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Formaldehyde in Baby Foods by HPLC-ELSD

Sushama Raju Ambadekar, Deepak Baburao Nikam*

Department of Chemistry, Institute of Science, Fort, Mumbai, India

Abstract Formaldehyde is a known carcinogen, hazardous for health. Formaldehyde is used in some of the food products as preservative to arrest microbial growth. A simple, precise, accurate, and sensitive Evaporative light scattering detection (ELSD) based High Performance Liquid Chromatography (HPLC) method is developed to analyze the selected baby foods samples. The chromatographic separation of formaldehyde was achieved with C18, 250 x 4.6 mm, 5 µm column. Mobile phase combination of 0.1% v/v formic acid in water and 0.1% v/v formic acid in acetonitrile is delivered in gradient mode for 15 min. run time at a flow rate of 1.0 mL/min. Formaldehyde lacks intrinsic chromophore, volatile in nature the sample was derivatized with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone, followed by ELSD detection. Method validation performed in accordance (ICH) Q2 (R1) guideline. A calibration curve plotted from 0.5 ppm to 5.0 ppm ($r > 0.9954$). %RSD for intra-day and inter day precision was < 5.0%. Limit of quantification (LOQ) for method was 0.5 ppm. Analysis of selected samples performed with validated method. Observed results are ranging from 0.6 ppm to 13.1 ppm.

Keywords Formaldehyde, Carcinogen, HPLC, ELSD

1. Introduction

Formaldehyde (CH2O) is also known as methanal, methylene oxide, oxymethylene, methylaldehyde, oxomethane, and formic aldehyde. Its Chemical Abstracts Service (CAS) registry number is 50-00-0. Pure formaldehyde is not available commercially but is sold as 30–50% (by weight) aqueous solutions. Formalin (37% CH2O) is the most common solution. 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 exposure is more than acceptable level" [1]. 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 seafood. 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 [2].

The World Health Organization (WHO) has established a Tolerable Daily Intake (TDI) of 0.15 mg/kg body weight for formaldehyde in drinking water [3]. Centre for food safety, the Government of Hong Kong has studied Formaldehyde levels observed in natural food. Findings of study are reported in Table 1.

Table 1. Levels of formaldehyde in natural food [4]

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

Presence of excess formaldehyde in any food product can result in adverse health effects. Hence, it is extremely important that quality and safety for all the food products is assured [5,6]. The dietary exposure per day shall be limited below 0.15 mg/kg body weight, tolerance limit set by WHO.

2. Materials and Method

All chemicals and solvents used in this study were of analytical / HPLC grade. A HPLC (Agilent Technologies, 1260 Infinity), Acetonitrile HPLC grade, Millipore Water, 2,4- Dinitrophenyl hydrazine, Formic acid HPLC grade, Formaldehyde AR grade, selected food products available

* Corresponding author:
deepaknikam76@gmail.com (Deepak Baburao Nikam)
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in market are used as test samples, these samples are purchased from the local market.

Chromatographic conditions

HPLC: Agilent Technologies, 1260 Infinity
Column: Inertsil ODS 3 C18, 250 mm x 4.6 mm, 5 µ
Flow: 1.0 mL/min.
Injection volume: 100 µL
Column temperature: 40°C
Diluent: 2, 4-DNPH solution: Acetonitrile (3:2)
Run time: 15 min.
Mobile phase: Water (A): Acetonitrile (B) in gradient mode

Gradient Program

Time (min.)	0.1% Formic acid (A)	0.1% Formic acid in Acetonitrile (B)	Flow (mL/min.)
Initial	80	20	1.0
10.0	20	80	1.0
12.0	80	20	1.0
15.0	80	20	1.0

Detector Parameters

Detection: Agilent 1260 Infinity Evaporative Light Scattering Detector
Evaporator temperature: 60°C
Nebulizer temperature: 50°C
Gas Flow rate: 1.60 SLM
Data rate: 80 Hz
LED Intensity: 100%
PMT gain: 10.0

Preparation of 2, 4-DNPH Solution

833 mg of 2, 4-DNPH was weighed and transferred in 200 mL volumetric flask. 170 mL of Acetonitrile added to the same flask followed by 28 mL Carbon tetrachloride and 2 mL ortho- Phosphoric acid. This solution was shaken well to dissolve the reagent. This solution was transferred to 500 mL separating funnel and 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 and 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% (Formalin) was weighed in 250 mL volumetric flask. Volume made upto 250 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, 1.0 mL of above solution is diluted to 100 mL with water.

