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Research Article

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Development and validation of a stability indicating RP-UPLC method for the determination of paracetamol and ibuprofen in tablet

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ABSTRACT

The proposed work describes the development and validation of a simple, precise and accurate method for the combination tablet formulation of Paracetamol and Ibuprofen by UPLC. Efficient separations of the Paracetamol and Ibuprofen were achieved in < 3 min by an isocratic elution with 20mM potassium dihydrogen phosphate buffer (pH 7.35 with dilute ortho phosphoric acid): acetonitrile (35:65 v/v) mobile phase at a flow rate of 0.25ml/min at 225 nm using a PDA detector. In UPLC, all of the analytes were resolved very well the resolution between Paracetamol and Ibuprofen was 5.4. The method was developed using different columns like UPLC @ BEH C8, C18; phenyl and HSS T3 with different length of the columns had studied under RP conditions. The final column chosen for the analysis was HSS T3 (100mm ×2.1, 1.8 µm) column which provided much stronger retention and resolution with good peak shape. The retention time of Paracetamol and Ibuprofen were 1.09 and 1.88 min, respectively. The linearity was obtained in the concentration range of 6.66-59.94 µg/ml and 8.0-72.0 µg/ml for Paracetamol and Ibuprofen with 0.9999 of correlation coefficients respectively. The specificity of the method was analyzed by the stress degradation study in acidic, alkaline, oxidative, thermal and photolytic conditions. The LOD for Paracetamol and Ibuprofen was found 0.84 and 0.52 µg/ml respectively. The LOQ was found 2.81 and 1.73 µg/ml for Paracetamol and Ibuprofen respectively. The validation follows the International Conference on Harmonization (ICH) guidelines.

Key words: Reversed phase; Isocratic elution; Stability indicating; UPLC-PDA; Method validation

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INTRODUCTION

Paracetamol is an aniline analgesics class of drug [1]. It is chemically known as an N-acetyl-p-aminophenol. It is often known as acetaminophen [2] which is prescribed to relieve the pain and to reduce the fever. It falls under the two classes such as an analgesic and an antipyretic [3-6]. Ibuprofen is chemically known as a (±) - 2 - (p - isobutyl phenyl) propionic acid and it is a nonsteroidal anti-inflammatory drug (NSAIDs) which is used to treat pain, fever and inflammation [7, 8]. The mechanism action of Paracetamol and Ibuprofen is to inhibit the cyclooxygenase (COX) [9, 10]. Paracetamol is not much effective as compared to Ibuprofen [11]. The combination of this medicine with considerably less gastrointestinal adverse effect than other NSAIDs [12]. The chemical structure of Paracetamol and Ibuprofen is given in the figure 1.

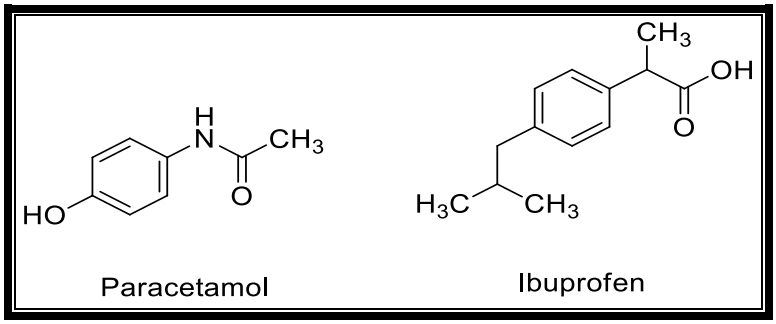

Figure 1: Chemical Structure of Paracetamol and Ibuprofen

The several analysis of Paracetamol and Ibuprofen in single or in combination dosage form were carried out by HPLC which containing the long period of run time, that minimizes their applications by throughput analysis. The recent approach is to develop a method by a new generation UPLC instrument which has very shorter run time and high throughput analysis. Simultaneous quantification of both the drugs; Paracetamol and Ibuprofen were performed and they were eluted out before 3.0 min., which shows a great advantage of shorter run time for the utilization in laboratories for the purpose of quality control as compared to conventional LC method.

