FAST, WIDE-RANGE SCREENING OF BANNED OR NOT REGULATED VETERINARY DRUGS IN URINE BY LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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INTRODUCTION

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

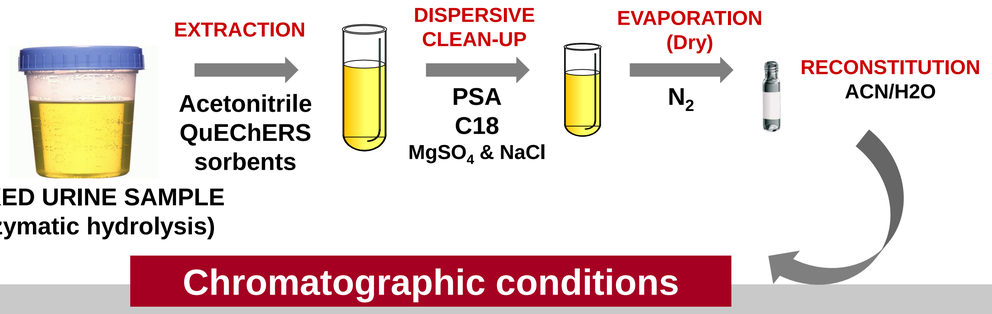
✓ **Low reporting levels in urine must be analytically achieved to successfully monitor their administration**

✓ **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



Chromatographic conditions

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

Inyection volume: 10 µL

Time (min)	0	5	8	8.5	10	10.5*
% A	100	50	50	5	5	100
% B	0	50	50	95	95	0

* Equilibration time: 3min

Mass conditions

System: Source: HESI II

Analyzer: Orbitrap Exactive™

Spray voltage: 3 kV
Sheath gas flow rate: 8
Auxiliary gas flow rate: 0
Skimmer voltage: 38 V
Heater temperature: 30 ºC
Capillary temperature: 250 ºC
Tube lens voltage: 120 V

Automatic gain control : 10^6(Balanced)

Resolving power (FWHM): 50.000 (High)

Mass range (m/z): 150-2000

➔ **Acquisition functions**

✓ Polarity switching: Positive and negative mode

✓ Pseudo MS/MS to obtain fragments for confirmation (HCD)

