

ORIGINAL ARTICLE

Measurement of melamine migration from melamine-ware products by designed HPLC method and the effect of food-type on the level of migration

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ABSTRACT

Melamine-ware is widely used around the world. There is a public health concern as regards the safety of melamine when exposed to food. This study was carried out to measure the level of melamine migration in melamine-ware products by HPLC method and the effect of food-type on the level of melamine migration. In food control laboratories in Iran, there is no common method to measure and monitor melamine migration, hence a method using HPLC technique was adopted and validated to solve this problem. The validation results showed the reliability with 94.9% accuracy and 95.3% precision. Furthermore, the limit of detection (LOD) and quantification (LOQ) were 0.145 and 0.435 µg/ml, which for a new method were within acceptable ranges. Melamine migrations from 4 most available melamine wares were measured. Distilled water, 3% acetic acid and 15% ethanol were used as food simulant at 30 °C for 90 min. Although melamine migration occurred in all samples and acidic conditions had a significant effect, the values were not higher than the European standard (30 µg/ml). The study revealed that the HPLC method was valid and could be applied and developed to measure melamine migration. However, precautions should be considered while choosing melamine-ware utensil as long-term exposure to this substance has a negative effect on health, especially on the kidneys.

KEY WORDS: melamine; migration; HPLC; food type

Introduction

Melamine, also known as tripolycyanamide or 2,4,6-triamino-1,3,5-triazine, is an industrial material used for the production of melamine resins (RAS, 2010; Johannes, 2005). It is generally used in the production of resins due to its durable thermosetting characteristic (Lokensgard & Richardson, 2003). Melamine ware is widely used around the world because of the toughness, heat resistance and low cost (RAS, 2010). It can decompose under high temperature; furthermore, factors such as temperature, acidity and frequent use significantly affect the level of melamine migration. This has raised concerns on the use

of melamine in packaging materials and utensils (Jacaab, 2007). The main safety concern regarding melamine-tableware is its possible migration into food (RAS, 2010) Direct contact with melamine can cause skin, eye and respiratory tract problems while (Jeong *et al.*, 2006) long-term exposure decreases fertility and high levels result in kidney damage (RAS, 2010; Kai-ching Hau *et al.*, 2009).

The No Observable Adverse Effects Level (NOAEL) of melamine was determined to be 63 mg/kg body weight/day, as to a 13-week toxicity assay by the National Toxicology Program (2009). The World Health Organization adopted a Take Daily Intake (TDI) of 0.2 mg/kg body weight/day. Few studies have been done to measure melamine migration and revealed that although the melamine-ware tested had low levels of migration, long term exposure can cause health problems (Kai-ching Hau *et al.*, 2009; WHO, 2009 Chik *et al.*, 2011) This study was carried out to measure the level of melamine migration in melamine-ware products using HPLC method and the effect of food-type on the level of migration.

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Materials and methods

Materials and devices

Four samples of Iranian melamine-ware products were purchased from the market. In this study, 3% acetic acid, distilled water and 15% ethanol were used as food simulants under test conditions of 90°C for 30 min. Other materials used for the study were: Melamine Standard (99.0% purity) (Sigma-Germany Lot number: 1422105v), acetonitrile HPLC grid (Merck-Germany), deionized water HPLC quality, 3% acetic acid HPLC Grid (Merck, Germany). HPLC machine (American Agilent Technologies) and ultrasonic devices (Elma- Germany), Diode-array detector (DAD) with XBD-C18 column (4.6 × 250 mm 5-micro) was used to measure melamine migration. The size of particles was 5 µm, injection volume was 100 µl, mobile phase was 30% water + 70% ethanol, and the flow rate was 1 ml per minute. Melamine was determined at 220 nm wavelength (Chien *et al.*, 2011)

Calibration curve

The calibration curve with a range from 0.5 to 10 µg/ml was prepared in deionized distilled water. To determine retention time, injection was done at 30 min as pilot. Then 6 different concentrations of melamine standard solution (0.5, 1, 2, 2.5, 5 and 10 µg/ml) were injected into the HPLC column (in three replicates). The calibration curve was obtained by plotting area ratios of melamine standard against solvent concentration using Microsoft Excel 2013 software. The flow rate was set at 1 ml/min with injection volume of 20 µl.

