Original Article

Automated Radiosynthesis, Quality Control, and Biodistribution of Ga-68 Pentixafor: First Indian Experience

Abstract

Background: Chemokine receptor CXCR4 is overexpressed in more than 27 different human tumors that make it a promising target in oncology. Ga-68 Pentixafor is the most promising positron emission tomography tracer for imaging CXCR4 receptors; hence, the present study was carried out to optimize the radiosynthesis of Ga-68-Pentixafor using fully automated method and the quality control (QC) checks were performed before being used as a clinical product. We also studied the normal biodistribution pattern of Ga-68-pentixafor intended for the use in variety of malignancies. **Materials and Methods:** We optimized the automated radio-synthesis of Ga-68 Pentixafor under good manufacturing practice conditions. A total of 62 productions were carried out in a span of 4 years. Extensive QC tests were performed to check for potency, identity, efficacy, and stability of the tracer. Biodistribution of Ga-68 Pentixafor was investigated in a healthy volunteer to determine normal range of standardized uptake value$_{maximum}$ (SUV_{max}) values in various organs. **Results:** The radiotracer was prepared successfully in 57/62 productions with radiochemical purity of >99%. Mean radiolabelling efficiency of 73.1% ± 7.7% (n = 57) was obtained with synthesis time approximatively of 34 min. The radiolabeled complex showed no signs of dissociation up to 4 h at the room temperature. Ga-68 Pentixafor upon incubation with human serum was found to be stable at 37°C for 4 h. The highest normal organ uptake was seen in urinary bladder (SUV_{mean} = 146.0), spleen (SUV_{mean} = 6.80) followed by kidneys (SUV_{mean} = 4.99). **Conclusion:** Using the automated radiosynthesis, Ga-68 Pentixafor exhibited good radiolabelling efficiency with excellent *in vitro* and *in vivo* stability and favorable biodistribution showing clinical applicability of the tracer.

Keywords: *CXCR4, Ga-68 pentixafor, normal biodistribution, positron emission tomography/computed tomography, quality control, radiolabelling*

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Introduction

Chemokine receptor, CXCR4 is the most widely expressed receptor on malignant tumors and their role in tumor biology has been studied extensively.[1,2] CXCR4 upregulation has been reported in more than 27 different epithelial, mesenchymal, and hematopoietic cancers.[3] CXCR4 and its ligand CCL12 (or stromal cell-derived factor-1 α) plays a salient role in oncology predominantly in tumor progression, local invasion, and distant metastasis.[4] CXCR4 expression has been primarily assessed using tissue sampling which is not accurate representation of the overall disease burden in the patient; hence, a more versatile approach was needed to map the overall CXCR4 overexpression.[5] CXCR4 targeting can be potential game changer in both imaging and treatment of certain oncological conditions. Noninvasive positron emission tomography (PET) imaging of CXCR4 has been recently explored as a complimentary diagnostic or prognostic biomarker leading to certain CXCR4 targeted theranostic approaches.[6] The most promising candidate that translated from preclinical settings to clinical scenario is Ga-68 Pentixafor. The initial proof of concept studies in-human showed promising results with high target to background ratio, metabolic stability, and high specificity for CXCR4 receptors.[7-9] In this study, we optimized the radiolabeling process of Ga-68 Pentixafor and validated its clinical usability through the essential quality control (QC) tests as laid down by the relevant international bodies/pharmacopoeias.

Materials and Methods

Radiosynthesis of (Ga-68 pentixafor)

Standardization of labelling of Ga-68 with Pentixafor was done under Good

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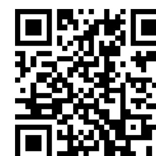

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manufacturing practice (GMP) condition in fully automatic synthesizer (Scintomics, Munich, Germany). This cassette-based GRP™ module was procured under the DST-FIST grant (Government of India). Three multiple stopcock manifolds cassette (ABX, Germany) that comes with in fitted Sep-Pak light C18 cartridge was used for the labelling process [Figure 1]. Various other standard reagents used were as follows:

1. Peptide – CPCR4.2 trifluoroacetate (20 µg)-(ABX, Germany)
2. PS-H + Cartridge
3. Sodium Chloride-(5M, 1.7 ml)
4. Ethanol – 5 ml
5. Ethanol/Water (1/1) – 2 ml
6. Phosphate buffer saline (PBS) – 20 ml
7. HEPES Buffer 1.5M
8. Water for injection (100 ml).

