Alasbimn Journal Year 4, Number 16 , July 2002

# Optimization of Labeling Conditions to Produce N-Isopropyl-p-[123I]-iodoamphetamine: A Radiopharmaceutical for Brain Perfusion Imaging.

Colturato, Maria Tereza; Muramoto, Emiko; Carvalho, Olga Gonçalves; Araújo, Elaine Bortoleti.; Silva, Constancia Pagano Gonçalves
Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN) Centro de Radiofarmácia - São Paulo -Brasil

**Correspondencia:**

Maria Tereza Colturato - radiopharmacist Centro de Radiofarmácia, CR Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP Av. Professor Lineu Prestes, 2242, Cidade Universitária, São Paulo, SP Brasil, CEP: 05508-000 e-mail: mtcoltur@net.ipen.br ebaraujo@net.ipen.br

**Cita/Reference:**
Colturato, Maria Teresa; et al. Optimization of Labeling Conditions to Produce N-Isopropyl-p-[123I]-iodoamphetamine: A Radiopharmaceutical for Brain Perfusion Imaging. Alasbimn Journal 4 (16): July 2002.

Índice/Index
Números anteriores Back issues
Enviar colaboraciones Instructions to authors
Sitios de interés Links
E-mail

## Abstract

Brain SPECT tracers, such as [$^{123}$I]-labeled amines and technetium-labeled compounds, offer high resolution SPECT images after intravenous injection using conventional rotating gamma cameras. The aim of this study was to optimize labeling conditions to produce N-isopropyl-p-[$^{123}$I]-iodoamphetamine) and to propose an easy, rapid and inexpensive chromatography quality control procedure to determine radiochemical purity. [$^{123}$I]-IMP was produced by isotopic exchange in presence of Cu(II) as catalyst and an excess of ascorbic acid as reducing agent. In order to obtain good radiochemical yield in a minimal reaction time, labeling reaction parameters were evaluated, with the study of different reaction temperatures and time, mass of ascorbic acid, reaction pH and [$^{131}$I]NaI/IMP ratio. [$^{123}$I]-IMP was obtained with radiochemical yield greater than 97% using 3 mg of IMP.HCl, 3.15 mol of $Cu^{++}$ and an excess of ascorbic acid in acid medium (pH 2.0) at 175$^{o}$C and 30 minutes. Radiochemical purity was determined by paper chromatography with high resolution. Biological distribution studies in animals showed high and persistent cerebral uptake and *in vivo* stability of the compound.

**Keyword:** N-isopropyl-p-[$^{123}$I]-iodoamphetamine; [$^{123}$I]-IMP; regional cerebral blood flow; labeling procedures, high labeling yield.

## Introduction

Brain perfusion SPECT (Single Photon Emission Computer Tomography) is a functional neuroimaging technique that allows noninvasive study of physiologic and physiopathologic events in the human brain. With the

appropriate technique and careful interpretation of the information provided, brain perfusion SPECT has proven potential for patient management (1, 2).

The ability of SPECT to detect regional cerebral blood flow (rCBF) variations in different conditions has favored the investigation of sensorial, motor and cognitive activities (neuroactivation studies) and the central effects of central nervous system (CNS) drugs (pharmacologic challenge) in both the normal and abnormal brain (1).

Several radiopharmaceuticals are commercially available for brain perfusion SPECT. The [$^{99m}$Tc]-labeled compounds hexamethylpropyleneamine oxime (HMPAO) and L,L-ethyl cysteinate dimer (ECD) have been the most successful and widely used for brain perfusion imaging, despite the fact that neither fulfils all the characteristics of an ideal radiopharmaceutical. Among the initially used [$^{123}$I]-labeled amines, isopropyl-[$^{123}$I]- amphetamine ([$^{123}$I]-IMP) has been the most frequently used (3). After crossing the intact blood-brain barrier, [$^{123}$I]-IMP binds to amphetamine receptors on neurons. This radiopharmaceutical has good characteristics for brain perfusion SPECT (4). However, its peak brain activity is reached as late as 20 min after injection, and it shows redistribution over time; that is, there is reuptake by the cerebral cortex that is not proportional to blood flow (4, 5).

