Radiofarmácia/Radioquímica

• Painel •

A GAS CHROMATOGRAPHY TECHNIQUE FOR ANALYSIS OF RESIDUAL SOLVENTS IN 18F-FDG PREPARATION.

Patrícia de A. Martins; José Luiz da Silva; João O. Junior; Neuza T.O. Fukumori; Margaret M. Matsuda.
Centro de Radiofarmácia, IPEN-CNEN – São Paulo, SP, Brasil.

Aim: Positron emission tomography (PET) with 18F-2-deoxy-2-fluoro-D-glucose (FDG) has been investigated as a means of detecting primary tumors in recent years. The analysis of 18F-FDG has focused on the detection of chemical, radiochemical and radionuclidic purities and little attention has been paid to the analysis of residual solvents involved in the preparation of 18F-FDG. Any residual solvent with potential toxic, physiologic or pharmacological effects must be evaluated. The aim of this study was to develop a quantitative method for residual acetonitrile, ethanol and isopropanol in 18F-FDG using gas chromatography. **Materials and methods:** Analysis was carried out on a Shimadzu 17AA gas chromatography equipped with a flame ionization detector (FID) and an auto-sampler. The injection was configured for split sample injection at a ratio 20:1 and operated at 250ºC. It was used a J&W DBWAX column 30 m x 0.25 mm and operated at a temperature range between 50-85ºC. Helium was used as the carrier gas (flow rate 2.0 mL/min). The detector was operated at 250ºC and injection sample volume was 1.0 µL. Standard solutions were prepared with high purity solvents in purified water. Calibration curves were prepared with concentration range of 0-600 ppm. Three injections of each standard were made to obtain the data. 50 samples of different batches were stored in sealed vials at room temperature and analyzed. **Results and discussion:** The analysis time was 3.75 min. The retention time for isopropanol, ethanol and acetonitrile were 2.20, 2.25 and 2.69 min, respectively. The USP 28 and FDA specified that the permissible levels of the residual solvents in the final preparation of 18F-FDG might not exceed 400 ppm for acetonitrile and 5000 ppm for ethanol and diethyl ether. In the considered process isopropanol is used instead of diethyl ether for cleaning. The correlation coefficients of the calibration curves were 0.9990 for isopropanol, 0.9988 for ethanol and 0.9979 for acetonitrile. In the 50 analyzed samples all the levels were below the allowable limit described for USP and FDA. The obtained range for isopropanol, ethanol and acetonitrile in the samples of 18F-FDG were 9.09-40.12 ppm, 27.23-515.28 ppm and 22.07-150.71 ppm, respectively. **Conclusion:** Gas chromatography is an excellent technique for determination of the residual solvents in the final preparation of 18F-FDG. The levels observed in the samples were in accordance with the permissible levels proposed for the USP and FDA.

• Tema Livre •

A SIMPLE METHOD FOR BONE MARROW-DERIVED MESENCHYMAL STEM CELLS LABELING WITH Tc-99m.

Gutfilen B.; Rodrigues C.; Goldenberg R.; Rezende G.; Mendonça C.A.G.; Carvalho A.C.C.; Fonseca L.M.B.
Hospital Universitário Clementino Fraga Filho e Instituto de Biofísica Carlos Chagas Filho – UFRJ.

Background: The ability to incorporate readily available radionuclides with optimal decay characteristics into tracer molecules has been the foremost consideration in development diagnostic radiopharmaceuticals. In this respect, 99mTc has become the mainstay of diagnostic nuclear medicine and in some chemical form is used in the majority of the diagnostic scans performed each year in hospitals. This preferential use of 99mTc radiopharmaceuticals reflects the ideal nuclear properties of the isotope, as well as its convenient availability from commercial generator columns. Employment of radiolabeled stem cells can provide many important contributions in the monitorization of cell delivery. It may also prove to constitute in the future an important ancillary procedure in the procession of the treatment of several chronic diseases. **Objective:** To evaluate homing and retention of the stem cells into human body organs. **Methods:** Approximately 10% of these cells were labeled with technetium 99m (370 MBq) by incubation in saline solution of SnCl2. Scintigraphic images were obtained one, three and twenty four hours after cell injection. **Results:** The radio labeling of stem cell with Tc-99m was obtained with high efficiency (89%). Imaging of 99mTc-stem cell following intravenous injection into normal rat showed the accumulation of radioactivity in liver, kidneys, lungs and spleen. When 99mTc-stem cells were injected into the heart of a chagasic patient, the radioactivity was accumulated in the liver, lungs and heart. When 99mTc-stem cells labeled were injected into hepatic artery by angiography in cirrhotic patients we could observe their homing by scintigraphy during 24 hours. **Conclusion:** 99mTc-stem cell is simple to prepare and uses a labeling agent for 24 hours distribution studies of injected stem cells.

• Tema Livre •

ASSESSMENT OF THE ANIMAL BIODISTRIBUTION OF SEVEN GLUCOSE APPENDED [99mTc]TECHNETIUM COMPLEXES IN A MURINE MELANOMA TUMOR MODEL.

Marques F.L.N.(1); Fisher C.(3); Okamoto M.R.Y.(1); Rodrigues J.B.S.(1); Otake A.(2); Storr T.(3); Orvig(3); Adam M.J.(4); Chammas R.(2); Buchpiguel C.A.(1).
(1)Center of Nuclear Medicine (LIM 43) and (2)Laboratory of Oncology (LIM 24) – Faculty of Medical Sciences, São Paulo University – Brazil; (3)Chemistry Department, University of British Columbia – Canada; (4) TRIUMF – Canada.

