Development and validation of a multi-residue HRMS method for the screening of antimicrobials drugs residues in eggs by LC-IMS-QToF and comparison with Q-Orbitrap technology

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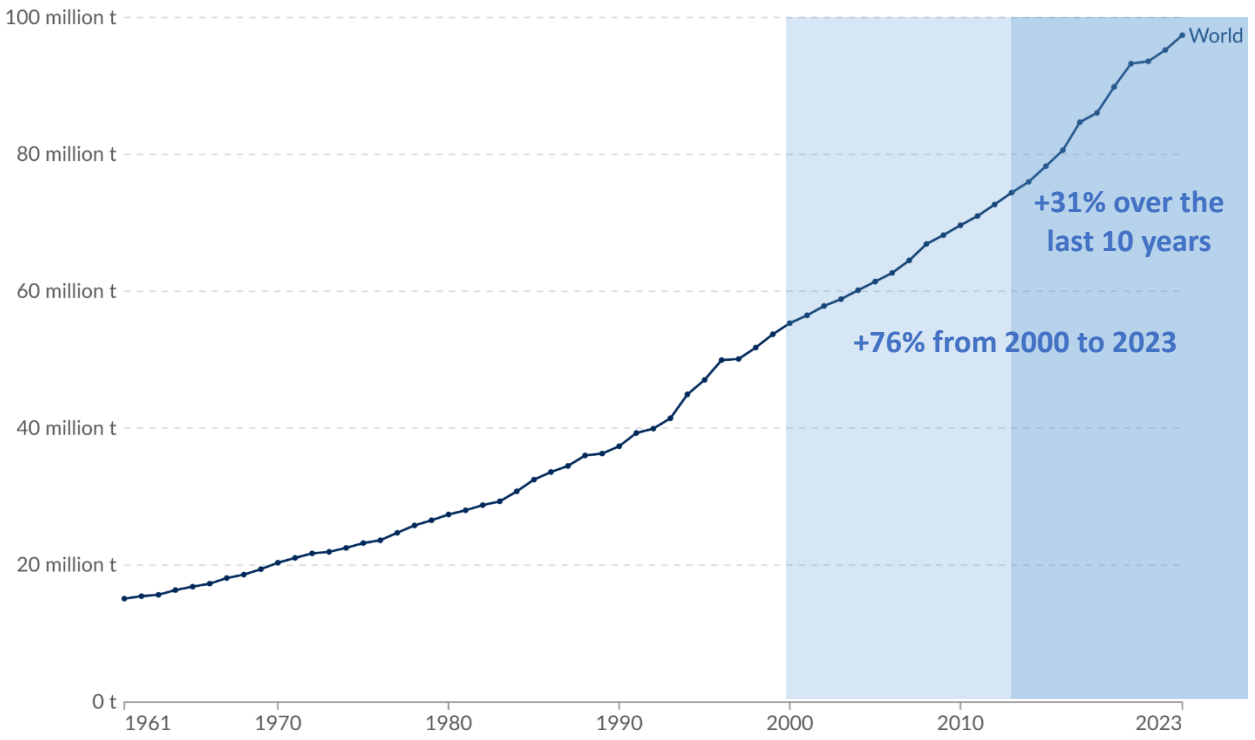
1 — Context & objectives

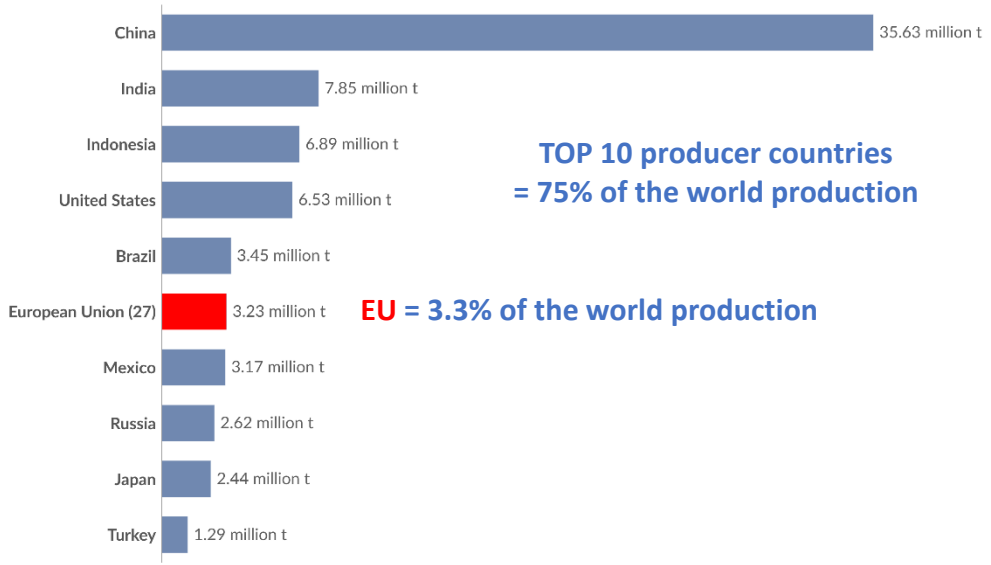
Context: Why control eggs?

Egg production, 1961 to 2023

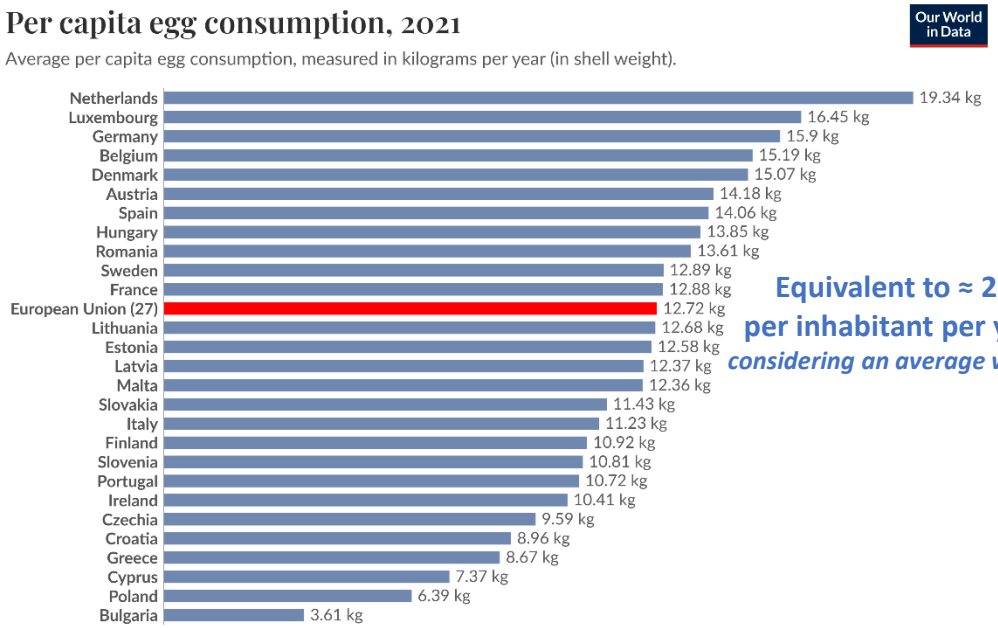


Data source: Food and Agriculture Organization of the United Nations (2025)
OurWorldinData.org/meat-production | CC BY
Note: Figures include eggs derived from all domesticated or farmed birds.

