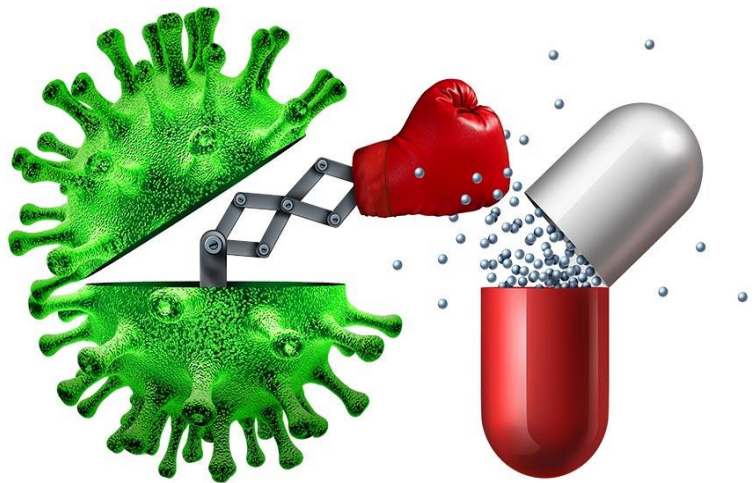


Antiviral drugs in food-producing animals

How to move towards official controls?

S. Sasse, A. Castiñeira-Landeira, M. Broeren, S. Sterk, A. Arrizabalaga-Larrañaga

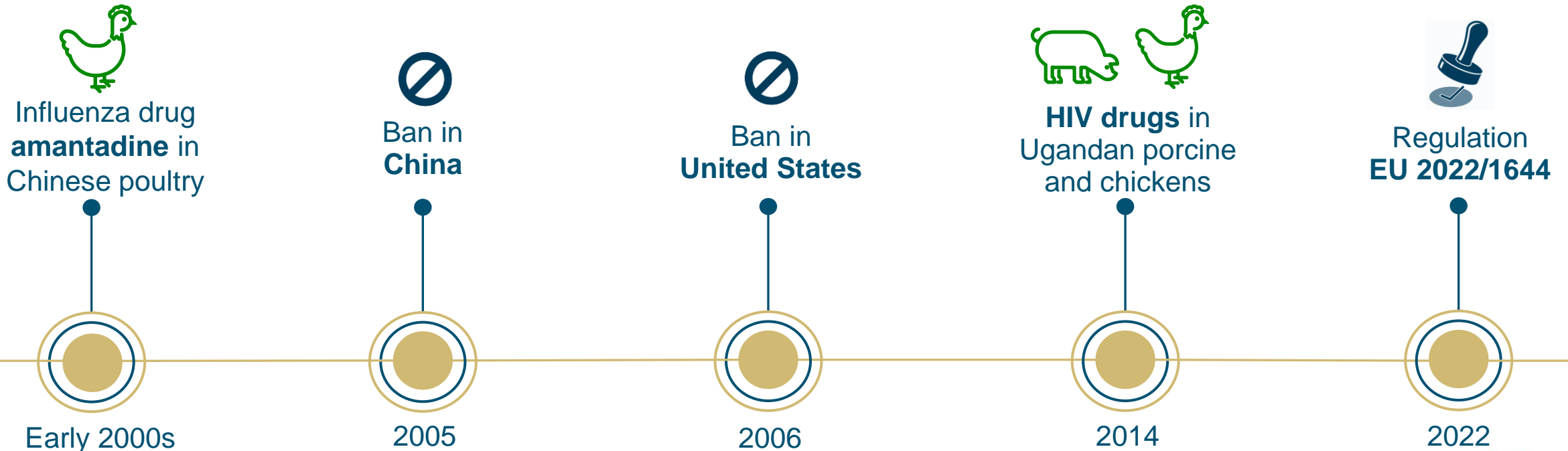


What do we know about antiviral drugs?

Antiviral drugs legally used to treat viral diseases in  Humans and  Companion animals under EU regulation No. 2019/6

↓ >100 antiviral drugs are available 

Have been misused in the past in food-producing animals



Why do we need to monitor antiviral drugs?

COMMISSION DELEGATED REGULATION (EU) 2022/1644 of 7 July 2022 supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council with specific requirements for the performance of official controls on the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof



In **ANNEX 1** of the regulation **ANTIVIRAL DRUGS** are included



under **GROUP A** – prohibited or unauthorized pharmacological active substances in food-producing animals



