

Radiochemical Purity of ^{68}Ga -BCA-Peptides: Separation of All ^{68}Ga Species with a Single iTLC Strip

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Abstract—In the present study, highly effective iTLC single strip method for the determination of radiochemical purity (RCP) of ^{68}Ga -BCA-peptides was developed (with no double-developing, changing of eluents or other additional manipulation). In this method iTLC-SG strips and commonly used eluent TFA_{aq} (3-5 % (v/v)) are used. The method allows determining each of the key radiochemical forms of ^{68}Ga (colloidal, bound, ionic) separately with the peaks separation being no less than 4 σ . $R_f = 0.0-0.1$ for ^{68}Ga -colloid; $R_f = 0.5-0.6$ for ^{68}Ga -BCA-peptides; $R_f = 0.9-1.0$ for ionic ^{68}Ga . The method is simple and fast: For developing length of 75 mm only 4-6 min is required (versus 18-20 min for pharmacopoeial method). The method has been tested on various compounds (including ^{68}Ga -DOTA-TOC, ^{68}Ga -DOTA-TATE, ^{68}Ga -NODAGA-RGD₂ etc.). The cross-validation work for every specific form of ^{68}Ga showed good correlation between method developed and control (pharmacopoeial) methods. The method can become convenient and much more informative replacement for pharmacopoeial methods, including HPLC.

Keywords—DOTA-TATE, ^{68}Ga , quality control, radiochemical purity, radiopharmaceuticals, iTLC.

I. INTRODUCTION

WIDE range of radiopharmaceuticals (RP) available today is a key reason for the expansion of nuclear medicine in the field of human health around the world. At the same time, production quality requirements applied to RP become more and more strict, especially since GMP came into force. RCP determination is an extremely important part of quality control (QC) in routine clinical practice, requiring usage of fast and informative methods. ^{68}Ga -BCA-peptide RPs are justly considered as cutting edge area of current studies and medical practice. During synthesis of ^{68}Ga -BCA-peptides (^{68}Ga -DOTA-TOC/-TATE/-NOC, ^{68}Ga -NODAGA-RGD₂, ^{68}Ga -PSMA-HBED-CC etc.) two major radiochemical impurities are formed: ^{68}Ga -colloid and unbound ionic ^{68}Ga . The amounts of each impurity and ^{68}Ga -BCA-peptide are strictly normalized according to pharmacopoeial standards. In The European Pharmacopoeia 8th Edition there is no method allowing separating all radiochemical impurities in ^{68}Ga preparations [1]. Normally it takes to use combination of two systems to separate them (iTLC-SG and 1 M ammonium

acetate/methanol 50:50 (v/v) – Ph. Eur. TLC method – and HPLC) [1].

Ph. Eur. TLC method does not allow separating ionic ^{68}Ga from ^{68}Ga -colloid. Of course, if the final stage of the RP synthesis is solid phase extraction, the ^{68}Ga -colloid is removed and never can be seen in the chromatogram (as well as it never can be seen by HPLC). However, if the RP synthesis occurs with the use of kits and GMP-grade generator eluate the ^{68}Ga -colloid (separately from ionic ^{68}Ga) determination is essential.

To date, it is well known that under conditions of Ph. Eur. TLC method ionic ^{68}Ga does not migrate with the solvent front, but remains at the origin of the chromatogram [2]-[5]. However, much to our surprise, in some papers the statement, that ionic ^{68}Ga has $R_f = 1.0$, can be seen (for example, [6]). Moreover, it appears that European Pharmacopoeia was affected with this misconception.

There is a number of published TLC methods for ^{68}Ga -BCA-peptides QC (see Table I). The aim of this study was to develop effective TLC method for the determination of RCP of ^{68}Ga -RP, allowing determining each of the key radiochemical forms of ^{68}Ga (colloidal, bound, ionic) separately.

II. MATERIALS AND METHODS

A. Chemicals and Reagents

Only deionized water 18.2 M Ω ·cm (Milli-Q Millipore or TKA Smart2Pure) was used. All chemicals and solvents were of high-purity or pharmaceutical grade and were purchased from Sigma-Aldrich or Panreac. GMP-grade RP precursors (DOTA-TATE, DOTA-TOC, DOTA-NOC, NODAGA-RGD, NODAGA-RGD₂, DOTA-RGD₂) were purchased from ABX Chemicals.

B. $^{68}\text{Ge}/^{68}\text{Ga}$ Radionuclide Generator

The germanium-68/gallium-68 generator, based on a TiO₂ solid phase (eluent – 0.1 M HCl), was obtained from Cyclotron Ltd (Obninsk, Russian Federation). Two generators with nominal activity of 1850 MBq (calibration dates: 2015-09-14, 2014-02-03) were used.

