ORIGINAL ARTICLE

The purity identification and radiolabeling of α-mangostin with technetium-99m

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ABSTRACT

Alpha-mangostin (AM) is a natural compound that has the greatest activity in breast cancer. Radiolabeling AM with technetium-99 m (Tc-99m) has a function as breast cancer radiotracer. This study is aimed to identify the purity of Tc-99m-labeled AM. The identification method was conducted by a validated radio-high-performance liquid chromatography (HPLC) to confirm the chemical purity of the compound when the thin layer of chromatography and paper chromatography were used to find out the radiochemical purity (RCP). The validated radio-HPLC method obtained was C18 column with methanol:water (90:10) as the mobile phase and ultraviolet (243 nm) tandem radioactive detector (Gabi Star). The result showed that the RCP was 70.6% ± 2.87%. The analytical method met the validation criteria according to ICH Q2 (R1); thus, it could be applied in the identification. Unfortunately, the ^{99m}Tc-AM identification using radio-HPLC showed that the expected complex was not yet formed perfectly because of chemical impurities.

Key words: ^{99m}Tc-alpha-mangostin, breast cancer, purity identification, radio-high-performance liquid chromatography

INTRODUCTION

Alpha-mangostin (AM) (1, 3, 6-trihydroxy-7-methoxy-2, 8-bis [3 methyl-2-butenyl]-9Hxanten-9-on) is one of the natural compounds containing xanthon derivatives which is isolated from mangosteen pericarp (*Garcinia mangostana* Linn.). AM is considerably appreciable to treat cancer. Muchtaridi and Wijaya reviewed the potentiality of AM as an anticancer.[1] The AM plays a role as anti-proliferative that suppresses tumor growth and metastasis in breast cancer of model rat. Breast cancer cells MCF-7 is inhibited by AM.[2] It induces apoptosis of cancer cells through mitochondrial pathways, cell cycle retention through induction of p21^{cip1}, and Akt dephosphorylation on breast cancer cells. Moreover, it inhibits the invasion and migration of cancer cells in the breast gland. The anti-proliferative activity of this compound against MC-7 adenocarcinoma cell apoptosis is demonstrated with IC_{50} value of 20 μM.[3,4] AM is a potentially anti-breast cancer agent with antagonistic activity to estrogen receptor α using *in silico* study.[5]

Based on the chemical structure, AM has an electron donor group in its molecule, thereby making it possible to be labeled with radioisotope technetium-99 m (Tc-99m).[6] The use of radioisotope Tc-99 m for radiotracer purposes has a significant advantage in comparison due to Tc-99m that has ideal properties with half-life of 6 h and low energy at 140 keV, easily obtainable, and has efficient price.[7]

In the development of radiopharmaceuticals, it is very important to have a high purity of labeled compound to get

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an effective product. A good radiopharmaceutical should have high radiochemical purity (RCP) (>90%).[8] On the other hand, the impurity might be occurred because of the presence of free radioisotopes, degradation products, or waste products during the reaction process. Therefore, besides the quality control of the products, the positive result of labeling process should be considered, for example, by using chromatography and electrophoresis. The chromatographies which are commonly used to separate radiolabeled compounds are thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).[9,10]

The objective of this study is to identify the purity of Tc-99m-labeled AM as a breast cancer radiotracer by using radio-HPLC. The radio-HPLC method should be validated initially; thus, the analytical method will always fulfill the expected results.[11]

MATERIALS AND METHODS

Materials

The materials used in this study were AM standard (isolated by the Faculty of Pharmacy Padjadjaran University with purity of 95%), $^{99m}TcO_4^-$ (from $^{99m}Tc/^{99}Mo$ generator was obtained from Hasan Sadikin Hospital), Na_2EDTA (Merck, Germany), $SnCl_2$ (Sigma-Aldrich, USA), sodium hydroxide (Merck, Germany), Whatman 31ET paper, and TLC-SG F_{254} (Merck, Germany). All solvents were purchased from Merck (Germany), except sodium chloride 0.9% and sterile aqua bidest (IPHA). Meanwhile, phosphate buffer pH 7.4 was produced in house.

The equipment to carry out this research consists of dose calibrator (Victoreen), vortex, single-channel analyzer (SCA) (Ortec), paper chromatography (PPC) apparatus, paper electrophoresis device, and radio-HPLC with ultraviolet (UV) detector tandem radioactive detector (Gabi Star).

