An Updated Review Article on Simultaneous Determination of Paracetamol and Ibuprofen in Tablets RP-HPLC Method

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Abstract- For the analysis of paracetamol and ibuprofen, a straightforward, precise, high performance liquid chromatographic (HPLC) approach was created and validated. Use Inertsil C18, 5, 150 mm x 4.6 mm for the C18 column. A mobile phase of acetonitrile/phosphate buffer (60:40, v/v, pH 7.0) was used, and the separation was carried out isocratically with UV detection at 260 nm. The internal standard was aceclofenac. Ibuprofen, paracetamol, and aceclofenac had retention times of 2.48, 4.45, and 6.34 minutes, respectively. - Column temperature 25 °C, Mobile Phase Flow: 1 mL / min. The new method's accuracy, precision, linearity, limit of detection, and limit of quantitation were all validated. This work sought to create and validate an HPLC method that is straightforward, precise, and picky. The suggested approach can be utilized for the determination of these drugs in combined dosage forms.

Key Words- Paracetamol, Validation, Dosage Form, Ibuprofen, Estimation

INTRODUCTION

Chemically speaking, ibuprofen is 2[4-(2-methyl propyl) phenyl] propanoic acid. The molecular weight is 206 and the structural formula is C13H18O2. This medication is non-steroidal anti-inflammatory (NSAID). As an analgesic, it is used to treat fever, primary dysmenorrheal symptoms, and arthritic symptoms. It is well known that ibuprofen has an ant platelet (blood-thinning effect). Chemically speaking, paracetamol is N-(4-hydroxyphenyl) acetamide. It functions as an antipyretic and non-opioid analgesic both centrally and peripherally. For the purpose of determining the presence of paracetamol with other medications either alone or in combination, numerous methods have been reported in the literature1–11. There isn't an RP-HPLC method available, though, for simultaneously estimating these medications in combination dosage forms. The market offers a fixed-dose combination of paracetamol (400 mg) and ibuprofen (325 mg) in tablet form.

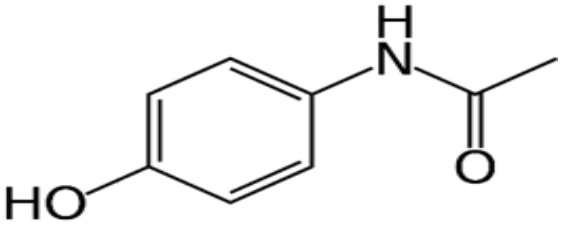

Fig No: 1 Paracetamol

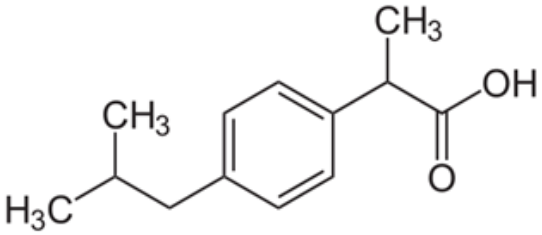

Fig No: 2 Ibuprofen

MATERIALS AND METHODS

1.1) Chemicals:

Acetonitrile, sodium hydrogen phosphate, and sodium dihydrogen phosphate in HPLC quality were purchased from Merck in India. Utilizing the Millipore Milli Q plus purification technology, high purity water was created.

1.2) Equipments:

To validate the analytical procedure, an High Performance Liquid Chromatography instrument with EZ Chrome software and a UV/Vis detector (Inertsil C_{18}, 5µ , 150 mm x 4.6 mm, make: Shimadzu

ltd, Japan) with the mobile phase containing acetonitrile and phosphate buffer in the ratio of 60:40 (v/v pH 7.0) at ambient temperature. Flow rate was kept at 1 ml/min and the elution was monitored at 260 nm.

1.3) Chromatographic Conditions:

The analysis was conducted using a High Performance Liquid Chromatograph system with LC solutions data handling system (Shimadzu-LC2010 with an auto sampler). Software called LC 2010 Solutions was used to record the data. Inertsil C18, 5, 150 mm x 4.6 mm, make: Shimadzu ltd, Japan), filled with Octadecyl silane chemically bonded to porous silica particles of 5 m diameter, was used for the purity determination. The mobile phase contained acetonitrile and phosphate buffer in the ratio of 60:40 (v/v pH 7.0) at room temperature. Elution was seen at 260 nm with a flow rate of 1 ml/min.

