Development and Validation of Colorimetric and RP-HPLC Methods for the Determination of Formaldehyde in Cosmetics

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MMR performed the analytical studies and wrote the first draft of the manuscript. Author SB designed the study, wrote the protocol, managed the analyses of the study and wrote the final draft of the manuscript. Author HOR managed the literature searches and author ASSR supervised the overall study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To develop and validate two assay methods for the determination of total and free formaldehyde content in cosmetics by Colorimetric and High Performance Liquid Chromatographic (HPLC) methods, respectively.

Study Design: Colorimetric and HPLC methods.

Place and Duration of Study: Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh; between February 2012 and June 2013.

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Methodology: In Colorimetric method the quantitation was performed by a UV/Vis spectrophotometer at 410 nm and by HPLC at 345 nm and the methods were linear over the concentration ranges of 4-12 mg/L ($R^2=$0.999) and 2-32 mg/L ($R^2=$0.999), respectively. Based on 2, 4-dinitrophenylhydrazine (DNPH) derivatization, free formaldehyde was analyzed using a Phenomenex C18 (250 mm × 4.6 mm i. d., 5 μm particle size) column in a binary separation mode with mobile phase consisting of acetonitrile and distilled water (45:55, v/v) and flow rate of 2.0 mL/min.

Results: The overall recovery rate of free formaldehyde was (95.33-98.37)±(0.29-1.6)% (RSD). Seventy five cosmetics of local and foreign brands were investigated. None of those products was labeled to contain formaldehyde. 18 products were labeled with specific formaldehyde donors (17 with DMDM hydantoin and 1 with imidazolidinyl urea). 64% of the products were formaldehyde positive among which 54% exceeded 0.05% limit of formaldehyde content for which EU dictates the mandatory formaldehyde donors labeling. The investigated amounts of the total formaldehyde and free formaldehyde were 190-5502 mg/L and 47-604 mg/L, respectively.

Conclusion: The methods were found valid as per in terms of suitability, linearity, accuracy, sensitivity, ruggedness, and robustness. The formaldehyde content found in the cosmetics was highly alarming and must be addressed by the authority in an immediate basis.

1. INTRODUCTION

Formaldehyde is a gas with a pungent smell. Pure formaldehyde is not available commercially but is sold as 30–50% aqueous solutions and 37% w/v formaldehyde water solution is termed as formalin [1].

Different types of antimicrobial preservatives are used in cosmetics. Formaldehyde and formaldehyde donors are widely used preservatives and represent an important group of skin sensitizers [2]. Formaldehyde donors are commonly used instead of formaldehyde, and release small amounts of formaldehyde over time [3]. These preservatives manifest antibacterial effect owing to their chemical compositions and on their decomposition formaldehyde is released [4]. Formaldehyde donor sensitization is ascribable to released formaldehyde [3].

The legally allowed maximum concentration of formaldehyde is 0.2% in cosmetics as authorized by the European Union (EU) [5]. In accordance with the EU, the maximum amount of these formaldehyde donors in cosmetics should be around 0.1–0.6% [1]. According to Annex VI of the Cosmetic Directive 76/768/EC, all finished products containing formaldehyde or substances in this Annex and which release formaldehyde must be labeled with the warning "contains formaldehyde" where the concentration of formaldehyde in the finished product exceeds 0.05% [6]. In Bangladesh, there is no regulation on the usage of formaldehyde or formaldehyde donors in cosmetics. There are limitations for the use of different formaldehyde donors in cosmetics which are delineated in Table 1 [7].

Table 1. Limit of contents of different formaldehyde donors in cosmetics

<table>
<thead>
<tr>
<th>Formaldehyde donors</th>
<th>Limit of content in cosmetics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazolidinyl urea</td>
<td>0.6</td>
</tr>
<tr>
<td>Diazolidinyl urea</td>
<td>0.5</td>
</tr>
<tr>
<td>DMDM hydantoin</td>
<td>0.6</td>
</tr>
<tr>
<td>Quaternium 15</td>
<td>0.2</td>
</tr>
<tr>
<td>Bronopol</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The acetylacetone colorimetric method is a widely applied standard procedure [8] and recommended in Europe for the determination of total formaldehyde [9]. The reaction, which is based on the Hantzsch synthesis, involves the cyclization of acetylacetone, ammonium acetate, and formaldehyde to form the dihydropyridine: 3, 5-diacetyl-1, 4-dihydrolutidine (DDL).

