Supplementary Information

Chemometric-Assisted Hydrophilic Interaction Chromatographic Method for the Determination of Gadolinium-Based Magnetic Resonance Imaging Contrast Agent in Liposomes

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Table S1. Results of variables screening for the development of method for determination of Gd-DTPA-BMA in liposomes by HILIC

Experiment	Independent variable	Dependent variable[a]									
		Signal-to-noise ratio	R_s	A_s	Peak height / mAU	Noise / mAU	N	Peak width / mm	Peak area / mm^2	t_R / min	k
1	NH_4Ac	2586043	11.8	1.44	9852825	3.81	3687	0.76	218788085	8.9	4.6
2	NH_4FA	8389613	6.9	1.44	10990393	1.31	4106	0.72	233993993	8.6	4.4
3	ACN 60%	10730254	7.5	1.25	16095381	1.50	3018	0.39	189478831	4.3	1.7
4	ACN 70%	8389613	6.9	1.44	10990393	1.31	4106	0.72	233993993	8.6	4.4
5	ACN 75%	9169528	15.6	1.71	6326974	0.69	4610	1.37	235782267	16.2	9.2
6	NH_4FA 5 mmol L^{-1}	16385674	8.1	1.42	10650688	0.65	3912	0.76	234068457	8.9	4.6
7	NH_4FA 10 mmol L^{-1}	8389613	6.9	1.44	10990393	1.31	4106	0.72	233993993	8.6	4.4
8	NH_4FA 15 mmol L^{-1}	3766828	7.8	1.45	10170436	2.70	4255	0.73	212774504	8.9	4.6
9[b]	pH 2.7[b]	–	–	–	–	1.60	–	–	–	–	–
10	pH 3.7	7788154	7.0	1.44	10436127	1.34	3377	0.72	239358814	8.8	4.6
11	pH 4.7	8389613	6.9	1.44	10990393	1.31	4106	0.72	233993993	8.6	4.4

[a]Values are expressed as mean (n = 3 samples, being 3 injections for each sample); [b]in this condition it was not possible to calculate the majority of the dependent variables evaluated due to the degradation of Gd-DTPA-BMA. Mobile phase used in each experiment: 1: ACN/NH_4Ac 10 mmol L^{-1}, pH 5.8, 70:30 v/v; 2: ACN/NH_4FA 10 mmol L^{-1}, pH 4.7, 70:30 v/v; 3: ACN/NH_4FA 10 mmol L^{-1}, pH 4.7, 60:40 v/v; 4: ACN/NH_4FA 10 mmol L^{-1}, pH 4.7, 70:30 v/v; 5: ACN/NH_4FA 10 mmol L^{-1}, pH 4.7, 75:25 v/v; 6: ACN/NH_4FA 5 mmol L^{-1}, pH 4.7, 70:30 v/v; 7: ACN/NH_4FA 10 mmol L^{-1}, pH 4.7, 70:30 v/v; 8: ACN/NH_4FA 15 mmol L^{-1}, pH 4.7, 70:30 v/v; 9: ACN/NH_4FA 10 mmol L^{-1}, pH 2.7, 70:30 v/v; 10: ACN/NH_4FA 10 mmol L^{-1}, pH 3.7, 70:30 v/v; 11: ACN/NH_4FA 10 mmol L^{-1}, pH 4.7, 70:30 v/v. R_s: resolution; A_s: asymmetry; N: number of theoretical plates; t_R: retention time; k: retention factor.

