molecules

MDPI

Supplementary materials for:

Preparation of Zirconium-89 Solutions for Radiopharmaceutical Purposes: Interrelation Between Formulation, Radiochemical Purity, Stability and Biodistribution

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Table S1. Radio-chromatograms of different zirconium-89 – oxalic acid preparations with iTLC-SG / CH_3OH-H_2O (1:1), 4% TFA (v/v) system.

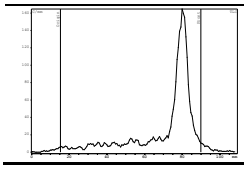
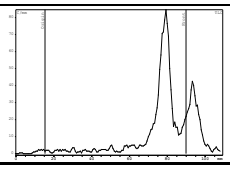
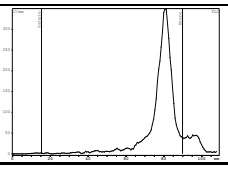
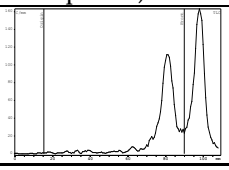
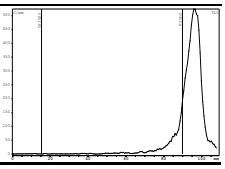
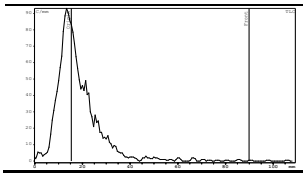
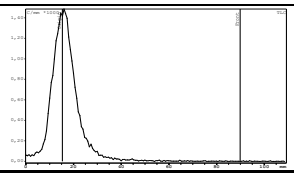
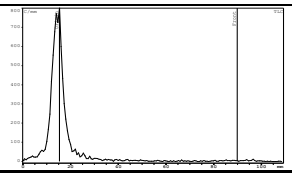
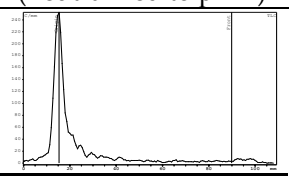
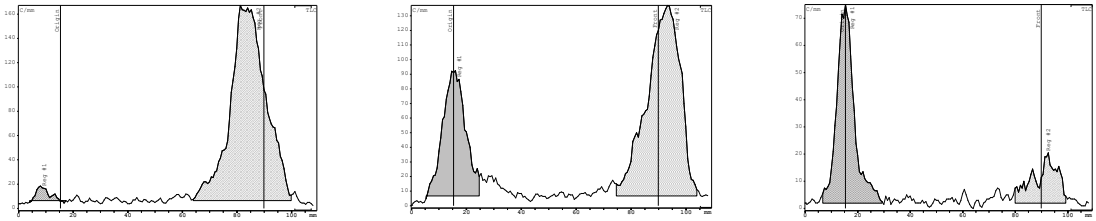
[^{89}Zr]Zr-oxalate 0.1 M oxalic acid	[^{89}Zr]Zr-oxalate 0.1 M oxalic acid (neutralized to pH~7)	[^{89}Zr]Zr-oxalate 0.27 M oxalic acid	[^{89}Zr]Zr-oxalate 0.27 M oxalic acid (neutralized to pH ~7)	[^{89}Zr]Zr-oxalate 1.0 M oxalic acid
				

Table S2. Radio-chromatograms of different zirconium-89 – HCl preparations with iTLC-SG / CH3OH-H2O (1:1), 4% TFA (v/v) system.

[^{89}Zr]$ZrCl_4$ in 5 M HCl	[^{89}Zr]$ZrCl_4$ in 1 M HCl	[^{89}Zr]Zr in 0.1 M HCl	[^{89}Zr]Zr in 0.1 M HCl (neutralized to pH 7)
			

These samples were prepared from [^{89}Zr]$ZrCl_4$ in 5 M HCl initial solution obtained by the manufacturer using oxalate-free method (with Dowex 1×8 anion-exchange resin)



(a) **(b)** **(c)**

Figure S1. Radio-chromatograms of different zirconium-89 preparations in HCl (iTLC-SG / 50 mM DTPA pH 7.0): (a) 5 M HCl; (b) 1 M HCl; (c) 0.1 M HCl.

