Method Development, Validation and Simultaneous Estimation of Ibuprofen and Carisoprodol by RP-HPLC and its Degradation Studies

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***Abstract*: A simple rapid reversed- phase high performance liquid chromatographic method has been developed and validated for estimation of Ibuprofen and Carisoprodol in bulk in pharmaceutical dosage form. Chromatographic separation of Ibuprofen and Carisoprodol was achieved on Waters Alliance -e2695, by using Waters Xterra RP18, (150x4.6mm, 3.5µm) column and the mobile phase containing 2.0 gm Hexane-1-Sulphonic acid is dissolved in 1lt water adjust pH-2.5 with OPA&ACN in the ratio of 50:50% v/v. The flow rate was 1.0 ml /min; detection was carried out by absorption at 240nm using a photodiode array detector at ambient temperature. Retention time for Ibuprofen and Carisoprodol was found to be 2.64 min, 3.13 min respectively. The proposed method was validated according to ICH guidelines. The number of theoretical plates and tailing factor for Ibuprofen and Carisoprodol were NLT 2000 and should not more than 2 respectively. Percentage Relative standard deviation of peak areas of all measurements are always less than 2.0. The method was found to be simple, precise, accurate, robust and suitable for quantitative analysis of Ibuprofen and Carisoprodol and study for its stability.**

***Keywords*: Ibuprofen, Carisoprodol, RP-HPLC, Validation, Degradation.**

Introduction:

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID).Chemically called 2-(4-Isobutylphenyl) propanoic acid.(1) Ibuprofen works by blocking the production of prostaglandins, substances that the body releases in response to illness and injury. Prostaglandins cause pain and swelling, or inflammation. (2)(3)They are released in the brain, and they can also cause fever. Ibuprofen's painkilling effects begin soon after taking a dose. side effects are vomiting, mild itching or rash, ringing in your ears dizzeness, bloating etc..,(4)(5)(6).

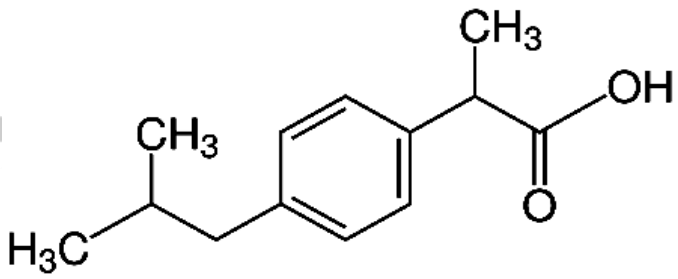

Fig 1: Structure for Ibuprofen

Carisoprodol (brand name Soma) is a skeletal muscle relaxant(8) approved by the FDA for use in painful musculoskeletal conditions on an acute basis. its mechanism of action remains unclear. Its sedative effects, which contribute to its therapeutic and recreational use, are generally attributed to the actions of its primary metabolite, meprobamate, at $GABA_A$ receptors ($GABA_AR$). side effects are insomnia, vomiting, abdominal cramps, headache, tremors, muscle twitching, hallucinations, and psychosis etc..,(9) Carisoprodol is contraindicated in acute intermittent porphyria and in patients with a knowncarbamate hypersensitivity because carisoprodol is metabolized to meprobamate.(10)metabolite meprobamate were studied through pharmacokinetic studies(11).

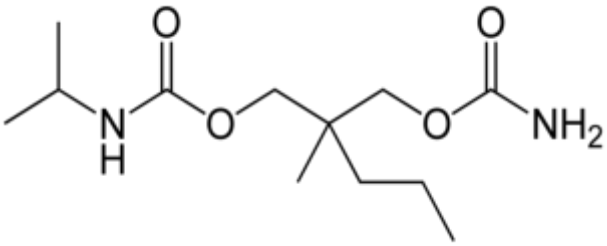

Fig 2 Structure for Carisoprodol

Experimental:

Materials and Methods: Reagents required HPLC graded acetonitrile: HPLC graded Hexane-1-Sulphonic Acid: HPLC graded Water: pure HCL:pure NAOH: pure Hydrogen peroxide: pure sodium bisulphate:HPLC graded Ortho Phosphoric acid.

Drugs used: Ibuprofen and Carisoprodol is formulated as oral tablet dosage form. Each tablet contains 400mg of Ibuprofen and 50mg of Carisoprodol was used.