Labelling procedure

The labelling process was controlled by the default software attached with automatic synthesizing module. synthesis sequence for labelling of Ga-68 peptides was loaded to module to carry out the whole process.

68Ge/68Ga generator (ITG, Schwaig, Germany) was eluted with 0.05M HCl (4 ml) to elute Ga-68. This eluent was transferred to the automatic synthesising module. Lyophilized precursor CPCR4.2 (20.0 µg) dissolved in variable concentrations of HEPES buffer was introduced into the reactor vial. The cassette and reactor vial were installed on GRP automatic module and dedicated synthesis sequence was loaded. 68Ga3+ ions were trapped on PS-H+ cation exchange cartridge followed by flush of nitrogen gas (4 bar). Activity was eluted using 1.7 ml of 5M NaCl into the preheated reactor at 125°C. Labelling process was carried out for 6 min at 125°C. After allowing the solution to cool, the reaction mixture was passed onto the light C-18 cartridge to separate unlabelled 68Ga3+ from the labelled product using 12 ml of water. Final elution of the labelled peptide was done using 2 ml of ethanol and transferred into sterile vacuum vial by passing through 0.22 µm Cathivex-GV filter (Millipore, Massachusetts, USA). The final product was obtained by diluting with 15.0 ml PBS buffer[10] [Figure 2].

Radiolabelling efficiency

For each production of the radioligand, radiolabelling efficiency was documented. Initial activity of the eluant (A0) at the start of synthesis was measured and decay corrected activity (Ad) for the time elapsed (34.0 min) was computed. Specific activity of the resultant radioligand (Ap) was measured after the end of synthesis. Decay corrected radiolabelling efficiency was calculated as:

Radiolabelling Efficiency = $(A_p/A_d) \times 100$

Optimizing the reaction volume with HEPES buffer

Buffer is essential component in determining the labelling efficiency and specific activity in a given radiolabelling procedure. (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) HEPES is a Zwitterionic buffering agent of choice for 68Ga-Complexations but the only limitation is of the concentration of buffer (200.0 µg) in the final injection volume.[11,12] Hence, we standardized the volume of HEPES to be used to dilute the peptide. A standard of 3 ml of 1.5M HEPES buffer is available as a part of the standard reagent kit. Variable volumes of 3 mL, 2.5 mL, 2 mL, and 1.5 mL of HEPES buffer were used in different productions. Radiolabelling yield was checked and documented for each volume of HEPES buffer.

Quality control of radiolabelled Ga-68 pentixafor

Since, Ga-68 Pentixafor is intended for human administration, it was imperative to perform strict QC checks before human administration. QC checks involved several parameters that ensure purity, potency, biological safety, specific identity, and radiopharmaceutical efficacy. Hence, the synthesized Ga-68 Pentixafor was subjected

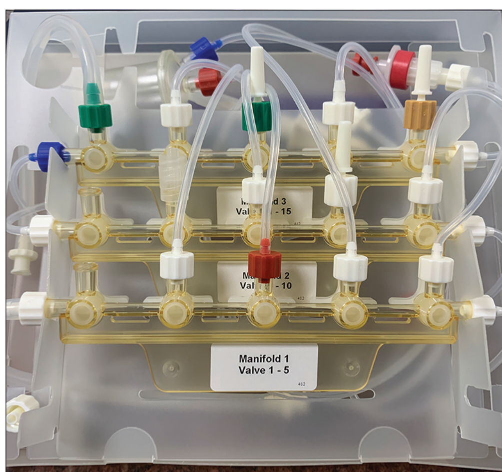

Figure 1: ABX-cassette with 3 manifolds used for automated synthesis of ^{68}Ga-pentixafor

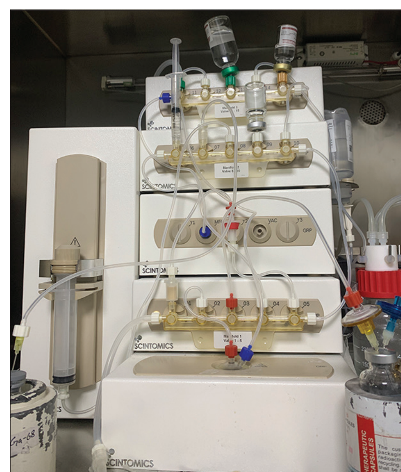

Figure 2: ABX-cassette mounted over the GRP™ module prior to carrying out the synthesis of ^{68}Ga-pentixafor

to the following QC tests before administration into the patients.

pH

A radiopharmaceutical should have an appropriate hydrogen ion concentration or pH in order to be suitable for human administration. Hence, for every batch of production of Ga-68 Pentixafor, pH was checked using narrow range pH paper. A drop of the final labelled product was spotted on the pH paper and the colour intensity was matched with the given reference color to document the pH.

