carbonate; the resolution factor is 1.5
- Linearity: the method was linear in the range 25-200 ng/mL
- Limit of detection (LOD): 6.1 ng/mL
- Limit of quantitation (LOQ): 18.4 ng/mL
- Repeatability: 5 concentrations (25, 50, 75, 100, 200 ng/mL) were analysed and the corresponding RSD values were 6.67%, 7.62%, 6.17%, 4.86%, 0.77%
- Accuracy: 5 concentrations were tested and mean of yield-% were: 25 ng/mL – 90%; 50 ng/mL – 102%; 75 ng/mL - 99%; 100 ng/mL - 104%; 200 ng/mL – 99%

The calibration curve determined for fluoride is shown in Figure 7. The mobile phase did not cause any peaks that would interfere with the analysis. Fluoride eluted very close to the water dip occurring in this analytical method, making analysis in some cases challenging (Fig. 8). The fluoride peak was identified by analysing a sample of irradiated ^{18}O-water and by comparing the retention time from the radioactive detector with the retention time from the conductivity detector using a reference standard.

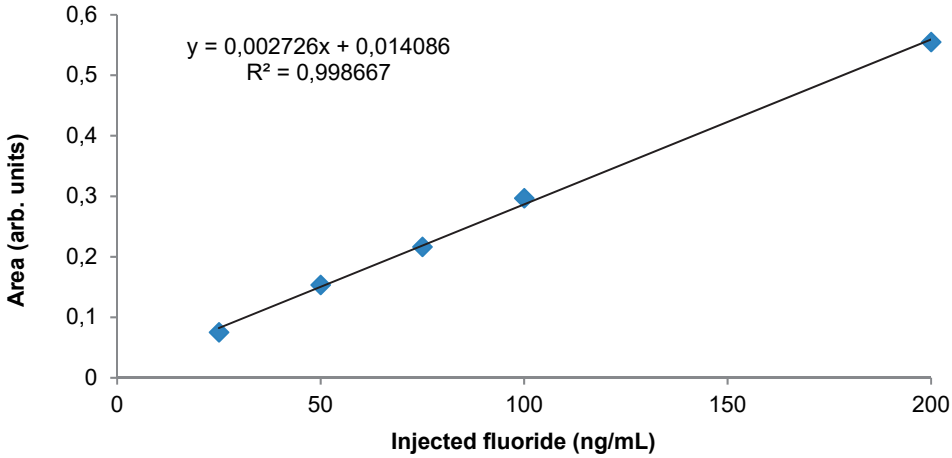


Figure 7. Calibration curve for fluoride. The linear regression parameters are shown.

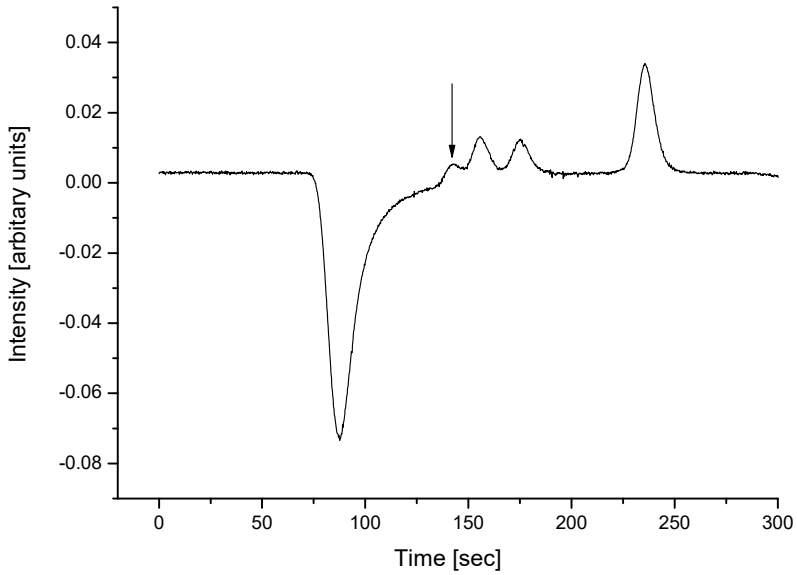


Figure 8. Representative ILC chromatogram of fluoride analysis when irradiated water was transported via PP tubing. The water dip and three sequential peaks are shown; fluoride is the first peak (arrow).

5.1.2. Qualification and validation in study II

Qualification of the synthesis device designed and built for [^{18}F]FTHA synthesis was successful. The following validation results for the HPLC method to analyse [^{18}F]FTHA were obtained:

- Specificity in UV detector: HPLC distinguished FTHA from the precursor and from two unknown impurities (Fig. 9). R_s´s for these peaks were 1.8, 3.7, and 1.8, respectively. For one impurity peak (apparently a decomposition product of FTHA), the $R_s = 0.82$ was under the acceptance limit $R_s = 1.5$, but the FTHA peak could be integrated reliably. No peaks resulted from either buffer or blank solution. HPLC could distinguish FTHA from the crude product's two unknown impurity peaks with the R_s of 5.4 and 1.9, respectively.
- Specificity when using radioactivity detector: HPLC distinguished [^{18}F]FTHA from the closest impurity peak appearing in the crude product. The R_s for this peak was 1.8. HPLC distinguished [^{18}F]FTHA from 1-2 other impurities seen in the chromatogram of the end product. The R_s was 8.7 to the nearest peak.
- LOD: 0.025 mg/mL.
- Repeatability when using UV detector: Reference (0.5 mg/mL) was injected 6 times. Relative standard deviation (RSD) for these injections was 2%.

