Technical Report: New IRA-Method for Quality Control of ^{99m}Tc-MAG3

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Introduction

The radiopharmaceutical chemistry group of the Institute of Radiophysics in Lausanne, Switzerland (IRA) has a continuous mandate from the Federal Office of Public Health (FOPH) for the market surveillance of radiopharmaceuticals in Switzerland. As part of this mandate, the labeling with kits for technetium-99m mercaptoacetyl-triglycine (^{99m}Tc -MAG3) has been tested regularly with the method described in the European Pharmacopoe (EP) for radiochemical purity (RCP) testing of ^{99m}Tc-MAG3 injection solutions. The EP method is a combination of HPLC and paper chromatography (PC). Usually a simplified method described in the authorized product information is used by nuclear medicine centers. The IRA reported that the simplified method – a solid phase extraction (SPE) method – tends to indicate lower RCP values than the EP method.
These findings initiated the development of a new quality control method based on Thin Layer Chromatography (TLC). This report summarizes results from several IRA reports, which have been prepared for the FOPH based on specific mandates for the development and validation.

The new method is based on two parallel chromatographic separations on iTLC-SG strips with two different eluents. Eluent 1 is a 60:40% mixture of ethyl acetate (EtAc) and methyl ethyl ketone (MEK), already described in the literature [4 to 6]. Eluent 2 is a 90:10% mixture of ethanol/water. The method is suitable for using a scanner, as well as, a dose calibrator or gamma counter.
The method is able to quantify all relevant impurities: free pertechnetate (^{99m}TcO$_4$), reduced colloidal Technetium (^{99m}TcO$_2$)$_n$, ^{99m}Tc-tartrate (transfer complex), precomplexes (^{99m}Tc-[MAG3]$_x$) and ^{99m}Tc-MAG2. The impurities are quantified in two distinct groups as summed parameters:
Eluent 1: ^{99m}TcO$_4$ and ^{99m}Tc-MAG2 migrate to the top part of the support (impurity group 1), whereas all other compounds stay at the origin.
Eluent 2: (^{99m}TcO$_2$)$_n$, ^{99m}Tc-tartrate and [^{99m}Tc-MAG3]$_x$ stay in the bottom part of the support (impurity group 2), whereas all other compounds move with the solvent front.
For each strip the sum of the targeted impurities is calculated as percentage after measuring both parts of the strip. The total sum of radiochemical impurities is derived by addition of the relative content of the two impurity groups.

The method was validated and compared to the EP method. The validation focused on specificity, accuracy, and robustness of the dual chromatographic method. As supporting information, the linearity and the limits of detection and quantitation of the employed measuring devices are reported. The accuracy was tested on samples produced using Mallinckrodt's TechneScan MAG3 labeling kits and Ultra-TechneKow generators. As starting point of this project, the impurity profile of such samples had been analyzed by HPLC (and PC) before the method developement was initiated. Those results are also presented in this summary.

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Materials and Methods

Analytical Instruments

HPLC radioactivity detectors: RAD Raytest Gabi with NaI probe
TLC scanners: Raytest MiniGita with BGO probe
Dose calibrators: Veenstra VDC-405 with nuclide setting for Tc-99m

Materials

^{99m}Tc-Generator: ULTRATECHNEKOW FM, Mallinckrodt

Mag3-Kit: TechneScan MAG3, Mallinckrodt

TLC strip: iTLC-SG Agilent no AT-SGI0001; PC strip: Whatmann 3MM Chr

HPLC Column: LiChrocart 250-4 LiChrospher 100RP-18, 5μm from Merck.

Solvents: Acetonitrile (ACN), ethyl acetate (EtAc), ethanol (EtOH), methyl ethyl ketone (MEK),
        methanol, and phosphate salts of analysis grade, water of distilled grade.
Chromatography chambers: Glass cylinders with inner diameters of 56 or 59 mm, lid with hook for
        hanging the strip in the chamber

Volumetric measuring devices: Measuring cylinders of 10 ml, Micro pipettes 0.5 – 5 ml, Syringes of 1 ml together with Needles of 22-25 *gauge*.

HPLC and PC Method (*Ph.eur.*)

HPLC Mobile phase: A: 0-10 minutes: Phosphate buffer pH 6.0 / Ethanol, 93:7
B: 10.1-25 minutes: Methanol / Water, 90:10
Flow rate: 1 ml/min

A typical chromatogram is shown below. ^{99m}Tc-tartrate elutes as the first peak followed by small peaks of precomplexes at around 2 min. ^{99m}TcO4 (at 2.9 min) and ^{99m}Tc-MAG2 (at 13.9 min) are the main impurities. For more details see the Pharm. Eur. monograph 1372 .

