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Intricacies of the Determination of the Radiochemical Purity of ^{68}Ga Preparations: Possibility of Sorption of Ionic ^{68}Ga Species on Reversed-Phase Columns

A. A. Larenkov*[a], A. Ya. Maruk[a], and G. E. Kodina[a]

[a] *State Research Center Burnasyan Federal Medical Biophysical Center, Federal Medical Biological Agency of Russia, Zhivopisnaya ul. 46, Moscow, 123182 Russia*
**e-mail: anton.larenkov@gmail.com*

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Abstract—The results of studying ^{68}Ga radiopharmaceuticals using various TLC and HPLC procedures are compared. The data obtained reliably show that a part of ^{68}Ga ionic species are irreversibly (under definite conditions) sorbed onto chromatographic columns packed with C18 reversed phase. The loss of ^{68}Ga ionic species in the analysis can reach 90%. The ^{68}Ga loss increases with an increase in pH of the preparation. At pH 2.5–3.0, the total loss of ^{68}Ga ionic species on the chromatographic column does not exceed 15%. At pH 4.0, it is 65 ± 7% on the average, and at pH 6.0 it reaches 87 ± 8%. This effect should be taken into account in analysis of any ^{68}Ga radiopharmaceuticals.

Keywords: radiopharmaceuticals, gallium-68, radiochemical purity, quality control, chromatography, HPLC, complexation, sorption

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The generator procedure for producing ^{68}Ga, making this radionuclide readily available for medical institutions, and optimum nuclear-physical characteristics of ^{68}Ga ($T_{1/2}$ = 67.8 min, 90% β^+) stimulate steady interest in ^{68}Ga radiopharmaceuticals [1, 2]. First, studies dealing with ^{68}Ga radiopharmaceuticals involved developing and bringing into clinical practice octreotide derivatives (^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-TATE, etc.) for diagnostics of neuroendocrine tumors. This was followed by the active development of ^{68}Ga radiopharmaceuticals based on agonists and antagonists of various receptors, including RGD peptides (for angiogenesis imaging based on the density of integrin receptors) and many other "vector" molecules. Since 2010, the interest in ^{68}Ga radiopharmaceuticals resumed. It was associated with the development of labeled low-molecular-mass inhibitors of prostate-specific membrane antigen (PSMA) for prostate cancer diagnostics [3]. The rapid progress of the ^{68}Ga radiopharmaceutical chemistry allows the development of new ^{68}Ga radiopharmaceuticals and their more active introduction into the clinical practice to be expected [4–6].

Determination of the radiochemical purity (RCP) of radiopharmaceuticals is an extremely important part of their quality control both in routine clinical practice and in the development of new radiopharmaceuticals, when searching for the optimum labeling conditions [7, 8]. In the latter case, accurate quantitative determination of the content of each impurity is particularly important. The RCP of radiopharmaceuticals based on ^{68}Ga, as well as on many other radionuclides, is commonly determined by TLC and HPLC.

The nature of gallium leads to the formation of two main radiochemical impurities in radiopharmaceuticals: hydrolyzed ^{68}Ga ("colloid") and so-called "free" ^{68}Ga [1]. By "free" ^{68}Ga the whole set of ^{68}Ga ionic species that have not underwent complexation and hydrolysis is meant.

In the course of experiments on the development of efficient methods for analysis of the radiochemical composition of various ^{68}Ga radiopharmaceuticals by TLC (iTLC) [9], we have noted an interesting effect. In some cases, determination of free ^{68}Ga in the samples by HPLC gave strongly underestimated results compared to TLC [10]. Correspondingly, the RCP determined by HPLC was overestimated. This effect was