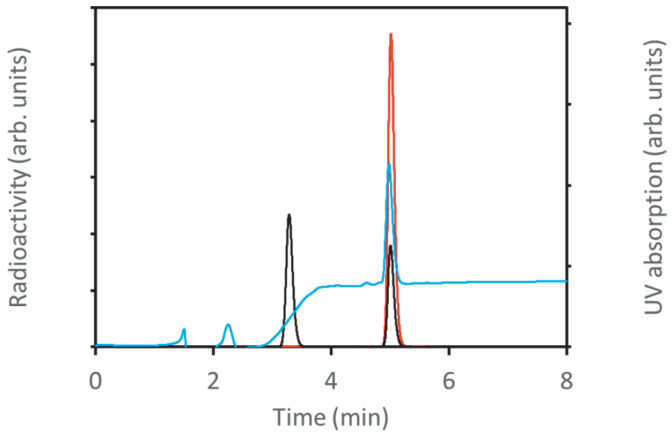


Figure 9. Representative chromatograms of FTHA, [^{18}F]FTHA and oxidative product of [^{18}F]FTHA. UV chromatogram of a FTHA reference, blue curve. Red curve, 14-(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA); black curve, oxidative products of [^{18}F]FTHA.

The NaI radiodetectors attached to the chromatographs were used for relative determinations and not for quantitation. Their linear range (5%) was determined separately. For quantitation of radioactivity isotope calibrators were used. These are calibrated regularly for linearity and quantitation according to in-house SOPs.

Four [^{18}F]FTHA syntheses were made to validate the synthesis procedure of [^{18}F]FTHA. All tests included in the validation (for specific results, see publication of study II) were within the specification limits. Radiochemical and chemical stability (i.e. shelf life) of the formulated product were determined to be 4 hours.

5.2. Molar activity of [^{18}F]fluoride and [^{18}F]fluciclatide

5.2.1. ILC analysis of [^{18}F]fluoride

In study I, 37 fractions A transported via different tubing materials were successfully analysed from the 45 fractions collected. In eight fractions analyses failed due to various reasons. Three samples per fraction were analysed by ILC for fluoride concentration. Some factors made the analysis of fluoride challenging at very low concentrations. Irradiated samples contained small organic acids (mainly acetate, formate or lactate) that eluted close to the fluoride peak. When using the carbonate-based eluent, a water dip appeared just before fluoride eluted. In some samples, the concentration of fluoride was even below the LOD. In these cases, repeated sample injections occasionally revealed some poor precision of the method, see "range" column in Table 6. The method was not linear below a concentration of 25 ng/mL. The results for the analysis of fraction A are presented in Table 6.

Table 6. Fluoride concentration in fractions A when using different tubing materials. Successfully collected fractions were analysed in triplicate by ILC. LOD = 6.1 ng/mL.

		Fraction A				
		analysed	injections	F^- concentration (ng/mL)		
Material	Sample	(vs. collected)	altogether	range	mean	SD
PEEK (1mm)	Non-irradiated	5(6)	15	3.0-12	6.6	2.4
	Irradiated	0(3)	0	-	-	-
PEEK 1	Non-irradiated	5(5)	15	6.0-10	8.4	1.3
	Irradiated	2(4)	6	22-65	41	20
PP 1	Non-irradiated	5(5)	15	1.0-95	24	36
	Irradiated	3(3)	7	19-54	35	19
PTFE 1	Non-irradiated	5(5)	15	2.6-32	14	10
	Irradiated	3(3)	9	53-70	62	6.7
ETFE 1	Non-irradiated	5(5)	14	1.8-21	8.8	6.4
	Irradiated	3(3)	8	69-120	95	18
No tubing	Irradiated	1(3)	3	6.8-12	9.0	2.8

5.2.2. HPLC analysis of [^{18}F]fluciclatide

The fractions B in study I were used to produce [^{18}F]fluciclatide. The identity of the end product was determined by HPLC by co-elution with a cold reference standard (same retention time for radioactive and UV peak).

Results show that the A_m of [^{18}F]fluciclatide was lower when PTFE was used as transport tubing as compared to PEEK tubing (Fig. 10). Concentration of fluciclatide was higher using fluorinated transport tubing compared to using non-fluorinated tubing. A significant difference was present using no tubing as compared to the utilization of non-fluorinated tubing, i.e. the latter showing a higher concentration. However, there was no significant difference between PTFE and ETFE tubing (Fig. 11).

The RCP of [^{18}F]fluciclatide was more than 97.7% in every synthesis, and the end volume was ~40 mL.

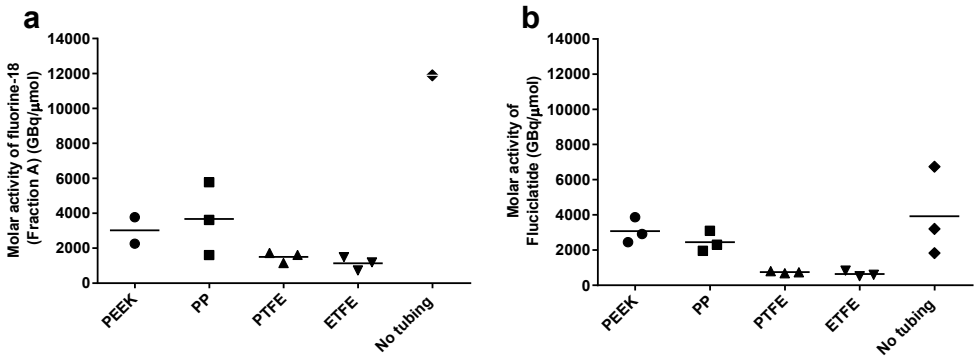


Figure 10. Molar activities as individual values and means for a) fluoride and b) fluciclatide as a function of transport tubing material. The tubing PEEK (1 mm) is omitted as no data are available for fluoride with this tubing.

