

# Simultaneous, Stability Indicating Method Development and Validation for Related Compounds of Ibuprofen and Paracetamol Tablets by RP-HPLC Method

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## Abstract

A simple, precise, accurate, simultaneous and stability-indicating RPLC method developed with an effective resolution for active pharmaceutical ingredients and marketed drug products. This method effectively separate all the related substances of Ibuprofen and Paracetamol along with impurities. This method is using in the estimation assay of Ibuprofen and paracetamol in drug substance also. The method was developed using RP18 Embedded polar phase column. A mobile phase used in this method was a mixture of acetonitrile and 0.1% v/v orthophosphoric acid in 55:45 v/v ratio. At 230 nm compounds will eluted and monitored. Ibuprofen and Paracetamol was subjected to the stress conditions of acid, base, oxidative, thermal and photolytic degradation. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating ability of the method. The developed method was validated as per USP and International Conference on Harmonization (ICH) guidelines. The current method has proven good linearity and accuracy over the range of all known impurities from LOQ to 150% of the target concentration. The degree of reproducibility as results obtained by deliberate changes in the method parameter and variety of condition has proven the method is robust and rugged.

**Keywords:** HPLC; Ibuprofen and Paracetamol; Validation; Reverse phase mode; Stability-indicating

## Introduction

Ibuprofen is ( $\pm$ ) - 2 - (p - isobutylphenyl) propionic acid [1] (Figure 1). It is a white powder and it is having a melting point of 74-77°C. It is very slightly soluble in < 1 mg/mL water and readily soluble in organic solvents like ethanol and acetone. It is under classification of nonsteroidal anti-inflammatory drug (NSAID) used for relief of symptoms of arthritis, fever as an analgesic (pain reliever), especially where there is an inflammatory component, and dysmenorrhea [2]. Nonsteroidal anti-inflammatory drugs such as ibuprofen work by inhibiting the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>). PGH<sub>2</sub>, in turn, is converted by other enzymes to several other prostaglandins (which

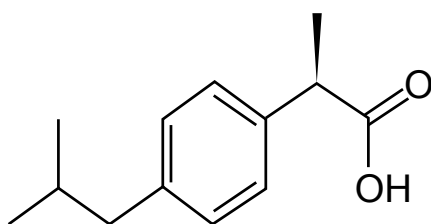


Figure 1: Structure of Ibuprofen.

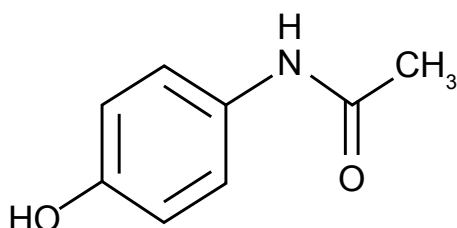


Figure 2: Structure of Paracetamol.

are mediators of pain, inflammation, and fever) and to thromboxane A<sub>2</sub> (which stimulates platelet aggregation, leading to the formation of blood clots) [3].

Paracetamol or Acetaminophen [4] is used as pain reliever and antipyretic fever reducer. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies [5] (Figure 2). The main mechanism proposed is the inhibition of cyclooxygenase (COX) and it is highly selective for COX-2. It is having Analgesic and antipyretic properties like NSAIDs. If inflammatory lesions have high level of peroxides that paracetamol shows limited anti-inflammatory property [6].

There are plenty of validated simultaneous analytical methods available for combinational dosage form of Ibuprofen and paracetamol assay method. But there is not much literature available for simultaneous related substance for combinational dosage form. In USP, related substance method available for Ibuprofen, Paracetamol and its various formulations for single conventional dosage form not for combination [1,4]. RP-HPLC assay method for Paracetamol and Ibuprofen tablets [7]. RP-HPLC assay method for Paracetamol, Ibuprofen and chlorzoxazone tablets [8]. Ibuprofen and Paracetamol determination by HPLC in soft capsule [9]. Paracetamol and its process related substance determination and rapid separation by using RP-

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HPLC with PDA detector [10]. Ibuprofen degradants was studied by oxidative and thermal treatments [11]. There are many studies available but there is no literature available simultaneous stability indicating method for the combined formulation. So the current work presents simultaneous stability indicating reverse phase HPLC method with PDA detection. Validation of the method was performed according to the requirements of United States Pharmacopeia and ICH guidelines for related substance determination, which includes accuracy, precision (repeatability and intermediate precision, ruggedness), selectivity, robustness, linearity and range. Additionally, in order to meet the regulatory guidance of the Federal Drug Administration/International Conference on Harmonization (ICH) [12-15] stability of mobile phase was established, standard, resolution solution and sample solution was injected freshly prepared solution hence instability of impurity solution. Degradation study was performed for active pharmaceutical ingredient, placebo and drug products in acid, base, peroxide, thermal, humidity, and water including photo stability.

