INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-5, Issue-3, July-Sept -2014 Coden : IJABPT Copyrights@2014

ISSN : 0976-4550

Received: 8th April-2014 *Revised: 30th April-2014* *Accepted: 12th May-2014*

Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL AND KETOPROFEN BY RP – HPLC TECHNIQUE

P.Haritha[1], B.Sreenivasa Rao*[2] and Y.Sunandamma[3]

[1]Research Scholar, Department of Chemistry, JNTUK, Kakinada, AP
[*2]Department of Chemistry, GITAM University, Visakhapatnam, AP
[3]Department of Chemistry, Vikrama Simhapuri University, Nellore, AP
Corresponding Author: battula_sr@gitam.edu

ABSTRACT: The present study describes a simple, accurate and precise RP-HPLC Technique for the simultaneous determination of Paracetamol and Ketoprofen in pharmaceutical dosage form. The method involves an isocratic elution of drug in a stationary phase of YMC, C4 (150 x 4.6 mm, 3 µm) column using a mobile phase composition of methanol and 0.1% (v/v) orthophosphoric acid in the composition ratio of 70:30 v/v with a flow rate of 0.9 mL/min at 254nm of detection. The injection volume is 10 µL. the method has been validated for specificity, linearity, range, precision, accuracy, limit of detection, limit of quantification, ruggedness and robustness. The retention times for Paracetamol and Ketoprofen are about 1.50 and 6.27 minutes respectively. Quantitative linearity was observed over the concentration range of 50 to 500 µg/mL for Paracetamol and 20.05 to 200.54 µg/mL for Ketoprofen respectively. The regression equations of concentration of Paracetamol and Ketoprofen are found to be y = 1319x+8671, y = 5280x + 29575 respectively where y is the peak area and x is the concentration of drug (µg/mL). The % recovery of Paracetamol and Ketoprofen are found to be in the range of 97% to104 %. All the validation parameters are within the acceptance range.

Keywords: RP-HPLC, Isocratic, Paracetamol, Ketoprofen

INTRODUCTION

Ketoprofen (Fig-1a) is one of the the propionic acid class of nonsteroidal anti-inflammatory drug (NSAID).Chemical formula $C_{16}H_{14}O_3$. Ketoprofen is used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis (arthritis caused by a breakdown of the lining of the joints) and rheumatoid arthritis (arthritis caused by swelling of the lining of the joints). Ketoprofen capsules are also used to relieve pain, including menstrual pain (pain that occurs before or during a menstrual period). Nonprescription Ketoprofen is used to relieve minor aches and pain from headaches, menstrual periods, toothaches, the common cold, muscle aches, and backaches, and to reduce fever. Ketoprofen is in a class of medications called NSAIDs. It works by stopping the body's production of a substance that causes pain, fever, and inflammation.

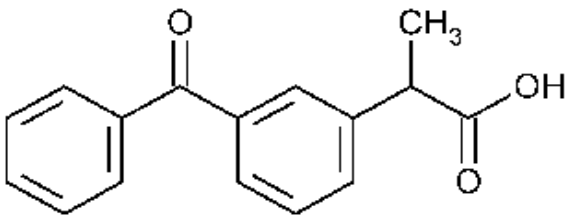


Figure 1a: Molecular Structure of Ketoprofen

Paracetamol (Fig-1b) chemically named N-acetyl-p-aminophenol, is a widely used pain reliever and antipyretic (fever reducer). Paracetamol (Figure 1b) is classified as a mild analgesic. 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. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity. The combination of both is used to relieve pain in case of post-operative conditions