Radionuclide purity

Radionuclide purity is defined as the percent of the total radioactivity in the form of the desired radionuclide present in a radiopharmaceutical. Radionuclide impurities result from undesired nuclear reactions or breakthrough of the parent radionuclide. These impurities result in undesired radiation burden to the patients.

Radionuclide purity was documented by determining the half-life (T1/2). Initial reading of radioactivity (A0) and activity after every 10 min (A10) were recorded. T1/2 was computed using the following formula:

$$T_{1/2} = 0.693t/2.03\ \mathring{A} \sim [Log\ A_0 - Log\ A_{10}]$$

Radiochemical purity

Radiochemical purity (RCP) of a radiopharmaceutical is defined as the fraction of the total radioactivity present in the desired chemical form in a radiopharmaceutical. The presence of radiochemical impurities can be because of various reasons including change in temperature, light or pH, decomposition due to radiolysis, etc., Hence, acceptable RCP in a diagnostic radiopharmaceutical is extremely important parameter to be checked.

RCP of the final labelled product was assessed using 10 cm × 1 cm silica gel instant thin-layer chromatography (SG-ITLC) strip with two different mobile phases:

1. A spot of 5.0 μL of the radiolabelled complex was spotted at the origin front (1.0 cm from the bottom) of the SG-ITLC strip. SG-ITLC strip was suspended in the mobile phase of 0.1 M aq. sodium citrate. The radioactivity profile and Rf were documented using TLC radioactivity scanner
2. Second mobile phase was made 1:1 (v/v) ratio of 1.0 M aq. NH4 OAc and MeOH. Similar SG-ITLC strip was used and scanned on TLC radioactivity scanner.

In vitro stability

Stability of radiopharmaceutical is the essential parameter to evaluate safety, quality during the validity period. Ideally, a PET radiopharmaceutical should not show the signs of dissociation or radiolysis with time. The *in vitro* stability of the radiolabelled formulation was documented by measuring the radiolabelling efficiency at five different postsynthesis time intervals of 15 min, 30 min, 1 h, 2 h, and 4 h.

Serum stability

The stability of the radiotracer with human serum needs to be checked for any dissociation of radiometal. Hence, the serum stability of radioligand was evaluated by incubating 0.8 mL of normal human serum with 0.2 mL of the radiolabelled complex in a vial at 37°C. The percent radiolabelling efficiency of the radio complex was documented at four different incubation intervals of 15-min, 1, 2, 3 and 4 hour, respectively.

Normal biodistribution of Ga-68 pentixafor

To understand and quantify the normal bio-distribution pattern of Ga-68 Pentixafor, whole body PET/computed tomography (PET/CT) was performed in one healthy volunteer. After the preparation and QC of the radioligand, 100.0 MBq of Ga-68 Pentixafor was injected intravenously. Whole body PET/CT images were acquired at 60 min postinjection. Low dose CT scan (current 20 mAs, voltage 120KeV, tube rotation 0.5 sec, pitch 0.98:1) was acquired without intravenous contrast enhancement. PET emission scan was acquired for 9 bed positions (3 min/frame). Attenuation correction was applied, and data were reconstructed using iterative reconstruction (2 iterations, 24 subsets).

Semi quantitative analysis was done by drawing Region of intrest (ROI's) to compute SUV_{mean} and SUV_{max} values. Fixed spherical ROI's (50 cm^3) were drawn over brain, nasopharynx, thyroid, aortic arch, lung, heart, liver, stomach, spleen, pancreas, kidneys, adrenal, intestines, rectum, bladder, muscle (gluteal, pectoral), bone (appendicular, axial), and vertebra, respectively.