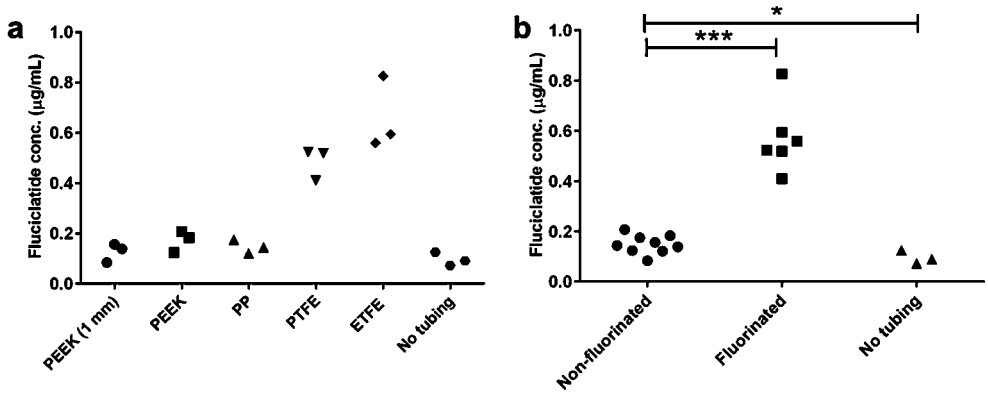


Figure 11. Fluciclatide concentration as a function of tubing material analysed by HPLC. Sample size, 3 per tubing material. a) Concentrations as a function of tubing materials b) Concentration as a function of non-fluorinated (PEEK, PP) and fluorinated (PTFE, ETFE) tubing materials; ***$p < 0.0001$. Concentrations between non-fluorinated (PEEK, PP) and no tubing; *$p < 0.05$.

5.2.3. Comparison of analytical methods

Molar activity and concentration for the fluoride and fluciclatide determined with ILC and HPLC respectively are presented in Figures 10, 11 and Tables 6, 7. The values were corrected to the time point EOB+60 min. A_m could not be determined in every case because in some fractions A the mass of fluoride was too low. It can be seen from the results that the A_m of the [^{18}F]fluoride was higher than the A_m of the [^{18}F]fluciclatide. There was a good ($y = 0.533x + 190$, $R^2 = 0.839$) linear correlation

between the A_m of fluoride versus A_m of fluciclatide. As the slope of the equation is < 1, it means that additional fluorine-19 is introduced in the preparation during the labelling process of [^{18}F]fluciclatide (Table 7 and Study I, Fig.6).

Table 7. Individual values for A_m's of fluoride and fluciclatide, and concentration of fluciclatide for each run.

Transfer tube	Run no.	A_m (GBq/μmol) at EOB + 60 min		Concentration of fluciclatide (μg/mL)
		Fraction A	Fluciclatide	
PEEK (1mm)	1	-	3350	0.14
	2	-	4240	0.16
	3	-	7130	0.08
PEEK1	1	3780	2450	0.18
	2	-	3870	0.12
	3	2260	2920	0.21
PP1	1	3620	590	0.12
	2	1610	1960	0.18
	3	5780	3100	0.14
PTFE1	1	1730	805	0.52
	2	1620	745	0.41
	3	1150	691	0.52
ETFE1	1	1490	827	0.59
	2	721	515	0.56
	3	1190	590	0.83
No tubing	1	11900	6740	0.07
	2	-	1830	0.09
	3	-	3210	0.13

5.3. Preparation of [^{18}F]FTHA

The synthesis procedure of [^{18}F]FTHA consisted of initial nucleophilic labelling reaction of the precursor containing protector groups, deprotection of the intermediate, preparative HPLC purification of the product, separation of HPLC eluent constituents from the product using SPE and formulation of the product for injection. The duration and temperature of the nucleophilic labelling reaction (5 min, 110 °C) and hydrolysis (3 min, 110 °C) of the protected intermediate have been optimized throughout the years we have prepared [^{18}F]FTHA. The preparative HPLC separation of the hydrolysate was changed to an SPE purification since this is more user-friendly as compared to the previously-used evaporation of HPLC solvent. Human serum albumin that interferes with the QC-tests, was replaced with a phosphate buffer-ascorbic acid mixture in the final formulation solution.

The results from successful clinical productions (n = 238) can be summarized as follows. The synthesis time was approximately 1 h. The synthesis results presented here refer to a longer period of time (01/2011-09/2018) than can be found in the

publication II (2011-2016). The RCY, based on the initial activity of [^{18}F]fluoride (6 - 40 GBq at EOB), was 13 ± 6.2% (EOB). The activity of [^{18}F]FTHA was 1.7 ± 0.8 GBq (range 0.03 to 4.9 GBq at EOS). RCP was better than 95.0% in all syntheses.

5.4. Preparation of [^{18}F]NaF

The [^{18}F]NaF was produced using a fully automated synthesis device. The synthesis was straightforward and the duration of the process took only 10 min. All batches fulfilled the specifications, i.e. appearance, identity, pH, sterility, bacterial endotoxins and RCP. The RCP exceeded 98.5% in every batch.

5.5. Animal studies

PET/CT-studies of the rats revealed that shortly after the injection of [^{18}F]NaF, the radioactivity was cleared rapidly from blood (Fig. 12a) with a high uptake being detected in kidneys. Subsequently, the excretion to the bladder increased (Fig. 12b).

