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ACCURATE QUANTIFICATION OF FORMALDEHYDE IN SELECTED COSMETICS BY FAST LIQUID CHROMATOGRAPHY (FAST LC)

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ABSTRACT

An accurate, rapid, specific and highly sensitive short run High performance fast liquid chromatographic (Fast LC) method was developed for the quantification of formaldehyde in selected cosmetic 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 cosmetic products including nail paints, lipsticks etc.

KEYWORDS: Formaldehyde, Fast LC, 2,4-dinitrophenylhydrazine, Cosmetic products, Validation, ICH.

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”.[1] 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.[2] 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.[3] 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.[4] 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%.[5,6,7,8,9] 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.[10,11] Due to lack of intrinsic chromophore or flurophore, volatility and reactivity, it is difficult to detect formaldehyde directly in complex matrices and quantification of formaldehyde requires sample pre-treatment.[12,13] 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.[14,15] Further, some of the methods are time consuming with longer chromatographic run time and longer sample preparation time.[16,17] 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.

Allowable levels of formaldehyde in cosmetic products are tabulated in Table 1.

Table 1: Allowable Levels of formaldehyde in Cosmetics.

Country	Regulation No.	Product	Allowable formaldehyde limit
European countries	Cosmetics Regulation (EC) No. 1223/2009, Article III[6,7]	Nail care products	5% (50000 ppm)
	Cosmetics Regulation (EC) No. 1223/2009, Article V[6,7]	Other products	0.2% (20000 ppm)
ASEAN countries	ASEAN Cosmetics Directive, Annex III[8]	Nail care products	5% (50000 ppm)
	ASEAN Cosmetics Directive, Annex IV[8]	Other products	0.2% (20000 ppm)
China	Safety and Technical Standards for Cosmetics, Table 3[9]	Nail care products	5% (50000 ppm)
	Safety and Technical Standards for Cosmetics, Table 4[9]	Other products	0.2% (20000 ppm)

The formaldehyde content in Cosmetic product shall be restricted below 0.2% and chronic exposure to formaldehyde must be avoided. Chronic exposure of formaldehyde may result in serious health hazards.

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 cosmetic products 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 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.

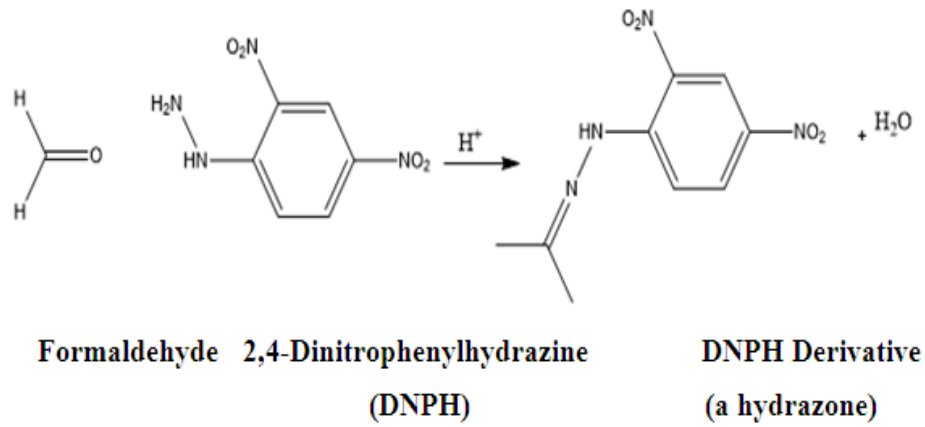

Figure 1: Reaction between 2, 4-Dinitrophenylhydrazine and formaldehyde.

