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# RP-HPLC Method Development and Its Validation for the Estimation of Mometasone Furoate in Pressurized Meter Dosage Form

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## ABSTRACT

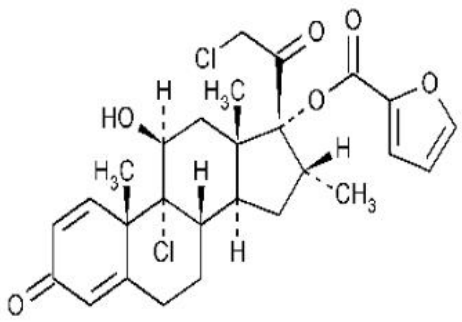
A simple, rapid, sensitive RP-HPLC method for the simultaneous determination of Momentasone furoate in pharmaceutical meter dosage forms was developed. Momentasone furoate has absorption maxima at 240 nm. For the estimation of Momentasone furoate the detection wavelength was taken as 240 nm. Mobile phase was Acetonitrile: water in ratio of 70:30, the flow rate was 1ml/min.Linearity for detector response was observed in the concentration range of 20 to 150 % of test concentration. Correlation coefficient (r) for calibration curve was found to be 0.99. Retention times were found to be 4.39 min. Percent recovery was found to be within the range of 99.4 – 99.8%. The percent RSD for the analyzed tablet and recovery studied was less than 2. The results of recovery studies were found to be linear in the range 20 to 150 % of test concentration. Results of the analysis were validated as per ICH guidelines. The developed method was found to be precise, selective and rapid for the determination of Momentasone furoate in bulk and in pharmaceutical dosage form.

**Keywords:** Momentasone furoate, Acetonitrile, Triple distilled water, RP-HPLC

**Introduction:-**

Mometasone furoate (MF) is a topical corticosteroid; it has anti-inflammatory, anti-pruritic, and vasoconstrictive properties. It is a Corticosteroids act by the induction of phospholipase A2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation i.e prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid [1, 2] Mometasone Furoate

9,21-Dichloro-17-[(2-Furanylcarbonyl)Oxy]-11-Hydroxy-16-Methyl Pregna-1,4 Dione



**Structure of Mometasone Furoate**

The present research was to develop and validate the method for analysis of the pressurized meter dosage form. The proposed methods were validated as per ICH guidelines. [9] Literature survey reveals that several analytical methods have been reported for the determination of Mometasone Furoate by RPHPLC [3, 4], HPTLC [5, 6] In nasal spray solution by HPLC [7],Meter dosage form by HPLC [8]

**Experimental**

**Instrumentation**

HPLC:Model: Agilent 1200 series Manufacturer: Agilent Technology Pump: Quaternary Pump G1311A,Injector: Auto sampler ALS G1313 A ,Detector: PDA Detector G1316 A Software: Chromeleon Software

**Materials and methods**

**Chemicals and Materials**

Methanol,Acetronitrile,Triple distilled water Tablets containing AMLB and INDA,Brand name: (AMLODAC-D)

**Preparation of Standard solution**

**Standard stock solution for Mometasone Furoate**

40 mg of Mometasone Furoate is accurately weighed and transferred into 100 ml volumetric flask, and add 50 ml of Acetonitrile sonicate till it dissolve. Make up with Acetonitrile and mix well.

**Working standard solution**

5ml of Mometasone Furoate standard stock solution was transferred in 100ml volumetric flask. The volume was made with diluent and mixed well.

**Table I: Optimized chromatographic conditions for Mometasone furoate**

| PARAMETER | CONDITIONS |
|---|---|
| Mobile phase | Acetonitrile : Water (70:30) |
| Pump mode | Isocratic |
| Stationary phase | Hiber Purosphere Star RP Column (150 mm x 4.6 mm , 5 µm particle size) |
| Flow rate (ml/min) | 1.0 |
| Run time (min) | 8.0 |
| Volume of Injection (µl) | 20 |
| Detection wavelength (nm) | 240 |
| Retention time (min) | 4.39 |
| Diluent | Acetonitrile:Water (50: 50) |

**Selection of Wavelength for Determination**

The working standard solution of Mometasone Furoate (20 µg/ml) were scanned individual in the range of 200-400 nm. The responses of standard solution measured with PDA detector and the

wavelength at 240 nm was selected for the RP-HPLC method.

