Simplified Multidose Preparation of Iodine-123-β-CIT: A Marker for Dopamine Transporters

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Iodine-123-β-CIT is a SPECT radioligand for dopamine and 5-HT transporters with potential use in Parkinson's disease, schizophrenia and cocaine addiction studies. At present, preparation of no-carrier-added (NCA) [^{123}I]β-CIT is achieved by iododestannylation of a trialkylstannyl precursor with sodium [^{123}I]iodide in the presence of oxidizing agent, followed by preparative HPLC. The purpose of this study was to develop a faster and simpler method for the routine preparation of this radiopharmaceutical. **Methods:** Purification of the labeled compound was accomplished by solid phase extraction (SPE) with a C-18 Sep-Pak Light cartridge, which removed unreacted iodide, reaction reagents, polar side products and tributylstannyl precursor. The tributylstannyl precursor was preferred as starting material over the trimethylstannyl precursor due to its higher lipophilicity, allowing better separation of the labeled product and precursor. A TLC method was developed to assess the radiochemical purity of the final product. **Results:** The method produced [^{123}I]β-CIT in high radiochemical yields (75% ± 4%), with high radiochemical purity (≥98%) and specific activity (>67000 Ci/mmole), in 1.5 hr. The final formulation was sterile and pyrogen free. **Conclusion:** The results obtained by solid phase extraction are consistent with those obtained by the HPLC method; with the advantage that the SPE method does not require solvent extraction, evaporation under reduced pressure or HPLC purification.

Key Words: tributylstannyl; iododestannylation; SPECT; β-CIT; DA transporters; solid-phase extraction

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Iodine-123β-CIT [2β-carbomethoxy-3β-(4-iodophenyl)-tropane, also designated RTI-55], is a SPECT radiotracer that labels dopamine (DA) and serotonin (5-HT) transporters associated with certain pathophysiological processes. It has been shown that [^{123}I]β-CIT labels DA transporters located on the neurons which degenerate in Parkinson's disease (*1*) as well as the 5-HT transporters of the frontal cortex (*2*), whose density decreases in patients with schizophrenia (*3*). DA transporters are also thought to be the primary site of action of cocaine (*4*). The DA, 5-HT and norepinephrine transporters are also the target of some antidepressant drugs (*5*).

β-CIT belongs to a group of metabolically resistant phenyltropane analogs synthesized by Clarke et al. (*6*). Iodine-123β-CIT was developed independently by Neumeyer et al. (*7*) and Carroll et al. (*8*) and its potential for in vivo labeling was confirmed by preliminary SPECT studies in nonhuman primates (*9,10*) and in patients with Parkinson's disease (*1*).

Our routine preparation of the no-carrier-added (NCA) [^{123}I]β-CIT was achieved by oxidative iodination of its trimethyl or tributylstannyl precursor with sodium [^{123}I]iodide in the presence of peracetic acid, followed by HPLC purification, to give a product with radiochemical purity of 97.5% ± 0.5% and specific activity of >10,000 Ci/mmole (*11*). This labeling procedure requires 3 to 3.5 hr and involves several steps, including extraction with organic solvent, evaporation under reduced pressure and purification by preparative HPLC.

To optimize this procedure for routine use, we prefer a faster and easier method for preparing [^{123}I]β-CIT. In this paper, we report a simplified procedure for the preparation of [^{123}I]β-CIT using solid-phase extraction (SPE). For convenience, the products prepared using HPLC purification and those using SPE are designated as [^{123}I]β-CIT-HPLC and [^{123}I]β-CIT-SP, respectively.

MATERIALS AND METHODS

The trialkylstannyl precursors of β-CIT [2β-carbomethoxy-3β-(4-tributylstannylphenyl)tropane, 1], [2β-carbomethoxy-3β-(4-trimethylstannylphenyl)tropane, 2] (Fig. 1) and nonradioactive β-CIT were obtained from Research Biochemicals International (Natick, MA). Iodination kits for both precursors were prepared as previously described (*11*) by dispensing 50 μl (50 μg) each of a 1 mg/ml chloroform solution of the alkylstannyl precursor into 1 ml V-vials, evaporating the solvent to dryness with a stream of N_2, and capping with 13 mm Teflon-lined stoppers. The vials were stored at 4°C protected from light.

