



## REVERSE ENGINEERING OF FORMALDEHYDE IN COSMETIC PRODUCTS USING RP-HPLC TECHNIQUE BY DERIVATIZATION WITH 2, 4-DINITROPHENYLHYDRAZINE

SUSHAMA R. AMBADEKAR, DEEPAK BABURAO NIKAM\* AND CHANDAN B. WARKAR

*Department of Chemistry, Institute of Science, Madam Kama road, Fort, Mumbai-400032, India.*

### ABSTRACT

Formaldehyde is a known carcinogen which is hazardous for health. Formaldehyde is used in cosmetic products to prevent microbial growth. Being a carcinogen, it is very much essential to monitor trace level formaldehyde in cosmetic products available in market. Hence, a simple, precise, accurate, and sensitive RP-HPLC method for determination of formaldehyde in cosmetic products was developed. The chromatographic separation of formaldehyde was achieved with C18, 250 x 4.6 mm, 5 µm column. The mobile phase Water: Acetonitrile (57:43) was delivered at a flow rate of 1.5 mL/min. Formaldehyde is not easily ionisable and cannot be easily analysed by Mass Spectrometry as well as Gas Chromatography. Formaldehyde lacks intrinsic chromophore or fluorophore and is volatile in nature which restricts direct detection of formaldehyde in complex matrices. To overcome these limitations, the sample was dissolved in selected solvent and derivatized with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone with UV absorbance maxima at 365 nm. Hence, detection was performed at 365 nm. Chromatographic run completed within 20 min. Developed method was subjected for validation to check suitability for quantification of formaldehyde in cosmetic samples. Validation is performed in accordance with International Conference on Harmonization (ICH) Q2 (R1) guideline. The calibration curve was found to be linear with correlation coefficient close to 1.0 over a concentration range of 0.4 to 4.0 ppm of formaldehyde. The RSD for intra-day and inter day precision was <5.0%. Limit of detection (LOD) of the method was found as low as 0.2 ppm and Limit of quantification (LOQ) of the method was found 0.4 ppm. Validation results proved that developed method is highly sensitivity, specific, linear, accurate, reproducible and robust for quantification of trace level (ppm) formaldehyde. Selected cosmetic samples were analysed with validated method. Observed results were reproducible. This method can be applied to determine formaldehyde content in other cosmetic samples such as creams, shampoos and other skin care products.

**KEYWORDS:** Formaldehyde, Carcinogen, RP-HPLC, 2,4-dinitrophenylhydrazine, Cosmetics, ICH.



**DEEPAK BABURAO NIKAM \***

Department of Chemistry, Institute of Science, Madam Kama road,  
Fort, Mumbai-400032, India

Received on: 06-05-2019

Revised and Accepted on: 19-06-2019

DOI: <http://dx.doi.org/10.22376/ijpbs.2019.10.3.p28-36>



[Creative commons version 4.0](https://creativecommons.org/licenses/by-nc-sa/4.0/)

## INTRODUCTION

Formaldehyde is a known carcinogen which is hazardous for health. The Occupational Health and Safety Administration (OSHA) guidelines have stated "Formaldehyde is a known carcinogen having potential to cause cancer in humans when present above the normal exposure level".<sup>1</sup> Formaldehyde is widely used as preservative in cosmetic products across the world. Formaldehyde or formaldehyde releasing preservatives are frequently used in cosmetic preparations such as nail paints, lipsticks, shampoos and other skin-care products for the prevention of microbial growth. The presence of formaldehyde in cosmetic products can lead to increase in toxic levels resulting in adverse health effects as cosmetic products are directly applied to skin.<sup>2</sup> Formaldehyde present in liquid form such as nail paints can be absorbed through skin. The effect of formaldehyde beyond threshold limiting value on the peoples exposed includes irritation of the eyes and upper respiratory tract, headache, nausea, drowsiness and allergic skin reaction.<sup>3</sup> Further, even in trace level, formaldehyde can be a potent allergenic agent as it reacts with amines present in the side chains of amino acids contained in proteins present in the respiratory tract. Chronic exposure of formaldehyde may result in serious health hazards such as cancer.<sup>4</sup> The maximum allowable limit for formaldehyde in cosmetic products are reported in Table 1. The maximum allowable limit for formaldehyde in nail care products is 5% and maximum allowable limit for formaldehyde in other cosmetic products is 2%.<sup>5</sup> Formaldehyde is not easily ionisable and cannot be easily analysed by Mass Spectrometry as well as Gas Chromatography. Formaldehyde can be analysed by Gas chromatography (GC) and Head space (HS) using FID detector. However, this technique is not suitable for trace level quantification.<sup>6,7</sup> Due to lack of intrinsic chromophore or fluorophore, volatility and reactivity, it is difficult to detect formaldehyde directly in complex matrices and quantification of formaldehyde requires sample pre-treatment.<sup>8,9</sup> High performance liquid chromatography (HPLC) methods are available for quantification of formaldehyde in products other than cosmetics are not specific for determination of formaldehyde in cosmetic samples.<sup>10,11</sup> Further, some of the methods are time consuming with longer chromatographic run time and longer sample preparation time.<sup>12,13</sup> It is extremely important that analytical technique used for quantification of formaldehyde shall be simple, short and highly sensitive to detect trace level (ppm level) of formaldehyde in cosmetic products to ensure quality and safety for cosmetic products available in the market.