Egg production, 2023



Per capita egg consumption, 2021



Data source: Food and Agriculture Organization of the United Nations (2024)
OurWorldinData.org/meat-production | CC BY
Note: Data refers to average per capita food supply at the consumer level, but does not correct for any wastages at the household level.

Eggs control is important because this matrix is consumed almost daily !

Context: Why use a Q-ToF HRMS system?

7.4.2017 EN Official Journal of the European Union L 95/1

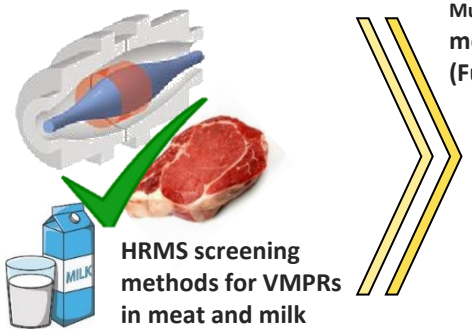
Regulation 2017/625 requires to ensure the control of VMPRs in food

REGULATIONS

REGULATION (EU) 2017/625 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 15 March 2017

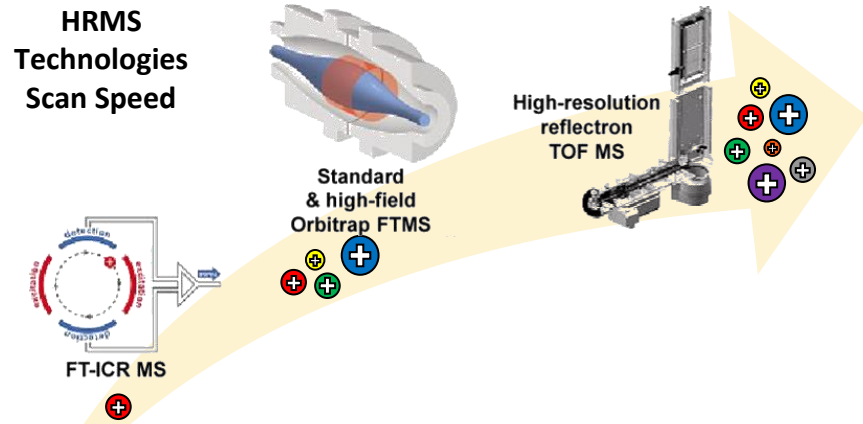
on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation)



HRMS screening methods for VMPRs in meat and milk (Orbital trap – FullScan vDIA)

Multi-VMPRs screening method for egg products (Full Scan HRMS - ToF)

- ❑ Including as many relevant Group B1 antibiotic residues as possible
- ❑ Using a single Full Scan High Resolution Mass Spectrometric instrument (UPLC-IMS-QTOF-MS)



LC-Q-ToF HRMS

- ❑ Acquisition speed
- ❑ Equipment miniaturization (reflectron)
- ❑ Increase in the number of equipped labs

Strategic context for the evolution of screening methods

- ❑ Regulatory to exposure strategic context
- ❑ Technology allows retrospective analyses

Objectives

Objective 01
Develop a full-scan HRMS method for broad VMPR analysis and high-throughput screening in egg products using an LC-IMS-QToF system

Objective 02
Validation according to Reg (EU) 2021/808 (screening method)

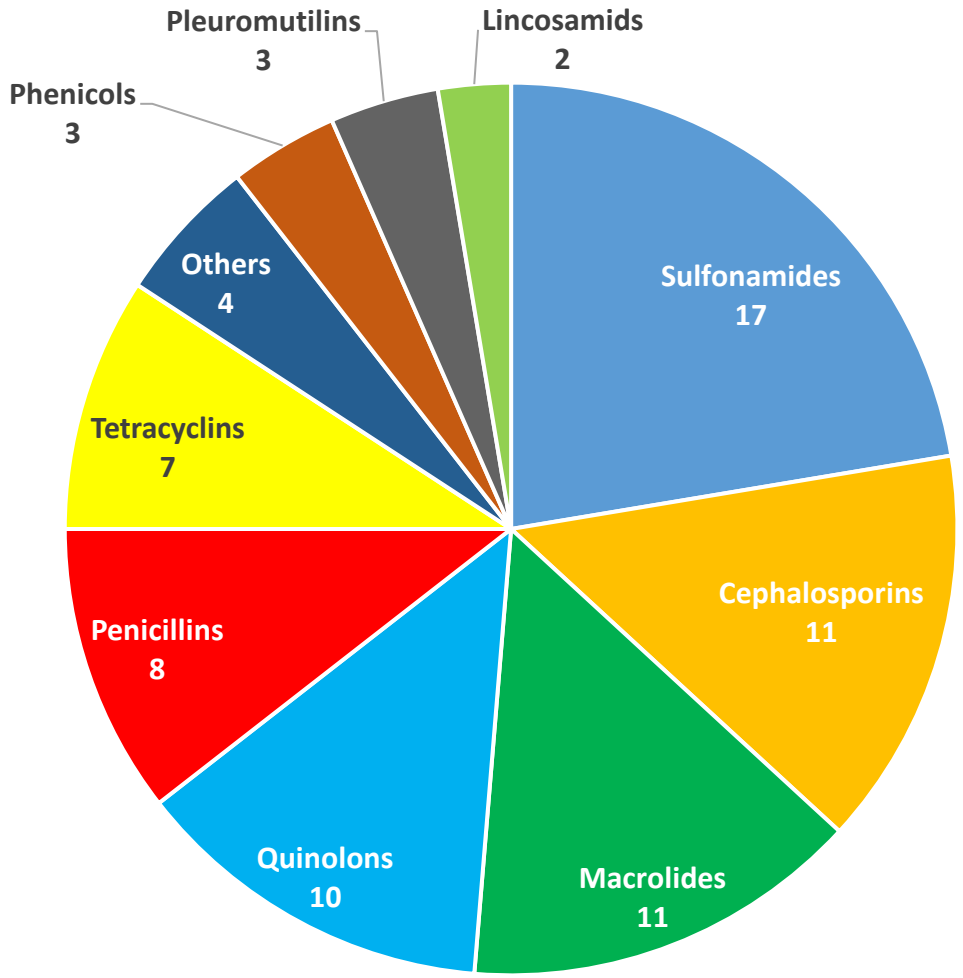
Objective 03
Study of the contribution of ion mobility