The PC method was also performed according to the EP monograph (eluent: ACN:water, 60:40).
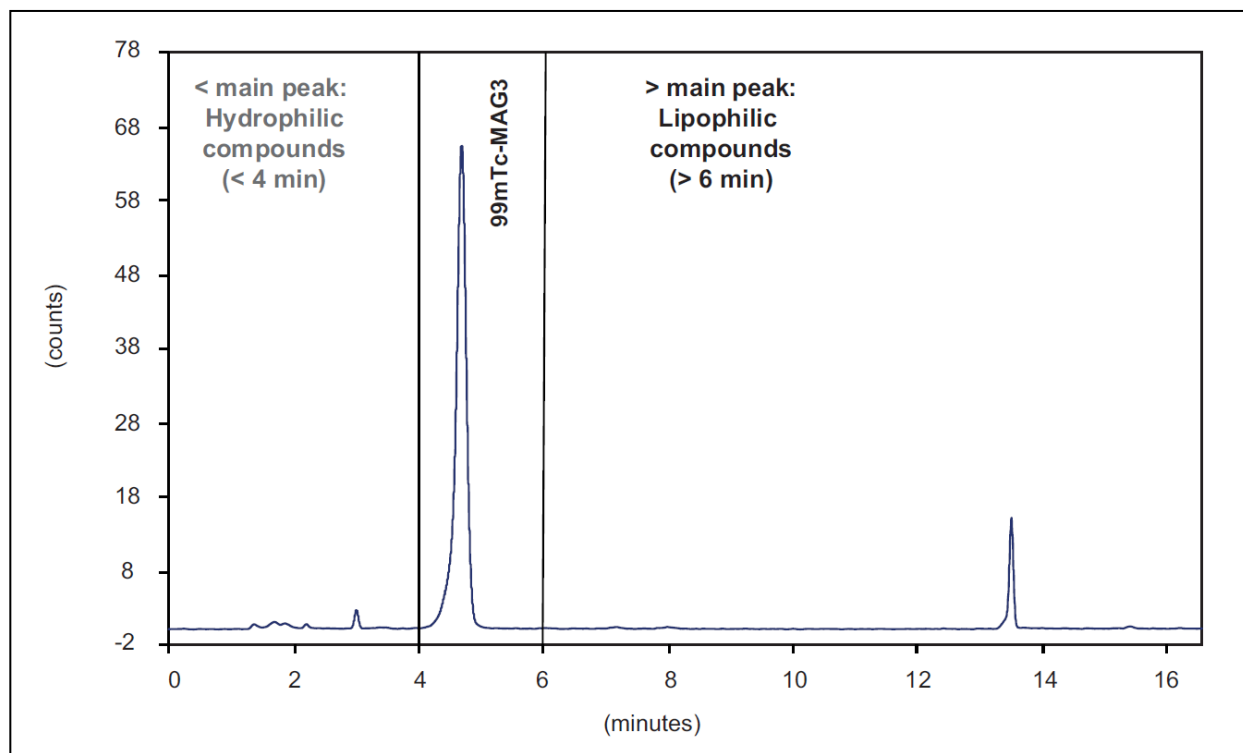
2-strip TLC Method (IRA)

Strips: iTLC-SG sheets are cut 2 - 2.5 cm wide x 10 cm long, and marked with solid pencil lines at 1.5 cm (Start) and 8.5 cm (Stop) from the bottom of the stripe. If a dose calibrator is used, an additional dotted line at 4.5 cm from the start as cutting mark between bottom and top part is required. The strips are conditioned for 1 hour at 110 °C. If a desiccator with silica gel is used, the strips can be stored for up to 2 weeks.

Eluents: Eluent 1 is a mixture of 6 ml ethyl acetate and 4 ml methyl ethyl ketone; eluent 2 is a mixture of 9 ml ethanol and 1 ml water. The components are filled into the chromatography tanks and mixed by gentle shaking. After covering the tanks with their lids, at least 10 minutes are allowed for equilibration.

New IRA-Method for Quality Control ^{99m}Tc-MAG3

<u>Sample application</u>: Approximately 10 μl of ^{99m}Tc-MAG3 sample on the start lines of strips 1 and 2. Using a 1 ml syringe with a 22 to 25 gauge needle this is equal to one drop. The applied aliquots are not dried before the strips are installed in the chromatographic tanks 1 and 2.

<u>Development</u>: Strip 1 is placed in in the tank containing eluent 1 and strip 2 in the tank containing eluent 2. The tanks are covered again. The migration time up to the stop mark is ~ 4 minutes for strip 1 and 13-15 minutes for strip 2. After the stop mark is reached, the strips are removed and allowed to dry.

<u>Quantification</u>: If a dose calibrator or gamma counter is used, cut the strip at the dotted line (4.5 cm from Start) and measure the activity of each part. If a scanner is used the regions of interest are set accordingly.

The radiochemical purity (RCP) of ^{99m}Tc-MAG3 (%) is calculated as described below:

$$\% \text{ impurity strip 1:} \frac{\text{Activity Top part} \times 100}{\text{Activity both parts}} \qquad \% \text{ impurity strip 2:} \frac{\text{Activity Bottom part} \times 100}{\text{Activity both parts}}$$

RCP [%] = 100 – (% impurities of strip 1 and 2)