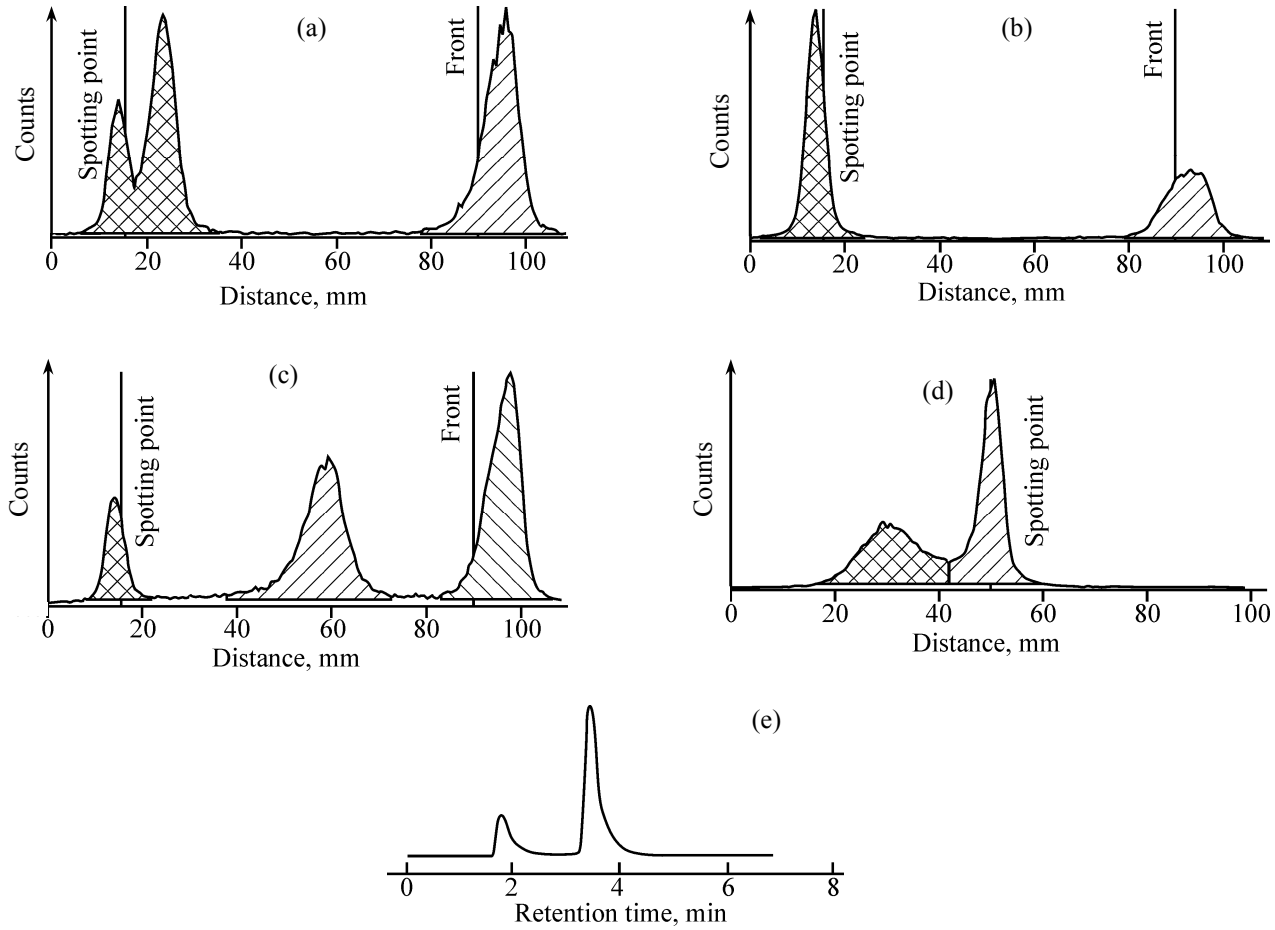

Fig. 1. Results of radiometry of ^{68}Ga-DOTA-TATE samples using different separation methods: (a) TLC: iTLC, 0.05 M H_3Citr; (b) TLC: iTLC, 1 M NH_4COOH in methanol–water mixture (1 : 1); (c) TLC: iTLC, 4% TFA (v/v); (d) electrophoresis: Whatman 3MM CHR paper, standard Veronal buffer; (e) HPLC: C_{18} column (150 × 3 mm), mobile phase 0.1% TFA in water (A), 0.1% TFA in acetonitrile (B), gradient chromatography: 0–10–15 min, 80–70–80% A.

particularly noticeable in the samples in which the content of ionic species was sufficiently high (>20%). Figure 1 shows as an example the results of analyzing the same [^{68}Ga]Ga-DOTA-TATE sample by different methods [10]. This sample was specially prepared under the conditions far from the optimum conditions for reaching high labeling yield [low precursor (DOTA-TATE) content, pH 5.5], so as to obtain a sample containing all the three major radiochemical forms of ^{68}Ga, characteristic of this class of radiopharmaceuticals.

TLC: iTLC, 0.05 M H_3Citr (Fig. 1a). The content of free ^{68}Ga (ionic species, R_f = 0.9–1.0) is 47%. The colloidal form of ^{68}Ga and [^{68}Ga]Ga-DOTA-TATE remain at the start of the chromatogram (R_f = 0.0–0.2, 53%).

TLC: iTLC, 1 M NH_4COOH in methanol–water mixture (1 : 1) (Fig. 1b). The content of [^{68}Ga]Ga-DOTA-TATE (R_f = 0.9–1.0) is 40%. The colloidal form of ^{68}Ga and free ^{68}Ga ionic species remain at the start of the chromatogram (R_f = 0.0–0.1, 60%).