**Preparation of Sample solution.**

One canister of Mometasone Furoate inhaler was taken. Prime the metering valve with wasting one dose. Shaken for at least 5 seconds and waste again. This procedure was repeated for further 4 times. The valve was washed with suitable solvent and tissue paper. Then kept the copper disc containing hole in centre in 100ml beaker. About 35ml diluents was added in beaker. The container was kept in inverted position and one dose was pressed in beaker. Shaken for at least 5 seconds and the second dose was taken. This procedure was repeated for further 8 times. This solution contains 20 µg/ml Mometasone Furoate. This solution was transferred in beaker solution into a 200ml volumetric flask. Copper disc was washed with diluents. The volume was made to mark with diluent.

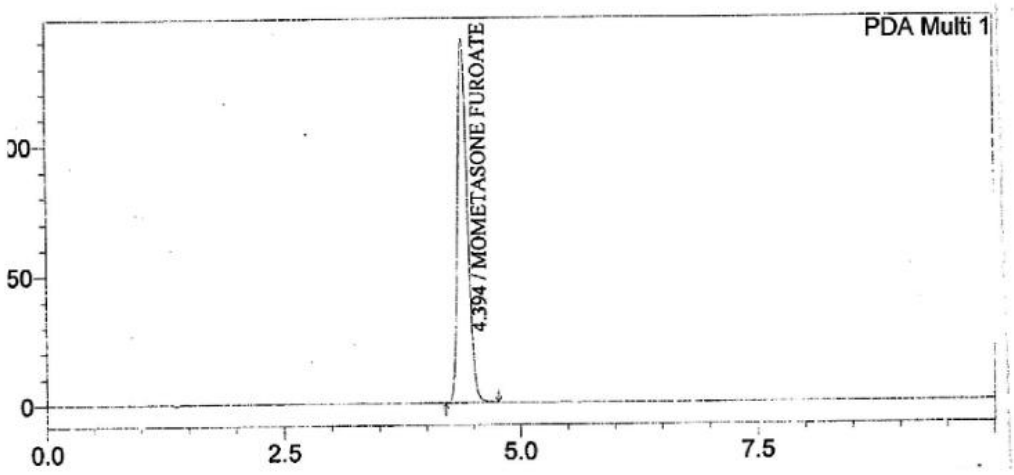



**Figure 1: Chromatogram of Mometasone Furoate using ACN: Water (70:30)**

**METHOD VALIDATION**

As per the ICH guidelines Q2R1, the method validation parameters studied were solution stability, specificity, linearity, accuracy, precision, limit of detection, limit of Quantitation, robustness and system suitability test.

**a. Specificity**

Specificity of an analytical method is its ability to measure the analytes accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions of Mometasone Furoate were compared, and peak purity spectra obtained from using photo diode array detector (PDA) were recorded in order to provide an indication of specificity of the method.

**b. Linearity**

The calibration curve was linear over the concentration range of 4-30 µg/ml for Mometasone Furoate (Table II)

**Table II: Result of Calibration readings for Mometasone Furoate**

| Linearity Range | Stock solution to be taken in mL | Dilute to volume (mL)with diluents | Final concentration in µg/mL (Formoterol Fumarate) | Area |
|---|---|---|---|---|
| 20% | 1 | 100 | 4 | 218144 |
| 40% | 2 | 100 | 10 | 426880 |
| 60% | 3 | 100 | 16 | 647313 |
| 80% | 4 | 100 | 20 | 8596417 |
| 100% | 5 | 100 | 24 | 1074175 |
| 150% | 7.5 | 100 | 30 | 1618439 |

