

## Effect of time and temperature on migration of melamine from melamine-ware products to foods

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**Accepted for publication:** 1 Dec 2017

### Abstract

**Background:** Melamine is an organic base material whose resin is used to prepare the food-related wares. The initial migration of melamine from tableware to food can be for the remained monomers, but the secondary migration is an important concern for breakage and damage of polymers. Previous studies showed that acidity, temperature, and time have effects on melamine migration. The present study was conducted to measure melamine migration from melamine-wares to food and to investigate the effects of temperature and time on migration using HPLC method.

**Methods:** Melamine-wares were purchased according to Iran National Standard guidelines. Four various tests were designed to examine the effects of time and temperature on melamine migration to the Acetic acid 3% as a food simulant. Exposures were done at temperatures 30 and 90 °C for 30 and 90 minutes. Migration was determined using HPLC method.

**Results:** In all samples, migration occurred but it was lower than the Specific Migration Limit (SML). Melamine was restricted by SML of 30 mg/kg (European Union standard). Findings indicated that the temperature and time had significant effects on migration. Temperature had specially a direct relationship with melamine migration.

**Conclusion:** Findings of the present study indicated that the independent variables, including temperature and time, had significant effects on migration, so precautions should be considered when using melamine wares for hot and acidic foods.

**Keywords:** Acetic Acid; HPLC; Melamine; Migration; Polymers

**Cite this article as:** Haghi E, Shakoori A, Alimohammadi M, Sadighara P. Effect of time and temperature on migration of melamine from melamine-ware products to foods. *SDH*. 2017;3(4):201-207. DOI: <http://dx.doi.org/10.22037/sdh.v3i4.19680>

### Introduction

Melamine is a chemical organic basis material that is usually found in white crystals rich of nitrogen (1). Melamine has a 5, 3-1 triazine framework. It is a trimer cyanamide 67% of whose mass is made of nitrogen like cyanamide (2, 3). Thermoset melamine resins are frequently used in plastics industries, textile cloths,

flooring, tableware manufacturing, etc. (3). Using melamine and its resin is very extensive in production of plastic wastes for its good resistance against high temperature, and using melamine-wares has been one of the most popular confrontation of human with them (4, 5).

Melamine is added to animals and even human foods to increase protein percentage (6); however, melamine is not confirmed by FAO/WHO Codex Alimentarius or national authorities as an additive to food products (6) and is described as a danger by swallowing, inhalation, or absorption through the skin. Chronic exposure can endanger health and cause liver damages, or probably cancer (1, 3). This materials casue food toxicity in low concentrations, but causes renal diseases and even death in infants and children in high concentrtrions. Melamine as a pollutant of various foods can make cyanoric acid complex in insoluble form to change urine pH that can crystalize and damage tissues such as bladder and causes melamine related kidney stones, and ultimately urinary blockage due to blockage of the urethra (7). There is no specific law in European Union (EU) for using melamine in non-plastic mateirals. Though, as melamine is the raw material of producing plastic products to serve food, specific migration limit (SML) was considered as an standard. SML for melamine was approved 30mg/kg by EU plastic unit that the derived SML from the toelrable daily amount for human and is for per kilogram of body weight (5, 8).

The initial migration of melamine from tableware such as plate and bowl to food can be for the remained monomers, but the secondary migration is an important concern for breakage and damage of polymers (9). Acidity and temperature effects on melamine was studied and it was found that melamine migration increases by increasing the temperature in solutions containing acetic acid that the effect of acetic acid containing solution has been more than that of water in terms of melamine precipitation (10, 11).

Since melamine is used in construction of the wares and dishes related to food, gastrointestinal tract can be considered as an important means of human exposure, and the bigger concern is that these containers are widely available for use by children in the market (11).

Thus, since using melamine in food-wares in contact with food products has extensively been used to preserve, transport, and serve foods in lower social classes, migration of melamine to food products is proposed as a dangerous and important issue in the safety and health of food products. The necessity of measuring melamine migration from food-wares is felt so as to act more consciously to control this danger in foods. The current study was conducted to measure the migration of melamine from melamine-ware products to 3% acetic acid, as a food simulant, and to study the effects of time and temperature on migration.

