J Radioanal Nucl Chem (2013) 298:1105–1109
DOI 10.1007/s10967-013-2512-0

Labeling of ursodeoxycholic acid with technetium-99m for hepatobiliary imaging

M. H. Sanad · M. El-Tawoosy

Received: 30 January 2013 / Published online: 23 April 2013

Abstract An adopted method for the preparation of high radiochemical purity ^{99m}Tc-ursodeoxycholic acid (UDCA) was conducted with a high radiochemical yield up to 97.5 %. The reaction proceeds well using 2 mg UDCA, 50 μg tin chloride in solution of pH 8 at room temperature for 30 min. The radiochemical yield was up to 97.5 % as pure as ^{99m}Tc-UDCA. Different chromatographic techniques (paper chromatography and electrophoresis) were used to evaluate the radiochemical yield and purity of the labeled product. Biodistribution studies were carried out in Albino Swiss mice at different time intervals after administration of ^{99m}Tc-UDCA. The uptake of ^{99m}Tc-UDCA in the liver gave the chance to diagnose it. The results indicate that the labeled compound cleared from the systematic circulation within 2 h after administration and majority of organs showed significant decrease in uptake of ^{99m}Tc-UDCA. Finally, the liver uptake was high and the results indicate the possibility of using ^{99m}Tc-UDCA for hepatobiliary imaging.

Keywords UDCA · Technetium-99m · Biodistribution · Hepatobiliary · Imaging

Introduction

Diagnostic radiopharmaceuticals contain γ-emitters whose radiation readily penetrates the body, thus permitting external detection and measurement. The pattern of radiation biodistribution allows a physician to evaluate both the morphology and functioning of organs [1, 2]. Technetium-99m is the most important radionuclide in diagnostic nuclear medicine. The preferential use of ^{99m}Tc-radiopharmaceuticals reflects the ideal nuclear properties of the isotope ($T_{1/2} = 6$ h, 140 keV γ-emitter), as well as its low cost and convenient availability from commercial generators [3–5]. In ^{99m}Tc-radiopharmaceuticals, the metal is bound to a transporting moiety that delivers the radioactivity to a specific site in the body determined by the properties of the transporter [6]. Current research is aimed to formulate new radiopharmaceuticals for hepatobiliary imaging studies. For human use, there are two different classes of ^{99m}Tc-complexes for hepatobiliary studies. These two classes are iminodiacetic acid and pyridoxalamino acid derivatives [7]. All the ^{99m}Tc-radiopharmaceuticals used for hepatobiliary imaging show similar pharmacokinetic properties in animals and human. They are effectively extracted from the blood by the liver and excreted into the bile. Furthermore, they assess disease of hepatocytic function and the functional status of the cystic duct and gallbladder. Biliary duct patency and hepatic diseases can be assessed by the scintigraphic procedure termed cholescintigraphy [8]. The majority of ^{99m}Tc hephatobiliary agents are iminodiacetic acid derivatives including ^{99m}Tc-disofenin (DISIDA) [9], ^{99m}Tc-mebrofenin [10–15], ^{99m}Tc-EHIDA [9], ^{99m}Tc-lidofenin [16, 17], ^{99m}Tc-JODIDA [11], ^{99m}Tc-IOIDA [8], ^{99m}Tc-BPIDA [18] and ^{99m}Tc-IIODIDA [19].

Ursodeoxycholic acid [3α,7β-dihydroxy-5β-cholan-24-oic acid] (UDCA) (Fig. 1) is one of the secondary bile acids, which are metabolic byproducts of intestinal bacteria. Primary bile acids are produced by the liver and stored in the gall bladder. When secreted into the intestine, primary bile acids can be metabolized into secondary bile acids by intestinal bacteria. Primary and secondary bile

M. H. Sanad (✉) · M. El-Tawoosy
Radioisotopes Production and Radioactive Sources Division, Labelled Compounds Department, Hot Laboratories Center, Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt
e-mail: msanad74@yahoo.com

