ISSN 0101-3084

CNEN/SP

ipen Instituto de Pesquisas Energéticas e Nucleares

DETERMINATION OF INORGANIC RADIOIODINE IN ^{131}I-ROSE BENGAL AND ^{131}I-BROMOSULPHTHALEIN

Iracelia Torres de Toledo e Souza, Nilda Sosa de Pereira and
Constancia Pagano Gonçalves da Silva

PUBLICAÇÃO IPEN 79 SETEMBRO/1985

SÃO PAULO

ISSN 0101-3084

PUBLICAÇÃO IPEN 79 SETEMBRO/1985

DETERMINATION OF INORGANIC RADIOIODINE IN ^{131}I-ROSE BENGAL AND ^{131}I-BROMOSULPHTHALEIN

Iracelia Torres de Toledo e Souza, Nilda Sosa de Pereira and
Constancia Pagano Gonçalves da Silva

DEPARTAMENTO DE PROCESSAMENTO

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

Série PUBLICAÇÃO IPEN

INIS Categories and Descriptors

B13.30

BROMOSULFOPHTHALEIN
CHROMATOGRAPHY
IODINE 131
ROSE BENGAL

Publicação aprovada pela CNEN em 15/05/85.

DETERMINATION OF INORGANIC RADIOIODINE IN ^{131}I-ROSE BENGAL AND ^{131}I-BROMOSULPHTHALEIN

Iracelia Torres de Toledo e Souza, Nilda Sosa de Pereira and
Constancia Pagano Gonçalves da Silva

ABSTRACT

A rapid miniaturized chromatographic system was developed for fast determination of the proportion of inorganic radioactive iodide from radiopharmaceuticals ^{131}I–Rose Bengal and ^{131}I–Bromosulphthalein.

Using 33% W/V aqueous solution of ammonium sulphate pH 7,5 as a solvent Rf values for radiopharmaceuticals, iodide, iodate correspond to Rf 0,0 0,5 0,9 respectively. The chromatographic quality control procedures are easy to use, rapid and can be incorporated in a routine quality control program.

DETERMINAÇÃO, POR CROMATOGRAFIA MINIATURIZADA, DE RADIOIODO INORGÂNICO EM SOLUÇÕES DE ROSA BENGALA – ^{131}I E BROMOSSULFALEINA ^{131}I

RESUMO

Desenvolvem-se um sistema rápido de cromatografia miniaturizada para controle radioquímico de Rosa Bengala – ^{131}I e Bromossulfaleina ^{131}I utilizando como solvente uma solução aquosa de sulfato de amônio 33%, pH 7,5. Os compostos marcados permanecem na origem enquanto os íons iodeto e iodato migram com Rf 0,5 e 0,9, respectivamente.

A cromatografia miniaturizada mostrou ser um método útil e fácil para a separação rápida de iodo inorgânico nos compostos iodados.

INTRODUCTION

Chromatographic quality control is an important facet of the daily quality control of a Radiopharmacy Department.

The electroforesis technique, conventional method[3,6], depends on the different migration rates of charged molecules in an electric field. Migration is primarily influenced by the polarity and magnitude of charge on a molecule and its size and shape, and also by the applied voltage, distance between electrodes, and duration of separation.

With the daily use of iodinated radiopharmaceuticals, a rapid and accurate chromatography system is needed to assess the radiochemical purity of the compounds.

The criteria applied in evaluating radiochemical quality control methods are of two types: The first consideration is the accurate and reprodutibility of the assay. The second criterion is that the procedure should be convenient for the assayer. Ideally, they should be simple, rapid and inexpensive to perform.

Inorganic iodide is the major radiochemical impurity of iodoorganic radiopharmaceuticals and may increase with storage.

The temperature is a physical agent that may influence the stability of the radiopharmaceuticals.

The United States Pharmacopoeia indicates that radiopharmaceuticals labelled with ^{131}I must not be used after 1 month from the date of standardization.

The purpose of this study is to develop a rapid, accurate and reproducible miniaturized chromatography system[1,8], wich could be used for commonly iodinated radiopharmaceuticals, after comparing it with electrophoresis method, which results in a linear function.

Lucka B. and Siuda A.[7,4] established that when aqueous solution of ammonium sulphate is used as solvent, iodide and iodate migrate on paper chromatograms at Rf 0,5 and Rf 0,9, respectively, whereas the labelled compound remains at the starting spot at Rf 0,0.