In the HPLC method, hydrazones are formed from DNPH by nucleophilic addition to the carbonyl group, followed by elimination of water in acidic solution which is then detected.

Formaldehyde can also be found in different drug substances as by-products while synthesizing them and can be detected by the proposed HPLC method [10].

As people are directly exposed to cosmetics almost every day, the potential health risks...
associated with the preservatives used are very high. The present study is intended to determine the amount of formaldehyde present in the cosmetics of marketed in Bangladesh which will bring about awareness among the general public of the country and will facilitate the establishment of a regulatory limit. To the best of our knowledge, this is the first paper for the analysis of formaldehyde in cosmetics in Bangladesh.

2. METHODOLOGIES

2.1 Types of Samples

Seventy five cosmetics were purchased from departmental stores, supermarkets and cosmetic shops in Dhaka, Bangladesh during 2012 and classified as following:

1. Rinse-off products: Shampoos, hair conditioners, face washes, and body cleaners.
2. Leave-on products: Skin creams and body lotions.

Among them, there were 34 domestic products including 23 leave-on and 11 rinse-off products; and 41 imports including 20 leave-on and 21 rinse-off products. No products were labeled 'formaldehyde contained' in these 75 cases. Out of these 75 products, 18 were labeled containing formaldehyde donors (such as DMDM hydantoin and imidazolidinyl urea) including 7 domestics and 11 imports. Four out of these 7 domestics were rinse-off products and rests of the 3 were leave-on products. Eight out of 11 imports were rinse-off products and rests of the 3 were leave on products. They are summarized in Table 2.

2.2 Purity Test of Formaldehyde

Analytical grade formaldehyde solution (37% purity) was purchased from Merck, Germany was used as standard. The standard was stored in a freezer at 0°C. To confirm the purity of the solution, the following test was performed [11].

One gram of formaldehyde solution was taken into a 100 mL volumetric flask and then diluted with distilled water to the mark. 50 mL of 0.1 N iodine solution and 20 mL of 1 N potassium hydroxide solution was taken in a volumetric flask and 10 mL of the diluted formaldehyde solution was added to it and this solution was kept into the dark chamber for about 15 minutes which was followed by an addition of 5 mL of 30% sulfuric acid solution. Titration of the excessive iodine was performed by 0.1 N sodium thiosulphate where 1 mL starch solution was used as indicator solution. To estimate the consumption of 0.1 N sodium thiosulphate, a blank test was performed using 10 mL of distilled water. Each mL of titrated 0.1 N iodine was found equivalent to 1.5013 mg formaldehyde.

So, Formaldehyde content

\[
\text{Formaldehyde content (\%) = } \frac{1.5013 \times (V_o - V) \times F}{\text{formaldehyde solution (g)}} \times 100\%
\]

Where, \(V\) is the depleted 0.1 N sodium thiosulphate solution (mL), \(V_o\) is the depleted 0.1 N sodium thiosulphate solution (mL) in blank test, and \(F\) is the valence of 0.1 N sodium thiosulphate solution.

2.3 Analysis of Total Formaldehyde by Colorimetric Method

2.3.1 Chemicals and reagents

Acetylacetone (Qualichems, India), ammonium acetate (BDH, England), acetic acid (NC-LR, Germany), sodium sulphate (Merck, Germany), and purified water were used for the analysis.

2.3.2 Solution preparation

2.3.2.1 Formaldehyde standard solution

37% formaldehyde solution was suitably diluted with distilled water to 4, 6, 8, 10, and 12 mg/L to be standard solutions.

2.3.2.2 Acetylacetone solution

Acetylacetone solution was prepared by dissolving 150 g of ammonium acetate in distilled water, followed by adding 3 mL of acetic acid and 2 mL of acetylacetone and diluting with distilled water again to a total volume of 1000 mL.

2.3.2.3 Ammonium acetate solution

Ammonium acetate solution was prepared by dissolving 150 g of ammonium acetate in distilled water, followed by adding 3 mL of acetic acid and diluting with distilled water again to a total volume of 1000 mL.