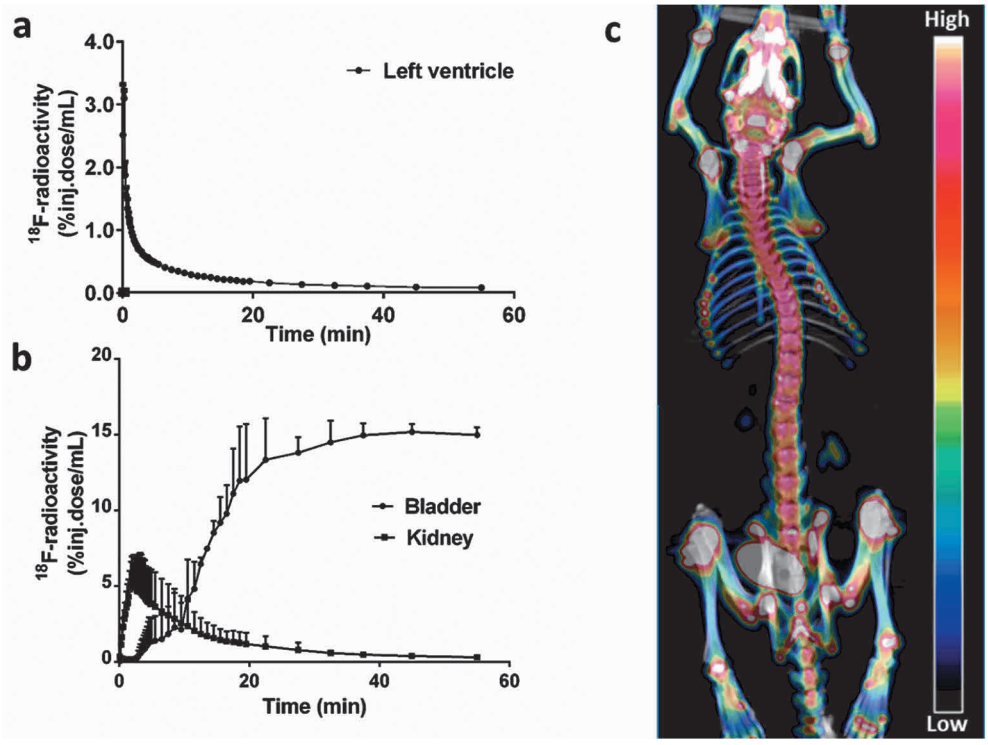


Figure 12. *In vivo* PET/CT results. a) Uptake in the left ventricle cavity exhibit the concentration of [^{18}F]fluoride in blood. b) Uptake in kidney and bladder shows the excretion of [^{18}F]fluoride via the urinary tract. c) Fused anterior 3D PET/CT image of in vivo biodistribution of [^{18}F]fluoride in as rat for the period of 20 - 50 min after [^{18}F]NaF injection.

A highest uptake of radioactivity among all measured bones could be observed in trabecular bone such as in tibia head ($p < 0.002$), and in mandible, lumbar vertebrae, and in parts of the pelvis. In the costochondral joints, the uptake was higher ($p = 0.0005$) than in other parts of the ribs. The lowest uptake was seen in compact bones, tibia, radius, rib and parietal bone (Fig. 12c and 13). The bone uptake measured *in vivo* correlated ($R^2 = 0.84$) with the ex vivo measurements (Fig. 14).

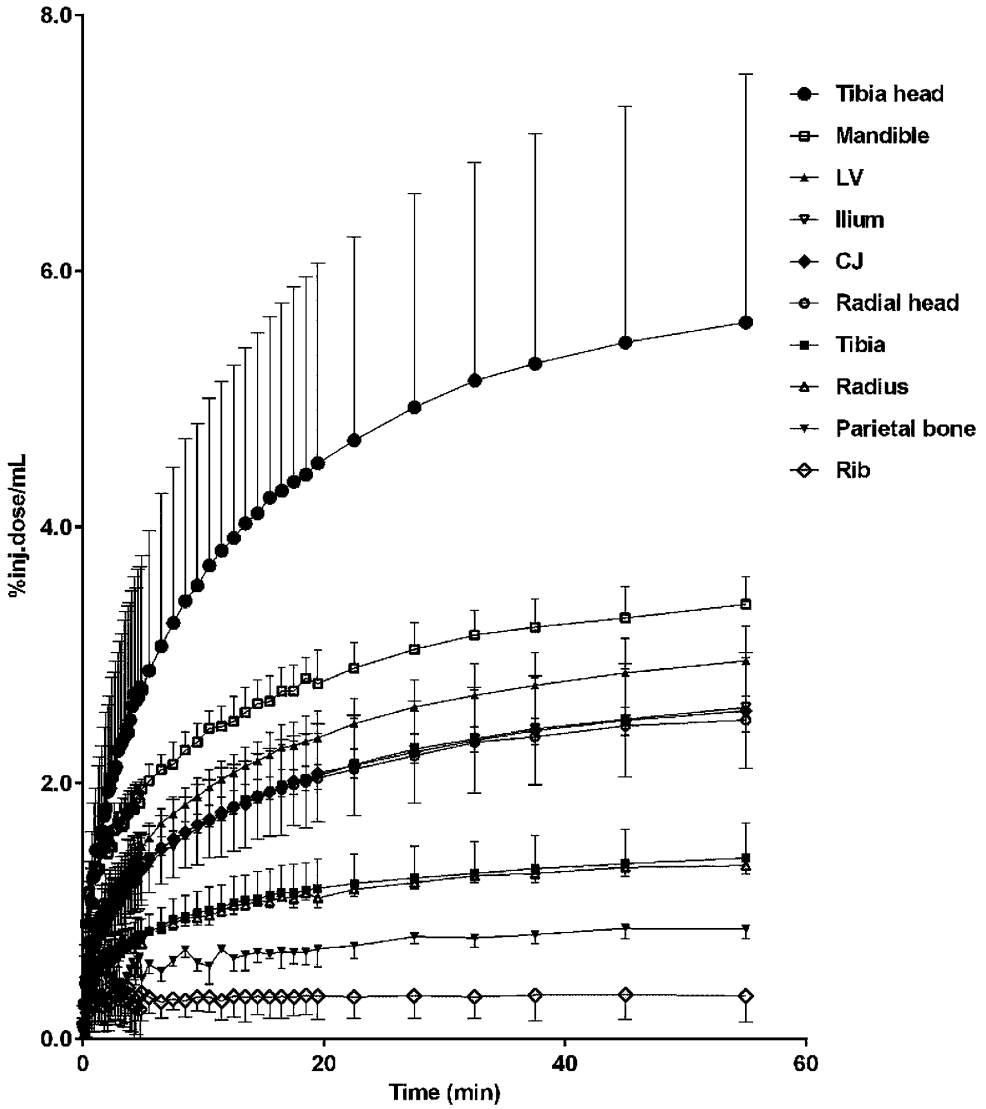


Figure 13. *In vivo* uptake (%inj.dose/mL) of [^{18}F]fluoride in various bone types. Values are from 3-5 rats and expressed as mean ± SD. LV = lumbar vertebrae, CJ = costochondral joint.

