Development and validation of a method for quantification of 15 antiviral drugs against influenza in poultry muscle using LC-MS/MS

CIFSQ 2021- 28/10/2021
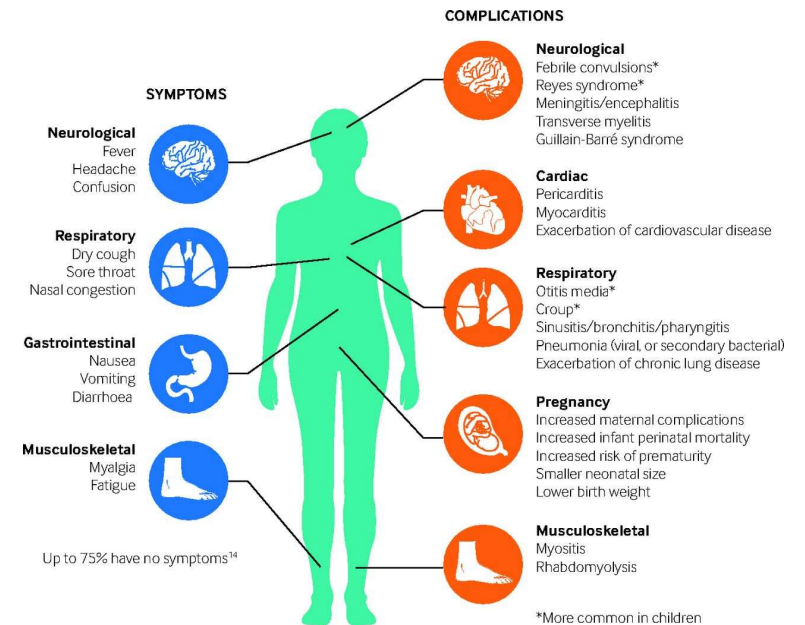Clément Douillet, Martin Danaher, Christopher Elliott

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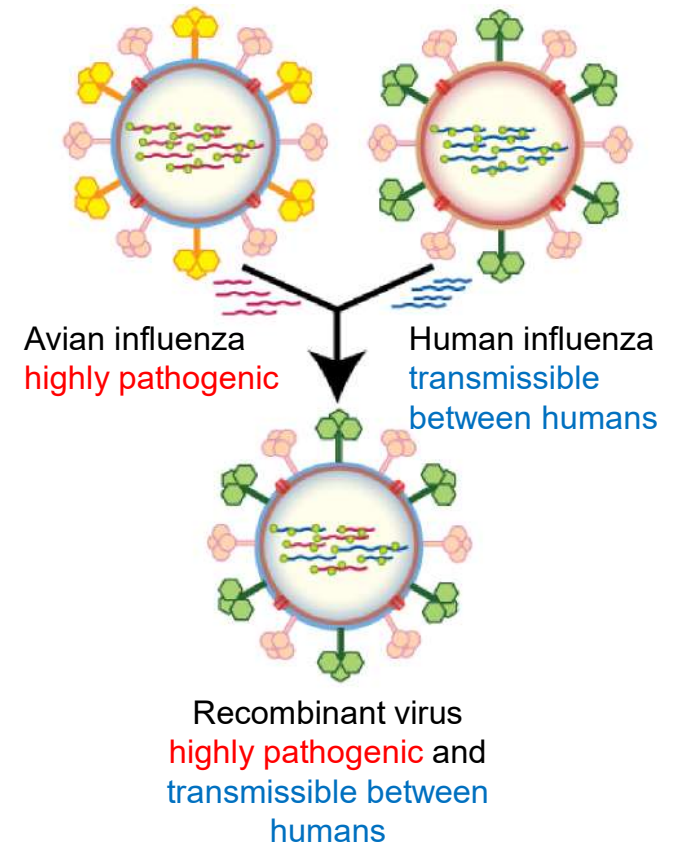
- Occurrence and conclusion

Influenza

- Three types of influenza: A, B and C.

- Symptoms: fever, headache, dry cough, nasal congestion.

- Seasonal epidemic form: between 300k and 500k deaths globally every year.

- Pandemic form from zoonotic origins every 10-50 years: deaths in millions.