2.3.2.4 25% sodium sulphate solution

Two hundred and fifty grams of sodium sulphate was taken into a 1000 mL volumetric flask,
Table 2. Types and sources of cosmetic samples

<table>
<thead>
<tr>
<th>Source</th>
<th>Leave-on products</th>
<th>Rinse-off products</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labeled with formaldehyde donors</td>
<td>No. of samples</td>
<td>Labeled with formaldehyde donors</td>
</tr>
<tr>
<td>Domestic</td>
<td>3</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Imported</td>
<td>3</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>43</td>
<td>12</td>
</tr>
</tbody>
</table>

followed by adding some amount of distilled water and shaken well until dissolved. Finally, it was diluted to the volume to prepare the solution.

2.3.3 Instrumentation

The total formaldehyde study employed UV/Visible spectrophotometer (Shimadzu UV-1650 PC, Shimadzu, Japan). After addition of acetylacetone and ammonium acetate, the solution was heated for 30 minutes at 40°C to complete the formation of DDL. Quantitation was performed by UV/Visible spectroscopy at 410 nm. A centrifuge machine (Heraceus Sepatech, Germany) was used in the analysis.

2.3.4 Preparation of standard calibration curve

(a) 5 mL of formaldehyde standard solutions in five different concentrations (4, 6, 8, 10 and 12 mg/L) were taken into 10 different test tubes in which two standard solutions of each five different concentrations were present. Same procedure was conducted for 5 mL of distilled water. (b) 5 mL acetylacetone solution was added into the 1st 5 test tubes of and 5 mL of ammonium acetate solution was added to the 2nd 5 test tubes. (c) All the test tubes were water-bathed at 40°C for 30 minutes and cooled down for 30 minutes. (d) Absorbance was measured at 410 nm. (e) Their absorbance were designated as $A_{S1}$, $A_{S2}$, $A_{S3}$, $A_{S4}$ and $A_{O1}$ respectively for the 1st 5 test tubes of series-1; and $A_{S5}$, $A_{S6}$, $A_{S7}$, $A_{S8}$ and $A_{O2}$ respectively for the 2nd 5 test tubes of series-2. (f) Further, their absorbance were designated as per as the Table 3. (g) The absorbance of water was designated as $A_O$, where $A_O = A_{O1}$, $A_{O2}$ (h) formaldehyde standard curve in this acetylacetone colorimetry approach was constructed by plotting (As-Ao) versus concentration using Microsoft Office Excel 2007 software.

2.3.5 Sample preparation

One gram sample was weighed and put in a centrifuge tube. 20 mL of 25% sodium sulfate solution was added to it and then the tube was shaken followed by the addition of distilled water to make the total volume of 40 mL. After that, the tube was water-bathed for 1 hour at 40°C and then was cooled down at room temperature. Then it was centrifuged for 10 minutes at 3500 rpm and the supernatant was filtered.

From the filtrate, 5 mL of sample solution was taken out twice and put into two new test tubes marked as Sample I and Sample II. 5 mL of acetylacetone solution was added in Sample I and 5 mL of ammonium acetate solution was added in Sample II. Both the test tubes were then water bathed for 30 minutes at 40°C and then again cooled down at room temperature for 30 minutes. Absorbance was measured for both Sample I and Sample II at 410 nm and designated as $A_{I}$ and $A_{II}$, respectively.

2.4 Analysis of Free Formaldehyde by HPLC Method

This procedure is based on dilution of the samples with THF-water in a ratio of 9:1. As formaldehyde does not possess chromophore, the developed HPLC method involves pre-column derivatization with DNPH and direct reversed phase HPLC. The formaldehyde derivative is stabilized in the reaction medium by addition of phosphate buffer. The method is also suitable for the direct evaluation of the formaldehyde donors used in cosmetics as preservatives.

UV/Vis spectroscopy is used for detection, with the absorption maxima of different hydrazones ranging from 340 to 427 nm. This method is widely used for the determination of free formaldehyde in different substances [10].
Table 3. Absorbance of various concentrations of formaldehyde standard solutions

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Absorbance, As (Absorbance with acetylacetone solution - Absorbance with ammonium acetate solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>As1 = Asi - Asa</td>
</tr>
<tr>
<td>6</td>
<td>As2 = Asii - Asb</td>
</tr>
<tr>
<td>8</td>
<td>As3 = Asiii - Asc</td>
</tr>
<tr>
<td>10</td>
<td>As4 = Asiv - Asd</td>
</tr>
<tr>
<td>12</td>
<td>As5 = Asv - Asf</td>
</tr>
</tbody>
</table>

### 2.4.1 Chemicals and reagents

Sodium hydroxide pellets (Merck, India), disodium hydrogen phosphate (Fisher Scientific, India), sodium dihydrogen phosphate (Fisher Scientific, India), DNPH (Merck, Germany), tetrahydrofuran (THF) (Fisher Scientific, USA), HPLC grade acetonitrile (RCI Labscan Ltd. Thailand) and HPLC grade water were used for analysis.

