Screen for Steroids using Gas Chromatography-Mass Spectrometry

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ABSTRACT

The purpose of this procedure is to serve as a general guide for the qualitative analysis of samples for the presence of anabolic-androgenic steroids (AAS). After the sample is extracted with acetonitrile, the extract is examined using gas chromatography with mass spectral detection (GC-MS). The mass spectra associated with the observed components of the sample are compared to mass spectral databases to tentatively identify the steroid. This identification must be confirmed by comparison of the retention time and mass spectrum to those of a standard, which is analyzed under the same conditions. Alternatively, the chromatographic method can be calibrated using a mixture of n-alkanes so that the retention times can be expressed as retention indices. This method corrects for differences in conditions across instruments, provided that similar chromatographic columns (USP G27, G36 and G41) are used. Characteristics (retention index, molecular weight) are presented for 92 steroids that have been encountered in the laboratory. Four types of consumer products that the Forensic Chemistry Center (FCC) typically encounters were analyzed to evaluate method effectiveness.

INTRODUCTION

There are medically valid uses of AAS such as in the treatment of androgen deficiency (for example, low testosterone), to lessen the effects of protein catabolism associated with prolonged use of corticosteroids, and in the treatment of metastatic breast cancer and some anemias [1]. However, the illicit use of these substances is of far greater concern. In 2013, a review estimating the lifetime prevalence of steroid abuse suggested that between 2.9 and 4 million people in the United States have abused AAS with the likely goal of increasing muscle mass and strength [2]. These substances are generally prohibited in competitive sports by the World Anti-Doping Agency (WADA) [3]. They are also listed under schedule III of the Controlled Substances Act when they meet certain criteria as defined in the Anabolic Steroids Control Act of 2004 and the Designer Anabolic Steroid Control Act of 2014. These criteria are: the substance is chemically related to testosterone; the substance is pharmacologically related to testosterone; the substance is not an estrogen, a progestin or a corticosteroid and the substance is not dehydroepiandrosterone (DHEA) [1].

As part of our role as an analytical resource in support of criminal investigations, the FCC frequently encounters AAS in a variety of formulations, from injectable oil-based solutions to tablets, capsules, and powders that are promoted as dietary supplements. There are challenges associated with this analysis, not the least of which are the large number of such substances, and the propensity for variations on the theme (designer steroids) [4]. The guidelines governing steroid nomenclature continue to evolve [5]. In addition, the labeling of steroid ingredients in products marketed as dietary supplements often does not follow standard steroid nomenclature rules and, at times, may mislead the consumer. Fortunately, as some recent publications illustrate [6,7], there is a well-developed set of broadly applicable and information-rich analytical methods centered on mass spectrometry, which possess adequate discriminating power to enable these substances to be detected, characterized and/or identified. The purpose of this document is to present a simple methodology which has proven to be generally useful for the detection and identification of AAS in a variety of matrices for forensic purposes. An in-house mass spectral database has also been developed to assist in the identification of emerging compounds whose spectra are not included in commercial reference libraries. This mass spectral library is regularly updated as new compounds are encountered and identified and is suitable for dissemination to field laboratories.

EXPERIMENTAL

Equipment

Four electron ionization source (EI, 70 eV) GC-MS systems with 5% diphenyl, 95% dimethyl-polysiloxane, 0.25 mm ID, 0.25 µm film thickness columns were used for method validation:

System 1. Agilent 6890N, 7683B injector, 5975i MSD, ChemStation D.02.00.275, with column, nominal length 35 m including 5 m guard, Phenomenex Zebron ZB-5HT Inferno, Catalog No. 7HG-G015-11GGA.
System 2. Agilent 7890A, Combi-PAL injector, 5975C XL MSD, ChemStation E.02.00.493, with column, nominal length 35 m including 5 m guard, Restek RTX-5MS, Catalog No. 12623-124.
System 3. Agilent 6890N, 7683B injector, 5973N MSD, ChemStation E.02.00.493, with column, nominal length 30 m (no guard), Restek RTX-5MS, Catalog No. 12623.
System 4. Agilent 6850, Combi-PAL injector, 5975C VL MSD, ChemStation E.02.00.493, with column, nominal length 30 m (no guard), Agilent HP-5MS, Catalog No. 19091-433E.

Pierce Reacti-Therm Heating Module No. 18870 with Reacti-Vap Evaporating Unit Model 18780.

Mass Spectral Libraries (multiple editions): NIST Mass Spectral Library, Wiley Registry of Mass Spectral Data, Wiley Mass Spectra of Designer Drugs, FCC user-generated mass spectral library.

Procedures

The method parameters listed in Table 1 are employed at FCC and are recommended for general screening. The lower initial oven temperature presented in Table 1 allows for detection of lower molecular weight components of interest. Steroid compounds do not typically fall into this category, therefore, when steroids are the only compounds of interest, a higher initial oven temperature is preferred. The oven temperature presented in Table 2 affords elution of steroid compounds within 30 minutes. For method validation, retention time indices for all 92 steroids were initially determined on four different GC-MS systems using the higher initial oven temperature and the parameters in Table 2. Retention time indices were determined using the general screening parameters on System #3 and were found to be consistent with the values determined from the initial method validation (Table 6). When additional components besides steroids are suspected or when the target analytes are unknown, it is recommended that a TMS-derivative be prepared and analyzed as described in the Sample Preparation section below.

Table 1. GC-MS Parameters Applicable to General Screening

Carrier Gas Settings (helium)	
Flow rate/nominal	1.0 mL/min
Average linear velocity	~30 cm/sec
Initial Pressure	varies (generally 9 to 14 psi)
Mode	constant flow
Injection parameters	
Mode	20:1 split
Volume (µL)	1
Temperature (ºC)	250
GC parameters	
Oven initial temperature	75 ºC
Initial time	1.0 min
Ramp rate	12 ºC/min
Final temperature	330 ºC
Final/Hold time	12.75 min
Run time	35 min
MSD transfer line temperature	280 ºC
MSD acquisition	
Mass range	40-550 Da
Scan mode	full
Ionization	EI
Solvent delay	3.5 min
Threshold (counts)	150
MS Quad	150 ºC
MS Source	230 ºC