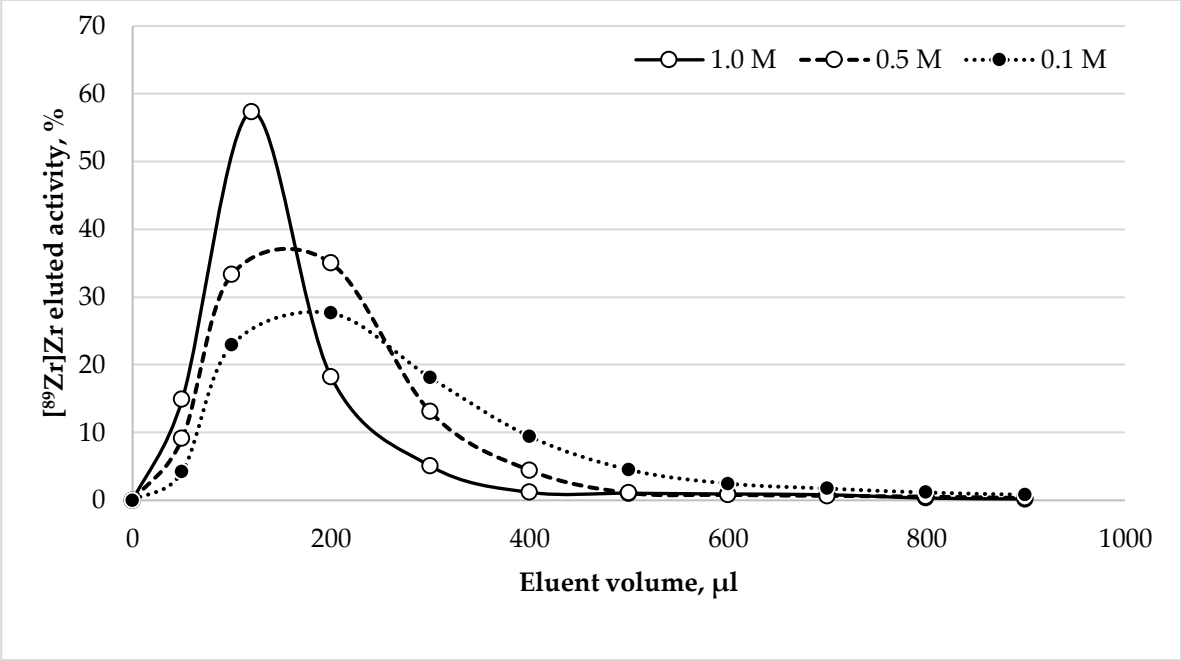
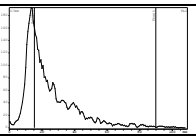
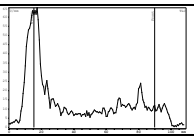
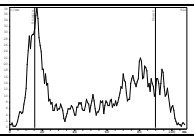
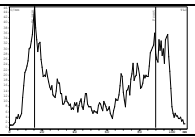
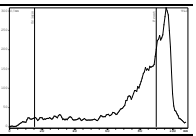
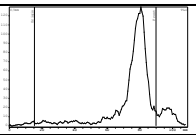
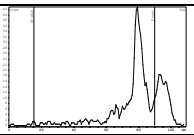
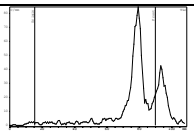
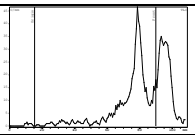
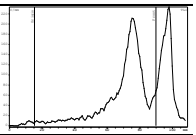


