Determination of Melamine in Milk by Ion Chromatography with UV Detection

INTRODUCTION

In 2008, melamine was found as a contaminant of milk and milk-containing products after the discovery of melamine contamination of pet food. These contaminations resulted in infant and pet deaths. Both deliberate contaminations originated in China, with some contaminated products exported to neighboring and more distant countries. Melamine was added to both products to increase their apparent protein content, as it was determined by a nonspecific total nitrogen test and melamine has a large amount of nitrogen per unit mass.

Dionex has designed two reversed-phase HPLC methods to determine the melamine adulteration of liquid and powdered milk.[1,2] One method is an ion-pairing HPLC method using an Acclaim®120 C18 column and the other method uses the Acclaim Mixed-Mode WCX column, where both the hydrophobic and cationic properties of melamine are used to affect the separation. As a cation, melamine can be separated by cation-exchange chromatography and therefore be determined by Ion Chromatography (IC). This Application Note (AN) shows how melamine can be determined in milk, powdered milk, and a milk-containing candy by IC using an IonPac® CS17 column and UV detection at 240 nm. This gives the analyst another chromatographic option, providing a selectivity that may be needed for some samples, or a way to increase sample throughput with existing laboratory instrumentation.

EQUIPMENT

Dionex ICS-3000 consisting of:

    DP Dual Pump

    DC Detector/Chromatography module with dual temperature zone equipped with

    6-port valve (injection valve)

    AM Automation Manager equipped with,

    10-port valve (high pressure valve)

    EG Eluent Generator

    AS Autosampler

    PDA-3000 Photodiode Array Detector*

Chromeleon® 6.8 Chromatography Data System

    * The Dionex VWD detector can also be used for this application. The photodiode array detector is required to confirm the melamine peak identity with the peak purity option.

Conditions

Column: IonPac CS17 Analytical,
4 × 250 mm (P/N 060557)

Guard: IonPac CG17 Guard,
4 × 50 mm (P/N 060560)

Concentrator: IonPac TCC-LP1,
4 × 35 mm (P/N 046027)

Eluent Source: EGC II MSA (P/N 058902)
with CR-CTC (P/N 066262)

Gradient: See chromatogram

Flow Rate: Pump 1:1.0 mL/min
Pump 2:1.0 mL/min

Inj. Volume: See chromatogram

Pressure: ~2100 psi

Detection: UV at 240 nm

REAGENT AND STANDARDS

Deionized water (DI), Type I reagent grade, 18 MΩ-cm
resistivity or better

Melamine (Sigma-Aldrich)

Acetic acid (Labscan)

PREPARATION OF SOLUTIONS AND REAGENTS
Eluent Solution

The eluent generator produces the eluent using the EluGen EGC II MSA cartridge and deionized water supplied by the pump, with the eluent concentration controlled by the Chromeleon software. Backpressure tubing must be added to achieve 2000–2500 psi backpressure that will allow the EG degasser to function properly. See the *ICS-3000 Ion Chromatography System Operator's Manual,* (P/N 065031-03) for instructions on adding backpressure.[3]

Standard Solutions
Stock Standard Solutions (1000 mg/L)

To prepare the 1000 mg/L melamine standard, dissolve 0.1 g of melamine in 100 mL of deionized water.

Secondary Standards

Prepare a 1 mg/L secondary standard from the stock standard. From this secondary standard, prepare the standard calibrations and MDL standards as follows.

1. Calibration standards

To prepare melamine standards at concentrations of 25, 50, 100, 200, 400, and 800 µg/L, add the appropriate volumes of 1 mg/mL standard to separate 100 mL volumetric flasks. For example, add 5.0 mL of 1 mg/L standard for the 50 µg/L standard. Bring to volume with deionized water. These six standards were used to calibrate one of the IC methods presented here, and for the second method, we prepared an additional 12.5 µg/L standard and calibrated with seven standards.

2. MDL standard

Prepare a 25 µg/L melamine standard by adding 2.5 mL of 1 mg/L melamine standard to a 100 mL volumetric flask and bring to volume with deionized water.

SAMPLE PREPARATION

OnGuard RP Preparation
Flush the OnGuard RP cartridge with 5 mL of methanol and then with 10 mL of deionized water at about 4 mL/min.

