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Identity confirmation of ^{99m}Tc-MAG3, ^{99m}Tc-Sestamibi and ^{99m}Tc-ECD using radio-LC-MS

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Abstract

Due to the low concentrations in which ^{99m}Tc-radiopharmaceuticals are obtained (4–40 ng/ml), confirmation of the identity of these tracer agents in the European Pharmacopoeia is generally performed only indirectly by assessment of their retention times on RP-HPLC. We have investigated whether it is possible to obtain more direct proof of the identity of technetium-99m labelled radiopharmaceuticals using radio-LC-MS. As representative examples, negatively charged ^{99m}Tc-MAG3, positively charged ^{99m}Tc-Sestamibi and neutral ^{99m}Tc-ECD were used. The three technetium-99m radiopharmaceuticals were prepared in several conditions to obtain variable relative amounts of radiochemical impurities and variable concentrations of the complexes (pico- to nanomolar). The preparations were analyzed on a reversed phase C18 HPLC column using a radio-LC-MS system equipped with a time of flight mass spectrometer with electrospray ionization in positive (^{99m}Tc-Sestamibi, ^{99m}Tc-ECD) or negative (^{99m}Tc-MAG3, ^{99m}Tc-ECD) mode. For each of the studied complexes, the main peak in the radiometric channel coincided with the expected molecular ion mass of the corresponding technetium complex in the mass spectrometer channel. The relative error on the measured accurate mass was in the range of 10 ppm. The identity of several radiochemical impurities of the three technetium complexes was also confirmed or established. It is concluded that radio-LC-MS can be a sensitive aid in quality control of 'no carrier added' radiopharmaceuticals.

Keywords: Radio-LC-MS; Radiopharmaceutical; ^{99m}Tc-MAG3; ^{99m}Tc-Sestamibi; ^{99m}Tc-ECD; HPLC

1. Introduction

Technetium-99m (^{99m}Tc) is the most frequently used radionuclide for in vivo imaging studies in Nuclear Medicine. It owes its popularity to its nearly ideal physical characteristics (i.e. short physical half-life ($T^{1/2}$) of 6 h; γ energy of 140 keV; no particulate emission during decay) and

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chemical properties (technetium easily forms complexes with a large number of ligands) combined with its continuous availability from a ^{99}Mo/^{99m}Tc generator at a reasonable cost.

Most ^{99m}Tc-radiopharmaceuticals consist of a ^{99m}Tc-chelate and are prepared ex tempore by the addition of a sterile solution of $^{99m}TcO_4^-$, obtained by elution of a ^{99}Mo/^{99m}Tc generator, to a sterile labelling kit. By reaction of Tc with the constituents of the kit, it is reduced from oxidation state VII (in $^{99m}TcO_4^-$) to a lower valence by the action of stannous ions and is then chelated by the ligand to form the desired technetium complex which is obtained in a short time (typically 5–30 min) in a high yield ($>90\%$). As this ex tempore preparation involves a modification of the chemical status of technetium-99m, the preparation should be analyzed (generally by radio-TLC) to verify the radiochemical purity prior to intravenous injection in patients.

The mass amount of technetium used in preparations for clinical use is typically in the pico- to nanomole range and consists of the sum of short-lived technetium-99m and its long-lived daughter technetium-99 ($T^{1/2} = 2.10^5$ years). Elucidation of the structure of technetium complexes is currently achieved by X-ray diffraction analysis of crystals of either the identical complex synthesized on milligram scale with long lived technetium-99 (thus radioactive and complicated to handle) or the analogous complex with stable rhenium, a chemical congener of Tc. Identity confirmation across ^{99m}Tc and ^{99}Tc or Re compounds is performed by co-injection on HPLC and comparison of UV and radiometric detector signal.

^{99m}Tc-MAG3 (^{99m}Tc-mercaptoacetyltriglycine, ^{99m}Tc-mertiatide) is a polar, negatively charged complex in which an oxotechnetium(V) core is bound to three deprotonated amide nitrogen atoms and one sulphur thiol atom [1] (Fig. 1). It is widely used as a renal function imaging agent and can be prepared from a commercial labelling kit (TechneScan MAG3®, Tyco Healthcare, Petten, The Netherlands). This labelling kit contains the ligand in its *S*-benzoyl protected form (to prevent thiol oxidation), stannous chloride and NaK-tartrate (a weak intermediate chelating agent). Potential radiochemical impurities that can be anticipated from 'the classic' exchange labelling procedure include $^{99m}TcO_4^-$ (starting product) and ^{99m}Tc-tartrate (intermediate complex).

^{99m}Tc-MAG3 can also be prepared by direct labelling (in the absence of an intermediate chelating agent) at alkaline pH starting from unprotected mercaptoacetyltriglycine. However, small variations in the pH during labelling have a profound influence on the radiochemical purity [2,3]. Labelling at pH <11 results in the formation of a so-called Tc-MAG3 precomplex, whereas labelling at pH >12 results in partial cleavage of a glycine moiety of the ligand and subsequent formation of Tc-MAG2 [4]. The structure of the Tc-MAG3 precomplex has not yet been elucidated, but it has been suggested that it is a complex in which technetium is coordinated by several MAG3 ligand molecules [2,3].

