

## STABILITY INDICATING RP-HPLC ASSAY OF HYDROCODONE AND IBUPROFEN IN TABLETS

RAMA KUMAR KANDULA<sup>1\*</sup>, RAJA SUNDARARAJAN<sup>1</sup>

<sup>1</sup>Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India 530045

Email: ramakumarkandula@gmail.com

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### ABSTRACT

**Objective:** The combination of hydrocodone and ibuprofen is prescribed to temporarily relieve severe pain. A new selective, precise and accurate stability indicating reverse-phase high-performance liquid chromatography (RP-HPLC) technique was developed for identification and estimation of hydrocodone and ibuprofen in tablets.

**Methods:** The analysis of hydrocodone and ibuprofen was executed on phase Zodiac C18 (250 × 4.6 mm, 5 μm) column with sodium dihydrogen phosphate (0.1M, pH5.0) and methanol (60:40, v: v) as a mobile phase with a flow rate of 1.0 ml/min. The detection and measurement were done at 234 nm. The stability of hydrocodone and ibuprofen was examined under different conditions recommended by ICH, including alkaline, acidic, neutral, oxidative, photolytic and thermal.

**Results:** Hydrocodone and ibuprofen are eluted at 4.019 min and 4.999 min, respectively. The assay method was linear (range: 3.75-11.25 μg/ml and 100-300 μg/ml) with  $R^2$  values of 0.9996 and 0.9999 for hydrocodone and ibuprofen, respectively. Method was accurate (recovery: 99.65% and 99.37% for hydrocodone and ibuprofen, respectively), precise (RSD: 0.262% and 0.261% for hydrocodone and ibuprofen, respectively), selective and robust. In degradation studies, peaks of degradants did not interfere with the peaks of hydrocodone and ibuprofen. This validated method was applied to assay the content of hydrocodone and ibuprofen in tablets.

**Conclusion:** This method can be opted in the routine quality control test of fixed-dose tablet combination comprising of hydrocodone and ibuprofen.

**Keywords:** Hydrocodone, Ibuprofen, Combination dosage form, Stress study, Analysis

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### INTRODUCTION

Hydrocodone, chemically known as (4R,4aR,7aR,12bS)-9-methoxy-3-methyl-1,2,4,4a,5,6,7a,13-octahydro-4,12-methanobenzofuro[3,2-e]isoquinolin-7-one (fig. 1), is a derivative of the baine or codeine with antitussive and analgesic effects [1-3]. As an antitussive agent, hydrocodone is used to relieve nonproductive cough symptoms. As an analgesic agent, it is used to relieve moderate or severe pain. In the central nervous system, hydrocodone activates primarily the  $\mu$ -opioid3 receptor which results in analgesia, respiratory depression, euphoria, physical dependence and cough suppression [4, 5].

Ibuprofen, chemically known as 2-[4-(2-methylpropyl) phenyl] propanoic acid (fig. 1), is a derivative of propionic acid belonging to the nonsteroidal anti-inflammatory class of drugs [6-8]. Ibuprofen has analgesic, anti-inflammatory and antipyretic activity. Ibuprofen is used to relieve pain from muscle aches, headache, arthritis, menstrual cramps and dental pain [9-11]. Also, ibuprofen is used to lessen fever. Ibuprofen, nonselectively, blocks the action of cyclooxygenase 1 and 2 enzymes [12, 13]. This results in reduced formation of prostaglandins (fever and pain mediators) and thromboxanes (blood clotting stimulators).