Reviews of literature appurtenant to analytical methods indicate that there is no any isocratic UPLC-PDA method is available for simultaneous quantification of Paracetamol and Ibuprofen in combination dosage form. The various methods were revealed for pharmaceutical dosage form, single or combined and biological fluids by HPLC [13-15], HPTLC [16], UV [17], FT-IR [18].

The analytical method for the determination of Paracetamol and Ibuprofen in combination in tablet dosage form was validated according to guideline of ICH Q2 (R1) [19].

EXPERIMENTAL SECTION

Materials and methods:
Working standards of Paracetamol and Ibuprofen were gifted by HETERO drugs Limited (Hyderabad, India) and combination tablet was purchased from the market. Analytical-grade hydrochloric acid, ortho-phosphoric acid, hydrogen peroxide (30 % v/v) and sodium hydroxide pellets were from Ranbaxy Fine Chemicals (New Delhi, India). Acetonitrile HPLC-grade and potassium dihydrogen phosphate were from Spectrochem Pvt. Ltd. (Mumbai, India), and HPLC grade water was prepared using Milli-Q Elix-3 water purification system. Nylon syringe filters (0.45 µm) were purchased from Millex-HN, Millipore (Mumbai, India).

Instrumentation:
The Waters Acquity™ UPLC chromatographic system used to perform development and validation (Waters, Milford, MA, USA). This system consists of a binary solvent manager (BMS), photodiode array detector, sample manager (SM) and column oven connected to a multi-instrument data acquisition and processing system Empower 2.1 version. A Sartorius CPA2P analytical microbalance (Gottingen, Germany), an ultra sonic bath SONICA Spinco used for degassing purpose from Spincotech Pvt. Ltd. (Mumbai, India). Milli-Q, Elix-3 water purification system (Millipore, Milford, USA) used as an HPLC grade water source.

Chromatography:
Chromatographic analysis was performed on HSS T3 (100 mm×2.1, 1.8µm) column. The mobile phase consists of 20mM potassium dihydrogen phosphate buffer: acetonitrile (pH 7.35 by ortho-phosphoric acid) in the ratio of (35:65 v/v) was used throughout the analysis. The flow rate was 0.25 ml/min, the injection volume was 1.2 µL, column temperature was 30ºC and detection was performed at 225 nm using a PDA detector.

Preparation of Phosphate buffer:
To prepare 20mM potassium dihydrogen phosphate buffer, 3.496gm was weighed and dissolved in 1 liter HPLC grade water and pH adjusted to 7.35 with ortho-phosphoric acid. It was filtered by 0.45µm filter and sonicated with an ultrasonic bath.

Mobile phase composition:
20mM potassium dihydrogen phosphate buffer (pH 7.35): acetonitrile in the proportion of (35:65 v/v).

Preparation of Diluents:
Mixer of water and acetonitrile in proportion of 50:50 v/v.

Preparation of Standard solution:
Standard stock solution of Paracetamol (333µg/ml) and Ibuprofen (400µg/ml) was prepared by transferring accurately weighed, 16.65 mg and 20 mg, of working standards into a 100 ml of volumetric flask, respectively. The 20 ml of mobile phase was added and the mixture was sonicated then the solution was diluted up to the 100 ml. Standard solutions were prepared by accurately transferring 2.5 ml of stock solution into 25 ml of volumetric flask to furnish the final concentration of Paracetamol (33.3µg/ml) and Ibuprofen (40µg/ml) and diluted with mobile phase up to mark.

Preparation of Sample solution:
To prepare a stock solution, 20 tablets were crushed and powdered and equivalent weight was taken to furnish the final concentration of Paracetamol (333µg/ml) and Ibuprofen (400µg/ml). Test solutions were prepared by accurately transferring 2.5 ml of stock solution and dilute with mobile phase up to 25 ml to obtain a final concentration of Paracetamol (33.3µg/ml) and Ibuprofen (40µg/ml). The mixture was further sonicated.

