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Simultaneous Determination of Ibuprofen, Paracetamol and Chlorzoxazone in Tablet Dosage Form by High Performance Liquid Chromatography (Hplc)

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Abstract :

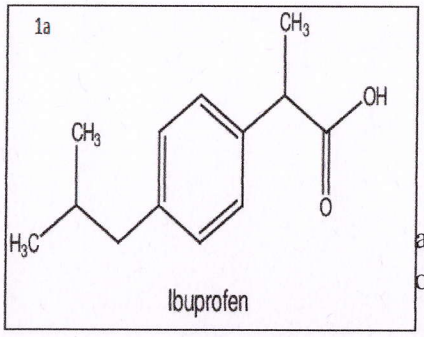
A simple, precise and rapid isocratic reverse phase high performance liquid chromatographic method is developed for the reverse phase high performance determination of Ibuprofen, Paracetamol, and Chlorzoxazone from tablet dosage form. The chromatographic separation was performed on a Inertsil C18 column (250mm × 4.6mm i.d 5µm particle size). Mobile phase consisted of a mixture of phosphate buffer (0.030M KH2PO4, pH adjusted to 2.9 using ortho phosphoric acid), Acetonitrile in the ratio of (40 : 60) at a flow rate of 1.0 mL/min. The wavelength was set at 240nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration was linear over the range of 40 – 120 µg/mL for Ibuprofen , 32.5 – 97.5 µg/mL for Paracetamol and 25 – 75 µg/mL for Chlozoxazone . The retention times were found as 2.7 mins for Paracetamol, 4.2 mins for Chlorzoxazone and 6.2 mins for Ibuprofen. This method was found to be accurate , precise and rapid and can be used for routine analysis.

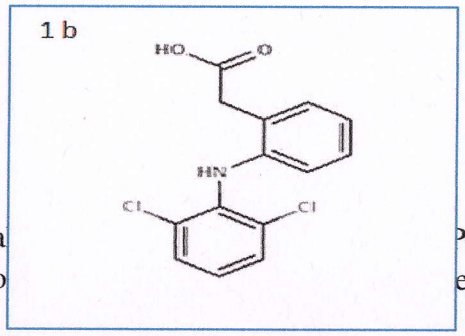
Keywords: Ibuprofen, Paracetamol, Chlorzoxazone, RP-HPLC, Validation

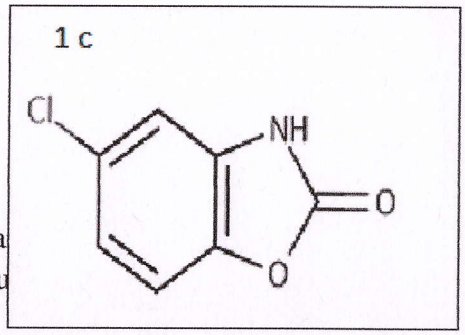
Introduction :

Ibuprofen (RS)-2-(4-(2-Methylpropyl)phenyl) propanoic acid) [Fig 1a] is the non steroidal anti inflammatory drug (NSAID) is used for treating pain, fever and inflammation. Paracetamol (p-hydroxy acetanilide) [Fig 1b] is a compound with analgesic and antipyretic properties. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding [1-4]. It affords quick relief of pain and wound edema [3,4]. Chlorzoxazone (5-chloro-2(3H)-benzoxazolone)[Fig1c] is a compound with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders.

Literature survey reveals that various analytical techniques viz, UV spectrophotometry [10,11]. Few HPLC methods[5-9] have been reported for the simultaneous determination of Ibuprofen, Paracetamol and Chlorzoxazone and Aceclofenac in combined dosage form. The aim of the present work was to develop and validate the rapid and sensitive high performance liquid chromatography (HPLC) method for simultaneous determination of Ibuprofen, Paracetamol and Chlorzoxazone .







certificates of analysis. Acetonitrile (HPLC grade), Potassium dihydrogen phosphate buffer (analytical grade) and orthophosphoric acid were purchased from Merck (Mumbai, India)

Standard stock preparation:

40mg of Ibuprofen, 32.5 mg of Paracetamol and 25mg of Chlorzaxone were accurately weighed and transferred to a 100cm3 volumetric flask. It was dissolved in a minimum quantity of methanol and then diluted up to the mark with methanol. The concentration of the solution obtained was 400µg/mL for Ibuprofen, 325 µg/mL for Paracetamol and 250µg/mL for Chlorzoxazone (Solution A). 2cm3 of this solution A was diluted to 10 cm3 in a volumetric flask with mobile phase. The concentration of the solution obtained was 80µg/mL for Ibuprofen, 65 µg/mL for Paracetamol and 50µg/mL for CHlorzoxazone .