METHODOLOGY

Rose Bengal and Bromosulphthalein are iodinated with ^{131}I in a substitution reaction using chloramine T[5] and ICl[2] as oxidizing agents for Rose Bengal and Bromosulphthalein, respectively.

PURIFICATION

The separation of the iodination products was effected through a IRA-400 exchange resin.

QUALITY CONTROL

1. Electrophoresis (conventional method). The support, Whatmann nº 1 (35 cm x 2 cm) paper strip, should be allowed soak in the buffer before applying the sample. It is necessary to add a carrier. The radioactivity distribution after electrophoretic separation for about 40 minutes is determined by a developer in order to localize the radioactive impurities.

2. Miniaturized Chromatography (new method). The chromatographic separation was developed using Whatmann 3MM (6,5 cm x 1 cm) paper strip as support and 33% W/V aqueous ammonium sulphate solution pH 7,5 as solvent.

^{131}I-Rose Bengal and ^{131}I-Bromosulphthalein were spotted 1 cm from the botton on paper strips. The strips then were immediately placed in a vial containing approximately 1 ml of a 33% W/V aqueous $(NH_4)_2SO_4$ solution pH 7,5. The chromatogram was developed for a distance of 5 cm during 10 minutes. The strips then were removed, dried, cut into 10 sections of 0,5 cm and counted for radioactivity distribution using gamma scintillation spectrometry. ^{131}I-Rose Bengal and ^{131}I-Bromosulphthalein are retained at the starting spot while iodide and iodate migrates at Rf 0,5 and Rf 0,9, respectively. A good separation of the specific radiochemical components was achieved. Consequently the strips were cut in 3 uniform sections.

Paper presented in the "IX Congreso de la Asociación Latino-Americana de Sociedades de Biologia y Medicina Nuclear – – ALASBIMN – 09 – 13 diciembre 1984, Montevideo, Uruguay".

STORAGE

Impurities may arise during preparation and storage of radiopharmaceuticals. Radiation induced decomposition is the most commonly mentioned cause of chemical break down during storage of radiopharmaceuticals. The magnitude of radiolytic effects varies with the energy adsorbed by the radiopharmaceuticals solution as well as with the specific activity of the preparation. The radiolytic effects can be reduced by lowering the temperature at which samples are stored. The study of the effect of temperature and time of storage on radiopharmaceuticals was made at 4°C and at ambient temperature during 4 weeks.

RESULTS

The chromatograms presented in Figure 1 and Figure 2 pertain to samples after iodination containing relatively large percentage of inorganic iodide, and after purification, containing small amount of inorganic iodide. The radioactive chromatographic profile is essentially the same.

