

DETERMINATION OF THE IONOPHORIC COCCIDIOSTAT MONENSIN IN CHICKEN TISSUES BY LIQUID CHROMATOGRAPHY- TANDEM MASS SPECTROMETRY AFTER WITHDRAWAL OF MEDICATED FEED

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Introduction

Coccidiostats are compounds that prevent or treat coccidiosis, a parasitic disease caused by the genus *Eimeria*. The group of polyether ionophores are coccidiostats widely spread representing the most important class used for poultry in France. At this time, coccidiostats are licensed in Europe as feed additives according to Regulation 1831/2003/EC, but their reevaluation by the European Food Safety Authority will lead to their ban or authorization with MRLs. A pharmacokinetic study in chicken was carried out in which 138 chickens were treated with feed containing monensin during 32 days. The objective was to verify the distribution of the concentrations for each tissue at the end of the treatment, and the depletion in tissues after treatment. A quantitative liquid chromatographic-tandem mass spectrometric method was developed for monensin in poultry muscle, liver, and fat. After extraction and clean-up, extracts are analysed in ESI positive mode. The precursor ion chosen was the $[M+NH_4]^+$ adduct and one product ion is recorded with selected reaction monitoring. Narasin was used as internal standard. The validation was performed for pharmacokinetic application, following the total error approach described in harmonization guidelines of the "Société Française des Sciences et Techniques Pharmaceutiques" (SFSTP).

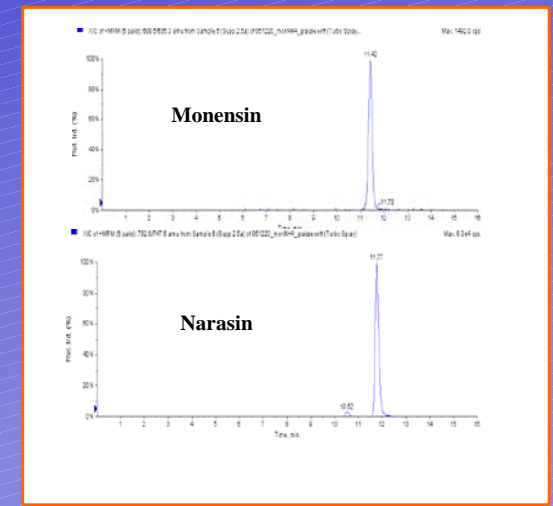
Method

Sample preparation

To 2 g of sample add 200 μ l internal standard solution at a concentration level of 50 μ g/kg, vortex mix and let in contact for 10 min. Add 6 ml of a mixture of methanol-water (87/13, v/v) and vortex mix. After placing the tube in an ultrasonic bath for 10 min, centrifuge the mixture for 10 min at 4000 g at 5°C. A 5 ml aliquot is purified on a C₁₈ SPE cartridge (Bond-Elut, 200mg, 3ml), preconditioned with 4 ml of methanol and 2 ml of water. Deposit the extract on the cartridge and rinse with 2 ml of a mixture of methanol-water (80/20, v/v). Collect in a 15-ml plastic tube with 4 ml of methanol after drying the cartridge. After evaporation to dryness under nitrogen at 45 °C, recovered the residue in 300 μ l of a mixture of acetonitrile-ammonium acetate 50 mM (80/20, v/v) and vortex briefly. Transfer to HPLC autosampler vials and inject 50 μ l (20 μ l for fat sample into the LC-MS/MS system).

Analysis

LC-MS / MS: HP1100 system in tandem with PE Sciex API 2000, mode ESI positive, SRM: **688.4 > 635.3 for monensin and 782.6 > 747.5 for narasin**. Column: Phenomenex, 150x2.1 mm (3 μ m) with guard column of the same material, flow of 0.30 ml/min. Gradient with water and acetonitrile each containing 0.1% formic acid.



Experimental design

Poultry breed: broiler chickens (Ross), weighting 1.9 ± 0.25 kg at the end of experiment.

Feed: adapted to the age containing 21.5 % to 18.5 % of crude protein

Treatment : monensin sodium at a concentration level of $121 \text{ mg} \cdot \text{kg}^{-1}$ per feed, (Lilly France)

Housing : 300 animals in one pen (15 m²), temperature (32°C to 19°C) and light (24h to 20h light/day) depending of the age.

