

Supplementary data: Towards a better quantification of cyanotoxins in fruits and vegetables: validation and application of a UHPLC-MS/MS-based method on Belgian products

Table s1: Validation results for eight microcystin congeners (MCs) and nodularin (NOD) in carrots, lettuce and strawberries at three concentration levels and on average. Included parameters are recovery, repeatability, reproducibility, measurement uncertainty (MU), average signal to noise for LOD, average signal to noise for LOQ, R².

Validation data Carrots								
Toxins	Spiked Concentration	Recovery (%)	Repeatability (%)	Reproducibility (%)	Measurement Uncertainty (%)	Average Signal to noise for LOD	Average Signal to noise for LOQ	R ²
MC-RR	1 µg/kg	82.00%	4.42%	13.20%	26.39%	133.07	174.79	0.99
	5 µg/kg	80.00%	5.30%	5.30%	10.61%			
	25 µg/kg	84.00%	8.30%	10.97%	21.94%			
	Average	82.00%	6.01%	9.82%	19.65%			
NOD	1 µg/kg	84.00%	3.69%	11.41%	22.82%	154.23	172.07	0.99
	5 µg/kg	81.00%	5.71%	7.38%	14.76%			
	25 µg/kg	82.00%	5.88%	7.02%	14.04%			
	Average	82.33%	5.10%	8.60%	17.21%			
MC-LA	1 µg/kg	85.00%	8.92%	13.50%	27.00%	50.08	76.93	0.99
	5 µg/kg	79.00%	7.16%	9.21%	18.42%			
	25 µg/kg	81.00%	8.11%	8.11%	16.23%			

	Average	81.67%	8.06%	10.27%	20.55%			
MC-LF	1 µg/kg	83.00%	3.51%	11.30%	22.59%	75.82	78.92	0.99
	5 µg/kg	74.00%	5.45%	14.37%	28.74%			
	25 µg/kg	78.00%	11.83%	11.83%	23.66%			
	Average	78.33%	6.93%	12.50%	25.00%			
MC-LR	1 µg/kg	86.00%	4.89%	12.26%	24.53%	63.12	68.34	0.99
	5 µg/kg	79.00%	5.48%	9.17%	18.35%			
	25 µg/kg	81.00%	7.60%	7.60%	15.24%			
	Average	82.00%	5.99%	9.68%	19.37%			
MC-LY	1 µg/kg	79.00%	10.87%	10.87%	21.74%	25.58	37.39	0.99
	5 µg/kg	77.00%	3.92%	7.20%	14.40%			
	25 µg/kg	81.00%	8.49%	8.49%	16.98%			
	Average	79.00%	7.76%	8.85%	17.71%			
MC-LW	1 µg/kg	76.00%	5.24%	6.50%	12.99%	62.44	68.68	0.99
	5 µg/kg	71.00%	5.96%	14.15%	28.31%			
	25 µg/kg	77.00%	12.77%	12.77%	25.55%			
	Average	74.67%	7.99%	11.14%	22.28%			
MC-YR	1 µg/kg	87.00%	8.27%	12.87%	25.73%	26.99	40.33	0.99
	5 µg/kg	79.00%	6.11%	10.59%	21.17%			
	25 µg/kg	80.00%	6.18%	6.18%	12.37%			
	Average	82.00%	6.86%	9.88%	19.76%			
	1 µg/kg	79.00%	7.86%	9.59%	19.18%	29.18	33.62	0.99

MC-WR	5 µg/kg	75.00%	8.87%	17.35%	34.71%			
	25 µg/kg	77.00%	8.98%	8.98%	17.96%			
	Average	77.00%	8.57%	11.97%	23.95%			
SUM	9 µg/kg	82.00%	3.72%	7.97%	15.93%	N.A.	N.A.	N.A.
	45 µg/kg	77.00%	5.69%	9.40%	18.79%			
	225 µg/kg	80.00%	8.46%	8.46%	16.93%			
	Average	79.67%	5.96%	8.61%	17.22%			
Validation data lettuce								
Toxins	Spiked Concentration	Recovery (%)	Repeatability (%)	Reproducibility (%)	Measurement Uncertainty (%)	Average Signal to noise for LOD	Average Signal to noise for LOQ	R ²
MC-RR	1 µg/kg	85.00%	7.76%	7.76%	15.51%	126.37	139.99	0.99
	5 µg/kg	87.00%	4.52%	9.59%	19.17%			
	25 µg/kg	84.00%	6.49%	13.73%	27.46%			
	Average	85.33%	6.25%	10.36%	20.71%			
NOD	1 µg/kg	84.00%	7.64%	11.54%	23.08%	105.79	129.43	0.99
	5 µg/kg	93.00%	5.41%	6.92%	13.84%			
	25 µg/kg	87.00%	6.29%	11.20%	22.41%			
	Average	88.00%	6.45%	9.89%	19.77%			
MC-LA	1 µg/kg	86.00%	7.52%	13.12%	26.23%	37.36	50.85	0.99
	5 µg/kg	90.00%	5.76%	7.64%	15.28%			
	25 µg/kg	85.00%	5.71%	10.68%	21.35%			