M. El-Tawoosy
e-mail: m_eltawoosy@yahoo.com

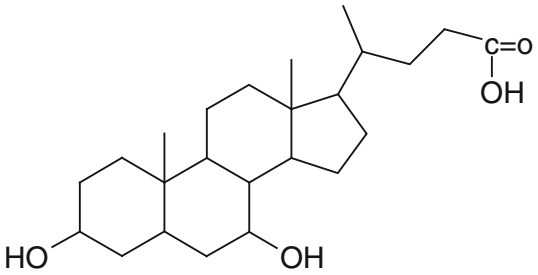

Fig. 1 Chemical structure of UDCA

acids help the body digest to fats. UDCA helps to regulate cholesterol by reducing the rate at which the intestine absorbs cholesterol molecules while breaking up micelles containing cholesterol. Because of this property, UDCA is used to treat (cholesterol) gallstones non-surgically. While some bile acids are known to be colon tumor promoters (e.g. deoxycholic acid), others such UDCA are thought to be chemopreventive, perhaps by inducing cellular differentiation and/or cellular senescence in colon epithelial cells [20]. It is believed to inhibit apoptosis [21]. It is the only FDA approved drug to treat primary biliary cirrhosis [22].

In this study, a simple method for ^{99m}Tc-labeling of UDCA has been investigated. The certain reaction parameters affecting the rate of the reaction including the function of the initial solvent, hydrogen ion concentration, substrate and reducing agent amounts and reaction time have been investigated. The method afforded a high radiochemical yield of pure ^{99m}Tc-UDCA.

Experimental

Materials

Drugs and chemicals

Technetium-99m was eluted as $^{99m}TcO_4^-$ from a $^{99Mo/99m}$Tc generator (radionuclidic and radiochemical purity 99.99 %, 1 Ci, Elutec, Brussels, Belgium). UDCA was obtained as a gift from Sigma Pharmaceutical Company-Egypt, Tin chloride was purchased from Sigma Chemical Company, USA. And all other chemicals were purchased from Merck and they were reactive grade reagent.

Animals

Swiss Albino mice weighing 20–30 g were purchased from the Institute of Eye Research Cairo, Egypt. The animals were kept at constant environmental and nutritional conditions throughout the experimental period and kept at room temperature (22 ± 2) °C with a 12 h on/off light schedule. Animals were kept with free access to food and water all over the experiment.

Methods

Labeling procedure

Accurately weighed 2 mg UDCA was transferred to an evacuated penicillin vial. Exactly 50 μg $SnCl_2$ solution was added and the pH of the mixture was adjusted to 8 using 0.1N NaOH and phosphate buffer then the volume of the mixture was adjusted to 1 ml by N2-purged distilled water. 1 ml of freshly eluted $^{99m}TcO_4^-$ (~400 MBq) was added to the above mixture. The reaction mixture was vigorously shaken and allowed to react at room temperature for 30 min. to complete the reaction [23, 24].

Quality control

The radiochemical yield and purity of ^{99m}Tc-complexes were determined by Paper chromatographic method (PC) and electrophoresis.

Paper chromatography of ^{99m}Tc-UDCA

Radiochemical yield of ^{99m}Tc-UDCA was determined by paper chromatography in which, the reaction mixture was spotted on ascending paper chromatography strips (10 × 1.5 cm). Free $^{99m}TcO_4^-$ in the preparation was determined using acetone as the mobile phase. Reduced hydrolyzed technetium was determined by using 2N HCl as a mobile phase to differentiate between reduced colloids which persist near the point of spotting and both complex and free, which move towards the front of chromatogram. After complete development, the strips were dried then cut into 0.5 cm pieces and their radioactivities counted in a well type Gamma counter: Scalar Ratemeter SR7, Nuclear enterprises LTD. USA.

Electrophoresis conditions

Electrophoresis was done with EC-3000 p-series programmable (E.C.apparatus corporation) power and chamber supply units using cellulose acetate strips. The strips were moistened with 0,05 M phosphate buffer pH 7.2 ± 0.2 and then were introduced in the chamber. Samples (5 μl) were applied at a distance of 10 cm from the cathode. The applied voltages were 300 v and standing time for one and half hours then the radioactivity values were continued. Developed strips were dried and cut into 1 cm segments and counted by a well-type NaI scintillation counter. The radiochemical yield was calculated as the ratio of the radioactivity of the labeled product to the total radioactivity.

Factors affecting % labeling yield

This experiment was conducted to study the different factors that affect labeling yield such as tin content as ($SnCl_2{\cdot}2H_2O$), substrate content, pH of the reaction, and reaction time. The experiment was repeated by keeping all variables constant except the factor under study, till the optimum conditions are achieved.

