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Illicit dehydrochloromethyltestosterone: how pure is it?

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Introduction

Dehydrochloromethyltestosterone (DHCMT) and methandienone are structurally related anabolic steroids. While synthesis procedure of illicitly produced DHCMT is not known, chlorination of methandienone could be one of the options. In this case the final product may be contaminated with the residual methandienone. This is of critical importance as methandienone could be detected longer [1] than DHCMT [2], and therefore administration of DHCMT by the cheating athletes may eventually lead to the adverse analytical findings for methandienone.

Illicit DHCMT preparations were analyzed for the presence of methandienone applying HPLC clean-up followed by gas chromatography – triple quadrupole mass spectrometry. The preparations tested include “Turinabol” (Hebei Genera Labs, 3 different packs), “Turinalin” (Cooper Pharma), “Oxastenon” (Sci Pharma Tech), “Turanabol” (British Dragon), “Turabolone” (Dynamic Development Laboratories), “Turinadrol-10” (Lyka, 4 different packs), “Turanabol” (Balkan Pharmaceuticals), “Oralturinabolan” (British Pharmaceuticals) and “Turinobol” (LSP).

The traces of methandienone were detected in every DHCMT product in the amount ranging from *ca.* 0.15 to 40 μg per tablet depending on manufacturer. One of the products contained pure methandienone instead of DHCMT.

Materials and Methods

Different DHCMT preparations were received from a local drug enforcement body. Each tablet was dissolved in 5 ml of methanol under sonication. After centrifugation 40 μl of the solution was supplemented with 15 μl of water and 5 μl of dehydropregnenolone acetate solution in methanol (50 ng/μl, retention time marker) and transferred into a vial for the HPLC clean-up.

An Agilent 1100 HPLC system equipped with preparative scale fraction collector was used in this study. A Waters SunFire C18 column (250 mm × 4.6 mm, 5 μm) protected by a guard column (20 mm × 4.0 mm) was maintained at 35°C for better retention time stability and to decrease backpressure was used. Gradient elution was applied as follows: 70% A (water) to 0% A within 0-20 min, then 100% B (acetonitrile) for 10 min, then 0% A to 70% A within 5 min, and equilibration at 70% A for 5 min. The eluent flow rate was 1 ml/min, injection volume – 50 μl, detector wavelength – 245 nm. Two fractions were collected corresponding to the retention time of DHCMT and methandienone. Fraction 1 (methandienone) was supplemented with 10 μl of methyltestosterone solution in methanol (30 ng/μl), evaporated to dryness at 50°C in vacuum and treated with 50 μl of MSTFA/NH_4I/dithiotreitol (1000/2/1.5 v/w/w) at 70°C for 30 min. The reaction mixture was transferred into a vial for the GC-MS analysis. Fraction 2 (DHCMT) was also collected for subsequent measurement of $\delta^{13}C/^{12}C$ values of DHCMT (data not shown).

GC-MS/MS system comprised a Trace GC Ultra gas chromatograph (Thermo Scientific, Italy) coupled to a TSQ Quantum GC triple quadrupole mass spectrometer (ThermoFisher Scientific, USA). The separation was done on HP-Ultra 1 column, 17 m × 0.2 mm × 0.11 μm (Agilent J&W, USA) with the temperature programming as follows: 177°C to 233°C at 4°C/min, then to 310°C at 20°C/min (held 4.15 min). One μl injections were done at 250°C in the split mode (1:20) with a carrier gas flow rate set to 0.6 ml/min (helium 99.9999%). Transfer line temperature was 300°C, the ion source was held at 250°C. The mass spectrometer was operated in the fullscan and SRM mode. The collision gas pressure was 0.13 Pa (or 1.0×10^{-3} Torr, argon 99.998%).

Results and Discussion

The problem of contamination of the sport nutrition products was raised in 2000 by Catlin et al. when the trace amounts of norandrostenedione were identified in over-the-counter (at that moment) androstenedione preparations [3]. Later, this issue was repeatedly discussed [4]. Alternatively, in present study we analyzed the *illicit* DHCMT preparations, as currently there is no any DHCMT product officially approved for human use, either as a nutritional supplement or pharmaceutical.

Direct GC-MS analysis of derivatized organic extracts prepared from tablets does not allow detecting trace amounts of methandienone as saccharides normally present as major constituents in pharmaceuticals coelute with and completely mask methandienone (Fig. 1,

left). Selectivity of GC-MS/MS could help, but sample size taken for analysis should still be restricted in order not to inject µg amounts of excipients into the sensitive equipment (Fig. 1A and 1B are the mass chromatograms plotted against the fragment ions of trimethylsilylated methandienone for DHCMT products #12 and #8, respectively, illustrating that in the latter case methandienone is undetectable without further clean-up and preconcentration steps).

We assumed that since saccharides due to their polarity are not retained in the reversed-phase HPLC, pure methandienone fraction may be collected. Importantly, HPLC also allows taking bigger sample aliquot and eventually decreasing the limit of detection.