Table 2. GC-MS Parameters Used for Method Validation

Equipment (instrument/column)	**System 1**	**System 2**	**System 3**	**System 4**
Carrier settings (carrier gas helium)				
Flow rate/nominal (mL/min)	1	0.8	0.7	0.6
Ave. linear velocity (cm/sec)	35	32.3	32	29
Pressure (psi)	15.4	11.3	8.3	6.6
Mode	constant flow	constant flow	constant flow	constant flow
Injection parameters				
Mode	20:1 split	20:1 split	20:1 split	20:1 split
Volume (µL)	1	1	1	1
Temperature (ºC)	250	280	250	250
GC parameters				
Oven initial temperature (ºC)	140	140	140	140
Initial time (minutes)	1.0	1.0	1.0	1.0
Ramp rate (ºC/min)	12	12	12	12
Final temperature (ºC)	330	330	330	330
Final/Hold time (minutes)	12.75	12.75	12.75	13
Run time (minutes)	~30	~30	~30	~30
MSD transfer line temperature (ºC)	280	280	280	280
MSD acquisition				
Mass range (Da)	40-550	33-533	40-550	40-600
Scan mode	full	full	full	full
Ionization	EI	EI	EI	EI
Solvent delay (minutes)	3.0	2.5	4.0	3.5
Threshold (counts)	150	100	150	100
MS Quad (ºC)	150	150	150	150
MS Source (ºC)	230	230	230	230

Reagents and Standards

1. Acetonitrile, HPLC grade: Fisher Scientific or equivalent
2. Methanol, HPLC grade: Fisher Scientific or equivalent
3. Hexanes, pesticide grade: Fisher Scientific or equivalent
4. Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA): Supelco or equivalent
5. Pyridine, certified A.C.S. grade: Fisher Scientific or equivalent
6. Oils
    a. Canola Oil; Kroger Co.
    b. Corn Oil; Kroger Co.
    c. Cottonseed Oil; Sigma Chemical Co.
7. Standards
    a. Steroid standards available from, but not limited to, the following manufacturers:
    Steraloids
    Toronto Research Chemicals

Sigma-Aldrich
U.S. Pharmacopeial Convention

b. Hydrocarbon Test Mix, C7-C40 Saturated n-alkanes, 1000 µg/mL in hexane; Sigma-Aldrich
c. Steroid Test Mix stock standard, 1 mg/mL in acetonitrile, used for system standardization:
    1. diethylstilbesterol; USP
    2. testosterone; Sigma
    3. methyltestosterone; Steraloids
    4. testosterone enanthate; Steraloids
    5. testosterone cypionate; Steraloids
    6. testosterone decanoate; Steraloids
d. Spiking solution, 1 mg/mL in acetonitrile
    1. 17β-estradiol 17-cypionate; Sigma
    2. formebolone; Istituto Famaceutico S.P.A.
    3. oxymetholone; Steraloids
    4. testosterone; Sigma
    5. dehydrochloromethyltestosterone; Steraloids

Standard Preparation

System Standardization: A dilution of the Steroid Test Mix was prepared in acetonitrile at a concentration of approximately 125 µg steroid/mL acetonitrile. For the optional establishment of retention indices, a solution of the saturated n-alkanes standard (C7-C40) was prepared from the Hydrocarbon Test Mix at a concentration of 125-250 µg n-alkane/mL in hexane. Both the Steroid Test Mix and Hydrocarbon Test Mix were stored in the refrigerator. No formal stability testing was performed to establish standard solution shelf life; however empirical laboratory evidence indicates that the steroids are stable in acetonitrile for 12 months or longer when refrigerated. Confirmatory standard solutions should be prepared in acetonitrile and analyzed at a concentration comparable to that of the component being identified.

Sample Preparation

General Considerations

Many steroids contain an ester and/or alcohol functional group. Due to the possible transesterification and methylation effect of methanol on these groups over time, acetonitrile is the preferred solvent, if the extract will be stored for an extended period of time. Both acetonitrile and methanol were used as the extraction solvents in the experiments presented here. Although no formal stability study was performed on the sample extracts, it is expected that they will perform similarly to the standard solutions. A confirmatory standard solution of the steroid component being identified should be prepared in acetonitrile or methanol at a comparable concentration.

Preparation for sample screening

1. If working with a known concentration of a steroid, whether liquid or solid, appropriate dilutions should be made with acetonitrile to achieve a concentration between 100 and 200 µg/mL.

2. If working with a liquid or solid of unknown concentration, use the following guidelines:

a. Oil/liquid
   Transfer approximately 1 mL to a glass scintillation vial.
b. Tablets/capsules
   Prepare a composite from an appropriate number of individual dosage units. Transfer the equivalent of one-half to one-unit content weight to a glass scintillation vial.
c. Bulk powder
   Transfer approximately 0.2-0.5 grams powder to a scintillation vial.

Add 5 mL extraction solvent and sonicate for 20 minutes. Filter through a 0.2-0.45µm Nylon or PTFE filter. Analyze. Depending on instrument sensitivity, oil and liquid samples can be prepared at a lower concentration of 5% (v/v) rather than the initial 20%.

3. When silyl derivatization is needed, proceed as follows [8]:
   a. Analyte of known concentration as stated on the label.
      To achieve an on-column concentration of approximately 100 to 200 µg/mL, transfer the appropriate amount of acetonitrile extract/sample preparation to an autosampler vial. Evaporate to dryness under a stream of dry air or nitrogen using a Reacti-Vap module set at approximately 70ºC. Add 200 µL pyridine and 200 µL BSTFA. Incubate at approximately 70ºC for 30 minutes. Analyze.
   b. Analyte of unknown concentration or total unknown.
      Transfer 40 µL of acetonitrile extract/sample preparation to an autosampler vial and proceed as described for derivatization of analytes of known concentration.

      Preparation of silylated extracts is seldom required for the identification of steroid compounds. Stability studies on a limited number of silylated samples and standards indicated no additional peak formation over a 48-hour period. When derivatization is warranted, whether it be for a steroid or a non-steroid compound, it is recommended that the silylated solutions be analyzed within 24 hours of preparation.

RESULTS AND DISCUSSION

General approach for the examination of the TIC of samples suspected of containing steroids

In the case of a targeted screen (e.g., a steroid is declared on the label), inspect the total ion chromatogram (TIC) for the sample and standard(s) analyzed. An identification may be declared if the sample contains a peak with a retention time and/or retention index matching the retention time and retention index of the respective standard within established limits (generally ± 2% for retention time and ±1% for retention index), and there is correspondence of full scan mass spectral characteristics [9, 10]. When comparing retention time, it is to be noted that the center of a peak shifts to longer retention times with increasing concentration, but the start of the leading edge remains the same. In cases of steroids where isomers (e.g., androstenediol) or diastereomers (e.g., 4-estren-3,17-diol) are encountered, the electron ionization (EI) spectra of the various forms are generally indistinguishable, but retention times/retention indices could differ, thus leading to a tentative identification.

In the absence of a label declaration, but with reasonable suspicion that a steroid may be present in a sample (information from CSO, OCI agent, or previous experience), the mass spectrum of each peak in the TIC should be searched against the available databases. A tentative identification may be declared if the sample contains a peak with a mass spectral match to entries in the databases. Due to the generally information-rich characteristics of steroid mass spectra, library match qualities will often be high (>90%) but lower match quality hits should be investigated when few search results are returned. In our experience at the FCC, the match quality can be heavily weighted toward higher mass fragments and sometimes the top spectral matches do not correspond well with the spectrum of the unknown. In these instances, the actual compound present in the sample extract could correspond to a lower match quality entry, hence it is imperative that the experienced analyst carefully compare the spectrum from the library with the spectrum of the unknown. The analysis is completed by running the appropriate confirmation standard, and by comparing retention times as well as mass spectra.