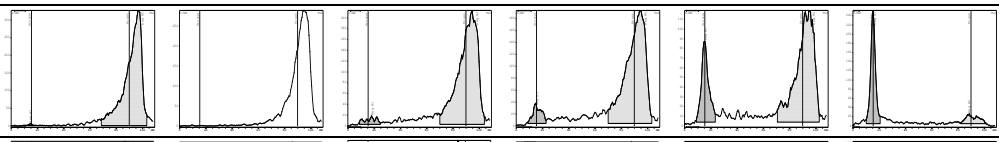
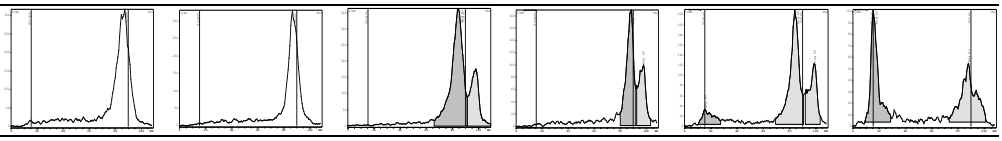
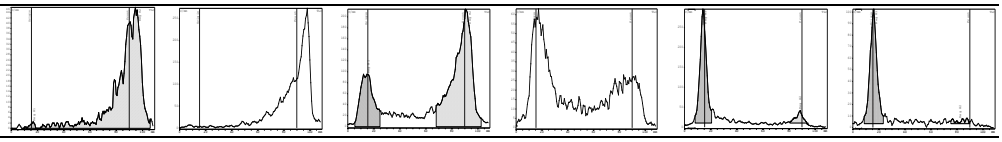
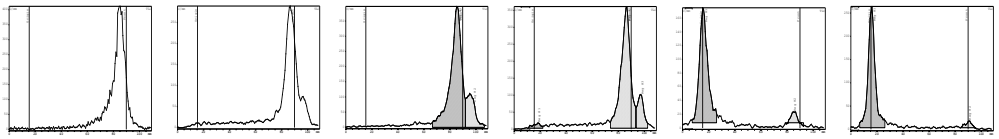
Figure S2. Elution profiles of ZR-resin column (50 mg, 50 mm × Ø2.1 mm) with oxalic acid of different concentrations.

Table S3. Radio-TLC analysis results of [^{89}Zr]Zr-oxalate preparations obtained with ZR-resin and solutions of different oxalic acid concentrations (final pH of all preparations was 6.5).

Oxalic acid concentration in the sample (moles/L)					
	0.1	**0.2**	**0.3**	**0.4**	**0.5**
method 1					
%§	100	~55	~40	~35	~10
method 2					
%§§	~10	~30	~35	~45	~50

§ % of zirconium-89 activity at the origin of chromatogram; §§ % of zirconium-89 activity at R*f* 1.0 peak.

Table S4. Radio-TLC analysis results for [^{89}Zr]Zr-oxalate preparations obtained without and after ZR-resin.

[^{89}Zr]Zr-oxalate in 0.1 M oxalic acid (without ZR-resin)						
pH	1	3	5	6	7	8
Method 1						
Method 2						
[^{89}Zr]Zr-oxalate in 0.1 M oxalic acid (after ZR-resin)						
pH	1	3	5	6	7	8
Method 1						
Method 2						

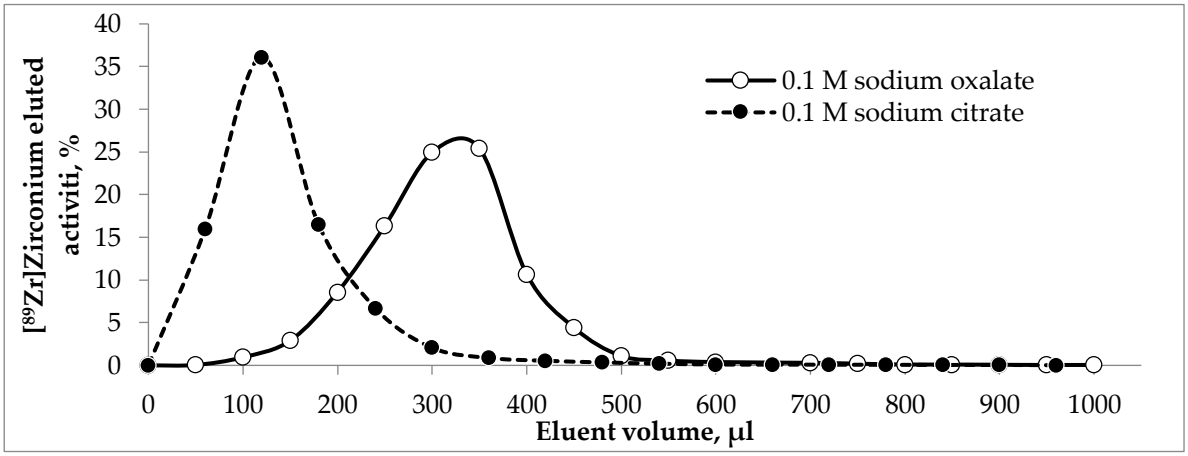


Figure S3. Chelex-100 column elution profile using 0,1 M Na_3Citr and 0.1 M $Na_2C_2O_4$ as eluents (50 mm ×Ø2.1 mm column, 60 mg of resin, load from 0.5 M oxalic acid medium (ZR-resin), eluent flow rate – 1 mL/min).