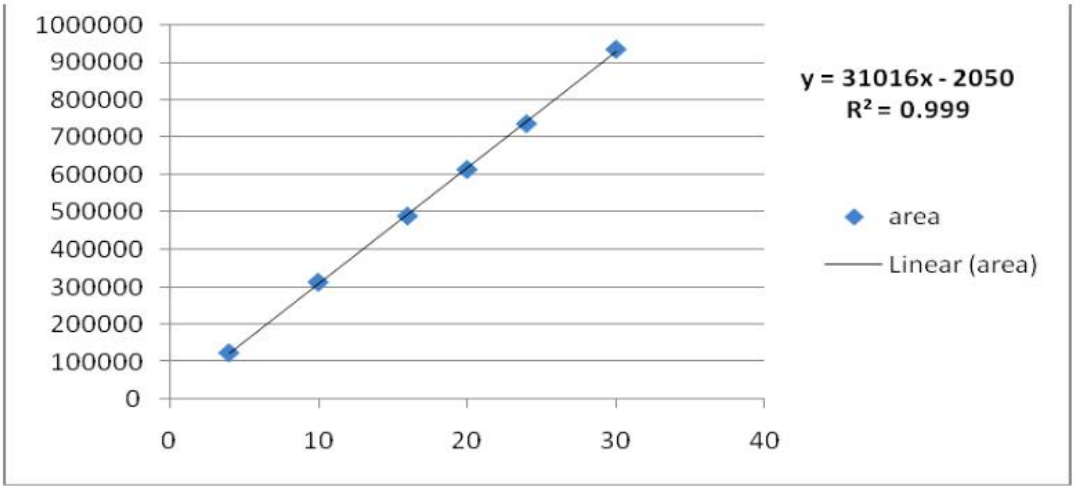



**Figure 2: Calibration curve of Mometasone furoate at 240nm**

**Table III: Statistical data for Mometasone furoate by RP- HPLC method**

| Parameter | Mometasone Furoate |
|---|---|
| Linear Range(µg/ml) | 4-30 |
| Slope | 31016 |
| Intercept | 2050 |
| Limit of Detection (µg/ml) | 3.98 |
| Limit of Quantitation (µg/ml) | 11.94 |

**c. Precision**

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It is indication of random error. The precision of analytical method is usually expressed as a standard deviation, relative standard error or co-efficient of variance of series of measurement.

**Intraday Precision:-** Variation of results within same day is called intraday precision.

**Procedure:-** Solutions containing 20 µg/ml Mometasone Furoate was analyzed 3 times on the same day and %RSD was calculated.

**Interday Precision:-** Variation of results amongst days is called intraday precision

**Procedure:-** Mixed solutions containing 20 µg/ml Mometasone Furoate was analyzed on 3 different days and % RSD was calculated. (Table IV)

**Table IV: Precision data for Mometasone furoate**

| Conc. µg/ml | Inter-day (n=3) | % RSD | Intra-day (n=3) | % RSD |
|---|---|---|---|---|
| 4 | 233580 | 0.21 | 233578.3 | 0.17 |
| 8 | 477738.7 | 0.34 | 478646.3 | 0.09 |
| 12 | 706236 | 0.10 | 706819.3 | 0.09 |

**d. Accuracy**

It is defined as the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It is measure of exactness of analytical method. Accuracy should be expressed as % recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. Accuracy should be established across the specified range of the analytical procedure. It was determined by calculating the recovery of Mometasone Furoate by standard addition method. (Table V)

**Table V: Determination of Accuracy**

| For Mometasone Furoate | | | | | |
|---|---|---|---|---|---|
| **Level** | **Amount of Drug added (mg )** | **Amount of Drug recovered (mg)** | **Recovery (%)** | **Mean (%)** | **% RSD** |
| 50 % | 2.0 | 1.9916 | 99.5 | 99.4 | 0.6 |
| | 2.0 | 1.9986 | 99.9 | | |
| | 2.0 | 1.9774 | 98.8 | | |
| 100 % | 4.0 | 4.0037 | 100.1 | 99.4 | 1.0 |
| | 4.0 | 3.9316 | 98.3 | | |
| | 4.0 | 3.9964 | 99.9 | | |
| 150 % | 6.0 | 5.9789 | 99.6 | 99.8 | 0.5 |
| | 6.0 | 5.9749 | 99.5 | | |
| | 6.0 | 6.0258 | 100.4 | | |