Confirmation of the identity of ^{99m}Tc-MAG3 in the monograph of the European Pharmacopoeia (Ph. Eur.) [5] is now performed indirectly by comparison of the retention time on RP-HPLC of the sample to be examined with the retention time of Tc-MAG3 obtained by labelling of the Ph. Eur. chemical reference substance *S*-benzyl-MAG3.

^{99m}Tc-Sestamibi (^{99m}Tc-hexakis(2-methoxy)-isobutyl isonitrile) is a lipophilic cationic complex, used for myocardial perfusion imaging, in which technetium(I) is bound by six methoxyisobutylisonitrile (MIBI) ligand molecules through the isonitrile carbon atoms (Fig. 1) [6].

^{99m}Tc-Sestamibi is prepared using a commercially available labelling kit (Cardiolite®, Bristol-Myers Squibb, Billerica). The MIBI ligand is volatile and is present in the Cardiolite® kit as the non-volatile $Cu(I)(MIBI)_4$ adduct in order to allow lyophilisation. During labelling, the MIBI ligands dissociate from the Cu(I) adduct and bind to reduced Tc(I). To prevent the formation of colloidal TcO_2, L-cysteine is present in the labelling kit and forms an intermediary polar ^{99m}Tc-cysteine complex. As a function of time, Tc is transchelated from its cysteine complex to eventually form the more stable Tc-Sestamibi complex. The exchange rate is enhanced by heating the labelling vial in a boiling water-bath.

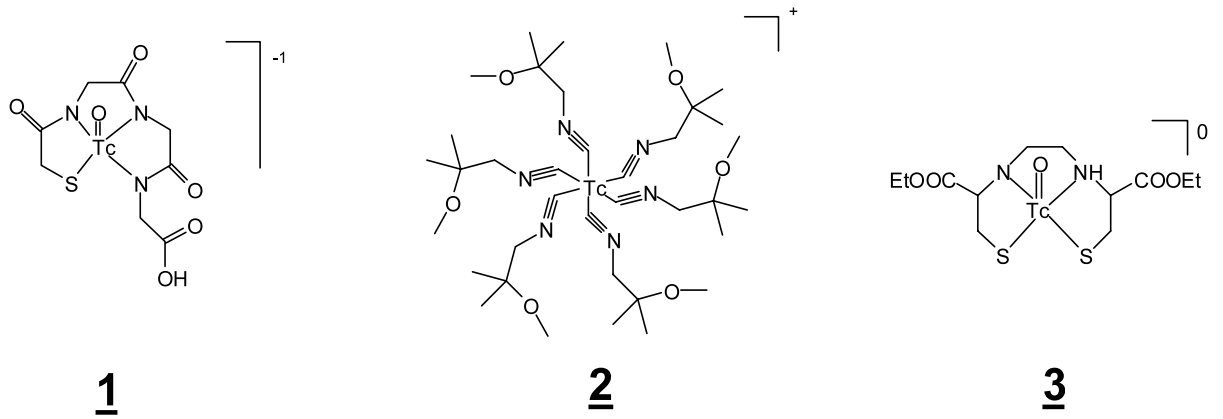

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Fig. 1. Structure of Tc-MAG3 (1), Tc-Sestamibi (2) and Tc-ECD (3).

^{99m}Tc-ECD (^{99m}Tc-*L*,*L*-ethylcysteinate dimer, ^{99m}Tc-bicisate, Fig. 1), is a neutral and lipophilic complex used for investigation of regional cerebral perfusion. In ^{99m}Tc-ECD, an oxotechnetium(V) core is chelated by two amine nitrogen atoms (one of which remains protonated) and two thiol sulphur atoms [7]. The ligand contains two ester functions, the integrity of which is very important to maintain the lipophilic nature of the ^{99m}Tc-bicisate complex.

^{99m}Tc-ECD can be prepared from a commercial labelling kit (Neurolite®, Bristol-Myers Squibb) at room temperature. Potential radiochemical impurities in ^{99m}Tc-ECD preparations include residual $^{99m}TcO_4^-$ (in case of incomplete reduction or partial reoxidation) and the complexes of ^{99m}Tc with, respectively, the mono-ester (ECM) and diacid derivatives (EC), generated by hydrolysis of the ligand or incomplete esterification during synthesis.

To our knowledge, only two reports have been published on the use of LC-MS for direct identity confirmation of ^{99m}Tc-labelled radiopharmaceuticals [8,9], but no reports are available with regard to the analysis of ^{99m}Tc-MAG3, ^{99m}Tc-Sestamibi or ^{99m}Tc-ECD preparations.

We have investigated whether it is possible to obtain direct identity confirmation of the three technetium-99m labelled radiopharmaceuticals using radio-LC-MS as an alternative to the indirect identification described in the Ph. Eur. Additionally, we have tried to identify radiochemical impurities of the three technetium preparations.

