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Determination of residual anabolic steroid in meat by gas chromatography–ion trap–mass spectrometer

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Abstract

The use of natural and synthetic anabolic steroids in animal fattening has been prohibited in Taiwan and many countries because of their potential toxic effect on public health. This paper describes a newly developed gas chromatography–ion trap–mass spectrometry (GC–IT–MS) method for the quantitative determination of various residual anabolic steroids in meat. Anabolic steroid was derivatized with *N*-methyl-*N*-trimethylsilytrifluoroacetamide prior to GC–IT–MS analysis. MS^2 was employed for quantitative measurement. In addition, 2D-estradiol was used as an internal standard. Quantitative determination was based on the ratio of peak area of steroid derivative to peak area of internal standard derivative. Good linearity of each compound, 0.03–1.0 μg/ml, was determined. Solvent extraction was used to extract residual anabolic compounds in meat samples and a solid phase extraction (SPE) procedure was utilized for sample cleanup and pre-concentration. The limits of detection of anabolic compounds approximately ranged from 0.1 to 0.4 μg/kg. The detection limit was comparable with or better than reported methods and was below the minimum required performance limits (MRPLs) established by the European Community (EC). The application of this newly developed method was demonstrated by analyzing various beef, pork, chicken and several animal internal organ samples from local markets.

Keywords: GC–IT–MS; Residual anabolic steroid; Meat

1. Introduction

Anabolic steroids have been used as therapeutic agents for the restoring muscle strength and increasing muscle size and as growth promoting agents for animals. However, the use of natural and synthetic anabolic steroids in food-producing animals has been prohibited in many countries because of the possible toxic effect on public health. Therefore, monitoring the residual content of these compounds has been applied to control the illegal use of steroids.

Immunoassays are often used for inspection of the residual anabolic compounds in meat products. Because of its sensitivity, the method is suitable for the screening of many samples for a small number of steroid substance [1]. Due to the lack of specificity of immunoassay assay, further confirmation analysis is needed to ascertain the test results. Methods utilizing liquid chromatography (LC) with fluorescence or electrochemical detection have been used for the determination of these chemicals in various animal meat and its byproducts [2–4]. These methods are simple and rapid but with limited sensitivity. LC–mass spectrometry (LC–MS) has also been used to measure the anabolic steroids in various samples [5–8]. These methods are with good sensitivity and accuracy; however, they are not accessible in all laboratories for routine analysis due to the high cost of equipment and the requirement of skillful operator. Some Gas chromatography–MS (GC–MS) methods were developed for detecting the residual anabolic compounds in various biological sample matrices (e.g. tissue, urine and blood) and in environmental samples [9–12]. Many of these reported assays focus on the analysis of some specific anabolic steroids or anabolic compounds.

The aim of this study is to develop a general and sensitive analytical method to detect anabolic steroids in meat (beef, pork and chicken) and animal internal organs. The analytical compounds were extracted from meat by acetonitrile; then isolated and pre-concentrated by solid phase extraction (SPE). The isolated anabolic steroids were derivatized with *N*-methyl-*N*-trimethylsilytrifluoroacetamide prior to $GC–MS^2$ measurement.

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2. Experimental

2.1. Chemicals and extraction column

Deionized water was from a Milli-Q system (Millipore, Bedford, MA, USA). HPLC grade acetonitrile and methanol and *n*-hexane were purchased from Mallinckrodt Baker Inc. (Paris, KT, USA). Acetic acid was obtained from Nacalai Tesque (Kyoto, Japan) and 1-propanol was from Riedel-de Häen AG (Seelze, Germany). Sodium sulfate anhydrous was obtained from J.T. Baker Inc. (Phillipsburg, NJ, USA). Hexestrol, diethylstilbestrol, dienestrol, androsterone, estradiol, zeranol, megestrol acetate, norgestrel, 17α-hydroxyprogesterone, α-zearalenol, 2D-estradiol, dithiothreitol (DTE), and *N*-methyl-*N*-trimethylsilytrifluoroacetamide (MSTFA) were purchased from Sigma (St. Louis, MO, USA). Trimethyliodosilane (TMIS) was obtained from Aldrich (Milwaukee, WI, USA). Bond Elut C_{18} SPE cartridge (10 ml, 500 mg) from Vairan Associates, Inc. (Harbor City, CA, USA) was used for sample pretreatment.