C. Measurement of Radioactivity

Measurement of ^{68}Ga absolute activity was performed by Atomlab™ 500 Dose Calibrator (Biodex, USA). Analysis of activity distribution on the chromatographic strips and electrophoresis plates was carried out by radio-TLC scanners Mini-Scan (Bioscan) and PET-MiniGita (Raytest, Germany).

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TABLE I
 SOME OF THE PUBLISHED TLC METHODS FOR ⁶⁸Ga-PEPTIDES RCP DETERMINATION

Method#	Stationary phase	Eluent	Rf value	Ref.
1	iTLC-SG	1.0 M ammonium acetate/methanol (1:1)	Rf (⁶⁸ Ga-peptide) ~ 0.8-1.0 Rf (⁶⁸ Ga-colloid) ~ 0.0-0.2 Rf (⁶⁸ Ga-ionic) ~ 0.0-0.2	[1]-[5], [7], [8], [10]
2	iTLC-SG	0.1 M Na-citrate buffer (pH 4.0 - 5.5)	Rf (⁶⁸ Ga-peptide) ~ 0.0-0.2 Rf (⁶⁸ Ga-ionic) ~ 0.9-1.0	[2], [3], [10], [13]
3	iTLC-SG	0.05-0.2 M citric acid	Rf (⁶⁸ Ga-peptide) ~ 0.0-0.2 Rf (⁶⁸ Ga-ionic) ~ 0.9-1.0	[4], [5], [7], [8]
4	iTLC-SG	methanol/saline (1:5)	-	[16]
5	iTLC-SG	0.1 % TFA in water/acetonitrile (1:1)	Rf (⁶⁸ Ga-peptide) ~ 0.4-0.7 Rf (⁶⁸ Ga-unbound) ~ 0.0	[9]
6	iTLC-SG	double-developed (DD): 1 – HCl pH 5.5, 2 – methanol	Rf (⁶⁸ Ga-colloid) ~ 0.0 Rf (⁶⁸ Ga-peptide) and Rf (⁶⁸ Ga-unbound) ~ 0.4	[5]
7	C ₁₈ RP-SG plates	methanol/saline (9:1)	Rf (⁶⁸ Ga-peptide) ~ 0.47	[12]
8	C ₁₈ RP-SG plates	methanol/saline (1:5)	-	[15]
9	C ₁₈ RP-SG plates	0.1% TFA/ methanol 30:70 (v/v)	Rf (⁶⁸ Ga-peptide) ~ 0.78 Rf (⁶⁸ Ga-unbound) ~ 0.01	[11]
10	Whatman №2 paper	50 % aqueous acetonitrile	Rf (⁶⁸ Ga-peptide) ~ 0.9 Rf (⁶⁸ Ga-colloid) ~ 0.0-0.1 Rf (⁶⁸ Ga-unbound) ~ 0.0-0.1	[3]
11	Whatman №2 paper	methanol/saline (5:1)	Rf (⁶⁸ Ga-peptide) ~ 0.9 Rf (⁶⁸ Ga-ionic) ~ 0.5	[14]
12	Whatman №2 paper	methanol/saline (1:5)	Rf (⁶⁸ Ga-peptide) ~ 0.15 Rf (⁶⁸ Ga-ionic) ~ 0.7	[17]

D. Radiolabeling of BCA-Conjugated Peptides and Reference Solutions' Preparation

Labeling of BCA-conjugated peptides was performed with automated or manual methods. For automated method, Modular-Lab PharmTracer (Eckert & Ziegler) with different preconcentration protocols [8], [9] was used. Labeling reaction was performed in 1.5 mL Eppendorf tubes in the case of peptide solutions, and in 5 mL glass vials for freeze-dried compositions. The pH level necessary for each specific peptide was achieved by adding required amount of aqueous 0.2 M sodium acetate solution or 1M HEPES buffer (necessary pH level depends on the nature of peptide or desired partition of ⁶⁸Ga between various radiochemical species). In each case the reaction mixtures were incubated at 95°C for 10 min. The Eppendorf tubes were heated in a Mixing Block MB-102 device (Bioer, China). For each preparation blank solution (peptide-free) was prepared, and for each batch of the experiments reference solutions were prepared in accordance with pharmacopoeial monographs [1].

E. Radiochemical Analysis of Preparations

TLC

The RCP of the preparations obtained (numerically equal to labeling yield in this study) and radiochemical composition of the preparations (including reference and blank solutions) were determined by thin-layer chromatography using different stationary phases – iTLC-SG strips (Agilent), silica TLC plates (105553, 105748; Merck Millipore), cellulose TLC plates (105552, 105574; Merck Millipore), C₁₈ RP-SG plates (115685, Merck Millipore), chromatography papers №1, №2 and №3MM (Whatman), – with various eluents. 100 × 15 mm strips (origin – 15 mm, solvent front – 90 mm) were used in all cases. Developing was performed immediately after spotting (except C₁₈ RP-SG plates and

nonaqueous solvents: strips were dried on air before developing). Analysis of activity distribution on the chromatographic strips was carried out with PET-MiniGita (Raytest, Germany).

