Formulation, Analytical Method Development and Validation of Anti-Arthritis Drugs by HPLC Method

[1]Prasad A. Badukale, [2]Vijay M. Waghulkar, [3]Monika P. Jadhao

[1]Student, [2]Professor, [3]Professor
[1]Department of Quality Assurance,
[1]Vidyabharti College of Pharmacy, Amravati, Maharashtra, India
prasadbadukale14@gmail.com

Abstract: This disquisition paper presents the development and evidence of a High- Performance Liquid Chromatography(HPLC) system for the estimation of Piroxicam and Esomeprazole, both of which are significant medicinal used in pain operation and gastric acid storing inhibition, singly. The study details the regular approach taken to establish a reliable logical system, including the characterization of the drugs, solubility studies, selection of chromatographic conditions, and the drug of mobile phases. The optimized HPLC system was validated in agreement with ICH guidelines for parameters including particularity, delicacy, perfection, linearity, limit of discovery, and robustness. The system demonstrated satisfactory performance characteristics, including a high degree of particularity and linearity across a wide attention range. The developed HPLC system is presented as a simple, rapid-fire- fire, and cost-effective logical tool suitable for routine quantitative and qualitative analysis of Piroxicam and Esomeprazole in pharmaceutical phrasings. The findings support the system's operation within the pharmaceutical sedulity, enhancing the quality control processes for these essential specifics.

***Index Terms*—High- Performance Liquid Chromatography (HPLC), Piroxicam, Esomeprazole, Method Development and Validation, Tablet**

I. INTRODUCTION:

Piroxicam is a monocarboxylic acid amide performing from the formal condensation of the carboxy group of 4- hydroxy acid 1,1-dioxide with the exocyclic nitrogen of 2- aminopyridine. A non- steroidal anti- seditious drug of the oxicam class, it's used to relieve pain and works by preventing the product of endogenous prostaglandins involved in the agreement of pain, stiffness, humaneness and lump. It has a part as an analgesic, a cyclooxygenase 1 asset, a non- steroidal anti-seditious drug, and an antirheumatic drug. It's a benzothiazine, a member of pyridines and a monocarboxylic acid amide. A cyclooxygenase-steroidal anti-seditious agent (NSAID) that is well established in treating rheumatoid arthritis and osteoarthritis and used for musculoskeletal conditions, dysmenorrhea, and postoperative pain. Its long half- life enables it to be administered formerly daily.[1-3][9-10]

Esomeprazole is a 5- methoxy- 2-{((4- methoxy -3,5-dimethylpyridin-2-yl) methyl) sulfinyl}- 1H- benzimidazole that has S configuration at the sulphur grain. An asset of gastric acid storing, it's used(generally as its sodium or magnesium navigator) for the treatment of gastro- oesophageal influx complaint, dyspepsia, peptic ulcer complaint, and Zollinger- Ellison pattern. It has a part as a histamine antagonist, an EC 3.6.3.10(H() K()- switching ATPase) asset, an anti- ulcer medicine and an EC 1.4.3.4(monoamine oxidase) asset. It's an enantiomer of a(R)- omeprazole. Esomeprazole, vended under the brand name Nexium, is a proton pump asset(PPI) drug used for the operation of gastroesophageal influx complaint(GERD), for gastric protection to help rush of stomach ulcers or gastric damage from habitual use of NSAIDs, and for the treatment of pathological hypersecretory conditions including Zollinger- Ellison(ZE) Syndrome.[4-8]

High-Pressure Liquid Chromatography is a particular type of Column Chromatography. The three primary components of HPLC are a column that contains packing material (stationary phase), a pump that circulates the mobile phase(s) through the column, and a detector that displays the molecules retention durations. The interactions between the stationary phase, the molecules being analysed, and the solvents) utilized affect the retention period.

Advantages of HPLC:

• Quick and effective separations (high resolution power).

• A constant watch on the column effluent.

• It may be used to separate and dissect extremely complicated combinations • Precise numerical measures.

• Excellently produced separations are achieved by adsorption, partition, ion exchange, and rejection columns.

