MSACL 2019

Poster number: #2c

Detection of F-2 Mycotoxin Zearalenone in Human Urine. Consumption of Contaminated Cereal Crops or Doping Offence?

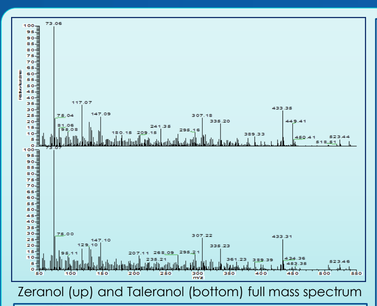
George-Mădălin Dănilă, Alice Madalina Pop, Cristina Stan, Ani Toboc
Romanian Doping Control Laboratory, Bucharest, Romania

INTRODUCTION

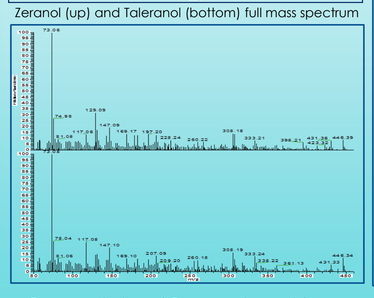
Zearalenone or F-2 mycotoxin is a heat-stable, potent estrogenic metabolic product of some *Fusarium* and *Gibberella* species, found in cereal crops like maize, oats, wheat, rice and barley [1]. Due to its similarity to naturally-occurring estrogens (17β-estradiol), zearalenone and its main metabolites (a-zearalenol, β-zearalenol, zeranol and taleranol) are considered by World Health Organization and European Commission as endocrine-disrupting chemicals and a possible cause of carcinogenesis [2]. In the 80's, Zeranol was used to rapidly grow animals for human consumption[3].
On the other hand, the use of Perfomance Enhancing Drugs (PEDs) is considered both dangerous for human health and unethical, and due to its anabolic effects, zeranol and taleranol are banned in sport and the presence of this metabolites must be clearly attributed to illegal use or unintended contamination [4].
The main objective of our laboratory was to develop a detection method for anabolic steroids zeranol / taleranol including several ion transitions to detect the presence of mycotoxin Zearalenone and its metabolites (a-zearalenol and β-zearalenol), in order to differentiate between an abuse of steroids and mycotoxin contamination .

METHOD DEVELOPMENT

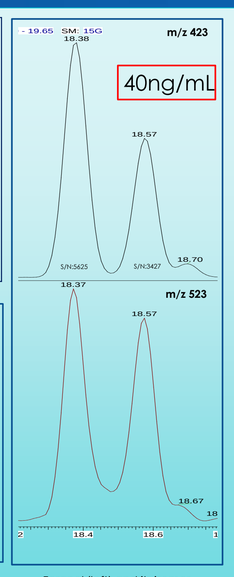
Single quadrupole MS → Triple quadrupole MS (evolution)



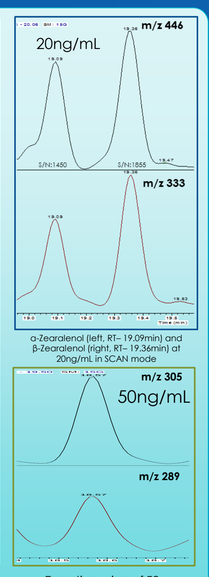
Zeranol (up) and Taleranol (bottom) full mass spectrum



α-Zearalenol (up) and β-Zearalenol (bottom) full mass spectrum



Zeranol (left) and its isomer Taleranol (right) at 40ng/mL in SCAN mode



α-Zearalenol (left, RT– 19.09min) and β-Zearalenol (right, RT– 19.36min) at 20ng/mL in SCAN mode

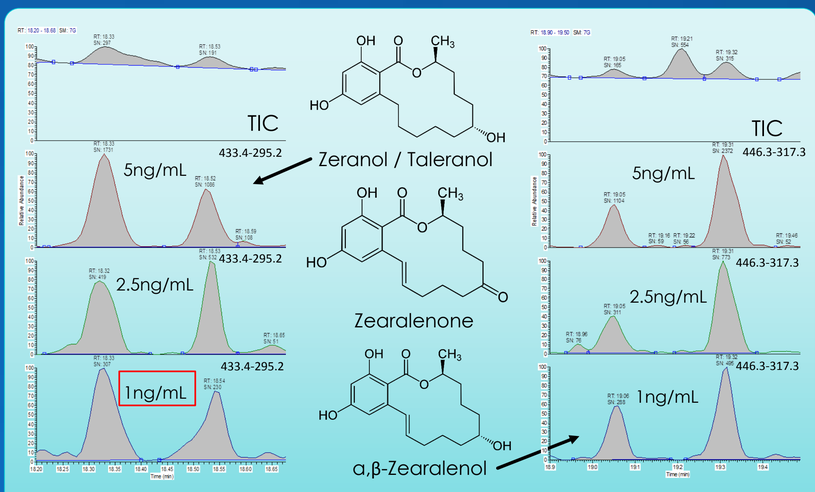
Excretion urine of F2-mycotoxin in SCAN mode, at ~50ng/mL