Preparation of solutions for mobile phase:

Solution A: Hexane-1-Sulphonic Acid (HSA) (HPLC grade)

Solution B: Acetonitrile (HPLC grade)

Mobile phase preparation: In this method mobile phase used is a mixture of solution A(HAS) and solution B (ACN) in the ratio of 50:50 v/v.

Diluent selection:
The diluents used in throughout the method was mobile phase in which the drugs are soluble.

Standard Solution Preparation:
400µg/ml of Ibuprofen and 50µg/ml of Carisoprodol are prepared by diluting 5 to 50ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation:
5 tablets (each tablet contains 400mg of Ibuprofen and 50mg of Carisoprodol) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Ibuprofen and Carisoprodol were prepared by dissolving weight equivalent to 400mg of Ibuprofen and 50mg of Carisoprodol in sufficient mobile phase. After that filtered the solution using 0.45-micron filter paper and sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 400µg/ml of Ibuprofen and 50µg/ml Carisoprodol.

Optimized chromatographic conditions

S.No.	PARAMETERS	CHROMATOGRAPHIC CONDITIONS
1.	Mobile phase	HSA : ACN(50:50)
2.	Column	Waters, X-terra RP18, 150mm x 4.6mm, 3.5µm.
3.	Flow rate	1.0 ml/min
4.	Column temperature	Room temperature
5.	Sample temperature	Room temperature
6.	Wavelength	240nm
7.	Injection volume	10 µl
8.	Run time	Ambient
9.	Retention time	2.64 mins Ibuprofen
		3.13 mins Carisoprodol

Results

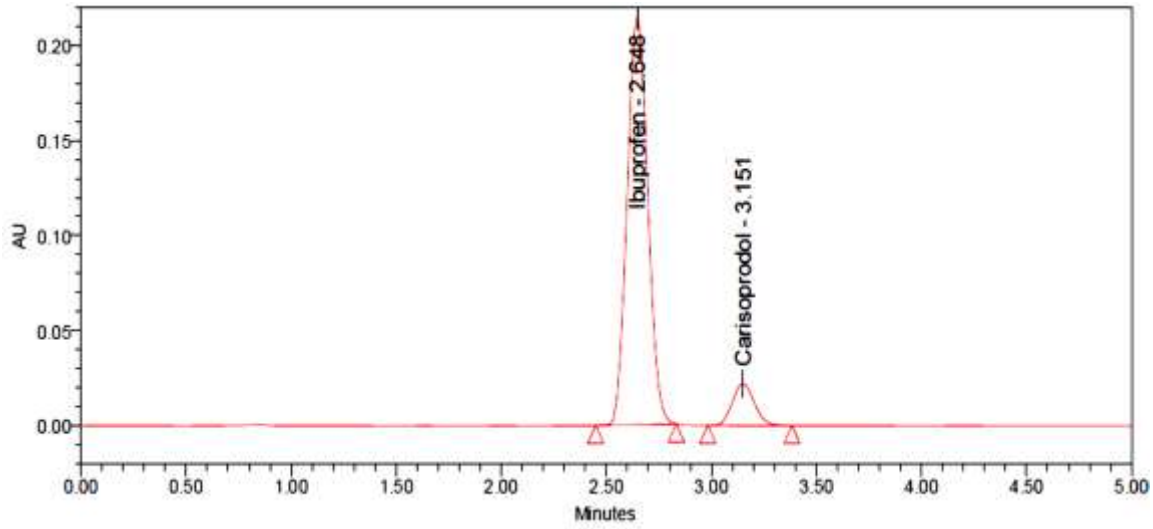

Fig 3 Typical Chromatogram of Standard

S.No	Name	Rt (min)	Peak Area
1	Ibuprofen	2.648	2912681
2	Carisoprodol	3.151	320382

Table no 1 Showing retention time & Peak Area of the standard drugs

Method Validation: As per ICH guidelines, the method validation parameters checked were linearity, precision, and accuracy, limit of detection and limit of quantification, robustness and degradation studies.

Accuracy

Accuracy data of Ibuprofen:

The accuracy study was performed for 50%, 100% and 150 % for Ibuprofen and carisoprodol. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation o f % recovery. The results are given in Table 2, and 3.

