Investigation of Vitamin E Ion Suppression in Certain Lipemic Serum Specimens

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Problem

- A Vitamins A&E assay was validated showing no interference by lipemia when diluting specimens with a lipemic pool or evidence of ion suppression in correlation sets (n=364 patient samples).
- Upon go-live, certain lipemic patient samples demonstrated ion suppression for Vitamin E and its internal standard, Vitamin E (phenyl-5,7-dimethyl-d6).

Std. Co...	mg/L	%Dev	Primary Area	Second. Area	IS Area
6.30	6.12	-2.8	121739	119990	183317
7.50	7.44	-0.8	131478	129039	162827
8.30	8.25	-0.6	133986	131787	149691
7.00	6.95	-0.7	105793	104246	140320
12.30	12.24	-0.4	177495	173607	133621
7.70	10.23	32.8	3090	2837	2785
1.60	2.00	25.2	768	742	3534
11.10	11.00	-7.0	115913	115754	89929
16.00	16.03	0.2	209724	208340	120565

Table 1. Discrepant results identified by low IS area relative to the rest of the set.

Method Information

- 150 µL serum combined with 20 µL IS in isopropanol and extracted by SLE with hexane, reconstituted in 150 µL methanol.
- 1.0 µL injection
- Acquity I-Class UPLC, column manager at 45°C
- Acquity BEH C18 UPLC column, 2.1x50 mm, 1.7 µm
- Waters TQ-XS and TQ-S micro MS
- Mobile Phase A: 0.1% formic acid, 2 mM ammonium acetate in water
- Mobile Phase B: 0.1% formic acid, 2 mM ammonium acetate in methanol
- 3.4 min method, linear gradient 90-100% mobile phase B at 0.5 mL/min
- Quantitative MRM acquisition

Troubleshooting Steps

Which samples are affected?

- Samples with ion suppression were all from patients receiving Total Parenteral Nutrition (TPN), which contains lipid emulsions in its mix of nutrients.

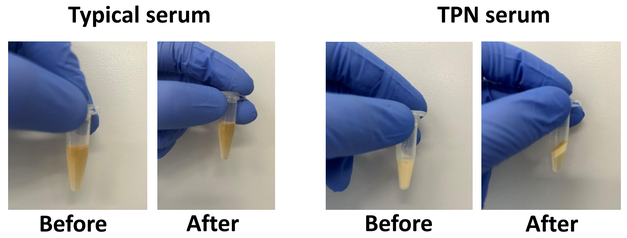

Fig. 1. Specimens before and after lipid separation by centrifugation at 13,000 rpm.

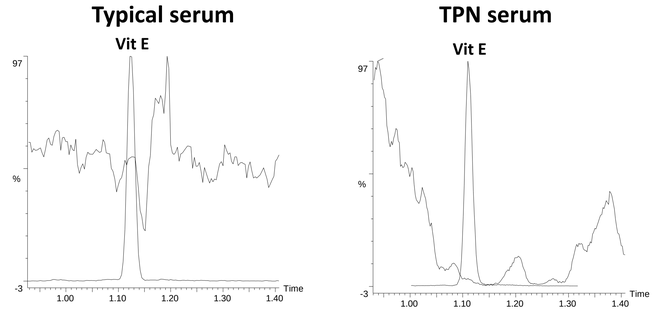

Fig. 2. Comparison of ion suppression profiles.

Typical serum

Hemolysis	Icterus	Turbidity
<15	<2	<20

TPN serum

Hemolysis	Icterus	Turbidity
<15	2	182

Table 2. Serum indices as measured by Vitros 4600 Chemistry Analyzer.

How does this affect results? Can we correct for this problem?

- Samples were diluted with standard (30.2 mg/L Vitamin E).
- An aliquot was centrifuged at 13,000 rpm and the lipid-free serum was extracted for comparison.
- Four specimens were used in the investigation, one of which is tracked here.

Sample Injected at 1.0 µL	Expected (mg/L)	Measured (mg/L)	% Deviation	IS Area
Neat patient specimen	--	3.5	--	77,857
1:5 Patient : Standard	24.9	24.0	-3.82	540,460
1:3 Patient : Standard	21.3	20.4	-4.46	631,303
1:1 Patient: Standard	16.8	16.0	-4.76	544,491
3:1 Patient : Standard	12.4	10.2	-17.42	304,231
5:1 Patient : Standard	8.8	7.5	-14.32	147,298
Neat patient specimen, lipid removed	--	3.0	-12.93	562,994

Table 3. Dilution series results showing decreasing intensity and higher negative bias as proportion of TPN serum increases.

Sample Injected at 0.2 µL	Expected (mg/L)	Measured (mg/L)	% Deviation	IS Area
Neat patient specimen	--	4.0	--	55,539
1:5 Patient : Standard	24.9	25.9	3.98	70,141
1:3 Patient : Standard	21.3	22.2	4.27	84,459
1:1 Patient: Standard	16.8	17.6	4.88	107,838
3:1 Patient : Standard	12.4	12.2	-1.53	87,632
5:1 Patient : Standard	8.8	8.6	-2.05	59,828
Neat patient specimen, lipid removed	--	3.2	-8.05	98,584

Table 4. Samples reinjected at 0.2 µL rather than the standard 1.0 µL.

- Ion suppression due to TPN led to a negative bias in results.
- Dilution and low volume injection both bring measured results closer to expected values.
- Lipid removal reduces the suppression as observed in recovered Internal Standard intensity, but also negatively affects analyte recovery.

Outcome

- If specimens in a clinical set show reduced IS area for Vitamin E, we reinject at the lower validated volume and report from this result.