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## **Plasma Levels of 19-Norsteroids after Oral and Sublingual Administration of Norandrostenedione**

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### **Introduction**

According to the Dietary Supplement Health and Education Act of 1996, products containing prohormones have been distributed as nutritional supplements in the USA. One of the most popular prohormones is norandrostenedione (19-nor-4-androstene-3,17-dione) which has been promoted as an oral anabolic supplement capable of converting to nandrolone and consequently increasing lean body mass and athletic performance.

Previous studies on norandrostenedione dealt with urine analysis or effects on athletic performance <sup>[1,2,3,4]</sup>. In order to assess a potential transformation of this hormone precursors into the active hormone, an evaluation of plasma levels of norsteroids is necessary. In a pilot study the conversion of norandrostenedione to nandrolone was proven by Machnik et al.<sup>[5]</sup>.

In the present investigation, a clinical trial was carried out to determine if and to what extent norandrostenedione is metabolised to nandrolone. Eight male subjects were participating in this investigation to examine the effects of orally administered supplements or sublingual formulations of norandrostenedione. Plasma total concentrations of the parent compound norandrostenedione, nandrolone, and the main metabolites norandrosterone and noretiocholanolone were determined by means of gas chromatography/mass spectrometry (GC/MS). Identification of unconjugated nandrolone in plasma samples was of particular interest in order to assess hormonal activity of the investigated nandrolone precursor.

## **Ethical requirements**

According to the German Drugs Act products containing prohormones as norandrostenedione are not classified as nutritional supplements but as drugs without marketing authorisation. Because of this status, the planned study was categorised as a phase-I study. Before starting this study ethical committee application, study protocol and informed consent form had been prepared and approved by the ethic committee of Cologne University. To comply with legal requirements registration at the local health authority and the Federal Institute for Drugs and Medical Devices (BfArM, Bonn) was applied.

Prospective volunteers underwent physical examinations. Eight subjects who met entrance criteria (as determined at a screening clinic visit from the medical history, vital signs, physical examinations, haematological tests, and clinical laboratory blood and urine chemical evaluation) were invited to participate. Prior to the beginning of the study, each participant read and signed a health history and informed consent that detailed the outline of the study designed in accordance with the Declaration of Helsinki.

## **Excretion studies**

### *Investigational drugs*

Two different nutritional supplements containing norandrostenedione were purchased via the Internet. Identity and purity of the supplements were verified before administration.

1. "19-Norandrostenedione" from Prolab Nutrition (11 Britton Drive, Bloomfield, CT U.S.A);  
100 mg 19- Norandrost-4-ene-3,17-dione per capsule, 60 capsules
2. „NorCycloDione“ from SportsOne (Wallingford, CT 06492, U.S.A.);  
25 mg 19- Norandrost-4-ene-3,17-dione per lozenge, 60 lozenges

### *Volunteers*

- 8 male, healthy volunteers
- age: 25 – 51 years (mean  $32.8 \pm 7.5$ )
- bodyweight: 67 – 99 kg (mean  $81.6 \pm 10.7$ )