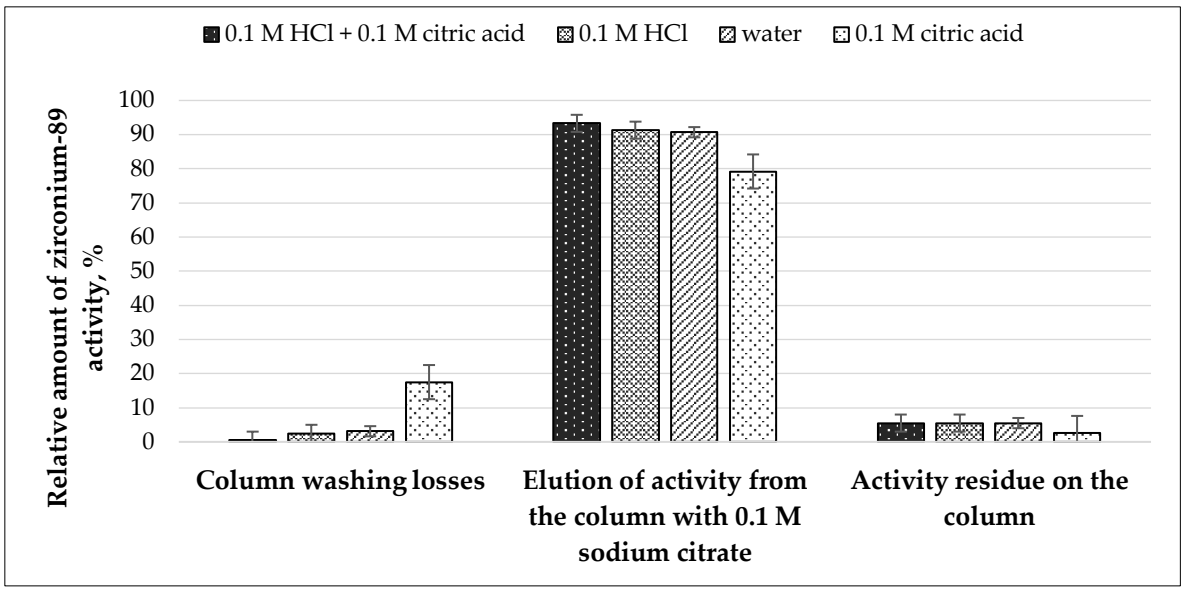
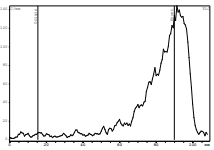
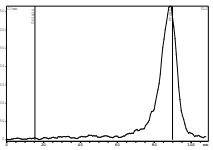
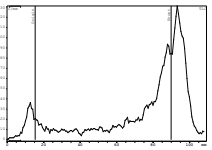
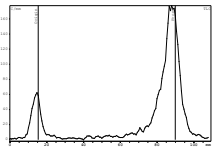
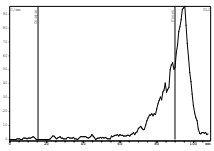
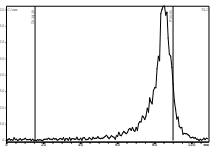
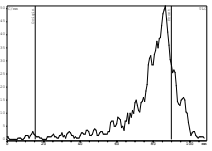
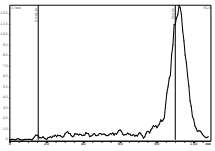


Figure S4. The effect of various solutions used for column wash on recovery efficiency of zirconium-89 with Chelex-100 resin (50 mm ×Ø2.1 mm column, 60 mg of resin, load from 0.5 M oxalic acid medium (ZR-resin), losses at the sorption step – 0.7 %, eluents flow rate – 1 mL/min).