RESULTS AND DISCUSSION

Method development:
Selection of the chromatographic parameters was depended upon the chemical and physical nature of the compounds. The analytical method was decided after several exploratory trials with different condition effecting UPLC analysis, for example diluents and mobile phase composition, organic solvent in the mobile phase, flow rate, detection wavelength and other chromatographic conditions. Preliminary trials with mobile phase comprising mixtures of water with methanol did not give good peak shape. The best peak shape was obtained by use of 20mM potassium dihydrogen phosphate buffer, adjusted to pH 7.35 with ortho-phosphoric acid and acetonitrile, mobile phase composition 35:65 (v/v). Acetonitrile was selected as an organic constituent of the mobile phase to reduce the retention time and buffer was preferred to reduce peak asymmetry and to achieve good peak shape. The optimized mobile phase enabled good resolution of Paracetamol and Ibuprofen and of compounds generated during force degradation. The final chromatographic condition was optimized and used for the further validation of the method; standard chromatogram obtains using the conditions are given in figure 2, where Paracetamol and Ibuprofen were eluted at 1.09 and 1.88 min., respectively. The three dimensional view of the chromatogram is shown in figure 3. The newly developed analytical method was validated according to the ICH guidelines [21], USP [22] and AOAC international [23].

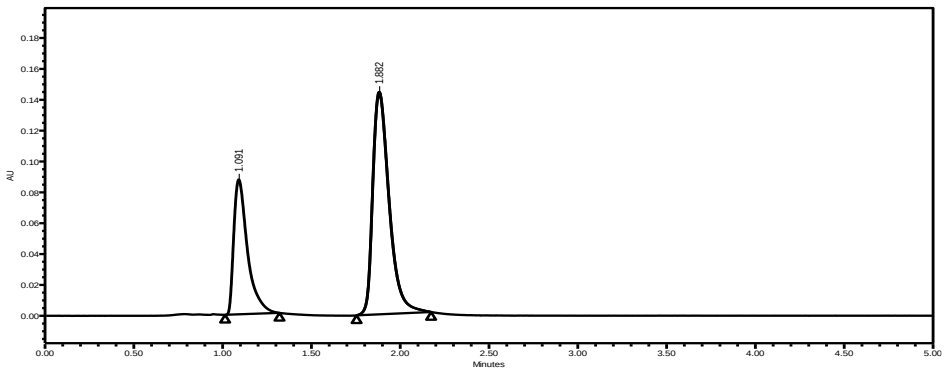

Figure 2: chromatogram of standard solution of Paracetamol and Ibuprofen

Solution stability:
Stability of the solution is the most significant parameter to get the reliable result throughout the validation. The solution stability study was performed using aged solution with compare to freshly prepared standard solution stored at room temperature and at 3-5ºC. To confirm the stability of the solution, percentage assay was calculated at different time interval such as initial, 6 h, 12 h, 24 h, 36 h and for 48 h. The results found satisfactory for 36 h, because at 48 h solution start degrading with % assay value less than 98% and % RSD found < 2.0 % for all the intervals except 48 h.

Forced Degradation
The stress studies were performed by degradation of Paracetamol and Ibuprofen in different chemical conditions like acidic, alkaline and oxidative; physical conditions, like thermal and photolytic.

