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VALIDATION OF THE HPLC TECHINQUE AS ANALYTICAL METHOD FOR THE DETERMINATION OF CHEMICAL AND RADIOCHEMICAL PURITY OF DOTAGA DRUGS IN CANCER TREATMENT

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ABSTRACT

The prostate-specific membrane antigen (PSMA) represents an ideal biomarker for molecular imaging. Various PSMAtargeted radioligands are available for prostate cancer imaging. In this study, labeling of PSMA I&T with 68Ga, as well as validation of the radiochemical purity of the synthesis product by reverse phase radio high-performance liquid chromatography (HPLC) method are intended. Since the standard procedure for the quality control (QC) was not available, definition of chemical and radiochemical purity of 68Ga-PSMA I&T was carried out according to the Q2 (R1) ICH guideline. The standard QC tests were analyzed with Scintomics 8100 radio-HPLC system equipped with a radioactivity detector. The method was evaluated in terms of linearity, precision and accuracy, LOQ, robustness parameters, and specificity. To assess the radiochemical and chemical purity of 68Ga-PSMA I&T, the developed method was validated to apply safely to patients. An excellent linearity was found between 1µg/mL and 30 µg/mL, with a limit of detection and limit of quantitation of 0.286 µg/mL and 0.866 µg/mL, respectively for 68Ga-PSMA I&T. The recovery was 96.8 ± 3.8%. The quality control of the final product was performed many times with validated radio-HPLC method and was found to comply with ICH requirements, thus demonstrating the accuracy and robustness of the method for routine clinical practice.

Keywords : *HPLC, DOTAGA, Radiochemical, cancer*

INTRODUCTION

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms that arise from cells of the endocrine and nervous systems. These tumors can originate from various areas of the body but are most commonly in the gastrointestinal or bronchopulmonary system. Many NETs may be characterized by a spectrum of overexpression of somatostatin receptors (SSTRs) on the cell surface. Radiolabeling of DOTA-conjugate peptides with the positron emission tomography (PET) tracer Ga-68 allows to detect SSTRs overexpressed on NET cells providing in vivo visualization of primary tumor and metastatic lesions. Recent availability of good manufacturing practice Good manufacturing practices (GMP)-grade generators for the production of the positron emission tomography (PET) tracer Ga-68 and publication of European Pharmacopoeia (Ph. Eur.) monograph (#2464) on Ga-68-chloride from generators have changed the imaging scenario. Finally, various new diagnostic agents based on Ga-68 labeling have been added to the clinical activity due to the use of automated synthesis systems. The continuing development of new tools for molecular imaging is not accompanied by a coherent effort in the development, standardization, and validation of quality control (QC) methods to guarantee high-quality

radiopharmaceutical production, especially in the routine clinical setting. Analytical methods play an essential role in the final product quality. However, the quality can only be reached if the analytical method undergoes an appropriate validation process. Analytical validation comprises a formal, systematic, and documented tool that measures the ability of an analytical method to provide reliable, accurate, and reproducible results. In this paper, we describe the validation process of a new selective and sensitive high-performance liquid chromatography (HPLC) method developed for determining chemical and radiochemical purity of Ga-68-DOTATATE (PET) tracer.

HPLC

The acronym HPLC stands for High Performance Liquid Chromatography. "Chromatography" is a separation technique, "chromatogram" is the chromatography result, and "chromatograph" is the chromatography apparatus. HPLC is a technique for disjointing, determinating, and quantifying each component in a mixture.Spectroscopy is used to identify and quantify the mixture, which is separated using the fundamental concept of column chromatography. Some of the major components of chromatographs include devices specialised in molecule separation known columns and high-performance pumps for supplying solvent at a steady flow rate, among the different technologies created for chromatography. The system once known as High Performance Liquid Chromatography became known simply as "LC" as associated technologies got more advanced. Ultra High Performance Liquid Chromatography (UHPLC), which is capable of high-speed analysis, is becoming increasingly popular and is being widely used nowadays.