Need for official controls
in the EU



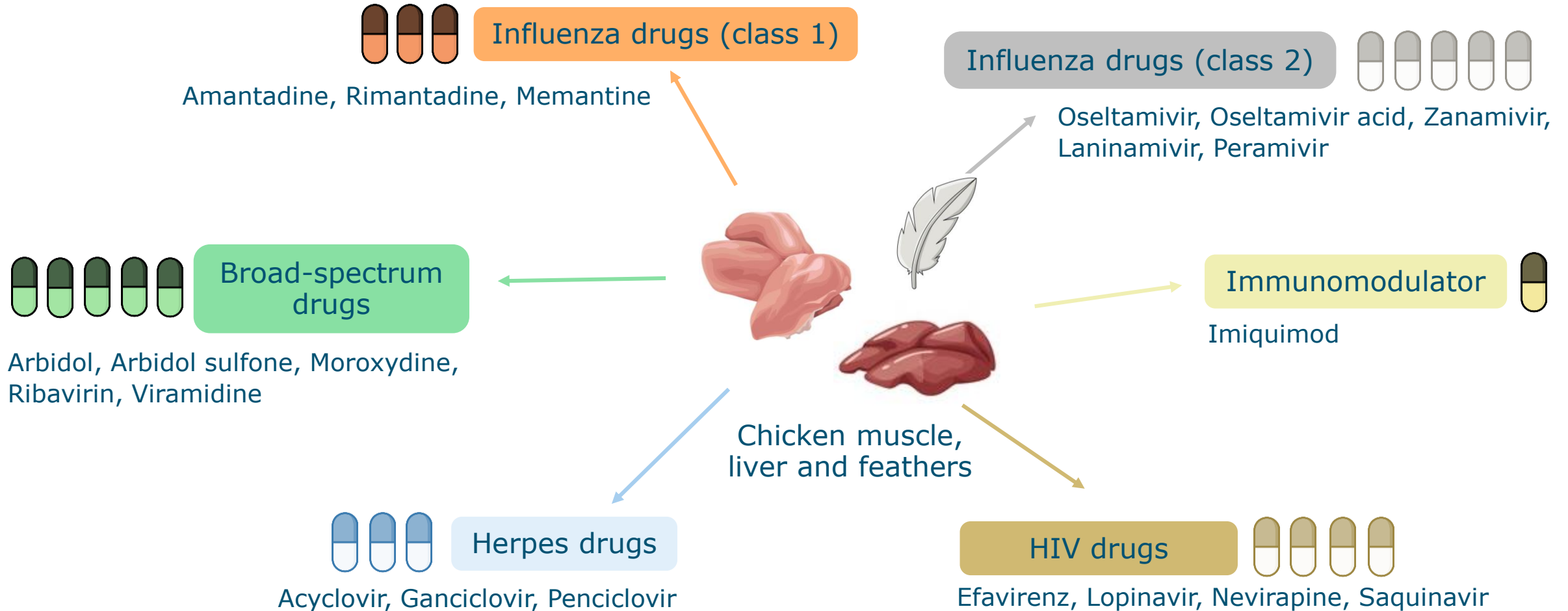
How did we move towards official controls in NL?

- 1 Selected Antiviral Drugs & Animal-derived Matrix
- 2 Developed Analytical Method
- 3 Validation of Analytical Method
- 4 Broiler Chicken Animal Study
- 5 Application of Analytical Method to Real Samples



Are antiviral drugs used in The Netherlands?

1. Selected Antiviral Drugs & Animal-derived Matrix



21 antiviral drugs from six different classes

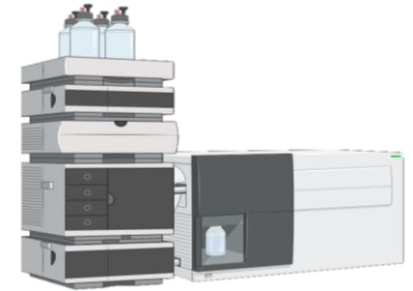
Very different chemical and physical properties !

2. Developed Analytical Method

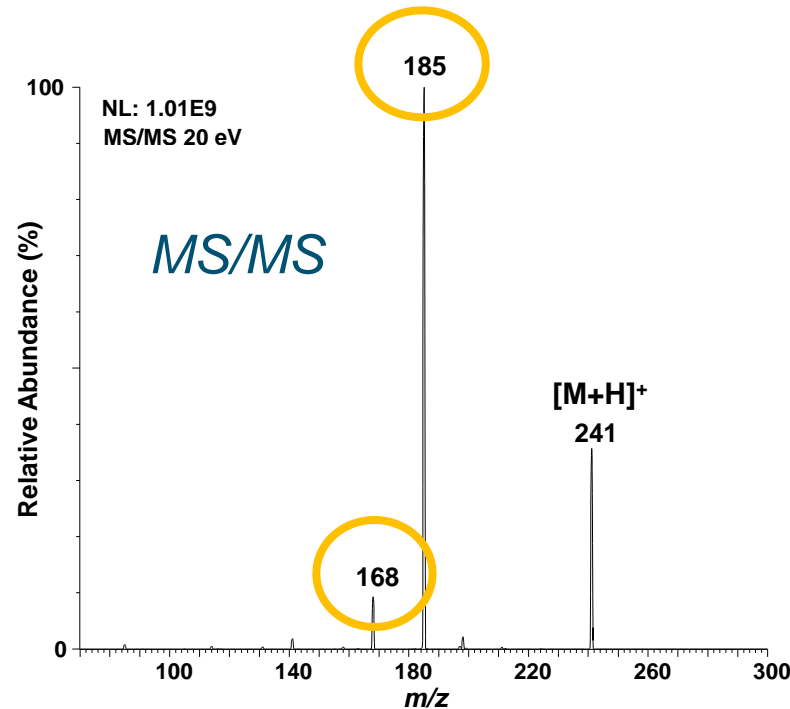
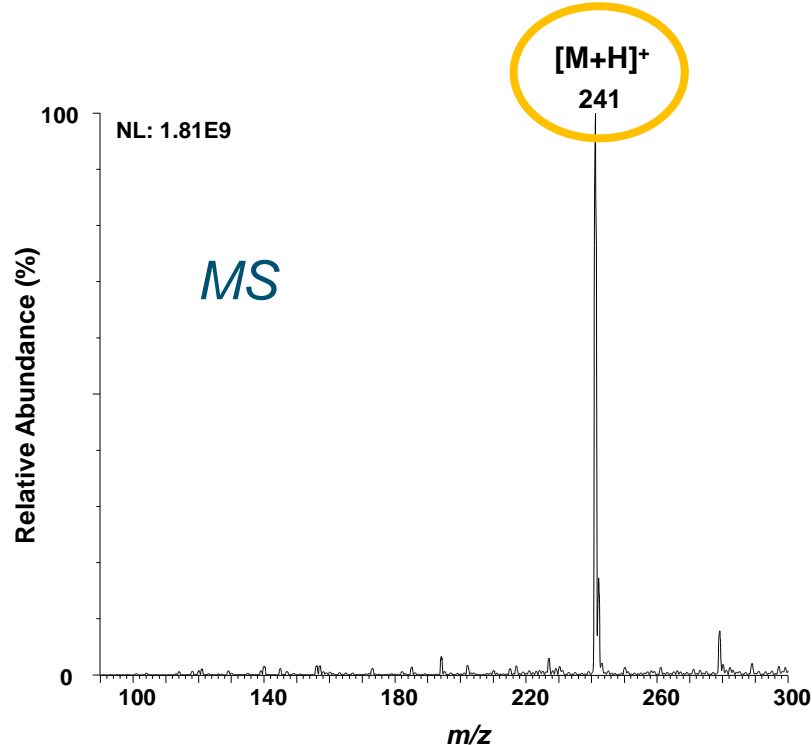


