COST-EFFECTIVE QUALITY CONTROL METHOD FOR RADIOCHEMICAL PURITY OF 99mTC-TECTROTYD USED IN A HOSPITAL RADIOPHARMACY UNIT

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Abstract: The new Radiopharmacy in Nuclear Medicine department in the Hospital and University Service of Kosovo apply the policy that all products administered into the human body, especially the new one are safe and show a constant high quality in producing the required effects. To ensure the efficacy of radiopharmaceuticals prepared at department in the Hospital and University Service of Kosovo, we introduced a cost-effective routine chromatographic method.

The radiochemical purity (RCP) of 99m-Technetium labelled radiopharmaceuticals (RP) is important to ensure optimal scintigraphic image quality. In a new hospital radiopharmacy unit it may not be possible to use compendial analytical methods or expensive equipment for radiochemical purity analysis, but all radiochemical analysis methods should however be validated against compendial or otherwise proven methods.

Our goal was to optimize the radiolabeling protocol for the regular use of ^{99m} Tc-Tektrotyd and to establish chromatographic method for quality control after labelling as a part of our daily diagnostic procedures for assessment of NET patients labelled with 99m-Technetium was for the first time used to identify medical problems related to overexpression of somatostatin receptors, particularly subtype 2 and, to a lesser extent subtype 3 and 5.

Methods: Tektrotyd or HYNIC – (D-Phe^{1,} Thyr^{3}-Octreotide) trifluoroacetate (Polatom) radiopharmaceutical were reconstituted with about 2 000 MBq of freshly eluted sodium pertechnetate as described by the manufacturer and spiked with eluate of the same generator to obtain a range of impurity concentrations. Samples of technetium- 99m Tektrotyd were spotted on 1x15 cm ITLC-SG strips and developed in appropriate mobile phases described by the manufacturer. Each strip was immediately cut into 30 pieces of 0.5cm and the radioactivity of each piece was measured in a dose calibrator (Capintec,Inc).

The percentage of RCP for each ITLC strip was calculated using the total the radioactivity and the radioactivity from each segment as the total present radioactivity from which the basic radioactivity was subtracted. The present plotted radioactivity in the obtained peaks corresponded to the distribution of radioactivity, i.e. to the present complex of 99mTc Tektrotyd.

Results and Discussion: The proposed method proved to be accurate and precise within the RCP range of approximately 90% to 100% in comparison of the producer requirements.

Conclusion: The proposed method is suitable as a reliable low-cost method for limited resource settings and small hospital radiopharmacy unit.

Keywords: Quality control, Radiochemical purity, Instant Thin Layer Chromatography Medium (ITLC), 99m Tc-Tektrotyd,

1. INTRODUCTION

Nuclear Medicine needs optimal quality radiopharmaceuticals for accurate diagnosis and therapy of diseases (IAEA 2008; Dondi 2011). As the use of Nuclear Medicine increases in developing countries, so does the need for radiopharmaceuticals.

The increased need for visualization of neuroendocrine tumors and the lack of PET did oblige us to introduce the 99mTc- Tektrotyd. 99mTc-Tektrotyd is a radiopharmaceutical for diagnostic use only. It is a radiopharmaceutical indicated for the diagnosis of pathological lesions in which somatostatin receptors are overexpressed (especially subtypes 2 and, to a lesser extent, subtypes 3 and 5) and which can be visualized with it. The physical characteristics of 99mTc are more suitable for gamma camera imaging, with lower patient radiation and better image quality with lower radiation doses.

With the increasing demand for radiopharmaceuticals (Faria 2015), it is of paramount importance to ensure that only safe and effective products are administered to patients (IAEA 2016). Even though most radiopharmaceuticals are used to be prepared in small scale units, quality assurance programs should be implemented (Elsinga et al. 2010). Internationally, guidelines for Good Radiopharmacy Practice recommend that only validated analytical methods should be used to evaluate product quality before patient use. Analytical Quality Control (QC) methods form part of quality assurance, helping to assess the quality of the product. According to Ajay et al., validation of analytical methods provides evidence that the method is suitable for it intended use, and thus helps to ensure provision of safe and effective final products (Ajay et al. 2012). The consequence of using unvalidated non-compendial methods could result in the administration of unknown substances, poor quality images and/or an unnecessary radiation exposure of patients (Amin et al. 2011; Vincenti et al. 2016).

