Flow Solution™ FS 3700 Automated Chemistry Analyzer

Determination of Nitrate and Nitrite in Milk and Milk Products Using Cadmium Reduction and FIA with In-line Dialysis per ISO 14673-3

Cartridge Part Number 331535CT

Scope and Application

This method is used for the determination of nitrate and nitrite in milk and milk products using cadmium reduction and FIA with in-line dialysis, according to **ISO 14673-3**. This method is also applicable to cheeses (hard, semi-hard, soft, and processed), milk powder, whey powder, liquid milk, and milk-based infant food.[1]

Method Performance

Nitrate

Range	0.5 mg/L – 5.0 mg/L
Rate	30 samples/hour
Precision	≤ 2% RSD at mid-range
Method Detection Limit (MDL)	0.016 mg/L

Nitrite

Range	0.025 µg/L – 0.400 µg/L
Rate	30 samples/hour
Precision	≤ 2% RSD at mid-range
Method Detection Limit (MDL)	0.0016 mg/L

The range may be lowered to 0.010 mg / L nitrite by installing a PEEK heater assembly P/N 33078, beneath the cartridge, in place of the last mixing coil (A001488). Connect the heater exit to the debubbler with a tubing assembly (A001525), and the heater entry to the last mix tee with a tubing assembly (A001525).

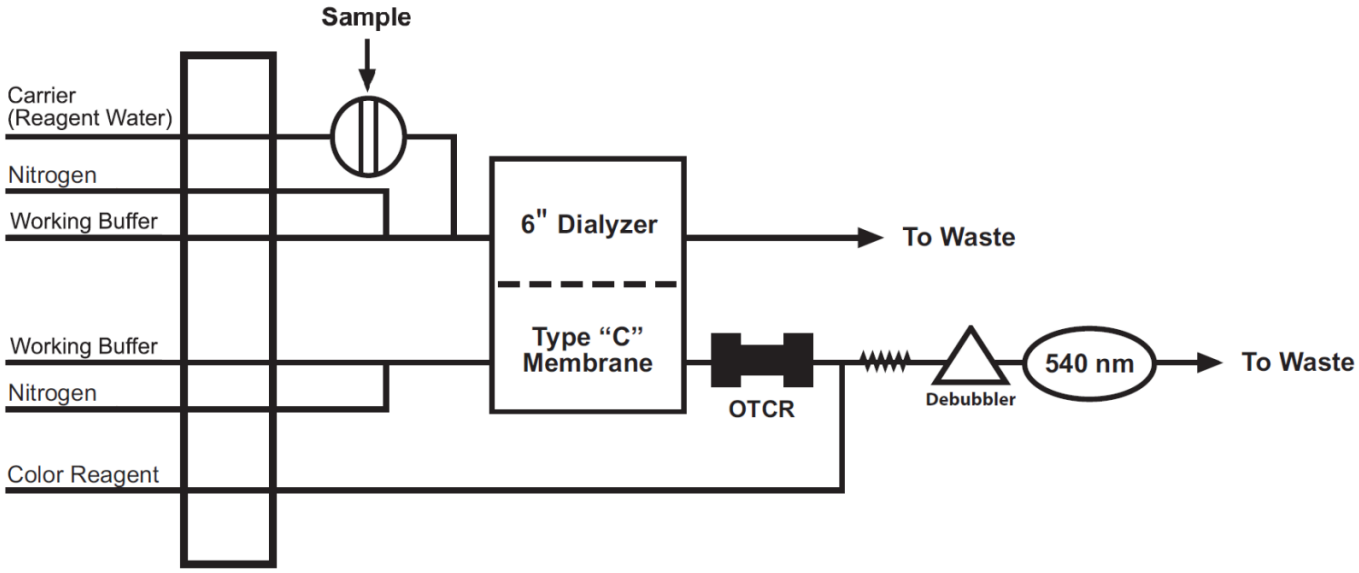


Figure 1. General flow diagram for the determination of nitrate and nitrite in milk and milk products using cadmium reduction and FIA with in-line dialysis.