Extraction protocol

- 2mL of urine
- Enzimatic hydrolysis
- LLE extraction
- Derivatization (MSTFA/NH4I/ Ethanethiol)
- 2μL on GC/MS/MS

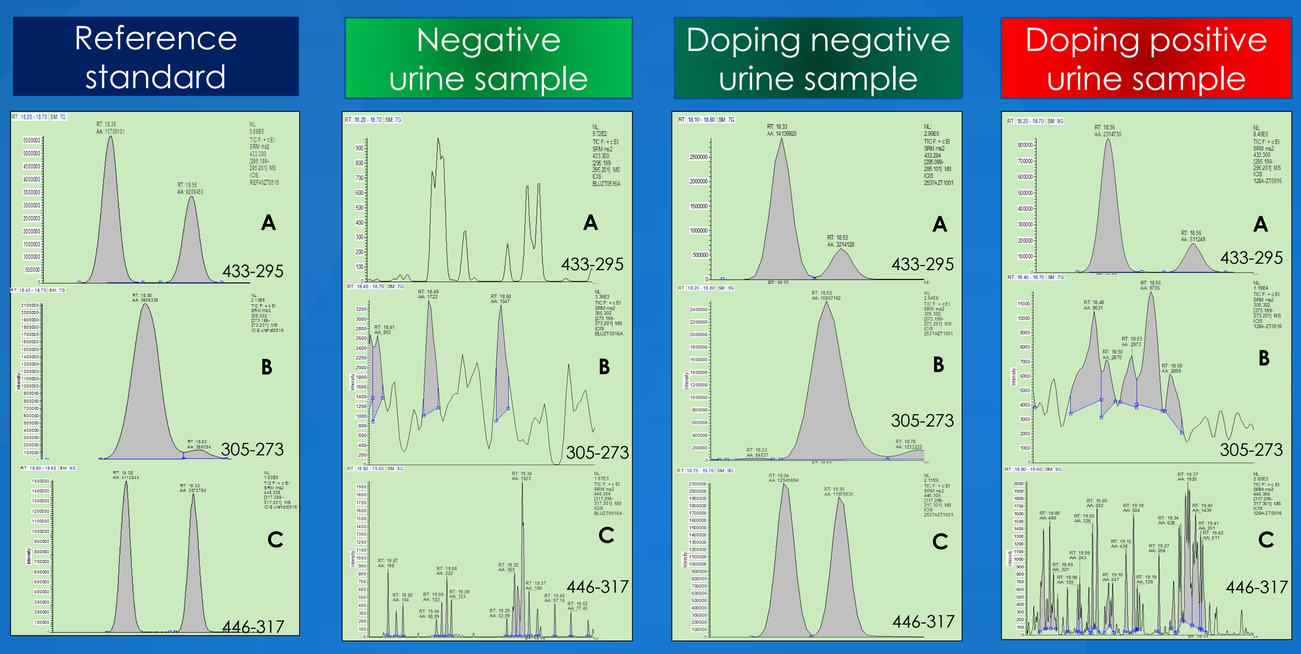
The method was developed for gas chromatography –tandem mass spectrometry (GC-MS/MS) GC Trace 1310 connected to a TSQ Quantum XLS Ultra from Thermo Scientific. The GC was equipped with an HP-Ultra 1 (17m x 200μm and 0.11μm film thickness) from J&W Scientific from Agilent Technologies (USA).
The temperature program was as follows: the initial temperature was 160°C hold time 2 min, increased by 5°C/min to 255°C and then by 30°C/min to 285°C (hold time 5 min) and finally by 60°C/min to a final temperature of 300°C (held 3.75 min). The transfer line temperature was set at 310°C. Helium was used as carrier gas (constant flow rate 1.03mL/min). A volume of 2μL of derivatized sample was injected in split mode (1:10) and compounds were detected after electron ionization (EI) at 40eV.

