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Method Development, Method Validation, Simultaneous Estimation of Paracetamol and Ibuprofen Solid Dosage Form by RP-HPLC Method

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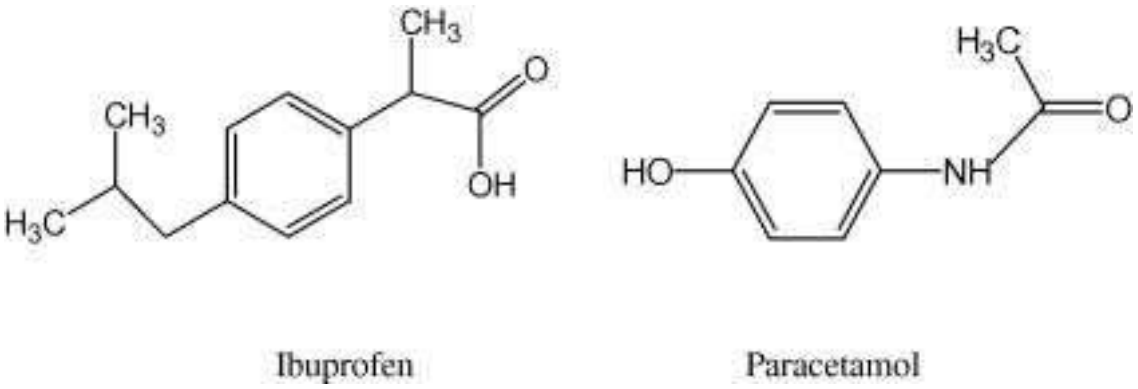
ABSTRACT: A reverse phase high performance liquid chromatography method is mostly used for the determination or simultaneous estimation of paracetamol and ibuprofen[1]. The method utilizes the C18 column with an isocratic mobile phase of phosphate buffer (pH- 6.8) and acetonitrile (65:35 v /v), at flow rate of 0.7ml/min and ambient temperature. The method developed was validated for different factors like specificity, accuracy, linearity, robustness, etc. RP-HPLC method is not reported for the simultaneous estimation of paracetamol and ibuprofen in combined doses or forms[2]. There is a dosage form or combination containing 400mg of paracetamol and 325mg of ibuprofen available as tablet in market.

KEYWORDS: Paracetamol, ibuprofen, RP-HPLC, Validation.

1. INTRODUCTION:

Paracetamol or Acetaminophen is an non- opioid analgesic and antipyretic agent used to treat fever and mild to moderate pain. Ibuprofen is the non-steroidal anti inflammatory agent belongs to the aryl propionic acid derivatives.[1,2]

Paracetamol and ibuprofen is mostly used as combinational solid dosage for the migrain, headache, and muscular pain, cold and fever. The molecular formula of ibuprofen is $C_{13}H_{18}O_2$ and molecular weight is 206.29g/mol, where as the molecular formula of paracetamol is $C_8H_9NO_2$ and molecular weight is 151.17g/mol. Some of the reports shows few techniques like HPLC and UV spectroscopy were reported for the estimation of ibuprofen and paracetamol individual pharmaceutical formulations. The main objective of this estimation was to develop an RP-HPLC method with the ultraviolet detection for simultaneous estimation of the ibuprofen and paracetamol in pharmaceuticals. The validation is done according to the ICH guidelines and as per United States Pharmacopoeia.[2,3]



Ibuprofen Paracetamol

Fig 1 : Structures of ibuprofen and paracetamol

2. EXPERIMENTAL :

2.1 Materials Required: paracetamol, ibuprofen, potassium dihydrogen phosphate, orthophosphoric acid or sodium hydroxide, HPLC- grade acetonitrile, HPLC-grade water (milli-Q or equivalent). Ortho phosphoric acid or sodium hydroxide is generally used for the pH adjustment and HPLC-grade acetonitrile is used as organic modifier.[5,6]

