Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1067 (2005) 303–309
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Simultaneous determination of anabolic steroids and synthetic hormones in meat by freezing-lipid filtration, solid-phase extraction and gas chromatography–mass spectrometry

Jungju Seo [a], Hye-Young Kim [a], Bong Chul Chung [b], Jongki Hong [a,*]

[a] *Hazardous Substance Research Team, Korea Basic Science Institute, 126-16 Anam-Dong, Sungbuk-Ku, 136-701 Seoul, South Korea*
[b] *Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul 130-650, South Korea*

Abstract

Estradiol, testosterone, progesterone, zeranol and diethylstilbestrol including estradiol metabolites were determined simultaneously in meat. Extraction of growth hormones was carried out by ultasonication using a methanol–water mixture. The growth hormones in the meat extract can be effectively separated from lipids by freezing-lipid filtration, followed by C_8-solid phase extraction (SPE). During freezing-lipid filtration, about 90% of lipids are removed without any significant loss of growth hormones. For further clean-up, silica- and aminopropyl-SPE were used. To enhance detection sensitivity, the growth hormones are derivatized with trimethylsilyl reagents. Quantitation using isotope-labelled internal standards was performed by gas chromatography–mass spectrometry in the selected ion monitoring mode. The method detection limits were 0.1–0.4 μg/kg for all growth hormones. Overall recoveries of synthetic and natural growth hormones were 68–106% with coefficients of variation of 5–16% for the complete procedure.

Keywords: Meat; Food analysis; Growth hormones; Freezing-lipid filtration; SPE clean-up; GC–MS

1. Introduction

Steroid hormones are widely used to improve feed conversion efficiency and promote growth rates in livestock production. The European Union has banned the use of anabolic hormones for growth promotion of cattle, whereas some other countries allow the restricted use of some hormones as growth promoters [1–5]. Many governmental regulatory agencies have dedicated much effort to develop analytical methods for monitoring hormones in domestic and import meat [6,7]. Since the early 1990s, the Korea Food and Drug Administration has also monitored growth hormones in the meat and natural and synthetic hormones including their metabolites have been selected as target compounds.

Analytical methods for the determination, screening and/or confirmation of hormones in various type of sample use gas (GC) and liquid (LC) chromatography as separation techniques [8–18]. Gas chromatography–mass spectrometry (GC–MS) is especially popular in this respect [19]. For the hormones with hydroxyl and/or keto-groups, the application of appropriate derivatization techniques and detection in the selected ion monitoring mode significantly improves the detection sensitivity and selectivity [20].

The conventional approach to determine anabolic steroids in meat involves extraction, followed by a multi-step purification using various sorbents. As extraction method, organic solvent extraction using agitation or shaking is commonly used. During the extraction of the hormones, a large amount of lipids is co-extracted with the target compounds due to their high solubility in organic solvents. During instrumental analysis, the lipids tend to adsorb in parts of the GC system such as the injection port and column, resulting in poor chromatographic performance.

The lipid components of meat are mainly phospholipids, triacylglycerolipids, phosphocholine lipids and cholesterol.

* Corresponding author. Tel.: +82 2 920 0790; fax: +82 2 920 0789.
E-mail address: jongki@kbsi.re.kr (J. Hong).

0021-9673/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.chroma.2004.12.063

They are soluble to varying degrees in various organic solvents and may be present in a sufficient quantity to adversely affect procedures used for clean-up of sample extracts. For the isolation of steroid hormones from lipid extracts, liquid–liquid partitioning with hexane or cyclohexane is commonly used [21,22]. Some of these methods are time-consuming and use large quantities of organic solvents. In addition, liquid–liquid partitioning can cause significant loss of some growth hormones [23,24]. Saponification [25] and acid treatment [26] have also been applied to remove fatty components. Moreover, various set of conditions have been developed according to the chemical properties of the growth hormones for the group separation by polar, phenolic and neutral steroids [27–30]. Downscaling sample size including hexane extraction and saponification, combined with GC–MS^2 has also been recommended to minimize lipid effects [23].

Recently, elimination of lipids by the freezing-lipid filtration method [31] has been applied for the determination of chlorinated pesticides with acetone–*n*-hexane (5:2, v/v) in fish extract. In this study, freezing-lipid filtration was used to eliminate lipids extracted from meat to analyze the growth hormones in the presence of high level of lipids. The aim of this study is to develop a new elimination method of lipids by using freezing-lipid filtration and SPE clean-up to quantify growth hormones in high lipid containing meat by GC–MS.

