INTRODUCTION

The use of pharmacologically active substances such as hormones, thyrostats, many β-agonists and other drugs, is prohibited in stock farming

Low reporting levels in urine must be analytically achieved to successfully monitor their administration

LC-MS/MS is the methodology of choice for confirmatory purposes but for multi-class and multi-component analysis this approach requires extensive compound-dependent parameter optimization

Full Scan approaches using high resolution mass spectrometry (HRMS) can provide a rapid wide-range screening of veterinary drugs overcoming the limitations of LC-MS/MS analysis

The aim of this study was to develop a UHPLC-HRMS method for the multi-residue screening of around 100 veterinary drugs banned or not regulated in bovine urine

EXPERIMENTAL

System: Autosample: Accela Open AS
UHPLC: Accela 1250 pump
Mobile phase: Eluent A: HAc 0.1%
Eluent B: Acetonitrile 0.1% HAc
Column: Hypersil Gold aQ (100 x 2.1 mm, 1.9 μ)
Elution mode: Gradient
Flow rate: 400 μL/min
Injection volume: 10 μL

Mass conditions

System: Source: HESI II
Analyzer: Orbitrap ExactiTm
Spray voltage: 3 kV
Sheath gas flow rate: 8
 Auxiliary gas flow rate: 0
Skimmer voltage: 38 V
Heat temperature: 30 °C
Capillary temperature: 250 °C
Tube lens voltage: 120 V

Acquisition functions

- Polarity switching: Positive and negative mode
- Pseudo MS/MS to obtain fragments for confirmation (HCD)

RESULTS

About 100 compounds

| β-agonists: | clenbuterol, brombuterol, ractopamine, citalerol, isoxsuprine, tulobuterol, chlorthalidone, omeprazole, ritodrine, terbutaline, metaproterenol, salbutamol, salmeterol, fenoterol, cimaterol, chlorthalbuterol, mabuterol, mapenterol, hydroxymethylceteroterol, clenpenterol, clensipenterol, clenbuterol, formoterol, clenbuterol-d9 and ractopamine-d5 |
| Steroids: | α-nortestosterone, β-nortestosterone, α-boldenone, β-boldenone, α-testosterone, hydroxynortestosterone, α-trembolone, β-trembolone, stanozolol, 16β-hydroxystanozolol, 3β-hydroxystanozolol, 4α-hydroxystanozolol, 4β-hydroxystanozolol, androstenedione, boldione, methyltestosterone, β-estrogesterone-d3, β-boldenone-d3, methyltestosterone-d3, 16β-hydroxystanozolol-d3 and β-trembolone-d3 |
| RAAs: | zearalenone, α-zearalenol, α-zearalenol-d3 and β-zearalenol-d4 |
| Sterilbene: | dihydroxystilbestrol, diestradiol, hexestrol, diethylstilbestrol-d6, dienestrol-d2 and hexestrol-d4 |
| Nitroimidazoles: | dimetridazole, metronidazole, ronidazole, ipronidazole, HMMNI, ipronidazole-OH, metronidazole-OH, dimetridazole-d3, ronidazole-d3 and ipronidazole-d3 |
| Corticosteroids: | dexamethasone, betamethasone, prednisone, methylprednisolone, flumethasone and dexamethasone-d4 |
| AINES: | phenylbutazone, oxyzephenylbutazone, naproxen, mefenamic acid and phenylbutazone-d10 |
| Thyrostats: | phenylthiouracil, mercaptoenbolimidazole, propylthiouracil, thiouracil, tapazol, methylthiouracil |
| Sedatives: | chlorpromazine, propionylpromazine, acarpromazine, carazolol, azaperone, azaperol, atenolol, haloperidol, ylazine, haloperidol metabolite II and chlorpromazine-d6 and atenolol-d7 |
| Anphencilos: | chloramphenicol, thiampenicol, florgenicol, forphenicol amino, chloramphenicol-d4 |
| Others: | dapsone, monacetyldapsone |

Database

Characterization of compounds

Elemental composition

Theoretical accurate mass (5 decimals)

Polarity (+/-)

Adducts (H+, CHICOO)

Expected Retention time (min)

Fragments

CONCLUSIONS

The developed method is simple, fast and wide-range for the screening of veterinary drugs banned or not regulated in bovine urine.

The performance characteristics have been determined according to the EU criteria for screening methods (2002/65/EC) and the guideline of the CRLs (2010). The validation was achieved analyzing 20 bovine urine blanks from different animals spiked at CCB levels.

In all the cases CCB levels were equal or lower than action limits or recommended by EU laboratories.

From our point of view, the use of high-resolution liquid chromatography combined with high resolution mass spectrometry is a powerful and reliable tool for identification and confirmation in multi-residue analysis. The information obtained from the combination of exact mass at high resolution (R ≥ 20000), isotopic pattern and fragments is highly specific, and should be taking into account in future for the establishment of new confirmation criteria in revision of the Decision 2002/65/EC, according to these new technologies.

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