Preparation of Sample solution:

Twenty tablets(Flezon MR) were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to one tablet containing 400mg of Ibuprofen , 325 mg of Paracetamol and 250mg of Chlorzoxazone was taken in a 100mL dilution flask. Then about 50mL of diluent was added to it. Then sonicated for 20-25mins at an ambient temperature with intermittent swirling, cooled and diluted upto the mark with diluent, mixed well. Then solution from the flask was filtered through syringe filter. This solution was used for further analysis.

Chromatographic conditions:

The chromatography was performed using Waters HPLC system having Waters 501 isocratic pump equipped with Waters TM 717plus autosampler and a Waters 486 tunable absorbance UV-detector. The data was recorded using Millenium32 chromatographic software. Separation was performed on a 250mm × 4.6mm i.d., 5 µ particle size Inertsil C18 column. Mobile phase consisted of a mixture of acetonitrile : buffer (40 : 60), pH 2.9 adjusted with ortho phosphoric acid. Flow rate was kept at 1.0mL/min. Wavelength was set at 240nm.

Method Validation:

The method was validated as per ICH guidelines[11] for specificity, linearity, quantification limit, precision, accuracy, recovery and stability. Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the exciepients at the retention times of IBU, PCT and CHZ. The accuracy of the method was determined by recovery experiments. The precision of the method was demonstrated by inter day and intraday variation studies, six repeated injections of standard and sample were made and percentage RSD was calculated. In the intra-day variation studies six repeated injections of standard and sample solution was carried out by injecting on the same day at different intervals and percentage RSD was calculated. In the inter day variation studies six repeated injections of standard and sample solution were made for three consecutive days and percentage RSD was calculated. The linearity of the method was demonstrated at seven concentration levels of the mixed standards of IBU, PCT and CHZ.

Results and Discussions

Optimization of the Chromatographic conditions

In order to develop an isocratic reverse phase HPLC method for the determination of IBU, PCT and CHZ. in combined dosage form the chromatographic conditions were

optimized. For better separation and resolution the different buffers were tried. It has been found that potassium dihydrogen phosphate buffer, pH 2.9 adjusted with orthophosphoric acid gave better peak shape than other buffers. The different compositions of mobile phase were changed for getting better separation of analytes. Thus the mobile phase composed of the mixture of buffer (0.03MKH2PO4, pH 2.9 adjusted with orthophosphoric acid) acetonitrile buffer (40 : 60 v/v) was finalized. The better separation, peak symmetry and reproducibility were obtained with Inertsil C18, 250mm x 4.6mm, 5µm column compared to Thermo BDS Hypersil C8, 150mm x 4.6mm, 5µm column. Both the analytes were gave better response at 240nm wavelength using UV detector. The flow rate kept was 1.0mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention times of IBU, PCT and CHZ. were found to be 2.8 mins, 4.1 and 6.2 mins respectively.

Validation:

The proposed method was showed short elution time and good separation between IBU, PCT and CHZ. The system suitability test was performed as per the USP and international conference of harmonization (ICH) guidelines to confirm the suitability and the reproducibility of the method. Six consecutive injections of the standard solution were performed and evaluated for repeatability, tailing factor, theoretical plates and resolution.
% RSD values were found to be 0.65, 0.50 and 0.78 for IBU, PCT and CHZ respectively. The tailing factor and theoretical plates were found to be perfectly within the limits.

The method was linear over the range 40–120µg/mL for Ibuprofen 32.5 – 97.5 µg/mL for Paracetamol and 25 – 75 µg/mL for Chlozoxazone. The calibration curve was constructed by plotting response factor against concentration of drugs (Fig 2a, Fig 2b and Fig 2c). The slope and intercept value for calibration curve was Y = 35473x + 38413 (r2= 0.9995) for Ibuprofen, Y = 42692x + 185382 (r2= 0.9927) for Paracetamol and Y = 43904x + 3130 (r2=0.9990) for Chlorzoxazone.
The results shows that an excellent correlation between response factor and concentration of drugs.

