INTRODUCTION:
Polmacoxib is 4-[3-(3-fluorophenyl)-4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl]benzenesulfonamide. Its molecular formula is C_{18}H_{14}FNO_{3}S and molecular weight is 361.39 gm/mol.

Structure

Polmacoxib, developed as CG100649 is also a selective cyclooxygenase-2 (COX-2) inhibitor, a type of non-steroidal antiinflammatory drug and acts as a potent inhibitor of several other CA isoforms (CA I and II), due to its aryl sulfonamide moiety, similar to celecoxib and valdecoxib. Unlike other NSAIDs, polmacoxib has a dual mode of action: inhibition of COX-2 and binding to carbonic anhydrase (CA) with high affinity. A key function of CA is to regulate the pH level in the body through the interconversion between carbon dioxide and bicarbonate. Where COX-2 and CA coexist, the high-affinity binding of polmacoxib to CA reduces the COX-2 inhibitory activity of polmacoxib. Preliminary experiments have shown the COX-2 inhibitory activities of polmacoxib with varying amounts of CA in the system. Lower intestine perforation caused by selective COX-2 inhibitors has been reported, but its incidence is extremely low. CG100649 in water is very low, and the different lattice energies of polymorphs give rise to different solubilities and dissolution rates. It is also Soluble in Acetone, DMSO, and DMF (Dimethyl formamide).

Reversed phase chromatography has found both analytical and preparative application in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase. The actual nature of the hydrophobic binding interaction itself is a matter of heated debate but the conventional wisdom assumes the binding interaction to be the result of a favourable entropy effect. The initial mobile phase binding conditions used in reversed phase chromatography are primarily aqueous which indicates a high degree of organised water structure surrounding both the solute molecule and the immobilised ligand. As solute binds to the immobilised hydrophobic ligand, the hydrophobic area exposed to the solvent is minimised.
Therefore, the degree of organised water structure is diminished with a corresponding favourable increase in system entropy.

In this way, it is advantageous from an energy point of view for the hydrophobic moieties, i.e. solute and ligand, to associate.

**MATERIAL AND METHODS**

**Instrumentation and Equipment**

In this experiment Waters Alliance2695 Separation Module integrated with Waters 2996 Photodiode Array Detector at 238nm using Phenomenex Luna C18, column (250mmx4.6mm), 5 µm was used and data was integrated using a Empower Pro software.

All the weighing in the experiments was done with Mettler Toledo analytical balance capable of measuring with an accuracy of 0.1 mg. The solubility was enhanced by sonication on an Ultrasonic bath.

Glass wares used in the entire process were thoroughly cleaned and calibrated.

**Reagents and Chemicals**

ACN (Thermo Fischer scientific) & Water (HPLC Grade)

**Reference standard and Test Samples**

Reference Standard and test samples of Polmacoxib were arranged from Indian Pharmacopoeia Commission (IPC) Ghaziabad.

**Methods**

| Chromatographic Condition | Property                  | Polmacoxib | Acceptance criteria |
|---------------------------|---------------------------|------------|---------------------|
| **Column**                | Phenomenex Luna C18, column (250mmx4.6mm), 5 µm |            |                     |
| **Organic Phase**         | ACN: Water (60:40)        |            |                     |
| **Detector**              | PDA detector              |            |                     |
| **Flow Rate**             | 1.0mL/min                 |            |                     |
| **Wavelength**            | 238nm                     |            |                     |
| **Injection Volume**      | 20µL                      |            |                     |
| **Temperature**           | Ambient                   |            |                     |
| **Diluent**               | Water and ACN in ratio 50:50 v/v |            |                     |

Table 1: System suitability parameters of Polmacoxib

| Sr. No. | Property                  | Polmacoxib | Acceptance criteria |
|---------|---------------------------|------------|---------------------|
| 1.      | Retention Time (RT)       | 8.12       |                     |
| 2.      | Tailing factor (T)        | 1.01       | NMT 2.0             |
| 3.      | Theoretical plates (N)    | 16336      | NLT 2000            |

From the data it was found that all the system suitability parameters for developed method were within the limit.

**Preparation of Standard stock Solution**

50.78mg of Polmacoxib RS was weighed and transfer into 50ml volumetric flask then added small volume of diluent and sonicate for 5 minutes to dissolve and dilute up to the mark with diluent.

**Preparation of Standard Solution**

Further pipette 10ml from the standard stock solution into 100ml volumetric flask and then dilute up to mark with diluent which possess the concentration of Polmacoxib RS 100 µg/ml.

**Preparation of Sample solution**

51.18mg of Polmacoxib was weighed accurately and transfer into 50ml volumetric flask then added small volume of diluent and sonicate to dissolve for 5-10 minutes and dilute up to the mark with diluent. Further pipette 10ml from this solution into 100ml volumetric flask and then dilute up to the mark with diluent.

The prepared solutions were stored at room temperature which possesses the concentration of 100 µg/ml.

**Method Validation**

The developed method was validated with respect to system suitability, specificity, linearity, precision, accuracy LOD, LOQ and robustness in the accordance of the ICH Q2 guidelines.

**Specificity and selectivity**

The developed method was found to be selective for Polmacoxib, since the injection of the blank solution confirmed the absence of interfering peak at RT examined substance at 238nm. The results obtained demonstrate that there was no interference from other material in the developed method and therefore confirm the specificity of the method.