SYMPTOMS

Neurological
Fever
Headache
Confusion

Respiratory
Dry cough
Sore throat
Nasal congestion

Gastrointestinal
Nausea
Vomiting
Diarrhoea

Musculoskeletal
Myalgia
Fatigue

Up to 75% have no symptoms[14]

COMPLICATIONS

Neurological
Febrile convulsions*
Reyes syndrome*
Meningitis/encephalitis
Transverse myelitis
Guillain-Barré syndrome

Cardiac
Pericarditis
Myocarditis
Exacerbation of cardiovascular disease

Respiratory
Otitis media*
Croup*
Sinusitis/bronchitis/pharyngitis
Pneumonia (viral or secondary bacterial)
Exacerbation of chronic lung disease

Pregnancy
Increased maternal complications
Increased infant perinatal mortality
Increased risk of prematurity
Smaller neonatal size
Lower birth weight

Musculoskeletal
Myositis
Rhabdomyolysis

*More common in children

Avian Influenza

■ Regular avian influenza outbreaks in poultry industry.

■ Constant mutation: new strains (e.g. H10N3).

■ Combination of avian influenza and human influenza can cause the next pandemic.

Avian influenza
highly pathogenic

Human influenza
transmissible
between humans
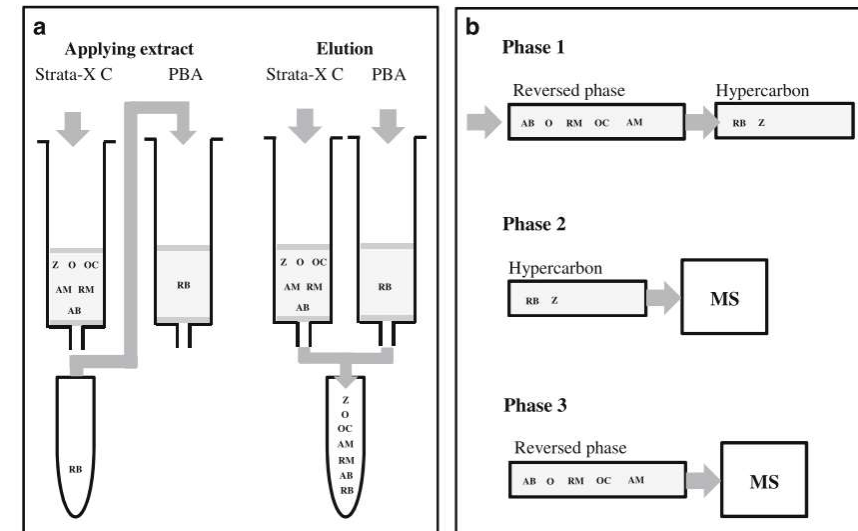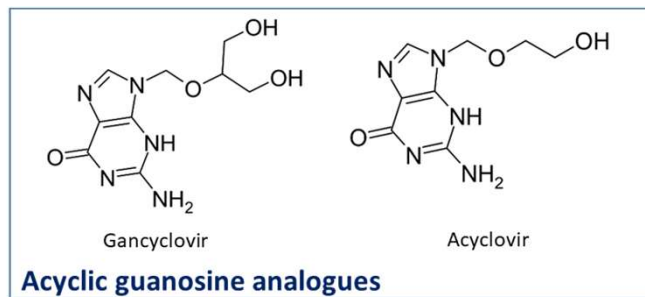
Recombinant virus
highly pathogenic and
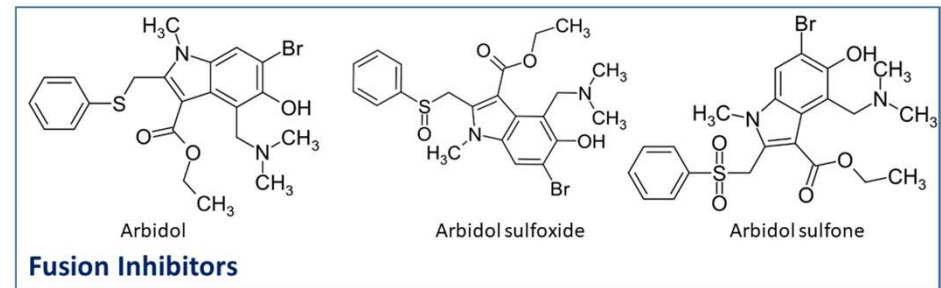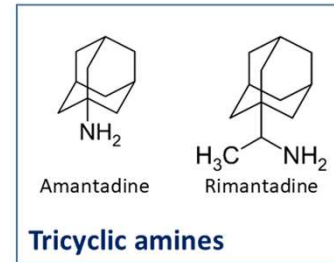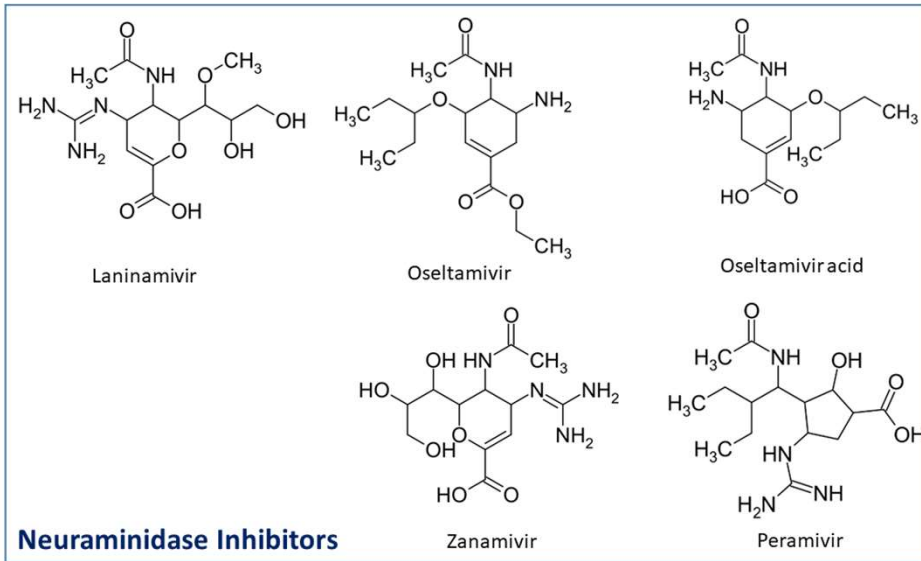transmissible between
humans

