

Supplementary Materials: Application of LC-MS/MS MRM to Determine Staphylococcal Enterotoxins (SEB and SEA) in Milk

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Table S1. Predicted peptide sequences of SEB (ETXB_STAAU) after *in silico* digestions using PeptideMass software. Only tryptic peptides (C-terminal K or R) without any missed cleavages are given. Mass is the monoisotopic mass (not including AM) of the uncharged peptide [M]. AM: Artificial modification.

Order number	Mass	Position	AM	Peptide Sequence	Length (number of amino acids)
1	2290.978	216–234	-	FIENENSFWYDMMPAPGDK	19
2	2129.931	108–124	Cys_CAM: 120	YVDVFGANYYYQCYFSK	17
3	1866.811	139–155	Cys_CAM: 140	TCMYGGVTEHNGNQLDK	17
4	1864.973	67–81	-	SIDQFLYFDLIYSIK	15
5	1837.864	201–215	-	LYEFNNSPYETGYIK	15
6	1586.817	53–66	-	VLYDDNHVSAINVK	14
7	1519.739	28–40	-	ESQPDPKPDELHK	13
8	1308.679	182–192	-	VTAQELDYLTR	11
9	1278.669	169–179	-	NLLSFDVQTNK	11
10	1272.582	126–136	-	TNDINSHQTDK	11
11	1191.517	240–248	-	YLMMYNDNK	9
12	1070.501	44–52	-	FTGLMENMK	9
13	966.5506	257–264	-	IEVYLTTK	8
14	950.469	85–92	-	LGNYDNVR	8
15	694.3406	163–168	-	VFEDGK	6
16	659.3875	193–197	-	HYLVK	5
17	624.2987	235–239	-	FDQSK	5
18	579.2807	249–253	-	MVDSK	5
19	575.3511	158–162	-	SITVR	5
20	561.2878	99–103	-	DLADK	5
21	522.2922	93–96	-	VEFK	4

Table S2. Predicted peptide sequences of SEA (ETXA_STAAU) after *in silico* digestions using PeptideMass software. Only tryptic peptides (C-terminal K or R) without any missed cleavages are given. Mass is the monoisotopic mass (not including AM) of the uncharged peptide [M]. AM: Artificial modification.

Order number	Mass	Position	AM	Peptide Sequence	Length (number of amino acids)
1	3327.664	206–235	-	GLIVFHTSTEPSVNYDLFGA QQQYSNTLLR	30
2	2306.04	80–98	-	GFFTDHSWYNDLLVDFDSK	19
3	2039.917	109–127	Cys_CAM: 120	VDLYGAYYGQCAGGTPNK	19
4	1913.895	242–257	-	TINSENMHIDIYLYTS	16
5	1742.886	66–79	-	ESHDQFLQHTILFK	14
6	1651.732	128–142	Cys_CAM: 130	TACMYGGVTLHDNNR	15
7	1434.654	191–202	-	YNLYNSDVFDGK	12
8	1385.738	173–184	-	NVTVQELDLQAR	12
9	1230.669	40–51	-	SELQGTALGNLK	12
10	1154.657	149–158	-	VPINLWLDGK	10

Order number	Mass	Position	AM	Peptide Sequence	Length (number of amino acids)
11	1128.626	159–168	-	QNTVPLETVK	10
12	1120.531	52–59	-	QIYYNEK	8
13	848.3996	28–34	-	SEEINEK	7
14	680.3613	186–190	-	YLQEK	5
15	619.3297	143–147	-	LTEEK	5
16	589.3191	99–103	-	DIVDK	5

Table S3. Quality criteria used for acceptance of the LC-MS/MS results.

Criteria	Accepted Limits or Descriptions
Retention time	RRT = RT of the IS $\pm$ 0.05
Mass spectral data criteria (Mention if any standards are followed)	According to EU-2002-657
Other applied criteria	1. The S/N * should be higher than 3 2. Visual control of chromatograph 3. At least one positive proteotypic peptide 4. Retention time of light peptides = Retention of heavy peptides $\pm$ 0.05 min 5. 2 transitions positive per proteotypic peptide 6. The intensity rank of the transitions of heavy peptides should match the intensity rank of the transitions of endogenous peptides

* S/N, signal to noise ratio.

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SP|P0A0L2|ETXA_STAAU
MKKTAFTLLLFIALTLTTSPLVNGSEKSEEINEKDLRKKSELQGTALGNLQIYYNEKA 60
SP|P0A0L1|ETXA_STAAW
MKKTAFTLLLFIALTLTTSPLVNGSEKSEEINEKDLRKKSELQGTALGNLQIYYNEKA 60
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SP|P0A0L2|ETXA_STAAU
KTENKESHDQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQC 120
SP|P0A0L1|ETXA_STAAW
KTENKESHDQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQC 120
*****

SP|P0A0L2|ETXA_STAAU
AGGTPNKTACMYGGVTLHDNNRLTEKKVPINLWLDGKQNTVPLETVKTNKKNVTQELD 180
SP|P0A0L1|ETXA_STAAW
AGGTPNKTACMYGGVTLHDNNRLTEKKVPINLWLDGKQNTVPLETVKTNKKNVTQELD 180
*****

SP|P0A0L2|ETXA_STAAU
LQARRYLQEKYNLYNSDVFDGKVQRGLIVFHTSTEPSVNYDLFGAQGQYSNTLLRIYRDN 240
SP|P0A0L1|ETXA_STAAW
LQARRYLQEKYNLYNSDVFDGKVQRGLIVFHTSTEPSVNYDLFGAQGQYSNTLLRIYRDN 240
*****

SP|P0A0L2|ETXA_STAAU KTINSENMHIDIYLYTS 257
SP|P0A0L1|ETXA_STAAW KTINSENMHIDIYLYTS 257

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Figure S1. Alignment of the SEA sequences using CLUSTAL O (1.2.1). This program aligned two selected ETXA_STAAU and ETXA_STAAW protein sequences delivering accurate alignments.