## Experimental

### Chemicals/Standards/Impurities

Acetonitrile HPLC grade was from Merck. Orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were from Merck. Standards and impurities include Ibuprofen, Ibuprofen Impurity-B, Paracetamol, 4-aminophenol and 4-chloroacetanilide. All impurities procured from Sigma-Aldrich grade.

### Apparatus

HPLC system (Waters system, USA) with a detector (PDA-G06296), equipped with a quaternary pump, auto sampler (F06SM4854A), column compartment (H03SMH 052 M) and empower software was employed during this study. The analysis was performed with 55 volumes 0.1% orthophosphoric acid and 45 volumes of acetonitrile in isocratic mode at the flow rate of 1.0mL/minutes through RP18 embedded polar stationary phase, 20 µL for about 60 minutes with the support of UV/PDA detection at 230 nm and the column compartment maintained at 25°C.

### Standard solutions and HPLC conditions

0.1% v/v orthophosphoric acid was prepared by dissolving 1.0mL of orthophosphoric acid dissolved in 1000 mL of water. 55 volumes of 0.1v/v orthophosphoric acid and 45 volumes of acetonitrile as a mobile phase this is degassed and filtered through 0.45 µ filter. Standard solution of paracetamol, 4-amino phenol, 4-chloroacetanilide, ibuprofen and ibuprofen impurity-B was prepared by using 45 volumes of 0.1% orthophosphoric acid and 55 volumes of acetonitrile as a diluent at the concentration of about 20 µg/mL.

### Sample solution

Sample solution was prepared by using diluent and the final concentration of about 20 mg/mL. 20 µL of standard and sample solution was analyzed with bracketing standard to maintain system suitability for each sequence.

## Results and Discussion

### Method development

Preliminary studies involved trying C8, C18, C18 embedded phase and Zorbax reversed-phase columns and testing some mobile phase compositions were conducted for the separation of paracetamol and ibuprofen with good resolution of its impurities. A RP18 column (5

µm, 250 mm, 4.6 mm id.) as a stationary phase with a mobile phase of acetonitrile/0.1%v/v orthophosphoric acid (45:55 v/v) at the flow of 1.0 mL/min and a detection wavelength of 230 nm afforded the best separation of paracetamol, ibuprofen and its impurities.

### Method validation

After method development, validation of the current test method for ibuprofen and paracetamol tablets was performed in accordance with United States Pharmacopeia requirements/ICH guidelines for related substance method the parameter includes precision, accuracy, linearity, LOD and LOQ, precision and accuracy at LOQ level, selectivity, specificity includes blank, placebo, known impurity interference and interference of degradants by degradation study. Robustness and ruggedness was also performed.

### System suitability

20 µL of standard solution six times injected into HPLC and recorded the chromatogram, % RSD of paracetamol, ibuprofen and its impurities peaks area was within the limit of 5.0% and resolution between paracetamol and 4-aminophenol, ibuprofen and ibuprofen impurity-B was not more than 2.0 for the entire activity.

### Precision

To evaluate precision 0.2% of impurity blend has been spiked in the sample preparation and analyzed and recorded the chromatogram, calculated percent RSD for the percent impurity of each individual impurity. The percentage impurity was found for 4-amino phenol, 4-chloro acetanilide, ibuprofen impurity-B and ibuprofen impurity-J is 0.195 to 0.207, 0.191 to 0.220, 0.190 to 0.215 and 0.198 to 0.219 and percent RSD of six replicate sample preparations was found 2.4, 4.9, 4.9 and 4.1 respectively. The results summarized in table 1 and blank, placebo, standard solution and impurity spiked sample preparation chromatogram shown in figures 3-6.