Results

Radiosynthesis of (Ga-68 pentixafor)

Each sequential production of Ga-68 Pentixafor under complete GMP environment was carried out using Automatic synthesize and ready to use kits. This automated radiolabeling process for the production of the final product was executed within 34 min. The final radio-labelled product was obtained in the product vial as 16.0 mL solution. The multiple step synthesis process was monitored through resultant chromatograms on the default software on the laptop attached with the automated module.

The representative chromatograms depicting the different chemical reactions and purification steps toward the final production of ^{68}Ga-Pentixafor using the automated chemistry synthesis module are presented in Figure 3.

Radiolabeling yield

A total of 62 productions of ^{68}Ga-pentixafor were synthesized and used for PET imaging in the study participants over a

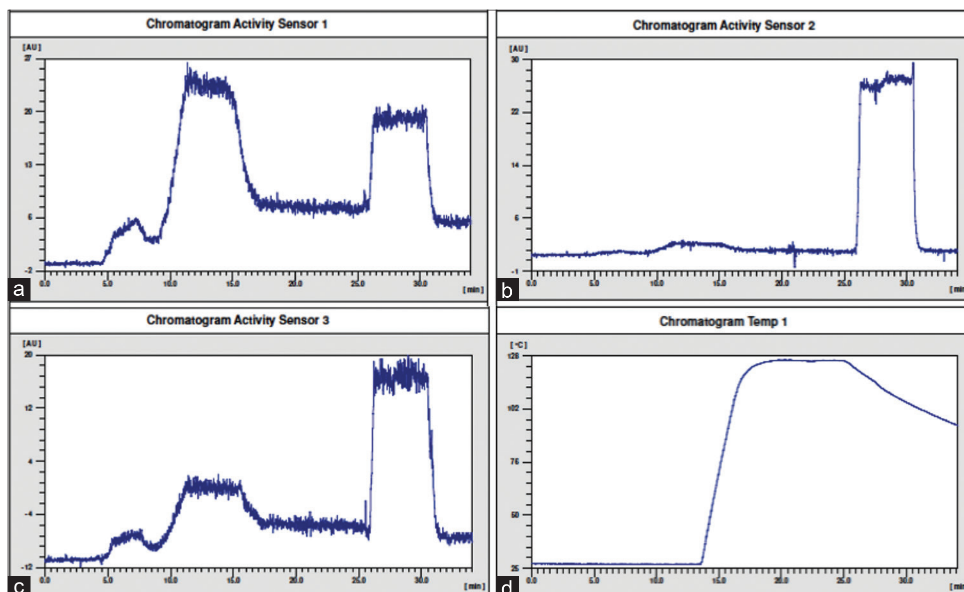

Figure 3: (a) The time activity curve from the radioactivity sensor (γ1) showing peak activity between 10 and 15 min depicting transfer of the activity of Ga-68 eluent to the reactor, (b) time activity curve from the radioactivity sensor (γ2) showing peak activity between 26 to 32 min depicting trapping of the final labelled product on the light C-18 cartridge, (c) time activity graph from the radioactivity sensor (γ3) showing peak activity between 26 to 32 min depicting transfer of the final labelled product to the product vial, (d) temperature-time graph showing peak temperature of 125°C from 14 to 25 min of the radiolabeling process

period of 4 years. The desired radiolabeling was achieved in 57/62 productions, whereas synthesis failed in 4/62 productions and in the remaining one production, a very poor radiolabeling of 17.0% was achieved and hence not used for patients' imaging. Overall, the mean radiolabeling efficiency of 73.1% ± 7.7% (n = 57) was obtained for the production of ^{68}Ga-pentixafor [Figure 4].

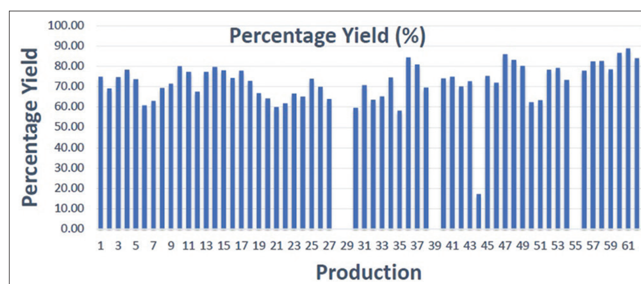

Figure 4: The graphical presentation of the radiolabeling efficiencies of the individual productions of Ga-68 pentixafor

Quality controls of Ga-68 pentixafor

pH

The mean pH of the solution of the final product of ^{68}Ga-Pentixafor was found to be 7.53 ± 0.43 (range 7.0–8.0, n = 57) which was very much in the physiological range (5.5–8.0) and hence was suitable and safe for human administrations.

