Original Article

Standardization and Clinical Use of a Single-vial Formulation of Technetium-99m-Trodat Using Autoclave Method

Abstract

Background: Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the substantia nigra. SPECT imaging using technetium-99m [^{99m}Tc] labeled trodat is the choice of imaging to differentiate PD from its other forms like drug-induced PD. **Aims and Objectives:** The main objective of our study was to prepare in-house sterile formulation of [^{99m}Tc]Tc-trodat and use in clinics. **Materials and Methods:** The labeling of trodat was standardized using glucoheptonate sodium salt (GHA), stannous chloride dihydrate (in 0.05 N HCl), and ethylenediaminetetraacetic acid (Na-EDTA). The preparation was mixed and autoclaved at 15 psi for 15 min. The standardised formulation was stored at 4°C, -20°C and -80°C and labeling with ^{99m}Tc was tested for up to 6 days. The radiochemical purity, chemical impurities, and endotoxin levels were tested. The frozen formulation was tested in swiss mice (n = 3) for biodistribution studies at 4 h. Around 18 ± 2 mCi was injected intravenously in each patient (n = 5) and the image was acquired at 4 h post-injection. **Results:** The radiochemical purity of the preparation was 98.3 ± 1.4% with a retention time of 16.8 ± 1.5 min as compared to 4.0 ± 0.5 min for free ^{99m}Tc. Animal distribution showed highest uptake in liver and dual excretion via hepatobiliary and renal system. [^{99m}Tc]Tc-trodat imaging was able to differentiate both caudate and putamen. **Conclusions:** In-house frozen preparation was advantageous, as it has decreased the chance of manual error as compared to daily make up formulations and economical as compared to commercially available kits.

Keywords: *Parkinson's disease, single-photon emission computed photography, technetium-99m, trodat*

Riptee Thakur, Chandana Nagaraj, Raman Kumar Joshi, Jitender Saini, Ravi Yadav[1], Pardeep Kumar

Departments of Neuroimaging and Interventional Radiology and [1]Neurology, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, India

Introduction

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta and the presence of intercellular protein alpha-synuclein.[1,2] The diagnosis and management of PD are very challenging due to the similarity of the idiopathic PD with other neurodegenerative disorders such as dementia with Lewy bodies, corticobasal degeneration, multiple system atrophy, and progressive supranuclear palsy.[3,4]

The imaging plays a decisive role in the diagnosis of PD, though routine diagnostic modalities such as computed tomography (CT) and magnetic resonance imaging are less helpful in the earlier stages. Thus, functional imaging like single-photon emission CT (SPECT) and positron emission tomography (PET) has played a major role in clinical diagnosis and understanding of the underlying pathophysiology of PD.[5,6] SPECT imaging is more economical as compared to PET in developing countries. There are several SPECT agents are available like Technetium -99m [^{99m}Tc]Tc-trodat, iodine-123 [123I]-ioflupane, [123I]-N-ω-fluoropropyl-2 β-carbomethoxy-3 β-(4-iodophenyl) norstropane (FP-CIT).[7,8] Due to the nonavailability of iodine-123 or easier availability of ^{99m}Tc, the latter is commonly used and ([^{99m}Tc]Tc-trodat is the choice of agent for SPECT imaging in the PD cases [^{99m}Tc]Tc-trodat is a successful imaging agent for dopamine transporter imaging.[9] A substantial number of studies have proved its role in the diagnosis of PD and its ability to differentiate it from various forms of PD.[10-12] [^{99m}Tc]Tc-trodat uptake in the striatum has a strong correlation with PD progression.[13]

Single-vial kits of trodat are commercially available, but the cost of the vial makes the

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Address for correspondence:
Dr. Pardeep Kumar, Department of Neuroimaging and Interventional Radiology, National Institute of Mental Health and Neurosciences, Bengaluru - 560 029, Karnataka, India.
E-mail: pardeep1182@gmail.com

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SPECT scan expensive for poor patients, especially in the developing country where the majority population is not covered by any insurance plan. Many of the laboratories do their in-house labeling for SPECT radiopharmaceuticals. A number of studies have reported in-house preparations, but some have multistep preparation;[14] in order to obtain high radiochemical purity, it recommends high-performance liquid chromatography (HPLC) separation or C18 Sep-Pak solid-phase cartridge purification which is not feasible in routine scenarios.[15] Some of the studies have formulated their formulation in a single-vial kit, but the unavailability of lyophilizer in the hospitals makes it difficult to produce in-house lyophilized kits.