Below typical chromatograms for a sample of ^{99m}Tc-MAG3 are shown for strip 1 (upper diagram) and strip 2 (lower diagram). The blue dotted line indicates the cutting line at 4.5 cm.
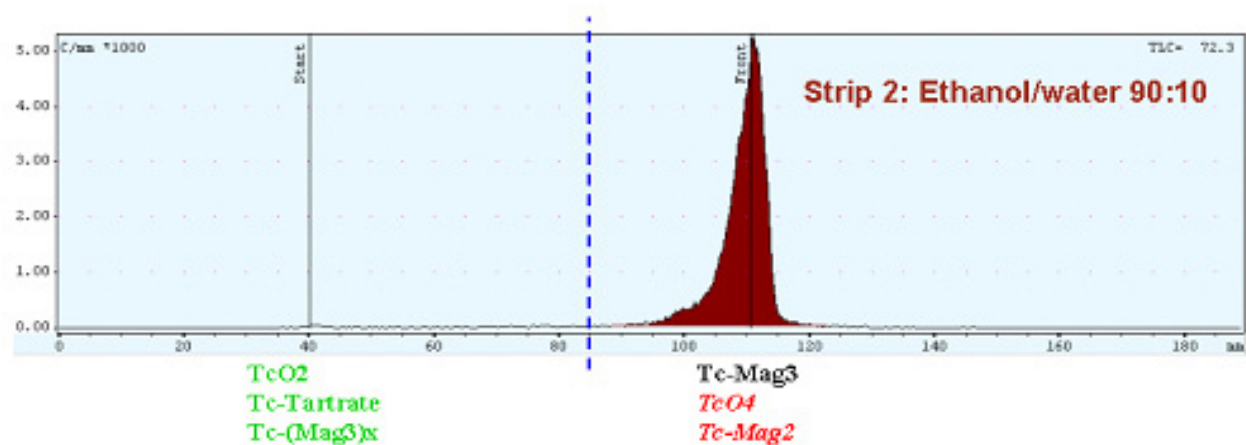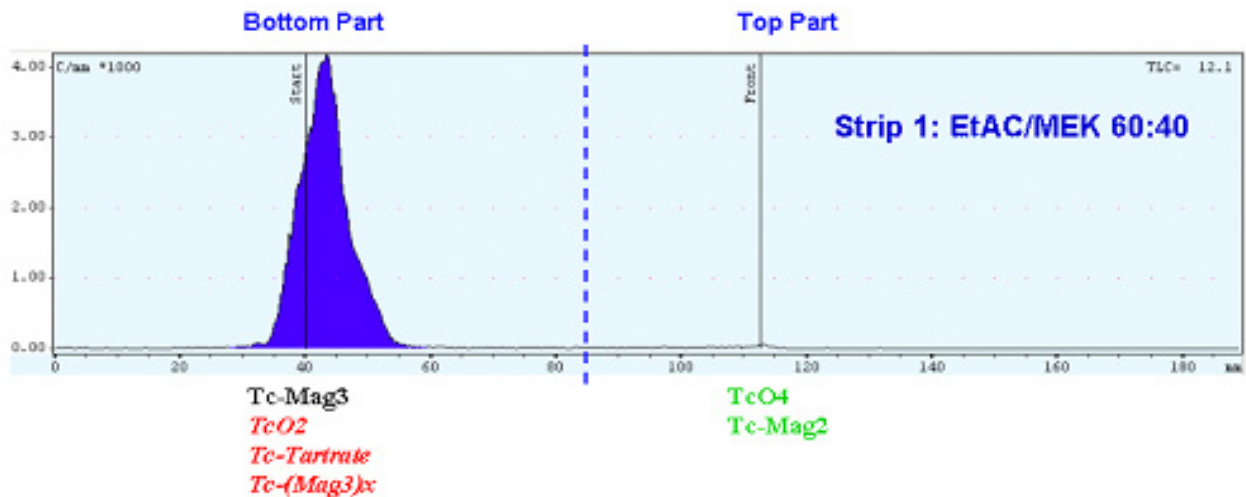
Radiochemical Impurity Profile of ^{99m}TcMAG3

At the beginning of this project, the radiochemical impurity profile of ^{99m}TcMAG3 solutions was determined using the combined HPLC/PC method of the EP in order to evaluate the significance of the potential impurities. The TechneScan kits used for this experiments had been 6 months beyond their shelf life to mimic a typical situation were increased impurities are to be expected. Table 1 shows results for three batches labelled at 100°C (regular method) and two batches labelled at only 75°C to mimic a typical deviation causing more radiochemical impurities. The second column reports the time span between end of labeling and analysis, which was varied to cover the potential influence of the shelf life of the labelled product on the impurity profile. Every batch was sampled at two different times. ^{99m}Tc-Tartrate and ^{99m}Tc-pre-complexes are reported as summed value of the respective HPLC-peaks in the third column. The highest single impurity value at the first sampling time of each experiment is shown in bold style.

Table 1: Impurity profiles of ^{99m}Tc-MAG3 at different reaction temperatures and sampling times

		HPLC results			PC result	HPLC+PC
Labeling Temperature	Time after labeling [hh:mm]	Tc-Tartrate & Tc(Mag3)$_x$ [%]	TcO$_4$ [%]	TcMAG2 [%]	(TcO$_2$)$_n$ [%]	Total [%]
100 °C (normal condition)	00:34	1.0	0.3	**1.7**	0.5	3.5
	04:30	<0.1	1.7	1.0	0.4	3.1
	00:49	0.5	0.5	**1.6**	0.4	3.1
	04:27	0.9	0.8	1.3	0.3	3.4
	02:30	1.4	0.5	**2.0**	0.3	4.3
	05:44	0.8	0.9	1.8	0.4	3.9
75 °C (to increase impurities)	02:37	**1.9**	0.8	1.4	0.8	5.3
	06:20	1.4	1.4	1.5	0.6	4.8
	04:05	**2.3**	1.4	2.2	0.8	6.7
	07:36	0.8	2.2	2.6	0.8	3.3

Under normal labelling conditions, ^{99m}Tc-MAG2 was the major impurity, whereas ^{99m}Tc-Tartrate (together with pre-complexes) was the major after labelling at 75°C.

The total of impurities was higher when the labeling temperature was only 75 °C. This was mainly due to a higher content of ^{99m}Tc-Tartrate (together with pre-complexes) and ^{99m}TcO4 (slower reaction at lower temperature), whereas ^{99m}Tc-MAG2 seems not to be influenced by the temperature.

At the second sampling time, the total of impurities was about 10% lower than at the first, in all but one case. Looking at the single impurities, ^{99m}TcO4 increases and ^{99m}Tc-Tartrate decreases in all cases. The early samples are more important, since the quality control is usually soon after the labeling.

In consequence, also a simple QC method must be able to account for the contributions of ^{99m}Tc-Tartrate and ^{99m}Tc-MAG2 to the radiochemical impurities in addition to ^{99m}TcO4 and $(^{99m}$TcO$_2)_n$.

Specificity of the new 2-strip TLC method

Specificity for free pertechnetate (^{99m}TcO$_4$)

Standard preparation: Saline eluate from ^{99m}Tc-generator

Results: With both eluents, ^{99m}TcO$_4$ migrates with the solvent front (see the following chromatograms). ^{99m}TcO$_4$ will be separated from ^{99m}Tc-MAG3 on strip 1.

