

Supplementary materials

Liquid chromatography-matrix-assisted laser desorption/ionization mass spectrometric imaging with sprayed matrix for improved sensitivity, reproducibility and quantitation

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Table S1 List of putative neuropeptides identified from blue crab PO by LC-MSI

platform with sprayed matrix (mass range from *m/z* 600 to 2500*)

<i>m/z</i>	Sequence	Neuropeptide Family
653.30		
657.27		
668.66		
673.28		
685.26		
695.29	NFLRFa	RFa
701.26		
717.32		
720.30		
733.30		
734.30		
743.39		
754.36	GQYAFGLa	AST-A
765.39		
770.34	GGAYSFGLa	AST-A
782.29		
784.33	FVGGSRYa	RYa
787.38		
796.40	NPYSFGLa	AST-A
797.35		
803.33		
810.41	AGPYSFGLa	AST-A
811.33		
815.37		
817.52	LRNLRFa	RFa
818.40		
820.38	ASNNLRFa	RFa
832.39	FYANRYa	RYa
834.37		
837.35		
838.38	GDPYAFGLa	AST-A
841.38	AGGAYSFGLa	AST-A
843.35		
845.40		
854.43	DGPYSFGLa	AST-A
863.42	pQGNFRFa	RFa
863.43	PSGFLGMRa	CabTRP
867.45		

m/z	Sequence	Neuropeptide Family
869.38		
876.36		
880.40		
883.42	SNPYSFGLa	AST-A
889.41		
891.40		
892.39	SGHYIFGLa	AST-A
894.40		
898.41	PDMYGFGLa	AST-A
899.52		
909.51	ARPYSFGLa	AST-A
911.41	ARAYDFGLa	AST-A
913.39		
915.55		
919.46		
921.44	PSM(O)RLRFa	RFa
931.48		
936.43		
937.54	PRVYSFGLa/ELNFLRFa	AST-A/RFa
939.53	TRPYSFGLa	AST-A
940.40	QRAYSFGLa	AST-A
948.56	PGVNFLRFa	RFa
950.50	APSGFLGM(O)Ra	CabTRP
956.37	PFCNAFTGCa	CCAP
959.52	SGFYAPRYa	RYa
964.54	TPSGFLGMRa	CabTRP
965.58	NRNFLRFa	RFa
970.62		
976.48	SGFYANRYa	RYa
978.34		
986.46		
987.58		
991.48		
992.54	APSGFLGM(O)Ra	CabTRP
1004.48	FSGTYNFGLa	AST-A
1008.52		
1012.65		
1014.58		
1021.59		
1022.63	GNRNFLRFa	RFa
1025.52		
1030.51	pEGFYSQRYa	RYa

m/z	Sequence	Neuropeptide Family
1036.51		
1043.57		
1046.54		
1052.49	SPRLTYFGLa	AST-A
1053.48	SDRNFLRFa	
1065.49	RDNFVLRFa	RFa
1068.46		
1068.58		
1074.58		
1083.53		
1090.51		
1092.49		
1094.67	ENRNFLRFa	RFa
1096.57		
1099.55		
1102.53		
1106.51		
1107.55	AGWSSMRGAWa	AST-B
1112.56		
1114.54	SSRFVGGSRYa	RYa
1115.53		
1117.51		
1119.62	SMPTLRLRFa	RFa
1123.58	AGWSSM(O)RGAWa	AST-B
1125.56	pQDLDHVFLR	myosuppressin
1126.68		
1128.53		
1129.53		
1130.56		
1131.50	DGPLAPFLRFa	RFa
1136.46		
1139.61		
1140.71		
1146.65	GYSKNYLRFa	RFa
1147.54	APQRNFLRFa	RFa
1148.71		
1150.58		
1155.58		
1158.63	YGNRSFLRFa	RFa
1172.61	AYNRSFLRFa	RFa
1182.59	TSWGKFQGSWa	AST-B
1186.61	FDAFTTGFGHS	Orcomyotropin