Radionuclide purity

The presence of any undesired radionuclide in the final formulation was determined by the radioactivity decay-based half-life determination method. Mean half-life as determined on ten random productions was found to be 66.95 ± 1.91 min. Thus, we found that the experimental half-life value was in excellent agreement (accuracy of about 99.0%) with the theoretical half-life value of 68 min for Ga-68.

Radiochemical purity

RCP of the radiolabeled complex was assessed by ITLC using two different solvent systems. Radio-chromatogram on both the mobile phase, i.e. 0.1M Sodium Citrate and 1M Ammonium acetate: Methanol (1:1) showed the presence of a single peak. The radiolabeled product of Ga-68 Pentixafor moved to solvent front and nothing was seen on the origin front. The retardation factor of Ga-68 Pentixafor was found in the range of 0.15–0.25. The area under the peak was 100.0% inferring that the final formulation had nearly 100.0% RCP. Radio-chromatogram of ^{68}Ga-Pentixafor in two different mobile media is shown in Figure 5.

In vitro stability

Once the formulation was successfully labelled and has shown good radiolabeling efficiency, it is further checked for *in vitro* stability over an extended period for up to 4 h. This test was performed to check the degree of dissociation, if any, of the radiolabeled product.

The *in vitro* stability of Ga-68 Pentixafor was checked for up to 240 min (4-h) by running the ITLC (as described above by using both the solvents) for estimating the radiolabeling efficiency periodically at six time points of 15, 30, 45, 60, 120, and 240 min, respectively. It was observed that the radiolabeled product remained stable for up to 4 h as no dissociation of the radiolabeled product

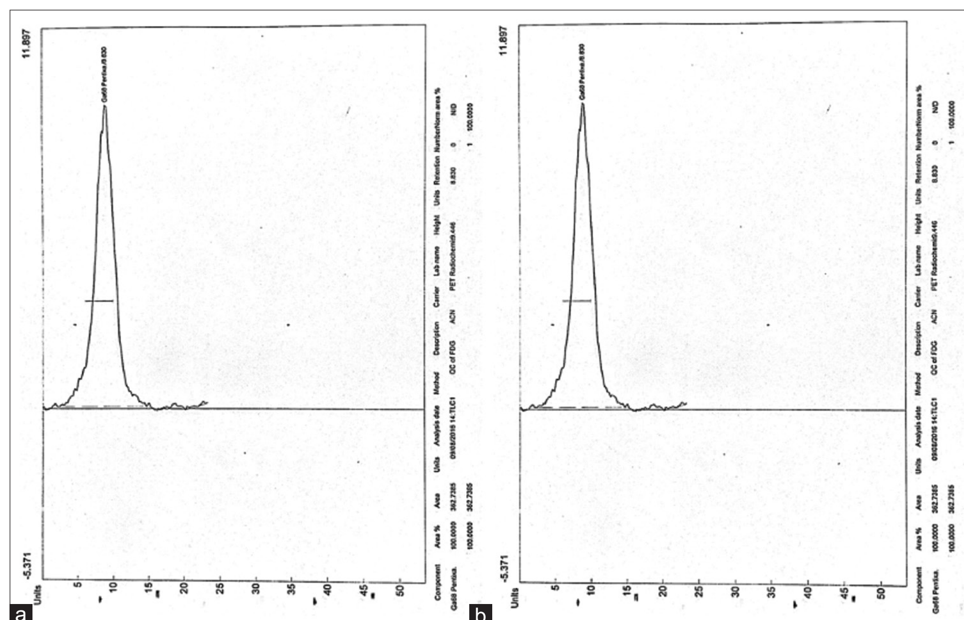

Figure 5: (a) Chromatogram for Ga-68 Pentixafor using 0.1M Na-Citrate as solvent and silica gel instant thin layer chromatography as stationary phase, (b) Chromatogram for Ga-68 Pentixafor using 1.0M Ammonium acetate: Methanol (1:1) as solvent and silica gel instant thin layer chromatography as stationary phase

of Ga-68 Pentixafor was observed for till this time point. This finding suggests that the radiolabeled complex can be used with confidence till 4 h. Radio-chromatograms of ^{68}Ga-Pentixafor acquired at different time points are shown in Figure 6.

