ISSN 0101-3084

CNEN/SP

ipen Instituto de Pesquisas Energéticas e Nucleares

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IPEN - PUB - 286

PUBLICAÇÃO IPEN 286 DEZEMBRO/1990

SÃO PAULO

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CNEN/SP
INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
SÃO PAULO – BRASIL

Série PUBLICAÇÃO IPEN

INIS Categories and Descriptors

B13.30

CHROMATOGRAPHY
IODINE 131
RADIOCHEMISTRY

IPEN - Doc - 3528

Aprovado para publicação em 29.08.89

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ABSTRACT

Different solvents were used in paper chromatographic methods to obtain the best system in routine radiochemical control for ^{131}I–MIBG produced at IPEN–CNEN/SP. The dates were compared with those obtained with eletrophoresis method in buffer acetate, pH = 4.5, 350V, during 40 minutes. The stability of the labeled compound stored under 4°C was studied during 15 days. Miniaturized chromatographic procedures were established using Whatman 3MM (8x1 cm) and n–butanol–:acetic acid:water (5:2:1) as a solvent. The Rf values were: 0.3 (I^-) and 1.0 (MIBG). The radiochemical purity was 99.3 and 99.2% (first day) obtained with eletrophoresis and miniaturized chromatographic procedures, respectively and, 84,7% after 15 days of its preparation. It is a rapid, practical and reproductive method.

* Paper to be presented at XI Congreso de La Asociación Latinoamericana de Sociedades de Biologia y Medicina Nuclear – ALASBIMN, held in Santiago – Chile, Oct. 08–11, 1989.

CROMATOGRAFIA MINIATURIZADA NO CONTROLE RADIOQUÍMICO ROTINEIRO DA MIBG–^{131}I*

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RESUMO

Estudaram–se sistemas cromatográficos em papel com diferentes solventes a fim de determinar o melhor método para o controle radioquímico rotineiro da MIBG–^{131}I produzido no IPEN–CNEN/SP. Compararam–se os resultados com aqueles obtidos por eletroforese realizada em tampão acetato, pH = 4,5, 350V, durante 40 minutos. Estudou–se também a estabilidade do produto marcado, estocado a 4°C durante 15 dias após a sua preparação. Estabeleceu–se a cromatografia ascendente em papel Whatman 3MM (1x8 cm) utilizando como solvente n–butanol: acido acético e H_2O (5:2:1) para o controle rotineiro. Os valores encontrados para os Rfs foram : 0,3 para o iodeto e 1,0 para a MIBG–^{131}I. A pureza radioquímica do produto marcado no dia da preparação foi de 99,3 e 99,2%, dados obtidos na eletroforese e cromatografia miniaturizada respectivamente, e de 84,7% 15 dias após a preparação. O método é realizado em tempo curto, é prático e reprodutível.

* Trabalho a ser apresentado no XI Congreso de La Asociación Latinoamericana de Sociedades de Biologia y Medicina Nuclear -- ALASBIMN, a realizar–se em Santiago – Chile, Out. 8–11, 1989.

1. INTRODUCTION

Radioiodinated metaiodobenzylguanidine (MIBG), an aromatic analog of neuron blocker guanethidine has been used clinically for detection and radiotherapy of catecholamine tumors[7,4] and imaging of adrenergically innervated organs such as the heart[3].

Radioiodinated MIBG is synthesized by an exchange technique that requires heating in the solid state at 140°C during 1 hour and 30 minutes followed by passage through anion exchange resin to remove unreacted radioiodide and iodate. Details of the radiosynthesis and the purity determination have been published, including the production into pharmaceutically acceptable form[1].

Paper chromatography and classical electrophoresis have been used as methods for the control of the radiochemical purity of labeled compounds in the field of radiopharmaceuticals. The classical eletrophoresis technique depends on the different migration rates of charged molecules in a electric field, the paper chromatography and thin layer on silica gel are an effective way on analyzing the inorganic radioiodide impurities. but these methods, although very accurate, are not very rapid for routine quality control.

Rapid miniaturized chromatography systems for ^{99m}Tc radiopharmaceuticals[2,8] including commercially available kits, have greatly simplified this task and allowed rapid and accurate radiochemical determination within a relatively short interval of time.

With the daily use of iodinated radiopharmaceuticals and the advent of shorter lived ^{123}I labeled compounds, a rapid and accurate system is needed to asses the radiochemical purity. Some chromatography procedures for specific iodinated compound have been established[5,6,9].