### Intermediate precision

To evaluate intermediate precision, precision experiment was done

% Impurity				
Paracetamol		Ibuprofen		
4-Amino phenol	4-Chloro acetanilide	Impurity-B	Impurity-J	
0.202	0.212	0.190	0.198	
0.205	0.204	0.195	0.199	
0.195	0.220	0.201	0.211	
0.204	0.199	0.215	0.205	
0.196	0.191	0.204	0.214	
0.207	0.204	0.214	0.219	
% RSD	2.4	4.9	4.9	4.1

Table 1: Precision.

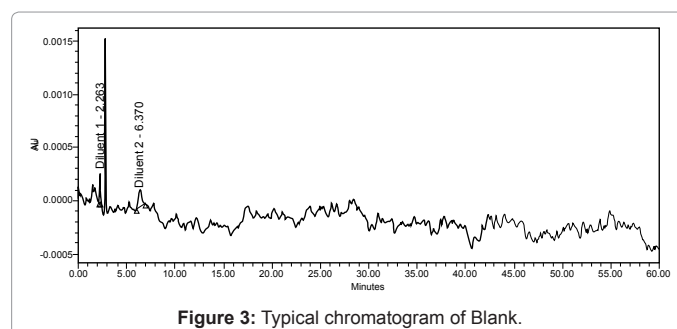


Figure 3: Typical chromatogram of Blank.

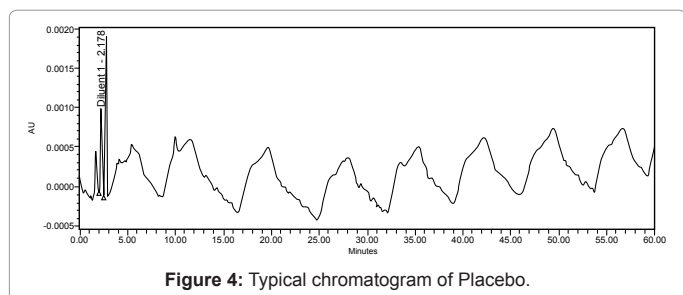


Figure 4: Typical chromatogram of Placebo.

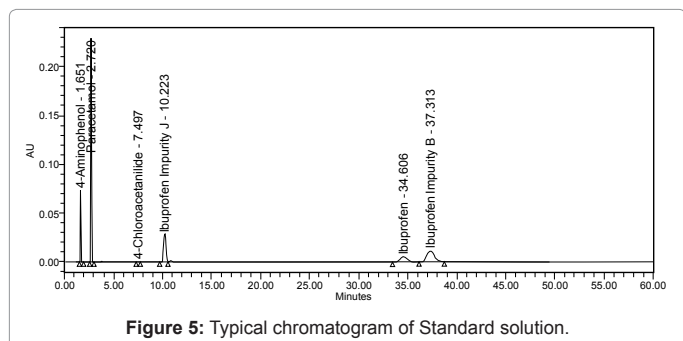


Figure 5: Typical chromatogram of Standard solution.

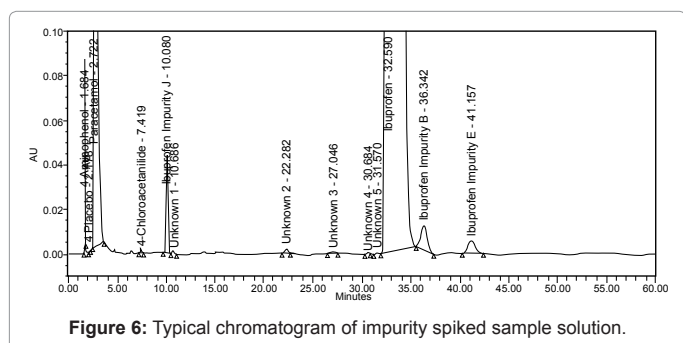


Figure 6: Typical chromatogram of impurity spiked sample solution.

