LC-MS/MS Procedure for the Screening of Veterinary Drugs in Foodstuffs

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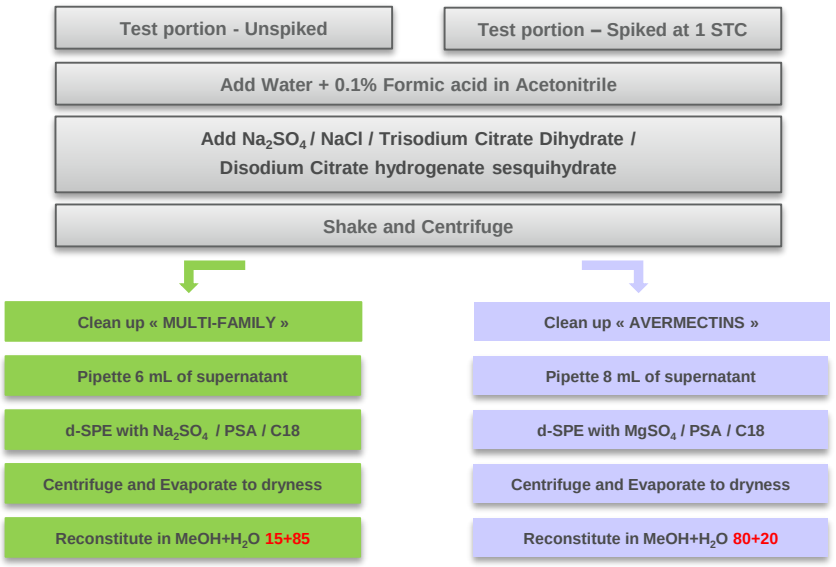
GOALS

- Develop & validate a LC-MS/MS procedure for the qualitative screening of >100 veterinary drugs in milk-, meat- and seafood-based products. Method to be adapted for heavy routine environments requiring short turn-around-times.
- Targets: 108 drugs belonging to 23 Families, namely Amphenicols (3), Avermectins (6), Benzimidazoles (14), Coccidiostats-ionophores (5) and non ionophores (6), Diaminopyrimidines (2), Lincosamides (2), Macrolides (8), Nonsteroidal anti-inflammatory drugs (NSAID) (5), Quinolones (18), Rifamycins (2), Salicylanilides (4), Sulfonamides (22), Tranquilizers (2) and others (9).
- For all analyte/matrix combinations, ensure that Screening Target Concentrations (STC) are at or below Maximum Levels stipulated in EU 37/2010 and CODEX regulations [1-2].

EXTRACTION PROCEDURE

Modified QuEChERS protocol comprising a dedicated clean up for AVERMECTINS. Large variations in matrix effects require each sample to be extracted twice: one as such and the other spiked at the Screening Target Concentration (STC).



- Acidified mixture of water/acetonitrile is required to efficiently promote protein precipitation.
- Na_2SO_4 **(instead of $MgSO_4$)** is employed as initial partitioning salt and in the d-SPE salt mixture **to avoid chelation of Quinolones** (MULTI-FAMILY method).
- Citrate salts are **mandatory** for an efficient buffering of extracts due to the broad range of compound pKas.
- Each resulting extract is divided in two portions which subsequently undergo different d-SPE cleanup to deal with AVERMECTINS **particular behavior**.
- Reconstitution of AVERMECTINS extract in high percentage organic solvent to avoid **adsorption** onto LC vials

INSTRUMENTAL PARAMETERS (Agilent 1290 Infinity & Sciex Qtrap 5500)

- LC column: Waters Acquity BEH C18, 2.1 x 100 mm, 1.8 µm
- Temperature: 40 °C
- Flow rate: 400 µL/min
- Injection volume: 10 µL
- Mobile phase A: 0.1 % Formic acid / 0.5 mM Ammonium formate in water
- Mobile phase B: 0.1 % Formic acid / 0.5 mM Ammonium formate in methanol
- Gradient flow
- Two LC runs: 16 min (MULTI-FAMILY) and 10 min (AVERMECTINS)

PARAMETERS	MULTI-FAMILY	AVERMECTIN
Ionisation type	Electrospray	Electrospray
Polarity	Positive / Negative switching	Positive
Ion spray voltage (IS)	5000 / -4500 V	5500 V
Temperature (TEM)	550 °C	**350 °C ***
Scheduled MRM	Enabled as Basic	Not used

*** Lower MS source temperature required to deal with Avermectins thermolability**

VALIDATION according to EU CRL 2010/01/20 [3]

- **Aim** is to check if samples are below or potentially above the Screening Target Concentration (STC).
- **Results** are either < *STC* (expressed in µg/kg) or *Suspect* (i.e. potentially > STC) to be further confirmed by a confirmatory quantitative analysis.
- **Response measured** as a relative comparison between Peak Area in Unspiked Sample **(A_{us})** *vs.* Peak Area in the related Spiked Sample **(A_s)**.

