International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH
Available online at:http://www.pharmaresearchlibrary.com/ijcps

Research Article

RP-HPLC Method Development and Validation for Estimation of Ibuprofen, Oxaprozin and Piroxicam in Their Combined Dosage Form

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Abstract

Analytical methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. Ibuprofen & Oxaprozin was freely soluble in water and alcohol. Piroxicam was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100 µg / ml. The % recovery of Ibuprofen, Oxaprozin and Piroxicam were found to be in the range of 99.22 % - 100.11 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Good agreement was seen in the assay results of Pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Ibuprofen, Oxaprozin and Piroxicam in Bulk drug and Pharmaceutical formulation.

Keywords: Ibuprofen, Oxaprozin and Piroxicam, HPLC.

Article History: Received 21 November 2022, **Accepted** 25 January 2023, **Available Online** 21 March 2023

Citation: D. Pavani, *et al. RP-HPLC Method Development and Validation for Estimation of Ibuprofen, Oxaprozin and Piroxicam in Their Combined Dosage Form, Int. J. of Chem. and Pharm. Sci., 11(1), 2023: 01-08.*

Contents:

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JOURNAL QR-CODE

1. Introduction

Drug name: Ibuprofen

Iupac name: 2-[4-(2-methylpropyl) phenyl] propanoic acid

Synonyms : Advil, Adran, 4-Isobutylhydratropic acid, (RS)-ibuprofen.

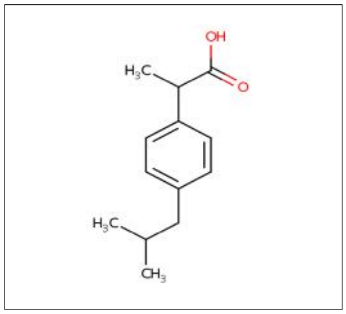
Solubility : Soluble in ethanol (25 mg/ml), chloroform (1:1), ether (1:2), acetone (1:1.5), aqueous solutions of alkali hydroxides and carbonates, dichloromethane, methanol (50 mg/ml), and ethyl acetate.

Description: Ibuprofen, a propionic acid derivative, is a prototypical nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties.

Melting point : 75-77.5^{0}C

C**AS NO** : 15687-27-1

Structure :



Molecular formula : $C_{13}H_{18}O_2$

Molecular weight : Average: 206.2808 Monoisotopic: 206.13067982 g/mol.

Bioavailability : 87–100% (oral), 87% (rectal)

Half-life : 2-4 hours

Protein binding : 90-99% to whole human plasma and site II of purified albumin, binding appears to be saturable and becomes non-linear at concentrations exceeding 20mcg/ml.

Dosage forms: tablet, solution, injection.

Dose : 200,400,600,800mg. 10,100mg/ml/5ml

Category : Anti-Inflammatory Agents, Non-Steroidal, Cyclooxygenase Inhibitors, Analgesics, Non-Narcotic.

Pharmacodynamics:

Ibuprofen is a nonsteroidal anti-inflammatory agent (NSAIA) or nonsteroidal anti-inflammatory drug (NSAID), with analgesic and antipyretic properties. Ibuprofen has pharmacologic actions similar to those of other prototypical NSAIAs, which are thought to act through inhibition of prostaglandin synthesis[1].

Mechanism of Action:

The exact mechanism of action of ibuprofen is unknown. Ibuprofen is a non-selective inhibitor of cyclooxygenase, an enzyme invovled in prostaglandin synthesis via the arachidonic acid pathway. Its pharmacological effects are believed to be due to inhibition cylooxygenase-2 (COX-2) which decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever and swelling. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Inhibition of COX-1 is thought to cause some of the side effects of ibuprofen including GI ulceration.

Pharmacokinetic Properties:

Absorption: ~ 80% absorbed from GI tract, Time to reach peak plasma concentration = 47 minutes (suspension), 62 minutes (chewable tablets), 120 minutes (conventional tablets)

Distribution:

Ibuprofen, like most drugs of its class, is highly protein bound (>99% bound at 20 µ g/mL). Protein binding is saturable and at concentrations >20 µ g/mL binding is non-linear. Based on oral dosing data there is an age- or fever-related change in volume of distribution for ibuprofen. Febrile children <11 years old have a volume of approximately 0.2 L/kg while adults have a volume of approximately 0.12 L/kg. The clinical significance of these findings is unknown.