m/z	Sequence	Neuropeptide Family
1189.60		
1194.55	VTWGKFQGSWa	AST-B
1199.62	NFDEIDRSGF	Ork
1204.58		
1205.57		
1208.55		
1209.65	TGWNKFQGSWa	AST-B
1211.56		
1213.74	DEIDRSGFGFA	Ork
1219.62		
1220.60	SGDWSSLRGAWa	AST-B
1222.65	GNWNKFQGSWa	AST-B
1224.64		
1227.57		
1230.71	SLKSDTVTPLLG	CPRP
1231.58		
1232.69	RSAQGLGKMER	CPRP
1236.61		
1237.70		
1238.64	SQPSKNYLRFa	RFa
1241.74		
1242.60		
1244.59	DLKSDTVTPLR	CPRP
1249.54		
1252.64		
1253.62		
1254.62		
1255.73		
1256.60	NFDEIDRSGFG	Ork
1268.60		
1270.60	NFDEIDRSGFA	Ork
1271.71	pQDLDHVFLRFa	RFa
1274.63		
1275.56		
1276.60		
1277.70		
1284.59		
1286.57	NFDEIDRSSFG	Ork
1287.81	ASLKSDTVTPLR	CPRP
1288.68	QDLDHVFLRFa	RFa
1293.64	STNWSSLRSAWa	AST-B
1300.60	NFDEIDRSSFA	Ork

m/z	Sequence	Neuropeptide Family
1306.69		
1308.79		
1315.60		
1316.67		
1318.78		
1320.70		
1322.68		
1322.74		
1328.67		
1328.68		
1330.78		
1334.63		
1341.69		
1342.68	DVRTPALRLRFa	RFa
1344.79		
1348.62		
1352.76		
1356.61		
1372.77	KIFEPLRDKNL	OTHERS
1378.60		
1380.66	NNNWTKFQGSWa	AST-B
1381.69	GYRKPPFNGSIFa	SIFa
1382.67		
1386.77		
1388.75		
1394.77		
1399.56		
1402.63		
1403.62	NFDEIDRSFGF	Ork
1404.72		
1408.74		
1410.75		
1413.64		
1415.83	RSAEGLGRMGRL	CPRP
1416.74		
1420.71		
1426.65		
1429.87		
1431.84		
1433.72	NFDEIDRSSFGF	Ork
1437.81		
1439.78		

m/z	Sequence	Neuropeptide Family
1445.85		
1449.65		
1451.80		
1453.77		
1455.80		
1458.74	RSAQGLGKMERLL	CPRP
1459.81		
1463.85		
1470.74	VPNDWAHFRGSWa	AST-B
1471.67		
1473.84		
1474.71	NFDEIDRSGFGFA	Ork
1475.71	DFDEIDRSGFGFA	Ork
1478.79	TPLGDLSGSLGHPVE	CPRP fragment
1486.85		
1489.82		
1492.68		
1495.76		
1500.84		
1502.70	NFDEIDRSGFGFV	Ork
1504.68	NFDEIDRSSFGFA	Ork
1506.71		
1508.83		
1514.72		
1515.58		
1516.88		
1517.76	NFDEIDRSGFGFN	Ork
1518.68		
1522.82		
1530.80		
1532.74	NFDEIDRSSFGFV	Ork
1534.69		
1544.81		
1547.66	NFDEIDRSSFGFN	Ork
1549.27		
1550.64		
1556.70		
1562.89		
1586.87	MFAPLAWPKGGARWa	AST-B
1589.77		
1593.61		

m/z	Sequence	Neuropeptide Family
1602.81		
1603.78		
1608.84		
1615.66		
1624.82		
1630.89		
1631.63		
1637.65		
1643.64		
1646.86		
1659.63		
1665.57		
1671.88		
1681.55		
1686.88		
1703.84		
1706.81		
1720.80		
1728.81		
1790.99		
1802.88		
1814.94		
1820.88		
1824.84		
1828.99		
1889.88		
1899.79		
1915.76		
1921.77		
1935.82		
1943.75		
1957.81		
2019.92		
2041.96		
2057.94		
2063.89		
2072.93		
2105.99		
2172.99		
2205.95		
2241.00		
2237.92		

m/z	Sequence	Neuropeptide Family
2370.11		
2392.09		
2415.22		
2441.09		
2446.15		
2468.16		