Antiviral drugs against influenza

■ Antiviral drugs against influenza developed for human use.

■ It is suggested that illegal use of antiviral drugs in farm causes development of drug-resistant influenza strains.

■ Consequently: Chinese and US authorities (FDA) have banned use of certain antiviral drugs in poultry farms.

■ Necessity of developing an analytical methodology to monitor antiviral drugs residue in poultry.

Existing methods

- Existing fast screening techniques.
- LC-MS/MS methods with very long/complicated procedures (SPE, two analytical columns) and/or not including all compounds of interest.
- Necessity to improve existing methods.
- Goal : develop a fast LC-MS/MS method including all relevant antiviral drugs against influenza.

Antiviral drugs selected

Laninamivir

Oseltamivir

Oseltamivir acid

Zanamivir

Peramivir

Neuraminidase Inhibitors

Amantadine

Rimantadine

Tricyclic amines

Ribavirin

Viramidine

Guanosine nucleoside analogue

Arbidol

Arbidol sulfoxide

Arbidol sulfone

Fusion Inhibitors

Favipiravir

Pyrazine-derived

Gancyclovir

Acyclovir

Acyclic guanosine analogues
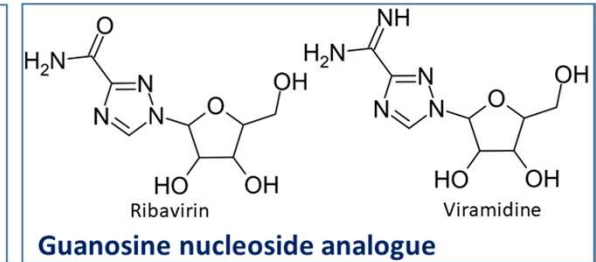
Antiviral drugs : properties

■ Broad range of polarity: from highly polar to non-polar compound.

■ Acid, basic and neutral compounds.

■ Broad range makes analytical method challenging.
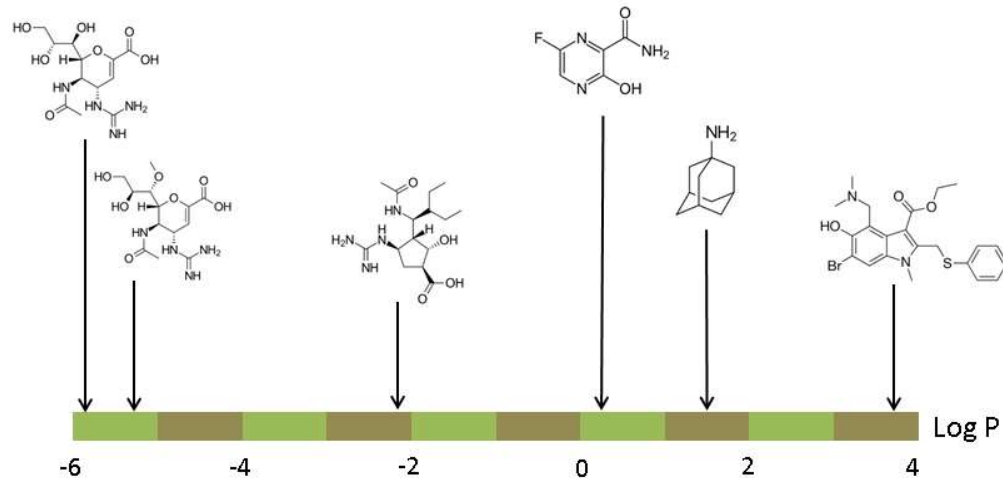
LC-MS/MS

