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Validated RP-HPLC method for determination of dexibuprofen: Application in pharmaceutical dosage and human serum

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ABSTRACT

A novel stability indicating, specific, precise and accurate, high performance liquid chromatographic method has been developed and validated for the determination of Dexibuprofen in pharmaceutical dosage forms of capsules and tablets, and in human serum. Acetonitrile: Buffer: Acetic Acid 0.1M (65:35:0.3 v/v/v) was the mobile phase at flow rate 1.0 ml/min using a Hibar® μBondapak® C_{18} column monitored at wavelength of 220nm. The calibration curve was linear with a correlation coefficient of more than 0.9995 for the drug. The averages of the absolute and relative recoveries were found to be 100.07% for Dexibuprofen with 10ng/ml limit of quantification and 3ng/ml limit of detection. The drug was subjected to stress conditions of hydrolysis (acid, base, oxidation, and thermal degradation). Maximum degradation was observed in base and 35% H_2O_2 while found relatively stable in the other stress conditions. The studies of forced degradation prove the stability indicating power of the method. The developed method was validated in accordance to ICH guidelines. The proposed High-performance liquid chromatographic method was successfully applied to quantify the amount of Dexibuprofen in bulk, dosage form and physiological fluid.

KEYWORDS

Dexibuprofen;
RP-HPLC;
Serum;
Stability indicating.

INTRODUCTION

Dexibuprofen (DBPN) is the pharmacologically effective (S+ Enantiomer of Ibuprofen) and chemically is 2-[4-(2-methylpropyl)phenyl] propanoic acid (Figure 1)[1]. In contrast to Ibuprofen the administration of dexibuprofen may offer the advantage of delivering the well-known antipyretic, analgesic and anti-inflammatory properties of ibuprofen in a more efficient way, with a better safety profile than the racemic ibuprofen formulations[2]. It is a widely used as nonsteroidal anti-inflammatory and analgesic agent for many years[3,4] and for the symptomatic treatment of osteoarthritis, primary dysmenorrhoea, muscularskeletal pain or dental pain and reduces gastric damage and improves analgesic and anti-inflammatory effect than racemic ibuprofen[5].

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Dexibuprofen is rapidly absorbed, and peak plasma concentrations is achieved within 1.5-2 hours of oral administration[6] It has been established there is a significant correlation between plasma Dexibuprofen levels and the resultant degree of pain relief, particularly 1 hour after administration[7].

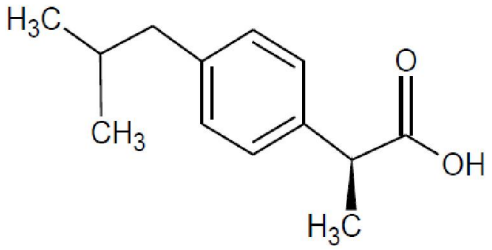

Figure 1 : Structure of dexibuprofen.

Literature survey reveals some analytical methods for Dexibuprofen including high-performance liquid chromatographic (HPLC) determination of Dexibuprofen in St.John's Wort[8-10], in human plasma and other physiologic fluids by HPLC/HPLC-mass spectrometry[11-16], by radian compression technique[17].

To the best of our research survey till now no such method is available which can determine Dexibuprofen so efficiently therefore an endeavor was made to develop a simple, sensitive and validated stability indicating RP-HPLC method using UV detection to determine Dexibuprofen in pharmaceutical dosage forms and human serum. The applicability of the method was confirmed for analysis in pharmaceutical products and for pharmacokinetic studies. The results of analysis were validated in accordance with ICH guidelines[18].

EXPERIMENTAL

The present method was designed to be easy to use, sensitive, rapid and simple sample preparation for the active ingredient. Separation and quantification in pharmaceutical drug formulations and blood were achieved with an isocratic elution.