A large variety of anabolic-androgenic steroids in different formulations has been encountered by the FCC. Dosage forms have included injectable oil-based solutions, tablets, capsules, and bulk powders. Three sample matrices (bulk powder, capsule, oil) were selected to evaluate the applicability of the method at a target level of 200 µg AAS per milliliter of sample solution. Five steroids with significant structural differences were selected to prepare a spiking solution, by combining approximately 10 mg of each of the standards in 10 mL of acetonitrile. Following the preparation scheme for samples of known concentration, capsule and bulk powder samples were weighed into scintillation vials and the appropriate volume of spiking solution added to each weighing. If necessary, the spiking solution was evaporated to dryness under a stream of air prior to the addition of extraction solvent. Canola, corn, and cottonseed oils were also selected for fortification. An aliquot equivalent to 1 mg each AAS was placed in a scintillation vial and taken to dryness. Approximately 1 gram of oil was added to the vial followed by the addition of extraction solvent. Each of the five steroids in the spiking solution was observed in the analysis of the fortified sample matrices. A summary of the fortified matrices is shown in Table 3.

Table 3. Spike Experiments

Matrix	Level of Fortification Observed (µg/mL)	Spike Concentration in the Matrix (mg/g)
Capsule composite	200	0.9
Capsule composite	200	3
Capsule composite	200	1
Corn oil	200	0.9
Canola oil	200	0.9
Cottonseed oil	200	1
Bulk powder	200	2
Bulk powder	200	2

Reporting

The labeling of steroid ingredients in dietary supplements can be ambiguous and misleading since standard steroid nomenclature rules are often not followed. In addition, for any given molecular formula there exist many possible stereo and positional isomers. These factors highlight the importance of using accurate, accepted nomenclature when reporting the presence of a steroid utilizing this approach. When a Schedule III steroid is identified, the primary name listed on the DEA controlled substances list should be used for reporting. If an AAS is identified that is not a scheduled substance, unambiguous terms should be used (IUPAC name, CAS #, etc.). The use of alternate names, synonyms, and common names are acceptable as long as the primary name (DEA name, IUPAC name) is listed first.

Creation and utilization of retention indices

The use of retention indices is not required, but it is helpful when dealing with complete unknowns, when distinguishing isomers that have the same mass spectra but different retention times, or when comparing data obtained from different instruments to look for common adulterants. This is advantageous when a number of analysts may be involved in an assignment, or when comparing data from one laboratory to another.

Prior to analyzing samples, each instrument was calibrated according to manufacturer and laboratory specifications with a standard autotune. The Hydrocarbon Test Mix and Steroid Test Mix were used to establish retention indices based on the non-isothermal Kovats' Retention Index formula [11]. Simplified, the Kovats' equation is

$$I_x = 100n + 100(t_x - t_n) / (t_{n+1} - t_n)$$

where t_n and t_{n+1} are the retention times of the reference n-alkane hydrocarbon eluting before and after the compound of interest "x"; t_x is the retention time of compound "x".

While retention times vary with the individual chromatographic system (e.g., column length, film thickness, diameter, carrier gas velocity and pressure, void time), retention indices are independent of these parameters, and allow for the comparison of values measured by different systems under varying conditions. Tables of retention indices can be used to tentatively identify components by comparing experimentally found retention indices with known values. The retention indices for the components in the steroid test mix were determined on the four GC-MS instruments described above. The absence of significant variability observed, as shown in Tables 4 and 5, demonstrates the ruggedness of this approach, and suggests the potential for utilizing the retention indices established in this LIB on similar chromatographic systems in other laboratories.

Table 4. Retention Index versus Instrument Variability

	System 1[a]	System 2	System 3[a]	System 4	SD[b]
Diethylstilbesterol	2364 (1)	2354	2381 (0.1)	2388	0.7
Testosterone	2728 (0.9)	2729	2759 (0.5)	2740	0.5
Methyltestosterone	2756 (0.8)	2757	2785 (0.3)	2766	0.5
Testosterone Enanthate	3421 (0.7)	3350	3452 (0.4)	3421	1.3
Testosterone Cypionate	3670 (0.9)	3653	3708 (0.4)	3678	0.6
Testosterone Undecanoate	3869 (0.9)	3849	3902 (0.2)	3874	0.6

[a] The number in parentheses denotes the variability in retention indices (RI) as a percent relative standard deviation established from runs of the test mix over a three-week period.

[b] This column denotes the percent relative standard deviation of RI established from the four different systems.

Table 5. Retention Time Variability on Systems 1 and 3

	System 1[a]	System 3[a]
Diethylstilbesterol	11.662 (0.003)	12.093 (0.01)
Testosterone	13.997 (0.003)	14.473 (0.01)
Methyltestosterone	14.162 (0.003)	14.628 (0.01)
Testosterone Enanthate	17.809 (0.004)	18.297 (0.01)
Testosterone Cypionate	19.529 (0.007)	20.177 (0.02)
Testosterone Undecanoate	21.372 (0.01)	22.100 (0.02)

[a] The number in parentheses denotes the variability in retention times (RT) as a percent relative deviation established from runs of the test mix over a three-week period from two different systems.

The Hydrocarbon and Steroid Test Mixes were analyzed weekly, over a three-week period on Systems 1 and 3, and once on Systems 2 and 4. As shown in Tables 4 and 5, little variation in retention time and calculated RI was observed for the instruments after three weeks, leading to the criterion for a monthly analysis of the Hydrocarbon Test Mix for establishing and evaluating future retention indices. Ninety-two steroid standards ranging in concentration from 100 µg/mL to 1.5 mg/mL were analyzed on System 3. The retention indices and mass spectral data for the standards are summarized in Table 6. All but one of the steroids analyzed using this method were easily observed without requiring derivatization. 20-Hydroxyecdysterone was not observed without derivatization. Stanozolol, although observed underivatized, is a good candidate for derivatization. The derivatized peak is sharp and symmetrical compared to the broad, poorly-shaped underivatized peak.

Representative samples of previously analyzed, steroid-containing matrices were selected for analysis, and non-derivatized extracts were prepared as described above. A comparison was made between the RI in the sample matrix and the RI of the standard. These values are shown in Table 7 and are indicative of how RI can be used as a tool for tentative identification of steroids, their esters, and possibly diastereomeric forms thereof. A tentative identification of the AAS observed in each sample was made by comparison of the mass spectrum of the compound to the mass spectra of standards available in commercial (NIST, Wiley, etc.) and user-generated mass spectral libraries. The identification was then confirmed by comparison of the retention time, retention index, and mass spectrum to those of a corresponding standard.