## MATERIALS AND METHODS

An Agilent 1200 series HPLC system with G1311A Quaternary/ Binary pump was used with a constant pump flow set at 1.5mL/min. The auto-sampler used was G1329A ALS with injection volume set at 20  $\mu$ L. The detector used was G1315D DAD with the wavelength set at 365 nm while separation was carried out on Water's Symmetry, C18, (250 x 4.6 mm) column packed with spherical Octadecylsilyl silica gel (5 $\mu$ m). The formaldehyde (37% purity) used was of Merck AR grade. All other chemicals and reagents i.e. 2,4-DNPH,

acetaldehyde, furfuraldehyde, benzaldehyde, acetonitrile used were of chromatography grade with >99% purity. The solvents were filtered through a 0.45  $\mu$ m membrane filter prior to use.

### **Preparation of Formaldehyde Standard Stock Solution (Solution C)**

250 mg of formaldehyde was weighed in 100 mL volumetric flask. Volume made up to 100 mL with water. 1 mL of this solution is diluted to 100 mL with water.

### **Preparation of Formaldehyde Standard Solution**

In 25 mL volumetric flask, 4 mL of Solution C and 16 mL of diluent was taken. This flask was kept for mechanical stirring for 30min. Further, the volume was made upto the mark with water and kept aside for 1hr. standing (Concentration of formaldehyde 4.0 ppm). From this solution, 20  $\mu$ L was injected into chromatograph under specific conditions.

### **Preparation of 2, 4-DNPH Solution**

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

### **Diluent**

Dilute three parts of the above 2, 4-DNPH solution with two parts of Acetonitrile.

### **Sample Preparation**

About 250 mg of sample was accurately weighed and transferred into 25 mL volumetric flask. 16 mL diluent and 4 mL water were added to it. This flask was kept for mechanical stirring for 30 minutes. and the volume was then made upto the mark with water and kept aside for 1 hr. standing. From this solution 20  $\mu$ L was injected into chromatograph under specific conditions.

### **Derivatization reaction using is 2,4-dinitrophenylhydrazine**

The derivatization reagent 2,4-Dinitrophenylhydrazine is used to form a dinitrophenylhydrazone derivative with formaldehyde. Formation of dinitrophenylhydrazone derivative is indicated by a yellow precipitate. This derivative has a absorption maxima at 365 nm.. The reaction between 2, 4-Dinitrophenylhydrazine and formaldehyde is shown in Figure 1.

### **Instrumental Parameters**

The chromatographic separation was performed on a reverse phase Waters Symmetry, C-18, (250 x 4.6 mm), 5 $\mu$  column. The mobile phase selected was simplest combination of aqueous and organic components. Mobile phase consisted of Water: Acetonitrile (57:43). The flow rate was set at 1.5 mL/min. Observed column back-pressure was about 65 bar. About 20  $\mu$ L of Standard and Sample solutions were injected and detection wavelength was set at 365 nm for determination of formaldehyde 2,4-Diphenylhydrazone.

## RESULT AND DISCUSSION

### Method Development

Different columns containing octyl and octadecylsilane stationary phase were tried for separation and resolution. However, Waters symmetry, C<sub>18</sub> 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 isocratic elution with Water: Acetonitrile in the ratio 57:43. The UV spectrum of formaldehyde derivative was scanned on photodiode array detector for selecting the optimum wavelength at 365 nm. Quantification of formaldehyde is done by External standard method. External standard method provides accurate quantification at trace level as concentration of standard preparation is matched with the levels observed in test sample. This eliminates the error arising from calibration curve method. The typical spectrum of formaldehyde dinitrophenylhydrazone derivative is given in Figure 2. The peak purity of formaldehyde was checked using photo diode array detector and it was found to be satisfactory for detecting the carcinogen with adequate sensitivity. A typical HPLC chromatogram for determination of formaldehyde from cosmetic sample is shown in Figure 3. The developed method was subjected for validation to check suitability for quantification of formaldehyde in cosmetic samples. Validation is performed in accordance with International Conference on Harmonization (ICH) Q2 (R1) guideline.<sup>14,15</sup> The calibration curve was found to be linear with correlation coefficient close to 1.0 over a concentration range of 0.4 to 4.0 ppm of formaldehyde. The RSD for intra-day and inter day precision was <5.0%. Limit of detection (LOD) of the method was found as low as 0.2 ppm and Limit of quantification (LOQ) of the method was found 0.4 ppm. Validation results proved that developed method is highly sensitivity, specific, linear, accurate, reproducible and robust for quantification of trace level (ppm) formaldehyde. Selected cosmetic samples were analysed with validated method. Observed results were reproducible which indicated that proposed analytical method is capable of estimating trace level (ppm) formaldehyde in cosmetic products available in market. Further, this method can be applied to determine formaldehyde content in other cosmetic samples such as creams, shampoos and other skin care products.