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: Acetonitrile.[18,19] Since formaldehyde do not have chromophore, quantification is done with derivatization technique. 2,4-

dinitrophenylhydrazine is used as derivatization reagent.[20] 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.[21,22] 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 cosmetic products is performed in accordance with ICH Q2 (R1) guideline.[23] Validation was performed for Specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), Linearity, Accuracy, Precision, Robustness, Solution stability and Filter study.[24] Method Validation Experimental Design and Results Summary is tabulated in Table 2. A typical HPLC chromatograms of Blank, Standard and Cosmetic 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 standard solutions in concentration range 0.008 (LOQ) to 0.06 ppm	R = 0.9994 % 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	Analysis of six homogeneous samples.	% RSD = 5.6%

Repeatability Intermediate precision	Comparison of results by two different analysts, analysed on different days and different HPLC's.	% RSD = 6.4% % RSD (Day 1 & Day 2) = 5.9%
Robustness Chromatographic variation Stability of Solutions Filter study	Deliberate changes in chromatographic conditions. Monitoring the area of Formaldehyde peak at selected time intervals, stored at 8°C. Suitability of different makes of 0.45µ nylon filters.	Robust for chromatographic changes. Standard solution and Sample solution are stable at 8°C for 20 hrs. Millipore make and Pall make 0.45µ nylon filters are suitable.

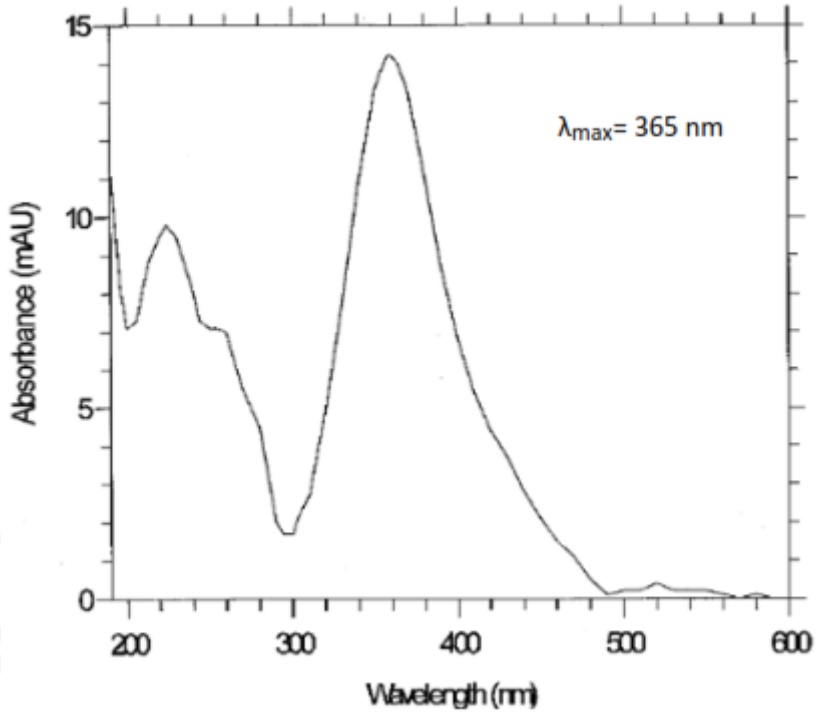

Figure 2: Specificity-UV spectrum of formaldehyde derivative.

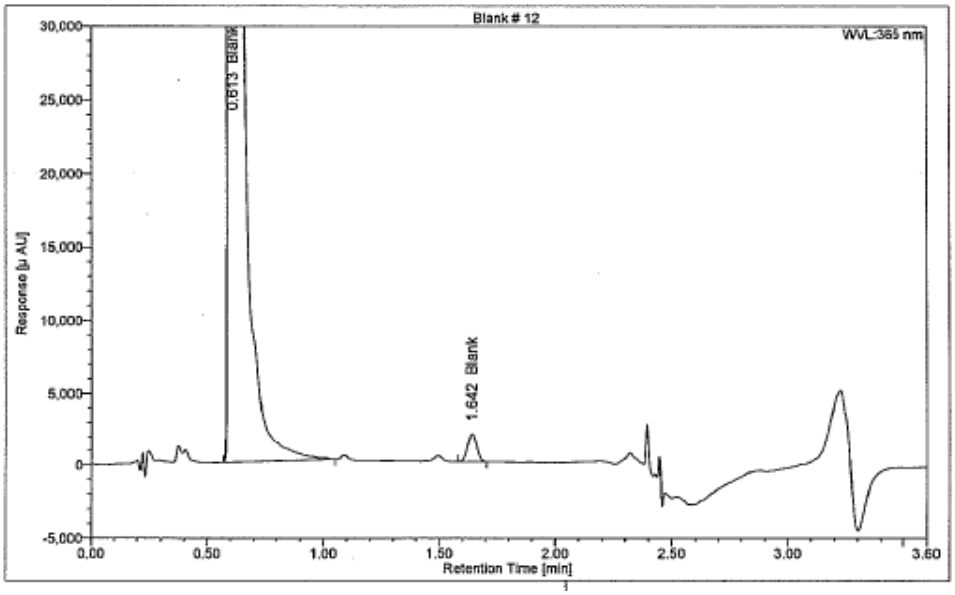

Figure 3: Specificity- Chromatogram of Blank Solution.