Liquid Milk Preparation
1. Mix 10 mL of liquid milk and 8 mL of deionized water.
2. Add 2 mL of 3% acetic acid and mix.
3. Pass the sample through a Whatman 2V filter.
4. Pass 5 mL of the filtered sample through a prepared OnGuard RP cartridge, discarding the first 3 mL and collecting the remaining sample into an AS vial.

Milk Powder and Candy Sample Preparation

Add about 5 g of sample to a 50 mL volumetric flask, dissolve, and bring to volume with deionized water. Take 10 mL of this sample and prepare by the same method as the liquid milk sample.

Samples Spiked with Melamine

To prepare a 50 µg/L melamine spiked sample, in step 1 of the sample preparation instead of adding 8 mL of deionized water to the sample, add 1 mL of 1 mg/L melamine secondary standard and 7 mL of deionized water. For the 100 µg/L standard, use 2 mL of 1 mg/L melamine and 6 mL deionized water.

Calculating Amount of Melamine in the Milk-Containing Candy Sample

The sample preparation involved dissolving 5.079 g of candy in 50 mL DI water and diluted 1:1.

Amount of melamine in 100 µL of the prepared candy sample:

$$= (13.78 \text{ µg/L}) \times (L/1{,}000{,}000 \text{ µL}) \times 100 \text{ µL}$$
$$= 13.78 \times 10^{-4} \text{ µg}$$

Amount of candy in the 100 µL injection:
$$5.079 \text{g}/50 \text{ mL} \times ((\text{mL}/1000 \text{ µL})/2) \times 100 \text{ µL}$$
$$= 5.079 \times 10^{-3} \text{ g}$$

Amount of melamine per g of candy:
$$= 13.78 \times 10^{-4} \text{ µg}/5.079 \times 10^{-3} \text{ g}$$
$$= 0.27 \text{ µg/g}$$

RESULTS AND DISCUSSION

Melamine is a cation and, therefore, can be separated from other compounds by cation-exchange chromatography. The IonPac CS17 column was designed for the separation of hydrophobic amines like melamine. While melamine is a cation at neutral pH, it is not fully ionized at pH 7 and therefore, suppressed conductivity does not provide a sensitive detection method for this compound. Sensitivity can be increased using the salt converter cation self-regenerating suppressor. However, more sensitivity and selectivity for melamine was found by using absorbance detection at 240 nm.

Milk and milk-based products can be difficult for chromatographic methods due to the large variety of compounds present that can interfere with the analytes of interest. To determine melamine, we used a sample preparation technique first developed for the IC determination of iodide in milk.[4] Despite this sample preparation, we found it difficult to determine melamine in the sample. Therefore, we first loaded the sample loop installed on the AM-HP1 with the prepared sample. Then, using deionized water, we moved the sample onto the cation-exchange concentrator installed on the injection valve, and then eluted from the concentrator, directly onto the IonPac CS17 column set. Figure 1 shows the schematic of this system configuration and Table 1 shows the valve programming that allows the sample delivery to the concentrator while washing unbound compounds to waste, and subsequent chromatography. This method ultimately proved more successful for melamine determination.
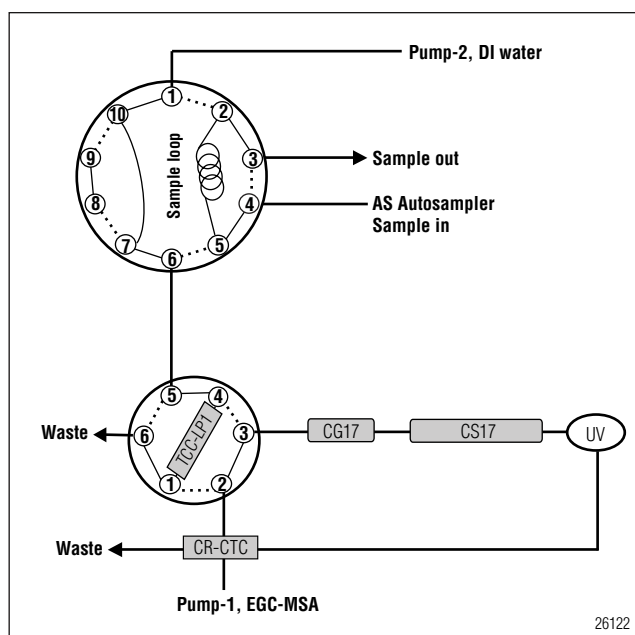

Figure 1. System configuration schematic.