Biodistribution of the labeled ^{99m}Tc-UDCA

In-vivo biodistribution studies were performed using 9 mice divided into three groups of three mice each. Each animal was injected in the tail vein with 0.2 ml solution containing 5–10 kBq of ^{99m}Tc-UDCA. The mice were kept in metabolic cages for the required time. Mice were killed by cervical dislocation at various time intervals (30, 60 and 120 min) after injection and the organs or tissues of interest were removed, washed with saline, weighted and their radioactivity were counted. Correction was made for background radiation and physical decay during the experiment [25, 26]. The weights of blood, bone and muscles were assumed to be 7, 10 and 40 % of the total body weight, respectively [27]. Differences in the data were evaluated with the Student t test. Results for p using the 2-tailed test are reported and all the results are given as mean ± SEM. The level of significance was set at $P < 0.05$.

Results and discussion

Paper chromatography

Radiochemical purity and stability of ^{99m}Tc-UDCA complex were assessed by ascending paper chromatographic method and electrophoresis condition. In case of ascending paper chromatographic method acetone was used as the developing solvent, free $^{99m}TcO_4^-$ moved with the solvent front ($R_f = 1$), while ^{99m}Tc-UDCA and reduced hydrolyzed technetium remained at the point of spotting. Reduced hydrolyzed technetium was determined by using 2N HCl as a mobile phase as where reduced hydrolyzed technetium remains at the origin ($R_f = 0$) while other species migrate with the solvent front ($R_f = 1$). The radiochemical purity was determined by subtracting the sum of the percent of reduced hydrolyzed technetium and free pertechnetate from 100 %. The radiochemical yield is the mean value of three experiments.

Electrophoresis

The paper electrophoresis pattern revealed that ^{99m}Tc-UDCA complex moved towards the cathode, indicating the cationic nature of this complex. But $^{99m}TcO_4^-$ moved considerably toward the anode, suggesting that it has a high negative charge.

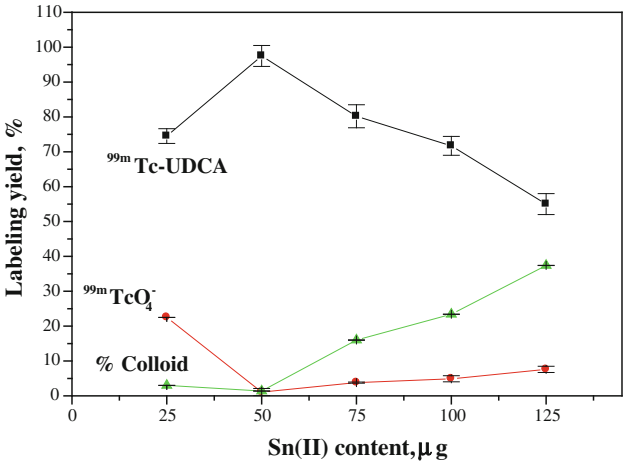

Fig. 2 Effect of $SnCl_2{\cdot}2H_2O$ concentration on the labeling yield of ^{99m}Tc-UDCA; reaction conditions: 2 mg UDCA, 25–125 μg of $SnCl_2{\cdot}2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4$ at pH 8, the reaction mixture was kept at room temperature for 30 min

Factors affecting labeling yield

Effect of tin chloride content

The majority of ^{99m}Tc-radiopharmaceuticals are prepared using $SnCl_2{\cdot}2H_2O$, [Sn(II)],for reduction of ^{99m}Tc from heptavalent to lower valence state, which facilitates its chelation by compounds of diagnostic importance. The effect of tin chloride as a reducing agent on the labeling of UDCA with ^{99m}Tc is illustrated in Fig. 2 at low amount of Sn(II), the radiochemical yield of ^{99m}Tc-UDCA complex was low (74.5 % at 25 μg) with the appearance of free pertechnetate (22.5 %) due to insufficient Sn(II) to reduce all pertechnetate present in the reaction mixture. Increasing the amount of Sn(II) to 50 μg led to an increase in labeling yield to 97.5 ± 0.5 %. By increasing the amount of reducing agent above 50 μg, until (125 μg), the labeling yield decreased again to 57.6 % due to colloid formation (37.4 %) [28].