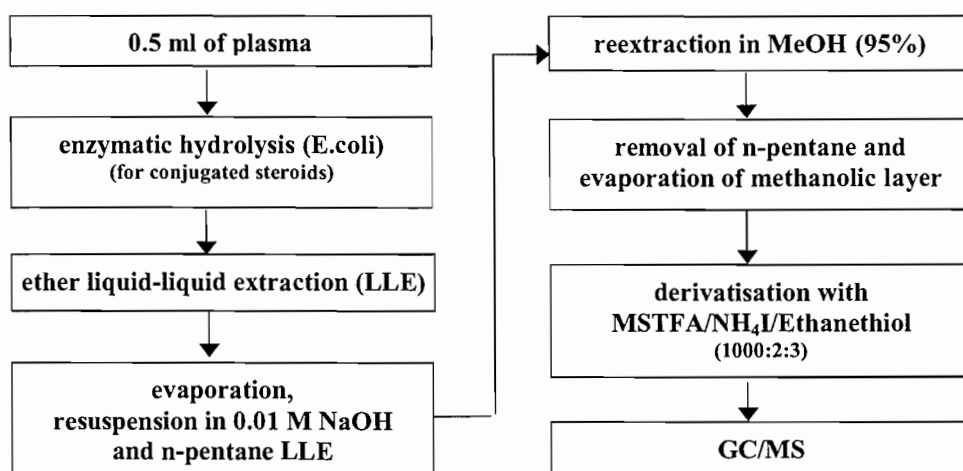
### Administration of supplements

One capsule of “19-Norandrostenedione” and, after a two-week washout period, one lozenge of “NorCycloDione” were administered to the volunteers, respectively. Blood (10 ml) was drawn from an indwelling catheter inserted to a forearm vein before administration and after 10, 20, 30, 45, 60, 90 minutes, 2 h, 3 h, 4 h, 6 h, and 8 h from the  $t = 0$  point. An additional sample was taken by venipuncture after 24 h. Samples were collected in EDTA tubes and immediately centrifuged. Plasma was frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis.

## Experimental

### Sample Preparation

In Scheme 1, the principle sample preparation procedure is depicted. For details, please refer to Machnik et al. <sup>[5]</sup>.



Scheme 1: Overview sample preparation

### GC/MS parameters

GC-MSD system:	Agilent 6890/5973
Injection mode:	2 $\mu\text{l}$ splitless
Carrier gas:	Helium (1ml/min flow)
Analytical column:	HP-5 MS, 0.25 mm i.d. , 0.25 $\mu\text{m}$ film thickness, length:17 m
Temperature program:	100° - 40°/min - 190° - 5°/min - 240° - 40°/min - 320° - 3 min
Acquisition mode:	SIM (selected ion monitoring)

Two fragment ions of the target analytes were selected for qualitative and quantitative purposes. They are represented in Table 1. To facilitate and optimise quantitative analyses deuterated analogues of nandrolone and noretiocholanolone were chosen, which were added to each plasma samples in defined amounts. Ions underlined were used for quantification.

**Table 1:** Fragment ions of norsteroids

norsteroid	<i>m/z</i>	<i>m/z</i>	internal standard	<i>m/z</i>	<i>m/z</i>
norandrostenedione	401	<u>416</u>	d <sub>3</sub> -nandrolone	406	<u>421</u>
nandrolone	403	<u>418</u>	d <sub>3</sub> -noretiocholanolone	<u>408</u>	423
norandrosterone	<u>405</u>	420			
noretiocholanolone	<u>405</u>	420			

## Results and Discussion

### *Validation of the analytical method*

To determine plasma concentrations, an existing analytical method <sup>[5]</sup> was adjusted and revalidated following the *FDA Guidance for Industry for Bioanalytical Method Validation* (2001)<sup>[6]</sup>. Validation parameters and results are listed in Table 2 and Table 3.

**Table 2:** Validation parameters and results for plasma total concentration (conjugated and unconjugated) norandrostenedione (ND), nandrolone (NT), norandrosterone (NA) and noretiocholanolone (NE)

	ND	NT	NA	NE
Working range	0.5 – 80 ng/ml	0.5 – 80 ng/ml	5 – 2000 ng/m	5 – 1000 ng/ml
Correl. coeff.(R <sup>2</sup> )	0.998	0.999	0.998	0.998
Mean recovery (%)	68.6 (5 ng/ml) 66.4 (50 ng/ml)	66.1 (5 ng/ml) 67.8 (50 ng/ml)	74.8 (10 ng/ml) 81.1 (500 ng/ml)	74.3 (10 ng/ml) 75.6 (500 ng/ml)
Accuracy (%)	9.17 (5 ng/ml) 6.96 (25 ng/ml) 3.02 (80 ng/ml)	5.54 (5 ng/ml) 7.61 (25 ng/ml) 1.67 (80 ng/ml)	5.94 (5 ng/ml) 6.91 (400 ng/ml) 10.97 (1200 ng/ml)	8.83 (5 ng/ml) 4.04 (400 ng/ml) 7.24 (1200 ng/ml)
Precision (CV, %)	4.62 (5 ng/ml) 1.06 (25 ng/ml) 2.94 (80 ng/ml)	2.51 (5 ng/ml) 2.24 (25 ng/ml) 1.58 (80 ng/ml)	12.94 (5 ng/ml) 2.50 (400 ng/ml) 3.30 (1200 ng/ml)	4.90 (5 ng/ml) 1.13 (400 ng/ml) 5.40 (1200 ng/ml)

