# ANALYTICAL METHOD VALIDATION AND CLEANING VERIFICATION OF FELODIPINE BY HPLC METHOD

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**Summary**

Cross contamination is a major problem in the manufacture of pharmaceutical formulations in multi drug formulation plant. To avoid cross contamination major importance has to be given to the cleaning activity of equipments used for the manufacturing purpose. The present study is undertaken to validate analytical procedure for felodipine, to perform cleaning verification studies by using worst case approach and to find the efficiency of cleaning process. The method for analysis of felopdine is chosen high performance liquid chromatography (HPLC) and has been validated according to ICH guidelines. In cleaning verification among tablets, felodipine, is identified as the worst case drug. The swab samples are taken from all equipments and analyzed. The results of cleaning verification is found to be well within the acceptable limits (based on 10 ppm criteria and maximum allowable carry over (MACO) approach. Thus the present study is found suitable for validation of analytical method and cleaning verification of felodipine tablet formulation.

**Keywords**

Felodipine, Analytical method validation, Cleaning verification.

## Introduction

Felodipine is a member of the dihydropyridine class of calcium channel antagonist. It reversibly competes with nitrendipine and or other calcium channel blockers for dihydropyridine binding sites, blocks voltage dependent $Ca^{++}$ currents in vascular smooth muscle and cultured rabbit atrial cells, and blocks potassium induced contracture of the rat portal vein[1]. Validation is a concept that has been evolving continuously since its first formal appearance in the United States in 1978. Because of this, it has an intangible quality that has led to confusion and controversy, resulting in a rash of different definitions[2]. According to the Rules Governing Medicinal Products in the European Community[3], validation is the; action of proving, in accordance with the principles of good manufacturing practice that any procedure, process,

equipment, material, activity or system actually leads to the expected results. Reproducible and accurate analytical results are a prerequisite throughout pharmaceutical development and manufacturing.[4,5] Achieving these depends on the use of valid and robust methods. Critical factors that should be evaluated include; accuracy (as evidenced by selectivity, specificity and lack of bias), precision, recovery, linearity and system suitability[6]. The validation of cleaning methods is an important element of both qualification and process validation for drug substance and drug product manufacture[7]. The objective is to minimize the possibility of significant cross- contamination. Present study is undertaken knowing importance of analytical method validation and cleaning verification for manufacture of felodipine tablet formulations.

## Materials and Methods

### Materials

Felodipine is procured from Astra Zeneca Pharma India Ltd, Bangalore. Acetonitrile (HPLC grade from Merk), phosphoric acid and methanol (AR grade from Ranbaxy), sodium dihydrogen phosphate (AR grade from Merk) and disodium hydrogen phosphate (AR grade from S.D. fine- chem, Ltd) were obtained from commercial sources and used as received.

### Methods

#### Analytical Method Validation

For analytical method, the instrument used is HPLC agilent – 1100 series make and column chosen is Lichrocart, C18, 5µ, 150 ×4.6 mm. Injection volume is 50µl and flow rate is kept at 1.0 ml/ minute. Mobile phase is prepared by transferring 400 ml of acetonitrile to a 1000 ml of volumetric flask. To this 200 ml of methanol is added and diluted to 1000 ml volume with phosphate buffer of pH 3.0. The solution is filtered through 0.45µm membrane and degassed in a sonicator for two minutes. Microbonda Pak RP- C18 (300 mm × 3.9 mm) and hypersil ODS ( 125 mm × 4.0 mm) are choices of columns for method development. It is found that λmax of analyte felodipine in mobile phase is 362nm at best resolution. The retention time of felodipine is about 6 minutes at flow rate of 1 ml per minute.

#### Specificity

Specificity is studied taking 400 ppm of felodipine as standard solution and mobile phase as blank solution. 50 µl of mobile phase and 50 µl of standard solution are injected in HPLC instrument separately, chromatographs are compared visually.

