



Development and validation of a LC-MS/MS method for the determination of 21 antiviral drugs in chicken muscle and liver

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Introduction

Since the 2000s, human antiviral drugs have been used at Chinese and Ugandan farms. The main concern is that the use of antiviral drugs in food-producing animals leads to less effective drugs for the treatment of human viruses. Recently, the ban of antiviral drugs in food-producing animals by Regulation (EU) 2022/1644 has increased the need for food control laboratories to develop analytical methods and perform official controls. In the present study, we covered multiple classes of antiviral drugs in chicken muscle and liver within a single LC-MS/MS method.

Analytical methodology

• Number of target compounds: 21



• Compound properties: Broad pKa and polarity range

• Selected matrix: Chicken muscle and liver

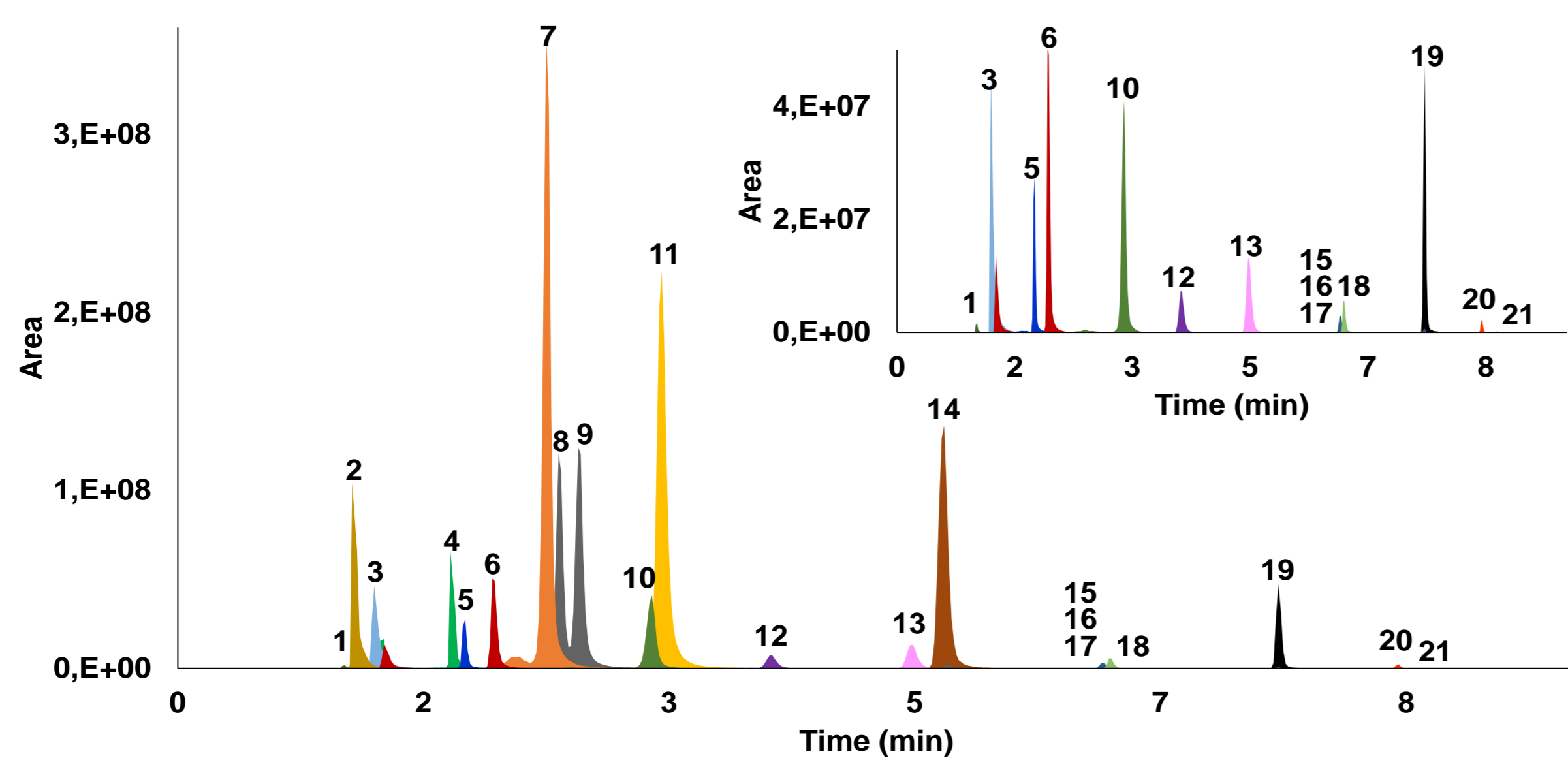


Figure 1. Chromatographic separation in 25 µg/L standard solution. 1. Efavirenz 2. Lopinavir 3. Nevirapine 4. Arbidol 5. Arbidol sulfone 6. Saquinavir 7. Imiquimod 8. Memantine 9. Rimantadine 10. Oseltamivir 11. Amantadine 12. Ribavirin 13. Acyclovir 14. Moroxydine 15. Penciclovir 16. Oseltamivir acid 17. Ganciclovir 18. Peramivir 19. Viramidine 20. Laninamivir 21. Zanamivir.

