Poster Number 04W1000

Simultaneous Measurement of Dehydroepiandrosterone (DHEA) and 17 Hydroxyprogesterone (17-OHP) as Biomarkers in Human Plasma using UPLC-MS/MS

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2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13–17, 2016
COLORADO CONVENTION CENTER, DENVER
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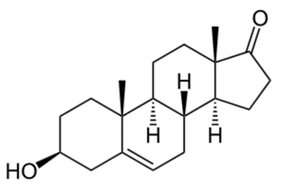
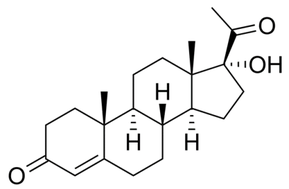
PURPOSE

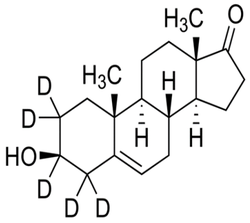
Dehydroepiandrosterone (DHEA) is a hormone produced by the body's adrenal glands. The body uses DHEA to make androgens and estrogens, the male and female sex hormones. 17-Hydroxyprogesterone (17-OHP) is an endogenous progestogen in the biosynthesis of other steroid hormones, including corticosteroids, androgens, and estrogens. In recent years, LC-MS/MS methods have been developed for determination of steroid hormones in human plasma. Many of them suffered from bioanalytical issues such as peak trailing and limited sensitivity. In this study, we have developed and validated a rugged method for simultaneous determination of DHEA and 17-OHP as biomarkers in human plasma in support of clinical studies.



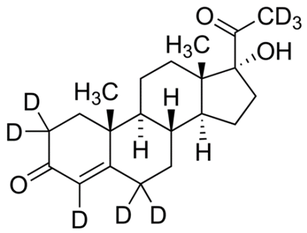
Dehydroepiandrosterone (DHEA)



17-Hydroxyprogesterone (17-OHP)



DHEA-d5 (IS1)



17-OHP-d8 (IS2)

METHOD

Sample Preparation:

Due to presence of endogenous DHEA and 17-OHP in human plasma, surrogate matrix of BSA in PBS solution was used for the preparation of calibration standards. Multiple lots of human plasma were screened and those lots with lower levels of DHEA and 17-OHP were pooled for the preparation of QC samples for the validation. The method utilized a liquid-liquid extraction and then derivatization procedure prior to LC-MS/MS analysis. Briefly, DHEA, 17-OHP and the added internal standards were extracted from 50 uL of human plasma using MTBE (methy-terbutyl ether). The organic layer was transferred and dried under nitrogen gas. The residue was reconstituted and derivatized with hydroxylamine. The resultant products were submitted for LC-MS/MS analysis.

Liquid Chromatography:

Pump:	Shimadzu LC-20AD
Autosampler:	Shimadzu SIL-20AC
System Controller:	Shimadzu CBM-20A
Analytical Column:	Phenomenex Hyperclone BDS C18, 5µ, 130A, 50x 2.0 mm
Gradient:	The analytes were eluted using a gradient of mobile phase A (0.1% formic acid in Water) and mobile phase B (0.1% formic acid in Acetonitrile) from 25% to 70%mobile phase B in 2.0 min.
Injection Volume:	5 µL

Mass Spectrometry:

MS System:	AB Sciex Triple Quard 5500	
Condition:	LC/(+)ESI-MS/MS,	
MRM transition:	DHEA (Derivatized):	304.1→253.1
	17-OHP (Derivatized):	361.1→112.1
	DHEA-d5 (Derivatized):	309.1→258.1
	17-OHP-d8 (Derivatized):	369.1→112.1