2. Experimental

2.1. Materials

$SnCl_2.2H_2O$ was obtained from Merck (Darmstadt, Germany), NaK-tartrate.$4H_2O$ from Acros (Geel, Belgium). *S*-benzoyl-MAG3 was synthesized according to a described procedure [10]. Cardiolite® and Neurolite® labelling kits were obtained from Bristol-Myers Squibb (Brussels, Belgium).

$Na^{99m}TcO_4$ was obtained by elution of a ^{99}Mo/^{99m}Tc generator (Ultratechnekow FM, Tyco Healthcare, Petten, The Netherlands). ^{99m}Tc-activity was measured in a Capintec CRC-35R dose calibrator (Veenstra, Joure, The Netherlands). The mass amount of Tc in the generator eluate is related to the activity of ^{99m}Tc, the age of the eluate and the time interval elapsed between the previous elution and the elution yielding the $Na^{99m}TcO_4$ that was used for the labelling experiments. The mass amount of Tc was calculated using algorithms derived for transient equilibrium generators [11].

2.2. Instruments

The radio-LC-MS system consisted of a Waters 2690 separation module (Waters, Milford, USA) connected to a RP C18 column (XTerra™ MS C18 3.5 μ, 2.1 × 50 mm, Waters) eluted at a flow rate of 300 μl/min. The column eluent was monitored for radioactivity using a radiometric detector (3-inch NaI(Tl) detector connected to a radiation analyzer module, The Nucleus, Oak Ridge). Finally, the column eluate was directed to a time-of-flight mass spectrometer (Micromass LCT, Manchester, UK) equipped with an orthogonal ESI probe.

In order to enable accurate mass calculations, the column eluate was mixed with a lock mass solution (*S*-benzoylmercaptoacetyltriglycine 0.1 mg/ml in methanol for ES− analysis, Kryptofix® \tf="DM8"\char178}}-
2.2.20.01$mg/mlinCH3CN${{\tf="BMa1"\cha-
/ml in $CH_3CN–H_2O$ (50:50, v/v) for ES+ analysis) infused with a Harvard Instruments 22 syringe pump (Harvard Instruments, Holliston) at a flow rate of 5 μl/min.

The relative error on accurate mass determinations was calculated by dividing the difference between theoretical and observed mass by the theoretical mass.

For the analysis of $^{99/99m}$Tc-MAG3 preparations the column was eluted with mixtures of acetonitrile (0′: 0%, 10′: 10%, 15′: 50%, 20′: 0%) and ammonium formate 0.1%. For the analysis of $^{99/99m}$Tc-Sestamibi, the column was eluted at a flow rate of 300 μl/min with gradient mixtures of acetonitrile and ammonium acetate 0.1 M (linear gradient from 0 to 80% acetonitrile in 15 min, then 80% acetonitrile until 20 min). For the analysis of $^{99/99m}$Tc-ECD preparations the column was eluted with mixtures of acetonitrile (0′: 0%; 20′: 70%) and 0.1% formic acid.

2.3. Radiolabelling

2.3.1. Preparation of ^{99m}Tc-MAG3

Standard ^{99m}Tc-MAG3 was prepared by addition of 1 ml generator eluate (450 megabecquerel (MBq), containing either 35 pmol or 0.3 nmol $^{99/99m}$Tc) to a labelling vial containing 1 mg *S*-benzoylmercaptoacetyltriglycine, 20 mg NaK-tartrate, 50 μg $SnCl_2.2H_2O$ in 12.5 μl HCl 0.05 M and 0.5 ml phosphate buffer (pH 7; 0.5 M). The labelling mixture was heated for 10 min in a boiling water-bath.

2.3.2. Preparation of ^{99m}Tc-MAG3 with increased amount of 'precomplex'

In a labelling vial, 7 mg of *S*-benzoyl-MAG3 was dissolved in 1 ml phosphate buffer (pH 11; 0.5 M). The mixture was incubated for 10 min at room temperature. A solution of 200 μg $SnCl_2.2H_2O$ in 50 μl HCl 0.05 M and 1 ml generator eluate (150 MBq, containing 0.6 nmol $^{99/99m}$Tc) were added and the mixture was incubated for 10 min at room temperature. Afterwards, the labelling reaction mixture was neutralized by addition of 1 ml of a phosphoric acid solution (0.5 M).

2.3.3. Preparation of ^{99m}Tc-MAG3 with increased amount of ^{99m}Tc-MAG2

In a labelling vial, 2 mg of *S*-benzoyl-MAG3 was dissolved in 1 ml phosphate buffer (pH 12; 0.5 M). The mixture was incubated for 10 min at room temperature, resulting in a slightly red opalescence and a characteristic odor. A solution of 200 μg $SnCl_2.2H_2O$ in 50 μl HCl 0.05 M and 1 ml generator eluate (85 MBq, containing 0.6 nmol $^{99/99m}$Tc) were added and the mixture was incubated for 10 min at room temperature. Finally, the labelling reaction mixture was neutralized by addition of 1 ml of a phosphoric acid solution (0.5 M).