In radiopharmacy, one of the most frequently used analytical criteria is radiochemical purity (RCP) of the product. The radiochemical purity of a preparation is the fraction of the total radioactivity in the desired chemical form of the radiopharmaceutical. During the labelling process, radiochemical impurities may be present as a result of incomplete radiolabelling or decomposition products due to the presence of oxidizing or reducing agents, radiolysis or change of temperature and pH (Saha 2010; Millar et al. 2009; Loveless 2009; Mambilima 2016).

RCP can be assessed by various analytical techniques, for example paper, thin layer, or liquid chromatography and electrophoresis. Radiochromatography involves the separation of components in solution (depending on their affinity with the chromatography materials), and the measurement of the distribution of radioactivity on the chromatogram (Saha 2010).

A variety of methods for quantification of distribution of radioactivity on radio-TLC plates are described in literature. Decristoforo and Zolle provide an overview of seven methods used for 99m-Tc radiopharmaceuticals, including cutting and counting in a scintillation counter, chromatogram scanning, analysis by linear analyzer and phosphor-imager autoradiography (Decristoforo et al. 2007). The methods differ regarding sensitivity, resolution and linearity, but differences in time required per analysis and the cost of equipment of each of these methods are considerable, making their adoption in resource developing countries challenging (Loveless 2009).

At Radiopharmacy in Nuclear Medicine department in the Hospital and University Service of Kosovo in Prishtina there is no chromatogram scanner or more advanced equipment with which the distribution of radioactivity or chromatograms of radiopharmaceuticals can be analyzed. Although the technical and analytical possibilities are limited, new methods and new radiopharmaceuticals are being introduced in our institution.

An alternative method was therefore sought, namely counting sections of the chromatography strips corresponding to the distribution of the different radiochemical species with a contamination monitor available in the Nuclear Medicine department in Prishtina.

Prior to employing it in daily practice, this proposed method had to be validated. The work described here was performed to validate a cost-effective method for quantifying the distribution of radioactivity on ITLC chromatography strips (Millar 2009).

2. MATERIAL

The study was conducted in the Department of Nuclear Medicine, in a laboratory for radiopharmacy using appropriate equipment that provides protection from ionizing radiation, suitable for the use of technetium-99m. The realization of this study is based on the idea of how to contribute to having a good safe new radiopharmaceutical product, using a radiopharmaceutical kit, 99mTc-Tektrotyd.

In this study we used:

- Tektrotyd (POLATOM), kit for preparation of radiopharmaceuticals (TEKTROTYD 2015),
- 99mTc –pertechnetate obtained off 99Mo / 99mTc generator - POLATOM with power of 16 GBq

And material for quality control of the final labelled radiopharmaceutical:

- ITLC SG pad - Merck - Silica gel impregnated pad,

- Methylethylketone (MEK),
- A mixture of acetonitrile and water in a volume ratio of 1: 1, 1 mL
- syringe with needle for subcutaneous injection,
- Appropriate equipment for measuring radioactivity (eg dose calibrator)

3. METHODS

For the preparation of 99mTc-Tektrotyd we used fresh sterile sodium pertechnetate (99mTs) solution for injection without oxidant (eluate of 99Mo/99mTs radionuclide generator) (up to 2000 MBq) in accordance with radiopharmaceutical preparation instructions (Figure 1).

Analysis and quality control of a labeled radiopharmaceutical 99mTc-Tektrotyd was performed by introducing validated and efficient methods based on detection and identification of 99mTc-Tectrotyd and determination of radiochemical purity (Parisella 2012).