2.1 HPLC System: High performance liquid Chromatography system (Shimadzu- Prominence, Japan) equipped with an auto sampler and UV- visible detector (Model- SPD 20A) used for the analysis. The data obtained is recorded by LC- solution software. Reversed – phase C18 column (150mm x 4.6mm,5µm,Phenomenex Inc.Japan).[6,7]

2.1 :Preparation of mobile phase:

Accurately weighed 1.75g of potassium dihydrogen phosphate dissolved in 900ml of pure water. The above solution is diluted with phosphoric acid or sodium hydroxide in order to maintain the pH- 6.8 ± 0.1 and makeup the volume up to 1litre. The prepared buffer is now mixed with the acetonitrile at the ratio of 65:35 v/v. now filtration is done by using 0.22 µm Millipore filter.

Nearly 20 tablets of Combiflam is taken which are composed of 325mg of ibuprofen and 400mg of paracetamol and weighed. Now the powder equivalent to the 25mg of paracetamol and 20mg of ibuprofen is weighed and transferred to the 25ml volumetric flask. The dilutions are made with the mobile phase to get a final concentration of the 25 µg/ml of paracetamol and 20 µg/ml of ibuprofen.[8,9]

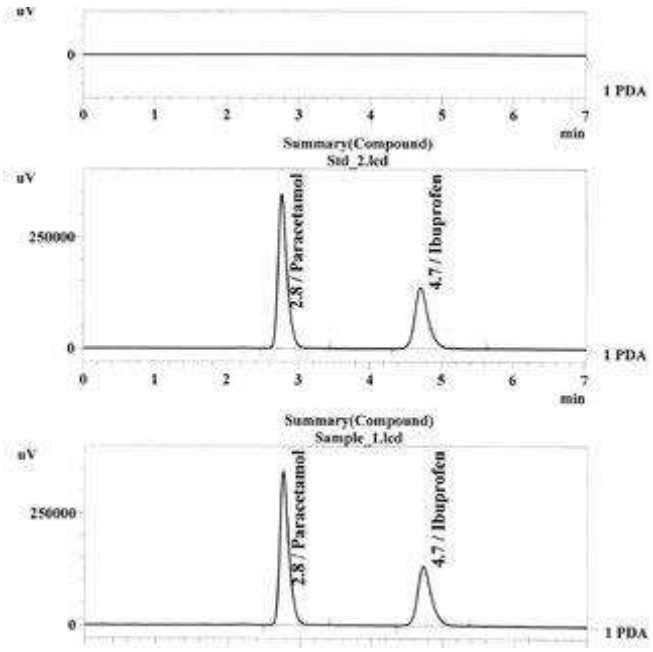


Fig 2: Chromatogram of Blank, standard, sample

2.2 : Chromatographic conditions :

The test is performed under the ambient temperature and isocratic conditions. The flow rate of mobile phase is 0.7ml/min. the system is maintained at the 25°C. The powder which ia dissolved is dissolved in mobile phase is sonicated to ensure dissolution and done filtration using 0.45 µm filter before injection. for the adequate sensitivity the injection volume is maintained i.e 20 µl. Total run time of the test is mostly about 10min to facilitate the rapid analysis. Typically paracetamol elutes at 4.3 to 5 minutes where as ibuprofen elutes earlier 2.0 to 3.0 minutes. For the simultaneous estimation UV detector is used at 220nm or 230nm.[16]

3. Simultaneous Estimation Equation :

For RP-HPLC simultaneous estimation, we use a system of linear equations derived from the beer lamberts law, where the absorbance of the mixture at two different wavelengths is used to determine the concentration of each component. The equations are in the form of

$A_1 = ax_1C(x) + ay_1C(y)$ and $A_2 = ax_2C(x) + ay_2C(y)$. Where:

- A_1 and A_2 = absorbances of the sample mixture at two selected wavelengths (λ_1 and λ_2).
- C(x) ,C(y) = concentrations of the two components (Component X and Component Y).
- ax_1 = Absorptivity of Component X at wavelength λ_1
- ay_1= Absorbitivity of component Y at wave length λ_1 .
- ax_2 = Absorptivity of Component X at wavelength λ_2.
- ay_2 = Absorptivity of Component Y at wavelength λ_2.