	Average	87.00%	6.33%	10.48%	20.96%			
MC-LF	1 µg/kg	81.00%	7.67%	8.75%	17.49%	55.52	68.48	0.99
	5 µg/kg	89.00%	6.01%	6.01%	12.02%			
	25 µg/kg	83.00%	7.40%	8.67%	17.35%			
	Average	84.33%	7.03%	7.81%	15.62%			
MC-LR	1 µg/kg	85.00%	5.48%	8.69%	17.39%	53.46	72.95	0.99
	5 µg/kg	86.00%	5.93%	8.38%	16.75%			
	25 µg/kg	81.00%	6.54%	15.24%	30.48%			
	Average	84.00%	5.99%	10.77%	21.54%			
MC-LY	1 µg/kg	87.00%	6.48%	11.64%	23.29%	27.24	35.25	0.99
	5 µg/kg	91.00%	7.38%	7.40%	14.80%			
	25 µg/kg	85.00%	6.12%	10.11%	20.22%			
	Average	87.67%	6.66%	9.72%	19.43%			
MC-LW	1 µg/kg	79.00%	6.36%	7.25%	14.50%	41.82	58.34	0.99
	5 µg/kg	85.00%	6.60%	6.57%	13.31%			
	25 µg/kg	81.00%	8.74%	8.74%	17.47%			
	Average	81.67%	7.23%	7.52%	15.10%			
MC-YR	1 µg/kg	83.00%	13.03%	14.25%	28.51%	26.14	32.56	0.99
	5 µg/kg	90.00%	4.45%	6.36%	12.72%			
	25 µg/kg	85.00%	7.00%	11.87%	23.75%			
	Average	86.00%	8.16%	10.83%	21.66%			
	1 µg/kg	80.00%	7.28%	20.35%	40.70%	25.79	25.90	0.99

MC-WR	5 µg/kg	84.00%	6.53%	12.51%	25.03%			
	25 µg/kg	79.00%	8.81%	17.60%	35.20%			
	Average	81.00%	7.54%	16.82%	33.64%			
SUM	9 µg/kg	83.00%	4.75%	9.26%	18.51%	N.A.	N.A.	N.A.
	45 µg/kg	88.00%	5.53%	6.96%	13.92%			
	225 µg/kg	83.00%	6.85%	11.41%	22.82%			
	Average	84.67%	5.71%	9.21%	18.42%			
Validation data Strawberry								
Toxins	Spiked Concentration	Recovery (%)	Repeatability (%)	Reproducibility (%)	Measurement Uncertainty (%)	Average Signal to noise for LOD	Average Signal to noise for LOQ	R ²
MC-RR	5 µg/kg	77.00%	2.32%	5.80%	11.60%	710.17	927.28	0.99
	10 µg/kg	82.00%	5.53%	12.73%	25.46%			
	25 µg/kg	76.00%	7.29%	7.29%	14.58%			
	Average	76.00%	4.61%	5.91%	11.82%			
NOD	1 µg/kg	77.00%	6.39%	8.03%	16.07%	212.13	259.61	0.99
	5 µg/kg	78.00%	4.52%	6.52%	13.03%			
	25 µg/kg	77.00%	3.65%	6.33%	12.65%			
	Average	77.33%	4.85%	6.96%	13.92%			
MC-LA	1 µg/kg	82.00%	9.91%	11.19%	22.37%	66.56	86.60	0.99
	5 µg/kg	78.00%	4.06%	6.58%	13.16%			
	25 µg/kg	77.00%	3.87%	5.37%	10.74%			

