Application Data Set from Shimadzu

● LCMSMS Analysis LCMSMS-001

Determination of melamine in milk powder by HPLC-tandem mass spectrometry

Abstract: A method was proposed for determination of melamine in milk powder with Shimadzu LCMS-8030 triple quadrupole mass spectrometer. Samples were extracted, separated with HPLC, and then qualitatively and quantitatively assayed using LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated satisfactory linearity for melamine in the concentration range of 0.01~0.5 mg/L with a correlation coefficient of calibration curve of 0.9998; precision tests were performed on standard solutions at concentrations of 0.01 mg/L, 0.1 mg/L and 0.5 mg/L, the %RSDs of retention time and peak area in 6 successive injections were below 0.64% and 4.34%, respectively, suggesting that the system was of good precision. The spike recovery test of samples spiked with 0.01 mg/kg of standard solution yielded a recovery of 104.0%. The method's LOQ was 0.005 mg/kg.

Key words: milk powder, melamine, triple quadrupole mass spectrometry

Melamine, chemical name of 1,3,5-Triazine-2,4,6-triamine, is an important nitrogen heterocyclic organic industrial chemical commonly used for the production of plastic, glue and flame retardant. It is frequently reported in recent years that law-breakers use melamine-containing raw material for diary product manufacturing. Triple quadrupole mass spectrometer is provided with multiple reaction monitoring (MRM) mode which can effectively eliminate matrix interference, therefore, it can be used for quantitative assay of trace amount of melamine. It is stipulated in China's national standard GB/T22388-2008 *Determination of Melamine in Raw Milk and Dairy Products* that LC-MS/MS method shall be used for determination of melamine in milk and dairy products. Furthermore, the method can achieve an LOQ of 0.01 mg/kg for melamine. In this paper， a method was proposed for quantitative assay of melamine in milk powder with Shimadzu LCMS-8030 triple quadrupole mass spectrometer.

Author: Hao Hongyuan, Shanghai Analysis Center. E-mail: sshhhy@shimadzu.com.cn

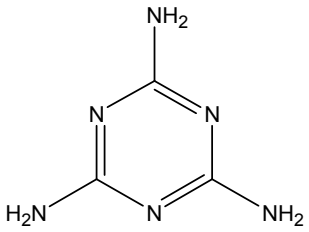

Fig.1 Chemical structure of melamine

1. Experiment

1.1 Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A_5 online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

1.2 Conditions of Analysis

LC conditions
Column: CAPCELL PAK CR (1:4) 2.0 mm I.D.×150 mm L., 5 μm
Mobile phase A: the mixture of 500 mL 10 mM ammonium acetate aqueous solution and 2 mL acetic acid.
Mobile phase B: acetonitrile
Elution mode: isocratic, A/B = 40/60 (v/v)
Flow rate: 0.2 mL/min
Column temperature: 40 °C
Injection volume: 10 μL

MS condition
Ionization mode: ESI (+)
Ionspray voltage: 4.5 kV
Nebulizing gas: Nitrogen 3.0 L/min
Drying gas: Nitrogen 15 L/min
Collision gas: Argon
DL temperature: 250 °C
Heater block temperature: 400 °C
Mode: multiple reaction monitoring (MRM), precursor ion at *m/z* 127.10, quantitative product ion at *m/z* 85.05, qualitative product ion at *m/z* 68.00.
Dwell time: 300 msec
Pause time: 3 msec
MRM parameters: see Table1.

Table 1 MRM parameters of melamine

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Melamine	127.10	85.05	-12.0	-20.0	-18.0
		68.00*	-12.0	-30.0	-27.0

* refers to qualitative ion.

1.3 Preparation of standard solutions and pretreatment of samples

Samples were subjected to pretreatment procedures as stipulated in GB/T22388-2008 *Determination of Melamine in Raw Milk and Dairy Products--Part II LC-MS/MS Method*.

Blank samples were subjected to the pretreatment procedures, and the resulted sample solution was used to progressively dilute 10 mg/L melamine stock solution into standard working solutions at concentrations of 0.01, 0.05, 0.1, 0.2, and 0.5 mg/L.

2. Results and Discussion

2.1 Chromatogram of melamine standard sample

The chromatogram of 0.1 mg/L melamine standard sample was as shown in Fig.2, in which the chromatographic peak at retention time of 5.62 min was melamine.

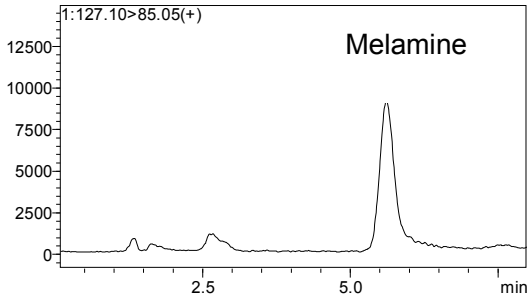

Fig.2 Chromatogram of 0.1 mg/L standard solution

2.2 Linearity

A series of standard working solutions at concentrations of 0.01, 0.05, 0.1, 0.2, and 0.5 mg/L were assayed using the analysis conditions specified in section 1.2. A calibration curve of equation Y = (1568.71)X + (-1426.24) was plotted (as shown Fig. 3) using concentration as abscissa and peak area as ordinate. The resulted curve was of good linearity with a correlation coefficient R of 0.9998. The concentration and peak area results of standard solutions are shown in Table 2.