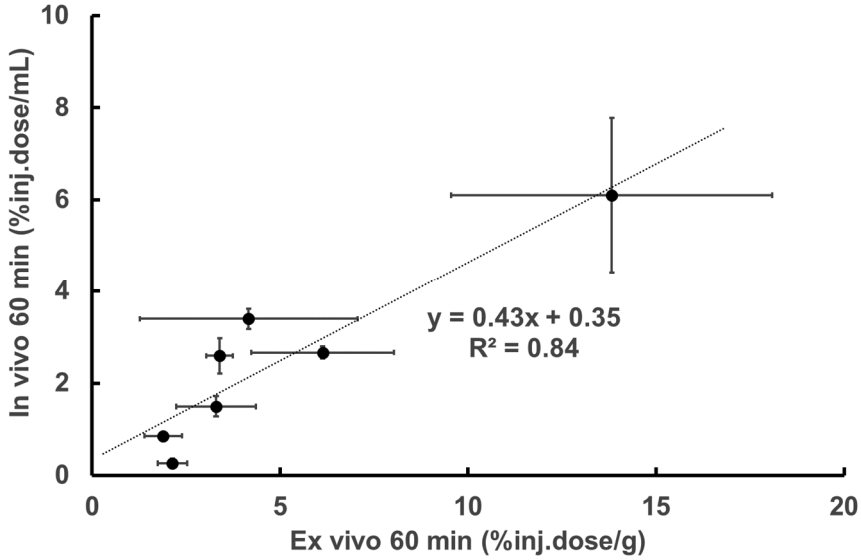


Figure 14. Correlation between *in vivo* 60 min (%inj.dose/mL) and *ex vivo* 60 min (%inj.dose/g) measurements of [^{18}F]fluoride uptake into parietal bone.

The mean bone perfusion (K_1) was higher in the tibia head ($p < 0.0001$) and the mandible ($p < 0.001$) and lower in the rib ($p < 0.01$) as compared to the other measured bones (Fig. 15a). The osteoblastic activity (K_i), was higher ($p \leq 0.001$) in tibia head compared to other bones, higher in mandible ($p < 0.001$ than in radius, parietal bone and rib, and ($p < 0.05$) in costochondral joint, radial head, and tibia. K_i values were also higher in lumbar vertebrae ($p < 0.001$) than in tibia, radius, parietal bone and rib, and ($p < 0.05$) in ilium, costochondral joint and radial head. The unidirectional extraction efficiency (K_i/K_1) to bone mineral was significantly lower ($p < 0.03$) in the radius, parietal bone and rib than in other measured bones (Fig. 15c). The rate constant from extravascular compartment to the bone bound compartment (k_3) was similar for all measured bones (Fig. 15d).

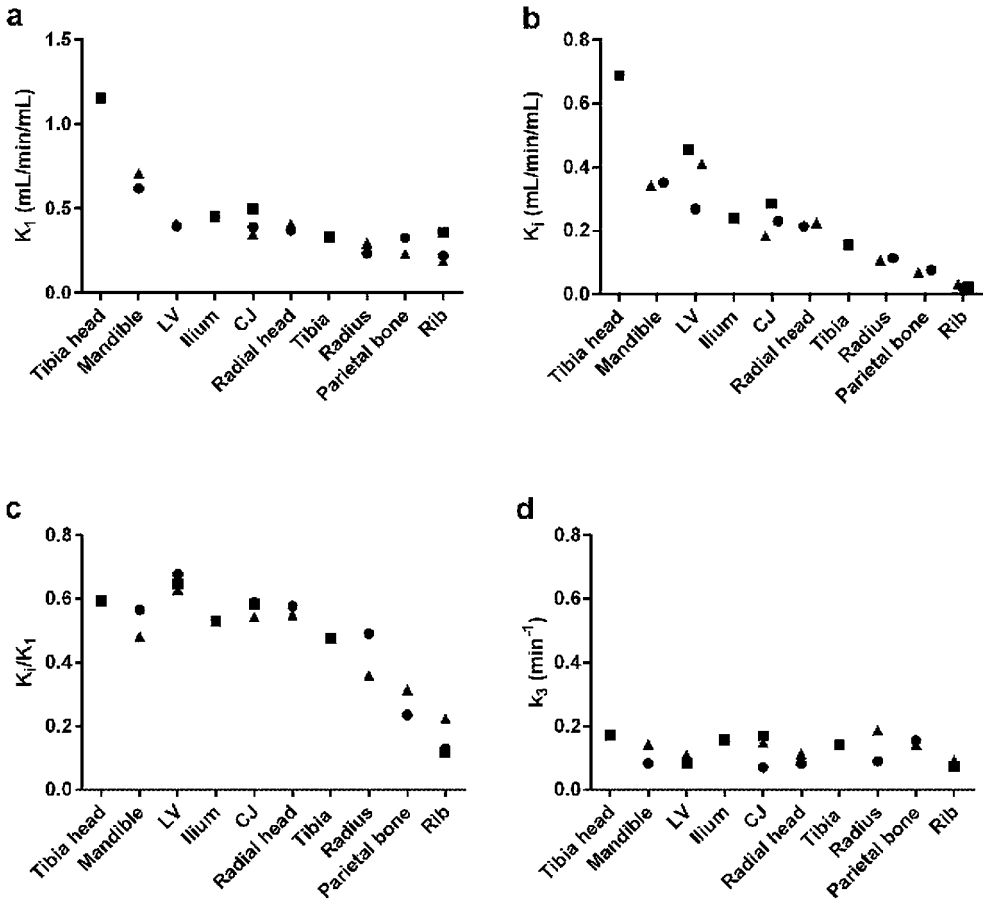


Figure 15. Kinetic values (K_1, K_i, K_i/K_1 and k_3) calculated using a three-compartment model of [^{18}F]fluoride uptake for different rat bones. a) Bone perfusion, K_1, b) bone osteoblastic activity, K_i, c) efficiency of unidirectional extraction from blood to bone, K_i/K_1, and d) rate constant from the extravascular compartment to the bone bound compartment. LV = lumbar vertebrae, CJ = costochondral joint.

The uptake in the different organs was statistically equivalent for males and females, and hence the ex vivo results were pooled together except for organs of the reproductive systems. The results from the *ex vivo* biodistribution studies confirmed that [^{18}F]fluoride was rapidly (Fig. 12a) cleared from blood and about 47 ± 4% of radioactivity in blood was present in erythrocytes. The amount remained similar for 4-6 h. The uptake of [^{18}F]fluoride in plasma proteins was insignificant. The uptake of [^{18}F]fluoride (%inj.dose/g) in different soft tissues was similar and the amount of activity seemed to follow the activity in blood (Fig. 16).