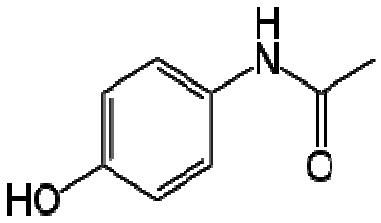


Figure 1b: Molecular Structure of Paracetamol

Literature survey revealed that UV detection method as per Ashraful SM et.al.(2011) and various other methods are available for estimation of Paracetomol as per Dimal AS et.al.(2011) and Patil et.al. (2010). Paracetomol in combination with other drug is reported by RP-HPLC method by Kumble RM et.al. (2012) and Pattan et.al.(2009). Ketoprofen in combination with other drug is reported by RP-HPLC method by Dvarak.J et.al.(2004),Mannucci C et.al.(1992) and Wong C et.al.(1992). Other methods are available for estimation of ketoprofen as per Blanko M et.al.(1998), Kormosh Z et.al.(2009),Labbozzetta S et.al.(2009).The present work describes a validated reverse phase HPLC method for simultaneous determination of Ketoprofen and Paracetomol tablets with high precise and sophisticated compared to earlier methods. The proposed method is validated as per ICH guidelines International conference on harmonization. IFPMA (2003), International Conference on Harmonization. Washington, DC (1996).

MATERIALS AND METHODS

Reagents and Chemicals

Orthophosphoric acid (AR grade, SD Fine chem limited), methanol (HPLC grade, Merck limited), Milli–Q water, Paracetamol (99.8% w/w procured from Unichem Laboratories Ltd), Ketoprofen (99.8% w/w procured from Wyeth Laboratories Ltd), All other chemicals are of the highest grade commercially available unless otherwise specified.

Instrumentation

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 70:30 % (v/v) of Methanol and 0.1% (v/v) Orthophosphoric acid operated on isocratic mode. The flow rate is 0.9mL/min. Chromatographic determination of Paracetamol and Ketoprofen was performed on YMC, C4 (150 x 4.6 mm, 3 µm) column .The wavelength of detection is 254 nm. The injection volume is 10µL.

Preparation of standard solutions, Calibration Standards & Quality Control Samples

Stock solutions of Paracetamol (10000 µg/mL) and Ketoprofen (6575µg/mL) were prepared separately in a volumetric flask using methanol and labeled accordingly. Suitable dilutions were then prepared using 50:50 %v/v methanol and Milli-Q water as diluent solution. For the linear calibration curve, eight non-zero standards were prepared using diluent solution in the concentration range of 50 to 500 µg/mL for Paracetamol and 20.05to 200.54 µg/mL for Ketoprofen. The calibration standard sample is then transferred into the auto sampler for analysis. Samples for specificity (Sample with Paracetamol alone, sample with Ketoprofen alone, Blank Sample and sample containing both the drugs) were also prepared accordingly.

For the preparation of quality control samples, a separate stock containing approximately the same concentration of the Paracetamol and Ketoprofen were prepared and labeled as quality control stocks. From these stocks, quality control samples containing Paracetamol and Ketoprofen were prepared at three concentration levels namely LQC, MQC and HQC so as to obtain low, medium and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples.

Assay

The assay of tablets containing Paracetamol and Ketoprofen is done using the procedure given in Indian Pharmacopoeia under tablets. The active ingredients in each of 10 dosage units is taken by random sampling and analyzed by the developed method. The tablets are said to be in compliance if the each individual content is 90 – 110 % of the average content or labeled claim.

For the current assay ten tablets were randomly taken and transferred separately into 100ml volumetric flasks and dissolved in 20 ml methanol. The solution was then ultrasonicated for 10min and then made up to volume. Required amount of solution is then taken and filtered through 0.45µ nylon membrane and diluted with diluent solution so that the resultant concentrations are within the calibration range of the developed method. The samples are then analyzed by using the validated method. The sample is then injected in triplicate.

