Original Article

Time-efficient HPLC Validation Methodology for the Qualitative Analysis of 68Ga PSMA-11 in Routine Clinical Usage under Isocratic Method

Sachin Tayal, Murari Gurjar, Varun Shukla, Manikandan Marappagounder Venkatachalam, Rohit Kumar, Yash Jain

Department of Nuclear Medicine and Molecular Imaging, Homi Bhabha Cancer Hospital and Mahamana Pandit Madan Mohan Malaviya Cancer Centre, Tata Memorial Centre, Homi Bhabha National Institute, Varanasi, Uttar Pradesh, India

Abstract

Background: Prostate-specific membrane antigen (PSMA) has shown to be a promising agent for prostate cancer imaging under PET-CT. With the automation in radiolabeling with 68Ga, using iTG 68Ge/68Ga generator, it has helped introduce various new diagnostic agents and achieve good manufacturing practices (GMP) simultaneously. However, before any radiopharmaceutical is put into clinical usage, it should always be checked for its radiochemical purity and other quality parameters before injecting in the patient. Chromatography techniques such as Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), and Thin-Layer Chromatography (TLC) are the most frequently utilized separation technique for purity analysis. A rapid quality control HPLC based methodology was required for radiopharmaceuticals. **Aim & Objective:** In our current setting, we conducted quality control analysis and standardized and validated HPLC method for the routine quality check of 68Ga-PSMA-11. **Materials and Methods:** The QC of 68Ga PSMA-11 was performed under ITLC and HPLC. **Results:** Linearity, accuracy, precision and specificity were assessed and quantified in accordance with International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (Q2 (R1) ICH) guidelines, which can be implemented in resource-limited settings to check the quality. **Conclusion:** The current HPLC based methodology is rapid, with a retention time of 2.24 min, rendering it a favorable analytical standard operating procedure for QC analysis of 68Ga-PSMA-11.

Keywords: *Gallium-68 prostate-specific membrane antigen-11, high-performance liquid chromatography, isocratic*

Introduction

Prostate cancer remains a significant health concern worldwide.[1,2] Although radiodiagnosis techniques such as magnetic resonance imaging and positron emission tomography (PET) scans have significantly improved detection and staging, challenges persist. However, prostate-specific membrane antigen (PSMA) has shown to be a promising agent for specific prostate cancer imaging[3,4] under PET-computed tomography. The radiolabeling of peptide (PSMA-11) with gallium-68 (^{68}Ga) is well suited for rapid pharmacokinetics, resulting in low radiation doses for patients.[5] ^{68}Ga-PSAM-11 proved a significant radiotracer in the management of prostate cancer patients.[6] Second, the automation in radiolabeling with ^{68}Ga has helped introduce various new diagnostic agents and achieve good manufacturing practices (GMPs) simultaneously.[7] Due to their generator-based production method, ^{68}Ga radiopharmaceuticals circumvent the substantial investment required for cyclotrons, which is necessary for ^{18}F-labeled radiotracers.[8] However, before any radiopharmaceutical is put into clinical usage, it should always be checked for its high radiochemical purity (RCP) and other quality parameters before injecting in the patient/clinical usage. Hence, various quality control (QC) parameters contribute to substantiating the safety, quality, and efficacy of the prepared radiopharmaceutical.[9] Successful QC clearance confirms that the radiotracer is suitable for injection with its high radiochemical yield and RCP.[5,10-15] Nonetheless, a multitude of factors can result in chemical and radiochemical impurities, such as incomplete labeling and degradation of radiotracers over time. Chromatography techniques such as Gas Chromatography

How to cite this article: Tayal S, Gurjar M, Shukla V, Venkatachalam MM, Kumar R, Jain Y. Time-efficient HPLC validation methodology for the qualitative analysis of 68Ga PSMA-11 in routine clinical usage under isocratic method. Indian J Nucl Med 2024;39:265-71.