Reagents and Calibrants

Chemical Name	CAS #	Chemical Formula	Part Number
Ammonium chloride	12125-02-9	NH_4Cl	
Ammonium hydroxide	1336-21-6	NH_4OH	
Brij®-35 (21% solution)	9002-92-0	$(C_2H_4O)_nC_{12}H_{26}O$	326126
Cupric sulfate pentahydrate	7758-99-8	$CuSO_4 \cdot 5H_2O$	
Ethylenediaminetetraacetic acid, disodium salt dihydrate (EDTA)	6381-92-6		
Hydrochloric acid	7647-01-0	HCl	
Imidazole	288-32-4	$C_3H_4N_2$	
1,3-Dimethylbarbituric acid	769-42-6	$C_6H_8N_2O_3$	
N-(1-Naphthyl) ethylenediamine dihydrochloride	1465-25-4	$C_{12}H_{14}N_2 \cdot 3HCl$	
Phosphoric acid	7664-38-2	H_3PO_4	
Potassium nitrate	7757-79-1	KNO_3	
Potassium nitrite	1310-73-2	KNO_2	
Sulfanilamide	63-74-1	$C_6H_8N_2O_2S$	
Water, deionized		H_2O	

Additionally, the following chemicals may be needed for sample preservation or treatment:

Chloroform	67-66-3	$CHCl_3$	

Summary of ISO 14673 - Part 3

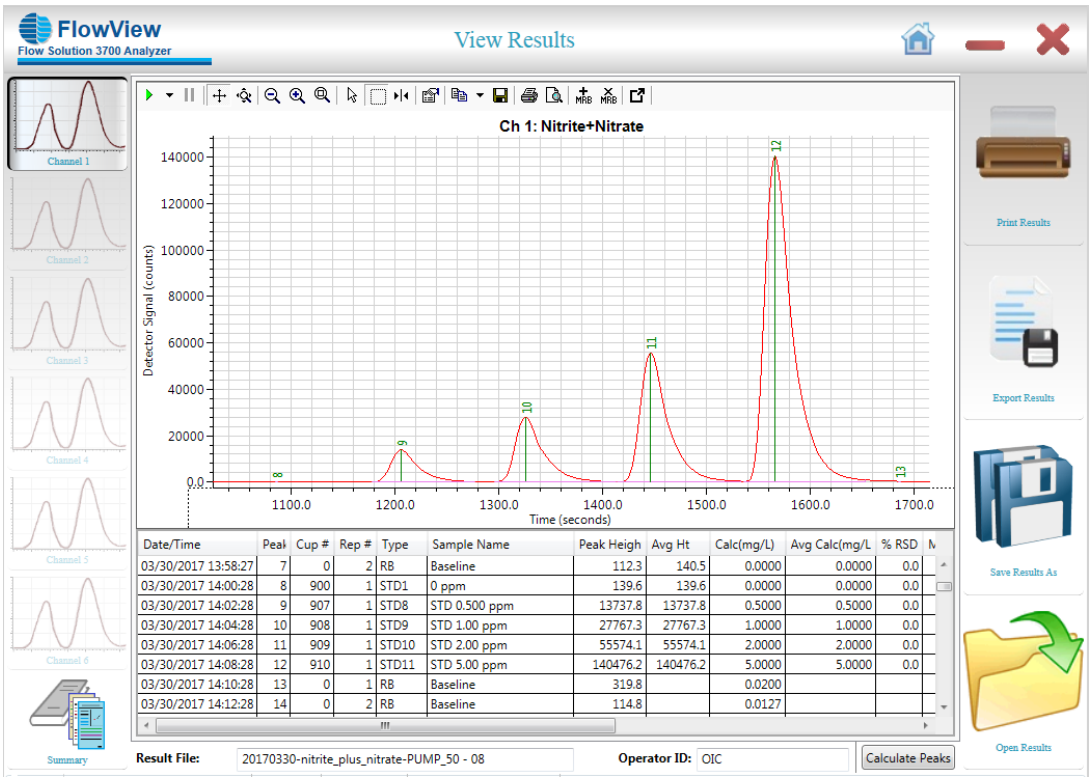
Method

- Prior to analysis, a test portion is suspended in a warm extraction buffer solution. Fat is separated by centrifuging and rapid cooling. Analyses are made of small portions of the de-fatted solution by **flow injection analysis (FIA)**. Inline dialysis is used to remove protein and remaining fat. The **nitrate** ions are reduced to **nitrite** ions by cadmium. The **nitrite** ions are reacted with sulfanilamide and N-(1-Naphthyl) ethylenediamine dihydrochloride to give a red-colored azo dye. The absorbance of the highly-colored azo dye that results is colorimetrically detected at 540 nm.
- Nitrite only may be measured by performing the same analysis as above, but without the cadmium reduction. Without the cadmium, nitrate is not reduced to nitrite and is not detected, since only nitrite forms the azo dye.
- Both nitrate and nitrite may be measured simultaneously by using a two channel flow analyzer. One channel is used to measure nitrate plus nitrite, while the second channel is used to measure nitrite only.
- For additional references, Patton[2] and Fox[3] provide discussions of the mechanisms and kinetics of the color forming reactions used in this method.

