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DEVELOPMENT AND VALIDATION OF NORETHINDRONE IN HUMAN PLASMA BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY/ MASS SPECTROMETRY

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ABSTRACT

Biological fluids like blood, serum, urine, tissue extracts or plasma may contain compounds like drugs and their metabolite, and Bio analysis is the term which is commonly used for the purpose of quantitative determination of this compound. An accurate Liquid chromatography with tandem mass spectrometry technique to quantify Norethindrone in dipotassium ethylene diamine tetra acetic acid human plasma over the concentration range 0.1660 ng/mL to 34.594 ng/mL as developed and validated. Norethindrone becomes extracted from plasma by means of the usage of solid Phase extraction method turned into accomplished on Acquity Ultra performance liquid chromatography ethylene bridged hybrid C18 (2.1 mm x 100 mm, 1.7 µm) column. This method is commonly used for sample analysis to support bio-equivalence/ bioavailability and/or pharmacokinetic studies involving formulations of Norethindrone.

Keywords: Bio-analytical method, Norethindrone, Ultra performance liquid chromatography-tandem mass spectrometry, Human plasma, Standard deviation, Lower and upper limit of Quantification

INTRODUCTION:

Norethindrone is in progesterone type of female chemical. It is utilized for substitution treatment. Norethindrone tablet is additionally utilized as an oral preventative item. Everyday portion of 0.35mg Norethindrone gives a consistent oral preventative routine **[1]**. Various techniques for the assurance of norethindrone without anyone else , as well as different pills/metabolites in assorted dose structures have been accounted for. These strategies couldn't be executed as such for the assurance of norethindrone in plasma. Norethindrone ought to be isolated from plasma proteins. Likewise, a few methodologies for the devotion of norethindrone in plasma have been progressed **[2]**. Norethindrone acetic acid derivation (NETA) has been utilized generally in women with rectovaginal endometriosis as long as a year follow-up, demonstrating a decent aggravation control, with average clinical (weight gain, decrease charisma) and hematological (lipid profile change) aftereffects and it seems a decent contender for long haul single medication the executives here **[3]**. The physicochemical properties of NET show its capability to parcel into natural rich parts. Past examinations have detailed amassing of NET in waterway residue **[4]**. Norethindrone acetic acid derivation has been accounted for to bring down plasma in hyper TGFA triglyceridemic people in any case, the bringing down of plasma TGFA levels during the post pregnancy time frame in sound ladies getting this progestin might address just an unconstrained re-visitation of basal level **[5]**.

INTRODUCTION TO LC-MS:

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS/MS):

LC-MS as the name recommends is a mix of two strategies; liquid chromatography and mass spectrometry. Liquid chromatography capacities to isolate out analytes in complex blends, fundamentally disentangling a blend. In liquid chromatography, the example is applied to a section (or fixed stage) by the versatile stage. The analytes in the example will have various affinities for the fixed stage and will tie feebly or unequivocally or on the other hand not in any manner relying upon that liking. Changing the mobile stage after some time, by for instance expanding the relative extent of one of the versatile stage parts, to lessen affinity to the stationary phase then causes the analytes to elute with the most fragile bound eluting first and the

most grounded last to give partition of the analytes **[6]**.

APPLICATION [7-11]:

1. Molecular Pharmacognosy.
2. Characterization and Identification of Compounds.
3. Quantitative Bioanalysis of various Biological Samples.
4. Qualitative and Quantitative Analysis of Complex Lipid Mixtures.
5. Phytoconstituents / Plant Metabolomics.
6. Automated Immunoassay in Therapeutic Drug Monitoring.
7. Two Dimensional (2-D) Hyphenated Technology.
8. Clinical chemistry and toxicology.
9. Proteomics.
10. Pharmacovigilance.

11. Organic/Inorganic Hybrid Nanoflowers

MATERIALS AND METHOD:

The Norethindrone (Working standard), Norethindrone D6 (Internal standard) are collected from MTR Lab, Chennai. The authentication of samples is done by melting and solubility studies. The UPLC-MS/MS containing liquid chromatography with Analyst software version 1.6.2 supplied by Applied Biosystems MDS as the software. The following solvents are used in the process. The Acetone-M, Water, Acetonitrile, TBME and Ethyl acetate are used in HPLC grade. The ammonium Formate and Ammonium Acetate are used in Analytical research grade.

