EVALUATION OF SELECTED COSMETIC PREPARATIONS FOR THE PRESENCE OF FORMALDEHYDE AND METHYL PARABEN BY NOVEL ANALYTICAL METHODS

M. Gandhimathi*, M. Jagadeeswaran, T.K.Ravi
Department of Pharmaceutical Analysis, College of Pharmacy,
Sri Ramakrishna Institute of paramedical Sciences,
Coimbatore- 641 044, Tamilnadu, India

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ABSTRACT

The preservatives are added to cosmetic preparations in order to prevent or inhibit the growth of micro organism and increase the shelf life of the product. Formaldehyde and methyl paraben are most widely used for this purpose and it is essential to evaluate their limit in newer cosmetic products since they were reported to be toxic while exceeding limits. In the present work formaldehyde was determined from Meswak tooth paste and herbal paste, using a colorimetric method. Methyl paraben was evaluated from Fair & Handsome cream, Dabur Red tooth paste and Ponds fairness cream by a reverse phase HPLC method. The colorimetric method developed for formaldehyde determination is based on aldehyde reaction with acetyl acetone at 40°C and measured at 413nm. An RP-HPLC method employed for methyl paraben was using C18 column with a mobile phase methanol: acetonitrile: water (25:15:60, v/v/v) and detection at 255nm. Preservatives were extracted by respective procedure and evaluated by the analytical methods. The amount of formaldehyde estimated from meswak paste was 0.0066ppm and from herbal paste it was 0.0273ppm. The amount of methyl paraben estimated was 0.036ppm, 0.069ppm and 0.016ppm respectively in Fair & Handsome cream, Dabur Red tooth paste and Ponds fairness cream. The estimated amounts of preservatives in selected cosmetics were found to be within the FDA limit. The two analytical methods developed for the preservatives are precise, accurate and sensitive. They can be employed to evaluate commercially available cosmetics in order to establish their safety for human consumption.

Corresponding author
M. Gandhimathi* M. Pharm. PhD.,
Asst. Professor, Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of paramedical Sciences, Coimbatore- 641 044, Tamilnadu, India
e-mail: gands72@yahoo.co.in

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INTRODUCTION

Cosmetics are used to protect, treat or improve the physical appearance or odor of human body. According to the US FDA cosmetics are applied for cleansing, beautifying, promoting attractiveness, or altering the appearance and they should not affect the body’s structure or functions [1]. Cosmetics are formulated with large number of additives of which the preservatives are class of substances used to prevent or inhibit the growth of microorganisms in cosmetic preparations and prolong the shelf life of the product. They maintain the product fresh and free from bacteria or fungal contamination [2]. A list of preservatives approved and their limits are recommended by FDA guidelines [3]. Every day trials have been carried out to bring newer cosmetics in the market [4]. Hence it is necessary to evaluate whether the preservatives present in products are within the limits. Formaldehyde and methyl paraben are widely employed in various cosmetic preparations. According to FDA the permissible limit of formaldehyde is 0.2%w/w and methyl paraben 1%w/w in cosmetics. Excess quantity of formaldehyde is reported to cause the respiratory problems and can trigger asthmatic attack, skin rashes, eye irritation, headache and fatigue. Cosmetic products that contain methyl paraben in excess than permissible limit may cause side effects like tumor in breast, toxicities such as skin and eye damage and male infertility [5]. For the determination of these preservatives in different types of products suitable analytical techniques are essential. In the current study two cosmetics that contain formaldehyde (Meswak tooth paste and herbal paste) and three preparations that contain methyl paraben were (Fair & Handsome cream, Dabur Red tooth paste and Ponds fairness cream) selected for their evaluation of limits and to assure the safety of products to use.

Few literatures available on formaldehyde [6-9] and methyl paraben [10] estimation from different cosmetic preparations. Cosmetic emulsions and suspensions were analyzed for methyl paraben using C18 column with UV detection [11]. However the reported methods cannot be applied for the selected cosmetic preparations as the extraction procedures, selectivity and sensitivity need to be established. Therefore the objective of this article is to describe development and validation of a simple colorimetric method for formaldehyde and an RP-HPLC method for the estimation of methyl paraben. These methods would be exploited for the estimation of preservatives in selected cosmetic preparation and to assure the safety of the products.

MATERIALS AND METHODS

Methyl paraben, formaldehyde, acetyl acetone were of AR grade and procured from S.D.Fine Chemical Ltd India. All the solvents and chemicals were obtained from Qualigens Fine Chemicals Ltd, Mumbai, India. A Jasco V-630 double beam UV/Vis spectrophotometer with pair of 1 cm glass cuvette was employed for colorimetric measurements. A Shimadzu HPLC system with LC 10AT Vp pump, SPDM10AT-detector with 20µl fixed volume Rheodyne injector was used in estimation of methyl paraben. Shimadzu digital electronic balance BL-220H and Remi mini spin centrifuge were employed in the study.