**Table VI: Reproducibility data for Mometasone furoate (20µg/ml)**

| Instrument 1 Area ± S.D(n=3) | Instrument 2 Area ± S.D(n=3) | Result of t-test* | Inference |
|---|---|---|---|
| 4784593 ± 534.36 | 477738.7 ± 1613.30 | 0.43 | Not significant difference |

* At 95% confidence interval, (t-Tabulated = 4.

**e. Limit of Detection and Limit of Quantitation:**

They were measured using standard deviation of Y-intercept and slop of calibration curve as per ICH guideline.

**f.System suitability**

The % RSD of peak area and retention time for the drug was within 2% indicating the suitability of the system. The efficiency of the column was expressed by number of theoretical plates for the 6 replicate injections. (Table VII)

**Table VII: System suitability parameters**

| Sr. No. | Parameters | Mometasone Furoate |
|---|---|---|
| 1. | Peak area ± % RSD | 476514.2 ± 0.59 |
| 2. | No. of theoretical plates ± %RSD | 5170 ± 1.48 |
| 3. | Retention time (min) ± %RSD | 4.32 ± 0.04 |
| 4. | Asymmetry ± %RSD | 1.03 ± 1.05 |

**g.. Accuracy (% recovery study)**

To study the accuracy 1 Canister were taken and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples.

**h. Specificity and selectivity:**

No any interfering peak from blank or placebo at the RT of Mometasone furoate was observed, hence the peak was concluded as pure. So the developed method is specific for estimation of Mometasone furoate.

**Table VIII: Specificity and Selectivity study**

| Study | Result |
|---|---|
| Specificity | Specific |
| Selectivity | Selective |

**i. Solvent suitability study**

Standard and sample solution stability was evaluated at room temperature for 48 hrs. It showed that both standard and sample solution was stable up to 48 hrs. at room temperature.

**Table IX: Solvent Suitability Study (Standard solution)**

| Time | Area Mometasone furoate (20 µg/ml) | RESULT % Mometasone furoate |
|---|---|---|
| 0 hr. | 478436 | 100 |
| 12.0 hrs. | 478042 | 99.92 |
| 24.0 hrs. | 477596 | 99.82 |
| 48.0 hrs. | 475598 | 99.41 |

**Table X: Solvent Suitability Study (Sample solution)**

| Time | Area Mometasone furoate (20 µg/ml) | RESULT % Mometasone furoate |
|---|---|---|
| 0 hr. | 469224 | 100 |
| 12.0 hrs. | 468821 | 99.91 |
| 24.0 hrs. | 468746 | 99.90 |
| 48.0 hrs. | 469927 | 100.15 |

**j.Robustness**

**(A) Variation in organic phase ratio of mobile phase:**

To demonstrate robustness of test method check system suitability parameters as mentioned in test method by injecting five replicate infections of mixed standard preparation into liquid chromatography with change in organic phase ratio of mobile phase from 100.0% (70.00% v/v) to 95.0% (66. 5% v/v) and 105.0% (73.5% v/v) of test method organic phase ratio.(Table XI)

**Table XI:Data indicating change in organic phase ratio of mobile phase study of Mometasone furoate**

| **Parameters** | **Observed Value** | | |
|---|---|---|---|
| | **As such Condition** | **Robustness** | |
| | **Organic phase ratio 100.0%** | **Organic phase ratio 95.0%** | **Organic Phase ratio 105.0%** |
| Relative standard deviation of Mometasone furoate Peak area | 0.2% | 0.2% | 0.3% |

**(B) Variation in flow rate:**

To demonstrate robustness of test method check system suitability parameters as mentioned in test method by injecting five replicate injections of mixed standard preparation into liquid chromatography with change in flow rate from 1.0 ml/minute to 0.9 ml/minute and 1.1 ml/minute.

