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Research Article

Development and validation of a RP-HPLC for simultaneous determination of Ibuprofen and Paracetamol in solid dosage forms: Application to dissolution studies

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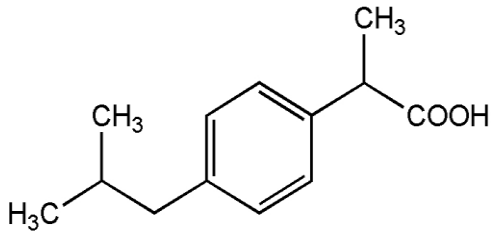
ABSTRACT

A reverse phase high performance liquid chromatographic (RP-HPLC) method suitable for simultaneous determination of Ibuprofen and Paracetamol in dissolution of solid dosage forms in pharmaceuticals has been developed. Chromatographic separation was performed on a Waters-Spherisorb C_8 (250 mm × 4.6mm, 5µm) column using a mobile phase of buffer (0.045M phosphoric acid, apparent pH adjusted to 4.0 ±0.2 with triethylamine) and acetonitrile in the ration of 30:70, *v/v* respectively. The effluent flow rate monitored at 1.0mL/minute, injection volume was 20µL and detected by ultraviolet at 225nm. The retention times of Ibuprofen and Paracetamol 2.7 and 3.8minutes, respectively. The total run time was 6minutes within which the drug product. The developed method has been validated for specificity, precision, linearity, accuracy, ruggedness and robustness. Additionally, the conditions of the dissolution test for Ibuprofen and Paracetamol tablets were presented by using: paddle at 150rpm stirring speed; medium volume of 900mL; temperature at 37±0.5°C; and pH 7.2 phosphate buffer used as dissolution medium. The average percentage drug release was found to be in between 95% to 105% within 30minutes for both drugs. The proposed analytical and dissolution method can be applied successfully for the quality control of commercial Ibuprofen and Paracetamol tablets and the comparison of in vitro dissolution of combination drug products.

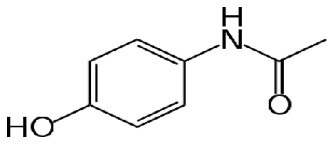
Keywords: Ibuprofen; Paracetamol; Dissolution test; Method development; validation.

1. INTRODUCTION

The systematic name of the Ibuprofen is - (RS)-2-(4-(2-methyl propyl) phenyl) propanoic acid (Fig. 1a). The molecular formula is $C_{13}H_{18}O_2$ and molecular weight is 206.29g/mol. Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID). It is used for relief of symptoms of arthritis, primary dysmenorrhea, fever and as an analgesic, especially where there is an inflammatory component. Ibuprofen is known to have an anti-platelet effect, though it is relatively mild and short-lived anti-platelet drug.



(a) Ibuprofen



(b) Paracetamol

Figure 1: Chemical structures of (a) Ibuprofen and (b) Paracetamol

The systematic name of the Paracetamol is N-(4-hydroxyphenyl) acetamide (Fig. 1b). The molecular formula is $C_8H_9NO_2$ and molecular weight is 151.17g/mol. The common name of Paracetamol is Acetaminophen. It is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies.

Literature survey reveals that few HPLC and UV Spectroscopic methods were reported for the estimation of Ibuprofen and Paracetamol individually as in pharmaceutical formulations (L. E.Briand et al, Maitreyi Zaveri et al, Dimitris Laitmer et al, A.J.Vyas et al, Yalcin Ozkan et al, Shaikh KA et al, Arunadevi S. Birajdar et al, Ramesh Sawant et al). Only one HPLC method has been reported for estimation of Ibuprofen and Paracetamol assay (Prasanna Reddy Battu et al). The review of the literature revealed that there is no RP-HPLC method

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available for determination of dissolution of this combination. Therefore aim of the present work was to develop simple, precise, accurate, linear, rugged and robust RP-HPLC method for simultaneous determination of Ibuprofen and Paracetamol in pharmaceutical dosage form and application of the method for dissolution study. The method was validated according to ICH guidelines (ICH-Q2 (R1)).

This paper describes a method for simultaneous determination of Ibuprofen and Paracetamol drugs in dissolution test of tablets. The procedure based on the use of reverse phase high performance liquid chromatography is simple, rapid and provides accurate and precise results.