Disadvantages of HPLC:

• There's presently not extensively available, sensitive discovery system

• Column performance is largely sensitive and contingent upon the Quilting system

• Extremely precious, have poor perceptivity for some motes, and are unfit to descry some because they're irreversibly adsorbed[10]

METHOD DEVELOPMENT & VALIDATION:

NEED OF ANALYTICAL METHOD DEVELOPMENT:

For the analysis of the enrolment batch and accelerated stability test samples to produce secure results in the fab, well- characterized and well vindicated logical procedures must be used. It's imperative to emphasize that every logical system possesses distinct attributes that differ depending on the analyte under disquisition. To give accurate confirmation data for different spots and parameters, as well as to show intra- and inter-laboratory responsibility, it's pivotal to assess the logical results when sample analysis for a specific study is conducted at multiple locales and in a marketable batch for mortal consumption system(s) in compliance with ICH recommendations styles are developed for new products when no sanctioned styles are available.

METHOD VALIDATION:

The word "Validation" comes from the Latin word "Strong ness." The power or robustness of a method, process, or piece of equipment to function is known as Validation. It is the act of demonstrating an approach's acceptance by confirmation and documenting it legally using evidence from science. The process of proving through laboratory testing that an analytical method's performance characteristics satisfy the demands of the planned analytical application is known as validation. Any new or modified procedure must be validated to make sure it can produce repeatable and trustworthy results when utilized by various operators using the same equipment in various laboratories. The method and its intended uses determine exactly what kind of validation program is needed.

Method Validation Parameter:

• Precision

• Accuracy

• Specificity

• Selectivity

• Linearity Range

• Limit of Detection (LOD)

• Limit of Quantitation (LOQ)

• Robustness

• Ruggedness

• System Suitability

II. MATERIALS AND METHODS:

Materials:

The drugs, chemicals, reagents, instruments, and filters used during the experiment.

Active Pharmaceutical Ingredients Used:

Table 1: Active Pharmaceutical Ingredients

Sr. No	Drug	Manufacturer/Supplier
1.	Piroxicam	APEX HEALTHCARE LIMITED 4710-4711, GIDC ESTATE, ANKLESHWAR, 393002, GUJARAT, INDIA
2.	Esomeprazole	DHAMTEC PHARMA AND CONSULTANTS B-204, SILVER SPRIGS, OPPOSITE MIDC OFFICE, TALOJA, NAVI MUMBAI, 410208, MAHARASHTRA, INDIA

Instruments Used:

Table 2: Instruments Used in Method Development

Sr. No	Name of Instrument	Model
1.	HPLC System	Younglin-HPLC System
2.	Detector System	Detector-UV detector (730D)
3.	Analytical Column	Hypersil-BDS, C 18 (250 mm*4.6 mm, 5 µm)
4.	Software	Autochrom 3000
5.	pH Meter	M Lab
6.	Injector	Manual
7.	Analytical Balance	Shimadzu Model-ATX224
8.	UV Spectrophotometer	Shimadzu UV1800 Spectrophotometer (Japan Corporation)
9.	Ultrasonicator	(Servewell Instrument) RC-System MU-1700

Solvents and Chemicals Used:

Table 3: Solvents and Chemicals Used in Method Development

Sr. No	Solvents & Chemicals:	Manufacturer
1.	Methanol (Gradient grade)	Qualigens (Thermo Fisher Scientific India Pvt. Ltd)
2.	Sodium Acetate Anhydrous (Extrapure)	Finar Chemicals Pvt. Ltd
3.	Glacial Acetic acid (HPLC grade)	Qualigens (Thermo Fisher Scientific India Pvt. Ltd)
4.	Water (HPLC grade)	Qualigens (Thermo Fisher Scientific India Pvt. Ltd)

Method:

Experimental Work:

Physical Characterization of Piroxicam (API):

Colour: White/Off-White/ Light Yellow

Odour: Odourless

Texture: Fine Powder

Taste: Bitter

Melting point: 198-200 °C

Physical Characterization of Esomeprazole (API):

Colour: White/Off-White/ Light Yellow

Odour: odourless

Texture: Fine Powder

Taste: Bitter

Melting point: 155 °C

Method Development & Validation

Optimization of Chromatographic Condition for The Estimation of Piroxicam and Esomeprazole

Solubility Studies:

Before Method Development, Solubility of drugs was tested in different solvents to obtain a suitable solvent which can be used for estimation of drugs in available dosage form. The Solubility of Piroxicam and Esomeprazole was best achieved in Methanol.

