Screening method for the identification of banned compounds in urine

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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.

Method

Enzymatic hydrolysis

Urine pH setting + β-glucuronidase/aryl sulfatase

1 hour at 50°C

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.

Results

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.

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References