Contents lists available at ScienceDirect

Heliyon

Research article

Determination of oxprenolol, methandienone and testosterone in meat samples by UHPLC-Q-ToF

A. Temerdashev [*], E. Dmitrieva, A. Azaryan, E. Gashimova

Department of Analytical Chemistry, Kuban State University, Krasnodar, 350040, Russia

ARTICLE INFO

ABSTRACT

The presence of some drugs in meat samples can cause threat to human health, therefore, its analysis is highly desirable for food safety purposes. In this work, a solid-phase extraction procedure for the determination of oxprenolol, a non-selective beta-blocker, and such anabolic agents as methandienone and testosterone in beef meat samples has been developed. Extraction conditions were optimized to achieve high sensitivity and accuracy of the results. The procedure was validated using meat samples free from target analytes. As a result, high selectivity and sensitivity were observed with the detection limits between 0.25 and 1.25 ng/g, and the results were not affected by matrix components. The proposed procedure was applied to the analysis of real beef samples purchased in the market, and the results have revealed the presence of contaminated samples. The concentration of oxprenolol in the contaminated sample was 7 ng/g, methandienone content in the sample was 30 ng/g, while testosterone level was 4 ng/g.

1. Introduction

An improvement of muscle growth for meat producing animals can be achieved in different ways. One of them is the use of anabolic steroids and some beta-blockers like oxprenolol [1–9] to increase feed conversion for economic reasons. However, they are strictly prohibited in most countries (including Europe and China) because of potential harm for human health, especially endocrine disrupting [3–8]. The most harmful effects resulting from the administration of anabolic steroids can be received by children since hormonal system plays a critical role in brain development and increases a risk of damage for some cognitive functions [9]. In adult population, exposure to anabolic agents increases neuronal spine densities in the hippocampus and amygdala—brain regions involved in learning and emotions (e.g., aggression) [10,11]. At least few weeks are necessary for the neuronal spine densities to return to normal levels in the amygdala, but not in the hippocampus. This suggests that pubertal steroid exposure could produce long-lasting structural changes in certain brain regions [11].

Another important problem of their usage is a growing risk of their presence in the environment. Steroid hormones and other pharmaceuticals can be excreted from humans and animals. Their residues can lead to sexual disorders, feminization, masculinization and infertility of the other organisms even after a contact with traces of some pharmaceuticals. It should be noticed that they can be also concentrated in fish and animals and then, according to the food chain, return to the human body [12–15]. Previously, numerous articles related to steroids determination in meat were published and discussed [13–40]. Boldenone, trenbolone, nandrolone, stanozolole and other steroid compounds are among the most commonly used drugs for these purposes. At the same time, every local

---

* Corresponding author.
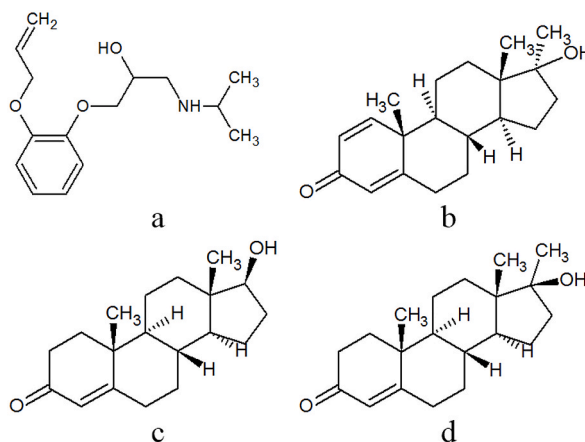*E-mail address:* temerdashevaz@gmail.com (A. Temerdashev).

Fig. 1. Chemical structures of studied analytes (a – oxprenolol, b – methandienone, c – testosterone) and internal standard (d – methyltestosterone).

market has its own specificity based on availability, legal status and price of steroid hormones and other veterinary drugs. For example, testosterone, methandienone and oxprenolol are much more commercially available compared to other drugs owing to their low cost in Russia. In Canada, trenbolone, progesterone, testosterone and zeranol are approved for veterinary purposes. In some cases, this specificity becomes critical, especially for athletes, because even traces of exogenous steroids or their metabolites could result in positive doping control test, since these compounds are stable at high temperatures [41–43]. According to the World Anti-Doping Agency (**WADA**) Prohibited List, steroid hormones are included in S1 section (anabolic agents) [44], which means that they are strictly prohibited for usage in- and out-of competition period. Athletes should be aware of the potential risks since competitions may take place in different regions and countries.

According to the currently available literature, liquid chromatography with tandem mass spectrometric detection (**LC-MS/MS**) is a commonly used technique for such residue analysis [1–7]. One of its main advantages is a possibility of fast polarity switching, which makes possible multi-target residue screening of hormones and veterinary drugs. However, even acquisition in multiple reaction monitoring (**MRM**) mode cannot guarantee uncompromised selectivity and accuracy of the analysis results. To prevent occurring of false-positive results, conformation by high-resolution mass spectrometry (**HRMS**) is required.

Therefore, the aim of this study is to develop a confirmation method for the determination of methandienone, testosterone and oxprenolol by UHPLC-HRMS in meat and establish its applicability in routine analysis of meat samples purchased in local markets of Krasnodar region (Russia) and the Republic of Adygea (Russia).