Preparation of Formaldehyde Standard Solution

In 50 mL volumetric flask, 18 mL diluent and 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 for 1 hr. standing (concentration of formaldehyde approx. 0.03 ppm).

Preparation of Sample solution

200 mg of crushed sample weighed and transferred in 50 mL volumetric flask. 18 mL of diluent and 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. Presence of formaldehyde is indicated by a yellow or red precipitate (known as a dinitrophenyl hydrazone). Thus, 2, 4-DNP was used as a diluent for sample preparation.

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

Method Development and Method Validation

The most widely used methods for the detection of formaldehyde are based on spectrophotometry, but other methods, such as colorimetry, fluorimetry, high-performance liquid chromatography, polarography, gas chromatography are also used. Organic and inorganic components in food products, other aldehydes and amines, can interfere with these methods of detection. ELSD is preferred for development of method for food products as it is considered as a universal detector due to the specificity of the detection method, which is based on the scattering of laser light on the non-volatile analyte particles. ELSD detector has advantage over other detectors for food samples analysis as other components, colors, additives, preservatives do not interfere in quantification, which is not possible with Gas chromatography detection and UV spectrophotometric detection [7]. Different HPLC columns with Octadecylsilane stationary phase were evaluated. However, C18, 250 x 4.6 mm, 5 µm column from Inertsil brand was found suitable. Similarly, different mobile phase compositions were tried but satisfactory separation and symmetrical peak was obtained by using gradient elution with selected composition of diluted formic acid by using gradient mode [8]. Formaldehyde do not have chromophore; quantification is done with derivatization technique. 2,4-dinitrophenyl hydrazine is used as derivatization reagent. Formaldehyde forms a hydrazone derivative upon reaction with 2,4-dinitrophenylhydrazine [9]. Use of derivatization technique enabled enhanced response for formaldehyde to

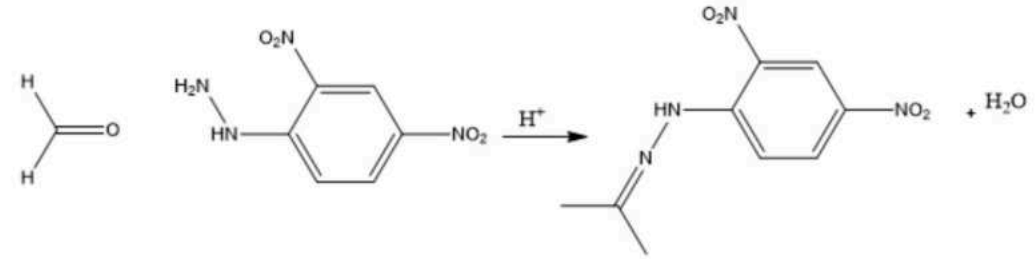
ensure trace level detection. The reaction between 2, 4-Dinitrophenyl hydrazine and formaldehyde is shown in figure 1.

Developed method is subjected to method validation. The sensitivity of the method is challenged by injecting lower concentration of formaldehyde. Analytical validation is performed for selected validation parameters Specificity, Limit of Quantification (LOQ), Linearity, Accuracy, Precision and Solution stability in accordance with ICH Q2 (R1) guideline [10,11]. Method validation experimental design and results summary is reported in Table 2.

Response obtained with ELSD detector is nonlinear. The concentration of unknown samples is obtained by taking the logarithm of the instrument readings, computing the corresponding logarithms of the concentrations from the calibration equation, then taking the anti-log to obtain the concentration. The linearity of the method is tested over a concentration range of 0.5 ppm (LOQ) to 5.0 ppm. Limit of Quantification (LOQ) results are reported in Table 3.