**Precision**

For precision study, standard solutions are made of 20000 ppm (S1), 4000 ppm (S2) and 160 (S3) ppm of felodipine and mobile phase is taken as blank solution. 50µl of blank solution is injected and all three concentrations of 50 µl (S1, S2, and S3) are injected three times each separately in HPLC system and chromatographs are taken. The average, standard deviation and relative standard deviation (RSD) of 3 injections of each of S1, S2 and S3 are calculated. Based on signal (S) to noise (N) approach 360 ppm of felodipine standard solution is prepared and mobile phase is taken as blank solution, 50 µl of standard solution is injected in HPLC and area of peak of interest is recorded, 50 µl of mobile phas is injected in HPLC system three times separately and chromatographed $\frac{S}{N}=\frac{2H}{h}$ is calculated considering H as height of peak of interest from the maximum of the peak to extrapolated baseline of signal observed over a distance equal to 20 times width at half height and considering h as range of the background noise in a blank chromatogram observed over a distance equal to 20 times width at half height of peak of interest situated equally around where this peak of interest would be found. The limit of detection (LOD) is calculated by taking equation LOD (cone) = $\frac{\text{cone.peakof interest} \times 3}{\frac{S}{N}}$ where conc, peak of interest is concentration of the peak of interest in the sample solution used to estimate LOD and S/N is for the peak of interest in the sample solution. Limit of quantification (LOQ) is calculated by using equation

$$\text{LOQ( cone)} = \frac{conc.\%\ peak\ of\ \text{interest} \times 10}{S/N}$$

LOQ concentration is prepared from stock solution and chromatographed to conform the same.

**Linearity and range**

The concentration at which LOQ is established becomes linearity level 1 (LI) solution and prepared form stock solution, L2, L3, L4, L5 and L6 contains 256 ppm, 1280ppm, 3200ppm, 8000 ppm and 20000 ppm solution.

Mobile phase is taken as diluents and blank solution. 50µl of mobile phase is injected in HLPC system, 50µl of LI is injected six times, 50 µl of L2,

L3, L4 and L5 are injected one time each and 50 µl of L6 is injected six times and all are chromatographed.

**Accuracy**

300 ppm of felodipine solution is prepared as standard solution (SS) and 4440 ppm solution is prepared as stock solution (ST). Three accuracy level solution (A1, A2 and A3) are prepared by taking 1.0 ml. of SS to 25 ml volumetric flask and adding 5 ml, 10ml and 20 ml of ST respectively and making up volume by mobile phase. 50µl of mobile phase and 50 µl of SS are injected separately in HPLC system and chromatographed, 50 µl of A1, A2, and A3 individually are injected three times in HPLC system and chromatographed.

**Intermediate Precision**

The ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by change in instrument, analyst and change to equivalent column. The parameter is to be studied under three different methods on the next day considering the same analyst on different instrument with the same column and different analyst on different instrument with the same column. Standard solution ST1, ST2 and ST3 are prepared of 20000 ppm, 1600 ppm and 256 ppm of felodipine solution respectively. Mobile phase is taken as blank solution, 50µl of mobile phase is injected and 50 µl of ST1, ST2, and ST3 are injected three times of each separately in HPLC system and chromatographed. Average standard deviation and relative standard deviation of 3 injections, each of ST1, ST2, and ST3 are calculated.

**Robustness**

The parameter is studied by altering composition of mobile phase. The ratio of acetonitrile, methanol and phosphate buffer pH 3.1 (pH altered from previous) is 39:21:40 for mobile phase (composition and pH both are altered). It is prepared with same procedure described earlier. Three standard solution F1, F2 and F3 are prepared of 20000 ppm, 1600 ppm and 256 ppm solution of felodipine respectively. 50µl of mobile phase is injected in HPLC system and chromatographed. 50µl of F1, F2 and F3 are injected three times of each separately and chromotographed. Average, standard deviation and relative standard deviation of three injections of each of F1, F2 and F3 are calculated.

**Analytical Method Validation of Felodipine in Swab Samples:**

**Swab Analysis :**

It is carried out by procedure and methods given for analytical validation by HPLC method described earlier.

