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# IDENTIFICATION, CONTROL AND QUANTIFICATION OF RESIDUAL SOLVENTS IN RADIOPHARMACEUTICAL PREPARATIONS

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## ABSTRACT

Radiopharmaceutical preparations for human diagnostics are isotonic, sterile and pyrogen-free solutions. These preparations can contain some solvents depending on the production method, final product purification or apparatus cleaning in the hot cells. A gas chromatography method has been developed for the measurement of residual solvents in radiopharmaceutical preparations for human applications in accordance with USP Classes II and III. The investigated solvents included diethyl ether, acetone, methanol, 2-propanol, ethanol, acetonitrile and benzyl alcohol. Analyses were carried out in a Shimadzu 17AA gas chromatograph equipped with a flame ionization detector (FID) and an auto-sampler. Excellent linearity was achieved and the obtained results in the analyzed samples were within the permissible levels proposed by ICH and USP. The described method was accurate in a single analysis, without sample pretreatment and suitable for the detection of residual solvents in radiopharmaceutical final products.

## 1. INTRODUCTION

According to US Pharmacopeia "Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The residual solvents are not completely removed by practical manufacturing techniques" [1].

Complete removal of residual solvent levels is not possible, so it is inevitable that they remain in the final product and even in small amounts can influence efficacy, safety and stability of the pharmaceutical products [2].

Residual solvents were evaluated for their possible risk to human health and placed into one of 3 classes as follows: Class I, solvents to be avoided, known human carcinogens, environmental hazards with unacceptable toxicities; Class II, solvents to be limited due to nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity but with potential adverse effects and should be limited; and Class III, solvents with low toxic potential to humans [1].

The determination of residual solvents can be performed by a large number of analytical techniques. However, the most popular and recommended technique employed is Gas Chromatography (GC) based on its selectivity and sensitivity, easy of use, simple sample

preparations and easy automation. Modern gas chromatography capillary columns can separate a large number of volatile components, identifying retention characteristics and detecting ppm levels [2].

A crucial step in GC analysis is the sample preparation, together with the capability, for an easy introduction of the sample in the gaseous phase into a chromatographic column separation and detection. Based on the different sample preparation types, GC procedures can be classified, mainly in direct injection, static headspace sampling and solid-phase microextraction [2].

Radiopharmaceuticals formulations are prepared as isotonic, sterile and pyrogen-free solutions and some solvents are required in the production procedures, final product purification and apparatus cleaning in the hot cells [3].

The quality control specifications for radiopharmaceutical preparations involve sterility tests, bacterial endotoxins tests, pH tests, radionuclidic purity, radiochemical purity and chemical purity, including the analysis of residual solvents. These impurities may degrade image quality and produce pharmacological or toxicological effects or allergic reactions [3].

The analysis of residual solvents by GC has been widely described with different injection techniques, columns and temperature profile. Klok and Windhorst described the analysis of 11 residual solvents in radiopharmaceuticals formulations containing up to 12% ethanol and Klick and Skold have published a general analytical procedure for the analysis of 42 residual solvents in drug susbstances [4-6].

The aim of this work was the development of an analytical method for determination of 7 solvents listed in the International Conference on Harmonization Guideline (ICH) and US Pharmacopeia (USP) based on Classes II and III and used in the radiopharmaceutical production at IPEN-CNEN/SP.

## 2. EXPERIMENTAL

### 2.1. Instrumentation

The experiments were performed in a Shimadzu 17AA-GC Gas Chromatograph (Shimadzu, Japan) equipped with flame ionization detection (FID) and an AOC-20i auto-sampler. Helium was used as the carrier gas with 2.0 mL $min^{-1}$ flow rate. Detector and injector were operated at 250 ºC with 100:1 split ratio sample injection and 1.0 µL sample volume.

Separation of diethyl ether, acetone, methanol, 2-propanol, ethanol and acetonitrile was evaluated in a 30 m x 0.25 mm i.d., 0.25 µm film thickness DBWAX column (J&W Scientific, USA) isothermically operated at 50 ºC for 5 minutes. Benzyl alcohol was determinated in the same column isothermically operated at 240 ºC for 3 minutes.

## 2.2. Standard Solutions and Reagents

The purified water used to prepare the standard solutions was obtained from the Milli-RX-45 water system (Millipore, USA).

Standard solutions for the working calibration curves were prepared by diluting high purity solvents with purified water. Initially, the retention times for each solvent were individually determinated.