### 2.4.2 Solution preparation

#### 2.4.2.1 THF solution

This solution was prepared by THF and distilled water in a ratio of 9:1 (v/v). 900 mL THF was taken in a 1000mL volumetric flask followed by addition of distilled water up to the mark.

#### 2.4.2.2 Formaldehyde standard solution

37% formaldehyde solution was diluted with THF to 2, 4, 8, 16, and 32 mg/L to be standard solutions.

#### 2.4.2.3 2 N hydrochloric acid solution

One hundred milliliter of distilled water was taken into a 500 mL volumetric flask followed by adding 98.65 mL of 37% hydrochloric acid (density 1.3) drop by drop and diluted to volume with distilled water.

#### 2.4.2.4 0.1% DNPH solution

Five hundred milligrams of DNPH was dissolved in 400 mL of 2 N hydrochloric acid solution in a 500 mL volumetric flask and further diluted with distilled water to the volume.

#### 2.4.2.5 0.1 M phosphate buffer (pH = 6.8)

An amount of 8.775 grams of disodium hydrogen phosphate and 3.519 grams of sodium dihydrogen phosphate were taken into a 500 mL volumetric flask. Some amount of distilled water was added and shaken until dissolved and finally diluted to volume with distilled water. It was then calibrated to ensure the pH.

### 2.4.3 Instrumentation

The free formaldehyde study employed HPLC (Shimadzu LC-20 AT integrated with Prominence software, Shimadzu, Japan) equipped with UV/Visible detector (Shimadzu SPD 20 A) and degasser (Shimadzu DGU 20 A3). A 20 μL sample solution was analyzed with a Phenomenex C18 (250 mm × 4.6 mm, 5 μm) column with Acetonitrile: Water (45:55, v/v) as mobile phase in a binary separation mood at ambient temperature and detected at the wavelength of 345 nm. The flow rate was 2.0 mL/min.

### 2.4.4 Derivatization procedure and measurement of derivatization time

The derivatization time was calculated by injecting a particular standard formaldehyde solution (here, 16 mg/L formaldehyde standard solution was used) at different time intervals ranging from 1 minute to 7 minutes. It was done in the following way:

One milliliter of standard 16 mg/L formaldehyde solution was mixed with 0.45 mL of 0.1% DNPH and shaken for 2 minutes. The solution was then mixed with 0.4 mL of 0.1 M phosphate buffer (pH 6.8) and 1.4 mL of 1 M sodium hydroxide. The mixture of the solution was shaken for 1 minute and then kept standing for 1 minute and then injected. Prior to injection, the final solution was
filtered using disposable syringe filtering cartridges with 0.45 µm nylon membranes. The same procedure was continued for the same standard formaldehyde solution and the final mixture was shaken for 1 minute and kept standing for 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, and 7 minutes respectively, before they were injected.

The derivatization time of the standard solution with DNPH was plotted against its corresponding peak area. From the plot it can be observed that, the peak area increases up to 4 minutes and then decreases again (Fig. 1).

So, for the further analysis of the standard and samples, the final mixture was shaken for 1 minute and then kept standing for 4 minutes prior to the injection.

2.4.5 Preparation of standard calibration curve

One milliliter of standard formaldehyde solution was mixed with 0.45 mL of 0.1% DNPH and shaken for 2 minutes. The solution was then mixed with 0.4 mL of 0.1 M phosphate buffer (pH 6.8) and 1.4 mL of 1 M sodium hydroxide. The mixture of the solution was shaken for 1 minute and then kept standing for 4 minutes and then injected. Prior to injection, the final solution was filtered using disposable syringe filtering cartridges with 0.45 µm nylon membranes. This procedure was carried on for all the five different concentrations of standard formaldehyde solution. The mean peak area for each concentration was calculated for six replicates. Formaldehyde standard curve in this HPLC approach was constructed by plotting peak area versus concentration using Microsoft Office Excel 2007 software.

2.4.6 Sample preparation

One gram of sample was dissolved in THF solution. Solutions were shaken to homogeneity and diluted properly. The solution was then filtered with Whatman no. 41 filter paper for further use.

