Method development of prohibited veterinary drugs in milk

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Background

The European Union established regulations to control and enforce veterinary drug use in food-producing animals (e.g. milk-producing bovine and caprine) due to the potential public health risk. Chloramphenicol (CAP), nitrofuran metabolites 3-amino-2-oxazolidinone (AOZ), 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ), semicarbazide (SEM), 1-aminohydantoin (AHD) and 3,5-dinitrosalicylic acid hydrazide (DNSH), three nitroimidazoles dimetridazole (DMZ), metronidazole (MNZ) and ronidazole (RNZ), chlorpromazine (CPZ), dapsone (DAP) and colchicine (COL) are prohibited veterinary drugs according to commission regulation (EU) no.37/2010 (Table 2). In this study, these substances, and 5 additional nitroimidazole(s) (metabolites); hydroxy metronidazole (MNZ-OH), 2-hydroxymethyl 1-methyl-5-nitroimidazole (DMZ-OH), ipronidazole (IPZ), hydroxy-ipronidazole (IPZ-OH) and ternidazole (TNZ) are referred to as prohibited pharmacologically active substances (PPAS).The analysis of PPAS requires highly sensitive, selective and accurate methods.

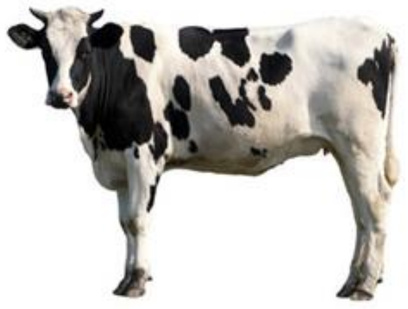
Objective

Bongers et al. (2021) have successfully combined five different classes of PPAS into one single method for milk. The objective of this study is to improve the efficiency of the method, expand the existing method by adding a sixth compound class (colchicine) of PPAS and optimized to lower levels of nitroimidazoles from 3.0 µg kg^{-1} to 1.0 µg kg^{-1} .

Method

500 ± 0.05 mg of homogenised raw bovine/caprine milk

20 µL internal standard solution (15-250 µg/L)



Chemical hydrolysis and derivatisation of the nitrofurans

Overnight at 37°C using 500 µL 0.5M HCl and 12.5 µL 100mM 2-nitrobenzaldehyde solution.

After cooling, 125 µL 0.3M trisodium phosphate solution and 75 µL 2M sodium hydroxide solution was added to adjust pH to 7.0 ± 1.0.

Clean-up

After centrifugation (10 min. at 3500g), the supernatant was applied to a 1 mL supported liquid extraction (SLE) column.

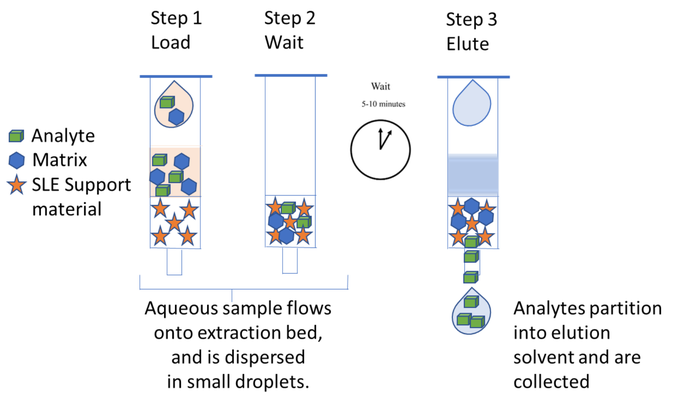


Figure 1: Principle of SLE extraction

Reconstitution

The sample was eluted into a 12 mL glass tube with 2 times 2.5 mL ethyl acetate. After evaporation under nitrogen at 40°C it was reconstituted in 100 µL methanol.