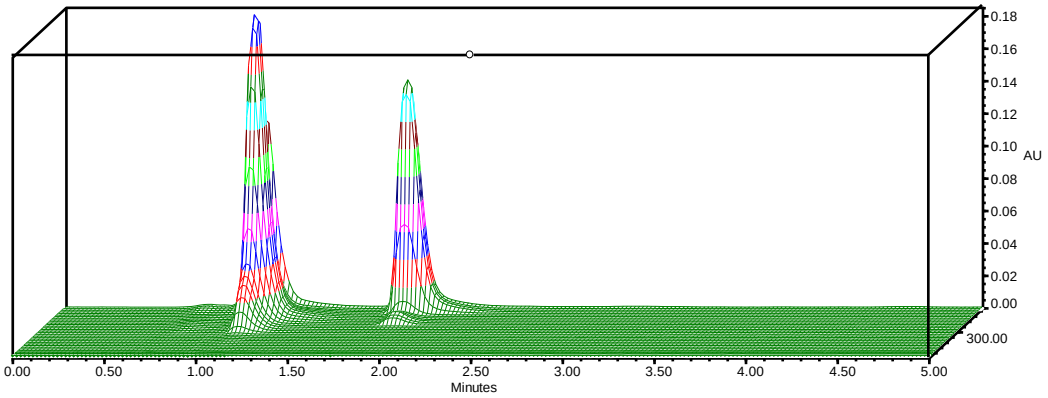

Figure 3: (3D) chromatogram of Paracetamol and Ibuprofen

Acidic degradation:

Equivalent weight of powdered of Paracetamol and Ibuprofen was taken and 3 ml 1N HCl were added in a 50 ml volumetric flask; it was put for degradation in water bath for 1 hour at 80^0 C, then it was neutralized with 3 ml, 1N NaOH and diluted up to mark with diluents. The concentration obtained was about 333.34 µg/ml of Paracetamol and 400.09 µg/ml of Ibuprofen. Then the solution was further diluted to furnish the final concentration of 33.34 µg/ml of Paracetamol and 40.09 µg/ml of Ibuprofen.

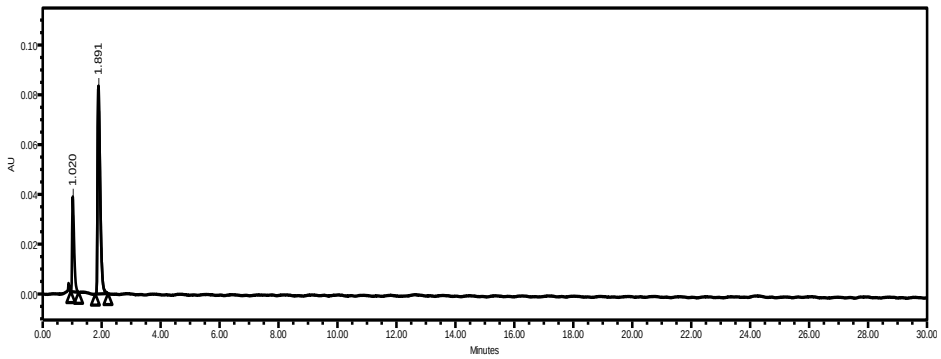

Figure 4: chromatogram of acidic degradation

Alkaline degradation:

Equivalent weight of powdered of Paracetamol and Ibuprofen was taken and 3 ml 0.1N NaOH were added in a 50 ml volumetric flask; It was put for degradation in water bath for 1 hour at 80^0 C, then it was neutralized with 0.1 N HCl, diluted up to the mark with diluents. The concentration obtained from that, was about 333.34 µg/ml of Paracetamol and 400.09 µg/ml of Ibuprofen.Then the solution was further diluted to furnish the final concentration of 33.34 µg/ml of Paracetamol and 40.09 µg/ml of Ibuprofen.

Oxidative degradation:

Equivalent weight of powdered of Paracetamol and Ibuprofen was taken and 3% 3 ml v/v H_2O_2, were added in a 50 ml volumetric flask and sonicated about 15 minutes in ultrasonic bath and the mixture was refluxed at 80°C for 1 hour. Then, the mixture was cooled to room temperature. The concentration obtained was about 332 µg/ml of Paracetamol and 399.80 µg/ml of Ibuprofen and 1 ml of this solution was taken into the 10 ml of volumetric flask and dilute up to the mark with diluents. The concentration obtained is about 32.3 µg/ml of Paracetamol and 39.80 µg/ml of Ibuprofen.