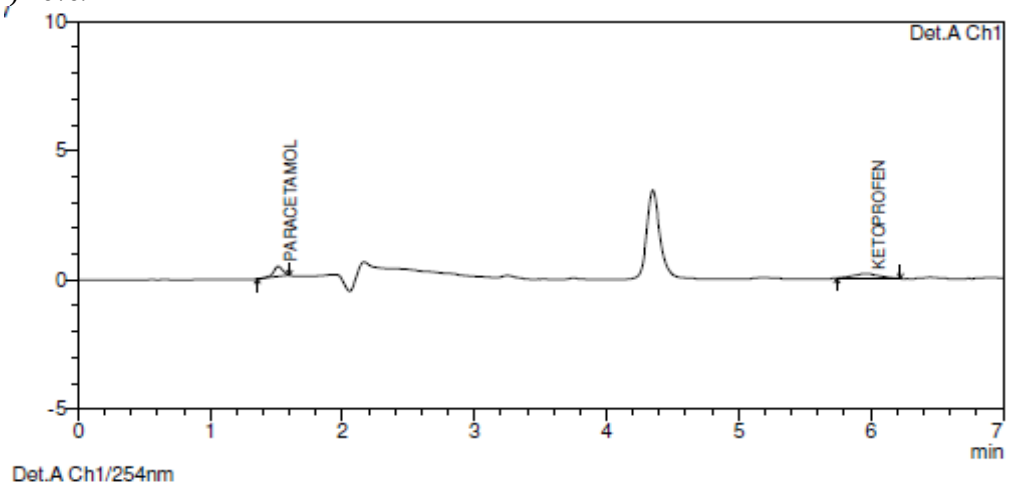
Method Validation

System Suitability

A sample containing mixture of Paracetamol (approximate concentration of 250µg/ml) and Ketoprofen (approximate concentration of 100.27µg/ml) was used as system suitability sample. System suitability was assessed by six replicate analysis. A percent coefficient of variation (% CV) less than 1 % for retention times for the drugs is taken as the acceptance criterion.

Detection and Quantification Limits (Sensitivity)

Limits of detection (LOD) and Limit of quantification (LOQ) (**Fig-2**) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 5, with precision (%CV) and accuracy with (±) 20%.



Chromatogram for LOD Sample:

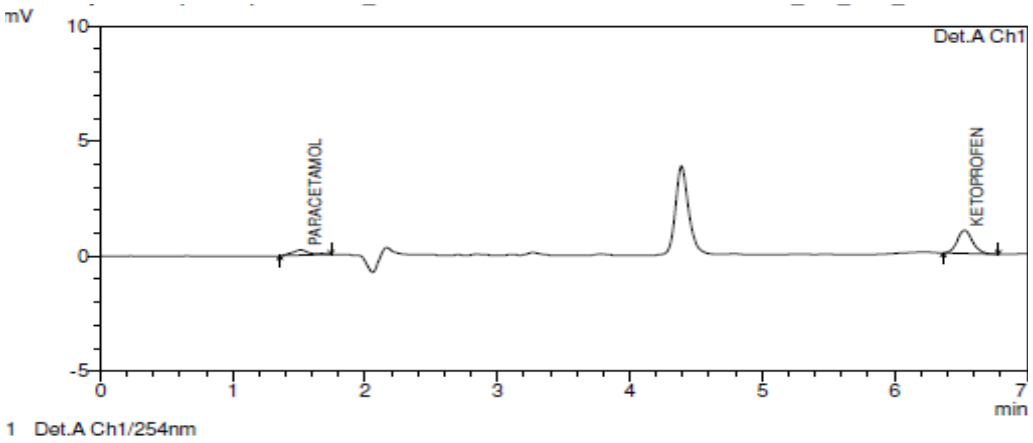


Figure-2 Chromatogram for LOQ Sample:

Linearity (Calibration Curve)

The Linearity of detector response to different concentrations of both the drugs was studied with a series of working standard solutions prepared by diluting the stock solution. The standard plots were then constructed between concentration Vs. Peak area using eight non-zero standards ranging from 50 to 500µg/mL for Paracetamol and 20.05 to 200.54 µg/mL for Ketoprofen. The linearity was evaluated by linear regression analysis, which was calculated by least square method. It is depicted in (**Fig- 3a and 3b**).

