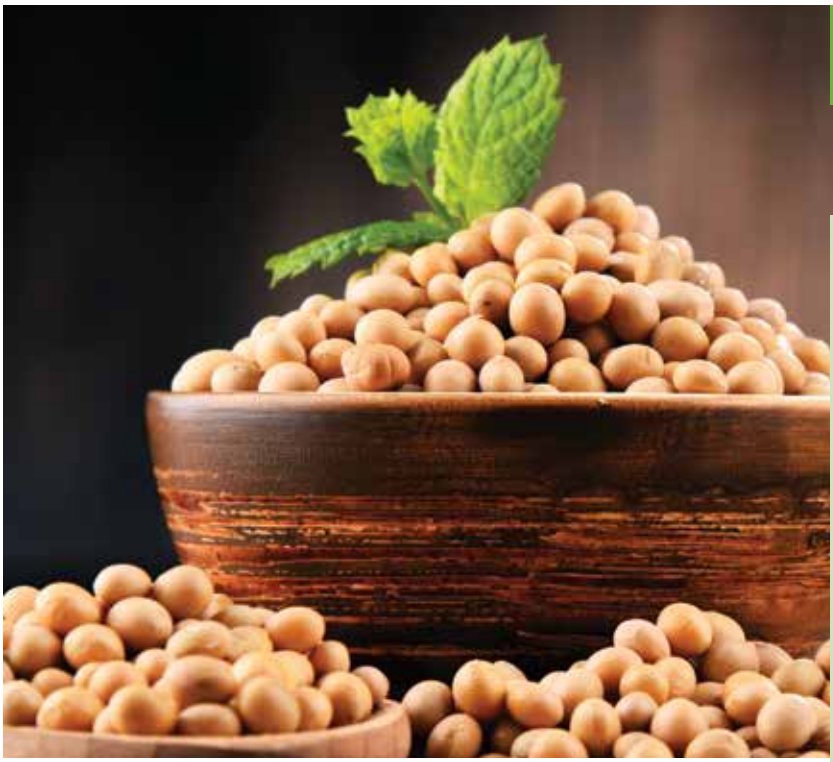

APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

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Estimation of Melamine in Soyabean meal matrix using PerkinElmer QSight® LC-MS/MS.

Introduction

Melamine is nitrogen rich organic compound, with the chemical formula $C_3 H_6 N_6$, which is normally used as an industrial chemical in plastics, glues, dinnerware, adhesive, molding compounds, coatings and flame retardants.[1,2] In milk, feed and other food product, protein content is one of the important quality parameters. Protein content in Food is invariably quantified using either the Kjeldahl wet chemistry or Dumas combustion methods.[5-10] This encourage, economically motivated adulteration in food and feed products to increase the apparent protein content. The migration of melamine used packaging material for food is other possibility of food contamination.[11,12] The intake of melamine contaminated food has been linked to kidney stones and other health problems.[11,12] The FSSAI implemented regulation on the levels of melamine permissible in powdered infant formula (1.0 mg/kg), liquid infant formula (0.15mg/kg) , other foods (2.5 mg/kg).[13]

To analyze these levels, chromatography techniques is specific and selective for quantification of melamine.[14-16] This application describes a specific, selective, robust LC-MS/MS method for the low ppb detection of melamine in soyabean meal.

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Experimental

The Chromatographic separation was done by a Perkin-Elmer LX-50 UHPLC System and detection was achieved using a PerkinElmer QSight® 220 triple quadrupole mass spectrometer, equipped with both ESI and APCI ionization sources. All instrument control, data acquisition and data processing were performed using the Simplicity 3Q™ software. All reagent and solvents were use of LC-MS grade.

Method Parameters

- Mobile phase A: Acetonitrile
- Mobile phase B: 20 mm Ammonium Acetate + 0.2 % acetic acid
- Column: Bronwlee SPP HILIC, 2.1 mm x 100 mm, 2.7
- µm (P/N N9308438)
- Injection volume: 5 µL
- Column temperature: 40°C.
- Flow: 0.4 ml/min

Table 1 - LC gradient program

Time (min)	A (%)	B (%)
0	97	3
0.1	97	10
4.5	20	80
5.0	3	97
6	97	3
7	97	3

Table 2 - MS parameters

ESI Voltage (Positive)	5500 V
Drying Gas	150
Nebulizer Gas	200
Source Temperature	350 °C
HSID Temperature	200 °C

Table 3 - MRM Transitions and Retention time of analytes:

Analyte Name	Q1 Mass	Q2 Mass	Dwell Time	Resolution	EV	CCL2	CC
Melamine_1	127	60	100	Unit	36	-44	-27
Melamine_2	127	85	100	Unit	36	-39	-23
Melamine_3	127	68	100	Unit	36	-45	-40

Standard Preparation

- Stock Solution (1 mg/ml): Weighed about 10 mg of melamine standard in 10 ml volumetric flask. Dissolved standard in minimum quantity of water and diluted to 10 ml by water.
- Preparation of Linear dilutions: For linearity study, serially diluted stock solution to prepare different concentration level using diluent acetonitrile-water (9:1 (v/v).

Extraction Protocol

- 2.0 g homogenized soymeal sample was weighed in 50 ml centrifuge tube.
- Added 10 ml acetonitrile: water (1: v/v) to the sample.
- Resulting solution was shaken/vortexed for 10 minutes.
- Then solution centrifuged for 10 minutes at 8500 rpm at room temperature.
- Transfered 0.1 ml of the aliquot in an eppendorf tube containing 25 mg of PSA and 0.9 ml of acetonitrile.
- Sample tube vortex, centrifuged at 10000 rpm for 5 minutes at 4° C.
- Allowed to settle solution and injected 5 ul of upper layer.

