Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

A new optimization strategy for MALDI FTICR MS tissue analysis for untargeted metabolomics using experimental design and data modeling

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Table S1 Fractional Factorial design matrix for ion transmission voltages optimizations in positive ion mode

Exp N°	Run Order	RF amplitude TOF	RF amplitude octopole	RF frequency Trans opt	Deflector plate	Funnel 1	Skimmer 1	Funnel RF amplitude	TOF	Capillary exit	Response: number of peaks
1	25	100	200	2	150	100	30	120	1.2	250	185
2	35	300	200	2	150	100	30	50	0.5	100	119
3	4	100	400	2	150	100	10	120	0.5	100	118
4	36	300	400	2	150	100	10	50	1.2	250	248
5	6	100	200	6	150	100	10	50	1.2	100	314
6	27	300	200	6	150	100	10	120	0.5	250	70
7	10	100	400	6	150	100	30	50	0.5	250	32
8	13	300	400	6	150	100	30	120	1.2	100	183
9	23	100	200	2	300	100	10	50	0.5	250	99
10	26	300	200	2	300	100	10	120	1.2	100	421
11	31	100	400	2	300	100	30	50	1.2	100	403
12	3	300	400	2	300	100	30	120	0.5	250	273
13	12	100	200	6	300	100	30	120	0.5	100	/
14	9	300	200	6	300	100	30	50	1.2	250	446
15	5	100	400	6	300	100	10	120	1.2	250	469
16	28	300	400	6	300	100	10	50	0.5	100	76
17	19	100	200	2	150	250	10	50	0.5	100	8
18	8	300	200	2	150	250	10	120	1.2	250	8
19	2	100	400	2	150	250	30	50	1.2	250	9
20	33	300	400	2	150	250	30	120	0.5	100	7
21	20	100	200	6	150	250	30	120	0.5	250	5
22	11	300	200	6	150	250	30	50	1.2	100	7
23	14	100	400	6	150	250	10	120	1.2	100	5
24	15	300	400	6	150	250	10	50	0.5	250	6
25	18	100	200	2	300	250	30	120	1.2	100	/
26	7	300	200	2	300	250	30	50	0.5	250	102
27	37	100	400	2	300	250	10	120	0.5	250	113
28	30	300	400	2	300	250	10	50	1.2	100	328
29	21	100	200	6	300	250	10	50	1.2	250	148
30	34	300	200	6	300	250	10	120	0.5	100	156
31	22	100	400	6	300	250	30	50	0.5	100	71
32	1	300	400	6	300	250	30	120	1.2	250	516
33	24	200	300	4	225	175	20	85	0.9	175	260
34	29	200	300	4	225	175	20	85	0.9	175	237
35	32	200	300	4	225	175	20	85	0.9	175	245
36	17	200	300	4	225	175	20	85	0.9	175	229
37	16	200	300	4	225	175	20	85	0.9	175	232

Table S2 Fractional Factorial design matrix for ion transmission voltages optimizations in negative ion mode

Exp N°	Run Order	Frequency octopole	RF frequency transfer optic	Deflector plate	Funnel 1	Funnel RF amplitude	Capillary exit	TOF	Response: number of peaks
1	3	2	2	-280	-200	60	-250	0.5	52
2	4	5	2	-280	-200	130	-250	1.2	30
3	13	2	4	-280	-200	130	-150	0.5	24
4	19	5	4	-280	-200	60	-150	1.2	/
5	6	2	2	-150	-200	130	-150	1.2	10
6	12	5	2	-150	-200	60	-150	0.5	9
7	16	2	4	-150	-200	60	-250	1.2	12
8	8	5	4	-150	-200	130	-250	0.5	14
9	11	2	2	-280	-100	60	-150	1.2	28
10	10	5	2	-280	-100	130	-150	0.5	18
11	17	2	4	-280	-100	130	-250	1.2	26
12	5	5	4	-280	-100	60	-250	0.5	11
13	15	2	2	-150	-100	130	-250	0.5	122
14	20	5	2	-150	-100	60	-250	1.2	/
15	2	2	4	-150	-100	60	-150	0.5	6
16	1	5	4	-150	-100	130	-150	1.2	/
17	9	2	2	-215	-150	95	-200	0.85	79
18	14	2	2	-215	-150	95	-200	0.85	83
19	18	2	2	-215	-150	95	-200	0.85	77
20	21	2	2	-215	-150	95	-200	0.85	78
21	7	2	2	-215	-150	95	-200	0.85	85

