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Comparison of chromatographic methods for quality control of DMSA complexes with ^{99m}Tc and ^{188}Re at (III) and (V) oxidation states

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Abstract

BACKGROUND: The reliable method for determination of identity and radiochemical purity (RCP) is of great importance in radiopharmaceutical development. This is especially relevant when more than one form of radiometal/ligand complex can be formed during radiolabelling, such as complexes of ^{99m}Tc or ^{188}Re with *meso*-2,3-dimercaptosuccinic acid (DMSA), where depending on the pH, metal can occur either at +3 or +5 oxidation state. The aim of our study was to evaluate possibilities for optimization of chromatographic systems leading to specific and reliable analytical method for determination of the identity and RCP of DMSA complexes with ^{99m}Tc or ^{188}Re.

MATERIAL AND METHODS: The commercial DMSA kits (POLATOM) were used for preparation of technetium-99m (III) and (V) complexes with DMSA. ^{99m}Tc(V)-DMSA complexes were prepared by addition of $NaHCO_3$ to the kit vial prior to ^{99m}Tc-eluate to obtain pH ~8. ^{188}Re(V)-DMSA was prepared either directly or using intermediate ^{188}Re(III)-EDTA complex added to DMSA. RCP was evaluated by TLC using: ITLC-SG developed in methylethylketon, SG60 coated plates developed in: n-BuOH/H_2O/CH_3COOH and n-PrOH/H_2O/CH_3COOH systems, and in H_2O. Comparative biodistribution studies were performed in normal Wistar rats.

RESULTS: Using silica gel plates and n-PrOH, H_2O and acetic acid in the developing solution, we observed that ^{99m}Tc/^{188}Re(III)-DMSA and ^{99m}Tc/^{188}Re(V)-DMSA complexes could be well separated from each other and from the impurities in the form of free pertechnetate/perrhenate. In vivo studies showed quite different biodistribution of ^{99m}Tc(III)- and ^{99m}Tc(V)-DMSA. The trivalent complex accumulated mainly in kidneys (>40%ID), while ^{99m}Tc(V)-DMSA revealed high excretion with urine and relatively high concentration in osseous tissue (ca. 2 %ID/g). Accumulation of this complex in kidneys was very low (ca. 2.5 %ID). Biodistribution pattern of ^{188}Re(V)-DMSA prepared directly was almost identical to that of ^{99m}Tc(V)-DMSA. Biodistribution results of the ^{188}Re preparation obtained using ^{188}Re(III)-EDTA intermediate indicated that the preparation contained the mixture of penta- and trivalent ^{188}Re complexes. The quite high accumulation of radioactivity in kidneys (23 %ID) gave evidence of the presence of ^{188}Re(III)-DMSA in this preparation, what was also confirmed by the results of TLC analysis performed using silica gel plate and n-propanol/water/acetic acid as developing system.

CONCLUSIONS: Based on our study, we have made recommendation on the suitable methods for investigations of RCP of DMSA complexes, i.e.: SG60 plates developed in the mixture of n-propanol/water/acetic acid, which enable determination

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of the tri- and pentavalent DMSA complexes, as well as, the pertechnetate/perrhenate impurity, and developed in water for determination of the colloidal residue.

KEY words: *meso*-2,3-dimercaptosuccinic acid (DMSA), ^{99m}Tc(III) and (V), ^{188}Re(V), complexes, identity, radiochemical purity, radiochromatography

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Background

Meso-2,3-dimercaptosuccinic acid (DMSA, succimer) is a water-soluble, organosulfur compound used for preparation of radiopharmaceuticals of great importance for nuclear medicine applications. Trivalent technetium-99m dimercaptosuccinic acid, ^{99m}Tc(III)-DMSA, is a widely used renal imaging agent. Pentavalent ^{99m}Tc-dimercaptosuccinic acid, ^{99m}Tc(V)-DMSA, is used for imaging of medullary and insular carcinoma of the thyroid [1–3], for some other soft tissue tumours [4–9], and skeletal metastases from breast carcinoma [10, 11]. $^{186/188}$Re(V)-DMSA is a radiotherapeutic analogue of ^{99m}Tc(V)-DMSA, and its use is well established in a variety of histologically different tumours and especially in bone metastases arising from breast and prostate carcinomas [12–14].

