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PROPOSAL OF AN IDENTIFICATION TEST FOR MIBI-TEC® LYOPHILIZED REAGENT USING INFRARED SPECTROSCOPY

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ABSTRACT

Quality Control is part of the Good Manufacturing Practices (GMP) responsible for the evaluation and release of pharmaceutical components, including active ingredients and excipients, to assure compliance with the specifications in pharmacopoeias. GMP for radiopharmaceuticals is regulated by RDC N. 17/2010 and RDC N. 63/2009 (ANVISA) establishing the minimum quality standards in industrial manufacture. Pharmacopoeias have the specifications for lyophilized reagents (LR) labeled with ^{99m}Tc regarding radiochemical purity, biodistribution and, in case of parenteral use, bacterial endotoxins and sterility tests must be performed. In radiopharmaceutical monographs, the necessity of non radioactivity assays for releasing lyophilized reagents are not well described, but ANVISA requires assays to assure the quality of the lyophilized powder, as active ingredient quantification and identification assays, stannous ion quantification and moisture determination. Infrared (IR) spectroscopy is widely used for identification of substances, mainly raw materials. The aim of this work is to propose an identification assay for MIBI-TEC® LR powder using IR spectroscopy. MIBI-TEC® batches were produced at IPEN/CNEN-SP and about 2 mg of the LR powder were mixed with 200 mg KBr, pressed at 80 kgf during 5 minutes into the holder to obtain a transparent pellet. The pellet was placed in ABB IR spectrometer, FTLA 2000 model, and a spectrum in the medium infrared region of 450 to 4000 cm^{-1} was acquired using Grams software. Tetramibi cuprous tetrafluoroborate, stannous chloride dihydrate, cysteine hydrochloride monohydrate, sodium citrate and mannitol had their respective IR spectrum recorded and the main characteristic absorptions were established for each formulation component. In the MIBI-TEC® spectrum, it was possible to observe a well characterized absorption in 2193 cm^{-1} which represents the presence of C≡N binding of tetramibi cuprous tetrafluoroborate active ingredient, free of interference, indicating that IR spectrum can be used as identification assay of the LR.

1. INTRODUCTION

ANVISA regulates Good Manufacturing Practices (cGMP) of radiopharmaceuticals through Resolutions RDC 17/2010 and 63/2009, establishing the minimum requirements to be followed to standardize the drug manufacture. GMP compliance is verified in the inspections and in the evaluation of dossier for marketing authorization of the pharmaceuticals [1, 2].

Quality Control (QC) is part of GMP, responsible for sampling and testing of raw materials, active ingredients, excipients and packaging materials to ensure that the required assays are performed and that qualitative and quantitative quality characteristics comply with the acceptance limits, before releasing for use and sale [1].

There are specifications for radiochemical purity, biodistribution, bacterial endotoxins and sterility for lyophilized reagents labeled with ^{99m}Tc in international and Brazilian pharmacopoeia monographs [3, 4, 5, 6].

However, the necessity of non radioactivity assays for releasing lyophilized reagent powder is not clearly established, albeit ANVISA has been requiring assays to assure the quality of the lyophilized powder as active ingredient quantification and identification assays, stannous ion quantification and moisture determination [6].

Identity is a quality attribute in pharmacopeial monographs and two general chapters containing identification procedures are frequently referenced in the monographs, i.e., General Chapter and Spectrophotometric Identification Tests. The identity test is intended to give confirmation with an acceptable degree of confidence that an active substance comply with the description on the label. In Spectrophotometric Identification Tests, Infrared (IR) and ultraviolet (UV) spectrophotometric tests are frequently mentioned [3].

The IR is divided into three regions: near-, mid- and far- IR, named for their relation to the visible spectrum and mid-IR is the most used for identity. The far-infrared, approximately 400-10 cm^{-1} (1000–30 μm), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The mid-IR, approximately 4000-400 cm^{-1} (30–1.4 μm) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The higher energy near-IR, approximately 14000-4000 cm^{-1} (1.4–0.8 μm) can excite overtone or harmonic vibrations [7].

More complex molecules have many bonds, and vibrations can be conjugated, leading to IR absorptions at characteristic frequencies that may be related to chemical groups. For example, the atoms in a CH_2 group, commonly found in organic compounds can vibrate in six different ways: symmetrical and antisymmetrical stretching, scissoring, rocking, wagging and twisting [7].

The IR spectrum of a sample is collected by passing a beam of IR light through the sample. Examination of the transmitted light reveals how much energy was absorbed at each wavelength. This can be done with a monochromatic beam, which changes in wavelength over time, or by using a Fourier transform instrument to measure all wavelengths at once. From this, a transmittance or absorbance spectrum can be produced showing at which IR wavelengths the sample absorbs [7].

Analysis of these absorption characteristics reveals details about the molecular structure of the sample. The IR absorption spectrum of a substance, compared with that obtained concomitantly for the correspondent USP reference standard, provides perhaps the most conclusive evidence of the identity of the substance that can be obtained from any single test.