Peak resolution value (Rs) was evaluated using Snyder's equation:

$$R_s = \frac{Rf_2 - Rf_1}{\sigma_2 + \sigma_1} Z_x \quad (1)$$

where Rf_1 , Rf_2 – retention factors of corresponding peaks; σ_1 , σ_2 – half-widths of peaks at 60.7 % height; Z_x – development length.

HPLC

HPLC analysis was carried out in a C₁₈-column 4.6 × 100 mm, 2 μm (Chromolith® Performance RP-18, Merck Millipore). Mobile phase: A: acetonitrile (HPLC-grade, Panreac); B: 0.1% TFA. Gradient: 80 – 70% B in 10 min. Temperature 40°C. Flow rate 1.0 mL·min⁻¹. Detection: NaI γ-radiation scintillation detector.

Gel Electrophoresis

Agarose gel electrophoresis experiments were carried out using Camag electrophoresis chamber with LKB 2103 power supply (Biochrom). In each case agarose gel was prepared using the same buffer solution, that was used for formulation of the test sample. Electrophoresis was performed at a constant current of 5 mA for 30-60 min, with a voltage of 400 V. The chamber was cooled by circulating tap water. Analysis of activity distribution on the plate was carried out with PET-MiniGita (Raytest).

III. RESULTS AND DISCUSSION

A big number of TLC systems with a variety of eluents and stationary phases has been studied in detail in attempt to cover all of the literature data found to be used for the analysis of RCP of ^{68}Ga -BCA-peptides (see Table I).

A detailed discussion of each of the systems tested will be discussed elsewhere. Shortly we can say that all these systems failed to achieve acceptable separation of all components with one chromatography run (in some cases, results obtained were contrary to published ones).

We shall restrict ourselves to considering here only few systems that use iTLC-SG strips as a stationary phase. Firstly, iTLC-SG strips are commercially available, easy to work with and widely used in the QC of RP (not only ^{68}Ga -based). Secondly, all other things being equal, the time of development with glass fiber sheets is smaller than that with the other stationary phases, including cellulose, silica gel and RP-C18 on the substrate, as well as Whatman №№ 1, 2, 3MM chromatography paper. Time is a limiting factor when dealing with short-lived radionuclides. And we have to bear in mind that time is an important parameter for comparison of different chromatographic systems even with the same stationary phase.

Method 1 (iTLC-SG – 1.0 M Ammonium Acetate/Methanol (1:1))

Using Pharmacopoeia method allows the relative content of ^{68}Ga -BCA-peptide to be reliably determined: peptide complex moves with the front of the eluent (R_f 0.9-1.0), and colloidal and ionic forms of ^{68}Ga stay at the start of the chromatogram (R_f 0.0-0.1). It would seem that's it. The method allows to determine the percentage of the radionuclide in the target chemical form, which is the RCP (according to General monograph 'Radiopharmaceutical preparations' [18], 'Radiochemical purity: the ratio, expressed as a percentage, of the radioactivity of the radionuclide concerned which is present in the RP preparation in the stated chemical form, to the total radioactivity of that radionuclide present in the RP preparation'). The method is highly reproducible and accurate.

Unfortunately, Method 1 does not permit to divide colloidal and ionic forms of ^{68}Ga to determine the percentage of each, as required by pharmacopoeial standards. Relatively long chromatographic run time is another disadvantage: for 75 mm developing length at the average 15 min are required. Also, we have to admit, that the peak corresponding to ^{68}Ga -BCA-peptide is often quite broad and tailed.

There is common misconception that when using Method 1 ionic forms of ^{68}Ga move to the front of the eluent with the peptide complex [6]. This fact can be considered as another kind of disadvantage. It should be noted, however, that this misleading standpoint is not completely unreasonable. The mobility of ionic forms of ^{68}Ga in this method depends on the acidity of the test solution. For unbound ^{68}Ga R_f = 0.0-0.1, when the concentration of hydrochloric acid in the initial [^{68}Ga]GaCl₃ solution (eluate) is from 0.05 to 0.1 M. This R_f is also true over the entire range of possible values of pH in preparations. However, when the concentration of HCl in

initial [^{68}Ga]GaCl₃ solution is 0.2 M, about 30-40% of the radioactivity on the chromatographic strip moves with the solvent front (R_f 0.9-1.0). And with 0.5 M HCl about 90% of ^{68}Ga move with the solvent front. Nevertheless, this range of HCl concentrations has no relation to the actual values of the acidity of RP.