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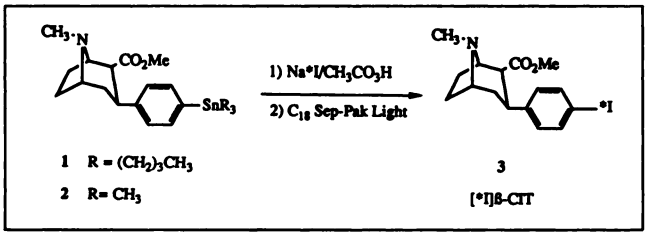

FIGURE 1. Radioiodination of β-CIT.

C-18 Sep-Pak Light cartridges (130 mg, 0.3 ml void volume) were obtained from Waters (Millipore Corp. Milford, MA) and were conditioned by washing with 5 ml absolute ethanol followed by 5 ml sterile water just before use. A technetium kit vial shield cover (Medi Physics, Arlington Heights, IL, 3 mm lead thickness. Half-value layer of ^{123}I for lead is 0.4 mm) was used to shield the Sep-Pak (Fig. 2). No-carrier-added sodium [^{123}I]iodide in 0.1 *M* NaOH (radionuclidic purity >99.8%) was obtained from Nordion International, Ltd. (Vancouver, B.C., Canada). Sodium [^{125}I]iodide was obtained from NEN/Dupont (N. Billerica, MA; specific activity 2,200 Ci/mmole). Peracetic acid (32 wt % solution in dilute acetic acid) was obtained from Aldrich Chemical Co. (St. Louis, MO) and diluted with sterile water before use. All other chemicals were reagent grade obtained from conventional sources and were used without further purification. Sterile water for irrigation USP, without bactericide (McGaw, Irvine, CA) was used throughout the processes.

Preparative and Analytical HPLC

A Spectra Physics 8800 ternary HPLC pump (San Jose, CA), Spectra Physics 200 uv detector (at 254 or at 225 nm) and a Canberra series 20 multichannel gamma detector (Canberra Industries; Meriden, CT) was used with the following systems:

System A: 4μ Novapak C-18 (3.9 × 250 mm) stainless steel column (Waters Associates, Milford, MA)//CH_3OH/H_2O/$(C_2H_5)_3N$ (75/25/0.2), at uv 254 nm and flow rates of 0.7 ml/min or 1.0 ml/min.

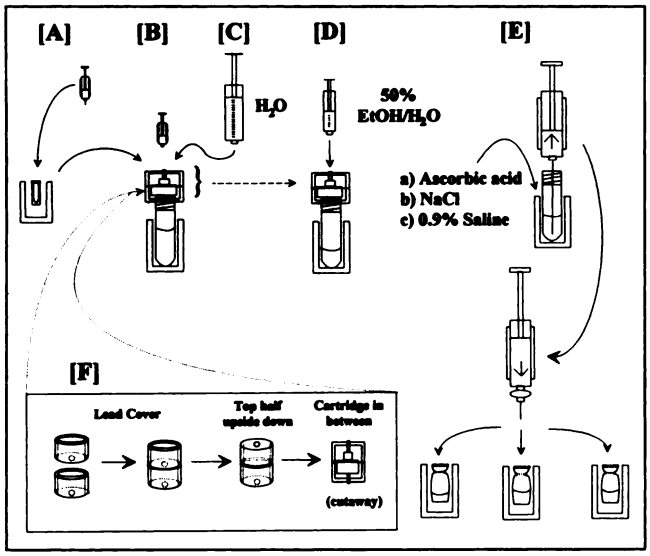

FIGURE 2. General procedure and formulation. (A) Reaction vial; (B) transfer to Sep-Pak; (C) elution of unreacted radioactive iodide; (D) elution of labeled β-CIT; (E) formulation; (F) Sep-Pak lead shielding.

System B: Waters RCM-C18 (8 × 10 mm) cartridge/ $CH_3OH/H_2O/(C_2H_5)_3N$ (95/5/0.2), uv 225 nm or 254 nm, flow rates 3 ml/min or 0.5 ml/min.

Radiolabeling Procedure for [^{123}I]β-CIT-HPLC and [^{123}I]β-CIT-SP

Using 0.5 ml insulin syringes (B-D, Lo-dose, U-100 28G1/2), the following items were added to the sodium [^{123}I]iodide 1 ml shipping vial through the septum: 50 μg (0.094 μmole) tributylstannyl precursor in 150 μl ethanol (sonicated for 3 min to dissolve), a volume of 0.5 *M* H_3PO_4 equal to one-fifth the volume of $Na^{123}I$/NaOH solution, rounded to the nearest 5 units and 50 μl of 0.02 *M* peracetic acid prepared freshly (100 μl 32% CH_3CO_3H in 2.4 ml water). After standing at room temperature for 30–45 min (depending on the $Na^{123}I$ solution volume) the reaction was quenched with 50 μl aqueous $NaHSO_3$ solution (10 mg/ml). For the [^{123}I]β-CIT-HPLC preparation, solvent extraction, evaporation under reduced pressure, HPLC purification and formulation were carried out as previously described (*11*).

