Dehydrochlormethyltestosterone: An Analytical Profile

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ABSTRACT: Analytical data (GC, GC/MS, FTIR, HPLC, ^{1}H- and ^{13}C- NMR) for the analysis and identification of dehydrochlormethyltestosterone ((17β)-4-chloro-17-hydroxy-17-methylandrosta-1,4-dien-3-one) is presented. Historical background is also included.

KEYWORDS: Dehydrochlormethyltestosterone, Chlorodehydromethyltestosterone, Turanabol, Turinabol, Anabolic Steroid, Controlled Substance, Analysis, Forensic Chemistry

Introduction

The Drug Enforcement Administration Mid-Atlantic Laboratory recently received a submission of steroids and steroid-related exhibits that were seized during a consent search of a residence in Winchester, Virginia. The exhibits included 15 bottles, each labeled "Turanabol," "Chlordehydromethyl-testosterone," and "Golden Triangle Pharmaceuticals" (see Photo 1). Despite identical appearances (same bottle type, labeling, lot number, and number of tablets (100)), six of the bottles contained nondescript orange capsules while nine bottles contained nondescript yellow capsules (see Photo 2, next page). Subsequent analyses confirmed that the orange capsules contained dehydrochlormethyltestosterone as the only active ingredient, while the yellow capsules contained primarily dehydrochlormethyltestosterone with minor amounts of stanozolol and methandrostenolone (see structures, next page). This is believed to be the first submission of dehydrochlormethyltestosterone to the DEA laboratory system (1).

Photo 1

Dehydrochlormethyltestosterone is a Schedule III controlled substance in the United States and is also listed in the 2006 Prohibited List/World Anti-Doping Code. It gained notoriety as a result of the East German Olympic doping scandals that were fully exposed after the fall of the Berlin wall (2). Data from

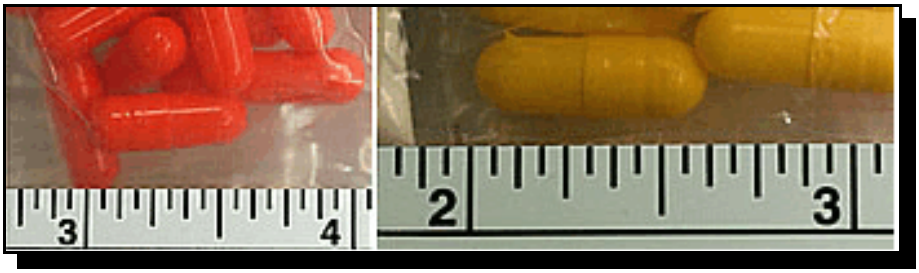
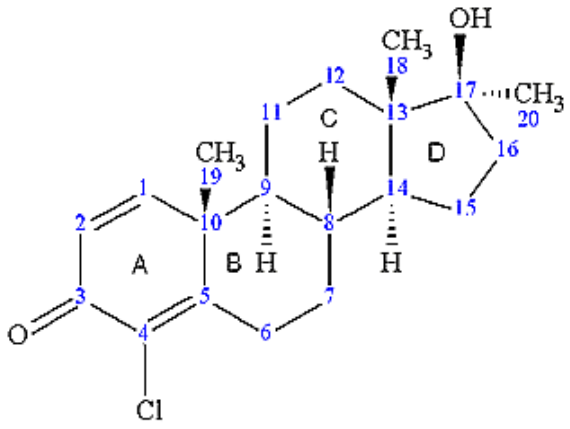
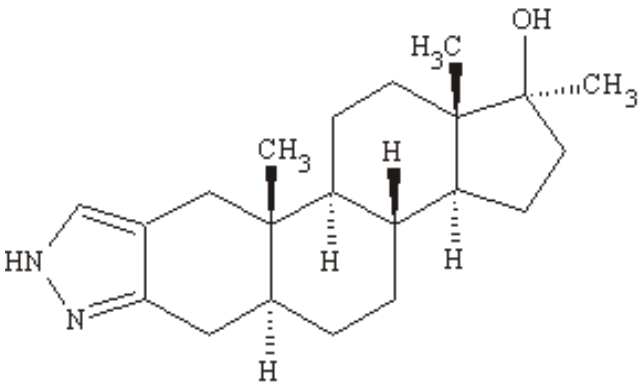


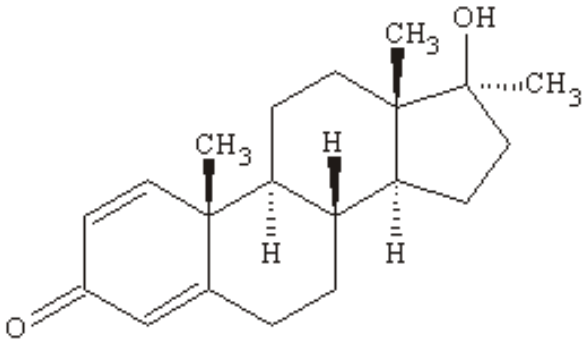
Photo 2 (Note that Both Types of Capsules are the Same Size)



Dehydrochlormethyltestosterone (with Labeling of Rings (A-D) and IUPAC Numbering of Carbons).



Stanozolol



Methandrostenolone

East German medical personnel involved in the doping indicated that dehydrochlormethyltestosterone produced dramatic increases in speed and strength, but with detrimental side effects such as deepening of the voice, increased acne, and body hair growth. Other, long term side-effects ranged from liver damage to severe gynecological disorders (2). This steroid is no longer legitimately produced, and appears to be available only as an illicitly-prepared product on the black market.

