

APPLIED CONSUMER SERVICES, INC.

Chemical Analysis for
Methyltestosterone

Report #: 27584

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Report to: Mr. Mitch Ross

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1.0 Aim of the Analysis

A spray blue bottle with security seal of 1 fluid ounce (30 ml) capacity and labeled S.W.A.T.S. (see Fig. 1-4) was brought to the laboratory to determine its content of methyltestosterone.

The S.W.A.T.S. bottle presented an unopened seal. Once the seal was broken and the bottle was opened, a light brownish liquid with suspended solids was identified as the content of the bottle. A volume of 30 ml is declared as the content of the bottle. The active principles of the product are presented as deer antler velvet extract, and stevia extract.

Figure # 1



Figure # 2



Figure # 3



(*) NIH. U.S. National Library of Medicine. TOXNET Toxicology Data Network.
toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+58-18-4

2.0 Method of analysis

By High Performance Liquid Chromatography (HPLC), according to United States Pharmacopoeia, Volume 38, National Formulary 33, Methyl Testosterone, page 8153. 2015.

For material and equipment used, sample preparation procedures, experiments performed, and analytical data obtained see Annexes 1-6.

3.0 Results

Results from our research and chemical analysis indicate:

1. No Methyltestosterone was detected in supplement product "S.W.A.T.S." (the minimum amount of Methyltestosterone that can be detected is 0.00026 mg/ml).
2. Steroid product, Methyltestosterone, is practically insoluble in water and poorly soluble in S.W.A.T.S. (approximately 0.08 mg/ml or 0.008% w/v).
3. From spiking (adding) of pure chemical Methyltestosterone (99.5%)* to the supplement:

- ✓ The “tainted product” (S.W.A.T.S. with added Methyltestosterone) becomes a liquid with numerous floating particles (suspension).
- ✓ In the event that all Methyltestosterone was dissolved in the S.W.A.T.S. solution, only a maximum of 0.04 mg of the steroid could be delivered with one serving size (three sprays).
- ✓ 250 serving sizes (750 individual sprays) of the “tainted product” are needed to match the dosage of a single tablet of Methyltestosterone.

* Note that the spiking of the Methyltestosterone is based on the minimum commercially available dose of Methyltestosterone on the market (10 mg per tablet).

4.0 Conclusions

- ✓ As per our knowledge, there is not a commercially available liquid form of Methyltestosterone.
- ✓ Since our experiment used the pure chemical Methyltestosterone (99.5%), inaccessible to the public, it is hard to imagine how the actual commercial steroid product (tablet form) can be physically combined with S.W.A.T.S.
- ✓ Adding the commercially available Methyltestosterone to the original S.W.A.T.S. spray liquid would turn the liquid into a very heavy suspension. This is due to the nature and low solubility of inactive ingredients (excipients) present in the commercially available tablet. This heavy suspension would clog the tip of the spray bottle and making it practically impossible to use the spray bottle.

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Annex 1. Materials and Equipment

- ✓ High Performance Liquid Chromatograph Dionex Ultimate 300, coupled to an Ultimate 3000 Autosampler.
- ✓ Column: Supelco Discovery HS C18, 25cm x 4.6 cm, 5 µm particle size, # 129842-06.
- ✓ Methyltestosterone, USP, NDC# 00216-4001-44, 99.5 % purity.
- ✓ Testosterone, Sigma, Lot 39H0638, 99.9% purity.
- ✓ Methanol, GFS, Lot C583810, 99.9 %.
- ✓ Acetonitrile, GFS, Lot C585106, 99.9%.
- ✓ Membrane filter, Supelco, Iso-Disc filter, N-13-4, Nylon, 13 mm x 0.45 µm

Annex 2. Sample preparation for methyltestosterone analysis

Sample preparations to study the content of methyltestosterone in S.W.A.T.S. and the ability of S.W.A.T.S. to solubilize methyltestosterone

- ✓ In a 15 ml vial a volume of 1.0 ml S.W.A.T.S. was added and mixed with 1.0 ml of methanol. The mixture was manually agitated and placed in an ultrasound mixer for 5 minutes. Re-agitated manually and then filtered through a 0.45 μm filter. Then 1.0 ml of this solution was taken to a final volume of 5 ml in a volumetric flask using methanol as solvent. Then it was analyzed for methyltestosterone.

- ✓ In a 15 ml vial a volume of 2.00 ml of S.W.A.T.S was mixed with 3.67 mg of methyltestosterone. The vial was closed and hand shaken for two minutes (to simulate a deliberate addition of the testosterone compound to S.W.A.T.S.). Then, the final mix was filtered through a 0.45 membrane filter and analyzed for methyl testosterone content.