Determination of radiochemical purity (RCP)

Radiochemical purity using Instant thin layer chromatography (ITLC) was performed on Silica gel strips with different mobile phases:

a. Acetone (Ac) to determine the percentage of the free $99mTcO_4^-$ fraction (Rf=0.9-1.0)

b. Acetonitryle (ACN) 50% for the 99mTc-colloid fraction (Rf=0.0 - 0.3)

Five µl of 99mTc-Tektrotyd samples was spotted on ITLC-SG strips. Within 5 minutes the strip was cuted in 30 pieces at 0.5 cm from the origin. The radioactivity from each portion of the strip was measured in NaI (Tl) crystal counter / dose calibrator. Each strip was placed flat in the bottom of a 10 cm deep container and the counter placed at the dose calibrator to ensure that the counting geometry was the same for all samples. Each count rate reading was recorded with background count rate subtracted. From these values, the percentage of radiochemical purity of each strip was calculated.

A drop of the same Tc-99m eluate used for kit reconstitution (and to spike the radiopharmaceuticals) was used to run a control analysis under the same conditions as those of the radiopharmaceutical samples.

The calculation the percentage of radioactivity of 99mTc-Tektrotyd using the following formula:

100% – (A +B).

Limit: minimum 90 per cent of the total activity.

Figure 1. Fume Hood - Safeflow- Class II Biohazard Safety Cabinet in which the procedure of labelling was performed

4. RESULTS

Preparation of technetium ^{99m}Tc- Tektrotyd kit was realized according to the aseptic procedure provided by the manufacturer Polatom, showed in the Figure 1 and Figure 2:

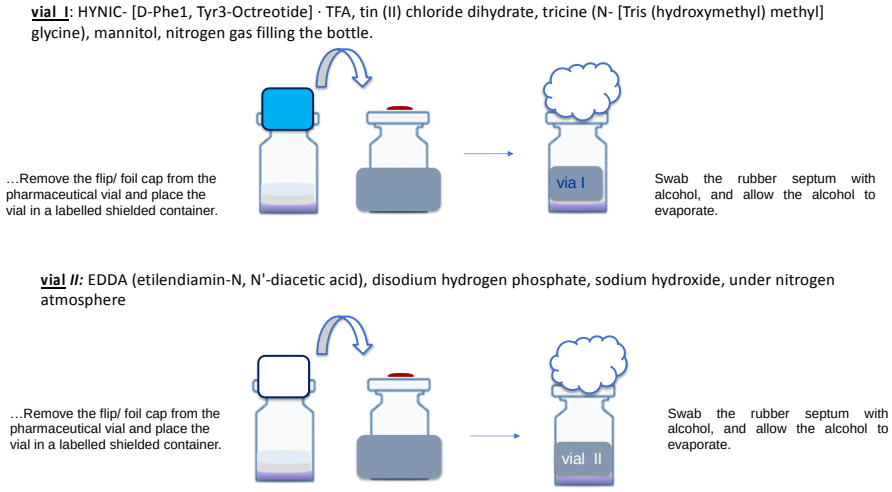

Figure 1. Method of preparation – the constitution of two vials

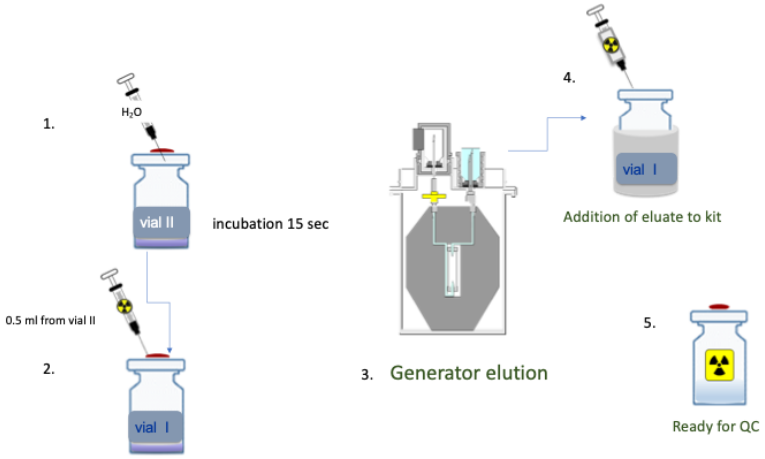

Figure 2. Procedure of labelling with 99mTc-pertechnetate

Quality control

Determination of radiochemical purity was performed using one of the alternative chromatographic procedures, A or B, as described.