Characterize antiviral drugs with mass spectrometry

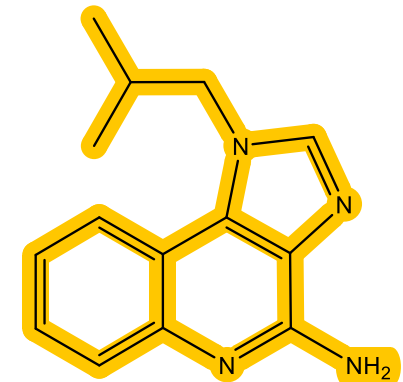
Tandem mass spectrometry was studied, and two most abundant and specific transitions were selected for each individual antiviral drug.



ESI (+) except 1 target compound, QqQ, MRM



Imiquimod



2. Developed Analytical Method



Separate antiviral drugs with Liquid Chromatography

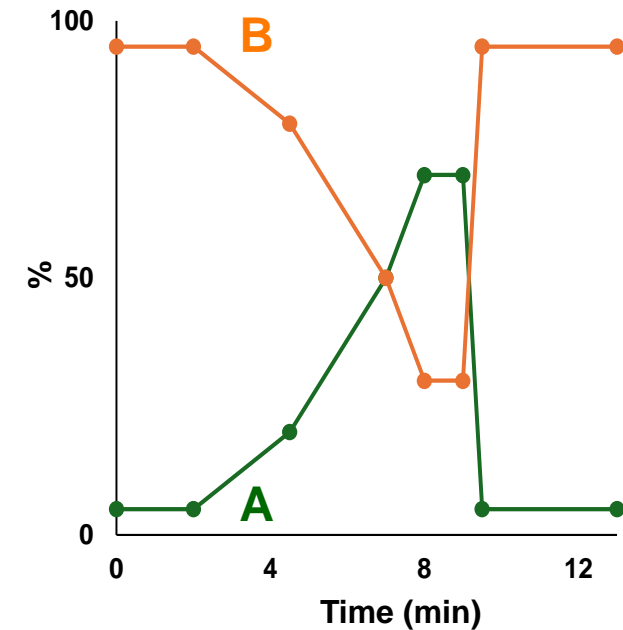
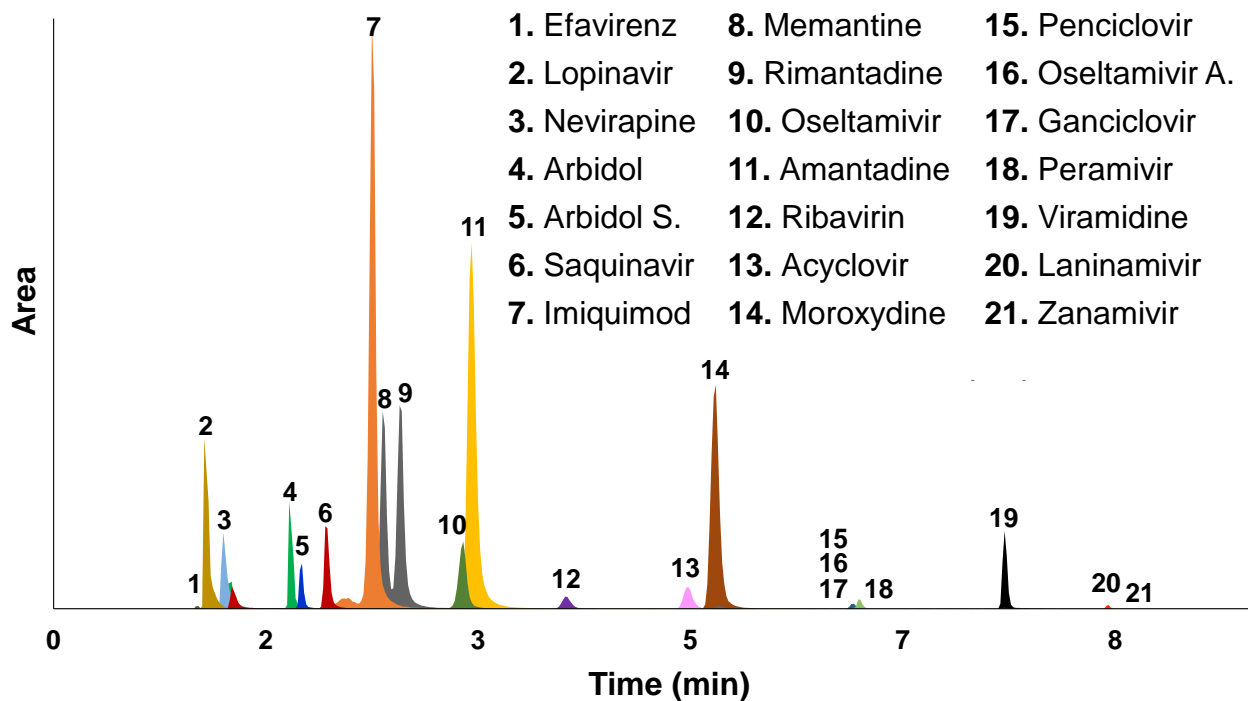
Column: BEH Amide (2.1 x 150 mm, 2.5 μm)