2.5 Analytical Method Validation Parameters

2.5.1 System suitability

To assess system suitability, the repeatability of six replicates of standard formaldehyde of concentration 8 mg/L were used by colorimetric method and 16 mg/L were used in case of HPLC method. The %RSD values were calculated in both cases.

Fig. 1. Effect of derivatization time on peak area
2.5.2 Linearity

In case of colorimetric method, the linearity was analyzed through the standard curves ranging from 4 to 12 mg/L by diluting appropriate amounts of formaldehyde standard solution (37%) with distilled water; whereas in case of HPLC method, it was from 2 to 32 mg/L; where formaldehyde standard solution was diluted with THF. In both cases, the solutions were prepared in triplicate. The linearity was then evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

2.5.3 Accuracy

For both standard and sample solutions, accuracy study of the method was carried out by HPLC method. In case of standard solution; standard solutions of formaldehyde, corresponding to 12.5, 25, 50, 100, and 200% of the nominal analytical concentration of formaldehyde (16 mg/L) were compared with reference standard solution of formaldehyde of known purity (16 mg/L), and the percent recoveries (mean ± %RSD of three replicates) of formaldehyde were calculated.

In case of sample solution, accuracy parameter was determined by the recovery test, which consisted of adding known amounts of formaldehyde to the samples’ solutions in the beginning of the process. This test was accomplished by assaying five different solutions, three replicates each, containing 2, 4, 8, 16, and 32 mg/L of formaldehyde standard solution added to a cosmetic sample solution (sample no. 69). Finally, the concentration was determined in three replicates and the percent recoveries (mean ± %RSD of three replicates) of formaldehyde were calculated.

2.5.4 Sensitivity

LOD and LOQ were determined using calibration curve method according to ICH Q2 (R1) recommendations. The LOD (k = 3.3) and LOQ (k = 10) of the proposed methods were calculated using the following equation:

\[ A = k\sigma/S \]

Where, A is LOD or LOQ, \( \sigma \) is the standard deviation of the response, and S is the slope of the calibration curve.

2.5.5 Ruggedness

Ruggedness was determined by analyzing the concentration of a sample (sample no. 69) in six replicates (Concentration ± %RSD) in case of colorimetric method and by analyzing six formaldehyde standard solutions having the concentration of 16 mg/L (%Recovery ± SD) in case of HPLC method by two analysts in the same laboratory under the same analytical conditions.

2.5.6 Robustness

To determine the robustness of the colorimetric method, the effect of change in the wavelength was studied at 405 and 415 nm instead of 410 nm by analyzing six formaldehyde standard solutions having the concentration of 8 mg/L, whereas in case of HPLC method, the effect of flow rate was studied at 1.9 and 2.1 mL/min instead of 2.0 mL/min. The effect of mobile phase composition was assessed at (ACN: Water = 40: 60, v/v) and (ACN: Water = 50: 50, v/v) instead of (ACN: Water = 45: 55, v/v). The %RSD of robustness testing under these conditions was calculated in all cases.

3. RESULTS AND DISCUSSION

3.1 Method Validation

Validation was performed for both colorimetric and HPLC methods.

3.1.1 System Suitability

The results (Mean ± %RSD of six replicates) obtained by the proposed two methods are shown in Table 4, suggesting the good performance of the system.

3.1.2 Linearity

The regression equations obtained were: \( Y = 0.027X - 0.066 \), where Y is absorbance and X is formaldehyde concentration in mg/L and \( Y = 30015X + 626 \), where Y is peak area of the derivatization product and X is formaldehyde concentration in mg/L for colorimetric and HPLC methods, respectively. The correlation coefficient of 0.999 (n = 5) in both cases proved excellent linearity over the concentration range of 4-12 mg/L (colorimetric method) and of 2-32 mg/L (HPLC method). Formaldehyde concentration was then determined from the regression equation.
3.1.3 Accuracy

The overall results of percent recoveries (mean ± %RSD) of formaldehyde in standard and sample solutions by HPLC method are evidenced in Table 5.

The overall recovery rate lies between 95.33% and 98.37% with an average of 96.85% and a RSD of 0.29 - 1.6% over concentrations ranging from 2 to 32 mg/L of formaldehyde indicating good accuracy of the proposed HPLC method.

