

Supporting Information for:

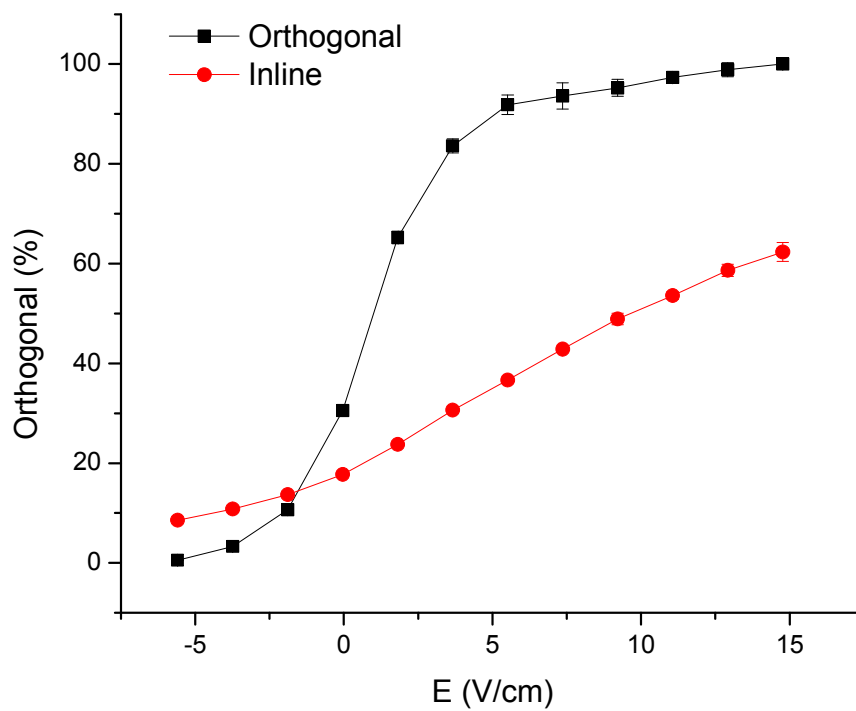
An Orthogonal Injection Ion Funnel Interface Providing Enhanced Performance for Selected Reaction Monitoring-Triple Quadrupole Mass Spectrometry

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Abstract

The supporting information includes two figures to help illuminate certain points in the text. The first figure (S1) depicts the ion current measurement at HPIF converging section in orthogonal and inline injection mode for the comparison to Figure 2c. The supplemental Table 1-4 shows the details of the experimental conditions and the quantitative MS analysis of low abundance peptides.

Supplemental Figure S1 Measurement of ion transmission efficiency at HPIF converging section in orthogonal and inline injection mode.



Supplemental Table S1. 9 peptide mixture used for performance evaluation

Peptide number	Peptide	Monitored mass (m/z)	charge
1	Bradykinin	530.7988	2
2	Human Angiotensin I	432.8998	3
3	Human Neurotensin	558.3105	3
4	Fibrinopeptide A	768.8499	2
5	Substance P	674.8634	2
6	Kemptide	386.743	2
7	Angiotensin II	523.7745	2
8	Melittin	712.4418	4
9	Renin	586.9824	3