Methods 2, 3 (iTLC-SG – 0.1 M Na-Citrate Buffer (pH 4.0 ÷ 5.5) or 0.05-0.2 M Citric Acid)

These systems were approved by a large number of researchers involved in ^{68}Ga -RP development. The methods allow accurate determining the content of unbound ionic ^{68}Ga in the sample. This is done by separating unbound ^{68}Ga in the form of citrate complex (R_f of 0.9-1.0) from ^{68}Ga -colloid and ^{68}Ga -peptide (R_f of 0.0-0.2). The method is highly reproducible, accurate and fast (2-4 min for 75 mm developing length). This system can be a worthy alternative to pharmacopoeial method, according to which the determination of unbound ionic ^{68}Ga is carried out using HPLC. In [13] the cross-validation work for unbound ionic ^{68}Ga showed good correlation between HPLC and iTLC method (r = 0.961). It is worth noting that the adequacy of using HPLC as a standard of comparison is highly questionable, since full recovery of the injected radioactivity is doubtful: apart from the fact that ^{68}Ga -colloid never can be seen, there is no confidence that no unaccounted sorption processes on the column does occur. For these reasons, in our work, aside from HPLC, gel electrophoresis was selected as a comparative method. According to our results, Methods 2 and 3 showed excellent correlation to electrophoresis in determining unbound ionic ^{68}Ga (r = 0.9994). Methods 2 and 3 still have some disadvantages. First of all, as in the previous case, all ^{68}Ga forms can't be separated. Secondly, ^{68}Ga -peptide still has slight mobility under these conditions (R_f being not higher than 0.2), which often leads to doubling of the peak at the origin (Fig. 1) if the developing length is bigger than 50 mm.

We underline that methods 1, 2 and 3 were chosen as control during validation of other chromatographic systems.

Method 4 (iTLC-SG – Methanol/Saline (1:5))

We could not find published reference R_f values for various forms of ^{68}Ga .

Based on [5], [14], [17] the Method 4 seemed to be very promising: analyzing of $^{68}\text{Ge}/^{68}\text{Ga}$ generator eluate (0.1 M HCl) resulted to the peak with R_f of 0.30-0.45 (albeit the peak is asymmetric and trailed), and for ^{68}Ga -DOTA-TATE the peak with R_f of 0.85-0.90 was obtained. Further experiments have shown that this is not entirely true. At a certain analogy with the Method 1, Method 4 proved to be pH-sensitive. With increasing of HCl concentration in the initial [^{68}Ga]GaCl₃ solution from 0.02 to 0.20 M, R_f varied from 0.1 to 1.0 (Fig. 2). Just as in the case of Method 1, in Method 4 over the entire range of possible values of pH in samples, R_f of unbound ^{68}Ga is 0.0-0.1, i.e. separation of the unbound and colloidal ^{68}Ga does not occur.