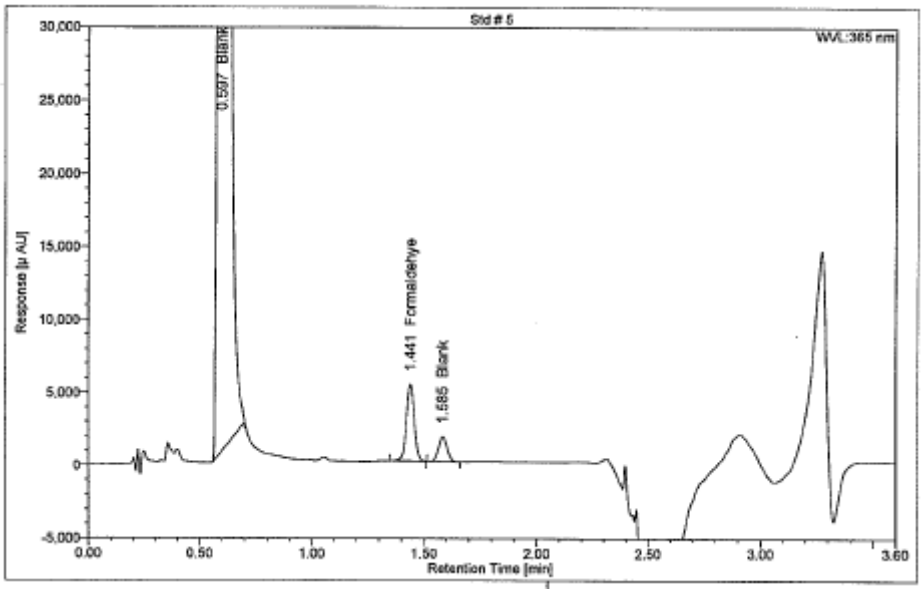

Figure 4: Specificity- Chromatogram of formaldehyde Standard Solution.

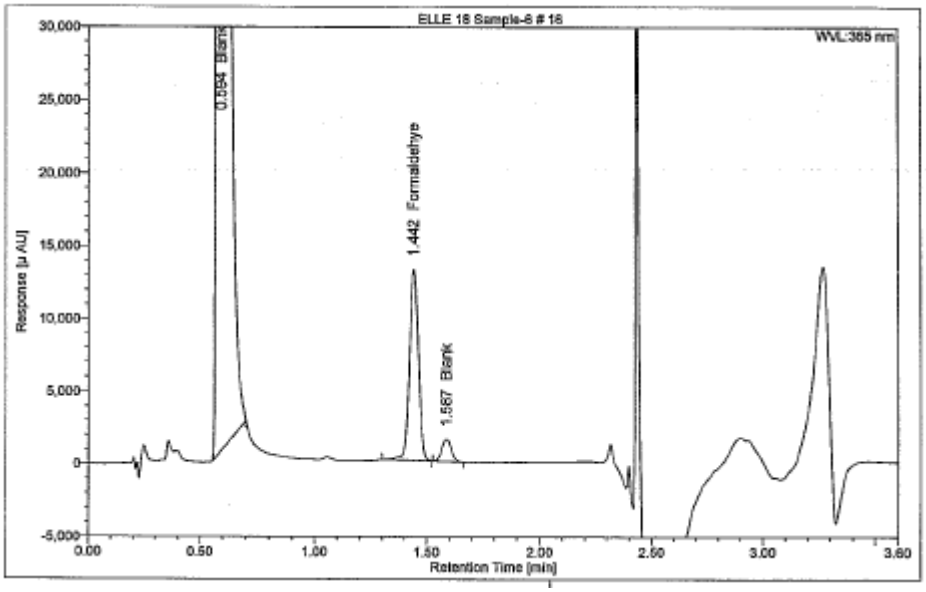

Figure 5: Specificity- Chromatogram of Cosmetic sample (ELLE 18 Nail paint).