% Impurity				
Paracetamol		Ibuprofen		
4-Amino phenol	4-Chloro acetanilide	Impurity-B	Impurity-J	
	0.212	0.202	0.199	0.199
	0.207	0.214	0.185	0.201
	0.201	0.201	0.211	0.214
	0.211	0.191	0.205	0.201
	0.190	0.199	0.201	0.210
	0.211	0.214	0.210	0.211
% RSD	4.2	4.4	4.7	3.1

Table 2: Intermediate Precision.

by different analyst, day, HPLC system, and column and recorded the chromatogram, calculated percent RSD for the percent impurity of each individual impurity. The percentage impurity was found for 4-amino phenol, 4-chloro acetanilide, ibuprofen impurity-B and ibuprofen impurity-J is 0.190 to 0.211, 0.191 to 0.214, 0.185 to 0.211 and 0.199 to 0.214 and percent RSD of six replicate sample preparations was found 4.2, 4.4, 4.7 and 3.1 respectively. The results summarized in table 2.

### Linearity

To evaluate linearity of the method, six levels calibration curve made includes LOQ level. Signal to noise ratio was observed about 10 for the concentration between 0.05 to 0.3 µg/mL for ibuprofen, paracetamol and its impurities. Hence linearity was established LOQ to 75 µg/mL

for ibuprofen, paracetamol and all the impurities. A plot of peak areas versus concentration was linear in the range from 0.05 to 0.75 µg/mL. The correlation coefficient (R) of 4-aminophenol, 4-chloroacetanilide, paracetamol, ibuprofen impurity - J, B, and ibuprofen was found 0.9986, 0.9983, 0.9991, 0.9963, 0.9991 and 0.9985 respectively. The results are summarized in table 3 and overall linearity graph for ibuprofen, paracetamol and its impurities was shown in figure 7.

### Accuracy

Accuracy of the method was studied for three levels from 50% to 150% by spiking 0.1% for 50% level from the target concentration of paracetamol impurities and 0.2%, 0.3% for 100%, 150% level from the target concentration of ibuprofen impurities spiked in sample preparation and analyzed with unspiked sample preparation, recorded the chromatogram. Six preparation for 50%, 150% level and triplicate preparation of median level concentration were done. Recovery was found 90.2% to 108.2% and RSD was found 2.4% to 8.1% is lowest and highest value. Results are summarized in table 4.

### Precision and accuracy at LOQ level

Precision and accuracy of the method was studied for the level

Concentration µg/mL	Ibuprofen, Paracetamol and its impurities (Area)					
	4-Amino phenol	4-Chloroacetanilide	Paracetamol	Imp - J	Imp - B	Ibuprofen
0.051	738	3062	3231	1179	870	1141
2.121	35695	127345	124388	49035	36174	47470
10.214	137817	613249	667165	246133	164202	228601
25.324	356489	1520453	1624544	595455	481906	586781
50.241	737088	3316470	3473300	1361502	856871	1224452
75.014	1185603	4523841	4862932	1784219	1279380	1678901
R	0.9986	0.9983	0.9991	0.9963	0.9991	0.9985

Table 3: Linearity.

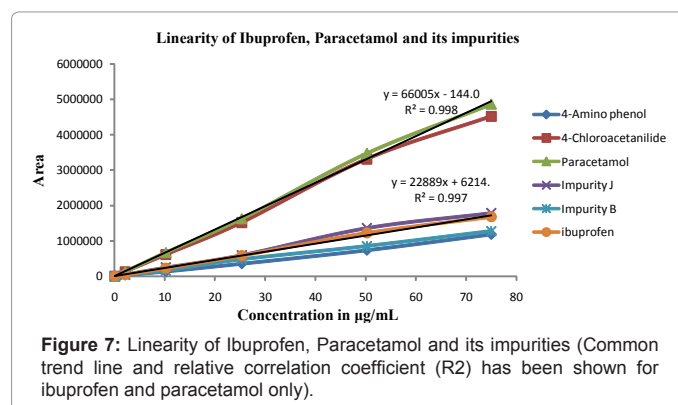


Figure 7: Linearity of Ibuprofen, Paracetamol and its impurities (Common trend line and relative correlation coefficient (R2) has been shown for ibuprofen and paracetamol only).