2. Materials and methods

2.1. Reagents

Testosterone, methandienone, oxprenolol and methyltestosterone analytical standards (>99%, Fig. 1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Varian Bond Elut C8 (100 mg, 1 mL) (Palo Alto, CA, USA), Waters Oasis HLB (30 mg, 1 mL) (Dublin, Ireland) and Phenomenex Strata C-18-E (100 mg, 1 mL) (Torrance, CA, USA) solid-phase extraction (**SPE**) cartridges were used in the experiments. HPLC-grade methanol and acetonitrile (both from J.T. Baker, Poland) as well as double-distilled water were used throughout the experiment.

2.2. Preparation of solutions

Standard solutions of the analytes at concentrations of 1 mg/mL were prepared in methanol and stored at 4 °C for a month. These solutions were diluted with methanol to prepare calibration (5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000 ng/mL) and quality control (**QC**) (50, 500, 2500 ng/mL) solutions in methanol. 1 mg/mL methyltestosterone (internal standard, **IS**) solution was prepared in methanol followed by its dilution with methanol to obtain a working solution (10 μg/mL). QC solutions were prepared separately from calibration solutions; all the solutions were stored at 4 °C for a month.

2.3. Instrumentation

To detect target analytes, a system consisting of a Bruker Elute ultra-high performance liquid chromatograph coupled to a Bruker MaXis Impact quadrupole time-of-flight (**Q-ToF**) mass spectrometer operating with an electrospray ionization (**ESI**) source was used in this study (Bremen, Germany). The separation was carried out using a core-shell Phenomenex Kinetex C18 analytical column (100 × 2.1 mm, 1.7 μm) (Torrance, CA, USA) with the respective guard column. The analytes were eluted using gradient elution mode with the mobile phase consisting of 0.1% formic acid solution in methanol (eluent A) and 0.1% formic acid solution in water (eluent B) at the
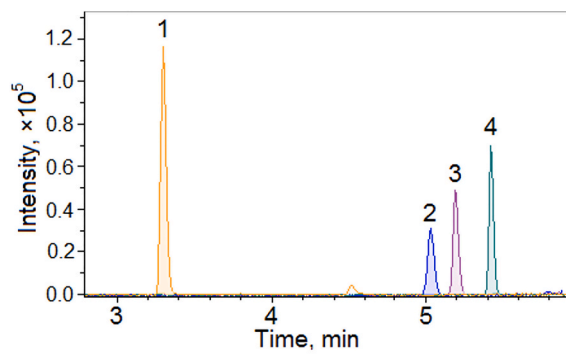
Fig. 2. The chromatogram of 25 ng/mL standard solution of studied analytes: 1 – oxprenolol, 2 – methandienone, 3 – testosterone, 4 – methyltestosterone.

Table 1
Conditions of UHPLC-MS detection of analytes.

Analyte	Monoisotopic mass, Da	$[M+H]^+$, Da	Mass measurement error, ppm	t_R, min
Oxprenolol	265.1678	266.1751	−1.1	3.2
Methandienone	300.2089	301.2162	0.7	4.7
Testosterone	288.2089	289.2162	2.4	4.9
Methyltestosterone[a]	302.2246	303.2319	1.0	5.2

[a] Internal standard.

flow rate of 0.4 mL/min. The gradient elution program was as follows: 0.0–1.0 min 5% A; 2.7–4.0 min 60% A; 5.0–7.5 min 90% A; 7.51–9.0 min 5% A. The column was thermostated at 40 °C; the injection volume was 10 μL. The chromatogram of standard solution of the analytes is given in Fig. 2.

The following conditions of mass spectrometric detection were used: ion source temperature: 250 °C; capillary voltage: 4 kV; end plate offset: 4 kV; nebulizing gas (nitrogen) pressure: 100 kPa; drying gas flow rate: 5 L/min; scan speed: 3 Hz; mass range, 150–1000 *m/z*; collision gas (nitrogen) pressure: 1.5 mTorr.A full scan mode was used to achieve high sensitivity of detection. To prevent false results and increase analysis accuracy, a mass tolerance window of 5 ppm was used. Conditions of UHPLC-MS detection are given in Table 1.

An Interscience BagMixer (Saint Nom la Bretêche, France) equipped with disposable 200-mL bags (bag composition: multilayer®, reinforced multicoated complex) was used for extraction.

2.4. Samples

Twenty beef chuck samples were purchased in the local markets of the Republic of Adygea and Krasnodar region, Russia, in January and February of 2022. The places of the markets are given in Fig. 3. These samples were transferred to the laboratory in a portable refrigerator at 4 °C and stored at −20 °C prior to analysis for no more than a month. According to veterinary authorities, the fat content in the studied samples was 9–13%, protein content – 27–35%, water ratio – 55–63%.

2.5. Preparation of beef meat samples for analysis

To prepare beef meat samples for analysis, the following optimized sample preparation procedure was used: 10 g of a meat sample (ten 1-g pieces) was transferred to a disposable bag; then, 20 mL of 30% methanol solution containing the internal standard (50 ng/mL methyltestosterone) was added, and the extraction procedure was performed for 5 min. The extract was transferred to a 15-mL Eppendorf tube made of high-density polyethylene and centrifuged for 10 min at 2000 rcf.

SPE was performed by conditioning a Waters Oasis HLB cartridge (30 mg, 1 mL) with 1 mL of methanol, equilibrating with 1 mL of water followed by loading 1 mL of a meat extract; the SPE cartridge was washed with 1 mL of 30% methanol solution and eluted with 0.5 mL of methanol; all the solutions were passed through the cartridge at a flow rate of 1 mL/min. The eluate was analyzed by the UHPLC-Q-ToF instrument.

2.6. Linearity and sensitivity

To establish a linear range, sample preparation was performed by using meat samples free from target analytes as well as model solutions (30% methanol solution (v/v)) to evaluate matrix effects.