For validating analytical procedures, the recommended protocol in the international conference of harmonized procedures which includes the limit of detection (LOD), limit of quantification (LOQ), linearity, precision, and accuracy was used (CEC, 1996).

The 3 replications in a day on 3 consecutive days were used to check for precision and accuracy. Three concentrations of 1, 2.5, and 10 µg/ml of stock solutions were prepared from melamine. Then 1 ml of each concentration was transferred to 3 test tubes and 9 ml of acetonitrile was then added to each of the 9 test tubes, shaken for 2 min, and dried with nitrogen at 35°C. 1 ml of mobile phase (30% distilled water + 70% ethanol) was added to the mixture and shaken for 2 min. The resultant mixture was then exposed to ultrasonic waves for 3 min and again shaken for 2 min. Finally, the mixture was injected into the HPLC column for analysis. For this method standard deviation must be <20% to accept its precision.

Sample preparation

Four Iranian standard brands of melamine-wares were purchased from the market. These brands were well known and commonly available. Three different tests were designed to examine the effect of acidity on melamine migration to food simulant. Therefore, 16 unique samples were prepared for injection and analysis in the HPLC column. 4×3=12 (4= number of melamine-wares; 3= condition tests).

Melamine wares were washed with distilled water, and dried in an air oven at 30°C. The food-simulating solvent was preheated to 90°C and poured to 1 cm below the upper edge of the wares. The samples were then placed in an oven to maintain the desired temperature of the food simulant for 30 minutes. Then, 1 ml of the sample was transferred to a test tube to which 9 ml acetonitrile was added; the mixture was dried by gas flow at 25°C. After this, 1 ml HPLC mobile phase (30% water + 70% ethanol) was added to the sample and thoroughly shaken for 2 min and then placed in an ultrasonic device for 2 min. The mixtures were then transferred to vials and prepared for injection.

Injection to the HPLC

The injections were performed according to the HPLC manufacturer's instructions. The flow rate was set at 6 min based on 3 min retention time. The other specifications were: 100 µl injection volume, 220 nm wavelength, mobile phase of 30% water + 70% ethanol and 6 min injection time.

Data analysis

SPSS (v.21) and Excel 2013 were used for data analysis. ANOVA test was also carried out.

Results

To obtain the calibration curve, the ratios of 6 different melamine standard concentrations (0.5, 1, 2, 2.5, 5 and 10 µg/ml) diluted with deionized distilled water were plotted against responses of the HPLC detector. The results are shown in Figure 1.

Standard chromatogram of melamine and chromatogram of samples are shown in Figures 2 and 3.

Validation of method

The LOD and LOQ of HPLC method were 0.145 and 0.435 ppm, respectively. A signal-to-noise ratio (S/N) was used to validate the LOD and LOQ. Correlation coefficient (R²) between concentration and response time was 0.9909, thus showing a linear relationship between the variables. Relative standard deviation was <20%, which was acceptable. Validation results are in Table 1.

Migration test

Quantification of melamine migration was conducted as previously described (see sample preparation section). The results of mean melamine migration (average of 3 repetitions) and significance comparison of the type of material (distilled water, acid and ethanol) and samples are shown in Tables 2 and 3.

Discussion

Since there are no common and routine methods to measure melamine migration from melamine-ware in food

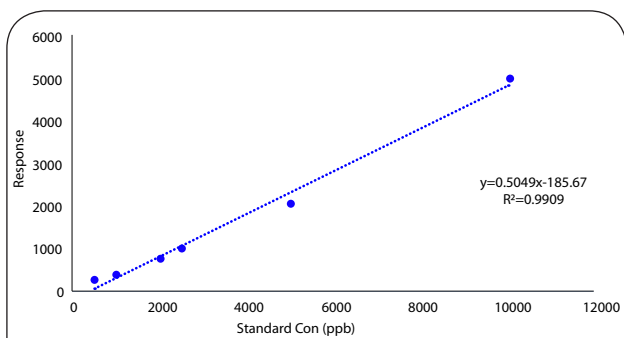


Figure 1. HPLC calibration curve.