Strip 1 (EtAc/MEK 60:40)

Strip 2 (EtOH/H$_2$O 90:10)
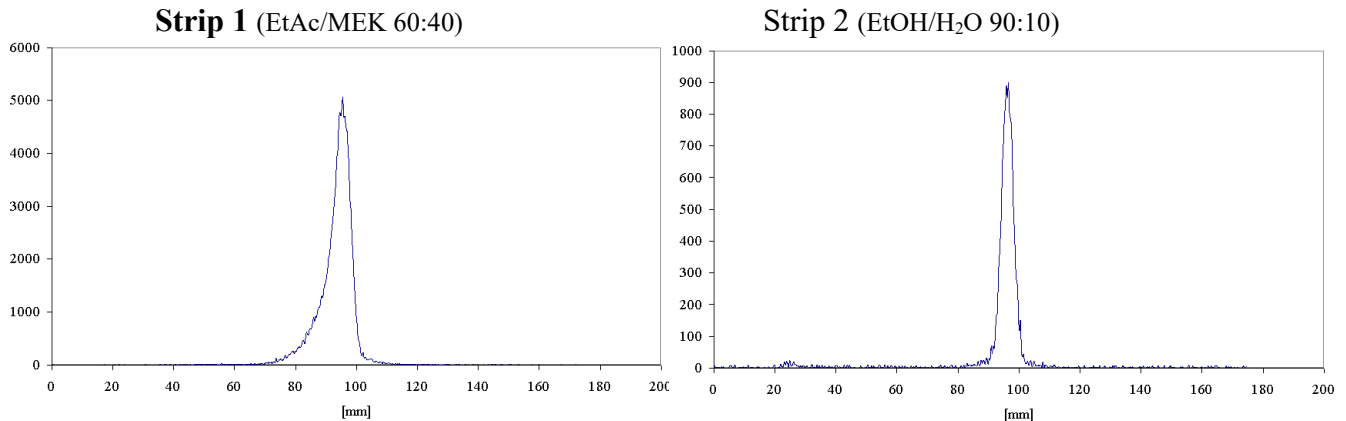
Specificity for reduced colloidal technetium (^{99m}TcO$_2$)$_n$

Standard preparation: To about 500 MBq of saline generator eluate 25 μl of a tin chloride solution (1.7 mg/ml) was added and slightly shaken for a few minutes

Results: Reduced colloidal technetium stays at the start with both eluents. (^{99m}TcO$_2$)$_n$ will be separated from ^{99m}Tc-MAG3 on strip 2.

Strip 1 (EtAc/MEK 60:40)

Strip 2 (EtOH/H$_2$O 90:10)
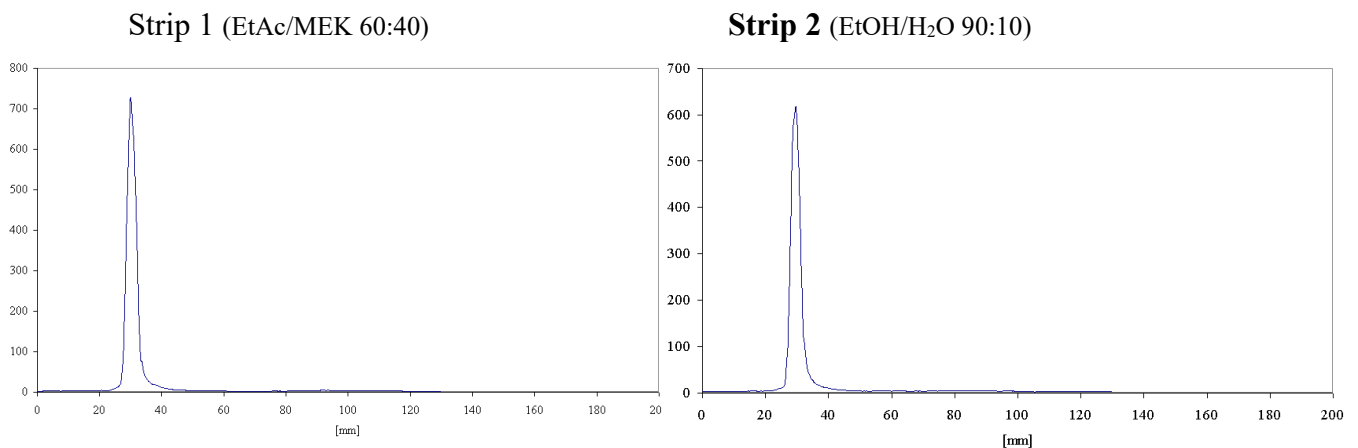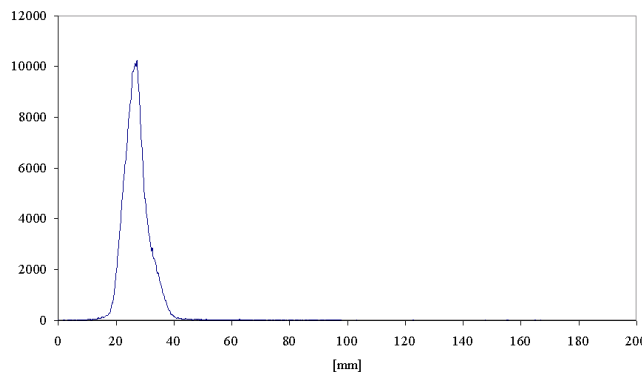
Specificity for ^{99m}Tc-tartrate

Standard Preparation: 0.4 g of Na-K tartrate were dissolved in 10 ml of MilliQ water (tartrate solution), followed by adding 25 µl of tin chloride solution (1.7 mg/ml) and ca. 500 MBq ^{99m}TcO$_4$.