2. Experiment

2.1. Reagents and chemicals

All solvents were of ultra residue-analysis grade and provided by J.T. Baker (Phillipsburg, NJ, USA). Most of the reference anabolic hormones were obtained from Sigma (St. Louis, MO, USA) and testosterone was obtained from Wako Chemical Industries (Tokyo, Japan). Diethylstilbestrol-d_8 and testosterone-d_3 were obtained from Cambridge Isotope Laboratory (Andover, MA, USA) and other internal standards were purchased from Sigma. The solid phase extraction (SPE) cartridges were from Supelco (Bellefonte, PA, USA) (Envi-ChromP: 0.5 g, C_{18}: 0.5 g) and Phenomenex (Torrance, CA, USA) (StrataNH_2 0.5 g, silica 1 g, C_8 1 g, 6 ml each). The derivatization reagent, *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA), was purchased from Pierce (Rockford, IL, USA). Trimethyliodosilane (TMSI) was obtained from Merck (Darmstadt, Germany). Ammonium iodide (NH_4I) and dithioerythritol (DTE) were purchased from Sigma.

2.2. Preparation of tissue samples and lipid determination

Various muscle tissues of beef cattle (lib loin, shank, brisket and flank) were collected to compare the influence of lipid contents in meat from markets in Seoul, Korea. Meat samples were homogenized (ca. 200 g) and stored at −20 °C until needed. After testing for the presence of anabolic hormones, only meat found to be free from anabolic hormones was used for recovery and spiking samples.

To determine the total intramuscular fat contents, meat (ca. 200 g) was homogenized in a household mixer. A 10 g aliquot of sample was mixed with sodium sulfate, allowed to remove water, and extracted for 24 h using hexane in a Soxhlet extractor. The extract was evaporated to dryness, and the lipid content determined gravimetrically.

2.3. Sample preparation

The method described in this paper used solvent extraction with methanol–water for sample extraction, lipid removal by freezing-filtration, SPE for sample purification, trimethylsilylation (TMS) for derivatization, and GC–MS for separation

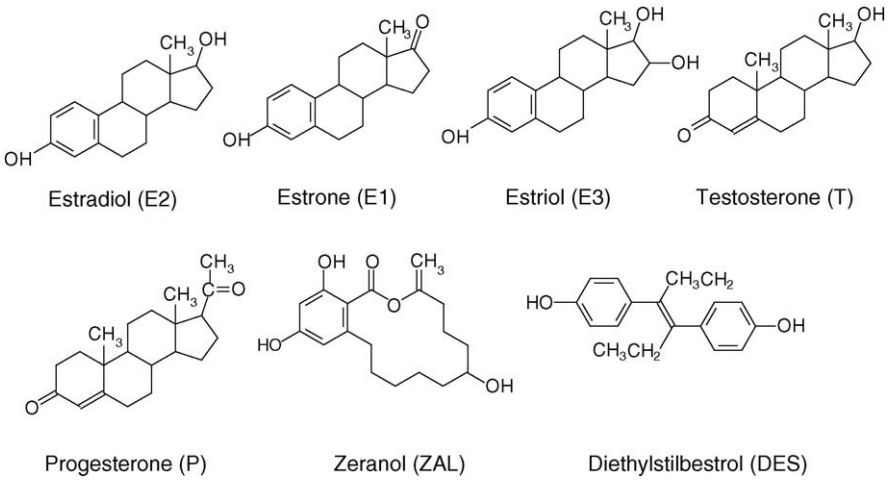

Fig. 1. Chemical structures of target hormones.

and quantification of synthetic and natural hormones (Fig. 1) in meat.

2.3.1. Extraction and delipidation of crude lipids

A 10 ml volume of acetate buffer (0.2 M, pH 5.2) was added to 10 g of ground beef. The samples were molten in a microwave oven (600 W, 1 min). Next, 50 ng of each internal standard and 50 ml of methanol were added. The extraction of the growth hormones was performed with a mixture of methanol–water using ultra-sonication for 10 min at 60 °C in a tightly sealed centrifuge tube. For the removal of lipids, the homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. Suspended and precipitated fat, obtained by storage to 4 °C for 1 h, were filtered and washed with cold methanol. The methanol–water extract was carefully evaporated using a vacuum evaporator because the presence of lipophilic compounds in the solvent led to bumping. The analytical procedure for the determination of growth hormones in meat is depicted in Fig. 2.