HPLC can only evaluate chemicals that are dissolved in solvents. HPLC separates chemicals dispersed in a liquid sample, allowing for qualitative and quantitative examination of which components are present in the sample and how much of each component is present.

In the 1960s, LC with low-pressure glass columns evolved into high-pressure chromatography (HPLC) using metal columns. As a result, it is a better version of TLC. Instead of allowing a solvent to drop through a column under gravity, it is pushed through at up to 400 atmospheres of pressure.

Principle of HPLC

- A separation column separates the stationary and mobile phases during purification.
- In a separation column, the stationary phase is a granular substance with very small porous particles.
- The mobile phase is a solvent or solvent combination that is pushed through the separation column under high pressure.
- The sample is loaded into the mobile flow regime from the pump to the separation column using a syringe through a valve with a linked sample loop, i.e. a tiny tube or capillary made of stainless steel.
- A chromatogram is generated in the HPLC software at the conclusion of this operation/run.
- The chromatogram allows the various compounds to be identified and quantified.

- As a result, owing to interactions with the stationary phase, the constituent components of a mixture migrate through the column at different speeds.
- Individual compounds are identified by an appropriate detector after exiting the column and transmitted as a signal to the computer's HPLC software.

Types of HPLC

Normal Phase

This method separates analyses based on their extreme nature. A liquid stationary stage and a non-polar portable stage are used in NP-HPLC. As a result, polar specimens are kept on the polar surface of the column pressing for longer than less polar ones.

Reverse Phase

The stationary stage is hydrophobic, whereas the versatile stage is a polar liquid, such as water-methanol or acetonitrile mixes. It is based on the hydrophobic collaboration rule, which states that the more nonpolar the substance, the more it will be retained.

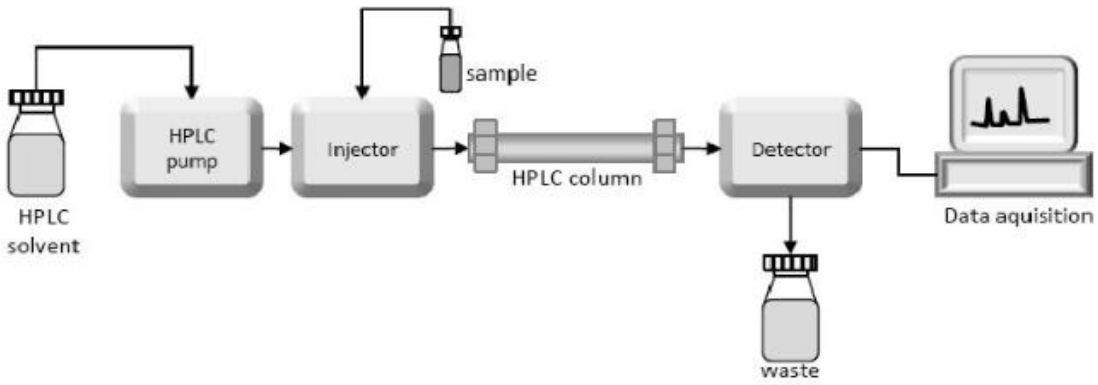
Size Exclusion

Molecules migrate into pores in a porous media and are segregated based on their size in comparison to the pore size. Large molecules elute first, followed by smaller ones.

Ion Exchange

The mobile phase is buffer, and the column packing comprises ionic groups. It is used to distinguish between anions and cations.

Parts of HPLC



Solvent Reservoir

A glass reservoir holds the mobile stage ingredient. In HPLC, the flexible stage, or dissolvable, is often a mixture of polar and non-polar liquid segments where specific fixations change depending on the specimen arrangement.

Pump

The pump system was developed as a result of the development of HPLC. The pump is located in the upper stream of the liquid chromatographic column and pumps eluent into the system from the solvent reservoir.

Injector

Next to the pump, there is an injector. The easiest way is to use a syringe to insert the sample into the eluent flow. Sampling loops are the most extensively utilised injection mechanism.