Table 2. Method Validation Experimental Design and Results Summary

Parameter	Experimental Design	Result
Specificity	Injection of Diluent, Placebo, Sample and Spiked sample solution	Specific, No interference from diluent and sample matrix.
Limit of Quantification (LOQ)	Measurement of Signal to noise ratio and %RSD for LOQ	LOQ = 0.5 ppm, Signal/noise ratio = 27 % RSD: 2.6%
Linearity	Evaluation of logarithmic calibration curve over a concentration range of 0.5 ppm to 5.0 ppm	R = 0.9954 y-intercept = 5.1918 Slope = 2.1942
Accuracy	Addition of known amount of Standard solution to test samples. Triplicate preparations for each level.	Mean: 98.6% Min: 87.2%, Max: 105.4% %RSD = 5.9%
Precision Repeatability Intermediate precision	Analysis of six homogeneous samples. Comparison of results by two different analysts, analyzed on different days.	% RSD Day 1 = 3.1% % RSD Day 2 = 1.7% % RSD (Day 1 & Day 2) = 2.4%
Stability of Solutions	Monitoring formaldehyde response at selected time interval, stored at 8°C.	Standard solution is stable for 36 hrs. Sample solution is stable for 30 hrs.



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

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

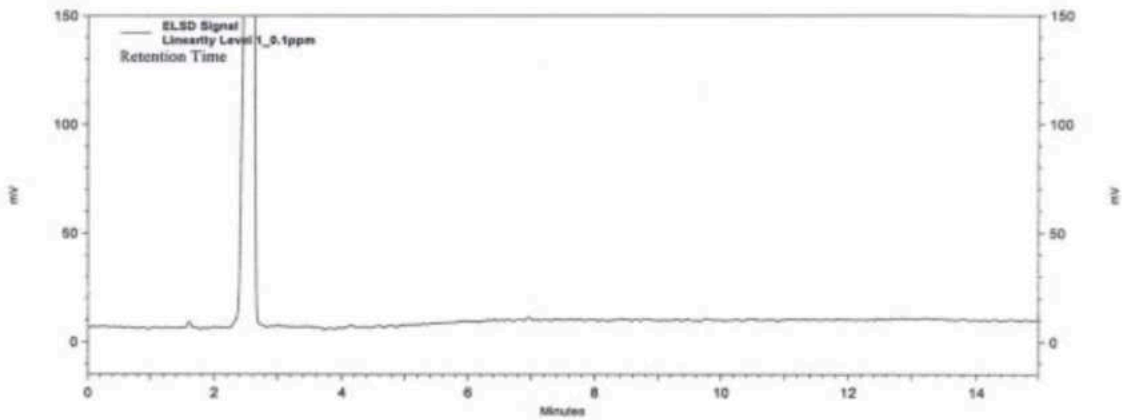


Figure 2. Chromatogram of Blank Solution

Table 3. Precision at Limit of Quantification (LOQ)

Injection No.	Area of Formaldehyde	S/N ratio
1	37840	25
2	39469	27
3	39359	24
4	38451	29
5	38460	27
6	36809	32
Mean	38398	27
RSD (%)	2.6%	-

A typical HPLC chromatograms of Blank, Standard and Food sample are shown in Figure 2, Figure 3 and Figure 4 respectively. Chromatogram for Limit of Quantification (LOQ) is represented in Figure 5. Linearity results are reported in Table 4 and a logarithmic linearity plot is represented in Figure 6. Results for Accuracy, Precision and Solution stability are reported in Table 5, Table 6, Table 7 and Table 8 respectively.

