WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 4, 777-790. Research Article ISSN 2277– 7105

RAPID MEASUREMENT OF FORMALDEHYDE IN SELECTED BABY FOODS BY FAST LIQUID CHROMATOGRAPHY

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Article Received on
22 Jan. 2020,

Revised on 12 Feb. 2020,
Accepted on 03 March 2020,

DOI: 10.20959/wjpr20204-17018

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ABSTRACT

A rapid, specific and highly sensitive short run High performance fast liquid chromatographic (Fast LC) method was developed for the quantification of formaldehyde in selected baby food products. The sample was dissolved in selected solvent and treated with 2,4-dinitrophenylhydrazine in order that formaldehyde is derivatized to form 2,4-dinitrophenylhydrazone with high UV absorbance. The separation was carried out using C18 column. The detection wavelength was 365 nm for the derivative of formaldehyde. The results showed that derivatization had no effect on the determination of formaldehyde. The limit of detection (LOD) for this method is as low as 0.005 ppm and limit of quantification (LOQ) for this method is as low as 0.008 ppm. This method is successfully validated and results obtained are positive for each parameter recommended by ICH Q2 B (R1) guidance. This method is applied for determination of formaldehyde in various foods including selected baby food products.

KEYWORDS: Formaldehyde, Fast LC, 2,4-dinitrophenylhydrazine, Baby foods, Validation, ICH.

INTRODUCTION

Formaldehyde is known carcinogen and hazardous for health. Occupational Health and Safety Administration (OSHA) has stated “Formaldehyde has the potential to cause cancer in humans when present above the normal exposure level”.[1] The World Health Organization (WHO) has established a Tolerable Daily Intake (TDI) of 0.15 mg/kg body weight for formaldehyde.[2] The TDI is the estimated amount of a substance that can be ingested daily (on body weight basis) over a lifetime without appreciable risk.

Formaldehyde is found naturally at low levels in a wide range of foods such as fruits, vegetables, mushrooms and seafoods (IPCS, 1989; HEXPOC, 2005).[3] It is also a normal product of human metabolism. Ingestion of a small amount of formaldehyde is unlikely to cause any acute effect. Acute toxicity after ingestion of large amount can cause severe abdominal pain, vomiting, coma, renal injury and possible death.[4] Presence of excess formaldehyde in any food product can result in adverse health effects.[5,6] Hence, it is extremely important that quality and safety for all the food products including baby foods is assured. Formaldehyde levels observed in natural foods are tabulated in Table 1.

Table 1: Levels of formaldehyde in natural food.

Food type	Formaldehyde Level (mg/kg)
Alcoholic beverage	0.02 – 3.8
Soft drinks	8.7
Brewed coffee	3.4 – 4.5
Instant coffee	10 – 16
Syrup	<1 – 1.5
Goat's milk	1
Cow's milk	< 3.3
Beef	4.6
Poultry	2.5 – 5.7
Carrot	6.7 – 10
Grapes	22.4

The total food consumed shall contribute not more than 9 mg (9000 ppm) formaldehyde per day for an individual with 60 kg body weight.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical / HPLC grade. A HPLC (Agilent Technologies, 1290 series), Acetonitrile HPLC grade, Millipore Water, 2,4-Dinitrophenylhydrazine, Formaldehyde AR grade, Various baby foods available in market are used as test samples. These samples are purchased from the local market. Column used is Zorbax XDB C18, 50 x 4.6mm, 3µ; make: Agilent.

Chromatographic conditions

HPLC : Agilent Technologies, 1290 series (Fast LC)

Column : Zorbax XDB C18, 50 mm x 4.6 mm, 3 µ

Flow : 2.0 mL/min.

Injection volume : 20 µL

Column temperature : 40°C

Detection : 365 nm

Diluent : 2, 4-DNPH solution: Acetonitrile (3:2)

Run time : 3.6 min.

Mobile phase : Water (A): Acetonitrile (B) in gradient mode

Gradient Program

Time (min.)	Water (A)	Acetonitrile (B)	Flow (mL/min.)
Initial	65	35	2.0
1.7	65	35	2.0
1.8	0	100	2.0
2.6	0	100	2.0
2.7	65	35	2.0
3.6	65	35	2.0

Preparation of 2, 4-DNPH Solution

833 mg of 2, 4-DNPH was weighed & transferred in 200 mL volumetric flask. 170 mL of Acetonitrile added to the same flask followed by 28 mL Carbon tetrachloride and 2 mL o-Phosphoric acid. This solution was shaken well to dissolve the reagent. This solution was transferred to 500 mL separating funnel & 200 mL water was added. Extraction was done by shaking well. The aqueous layer was separated. This solution was used for preparation of diluent.