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%**	**-**

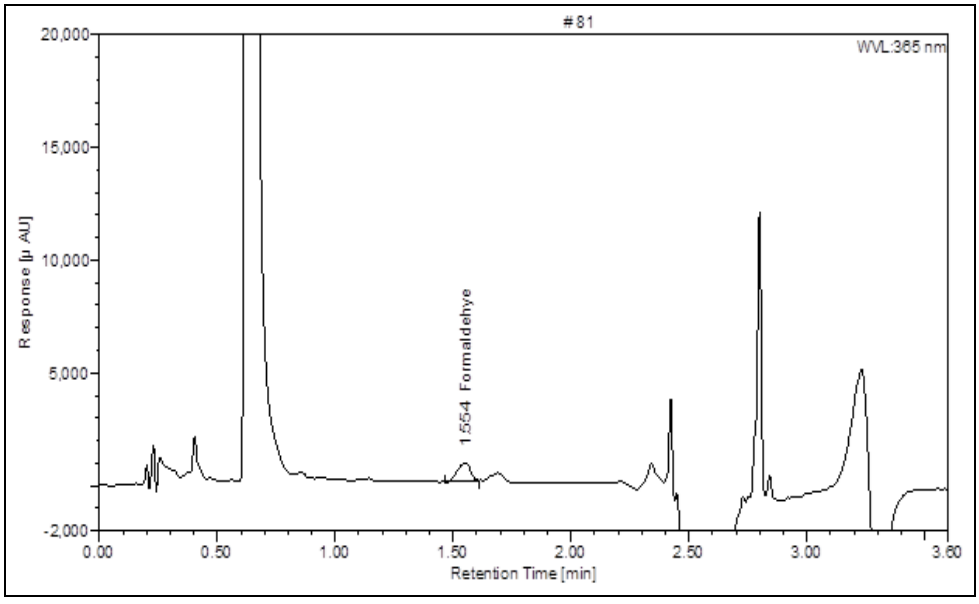

Figure 6: Chromatogram of Limit of Quantification (LOQ).

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

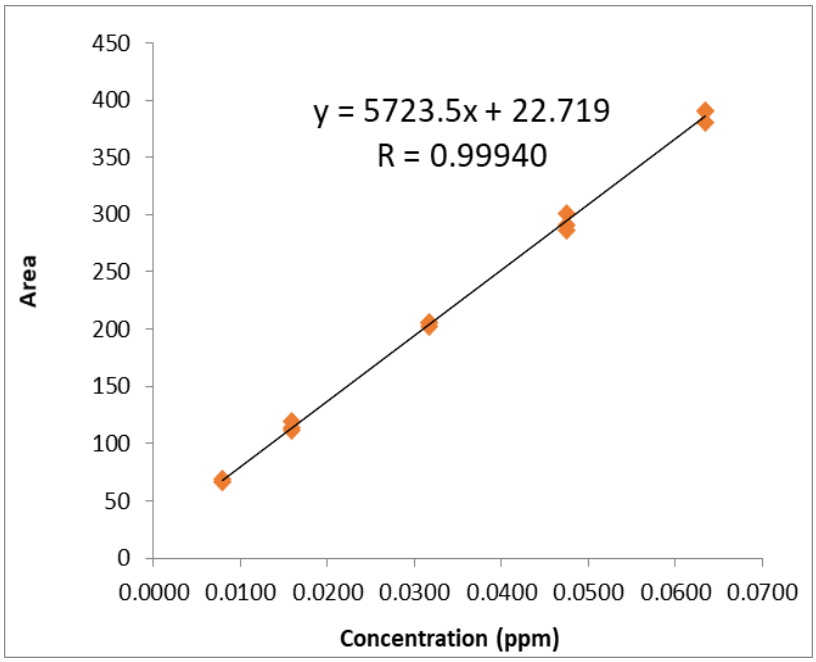

Figure 7: Linearity_Calibration curve for formaldehyde.