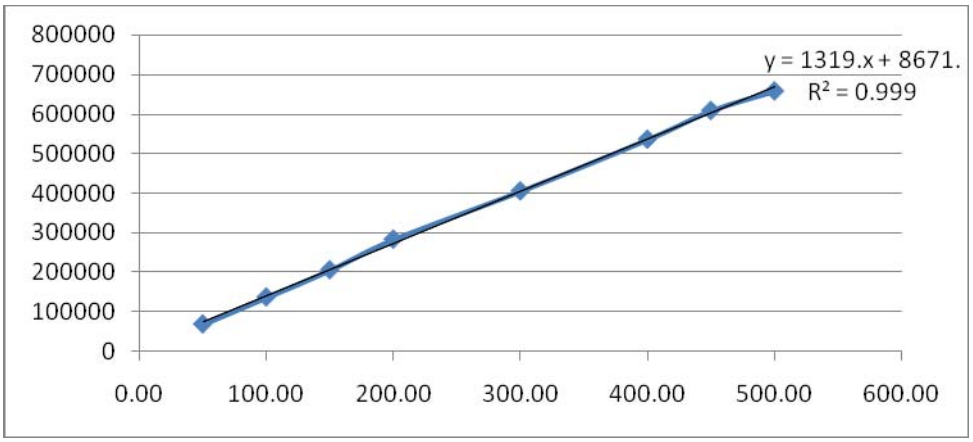


Figure- 3a. Linear calibration curve of Paracetamol

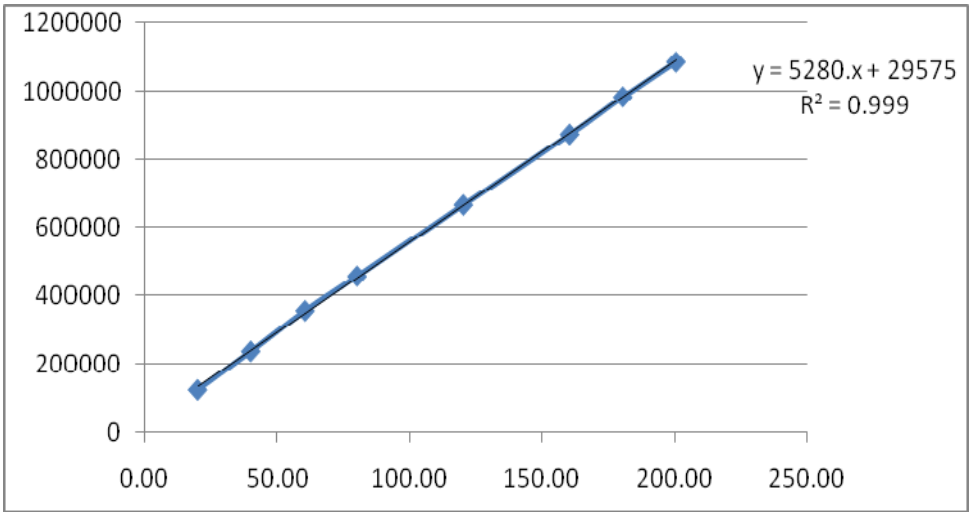


Figure-3b: Linear calibration curve of Ketoprofen

Accuracy and Precision

According to ICH guidelines, repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e. three concentrations and three replicates of each concentration).Precision was studied to find out intra and inter day variations of the proposed method at three different levels. The %CV values less than 2% indicate that the method was precise.

Specificity

For demonstration of specificity, 4 samples namely blank sample, sample containing Paracetamol alone, sample containing Ketoprofen alone and sample containing the mixture of Paracetamol and Ketoprofen were prepared separately. Specificity of the method was determined by comparing results of all the samples (**Figure -4**). The developed method is said to be specific if the % interference calculated as peak area (if any) at the retention time of each of the analytes in the blank sample is less than 20% of peak area at the corresponding retention times of each of the drugs in the lowest calibration standard. Sample Specificity is also observed in the degradation study of the drug. None of the degraded products must interfere with the quantification of the drug.