HPLC results: After a few minutes of reaction time, the solution was analyzed by HPLC to confirm the quantitative formation of ^{99m}Tc-tartrate (see blue trace in the following chromatogram). Afterwards an aliquote of the solution was heated for a few minutes and again analyzed (see red trace). The latter experiment demonstrates the weak stability of this transfer complex.
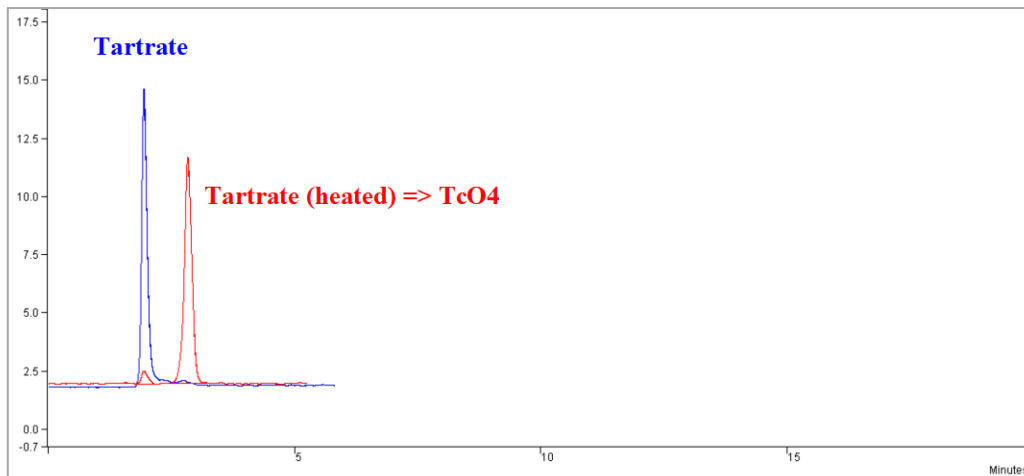

TLC Results: ^{99m}Tc-tartrate stays in the lower part of both strips and will be separated from the ^{99m}Tc-MAG3 on strip 2. The minor peak at 100 mm on strip 2 is due to ^{99m}TcO$_4$ which must have been released from the weak tartrate complex in the standard solution. Since the composition in this standard solution deviates from ^{99m}Tc-MAG3 labelling solutions, it stays unknown if a minor release would also occur in such samples. However, since ^{99m}Tc-tartrate will be only present in small amounts (less than 5%), a minor artifical release from this small pool during the TLC chromatography will not be significant for the quantification of the radiochemical purity. This assumption is confirmed by the accuracy results (see there).

Strip 1 (EtAc/MEK 60:40)   **Strip 2** (EtOH/H$_2$O 90:10)
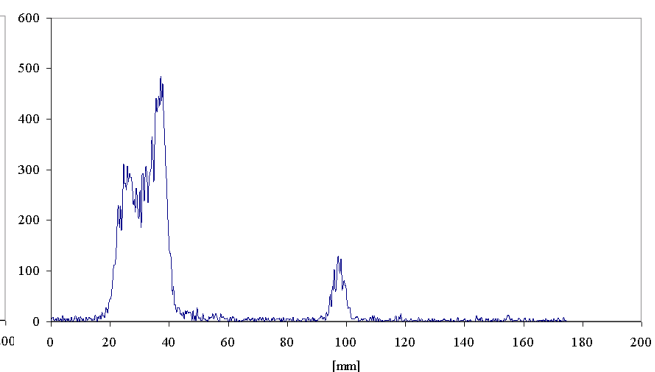
Specificity for ^{99m}Tc-MAG2

Standard Preparation: 5 mg of S-benzyl-Mag2 were dissolved in 5 ml of 1-mM-NaOH by heating at 80 °C for 5 minutes. 1 ml of this solution was added to a glass vial with a rubber septum. The vial had been purged with nitrogen before. Subsequently, 1 ml of tartrate solution (4.7 g/ml), 25 µl of tin chloride solution (1.7 mg/ml) and about 500 MBq of saline generator eluate were added to the same vial. After a few minutes of reaction time, the final solution was analyzed by HPLC to confirm the formation of ^{99m}Tc-MAG2.

HPLC Results: The major peak at about 13.5 min confirms the almost quantitative formation of the ^{99m}Tc-MAG2 complex. Only a small amount of free ^{99m}TcO4 was detected at about 2.5 min.
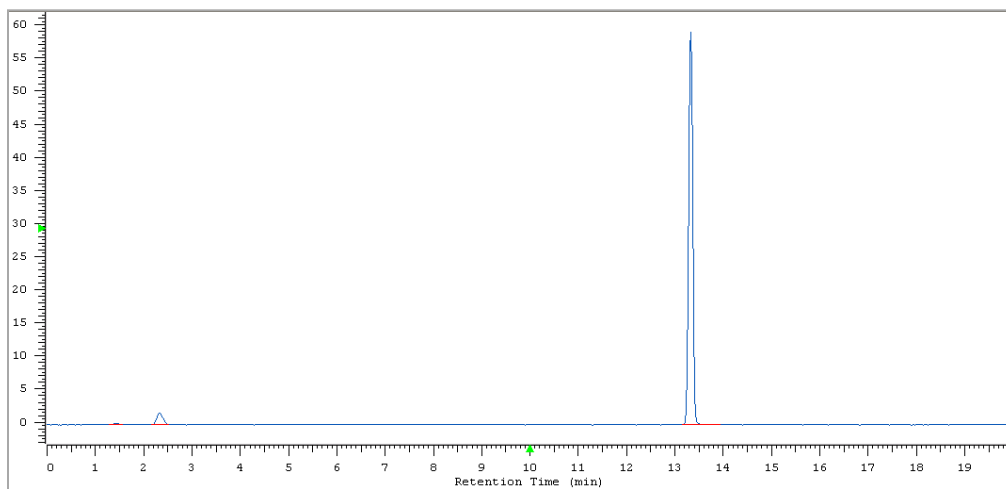
TLC Results: ^{99m}Tc-MAG2 migrates to the front of both strips and can be quantified as impurity on the upper part of strip 1.