Material and reagents

Dexibuprofen (DBPN) was kind gift from National Pharmaceuticals, whereas sulphuric acid, sodium heptane sulphonate, Acetonitrile (MeCN) HPLC grade, Acetic Acid (AcOH) analytical grade were purchased from Merck (Germany). The pharmaceutical dosage forms containing DBPN were obtained from commercial source including Ibusoft® Capsules 200mg/Caps. (Zafa pharmaceutical laboratories (pvt) ltd.), Prophed Forte® Tablet 400mg/Tab. (Macter international (pvt) ltd.), Rhinoff Forte® Tablet 400mg/Tab.(Atco Laboratories Limited). Distilled water was obtained by passage through RO plant (Waterman, Pakistan) and was further filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA).

Instruments

A UV-visible Shimadzu 1650 PC spectrophotometer with UV Probe software, ultrasonic cleaner (Elmasoni E 60 H), Jenway 3240 pH meter and Sartorious TE2145 analytical balance were used in the research work. For chromatography a SIL 10A auto injector HPLC system comprising of SCL 10A system controller, SPD 20A prominence UV/VIS detector, and Shimadzu LC 20 AT pump with LC Solutions software was used. Separation was performed on a Hibar® µBondapak® ODS C18 HPLC column, (4.6 x 250 mm; 5 µm bead size) maintained at 25°C. Throughout the work only amber glass flasks were used to avoid light effect on the solutions of DBPN standards and samples.

Chromatographic conditions

The HPLC analysis was carried out at ambient temperature. The compound was chromatographed isocratically with a mobile phase consisting of MeCN (HPLC grade): Acetate Buffer: AcOH 0.1M (65:35:0.3 v/v/v) with the pH adjusted if required to 5.5 ± 0.1 using AcOH 0.1M. The mobile phase was filtered by passing through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA). The flow rate was 1.0 ml/min, and the injected volume was 20 µL. The effluent was monitored spectrophotometrically at wavelength of 220nm. Mobile Phase was used as dilution solvent.

ANALYTICAL PROCEDURE

Standard preparation

In a 100ml volumetric flask, accurately 100 mg of DBPN was weighed and dissolved in 70ml of dilution solvent sonicated for 5 minutes and then added by up to the mark with dilution solvent to get stock solution-A containing 1000µg/ml of DBPN. The solution-A was

used in further analytical steps. For standard preparation 10ml of stock solution-A was diluted in 20ml flask to get working solution of 500µg/ml was then filtered through 0.45µm filter paper and injected into the HPLC system and consequently used in the proposed analytical procedure.

Sample preparation

(a) Sample preparation for products

For making a sample from commercially available capsules and tablets, 10 capsules were opened and content was mixed to get an evenly homogenized sample. Similarly for tablets, 10 tablets were grind to fine powder and then weighed the sample accurately equivalent to 100mg DBPN was taken in 100ml volumetric flask and 70 ml of diluent was added. The sample was sonicated for 05 minutes and then added diluent up to the mark, placed on stirrer for 10 minutes. The sample was then filtered through 0.45µm filter paper and injected into the HPLC system.

(b) Sample preparation for serum

Blood samples were collected from healthy volunteers in evacuated glass tube through an indwelling cannula placed on forearm vein by a trained clinical laboratory technician. The volunteers were not involved in any medication, smoking, and strenuous activity. The blood was shaken and centrifuged at 10,000 rpm for 10 min to separate out plasma. MeCN was added to plasma (90:10, v/v) and centrifuged at 10,000 rpm for 10 min to deprotonate it. The supernatant serum thus obtained was filtered and used for the analysis and was stored at 20°C. For making working sample 10ml of stock solution-A was taken in 20ml flask, followed by 5ml of serum. The sample thus obtained was stir for 10 minutes and then added diluent up to the mark and was filtered through 0.45µm filter paper and injected into the HPLC system. Triplicate solutions were made for each working solution for analysis in serum.