Recovery level	Accuracy Ibuprofen			
	Amount taken (mcg/ml)	Area	%Recovery	%RSD
50%	200.0	1460582	100.7	0.54
	200.0	1404716		
	200.0	1438218		
100%	400.0	2844501	100.2	0.12
	400.0	2816991		
	400.0	2881988		
150%	600.0	4271373	100.0	0.05
	600.0	4272024		
	600.0	4187242		

Table no 2 Accuracy data for Ibuprofen

Accuracy data for Carisoprodol:

Recovery level	Accuracy Carisoprodol			
	Amount taken (mcg/ml)	Area	%Recovery	%RSD
50%	25.0	162463	100.07	0.05
	25.0	164284		
	25.0	161312		
100%	50.0	308443	100.12	0.14
	50.0	307618		
	50.0	307284		
150%	75.0	472031	100.05	0.26
	75.0	464570		
	75.0	456456		

Table no 3 Accuracy data for Carisoprodol

Precision

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

Method precision data for 400mg +50mg

S.No.	Ibuprofen		Carisoprodol	
	Area	% Assay	Area	% Assay
1	2854025	100.07	305295	100.1
2	2874457	101.3	305997	100.2
3	2843183	100.4	309917	100.6
4	2853887	101.1	307131	100.2
5	2876320	102.1	303496	99.9
6	2812192	101.0	308831	100.5
Avg	2845621	101.1	308456	100.3
SD	284.36	0.583	251.32	0.259
%RSD	0.46	0.58	0.75	0.26

Table no 4 Method Precision data for Ibuprofen

Intermediate Precision:

Intermediate precision data of Ibuprofen and Carisoprodol:

S.No.	Ibuprofen		Carisoprodol	
	Area	% Assay	Area	% Assay
1	2854025	100.7	317104	100.9
2	2874457	101.3	314776	100.1
3	2843183	100.4	317119	101.0
4	2853887	101.1	316629	101.2
5	2876320	102.1	313471	100.4
6	2812192	101.0	310563	100.6
Avg	2828652	101.1	310598	100.7
SD	284.36	0.583	254.36	0.41
%RSD	0.46	0.58	0.75	0.41

Table no 5 Method Precision data for Carisoprodol

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD and LOQ were determined as signal to noise ratio. LOD of Ibuprofen and Carisoprodol was calculated by using the concentrations of 0.40 µg/mL,0.05 µg/mL, respectively.LOQ was calculated by using the concentrations of 4.00 µg/mL of Ibuprofen, 0.50 µg/mL of Carisoprodol.

S.No.	Sample name	LOD		LOQ	
		Conc. (µg/ml)	S/N	Conc. (µg/ml)	S/N
1.	Ibuprofen	0.4001	5	4.005	22
2	Carisoprodol	0.0505	7	0.505	27

Table no 6 LOD and LOQ data for Ibuprofen and Carisoprodol

Robustness:

Small changes were done in the optimized conditions like change in the flow rate, organic phase and pH Variation and it was observed that there is no change in the response of the drugs regarding peak area of individual drugs.

Robustness		Ibuprofen	Carisoprodol
		% RSD	% RSD
Flow Rate	1.2 ml/min	0.35	0.25
	0.8 ml/min	0.27	0.46
Organic Phase	55:45 v/v	0.21	0.35
	45:55 v/v	0.49	0.61
pH Variation	2.70	0.21	0.15
	2.30	0.20	0.46

Table no 7 Robustness data for Ibuprofen and Carisoprodol

Linearity:-

Linearity was observed in the range of 40.01-600.15µg/mL, 5.01-75.15µg/mL,for Ibuprofen and Carisoprodol. Correlation coefficient was found to be 0.999 and slope was calculated.