2.3.4. Preparation of ^{99m}Tc-Sestamibi

A Cardiolite® labelling kit was reconstituted by the addition of 3 ml generator eluate containing either 0.21 GBq ^{99m}Tc (17 pmol Tc, 'low concentration') or 14.73 GBq ^{99m}Tc (2.6 nmol Tc, 'high concentration') and heating for 10 min in a boiling water-bath. For analysis, 10 μl of the preparation was applied on the radio-LC-MS system.

2.3.5. Preparation of ^{99m}Tc-ECD

A Neurolite® kit was reconstituted according to the instructions of the manufacturer. A high concentration of ^{99m}Tc (3.7 GBq/ml, conc. of $^{99/99m}$Tc = 2.5 pmol/l) was added. The solution was incubated at room temperature for 30 min and

40 μl of the preparation was applied on the radio-LC-MS system for analysis.

2.3.6. Alkaline hydrolysis of ^{99m}Tc-ECD

Some 200 μl NaOH 1 M was added to 700 μl of the standard labelling mixture. After mixing and incubation for 15 min at room temperature, the mixture was neutralized by the addition of 200 μl of H_3PO_4 0.5 M.

3. Results and discussion

In general, the peaks corresponding to the molecular ion masses of the studied technetium complexes are relatively small compared to mass peaks originating from background or from the presence of much higher concentrations of chemicals present in the labelling reaction mixture that coelute with the technetium complexes on RP-HPLC. The radiometric trace, however, specifically detects the presence of technetium-99m in the column eluate and is used as a guide to delineate the time interval on the mass spectrometric chromatogram in which the molecular ion of the technetium labelled compound is to be found.

3.1. Tc-MAG3

Tc-MAG3 was prepared by a standard exchange labelling procedure using a high or low amount of technetium. The radio-LC-MS chromatogram obtained by analysis of a standard preparation containing a low amount (35 pmol) of Tc-MAG3 is shown in Fig. 2(C). The radiometric channel only shows two peaks, one corresponding to Tc-MAG3 (retention time (Rt) = 8.7 min) and the other corresponding to the so-called Tc-MAG3 precomplex, an earlier eluting impurity (Rt = 7.6 min).

The mass spectrometer total ion current shows a large peak at Rt = 14.9 min corresponding to *S*-benzoyl-MAG3. Close examination of the mass channel spectrum shows the presence of a small peak (Rt = 2.1 min) which corresponds to deprotected mercaptoacetyltriglycine (data not shown).

As can be expected, the analysis of a preparation containing a higher concentration of Tc shows a better signal/noise ratio and due to the larger amount of ions detected and the associated increased statistics, the accurate mass determination shows a relative error of only 10 ppm (Table 1). For these analyses only 10 μl of the preparation was injected but if required, solutions with a lower concentration of the tracer agent can be analyzed by injecting larger volumes. Radio-LC-MS can thus be used for the direct confirmation of the identity of Tc-MAG3 in routine preparations.

The starting product TcO_4^- (which can be present in the case of incomplete reduction or in the case of (partial) reoxidation) elutes at a retention time of 2.1 min (radioactivity channel) and the background subtracted mass spectrum over this peak (data not shown) shows the presence of a peak corresponding to the pertechnetate ion (Table 1). The accurate mass measured approximates the theoretical mass with a relative error of 11 ppm. Since TcO_4^- is a potential impurity in all technetium-99m labelled radiopharmaceuticals, this radio-LC-MS system can be applied for the identification of this compound in any ^{99m}Tc-labelled tracer agent.

To obtain a preparation with an increased amount of Tc-MAG2, Tc-MAG3 was prepared by direct labelling in phosphate buffer (pH 12; 0.5 M) [3]. On subsequent RP-HPLC analysis, we detected a peak at Rt = 14.7 min on the radioactivity channel, the background subtracted mass spectrum of which corresponds to that of Tc-MAG2 (Fig. 2B). Again, the determined accurate mass was close to the theoretical ion mass (relative error of 9 ppm, Table 1) and the single ion mass chromatogram (316.93–316.98 kDa) had an identical retention time and peak shape as the peak observed in the radioactivity channel (data not shown).

Direct labelling of Tc-MAG3 at pH 11 using a high amount of the ligand following a reported procedure [3] yielded a preparation containing a large amount (80%) of so-called Tc-MAG3 precomplex, which has a retention time of 7.6 min in the radioactivity channel (Fig. 2A). The structure of this complex has not been elucidated but this precomplex is known to rapidly convert to Tc-MAG3 at pH >12. It is also larger in size than Tc-MAG3 as it has a shorter retention time on