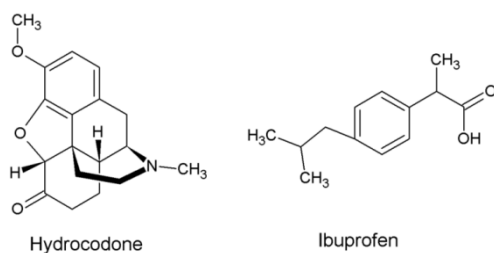


Fig. 1: Structures of drugs selected

The hydrocodone and ibuprofen combination is available as a tablet formulation with brand names Vicoprofen (hydrocodone 7.5 mg and

ibuprofen 200 mg), Ibudone (hydrocodone 5/10 mg and ibuprofen 200 mg) and Reprexain (hydrocodone 2.5/5/10 mg and ibuprofen 200 mg). Hydrocodone and ibuprofen combination medication is used to relieve short-term severe pain [14, 15]. This combination medication works by decreasing molecules which produce fever, pain and inflammation. No analytical technique was found in the literature review to quantify hydrocodone and ibuprofen simultaneously. Therefore, the aim of the current investigation was to develop a novel, rapid, accurate and sensitive stability indicating RP-HPLC method for the analysis of hydrocodone and ibuprofen simultaneously in pure and tablet formulations.

### MATERIALS AND METHODS

#### Instrumentation

Chromatographic assay of hydrocodone and ibuprofen was implemented on Waters HPLC Alliance 2695 model with quaternary pump, column thermostat, auto sampler and photodiode array detector (2998 model). Chromatographic data procurement and study were done using the Empower 2.0 version (Waters, USA).

#### Conditions

Chromatographic assay was done on a reversed phase Zodiac C18 (250 × 4.6 mm, 5 μm) column. The mobile phase, comprising of 0.1M sodium dihydrogen phosphate (pH 5.0, tuned with phosphoric acid) and methanol (60:40, v: v), was filtered via 0.45 μm membrane filter, degassed, and delivered with a flow rate of 1.0 ml/min. The sample injection volume and detection wavelength were optimized at 10 μl and 234 nm, respectively.

#### Requirements

Water for this HPLC assay was made by Millipore water purification system (Millipore, MA, USA). Reference standards of hydrocodone and ibuprofen were procured from Rainbow pharma training labs, Hyderabad, India. Vicoprofen (Abbvie Inc., USA) tablets with label claim 7.5 mg hydrocodone and 200 mg ibuprofen was procured from the provincial pharmacy store. Analytical grade chemicals (NaOH, HCl, H<sub>2</sub>O<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub> and phosphoric acid) are from Sd. Fine

Chemicals Ltd., Mumbai, India. HPLC grade solvent, methanol was obtained from Merck India Ltd, Mumbai, India.

### Standard solutions

Hydrocodone (7.5 mg) and ibuprofen (200 mg) reference standards were dissolved in the mobile phase to an ultimate concentration of 75 µg/ml hydrocodone and 2000 µg/ml ibuprofen (stock solution). Calibration solutions at five level concentrations (hydrocodone-3.75, 5.63, 7.5, 9.375, 11.25 µg/ml; ibuprofen-100, 150, 200, 250 and 300 µg/ml) were prepared in mobile phase with the above stock solution. Validation solution with a concentration of 7.5 µg/ml hydrocodone and ibuprofen 200 µg/ml was prepared in mobile phase with the above stock solution.

### Construction of linearity graph

To construct linearity graph, 5 distinct concentration solutions (hydrocodone-3.75, 5.63, 7.5, 9.375, 11.25 µg/ml; ibuprofen-100, 150, 200, 250 and 300 µg/ml) were prepared. These solutions were analyzed with the outlined HPLC conditions above. Hydrocodone and ibuprofen linearity graphs were made by using the area of peak of drug against corresponding drug concentrations. Regression analyzes were completed with the chromatographic data. The processed linearity graphs and regression equations were then used to quantify the unknown content of hydrocodone and ibuprofen.

### Extraction and assay of hydrocodone and ibuprofen combination in tablet

Weigh precisely homogenized tablet sample powder equal to 7.5 mg hydrocodone and 200 mg ibuprofen into a 100 ml standard flask. Added 50 ml of the mobile phase. Sonicated in ultrasonicator for 10 min to extract the drugs completely. Filter the solution using 0.45-micrometer membrane filter. Then, the volume was completed to 100 ml with mobile phase. This is a stock tablet solution with a concentration of 75 µg/ml hydrocodone and 2000 µg/ml ibuprofen. Test tablet solution was made by diluting 1 ml of stock tablet solution to 10 ml using a mobile phase. The final concentration achieved is 7.5 µg/ml hydrocodone and 200 µg/ml ibuprofen. Injected 10 µl of test tablet solution into the HPLC system. Peak areas of hydrocodone and ibuprofen were measured and contents of hydrocodone and ibuprofen in tablet were evaluated via regression equation or linearity graph.