According to Camargo et al, this redistribution property may be used with advantage, in the evaluation of response to therapy in patients with stroke. The theoretical approach consist of simultaneously injecting [$^{123}$I]-IMP and [$^{99m}$Tc]-labeled tracer at the time of admission and imaging the patient only once, later (4-6h) with simultaneous acquisition of $^{123}$I and $^{99m}$Tc images (2).

The cerebral distribution properties of [$^{123}$I]-IMP were recently applied for the early diagnosis of Creutzfeldt-Jakob disease (6). A different but not less important application of the radiopharmaceutical refers to the diagnosis of uveal malignant melanoma (7).

Since SPECT needs large doses of [$^{123}$I]-IMP careful optimization of the labeling and quality control methods was required for best utilization of the expensive $^{123}$I and maximum target/nontarget ratios. Published procedures were not fully satisfactory in these respects (8, 9, 10, 11). Labeling methodology frequently employs high reaction time and purification steps. This work intend to optimize labeling conditions and propose an easy, rapid and inexpensive chromatography quality control procedure to determine the radiochemical purity of the labeled [$^{123}$I]-IMP.

## Materials and Methods

N-isopropyl-p-[$^{123}$I]-iodoamphetamine was produced by isotopic exchange in presence of Cu(II) as catalyst and an excess of ascorbic acid as reducing agent of Cu(II). All labeling optimizing experiments were developed with [$^{131}$I]NaI solution obtained from Nordium (Canada) and processed at IPEN-CNEN/SP. Ideal labeling conditions were reproduced using carrier free $^{123}$I obtained by the reaction $^{124}$Xe(p,2n)$^{123}$Cs ®$^{123}$Xe ®$^{123}$I in the CV-30 cyclotron at IPEN-CNEN/SP, with 30MeV protons and enriched $^{124}$Xe gas target.

**a) 131-Iodine labeling of N-isopropyl-p-iodoamphetamine hydrochloride**: Basic reaction conditions were performed as published procedures with some modifications (11). The N-isopropyl-p-iodoamphetamine hydrochloride (Emka Chemie) (3 mg/50 ¼L) was placed in a tightly-sealed glass vessel followed by the addition of 50 ¼L of

$CuSO_4.5H_2O$ (3,15 m of 96% acetic acid solution), 2 mg of ascorbic acid and 10-50 ¼L of [$^{131}$I]NaI solution (37-370 MBq). The reaction was conducted at 175 $^{o}$C for 20 minutes, using a copper heating block. The pH of the reaction mixture was 2.0. In order to obtain good radiochemical yield in a minimal reaction time, labeling reaction parameters were evaluated, with the study of different reaction temperatures and time, mass of ascorbic acid, reaction pH and [$^{131}$I]NaI/IMP ratio.

**b) Determination of radiochemical purity:** Radiochemical purity of the labeling mixtures were determined by chromatographic method using Whatmann 3MM paper (13 x 1 cm) and chloroform:methanol:acetic acid (85:15:1) as solvent. In this system, the $R_f$ value of the [$^{131}$I]-IMP is 0.9-1.0 and of the radioiodine is 0.0-0.1. The HPLC elution profile of the labeled product was conducted with HPLC system (Waters Instruments), equipped with a radioisotope detector (Radiomatic 150TR, Packard) and a variable wave length U.V. spectrophotometer set at 254 nm, using a RP-column ($C_{18}$, 10 ¼m, 250 x 4,6 mm, Whatman) eluted with ethanol:water:amonium acetate:acetic acid (55:43:1:1) at a flow rate of 1 mL/min (10).
>
**c) Biodistribution studies:** Biological distribution studies were performed using Swiss female mice (15-25g).The animals received 1.11 1.48 MBq/100 ¼L of [$^{131}$I]-IMP intravenously via tail vein. Blood samples were collected by an orbital bleed at different times after the dose administration, the animals were sacrificed and the tissues of interest removed, washed, weighed and counted for $^{131}$I activity, using a gamma counter (Packard). The percent injected dose was calculated by comparing the activity in each tissue with injection standards of suitable count rate. Planar scintilographic images (Gammatomo MB 9300) were obtained from a rabbit, 20 and 60 minutes after the radiopharmaceutical administration. All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

## Results

In the optimization of IMP labeling conditions, the influence of reaction temperature (figure 1) and reaction time (figure 2) in the radiochemical yield were determined.