The extract of a meat sample free from target analytes was obtained under conditions optimized above, i.e., using 10 g of the sample

Fig. 3. Sampling area of beef samples.

Table 2
Figures of merit of the proposed procedure.

Analyte	LOD, ng/g	LOQ, ng/g	Linear range, ng/g	R^2
Oxprenolol	0.25	0.5	0.5–250	0.998
Methandienone	1.25	2.5	2.5–250	0.995
Testosterone	0.5	1.25	1.25–250	0.996

and 20 mL of 30% methanol solution as an extraction solvent. This extract as well as model solutions were spiked with the analytes to obtain the final concentrations of 0.25, 0.5, 1.25, 2.5, 5, 12.5, 25, 50, 125, 250 ng/g; the concentration of the internal standard (methyltestosterone) was fixed at 100 ng/g. Then, these samples were passed through the SPE procedure under previously optimized conditions.

The detection and quantification limits evaluated by using the signal-to-noise ratios of 3 and 10, respectively, as well as linear ranges, i.e., the concentration ranges with deviations from nominal concentrations less than 15%, are presented in Table 2. The slopes of the calibration curves obtained for model and real solutions had negligible difference, which shows insignificant contribution of matrix components to the results of the quantification.

2.7. Accuracy, precision calculation

To access accuracy and precision of the proposed procedure, QC solutions of low, medium and high concentrations with final concentrations of 2.5, 25 and 125 ng/g were analyzed within one day and on three consecutive days ($n = 15$). To prepare QC solutions of these concentrations, a meat sample extract was obtained and then spiked with the studied analytes at three concentration levels. Then, these solutions were passed through the SPE procedure and analyzed.
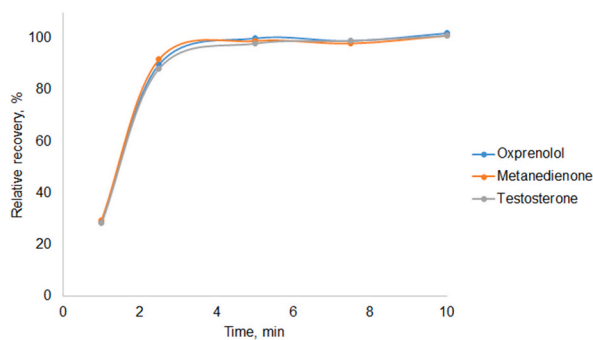
Fig. 4. Optimization of extraction time.

Accuracy was calculated by using equation (1):

$$\text{bias}\ (\%) = \left(\left(c_{observed} - c_{nominal} \right) / c_{nominal} \right) \times 100 \tag{1}$$

while precision was expressed as the coefficient of variation (**CV, %**).

3. Results and discussion

To optimize sample preparation conditions, extraction of analytes from meat samples using a solvent mixture and SPE of the obtained extract were successively optimized.

3.1. Optimization of extraction conditions

3.1.1. Extraction solvent composition

In the first step of the sample preparation procedure, the analytes were extracted from meat samples using a solvent mixture. Owing to sufficient solubility of the studied analytes in methanol as well as its compatibility with reversed-phase sorbents, different methanol–water solutions spiked with the internal standard were tested to quantitatively extract them from the meat sample. As a preliminary study, the efficiency of methyltestosterone extraction from meat after injection and without injection into a meat sample was tested and compared. According to the observed data, no significant difference in peak areas was observed. The same methodology of internal standard solution usage was previously described in Ref. [7]. Since this solution was further subjected to SPE with a hydrophobic sorbent, high methanol contents in the solution would not allow to adsorb analytes (Section 3.2.3). Therefore, the content of methanol in the extraction solvent was limited to 30% (v/v) to avoid the breakthrough of target analytes in SPE, which was especially relevant for oxprenolol.

The experiment was carried out as follows: the mixture of target analytes was injected in a 10-g meat sample by a syringe to achieve the final concentration of 1 µg/g; this concentration of analytes was selected to exclude the possibility of analytes breakthrough in real sample analysis. Then, the sample was transferred into a disposable bag followed by the addition of 20 mL of water–methanol mixture (methanol content of 0, 5, 10, 15, 20, 25, 30%, v/v) containing the internal standard (50 ng/mL), and the extraction was performed for 10 min. The extract was transferred to a 15-mL Eppendorf tube and centrifuged for 10 min at 2000 rcf. Finally, SPE under optimized conditions was carried out.

The results showed that 30% methanol solution provided analyte recoveries in the range of 80–97%, therefore, this extraction solvent composition was used in further experiments.

3.1.2. Extraction solvent volume

After the optimization of extraction solvent type, different sample to extraction solvent ratios were tested. For this, 10-g meat samples spiked with target analytes at 1 µg/g by a syringe were placed in disposable bags, and different solvent volumes (containing methyltestosterone at 50 ng/mL) between 20 and 60 mL with a 5-mL step were tested. Volumes lower than 20 mL were not used owing to the technical features of the BagMixer. The extraction process in the bags was performed in the same manner as in the previous section followed by SPE under optimized conditions. The volume of 20 mL was sufficient for the extraction of the analytes, while higher volumes resulted in increased dilution and, as a result, decreased sensitivity; therefore, the sample to solvent ratio of 1:2 was selected to decrease the analytes dilution.