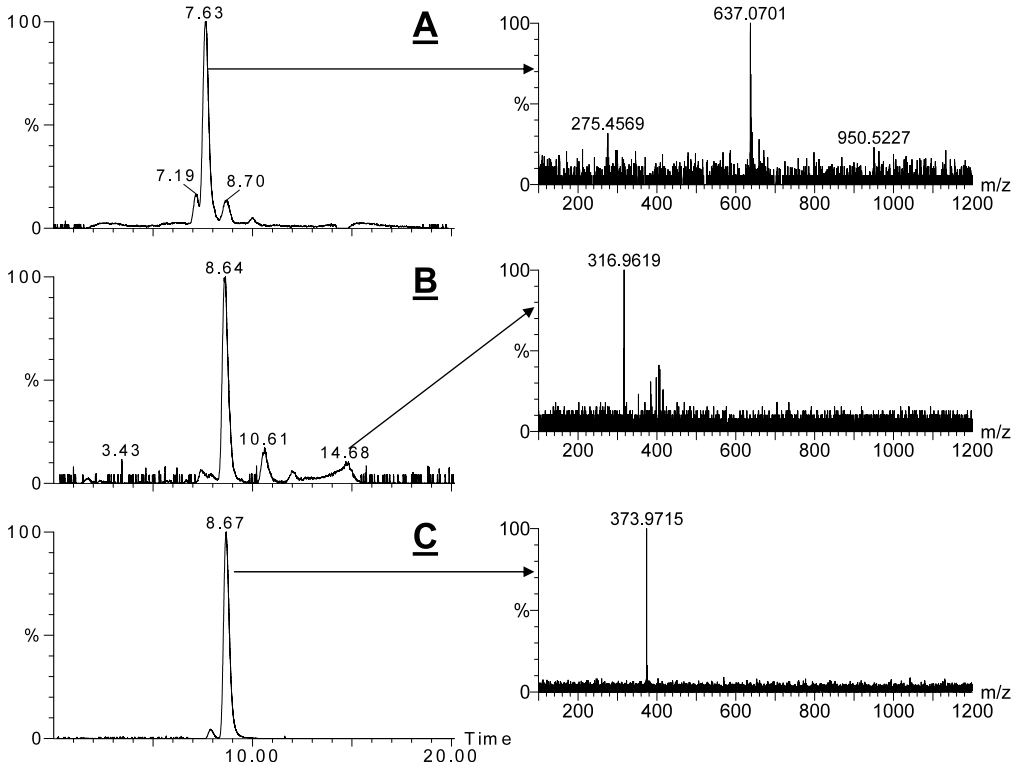

Fig. 2. Radio-LC-MS chromatograms and background subtracted mass spectra (ES−) of a standard Tc-MAG3 labelling reaction mixture. (A) ^{99m}Tc-MAG3 preparation containing a high amount of Tc-MAG3 precomplex. (B) ^{99m}Tc-MAG3 preparation containing Tc-MAG2. (C) ^{99m}Tc-MAG3 preparation.

Table 1
Compounds analyzed with their ion formula, theoretical mass, experimentally determined mass and relative mass error

Compound	Detection mode	Ion formula	Theoretical mass	Experimental mass	Relative mass error (ppm)
Tc-MAG3	ES−	$C_8H_9N_3O_6STc$	373.9274	373.9402	34
Tc-MAG3	ES−	$C_8H_9N_3O_6STc$	373.9274	373.9312	10
Tc-MAG2	ES−	$C_6H_6O_5N_2STc$	316.9059	316.9088	9
TcO_4^-	ES−	TcO_4	162.8859	162.8877	3
Tc-MAG3 precomplex	ES−	$C_{16}H_{22}N_6O_{11}S_2Tc$	636.9850	636.9818	5
MAG3	ES−	$C_8H_{13}N_3O_5S$	262.0503	262.0476	10
Tc-Sestamibi	ES+	$C_{36}H_{66}N_6O_6Tc$	777.4095	777.4089	0.77
$Cu(I)(MIBI)_2$	ES+	$C_{12}H_{22}CuN_2O_2$	289.0933	289.0972	13.4
Tc-ECD	ES+	$C_{12}H_{22}O_5N_2S_2Tc$	437.0021	437.0043	4.8
Tc-ECD	ES−	$C_{12}H_{20}O_5N_2S_2Tc$	434.9876	434.9818	15.2
Tc-ECM	ES−	$C_{10}H_{16}O_5N_2S_2Tc$	406.9563	406.9588	6.1
Tc-EC	ES−	$C_8H_{11}O_5N_2S_2Tc$	378.9250	378.9119	34.6
ECD	ES+	$C_{12}H_{25}O_4N_2S_2$	325.1250	325.1215	10.8
ECD-disulfide	ES+	$C_{12}H_{23}O_4N_2S_2$	323.1094	323.0986	33.4
Sn-ECD	ES+	$C_{12}H_{21}O_4N_2S_2Sn$	440.9958	440.9948	2.3
Sn-EC	ES−	$C_8H_{11}O_4N_2S_2Sn$	382.9186	382.9009	46.2

size-exclusion chromatography [3]. The precomplex was therefore suggested to consist of Tc coordinated by four thiolate groups from different MAG3 molecules in analogy with a precomplex of Tc-DADS (DADS is a diamido dithiol Tc-chelating tetraligand [12]). Due to its instability (conversion to Tc-MAG3) it may not be possible to determine its structure using X-ray diffraction techniques.