Objective: In this work we prepared seven carbohydrate-appended [99mTc]technetium complexes, including two bisoxo-bisdiamine ([99mTc(O)2(Ldam)2]+), two diamine-tricarbonyl ([99mTc(Ldam)(CO)3]+) and three 2,2´-dipicolylamine-tricarbonyl ([99mTc(Ldpy)(CO)3]+) complexes, as candidate for (18F)FDG substitution in nuclear medicine imaging in tumor detection. The biodistribution of each one was assessed in a tumor animal model. **Methods:** Ligands Len = 2,3-diamino-1-propyl-ß-D-glucopyranosyl, Lpen = 1,3-diamino-2-propyl-ß-D-glucopyranosyl, Lpy = 2-(bis(2-pyridinylmethyl)amino)ethyl- ß-D-glucopyranosyl, LpyN = 2-(bis(2-pyridinylmethyl)amino)acetyl-a-D-1-amineglucopyranosyl and LpyS = 2-(bis(2-pyridinylmethyl)-amino)ethyl- ß-D-2-thioglucopyranosyl were synthesized and characterized previously. [99mTc(O)2(Len)2]+ and [99mTc(O)2(Lpen)2]+ complexes were prepared by the reduction of TcO4- with Sn2+ in alkaline medium; [99mTc(Len)(CO)3]+, [99mTc(Lpen)(CO)3]+, [99mTc(Lpy)(CO)3]+, [99mTc(LpyN)(CO)3]+ and [99mTc(LpyS)(CO)3]+ were prepared from an Isolink® kit, through transchelation. Radiochemical purity was determinated by HPLC or thin layer chromatography. The biodistribution of each complex was assessed in a C57BL6 mouse with implanted B16F10 murine melanoma tumor cells. Animals (n=3 to 5) were anesthetized, killed and organs were excised at 15, 60, 120 and 240 minutes after complexes injection and the concentration of the compounds was calculated as% dose/g organ. **Results:** Radiochemical purity of all complexes were over 95%. All complexes showed insignificant cardiac and cerebral uptake, the main organs of FDG metabolism. In general, [99mTc(Ldam)(CO)3]+ and [99mTc(Ldpy)(CO)3]+ complexes have hepatobilary system as principal elimination route, whereas [99mTc(O)2(Ldam)2]+ complexes were eliminated by the kidneys. Best data for tumor concentration (a) and tumor/blood ratio (b), were taken at 120 minutes, and the best results were given by the following complex: [99mTc(O)2(Lpen)2]+ (a) = 1.30±0.40% and (b) = 1.39 ± 0.57%; [99mTc)Len(CO)3]+ (a) = 1.22±0.61% and (b) = 1.88 ± 0.66%; [99mTc(Lpyr)(CO)3]+, (a) = 0.43 ± 0.12% and (b) = 1.53 ± 0.68%. Our general results permit to observe that less lipophilic complexes ([99mTc(O)2(Lpen)2]+) and ([99mTc(Len)(CO)3]+) have

higher tumor accumulation in relation to more lipophilic ([99mTc (Lpyr)(CO)3]+) but, on the other hand, the last one has a fast blood pool washout. **Conclusion:** The concentration of these complexes in the tumor and tumor/blood ratio, is comparable to data published in the literature for other glucose appended 99mTc complex, but it is improbable they will be used in clinical trials due to the high uptake in surrounding organs. The insignificant cardiac and cerebral concentrations suggest that these compounds were not metabolized as glucose or (18F)FDG. So, research in this area must be continued to understand the uptake mechanisms and to rationalize the development of new products.

• Painel •

DEVELOPMENT OF IODINATION METHODOLOGIES FOR THE OBTENTION OF 5-[123I]IODOURACIL AND IODOAROMATICS COMPOUNDS.

Cíntia de Andrade Custódio; Simon John Garden; Léa Mírian Barbosa da Fonseca; Maria Verônica Fonseca Torres de Oliveira.
Instituto de Engenharia Nuclear – IEN/CNEN; Instituto de Química – IQ/UFRJ; Departamento de Radiologia/Medicina Nuclear – HU/UFRJ.

The substance 5-iodouracil is a derivative of the heterocyclic base, pyrimidine. Uracil is a pyrimidine base found in RNA, whereas 5-iodouracil may act as an analog of thymine, a pyrimidine base found in DNA. Aromatic halides have been used in organic synthesis for more than 100 years and they are therefore important intermediates in synthetic organic chemistry. Aromatic halides are used as intermediates for the obtention of other functional groups on the aromatic ring either by substitution reactions or via aromatic organometallic reagents. **Aim:** This study aimed to investigate the development of radiochemical synthetic methodologies for the obtention of 5-[123I]iodouracil. As well as the synthesis of 5-[123I]iodouracil, the iodination of aminobenzonitrile derivatives was investigated using potassium dichloroiodate as the iodination reagent. As well as the preparation of the 123I labeled molecule, this study aimed to investigate the use of the molecule as radioactive tracer for imaging in tumor cells. **Methods:** The radiochemical synthetic methodologies used were electrophilic substitution and isotopic and non-isotopic exchange reactions. **Results and discussion:** Inexpensive and readily available oxidants were employed in the electrophilic substitution reactions: chloramine T, Oxone®, ammonium and cerium nitrate, trichloroisocyanuric acid were purchased, and potassium dichloroiodate was synthesized. The optimal reaction conditions were generally mild and resulted in good radiochemical yields. For the exchange reactions, isotopic and non-isotopic methodologies were developed. In order to develop the non-isotopic exchange 5-bromouracil was prepared, so as to obtain the product of interest in a carrier free state. In this study it was found that the exchange methodologies gave inferior radiochemical yield when compared with electrophilic substitution reactions. The iodination of aminobenzonitrile derivatives was investigated using potassium dichloroiodate as the iodination reagent. The results obtained confirmed the versatility of this reagent and the reactions gave satisfactory chemo- and regio-selective results. **Conclusions:** The iodinated aminobenzonitrile derivatives could be used as precursors for the synthesis of radiopharmaceuticals used in imaging. The labeled molecule, 5-[123I]iodouracil, was found to have good prospects for use in imaging in Nuclear Medicine.

• Tema Livre •

111In-DTPA-OCTREOTIDE: PRODUCTION AND QUALITY CONTROL.

Barboza M.F.; Fukumori N.T.O.; Herrerias R.; Souza A.A.; Pires J.A.; Cerqueira Filho A.; Couto R.; Campos D.G.; Araujo E.B.; Mengatti J.; Lima E.N.
IPEN-CNEN/SP; Hospital do Câncer.