Thermal degradation:

The powdered drugs were kept at 80° C for 48 hours. Then Equivalent weight of powdered of Paracetamol and Ibuprofen was taken and dissolved in a 50 ml of volumetric flask with diluents and sonicated with ultrasonic bath then the mixture was cooled to room temperature and diluted up to the mark. The concentration obtained was about 333.34 µg/ml of Paracetamol and 400.04 µg/ml of Ibuprofen and was taken 1 ml of this solution and transferred into 10ml volumetric flask and diluted up to the mark with diluents. The concentration obtained was about 33.34 µg/ml of Paracetamol and 40.04 µg/ml of Ibuprofen.

Photolytic degradation:

The powdered of Paracetamol Ibuprofen was exposed to sunlight for 48 hours. Then Equivalent weight of powdered of Paracetamol and Ibuprofen was taken and dissolved in a 50 ml of volumetric flask with diluents and sonicated then the mixture was cooled to room temperature and diluted up to the mark. The concentration obtained was about 332.6 µg/ml of Paracetamol and 400.04 µg/ml of Ibuprofen and from that 1 ml of solution was taken into 10 ml of volumetric flask and diluted up to the mark with diluents. The concentration obtained was about 32.36 µg/ml of Paracetamol and 40.04 µg/ml of Ibuprofen. The chromatogram obtain from the study is shown in figure 5. The results of degradation study are shown in table 1.

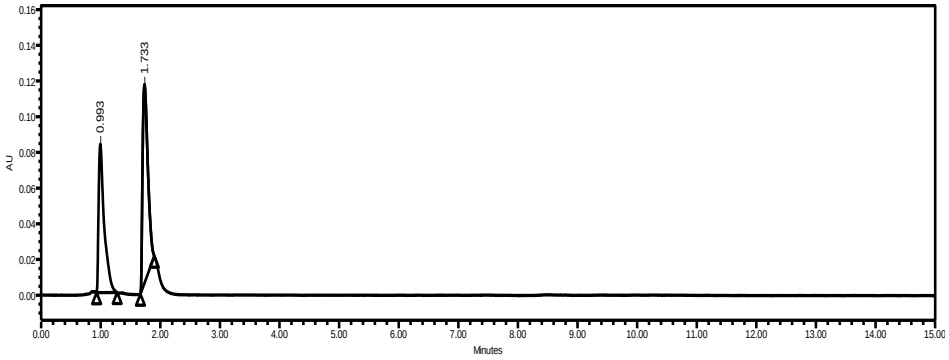

Figure 5: chromatogram of photolytic degradation.

Table 1: Summary of degradation study

Sr. No	Condition	Paracetamol		Ibuprofen	
		Peak area	Total Degradation %	Peak area	Total Degradation %
1	Acidic (1N HCl)	387484	58.41	1096667	9.52
2	Alkaline (0.1N NaOH)	295845	5.21	974525	47.89
3	Oxidative (3% H_2O_2)	792274	37.01	1617443	38.11
4	Thermal (80° C)	549671	30.82	929660	34.44
5	Photolytic (sunlight,48h)	574835	18.3	954082	38.4

Specificity:

The specificity of the method was determined by checking for interference with the analytes from placebo, diluents, mobile phase and degradation products. The specificity of the components was confirmed by measuring peak purity for Paracetamol and Ibuprofen during the force degradation study. There was no interference from any degradation product peak with the drug peaks. Figure (6 and 7) shows the peak purity chromatographs of Paracetamol and Ibuprofen, respectively for tablet solution.

