SHORT COMMUNICATION

Quality control of Actinium-225 radiopharmaceuticals: Current challenges and solutions in Malaysia

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ABSTRACT

Development of Prostate Specific Membrane Antigen (PSMA)-targeted radiopharmaceuticals for theranostics has changed the treatment landscape for patients with metastatic castration-resistant prostate cancer (mCRPC). The emerging use of [^{225}Ac]Ac-PSMA-RLT has been effective and safe for the treatment of mCRPC. Nevertheless, challenges with the nuclear recoil of [^{225}Ac]Actinium radionuclides, which may release the daughter radionuclide from the radiopharmaceutical and lead to unnecessary irradiation of other organs, poses threats such as organ dysfunction. Therefore, this short communication aims to highlight the current situation in Malaysia and explain the solutions by using a risk-based approach analysis for the in-house preparation.

KEYWORDS:
^{225}Actinium; PSMA; metastatic Castration-Resistance Prostate Cancer (mCRPC); quality control; radiopharmaceutical

INTRODUCTION

Metastatic Castration-Resistance Prostate Cancer (mCRPC) occurs when there is spread of prostate cancer in the body despite optimised pharmacological therapy and achieving castration levels of testosterone hormone to control the disease. Treatment is usually palliative at this point, however, the advent of using radiopharmaceuticals to treat mCRPC has brought new hope for improved progression free survival and overall survival. The development of mCRPC therapy has gone further from [^{177}Lu]Lu-PSMA-RLT to [^{225}Ac]Ac-PSMA-RLT since the establishment of [^{68}Ga]Ga-PSMA-11 as the theragnostic twin.[1,2] In prostate cancer, PSMA is overexpressed 100- to 1,000 times more than in normal cells, making it an interesting target for imaging and therapeutic tools and enabling this "image and treat" or also known as " treat what you see" strategy to become an important approach for personalised patients care.[3]

The use of [^{225}Ac]Ac-PSMA-RLT is found to be efficacious and safe for the treatment of mCRPC.[4] Following the Letter from Kleynhans & Duatti to EJNMMI Radiopharmacy and Chemistry volume 7, Article number: 23 (2022)[5] that has stated the interest and the number of clinical studies published on the use of [^{225}Ac]Ac-PSMA-RLT continue to increase in recent years. The main matter is largely related to the ''true'' molecular identity of 225Ac-radiopharmaceuticals. Generally, the molecular/chemical identity is confirmed using a reference standard containing a stable isotope of the radionuclide.

However, in the case of [^{225}Ac]Ac-radiopharmaceuticals, the lack of a stable isotope necessitates cross-validation methods using high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC) methods. In addition, the radiochemical purity (RCP) of [^{225}Ac]Ac-radiopharmaceuticals can only be measured through its daughter product that emits photons; ^{221}Fr (^{218}keV) or 213Bi (^{440}keV), that is measurable until it reaches equilibrium after 6 half-life of both daughter nuclides. In practice, [^{221}Fr]Fr is commonly used for detection as the secular equilibrium between [^{225}Ac]Ac and [^{221}Fr]Fr can be achieved within 30 minutes post-radiolabelling, as depicted in Figure 1. Inherently, another issue with the use of [^{225}Ac]Ac radionuclides includes the nuclear recoil effect that causes the release of the daughter radionuclide from the radiopharmaceutical and may lead to unnecessary irradiation of other organs that may subsequently cause severe radiotoxic effects such as organ dysfunction.[6]

Nevertheless, the quality control practice in Malaysia for in-house preparation for [^{68}Ga]Ga and [^{68}Lu]Lu-labelled radiopharmaceuticals are generally radiochemical yield (RCY), radionuclidic purity and pH, neglecting the chemical identity of the labelled compound. The concern in the case of [^{225}Ac]Ac-labelled radiopharmaceuticals was due to the nuclear recoil effect that may cause radiolysis. Therefore, correct analytical methods are critical to identify free [^{225}Ac]Ac, [^{225}Ac]Ac-DTPA, radiolysed [^{225}Ac]Ac-labelled, and labelled [^{225}Ac]Ac-radiopharmaceuticals as presented in Figure 2. Hooijman et al. were able to separate and identify free [^{225}Ac]Ac, [^{225}Ac]Ac-DTPA, and labelled [^{225}Ac]Ac-radiopharmaceuticals, however, could not identify the radiolysed [^{225}Ac]Ac-labelled using the Radio-TLC method.[7]