Table 1. Valve Switching Program			
Retention Time (min)	AM_HP1	Injection Valve	Note
−5.0	A	Load	
−3.0	B	Load	AS loads the sample to sample loop before the AM_HP1 switches from A to B.
0.0	A	Inject	
End run	A	Inject	The end of runtime depends on the gradient.

Prior to quantitative sample analysis, we calibrated the method as described in the section *Secondary Standards,* earlier in this application note. Figure 2 shows the chromatography from the calibration, which was linear with a correlation coefficient of 0.9998. To estimate the minimum detection limit, we made seven injections of the 25 µg/L standard. Figure 3 shows the seven injections along with the blank, an injection of water. The blank shows that there are no peaks from the water or chromatography system interfering with melamine determination. Table 2 shows the data from the MDL experiment and that the MDL estimate was 4.4 µg/L.
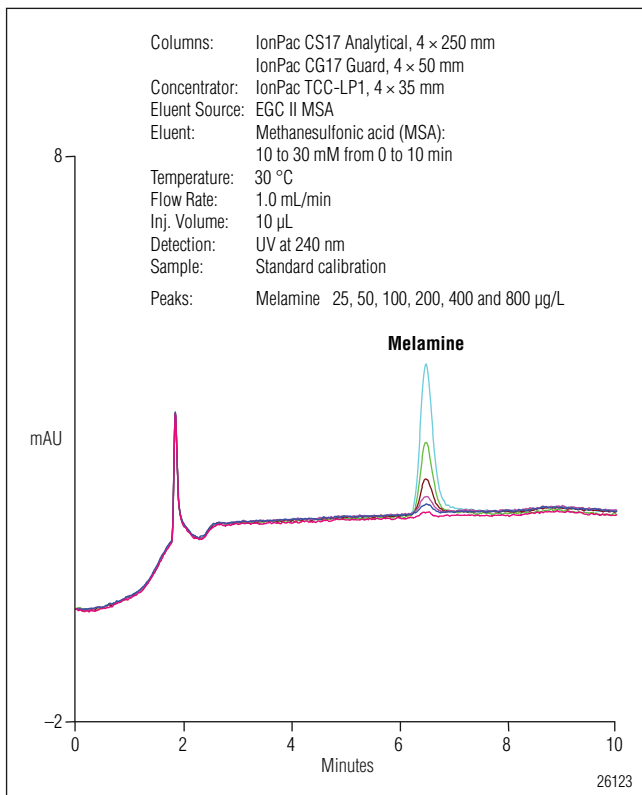
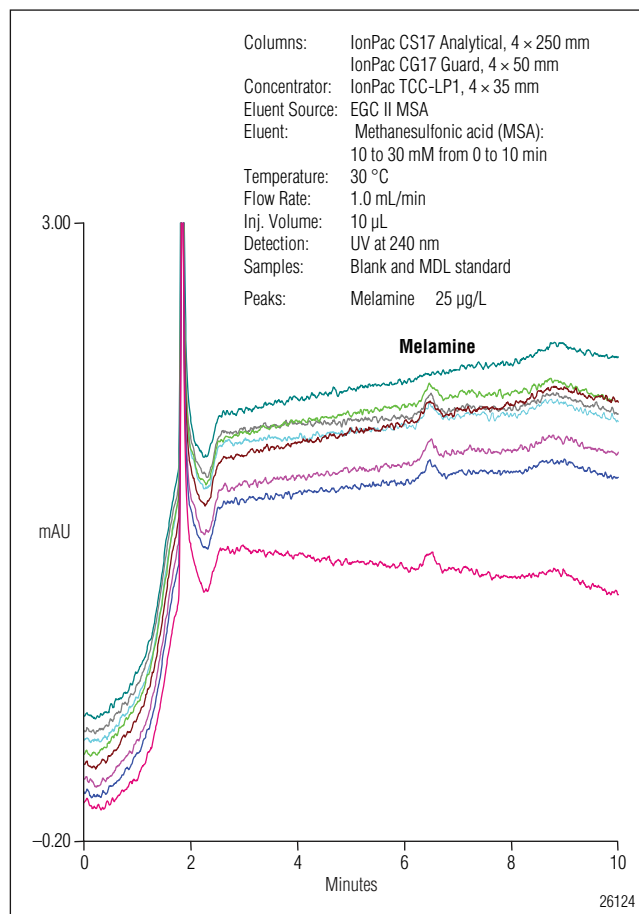Figure 2 Chromatograms of six melamine standards used for calibration.

