RIKILT
INSTITUTE OF FOOD SAFETY
WAGENINGEN UR

Multiresidue method for the simultaneous determination of benzimidazoles in bovine milk by on-line SPE-LC-MS/MS

Frédérique L. van Holthoon and Tina Zuidema

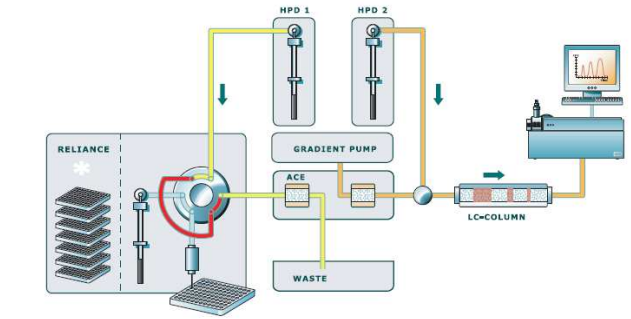

Figure 1. On-line SPE configuration (Symbiosis Pharma™, Spark Holland).

Introduction

Sensitivity, selectivity and specificity of methods for the analysis of residues in animal matrices have greatly increased with the introduction of LC-MS/MS. However matrix effects remain a source of sensitivity loss. Suitable clean-up practice is necessary to lose matrix effects that can cause suppression or enhancement of the MS signal. Techniques like liquid-liquid extraction and SPE are widely used. However both are very laborious and tedious. Additionally, method development is very time-consuming.

Nowadays, on-line SPE-LC-MS/MS has increased interest, especially in pharmaceutical, environmental and clinical applications. It offers many advances over off-line techniques, such as speed, lower cost, automation, and efficiency.

This poster describes the developement of one of the first applications in the residues field.

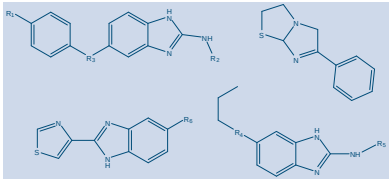

Figure 2. Basic benzimidazole structure. Rest groups can consist of -OH, H, -CO, -COH, -S, -SO, -SO2 and/or -CO_2C

Method

Milk (1 ml) is deproteinated by adding acetonitril (250 µl). After ultrasonication, the sample is centrifuged at 4 °C for 30 min. at 40000 g. The supernatant is removed and transferred to a glass vial. The vials are placed inside the Symbiosis and analysed by SPE-LC-MS/MS (see table 1).

Table 1. on-line SPE program

Step 1:	Move cartridge	Left to other clamp	
Step 2:	Elution	500 µl FA/MeOH (1/100)	100 µl/min
Step 3:	New cartridge	left	
Step 4:	Valve Wash	1000 µl FA/H2O/ACN (1/50/50)	5000 µl/min
Step 5:	Solvation	1000 µl MeOH	5000 µl/min
Step 6:	Equilibration	1000 µl NH_3/H2O/MeOH (1/90/10)	5000 µl/min
Step 7:	Start Autosampler		
Step 8:	Sample Extraction	1000 µl NH_3/H2O/MeOH (1/90/10)	2000 µl/min
Step 9:	Wash Cartridge	1000 µl NH_3/H2O/MeOH (1/90/10)	5000 µl/min
Step 10:	Clamp Flush	500 µl FA/H2O (1/100)	5000 µl/min
Step 11:	Clamp Flush	75 µl FA/MeOH (1/100)	5000 µl/min
Step 12:	Move Cartridge	right to tray	

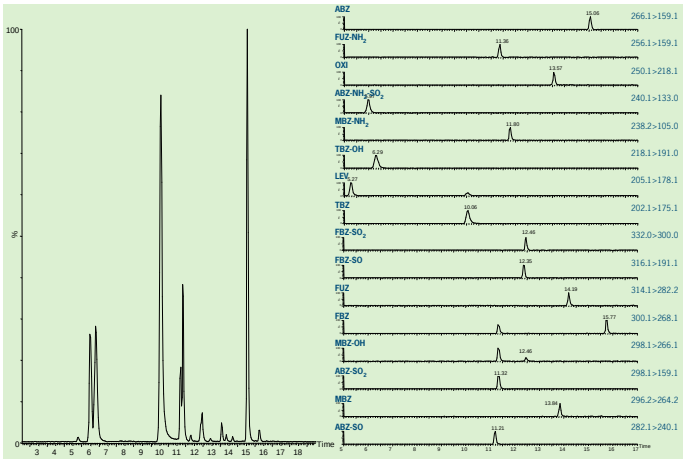

Figure 3. LC-MS/MS chromatogram obtained for a processed milk sample fortified with a mixture of 16 benzimidazoles at 2*MRL level (2-100 µg/kg).
Left panel: TIC, Right panel: selected ion traces.

Table 2. Validation in milk; performance parameters obtained at MRL (n=7) on day 3. RSD_r = intra-day precision; A = accuracy; r^2 = linearity, R = recovery

	MRL	Off-line				On-line			
	µg/kg	RSD_r	A	r^2	R	RSD_r	A	r^2	R
ABZ	100	1.00	106	0.998	33	2.7	106	0.999	38
ABZ-SO	100	7.6	97	1.000	67	2.3	103	0.998	82
ABZ-SO_2	100	7.7	96	1.000	79	2.8	109	0.998	74
ABZ-NH_2-SO_2	100	7.3	92	1.000	69	4.4	107	1.000	83
FBZ	10	12.2	88	0.996	15	3.9	107	0.998	22
FBZ-SO	10	9.0	103	1.000	69	6.8	108	1.000	68
FBZ-SO_2	10	11.2	86	0.998	79	6.7	104	1.000	51
FUZ	2	16.5	98	0.998	63	12.5	93	0.997	53
FUZ-NH_2	2	7.2	98	0.992	59	8.1	112	0.993	79
FUZ-OH	2	6.6	106	0.992	57	10.9	102	0.998	79
MBZ	2	8.5	95	1.000	77	5.0	108	0.996	51
MBZ-OH	2	8.6	97	0.998	85	8.5	103	0.999	59
MBZ-NH_2	2	16.2	90	0.995	78	19	105	0.990	59
TBZ	100	9.3	93	1.000	66	3.8	106	0.998	73
TBZ-OH	100	6.5	96	1.000	75	6.6	89	0.995	44
LEV	2	14.1	100	0.999	65	7.5	113	0.998	67
OXI	2	10.8	100	1.000	58	7.4	104	0.999	44

Results

All major benzimidazoles could be analysed simultaneously with their relevant metabolites using this multiresidue method. The method was validated according to the revised European Union requirements and all parameters were found conform the criteria (European Decision 2002/657/EC and Guideline SANCO/2004/2726rev1).

Simultaneous with on-line validation experiments, an off-line SPE comparison experiment was performed (table 2). Generally, on-line SPE shows less intra-day variation but other performance parameters closely match between both ways of cleaning-up milk extracts.

Conclusions

This method will be applied in the Dutch monitoring program for residues of benzimidazole veterinary drugs in raw milk.

RIKILT – Institute of Food Safety
P.O. Box 230
NL-6700 AE Wageningen, The Netherlands
Phone: +31 317 47 54 22 • Fax: +31 317 41 77 17
Internet: www.rikilt.wur.nl