Strip 1 (EtAc/MEK 60:40)

Strip 2 (EtOH/H$_2$O 90:10)
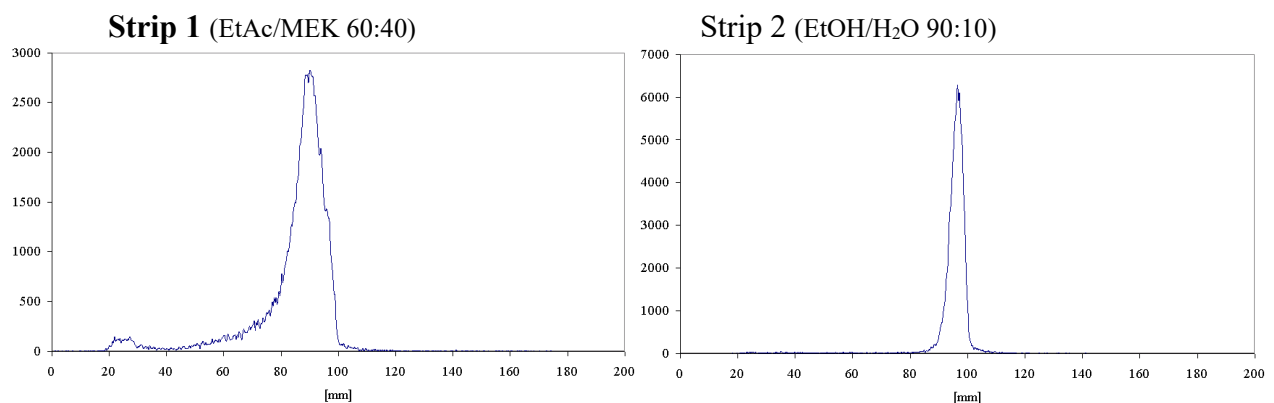
Specificity for Pre-Complexes (^{99m}Tc-(Mag3) $_x$)

Standard Preparation: Pre-complexes are formed transiently during labeling and are not available as isolated compounds. In order to artificially increase the amount of these compounds, a labeling with a TechneScan MAG3 kit was carried out at 75 °C. For direct comparison, an aliquot of the same starting solution was heated at 100 °C.

TLC Results: The following overlay of both chromatograms with eluent 2 show more counts in the bottom part for the sample which has been heated at 75 °C compared to the sample heated at 100 °C.

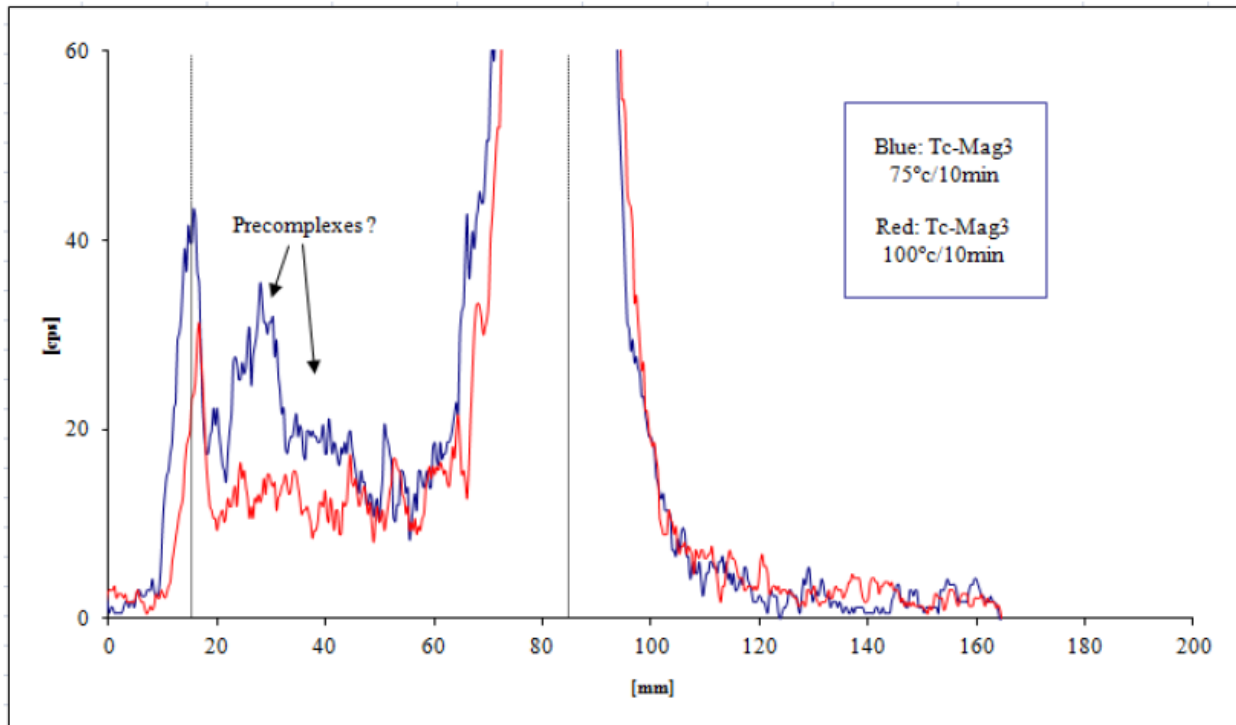*Figure 13: separation by thin-layer chromatography: [Tc-(Mag3)x]*

Peaks between 20 and 40 mm are likely to be stemming from pre-complexes, since it was shown by HPLC analysis that their content increases slightly at lower reaction temperatures. In such a case, pre-complexes would be quantified as impurities in the lower part of strip 2.

Accuracy

Detection Accuracy and Sensitivity of TLC Scanner and Dose Calibrator

A deposited activity of about 1 MBq in 10 µl is the target value according to the TechneScan MAG3 SPC. Therefore, the detection accuracy of the equipment in the required range of activities was tested using a generator eluate diluted to a volume activity of about 1 MBq in 10 µl of Tc-99m.

Six paired samples of activities corresponding to a ratio of 97% to 3%, as well as six pairs corresponding to a ratio of 94% to 6% were deposited on TLC support samples (strips of 2 x 3 cm). The amount of each deposit was determined by weighing in order to yield the best possible accuracy of the reference value (1st column in the table shown below).

For testing the TLC scanner, the low and high activity spots were positioned on the measuring plate with a distance of 7 cm in order to simulate the influence of the high activity on the low activity quantification due to the potential increase in background signals.

From the measured counts (scanner), respectively, activities (dose calibrator) the percentage of the lower activity spot - mimicking the impurity - was calculated.

Table 2: Detection accuracy at 3% and 6% levels of impurity at a sample activity concentration of 1 MBq/µl and a sample application of 10 µl.

