METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF 7DM IMPURITY IN MOMETASONE Furoate

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ABSTRACT
Objective: Objective of the present analytical research work was to develop and subsequent validate Reverse phase High Performance Liquid Chromatographic method for estimation of 7DM impurity content in Mometasone Furoate. Methods: Numerous HPLC condition were tested for estimation of 7DM, the result were achieved by using Intersil ODS C18 (50mm×4.6mm×3µm) column and mobile phase containing 0.1% TEA adjusted to pH 3 with TFA and 100% ACN in gradient mode at flow rate 1.5ml/min. at temperature 45°C with UV detection at 242nm. Results: In this method the linear response was observed in range of 1.5-7.5µg/ml. with correlation coefficient of 1. Accuracy and precision were found to be satisfactory within limit for this method. The proposed method has adequate specificity and suitability. Interpretation and conclusion: The simple, sensitive, precise, rapid and specific method were developed and validated statistically for estimation of 7DM impurity in Mometasone Furoate.

KEYWORDS: Mometasone Furoate; 7DM impurity; gradient mode; RP-HPLC.

INTRODUCTION
Mometasone Furoate (MF) is chemically known as (11β, 16α)-9, 21-dichloro-11-hydroxy-16-methyl-3, 20-dioxopregna-1, 4-dien-17-2-furoate is topical corticosteroid (figure 1). It has anti inflammatory antipruritic and vasoconstrictive properties. It is corticosteroid which helps
to decrease inflammation and control various asthmatic symptoms. Corticosteroids act by the induction of phospholipase A2 inhibitory proteins, collectively called as lipocortins.\textsuperscript{[1-2]}

Literature survey reveals that several analytical methods have been published for the estimation of mometasone furoate alone or combination with other drugs. However no HPLC method has been reported for estimation of 7dm impurity content in mometasone furoate. Therefore attempt were made to develop RP-HPLC analytical method for estimation of 7DM impurity in Mometasone Furoate.\textsuperscript{[3-4]}

**MATERIALS AND METHODS**

**Instrument**- A HPLC (Agilent 1200 series) with auto intelligent HPLC pump and FLD/DAD detector with chromelon software version 6.80 is used for this study. Water for HPLC is generated using Milli-Q plus water purification system (Millipore, Milford, MA, USA).

**Materials and reagents**- active pharmaceutical ingredient of mometasone Furoate were supplied by Cipla Ltd. Mumbai, India. HPLC grade acetonitrile were used of RANKEN INDIA Mumbai, HPLC grade methanol, Triethylamine and Trifluoroacetic acid were obtained from Merck specialties Pvt. Ltd India.

**Preparation of standard solution**

7DM stock solution

40mg of 7DM std. were weigh accurately and transferred in 20ml volumetric flask diluted and add sufficient amount of diluent up to mark and sonicate it to dissolve it completely. Take 5ml of above solution and dilute it up to 100ml with diluent (100pmm).

Reference solution

Take 1ml of solution from above 7DM std. stock solution in 20ml volumetric flask and adjust the volume up to mark with diluent (5pmm).

Test solution

Weigh and transfer 100mg of Mometasone Furoate sample in 20ml volumetric flask add sufficient amount of diluent and sonicate to dissolve and make up to mark with diluent (5000ppm).

**Chromatographic condition**

Chromatographic separation was operated at 45\textdegree C on reverse phase Intersil ODS C18 column. The mobile phase consists of two solvent. Solvent A (100% Acetonitrile) and Solvent B
(0.1% TEA in purified water, adjusted to pH 3 with TFA). Gradient elution system was used which consist of solvent A and solvent B. the program of gradient elution is shown in Table 1.

**Optimization of HPLC method**

The HPLC procedure was optimized with the view to develop estimate 7DM content in Mometasone Furoate. Reverse phase column [Intersil ODS C18 (50mm×4.6mm×3µm) column] was selected on the basis of polarity of drug for analysis. Following parameter were optimized for development of method i.e. column, wavelength, mobile phase concentration, solvent, flow rate, concentration of buffer. It was observed that at wavelength 242nm drug could be detected with no mobile phase interference, good separation, sensitivity and consistent baseline. The feasibility of various combinations of solvents such as acetonitrile, methanol, buffer and water with altered flow rates and concentrations was investigated for complete chromatographic resolution of above used drugs with best sensitivity, efficiency and peak shape. Optimized parameters for HPLC method are shown in table 2.