(c) Sample preparation for degradation studies of drugs

For this purpose 25ml of stock solution-A was diluted in four individual 50 ml volumetric flasks and 15 ml of degrading agent was added to each flask individually, with exception of one to which only diluent was added; these included 0.1 N HCl, 0.1 N NaOH, 35%H_2O_2 and then to each flask diluent was added up to the mark. All the four samples were placed in water bath at 60°C for one hour. The samples were then filtered through 0.45µm filter paper and injected into the HPLC system.

Stability studies

For stability studies the commercially available samples (tablets and capsules) were placed at accelerated conditions of temperature that is at 40°C with 75% relative humidity and at ambient conditions of 30°C temperature with 65% relative humidity in environmental chamber for six months. The stability protocol was followed for six months and assays were made as described in analytical procedure.

Method validation

The method validation was performed in following ICH guidelines[18] according to which various procedures were performed including specificity, linearity, range, accuracy, intra-day and inter-day precision etc.

To study the linearity of standard solutions, twenty dilutions were prepared from stock solution-A to give standard solutions in range of 10% to 200 % of drugs content. The standard calibration curve was generated using regression analysis. For specificity commonly used excipient in dosage forms preparation were spiked in a pre-weighed quantity of drug and then peak areas were measured and calculations done to determine the quantity of the drug recovered.

The precision was done by analyzing corresponding standard daily for a period of three days i.e. Inter-day precision, and three times a day with an interval of 08 hours (Intra-day precision) against freshly prepared standard solutions. For determining accuracy the reference standards were accurately weighed and added to the sample, to get three different concentration levels i.e. 80% -100% -120% of the ingredients. At each level, samples were prepared in triplicate and the recovery percentage was determined.

Limit of detection and quantification (LOD & LOQ) for the method were established by sequentially diluting the standard solutions at decreasing concentrations, in the range of 100ng/ml to 1ng/ml and injected onto the chromatographic system. The limit of detection was defined as the concentration for which a signal-to-noise

ratio of 3 was obtained and, for quantification limit; a signal-to-noise ratio of 10 was considered.

The robustness was studied by analyzing the same samples by deliberate variation in the method parameters, such as in the chromatographic conditions, like mobile phase, pH, flow rate, temperature etc. System suitability of the method was evaluated by analyzing the symmetry of the standard peaks, resolution and theoretical plates of the column.

RESULTS AND DISCUSSION

The HPLC method development and its validation are the utmost requirements for any drug available in the market to have high quality products. A few methods are available for determination of the DBPN as described earlier, but many of them are used only for certain definite objectives and none can be generalized for its determination in form of pharmaceutical products and serum. Similarly none of them is as much sensitive as ours is; in terms of its Precision, accuracy, %recovery, limit of detection (LOD) and limit of quantification (LOQ). Moreover this method is sensitive enough to be used for pharmacokinetic studies as its LOD & LOQ are in nanogram range.

Method development & optimization

For developing an efficient method for analysis, parameters, such as detection wavelength, mobile phase composition, optimum pH and concentration of the standard solutions were comprehensively studied. All the ingredients were diluted in dilution solvent and then run through UV spectrophotometer in UV range of 190nm – 400nm to get maximal wavelengths, where maximum absorbance was gained i.e. 220nm at which the molecule gave a satisfactory absorbance and representable chromatogram. Mobile phase was selected in terms of its components and their proportions. The chromatographic parameters were evaluated using a Hibar® µBondapak® C_{18} column, the mobile phase composed of MeCN: Buffer of given proportion which promoted a short run time (10 min) without any interference, so this condition was adopted in subsequent analysis.

Validation studies

The linearity was determined in the range of 10%-200%. The assay was judged to be linear as the correlation coefficient was greater than 0.9995 calculated by the least-square method. A linear correlation was found between the peak areas and the concentrations, in the assayed range. The regression analysis data are presented in TABLE 1 and Figure 2.