Results

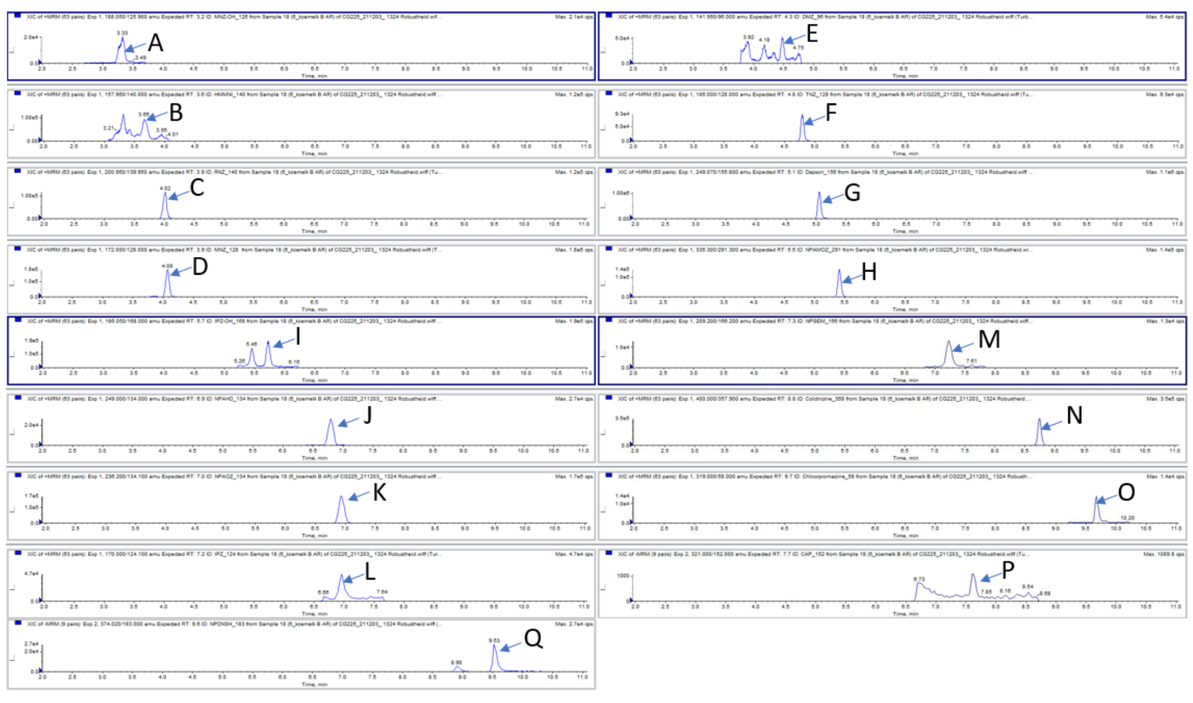


Figure 2. chromatogram of all compounds. A: MNZ-OH 126 m/z, B: DMZ-OH 140 m/z, C: RNZ 140 m/z, D: MNZ 128 m/z, E: DMZ 96 m/z, F: TNZ 128 m/z, G: DAP 156 m/z, H: NPAMOZ 291 m/z, I: IPZ-OH 168 m/z, J: NPAHD 134 m/z, K: NPAOZ 134 m/z, L: IPZ 124 m/z, M: NPSEM 166 m/z, N: COL 358 m/z, O: CPZ 58 m/z, P: CAP 152 m/z, Q: NPDNSH 183 m/z.

Conclusions

This multi-class method was optimized and expanded, whereby a sixth compound class (colchicine) was added. The sample preparation has been accelerated and simplified by the use of a supported liquid extraction (SLE). The improved method consists of an acid hydrolysis combined with a derivatization step, followed by SLE and UHPLC-MS/MS analysis.

The method was developed for bovine and caprine milk with target values of 0.1 µg kg^{-1} for chloramphenicol, 0.5 µg kg^{-1} for the nitrofurans, 1.0 µg kg^{-1} for the nitroimidazoles, 2.0 µg kg^{-1} for chlorpromazine, 5.0 µg kg^{-1} for dapsone and 6.0 µg kg^{-1} for colchicine. This improved method demonstrated that six different compound classes were successfully combined into single multi-class method at low levels.

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