	Average	79.00%	5.95%	7.71%	15.42%			
MC-LF	1 µg/kg	74.00%	7.61%	7.61%	15.23%	88.72	89.63	0.99
	5 µg/kg	76.00%	3.02%	5.82%	11.64%			
	25 µg/kg	76.00%	4.04%	5.06%	10.12%			
	Average	75.33%	4.89%	6.16%	12.33%			
MC-LR	1 µg/kg	79.00%	7.06%	7.16%	14.32%	66.36	89.14	0.99
	5 µg/kg	77.00%	5.13%	6.05%	12.11%			
	25 µg/kg	75.00%	4.84%	6.73%	13.46%			
	Average	77.00%	5.68%	6.65%	13.29%			
MC-LY	1 µg/kg	77.00%	6.50%	8.38%	16.76%	35.86	53.57	0.99
	5 µg/kg	74.00%	3.64%	8.15%	16.30%			
	25 µg/kg	74.00%	4.78%	6.89%	13.79%			
	Average	75.00%	4.97%	7.81%	15.62%			
MC-LW	1 µg/kg	75.00%	7.95%	13.47%	26.94%	76.89	81.55	0.99
	5 µg/kg	73.00%	3.79%	8.66%	17.32%			
	25 µg/kg	73.00%	5.75%	8.14%	16.29%			
	Average	73.67%	5.83%	10.09%	20.18%			
MC-YR	1 µg/kg	73.00%	8.09%	12.77%	25.54%	33.53	40.28	0.99
	5 µg/kg	74.00%	4.10%	7.88%	15.75%			
	25 µg/kg	74.00%	4.27%	6.81%	13.61%			
	Average	73.67%	5.49%	9.15%	18.30%			
	1 µg/kg	77.00%	11.54%	11.54%	23.07%	33.72	32.12	0.99

MC-WR	5 µg/kg	73.00%	6.01%	9.19%	18.38%			
	25 µg/kg	73.00%	5.28%	9.27%	18.54%			
	Average	74.33%	7.61%	10.00%	20.00%			
SUM	8 µg/kg	77.00%	6.89%	6.89%	13.78%	N.A.	N.A.	N.A.
	45 µg/kg	76.00%	3.35%	5.95%	11.90%			
	225 µg/kg	75.00%	3.86%	5.31%	10.63%			
	Average	76.00%	4.54%	5.90%	11.79%			

Table s2: Overview of samples taken from the Belgium market showing separate results, origin, sample type and sample annotation.

Sample annotation	Sample type	origin	Sample date	Total concentration MCs (µg kg ⁻¹)
S22FD00063	Strawberries	Hoogstraten, Belgium	29/06/2021	<LOD
S22FD00109	Strawberries	Hoogstraten, Belgium	30/06/2021	<LOD
S22FD00110	Strawberries	Belgium	30/06/2021	<LOD
S22FD00111	Strawberries	Belgium	30/06/2021	<LOD
S22FD00112	Strawberries	Hoogstraten, Belgium	30/06/2021	<LOD
S22FD00113	Strawberries	Hoogstraten, Belgium	30/06/2021	<LOD
S22FD00114	Strawberries	Sint-Katelijne Waver, Belgium	30/06/2021	<LOD
S22FD00115	Radish	Nederland	27/07/2021	<LOD
S22FD00116	Radish	Belgium	27/07/2021	<LOD
S22FD00117	Radish	Belgium	27/07/2021	<LOD

S22FD00118	Radish	Belgium	27/07/2021	<LOD
S22FD00119	Radish	Nederland	27/07/2021	<LOD
S22FD00120	Lettuce	Belgium	10/08/2021	<LOD
S22FD00121	Lettuce	Belgium	10/08/2021	<LOD
S22FD00122	Lettuce	Belgium	10/08/2021	<LOD
S22FD00123	Lettuce	Sint-Katelijne Waver, Belgium	10/08/2021	<LOD
S22FD00124	Lettuce	Sint-Katelijne Waver, Belgium	10/08/2021	<LOD
S22FD00125	Lettuce	Belgium	15/08/2021	<LOD
S22FD00126	Lettuce	Belgium	16/08/2021	<LOD
S22FD00127	Lettuce	Belgium	17/08/2021	<LOD
S22FD00128	Lettuce	Belgium	18/08/2021	<LOD
S22FD00129	Lettuce	Belgium	17/11/2021	<LOD
S22FD00130	Lettuce	Belgium	17/11/2021	<LOD
S22FD00131	Lettuce	Belgium	17/11/2021	<LOD
S22FD00132	Lettuce	Belgium	17/11/2021	<LOD
S22FD00133	Potato	Belgium	10/08/2021	<LOD
S22FD00134	Potato	Germany	10/08/2021	<LOD
S22FD00135	Potato	Spain	10/08/2021	<LOD
S22FD00136	Potato	Belgium	10/08/2021	<LOD
S22FD00137	Potato	Belgium	10/08/2021	<LOD