TLC: iTLC, 4% CF_3COOH (TFA) (v/v) (Fig. 1c). The newly developed method. The colloidal form of ^{68}Ga remains at the start of the chromatogram (R_f = 0.0–0.1, 13%), [^{68}Ga]Ga-DOTA-TATE stops near the center of the chromatogram (R_f = 0.55, 40%), and the ionic species move with the solvent front (R_f = 0.9–1.0, 47%). The results obtained by this method fully agree with the results obtained by other TLC methods.

Electrophoresis: Whatman 3MM CHR paper, standard Veronal buffer (Fig. 1d). This is a nontrivial method; it is not used in routine analysis of ^{68}Ga radiopharmaceuticals. It was used for additionally con-

firming the content of ^{68}Ga ionic species in the sample. The relative area of the peak that moved from the start of the electrophoregram is about 45% (which confirms the correctness of the determination of the free ^{68}Ga content).

HPLC: C18 column (150 × 3 mm). Mobile phase: 0.1% TFA in water (A), 0.1% TFA in acetonitrile (B), gradient chromatography: 0–10–15 min, 80–70–80% A (Fig. 1e). This method is the closest analog of the method of European Pharmacopoeia [11] for monitoring the quality of [^{68}Ga]Ga-DOTA-TATE. The retention factor of free ^{68}Ga was 1.05 (21%), and that of [^{68}Ga]Ga-DOTA-TATE, 2.07 (79%).

Because this effect was frequently observed in experiments with various ^{68}Ga radiopharmaceuticals, we attempted to determine whether it has a chemical origin or is associated exclusively with shortcomings of

EXPERIMENTAL

^{68}Ge/^{68}Ga generator. We used two ^{68}Ge/^{68}Ga generators based on TiO_2 modified with 4% ZrO_2, (Cyclotron, Obninsk, Russia) with the initial activity of 740 MBq (6 and 12 months after fabrication).

Chemicals and precursors. Only deionized water was used (resistivity 18 MΩ cm, TKA Smart2Pure, Germany). All the chemicals were of pharma, analytical, or HPLC grade, or were indicated as high-purity by the producers (Panreac, Spain; Sigma–Aldrich, the United States). The chelators, 1,4,7-triazacyclononane-*N*,*N*',*N*''-triacetic acid (NOTA) and 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA), were supplied by CheMatech (France), and the conjugates, DOTA-TATE and PSMA-617, by ABX (Germany).

Measurement of the ^{68}Ga activity. The absolute activity of ^{68}Ga was measured with RIS-A1 (Amplituda Scientific and Technical Center, Russia) and Atomlab™ 500 (Biodex, the United States) dose calibrators. The relative activity (counting rate) of the samples was measured with an RFT 20046 radiometer equipped with an NaI detector (Veb Robotron-Messelektronik, Germany), and also with a 2480 Wizard^2 automatic γ-counter (Perkin Elmer, the United States) equipped with an NaI(Tl) detector.

Purification and concentration of the eluate from the ^{68}Ge/^{68}Ga generator, preparation of radiopharmaceuticals of required quality. The eluate from the ^{68}Ge/^{68}Ga generator was conditioned (purified and concentrated) by the HCl–ethanol [12] or HCl–acetone [13] method with a Modular-Lab PharmTracer module (Eckert & Ziegler, Germany). The [^{68}Ga]Ga-DOTA-TOC/TATE and [^{68}Ga]Ga-DOTA-PSMA (PSMA-617) radiopharmaceuticals of required quality (RCP ≥ 95 %) were also prepared with this automated module.

Preparation of samples containing no chelating molecules. To minimize the ^{68}Ga hydrolysis in the samples containing no chelating molecules at pH ≥ 2, the eluate from the ^{68}Ge/^{68}Ga generator was mixed with 0.2 M sodium acetate or 0.1 M HEPES {2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid} solution (pH 7.5 or 12) in various ratios, so as to obtain the required pH value. Prior to starting the analysis, the samples were kept at room temperature with continuous stirring for 10–15 min.

Preparation of model samples. To obtain model samples of labeled compounds [^{68}Ga]Ga-DOTA-TOC/TATE and [^{68}Ga]Ga-DOTA-PSMA, we added preliminarily prepared ^{68}Ga solutions containing buffer agents only (sodium acetate or HEPES) or the eluate from the ^{68}Ge/^{68}Ga generator of required activity to the samples with RCP 95–100%. By this procedure, we prepared samples differing both in the ^{68}Ga speciation and in pH.

TLC. Several different chromatographic systems were used for determining the radiochemical purity (and/or ratio of chemical species) of ^{68}Ga preparations by TLC (Table 1). In most cases, silica gel impregnated fiberglass strips (iTLC-SG, Varian and Thermo Fisher Scientific, the United States) were used as a stationary phase.