Selection of Wavelength:

An UV spectrum of 10 µg/ml Piroxicam in Methanol and Esomeprazole in Methanol was recorded by scanning in the range of 200 nm to 400 nm. The wavelength which gives good response and showed optimal absorbance for drugs was selected. The UV spectrum a wavelength of 295nm for Esomeprazole and a wavelength of 330nm for Piroxicam was selected.

Selection of Chromatographic Method:

Proper chromatographic method selection depends on the nature of sample properties like ionic/ ionizable/neutral character, its molecular weight and solubility and different physio-chemical properties. The drug selected for the study was high in affinity towards organic solvents. Hence Reverse Phase HPLC or lon-Pair or lon Exchange Chromatography must be used. The RP-HPLC method was selected for the initial separations because of its suitability and simplicity.

Identification and Characterization of Drug:

Before commencing the experimental work, it is necessary to determine the different physical and chemical property of the drugs which provide information regarding the purity and nature of drugs.

Selection and Procurement of Drug:

Piroxicam and Esomeprazole were selected as drug candidates for Method Development and Validation.

The Piroxicam drug was received as gift sample from Apex Healthcare Limited4710-4711, GIDC Estate, Ankleshwar, 393002, Gujarat, India and The Esomeprazole drug was purchased from Dhamtec Pharma and Consultants, B-204, Silver Sprigs, Opposite MIDC Office, Taloja, Navi Mumbai, 410208, Maharashtra, India. Procured drug was analyzed for different physical properties viz Colour, Odour, Melting Point, etc.

Physio-Chemical Characterization:

The Physio-Chemical Characterization of drug molecules is important regarding its purity, identification in development and validation of analytical method. The various tools used for characterization of drug molecules includes Melting Point, UV spectroscopy, Solubility Study, etc.

SPECTROSCOPIC STUDIES:

Determination of Isosbestic Point:

Stock (10 µg/ml) of Piroxicam and Esomeprazole was prepared by using Methanol. The solution was kept in Quartz Cuvette and the UV spectrum was recorded in the range of 200-400nm Shimadzu UV- visible Spectrophotometer (UV-1800). It showed Isosbestic Point at 310nm, 293nm, 229.60nm using UV- Visible Spectrophotometer.

Selection of Mobile Phase:

The pure drug of Piroxicam and Esomeprazole was injected into the HPLC system and run in different solvent system. Each mobile phase was allowed to equilibrate with stationary phase until steady base line was obtained. Different mobile phase like ACN: Water, Methanol: Ammonium Dihydrogen Orthophosphate, Methanol: Ammonium Formate, Methanol: Sodium Acetate diluted with Acetic Acid solution, various proportions were tried to get a stable peak each mobile phase and sonicate on ultrasonic bath and then filter through 0.45 µm filter paper. After Trials the final combination of the solvent selected was Methanol, Sodium Acetate diluted with Acetic Acid solution in the ratio 77:23 that gives sharp peak and good resolution.

Buffer Preparation:

Weigh Accurately 1.64 gm of Sodium Acetate (AR grade) and then Transfer it into 1 Litre Reagent Bottle. Add 900ml HPLC Grade Water to the Reagent Bottle and Shake Well. Then Adjust the pH to 6.6 with Dilute Acetic Acid. Then make up the volume up to 1000ml with HPLC Grade Water and Shake Well.

Mobile Phase Preparation:

Prepare a Homogeneous Mixture consisting of 770ml HPLC Grade Methanol and 230 ml of Buffer and Shake Well. Then Filter the Mobile Phase through 0.2 µm Nylone 6,6-Membrane Filter Paper, and Sonicate the Mobile Phase for 5 minutes.

Stock Solution:

Weigh Accurately 10 mg of Esomeprazole and 10 mg of Piroxicam and then Transfer it into 100ml Glass Volumetric Flask. Dissolve the powder (API) and dilute it up to the mark with the Diluent and Shake Well. Then Sonicate it for 2 minutes. (Concentration: - 100 µg/ml Esomeprazole, 100 µg/ml Piroxicam)

Preparation of Standard Solution:

Pipette out 2ml from the Stock Solution and Dilute it into 20 ml with the help of Diluent and Shake Well. Then Sonicate it for 2 mins and filter it through 0.2 µm Syringe Filter, before Inject.