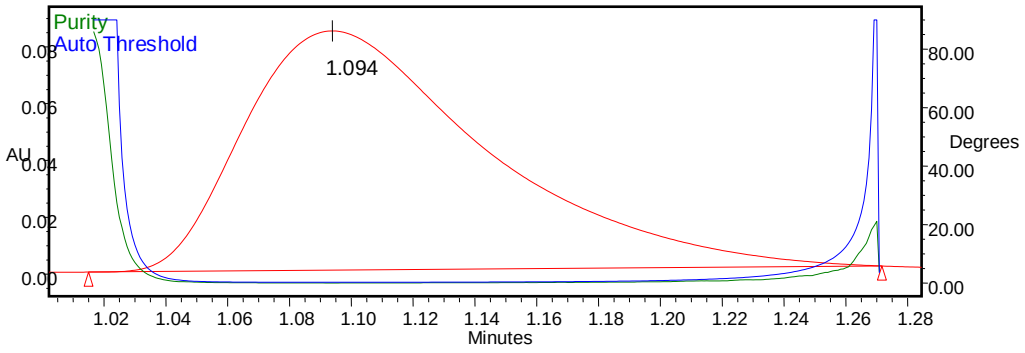

Figure 6: Peak purity chromatogram of Paracetamol

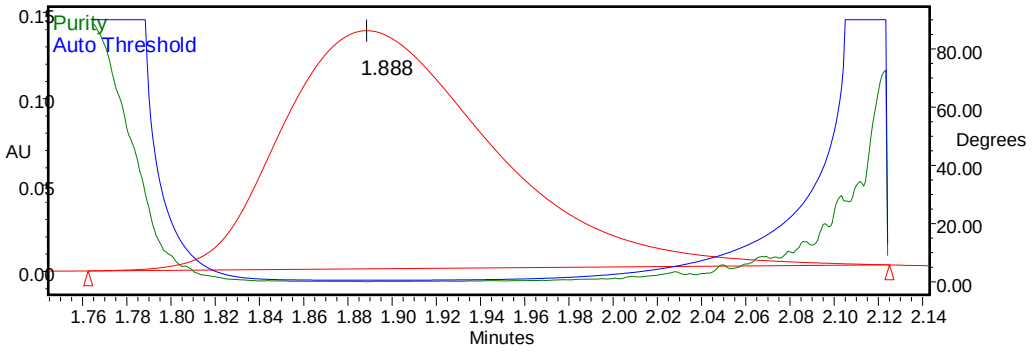

Figure 7: Peak purity chromatogram of Ibuprofen

Accuracy:

Accuracy was performed by using three different amounts (corresponding to 50, 100 and 150% of the test preparation concentrations) of Paracetamol and Ibuprofen to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was injected in duplicate. Accuracy was calculated for Paracetamol for three different % set, 100.24-100.90 and 99.79-99.93 for Ibuprofen. The results of accuracy study are shown in table 2.

Table 2: Results of Accuracy study

Sample	Level %	Peak area*	% Recovery*	% RSD
Paracetamol	50	201367	100.9	0.92
	100	402727	100.24	0.63
	150	595720	100.45	0.57
Ibuprofen	50	513114	99.93	0.02
	100	1025935	98.84	1.15
	150	1538626	99.79	0.13

*(*Mean of three set of each % level concentration)*

Precision:

The % RSD value for Paracetamol and Ibuprofen drugs peak area for different sets of precision (method precision and Intermediate precision) were 0.481 and 0.256 respectively.

Hence the value of %RSD for each set of precision was not more than 2.0 that proved the method is highly precise and % assay value was found between the 98.0% to 102 %.