Metabolism:

R-enanatiomer undergoes extensive enantiomeric conversion (53-65%) to the more active S-enantiomer in vivo. Metablized by oxidation to 2 inactive metabolites: (+)-2[4′-(2-hydroxy-2-methylpropyl) phenyl] propionic acid and (+)-2-[4′-(2-carboxypropyl) phenyl] propionic acid. Very small amounts of 1-hydroxyibuprofen and 3-hydroxyibuprofen have been recovered from urine. Cytochrome P450 2C9 is the major catalyst in the formation of oxidative metabolites. Oxidative metabolites may be conjugated to glucuronide prior to excretion.

Elimination:

Ibuprofen is rapidly metabolized and eliminated in the urine. The excretion of ibuprofen is virtually complete 24 hours after the last dose. It has a biphasic plasma elimination time curve with a half-life of approximately 2.0 hours. There is no difference in the observed terminal elimination rate or half-life between children and adults, however, there is an age-or fever-related change in total clearance. This suggests that the observed change in clearance is due to changes in the volume of distribution of ibuprofen[2-5].

Adverse Effects:

Nausea, dyspepsia, gastrointestinal ulceration/bleeding, raised liver enzymes, diarrhea, constipation, nosebleed, headache, dizziness, rash, salt and fluid retention, and hypertension. Infrequent adverse effects include: esophageal ulceration, heart failure, hyperkalemia, renal impairment, confusion, and bronchospasm. Ibuprofen can exacerbate asthma, sometimes fatally.

Storage: Store at room temperature

Oxaprozin

IUPAC Name: potassium 3-(4,5-diphenyl-1,3-oxazol-2-yl) propanoate

Chemical formula: $C_{18}H_{14}KNO_3$

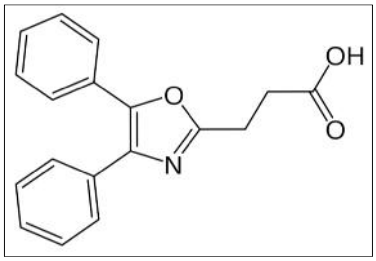
Molecular weight : 331.412

Cas No : 174064-08-5

Category : Analgesics, Non-Narcotic, Anti-Inflammatory Agents.

Mechanism of action:

Anti-inflammatory effects of Oxaprozin are believed to be due to inhibition of cylooxygenase in platelets which leads to the blockage of prostaglandin synthesis. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Oxaprozin is a non-selective NSAID, with a cell assay system showing lower COX-2 selectivity implying higher COX-1 selectivity.

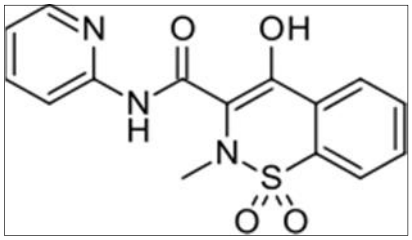


Brand name: Daypro

Piroxicam

IUPAC Name: 2-methyl-1, 1-dioxo-3-[(pyridin-2-yl) carbamoyl]-2H-1lambda6,2-benzothiazin-4-yl (2E)-3-phenylprop-2-enoate

Chemical formula: $C_{24}H_{19}N_3O_5S$



Molecular weight: 461.49

Cas No : 90101-16-9

Category: Agents causing hyperkalemia, Agents that produce hypertension.

Mechanism of action: Droxicam is converted to Piroxicam via hydrolysis of the ester group in the intestine 2. Droxicam administration inhibits the synthesis of prostaglandins by cyclooxygenase enzymes.

Brand name: Feldene

2. Materials and Methods

Selection of wavelength

10 mg of Ibuprofen, Oxaprozin and Piroxicam was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Ibuprofen, Oxaprozin and Piroxicam. The isobestic point was taken as detection wavelength.

Selection of column

Heart of HPLC made of 316 grade stainless steel packed with stationary phase. Silica based columns with different cross linking's in the increasing order of polarity are as follows:

Non-polar----------moderately polar--------Polar

C_{18}< C_8< C_6< Phenyl < Amino <Cyano< Silica

- In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material.
- Column is selected based on solubility, polarity and chemical differences among analysts and Column selected: i.e Agilent (4.6×150mm)5µ.
- Reasons : Better separation,
  Good tailing factor.

Selection of solvent delivery system

- Always preferable solvent delivery system.
- More chance of getting reproducible result on retention time of analytes.
- More economic than gradient technique.

Selection of flow rate

Acceptable limit: - Not more than 2.5 ml/min

- Flow rate selected was 1ml/min
- Flow rate is selected based on
  1. Retention time
  2. Column back pressure
  3. Peak symmetry.
  4. Separation of impurities.