Recent advances in receptor mediated-tumor imaging have resulted in the development of somatostatin analogues. Somatostatin binds with high affinity to all hSSTr-subtypes and undergoes rapid "in-vivo" degradation compared with octreotide. Octreotide, an octapeptide analog of somatostatin, has a longer biological half-life, which makes it more suitable for labeling and imaging. Ocrteotride can be radioiodinated or labeled with radiometals: In-111; Ga-67; Cu-64; Y-90 and Tb-161.The successful use of radiolabeled somatostatin analogues in imaging promoted further studies in utilizing them in radiopeptide therapy. The aim of this work is to establish and validate the labelling, the quality control procedures and evaluate the "in-vitro" stability for routine production and clinical application of 111In-DTPA-Tyr3-Octreotide (111In-DTPA-Oct)-. The labeling of DTPA-Oct with In-111 (111InCl3) was performed in a "glove-box" under GMP condition, with 1850 - 3700 MBq of 111InCl3 at pH 4.5, using radionuclide:peptide ratios of 122 MBq/10 µg in sodium acetate buffer, at room temperature for 30 minutes. All solutions were prepared with WFI water. Radiochemical purity was determined by ITLC-SG using 0.1M sodium citrate, pH 5.5, as solvent. The labeled peptide migrates from the origin Rf = 0.4 –0.5 and the radionuclide migrates with the solvent front Rf = 1.0. Radiochemical purity was also determined using Sep-Pack silica cartridge. The free radionuclide was eluted with 5 mL of 0.1M sodium acetate, pH 5.5, and the labeled peptide with 5 mL of methanol. The stability of the final product was evaluated immediately, 24 and 48 hours kept under refrigeration. Sterility and pyrogen tests are performed by the microbiology procedures in different culture medium and the apirogenicity by the "in-vitro" Limulus test (LAL). The final product presents the following characteristics: radioactive concentration of 185 MBq/mL; chemical concentration of 15–16 µg/mL; specific activity of 12.21 MBq/µg; validation and calibration time of 48 hours. The stability of the radiolabeled peptide (111In-DTPA-Oct) was high even 48 hours under refrigeration, exceeding a radiochemical purity of 98%, determined in both systems. Sterility and pyrogen tests were negative in all the delivered vials, which are considered suitable for clinical applications. The efficient procedure to obtain 111In-DTPA-Oct was confirmed in the first clinical groups.

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INVASIVE EVALUATION OF 99mTc(CO)3-THYMIDINE ANALOG IN A LUNG CANCER MODEL.

Rodrigo L.S.R. Santos; Bluma L. Faintuch; Rodrigo Teodoro; Emiko Muramoto; Ivane V.S. Nunes.
Centro de Radiofarmácia, Instituto de Pesquisas Energéticas e Nucleares – São Paulo, SP, Brasil.

Introduction: The use of [99mTc(CO)3]+ as a radiopharmaceutical precursor opens new routes in the labeling of biomolecules. Labeled thymidine is used for tumor imaging, since it is incorporated into DNA and therefore provides a measure of cell proliferation. For the current study thymidine was functionalized at the C5'position of the sugar moiety with the tridentate iminodiacetic acid chelator for complexation and radiolabeling with 99mTc(I)-tricarbonyl core. A lung cancer model was selected because this is one of the most lethal of cancers worldwide causing up to 3 million deaths annually. **Aim:** The aim was a biodistribution study of the complex 99mTc(CO)3-thymidine analog in nude mice bearing lung cancer tumor. **Methods:** The preparation of the organometallic technetium precursor was done under mild reaction following the procedures of Alberto et al. (1998) protocol, where gaseous carbon monoxide and sodium borohydride were used. Then the ligand iminodiacetic acid thymidine was radiolabeled with previously prepared 99mTc-carbonyl. Athymic male nude mice were inoculated with a human non-small-cell lung carcinoma cell line (A549). Ten days after the inoculation the radioactive complex was injected and one and half hour after the administration of the drug the animals were sacrificed and the invasive studies performed. **Results:** Yield of the [99mTc(CO)3]+ was 98.3 ± 0.8%. Radiochemical purity of [99mTc(CO)3]-thymidine analog was 97.3 ± 0.4%. Biodistribution studies in mice bearing tumor showed

the highest uptake by intestine, followed by liver and kidneys. It was observed that blood clearance was not very fast after 1.5 hour. Tumor/blood and tumor/muscle ratios were 0.2 and 1.4 respectively. Uptake by the tumor was 0.3 ± 0.02%ID/g. **Conclusion:** Despite the good radiochemical profile of the complex, the uptake in lung tumor was low. Other tumor models should be used in the search for better results.

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PREPARAÇÃO E CONTROLE DE QUALIDADE DO GLUCARATO-99mTc.

A.C. Camargo(1); R. Herrerias(2); R.M. Couto(2); E. Muramoto(2); J. Mengatti(2); C.P.G. Silva(2); M.F. Barboza(2).
(1)Hospital Israelita Albert Einstein – HIAE/SP; Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN/SP(2) – Brasil.