In the absence of a match, the following discussion, referring to Figures 1 through 6, offers some guidance for identification of potential steroids [7, 12].

1. The presence of a series of clusters of ions between 60 and 200 Daltons and approximately 14 Daltons apart, Fig. 2, is indicative of a steroid.

2. Steroids with the same A and B ring structures as testosterone, Fig. 1, contain a very intense ion at m/z = 124, Fig. 3. In most cases this ion is the most intense. Its absence is also generally an indication that the structure of rings A or B of the unknown differs from that of testosterone [6].

3. A loss of 42 ($-CH_2CO$) from the molecular ion is also an indicator of a testosterone-like A ring structure, where the double bond could also be between C1 and C2, Fig. 3. It is postulated that this fragment results from the elimination of C2 and C3 from ring A [6].

4. A loss of 44 ($-CH_2CHOH$) from the molecular ion is an indicator of 17-keto steroids. It results from the elimination of C16 and C17 from ring D, Fig. 4 [6].

5. A very intense (generally the most intense) ion at m/z = 122 is indicative of 3-keto-1,4-diene (e.g. boldenone), Fig. 5 and 3-keto-1-ene (e.g. 1-dehydromethandrostenolone) type steroids, Fig. 6 [6].

6. The retention times of the esters are longer than those of the corresponding steroid. If the EI spectrum for the unknown matches a steroid in the mass spectral library, and the retention time seems to be too long for that steroid, the peak may be from an ester of the steroid. This is due to the fact that for the esters of some steroids, the molecular ion is absent or may be of very low intensity, and the spectra are very similar to those of the unesterified steroids. In such cases, check for molecular ions that correspond to esters of the steroid by plotting extracted ion chromatograms. Note that the molecular mass of an ester of a steroid is equal to the mass of the steroid plus the mass of the acid less 18 for the loss of water during the ester formation.

If a tentative identification is assigned based on the above observations or a library match, the identity must be confirmed by analyzing a standard and comparing the retention time and mass spectrum to that of the unknown compound.

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Table 6. Standards Analyzed using System 3

	CAS Number	Molecular Wt.	RI	Molecular Ion Present
5α-androstane (etioallocholane)	438-22-2	260	2063	yes
2,(5α)-androsten-17-one	963-75-7	272	2328	yes
2,(5α)-androsten-17β-ol	2639-53-4	274	2338	yes
desoxymethyltestosterone (madol)	3275-64-7	288	2366	yes
4-estren-3α-17β-diol	35950-87-9	276	2521	yes
5(10)-estren-3β,17β-diol	49933-32-2	276	2526	yes
4-estren-3β-17α-diol	not available	276	2528	yes
9(11)-dehydro DHEA	62509-26-6	286	2545	yes
epietiocholanolone	571-31-3	290	2571	yes
dehydroandrosterone	2283-82-1	288	2588	yes
4-androstenediol	1156-92-9	290	2600	yes
prasterone (DHEA)	53-43-0	288	2605	yes
5α-androstan-3α-ol-17-one	53-41-8	290	2611	yes
5-androstene-3β-17β-diol	521-17-5	290	2617	yes
dihydroandrostanediol	1852-53-5	292	2617	yes
5-androstenediol	521-17-5	290	2618	yes
17β-dihydroandrosterone	571-20-0	292	2626	yes
19-norandrostendione	734-32-7	272	2673	yes
1, 5(α)-androsten-3,17-dione	571-40-4	286	2675	yes
nandrolone	434-22-0	274	2686	yes
1-dehydroandrostanolone	65-06-5	288	2688	yes
methylandrostanolone (mestanolone)	521-11-9	304	2699	yes
estrone	53-16-7	270	2703	yes
9-dehydrotestosterone	2398-99-4	286	2711	yes
dehydromethandrostenolone	65-04-3	302	2717	yes
methasterone (superdrol)	3381-88-2	318	2717	yes
17β-estradiol	50-28-2	272	2718	yes
mesterelone	1424-00-6	304	2733	yes
6-dehydronandrolone	14531-84-1	272	2737	yes
4-androsten-3,17-dione	63-05-8	286	2740	yes
epitestosterone	481-30-1	288	2748	yes
norethindrone	68-22-4	298	2749	yes
testosterone	58-22-0	288	2752	yes
formestane	566-48-3	302	2753	yes
androstatrienedione (ADT)	633-35-2	282	2757	yes
6-dehydroandrostenedione	633-34-1	284	2757	yes
4,9(11)-estradien-3,17-dione	not available	270	2763	yes
androstadiendione	897-06-3	284	2773	yes

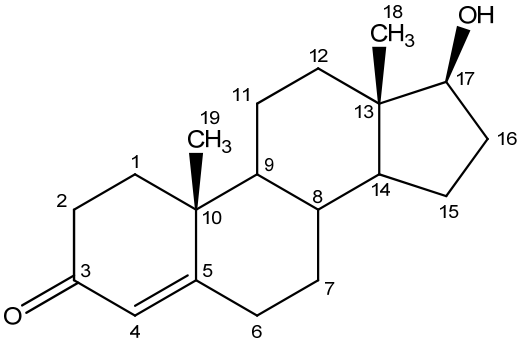
	CAS Number	Molecular Wt.	RI	Molecular Ion Present
methyltestosterone	58-18-4	302	2781	yes
6-dehydrotestosterone	2484-30-2	286	2781	yes
ethinyl estradiol	57-63-6	296	2791	yes
androsterone propionate	not available	347	2793	yes
boldenone	846-48-0	286	2795	yes
pregnenolone	145-13-1	316	2798	yes
trenbolone	10161-33-8	270	2805	yes
methandrostenelone	72-63-9	300	2818	yes
7β-hydroxyandrostenediol	2697-85-0	306	2841	yes
norethandrolone	52-78-8	302	2851	yes
testosterone acetate	2363-59-9	328	2872	yes
methenolone	153-00-4	302	2876	yes
oxandrolone	53-39-4	306	2881	yes
5-androsten-3β-ol-7,17-dione	566-19-8	302	2882	yes
4-androsten-3,6,17-trione	2243-06-3	300	2886	yes
6α-hydroxyandrostenedione	24704-84-5	302	2888	yes
5α-androstan-3,6,17-trione	2243-05-2	302	2904	yes
6β-hydroxyandrostenedione	63-00-3	302	2907	yes
trenbolone acetate	10161-34-9	312	2909	yes
oxymetholone	434-07-1	332	2913	yes
dromostanolone propionate	521-12-0	360	2923	yes
progesterone	57-83-0	314	2936	yes
norebolethone	797-58-0	316	2948	yes
5-androsten-3β-ol-7,17-dione acetate	1449-61-2	344	2984	yes
testosterone propionate	57-85-2	344	2985	yes
methenolone acetate	434-05-9	344	2989	yes
tetrahydrogestrinone (THG)	618903-56-3	312	3026	yes
boldenone propionate	not available	342	3031	yes
fluoxymesterone	76-43-7	336	3066	yes
betamethasone	378-44-9	392	3082	yes
dehydrochloromethyltestosterone	2446-23-3	334	3082	yes
estradiol dipropionate	113-38-2	384	3167	yes
17β-estradiol-17-valerate	979-32-8	356	3172	yes
testosterone valerate	3129-43-9	373	3207	yes
hydroxymesterone	2668-66-8	344	3237	yes
stanozolol	10418-03-8	328	3266	yes
testosterone isocaproate	15262-86-9	386	3282	yes
testosterone enanthate	315-37-7	400	3347	yes
stigmasterol	83-48-7	412	3342	yes
dromostanolone enanthate	not available	417	3379	yes
formebolone	2454-11-7	344	3380	yes
17β-estradiol-17-enanthate	4956-37-0	384	3406	yes