As with nearly all anabolic steroids, dehydrochlormethyltestosterone has multiple name variations, including (but not limited to): Dehydrochloromethyltestosterone, chlordehydromethyltestosterone, chlorodehydromethyl-testosterone, 4-chlorodehydromethyltestosterone, 4-chloromethandienone, 4-chlor-1-dehydro-17α-methyl-testosterone, 1,4-androstadien-4-chloro-17α-methyl-17β-ol-3-one, and 4-chloro-17β-hydroxy-17α-methyl-androst-1,4-dien-3-one. The most common trade name for dehydrochlormethyltestosterone, Oral-Turinabol®, is often abbreviated as "OT" in both the scientific literature and on internet websites dedicated to anabolic steroid use/abuse (2,3).

Not surprisingly, most of the scientific literature dedicated to the analysis of dehydrochlormethyltestosterone has a toxicological focus (that is, analysis of biological fluids for dehydrochlormethyltestosterone metabolites for detection of doping (4,5). Although there are a number of reports of submissions of dehydrochlormethyl-testosterone to forensic and crime laboratories (6), complete forensic analysis of dehydrochlormethyltestosterone has not been previously reported, and even standard reference texts in the field (e.g., 7,8,9) do not contain data for this compound. Herein, we report analytical data (GC, GC/MS, FTIR-ATR, HPLC, and ^{1}H- and ^{13}C- NMR) for the analysis and identification of the title steroid. In addition, because this is the first comprehensive report for this steroid, an in-depth analysis of the NMR data is presented.

Experimental

Standard: A reference standard of dehydrochlormethyltestosterone was obtained from Steraloids (Newport, RI).

Gas Chromatography (GC): GC screening was conducted using an Agilent 6890N (Waldbronn, Germany) equipped with flame ionization detector (FID). The sample was dissolved in methanol and injected into the instrument using the parameters below.

Instrument	Agilent 6890N
Column	HP-5 (5 % phenyl/95 % methyl silicone); 12 m x 0.2 mm i.d. x 0.33 µm thickness
Carrier Gas	Helium at 1.0 mL/min
Temperatures	Injector: 270 °C Detector: 280 °C Oven Program: 175 °C for 1 min 15 °C/min to 280 °C Hold at 280 °C for 4 min
Injection Parameters	Split ratio = 60:1, 1 mL injected

Gas Chromatography/Mass Spectrometry (GC/MS): An Agilent 6890N gas chromatograph equipped with an Agilent 5973 Mass Selection Detector (MSD) (Waldbronn, Germany) was used in the electron ionization (EI) mode to obtain mass spectra of samples and standards. Instrumental parameters are listed below. Agilent's MS Interpreter (Version 0.9) was used to derive the relative abundances of the molecular ion cluster.

Instrument	Agilent 6890N with Agilent 5973 Mass Selection Detector
Column	HP-5MS (5 % phenyl/95 % methyl silicone); 15 m x 0.25 mm x 0.25 µm thickness
Carrier Gas	Helium at 1.0 mL/min
Temperatures	Injector: 280 °C Oven Program: 150 °C for 0.5 min 30 °C/min to 300 °C Hold at 300 °C for 1.5 min
Injection Parameters	Split ratio = 75:1, 1 mL injected

Detector	Quadrupole Mass Detector
Temperatures	Transfer Line: 280 °C MS Quad: 150 °C MS Source: 230 °C
Acquisition Mode	Scan
Solvent Delay Time	0.5 minutes
Scan Parameters	Mass Range: 40 - 450 amu Sample #: 3 (2n = 8 samples taken at each mass) Resulting Scan Rate = 1.84 scans/sec

Fourier Transform Infrared Spectrometer - Attenuated Total Reflectance (FTIR-ATR): Infrared spectroscopy was performed using a Thermo Nicolet Nexus 670 Fourier Transform Infrared Spectrometer (FTIR) (Madison, WI) equipped with a Golden Gate Attenuated Total Reflectance (ATR) detector. The sample was prepared by extraction of the capsule matrix with methanol followed by evaporation. The IR spectrum was collected by averaging 24 scans with a resolution of 4.0 wavenumbers (cm^{-1}).

High Performance Liquid Chromatography (HPLC): HPLC was conducted using an Agilent 1100 Series instrument (Waldbronn, Germany) using ultraviolet (UV) detection. The sample was dissolved in methanol and injected into the instrument using the parameters below (10).

Instrument	Agilent 1100 Series
Column	Waters Xterra RP18 (4.6 x 150 mm, 3.5 mm)
Mobile Phase	80 % Water (W): 20 % Acetonitrile (A) hold for 3 min Ramp to 55 % W: 45 % A for 2 min and hold for 8 min Ramp to 35 % W: 65 % A for 3 min and hold for 10 min Ramp to 10 % W: 90 % A for 5 min and hold for 9 min
Temperature	45 °C
Detection Wavelength	225 nm
Injection Volume	5 mL
Injection Solvent	Methanol

Nuclear Magnetic Resonance (NMR) Spectroscopy: One and two dimensional (1D and 2D) NMR experiments were performed on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac pulse field gradient (PFG) indirection detection probe (Varian Inc., Palo Alto, CA). Standard Varian pulse sequences were employed. The sample and standard were prepared in deuterated methanol (CD_3OD) with tetramethylsilane (TMS) added (approximately 0.05 % v/v) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, WI). The ^{1}H-NMR spectrum of the standard was obtained with 8 scans using a 45 second delay, 90 ^{O} pulse, 2 second acquisition time, and oversampling of 6. The ^{13}C-NMR spectrum of the standard was obtained with proton decoupling; 2,000 scans were acquired, using a 1 second delay, 45 ^{O} pulse, 1.2 second acquisition time, and oversampling of 3. Samples were maintained at 25 ^{O}C. Standard Varian gradient versions of 2D NMR experiments were performed to help make assignments, including homonuclear COSY (2 - 4 bond proton-proton through bond correlations), NOESY (proton - proton spatial nearness correlations for protons < 4 angstroms apart), heteronuclear HSQC (proton to directly bonded carbon correlations), and HMBC (2, 3, or 4 bond proton to carbon correlations). Structural elucidation was performed utilizing Applied Chemistry Developments (ACD/Labs, Toronto, Canada) software (HNMR Predictor, CNMR predictor, and Structure Elucidator).

