Confirmatory method for the determination of acidic and basic NSAIDs in milk by UPLC-MS/MS

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Introduction

To date, most methods available for non-steroidal anti-inflammatory drugs (NSAIDs) in milk consider either basic or acidic NSAIDs because of their different chemical characteristics. Therefore, two separate sample preparation procedures are applied very often to control the MRLs of NSAIDs in milk or their potential misuse.

QuEChERS is a comprehensive extraction method commonly used in pesticide residue analysis covering compounds with very heterogeneous chemical characteristics. For this reason the application of QuEChERS for the extraction of acidic as well as basic NSAIDs in milk appears promising. In addition, the NSAID analysis should comprise a hydrolysis and a clean-up step. A hydrolysis step potentially releases residues from matrix components[1, 2]; the clean-up step is expected to remove major interferences causing e.g. matrix effects.

➢ Objective

Development and validation of a confirmatory method for the simultaneous determination of in total 31 acidic and basic NSAIDs in milk by UPLC-MS/MS.

Method

Weigh sample
- 2 g of fresh milk or the corresponding amount of lyophilized material

Hydrolysis
- Buffer sample: 200 µL 3,3 M NaOAc buffer (pH 6)
- Add 20 µL β-glucuronidase/ aryl sulfatase
- Incubate in water bath at 37 °C for 1 h

Extraction
- Add 2 mL of ACN
- Add QuEChERS salts: 0.8 g $MgSO_4$, 0.2 g NaCl, 0.1 g Na_2H-Citrate x1.5 H_2O, 0.2 g Na_3-Citrate x 2 H_2O
- 15 min overhead shaker, centrifugation

Clean-up
- 1 mL aliquote of the ACN layer + 250 mg C_{18}
- 5 min overhead shaker, centrifugation

Measurement
- Instrumentation: 1290 Infinity (Agilent) + 6500QTrap (Sciex)
- Column: Waters Aquity UPLC HSS C_{18} (100 x 2.1 mm, 1.8 µm)
- Eluents: (A) H_2O; (B) ACN: H2O 99.5: 0.5 (v/v) both with 5 mM NH_4Ac, 0.05 % AcOH

Validation

The validation study (alternative approach) was conducted in accordance with 2002/657/EC[3]. With the help of the InterVAL Plus software, version 3.4.0.0 (QuoData, Dresden, Germany), the study was designed and evaluated considering changes that may occur during routine analysis. In the course of the validation experiment milk of in total five different cows was used for the spiking experiments and seven factors were systematically varied on two levels in order to demonstrate the ruggedness of the method (Table 1).

Table 1: Description of factors and factor levels

Factor	Factor level (-)	Factor level (+)
Kind of matrix	fresh	lyophilised
Storage of extract	no storage	2-3 days at -20 °C
Removal of SPE material	15 min after extraction	immediately after extraction
Operator	occasional	routine
QuEChERS salt mix	bought	weighed
Sepra C18-E SPE bulk material	batch A	batch B
UPLC column	column A	column B

The concentration levels validated, covered at least a range from 0.5x MRL to 1.5x MRL for authorised drugs. Concentrations as low as possible were validated for substances without MRL. The four target concentration levels validated are displayed in Table 2.

Results and discussion

Most of the 31 analytes are well separated under the chromatographic conditions shown (Figure 1).

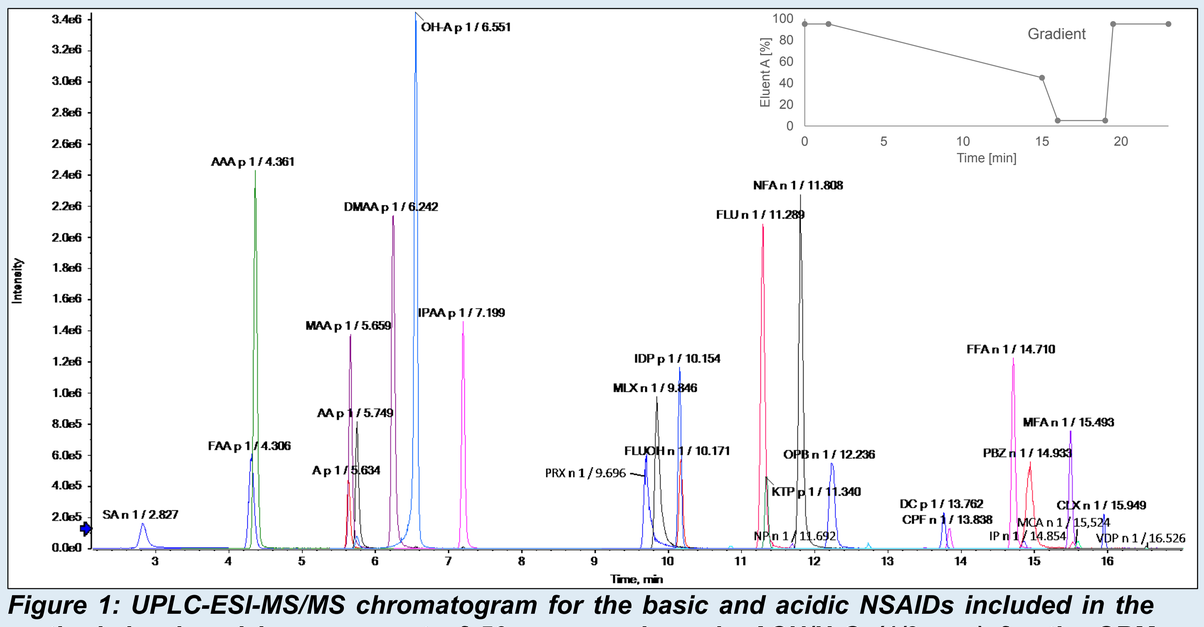

Figure 1: UPLC-ESI-MS/MS chromatogram for the basic and acidic NSAIDs included in the method developed in an amount of 50 pg on column in ACN/H_2O (1/9, v:v) for the SRM transition of the quantifier (n: negative ionisation mode, p: positive ionisation mode).