3.1.3. Extraction time

The extraction times in the Interscience BagMixer were evaluated between 1 and 10 min (1, 2.5, 5, 7.5, 10 min). For this experiment, 10 g of a meat sample spiked with analytes at 1 µg/g was transferred to the bag, and 20 mL of 30% methanol solution (v/v) containing 50 ng/mL methyltestosterone was added. The recoveries increased up to 5 min and then a plateau was observed (Fig. 4). Therefore, a 5-min extraction was used in subsequent experiments.
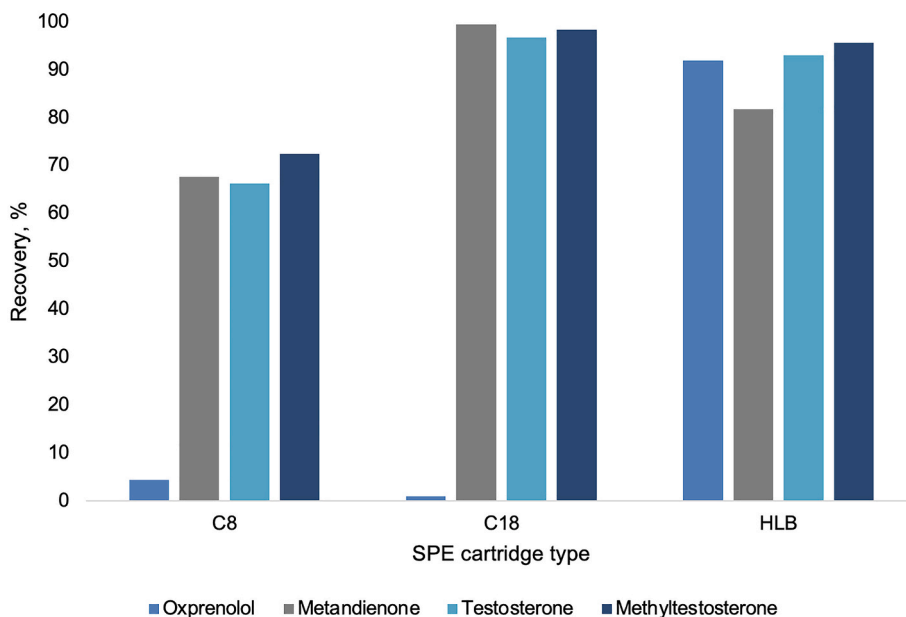
Fig. 5. Analyte recoveries from different SPE cartridges.

3.2. Optimization of solid-phase extraction conditions

3.2.1. SPE cartridge type

Three SPE cartridge types were studied in this work: Varian Bond Elut C8 (100 mg, 1 mL), Waters Oasis HLB (30 mg, 1 mL) and Phenomenex Strata C-18-E (100 mg, 1 mL).

The experiments were carried out following the manufacturer recommendations; in all cases, methanol–water systems were used during the SPE procedure. When octyl and octadecyl sorbents were used, a breakthrough of oxprenolol was observed during the sample loading. In contrast, HLB cartridges allowed to quantitatively extract target analytes at QC high (125 ng/g) (Fig. 5); therefore, they were used in the following experiments.

3.2.2. Sample volume

The sorbent capacity was tested by passing different volumes of meat extracts (1–3 mL) containing target analytes and the internal standard at 250 ng/g through the SPE cartridges. The SPE cartridge was conditioned with 1 mL of methanol and equilibrated with 1 mL of water. The sample was loaded (1–3 mL) and washed with 1 mL of 30% methanol solution, dried under the air stream with subsequent elution using 1 mL of methanol. The volume up to 2 mL could be passed through the cartridge with sufficient analyte recoveries of 73–86%; at the same time, when 1 mL of the sample was loaded, the recoveries of 89–96% were observed.

3.2.3. Washing solvent composition

To achieve the highest removal of interfering substances, various methanol–water mixtures were studied (0–50% methanol with a 5% step, v/v). At methanol contents higher than 30%, the breakthrough of oxprenolol occurred. Therefore, 30% methanol solution was used in all experiments.

3.2.4. Eluent volume

Different methanol volumes between 0.5 and 3 mL with a step of 0.5 mL were studied to achieve the complete removal of the analytes from the SPE sorbent. A 0.5-mL aliquot of methanol was sufficient for the quantitative removal of the analytes (>98%). Consequently, the analytes were eluted with this methanol volume.3.3. Method validation.

The proposed procedure was validated considering the FDA Bioanalytical Method Validation guidance [45] in terms of linearity and sensitivity (section 2.6), accuracy and precision (section 2.7), selectivity, recovery and stability.

3.2.5. Accuracy, precision and recovery

Accuracy and precision values were established not to exceed 15%, which agreed with the FDA criteria.

Recoveries were calculated by comparison of analyte peak areas obtained after analysis of standard solutions as well as real samples spiked with target analytes that were passed through the proposed SPE procedure. Quantitative recoveries between 89% and 96% were also observed for all analytes.

Moreover, matrix effects were evaluated by comparison of the results obtained for real and model solutions spiked with target analytes at the three concentration levels which were passed though the sample preparation procedure under the optimized conditions.

Table 3
Results of QCs analysis by the proposed procedure.

Analyte	QC concentration, ng/g	Interday		Intraday		Matrix effect, %	Recovery, %
		Accuracy, %	Precision, %	Accuracy, %	Precision, %		
Oxprenolol	2.5	−17.8	16.2	−18.6	18.5	11.2	96
	25	−11.5	10.7	−12.1	13.6	7.2	94
	125	−5.6	9.6	−5.9	9.5	6.4	93
Methandienone	2.5	−10.4	14.1	−11.6	12.7	9.1	95
	25	−8.3	9.5	−8.9	10.5	2.7	93
	125	−4.6	8.8	−5.0	9.8	2.2	89
Testosterone	2.5	−12.4	13.9	−13.3	16.2	9.3	95
	25	−9.8	9.7	−10.4	11.3	5.4	94
	125	−5.2	9.1	−5.7	10.4	1.9	91

The results obtained are given in Table 3.