## Methods

### *Preparation of sample*

First, four brands of melamine-wares, prepared according to Iran National Standard guidelines, were selected to prepare the sample which had the maximum sale in market and consequently the highest consumption. Four various tests were designed to examine the effects of time and temperature on melamine migration to the food simulant. Therefore, 16 unique samples were prepared for injection and were thus analyzed using this device.

$$4 \times 4 = 16$$

4 = number of melamine-wares

4 = various tests

First, wares were fully washed using distilled water to prepare sample, then they were fully dried in 30°C and were filled up to 1 cm below bowl edge using food simulant solutions that were heated up to the previously mentioned temperature. Next, they were put in the oven in order to maintain the desired temperature of the food simulant based on the required time (30-90 min). Then, 1 ml sample was transferred to the test tube on the next preparation step using sampler, and then 9 ml acetonitrile was added to it and was fully dried using gas flow in 25°C. After full drying of samples, 1 ml of mobile phase

was added to HPLC device including 30% water + 70% ethanol. They were fully shaken for 2 min and then put under ultrasonic device. After that, they were transferred to the specific vials and prepared for injection to the device. These steps were equally done for all samples.

#### *Materials*

Melamine standard (Sigma-Germany Lot number: 1422105v -)

Acetonitrile HPLC Grid (Merck-Germany)

Deionized water quality HPLC

Acetic acid 3% HPLC Grid (Merck, Germany)

All are suppliers.

HPLC machine (American Agilent Technologies)

Ultrasonic devices (Elma- Germany)

#### *Methodology*

Modified CEN (CEN 2005b) method was used in the current study. The used HPLC device has detector of 1260 Infinity II Diode Array Detector (DAD) and column (XDB-C18 4.6X250mm 5-micro). Particle size was 5  $\mu\text{m}$ , injection volume was 100  $\mu\text{l}$ , mobile phase was 30% water+70% ethanol, and flow was 1 ml/min. Adsorption of 220 nm was used to determine melamine (11).

#### *Preparation of solutions*

First, 50 mg standard melamine was poured in 500 ml volumetric balloon, and 40 ml distilled water was added. Balloon was put in ultrasonic water bath in 70°C for 15-30 min to solve the melamine. The solution was cooled in room temperature, then the volumetric balloon was filled with distilled water to the marked line, and then 12 different concentrations (25-1000000 ngr/ml) were prepared from the standard melamine.

Moreover, deionized distilled water and grid HPLC ethanol was used to prepare mobile phase to inject in HPLC. Their ratio in mobile phase was 30% distilled water and 70% ethanol.

#### *Drawing calibration curve*

First, a pilot injection with a 30-minute injection time was made to determine the

retention time, then 6 different concentrations (500-1000-2000-2500-5000-10000 ng/ml) of melamine standard solution were injected to the device (in three repetitions), and calibration curve was drawn.

#### *Validation Method*

The offered protocol in the international conference of harmonized procedures for validating analytical procedures, which includes the Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, precision, and accuracy, was used to validate the method (12).

Three replications on a day, repeated for three consecutive days, were used to check the precision and accuracy. To do so, three concentrations of 1000, 2500, and 10000 ng/ml were selected from melamine stock solutions, and then 1 ml of each concentration was transferred to the 3 test tubes. Then, 9 ml of acetonitrile was transferred to each of the 9 test tubes and were shaken for 2 min, and were dried using nitrogen flow in 35°C. Then, 1 ml of mobile phase (30% distilled water+70% ethanol) was added and shaken for 2 min, and then was exposed to ultrasonic waves for 3 min and again shaken for 2 min. Finally, it was injected to the device to be read. In this protocol, that is protocol of preparing samples to inject to HPLC device, less than 20% standard deviation must be considered to provide the acceptable precision.

#### *Injection to the HPLC device*

Samples were injected in to the device based on the mentioned method. The time considered for each injection was 6 min, considering the 3 min retention time.

Method specifications are as following:

Injection volume: 100  $\mu\text{l}$

Wavelength: 220 nm

Mobile phase: 30% water+70% ethanol

Injection time: 6 minutes

#### *Data analysis*

Data was analyzed using IBM SPSS Statistics for Windows, Version 21.0. and Microsoft Excel 2013. Analysis of variance (ANOVA) was used to analyze data.