This study was initiated to develop a rapid and exact miniaturized chromatography system which could be used into a daily quality control program of ^{131}I–MIBG production, after comparing it with electrophoresis method.

2. METHODOLOGY

The miniaturized chromatography procedures were developed using Whatman 3MM (8x1 cm) paper strip as support and the followed solvents as mobile phase:

1) ethyl acetate, ethanol (1:1),
2) ethanol, ethyl acetate and ammonium hidroxide (20:20:1),
3) ethanol, ammonium hidroxide (3/1), and
4) n–butanol, acetic acid and water (5:2:1).

The paper were stopped 1cm from the bottom, the strips were placed in a vial containing approximately 0.5 ml of solvent. The chromatogram was developed for a distance of 7.5cm during 10–30 minutes. The strip was removed, dried and cut into 2 segments (in the middle between the origin and the solvent front). Each segment were counted separately in a gama well counter (ANSR – Abbot Lab.). The counts of each part were expressed as a percentage of the total counts of the strip.

The conventional method (electrophoresis). The support, Whatman nº 1 (30x2 cm) paper strip, and solvent acetate buffer pH 4.5 was developed in 350 V for about 40 minutes. The radioactivity distribution after electrophoretic separation was determined using gamma scintillation spectrometry.

Samples of ^{131}I–MIBG stored under 4°C were analyzed during 15 days after its preparation, using miniaturized chromatography system (Whatman 3MM strip and n–butanol, acetic acid and water as solvent).

3. RESULTS AND CONCLUSIONS

Figure 1 shows the graphic representation of chromatographic strip activities distribution for ^{131}I–MIBG. Four specific solvents were used for the labeling efficiency and Rf values determining:

1) ethyl acetate: ethanol (1:1) Rf I^- = 0.3; MIBG = 1.0,
2) ethanol, ethyl acetate, ammonium hidroxide (20:20:1) Rf I^- = 0.5 and MIBG = 1.0,
3) ethanol, ammonium hidroxide (3/1) Rf I^- = 0.75 and MIBG = 0.87.

As the date shows, a good separation between unreated iodide and ^{131}I–MIBG is observed only in solvent 1) but without a reproducibility.

However, Figure 2 illustrates a good separation of chromatography strips activities distribution for iodide and MIBG, using Whatman 3MM (8x1 cm) as support and solvent 4) n–butanol,acetic acid and water (5:2:1) as solvent. developed during 30 minutes. The Rf values for I^- = 0.3 and MIBG = 1.0. A good separation and reproducibility (Table I) are achieved between these radiochemical species. With this technique the mean free $^{131}I^-$ concentration in ^{131}I–MIBG was compared with that obtained with the electrophoresis systems. A summary of the chromatographic systems used for ^{131}I–MIBG is found in Table II. As indicated, the mean free ^{131}I iodine concentration in ^{131}I–MIBG was 1.05 and 0.98% respectively, with a correlation coefficient of 0.9836 (Figure 4).

Table III shows the radiochemical stability of ^{131}I–MIBG stored under 4°C during 15 days. The radiochemical yield was 99.13 and 84.78% in the first and 15 days after preparation , respectively.

The method presented is a rapid, inexpensive, and chemically reliable process that may be used in a nuclear medicine department where it is desired to test ^{131}I–MIBG for labeling efficiency before administration to patients. The materials necessary to perform this test are simple; the extra cost, time and effort are minimal. This allows good separation between any unreated ^{131}I and the specific radiopharmaceutical, with an excellent reproducibility (Table I) with a (coeficient of variance) CV = 0,20%.

The authors wish to thank Suely Dall'Evedore and Alfredo dos Santos for valuable technical assistence.

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TABLE I – REPRODUCTIBILITY OF THE METHOD OBTAINED WITH THE SAME SAMPLE AND SIMULTANEOUS RUNS (15) FOR ^{131}I–MIBG

Runs	^{131}I–MIBG %	IODIDE (I^-) %
1	99.18	0.82
2	99.27	0.73
3	99.18	0.82
4	99.21	0.79
5	99.40	0.70
6	99.25	0.75
7	99.38	0.62
8	99.17	0.83
9	99.30	0.70
10	99.31	0.69
11	99.10	0.90
12	99.18	0.82
13	99.45	0.55
14	99.85	0.15
15	99.65	0.35
	$\bar{x}$ = 99.32 SD = 0.20 CV = 0.20%	$\bar{x}$ = 0.67 SD = 0.20