Reasons:

- For earlier elution of analyte and elution of all impurities within 6.0 min.
- Information from the reference method in literature.

Selection of diluent

- Selection of diluent is based on the solubility of the analyte
- Diluent selected: Methanol : phosphate buffer pH 3 (55 : 45v/v)

Reason: good peak area, retention time, peak symmetry

Selection of column temperature:

- Preferable temperature is ambient or room temperature.

Reasons:

- To elute all impurities along with analyte with in 10.0 min of run time.
- Less retention time
- Good peak shape
- Higher theoretical plates.
- Good resolution.

Selection of test concentration and injection volume

Test concentration is finalized after it is proved that API is completely extractable at the selected test concentration.

- Test concentration is fixed based upon the response of API peak at selected detector wavelength.
- And the test concentration selected is 10 ppm.
- Injection volume selected was 10µL.

3. Results and Discussion

Table 1: Chromatogram values for System suitability of Ibuprofen

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	2.405	1250763	2487	1.62
2	2.406	1247867	2489	1.58
3	2.406	1255849	2496	1.64
Mean		1251360		
SD		3850.679		
% RSD		0.30722		

Acceptance Criteria:

1). Tailing factor Obtained from the standard injection is 1.7

2). Theoretical Plates Obtained from the standard injection is 2496

Table no 2: Chromatogram values for System suitability of Oxaprozin

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.417	940627	2271	1.52	3.02
2	2.418	931161	2243	1.46	3.07
3	2.417	940306	2262	1.45	3.04
Mean		937364.7			
17SD		5374.93			
% RSD		0.473409			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.51

2) Theoretical Plates Obtained from the standard injection is 2281

Table no-3: Chromatogram values for System suitability: Piroxicam

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	7.087	933579	2504	1.22	11.84
2	7.088	934565	2592	1.23	12.23
3	7.087	920436	2564	1.24	13.14
Mean		929157.7			
SD		7597.932			
% RSD		0.827823			

Acceptance Criteria:

1) Tailing factor Obtained from the standard injection is 1.25

2) Theoretical Plates Obtained from the standard injection is 2594

Linearity

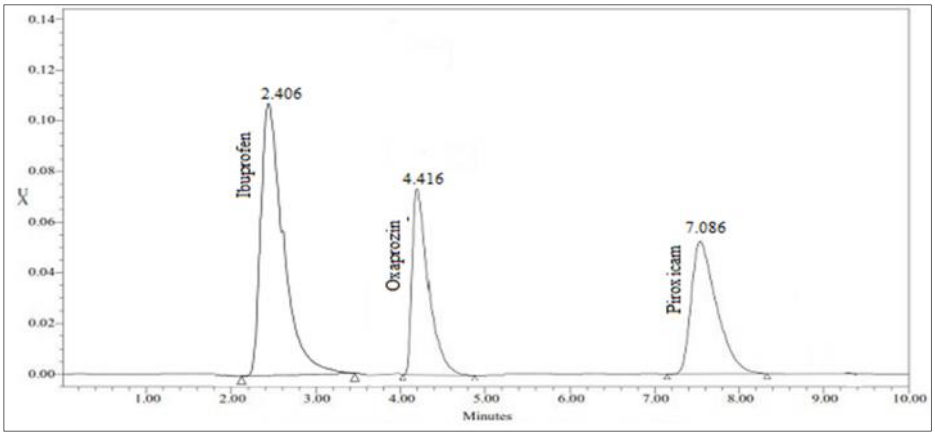


Fig.No.1. Chromatogram for Linearity level 2

Table 4: Linearity results for Ibuprofen

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	10 ppm	339286
2	II	20 ppm	667774
3	III	30 ppm	986474
4	IV	40 ppm	1339994
5	V	50 ppm	1639065
Correlation Coefficient			0.999

Table 5: Linearity results for Piroxicam

S.No	Linearity Level	Concentration (µg/ml)	Area
1	I	12.5	231737
2	II	25	453615
3	III	37.5	659796
4	IV	50	895191
5	V	62.5	1094356
Correlation Coefficient			0.999

Table no-6: Calibration parameters for Ibuprofen, Oxaprozin and Piroxicam

Parameter	Results for Ibuprofen	Results for Oxaprozin	Results for Piroxicam
Slope	18718	14315	70355
Intercept	65497	49120	47086
Correlation co-efficient	0 .9993	0.99918	0.99902

Acceptance criteria:

1. Correlation Coefficient should be not less than 0.9990.
2. % RSD of peak areas for Solution 1, 2, 3, 4 and 5 should be not more than 2.0 %.
3.