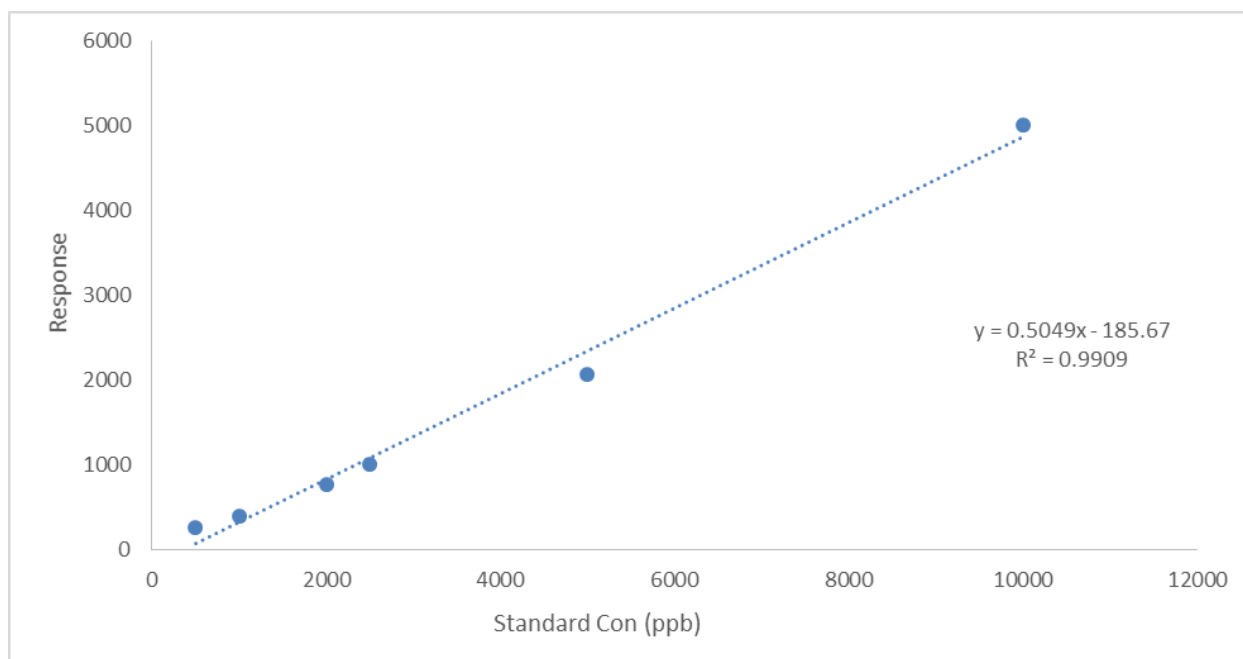


Figure 1 Calibration curve of six different concentrations of standard melamine solution

## Results

### *Calibration curve*

Six different concentrations (500, 1000, 2000, 2500, 5000, and 10000 ng/ml) from standard melamine solution were injected to device in deionized distilled water to draw calibration curve (Figure 1).

### *Method validation*

The LOD detection limit and LOQ ML in quantitative measurement of melamine using HPLC was obtained by calculating the ratio of signal to noise by which LOD ML was 145 Parts per billion (ppb), and LOQ ML was 435 ppb.

Moreover, since the acceptable correlation coefficient to confirm the linearity of this relationship was bigger than 0.99, analysis of the relationship between concentration and response in six various concentrations from standard melamine solution showed correlation coefficient of  $R^2=0.9909$ , and a

linear relationship was obtained between concentration and response (Table 1).

Repetitions on a single day (three repetitions) and repetitions on three consecutive days were used to examine the accuracy and precision, and then the results were recorded after preparation of samples and injection to the device. Later, accuracy, precision and relative standard deviation (RSD) were calculated whose results are shown in table 1. RSD was found to be acceptable (RSD<20%).

### *Measuring samples migration*

Four melamine samples were taken on which four various tests were conducted to examine the effects of time and temperature on migration of melamine from such wares to food simulant products. In this regard, samples and various tests were coded as following (Table 3).