Coden : IJABPT, Copyrights@2014, ISSN : 0976-4550

Figure-4a: Blank Chromatogram

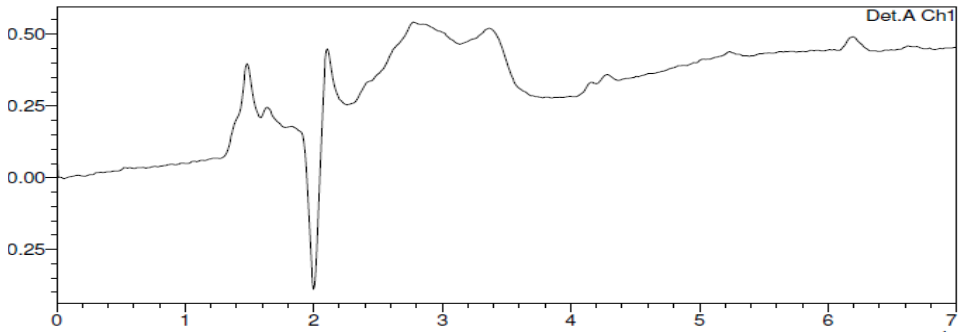


Figure-4b:Chromatogram of Paracetamol alone

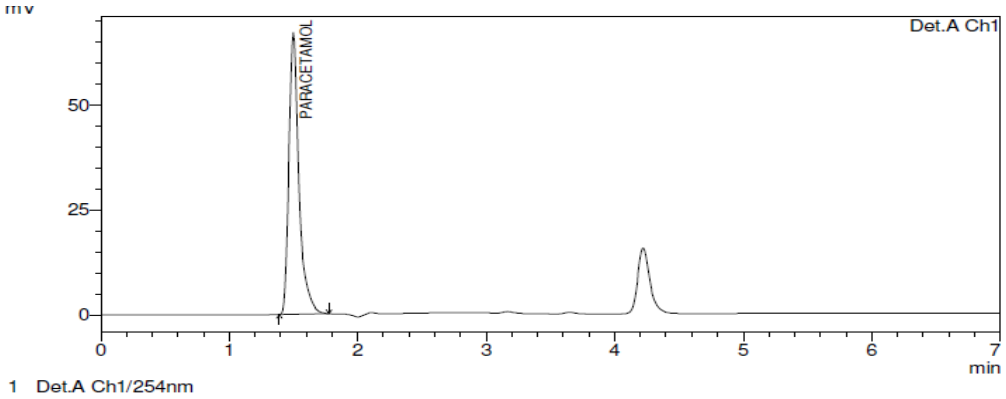


Figure-4c: Chromatogram of Ketoprofen alone

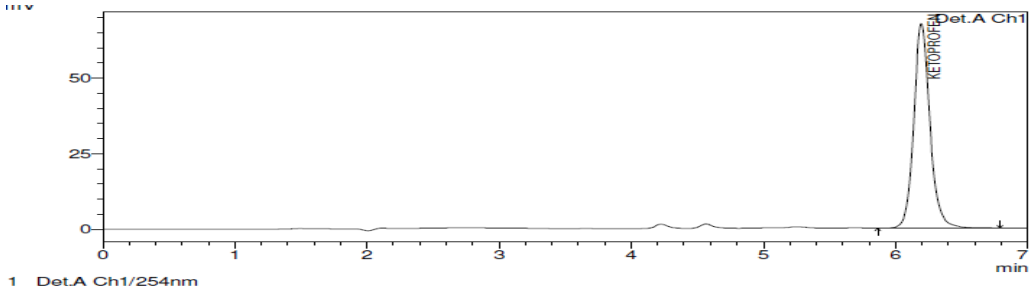


Figure-4d: Chromatogram of both Paracetamol and Ketoprofen

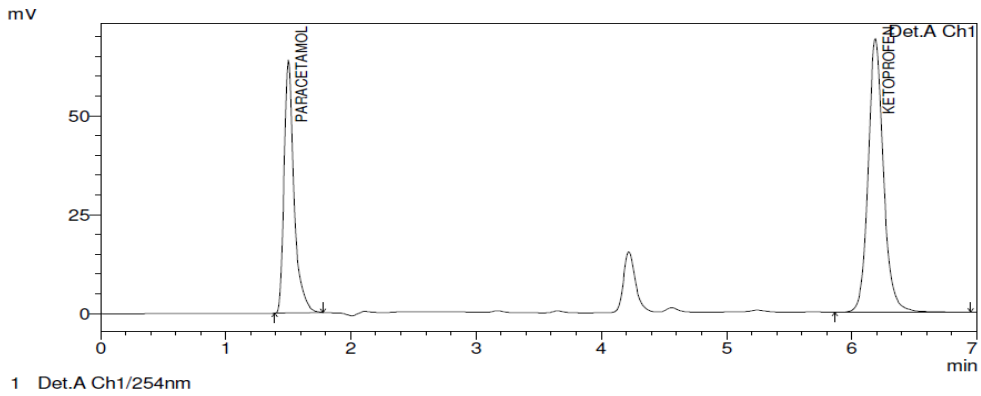


Fig-4 :Comparison of (a)Blank Chromatogram,(b)Paracetamol alone, (c)Ketoprofen alone and(d) sample containing both Paracetamol and Ketoprofen

Stability

The stability of the drug is determined by placing the MQC samples for the short term stability at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hrs was studied and established.

Stress Degradation Studies

For Stress Degradation Analysis, 1mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100µL of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 5% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for Photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples. The analysis is performed in triplicate.

RESULTS AND DISCUSSION

Method Development and Validation

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, YMC C4, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 have been tried with different buffer salts such as ammonium Formate, orthophosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran is done. However less tailing and high theoretical plates are obtained with YMC, C4 (150 x 4.6 mm,3µm)column .The final mobile phase composition consisted of (70:30 v/v) of Methanol and 0.1% Orthophosphoric acid on isocratic mode. The flow rate of the method is 0.9 mL/min. Calibration standards were prepared in diluents solution containing 50:50 % v/v of Methanol and Milli-Q water. The wavelength of detection is 254nm. The column temperature is maintained at 25^{O}C. At the