For [^{123}I]β-CIT-SP preparation, 500 μl saturated $NaHCO_3$ was added to the reaction vial through the septum and the whole reaction mixture was taken up in a lead shielded 1 ml syringe equipped with a 20-g 1/2 needle (0.9 × 40 mm). After removing the needle, the syringe was attached to a lead-shielded, conditioned C-18 Sep-Pak Light cartridge (kept filled with water until use), which was resting on top a 50-ml sterile conical plastic tube placed inside a lead pot (Fig. 2).

The reaction mixture was added slowly to the cartridge by hand (estimated flow: 1 ml/min). Reaction vial and transfer syringe were rinsed with 1.0 ml sterile water and the wash was added to the Sep-Pak. The 1 ml transfer syringe was removed from the cartridge and replaced with a 20 ml syringe filled with sterile water, eluting the cartridge (flow: 3–4 ml/min). The receiver tube was removed, capped and assayed. A fresh sterile receiver tube was placed under the cartridge, the 20 ml syringe was replaced with a 10-ml syringe containing 7 ml of 50/50 EtOH/water (freshly prepared by mixing 10 ml USP ethanol with 10 ml sterile water) and the [^{123}I]β-CIT was slowly eluted (flow: 1 ml/min).

Formulation

L-ascorbic acid (100 μg; 5.7 μmole; 1 mg/ml) plus 33 mg (0.56 mmole) NaCl and 18 ml of sterile saline solution were added to the tube containing the labeled product. The tube was capped and swirled gently and the solution was taken up in a lead shielded 30 ml syringe using a spinal needle (Quincke type point 18 G 3 1/2; BD, NJ) and filtered (0.2 μ membrane filter, Acrodisc-13, Gelman Sciences, Ann Arbor, MI) into three sterile septum closed 10 ml vials (Lypho Med SV-10, Rosemont, IL) placed in lead pots, to get three doses of the final product, each containing [^{123}I]β-CIT in 13.4% EtOH/8.3 ml 0.9% saline (pH 5.3–5.5).

Quality Control

From each vial, 0.5 ml was removed aseptically with a 0.5 ml insulin syringe and counted (mCi counted × 2 = specific concentration in mCi/ml). The final volume was calculated by counting the remaining radioactivity in the vial (correcting for glass attenuation): corrected mCi / spec Conc = total volume.

From the 0.5-ml sample, an aliquot was used for quality control (radiochemical purity) by TLC, using a 2 × 10 cm aluminum backed silica gel 60 F_{254} strip (EM Science, NJ), hexane/ether/ isopropylamine (70:30:2) and a UV GL-25 mineralight lamp (UVP Inc, San Gabriel, CA). The sample was placed 1.5 cm above the lower edge of the strip, an ethanolic solution of cold β-CIT was

spotted alongside and the chromatogram was developed until the solvent reached 1 cm below the upper edge; the strip was scanned using a homemade TLC scanner equipped with a NaI scintillation detector (calibrated for ^{123}I and ^{125}I). Iodine-123-β-CIT had R_f = 0.54 and [^{123}I]iodide stayed at the origin. The strip was cut into three fragments one cm below and one cm above the cold β-CIT spot, wrapped in plastic film and counted in a well counter.

Radiochemical Purity Calculation

Counts middle fragment divided by the sum of the fragments × 100 = % [^{123}I]β-CIT.

The radiochemical purity of both preparations ([^{123}I]β-CIT-SP and [^{123}I]β-CIT-HPLC) was measured by HPLC (System A, 30 μl sample) immediately after formulation and again after storage at room temperature for 26–70 hr. In the case of [^{125}I]β-CIT-SP and [^{125}I]β-CIT-HPLC preparations, the stability was measured from 24 hr to 5 wk stored at 0–4°C.

Sterility and Pyrogenicity Tests

Pyrogenicity was measured with the Limulus Amebocite Lysate test (LAL; Endosafe, Charleston, NC), after 1:1 dilution of the sample with saline solution. Sterility was confirmed by lack of growth in two media: fluid thioglycolate and casein soya (Remel, Lexena, KS).

