

## Determination of Melamine Residue in Water Samples by UPLC-MS/MS

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### Abstract

In this application note we determine the melamine contamination of water samples by UPLC-MS/MS.

### Benefits

The analyses can be fulfilled in 1.0 min with a recovery of  $101.8 \pm 10.7\%$  and a precision of 3.2%. With the range covering three magnitudes, the linear coefficient is greater than 0.99 and the detective limit is up to 0.4 ng/L.

### Introduction

The melamine contamination of milk that occurred in 2008 was undoubtedly a serious food safety situation in China as safe drinking water is an important part of food safety. Since both the drinking water and the food chain may cause a direct or indirect impact on human health, it is urgent to carry out the study on the analysis method of melamine residue in water samples.

### Experimental

### Equipment and Reagents

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An Ultra Performance Liquid Chromatography/tandem mass spectrometry (ACQUITY/Quattro Premier); MassLynx 4.1 workstation; solid-phase extraction (Zymark); Waters Oasis MCX SPE column (200 mg, 6 mL); pressure blowing concentrator (Organomation).

Methanol and acetonitrile are both in liquid chromatography pure (Fisher); water is obtained from a Millipore water purification system; ammonium acetate is in guaranteed grade (Merck), hydrochloric acid and ammonia are in analytical pure (purchased from East China Pharmaceutical Company); and the standard reference is purchased from J&K.

## Sample of the Pretreatment Methods

Solid-phase extraction: Add 5 mL of methanol and 5 mL water, at the speed of 5 mL/min to the column to make it active. Acidification of water sample: Add 0.25 ml of concentrated hydrochloric acid to 500 ml of water. Add the water sample with the speed of 4 mL/min and the volume of 500 ml. Rinse the sample with 5 mL of water and methanol respectively. Elute with 5 mL of methanol (containing 5% of ammonia) twice and concentrate the eluent with a pressure blowing concentrator until it is almost dried up. Dilute to 1 mL with mobile phase and filter it. Inject.

## Chromatographic Conditions

UPLC-MS method: column: UPLC column (ACQUITY UPLC BEH HILIC, 1.7  $\mu$ m, 2.1 50 mm); column temperature: 40 °C; mobile phase: a mixture of acetonitrile and water (containing 10 mM of ammonium acetate) (90:10); flow speed: 0.4 ml/min.

The target contaminator is quantitatively analyzed with the MRM mode. Detect the target with ESI<sup>+</sup>. Capillary voltage: 3.0 KV, source temperature: 120 °C, the temperature of desolvent gas: 400 °C, flow speed: 800 L/h, cone gas flow: 50 L/h; argon flow speed: 0.38 mL/min at the MRM mode; cone current: 40 V, CID: 17; characteristic ions pair: 127>85, as in the following Figure 1.