We have found that the mass spectrum over the Tc-MAG3 precomplex peak (observed in the radioactivity channel) shows an ion mass of 637 kDa (Fig. 2A). This is in agreement with a complex in which an oxo-technetium core is coordinated by two deprotected MAG3 ligand molecules (Fig. 3). The accurate mass determination supports this structure (relative error of 5 ppm) and the single mass chromatogram (636.709–637.391 kDa) also shows a peak with identical retention time as the peak observed on the radioactivity channel (results not shown).

As the Tc-MAG3 precomplex is more polar than Tc-MAG3, the two carboxyl groups are probably not involved in the coordination of technetium. As thiol groups are known to strongly coordinate technetium, it can be anticipated that the two mercapto groups of the ligand molecules and their adjacent amide nitrogen atoms coordinate technetium as proposed in Fig. 3. However, the exact structure remains speculative.

3.2. Tc-Sestamibi

The radiometric trace of the RP-HPLC analysis of the labelling reaction mixtures of Tc-Sestamibi essentially shows one major peak for both the high and the low technetium concentration preparation

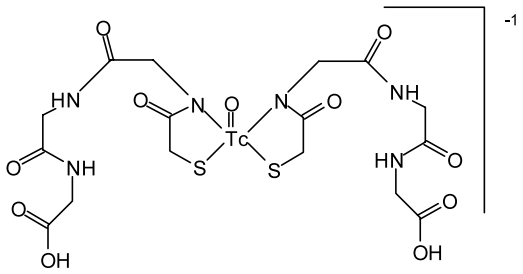

Fig. 3. Proposed structure of Tc-MAG3 precomplex.

(Fig. 4A). The background subtracted mass spectrum over this peak area shows the presence of the molecular ion peak of Tc-Sestamibi (Fig. 4A). The single ion mass chromatogram (Fig. 4B) corresponding to the molecular ion mass of Tc-Sestamibi, shows a peak with identical shape and retention time as the peak observed in the radioactivity channel. Even for the low concentration preparation (60 femtomol of Tc-Sestamibi injected into the radio-LC-MS system), the mass spectra over the Tc-Sestamibi peak area allowed to determine the accurate mass with a small relative error (0.7 ppm at high concentration, 4.6 ppm at low concentration) (Table 1). Compared to other technetium complexes (Tc-ECD, Tc-MAG3, Tc-exametazime) which have been analysed using the same radio-LC-MS system, the molecular ion of Tc-Sestamibi was detected with a 10-fold higher sensitivity.

The amount of Tc-Sestamibi in the low concentration preparation was too small to be visible on the total ion current (TIC) chromatogram (Fig. 4C) which shows a main peak, the mass of which corresponds to a copper adduct with two molecules of 2-methoxyisobutylisonitrile (Fig. 4C). This is surprising, as the package insert of the Cardiolite® labelling vial mentions the presence of a Cu(I) adduct with four molecules of 2-methoxyisobutylisonitrile. Repeated analyses using different ionisation parameters (lower desolvation temperature, lower cone voltage, lower capillary voltage) did not yield the molecular ion of the described $Cu(I)(MIBI)_4$ adduct. Although it cannot be ruled out that this is an artefact of the mass spectrometric analysis, this finding seems to suggest that the $Cu(I)(MIBI)_4$ adduct was converted to $Cu(I)(MIBI)_2$ possibly during lyophilisation of the labelling vial. The TIC chromatogram further shows four peaks (Fig. 4C) the mass spectra of which are not shown. So far, we have not been able to identify these compounds, but mass spectra over the peaks eluting at 8.5 and 9.3 min show the presence of a mass peak exactly matching the molecular ion mass of, respectively, four and six MIBI molecules (data not shown). The impurities are not generated by thermal decomposition during the 10 min heating step as they were found