For the further delipidation, C_8-SPE column was conditioned with 4 ml methanol and 6 ml deionized water. The extract was quantitatively passed to the top of the column and applied weak vacuum using a sample preparation unit coupled to a vacuum source. The flask and cartridge were rinsed with 4 ml deionized water, followed by 2 ml methanol–water (40:60, v/v). Elution of all growth hormones including estriol was carried out within 4 ml methanol.

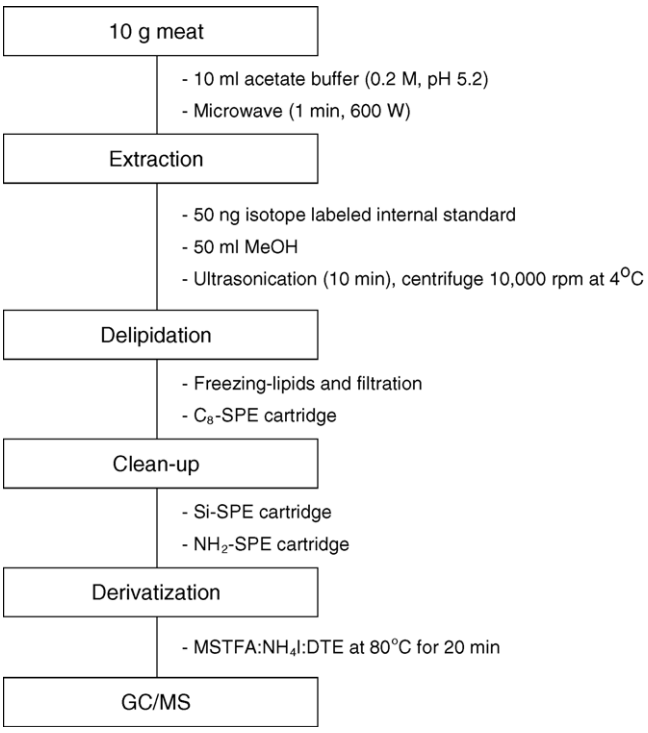

Fig. 2. Analytical procedure for the extraction, clean-up, and derivatization of growth hormones in meat.

2.3.2. SPE clean-up

The dried residue was dissolved in 3 ml *n*-hexane/dichloromethane (60:40, v/v) and applied to a silica-SPE cartridge, which had been conditioned with 6 ml *n*-hexane. After rinse with 3 ml *n*-hexane–ethyl acetate (75:25, v/v), the growth hormones were eluted with 10 ml *n*-hexane–ethyl acetate (25:75, v/v). Eluent was dried under a nitrogen flow and redissolved in 2 ml ethyl acetate–methanol (80:20, v/v) to apply aminopropyl (NH_2)-SPE cartridge.

An NH_2-SPE cartridge was conditioned with 4 ml ethyl acetate–water (water saturated 25:0.68, v/v), followed by 4 ml ethyl acetate–methanol (80:20, v/v). After sample loading on the NH_2-SPE cartridge, the growth hormones were collected with 4 ml ethyl acetate–methanol (80:20, v/v) in a 10 ml tapered glass tube.

2.3.3. Trimethylsilyl (TMS) derivatization

To derivatize the hydroxyl groups and active ketone groups, 40 μl of derivatizing mixture containing MSTFA/NH_4I/DTE (1000:4:2, v/w/w) were added to dried residue. The reaction tube was mixed thoroughly using vortex mixer, followed by heating at 80 °C for 20 min. The resulting solution was analyzed by GC–MS.

2.4. GC–MS and fast atom bombardment (FAB) analysis

The instrument used was an Agilent (Palo Alto, CA, USA) 6890 plus gas chromatograph equipped with a 5973 mass selective detector quadrupole mass spectrometer. Samples were injected by an Agilent 7683 autosampler. The samples were introduced by split mode at a split ratio of 1:10. The injector temperature was set at 250 °C. The GC column was DB-1MS (30 m × 0.25 mm i.d., with 0.25 μm film thickness) with dimethylpolysiloxane phase (J&W Scientific, Folsom, CA, USA). The GC oven temperature was initially maintained at 120 °C for 2 min and then programmed to 250 °C at a rate 15 °C/min, then to 300 °C at 5 °C/min and maintained for 5 min. The temperature of the direct transfer line was maintained at 280 °C. Helium (99.999%) was used as carrier gas at 1 ml/min. The source and quadrupole temperatures were 230 and 150 °C, respectively. The mass selective detector was operated in the electron ionization mode using selected ion monitoring (SIM). The dwell time of each ion was set at 50 ms. The selected ions of target analytes and their respective internal standards used in SIM mode are listed in Table 1.