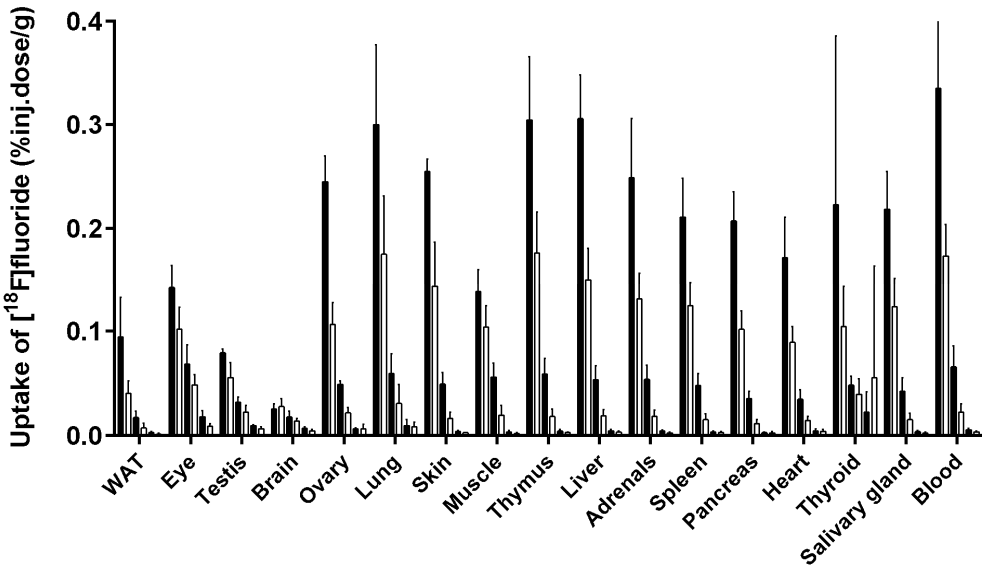


Figure 16. Biodistribution of [^{18}F]fluoride in rat soft tissues at 15, 30, 60, 120, 240 and 360 min after injection of [^{18}F]NaF. Values (n = 6/time group, except n=3/time group for testis and ovaries) represent the mean value as % of the injected dose/g tissue (%inj.dose/g) and SD. WAT = white adipose tissue.

The calculated organ-to-blood uptake ratios in most soft tissues studied were basically the same, meaning that the clearance rate of [^{18}F]fluoride resembled that in blood. However, the organ-to-blood ratios calculated for eyes, brain, lungs, testes and ovaries exhibited increased uptake of [^{18}F]fluoride over the measured 6 h time course (Fig. 17).

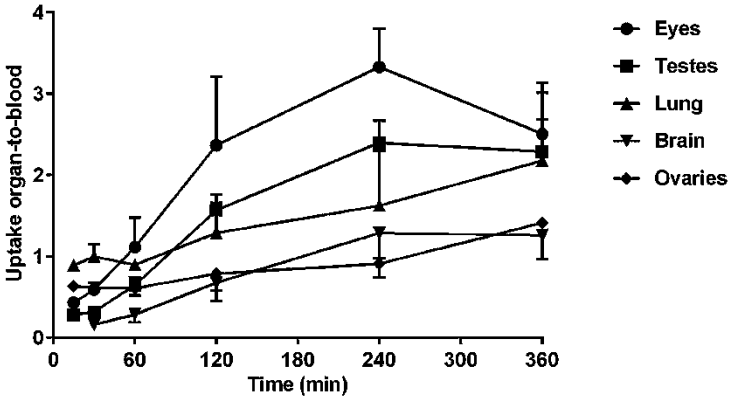


Figure 17. *Ex vivo* organ-to-blood ratios of [^{18}F]fluoride at 15, 30, 60, 120, 240, and 360 min after injection. Ratios increase in the eyes, testes, lung, brain, and ovaries.

The urinary tract was the main route of excretion of the tracer. The amount of total excreted radioactivity in urine between female and male rats was measured. Notably, the females had more rapid excretion than males (p = 0.0001) (Fig. 18a). In addition [^{18}F]fluoride was found in the contents of the small intestine until 120 min after tracer injection; in the contents of the large intestine, it appeared more slowly with the highest value found at the last time point 360 min (Fig. 18b).

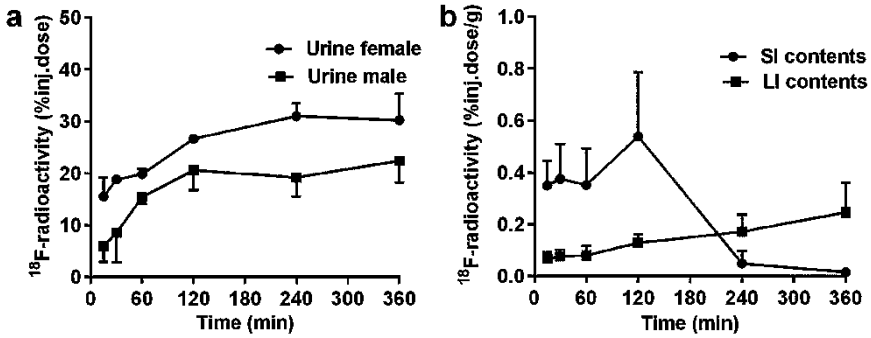


Figure 18. *Ex vivo* uptake of [^{18}F]fluoride at 15, 30, 60, 120, 240, and 360 min after tracer injection. (a) The total amount of [^{18}F]fluoride excreted in the urine as % of injected dose, presented separately for males (n = 3) and females (n = 3) showing faster [^{18}F]fluoride excretion for females (p = 0.0001). (b) Uptake in the small intestine (SI), and large intestine (LI) as %inj.dose/g (n = 6/time point). Results are averages ± SD at each time point.