4. Method Validation :

Validation is done according to the United States of Pharmacopoeia and ICH guidelines with respect to the accuracy, precision, linearity, robustness, specificity, limit of detection (LOD), limit of quantification(LOQ), force degradation, etc.

4.1 Accuracy :

Accuracy of the any analytical method represents the nearness of values between the obtained value and expected value. Accuracy of the present experiment is done by assessing the spiked samples of paracetamol and ibuprofen with known concentrations (80, 100, 120%). Accuracy results of ibuprofen and paracetamol are tabulated in table1.[15]

4.2 Precision :

The precision of assay was assessed with respect to repeatability and reproducability. The precision studies are done as inter-day and intra-day repeatability of responses on various HPLC machines and columns. Inter- day and intra- day resembles the %

drug release and it is expressed as %RSD. [%RSD= standard deviation/ mean x 100]. Results of precision are tabulated in the table2.[2,5]

4.3 Linearity :

It is the factor which describes the linear responses for both paracetamol and ibuprofen. For this studies the different concentrations of paracetamol and ibuprofen across the concentration range are used. About 15 to 70 µg/ml of ibuprofen and 20 to 80 µg/ml of paracetamol concentration ranging levels are selected. The calibration curve was developed by plotting the response against the concentration of drugs. The precision results are tabulated in the table3.[1,2,14,16]

4.4 Solution Stability :

Majorly the solution form of most of the drugs varies upon repeated administration, the peaks of the drugs may alter. Here in this experiment the stability of the drugs are demonstrated by diluting the paracetamol and ibuprofen in suitable solvent and mobile phase. And transfer solutions into suitable sized vials and maintain temperature and also keep in the refrigerator for a week. The samples are estimated by the HPLC technique of every

3 days in order to check stability. The samples are said to be stable if there is no change in peaks or retention times are observed.[6,7]

4.5 System Suitability :

System suitability is the major aspect in order to test the instruments, reagents, columns, etc. are adequate or

suitable for the intended analysis. Several parameters are evaluated like retention times, tailing factor, theoritical plates. Generally the system suitability studies are done before the sample analysis. The system suitability results are tabulated in the table4. [7,8]

4.6 Limit of Detection and Limit of Quantification : The low Concentrations of the standard drugs are used for the determination of limit of detection and limit of quantification using the developed RP-HPLC method. LOD is the lowest amount of analyte present in the sample, where as the LOQ is the lowest concentration that can be estimated with acceptable accuracy and precision. The LOD of the paracetamol is 6ng/ml, where as ibuprofen is 10ng/ml. The LOQ of the paracetamol is about 15ng/ml where as LOQ of ibuprofen is 25ng/ml.[12]

4.6 Robustness :

It is the indication of the reliability of the analytical method during normal usage. The following parameters in RP-HPLC like wavelength, mobile phase composition, flow rate, solvent ratio etc. if there are no alterations in the above factors then method is said to be reliable. The results of the robustness of paracetamol and ibuprofen are tabulated in table5.[16]

RESULTS : Table 2: Precision Results of Intraday and Interday

S. NO.	Intraday (% drug release)		Interday (% drug release)	
	Ibuprofen	**Paracetamol**	**Ibuprofen**	**Paracetamol**
1.	100	99	100	99
2.	100	97	101	99
3.	101	98	101	99
4.	101	98	101	99
5.	101	99	101	99
6.	101	98	100	99
Mean	101	98	101	99
%RSD	0.6	0.5	0.6	0.0