A fast atom bombardment (FAB) mass spectrometer was used to determine the nature of the lipids extracted from meat and to evaluate SPE clean-up efficiency. FAB mass spectra were obtained with a JEOL JMS-SX102A (Akishima, Tokyo, Japan) high-resolution mass spectrometer (B/E configuration) operating at an acceleration voltage 10 kV with a mass range of 50–2000 amu. Xenon and 3-nitrobenzylalcohol were used as the atom beam and matrix, respectively.

Table 1
Selected ions (*m*/*z*) and retention times (min) of target analytes and their corresponding isotope-labelled internal standards (IS) in SIM mode

Compounds	Abbreviation	RT (min)	Quant. ion (*m*/*z*)	Confirm. ion (*m*/*z*)
Group I				
Diethylstilbestrol-$(OTMS)_2$	DES	12.95	412	397
DES-d_8-$(OTMS)_2$	IS1	12.91	420	405
Group II				
17α-Estradiol-$(OTMS)_2$	E2α	15.41	416	285
17α-Testosterone-$(OTMS)_2$	ET	15.47	432	417
Estrone-$(OTMS)_2$	E1	15.56	414	155
17β-Testosterone-$(OTMS)_2$	T	15.97	432	417
17β-Estradiol-$(OTMS)_2$	E2β	15.80	416	285
Estradiol-d_3-$(OTMS)_2$	IS2	15.77	419	285
Testosterone-d_2-$(OTMS)_2$	IS3	15.96	434	419
Group III				
Zeranol-$(OTMS)_3$	ZAL	17.34	538	433
Taleranol-$(OTMS)_3$	β-ZAL	17.49	538	433
Estriol-$(OTMS)_3$	E3	18.02	504	345
Progesterone-$(OTMS)_2$	P	18.19	458	443
Norprogesterone-$(OTMS)_2$	IS4	17.78	444	429

3. Results and discussion

3.1. Extraction

Both the selection of the solvent and the extraction method can be critical in obtaining a satisfactory recovery of growth hormones from meat. The hormones are only sparingly soluble in non-polar solvents. Moreover, large amounts of lipids were co-extracted when a non-polar solvent such as dichloromethane or hexane was used. Chloroform is the solvent commonly used to extract neutral lipids from animal tissues [32] rather than steroid hormones. Acetone or acetonitrile cannot penetrate meat that has aggregated during ultrasonic agitation. Since the use of only methanol caused precipitation of protein, deproteinization step with water was necessary to improve the extraction efficiency and prevent blocking of SPE cartridge. Methanol–water as extraction solvent was suitable for the extraction of growth hormones including highly polar steroids and reduced the lipid extraction.

3.2. Delipidation

Triacylglycerols and phosphospholipids are major lipids in meat tissue [33]. They cannot easily be eliminated during the several clean-up steps. Delipidation by *n*-hexane causes a significant loss of progesterone. This study proposes a new delipidation method by freezing-lipid filtration without the *n*-hexane wash and without time-consuming column chromatography or saponification. The difference in freezing points between lipids (below about 40 °C) and growth hormones (127–282 °C) in methanol (−98 °C) is significant enough to separate lipids easily by centrifugation at below 4 °C in cold methanol solution. Most of the neutral lipids in the extract were then precipitated as a freezing fat lump. The freezing lipids could be eliminated by simple filtration. Approximately 90 ± 2% ($n = 5$) of lipids were eliminated by this process without any significant loss of growth hormones. The removal efficiency of lipids was determined gravimetrically from the weight of the total lipids and the removed lipids by freezing-lipid filtration. This method is very simple and can greatly reduce the use of extraction solvent, time and effort.

Because of the good solubility of methanol for the polar lipids such as phospholipids, glycoproteins and cholesterol [34], the polar lipids are not effectively eliminated by only freezing-lipid filtration. After freezing-lipid filtration, the extract still contains about 10 ± 2% ($n = 5$) of lipids, equivalent to about 0.2 g in 10 g of meat. Although a significant amount of lipids was eliminated by freezing-lipid filtration, the amount of residual lipids was five orders higher than that of the target analytes at the ng/g level in the extract. Thus, the relatively high amount of the remaining methanol-soluble lipids should be eliminated by column chromatography.