Figure 3. Chromatograms of a water injection (blank) and seven consecutive injections of 25 µg/L melamine.

After method qualification, we evaluated the melamine content of milk, milk powder, and a milk-containing candy. Only the candy was known to contain melamine. Figures 4 and 5 together with Tables 3 and 4 show the results of the determinations of melamine in milk and milk powder. The analysis shows that neither sample contained melamine.

To demonstrate that melamine was not lost during sample preparation, melamine was added to each sample prior to sample preparation. One portion of each sample was spiked with 50 µg/L melamine and a second portion was spiked with 100 µg/L melamine. The chromatography in Figures 4 and 5 and the quantitative results in Tables 3 and 4 show that melamine was recovered from both samples with recoveries greater than 90%.

Table 2. Data from Seven Consecutive Injections of 25 µg/L Melamine	
Injection No.	Height (mAU)
1	0.0906
2	0.1071
3	0.0959
4	0.0924
5	0.0948
6	0.0969
7	0.0999
Average:	0.0968
RSD:	5.63
MDL (µg/L):	4.4

Columns: IonPac CS17 Analytical, 4 × 250 mm
                IonPac CG17 Guard, 4 × 50 mm
Concentrator: IonPac TCC-LP1, 4 × 35 mm
Eluent Source: EGC II MSA
Eluent: Methanesulfonic acid (MSA):
                10 to 30 mM from 0 to 10 min
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: UV at 240 nm
Sample: 1. Milk
                2. Milk + 50 µg/L melamine
                3. Milk + 100 µg/L melamine

Peaks: Melamine   µg/L
                1.   —
                2.   50.88
                3.   102.38

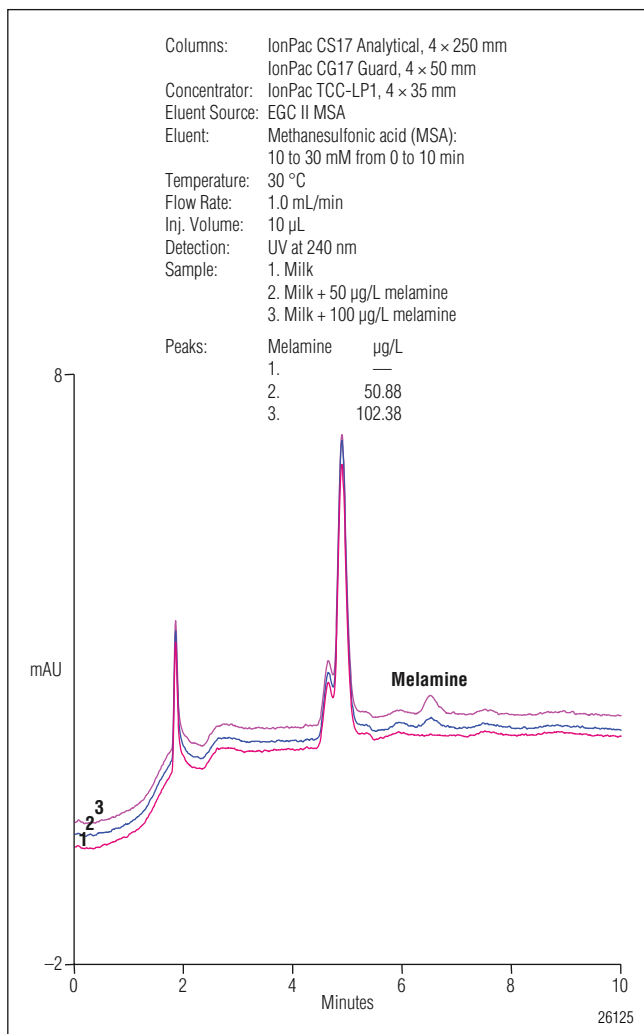*Figure 4. Chromatograms of milk and milk spiked with melamine. Milk (1); milk + 50 µg/L melamine (2) ; and milk + 100 µg/L melamine (3).*

Columns: IonPac CS17 Analytical, 4 × 250 mm
                IonPac CG17 Guard, 4 × 50 mm
Concentrator: IonPac TCC-LP1, 4 × 35 mm
Eluent Source: EGC II MSA
Eluent: Methanesulfonic acid (MSA):
                10 to 30 mM from 0 to 10 min
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: UV at 240 nm
Samples: 1. Milk powder
                2. Milk powder + 50 µg/L melamine
                3. Milk powder + 100 µg/L melamine