3.2.6. Selectivity and cross-contamination

The investigation of selectivity was performed by using blank samples of meat (the sample was analyzed before spiking; if peaks corresponding to analytes were not observed, it could be used as a blank) with their further spiking at different concentration levels.

To prevent cross-contamination, the extraction procedure was conducted using disposable 200-mL bags with an integrated paper filter from Interscience (Saint Nom la Bretêche, France).

3.2.7. Stability

The stability of processed samples placed in the autosampler was evaluated for 24 h. The samples in vials were thermostated at 5 °C in the darkness. The deviations in the results did not exceed 15%, which indicates high stability of the prepared solutions. However, the long-term stability test of QC samples showed that the meat extracts degraded even after the first freeze-thaw cycle and could not be used thereafter.

The stability of 1 mg/mL standard solutions of analytes prepared in methanol was evaluated for a month. The results showed that these solutions were stable within this period of time, i.e., the deviations in the results were less than 15%.

3.2.8. Carryover

Carryover was evaluated by analysis of a blank meat sample after high quality control (**HQC**). The peaks corresponding to target analytes were not present in the chromatogram of the blank solution after HQC analysis, which means the lack of carryover. **3.4. Comparison of the proposed and reported methods**.

Over the years, numerous analytical methods were developed to determine different classes of contaminants in products. In the last two decades, GC-MS and LC-MS/MS were mostly used for these purposes. Conventional UHPLC with triple quadrupole mass spectrometry by using liquid-liquid (**LLE**) or organic solvent extraction is the most commonly used and simple routine method which could be applied to the determination of steroids and other pharmaceuticals at μg/g and ng/g levels [41]. The same sensitivity with high selectivity could be achieved by using GC-MS(/MS) because of thermal stability of most steroid hormones [36,37,41,43]. To achieve higher sensitivity, SPE [30,31,34] or molecularly imprinted sorbents can be used [37]; however, it leads to a significant increase in the analysis cost. At the same time, it allows to control selectivity of the sample preparation and leads to higher accuracy and precision of the analysis [36,37]. Another way to increase selectivity is the use of HRMS instruments which have become commercially available even for a routine analysis [28]. Unfortunately, matrix effects appearing during sample analysis (suppressed or increased ionization) cannot be overcome when ESI source is used. Atmosphere pressure chemical ionization (**APCI**) source can be used to achieve higher reproducibility [29]; however, it will dramatically decrease the number of possible contaminants which could be simultaneously determined.

Meat is a complex matrix, and its direct analysis can result in high matrix interferences and false results due to ionization suppression or enhancing. Also, direct analysis of such extracts by using UHPLC can damage the analytical column. To prevent it, sample clean-up by using SPE is a necessary sample preparation step. In this research, no enzymatic or mineral hydrolysis was used because commercially available injection forms of the analytes are unconjugated and their presence in the samples may be a sign of their recent use. It can also clearly show a potential threat for an athlete because these compounds are stable even after heating. As a result, sensitive analysis of meat samples is required for doping control purposes. Moreover, the presence of these compounds can be harmful to human health [37], which makes this procedure essential for food safety purposes.

Previously published papers [35–37,40] were aimed at targeted determination of anabolic steroids. An apparent advantage of such approach is the possibility to achieve low detection limits; however, it significantly complicates screening of other xenobiotics, which are equally important to determine in food. The proposed approach based on the application of high-resolution mass spectrometry coupled with the sample preparation procedure adopted for different classes of compounds allows to significantly extend the list of detected indicators and apply non-targeted screening methodology, which plays an important diagnostic role in doping control. Thus, when routine testing of products is carried out, possible manipulations in determining the source of prohibited substances in biological fluids may be avoided.
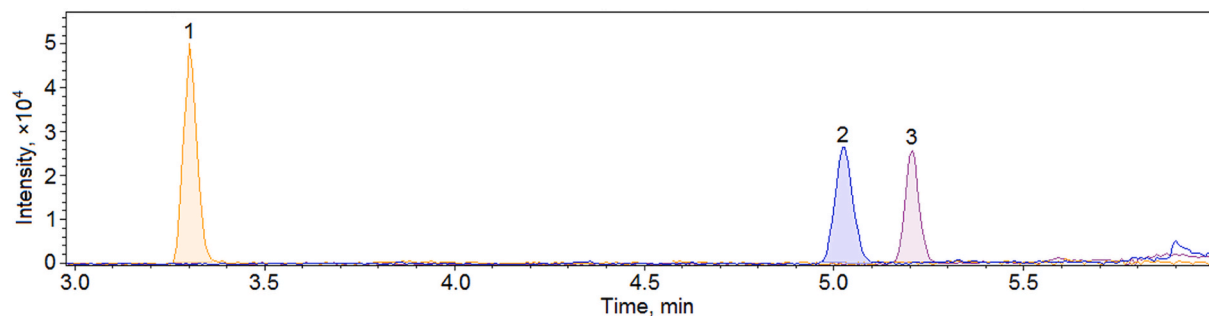
Fig. 6. Extracted ion current (EIC) chromatograms of the contaminated meat samples: 1 – oxprenolol (7 ng/g, sample "Krasnodar 1"), 2 – methandienone (4 ng/g, sample "Adygea 2"), 3 – testosterone (30 ng/g, sample "Adygea 3").