The aim of this work is to propose an identification assay for MIBI-TEC® lyophilized reagent powder using IR spectroscopy.

2. EXPERIMENTAL

Separately, about 2 mg of the lyophilized reagent and each reagent of the formulation was mixed with 200 mg KBr, pressed at 80 kgf during 5 minutes into the holder to obtain a transparent pellet.

Twenty scans were performed to obtain the IR spectrum in ABB Fourier Transform IR (FTIR) spectrophotomer (FTLA 2000 model, Canada), which presents 4 cm^{-1} resolution. The FTIR spectra in the region of 450 to 4000 cm^{-1} were acquired using Grams software. The background spectrum was obtained against the air and subtracted from the sample spectrum. MIBI-TEC® batches were produced at IPEN-CNEN/SP, containing tetramibi cuprous tetrafluoroborate (or tetrakis (2-MIBI) copper (I) tetrafluoroborate), stannous chloride dehydrate as reducing agent and other adjuvants such as cysteine hydrochloride monohydrate, sodium citrate and mannitol and each component of the formulation had the spectrum and the main absorption peaks recorded.

3. RESULTS AND DISCUSSION

Infrared spectroscopy is widely used in both industry and research and as a simple and reliable technique for quality control. An IR spectrum is represented by a plot of wavenumber (cm^{-1}) (X-axis) vs transmittance (%) (Y-axis). The frequency scale at the bottom of the chart is given in units of reciprocal centimeters (cm^{-1}) because the numbers are more manageable. Different types of bonds, and thus different functional groups, absorb IR radiation of different wavelengths.

Fig. 1 illustrates the IR spectrum of a sample of MIBI-TEC® lyophilized reagent powder.

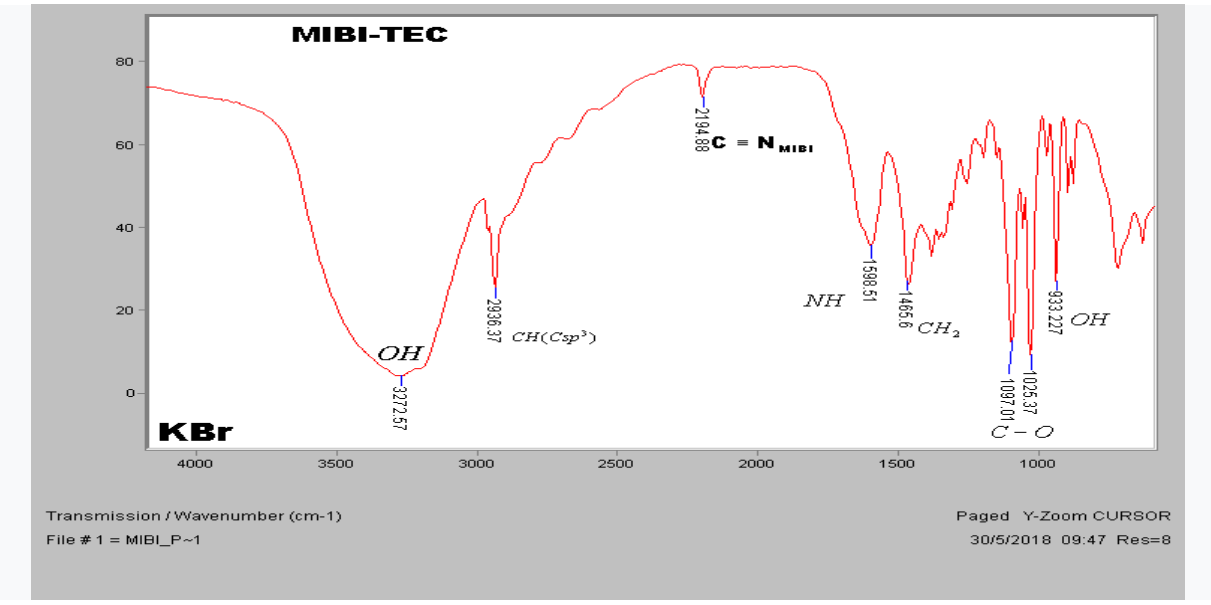

Figure 1: IR spectrum of MIBI-TEC® lyophilized reagent powder

Fig. 2 shows IR spectrum of (A) tetramibi cuprous tetrafluoroborate, (B) L-cysteine hydrochloride monohydrate, (C) tri-sodium citrate and (D) mannitol.

