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High resolution orbitrap mass spectrometry in comparison with tandem mass spectrometry for confirmation of anabolic steroids in meat

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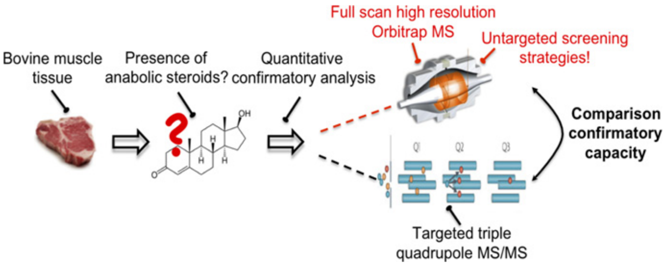
HIGHLIGHTS

- ▶ HRMS has great potential in residue analysis of steroids in animal derived matrices.
- ▶ HRMS enables untargeted screening strategies for new, initially unknown steroids.
- ▶ Good linearity, precision and selectivity were observed upon HRMS analysis.
- ▶ Sensitivity of HR-Orbitrap-MS was generally lower compared to QqQ method.

GRAPHICAL ABSTRACT



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ABSTRACT

A prominent trend which has been observed in recent years in the analysis of veterinary drugs and growth-promoting agents is the shift from target-oriented procedures, mainly based on liquid chromatography coupled to triple-quadrupole mass spectrometry (LC-QqQ-MS), towards accurate mass full scan MS (such as time of flight (ToF) and Fourier Transform (FT) Orbitrap MS). In this study the applicability of high resolution single-stage-Orbitrap-MS for confirmatory analysis of growth-promoting agents in meat was compared to that of a QqQ-MS. Validation according to CD 2002/657/EC demonstrated that steroid analysis based on Orbitrap MS, operating at a resolution of 50,000 FWHM, is indeed capable to compete with QqQ-MS in terms of selectivity/specificity, while providing excellent linearity (for most compounds >0.99) but somewhat inferior sensitivity. Indeed, CC_{α}s reached from 0.04–0.88 $\mu g\,kg^{-1}$ for the 34 anabolic steroids upon MS/MS detection, while upon Orbitrap MS detection a range of 0.07–2.50 $\mu g\,kg^{-1}$ was observed. Using QqQ-MS adequate precision was obtained since relative standard deviations, associated with the repeatability and intra-laboratory reproducibility, were below 20%. In the case of Orbitrap MS, for some compounds (i.e. some estrogens) this threshold was exceeded and thus poor precision was observed, which is possibly caused by the lack in sensitivity. Overall, it may be concluded that Orbitrap-MS offers an adequate performance in terms of linearity and precision but lacks in sensitivity for some of the compounds.

1. Introduction

Because of their potential anabolic effects, some steroids have been used to boost the mass and quality of animal carcasses in food production for economic reasons [1,2]. Although there are a number of steroid preparations authorized for this purpose in countries

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such as the USA, the use of growth promoters is banned within the EU [3]. The reasons for this ban were highlighted in two reports from the European Commission in 1999 and 2002, which concluded that the presence of hormones in meat products might potentially be harmful to human health through endocrine disrupting or carcinogenic mechanisms. As a result, national plans of the individual Member States were developed to monitor the abuse of anabolic steroids. In this context, the development of sensitive, specific and multi-residue analytical methods, allowing an adequate control of the possible illegal use of growth promoters in meat production, is requisite. These analytical methods must be in compliance with the criteria of CD 2002/657/EC [4].

From literature, it may be concluded that LC-triple quadrupole tandem MS (QqQ-MS/MS) is currently the preferred method for residue analysis [5,6]. The majority of current LC–MS based hormone and veterinary drug residue analyses relies on the high sensitivity and selectivity of the selected reaction-monitoring (SRM) mode of QqQ-MS. Besides, the introduction of UHPLC and fast-switching QqQ-MS instruments significantly increased the number of analytes that can be detected in one run. Indeed, modern instruments produce high signal-to-noise (S/N) ratios, even when relying on short SRM dwell times [7]. This permits monitoring of an increasing number of transitions and thus compounds. Further increasing the number of analytes to be considered, requires monitoring at specific retention time windows, also called "timed SRM" or "scheduled SRM". In each retention time window, the number of transitions to be acquired can as such be kept relatively low (<10). Using this approach, selective and sensitive analysis of a larger number of compounds (max. 100–120) may be achieved within one single LC-QqQ-MS run. This approach requires, however, frequent readjustments due to small shifts in retention time. In recent years many (UHP)LC–MS/MS applications have been described for the analysis of anabolic steroids in various biological samples including urine or serum from bovine and equine origin, bovine hair and kidney fat [8–18], since these tend to be the matrices of choice when monitoring for anabolic steroids. For controls at retail level and for products imported in the EU, it is, however, necessary to have analytical methods applicable to meat samples. Several methods on the determination of anabolic steroids in muscular tissue of animal origin have been reported [19–26], monitoring a selection of the existing anabolic steroids (10 to 20), sometimes in combination with corticosteroids. Recently, a new QqQ-MS based method for the simultaneous analysis of 34 anabolic steroids was reported [27].