EXPERIMENTAL METHODOLOGY

Preparation of solutions

Preparation of acetyl acetone solution: Ammonium acetae 15gm was dissolved in 0.3ml of acetic acid and 0.2ml of acetyl acetone and diluted up to 100 ml with water.

Preparation of stock solution of formaldehyde: A stock solution of formaldehyde containing 30µg/ml was prepared in water.

Preparation of stock solution of methyl paraben: A stock solution of methyl paraben containing 10mg/ml was prepared in methanol and successive dilutions were made to get a 10 µg/ml solution in methanol.
Analytical Method development and validation

A colorimetric method was developed for formaldehyde estimation, in which concentration and volume of acetyl acetone, heating time were optimized. Selection of appropriate wavelength for analysis was crucial as it plays role in sensitivity of the method.

In RP-HPLC for the estimation of methyl paraben from cosmetics, a mobile phase system which retains the component was ideally fixed, chromatographic conditions were optimized so that the peak is ideal for quantification.

Both the methods were validated as per ICH guidelines for precision, accuracy, linearity, system suitability, selectivity, ruggedness and robustness.

Selection of cosmetic preparations:

Two different cosmetic preparations such as Dabur herbal toothpaste (B.No: BD0127) and Dabur Meswak toothpaste (B.No: BD0302) which contain formaldehyde were selected for the study. The cosmetic preparation used for the estimation of methyl paraben were Fair & Handsome cream (B.No: AP430, AP212), Dabur Red tooth paste (B.No: RU0430, BD1151) and Ponds beauty lightening cream (B.No: B035, B075). From each batch/product six samples were chosen to analyze for preservative using optimized analytical methods.

Extraction of formaldehyde from cosmetic preparations: To the cosmetic preparations (0.1 gm each), 7 ml of dichloromethane and 10 ml of 0.002M HCl were added and heated in a water bath for 30 min. After cooling, the HCl layer was separated and centrifuged for 15 min at 3000 rpm. The solution was then filtered and filtrate was used for analysis.

Extraction of methyl paraben from cosmetic preparations: A quantity of 0.5gm of cosmetic preparation was taken in a centrifuge tube, added with 5 ml of acetonitrile and sonicated for 12 min. To this 5 ml of acetonitrile was added sonicated for 10 min and centrifuged at 3000 rpm for 15 min. A volume of 1ml from this is evaporated to dryness under nitrogen stream. The residue is reconstituted with 3ml of mobile phase and 1ml of this is diluted to 10ml with same and injected into HPLC system.

Analysis of standards of preservatives and extracts of cosmetics:

The working standards of formaldehyde solutions were scanned between 400-800 nm after treating with acetyl acetone. The extracts of tooth paste were also treated in same way and spectra recorded and absorbance was noted at 413 nm.

For the analysis of methyl paraben, the standard solutions and extracts of three different cosmetics were injected into a chromatographic system of steady baseline in above mentioned conditions. The peak areas were noted after integration and amount was calculated.

RESULTS AND DISCUSSIONS

Evaluation of formaldehyde

Two cosmetics containing formaldehyde were selected to evaluate formaldehyde content from them and they were Dabur herbal toothpaste (B.No: BD0127) and Dabur Meswak toothpaste (B.No: BD0302). To evaluate formaldehyde a colorimetric method was developed using standard formaldehyde, which is based on the reaction of aldehyde group in formaldehyde with acetyl acetone as a chromogen. After heating the mixture it turn to yellow color and it was scanned between 400-800 nm in a double beam UV-Visible spectrophotometer.
Figure 1 represents the absorption spectra of formaldehyde showing the λ max at 413nm. The volume of chromogen, heating time and temperature were optimized as 5ml, 30min and 40°C, respectively. These conditions were fixed as absorbance was highest for the yellow chromogen developed by formaldehyde.

![Absorption Spectra of Formaldehyde](image1)

**Figure 1:** Spectra of formaldehyde treated with acetyl acetone

The method obeyed Beer’s law in the linear concentration of 0.1-1.2µg/ml with correlation coefficient of 0.9998. The precision of method was confirmed by repeatability, inter day and intraday precision studies which gave %RSD values less than 0.5. The stability of standard formaldehyde solution was found stable for 8 hrs in room temperature and 16 hrs under refrigeration.

The selectivity of method was proven by comparing the spectra of extract of placebo prepared. The overlay of blank extract of placebo and formaldehyde extract of paste are shown in figure 2. The placebo spectra were found not interfering with the spectra of formaldehyde extracted from pastes which confirms the selectivity of the method. The accuracy of the method was confirmed by recovery studies by standard addition method and the average value of recovery was 105.33%.