S22FD00138	Potato	Belgium	10/08/2021	<LOD
S22FD00139	Potato	Belgium	10/08/2021	<LOD
S22FD00140	Potato	Belgium	17/11/2021	<LOD
S22FD00141	Potato	Belgium	17/11/2021	<LOD
S22FD00142	Potato	Spain	17/11/2021	<LOD
S22FD00143	Potato	Belgium	17/11/2021	<LOD
S22FD00144	Onion	Belgium	10/08/2021	<LOD
S22FD00145	Onion	Belgium	10/08/2021	<LOD
S22FD00146	Onion	Belgium	10/08/2021	<LOD
S22FD00147	Onion	Belgium	10/08/2021	<LOD
S22FD00148	Onion	Belgium	10/08/2021	<LOD
S22FD00149	Onion	Belgium	15/08/2021	<LOD
S22FD00150	Onion	Belgium	16/08/2021	<LOD
S22FD00151	Onion	Belgium	17/08/2021	<LOD
S22FD00152	Onion	Belgium	17/08/2021	<LOD
S22FD00153	Onion	Belgium	17/08/2021	<LOD
S22FD00154	Onion	Belgium	17/08/2021	<LOD
S22FD00155	Carrots	Belgium	10/08/2021	<LOD
S22FD00156	Carrots	Belgium	10/08/2021	<LOD
S22FD00157	Carrots	Belgium	10/08/2021	<LOD

S22FD00158	Carrots	Belgium	10/08/2021	<LOD
S22FD00159	Carrots	Belgium	10/08/2021	<LOD
S22FD00160	Carrots	Belgium	10/08/2021	<LOD
S22FD00161	Carrots	Belgium	10/08/2021	<LOD
S22FD00162	Carrots	Belgium	10/08/2021	<LOD
S22FD00163	Carrots	Belgium	10/08/2021	<LOD
S22FD00164	Carrots	Belgium	17/11/2021	<LOD
S22FD00165	Carrots	Belgium	17/11/2021	<LOD
S22FD00166	Carrots	Belgium	17/11/2021	<LOD
S22FD00167	Carrots	Belgium	17/11/2021	<LOD
S22FD00168	Tomato	Belgium	27/07/2021	<LOD
S22FD00169	Tomato	Belgium	27/07/2021	<LOD
S22FD00170	Tomato	Belgium	27/07/2021	<LOD
S22FD00171	Tomato	Belgium	27/07/2021	<LOD
S22FD00172	Tomato	Belgium	27/07/2021	<LOD
S22FD00173	Tomato	Belgium	27/07/2021	<LOD
S22FD00174	Tomato	Belgium	27/07/2021	<LOD
S22FD00175	Tomato	Belgium	27/07/2021	<LOD
S22FD00176	Tomato	Belgium	27/07/2021	<LOD
S22FD00177	Tomato	Belgium	27/07/2021	<LOD

S22FD00178	Tomato	Belgium	27/07/2021	<LOD
S22FD00179	Tomato	Belgium	27/07/2021	<LOD
S22FD00180	Tomato	Belgium	27/07/2021	<LOD
S22FD00181	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00182	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00183	Cherry tomato	Hoogstraten, Belgium	27/07/2021	<LOD
S22FD00184	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00185	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00186	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00187	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00188	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00189	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00190	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00191	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00192	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00193	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00194	Cherry tomato	Belgium	27/07/2021	<LOD
S22FD00195	Chicory	Belgium	29/06/2021	<LOD
S22FD00196	Chicory	Belgium	30/06/2021	<LOD
S22FD00197	Chicory	Belgium	30/06/2021	<LOD

S22FD00198	Chicory	Belgium	30/06/2021	<LOD
S22FD00199	Chicory	Belgium	30/06/2021	<LOD
S22FD00200	Chicory	Belgium	30/06/2021	<LOD
S22FD00201	Chicory	Belgium	30/06/2021	<LOD
S22FD00202	Chicory	Belgium	30/06/2021	<LOD
S22FD00204	Chicory	Belgium	30/06/2021	<LOD
S22FD00205	Chicory	Belgium	30/06/2021	<LOD
S22FD00206	Chicory	Belgium	30/06/2021	<LOD
S22FD00207	Chicory	Belgium	30/06/2021	<LOD
S22FD00208	Chicory	Sint-Katelijne Waver, Belgium	30/06/2021	<LOD
S22FD00211	Chicory	Sint-Katelijne Waver, Belgium	30/06/2021	<LOD
S22FD00212	Chicory	Belgium	30/06/2021	<LOD

