Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

Department 5
European Reference Laboratory for Residues of Pharmacologically Active Substances (EURL)

European Commission

Multi laboratory validation study for a confirmatory method for NSAIDs in milk

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Introduction

In consequence of animal treatment, residues of drugs may enter the human food chain. The development of reliable analytical methods is required to ensure human food safety.

The EURL Berlin developed and in-house validated confirmatory method for in total 34 basic and acidic NSAIDs in milk.

The method consists of an enzymatic hydrolysis with β-glucuronidase, a QuEChERS extraction, a clean-up by dispersive SPE, and the measurement by LC-MS/MS.

As next step, the EURL Berlin and QuoData tried to combine the alternative validation approach according to Commission Decision 2002/657/EC with a classical method validation study (interlaboratory comparison). Accordingly a factorial design inter-laboratory study was organised by the EURL in order to promote the harmonisation of official residue control and the application of comprehensively validated methods

Description of the study

Study: in-house method validation + inter-laboratory method validation of NSAIDs in milk around MRL-levels /MRPLs

Participants: 18 laboratories
(11 NRLs, 6 German RFL and 1 lab in a third country)

Test material : - blank milk from 8 different cows
- incurred material containing diclofenac, flunixin hydroxide, methylaminoantipyrine

Study design

Tab. 1: Study design for one of the participants (example) *The factors that might have an influence on the analytical result are systematically varied on 2 levels.*

Run	Sample	Factor-level combinations			
		Texture of milk	Operator	Analysis of final extract	HPLC column
1	P160094	lyo	occasional	directly	batch A
2	P160104	liquid	routine	after 2 - 3 days	batch A
3	P160096	liquid	occasional	after 2 - 3 days	batch B
4	P160185	lyo	routine	directly	batch B
5	P160106	liquid	occasional	directly	batch B
6	P140433	lyo	routine	after 2 - 3 days	batch B
7	P140428	lyo	occasional	after 2 - 3 days	batch A
8	P160109	liquid	routine	directly	batch A

Evaluation

In-house validation: InterVAL (provided by QuoData GmbH)

Inter-laboratory method validation: The statistical analysis is based on a mixed linear model with random and systematic effects (InterVAL based QuoData GmbH internal evaluation)

Tab. 2: Analytes and spike levels *(maximum residue limit (MRL) or recommended concentration (RC) for a selection of the analytes covered by the method.*

NSAID	Limit (µg/kg)	Spike-levels
Diclofenac (DC)	0.1 (MRL)	0.05 – 0.10 – 0.15 – 0.30 – 0.60
Flunixin hydroxide (FLUOH)	40 (MRL)	25 – 50 – 75 – 150 – 300
Ibuprofen (IP)	10 (RC)	5 – 10 – 15 – 30 – 60
Mefenamic acid (MFA)	10 (RC)	5 – 10 – 15 – 30 – 60
Meloxicam (MLX)	15 (MRL)	5 – 10 – 15 – 30 – 60
Methylaminoantipyrine (MAA)	50 (MRL)	25 – 50 – 75 – 150 – 300
Naproxen (NP)	10 (RC)	1 – 2 – 3 – 6 – 12
Oxyphenbutazone (OPB)	5 (RC)	1 – 2 – 3 – 6 – 12
Phenylbutazone (PBZ)	5 (RC)	1 – 2 – 3 – 6 – 12
Salicylic acid (SA)	9 (MRL)	5 – 10 – 15 – 30 – 60
Tolfenamic acid (TFA)	50 (MRL)	25 – 50 – 75 – 150 – 300

Results

The following three figures show the results across laboratories exemplarily for Meloxicam (MLX).

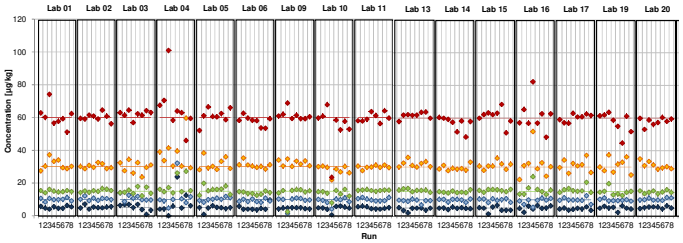

Fig. 1: Visualisation of the laboratory results for all runs and all spike levels (MLX) (spike levels can be distinguished by the following colours: dark blue – 5 µg/kg; light blue – 10 µg/kg; green: 15 µg/kg; orange – 30 µg/kg; red – 60 µg/kg)

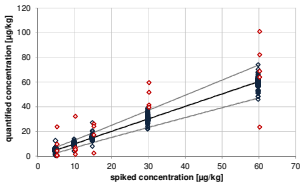

Fig. 2: Overall curve (black line) with 95 % prediction interval (grey lines) and quantified MLX concentrations of all laboratories (diamonds = outliers); 120 measurements per concentration

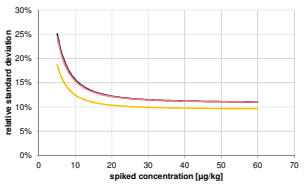

Fig. 3: Relative standard deviations for MLX (black = reprod. s.d.; red = intermediate s.d.; orange = repeat. s.d.)

Tab. 3: Summary of results for all NSAIDs *(critical concentrations and factorial effects)*

NSAID	Level of interest [µg/kg]	Proportion of variance components on the reproducibility standard deviation [%]							Inter-mediate reprod.s.d. [%]	CCα [µg/kg]	
		Repeat-ability	Run	Lab	Operator	Milk	HPLC Analysis	HPLC Column		min	max
DC	0.1	26	46	9	3	13	3	0	33	0.119	0.278
FLUOH	40	45	29	25	0	0	1	0	22	46.9	98.6
IP	5	17	63	17	0	1	0	2	46	6.66	20.1
MAA	50	64	18	11	0	1	1	4	23	55.1	105.6
MFA	5	26	20	0	25	0	21	8	42	6.48	54.3
MLX	15	69	14	2	8	3	2	3	13	16.8	33.4
NP	6	87	3	2	0	1	4	3	20	6.63	10.8
OPB	2	52	24	0	22	1	0	0	36	2.31	8.90
PBZ	2	31	39	1	11	2	5	11	24	2.30	4.82
SA	9	4	66	0	0	16	14	0	102	10.4	55.8
TFA	50	22	44	25	2	3	1	2	20	59.2	130.8

Discussion

The preliminary evaluation confirms principle applicability of this alternative multi laboratory validation approach

- The method itself is proven to provide robust method performance data in a laboratory comprehensive validation study
- Participating laboratories benefit from a complete in-house validation based on their own results
- Main source of error is repeatability, factorial effect play a minor role
- Systematic deviation between the laboratories seems to play a minor role

Further studies are required to investigate whether this approach may be successfully for other compounds and matrices.

Acknowledgment

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