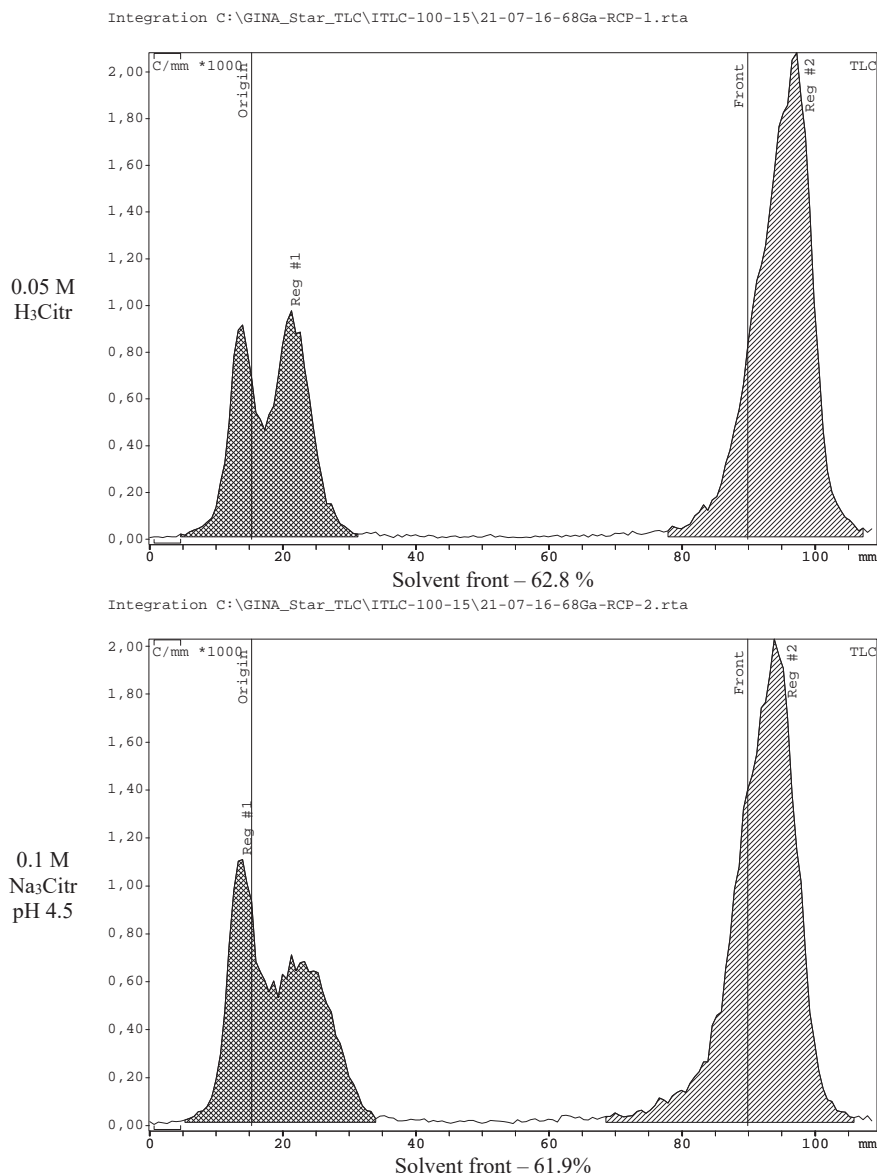


Fig. 1 Radio-chromatograms of ^{68}Ga -DOTA-TATE preparation with high amount of free ionic ^{68}Ga in citric-containing solvents: doubling of the peak at the origin is observed

It should be particularly noted that the use of the $^{68}\text{Ge}/^{68}\text{Ga}$ generator eluate is often inadequate for determining the chromatographic behavior of ionic ^{68}Ga in radiopharmaceutical preparations. However, Method 4 allows determining the content ^{68}Ga -BCA-peptide (RCP, just as in Method 1) reliably and reproducibly. The peak corresponding to ^{68}Ga -BCA-peptide is fairly narrow and symmetrical. Furthermore, the system is very fast: the time required for developing a 75 mm strip takes 2.5-3.0 times less than in Method 1 (see Table II). Thus, the system can become a good and more convenient alternative to Pharmacopoeia method.

Method 5 (*i*TLC-SG – 0.1 % TFA in Water/Acetonitrile (1:1))

Method 5 was described in [9] for the analysis of ^{68}Ga -DOTA-TOC RCP: R_f (free ^{68}Ga) = 0.0, R_f (^{68}Ga -DOTA-TOC) = 0.4-0.7. To our great surprise, a detailed simulation of the [9] method of ^{68}Ga -DOTA-TOC synthesis, as well as other routes of synthesis of ^{68}Ga -peptides (^{68}Ga -DOTA-TATE, ^{68}Ga -NODAGA-RGD₂), led to the obtaining of entirely different results: R_f (^{68}Ga -colloid) = 0.0, R_f (^{68}Ga -ionic) = 0.6 and R_f (^{68}Ga -peptide) = 1.0. Although various ^{68}Ga forms correspond to different R_f values, effective separation was not achieved (Fig. 4 E). The reason was the fact that peak corresponding to unbound ^{68}Ga is strongly asymmetrical and tailed, which leads to its poor resolution with ^{68}Ga -colloid

peak. ^{68}Ga -peptide peak is also quite broad and not fully resolved from ionic ^{68}Ga peak. Thus, Method 5 proved to be

ineffective for the separation of all chemical forms in ^{68}Ga RP preparations.