reported flow rate, peak shape was excellent; however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape. Hence 0.9 mL/min was optimized flow rate decreasing the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. To evaluate the feasibility of the experiment under regular lab conditions, the assessment of the stability of Paracetamol and Ketoprofen under room temperature and under normal light conditions is done.

Method Validation

System Suitability

The % CV of the peak area for both drugs is within the acceptable criteria (**Table-1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 8032.8±106.30 for Paracetamol and 55003.2±527.44 for Ketoprofen. The USP tailing factor was 1.197 ± 0.0082 for Paracetamol while that of Ketoprofen is 1.12 ± 0.00.

Table-1a: System suitability for Paracetamol

Sample ID	Peak Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	1.50	343890	7975	1.21
2	1.50	349607	7948	1.20
3	1.50	345856	7909	1.20
4	1.50	353890	8056	1.19
5	1.50	354491	8139	1.19
6	1.50	353989	8170	1.19
MEAN	**1.500**	**350287.2**	**8032.8**	**1.197**
STDEV	**0.0000**	**4590.81**	**106.30**	**0.0082**
%CV	**0.00**	**1.31**	**1.32**	**0.68**

Coden : IJABPT, Copyrights@2014, ISSN : 0976-4550

Table-1b: System suitability for Ketoprofen

Sample ID	Peak Retention Time	Peak Area	Theoretical Plates	Tailing Factor
1	6.22	568955	54442	1.12
2	6.21	578133	54642	1.12
3	6.21	573380	54563	1.12
4	6.21	588122	55165	1.12
5	6.20	587237	55554	1.12
6	6.19	585434	55653	1.12
MEAN	**6.207**	**580210.2**	**55003.2**	**1.120**
STDEV	**0.0103**	**7961.24**	**527.44**	**0.00**
%CV	**0.17**	**1.37**	**0.96**	**0.00**

Determination and Quantification Limits (Sensitivity)

Figure-2 represents the chromatogram of limit of detection and limit of quantification. The method is found to be sensitive which can be determined from the data obtained from the (**Table-2)**.