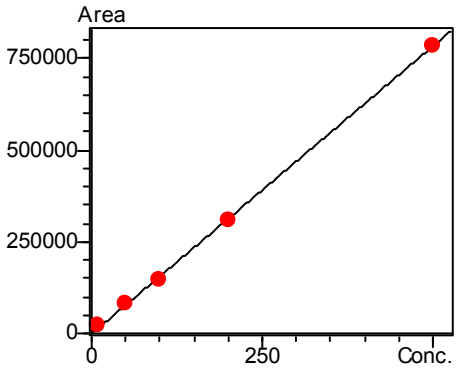

Fig. 3. Calibration curve of melamine

Table 2. Concentrations and peak areas of standard solutions.

Conc. (mg/L)	Area
0.01	19666
0.05	81499
0.1	148105
0.2	786149
0.5	306538

2.3 Precision test

The method's precision was assessed by 6 successive assays to standard samples at concentrations of 0.01 mg/L, 0.1 mg/L and 0.5 mg/L, respectively. The resulted %RSDs of retention time and peak areas are shown in Table3.

Table 3 Reproducibility test data of melamine (n=6)

Conc.(mg/L)	%RSD (RT)	%RSD (Area)
0.01	0.64	4.34
0.1	0.18	3.80
0.5	0.34	3.18

2.4 Accuracy test

In order to assess the method's accuracy, samples spiked with 0.01 mg/kg standard were assayed for determination of spike recovery. The MRM chromatogram of blank sample is shown in Fig.4, suggesting that the sample contained trace amount of melamine at concentration of 2.736 μg/kg. The chromatogram of samples spiked with 0.01 mg/kg standard is shown in Fig.5, showing that the average recovery of two samples spiked with 0.01 mg/kg standard was 104.0%. Samples spiked with 3 mg/kg standard were processed in duplicate and one-fifth of the filtrate obtained at the step of solid-phase extraction was purified. The calculated average recovery was 99.9%, meeting the requirements stipulated in China's national standard that "at the spiked level of 0.01 mg/kg~0.5 mg/kg, the recovery shall be in the range of 80%~110%".

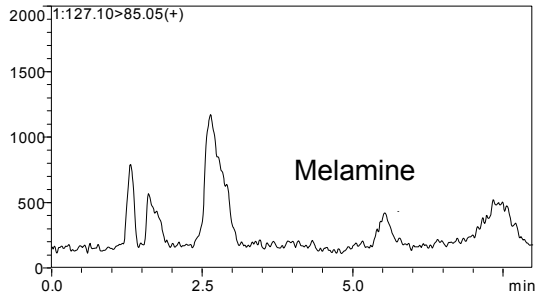

Figure 4. MRM chromatogram of a blank sample

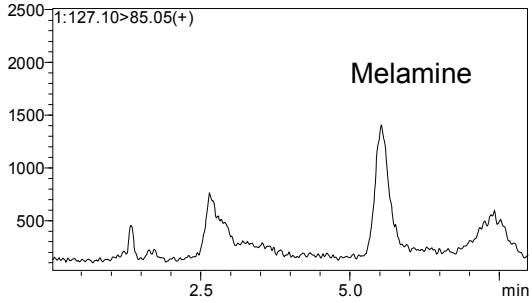

Fig.5 MRM chromatogram of a sample spiked with 0.01 mg/kg of standard

2.5 Sensitivity test

Standard working solutions of melamine (0.01, 0.05, 0.1, 0.2 and 0.5 mg/L) were analyzed and workstation software was used to calculate the LOD (S/N=3) of melamine to be 0.0017 mg/L and LOQ (S/N=10) to be 0.0052 mg/L. 0.005 mg/L standard solution of melamine was prepared and injected for analysis, yielding the MRM chromatogram as shown in Fig. 6, where the S/N=14.04. Therefore 0.005 mg/L can be deemed as the LOQ and the method's LOQ can be calculated in light of the pretreatment procedures to be 0.005 mg/kg.

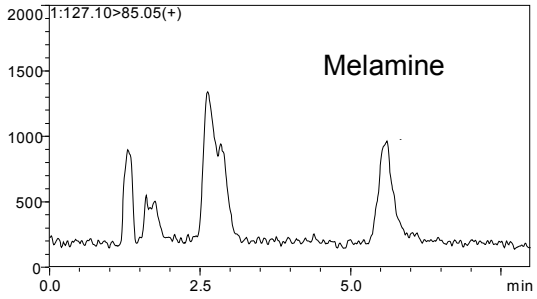

Fig.6 Chromatogram of 0.005 mg/L melamine

3. Conclusion

A method that met the requirements stipulated in China's national standard was proposed for determination of melamine in milk powder with Shimadzu LCMS-8030 triple quadrupole mass spectrometer. The calibration curve had a correlation coefficient higher than 0.999. The %RSDs of retention time and peak areas of standard working solutions at 3 different levels were below 0.64% and 4.34%, respectively. The method's LOQ was 0.005 mg/kg and spike recovery of 0.01 mg/kg sample was 104.0%. Shimadzu LCMS-8030 triple quadrupole mass spectrometer can satisfactorily meet the requirements for determination of melamine in dairy product.