Therefore, we have started in-house preparation of the [^{99m}Tc]Tc-Trodat which is economically cheaper than any commercially available kit. In the present study, we have standardized the procedure of labeling, devised a method which ensures the sterility of the preparation, and also achieved success in formulating a single-frozen vial. We have standardized the preparation, characterized by HPLC and thin-layer chromatography (TLC), and performed other quality control tests.

Materials and Methods

Chemicals and reagents

^{99m}Tc was purchased from the regional center of the board of radioisotope and technology, Kidwai Hospital, Bengaluru, India. Trodat was procured from ABX, Germany, sterile 0.22µ filters were procured from Waters India, and sterile vials were procured from Esente Healthcare, India. All other chemicals such as stannous chloride, sodium glucoheptonate (GHA), sodium-ethylene diamine tetra-acetic acid (Na-EDTA), sodium chloride, acetonitrile, ethanol, and all other solvents were purchased from Loba Chemie (Mumbai, India) and were used without any purification. HPLC system consists of a binary HPLC pump equipped with an ultraviolet/visible (UV) detector (Dionex, Thermo Fisher, California, USA) and a radioactivity detector (Carroll and Ramsey Associates, California, USA). The HPLC analysis was done on C-18, Shim-pack GWS (5 µm, 4.6 × 250) (Shimadzu, Kyoto, Japan) column using a flow rate of 1 mL/min and UV detection at 280 nm with the following solvent (acetonitrile/water) gradient. Water/0.1% trifluoroacetic acid (solvent A) and acetonitrile/0.1% trifluoroacetic acid (solvent B) as mobile phase: 0-5 min-5% solvent B, 5-20 min- 5-100% solvent B, 20-25 min- 100% solvent B, 25-30 min - 100-5% solvent B. TLC was done using normal saline as mobile phase on silica gel-coated aluminum strip (Merck, F254) and scanned on TLC scanner (EZ-SCAN) with multimode radiation detector (OMNI-RAD, California, USA). Gas chromatography was done on Scion GC-436 (Scion 436 GC, The Netherlands) equipped with flame-ionization detection (FID) to evaluate ethanol levels in the final preparation. The column was operated initially at 40°C for the first 3 min and then rose 50°C/min up to 8 min and the final temperature was set at 240°C and the column was BR-200 ms, 0.32 mm ID. The makeup gas consists of helium (28 mL/min), 0 air (300 mL/min), and hydrogen gas (30 mL/min) flow at the rate of 2 mL/min. The endotoxin test was done with Endosafe NexGen-PTS (Charles River, MA, USA) using cartridges. The dilution of the preparation for the test was standardized to 1:100 and 25 µL of the diluted sample was loaded into the wells of cartridge and the test was done. The sterility test was done using tryptic soy broth. SPECT-CT was performed on a Siemens Symbia system (Munich, Germany).

Radiochemistry

10 mg of trodat was dissolved in 2.0 mL of ethanol (stock solution) containing 10% 1N HCl (ethanolic HCl) to form a stock solution of 5 µg/µL. The stock solution was stored at −80°C. On the day of experiment, the solution was taken out and allowed to attain the room temperature. Stannous chloride dihydrate ($SnCl_2.2H_2O$) was prepared fresh each time as 10 mg/mL in 0.1 N HCl and filtered through a 0.22 µ filter for the preparation. Freshly prepared sodium GHA was prepared at a concentration of 50 mg/mL (in deionized water) and sodium-Na-EDTA at 10 mg/mL (in deionized water). The radiolabeling method was standardized for the concentration of GHA (0–5 mg) and EDTA (0–1 mg), and for standardization, 25 µg of trodat was used in each synthesis. The temperature ranged from 37° to 121°C (37°C –100°C was attained in the water bath and 121°C in autoclave). We have also tested the effect of storage of trodat (−80°C, −20°C, and 4°C) on labeling. To this formulation, 45 ± 3 mCi of (^{99m}Tc) Tc in a volume of 500 µL of saline was added and the vial was sealed with rubber cap and aluminum seal. The vial was kept in the autoclave and autoclaved at 121°C under 15 psi for 15 min.