Diluent

2, 4-DNPH solution: Acetonitrile (3:2).

Preparation of Blank

10 mL diluent & 6 mL water was taken into 20 mL volumetric flask. This flask was kept for mechanical stirring for 30 min. Volume made upto the mark with water and kept aside for 1 hr. standing.

Standard Stock Solution

205 mg of formaldehyde (37%) was weighed in 250 mL volumetric flask. Volume made upto 100 mL with water. 10 mL of this solution diluted to 100 mL with water. Transferred 1 mL of resultant solution to 100 mL volumetric flask and diluted up to the mark with water. Further, 1 mL of above solution is diluted to 100 mL with water.

Preparation of Formaldehyde Standard Solution

In 50 mL volumetric flask, 18 mL diluent & 2 mL of Standard stock solution of formaldehyde solution was taken. This flask was kept for mechanical stirring for 30 min. Volume made upto the mark with water and kept aside for 1 hr. standing (concentration of formaldehyde approx. 0.03 ppm w.r.t. test solution concentration).

Preparation of Sample solution

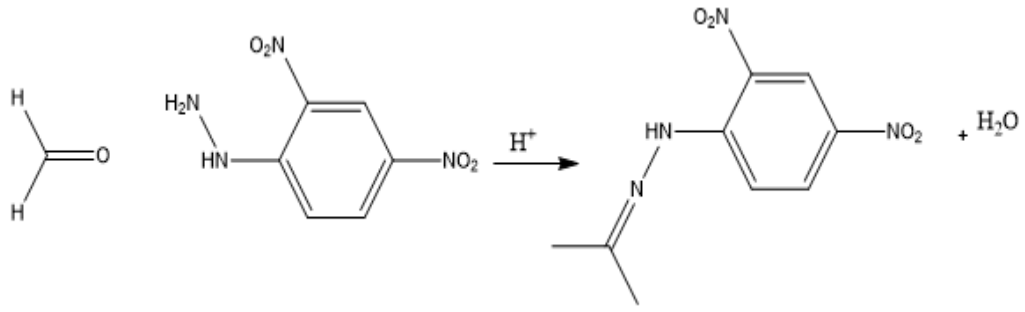
200 mg of crushed sample weighed and transferred in 50 mL volumetric flask. 18 mL of diluent & 2 mL of water was added to the flask. This flask was kept for mechanical stirring for 30 min. Volume made upto the mark with water and kept aside for 1 hr. standing.

Note: Sample preparation can be adjusted to obtained the area of sample solution within range of calibration curve.

Derivatization reaction used is 2,4-dinitrophenylhydrazine

2,4-Dinitrophenylhydrazine used to detect the carbonyl functionality of formaldehyde. A positive test is signaled by a yellow or red precipitate (known as a dinitrophenylhydrazone). Thus, 2, 4-DNP was used as a diluent for sample preparation. The reaction between 2, 4-Dinitrophenylhydrazine and formaldehyde is shown in figure 1.

Note: Store Standard stock solutions, Standard solution and Sample solution at 8°C, immediately after preparation.



Formaldehyde 2,4-Dinitrophenylhydrazine DNPH Derivative (DNPH) (a hydrazone)

Figure 1: Reaction between 2, 4-Dinitrophenylhydrazine and formaldehyde.

Method Development and Method Validation

Different HPLC columns containing Octyl and octadecylsilane stationary phase were tried for separation and resolution. However, Agilent Zorbax XDB C18, 50 x 4.6, 3 µ column was

found satisfactory over the other columns. Similarly, several mobile phase compositions were tried but satisfactory separation and symmetrical peak was obtained by using gradient elution with selected composition of Water[7,8]: Acetonitrile. Since formaldehyde do not have chromophore, quantification is done with derivatization technique. 2,4-dinitrophenylhydrazine is used as derivatization reagent.[9] Formaldehyde form a hydrazone derivative upon reaction with 2,4-dinitrophenylhydrazine. The UV spectrum of formaldehyde derivative was recorded on photo diode array detector for selecting the optimum wavelength at 365 nm.[10,11] The UV spectrum of formaldehyde derivative is given in Figure 2. The peak purity of formaldehyde was checked using photo diode array detector and was found to be satisfactory for detecting the carcinogen with adequate sensitivity. This method is subjected to method validation to evaluate performance of the method.