2.2. Standard solution

The anabolic steroid stock solutions (100 μg/ml) of each were dissolved in methanol. The stock standards were stored at 4 °C. Working standards were prepared weekly.

2.3. Apparatus and condition

A Microfuge-11 centrifuge (Beckman Instruments, Inc., Fullerton, MA, USA), a Vac Elute SPS24 vacuum station (Varian Inc., Walnut Creek, CA, USA) and a N-EVAP-111 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA, USA) were used for sample preparation.

A Varian CP-3800 GC and a Varian Saturn 2000 GC/MS/MS were used for this study. A DB-5 GC column (0.25 μm film thickness, 0.25 mm × 30 m; Agilent Technologies, Inc., Wilmington, DE, USA) was used for analysis. The injector temperature was maintained at 290 °C. Splitless injection (1 min) was employed and the injection volume was 1 μl. The oven temperature was set at 180 °C initially for 1 min, increased to 240 °C at 6 °C/min, held for 2 min, then increased to 290 °C at 6 °C/min. and held for 10 min at 290 °C. GC–MS transfer line was set 290 °C. Carrier gas was helium and the flow rate was set at 1 ml/min. Electron impact ionization (70 eV) was utilized and the ion source temperature was set at 200 °C. Positive mode was used for detection.

Saturn revision 5.5 software was used for GC–ion trap–MS control and data analysis. For the MS full scan, the condition for data acquisition was under the following condition: *m*/*z* range, 50–650; scan time, 1 s; solvent delay, 6.5 min. Also, the full scan MS–MS acquisition method parameters (3 scans/s, several scan segments with scan events depending on the anabolic steroids to be analyzed; mass range depending upon the selected precursor ion; excitation amplitude between 0.38 and 1.00 V) were employed. Multiple reaction monitoring was employed for quantitative measurement.

2.4. Derivatization of anabolic steroids

The derivatization reaction followed the reported procedures [13,14]. The reaction reagent was prepared as follows: about 0.05 mg DTE were dissolved in a 1 ml of MSTFA with 0.05 ml of TMIS mixture. This reaction reagent was prepared weekly and stored in a tightly sealed brown glass bottle at 4 °C. The anabolic steroid standard solution (100 μl) was dried under nitrogen on a 50 °C block heater (Supelco, Bellefonte, PA, USA) and then dissolved with 100 μl of derivatization reaction reagent in a Reacti-vial (Pierce & Warriner, Chester, UK). The solution was mixed with a vortex and heated at a 60 °C block heater for 30 min. The derivatives were ready for analysis.

2.5. Extraction procedure

Hexane was used to remove the non- or less-polar endogenous substances of meat that were extracted by acetonitrile. 1-Propanol was added to the extract and the mixture was concentrated to dryness and re-dissolved in 3 ml of water–methanol (40/60). A C_{18} SPE cartridge was utilized to remove potential interference and further cleanse the extract. The SPE cartridge was conditioned with methanol, water and acetic acid (0.1%) before loading the sample. Water was utilized to wash the potential interference components and acetonitrile was used to elute the compounds and then collected. This acetonitrile solution was evaporated to dryness under nitrogen gas and then re-constituted with methanol. Detailed sample preparation procedure is shown in Fig. 1. This solution was transferred to a Reacti-vial, evaporated to dryness and the derivatizing mixture was added. The derivatization procedure has been discussed in previous section.

3. Results and discussion

3.1. Derivatives of anabolic steroid derivatives

A commonly used trimethyl-silyation (TMS) derivatization method for ketosteroids was employed for this study [13]. The keto group easily reacted to enol ether by the addition of TMIS and then reacted with the derivatization reagent MSTFA [14]. Dithioerythritol was added to stabilize the derivatization mixture. Table 1 presents the TMS derivatized products and the fragment ions detected by GC–MS. The MS results clearly provide the information of derivatization products of these steroids and multiple derivatized products were found in these compounds. For hexestrol, deithylstilberstrol, and dienestrol, both hydroxyl groups reacted and bis-*O*-TMS derivatives were produced. Androsterone,

