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ABSTRACT

O-iodohippuric acid labelled with ^{131}I is one of the most frequentely used radiopharmaceuticals in Nuclear medicine for kidney function examination. The kit is constituted by 2 vials: 1) 120 mg of 2) buffer phosphate EDTA pH = 6.0. The o-iodohippuric acid is labelled by adding ^{131}INa (or ^{123}INa) solution up to 10 mCi. The vial is then heated for 30 minutes in boiling water (100°C) and after cooling, 0,5mL of buffer phosphate is added resulting a pH 6.0 ready solution. A rapid quality control is made using Whatman 3 MM paper chromatography with chloroform/glacial acetic acid (90:10) as solvent. The radiochemical yields were: 98.7 and 97.7% (the first and 15th day). The kit was kept under 4°C for 60 days. The radiochemical purity was determinate during this period of time, verifying that the radiopharmaceutical was stable with a high yield. The preparation of this kit shows and ideal radiopharmaceutical, in liquid form, for labelling "in situ" with short-lived radionuclides(^{123}I).

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PREPARAÇÃO DE CONJUNTO DE REATIVO DO ÁCIDO O-IODOHIPPURICO PARA MARCAR COM ^{131}I E/OU ^{123}I (*)

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RESUMO

O ácido o-iodohippurico (hippuran) marcado com radionuclídeo do iodo é um dos radiofármacos usado com maior frequência para avaliar a função renal. Prepararam-se conjuntos de reativos, do hippuran, para marcar com ^{131}I. O conjunto de reativo consta de dois frascos contendo 1) 120 mg de ácido o-iodohippúrico e 2 µg de $CuCl_2$ em 2 mL, pH = 4,6 - 4,9. 2) Solução tampão fosfato EDTA pH = 6,0. Adicionaram-se 0,1-0,2mL de uma solução de Na ^{131}I no frasco 1 levando-o em banho maria (100°C) durante 30 minutos. Após esfriamento adicionaram-se 0,5 mL de solução 2 a fim de acertar o pH a 6,0. Realizaram-se controle de pureza radioquímica em papel Whatman 3 MM (6,5 x 1cm) em clorofórmio a ácido acético (90:10) como solvente. Os rendimentos da pureza radioquímica foram 98,7 e 97,7% no primeiro e 15º dia, respectivamente, após a marcação. O conjunto de reativo de hippuran foi conservado a 4°C durante 60 dias, neste período de tempo realizaram-se determinações da pureza radioquímica, verificando que o fármaco manteve-se estável apresentando um alto rendimento de marcação. Este produto possue características adequadas para ser formulado em forma líquida, para marcação "in situ" com radioisótopos de meia-vida curta(^{123}I).

(*) Trabalho a ser apresentado no 3º Congresso Geral de Energia Nuclear - de 22 a 27 de julho/90 - Rio de Janeiro/RJ.

INTRODUCTION

The use of ^{131}I-o-iodohippuric acid (hippuran; o-IHA) has long been established for the investigation of kidney function in Nuclear Medicine.

The renogram using ^{131}I-hippuran gives information about the renal blood flow, urinary tract potency and urinary flow. It may be used in renal scanning to provide information on the distribution of this compound. The first report in renal scanning was made by Tubis et al. in 1960(11). This information is of special value for diagnostic purpose in cases of transplated kidneys and for the detections of abnormal renal status that may be obtained quickly and easily.

Up to 1972, the literature on labelling o-iodohippuric acid (O-IHA) has been summarized(3). In the meantime, Thakur et al.(9), Gillet et al.(4) as well as Nijl et al.(2) have reported methods for ^{123}I labelling of o-IHA using the so-called "melt-method".

Radiochemical purity of o-IHA is important regarding the patient's radiation dose, especially in view of the high thyroid exposure from free iodide. Two different radiochemicals impurities were found in all hippuran samples: a) radioiodide and b) radiochemical impurity due to labelled o-iodobenzoic acid. There are two reasons for the radiodide impurity; 1) residues from manufacturing, since o-IHA is labelled with radioiodine by isotopic exchange and 2) decay products due to the "in vitro" instability. Labelled o-iodobenzoic acid is due to an impurity of the initial material o-IHA.

Various procedures for the separation and purification of labelled o-IHA after exchange method were described (1-13).

Hawking, 1982(5), Rovnij, 1985(7) and Hinkel, 1988(6) published a rapid method for the preparation of ^{123}I o-IHA in a kit form with high labelling efficiency and radiochemical purity.