Analytical validation of method developed for quantification of formaldehyde in food products is performed in accordance with ICH Q2 (R1) guideline.[12] Validation was performed for Specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), Linearity, Accuracy, Precision, Robustness, Solution stability and Filter study.[13] Method Validation Experimental Design and Results Summary is tabulated in Table 2. A typical HPLC chromatograms of Blank, Standard and food sample for determination of formaldehyde are shown in Figure 3, Figure 4 and Figure 5 respectively. Limit of Quantification (LOQ) results are reported in Table 3 and chromatogram for Limit of Quantification (LOQ) is represented in Figure 6. The linearity of the method is tested over a concentration range of 0.008 ppm (LOQ) to 0.06 ppm. Linearity results are reported in Table 4 and linearity plot is represented in Figure 7. Results for Accuracy, Precision, Robustness, Solution stability and Filter study are tabulated in Table 5, Table 6, Table 7, Table 8/9 and Table 10 respectively.

Table 2: Method Validation Experimental Design and Results Summary.

Parameter	Experimental Design	Result
Specificity	Injection of Diluent, Formaldehyde Standard solution, Acetaldehyde, Furfuraldehyde, Spiked sample solution.	Specific, No interference from diluent and sample matrix.
Limit of Detection (LOD) and Limit of Quantification (LOQ)	Injections of series of dilutions of Formaldehyde Standard solution. Measurement of Signal to noise ratio and %RSD for LOQ.	LOD = 0.005 ppm; Signal / noise ratio = 6 LOQ = 0.008 ppm, Signal / noise ratio = 16 % RSD: 2.2%
Linearity	Triplicate injection of Formaldehyde	R = 0.9994

	standard solutions in concentration range 0.008 (LOQ) to 0.06 ppm	% y-intercept = 11.12% Slope = 5723.5
Accuracy (LOQ-25%, 50%, 100%, 150 and 200%)	Addition of known amount of Standard solution to test samples. Triplicate preparations for each level.	Mean: 93% Min: 90%, Max: 98% %RSD = 2.5%
Precision Repeatability Intermediate precision	Analysis of six homogeneous samples. Comparison of results by two different analysts, analysed on different days and different HPLC's.	% RSD = 5.6% % RSD = 6.4% % RSD (Day 1 & Day 2) = 5.9%
Robustness Chromatographic variation	Deliberate changes in chromatographic conditions.	Robust for chromatographic changes.
Stability of Solutions	Monitoring the area of Formaldehyde peak at selected time intervals, stored at 8°C.	Standard solution and Sample solution are stable at 8°C for 20 hrs.
Filter study	Suitability of different makes of 0.45µ nylon filters.	Millipore make and Pall make 0.45µ nylon filters are suitable.

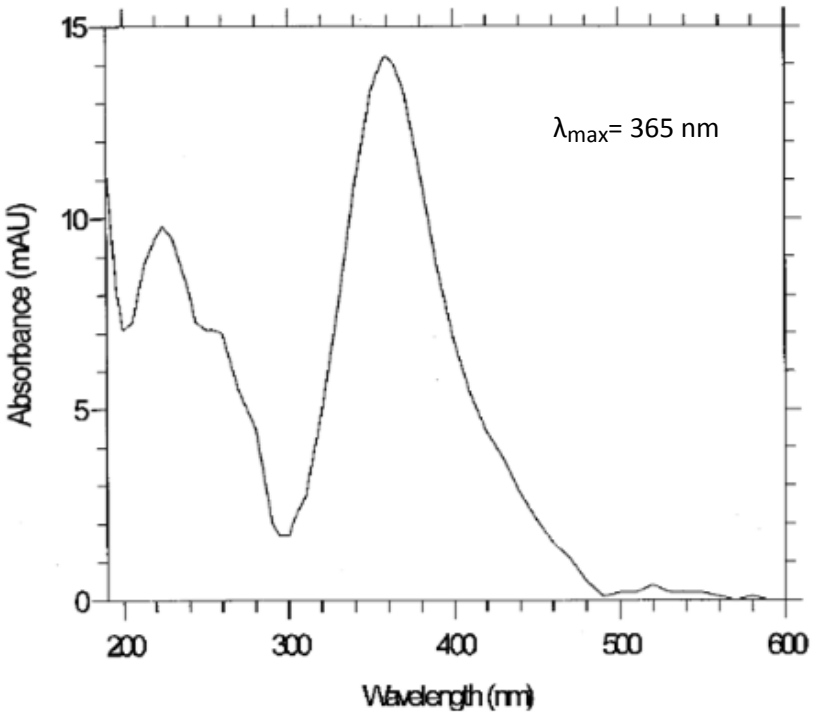


Figure 2: Specificity-UV spectrum of formaldehyde derivative.