Supplemental Table S2. MS¹ Quantitative measurements using the 9 peptide mixture

Interface	Peptide Number ^a	Concentration (nmol)											
		0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	200	400	
Orthogonal	1	Intensity	1.1E+06	1.3E+06	1.7E+06	2.5E+06	4.4E+06	7.4E+06	1.4E+07	2.7E+07	5.6E+07	9.8E+07	
		CV(%)		6.70%	3.20%	2.95%	1.95%	4.24%	1.95%	3.26%	0.68%	1.25%	1.09%
		S/N ^b	<3	6.9	9.3	13.2	18.2	29.6	52.1	87.2	81.0	126.1	222.3
	2	Intensity			2.0E+06	3.1E+06	5.4E+06	1.1E+07	1.9E+07	4.1E+07	7.0E+07	1.9E+08	3.0E+08
		CV(%)			3.63%	3.56%	1.27%	11.93%	4.17%	1.33%	3.67%	3.20%	0.98%
		S/N		<3	6.8	14.0	33.8	64.6	71.2	115.4	204.3	328.5	230.2
	3	Intensity		7.6E+05	1.1E+06	1.6E+06	2.6E+06	5.5E+06	9.1E+06	1.7E+07	3.0E+07	7.2E+07	1.4E+08
		CV(%)		1.28%	3.70%	1.00%	2.16%	7.81%	2.33%	2.27%	4.77%	3.29%	2.02%
		S/N	<3	5.0	11.0	17.8	33.5	79.7	112.9	124.3	118.9	119.2	88.5
	4	Intensity		3.6E+05	4.9E+05	8.0E+05	1.4E+06	2.6E+06	4.9E+06	8.5E+06	2.5E+07	4.4E+07	
		CV(%)		4.12%	5.52%	0.71%	16.94%	1.71%	2.70%	3.37%	2.69%	3.08%	
		S/N		<3	4.9	8.3	22.6	28.6	42.5	88.2	104.6	311.2	300.4
	5	Intensity		5.4E+05	5.6E+05	7.7E+05	1.1E+06	1.9E+06	3.3E+06	6.1E+06	1.2E+07	2.6E+07	4.5E+07
		CV(%)		6.07%	4.16%	3.83%	5.41%	4.08%	3.40%	2.32%	4.56%	0.84%	1.65%
		S/N		6.1	6.2	11.0	12.6	24.9	29.3	44.5	83.8	64.0	60.2
	6	Intensity				2.8E+06	4.1E+06	7.8E+06	1.3E+07	2.5E+07	4.3E+07	1.0E+08	2.0E+08
		CV(%)				4.68%	0.95%	10.65%	4.13%	1.61%	3.65%	1.62%	2.23%
		S/N			<3	8.8	16.7	36.5	56.8	95.1	193.0	345.6	272.2
7	Intensity		1.1E+06	1.4E+06	1.7E+06	2.5E+06	4.5E+06	7.2E+06	1.4E+07	2.4E+07	5.3E+07	9.2E+07	
	CV(%)		9.07%	2.77%	4.14%	0.69%	16.79%	1.83%	1.63%	2.37%	1.65%	1.57%	
	S/N	<3	8.6	11.6	14.5	30.1	68.5	98.1	163.6	258.7	374.8	490.7	
8	Intensity					1.0E+06	1.6E+06	3.0E+06	5.0E+06	9.0E+06	1.9E+07	3.1E+07	
	CV(%)					6.52%	13.99%	5.48%	3.81%	5.44%	1.57%	1.37%	
	S/N				<3	20.2	22.0	38.0	42.9	62.5	114.1	146.1	
9	Intensity				8.5E+05	1.3E+06	2.4E+06	4.1E+06	8.0E+06	1.4E+07	3.3E+07	6.3E+07	
	CV(%)				0.14%	7.05%	13.27%	3.82%	2.93%	1.11%	1.44%	0.78%	
	S/N			<3	9.1	14.6	19.2	32.3	51.7	72.2	113.3	117.4	
Inline	1	Intensity	1.2E+06	1.2E+06	1.6E+06	1.9E+06	3.1E+06	5.8E+06	1.1E+07	1.9E+07	3.8E+07	8.3E+07	
		CV(%)	0.88%	9.03%	1.31%	2.11%	3.05%	1.63%	5.25%	3.01%	1.47%	4.13%	
		S/N ^b	<3	5.9	7.7	9.4	14.6	39.4	42.8	80.1	129.8	141.8	268.9
	2	Intensity				2.5E+06	3.7E+06	6.2E+06	1.1E+07	2.3E+07	4.5E+07	9.1E+07	2.1E+08
		CV(%)				2.19%	4.49%	2.69%	6.06%	4.99%	6.42%	2.55%	2.83%
		S/N			<3	3.7	14.4	21.2	32.7	66.8	123.3	217.1	277.2