### Method Validation

The suitability of the proposed analytical procedure was demonstrated by statistically evaluating the following validation characteristics:

### Specificity

Specificity is the ability of the method to resolve the principal peak from the other peaks. Hence to prove specificity Blank (diluent), Formaldehyde standard, Acetaldehyde standard, Furfuraldehyde standard, Benzaldehyde standard, Sample solution and Spiked sample solution were subjected to derivatization and derivatized samples injected individually into the chromatograph. The peak of formaldehyde was found to be well resolved from the other peaks in the sample solution. The typical chromatograms are given in Figure 3 and Figure 4.

### Linearity and range

The linearity was evaluated over five different concentration levels of formaldehyde. The range of linearity was selected from 0.4 to 6.0ppm. Each solution was analysed in triplicate and the average values were accepted as representative. The linearity of calibration curve is represented in Figure 5. The regression data obtained for formaldehyde is represented in Table 2.

### Accuracy

The accuracy of the method was determined by calculating recoveries of formaldehyde by standard addition method. The known amount of standard solutions of formaldehyde (10-LOQ, 25, 50, 100 and 150%) were added to previously quantified samples of cosmetic sample. The accuracy was determined from the percentage formaldehyde recovered against formaldehyde added to the cosmetic sample. Table 3 represents recovery results of the formaldehyde for samples spiked at selected concentrations.

### Precision

The precision studies included intra-day and inter-day (intermediate) precision experiments. The precision was evaluated by analysing six independent Spiked sample solutions using the proposed method on the same day and different day. Precision results are tabulated in Table 4.

### Limit of detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of the analytical procedure was determined based on signal to noise ratio by comparing measured signals from samples with known low concentrations of formaldehyde with those of blank and establishing the minimum concentration at which the formaldehyde can be reliably detected and quantified. As per ICH Q2 (R1), a signal to noise ratio of minimum 3:1 is considered acceptable for limit of detection (LOD) and a signal to noise ratio of minimum 10:1 is considered acceptable for limit of quantitation (LOQ).<sup>14</sup> Signal to noise ratio of 6 was observed for 0.2 ppm formaldehyde solution. Signal to noise ratio of 16 was observed for 0.4 ppm formaldehyde solution. Hence, 0.2 ppm was considered as LOD and 0.4 ppm was considered as LOQ. Results for LOQ are tabulated in Table 5.

### Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

- Variation in the flow rate by  $\pm 0.3$  mL/min
- Variability from column initial temperature  $\pm 5^\circ\text{C}$
- Change in wavelength
- Change in Mobile Phase ratio
- Column from different lots and different makes, different dimensions of columns

The results of the analysis with above variations are summarized in Table 6. These results indicated that method is robust for all above variations.

**Stability of Solution**

Standard solution and Sample solution was stored at room temperature and injected at regular intervals. Standard solution and Sample solution was found stable for 42 and 35 hours respectively as % change was less than 5%. This confirmed the stability of formaldehyde in the solvents used for the analysis. The results of the solution stability study are summarized in Table 7.

**Method application**

About 250 mg of each of selected cosmetic samples were accurately weighed and transferred in 25 mL volumetric flask. 16 mL diluent and 4 mL water were

added. This flask was kept for mechanical stirring for 30 min. and the volume was made up to the mark with water and kept aside for 1 hr. standing. From this solution 20 µL was injected into HPLC the formaldehyde peak was identified based on retention time of formaldehyde obtained in Standard solution. This method was applied for determining formaldehyde present in the marketed cosmetic formulations. The results of the same are expressed in Table 8. The typical bar graph showing formaldehyde in different marketed cosmetic products is given in Figure 6.