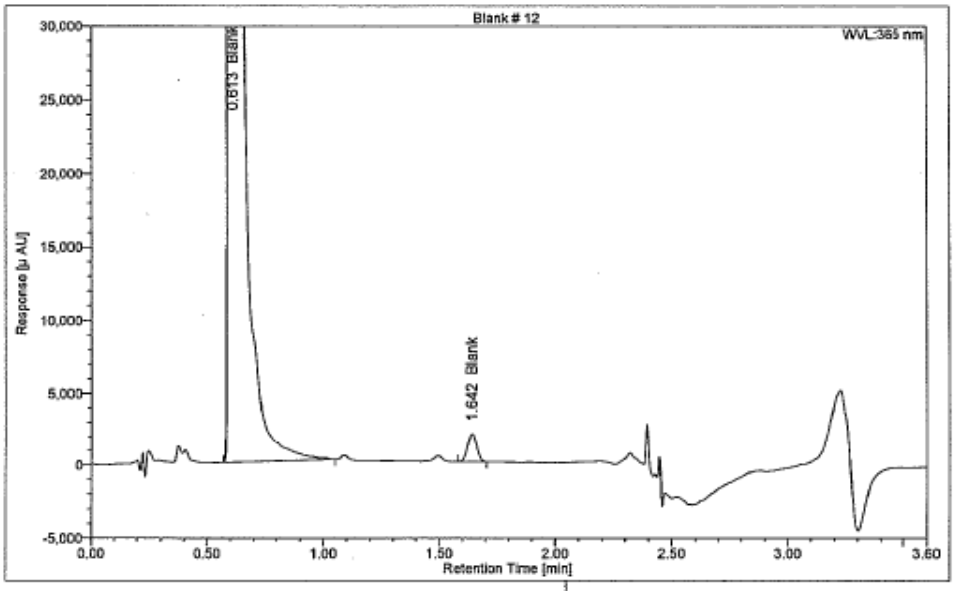


Figure 3: Specificity- Chromatogram of Blank Solution.

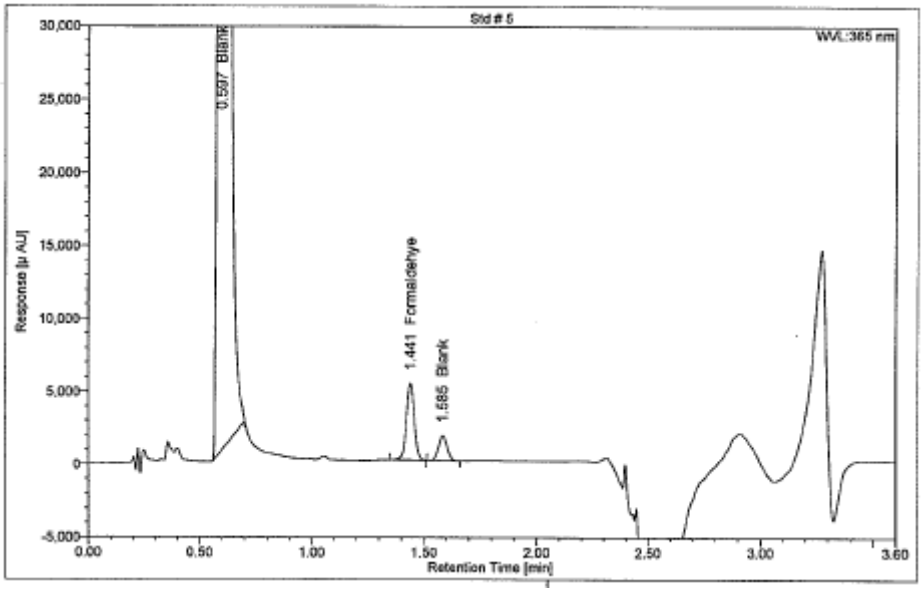


Figure 4: Specificity- Chromatogram of formaldehyde Standard Solution.

