Detection of Melamine Residue in Raw Milk and Milk Related Products by UV spectrophotometry

T.ANANTHAKUMAR, A.JERAD SURESH, V.NIRAIMATHI*

Department of Pharmaceutical Chemistry, College Of Pharmacy,
Madras Medical College, Chennai-600 003, India.
*Email id: vnmpg2@gmail.com
Phone No.:+91-9444563880

ABSTRACT

Melamine is a toxic triazine used as an adulterant in milk & milk related products to increase the protein content. Spectrophotometric method has been developed using 0.1M sodium hydroxide as solvent to detect the melamine residue in raw milk and milk related products. As the amount of melamine residue present in samples would be of nanogram level which is beyond detection by UV spectrophotometer, standard addition method was adopted. The melamine was extracted by precipitation of milk protein at its isoelectric point with dilute acids. Samples were purified by using membrane filter of 0.45µm pore size, a known concentration of melamine standard solution was added to this filtrate and the absorbance was measured spectrophotometrically. The linearity curve was constructed for the concentration of melamine ranging from 3 - 8µg/ml.The correlation co-efficient was found to be 0.9926. In this study 31 milk samples (raw milk and milk related product) were analysed by standard addition method. It was observed that all the 31samples were found to contain melamine residue. This is the first study to confirm the existence of melamine residue in pasteurized milk marketed in Tamilnadu.

KEYWORDS: UV spectrophotometer, hydrochloric acid, melamine & milk.

INTRODUCTION

Melamine being a polar organic compound has a 1,2,3- triazine nucleus. It is a main ingredient in the production of plastics plates, in finishers for paper, in fertilizer, as a flame retardant, in the manufacture of wrinkle-free textiles Garber [1]. There were continuous articles related to melamine in milk and milk related product from 2007 to till date in various places across the world, particularly China, United States, Canada and Pakistan In early 2007, it became a topic of significance as hundreds of pet deaths occurred due to pet food contamination. In September 2008 [2], the World Health Organization (WHO) reported that there have been six infant deaths, and more than 1lakh of infants and young children in China were hospitalized for urinary problems [3], with possibility of renal tube blockages and possible kidney stones which are attributed to the consumption of melamine contaminated infant formula and related dairy products. In 2013 researchers confirmed that melamine tableware releases melamine when used to serve hot foods. In veterinary medicine cyromazine is used as an ectoparasiticide and is added to animal feed to chase fly from the manure and also used as pesticide. Some data have shown that the carry-over occurs from feed to products of animal origin including milk, eggs, meat, and fish [4]. Contamination of melamine into the food chain at baseline level is possible as it is present in the environment due to the widespread use of melamine-containing materials. Several detection methods for melamine have been reported, which includes GC, HPLC, GC-MS, and LC-MS, All the reported methods consume large quantities of organic solvents as mobile phases [5, 8, 9-10] and much costlier for regular analysis. Risks associated with these solvents extend beyond direct implications on the health of humans, wildlife and the ecosystem. The present study is eco-friendly, makes use of green chemistry approach and less expensive. The work presented is first of its kind to be reported in TAMIL NADU.

MATERIALS AND METHODS

Chemicals, Reagents & Equipment: Standard Melamine (99 % purity) was purchased from Sigma-Aldrich, Ultra-pure water was obtained from a Millipore system, disposable syringe of pore size 0.45 µm, ultrasonic bath, centrifuge, UV Spectrophotometer.

Instruments: Determination of pH by using pH meter and absorbance measurements were carried out using UV spectrophotometer (Make: shimadzu ; model: 1650PC)

Sample Collection: A total number of 31 Samples comprising of raw milk, pasteurized milk, flavored milk, curd and powdered milk sample were collected from various places in TAMIL NADU.