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Results and Discussion

Gas Chromatography (GC): The chromatogram is not shown. Methandrostenolone, dehydrochlormethyltestosterone, and stanozolol eluted at 7.96, 9.32, and 10.29 minutes, respectively. The peak shape for stanozolol was broad in comparison to the other steroids. The mixture in the yellow tablets was not formally quantitated, but was estimated as roughly 100 : 5 : 2.5 dehydrochlormethyltestosterone : methandrostenolone : stanozolol. Table 1 lists the relative retention times for cocaine, heroin, and six other steroids with similar chromatography.

Table 1. GC Relative Retention Times.

Drug (GC)	RRt
Cocaine	0.580
Mesterolone	0.813
Testosterone	0.822
Heroin	0.829
Methyltestosterone	0.836
Methandrostenolone	0.854
Testosterone Acetate	0.882
Fluoxymesterone	0.990
Dehydrochlormethyltestosterone	**1.000**
Stanozolol	1.103
Testosterone Isocaproate	1.188

Gas Chromatography/Mass Spectrometry (GC/MS): The mass spectra of dehydrochlormethyltestosterone, methandrostenolone, and stanozolol are shown in Figures 1 - 3, respectively. Dehydrochlormethyltestosterone displayed a molecular ion at *m/z* 334. Analysis of the molecular ion cluster (i.e., for $C_{20}H_{27}O_2Cl$) revealed close agreement with the theoretical values obtained from the MS Interpreter program, confirming the molecular formula and the presence of a chlorine atom (Table 2). Of note, the spectra did not give a satisfactory match with any compound in the instrument's database, indicating both that the compound is not a different steroid and that dehydrochlormethyltestosterone is not entered.

Table 2. Theoretical versus Actual Values for the Relative Abundances for the Molecular Ion Cluster (i.e., for $C_{20}H_{27}O_2Cl$).

Mass (amu)	Theoretical (Relative Abundance)	Experimental (Relative Abundance)
334	100.00	100.00
335	22.73	22.87
336	34.84	34.92
337	7.53	7.41
338	0.93	0.83

Fourier Transform Infrared Spectrometer - Attenuated Total Reflectance (FTIR-ATR): The IR spectrum of the reference standard is shown in Figure 4. The spectrum displayed major absorbances for O-H (3485 cm^{-1}), C-H (2947 cm^{-1}) and C=O (1655 cm^{-1}). Comparison of the reference standard with the sample is shown in Figure 5. The direct comparison did not show a high quality match; it is suspected that either polymorphism or the presence of other soluble capsule materials in the extract caused the differences in the spectra. Again, neither spectrum gave a satisfactory match with any compound in the instrument's database, indicating both that the compound is not a different steroid and that dehydrochlormethyltestosterone is not entered.

High Performance Liquid Chromatography (HPLC): The chromatograms for dehydrochlormethyltestosterone standard and a mixture of dehydrochlormethyltestosterone and stanozolol standards (roughly 5 : 95) are shown in Figure 6. The two peaks resulting from the dehydrochlormethyltestosterone - stanozolol mixture did not resolve using this method (inset in Figure 6). However, they are resolved by GC or GC/MS, enabling each to be identified. Table 3 lists the relative retention times of a series of similarly sized steroids. Figure 7 shows the UV spectrum of dehydrochlormethyltestosterone.

Table 3. HPLC Relative Retention Times (Asterisks denote steroids analyzed at an earlier date; the retention times were adjusted relative to the dehydrochlormethyltestosterone).

Drug (LC)	RRt
Fluoxymesterone	0.64
Boldenone*	0.70
Nandrolone*	0.73
Methandrostenolone	0.75
Testosterone	0.79
Methyltestosterone	0.87
Dehydrochlormethyltestosterone	**1.00**
Stanozolol	1.02
Testosterone Acetate	1.37
Methenolone Acetate*	1.46
Nandrolone Propionate*	1.52
Testosterone Propionate*	1.77
Nandralone Phenylpropionate*	1.86
Testosterone Phenylpropionate*	1.98
Testosterone Isocaproate*	2.14
Testosterone Cypionate*	2.34
Methenolone Enanthate*	2.40
Nandralone Decanoate*	2.58
Testosterone Decanoate*	2.62
Testosterone Undecylanate*	2.68