2. EXPERIMENTAL

2.1. Materials

Ibuprofen and Paracetamol working standards and tablets were manufactured by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade acetonitrile, analytical grade potassium dihydrogen phosphate, sodium hydroxide, triethylamine and ortho phosphoric acid were purchased from Merck, Mumbai; HPLC grade water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

2.2. Equipment

The dissolution test carried out by using DISSO2000 model of LABINDIA dissolution apparatus. Agilent 1200 series integrated high performance liquid chromatographic system was used for this experiment. Agilent 1200 series system equipped with Agilent 1200 series quaternary pump, Agilent 1200 series auto sampler, Agilent 1200 series variable wavelength detector, Agilent 1200 series Column thermostat and controlled by Empower2 software.

2.3. Chromatographic conditions

The chromatographic separation was achieved on a Waters-Spherisorb C8 (250 mm × 4.6mm i.d. with 5µm) column using a mobile phase of buffer (0.045M phosphoric acid, apparent pH adjusted to 4.0 ± 0.2 with triethylamine) and acetonitrile in the ratio of 30:70, *v/v* respectively. The effluent flow rate monitored at 1.0mL/min, injection volume was 20µL and detected by ultraviolet detector at 225nm. The column temperature was maintained at 35ºC and the retention times of Ibuprofen, Paracetamol were found 2.7 and 3.7min, respectively. The total run time was 6minutes within which the drug product (Fig. 2).

Figure.2: A typical HPLC chromatogram of *Ibuprofen and Paracetamol* (*Fig.3, Fig.4, Fig.5, Fig.6)* blank, finished product, placebo preparation and all peaks overlaid.

2.4. Preparation of standard solution

A stock solution of Ibuprofen and Paracetamol dissolution standard (1.0mg/mL of Ibuprofen and 0.9mg/mL of Paracetamol) was prepared by dissolving an appropriate amount in pH 7.2 phosphate buffer and along with addition of 2mL of methanol. Final working standard (0.1mg/mL of Ibuprofen and 0.09mg/mL of Paracetamol) was prepared from above stock solution in pH 7.2 phosphate buffer for dissolution test of tablets of Ibuprofen and Paracetamol.

2.5. Preparation of dissolution medium

Dissolved 6.8g of mono basic potassium phosphate in 200mL of water and added 173.5mL of 0.2M sodium hydroxide and diluted with water to 1000mL.

2.6. Degassing of the dissolution medium

Heated the dissolution medium to about 41°C, immediately filtered under vacuum using a filter having of 0.45µm size, with vigorous stirring and continued stirring under vacuum for 5min.

2.7. Bath preparation

150rpm speed of the motor, the constant temperature bath at 37°C. Placed 900mL volume of dissolution medium in each of six vessels of dissolution apparatus, which previously have been immersed in the constant temperature bath, and allowed the medium to come to a temperature of 37.0 ± 0.5°C.

2.8. Dissolution test of tablets

The dissolution study was performed following the USP, Apparatus 2 (paddle) method (LABINDIA DISSO2000, USA).The paddle speed and bath temperature were set to 150rpm and 37±0.5°C, respectively. Each test was carried out in 900mL of pH 7.2 phosphate buffer as dissolution medium for the sink condition. Accurately weighed tablets containing the equivalent of 400mg of Ibuprofen and 325mg of Paracetamol were placed in the dissolution medium. Then, 10mL aliquot samples were withdrawn at the specified time (i.e.30min) and were filtered using 0.4µm Nylon 66 syringe filters. Equal volumes (20µL) of these solutions were injected into the chromatograph by auto sampler and peak areas were measured.

2.9. Sample preparation

Transferred 5mL above sample solution into 20mL of volumetric flask and diluted upto volume with pH 7.2 phosphate buffer (0.111mg/mL of Ibuprofen and 0.0902 mg/mL of Paracetamol). This solutions were filtered using 0.45µm (Nylon 66- membrane) filter and injected into liquid chromatographic system.