**Table 1**  
**Allowable formaldehyde limit in Cosmetics**

Country	Regulation No.	Product	Allowable formaldehyde limit
European countries	Cosmetics Regulation (EC) No. 1223/2009, Article III <sup>16,17</sup>	Nail care products	5 % (50000 ppm)
	Cosmetics Regulation (EC) No. 1223/2009, Article V <sup>16,17</sup>	Other products	0.2 % (20000 ppm)
ASEAN countries	ASEAN Cosmetics Directive, Annex III <sup>18</sup>	Nail care products	5 % (50000 ppm)
	ASEAN Cosmetics Directive, Annex IV <sup>18</sup>	Other products	0.2 % (20000 ppm)
China	Safety and Technical Standards for Cosmetics, Table 3 <sup>19</sup>	Nail care products	5 % (50000 ppm)
	Safety and Technical Standards for Cosmetics, Table 4 <sup>19</sup>	Other products	0.2 % (20000 ppm)

**Table 2**  
**Regression analysis data**

Parameters	Result
Correlation Coefficient (r)	0.9998
Intercept (y)	17.893
% Intercept (y)	0.9
Slope (m)	507.95

**Table 3**  
**Accuracy regression analysis data**

Level (%)	Formaldehyde (ppm)	% Recovery	% RSD
10 (LOQ)	0.4	99, 83, 104	11.5
25	1.0	94, 99, 96	2.6
50	2.0	98, 97, 99	1.0
100	4.0	97, 98, 98	0.6
150	6.0	98, 96, 99	1.6

**Table 4**  
**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	38.1	38.4
2	38.8	39.3
3	39.5	40.2
4	40.5	39.9
5	39.7	37.4
6	37.6	41.4
Mean	39.0	39.4
SD	1.07	1.41
% RSD	2.8	3.6
% RSD (Day 1 & Day 2)	3.1	
Difference	-	0.4

**Table 5**  
**Results for Limit of quantification (LOQ)**

	Area of Formaldehyde	(S/N)
1	229.9061	17
2	218.2712	16
3	215.2305	16
4	209.7687	16
5	216.2619	16
6	216.6183	16
Mean	217.6761	16
RSD (%)	3.1%	-

**Table 6**  
**Results of Robustness study**

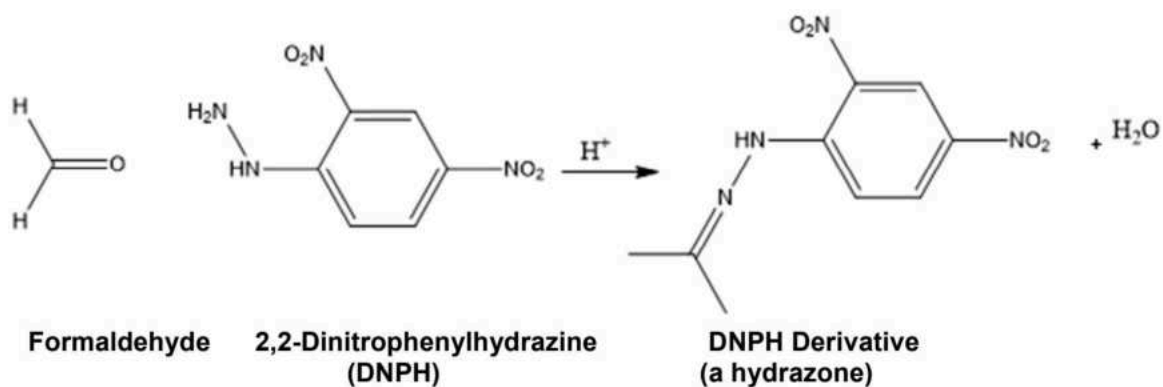
Robustness condition	Formaldehyde Retention time (min.)	Tailing Factor of Formaldehyde
Normal as per method	12.875	1.2
Flow rate 1.3 mL/min.	14.889	1.2
Flow rate 1.8 mL/min.	10.664	1.2
Column Temperature: 20°C	13.865	1.2
Column Temperature: 30°C	11.949	1.2
Wavelength 363 nm	12.886	1.2
Wavelength 367 nm	12.877	1.2
Mobile Phase: Water: Acetonitrile (67:33)	29.061	1.1
Mobile Phase: Water: Acetonitrile (47:53)	6.860	1.1
Column: Inertsil ODS 3V 250 x 4.6 mm, 5 $\mu$	13.929	1.0
Column: YMC ODS AM 250 x 4.6 mm, 5 $\mu$	10.495	1.1