Nuclear Magnetic Resonance (NMR) Spectroscopy: The ^{1}H-NMR spectrum of the reference standard are shown in Figures 8a - b. Spectral assignments are summarized in Table 4 (next page). The proton, carbon, and HSQC experiments showed that the unknown molecule contained 20 carbons and 26 non-exchangeable hydrogens. There were 6 quaternary, 5 methine, 6 methylene, and 3 methyl carbons. Adding the carbons (20), non-labile protons (26), oxygens (2), and chlorine (1 based on the MS data), gives a molecular weight of 333 Daltons. The remaining mass (1 Dalton) is due to an exchangeable proton. Using the HMBC NMR data, it was determined that there is one carbonyl carbon (180 ppm), 1 - 3 bonds from 4 alkene carbons at 126.5, 128.6, 158.9, and 166.1 ppm, two of which are protonated, with the hydrogens (6.32 and 7.33 ppm) coupled to each other (J = 10.1 Hz). This corresponds well to a doubly conjugated ketone on ring "A" with the carbonyl at position 3, protonated alkene carbons at positions 1 and 2, and quaternary alkene carbons at positions 4 and 5 (meaning position 4 has a substituent, presumably the chlorine). Far removed is a quaternary carbon at 82.0 ppm, indicating that it is bonded to oxygen (likely bonded to the exchangeable proton). Assuming that this is a common steroid ring structure, placement of the carbon (82 ppm) bonded to oxygen would be at the 17 position. This accounts for all but the methyl group, and since the 82 ppm carbon is a quaternary carbon, the methyl group is attached at the 17 position. Comparison of the experimental data with that predicted with the ACD software showed very good agreement. In addition, comparison of the ^{1}H-NMR spectrum of the unknown to methandrostenolone showed they were nearly identical below 2.0 ppm, indicating that the B, C, and D rings are the same.

Table 4. NMR Data and Assignments.

Position	Carbon	Proton			
	ppm	ppm	#H	Type	Coupling Constants (*J*) (Hz)
1	158.9	7.33	1	bd	10.1
2	126.5	6.32	1	d	10.1
3	180.2	-	-	-	-
4	128.6	-	-	-	-
5	166.1	-	-	-	-
6a	30.1	2.4	1	td	13.6(x2), 5.2
6b	30.1	3.27	1	dt	13.6, 3.2(x2)
7a	33.5	0.99	1	m	-
7b	33.5	2.02	1	abdq	13.6, ~3.9, ~3.9, 3.2
8	37.5	1.84	1	m	-
9	54.9	1.06	1	m	-
10	48.1	-	-	-	-
11a (or 15)	24.1	1.33	1	m	-
11b (or 15)	24.1	1.81	1	m	-
12a	32.7	1.31	1	m	-
12b	32.7	1.61	1	m	-
13	47.0	-	-	-	-
14	51.0	1.23	1	m	-
15a (or 11)	24.4	1.39	1	m	-
15b (or 11)	24.4	1.63	1	m	-
16a	39.1	1.68	1	m	-
16b	39.1	1.87	1	m	-
17	82.0	-	-	-	-
18	14.7	0.94	3	s	-
19	19.6	1.35	3	s	-
20	26.1	1.16	3	s	-

b = Broad, d = Doublet, m = Multiplet, abdq = Broad Doublet of Quartets, s = Singlet, and t = Triplet. Many coupling constants could not be determined due to the complexity of the ^{1}H-NMR spectrum.

Final confirmation of the compound's identity was achieved via comparison of mass spectral fragmentation patterns, GC retention times, and proton and carbon NMR spectra, with the reference standard.

Acknowledgements

The authors gratefully acknowledge Forensic Chemist Esther Chege and Senior Forensic Chemist Charles Matkovich, both of the Mid-Atlantic Laboratory, for their assistance running NMR experiments.

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[References Continued on Page 65.]

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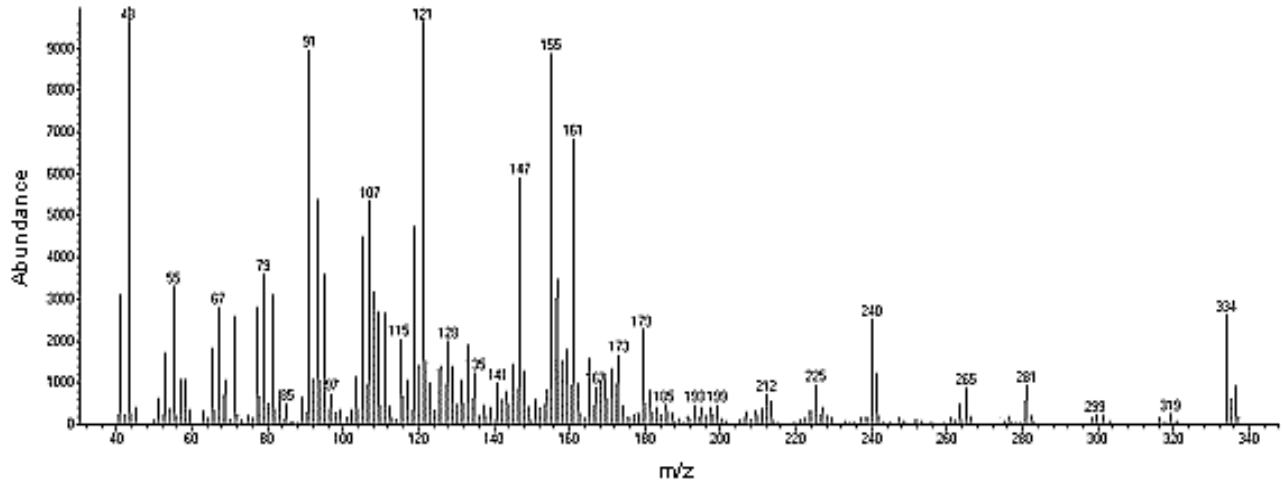

Figure 1. Mass Spectrum of Dehydrochlormethyltestosterone.