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 0.45m nylon filters	3237.7695	32.93
Value prep 0.45m nylon filters	3330.7930	32.10
MDI 0.45m 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 cosmetic products available in market are analysed for formaldehyde content. Results for these cosmetic products are reported in table 11 and represented in Figure 8.

Table 11: Formaldehyde content in Cosmetic products.

Brand	Sample Type	B. no.	Formaldehyde in Cosmetic product (ppm)
HD+	Nail paint	MH-101315	1185
PREFIX	Nail paint	MH/101395	1146
Elle 18	Nail paint	B 562	4080
HD Velvet	Nail paint	KDC-376	2310
CEREMA	Nail paint	MH/101320	3606
Pepsodent	Toothpaste	B180	186
Closeup	Toothpaste	B900	2115
Colgate	Toothpaste	B18	375
Dabar Red gel	Toothpaste	BD0076	1080
TRESemme	Shampoo	A8100019M16	203
L'OREAL	Shampoo	B507757N	608
Clinic plus	Shampoo	B708	2865
Mediker	Shampoo	KKB0029	870
Pantene	Shampoo	B715911	905
Sunsilk	Shampoo	0140319B09	1100

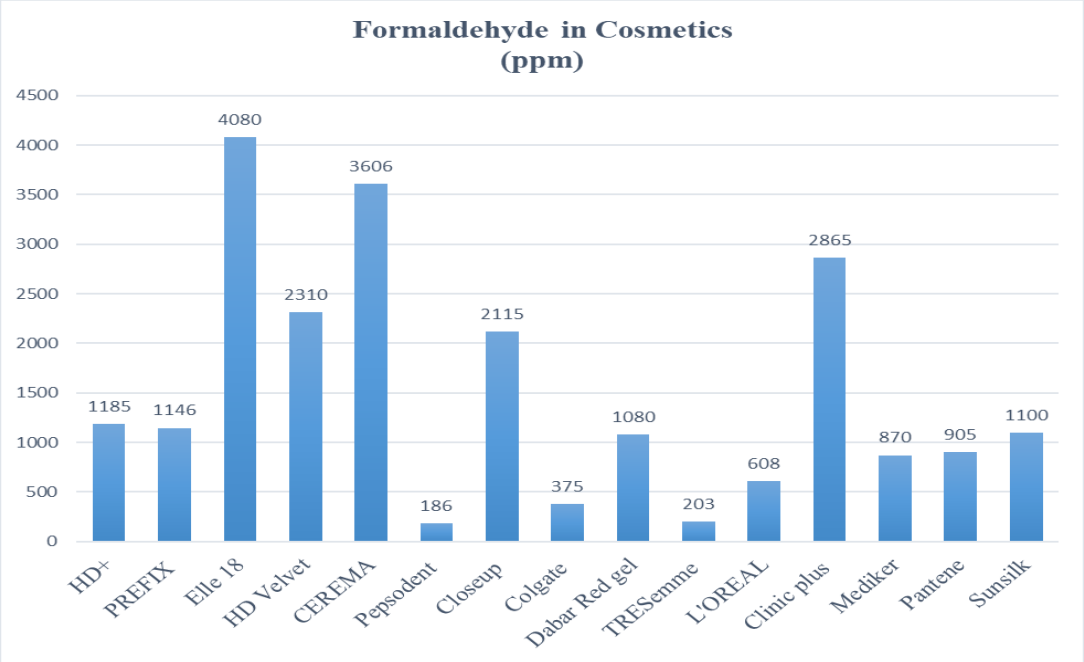

Figure 8: Formaldehyde in Cosmetic products.

CONCLUSION

This paper presents the development and validation of a simple gradient High Performance Fast Liquid Chromatography (Fast LC) procedure suitable for the analysis of formaldehyde in selected cosmetic products. It is demonstrated that developed analytical procedure is sensitive, accurate, precise, and robust as 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, 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 of the cosmetic products is close to 5000 ppm, which is significant compared to tolerance levels defined by European and Asian regulatory authorities. In such case, Quantitative determination of the formaldehyde levels in cosmetic products is very important as chronic exposure to Formaldehyde can result is serious health hazards.

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