The aim of this work is the development of sample kit, based in Rovnij report(7), for the preparation of o-IHA, in a liquid form, for labelling with ^{123}I or ^{131}I and the procedures of the radiochemical quality control to establish the validity of this radiopharmaceutical of known value in the study of kidney function.

MATERIAL AND METHOD

Each vial contain: A - 120 mg of o-IHA from Mallinckrodt, dissolved in 1.9 Ml distilled water and 20 µg $CuCl_2$ in 0.1 mL 0.01N HCl, pH of this solution 4.6 - 4.9 B-phosphate buffer solution, pH 6.0 . These solutions were sterilized by Millipore filtration (0.22 µ) and stored under 4°C during 60 days.

The o-IHA from Mallinckrodt was tested in HPLC separation method to determine the contaminant, o-iodobenzoic acid. CG Instrumentos Científicos C.G. Ltda. Mod. 480C, C-18 column reverse phase and 435-B u.v. detector were used; and the eluent was: methanol: acetic acid: water (30:0.1:70) at a flow rate of 1.0 mL/min(12).

The ^{131}INa used for labelling was from IPEN-CNEN/SP., 0.2 - 0.1 mL of radioiodine (^{131}I) in 0.01N NaOH was added to the vial A, the vial is heated during 30 minutes at 100°C in boiled water and allowed to cool at room temperature. Then 0.5 mL of solution B is added.

Chromatographic technique was used to asses the radiochemical purity (8). The chromatographic procedure was developed at room temperature during 10 minutes using Whatmann 3 MM (6,5 x 1cm) paper strip as support and chloroform: glacial acetic acid (90:10) as solvent. With this system, ^{131}I-o-IHA migrated to the solvent front, where as the inorganic iodide remained at the origin. Each segment of the strip were counted in scintillation counter (ANSR.Gamma Counter Abbot Lab.) and the results were expressed as a percentage of the total counts on the strip.

RESULTS AND CONCLUSION

The percentual of inorganic iodide and o-IHA are presented in Table I as function of time after labelling with ^{131}I, using 1-10 mCi in 0.1 - 0.2 mL 0,01N NaOH. The data show a radiochemical purity of ^{131}I-o-IHA stored at 4°C from 98.6 to 98.2% during a period of 15 days and the percentage of free iodide increased from 1.4-1.8%, however, these values are little different from those stored at room temperature (from 98.4 - 98.2% during the same period of time).

As indicated in Table II the percentage of free iodide does not differ significantly with different activities of ^{131}I used in the labelling test of o-IHA kit. These values were from 1.2 to 1.7% of free iodide using 1 and 10 mCi of ^{131}I Na, respectively.

As shown in Table III, the radiochemical purity of ^{131}I-o-IHA from the kit prepared in Cuba by the group of Ronij et al.(7) and that of the IPEN-CNEN/SP is not different (98.4 - 98.3% respectively in the first day of labelling). The percentage of free iodide (2.1%) found in the kit from Cuba 15 days after labelling is perhaps due to the inadequate transportation to Brazil.

Table IV shows the stability of the kit stored under 4°C during 60 days. The kit kept in this condition was labelled with 1 mCi of ^{131}I in 0.1 mL 0.01N NaOH and the percentage of free iodide found during 10 days after labelling increased from 1.24% in the first day to 1.96% after 10 days and not more than 2%, these data are in agreement with the limit (3%) described in monographies of the Pharmacopoeia (10).

The high-pressure liquid chromatographic (HPLC) analysis indicated that the o-IHA used in the kit preparation is free from o-iodobenzoic acid contaminant (12), as show Figure 1.

Any procedure for both ^{131}I or ^{123}I labelling o-IHA has to meet the following criteria: 1) the radiochemical yield should be close to 100% to keep activity losses low, 2) the chemical procedures used for labelling should be fast and simple and 3) the method should lead to reproducible results to make it suitable for routine production.

The kit presented in this paper, in a liquid form for preparing ^{131}I-o-IHA yields a radiochemical pure agent. The method is quick and easy resulting a product ready for use, 60 minutes after addition of radioiodine to the reaction vial.

The kit has prove satisfactory with a shelf life 2 month (stored under 4°C) and with a high yield for radiochemical purity after 15 days of labelling

TABLE 1

Radiochemical purity of ^{131}I-Hippuran as a function of time after labelling

Time Period (days)	Room Temperature		4°C	
	% Hippuran	% Iodide	% Hippuran	% Iodide
1	98.4	1.6	98.6	1.4
3	98.6	1.4	98.6	1.4
7	98.5	1.5	98.3	1.7
10	98.3	1.7	98.6	1.4

labelled with ^{131}I-Na (10 mCi) in 0.2-0.1 0.01N NaOH (n=5).