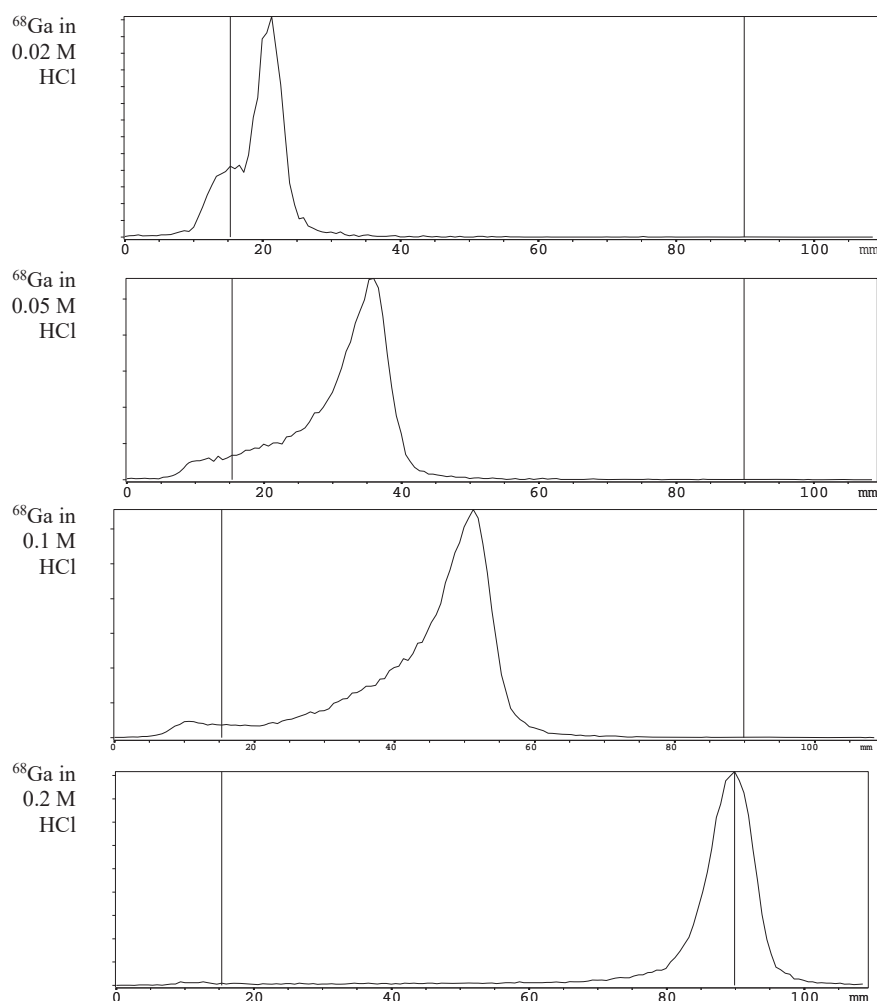


Fig. 2 Radio-chromatograms of ^{68}Ga solutions with different concentration of HCl (iTLC-SG – methanol/saline (1:5) system)

TABLE II
CHROMATOGRAPHY RUN TIME FOR iTLC-SG STRIP (75 MM) IN DIFFERENT SOLVENTS

iTLC-SG in	18°C	25°C
0.05 M citric acid	4'00"-4'10"	2'30"-2'45"
saline	4'00"-4'10"	2'45"-3'00"
water	4'10"-4'15"	2'40"-2'50"
TFA, 4%	5'45"-5'50"	3'45"-4'00"
methanol/saline (1:5) v/v	6'45"-6'55"	4'30"-5'00"
methanol	7'15"-7'25"	4'45"-4'55"
0.1 % TFA in water/acetonitrile (1:1) v/v	7'40"-7'50"	5'00"-5'15"
1.0 M ammonium acetate/methanol (1:1) v/v	18'00"-18'30"	12'00"-12'30"

The reason of non-compliance of our results with previously published was not found so far.

Method Developed

It was found that separation of all forms of ^{68}Ga presented in RP preparations based on BCA-conjugated-peptides is

possible using chromatographic system iTLC-SG – TFA water solutions (1 to 5% (v/v)). This method allows to achieve R_f (^{68}Ga -peptide) \approx 0.5, while R_f (^{68}Ga -colloid) = 0.0 and R_f (^{68}Ga -ionic) = 1.0. For every given compound certain concentration of TFA is needed. For example, for ^{68}Ga -DOTA-TATE/-TOC/-NOC R_f (^{68}Ga -peptide) = 0.55 is observed when using 4 % TFA (Figs. 3, 4 F).

The method has been tested on various ^{68}Ga -BCA-peptides (including ^{68}Ga -DOTA-TOC, ^{68}Ga -DOTA-TATE, ^{68}Ga -NODAGA-RGD₂) and mixtures (model preparations) with different values of RCP. Different values of RCP came from the imitative variation of content of ^{68}Ga -colloid and ionic ^{68}Ga .

Content of every specific form of ^{68}Ga was validated using control methods and showed good correlation:

- ^{68}Ga -BCA-peptide content, compared to Methods 1 – $r = 0.9985$;
- free ^{68}Ga content, compared to Method 2, 3 – $r = 0.9998$;

- ^{68}Ga -colloid content, compared to Methods 1, 2, 3 – $r = 0.9991$.

For greater reliability of our results, additional control methods were used: gel electrophoresis and HPLC. Data on the correlation of this method with HPLC were excluded from this paper and will be considered elsewhere. Gel electrophoresis confirmed the accuracy of the developed method during determination of content of ionic unbounded ^{68}Ga .

Being three-component mixtures (colloid, peptide-bound, free ^{68}Ga) analyzed with the developed method, three narrow and symmetrical peaks are observed. Provided sufficient development length, this ensures a high separation factor (and hence high accuracy of the values obtained). For example, for octreotide derivatives (^{68}Ga -DOTA-TATE/-TOC/-NOC) $R_f = 0.55$ (iTLC-SG - 4% TFA). If the development length is 75 mm, for the peaks of ^{68}Ga -colloid and ^{68}Ga -peptide $R_s = 2.0$ (8σ), and for the peaks of ^{68}Ga -peptide and free ^{68}Ga $R_s = 1.5$ (6σ).