Serum stability

The stability of the radiolabeled product of Ga-68 Pentixafor is also needed to be determined in human serum samples before its intravenous administration as the same was intended to be used in patients.

The radiolabeled product of Ga-68 Pentixafor was incubated with human serum samples and its stability was checked by ITLC scanner method at varying time points of 15 min, 1 h, 2 h, and 4 h. The product was found to be remaining stable in serum till 4 h [Figure 7]. This demonstrated that the product after administration to patients does not dissociate and is available in blood for specific binding at the intended receptors/targets till 4 h. Although, for most of the PET procedures, the imaging is done at 1 h after the tracer administration, yet longer serum stability of the product provides as additional advantage when delayed imaging is needed beyond 1 h, especially in dosimetry studies with new investigational radiopharmaceuticals.

Reaction volume optimization for improving the radiolabeling yield

Optimizing the reaction volume is critical to achieve an optimal radiolabeling yield and efficiency. The reaction volume was titrated with HEPES buffer and it was found that the use of 1.5 mL of buffer provided highest radiolabeling yield of 76.75% ± 6.00%. The use of other three higher buffer volumes of 2.0 mL, 2.5 mL, and

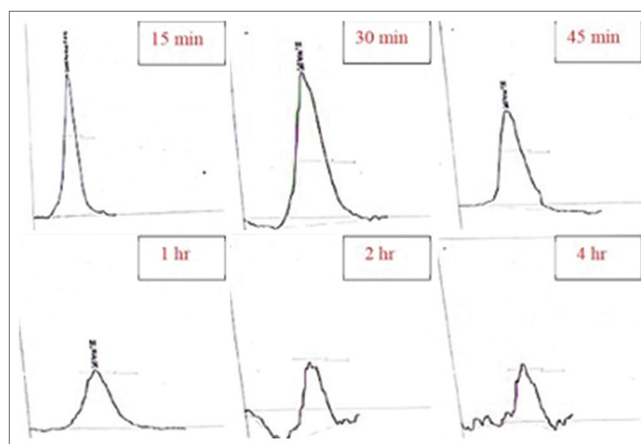

Figure 6: Radio-chromatograms of Ga-68 pentixafor showing single peak at different time intervals of 15-min, 30-min, 45-min, 1h, 2-h, and 4-h demonstrating no dissociation of the product

3.0 mL provided significantly ($P < 0.001$) lower percent radiolabelling yields of 67.96 ± 7.45, 69.38 ± 6.83, and 67.40 ± 7.23, respectively [Figure 8]. HEPES buffer of 1.5 mL volume was used in the final reaction. Therefore, HEPES buffer volume of 1.5 mL is precisely used, which persistently yielded a radiolabeling yield of close to 80.0%.

Normal physiological biodistribution of Ga-68 pentixafor

An understanding of the normal physiological biodistribution of a new investigational radiotracer is critical from the point of view of investigating the patients' safety, radiation absorbed doses to body organs and route of tracer excretion which has significance in the accurate image interpretation as normal or abnormal.

The whole-body PET data were reconstructed and displayed as cross-sectional, coronal and sagittal images to visualize the physiological distribution in all the organs and in the entire body. The reconstructed data sets of images [Figure 9] were subjected to a semi-quantitative analysis and the SUV_{mean} and SUV_{max} values as a measure of the tracer uptake in various organs and regions were evaluated. The maximum intensity projection (MIP) image demonstrated highest tracer accumulation in the urinary bladder, spleen followed by kidneys and in the nasopharynx. The axial skeleton showed moderate and in-homogenous tracer uptake. A representative whole body Ga-68 Pentixafor MIP PET image in a healthy volunteer acquired at 1 h after tracer administration is presented in [Figure 10]. The image quantification indicated that the SUV_{mean} and SUV_{max} values for urinary bladder, spleen, kidneys, nasopharynx, and liver were (146.0; 239.0), (6.80; 10.10), (4.99; 20.55), (3.80; 8.30), and (1.30; 3.20), respectively [Table 1 and Figure 11].