TABLE II – COMPARISION OF PORCENTUAL VALUES OF *INORGANIC IODIDE IN THE ASSAY OF* ^{131}I–MIBG BY ELETROPHORESIS AND MINIATURIZED CHROMATOGRAPHY

Sample (^{131}I–MIBG)	Porcentual Value of Inorganic Iodide	
	Classical Eletrophoresis	Miniaturized Chromatography
1	0.89	0.91
2	0.88	0.90
3	0.77	0.87
4	1.25	1.50
5	1.15	1.30
6	1.00	1.05
7	0.97	1.04
8	1.20	1.21
9	0.88	0.89
10	0.87	0.88
	$\bar{x}$ = 0.98	$\bar{x}$ = 1.05

Correlation Coefficient = 0.9836

TABLE III – QUALITY CONTROL OF ^{131}I–MIBG DURING 2 WEEKS AFTER STANDARDIZATION BY MINIATURIZED CHROMATOGRAPHIC PROCEDURE

Sample	Days				
	1	3	6	8	15
1	99.2	98.5	92.4	88.5	84.6
2	99.0	95.3	91.9	87.6	84.7
3	99.4	97.2	92.1	88.7	85.2
4	99.0	96.6	92.2	89.6	83.0
5	99.8	96.5	90.1	88.8	85.8
6	99.7	97.1	92.3	88.5	84.5
7	99.1	97.2	92.8	86.9	83.7
8	98.3	96.8	91.8	87.8	84.3
9	98.0	94.1	90.5	88.3	86.3
10	99.8	97.7	92.3	88.1	85.7
Mean Value	99.13	96.70	91.84	88.28	84.78