Table S5. Radio-chromatograms of different [^{89}Zr]Zr-citrate preparations.

	pH			
	4.5		**6.5**	
	Method 1	*Method 2*	*Method 1*	*Method 2*
[^{89}Zr]Zr-citrate in 0.1M citric acid				
[^{89}Zr]Zr-citrate in 1.0 M citric acid				

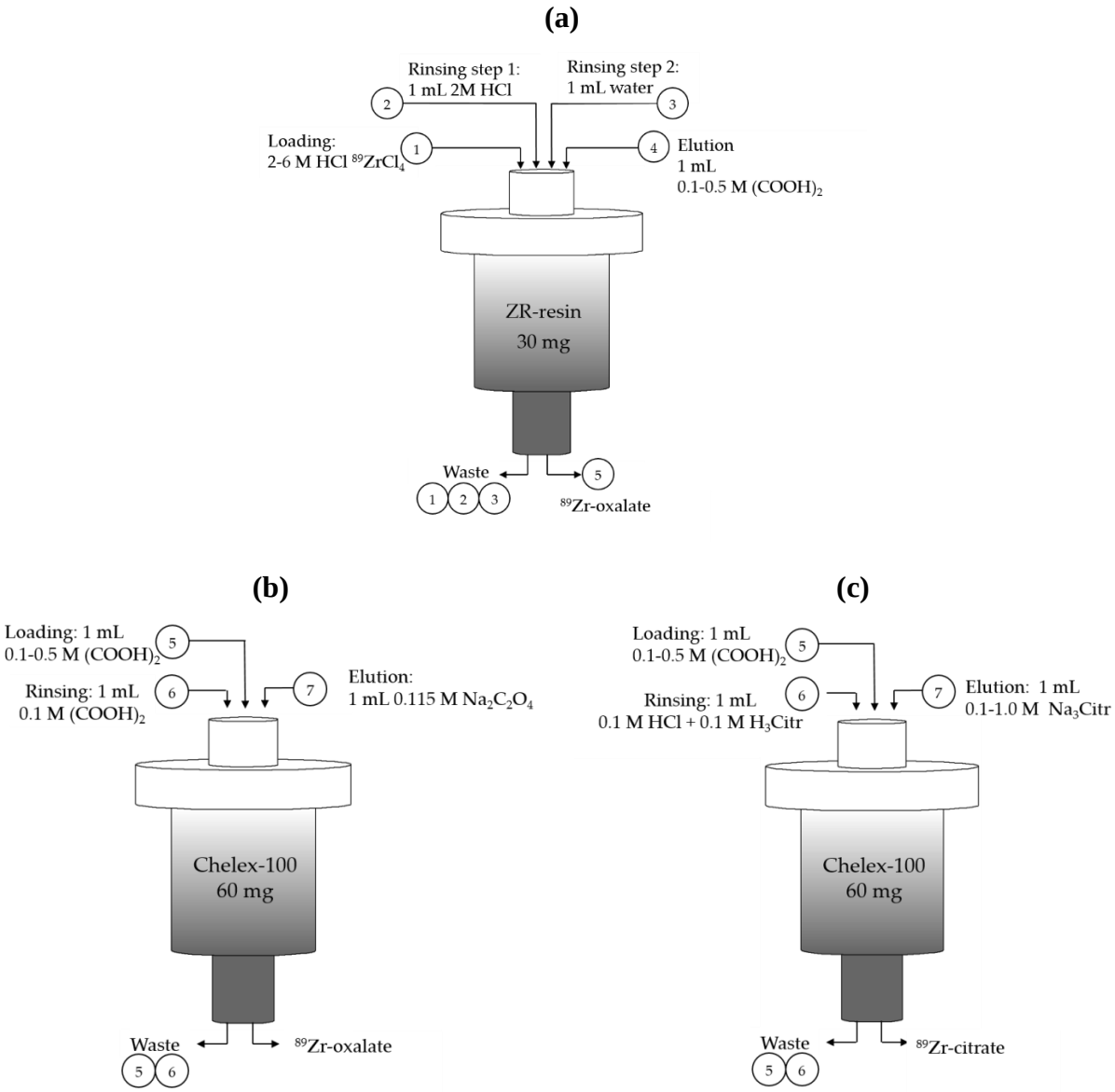


Figure S5. The scheme of methods proposed for preparation of zirconium-89 solutions for radiopharmaceutical purposes: **(a)** using ZR-resin only (zirconium-89 is obtained in 0.1-0.115 M oxalic acid); **(b)** using combination of ZR-resin and Chelex-100 (zirconium-89 is obtained in 0.1-0.116 M sodium oxalate); **(c)** using combination of ZR-resin and Chelex-100 (zirconium-89 is obtained in 0.1-1.0 M sodium citrate).