3.1.4 Sensitivity

The LOD and LOQ of formaldehyde were found 0.8 mg/L and 2.4 mg/L, respectively, by colorimetric method and were found 0.5 mg/L (S/N = 3.217) and 1.5 mg/L (S/N = 9.845), respectively, by HPLC method (Figs. 2 and 3).

Table 4. System suitability of the proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Colorimetric method</th>
<th>HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.153±0.64</td>
<td>489051±0.36</td>
</tr>
<tr>
<td>Peak area</td>
<td></td>
<td>489051±0.36</td>
</tr>
<tr>
<td>Retention time</td>
<td>15.32±0.66</td>
<td>15.32±0.66</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.167±1.04</td>
<td>1.167±1.04</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>7767±0.075</td>
<td>7767±0.075</td>
</tr>
</tbody>
</table>

*Mean ± % RSD of six replicates

Table 5. Accuracy studies of formaldehyde in standard and sample solutions

<table>
<thead>
<tr>
<th>Concentration of formaldehyde added (mg/L)</th>
<th>Recovery (Mean ± %RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard solution</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>95.67±1.59</td>
</tr>
<tr>
<td>4</td>
<td>97.33±1.19</td>
</tr>
<tr>
<td>8</td>
<td>95.33±1.60</td>
</tr>
<tr>
<td>16</td>
<td>98.09±0.69</td>
</tr>
<tr>
<td>32b</td>
<td>97.50±0.89</td>
</tr>
<tr>
<td>Sample solution (Sample no. 69)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>96.67±0.59</td>
</tr>
<tr>
<td>4</td>
<td>98.17±0.29</td>
</tr>
<tr>
<td>8</td>
<td>96.95±0.60</td>
</tr>
<tr>
<td>16</td>
<td>98.37±0.34</td>
</tr>
<tr>
<td>32b</td>
<td>98.13±0.52</td>
</tr>
</tbody>
</table>

*Mean and RSD of three replicates; bSuitably diluted to the linearity range

Fig. 2. LOD chromatogram
3.1.5 Ruggedness

The results obtained by colorimetric method (concentration ± %RSD of six assay samples) and the results obtained by HPLC method (% Recovery ± Standard Deviation (SD) of six assay samples) are given in Tables 6 and 7 respectively, indicating the ruggedness of the proposed methods.

3.1.6 Robustness

The % RSD of robustness testing under different altered conditions is given in Table 8, indicating that the proposed methods are robust.

3.2 Quantitation of Formaldehyde by Colorimetric Method

Different types of cosmetics of both home and abroad were bought and primarily subjected to colorimetric analysis. Formaldehyde donors liberated formaldehyde in different extent. Those that partially liberated formaldehyde were called bonded formaldehyde and that liberated formaldehyde freely were called free formaldehyde. Colorimetric method was used to determine the total formaldehyde, that is, the summation of bonded and free formaldehyde content in the cosmetics.

Solution concentration was obtained by substituting the absorbance of (AI-AII) into the regression equation. Total formaldehyde content in samples was calculated by multiplying the concentration with the volume and dividing by the sample weight.

3.2.1 Formaldehyde content survey on 75 cosmetic products

Forty eight out of 75 products, that is, 64% of the products were found to be formaldehyde positive. The general findings of formaldehyde content by colorimetric method are summarized in the Table 9.

Eighteen out of 34 domestic and 30 out of 41 imported products were found formaldehyde positive. The measured formaldehyde content in the domestic products ranged from 267 to 4841 mg/L; whereas, in case of imported products it ranged from 190 to 5502 mg/L.

Out of 48 formaldehyde positive products, 26 exceeded 0.05% limit of formaldehyde content, for which EU dictates the mandatory formaldehyde donor labeling. That is, 54% of the formaldehyde positive products were above 0.05% limit.

Seventeen out of 18 products labeled with formaldehyde donors were found formaldehyde positive. 17 contained DMDM hydantoin and 1 contained imidazolidinyl urea. The formaldehyde content of the products labeled with DMDM hydantoin and imidazolidinyl urea ranged from 1163 to 5502 mg/L and 2533 mg/L, respectively.

3.2.2 Different types of formaldehyde positive cosmetic products

Cosmetic products such as shampoos, conditioners, face washes, lotions, creams, and hair gels were analyzed. They are summarized in Table 10.