The radiolysed [^{225}Ac]Ac-labelled radiopharmaceutical can only be analysed using the HPLC method, as illustrated in Figure 3. Due to the time required for equilibrium between [^{225}Ac]Ac and [^{221}Fr]Fr, a fraction collector is needed to do such an analysis.[8] The collected fractions are then measured using

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Table I: Hazard Analysis and Critical Control Point (HACCP) Matrix for in-house [225Ac]Ac radiopharmaceutical preparation

Process Steps	Potential Hazard	Critical Limit	Risk Level	Corrective Action	Frequency
Receipt of Starting Materials					
Receiving of radionuclide source	Long live radionuclidic & radioisotopic impurities	Depending of [225Ac]Ac production route: i. ^{229}Th/^{225}Ac generator: • [^{229}Th]Th < 0.009% • [^{225}Ra]Ra < 0.002% ii. Irradiation of [232Th]Th (spallation reaction): • [^{227}Ac]Ac ≤2%	High	Check and verify the [^{225}Ac]Ac Certificate of Analysis (COA)	Each delivery
Receiving of precursors (Eg. PSMA, DOTA-TATE)	Risk of microbial contamination	• GMP grade • Quantity of precursors clearly stated	High	Check and verify the Certificate of Analysis (COA)	Each delivery
Receiving of other starting material (Eg. Ascorbic acid, DTPA solution, Sodium acetate buffer, Hydrochloric acid)	Risk of microbial and metal contamination	• GMP grade • Trace free metals • Quantity/Concentration	Moderate	Check and verify the Certificate of Analysis (COA)	Each delivery
Radiolabeling Process					
Addition of quenchers	Radiolysis effect	• Highly recommended to be added in critical steps such as radiolabeling and dilution	Moderate	personnel - ufficient amount of ascorbate is added - verified by secondary personnel - record in batch preparation record	Each preparation
Labeling Buffer	Unsuitable labelling pH resulted in low RCP	• Sodium Acetate buffer ~ pH 5 • Tris (hydroxymethyl) aminomethane buffer ~ pH 9	High	Documentation and personnel - correct buffer is use - verified by secondary personnel - record in batch preparation record	Each preparation
Heating condition *relevant for DOTA chelators	Low RCP and unlabeled [^{225}Ac]Ac	• Ensure correct temperature and time : dry bath incubator <100°C	High	Documentation and personnel - correct temperature and time - verified by secondary personnel - record in batch preparation record	Each preparation
Addition of DTPA to complex free [^{225}Ac]Ac in final product	Radiotoxic effect of daughter nuclide due to recoil effect	• to be added in final product	High	Documentation and personnel - sufficient amount of DTPA is added - secondary personnel must check that DTPA is added - check the COA for the correct amount of DTPA	Each preparation
Quality Control					
Time for analysis	Inaccurate analysis lead to wrong interpretation	• Ensure secular equilibrium is achieved ~ 30 minutes waiting time	Low	Documentation - Can be identified from preparation time and analysis time (more than 30 minutes)	Each preparation
Physical appearance pH analysis	Risk of viable and non-viable particulate contamination	• Clear, colourless and free of particulate matter	Low	- visual inspection behind lead glass	Each preparation

Table I: Hazard Analysis and Critical Control Point (HACCP) Matrix for in-house [225Ac]Ac radiopharmaceutical preparation

Process čteps	Potential Hazard	Critical Limit	Risk Level	Corrective Action	Frequency
pH analysis	Irritation at injection site	• pH range 4.5-5.5	Low	- pH paper or calibrated pH meter	Each preparation
Radiochemical Yield *ratio (%) between labelled and free [^{225}Ac]Ac and/or [^{225}Ac]Ac-DTPA	Risk of impurities (free [^{225}Ac]Ac) present in final dose	• Radiochemical yield (RCY) ≥ 98%	High	- perform using radio TLC	Each preparation
Osmolality	Introduce pain at injection site	• < 600 mOs/kg	Low	- perform using calibrated osmometer; - osmolality data can be provided by manufacturer	• Validation phase • Changes in formulation
Radiochemical Purity	Risk of impurities (radiolysed [^{225}Ac]Ac-compound) in final product	• Radiochemical purity (RCP) ≥ 95%	High	- perform using HPLC with fraction collector - HPLC data can be provided by manufacturer	• Validation phase • Changes in starting materials / preparation/ process / analytical equipment
Validation of the analytical method	Inaccurate analysis lead to wrong interpretation	Radio-TLC and HPLC • Specificity & Range • Accuracy • Precision • Limit of Detection • Limit of Quantitation	High High	- to be performed at initial stage / validation phase	• Validation phase • Periodically (eg. Performance Qualification)
Stability study	Risk of impurities (free [^{225}Ac]Ac and radiolysed [^{225}Ac]Ac) present in final dose over time	Over the specified period of study: • Radiochemical purity (RCP) ≥ 95% • Radiochemical yield (RCY) ≥ 98%		- stability study & report can be provided by manufacturer	• Validation phase • Changes in starting materials / preparation process /

a gamma counter, and the chromatographic separation is analysed.