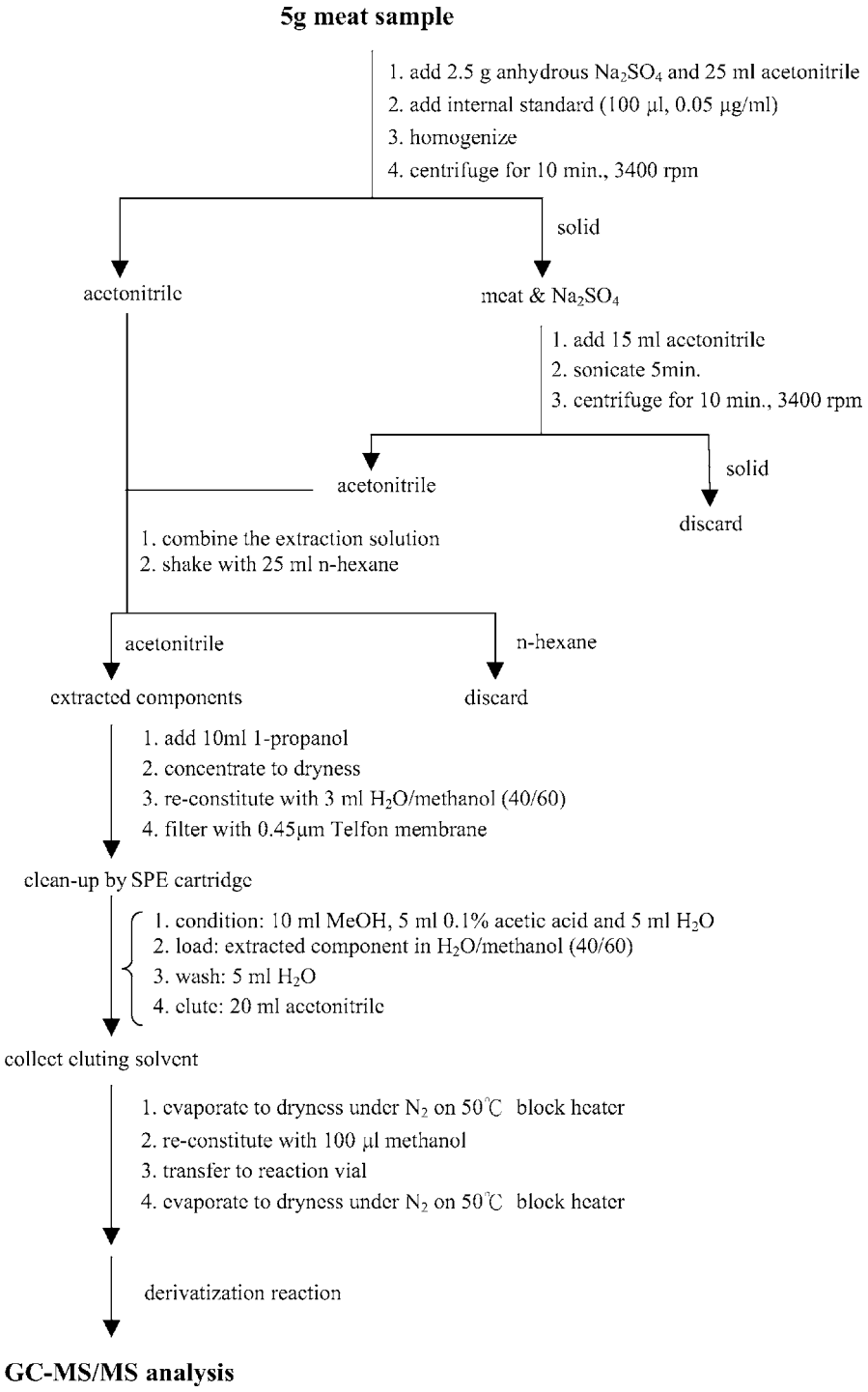

Fig. 1. Extraction procedure.

norgestrel and 17α-hydroxyprogesterone gave bis- or tris-*O*-TMS-derivatized products at all hydroxyl groups and enolic hydroxyl groups taoutomerized from the carbonyl groups as expected. Both zeranol and zearalenol have two acidic phenolic hydroxyl groups as well as one ordinary hydroxyl group. Therefore, tris-*O*-TMS derivatized products were obtained. For megestrol acetate, bis-*O*-TMS was formed. Obviously, the ester carbonyl groups of these compounds did not react. Similar results were found in previously published reports [10,13,15]. For most of the compounds examined, molecular ions of TMS derivatized products were detected in MS. For hesestrol, androsterone and zeranol, $[M–CH_3]^+$ ion was observed. $[M–CH_3COOH]^+$ ion was detected in megestrol acetate.