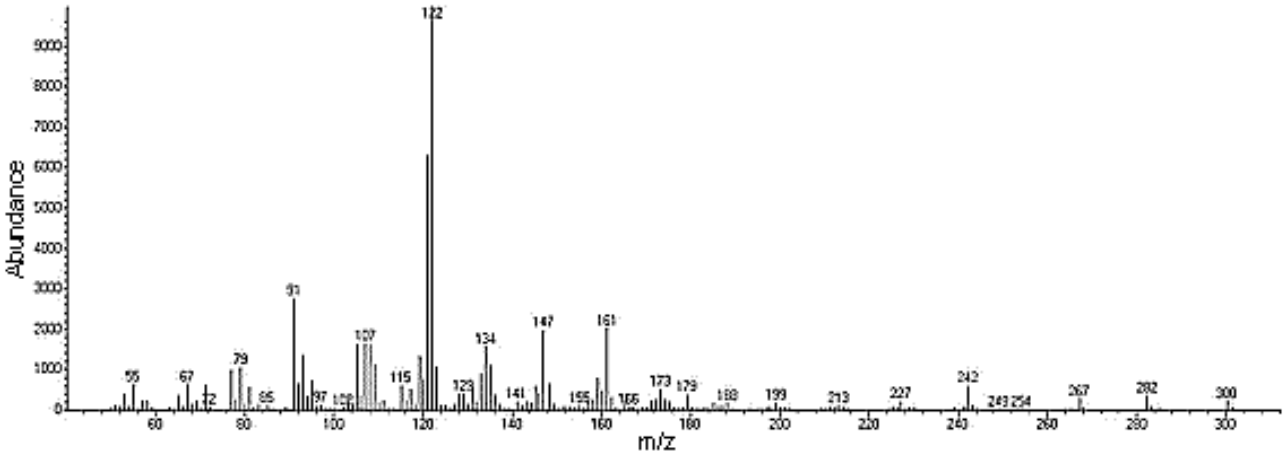

Figure 2. Mass Spectrum of Methandrostenolone.

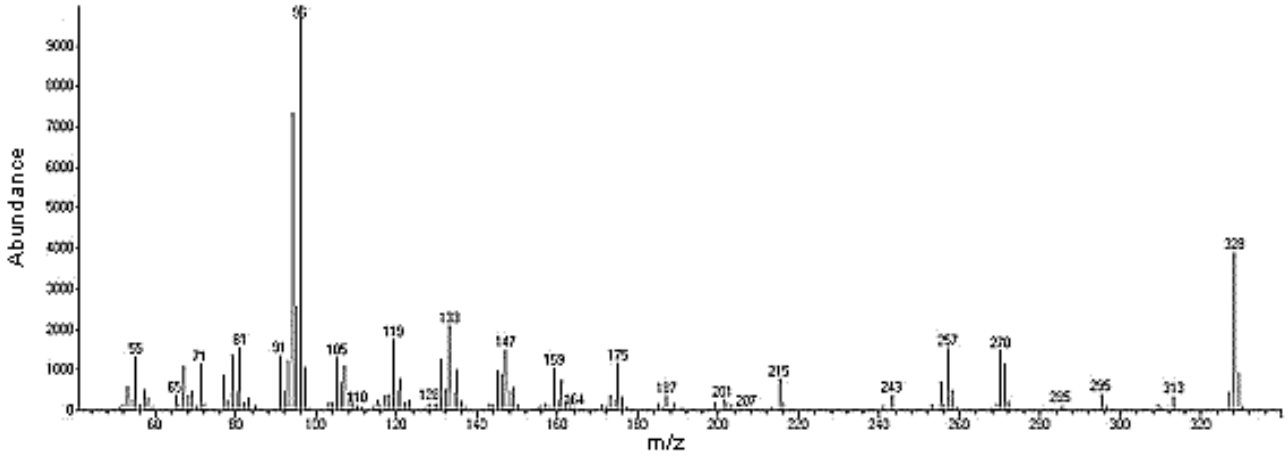

Figure 3. Mass Spectrum of Stanozolol.

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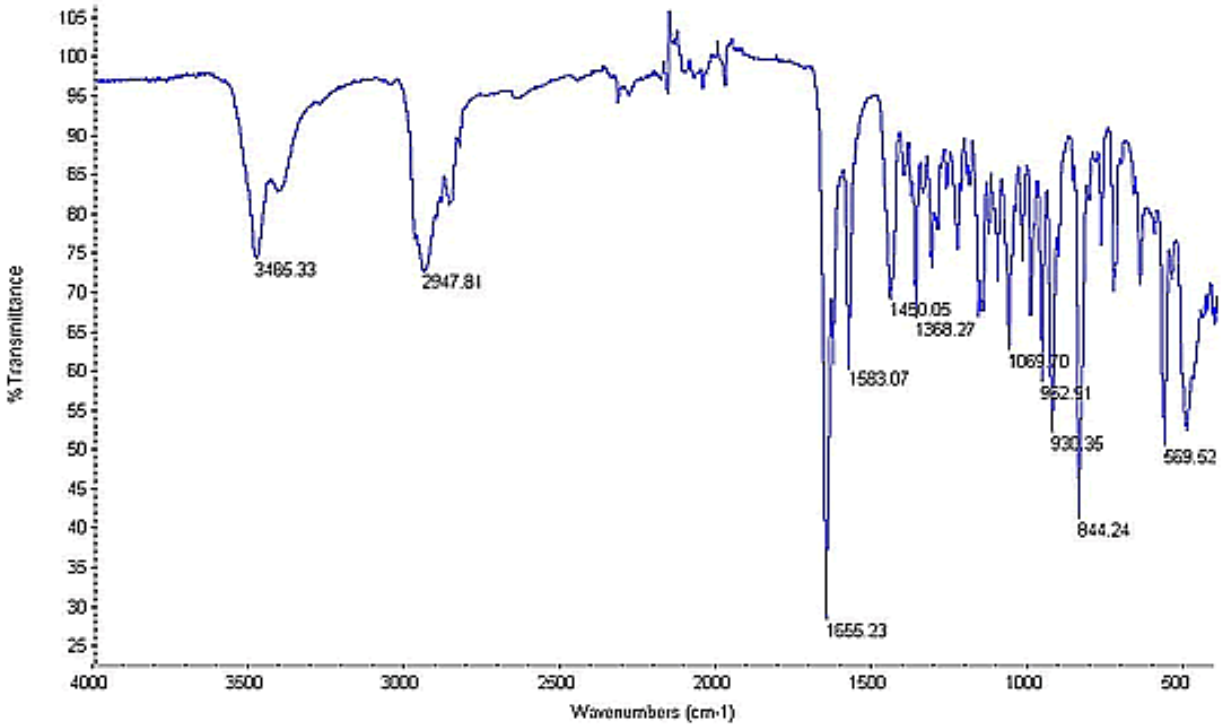