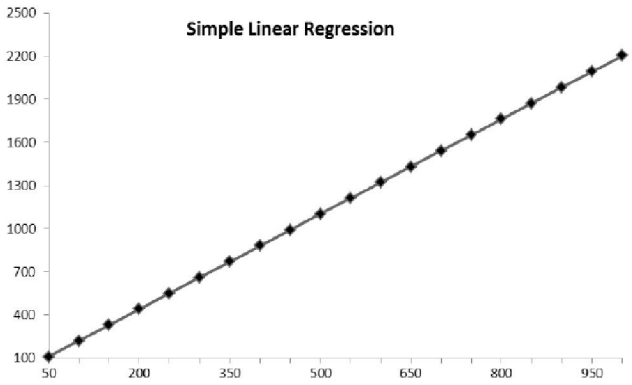

Figure 2 : Linearity graph of the method for DBPN.

TABLE 1 : Calibration curve data and validation parameters.

Parameter	Inference
	DBPN
Linearity range (µg /ml)	50-1000
Intercept(c)	0
Standard Regression equation (y=mx+c) Slope(m)	2202.41 x + 0
Correlation coefficient(R2)	0.99961
Limit of detection (LOD) (ng /ml)	3
Limit of quantification (LOQ) (ng /ml)	10

Chromatogram shown in Figure-3a proves specificity or selectivity of the assayed method, as the chromatograms in samples were found identical with standard chromatogram (Figure 3b) and no interference peak was observed. Peak purities higher than 98.0% were obtained in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with peak of interest. The chromatogram obtained with the mixture of the product's excipient proves that there is no interference from excipient and peak of interest fulfill all the requirements of symmetrical peak, and hence the specificity is confirmed.

The precision of an analytical method is the degree of coherence among individual test results when the method is applied repeatedly to multiple sampling of homogeneous bulk. Intra-day precision of the method was evaluated at three different independent concentrations i.e. 80%, 100%, and 120% for the drugs (n=3)

by determining their assays. Inter-day precision of the method was tested for 3 days at the same concentration levels. Solutions for calibration curves were prepared every day on freshly basis. Since the inter-day and intra-day precision obtained %RSD was less than 2% it assures that the proposed method is quite precise and reproducible as shown in TABLE 2.

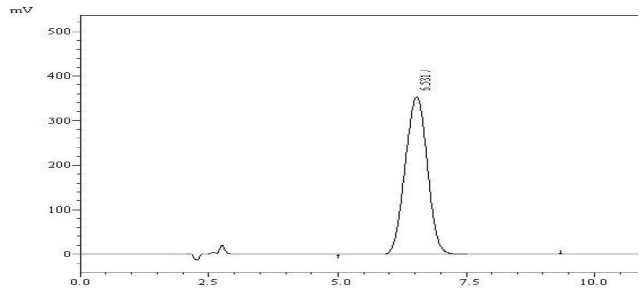

Figure 3a : Typical chromatogram of physiological sample.

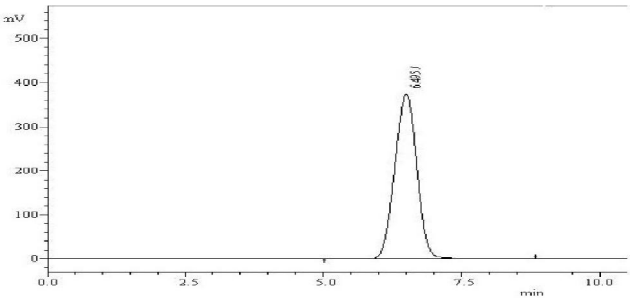

Figure 3b : Typical chromatogram of standard.

TABLE 2 : Inter-day and intra-day precision and recovery studies.