**Table 7**  
**Results of Stability of Standard solution and Sample solution**

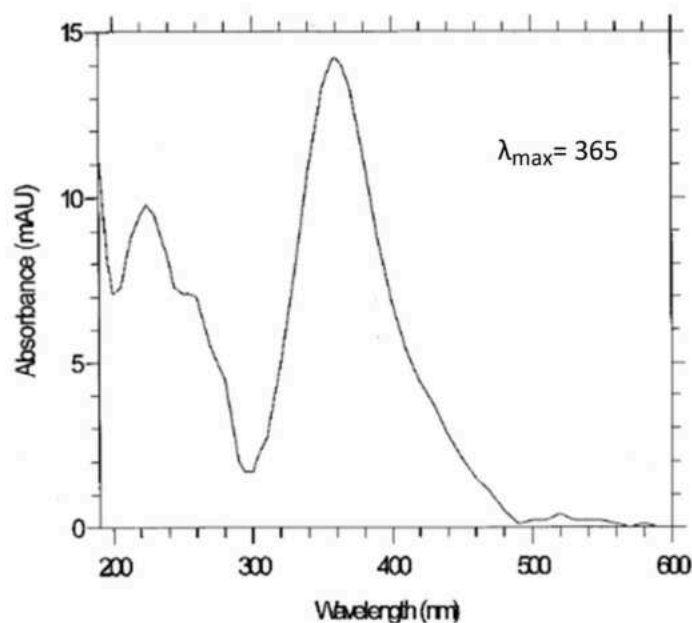
Time (Hours)	Area of Formaldehyde derivative in Standard solution	% change w.r.t. 0 hr	Area of Formaldehyde derivative in Sample solution	Formaldehyde Content (ppm)	% change w.r.t. 0 hr
0	2967.2	N.A.	7524	92.70	N.A.
5	2962.2	0.2	7681	94.63	2.1
11	2942.6	0.8	7706	94.94	2.4
17	2947.2	0.7	7764	95.66	3.2
24	2941.8	0.9	7801	96.12	3.7
32	2942.8	0.8	7854	96.77	4.4
35	2948.2	0.6	7893	97.25	4.9
42	2946.8	0.7	7955	98.01	5.3

**Table 8**  
**Data showing the amount of formaldehyde in marketed products**

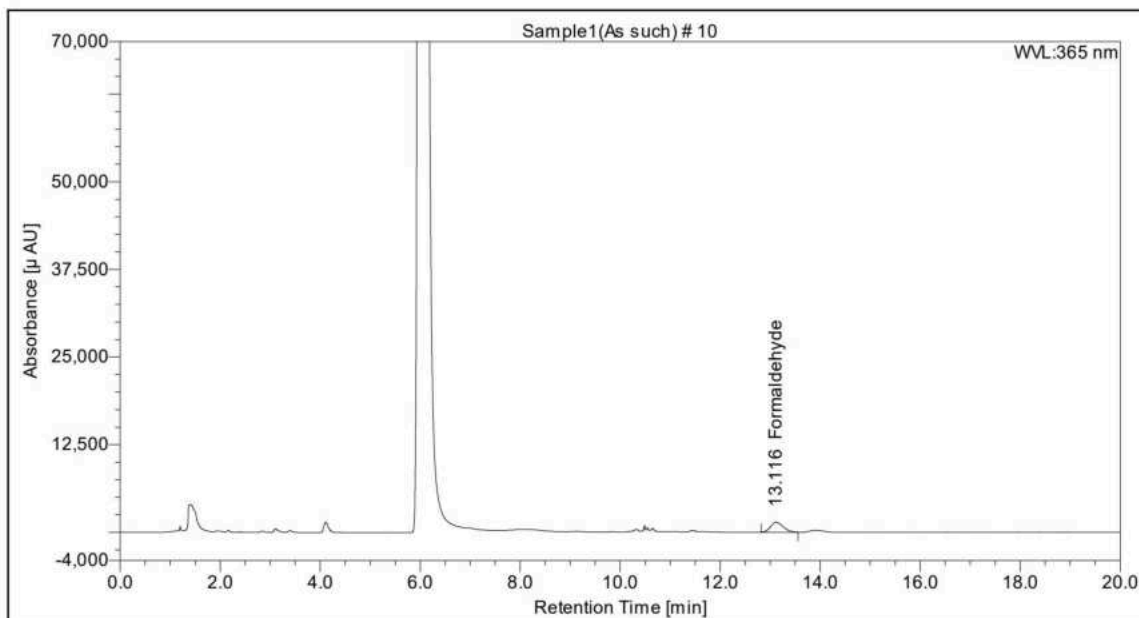
Brand	Batch no. / Lot no.	Formaldehyde content (ppm)
Elle 18_Nailpaint	498	3462
Tips 21_Nailpaint	KDC/267	1745
L'oreal_Nailpaint	16F401	0
Galaxy_Nailpaint	KDC/447	4729
Etios_Nailpaint	KDC/376	146
Alka_Nailpaint	KDC/324	103
Mayuri_Nailpaint	KDC/441	5100
Dolly_Nailpaint	KDC/672	166
Priya Silver_Nailpaint	ML/986	154
Revlon_Nailpaint	F1112401	0
ADS_Lipstick	AO501	3089
Baby Doll_Lipstick	RBI B3429	62
Crystal_Lipstick	K21209	15
Elle18_Lipstick	M DNH/C/33	66