Among the cosmetics tested, all the shampoos and conditioners were formaldehyde positive. The measured formaldehyde content for shampoos and conditioners ranged from 190 to 5502 mg/L and 213 to 1899 mg/L, respectively. The ranges of formaldehyde content in case of face washes, lotions, creams and hair gels were 240 to 4530 mg/L, 228 to 4000 mg/L, 267 to 2533 mg/L, and 240 to 2172 mg/L, respectively.

75% shampoos, 33.33% conditioners, 42.86% face washes, 33.33% lotions, and 10.53% creams tested contained formaldehyde greater than 0.05% limit. Only one domestic hair gel contained formaldehyde greater than 0.05% limit and the measured formaldehyde content was 2173 mg/L.

3.3 Quantitation of Free Formaldehyde by HPLC Method

The derivatization procedure was conducted and the stability of the derivative was calculated. As shown in Fig. 4, the maximal value can be obtained in four minutes.

Derivatization of formaldehyde could be done with p-Nitrophenylhydrazine, dansyl hydrazine, N-methyl-2,4-dinitrophenylhydrazine, N-methyl-4-hydrazino-7-nitrobenzofuran, or N-methyl-4-N',N'-dimethylamino-6-(4'-methoxy-1'-napthyl)-1,3,5-triazine-2-hydrazine instead of DNPH, followed by analyzing with HPLC-UV/Vis spectrophotometer. But HCHO-DNPH complex formation was chosen, as the hydrazone complex formed in this way was quite stable from a wide range of carbonyls and it was an internationally recognized procedure. This was
also supported by Pal and Kim, and they claimed this approach as very reliable and robust [12].

Sample derivatives were analyzed in HPLC and compared with the retention time of standard formaldehyde for qualification. The formaldehyde-DNPH product peak, which had retention time about 15 min, was detected as a single peak at the wavelength of 345 nm.

Solution concentration was obtained by substituting the peak area into the regression equation. Finally, the free formaldehyde content was calculated by multiplying the concentration with the dilution factor.

The HPLC chromatogram of blank, 16 mg/L standard formaldehyde solution, and sample no. 69 derivatized with DNPH is shown in Fig. 4.

Eleven products found to be formaldehyde positive in colorimetric analysis were tested for the determination of free formaldehyde which is shown in Table 11.

The highest and the lowest free formaldehyde content was found in a shampoo+conditioner and facial foam, with the measured free formaldehyde content of 604 mg/L and 47 mg/L, respectively.

3.4 Comparison of Analysis by Colorimetric and HPLC Methods

The comparison between the free formaldehyde and total formaldehyde of various cosmetics is shown in Fig. 5.

![Graph showing comparison]

**Table 6. Ruggedness of the colorimetric method**

<table>
<thead>
<tr>
<th>Analyst-1 concentration (mg/L) ± %RSD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Analyst-2 concentration (mg/L) ± %RSD&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample no. 69 5502±0.45</td>
<td>5503±0.63</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentration (mg/L) ± %RSD of six samples

**Table 7. Ruggedness of the HPLC method**

<table>
<thead>
<tr>
<th>Added amount of standard formaldehyde (mg/L)</th>
<th>Analyst-1 (% recovery ± SD&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Analyst-2 (% recovery ± SD&lt;sup&gt;a&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample no. 69 16</td>
<td>98.4±0.41</td>
<td>98.72±0.35</td>
</tr>
</tbody>
</table>

<sup>a</sup>% of Recovery ± SD of six samples.

**Table 8. Robustness of the method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Changed conditions</th>
<th>Amount of standard formaldehyde solution (mg/L)</th>
<th>Amount of formaldehyde (mg/L) detected (Mean±%RSD)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorimetric Method</td>
<td>Change in wavelength (nm)</td>
<td>405</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>410</td>
<td>8</td>
<td>8.06±0.55</td>
</tr>
<tr>
<td></td>
<td>415</td>
<td>8</td>
<td>7.93±0.77</td>
</tr>
<tr>
<td>HPLC Method</td>
<td>Change in mobile phase composition</td>
<td>ACN : Water = 40 : 60</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>ACN : Water = 45 : 55</td>
<td>16</td>
<td>15.89±0.98</td>
</tr>
<tr>
<td></td>
<td>ACN : Water = 50 : 50</td>
<td>16</td>
<td>15.96±1.39</td>
</tr>
<tr>
<td></td>
<td>Change in flow rate (mL/min)</td>
<td>1.9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>16</td>
<td>15.89±0.98</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
<td>16</td>
<td>16.09±1.41</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± %RSD of six replicates
Table 9. Detection of formaldehyde in purchased samples

