Screening method for the identification of banned compounds in urine

Robin S Wegh, Stanislava Vonsovic, Tina Zuidema

Introduction

After the positive findings of banned antibiotics in 2013 and 2014 in The Netherlands the need for a screening method that can determine all banned veterinary drugs mentioned in Table 2 of EC 37/2010 became urgent. Within the Dutch National Residue Control Plan a large variety of compounds is already monitored in several matrices including the banned compounds. However, in most cases methods are focussing on one compound (i.e. chloramphenicol) or a group of compounds (i.e. nitrofurans) in tissues, milk and eggs. To determine in an early stage whether a banned compound has been used, monitoring and investigating non-invasive materials is necessary. The research presented focusses on the development of a monitoring method for the screening of those banned compounds in urine.

Objective

The objective is to develop a monitoring method for the screening of banned compounds: chloramphenicol, chlorpromazine, colchicine, dapson, dimetridazole, metronidazole, ronidazole, furazolidone, furaltadone, nitrofurantoin and nitrofurazon (see figure 1) in urine.

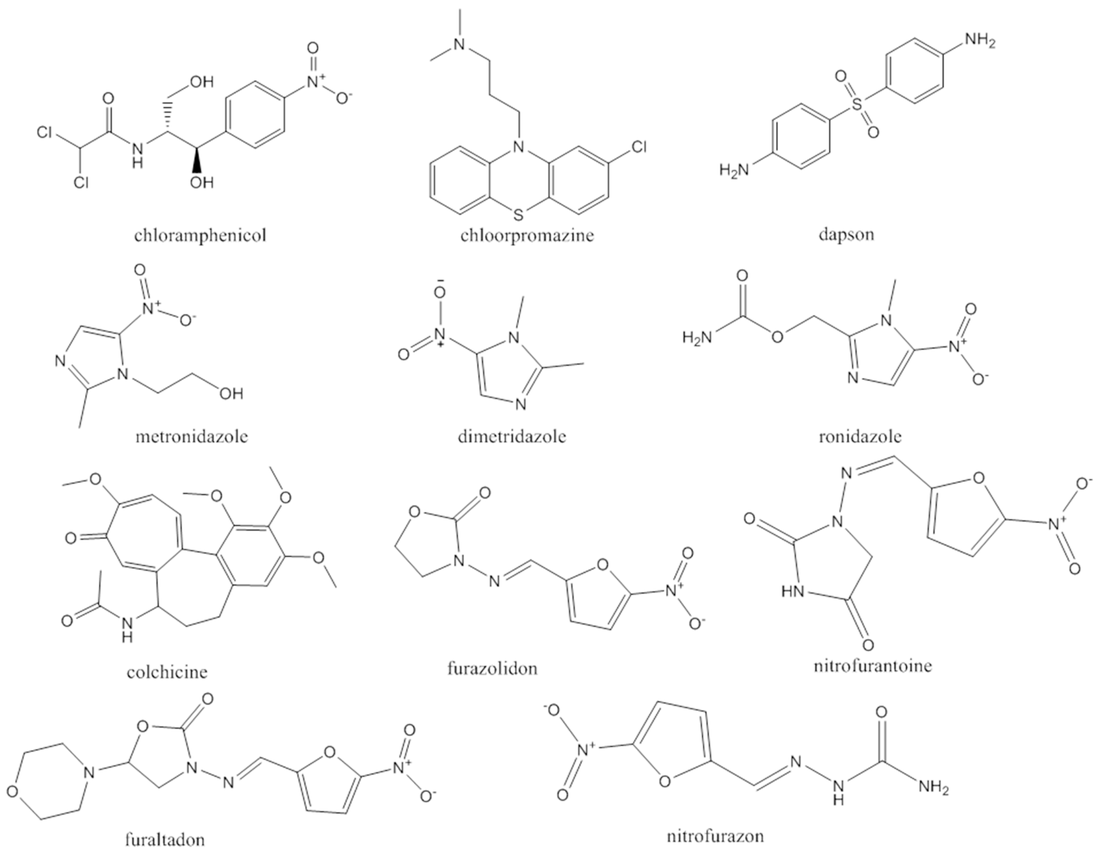

Figure 1. Molecular structures of the selected compounds (Table 2 of EC 37/2010)

Method

Enzymatic hydrolysis

Urine  pH setting + β-glucuronidase/arylsulfatase

Chemical hydrolysis and derivatization

Overnight at 37°C using HCl/NBA (2-nitrobenzaldehyde)

Clean-up

Clean-up using ACN and a QUECHERS kit followed by dispersive SPE

LC-MS/MS analysis

Analytical column: Acquity BEH C18 column (2.1 x 100mm, 1.7µm)
Detection was carried out using the AB Sciex Q-trap 6500.