Table S2. Coefficients of the mathematical model obtained by Box-Behnken and summary of ANOVA

Independent variable	Dependent variable					
	Signal-to-noise ratio		R_s		A_s	
	Coefficient	*p*-value	Coefficients	*p*-value	Coefficients	*p*-value
X_1	229221	0.03514	2.09589	0.00166	0.00169	0.10157
X_1^2	−1521253	0.00046	0.49960	0.01551	0.00319	0.01758
X_2	−680585	0.00418	1.69384	0.00254	0.05653	0.00011
X_2^2	−514916	0.00396	−0.23383	0.06546	0.00610	0.00492
X_3	−1814373	0.00059	−0.66067	0.01636	0.01092	0.00284
X_3^2	−542513	0.00357	−0.48766	0.01626	0.00026	0.59944
X_1 X_2	382332	0.02564	0.68013	0.03022	0.00784	0.01088
X_1 X_3	−1801360	0.00120	0.90450	0.01742	0.01104	0.00553
X_2 X_3	56650	0.45995	0.21786	0.21355	−0.01400	0.00345
Intercept	5686189	0.00004	4.00106	0.00030	1.26278	0.00000
Pure error	155887×10^5		0.0585495		0.0000027	
r	0.9530		0.9729		0.9794	
r^2	0.9082		0.9465		0.9592	

R_s: resolution; A_s: asymmetry; X_1: pH; X_2: ACN ratio (in percentage); X_3: buffer concentration (mmol L^{-1}); $p < 0.05$ (ANOVA); r: correlation coefficient; r^2: coefficient of determination.

Table S3. Results from Box-Behnken experimental design of development and optimization of the method for determination of Gd-DTPA-BMA in liposomes by HILIC.

Independent variable		Levels		
		−1	0	1
X_1	pH	3.7	4.2	4.7
X_2	ACN ratio / %	60	65	70
X_3	Buffer concentration / (mmol L^{-1})	5	15	25

Experiment	Experimental condition			Dependent variable[a]						
	X_1	X_2	X_3	Peak height / mAU	Baseline noise / mAU	N	Peak width / mm	Peak area / mm^2	t_R / min	k
1	4.7	70	15	6541768	0.91	4086	0.76	139983991	8.9	4.6
2	4.7	60	15	13132828	1.67	4132	0.34	131824176	4.3	1.7
3	3.7	70	15	5955899	1.48	3359	0.76	135430549	8.7	4.5
4	3.7	60	15	12616128	2.02	3438	0.38	139555792	4.2	1.7
5	4.7	65	25	9659601	5.77	4012	0.49	134050337	5.8	2.6
6	4.7	65	5	9906433	1.03	3774	0.50	143104879	5.8	2.6
7	3.7	65	25	9715330	1.44	3339	0.52	145532548	5.7	2.6
8	3.7	65	5	9160782	1.22	3058	0.53	144444010	5.7	2.6
9	4.2	70	25	6350614	2.73	3872	0.73	132688574	8.5	4.3
10	4.2	70	5	6178102	1.20	3467	0.78	137785630	8.5	4.3
11	4.2	60	25	14248215	4.08	4067	0.34	142228701	4.2	1.6
12	4.2	60	5	13050675	2.00	3504	0.37	142678121	4.2	1.7
13[b,c]	4.2	65	15	10461231	4.66	3749	0.50	151422350	5.7	2.6

[a]Values are expressed as mean of 3 injections; [b]values are expressed as mean (n = 3 samples, being 3 injections for each sample); [c]central point. X_1: pH; X_2: ACN ratio (in percentage); X_3: buffer concentration (mmol L^{-1}); N: number of theoretical plates; t_R: retention time; k: retention factor.

Table S4. Effect of the flow-rate in the parameters t_R, peak height, N and R_s, using the chromatographic conditions of the developed method

Flow / (mL min^{-1})	Dependent variable[a]				Increase compared to flow of 1.0 mL min^{-1} / %			
	t_R / min	Peak height / mAU	N	R_s	t_R / min	Peak height / mAU	N	R_s
0.6	7.1	13939706	4047	3.56	68	12	29	12
0.7	6.1	13506172	3745	3.44	44	7	19	9
0.8	5.3	13405633	3552	3.36	26	8	13	6
0.9	4.7	12687237	3337	3.23	11	2	6	2
1.0	4.2	12472545	3140	3.17	–	–	–	–

[a]Values are expressed as mean of 3 injections. t_R: retention time; N: number of theoretical plates; R_s: resolution.