Synthesis of ^{99m}technetium-alpha-mangostin

Labeling Tc-AM was based on indirect labeling using Na_2EDTA as co-ligand and $SnCl_2$ as reducing agent. To obtain an optimum condition, the experiment was carried out by various parameters, including the pH level, the amount of reducing agent ($SnCl_2$), the amount of ethylenediaminetetraacetic acid (EDTA), the amount of AM, and the incubation time. The optimum results for each parameter determined other parameters until the optimum formula obtained. The optimum formula was carried out by adding 300 μg AM, 90 μg $SnCl_2{\cdot}2H_2O$ and 150 μg Na_2EDTA, and 10 μL chloride acid 0.1 M (to set the optimum pH 9). After the addition of all reagents, 400 μL ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) with activity of ± 0.3 mCi was put into a vial. The final volume was carried out into 1 mL by adding NaCl 0.9% and the incubation lasted for 5 min in room temperature.

Radiochemical purity analysis of technetium-α-mangostin

RCP was determined using a TLC and PPC. To separate impurities of ^{99m}Tc-reduced ($^{99m}TcO_2$) at Rf = 0, TLC-SG F_{254} (10 cm × 1 cm) was used as stationary phase, whereas ethanol: water: ammonia (2:7:1) as mobile phase. With that regard, to separate the impurities of ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) at Rf = 1, Whatman 31ET paper was used as a stationary phase and acetonitrile: water (1:1) as mobile phase. Both TLC and PPC were marked every 1 cm, and 2 ml of the labeled compound was spotted on the strip and eluted. Every 1 cm segment of chromatogram strips was cut and measured by SCA with NaI (Tl) scintillation counter to determine the distribution of radioactivity.

$$\%RCP = 100\% - (\%\ ^{99m}TcO_2 + \%\ ^{99m}TcO_4^-)$$

$$\%^{99m}TcO_2 = \frac{\text{The amount of radioactivity in Rf0}}{\text{Total amount radioactivity}} \times 100\%$$

$$\%^{99m}TcO_4^- = \frac{\text{The amount of radioactivity in Rf1}}{\text{Total amount radioactivity}} \times 100\%$$

Paper electrophoresis

Paper electrophoresis was carried out using cellulose acetate paper (13 cm × 1 cm). Two microliters of the labeled compound was spotted in the middle of the paper. Electrophoresis was observed for 1 h at 200 V and 10 A per paper. Every 1 cm segment of cellulose acetate papers was cut and measured by SCA to determine the distribution of radioactivity.

Identifying chemical purity using radio-high-performance liquid chromatography

The radio-HPLC method was initially validated by measuring some parameters according to ICH (2005) criteria, such as system suitability, specificity, linearity, precision, accuracy, limit of detection (LoD), and limit of quantification (LoQ).

The validated HPLC system (Agilent 1200 Infinity Series) with UV and radioactive (Gabi Star Raytest, Germany) detectors was set at maximum wavelength 243 nm. The SGE Analytical Enduro® C18 column (4.6 mm × 250 mm, 5 μm) was used as the stationary phase and methanol: water (90:10 v/v) as the mobile phase with 1.0 mL/min flow rate and 20 μL for injection volume in 15 min. The Tc-AM was injected as well as the controls (AM, Tc-99m and ^{99m}Tc-EDTA) to the HPLC system.

RESULTS AND DISCUSSION

The radiochemical purity analysis of technetium-alpha-mangostin

The optimum conditions of Tc-AM radiolabeling must be done to obtain the high RCP of the labeling compounds. Some factors affect the purity of the labeled compounds, including the reaction pH, the amount of $SnCl_2$-EDTA and AM, and the incubation time. The result of the optimum conditions of Tc-AM radiolabeling is shown in Table 1.

The optimal pH must be within the acceptable pH range in the form of intravenous injection preparations between 3 and 10.5. The optimum pH used was set at 9 with a clear appearance.