Reference Value [%]	TLC SCANNER Results		DOSE CALIBRATOR Results	
	[%]	Difference to Reference [%]	[%]	Difference to Reference [%]
3.1	3.4	0.3	3.2	0.1
3.1	3.3	0.2	3.9	0.8
3.0	3.1	0.1	3.0	0.0
3.0	3.3	0.3	3.1	0.1
3.1	3.5	0.4	3.1	0.0
3.1	3.2	0.1	3.2	0.1
6.1	6.8	0.7	6.6	0.5
6.1	6.4	0.3	6.9	0.8
6.0	6.3	0.3	6.4	0.4
6.0	6.5	0.5	6.1	0.1
6.3	6.8	0.5	6.4	0.1
6.3	6.3	0.0	6.3	0.0

For both instruments, the deviation between reference and measured value was always below 1% and all deviations were on the conservative side (overestimation of impurity). This confirms the suitability of our systems to test the accuracy of the method in the most critical range of 94-97% radiochemical purity at the target activity concentration. The excellent accuracy of the detection confirms also that the linearity and sensitivity (quantitation limit) of both systems were sufficient for the purpose of validating the accuracy of the TLC method.

Accuracy of the new 2-strip TLC method

On three consecutive days one batch of ^{99m}Tc-MAG3 was prepared and analyzed with the new 2-strip TLC method as well as the combined EP method (HPLC + PC). Six TLC procedures were performed for each sample; three of them were quantified with the scanner and the other three with the dose calibrator. For the three replicates, three different batches of iTLC sheets were used, because the experiments were also part of the robustness validation. Since no significant differences were observed between the iTLC batches, it is justified to treat these data sets as replicates for the accuracy validation. Timewise, sampling and analysis of all seven samples (6 TLC, 1 HPLC) was as closely together as possible

A planned variation between the three days was the duration between preparation and starting the analysis of approximately 1, 2 and 8.5 hours, in order to compare the method also at potentially different levels of impurities. We assumed that a longer waiting time would lead to some the accumulation of impurities. The results are shown in the following table.

Table 3: *Comparision of RCP values as determined by the new TLC method with quantification by scanner or dose calibrator against the EP method as reference*

Sample (storage time before analysis)	Radiochemical Purity (%)		
	EP method	Two-strip TLC method (SD of n=3)	
	HPLC + PC	Scanner	Dose calibrator
1 hr	96.9	96.6 ± 0.3	96.6 ± 0.1
2 hr	97.0	96.6 ± 0.1	97.1 ± 0.3
8.5 hr	96.0	95.0 ± 0.2	96.1 ± 0.2

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Whereas no substantial difference was found in the RCP between 1 and 2 hours of storage, the sample at 8.5 hours had a slightly lower RCP value. Compared to the EP method the values obtained by the new TLC method are very similar, independent of the radioactivity detection device used. Out of 18 single results only one showed a difference of more than 1% (1.2%) to the reference values. The standard variations were comparable for both detection methods.

On average over the nine tests each, the dose calibrator values were closer to the EP method than the ones from the scanner (0.06% vs. -0.53%). The scanner seems to overestimate the impurities slightly, probably due to counts for the impurity peak coming from the nearby product activity.

Overall, the accuracy of the new 2-strip TLC method for determining the radiochemical purity is excellent and the method is successfully validated in this respect.

Robustness of the new 2-strip TLC method

Eluent Composition

Undeliberate variations in eluent composition may modify the separation between the compounds. The solvent mixtures are prepared with 10 ml graduated cylinders which are graduated every 0.1 ml. Variations of ± 0.5 ml for Eluent 1 and ± 0.3 ml for Eluent 2 were tested for their influence on the results of radiochemical purity.

In order to study the influence with higher sensitivity, the labeling was performed at only 70 °C to yield a somewhat higher impurity level than normal. Three serial replicates were performed; they differ in the time span after end of labelling and starting the analysis (Dt label).

Table 4: Variation of Eluent 1 and its influence on the sum of impurities

Sample	Labeling	Time	Dt label [h]	Strip 1: EtAc/MEK 55:45 [%]	60:40 [%]	65:35 [%]	Average [%]	STD k=2 [%]
Tc-Mag3	70°c/10min	T1	2:31	6.5	6.1	6.0	**6.2**	0.2
		T2	2:52	6.6	6.5	6.0	**6.4**	0.3
		T3	3:13	6.7	6.4	6.6	**6.6**	0.1
		Average		6.6	6.3	6.2	**6.4**	
		STD *(k=2)*		0.1	0.2	0.3	**0.3**	

The sum of impurities found with 40% versus 35% MEK do not significantly differ (6.3 vs. 6.2%). At 45%, a slightly higher impurity level was determined (6.6 vs. 6.3%). The difference is at the border of being statistically significant.

Table 5: Variation of Eluent 2 and its influence on the sum of impurities

Sample	Labeling	Time	Dt label	Strip 2: EtOH/H2O 87:13 [%]	90:10 [%]	93:7 [%]	Average [%]	STD k=2 [%]
Tc-Mag3	70°c/10min	T1	1:07	5.0	5.3	5.5	**5.3**	0.3
		T2	1:20	4.9	5.1	5.3	**5.1**	0.2
		T3	2:14	4.6	5.5	5.0	**5.0**	0.4
		Average		4.8	5.3	5.3	**5.1**	
		STD *(k=2)*		0.2	0.2	0.3	**0.3**	

The results for 10% and 7% H_2O do not significantly differ (5.3 vs. 5.3%). At 13% H_2O, a slightly lower impurity level was determined (4.8 vs. 5.3%). The difference of 0.5% is just statistically significant with a *k* value of 2.

The results indicate only a very moderate influence of variations in the eluent composition which may be expected in routine use. The use of a micro pipette for the addition of the volume of the

minor solvent (as recommended in our method) should considerably reduce the risk of a significant error.