Current situation and solution in Malaysia

The preparation of in-house radiopharmaceuticals follows a risk-based approach. Risk assessment is necessary to determine the level of validation when introducing a new radiopharmaceutical compound. Generally, therapeutic radiopharmaceuticals follow a stringent requirement. In addition, in this case where there is no individual monograph for [^{225}Ac]Ac-labelled radiopharmaceuticals, the validation of analytical method and stability study are required to be done initially before it is adopted into the clinical settings.[9] This is to ensure that the patient's safety is not compromised as the routine quality control test in local hospital radiopharmacy is based solely on three general tests. Table 1 represents the Hazard Analysis and Critical Control Point (HACCP) matrix that can be considered for in-house [^{225}Ac]Ac radiopharmaceutical preparation starting from receiving of [^{225}Ac]Ac until the quality control analysis of final [^{225}Ac]Ac-radiopharmaceutical preparation.

The RCP and RCY analysis validation for [^{225}Ac]Ac-labelled radiopharmaceuticals has been published using HPLC and Radio TLC methods. Therefore, to the utmost knowledge and the responsibility of the radiopharmacist to identify the method used for [^{225}Ac]Ac-labelled radiopharmaceutical preparation since certain analysis cannot be performed without sophisticated equipment. Identifying radiolysed [^{225}Ac]Ac-labelled using the HPLC method can be tedious without a fraction collector. The manual collection method can be done by disconnecting the outlet from the UV detector and collecting using vials separated by time per fraction (0.5 minutes, 1.0 minutes). However, this method may pose a risk of radiation exposure to the analyst. Thus, due to the lack of equipment, specifically, HPLC with a fraction collector, proper radiation protection procedures, including its documentation, are required to prevent unnecessary exposure to ionising radiation during [^{225}Ac]Ac radiopharmaceutical quality control analysis.

Nevertheless, radiolysis can be prevented with the usage of an appropriate and sufficient amount of antioxidant. Hence, proper procedure and documentation should be considered to