Column temperature: 40 °C

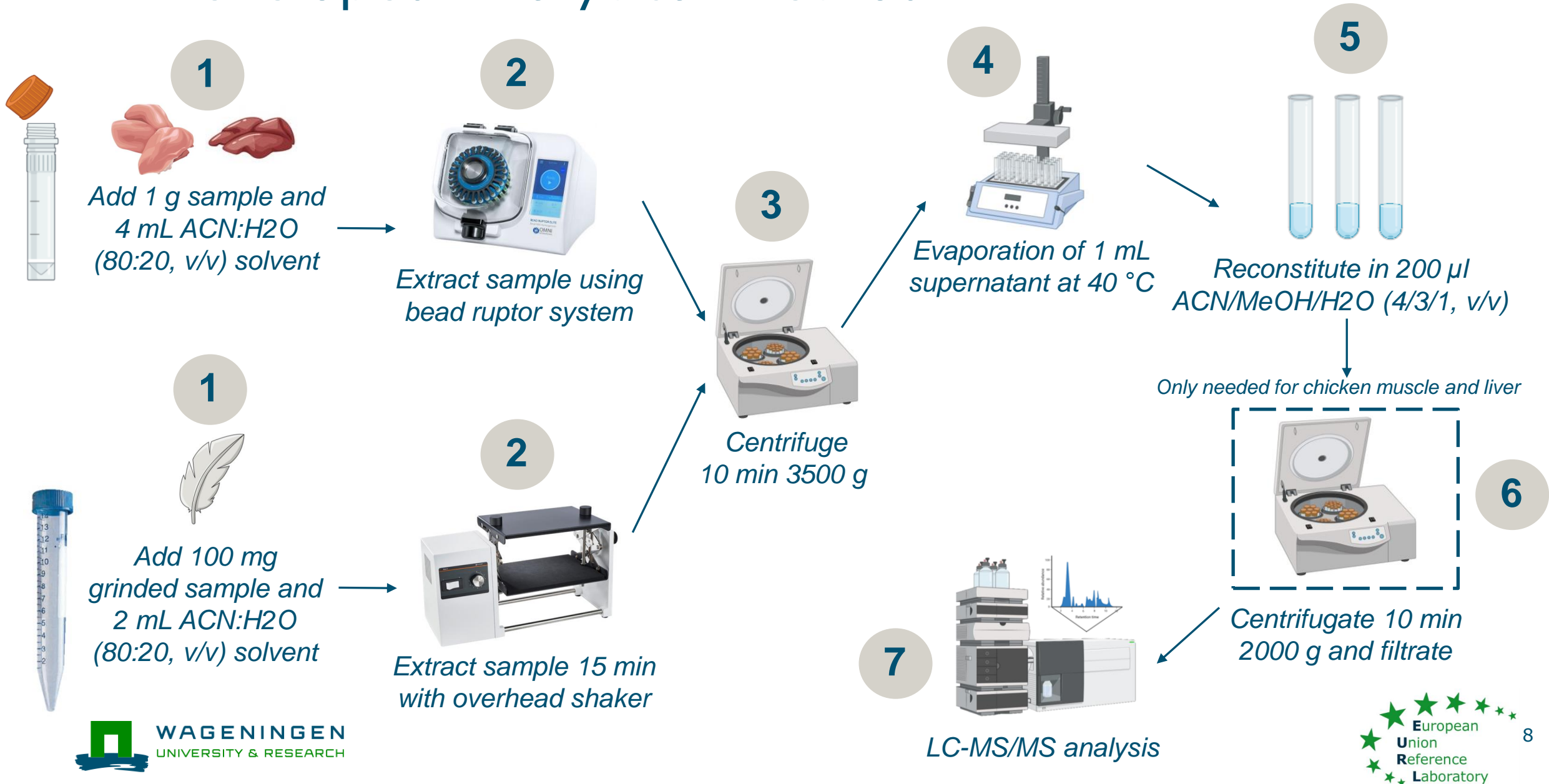
Flow: 0.3 mL min⁻¹

Mobile phase A: 0.1% formic acid and 2 mM ammonium acetate in MQ

Mobile phase B: 0.1% formic acid and 2 mM ammonium acetate in ACN/MQ (95/5; v/v)



2. Developed Analytical Method



3. Validation of Analytical Method

Validation according to Commission Implementing Regulation (EU) 2021/808

Antiviral drug	LCL (µg/kg) muscle and liver	LCL (µg/kg) feathers
Acyclovir*	0.5	5
Amantadine*	0.15	10
Arbidol*	1	1
Arbidol sulfone	1	20
Efavirenz	10	50
Ganciclovir*	5	50
Imiquimod	0.15	2
Laninamivir	20	100
Lopinavir*	0.15	1
Memantine*	0.15	2
Moroxydine*	0.5	2
Nevirapine	1	2
Oseltamivir	0.5	1
Oseltamivir acid*	2	100
Penciclovir*	2	100
Peramivir	5	10
Ribavirin*	25	40
Rimantadine	1	10
Saquinavir*	0.15	2
Viramidine	5	20
Zanamivir*	50	100

*Isotopically-labelled internal standard included

Validation levels
1*LCL – 2*LCL – 3*LCL



3-day validation



1-day validation



1-day validation
(is currently done)

Validation criteria according (EU) 2021/808

Do the samples fulfil the criteria for
trueness, repeatability and
reproducibility?

Yes

Quantitative

No

Qualitative

Do the samples fulfil the criteria for
max. deviation ion ratio and
retention time?