The specified parameters for chromatography in order to save time the development length can be reduced to 50 mm, which corresponds to the resolution of 6σ ($R_s = 1$, only 3% of the area adjacent peaks overlap).

It is important that in the system iTLS-SG - 4% TFA R_f value is reproducibly equal to 1.0 for the ionic forms of ^{68}Ga ,

when using different concentrations of hydrochloric acid in the initial $[^{68}\text{Ga}]\text{GaCl}_3$ solution (0.02 to 0.5 M HCl) and throughout all the range of possible pH values in the preparations. The same holds true when using 1 to 5% TFA as eluent.

Regarding ^{68}Ga complexes with BCA-conjugated peptides, depending on their molecular weight, the nature of the chelator and the peptide fragment, corresponding R_f value will vary with the TFA concentration in the eluents. The general trends are:

- the more TFA concentration in the eluent, the greater R_f value for a single complex;
- at a given concentration of TFA in eluents, the lower the molecular weight of the complex, the greater R_f value will correspond to it (in a rough approximation).

For example, when using 1, 2, 3, 4 and 5% TFA solutions as eluents, for ^{68}Ga -DOTA-TATE R_f values are 0.20, 0.28, 0.46, 0.55, 0.63 (± 0.02) respectively. In these conditions, there are no significant differences in shape (width and symmetry) of these five chromatographic peaks.

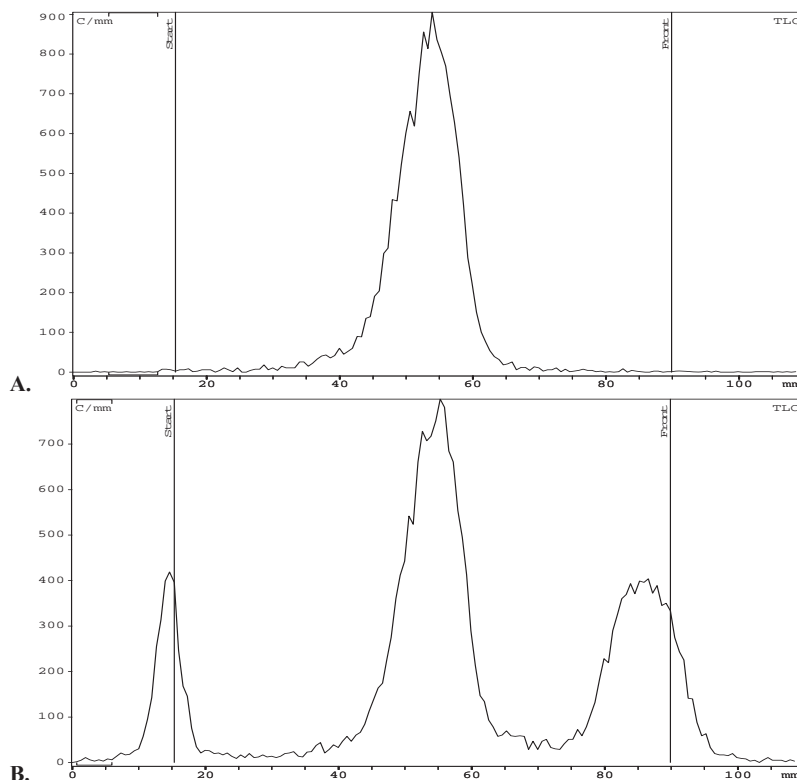
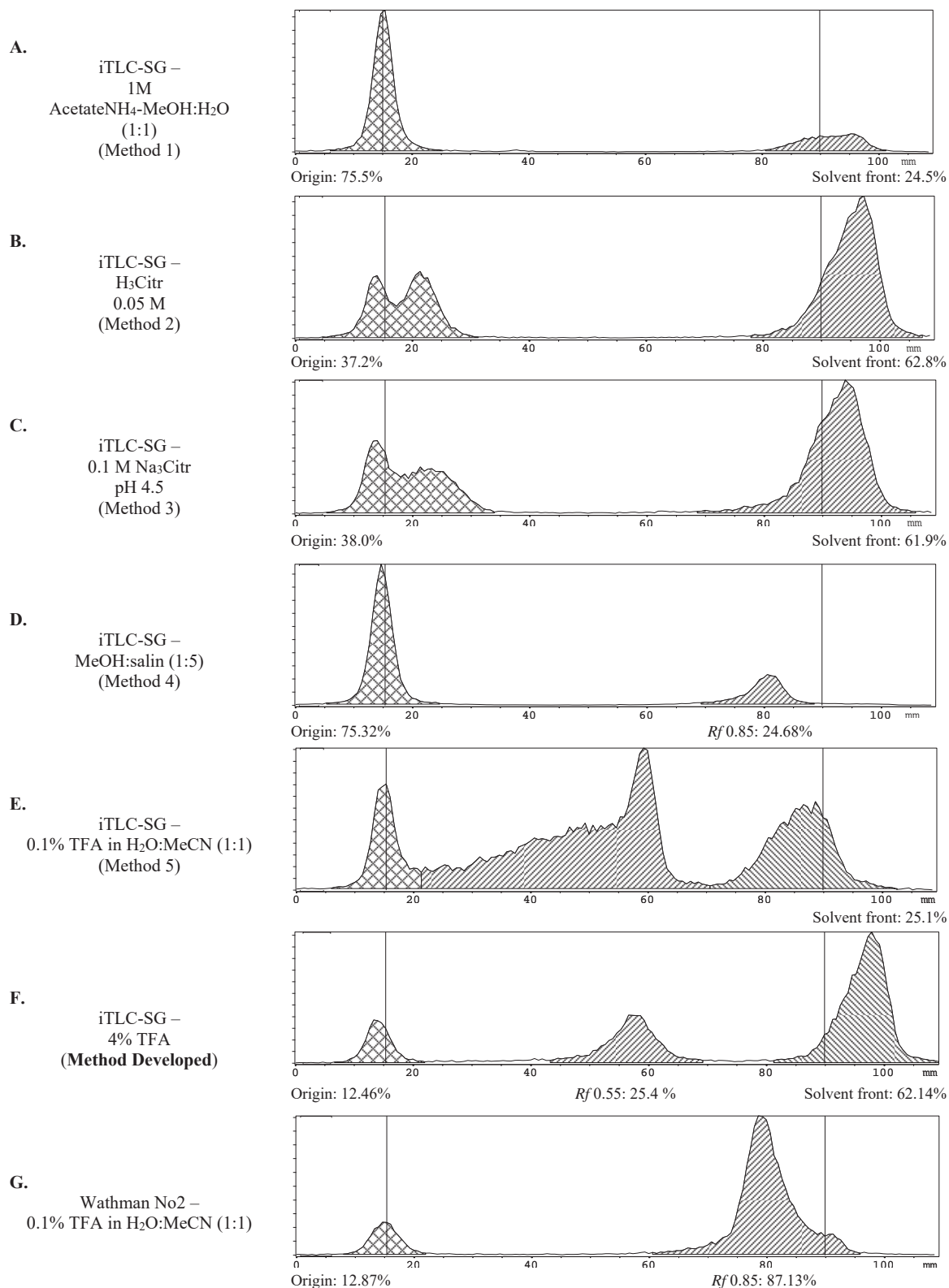