3. RESULTS AND DISCUSSION

3.1. Method development and optimization of assay dissolution test method

The method was optimized by using RP-HLC UV detector. The main target of the RP-HPLC method is to get the simultaneous determination of Ibuprofen and

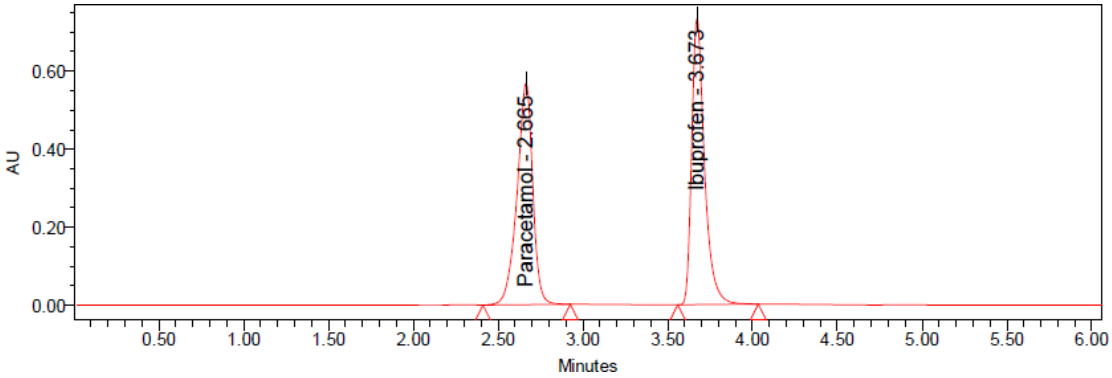

Figure 2: A typical chromatogram of Paracetamol and Ibuprofen

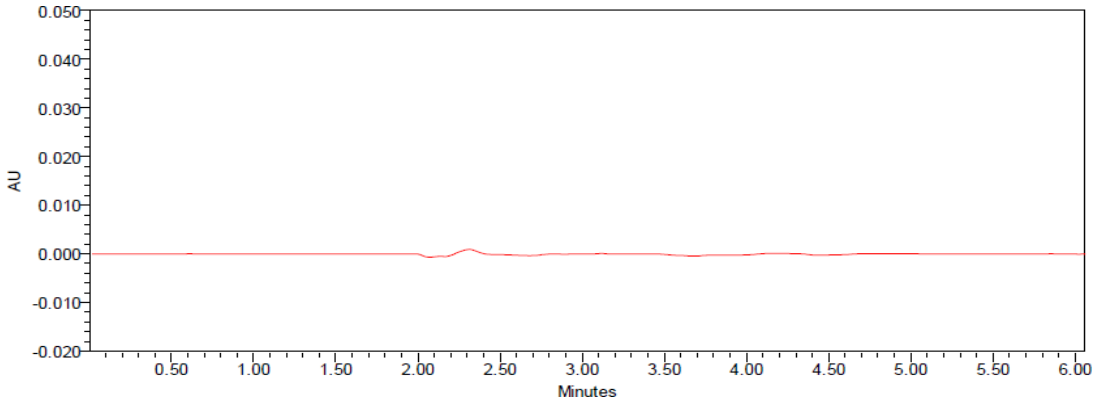

Figure 3: A typical chromatogram of blank

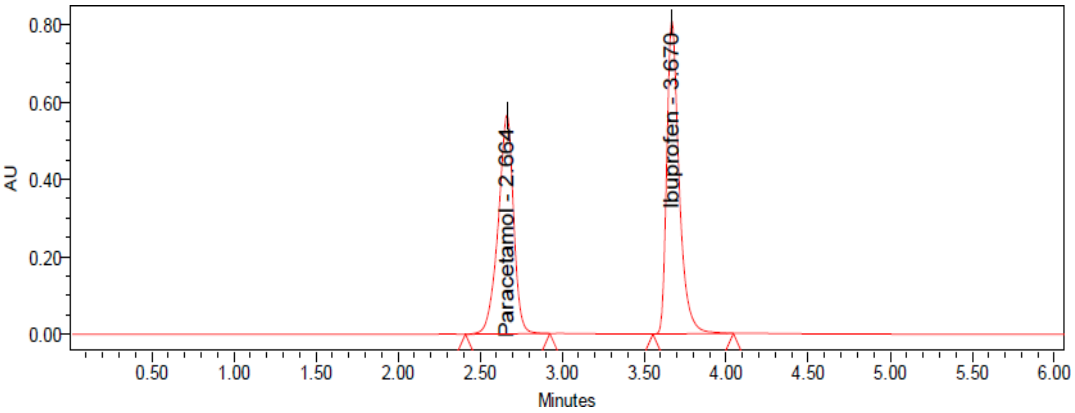

Figure 4: A typical chromatogram of finished product

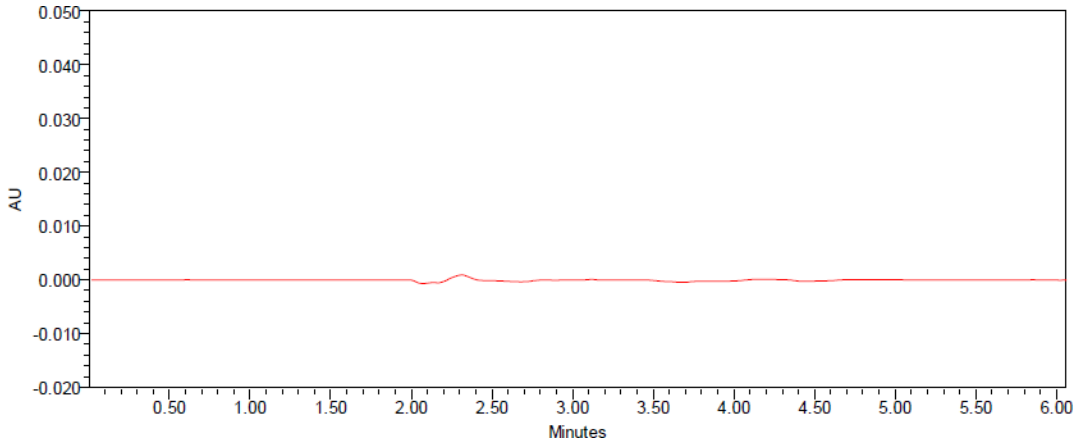

Figure 5: A typical chromatogram of placebo of finished product

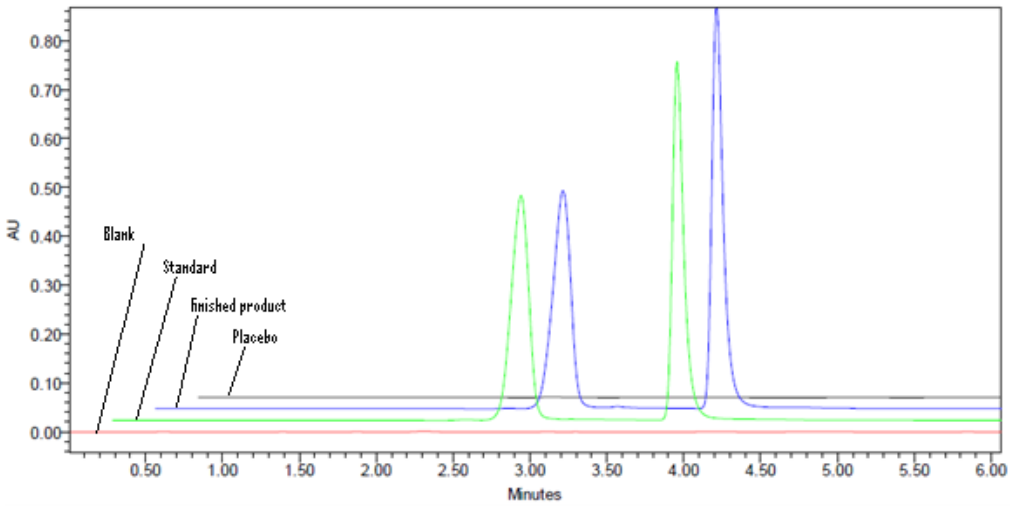

Figure 6: A typical chromatogram all peaks overlaid

Paracetamol in dissolution test of the formulation of solid dosage forms. The samples were run using different stationary phases like C_{18}, C_8, and mobile phases containing different buffers like phosphate with different pH (2-7.5) and using organic modifiers like acetonitrile and methanol in the mobile phase. But the separation was satisfactory in the adopted chromatographic conditions only. This indicates that the isocratic with 70% acetonitrile as organic modifier in mobile phase was successful in the simultaneous determination of Ibuprofen and Paracetamol in dissolution test of solid dosage forms in pharmaceuticals.

3.2. Method validation

Method validation was performed as per ICH guidance for simultaneous determination of Ibuprofen and Paracetamol in tablets of pharmaceutical formulations. The following validation characteristics were addressed: system suitability, system precision, linearity, precision, accuracy, robustness, ruggedness and specificity.

3.2.1. System suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: tailing factor, theoretical plates and resolution. The system suitability method acceptance criteria set in each validation were: tailing factor ≤ 2.0, theoretical plates > 2500 for both peaks and >2.5 resolution in between Paracetamol and Ibuprofen peaks (table.1).