The reliable method for determination of identity and radiochemical purity (RCP) is of great importance in radiopharmaceutical development. This is especially relevant when more than one form of radiometal/ligand complex can be formed during radiolabelling, and ^{99m}Tc or ^{188}Re complexes with DMSA present such a case. Under a wide range of pH several different entities of radiolabelled DMSA can be formed with quite different biological behaviour. In DMSA complexes with ^{99m}Tc, depending on pH, the metal can occur at +3 or +5 oxidation state. Preliminary structural analysis by NMR [15] suggests that in acidic media (pH around 3), a hexacoordinated asymmetric bis-complex is formed in which one molecule is bound to technetium via two -S- bridges and one -O- bridge, while the other is bound via one -S- bridge and two -O- bridges, and one –SH remains free (Figure 1). In an alkaline medium (pH ≥ 8), all of the free –SH groups of dimercaptosuccinic acid (DMSA) are available, forming a pentacoordinated bis-complex with the metal central ion coordinated by four thiolates of two DMSA ligands and an apical oxo group. ^{99m}Tc(V)-DMSA, as well as ^{188}Re(V)-DMSA, are in fact a mixture of three stereoisomers arising from different orientations of the 4 carboxylate groups of the complex $[TcO(DMSA)_2]^{-1}$ (Figure 2).

Although there is the monograph No. 0643 in the European Pharmacopoeia [16] describing RCP test for ^{99m}Tc-succimer (^{99m}Tc(III)-DMSA) using ITLC SG plate and methylethylketone as mobile phase, this method is not specific to distinct possible

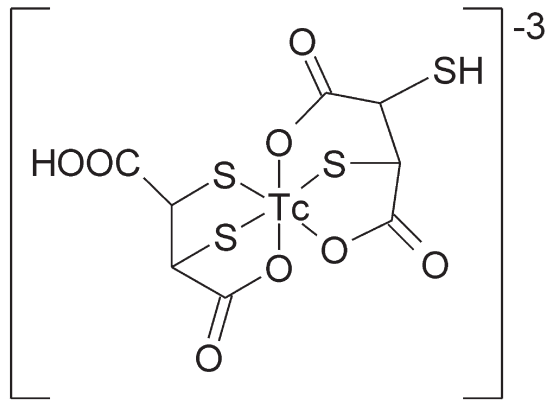

Figure 1. Suggested structure of bisDMSA complex with trivalent ^{99m}Tc

forms of the complex and allows only the determination of free ^{99m}Tc-pertechnetate. Under these chromatographic conditions both ^{99m}Tc(III)-DMSA and ^{99m}Tc(V)-DMSA are retained at the origin, the same as colloidal residues of ^{99m}Tc, and only ^{99m}Tc-pertechnetate moves with the solvent. Although this kind of information may be considered sufficient for routine quality control purposes of radiopharmaceutical obtained from a commercially prepared kit with marketing authorization, its practical utility for testing ^{99m}Tc(V)-DMSA preparations is hardly acceptable. Up to date, biodistribution studies have been involved to confirm potential clinical efficacy of the radioactive DMSA preparations. However, in order to reduce the number of experiments in laboratory animals, routine biodistribution testing should be replaced by other suitable physico-chemical or *in vitro* methods. Development of such methods for comprehensive investigation of the radioactive DMSA complexes is still needed.

The aim of this work was to evaluate the currently available chromatographic systems and their optimization in order to provide specific and reliable analytical methods for investigation of DMSA complexes with ^{99m}Tc or ^{188}Re.

Material and methods

Chemicals

The kit for preparation of technetium-99m succimer injection (^{99m}Tc-DMSA MTcK-12, NCNR RC POLATOM, Poland) was used for preparation of technetium-99m (III) and (V) complexes with DMSA.