Peaks: Melamine   µg/L
                1.   —
                2.   49.03
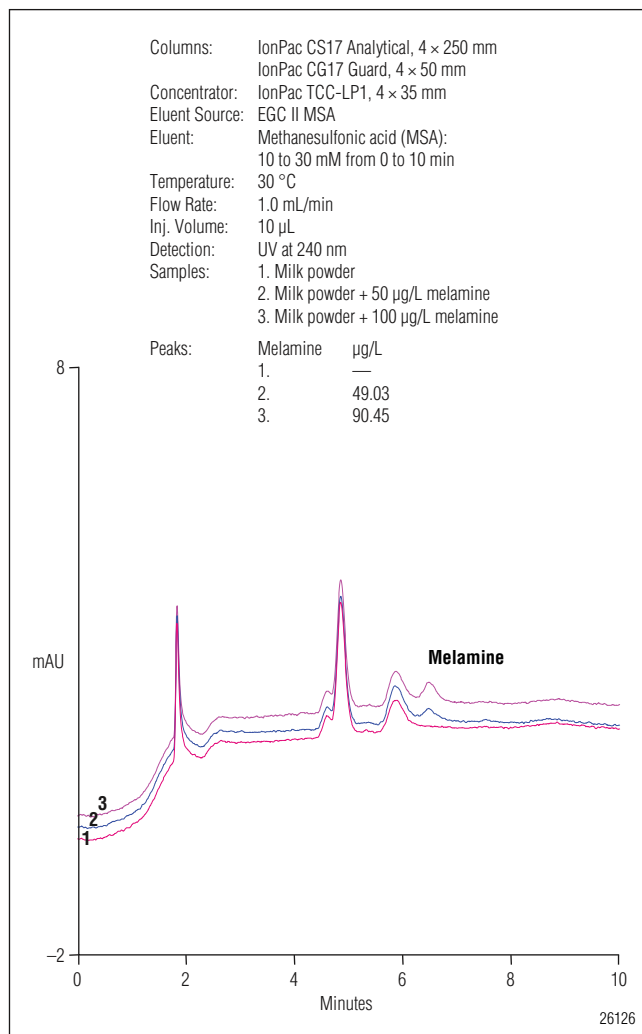   3.   90.45

Figure 5. Chromatograms of milk powder and milk powder spiked with melamine. Milk (1); milk + 50 µg/L melamine (2); and milk + 100 µg/L melamine (3).

Table 3. Recovery of Melamine in the Milk Sample			
	Amount (µg/L)		
Injection #	Liquid Milk	Liquid Milk + 50 µg/L Melamine	Liquid Milk + 100 µg/L Melamine
1	ND	53.58	96.20
2	ND	53.12	110.31
3	ND	44.42	93.54
4	ND	52.00	100.33
5	ND	51.27	111.51
Average:	NA	50.88	102.38
RSD:	NA	7.32	7.98
% Recovery:	NA	101.8	102.4

Table 4. Recovery of Melamine in the Milk Powder Sample			
	Amount (µg/L)		
Injection #	Milk Powder	Milk Powder + 50 µg/L Melamine	Milk Powder + 100 µg/L Melamine
1	ND	49.56	92.30
2	ND	56.27	89.74
3	ND	45.07	88.04
4	ND	49.16	88.74
5	ND	45.07	93.44
Average:	NA	49.03	90.45
RSD:	NA	9.35	2.57
% Recovery:	NA	98.06	90.45

The analysis of the melamine-containing candy sample proved more difficult. Melamine was not completely resolved from another peak. This was not observed in the milk and milk powder samples. To resolve these two peaks, we changed the mobile phase composition from a 10 min 10–30 mM MSA gradient to 5 mM MSA for 20 min. Due to the use of a RFIC system, this mobile phase change and other changes made to arrive at the final method did not require the preparation of new eluents. We simply used the Chromeleon chromatography workstation to instruct the eluent generator to prepare a new mobile phase.

Our initial chromatography of the candy sample also suggested that there was only a small amount of melamine in the sample. Therefore, when we calibrated the system for the new separation method, we added a lower concentration standard (12.5 µg/L) to the calibration and increased the injection volume from 10 to 100 µL. The calibration was linear with a correlation coefficient of 0.9997.

