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^{68}Ge content quality control of ^{68}Ge/^{68}Ga-generator eluates and ^{68}Ga radiopharmaceuticals – A protocol for determining the ^{68}Ge content using thin-layer chromatography

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HIGHLIGHTS

- ^{68}Ga is analytically separated from ^{68}Ge using TLC.
- ^{68}Ge-breakthrough can be determined in ^{68}Ga-eluate and radiopharmaceutical.
- ^{68}Ge breakthrough determination takes place within 1 h.

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ABSTRACT

^{68}Ge breakthrough from a ^{68}Ge/^{68}Ga-generator appears to be one of the most critical parameters for the routine clinical application of this generator and ^{68}Ga-radiopharmaceuticals. We report a TLC-based (thin-layer chromatography) protocol which allows the ^{68}Ge breakthrough of a generator to be determined within 1 h post-initial elution. The protocol can also be adapted to allow the ^{68}Ge content of a ^{68}Ga-radiopharmaceutical preparation to be determined prior to in vivo application.

1. Introduction

Commercially available ^{68}Ge/^{68}Ga-generators offer initial activities of 0.74 (20 mCi), 1.11 (30 mCi), 1.85 (50 mCi) or more GBq of ^{68}Ge. During the first few weeks of use, the ^{68}Ga elution yield of these generators is typically 60–90%, which includes ^{68}Ge breakthrough between 10^{-2}% and 10^{-4}% (Roesch and Riss, 2010; Loktionova et al., 2011). The breakthrough refers to the actual ^{68}Ge content of the eluate, and is reported as the ^{68}Ge activity in the eluate relative to that on the generator column (other values express this as the co-eluted ^{68}Ge activity relative to ^{68}Ga in the eluate (Breeman et al., 2011)). Many consider the problem of ^{68}Ge-breakthrough to be one of the more significant challenges to the wider clinical acceptance and application of ^{68}Ga and its generator. ^{68}Ge-breakthrough is a sensitive parameter for ^{68}Ga-radiopharmaceuticals considering the quality control requirements governing their routine clinical application (Breeman and Verbruggen, 2007; Breeman et al., 2011). A recent report by Velikyan et al. proposed that the ^{68}Ge content in a ^{68}Ga-radiopharmaceutical is already below the toxic level for humans (Velikyan et al., 2013). In spite of this, current European Pharmacopeia Standards stipulate that the ^{68}Ge content in generator eluate should not exceed 0.001% (European Pharmacopeia, 2013). Therefore, not only must ^{68}Ge be removed, but a method is required to quantify the ^{68}Ge content of a ^{68}Ga-radiopharmaceutical.

^{68}Ge decays exclusively via electron capture and is therefore only detectable (using radiochemical means) indirectly by the positron emission of its daughter nuclide, ^{68}Ga. However, using this method it is not possible to detect ^{68}Ge in the presence of an excess of ^{68}Ga. Recently, we reported a protocol that allowed the ^{68}Ge breakthrough to be isolated online, through the use of strong cation-exchanger resins, and quantified via the decay of its daughter radionuclide, ^{68}Ga (Eppard et al., 2013). Using this protocol it was possible to determine the ^{68}Ge-breakthrough within one (or 1\2) half-life of ^{68}Ga after the initial elution.

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For a "fresh" 1.11 GBq (30 mCi) generator, the ^{68}Ge activity in the eluate is between 111.00 and 1.11 kBq (3000–30 nCi) (for breakthrough levels of 10^{-2}–10^{-4}%) within 0.67–1.00 GBq (18–27 mCi) of ^{68}Ga. The eluted ^{68}Ga decay according to Eq. (1) based on its half-life ($t_{1/2}$ of ^{68}Ga=67.71 min) is given as follows:

$$A^t_{Ga} = A^0_{Ga}(e^{-\lambda t}) \quad (1)$$

Using $\lambda = (\ln 2)/(t_{1/2})$ leads to

$$\ln(A^t_{Ga}) = \ln(A^0_{Ga}) - \frac{\ln 2}{t_{1/2}}t \quad (2)$$

$$\approx \ln(A^0_{Ga}) - 0.01t \quad (3)$$

where $A^0_{Ga} = {}^{68}$Ga activity immediately after elution and $A^t_{Ga} = {}^{68}$Ga activity at time t (in minutes) after elution, and $\lambda = (\ln 2)/t_{1/2}$.