As doenças cardiovasculares encontram-se entre as maiores causas de morbidade e mortalidade no adulto. O infarto agudo do miocárdio continua sendo freqüente, apesar dos avanços da medicina preventiva. O diagnóstico baseia-se normalmente na tríade: dor anginosa típica, alterações do eletrocardiograma e elevação das enzimas cardíacas no soro. Muitos casos podem, no entanto, cursar sem a síndrome completa. A dificuldade do diagnóstico diferencial é por si só causa adicional de eventos fatais. Portanto, o desenvolvimento de ensaios não-invasivos para a detecção dos danos do miocárdio é de extrema importância. Os danos celulares podem ser identificados pela técnica cintilográfica utilizando, entre outros radiotraçadores, o glucarato-99mTc (GLA-99mTc). O ácido glucárico, também conhecido como ácido sacárico, é um ácido dicarboxílico análogo da glicose. Ele é estruturalmente similar à frutose e penetra na célula através do sistema de transporte D-frutose, sendo desprezível em condições não isquêmicas. O objetivo principal foi estudar as condições de preparação, controle de qualidade, estabilidade e biodistribuição do reagente liofilizado GLA marcado com 99mTc. O reagente de GLA foi preparado sob a forma liofilizada, em condições assépticas sob fluxo laminar. Cada frasco contém: 12,00mg de ácido glucárico; 0,50mg de SnCl2.H2O; 0,50mg de ácido gentísico com pH final igual a 5,0. A pureza radioquímica foi avaliada em dois sistemas cromatográficos: 1) papel Whatman 3MM como suporte e acetona como solvente (Rf = 1,0 para 99mTcO4 e Rf = 0,0 para 99mTcO2-/GLA-99mTc); e 2) ITLC-SG (fibra de vidro) como suporte e cloreto de sódio 0,9% como solvente (Rf = 1,0 para 99mTcO4/GLA-99mTc e Rf = 0,0 para 99mTcO2). Foram obtidos resultados maiores que 97% aos 30, 60 e 120 minutos após a marcação utilizando 37, 370, 1850 e 3700MBq/ 3-5mL de 99mTcO4. O produto manteve-se estável por 12 meses, armazenado a temperatura de 2°C a 80°C. A pureza radioquímica verificada nos ensaios de estabilidade em plasma aos 30, 60 e 120 minutos de incubação foi superior a 98%. A biodistribuição em camundongos Swiss demonstrou rápido clareamento sangüíneo, eliminação renal elevada e baixa captação em órgãos adjacentes e sistema ósseo, confirmada através das imagens cintilográficas em ratos Wistar aos 30, 120 e 360 minutos após a injeção de 18,5MBq de GLA-99mTc. O produto apresentou pureza radioquímica, estabilidade e biodistribuição adequadas para sua implantação nos ensaios clínicos, estando em fase de validação os processos de liofilização e controle de qualidade, a fim de estender o protocolo em produção rotineira.

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PREPARATION AND QUALITY CONTROL OF 18F-FDG.

Barboza M.F.; Fukumori N.T.O.; Herrerias R.; Bruzinga W.; Bambalas E.; Pires J.A.; da Silva J.L.; Oliveira I.M.; Osso J.A.; Mengatti J.
Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN/SP.

The most important radiopharmaceutical used routinely worldwide in clinical PET studies is the 2-[18F]fluoro-2-deoxi-D-glucose (18F-FDG), for brain, heart and tumors studies, as well as in basic research. The compound has provided a valuable tool for the study the glucose metabolism in both normal and disease tissue. The synthesis is achieved by a nucleophilic substitution reaction in automatic module available for production. The main advantages of this method are the high purity of the final product, the reduced synthesis time and the decrease radiation exposition to the workers. The aim of this work is to describe the procedure developed and validated, for the routine production and quality control of 18F-FDG. The 18F- is obtained by the nuclear reaction 18O(p,n)18F using enriched H218O (97%). At the end of bombardment the fluoride is transferred directly to the automatic module. All the reagents are with ultra-pure degree and provided as a "reagents kit" that must be fit 15-20 minutes before the start of the synthesis. The automatic synthesis is achieved in 25 minutes. The impurities are trapped automatically and the labeled precursor is washed away and sterilized by 0.22 mm Millipore filter. The resulting neutral eluent (16 ± 0.6) ml of 18F-FDG is dispensing in a sterile glass vial. Thin layer chromatography system is carried out for radiochemical and chemical determination, in TLC using acetonitrile:H2O (95:5) and NH4OH: MeOH (1:9) as solvents, respectively. Stability of 18F-FDG is determined immediately and 10 hours at the end of synthesis (EOS). Sterility and pyrogen tests are performed by the microbiology procedures outlined in the pharmacopoeias in different culture medium. The apirogenicity is evaluated using the "in-vitro" Limulus test (LAL). The yield of synthesis was higher than 55%. The radiochemical purity of 18F-FDG were (99.04 ± 0,96)% and (95.91 ± 4,09)%, immediately and 10 hours EOS, respectively. The Kriptofix level was below the detection limit of color spot test. Sterility and pyrogen tests were negative in all delivered vials. During the first five months in 2006, the Radiopharmacy Center has produced 92,500 – 110,000 MBq/batch of 18F-FDG at the end of synthesis (EOS) and distributed 3,201 doses at nuclear medicine services in Brazil.

• Painel •

PREPARATION AND QUALITY CONTROL OF 99mTc-DMSA.

Dulcila M. Lessa Bernardes; Cláudia Elisabete Castanheira; José Alberto de Castro; Emiko Muramoto; Cláudia Regina Pereira; Domingos Gomes de Campos; João Alberto Osso Júnior.
Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP).

99mTc-dimercaptosuccinic acid (DMSA) is considered an excellent kidney imaging agent and is indicated for evaluation of renal parenchymal disorders. After intravenous injection in humans, 99mTc-DMSA becomes loosely bound to plasma protein (75% at 1 hour after injection, increasing to 90% by 24 hours), with little or no diffusion into red cells. Renal excretion is slow, with only 16% of the dose in urine 2 hours after injection. This report describes the validation of a new formulation of DMSA for routine production and quality control, in lyophilized form for labeled with 99mTc with 37 at 3,700 MBq. The process was done under vacuum and low temperature in Super Modulyo –"Edwards" lyophilizator and each lyophilized vial contains: 1.0 mg of DMSA; 0.44 mg SnCl2.H2O; 0.7 mg ascorbic acid and 50.0 mg inositol, pH = 2,5. The radiochemical purity was evaluated by thin layer chromatography system in Whatman 3MM paper(1 x 8 cm) and TLC-SG (Al) 1.5 x 12.5 cm, using acetone and 0.9% NaCl as solvents, respectively, at 30, 60, 120 and 240 minutes after labeling. The Rf value in acetone is 1.0 for 99mTcO4- and 0.0 for 99mTcO2- / 99mTc-DMSA and the Rf value in 0.9% NaCl is 1.0 for 99mTcO4- / 99mTc-DMSA and 0.0 for 99m TcO2-. The stability was evaluated during 6 months and the validation performed in 6 batches. The sterility and pyrogen tests were performed by the microbiology procedures outlined in the pharmacopoeias and by the "in-vitro" Limulus test, respectively. Biological distribution was evaluated in Wistar rats by i.v. of 8.8MBq / 0.100 mL. The% dose / organ in different tissues was determined at 1 h after dose. The method was validated for routine production at Radiopharmacy Center, with a stability of 6 month kept at 2–8ºC and with a radiochemical purity higher than 90%. The biological distribution in rats