For ^{188}Re(V)-DMSA preparation two preformulated kits were developed:

Kit 1 for direct preparation of ^{188}Re(V)-DMSA consisting of two vials:

— vial 1-1: 2 mg DMSA and 1 mL of 0.05 M carbonate buffer of pH 9.0 (freeze-dried);

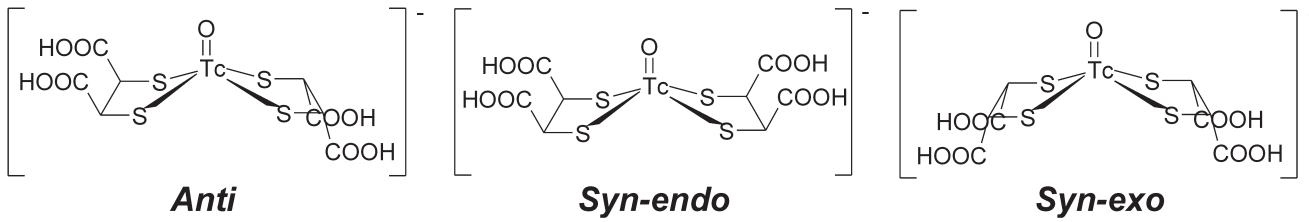

Figure 2. Stereoisomers of pentavalent ^{99m}Tc (^{188}Re) complex with DMSA

— vial 1-2: 2 mL solution of stannous chloride in 1 M HCl (2.0 mg/mL).

Kit 2 for indirect radiolabelling:

— vial 2-1: 5.0 mg EDTA, 5.0 mg mannitol and 1.0 mg stannous chloride (freeze-dried);
— vial 2-2: 0.5 mg DMSA in 1 mL of 0.1 M phosphate buffer of pH 7.4 (freeze-dried);
— vial 2-3: 1 mL solution of 0.1 M HCl.

The eluate of sodium pertechnetate (^{99m}Tc) was obtained from *Technetium (^{99m}Tc) Generator POLGENTEC* and the eluate of sodium perrhenate (^{188}Re) was obtained from ^{188}W/^{188}Re generator [17] (both radionuclide generators produced by NCNR RC POLATOM, Poland).

All other chemicals and materials were used as supplied and were of analytical or HPLC grade unless otherwise stated.

Preparation of DMSA complexes

^{99m}Tc(III)-DMSA was prepared following manufacturer's instruction for ^{99m}Tc-labelling. For preparation of ^{99m}Tc(V)-DMSA complexes, 0.18 mL of 7% $NaHCO_3$ was added to the commercial DMSA kit vial to obtain pH ~8, followed by 4.82 mL of the eluate of sodium pertechnetate (^{99m}Tc) [18].

^{188}Re(V)-DMSA preparations were obtained either by the direct labelling method [19] or using the pre-formed complex of ^{188}Re-EDTA, which was added to DMSA at pH 7.4 [20]. For both methods, kits formulations were prepared (Kit 1 and Kit 2) as described in paragraph on Chemicals.

Radiolabelling of DMSA with ^{188}Re:

— using Kit 1: 1 mL of $^{188}ReO_4^-$ eluate was added to the 1-1 vial, followed by 1 mL solution (2.0 mg/mL) of stannous chloride in 1 M HCl (vial 1-2). The mixture was incubated for 30 min at 95 °C.
— using Kit 2: ^{188}Re(III)-EDTA precursor was prepared by adding 3-5 mL of $^{188}ReO_4^-$ eluate to the 2-1 vial followed by 0.5 mL of 0.1 M HCl solution from the vial 2-3. The mixture was incubated for 20-30 min at room temperature. Then, 1 mL of such obtained solution was transferred to the 2-2 vial and the content was incubated for 10 min at RT.