The total ^{68}Ga activity in the generator eluate is represented by both the eluted ^{68}Ga decay and ^{68}Ga generation from the co-eluted ^{68}Ge. Thus, the equation is applied as follows:

$$A^t_{Ga} = A^0_{Ga}(e^{-\lambda_{Ga}t}) + \frac{\lambda_{Ga}}{\lambda_{Ga}-\lambda_{Ge}}A^0_{Ge}(e^{-\lambda_{Ge}t} - e^{-\lambda_{Ga}t}) \quad (4)$$

where $A^0_{Ge} = {}^{68}$Ge activity co-eluted (=breakthrough), $A^t_{Ga} = {}^{68}$Ga activity at time t after elution.

If there is no ^{68}Ga present ($A^0_{Ga} = 0$) with the ^{68}Ge-breakthrough (i.e. ^{68}Ga in the eluate is removed), then Eq. (4) simplifies to Eq. (5). In this case increasing ^{68}Ga activities are measured, due to decay of ^{68}Ge-breakthrough, according to the secular radionuclide generator equilibrium (Rösch and Knapp, 2003)

$$A^t_{Ga} = A^0_{Ge}(1 - e^{-\frac{\ln 2}{t_{1/2}}t}) \quad (5)$$

The saturation point or equilibrium is reached when

$$A^{equ}_{Ga} = A^{equ}_{Ge} \quad (6)$$

Therefore, the ^{68}Ga activity from an initially pure ^{68}Ge sample measured at 28.10 and 67.71 min will represent ¼ and ½ of the total initial ^{68}Ge activity, respectively. Therefore, it is possible to determine the amount of ^{68}Ge present by multiplying the measured ^{68}Ga activities at 28.10 and 67.71 min by a factor of 4 and 2, respectively.

For the quality control of ^{68}Ga-radiopharmaceuticals, TLC is commonly used to analyze the labeling yield and product purity of ^{68}Ga species based on the different R_f values of individual ^{68}Ga species. Similarly, Ge(IV) and Ga(III) species may behave differently depending on the chosen stationary and mobile phases (Mirzadeh and Lambrecht, 1996). Provided that there is sufficient separation between the different species on a TLC plate, it is possible to determine the ^{68}Ge content easily and quickly.

Of course, the ^{68}Ge species is not visible directly after TLC plate development. It requires time to decay to its daughter radionuclide via electron capture before the positron emission of generated ^{68}Ga is detectable. Thus, the ^{68}Ge TLC 'spot' is allowed to develop for a known amount of time (1\4 or 1\2 of a ^{68}Ga half-life) to generate ^{68}Ga before analyzing the TLC plate. 'Spots' pertaining to the different metallo-radionuclides are easily identified because the ^{68}Ga species exhibits decreasing radioactivity, while the ^{68}Ge species TLC spot increases in activity for ~10 h past generator elution (assuming perfect separation of these species).

Fig. 1 shows a theoretical decay profile of a sample containing equal activities of ^{68}Ge/^{68}Ga analyzed by TLC. The ^{68}Ge and ^{68}Ga species were assigned arbitrary R_f values of 0.2 and 0.8, respectively. The calculated activities of each spot are shown at t=0, 33.85 (1/2 of a single ^{68}Ga half-life) and 67.7 min (one ^{68}Ga half-life) post-elution. There is a relationship between the rate at which ^{68}Ge decays for the ^{68}Ge spot and the initial ^{68}Ge amount.