**Cleaning Validation :**

Felodipine is selected as worst case on the basis that it is most potent of all products and it has least solubility and more difficulty to clean. In the present study residue limits established is not more than 0.001 % which is carried over to the next product.

**Establishing Limits and Acceptance Criteria Based on Medical Dose Combination with Safety Factor :**

$$\text{Worst case} = \frac{\text{Smallest batch size of any other product in the group}}{\text{largest formula daily dose of any other product in the group}}$$

$$= \frac{50400000\ \text{mg}}{1043\ \text{mg per dose}} = 4832215\ \text{doses}$$

Maximum allowable carry over (MACO) =

$$\frac{\text{Minimum batch size of next product (MBS)} \times \text{Minimum therapeutic dose (MTD)}}{\text{Safety factor (SF)} \times \text{Single largest daily dose for the next batch (SDD)}}$$

SF is usually taken as 1000.

The MACO for felodipine tablet 5 mg is found 1930.0mg in present study.

Sampling is done for various equipments involved in felodipine tablet manufacture after they are cleaned according to standard operating procedures (SOP) used fro cleaning equipment and swab sampling method is used in this study.

The next batch of manufacture is metoprolol tartarate I.P. (100mg tablets) of batch size of six lakhs, total weight of materials used in batch is 187800 g and average weight of one tablet is 0.313 g.

**Table 1**

**MATRIX FOR ESTABLISHING THE WORST CASE PRODUCT**

**COMBINED MATRIX FOR POTENCY AND BATCH SIZE INFORMATION**

| NAME OF THE ACTIVE | MAX. BATCH SIZE (Kg) | MIN. THERAPEUTIC | SINGLE LARGEST DAILY DOSE (mg) |
|---|---|---|---|

| INGREDIENT | | DOSE (mg) | |
|---|---|---|---|
| METOPROLOL TARTRATE | 346.00 | 100.0 | 450.0 |
| TERBUTALINE SULPHATE | 141.00 | 7.5 | 15.0 |
| ISOSORBIDE-5-MONO NITRATE | 214.00 | 20.0 | 120.0 |
| FELODIPINE | 102.00 | 2.5 | 20.0 |
| RAMIPRIL | 38.00 | 1.25 | 20.0 |
| LISINOPRIL | 63.00 | 2.5 | 40.0 |

## Results and Discussions

The result of analytical method validation of felodipine by HPLC are depicted in Table 2.

**Table 2**

**SUMMERISED RESULTS OF ANALYTICAL METHOD VALIDATION OF FELODIPINE**

| S.No. | PARAMETERS | ACCEPTANCE CRITERIA | RSD % | RESULTS |
|---|---|---|---|---|
| 1 | Accuracy | Should be accurate across 50 -150 % Spiking | - | The method is found to be accurate across 50 – 150 % of the spiked data. |
| 2 | Precision | RSD should be with in 2% | 0.7015<br>0.7378 | The value of RSD is well under the limits. |
| 3 | Linearity & Range | Percentage curve fitting should not be less than 99.97 % across the range of 50-150% | - | The percentage curve fitting is found to be 99.99% |
| 4 | Specificity | Placebo should not interfere in analysis | - | Complied |
| 5 | Limit of Detection (LOD) | The signal to noise ratio should be 3 :1 | - | The LOD = 4.5ng/ml & Signal: Noise = 3: 1 |
| 6 | Limit of Quantification (LOQ) | The signal to noise ratio should be 10 :1 and RSD should be within 2% | 1.0596 | The method is found to be complied. |

| | | | | |
|---|---|---|---|---|
| 7 | System Suitability | Theoretical plates/meter should not be less than 1500 | - | Found to be 15999.52 |
| | | Theoretical plates/column should not be less than 1500 | - | Found to be 1999.94 |
| | | Tailing factor should be close to 1 | - | Found to be 1 |
| 8 | Ruggedness | No significant difference between two analysts | - | The method is found to be Rugged |
| 9 | Robustness | No significant difference between two extreme conditions | - | The method is found to be Robust |

RSD=Relative Standard Deviation

The proposed method is found to be simple, accurate, precise linear, rugged and rapid. Hence this method is suitable for quality control of raw material, formulation and in residual analysis.