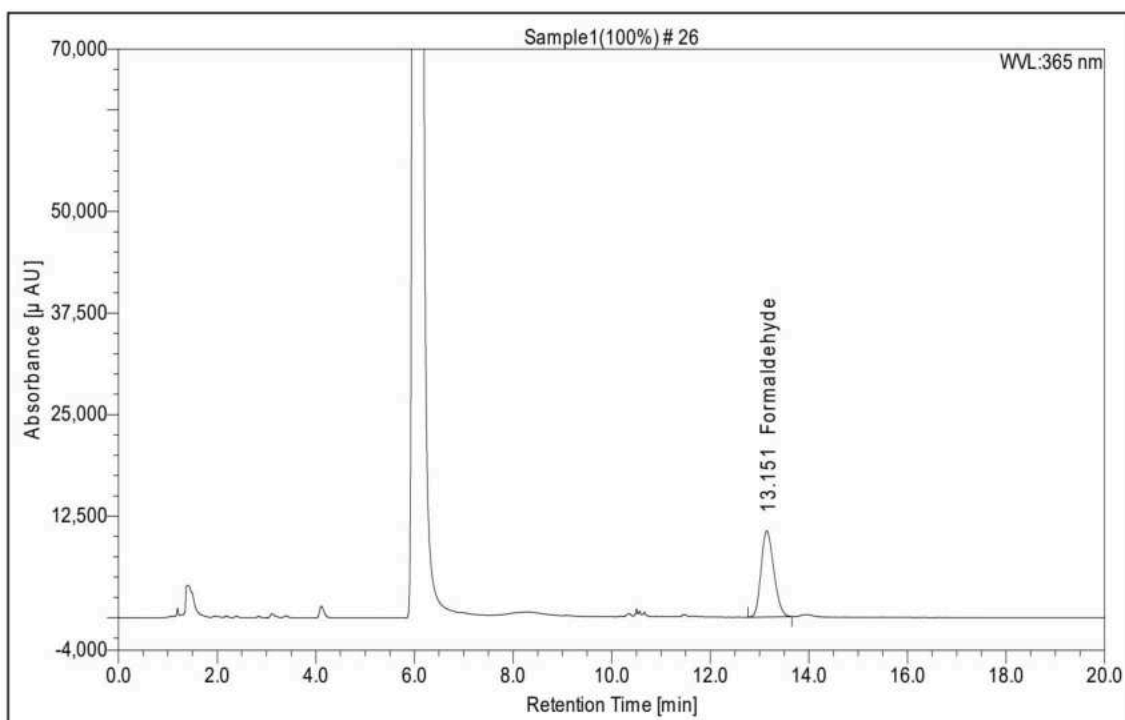
**Figure 1**  
**The reaction between 2, 4-Dinitrophenylhydrazine and Formaldehyde**



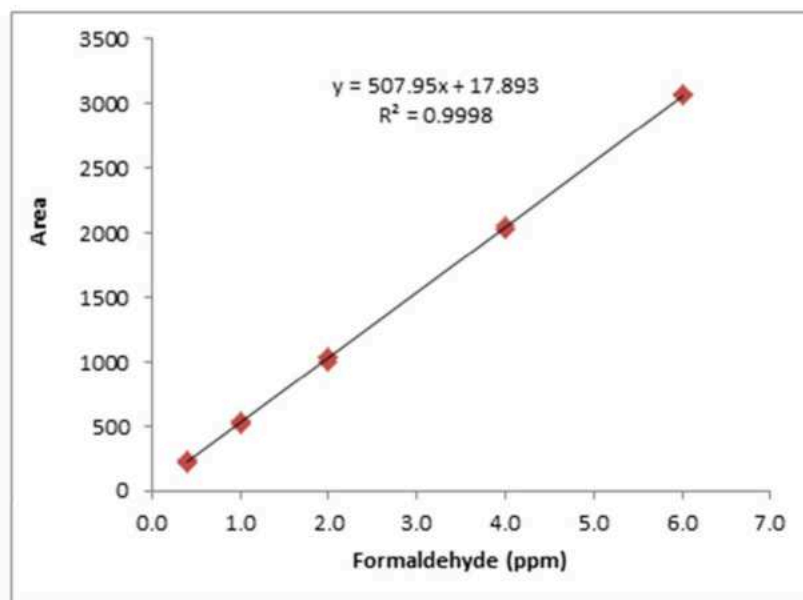
**Figure 2**  
**UV spectrum of formaldehyde derivative scanned on photo diode array**