■ Method was developed on a LC-MS/MS instrument: Waters Acquity I-Class coupled with a Xevo TQ-XS.

■ Provide suitable selectivity and sensitivity for confirmatory quantitative analysis of residue traces.

Mass Spectrometry optimisation

- Electrospray source of ionisation was compared to Unispray.
- Electrospray gave better sensitivity at the desired flow and chromatography mode

- Source parameters were optimised and most suitable MS transitions were selected.
- Positive mode and protonated molecules [M+H]$^+$ were chosen for this method

Chromatography: C18 columns

■ Wide range of C18 columns experimented.

■ Little to no retention for most polar compounds (Zanamivir, Ganciclovir, Ribavirin).

■ Not adapted to polar analytes.


CSH C18

EC C18
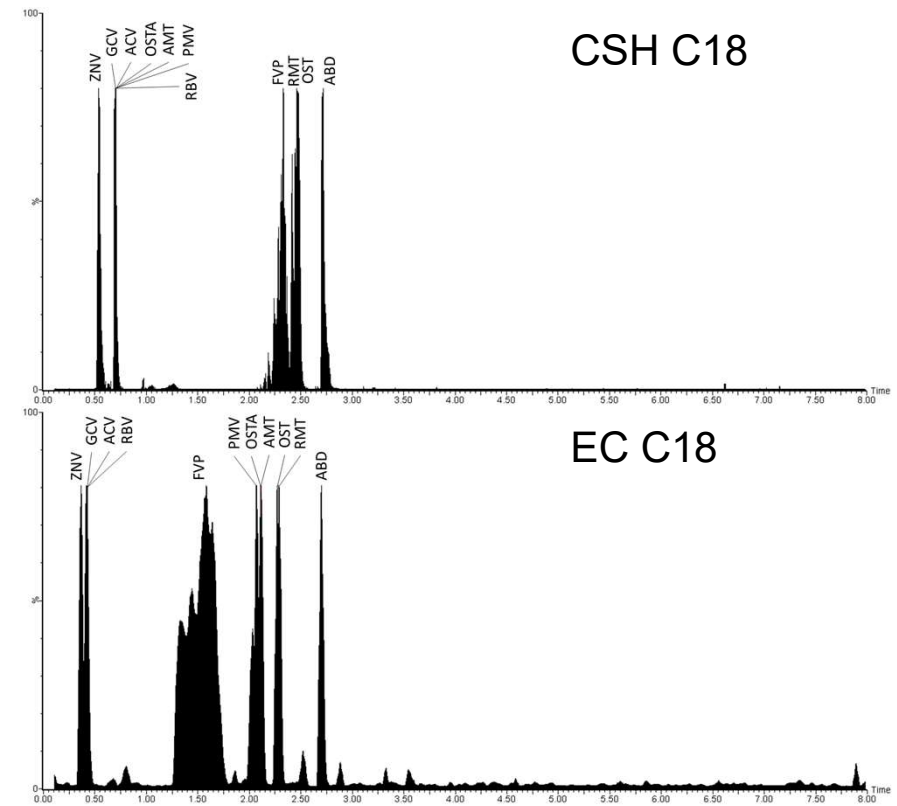
Chromatography: alternative to C18

- Alternative reversed-phase columns screened.

- Best retention for polar compounds: Selectra PFPP, better retention factor.