### Forced degradation studies

Stability studies on hydrocodone and ibuprofen stability were conducted to demonstrate how the quality of hydrocodone and ibuprofen varies under the effect of a number of conditions like hydrolysis (with acid, base and water), oxidation (with hydrogen

peroxide), temperature and photo [16]. These studies also give information on specific conditions for the storage of drugs [17].

Tablet test solution (10 ml, 75 µg/ml hydrocodone and ibuprofen 2000 µg/ml) was exposed to 0.1 N hydrochloric acid (10 ml), 0.1N sodium hydroxide (10 ml), deionized water (10 ml) and 30% hydrogen peroxide solutions (10 ml) to assess the degree of degradation by acid, base, neutral and oxidation, respectively. Following sonication for 30 min at 27±2 °C, degraded samples were completed to 100 ml with the mobile phase (concentration: hydrocodone 7.5 µg/ml and ibuprofen 200 µg/ml). Samples are filtered with 0.45 micrometer membrane filter after degradation. Injected 10 µl of the degraded sample into the HPLC system. Peak areas of hydrocodone and ibuprofen were measured to assess the percent degradation. Tablet sample powder equal to 7.5 mg of hydrocodone and 200 mg of ibuprofen was exposed separately to 105 °C for 30 min in hot air oven and to sunlight, for 24 hr to evaluate the extent of thermal and photo degradation, respectively. After exposure, the samples solutions are prepared and analyzed following steps described in section "Extraction and assay of hydrocodone and ibuprofen combination in the tablet". The peaks of hydrocodone and ibuprofen were tested for peak purity in all the degraded samples.

### Method validation

The method's selectivity, linearity, sensitivity, specificity, precision, robustness and accuracy were validated in accordance with ICH rules [18, 19].

### RESULTS AND DISCUSSION

During the development of HPLC conditions to assay hydrocodone and ibuprofen simultaneously, different columns (Waters C18, Supelco C18, Thermo, C18 and Zodiac C18) and mobile phases (orthophosphoric acid: methanol, 0.1M potassium dihydrogen phosphate: methanol, 0.1M sodium dihydrogen phosphate: methanol) with different ratios were investigated to achieve good separated peak shapes, high sensitivity and apt running time. Hydrocodone and ibuprofen were eluted on Zodiac C18 (250 × 4.6 mm, 5 µm) column in an isocratic mode with sodium dihydrogen phosphate (0.1M, pH 5.0, 60%) and methanol (40%) as a mobile phase. The elution time for hydrocodone and ibuprofen was 4.019 min and 4.999 min, respectively. These conditions played a good role in achieving good peak symmetry, less retention time, acceptable resolution and high sensitivity. The maximum intensity of hydrocodone and ibuprofen peaks were observed at 234 nm. Therefore, 234 nm was chosen as the detection wavelength for quantifying hydrocodone and ibuprofen. The hydrocodone and ibuprofen chromatogram is presented in fig. 2.

