# A COMPREHENSIVE METHOD FOR THE DETERMINATION OF 20 COCCIDIOSTATS RESIDUES IN VARIOUS FOOD MATRICES

**Konrad Pietruk, Małgorzata Olejnik, Iwona Szymanek-Bany, Teresa Szprengier-Juszkiewicz**

**Department of Pharmacology and Toxicology, National Veterinary Research Institute**

**Partyzantow 57, Pulawy, Poland, e-mail: konrad.pietruk@piwet.pulawy.pl**

## INTRODUCTION

To minimize risk of occurrence of residues of coccidiostats maximum limits (MLs) were established. As a result of this regulations there is a need for reliable analytical methods for the determination of all regulated coccidiostats in a wide range of matrices.

Developing effective sample preparation is one of the most crucial steps in the method development. Appropriate sample preparation protocol should be easy and time effective but also should provide as high recovery and precision as possible.

We here present a versatile, LC-MS/MS based method for the determination of coccidiostats residues in food matrices. The procedure enables the determination of 20 coccidiostats in a wide range of matrices: muscle, liver, milk and eggs.

## MATERIAL AND METHOD

Eggs

Milk

Muscle

Liver

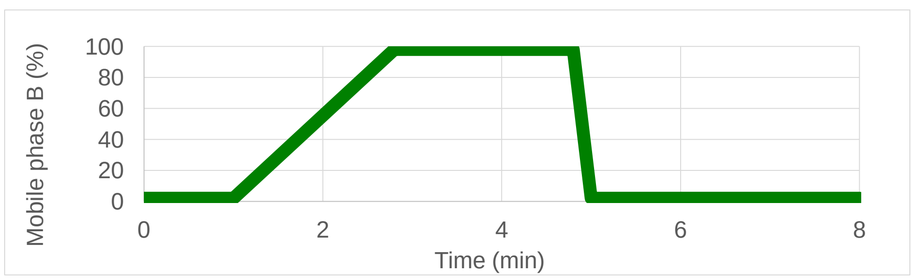
### Sample preparation

| | |
|---|---|
| 5 ml 90% ACN | 5 ml 80% ACN |
| SiOH 500 mg SPE | SiOH 500 mg SPE |
| | **200 mg C18 d-SPE** |
| Freezing | Freezing |
| Evaporation | Evaporation |
| Reconstitution in 50% ACN | Reconstitution in 50% ACN |

### UHPLC-MS/MS analysis

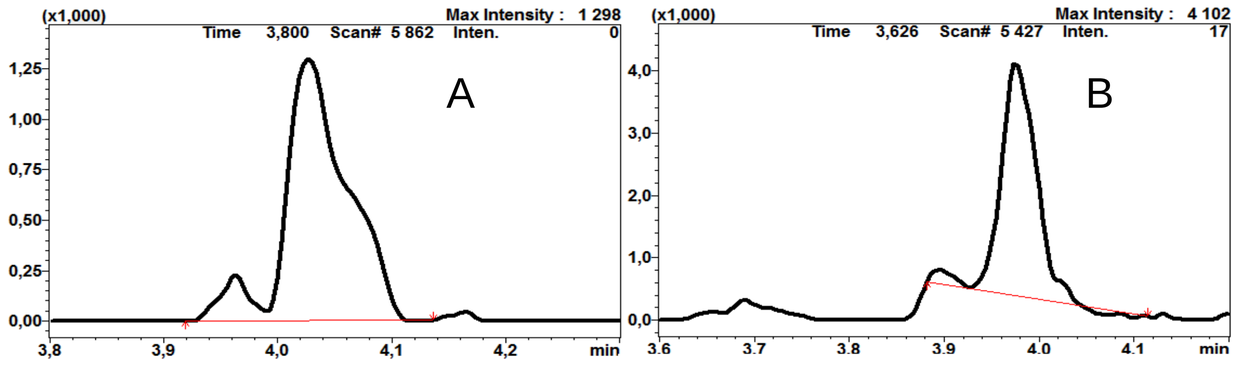

**Shimadzu Nexera X2 + Shimadzu LCMS-8050**

A: 0.01 M $HCOONH_4$, pH 4.0
B: MeCN
Flow rate: 0.6 mL/min
Agilent Zorbax Eclipse Plus C18 RRHD 1.8 µm, 2.1 x 50 mm
**Run time: 8 min**
Column temperature: 40°C
Injection volume: 5 µL




ESI (+)/ ESI(-)
**20 compounds**
2 transitions per compound
$[M+H]^+$, $[M-H]^-$, $[M+Na]^+$ parent ions
**Time scheduled MRM method applied for higher sensitivity**