Preparation of standards of Melamine: A 1000 µg/ml melamine stock standard was prepared by accurately weighing 100 mg of melamine into a 100 ml volumetric flask with distilled water. The standard stock solution

was diluted appropriately to contain varied concentration ranging from 3 - 8.00 µg/mL with 0.1M sodium Hydroxide.

Sample preparation for raw milk, pasteurized milk, flavored milk & curd Sample: 100ml of sample was taken, acidified with dilute hydrochloric acid to a pH - 4.6 & centrifuged (setting rpm to 6000 for 30 minutes). Two known concentration (3ppm & 8ppm) of melamine standard was added to each of sample solution and made up to desired concentration with 0.1M sodium Hydroxide.

Sample Preparation for Pasteurized milk: 2g of powdered milk sample was homogenized with 100ml of water, acidified with dilute hydrochloric acid to a pH - 4.6 & centrifuged (setting rpm to 6000 for 30 minutes). Two known concentration (3ppm & 8ppm) of melamine standard was added to each sample solution and made up to desired concentration with 0.1M sodium Hydroxide.

Method: The standard solution of melamine was scanned between 200 – 400 nm using 0.1M sodium hydroxide as blank. The maximum absorbance was observed at 219 nm. Subsequently, absorbance of varied concentration of standard were measured at 219 nm and a linearity curve was constructed. The sample absorbance was carried out by addition of known quantity of standard i.e 3ppm & 8ppm at the same wavelength.

RESULT AND DISCUSSION

The official procedure for isolation of melamine from milk and milk related product consists of two steps, sample extraction and purifying the sample extract on an activated SPE column; and then drying the purified extract with N_2 at 50 °C. Therefore it is necessary to find an efficient and simple way to prepare both milk and milk related product. The optimized procedure used in this study is simple and efficient, and does not require purification by SPE and sample drying; it requires only precipitation with dilute hydrochloric acid, subsequent centrifugation and filtration, which is suitable for both liquid milk and milk powder products.

Both spiked sample and standard exhibited maximum absorbance at a wavelength of 219 nm. The difference in absorbance between spiked sample and standard indicated the presence of melamine residue in sample. The entire 31 samples were found to contain melamine residue. WHO in 2008 has also specified that the tolerable daily intake of melamine is 0.2mg/kg [7].

CONCLUSION

The present study showed that all the marketed milk and milk related product contain melamine residue. The samples were found to contain approximately 0.05 – 0.1 ppm .As per Food Safety and Standard Authority of India the infant formula should not contain melamine more than 1ppm and other foods more than 2.5ppm [6]. Consumption of foods containing low levels of melamine does not constitute health risk for consumers. Recent product recalls and food safety incidents due to melamine adulteration or contamination have caused a worldwide food security concern. This has led to many methods being developed to detect melamine in foods, but few methods have been reported that can rapidly and reliably measure melamine in environmental samples. The proposed method is simple, and less expensive. In this study only raw milk and few milk related product have been analyzed for melamine residue, it is recommended that all milk related products should be analyzed for melamine residue in Tamilnadu, so has to ensure safe consumption. The proposed method could be used for detection of melamine.