Objective 04
Performance comparison between two HRMS systems: Orbitrap VS ToF

2 — Materials & Methods

Field of application: Analytes of interest in eggs

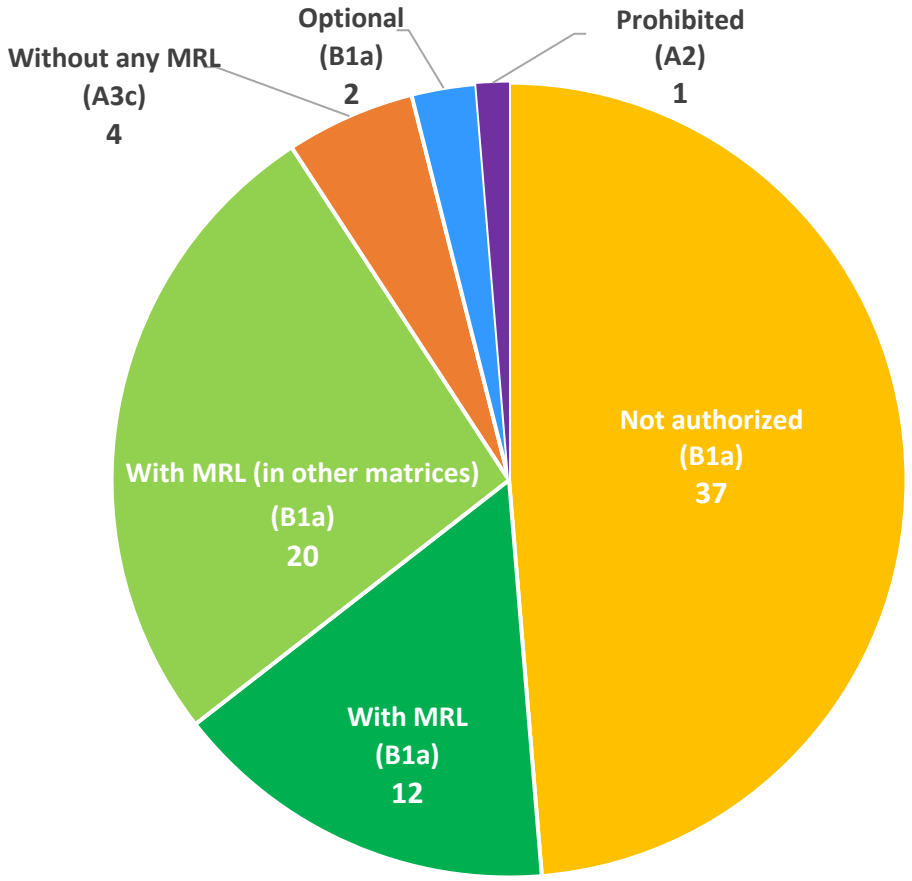
Antibiotics from Group B1a & A3c



- Analytes choices were based on NRMP recommendations
- 10 family classes
- 76 analytes
- High variability of physico-chemical properties

Field of application: Analytes of interest in eggs

Regulatory status of antibiotics of interest



B1a (table 1 of reg 37/2010): specified as "not authorized for laying hens »
CCβ as low as possible -> at or above LCL

A3c (not in reg 37/2010): substances without any MRL
CCβ as low as possible -> at or above LCL

B1a (table 1 of reg 37/2010): authorized for laying hens (MRL in eggs)
0.1 MRL ≤ **CCβ** ≤ 0.5 MRL (MRLs ranged from 25 to 200 µg/kg)

B1a (table 1 of reg 37/2010): no MRL in eggs
MMPR = 0.25 x Lowest MRL of all other matrices (cascade MRL)
CCβ < MMPR (MMPRs ranged from 2.5 to 50 µg/kg)

A2 (table 2 of Reg 37/2010): prohibited substances
CCβ < MMPR = 5 µg/kg (Dapsone)

B1a (table 1 of reg 37/2010): optional MRL substances in eggs (metabolites)
0.1 MRL ≤ **CCβ** ≤ 0.5 MRL

Methods: Analytical system

UPLC Acquity H-CLASS
Binary Solvent Manager
Limit Pressure: 1250 bars

Ion Source: ESI or UniSpray
Zspray interface
LockSpray: Leucine Enkephalin (internal calibration for mass and CCS); mass accuracy < 3 ppm

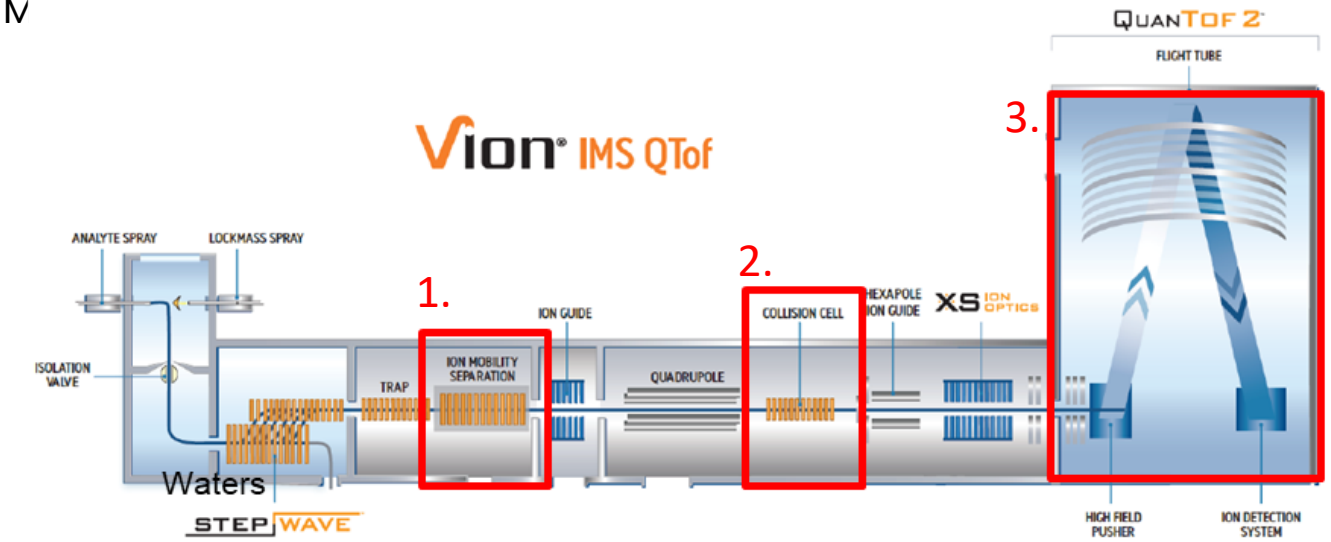
Resolution > 50 000 (MS, MS/MS) m/z 956 (40Hz)
> 44 000 (MS, MS/MS) m/z 785 (40Hz)
> 35 000 (MS, MS/MS) m/z 152 (40Hz)