Radioanalytical methods

Several chromatographic systems were tested:

— European Pharmacopoeia method [16].
— ITLC-SG (P/N 61886, *PALL*) strips and methylethylketone (MEK) as mobile phase.
— TLC on silica gel plates as proposed by Westera et al. [21]: silica gel TLC plates (Kieselgel 60 DC-Plastikfolien; 105748; Merck) and n-butanol/ water/acetic acid (3/3/2 *V/V/V*) as mobile phase;
— TLC on silica gel plates — modifications: silica gel TLC plates (Kieselgel 60 DC-Plastikfolien; 105748; Merck) and several compositions of mobile phase: n-propanol / water / acetic acid: (4/3/1 *V/V/V*), (4/2/1 *V/V/V*), (4/1/1 *V/V/V*), etc.
— TLC on silica gel plates and water as mobile phase [22].

Distribution of radioactivity on the developed and dried chromatographic strips/plates was analyzed using linear gamma Radio-TLC Scanner (*BioScan*) and by autoradiography using Cyclone Plus Storage Phosphor Scanner (*Perkin Elmer*).

In vivo studies

Biodistribution studies were performed in normal Wistar rats (male, weighing 190 ± 10 g) according to the procedure described in Ph. Eur. monograph No. 0643 [16]. Briefly, ca. 12 MBq of the ^{99m}Tc- and 9-21 MBq of ^{188}Re-labelled preparations, respectively, in 0.2 mL volume were injected intravenously (*i.v.*) through the tail vein. Five rats were used per each tested preparation. The rats were sacrificed at 1 h post injection (*p.i.*) and the tissues and organs were excised, rinsed with saline, weighed and counted in a scintillation gamma counter supplied with an adapter for the whole-body measurement. Distribution of the activity in different organs was expressed as percentage of injected radioactivity dose per organ (%ID) and as percentage of injected dose per gram of tissue (%ID/g).

All animal experiments were performed after approval by The IVth Local Animal Ethics Committee in Warsaw and were carried out in accordance with the principles of good laboratory practice.

Results and discussion

Table 1 shows the results of RCP determination of different DMSA preparations using selected TLC systems. In the chromatographic system recommended by the Ph. Eur. monograph for RCP testing of ^{99m}Tc-succimer [16], ^{99m}Tc(III)-DMSA complex remains at the application point, the same like most of the technetium-99m radiopharmaceuticals, and an unbound ^{99m}Tc in the form of pertechnetate migrates with the solvent front. This method enables quantitative determination of technetium-99m in the form of pertechnetate only. Therefore, the biodistribution testing of the ^{99m}Tc-DMSA in normal rats is required by the Ph. Eur. monograph to assure that the preparation is suitable for renal scanning.

Vanlić-Razumenić and Petrović [22] proposed quite smart method using silica gel plates and water as mobile phase for determination of the colloidal forms of ^{99m}Tc or ^{188}Re in DMSA preparations. Our investigations confirmed that using this method the pertechnetate (perrhenate), as well as, the pentavalent and trivalent complexes of ^{99m}Tc and ^{188}Re with DMSA migrate with the solvent front while colloidal forms are retained at the origin. Hence, the method can be recommended for investigation of the colloidal form of the radionuclide in DMSA preparations, although a small unspecific retention of radioactivity at the application point (ca. 0.3%) should be taken into account (Table 1).

The TLC method proposed by Westera et al. [21] utilizing silica gel coated plates and n-BuOH/H_2O/acetic acid (3/3/2 *V/V/V*) allows separation of the trivalent and pentavalent DMSA complexes (Figure 3A) in a chromatographic process lasting 2 h. This chromatographic system enables also detection of colloidal forms of ^{99m}Tc and/or ^{99m}Tc(III)-DMSA complex in ^{99m}Tc(V)-DMSA preparations and vice versa. A rather long time of chromatographic process (ca. 2 h) is a main disadvantage of the method. Therefore, in the present study we tested also some modifications using the same silica gel plates (Kieselgel 60 Plastikfolien, Merck) and varying compositions of the mobile phase. The n-butanol has been replaced by n-propanol as it is a solvent better soluble in water. Changes in the water and acetic acid contents were tested with regard to the separation power of the system, as well as the time needed to complete analysis (Figure 3B and 3C).