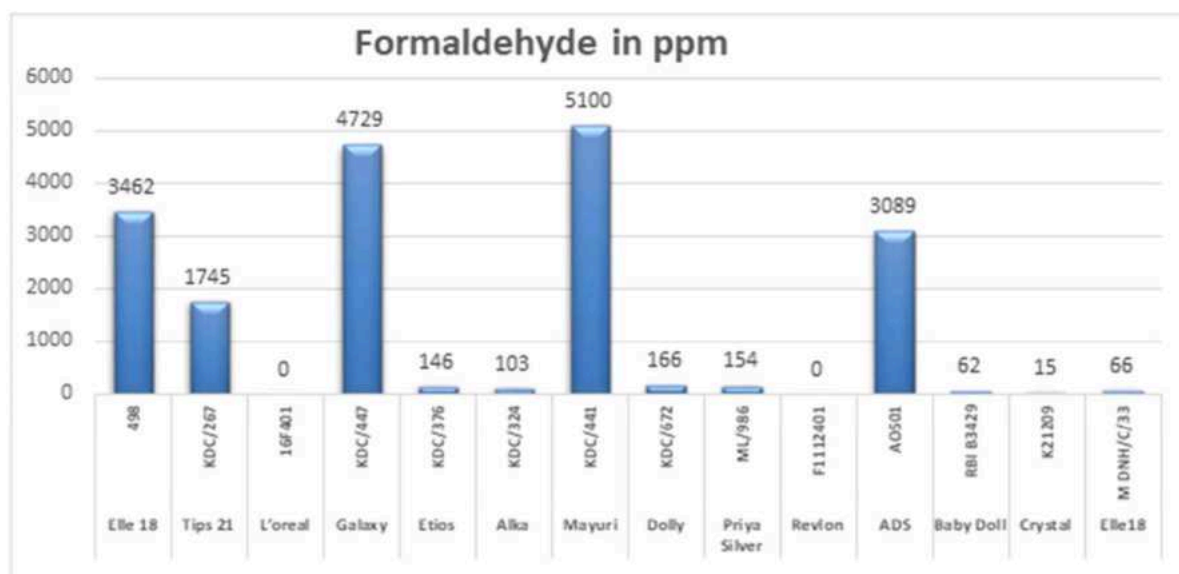
**Figure 3**  
*The typical chromatogram of formaldehyde in Cosmetic sample\_Normal*



**Figure 4**  
*The typical chromatogram of formaldehyde in Cosmetic sample\_Spiked*



**Figure 5**  
*Linearity calibration curve for formaldehyde*



**Figure 6**  
*Typical bar graph showing formaldehyde in marketed cosmetic products*

## CONCLUSION

Method developed for quantification of formaldehyde in cosmetic product is suitable for intended application. Method provides selective quantification of formaldehyde in presence of other aldehydes without interference from blank. This method is validated successfully. Validation data proved that method is Specific, Linear, Accurate, Reproducible and Robust. Method is highly sensitive and capable of determination of formaldehyde in cosmetic samples at trace level as LOQ of the method is as low as 0.4 ppm. This method is successfully applied for quantification of formaldehyde in selected cosmetic samples. Observed results are reproducible. Thus, the proposed RP-HPLC method is novel and very simple chromatographic method for the estimation of trace level (ppm) formaldehyde in cosmetic products including nail paints, lipsticks etc. This method can be applied to determine formaldehyde content in

other cosmetic samples such as creams, shampoos and other skin care products. Further, this method can be used for determination of formaldehyde in chemicals, pharmaceutical products, and food products if sample is soluble in the derivatization solvent and sample matrix does not exhibit absorbance at 365 nm.

## AUTHORS CONTRIBUTION STATEMENT

Prof. Sushama Ambadekar suggested the research topic and guided me for method development and validation study. Chandan Warkar assisted in validation study and selection of market samples. Analysis of marketed samples is done by Deepak Nikam. Chandan Warkar performed calculations for experimental data obtained from validation study and analysis of marketed samples. All authors discussed the results and commented on the manuscript. Deepak Nikam drafted the final manuscript.



**ACKNOWLEDGMENTS**

Authors express sincere thanks to the Director & Head of Chemistry Department, Institute of Science, Mumbai for guidance, encouragement and providing laboratory facilities.