	3	Intensity		1.1E+06	1.4E+06	1.8E+06	2.9E+06	5.3E+06	1.0E+07	1.9E+07	3.7E+07	8.9E+07	
		CV(%)		9.25%	0.95%	5.72%	0.82%	3.95%	4.48%	2.04%	3.91%	3.01%	
		S/N		<3	7.8	7.5	21.8	36.6	61.5	118.2	153.6	139.1	109.7
	4	Intensity		4.1E+05	5.6E+05	6.1E+05	8.6E+05	1.5E+06	2.8E+06	5.3E+06	1.2E+07	2.6E+07	
		CV(%)		14.68%	5.43%	11.15%	1.36%	4.26%	2.75%	6.13%	1.97%	2.37%	
		S/N		<3	7.5	4.3	11.4	22.4	28.1	47.9	83.5	157.1	235.5
	5	Intensity					8.7E+05	1.3E+06	2.5E+06	4.0E+06	7.3E+06	1.5E+07	3.3E+07
		CV(%)					6.26%	2.14%	2.17%	3.73%	4.41%	2.14%	2.31%
		S/N				<3	7.4	16.7	22.6	33.0	38.7	54.6	31.8
	6	Intensity				3.8E+06	5.4E+06	9.7E+06	1.7E+07	3.1E+07	6.1E+07	1.4E+08	
		CV(%)				2.97%	1.85%	4.89%	2.42%	6.93%	2.17%	2.17%	
		S/N				<3	11.4	21.9	29.3	60.2	94.5	209.7	555.0
7	Intensity				1.3E+06	1.6E+06	2.4E+06	4.4E+06	8.3E+06	1.6E+07	3.1E+07	6.8E+07	
	CV(%)				2.19%	1.65%	1.79%	3.60%	3.42%	1.53%	1.95%	4.07%	
	S/N			<3	5.4	18.4	28.5	40.9	65.5	119.0	243.3	400.9	
8	Intensity				6.7E+05	7.9E+05	9.7E+05	2.1E+06	3.1E+06	6.4E+06	1.7E+07		
	CV(%)				9.30%	3.95%	7.97%	8.80%	4.16%	4.53%	4.30%		
	S/N				<3	9.0	10.7	12.1	31.0	40.3	83.3	158.9	
9	Intensity				1.0E+06	1.4E+06	2.5E+06	5.0E+06	9.2E+06	1.8E+07	4.1E+07		
	CV(%)				5.37%	1.58%	7.54%	2.30%	7.24%	4.57%	3.19%		
	S/N				<3	8.7	14.7	17.5	29.2	34.5	47.7	51.0	
Standard	1	Intensity					9.2E+06	1.4E+07	2.2E+07	3.2E+07	5.5E+07	1.0E+08	
		CV(%)					17.67%	3.22%	6.02%	2.43%	5.03%	1.38%	
		S/N ^b					>20%	<3	13.2	18.2	29.6	52.1	87.2
	2	Intensity						1.1E+07	1.9E+07	3.2E+07	5.0E+07	9.2E+07	1.9E+08
		CV(%)						>20%	18.05%	2.74%	7.04%	3.46%	7.44%
		S/N						<3	14.0	33.8	64.6	71.2	115.4
	3	Intensity						1.1E+07	1.6E+07	2.7E+07	4.0E+07	6.9E+07	1.2E+08
		CV(%)						>20%	16.22%	1.92%	5.36%	5.39%	12.13%
		S/N							17.8	33.5	79.7	112.9	124.3
	4	Intensity							3.5E+06	3.6E+06	5.3E+06	8.3E+06	
		CV(%)							10.99%	8.88%	9.25%	8.50%	
		S/N							28.6	42.5	88.2	104.6	
	5	Intensity						4.3E+06	7.9E+06	9.3E+06	1.2E+07	1.8E+07	3.5E+07
		CV(%)						>20%	19.76%	4.47%	8.21%	7.07%	1.11%
		S/N							11.0	12.6	24.9	29.3	44.5
	6	Intensity							3.4E+07	4.5E+07	6.6E+07	1.1E+08	
		CV(%)							4.11%	5.76%	4.04%	1.91%	
		S/N							36.5	56.8	95.1	193.0	
7	Intensity						1.4E+07	2.0E+07	2.6E+07	4.5E+07	8.4E+07		
	CV(%)						>20%	4.15%	6.53%	6.64%	1.53%		
	S/N							30.1	68.5	98.1	163.6		
8	Intensity							4.2E+06	5.5E+06	6.3E+06	1.1E+07		
	CV(%)							>20%	7.88%	13.59%	13.85%		
	S/N							20.2	22.0	38.0	42.9		
9	Intensity							6.7E+06	8.7E+06	1.1E+07	1.9E+07		
	CV(%)							>20%	8.82%	8.56%	8.36%		
	S/N							14.6	19.2	32.3	51.7		