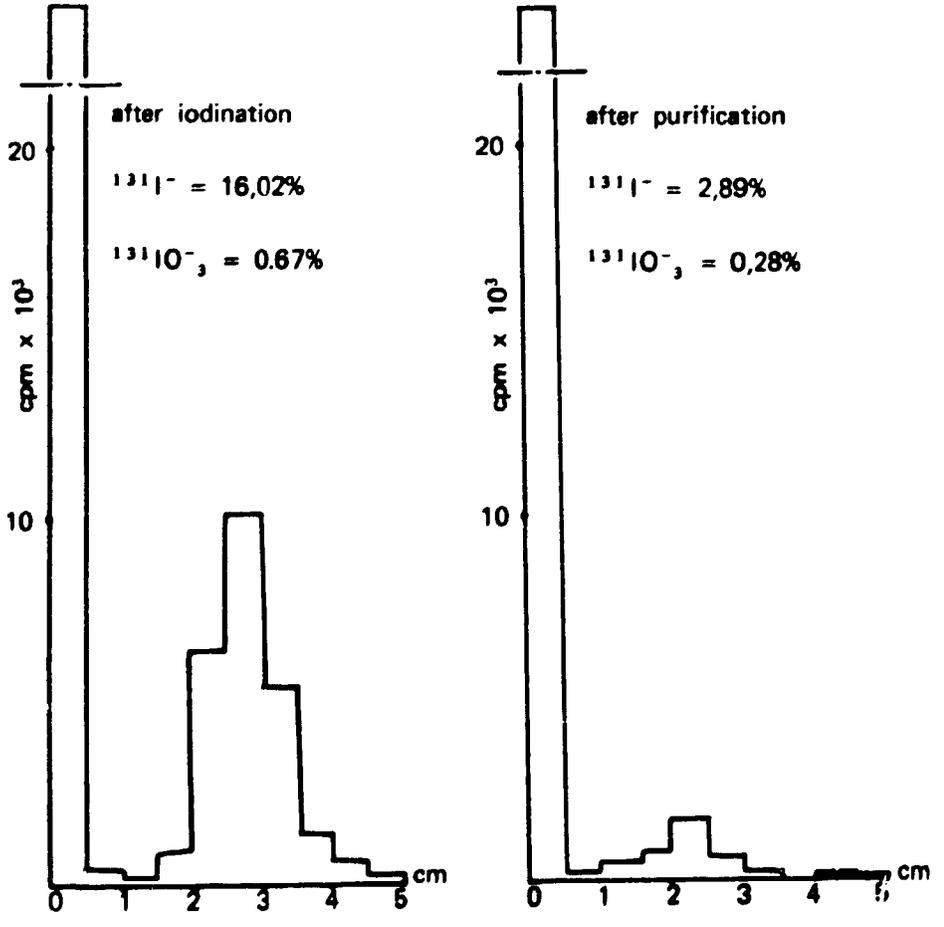

Figure 1 – Chromatographic Strip Activity Distribution for ^{131}I-Rose Bengal

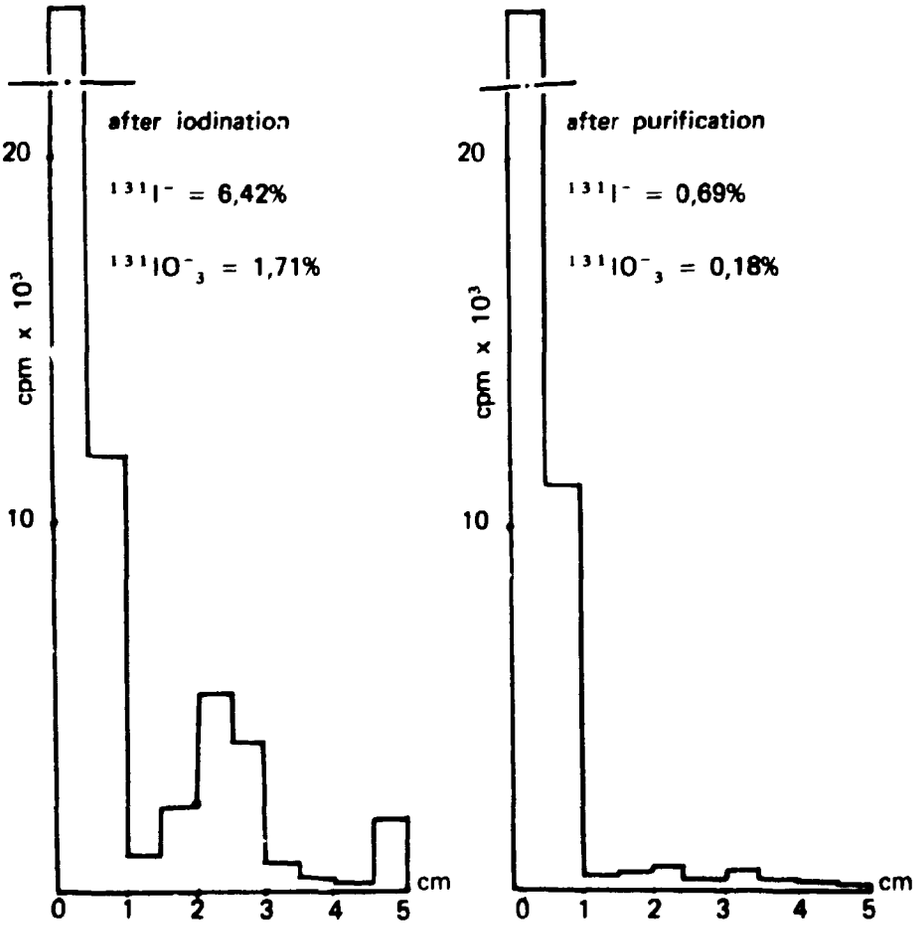

Figure 2 – Chromatographic Strip Activity Distribution for ^{131}I-Bromosulphthalein

The percentual of impurities ($^{131}I^-$ + $^{131}IO^-_3$) in ^{131}I-Rose Bengal and ^{131}I-Bromosulphthalein are shown in Table I and Table II. The mean values of the iodide determinations were in agreement with the data obtained by paper electrophoresis in a routine quality control procedure.