- Still very low retention, improved by use of ion pairing (HFBA) but was not practical.
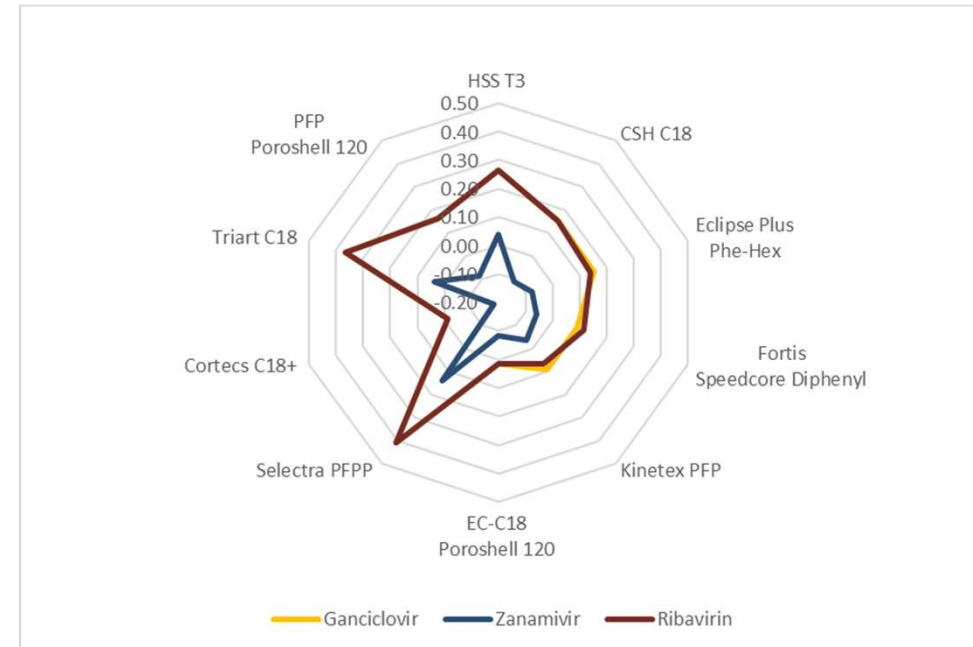
Chromatography: HILIC

- HILIC is the best chromatography mode for polar compounds.

- Best results were found with columns driven by partitioning mechanism.

- BEH Amide (A) gave more appropriate retention to non-polar or neutral compounds than HILIC-Z (B).

BEH Amide

HILIC-Z

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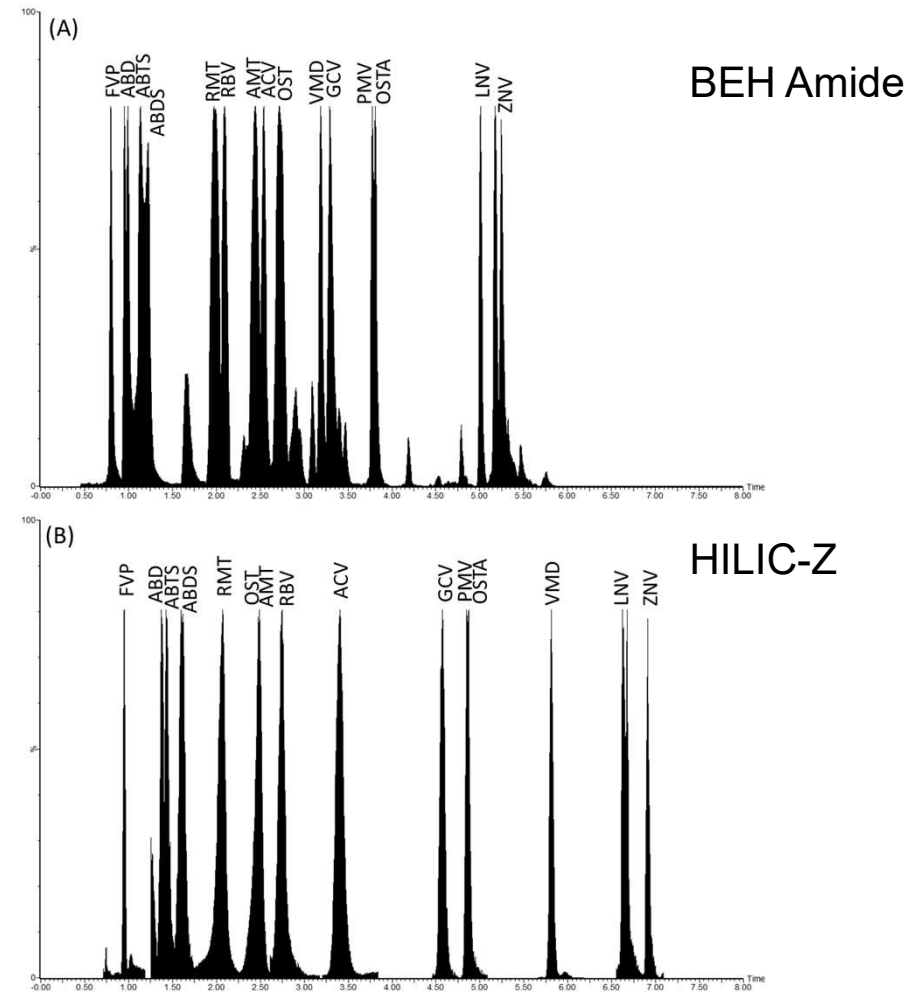
# Chromatography: ion suppression