Yes

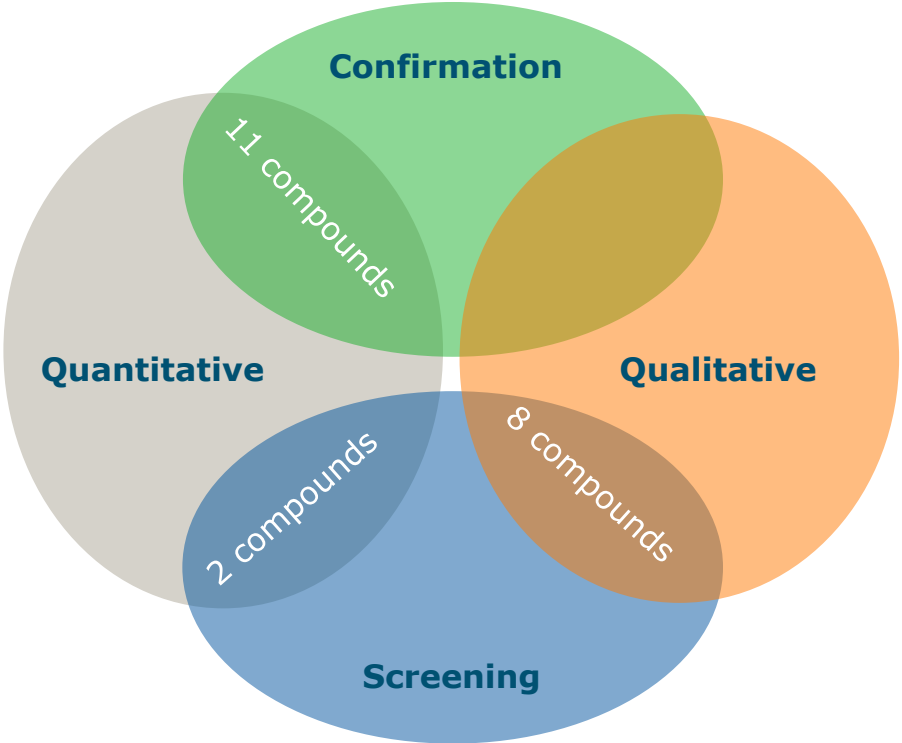
Confirmation

No

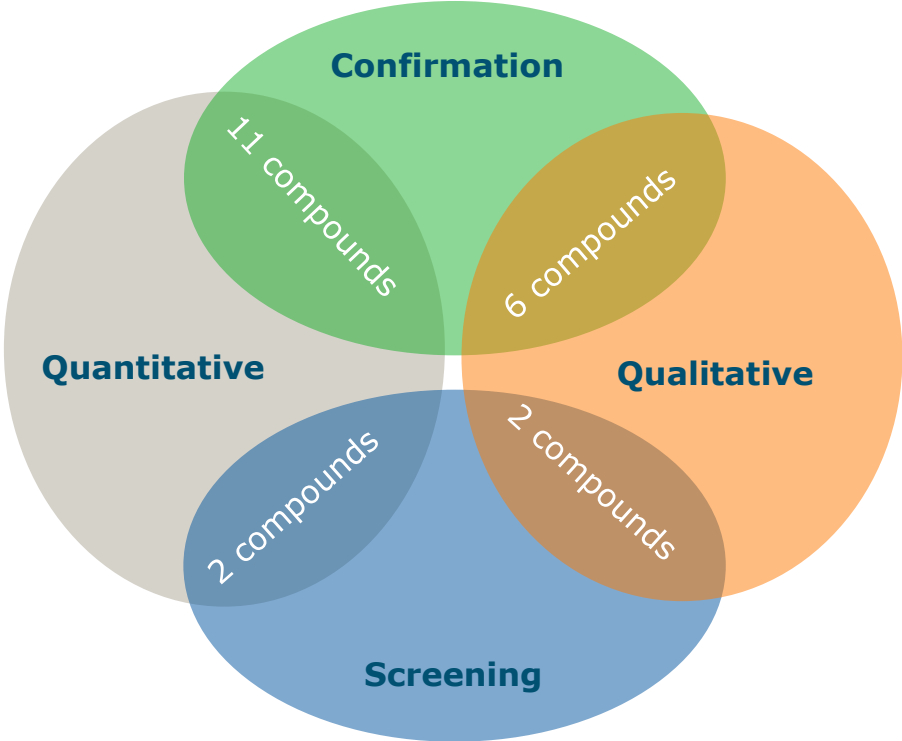
Screening

3. Validation of Analytical