Table -2: Sensitivity of Paracetamol & Ketoprofen

LOD: Paracetamol

SR NO	Paracetamol	
	Retention Time	Peak Area
1	1.51	1853
2	1.51	2936
3	1.51	1934
MEAN	**1.5**	**2241.0**
ST DEV	**0.00**	**603.25**
% CV	**0.00**	**26.92**

LOD: Ketoprofen

SR NO	Ketoprofen	
	Retention Time	Peak Area
1	5.96	2402
2	5.97	2601
3	5.97	2428
MEAN	**6.0**	**2477.0**
ST DEV	**0.01**	**108.17**
% CV	**0.10**	**4.37**

LOQ Paracetamol

SR NO	Paracetamol	
	Retention Time	Peak Area
1	1.51	2185
2	1.51	2137
3	1.52	2158
MEAN	**1.5**	**2160.0**
ST DEV	**0.01**	**24.06**
% CV	**0.38**	**1.11**

LOQ Ketoprofen

SR NO	Ketoprofen	
	Retention Time	Peak Area
1	6.53	8074
2	6.54	7921
3	6.54	7940
MEAN	**6.5**	**7978.3**
ST DEV	**0.01**	**83.39**
% CV	**0.09**	**1.05**

Linearity

The linearity was demonstrated in triplicate. The results of the best fit line (y = mx + c) for the triplicate analysis is given in **Table-3**.

Table-3: Results of best-fit line for triplicate analysis for Paracetamol (above) and Ketoprofen (below)

Paracetamol			
Curve	**Slope**	**Intercept**	**r^2**
1	1319	8671	0.999
2	1335	8682	0.998
3	1320	8652	0.999
Mean	1324	8668	0.9986
Ketoprofen			
Curve	**Slope**	**Intercept**	**r^2**
1	5280	29575	0.999
2	5260	29478	0.998
3	5272	29560	0.997
Mean	5270.6	29537	0.998

The accuracy of the calibration standards was evaluated from the back calculated concentrations **(Table-4)**. All the standards were found to be within the range of 91 – 104 %.

Table- 4: Linearity and Range for Paracetamol (above) and Ketoprofen (below)

Paracetamol					
Sample ID	**Concentration (Microgram/mL)**	**Retention Time**	**Peak Area**	**Back Calc Concentration**	**% Accuracy**
Blank	Blank	**NA**	**0**	NA	**NA**
CC - 01	50.00	1.48	69151	45.85	91.71
CC - 02	100.00	1.49	137304	97.52	97.52
CC - 03	150.00	1.49	206271	149.81	99.87
CC - 04	200.00	1.49	283757	208.56	104.28
CC - 05	300.00	1.50	405579	300.92	100.31
CC - 06	400.00	1.51	536352	400.06	100.02
CC - 07	450.00	1.51	609083	455.20	101.16
CC - 08	500.00	**1.51**	658606	492.75	98.55
	Blank	**NA**	0	**NA**	**NA**

Ketoprofen					
Sample ID	**Concentration (Microgram/mL)**	**Retention Time**	**Peak Area**	**Back Calc Concentration**	**% Accuracy**
Blank	0.00	**NA**	**0**	NA	NA
CC - 01	20.05	6.17	126460	18.35	91.52
CC - 02	40.11	6.17	239267	39.71	99.01
CC - 03	60.60	6.17	357897	62.18	102.61
CC - 04	80.22	6.18	459205	81.37	101.43
CC - 05	120.32	6.18	667164	120.76	100.36
CC - 06	160.43	6.16	874072	159.94	99.70
CC - 07	180.48	6.18	983390	180.65	100.09
CC - 08	200.54	**6.19**	1085227	199.93	99.70
	Blank	**NA**	**0**	**NA**	**NA**