This approach can also be applied to determine the ^{68}Ge content of a ^{68}Ga-labeled compound, such as ^{68}Ga-labeled peptides, to determine ^{68}Ge traces remaining in the final injectable solution. A TLC system capable of discriminating between the ^{68}Ge, the ^{68}Ga-radiopharmaceutical and uncomplexed ^{68}Ga, is required. This approach may not be universal in terms of the conditions used for TLC, with different radiopharmaceuticals requiring tailor-made TLC systems. In this report ^{68}Ga-DOTATOC, which is used for the diagnostic imaging of neuroendocrine tumors, was selected as a proof-of-principle study.

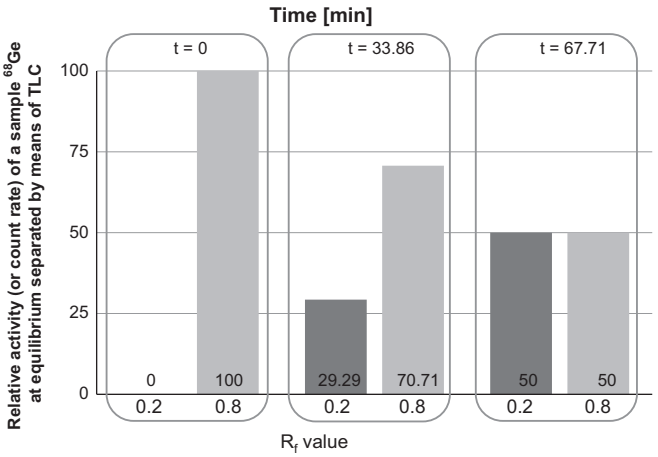

Fig. 1. Theoretical profile of a ^{68}Ge/^{68}Ga sample at equilibrium analyzed by TLC. The ^{68}Ge and ^{68}Ga species are assigned arbitrary R_f values of 0.2 and 0.8, respectively. At t=0, there is no activity for the ^{68}Ge spot and maximum activity for the ^{68}Ga spot (=100%). [*Note*: this neglects nuclear transformations that occur during the TLC development and scanning (~10 min)]. Approximately 33.9 min later, a second scan detects ^{68}Ga activity at the ^{68}Ge species spot equal to 29% saturation (Eq. (2)), whereas the activity originating from the initial ^{68}Ga spot (R_f=0.8) decreases to 71% of its initial value. A final scan of the TLC plate at t=67.71 min represents equal activities, i.e. the ^{68}Ga activity detected at the ^{68}Ge spot equals 1\2 of the initial ^{68}Ge present.

Table 1
TLC mobile phases and R_f-values for ^{68}Ga, ^{68}Ge and ^{68}Ga-DOTATOC.

	Mobile phase	R_f-values		
		^{68}Ga(III)	^{68}Ge(IV)	^{68}Ga-DOTATOC
(a)	0.1 M citric buffer (pH=4)	1	1	0.1–0.2
(b)	5% NaCl:MeOH:25% NH_3 (3:1:1)	0	0.4–0.6	0.1–0.2
(c)	2 M HCl:acetone (1:1)	1	0.1	0.4
(d)	0.01 M $NaC_4H_5O_6$:MeOH (3:1)	0	0.4–0.9	0.1–0.2
(e)	Cyclohexanone:2 M HCl (20:1)	0.4–0.5	0.1	0

2. Methods

^{68}Ge/^{68}Ga generator: A two-year old ^{68}Ge/^{68}Ga generator (Eckert & Ziegler Strahlen- und Medizintechnik AG, Berlin, Germany) with a ^{68}Ga yield of ~100 MBq and ^{68}Ge breakthrough of ~85 kBq was used. The generator was eluted with 5 mL of 0.1 N HCl in all cases. Another generator was used as a source of ^{68}Ge activity, and to obtain a stock solution of ^{68}Ge/^{68}Ga at equilibrium (1.35 MBq ^{68}Ge/^{68}Ga in 5 mL 0.1 N HCl).