showed an uptake higher than 40% and 6% of injected dose in kidney and rate kidney/liver+spleen, respectively. Sterility and pyrogen tests were negative in all the delivered lyophilized vials. During the first 6 months in 2006, were distributed more than 1,200 lyophilized "kits" of DMSA at clinics and hospitals of nuclear medicine in Brazil.

• Painel •

PREPARATION OF 90Y-CITRATE (90Y-CIT) FOR SYNOVECTOMY.

Couto R.M.; Araujo E.B.; Souza A.A.; Mengatti J.; Barboza, M.F.
Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP).

Radyosinovectomy is a therapy used to relieve pain and inflammation from rheumatoid arthritis and relates diseases. It has been shown that the intra-articular injection of a radioisotopic B-emitter can be used to control synovial inflammation. Synoviorthesis with radioisotopes is indicated when joint inflammation is not totally controlled by drug prescription. Ideal radiopharmaceuticals for this type of treatment would be one, which is pharmaceutically stable, which destroys only the synovial membrane and which is confined to the intra-articular space. Several publications have shown the efficacy of Y-90 for treatment of the knee joint in rheumatoid arthritis. The ideal particle size range was considered to be from 2 - 10 microns and the dose of 148MBq seems to be efficient. The aim of this work was to study the preparation, the quality control and the stability of 90Y-citrate (90Y-Cit) for synovectomy of knee joint. The labeling process is carried out as described previously in literature using 90YCl3 from Nordion. The 90YCl3 solution is evaporated to nearly dryness at 150 C. After cooled at room temperature is added 1.5mL /2mM Y(NO3)3, 0.1mL /10mM sodium citrate and 2.4mL of sterile water for injection USP. The pH was adjusted to 7.0 with 0.1N NaOH. The solution was gently agitated via rotation, heated at 100 C for one hour and then cooled at room temperature. Radiochemical purity was determined by paper chromatograph system in Whatman 3MM and in TLC-AL, using 0,9% saline solution as a solvent, at 30, 60 and 120 minutes after labeling. In these systems the Rf of 90Y-Cit = 0.0 while the Rf of 90YCl3 = 1.0. The final product showed radiochemical purity greater than 99% with particle size less than 5 microns. The 90Y-Cit was stable for 5 days at room temperature. Further studies on radiochemical purity, physical, biological and chemical evaluation will be make to compare characteristics and efficacy of 90Y-Cit and the 90Y-HA for synovectomy.

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PREPARATION OF HIDROXIAPATITE (90Y-HA) FOR SYNOVECTOMY.

Couto R.M.; Araujo E.B.; Souza A.A.; Mengatti J.; Barboza M.F.
Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP).

It is estimated that about 3% of the population worldwide is affected by rheumatoid arthritis. Radiation synovectomy is a method of treatment in non-surgical operation damages, by intra-articular application of b-emitting radioisotopes. An ideal radiopharmaceutical for this type of treatment would be one, which is pharmaceutically stable; which destroys only the synovial membrane and which is confined to the intra-articular space. There are several radionuclides available for this treatment such as Y-90; Sm-153; Dy-165; Ho-166 and Re-188/186. However, Ytrium-90 is often believed to be among the most useful of the radionuclides that have been considered for therapeutic applications, with a half-life of 64.1h and beta rays of high-energy 2.3MeV, with no gamma rays, and decays to a stable daughter (90Zr). The aim of this work is to establish the methodology of preparation, the quality control and stability of 90Y-hydroxyapatite (90Y-HA) for synovectomy. The labeling process was carried out as described previously in literature using 90YCl3 from Nordion. In a conical glass vial containing 40 mg of HA from Bio-Rad, with particles in the desirable size range (20mm), dissolved in 0.75 mL sterile water, is added 74–370 MBq of 90Y in citrate form. The vial is sealed and mixed for 30 minutes at room temperature. The suspension is centrifuged twice at 1000 rpm for 3–5 minutes, the liquid was discarded and the precipitated resuspended with 5 mL of saline solution. The final precipitate (90Y-HA) is resuspended in 5–8 mL of sterile saline solution (pH = 6.0), sealed and autoclaved for 30 minutes at 121ºC. The percentage of bound activity is determined by measuring the activity of particles (90Y-HA) and supernatant (90Y+++) solution in a dose calibrator in order to calculate the yield of labeling procedure. The radiochemical quality control is evaluated by chromatography system using ITLC-SG and Whatman 3MM paper (1 x 10cm) as support and 0.9% and 8,4% saline solution and acetate buffer as solvents. Radiochemical purity was carried out 30; 120; 240 minutes after labeling to assess the stability of the 90Y-HA. Filters of different sizes (1.2; 5; 8 and 12 mm) were used for particle size determination. The labeling yield of 90Y- HA was (92.1 ± 1.4)% (n = 9). The final product presents a radiochemical purity > 98,9% with particle size > 8mm and "in-vitro" stability of 5 days at room temperature.

• Painel •

PRODUÇÃO E AVALIAÇÃO DE KIT PARA OBTENÇÃO DO 99mTc-HEDP.

Radin A.(1); Rosane F.B.(2); Fukuda A.M.(2); Prando S.(1); Marques F.L.N.(1).
(1)Centro de Medicina Nuclear (LIM 43), Depto. de Radiologia – FMUSP – Brasil; (2)Faculdade de Ciências Farmacêuticas – PUC-Campinas – Brasil.