Annex 3. Preparation of standard and HPLC system suitability solution

Testosterone and methyltestosterone were weighed in the same volumetric flask and dissolved to a volume of 25.0 ml with methanol, as indicated in the table:

Compound	Purity (%)	Weight (g)	Total Volume (mL)	Concentration (mg/mL)
Methyltestosterone	99.5	0.00669	25.0	0.2663
Testosterone	99.9	0.00969	25.0	0.3872

Then, 0.20 ml of above solution was taken to a final volume of 5.0 ml in a volumetric flask, to give a concentration of 10.7 µg/ml and 15.5 µg/ml of methyltestosterone and testosterone respectively, as system suitability solution.

Specific gravity of methanol at 25 °C = 0.78697 g/mL

$$\text{Methyltestosterone} = \frac{(0.00669\text{g}) \times (0.995)}{(25\text{ml}) \times (0.78697\text{g/mL})} = 0.3383\text{mg/g}$$

$$\text{Testosterone} = \frac{(0.00969\text{g}) \times (0.999)}{(25\text{ml}) \times (0.78697\text{g/mL})} = 0.4920\text{mg/g}$$

As a quantitation standard an amount of 0.60470 g of above solution was taken and diluted to 10.0 ml to obtain a final concentration of 0.0205 mg/ml of methyltestosterone.

Finally, to estimate a limit of quantitation for this method used, a dilution of the concentrated solution of methyltestosterone was made, as follows:

$$\text{Methyltestosterone} = \frac{(0.11793\text{g}) \times (0.3383\text{mg/g})}{(10\text{ml})} = 0.00399\text{mg/mL}$$

Annex 4. Amount of S.W.A.T.S. per dose

According to the manufacturer the recommended dose of the product is 3 sprays per dose, two or more times per day. What follows is the amount of S.W.A.T.S. delivered per dose: 0.50403 g

Sample	Weight (g)
3 sprays	0.49887
3 sprays	0.50350
3 sprays	0.50971
Average:	0.50403

Annex 5. Suitability test results for the HPLC instrument.

Analyte	Retention Time (min)	Resolution (EP)	Asymmetry	Plates
Testosterone	7.240	3.35	1.94	6234
Methyl Testosterone	8.560		1.90	6538

Retention Time (min)	Area (mAU/min)	Height (mAU)
8.560	53.800	172.84
8.560	53.745	171.58
8.560	53.546	171.37
8.560	53.748	171.74
8.560	53.606	171.67
Average:	53.689	171.83
Std. Dev.(%)	0.20	0.34

Annex.6 Analytical Data and Discussion

Sample	Retention Time (min)	Area (mAU/min)	Height (mAU)
0.0205 mg/mL methyltestosterone	8.560	53.689	171.837
Blank	N/A	N/D	N/D
2.0 ml S.W.A.T. S.+ 3.67 mg methyltestosterone[0.5/5]	8.540	21.247	58.218
S.W.A.T.S. only(1.0 ml S.W.A.T.S. + 1.0 ml methyl alcohol[1/5])	N/A	N/D	N/D
0.00399 mg/ml methyltestosterone	8.567	10.993	35.119

N/A: not applicable, N/D: not detected

Limit of Quantitation (LOQ) = $10 \times \text{blank value} \times [\text{concentration of diluted standard stock solution}] / \text{Signal intensity or standard stock solution}$

LOQ = $10 \times 0.231 \text{ mAU} \times 0.00399 \text{ mg/ml} / 35.119 \text{ mAU} = 0.00026 \text{ mg/ml}$ methyl testosterone. That is the minimum amount of methyltestosterone the instrument can detect.

Then, in the S.W.A.T.S. sample, the absence of methyl testosterone can be estimated as less than: $0.00026 \text{ mg/ml} \times (5.0 \text{ ml} / 1.0 \text{ ml}) (2.0 \text{ ml} / 1.0 \text{ ml}) = < 0.0026 \text{ mg/ml}$ methyl testosterone which, for a total available volume of 30 ml in the S.W.A.T. spray bottle indicates a limit of no more than 0.078 milligrams of methyltestosterone, if present.

For the methyltestosterone spiked S.W.A.T.S. sample, a concentration of 0.008097mg/ml was found, and, by correcting this value for the dilution used for analysis (0.50 ml to 5.0 ml total) a concentration of 0.08097mg/mL of methyltestosterone is obtained, indicating the very poor solubility of methyltestosterone in the S.W.A.T.S product.

Then, if the addition of methyltestosterone to a S.W.A.T.S. bottle is intentionally attempted, no more than 2.429mg of methyltestosterone could be dissolved in the 30 ml of the liquid present in a S.W.A.T.S. bottle, independently of the amount added in solid form.