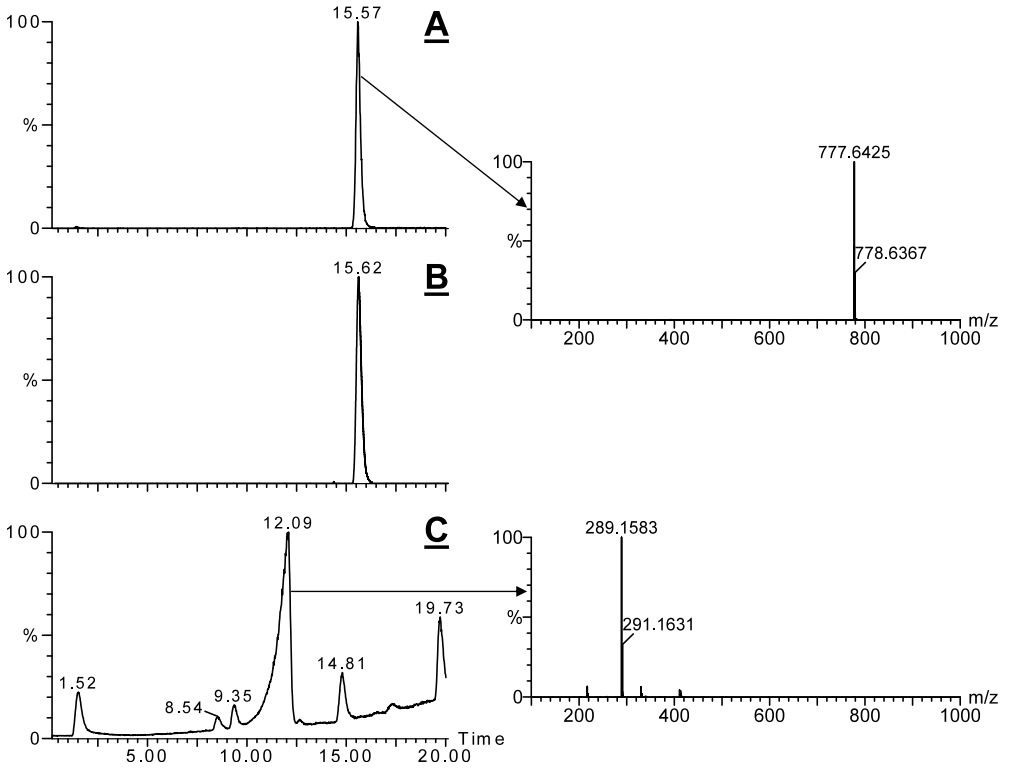

Fig. 4. ^{99m}Tc-Sestamibi preparation. (A) Radio-LC-MS chromatogram and background subtracted mass spectrum (ES+). (B) Single ion mass chromatogram (776.504–779.801 kDa). (C) Total ion current chromatogram and background subtracted mass spectrum (ES+) of Cu(I)(MIBI)$_2$.

already in the labelling reaction mixture analysed prior to the heating step.

It also should be noted that the radiometric trace of the analysis showed that already 90% of technetium-99m in the labelling reaction mixture is present as ^{99m}Tc-Sestamibi prior to the heating step.

3.3. Tc-ECD

The RP-HPLC analysis of a standard preparation of ^{99m}Tc-ECD essentially shows only one peak on the radioactivity channel (Fig. 5B). The background subtracted mass spectra in both ES positive mode (Fig. 5B) and ES negative mode (results not shown) show one peak corresponding to the molecular ion mass of Tc-ECD, besides a smaller peak which corresponds to a fragment of Tc-ECD.

The single ion mass chromatograms recorded in both ES+ mode and ES− mode (data not shown), corresponding to the molecular ion mass of Tc-ECD, show a peak with identical shape and retention time as the peak observed in the radioactivity channel. The accurate mass determined in ES+ and ES− mode is in good agreement with the theoretical mass of Tc-ECD (relative mass error ES+ 4.8 ppm, ES− 15.2 ppm) (Table 1). Detection in ES+ mode was 1.5 times more sensitive than in ES− mode. The ^{99m}Tc-ECD preparation contained a relatively large amount of technetium (800 nmol/ml), but from the signal to noise ratio of the obtained spectrum it can be predicted that preparations containing a 10-fold lower amount of technetium are still amenable for identity confirmation using radio-LC-MS.

The ES+ total ion current trace of a ^{99m}Tc-ECD preparation shows a peak, the mass of which