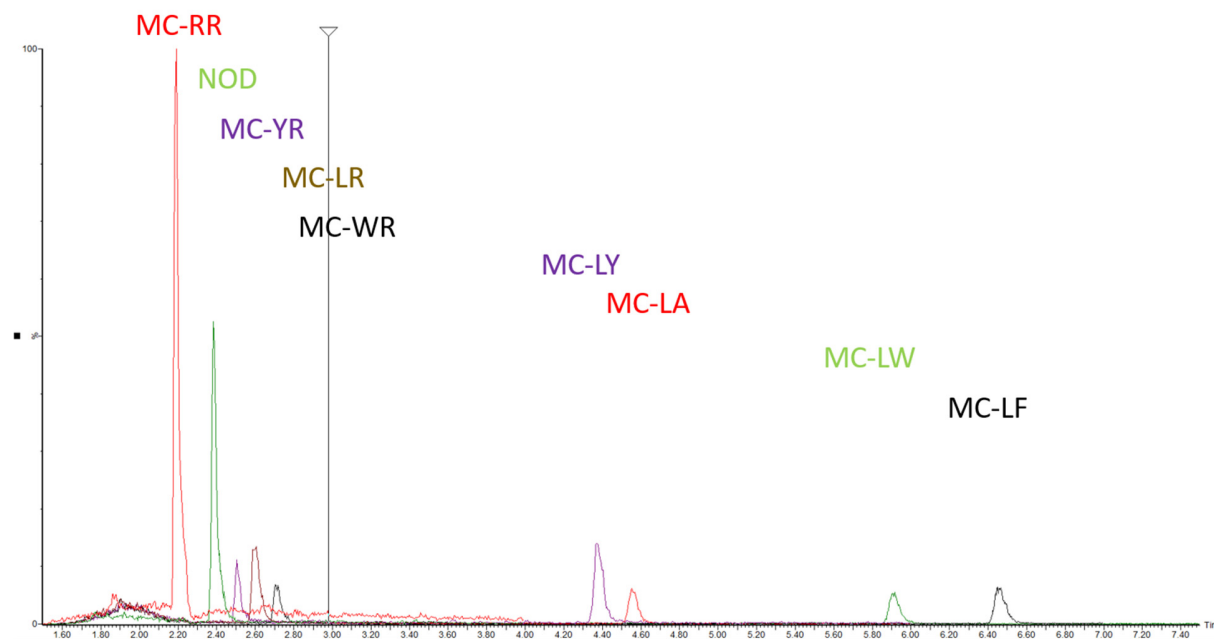


Figure S1: The elution peaks for 8 microcystin congeners and NOD in carrot matrix at validation level 5 ng g^{-1} . The peaks are presented together at representable ratios based on the peak intensities by overlaying the chromatograms of the different toxins. However, during analysis, the chromatograms for each toxin are analyzed separately.