ACKNOWLEDGMENTS

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REFERENCES

[1] World Health Organization, Melamine and Cyanuric Acid Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food, Updated 30 October 2008.
[2] E. Braekevelt, et al., Determination of Melamine, Am-meline, Ammelide and Cyanuric Acid in Infant Formula purchased in Canada by Liquid Chromatography-Tandem Mass Spectrometry, Vol. 28, No. 6, 2011, pp. 698-704.
[3] C.A. Brown, K.S. Jeong, R.H. Poppenga, B. Puschner, D.M. Miller & A.E. Ellis, Journal of Veterinary Diagnostic Investigation, 2007, 19, 525-531.
[4] A. Filazi et al.,Determination of melamine in milk and dairy products by high performance liquid chromatography, journal of dairy science, Volume 95, Issue 2, Pages 602–608, February 2012 .
[5] Naoto, et al., A 100% Water Mobile Phase HPLC-PDA Analysis of Melamine and Related Analogues, American Journal of Analytical Chemistry, February 21, 2012; revised March 25, 2012;
[6] Food Safety and Standard Authority of India, Advisory No.;2/FSSAI/2008.
[7] World Health Organization, Melanie and Cyanuric Acid: Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food, Updated 30 October 2008.
[8] Hua Kuang, Wei Chen, Wenjing Yan, Liuang Xu, Yingyue Zhu, Liqiang 3iu, huaqin Chu, Chifang Peng, Libing Wang, Nicholas A. Kotov, Chuanlai Xu, Biosensors and bioelectronic, 2011, 26, 2032-2037.
[9] Xiao- Lin Zheng, Bing-Sheng Yu, Ke-Xian Li, Ying-Na Dai, Food Control, 2012, 23, 245-250.
[10] H., Sun, L. Wang, L. Ai, S. Liang, & H. Wu, Food Control, 2010, 21, 686-691.