Method - Results

Chicken muscle

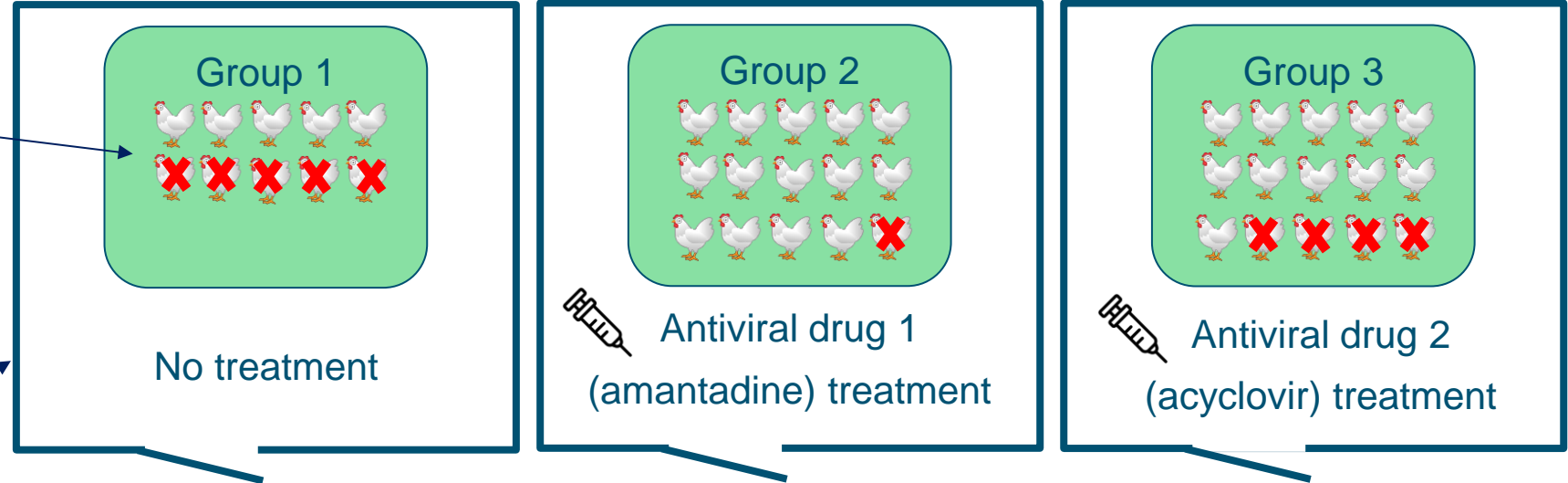


Chicken liver



4. Broiler chicken animal study