Column

The separation takes place within the column. Instead of glass columns, contemporary columns are frequently manufactured in a stainless steel housing. In comparison to calcium carbonate, silica or polymer gels are commonly utilised as packing materials.

Detector

The separation of analytes takes place inside the column, and the separation is seen using a detector. When no analyte is present, the eluent has a constant composition. While the presence of analyte alters the eluent's composition. These differences are measured by the detector. This disparity is measured using an electrical signal. Different kinds of detectors are available.

Data Collection

Signals from the indicator might be collected via outline recorders or electronic integrators with varying degrees of multi-sided fidelity and the ability to analyse, store, and reprocess chromatographic data. The PC coordinates the identifier's reaction with each component and records it in a chromatograph that is simple to read and understand.

Uses of HPLC

- Purification of water.
- Impurity detection in the pharmaceutical industry.
- Trace components are pre-concentrated.
- Chromatography based on ligand exchange.
- Protein chromatography via ion exchange.
- Carbohydrate and oligosaccharide anion-exchange chromatography at high pH.

Applications of HPLC

- Drug evaluation
- Synthetic polymer analysis
- Pollution analysis in environmental analytics
- Drug determination in biological matrices
- Isolation of high-value goods

OBJECTIVE

1. to study on dotaga drugs in cancer treatment
2. to study on software interface module diagram of the automated scintomics radiosynthesis system

METHODOLOGY

Reagents

All reagents to be used for synthesis and QC were purchased from Merck in high purity pharmaceutical grade. Kit equipment for the synthesis of 68Ga peptides using cationic purification were obtained from ABX D-01454 Radeberg (Germany). The kit contains chemicals, hardware, and the cassette required for radiosynthesis of 68Ga peptides the Scintomics GRP synthesizer using cationic purification. The kit components are: Cassette, PS-H+ cartridge, 5M sodium chloride solution, ethanol, ethanol/water (1/1), phosphate buffered saline, 1.5M HEPES buffer solution, and water for injections. The cassette is a disposable cassette and therefore is made for single use. Reference PSMA I&T peptide was purchased from ABX D-01454 Radeberg (Germany) and stored at –20 °C. Dilutions of PSMA I&T were prepared with Farmako brand sterile water (1:1). Hydrochloric acid (0.6 M ultrapure HCl) and 1.5 M HEPES (2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid) buffer solution were obtained from ABX D-01454 Radeberg (Germany).

Instruments

68GaCl3 was used for synthesis of 68Ga-PSMA I&T (Figure 1). 68Ga activity was measured in a Comecer VDC-606 dose calibrator. The radiochemical purity of 68Ga-PSMA I&T solution was controlled using Scintomics 8100 radio-HPLC system. The HPLC system was equipped with a UV and a gamma detector. The radio-HPLC system consists of Agilent 8100 quaternary pump (Germany) [Operating principle: parallel dual-plunger pump; low-pressure gradient, number of solvents: up to 4 solvents, gradient formation: 4-channel mixing valve, composition precision: 99.5% for 2.5 AU (acetone, 254 nm), flow cell vol: 7 µL (analytical)], and radioactivity detector Berthold Technologies; DataApex Clarity program (Prague, Czech Republic).

Labelling of PSMA I&T with 68Ga in automated synthesis module

There is a specific synthesis cassette for the production of PSMA I&T and these cassettes are sterile and single-use. All components and the cassette are manufactured according to GMP Standards for APIs. In the studies, commercial 68Ge/68Ga generator modified with tin oxide (SnO_2) was used in an iThemba

Labs brand polyethylene column. A cation exchange cartridge (PS H+, not preconditioned) was used to remove trace metals in GaCl3 solution eluted from 68Ge/68Ga generator. GaCl3 eluted from the PS-H+ cartridge with 5.0 M NaCl was added to the reaction vial containing 25 µg peptide dissolved in HEPES buffer. The eluate was buffered with HEPES solution (pH 5) to prevent formation of 68Ga(OH)3 at higher pH values. The mixture was then heated for 15 min at 90 ºC for labeling of the peptide with 68Ga(III). The solution of 68GaPSMA I&T in the reaction vial was passed through the C18 ion exchange cartridge to remove the unbound free 68Ga