Address for correspondence:
Mr. Sachin Tayal, Mahamana Pandit Madan Mohan Malaviya Cancer Centre, A Unit of Tata Memorial Centre, BHU Campus, Sunadar Bagiya, Varanasi - 221 005, Uttar Pradesh, India.
E-mail: sachintayal7@gmail.com

Received: 06-04-2024
Revised: 08-06-2024
Accepted: 14-06-2024
Published: 18-11-2024

Access this article online

Website: https://journals.lww.com/ijnm

DOI: 10.4103/ijnm.ijnm_42_24

Quick Response Code:

(GC), High-Performance Liquid Chromatography (HPLC), and Thin-Layer Chromatography (TLC) are the most frequently utilized separation technique for purity analysis.[16-18] In our current setting, we conducted QC analysis and standardized and validated the HPLC method for the routine quality check of ^{68}Ga-PSMA-11. A reverse-phase HPLC method was validated within our laboratory for the qualitative and quantitative analysis of ^{68}Ga-PSMA-11. Linearity, accuracy, precision, and specificity were assessed and quantified in accordance with Q2 (R1) ICH guidelines, which can be implemented in resource-limited settings to oversee their quality.

Materials and Methods

Chemical and reagents

Pharmaceutical-grade chemicals and reagents were used for the synthesis of radiopharmaceutical products. Reference standard (500 µg) PSMA-11 peptides were purchased from ABX advanced biochemical compounds, Germany. We use an automated cassette-based synthesis module, a GMP-certified module to synthesize pure radiopharmaceutical products.[19] Specialized cassettes with consumables (HCl, saline, ethanol bottles, and C18 ion exchange cartridges) are required to synthesize ^{68}Ga-based radiopharmaceuticals, which were also supplied by ABX advanced biochemical compounds, Germany. For RCP, QC experiments were performed under instant TLC medium (ITLC) and HPLC. HPLC grade solvents, acetonitrile (ACN), methanol, and trifluoroacetic acid (TFA) were procured from Merck, India. Ultra-pure water ≥18.2 MΩ was obtained from PURELAB® flex × 2 plus water purification system (ELGA LabWater, USA). ITLC paper strips were purchased from Sigma-Aldrich.

Instrument and equipment

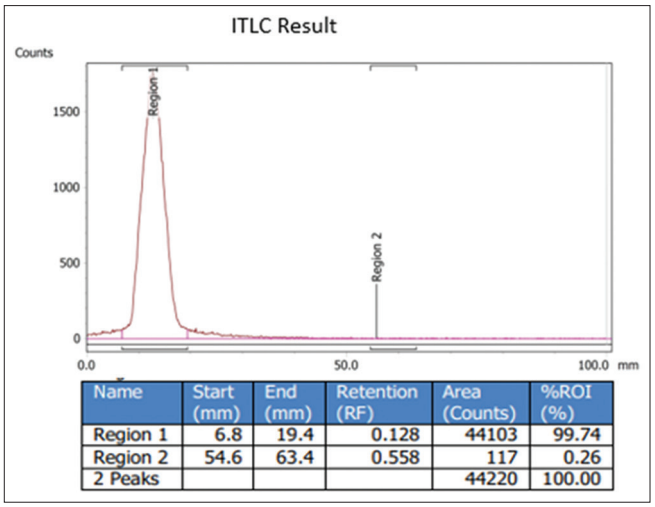
iQS-TS Synthesis module, ITG, Germany, is an automated cassette-based synthesis module approved by a European agency as a GMP-certified module, and the itG ^{68}Ge/^{68}Ga generator has a nonmetallic column (silica gel [SG]-modified dodecyl gallate) which does not require prepurification steps unlike metallic column ^{68}Ge/^{68}Ga generator.

Synthesis and quality control procedures

The QC of ^{68}Ga-PSMA-11 was performed under ITLC and HPLC.

Instant thin-layer chromatography

RCP assessment was performed on TLC SG 60 F_{254} aluminum sheets with an automatic peak finder with high sensitivity and accuracy (LabLogic Scan-RAM/Radio-TLC Scanner). An SG-ITLC strip measuring 10 cm × 1 cm and a 0.5 M sodium citrate buffer served as the solid and mobile phases, respectively. A drop of the final product was applied to the SG-ITLS strip at the point of spotting (PS), and the chromatogram was developed up to the solvent front (SF). ^{68}Ga-labeled radiopharmaceuticals remained at PS, while the unlabeled fraction ^{68}Ga migrated to SF [Figure 1]. Counting was performed in LabLogic Scan-RAM/Radio-TLC Scanner with the help of Laura™ radiochromatography data collection and analysis software version v6.1.1.20 andRCP was calculated [Figure 1], as shown in equation 1:[9,20]



Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)
Region 1	6.8	19.4	0.128	44103	99.74
Region 2	54.6	63.4	0.558	117	0.26
2 Peaks				44220	100.00

Figure 1: Graph obtained under instant thin-layer chromatography and its result. ITLC: Instant thin-layer chromatography

$$\%RCP = \frac{Cnt(PS) \times 100}{Cnt(PS) + Cnt(SF)} \quad \text{Equation 1}$$

where %RCP = Percentage RCP; Cnt (PS) = Count at PS; Cnt (SF) = Count at SF.

High-performance liquid chromatography

The equipment [Figure 2] consists of a solvent delivery system (S-1125 HPLC pump system-plus), an oven, a manual injection setup comprising a rotary valve, an Ultraviolet-Visible detector (S-3245 UV/Vis), and a gamma detector (S-3700). Data analysis was conducted using ChromStar workstation v. 7.0 software. Separation was achieved at 25°C utilizing an Acclaim 120, C18, 3 µm 120 Å (3.0 mm × 150 mm) column (Thermo Scientific, USA). The mobile phase comprised (a) water + 0.1% TFA, and (b) ACN + 0.1% TFA, delivered at a rate of 0.4 mL/min. Isocratic elution was performed to achieve separation, maintaining a mobile phase composition of 55% (a) water + 0.1% TFA and 45% ACN + 0.1% TFA throughout the runtime. Manual injection of 20 µL was performed, with detection wavelength set at 220 nm, and ^{68}Ga radiotracer quantified by HPLC using a sodium iodide (NaI)-based detector.

Synthesis of radiopharmaceuticals

The routine production of ^{68}Ga-labeled radiopharmaceuticals was conducted using the ITG ^{68}Ge/^{68}Ga generator, employing a metal-free modified silica matrix and having an automated cassette-based synthesis module. The synthesis process can be categorized into four main sections: mechanical testing,

Figure 2: High-performance liquid chromatography system with its components and software

presynthesis procedures, radiopharmaceutical synthesis, and filter integrity assessment.[9] This generator allows elution with lower molarity HCl, ensuring consistently high labeling yields for various molecular imaging agents.[9,21] This module automates the elution, formulation, and purification processes of radiopharmaceuticals.

Preparation of reagents

The initial peptide stock, consisting of 500 µg of PSMA-11, was dissolved in 3 mL of ultrapure water. Subsequently, 30 µL aliquots were dispensed into 1 mL polypropylene tubes (Eppendorf tube) under aseptic conditions and stored at − 20°C. On the day of synthesis, one tube containing PSMA-11 peptide was retrieved and allowed to equilibrate to room temperature before use.[9]

Radiopharmaceutical synthesis

The synthesis is initiated on the software platform, with the entire process lasting approximately 17 min. The synthesis encompasses various steps as outlined in a previous paper.[9] A schematic flowchart illustrating these steps can be found in a previous paper.[15]

Quality control of radiopharmaceuticals under high-performance liquid chromatography

In the present study, our primary emphasis focuses on validating the HPLC method for QC purposes. The The analytical method for assessing the chemical and radiochemical purity of 68Ga-PSMA-11 was validated according to the Q2 (R1) ICH guideline. The validation process was assessed through the evaluation of specificity, linearity, accuracy, and precision.[22,23] In addition to this, ITLC assessment, pH determination (utilizing pH strips), and verification of radionuclide purity (via examination of principal γ photons) using γ-ray spectrometry[24] was conducted as mentioned in work done by Tayal *et al.*[9]

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants, and matrix. The assay's specificity was evaluated by repeated measurement of the content or potency of the analyte obtained from the final product, ^{68}Ga-PSMA-11 solution with different concentrations of radioactivity.

Linearity

Linearity is the ability of an analytical procedure to produce test results that are directly proportional to the concentration of the analyte within the standard curve range. To cover a range of ^{68}Ga-PSMA-11 concentration values, three sets of calibration standards of radioactivity concentration in (MBq) ranging from 100% to 12.5% were injected into the HPLC systems under the above-specified chromatographic conditions (injection volume was 20 µl) and analyzed for a linearity analysis. Using the peak area of the (^{68}Ga-PSMA-11) analyte, (y) versus the amount (x), the calibration curve was created for evaluating the slope, intercept, and regression coefficient (R^2) with the help of linear regression.