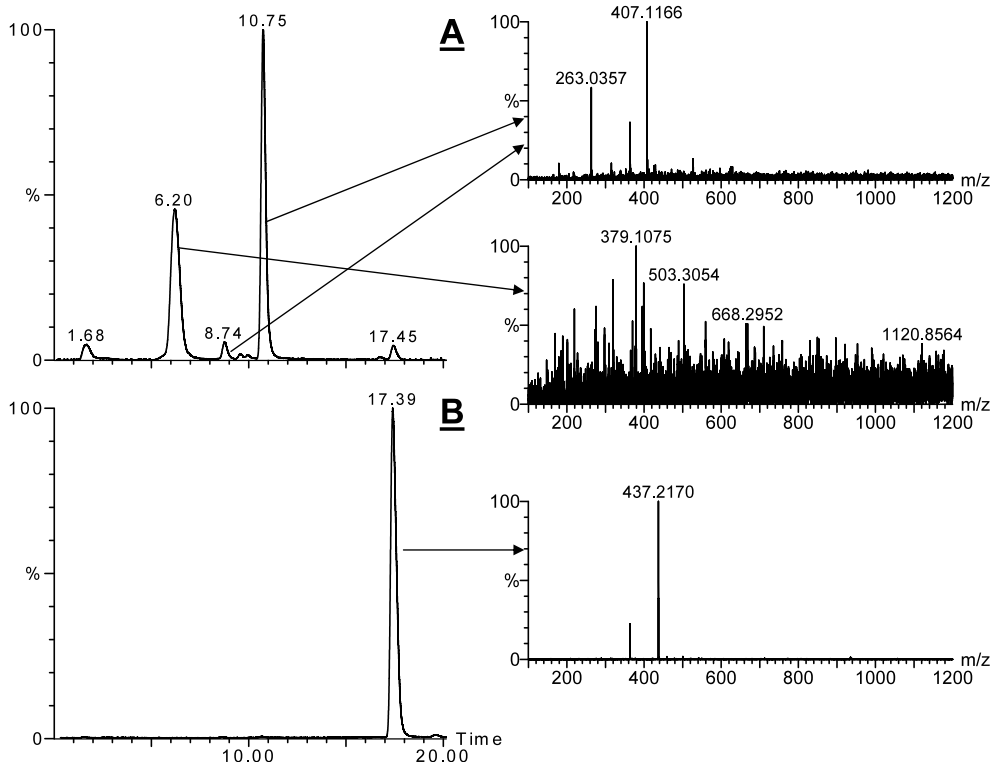

Fig. 5. ^{99m}Tc-ECD. (A) Radio-LC-MS chromatogram and background subtracted mass spectrum (ES+) of ^{99m}Tc-ECM (Rt = 10.75 min) and ^{99m}Tc-EC (Rt = 6.2 min). (B) Radio-LC-MS chromatogram and background subtracted mass spectrum (ES+) of ^{99m}Tc-ECD (Rt = 17.39 min).

corresponds to the unlabelled ligand besides a relatively important peak of its disulfide, generated by oxidation of the two thiols to a disulfide. These compounds were not observed in ES− mode, in contrast to Tc-ECD, in which binding of technetium to the amino group apparently facilitates deprotonation of the latter (data not shown).

Alkaline hydrolysis of ECD or incomplete esterification of ethylene dicysteine yields the monoacid mono-ester (ECM) and the diacid (EC) derivatives. Technetium labelling of ECD labelling kits containing these degradation products would result in the formation of the corresponding complexes Tc-ECM and Tc-EC. ^{99m}Tc-ECM can occur as a pair of two diastereomers with the oxo-technetium core in syn or anti position relative to the residual ester group [13]. We have subjected a Tc-ECD preparation to alkaline hydrolysis in order to obtain relatively large amounts of Tc-ECM and Tc-EC.

The radiometric trace of the radio-LC-MS analysis of the mixture after alkaline hydrolysis (Fig. 5A) shows the presence of three ^{99m}Tc-labelled hydrolysis products, besides a small amount of residual ^{99m}Tc-ECD. Mass spectrometric detection in the ES+ mode could not assign a molecular ion mass to the hydrolysis products that contain a carboxylate group, which probably prevents protonation of the amino group. In ES− mode, the peak with Rt = 10.8 min on the radiometric channel shows a spectrum corresponding to Tc-ECM (Fig. 5A). Another peak (Rt = 8.7 min) shows a spectrum at low intensity also corresponding to the molecular ion of Tc-ECM (probably the other diastereomer of Tc-ECM). The retention time and the relative proportions of the two peaks

on the single mass chromatogram corresponding to the molecular ion mass of Tc-ECM are in good agreement with those on the radiometric channel (data not shown).

The peak eluting at $Rt = 6.2$ min shows a spectrum including a relatively small peak which can be assigned to Tc-EC (Fig. 5A). In the chromatogram obtained on the radiometric channel, the proportion of Tc-EC is nearly equal to that of the largest Tc-ECM peak, but the mass spectrometric response is much weaker. This indicates that Tc-EC, although possessing two carboxylate groups, is less efficiently ionized than its mono-ester derivative.

Close inspection of the mass spectra obtained by analysis of the Tc-ECD preparation after hydrolysis further shows small amounts of probably Sn^{4+}-complexes with ECD and EC, eluting after the corresponding complexes with technetium. By coincidence, the molecular masses of the oxotechnetium(V) core and the ^{116}Sn isotope are identical (data not shown). The presence of such tin complexes may reduce the effective specific activity of technetium-99m labelled receptor tracers that consist of a conjugate of a receptor binding agent with a diamino dithiol Tc-chelate. It may indeed be possible that the corresponding tin complexes have a comparable affinity for the targeted receptor, in which case it may be necessary to purify such ^{99m}Tc-complexes with HPLC prior to biological evaluation or clinical utilization [14,15].

4. Conclusion

The high sensitivity of radio-LC-MS allows the use of this technique for the direct confirmation of the identity of ^{99m}Tc-MAG3, ^{99m}Tc-Sestamibi and ^{99m}Tc-ECD in standard preparations. It further enabled us to provide new data with regard to the structure of the Tc-MAG3 precomplex. The ^{99m}Tc-Sestamibi preparation was found to contain several chemical side products that could not yet be identified. Analysis of a preparation of ^{99m}Tc-ECD subjected to alkaline hydrolysis also identified ^{99m}Tc-EC and two diastereomers of ^{99m}Tc-ECM.

Radio-LC-MS provides structural information useful for both regulatory and research purposes and this analytical technique is likely to become indispensable in both the development of new ^{99m}Tc-radiopharmaceuticals and the confirmation of the identity of ^{99m}Tc-radiopharmaceuticals for clinical use.

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