Calibration curve was prepared in the range of 30-300 μg $mL^{-1}$ for diethyl ether, acetone, methanol, 2-propanol, ethanol and acetonitrile and 50-500 μg $mL^{-1}$ for benzyl alcohol. The intensity data were based on an average of three replicates.

## 2.3. Sample Preparation

Samples from five different batches $^{131/123}$I-MIGB (meta-iodobenzylguanidine-iodine-131/123); $^{131}$I-HIPPURAN (iodohippurate-sodium-iodine-131); $^{131}$I-LIPIODOL (Ethiodised-oil-iodine-131); $^{131}$I-SAH (human serum albumin-iodine-131), $^{18}$F-FDG (2-deoxy-2-fluoro-D-glucose-fluoro-18); $^{153}$Sm-EDTMP (ethylenediaminetetramethylene phosphonic acid-samarium-153), $^{51}$Cr-EDTA (ethylenediamine tetraacetic acid-chromium-51), $^{111}$In-DTPA (pentate-indium-111), $^{90}$Y-HA (hydroxiapatite-ytrium-90) and $^{177}$Lu-DOTATATE [DOTA(1,4,7,10-tetraazacyclododecane-*N*-,*N*-,*N*-,*N*-tetraacetic acid)D-Phe1-Tyr3-octreotate] were diluted to 1:5 with purified water and analyzed [7].

# 3. RESULTS AND DISCUSSION

It was used direct-injection sample preparation because of its simplicity, reliability and easy operation. The sample was dissolved and extracted with a high-boiling-point solvent with advantage that the diluent solvent was eluted later, without interference in the analyte peaks. An aliquot was transferred into a vial and a standard auto-samppler injected the solution directly into the heated port of the GC. The injection mode was split [2].

Fig. 1 and Fig. 2 show the chromatogram of 7 residual solvents commonly used in the radiopharmaceutical production. DBWAX column satisfactorily separated the solvents and the resolution peak was above 1.61 in all chromatograms of standard solutions and samples. The solvent list (Table 1) includes some solvents that have low severe toxicity but with potential adverse effects (Class II) and solvents with low toxic potential (Class III) [1]. The benzyl alcohol is not classified in US Pharmacopeia, but it was included in the study because it has been used in the radiopharmaceutical production.

**Table 1. ICH Classes II and III residual solvents [1]**

| Class | Compound | Concentration Limit μg $mL^{-1}$ (ppm) |
|---|---|---|
| II | Acetonitrile | 410 |
| | Methanol | 3000 |
| III | Acetone | 5000 |
| | Ethanol | 5000 |
| | 2-Propanol | 5000 |
| | Ethyl Ether | 5000 |
| Others | Benzyl Alcohol | 5000 |

The solvent concentration (**x**) in the samples was calculated by using the Equation 1 expressed as:

$$\textbf{Intensity} = \textbf{A} + \textbf{B}\ [\textbf{solvent (ppm)}] \quad (1)$$

where the concentration of the analyte to be measured is directly proportional to the analytical signal. **A** is a coefficient linear, the constant that describes the background and **B** is a coefficient angular (slope) and represents the sensitivity [8].

The method was linear for all solvents within a determinate range and the correlation coefficients ($R^2$) were all above 0.99. An overview of the results is showed in Table 2.

The limit of detection (LOD) was estimated based on the standard deviation of the blank and the slope, as expressed by the Equation 2:

$$\textbf{L. O. D.} = \frac{\textbf{s} \times \textbf{3}}{\textbf{Slope}} \quad (2)$$

where **s** is the standard deviation of the response (A). The slope **B** was calculated from three calibration curves in the range 2 to 10 μg $mL^{-1}$ [8].