Method for preparation of tablet mixture of Piroxicam & Esomeprazole:

Preparation of Tablets of Piroxicam and Esomeprazole

The tablets were prepared by Direct Compression Technique. The composition of the tablets was showed in Table. All the excipients were passed through sieve no.30. The required ingredients were accurately weighed and mixed thoroughly and dry blended with talc and magnesium stearate for 5 min. The resulting blends were subjected to the micromeritic properties and compressed by using 8 mm flat face punch using multi station Tablet Punching Machine.

Table 4: Formulation of Different Batches

Sr. No.	Ingredients	Batch				
		F1	F2	F3	F4	F5
1.	Piroxicam	20	20	20	20	20
2.	Esomeprazole	20	20	20	20	20
3.	Microcrystalline Cellulose	140	145	150	155	160
4.	Mannitol	35	35	30	30	25
5.	Povidone K30	25	20	20	15	15
6.	Talc	5	5	5	5	5
7.	Magnesium Stearate	5	5	5	5	5

III. RESULTS AND DISCUSSION:

Optimization of Chromatographic Conditions:

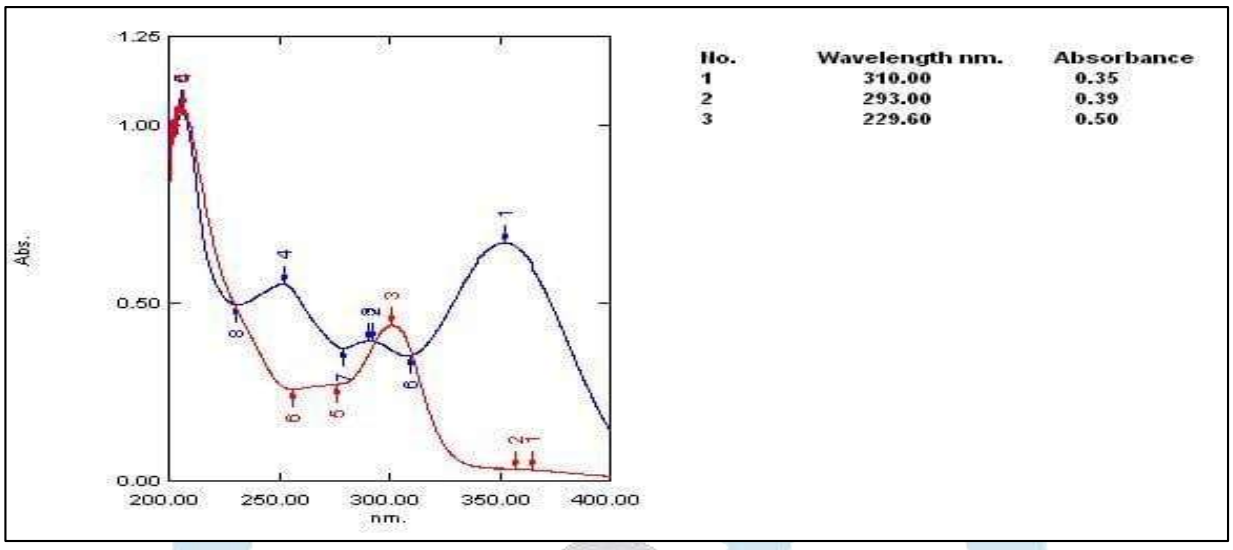


Figure 1: Isosbestic Point of Piroxicam and Esomeprazole

Table 1: Post Compression Evaluation of Tablet Batches:

Batch No.	Weight Variation (in mg) (±SD)	Hardness (in kg/cm^2) (±SD)	Thickness (in mm) (±SD)	Friability (±SD)	Disintegration time (in min)
F1	251±1.32	4.6±0.3	3.551±0.010	0.485±0.002	4.60±0.03
F2	249.7±1.73	4.9±0.4	3.495±0.005	0.480±0.006	4.58±0.04
F3	250.2±1.56	5.0±0.2	3.503±0.012	0.492±0.001	4.63±0.02
F4	250.8±1.89	4.8±0.1	3.581±0.009	0.486±0.003	4.61±0.05
F5	249.3±2.15	5.2±0.5	3.571±0.011	0.483±0.004	4.55±0.07