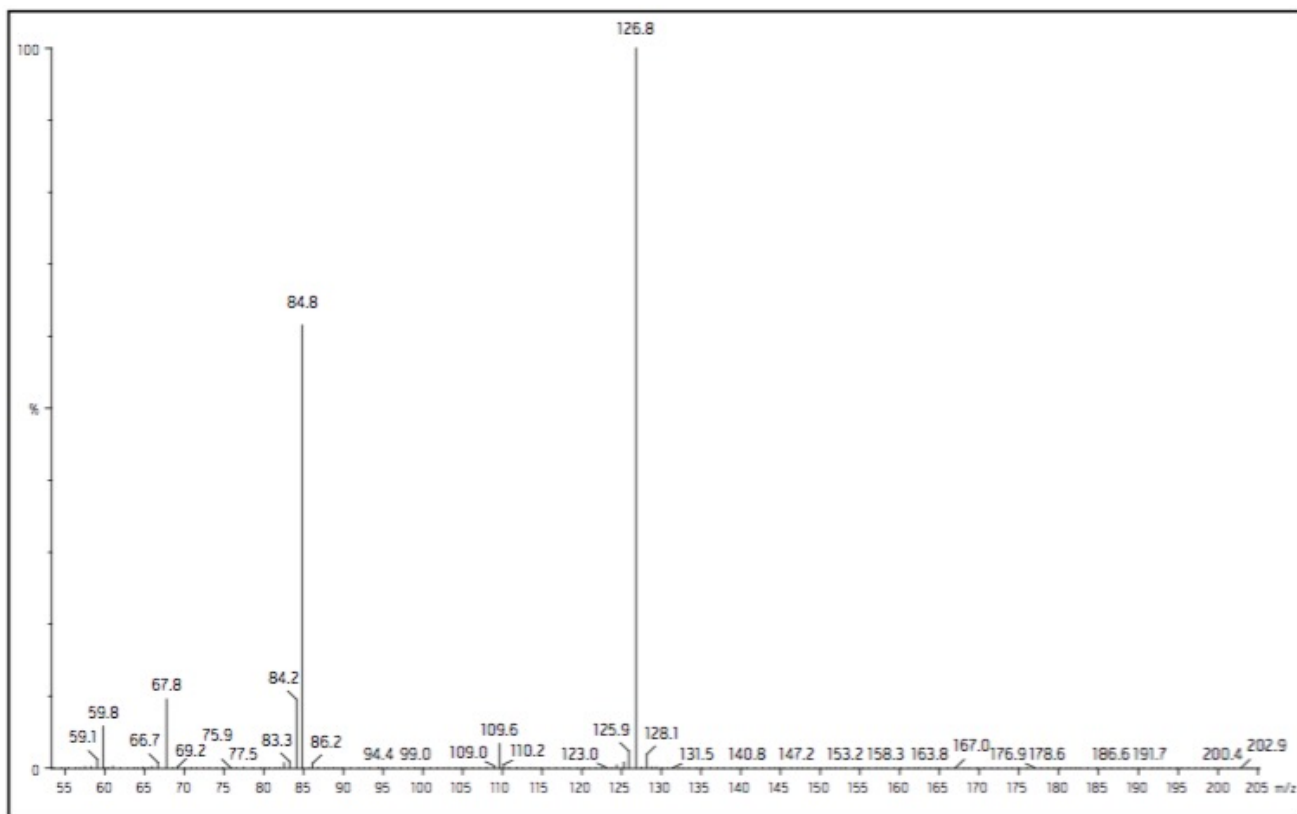


Figure 1. Mass spectra of melamine.

## Results and Discussion

### Accuracy

Add 6.0 µg of target substance to 500 ml of testing water, and the recovery of a series of pre-sampling is  $101.8 \pm 10.7\%$  ( $n=6$ ), as showed in Table 1.

Analytes	Precision %( $n=6$ )	Recover% ( $n=6$ )	Detective limit (ng/L)	Linearity
Melamine	3.2	$101.8 \pm 10.7$	0.4	$y = 1350.3x + 161822$ , $r=0.9925$

Table 1. Performance of this method.

### Precision

Inject 1.0 µg/ml of standard solution for 6 times repeatedly and the RSD of peak area is 3.2%.

## Detective limit

The signal/noise ratio is 15 for 1.0 ng/mL of standard solution and 3 for 0.2 ng/mL of standard solution, as shown in Figure 2. Taking the pre-sampling procedure into consideration, the quantitative detective limit of this method is 0.4 ng/L.