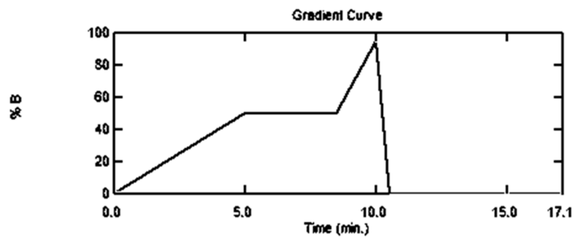

Figure 2. Gradient solvent A, 1 M ammoniumformate and 0.16% formic acid; solvent B, 1 M ammoniumformate and 0.16% formic acid in MeOH

Results

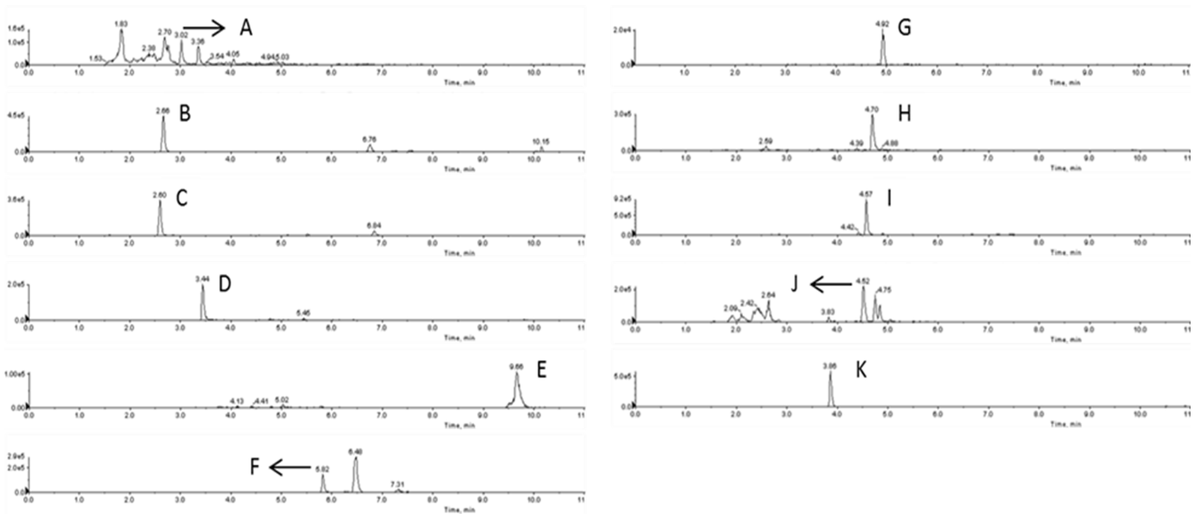

Figure 3. chromatogram of all compounds. A: dimetridazole 95.8 m/z, B: metronidazole 127.9 m/z, C: ronidazole 139.9 m/z, D: dapson 155,8 m/z, E: chlorpromazine 58.0 m/z, F: colchicine 357.9 m/z, G: chloramphenicol 152.0 m/z, H: NPSEM 166.2 m/z, I: NPAOZ 134.1 m/z, J: NPAHD 134.0 m/z, K: NPAMOZ 291.3 m/z.

During method development it was observed that it is possible to combine the enzymatic and chemical hydrolysis in one method. The enzymatic hydrolysis has to be performed prior to the chemical hydrolysis because of the stability of the used enzymes.

Conclusions

A very effective analytical procedure for qualitative analysis of the banned compounds mentioned in Table 2 of EC 37/2010 in urine was developed. The method is able to detect chloramphenicol at a level of 0.15 µg/l and for chlorpromazine, colchicine, dapson, dimetridazole, metronidazole, ronidazole, furazolidone, furaltadone, nitrofurantoin and nitrofurazon at 0,5 µg/l. At the moment the developed method is validated according Commission Decision 2002/657/EC.

Acknowledgements

This research was funded by the Dutch Ministry of Economic Affairs under its statutory tasks.

RIKILT Wageningen UR
P.O. Box 230, 6700 AA Wageningen
Contact: Robin.Wegh@wur.nl
www.wageningenUR.nl/en/rikilt

References

[1] 2002/657/EC,in:Off.J.Europ.Commun., 2002, pp.8–36.
[2] COMMISSION REGULATION (EU) No 37/2010, Table 2.
[3] Berendsen BJA, Essers ML, Stolker AAM, Nielen MWF, J. of Chrom. A. 2011;1218:7331-7340