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# RADIOCHEMICAL STABILITY OF RADIOPHARMACEUTICAL PREPARATIONS

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## ABSTRACT

The "*in vitro*" stability studies of the radiopharmaceutical preparations are an essential requirement for routine practice in nuclear medicine and are an important parameter for evaluating the quality, safety and efficacy required for the sanitary registration of pharmaceutical products. Several countries have published guidelines for the evaluation of pharmaceutical stability. In Brazil, the stability studies should be conducted according to the Guide for Conducting Stability Studies published in the Resolution-RE n. 1, of 29$^{th}$ July 2005. There are also for radiopharmaceutical products, two specific resolutions: RDC-63 regulates the Good Manufacturing Practices for Radiopharmaceuticals and RDC-64 provides the Registration of Radiopharmaceuticals, both published on the 18$^{th}$ December 2009. The radiopharmaceutical stability is defined as the time during which the radioisotope can be safely used for the intended purpose. The radiochemical stability can be affected by a variety of factors, including storage temperature, amount of radioactivity, radioactive concentration, presence or absence of antioxidants or other stabilizing agents. The radiochemical stability studies must be established under controlled conditions determined by the effective use of the product. The aim of this work was to evaluate the radiochemical stability of labeled molecules with $^{131}$I, $^{123}$I, $^{153}$Sm, $^{18}$F, $^{51}$Cr, $^{177}$Lu and $^{111}$In as well as $^{67}$Ga and $^{201}$Tl radiopharmaceuticals. Radiochemical purity was evaluated after production and in the validity period, with the maximum activity and in the recommended storage conditions. The analyses were carried out by thin-layer silica gel plate, paper chromatography and gel chromatography. The experimental results showed to be in accordance with the specified limits for all the analysed products.

## 1. INTRODUCTION

The earliest works relating stability of pharmaceuticals go back to the 50's, when the marketing of new products were expressive. Up to 1984, there was no interference from regulations issued by health organizations in the evaluation of the stability of drugs. In order to attend the international trade and globalization, the health authorities harmonized the requirements necessary for the registration of drugs, including the stability studies in different climate zones [1].

Stability is defined as the time during which a drug maintains its characteristics within the specified limits and the safety and efficacy are the same as when it was developed and during storage period [2]. The stability of pharmaceutical products depends on several factors such as temperature,

humidity, light and others associated to the physical and chemical properties of the active substances, excipients, pharmaceutical form, as well as the manufacturing process, type and properties of the packaging materials [3]. There are five conditions that can affect the stability of drugs: chemistry, physics, microbiology, toxicology and therapeutics and these aspects should be verified for investigation of possible degradation products that may alter the safety and efficacy of the drug [2].

In Brazil, the stability studies of pharmaceutical products, classified as accelerated, monitoring and long duration must be conducted according to the Resolution-RE n. 1, 29th July 2005 - Guide for Performing Stability Studies of the National Sanitary Agency (ANVISA), in order to predict, determine or monitor the validity period of a drug. The stability study should consider the market for which the product is destined, that is, the climate zone of the region where it is going to be commercialized. Brazil is situated in the hot / humid climate zone IV, with storage conditions of 30 °C temperature and 70% relative humidity [1].

Radiopharmaceuticals are compounds with no pharmacological action, which have in their composition a radionuclide, and are used in nuclear medicine for diagnosis and therapy of various diseases. The physical-chemical characteristics of a radiopharmaceutical determine its pharmacokinetics, i.e., its fixation on the target organ, metabolism and excretion of the organism, whereas the physical characteristics of the radionuclide determine the application of the compound in diagnosis or therapy. The nuclear medicine makes use of the radiopharmaceuticals that allow the evaluation of not only the morphology but also the function of several organs. From the patient viewpoint, the technique is simple and only requires intravenous or oral administration of the radiopharmaceutical and the adverse reactions are rare [4].

Due to the growing importance of radiopharmaceuticals in Brazil, ANVISA published two new resolutions on the 18th December 2009 to regulate the radiopharmaceutical production: RDC-n. 63 - Good Manufacturing Practices (GMP) for Radiopharmaceuticals, to complement RDC-n. 17 of 4th August 2003 [5] and RDC-n. 64 - Registration of Radiopharmaceuticals.

