INTRODUCTION

In this method development, three components are separated and quantified simultaneously. These three compounds contain aceclofenac, methyl salicylate, and benzyl alcohol. Of these three, aceclofenac is the active drug while other two are preservatives.

The proposed method is offering simultaneous estimation of one active drug along with two preservatives simultaneously.

Aceclofenac is widely used anti-inflammatory drug and analog of diclofenac. It is used for the relief of pain and inflammation, chemically \((2-\{2,6\text{-dichlorophenyl}\}\text{ amino phenylacetooxyacetic acid})\), is a crystalline powder with a molecular weight of 354.19. It is practically insoluble in water with good permeability. According to the biopharmaceutical classification system (BCS), aceclofenac falls under the BCS Class II, poorly soluble and highly permeable drug.[1] The structure of aceclofenac, methyl salicylate, and benzyl alcohol is shown in Figures 1-3, respectively.

Methyl salicylate[2] is commonly used as a fragrance in foods, beverages, and liniments, whereas benzyl alcohol[3] is widely used as a preservative in pharmaceutical topical preparation. Benzyl alcohol is a aromatic alcohol with the formula \(C_6H_5CH_2OH\).

Literature survey revealed that many methods of analysis were reported for the analysis of aceclofenac for individual drug as well as with combination with other drugs,[4-19] but no method is reported for simultaneous determination of aceclofenac, methyl salicylate, and benzyl alcohol in cream formulations using liquid chromatography. The proposed method is very simple, accurate, and precise, so can be easily adopted for routine analysis.
and validated as per ICH guideline.[20-23] Hence, the proposed method is advantageous over reported method and can be conveniently adopted for routine quality analysis in pharmaceutical industries.

**EXPERIMENTAL**

**Chemicals and Reagents**

Methyl salicylate, benzyl alcohol, and aceclofenac standards were provided by Cipla Limited as a gift samples. Cream samples were procured from market. Milli-Q grade water, orthophosphoric acid (GR Grade), triethylamine (GR Grade), and acetonitrile (high-performance liquid chromatography [HPLC] grade) were used. Autosampler HPLC Dionex along with ultraviolet (UV) and photodiode array detector was used.

**Chromatographic Conditions for Developed Method**

About 0.01M monobasic phosphate adjusted to pH 2.95 with orthophosphoric acid and acetonitrile in the ratio of 1:1 is used as a mobile phase for the separation of three components, i.e., aceclofenac, methyl salicylate, and benzyl alcohol.

Column: L1 250 × 4.6 mm, 5 µ

Column oven temperature: 37°C

Wavelength: 215 nm

Injection volume: 20 µL

Flow rate: 1.0 mL/min.

Retention times: Aceclofenac, methyl salicylate, and benzyl alcohol eluted at about 15 min, 11.9 min, and 4.3 min, respectively.

Diluent: Buffer and methanol in the ratio 22:78.

**Preparation of Standard Solutions**

Standard solution in diluents containing aceclofenac (75 µg/mL), benzyl alcohol (50 µg/mL), and methyl salicylate (50 µg/mL) sonicated for 5 min to dissolve.

**Preparation of Sample Solution**

Dissolved cream sample in diluents by 45 min sonication and made dilutions to achieve a solution which contains aceclofenac (75 µg/mL), benzyl alcohol (50 µg/mL), and methyl salicylate (50 µg/mL). Nylon syringe filter of 0.45 µm used for filtration.

**RESULTS AND DISCUSSION**

**Method Development**

Method for the determination of aceclofenac, methyl salicylate, and benzyl alcohol in cream formulations was developed and finalized after detailed study of developmental parameters such as solubility, selection of diluents, UV detection wavelength, selection of working pH range for mobile phase, choice of buffer, buffer concentration, selection of columns, and final method optimization.

Solubility of compound/s to be used in method development was checked in HPLC compatible solvents such as methanol, acetonitrile, tetrahydrofuran, isopropyl alcohol, and water as well as mixture of solvents and water. Solubility in acidic and basic media was also checked. On the basis of solubility study and some initial trials, mobile phase was used as diluents for this method development. All three compounds showing comparatively better response at 215 nm UV wavelength; therefore, 215 nm wavelength was selected for this method after comparative study.

Initially, trials were conducted using acidic and basic pH of mobile phase and after comparative study, it was
observed that acidic pH is favorable for the separation of these three compounds. Therefore, 0.01 molar monobasic phosphate buffer adjusted to pH 2.95 was selected as a buffer and acetonitrile used in mobile phase in the ratio of 1:1 v/v.