Active Drugs	Nominal Concentration (µg /ml)	Day 1	Day 2	Day 3	Mean	Inter-Day RSD%
DBPN	400	99.74%	100.38%	98.86%	99.66%	0.765
	500	100.65%	100.96%	100.00%	100.53%	0.487
	600	101.57%	98.95%	99.51%	100.01%	1.380
	Mean	100.65%	100.09%	99.46%	100.07%	
	Intra-Day RSD%	0.91	1.03	0.58	0.44	

The accuracy was investigated by means of addition of reference standards to a mixture of the products excipients. Recovery studies of the drug were carried out for the accuracy parameter at three different concentration levels i.e. multiple level recovery studies. A known amount of API standard was added into pre-analyzed sample and subjected to the proposed HPLC method. The mean recovery (n = 9) was 99.46% - 100.65% (RSD=0.84%), demonstrating the accuracy of the method. Percentage recoveries for marketed products were found to be within the limits TABLE 3.

TABLE 3 : Contents of DBPN, in pharmaceutical dosage forms.

SAMPLES	Content (%) ± S.D.
	DBPN
IBUSOFT® Capsules	99.75%± 0.19
PROPHED FORTE® Tablet	98.91 %± 0.81
RHINOFF FORTE® Tablet	100.12%± 0.51

S.D. = Standard deviation

The statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. The parameters used in system suitability test were symmetry of peak, tailing factor, resolution and RSD of peak area for replicate samples. Thus, the method showed to be robust for changes in mobile phase MeCN proportion, mobile phase pH, flow rate, and column temperature TABLE 4.

TABLE 4 : Robustness of the method.

Chromatographic Conditions	Variation	Retention time
		DBPN
Temprature (ºC)	22	5.781
	30	7.019
Flow rate (ml/min.)	0.8	10.949
	1.2	3.960
Amount of MeCN (%)	48	5.972
	52	5.398

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light hence enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies. The proposed assay method was applied to stability study of commercially available products, for which the samples were placed at previously mentioned conditions. Stability study was performed according to stability protocol as in TABLE 5. Samples were analyzed and percentage of contents was measured. According to the

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results obtained DBPN was found to be stable at applied conditions of temperature and relative humidity, and were accurately analyzed with the proposed method.

TABLE 5 : Summary of stability studies.

TEST (Claimed content)	INTERVAL							Mean	RSD%
	Initial	1Month	2Month	3Month	4Month	5Month	6Month		
Studies at Accelerated (40ºC+75%H)									
IBUSOFT® Capsules	100.351	99.961	99.476	99.265	98.971	98.432	98.157	99.044	0.794
PROPHED FORTE® Tablet	99.973	99.894	99.918	99.389	98.991	98.765	98.713	99.378	0.518
RHINOFF FORTE® Tablet	100.013	99.989	99.913	99.873	99.894	99.561	99.231	99.782	0.264
Stability Studies at Long Term (30ºC+65%H)									
IBUSOFT® Capsules	100.351	100.135	100.384	100.174	99.853	99.239	99.472	99.876	0.443
PROPHED FORTE® Tablet	99.973	99.978	99.938	99.919	99.881	99.873	99.784	99.907	0.063
RHINOFF FORTE® Tablet	100.013	100.131	99.987	99.978	99.853	99.713	99.635	99.901	0.164