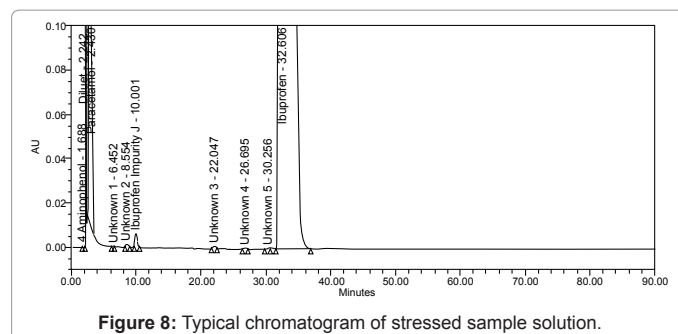


Figure 8: Typical chromatogram of stressed sample solution.

Levels	% Impurity			
	Paracetamol		Ibuprofen	
	4-Amino phenol	4-Chloro acetanilide	Impurity-B	Impurity-J
50%	97.4	90.2	95.2	99.2
	95.6	96.8	90.2	93.2
	106.2	107.5	107.2	95.6
	108.2	103.5	105.3	100.2
	102.5	103.5	102.5	103.5
	90.2	108.2	107.5	108.2
<b>% RSD</b>	<b>6.8</b>	<b>6.8</b>	<b>7.0</b>	<b>5.4</b>
100%	99.6	96.8	95.6	96.2
	98.5	98.5	98.5	103.2
	102.3	103.2	100.2	105.2
<b>% RSD</b>	<b>2.0</b>	<b>3.3</b>	<b>2.4</b>	<b>4.7</b>
150%	96.5	90.2	91.2	93.2
	90.2	106.2	90.2	95.1
	106.2	107.2	103.5	106.2
	107.2	102.5	106.2	102.3
	103.2	107.3	107.8	107.2
	101.2	105.2	107.2	108.2
<b>% RSD</b>	<b>6.4</b>	<b>6.4</b>	<b>8.1</b>	<b>6.3</b>

Table 4: Accuracy.

	% Impurity & Amount recovered in µg/mL							
	Paracetamol				Ibuprofen			
	4-Amino phenol		4-Chloro acetanilide		Impurity-B		Impurity-J	
	A	P	A	P	A	P	A	P
LOQ	108.2	0.0541	109.2	0.0546	90.2	0.0451	107.8	0.0539
	106.2	0.0531	108.2	0.0541	91.3	0.0457	105.2	0.0526
	93.5	0.0468	101.2	0.0506	97.8	0.0489	90.2	0.0451
	98.2	0.0491	94.2	0.0471	102.3	0.0512	108.2	0.0541
	95.4	0.0477	93.5	0.0468	107.2	0.0536	102.9	0.0515
	101.2	0.0506	102.3	0.512	105.2	0.0526	106.2	0.0531
<b>% RSD</b>	<b>5.8</b>		<b>6.6</b>		<b>7.2</b>		<b>6.5</b>	

A-Accuracy; P-Precision (Amount recovered in µg/mL)

Table 5: Precision and Accuracy at LOQ level.

of LOQ at the concentration. The LOQ was found 0.05 to 0.3 µg/mL, henceforth precision and accuracy was performed by spiking all the impurities at the concentration of 0.05 µg/mL at six replicate samples prepared and analyzed, recorded the chromatogram. Percent recovery and amount recovered was found 90.2 to 109.2, 0.0451 µg/mL to 0.0546 µg/mL and RSD was found 5.8 and 7.2 is the lowest and highest value. Results are summarized in table 5.

### Range

Range of the method was proven by precision, linearity and accuracy experiment. Hence the data has been captured from section of precision, linearity and accuracy. Overall summary of the extreme range of the method and acceptance criteria was summarized in table 6.

### Selectivity (stability indicating evaluation)

Selectivity of the method was demonstrated by enhancing degradation of drug products under stress conditions acid, base, peroxide, 90% humidity, thermal, and UV light. Sample was analyzed and recorded the chromatogram up to 90 minutes. Degradation was found 0.5% to 5.3%, and the maximum degradation found in base degradation. Mass balance was satisfied from the degradation study report, the peak purity of ibuprofen and paracetamol was passing for all type of stressed samples. Detailed stress conditions and results are summarized in table 7 and chromatogram of stressed sample, peak

purity angle and threshold of paracetamol and ibuprofen was shown in figures 8-10.