The limit of quantification (LOQ) was calculated as follows (Equation 3):

$$\textbf{L. O. Q.} = \frac{\textbf{s} \times \textbf{10}}{\textbf{Slope}} \quad (3)$$

**Table 2. Correlation Coefficients, L.O.D. and L.O.Q. Parameters of the Analyzed Solvents**

| Compound | Retention Time (min) | Correlation Coefficients ($R^2$) | LOD μg $mL^{-1}$ (ppm) | LOQ μg $mL^{-1}$ (ppm) |
|---|---|---|---|---|
| **Ethyl Ether** | 1.37 | 0.9970 | 0.52 | 1.07 |
| **Acetone** | 1.71 | 0.9992 | 0.47 | 1.25 |
| **Methanol** | 2.07 | 0.9984 | 0.93 | 1.76 |
| **2-Propanol** | 2.27 | 0.9996 | 0.63 | 1.76 |
| **Ethanol** | 2.37 | 0.9992 | 0.68 | 1.60 |
| **Acetonitrile** | 2.94 | 0.9995 | 0.20 | 0.76 |
| **Benzyl Alcohol** | 1.30 | 0.9996 | 11.20 | 15.09 |

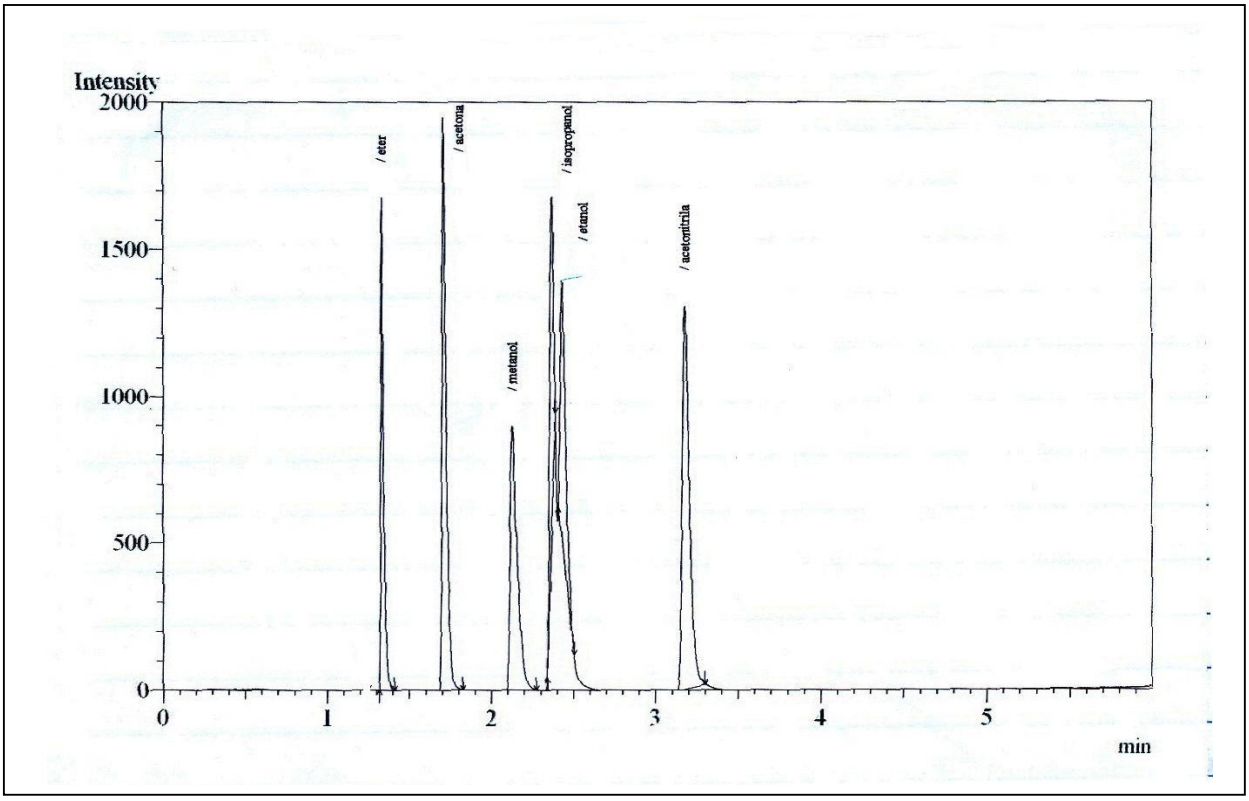


**Figure 1. Ethyl ether, acetone, methanol, 2-propanol, ethanol, acetonitrile peak profile. Experimental conditions: DBWAX column (30 m x 0.25 mm i.d., 0.25 μm film thickness); oven temperature: 50 ºC for 5 minutes; FID detector and injector at 250 ºC; 2.0 mL $min^{-1}$ helium flow rate; 100:1 split ratio and 1.0 μL sample volume.**