TABLE 2

Radiochemical purity of ^{131}I-Hippuran as a function of ^{131}I-Na Activity (N=5)

Activity (mCi)	% Hippuran	% Iodide
1	98.8 ± 0.3	1.2
2	98.6 ± 0.4	1.4
3	98.4 ± 0.5	1.6
5	98.7 ± 0.3	1.3
10	98.3 ± 0.6	1.7

TABLE 3

Comparasion of radiochemical purity of ^{131}I- Hippuran (prepared in kit form by .OR-Cuba and IPEN-CNEN/SP) as a function of time after labelling.

Time (day)	CUBA		IPEN-CNEN/SP	
	% Hippuran	% Iodine	% Hippuran	% Iodine
1	98.4	1.6	98.3	1.4
5	98.3	1.7	98.5	1.5
10	98.1	1.9	98.4	1.6
15	97.9	2.1	98.2	1.8

Labelled with 1 mCi ^{131}I-Na (n=3)

TABLE 4

Radiochemical purity of ^{131}I- Hippuran 60 days after kit preparation stored under 4°C, as a function of time after labelling.

Time Labelling (days)	% Hippuran	% Iodide
1	98.76	1.24
3	98.73	1.27
5	98.60	1.40
10	98.04	1.96

Labelled with 1 mCi ^{131}I-Na (n=3)

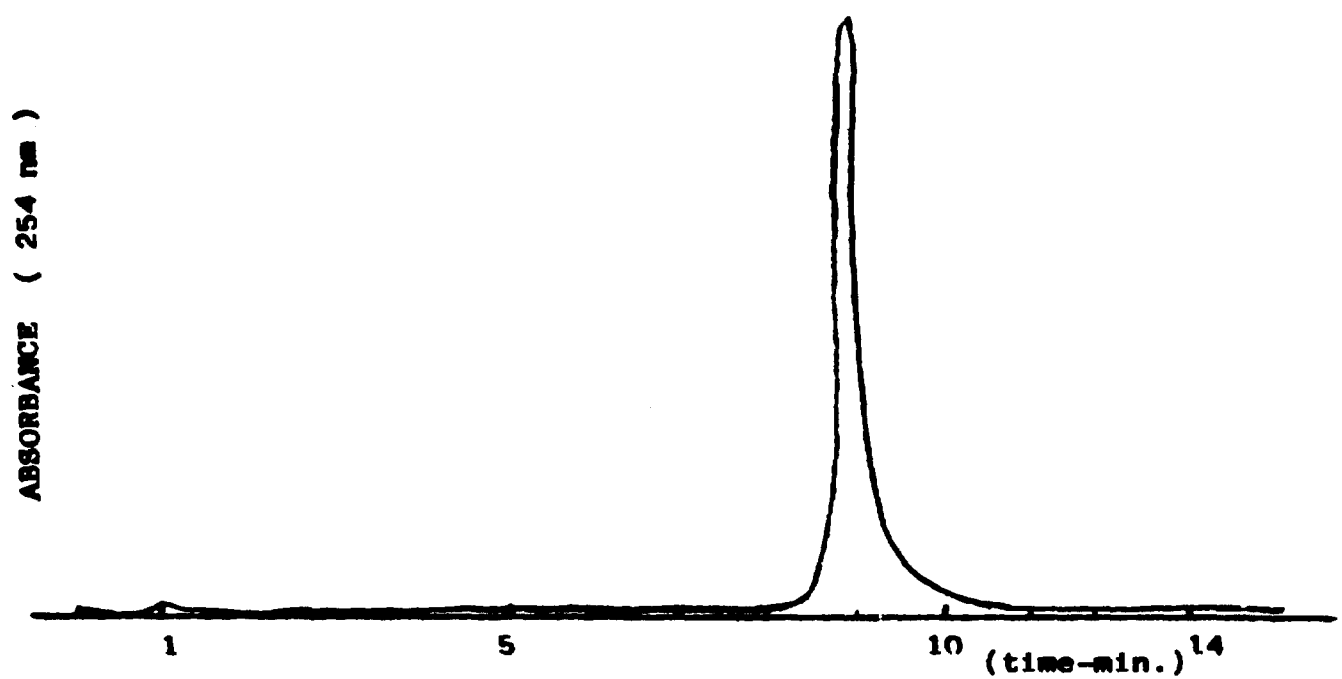

FIGURE 1 - Ultraviolet chromatogram of a test solution of o-IHA kit in HPLC, flux 1 mL/min., 254 nm, MeOH/H_2O/Acetic Acid (30:70:0.1, pH=3.9) as eluent.

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