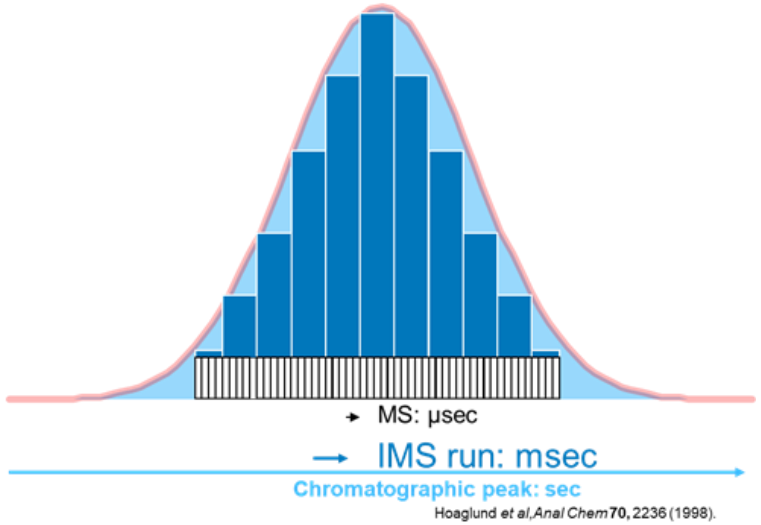
Acquisition mode: HD-MS^E (IMS and IDA):
Ionic Mobility couple to Low and high energy full scan acquisition

Methods: HDMS^E acquisition mode

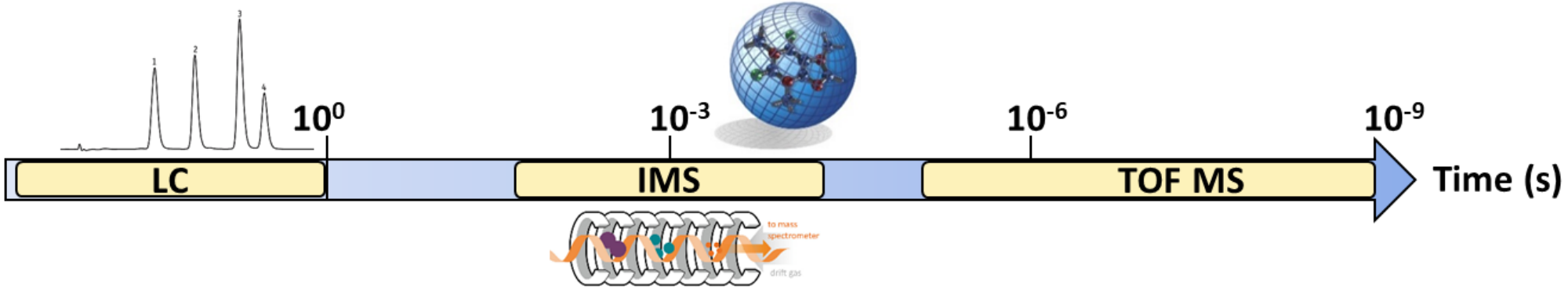
1. **IMS :** Determination of Drift Time (CCS values) from precursors ions
2. **MS^E :** Fragmentation at Low and High ramping Energy
3. **MS :** M

HDMS^E = IMS + MS^E)