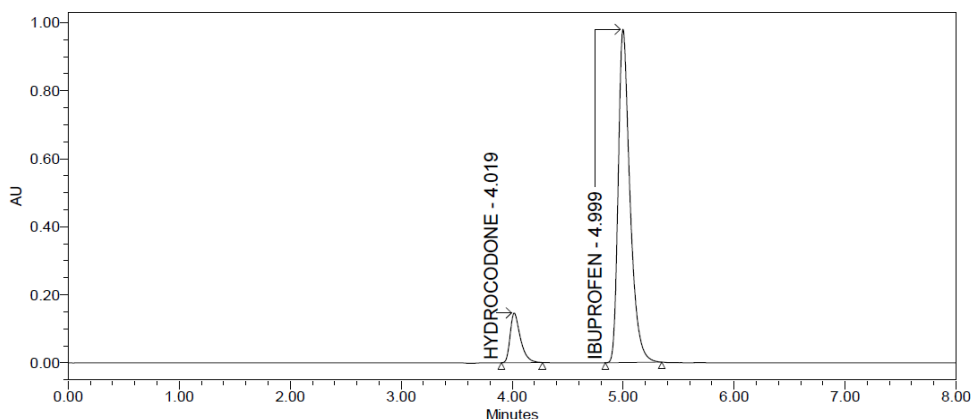


Fig. 2: Optimized chromatogram of hydrocodone and ibuprofen

### Method validation

#### Selectivity

During selectivity testing, chromatograms of standard (7.5 µg/ml hydrocodone and 200 µg/ml ibuprofen), tablet sample (7.5 µg/ml

hydrocodone and 200 µg/ml ibuprofen), blank mobile phase and blank placebo were screened for probable interference. No potential interfering peaks were observed at the retention times of hydrocodone and ibuprofen in the blank mobile phase and placebo chromatograms, hence the method is proved selective (fig. 3).

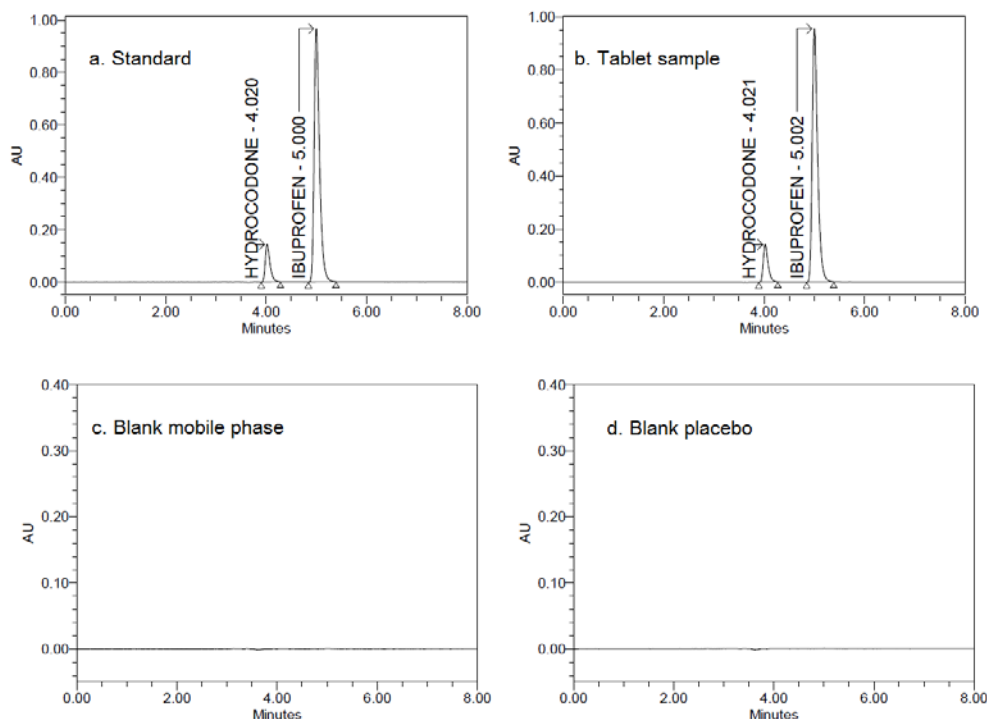


Fig. 3: Chromatograms of (a) standard (b) tablet sample (c) blank mobile phase (d) blank placebo

#### Linearity and sensitivity

The linearity curves for hydrocodone ranging from 3.75 to 11.25  $\mu\text{g/ml}$  and for ibuprofen ranging from 100 to 300  $\mu\text{g/ml}$  were linear and regression coefficients ( $R^2$ ) are calculated using least square regression analysis. The characteristic linear regression equations are: hydrocodone,  $y = 12440x - 1408$  ( $R^2 = 0.9996$ ); ibuprofen,  $y = 35776x - 2619$  ( $R^2 = 0.9999$ ).