- HILIC gave better ion suppression results than reversed-phase for polar compounds.
- Very high ion suppression zone around the dead time in reversed-phase mode.

Matrix effects
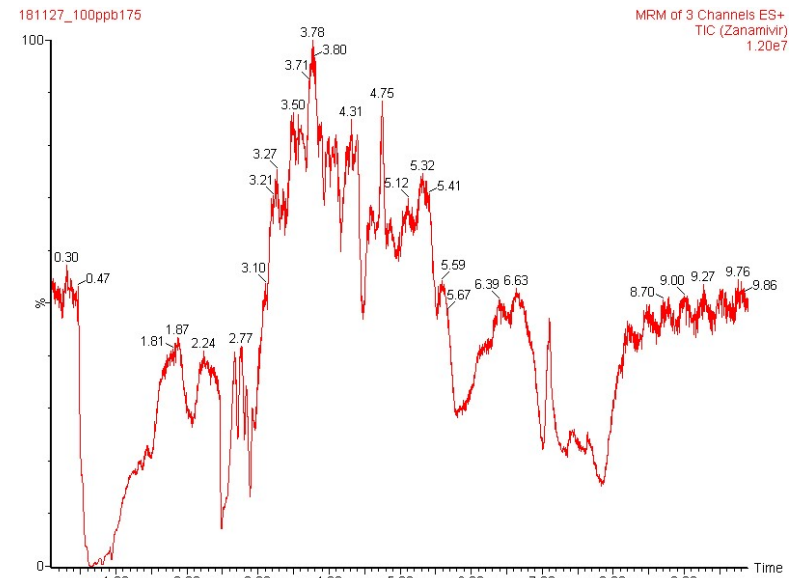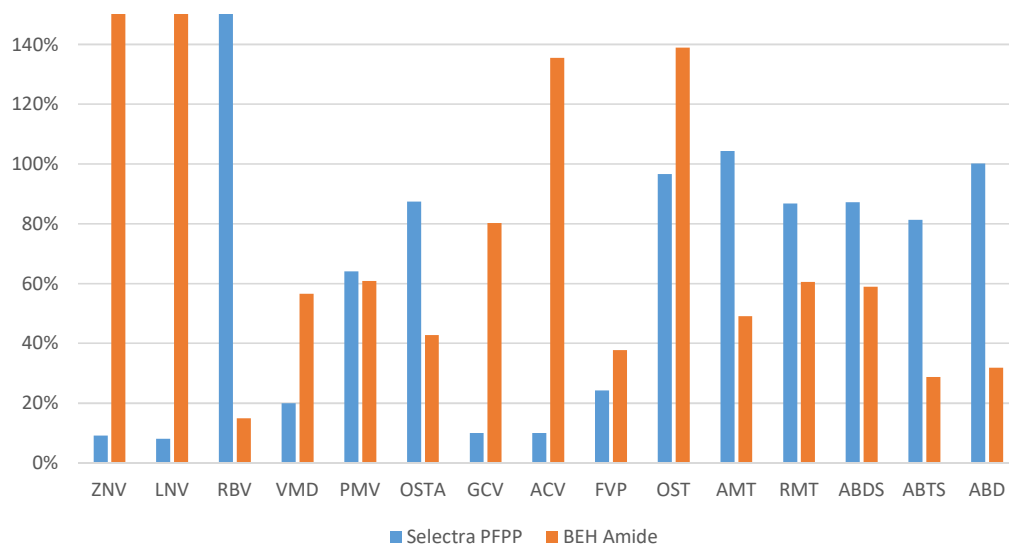
Sample preparation: sample extraction

- QuEChERS is widely used in drug residue food analysis but was found ineffective at extracting highly polar compounds.

- Acetonitrile, methanol and isopropanol were tried.