Deposited Volume

The volume of sample deposited on the chromatographic strip may modify the separation between the compounds. Too large volumes can greatly widen the peaks and reduce the resolution between the compounds. The presence of a large amount of water (the main medium of a Tc-Mag3 label) can also interfere with separation.
In order to have a higher amount of impurities, a waiting period of 15 minutes was introduced before applying 100 °C, in case of the experiment for strip 1.

The following chromatogram overlays for both strips show that the influence of deposited volumes of 10, 15 and 20 µl are moderate. The influence was greater on Strip No. 1, where the majority of the deposited activity is not migrating. Increasing the deposited volume widens the
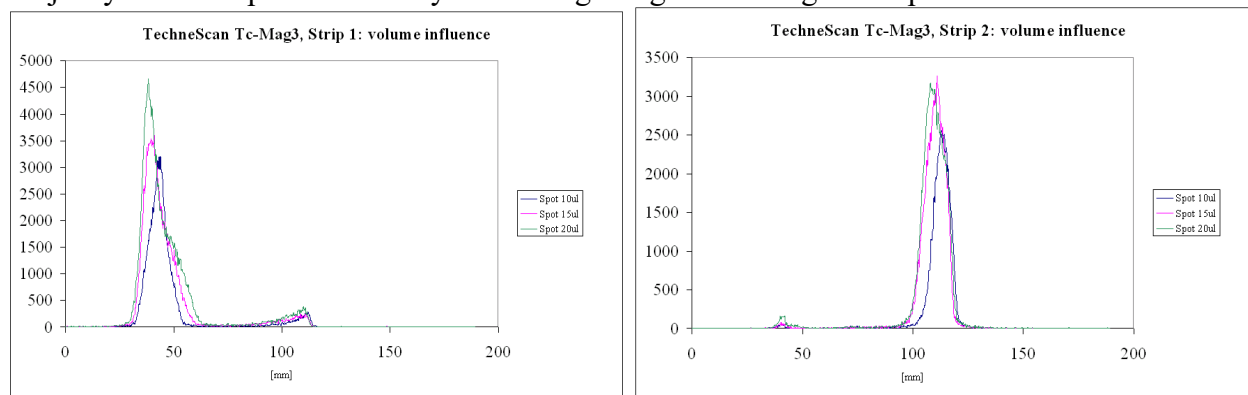
base of the ^{99m}Tc-MAG3 peak. The influence on strip 1 was tested in three replicates (Table 6). The impurity results on strip 2 at 10, 15, and 20 µl were determined only once (Table 7).

Table 6: Variation of volume deposited, Strip 1

RPh	Labelling	Strip	Chromato	Spot volume			Average	STD
				10 ul [%]	15 ul [%]	20 ul [%]	[%]	k=2 [%]
Tc-Mag3	23°c/15 min	1	1	7.8	7.0	7.4	7.4	0.8
	then		2	7.5	6.6	6.8	7.0	0.9
	100°c/10min		3	7.3	7.0	6.6	7.0	0.8
			Average	7.5	6.9	6.9	7.1	
			STD (k=2)	0.5	0.5	0.9	0.8	

The differences in the determined RCP value are not statistically significant at $k = 2$. However, due to the observed peak broadening it is advisable to limit the sample volume to 10 µl.

Table 7: Variation of volume deposited, Strip 2

RPh	Labelling	Strip	Chromato	Spot volume			Average	STD $k=2$
				10 ul [%]	15 ul [%]	20 ul [%]	[%]	[%]
Tc-Mag3		2	1	2.0	1.8	2.1	**2.0**	0.4

The results show no significant influence of the deposited volumes.

Batch of the iTLC-SG material

Differences between batches of chromatographic material may influence the separation.
Three batches of iTLC-SG produced in 2013, 2015 and 2016 were compared in parallel on three consecutive days. The results shown in Table 8 and 9 are the mean ± standard deviation from serial triplicates from the one labeling batch of the day. The TLC scanner was used for quantification of the impurity fraction.

Table 8: Variation of iTLC-SG Batch for Strip 1

Trial Date	Radiochemical Impurity fraction (%)		
	Batch 2013	Batch 2015	Batch 2016
27.04.2016	1.7 ± 0.4	1.4 ± 0.3	1.4 ± 0.6
28.04.2016	1.4 ± 0.3	1.3 ± 0.2	1.5 ± 0.4
29.04.2016	2.8 ± 0.2	2.6 ± 0.2	2.5 ± 0.1
Mean	**2.0 ± 0.6**	**1.8 ± 0.6**	**1.8 ± 0.5**

Table 9: Variation of iTLC-SG Batch for Strip 2

Trial Date	Radiochemical Impurity fraction (%)		
	Batch 2013	Batch 2015	Batch 2016
27.04.2016	1.7 ± 1.0	1.8 ± 0.4	1.8 ± 0.6
28.04.2016	2.0 ± 0.4	1.8 ± 0.5	1.8 ± 0.4
29.04.2016	1.3 ± 0.3	1.3 ± 0.1	1.2 ± 0.2
Mean	**1.7 ± 0.3**	**1.6 ± 0.2**	**1.6 ± 0.3**

According to the results shown above, the batches from 2015 and 2016 are more similar. This may be due to an aging effect on the batch from 2013. However, comparing the differences between the means and considering the respective standard deviations it can be concluded that the differences are not significant.

Conclusion

The 2-strip thin layer chromatography method, comprising 2 separations on the same type of support with 2 different solvent mixtures, was successfully validated for the determination of the radiochemical purity of Mallinckrodt TechneScan MAG3 (technetium mertiatide).

The method has been found to be specific for the main impurities of ⁹⁹ᵐTc-MAG3 in the sense that all impurities can be separated from ⁹⁹ᵐTc-MAG3 by one of the two TLC separations, without being counted twice as impurity.

The accuracy was validated by comparing the results for the radiochemical purity the good results with the reference method from the European Pharmacopoe

The robustness of the method against variations of eluent composition, volumes deposited, and iTLC-SG batches seems sufficient for routine use.

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