6. Discussion

The idea of this thesis has been to present the whole production process of ^{18}F-labelled radiopharmaceuticals emphasis on different important aspects in the process. The molar activity is an important parameter that should not be overlooked in the preparation of radiopharmaceuticals. While producing fluoride-18, the sources of stable fluorine-19 should be minimized in the production process. The study I shows that the tubing used in the transport should be made out of non-fluorinated material to decrease the amount of stable fluorine and increase the A_m. The study II gives a good overview on taking a new radiotracer into a use. This requires the qualification of the synthesis device, validation of the synthesis process and the analysis method used in the quality control. The experience we have gained over the years on producing [^{18}F]FTHA for clinical use is of importance. The last step in production process is the use of the radiotracer. The study III gives new information on the pharmacokinetics of [^{18}F]fluoride in bone. In the following subchapters the results in all three studies are discussed in detail.

6.1. Qualification and validation

Validation is defined as "the action of providing that any procedure, process, equipment, material, activity or system actually leads to expected results, with the aim to contribute to guarantee the quality of a radiopharmaceutical" (Todde et al., 2017). Qualification, compared to validation, can be viewed as a more practical set of procedures that are aimed to show that the system has been acceptably installed, works as expected and leads to results that are wanted (Todde et al., 2017). In recent years, validation has increasingly become an essential part in the preparation of PET radiopharmaceuticals e.g. when starting the production of a new tracer or initiating a new QC analysis method. It is vital to ensure the safety of the PET radiopharmaceutical to the patient, and in order to maintain the high quality of these products, the quality has to be "built into the process". It is essential for everyone taking part in the production of radiopharmaceuticals to understand that quality is not something that can be added to the finished product nor can it be guaranteed simply by end-product testing (FDA, 2011). For example, the sterility analysis of the end product is done in many cases after the patient has been injected with the

radiopharmaceutical so the process itself has to be such that the outcome will always be a sterile end product.

The regulations and guidelines on validation are diverse as they have been published by many parties (authorities and international organisations). The most important document for those working in the European Union preparing radiopharmaceuticals is the Annex 15 "Qualification and validation" in the Eudralex Volume 4 (European Commission, 2015).

The validation of the ILC method in study I revealed that the method was not linear when the concentration of fluoride was less than 25 ng/mL, which was considered as slightly problematic in some cases as the fluoride concentration in total water bolus samples was very low. Despite this limitation, the method was assessed as being adequate for fluoride analysis as the LOQ was 18.4 ng/mL and LOD was 6.1 ng/mL. The RSD values for the repeatability measurements of the 3 lowest concentrations (25, 50, 75 ng/mL) were less than 10%, whereas with the two highest concentrations (100, 200 ng/mL) RSD-values were less than 5%. Results for accuracy measurements, specification 95% ≤ mean ≤ 105%, were acceptable with the four highest concentrations but rejected at a concentration of 25 ng/mL (90%). Despite these failings in the validation, the method was considered to be sufficiently reliable for the analysis of fluoride samples. Further development of ILC will most probably bring increased sensitivity, e.g. the hydroxide-eluent method developed by Thermo Fisher (Hydroxide-Selective Anion-Exchange IC, Thermo Fisher Scientific)

In study II, the assessment of the synthesis device used for [^{18}F]FTHA-synthesis was successful. During the development of the synthesis method, [^{18}F]FTHA was found to decompose by radiolysis. To prevent this phenomenon, ascorbic acid was added to the formulation solution. After the incorporation of these changes, the analytical HPLC method and the synthesis method were validated. The process validation batches fulfilled the product specifications and the process was found to be appropriate for the production of [^{18}F]FTHA.

6.2. Considerations on molar activity

The preparation of PET radiopharmaceuticals is a process consisting of many sequential steps that start with the production of the radioactive label in a cyclotron. In the case of [^{18}F]fluoride, this is done by bombarding ^{18}O-enriched water with protons. An aqueous solution of [^{18}F]fluoride is obtained and the water is transported via a system consisting of tubing and valves made of different materials so that it can be used in the next step i.e. the synthesis of the labelled compound. During this transportation process, the irradiated aqueous fluoride can interact with the materials through which it passes and with which it is in contact. Certain processes like radiolytic degradation can exert an unfavourable influence on the

molar activity. Before embarking on the synthesis of a radiotracer, a given amount of radioactivity with sufficient molar activity is needed. According to literature molar activity is considered high when it exceeds 37 GBq/μmol (1000 Ci/mmol) (Lapi and Welch, 2013). This value is low, as very potent tracers can cause partial occupancy for e.g. receptors and transporter systems at this level of A_m. Careful consideration should also be given to animal experimentation, when in most cases the injected radioactivity doses are 10-100 times higher per mass of the subject as compared to human doses (Keller et al., 2019).

In study I, the effect of the transport material to the molar activity was examined. Fluoride concentrations were analysed in irradiated and non-irradiated water samples transported via fluorinated (PTFE, ETFE) and non-fluorinated (PEEK, PP) tubing or using no tubing at all. The working hypothesis was that the fluorinated materials (PTFE, ETFE) would be a source of stable fluoride. Nonetheless, it should also be realized that the manufacturing process of a non-fluorinated material, PEEK, consists of polymerization of a fluoride-containing compound. During the process, fluorine is eliminated but the possibility exists of contamination of the polymer with fluoride.

The results in Table 6 show that there was a significant difference in fluoride concentrations between the irradiated and non-irradiated fractions for PEEK ($p = 0.0133$), PTFE ($p = 0.0005$) and ETFE ($p = 0.0002$) indicating that ionizing radiation had interacted with the materials used in the transport system of target water. The radioactivity-induced processes that lead to release of fluorine-19 are not known in detail, but most probably include radical formation induced by beta- and gamma radiation. Analysis of the fluoride concentrations after transport in tubing made from PEEK or PP or alternatively PTFE or ETFE showed that there were no significant differences within the groups of fluorinated or non-fluorinated materials.

The A_m of the [^{18}F]fluoride was compared with the A_m of [^{18}F]fluciclatide. The result showed that the A_m of [^{18}F]fluoride was higher than that of [^{18}F]fluciclatide indicating that stable fluoride had also been introduced during the labelling synthesis.