Signal to noise ratio criteria of 10 and 3 was used to calculate limit of quantification and limit of detection, respectively. The detection and quantification limits were calculated as 0.048  $\mu\text{g/ml}$  and 0.162

$\mu\text{g/ml}$  for hydrocodone, 0.192  $\mu\text{g/ml}$  and 0.642  $\mu\text{g/ml}$  for ibuprofen, respectively. The values show adequate method sensitivity.

#### Precision and accuracy

Accuracy and precision were checked by evaluating five replicates of standard solution (7.5  $\mu\text{g/ml}$  of hydrocodone and 200  $\mu\text{g/ml}$  of ibuprofen). Precision was described by the percent relative standard deviation while the accuracy was described by the percentage of concentration calculated [20, 21]. The method was proven to be precise ( $\leq 2$ ) and accurate (nearer to 100%).

Table 1: Summary of precision and accuracy study

Drug	Concentration ( $\mu\text{g/ml}$ )	Mean peak area (mAU)*	Percent RSD of peak area*	Mean concentration determined*	Mean percentage of concentration*
Hydrocodone	7.5	929579	0.262	7.47	99.65
Ibuprofen	200	7146683	0.261	198.75	99.37

\*Mean of six values

#### Recovery

Recovery was checked by the standard addition technique. This test was done by fortifying pre-analyzed tablet sample solution with known amounts of hydrocodone (3.75, 7.5 and 11.25  $\mu\text{g/ml}$ ) and

ibuprofen (100, 200 and 300  $\mu\text{g/ml}$ ). Each concentration level was evaluated in triplicate by the proposed method. Good mean recoveries of hydrocodone and ibuprofen at 3 fortified concentration levels indicated the good accuracy of this method and also the absence of hindrance from the tablet excipients (table 2).

Table 2: Summary of recovery study

Level (%)	Hydrocodone concentration			RSD (%)
	Added ( $\mu\text{g/ml}$ )	Determined ( $\mu\text{g/ml}$ )	Recovered (%)	
50	3.75	3.74	99.68	0.011
100	7.5	7.47	99.63	0.025
150	11.25	11.22	99.75	0.095
	Ibuprofen concentration			
50	99.0	99.49	100.49	0.068
100	198.0	198.73	100.37	0.040
150	297.0	298.64	100.55	0.145

\*Mean of three values

**System suitability**

System suitability was investigated by five replicate analyses of hydrocodone (7.5 µg/ml) and ibuprofen (200 µg/ml) standard

solution. The approval criteria were: ≤2% RSD for peak area and retention time of hydrocodone and ibuprofen; >2000 for plate count; ≤2 for peak tailing; ≥2 for resolution. The values obtained are within the approval criteria (table 3).

**Table 3: System suitability report summary**

Parameter	Hydrocodone	Ibuprofen
Precision RSD for peak area*	0.223	0.303
Precision RSD for retention time*	0.503	0.346
Resolution*	-	5.278
Peak tailing*	1.486	1.436
Plate count *	9223	11028

\*Mean of five values

**Robustness**

Robustness was investigated through deliberate variations in the pH of mobile phase (±0.1 unit), percentage of methanol content (±5%),

wavelength (±2 nm), flow rate (±0.1 ml/min) and column temperature (±2 °C). It was noted that there were no significant changes in peak tailing, resolution and plate count demonstrated that the method developed was robust (table 4).