Table 1
Derivatives of anabolic steroids and their fragment ion detected by GC–MS

Component	Mol. weight	Number of oxygen containing group	Derivative[a]	TMS-ether derivative fragment ion detected
Hexestrol	270	2	bis-*O*-TMS (414)	$[M–CH_3]^+$
Diethylstilbestrol	268	2	bis-*O*-TMS (412)	$[M]^+$
Dienestrol	266	2	bis-*O*-TMS (410)	$[M]^+$
Androsterone	290	2	bis-*O*-TMS (434)	$[M–CH_3]^+$
Estradiol	272	2	bis-*O*-TMS (416)	$[M]^+$
Zeranol	322	4	tris-*O*-TMS (538)	$[M–CH_3]^+$
Megestrol acetate	384	3	bis-*O*-TMS (528)	$[M–CH_3COOH]^+$
Norgestrel	312	2	bis-*O*-TMS (456)	$[M]^+$
α-Zearalenol	320	4	tris-*O*-TMS (536)	$[M]^+$
17α-Hydroxylprogesterone	330	3	tris-*O*-TMS (546)	$[M]^+$

[a] Molecular weight in parenthesis.

3.2. GC–MS^2 results

A temperature programming GC separation was performed to separate these anabolic steroid derivatives. MS^2 was employed in detection to enhance the selectivity and sensitivity. A typical GC-reconstructed ion chromatogram (RIC) of anabolic steroid spiked beef sample extract is shown in Fig. 2. The retention time of each derivative is summarized in Table 2. Although deithylstilbestrol/dienestrol, estradiol/2D-estradiol (internal standard) and zeranol/megestrol acetate pairs were not baseline separated, the determination of these compounds were accomplished by the different characteristic fragmented ions of each component. There were many endogenous components detected in the beef extract sample but they did not appear to interfere with the determination of anabolic steroids by MS^2. This is attributed to good selectivity of tandem mass detection. For these compounds, the alkyl group or siloxy group at side chain is easily to be cleaved in MS^2. The characteristic fragmentation of anabolic steroid derivative has been reported [13,15]. Similar results were observed in this study. Three major characteristic product ions of each