Single-vial frozen formulation

All the constituents were added to a sterile vial (10 mL) and kept stored at 4°C, −20°C, and −80°C. The vial was labeled on the 2nd, 4th, and 6th days of the storage by direct addition of (^{99m}Tc) Tc (diluted with normal saline to make a final volume of 500 µL). The preparations were labeled by autoclaving at 15 psi for 15 min.

Quality control

Physical appearance

The physical appearance of the preparation solution was tested by physical inspection by a naked eye through a lead glass shield.

Radiochemical purity

After incubation, around 10 µL of the preparation was diluted with 200 µL of normal saline and injected into HPLC injector and HPLC was started. 5 µL from the same

dilution was put on TLC strip and TLC was run in normal saline. Once the TLC run complete, the TLC strip was cut into two halves to take counts. A comparison of HPLC and TLC was done to evaluate in any variation between the results of two techniques.

pH

The pH of the radiopharmaceuticals was checked by two methods: one using litmus paper with visual analysis and pH meter.

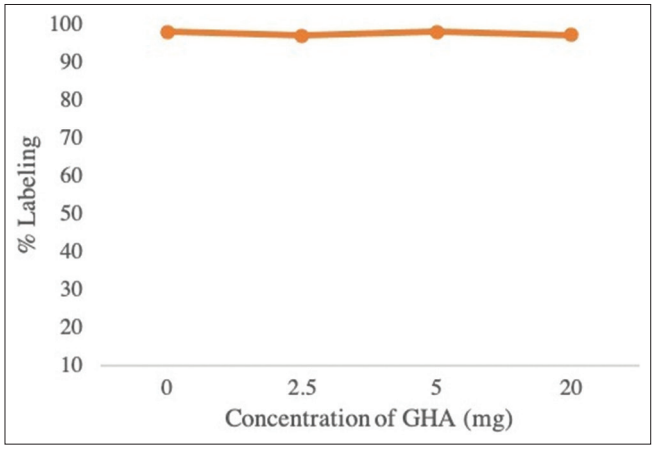

Figure 1: Effect of concentration of the glucoheptonate on the labeling of the TRODAT with (Technetium-99m). GHA: Glucoheptonate

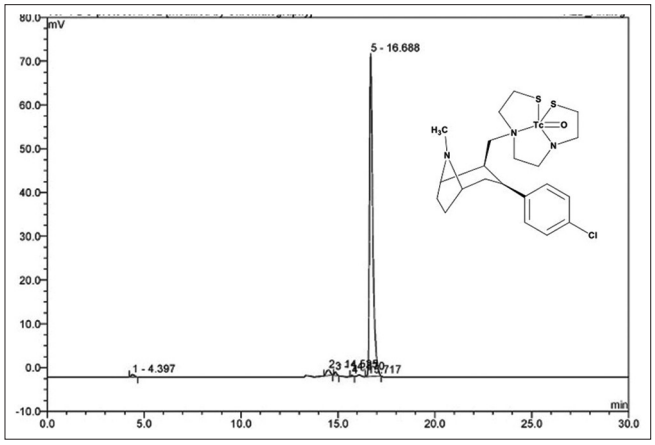

Figure 2: Radio-high-performance liquid chromatography peak for (Technetium-99m [^{99m}Tc])-Trodat at 16.6 min and free (^{99m}Tc) at 4.3 min

Residual solvent

1 μL of the preparation was injected into the injector of gas chromatography (GC) and the method was run for 8 min. It is used to analyze the amount of ethanol content in the final preparation.

Endotoxin test

The endotoxin test was carried out using Endosafe PTS cartridges. The dilution factor of 100 was made for each preparation, and 25 μL of the sample was added to each well. The results were displayed on the screen in 20 min.

Sterility test

10 μL of the [^{99m}Tc]Tc-Trodat was added to the 10 mL of tryptic soy broth in a sterile test tube and was incubated at 37°C. It was daily monitored for the appearance of any turbidity in the media for 14 days.