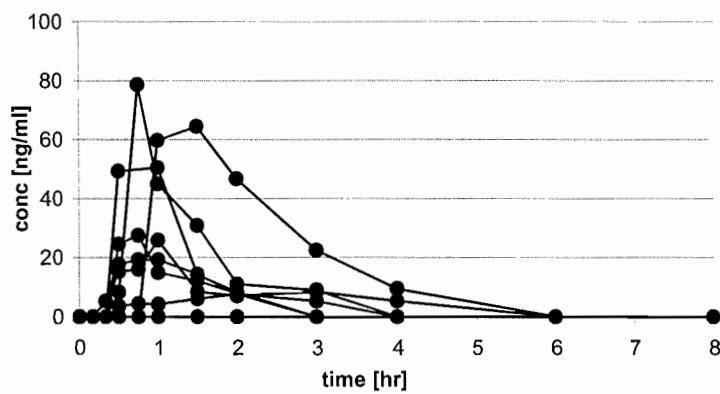
**Table 3:** Validation parameters and results for determination of unconjugated nandrolone (NT) in plasma

	NT (unconjugated)
Working range	0.5 – 5 ng/ml
Correl. coeff.(R <sup>2</sup> )	0.999
Mean recovery (%)	61.8 (2.5 ng/ml)
Accuracy (%)	9.88 (0.5 ng/ml) 4.85 (5 ng/ml)
Precision (CV, %)	7.44 (0.5 ng/ml) 1.65 (5 ng/ml)

**Plasma concentrations of norsteroids after administration of one capsule of „19-Norandrostenedione“ (100 mg norandrostenedione)**

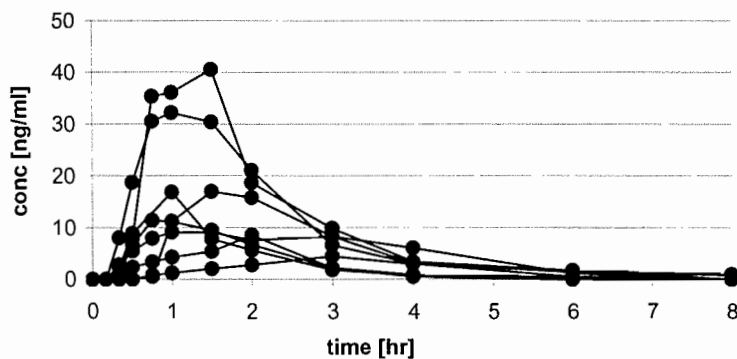
*Plasma total concentrations (unconjugated and conjugated steroids)*

Oral ingestion of a 100 mg capsule of norandrostenedione resulted in maximum plasma total concentrations ranging from 3.6 to 78.7 ng/ml for the parent compound. Norandrostenedione (ND) concentrations started rising about 30 min after administration and peaked between 45 min and 3 hrs (Fig. 1).



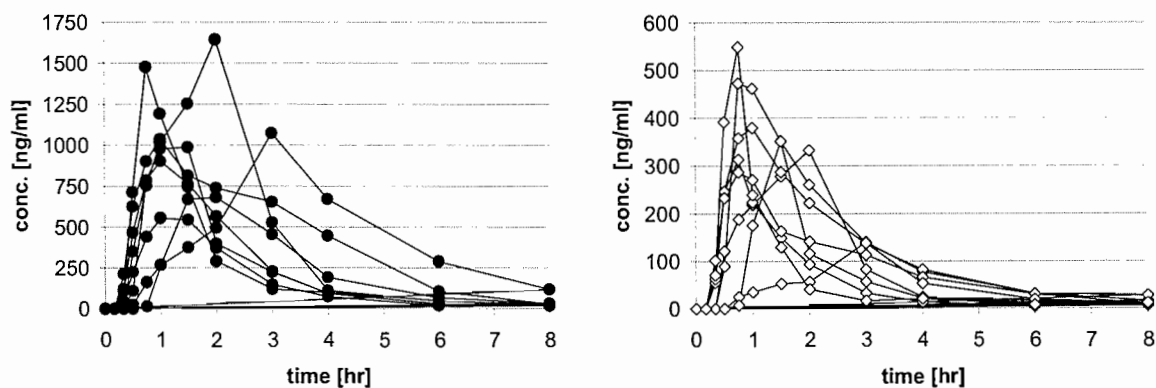
**Figure 1:** Plasma total concentrations of ND after administration of a 100 mg ND capsule (eight volunteers)

Nandrolone was detected in plasma samples of all volunteers. Maximum values varied between 4.5 and 40.1 ng/ml as demonstrated in Figure 2.