### Robustness

Robustness of the current method was investigated by analyzing the standard solution and established system suitability with the deliberate variation of mobile phase organic variation, flow rate and column temperature at 10 percentage level from the original value. RSD of five replicate injections of standard solution was found below 5.0% for all

Parameter	Peak Name	Result	Acceptance Criteria
Linearity	4-Amino phenol	0.9986	R ≥ 0.995
	4-Chloroacetanilide	0.9983	
	Paracetamol	0.9991	
	Ibuprofen impurity-J	0.9963	
	Ibuprofen impurity-B	0.9991	
	Ibuprofen	0.9985	
Precision	4-Amino phenol	2.4	%RSD of 6 sample preparations NMT 15.0
	4-Chloroacetanilide	4.9	
	Ibuprofen impurity-B	4.9	
	Ibuprofen impurity-J	4.1	
Accuracy	4-Amino phenol	95.6% to 108.2	Recovery 85.0% to 115.0%
	4-Chloroacetanilide	90.2% to 108.2	
	Ibuprofen impurity-B	90.2% to 107.8	
	Ibuprofen impurity-J	93.2% to 108.2	

Table 6: Range.

Stress condition	% Assay	% Net degradation
6N Hydrochloric acid/Reflux 1 Hrs	95.4	4.6
6N Sodium hydroxide/Reflux 1 Hrs	94.7	5.3
6N Hydrogen peroxide/Reflux 1 Hrs	98.2	1.8
Water/Reflux 1 Hrs	99.0	1.0
90% Humidity/7 Days	99.5	0.5
Thermal 105°C/8 Hrs	99.3	0.7
UV light	99.2	0.8

Peak purity was passed for ibuprofen and paracetamol peak for all type of stressed samples.

Table 7: Selectivity & Specificity.

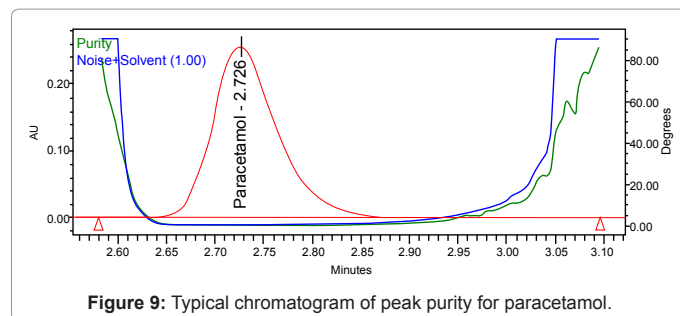


Figure 9: Typical chromatogram of peak purity for paracetamol.

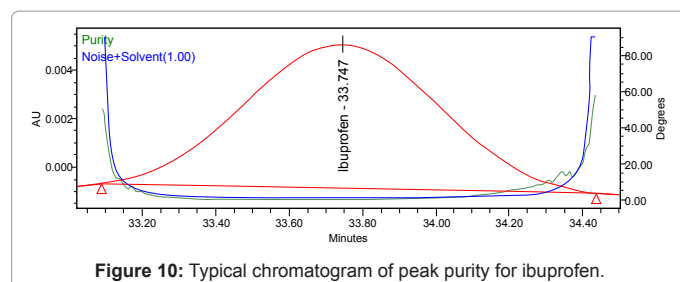


Figure 10: Typical chromatogram of peak purity for ibuprofen.

Chromatographic condition		Resolution of 4-aminophenol and Paracetamol	Resolution of Ibuprofen and Ibuprofen impurity B
Flow mL/Minutes	0.9	7.7	1.6
	1.0	7.9	1.7
	1.1	7.6	1.6
Column temperature(°C)	22	7.7	1.6
	25	7.9	1.7
	28	7.6	1.6
Mobile phase variation (%)	90	6.3	2.0
	100	7.9	1.7
	110	6.2	1.9

**Table 8:** Robustness.

the chromatographic condition and all peaks in standard solutions. Resolution between 4-aminophenol and paracetamol peak was found 6.2 to 7.9 and resolution between ibuprofen and Ibuprofen impurity B was found 1.6 to 2.0. The results are summarized in table 8.

## Conclusion

A simple, accurate, precise, simultaneous and stability-indicating RP-HPLC method was developed and validated for the routine analysis of related compounds of ibuprofen and paracetamol combined tablet formulation. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability-indicating.

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