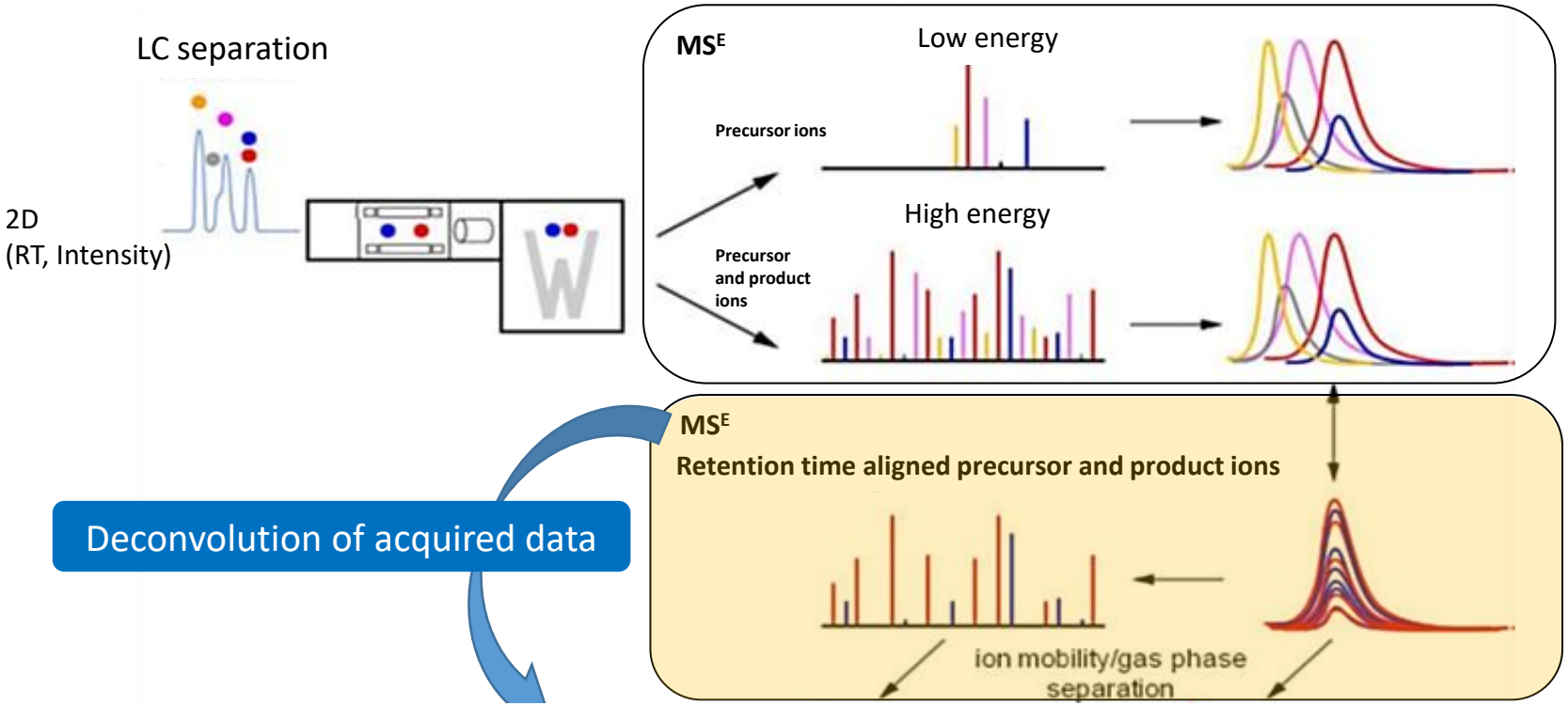


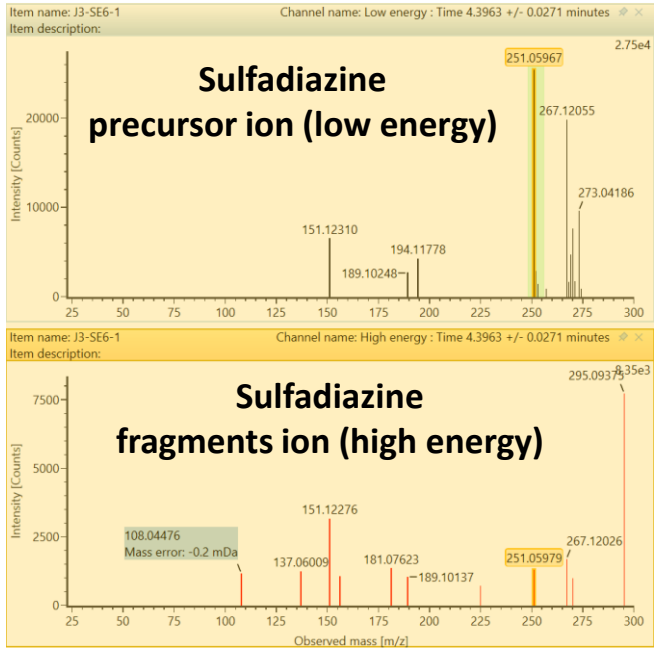


Precursors and Products Ions have the same CCS



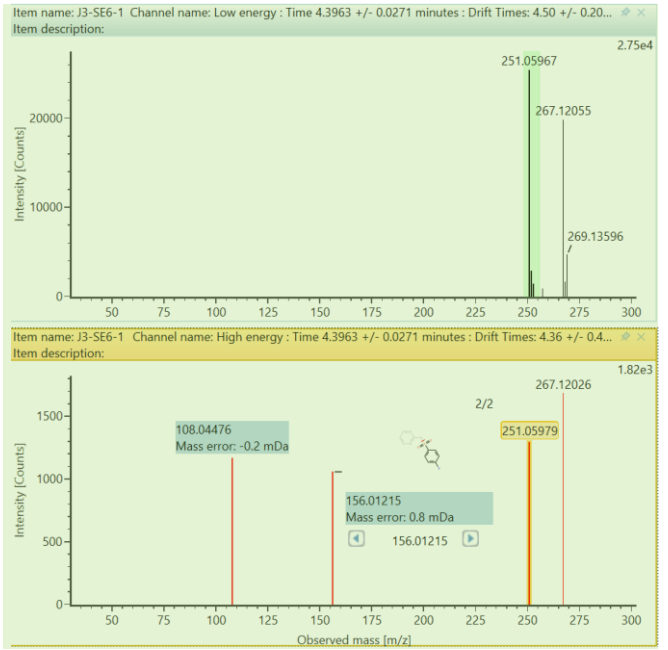
Methods: HDMS^E acquisition mode





MS^E

IMS



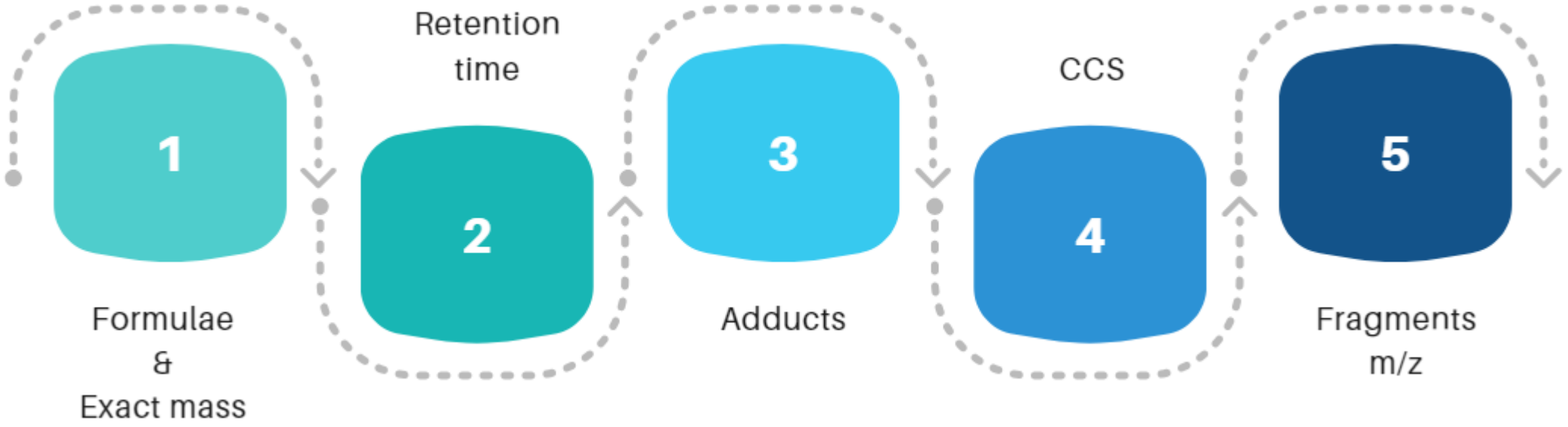
HDMS^E

Methods: Database set up

	Component name	Item tags	Expected RT (min)	Expected neutral mass (Da)	Adducts	Expected fragment (m/z)	Formula
1	Cefacetrile	Bêta lactamines	4.55	339.0525	+Na		C13H13N3O6S
2	Valnemulin	Pleuromutilines	7.97	564.3597	+H		
3	Tylosin	Macrolides et apparentés	7.50	915.5192	+H		
4	Tulathromycin B	Macrolides et apparentés	3.61	805.5664	+H		
5	Benzylpenicillin (Penicillin G)	Bêta lactamines	7.20	334.0987	+H		
6	Sulfanilamide	Sulfamides	1.91	172.0306			C6H8N2O2S
7	Sulfathiazole	Sulfamides	4.55	255.0136	+H	156.0114, 92.0495, 108.0449	C9H9N3O2S2
8	Tetracycline	Tétracyclines	5.10	444.1533	+H	410.1234, 392.1129, 154.0499	C22H24N2O8
9	Sulfachloropyridazine	Sulfamides	5.90	284.0135	+H		C10H9ClN4O2S
10	Novobiocin	Autres - Divers	9.32	612.2319	+H	189.0910, 396.1442, 218.1023	C31H36N2O11
11	Sulfaclozine	Sulfamides	6.01	284.0135	+H	130.0167, 191.9629, 94.0651	C10H9ClN4O2S
12	Cefalexine						
13	Sulfamethoxa:						
14	Florfenicol am						

Add Delete

	Adduct	Mass	Charge	CCS
1	+Na	22.98...	1	168.5...



Methods: Sample preparation

1	TEST PORTION	1g +/- 0.02 g of eggs