The optimization of reducing agent used is the important step because the more redactor is used, the more $^{99m}TcO_2$ is formed as a result of reduction process of $^{99m}TcO_4^-$ to the lower oxidation state. Nonetheless, if less redactor is used, the reduction process will not run perfectly; therefore, the $^{99m}TcO_4^-$ is still original.[12]

The optimum amount of $SnCl_2 \cdot 2H_2O$ was 90 μg and EDTA was 150 μg. If the amount of $SnCl_2$ is more than 90 μg, it will produce more $^{99m}TcO_2$ impurities because more Tc (VII) will be reduced and form Tc (IV) and vice versa.[13] However, the strength of this reducing agent will generate impurities of radiocolloids during the labeling process by this method.[14,15]

Table 1: The optimum formulation of Tc-AM

Parameters optimization	Amount
$Na^{99m}TcO_4$	400 μL; 0,3 mCi
α-mangostin	300 μg
pH	9
$SnCl_2 \cdot 2H_2O$	90 μg
Na_2EDTA	150 μg
Incubation time	5 min

The optimum amount of AM was 300 μg. RCP will increase with the presence of ligand (AM) when compared to labeling process without AM (^{99m}Tc-EDTA as control). It means that the absence of AM increases the amount of $^{99m}TcO_2$ impurities because Tc (VII) will be reduced and unbind to the ligand (AM). As a result synthesis of ^{99m}Tc-Ketoconazol, the amount of additional ketoconazole poor, thereby producing more impurities which make the efficiency of radiolabeling decreased.[16]

Finally, the best incubation time for this radiolabeling reaction was set in 5 min, with purity 70.60% ± 2.87%. The fast incubation time on strong reducing agent leads to the increase of $^{99m}TcO_2$ impurities.[12] On the other hand, pH 9 showed a clear solution, making it eligible for intravenous preparation.

The RCP analysis of the optimum conditions of Tc-AM was measured through the PPC and TLC. The RCP graphics for both are shown in Figures 1 and 2. As shown in both figures, TLC and PPC could not separate ^{99m}Tc-AM and ^{99m}Tc-EDTA. Furthermore, electrophoresis and HPLC were needed to ensure that ^{99m}Tc-AM and ^{99m}Tc-EDTA were separated.

The electrophoresis was conducted by analyzing Tc-AM and $^{99m}TcO_4^-$ and ^{99m}Tc-EDTA as controls. The result was a radioactive migration pattern of the sample which consequently compared to the controls. The results showed that the three samples had different migration pattern;

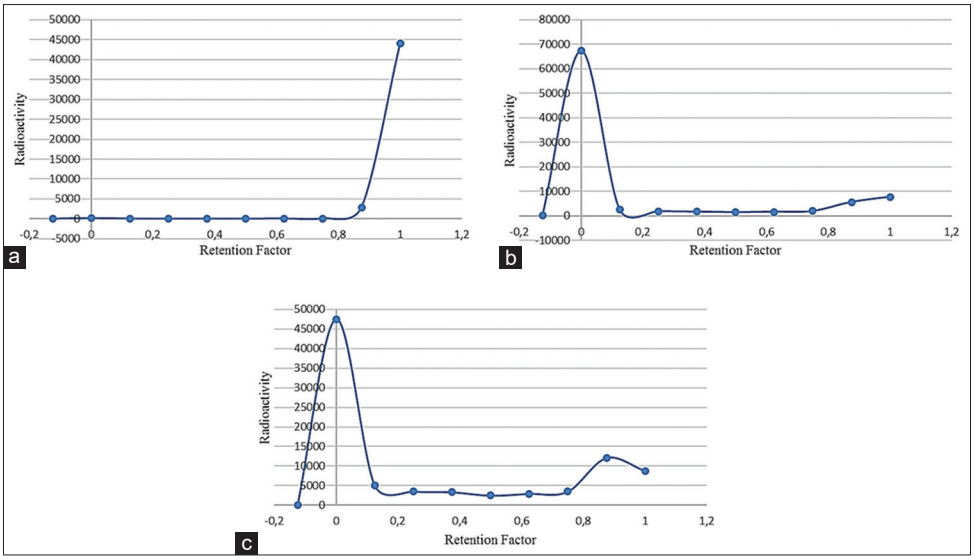

Figure 1: The radiochemical purity graphics of (a) $^{99m}TcO_4$ (b) technetium-alpha-mangostin (c) ^{99m}technetium-ethylenediaminetetraacetic acid in paper chromatography

hence, it can be concluded that the formation of compounds was different [Figure 3].

The peak of control $^{99m}TcO_4^-$ at the range of migration 4 cm, the peak of Tc-AM at range of migration 0 cm, while Tc-EDTA control showed several peaks. To sum up, Tc-AM has been formed. The differences in migration patterns are based on electronegative sample. The negative charge compound will move toward the anode and vice versa.[17] However, electrophoresis still could not ensure this separation; thus, HPLC has played an important role to confirm that the separation has been succeed. Prior to use HPLC as a method, it has to be validated by measuring the validation parameters, such as system suitability, specificity, linearity, accuracy, precision, LoD, and LoQ.