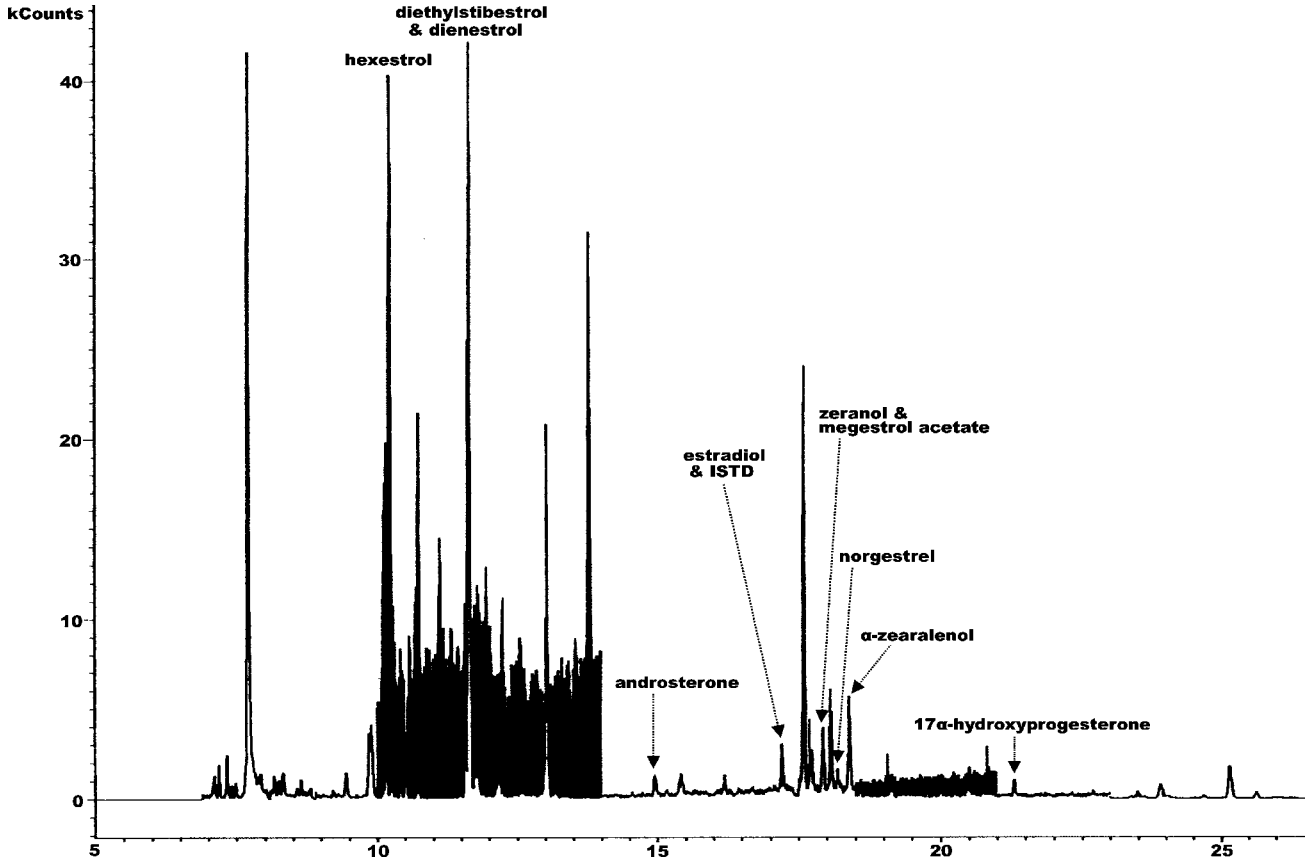

Fig. 2. A typical GC–MS reconstructed ion chromatogram of anabolic steroid (1 μg/kg) and internal standard (1 μg/kg) spiked beef sample extract.

Table 2
Retention time and GC–MS^2 diagnostic ions of steroid derivatives

Component	Retention time (min)	GC–MS^2 precursor ion (m/z)	GC–MS^2 diagnostic ions
Hexestrol	10.18	207	207, 191, 179
Diethylstilbestrol	11.58	412	397, 383, 217
Dienestrol	11.59	410	395, 381, 245
Androsterone	14.85	419	419, 329, 239
Estradiol	17.18	416	326, 285, 244
Zeranol	17.86	433	433, 415, 389
Megestrol acetate	17.88	468	468, 453, 353
Norgestrel	18.11	456	427, 316, 301
α-Zearalenol	18.43	446	414, 324, 295
17α-Hydroxylprogesterone	21.26	546	457, 441, 351

Table 3
Linearity and detection limit of measurement

Component	Quantitation ions (m/z)	Linearity[a]	r^2	Detection limit (ng/g)
Hexestrol	179	$Y = 84.74X - 0.12$	0.9986	0.2
Diethylstilbestrol	383	$Y = 59.65X - 0.39$	0.9973	0.1
Dienestrol	395	$Y = 19.05X + 0.14$	0.9985	0.1
Androsterone	329	$Y = 13.01X - 0.16$	0.9988	0.2
Estradiol	285 + 326	$Y = 19.49X - 0.02$	0.9962	0.1
Zeranol	389	$Y = 14.56X - 0.23$	0.9971	0.1
Megestrol acetate	453	$Y = 11.31X - 0.16$	0.9943	0.2
Norgestrel	316 + 427	$Y = 5.11X + 0.01$	0.9877	0.4
α-Zearalenol	324 + 414	$Y = 25.25X + 0.57$	0.9852	0.1
17α-Hydroxylprogesterone	441 + 457	$Y = 6.21X - 0.05$	0.9968	0.4

[a] Linear range: 0.03–1.0 μg/ml, Y: concentration of analyte (μg/ml), X = peak area of analyte/peak area of ISTD.

derivative were selected as diagnostic ions (as shown in Table 2) for the identification of these components. This is important since co-elution often occurs in complex matrix sample. Additional confirmation ions of target compound are often needed to ascertain the accuracy of analytical result.

