National Food Institute

Analytical approach suitable for

Determination of 1,2-dihydroxybenzene in food (meat, cheese and fish) with smoke flavour

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The EU Reference Laboratory for Processing Contaminants was asked about suitable methods and expected achievable Limits of Quantifications (LOQs) for determination of 1,2-dihydroxybenzene in foods, including meat, cheese and fish, The compounds are relevant for smoked, via a smoking process or smoke flavour, foods.

Conclusion

The EURL-PC has tested a simple extraction and clean-up approach using LC-MS/MS for the determination of 1,2-dihydroxybenzene in food (meat, cheese and fish) and has achieved acceptable results. The lowest tested level was 10 µg/kg, which should therefore be considered to be the preliminary Limits of Quantification (LOQ) for the compound.

Limitation

This approach has been tested, but not finally validated due to a very short deadline given for the work. Therefore, appropriate validation should be performed by laboratories using the described approach.

Scope

Foods – the approach has been test on meat, cheese and fish.

Methodology

The determination of 1,2-dihydroxybenzene has been included in this document.

Compound	CAS No.
1,2-dihydroxybenzene / benzene-1,2-diol	120-80-9
1,2-dihydroxybenzene-D6 / benzene-1,2-diol-D6 (IS)	202656-22-2

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1. A representative sample should be homogenised and 2 g of sample is transferred to a 15 mL centrifuge tube.
2. Add 3 mL water and vortex for 15 seconds.
3. Add internal standard (0.2 µg of 1,2-dihydroxybenzene-D6)
4. Add 5 mL 0.1% formic acid in methanol and vortex for 15 seconds.
5. Centrifuge using 3500g for 10 min. (4°C)
6. Transfer the liquid to a centrifuge tube and add 3 mL heptane
7. Rotate on tube rotator for 15 min at low speed
8. Centrifuge using 3500g for 5 min. (4°C)
9. Remove the upper heptane phase
10. Evaporate (gently) 4 mL of sample to 1.5 mL at 40°C with nitrogen
11. Transfer the final extract into a filter vial (0.45µm) and analyse using LC-MS/MS.
12. Prepare aqueous calibration samples in LC-vials with 0; 1; 2; 5; 10; 20 and 50 ng/mL with 50 ng/mL internal standard

Instrumentation

LC-MS/MS instrument equipped with a Kinetex F5 column, 150 mm x 2.1 mm, 1.7 µm

Flowrate: 0.2 mL/min.

Injection: 2 µL

Column temperature: 30°C

Solvent and gradient:

Time (min)	% Mobile phase A (0.05% acetic acid)	% Mobile phase B (acetonitrile)
0	98	2
0.3	80	20
6	20	80
8	20	80
8.1	98	2
15	98	2

MS parameters[1]

Ionisation: negative electrospray
Spray voltage: 3 kV
Cone temperature and gas flow: 350°C, 20
Probe temperature and gas flow: 300°C, 40
Nebuliser gas flow: 50

Compounds	**RT [min.]**	**Precursor [m/z]**	**Product [m/z]**	**Collision energy (eV)**
1,2-dihydroxybenzene	7.5	109	91 (Quantifier) 108 (Qualifier) 81 (Qualifier)	17 4 13
1,2-dihydroxybenzene-d6 (IS)	7.4	113	94 66	21 24

[1] Bruker EVOQ Elite mass spectrometer

Results from preliminary test of the method

Two concentration levels (10 µg/kg and 50 µg/kg) in meat, fish tissue and cheese has been tested for estimating trueness, precision and LOQ. Four replicates for each sample type and concentration level are analysed, repeated on two days. Content in the blank sample material was subtracted from the spike level.

Precision below 12% was achieved and mean recoveries are in the range 86 – 116%. Absolut recovery is lowest in cheese (23-33%) and highest in meat (42-57%). Therefore, use of correction is essential for example as described by use of internal standard.

Sample type	N	Level	Reproducibility	Repeatability	Recovery	Abs. recovery
Fish muscle	8	10 µg/kg	4%	2%	97%	33%
	8	50 µg/kg	2%	2%	102%	23%
Meat	8	10 µg/kg	11%	6%	86%	57%
	8	50 µg/kg	7%	3%	101%	42%
Cheese	8	10 µg/kg	6%	4%	116%	40%
	8	50 µg/kg	8%	3%	109%	35%

Ion ratio (for qualifier ions) for all spiked samples are comparable to ion ratio in the standards (using 20% tolerance). In the sample of cheese a peak eluted just before 1,2-dihydroxybenzene, but the two peaks were well separated.

Limit of quantification calculated as 6 times repeatability standard deviation is below 4 µg/kg for all sample types but since the lowest tested spike level is 10 µg/kg this level is established as the preliminary LOQ.

Sample type	LOQ (6*SDr)	LOQ (Lowest tested level)
Fish muscle	1.2	10
Meat	3.3	10
Cheese	3.9	10