**Table 4: System suitability report summary during the robustness test**

Value investigated	Hydrocodone			Ibuprofen		
	Plate count	Tailing factor	Resolution	Plate count	Tailing factor	Resolution
<b>Mobile phase pH</b>						
Normal	9223	1.48	-	11028	1.43	5.27
-0.1 unit	9164	1.50		10937	1.44	5.26
+0.1 unit	9064	1.49		10876	1.44	5.25
<b>Methanol percentage in the mobile phase</b>						
Normal	9223	1.48	-	11028	1.43	5.27
-5%	8517	1.44		10301	1.40	5.17
+5%	9340	1.51		11131	1.45	5.34
<b>Flow rate</b>						
Normal	9223	1.48	-	11028	1.43	5.27
-0.1 ml/min	8517	1.44		10301	1.40	5.17
+0.1 ml/min	8918	1.47		10309	1.42	5.15
<b>Wave length</b>						
Normal	9223	1.48	-	11028	1.43	5.27
-2 nm	9254	1.50		11094	1.44	5.28
+2 nm	9757	1.45		11366	1.41	5.39
<b>Column temperature</b>						
Normal	9223	1.48	-	11028	1.43	5.27
-2 °C	9340	1.51		11131	1.45	5.34
+2 °C	9815	1.53		11580	1.45	5.49

**Degradation studies**

After treatment with 0.1N NaOH, hydrocodone and ibuprofen showed 2 degradation peaks with retention times of 1.482 min and 5.825 min. The percent degradation of hydrocodone and ibuprofen were 1.04 % and 15.9%, respectively. Following treatment with 0.1N HCl, hydrocodone and ibuprofen showed 5 degradation peaks with retention times of 1.731 min, 2.707 min, 3.431 min, 4.728 min, 5.776 min. The percent degradation of hydrocodone and ibuprofen were 3.28 % and 1.26%, respectively. On hydrolysis with water, hydrocodone and ibuprofen showed 2 degradation peaks with retention times of 3.431 min and 6.296 min.

The percent degradation of hydrocodone and ibuprofen were 0.76 % and 1.97%, respectively. In oxidation condition with hydrogen peroxide, hydrocodone and ibuprofen showed 4 degradation peaks with retention times of 2.186 min, 3.330 min, 3.440 min and 5.781 min. The percent degradation of hydrocodone and ibuprofen were 4.59 % and 7.38%, respectively. In dry heat situation, hydrocodone and ibuprofen showed 6 degradation peaks with retention times of 1.458 min, 1.617 min, 2.655 min, 3.420 min, 5.821 min and 6.980 min. The percent degradation of hydrocodone and ibuprofen were 1.25 % and 0.79%, respectively. After degradation with sunlight, hydrocodone and ibuprofen showed 4 degradation peaks with the retention time of 1.654 min, 2.124 min, 2.588 min and 3.434 min.

The percent degradation of hydrocodone and ibuprofen were 2.48 % and 0.83%, respectively. The representative chromatograms of hydrocodone and ibuprofen in all degradation conditions are shown in fig. 4.

Peak purity/homogeneity test for hydrocodone and ibuprofen peaks was done to evaluate the interference of degradants at the retention times of hydrocodone and ibuprofen by using photodiode array detection. The results (purity angle is less than purity threshold) indicated that additional peaks were not co-eluting with hydrocodone and ibuprofen peaks (table 5). This demonstrates the ability of the proposed method to assess unequivocally hydrocodone and ibuprofen in the presence of degradants. The assay results of hydrocodone and ibuprofen in all conditions of degradation was summarized in table 5. All the results proved the stability indicating power and specificity of the developed RP-HPLC method.

**Application to the tablet for the analysis of hydrocodone and ibuprofen**

The hydrocodone and ibuprofen contents of the tablet samples analyzed are reported in table 6. The exposed values signify the good accuracy and precision of the method to quantify hydrocodone and ibuprofen simultaneously in tablets. There was no interference from excipients normally found in tablets.