Accuracy

The accuracy of the obtained concentrations compared to the known concentration was assessed in this method. The accuracy was expressed by the percentage of recovery (R%), calculated as the ratio of found concentration to actual concentration. The quantitative recovery values that are higher than 95% are an acceptance criterion for expressing high accuracy. For determination of accuracy, we performed three sets of runs with radioactivity concentration in (MBq) ranging from 100% to 12.5% being injected into the HPLC systems under the above specified chromatographic conditions. ^{68}Ga-PSMA-11 being a radionuclide showed the decay inevitably leading to a decrease over time of the radioactivity and recalculating the obtained peak area values with the below-mentioned decay equation:

$$lnA_0 = lnA + \lambda t_{1/2} \qquad \text{Equation 2}$$

A_0 = corrected peak area; A = measured peak area; and $t_{1/2}$ = half-life (^{68}Ga = 67.63 min).

Precision

Precision or repeatability was calculated based on the content of ^{68}Ga-PSMA-11, and the statistical parameter of concern evaluated was coefficient of variation (CV%), which is determined using the equation: CV% = s/m × 100, where m is the average of the concentrations and s is the standard deviation. Three different radioactivity concentrations in (MBq) of ^{68}Ga-PSMA-11 ranging from 75% to 25%, (HQC – 75%, MQC – 50%, and LQC – 25%) were prepared and injected into the HPLC system to measure repeatability or intermediate precision using the

regression equation with 6 replicates and 12 replicates for each concentration on intraday and interday, respectively. Normalized peak area values after decay correction were compared to ensure consistent statistical analysis.

Results

Quality control results

The QC tests for ^{68}Ga-PSMA-11 are illustrated in Table 1. The QC experiment methodology is described in Table 1.[9]

Specificity

The concentration changes did not affect the retention time and show evidence of any interference from another compound (single peak) as depicted in Figure 3a. The average recovery obtained from the accuracy experiment range was 99.99%–100%, while the range of CV came to be 0.766%–2.239%. Therefore, the method was found to have acceptable specificity for the assay of ^{68}Ga-PSMA-11 with high accuracy.

Linearity

The regression analysis of three independent runs of ^{68}Ga-PSMA-11 indicated no deviation with an R^2 value to be 0.9983, as shown in Figure 3b. Linearity was ≥0.99% and met acceptance criteria.

Accuracy

The accuracy of the obtained concentrations in comparison to the known concentration was assessed for the procedure. The accuracy range came out to be between 99.99% and 100%, while the range of CV is 0.766%–2.239%, as shown in Figure 3c. The outcome shows good accuracy for varying radioactivity concentration in MBq (100%–12.5%) of ^{68}Ga-PSMA-11.

Table 1: Quality control results from gallium-68 prostate-specific membrane antigen-11 radiolabeled formulated product

QC results ^{68}Ga-PSMA-11 radio-labeled product			
QC data	**Run - 1**	**Run - 2**	**Run - 3**
Appearance	Colorless	Colorless	Colorless
Particulate matter	Not observed	Not observed	Not observed
Radionuclidic identity (min)	67.71	67.71	67.71
Radionuclidic purity (%)	100.00	100.00	100.00
Radiochemical identity (min)	2.10	2.11	2.10
Radiochemical purity (%)	99.45	99.78	99.75
^{68}Ge impurity (%)	4.9×10^{-5}	4.7×10^{-5}	4.8×10^{-5}
pH	5.5	5.5	5.5
Yield (%)	63.34	63.71	64.01
Activity (MBq)	463	459	464
Final volume (mL)	10	10	10