3.2.2. System precision

In the all cases, the relative standard deviation (R.S.D) for the analyte peak area for five consecutive injections was < 2.0%. A chromatogram obtained from the standard solution is presented in Figure 2.

3.2.3. Precision (inter-day precision and intra-day precision)

The method precision was demonstrated by the dissolution of assay (drug release) of series of six samples, prepared as described in the dissolution test, on two

Table 1: System suitability results

Conditions: 1.0mL/min, pH 4.0, O.P 100%, Temp-35°C	Resolution	Tailing factor		Plate counts		%R.S.D	
		Para	Ibup	Para	Ibup	Para	Ibup
	8.9	1.0	1.4	4099	13616	0.1	0.9

Para = Paracetamol; Ibup = Ibuprofen

Table 2: Precision results of intraday and inter day

S. No.	Intraday (%drug release)		Inter day (%drug release)	
	Paracetamol	Ibuprofen	Paracetamol	Ibuprofen
1.	99	100	99	100
2.	97	100	99	101
3.	98	101	99	101
4.	98	101	99	101
5.	99	101	99	101
6.	98	101	99	100
Means	98	101	99	101
%R.S.D	0.5	0.6	0.0	0.6

Table 3: Linearity results of Paracetamol and Ibuprofen

Conc. in %	Ibuprofen		Paracetamol	
	Conc. in µg/mL	Area	Conc. in 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
Correlation coefficient (r)		0.999	0.999	
Slope (m)		38998	39395	
Intercept (c)		51536	24917	
Bias at 100% level		1.1	0.6	

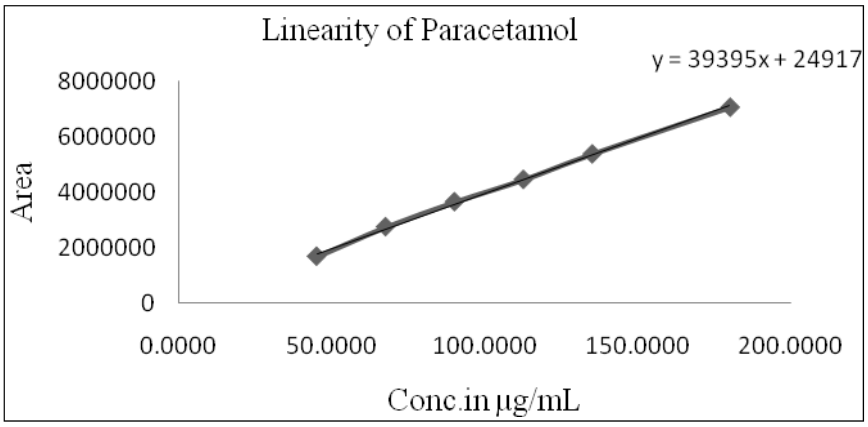

Figure 7: Linearity graph of Paracetamol

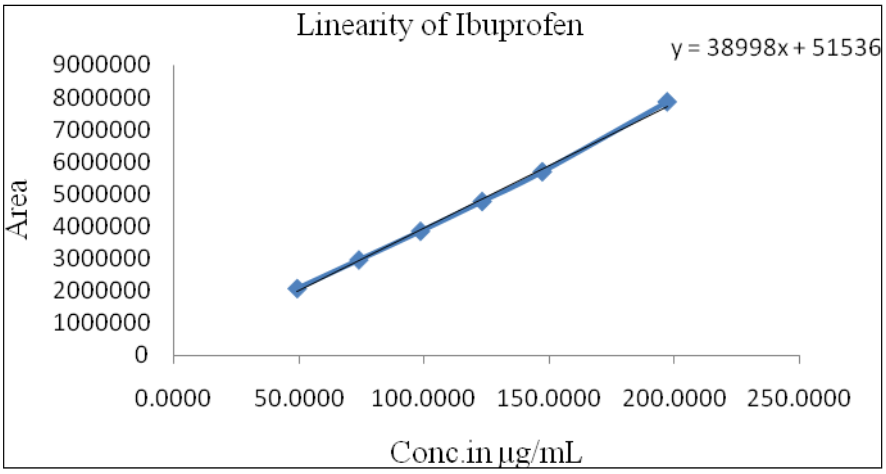

Figure 8: Linearity graph of Ibuprofen

consecutive days. The inter and intraday means and relative standard deviation were calculated (table.2).