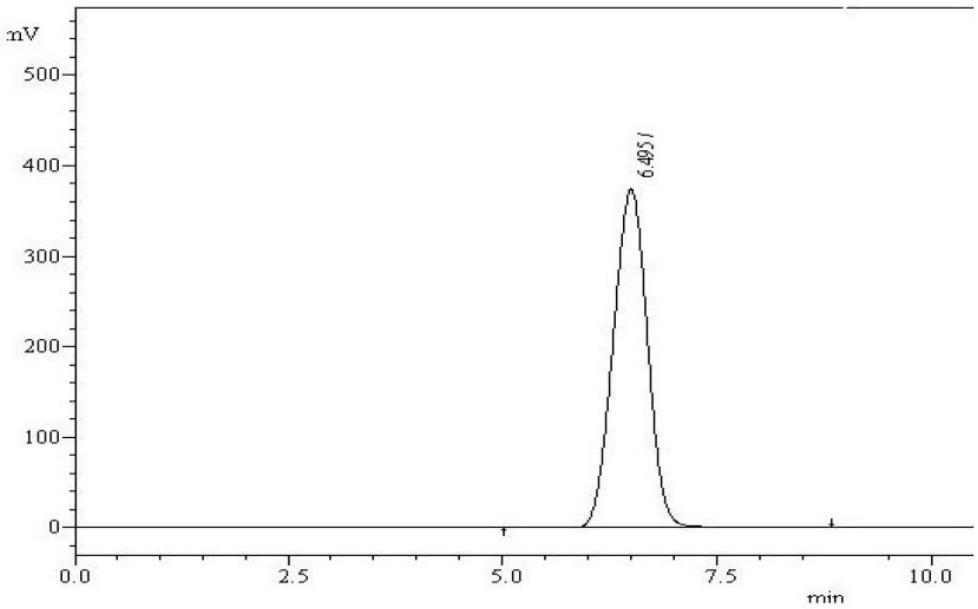

Figure 4a : Chromatogram of heat treated sample.

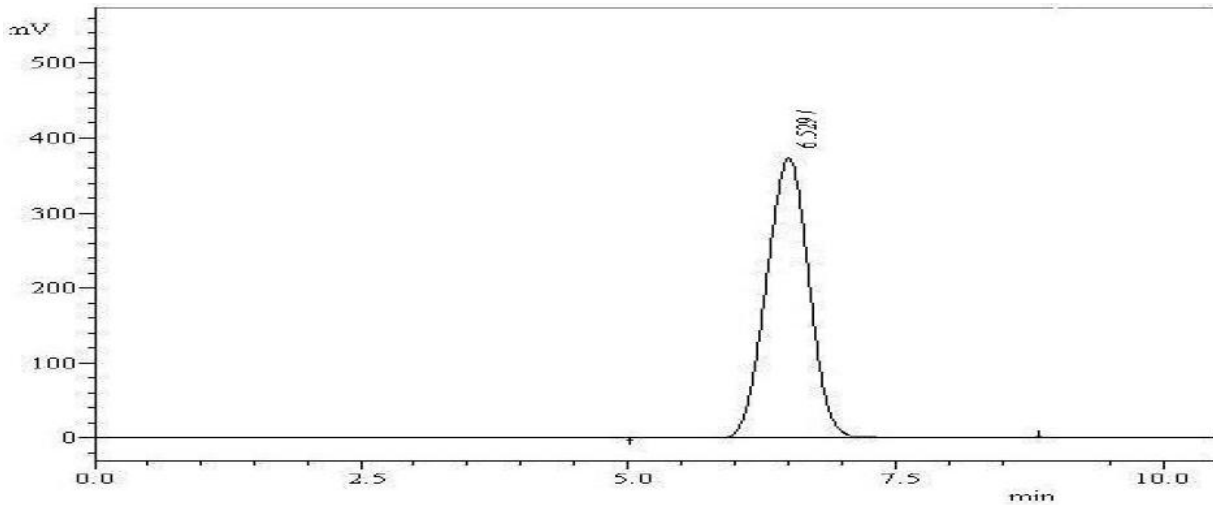

Figure 4b : Chromatogram of acid treated sample.