3.3. Quantitative analysis

GC–MS^2 and multiple reaction monitoring (MRM) were employed for quantitative measurement. 2D-Estradiol was used as an internal standard for calibrating the variations caused by instrumental, sample preparation and derivatization reaction. Quantitative determination was performed based on the ratio of peak area of each steroid derivative to peak area of internal standard derivative. The linearity of each compound measured by this newly developed GC–MS^2 method was evaluated by a series of steroid standard solution (0.03, 0.05, 0.1, 0.5 and 1 μg/ml). The linearity was good from 0.03 to 1.0 μg/ml and acceptable correlation over the range examined was determined (as shown in Table 3).

Table 4
Intra- and inter-day precision data of the determination of steroid

	Intra-day				Inter-day			
	0.05 (μg/ml) ave ± S.D.[a]	CV[b] (%)	0.5 (μg/ml) ave ± S.D.	CV (%)	0.05 (μg/ml) ave ± S.D.	CV (%)	0.5 (μg/ml) ave ± S.D.	CV (%)
Hexestrol	0.054 ± 0.004	7.4	0.52 ± 0.01	1.9	0.051 ± 0.003	6.0	0.51 ± 0.01	3.9
Diethylstilbestrol	0.053 ± 0.002	3.8	0.51 ± 0.02	3.9	0.051 ± 0.002	3.9	0.52 ± 0.01	1.9
Dienestrol	0.048 ± 0.003	6.2	0.52 ± 0.03	5.8	0.052 ± 0.004	7.7	0.52 ± 0.02	3.8
Androsterone	0.051 ± 0.002	3.9	0.51 ± 0.01	2.0	0.051 ± 0.001	2.0	0.50 ± 0.02	4.0
Estradiol	0.049 ± 0.001	2.0	0.51 ± 0.01	2.0	0.050 ± 0.002	4.0	0.48 ± 0.01	2.1
Zeranol	0.050 ± 0.002	4.0	0.51 ± 0.02	3.9	0.052 ± 0.003	5.8	0.50 ± 0.02	4.0
Megestrol acetate	0.056 ± 0.004	7.1	0.48 ± 0.03	6.3	0.055 ± 0.003	5.5	0.49 ± 0.02	4.1
Norgestrel	0.055 ± 0.004	7.3	0.47 ± 0.03	6.4	0.056 ± 0.004	7.1	0.48 ± 0.01	2.1
α-Zearalenol	0.048 ± 0.002	4.2	0.54 ± 0.02	3.7	0.048 ± 0.002	4.2	0.52 ± 0.02	3.8
17α-Hydroxylprogesterone	0.058 ± 0.005	8.6	0.46 ± 0.02	4.3	0.056 ± 0.004	7.1	0.48 ± 0.03	6.2

[a] ave ± S.D.: average ± standard deviation (μg/ml) ($n = 3$).
[b] CV: coefficient of variation.

Table 5
Recovery of steroid added sample

Component	Beef ave ± S.D.[a]	Pork ave ± S.D.	Chicken ave ± S.D.	Pig liver ave ± S.D.	Chicken liver ave ± S.D.
Hexestrol	85.7 ± 3.1	84.6 ± 3.0	104.3 ± 3.1	101.2 ± 5.6	91.2 ± 6.0
Diethylstilbestrol	85.1 ± 2.1	80.1 ± 3.1	85.7 ± 3.3	96.2 ± 7.5	89.4 ± 7.4
Dienestrol	89.2 ± 2.4	89.5 ± 4.0	99.6 ± 2.4	79.3 ± 6.5	84.2 ± 2.8
Androsterone	83.4 ± 2.9	91.0 ± 4.2	87.3 ± 5.9	87.5 ± 2.8	99.5 ± 8.9
Estradiol	82.3 ± 3.0	95.8 ± 4.1	95.2 ± 3.0	91.2 ± 9.1	91.4 ± 4.5
Zeranol	85.8 ± 5.6	95.3 ± 2.7	90.2 ± 5.6	100.4 ± 3.2	99.1 ± 7.9
Megestrol acetate	97.7 ± 7.0	96.1 ± 9.2	95.5 ± 3.0	92.6 ± 3.8	84.0 ± 6.9
Norgestrel	93.0 ± 6.1	93.7 ± 8.3	91.3 ± 8.0	81.2 ± 5.2	86.2 ± 8.2
α-Zearalenol	93.6 ± 3.0	94.6 ± 4.6	101.3 ± 3.0	86.3 ± 5.4	84.3 ± 3.0
17α-Hydroxylprogesterone	82.5 ± 5.4	102.4 ± 7.0	100.7 ± 5.4	83.6 ± 2.2	91.9 ± 4.4

[a] ave ± S.D.: average ± standard deviation ($n = 3$).