The radiopharmaceutical stability is defined as the time during which the radioisotope can be safely used for the intended purpose. The radiochemical stability studies must be conducted to represent the effective use of the product. The radiochemical and radionuclidic controls are important assays to be performed in the radiopharmaceutical stability studies. Thin-layer and paper chromatography methods are the most widely used techniques for radiochemical control and the methods must be quick, easy to perform, able to separate all possible components without changes in the sample composition, accurate and sensitive [4].

The aim of this work was to evaluate the radiochemical stability of labeled molecules with $^{131}$I, $^{123}$I, $^{153}$Sm, $^{18}$F, $^{51}$Cr, $^{177}$Lu and $^{111}$In as well as $^{67}$Ga and $^{201}$Tl radiopharmaceuticals produced at IPEN-CNEN/SP.

## 2. EXPERIMENTAL

### 2.1. Samples

Samples from three different batches of $^{51}$Cr-EDTA (ethylenediamine tetraacetic acid-chromium-51); $^{18}$F-FDG (2-deoxy-2-fluoro-D-glucose-fluoro-18); $^{67}$Ga-Citrate (gallium-67 citrate); $^{131/123}$I-MIGB (meta-iodobenzylguanidine-iodine-131/123); $^{111}$In-Octreotide (pentate-indium-111); $^{177}$Lu-DOTATATE [DOTA(1,4,7,10-tetraazacyclododecane-*N*-,*N*-,*N*-,*N*-tetraacetic acid)D-Phe1-Tyr3-octreotate]; $^{131/123}$I (sodium iodine-131/123); $^{153}$Sm-EDTMP (ethylenediaminetetramethylene

phosphonic acid-samarium-153); $^{153}$Sm-HA (hydroxiapatite-samarium-153) and $^{201}$TlCl (thallium-201 chloride) were produced at IPEN-CNEN/SP and analysed.

The radiochemical stability studies were conducted for each product after the production and during the validity period, at the recommended storage conditions and in the maximum activity/volume. These parameters for each radiopharmaceutical are described in Tab. 1.

**Table 1. Parameters for Stability Studies of Radiopharmaceuticals**

| PRODUCT | TIME PERIOD | STORAGE TEMPERATURE (°C) | MAXIMUM ACTIVITY / VOLUME (MBq/mL) |
|---|---|---|---|
| **$^{51}$Cr-EDTA** | 15 days | 2-8 | 555 / 14.3 |
| **$^{18}$F-FDG** | 0, 5, 10 hours | ≤ 30 | 7104 / 2.4 |
| **$^{67}$Ga-Citrate** | 10 days | ≤ 30 | 2590 / 11.6 |
| **$^{131}$I-MIBG** | 5 days | ≤ -15 | 555 / 7.3 |
| **$^{123}$I-MIBG** | 48 hours | ≤ -15 | 740 / 1.7 |
| **$^{111}$In-Octreotide** | 48 hours | ≤ -15 | 555 / 0.8 |
| **$^{177}$Lu-DOTATATE** | 48 hours | ≤ -15 | 7400 / 1.1 |
| **Na $^{131}$I** | 15 days | ≤ 30 | 7400 / 1.2 |
| **Na $^{123}$I** | 48 hours | ≤ 30 | 740 / 1.0 |
| **$^{153}$Sm-EDTMP** | 48 hours | ≤ -15 | 7400 / 7.3 |
| **$^{153}$Sm-HA** | 48 hours | ≤ 30 | 7400 / 3.2 |
| **$^{201}$TlCl** | 10 days | ≤ 30 | 1110 / 8.6 |

## 2.2. Quality Control

Radiochemical purity was carried out by thin-layer-silica-gel-alumina (TLC-Al, Merck, Germany) plate, paper chromatography (PC, W3MM or W1MM, Whatman) and gel chromatography (Sephadex C-25, Pharmacia). Tab. 2 describes the mobile and stationary phases used to determine the radiochemical purity in the products.