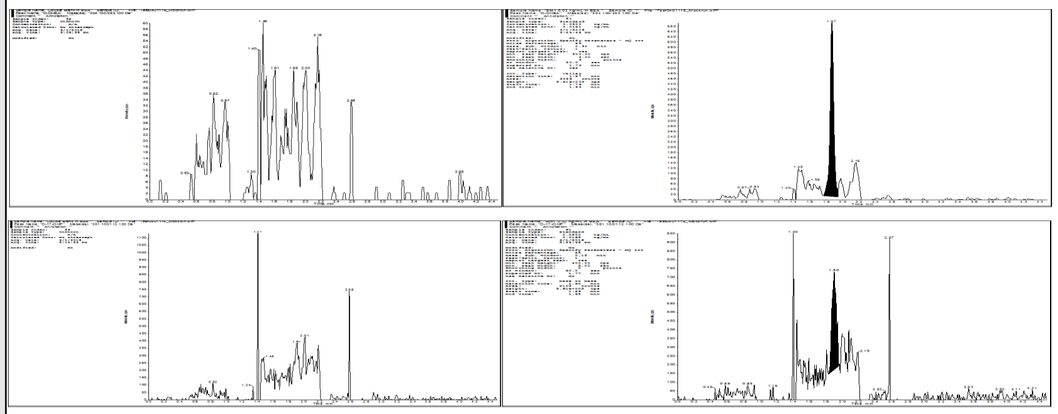


Fig 1. Ion Chromatograms of Blank BSA-PBS (upper and lower left), 0.02 ng/mL of DHEA (upper right) and 0.02 ng/mL of 17-OHP (lower right) extracted from BSA-PBS

RESULTS

The chromatograms of LLOQ samples of DHEA and 17-OHP are shown in Fig 1.

Using derivatization, the selectivity and sensitivity for both DHEA and 17-OHP are greatly improved.

Excellent linearity was obtained with a correlation coefficient ≥ 0.9958 for DHEA and ≥ 0.9981 for 17-OHP. A typical calibration curve for DHEA and 17-OHP are presented in Fig. 2.

The accuracy and precision of 5 levels of QC samples are presented in tables below. The data indicated using derivatization for simultaneous quantitation of DHEA and 17-OHP provides reliable results.

	DHEA Nominal Concentrations* (ng/mL)				
	LLOQ in BSA	LLOQ in Plasma	LQC in Plasma	MQC in Plasma	HQC in Plasma
	0.0200	0.0700	0.1100	0.5500	8.0500
	DHEA Assayed Concentrations (ng/mL)				
	0.0184	0.0740	0.1090	0.5610	8.5100
	0.0178	0.0623	0.1040	0.4980	8.4600
	0.0175	0.0710	0.1050	0.5110	8.5500
	0.0205	0.0700	0.0981	0.5020	8.8500
	0.0207	0.0772	0.1020	0.5310	8.6100
	0.0227	0.0625	0.1080	0.4850	8.6500
Mean	0.0196	0.070	0.104	0.515	8.61
SD	0.0020	0.006	0.004	0.027	0.14
CV	10.4%	8.7%	3.8%	5.3%	1.6%
RE	-2.0%	-0.7%	-5.1%	-6.4%	6.9%

* DHEA endogenous concentration in pooled human plasma is 0.0500 ng/mL.

	17-OHP Nominal Concentrations* (ng/mL)				
	LLOQ in BSA	LLOQ in Plasma	LQC in Plasma	MQC in Plasma	HQC in Plasma
	0.0200	0.150	0.190	0.630	8.13
	17-OHP Assayed Concentrations (ng/mL)				
	0.0172	0.156	0.192	0.613	8.64
	0.0177	0.155	0.189	0.608	8.56
	0.0178	0.149	0.159	0.606	8.49
	0.0200	0.147	0.156	0.615	8.43
	0.0212	0.143	0.198	0.603	8.46
	0.0174	0.157	0.183	0.608	8.72
Mean	0.0186	0.151	0.180	0.609	8.55
SD	0.0016	0.006	0.018	0.004	0.11
CV	8.9%	3.8%	9.9%	0.7%	1.3%
RE	-7.3%	0.8%	-5.5%	-3.4%	5.2%

* 17-OHP endogenous concentration in pooled human plasma is 0.130 ng/mL.

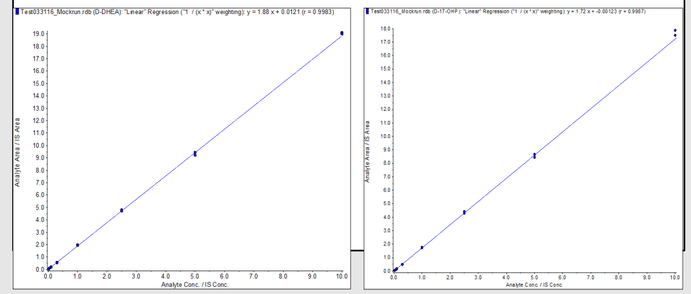


Fig 2. Calibration Curves of DHEA (left) and 17-OHP (right)

CONCLUSION

A sensitive and reproducible UPLC-MS/MS method has been validated for the simultaneous quantitation of steroid hormone DHEA and 17-OHP in human plasma with a LLOQ of 0.02 ng/mL by using a 0.05 mL plasma. The validated method has been successfully used for the analysis of clinical study samples.