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Identification And Evaluation Of Some Known Impurities In Active Pharmaceutical Ingredients Of Polmacoxib Using Rp-Hplc

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Abstract:

A Novel, efficient and convenient reversed-phase high-performance liquid chromatography method was developed for Polmacoxib (PCB) drug in the presence of its impurities. Successful separation of Palmacoxib drug from the its impurities was achieved on Zorbax SB-C8 250 x 4.6 mm, 5.0 µm with gradient elution of Acetonitrile:Methanol as a mobile phase. The Ultraviolet detection was monitored at a wavelength of 240 nm at flow rate 1.0 mL/minute. The validation of proposed method was carried for linearity, precision, accuracy, limit of detection, limit of quantification and robustness were determined in accordance with ICH guidelines. The method has good specificity and specified impurities can be effectively separated with good resolution. The proposed method is found to have linearity in theitech 4-20 µg/mL concentration range of Polmacoxib with correlation coefficients of not less tuuticahan 0.999. The limit of detection for the drug and impurities are 0.81µg/ml , 0.45µg/ml and 0.65µg/ml and the limit of quantification for the drug and impurities are 2.75µg/ml, 1.50µg/ml and 2.20 µg/ml respectively. The method successfully estimated the drug in formulation tablet in the presence of known impurities. The proposed method can be applied for quality control studies of other drugs with the competence of simplicity, accuracy, robustness, good selectivity, and high sensitivity.

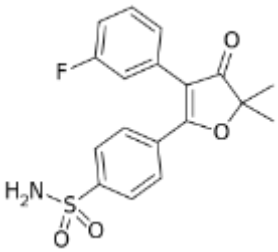
Keywords: Polmacoxib, Impurity, RP-HPLC.

Introduction:

Polmacoxib is a nonsteroidal anti-inflammatory drug. Proven to be potent for osteoarthritis. It was developed and approved in South Korea[22]. Polmacoxib inhibits the enzymes and COX-2 inhibitors. Drug proven to have some effects on prostaglandin metabolites during urinary tractions. Both Polmacoxib along with celecoxib also suggested to have cardiovascular risk profile[23]. In addition to that the relationships of Polmacoxib to other cox inhibitor drugs have been guided by clinical development strategies Subject to their anti-inflammatory activity and COX-2 Inhibition, along with severe side effects such as heart failure, leads to development of several new cox-2 inhibitors, in which Polmocoxib stand in front ahead and found to be the most promising and 16-fold efficient

that Etoricoxib with not admissible side effects such as heart failures. The relationships of Polmacoxib to other cox inhibitor drugs have been guided by clinical development strategies.

The structural formula of Polmacoxib is:



Polmacoxib

Impurity profiling:

Impurity profiling is a group of analytical activities for detection, isolation, identification/structure elucidation, and quantitative determination of organic and inorganic impurities and residual solvents in bulk drugs and pharmaceutical formulations. The impurity profiling identification plays vital role in identifying the related impurities arose in drug substance and drug product, for its best control and efficacy.

Several methods for impurity identification, isolation and characterization are Gas chromatography, Flash chromatography, Column chromatography, TLC, GC, Capillary electrophoresis (CE), HPLC, HPTLC.

Materials and Methods:

High performance liquid of Waters2690 separation module equipped with Auto Sampler and PDA detector with Column (Zorbax SB-C8 250 x 4.6 mm, 5.0 µm).

Preparation of Buffer: Prepare a solution 1.0 mL of Orth phosphoric acid (88%) acid dissolved in 1000 mL of water and mix. Filter and degas through 0.45µm membrane filter paper.
Preparation of Solvent-A: Use buffer as solvent-A.

Preparation of Solvent-B: A mixture of acetonitrile and water in the ratio 80:20 % (v/v) has to be Filter and degas through 0.45µm membrane filter paper.