Synthesis of ^{68}Ga-DOTATOC: DOTATOC (21 nmol) was labeled using 0.4 mL of the ^{68}Ge/^{68}Ga generator eluate (not post-processed) and 0.6 mL of 0.2 M CH_3COONa buffer (pH=4) for 10 min at 95 °C. The ^{68}Ga-labeled DOTATOC R_f value was determined for solvents a)–e).

Thin-layer chromatography: Table 1 shows the five mobile phases investigated for separating ^{68}Ge and ^{68}Ga on TLC plates (65 mm, Silica Gel 60 TLC strips, MERCK, Darmstadt, Germany).

The four mobile phases recommended by Mirzadeh and Lambrecht (1996), and the widely used 0.1 M citrate buffer (pH=4) were used. Freshly prepared solutions were used in all cases, which is especially important for mobile phases c) and e), due to self-condensation of the organic components.

For TLC analyses, 4 μL aliquots from either the generator eluate or solution with the ^{68}Ga-labeled product were spotted on TLC plates. Following TLC elution, the ^{68}Ge and ^{68}Ga distributions were detected using an instant-imager (Instant Imager®, Packard Canberra, Schwadorf, Austria) at 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, and 30 h after TLC development.

Radioactivity measurements: For the quantitative analysis of low ^{68}Ge activities, a sensitive detection system and short acquisition time are mandatory. Therefore, the detection limits of two different instant imagers (Instant Imager®, Packard Canberra and Rita Star®, Raytest-Isotopenmessgeräte GmbH, Straubenhardt, Germany) were investigated. Ten dilutions were made from a stock solution containing 0.118 MBq ^{68}Ge in 5 mL (Table 2). The assessment was performed by spotting known activities of ^{68}Ge on a cellulose strip and recording the ^{68}Ge activity, using both imagers, after 10 and 30 min. For this aliquots 4 μL were taken from each dilution and spotted on a single cellulose strip (150 mm × 10 mm). The cellulose strip was not developed.

3. Results and discussion

A TLC system to differentiate between ^{68}Ga (30–50 kBq for this setup) and the relative small amount of ^{68}Ge (~34 Bq for this setup) present in the generator eluate is required to allow the ^{68}Ge content to be determined. With the exception of 0.1 M citrate buffer, all of the investigated mobile phases could be used to separate the ^{68}Ga and ^{68}Ge species on TLC plates. The recorded R_f values of the different species using each mobile phase are summarized in Table 1. Fig. 2 shows the positions of the ^{68}Ge and ^{68}Ga species on a TLC plate developed using mobile phase b). As expected, the measurements directly after TLC plate development almost exclusively show the initial ^{68}Ga activity (not shown). To detect ^{68}Ge, follow-up scans are required. These follow-up scans were recorded at 1 (L1), 10 (L2) and 24 h (L3) after TLC development.

While the activity of the ^{68}Ga spot decreased, that of the ^{68}Ge spot increased consistent with the generation, and subsequent decay, of its daughter radionuclide. The activity level 67.7 min after the TLC plate development represents half of the total ^{68}Ge activity at the start. Therefore, using the calibration between absolute activity and observed count rate, it is possible to quantify the ^{68}Ge breakthrough relatively quickly, i.e. approximately 1 h after generator elution or labeling.

When this protocol is applied to determine the ^{68}Ge content of a prepared radiopharmaceutical, the chosen TLC system should

Table 2
Dilutions of the ^{68}Ge stock solution. An aliquot represents 4 μL from 400 μL of ^{68}Ga (=1%).

Nr	Aliquot (Bq)	Aliquot (mol)
1	47.200	2.647×10^{-15}
2	23.600	1.324×10^{-15}
3	11.8.00	6.617×10^{-16}
4	5.900	3.309×10^{-16}
5	2.950	1.655×10^{-16}
6	1.475	8.272×10^{-17}
7	0.738	4.136×10^{-17}
8	0.369	2.068×10^{-17}
9	0.184	1.034×10^{-17}
10	0.092	5.170×10^{-18}