The electrospray ion signal was studied for coeluting compounds, showing no significant effect on their individual responses.

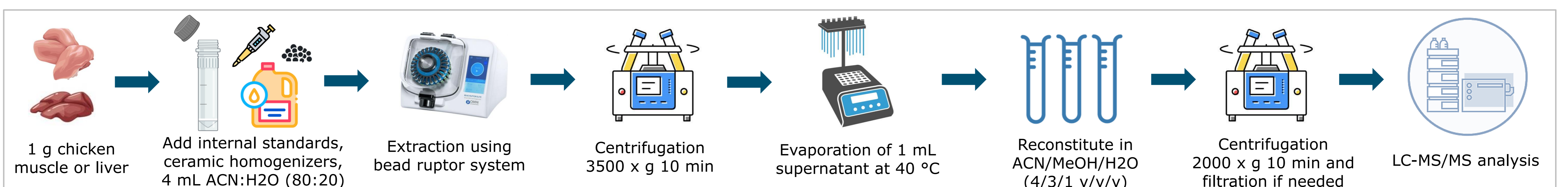


Figure 3. Sample preparation method. Recoveries for the 21 antiviral drugs are between **77-110%** in chicken muscle and **58-98%** in chicken liver.

Validation according to Regulation (EU) 2021/808

Fully validated: chicken muscle; **Partially validated:** chicken liver.

Twelve isotopically labelled internal standards were used to compensate for matrix effects and recoveries.

Table 2. Performance characteristics of chicken muscle at lowest calibration level (LCL)

Antiviral drugs classes	LCL (µg/kg)	Accuracy%	RSDr%	RSDrl%	CCa
Influenza (I)	0.15-1	98-104	7.0-9.5	7.4-11	0.18-1.3
Influenza (II)	0.5-50	92-103	12-42	13-47	0.65-7.1
Herpes	0.5-5	94-103	7.5-14	7.5-14	2.6-6.4
Broad-spectrum	0.5-25	81-123	8.8-24	9.0-45	0.60
Antiretroviral	0.15-10	81-125	14-38	15-46	0.20
Immunomodulator	0.15	102	12	12	0.19

Table 1. Parameters of Liquid chromatography (LC) – Mass spectrometry (MS) method

Liquid Chromatography		Mass spectrometry	
Column	BEH Amide (2.1x150 mm, 2.5 µm)	Ionization source	Electrospray
Mobile phase	0.1% formic acid and 2 mM ammonium acetate in (A) Milli-Q and (B) ACN/Milli-Q (95:5)	Ionization mode	Positive and Negative
Run time	13 minutes	Acquisition mode	Multiple reaction monitoring