Stability studies

For testing *ex vivo* stability, around 2.0 mL of blood was collected in a heparinized vial from the human volunteers. The blood was centrifuged at 3000 rpm for 10 min at room temperature to separate the plasma. Twenty microliters of [^{99m}Tc]Tc-Trodat was mixed with 180 μL of plasma and incubated for 60 and 240 min at 37°C in a water bath. An equal volume of separated plasma and chloroform/methanol (4:1, v/v) was mixed and centrifuged at 6000 × *g* for 5 min to precipitate the proteins. The supernatant was used for HPLC analysis.

For testing *in vivo* stability, a blood sample was randomly drawn from patients after intravenous injection of 18 ± 2 mCi of [^{99m}Tc]Tc-Trodat at 1 and 4 h postinjection. Blood samples (into heparinized vials) were collected and processed in the same manner as described above.

Animal biodistribution studies

Animal biodistribution studies were carried out in normal Swiss albino mice (n = 3) after the institute's animal ethics committee approval. A group of mice (15–20 weeks, 25–35 g) were injected intravenously (in tail vein) with 4–6 MBq of [^{99m}Tc]Tc-Trodat diluted with saline (in a total volume of 200 μL), and the mice were sacrificed at 240 min postinjection. Mice were then euthanized by carbon dioxide

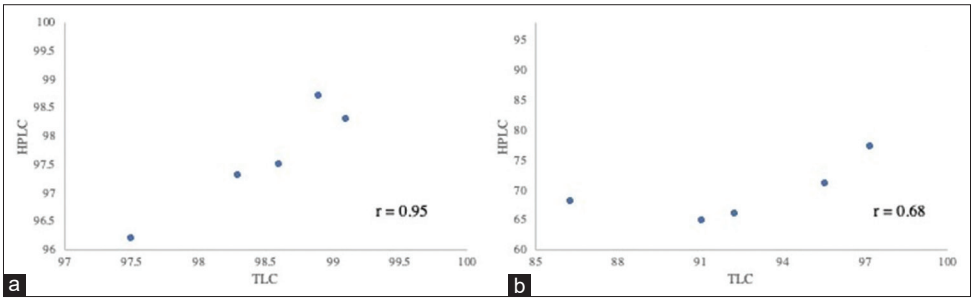

Figure 3: Correlation between high-performance liquid chromatography (HPLC) and thin-layer chromatography technique for measuring labeling efficiency (a) when HPLC showed labeling >95% (b) when HPLC showed labeling efficiency <90%

inhalation, and tissues were dissected, washed free of blood, dried, and weighed. Concomitant radioactivity was counted together with a (^{99m}Tc) standard solution and the injected dose per gram (% ID/g) was then calculated.

Single-photon emission computed photography–computed photography imaging

All necessary permissions and hospital committee approval were taken to use in-house [^{99m}Tc]Tc-Trodat in the patients. Briefly, 18 ± 2 mCi (666 ± 74 MBq) of [^{99m}Tc] Tc-Trodat was injected into each patient (n = 5), and the SPECT-CT scan was acquired at 30 min (to visualize whole-body distribution and evaluate *in vivo* stability) and 4 h postinjection. A dual-head gamma camera SPECT/CT equipped with low energy high resolution was used for imaging in the supine position, with the fixed head using a head holder. Along with SPECT, CT was acquired to localize the anatomical details of the brain. Imaging acquisition was performed in the matrix size of 128 × 128, zoom of 1.23, and 360° arcs with 64 projections and 60 s per projection. Images were reconstructed using FLASH 3D (8 iterations 8 subsets and 6 iterations 16 subsets) using Gaussian filter with full width half maximum 8 mm. All data sets were corrected for photon attenuation using Chang's first-order correction with attenuation coefficient of 0.12/cm applied manually for all projection angles.

The analysis of the images was done by drawing region of interest (ROI) on caudate, putamen, and the occipital area (as a background) and their ratio was calculated. The average count per pixel was calculated for each striatum and occipital ROI.