Objetivos: Complexos de tecnécio com difosfonatos são largamente utilizados em cintilografia óssea. Dois produtos são aprovados pelo FDA-USA para uso em humanos, o 99mTc-MDP, o qual é produzido no Brasil, e o 99mTc-HEDP, que somente pode ser obtido sob importação. Neste trabalho, buscamos sintetizar o ligante 1-hidroxi-1,1-difosfonato-etano (HEDP), preparar o complexo de tecnécio e comparar a biodistribuição deste com o 99mTc-MDP. **Métodos:** O ligante foi sintetizado pela reação entre tricloreto de fósforo e ácido acético, seguido por neutralização com NaOH, e foi caracterizado por análise elemental e RMN. Kit para marcação com [99mTc]tecnécio foi preparado com 5,0 mg do ligante, 0,75 mg de SnCl2.2H2O e 1,5 mg de ácido ascórbico, a pH = 6,0. Marcações foram realizadas utilizando atividades de até 18,5 GBq (500 mCi) e a eficiência de marcação e estabilidade da ligação foi avaliada por cromatografia em ITLC-SG utilizando solução fisiológica e acetona como fases móveis. Imagens estáticas da biodistribuição do produto, em coelhos Nova Zelândia, foram adquiridas nos tempos de 1, 2 e 3 horas após a administração do radiofármaco, utilizando câmara à cintilação LEM-Ziemens, equipada com colimador LEAP. Para comparação, imagens de 99mTc-MDP foram realizadas utilizando os mesmos parâmetros e as imagens foram analisadas por dois profissionais com experiência na área. **Resultados:** A síntese do ligante forneceu 67% de rendimento, com o produto apresentando análise elemental teórico C = 7,40%; H = 3,40% e obtido C = 7,67%; H = 2,87% e 1H-RMN (D2O, 300 MHz), δ = 1,6 (t, 3H). A eficiência de marcação para o produto marcado com 500 mCi (n = 5) foi de 98,76 ± 1,15%, após 30 minutos, e 98,95 ± 0,87%, após 14 horas. A relação entre a captação no osso e partes moles, para os tempos de 2 e 3 horas, foram de: 1,68 e 2,50 para o 99mTc-MDP e 1,86 e 2,82 para o 99mTc-HEDP. Análise visual não permitiu diferenciar entre a utilização dos dois produtos. **Conclusões:** A síntese do ligante é de relativa facilidade e o produto obtido apresentou características adequadas. O kit pôde ser marcado com alta atividade, superior àquela definida para o MDP, fornecendo produto com alta pureza radioquímica e estabilidade. A qualidade das imagens é equivalente, demonstrando que o kit produzido pode ser uma alternativa ao uso do 99mTc-MDP, principalmente em clínicas em que são realizadas grande Tema de cintilografias ósseas.

• **Painel** •

PRODUCTION OF I-131 BY DRY-DESTILLATION OF IRRADIATED TELLURIUM OXIDE.

G. Barrio; L.A. Villela; R.L. Bezerra; J.L. Correia; J.A. Osso.
Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN/SP.

One of the more important radioisotopes for use in Nuclear Medicine is I-131 It can be produced in nuclear reactors, by two different reactions: fission of U-235 or neutron activation of Te in different forms, such as telluric acid, tellurium oxides and the elementary tellurium. The reaction of choice was the neutron activation of Te because it provides I-131 with high radioactive concentration, high specific activity and lower amount of waste (radioactive or not) when compared to the U-235 fission. The irradiation parameters that could be varied were the mass of the target, the neutron flux and length of irradiation. The objective of this work was to study the production of I-131 using the dry destillation technique for its separation from targets of tellurium oxide (TeO2) irradiated at IPEN's IEA-R1 Nuclear Reactor. After the irradiation the targets were heated inside a resistive oven at temperatures higher than its melting point for an adequate period of time. In this condition, I-131 is sublimated and carried by a flow of oxygen gas and further trapped onto water cooled diluted NaOH solution (pH 11). The variables studied in this procedure were the time and the temperature of destillation and the effect of the mass of the target to be processed. The results shown that the best conditions of destillation occurred with oven temperatures between 800ºC and 750ºC and with the destillation time between 2h and 4h. For the temperature of 750ºC, the total I-131activity produced was in average 468.346 MBq (12658 mCi), while at 800ºC the average value was 361.453 MBq (9769 mCi). The distillation apparatus could handle up to 3 targets (150 g of TeO2). Quality control studies showed that the I-131 produced had the proper conditions to be used in Nuclear Medicine. Nowadays a total activity of nearly 777 GBq (21 Ci) of I-131 can be produced every week using this technology that represents about 60% of the total demand of I-131.

• **Tema Livre** •

PRODUCTION OF 177Lu-DOTA-TYR3-OCTREOTATE TO CLINICAL APPLICATION IN NEUROENDOCRINE TUMORS.

Caldeira Filho J.S.; Herrerias R.; Barboza M.F.; Fukumori N.T.O.; Lima E.N.; Araújo E.B.
Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN/SP.

Radiolabeled receptor-binding peptide is an important class of radiophamaceuticals for tumour diagnosis and therapy. The new somatostatin analogue Tyr3-octreotate has an increased receptor affinity compared with octreotide and Tyr3-octreotide. Suitable radionuclide as Lutetium -177 (Lu-177) with a medium energy • emitter (0.5 MeV), a low abundance • and half life of 6.7 days is one of the most frequently radioisotope used in peptide receptor radiotherapy (PRRT). The aim of this work is to validate the preparation and quality control of Tyr3-octreotate labeled with Lu-177, using DOTA as chelating agent, for clinical application. The labeling of DOTA-Tyr3-Octreotate with Lu-177 was performed, under GMP condition, in a "glove-box" with 177LuCl3 (IDB-Holland) at pH 4.5, using radionuclide:peptide ratio of 279 MBq/18 µg in sodium acetate buffer, at 100ºC for 30 minutes. Radiochemical purity was determined by ITLC-SG in 0.1M sodium citrate, pH 5.5, as solvent. The labeled peptide migrates from the origin Rf = 0.1-0.3 and the radionuclide migrates with the solvent front Rf = 1.0. The stability of the final product was evaluated immediately and for 3 days, kept under freezing condition. Sterility and pyrogen tests were performed by microbiology procedures in different culture medium and the apirogenicity by the "in-vitro" Limulus test (LAL). The 177Lu-DOTA-Tyr3 - Octreotate was stable for 3 days with a radiochemical purity of (98.6±.3)%; (98.8±.6)% and (98.5±0.5)%; first day, 24 and 48 hours, respectively, kept under freezing condition. Sterility and pyrogen tests were negative, which are considered suitable for clinical applications. The clinical study were successfully performed and the scintigraphic images were compared with 111In-DTPA-Oct, showing a similar distribution in the same patient.

