Sciex

Clinical

Application Note : 2106

Analysis of Total Vitamin C in Plasma:

Vitamin C Quantification in Plasma at 9 Seconds per Sample Using Luxon Ion Source®

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Introduction

Vitamin C (Ascorbic acid, AA) is an important compound that engages in many physiological activities in living organisms (synthesis of collagen, acts as an antioxidant, metabolisms and synthesis of adrenaline, prevention, and treatment of scurvy and cold).

In the presence of moisture, air, heat, light and oxygen, ascorbic acid transforms into its oxidized form, dehydroascorbic acid (DHAA). The total Vitamin C concentration in a plasma sample is defined as the sum of AA and DHAA.

Our goal for this application note is to use an automated sample preparation method for the quantification of total vitamin C in plasma using a single operation with the Luxon Ion Source®, based on the LDTD technology.

LDTD-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled quantification method. To develop this application, we focused on performing a quick and simple sample preparation. Vitamin C is analyzed and results are obtained in less than 9 seconds per sample.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing more precision, accuracy, and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic saturation characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Automated Sample Extraction

Due to the instability of ascorbic acid, plasma samples were spiked and stabilized with a sulfuric acid solution (0.2N). A mixture of plasma: H_2SO_4 (0.2N) / 1:1 was used. Stabilized samples were transferred into barcoded tubes, readable by the Azeo extraction system.

Each barcoded vial was scanned by the Azeo Liquid Handler and an automatic batch file was created. The Azeo extraction system (**Figure 3**) is used to extract the samples using the following conditions:

- 40 µL of stabilized plasma sample were transferred from the vials to a deep-well plate.
- 20 µL of Internal standard (Ascorbic acid-13C6, 50 µg/mL in H_2SO_4 (0.2N)) were added to each sample.
  - Mix
- 4.5 µL of plasma/IS, 30 µL of extraction buffer (KH_2PO_4, 1 mM) and 300 µL of acetonitrile were added to a second deep-well plate.
  - Mix
  - Centrifuge 5 minutes/5000 rpm
- Spot 4 µL of the upper layer phase onto a LazWell™ 96 plate
  - Dry 3 minutes at 40°C in the Aura LazWell Dryer

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters

LDTD
Model: Luxon S-960, Phytronix
Carrier gas: 3 L/min (air)
Laser pattern:

- 6-second ramp to 45% power

MS/MS
MS model: QTrap® System 5500, Sciex
Scan Time: 50 msec
Total run time: 9 seconds per sample
Ionization: APCI
Analysis Method: Negative MRM mode

Note: All ascorbic acid is transformed to DHAA in the APCI source. No additional step of reduction or oxidation is needed during the sample preparation.

Table 1 - MRM transitions for Luxon-MS/MS

	Transition	CE
Vitamin C (Qual)	173 → 99	-10
Vitamin C (Quan)	173 → 113	-15
Ascorbic acid-13C_6	179 → 103	-10

Results and Discussion

Validation Test

Calibration curves ranging from 3 to 100 µg/mL and QCs were prepared in an AA-depleted plasma (plasma kept at room temperature/exposed to light for 4 days). Replicate extractions were deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.995

Table 2 shows the inter-day correlation coefficients for Vitamin C. Values greater than 0.999 are obtained. **Figure 4** shows a typical calibration curve result for Vitamin C.

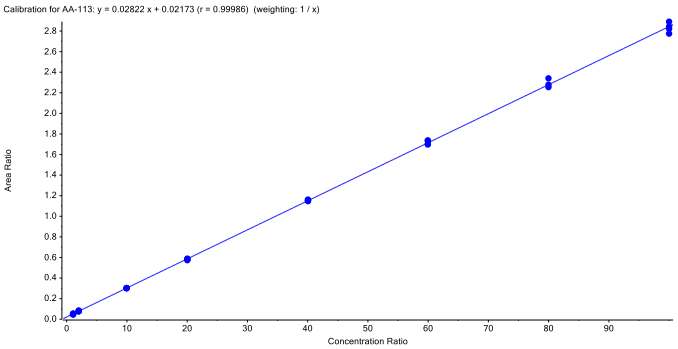

Figure 4 – Vitamin C calibration curve

Table 2 – Inter-day calibration curve correlation coefficients

	Vitamin C
Curve 1	0.99986
Curve 2	0.99981
Curve 3	0.99885
Curve 4	0.99972
Curve 5	0.99785

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria were used:

- Each concentration must not exceed 15% CV
- Each concentration must be within 100 ± 15% of the nominal concentration

DHAA was spiked at 50 µg/mL and analyzed as a QC. **Table 3** shows the intra-run precision and accuracy results for DHAA-QC. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

For the inter-run precision and accuracy experiment, each QC was analyzed in sextuplicate, on five different days. **Table 4** shows the inter-run precision and accuracy results for Vitamin C. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

Table 3 – Intra-Run Precision and Accuracy of Vitamin C

Vitamin C	QC-M (DHAA)
Conc (µg/mL)	50.0
N	6
Mean (µg/mL)	42.8
%CV	2.8
%Nom	85.6

Table 4 - Inter-Run Precision and Accuracy of Vitamin C

Vitamin C	QC-L	QC-M	QC-H
Conc (µg/mL)	3.0	50.0	75.0
N	30	30	30
Mean (µg/mL)	2.9	49.7	76.2
%CV	6.0	3.6	3.4
%Nom	96.2	99.3	101.6

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 1 day, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision and accuracy of QC samples are reported in **Table 5**. All the results are within the acceptable criteria range for 1 day at 4°C.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 1 hour before analysis. The precision and accuracy results of QC samples are reported in **Table 5**. All the results are within the acceptable criteria range for 1 hour at room temperature.

Table 5 - Wet and Dry Stability of Vitamin C

Parameters	Dry stability (1 hour / RT)			Wet stability (1 day / 4°C)		
QC	QC-L	QC-M	QC-H	QC-L	QC-M	QC-H
Conc. (µg/mL)	3.0	50.0	75.0	3.0	50.0	75.0
N	6	6	6	6	6	6
Mean (µg/mL)	3.1	49.4	76.6	3.2	49.8	80.6
%CV	3.4	2.5	1.2	6.3	3.2	2.9
%Nom	101.7	98.7	102.1	105.0	99.6	107.5

Evaluation of Matrix Effect

Ascorbic acids are spiked in six (6) different AA-depleted plasma samples at QC-M level and stabilized. After extraction, concentrations are evaluated against a calibration curve. Replicate extractions are deposited onto a LazWell™ plate and dried before analysis. The precision and accuracy criteria are used.

Results are shown in **Table 6**. Each concentration does not exceed 15% CV and the mean concentration is within ±15% of expected value.

Table 6 - Matrix Effect Evaluation for Vitamin C

Vitamin C	M1	M2	M3	M4	M5	M6
Exp. conc.(µg/mL)	50	50	50	50	50	50
Calc. conc (µg/mL)	50.3	50.7	49.4	50.6	57.1	49.9
N	3	3	3	3	3	3
%CV	1.1	0.7	2.0	1.8	9.1	1.2
%Nom	100.5	100.9	98.7	101.3	114.3	99.8

Conclusion

The Luxon Ion Source® combined with Sciex QTrap® 5500 mass spectrometer system allows the ultra-fast (**9 seconds per sample**) analysis of total Vitamin C in plasma.

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