	CAS Number	Molecular Wt.	RI	Molecular Ion Present
methenolone enanthate	303-42-4	414	3559	yes
trenbolone enanthate	not available	382	3483	yes
testosterone caproate	10312-45-5	386	3330	yes
17β-estradiol-17-cypionate	313-06-4	396	3657	yes
17β-estradiol-3-benzoate	50-50-0	376	3657	yes
testosterone cypionate	52-20-8	412	3704	yes
nandrolone decanoate	360-70-3	428	3725	yes
testosterone decanoate	5721-91-5	442	3783	yes
nandrolone phenylpropionate	62-90-8	406	3791	yes
testosterone phenylpropionate	1255-49-8	420	3855	yes
testosterone undecanoate	5949-44-0	456	3898	yes
boldenone undecylenate	13103-34-9	452	3933	yes
nandrolone laurate	26490-31-3	456	3943	yes
20-hydroxyecdysone	5289-74-7	481	3994	no

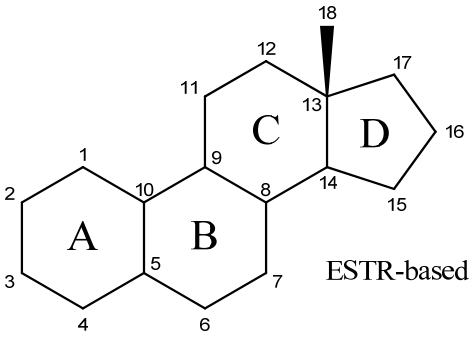
Table 7. Steroids Encountered in Dietary Supplements, Approved Dosage Forms, Oils, and Bulk Powders

Matrix	Observed Compound	Retention Index in the Matrix	Retention Index of the Standard
oil	nandrolone decanoate	3720	3725
oil	testosterone cypionate	3698	3704
oil	trenbolone enanthate	3483	3483
oil	testosterone cypionate	3708	3704
oil	dromostanolone propionate	2919	2923
oil	testosterone enanthate	3447	3447
oil	testosterone enanthate	3438	3447
oil	trenbolone enanthate	3476	3483
oil	testosterone enanthate	3435	3447
oil	nandrolone decanoate	3766	3725
capsule composite/supplement	prasterone (DHEA)	2595	2605
capsule composite/supplement	prasterone (DHEA)	2591	2605
capsule composite/supplement	1,4,6-androstrien-3,17-dione/ 4-androsten-3,6,17-trione	2745/2876	2757/2886
capsule composite/supplement	dehydrochloromethyltestosterone	3071	3082
capsule composite/supplement	methasterone	2709	2717
tablet composite/supplement	stanozolol	3236	3266
tablet composite/dosage form	ethinyl estradiol	2790	2791
tablet composite/dosage form	ethinyl estradiol	2788	2791
tablet composite/dosage form	17β-estradiol	2711	2718
bulk powder	dehydrochloromethyltestosterone	3085	3082
bulk powder	desoxymethyltestosterone (madol)	2360	2366
bulk powder	methasterone (superdrol)	2710	2717
bulk powder	dehydrochloromethyltestosterone	3084	3082