**Figure 2:** Plasma total concentrations of nandrolone after administration of a 100 mg ND capsule (eight volunteers)

Oral ingestion of a 100 mg capsule of ND resulted in maximum total plasma concentrations of the main metabolites norandrosterone (NA) and noretiocholanolone (NE) ranging from 555.4 to 1644.8 ng/ml and 140.7 to 549.0 ng/ml, respectively (Fig. 3).

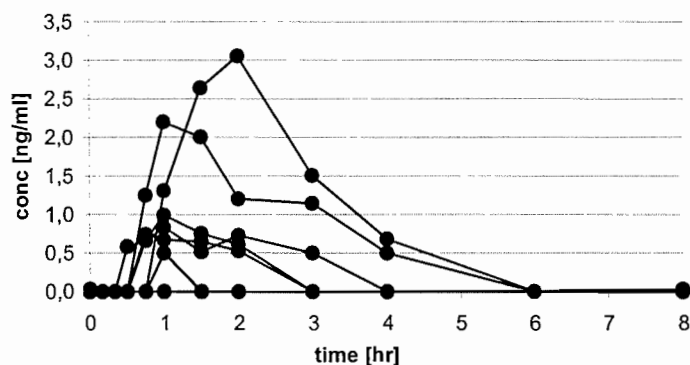


**Figure 3:** Plasma total concentrations of NA (●, left) and NE (◇, right) after administration of a 100 mg ND capsule (eight volunteers)

*Plasma concentrations of unconjugated nandrolone*

Plasma samples of all volunteers showed low amounts of unconjugated nandrolone after application of 100 mg norandrostedione (one capsule of *19-Norandrostedione*).

Maximum values up to 3.1 ng/ml were determined (Fig. 4). Nandrolone concentrations in plasma samples of two volunteers were very low (< 0.5 ng/ml) and could not be sufficiently quantified.



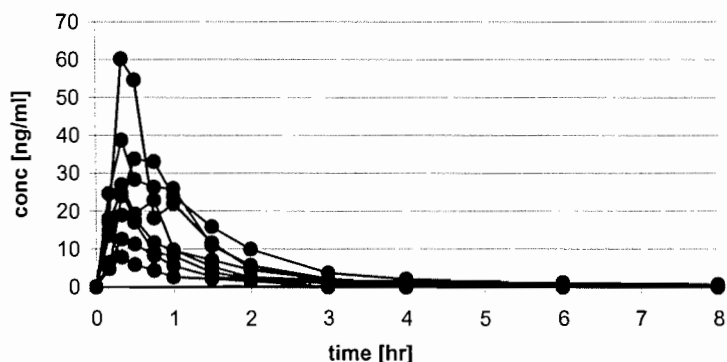
**Figure 4:** Plasma concentrations of unconjugated nandrolone after administration of a 100 mg ND capsule (eight volunteers)

**Plasma concentrations of norsteroids after administration of one lozenge of „NorCycloDione“ (25 mg 4-norandrostenedione)**

Application of this supplement is accomplished by dissolving the lozenge under the tongue. This so called sublingual administration provides the advantage of bypassing the first-pass effect of the liver because active ingredients can be resorbed through the oral mucosa. As a consequence, fast invasion and increased bioavailability is possible.

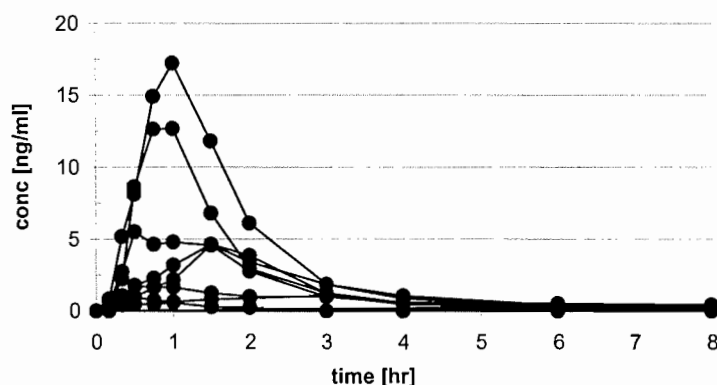
*Plasma total concentrations*

Administration of NorCycloDione lozenges resulted in fast and efficient resorption of norandrostenedione. Maximum plasma total concentrations of the parent compound were reached within 30 minutes and ranged between 12.5 and 60.2 ng/ml (Fig. 5).