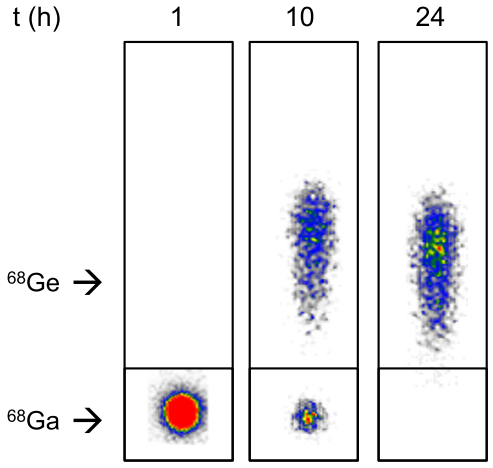

Fig. 2. The same TLC plate with ^{68}Ge and ^{68}Ga developed using mobile phase (c) and measured at 1, 10 and 24 h after TLC development.

provide sufficient separation of ^{68}Ge species, uncomplexed and complexed ^{68}Ga (the radiopharmaceutical). In the case of ^{68}Ga-DOTATOC, which can be prepared with a radiochemical yield of 99%, this translates to activity rations of 1:100:10,000 for the three species. As shown in Fig. 3, mixtures b), c) and d) were most suitable for the resolution of ^{68}Ga (L1), ^{68}Ga-DOTATOC (L2) and ^{68}Ge (L3), respectively.

Fig. 4 depicts a population distribution map of a TLC plate, spotted with a solution containing ^{68}Ga-DOTATOC and ^{68}Ge (0.5 mL of stock solution), imaged at 0, 2, 4 and 6 h after TLC development with mobile phase c). The activity of the ^{68}Ga-DOTATOC spot (R_f=0.4) decreased at a rate corresponding to the ^{68}Ga half-life. In parallel, the ^{68}Ge spot (R_f=0.1) showed an increasing count rate, corresponding to the half-life of ^{68}Ga generation in situ. The calibration between the observed activity (on the TLC) and the absolute activity of ^{68}Ge allowed the ^{68}Ge content in the ^{68}Ga-DOTATOC sample to be quantified 1 h after labeling.

The Packard Canberra Instant Imager® detected ^{68}Ge levels as low as 5.90 and 2.95 Bq (10^{-16} mol) after 10 and 30 min, respectively. By comparison, the Raytest-Isotopenmessgeräte GmbH Rita Star® was considerably more sensitive, detecting activities of 0.74 and 0.36 Bq (10^{-17} mol) at the same time points. For each imager, linear regression was used to determine a unique linear calibration equation, which allows the ^{68}Ge content to be quantified (Fig. 5).

The linear regression from the Instant Imager® (Packard Canberra) calibration, after 10 min measurement, yields the following equation:

$$y = 2.6344x + 0.4167 \quad (7)$$

$$x = \frac{y - 0.4167}{2.6344} \quad (8)$$

where y=counts per minute measured over 10 min and x=activity in Bq spotted onto the TLC plate.

4 μL sample from the initial ^{68}Ga eluate was spotted onto a TLC plate and developed with mobile phase b). Fig. 6a shows the TLC measured directly after development. Uncomplexed ^{68}Ga remains at the starting point (R_f=0). The TLC was then cut at R_f=0.3 and the upper section re-measured for 10 min (Fig. 6b). The upper section contains the ^{68}Ge species, and is detected via the decay of its daughter radionuclide. The measured value for counts per minute is inserted in Eq. (8), which leads to value of 12.75 Bq ^{68}Ge in 4 μL aliquot, which corresponds to 15.93 kBq ^{68}Ge in the initial eluate (5 mL). Inserting this value into Eq. (5) gives the absolute ^{68}Ge-breakthrough in the initial eluate as 147.83 kBq.

