Development of a UPLC-MS/MS Method for the Determination of Norethindrone from Human Plasma Lan Li, Jinlan Dong, Tracey Paxon, Lin Zhang, Yuan-Shek Chen, Ben Hsu QPS, LLC, Newark, Delaware

Norethindrone is a form of progesterone, a female hormone. It binds to the progesterone intracellular receptors in the reproductive system to inhibit ovulation and change the normal cycle of cervical mucus and endometrium. For these reasons, norethindrone is used to prevent pregnancy, to treat menstrual disorders, endometriosis, and other diseases caused by a hormone imbalance. Due to a lack of easily ionized structure moiety for mass spectrometry, quantification of norethindrone at midpicogram level is challenging, and analytical methods often require large sample volume and complicated sample processing procedures. In our approach, the MS sensitivity of norethindrone was enhanced by utilizing a low cost derivatization agent. With a one-step liquid-liquid extraction on Tomtec, we achieved a lower limit of quantification (LLOQ) of 50 pg/mL, with 50 μ L of sample consumption.





range of 50 to 10,000 pg/mL.



Results



BIGANALYSIS

Assay Range: 0.05-10 ng/mL Linearity: 0.0918119754 Intercept: -0.000660934

 \mathbf{R}^2 : 0.9968

LLOQ QC		L, M and HQC			
Intra-run	Inter-Run	Intra-run	Inter-Run		
2.9 to 5.2	4.7	0.7 to 5.3	2.0 to 3.8		
3.2 to -2.6	-4.6	-9.6 to -3.5	-8.4 to -6.0		

Possessed Sample Stability at 4°C

%RE		Time (hours)	Concentrations (ng/mL)		
			0.150 (LQC)	8.00 (HQC)	
-10.0			0.171	8.54	
-4.4			0.171	8.49	
-7.0		123	0.176	8.52	
-0.2			0.163	8.41	
-1.8			0.159	8.59	
-13.0			0.157	8.46	
		n	6	6	
		Mean	0.166	8.50	
		S.D.	0.00760	0.0631	
		%CV	4.6	0.7	
		%RE	10.7	6.3	

ng/mL	1.00/2.50 ng/mL	8.00/2.50 ng/mL
3	86.6	87.5
	84.8	
6	NA	0.978

Summary

1. We successfully developed a low-cost derivatization method for Norethindrone utilizing Hydroxylamine to increase the MS sensitivity to achieve an LLOQ at 50 pg/mL.

2. With the one-step Liquid-Liquid extraction on Tomtec and derivatization, a LC-MS/MS method was successfully validated for Norethindrone in human plasma at an assay