- Best compromise was found with [acetonitrile:water] 80:20 *v:v*, best overall extraction efficiencies (>40%) and relatively clean extracts.
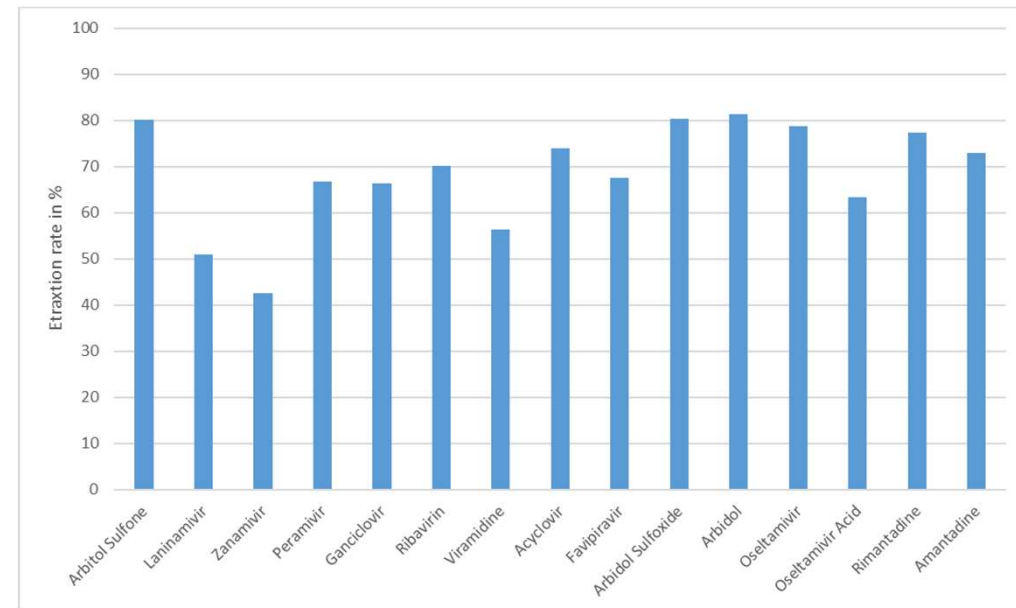
Sample preparation: clean-up

■ Various standard clean-up procedure were tested and found inefficient:

■ Ultrafiltration did not provide additional clean-up.

■ Freezing step trapped polar compounds in ice.

■ Several dspe sorbent were not beneficial.

■ Dilute and shoot was chosen prior to LC-MS/MS analysis. Best final solvent mixture was [acetonitrile:methanol:water] 50:25:25 *v:v:v*.

Method performance: Validation

- Method was validated according to EU guidelines 2021/808.
- All criteria were validated.
- Reproducibility trueness were ranging from 84% to 127% and reproducibility precision from 2.8% to 22.7%.
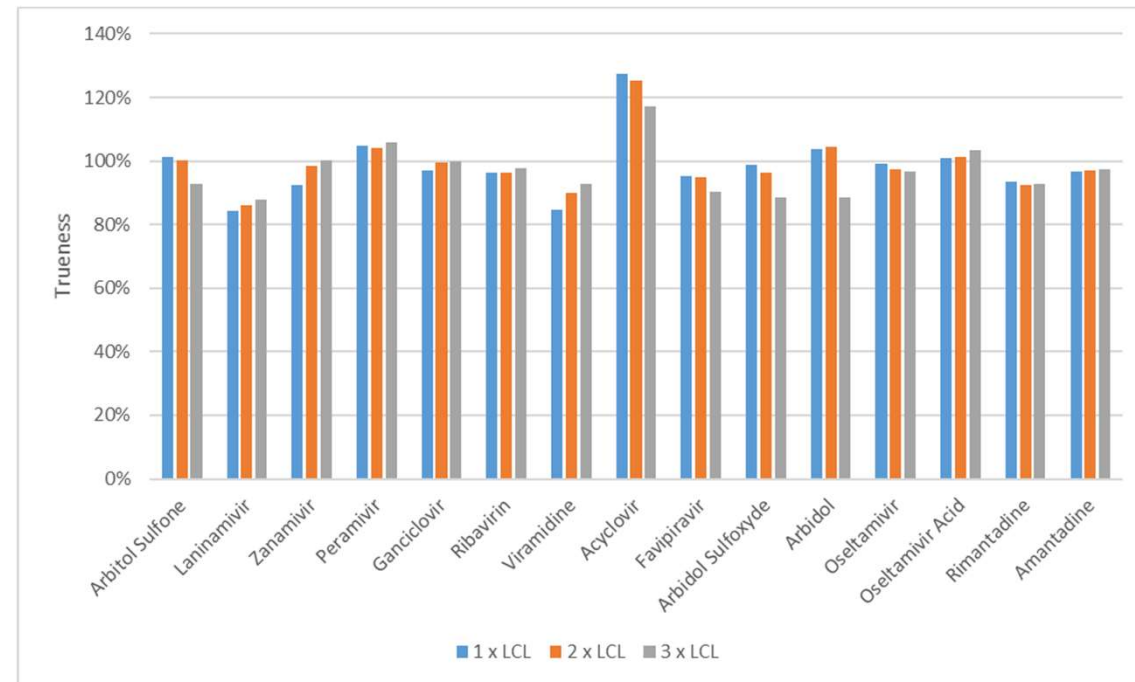
Method performance: LOQ/LOD