In parallel, the RCP was determined by TLC using at least three systems indicated in Table 1. The results thus obtained coincided with 2% accuracy in all the cases. System 3 [11] was used in analysis of samples containing radiopharmaceutical precursors (DOTA-TATE, DOTA-PSMA) and chelators (DOTA, NOTA). The activity distribution along chromatographic strips was determined with a miniGITA radiochromatogram scanner (Raytest, Germany).

HPLC. HPLC measurements were performed with a Knauer Smartline liquid chromatograph (Germany) equipped with an fLumo radiometric detector (Berthold, Germany). The mobile phase (eluent) consisted

Table 1. Description of the chromatographic systems used in TLC analysis

System	Description	R_f			
		hydrolyzed ^{68}Ga (colloid)	[^{68}Ga]Ga-DOTA-TATE/PSMA	^{68}Ga ionic species	[^{68}Ga]Ga-DOTA/NOTA
1	iTLC-SG, 0.05 M H_3Citr and iTLC-SG, 0.1 M Na_3Citr (pH 4–5)	0.0	0.0–0.2	0.9–1.0	0.9–1.0
2	iTLC-SG, 3–5% TFA (v/v)	0.0	0.4–0.6	0.9–1.0	0.9–1.0
3	iTLC, 1 M MH_4COOH in methanol–water mixture (1 : 1)	0.0	0.9–1.0	0.0–0.1	0.9–1.0
4	Whatman 2 CHR, 0.1% TFA in water–acetonitrile mixture (1 : 1)	0.0	0.9–1.0	0.8–1.0	0.9–1.0

of a mixture of a 0.1% aqueous TFA solution (solution A) and 0.1% TFA solution in acetonitrile for liquid chromatography (solution B). These solutions were chosen as the most widely used in analysis of ^{68}Ga-labeled compounds [7, 11, 14]. The column thermostat temperature was 40°C in all the cases.

The following conditions were used for analysis of the labeled ^{68}Ga conjugates ([^{68}Ga]Ga-DOTA-TATE, [^{68}Ga]Ga-PSMA-617). Chromatography was performed in the gradient mode in accordance with the following programs: 0–10–15 min, 80–70–80% solution A for [^{68}Ga]Ga-DOTA-TATE; 0–5–8–10–11–16 min, 0–0–50–50–0–0% solution A for [^{68}Ga]Ga-PSMA-617. In the course of these experiments, the results were reproduced on three columns packed with the sorbent with reversed C18 phase (particle size 5 μm, pore size 100 Å: 100 × 4.6 mm, Chromolith Performance, Merck, Germany; 150 × 3 mm, Luna, Phenomenex Inc., the United States; and 150 × 4.6 mm, ACE, Advanced Chromatography Technologies Ltd., the United Kingdom). The eluent flow rate was 1 mL min^{-1} for the Chromolith Performance and ACE columns and 0.5 mL min^{-1} for the Luna column.

The samples containing no radiopharmaceutical precursors were analyzed using the Chromolith Performance and ACE columns in the isocratic mode (100, 90, or 70% A). The eluent flow rate in all these experiments was 1 mL min^{-1}.

Procedure for determining the ratio of the activity eluted from the chromatograph to the injected activity (recovery). Five replicate samples were measured for each preparation in one series of experiments. Simultaneously with the sample injection into the liquid chromatograph, the ^{68}Ga speciation in the sample was analyzed by TLC (systems, 1, 2, and 4, Table 1) to take into account the relative content of the ^{68}Ga colloidal form, which is not determined by HPLC. The eluate (~10 mL for samples without radiopharmaceutical precursors) was collected from the injection moment until the end of the measurement (elution time of the activity peak + time required for the sample to travel the distance from the radioactivity detector to the chromatograph outlet). Aliquots of the same preparation with the volume equal to that of the chromatograph loop (20 μL) were used as reference samples. The aliquots were taken with a calibrated Eppendorf Reference 2 pipette (Eppendorf AG, Germany). The recovery (r, %) was determined as the ratio of the mean counting rate of the samples collected at the chromatograph outlet ($\bar{I}_j$, cps, n = 5–20) to that of the reference samples ($\bar{I}_i$, cps, n = 5–20) (minus the percentage of the ^{68}Ga colloidal form), using the formula:

$$r = 100\% \times \bar{I}_j/[\bar{I}_i\omega(^{68}Ga^{ion})],$$

where $\omega(^{68}Ga^{ion})$ is the fraction of ^{68}Ga ionic species in the preparation, determined by TLC.