Table 1. Validation result of six different concentrations of standard melamine solution

Parameter	Result
Retention Time	3.2 min
Accuracy (% Recovery)	%94.9
Precision	%95.3
Slope	0.5049
Intercept	-185.67
Linearity range (ng/ml)	500 – 10000
Standard equation regression	$y=0.5049x-185.67$
Correlation Coefficient	$R^2=0.9909$
LOD (ng/ml)	145
LOQ (ng/ml)	435

Table 2. Test coding

Test code	Test conditions
1	Acetic acid (3%) 30 °C - 30 min
2	Acetic acid (3%) 30 °C - 90 min
3	Acetic acid (3%) 90 °C - 30 min
4	Acetic acid (3%) 90 °C - 90 min

Tables 3. Melamine Migration results (ppb) (average of triplicate specimens)

	Sample 1	Sample 2	Sample 3	Sample 4
Test 1	1061.0262	1504.7161	3002.1694	4210.432
Test 2	2142.5203	1223.4484	4382.758	2861.5356
Test 3	2477.2684	3121.0149	5969.3454	504.43313
Test 4	3224.0143	5648.4626	6813.1485	6323.9012

## Discussion

Melamine was proved to migrate into food stimulant from all samples. None of the samples exceeded the SML, i.e. 30 mg/kg (EU standard for melamine migration). Our findings indicated that the independent variables including temperature and time had significant effects on migration. In addition,  $R^2$  coefficient (96.4%) verified these results.

Furthermore, studying the results related to each variable individually showed that temperature was an important factor in migration of melamine to food simulants. This effect was direct so that increasing temperature from 30 to 90°C while the other variables were constant caused

melamine migration in 97% cases.  $P$  value at the level of 0.04 showed this effect was significant hence temperature can be considered as an important and principal factor on melamine migration from food-wares. As for the time variable, in 86% of samples, increasing time, when the other variables were constant, promoted migration from melamine-ware to food simulants.

Chao-Yi Chien et al. measured melamine migration from different material-made tableware using LC-MS/MS device. Test samples were filled with pre-warmed distilled water or 3% acetic acid as the simulant and the temperatures were from 20°C to 90°C for 15 or 30 minutes.

They found high melamine migration levels from all melamine-made samples containing 3% acetic acid in water bath of 90°C for 30 min, whereas melamine was detectable in none of other material-made samples in the same condition (13). Moreover, Chik et al. measured melamine migration from 41 samples of food serving dishes to food simulant. The samples were exposed to two types of food simulants (3% acetic acid and distilled water) at three test conditions (25°C (room temperature), 70 and 100°C) for 30 min. They used LC-MS/MS device. This study suggested that excessive heat and acidity may directly affect melamine migration from melamine-ware products. The melamine migration was observed in all samples less than SML (10). In another investigation, Bradley et al. studied migration of melamine and formaldehyde from melamine food contact articles using LC-MS devices. The test conditions were exposure to the simulant for 2 hours at 70°C. The food simulant was 3% aqueous acetic acid; melamine migration occurred in 43 out of 50 samples, which were less than standard migration value (5).

Lund et al. measured the melamine migration from 10 samples of melamine-ware utensils (such as bowl, jug, mug, ladle, and different cups and plates). The samples were exposed to the food stimulant 3% acetic acid for 2 h at 70°C. They used UV-HPLC method and could separate melamine from 7 of 10 samples: the migration was less than the standard value (14). Bradley et al. compared the migration of melamine from melamine-formaldehyde plastics (melaware) into various food simulants and foods themselves. The test conditions used were repeated exposure to the simulant for 2 hours at 70 °C. They used HPLC device and observed the very strong influence of time and especially temperature on melamine migration in exposure of microwave heating of food simulants or beverage in the melaware articles (15). Also, LU et al. examined the

melamine migration from dairy products package in 37 samples. The simulating solutions were distilled water, 3% acetic acid, n-hexane, and 15% ethanol. The HPLC device was used and low level of melamine was found in 3 out of the 6 melamine resin containers (16).

All these studies were consistent with our findings and showed that migration occurred from food wares to food simulants and temperature, time, and acidity were effective in this process. Acid can resolve melamine well and temperature and long time would help this action.

Although, melamine migration occurred less than EU standard in all samples (SML=30mg/kg), the results clearly indicated that there is a chance of human exposure to melamine through migration from food contact materials, so precautions should be considered when choosing dishes, because long term usage of these dishes, particularly to serve hot and acidic foods, can make defects by melamine toxicity and endanger public health. These type of dishes are used particularly by lower social classes and children who are sensitive to toxicity by melamine, which calls for more attention of public health authorities to promote social awareness in the selection of utensil and food dishes specifically for children.

#### *Conflict of interest*

Authors declare no conflict of interests.

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