3.2.4. Linearity

Standard curves were constructed using seven standard concentrations in a range of 45.0549µg/mL to 180.2196µg/mL for Paracetamol and 49.2450µg/mL to 196.9800µg/mL for Ibuprofen. This concentration range corresponds to levels of 50% to 200% of target concentration of Paracetamol and Ibuprofen. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression demonstrated by linear regression equation was y = *m x + c*, where 'y' is the peak area ratio of Ibuprofen and Paracetamol, 'm' *is* the slope, 'c' is the intercept and 'x' is concentration of the measured solution in µg mL^{-1} , respectively. The results show that an excellent correlation existed between the peak area and concentration of the analyte (table.3).

3.2.4. Accuracy

The accuracy of the test method was evaluated by simultaneous determination of recovery of Ibuprofen and Paracetamol in dissolution test of tablets on five concentration levels (i.e.50% to 125%) of target concentration. The excellent individual recoveries were

Table 4: Accuracy results of Paracetamol and Ibuprofen

%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
125	113.7182	111.8761	98.4	137.8037	136.4500	99.0

Table 5: Robustness study results

Condition	Resolution	Tailing factor		Plate counts		% R.S.D	
		Para	Ibup	Para	Ibup	Para	Ibup
Flow rate in mL/min (±0.2)							
0.8mL/min	8.8	1.0	1.5	3952	14679	0.1	0.8
1.2mL/min	7.3	1.0	1.4	8095	3658	01	0.3
pH of buffer (±0.2)							
pH 3.8	8.1	0.9	1.4	4595	12865	0.0	0.6
pH 4.2	6.9	0.9	1.5	4020	12772	0.1	0.2
Organic phase in mobile phase (±10%)							
O.P-90%	7.9	0.9	1.4	4388	12293	0.1	1.1
O.P-110%	7.4	0.9	1.3	3512	13968	0.1	0.7
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

Para = Paracetamol; Ibup = Ibuprofen

made at each added concentration level of Ibuprofen and Paracetamol. The mean recoveries were found in the range of 95.0% to 105.0% (table.4).

3.2.5. Solution stability and mobile phase stability

The solution stability of dissolution test of Ibuprofen and Paracetamol tablets have been carried out by leaving the test solution in tightly capped volumetric flask at room temperature and as well as refrigerator for 48 hours. The mobile phase stability was also carried out by assaying the freshly prepared standard solution for 6hours interval upto 48hours. The mobile phase preparation was kept constant during the study period. The percentage of R.S.D of assay of Ibuprofen and Paracetamol were calculated and found < 2.0% for the study period during mobile phase and solution stability experiments.

3.2.6. Specificity and selectivity

The specificity of test method was established by injecting placebo preparations of Ibuprofen and Paracetamol tablets without main drugs in the formulation. No, interference found due to ingredients which were used in the formulation, this indicates that excipients did not interfere with main analytes in tablets of the pharmaceutical formulation. Figure 4 shows that the method was sufficiently specific to the drug.

Intermediate precision was performed to confirm that separation of Ibuprofen and Paracetamol satisfactory under conditions mentioned above. The system suitability parameters were within the limits; this indicates that the method remains selective under tested conditions.

3.2.7. Ruggedness and Robustness

The ruggedness of test method was determined by using different instruments, different days, different columns and different HPLC (Agilent 1200 series) systems. Typical variations in the liquid chromatography conditions were used to evaluate the robustness of the assay of dissolution test method. In this study, the chromatographic parameters were monitored as follows; retention time, tailing factor, theoretical plates and resolution. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

The effect of variation in mobile phase buffer pH was studied at pH 3.8 and pH 4.2 instead of pH 4.0. Similarly, the effect of variation in mobile phase composition was changed by 10% organic phase in mobile phase to 90% (63) and 110% (77) instead of buffer (pH 4.0)-acetonitrile 30:70, (*v*/*v*) respectively. The effect of variation in column temperature was studied at 30°C and 40°C instead of 35°C. The mobile phase flow rate was 1.0mL min−1. This was changed by 0.2 units to 0.8 and 1.2mL min−1 and the effect was studied. The filter validation has been studied and similarity factor of standards versus tests of Ibuprofen and Paracetamol were found in the range of 0.98 to 1.02 (table.5).

4. CONCLUSIONS

The reverse phase high performance liquid chromatography method proved to be simple, linear, precise, accurate, robust, rugged and specific. The total runtime was 6min within which the drugs and their formulation products were separated. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be used for simultaneous determination Ibuprofen and Paracetamol in dissolution test of the formulation.

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