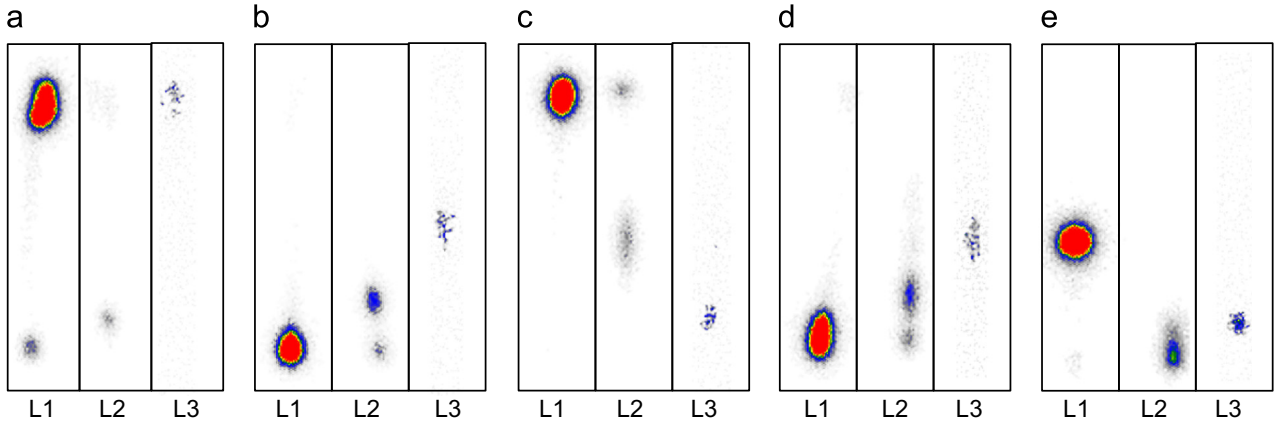

Fig. 3. TLC plates developed using mobile phases (a)–(e) (Table 1). Lanes: L1 = ^{68}Ga; L2 = ^{68}Ga-DOTATOC and uncomplexed ^{68}Ga; L3 = ^{68}Ge.

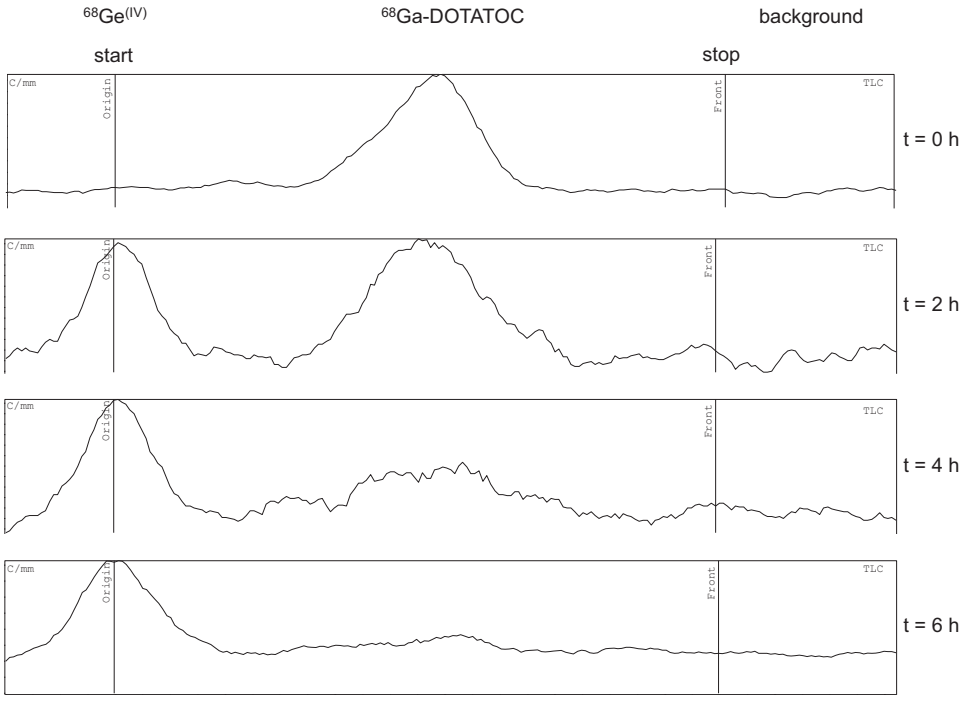

Fig. 4. TLC analysis of a ^{68}Ga-DOTATOC preparation developed using the TLC solvent mixture (c), 2 M HCl/acetone (1:1) and measured at 0, 2, 4 and 6 h after development. The *y*-axis represents the maximum relative count-rates.