Interferences

- Turbid samples may interfere with the photometric detector's ability to measure the true absorbance of the sample. Filter turbid samples prior to analysis.
- Iron, copper, and other metals may interfere with the analysis by binding with the nitrate and/or nitrite in the sample, thus blocking the color formation reaction. The imidazole buffer eliminates this interference.
- Samples that are outside the functional pH range of the ammonium chloride buffer may affect the results obtained from this method. Adjust the pH of these samples to within a range of 5–9 using either concentrated hydrochloric acid (HCl) or sodium hydroxide (NaOH).
- Sample treatment is described in **Sample Handling and Preservation**.
- Oil and grease will coat the cadmium surface, thus reducing its reduction efficiency. Extract samples containing large concentrations of oil and grease with an appropriate organic solvent.
- Sulfide in the presence of cadmium will form cadmium sulfide (CdS), which will inhibit nitrate reduction. Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts.
- Dissolved oxygen and carbonate can react with cadmium to form cadmium hydroxide ($Cd(OH)_2$) and cadmium carbonate ($CdCO_3$) precipitants. Additionally, dissolved oxygen competitively inhibits the reduction of nitrate to nitrite. Care must be taken to ensure that the **pH** never exceeds **8.5**. Also, degass all reagents prior to analysis.
- Chlorine may reduce the reduction efficiency of the cadmium reactor. Samples that may contain residual chlorine should be tested for reduction efficiency through the analysis of Matrix Spike/Matrix Spike Duplicate (MS/MSD) samples. When necessary, dechlorinate samples with sodium thiosulfate ($Na_2S_2O_3$).
- Norwitz and Keliher, as well as Nydahl, have compiled a comprehensive study of interferences in the spectrophotometric analysis of nitrate and nitrite.[6,7,8]
- Method interferences can be caused by contaminants in the reagents, reagent water, and glassware, which may bias the results. Take care to keep all such items free of contaminants.