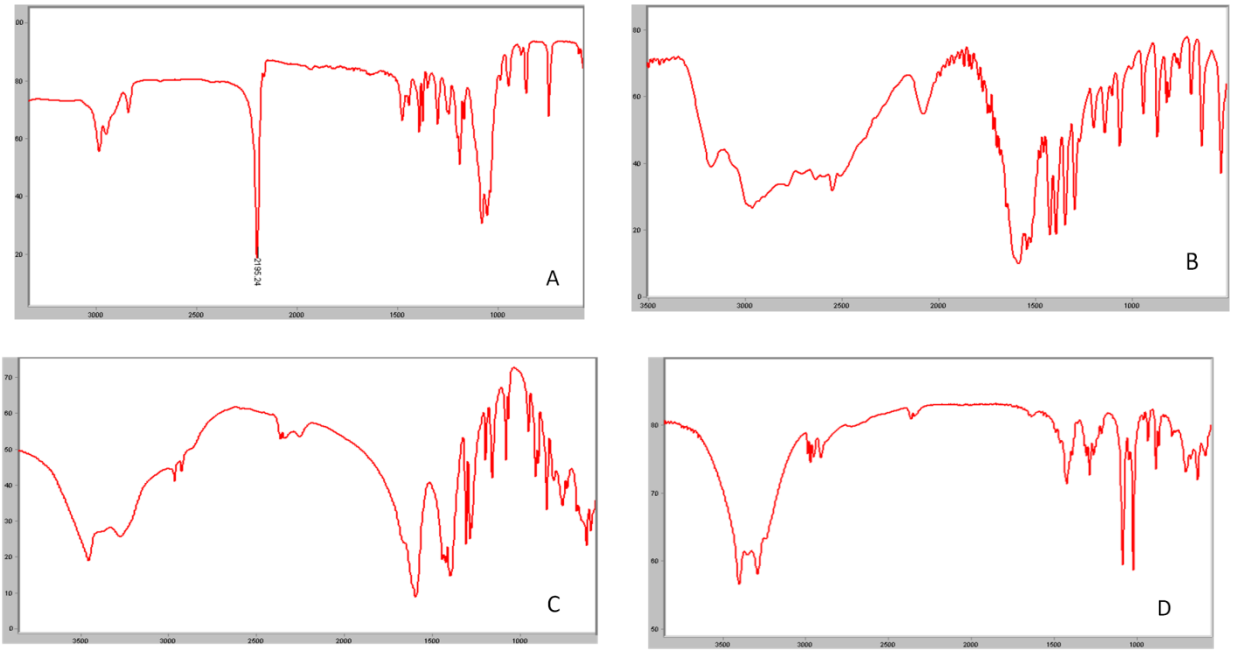

Fig. 2: IR spectrum of (A) tetramibi cuprous tetrafluoroborate, (B) L-cysteine hydrochloride monohydrate, (C) tri-sodium citrate and (D) mannitol in KBr pellet

Table 1 relates the main IR absorption peaks (cm^{-1}) for each component of the formulation of MIBI-TEC® lyophilized reagent.

Table 1: Main IR absorption peaks (cm^{-1}) for each component of the formulation of MIBI-TEC® lyophilized reagent

Component of the formulation	Wavenumber (cm^{-1})
Tetramibi cuprous tetrafluoroborate	2984, 2198, 1474, 1391, 1372, 1298, 1187, 1078, 1052, 857, 743
L-cysteine hydrochloride monohydrate	3177, 2963, 2550, 2080, 1587, 1423, 1393, 1345, 1296, 866, 636
D-mannitol	3398, 3287, 1419, 1281, 1081, 1020, 882, 701, 631
Tri-sodium citrate	3453, 3272, 2924, 2359, 1591, 1393, 1305, 1193, 1156, 1079, 907, 843

In the IR spectrum of MIBI-TEC® lyophilized reagent powder, a well characterized absorption in 2193 cm^{-1} represents the presence of C≡N binding (Fig. 3) in tetramibi cuprous tetrafluoroborate (Fig. 1 and 2A), free of interference from the absorptions of other components of the formulation, allowing to identify the active ingredient.

Other absorption wavenumbers, not specific for one reagent, are: 3279 cm^{-1} represents - OH axial deformation of mannitol and tri-sodium citrate, 2931 cm^{-1} is the axial deformation CH (Csp^3) of citrate, MIBI and cysteine; 1593 cm^{-1} is the axial deformation NH of cysteine, 1456 cm^{-1} is the angular deformation CH_2 of cysteine; 1091 cm^{-1} and 1023 cm^{-1} are the C-O axial deformation of mannitol.

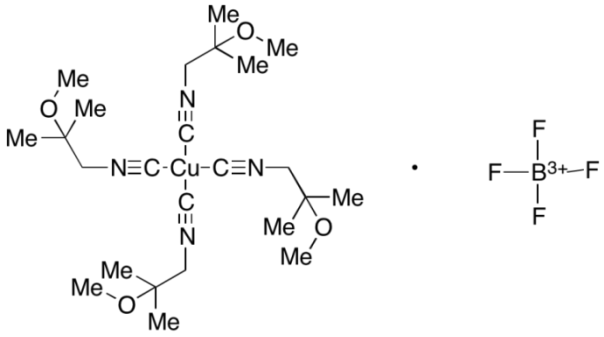

Figure 3: Chemical structure of tetramibi cuprous tetrafluoroborate [8]

4. CONCLUSIONS

Eventhough there is not a pharmacopeial IR standard spectrum of MIBI-TEC® lyophilized reagent powder, in this work it was possible to establish an identification test for the active ingredient of the radiopharmaceutical, to comply with one of the requirements for the stability studies and registration report for marketing authorization at ANVISA.

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