**Method validation**

The method was validated according to the ICH guidelines. The following parameters like range and linearity, accuracy, precision, limit of detection (LOD) and quantification (LOQ) were addressed.

**System suitability and specificity**

System suitability and specificity is pharmacopoeial requirement and used to verify whether resolution and reproducibility of chromatographic system are adequate for analysis to be done.

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest without interferences from blank and other components. The test was performed by collecting data from injection of standard drug solution, blank solution, sample solution and resolution solution.

**Linearity**

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the samples.
Accuracy and recovery
Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The true value is that result which would be observed in the absence of error. Accuracy may often be expressed as percent recovery by assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method that is true for all practical purpose.

Limit of detection (LOD)
It is lowest amount of analyte in sample that can be detected, but not necessarily quantitated, under the stated conditions. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 3:1.

Limit of Quantitation (LOQ)
It is lowest amount analyte in sample that can be determined with acceptable precision. It is generally expressed as the concentration of the analyte sample. It is usually regarded as the amount for which the SNR is 10:1.

Precision
The precision of an analytical method is the closeness of agreement (degree of scatter) between series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed conditions. Precision study was done with six measurements at working concentration of the test solution and standard solution.

RESULT AND DISCUSSION
Linearity
The linearity of the method was tested using the 7DM stock solution described above. Plot of the concentration against response were linear in the range of 1.5-7.5μg/mL (figure 2) the mean regression equation was \( y = 25732x + (1264.68) \). The correlation coefficient was 1. The system suitability parameter was given in Table 3.

Specificity and suitability
Specificity and System suitability parameters were analyzed on freshly prepared standard solutions of 7DM impurity and Mometasone Furoate (figure 3, 4). Results shows there is no interference of any other peaks from sample and any other impurities or degradation product at the retention time of 7DM peak.
Accuracy
The results of accuracy studies (Table 4) show that the method is accurate within the desired range. The RSD was calculated for each recovery solution and all the results are within limits (98-104%).

Precision
The precision (repeatability) of an analytical method refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment. Repeatability of the method was evaluated by calculating the RSD of the peak areas of five replicate injections for the standard concentration (100%) of mometasone furoate, which were found to be 0.2%. (Table 5).

Limit of detection (LOD) and limit of Quantitation (LOQ)
Three set of known concentrations were prepared. LOD and LOQ values were found to be 0.037µg/ml and 0.112µg/ml respectively. LOD and LOQ were calculated by using formulae as

\[
LOD = 3.3 \frac{SD}{S}
\]

\[
LOQ = 10 \frac{SD}{S}
\]

Were S is value of slope of calibration plot and SD is calculated by using value of y intercept of regression equation.

Table 1: Gradient Elution Flow Run.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>4.00</td>
<td>75</td>
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<tr>
<td>8.00</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>10.00</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>10.01</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>12.00</td>
<td>75</td>
<td>25</td>
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</table>

Table 2: Optimized Parameters for Hplc

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Specifications</th>
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<tbody>
<tr>
<td>Instrument</td>
<td>Agilent HPLC 1200 series</td>
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<tr>
<td>Column</td>
<td>Intersil ODS (50mm×4.6mm×3µm)</td>
</tr>
<tr>
<td>Method</td>
<td>Gradient</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile: 0.1%TEA solution of pH 3 with TFA.</td>
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</table>
Column temperature 45°C
Flow rate 1.5ml/min
Detection wavelength 242nm
Run time 12min
Injection volume 20µl/ml

METHOD.

Table 3: System Suitability Parameter.

<table>
<thead>
<tr>
<th>Replicate Injection No.</th>
<th>Retention Time (minutes)</th>
<th>Area (USP)</th>
<th>Tailing</th>
<th>Theoretical Plates</th>
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</thead>
<tbody>
<tr>
<td>01</td>
<td>6.191</td>
<td>129505</td>
<td>0.8</td>
<td>42230</td>
</tr>
<tr>
<td>02</td>
<td>6.193</td>
<td>130010</td>
<td>0.8</td>
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<td>03</td>
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<tr>
<td>04</td>
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<td>129683</td>
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<td>05</td>
<td>6.191</td>
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<td>0.8</td>
<td>41930</td>
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<tr>
<td>Average</td>
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<td>129750</td>
<td>0.8</td>
<td>42230</td>
</tr>
<tr>
<td>SD</td>
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<td>241.887</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>% RSD</td>
<td>0.0</td>
<td>0.2</td>
<td>NA</td>
<td>NA</td>
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Table 4: % Recovery Result For Different Accuracy Levels In Accuracy Study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Level of % Recovery</th>
<th>Amount added in mg/ml</th>
<th>Amount recovered in µg</th>
<th>% Recovered</th>
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<tr>
<td>1</td>
<td>LOQ</td>
<td>0.03</td>
<td>0.031</td>
<td>104</td>
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<tr>
<td>2</td>
<td>50</td>
<td>0.05</td>
<td>0.050</td>
<td>101</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.10</td>
<td>0.100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>0.15</td>
<td>0.151</td>
<td>101</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>101.5</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 5: System Suitability Summary For Replicate Area Of Standard Solution In Precision Study.