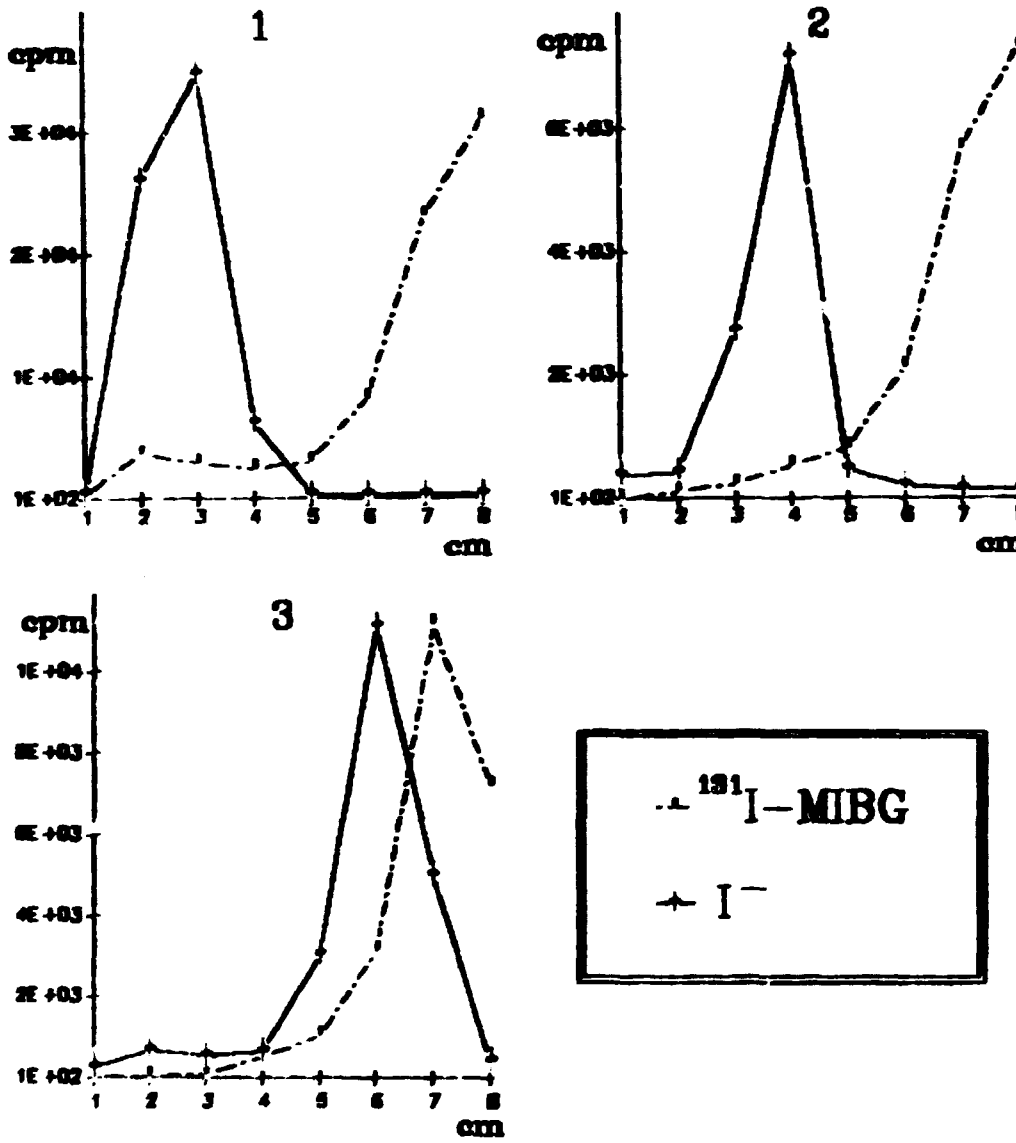

FIGURE 1 – GRAPHIC REPRESENTATION OF CHROMATOGRAPHIC STRIPS ACTIVITIES DISTRIBUTION FOR ^{131}I–MIBG. MINIATURIZED SYSTEMS: USING WHATMAN 3MM (8X1cm) AND DIFFERENTS SOLVENTS

1) ETHYL ACETATE, ETHANOL (1:1) Rf I^- = 0.3 and MIBG^1 = 1.0
2) ETHANOL, ETHYL ACETATE, AMMONIUM HIDROXIDE (20:20:1) Rf I^- = 0.3 and ^{131}I–MIBG = 1.0
3) ETHANOL, AMMONIUM HIDROXIDE (3/1) Rf I^- = 0.75 and ^{131}I–MIBG = 0.87

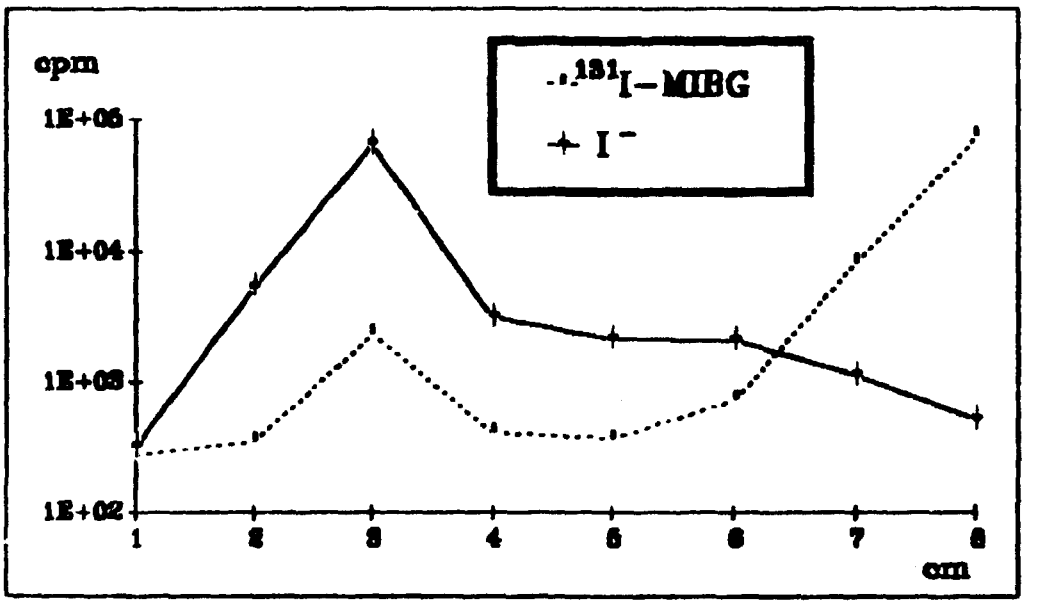

FIGURE 2 – GRAPHIC REPRESENTATION OF CHROMATOGRAPHIC STRIPS ACTIVITIES DISTRIBUITION FOR ^{131}I–MIBG, USING WHATMAN 3MM (15x1cm), (8x1cm) AND N–BUTANOL; ACETIC ACID AND H_2O (5:2:1) AS A SOLVENT