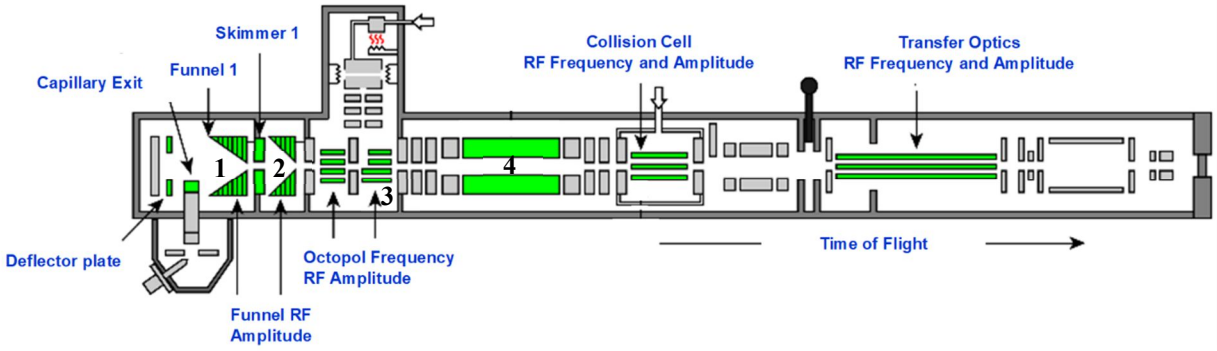

Fig. S1 Schematic diagram of the FTICR instrument (SolariX XR, Bruker, Bremen, Germany)

Briefly, a two-stage ion-funnel (1,2) focuses the ions into a RF hexapole (3) ion guide. Ions then pass through a quadrupole (4) to reach a multifunctional RF hexapole ion trap where they can be accumulated. Ions are then guided via a RF only hexapole into the ICR cell. First, the deflector plate directs the ions towards the two-stage ion-funnel. This parameter will increase the voltages on the MALDI plate as the voltage applied to the MALDI target is relative to the deflector plate. The capillary exit voltage lies between the deflector plate and funnel 1 voltage. The funnel 1 voltage is applied to the entrance of funnel 1, between the capillary exit and skimmer 1. The funnel RF amplitude is the peak-to-peak voltage applied to funnel 1 and funnel 2. The skimmer 1 lens lies between funnel 1 and funnel 2 in the ion source region. The skimmer 1 voltage is applied at the exit of funnel 1 and is connected to the resistor-divider series on funnel 1. The funnel 1 voltage and skimmer 1 voltage define the beginning and end, respectively, of the resistor-divider of ion funnel 1 and thus control the funnel 1 voltage gradient. The skimmer 1 voltage is critical as it is the last region in the source where the pressure is high enough that ions can undergo collisions with the background gas, causing fragmentation. The octopole frequency is applied to the divided octopole that is between skimmer 2 and the quadrupole. The RF amplitude octopole is the peak-to-peak voltage applied to the divided octopole that is between skimmer 2 and the quadrupole. The amplitude Time-Of-Flight sets the peak-to-peak RF voltage applied to the ICR transfer hexapole rods. The transfer optics frequency sets the frequency applied to the ICR transfer hexapole rods. The time-of-flight corresponds to the time taken by the ions to travel after ejection from the collision cell and before being trapped into the ICR cell. It has a significant impact on the observed *m/z* range. In general, low *m/z* ions require shorter TOF whereas high *m/z* ions require longer TOF.