• **Painel** •

QUALITY ASSURANCE IN RADIOPHARMACEUTICAL PRODUCTION.

Elaine Bortoleti de Araújo; Maria Tereza Colturato.
Nuclear and Energetic Research Institute – IPEN-CNEN.

Radiopharmaceuticals must be manufactured in accordance with the basic principles of Good Manufacturing Practices (GMP) for sterile pharmaceutical products as recomended by the World Human Organization (WHO). **Objective:** The aim of this paper is to discuss the Quality Assurance in radiopharmaceutical production based on WHO recommendations and in USA and Europe regulations. **Methods and results:** Quality Assurance is a wide ranging concept which covers all matters which individually or collectively influence the quality of a product. GMP means the part of Quality Assurance which ensures that products are consistently produced and controlled in accordance with the quality standards appropriate to their use. The GMP in USA are part of the "Code of Federal Regulations" – CFR 21, parts 210 and 211. Radioactive drugs are regulated to the same extent that other drugs. The European Comission adopt the Directive 2003/94/C to regulates GMP. The GMP in Brazil were published in the Resolution ANVISA, RDC 210, 2003. Some aspects of the GMP applied to radiopharmaceutical production are of special interest: Personnel: personnel should be trained in GMP, safe handling of radioactive materials and radiation safety procedures. Premises and equipment: Laboratories for the handling of radioactive materials must be specially designed to take into consideration aspects of radiation protection, cleanliness and sterility. The production of sterile radioactive products should be carried out under negative pressure surrounded by a positive pressure zone ensuring that appropriate air quality requirements are met. Production: Careful consideration should be given to the validation of the process, process control and monitoring of the established parameters, specially from the environment. Quality Control and Quality Assurance: principal responsibilities: (a) instructions for each test /analysis and revision of procedures/specifications; (b) identification and segregation of test samples to avoid mix-ups and cross-contamination; (c) environmental monitoring and equipment and process validation for evaluating the adequacy of the manufacturing conditions; (d) release or rejection of starting materials, intermediate products, packaging and labelling materials, and each batch of finish preparation; (e) evaluation of stability of the finished products and establishment of expiry dates; (f) retaining samples of radiopharmaceuticals products and keeping adequate records of the distribution. **Conclusion:** Because of their short half-lives, many radiopharmaceuticals are released and administered to patients shortly after their production, so that quality control (e.g. tests for sterility, endotoxin, radionuclidic purity, etc) may sometimes be retrospective. The implementation of and compliance with the Quality Assurance Programme are therefore essential.

• **Painel** •

STUDY OF PREPARATION OF GENERATORS OF 99 Mo-99mTc BASED TO GELS OF MOLYBDENUM WITH ZIRCONIUM, TITANIUM, CERIUM AND HAFNIUM.

Vanessa Moraes; Barbara Marczewski; Carla Roberta Dias; Patrícia de Andrade Martins; João Alberto Osso Junior.
Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN.

Generator of 99Mo-99mTc is a system formed with these two radioisotopes, where the molybdenum, by radioactive decay, produces the technetium that must be separated from molybdenum. 99Mo can be produced by several nuclear reactions in particles accelerators or nuclear reactors. 99mTc has ideal nuclear properties for organ imaging in nuclear

medicine, due to its nuclear characteristics: short half-life (6.04 h), emission of gamma radiation of low energy (140 keV), absence of beta emission, 100% of decay by isomeric transition for 99Tc. This work presents the preparation of gel generators of molybdenum with zirconium, titanium, cerium and hafnium and characterization of the gels: mass ratio between molybdenum and cation, particles size and elution percentage of 99mTc after irradiating the gels. Gels had been prepared in different temperatures (25 and 50 ºC), NaOH concentrations (2 and 4 mol/L), mass ratio (Mo/Zr = 3.29, Mo/Ti = 1.80 and 2.25, Mo/Ce = 0.31 and 0.38, Mo/Hf = 0.24) and final pH of 3.5 and 4.5. The analysis of the results proved that these gels are adequate for preparation of the generators of 99Mo-99mTc: Zr: Mo/Zr = 3.29, NaOH concentration = 2 mol/L, 50ºC and final pH = 4.5 Ti: Mo/Ti = 2.25, NaOH concentration = 2 mol/L, 25ºC and final pH = 3.5 Ti: Mo/Ti = 2.25, NaOH concentration = 4 mol/L, 50ºC and final pH = 3.5 Hf: Mo/Hf = 0.24, NaOH concentration = 4 mol/L, 50ºC and final pH = 4.5 Percentages of molybdenum in the molybdenum with zirconium gels and the two molybdenum with titanium gels are similar, which is not observed in the molybdenum with hafnium gel, since the molybdenum percentage is lower. If the activation of the molybdenum during the irradiation is considered, the totality of 99Mo produced will be similar in the molybdenum with zirconium and molybdenum with titanium gels and will be lower in the molybdenum with hafnium. An adequated gel for the preparation of the molybdenum generators must possess particles of size between 0.106 and 0.150 mm and all gels are adequate. 99mTc elution is a process that consists of passing saline solution through the irradiated gel to remove the 99mTc and the elution yield are high and similar for all gels, demonstrating good performance. The results have shown a good performance of molybdenum with titanium gels and molybdenum with hafnium gels, when compared with the molybdenum with zirconium gels.