Results and Discussion

The final preparation protocol was established by varying the concentration of GHA (0–20 mg) and EDTA (1 mg) [Figure 1]. It was observed that the trodat can also be bound directly to (^{99m}Tc) with a labeling efficiency of 70% ± 10%. The low labeling and greater standard deviation explained the role of a weak chelating agent like GHA and EDTA in the preparation. Trodat can be labeled in the presence of the GHA or EDTA with a minimal concentration of 2.5 mg or 0.5 mg, respectively, but it was observed that the absence of GHA in the preparation decreased the *in vivo* stability of the [^{99m}Tc]Tc-Trodat complex. Therefore, we have adopted to use both GHA and EDTA in our preparation. Various combinations were used, and finally, the combinations of the GHA: EDTA in the ratio of 5:1 with a minimal concentration of 2.5 mg of GHA were standardized. The minimum concentration of trodat molecule used for labeling was 25 µg. The effect of storage of trodat was also evaluated, and it was observed that trodat stored at −20°C and 4°C showed low radiochemical purity. The radiochemical purity was 75.5% ± 5.6% and 81.6% ± 6.5% for trodat stored at −20°C and 4°C, respectively. Therefore, the trodat shall be stored at −80°C for getting high radiochemical purity. The radiochemical purity as analyzed by radio-HPLC was 98.3% ±1.4% with a retention time of 16.8 ± 1.5 min as compared to 4.2 ± 0.5 min for

Table 1: Labeling efficiency of the formulation after storing under various conditions

Storage temperature (°C)	Labeling efficiency		
	Day 1	Day 2	Day 6
4	73±4	53±3	44±2
−20	79±2	66±4	23±3
−80	98±1	97±2	89±3

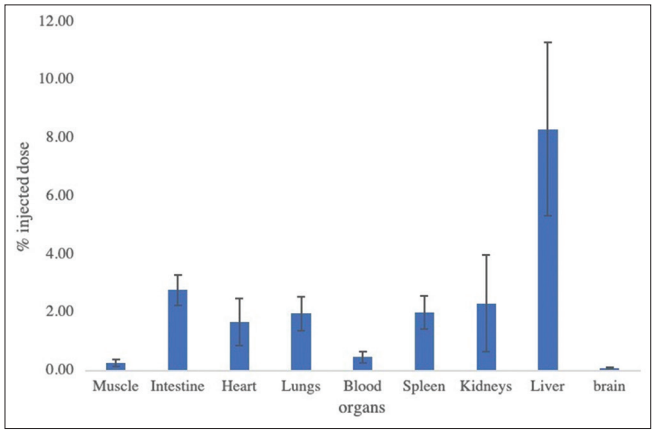

Figure 4: Biodistribution of (Technetium-99m)-TRODAT in mice at 240 min postinjection

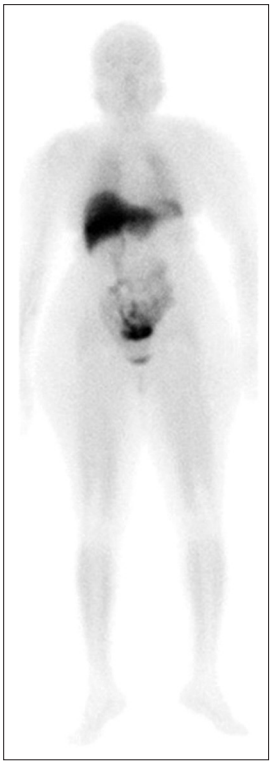

Figure 5: (Technetium-99m)-TRODAT biodistribution in humans

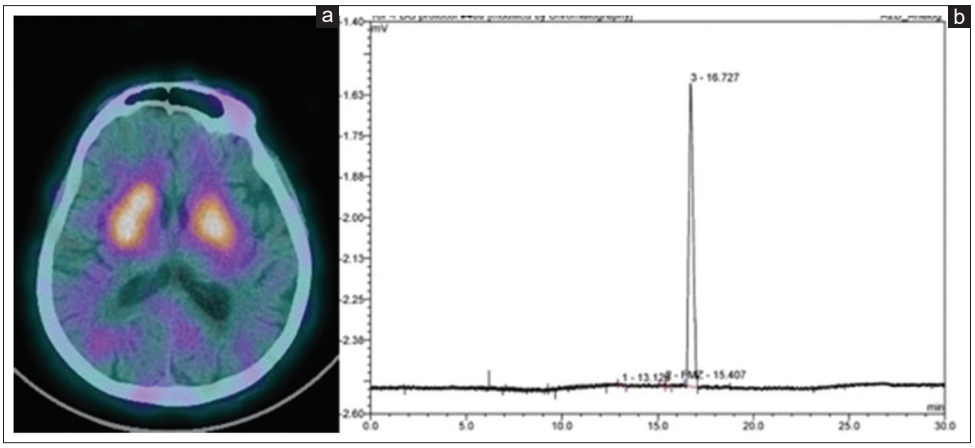

Figure 6: (a) Uptake in brain, uptake can be seen in caudate and putamen regions. (b) Radio-high-performance liquid chromatography peak for (Technetium-99m)-TRODAT proving *in vivo* stability at 4 h