Effect of substrate amount

The influence of UDCA amount as a substrate on the labeling yield using 50 μg of $SnCl_2{\cdot}2H_2O$ (tin chloride) was shown in Fig. 3. The increase of the amount of UDCA was accompanied by a significant increase in the labeling yield, where it reached above 79 % at 1 mg of UDCA. Increasing the amount of UDCA from 1 to 2 mg produced significant increase in the labeling yield (97.5 %). Increasing the amount of starting material is usually increases the total amount of incorporated ^{99m}Tc since

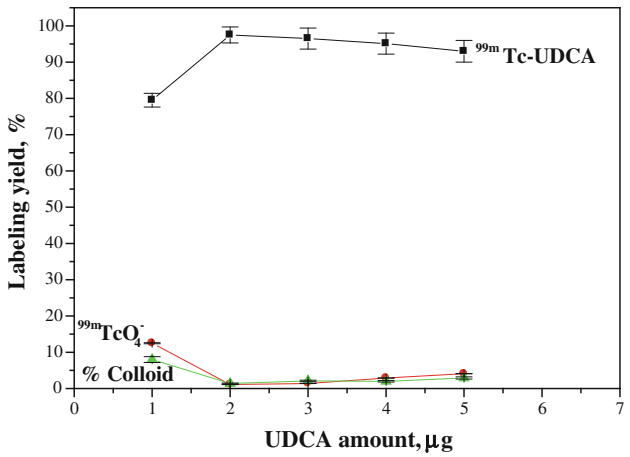

Fig. 3 Percent labeling yield of ^{99m}Tc-UDCA as a function of substrate amount; reaction conditions: 1–5 mg UDCA, 50 µg of $SnCl_2{\cdot}2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4$ at pH 8, the reaction mixture was kept at room temperature for 30 min

there is a minimum limit to the volume used [27]. 2 mg of UDCA was required to obtain maximum labeling yield. Below this amount there will be a significant decrease in the yield apparently because of insufficient amount of the ligand to bind all the reduced technetium and the amount of colloid increased. On the other hand, using higher amount did not significantly affect labeling yield.

Effect of pH

In order to reach the suitable pH value for maximum radiochemical yield, labeling of UDCA with ^{99m}Tc was carried out at different pH ranging from 7 to 11 as shown in Fig. 4, it is observed that ^{99m}Tc-UDCA is considered negligible from 2 to 6 due to its degradation in acidic medium. The test was performed using 2 mg of UDCA, 0.5 m1 of each buffer at different pH values at 30 min reaction time. As shown in Fig. 4, pH 8 is the optimum pH at which the maximum yield was obtained (97.5 %). At pH 10 and 11 the yield was 68.5, 60.4 %, respectively.

Effect of reaction time

Figure 5 shows the relationship between the reaction time and the yield of ^{99m}Tc-UDCA. Radiochemical yield was significantly increased from 80 to 97.5 % with increasing reaction time from 1 to 30 min, respectively. Extending the reaction time to 60 min produced no significant change of the radiochemical yield.

In vitro stability of ^{99m}Tc-UDCA

In the present experiment, a slight decrease in the stability of ^{99m}Tc-UDCA from 97.0 to 96 % at 2 and 6 h post labeling was observed. Further significant decrease in the yield was observed at 12 and 24 h post labeling, as the yield was 94.8 % (Table 1).

Biodistribution of ^{99m}Tc-UDCA in mice

Biodistribution study of ^{99m}Tc-UDCA in normal mice showed that ^{99m}Tc-UDCA was distributed rapidly in blood, kidney, liver and intestine at 30 min post injection. After 1 h, ^{99m}Tc-UDCA uptake was significantly increased in blood, stomach, intestine and bone while, it decreased in liver, heart and muscle. At 2 h post injection, the majority of tissues and organs showed significant decrease in ^{99m}Tc-UDCA uptake. On the other hand, lung, intestine