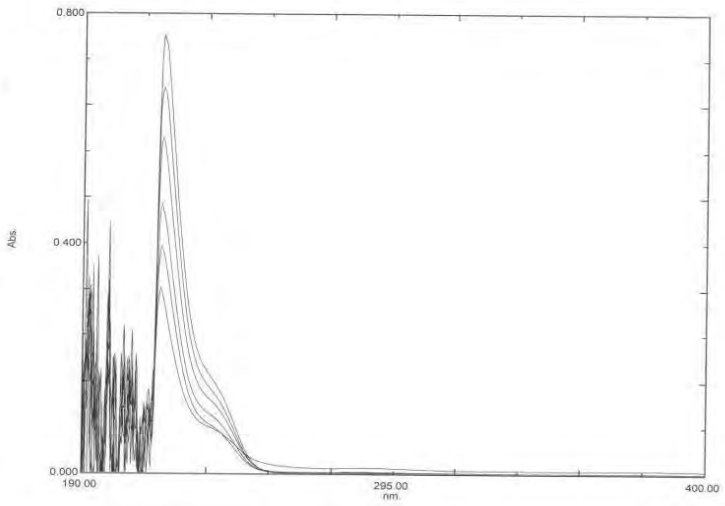

Figure 1: Overlain spectrum of melamine standard

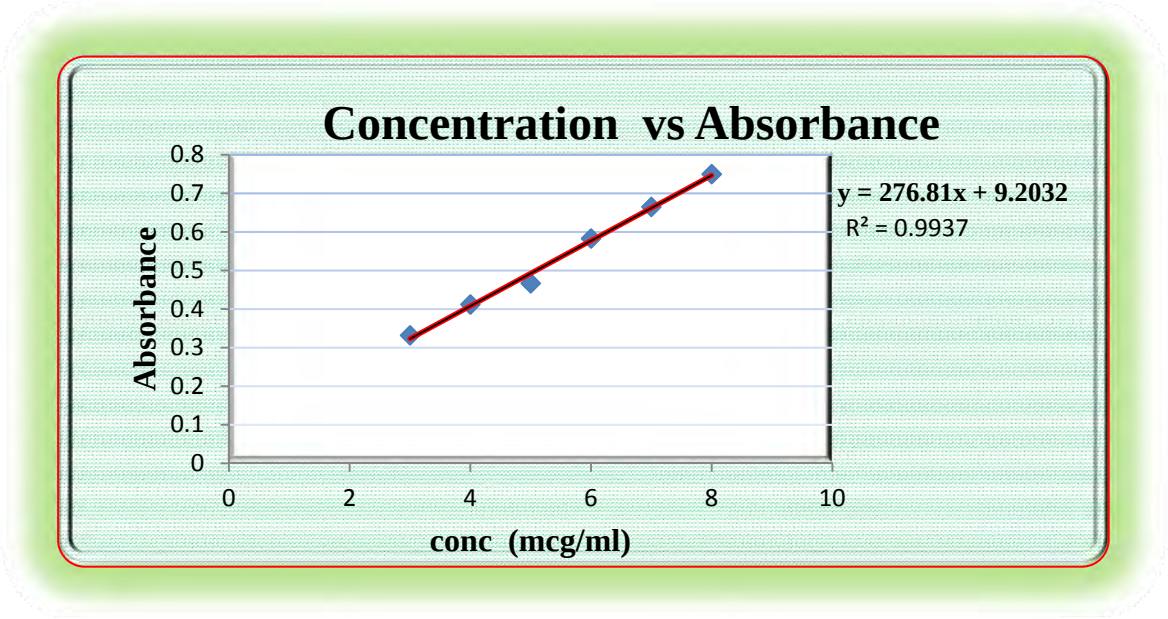

Figure 2: Calibration curve for melamine standard

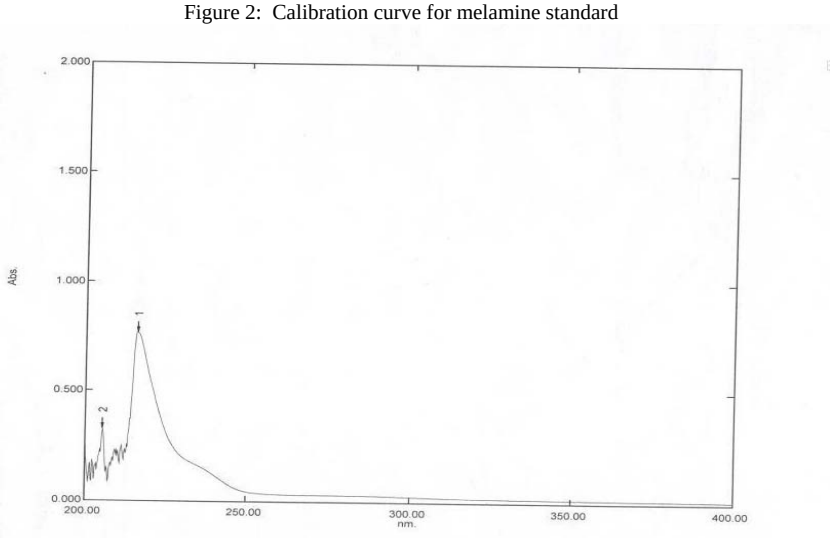

Figure 3: Spectrum of Rawmilk sample

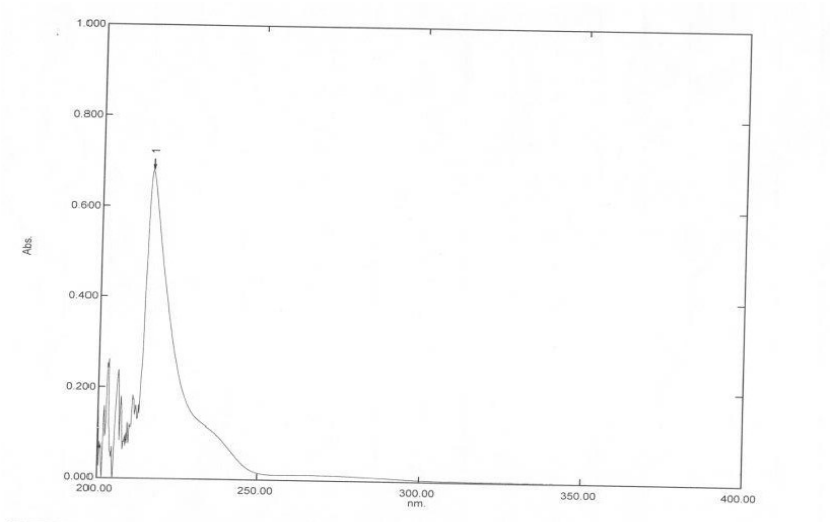

Figure 4: Spectrum of Pasteurized milk sample

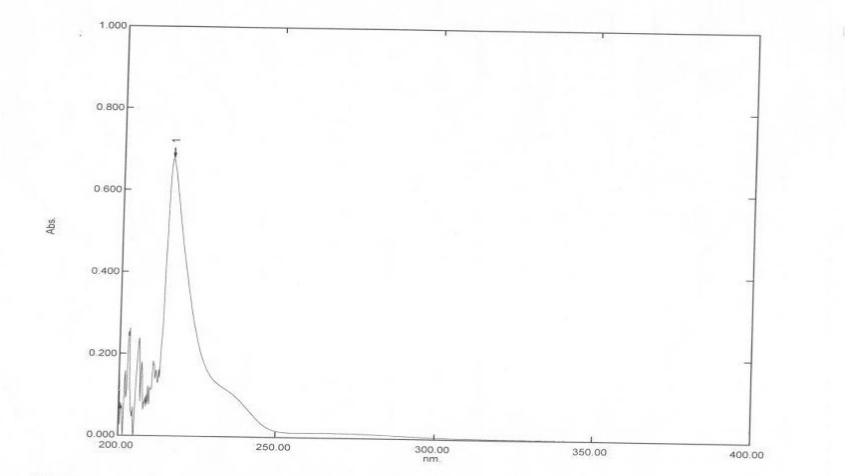

Figure 5: spectrum of Powdered milk sample

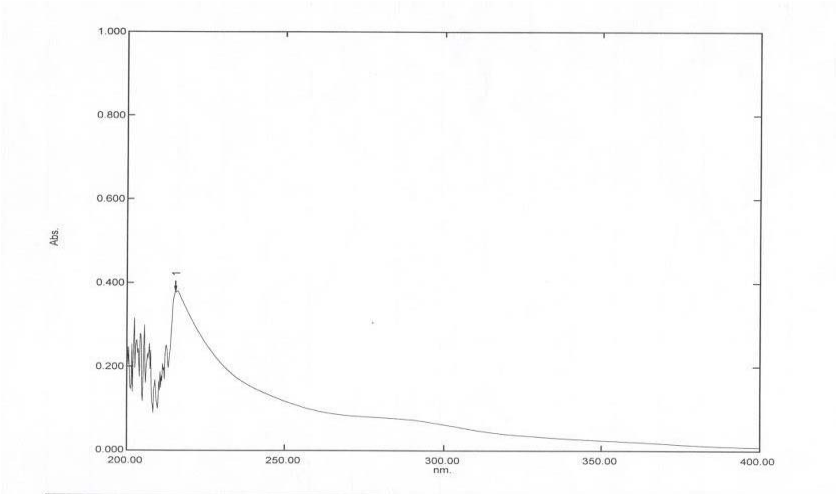

Figure 6: spectrum of Flavord milk sample

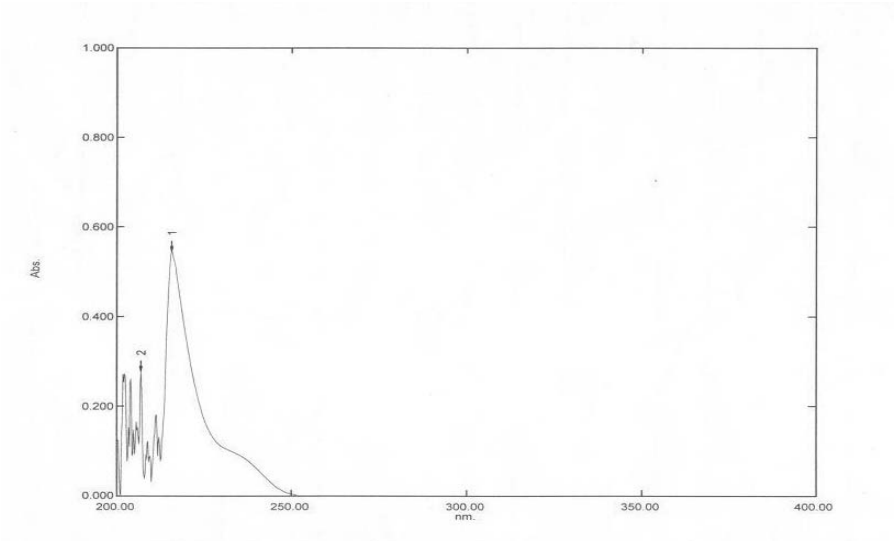

Figure 7: spectrum of Curd sample

Table 1: Detection of melamine in Raw milk Samples