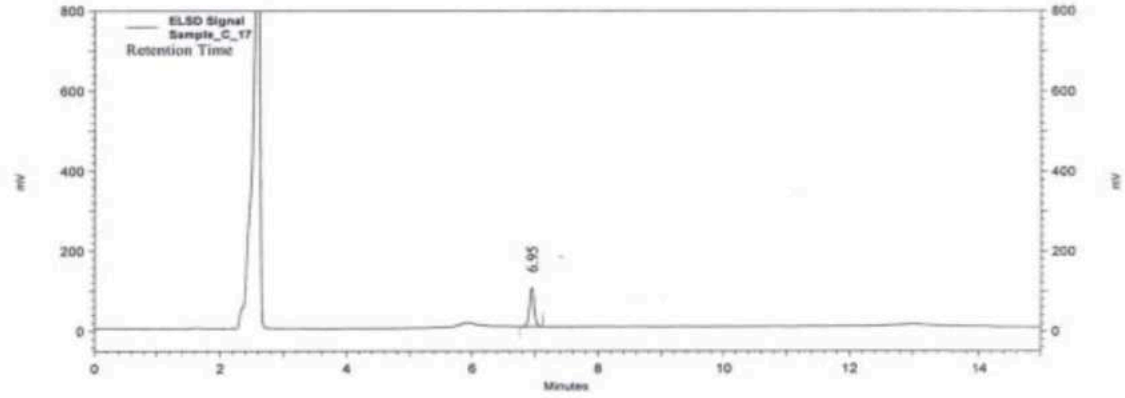


Figure 3. Chromatogram of formaldehyde Standard

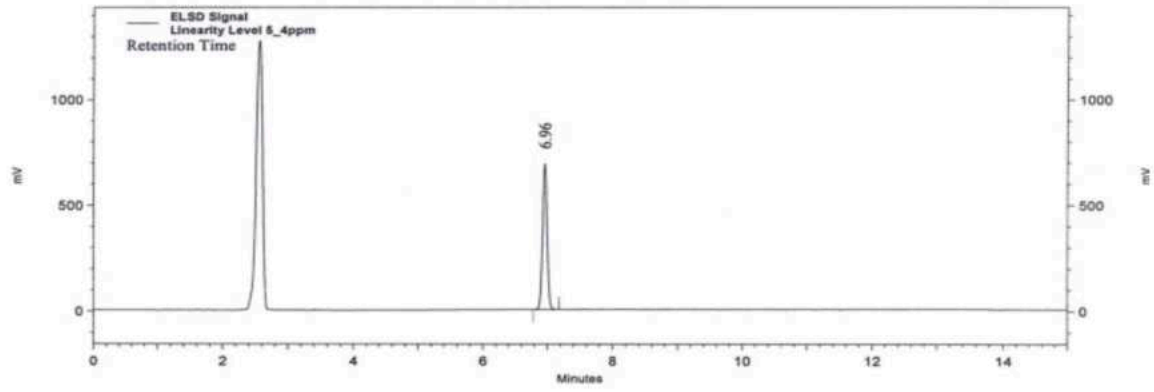


Figure 4. Chromatogram of food sample

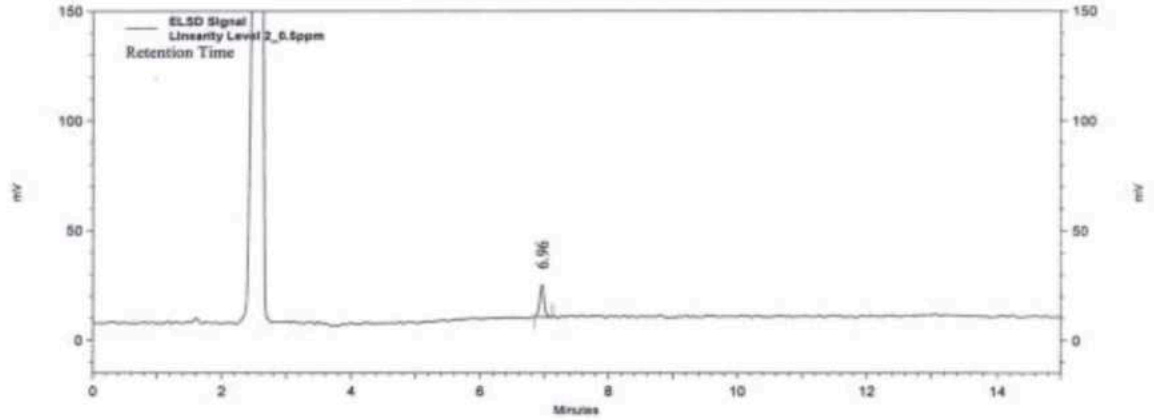


Figure 5. Chromatogram of Limit of Quantification (LOQ)