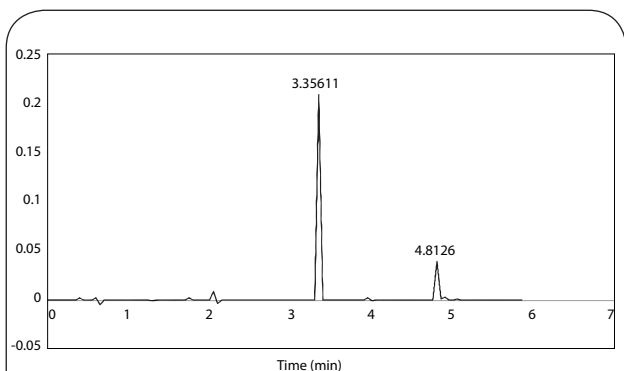


Figure 2. Standard chromatogram of melamine.

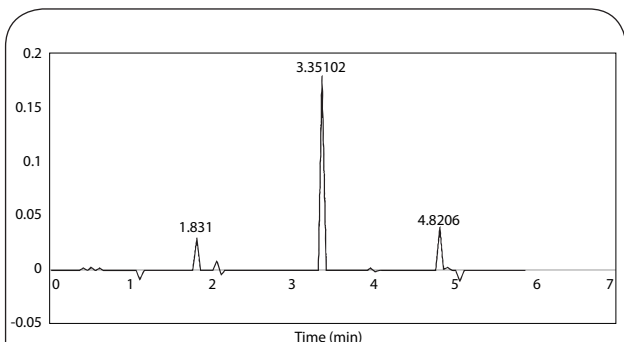


Figure 3. Separation chromatogram of samples.

Table 1. Validation results.

Parameter	Result
Retention Time	3.2 min
Accuracy (% Recovery)	94.9 %
Precision	95.3%
Slope	0.5049
Intercept	-185.67
Linearity range (µg/ml)	0.5–10
Standard equation regression	$y = 0.5049x - 185.67$
Correlation Coefficient	$R^2 = 0.9909$
LOD (µg/ml)	0.145
LOQ (µg/ml)	0.435

Table 2. Mean melamine migration of samples (average of 3 repetitions).

Sample	Test	Mean Result ± SD (ppm)	p-value
Sample 1	Water 30°C – 90 min	2.14252 ± 0.00057735	<0.001
	Acetic acid (3%) 30°C – 90 min	2.84767 ± 0.000416333	
	Etanol (15%) 30°C – 90 min	5.4008 ± 0.0019615	
Sample 2	Water 30°C – 90 min	1.33437 ± 0.005445487	<0.001
	Acetic acid (3%) 30°C – 90 min	2.37823 ± 0.005795113	
	Etanol (15%) 30°C – 90 min	1.22344 ± 0.003695042	
Sample 3	Water 30°C – 90 min	2.20392 ± 0.001078579	<0.001
	Acetic acid (3%) 30°C – 90 min	4.38275 ± 0.001249	
	Etanol (15%) 30°C – 90 min	1.17591 ± 0.000493288	
Sample 4	Water 30°C – 90 min	2.86153 ± 0.003415162	<0.001
	Acetic acid (3%) 30°C – 90 min	3.70930 ± 0.02988650	
	Etanol (15%) 30°C – 90 min	1.94048 ± 0.001692139	

control laboratories in Iran, it was vital to design a valid method using HPLC to resolve this challenge. Validation results of 94.9% accuracy and 95.3% precision showed that HPLC analysis is a reliable technique to determine melamine migration and can consequently be applied and developed for use in food control laboratories in Iran. The analysis of melamine migration confirmed and indicated that the designed method for this study was significant at $p < 0.001$.