Fig. 3 Radio-chromatograms obtained in iTLC-SG – 4% TFA system for A. RP preparation of ^{68}Ga -DOTA-TATE (RCP 99%); B. Model preparation of ^{68}Ga -DOTA-TATE (RCP 53 %)

Fig. 4 Radio-chromatograms obtained in different TLC systems for the same ⁶⁸Ga-DOTA-TATE model preparation

At the same time, when using 4% TFA, ^{68}Ga -NODAGA-RGD, ^{68}Ga -NODAGA-RGD₂ complexes have R_f values of 0.7, 0.52. Nature of the chelating agent also has a significant impact on R_f of the complexes. For example, when using 4% TFA solution $R_f = 0.52$ for ^{68}Ga -NODAGA-RGD₂ ($M = 1707.8$ g/mol) and $R_f = 0.33$ for ^{68}Ga -DOTA-RGD₂ ($M = 1704.8$ g/mol).

R_f values for ^{68}Ga -colloid (0.0) and unbound ionic forms of ^{68}Ga (1.0) remain constant at various concentrations of TFA in the solvent and different values of pH of the test sample and may serve as a kind of reference points in the selection of optimal separation conditions for a particular labeled peptide. Other words, the system is versatile enough to carry an adequate analysis of a large number of RP based ^{68}Ga -BCA-peptides.

IV. CONCLUSION

A number of published TLC methods for ^{68}Ga -BCA-peptides QC was evaluated. All these systems failed to achieve acceptable separation of all components.

New method for determination of RCP of ^{68}Ga -BCA-peptides was developed. The method is easily performed, fast and informative and can be considered as an alternative to pharmacopoeial method.

It also was found that RP chemistry of gallium-68 has some uncertainties, which must be considered when carrying out QC procedures as well as when developing new RP. One of the underexamined points is the existence of so-called 'free ^{68}Ga ' in few different ionic forms (not only Ga^{3+}). This fact affects chromatographic behavior of ^{68}Ga , and it's should be taken into account when HPLC analysis is considered. Behavioral features of ^{68}Ga in terms of HPLC and other chromatographic techniques are the subject of further studies.

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