**Figure 5:** Plasma total concentrations of ND after administration of a 25 mg ND lozenge (eight volunteers)

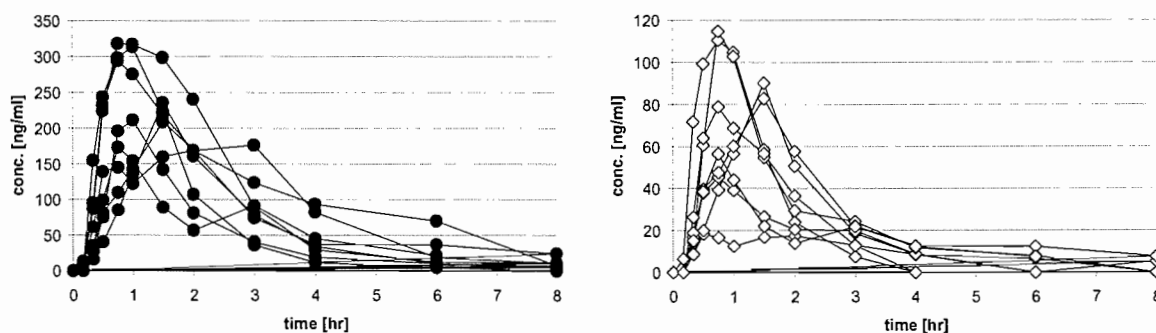
Nandrolone was detectable in plasma samples of all volunteers. Maximum plasma total concentrations of generated nandrolone were determined between 0.7 and 17.2 ng/ml (Fig. 6).



**Figure 6:** Plasma total concentrations of nandrolone after administration of a 25 mg ND lozenge (eight volunteers)



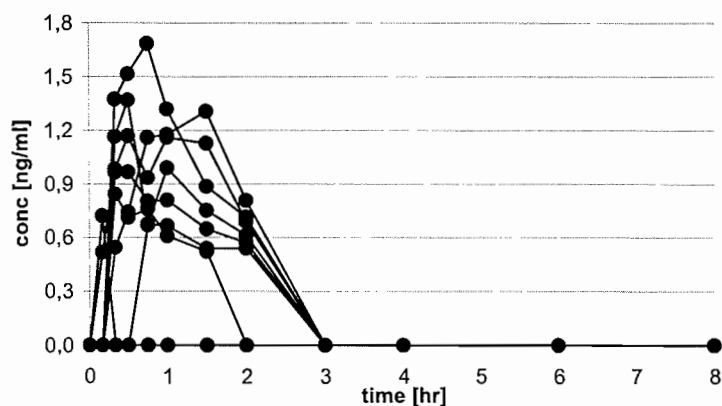
Corresponding to the fast absorption of norandrostenedione after administration of the lozenges, concentrations of the main metabolites norandrosterone and noretiocholanolone increased earlier than after administration of capsules. Maximum values for NA varied between 169.1 and 318.2 ng/ml and for NE between 18.5 and 114.9 ng/ml (Fig. 7).



**Figure 7:** Plasma total concentrations of NA (●, left) and NE (◇, right) after administration of a 25 mg ND lozenge (eight volunteers)

#### *Plasma concentrations of unconjugated nandrolone*

After administration of a single lozenge *NorCycloDione* (25 mg norandrostenedione), plasma samples of the volunteers contained maximum concentrations of unconjugated nandrolone in amounts up to 1.7 ng/ml (Fig. 8).