During the accumulation and the depletion phases, 6 or 10 chickens were slaughtered every 2 hours.

day 0 to day 33: Premix distribution

day 32 to day 33 : 70 chickens slaughtered to determine the steady state concentrations

day 33 to day 36: 68 chickens slaughtered to evaluate the concentration during the depletion study

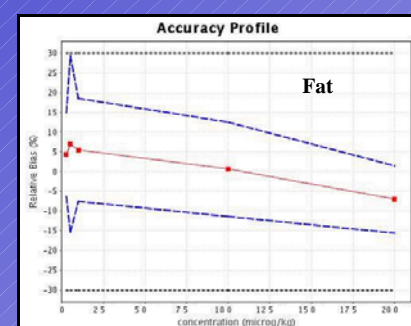
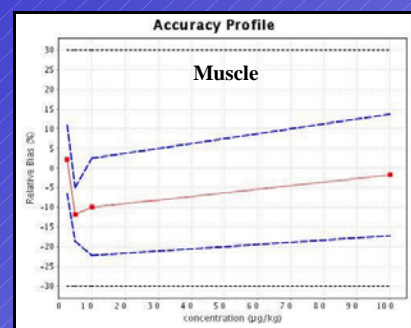
Validation

- Two kinds of samples were prepared : **calibration standards (SC)** and **validation standards (SV)**.

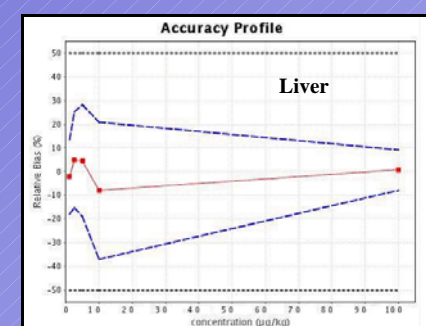
- SC: 2 calibration standard's series of 5 concentration levels replicated on 3 different days.

- SV: same concentration levels as the SC, with 3 repetitions at each level.

Tissues	Validated range (μ g/kg)	Trueness (%)	Precision (RSD %)	
			Repeatability	Intermediate precision
Muscle	2.5 - 100	88.19 - 102.20	2.77 - 5.22	4.61 - 10.85
Liver	1 - 100	92.04 - 105.00	3.48 - 11.79	3.48 - 11.79
Fat	2.5 - 200	93.00 - 107.00	1.94 - 6.49	2.79 - 8.07



- Concept : based on the **total error measurement** (systematic and random errors) proposed by the SFSTP.
- Easy visualised by **accuracy profiles** characterised by the two-sided 95% β -expectation tolerance intervals calculated at each concentration level.
- Regression models: the best models were chosen according to the best accuracy profiles.



Results

Matrix	Steady state Day 32	Depletion phase Day 33				
	Time (hours)	Time schedule (hours)				
	12 h after night (5 p.m.)	Morning (light on 5 a.m.)	0 (end of treatment)	+2	+4	+6
Muscle	8.33 \pm 4.86	7.91 \pm 5.05 (n=4)	5.77 \pm 2.01 (n=5)	6.35 (n=1)	ND	ND
Liver	12.78 \pm 13.11	9.0 \pm 7.79 (n=10)	16.98 \pm 6.44 (n=6)	4.94 \pm 3.90 (n=6)	3.82 \pm 1.83 (n=5)	1.48 \pm 1.26 (n=2)
Fat	67.05 \pm 49.23	15.65 \pm 9.32 (n=10)	49.13 \pm 20.54 (n=6)	29.23 \pm 7.02 (n=4)	28.75 \pm 10.23 (n=6)	10.52 \pm 7.76 (n=6)

Mean concentration (\pm sd) of monensin at steady state and in depletion phase after feed withdrawal

Conclusion

A method was **developed** and **validated** according to the guidelines of the SFSTP in order to quantify monensin accurately in edible tissues of chickens for pharmacokinetic studies. An animal experiment was performed to determine concentration level in tissues after withdrawal of the medicated feed. **The main tissue is fat** where monensin is observed in all animals **6 hours** after the end of treatment. Monensin **decreased rapidly in muscle** and not anymore detected at 4 hours. It's still **detected in two animals only in liver at 6 hours**.