Table S6. Radio-TLC analysis results of [^{89}Zr]Zr-oxalate preparations during their storage.

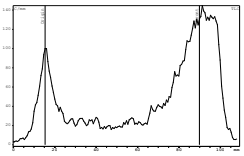
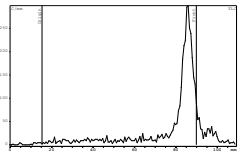
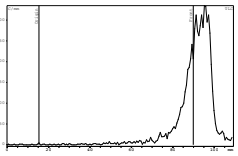
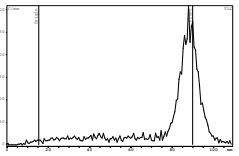
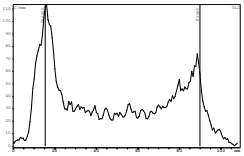
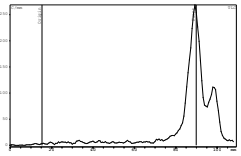
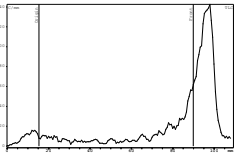
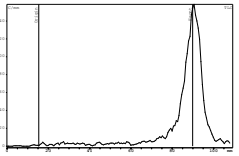
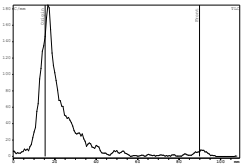
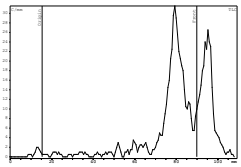
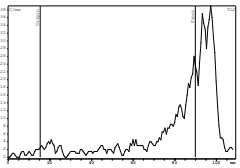
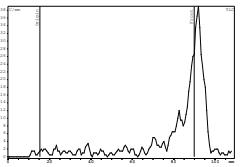
	[^{89}Zr]Zr-oxalate in 0.1 M oxalic acid pH 6			
	Only ZR-resin		**ZR-resin + Chelex-100 resin**	
	Method 1	*Method 2*	*Method 1*	*Method 2*
Day 1				
Day 5				
Day 10				

Table S7. Examples of analysis of the products of zirconium-89 reactions with DFO.