Table S3 Fractional Factorial design matrix for MALDI and detection parameters optimizations in positive ion mode

Exp N°	Run Order	Size	Scans	Shots	Power	Weighted resolution	Number of peaks	Mean error (ppm)
1	5	2	1	50	18	94364	91	0.125
2	8	2	20	150	24	93350	136	0.260
3	9	2	40	250	30	85607	146	/
4	2	4	1	150	30	179427	228	0.194
5	11	4	20	250	18	172418	216	0.182
6	13	4	40	50	24	141419	156	0.205
7	1	8	1	250	24	273884	366	0.093
8	7	8	20	50	30	308982	286	0.098
9	12	8	40	150	18	247360	293	0.058
10	3	4	20	150	24	157376	249	/
11	4	4	20	150	24	162151	216	0.211
12	14	4	20	150	24	157357	/	0.199
13	10	4	20	150	24	160006	249	0.152
14	6	4	20	150	24	155438	248	0.195

.

Table S4 Fractional Factorial design matrix for MALDI and detection parameters optimizations in negative ion mode

Exp N°	Run Order	Size	Scans	Shots	Power	Weighted resolution	Number of peaks	Mean error (ppm)
1	1	2	1	50	22	99881	231	0.294
2	2	2	20	150	31	104178	278	0.397
3	5	2	40	250	40	110176	244	1.085
4	8	4	1	150	40	/	/	/
5	11	4	20	250	22	221766	574	0.123
6	12	4	40	50	31	200938	434	0.240
7	7	8	1	250	31	456644	973	0.404
8	6	8	20	50	40	411187	662	0.934
9	4	8	40	150	22	398823	1314	0.032
10	10	4	20	150	31	237075	425	0.450
11	14	4	20	150	31	238060	505	0.505
12	9	4	20	150	31	204409	412	0.455
13	3	4	20	150	31	226791	431	0.435
14	13	4	20	150	31	183116	437	0.612

Table S5 MLR model regression coefficients (Ion transfer, positive ionization mode)

Number of peaks	Coefficient	SD	*p*
Constant	1.9657	0.071213	3.02E-20
RF amplitude TOF	0.0347767	0.0770904	0.655794
RF amplitude octopole	-0.0110429	0.0770904	0.887245
RF frequency Trans opt	-0.0324444	0.0759513	0.672908
Deflector plate	0.429251	0.0770904	8.64E-06
Funnel 1	-0.375178	0.0759513	4.36E-05
Skimmer 1	0.0135934	0.0770903	0.861458
Funnel RF amplitude	0.0489785	0.0770904	0.53098
TOF	0.164805	0.0759513	0.039723
Capillary exit	-0.0231362	0.0770903	0.76657

N = 35	Q2 =	0.441		
DF = 25	R2 =	0.717	RSD =	0.414
	R2 adj. =	0.616		
			Confidence =	0.95

DF: Degrees of Freedom, SD ; Standard Deviation, R2: cumulative modeled variation, Q2: cumulative predicted variation, RSD: Residual Standard Deviation.

Table S6 MLR coefficient regression (Ion Transfer, negative ionization mode)

Number of peaks	Coefficient	SD	*p*
Constant	19.049	4.5093	0.00175936
Frequency octopole	-17.6778	3.88795	0.00106349
RF frequency transfer optic	-30.3826	4.30638	3.48E-05
Deflector plate	9.57286	4.37507	0.0535121
Funnel 1	14.3229	4.37507	0.00837705
Funnel RF amplitude	4.36809	4.20479	0.323347
Capillary exit	-25.9793	4.63965	0.000227879
TOF	-10.5245	4.34086	0.0357839

N = 18	Q2 =	0.5		
DF = 10	R2 =	0.897	RSD =	14.84
	R2 adj. =	0.825		
			Confidence =	0.95

DF: Degrees of Freedom, SD ; Standard Deviation, R2: cumulative modeled variation, Q2: cumulative predicted variation, RSD: Residual Standard Deviation.