Validation method and characterization of technetium-α-mangostin using radio-high-performance liquid chromatography

The results of system suitability and validation parameters are shown in Tables 2 and 3. The chromatogram of specificity test between AM and blank is shown in Figure 4.

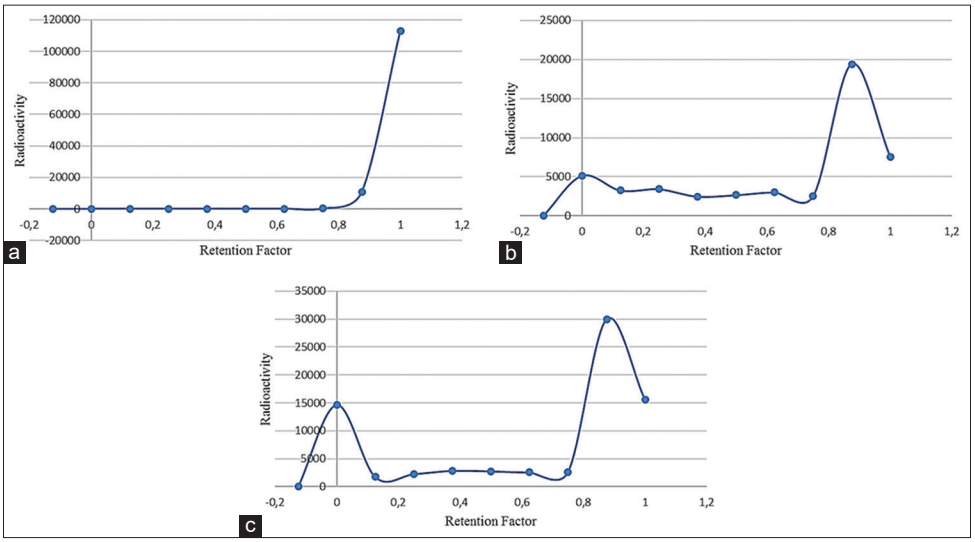

Figure 2: The radiochemical purity graphics of (a) $^{99m}TcO_4$ (b) ^{99m}technetium-alpha-mangostin (c) ^{99m}technetium-ethylenediaminetetraacetic acid in thin-layer chromatography paper electrophoresis

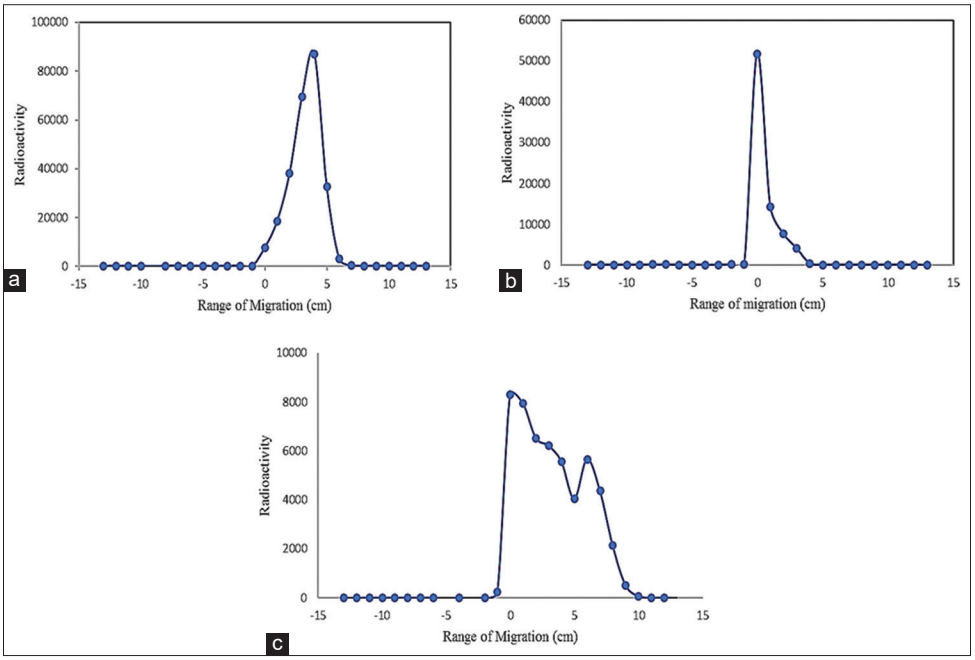

Figure 3: The different electrophoresis migration of (a) $^{99m}TcO_4$ (b) technetium-alpha-mangostin (c) ^{99m}technetium-ethylenediaminetetraacetic acid