3.3. Real sample analysis

Twenty meat samples were analyzed by the proposed procedure. Analyzed samples were purchased in different districts of the Krasnodar region (n = 9) and the Republic of Adygea (n = 11); the samples were labelled as "Adygea N″" and "Krasnodar N″" according to the regional center name (Fig. 3). As a result, three positive samples were found with concentrations within the linear range of the procedure: "Adygea 2" containing methandienone at 4 ± 1 ng/g, "Adygea 3" with testosterone content of 30 ± 4 ng/g and "Krasnodar 1" contaminated with oxprenolol at 7 ± 2 ng/g. Overlayed chromatogram of contaminated meat samples is given in Fig. 6. High detected concentrations of these substances may be caused by their use by farmers.

It should be noted that methyltestosterone used in this study as an internal standard can also be used in animals as a muscle growth promoting substance. Therefore, to avoid obtaining understated results, the absence of methyltestosterone in the analyzed samples should be confirmed.

4. Conclusions

A rapid, easy and reliable method for steroids determination in meat using UHPLC-HRMS was validated. Optimization of sample preparation conditions has revealed that SPE with HLB cartridges is suitable for the quantitative extraction of oxprenolol, methandienone and testosterone from meat samples with high accuracy, sensitivity and precision. The use of 30% methanol solution for the extraction of these analytes from meat samples and as a washing solution leads to low matrix effects with the lack of analytes breakthrough. As a result, this procedure is suitable for the analysis of beef meat samples to determine trace analyte concentrations.

Author contribution statement

Azamat Temerdashev: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Catherine Dmitrieva: Performed the experiments; Wrote the paper.

Alice Azaryan: Performed the experiments.

Elina Gashimova: Conceived and designed the experiments; Analyzed and interpreted the data.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare no competing interests.

References

[1] K. Walpurgis, A. Thomas, H. Geyer, U. Mareck, M. Thevis, Dietary supplement and food contaminations and their implications for doping controls, Foods 9 (8) (2020) 1–21, https://doi.org/10.3390/foods9081012.

[2] M. Pedersen, J.H. Andersen, Confirmatory analysis of steroids in muscle using liquid chromatography-tandem mass spectrometry, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess. 28 (4) (2011) 428–437, https://doi.org/10.1080/19440049.2010.549153.

[3] L. Vanhaecke, L. Van Meulebroek, N. De Clercq, J. Vanden Bussche, High resolution orbitrap mass spectrometry in comparison with tandem mass spectrometry for confirmation of anabolic steroids in meat, Anal Chim Acta [Internet] 767 (1) (2013) 118–127, https://doi.org/10.1016/j.aca.2013.01.009.

[4] S.H. Jeong, D. Kang, M.W. Lim, C.S. Kang, H.J. Sung, Risk assessment of growth hormones and antimicrobial residues in meat, Toxicol. Res. 26 (4) (2013) 301–313, https://doi.org/10.5487/TR.2010.26.4.301.

[5] J.P. Scarth, A. Clarke, P. Teale, A. Mill, R. Macarthur, J. Kay, Detection of endogenous steroid abuse in cattle: results from population studies in the UK, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess. 28 (1) (2011) 44–61, https://doi.org/10.1080/19440049.2010.539628.

[6] Y. Shahbazi, H. Malekinejad, H. Tajik, Determination of naturally occurring estrogenic hormones in cow's and river buffalo's meat by HPLC-FLD method, J Food Drug Anal [Internet] 24 (3) (2016) 457–463, https://doi.org/10.1016/j.jfda.2016.02.014.

[7] Y. Yang, B. Shao, J. Zhang, Y. Wu, H. Duan, Determination of the residues of 50 anabolic hormones in muscle, milk and liver by very-high-pressure liquid chromatography-electrospray ionization tandem mass spectrometry, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 877 (5–6) (2009) 489–496, https://doi.org/10.1016/j.jchromb.2008.12.054.

[8] P. Marchand, B. Le Bizec, C. Gade, F. Monteau, F. André, Ultra trace detection of a wide range of anabolic steroids in meat by gas chromatography coupled to mass spectrometry, J. Chromatogr. A 867 (1–2) (2000) 219–233, https://doi.org/10.1016/S0021-9673(99)01114-0.

[9] B.S. Keenan, G.E. Richards, J.S. Dallas, R. Smith, Androgen-Stimulated Growth : (November):996–1001.Cunningham RL, Claiborne BJ, McGinnis MY. 2007. Pubertal exposure to anabolic androgenic steroids increases spine densities on neurons in the limbic system of male rats, Neuroscience 150 (3) (2014) 609–615, https://doi.org/10.1016/j.neuroscience.2007.09.038.

[10] R.D. Romeo, H.N. Richardson, C.L. Sisk, Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential, Neurosci. Biobehav. Rev. 26 (3) (2002) 381–391, https://doi.org/10.1016/S0149-7634(02)00009-X.

[11] K. Kołecka, M. Gajewska, M. Caban, From the pills to environment – prediction and tracking of non-steroidal anti-inflammatory drug concentrations in wastewater, Sci. Total Environ. 825 (2022), https://doi.org/10.1016/j.scitotenv.2022.153611.

[12] J. Chen, S.S. Liu, Y.J. Wang, J. Li, Y.S. Liu, F. Yang, G.G. Ying, Optimized constructed wetlands enhance the removal and reduce the risks of steroid hormones in domestic wastewater, Sci. Total Environ. (2021), 143773, https://doi.org/10.1016/j.scitotenv.2020.143773 [Internet]. 757.