^a The peptide information according to the peptide number is described in Table 1.

^b Average S/N for 3 SRM measurements. S/N calculation is described in Methods.

Supplemental Table S3. Set of 18 spiked-in surrogate peptides selected from the four proteins mixture.

Peptide number	Peptide sequence*	Precursor mass (m/z)	Precursor charge	Product mass (m/z)	Observed Product charge	Fragment	Collision energy
1	AFKDEDTQAMPFR	522.581258	3	631.325997	1	y5	14
				560.288883	1	y4	13
				429.248399	1	y3	13
				766.408147	1	y7	15
2	DFPIANGER	514.75539	2	556.271319	1	y5	20
				383.707711	2	y7	14
3	DGPLTGTYSR	495.24994	2	607.30737	1	y5	17
				506.259692	1	y4	15
				409.225737	2	y7	14
4	EDLIAYLK	486.778225	2	728.479637	1	y6	15
				615.395573	1	y5	15
				502.311509	1	y4	13
5	GGLEPINFQTAADQAR	849.427684	2	1341.678508	1	y12	25
				1131.54168	1	y10	28
				671.342892	2	y12	24
6	HGFLPR	368.710057	2	599.353926	1	y5	14
				542.332463	1	y4	14
7	IVGGWECEK	543.262416	2	263.660507	2	z4	20
				972.433492	1	y8	18
				873.365078	1	y7	17
8	LFTGHPETLEK	427.230593	3	724.396695	1	y6	15
				583.80022	2	y10	11
				510.266013	2	y9	11
9	LSEPAELTDAVK	640.844824	2	951.523687	1	y9	18
				854.470923	1	y8	23
				541.307152	1	y5	15
10	LWSAEIPNLYR	686.368379	2	1072.566104	1	y9	19
				785.454369	1	y6	23
				672.370305	1	y5	19
11	TGNLHGLFGR	589.818856	2	696.381538	1	y6	22
				559.322626	1	y5	23
				510.784285	2	y9	19
12	TQDENPVVHFFK	490.248535	3	881.512334	1	y7	18
				685.391156	1	y5	15
				586.322743	1	y4	19
13	VDEDQPFPAVPK	675.344991	2	763.459236	1	y7	17
				519.338058	1	y5	20
				252.179767	1	y2	18
14	VEADIAGHGQEVLR	539.625856	3	824.486397	1	y7	22
				759.400938	2	y14	15
				694.879642	2	y13	14
15	VLAIHDLNEDQLR	515.946818	3	784.382326	1	y6	21
				723.882381	2	y12	14
				667.340349	2	y11	15
16	VLVLDTDYKK	601.349545	2	989.539337	1	y8	19
				890.470923	1	y7	20
				777.386859	1	y6	20
17	VYVEELKPTPEGDLLEILLQK	774.429186	3	1030.074269	2	y18	18
				980.540063	2	y17	19
				794.955437	2	y14	17
18	YEINVLK	458.759944	2	624.40669	1	y5	16
				511.322626	1	y4	16
				265.118283	1	a2	17

*Amino acids in bold are labeled with heavy stable isotopes. Lysine (K) is 8 Da heavier and arginine (R) is 10 Da heavier.

Supplemental Table S4. Comparison of detection limits for 18 peptides spiked into a *Shewanella* lysate

Peptide sequence	LOD (amol)			Linearity		
	Orthogonal	Inline	S-lens	Orthogonal	Inline	S-lens
AFKDEDTQAMPFR	<u>500*</u>	1000	2000	0.9981	n/a	n/a
DFPIANGER	125	125	<u>15.625</u>	0.9977	<u>0.9985</u>	0.979
DGPLTGTYSR	<u>125</u>	250	<u>125</u>	<u>0.9921</u>	0.991	0.9847
EDLIAYLK	<u>250</u>	<u>250</u>	500	0.987	0.9905	<u>0.9955</u>
GGLEPINFQTAADQAR	<u>31.25</u>	62.5	<u>31.25</u>	<u>0.9967</u>	0.9947	0.9967
HGFLPR	<u>125</u>	<u>125</u>	500	<u>0.9997</u>	0.9938	0.9958
IVGGWECEK	<u>125</u>	<u>125</u>	1000	<u>0.9983</u>	0.9979	n/a
LFTGHPETLEK	<u>125</u>	250	250	<u>0.9992</u>	0.9942	0.996
LSEPAELTDAVK	<u>31.25</u>	62.5	<u>31.25</u>	0.9959	<u>0.9992</u>	0.9939
LWSAEIPNLYR	<u>125</u>	<u>125</u>	1000	<u>0.9843</u>	0.9919	n/a
TGPNLHGLFGR	<u>125</u>	250	500	0.9858	0.9871	<u>0.9987</u>
TQDENPVVHFFK	<u>31.25</u>	62.5	62.5	0.9963	0.9936	<u>0.9971</u>
VDEDQPFPAVPK	<u>15.625</u>	62.5	31.25	<u>0.9996</u>	0.9991	0.9978
VEADIAGHGQEVLR	500	500	500	0.9641	0.9631	<u>0.9746</u>
VLAIDLHNLNEDQLR	62.5	62.5	62.5	0.99	0.9928	<u>0.9956</u>
VLVLDTDYKK	250	<u>125</u>	250	0.9972	0.9978	<u>0.9998</u>
VYVEELKPTPEGDLEILLQK	<u>1000</u>	<u>1000</u>	2000	n/a	n/a	n/a
YEINVLR	31.25	31.25	31.25	<u>0.999</u>	0.9976	0.9955

*The best LOD obtained by orthogonal, inline ion funnel or S-lens for each peptide is underlined.

*The mass spectra of lowest detectable concentration for each peptide was manually inspected to ensure the S/N is greater than 3.