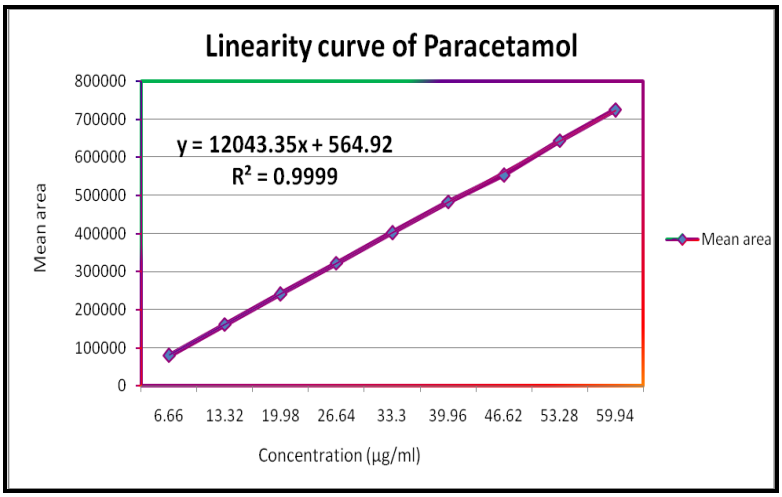

Figure 7: Linearity curve of Paracetamol

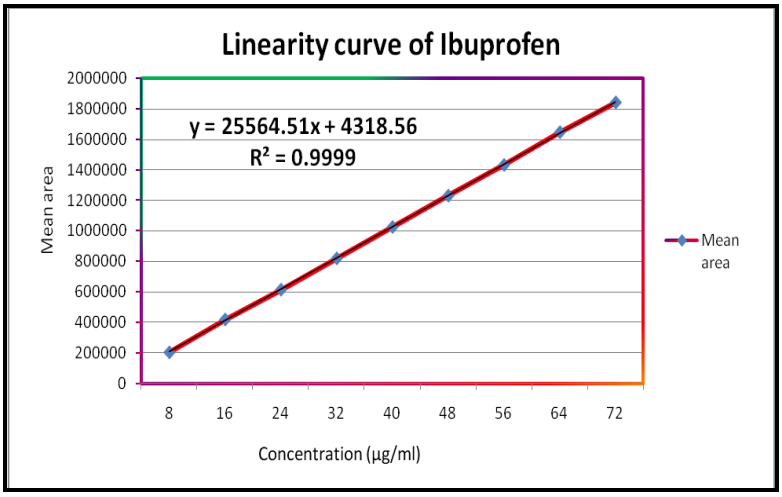

Figure 8: Linearity curve of Ibuprofen

Linearity:
Paracetamol and Ibuprofen showed linearity in the range of 6.66-59.94 µg/ml and 8-72 µg/ml respectively. The linear regression equation were y=12043.35x+564.92 and y= 25564.51x+4318.56, correlation coefficient 0.9999for both the drug (figure 7 and 8), where x axis is the concentration in µg/ml and y axis is the peak area in absorbance units.

Limit of detection (LOD) and limit of quantification (LOQ):
The limits of detection and quantification were evaluated based on the comparison of standard deviation of the peak area and the slope of calibration curve of Paracetamol and Ibuprofen. However, the equations used for the LOD and LOQ were 3σ/S and 10σ/S, respectively. LOD for Paracetamol and Ibuprofen were 0.84 ppm and 0.52 ppm, respectively; the LOQ were 2.81 ppm and 1.73 ppm, respectively.

Robustness:
The analytical method validation parameter of robustness was carried out by several changes in flow (±0.01mL/min), mobile phase proportions (±2.0), the pH of the mobile phase (±0.01), wavelength (±3.0 nm), column temperature (±2.0ºC) and different column lot. There was no significance change observed in % assay and retention time. Furthermore it was confirmed by the variations in theoretical plates, asymmetry and % RSD. Results derived from the study were within the criteria of acceptance. So the method is highly robust.

System suitability:
System suitability study was verified by measurement of peak asymmetry (A < 2.0), resolution (Rs > 3.0) and number of theoretical plates (N > 1500) after chromatography of standard solution. The values of these properties were in accordance with in-house limits (table 3).

Table 3: System suitability results

Sr. No	Parameter	Paracetamol	Ibuprofen
1	Tailing factor (T_f)	1.786	1.449
2	Resolution (R_s)	5.388	
3	Retention time (Min)	1.091	1.882
4	Theoretical plates (N)	1573	2541

CONCLUSION

There are several methods were reported for the determination of Paracetamol and Ibuprofen, but there is no any RP-UPLC method revealed, so the recent work is useful for stability indicating, simultaneous determination of Paracetamol and Ibuprofen in combined dosage form. It is a unique isocratic RP-UPLC method with PDA detector. Both the drugs show the higher response and area of peak at the 225 nm of wavelength with shorter run time. This method gives higher and speedy throughput analysis. Stress degradation studies were carried out by conditions such as acidic, alkaline, oxidative, thermal and photolytic for specificity. By the study of validation parameters, it is confirmed that the results obtained from this analytical method by UPLC- PDA are reliable, within the acceptance criteria and the proposed method is immensely robust as well as rugged as per the ICH guideline.

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