**Table 2. Mobile and Stationary Phases for Radiochemical Purity Control**

| PRODUCT | MOBILE PHASES | STATIONARY PHASES |
|---|---|---|
| **$^{51}$Cr-EDTA** | 33% (p/v) Ammonium sulphate | W3MM (9 cm x 1 cm) |
| **$^{18}$F-FDG** | 95% Acetonitrile:Water | TLC-Al (12.5 cm x 1.5 cm) |
| **$^{67}$Ga-Citrate** | 0.16 mol $L^{-1}$ Sodium acetate | W3MM (9 cm x 1 cm) |
| **$^{131}$I-MIBG/$^{123}$I-MIBG** | (5:2:1)Buthanol-1:Concentraded Acetic Acid :Water | W3MM (8 cm x 1 cm) |
| **$^{111}$In-Octreotide** | 0.2 mol $L^{-1}$ EDTA | TLC-Al (12.5 cm x 1.5 cm) |
| **$^{177}$Lu-DOTATATE** | 0.1 mol $L^{-1}$ Sodium citrate buffer | TLC-Al (12.5 cm x 1.5 cm) |
| **Na $^{131}$I/Na $^{123}$I** | 85% Methanol:Water | W3MM (12.5 cm x 1.5 cm) |
| **$^{153}$Sm-EDTMP** | 0.9% (p/v) NaCl | Sephadex C-25 column |
| **$^{153}$Sm-HA** | 0.9% (p/v) NaCl | W1MM (12.5 cm x 1.5 cm) |
| **$^{201}$TlCl** | Acetone | W3MM (8 cm x 1 cm) |

Each product was applied with a glass capillary on the chromatography plate and immediately placed in a pre-saturated chamber with the mobile phase, in triplicate.

After migration of the mobile phase 1 cm from the top, the plates were dried; cut into 2, 7 or 10 pieces and each segment had the radioactivity measured in a gamma counter (Perkin Elmer Gamma Counter, Perkin Elmer) during 0.20 minutes using each radioisotope energy window. The gamma counter data were expressed in counts per minute (cpm).

$^{153}$Sm-EDTMP radiochemical purity was evaluated in Sephadex C-25 column (2.0 cm x 2.5 cm). The sample was loaded into the column and the total activity was measured in a dose calibrator (CRC-15, Capintec). After the elution with 25 mL 0.9% (p/v) NaCl, the radioactivity of the column was measured. The impurity remained in the column.

## 3. RESULTS AND DISCUSSION

In this study, the stability of 12 radiopharmaceutical compounds with radionuclides of half-life, ranging from hours to days, was evaluated.

The stability study was performed to comply with the requisites of RE n.1, evaluating the radiochemical purity in the maximum activity and volume of products of three batches of each radiopharmaceutical. Radiochemical impurities can arise from the decomposition of the radiopharmaceutical during the production process, in the labeling step, by the variation of the temperature and pH, light, presence of oxidizing or reducing agents and also by radiolysis [6, 7, 8].

pH is an important parameter that has a dramatic impact on ionic equilibrium and solubility of radiometals. For example, the oxidation state of $^{201}$Tl ions are strongly influenced by pH. An alkaline pH favors the formation of the desired thallium ions ($Tl(OH)_2^+$), whereas a low acidic pH favors the formation of thallic ions ($Tl^{+3}$), which may form complex ions that give undesired thyroid and RBCs uptake. In the preparation of $^{111}$In and $^{177}$Lu-labeled antibodies, the pH range 4.5-6 is adequate for efficient labeling and to maintain the stability of the carrier molecule, and also prevent hydrolysis of $^{111}$In ions. In the $Na^{131}I$/ $Na^{123}I$ production, the volatilization of iodine occurs as the result of the oxidation of iodide by dissolved oxygen in slightly acidic solution and the use of alkaline pH or addition of reducing agents decrease significantly the volatility rate [8].

Radiolysis generates free radicals and it is one of the main factors for the degradation of radiolabeled preparations. The radiolysis can lead to breakage of chemical bonds between the radionuclide and the molecule, promoting the occurrence of radiochemical impurities. The radiochemical purity need to be evaluated because the impurity may become the predominant tracer circulating in the bloodstream causing excessive body background, masking the diagnostic of the disease, and increasing the radiation dose to the patient [7, 8].

The generation of free radicals by radiolysis is an important problem to be overcome in $^{131}$I products. In $^{131}$I-MIBG formulations, the radiolysis can generate free $^{131}$I as radiochemical impurity [8].

Tab. 3 shows the radiochemical purity results of the analysed products in the maximum activities in the validity period and the limits of acceptance for the radiopharmaceuticals reported in U.S. Pharmacopeia [9].