* The sequence and family were matched to previously identified neuropeptides

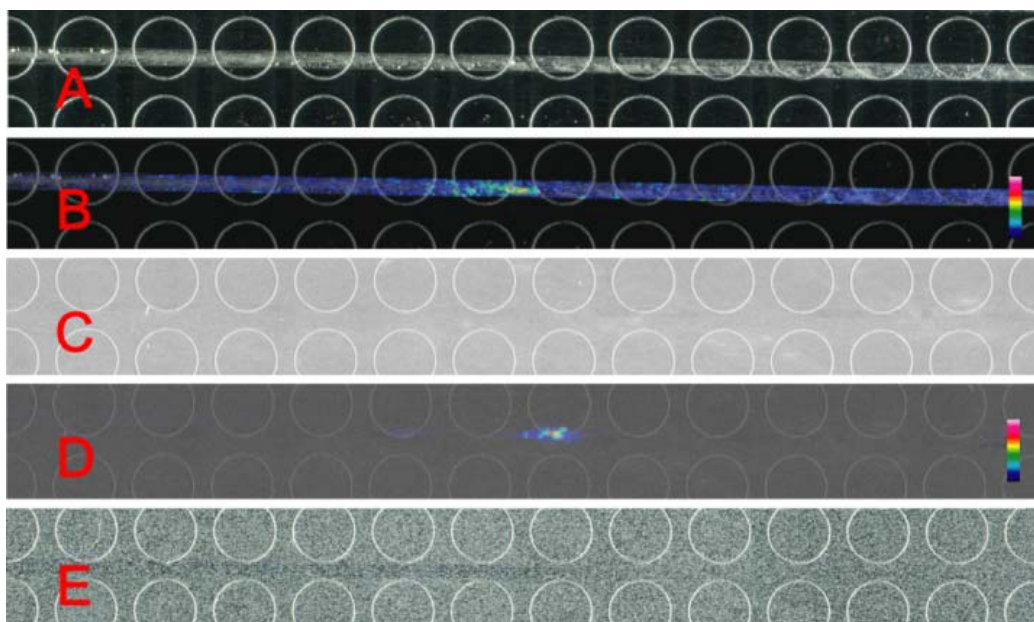


Fig. S1 Comparison of sprayed matrix and capillary-delivered matrix for LC-MSI.

A: The LC trace collected on MALDI plate with matrix delivered by capillary and mixed with LC eluent. “Marble like” surface can be observed due to the irregular size of matrix crystal. B: The image of m/z 1292.9 with LC-MSI analysis using capillary delivered matrix. C: The LC trace collected on the MALDI plate (dried LC trace invisible covered by highly uniform matrix) with matrix sprayed by a sprayer. D: The image of m/z 1292.9 with LC-MSI analysis using sprayed matrix. Less peak diffusion and significantly reduced background noise was obtained compared to panel B. E: The LC trace collected on MALDI plate and with matrix sprayed via “air brush”.

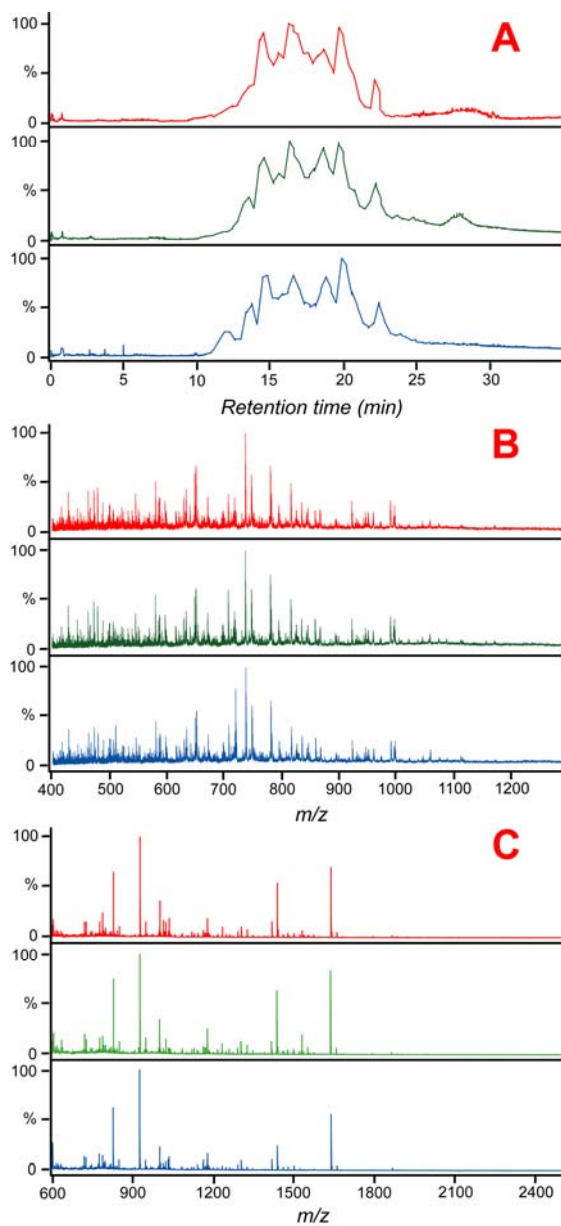


Fig. S2 Reproducibility of monolithic RPLC column on ESI MS and MALDI MSI with BSA tryptic peptides.

A: The total ion chromatograms of three LC runs on consecutive days. B: Mass spectra of three LC-MS runs on consecutive days with ESI-QTOF detection. C: Mass spectra of three LC-MS runs on consecutive days with MALDI MSI detection.