Name of Sample	No. of Sample	Absorbance of Standard Solution at 219 nm		Absorbance of Spiked sample at 219 nm		Difference in absorbance (due to melamine residue in sample)	Standard deviation
		Standard concentration	Absorbance #	Concentration of standard in spiked milk sample	Absorbance #		
Raw Milk sample	RMS 1	3 ppm	0.322	3 ppm	0.394	0.072	0.004
		8 ppm	0.762	8 ppm	0.838	0.076	0.005
	RMS 2	3 ppm	0.332	3 ppm	0.395	0.063	0.003
		8 ppm	0.776	8 ppm	0.83	0.054	0.008
	RMS 3	3 ppm	0.336	3 ppm	0.399	0.063	0.005
		8 ppm	0.769	8 ppm	0.828	0.059	0.008
	RMS 4	3 ppm	0.328	3 ppm	0.397	0.069	0.005
		8 ppm	0.77	8 ppm	0.840	0.070	0.010
	RMS 5	3 ppm	0.336	3 ppm	0.405	0.069	0.006
		8 ppm	0.776	8 ppm	0.852	0.076	0.007
	RMS 6	3 ppm	0.33	3 ppm	0.389	0.059	0.004
		8 ppm	0.78	8 ppm	0.838	0.058	0.003

Mean of three determinations, RMS-Raw milk sample

Table 2: Detection of melamine in Flavored milk Samples

Name of Sample	No. of Sample	Absorbance of Standard Solution at 219 nm		Absorbance of Spiked sample at 219 nm		Difference in absorbance (due to melamine residue in sample)	Standard deviation
		Standard concentration	Absorbance #	Concentration of standard in spiked milk sample	Absorbance #		
Flavored Milk sample	FMS 1	3 ppm	0.33	3 ppm	0.394	0.064	0.002
		8 ppm	0.77	8 ppm	0.833	0.063	0.006
	FMS 2	3 ppm	0.329	3 ppm	0.396	0.067	0.004
		8 ppm	0.772	8 ppm	0.838	0.066	0.009
	FMS 3	3 ppm	0.328	3 ppm	0.389	0.061	0.006
		8 ppm	0.771	8 ppm	0.889	0.118	0.005
	FMS 4	3 ppm	0.331	3 ppm	0.401	0.070	0.008
		8 ppm	0.769	8 ppm	0.849	0.080	0.006