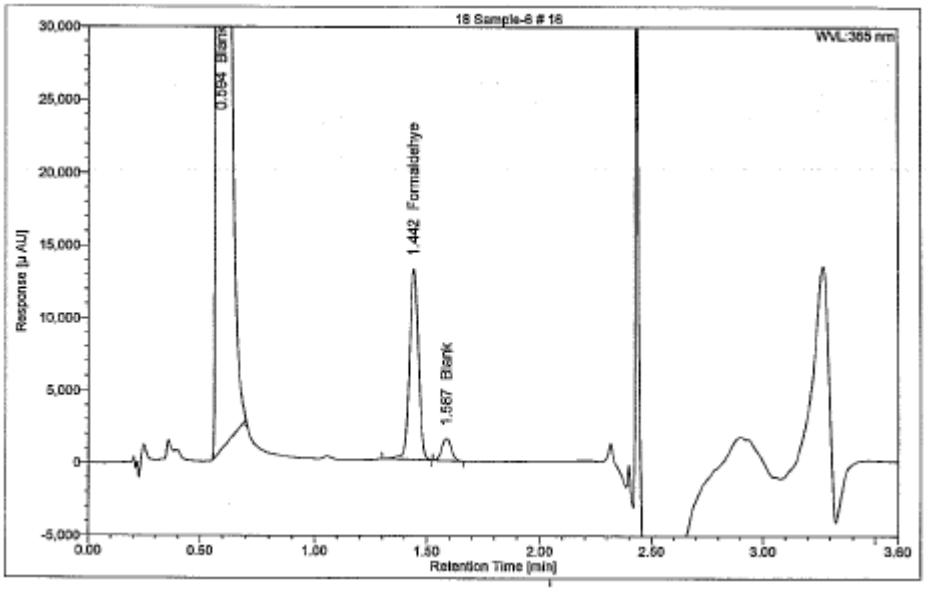


Figure 5: Specificity- Chromatogram of food sample (Lay's).

Table No. 3: Precision at Limit of Quantification (LOQ).

Injection No.	Area of Formaldehyde	S/N ratio
1	72.53454	17
2	71.66927	16
3	68.35475	16
4	69.35915	16
5	69.42895	16
6	70.04648	16
Mean	**70.23219**	**16**
RSD (%)	**2.2%**	**-**

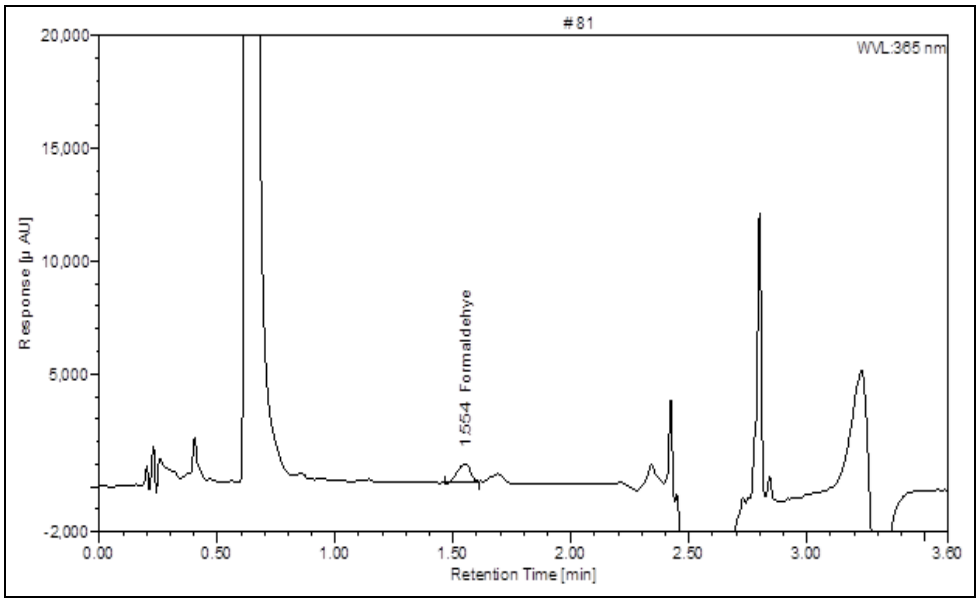


Figure 6: Chromatogram of Limit of Quantification (LOQ).

Table 4: Linearity regression analysis data.