2	2-FOLD LLE	• 3mL of citric acid 5mM in H2O/MeCN (25/75 ; v/v) • 3mL of MeCN
3	EXTRACTS CONCENTRATION	Addition of 50 µL of DMSO Evaporation under N2
4	FINAL RECONSTRUCTION	50µL of DMSO + 950µL of 0.2M ammonium acetate
5	FILTRATION	0.45µm

3 — Validation design

Methods: Validation plan

STUDIED CONCENTRATIONS	LVL 1	LVL 2	LVL 3	LVL 4	LVL 5	LVL 6	RV min	RV max
MRL SUBSTANCES [2.5 - 2000]	0.10 MRL	0.25 MRL	0.50 MRL	**1.00 MRL**	1.50 MRL	2.00 MRL	**25 µg/Kg**	**1000 µg/Kg**
IF NO MRLS IN EGGS [1.0 - 400]	0.10 cMRL	**0.25 cMRL (MMPR)**	0.50 cMRL	1.00 cMRL	1.50 cMRL	2.00 cMRL	**2.50 µg/Kg**	**50 µg/Kg**
PROHIBITED OR UNAUTHORIZED SUBSTANCES [2.0 - 40]	**2.00 µg/Kg (LCL)**	5 µg/Kg	10 µg/Kg	20 µg/Kg	30 µg/Kg	40 µg/Kg	**MMPR or RPA** If applicable	-

Parallel validations performed on 3 validation days

n = 21 for each level studied

METHOD VALIDATION

Multiple comparisons of systems & acquisition modes

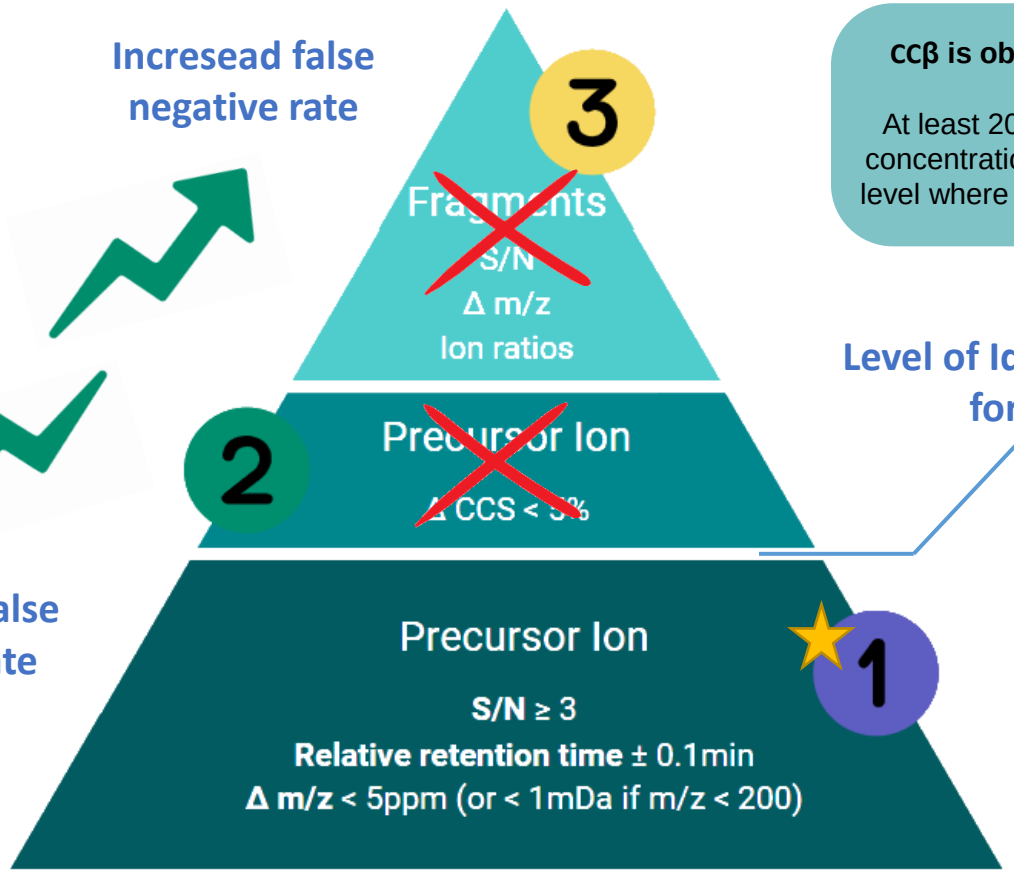
Methods: CCβ determination

CCβ is obtained using method 2 of Reg (EU) 2021/808

At least 20 spiked blank samples are analysed at different concentrations levels. CCβ corresponds to the concentration level where the percentage of false-negative results are ≤ 5%

Incresead false negative rate

Increased false positive rate



Level of Identification for CCβ

74 analytes

6 levels of conc.