**Table XII: Data indicating change in flow rate study of Mometasone furoate**

| Parameters | Observed Value | | |
|---|---|---|---|
| | As such Condition | Robustness | |
| | 1.0 ml/minute | 0.9 ml/minute | 1.1 ml/minute |
| Relative standard deviation of Mometasone furoate Peak area | 0.1% | 0.2% | 0.6% |

**(C) Variation in Temperature:**

To demonstrate robustness of test method check system suitability parameters as mentioned in test method by injecting five replicate injections of mixed standard preparation into liquid

**Table XIII: Data indicating change in Temperature study of Mometasone furoate**

| Parameters | Observed Value | | |
|---|---|---|---|
| | As such Condition | Robustness | |
| Relative standard deviation of Mometasone furoate Peak area | 0.5% | 1.7% | 0.5% |

**Application of developed method to pharmaceutical formulation**:

The proposed validated method was successfully applied to determination of Mometasone furoate in its Inhaler dosage form.

**Table XIV: Assay Results of Marketed Dosage Form**

| Formulation | Actual concentration µg/ml Mometasone furoate | Amount obtained µg/ml Mometasone furoate | % Mometasone furoate |
|---|---|---|---|
| Inhaler | 20 | 19.89 | 99.10 |

**Table XV: Peak Purity Index**

| Drug | Purity Threshold | Purity Criteria |
|---|---|---|
| Mometasone furoate | 999.946 | Pass |

**Table XVI: Summary of Validation Parameters**

| Parameters | Mometasone furoate |
|---|---|
| Recovery % | 99.4 – 99.8 |
| Repeatability (%RSD, n=6) | 0.1366 |
| Precision(%RSD)<br>Intra-day (n=3)<br>Inter-day (n=3) | <br>0.09 - 0.33<br>0.08 – 0.17 |
| Specificity | Specific |
| Solvent suitability | Solvent suitable for 48 hrs. |

**Discussion**

Calibration data for Mometasone furoate is shown in Table VIII. The calibration curve for Mometasone furoate was prepared by plotting area and concentration. (Figure.1) Linear equation for Mometasone furoate: y = 31016x + 2050.The developed HPLC method was validated. The linear range, correlation coefficient, detection limit and standard deviation for Mometasone furoate by HPLC method are shown in Table. Various system suitability test parameters were calculated and are shown in Table IX; X.

Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 99.4% – 99.8% for Mometasone furoate. Precision was calculated as repeatability, intra and inter-day variation & reproducibility for Mometasone furoate. The method was found to be precise with % RSD 0.10 - 0.34 for intra-day (n=3) and % RSD 0.09 – 0.17 for inter-day (n=3) for Mometasone furoate. The method was also found to be specific as no interference observed when the drug was estimated in presence of excipients. Chromatograms of blank, placebo, standard & sample preparation. Peak purity data is shown in table XV. The method was also robust as there was no change in area up to 48 hours of preparation of solution in Mobile phase. The LOD and LOQ for Mometasone furoate was found to be 3.98 (µg/ml) and 11.94 (µg/ml) respectively. Summary of validation parameters is tabulated in Table XII. Marketed Dosage Forms were analyzed by the proposed method and assay results of marketed formulations were shown in Table.XVI.

**Conclusion:**

The validated RP-HPLC method developed here proved to be simple, fast, accurate, precise and sensitive. Thus method described enables to the quantification of Mometasone Furoate. There is also simplicity of sample preparation and the low costs of solvent used. Peak purity of standard and sample preparation was resolved. So the method described is specific. Hence, this method can be used for analysis of Mometasone Furoate in pharmaceutical dosage form in quality control department for routine analysis.

**Acknowledgements:-**

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