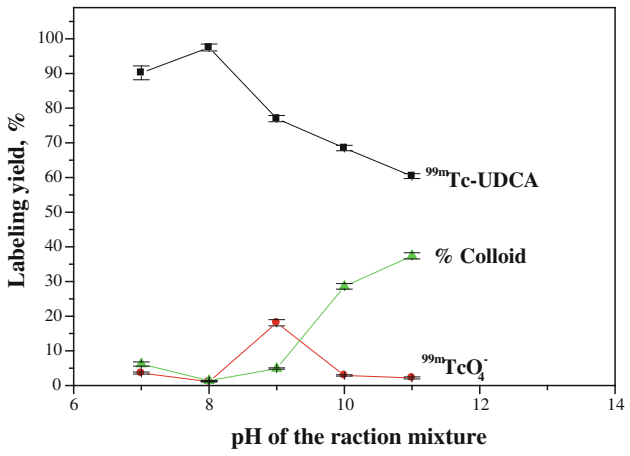

Fig. 4 Effect of pH on the labeling yield of ^{99m}Tc-UDCA, 2 mg of UDCA, 50 µg of $SnCl_2{\cdot}2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4$ at 7-11 pH, the reaction mixture was kept at room temperature for 30 min

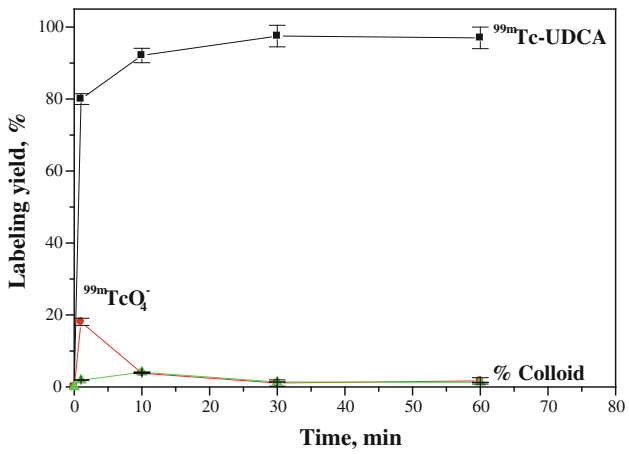

Fig. 5 ^{99m}Tc-UDCA yields vs. reaction time, 2 mg UDCA, 50 µg of $SnCl_2{\cdot}2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4$ at pH 8, the reaction mixture was kept at room temperature at different time post labeling

Table 1 Effect of time on the Stability of ^{99m}Tc-UDCA

Time post labeling (h)	Labeled compound (%)	Free ^{99m}Tc (%)	Colloid (%)
1	97.0 ± 0.50	1.7 ± 0.29	1.3 ± 0.13
2	96.1 ± 0.24	1.9 ± 0.24	2.0 ± 0.07
6	96.5 ± 0.25	1.9 ± 0.44	1.6 ± 0.03
12	94.6 ± 0.33	2.6 ± 0.61	2.8 ± 0.20
24 h	94.8 ± 0.71	2.3 ± 0.21	2.9 ± 0.01

Values represent the mean ± SEM. $n = 3$

Table 2 Biodistribution of ^{99m}Tc-UDCA in mice

Organs and body fluids	Percent I.D./g organ		
	Time post injection, min		
	30	60	120
Blood	5.04 ± 0.01	0.37 ± 0.02[a]	0.20 ± 0.004[a]
Bone	0.35 ± 0.02	0.46 ± 0.04[a]	0.84 ± 0.005[a]
Muscle	0.92 ± 0.07	0.74 ± 0.05[a]	0.35 ± 0.001
Liver	15.37 ± 0.19	12.07 ± 0.15[a]	3.03 ± 0.16[a]
Intestine	11.81 ± 0.50	14.38 ± 0.30	22.10 ± 0.70[a]
Kidney	10.26 ± 0.40	14.26 ± 0.30	3.59 ± 0.20[a]
Lung	2.19 ± 0.10	2.67 ± 0.12[a]	3.81 ± 0.20[a]
Stomach	1.42 ± 0.20	2.75 ± 0.09	1.80 ± 0.16[a]
Heart	1.42 ± 0.15	0.98 ± 0.05[a]	0.91 ± 0.05[a]
Spleen	0.71 ± 0.01	0.66 ± 0.04[a]	0.69 ± 0.02
Urine	25.41 ± 1.50	49.61 ± 1.52	53.33 ± 1.51

Values represent mean ± SEM. $n = \mathbf{5}$

[a] Significantly different from the previous value of each organ using unpaired Student's t test ($P < 0.05$)

and stomach showed significant increase in ^{99m}Tc-UDCA uptake (Table 2). The uptake in liver was 15.37, 12.0 and 3.0 % at 30 min, 1 and 2 h respectively. This uptake may be useful for radioimaging of the liver.