Table 1. Typical retardation factors (Rf) for radioactive entities in the DMSA preparations determined in different TLC systems

Preparations	ITLC-SG;MEK (developing time ca. 10 min)	SG60; H2O	SG60; BuOH/ H_2O/CH_3COOH (3/3/2) (developing time ca. 2 h)	SG60; PrOH/H_2O/CH_3COOH (4/3/1) (developing time ca. 1 h 20 min)
	Rf (% activity)	Rf (% activity)	Rf (% activity)	Rf (% activity)
^{99m}Tc-pertechnetate/^{188}Re-perrhenate	0.9–1.0 (100%)	0.9–1.0 (99.7%)	0.8–0.9 (100%)	0.8–0.9 (100%)
^{99m}Tc/^{188}Re-colloid	0.0 (100%)	0.0 (100%)	0.0 (100%)	0.0 (100%)
^{99m}Tc(V)-DMSA	0.0–0.1 (100%)	0.9–1.0 (99.7%)	0.6–0.7 (> 90%)	0.6 (> 97%)
^{99m}Tc(III)-DMSA	0.0–0.1 (100%)	0.9–1.0 (> 97%)	0.0–0.4 (56%) 0.5–0.6 (26%) 0.7 (17%)	0.0–0.4 (86%) 0.5 (5%) 0.6 (9–10%)
^{188}Re(V)-DMSA — Kit No. 1	0.0–01 (100%)	0.9–1.0 (> 99.7%)	0.6–0.7 (100%)	0.6 (100%)
^{188}Re(EDTA)DMSA — Kit No. 2	–	–	0.0 (22%) 0.4 (40%) 0.6–0.7 (44%)	0.0 (17%) 0.5 (28%) 0.6 (38%)