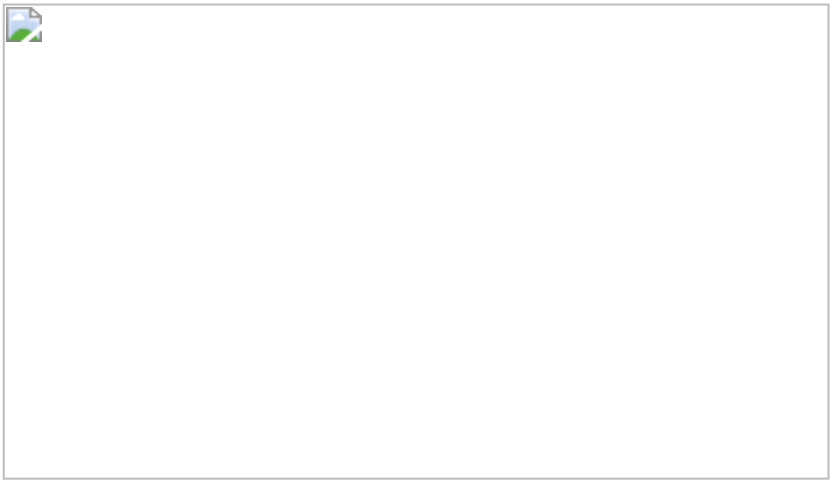

**Figure 1.** Radiochemical yield of [$^{131}$I]-IMP: influence of reaction temperature

(20 minutes; 2 mg ascorbid acid; pH 2)

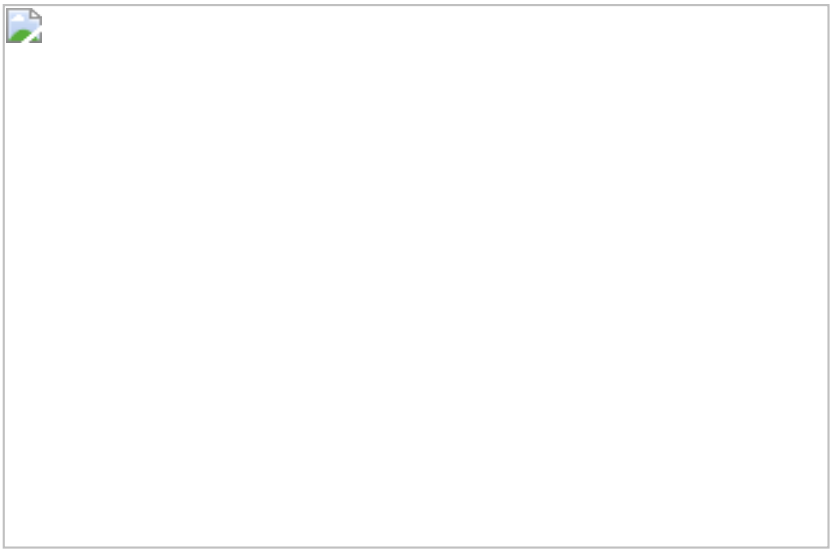

**Figure 2.** Radiochemical yield of [$^{131}$I]-IMP: influence of reaction time

(175$^{o}$C; 2 mg ascorbid acid; pH 2)

Figure 3 represents the variation of radiochemical purity using ascorbic acid solution with different concentrations

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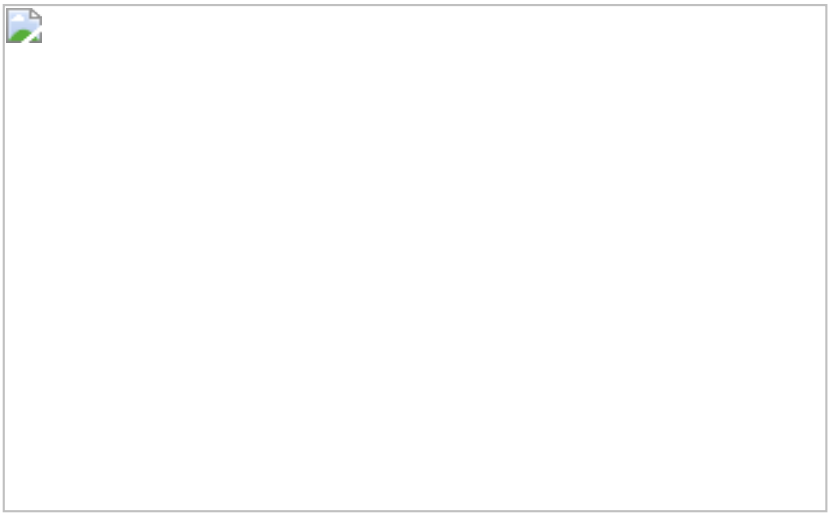


