COMPARATIVE METHOD VALIDATION FOR CLOSANTEL IN CATTLE AND SHEEP MILK ACCORDING TO EU VOLUME 8 AND VICH GL 49 GUIDELINES

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Introduction

Depletion studies of veterinary drugs

Analytical method for quantification of residues in animal matrices needed

Method validation?

→ **EU Volume 8 guidelines**

→ **VICH GL 49**

Aims

1/ Develop a sensitive LC-MS/MS method for determination of closantel (CLO) in bovine and ovine colostrum and tank milk

2/ Validate the method according to EU guidelines [1] alone (bovine) or in conjunction with the VICH GL49 guidelines [2] (ovine)

3/ Compare validation parameters and acceptance criteria of both guidelines
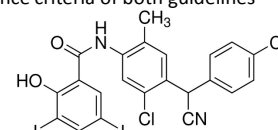
Materials & Methods

LC-MS/MS method

Sample preparation:
5 g milk + 12.5 µL of 10 µg/mL $^{13}C_6$-CLO + 15 mL ACN/H2O (80/20, v/v)
Vortex mixing – shaking – centrifugation
SPE extraction (Oasis® MAX®) – elution 5% formic acid in MeOH
Evaporation to dryness (50°C, N_2)
Resuspension: 125 µL ACN + 125 µL H_2O

HPLC conditions:
Alliance type 2695 HPLC (Waters)
Stationary phase: Zorbax® Eclipse Plus C18 column (Agilent)
Mobile phases: (A) 1 mM ammonium acetate in H_2O (B) ACN

MS/MS conditions:
Quattro Ultima® triple quadrupole MS (Micromass)
positive ESI mode
SRM transitions for CLO: m/z 660.7 > 344.6, 660.7 > 314.8*
SRM transition for $^{13}C_6$-CLO : m/z 666.8 > 350.7*
*: quantification ion

Results

Linearity:
correlation coeff ≥ 0.99, goodness-of-fit coeff ≤ 10%

Accuracy and precision:
Results fell within specified ranges [1],[2]

LOD: 0.32-1.27 µg/kg

LOQ: 10 µg/kg

Specificity:
No interfering peaks

Stability:
Working solutions of CLO and $^{13}C_6$-CLO (2-8°C): > 99 and 91 days
In extract: CLO was stable for ≥ 2 days (2-8°C)
In matrix: CLO was stable for > 90 days in sheep and for > 180 days in cattle milk (≤ -15°C)

EU Volume 8 ⟺ VICH GL 49

Figure 1. Chemical structure of closantel

Table 1. Overview of the acceptance criteria for different method validation parameters specified by EU Volume 8 [1] and VICH GL49 [2] guidelines

Parameter	EU Volume 8		VICH GL49	
Linearity	no criteria		- Calibration curve: encompass ≥ 5 different concentrations - Matrix-matched calibration samples are subject to accuracy and precision acceptability ranges	
Accuracy	-30% to +10%	> 1 µg/kg	-30% to +10% -20% to +10%	≥ 10 µg/kg < 100 µg/kg ≥ 100 µg/kg
Within-run precision	20% 15%	≥ 10 µg/kg < 100 µg/kg ≥ 100 µg/kg	15% 10%	≥ 10 µg/kg < 100 µg/kg ≥ 100 µg/kg
Between-run precision	As low as achievable 23%	< 100 µg/kg 100 µg/kg	23% 16%	≥ 10 µg/kg < 100 µg/kg ≥ 100 µg/kg
LOD	Several methods are valid. A scientific justification is needed		Several methods are valid. A scientific justification is needed	
LOQ	- Several methods are valid. A scientific justification is needed - The method has to meet accuracy and precision criteria at LOQ level - MRL should significantly exceed LOQ		Several methods are valid. A scientific justification is needed	
Specificity	no criteria		S/N blank sample < 20% of S/N LOQ	
Applicability and practicability	- The method utilizes commercially available standards, reagents, and equipment - The method should be designed to be performed safely by trained analysts - Analyze a sufficiently large number of samples within reasonable time-periods		no criteria	
Susceptibility to interference	Any possible interfering matrix components should be investigated		no criteria	
Stability	Should be tested: - In solvent during storage - In matrix during storage/sample preparation - In extract during storage/analysis		Should be tested: - In matrix during storage (2 different concentrations, in triplicate) - In extract during storage/analysis	

Discussion

➢ Both guidelines cover a similar set of parameters for linearity, accuracy, precision, LOD and LOQ
   ➢ Acceptance criteria might differ: accuracy and precision
   ➢ No specific criteria are stipulated: LOD and LOQ
➢ Only one of both guidelines stipulates acceptance criteria:
   ➢ EU Volume 8 [1]: applicability, susceptibility and practicability
   ➢ VICH GL 49 [2]: linearity, specificity, analyte stability
➢ None of both guidelines mention following parameters
   ➢ Signal suppression/enhancement
   ➢ Extraction recovery

More effort is needed to harmonize different guidelines

References

[1] EU Volume 8, Eudralex, 2005.
[2] VICH GL 49, MRK (Metabolism and Residue Kinetics), 2012.
Further information: Devreese *et al.*, 2014 – *Journal of Chromatography A, in press.*