An inherent limitation of targeted LC–MS/MS approaches is, however, the inability to detect residues such as novel illegally administered anabolic steroids, designed with the aim not to be detected by control agencies. An attractive alternative is the use of full scan MS approaches, which offer the possibility to simultaneously analyze a virtually unlimited number of compounds. Furthermore, the retrospective "post-targeted" evaluation of old data offers the possibility to screen for non-"a priori" selected analytes. Moreover, their accurate-mass capabilities support the reconstruction of highly selective, accurate-mass chromatograms of target residues in complex matrices. To allow the detection of residues at the by legislation demanded low ppb ($\mu g\,kg^{-1}$) or ppt ($ng\,kg^{-1}$) concentration ranges, very sensitive full scan analyzers are required. In comparison with the unit-resolution scanning mass spectrometric techniques, the medium (up to 15,000 full width half maximum (FWHM)) or high resolution (up to 100,000 FWHM) of the current full-scan analyzers significantly affects the selectivity and provides as such the required sensitivity gain. However, it should be noticed that accurate-mass determination without proper mass resolution criteria might lead to false compliant (false negative) results, both in MS screening and MS/MS confirmation. For example, when medium resolution LC-Q-ToF-MS was used for the analysis of the anabolic steroid stanolozol, a lack in mass-resolving power was demonstrated [28]. Indeed, the currently published work relies on ToF instruments with mass deviations of <5 ppm and resolutions of maximally 15,000 FWHM. This resolution may lead to inaccurate mass measurements in complex matrices due to unresolved background matrix interferences. Co-elution of isobaric compounds may cause significant deviations in exact mass measurements. Especially this mass resolution is of crucial importance in the successful identification of residues or contaminants in samples containing high amounts of complex matrix co-extracts. In this context, the high resolving power (up to 100,000 FWHM) of the Fourier Transform Orbitrap mass spectrometric technology provides high mass accuracy (mass deviations <2 ppm) resulting in both high selectivity and sensitivity for complex sample analysis.

Therefore, in this study the aim was to compare the acknowledged confirmatory capacity of a triple quadrupole mass analyzer (QqQ-MS) to that of a single-stage Orbitrap mass analyzer. To this extent, the high-throughput detection, confirmation and quantification of 34 anabolic steroids in muscle tissue, covering the classes of gestagens, estrogens (including stilbenes) and androgens, both the synthetic as well as the natural derivates, extracted at their respective recommended concentration/action limit (0.5–10 $\mu g\,kg^{-1}$) from bovine muscle, was performed by using these two distinct mass analyzers, preceded by UHPLC separation.

2. Materials and methods

2.1. Reagents and chemicals

Standards of 17β-nortestosterone, fluoxymesterone, progesterone, estrone, estriol, 17α-estradiol, 17β-estradiol, 17α-hydroxyprogesterone, 17β-ethinylestradiol, diethylstilbestrol, dienestrol, α-zearalanol, β-zearalanol, 17α-testosterone, 17β-testosterone, hexoestrol, norgestrel, acetoxyprogesterone, medroxyprogesterone acetate, methyltestosterone and the internal standard androstadieendione were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methylboldenone (or methandienone), norethandrolone, α-nortestosterone, methandriol, β-boldenone, 4-androstenedione and caproxyprogesterone were provided by Steraloids Inc. (Newport, RI, USA). α-boldenone and the internal standards 17β-estradiol-d3, medroxyprogesteron acetate-d3, methyltestosterone-d3, 17β-testosterone-d2 and hexoestrol-d4 were obtained from RIKILT (Wageningen, The Netherlands). β-trenbolone, flugestone acetate, trenbolone acetate, megestrol acetate, chlormadinone acetate and melengestrol acetate were kindly provided by WIV (Brussels, Belgium). Solvents were of analytical grade when used for extraction and purification steps, and of LC–MS Optima grade for UHPLC-MS application. They were obtained from VWR International (Merck, Darmstadt, Germany) and Fisher Scientific UK (Loughborough, UK), respectively.

Primary stock solutions were prepared in methanol at a concentration of 1000 ng μL^{-1}. Working solutions were prepared by 100 and 1000 times dilution in methanol/water (50/50). When necessary, sonication was applied to ensure the complete dissolution of the substances. Solutions were stored in dark glass bottles at −20 °C.

2.2. Instrumentation

The LC system consisted of a Thermo Fisher Scientific (San José, CA, USA) Accela UHPLC pumping system, coupled with an Accela Autosampler and Degasser. Chromatographic separation was achieved by reversed phase chromatography and gradient elution. Separation of the anabolic steroids was carried out on