1.4) Solubility studies:

Paracetamol Insoluble in diethyl ether, but soluble in alkali hydroxide solutions, water (1:70, 1:20 at 100°C), ethanol (1:7), acetone (1:13), chloroform (1:50), glycerol (1:40), methanol (1:10), and propylene glycol (1:9). The pH of a saturated aqueous solution is 6.

The ibuprofen majority of organic solvents, such as ethanol (66.18 g/100 mL at 40 °C for 90% EtOH), methanol, acetone, and dichloromethane, are far more soluble than water in terms of ibuprofen than these other solvents.

1.5) Preparation of Standard Solution:

Ibuprofen and paracetamol were produced as standard stock solutions (1 mg/ml) by separately dissolving 25 mg of each medication in 25 ml of acetonitrile. The solutions were appropriately diluted with mobile phase to create a mixed standard solution that contained internal standards of 25 g/ml of paracetamol, 20 g/ml of ibuprofen, and 30 g/ml of aceclofenac.

1.6) Mobile Phase:

Mobile phase: water / acetonitrile mixture in 80/20 ratio.

1.7) Preparation of Sample Solution:

Twenty tablets of Comb flam, manufactured by Aventis ltd. in Mumbai, each containing 325 mg of ibuprofen and 400 mg of paracetamol, were carefully weighed. A 25 ml volumetric flask was then filled with powder corresponding to 25 mg of paracetamol. The medicines were extracted into acetonitrile, the volume was set at 25 ml, the mixture was vortexed, and then the mixture was filtered through a 0.45 membrane filter. In order to obtain a final concentration of 25 mg/ml of paracetamol and 20 mg/ml of ibuprofen, as well as 30 mg/ml of aceclofenac as an internal standard, from this solution, additional dilutions were made using mobile phase. This solution was then utilized for the estimation. Calculations were made on the drug concentration

1.8) Method Validation :

The development of reference techniques and the evaluation of a laboratory's capability to produce reliable analytical records both depend heavily on method validation. The context of the procedure that produced the chemical data has been used to place validation. In an effort to prevent their improper use and ensure scientific accuracy and consistency, analytical method validation, thinking about the most pertinent procedures for examining the best parameters of analytical methods, and using a variety of pertinent overall performance indicators, including selectivity, specificity, accuracy, precision, linearity, range, limit of detection (LOD), limit of quantification (LOQ), ruggedness, and robustness are heavily discussed.

1.9) Parameters :

- Specificity
- System Suitability
- Precision
- Accuracy
- Linearity
- Range
- LOD (Limit of Detection)
- LOQ (Limit of Quantitation)

2.0) Specificity:

The capacity of an analytical method to measure an analyte accurately in the presence of interferences that may be anticipated to be present in the sample matrix is known as selectivity. Chromatographic blanks from a sample that is known to be analyte-free are examined within the anticipated time window of the analyte peak in order to determine the selectivity. Additionally, the

raw data for selectivity will be saved in the acceptable forms.

2.1) System Suitability:
Chromatographic methods include system suitability tests that are used to confirm that the system's resolution and repeatability are sufficient for the intended analysis.

2.2) Precision:
The degree of consistency between individual test results when a technique is done repeatedly to various samples is known as a method's precision. A homogeneous batch of samples from several samplings is used to analyse a set of standards that are injected or to quantify precision. Precision as relative standard deviation (% rsd) is determined using the measured standard deviation (SD) and Mean values.

Accuracy:
The degree to which test findings produced by an analytical method correspond with the actual value is considered the method's accuracy.
By adding a known concentration of analyte standard to the sample matrix of interest and analysing the sample using the "method being validated," accuracy is determined. From matrix to matrix, the process and computation for Accuracy (as% recovery) will differ, and it will be provided in the appropriate research plan or study plan amendment.

Linearity:
The linearity of an analytical method is its capability to elicit check consequences which might be at once, or with the aid of well described mathematical adjustments, proportional to the concentration of analytes in within a given range. Linearity is determined by injecting a series of standards of stock solution/diluted stock solution using the solvent/mobile phase, at a minimum of five different concentrations in the range of 50–150% of the expected working range

LOD (Limit of Detection):
The term LOD is defined as the lowest concentration at which the instrument is able to detect but not quantify and the noise to signal ratio for LOD should be 1:3. The term LOQ is defined as the lowest concentration at which the instrument is able to detect and quantify. The noise to signal ratio for LOQ should be 1:10. Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ) from Detector Linearity experiments (applicable to only instrument sensitivity).