Interestingly, the radio-HPLC can be used to identify the chemical purity of Tc-AM. The chemical impurities include nonradioactive materials, such as raw materials, solvents, and other compounds within the preparation process.[18] The HPLC analytical method should be validated first to attain the expected results. According to Table 2, the measured system suitability with AM standard injection has consistent retention time with relative standard deviation 0.94% from six injections. While in Table 3, another validation parameters seemingly meet the requirements criteria from the International Conference of Harmonization Q2 (R1);[19] thus, the HPLC method could be used to analyze the compounds.

The test results in Table 4 show that the AM peak is still appeared in the Tc-AM chromatogram (in 6.68 min). It is apparent that Tc-AM has chemical impurity meaning that the reaction between ligands (AM) and radioisotope (Tc-99m) is not perfectly occurred. By comparing the migration pattern of each sample, it proves the application of the system to analyze the Tc-AM could not separate Tc-AM and ^{99m}Tc-EDTA control because both of them have the same chromatograms (at 1.60 and 1.67 min). At this point, the new HPLC system which could separate Tc-AM and ^{99m}Tc-EDTA should be found. The chromatograms of Tc-AM are shown in Figure 5.

CONCLUSION

The analytical method meets the validation criteria according to ICH Q2 (R1); thus, it can be applied in the identification. However, the purity identification of Tc-AM using radio-HPLC shows that the expected complex is not yet formed perfectly because of chemicals impurities. This result is in line with the RCP analysis result that produces the purity of 70.6% ± 2.87%.

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Table 2: The high-performance liquid chromatography system suitability

Parameters	Requirements	Results
Capacity factor	<2	3.125
Plate number	>2000	6225
Tailing factor	≤2	1.17
HEPT	Low value	0.040

HEPT: Height equivalent to the theoretical plate

Table 3: The validation method

Parameters	Requirements	Results
Linearity	$R^2 \geq 0.999$	$R^2 = 0.9992$
Accuracy (%)	Percentage recovery 98-102	99.00-101.64±0.002-0.015
Precision (%)	Percentage RSD ≤2	0.30-1.47
LoD	-	29.34 μg/mL
LoQ	-	88.92 μg/mL
Specificity	-	No intervention

LoD: Limit of detection, LoQ: Limit of quantification, RSD: Relative standard deviation

Table 4: Informative retention time values of Tc-AM and controls

Sample	tR UV (min)	tR Gabi star (min)
Technetium-99m	-	1.77
^{99m}Tc-EDTA	1.60	1.67
EDTA	1.60	-
α-mangostin	6.65	-
Tc-AM	1.60; 6.68	1.67

tR: Retention time, EDTA: Ethylenediaminetetraacetic acid, Tc-AM: technetium-alpha-mangostin

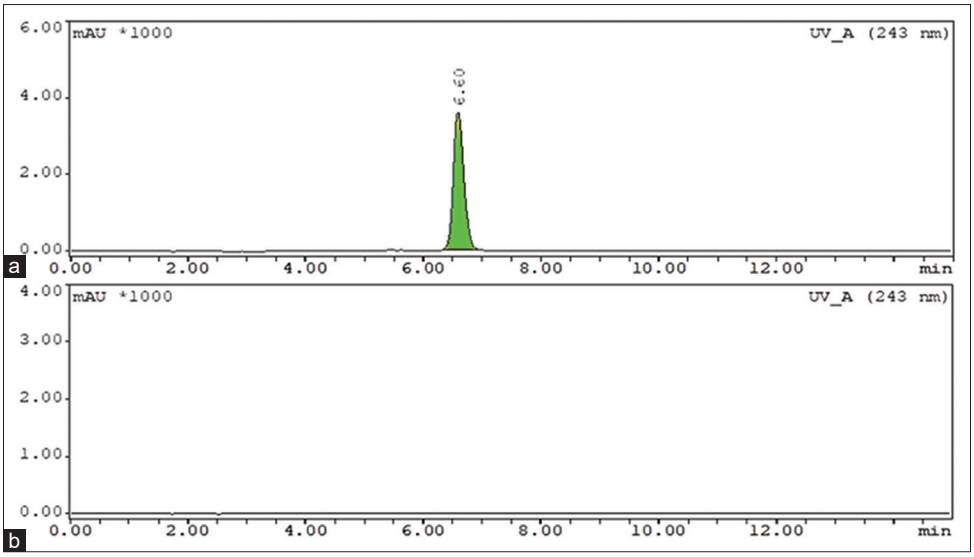

Figure 4: High-performance liquid chromatography chromatograms of (a) alpha-mangostin standard (b) blank