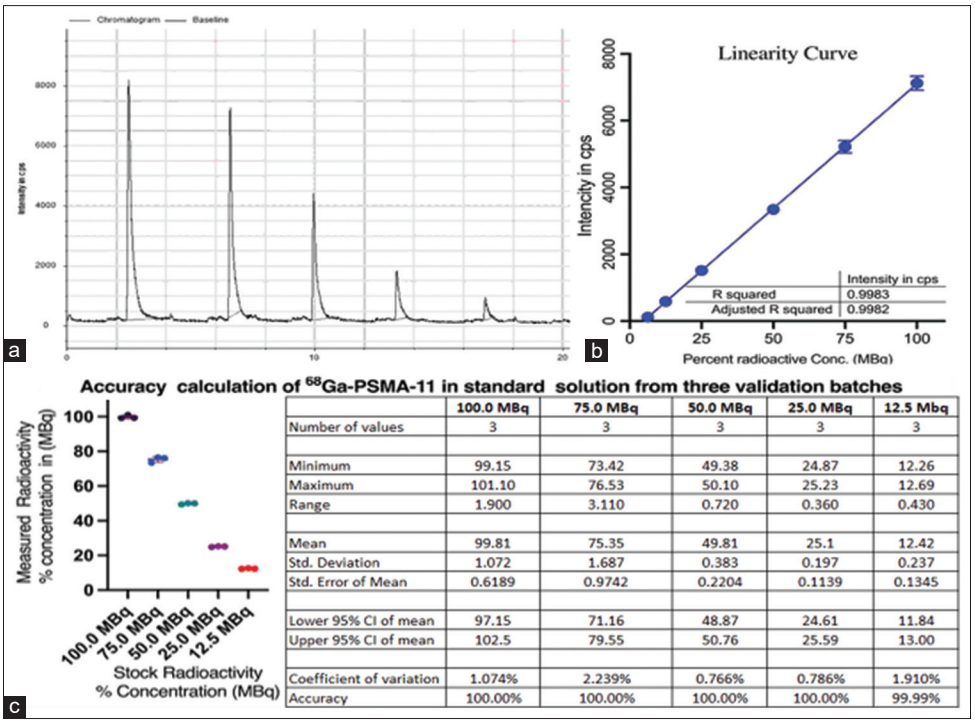
PSMA 11: Prostate-specific membrane antigen-11, QC: Quality control, ^{68}Ga: Gallium-68

Precision

The method was considered precise, obtaining coefficients of variation ranging between 0.6306% and 1.316% for intraday and 1.562%–2.472% for the interday precision, as shown in Figure 4a and b. The intraday and interday accuracy range was 99.98%–100% and 99.99%–100%, respectively.

Discussion

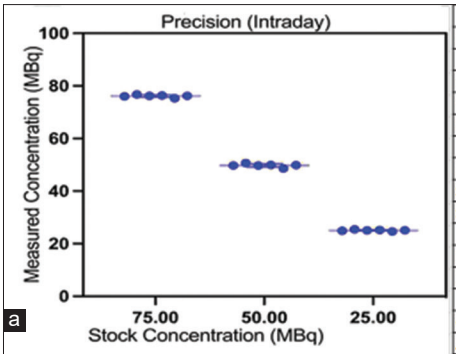
Radiotracers directed at the PSMA are employed in clinical settings for PET scans to visualize the disease burden of prostate cancer. FDA approval for ^{68}GA-PSMA came a decade after its discovery in 2010.[24] Despite the ongoing advancements in imaging technology, the progress in developing, standardizing, and validating QC parameters for in-house prepared radiopharmaceuticals has not kept pace.[25] The absence of consensus guidelines in India regarding the validation of radiopharmaceutical QC methods prompted us to develop and utilize an isocratic radio-HPLC method to validate the cassette-based production of ^{68}Ga-PSMA-11 at our institute. The process is straightforward, employing the isocratic method for QC testing of synthesized ^{68}GaPSMA-11. Moreover, the mobile phase used in our study has been previously employed for various PSMA variants by other researchers.[20,26-28] The optimal amount of starting peptide per production of ^{68}Ga-PSMA-11 was 16 µg at our facility. The peak intensity and retention time observed for both PSMA-11 and ^{68}Ga-PSMA-11 under ultraviolet and radionuclide detectors were relatively similar. Our method not only demonstrated favorable analytical characteristics but was also validated for linearity, accuracy, and precision, yielding results indicative of excellent reproducibility and method robustness. Therefore, it is advisable to adhere to GMPs and implement a validation method using analytical techniques for radiopharmaceutical analysis. This practice instills confidence in a clinical setting when conducting studies with in-house labeled radiotracers, ensuring patient safety and optimal image quality while accurately quantifying radiotracer accumulation for planning therapeutic studies. Furthermore, the integration of automation has significantly enhanced reproducibility while concurrently reducing operator variability.[7,29] The iTG ^{68}Ge/^{68}Ga generator had a stable production, and it is highly encouraged to have regular elution and at least one elution before preparing the radiopharmaceutical after a gap of few days. Our study concurs with Assadi and Dadgar[30] that ^{68}Ga obtained from a generator system serves as a cost-effective tracer compared to cyclotron-produced alternatives. The radiopharmaceutical production interval of every 3 h is particularly advantageous. Our obtained RCP consistently exceeded 95%, aligning with earlier findings.[8,31] Previously established validation methods by Migliari *et al*.[26] and according to Pharmeuropa 2017 involved retention times of 7 min and 16 min, respectively. In our study, we



