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Coccidiostats in egg and milk (EC/124/2009)

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Introduction

The occurrence of unavoidable carry-over of feed additives like coccidiostats and histomonostats in non-target feed may result in the presence of residues of these substances in animal products like milk and eggs. Recently maximum levels (MLs; table 1) for these substances were laid down by the EU in Commission Regulation (EC) 124/2009[1].

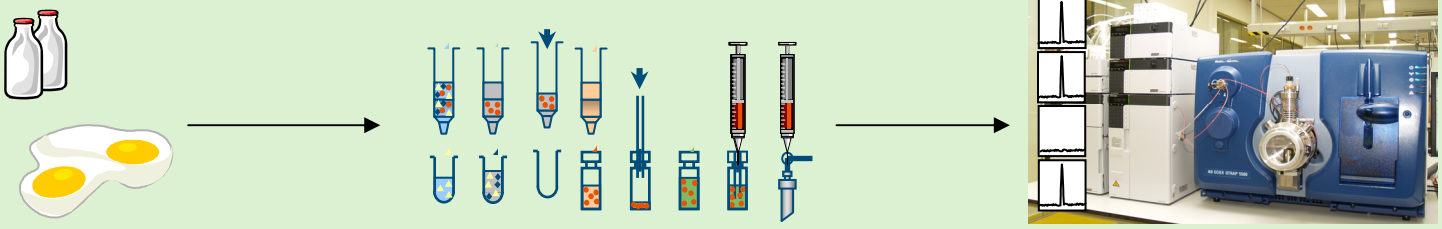
For monitoring purposes the available analytical methods have to be optimized to be able to detect the new low – in comparison with MRLs – MLs and to add new analytes and matrices.

Table 1. *Maximum levels in food in µg/kg according to EC/124/2009*[1]

Compound	Eggs	Milk	Liver	Kidney	Skin/fat	Other
Narasin	2	1	50			5
Lasalocid-Na		1	50	50		5
Salinomycin-Na	3		5			2
Monensin-Na			8			2
Semduramicin						2
Maduramicin						2
Robenidine	25		50	50	50	5
Decoquinate						20
Halofuginone	6	1	30	30		3
Nicarbazin	100	5	100	100		25
Diclazuril	2		40	40		5

Experimental

Egg or milk samples were homogenized. Samples were extracted twice with acetonitrile. After centrifugation the acetonitrile layer was diluted with water and ammonia and concentrated on an Oasis® HLB solid phase extraction cartridge. After evaporation under N_2 and reconstitution in water/methanol, the final extract was injected on an LC-MS/MS system. The analysis was carried out using an Agilent Zorbax Eclipse XDB-C8 analytical column using a mobile phase of acetic acid, water and methanol. The mass spectrometer operated in ESI+ or ESI- mode (depending on the compound) and data acquisition was performed in multiple reaction monitoring mode (MRM).



Validation

For validation the following parameters (according to EU legislation) are important: accuracy, repeatability, within-lab reproducibility, specificity, decision limit (CCα), detection capability (CCß), linearity, ruggedness and stability [2]. CCα and CCß were determined by analysing 20 blank eggs and 20 eggs fortified at 0.5, 1.0 and 1.5 ML (for results see table 2). Ruggedness was determined by introducing small variations in the sample pre-treatment procedures. The stability of the coccidiostats in stock solutions was determined by storing batches at +4°C and -70°C for different periods of time. The stability of the extracts was tested at +4°C for 1 week.
The developed method proved to be robust, specific and suitable to quantify and identify all coccidiostats from 0.25 * ML in egg, with the exception of halofuginone and semduramicin. To quantify these compounds multi-level standard addition has to be applied.

Table 2. *Validation results for egg at ML (n=21) according to the EU procedure*[2]

Compound	Target value (µg/kg)	Acc. (%)	RSD_r (%)	RSD_R (%)	CCα (µg/kg)	CCβ (µg/kg)
Narasin	2	108	14	16	2.6	3.1
Lasalocid-Na	5	97	9.3	15	6.2	7.3
Salinomycin-Na	3	96	12	17	3.8	4.6
Monensin-Na	2	106	8.4	8.6	2.3	2.6
Semduramicin	2	131	23	34	3.5	4.9
Maduramicin	2	87	7.8	10	2.3	2.6
Robenidine	25	104	2.0	2.8	26.2	27.4
Decoquinate	20	104	4.0	4.1	21.4	22.8
Halofuginone	6	117	15	16	7.8	9.6
Nicarbazin (DNC)	100	101	2.6	3.8	106	113
Diclazuril	2	104	2.7	3.1	2.1	2.2

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[1] EC/124/2009, L40, 7-11.
[2] Commission Decision 2002/657/EC (2002), L221, 8-36.

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