**Figure 3.** Radiochemical yield of [$^{131}$I]-IMP: influence of ascorbic acid concentration

(175$^{o}$C; 30 minutes; pH 2)

The presence of acetic acid in the reaction mixture confers the acidic medium for the nucleophilic substitution. However, the radiochemical yield varied from 98.01 ± 0.38 % (N=6) at pH 2 to 94.33 ± 0.14 % (N=6) at pH 4.0 and 86.06 ± 0.42 % (N=3) at pH 6.

We also studied the influence on radiochemical yield with increasing $^{131}$I activity using 3 mg of IMP.

Figure 4 summarizes the results obtained in this study .

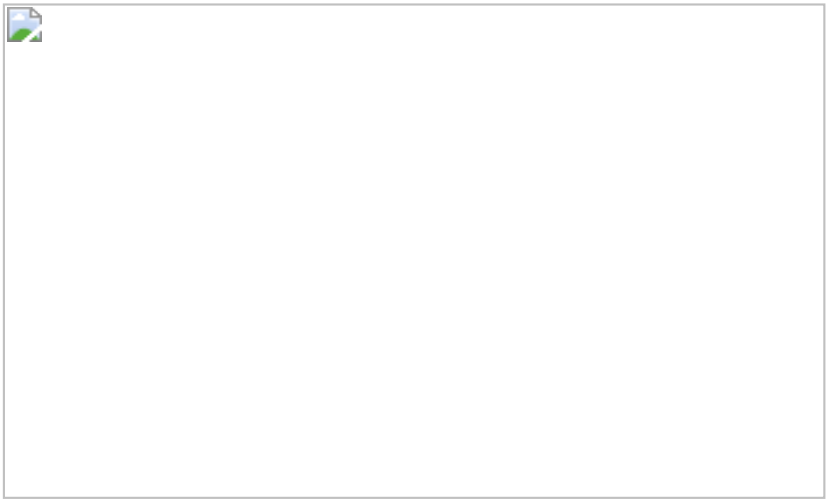


**Figure 4.** Radiochemical yield of [$^{131}$I]-IMP: influence of [$^{131}$I]NaI/IMP ratio

(175$^{o}$C, 30 minutes; 2 mg ascorbid acid; pH 2)

The chromatographic system employed using Whatmam 3MM paper presented good resolution in the determination of the percent of unlabeled radioiodine as showed in a typical chromatogram for reaction mixture (figure 5), in which peak 1 represents the free iodine and peak 2 the [$^{131}$I]-IMP.

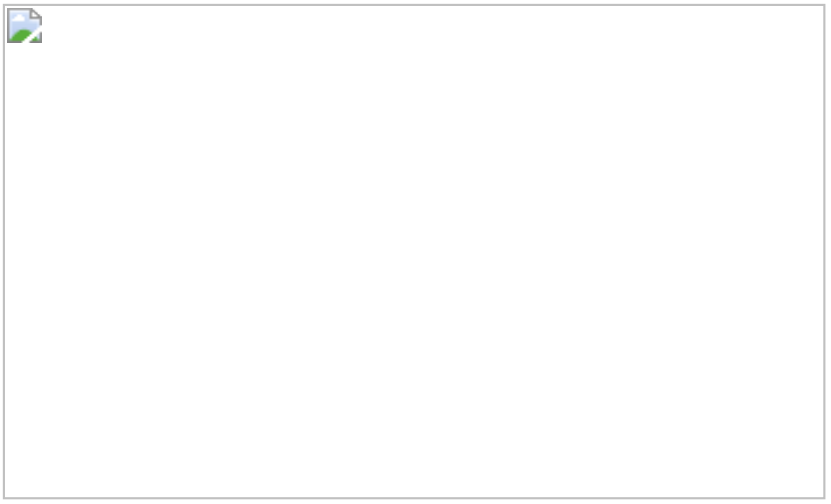


**Figure 5.** Radiochromatogram of the [$^{131}$I]-IMPreaction mixture using paper chromatography

Figure 6 shows HPLC chromatogram of the [$^{131}$I]-IMP reaction mixture with two peaks with Rt 3.48 and 7.21 minutes that corresponds to free radioiodine and [$^{131}$I]-IMP, respectively.