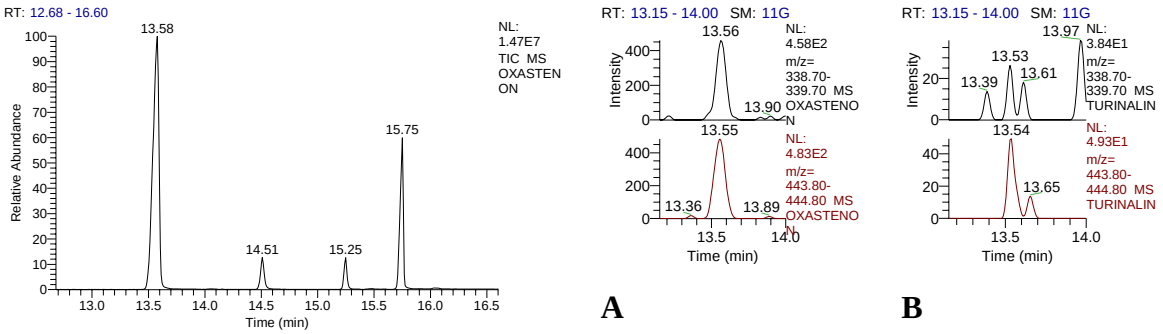

Fig. 1. GC-MS chromatogram of trimethylsilylated extract from DHCMT product; 13.58 and 15.25 min – saccharides, 15.75 min – DHCMT. Methandienone 2TMS elutes at 13.55 min.

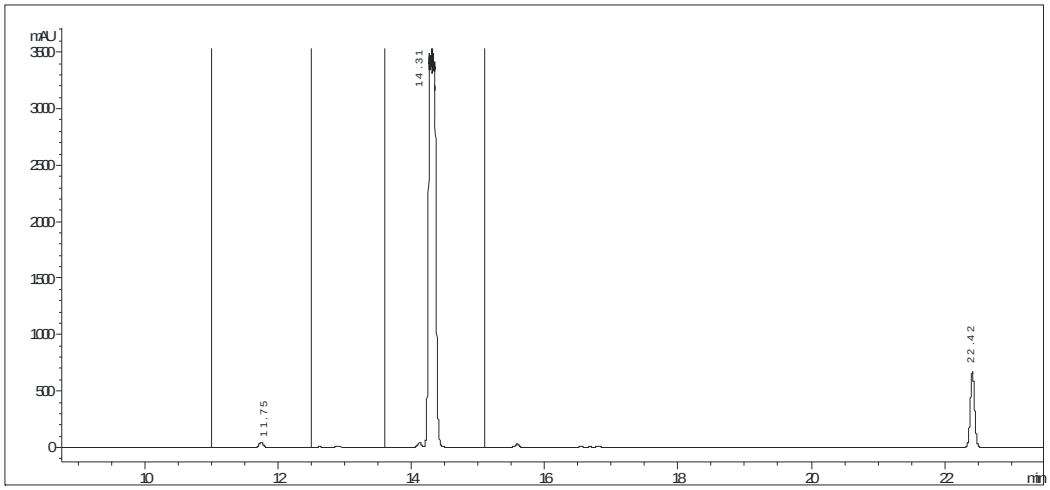

Fig. 2. HPLC-UV chromatogram of the extract from DHCMT product; 11.75 min – methandienone, 14.31 – DHCMT; 22.42 min – retention time marker.

After HPLC clean-up (Fig. 2) we identified methandienone traces in **every** DHCMT product using GC-MS/MS in SRM mode (limit of detection 0.01 ng per HPLC fraction), in the amount ranging from *ca.* 0.15 to 40 µg per tablet depending on the manufacturer

(Table 1). The content of methandienone was estimated against a calibration curve bracketing the concentration range 0.1-200 ng methandienone per HPLC fraction. The content of DHCMT in tablets was also estimated using HPLC-UV detection comparing absolute peak areas for a DHCMT product and the standard mixture with known amount of DHCMT.

Table 1. List of DHCMT products analyzed. Declared content is 10 mg/tab, except #9 (5 mg)

#	Product Name	Manufacturer	DHCMT, mg/tab	MA, µg/tab
1	Turinabol #1	Hebei Genera Labs	8.1	3.4
2	Turinabol #2	Hebei Genera Labs	10.0	2.3
3	Turinabol #3	Hebei Genera Labs	6.5	2.7
4	Turinadrol #1	Lyka	9.6	1.5
5	Turinadrol #2	Lyka	10.1	1.6
6	Turinadrol #3	Lyka	9.4	2.0
7	Turinadrol #4	Lyka	8.5	2.4
8	Turinalin	Cooper Pharma	8.3	3.4
9	Turabolone	Dynamic Development Labs.	4.6	0.14
10	Oralturinabolan	British Pharmaceuticals	9.0	0.60
11	Turanabol	Balkan Pharmaceuticals	9.9	37.4
12	Oxastenon	Sci Pharma Tech	10.0	15.7
13	Turanabol	British Dragon	10.5	21.5
14	Turinobol	LSP	ND	10700

As is seen from Table 1 data, most of DHCMT products contain methandienone in the amount of 1.5-3.5 µg per tablet, while some of them – more than 15 µg per tablet. Only two products (#10, 11) were relatively pure. Interesting to note that product #14 contained methandienone instead of DHCMT.

References

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