Table 3 : Linearity Results of Paracetamol and Ibuprofen

% Conc.	Ibuprofen		Paracetamol	
	Conc. (µg/ml)	**Area**	**Conc. (µg/ml)**	**Area**
50%	49.2450	2065486	45.0549	1683881
75%	73.8675	2952450	67.5824	2751828
100%	98.4900	3842565	90.1098	3655200
125%	123.1125	4766603	112.6373	4458302
150%	147.1125	5688024	135.1647	5381590

200%	196.9800	7856440	180.2196	7067887
Corelation coefficient (r)		0.999	0.999	
Slope (m)		38998	39395	
Intercept (c)		51536	24917	
Bias at 100% level		1.1	0.6	

Fig 4 : Linearity Graph of Paracetamol (A) and Ibuprofen (B)

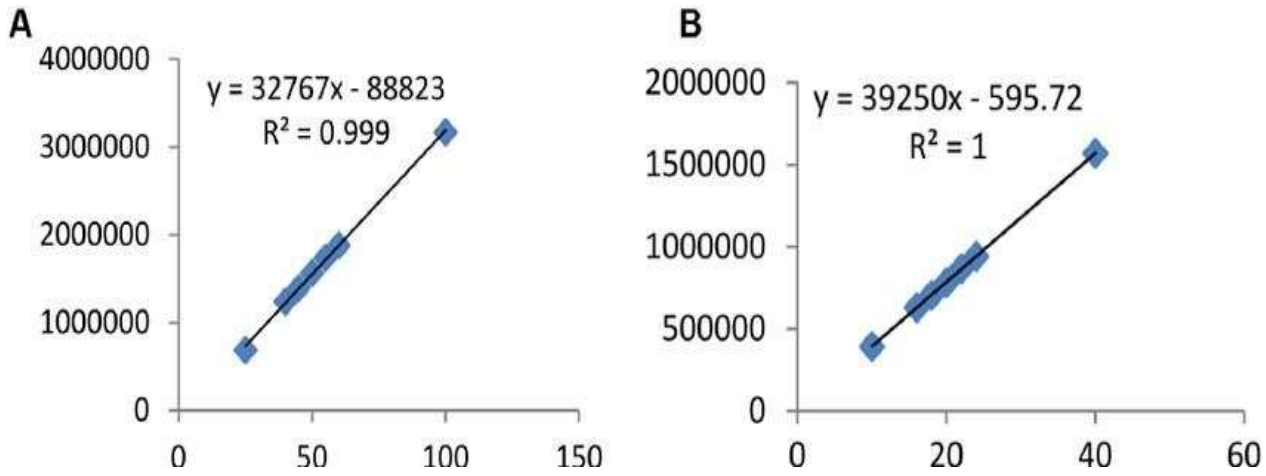


Table 4: Results of System Suitability

Conditions: 1.0ml/min, pH-4.0, O.P100%,Temp-35°C	Resolution	Plate Counts		Tailing Factor		%RSD	
		Ibu	**para**	**Ibu**	**Para**	**Ibu**	**Para**
	8.9	13616	**4099**	**1.4**	**1.0**	**0.9**	**0.1**

Ibu= Ibuprofen, Para = Paracetamol

Table 5: Results of Robustness

		Tailing factor		Plate counts		%RSD	
Condition	Resolution	**paraIbu**		**Para**	**Ibu**	**Para**	**Ibu**
Flow rate in ml/min (± 0.2)							
0.8ml/min	8.8		1.01.5	3952	14679	0.1	0.8
1.2ml/min	7.3		1.01.4	8095	3658	0.1	0.3
Column Temperature (±5°C)							
30°C	8.7	1.0	1.4	4209	12641	0.0	1.0
40°C	7.5	0.9	1.4	3559	13667	0.0	0.7