**System Suitability**

System Suitability tests are an integral part of method development and were used to ensure adequate performance of the chromatographic system. Retention Time (RT), tailing factor, peak asymmetry, and theoretical plates (T) were evaluated. The results are shown here in Table 1.

Chromatogram: Blank of Polmacoxib
**Linearity and Range**

Linearity of the developed method demonstrates the ability of method to produce a result which is directly proportional to concentration of analyte in the sample. The amount of Polmacoxib was prepared for linearity in the range of 80-120 ppm. The amount of polmacoxib in five different concentrations is 80 ppm, 90 ppm, 100 ppm, 110 ppm and 120 ppm respectively. The graph was plotted between concentrations versus area of peak. The Polmacoxib shows good correlation coefficient ($r^2 = 0.999$) and the proposed method was linear in concentration range 80-120 ppm.

**Table 2: Linearity of Polmacoxib**

| S. No. | Compound | Values of X and Y variables | Correlation co-efficient |
|-------|----------|----------------------------|--------------------------|
| 1.    | Polmicoxib | Variable 1 2 3 4 5 | 0.999 |
|       |          | X 80 90 100 110 120 |               |
|       |          | Y 4737372 5276776 5887575 6470577 7033314 |               |

Note: X is the concentration of the respective component in µg/ml, Y is the peak response of the respective component in area counts.

**Linearity Curve**

Calibration curve was constructed between concentrations versus peak area. Results were recorded for equation of line, correlation coefficient and intercept were determined. Where, $Y$: area $X$: Unknown concentration $m$: Slope of graph $c$: Intercept.

**Precision**

It reveals the data regarding closeness between the series of measurements. The precision of the developed method was verified by system precision and method precision. A homogenous sample 100 µg/mL of Polmacoxib was prepared under prescribed conditions and estimation was carried out. The results are expressed in the form of standard deviation and RSD value. Table 2 shows the result of system precision and method precision respectively and the developed method is highly precise as % RSD is less than 2%.

**Table 3: Calculation of %RSD for Polmacoxib (System Precision)**

| S. No. | Compound (Polmacoxib) | No. of Injections | Concentration (µg/ml) | Area count |
|--------|-----------------------|-------------------|-----------------------|------------|
| 1.0    | Reference Standard (RS)| 1 2 3 4 5 6       | 80.0                  | 4737372    |
|        |                       |                   | 90.0                  | 5276776    |
|        |                       |                   | 100.0                 | 5887575    |
|        |                       |                   | 110.0                 | 6470577    |
|        |                       |                   | 120.0                 | 7033314    |
The best results were found for Polmacoxib. According to earlier mentioned parameters, LOD and LOQ (Limit of Detection and Limit of Quantification) were estimated for Polmacoxib for each level solution was prepared from solution of Polmacoxib for determination of LOQ and LOD. The signals of sample and blank sample of LOD and LOQ were obtained was 3.07%. The study proves that the method is robust under ± 2 wavelength, ± 10% flow rate and ± 10% increase and decrease in organic phase and at the different column (Inertsil ODS-3, column (250mmx4.6mm), 5 micron. There is no significant change in recovery of Polmacoxib. The % RSD shown in table 8 negligible changes were observed during robust condition. So we can say that the developed method is robust.

Table 6: Summary of assay of Polmacoxib

| S. No. | Level  | % Average Assay (ODB) | %RSD |
|-------|--------|-----------------------|------|
| 1     | 80%    | 100.39                | 0.08 |
| 2     | 100%   | 99.97                 | 0.27 |
| 3     | 120%   | 99.89                 | 0.29 |

The percentage of assay value of polmacoxib in the range of 99.22-100.0 % and the % RSD of assay value obtained was in the range of 0.08-0.37 %. The study proves that the method is accurate for the estimation of Polmacoxib assay over the range of 80-120% of target concentration

LOD and LOQ (Limit of Detection and Limit of Quantification)

Limit of detection (LOD) and Limit of Quantification (LOQ) reveal information regarding concentration of analyte that yields signal-to-noise around 1 to 10. Serial dilutions are made from solution of Polmacoxib for determination of LOQ and LOD. The samples were injected in HPLC and compare the signals of sample and blank sample of LOD and LOQ. According to earlier mentioned parameters, LOD and LOQ were estimated for Polmacoxib 0.5 µg/ml and 1.5 µg/ml.

Table 7: LOD & LOQ studies of Polmacoxib

| Drug     | LOD   | LOQ   |
|----------|-------|-------|
| Polmacoxib | 0.5 ppm | 1.5 ppm |
CONCLUSION

The HPLC method was successfully developed and validated on a Waters 2996 alliance for simultaneous determination of Polmacoxib. The method for simultaneous determination has not been reported before. This present method is novel for the determination of drug at a single wavelength, 10µL injection capacity and Phenomenex Luna C18 5µm 4.6*150mm column. It was the found that the method is sufficiently simple, rapid and as well as precise, accurate, linear, robust which compiles the ICH guidelines. The entire experimentation was proved that the developed HPLC method shows good resolution, linearity and RSD values (less than 2%). Which indicate the method is suitable for the determination of Polmacoxib.

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