C_8, C_{18}, and styrene/divinylbenzene co-polymeric SPE were tested with various ratios of methanol and water. For C_{18} and styrene/divinylbenzene co-polymeric SPE, polar steroids such as estriol showed poor elution efficiency. When methanol–water (1:1, v/v) was applied as elution solvent on C_8-SPE, the recovery of estriol was unsatisfactory. All analytes were completely eluted from C_8-SPE with 4 ml of pure methanol, 100% eluent with 95–106% of recoveries. To test the separation ability of C_8-SPE for the lipids, each methanol collection and hexane extract of the remaining C_8-SPE were analyzed. Although FAB-MS data are not shown here, the major components of the lipids trapped by the C_8-SPE cartridge were found to be triacylglycerol with fatty acids and glycerophosphocholines. Fig. 3 shows the elution patterns of steroid hormones and lipids on the C_8-SPE cartridge: the methanol-extractable lipids were effectively separated from the steroid hormones.

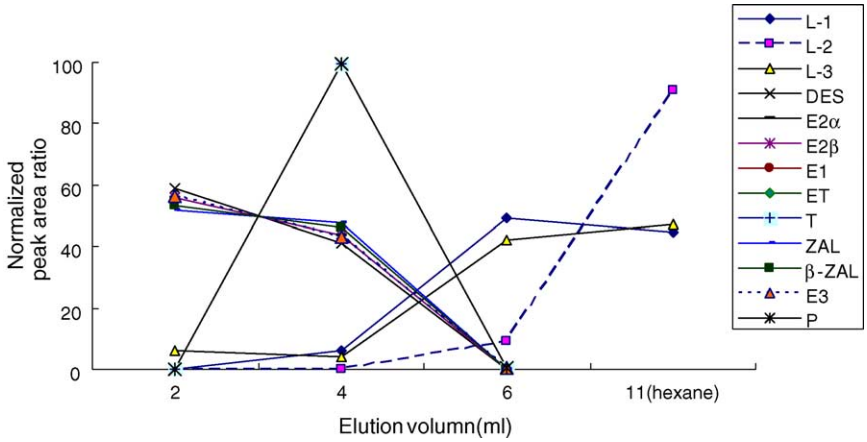

Fig. 3. Elution patterns of growth hormones on C_8-SPE with hexane 5 ml following methanol 4 ml elution: L-1; glycophospholipids, L-2; triacylglycerols, L-3; glycerophosphocholines.

3.3. Clean up by SPE cartridges

Although most of the lipids were removed through freezing-lipids filtration and C_8-SPE, a relatively high amount of polar interferences and steroid-like lipids such as cholesterol were still present in the extract. Additional clean-up was required to reach the ng/g level quantitation in the meat. Silica SPE with *n*-hexane–ethyl acetate (25:75, v/v) as eluent was suitable for the simultaneous elution of phenolic steroids, non-polar neutral steroids and more polar estriol. On the other hand, estriol could not effectively be separated from other steroids when a less polar solvent system, hexane–ethyl acetate (85:15 or 60:40, v/v), was used as eluent. Recoveries of all target compounds were 86–103% on silica-SPE cartridge.

Silica modified with polar aminopropyl groups has been used successfully for the clean-up of various extracts from complex matrices such as sludge, biological tissues and food [35]. With a silica-SPE clean-up alone, the extract was not sufficiently clean to be injected directly on the GC–MS. Aminopropyl SPE could effectively remove acidic compounds extracted from meat. The acidic compounds that were not eliminated by both C_8- and silica SPE caused serious interferences in GC–MS. For all target analytes the recoveries were over than 90%. The dual SPE clean-up gave a clean total ion chromatogram during the entire GC run.

3.4. Derivatization

Because of their thermal instability and low volatility, derivatization of growth hormones is necessary for their detection at sub-ng/g levels by GC–MS analysis. TMS derivatization of keto and alcohol groups was rapidly and simply achieved by using NH_4I instead of TMSI as catalyst [36,37]. The reaction mixture MSTFA/NH_4I/DTE was easy to prepare and remained stable for more than 4 weeks. Although this TMS derivatization of growth hormones can be performed at room temperature, under these conditions repeatability is often poor. Derivatization at 80 °C for 20 min was able not only to decrease the uncertainty of quantitation but also to enhance the analyte detectability.