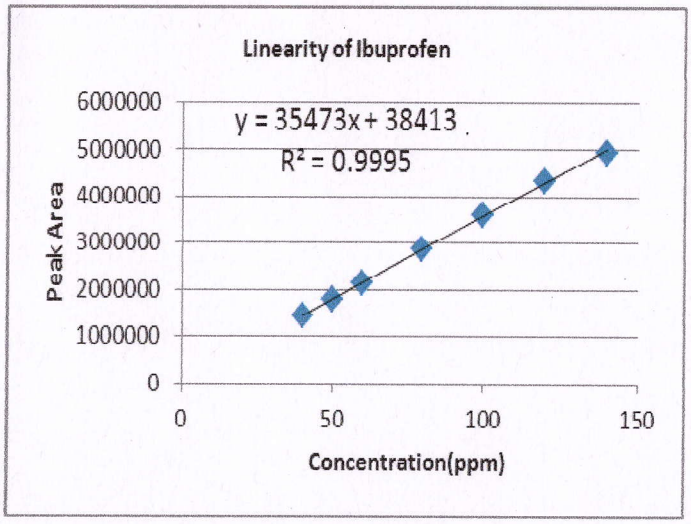


Figure 2a Linearity Of Ibuprofen

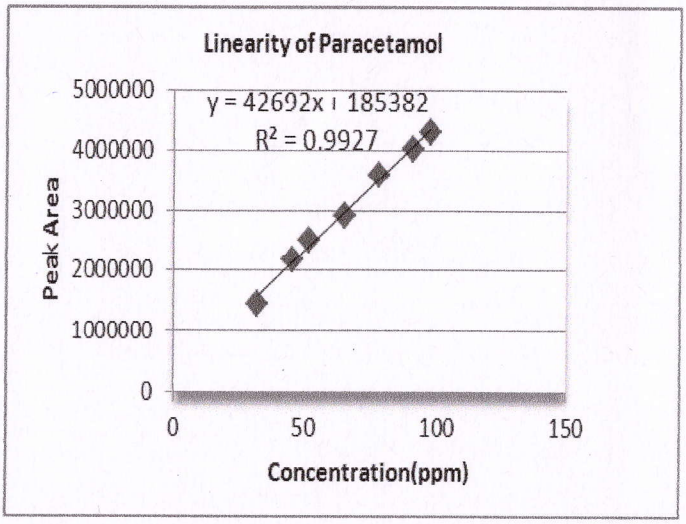


Figure 2b Linearity Of Paracetamol

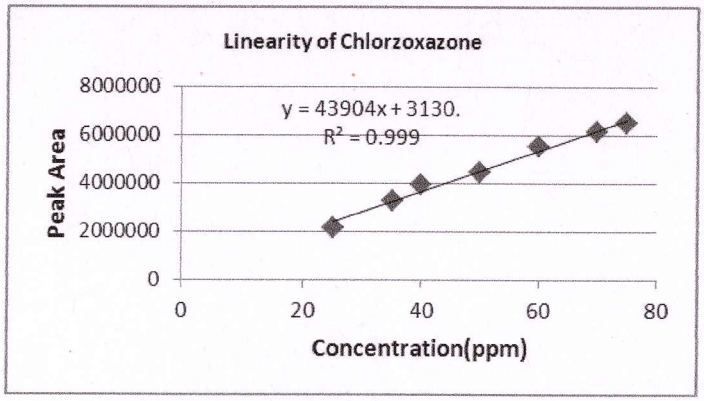


FIGURE 2C LINEARITY OF CHLORZOXAZONE

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The limit of quantification (LOQ) and limit of detection (LOD) was established at a signal-to-noise ratio. The LOQ and LOD of IBU, PCT and CHZ were experimentally determined. The LOD of IBU, PCT and CHZ was found to be 0.0030 µg/mL,0.078 µg/mL and 0.095 µg/mL respectively. The LOQ of IBU, PCT and CHZ was found to be 0.0125 µg/mL, 0.085 µg/mL and 0.095 µg/mL respectively.

The system precision study was performed to determine the repeatability of the method. Six samples of standard were prepared at 100% level and assayed according to the procedure. The method precision study was performed to determine the reproducibility of the method. Six samples of tablets were prepared at 100% level and assayed according to the procedure. The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10%, 20% and 30% of the standard drug of analytes were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for IBU, PCT and CHZ from the combination formulation are shown in Table 1. The results are well within the acceptance limit and hence the method is accurate.