With this method, melamine migration was detected in all samples at values less than 30 mg/kg, which is

the European Union standard. The results of the study also indicated that under the same conditions (30°C and 90 min) acidity affected melamine migration, hence higher values were obtained for acidic foods. The mean differences (using ANOVA test) in melamine migration were significant among acidic, alcoholic and neutral food stimulants. The p -values were significant at levels of less than 0.001. Migration to alcoholic food simulant was less because melamine does not dissolve completely in alcohol. Our finding was consistent with studies of other authors (LU *et al.*, 2009; Chao-Yi Chien *et al.*,

Table 3. Significance comparison of the type of material (distilled water, acid and ethanol).

Brand	Temperature and time	Simulant (I)	Simulant (J)	Mean difference (I-J)	p value	95% Confidence interval for differences	
						Upper bound	Lower bound
Sample 1	30°C - 90 min	Water	Acetic acid	1.219*	0	1.430	1.008
			Ethanol	-0.468*	0	-.257	-.679
		Acetic acid (3%)	Water	1.687*	0	1.898	1.476
			Ethanol	0.468	0	.679	.257
		Ethanol (15%)	Water	-1.219*	0	-1.008	-1.430
			Acetic acid	-1.687*	0	-1.476	-1.898
Sample 2	30°C - 90 min	Water	Acetic acid	-0.368*	0	-0.156	-0.579
			Ethanol	-0.162	0.196	0.049	-0.373
		Acetic acid (3%)	Water	0.206	0.059	0.417	-0.006
			Ethanol	0.368*	0	0.579	0.156
		Ethanol (15%)	Water	-0.206	0.059	0.006	-0.417
			Acetic acid	0.162	0.196	0.373	-0.049
Sample 3	30°C - 90 min	Water	Acetic acid	0.378*	0	0.590	0.167
			Ethanol	-0.492*	0	-0.281	-0.704
		Acetic acid (3%)	Water	0.871*	0	1.082	0.660
			Ethanol	0.492*	0	0.704	0.281
		Etanol (15%)	Water	-0.871*	0	-0.660	-1.082
			Acetic acid	-0.378*	0	-0.167	-0.590
Sample 4	30°C - 90 min	Water	Acetic acid	-0.047	1.000	0.164	-0.258
			Ethanol	0.525*	0	0.736	0.314
		Acetic acid (3%)	Water	0.047	1.000	0.258	-0.164
			Ethanol	0.572*	0	0.783	0.361
		Etanol (15%)	Water	-0.572*	0	-0.361	-0.783
			Acetic acid	-0.525*	0	-0.314	-0.736

2011; Chik *et al.*, 2011). Chik et al. measured melamine migration from 41 samples of food serving dishes to food simulants. In that study, samples were exposed to two types of food simulants (3% acetic acid and distilled water) under three test conditions (25, 70 and 100 °C) for 30 min, using LC-MS/MS device. The results of their study suggested that excessive heat and acidity may directly affect melamine migration from melamine-ware products. Melamine migration observed in all samples was less than the specific migration limit (SML). In addition, Lu et al. examined melamine migration from dairy products packaged in 37 samples. The simulating solutions were distilled water, 3% acetic acid, n-hexane and 15% ethanol. It was found that the migration in 15% (v/v) ethanol aqueous solution and 3% (w/v) acetic acid aqueous solution were greater than in distilled water (Lu *et al.*, 2009). In a similar study, Chao-Yi Chien et al. measured melamine migration from different material-made tableware by LC-MS/MS device. Test samples were filled with pre-warmed distilled water and 3% acetic acid as the simulant at temperatures ranging from 20 to 90 °C for 15 to 30 min. They reported high melamine migration levels from the melamine-made samples containing 3% acetic acid in a water bath of 90 °C for 30 min, whereas melamine was not

detected in other material-made samples under the same conditions (Chao-Yi Chien *et al.*, 2011).

Conclusion

All over the world, there is growing public concerns about the safety of food contact materials. This study investigated a method to measure melamine migration from melamine-ware to food and showed that under experimental conditions acidity affected melamine migration towards higher levels but there were low levels of melamine migration (less than specific migration limit) from melamine-ware samples to simlants . However, long term exposure to melamine and its effect on public health should be considered. Therefore, precautions should be taken with regard to choosing melamine wares for exposure to food stuff.

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