To evaluate the usefulness of this protocol for determining the ^{68}Ge content of a ^{68}Ga-DOTATOC preparation, 1 mL solution of the radiopharmaceutical was spiked with 0.4 mL of the ^{68}Ge stock solution. 4 mL aliquot was spotted onto a TLC plate and developed using mobile phase d). Due to regular fluctuations in the ^{68}Ge breakthrough and dilution effects, a known amount of ^{68}Ge was added to the ^{68}Ga-DOTATOC solution instead of measuring the ^{68}Ge breakthrough directly.

Fig. 7a shows the entire TLC plate scanned immediately after development. ^{68}Ga-DOTATOC remained near the starting point at R_f = 0.1. The TLC plate was then cut at R_f = 0.3–0.35, and scanned for 10 min (Fig. 7b) to measure the activity resulting indirectly from spotted ^{68}Ge. The measured counts per minute was inserted into Eq. (8) to give a ^{68}Ge content in 4 μL aliquot of 106.42 Bq. Using the dilution factor this translates to an initial ^{68}Ge content of 37.25 kBq in the 1 mL ^{68}Ga-DOTATOC solution.

The actual ^{68}Ge breakthrough contained in the original ^{68}Ga-DOTATOC solution is 1.41 kBq, which was calculated by subtracting the ^{68}Ge spike (35.84 kBq) from the actual ^{68}Ge content. Inserting this value into Eq. (5) gives an absolute ^{68}Ge content of 13.01 kBq.

The ^{68}Ge breakthrough in the generator eluate and ^{68}Ga-DOTATOC solution was determined under standard conditions. The overall protocol, which includes TLC development and measurement, takes less than 30 min. Therefore, it is possible to quantify the ^{68}Ge content in a generator eluate or radiopharmaceutical preparation prior to their further application.

4. Conclusions

A TLC based protocol has been developed which allows the ^{68}Ge content of generator eluates and ^{68}Ga-DOTATOC preparations to be determined half an hour after TLC development. The method relies on the resolution of the ^{68}Ge and ^{68}Ga species on TLC with ^{68}Ge quantification made possible via the decay of its daughter radionuclide. This novel protocol allows for the rapid and reliable

