Efficiency improvement in the analyses for the Dutch national monitoring plan

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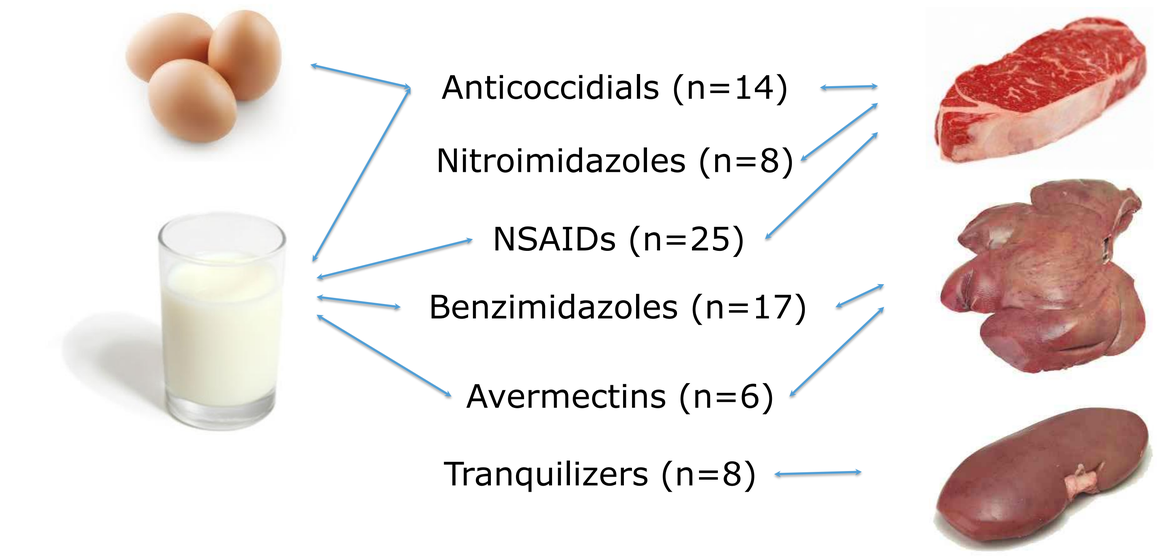
Background

Within the Dutch national monitoring plan a large variety of analytes is monitored in several matrices. Conventionally most antibiotics are screened using bioassays and for other analytes methods are operational for each individual analyte group. Especially the latter yields a very cost inefficient workflow. Combining these methods into a single workflow significantly increases sample throughput and is therefore more cost efficient.

Objective

- The development of a single workflow for the analysis of avermectins, anticoccidials, benzimidazoles, nitroimidazoles, NSAIDs and tranquilizers in egg, liver, kidney, milk and/or muscle.