<table>
<thead>
<tr>
<th>Source</th>
<th>Leave-on products</th>
<th>Rinse-off products</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formaldehyde</td>
<td>No. of Samples</td>
<td>Formaldehyde</td>
</tr>
<tr>
<td></td>
<td>content &gt;0.05%</td>
<td>Formaldehyde</td>
<td>content &gt;0.05%</td>
</tr>
<tr>
<td>Domestic</td>
<td>3</td>
<td>23</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 10. Presence/absence of formaldehyde in different types of cosmetics

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Formaldehyde content &gt;0.05%</th>
<th>Formally positive products</th>
<th>Total number of products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Domestic</td>
<td>Imported</td>
<td>Total</td>
</tr>
<tr>
<td>Shampoo</td>
<td>8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Conditioner</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Face wash</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Lotion</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Cream</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hair gel</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 3. LOQ chromatogram
Fig. 4. HPLC Chromatograms of Blank (A); 16 mg/L Standard formaldehyde Solution (B); and sample no. 69 derivatized with DNPH (C)
Table 11. Free formaldehyde content in samples tested

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Product type</th>
<th>Free formaldehyde concentration (mg/L)*</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>Shampoo+conditioner</td>
<td>604</td>
<td>UK</td>
</tr>
<tr>
<td>71</td>
<td>Shampoo</td>
<td>363</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>6</td>
<td>Facial scrub</td>
<td>289</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>52</td>
<td>Cream</td>
<td>285</td>
<td>India</td>
</tr>
<tr>
<td>47</td>
<td>Body lotion</td>
<td>236</td>
<td>India</td>
</tr>
<tr>
<td>42</td>
<td>Shampoo</td>
<td>232</td>
<td>Thailand</td>
</tr>
<tr>
<td>14</td>
<td>Body lotion</td>
<td>209</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>43</td>
<td>Body lotion</td>
<td>126</td>
<td>India</td>
</tr>
<tr>
<td>49</td>
<td>Cream</td>
<td>92</td>
<td>India</td>
</tr>
<tr>
<td>21</td>
<td>Body lotion</td>
<td>86</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>58</td>
<td>Facial foam</td>
<td>47</td>
<td>India</td>
</tr>
</tbody>
</table>

a Mean of three replicates

Fig. 5. Comparison of free and total formaldehyde content in various cosmetics

The highest free formaldehyde concentration showed by the 2 samples in HPLC method, were also the highest two in colorimetric method. So, it can be inferred that, the HPLC data was consistent with the colorimetric approach. The measured free formaldehyde content was 1.16 to 60.81 times smaller than the corresponding total formaldehyde content measured by colorimetric method. A similar type of study was also conducted by Groot and Veenstra [13] about the presence of formaldehyde-releasers in cosmetics in the USA and in Europe, they revealed that in 25% of their 496 examined skin care products, formaldehyde-releasers were present. Moreover, in another study conducted by Monakhova Y B et al. [14] stated that 105 of their analyzed hair straightening cosmetic samples contained formaldehyde concentrations in the range of 1.2-8.8% although most of these products had been advertised as “formaldehyde-free”. Along with others, these two studies strongly support the findings of our researches and this alarmingly high formaldehyde content in our investigated samples, we postulate a strong allergic potential of these cosmetic products and the use of such unregulated formulations may exceed limits of the carcinogenic chemical [13,14].

4. CONCLUSION

This study represents suitable, linear, accurate, sensitive, rugged, and robust colorimetric and HPLC methods for the determination of formaldehyde in cosmetics. Colorimetric method was used to determine the total formaldehyde content which seems to be very much non-
specific. HPLC method precisely determined the free formaldehyde content present in the cosmetics which gives an idea on the health effects of formaldehyde. This study revealed high levels of formaldehyde in the cosmetics marketed in Bangladesh which is very much alarming for the consumers who are directly exposed to these products. As there is no regulation on the usage of formaldehyde or formaldehyde donors in cosmetics in our country, immediate measures should be taken to establish a regulatory limit and bring all the cosmetics available in our country under this regulation.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

**REFERENCES**

6. The Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers SCCNFP/587/02, final, a clarification on the formaldehyde and para formaldehyde entry in directive 76/768/EEC on cosmetic products, (adopted December 17, 2002).

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