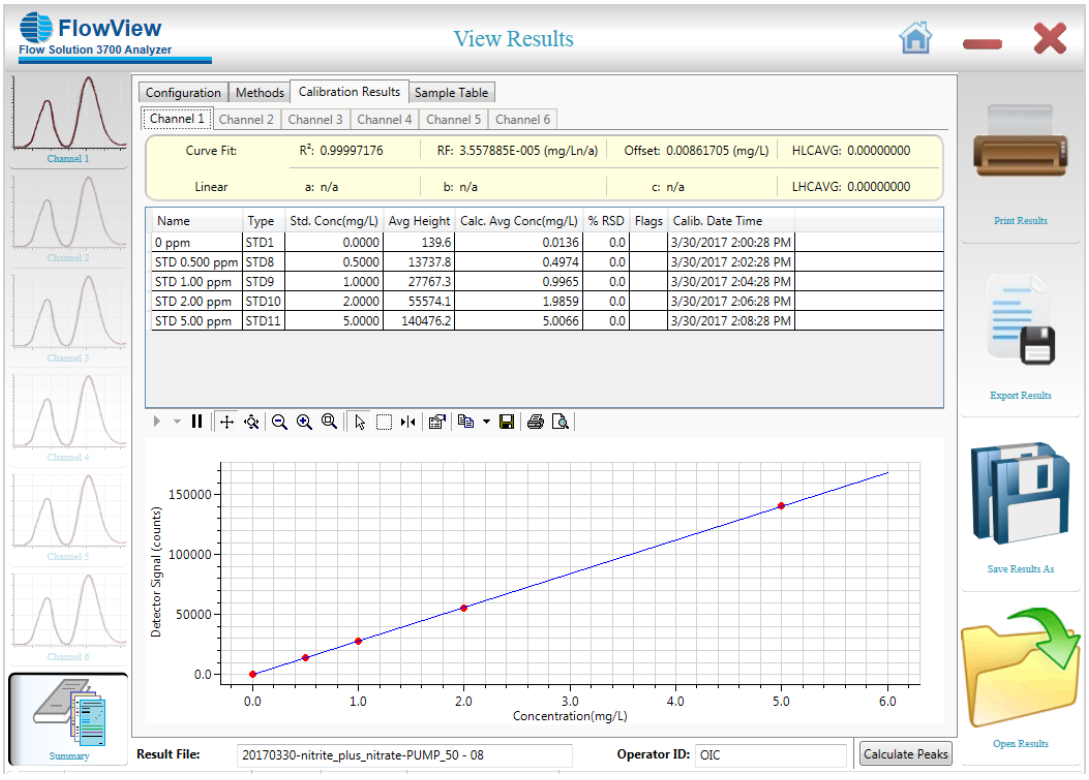
Nitrate



Date/Time	Peak	Cup #	Rep #	Type	Sample Name	Peak Heigh	Avg Ht	Calc(mg/L)	Avg Calc(mg/L	% RSD
03/30/2017 13:58:27	7	0	2	RB	Baseline	112.3	140.5	0.0000	0.0000	0.0
03/30/2017 14:00:28	8	900	1	STD1	0 ppm	139.6	139.6	0.0000	0.0000	0.0
03/30/2017 14:02:28	9	907	1	STD8	STD 0.500 ppm	13737.8	13737.8	0.5000	0.5000	0.0
03/30/2017 14:04:28	10	908	1	STD9	STD 1.00 ppm	27767.3	27767.3	1.0000	1.0000	0.0
03/30/2017 14:06:28	11	909	1	STD10	STD 2.00 ppm	55574.1	55574.1	2.0000	2.0000	0.0
03/30/2017 14:08:28	12	910	1	STD11	STD 5.00 ppm	140476.2	140476.2	5.0000	5.0000	0.0
03/30/2017 14:10:28	13	0	1	RB	Baseline	319.8		0.0200		
03/30/2017 14:12:28	14	0	2	RB	Baseline	114.8		0.0127		

Figure 3. Calibration Series for Determination of Nitrate and Nitrite in Milk and Milk Products Using Cadmium Reduction and FIA with In-line Dialysis