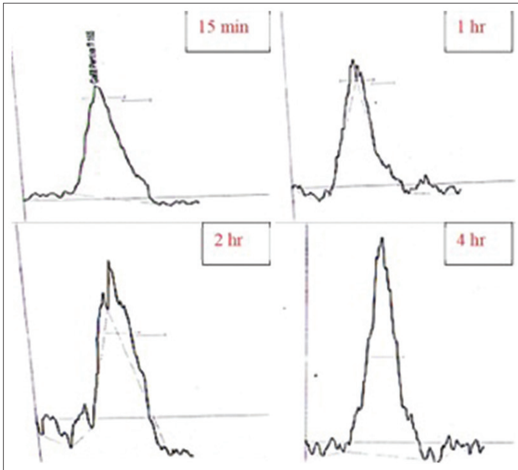

Figure 7: Radio-chromatogram of ^{68}Ga-pentixafor incubated with human serum samples demonstrating stability (single peak) at different time points

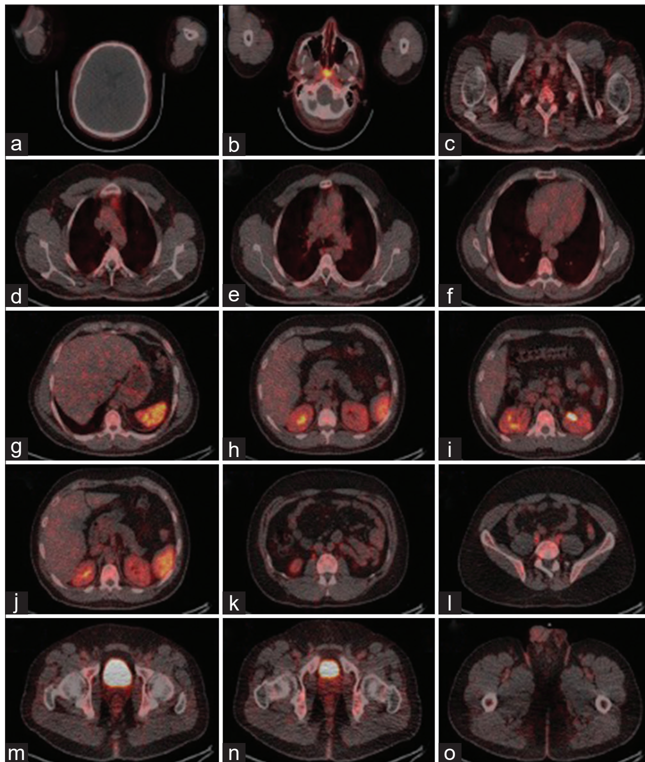

Figure 9: ^{68}Ga-Pentixafor positron emission tomography/computed tomography transaxial images showing accumulation of Ga-68 Pentixafor in normal organs (a) brain (b) nasopharynx (c) thyroid (d) aortic arch (e) lungs (f) myocardium (g) stomach, liver and spleen (h) pancreas (i) adrenals (j) kidneys (k) L1 vertebra (l) cecum (m) urinary bladder (n) gluteal muscle (o) femur. The SUV_{mean} and SUV_{max} values of the tracer on the transaxial images over these regions were computed and reflected the normal physiological uptake pattern of the tracer

Discussion

Ga-68 Pentixafor is the most promising CXCR4-targeted high-affinity nuclear probe, developed by Wester's group from Germany[8,13] Prior to using any new radiotracer in patients, a comprehensive standardization and optimization of the labelling procedure, extensive QC testing needs to be performed. This is the first study to report the automated synthesis of Ga-68 Pentixafor with

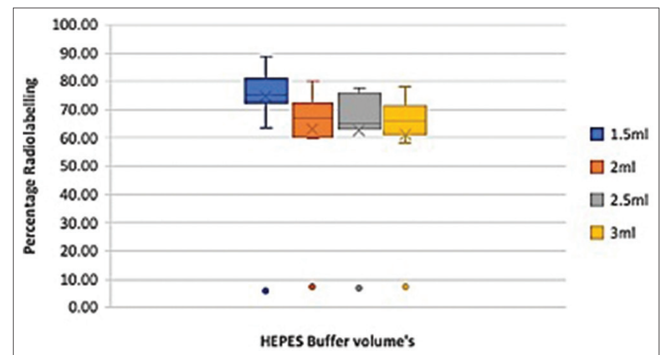

Figure 8: Box-and-whisker plots demonstrating that the maximum radiolabeling yield was achieved when the HEPES buffer volume was adjusted to 1.5 mL