On the basis of literature survey, the study has been conducted by using 250 × 4.6 mm, 5 µ and 150 × 4.6 mm, 5 µ columns. After very extensive study and comparisons, Inertsil ODS-3V, 250 × 4.6 mm, 5 µ column was selected for method development.

Finally, method was optimized by making required changes in mobile phase composition, flow rate, column oven temperature, standard, and sample concentration. Effect of each individual parameter was studied on separation.

After several trials and optimization, final parameters selected which gives better separation between aceclofenac, methyl salicylate, and benzyl alcohol with good peak shapes and resolution between all three peaks.

Representative chromatogram is as shown in Figure 4.

To validate the method, parameters such as specificity, linearity, precision, accuracy, robustness, solution stability, filter compatibility, and system suitability were performed as per ICH guideline.

System Suitability
System suitability of analysis was confirmed by injecting six replicates of standard solution containing aceclofenac, methyl salicylate, and benzyl alcohol. Mean of area response, standard deviation, and % relative standard deviation (% RSD) were calculated.

System suitability data are shown in Table 1.

Specificity
Interference from diluents and placebo was checked at the retention time of aceclofenac, methyl salicylate, and benzyl alcohol peaks. It was observed that there is no peak interference at the retention time of aceclofenac, methyl salicylate, and benzyl alcohol from the diluents and placebo solution. For identification purpose, retention time of aceclofenac, methyl salicylate, and benzyl alcohol peaks in sample solution matches the retention time of aceclofenac, methyl salicylate, and benzyl alcohol peaks in standard solution. This result shows that the method is specific enough for the determination of aceclofenac, methyl salicylate, and benzyl alcohol without any interference.

Linearity
Linearity was performed by preparing five solutions of different concentrations in the range of 60–160% of working concentration of aceclofenac, methyl salicylate, and benzyl alcohol, i.e., 60%, 80%, 100%, 120%, and 160%. Correlation coefficient obtained from graph was close to 1.000 for all the compounds. Linearity data with correlation coefficient are shown in Table 2.

Calibration curves for aceclofenac, methyl salicylate, and benzyl alcohol are shown in Figures 5–7, respectively.

Precision/Repeatability
For precision, prepared six different samples of same concentration and calculated for the content of aceclofenac, methyl salicylate, and benzyl alcohol. RSD and content of all components shows the method is repeatable.

<table>
<thead>
<tr>
<th>Standard replicates</th>
<th>Area response</th>
<th>Benzy alcohol</th>
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<tbody>
<tr>
<td></td>
<td>Aceclofenac</td>
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<tr>
<td>Standard_1</td>
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<td>RSD</td>
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RSD: Relative standard deviation

<table>
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<th>Area response</th>
<th>Benzy alcohol</th>
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<td>Correlation coefficient</td>
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</table>

Figure 4: Chromatogram standard solution
Accuracy or Recovery

The accuracy study was performed by spiking standard solutions into placebo. The recovery values were determined by calculating the amount added and the amount recovered. Recovery values for all three components were between 98% and 102%.

Recovery results are shown in Table 3.

Robustness

Robustness study confirmed that there is no significant impact on results when altered chromatographic condition, i.e., flow rate and column oven temperature and analyzed standard solutions and system suitability parameter RSD was evaluated. Percent RSD of area response and retention time was below 2%. Robustness results are shown in Table 4.

Filter Compatibility

Filter compatibility of method was confirmed by comparing centrifuged sample with filtered samples and observed difference between centrifuged sample and filtered samples through nylon 0.45-micron filter was not more than 2.0%. It was observed that nylon filter paper does not absorb compound of interest during filtration of sample solutions. Observations of filter study are reported in Table 5.

Solution Stability

Solution stability was performed by comparing results of freshly prepared sample solution and stored sample solution at room temperature for 24 h. It was observed that solution was stable for 24 h.

CONCLUSION

Literature survey reveals that there is no method reported for simultaneous determination of aceclofenac, methyl salicylate, and benzyl alcohol content from pharmaceutical cream samples using reverse phase HPLC. The method developed gives better separation between aceclofenac, methyl salicylate, and benzyl alcohol with good peak shapes and resolution between all three peaks.

The proposed method is very simple, accurate, and precise. Hence, this validated method can be easily and conveniently adopted for routine analysis of...
aceclofenac, methyl salicylate, and benzyl alcohol content from pharmaceuticals.

ACKNOWLEDGMENT

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REFERENCES