Supplementary Material

Meat authentication: A new HPLC-MS/MS based method for the fast and sensitive detection of horse and pork in highly processed food

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Supplementary Table 1– Commercial Samples

Sample #	Declaration	Date of purchase	Species declared	Source	Packing - Storage
C_1	Corned Beef A	Mar-12	Beef	Supermarket Münster	Canned - Shelf
C_2	Corned Beef B	Dec-13	Beef	Supermarket Münster	Canned - Shelf
C_3	Corned Beef C	Dec-13	Beef	Supermarket Münster	Canned - Shelf
C_4	Mini Salami A	Nov-13	Beef and Pork	Supermarket Münster	Plastic / aluminium wrapping - Shelf
C_5	Pork in jus	Mar-12	Pork	Supermarket Münster	Canned - Shelf
C_6	Salami A	Jan-14	Beef	Supermarket Münster	Plastic wrapping - Shelf
C_7	Salami B	Oct-13	Deer and Pork	Supermarket Münster	Plastic wrapping - Shelf
C_8	Salami C	Oct-13	Pork and Wild Boar	Supermarket Münster	Plastic wrapping - Shelf
C_9	Mini Salami B	Jan-14	Pork	Supermarket Münster	Plastic / aluminium wrapping - Shelf
C_10	Bolognese Sauce A	Jan-14	Beef and Pork	Supermarket Münster	Glas - Shelf
C_11	Bolognese Sauce B	Jan-14	Beef and Pork	Supermarket Münster	Glas - Shelf
C_12	Frikadeller	Jan-14	Pork	Supermarket Münster	Plastic wrapping - Shelf
C_13	Hamburger Patty	Jan-14	Beef	Supermarket Münster	Plastic wrapping - Frozen
C_14	Spaghetti Bolognese (Sauce and Noodles)	Jan-14	Beef and Pork	Supermarket Münster	Plastic wrapping - Chilled
C_15	Lasagnese Bolognese	Jan-14	Beef and Pork	Supermarket Münster	Paper - Frozen
C_16	Meatball in sauce	Jan-14	Beef and Pork	Supermarket Münster	Plastic wrapping - Frozen
C_17	Mini Salami C	Jan-14	Horse	Farmer´s Market Münster	unpacked - Shelf
C_18	Frikandeln A	Jan-14	Pork	Supermarket Münster	Plastic wrapping - Frozen
C_19	Frikandeln B	Jan-14	Pork and Chicken	Supermarket Ensche (NL)	Plastic wrapping - Frozen
C_20	Frikandeln C	Jan-14	Pork and Chicken	Supermarket Ensche (NL)	Plastic wrapping - Frozen
C_21	Frikandel D	Jan-14	Pork and Chicken	Supermarket Ensche (NL)	Plastic wrapping - Frozen
C_22	Frankfurter Sausage	Jan-14	Horse	Farmer´s Market Münster	unpacked - Chilled
C_23	Salami D	Jan-14	Horse	Farmer´s Market Münster	unpacked - Shelf

Supplementary Table 2 – MS and HPLC conditions

Turbo V Source Paratemeters

Parameter	Value
Curtain Gas	35
Source Gas 1	40
Source Gas 2	40
Spray Voltage [V]	5500
Source Temperature [°C]	400

HPLC-Conditions

Injection Volume [µL]	45
Oven Temperature [°C]	45
Autosampler Cooling Temp [°C]	4
Flow [mL|min]	0.3

HPLC-Gradient

Time[min]	ACN (0.1 % FA) [%]	0.1 % FA [%]
0	3	97
22	28.4	71.6
23	100	0
28	100	0
29	3	97
35	3	97