Table 4. Linearity regression analysis data

Set	Concentration levels	Final conc. (ppm)	Area	Logarithm of Concentration	Logarithm of Area
1	12.5% (LOQ)	0.5	37840	-0.30103	4.5779511
	25%	1.0	122705	0.00000	5.0888623
	50%	2.0	765072	0.30103	5.8837023
	100%	4.0	3470257	0.60206	6.5403616
	125%	5.0	5368816	0.69897	6.7298785
2	12.5% (LOQ)	0.5	39469	-0.30103	4.5962561
	25%	1.0	126943	0.00000	5.1036088
	50%	2.0	762879	0.30103	5.8824557
	100%	4.0	3391772	0.60206	6.5304267
	125%	5.0	5166749	0.69897	6.7132174
3	12.5% (LOQ)	0.5	39359	-0.30103	4.5950441
	25%	1.0	119298	0.00000	5.0766332
	50%	2.0	740720	0.30103	5.8696541
	100%	4.0	3465894	0.60206	6.5398153
	125%	5.0	5157947	0.69897	6.7124769

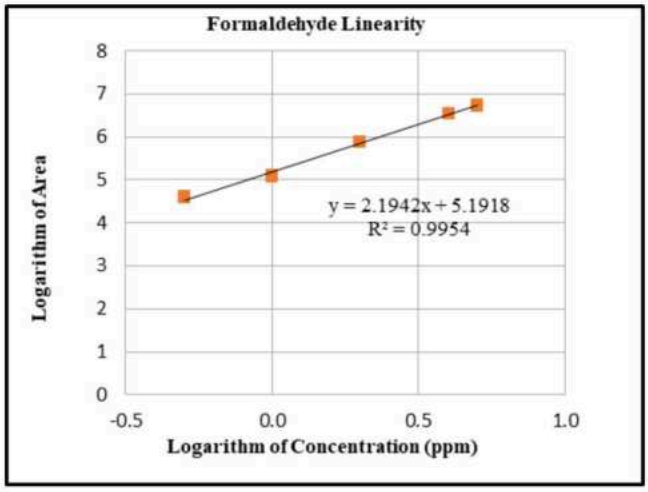


Figure 6. Linearity_Logarithmic Calibration curve for formaldehyde

Table 5. Accuracy regression analysis data

Concentration level	Concentration (ppm)	% Recovery in triplicate
12.5% (LOQ)	0.5	103.4, 105.4, 105.2
25%	1.0	88.3, 89.7, 87.2
50%	2.0	101.7, 101.6, 100.2
100%	4.0	101.3, 100.2, 101.2
125%	5.0	98.9, 97.2, 97.1
	Mean	98.6
	Min.	87.2
	Max.	105.4
	Std. Dev.	0.06
	% RSD	5.9

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	3.97	3.92
2	3.97	3.94
3	3.75	4.06
4	4.10	3.90
5	4.02	4.05
6	3.87	4.01
Mean	3.95	3.98
Standard Deviation	0.12	0.07
% RSD	3.1	1.7
% RSD (Day 1 & Day 2)	2.4	
Difference (ppm)	0.03	

Table 7. Results of Standard Solution stability at 8℃

Time interval	Area	Area	% Change
0 Hr.	3229152	100.0	-
36 Hr.	3391772	105.0	5.0
% Change is < 10.0%			

Table 8. Results of Sample Solution stability at 8℃

Time interval	Area	Formaldehyde content (ppm)	% Change
0 Hr.	1727152	2.95	-
30 Hr.	1630327	2.87	2.8
% Change is < 10.0%			

3. Results and Discussion

The developed HPLC based ELSD method is used for quantification of trace level formaldehyde in selected baby food products, picked from market. Samples were selected to include most consumed brands in various food categories such as Biscuits, Jam, Ketchup, Dairy product, and Food supplements. The higher sensitivity of the method is indicated by sharpness of the peak (higher signal to noise ratio) and the concentration of the sample. As the evaporative light scattering detector response increases exponentially with an increase in formaldehyde concentration, both the sharpness of the peak and higher area response provides accuracy and reproducibility for quantification of formaldehyde at trace level.