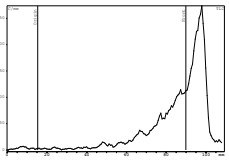
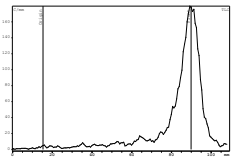
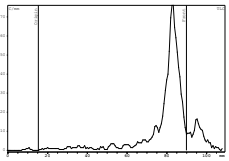
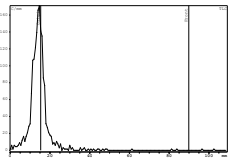
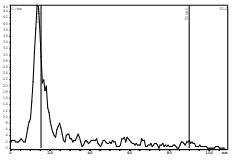
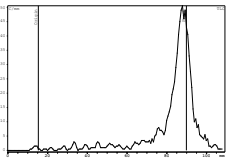
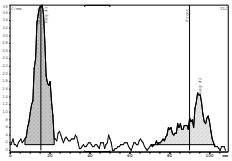
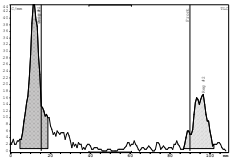
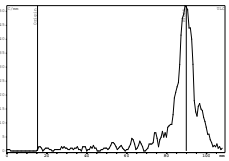
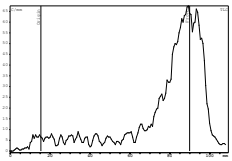
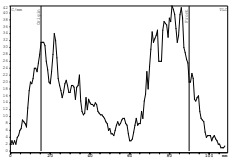
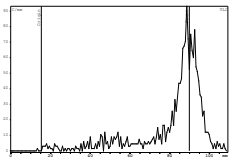
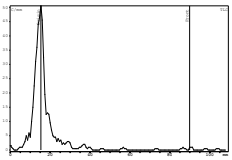
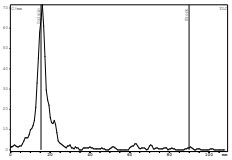
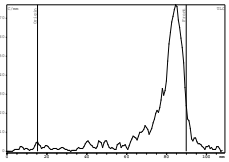
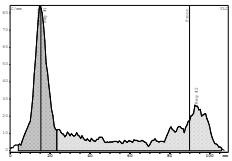
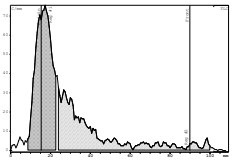
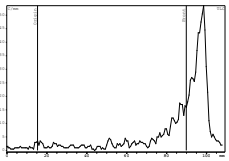
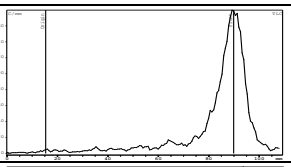
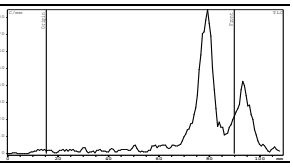
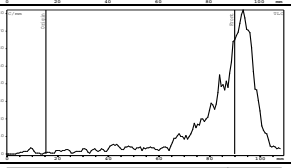
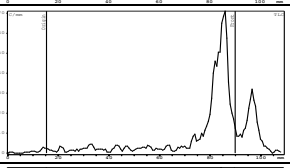
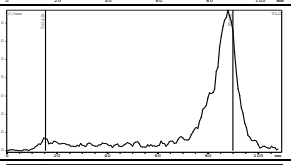
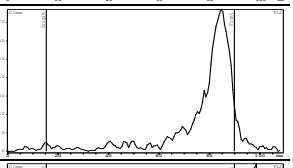
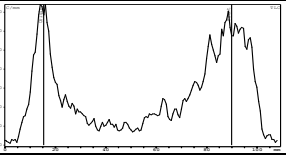
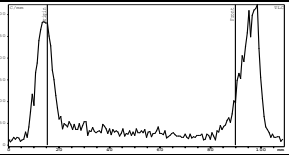
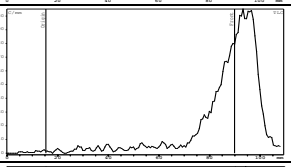
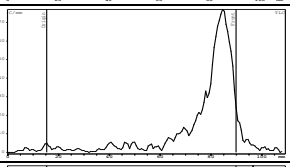
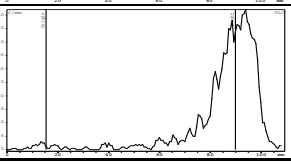
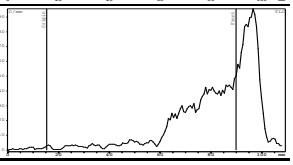
[^{89}Zr]zirconium formulation	Reaction mixture	Radio-chromatograms		
		iTLC-SG / 50 mM DTPA (pH 7) *method 1*	**iTLC-SA / 50 mM EDTA (pH 5)**	**iTLC-SG / CH_3OH-H_2O (1:1), 4% TFA (v/v)** *method 2*
[^{89}Zr]Zr-oxalate (0.1 M oxalate anion) pH 6	*pure*			
	+ 5.0 μg DFO			
	+ 0.25 μg DFO			
[^{89}Zr]Zr-citrate (0.1 M citrate anion) pH 6	*pure*			
	+ 5.0 μg DFO			
	+ 0.1 μg DFO			

Table S8. The results of the analysis of various preparations of zirconium-89 while keeping the samples at room temperature and when heated.