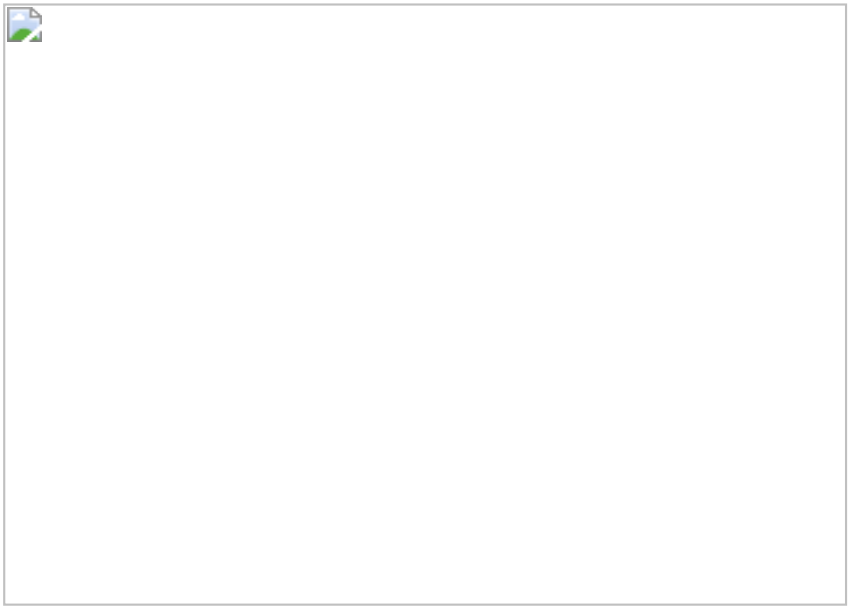


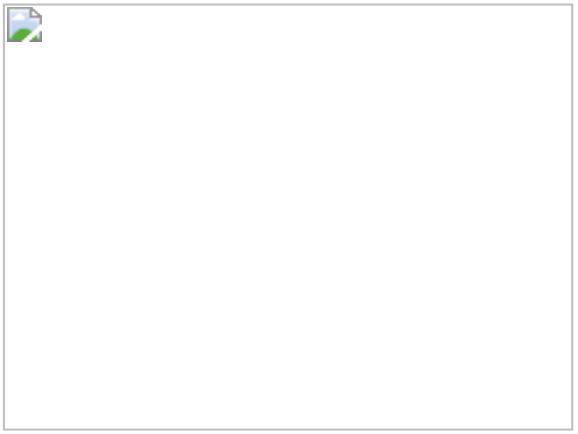
**Figure 6.** HPLC chromatogram of [$^{131}$I]-IMP (RP-column $C_{18}$, 10 ¼m, 250 x 4,6 mm); ethanol:water:amonium acetate:acetic acid (55:43:1:1) eluent and flow rate of 1 mL/min

Table 1 and figure 7 show the results obtained on biodistribution studies in mice (invasive study) and in rabbit, respectively.

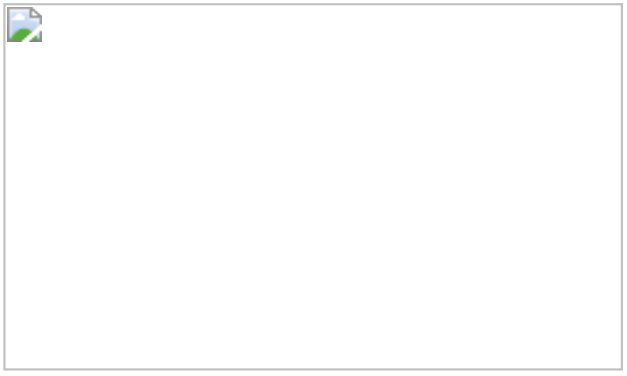
**Table 1.** Biodistribution studie of [$^{131}$I]-IMP in mice