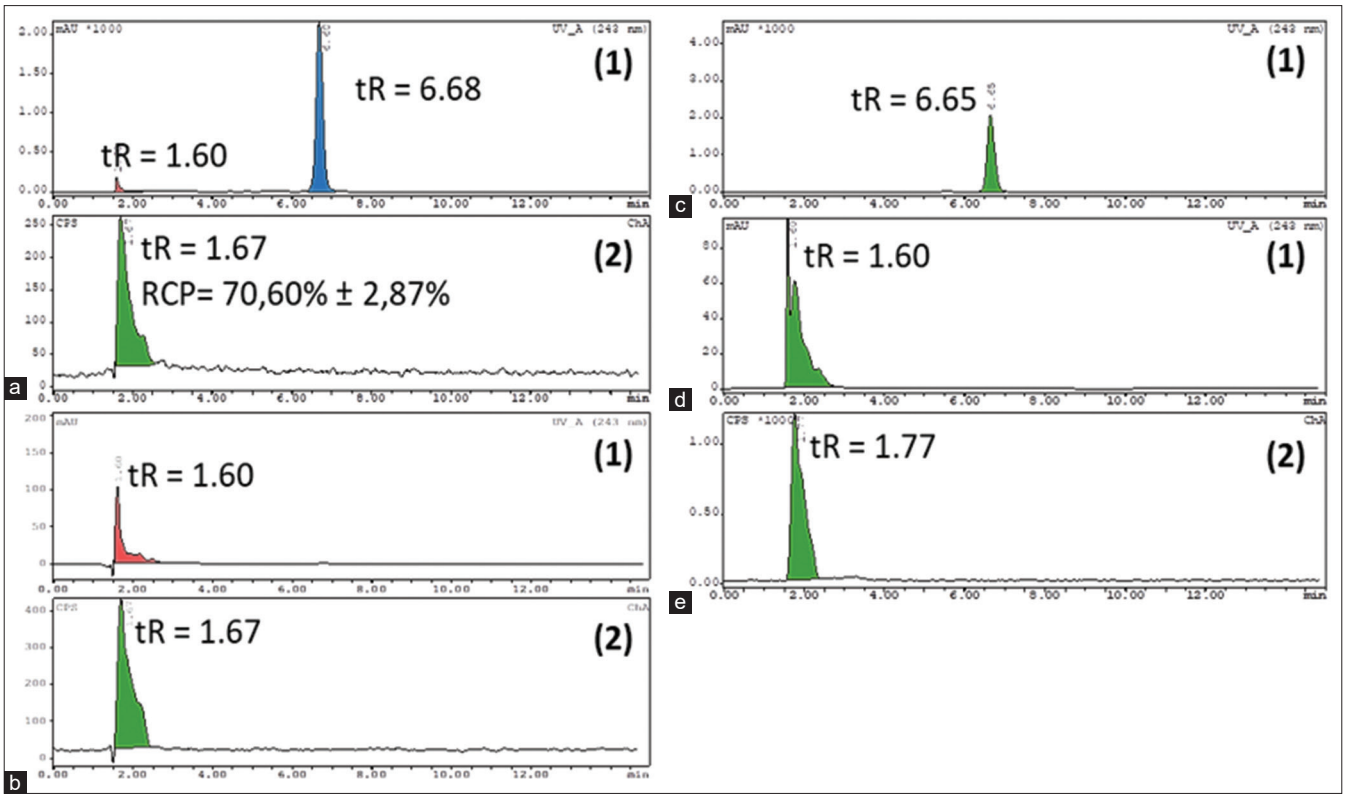

Figure 5: High-performance liquid chromatography chromatograms of (a) technetium-alpha-mangostin, (b) ^{99m}Tc-ethylenediaminetetraacetic acid, (c) alpha-mangostin, (d) ethylenediaminetetraacetic acid, (e) Tc-99m, when the (1) shown the chromatograms from ultraviolet detector and (2) from radioactive detector (Gabi Star)

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Conflicts of interest

There are no conflicts of interest.

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