In accordance with Commission Decision 657/2002/EC the method has been fully validated for 30 NSAIDs in milk. The validation data in Table 2 show that the method is fit for purpose.

Table 2: Validation parameters according to Commission Decision 657/2002/EC calculated with the InterVAL software.

Analyte	Target NSAID mass fractions [µg/kg]	Limit [µg/kg]	CCα [µg/kg]	CCβ [µg/kg]	Recovery at CCα [%]	RSD_r [%]	RSD_{wR} [%]
Antipyrine	1 - 2 - 3 - 4	- -	1.30	1.52	92.1	4.7	8.1
Aminoantipyrine	25 - 50 - 75 - 100	- -	41.25	56.93	95.2	7.7	16.3
Acetylaminoantipyrine	20 - 40 - 60 - 80	- -	23.17	25.61	100.4	2.0	4.8
Celecoxib	5 - 10 - 15 - 20	- -	7.63	10.83	100.4	14.2	16.5
Carprofen	1 - 2 - 3 - 4	- -	1.26	1.48	99.1	7.5	8.6
Diclofenac	0.05 - 0.1 - 0.15 - 0.2	MRL 0.1	0.12	0.14	101.3	7.2	9.0
Dimethylaminoantipyrine	1 - 2 - 3 - 4	- -	1.15	1.27	100.1	1.9	4.8
Formylaminoantipyrine	1 - 2 - 3 - 4	- -	1.33	1.57	107.5	5.4	8.0
Firocoxib	5 - 10 - 15 - 20	- -	6.95	8.44	97.9	8.7	10.7
Flufenamic acid	1 - 2 - 3 - 4	- -	1.42	1.74	100.6	8.2	10.8
Flurbiprofen	7.5 - 15 - 22.5 - 30	- -	10.44	13.49	98.1	9.7	14.7
Flunixin	1 - 2 - 3 - 4	- -	1.18	1.32	101.1	3.8	5.4
Flunixin-hydroxy	20 - 40 - 60 - 80	MRL 40	43.52	47.24	99.3	2.6	4.5
Indoprofen	1 - 2 - 3 - 4	- -	1.35	1.69	112.1	10.7	11.6
Ibuprofen	5 - 10 - 15 - 20	RC 10	6.09	6.91	100.2	5.4	6.4
Isopropylaminoantipyrine	1 - 2 - 3 - 4	- -	1.25	1.45	100.8	4.4	7.5
Ketoprofen	1 - 2 - 3 - 4	- -	1.14	1.25	99.4	2.5	4.6
Methylaminoantipyrine	25 - 50 - 75 - 100	MRL 50	57.64	67.65	92.6	6.7	8.8
Meclofenamic acid	5 - 10 - 15 - 20	-	6.64	8.22	101.7	9.3	10.6
Mefenamic acid	1 - 2 - 3 - 4	RC 10	1.43	1.73	100.7	9.4	10.3
Meloxicam	7.5 - 15 - 22.5 - 30	MRL 15	16.35	17.82	101.2	3.3	4.8
Niflumic acid	5 - 10 - 15 - 20	- -	5.93	6.68	101.8	4.2	5.6
Naproxen	1 - 2 - 3 - 4	RC 10	1.32	1.59	101.1	8.8	9.6
Hydroxyantipyrine	25 - 50 - 75 - 100	- -	-	-	-	-	-
Oxyphenbutazone	1 - 2 - 3 - 4	RC 5	1.19	1.34	101.4	2.9	5.6
Phenylbutazone	1 - 2 - 3 - 4	RC 5	1.24	1.42	97.5	4.3	7.0
Piroxycam	1 - 2 - 3 - 4	- -	1.32	1.55	100.2	6.0	8.3
Rofecoxib	7.5 - 15 - 22.5 - 30	- -	9.91	11.88	100.2	8.0	9.6
Salicylic acid	20 - 40 - 60 - 80	- -	23.14	25.55	100.0	1.9	4.9
Tolfenamic acid	25 - 50 - 75 - 100	MRL 50	59.44	69.47	102.0	6.5	8.7
Vedaprofen	7.5 - 15 - 22.5 - 30	- -	10.00	11.86	101.0	6.3	9.1

MRL: maximum residue limit, RC: recommended concentration, RSD_r: repeatability, RSD_{wR}: within-laboratory reproducibility

The method proved to be rugged against changes with regard to the operator, the extraction procedure, the clean-up procedure, the storage of the final extracts and the application of HPLC columns from different batches.

Conclusion

Taken all together, a LC-MS/MS-based method for the simultaneous analysis of acidic and basic NSAIDs in milk was successfully developed for 31 NSAIDs and fully validated for 30 NSAIDs in accordance with 657/2002/EC. The method comprises a hydrolysis step, an extraction by QuEChERS in combination with dispersive SPE and allows a reliable quantification of the NSAIDs validated.

Acknowledgements

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Literature

[1]Cooper, J. et al. (2001). J Chromatogr B Biomed Sci Appl 757, 221-227. [2]Jedziniak, P. et al. (2013). J. Vet. Pharmacol. Ther. 36, 571-575. [3]Commission Decision 2002/657/EC, Off. J. Eur. Commun. 2002, No L 221/8

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