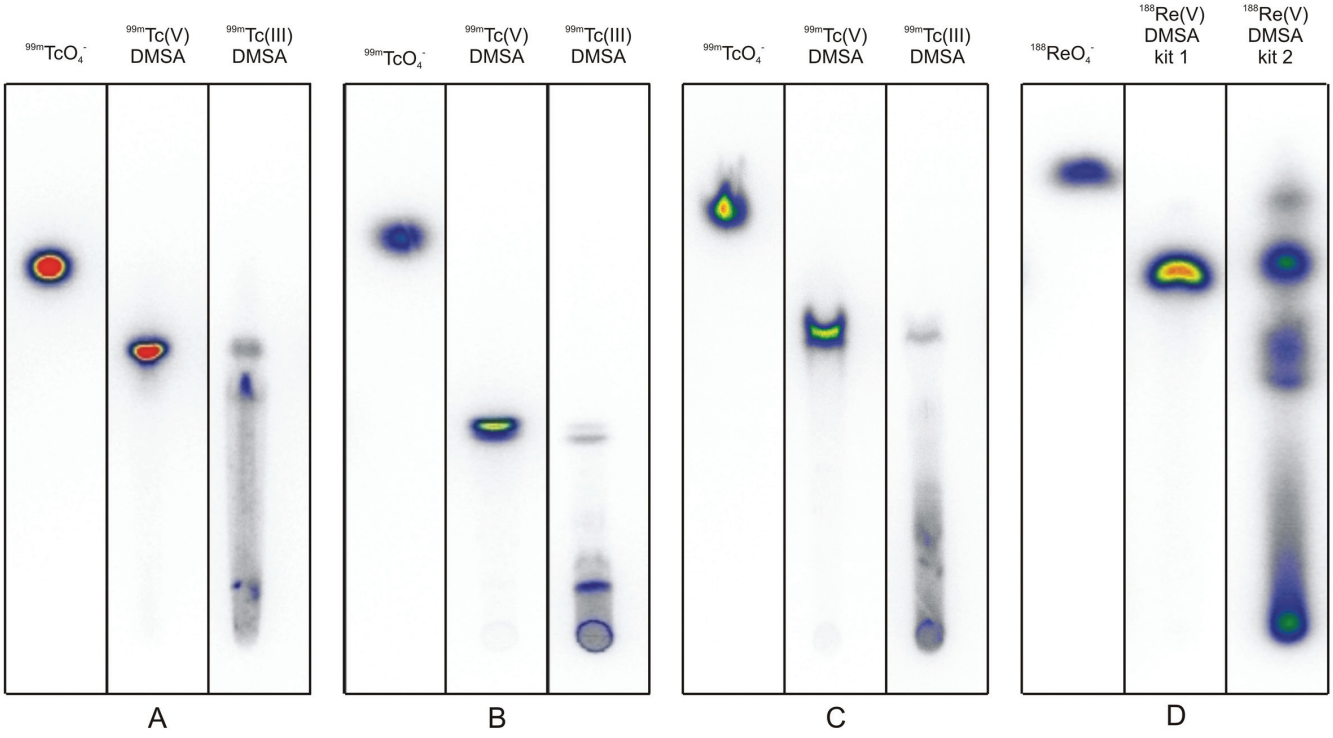

Figure 3. The TLC autoradiograms of ^{99m}Tc- and ^{188}Re-DMSA complexes, obtained using silicagel 60 plates developed in various mobile phases: A. n-BuOH/ H_2O/AcOH (3/3/2 *V/V/V*) (the method published by Westera et al. [21]); B. n-PrOH/H_2O/AcOH (4/1/1 *V/V/V*); C. n-PrOH/H_2O/AcOH (4/3/1 *V/V/V*); D. n-PrOH/H_2O/AcOH (4/3/1 *V/V/V*)

Modification of the chromatographic system by using silica gel plates and n-PrOH/H_2O/AcOH (4/3/1 *V/V/V*) as mobile phase showed excellent resolution power for the DMSA complexes with ^{188}Re (Figure 3D). The system was a little bit faster (ca. 1 h 20 min vs. ca. 2 h) and revealed good separation ability with regard to differentiation between various radiochemical entities present in the DMSA preparations. It was clearly seen, especially for ^{188}Re-DMSA preparation obtained by the indirect method using Kit 2 and intermediate ^{188}Re(III)-EDTA complex, which always resulted in a mixture of radioactive entities, *i.e.*: ^{188}Re(V)-DMSA, ^{188}Re(V)-EDTA/DMSA, ^{188}Re(III)-EDTA, etc.

Blower et al. [13] have proposed RP-HPLC method, which can separate anti, syn-endo and syn-exo stereoisomers (Fig. 2) of the pentavalent ^{99m}Tc- or ^{188}Re-DMSA complexes. We also examined this HPLC method for investigations of radioactive DMSA complexes. However, in spite of observation of characteristic set of peaks corresponding to the stereoisomers, the radiochromatograms obtained did not provide complete information on all radio-

Table 2. Biodistribution results of the radioactive DMSA preparations in Wistar rats 1 h post intravenous injection (mean %ID ± SD; n = 5)

Organ/tissue	^{99m}Tc(III)-DMSA	^{99m}Tc(V)-DMSA	^{188}Re(V)-DMSA[a] (Kit No. 1)	^{188}Re-DMSA[b] (Kit No. 2)
Blood (%ID/g)	0.59 ± 0.04	0.31 ± 0.05	0.34 ± 0.04	1.00 ± 0.17
Femur (%ID/g)	0.72 ± 0.10	1.91 ± 0.43	1.58 ± 0.16	0.55 ± 0.08
Liver	3.86 ± 0.51	1.23 ± 0.15	1.3 ± 0.1	5.8 ± 0.5
Kidneys	42.87 ± 1.35	2.48 ± 0.10	2.1 ± 0.2	23.0 ± 1.2
Lungs	0.60 ± 0.12	0.24 ± 0.04	0.5 ± 0.2	0.7 ± 0.1
Stomach	0.37 ± 0.09	0.28 ± 0.08	0.3 ± 0.0	0.6 ± 0.1
Urine	4.09 ± 5.37	35.57 ± 18.57	46.0 ± 2.4	28.7 ± 6.8
Carcass	35.91 ± 5.94	48.52 ± 7.41	32.3 ± 2.7	26.5 ± 0.9