RESULTS AND DISCUSSION

To make sure that the radioactivity is not retained in the injection loop and in other parts of the chromatograph and that there are no other instrumental errors, we first performed experiments to evaluate the reproducibility and recovery. To this end, we used samples containing both ^{68}Ga ionic species and stable charged and neutral ^{68}Ga complexes. In the first case, we used samples of the initial eluate from the ^{68}Ge/^{68}Ga generator and of purified and concentrated ^{68}Ga solution [12, 13] ($^{68}GaCl_3$ solution in 0.1 M HCl). In the second case, we used solutions of ^{68}Ga-NOTA and ^{68}Ga-DOTA. The choice of cyclic chelators NOTA and DOTA was governed by high stability of their com-

plexes with Ga(III), on the one hand, and by the fact that the ^{68}Ga complex was recommended by the European Pharmacopoeia as a reference sample in the quality control of ^{68}Ga-DOTA-TOC [11], on the other hand. To minimize the instrumental errors associated with the attainment of the equilibrium between two successively analyzed samples in the column, we used the isocratic elution mode.

The experimental data (Table 2) show that, in the cases of both the initial eluate from the ^{68}Ge/^{68}Ga generator and the solution of ^{68}Ga-NOTA, the injected activity leaves the chromatograph virtually completely; the error does not exceed 1.5%.

Thus, apparently, all the losses observed in the further experiments are due specifically to prolonged retention or nonspecific sorption on the chromatograph column.

When analyzing ^{68}Ga-NOTA solutions, the chromatogram has a single symmetrical peak with the retention factor of 1.18 (uncharged complex). It should be noted that, when analyzing the eluate from the ^{68}Ge/^{68}Ga generator by HPLC, we repeatedly observed an unusual phenomenon: separation of the eluate into two peaks with the retention factors of 1.05 ± 0.05 and 1.41 ± 0.10. The ratio of these peaks, as a rule, is constant for the samples obtained in the course of the same generator run (elution and conditioning). However, for the samples obtained in different generator runs, the ratio of these peaks varied up to obtaining a single peak in the chromatogram, as a rule, with the retention factor of 1.05 ± 0.05. Examples of various chromatograms obtained in HPLC analysis of the eluate from the ^{68}Ge/^{68}Ga generator in the isocratic mode (100% A) are shown in Fig. 2.

Such chromatographic behavior can be caused both by the existence of ^{68}Ga in different ionic forms, some of which can be shortly retained on the reversed phase, and by other, yet unclear, factors. Until now, we attempted to determine the influence exerted on the ratio of these peaks by the following factors: time of the previous generator elution, eluate conditioning, eluate dilution, etc. No regular trends were revealed in these experiments. However, in the context of this study it is important that, irrespective of variations in the chromatographic behavior, the eluate from the ^{68}Ge/^{68}Ga generator in 0.1 M HCl is completely eluted from the chromatographic column; i.e., the peak ratio does not influence *r*.

Table 2. Results of studying the reproducibility and recovery *r*. Numerator: reference samples (pipette); denominator, test samples (chromatograph); (δ) relative error of a series of replicate measurements, %; (Δ) difference between the injected activity and activity at the outlet, %

Parameter	Eluate from the ^{68}Ge/^{68}Ga generator ($^{68}GaCl_3$ in 0.1 M HCl)	^{68}Ga-NOTA solution (acetate buffer, pH 5.0, RCP ≥ 99.0%)
n	20/20	10/10
δ, %	0.93/0.81	0.85/0.79
Δ, %	1.5	0.81

In the second (main) step of the study, we examined the dependences of *r* in HPLC on the acidity of the samples containing no chelating molecules. As such samples we took mixtures of the eluate from the ^{68}Ge/^{68}Ga generator with a 0.2 M sodium acetate or 0.1 M HEPES solution (pH 12). These solutions were

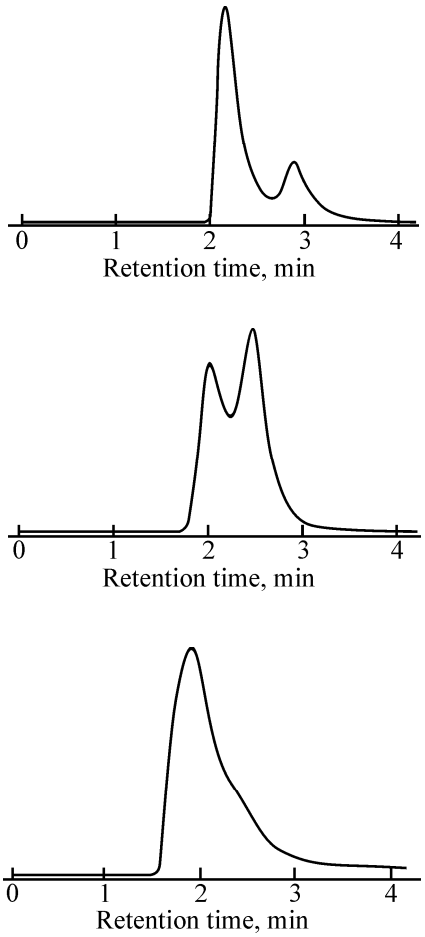

Fig. 2. Examples of chromatograms obtained in analysis of the eluate from the ^{68}Ge/^{68}Ga generator in the isocratic mode (100% A).