Validation scheme:

Samples

Milk-based products
Milk fractions (16), infant formulas & milk powders (15), milk-containing infant cereals (5)

Meat-based products & seafood
Meat & seafood powders (10), Meat & seafood fresh and cooked (10), meat containing baby foods (11)

Experimental plan

- Sixty seven (67) samples
- Spiked at 0, 1 and 2 x STC
- Three Analysts involved
- Over 15 days

Quality criteria

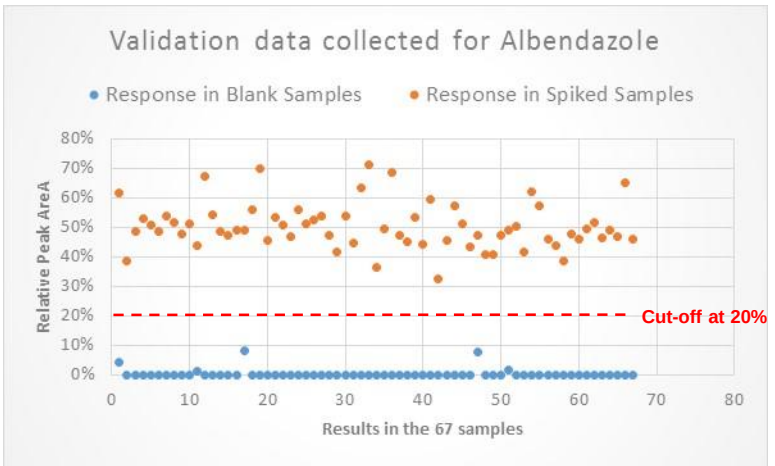
Cut-off level*
False suspect rate: <10-20%
False negative rate: < 5 %
Retention time: < 0.2 min
Identification: 2 SRMs

***Cut-off determination**: The cut-off value is the value above which a sample is classified as suspect

✓ Response in blank sample = (Peak area in unspiked sample / Peak area in 1 STC) * 100

✓ Response in spiked sample = (Peak area in 1 STC sample / Peak area in 2 STC sample) * 100

→ *cut-off level must be set to ensure that highest response in blank samples is below lowest response in spiked samples (see example for Albendazole below)*



RESULTS

Family	Nb of compounds	STC (µg/kg)	Cut-off (%)	FP rate (%)	FN rate (%)
Amphenicols	3	0.3 to 10	20%	**100 % PASSED** All FP < 3 %	**98 % PASSED** ALL FN < 4 % Not passed for* Clorsulon Tulathromycin A
NSAID	5	5			
Avermectins	6	10			
Benzimidazoles	14	2 to 5			
Coccidiostats / ionophores	5	15			
Coccidiostats / non ionophores	6	15			
Diaminopyrimidines	2	15			
Lincosamides	2	15			
Macrolides	8	15			
Quinolones	18	10			
Rifamycins	2	15			
Salicylanilides	4	10 to 15			
Sulfonamides	22	5 to 10			
Tranquilizers	2	15			
Others	9	5 to 15			

* Clorsulon and Tulathromycin A gave FN > 5% due to the absence of SRM2 signal

CONCLUSIONS

- **Straightforward workflow, suitable for routine environement, applicable to all milk-, meat- and seafood-based products surveyed**
- **106 out of 108 compounds fulfill the analitycal requirement with both FP/FN rates < 5%**

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[1] Commission Regulation (EU) No 37/2010 on Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origins
[2] Codex Alimentarius Commission Maximum Residue Limits for Veterinary Drugs in Foods
[3] EU Community Reference Laboratories (CRL) Guidelines for the validation of screening methods for residues of veterinary medicines; 2010-01-20

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