[13] J. Chen, Y.S. Liu, W.J. Deng, G.G. Ying, Removal of steroid hormones and biocides from rural wastewater by an integrated constructed wetland, Sci Total Environ [Internet 660 (2019) 358–365, https://doi.org/10.1016/j.scitotenv.2019.01.049.

[14] C. Blasco, C. Van Poucke, C. Van Peteghem, Analysis of meat samples for anabolic steroids residues by liquid chromatography/tandem mass spectrometry, J. Chromatogr. A 1154 (1–2) (2007) 230–239, https://doi.org/10.1016/j.chroma.2007.03.090.

[15] J. Scarth, C. Akre, L. van Ginkel, B. le Bizec, H. de Brabander, W. Korth, J. Points, P. Teale, J. Kay, Presence and metabolism of endogenous androgenic-anabolic steroid hormones in meat-producing animals: a review, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess. 26 (5) (2009) 640–671, https://doi.org/10.1080/02652030802627160.

[16] R. Ahmadkhaniha, A. Shafiee, N. Rastkari, F. Kobarfard, Accurate quantification of endogenous androgenic steroids in cattle's meat by gas chromatography mass spectrometry using a surrogate analyte approach, Anal. Chim. Acta 631 (1) (2009) 80–86, https://doi.org/10.1016/j.aca.2008.10.011.

[17] J. Seo, H.Y. Kim, B.C. Chung, J. Hong, Simultaneous determination of anabolic steroids and synthetic hormones in meat by freezing-lipid filtration, solid-phase extraction and gas chromatography-mass spectrometry, J. Chromatogr. A 1067 (1–2) (2005) 303–309, https://doi.org/10.1016/j.chroma.2004.12.063.

[18] M. Doué, E. Bichon, G. Dervilly-Pinel, V. Pichon, F. Chapuis-Hugon, E. Lesellier, C. West, F. Monteau, B. Le Bizec, Molecularly imprinted polymer applied to the selective isolation of urinary steroid hormones abuse: an efficient tool in the control of natural steroid hormones abuse in cattle, J Chromatogr A [Internet 1270 (2012) 51–61, https://doi.org/10.1016/j.chroma.2012.10.067.

[19] G. Kaklamanos, G. Theodoridis, T. Dabalis, Determination of anabolic steroids in bovine urine by liquid chromatography-tandem mass spectrometry, J Chromatogr B Anal Technol Biomed Life Sci [Internet] 877 (23) (2009) 2330–2336, https://doi.org/10.1016/j.jchromb.2009.03.033.

[20] L. Vanhaecke, J Vanden Bussche, K. Wille, K. Bekaert, H.F. De Brabander, Ultra-high performance liquid chromatography-tandem mass spectrometry in high-throughput confirmation and quantification of 34 anabolic steroids in bovine muscle, Anal Chim Acta [Internet] 700 (1–2) (2011) 70–77, https://doi.org/10.1016/j.aca.2010.10.006.

[21] S. Hartmann, H. Steinhart, Simultaneous determination of anabolic and catabolic steroid hormones in meat by gas chromatography-mass spectrometry, J. Chromatogr. B Biomed. Appl. 704 (1–2) (1997) 105–117, https://doi.org/10.1016/S0378-4347(97)00460-X.

[22] G.J. Zhang, B.H. Fang, Y.H. Liu, X.F. Wang, L.X. Xu, Y.P. Zhang, L.M. He, Development of a multi-residue method for fast screening and confirmation of 20 prohibited veterinary drugs in feedstuffs by liquid chromatography tandem mass spectrometry, J Chromatogr B Anal Technol Biomed Life Sci [Internet 936 (176) (2013) 10–17, https://doi.org/10.1016/j.jchromb.2013.07.028.

[23] L. Vanhaecke, L. Van Meulebroek, N. De Clercq, J. Vanden Bussche, High resolution orbitrap mass spectrometry in comparison with tandem mass spectrometry for confirmation of anabolic steroids in meat, Anal Chim Acta [Internet] 767 (1) (2013) 118–127, https://doi.org/10.1016/j.aca.2013.01.009.

[24] A. Azzouz, B. Souhail, E. Ballesteros, Determination of residual pharmaceuticals in edible animal tissues by continuous solid-phase extraction and gas chromatography-mass spectrometry, Talanta [Internet] 84 (3) (2011) 820–828, https://doi.org/10.1016/j.talanta.2011.02.016.

[25] B. Le Bizec, P. Marchand, D. Maume, F. Monteau, F. André, Monitoring anabolic steroids in meat-producing animals. Review of current hyphenated mass spectrometric techniques, Chromatographia 59 (SUPPL) (2004) 3–11, https://doi.org/10.1365/s10337-004-0245-5.

[26] X.M. Liu, X.L. Xu, X.M. Nie, X.S. Feng, F. Zhang, A holistic strategy for discovering structural analogues of drug residues in meat using characteristic structural fragments filtering by high-resolution Orbitrap mass spectrometry, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess [Internet] 38 (1) (2021) 81–94, https://doi.org/10.1080/19440049.2020.1825828.

[27] Y. Yang, B. Shao, J. Zhang, Y. Wu, H. Duan, Determination of the residues of 50 anabolic hormones in muscle, milk and liver by very-high-pressure liquid chromatography-electrospray ionization tandem mass spectrometry, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 877 (5–6) (2009) 489–496, https://doi.org/10.1016/j.jchromb.2008.12.054.