21 replicates

3 acquisition modes

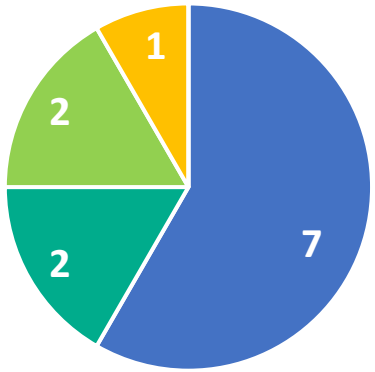
27,972 chromatograms were checked

4 – Results

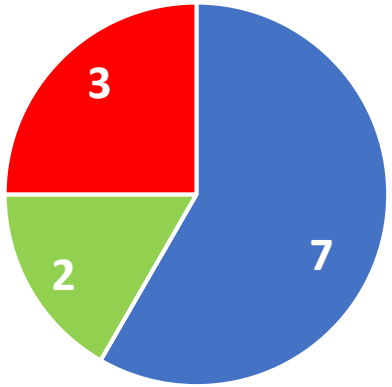
Results: MRL Substances

n = 12 : MRLs ranged from 25 to 1000 ng/g

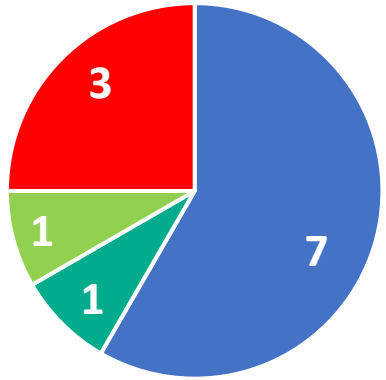
Using **HDMS^E** acquisition mode (Vion)



Using **MS^E** acquisition mode (Vion)



Using **FullScan vDIA** acquisition mode (QExactive)



■ CCβ = 0.1 MRL ■ CCβ = 0.25 MRL ■ CCβ = 0.5 MRL ■ CCβ = 1.0 MRL ■ CCβ > MRL

Epi-CTC (MRL = 200)

Erythromycin (MRL = 150)
Tylosin (MRL = 200)
Tylvalosin (MRL = 200)
High-mass calibration (Day 1)

CTC (MRL = 200)
Epi-CTC (MRL = 200)
OTC (MRL = 200)

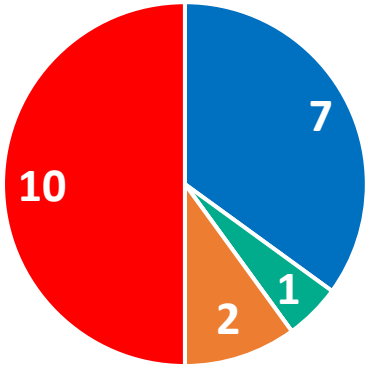
⇒ **Similar results**
Tetracyclins(x6) & (macrolides) having best results using MS^E

⇒ **Similar results**
Tetracyclins (x6) having best results using MS^E

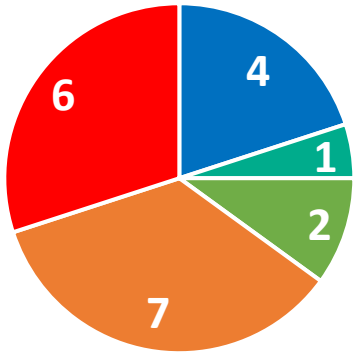
Results: Substances using a Cascade MRL

n = 20 : MRLc ranged from 10 to 200 ng/g

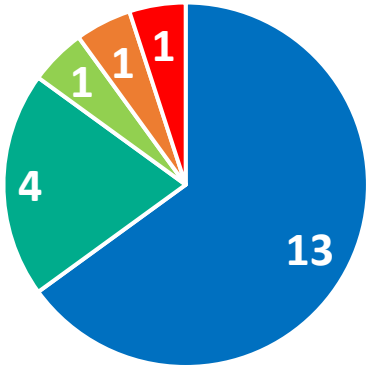
Using **HDMS^E** acquisition mode (Vion)



Using **MS^E** acquisition mode (Vion)



Using **FullScan vDIA** acquisition mode (QExactive)



■ CCβ = 1/2 MMPR ■ CCβ = 0.25 MRLc = MMPR ■ CCβ = 0.5 MRLc ■ CCβ = MRLc ■ CCβ > MRLc

6x Cephalosporins (MRLc = 20-100)
1x Penicillin (Nafcilline ; MRLc = 30)
1x Pleuromutilin (Valnemulin ; MRLc = 50)
2x Others (MRLc = 50-60)

Baquiloprim (MRLc = 10)
Rifaximine (MRLc = 60)
DCCD (MRLc = 10)
3x Macrolids: High-mass calibration (Day 1)

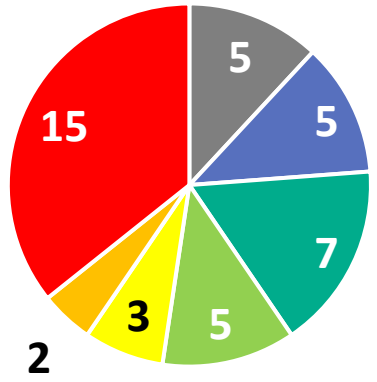
DCCD (MRLc = 100)
Cephalonium (MRLc = 20)

⇒ **HDMS^E: seems to be the least efficient VS MS^E**
⇒ **FullScan vDIA: seems to be the best efficient VS MS^E**

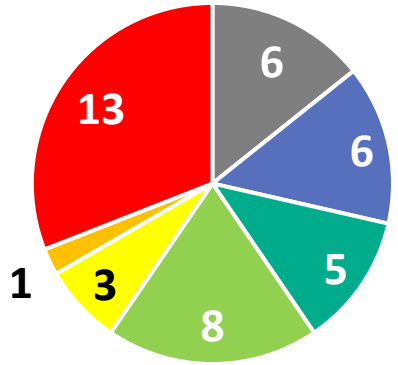
Results: Prohibited & Unauthorized substances

n = 42 CCβ evaluation performed from 2 to 40 ng/g

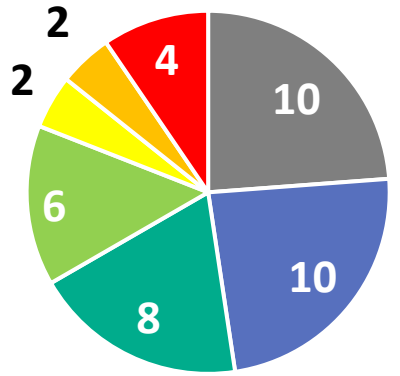
Using **HDMS^E** acquisition mode (Vion)



Using **MS^E** acquisition mode (Vion)



Using **FullScan vDIA** acquisition mode (QExactive)



CCβ = 2 ng/g | CCβ = 5 ng/g | CCβ = 10 ng/g | CCβ = 20 ng/g | CCβ = 30 ng/g | CCβ = 40 ng/g | CCβ > 40 ng/g

