A Sensitive Liquid Chromatography–Tandem Mass Spectrometry Method for the Measurement of Nestorone® in Human Serum

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

INTRODUCTION

- Nestorone® (NES) is a synthetic progestin being developed by Population Council and NICHD for female and male contraception.
- NES does not bind to the androgen or the estrogen receptor and may have less adverse effects than androgenic progestin commonly used for female contraception.
- NES was previously measured in serum by radioimmunoassay that has non specific interference leading to detectable NES levels in men when no NES was administered.
- The objective of this study was to develop and validate a highly sensitive LC MS/MS method for the detection of serum Nestorone levels for research and clinical purposes in men.

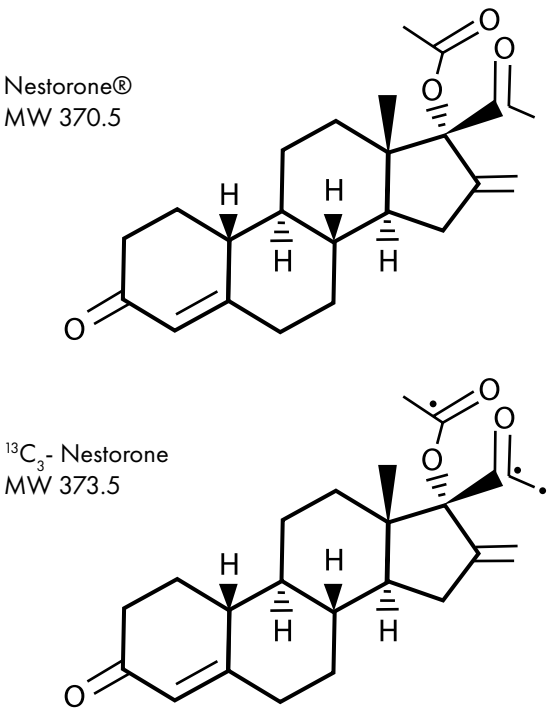


Figure 1. Calibrator/Internal Standard

MATERIALS AND METHODS

Mass spectrometric conditions on API 5000:
Optimized mass parameters used positive MRM mode.
The selected parent/product ions m/z are 371.4/253.1 for NES and 374.4/253.1 for internal standard (IS) $^{13}C_3$ Nestorone, respectively.

Liquid chromatographic conditions:
Kinetex C18 column (1.7µm,100 mm × 3 mm) with a gradient mobile phase from 45%B to 100%B for 5 minutes (B / A = 100%MeOH / H 2 O with 0.1% formic acid) delivered at 0.6 mL/minute, total run time: 7 minutes

Table 1. Sample Preparation

Protein Precipitation	• Add 25 µL internal standard (IS) into 200 µL of serum samples, calibrators and QC; vortex well • Add 750 µL acetonitrile with 1% formic acid and mix well • Centrifuge at 14,000 rpm for 3 minutes • Transfer supernatant
Condition	• Aspirate and Dispense HybridSPE- XTR tips with 1000 µL acetonitrile
Extraction	• Aspirate/dispense the supernatant 2x with HybridSPE -XTR tips binding interferents to sorbent
Prep for injection	• Dry down the extracted samples • Reconstitute with 40% MeOH in 0.1% formic acid H_2O for the analysis

The range of calibration curve: 10 ~ 30,000 pg/mL for Nestorone in human serum samples.

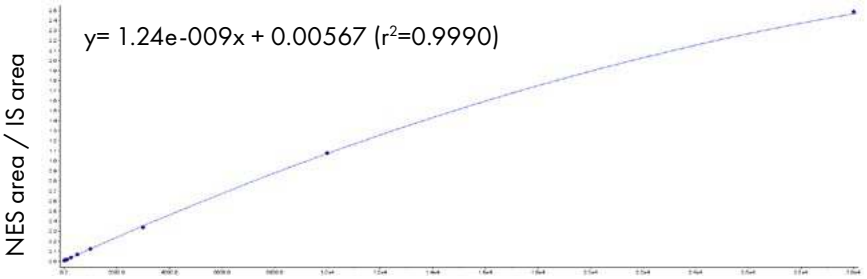
RESULTS

Nestorone (pg/mL)	Intra-assay precision (n=10)		Inter-assay precision (n=9 days)	
	Mean ± SD	%CV	Mean ± SD	%CV
QC1	27.1 ± 1.1353	4.2	28.3 ± 2.734	9.6
QC2	141.2 ± 12.505	8.9	158.4 ± 3.909	2.5
QC3	434.7 ± 12.774	3.2	457.0 ± 21.96	4.8
QC4	1509.0 ± 65.44	4.3	1581.6 ± 40.35	2.3
QC5	7546 ± 304.43	4.0	7405 ± 230.39	3.1
QC6	24090 ± 371.20	1.8	24611 ± 1393	5.7

Nestorone (pg/mL)	%Accuracy (n=9 days)	%CV
QC1	99.7	9.6
QC2	104.7	2.5
QC3	102.4	4.8
QC4	98.0	2.3
QC5	93.6	3.1
QC6	100.8	5.7

Analyte (100 pg/mL)	% Matrix Effect						
	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Average
Nestorone	66.1	85.2	72.9	78.9	82.9	69.9	76.0
$^{13}C_3$-NES	51.3	68.9	67.2	80.4	78.4	58.8	64.4

Nestorone	LLOQ (n=6) Signal/Noise									
Serum	1	2	3	4	5	6	X	SD	%CV	% Accuracy
10 pg/mL	10.2	10.9	13.1	13.7	10.0	13.2	11.85	1.6646	14.0	-
Measured	8.72	9.05	9.93	9.88	9.74	10.1	9.57	0.5529	5.8	95.7



CONCLUSIONS

A sensitive LC MS/MS assay for human serum Nestorone® was developed an validated which can be applied to clinical and research studies.

ACKNOWLEDGEMENT

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