Selected baby food products contain additives such as food colours, flavours and preservatives. Presence of these additives did not affect the performance, accuracy and sensitivity of the method as ELSD detection is unique and eliminates possibility of interference from these components unlike UV detection or GC FID detection. The various randomly selected baby food products available in market are analysed for formaldehyde content. The results for selected baby food are reported in table 9.

Table 9. Results of Baby food

Brand	B. no.	Formaldehyde in baby food (ppm)
Kellogg's chocos	M3A26	3.9
Kurkure	N222B	2.9
Lay's	N275B	0.6
Little Heart's	BO91234	4.9
Kissan Jam	AO501	13.1
Kissan Ketchup	RBI B3429	3.5
Dairy Milk	K21209	3.5
Bournvita	W20317	6.3
Horlik's	D072234	8.9

Figure 7 represents comparative analysis results for formaldehyde content in selected baby food.

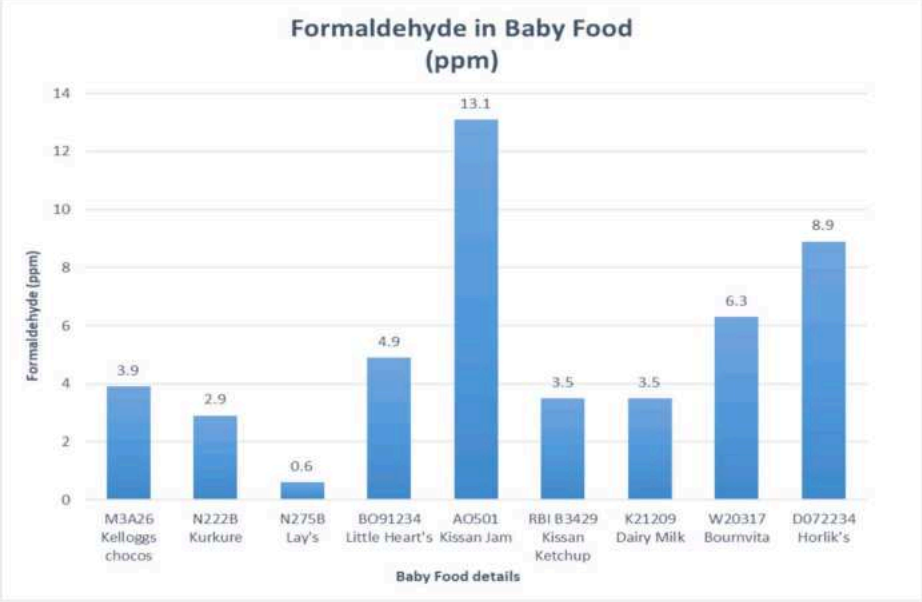


Figure 7. Formaldehyde in Baby food

4. Conclusions

This paper presents the development and validation of a simple High Performance Liquid Chromatography based ELSD method suitable for the analysis of formaldehyde in selected baby foods available in market. It is demonstrated that the analytical procedure developed is sensitive, accurate and precise with good stability in selected solvent, as results for selected validation parameters meet the requirements of ICH Q2 (R1) guideline. Specificity of the method was not compromised due to presence of additives such as food colours, flavours and preservatives in selected food products, which proved the advantages of ELSD detector over other commonly used analytical techniques such as Gas chromatography and UV spectrophotometry. Results observed for test samples were precise and reproducible. A very good linear fit of log ELSD response against log formaldehyde concentration is observed. The formaldehyde derivatization reaction with 2,4-dinitrophenylhydrazine and detection by ELSD detector 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.

Further, this study has revealed presence of higher amount of formaldehyde content in some of the tested food products. In such case, Quantitative determination of the formaldehyde

levels in food products is very important as chronic exposure to Formaldehyde can result is serious health hazards. Accurate results obtained by using developed method will enable control of formaldehyde content in various Food products, Cosmetic products, Consumer products and Pharmaceutical preparations within allowable tolerance levels defined by Occupational Health and Safety Administration (OSHA) and World Health Organization (WHO).

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