Set	Concentration levels	Final conc. (ppm)	Area
1	25% (LOQ)	0.008	66.95235
	50%	0.016	119.45010
	100%	0.032	202.06794
	150%	0.048	291.33026
	200%	0.063	380.12417
2	25% (LOQ)	0.008	66.98878
	50%	0.016	113.36088
	100%	0.032	205.44779
	150%	0.048	286.88845
	200%	0.063	390.41312
3	25% (LOQ)	0.008	68.71295
	50%	0.016	111.77492
	100%	0.032	205.20667
	150%	0.048	301.22235
	200%	0.063	390.81490

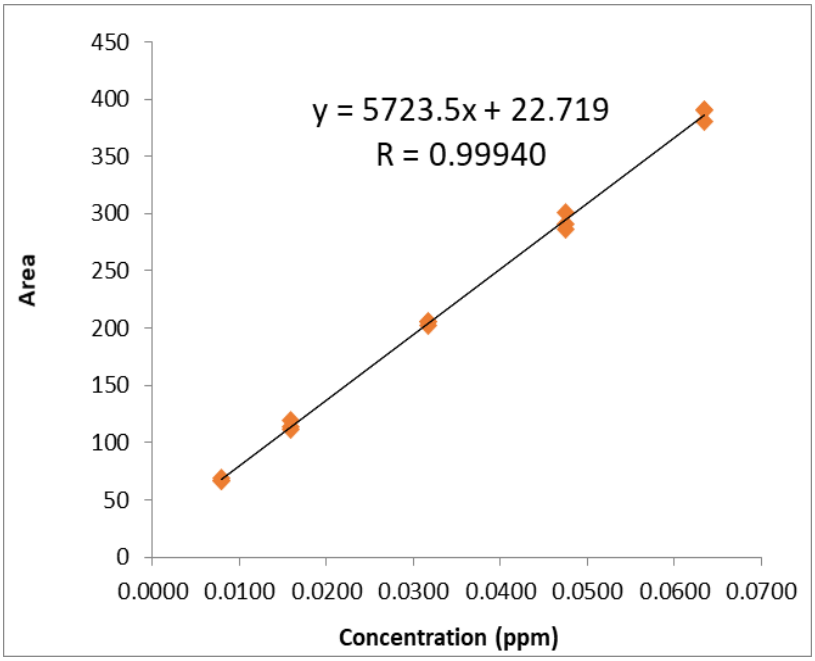


Figure 7: Linearity_Calibration curve for formaldehyde.

Table 5: Accuracy regression analysis data.

Set	Concentration levels	Concentration (ppm)	% Recovery
1	25% (LOQ)	0.008	94
	50%	0.016	90
	100%	0.032	98
	150%	0.048	96
	200%	0.063	92

2	25% (LOQ)	0.008	90
	50%	0.016	93
	100%	0.032	93
	150%	0.048	94
	200%	0.063	91
3	25% (LOQ)	0.008	92
	50%	0.016	90
	100%	0.032	94
	150%	0.048	94
	200%	0.063	91
		Mean	93
		Min.	90
		Max.	98
		Std. Dev.	2.3
		% RSD	2.5

Table 6: Statistical evaluation of the Formaldehyde Content data obtained in Method precision (Day 1) and Intermediate precision (Day 2).

Formaldehyde Content (ppm)		
Sample no.	**Day 1**	**Day 2**
1	4.22	3.80
2	3.76	3.90
3	4.31	3.96
4	3.80	4.00
5	3.97	3.98
6	3.91	3.77
Mean	4.0	3.9
Standard Deviation	0.22	0.24
% RSD	5.6	6.4
% RSD (Day 1 & Day 2)	5.9	
Difference	0.1	

Table 7: Results of Robustness study.

Robustness condition	**Formaldehyde Retention time (min.)**	**Formaldehyde Tailing Factor**
Normal Column: Agilent Zorbax XDB C18, 50 x 4.6, 1.8 µ	1.475	1.2
Flow rate 1.8 mL/min	1.510	1.2
Flow rate 2.2 mL/min	1.490	1.2
Column Temperature:35^{o}C	1.657	1.2
Column Temperature:45^{o}C	1.383	1.2
Wavelength 363nm	1.512	1.2
Wavelength 367nm	1.509	1.2
Gradient B: 40%	1.077	1.1
Gradient B: 33%	1.707	1.2

Column: Supelco Asentis75*4.6, 2.7µ	1.325	1.0
Column: Agilent SB C18 50*4.6, 1.8µ	1.467	1.1
Column: Water XTerra 50*4.6, 5µ	2.019	1.2

Table 8: Results of Standard Solution stability at 8°C.