- **Highlighted row indicates the best batch of tablets in all batches**

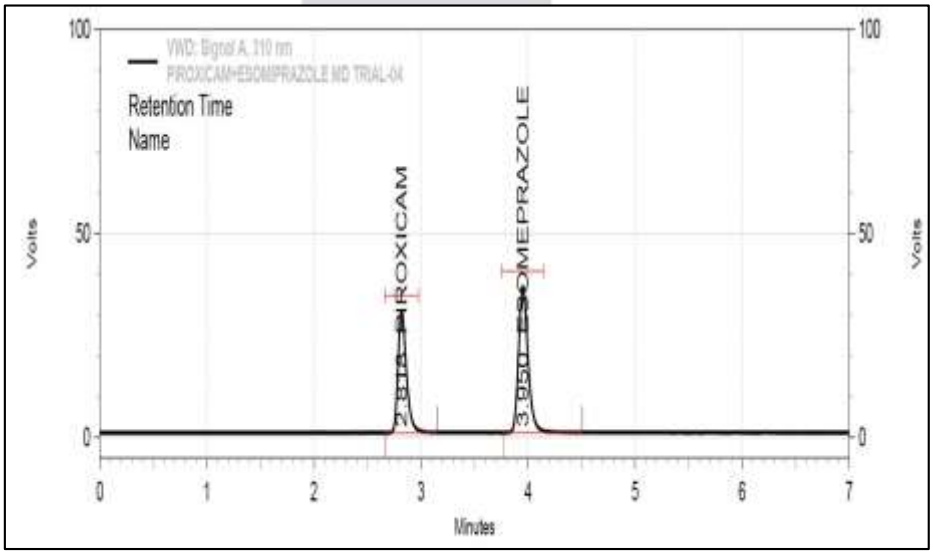


Figure 2: Method Development of Piroxicam and Esomeprazole

PARAMETERS	SPECIFICATION
HPLC system	Younglin-HPLC System
Analytes	Piroxicam and Esomeprazole
Column	Hypersil-BDS, C_{18} (250mm x 4.6mm, 5 um)
Pump	Pump – SP930 D
Mobile Phase	MeOH: Buffer (77:23) (0.02 M Sodium Acetate pH 6.6 with dilute Acetic Acid solution)
Detection Wavelength	310 nm
Flow rate	1.1 ml/min
Temperature	Ambient
Injection volume	20 µL
Run time	7 minutes
Sample Concentration	10 ppm

Method Validation:

1. Accuracy:

Piroxicam	%Mean recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	100.93	0.8096	**0.80**
Accuracy at 100 %	99.19	0.4457	**0.45**
Accuracy at 120 %	100.24	0.6212	**0.62**

Esomeprazole	% Mean recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	100.71	1.1446	**1.14**
Accuracy at 100 %	101.16	1.3780	**1.36**
Accuracy at 120 %	99.72	0.5295	**0.53**

Remark: % Mean recovery observed within acceptance criteria, also % RSD of recovery observed within acceptance criteria; hence accuracy is justified.

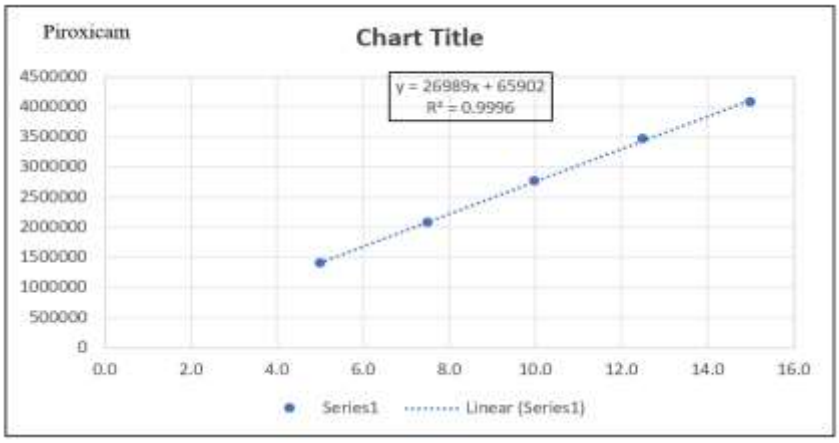
2. Precision:

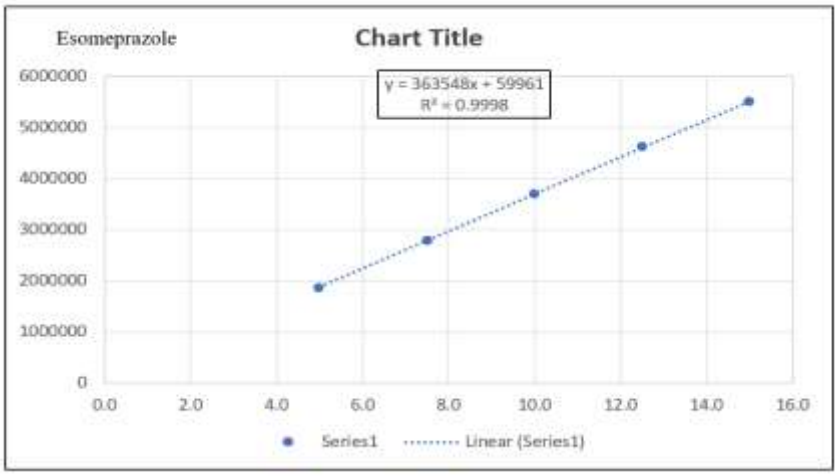
Remark:

A. Overall% RSD for results of set-1 and set-2 performed in single day observed within acceptance criteria, so method is precise in terms of repeated analysis in single day, hence intraday precision is justified.

B. Overall% RSD for results obtained of Day-1 and Day-2 analysis i.e. performed in different days observed within acceptance criteria, so method is precise in terms of repeated analysis in different days, hence interday precision is justified.

3. Linearity:





Remark: Correlation coefficient observed within acceptance criteria; hence method is linear and linearity is justified.

4. Robustness:

Robustness changes in method parameters for Piroxicam & Esomeprazole			
		Piroxicam	Esomeprazole
Name	**Preparations**	**%Assay**	**%Assay**
Original method parameters	Test prep-1	99.03	100.88
Original method parameters	Test prep-2	99.87	100.21
Pump, Flow 1.0 ml/min	Test prep	101.08	98.78
Pump, Flow 1.2 ml/min	Test prep	100.96	100.87
Mobile phase ,Buffer pH 6.5	Test prep	98.04	98.62
Mobile phase ,Buffer pH 6.7	Test prep	100.43	100.36
Mean		99.90	99.95
SD		1.1844	1.0084
%RSD (NMT 2)		**1.19**	**1.01**

Remark: Overall% RSD of results with change in pump flow rare and change pH of buffer in mobile phase observed within acceptance criteria, method is robust in terms of slight change in internal method parameters, hence Robustness is justified.

5. Limit of Detection (LOD) & Limit of Quantification (LOQ):

	Piroxicam	Esomeprazole
Conc. (ppm or ug/ml)	**Area**	**Area**
5.00	1407399	1869262
7.50	2086690	2795865
10.00	2769731	3691307
12.50	3471751	4618437
15.00	4088512	5502323
STEYX	24495	12808
SLOPE	269891	363548
LOD (ug/ml)	0.30	0.12
LOQ (ug/ml)	0.91	0.35

IV. SUMMARY & CONCLUSION:

Summary:

Validation Parameter	Acceptance Criteria	Results
Accuracy	% RSD should be **NMT 2%**	All the Results are observed within the Acceptance criteria.
Precision	% RSD of mean concentration should be **NMT 2%**	
Assay	% Assay should range between **95 – 105 %**	
Linearity	Coefficient of correlation (R^2) should be **NLT 0.99**	The Results complies all the Acceptance Criteria Conditions.
Limit of Detection (LOD)		
Limit of Quantification (LOQ)		
Specificity		Hence the method is suitable, specific, justified with the Acceptance Criteria.
Robustness	% RSD should be **NMT 2%**	
System Suitability Studies	RSD should be **NMT 2%** Theoretical plates should be **MT 2000.** Tailing Factor should be **NMT 2.**	

Conclusion:

The developed HPLC method was found to be simple, accurate, sensitive, precise, specific, economical and rapid. This HPLC method is very simple involving no complicated sample preparations. The drugs were well validated and optimized chromatographic conditions indicating the selective nature of developed HPLC method. This HPLC method was found to be highly specific. The

developed HPLC method was found to be linear over wider concentration range. Therefore, the developed HPLC method can be applied for routine quantitative and qualitative analysis of Piroxicam and Esomeprazole in pharmaceutical formulations like tablets. This HPLC method was validated as per the ICH guidelines. The developed HPLC method can be employed for pharmaceutical preparations within pharmaceutical industry.

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