■ Eight matrix matched calibrants and the zero level were used to build a calibration curve, all R^2 were ≥0.988.

■ LOQ ranged from 0.03 to 10.46 µg/kg.

Analyte	Curve range (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Arbidol Sulfoxyde	0.1 - 0.8	0.01	0.03
Arbidol	0.1 - 0.8	0.01	0.04
Arbitol Sulfone	0.5 - 4	0.11	0.35
Acyclovir	0.5 - 4	0.07	0.22
Rimantadine	0.5 - 4	0.03	0.11
Oseltamivir	0.5 - 4	0.05	0.18
Amantadine	1 - 8	0.06	0.19
Zanamivir	2 - 16	0.16	0.55
Peramivir	2 - 16	0.19	0.62
Ganciclovir	2 - 16	0.19	0.62
Viramidine	2 - 16	0.08	0.26
Oseltamivir Acid	2 - 16	0.28	0.92
Laninamivir	10 - 80	0.44	1.47
Ribavirin	25 - 200	2.48	8.26
Favipiravir	25 - 200	3.14	10.46

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Occurrence in Irish market

■ A total of 120 poultry products were selected from Irish market.

■ Mix of EU/UK and outside UK/EU origin and mix of cooked and raw products.

■ No trace of antiviral drug against influenza was found.

■ EU/UK ■ Outside of EU/UK

■ Cooked/Processed ■ Raw

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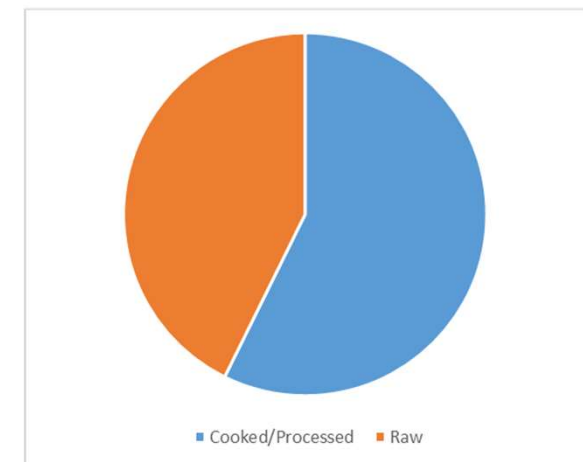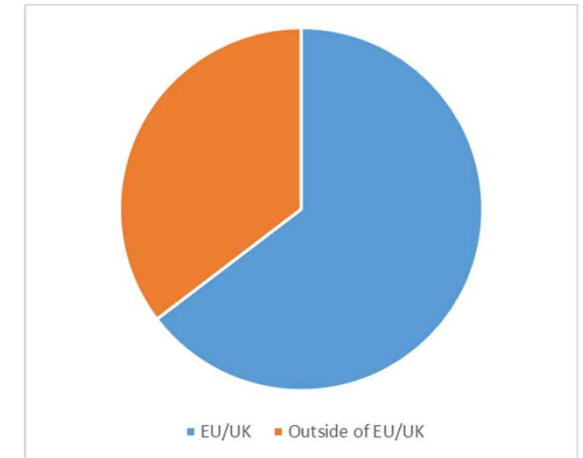
Conclusion

- LC-MS/MS method for the confirmatory and quantitative analysis of 15 antiviral drug residues in avian muscle.

- Fast and easy method.

- Method to monitor antiviral drug residues to better control the risk of resistant avian influenza strains generation.

- Sensitive method with LOQ ranging from 0.03 to 10.46 µg/kg.

Thank you for your attention
Any questions?