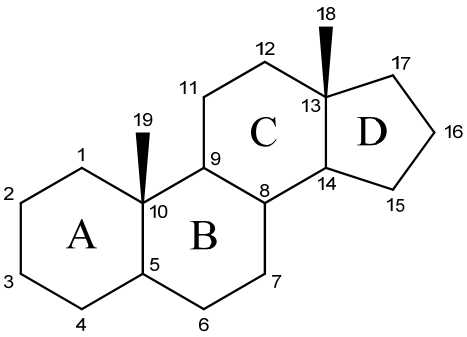
Figure 1. Common Steroid Structures



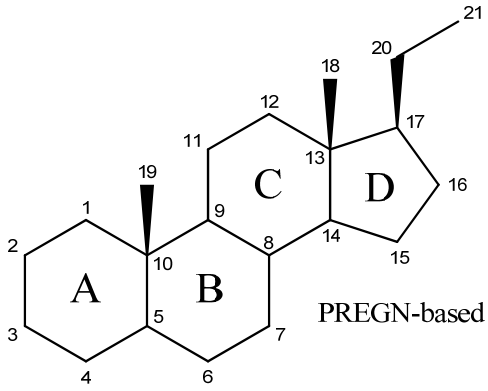
Testosterone



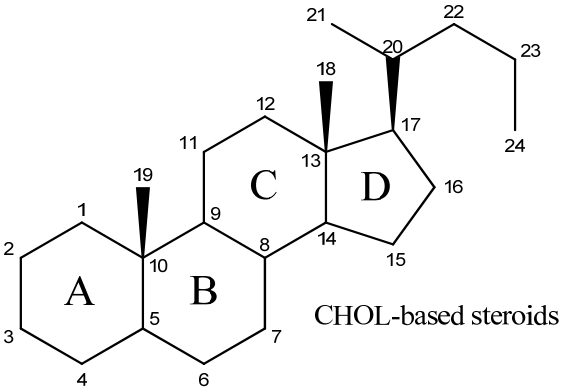
ESTR-based steroids



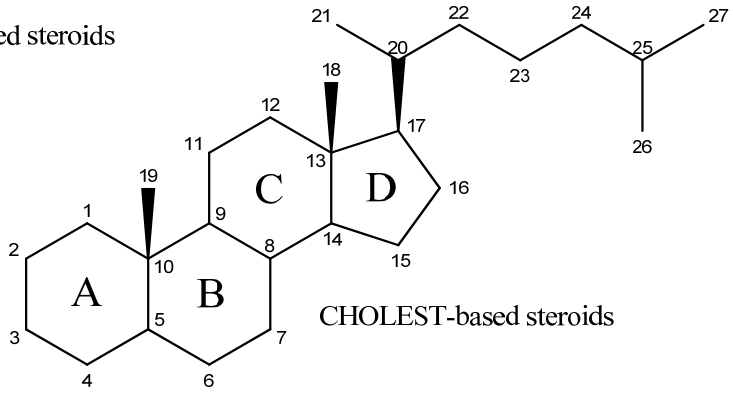
ANDROST-based steroids



PREGN-based steroids



CHOL-based steroids



CHOLEST-based steroids

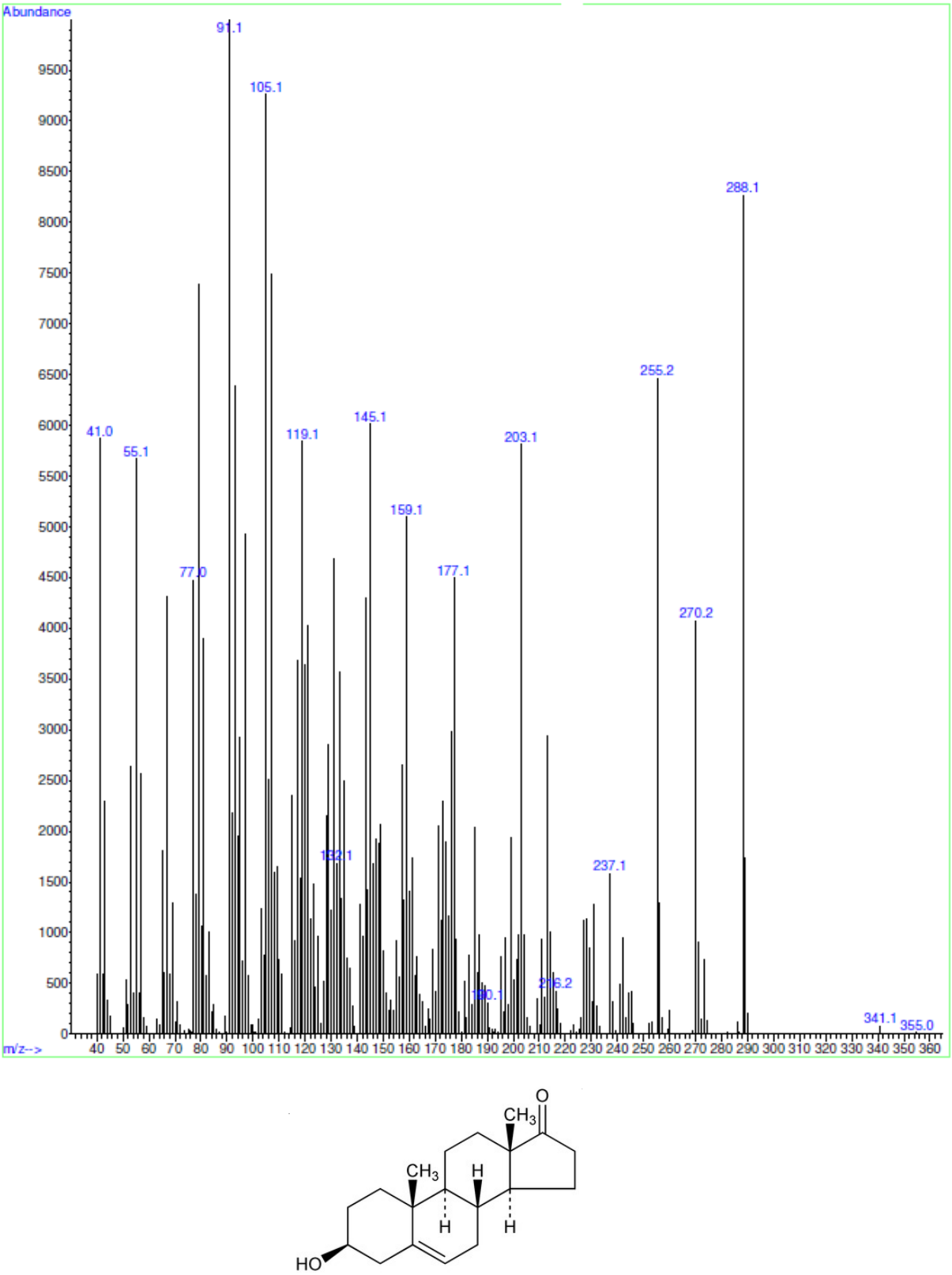


Figure 2. Prasterone (DHEA)