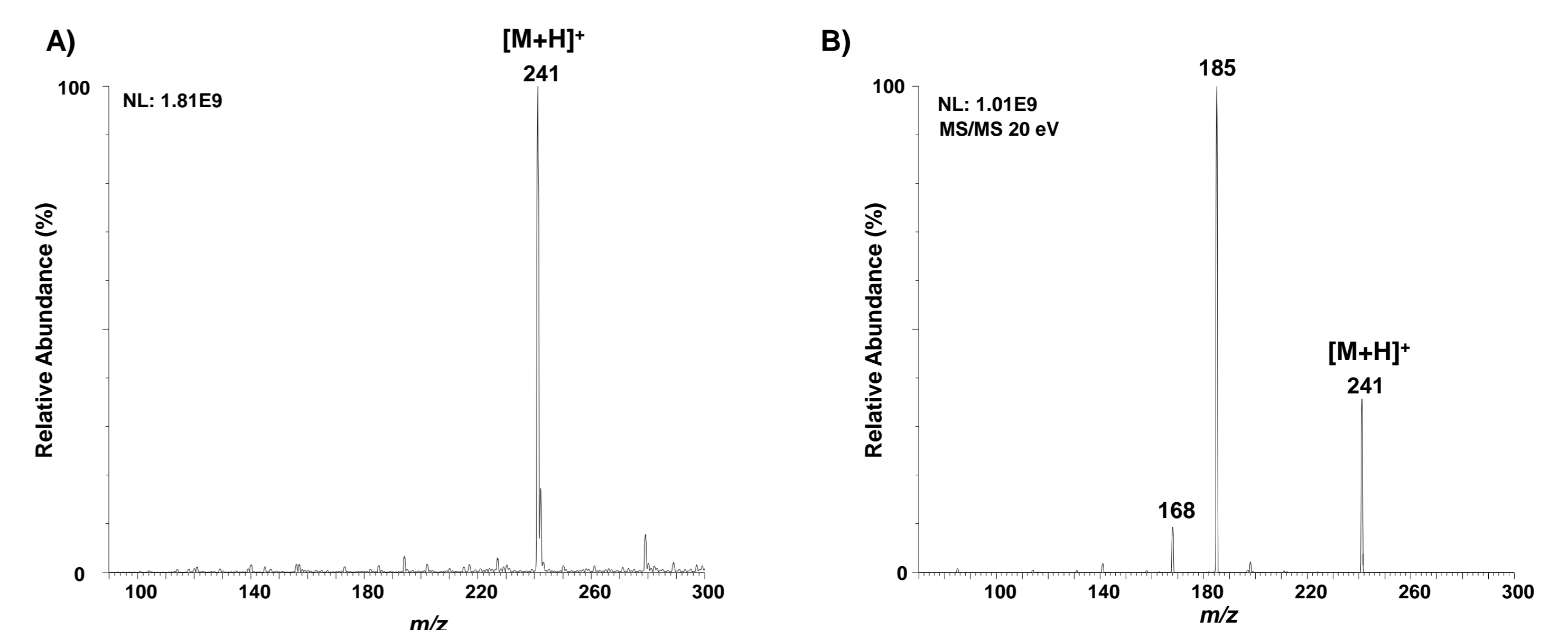
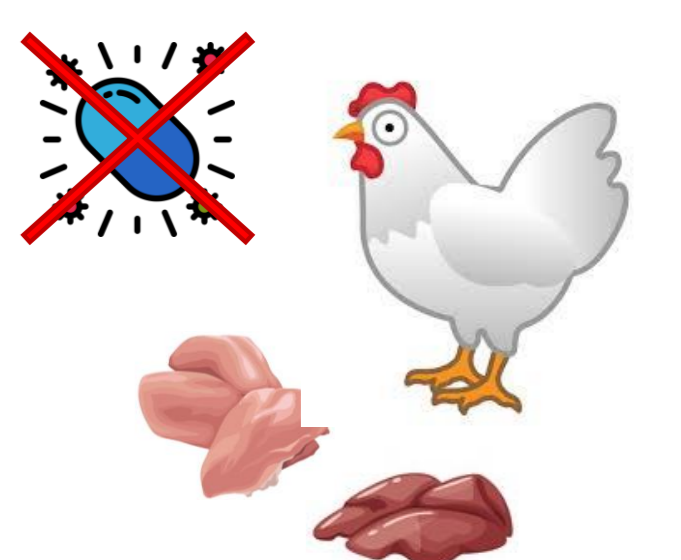


Figure 2. Mass spectra of imiquimod (A) Full scan (B) Product ion scan.

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

Application of analytical method

The method was applied to 10 chicken muscle and 10 chicken liver samples from the Dutch National Residue Control Plan and resulted in no detected antiviral residues.



Conclusions and Perspectives

- The developed and validated LC-MS/MS method is suitable for the monitoring of antiviral drugs abuse in chickens.
- Official controls are needed to collect data on whether antiviral drugs are used in poultry in Europe.

Acknowledgements

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