Mean of three determinations, FMS- Flavored milk Samples

Table 3: Detection of melamine in Pasteurized milk Samples

Name of Sample	No. of Sample	Absorbance of Standard Solution at 219 nm		Absorbance of Spiked sample at 219 nm		Difference in absorbance (due to melamine residue in sample)	Standard deviation
		Standard concentration	Absorbance #	Concentration of standard in spiked milk sample	Absorbance #		
Pasteurized Milk sample	PMS 1	3 ppm	0.33	3 ppm	0.394	0.064	0.003
		8 ppm	0.769	8 ppm	0.83	0.061	0.006
	PMS 2	3 ppm	0.319	3 ppm	0.392	0.073	0.005
		8 ppm	0.772	8 ppm	0.839	0.067	0.004
	PMS 3	3 ppm	0.332	3 ppm	0.396	0.064	0.009
		8 ppm	0.759	8 ppm	0.839	0.080	0.003
	PMS 4	3 ppm	0.338	3 ppm	0.386	0.048	0.008
		8 ppm	0.779	8 ppm	0.849	0.070	0.004
	PMS 5	3 ppm	0.346	3 ppm	0.389	0.043	0.007
		8 ppm	0.766	8 ppm	0.862	0.096	0.010
	PMS 6	3 ppm	0.334	3 ppm	0.369	0.035	0.003
		8 ppm	0.787	8 ppm	0.848	0.061	0.004
	PMS 7	3 ppm	0.333	3 ppm	0.379	0.046	0.004
		8 ppm	0.789	8 ppm	0.84	0.051	0.008

Mean of three determinations, PMS- Pasteurized milk Samples

Table 4: Detection of melamine in Curd Samples

Name of Sample	No. of Sample	Absorbance of Standard Solution at 219 nm		Absorbance of Spiked sample at 219 nm		Difference in absorbance (due to melamine residue in sample)	Standard deviation
		Standard concentration	Absorbance #	Concentration of standard in spiked milk sample	Absorbance #		
Curd sample	CS 1	3 ppm	0.336	3 ppm	0.413	0.077	0.003
		8 ppm	0.773	8 ppm	0.846	0.073	0.005
	CS 2	3 ppm	0.334	3 ppm	0.386	0.052	0.009
		8 ppm	0.772	8 ppm	0.845	0.073	0.001
	CS 3	3 ppm	0.329	3 ppm	0.396	0.067	0.003
		8 ppm	0.78	8 ppm	0.836	0.056	0.003
	CS 4	3 ppm	0.339	3 ppm	0.422	0.083	0.002
		8 ppm	0.76	8 ppm	0.869	0.109	0.009

Mean of three determinations, CS- Curd Samples

Table 5: Detection of melamine in Powdered milk Samples

Name of Sample	No. of Sample	Absorbance of Standard Solution at 219 nm		Absorbance of Spiked sample at 219 nm		Difference in absorbance (due to melamine residue in sample)	Standard deviation
		Standard concentration	Absorbance #	Concentration of standard in spiked milk sample	Absorbance #		
Powdered Milk sample	PMS 1	3 ppm	0.326	3 ppm	0.384	0.058	0.005
		8 ppm	0.776	8 ppm	0.832	0.056	0.003
	PMS 2	3 ppm	0.318	3 ppm	0.398	0.08	0.006
		8 ppm	0.769	8 ppm	0.828	0.059	0.005
	PMS 3	3 ppm	0.329	3 ppm	0.386	0.057	0.003
		8 ppm	0.77	8 ppm	0.849	0.079	0.008
	PMS 4	3 ppm	0.332	3 ppm	0.396	0.064	0.007
		8 ppm	0.762	8 ppm	0.847	0.085	0.002
	PMS 5	3 ppm	0.331	3 ppm	0.409	0.078	0.010
		8 ppm	0.762	8 ppm	0.869	0.107	0.006
	PMS 6	3 ppm	0.326	3 ppm	0.359	0.033	0.003
		8 ppm	0.769	8 ppm	0.856	0.087	0.005
	PMS 7	3 ppm	0.328	3 ppm	0.369	0.041	0.004
		8 ppm	0.763	8 ppm	0.843	0.080	0.006
	PMS 8	3 ppm	0.332	3 ppm	0.369	0.037	0.007
		8 ppm	0.769	8 ppm	0.862	0.093	0.003
	PMS 9	3 ppm	0.319	3 ppm	0.359	0.040	0.003
		8 ppm	0.77	8 ppm	0.898	0.128	0.008
	PMS 10	3 ppm	0.334	3 ppm	0.369	0.035	0.004
		8 ppm	0.769	8 ppm	0.849	0.080	0.008

Mean of three determinations, PMS- Powdered milk Samples