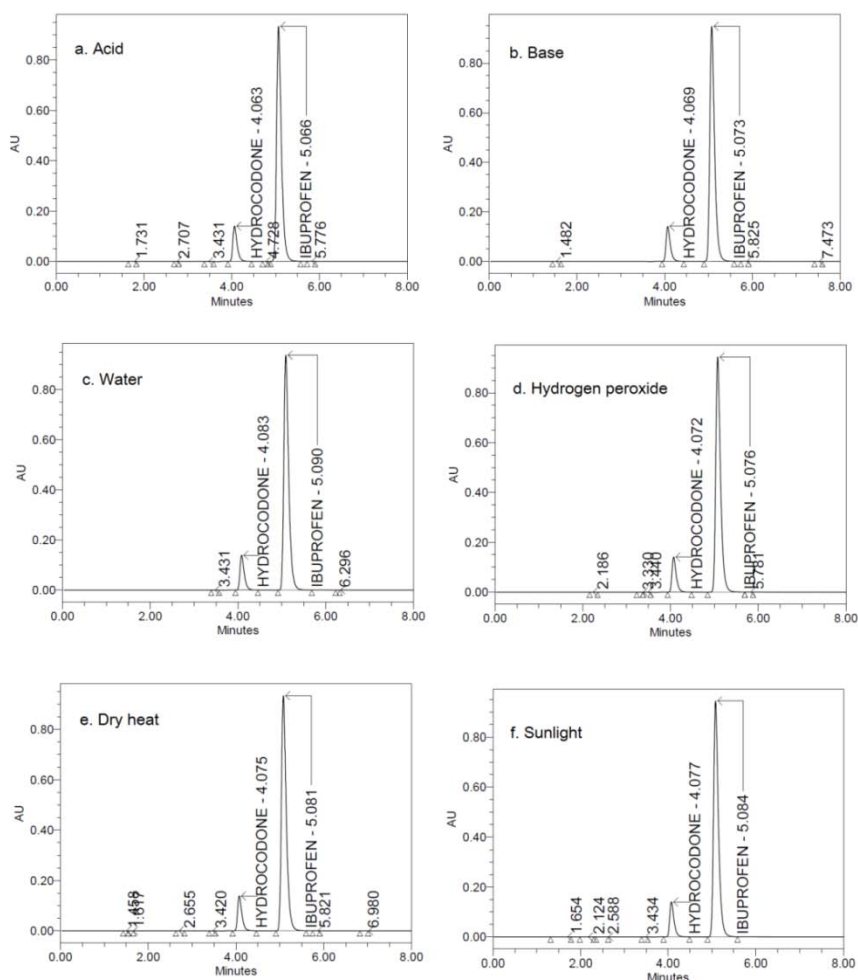


Fig. 4: Chromatograms of hydrocodone and ibuprofen after treatment with (a) Acid (b) Base (c) Water (d) hydrogen peroxide (e) Dry heat (f) Sunlight

Table 5: Summary of peak purity test, and percent assay of hydrocodone and ibuprofen

Degradation with	Ibuprofen			Hydrocodone		
	% Assay	Purity angle	Purity threshold	% Assay	Purity angle	Purity threshold
Acid	98.74	0.715	0.748	96.72	0.338	0.453
Base	84.10	0.309	0.64	98.96	0.234	0.462
Oxidation	92.62	0.421	0.65	95.41	0.333	0.559
Thermal	99.21	0.326	0.857	98.75	0.221	0.454
Photo	99.17	0.394	0.634	97.52	0.232	0.46
Water	98.03	0.299	0.543	99.24	0.201	0.355

Table 6: Assay of hydrocodone and ibuprofen content in dosage form

Drug	Labelled claim (mg)	Determined value (mg)*	Recovered (%)	RSD (%)
Hydrocodone	7.5	7.48	99.69	0.605
Ibuprofen	200	200.94	100.47	0.912

\*Average of three values

## CONCLUSION

A stability indicating RP-HPLC coupled with photodiode array detection method was developed to quantitatively assay hydrocodone and ibuprofen simultaneously in tablets. Validation of the method revealed good linearity, adequate sensitivity, satisfactory selectivity, specificity, accuracy and precision. The developed method is fit for routine use in quality control laboratories and in hydrocodone and ibuprofen stability studies.

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## AUTHOR'S CONTRIBUTION

This paper is the research work of RKK under the guidance of RS.

**CONFLICTS OF INTERESTS**

No conflicts of interest exist

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