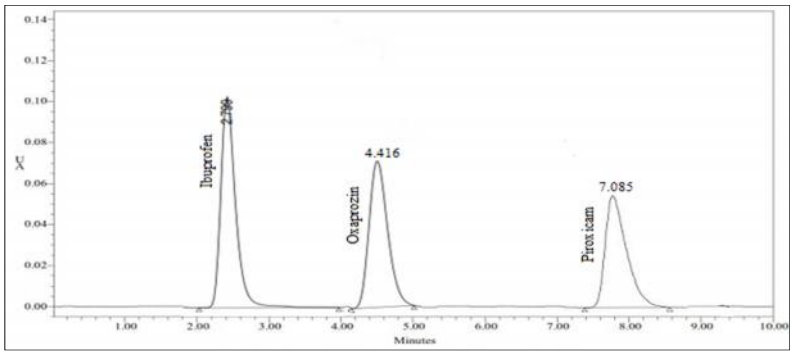


Fig.No.2. Sample Chromatograms for precision injection-1

Table 7: Sample Chromatogram values for Repeatability Ibuprofen

Injection No	Peak Area	R_t
1	935035	4.416
2	929353	4.417
3	930459	4.619
4	932389	4.418
5	922057	4.417
Avg	927458.6	
SD	4865.16	
% RSD	0.4232	

Table no-8: Chromatogram values for intermediate Precision: Ibuprofen

Injection No	Peak Area	R_t
1	912412	4.416
2	913062	4.417

3	909642	4.418
4	916881	4.419
5	914005	4.418
Mean	913200.4	
SD	2621.886	
% RSD	0.387	

Table no-9: Chromatogram values for intermediate Precision: Oxaprozin

Injection No	Peak Area	R_t
1	914922	7.086
2	909335	7.085
3	913266	7.086
4	909418	7.087
5	911496	7.086
Mean	911687.4	
SD	2432.859	
% RSD	0.4668	

Table no-10: Chromatogram values for intermediate Precision: Piroxicam .

Sample No.	Spike Level	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.89%
		15	14.86	99.0%	
		15	14.99	99.79%	

Table 11: Chromatogram Values for Accuracy of Ibuprofen.

Sample No.	Accuracy	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 12: Chromatogram Values for Accuracy of Oxaprozin

Sample No.	Spike Level	Amount added(mg)	Amount found(mg)	% Recovery	Mean % Recovery
1	50 %	10	9.8	98%	100%
		10	10.2	102%	
		10	10	100%	
2	100 %	20	19.8	99%	100%
		20	20.2	101%	
		20	20	100%	
3	150 %	30	29.6	98.66%	99.33%

		30	30	100%	
		30	29.8	99.33%	

Table 13: Robustness results for Ibuprofen (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min R_t	1ml/min R_t	0.1.2m l/min R_t
1	**Oxaprozin Robustness Results**	2.475	2.482	2.488
USP Plate count		2279	2232	2185
USP Tailing		1.47	1.49	1.51

Table 14: Robustness results for Oxaprozin (flow rate):

S.No	Drug	Flow Rate ml/min		
		0.8ml/min R_t	1ml/min R_t	1.2 m l/min R_t
1	**Piroxicam Robustness Results**	3.488	3.190	2.877
USP Plate count		2346	2556	2096
USP Tailing		1.28	1.24	1.27

Table 15: Robustness results for Oxaprozin

S.No	Drug	Mobile phase		
		More organic R_t	Organic R_t	Less organic R_t
1	**Piroxicam Robustness Results**	7.087	7.086	7.086
USP Plate count		2482	2556	2030
USP Tailing		1.21	1.23	1.32

Table no-14: Results of LOD

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Ibuprofen	371877.10	563365963	2.03
Oxaprozin	431401.80	476884400	0.0365
Piroxicam	287058.10	376884400	1.84

Table no-15: Results of LOQ

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Ibuprofen	371877.10	563365963	4.53
Oxaprozin	431401.80	476884400	5.75
Piroxicam	287058.10	376884400	3.84

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula.

4. Conclusion

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool[4-6]. Ibuprofen & Oxaprozin was freely soluble in water and alcohol. Piroxicam was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH3) was chosen as the mobile phase. The run time of the HPLC procedure was 10 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100 µg / ml. The % recovery of Ibuprofen, Oxaprozin and Piroxicam were found to be in the range of 99.22 % - 100.11 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from

insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Good agreement was seen in the assay results of Pharmaceutical formulation by developed method[7-11]. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Ibuprofen, Oxaprozin and Piroxicam in Bulk drug and Pharmaceutical formulation.

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