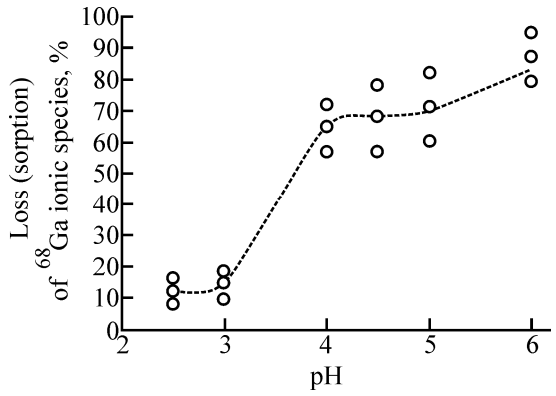

Fig. 3. pH dependence of the loss (sorption) of ^{68}Ga ionic species on an ACE column (buffer: 0.2 M solution of sodium acetate).

chosen because acetate and HEPES are the most frequently used buffer agents in studies on the development of new ^{68}Ga radiopharmaceuticals [7]. The dependence of the radioactivity loss in the course of the HPLC analysis on the acidity of the samples prepared using a 0.2 M sodium acetate solution is shown in Fig. 3.

As can be seen, when using the acetate buffer, partial sorption of ^{68}Ga ionic species on the reversed-phase HPLC column is observed even at low pH values (2.5–3): 12 ± 6% (compare it to the complete elution of the activity from the chromatograph when studying samples of the eluate from the ^{68}Ge/^{68}Ga generator and of the complexes ^{68}Ga-NOTA and ^{68}Ga-DOTA). The percentage of the ^{68}Ga ionic species sorbed on the column sharply increases with increasing pH. At pH 4.0 (the most frequently used value in synthesis of ^{68}Ga radiopharmaceuticals), the loss of the ^{68}Ga ionic species at the chromatograph outlet was 65 ± 7% on the average, and at pH 6.0 it reached 87 ± 8%. The results of these experiments were reproduced on different chromatographic columns (ACE and Cromolith).

The data of [15] suggest possible formation of [^{68}Ga]Ga–acetate associates, which can be retained on the column, at low concentrations of gallium ions (the molar concentration of gallium in the eluate from the ^{68}Ge/^{68}Ga generator is ~10^{-3} nM) and relatively high acetate concentrations (0.07–0.2 M). In this case, it is logical to assume that retention of such associates can be reduced by adding acetonitrile into the eluent. Therefore, we performed a series of HPLC experiments using A : B ratios of 90 : 10 and 70 : 30. As in the previous experiments, chromatography was performed in the isocratic mode. Experiments were performed with a ^{68}Ga solution in acetate buffer with pH 4.5. However, as we found, the loss in the chromatograph column only increased on adding acetonitrile to the HPLC eluent. For example, in one of the experimental series the loss was 58 ± 2% without acetonitrile, 66 ± 3% with 10% acetonitrile, and 80 ± 5% with 30% acetonitrile in the eluent.

It is impossible to study this effect in batch sorption experiments because of virtually zero wetting of octadecyl-modified silica gel with aqueous solutions. Therefore, to confirm the effect observed on an HPLC column, we performed dynamic experiments using ready commercial C_{18} cartridges (CHROMAFIX® C_{18}-S, Macherey-Nagel). Both the initial (dry) cartridges and the cartridges preliminarily activated with 5 mL of a 0.1% aqueous TFA solution (for more accurate simulation of the process occurring in HPLC) were used. The fraction of the activity retained on the C_{18}-S cartridge when using the eluate from the ^{68}Ge/^{68}Ga generator and the ^{68}Ga solution (acetate, ^{68}Ga; content of ^{68}Ga ionic species ≥99%, pH 5.5) was, respectively, 1.9 ± 0.7% (n = 5) and 18 ± 4% (n = 10) for dry cartridges and 2.7 ± 0.9% (n = 5) and 49 ± 17% (n = 10) for activated cartridges. As can be seen, even on small reversed-phase cartridges the behavior of the ^{68}Ga ionic species is the same as in HPLC. These results additionally confirm the chemical nature of the effect observed.

HEPES is another buffer agent for preparing ^{68}Ga radiopharmaceuticals, used along with sodium acetate. The majority of authors believe that HEPES is preferable in synthesis of ^{68}Ga radiopharmaceuticals, because it ensures the highest labeling yields. Therefore, we also performed experiments using this buffer agent.