Results and Discussion

Melamine was analysed using a normal phase gradient on a hydrophilic interaction liquid chromatography column (HILIC). Limit of detection (LOD) for melamine standard obtained at 1 µg/kg. Linearity study was carried out over a range of 1 to 100 ug/kg. Calibration curve of melamine (MRM transition 127/85) obtained with coefficients of regression, R2= 0.9989, shown in figure-1. Reproducibility and accuracy of the developed method was studied by injecting 5 replicate injections and spiking at 50 ug/kg concentration level. The accuracy at 50 ug/kg level using 5 replicate injections observed well within acceptance criteria (%RSD = 4.23), which is shown in figure-2.

Figure 1-Melamine Standard linearity curve from 1 to 100 ug/kg

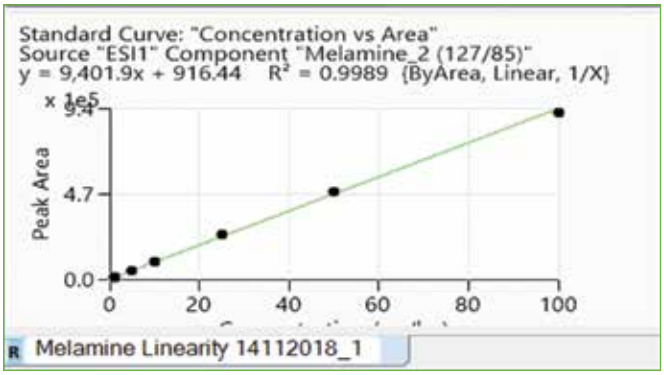
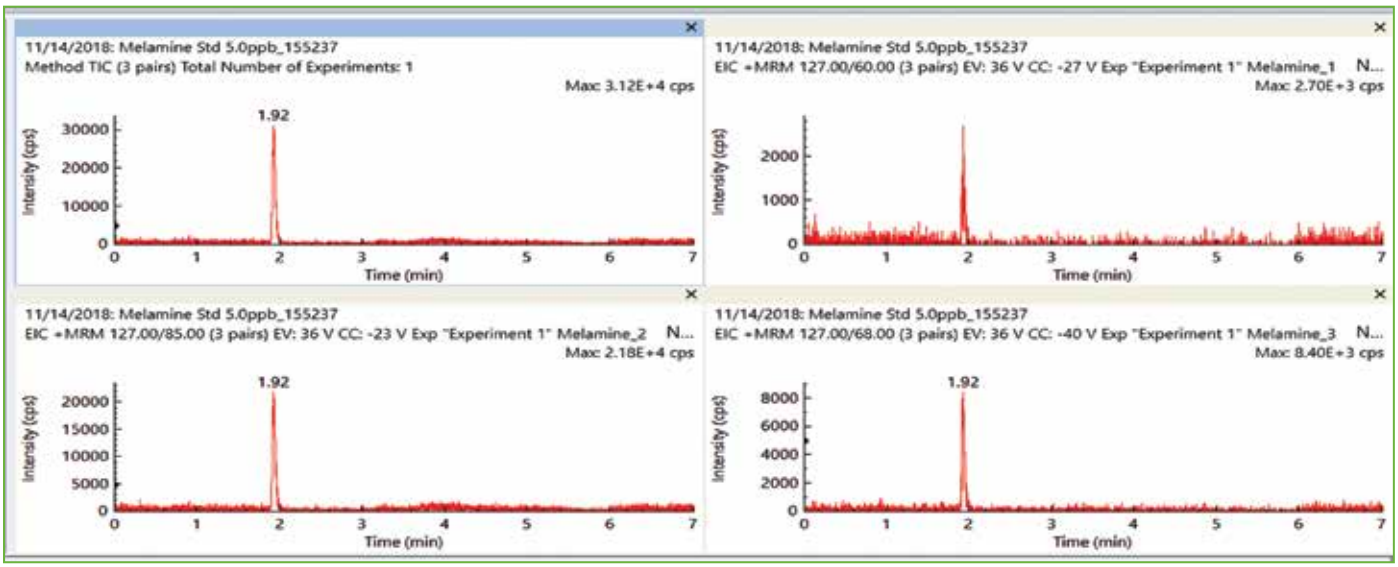


Figure 2- Melamine % RSD and % recovery calculation in soymeal at 50 ug/kg

Name	Spike_50 µg/kg						Average % recovery	% Recovery	STD DEV	% RSD
	Spk_01	Spk_02	Spk_03	Spk_04	Spk_05	Spk_06				
Melamine	50.86	46.46	46.75	48.25	47.74	44.18	47.37	81.741	2.02	4.23

Figure 3- TIC and Chromatograms



- Simple method developed for soymeal to meet FSSAI regulation at 50 time lower than MRL.
- Lowest detection for melamine in soymeal 50 ug/kg.
- Standard linearity range (1 ppb -100 ppb) > 0.99
- Reproducibility check at 50 ppb spiked level- < RSD 10%
- Recovery of melamine obtained between 80% to 110%

Conclusion

The simple sample preparation method was developed without use of QuEChERS. The analysis results demonstrate that melamine in soyabean meal products can successfully be analyzed by QSight® LC-MS/MS well below the limits required by FSSAI regulation.

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