The correlation coefficient between the two methods was r = 0,9518 for ^{131}I-Rose Bengal and r = 0,9615 for ^{131}I-Bromosulphthalein, Figure 3 demonstrates with the data contained in Table I end Table II the linear regression obtained by the relationship between the variables.

Table I

Comparison of Percentual Values of Impurities ($^{131}I^-$ + $^{131}IO^-_3$) of ^{131}I-Rose Bengal by Electrophoresis and Miniaturized Chromatography

DATA	SPEC. ACTIVITY µCi/mg	ELECTROPHORESIS % ($^{131}I^-$ + $^{131}IO^-_3$)	CHROMATOGRAPHY
29.11.83	55	1.55	1.47
07.02.84	273	2.45	1.98
09.03.84	278	1.86	2.01
03.04.84	287	4.05	3.46
17.04.84	191	3.56	3.19
02.05.84	206	3.20	3.27
15.05.84	171	3.26	3.45
29.05.84	183	4.03	4.12
12.06.84	201	3.75	3.57
26.06.84	174	3.53	3.37
mean value r = 0.9518	201	3.12	2.98

Table II

Comparison of Percentual Values of Impurities ($^{131}I^-$ + $^{131}IO^-_3$) of ^{131}I-Bromosulphthalein by Electrophoresis and Miniaturized Chromatography

DATA	SPECIF. ACTIVITY µCi/mg	ELECTROPHORESIS % ($^{131}I^-$ + $^{131}IO^-_3$)	CHROMATOGRAPHY
21.11.83	710	1.69	1.59
05.12.83	598	0.55	0.76
13.03.84	489	1.30	1.37
11.04.84	538	0.51	0.52
24.04.84	600	0.23	0.26
09.05.84	719	1.58	1.25
22.05.84	472	1.05	1.28
04.06.84	548	2.14	2.05
18.06.84	483	0.98	0.84
03.07.84	331	1.10	1.01
mean values r = 0.9615	549	1.11	1.09

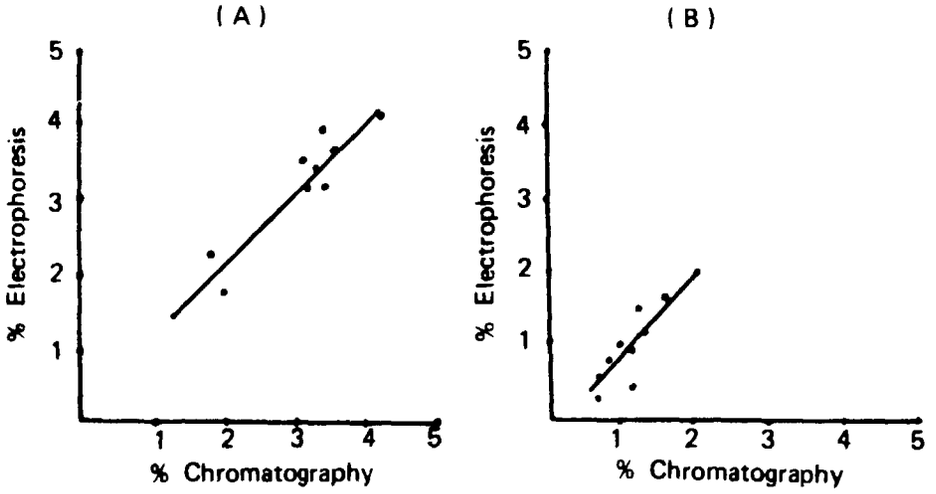

Figure 3 – Graphic Representation of Percentual Results of Impurities ($^{131}I^- + {}^{131}IO^-_3$) Electrophoresis X Miniaturized Chromatograph for ^{131}I-Rose Bengal (A) and ^{131}I-Bromosulphthalein (B)

Table III and Table IV indicate tha percentual of impurities ($^{131}I^- + {}^{131}IO^-_3$) in ^{131}I-Rose Bengal and ^{131}I-Bromosulphthalein obtained during 4 weeks after standardization.