**Table 3. Results of the Radiochemical Stability Studies**

| PRODUCT | RADIOCHEMICAL PURITY (%) | | USP LIMIT [9] |
|---|---|---|---|
| **$^{51}$Cr-EDTA** | Initial = | 99.43 ± 0.69 | ≥ 95% |
| | 15 days = | 99.77 ± 0.04 | |
| **$^{18}$F-FDG** | Initial = | 99.06 ± 0.38 | ≥ 90% |
| | 5 hours = | 94.24 ± 2.70 | |
| | 10 hours = | 93.84 ± 3.05 | |
| **$^{67}$Ga-Citrate** | Initial = | 99.14 ± 0.80 | ≥ 97% |
| | 10 days = | 98.30 ± 0.59 | |
| **$^{131}$I-MIBG** | Initial = | 98.62 ± 0.74 | ≥ 95% |
| | 5 days = | 98.78 ± 0.47 | |
| **$^{123}$I-MIBG** | Initial = | 98.53 ± 0.65 | ≥ 95% |
| | 48 hours = | 97.88 ± 0.62 | |
| **$^{111}$In-Octreotide** | Initial = | 99.68 ± 0.15 | ≥ 95% |
| | 48 hours = | 99.64 ± 0.09 | |
| **$^{177}$Lu-DOTATATE** | Initial = | 99.72 ± 0.29 | ≥ 95% |
| | 48 hours = | 99.80 ± 0.25 | |
| **Na$^{131}$I** | Initial = | 99.61 ± 0.16 | ≥ 95% |
| | 10 days = | 99.53 ± 0.13 | |
| **Na$^{123}$I** | Initial = | 99.43 ± 0.12 | ≥ 95% |
| | 48 hours = | 99.34 ± 0.27 | |
| **$^{153}$Sm-EDTMP** | Initial = | 99.47 ± 0.53 | ≥ 98% |
| | 48 hours = | 99.84 ± 0.10 | |
| **$^{153}$Sm-HA** | Initial = | 99.73 ± 0.11 | ≥ 95% |
| | 48 hours = | 99.74 ± 0.08 | |
| **$^{201}$TlCl** | Initial = | 99.52 ± 0.57 | ≥ 95% |
| | 10 days = | 99.61 ± 0.55 | |

All the results were above the limits of radiochemical purity proposed by USP [9].

$^{51}$Cr-EDTA was stored at 2-8 °C for 15 days without significant differences in the radiochemical purity in relation to the day of production.

The radiochemical purity of $^{67}$Ga-Citrate, $^{201}$TlCl and Na$^{131}$I radiopharmaceuticals 10 days after the production, stored at temperature ≤ 30 °C was higher than 98%. Na$^{123}$I also maintained the radiochemical stability two days after production.

Using the automatized module for synthesis of $^{18}$F-FDG, chemical, radiochemical and radionuclidic impurities were retained on the cartridges and purification columns to obtain the final product [10]. The storage temperature for this product was ≤ 30 °C and 10 hours after production, the stability results of radiochemical purity were > 93%, 6% lower than the initial analysis.

For products in which the temperature affects the labeling efficiency, dried ice was put in the transportation package with a caution to place the vial inside the lead protection in the freezer of the customer soon after receiving and up to use time. It was observed that the temperature inside the vial with dried ice was lower than in a freezer. For $^{131}$I-MIBG for diagnostic application, no effect due to radiolysis was detected by keeping the vial in the freezer (at ≤ -15 °C) for five days. The

stability of $^{111}$In and $^{177}$Lu-labeled antibodies can decrease with time and temperature but $^{111}$In-Octreotide, $^{177}$Lu-DOTATATE stored at ≤ -15 °C maintained the labeling efficiency > 99% [8].

$^{153}$Sm-HA radiochemical purity was >99% after 2 days at ≤ 30 °C.

## 4. CONCLUSIONS

The stability is an essential parameter to evaluate the quality, safety and efficacy of pharmaceutical and radiopharmaceutical products during their validity period. However, due to the peculiarity of radiopharmaceuticals, the design of the stability studies has been laborious involving the participation of the Production, Quality Control and Quality Assurance Groups.

The experimental results of radiochemical purity were in accordance with the specified limits for all analysed products, and it was shown the importance of maintaining the storage condition recommended by the manufacturers in order to guarantee their quality.

New stability studies shall be conducted considering the national regulations and aiming to establish the basis of a reference study for radiopharmaceuticals.

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