Specific activity was measured by comparing the HPLC uv response of the sample with that of known concentrations of β-CIT. Radiochemical yield was calculated by dividing the radioactivity in the final purified product, corrected for decay, by the amount of starting radioactive sodium iodide. Radioactivities were measured in the gamma ionization chamber calibrated for each geometry and vessel configuration.

HPLC Determination of Tributylstannyl Precursor in the Final Preparation

The amount of tributylstannyl precursor present in the 50% EtOH/H_2O collected eluate was determined by HPLC (System B, uv 225 nm, 3 ml/min) by mimicking the reaction procedure without radioactive iodide. After evaporation of the eluate under reduced pressure, the residue was injected into the HPLC in 200 μl MeOH/H_2O (95:5) and the uv area was compared against that of a series of known standards (0.05–1 μg) run on the same day under the same HPLC conditions. A Beer's law plot of the standards was linear in this range [$y = (1.39 \times 10^5)x + 1621$, $r = 0.999$].

Elution of Tributylstannyl and Trimethylstannyl Precursor Using Ethanol/Water Mixtures

Elution from Sep-Pak Light of each of the two precursors was carried out, with either 0.7 or 1.0 ml 100% ethanol, 3.3 ml of 70/30 ethanol/water, 7 or 10 ml of 50% ethanol/water. The eluted fractions were evaporated under reduced pressure and the residue was analyzed by HPLC (System B, uv 225 nm/3 ml/min for tributylstannyl; uv 254 nm/0.5 ml/min for trimethylstannyl), comparing the uv peak areas to those of known standards (0.05–4 μg for tributylstannyl and 1–4 μg for trimethylstannyl). A second elution with 5 ml of 100% ethanol was used to recover the remaining precursor. These second eluted fractions were analyzed similarly by HPLC.

Binding Assay

In vitro determinations of K_D and B_{Max} for both [^{125}I]β-CIT-SP and [^{125}I]β-CIT-HPLC were performed using striatum and cortex rat tissue homogenates (anterior to coronal section, including frontal, parietal and cingulate cortex), prepared as previously described (*12*); final tissue dilution was 1/6000 g/ml for striatal and 1/1600 g/ml for cortical tissue. Incubation (90 min at 22°C) was carried out in triplicate, mixing 100 μl [^{125}I]β-CIT (0.02 nmole), with either 100 μl buffer (128 m*M* NaCl, 3 m*M* KCl, 1.25 m*M* $NaH_2PO_4 \cdot H_2O$, 10 m*M* D-glucose, 2 m*M* $CaCl_2$, 2 m*M* $MgSO_4$, 15 m*M* $NaHCO_3$) or 100 μl nonradioactive β-CIT (10^{-14} *M* to 10^{-6} *M*) and 800 μl of tissue solution. Incubation was terminated by rapid filtration through GF/B filters on a 48-channel Cell Harvester. Filters were rapidly washed three times with 5 ml ice-cold buffer and counted in a COBRA 5010 gamma counter (Packard, Meriden, CT) with an efficiency of 80%.

Experiments (n = 3) were analyzed by weighted nonlinear regression analysis using the program LIGAND (Munson and Rodbard, 1980; NIH, Bethesda, MD) on a 80486 IBM PC. A one site and a two site model were tested with all parameters. The N parameter (nonspecific/free) was fixed to the measured value (binding remaining in the presence of 1 μmole β-CIT). K_D values were expressed in nM and B_{Max} values were expressed as pmole/g of tissue.

A note of caution: To achieve good purification of the radiolabeled product by the SPE method, three steps are essential: the conditioning of the Sep-Pak cartridge before its use, the elution flow rate and the storage of unused cartridges. The first two steps are described in this paper. The last one usually involves storing the pouch firmly closed in a desiccator (refer to manufacturer's instructions).

RESULTS

The simplified method for the preparation of [^{125}I] or [^{123}I]β-CIT reported in this paper resulted in radiochemical yields of 75% ± 4% (n = 3) for the [^{123}I] and 91% ± 7% (n = 3) for the [^{125}I]β-CIT, with radiochemical purity range 98%–99% for either preparation. The labeled product was stable (98%–99%) for at least 3 days for ^{123}I and 5 wk for ^{125}I labeled product.