Under the above conditions, the growth hormones with a keto group were easily converted into enol-OTMS derivatives. Especially for progesterone and norprogesterone, two progesterone-$(OTMS)_2$ derivatives were produced during trimethylsilylation, owing to the formation of different iso-

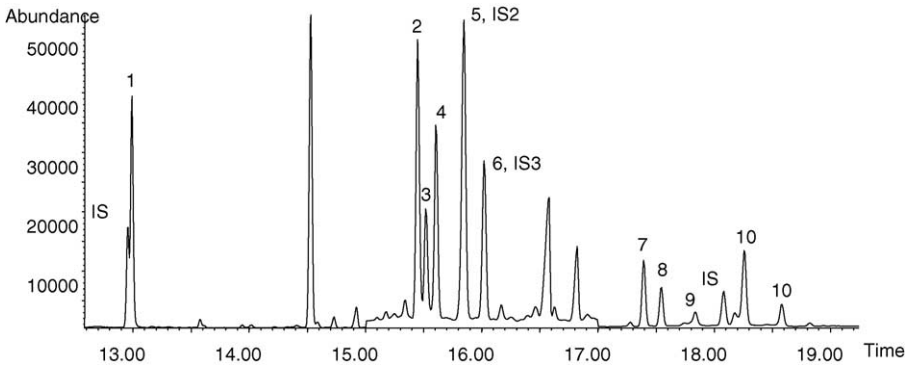

Fig. 4. Total ion chromatogram of trimethylsilylated growth hormones extracted from spiked meat (fortified 5 μg/kg). Peak identity: 1; DES-$(OTMS)_2$, 2; E2α-$(OTMS)_2$, 3; ET-$(OTMS)_2$, 4; E1-$(OTMS)_2$, 5; E2β-$(OTMS)_2$, 6; T-$(OTMS)_2$, 7; ZAL-$(OTMS)_3$, 8; β-ZAL-$(OTMS)_3$, 9; E3-$(OTMS)_3$, 10; P-$(OTMS)_2$.

Table 2
MDLs and recoveries of steroid hormones from spiked meat ($n = 5$)

Compound	MDL (μg/kg)	Mean recovery (RSD) (%)	
		0.5 μg/kg spiked	5 μg/kg spiked
Diethylstilbestrol	0.3	91 (13)	68 (5)
17α-Estradiol	0.1	85 (5)	82 (7)
17β-Estradiol	0.2	129 (10)	84 (7)
Estrone	0.1	97 (8)	77 (10)
Estriol	0.4	70 (15)	68 (16)
Epitestosterone	0.1	96 (3)	82 (9)
Testosterone	0.1	100 (2)	77 (8)
Zeranol	0.2	83 (18)	68 (9)
Taleranol	0.1	93 (12)	106 (6)
Progesterone	0.3	80 (7)	96 (8)

meric enol ethers at the C17 position of the side chain. The isomer pattern was reproducible and was also useful for the identification of progesterone [30].

3.5. Method evaluation

The isotope-labelled internal standards and growth hormone standards were spiked into the control meat, and then extracted, purified, derivatized, and analyzed by the proposed method. A typical total ion chromatogram of the analysis of spiked meat is given in Fig. 4. No significant interferences were observed in this chromatogram. The ratios of the peak areas of the growth hormones and the corresponding internal standards were determined. The calibration curves were generated by a least-squares linear regression analysis. The correlation coefficients for the trimethylsilyl-derivatized growth hormones were higher than 0.994.

The method detection limits (MDLs) and analyte recoveries from 0.5 and 5 μg/kg spiked meat are listed in Table 2. Recoveries were 68–106% with RSD of 5–16% at the higher spiking level. The MDLs of all growth hormones in the meat tested were 0.1–0.4 μg/kg ($n = 7j$ 99% confidence). This ensures a reliable determination at levels lower than the National Minimum Required Performance Limits (2–50 μg/kg for the xenohormones) of EU [38] and the U.S. residue limits for veterinary drugs (0.12–3 μg/kg for natural hormones) [4].

