

Simultaneous determination of 24 benzimidazoles in egg by ultrafiltration, off-line SPE and on-line SPE

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Introduction

Benzimidazoles are veterinary drugs widely used for prevention and treatment of parasitic infections in agriculture. Metabolites of benzimidazoles have been reported in several matrices including eggs^{1,2}. To date flubendazole (Figure 1) is the only anthelmintic that has an established MRL of 400 µg kg⁻¹ in egg³. A target value of 50 µg kg⁻¹ was established for all other anthelmintics.

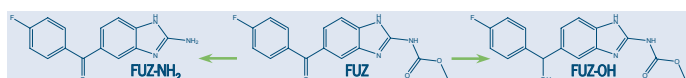


Figure 1. Structures of flubendazole (FUZ) and its metabolites, hydrolysed flubendazole (FUZ-NH₂) and reduced flubendazole (FUZ-OH).

Method development

Ultrafiltration, off-line and on-line SPE protocols were based on the on-line SPE milk method, however when the method was transferred to benzimidazoles in egg, some details of the procedure were optimised (see table 1). The method was expanded with triclabendazole and several deuterated internal standards. Egg was extracted by adding acetonitrile twice and vortexed immediately, followed by centrifugation.

Table 1. Experiments performed to optimise sample pretreatment for the analysis of benzimidazoles in egg.

	Technique	Milk	Milk→Egg	Egg→Egg
1	Ultrafiltration	Ultrafilter	30 kDa	10 kDa
2		Acetonitrile on UF	20%	50%
3	Off-line SPE	Reconstitution	Evaporation	Dilution 10x
4			Evaporation	Dilution 20x
5	On-line SPE	Dilution	Dilution	Evaporation
6		Extraction with FA	0%	5%
7		Extraction time	15 min.	60 min.

Conclusions method development

All three evaluated methods could be used to analyse egg samples and the validation characteristics of all these methods met the EU criteria⁴. However, the ultrafiltration and on-line SPE methods entailed less sample pretreatment and therefore intraday precision was lower for both methods. The changes on the original milk method shown in Table 1 proved to be unsuccessful. Therefore, the ultrafiltration procedure was used to study egg samples. This method was easy to implement and showed low intraday precision for all relevant analytes.

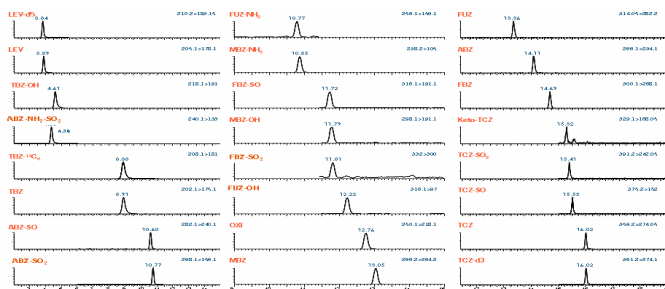


Figure 2. Selected ion traces of a LC-MS/MS chromatogram obtained for a processed egg sample fortified with a mixture of 24 benzimidazoles at ½ MRL (target) level with on-line SPE.

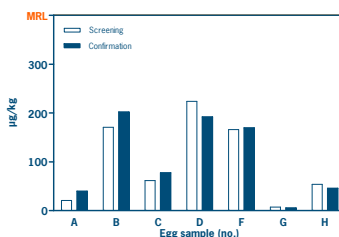


Figure 3. Screening vs. confirmation results of pooled egg samples (flubendazole, FUZ).

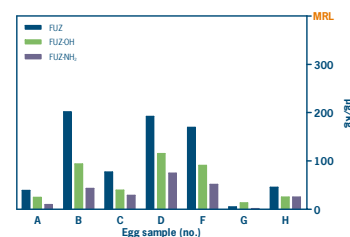


Figure 4. Composition of suspect pooled house samples found during monitoring of egg samples.

Results

The developed ultrafiltration method was used to screen several pooled egg samples (Figure 3). Only FUZ (24-105 µg kg⁻¹) and metabolites were detected in some of these pooled samples, but all concentrations were under the MRL level of 400 µg kg⁻¹ (Figure 4). When these pooled samples were analysed as separate houses, content varied between 46 and 105 µg kg⁻¹ FUZ for sample B and 0-209 µg kg⁻¹ FUZ for sample F and (Figure 5). Subsequent experiments showed that the detected metabolites are formed in the egg and are not an artefact of extraction or MS conditions. A sample consists on average of 49% of FUZ, 34% of FUZ-OH and 17% FUZ-NH₂ (Figure 4). No other benzimidazoles were detected and triclabendazoles were not included in this study.

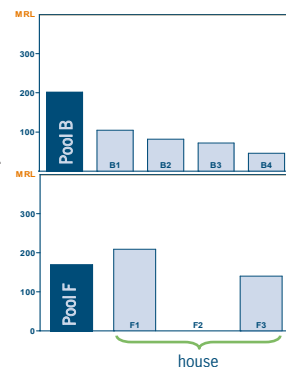


Figure 5. Flubendazole content of individual houses making up pooled samples B and F.



Future

Recently, several new deuterated benzimidazoles have become available. The method will be further enhanced using these deuterated compounds as internal standards and the method will be validated according to the guidelines stated in European Decision 2002/657/EC⁴.

References

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