With HPLC system A, flow 1.0 ml/min, the retention time of cold standard β-CIT was 10.2 min, and [^{125}I] or [^{123}I]β-CIT was 10.9 min (difference due to elapsed time between uv and gamma detector). Using silica gel TLC, cold β-CIT and labeled β-CIT had R_f = 0.54. Under the same conditions, unreacted radioactive iodide had HPLC retention time of 2.3 min and TLC R_f = 0.0. Both methods (HPLC and TLC) gave the same radiochemical purity.

Specific activity for [^{125}I]β-CIT-SP and [^{125}I]β-CIT-HPLC was found to be close to the theoretical maximum and showed no significant difference between both preparations (SP 2,000 Ci/mmole and HPLC 2,060 Ci/mmole). For [^{123}I]β-CIT-SP product, the specific activity was >67,000 Ci/mmole (limit of detection with our HPLC system A).

Pyrogenicity and Sterility

The LAL test showed a sensitivity of 0.125 to 0.250 Eu/ml. Since the presence of 13.4% ethanol in the final formulation of [^{123}I]β-CIT inhibited the coagulation of the internal control (100 μl of the 10 Eu/ml endotoxin standard plus 100 μl of the formulation); the test was run diluting the sample 1:1 with saline solution, in which case coagulation for the internal control occurred. The [^{123}I]β-CIT-SP product produced no coagulation after 1:1 dilution (pyrogen-

TABLE 1
Amount of Precursor in Sep-Pak Light Elution from 50 Microgram

Precursor	% EtOH	Volume (ml)	Precursor in eluate (μg)
β-CTSnMe$_3$	100	0.7	43.0
	70	3.3	38.0
	50	10.0	12.0
β-CTSnBu$_3$	100	0.7	34.0
	70	3.3	3.9
	50	10.0	0.10
	50	7.0	0.08

*HPLC System C-(tributylstannyl: 225 nm/3.0 ml/min; trimethylstannyl: 254 nm/0.5 ml/min.)

free, <0.5 Eu/ml) and was sterile after two weeks of incubation.

Precursor Choice

Both precursors, tributylstannyl and trimethylstannyl, were tried as starting material for the SPE method. Under the HPLC and Sep-Pak conditions described, the tributylstannyl gave better separation from the labeled β-CIT than the trimethylstannyl precursor with longer retention in a C_{18}-HPLC column or C18-Sep-Pak. For example, the trimethylstannyl precursor eluted with HPLC system A in 40.2 min at flow 1.0 ml/min, whereas the tributylstannyl precursor did not elute in this system even after four hours of elution at the same flow. With system B, trimethylstannyl precursor eluted in 12.8 min at flow 0.5 ml/min, whereas tributylstannyl eluted at 8.6 min but with flow 3 ml/min.

Choice of Cartridge

The Waters C18 Sep-Pak Light cartridge, chosen for purification of the reaction mixture in the SPE method allowed initial aqueous wash out of unreacted radioactive iodide (identified by TLC and HPLC) and polar byproducts, with retention of [^{125}I] or [^{123}I]β-CIT and alkylstannyl precursor. Subsequent elution with 50% ethanol/water recovered the labeled β-CIT with most of the precursor remaining in the Sep-Pak cartridge.

Table 1 shows the measured amounts (by HPLC) of alkylstannyl precursor present in the final formulation when the reaction was carried out with 50 μg of either the trimethylstannyl or the tributylstannyl precursor and the labeled product was eluted with 50%–100% ethanol. These results show the advantage of tributylstannyl over trimethylstannyl precursor for the SPE method.

From Table 1, the amount of tributylstannyl precursor present when the labeled β-CIT was eluted with 7 ml of 50% EtOH/H_2O was 0.08 μg. The minimum amount detectable from standards, with our HPLC system at 225 nm, was 0.05 μg. Most of the tributylstannyl precursor (40–45 μg) was recovered from the Sep-Pak when the 50% EtOH/H_2O elution was followed by 5 ml of 100% EtOH.

For both the tributylstannyl and trimethylstannyl precursors, higher uv absorbance was observed at 225 nm. This wavelength was particularly appropriate to measure amounts below 0.5 μg, which is the limit of detection for our HPLC system at 254 nm. Since the amount of trimethylstannyl precursor present in the 10 ml 50% EtOH/H_2O eluate was in the order of 12 μg, uv areas for this precursor were accurately measured at 254 nm.

In Vitro Binding

The in vitro binding of [^{125}I]β-CIT prepared by the SPE method was compared to that of [^{125}I]β-CIT purified by HPLC. The in vitro binding parameters of the two preparations were similar and comparable to previously published values (*12*). The striatum binding was best fitted by a two site model, while the cortical binding was compatible with binding to one population of sites. No significant differences between the two preparations were observed for any of these parameters (t-tests) (Table 2).