Accuracy calculation of ^{68}Ga-PSMA-11 in standard solution from three validation batches

	100.0 MBq	75.0 MBq	50.0 MBq	25.0 MBq	12.5 Mbq
Number of values	3	3	3	3	3
Minimum	99.15	73.42	49.38	24.87	12.26
Maximum	101.10	76.53	50.10	25.23	12.69
Range	1.900	3.110	0.720	0.360	0.430
Mean	99.81	75.35	49.81	25.1	12.42
Std. Deviation	1.072	1.687	0.383	0.197	0.237
Std. Error of Mean	0.6189	0.9742	0.2204	0.1139	0.1345
Lower 95% CI of mean	97.15	71.16	48.87	24.61	11.84
Upper 95% CI of mean	102.5	79.55	50.76	25.59	13.00
Coefficient of variation	1.074%	2.239%	0.766%	0.786%	1.910%
Accuracy	100.00%	100.00%	100.00%	100.00%	99.99%

Figure 3: (a) Linearity of gallium-68 prostate-specific membrane antigen-11 (^{68}Ga-PSMA-11) (product of single elution) was determined using serially diluted concentrations injected for high-performance liquid chromatography run at different time points; (b) Linear regression curve for ^{68}Ga-PSMA-11 radioactivity concentration in (MBq) ranging from 100% to 12.5%; (c) Accuracy shown in aqueous standard solution of ^{68}Ga-PSMA-11. CI: Confidence interval, ^{68}Ga-PSMA-11: Gallium-68 prostate-specific membrane antigen-11



	75.0 MBq	50.0 MBq	25.0 MBq
Number of values	6	6	6
Minimum	75.31	48.61	24.64
Maximum	76.76	50.64	25.43
Range	1.450	2.030	0.790
Mean	76.15	49.75	25.05
Std. Deviation	0.4799	0.6547	0.2688
Std. Error of Mean	0.1959	0.2673	0.1097
Lower 95% CI of mean	75.64	49.06	24.77
Upper 95% CI of mean	76.65	50.44	25.34
Coefficient of variation	0.6306%	1.3160%	1.0730%
Accuracy	99.98%	99.99%	100.00%

	75.0 MBq	50.0 MBq	25.0 MBq
Number of values	12	12	12
Minimum	71.74	48.61	24.03
Maximum	76.76	51.87	25.95
Range	5.020	3.262	1.920
Mean	74.73	50.03	25.14
Std. Deviation	1.848	0.7804	0.4960
Std. Error of Mean	0.5333	0.2256	0.1432
Lower 95% CI of mean	73.68	49.59	24.86
Upper 95% CI of mean	75.77	50.47	25.42
Coefficient of variation	2.472%	1.562%	1.973%
Accuracy	100.00%	100.00%	99.99%

Figure 4: Precision and accuracy estimation. (a) Intraday run calculation of gallium-68 prostate-specific membrane antigen (^{68}Ga-PSMA-11) in standard solution from six validation batches. (b) Interday calculation of ^{68}Ga-PSMA-11 in standard solution from 12 sets of validation batches. CI: Confidence interval

have developed a novel method to expedite this process, achieving a retention time of 2.245 min for rapid validation and release for clinical application[26] under isocratic conditions. There is also HPLC-based method validated assay for ^{68}Ga-PSMA-11 analysis, providing estimation in 7-min retention time and a relatively narrower linearity

range.[26] A recently published EP monograph utilized a TFA/water/ACN mixture as a mobile phase with a 16-min runtime (Pharmeuropa, 2017). Hence, our suggested assay demonstrates superiority in assay time, a critical factor during the quality assessment of radiopharmaceuticals.