In contrast to the solutions in acetate buffer, samples with HEPES (pH 3–7) containing no chelating agents are characterized by gradual formation of colloidal ^{68}Ga, up to complete hydrolysis of all the ^{68}Ga ionic species. This fact severely complicated the experiments and data processing. Nevertheless, the experimental data show that the effect observed when using acetate buffer is also observed with HEPES. However, in contrast to acetate buffer, we were unable to obtain explicit pH dependence of the fraction of the ^{68}Ga ionic species retained on the column. For the observed samples, the mean loss of the ^{68}Ga ionic species

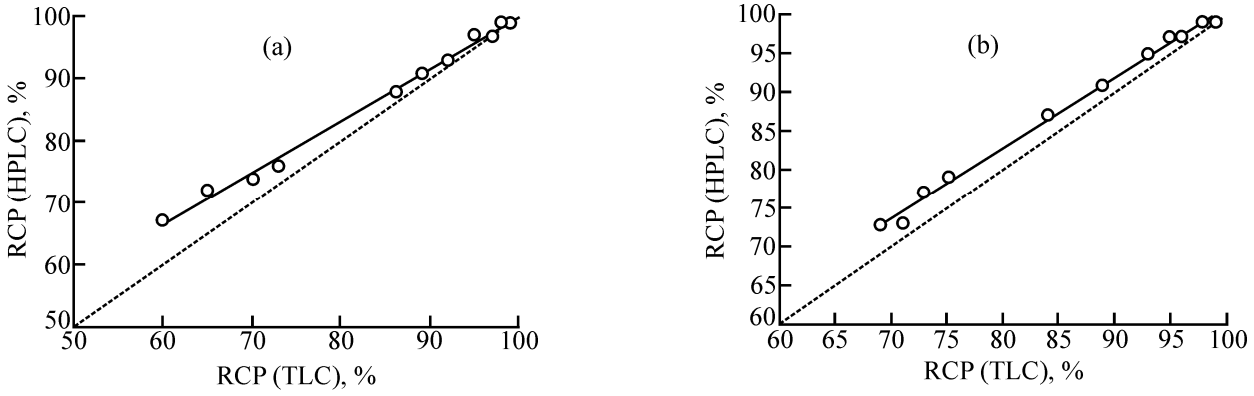

Fig. 4. Correlation between the TLC and HPLC data on RCP of (a) [^{68}Ga]Ga-DOTA-TATE and (b) [^{68}Ga]Ga-DOTA-PSMA samples at pH 1.0–2.5. Regression equations: (a) $y = 0.8387x + 16.187$ ($R^2 = 0.9952$) and (b) $y = 0.9036x + 10.525$ ($R^2 = 0.995$).

on the chromatographic column at pH in the range 3–6 was 47 ± 10%.

Comparison of the data obtained using acetate buffer (Fig. 3) and HEPES suggests that the observed effect may be due not only to the transformation of cationic gallium species ($^{68}Ga^{3+}$) into anionic species ($[^{68}Ga(OH)_4]^-$) via a series of intermediate species [16, 17], but also, probably, to the formation of ^{68}Ga associates with the acetate anion [15].

Comparative analysis of the data obtained for the model samples of [^{68}Ga]Ga-DOTA-TATE and [^{68}Ga]Ga-DOTA-PSMA (PSMA-617) by TLC (systems 1–3, Table 1) and HPLC shows that the results correlate with each other well at pH in the range from 1.0 to 2.5 (Fig. 4). Nevertheless, the equation of the regression line does not pass through the origin: The HPLC data are somewhat overestimated relative to the TLC data at low values of RCP (at high content of ^{68}Ga ionic species). However, this overestimation is not regular. With increasing pH, this tendency becomes steady and significant. The overestimation of the HPLC data is caused by the same effect, partial retention of ^{68}Ga ionic species on the chromatographic column.

The RCP values of model [^{68}Ga]Ga-DOTA-TATE samples prepared using acetate buffer, evaluated with TLC and HPLC, are compared in Fig. 5. In this case, RCP was determined as the percent ratio of the complex [^{68}Ga]Ga-DOTA-TATE and free ^{68}Ga (without taking into account the colloidal form).

As seen from the experimental data, the difference between the TLC and HPLC results becomes significant already at pH 3 and increases with pH, which agrees with the results of the experiment performed without precursor (Fig. 3). At pH 5, there is no difference between the TLC and HPLC data, because under these conditions the complexation occurs at the highest rate and with the highest yield, so that it is extremely difficult to adequately simulate the presence of Ga ionic species in this point.

Figure 6 shows the results of determining RCP of model [^{68}Ga]Ga-DOTA-TATE samples prepared using HEPES as a buffer. As can be seen, with HEPES the dependence is similar. In the presence of chelating agents (radiopharmaceutical precursors), the tendency toward retention of ^{68}Ga ionic species on the reversed phase at different pH values is preserved, but the degrees of retention are somewhat different. Most probably, the presence of one or another chelating agent influences the distribution of ^{68}Ga ionic species in the solution and their relative amount.