The proposed analytical procedure was used to determine, the growth hormones in meat purchased in the market. No meat sample was found to contain an abnormal concentration of natural or synthetic hormones. The concentrations of the estrogens 17β-estradiol, estriol and estrone were below 0.1 μg/kg, and those of testosterone and epitestosterone below 0.2 μg/kg. DES and zeranol were not detected in edible meat. The concentration of progesterone was 0.3–36.5 μg/kg. It has already been reported that progesterone is naturally present in female cattle from 0.5 up to 40 μg/kg [39]. The progesterone levels in beef, generally, vary with the fat content, breed, sex and age as well as environmental factors. For a positive meat sample containing progesterone (8.9 μg/kg) and testosterone (0.2 μg/kg), the ion chromatograms of testosterone-$(OTMS)_2$ and progesterone-$(OTMS)_2$ are shown in Fig. 5. Two peaks were observed in the GC–MS chromatogram of progesterone. The isomer patterns of progesterone-$(OTMS)_2$ and the internal standard, norprogesterone-$(OTMS)_2$ were diagnostically used for the confirmation of progesterone. The detection and identification of other hormones was achieved on the basis of retention times and selected ion ratios with isotope-labelled internal standards in GC–MS–SIM.

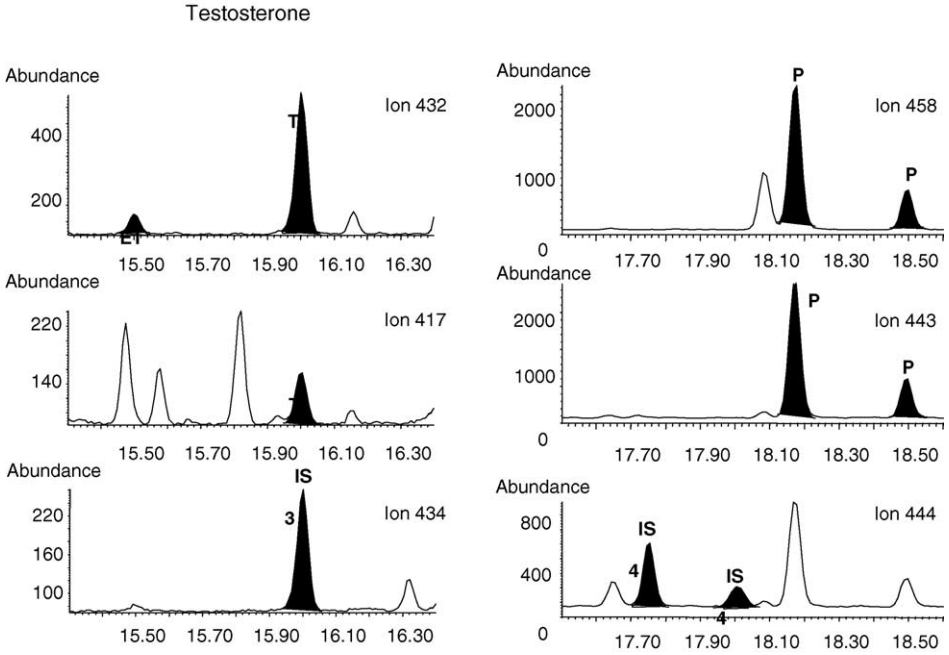

Fig. 5. Extracted ion chromatograms of positive meat sample containing testosterone (0.2 μg/kg) and progesterone (8.9 μg/kg).

4. Conclusions

Freezing-lipid filtration effects an efficient removal of lipids extracted from meat without any significant loss of growth hormones. For a satisfactory final result, the freezing-lipid filtration has to be combined with multiple SPE was simple, consumes little organic solvent, and gives results comparable to those of traditional preparation methods. Hence, the method can be used as a rapid screening tool for the determination of hormones in meat, on the basis of TMS derivatization and GC–MS analysis with isotope-labelled hormones as internal standards. All analytical performance data were fully satisfactory at the analyte concentrations of interest. Future work will explore the possibility of applying the method to determine growth hormones in the presence of high levels of lipids in other types of seafood, milk and butter.

Acknowledgement

We acknowledge financial support from the "Monitoring of Growth Hormones in Meat" project of the Korea Food and Drug Administration.