<table>
<thead>
<tr>
<th>Replicate Injection No.</th>
<th>Retention Time (minutes)</th>
<th>Area (USP)</th>
<th>Tailing</th>
<th>Theoretical Plates</th>
</tr>
</thead>
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<td>1.3</td>
<td>37645</td>
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<td>6.194</td>
<td>130615</td>
<td>1.3</td>
<td>37680</td>
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<td>6.193</td>
<td>131335</td>
<td>1.3</td>
<td>37646</td>
</tr>
<tr>
<td>05</td>
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<td>1.3</td>
<td>37640</td>
</tr>
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<td>Average</td>
<td>6.193</td>
<td>130991</td>
<td>1.3</td>
<td>37646</td>
</tr>
<tr>
<td>STDEV</td>
<td>0.001</td>
<td>310.536</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.0</td>
<td>0.2</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Figure 1: Structure of mometasone furoate.

Figure 2: linearity and range of 7DM, $R^2 = 1$

Figure 3: chromatogram of standard 7DM impurity.
Table and figure title and legends.

TABLE 1: GRADIENT ELUTION FLOW RUN
Flow time is 12 minute.

TABLE 2: OPTIMIZED PARAMETERS FOR HPLC METHOD
TEA is triethylamine, TFA is trifluoroacetic acid.

TABLE 3: SYSTEM SUITABILITY PARAMETER
SD is standard deviation, NA represents not applicable.

TABLE 4: %RECOVERY RESULT FOR DIFFERENT ACCURACY LEVELS IN ACCURACY STUDY
LOQ is limit of Quantitation, % RSD represent percent relative standard deviation.

TABLE 5: SYSTEM SUITABILITY SUMMARY FOR REPLICATE AREA OF STANDARD SOLUTION IN PRECISION STUDY
USP represent united state pharmacopeia.

Figure 1: Structure of mometasone furoate.
Figure 2: linearity and range of 7DM, $R^2 = 1$
Figure 3: chromatogram of standard 7DM impurity.
Figure 4: chromatogram of Mometasone furoate sample.

DISCUSSION
This method describes a reverse phase High performance liquid chromatographic procedure employing a C18 column by gradient method and mobile phase comprising of 100% acetonitrile and 0.1% triethylamine in purified water of pH 3 adjusted with TFA solution. Mometasone Furoate and 7DM are almost insoluble in aqueous solutions whereas they are
freely soluble in organic solvents like methanol and acetonitrile. Mobile phases containing acetonitrile: 0.1% triethylamine solution of pH 3 were resulted in satisfy resolution of 7DM and mometasone Furoate. Best resolution was obtained using column temperature at 45°C that gives peak sharpness. The sampling wavelength was selected after scanning the drug solutions in the mobile phase having concentration of 100μg/ml in the UV range of 200–400 nm on a UV/Visible spectrophotometer and 242 nm was selected as suitable wavelength for estimation.

Accuracy, reproducibility and precision of proposed method were further confirmed by percent recovery values. The proposed method was validated as per the ICH guidelines. Linearity was determined at different concentration 7DM impurity were showed linearity in the range of 1.5-7.5μg/ml, with correlation coefficient of 1.00. Limit of detection (LOD) and Limit of Quantitation (LOQ) were determined by standard deviation of response and slope of calibration curve. LOD and LOQ were found to be 0.037μg/ml and 0.112μg/ml.

The reproducibility of sample was expressed in terms of ±S.D. and % R.S.D. There was no interference from the other impurities present in Mometasone Furoate. Percent recovery values were close to 100 with low values of relative standard deviation the drug content.

ACKNOWLEDGEMENT
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REFERENCES


Books and Other Monographs