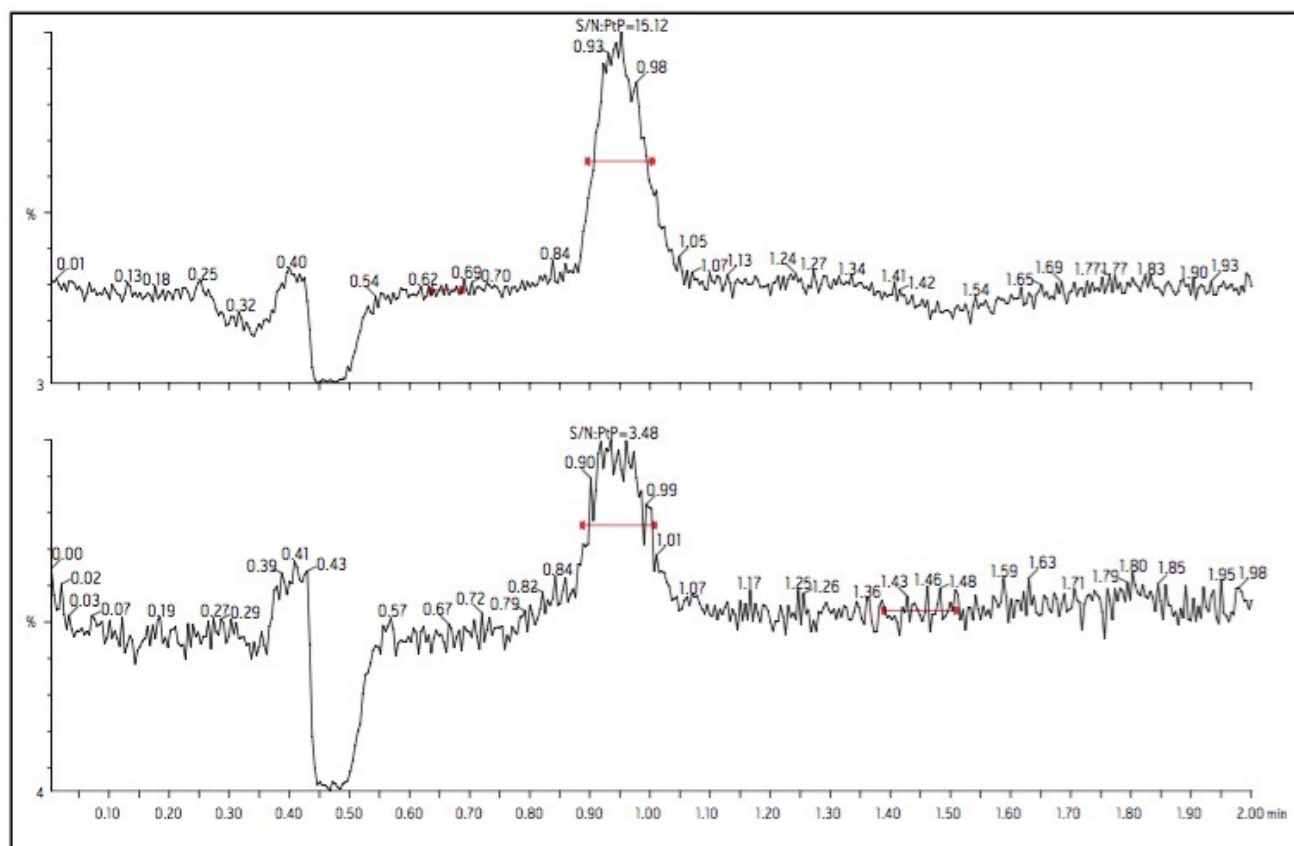


Figure 2. The S/N of melamine in different concentration.

## Linearity

Prepare 4 standard solutions with different concentrations ranging from 1.2 ng/mL to 2400 ng/mL, the equation for the standard curve is  $y=1350.3x+161822$ ,  $R=0.9925$ .

## Blank

The blank is lower than the detective limit.

## Determination of sample

The water sample is obtained from a reservoir in Zhejiang province with a concentration of melamine of  $<0.4$

ng/L. Add some standard solution to this water sample and analyze the content of melamine. The concentration of melamine is 0.03 µg/L and the total ion flow is showed in Figure 3.

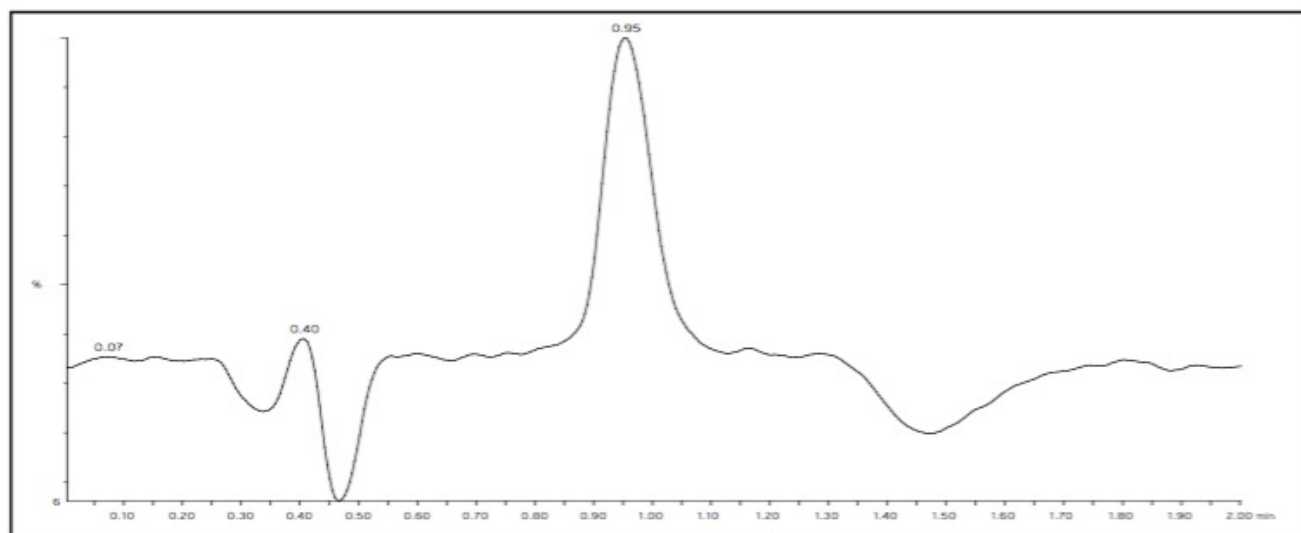


Figure 3. TIC of testing sample.

## Conclusion

The UPLC-MS/MS method used for the determination of melamine residue in water sample has been proved to be practicable. The analyses can be fulfilled in 1.0 min with a recovery of  $101.8 \pm 10.7\%$  and a precision of 3.2%. With the range covering three magnitudes, the linear coefficient is greater than 0.99 and the detective limit is up to 0.4 ng/L. All of these indicated that this method meets the requirement of practical analyses.

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