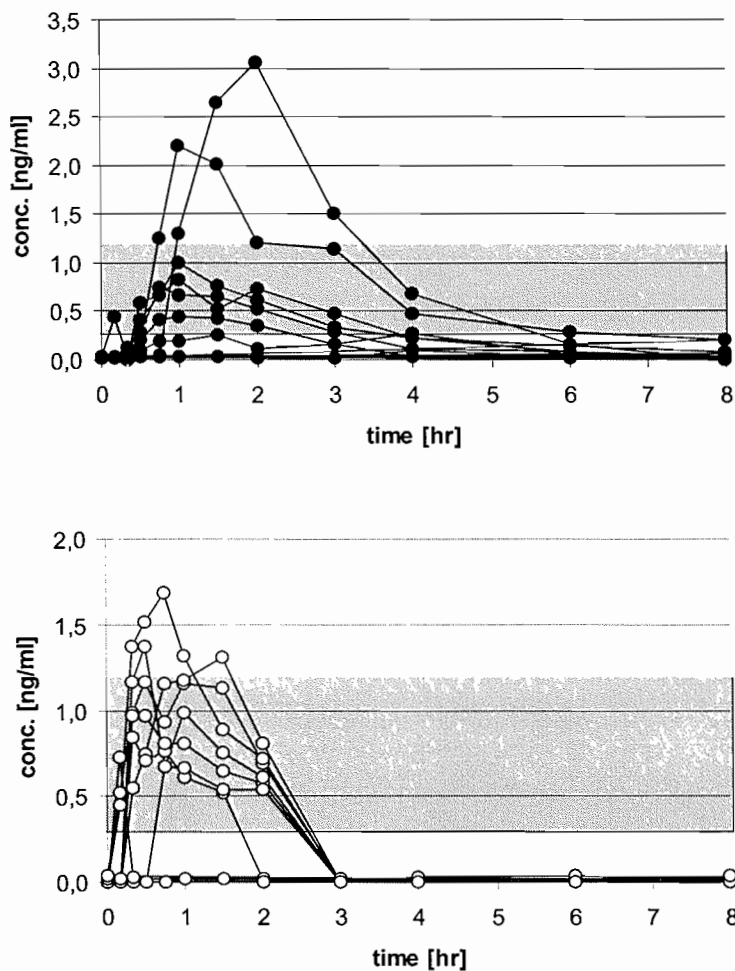


**Figure 8:** Plasma concentrations of unconjugated nandrolone after administration of a 25 mg ND lozenge (eight volunteers). Values of one volunteer could not be sufficiently quantified as nandrolone concentrations were lower than 0.5 ng/ml.

## Estimation of hormonal activity

Studies concerning common therapeutic applications as well as male fertility control showed concentrations of nandrolone up to 8 ng/ml after *i.m.* injections of nandrolone esters. Testosterone suppression was determined as an evidence for nandrolone activity. Plasma testosterone did not rise until nandrolone concentrations declined to 0.3-1.2 ng/ml<sup>[7,8,9]</sup>. The hatched area in Figure 9 represents these minimum concentrations of unconjugated nandrolone, which are considered physiologically relevant if maintained constantly.

Within the 100 mg norandrostenedione-study six out of eight volunteers reached these threshold levels as shown in Figure 9. Two volunteers exceeded this concentrations remarkably. After administration of 25 mg norandrostenedione lozenges, maximum concentrations of all volunteers were determined at levels possibly causing interference in physiological processes.



**Figure 9:** Plasma concentrations of unconjugated nandrolone (eight volunteers) after administration of 100 mg ND capsules (●, upper) and 25 mg ND lozenges (○, lower)

## Conclusion

In contrast to urine analysis, blood analysis enables the detection of the applied prohormone norandrostenedione itself and the intermediately formed nandrolone in addition to the main metabolites norandrosterone and noretiocholanolone. The obtained data demonstrate that the administration of a single dose of the respective supplements generates physiologically relevant plasma concentrations of unconjugated nandrolone over a short period. Physiological intervention might be possible if these plasma levels are maintained constantly. Until now, the use of prohormones is completely unregulated, and amounts greater than those recommended by the manufacturers can be easily consumed. This may result in higher nandrolone plasma concentrations for longer periods of time.

But soon a change in legislation will become apparent. By adoption of the Anabolic Steroid Control Act of 2004 the U.S. Congress voted to ban all prohormones, including norandrostenedione, from the nutritional supplement market, and reclassify these compounds as Schedule III Anabolic Steroids <sup>[10]</sup>.

### *Acknowledgement*

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