[89Zr]zirconium formulation	Temperature conditions	Radio-chromatograms	
		iTLC-SG / 50 mM DTPA (pH 7) ***method 1***	**iTLC-SG / CH_3OH-H_2O (1:1), 4% TFA (v/v)** ***method 2***
[89Zr]Zr-oxalate (0.1 M oxalate acid) pH 5.5	*room temperature*		
	95°C, 30 min		
[89Zr]Zr-citrate (0.1 M sodium citrate) pH 5.5	*room temperature*		
	95°C, 30 min		
[89Zr]Zr-citrate (1.0 M sodium citrate) pH 5.5	*room temperature*		
	95°C, 30 min		

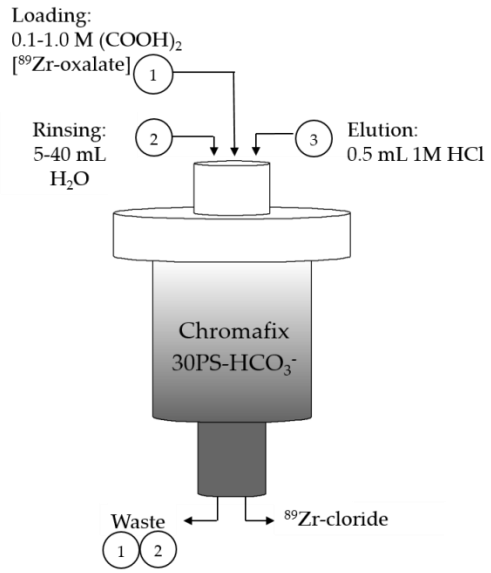
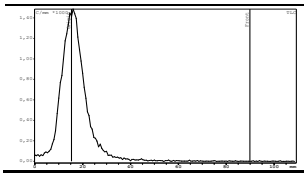
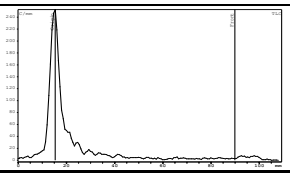
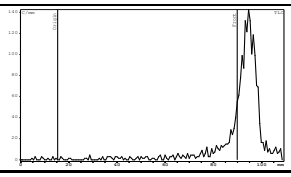
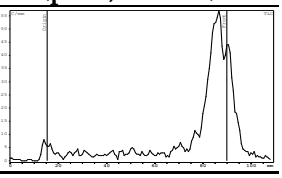


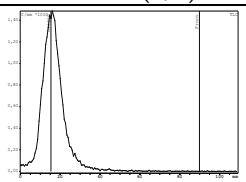
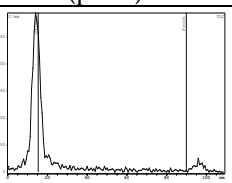
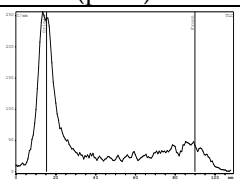
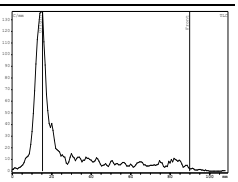
Figure S6. The scheme of zirconium-89 reconstitution from oxalic acid solution into hydrochloric acid solution using Chromafix 30PS-HCO_3 anion-exchange cartridge.

Table S9. Radio-TLC results (method 2) of zirconium-89 samples obtained in 1.0 M HCl using oxalate-free technology and using the reconstitution method from oxalic acid solution.

[^{89}Zr]$ZrCl_4$ in 1 M HCl§	**[^{89}Zr]Zr in 1.0 M HCl (neutralized to pH 6)§**	**[^{89}Zr]Zr in 1.0 M HCl after reconstitution**	**[^{89}Zr]Zr in 1.0 M HCl after reconstitution (pH 6, HEPES)**
			

§ These samples were prepared from [^{89}Zr]$ZrCl_4$ in 5 M HCl initial solution obtained by the manufacturer using oxalate-free method (with Dowex 1×8 anion-exchange resin);.

Table S10. Radio-chromatograms in various systems. Zirconium-89 samples obtained in 1.0 M HCl using oxalate-free technology and those reconstituted from oxalic acid solution (pH = 6).

	Radio-TLC systems			
Sample characteristics	iTLC-SG / CH_3OH-H_2O (1:1), 4% TFA (v/v)	iTLC-SG / 50 mM DTPA (pH 7)	iTLC-SA / 50 mM EDTA (pH 5)	iTLC-SA / 0.5 M citric acid
[^{89}Zr]Zr in 1.0 M HCl (oxalate-free, neutralized to pH 6)				
[^{89}Zr]Zr in 1.0 M HCl after reconstitution (pH 6, HEPES)