Time interval (Hr.)	Area	Area w.r.t 0 hr.	% Change
0	190.55438	100.0	-
1	191.32126	100.4	-0.4
2	189.53393	99.5	0.5
3	187.74246	98.5	1.5
4	187.44602	98.4	1.6
5	188.81244	99.1	0.9
14	187.47722	98.4	1.6
16	186.36651	97.8	2.2
18	184.65830	96.9	3.1
20	182.83071	95.9	4.1
% Change is < 10.0%			

Table 9: Results of Sample Solution stability at 8°C.

Time interval (Hr.)	Area	Formaldehyde content	% Change
0	33.62930	3.089	-
1	34.31148	3.151	2.0
2	34.09604	3.131	0.6
3	32.70662	3.004	4.1%
% Change is < 10.0%			

Table 10: Results of Filter Study.

Filter Details	Area of Formaldehyde from Sample solution	Concentration of Formaldehyde (ppm)
Millipore 0.45µ nylon filters	3237.7695	32.93
Value prep 0.45µ nylon filters	3330.7930	32.10
MDI 0.45µ nylon	3267.8932	32.63
No significant change by using any of the above filters		

RESULTS AND DISCUSSION

The method is developed on conventional HPLC and scaled downed on Agilent, Fast LC to achieve total run time of 3.6 min. This was achieved using available statistical tools and shorter column. Modified method was found suitable for analysis of selected cosmetic products. Specificity of the method was unaffected. The various randomly selected food products available in market are analysed for formaldehyde content. Results

for these baby food products are reported in table 11 and represented in Figure 8.

Table 11: Results of Baby food products.

Brand	B. no.	Formaldehyde In Baby food (ppm)
Kellogg's chocos	M3A26	4.7
Kurkure	N222B	1.6
Lay's	N275B	0.9
Little Heart's	BO91234	5.4
Kissan Jam	AO501	10.1
Kissan Ketchup	RBI B3429	3.4
Dairy Milk	K21209	2.9
Bournvita	W20317	7.9
Horlik's	D072234	8.6
Boost	26JAIP 184	8.6

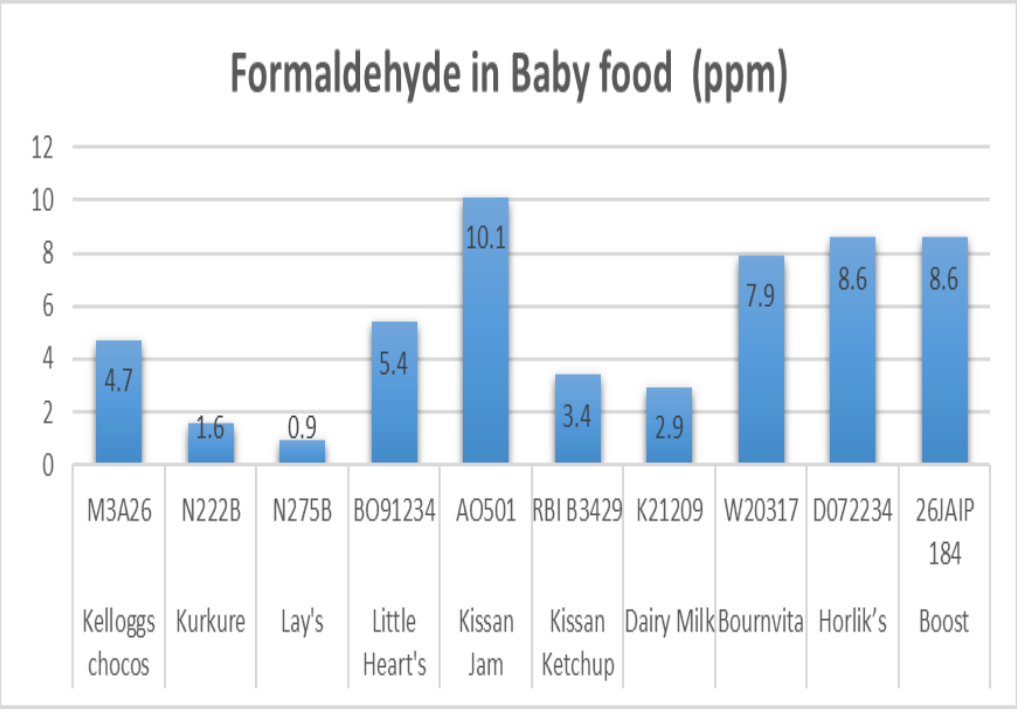


Figure 8: Formaldehyde in Baby food products.