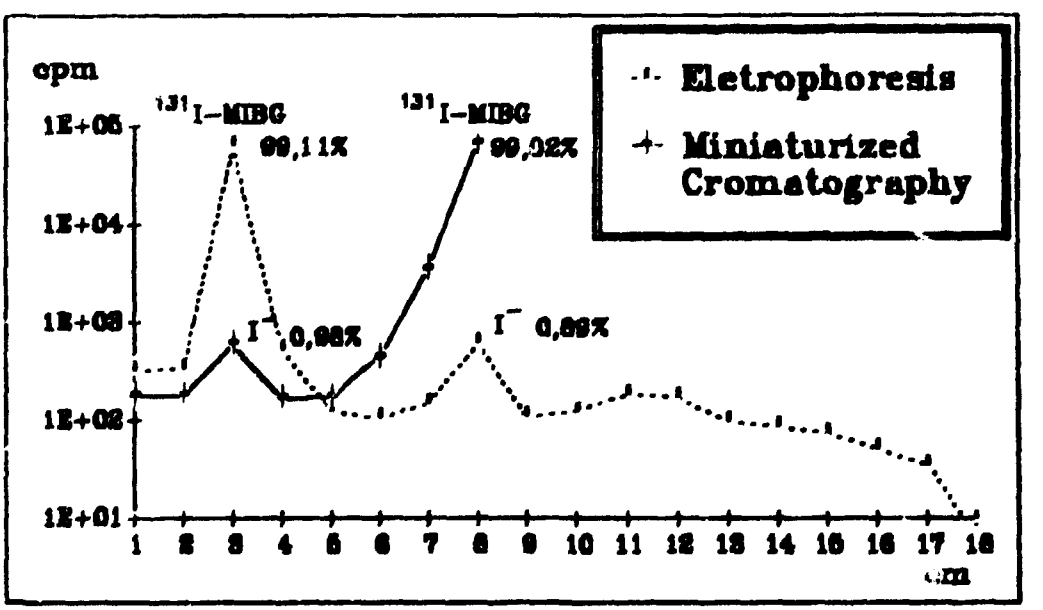

FIGURE 3 – GRAPHIC REPRESENTATION OF CHROMATOGRAPHIC STRIP ACTIVITY DISTRIBUITION FOR ^{131}I–MIBG (ELETROPHORESIS AND MINIATURIZED CHROMATOGRAPHY SYSTEMS)

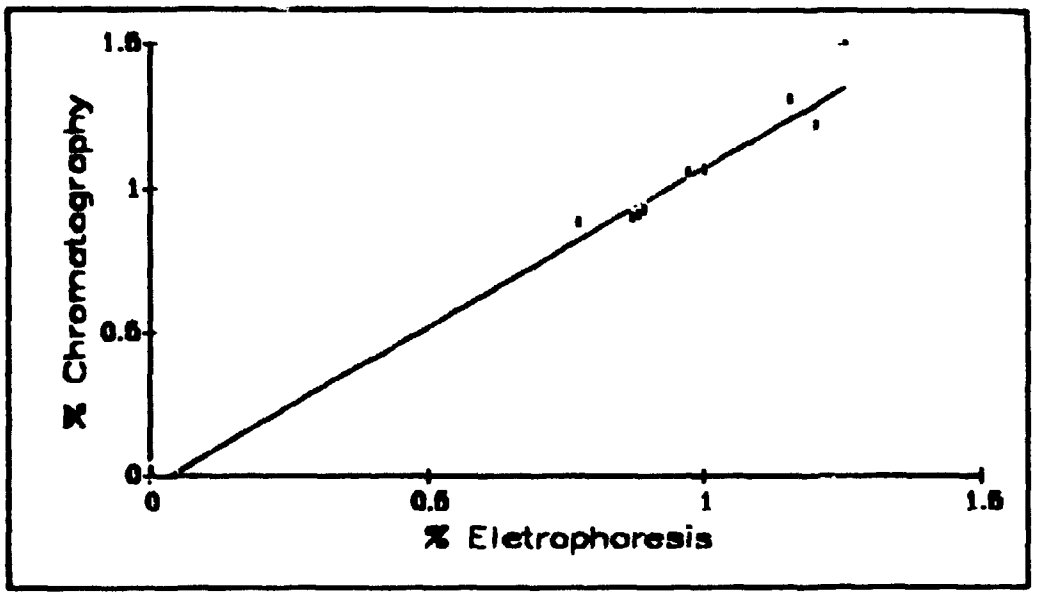

FIGURE 4 – GRAPHIC REPRESENTATION OF PERCENTUAL RESULTS OF IMPURITIES ELETROPHORESIS VERSUS MINIATURIZED CHROMATOGRAPHY FOR ^{131}I–MIBG
(Correlation Coefficient = 0.9836)