Compound	Single MS ions	MS/MS transitions (eV)
Zeranol / Taleranol	538 (M+3TMS), 523, 449, 433	433.4-295.2 (21), 433.4-389.3 (9), 433.4-309.3 (7)
Zearalenone	462(M+2TMS), 429, 305, 260	305.3-273.2 (20), 305.3 – 289.2 (20)
α-Zearalenol / β-zearalenol	446(M+2TMS), 431, 333, 305	446.3-317.3 (10), 446.3-333.3 (10)



Using a GC-MS/MS system, low concentrations of zeranol and taleranol can be detected and confirmed. This is the case also for the mycotoxin metabolites. Here, we analyzed 3 urine samples spiked with 5, 2.5 and 1ng/mL, respectively, of zeranol and taleranol, and α- and β-zearalenol. Even at 1ng/mL (LOD), the peaks are still present at a S/N ratio above 200 for all the compounds. This detection capability is due to the fact that when using QQQ systems, we increase method sensitivity by optimizing a specific fragmentation from a precursor to a product ion.

CASE STUDY



Monitored ion transitions (MRM) mode for A - Zeranol/Taleranol, B - Zearalenone PC, C - α/β - zearalenol, in a spiked negative urine sample, a negative urine sample (neither steroids, nor mycotoxin detected), an negative urine sample according to WADA documents and a positive urine sample for Zeranol / Taleranol steroids.

Our validated method for the detection of zeranol/taleranol in conjuction with zearalenone and its metabolites was used in a case study with a real, mycotoxin contaminated urine sample.

In the case of a presumptive analytical finding (PAAF) for Zeranol /Taleranol, we established in our confirmation procedure that together with the suspicious sample, it will be analyzed a spiked negative urine with all reference standards: the anabolic steroids Zeranol/Taleranol, and Zearalenone and its metabolites (a-zearalenol and β-zearalenol).

From a doping control point of view, we can distinguish two situations when a urine sample is reported negative:

i) Neither Zeranol/Taleranol, nor Zearalenone and/or its metabolites are present;

ii) Zeranol/Taleranol present, but also Zearalenone and /or its metabolites in concentrations slighlty higher than the anabolic steroids. The metabolic path of zeranol does not produce important quantities of Zearalenone and its metabolites . After the ingestion of contamination cereals with F2-mycotoxin only small concentrations of steroids Zeranol/Taleranol are observed [5]. In our case study (doping negative urine sample), Zeranol concentration was 11ng/mL, Zearalenone concentration was 55ng/mL, and its metabolites: 43ng/mL for a-zearalenol and 10ng/mL for b-zearalenol.

A positive analytical finding for Zeranol is considered when only Zeranol/Taleranol are present in the sample. From our laboratory's experience, all our Zeranol/Taleranol findings were related with consumption of contaminated cereal crops.

CONCLUSIONS

For doping control purposes, the detection of zeranol/taleranol, in conjunction with zearalenone and/or its metabolites a,β-zearalenol is considered a negative finding because the presence of steroids zeranol and taleranol can be attributed to the contamination of food with fungal mycotoxin Zearalenone.
In order to differentiate between an abuse of steroids and mycotoxin contamination, we developed and validated a sensitive method to detect low concentrations of steroids (LOD – 1ng/mL), including the evaluation of metabolic pattern of Zearalenone[5].
Using the GC-MS/MS system our laboratory is able to detect very low concentrations of steroids (LOD - 1ng/mL) compared to a classic single-quad MS (LOD is 40 times lower).
In the case of an abuse of this steroids, the metabolic pattern changes considerably and is in accord with the literature describing the *in vivo* metabolism of zeranol / taleranol [6]. The occurrence of this mycotoxin contamination is a worldwide phenomenon and it is found particularly in wheat, barley and corn – cereals that can be part of an athlete meal.
Nevertheless, when zeranol / taleranol is detected in doping control samples, the need to differentiate between an abuse of steroids and mycotoxin contamination becomes important and must be done accordingly.

REFERENCES:

[1] T. Tanaka, et al., Worldwide Contamination of Cereals by the Fusarium Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone. 1. Survey of 19 Countries, J. of Agric. Food Chem., American Chemical Society, 1988, 36 (5), 979;
[2] European Commission 2007, Commission regulations (EC) No. 1126/2007 on maximum levels of certain contaminants in foodstuff as regards as regards Fusarium toxins in maize and maize products, Off. J. Eur. Union, 14-17;
[3] R.S. baldwin, et al., Zearnol: Areview of metabolism, toxicology, and analytical methods for detection of tissue residues, Reaul. Toxicol. Pharm., 1983, 3, 9;
[4] World Anti-doping Agency Prohibited List 2018, https://www.wada-ama.org/sites/default/files/wada_2019_english_prohibited_list.pdf;
[5] M. Thevis, et al., Zeranol:doping offence or mycotoxin? A case-related study.,Drug Test.Analysis, 2011, 3, 777;
[6] M. Kleinova, et al., Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers., J. Agr. Food Chem. 2002, 50, 4769;