5x Penicillins
5x Sulfonamids
2x Macrolids
2x Amphenicols (FF & FFA)
1x Cephalospirin (Cefuroxim)

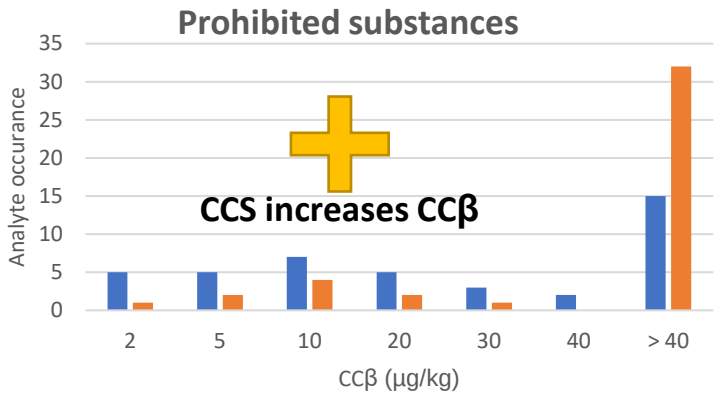
5x Penicillins
4x Macrolids: High-mass calibration (Day 1)
2x Sulfonamids (SDMZ & Sulfanilamide)
1x Amphenicol (FF)
1x Cephalosporin (Cefuroxim)

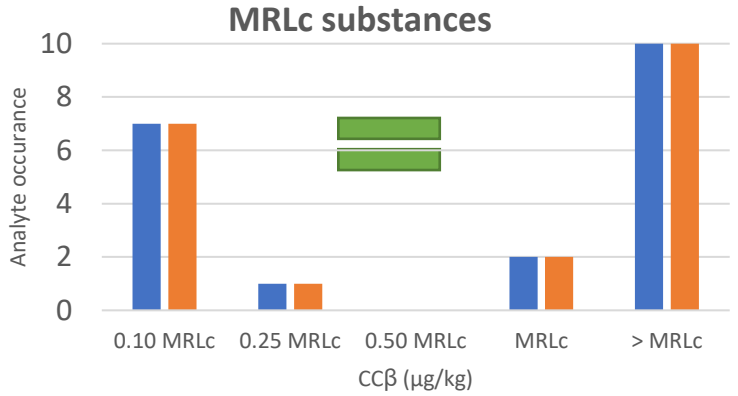
3x Quinolons (Difloxacin, Nalidixic & Oxolinic acid)
1x Penicillin (Cloxacillin)

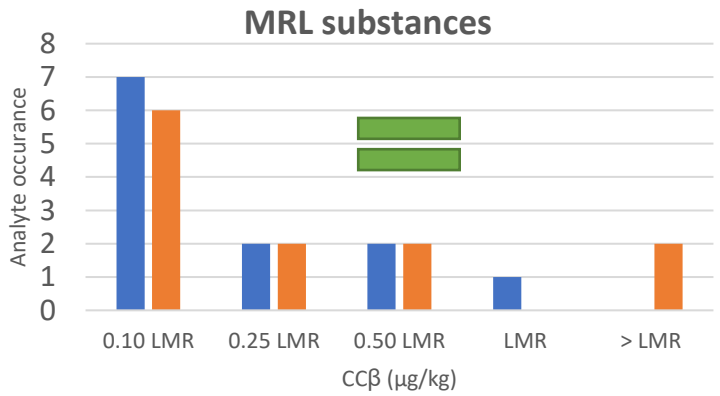
⇒ **HDMS^E: seems to be the least efficient VS MS^E**

⇒ **FullScan vDIA: seems to be the best efficient VS MS^E**

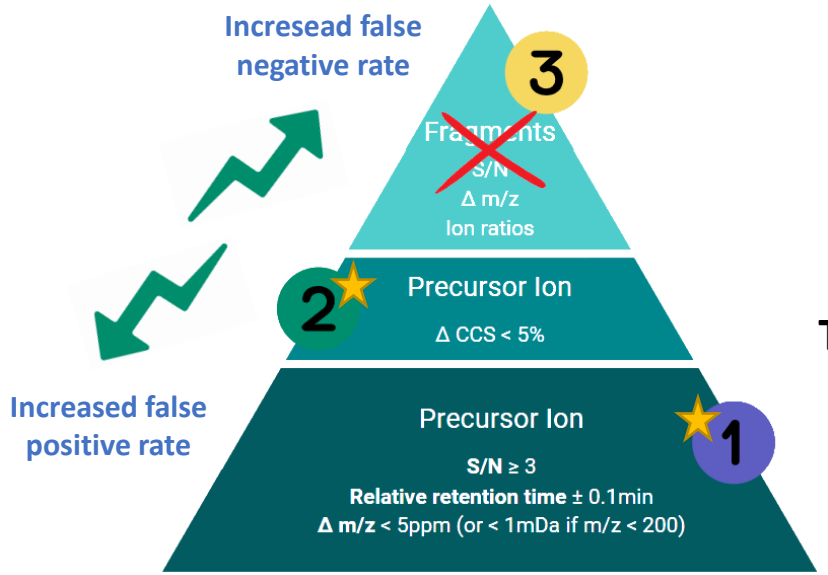
Results: HDMS^E – CCβ using or not CCS as criterion







Without CCS With CCS



⇒ **Addition of CCS as a criterion increases CCβ values of prohibited substances**

⇒ **No differences for MRL or MRLc substances**

The CCS can be an interesting identification criterion for complexe matrices, but only if the concentration levels of interest are sufficient.

5 — Conclusion

Conclusion:

- **Importance of monitoring antibiotics in eggs:**
  widely consumed ; many antibiotics have no MRL or not authorized for laying hens

- **SOP for screening:** LLE & LC-HRMS method has been fully developed & validated (2021/808)

- **Comparison between HDMS^E VS MS^E:**

	Specificity	Data interpretation	Sensitivity	FNR	FPR	Suitable for
MS^E (without IMS)	-	-	+	-	+	Screening
HDMS^E (with IMS)	+	+	-	+	-	Confirmation

- **Comparison between Vion (Q-ToF) and Q-Exactive$^+$ (Q-Orbitrap) systems:**
  Q-Exactive$^+$ system seems to be the one offering the lowest detection limits for VMPRs in eggs
  - v-DIA use three fixed CE values (low, medium & high) for Product Ions
  - MS^E use low to high ramping CE to Products Ions
  - MS^E detection was carried out using a UniSpray source: very fast soiling

Thank you for your attention!

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