Bioanalytical LC-MS/MS Method for the Determination of a Vitamin E Analog in Human Plasma

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OVERVIEW

- An LC–MS/MS method was developed and validated for the quantification of a Vitamin E Analog with analog ISTD.
- Analog ISTD was fragmented in source then MS/MS performed.
- Stable method on reverse phase column.
- Carryover and solubility controlled with proper recon and mobile phase methanol concentrations.

INTRODUCTION

The goal was to produce a rugged bioanalytical method for determination of a Vitamin E Analog with unlabeled internal standard. Concerns included lipid build-up on the HPLC column along with internal standard stability in source and Q1 of the mass spectrometer. The R-group on the analog internal standard required fragmentation in source followed by MS/MS to gain proper stability and compound tracking.

METHODS

An aliquot of human plasma (EDTA) containing the analyte and internal standard was extracted using a solid phase extraction procedure. The extracted samples were analyzed using an HPLC equipped with an AB SCIEX API 4000 triple quadrupole mass spectrometer using an ESI source.

Negative ions were monitored in the multiple reaction monitoring (MRM).

System setup:

Mobile Phase: 90:10 Methanol: (0.1 Triethylamine pH 4.7)
Column: phenyl column

90:10 Methanol: Water as recon provided the best solubility and column performance.
C18 columns with 100% organic mobile phase produced suppression after limited number of injections.

Table 1. Solubility of Compound and Internal Standard in Lower and Higher Methanol.

	50:50 MeOH:H2O		Added additional 50 uL MeOH	
	Compound	ISTD	Compound	ISTD
STD A 1 1	722.344	2027.623	607.529	60870.6
STD A 2 1	450.192	1992.267	755.396	65982.93
STD B 1 1	589.985	1189.676	2469.642	99130.25
STD C 1 1	690.754	3043.298	4612.777	96690.56
STD D 1 1	554.285	1928.79	4262.813	40506.76
STD E 1 1	1022.588	1277.65	33650.21	69690.13
STD F 1 1	2961.733	2607.429	78411.09	51463.04
STD G 1 1	5020.644	1681.133	172798.4	54133.16
STD H 1 1	8826.535	1641.984	150842.2	25408.52
STD I 1 1	17735.37	2089.573	779231.7	71749.9
STD J 1 1	15814.56	1393.735	1003417	71469.95

RESULTS

Table 2. Calibration Curve Standard Concentrations.

Batch	STD B 5.00 ng/mL	STD C 10.0 ng/mL	STD D 25.0 ng/mL	STD E 50.0 ng/mL	STD F 100 ng/mL	STD G 200 ng/mL	STD H 500 ng/mL	STD I 1000 ng/mL	STD J 1250 ng/mL
41	4.99	9.93	24.9	51.6	106	204	482	964	1220
42	4.96	10.0	26.1	49.4	97.0	196	492	1000	1290
45	4.95	10.1	25.1	51.1	103	197	510	981	1190
51	5.11	9.72	23.8	*53.0	105	188	489	978	1380
Mean	5.00	9.94	25.0	50.7	103	196	493	981	1270
SD	0.0737	0.161	0.943	1.15	4.03	6.55	11.9	14.8	84.5
% CV	1.5	1.6	3.8	2.3	3.9	3.3	2.4	1.5	6.7
% Bias	0.0	-0.6	0.0	1.4	3.0	-2.0	-1.4	-1.9	1.6
n	4	4	4	3	4	4	4	4	4

Table 3. Matrix Effect.

No significant matrix effect was observed in any of the 10 human plasma (EDTA) lots that were fortified with Vitamin E Analog at the concentration of the LLOQ (5.00 ng/mL) or high QC (900 ng/mL) samples.