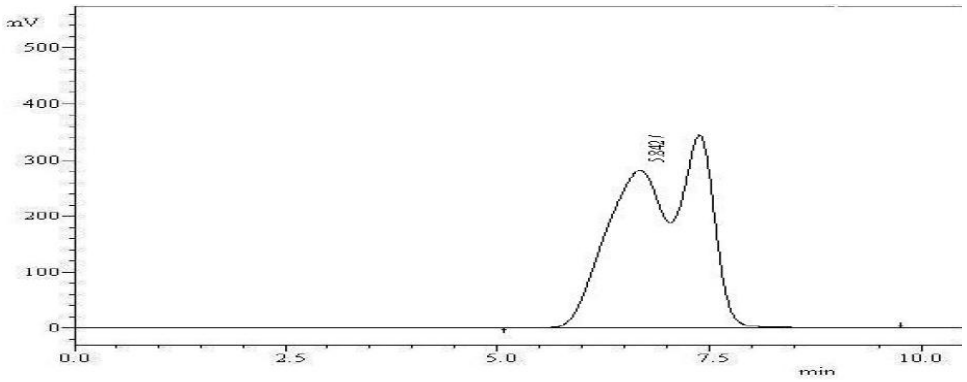

Figure 4c : Chromatogram of base treated sample.

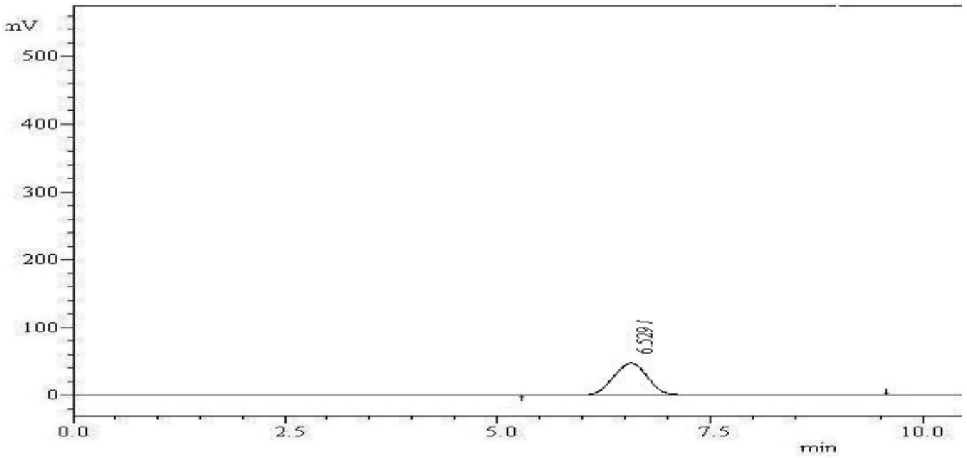

Figure 4d : Chromatogram of H2O2 treated sample.

During the degradation study upon treatment of DBPN with base (0.1M NaOH), acid (0.1M HCl), hydrogen peroxide (35%) and heat it was observed that DBPN is fairly stable to acid and heat (Figure 4a & 4b) and no decarboxylation occurs to DBPN up to the temperature given. Further in Figure-4c, Base treated sample, degraded peaks of DBPN is the Clear evidence of base hydrolysis of carboxylic group[19]. Therefore the results so obtained can't be clearly calculated as to which percentage the active ingredient has been degraded. So far as action of oxidation stress is concerned Figure-4d shows that the DBPN is demolished to a great extent and its absorbance capability is severely damaged by this condition, which is very efficiently detected by the proposed method.

TABLE 6 : Summary of forced degradation results.

Stress conditions	Time / h	Assay of active Substance	Degradation (%)
Acid hydrolysis (0.1M HCl)	1	96.20%	3.30%
Base hydrolysis (0.5M NaOH)	1	99.57%*	0.43%*
Thermal (60ºC)	1	96.20%	3.80%

*** Not determined correctly**

CONCLUSION

The proposed new HPLC method described in this paper provides a simple, convenient and reproducible approach for the identification and quantification of Dexibuprofen in bulk, human serum, and pharmaceutical formulations with good presentation. In addition, this method has the potential application to clinical research of drug combination. Analytical results are accurate and precise with good recovery and lowest detection limit values. In short, the developed method is simple, sensitive, easy, and efficient having short chromatographic time and can be used for routine analysis in QC laboratory and therapeutic monitoring.

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