• Painel •

STUDY OF THE VIABILITY OF THE PRODUCTION OF 177Lu IN NUCLEAR REACTOR.

Silva G.P.; Osso Junior J.A.
Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN.

The beta- emitter 177Lu is an important radioisotope, for research and investigational purposes as a diagnostic and radiotherapy agent in the treatment of several malignant tumors. The nuclear properties of 177Lu are advantageous compared to other therapeutic radionuclides, e.g. 90Y, and it can label several biomolecules, such as peptides. The objective of this work is to study the production of 177Lu in the nuclear reactor located at IPEN using the two different methods: direct and indirect route. In the first reaction, Lu2O3 is irradiated in the reactor producing 177Lu. The second route employs targets of Yb2O3 are irradiated in the reactor producing 177Yb that decays to no-carried-added 177Lu. This paper shows the results of the production yields of 177Lu using the two nuclear reactions, and the extrapolations to real production conditions. Targets of Lu2O3 and Yb2O3 were irradiated in the nuclear reactor under different neutron fluxes and irradiation times. After the irradiation the targets were analyzed by g-ray spectroscopy using a hiperpure Ge detector. The direct method gives a lower specific activity 4,92x E+2 GBq/g compared to the indirect one, 7,14xE+1 GBq/g, but the later can not achieve the total activity required for a routine production.

• Painel •

Tc-99m DIRECT RADIOLABELING OF MONOCLONAL ANTIBODIES: REDUCING AND PURIFICATION OF ANTIBODIES.

Carla Roberta Dias; Marycel Figols de Barboza; Vanessa Moraes; Barbara Marczewski; João Alberto Osso Junior.
Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN.

Monoclonal antibodies (Mabs) have been used for diagnostic and therapeutic treatment of some types of tumors. Nuclear medicine is one the best tools among the diagnostic modalities in the cancer diagnosis and the radionuclide technetium-99m (Tc-99m) is extensively used in radiodiagnostics in nuclear medicine. The direct labelling method of Mabs with Tc-99m depends on the free disulfide bridges (-SH) obtained after the reduction of the antibody (Ab). The objective of this work was to study the reduction process of Mabs and their purification before labelling with Tc-99m. The -SH bridges of the Ab molecule were broken by using the reducing agent 2-mercaptoethanol (2-ME) and converting them into free -SH groups. The mixture of Ab (CEA-1 and EGF/R3) and 2-ME was incubated at room temperature and after the reaction time the resulting solution was purified on a Sephadex PD-10 column using phosphate buffered saline (PBS)(pH 7.4) purged with nitrogen as mobile phase and fractions of 1 mL were collected (12 fractions). These fractions were analysed by HPLC using a Protein-Pak Diol (OH) column and PBS as solvent, measuring the UV signal at 254 nm. The results showed that the reduction time of 30 min is enough when the native Ab is a fresh one. After some storage time, the reduction time had to be increased in order to improve the labelling efficiency. A relation with the mass of Ab was established as follows: for 5 mg of Ab the reduction time was 1 hour and for 10 mg of Ab was 2 hours. The HPLC showed a good separation between the Ab peaks (Retention time of 7.84 min for CEA1 and 6.67 min for EGF/R3) and the 2-ME peak (Retention time of 12.22 min). In the purification system, the reduced Ab was in the fractions 3 to 5 and sometimes it appeared in the fraction 6 together with 2-ME. From the fraction 7 on, only 2-ME appeared in the HPLC analysis. As conclusion, the Ab reducing process must produce a number of free -SH bridges enough to label it with Tc-99m with a good radiochemical yield. The HPLC analysis is very important to make sure that the right fractions of the purification process are collected for further use, otherwise 2-ME will be present, a contaminant that interfers in the labelling reaction.

• Painel •

UV-VIS SPECTROPHOTOMETRIC QUANTIFICATION OF [Cu(MIBI)4]BF4 IN LYOPHILIZED KIT FOR [99mTc]TECHNETIUM LABELING.

Marques F.L.N.(1); Lima K.S.(2).
(1)Center of Nuclear Medicine (LIM43), Faculty of Medical Sciences; (2)Faculty of Pharmaceutical Science, São Paulo University – Brazil.

Objectives: Before the use as radiopharmaceuticals, lyophilized kit for [99mTc]technetium labeling are considered pharmaceuticals preparations. By the way, biological and physico-chemical analysis must be performed, including quantification of the major component. Although the HPLC is the choice method for this analysis, sometimes UV-VIS spectrophotometry is the used method, once it is low cost and fast analytical procedure. In this work we assessed the UV-VIS spectrophotometric method for quantification of [Cu(MIBI)4]BF4 in lyophilized preparations used for obtention of the radiopharmaceutical [[99mTc](MIBI)6]+, the important agent for myocardial perfusion studies. **Methods:** Standard of [Cu(MIBI)4]BF4 was synthesized and characterized by 1H-NMR, Infrared spectrometry, elemental analysis, melting point and HPLC. A calibration curve was generated from concentration between 5 µg to 80 µg/mL, in water, with absorbance measured at 218 nm, using water as reference. Vials content were evaluated dissolving lyophilized products Cardiolite® (n = 6), MIBI-CMN prepared at CMN-FMUSP (n = 6) and MIBI-IPEN prepared at IPEN-CNEN/SP (n = 2) in water and samples were measured at 218 nm using the others kit components as reference. **Results:** Analytical data for [Cu(MIBI)4]BF4 standard are in agreement with structure and purity for desired compound. The calibration curve showed linear regression of R2 = 0,9986 and concentration of the [Cu(MIBI)4]BF4 in the samples were: 1.07±0.03 mg for Cardiolite®, 0.97±0.07 mg for MIBI-CMN and 1.07±0.04 mg for MIBI-IPEN. **Conclusions:** The use of UV-Vis spectrophotometry for quantification of [Cu(MIBI)4]BF4 in lyophilized preparations allowed a fast and inexpensive analysis, and the results obtained are in concordance with established values of concentration and deviation of 1,00 ±10% mg of [Cu(MIBI)4]BF4 for all products.