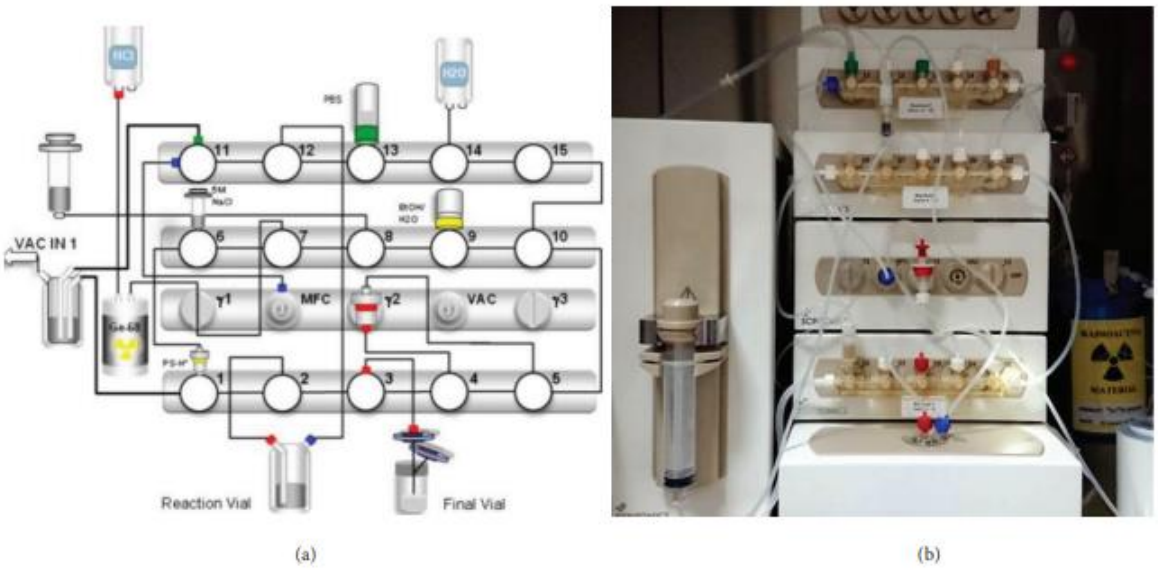


Figure 1. (a) Software interface module diagram of the automated Scintomics radiosynthesis system used to prepare 68Ga-PSMA I&T, (b) Original Scintomics radiosynthesis module equipped with cassette for the labelling of PSMA I&T peptide.

ions. The retained 68Ga-PSMA I&T was eluted from the C18 ion exchange cartridge with 2.0 mL ethanol/water (1/1). The product was passed through the 0.22-µm filter syringe and collected in the final vial.

Quality control experiments

Th hydrophobic part of PSMA forms three diastereomers, RR, RS, and SS configurations at its amine nitrogens during chelating of gallium-68 with PSMA. The RR configuration is known as a thermodynamically favorable isomer. The other diastereomers have the same biological activity and radiochemical stability after the labeling procedure . In addition to the requirements described above, a QC test is therefore required. The standard QC tests for 68Ga-PSMA I&T were carried out after each synthesis. The radiochemical purity of 68Ga-PSMA I&T solution was identified with Scintomics 8100 radioHPLC system equipped with a radioactivity detector, under working conditions maintained with a flow rate of 0.6 mL/ min and column temperature at room temperature. An isocratic separation was performed using a mobile phase including 30% acetonitrile and 0.1% trifluoroacetic acid (TFA) in water. For the detection of chemical impurities, the samples were also monitored with a UV detector at 220 nm and radio-detector. The injection volume was 20 µL and detection time determined as 15 min.