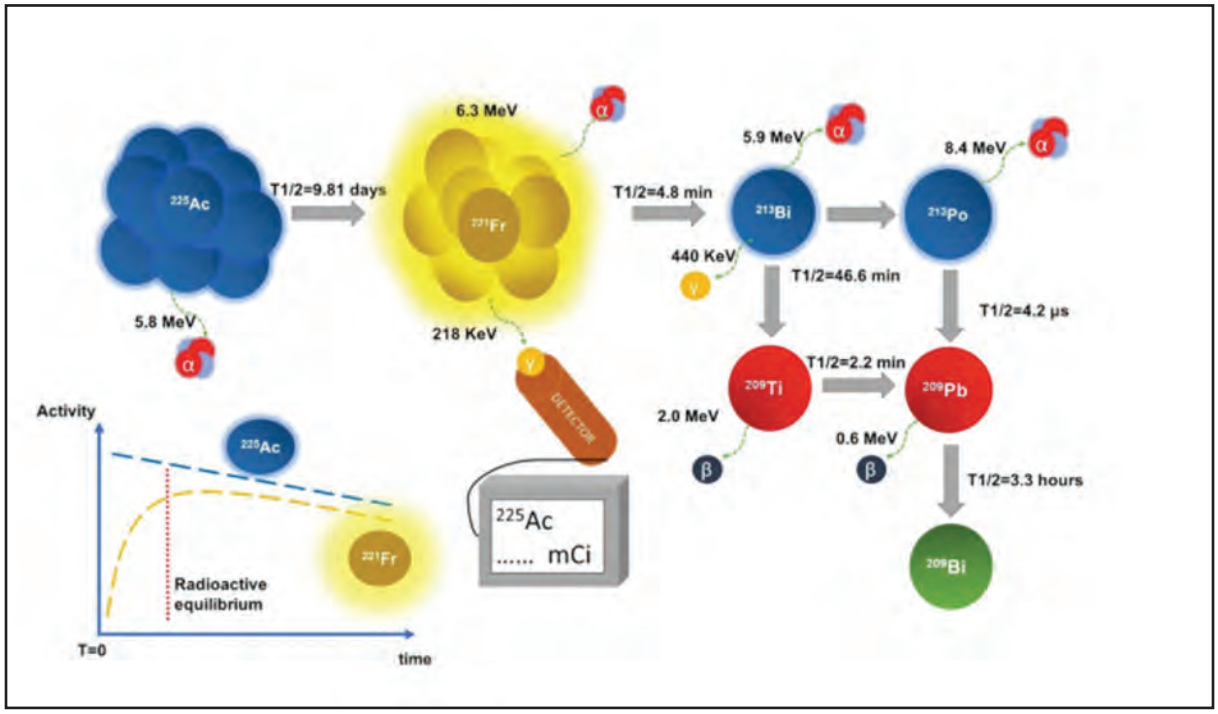

Fig. 1: Summary of the [^{225}Ac]Ac decay, which produces four alpha particles. The activity is measurable after radioactive equilibrium

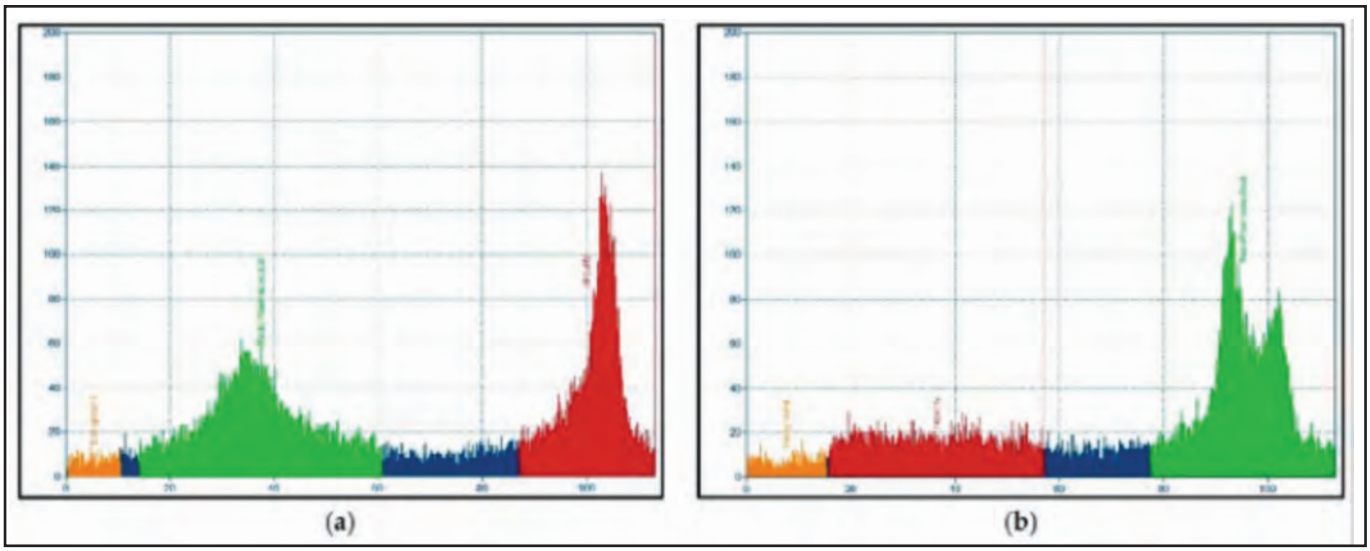

Fig. 2: Radio TLC analysis of [^{225}Ac]Ac and [^{225}Ac]Ac-DTPA, [^{225}Ac]Ac-PSMA-I&T using mobile phases sodium citrate (a) and acetonitrile/water (b). The colored chromatogram represents ([^{225}Ac]Ac-PSMA-I&T, green), impurity ([^{225}Ac]Ac and/or [^{225}Ac]Ac-DTPA, red), background orange), non-selected area blue). Adapted with from (Hooijman, Chalashkan et al. 2021)
The radiolysed [^{225}Ac]Ac-labelled radiopharmaceutical

ensure it is introduced in the preparation. This also applies to the peptide used for the preparation where wrong or insufficient peptide amount should be prevented as the molecular/chemical identity of [^{225}Ac]Ac-labelled radiopharmaceutical is not performed. The addition of DTPA to complex free [^{225}Ac]Ac is important to avoid the injection of free [^{225}Ac]Ac into a patient. Hence, this should also be documented as proof that it has been introduced during preparation.

A major challenge for [^{225}Ac]Ac radiopharmaceutical preparation is the ability to accurately quantify RCY and RCP given the time required for [^{225}Ac]Ac to reach secular equilibrium. Therefore, the limit of detection (LOD) and quantification (LOQ) for the analytical method should be defined to ensure that non-detectable free [^{225}Ac]Ac should be calculated based on the LOD or LOQ.