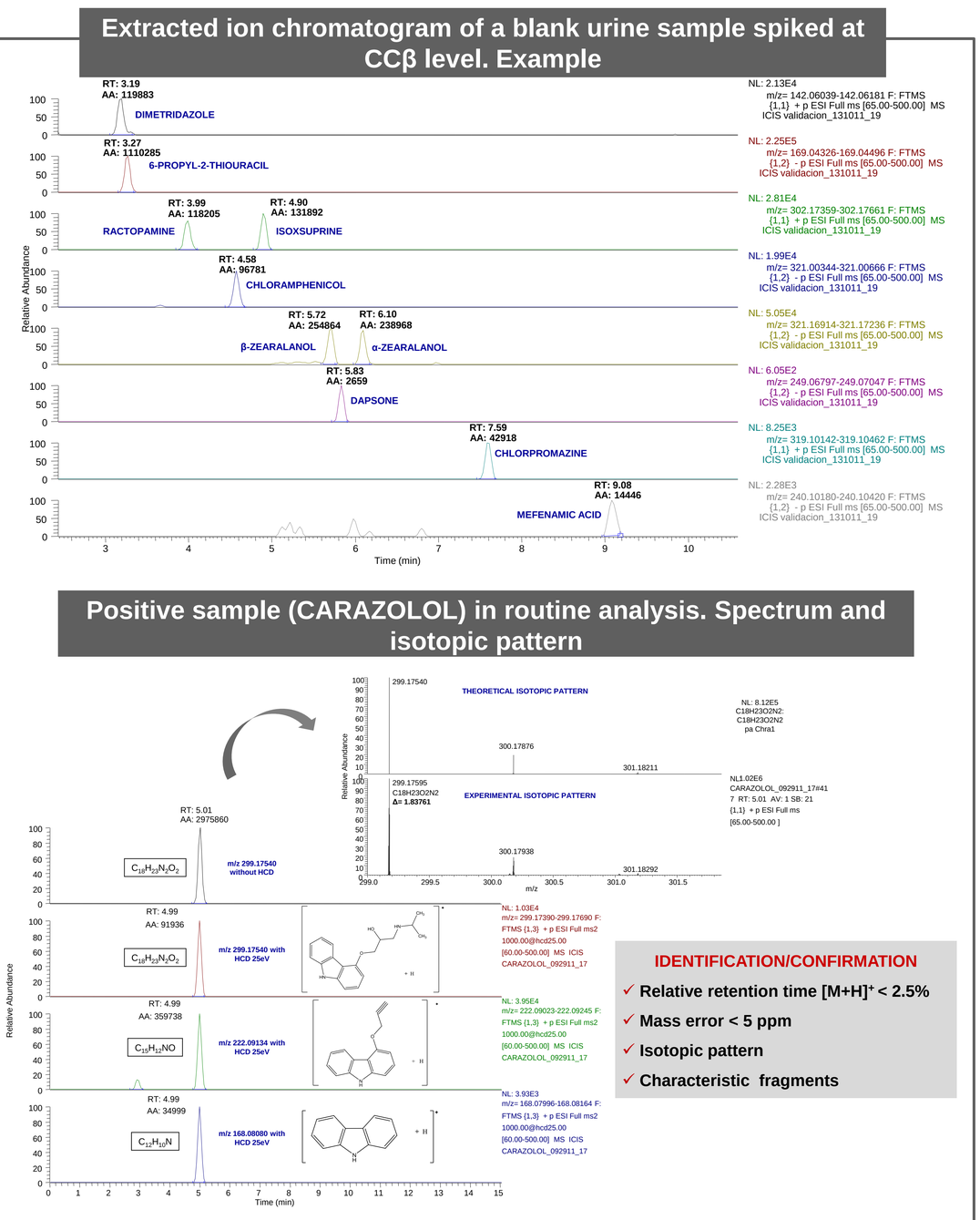
RESULTS ➡ About 100 compounds

- **β-agonists:** clenbuterol, brombuterol, ractopamine, zilpaterol, isoxuprine, tulobuterol, chorprenaline, cimaterol, ritodrine, terbutaline, metaproterenol, salbutamol, salmeterol, fenoterol, cimbuterol, chrolbrombuterol, mabuterol, mapenterol, hydroxymethylclenbuterol, clenpenterol, clenisopenterol, clencyclohexerol, clenhexerol, formoterol, clenbuterol-d9 and ractopamine-d5
- **Steroids:** α-nortestosterone, β-nortestosterone, α-boldenone, β-boldenone , α-testosterone, hydroxystanozolol, α-trenbolone, β-trenbolone, stanozolol, 16β-hydroxystanozolol, 3'-hydroxystanozolol, 4α-hydroxystanozolol, 4β-hydroxystanozolol, androstenedione, boldione, methyltestosterone, β-nortestosterone-d3, β-boldenone-d3, methyltestosterone-d3, 16β-hydroxystanozolol-d3 and β-trenbolone-d3
- **RALs:** zearalanone, α-zearalenol, β-zearalenol, α-zearalanol, β-zearalanol, zearalenone, α-zearalanol-d4 and β-zearalanol-d4
- **Stilbenes:** diethylstilbestrol, dienestrol, 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, prednisolone, methylprednisolone, flumethasone and dexamethasone-d4
- **AINES**: phenylbutazone, oxyphenylbutazone, naproxen, mefenamic acid and phenylbutazone-d10.
- **Thyrostats**: phenylthiouracil, mercaptobenzimidazole, propylthiouracil, thiouracil, tapazol, methylthiouracil
- **Sedatives**: chlorpromazine, propionylpromazine, acepromazine, carazolol, azaperone, azaperol, atenolol, haloperidol, xylazine, haloperidol metabolite II and chlorpromazine-d6 and atenolol-d7
- **Anphenicols:** chloramphenicol, thianphenicol, florphenicol, florphenicol amine, chloramphenicol-d4
- **Others**:, dapsone, monoacethyldapsone…

✓ **Database** ➡ **Characterization of compounds**

Elemental composition	Theoretical accurate mass (5 decimals)
Polarity (+/-)	Expected Retention time (min)
Adducts (H^+, $CH3COO^-$)	Fragments

Extracted ion chromatogram of a blank urine sample spiked at CCβ level. Example



Positive sample (CARAZOLOL) in routine analysis. Spectrum and isotopic pattern

IDENTIFICATION/CONFIRMATION

✓ **Relative retention time $[M+H]^+$ < 2.5%**

✓ **Mass error < 5 ppm**

✓ **Isotopic pattern**

✓ **Characteristic 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 (2002/657/EC) and the guideline of the CRLs (2010). The validation was achieved analyzing 20 bovine urine blanks from different animals spiked at CCβ levels.**

➔ **In all the cases CCβ 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 2002/657/EC, according to these new technologies.**

Acknowledgements. Thermo SCIENTIFIC