LOQ (Limit of Quantitation):
Prepare a series of standard solutions (at least five concentrations should be prepared to cover working concentrations needed for routine analysis), analyse each solution at least twice, and then record the instrument's reaction. The Limit of Quantification (LOQ) is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision [24]. However, the determination of LOQ depends on the predefined acceptance criteria and performance requirements set by the IA developers

RESULTS AND DISCUSSION

The ideal chromatographic conditions allowed for the recording of a constant baseline. Ibuprofen, paracetamol, and aceclofenac were shown to have retention times of 3.78, 5.25, and 6.44 min, respectively. Figure 1 shows a typical chromatogram of a sample solution. Detection was done at 260 nm. Six times of the test process were performed, and the mean peak area ratio and mean weight of the reference medications were then computed. Table 1 shows the percentage of each drug detected in the formulation, as well as the mean and standard deviation. The analysis's findings demonstrate that the dosage matched the formulation's label promise quite well.
The approach was approved in accordance with ICH standards. Recovery experiments determined the method's accuracy. Six recovery trials were conducted, and table 1 shows the percentage recovery that was computed for each study. Added RSD were calculated using the data that were obtained. In the interday variation investigations, response factors for drug peaks and % RSD were computed after six repeated injections of standard and sample solutions over the course of three days. The data gathered showed that the devised HPLC technique was accurate.

Linearity:
The method's linearity was tested at seven different concentration levels, ranging from 10 to 70 g/ml for ibuprofen and 20 to 80 g/ml for paracetamol. Plotting

the response factor against the medication concentration allowed for the construction of the calibration curve. The Limit of Detection and The Slope recoveries of standard drugs were found to be accurate.

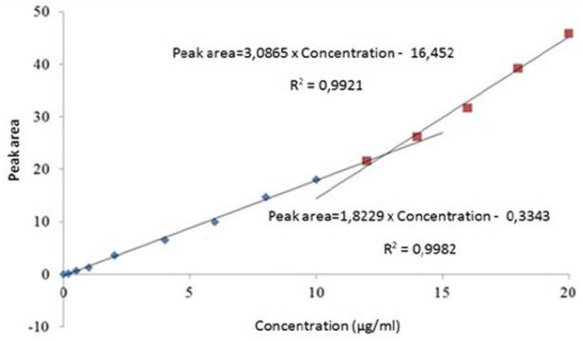

Fig No: 33 Straight Line for Paracetamol determination

System Suitability:

The system suitability studies were carried out to determine theoretical plate/meter, resolution factor, asymmetric factor and tailing factor. The results were given in the Table 2. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ±3% standard deviation range during routine performance of the method.

Precision:

The method's accuracy was shown by the inter day 0.998) for ibuprofen. The findings indicate that the study was outstanding and intraday fluctuation. Repeated injections of standard and sample solutions with drug concentrations within the previously mentioned range were used in the intraday trials, where there is a six correlation between response factor and concentration. Made, as well as the percentage and drug peak response component

LOD and LOQ:

The new RP-HPLC method's Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by injecting progressively lower amounts of the standard solutions. The LOD is the analyte concentration at which a detectable response can be obtained (signal-to-noise ratio of 3). The LODs for ibuprofen and paracetamol were discovered to be 6ng/ml and 10ng/ml, respectively. The LOQ is the analyte concentration at which the response can be precisely quantified at (signal-to-noise ratio of 10). For paracetamol and ibuprofen, the LOQ was 15 ng/ml and 25 ng/ml, respectively.

RUGGEDNESS

The experiment was conducted on various instruments, including Shimadzu HPLC (LC-2010), Agilent HPLC, by various operators using various columns of the same type, Intersil C18, and Hypersil C18, in order to assess the robustness of the procedure. By making small adjustments to the chromatographic conditions, the robustness of the procedure was evaluated. The fact that there were no obvious alterations in the chromatograms showed how tough and reliable the established HPLC process was

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 hours at room temperature. The results show that for both solutions, the retention time and peak area of paracetamol and ibuprofen remained almost unchanged(%RSD <2) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 hours, which was sufficient to complete the whole analytical process.