The precision of this method was measured by replicated analyses of standard solutions and the results are summarized in Table 4. Standards were prepared and analyzed each day. A total of three series of standards were examined over a week-long period and each standard was measured in triplicate. The intra- and inter-day precisions showed a coefficient of variance (CV) ranging from 1.9 to 8.6 and 1.9 to 7.7%, respectively.

3.4. Sample preparation

The enzymatic hydrolysis to cleave steroid glucuronide or sulfate conjugate was suggested in previously reported method [16]. However, some references reported that no significant hormone liberation by enzymatic hydrolysis was determined in muscle and fatty tissues and meat [17,18]. Therefore, the enzymatic hydrolysis procedure was not performed in this study.

Due to the complex nature of animal tissue sample, a pre-treatment procedure is often required to remove protein, fat, and potential interferences from sample matrix. Solvent extraction combined with solid phase extraction (SPE) has been demonstrated as an effective sample pre-treatment procedure for a variety of meat samples [19,20]. In addition, twin SPE (amino and silica) cartridge system has been utilized for the pre-treatment of anabolic steroids in hair and meat sample prior to GC-analysis [15,21]. In this study, a new sample preparation procedure which used single SPE column was developed (as shown in Fig. 1).

In addition, it was found that TMS-derivatives were sensitive to hydrolysis; thus, extra precautions must be taken. The stability of meat extract derivatives under different storage conditions was examined. There was no degradation observed in 3 days if kept in a desiccator at 4 °C in a sealed vial before injection.

3.5. Recovery

In order to examine the applicability of this newly developed procedure, the recoveries of spiked steroids (1 ng/g) in

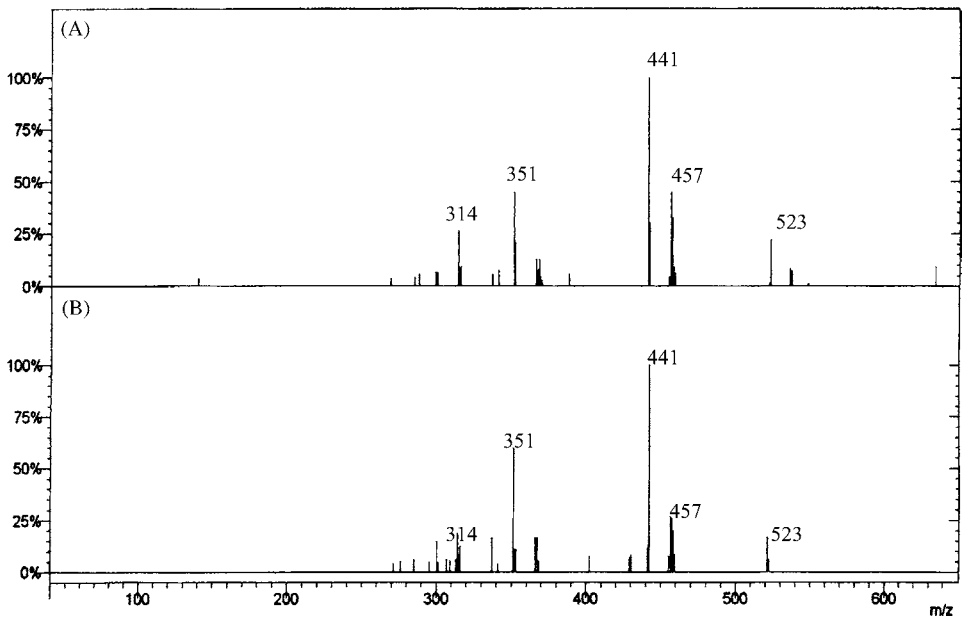

Fig. 3. The MS^2 spectra of: (A) 17α-hydroxylprogesterone standard; (B) 17α-hydroxylprogesterone from chicken liver extract.

various meat (beef, pork and chicken) and animal internal organ (chicken liver and pig liver) samples were examined. The results are summarized in Table 5. Good recoveries ranging from 79.3 to 104.3% were determined, which indicated that this sample preparation method was suitable for the analysis of steroid in various meat samples. In addition, the detection limit of each steroid ranged approximately from 0.1 to 0.4 ng/g (as shown in Table 3) based on a signal-to-noise ratio of 3. The detection limit was comparable with or better than reported GC–MS methods [12,15] and was below the minimum required performance limits (MRPLs) established by the EC.