Linearity of detector response for Ibuprofen and Carisoprodol:

S.No.	Conc.(µg/ml) Ibuprofen	Conc.(µg/ml) Carisoprodol	Area		Acceptance criteria
			Ibuprofen	Carisoprodol	
1	40.01	5.01	303014	31363	$R^2 < 0.999$
2	100.03	12.53	739150	79789	
3	200.05	25.05	1461298	157930	
4	400.10	50.10	2880850	315914	
5	500.13	62.63	3496077	402890	
6	600.15	75.15	4289562	484618	

Table no 8 Linearity data for Ibuprofen and Carisoprodol

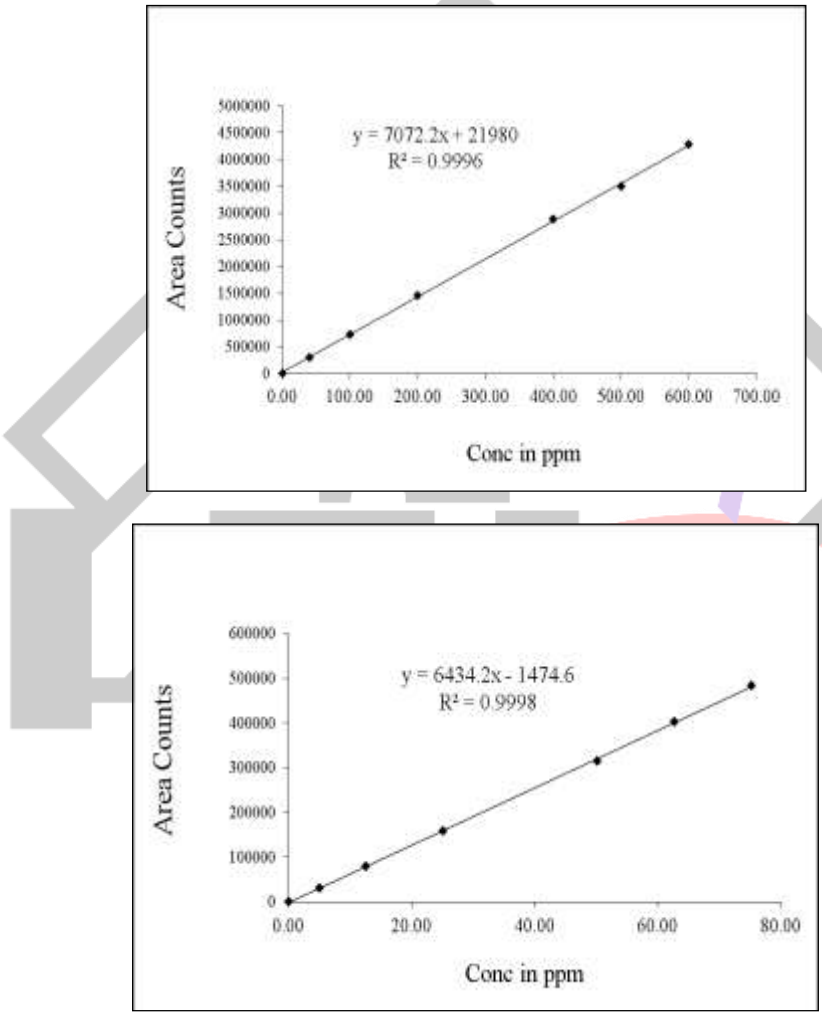

Fig 4 Linearity graphs of Ibuprofen and Carisoprodol

S.No	Linearity Parameters	Ibuprofen	Carisoprodol
1.	Linearity range	40-600 µg/ml	5-75 µg/ml
2.	Correlation coefficient	0.999	0.999
3.	Y intercept	7072x + 21980	6434x + 1474

Degradation Studies

Sample solution was prepared by transferring the powder equivalent to the weight of 400 ,and 50 mg of Ibuprofen and Carisoprodol are prepared by diluting 5 to 50 ml with mobile phase. futher dilutions are prepared 400µg/mL and 50µg/mL for Ibuprofen and Carisoprodol respectively.

Degradation Studies Data:

Table no 9 Degradation studies data for Ibuprofen and Carisoprodol

S.No	Degradation Parameters	%Recovery		%Degradation	
		Ibuprofen	Carisoprodol	Ibuprofen	Carisoprodol
1	Control	100.6	100.3	-0.6	-0.3
2	Acid Degradation	88.1	87.8	12.5	12.5
3	Alkali Degradation	81.8	82.7	18.8	17.6
4	Peroxide Degradation	92.0	85.1	8.6	15.2
5	Reduction Degradation	87.6	85.5	13	14.8
6	Thermal Degradation	80.2	79.4	20.4	20.9
7	Photolytic Degradation	82.8	82.3	17.8	18

Discussion:

Several analytical procedures have been proposed for the quantitative estimation Ibuprofen and Carisoprodol separately and in combination with other drugs. So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Ibuprofen and Carisoprodol. validated parameters such as precision, accuracy, linearity, LOD, LOQ, robustness,and degradation studies were within limits. By using simple mobile phase the retention time obtained is 2.64 min, 3.13min for ibuprofen and carisoprodol respectively. Degradation studies were performed for the drugs by exposing to extreme conditions but very less degradation was observed.