Preparation of stock solution:

Weigh accurately about 5.0 mg of Methyl impurity, (Imp-A) 5.0 mg FCS impurity(Imp- B) and 25mg PCB reference standard into a 25 mL volumetric flask, dissolve it and dilute with diluent and sonicate.

Standard Solution Preparation:

Weigh accurately about 5.0 mg of Methyl impurity, (Imp-A) 5.0 mg FCS impurity (Imp- B) and 25mg PCB reference standard into a 25 mL volumetric flask, dissolve it and dilute with diluent and sonicate. Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Method development and validation:

Linearity:

Weigh accurately about 5.0 mg of Methyl impurity, (Imp-A) 5.0 mg FCS impurity (Imp-B) and 25mg PCB reference standard into a 25 mL volumetric flask, dissolve it and dilute with diluent and sonicate. Take 5 different concentration (0.2, 0.4, 0.6, 0.8, 1.0) 1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluents. Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same. Inject the standard solution, Accuracy - 50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Polmacoxib and calculate the individual recovery and mean recovery values.

Precision:

Weigh accurately about 5.0 mg of Methyl impurity, (Imp-A) 5.0 mg FCS impurity (Imp- B) and 25mg PCB reference standard into a 25 mL volumetric flask, dissolve it and dilute with diluent and sonicate. Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specifiedlimits.

Intermediate Precision/ Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method,precision was performed on different day within the laboratory. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Robustness:

Robustness was studied by deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

Limit of Detection:

Preparation of 0.82µg/ml solution:

Weigh accurately about 5.0 mg of Methyl impurity, (Imp-A) 5.0 mg FCS impurity (Imp- B) and 25mg PCB reference standard into a separate 25 mL volumetric flask, dissolve it and dilute with diluent and sonicate. Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Finally pipette 1.35ml of Polmacoxib, 3.8ml of Imp-A solution and 5.4ml of the Imp-B solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Limit of Quantification:

Preparation of 2.70µg/ml solution:

Weigh accurately about 5.0 mg of Methyl impurity, (Imp-A) 5.0 mg FCS impurity (Imp- B) and 25mg PCB reference standard into a separate 25 mL volumetric flask, dissolve it and dilute with diluent and sonicate. Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask

and dilute up to the mark with Diluents. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Finally pipette 4.5ml of Polmacoxib, 1.3ml of Imp-A solution and 1.8ml of the Imp-B solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Results and Discussion:

Linearity:

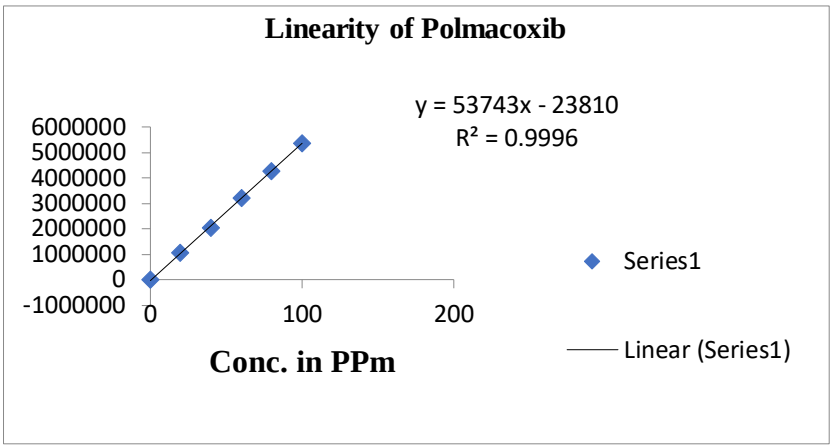


Fig: Calibration graph for Polmacoxib

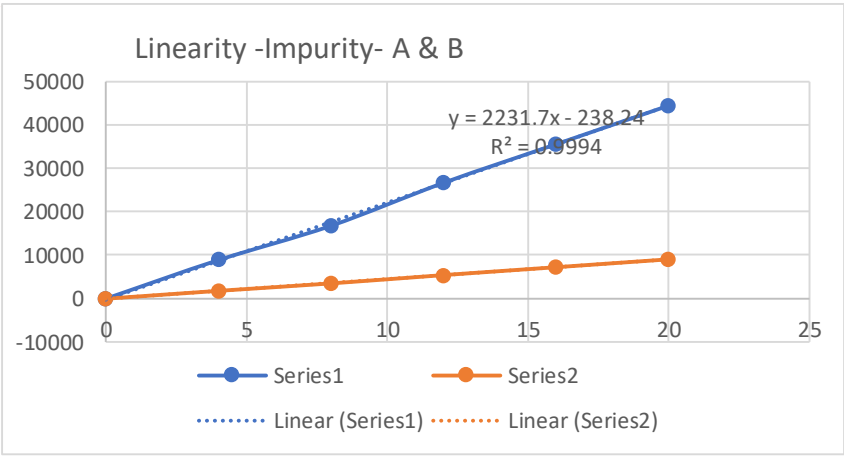


Fig: Calibration graph for Impurity A and B

Parameters	Polmacoxib	Impurity A	Impurity B
Slope (m)	53743	2231.7	445.87
Intercept (c)	23810	238.24	24.04
Correlation coefficient (R^2)	0.999	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

The correlation coefficient obtained was 0.999 which is in the acceptance limit.

ACCURACY:

Accuracy (recovery) data for Polmacoxib

%Concentration Polmacoxib (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	1607995	12.5	12.48	99.80	99.72
100%	3208527	25	24.89	99.57	
150%	4823985	37.5	37.43	99.80	

Accuracy (recovery) data for Impurity A

%Concentration Impurity A (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	13209	2.5	2.47	98.76	99.46
100%	26701	5	4.99	99.82	
150%	40042	7.5	7.48	99.80	

Accuracy (recovery) data for Impurity B

%Concentration Impurity B (at specification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	2661	2.5	2.49	99.43	99.51
100%	5317	5	4.97	99.33	
150%	8011	7.5	7.48	99.78	

Acceptance Criteria:

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

PRECISION:

Results of Precision for Polmacoxib

Injection	Area	Area	Area
Injection-1	3215990	26695	5342
Injection-2	3208990	26695	5342
Injection-3	3215990	25695	5342
Injection-4	3215990	26695	5442
Injection-5	3115990	26695	5342
Injection-6	3215990	26695	5342
Average	3198157	26528.33	5358.667
Standard Deviation	40350.55	408.2483	40.82483
%RSD	1.2	1.5	0.7

Acceptance criteria:

%RSD for sample should be NMT 2

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

INTERMEDIATE PRECISION:

Results of Intermediate precision for Polmacoxib

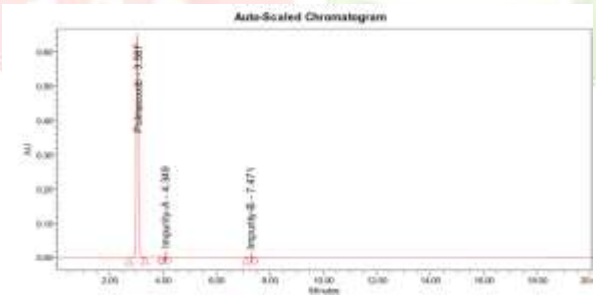
Injection	Area	Area	Area
Injection-1	3115990	26695	5342
Injection-2	3208990	26695	5342
Injection-3	3225990	25695	5342
Injection-4	3215990	26695	5442
Injection-5	3115990	25695	5542
Injection-6	3214990	26695	5342
Average	3182990	26361.67	5392
Standard Deviation	52184.29	516.3978	83.666
%RSD	1.6	1.9	1.5

Acceptance criteria:

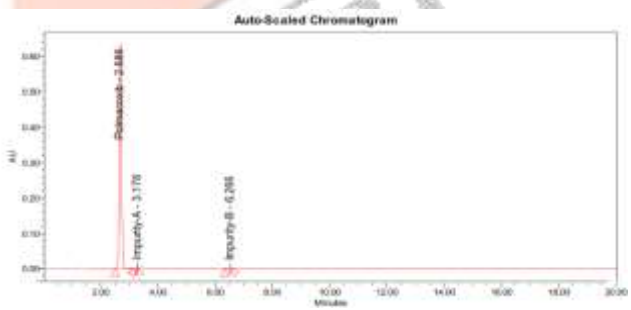
%RSD of five different sample solutions should not more than 2
The %RSD obtained is within the limit, hence the method is rugged.

ROBUSTNESS:

Variation in flow:

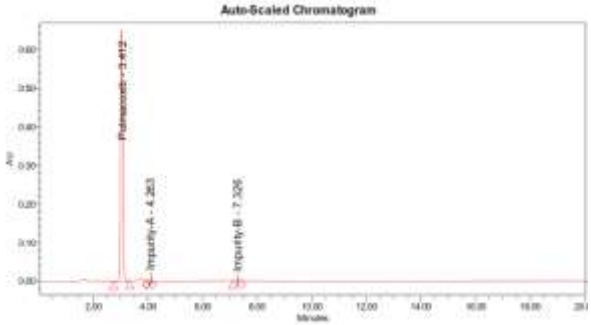


Chromatogram showing less flow

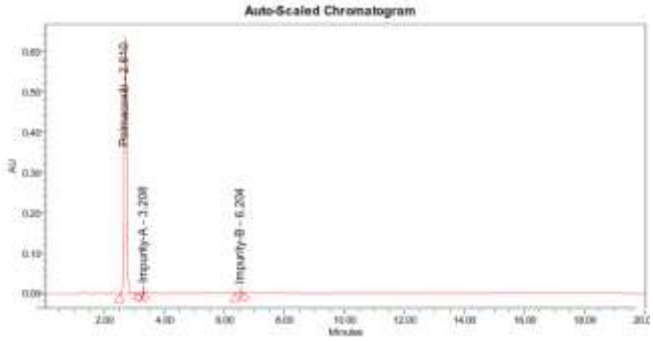


Chromatogram showing more flow

Variation of mobile phase organic composition:



Chromatogram showing less organic composition



Chromatogram showing more organic composition

Acceptance criteria:

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the methodis robust.

LIMIT OF DETECTION:

The LOD values of polmacoxib, Impurity A and Impurity B was found to be 0.81, 0.45, 0.65 µg/ml respectively. Signal to noise ratio shall be 3 for LOD solution. The result obtained is within the limit.

LIMIT OF QUANTIFICATION:

The LOQ values of polmacoxib, Impurity A and Impurity B was found to be 2.75, 1.50, 2.20 µg/ml respectively. Signal to noise ratio shall be 10 for LOQ solution. The result obtained is within the limit.

CONCLUSION:

The Polamcoxib drug along with the known impurities Methyl Impurity: 4-(3-fluorophenyl)-2,2-dimethyl-5-(4-(methylthio)phenyl)furan-3(2H)-one (Imp-A), FCS Impurity : Sodium 4-(3-(3-fluophenyl)-5, 5-dimethyl-4-oxo-4,5-dihydrofuran-2-yl) benzenesulfinate (Imp-B) has been evaluated using suitable analytical RP-HPLC method development and validation.

From the above work, it is concluded that Polmacoxib has and the corresponding process related and degradation impurities have been controlled with in process controls and with a suitable analytical method been synthesized. Further the structures of these impurities have been evaluated for confirmation. Further the process didn't involve any

organic bases such as alkyl amines, so that it may be concluded that the possiblez formation of NSA (nitrosamine impurities) is negligible.

The present suggested method can be further used to evaluate more unknown impurities when combined with other suitable drug combinations.