Sample preparation

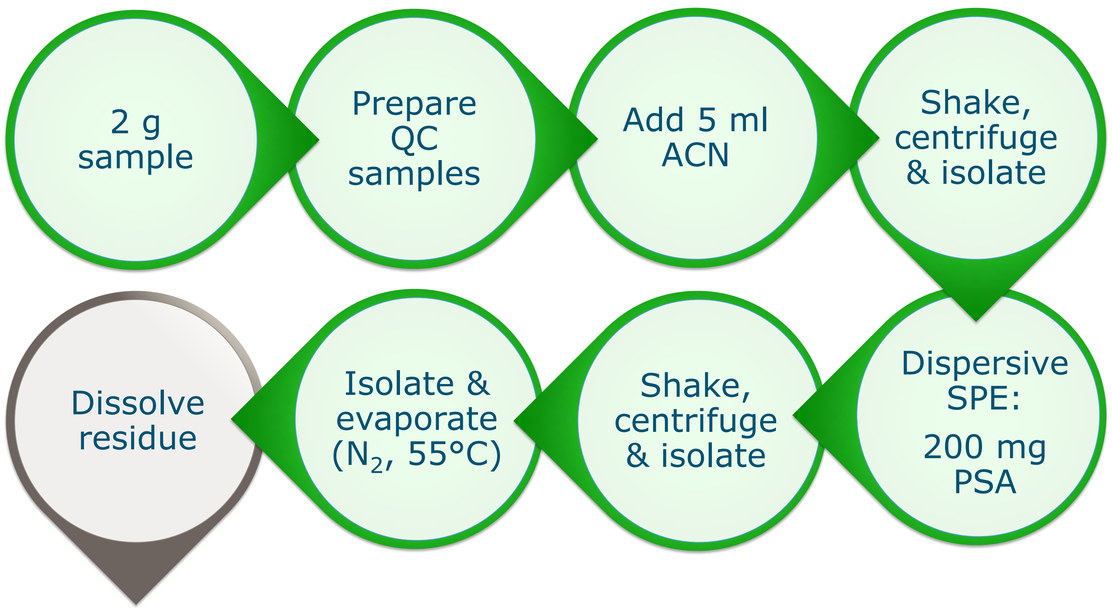


Table 1. Composition of the final extract and injection volume per analyte/matrix combination

Analyte group	Solvent, volume	Injection volume
Anticoccidials	MeOH, 200 µL	20 µL
Nitroimidazoles	H_2O, 200 µL	10 µL
NSAIDs	MeOH, 800 µL	5 µL
Benzimidazoles	MeOH, 200 µL	4 µL
Avermectine, liver	Liver: MeOH, 200 µL	4 µL
Avermectine, milk	H_2O/MeOH (1:1 v/v), 200 µL	10 µL
Tranquilizers	MeOH, 200 µL	4 µL

Discussion

- Acetone resulted in higher extraction recoveries compared to acetonitrile. However, matrix effects were more pronounced compromising quantitative performance and detection limits.
- Matrix effect were reduced by using dSPE with primary secondary amine (PSA). C_{18} was less effective in cleaning the raw extract.
- The balance between the composition of the final extract, dilution of the final extract and the injection volume proofed to be critical (table 1 for optimal results).

Validation results

Table 2. Summary of validation results according to CD 2002/657/EC

Analyte group, matrix		LoD (µg/kg)	Trueness (%)	RSD_{RL} (%)
Anticoccidials, muscle	Qualitative	≤ 0.5*MRL		
Anticoccidials, milk	Qualitative	≤ 0.5*MRL		
Anticoccidials, egg	Qualitative	≤ 0.5*MRL		
Nitroimidazoles, muscle	Quantitative	≤ 1.0	83 – 105	2.7 – 17
NSAIDs, muscle	Quantitative	≤ 0.5*MRL	99 - 104	2.6 – 18
NSAIDs, milk	Quantitative	≤ 0.5*MRL*	87 - 105	2.9 - 22
Benzimidazoles, liver	Quantitative	< 0.5*MRL		
Benzimidazoles, milk	Quantitative	< 0.5*MRL	92 - 104	2.9 – 16
Avermectine, liver	Qualitative	7.5 – 10	94 - 104	4.1 - 18
Avermectine, milk	Qualitative	0.1 - 10		
Tranquilizers, kidney	Quantitative	< 0.5*MRL	97 – 102	3.8 – 14

* MRL of diclofenac in milk (0.1 µg/kg) only achievable using AB-Sciex Qtrap 6500 mass spectrometer

Instrumentation

- LC column: Acquity UPLC HSS-T3, 1.8 µm, 100 x 2.1 mm
- Gradient elution
- Mobile phase A: NH_4COOH (1M)/HCOOH/H_2O (2:0,16:1000 v/v/v)
- Mobile phase B: NH_4COOH (1M)/HCOOH/MeOH (2:0,16:1000 v/v/v)
- Flow: 0.4 ml min^{-1}
- Mass spectrometer: Waters Quattro Premier, SRM
- Ionisation: Electrospray

Conclusions

- A single workflow was developed for the analysis of anticoccidials, nitroimidazoles, NSAIDs, benzimidazoles, avermectins and tranquilizers in various matrices.
- The method consists of a solvent extraction combined with dSPE and is applicable for qualitative analysis of anticoccidials and avermectins and for quantitative analysis of most nitroimidazoles, NSAIDs, benzimidazoles and tranquilizers included.
- All methods are performed using the same procedure, chemicals and LC-MS/MS system, increasing sample throughput and flexibility.
- The developed workflow resulted in significant improvement in laboratory efficiency and reduction of costs in carrying out the Dutch national monitoring plan.

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