A Sensitive Liquid Chromatography–Tandem Mass Spectrometry Method for the Measurement of 11β-MNT and 11β-MNTDC in Human Serum

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HIGHLIGHTS: Reduce manual sample prep. time HybridSPE® - XTR

INTRODUCTION

11-beta-methyl-19-nortestosterone (11β-MNT) is the active androgen derived from 11-beta-methyl-nortestosterone dodecylcarbonate (11β-MNTDC). 11β-MNT is being developed as a candidate drug of male hormonal contraception. 11β-MNT like dimethandronlone (DMA) binds to both the androgen and progesterone receptor and may provide the suppression of spermatogenesis with one compound instead of two agents, testosterone and a progestin. The objective of this study was to develop and validate a highly sensitive LC-MS/MS method for the simultaneous detection of serum 11β-MNT and 11β-MNTDC levels for research and clinical purposes in men.

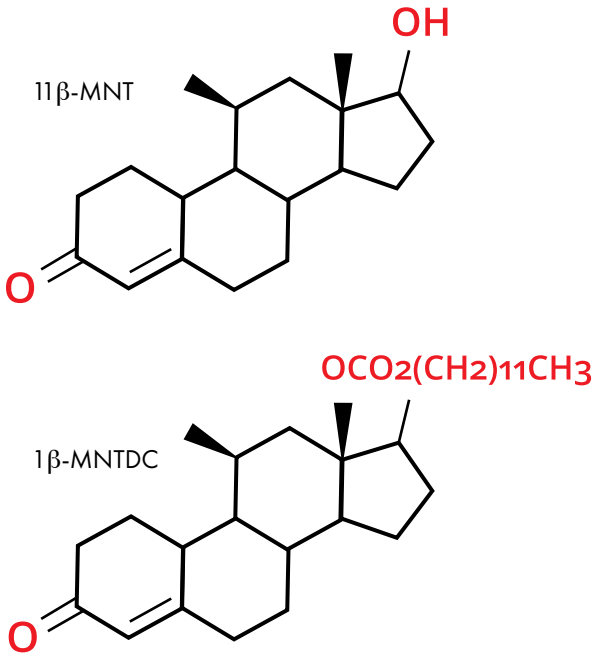

Figure 1. 11β-MNT and 11β-MNTDC

MATERIALS AND METHODS

Optimized mass parameters used negative MRM mode. The selected parent/product ions m/z are 289.3/109.1 for 11β-MNT and 501.3/271.1 for 11β-MNTDC, and 295.2/113.0 for internal standards (IS) d6-MNT and 501.2/277.2 for d6-MNTDC,respectively. An Accucore PFP column (2.6μ,150 mm×2.1 mm) was used with a gradient mobile phase from 48%B to 60%B for 8 minutes and then to 100%B for 3 min (B / A = 100%MeOH / H_2O with 0.1% formic acid) delivered at 0.5mL/min. Total run time was 15 minutes. Sample preparation was performed following the method in table 1. The range of calibration curve was 0.5 (0.1) ~ 200 ng/mL for 11β-MNT, and 0.5 ~ 2,000 ng/mL for 11β-MNTDC.

Table 1. Sample Preparation

Protein Precipitation	• Add 25μL internal standard (IS) into 100μL of serum samples or calibrators and vortex well • Add 300 μL acetonitrile and mix well • Centrifuge at 14,000 rpm for 3 minutes • Transfer supernatant
Condition	• Aspirate and Dispense HybridSPE® XTR Tips with 500μL acetonitrile
Extraction	• Aspirate and dispense the supernatant 2x with HybridSPE Tips binding interferents to sorbent • Transfer clean sample to a new glass vial
Prep for injection	• Dry down the extracted samples • Reconstitute with 40% MeOH in 0.1% formic acid H_2O for the analysis

RESULTS

Table 2.

	11β-MNT (100 ng/mL)	11β-MNTDC (1000 ng/mL)
Matrix Effect (n=3)	102.2%	114.8%
% CV	2.7	5.2
	IS (100 ng/mL)	**IS (600 ng/mL)**
Matrix Effect (n=3)	101.5%	116.4
% CV	3.2	5.8
Correlation Analyte Conc. / IS Conc.	y=0.2100x-0.00952 r^2=0.9956	y= 0.0135x-0.00247 r^2=0.9966
	11β-MNT (0.5 ng/mL)	**11β-MNTDC (0.5 ng/mL)**
LLOQ (n = 5) signal/noise	12.3 ± 0.451	13.3 ± 1.620
%CV	3.65	12.2

Table 3.

11β-MNT (ng/mL)	% Accuracy ± SD (n=6, 3 days)	%CV
1.5	102.5 ± 0.069	4.5
20	105.5 ± 1.130	5.4
80	99.4 ± 7.403	9.3
11β-MNTDC (ng/mL)	**% Accuracy ± SD (n=6, 3 days**	**%CV**
3.0	101.4 ±0.208	6.8
60	104.8 ± 6.281	10.0
800	92.4 ± 71.98	9.7

CONCLUSIONS

A sensitive LC-MS/MS assay for human serum 11β-MNT and 11β-MNTDC was developed and validated which can be applied to clinical and research studies.