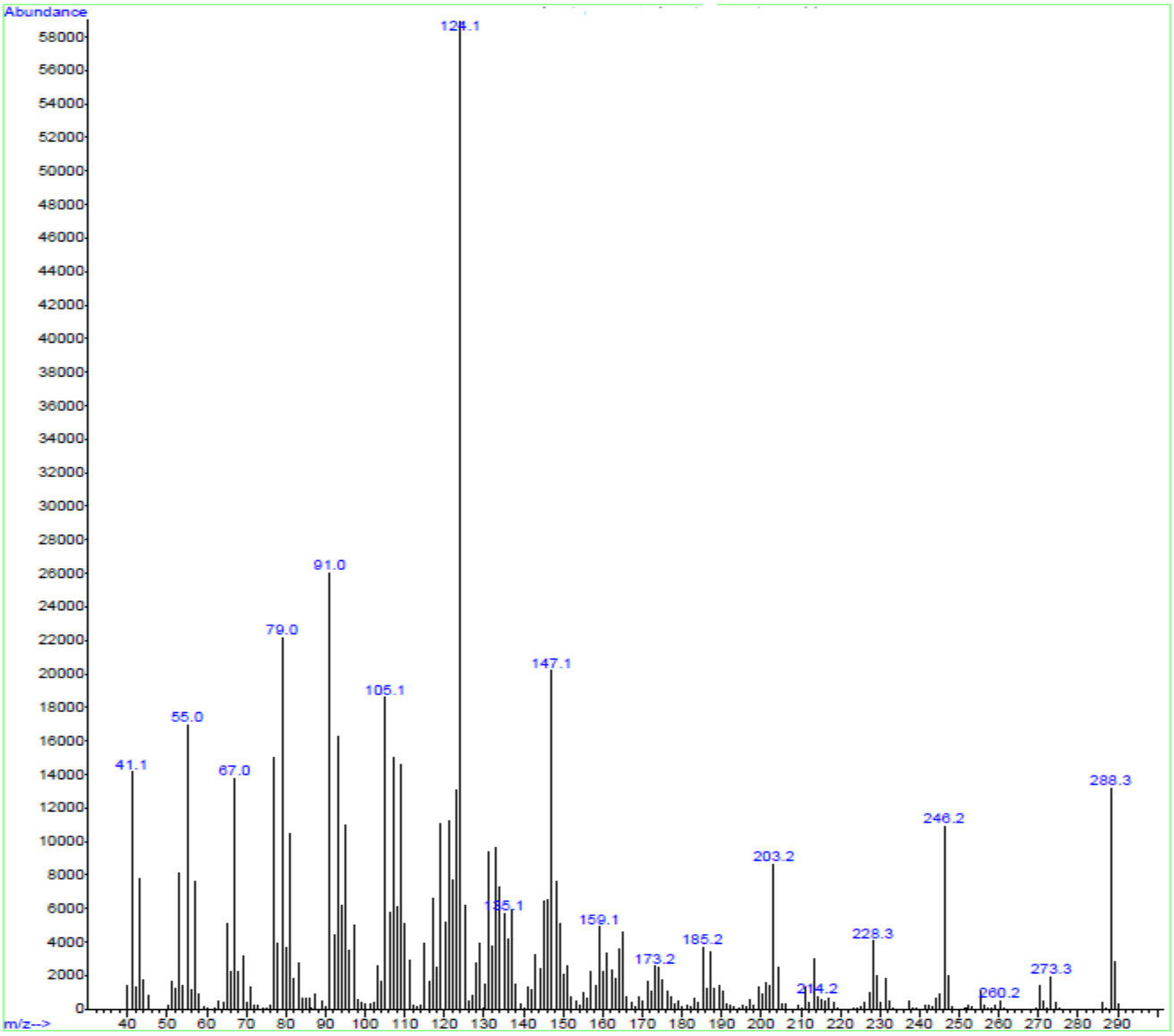


Figure 3. Testosterone

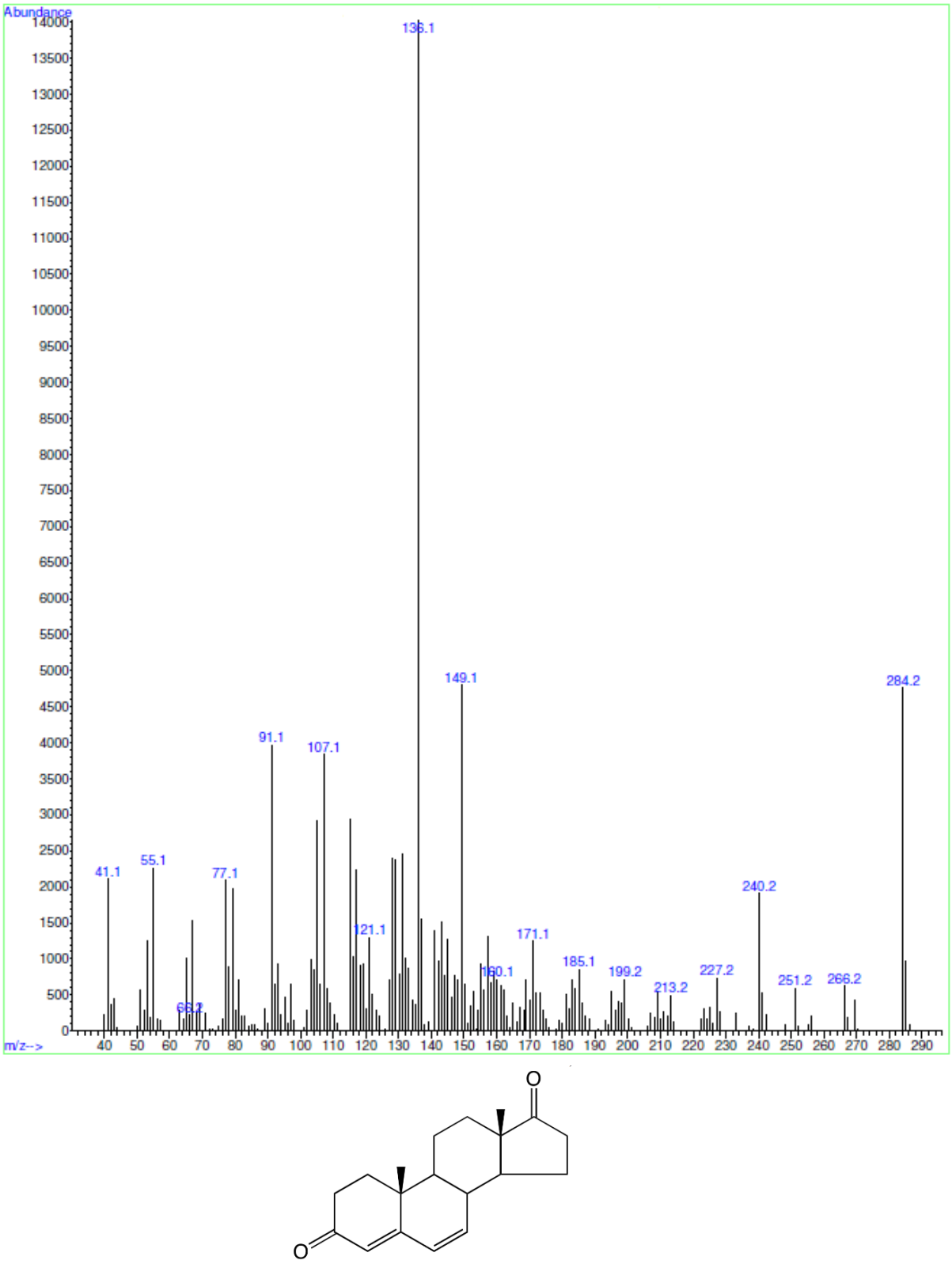
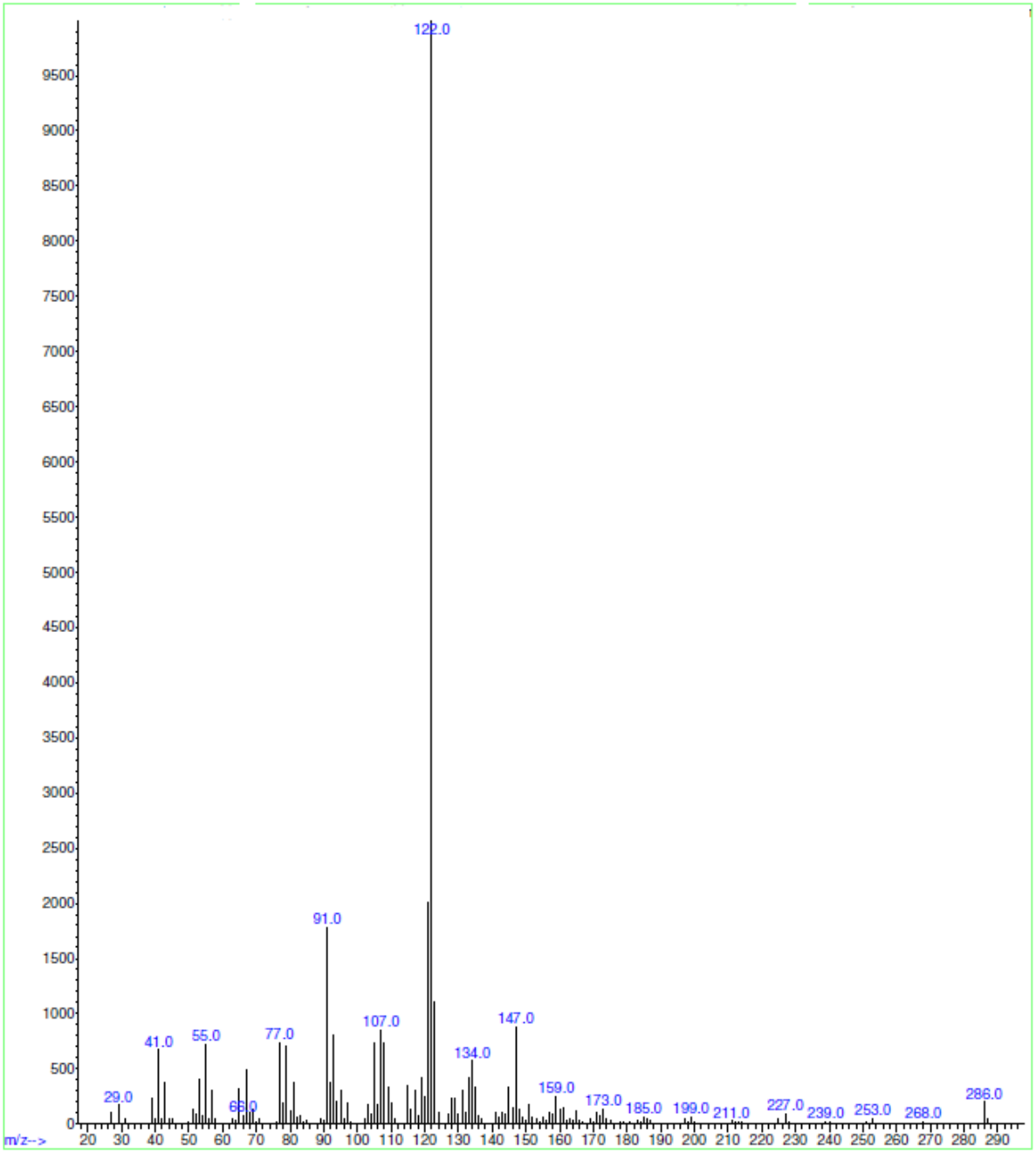


