1.0 Purpose

The purpose of this procedure is to describe a method for the semi-quantitative analysis of nitrate (NO_3^-) dietary supplement raw materials and finished products using the MQuant™ Test Strips by EMD Millipore Corporation.

2.0 Scope

The MQuant™ Test Strip method for nitrate determination has been demonstrated to be effective for quantifying nitrate in plants, fruit juices, foods and water sources not to include seawater. See product 110020 MQuant™ Nitrate Test Label for range of sensitivity. Nitrites (NO_2^-) can interfere with the analysis of nitrates. This method is based on a diazotization reaction first described by Griess in 1879. This method incorporated the instructions given with the test strips, MQuant™ Nitrate Test. This method was validated under protocol MV-LAB-15-037.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure current with latest Ion Labs practices.

4.0 Definitions

4.1 **NO_3^-** – Nitrate Ion

4.2 **NO_2^-** – Nitrite Ion

4.3 **Sulfamic acid** – also known as amidosulfuric acid or NH_2SO_3H

4.4 **QC** – Quality Control

5.0 References

5.1 MV-LAB-15-037, Validation Protocol, Semi-Quantitative Determination of Nitrates using MQuant™ Nitrate Test Test-Strips, July 2015

5.2 Griess, P. *Chem. Ber.*, v.12, 426(1879).

5.3 MQuant™ Nitrate Test Instructions, EMD Millipore Corporation, August 2012

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Reagents

6.1.1 Millipore Deionized Water

6.1.2 Sulfamic Acid

6.1.3 Nitrate traceable standard at 1mg/mL in water.

6.1.4 Sodium acetate

6.1.5 Tartaric Acid

6.1.6 MQuant™ Nitrate Test Strips, EMD Millipore, Cat# 1.10020.0001

Note: Store in a closed vial at 2° to 8° C when not in use

6.2 Supplies and Glassware

6.2.1 50mL, 100mL and 500mL volumetric flasks

6.2.2 200uL, 1mL and 10mL pipette tips

6.2.3 22mL screw cap vials

6.2.4 1.5mL and 2.0mL micro centrifuge tubes

6.2.5 Weigh Paper and weigh boats

6.3 Equipment

6.3.1 Analytical Balance

6.3.2 Mortar and Pestle

6.3.3 Wrist Action Shaker

6.3.4 Sonicator

6.3.5 Vortex

6.3.6 Stir Plate

6.3.7 200µL, 1mL, and 10mL Pipette- adjustable

6.3.8 Microcentrifuge

6.3.9 pH meter

6.3.10 Timer / Stopwatch

7.0 Procedure

7.1 Reagent preparation

7.1.1 **10% sulfamic acid solution** is prepared by adding 5g of sulfamic acid to a 50mL volumetric flask, adding Millipore Water to 2/3 final volume and vortex to dissolve. Bring up to 50mL final volume before use.

7.1.2 **1M Sodium Acetate (MW 82.03g/mol)** is prepared by adding 4.102g sodium acetate to a 50mL volumetric flask, adding Millipore Water to 2/3 final volume and vortex to dissolve. Bring up to 50mL final volume before use.

7.1.3 **1M Tartaric Acid (MW 150.09g/mol)** is prepared by adding 1.501g of tartaric acid to a 10mL volumetric flask, adding Millipore Water 2/3 final volume and vortex to dissolve. Bring up to 10mL final volume before use.

7.1.4 **Nitrate Standard-** Standardized to 1mg/mL.

7.2 Sample preparation

7.2.1 For finished products, 10 or more dosage units can be pooled and ground by mortar and pestle as necessary.

7.2.2 Based on the fill weight or tablet weight per dose weigh a portion of the pooled dosages to generate a nitrate concentration that is within the validated range.

7.2.3 Samples can be dissolved in Millipore H_2O at any volume starting from 10mL. To manage large volumes the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted to bring the analyte concentration into the range of measurement. The final diluted sample must be centrifuged before analyzing. See Attachment 1 for validated assay range.

7.2.4 Check the pH of the final sample. The pH must be > 1 and < 12. If the pH is < 1 add 1M sodium acetate until the pH is within a suitable range. If the pH is > 12 add 1M tartaric acid until the pH is within a suitable range. If required titrant volume exceeds 200uL, prepare fresh sample and adjust pH at 2/3 final volume before bringing up to final volume.

7.3 Standard preparation

7.3.1 Using a standardized traceable standard with a stock concentration of 1mg/mL nitrate determine the dilution needed to create a standard at an equivalent concentration to the sample. Make dilutions using Millipore Water as diluent.

7.4 Testing

7.4.1 Immerse both reaction zones of the test strip in the pretreated sample (15°C to 25°C) for 1 second.

7.4.2 Shake off excess liquid from the strip and after 1 minute determine which color field on the label the color of the NO_3^- reaction zone matches more closely.

7.4.2.1 The color of the reaction zone may continue to change after the specified reaction time has elapsed. This must not be considered in the measurement.

7.4.3 Verify that the diluted standard coincides with the same color field.

7.4.4 If the NO_2^- alert zone changes color it is necessary to eliminate the NO_2^- before determining nitrate content.

7.4.4.1 To five mL of sample (pH <10) add 5 drops (200uL) of a 10% aqueous sulfamic acid solution and shake several times.

7.4.4.2 Repeat nitrate measurement.

7.4.5 If the color of the reaction zone is equal to or more intense than the darkest color on the scale, repeat the measurement using fresh, diluted samples until a value of less than 500mg/L NO_3^- is obtained.

7.5 Requirements

7.5.1 If the reaction zone of the sample matches the standard then the sample meets the claim.

7.5.2 If the reaction zone of the sample is darker or lighter than the standard then the approximate concentration can reported as > (claim) or < (claim) respectively. Note: The resolution of the color scale is not suitable to extrapolate a calculated value when the color of the sample does not match the color of the standard.

7.5.3 The nitrite reaction zone must be negative to report result

7.5.4 The pH must be verified to be between pH 1 and 12.

7.5.5 The presence of nitrate is verified by a color change in the range of 548nm (purple) when the nitrite reaction zone is negative.

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/05/16	New	16-0015	N. Zhang
1	01/29/19	Scheduled review: updated responsibilities.	19-0116	J. Maignan
2	01/16/23	Scheduled review: update responsibilities. Change logo. Update document format.	CC-23-0023	K. Burris

9.0 Attachments

9.1 Attachment 1 – Troubleshooting: Table of Common Interferences

Attachment 1 – Troubleshooting: Table of Common Interferences

Concentrations of foreign substances known to cause interference with assay					
Int	**mg/L**	**Int**	**mg/L**	**Int**	**mg/L**
Ag^{+}	50	Fe3+	250	NO_2^-	0.5
Al^{3+}	1000	$[Fe(CN)_6]^{4-}$	100	Pb^{2+}	1000
Ba^{2+}	1000	$[Fe(CN)_6]^{3-}$	100	PO_4^{3-}	1000
Ca^{2+}	1000	Hg^{+}	50	S^{2-}	25
Cl^-	1000	Hg^{2+}	100	SCN^-	100
CN^-	1000	K^{+}	1000	SO_3^{2-}	500
Co^{2+}	1000	Mg^{2+}	1000	SO_4^{2-}	1000
CrO^{4+}	20	Mn^{2+}	1000	$S_2O_3^{2-}$	250
Cu^{2+}	1000	MnO_4^-	10	Zn^{2+}	1000
Fe^{2+}	500	Ni^{2+}	1000		