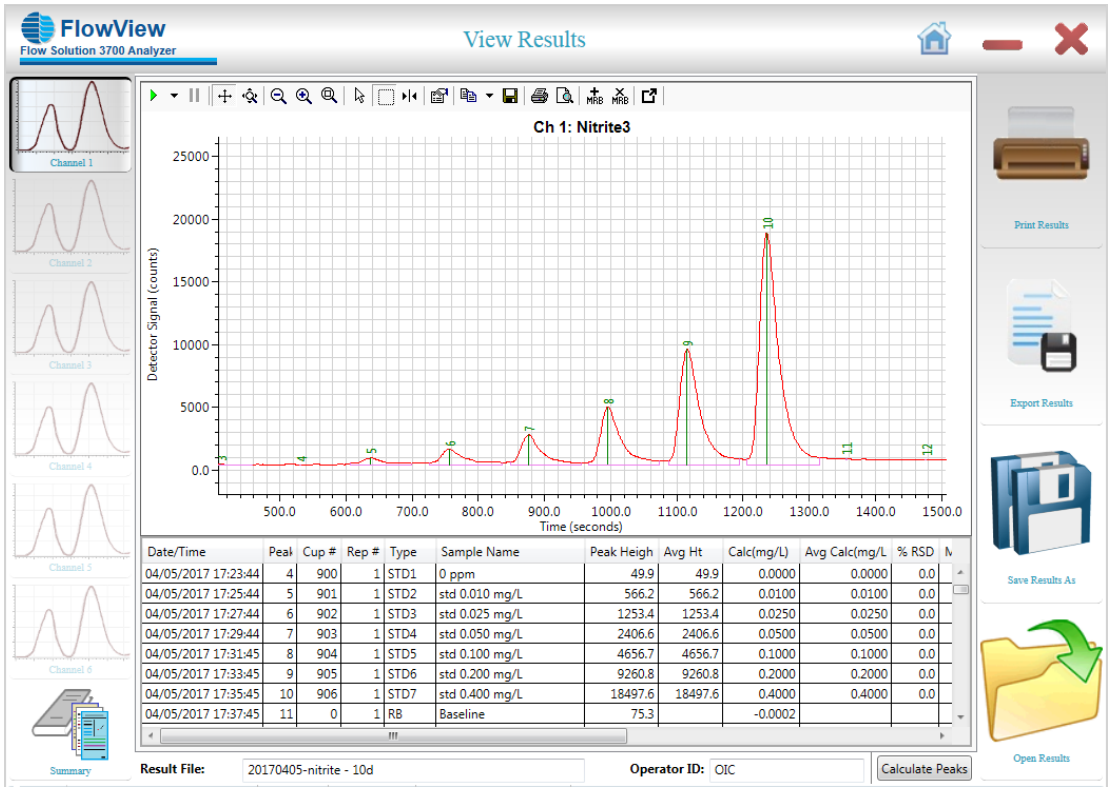
Nitrate



Name	Type	Std. Conc(mg/L)	Avg Height	Calc. Avg Conc(mg/L)	% RSD	Flags	Calib. Date Time
0 ppm	STD1	0.0000	139.6	0.0136	0.0		3/30/2017 2:00:28 PM
STD 0.500 ppm	STD8	0.5000	13737.8	0.4974	0.0		3/30/2017 2:02:28 PM
STD 1.00 ppm	STD9	1.0000	27767.3	0.9965	0.0		3/30/2017 2:04:28 PM
STD 2.00 ppm	STD10	2.0000	55574.1	1.9859	0.0		3/30/2017 2:06:28 PM
STD 5.00 ppm	STD11	5.0000	140476.2	5.0066	0.0		3/30/2017 2:08:28 PM

Figure 4. Calibration Curve and Statistics for Determination of Nitrate and Nitrite in Milk and Milk Products Using Cadmium Reduction and FIA with In-line Dialysis