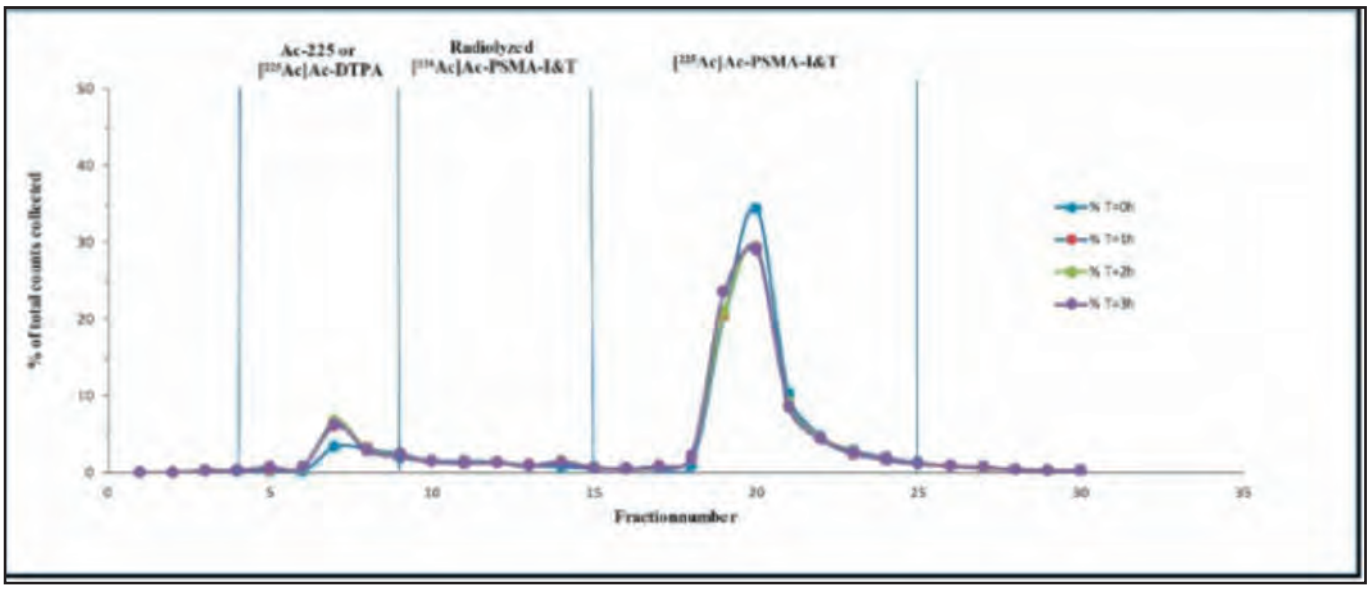

Fig. 3: HPLC fractions are measured using a gamma counter where the x-axis represents the fraction number based on [221Fr]Fr measurements and they-axis % of total counts, measured for 0, 1, 2, and 3 h: Non-optimized synthesis (RCY < 85%) with radiolysed [225Ac]Ac-labelled present in between peaks (10–15). Adapted with from (Hooijman, Chalashkan et al. 2021)

Extension of [225Ac]Ac-labelled radiopharmaceutical stability should only be considered after validation using HPLC. This is largely due to the possibility of an increase in radiolysed [225Ac]Ac -labelled present in the product. Thus, without a proper stability study, any [225Ac]Ac-labelled radiopharmaceutical should be discarded after expiration. Nevertheless, the need to extend the stability of [225Ac]Ac-labelled radiopharmaceutical can be prevented if patient preparation is done in a timely manner.

Furthermore, the outcome from the WARMTH Act study conducted on 488 men with mCRPC and a total of 1174 cycles of [225Ac]Ac-PSMA-RLT was a median overall survival of 15.5 months. Most importantly, no serious adverse events or treatment-related deaths were reported.[10] The most common adverse event was xerostomia as seen in other studies.[4] Nonetheless, ensuring the safety and efficacy of the [225Ac]Ac-labelled radiopharmaceutical preparation is critical. Such preparation should only be used in-house and approved by an authorized person.

CONCLUSION

The present work summarizes potential hazards and a practical approach for in-house preparation of [225Ac]Ac-labelled radiopharmaceutical using the Hazard Analysis and Critical Control Point tool. This document can also guide local authorities in documenting, evaluating, and approving the preparation procedure. In addition, closing the gap between research and clinical institutions should be considered to intensify the development of Targeted Alpha Therapy and other radiopharmaceuticals in Malaysia.

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