## RESULTS



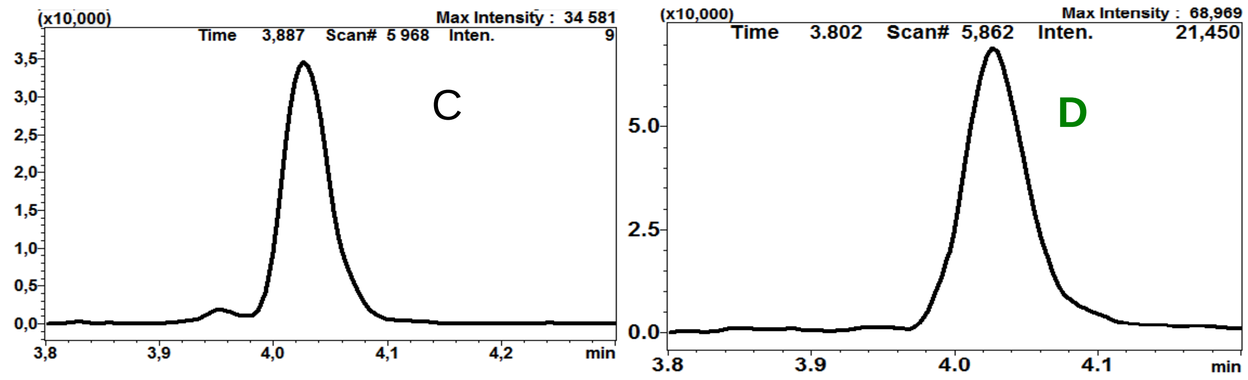




Figure 1. Development of sample preparation protocol - salinomycin in liver sample spiked at 2.5 µg/kg (0.5 ML).
A - SiOH cartridges followed by freezing (milk ang eggs sample prepration protocol)
B - SiOH cartridges followed by freezing and d-SPE - 250 mg of ENVI-Carb
C - SiOH cartridges and freezing followed by Strata-X cartridges
**D - SiOH cartridges followed by freezing and d-SPE - 200 mg of C18 sorbent - allowed to obtain best reproducibility and sensitivity**

Table 1. Validation results

| *Analyte* | *Eggs* | | | | |
|---|---|---|---|---|---|
| | *MRL/ML µg/kg* | *Reproducibility CV(%)* | *Recovery %* | *CCα µg/kg* | *CCβ µg/kg* |
| Amprolium* | 20 | 18.7 | 107.2 | 27.0 | 33.8 |
| Decoquinate | 20 | 20.4 | 102.0 | 23.5 | 27.9 |
| Diclazuril | 2 | 26.1 | 104.5 | 2.63 | 3.55 |
| Halofuginone | 6 | 3.1 | 100.3 | 6.19 | 6.64 |
| Lasalocid | 150 | 21.4 | 105.4 | 203 | 277 |
| Maduramicin | 12 | 14.3 | 112.0 | 15.8 | 22.1 |
| Monensin | 2 | 15.2 | 101.8 | 2.60 | 3.09 |
| Narasin | 2 | 17.5 | 109.2 | 2.48 | 3.25 |
| Nicarbazin | 300 | 10.0 | 94.9 | 347 | 406 |
| Robenidne | 25 | 13.6 | 104.3 | 32.9 | 41.1 |
| Salinomicin | 3 | 12.7 | 101.1 | 4.08 | 5.60 |
| Semduramicin | 2 | 17.9 | 107.6 | 2.87 | 3.95 |
| Toltrazuril* | 25 | 12.1 | 99.8 | 31.5 | 40.2 |
| Toltrazuril sulfoxide* | 25 | 14.1 | 92.1 | 31.4 | 39.4 |
| Toltrazuril sulfone* | 25 | 14.1 | 92.8 | 29.4 | 36.7 |
| Arprinocid* | 5 | 13.5 | 110.6 | 6.32 | 8.28 |
| Ethopabate* | 5 | 9.5 | 99.5 | 6.05 | 7.19 |
| Clazuril* | 5 | 18.7 | 91.6 | 5.69 | 7.10 |
| Clopidol* | 10 | 17.8 | 110.3 | 14.6 | 19.4 |
| Nequinate* | 5 | 15.1 | 95.1 | 5.72 | 7.06 |

* *Coccidiostats without regulation - target levels for validation selected by authors.*

## CONCLUSION

A sensitive and reliable method for the simultaneous determination of twenty coccidiostats in a wide range of food products was developed. Developed sample preparation protocol is simple and effective, even in case of challenging matrices such as muscle and liver.

The validation experiment has proven fitness of the method for confirmatory analysis. Presented method can be used for routine analysis of coccidiostats residues in food matrices.

**EuroResidue VIII, Conference on Residues of Veterinary Drugs in Food**

**Egmond aan Zee, The Netherlands, 23-25 May, 2016**