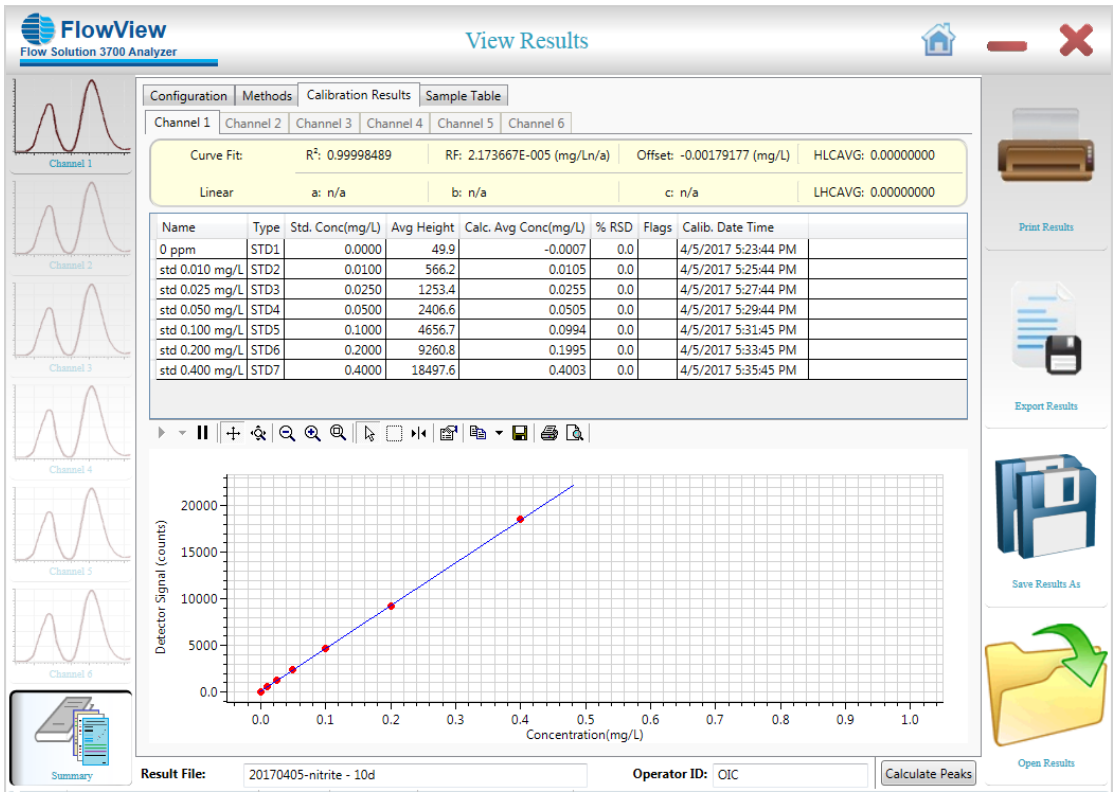
Nitrite



Date/Time	Peak	Cup #	Rep #	Type	Sample Name	Peak Heigh	Avg Ht	Calc(mg/L)	Avg Calc(mg/L	% RSD
04/05/2017 17:23:44	4	900	1	STD1	0 ppm	49.9	49.9	0.0000	0.0000	0.0
04/05/2017 17:25:44	5	901	1	STD2	std 0.010 mg/L	566.2	566.2	0.0100	0.0100	0.0
04/05/2017 17:27:44	6	902	1	STD3	std 0.025 mg/L	1253.4	1253.4	0.0250	0.0250	0.0
04/05/2017 17:29:44	7	903	1	STD4	std 0.050 mg/L	2406.6	2406.6	0.0500	0.0500	0.0
04/05/2017 17:31:45	8	904	1	STD5	std 0.100 mg/L	4656.7	4656.7	0.1000	0.1000	0.0
04/05/2017 17:33:45	9	905	1	STD6	std 0.200 mg/L	9260.8	9260.8	0.2000	0.2000	0.0
04/05/2017 17:35:45	10	906	1	STD7	std 0.400 mg/L	18497.6	18497.6	0.4000	0.4000	0.0
04/05/2017 17:37:45	11	0	1	RB	Baseline	75.3		-0.0002		

Figure 5. Calibration Series for Determination of Nitrate and Nitrite in Milk and Milk Products Using Cadmium Reduction and FIA with In-line Dialysis

Nitrite



Name	Type	Std. Conc(mg/L)	Avg Height	Calc. Avg Conc(mg/L)	% RSD	Flags	Calib. Date Time
0 ppm	STD1	0.0000	49.9	-0.0007	0.0		4/5/2017 5:23:44 PM
std 0.010 mg/L	STD2	0.0100	566.2	0.0105	0.0		4/5/2017 5:25:44 PM
std 0.025 mg/L	STD3	0.0250	1253.4	0.0255	0.0		4/5/2017 5:27:44 PM
std 0.050 mg/L	STD4	0.0500	2406.6	0.0505	0.0		4/5/2017 5:29:44 PM
std 0.100 mg/L	STD5	0.1000	4656.7	0.0994	0.0		4/5/2017 5:31:45 PM
std 0.200 mg/L	STD6	0.2000	9260.8	0.1995	0.0		4/5/2017 5:33:45 PM
std 0.400 mg/L	STD7	0.4000	18497.6	0.4003	0.0		4/5/2017 5:35:45 PM

Figure 6. Calibration Curve and Statistics

151 Graham Road
PO Box 9010
College Station, Texas
77842-9010

(979) 690-1711
(800) 653-1711 USA/Canada
(979) 690-0440

www.oico.com
E-mail: oi-info@xyleminc.com