Conclusion

The current HPLC-based methodology is rapid, with a retention time of 2.24 min, rendering it a favorable analytical standard operating procedure for QC analysis of ^{68}Ga-PSMA-11. In conclusion, our study indicates that this method is easily reproducible and dependable for assessing RCP during cassette-based ^{68}Ga-PSMA-11 production, ensuring the reliability of its results based on isocratic flow parameters of mobile phase.

Limitation

We evaluated the graph only under radio detector of HPLC for the isocratic method. We wanted to have a faster method of isocratic protocol to minimize radioactive decay.

Acknowledgment

The authors are thankful to Ms. Ankita Pal, Biostatistician, for her assistance in the manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, *et al.* EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol 2014;65:467-79.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225-49.
3. Lütje S, Heskamp S, Cornelissen AS, Poeppel TD, van den Broek SA, Rosenbaum-Krumme S, *et al.* PSMA ligands for radionuclide imaging and therapy of prostate cancer: Clinical status. Theranostics 2015;5:1388-401.
4. Jadvar H. PSMA PET in prostate cancer. J Nucl Med 2015;56:1131-2.
5. Gillings N, Hjelstuen O, Ballinger J, Behe M, Decristoforo C, Elsinga P, *et al.* Guideline on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. EJNMMI Radiopharm Chem 2021;6:8.
6. Afshar-Oromieh A, Avtzi E, Giesel FL, Holland-Letz T, Linhart HG, Eder M, *et al.* The diagnostic value of PET/CT imaging with the (68) Ga-labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. Eur J Nucl Med Mol Imaging 2015;42:197-209.
7. Aslani A, Snowdon GM, Bailey DL, Schembri GP, Bailey EA, Roach PJ. Gallium-68 DOTATATE production with automated PET radiopharmaceutical synthesis system: A three year experience. Asia Ocean J Nucl Med Biol 2014;2:75-86.
8. Nanabala R, Anees MK, Sasikumar A, Joy A, Pillai MR. Preparation of [(68) Ga] PSMA-11 for PET-CT imaging using a manual synthesis module and organic matrix based (68) Ge/(68) Ga generator. Nucl Med Biol 2016;43:463-9.
9. Tayal S, Ali A, Kumar V, Jha AK, Gandhi A. Installation and optimization of (68) Ga synthesis module for clinical use: An institutional experience. Indian J Nucl Med 2021;36:282-7.
10. Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, *et al.* Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. Eur J Nucl Med Mol Imaging 2010;37:1049-62.
11. Gillings N, Todde S, Behe M, Decristoforo C, Elsinga P, Ferrari V, *et al.* EANM guideline on the validation of analytical methods for radiopharmaceuticals. EJNMMI Radiopharm Chem 2020;5:7.
12. Li X, Yu W, Wollenweber T, Lu X, Wei Y, Beitzke D, *et al.* [(68) Ga] Pentixafor PET/MR imaging of chemokine receptor 4 expression in the human carotid artery. Eur J Nucl Med Mol Imaging 2019;46:1616-25.
13. EDQM, Guide for the elaboration of monographs on Radiopharmaceutical Preparations, Radiopharmacy_Committee, E.E.A. and N. Medicine (Eds), 2018. Strasbourg, France: European Pharmacopoeia, pp. 40. Available from: https://www.edqm.eu/en/d/66901. [Last accessed on 2024 Apr 06].
14. IAEA, Quality Control in the Production of Radiopharmaceuticals. 2018. IAEA-TECDOC-1856, IAEA, Vienna, Austria.
15. EudraLex, Good Manufacturing Practice (GMP) guidelines. 2023. Available from: https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en. [Last accessed on 2024 April 06].
16. de Blois E, de Zanger RM, Chan HS, Konijnenberg M, Breeman WA. Radiochemical and analytical aspects of inter-institutional quality control measurements on radiopharmaceuticals. EJNMMI Radiopharm Chem 2019;4:3.
17. Levin, S., High Performance Liquid Chromatography (HPLC) in the Pharmaceutical Analysis, In: Gad SC, editor. Pharmaceutical Sciences Encyclopedia: Drug discovery, development, and manufacturing. New Jersey: John Wiley & Sons, Inc.; 2010. pp. 34.
18. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. A rapid HPLC method for the determination of losartan in human plasma using a monolithic column. Arzneimittelforschung 2005;55:569-72.
19. ITM. Fully automated Theranostics Synthesizer for radiolabeling of targeting molecules. 2021; Available from: https://www.itm-radiopharma.com/products/radiolabeling-quality-control-equipment/iqs-theranostics-synthesizer/. [Last accessed on 2024 April 06].
20. Hooijman EL, Chalashkan Y, Ling SW, Kahyargil FF, Segbers M, Bruchertseifer F, *et al.* Development of [(225) Ac] Ac-PSMA-I and T for targeted alpha therapy according to GMP guidelines for treatment of mCRPC. Pharmaceutics 2021;13:715.
21. Amor-Coarasa A, Kelly JM, Gruca M, Nikolopoulou A, Vallabhajosula S, Babich JW. Continuation of comprehensive quality control of the itG (68) Ge/(68) Ga generator and production of (68) Ga-DOTATOC and (68) Ga-PSMA-HBED-CC for clinical research studies. Nucl Med Biol 2017;53:37-9.
22. EMA., ICH Guideline Q2(R1) Validation of Analytical Procedures: Text and Methodology, 1995. Avaiable from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r1-validation-analytical-procedures-text-methodology-step-5-first-version_en.pdf. [Last accessed on 2024 April 06].
23. EMA., ICH Q8 (R2) Pharmaceutical development - Scientific guideline, 2014. Avaiable from: https://www.ema.europa.eu/en/