For four 99mTc-Tektrotyd samples RCP was 93 ± 0,43, under the limit of 90% to 100% as required , with activities between 2 and 3 MBq. The mean RCP of triplicate analyses at each sample RCP level for this radiopharmaceutical is shown in Table 1.

Table 1 Radiochemical purity of 99mTc-Tektrotyd

Series	99mTc-Tektrotyd (complex)	99mTc O_4^- (free unbound radionuclide)	99mTc colloid species
1	94,44 ± 0,39	0,67 ± 0.38	4,89 ± 0.32
2	93,44 ± 0,41	1,45 ± 0.43	5,11 ± 0.35
3	93,74 ± 0,44	1,29 ± 0.30	4,97 ± 0.29
4	93,66 ± 0,46	0,69 ± 0.41	5,65 ± 0.31
Mean ± CV	93± 0,43	1,03 ± 0.40	5,16 ± 0.34

Mean ± CV; N=3; CV - coefficient of variation

Obtained results for the percentage of the radioactive complex of 99mTc-Tektrotyd and percent of free unbound radionuclide and 99mTc colloid species are showed on and Figure 3 and Figure 4.

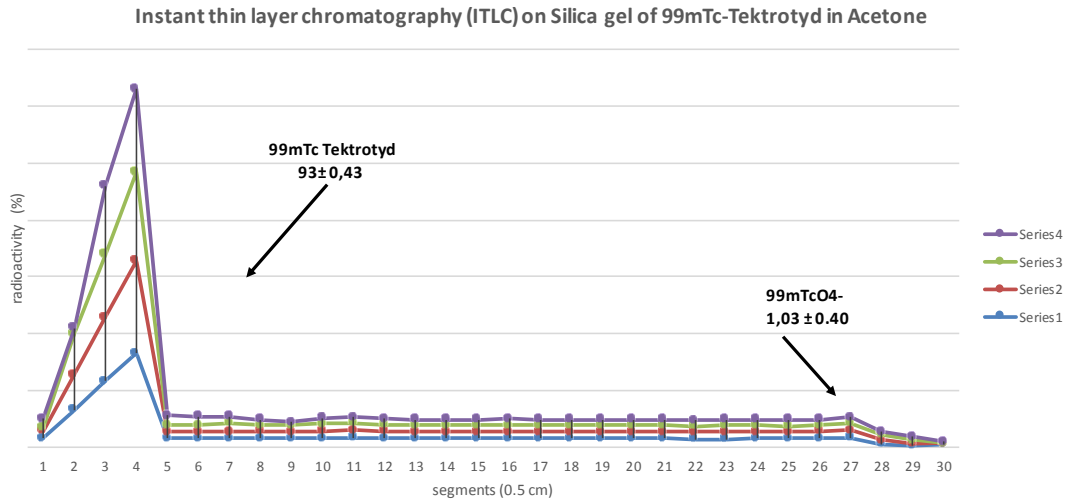

Figure 3. Instant ITLC showing 99mTc-Tektrotyd in Aceton as a developing medium

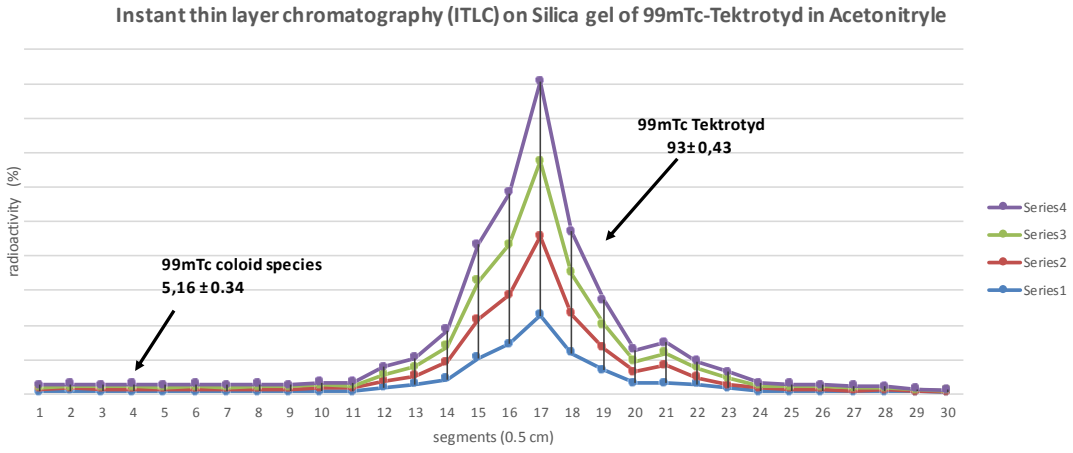

Figure 4. Instant ITLC showing 99mTc-Tektrotyd in Acetonytrile as a developing medium