Supplentary Table 3: MRM parameters and MRM^3 paremeters

Marker Peptide # (see Table XY)	charge state parent ion / fragment ion	Q1	Q3	Dwell time (ms)	CE [V]	CXP [V]	EP [V]	DP [V]
1	(+)2/b2	453.8	279.1	30	19.0	10.0	10.0	100.0
1	(+)2/b3	453.8	392.2	30	19.0	10.0	10.0	100.0
1	(+)2/y3	453.8	402.3	30	47.0	11.0	10.0	100.0
1	(+)2/b4	453.8	505.3	30	19.0	10.0	10.0	100.0
1	(+)2/b5	453.8	619.3	30	47.0	11.0	10.0	100.0
1	(+)2/y6	453.8	743.4	30	35.0	8.0	10.0	100.0
2	(+)2/y3	534.4	375.3	30	28.0	10.0	13.0	100.0
2	(+)2/y4	534.4	522.1	30	39.0	10.0	13.0	100.0
2	(+)2/y5	534.4	635.2	30	39.0	10.0	13.0	100.0
2	(+)2/y6	534.4	782.4	30	39.0	10.0	13.0	100.0
2	(+)2/y7	534.4	853.3	30	39.0	10.0	13.0	100.0
3	(+)3/b7 (+2)	376.1	322.2	30	28.0	10.0	5.0	100.0
3	(+)3/b8 (+2)	376.1	392.8	30	34.1	10.0	5.0	100.0
3	(+)3/y4	376.1	477.2	30	28.0	10.0	5.0	100.0
3	(+)3/y5	376.1	576.4	30	34.1	10.0	5.0	100.0
3	(+)3/y6	376.1	647.2	30	34.1	10.0	5.0	100.0
4	(+)2/b2	582.8	277.1	30	32.0	10.0	10.0	100.0
4	(+)2/y5	582.8	589.3	30	32.0	10.0	10.0	100.0
4	(+)2/y6	582.8	646.3	30	32.0	10.0	10.0	100.0
4	(+)2/y7	582.8	759.4	30	32.0	10.0	10.0	100.0
4	(+)2/y8	582.8	888.5	30	41.5	10.0	10.0	100.0
5	(+)2/b2	508.3	213.2	30	41.0	10.0	10.0	100.0
5	(+)2/y5	508.3	574.3	30	41.0	10.0	10.0	100.0
5	(+)2/y6	508.3	689.4	30	41.0	10.0	10.0	100.0
5	(+)2/y7	508.3	803.4	30	31.3	10.0	10.0	100.0
5	(+)2/y8	508.3	902.5	30	31.3	10.0	10.0	100.0

MRM^3: Peptide Marker 4 582.8-->646.3-->345.0

Parameter	Start	Stop
AF2	0.2	0.2
AF3	4.19	4.21
CE	22	22
CES	3	3
DP	100	100
EP	6	6
EXB	-144	-142

MRM^3: Peptide Marker 1 453.8-->743.4-->628.8

Parameter	Start	Stop
AF2	0.1	0.11
AF3	1.508	4.284
CE	32	38
CES	13	17
DP	100	100
EP	10	10
EXB	-158	-120

Detailed description of the MS experiments used in this publication

In this study a HPLC-ESI-MS/MS system was used, as our HRMS system is less sensitive.

One of the characteristics of collisional-induced dissociation (CID)-based MS/MS experiments of peptides is the excellent predictability of the fragmentation pattern. CID nearly exclusively leads to fragmentation of the amide bond, resulting in the formation of b-ions (the charge resides on the N-terminal fragment of the peptide), and y-ions (charge is located on the C-terminal part).

The MRMPilot software supports automated method development by the prediction of multiple reaction monitoring (MRM) transitions based on the CID fragmentation pattern of peptides.

To the best of our knowledge no official guidelines are in place that define the minimal number of MRM transitions per peptide required for specific detection. For all peptides included in this study, we detected at least four MRM-transitions. A reduction of peptide concentration results in a reduced number of MRM transitions as MRMs with lower signal intensities are no longer detectable. To ensure specificity down to the LOD, we defined that at least three MRM-transitions, matching retention time and matching signal intensities of detected MRMs are required for specific detection.

To reach the best LOD, we took advantage of the MRM^3 capabilities of the linear ion trap (Qtrap ©). In the MRM^3 mode, the ion trap performs a secondary MRM experiment by collecting a specific fragment ion (e.g. *m/z* 743.2 from MRM transition*/z* 453.8→ 743.2) and further fragments it (e.g. *m/z* 453.8→ 753.8→ 628.3). As the ions are collected in the linear ion trap prior to MRM^3 fragmentation, this experiment further increases sensitivity and is very specific. Using the QTrap, lacking MRM-transitions can therefore be replaced by MRM^3 experiments to ensure specificity at low concentrations.