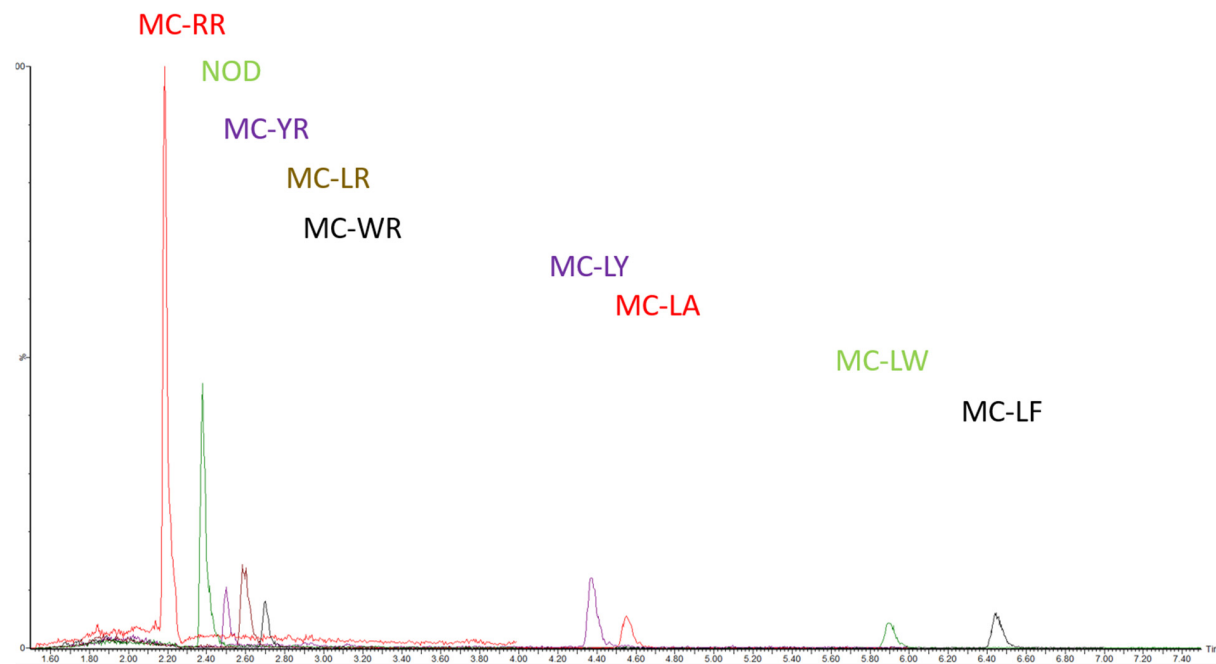


Figure S2: elution peaks for 8 microcystin congeners and NOD in lettuce matrix at validation level 5 ng g^{-1} . The peaks are presented together at representable ratios based on the peak intensities by overlaying the chromatograms of the different toxins. However, during analysis, the chromatograms for each toxin are analyzed separately.

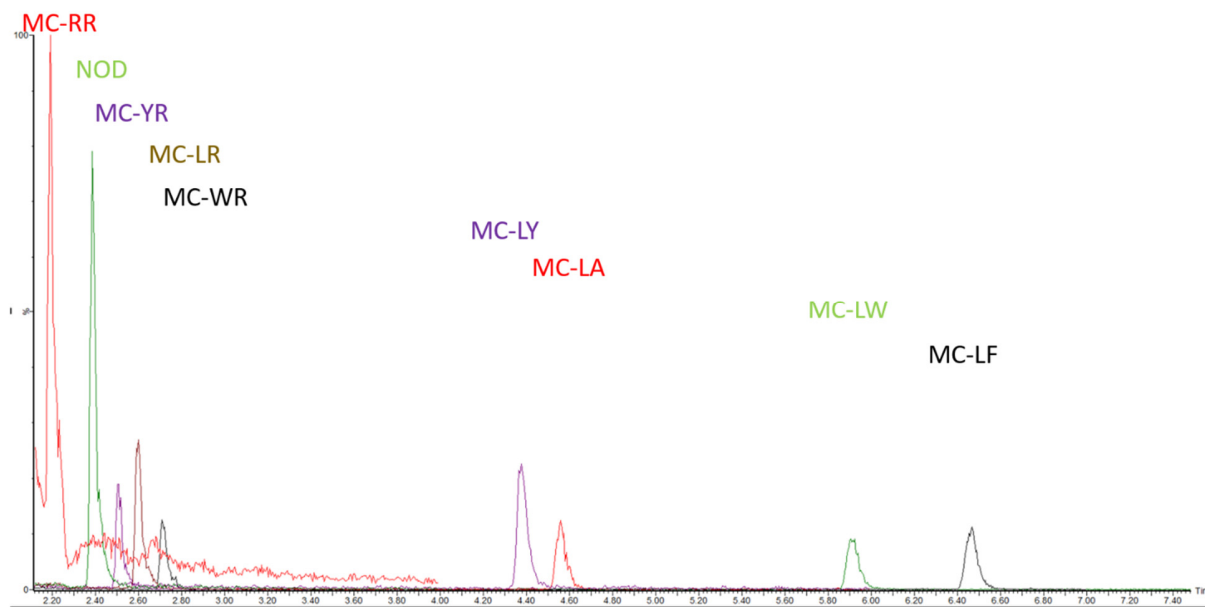


Figure S3: elution peaks for 8 microcystin congeners and NOD in lettuce matrix at validation level 5 ng g^{-1} . The peaks are presented together at representable ratios based on the peak intensities by overlaying the chromatograms of the different toxins. However, during analysis, the chromatograms for each toxin are analyzed separately.