Figure 4. 6-Dehydroandrostenedione



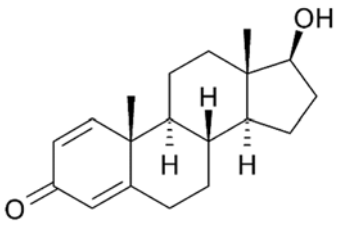


Figure 5. Boldenone

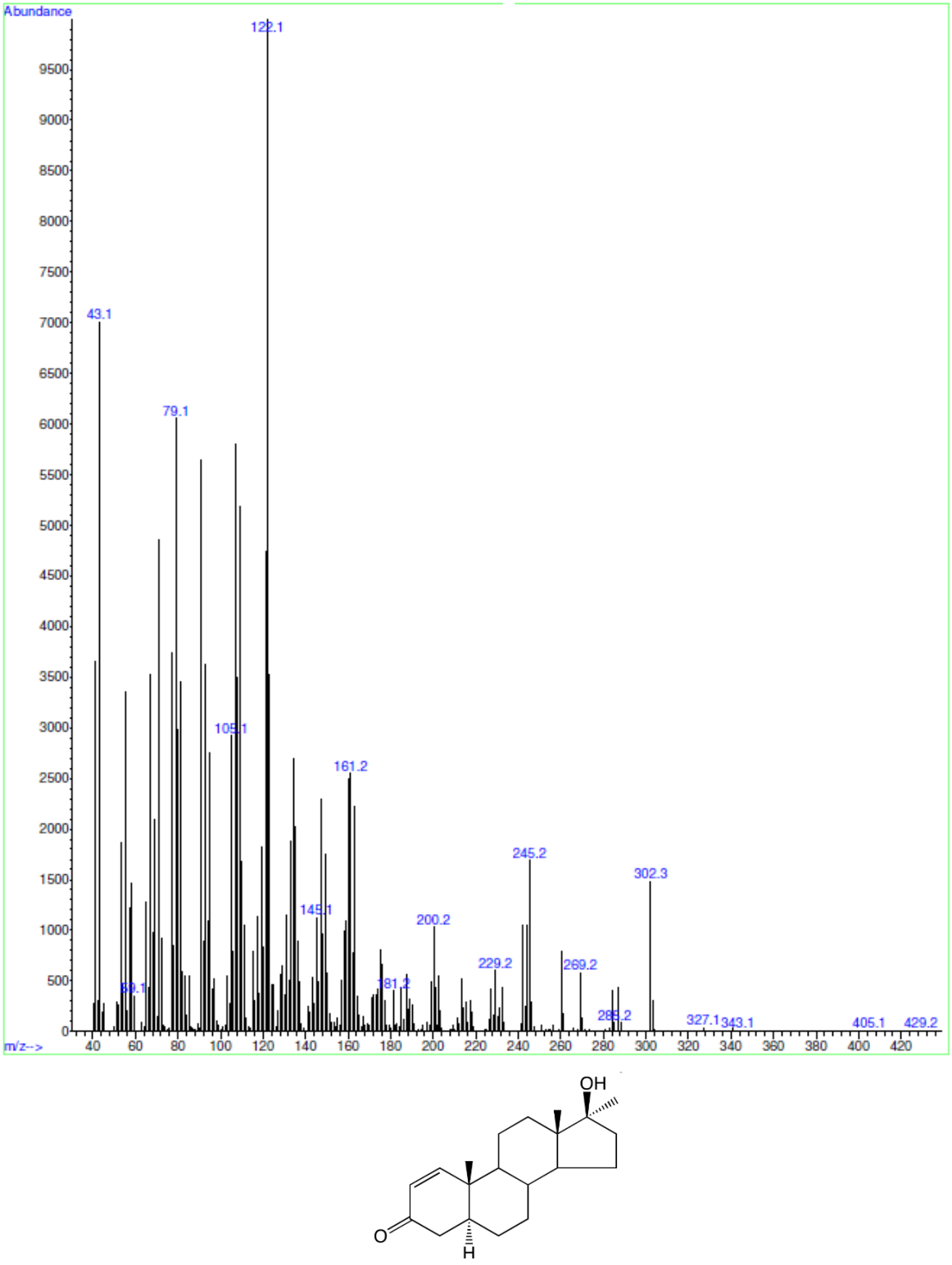


Figure 6. 1-Dehydromethandrostenolone