**REFERENCES**

- Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1910.1048. 1998. Available from: <https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1048>
- 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.
- World Health Organization Environmental Health Criteria for Formaldehyde. Geneva, Switzerland: World Health Organization,; 1989. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc89.htm>
- 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. Available from: <https://www.cdc.gov/niosh/docs/97-119/pdfs/97-119.pdf>
- Formaldehyde in Cosmetic products. SGS Hong Kong. 2016 [cited 1BC Feb 16]. Available from: <https://www.sgsgroup.com.hk/en/news/2016/02/formaldehyde-in-cosmetic-products>
- Daoud Agha Dit Daoudy B, Al-Khayat MA, Karabet F, Al-Mardini MA. A Robust Static Headspace GC-FID Method to Detect and Quantify Formaldehyde Impurity in Pharmaceutical Excipients. *J Anal Methods Chem.* 2018;2018:1–8. DOI: 10.1155/2018/4526396
- Dojahn JG, Wentworth WE, Stearns SD. Characterization of Formaldehyde By Gas Chromatography Using Multiple Pulsed-Discharge Photoionization Detectors and a Flame Ionization Detector. *J Chromatogr Sci.* 2001;39(2):54–8. DOI: 10.1093/chromsci/39.2.54
- Michels JJ. Improved measurement of formaldehyde in water-soluble polymers by high-performance liquid chromatography coupled with post-column reaction detection. *J Chromatogr A.* 2001;914(1–2):123–9. DOI: 10.1016/S0021-9673(00)01267-X
- Sebaei AS, Gomaa AM, El-Zwairy AA, Emara EA. Determination of Formaldehyde by HPLC with Stable Precolumn Derivatization in Egyptian Dairy Products. *Int J Anal Chem.* 2018;2018:1–5. DOI: 10.1155/2018/2757941
- Daniela C, Rusmarilin H, Sinaga H. Aloe vera and

**CONFLICT OF INTEREST**

Conflict of interest declared none.

- lemon juice capability in decreasing formaldehyde content in tofu sumedang with cold storing. *IOP Conf Ser Earth Environ Sci.* 2019;260:12089. DOI: 10.1088/1755-1315/260/1/012089
- Martínez-Espinoza E.T., Morales-López G., Segoviano-Garfías J.J.N. M-SM. Determination of Formaldehyde Content in Footwear Samples using a HPLC-UV technique. *Int J Sci Res Publ.* 2015;5(11):225–9. Available from: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.736.2766&rep=rep1&type=pdf#page=234>
- Bhowmik S, Begum M, Hossain MA, Rahman M, Alam AKMN. Determination of formaldehyde in wet marketed fish by HPLC analysis: A negligible concern for fish and food safety in Bangladesh. *Egypt J Aquat Res.* 2017;43(3):245–8. DOI: 10.1016/j.ejar.2017.08.001
- Wahed P, Razzaq MA, Dharmapuri S, Corrales M. Determination of formaldehyde in food and feed by an in-house validated HPLC method. *Food Chem.* 2016;202:476–83. DOI: 10.1016/j.foodchem.2016.01.136
- Borman P, Elder D. Q2(R1) Validation of Analytical Procedures. ICH Quality Guidelines. John Wiley & Sons, Inc.; 2017. p. 127–66. DOI: 10.1002/9781118971147.ch5
- Bliesner. DM. Validating Chromatographic Methods. A Practical Guide; 2009. Available from: <https://www.wiley.com/en-us/Validating+Chromatographic+Methods%3A+A+Practical+Guide-p-9780471741473>
- Regulation (EC) no 1223/2009 of the European parliament and of the council of 30 November 2009 on cosmetic products. Official Journal of the European Union. 2009 [cited 1BC Dec 12]. Available from: [https://ec.europa.eu/health/sites/health/files/endo\\_crine\\_disruptors/docs/cosmetic\\_1223\\_2009\\_regulation\\_en.pdf](https://ec.europa.eu/health/sites/health/files/endo_crine_disruptors/docs/cosmetic_1223_2009_regulation_en.pdf)
- EU Cosmetic Directive 76/768/EEC. Annex III. 2003. Available from: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1976L0768:20100301:en:PDF>
- ASEAN Cosmetics Directive . Annex III, Annex IV. 2018 [cited 1BC Jun 6]. Available from: [https://www.hsa.gov.sg/content/dam/HSA/HPRG/Cosmetic\\_Products/Annexes\\_of\\_the\\_ASEAN\\_Cosmetic\\_Directive\\_\(updated\\_June\\_2018\).pdf](https://www.hsa.gov.sg/content/dam/HSA/HPRG/Cosmetic_Products/Annexes_of_the_ASEAN_Cosmetic_Directive_(updated_June_2018).pdf)
- China Food and Drug Administration. Safety and Technical standards for Cosmetic. 2015 [cited 1BC Dec 1]. Available from: <https://link.springer.com/content/pdf/10.1007%2F978-3-319-15576-0.pdf>