References

[1] Commission of the European Communities, Council Directive 96/22/EC, Off. J. Eur. Communities: Legis. L125 (1996) 3.
[2] The EU's Ban on Hormones-Treated Meat, CRS Report for Congress, RS20142, 2000.
[3] Blue book, FSIS National Residue Program, FSIS USDA, Washington, DC, 2000.
[4] CFR556.240, CFR556.710, CFR556.540, CFR556.749, CFR556.760, Code of Federal Regulations, Title 21, vol. 11, 2002.
[5] FAO/WHO Joint Expert Committee on Food Additives (JECFA), Residues of some veterinary drugs in animal and food, FAO food and nutrition paper, 41/100, 1997.
[6] D. Behrendt, Microchem. J. 67 (2000) 31.
[7] S.A. Hewitt, M. Kearney, J.W. Currie, P.B. Young, D.G. Kennedy, Anal. Chim. Acta 473 (2002) 99.
[8] A. Koole, Multi-Residue Analysis of Growth Promoters in Food-producing Animals, Universal Press, Veenendaal, The Netherlands, 1998.
[9] O. Nozaki, J. Chromatogr. A 935 (2001) 267.
[10] K. Shimada, K. Mitamura, T. Higashi, J. Chromatogr. A 935 (2001) 141.
[11] S. Hartmann, L. Lacorn, H. Steinhart, Food Chem. 62 (1) (1998) 7.
[12] D.S. Hage, Anal. Chem. 71 (1999) 294R.
[13] B.G. Wolthers, G.P.B. Kraan, J. Chromatogr. A 843 (1999) 247.
[14] P.E. Joos, M.V. Ryckeghem, Anal. Chem. 71 (1999) 4701.
[15] C. Lai, C. Tsai, F. Tsai, J. Wu, W. Lin, C. Lee, J. Clin. Lab. Anal. 16 (2002) 20.
[16] R. Draisci, L. Palleschi, E. Ferretti, L. Lucentini, P. Cammarata, J. Chromatogr. A 870 (2000) 511.
[17] M.J.L. Alda, D. Barcelo, J. Chromatogr. A 892 (2000) 391.
[18] R. Draisci, C. Marchiafava, L. Palleschi, P. Cammarata, S. Cavalli, J. Chromatogr. B 753 (2001) 217.
[19] J. Jodlbauer, P. Zollner, W. Lindner, Chromatographia 51 (11–12) (2000) 681.
[20] J. Segura, R. Venture, C. Jurado, J. Chromatogr. B 713 (1998) 61.
[21] F. Smets, Ch. Vanhoenackere, G. Pottie, Anal. Chim. Acta 275 (1993) 147.
[22] L.V. Look, P. Deschuytere, C.V. Peteghem, J. Chromatogr. 489 (1989) 213.
[23] S. Impens, D. Courtheyn, K.D. Wasch, H.F. De Brabander, Anal. Chim. Acta 483 (2003) 269.
[24] T. Hamoir, D. Courtheyn, H. De Brabander, P. Delahaut, L. Leyssens, G. Pottie, Analyst 123 (1998) 2621.
[25] A.A.M. Stolker, P.W. Zoontjes, L.A. van Ginkel, Analyst 123 (1998) 2671.
[26] M.V. Puymbroeck, L. Leyssens, D. Vanderzande, J. Gelan, J. Raus, Analyst 123 (1998) 2449.
[27] D. Maume, Y. Deceuninck, K. Pouponneau, A. Paris, B. Le Bizec, F. Andrè, Acta Pathol. Microbiol. Immunol. Scand. 109 (2001) 32.
[28] S. Hartmann, H. Steinhart, J. Chromatogr. B 704 (1997) 105.
[29] P. Marchand, B. le Bizec, C. Gade, F. Monteau, F. Andrè, J. Chromatogr. A 867 (2000) 219.
[30] S. Fritsche, G. Schmidt, H. Steinhart, Eur. Food Res. Technol. 209 (1999) 393.
[31] J. Hong, H.Y. Kim, D. Kim, J. Seo, K. Kim, J. Chromatogr. A 1038 (2004) 27.
[32] J. Folch, M. Lees, G.H.S. Stanley, J. Biol. Chem. 226 (1957) 497.
[33] A.E.O. Malau-Aduli, B.D. Siebert, C.D.K. Bottema, W.S. Pitchford, J. Anim. Physiol. Anim. Nutr. 83 (2000) 113.
[34] T.P.L. Ferraz, M.C. Fiúza, M.L.M. dos Santos, L. Pontes de Carvalho, N.M. Soares, J. Biochem. Biophys. Methods 58 (2004) 187.
[35] M. Hennion, J. Chromatogr. A 856 (1999) 3.
[36] M.N. Bakola-Christianopoulou, V.P. Papageorgiou, K.K. Apazidou, J. Chromatogr. 645 (1993) 293.
[37] M.N. Bakola-Christianopoulou, K.K. Apazidou, L. Psarros, Appl. Organomet. Chem. 11 (1996) 205.
[38] S. Impens, K.D. Wasch, M. Cornelis, H.F. De Brabander, J. Chromatogr. A 955 (2002) 281.
[39] S. Fritsche, H. Steinhart, Eur. Food Res. Technol. 209 (1999) 153.