ich-q8-r2-pharmaceutical-development-scientific-guideline. [Last accessed on 2024 April 06].

24. Hennrich, U. and M. Eder, [(68)Ga]Ga-PSMA-11: The First FDA-Approved (68)Ga-Radiopharmaceutical for PET Imaging of Prostate Cancer. Pharmaceuticals (Basel), 2021;14:713.
25. Sammartano A, Migliari S, Scarlattei M, Baldari G, Ruffini L. Validation of quality control parameters of cassette-based gallium-68-DOTA-Tyr3-octreotate synthesis. Indian J Nucl Med 2020;35:291-8.
26. Migliari S, Sammartano A, Scarlattei M, Serreli G, Ghetti C, Cidda C, *et al.* Development and validation of a high-pressure liquid chromatography method for the determination of chemical purity and radiochemical purity of a [(68) Ga]-labeled Glu-Urea-Lys (Ahx)-HBED-CC (positron emission tomography) tracer. ACS Omega 2017;2:7120-6.
27. Schottelius M, Wurzer A, Wissmiller K, Beck R, Koch M, Gorpas D, *et al.* Synthesis and preclinical characterization of the PSMA-targeted hybrid tracer PSMA-I and F for nuclear and fluorescence imaging of prostate cancer. J Nucl Med 2019;60:71-8.
28. Iudicello A, Boschi S, Ghedini P, Lohr F, Panareo S. Optimization of precursor preparation in PSMA-11 radiolabeling to obtain a highly reproducible radiochemical yield. Pharmaceuticals (Basel) 2022;15:343.
29. Aerts J, Ballinger JR, Behe M, Decristoforo C, Elsinga PH, Faivre-Chauvet A, *et al.* Guidance on current good radiopharmacy practice for the small-scale preparation of radiopharmaceuticals using automated modules: A European perspective. J Labelled Comp Radiopharm 2014;57:615-20.
30. Assadi M, Dadgar H. Quality control and GMP synthesis of (68) Ga-prostate-specific membrane antigen-11 for detection of low-and high-grade prostate cancer. World J Nucl Med 2020;19:93-8.
31. Fuscaldi LL, Sobral DV, Durante AC, Mendonça FF, Miranda AC, da Cunha ML, *et al.* Standardization of the [(68) Ga] Ga-PSMA-11 radiolabeling protocol in an automatic synthesis module: Assessments for PET imaging of prostate cancer. Pharmaceuticals (Basel) 2021;14:385.