5. DISCUSSION

Many Nuclear Medicine units and radiopharmacies strive to upgrade their quality management systems, aiming at harmonization and standardization to meet internationally recommended Good Practice guidelines as far as possible. Guidelines for radiopharmacy practice have been published, amongst others by the IAEA to assist with standardization of radiopharmacies (IAEA 2008a, IAEA 2008b). In developing countries, it is not always possible to

meet all good radiopharmacy practice requirements. There is therefore a risk that radiopharmaceuticals prepared or compounded in such sub-optimal facilities may not meet the required safety and efficacy standards.

Recommended analytical methods are supplied with package inserts from radiopharmaceutical kits and by pharmacopoeia monographs. In radiopharmacies with limited funding, it may be difficult to follow the recommended analytical methods due to lack of adequate equipment and limited availability of consumables. Other disadvantages of many of the recommended methods include the time required to complete the tests, which delays administration of the short-lived products to patients, and use of relatively large volumes of solvents when solid phase extraction (SPE) or HPLC are used (Seetharaman et al 2004). Thus, there is a clear need for practical, simple, faster (Hammes et al. 2004; Mihon et al. 2016) and low-cost methods. Any alternative methods should be validated prior to use (Leonardi et al. 2012).

The current study describes the validation of a cost-effective quantification method for instant thin layer radiochromatography strips available at Nuclear Medicine Department, Hospital and University Clinical Service of Kosovo in Prishtina.

In validation of analytical methods, reference standards are generally used for comparison purpose. Certified reference standards for 99mTc impurities do not exist in the radiopharmaceutical industry due to the short half-life and short shelf life of the radiolabeled complexes as well as absence of a stable isotope of 99mTc. In our study, samples containing varying concentrations of 99mTc-Tektrotyd were obtained by spiking with adequate quantities of sodium pertechnetate and measured with a validated method for comparison with the proposed alternative counting method. Seetharaman et al. used a similar approach to determine linearity of their method (Seetharaman et al. 2006).

Limitations of this study include the non-availability of reference standards for Tc-99m labelled compounds since there are no stable isotopes of technetium (Todde et al. 2014).

In validation studies, sample preparation should be done very carefully, and effects like possible adhesion of impurities to containers used in preparing samples should be considered in the design of the tests. Furthermore, spiking samples with pertechnetate may result in additional labeling of the Tc-99m-radiopharmaceutical, which may cause an additional uncertainty in the outcome of the experiments.

6. CONCLUSION

Through the preparation of this study it is expected to obtain results that will aim to increase the quality of diagnosis and follow-up therapy of neuroendocrine tumors.

The introduction of a new radiopharmaceutical 99mTc-Tectrotyd containing the radioactive isotope 99m-Technetium, available in all nuclear medical institutions, enables timely diagnosis even where positron emission tomography is unavailable. In order to achieve high specific binding and safety after application of this relatively new radiopharmaceutical, it is necessary to ensure proper quality control, which will guarantee the same. The value of the successful validation of the proposed method is twofold: Firstly, it has increased the awareness of the importance of validation among staff in our unit, and secondly, a cost-effective method is now available and can be used in any other low-income Nuclear Medicine units.

From a practical point of view, we hope that this research will indicate the importance of using new radiopharmaceuticals labeled with technetium-99m, as in this case 99mTc-Tectrotyd, in diagnosing and monitoring patients with neuroendocrine tumors, especially for the diagnosis of which somatostatin receptors are overexpressed (especially subtype 2 and, to a lesser extent, subtype 3 and 5) and which can be visualized with it.

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