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[3,4]; 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 all 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 sulftase
- Incubate in water bath at 37 °C for 1 h

**Extraction**
- Add 2 mL of ACN
- Add QuEChERS salts: 0.8 g MgSO4, 0.2 g NaCl, 0.1 g Na2H-Citrate x1.5 H2O, 0.2 g Na2C-Citrate x 2 H2O
- 15 min overhead shaker, centrifugation

**Clean-up**
- 1 mL aliquot of the ACN layer + 250 mg C18
- 5 min overhead shaker, centrifugation

**Measurement**
- Instrumentation: 1290 Infinity (Agilent) + 6500QTrap (Sciex)
- Column: Waters Acquity UPLC HSS C18 (100 x 2.1 mm, 1.8 μm)
- Eluents: (A): 300 - (B): AHC; 95.0 ± 0.5 (v/v) both with 5 mM NH4Ac, 0.05 % AcOH

Validation

The validation study (alternative approach) was conducted in accordance with 2002/657/EC[5]. With the help of the InterVAL Plus software, version 3.4.0 (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 (−) |
| Heated matrix | fresh | lyophilized |
| 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 | Seprac 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 µg on column in ACN/H2O (1/9, v/v) for the SRM transition of the quantifier (n: negative ionisation mode, p: positive ionisation mode).](image)

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 for the simultaneous analysis of acidic and basic NSAIDs in milk by UPLC-MS/MS.](image)

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**Concluding remarks**

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.

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**Literature**


**EURL for Residues of Pharmacologically Active Substances**

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