[28] X.M. Liu, X.L. Xu, X.M. Nie, X.S. Feng, F. Zhang, A holistic strategy for discovering structural analogues of drug residues in meat using characteristic structural fragments filtering by high-resolution Orbitrap mass spectrometry, Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess [Internet] 38 (1) (2021) 81–94, https://doi.org/10.1080/19440049.2020.1825828.

[29] L. Vanhaecke, J Vanden Bussche, K. Wille, K. Bekaert, H.F. De Brabander, Ultra-high performance liquid chromatography-tandem mass spectrometry in high-throughput confirmation and quantification of 34 anabolic steroids in bovine muscle, Anal Chim Acta [Internet 700 (1–2) (2011) 70–77, https://doi.org/10.1016/j.aca.2010.10.006.

[30] P. Marchand, B. Le Bizec, C. Gade, F. Monteau, F. André, Ultra trace detection of a wide range of anabolic steroids in meat by gas chromatography coupled to mass spectrometry, J. Chromatogr. A 867 (1–2) (2000) 219–233, https://doi.org/10.1016/S0021-9673(99)01114-0.

[31] A. Azzouz, B. Souhail, E. Ballesteros, Determination of residual pharmaceuticals in edible animal tissues by continuous solid-phase extraction and gas chromatography-mass spectrometry, Talanta [Internet] 84 (3) (2011) 820–828, https://doi.org/10.1016/j.talanta.2011.02.016.

[32] A. Nili-Ahmadabadi, F. Rezaei, A. Heshmati, A. Ranjbar, A. Larki-Harchegani, Steroid hormone exposure as a potential hazard in milk consumers: a significant health challenge in Iran, J. Food Qual. 2021 (2021), https://doi.org/10.1155/2021/5595555.

[33] R. Islam, N. Sultana, U. Ayman, M.R. Islam, M.A. Hashem, Role of steroid growth promoter on growth performance and meat quality traits in broiler, Poultry Sci. 101 (7) (2022) 1–13, https://doi.org/10.1016/j.psj.2022.101904.

[34] S. Mokh, F. Moussa, E.E.L. Khoury, R. Nassar, N. Bernabò, M. Al Iskandarani, Development of a new liquid chromatography-tandem mass spectrometry method for the determination of hormones in bovine muscle, J. Pharm. Biomed. Anal. 190 (2020), https://doi.org/10.1016/j.jpba.2020.113550.

[35] Z. Zeng, R. Liu, J. Zhang, J. Yu, L. He, X. Shen, Determination of seven free anabolic steroid residues in eggs by high-performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. Sci. 51 (3) (2013) 229–236, https://doi.org/10.1093/chromsci/bms132.

[36] B Le Bizec, J.P. Antignac, G. Pinel, Chapter 11 analytical strategies to control the illegal use of banned growth promoters in meat producing animals, Compr. Anal. Chem. 51 (8) (2008) 339–361, https://doi.org/10.1016/S0166-526X(08)00011-1.

[37] B.B. Hirpessa, B.H. Ulusoy, C. Hecer, Hormones and hormonal anabolics: residues in animal source food, potential public health impacts, and methods of analysis, J. Food Qual. 2020 (2020), https://doi.org/10.1155/2020/5065386.

[38] J.L. York, R.H. Magnuson, K.A. Schug, On-line sample preparation for multiclass vitamin, hormone, and mycotoxin determination in chicken egg yolk using LC-MS/MS, Food Chem [Internet 326 (April) (2020), 126939, https://doi.org/10.1016/j.foodchem.2020.126939.

[39] L. Huml, J. Tauchen, S. Rimpelová, B. Holubová, O. Lapčík, M. Jurášek, Advances in the determination of anabolic-androgenic steroids: from standard practices to tailor-designed multidisciplinary approaches, Sensors 22 (1) (2022), https://doi.org/10.3390/s22010004.

[40] Y. Jiafeng, S. Decheng, L. Xiaoyong, L. Yang, L. Guangyu, B.S. Min, Multiresidue determination of 19 anabolic steroids in animal oil using enhanced matrix removal lipid cleanup and ultrahigh performance liquid chromatography-tandem mass spectrometry, Anal. Methods 13 (21) (2021) 2374–2383, https://doi.org/10.1039/d1ay00437a.

[41] M. Polet, W. Van Gansbeke, P. Van Eenoo, K. Deventer, Efficient approach for the detection and identification of new androgenic metabolites by applying SRM GC-CI-MS/MS: a methandienone case study, J. Mass Spectrom. (2016), https://doi.org/10.1002/jms.3781 (February):524–534.

[42] M. Mazzarino, F.L. Khevenhüller-Metsch, I. Fiacco, M.K. Parr, X. de la Torre, F. Botrè, Drug–drug interaction and doping: effect of non-prohibited drugs on the urinary excretion profile of methandienone, Drug Test. Anal. 10 (10) (2018) 1554–1565, https://doi.org/10.1002/dta.2406.

[43] DB. da J. Neves, E.D. Caldas, GC–MS quantitative analysis of black market pharmaceutical products containing anabolic androgenic steroids seized by the Brazilian Federal Police, Forensic Sci Int [Internet 275 (2017) 272–281, https://doi.org/10.1016/j.forsciint.2017.03.016.

[44] World Anti-Doping Agency (WADA), Motreal; [accessed 2022 May 6], https://www.wada-ama.org/sites/default/files/resources/files/2022list_final_en.pdf, 2022.

[45] Food and Drug Administration (FDA), Silver Spring (MD); [accessed 2022 May 6], https://www.fda.gov/media/70858/download, 2018.