We have now been using PP tubing as the transport line material for 5 years in the production of ^{18}F-labelled radiotracers and in our experience, the PP tubing is better than the PEEK tubing. The transport of material proceeds smoothly and the tubing has to be changed less frequently. This is a good indication that the matter has to be taken into account when selecting the tube material.

Even though a clear effect of fluorine-19 contamination from the fluorinated tubing was seen, the A_m's as such were still relatively high. Study I was carried out using initially virgin tubing and using the tubing only for the experiments described. An important limitation of the study presented in paper I is that long term exposure of tubing to radioactivity is not addressed. Especially for the fluorinated tubing it

would be very interesting to follow long term behaviour of A_m of fluoride as a function of exposure to fluoride-18. Hypothetically, the fluorine-19 release from the tubing are expected to cumulatively increase as a function of radiation exposure.

6.3. Synthesis of [^{18}F]FTHA

During the time period 01/2011-09/2018, we have made 256 preparations of [^{18}F]FTHA, 93% of which have been successful. In most cases, leaks in the ethanol vial or in the collection vial situated before the SFU or an insufficient amount of precursor or Kryptofix 2.2.2 have been the reasons for an unsuccessful synthesis. A total of 185 syntheses were done for clinical use (265 patients) with the rest being utilized for tests, QC purposes or preclinical studies. The synthesis as a whole takes approximately 1 h.

In the last eight years that we have produced [^{18}F]FTHA, we have made ten controlled changes to the synthesis process of [^{18}F]FTHA and documented them according to the GMP regulations followed in Turku PET Centre. Synthesis automation (opening and closing valves according to certain patterns, based on information received from transducers) has been the major reason for these changes. The rest of the changes concerned materials used in the synthesis or in the equipment, a specification change in the end product with also two changes being made to the actual synthesis device. An oil bath which had been initially used to heat the reaction vessel was changed to an easier to control air bath. The air bath worked well for many years, but last year there was a drop in the [^{18}F]FTHA yield due to a malfunction in the air bath. As a result of these synthesis difficulties, the oil bath was reintroduced into the synthesis device and the synthesis yield returned to the normal level.

6.4. Animal studies with [^{18}F]NaF

The results of Study III are important to both clinical and experimental settings. The findings on the pharmacokinetic properties of [^{18}F]fluoride in different bones as a function of time help to understand the behaviour of [^{18}F]fluoride in different clinical applications. Also the results of free [^{18}F]fluoride uptake in soft tissues improve our understanding if ^{18}F-labeled tracers defluorinate; this information might also be important on kinetic modeling approaches.

7. Conclusions

The major conclusions of the work presented in this thesis are as follows:

- The utilization of a fluorinated material, such as PTFE or ETFE, as the tubing to transport [^{18}F]fluoride from the cyclotron target chamber to a synthesis device is not recommended, especially if a high molar activity is desired. The interaction between ionizing radiation and the tubing material releases stable fluoride from the walls of the tubing, diminishing the molar activity of [^{18}F]fluoride. Thus, it is recommended to utilize non-fluorinated material, such as PEEK or PP, as the transport line material. In our experience, PP is the preferable tubing material due to its better transport properties and minor maintenance requirements.
- An automated synthesis device including a control system was constructed for use in a nucleophilic multistep synthesis. The device was situated in a hot cell in the clean room area. An improved and fully automated synthesis method for the production of [^{18}F]FTHA was established. The process fulfils GMP and radiation safety regulations. The synthesis device was evaluated extensively, with the synthesis procedure, HPLC analysis of the end product, and cleaning of the synthesis all being validated. The outcome is a user-friendly process that provides enough material for the clinical studies being conducted at the Turku PET Centre.
- Bone perfusion and osteoblast activity are highest in trabecular bone and, therefore, the uptake of [^{18}F]fluoride is higher at these sites than in cortical bone. However, the rate constant from the extravascular compartment to the bone-bound compartment is similar for all measured bones. In soft tissues, an increased organ-to-blood ratio was found in the brain, eyes, lungs, ovaries, testes, and to some extent thyroid within the 6 h follow-up. Understanding the pharmacokinetics of [^{18}F]fluoride in various bones and soft tissues helps to evaluate and analyse ^{18}F-labeled radiotracers that release [^{18}F]fluoride in their metabolic pathways. The same applies when [^{18}F]NaF is used in human bone or calcification studies.

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Turku, in April 2019

Nina Sajo

9. Abbreviations

A_m	Molar activity
A_s	Specific activity
CT	Computed tomography
cGRPP	Current good radiopharmacy practice
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
[^{18}F]FTHA	14-(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid
ETFE	Ethylene tetrafluoroethylene
EANM	European Association of Nuclear Medicine
EC	Electron capture
EDQM	European Directorate for the Quality of Medicines and Healthcare
EMA	European Medicines Agency
EOB	End of bombardment
EOS	End of synthesis
EU	European Union
FEP	Fluorinated ethylene propylene
Fimea	Finnish Medicines Agency
FDA	Food and Drug administration
GMP	Good manufacturing practice
HDPE	High-density polyethylene
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human use
ILC	Ion liquid chromatography
IMP	Investigational medicinal product
IQ	Installation qualification
LI	Large intestine
LOD	Limit of detection
MET	Method description
MRI	Magnetic resonance imaging
N_{av}	Avogadro constant

n.c.a	Non-carrier added
NIMP	Non-investigational medicinal product
OQ	Operational qualification
PEEK	Polyether ether ketone
PET	Positron emission tomography
PFA	Perfluoroalkoxy
Ph. Eur.	European Pharmacopoeia
PIC/S	Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme
PP	Polypropylene
PQ	Process qualification
PTFE	Polytetrafluoroethylene
QC	Quality control
R_s	Resolution factor
RCP	Radiochemical purity
ROI	Origin of interest
RSD	Relative standard deviation
SD	Standard deviation
SFU	Sterile filtration unit
SI	Small intestine
SOP	Standard operating procedure
SPE	Solid phase extraction
TAC	Time activity curve
TWB	Total water boluses
UPW	Ultra-pure water
VOI	Volume of interest
WHO	World Health Organisation

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