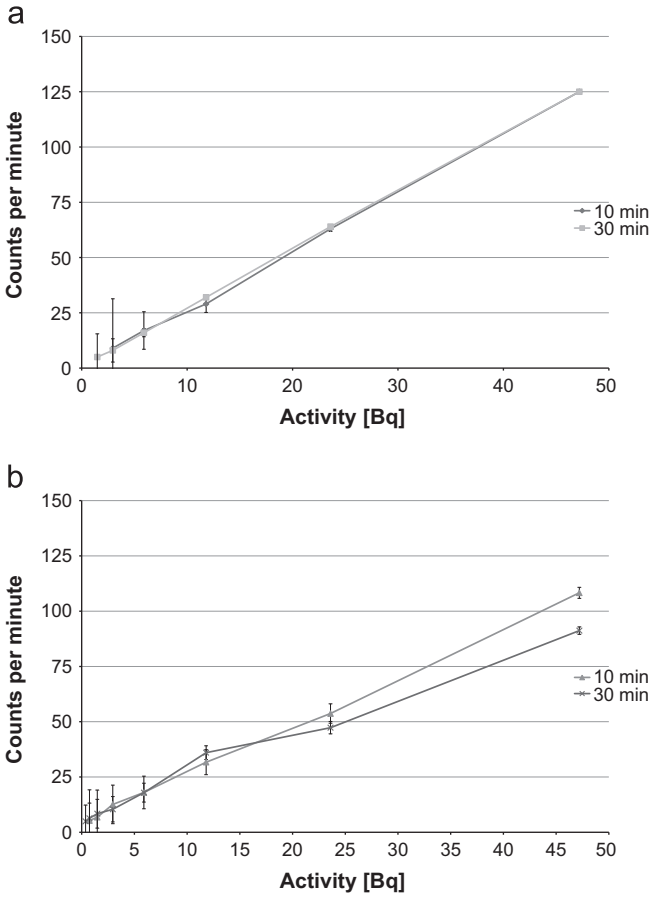

Fig. 5. Calibration of the ^{68}Ge-breakthrough quantification for the a) Instant Imager® (Packard Canberra) and b) Rita Star® (Raytest – Isotopenmessgeräte GmbH) after 10 and 30 min duration measurements.

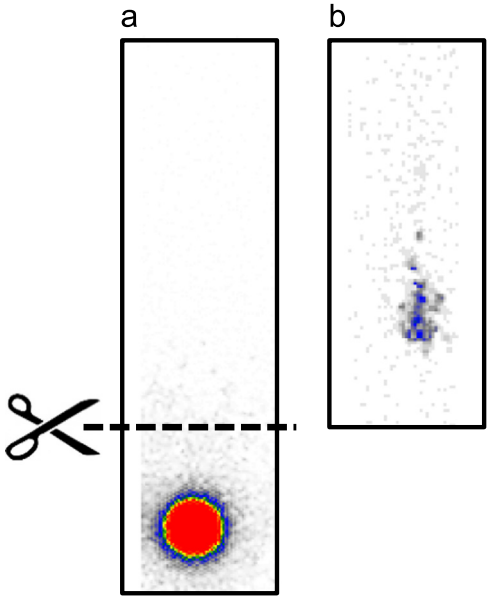

Fig. 6. TLC analysis of the initial eluate developed using the mobile phase (b), 5% NaCl:MeOH:25% NH_3 (3:1:1) and measured (a) directly after TLC development and (b) directly after cutting the TLC at R_f=0.3.

determination of ^{68}Ge activity levels in a ^{68}Ge/^{68}Ga-generator eluate without the use of γ-spectroscopy. The protocol may also

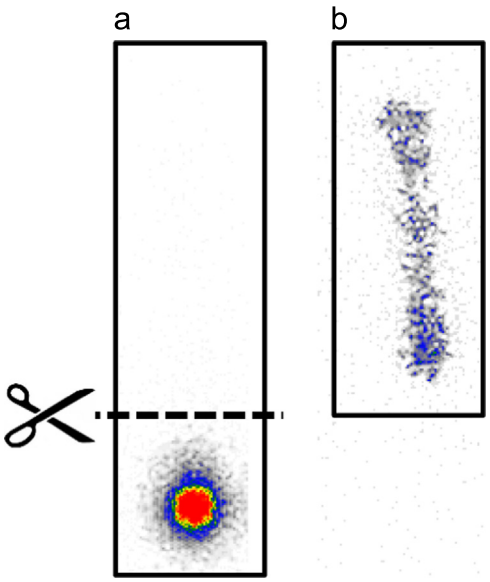

Fig. 7. TLC analysis of ^{68}Ga-DOTATOC preparation (containing ^{68}Ge spike) developed in mobile phase (d), 0.01 M $NaC_4H_5O_6$:MeOH (3:1), measured (a) directly after TLC development and (b) directly after cutting the TLC plate at R_f=0.3–0.35.

suitable for the quality control for ^{68}Ge content of individual radiopharmaceutical preparation. This has been demonstrated for ^{68}Ga-DOTATOC, where the ^{68}Ge content can be determined prior to administration of the radiopharmaceutical. Therefore, this novel protocol permits the analytical quantification of the ^{68}Ge content in a ^{68}Ge/^{68}Ga-generator eluate and ^{68}Ga-radiopharmaceutical within a time frame suitable for their further application.

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