| | % Administered Dose/Gram | | | | | |
|---|---|---|---|---|---|---|
| **Organ** | **Time After Dose Administration (hours)** | | | | | |
| | **0.25** | **0.5** | **1** | **2** | **6** | **24** |
| **Lung** | 31.53±2.64 | 23.70±3.03 | 23.73±4.16 | 23.65±3.50 | 20.11±0.46 | 0.45±0.16 |
| **Liver** | 13.86±1.44 | 14.27±1.84 | 13.86±1.18 | 12.26±1.15 | 9.64±0.91 | 0.24±0.06 |
| **Intestines** | 5.90±1.10 | 6.70±0.58 | 6.74±1.04 | 6.09±0.25 | 5.41±0.79 | 0.09±0.01 |
| **Kidney** | 13.95±2.23 | 12.98±0.99 | 11.72±1.00 | 11.95±1.90 | 12.76±0.61 | 0.37±0.10 |
| **Brain** | 11.67±1.44 | 11.84±1.23 | 12.38±1.34 | 11.29±0.93 | 9.37±0.94 | 0.24±0.03 |
| **Thyroid*** | 0.13±0.02 | 0.15±0.02 | 0.29±0.02 | 0.46±0.06 | 0.72±0.08 | 0.31±0.04 |
| **Blood**** | 29.24±3.10 | 26.37±2.54 | 26.31±3.20 | 23.55±2.86 | 20.50±2.01 | 8.02±1.53 |

*% administered dose/organ ** administered dose in total blood



**(A)**



**(B)**

**Figure 7.** Planar scintigraphy image of rabbit brain after [$^{123}$I]-IMP administration : (A) 20 minutes (frontal) and (B) 60 minutes (lateral)

## Discussion

Cu (II) salts have often been used for the nucleophilic isotopic exchange of aryl bound iodine, such as the radioiodination of N-isopropyl-p-iodoamphetamine (IMP) (8,10,11).

When applying labeling methods using Cu(II) salts in acetic acid, the labeling yield does not exceed 60% and HPLC analysis shows different cold and labeled side products (12). The addition of reducing agents like Sn(II) or ascorbic acid to the initial reaction mixture containing Cu(II) in situ, allows the use of temperature with a high labeling yield. The use of ascorbic acid in excess allow the formation of Cu(I) *in-situ*, which may form an Ar-Cu-I complex, inducing the isotope nucleophilic substitution reaction (10,11,12).
In this study, the optimization of the reaction parameters made possible the procurement of [$^{123}$I]-IMP with high radiochemical yield (> 97 %) using 3 mg of IMP.HCl, 3.15 ¼mol of $Cu^{++}$, an excess of ascorbic acid (11.36 ¼g), in acetic acid medium (pH 2.0) and minimal reaction volume in a sealed flash heated at 175$^{O}$C for 30 minutes.
The high radiochemical yield obtained confirmed by HPLC analysis of the labeled product, allows the utilization of [$^{123}$I]-IMP without purification step. The labeled IMP remains stable for 24 hours with radiochemical yield greather than 95% . Some chromatographic systems previously described for the determination of radiochemical purity of labeled IMP (10,11,12), are based in thin layer chromatography (TLC) with silica gel support. In our experiments, comparative studies using TLC and Whatman 3MM paper chromatography and the solvent mixture described in the methodology revealed the viability of paper in separating labeled IMP from free radioiodine.

The evaluation of radiochemical purity using paper chromatography represented an easy, rapid and efficient way to be used in routinary production, with high resolution as confirmed by HPLC analysis.
The product crossed the intact blood brain barrier as evidenced in animals studies, with good cerebral uptake that remains almost inalterated in the first 2 hours.
High *in vivo* stability of the compound when considering the possibility of dehalogenation was confirmed by the low thyroid uptake. The considerable amount of lung uptake of IMP was previously reported both in animals and in human subjects. Uptake in the blood and other organs were compatible with previously published results (13, 14, 15).

## Conclusion

The optimization of labeled parameters and quality control made possible to obtain N-isopropyl-p-[$^{123}$I]-amphetamine in high radiochemical yield and *in vitro* and *in vivo* stability, with good cerebral uptake.

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Abstract | Introduction | Materials and Methods | Results | Discussion | Conclusion | References | Print