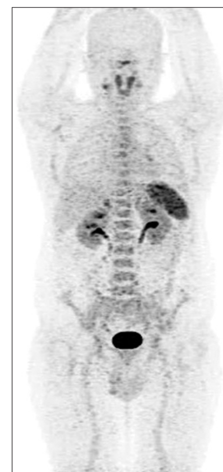

Figure 10: Whole body Ga-68 Pentixafor positron emission tomography/ computed tomography maximum intensity projection image in a healthy male volunteer showing accumulation of the radiotracer in kidneys, urinary bladder, spleen, nasopharynx and moderate and inhomogeneous uptake in the entire axial skeleton. No visible uptake was noted in brain

Table 1: Standardized uptake value$_{mean}$ and standardized uptake value$_{maximum}$ values as a measure of 68 Ga-pentixafor uptake in various organs, tissues and bone in a normal male human volunteer

Organs	SUV (max)	SUV (avg)
Brain left lobe	0.1	0
brain right lobe	0.2	0
Nasopharynx	8.3	3
thyroid left lobe	1.8	0.6
Thyroid right lobe	1.6	0.6
Aortic arch	3.9	1.5
Left lung	1.9	0.9
Right lung	1.9	0.8
Heart	3.7	1.5
Liver	3.7	1.3
Stomach	5.4	1.3
Spleen	12	5.9
Pancreas	2.6	0.9
Right kidney	11	4.1
Left kidney	22	4
Right Adrenal	4.2	2.1
Left Adrenal	4	1.8
Rectum	3	1.5
Cecum	2.2	1.1
Bladder	239	146
Appendicular bone	2.6	0.4
Axial bone	2.3	1.6
Glutel Muscle	1.5	0.5
Right pectoral muscles	0.8	0.3
Left Pectoral Muscles	0.8	0.4
L1 vertebra	6.2	2.5

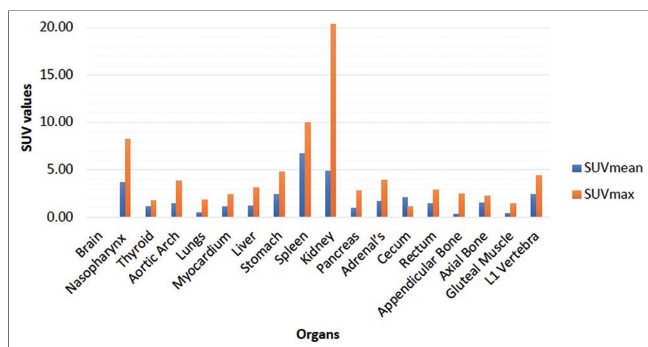

Figure 11: The histogram for SUV$_{mean}$ and SUV$_{max}$ values of Ga-68 Pentixafor in different organs (the values for urinary bladder going out of the chosen scale were excluded) in a normal human volunteer

substantial number of production and its extensive QC checks. The automated synthesis of Ga-68 Pentixafor was easy, yielding the final product within 34 min. Excellent radiolabelling yield was obtained that was in consonance with other clinically approved ^{68}Ga-labelled products which had been in use for human PET imaging. In our expanded panel of QC tests on Ga-68 Pentixafor product, very high RCP of nearly 100.0% was obtained and the product demonstrated excellent *in vivo* and *in vitro* stability. We are also the first one to document the normal biodistribution in a healthy volunteer. This is important information for generating ^{68}Ga-Pentixafor PET database for documenting the normal distribution of CXCR4 expression in human body organs' and for reporting its excretory pattern.

Conclusion

Ga-68 Pentixafor can be labelled with ease using an automated synthesizing module and the final product exhibited excellent metabolic stability implicating its clinical use with greater reliance. The understanding of its normal biodistribution in the human body is obligatory for gaining confidence in identifying the abnormal tracer foci on PET imaging. Since, there are more than 30 human malignancies which are known to over-express CXCR4 receptors; therefore, the applications of Ga-68 Pentixafor PET/CT targeting these receptors can be potential game changer in oncology in both imaging and theranostics.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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