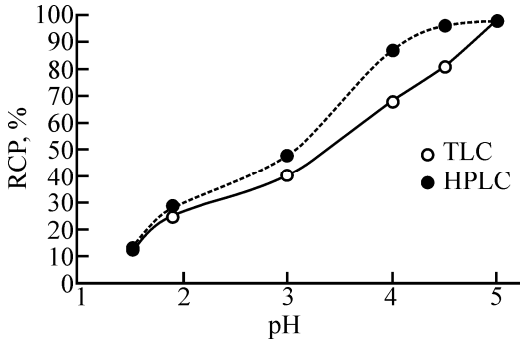

Fig. 5. Comparison between the TLC and HPLC data on RCP of model [^{68}Ga]Ga-DOTA-TATE samples at different pH values (acetate buffer).

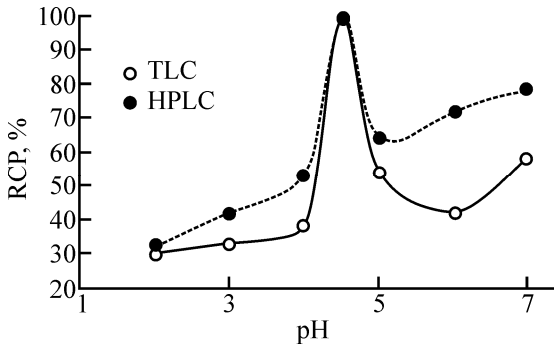

Fig. 6. Comparison between the TLC and HPLC data on RCP of model [^{68}Ga]Ga-DOTA-TATE samples at different pH values (HEPES).

Further studies are required to determine whether the observed effect is associated with true sorption (similar to nonexchange sorption of gallium on ion-exchange resins [12, 18]), formation of associates of ^{68}Ga with buffer agents [15, 19], or hydrolysis of certain ^{68}Ga ionic species on contact with the reversed phase of the column.

We are aware of only a single paper [14] in which similar effects are observed. In that paper, the correlation between the results of determining the free ^{68}Ga in ^{68}Ga-DOTA-NOC by TLC [iTLC (Varian iTLC-SG), 0.1 M citrate buffer, pH 4–5] and HPLC is evaluated. In the RCP range from 85 to 100%, the TLC and HPLC data correlate with $R = 0.961$. Nevertheless, the published results do not refute our data. On the one hand, in [14] the content of free ^{68}Ga in the samples was varied by adding various amounts of the eluate from the ^{68}Ge/^{68}Ga generator ($^{68}GaCl_3$ in 0.05 M HCl) to ready ^{68}Ga-DOTA-NOC sample. The ready ^{68}Ga-DOTA-NOC sample was prepared by conditioning through a C_{18} cartridge; it was an isotonic NaCl solution with a minor ethanol amount, and its volume was 6 mL. Thus, apparently, addition of the initial eluate from the ^{68}Ge/^{68}Ga generator to this sample shifts the pH toward more acidic values (most probably, in the range 1.5–3.0). According to our data, in this pH range the sorption of ^{68}Ga ionic species on the reversed phase is extremely low, and the correlation between the TLC and HPLC data is good. On the other hand, according to the data of [14], the higher is the content of free ^{68}Ga in the sample, the larger is the difference between the results of its determination by TLC and HPLC: 98.5% TLC–98.7% HPLC and 87.6% TLC–84.8% HPLC. Also, as assumed above, the observed effect depends not only on pH of the sample, but also on the relative amount of ^{68}Ga ionic species.

Thus, we have shown that ^{68}Ga ionic species can be captured to a considerable extent on the reversed-phase HPLC column at pH in the range from 2 to 6. Hence, HPLC does not ensure the required reliability in analysis of ^{68}Ga radiopharmaceuticals. This is particularly important when radiopharmaceuticals that are not subjected to additional purification (e.g., by solid-phase extraction) are concerned, such as, e.g., radiopharmaceuticals prepared from freeze-dried samples. Therefore, it is preferable to determine the ^{68}Ga speciation in its preparations using TLC.

It should be noted in addition that, as we showed previously [9], the use of acid solutions of ^{68}Ga (initial eluate of the ^{68}Ge/^{68}Ga generator or conditioned ^{68}Ga solutions) as reference samples in analytical procedures is not appropriate. pH not only plays the key role in synthesis of target radiopharmaceutical complexes, but also affects the behavior of ^{68}Ga ionic species and, as a consequences, even the analysis results.

With all the advantages of the HPLC method, it should be taken into account that this method, when applied to radiopharmaceuticals based on ^{68}Ga and, possibly, also on other metal radionuclides (e.g., ^{111}In, ^{177}Lu, etc.), can give inadequate data on the content of ionic species that have not underwent complexation.

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