3.6. Real sample

Twelve meat (pork, chicken, lamb and beef) and five animal internal organ (chicken liver, pig liver) samples purchased from local supermarkets were examined by this newly developed method. Four of the meat samples were positively tested by immunoassays method; however, no residual steroid was measured in all meat samples by this newly developed method. These samples have been further examined by LC–MS and no residual steroid was found. It has been known that immunoassay has a tendency to produce false results and this newly developed method is a good confirmation method to assure the accuracy of analysis. Approximately 4 μg/kg of 17α-hydroxylprogesterone was determined in one of the chicken liver samples. The GC–MS^2 spectra of standard and sample were shown in Fig. 3 and good agreement between these two spectra was observed. It demonstrated the capability of this newly developed method for the analysis of anabolic steroid in animal tissue samples.

4. Conclusion

In this paper, an analytical method to determine eleven commonly used anabolic steroids in meat was developed. The steroid was extracted from meat by a solvent extraction/SPE procedure and derivatized by MSTFA prior to GC–MS^2 measurement. Good linearity and precision of this newly developed assay were determined. In addition, a newly developed sample pre-treatment procedure effectively removed the potential matrix interferences from endogenous substances of meat. Good recoveries ranging from 79.3 to 104.3% were obtained. The detection limit of this assay was comparable with or better than reported GC–MS methods and was below the minimum required performance limits (MRPLs) established by the EC. This newly developed method has been successfully applied to analyze various meat and animal internal organ samples.

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References

[1] C.J.M. Arts, M.J. Van Braak, H. Van den Berg, R. Schilt, P.L.M. Berende, J.M.P. Den Hartog, Arch. Lebensmittelhyg 41 (1990) 58.
[2] R. Gonzalo-Lumbreras, R. Izquierdo-Hornillos, J. Chromatogr. B 794 (2003) 215.
[3] R. Verbeke, J. Chromatogr. 177 (1979) 69.
[4] H. Yamada, K. Yoshizawa, T. Hayase, J. Chromatogr. B 775 (2002) 209.
[5] M. Fiori, E. Pierdominici, F. Longo, G. Brambilla, J. Chromatogr. A 807 (1998) 219.
[6] H. Shibasaki, T. Furuta, Y. Kasuya, J. Chromatogr. B 692 (1997) 7.
[7] H. Zhang, J. Henion, Anal. Chem. 71 (1999) 3955.
[8] G. Singh, A. Gutierriz, K. Xu, I.A. Blair, Anal. Chem. 72 (2000) 3007.
[9] K. Shimada, K. Mitamura, M. Shiroyama, K. Yago, J. Chromatogr. A 847 (1999) 171.
[10] S. Hartmann, H. Steinhart, J. Chromatogr. B 704 (1997) 105.
[11] H.J. Leis, G. Fauler, G.N. Rechberger, W. Windischhofer, J. Chromatogr. B 794 (2003) 205.
[12] W.H. Ding, C.C. Chiang, Rapid Commun. Mass Spectrom. 17 (2003) 56.
[13] B. Le Bizec, M.P. Montrade, F. Monteau, F. Andre, Anal. Chim. Acta 275 (1993) 123.
[14] M. Donike, J. Zimmermann, J. Chromatogr. 202 (1980) 483.
[15] S. Impens, K. De Wasch, M. Cornelis, H.F. De Brabander, J. Chromatogr. A 955 (2002) 281.
[16] B. Bergner-Lang, M. Kachele, Dtsch. Lebensm. Rundsch. 77 (1981) 305.
[17] F. Busico, G. Moretti, G.P. Cartoni, F. Rosati, J. High Resolut. Chromatogr. 15 (1992) 94.
[18] E.A.I. Daeseleire, A. De Guesquiere, C.H. Van Peteghem, Chromatogr. Sci. 30 (1992) 409.
[19] M.R. Fuh, S.Y. Chu, Anal. Chim. Acta 499 (2003) 215.
[20] M.R. Fuh, S.A. Chan, Talanta 55 (2001) 1127.
[21] Y. Gaillard, F. Vayssette, A. Balland, G. Pepin, J. Chromatogr. B 735 (1999) 189.