Conclusion:

Method to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Ibuprofen and Carisoprodol in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

References:

[1] https://pubchem.ncbi.nlm.nih.gov/compound/ibuprofen

[2] http://www.ijpba.info/ijpba/index.php/ijpba

[3] https://www.medicinenet.com/acetaminophen_vs_ibuprofen_side_effects/article.htm#which_one_is_better_for_pain_relief_acetaminophen_or_ibuprofen

[4] https://www.medicalnewstoday.com/articles/161071#side-effects

[5] https://www.edinformatics.com/interactive_molecules/info/ibuprofen.htm

[6] https://www.medicinenet.com/ibuprofen/article.htm#what_are_the_side_effects_of_ibuprofen

[7] https://en.wikipedia.org/wiki/Ibuprofen

[8] https://www.drugs.com/carisoprodol.html

[9] https://www.drugs.com/sfx/carisoprodol-side-effects

[10] https://www.pediatriconcall.com/drugs/carisoprodol/369

[11] https://www.drugs.com/pro/carisoprodol.html

[12] https://en.m.wikipedia.org/wiki/Carisoprodol

[13] Jayana Chaitanya, Shane Lobo, et.al, Analytical method development and validation of Carisoprodol in bulk drug and formulation by RP-HPLC method: Journal of chemical and pharmaceutical sciences (JCPS) Volume 10 Issue 2, April - June 2017, P:840-846.

[14] Chandani Patel, Laxman Prajapati, et.al, simultaneous estimation Of Ibuprofen and Carisoprodol in synthetic mixture by HPTLC: (IJLSR): Sr No: 2 DOI: 10.13040/IJPSR.0975-8232.IJLSR.1(11).316-21 30 November, 2015 Page No: 316-21.

[15] Shantaram G. Khanagea et.al, development and validation of reversed phase high performance liquid chromatographic method for simultaneous estimation of Paracetamol, Caffeine and Carisoprodol in Tablet formulation. Eurasian Journal of Analytical Chemistry , 2015, 10(3): 163-179.

[16] Sovan Pattanaik1, Sangeetha Mukhi, et.al, assay method development and validation of Ibuprofen tablets by HPLC: Pelagia research Library Der Pharmacia Sinica, 2013, 4(4): 91-96.

[17] Jahan, Md. Sarowar ; Islam et.al, a study of method development, validation, and forced degradation forsimultaneous quantification of Paracetamol and Ibuprofen in pharmaceutical dosage form by RP-HPLC method. Analytical Chemistry Insights, volume 9, (issn: 1177-3901, eissn: 1177-3901), 2014, pages 75-81.

[18] Juti Rani Devi, Bidyut Das preparation, characterization, and evaluation of physical mixtures of Ibuprofen for solubility enhancement : IJPPR Human Journals, Research Article, December- 2017 , Vol. 11 (1): 262-278.

[19] Narendra Nyola and Govinda Samy et.al, simultaneous estimation of Ibuprofen and Famotidine in pure and combination dosage form by RP-HPLC: Journal of Applied Pharmaceutical Science 02 (05); 2012: 79-83.

[20] V.Rajani Sekhar, Y. Padmanabha Reddy, et.al, RP-HPLC and UV-derivative spectrophotometry technique for the simultaneous estimation of ibuprofen and famotidine in pharmaceutical dosage form: Pelagia research Library Der Pharmacia Sinica, 2013, 4(2): 160-170.

[21] Allabasha Mumtaz, Mrs. Iffath Rizwana, simultaneous estimation of Paracetamol, Ibuprofen and Famotidine by using RP-HPLC method: Indo American Journal of Pharmaceutical Research, 2015, ISSN NO: 2231-6876, P: 2964-2981.

[22] Prasanna Reddy Battu and MS Reddy , RP-HPLC method for simultaneous estimation of Paracetamol and Ibuprofen in tablets: ajrc research article, 2009, p:70-72.