RESULT AND DISCUSSION:

Table 1: Specificity and selectivity for norethindrone and internal standard

Plasma lot ID	Specificity (Blank)		Selectivity (Spiked LLOQ)		% Interference in Blank		Area Ratio	S/N Ratio (≥5)
	Analyte	IS peak	Analyte	IS peak	Analyte (<20%)	IS (<5%)	Analyte/IS	Analyte
MAT-19-0625-I	21	8	225	9194	9.3333	0.0870	0.0245	58.500
MAT-19-0626-I	12	6	237	9314	5.0633	0.0644	0.0255	72.941
MAT-19-0627-I	5	6	213	9133	2.3474	0.0657	0.0234	66.439
MAT-19-0628-I	5	5	231	9482	2.1645	0.0527	0.0243	35.705
MAT-19-0629-I	2	12	170	8889	1.1765	0.1350	0.0192	42.250
MAT-19-0630-I	13	8	166	8770	7.8313	0.0912	0.0190	47.708
MAT-18-0469(H)-IX	3	6	206	9098	1.4563	0.0659	0.0266	11.677
MAT-18-0470(L)-IV	17	1	176	8945	9.6591	0.0112	0.0197	64.902
MAT-19-0604(HEP)-II	14	8	234	8618	5.9829	0.0928	0.0271	135.429
			Mean	9049.22222	5.00163	0.07400	0.02281	
						SD	0.002923	
						%CV	12.81	

Acceptable Range/Criteria (%CV of Area Ratio) should be ≤20

Table 2: IS normalized matrix factor for norethindrone at LQC level

Plasma Lot ID	Aqueous sample		Spiked sample		Matrix Factor			Area Ratio	
	Analyte Area	IS Area	Analyte Area	IS Area	Analyte	IS	IS Normalized	Aqueous Sample	Spiked Sample
MAT-19-0625-I	312	7249	436	9368	1.11	1.10	1.01	0.0431	0.0465
MAT-19-0626-I	263	7137	444	9435	1.13	1.10	1.03	0.0368	0.0470
MAT-19-0627-I	338	7793	437	9288	1.11	1.09	1.02	0.0433	0.0470
MAT-19-0628-I	468	9885	452	9142	1.15	1.07	1.07	0.0474	0.0495
MAT-19-0629-I	476	9953	522	9973	1.33	1.17	1.14	0.0478	0.0524
MAT-19-0630-I	500	9295	435	9316	1.11	1.09	1.02	0.0538	0.0467
MAT-18-049(H)-IX			390	9291	0.99	1.09	0.91		0.0420
MAT-18-0470(L)-IV			399	8904	1.02	1.04	0.98		0.0448
MAT-19-0604(HEP)-II			422	8819	1.07	1.03	1.04		0.0478
Mean	392.8333	8552.00000				Mean	1.02444	0.04537	0.04708
						SD	0.062272		
						%CV	6.08		

Acceptable Range/Criteria (%CV of Area Ratio) should be ≤15.

Table 3: IS normalized matrix factor for norethindrone at HQC level

Plasma lot ID	Aqueous sample		Spiked Sample		Matrix factor			Area Ratio	
	Analyte Area	IS Area	Analyte Area	IS Area	Analyte	IS	IS Normalized	Aqueous Sample	Spiked Sample
MAT-19-0625-I	312	7249	436	9368	1.11	1.10	1.01	0.0431	0.0465
MAT-19-0626-I	263	7137	444	9435	1.13	1.10	1.03	0.0368	0.0470
MAT-19-0627-I	338	7793	437	9288	1.11	1.09	1.02	0.0433	0.0470
MAT-19-0628-I	468	9885	452	9142	1.15	1.07	1.07	0.0474	0.0524
MAT-19-0629-I	476	9953	522	9973	1.33	1.17	1.14	0.0478	0.0524
MAT-19-0630-I	500	9295	435	9316	1.11	1.09	1.02	0.0538	0.0467
MAT-18-0469(H)-IX			390	9291	0.99	1.09	0.91		0.0420
MAT-18-0470(L)IV			399	8904	1.02	1.04	0.98		0.0420
MAT-18-060(HEP)II			422	8819	1.07	1.03	1.04		0.0478
Mean	392.83333	8552.00 000				Mean	1.02444	0.04537	0.04708
						SD	0.062272		
						%CV	6.08		

Acceptable Range/Criteria (%CV of Area Ratio) should be ≤15

Table 4: Carry over test for norethindrone and norethindrone d6

Sample ID	Analyte Peak Area	IS Peak Area
Extracted blank	0	20
Extracted LLOQ+IS	203	19836
Extracted ULOQ+IS	45160	20578
Extracted Blank I	1	1
Extracted Blank II	6	0
% Carry Over in Blank I	0.49	-0.09
% Carry Over in Blank II	2.96	-0.10

Acceptable Range/Criteria (%CV of Area Ratio) should be CC≤20 & IS < 5

Table 5: Intra batch precision and accuracy of norethindrone

QC ID	LOQQC	LQC	INTQC	MQC	HQC
Actual Concentration (ng/mL)	0.1670	0.4680	2.9250	12.9980	25.9960
Calculated concentration (ng/mL)	0.1849	0.5228	2.7225	12.6899	26.2374
	0.1282	0.4706	2.8478	13.2840	26.6321
	0.1673	0.4488	3.1696	13.2302	22.7187
	0.1849	0.4539	3.0705	13.1474	25.4908
	0.1409	0.5647	3.1557	12.2821	25.6536
	0.1320	0.4717	2.7367	12.3606	22.7120
Mean	0.15637	0.48875	2.95047	12.83237	24.90743
SD	0.025978	0.045516	0.206278	0.448808	1.746474
%CV	16.61	9.31	6.99	3.50	7.01
%Nominal	93.63	104.43	100.87	98.73	95.81

Acceptable Range/Criteria (%CV of Area Ratio) should be LOQQC ≤20 & LQC, INTQC, MQC&HQC ≤ 15.