RESULTS AND DISCUSSION

Labelling of PSMA I&T with 68Ga

All synthesis studies were conducted in a radiopharmaceutical laboratory at Department of Nuclear Medicine, in Pamukkale University Hospital. The synthesis of 68Ga-PSMA I&T was performed a total of 150 times between March 2018 and January 2019 in our laboratory. The reagent and hardware kit manufactured by ABX for Scintomics is produced after bioburden testing. All reagents used are sterile and the synthesis process is carried out under GMP conditions. Therefore, no additional QC is required for method validation. The activity of the 68Ga gallium labeled PSMA peptide depends on the activity eluted from the 68Ge/68Ga generator. For the 30 mCi of eluate the Scintomics GRP automated synthesis module can produce around 18–20 mCi of product. At the end of synthesis, the total volume of the final product is 16 mL. The labeling efficiency was found to be >99%. The automated synthesis was performed within 32 min. Postsynthesis of the product after 45 min (after QC) remained at a total activity of 68% that is convenient for clinical application. The pH of the final product was determined to be in between 6 and 7.

As the synthesis yield decreased, the dose and number of patients were adjusted. From the synthesis of the 68Ge/68Ga generator in the first months, sufficient activity was obtained for three patient doses (185 MBq to 259 MBq per dose).

Physical and chemical properties of 68Gallium and PSMA I&T

68Ga(III) as a positron emitter is readily obtained from modern generators and has a short half-life of 68 min . It is used for labeling ligands having five or six coordination sites and the labeled complexes are stable at the physiological pH. Like metallic elements, transition metals also require chelating agents. After labeling a molecule with a metal or transition metal chelator, a molecular structure appears whose final chemical structure is affected by the chelate. Therefore, large molecules such as peptides, proteins, or antibodies are preferred over radiolabeling with metal or transition metal. For radiopharmaceutical preparation with metal or transition metal, the appropriate chelating agent must be present for each metal or transition metal. Gallium is in the IIIA group of the periodic table. It is in the form of three ions and a chelation such as 1,4,7-triazacyclonona-1,4,7-triacitic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) is required [23,24]. These two chelating agents are widely used in routine PET imaging with 68Ga. Prostate-specific membrane antigen (PSMA) is the biological target for therapeutics and diagnostics. It is a type II transmembrane protein with a short intracellular area (amino acids 1–18), a transmembrane area (amino acids 19–43), and a large extracellular area (amino acids 44–750) [25–28]. Also, PSMA ligands with DOTA-derived chelators have been developed, which can be labeled with diagnostic compounds. Recently, EuK-Sub-kf-(3-iodo-y-) DOTAGA (PSMA I&T) (Figure 2) from these diagnostic nuclides is of interest.

DISCUSSION OF QUALITY CONTROL

Although the 68Ga-PSMAI&T synthesized for the patient has a thermodynamically preferred diastereomer, the other diastereomer is also known to be present in the formulation. Thus, there is a need

for a QC test to assess the diagnostic efficiency and purity of the final product. The QC was evaluated with the analysis carried out by high-performance liquid chromatography (HPLC).

CONCLUSIONS

Here, we developed and validated a simple and rapid isocratic radio-HPLC method with high precision for quality control testing of synthesized 68Ga-PSMA I&T radiopharmaceuticals for its use in routine PET imaging in prostate cancer patients. The absence of specific literature about the quality controls of 68Ga-PSMAI&T has led us to use isocratic radio-HPLC method for validation of the produced radiopharmaceutical to implement it in the clinical setting. The bioburden and endotoxin levels of the 0.05 M hydrochloric acid are controlled and specified on the certificate of analysis. We consider the verification of this certificate to be sufficient; therefore, we did not perform a routine assay for bioburden or bacterial endotoxins on the 68Ga eluate. Definition of chemical and radiochemical purity of 68Ga-PSMA I&T was carried out according to the Q2 (R1) ICH guideline. The method was validated in terms of linearity, precision and accuracy (recovery), LOD and LOQ, robustness, and specificity parameters. In addition, samples can be analyzed quickly due to the short analysis time (less than 10 min) required. As a result, it was concluded that the proposed method was applicable and reliable in determining the radiochemical purity of 68Ga-PSMA I&T from the validation results obtained in this study.

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