Table S7 MLR coefficient regression (MALDI and detection parameters, positive ionization mode)

Weighed resolution	Coefficient	SD	*p*
Constant	5.2452	0.0157276	9.97E-20
Size	0.225309	0.0224159	3.43E-06
Scans	-0.0324148	0.0231122	0.194306
Shots	-0.0014311	0.0231165	0.951987
Power	0.0119354	0.0231165	0.618081

N = 14	Q2 =	0.581		
DF = 9	R2 =	0.92	RSD =	0.05662
	R2 adj. =	0.884		
			Confidence =	0.95

Mean error	Coefficient	SD	*p*
Constant	0.165698	0.00597352	2.03E-08
Size	-0.0842173	0.00903859	3.40E-05
Scans	0.0376356	0.0100036	0.00705458
Shots	0.036971	0.00995321	0.00751035
Power	0.0503044	0.00995321	0.00147306

N = 12	Q2 =	0.827		
DF = 7	R2 =	0.93	RSD =	0.01969
	R2 adj. =	0.89		
			Confidence =	0.95

Number of peaks	Coefficient	SD	*p*
Constant	237.37	8.86558	4.07E-09
Size	89.3884	12.276	8.54E-05
Scans	-15.3616	12.6356	0.258738
Shots	32.5	12.6378	0.0330429
Power	10	12.6378	0.451603

N = 13	Q2 =	0.584		
DF = 8	R2 =	0.885	RSD =	30.96
	R2 adj. =	0.828		
			Confidence =	0.95

DF: Degrees of Freedom. SD ; Standard Deviation, R2: cumulative modeled variation, Q2: cumulative predicted variation, RSD: Residual Standard Deviation.

Table S8 MLR coefficient regression (MALDI and detection parameters, negative ionization mode)

Weighed resolution	Coefficient	SD	*p*
Constant	267450	5272.78	2.53E-11
Size	157375	7212.84	2.05E-08
Scans	-13485.5	8361.8	0.145465
Shots	12763.2	7418.99	0.123681
Power	5873.8	8390.86	0.503757

N = 13	Q2 =	0.966		
DF = 8	R2 =	0.984	RSD =	1.82E+04
	R2 adj. =	0.976		
			Confidence =	0.95

Mean error	Coefficient	SD	*p*
Constant	-0.42701	0.0245613	1.22E-07
Size	-0.184973	0.0335983	0.000570081
Scans	-0.272141	0.0389503	0.000114126
Shots	-0.0145316	0.0345586	0.685194
Power	0.569832	0.0390857	4.80E-07

N = 13	Q2 =	0.933		
DF = 8	R2 =	0.969	RSD =	0.08465
	R2 adj. =	0.954		
			Confidence =	0.95

Number of peaks	Coefficient	SD	*p*
Constant	2.7123	0.0130737	3.26E-16
Size	0.280505	0.017884	2.72E-07
Scans	0.0526511	0.0207328	0.0347375
Shots	0.052076	0.0183951	0.0221172
Power	-0.0933452	0.0208049	0.00203763

N = 13	Q2 =	0.84		
DF = 8	R2 =	0.971	RSD =	0.04506
	R2 adj. =	0.957		
			Confidence =	0.95

DF: Degrees of Freedom, SD ; Standard Deviation, R2: cumulative modeled variation, Q2: cumulative predicted variation, RSD: Residual Standard Deviation.

Table S9 Parameters values for positive and negative ion modes before and after optimizations

Factor	Abbreviation	Unit	Value before optimization		Value after optimization	
Optimized ion transmissions voltages						
			Positive	**Negative**	**Positive**	**Negative**
RF amplitude TOF	RFt	Vpp	200	-100	298	-100
RF amplitude octopole	RFo	Vpp	200	250	200	250
Frequency Transfer Optic	RFf	MHz	2	2	2	2
RF Frequency octopole	Fo	Vpp	2	2	2	2
Deflector plate	Def	V	180	-150	299	-151
Funnel 1	Fun	V	100	-100	100	-158
Skimmer	Ski	V	10	-15	30	/
Funnel RF amplitude	Fu2	V	200	100	120	60
Time-Of-Flight	TOF	ms	0.5	0.5	0.85	1.08
Capillary exit	CaE	V	150	-120	104	-249
Optimized MALDI parameters						
			Positive	**Negative**	**Positive**	**Negative**
Size	/	M	2	2	8	8
Scan	/	/	1	1	1	29
Shot	/	/	500	200	221	240
Power	/	%	20	30	24	22