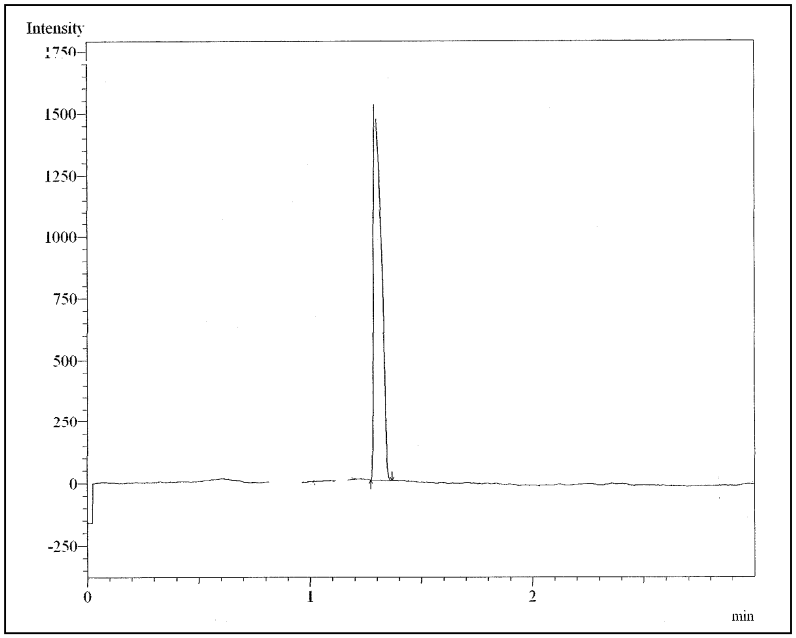


**Figure 2. Benzyl alcohol peak profile. Experimental conditions: DBWAX column (30 m x 0.25 mm i.d., 0.25 μm film thickness); oven temperature: 240 ºC for 3 minutes; FID detector and injector at 250 ºC; 2.0 mL min$^{-1}$ Helium flow rate; 100:1 split ratio and 1.0 μL sample volume.**

$^{153}$Sm-EDTMP, $^{90}$Y-HA, $^{177}$Lu-Dotatate and $^{111}$In-DTPA samples presented no residual solvents.

Benzyl alcohol preservation of radiopharmaceuticals may be problematic for two additional reasons. First, it is a vasodilator and therefore should not be used with a radiophamaceutical intended for regional blood flow measurements. Second, it undergoes radiation decomposition with the production of a precipitate, benzoic acid, in solutions of high radioactive concentrations [3]

Benzyl alcohol was found in the range 400 - 4,500 ppm in $^{131/123}$I-MIGB, $^{131}$I-HIPPURAN, $^{131}$I-SAH and $^{51}$Cr-EDTA.

2-propanol is used for cleaning the apparatus in the hot cells and in the auto-synthetic system. $^{18}$F-FDG, $^{131}$I-HIPPURAN, $^{131}$I-MIGB and $^{123}$I-MIBG presented, respectively, 32.45 ± 7.54; 4345 ± 262; 2497 ± 516 and 972 ± 75 ppm.

In $^{131}$I-LIPIODOL production, diethyl ether and acetone are used as catalysts in the labeling reactions and were detected in the range of 900 – 4,300 and 24 - 2,500 ppm, respectively. $^{131}$I-LIPIODOL and $^{131}$I-HIPPURAN are processed in the same hot cell and the last one presented acetone in the 9.42 ± 1.72 ppm concentration.

Ethanol has been used in the solid-phase extraction procedure after purification of many radiopharmaceuticals and raw material dissolution, while acetonitrile has been used as the solvent for the precursor in $^{18}$F-FDG production. $^{18}$F-FDG presented more than 405.15 ± 88.03 and 142.77 ± 29.23 for ethanol and acetonitrile, respectively.

It was observed that the results were below the permissible limits proposed by ICH and USP.

## 4. CONCLUSIONS

The determination of residual solvents is very important because of the potential risk to human health from the toxicity and may also affect the physicochemical properties and stability of not only raw materials but also drug products. Any residual solvent with potential toxic, physiologic or pharmacological effects must be evaluated with their appropriate limits.

An ideal residual solvent method permits identification and quantification of the components in a single analysis.

The described method using direct-injection sample preparation is a fast and accurate analysis technique, without needing sample pretreatment or special injection techniques and suitable for detection of residual solvents in radiopharmaceutical preparations, including those of short half-life.

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