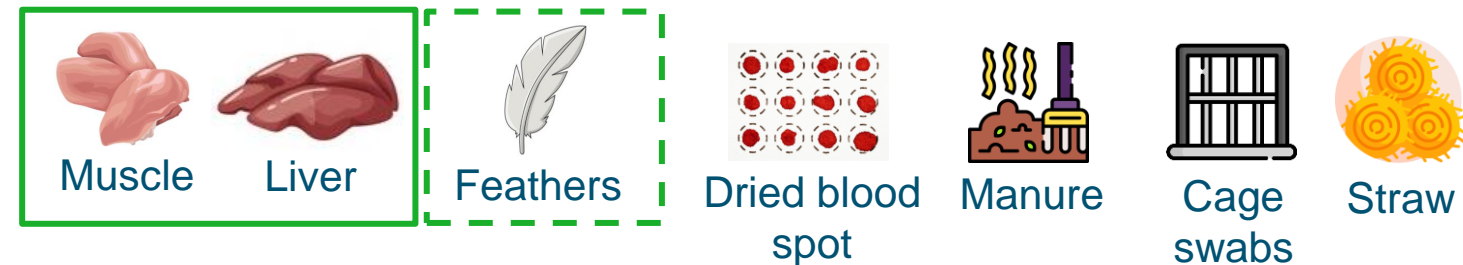
Design



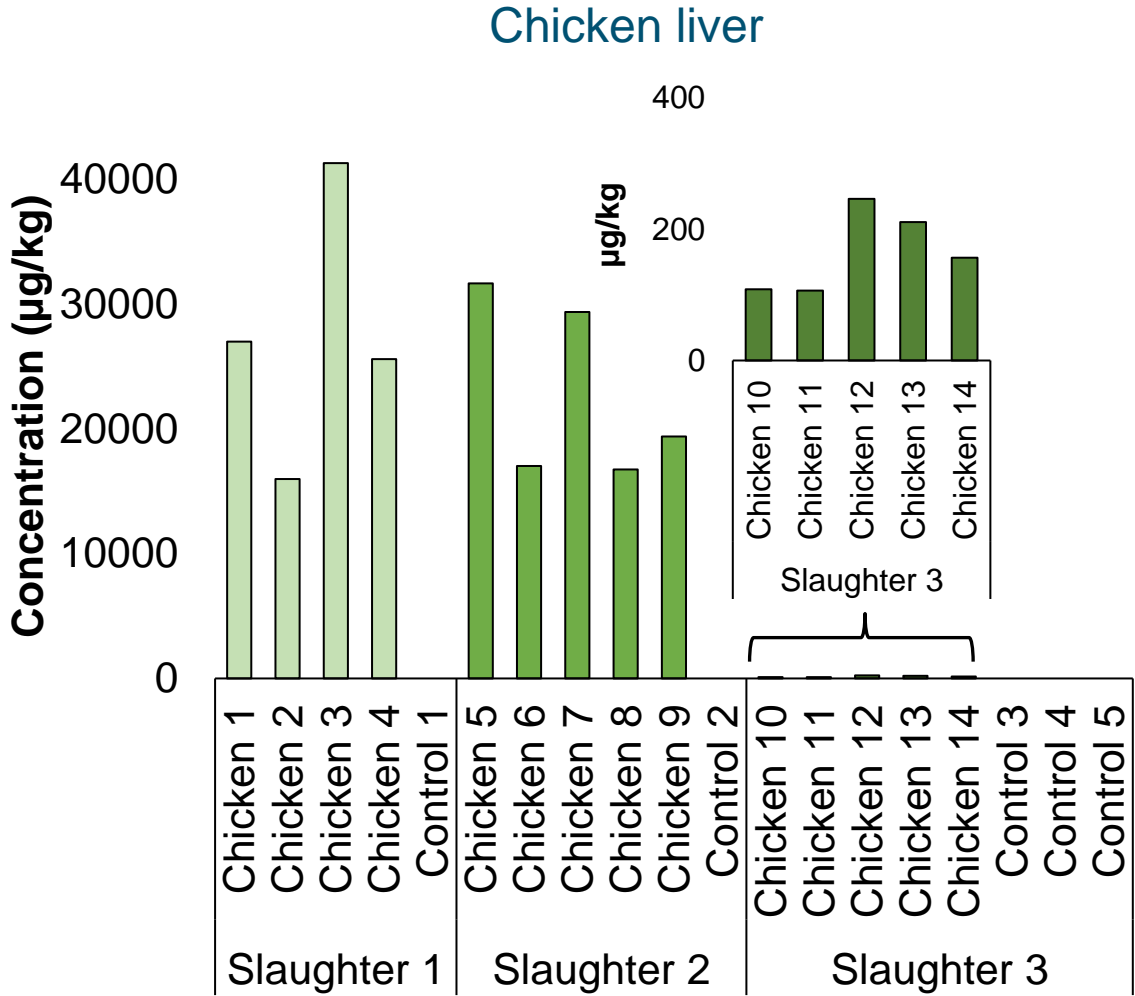
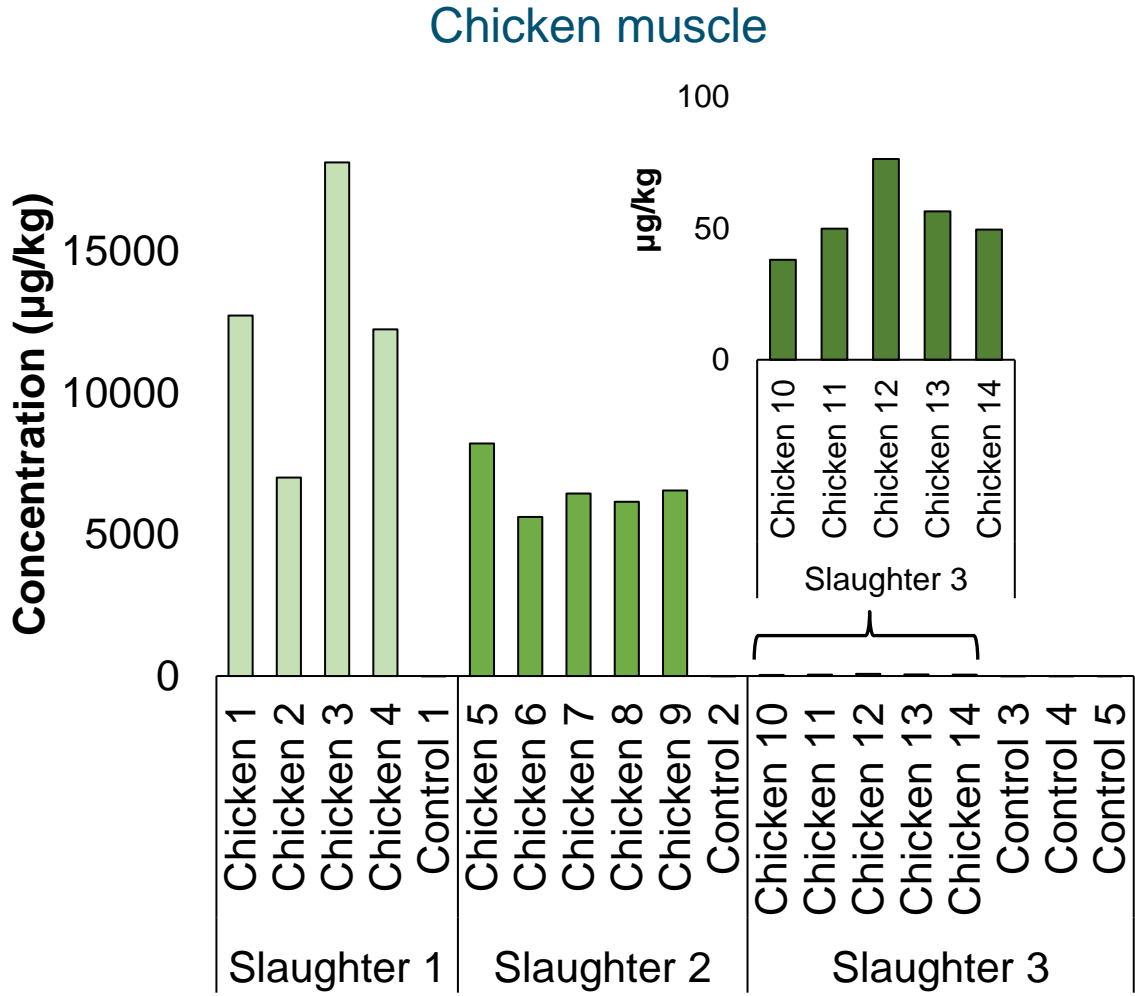
Timeline