		LLOQ		High	
Batch	Lot#	5.00 ng/mL	% Dev.	900 ng/mL	% Dev.
42	1	5.23	+4.6	921	+2.3
	2	5.23	+4.6	902	+0.2
	3	5.51	+10.2	920	+2.2
	4	5.32	+6.4	935	+3.9
	5	5.66	+13.2	915	+1.7
	6	5.21	+4.2	894	-0.7
	7	4.67	-6.6	908	+0.9
	8	5.81	+16.2	929	+3.2
	9	5.93	+18.6	1010	+12.2
	10	5.08	+1.6	942	+4.7
Mean		5.37		928	
% CV		6.9		3.5	
% Theoretical		107.4		103.1	
n		10		10	

Table 4. Hemolyzed Sample Integrity.

No significant interference for compound was observed in any of the 3 hemolyzed human plasma (EDTA) lots (fortified with 2% whole blood) that were fortified at the concentration of the LLOQ (5.00 ng/mL) and high QC (900 ng/ml) levels.

		LLOQ		High	
Batch	Lot#	5.00 ng/mL	% Dev.	900 ng/mL	% Dev.
42	1	4.80	-4.0	854	+5.1
	2	4.96	-0.8	865	+3.9
	3	5.67	+13.4	975	+8.3
Mean		5.14		898	
% CV		9.0		7.5	
% Theoretical		102.8		99.8	
n		3		3	

Table 5. Lipemic Sample Evaluation.

No significant interference for the compound was observed in any of the 3 lipemic human plasma (EDTA) lots at the LLOQ (5.00 ng/ml) and high QC (900ng/ml) levels.

		LLOQ		High	
Batch	Lot#	5.00 ng/mL	% Dev.	900 ng/mL	% Dev.
47.48*	1	4.34	-13.2	1020	+13.3
	2	5.42	+8.4	908	+0.9
	3	4.90	-2.0	973	+8.1
Mean		4.89		967	
% CV		11.0		5.8	
% Theoretical		97.8		107.4	
n		3		3	

Table 6. Recovery Data of Vitamin E Analog.

Theoretical Concentration: Batch	15.0 ng/mL Concentration Extracted	Unextracted	80.0 ng/mL Concentration Extracted	Unextracted	900 ng/mL Concentration Extracted	Unextracted
43	14.1	14.0	78.0	78.0	881	852
	14.5	13.5	74.0	80.2	856	846
	13.0	13.9	73.2	79.0	906	815
	14.5	14.0	75.7	77.6	837	819
	13.5	14.5	77.1	79.7	838	824
	12.5	13.8	72.2	77.4	854	812
Mean	13.7	14.0	75.0	78.7	862	828
% CV	6.0	2.3	3.0	1.5	3.1	2.0
% Recovery	98		95		104	
n	6	6	6	6	6	6

Table 7. Carryover Evaluation.

Batch	% Detector Response Compound Beginning of Batch	End of Batch	% Detector Response ISTD Beginning of Batch	End of Batch
41	0.0346	0.0241	0	0
42	0	0	0	0
45	0	0.0160	0	0
51	0.0250	0.0220	0	0

Figure 1. Representative Chromatograms of Vitamin E Analog Compound ULOQ (Retention time 1.5 minutes).

Figure 2. Representative Chromatograms of Vitamin E Analog ISTD (Retention time 2.14 minutes) separated from naturally occurring Vitamin E (Retention time 2.47 minutes).

Figure 3. Representative Chromatograms of Blank Sample with endogenous Vitamin E (Retention Time 2.49 minutes)

Figure 4. Representative Chromatograms of Vitamin E Analog Compound LLOQ (Retention Time 1.5 minutes).

CONCLUSIONS

A rugged bioanalytical method for the determination of a Vitamin E Analog with unlabeled internal standard was produced. With the use of 90% methanol and triethylamine, we were able to separate the ISTD from naturally occurring Vitamin E in the ISTD channel. Solubility and suppression were controlled by proper recon and mobile phase methanol concentrations. A stable method with a limit of detection of 5 ng/ml passed all validation requirements and was used in preliminary production testing.

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