Table 1 : Paracetamol and Ibuprofen Accuracy Results

%Spike level	Paracetamol			Ibuprofen		
	µg/mL Added	µg/mL Found	Mean recovery (n=3)	µg/mL Added	µg/mL Found	Mean recovery (n=3)
50	45.8541	46.1422	100.6	56.8714	56.9806	**100.2**
70	64.1957	65.5087	102.0	80.9323	81.2157	**100.4**
90	80.7032	80.5210	99.8	98.4312	99.4574	**101.0**
100	**89.8740**	**89.4451**	**99.5**	**109.3680**	**110.0983**	**100.7**

CONCLUSION :

Reverse phase high performance liquid chromatography was a simple, easy, robust, accurate, reliable. The simultaneous estimation of paracetamol and ibuprofen by the RP-HPLC has been demonstrated to be selective and reproducible analytical approach for pharmaceutical analysis. The reported data is confidential and data is satisfactory. All the factors of method validation are evaluated according to the united states pharmacopoeia and ICH guidelines. RP-HPLC is the preferred technique for the method validation for most of the drugs which can give the satisfactory results.

REFERENCES :

1. British pharmacopoeia, Vol II, London: Her mejesty's Stationary office 1998, p. 1854

2. Indian pharmacopoeis Vol II, New Delhi, The controller of Publications. Govt of India, 1996, p. 554.

3. Ortega-Barrales, P., Padilla-Weigand,
R. and Molina-Diaz, A., Anal.Sci., 2002, 18, 1241.

4. Dinc, E., Yucesoy, C and Onu, F, J. Phar. Biomed. Anal., 2002, 15, 1091.

5. ICH, Q2A Text on Validation of Analytical Procedures, International Conference on Harmonization. October 1994.

6. Jahan ms, Islam Mj, Begum R, Kayesh R. A study of method development, method validation, and forced degradation of simultaneous quantification of paracetamol and ibuprofen by RP-HPLC. Analytical Chemistry Insights .2009; 2:700072.

7. Battu P, Reddy M. RP-HPLC method for simultaneous estimation of paracetamol and ibuprofen in tablets. *Asian J Res Chem*. 2009;2:70–72.

8. Hardman JG, Limbird LE, Gilman AG. *Goodman C Gilman's the pharmacological basis of therapeutics*. 10th ed. New York, USA: McGraw-Hill; 2001.

9. Brune K, Hinz B. Paracetamol, ibuprofen, or a combination of both drugs against knee pain: an excellent

new randomised clinical trial answers old ques- tions and suggests new therapeutic recommendations. *Ann Rheum Dis*. 2011;70: 1521–1522.

10. Hasan, N.Y., Abdel-Elkawy, M., Elzeany, B.E. and Wagieh, N.E., Farmaco., 2003, 58, 91.

11. EI-Saharty, Y.S., Refaat, M. and EI- Khateeb, S.Z., Drug. Develop. Ind. Pharm., 2002, 28, 571.

12. Gnana RM, Geetha G, Sangaranarayanan A. Simultaneous, stability indicating method development and validation for related compounds of ibuprofen and paracetamol tablets by RP- HPLC method. *J Chromatograph Sep Tech*. 2012;3:3-8

13. Sahoo S, Sahu R, Patra S. A study of method development, method validation, and forced degradation of simultaneous quantification of paracetamol and ibuprofen by RP- HPLC. Sci. pharm. 2014;82(3):563- 579.

14. Tsvetkova BG, Pencheva IP, Zlatkov AB, Peikov PT. Development and Valida- tion of RP-HPLC method for simultaneous determination of paracetamol and ibuprofen in fixed dose combinations. *Int J Pharm Sci Rev Res*. 2012;16:13–16.

15. Szepesi G. Selection of high- performance liquid chromatographic methods in pharmaceutical analysis. III. Method validation. *J Chromatogr*. 1989;464: 265–278.

16.Lakka NS, Goswami N, Balakrishna P. Development and validation of a RP- HPLC for simultaneous determination of Ibuprofen and Paracetamol in solid dos- age forms: application to dissolution studies. *Int J Res Pharm Sci*. 2011;2: 331– 337.