Figure 4. The Infrared Spectrum (FTIR-ATR) of Dehydrochlormethyltestosterone Reference Standard.

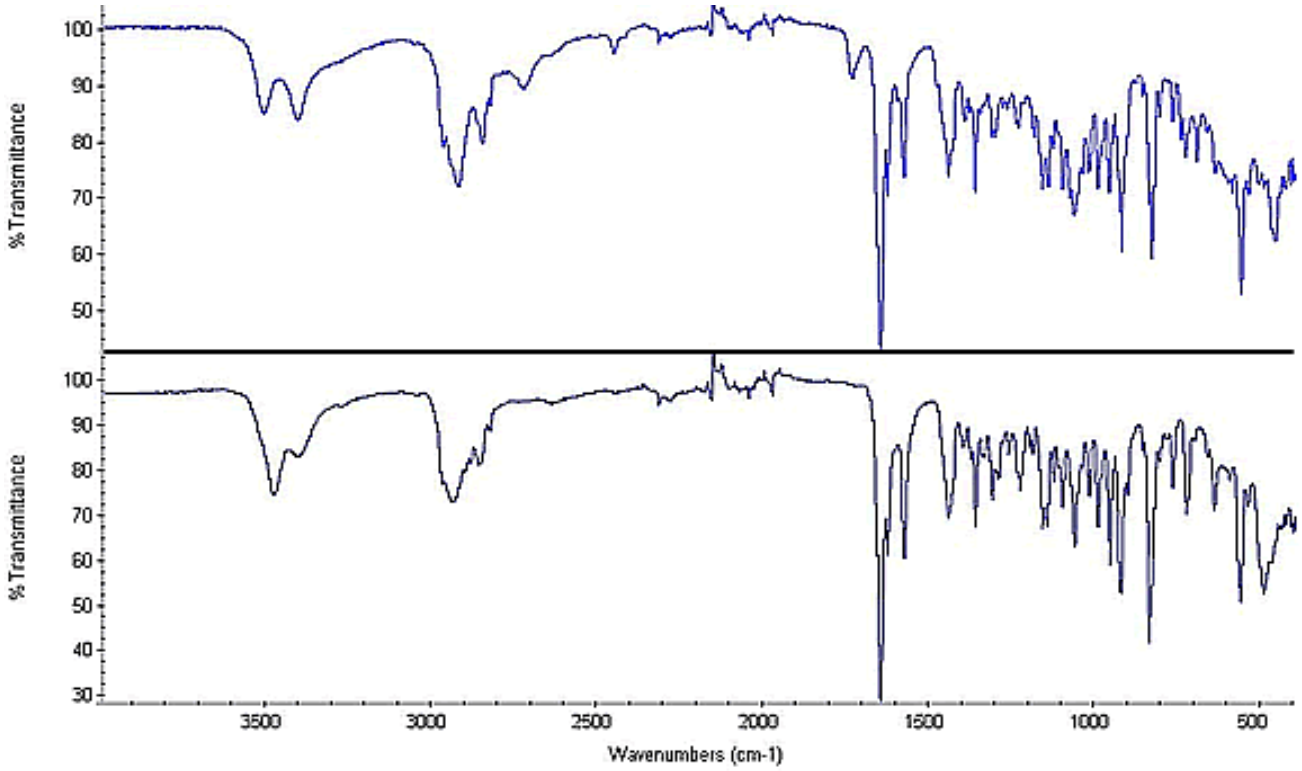

Figure 5. Infrared Spectrum (FTIR-ATR) of Sample of Orange Colored Capsule's Methanol Soluble Materials (Upper Trace) Compared to the Reference Standard (Lower Trace).

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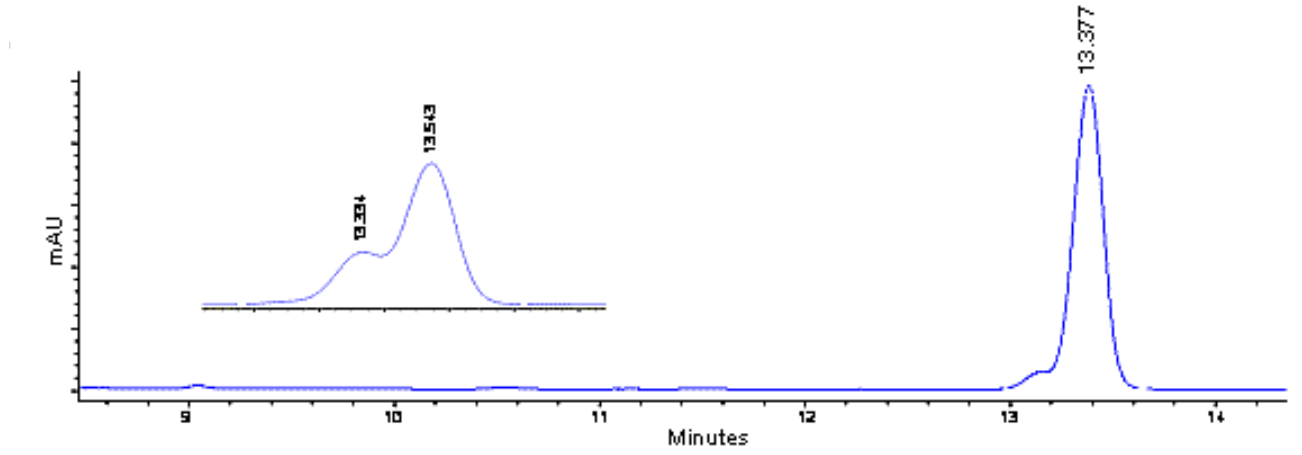