![Overlay Spectra of Formaldehyde Extracts](image2)

**Figure 2:** Overlay Spectra of formaldehyde extracts from cosmetics and blank placebo
Formaldehyde present in the cosmetics needs to be extracted before colorimetric estimation and hence extraction procedure was also optimized. The type and volume of organic solvent, acidification with HCl and centrifugation were the parameters considered for optimization. Among various solvents tried dichloromethane (7ml) and 10 ml of HCl (0.002M) were selected. After addition of these to the cosmetics, the mixture was heated for 30 min, after cooling they were centrifuged for 15 min at 3000 rpm. The filtrate of this was treated with acetyl acetone and spectrum was recorded from the measured absorbance the amount of formaldehyde was calculated. The amount of formaldehyde estimated was 0.0066 ppm from meswak paste and 0.0273 ppm from herbal paste.

**Evaluation of methyl paraben**

Three herbal preparations named Fair & Handsome cream (B.No: AP430, AP212), Dabur Red tooth paste (B.No: RU0430, BD1151) and Ponds beauty lightening cream (B.No: B035, B075) were chosen to evaluate methyl paraben. A validated RP-HPLC method was developed using Phenomenex C\(_{18}\) (150× 4.6mm, 5µm) column. The mobile phase was optimized to get a symmetrical peak with good chromatographic characters. Many solvents were tried and finally system consisted of methanol: acetonitrile: water (25:15:60) %v/v/v was fixed, which was flowing at 0.8ml/min and methyl paraben was detected at 255nm. An ideal chromatogram of methyl paraben is shown in figure 3. This shows good retention of methyl paraben with Rt of 11.11 min, separated from interferences that eluted before 3 min.

![Figure 3: Ideal chromatogram of methyl paraben standard in fixed chromatographic conditions](image)

The linearity of the method was confirmed between the concentrations of 1-6 µg/ml with ‘r’ value of 0.9990. The precision was assured with %RSD less than 0.5 in repeatability, inter day and intraday precision studies. The stability of methyl paraben solution was found stable for 8 hrs in room temperature and 24 hrs under refrigeration. The system suitability parameters like tailing factor (1.30), plate count (6182), resolution (6.9) were ascertained to be ideal for the evaluation of methyl paraben from cosmetics.

To extract methyl paraben from cosmetic preparations, a liquid-liquid extraction procedure for was optimized using different organic solvents and finally acetonitrile was fixed. After centrifugation the samples were evaporated, reconstituted and injected to the optimized chromatographic system from the peak area of extracts of cosmetic samples the amount of methyl paraben was calculated. The chromatogram of methyl paraben
extracted from Dabur red (BD1151) is shown in figure 4. The interferences of paste found eluted well before methyl paraben and enabled the successful estimation with high accuracy. The extracted sample was found to be stable up to not less than 6 hr in room temperature and 36 hrs under refrigeration. The amount of methyl paraben estimated from three cosmetics of two different batches is shown in table 1. The amount of methyl paraben was found to be within the FDA permissible limits.

![Figure 4: The chromatogram of methyl paraben extracted from Dabur red (BD 1151)](image)

<table>
<thead>
<tr>
<th></th>
<th>Fair &amp; handsome cream (15gm)</th>
<th>Dabur red tooth paste (50gm)</th>
<th>Ponds lightening cream (15gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch I</td>
<td>0.036 gm</td>
<td>0.069 gm</td>
<td>0.016 gm</td>
</tr>
<tr>
<td>Batch II</td>
<td>0.032 gm</td>
<td>0.063 gm</td>
<td>0.017 gm</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Evaluation of two preservatives, formaldehyde by colorimetry and methyl paraben by RP-HPLC method was carried out successfully from selected cosmetic preparations. Both methods were developed and validated to prove the suitability for the purpose intended. The extraction procedures were optimized and methods were found to be highly selective as they were free from interference of the matrix. The amounts of preservatives obtained from cosmetic preparations were calculated in ppm. The FDA limit of formaldehyde is 0.2% ppm and methyl paraben is 1% ppm. The amount of formaldehyde estimated from meswak paste was 0.0066ppm and herbal paste 0.0273ppm. The amount of methyl paraben estimated was fair & handsome cream 0.036ppm, toothpaste 0.069ppm and ponds cream 0.016ppm, which are found to be within the limit. The methods developed here are ensuring the limits of preservatives added to selected cosmetics and confirm that they can be used safely. Further the methods were validated by ICH guidelines and can be successfully employed to estimate these preservatives from the selected cosmetic preparations as a quality control procedure.

**AUTHOR’S STATEMENT**

**Competing Interests**

The Authors declare no conflicts of interest
REFERENCE