[a]^{188}Re-DMSA obtained from Kit 1, DMSA (carbonate buffer pH = 9). Injection per animal: 9 MBq (50 μg DMSA)/0.2 mL (n = 5, male, 196 g ± 13); [b]^{188}Re-DMSA (Kit 2, DMSA in phosphate buffer pH = 7.5 + EDTA). Injection per animal: 21.5 MBq (14.3 μg DMSA)/0.2 mL; (n = 5, male, 192 g ± 9)

chemical forms present in the ^{99m}Tc- or ^{188}Re-DMSA preparations. This was due to the fact that the trivalent complexes were strongly retained or precipitated on the chromatographic column, as in the case of the ^{99m}Tc-colloidal forms, and the calculation of radioactivity balance showed that usually not more than 10% of the injected ^{99m}Tc(III)-DMSA was eluted from the column. Additionally, it is worth to note that a quantitative contribution of particular stereoisomers, as seen on the RP-HPLC radiochromatograms, does not have any practical utility since no significant differences in organ distribution and kinetics in-vivo were observed for the separated isomers, which would favour the use of purified stereo isomers over the isomeric mixture of ^{188}Re(V)-DMSA [23].

The results of biodistribution of ^{99m}Tc(III)- and ^{99m}Tc(V)-DMSA in normal Wistar rats are presented in Table 2. As required by the specification given in the Ph. Eur. monograph [16], the trivalent complex accumulated mainly in kidneys (> 40 %ID) and showed low excretion with urine. Differently, ^{99m}Tc(V)-DMSA complex revealed high excretion with urine (>35 %ID 1h *p.i.v.*) and relatively high concentration in osseous tissue (ca. 2 %ID/g). Accumulation of this complex in kidneys was very low (ca. 2.5 %ID). The biodistribution pattern of ^{188}Re(V)-DMSA prepared from Kit 1, which usually enabled preparation of the complex of high radiochemical purity (93-98%), was almost identical to that of ^{99m}Tc(V)-DMSA. For ^{188}Re preparation obtained using Kit 2, the biodistribution results indicated that the preparation contained the mixture of pentavalent and trivalent ^{188}Re complexes. The quite high accumulation of radioactivity in kidneys (23 %ID) confirmed the presence of ^{188}Re(III)-DMSA in this preparation, what was also determined by the TLC analysis performed using silica gel plate and n-propanol/water/acetic acid as developing system.

Conclusions

In order to predict the in vivo efficacy of DMSA based radiopharmaceuticals for diagnostic or therapeutic applications, not only the information on radiochemical impurities such as the pertechnetate/perrhenate and colloidal residues of radiometal is crucial but also on the tri- or pentavalent DMSA complexes, since the contribution of each of them influences the biodistribution of radiopharmaceutical. We have shown that a well-matched constituents and radiolabelling conditions enable preparation of pentavalent ^{99m}Tc/^{188}Re-DMSA complexes of very high radiochemical purity (> 95%). To properly predict biological behaviour of the DMSA preparations, reliable analytical investigations are necessary before its clinical application. Among the tested chromatographic systems none can be considered alone as universal for determination of identity and radiochemical purity of DMSA complexes. Based on our study, for RCP testing of DMSA radiopharmaceuticals we recommend TLC using SG60 coated plates and n-propanol, water and acetic acid in the developing solution, because this chromatographic system enables separation of the tri- and pentavalent DMSA complexes, as well as the impurities in the form of the pertechnetate or perrhenate ions. If combined with the TLC method for the determination of the colloidal residues [22], these two chromatographic systems will give comprehensive identification of all radiochemical entities, which may be present in ^{99m}Tc(^{188}Re)-DMSA preparations.

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