free (^{99m}Tc) [Figure 2]. The TLC results showed a retention factor of 0.8–1.0 for free (^{99m}Tc) and 0.0–0.1 for [^{99m}Tc] Tc-Trodat. The comparison of two techniques (HPLC and TLC) showed that results are in concordance (r = 0.95) with each other for preparation when radiochemical purity was >95% on HPLC evaluation [Figure 3a]. Any preparation that has <95% radiochemical purity on HPLC and TLC showed an overestimation of the labeled product and showed a moderate correlation (r = 0.68) between the two techniques [Figure 3b]. The other drawback of the TLC is that there is no robust method to distinguish various complexes formed during the reaction like (^{99m}Tc) Tc-stannous colloid, (^{99m}Tc) Tc-GHA, or (^{99m}Tc) Tc-EDTA from the retention time of the final product. Therefore, it is very important to adopt TLC as a method for estimating radiochemical purity only after the establishment of the labeling protocol and evaluation of the radiochemical purity by radio-HPLC. All three preparations were standardized and yielded more than 95% radiochemical purity. The labeling efficiency of frozen vials is displayed in Table 1. The frozen formulations which were stored at −80°C and used on the 2nd day showed satisfactory results. Therefore, the frozen vial can be prepared 2 days before imaging and can be used on the day of imaging. This was done to remove any manual (systematic) error that occurred during the preparation. The kits stored at 4°C and −20°C showed low labeling efficiency. The same results were observed when fresh labeling was done. This may be due to the fact that the trodat precursor was not stable at 4°C and −20°C. The preparation was standardized to label in the autoclave to maintain the sterility of the final formulation. Although it was observed that labeling can be achieved at 70°C, autoclaving was found to be a better option to get a sterile formulation. The endotoxin test showed that the level of endotoxin was <1 endotoxin unit per dose, and tryptic soy broth showed no turbidity till the 14th day. This ensured that the preparation was free of any bacterial endotoxins and safe for patient use.

The biodistribution studies [Figure 4] in mice showed maximum %ID of 8.29 ± 2.98 in the liver due to metabolic degradation of the trodat in the hepatocytes. The major excretion route of the radiotracer was through dual pathway, i.e., hepatobiliary route; therefore, the intestine showed high %ID of 2.76 ± 0.53 and through kidneys (2.31 ± 1.65) followed by spleen, lungs, and heart. The brain showed %ID of 0.09 ± 0.02.

The quality of the images was assessed by the nuclear medicine physician (CN). The whole-body image showed a diffuse tracer uptake in the brain, nasal mucosa, bilateral lungs, liver, and intestines in whole-body sweep images at 30 min [Figure 5]. The tracer showed a good uptake in the caudate and putamen regions of the brain. The image quality was satisfied and tracer was stable under *in vivo* conditions [Figure 6]. As per literature, SPECT tracers like [123I]-FP-β-CIT have higher accuracy and superior sensitivity in detecting disease progression than [^{99m}Tc] Tc-Trodat[16] but (^{99m}Tc) being easily available and cheaper radionuclide suited better to our clinics where patients cannot afford expensive scans. Our formulation has high radiochemical purity and provides better trodat images at an economical cost.

Conclusions

[^{99m}Tc]Tc-Trodat was produced with high radiochemical purity and safe for human use. A composition of a weak chelating agent plays a significant role in labeling trodat with (^{99m}Tc) and influences *in vivo* stability. Weaker chelating agents play an important role in the labeling and stability of the complex. The in-house preparation makes trodat scan economical for poor patients. The future goal is to prepare lyophilized vials to make labeling more convenient and error-free.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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