CONCLUSION

This paper presents the development and validation of a simple isocratic High Performance Fast Liquid Chromatography (Fast LC) procedure suitable for the analysis of formaldehyde in selected baby food products. It is demonstrated that the analytical procedure developed is sensitive, accurate, precise, and robust with good stability in selected solvent, as results for all validation parameters meet the requirements of ICH Q2 (R1) guideline.

The formaldehyde derivatization reaction with 2,4-dinitrophenyl- hydrazine and detection at 365 nm are expected to be applicable to analysis of formaldehyde in other test samples such as various Food products, Cosmetic products, Consumer products and Pharmaceutical preparations available in market as long as these products disintegrates or are soluble in water. Sample preparation procedure can be modified including diluent used to ensure complete disintegration of sample matrix. Also, components of these products should not demonstrate significant UV absorption above 300 nm.

Further, this study has revealed that Formaldehyde content observed in some the baby food products is on the threshold of allowable tolerance levels defined by Occupational Health and Safety Administration (OSHA) and World Health Organization (WHO). In such case, Quantitative determination of the formaldehyde levels in baby foods is very important as chronic exposure to Formaldehyde can result is serious health hazards.

ACKNOWLEDGEMEMNT

I wish to acknowledge the guidance provided by my research guide Dr. Sushama R. Ambadekar. I would also like to show my deep appreciation to technical and support staff of Institute of Science, Mumbai for helping me in conducting my work for this research paper.

REFERENCES

1. Occupational Safety and Health Administration (OSHA). Occupational Safety and Health Standards, Toxic and Hazardous Substances. Code of Federal Regulations 29 CFR 1910.1048. 1998.
2. CICAD (2002). Concise International Chemical Assessment Document 40. Formaldehyde. World Health Organisation. Geneva.
3. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to Use of formaldehyde as a preservative during the manufacture and preparation of food additives Question Nº EFSA Q-2005-032. The EFSA Journal, 2006; 415: 1-10.
4. Food Safety Focus (6th Issue, January 2007) – Incident in Focus. Formaldehyde in Food. Reported by Mr. Arthur YAU, Scientific Officer, Risk Communication. Centre for Food Safety, The Government of the Hong Kong Special Administrative Region.
5. U.S. Environmental Protection Agency. Health and Environmental Effects Profile for Formaldehyde. EPA/600/x-85/362. Environmental Criteria and Assessment Office, Office

of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH. 1988.

6. U.S. Department of Health and Human Services. Registry of Toxic Effects of Chemical Substances (RTECS, online database). National Toxicology Information Program, National Library of Medicine, Bethesda, MD. 1993.
7. Lloyd R. Snyder, Joseph L. Glajch, and Joseph J. Kirkland; Practical HPLC Method Development, 2nd Ed, Published March 17th 1997, Wiley-Interscience.
8. Fundamental of analytical chemistry – Skoog, Douglas A, West, Donald M. Md. Zafar Iqbal, Senad Novalin, Analysis of formose sugar and formaldehyde by high-performance liquid Chromatography, Journal of Chromatography A, 2009; 1216: 5116–5121.
9. ZHAO J. FAN B. 2006. Spectrophotometric Determination Method of Formaldehyde [J]. Guangdong Trace Elements Science, 13(2): 17-22. ISSN 1006-446X
10. SINGH R. B. JAIN P. SINGH R. P. 1982. Hydrazones as analytical reagents: a review. Talanta, 29(2): 77-84. ISSN 0039-9140
11. Validation of Analytical Procedures: Text and Methodology Q2 (R1).
12. Validating Chromatographic Methods: A Practical Guide, David M. Bliesner.
13. Koivusalmi, E., Haatainen, E. & Root, A. (1999) Anal chem., 71: 86-91.
14. Michels, J.J. Improved measurement of formaldehyde in water-soluble polymers by high-performance liquid chromatography coupled with post-column reaction detection. J. CHROMATOG. A., 2001; 914: 123-129.
15. Soman, A., Qiu, Y. & Chan Li, Q (2008) J. Chromatog. Sci., 46: 461-465.
16. Jones, S.B., Terry, C. M., Lister, T.E., & Johnson D. C. (1999) Anal Chem., 71: 4030-4033.