Table 1. % Recovery of Ibuprofen, Paracetamol and Chlorzoxazone

Sr.No	% Added	Original amount	Added amount	Total amount	Mean (n =5)	% Recovery	S.D	% R.S.D
Amount of Ibuprofen in mg								
1	0	80	0	80	80.23	100.28	0.162	0.203
2	10	80	8.10	88.10	87.90	99.77	0.141	0.160
3	20	80	16.30	96.30	96.85	100.57	0.388	0.402
4	30	80	24.55	104.55	104.20	99.66	0.247	0.237
Amount of Paracetamol in mg								
1	0	65	0	65	65.05	100.07	0.035	0.054

2	10	65	6.50	71.50	71.10	99.44	0.028	0.396
3	20	65	13.0	78.00	77.92	99.89	0.056	0.072
4	30	65	19.50	74.50	74.59	100.12	0.063	0.085

Amount of Chlorzoxazone in mg

Sr.No	% Added	Original amount	Added amount	Total amount	Mean (n =5)	% Recovery	S.D	% R.S.D
1	0	50	0	50	49.48	98.96	0.367	0.739
2	10	50	5.05	55.05	54.95	99.81	0.070	0.128
3	20	50	10.20	60.20	61.08	101.46	0.622	1.026
4	30	50	15.30	65.30	64.85	99.31	0.318	0.188

The specificity of the method was determined by exposing 100% sample solution of Ibuprofen, Paracetamol and Chlorzoxazone. The Chromatogram of the sample solution shows that there should not be any interference of the placebo at the retention times of the analytes. It is shown in fig 3

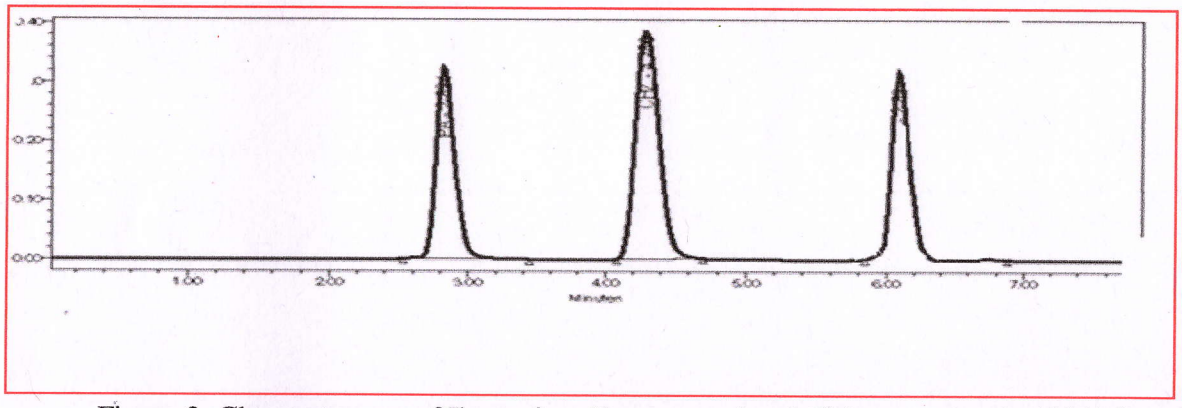


Figure 3. Chromatogram of Ibuprofen , Paracetamol and Chlorzoxazone in 100% Sample solution

Method applications:

The validated HPLC method was applied to the simultaneous determination of IBU, PCT, and CHZ in tablet dosage form. The samples were analysed and the assay results are as per the label claim shown in Table 2.

Drug	Label Claim mg	Amount found (n = 7)	S.D	% RSD	% Assay
Ibuprofen	400	400.50	0.353	0.088	100.12
Paracetamol	350	349.28	0.509	0.145	99.79
Chlorzoxazone	250	251.54	1.088	0.434	100.61

Table 2 Results of Assay Experiments

Conclusion:

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and is suitable for simultaneous quantification of Ibuprofen, Paracetamol and Chlorzoxazone The proposed method gives a good resolution among the analytes. The method is very simple, rapid and no complicated sample preparation is needed. High percent of recovery shows the method is free from interference of excipients present in the formulations and the method is accurate.

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