Conclusion

^{99m}Tc-UDCA was prepared easily at pH 8 using 50 μg $SnCl_2 \cdot 2H_2O$ as a reducing agent with a labeling yield of 97.5 ± 0.3 %. ^{99m}Tc-UDCA complex was formed once the addition of ^{99m}Tc to the reaction mixture and the formed complex was stable up to 6 h, which shows high stability time. The data obtained from the biodistribution of ^{99m}Tc-UDCA reflect the rapid uptake in the liver which was enough to give an imaging picture.

References

1. Giglio J, Patsis G, Pirmettis I et al (2008) Eur J Med Chem 43:741
2. Kowalsky R, Fallen S (2004) Radiopharmaceuticals in nuclear pharmacy and nuclear medicine. American Pharmacists Association, Washington, DC
3. Mustapha A, Eric B, Nouzha H et al (2004) Tetrahedron 60:1167
4. Dilworth JR, Parrot SJ (1998) Chem Soc Rev 27:43
5. Jurisson S, Lydon JD (1999) Chem Rev 99:2205
6. Johannsen B, Spies H (1997) Transit Met Chem 22:318
7. Volkert AW, Jurisson S (1996) Technetium-99m chelates as radiopharmaceuticals. Springer, Berlin
8. Young DH, Beom SJ, Sang MC et al (2004) Appl Radiat Isot 61:1273
9. Schwarzrock R, Kotzerke J, Hundeshagen H et al (1986) Eur J Nucl Med 12:346
10. Camuzzini GF, Angeli BD, Angeli B et al (1984) J Nucl Med Allied Sci 28:167
11. Chauhan UPS, Pishra P, Chander J (1993) Appl Radiat Isot 44:843
12. Chiotellis E, Varvarigou A (1980) Int J Nucl Med Biol 7:1
13. Johnson K, Alton HM, Chapman S (1998) Pediatr Radiol 28:937
14. Nunn AD, Loberg MD, Conley RA (1983) J Nucl Med 24:423
15. De Graaf W, Häusler S, Heger M et al (2011) J Hepatology 54:738
16. Callery PS, Faith WC et al (1976) J Med Chem 19:962
17. Jonas E, Hultrantz R, Slezak P et al (2001) J Nucl Med Commun 22:127
18. Motaleb MA, El-Said H, Atef M, Abd-Allah M (2012) Radiochemistry 54(3):274
19. Brbori JS, Jovanovi MS, Popovi G, Kapetavovi V, Vladimirov S (2006) J Serb Chem Soc 71(1):55
20. Akare S, Jean-Louis S, Chen W, Wood DJ, Powell AA, Martinez JD (2006) Int J Cancer 119(12):2958
21. Amaral JD, Viana RJ, Ramalho RM, Steer CJ, Rodrigues CM (2009) J Lipid Res 50(9):1721
22. Jackson H, Solaymani-Dodaran M, Card TR, Aithal GP, Logan R, West J (2007) Hepatology 46(4):1131
23. Zmbova B, Kastie K, Djokie DK, Obradorie V (1987) Isotopenpraxis 23(7):278
24. Loberg MD, Cooper M, Harvey P (1978) J Nucl Med 19:635
25. Challeryes M, Banerjee S (1991) J Nucl Med Biol 18(3):363
26. Pandalai PK, Bulcao CF, Merrill WH, Akhter SA (2006) J Thorac Cardiovasc Surg 31:975
27. Hanson J, Reynaud D, Qiao N, Devel P, Moray AL, Renard JF, Kelley LP, Winum JY, Montero JL, Kinsella BT, Pirotte B, Pace-Asciak CR, Dogne JM (2006) J Med Chem 49(12):3701
28. Hafez TM (2010) Molecular modeling, synthesis, quality control and biological evaluation of some antimicrobial agents labeled with ^{99m}Tc, PhD thesis, Faculty of Pharmacy, Ain Shams University, Cairo