Samples collected during course of study



4. Broiler chicken study – results amantadine treatment

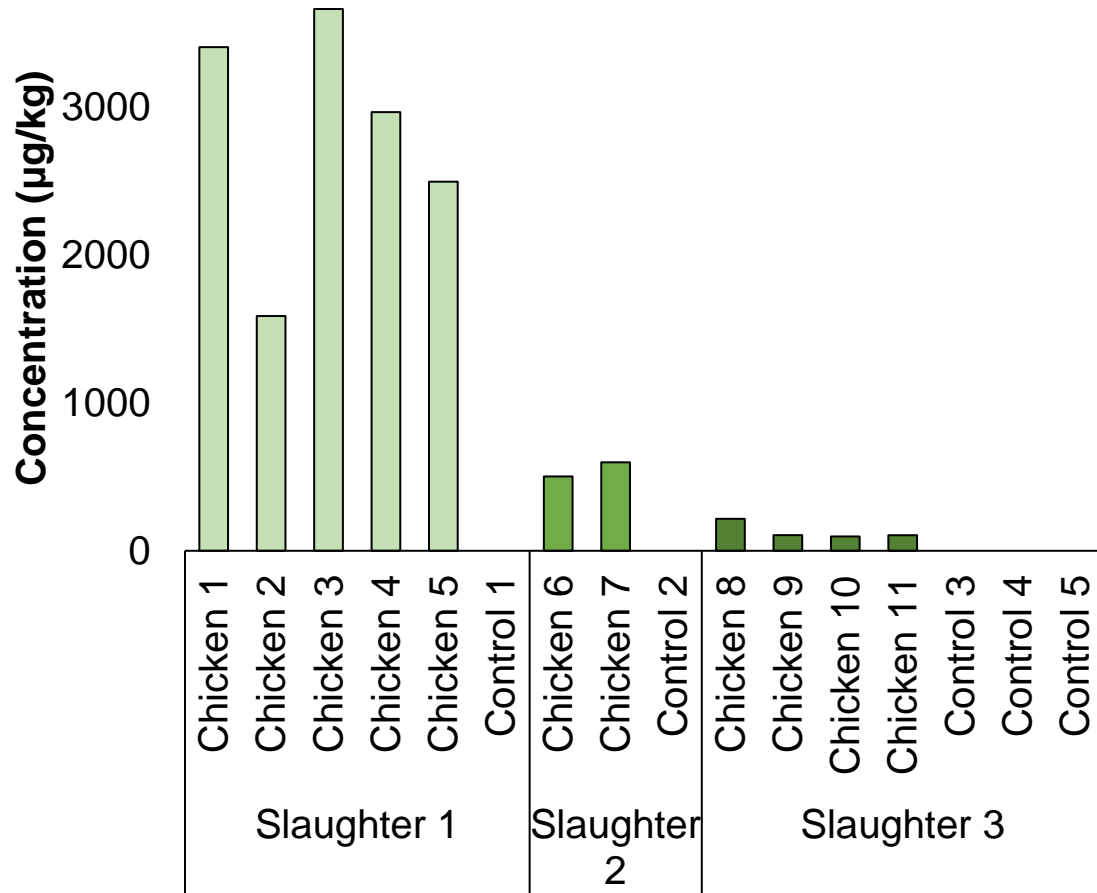


Average liver/muscle ratio = 2.9

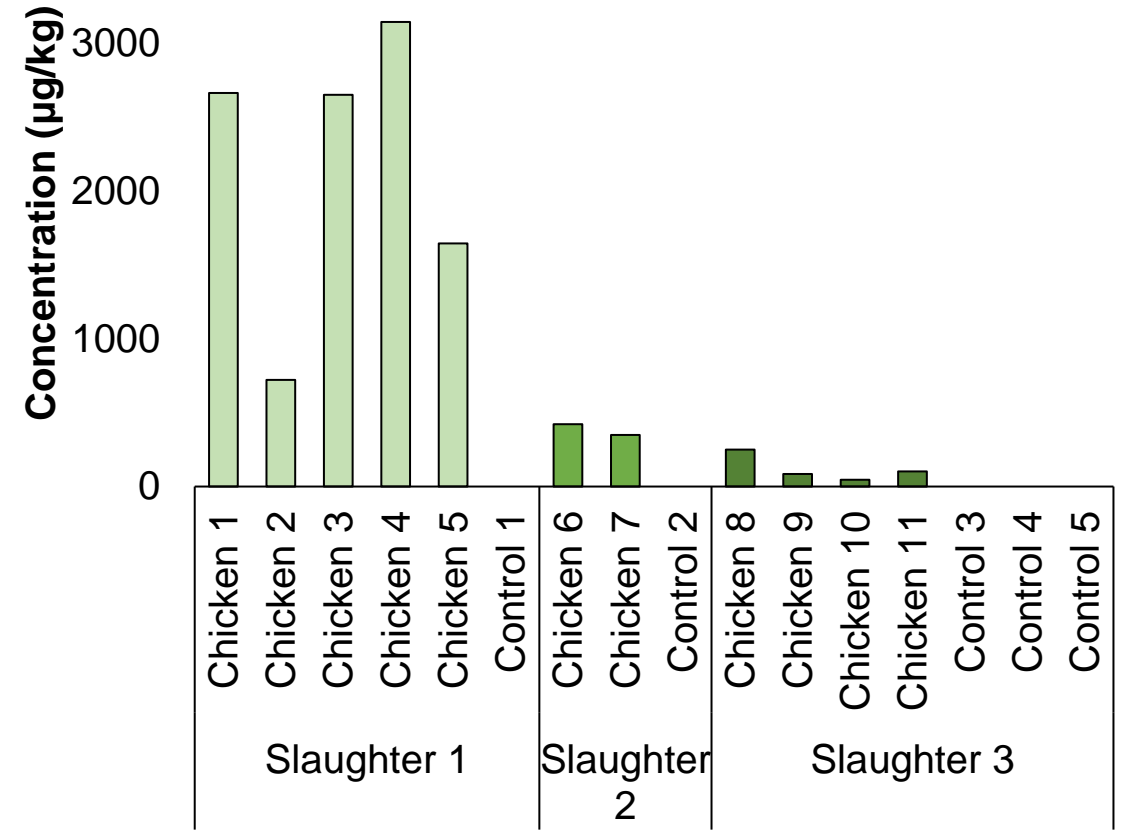
Amantadine was also detected in feathers!

4. Broiler chicken study – results acyclovir treatment

Chicken muscle



Chicken liver



Average liver/muscle ratio = 0.8

Acyclovir was also detected in feathers!

5. Application of Analytical Method to Real Samples

- Since 2025, the developed and validated LC-MS/MS method for antiviral drugs is applied in the **Dutch National Control Plan** for **official controls**.
- Additionally, a **survey** is currently done to examine if antiviral drugs are present in **imported chicken meat from Asia**.
- Until now, **no** antiviral residues were found.
- But... important to continue **data collection**.



Conclusions

- A **simple and fast** LC-MS/MS method for a **broad range** of 21 antiviral drugs in chicken muscle and liver was successfully developed, validated and is currently applied for official controls.
- The **broiler chicken animal study** showed that:
 - Antiviral drugs **can end up** in chicken meat, liver and feathers.
 - Antiviral drugs can remain in chicken meat and liver **at least 1 week** after the last dosage.
 - The **rate of decline** and **matrix of choice for analysis** based on concentration ratio differ between the two antiviral drugs tested.

What's next?



Continue official controls



Validate method and analyse all samples from broiler chicken animal study



Extend scope of analysis



Pig animal study for antiviral drugs



Ana Castineira
Landeira



Melissa Broeren



Ane Arrizabalaga
Larranaga



Saskia Sterk



WFSR at
TRACES 2025



Thank you for your attention!



Ministry of Agriculture, Nature and
Food Quality of the Netherlands



Publication
method antiviral drugs