Table III

Quality Control of ^{131}I-Rose Bengal During 4 Weeks After Standardization by Miniaturized Chromatographic Procedure

DATE	SPECIF. ACTIVITY μCi/mg	% ($^{131}I^- + {}^{131}IO^-_3$) 4°C temperature Week				% ($^{131}I^- + {}^{131}IO^-_3$) ambient temperature Week			
		1	2	3	4	1	2	3	4
29.11.83	55	1.57	2.14	2.29	3.50	1.91	3.14	3.18	4.29
07.02.84	273	3.13	3.16	3.19	3.93	3.57	3.63	3.99	4.43
09.03.84	278	2.29	2.29	2.70	2.80	3.08	3.16	4.21	4.24
03.04.84	287	3.18	4.28	4.35	4.66	3.62	4.54	4.83	5.28
15.05.84	171	3.42	3.53	3.56	3.74	3.73	4.05	4.18	4.47
26.06.84	174	3.19	3.33	3.57	3.65	3.61	4.10	4.36	4.43
mean value	206	2.79	3.12	3.27	3.71	3.25	3.77	4.12	4.52

Table IV

Quality Control of ^{131}I-Bromosulphthalein During 4 Weeks Standardization by Miniaturized Chromatographic Procedure

DATA	SPECIF. ACTIVITY	% ($^{131}I^-$ + $^{131}IO^-_3$) 4°C temperature Week				% ($^{131}I^-$ + $^{131}IO^-_3$) ambiente temperature Week			
		1	2	3	4	1	2	3	4
21.11.83	710	2.79	6.28	6.60	8.32	2.94	6.55	6.96	8.45
05.12.84	598	1.37	5.19	6.91	7.35	1.72	6,54	7.82	8.10
13.03.85	489	4.62	6.19	8.97	9.29	4.94	6.86	10.03	11.02
11.04.84	538	3.40	5.72	6.78	7.46	3.78	6.82	7.30	9.78
22.05.84	472	3.74	4.91	6.24	8.80	3.78	5.25	6.79	8.80
18.06.84	483	3.41	4.86	6.64	8.21	3.84	5.27	6.96	8.96
mean values	548	3.22	5.52	7.02	8.23	3.50	5.88	7.64	9.18

RESULTS AND CONCLUSIONS

The percentual of inorganic iodide for ^{131}I-Rose Bengal was approximately 3% after iodination and purification and a mean value of 4,52% after 4 weeks from standardization. For ^{131}I-Bromosulphthalein, they were about 1% and 9,18%, respectively.

The greater stability of ^{131}I-Rose Bengal in relation to that ^{131}I-Bromosulphthalein is probably due to its lower specific activity, mean value 206 μCi/mg versus mean value 548 μCi/mg.

The percentual of impurities ($^{131}I^-$ + $^{131}IO_3$) presented in Table III and Table IV was similar during storage of the radiopharmaceuticals at 4°C and at ambient temperature.

The miniaturized chromatography system for iodinated radiopharmaceuticals provides a rapid and easy method to assess the radiochemical purity and can be incorporated in a routine quality control program.

REFERÊNCIAS BIBLIOGRÁFICAS

1. COLOMBETI, L. G.; MOERLIEN, S.; PATEL, G. C.; PINSKY, S. M. Rapid determination of oxidation state of unbound ^{99m}Tc and labelling yield in ^{99m}Tc labelled radiopharmaceuticals. *J. Nucl. Med.* 17:805-809.

2. HUNTER, W. M. & GREENWOOD, F. C. Preparation of odine-131 labelled human growth hormone of high specific activity. *Nature,* 194:495-6, 1962.

3. INTERNATIONAL ATOMIC ENERGY AGENCY. *Analytical control of radiopharmaceuticals: proceedings of a panel on. . . , held in Vienna, 7-17 July 1969. Vienna, 1970.*

4. *LUCKA, B.* & SIUDA, A. Separation of inorganic iodine in the analysis of some iodoorganic radiopharmaceuticals. *J. Labelled Compd. Radiopharm.,* 18:1471-7, 1981.

5. McFARLANE, A. S. Efficient trace labelling of proteins with iodine. *Nature,* 182:53-

6. PHARMACOPEIAL CONVENTION, UNITED STATES. The pharmacopeia of the United States of America: meeting held in Washington D. C. April 8-10, 1970. Official from July 1, 1975.

7. SIUDA, A. & LUCKA, B. Determination of inorganic radioiodine in solutions of ^{125}I labelled proteins. *J. Labelled Compd. Radiopharm.*, 18:915-9, 1981.

8. ZIMMER, A. M. & PAVEL, D. G. Rapid miniaturized chromatographic quality control procedures for iodinated radiopharmaceuticals. *Am. J. Hosp. Pharm*, 35:426-8. 1978.