Figure 6 and Table 5 show the results of the analysis of the candy sample for melamine. The candy sample contained melamine with a concentration of about 14 µg/L in the prepared sample, or 0.27 µg/g in the candy. To assess the accuracy of this determination, we prepared two spiked candy samples with (a) a 10 µg/L spike, and (b) a 20 µg/L spike. Melamine was recovered from both samples suggesting that the method is accurate. After installing the photodiode array detector on our system, we also confirmed that the melamine peak in the candy sample was a spectral match to the melamine standard.

This IC method accurately determined melamine in milk, milk powder, and a milk-containing candy after a simple sample preparation. As this method uses a RFIC system, the analyst does not have to prepare eluents and can easily change the mobile phase for samples where unknown peaks coelute with melamine.
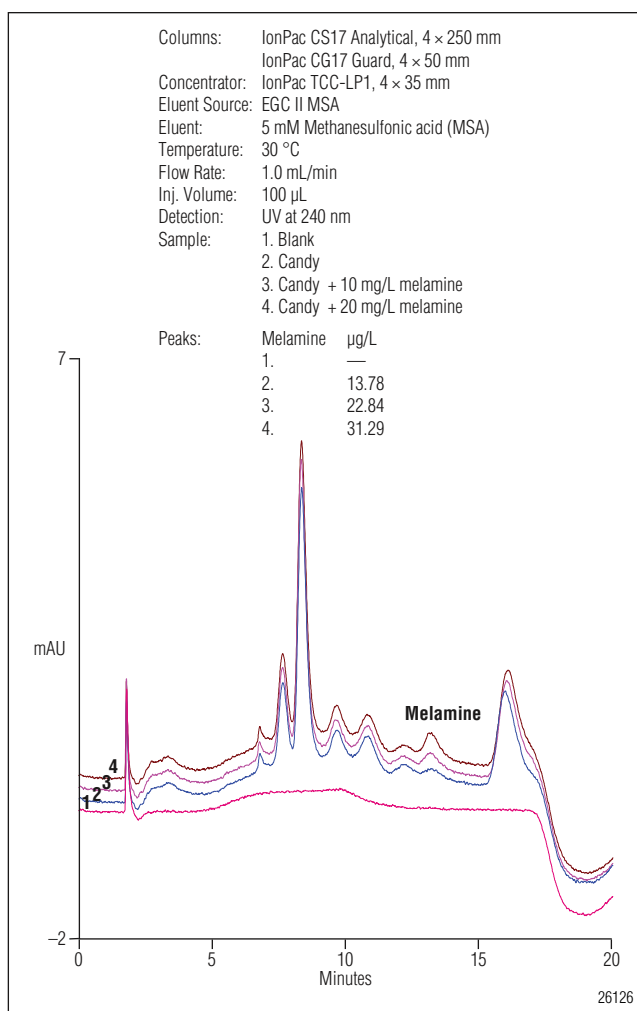

Figure 6. Chromatograms of milk-containing candy and candy spiked with melamine. Blank (1); candy (2); candy + 10 µg/L melamine (3); and candy + 20 µg/L melamine (4).

Table 5. Recovery of Melamine in the Candy Sample			
	Amount (µg/L)		
Injection #	**Candy**	**Candy + 10 µg/L Melamine**	**Candy + 20 µg/L Melamine**
1	14.27	23.16	28.86
2	15.31	25.46	31.47
3	12.70	21.96	31.23
4	12.49	21.59	32.56
5	14.10	22.05	32.32
Average:	13.78	22.84	31.29
RSD:	8.51	6.91	4.69
% Recovery:		90.66	87.55

REFERENCES

1. *Rapid Determination of Melamine in Liquid Milk and Milk Powder by HPLC on the Acclaim Mixed-Mode WCX-1 Column with UV Detection.* Application Note 221 (LPN 2181, March 2009), Dionex Corporation, Sunnyvale, CA.

2. *Determination of Melamine in Milk Powder by Reversed-Phase HPLC with UV Detection.* Application Note 224 (LPN 2184, March 2009), Dionex Corporation, Sunnyvale, CA.

3. *ICS-3000 Ion Chromatography System Operator's Manual,* Document No. 065031-03. Dionex Corporation, Sunnyvale, CA

4. *Determination of Iodide in Milk Products.* Application Note 37 (LPN 0702-03, October, 2004), Dionex Corporation, Sunnyvale, CA.

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