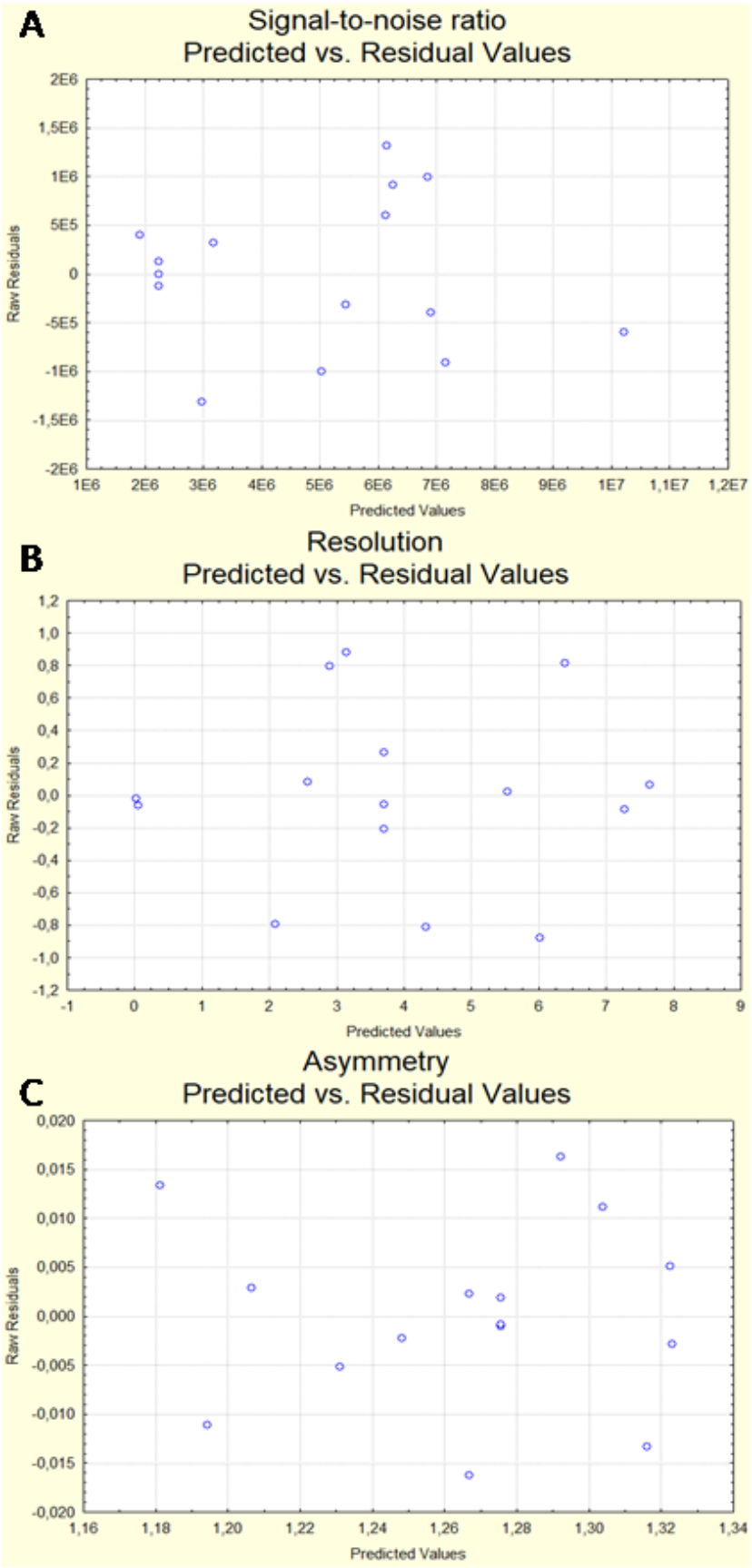

Figure S1. Graphs of distributions residuals from the analysis of dependent variables: signal-to-noise ratio (A), R_s (B) and A_s (C).