Table 6: Inter batch or total precision and accuracy and norethindrone

QC ID	LOQQC	LQC	MQC	INTQC	HQC
Actual concentration (ng/mL)	0.1670	0.4680	2.9250	12.9980	25.9960
Calculated concentration (ng/mL)	0.1760	0.5696	2.8388	12.6475	25.6889
	0.2382*	0.5218	2.8029	12.5710	25.1125
	0.2279	0.4058	2.7216	12.6610	25.7036
	0.1899	0.4378	2.8412	13.0158	25.1658
	0.1636	0.4279	2.8080	12.6438	23.9767
	0.1507	0.4397	2.9155	13.0013	24.7626
Mean	0.15918	0.47699	2.93771	12.91758	25.48752
SD	0.029215	0.048720	0.166643	0.348950	1.267134
%CV	18.35	10.21	5.67	2.70	4.97
%Nominal	95.32	101.92	100.43	99.38	98.04

Acceptable Range/Criteria (%CV of Area Ratio) should be LOQQC ≤20 & LQC, INTQC, MQC&HQC ≤ 15

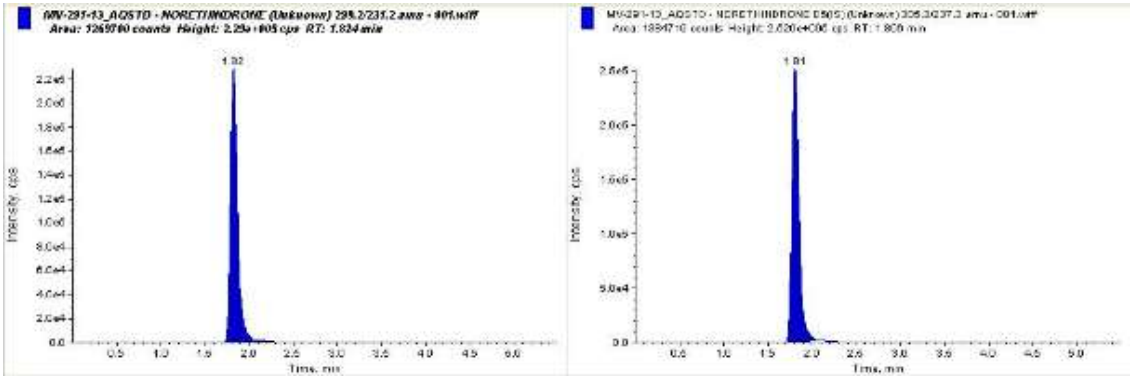

Figure 1: Chromatogram of an Aqueous Standard Solution for Norethindrone

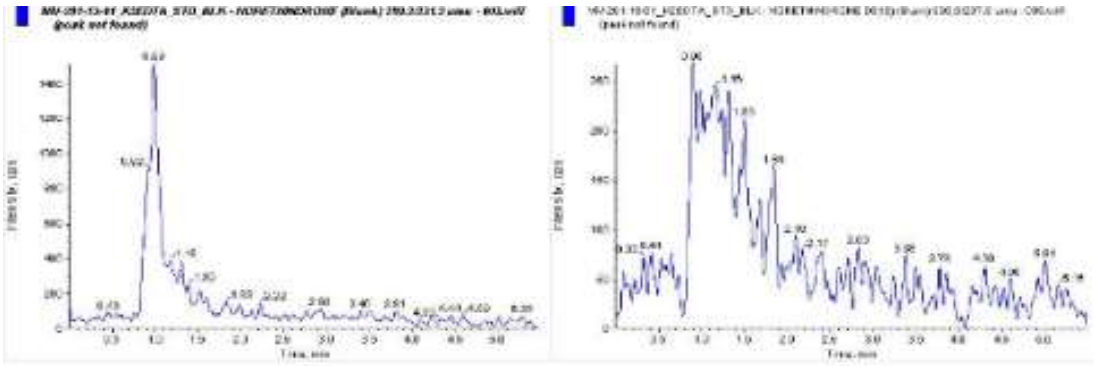

Figure 2: Chromatogram of a Standard Blank for Norethindrone

CONCLUSION:

Method Validation experiments (Specificity & Selectivity, IS Normalized Matrix Factor, Precision & Accuracy batches and Carry over Test) met the acceptance criteria. Hence the method is suitable for Norethindrone sample analysis on different instrument (Acquity UPLC with TQMS from Waters) to support bioequivalence/bioavailability for Pharmacokinetic studies.

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