Figure 6. The HPLC UV Chromatogram (225 nm Detection) for Dehydrochlormethyltestosterone Standard (13.377 Minutes). The Inset Shows the Chromatogram for the 5 : 95 Mixture of Dehydrochlormethyltestosterone (13.334 minutes) and Stanozolol (13.543 minutes) Standards.

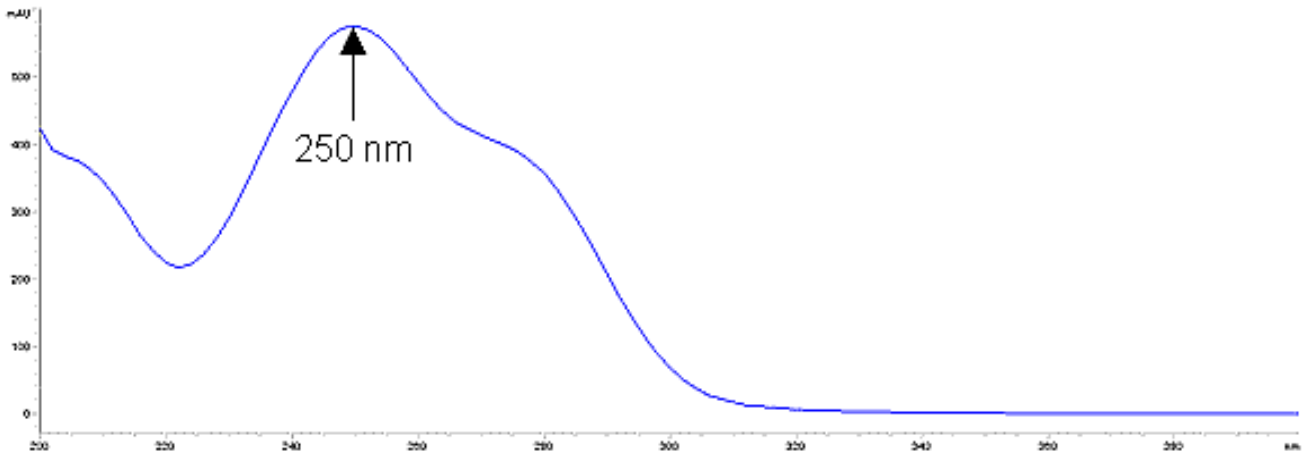

Figure 7. HPLC UV Spectrum of Dehydrochlormethyltestosterone in Methanol.