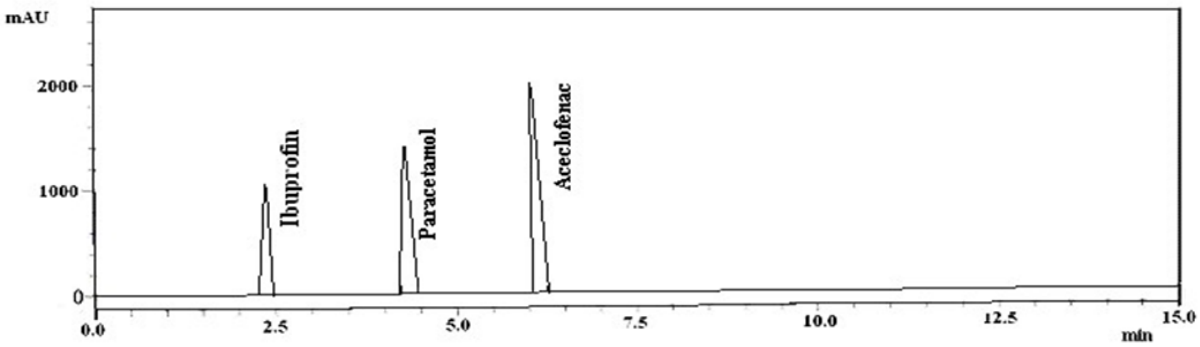

Fig No: 4 Chromatogram of Sample Solution

Drug	Labeled	Found	% Label Claim	% Recovery
Paracetamol	400	399.06± 1.045	99.80± 1.020	98.90± 0.815
Ibuprofen	325	324.01± 1.135	96.05± 1.095	96.01± 0.580

Table No: 1 Results of analysis of formulation and recovery studies

Parameters	Paracetamol	Ibuprofen
Linearity range	20 to 80 µg/ml	10 to 70 µg/ml
Regression equation Y = mx +c^{+}	y = 0.0071x-0.001	y = 0.0061x+0.002
Correlation coefficient	0.999	0.998
Theoretical plate/meter	26458	28764
Resolution factor	1.30	1.30
Asymmetric factor	0.90	1.01
Tailing factor	1.2	1.0
LOD (ng/ml)	6	10
LOQ (ng/ml)	15	25

Table 2: Validation and System Suitability Studies

CONCLUSION

The method was validated as per ICH guidelines. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery were calculated and presented in table 1. From the data obtained, added RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

Thus the proposed RP-HPLC method for the simultaneous estimation of paracetamol and ibuprofen in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitabnle for the quality control of the raw materials, formulations and dissolution studies.

REFERENCE

[1] Jensen, L.S., Valentine, J., Milne, R.W. and Shimada, K., J. Pharm. Biomed. Anal., 2004, 34, 585.

[2] Ohta, M., Kawakami, N., Yamato, S. and Shimada, K. J.Pharm Biomed. Anal., 2003, 15, 1759.

[3] KUNTHEAVY, R., ING-LORENZINI, DESMEULES, JA., BESSON, M., etall Two-dimensional chromatography- ion trap mass spectrometry for the simultaneous determination of ketorolac enantiomres and paracetamol în human plasma, Application to a pharmacokinetic study, Journal of Chromatography A.

[4] REMY, C., MARRET, E., BONNET, F., Actualite du paracétamol,Evaluation et traitement de la douleur 2006, p. 639-648.

[5] SORODOC, L., Toxicologie clinica de urgenta vol I, Iasi, EdituraJunimea, 2005.

[6] SIN, B., KOOP, K., LIU, M., YEH, JY., THANDI, P., - IntravenousAcetaminophen for renal colic în the emergency department:Where do we stand? Am J Ther, 2016 oct 24.

[7] FARNIA, MR., BABAEI, R., SHIRANI, F., MOMENI, M., et al Analgesiceffect of paracetamol combined with low- dose morphine versus morphine alone on patients with biliary colic: a double blins randomized controlled trial, World J Emergency Medical 2016; 7 (1) p. 25

[8] FDA, Guidance for Industry, Validation of Analitical Proceduresand Methods Validation for Drugs and Biologics, USA, 2015. 11. MALESKER, MA., BRUCKNER, AL., LOGGIE, B., HILLEMAN, DE., IV Acetaminophen: Assessment of Medication Utilization Evaluation Data in Peri-operative Pain Management. Journal of Surgery 2015; 10(4) p. 230-234 doi: 10.7438/1584-9341-10-4-36284272 (2001)

[9] Therapeutic effervescent compisition U.S. Patent No-4687662

[10] S.I.Saleh in “An approach to the direct compressible effervescent tablets” Lab

Pharmacotech, Fac, Pharma f-67048, strasboug celex 1983

[11] Swierkosz T.A. Jordan L. Mcgough.K.(2002) 'Action of paracetamol on COX in tissue and cell.

[12] L.Kalantzi, C.Reppas, J.B. Dressman, G.L.Amidon, H.E.Junginger in "Journal of Pharmaceutical Science" 95.