Preparation Time

The time required for the synthesis of [^{123}I]β-CIT by the SPE method was 1.5 hr; for [^{125}I]β-CIT, which does not need final formulation, 1 hr was required.

DISCUSSION

Based on the high lipophilicity of the tributylstannyl precursor relative to β-CIT, we have developed a simplified method for the preparation of [^{125}I] or [^{123}I]β-CIT without the time consuming steps and equipment requirements of the previously reported procedure (*11*) (ie: sol-

TABLE 2
In Vitro Binding Parameters for [^{125}I]β-CIT-SP and [^{125}I]β-CIT-HPLC Using Rat Tissue Homogenates.

	Cortex*		Striatum†			
Method	K_D‡	B_{Max}‡	K_{high}‡	K_{low}‡	$B_{Max\text{-}high}$‡	$B_{Max\text{-}low}$‡
[^{125}I]β-CIT-HPLC	0.10 ± 0.02	13 ± 1.5	0.16 ± 0.03	55 ± 26	91 ± 13	624 ± 231
[^{125}I]β-CIT-SP	0.08 ± 0.02	10 ± 1.8	0.20 ± 0.05	69 ± 27	96 ± 19	492 ± 197

*Two site model.
†One population of sites.
‡n*M*.
§pmole/g tissue.

vent extraction, evaporation under reduced pressure and HPLC purification).

To simplify the preparation, the removal of unreacted radioactive iodide, polar side products and reaction reagents was achieved by passing the reaction mixture through a C18 solid-phase extraction cartridge, eluting with 20 ml of water (volume large enough to assure complete elimination of those reaction components). A second elution with 7 ml 50% EtOH/H_2O recovered radiolabeled β-CIT.

Of the two alkylstannyl precursors, tributylstannyl was found to be more appropriate for the SPE method. Increasing the elution volume to 10 ml 50% ethanol/water still recovered the radiolabeled product with small amount of precursor (0.1 μg). By contrast, 12 μg of trimethylstannyl coeluted under the same conditions. Attempts to recover the radiolabeled β-CIT with a small volume of 100% ethanol (0.7 ml) produced much larger coelution of either of the two alkylstannyl precursors.

A typical preparation beginning with 33 mCi of $Na^{123}I$ produced over 24 mCi of ^{123}I labeled material, sufficient for three doses of 8 mCi each. The recommended human dose for [^{123}I]β-CIT, based on biodistribution studies in humans, is not to exceed 14 mCi, which deposits approximately 5 cGy to the lungs (dose-limiting organ) (*13*). The amount of tributylstannyl precursor present in each dose prepared this way was less than 0.03 μg.

In the final formulation of [^{123}I]β-CIT, sodium chloride (33 mg) is added to adjust isotonicity and ascorbic acid (100 μg) as antioxidant, resulting in a preparation containing 13.4% ethanol in 25 ml 0.9% NaCl. The product from the simplified preparation was sterile, pyrogen-free and suitable for in vivo studies. For in vitro studies with [^{125}I]β-CIT, the 50% ethanol/water fraction can be used directly without further dilution (with optional addition of 100 μg of ascorbic acid).

The results from the in vitro binding study indicate that [^{125}I]β-CIT-SP behaves similarly to [^{125}I]β-CIT-HPLC; K_D and B_{Max} in rat cortex and caudate for both preparations showed no significant difference between them. The total density of DA transporter sites reported in this study (600–800 nmole) is of the same magnitude reported previously by us (863 nmole; (*12*)) and others (730 nm; (*14*)) in rat striatal homogenate membranes using [^{125}I]β-CIT, and are comparable to site densities measured with cocaine estimated at 340 nmole with [^{3}H]cocaine (*15*), and with [^{3}H]CFT (459 nmole; (*16*)).

CONCLUSION

This study reports a method for preparing [^{123}I]β-CIT or [^{125}I]β-CIT that can be completed in 1.5 hr or less. The results obtained by SPE are consistent with those obtained by the HPLC method, with the advantage that the SPE method does not require solvent extraction, evaporation under reduced pressure or HPLC purification. We likewise propose a TLC system for the determination of the radiochemical purity of the final formulation as well as a practical way to shield the Sep-Pak Light. Our studies suggest that [^{123}I]β-CIT prepared by the SPE method is suitable for in vivo use.

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