Analytical method validation of felodipine in swab samples is carried out to establish documented evidence, which provides a high degree of assurance that analytical method is suitable for the estimation of felodipine in swab cleaning verification samples. Results are conforming and close to Table 2. It is found from results that method is suitable.

**Table 3**

**OBSERVATION OF CLEANING VALIDATION (SWAB SAMPLES)**

| **Equipment and its surface area (Square decimeter)** | **Equipment part Swabbed** | **Peak Area** | **Concentration of drug per Swab (μg/dm$^2$)** | **Total concentration of drug in μg/dm$^2$** | **Concentration of drug as per surface area of equipment in μg including correction factor** | **The residue of drug which can pass into next batch in μg/dm$^2$** |
|---|---|---|---|---|---|---|
| SIFTER (194.35) | HOPPER | 2215 | 22.7 | 143.0 | 143.0 × 194.35 = 27792<br>27792 × 1.43 (Correction factor) = 39742 | 205136.4 |
| | RING | NIL | NIL | | | |
| | BOWL | 11637 | 119.5 | | | |
| | MESH | NIL | NIL | | | |
| RAPID MIXER GRANULATOR (542.08) | LID | NIL | NIL | NIL | NIL | NIL |
| | BOWL | NIL | NIL | | | |
| | CHOPPER | NIL | NIL | | | |
| | IMPELLER | NIL | NIL | | | |
| | DISCHARGE PORT | NIL | NIL | | | |
| FLUID BED DRIER (251.90) | WINDOW | NIL | NIL | NIL | NIL | NIL |
| | RETARDIGN DRUM | NIL | NIL | | | |
| | BOWL | NIL | NIL | | | |
| | MESH | NIL | NIL | | | |
| MULTI MILL (110.28) | HOPPER | NIL | NIL | NIL | NIL | NIL |
| | MESH | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | DISCHARGE PORT | | | | | |
| DOUBLE CONE BLENDER (645.43) | UPPER LID | NIL | NIL | | | |
| | BOWL | | | | | |
| | BOTTOM LID | | | | | |
| COMPPESSION MACHINE (84.48) | TURRETT | NIL | NIL | NIL | NIL | NIL |
| | DIEBORE | NIL | NIL | | | |
| | DIE CAVITY | NIL | NIL | | | |
| | DELIVERY CHUTE | NIL | NIL | | | |
| | HOPPER | NIL | NIL | | | |

The total residue of drug which can pass into next batch = 39.7 mg.

**Table 4**

**SUMMARIZED RESULTS OF CLEANING VERIFICATION**

| **SL NO.** | **SAMPLING METHOD** | **EQUIPMENT** | **ACCEPTANCE CRITERIA (mg)** | **MACO (mg)** | **TOTAL RESIDUAL CARRY OVER (mg)** | **RESULT** |
|---|---|---|---|---|---|---|
| 1. | SWAB METHOD | SIFTER | 1930 | 39.7 | 39.7 | It is found to be within the acceptance limit |
| 2. | | RAPID MIXER GRANULATOR | | 0 | | |
| 3. | | FLUID BED DRIER | | 0 | | |
| 4. | | MULTI MILL | | 0 | | |
| 5. | | DOUBLE CONE BLENDER | | 0 | | |
| 6. | | COMPRESSION MACHINE | | 0 | | |
| 7. | | STRIP PACKING MACHINE | | 0 | | |

MACO=Maximum allowable carry over

The cleaning verification of the equipments used in manufacturing process of felodipine tablet is carried out to provide documented evidence with high degree of assurance and presented in Table 3 and Table 4. Samples for analysis are obtained by swab method. The total residual carry over of felodipine is found to be 39.7 mg. The result is found to be well within, the acceptance criteria of 10 ppm and MACO limits of 1930 mg. It is concluded that the cleaning procedure followed is appropriate.

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