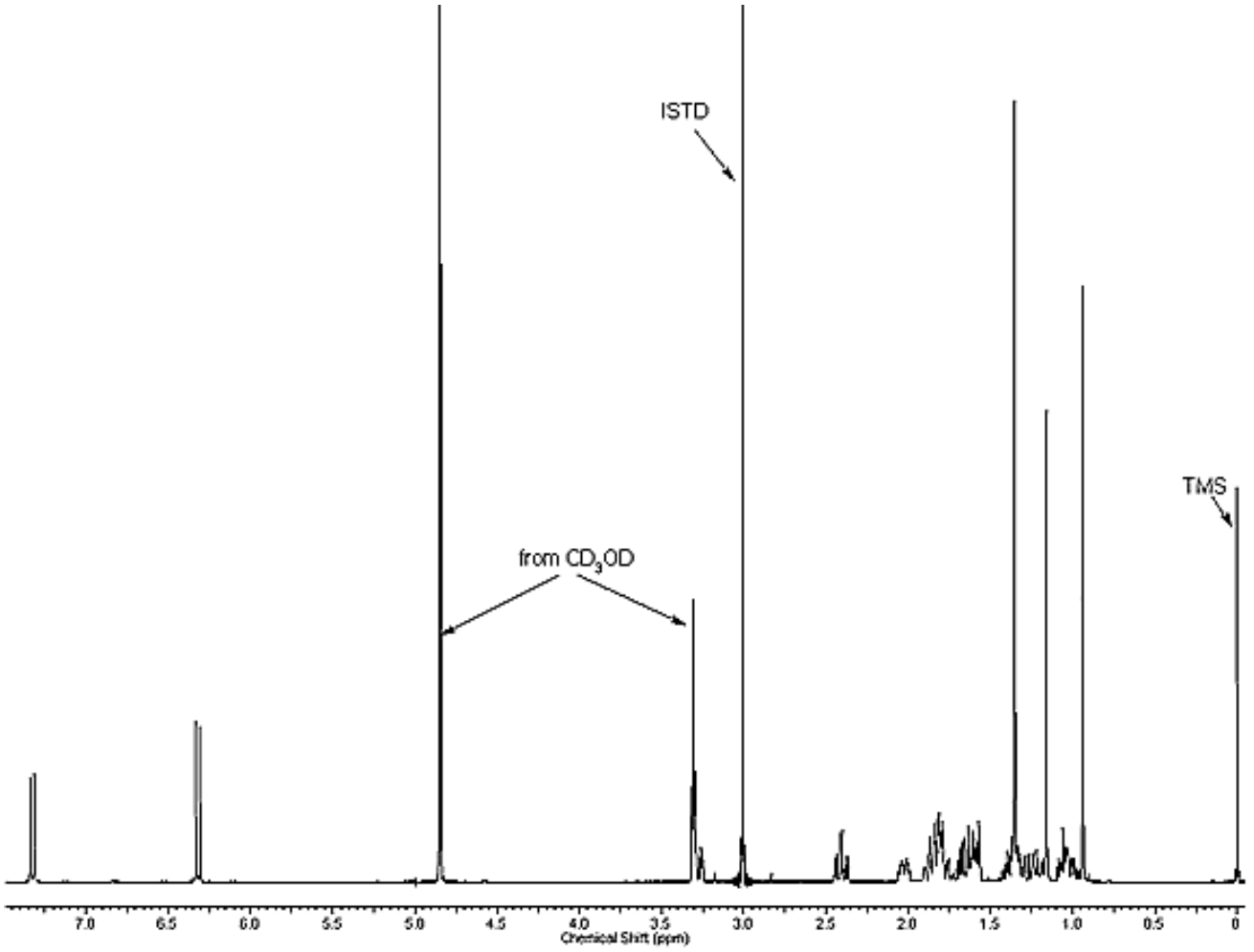

Figure 8a. ^{1}H-NMR of Dehydrochlormethyltestosterone Reference Standard in CD_3OD. Dimethylsulfone (Listed as ISTD) was used to Quantitate the Standard.

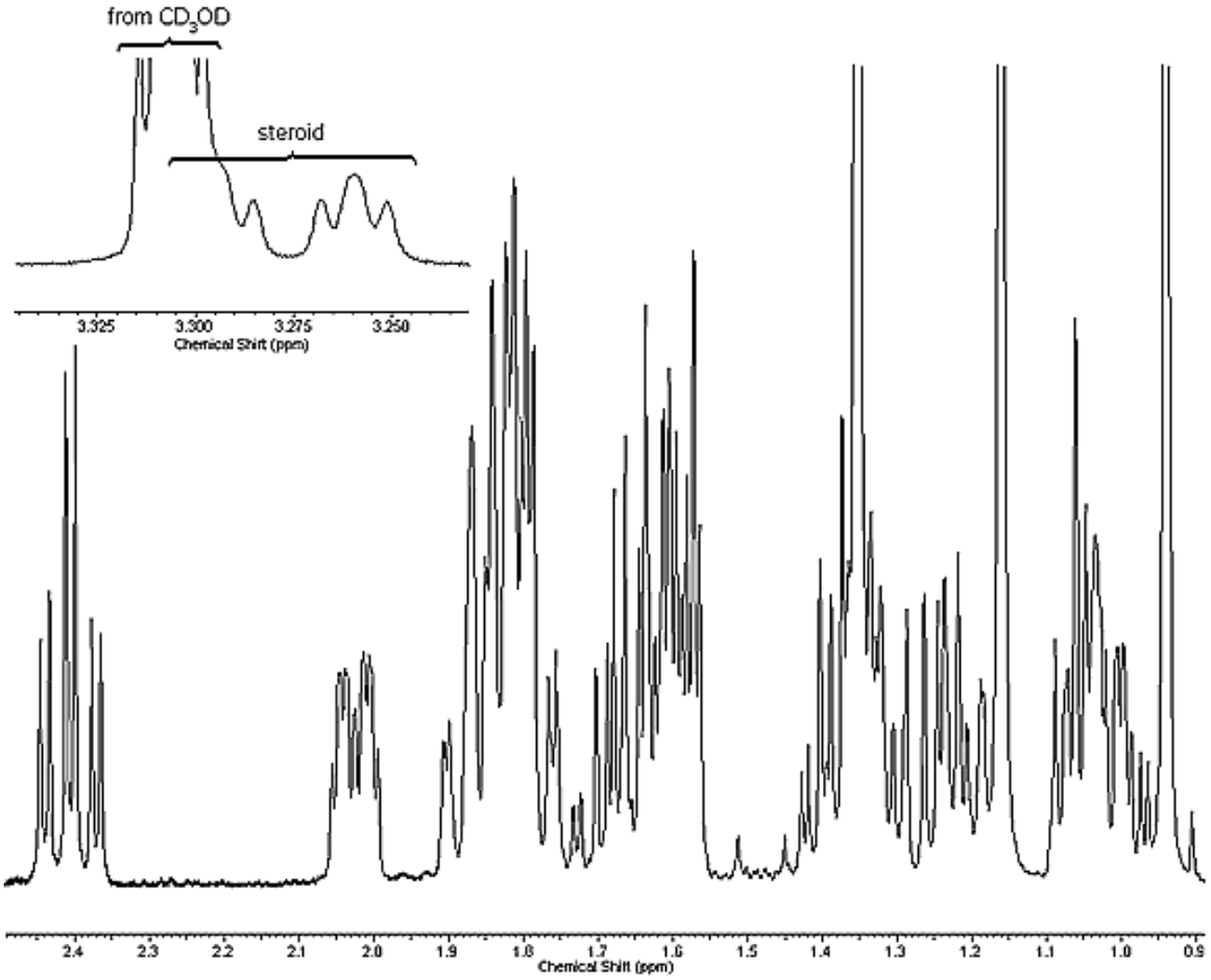

Figure 8b. Alkyl Region of Proton Spectrum of Figure 8a, Expanded to Show Peak Splitting Patterns.

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