Accuracy and Precision

Accuracy and precision calculated for the QC samples during the intra- and inter –day run are given in the **Table-5**.The intra-day (day-1) and inter-day accuracy for Paracetamol ranged from 99.62 – 104.81 %. while that of Ketoprofen ranged from 103.52 – 109.69 %. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

Table 5a: Results of inter and intra-day accuracy & precision for Paracetamol

Paracetamol	Nominal concentration(µg/mL)		
	125(LQC)	250(MQC)	375(HQC)
DAY 1 (Intra day)			
MEAN (n=6)	102.43	101.57	104.81
STDEV	1.93	0.55	3.29
% CV	1.88	0.54	6.82
DAY 2			
MEAN (n=6)	101.86	100.22	101.32
STDEV	2.12	0.23	4.62
% CV	2.08	0.22	4.55
DAY 3			
MEAN (n=6)	99.72	99.62	100.24
STDEV	1.43	0.67	3.21
% CV	1.43	0.67	3.20

Table 5b: Results of inter and intra-day accuracy & precision for Ketoprofen

Ketoprofen	Nominal concentration(µg/mL)		
	50.03(LQC)	100.27(MQC)	150.40(HQC)
DAY 1 (Intra day)			
MEAN (n=6)	109.69	105.06	106.50
STDEV	4.75	0.81	3.37
% CV	4.33	0.77	3.16
DAY 2			
MEAN (n=6)	106.28	104.20	103.52
STDEV	3.22	0.62	3.52
% CV	3.02	0.59	3.40
DAY 3			
MEAN (n=6)	105.12	106.94	104.23
STDEV	4.24	0.39	4.92
% CV	4.03	0.36	4.72

Specificity

Specificity was determined by comparison of the Blank chromatogram with that of the Standard chromatogram (**Figure-4**)

Room Temperature Stability

Stability studies were done for short term stability up to 12 hrs on the bench top for the MQC levels conditions. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 98.46% for Paracetamol and 99.10 % for Ketoprofen. The results are tabulated in **Table-6**.

Stress Degradation

Stress studies on Paracetamol indicated the stability of drug under oxidative stress,light(UV) , acid and alkaline(0.1N NaOH) conditions (**Figure-5a**).This has been clearly demonstrated by the help of overlap spectra of all the stress samples as compared with that of freshly prepared sample of similar concentration **(Figure-5a)**. For all the stress conditions, the Paracetamol content was within 99.56 –101 % indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Stress studies revealed that Ketoprofen is not susceptible to degradation under oxidative stress, light (UV) acid stress conditions (**Figure-5b**). However, in alkaline conditions (0.1N NaOH) , the drug was unstable and the degradation peak eluted earlier accompanied with a drastic peak distortion and increased tailing. Except for alkaline conditions, the drug content was within 100.16–100.17 % for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Table- 6a: Room Temperature Stability of Paracetamol (n=6)

FRESH SAMPLE Paracetamol

SR NO	SAMPLE ID	DRUG	
		RETENTION TIME	PEAK AREA
1	FRESH SAMPLE	1.50	270160
2	FRESH SAMPLE	1.50	271975
3	FRESH SAMPLE	1.50	269901
4	FRESH SAMPLE	1.51	271909
5	FRESH SAMPLE	1.51	274401
6	FRESH SAMPLE	1.51	275527
MEAN			**272312.17**
STDEV			**2254.78**
% CV			**0.83**

STABILITY SAMPLE Paracetamol

SR NO	SAMPLE ID	DRUG	
		RETENTION TIME	PEAK AREA
1	STABILITY SAMPLE	1.51	247472
2	STABILITY SAMPLE	1.51	270120
3	STABILITY SAMPLE	1.51	273364
4	STABILITY SAMPLE	1.51	271729
5	STABILITY SAMPLE	1.51	274218
6	STABILITY SAMPLE	1.51	271860
MEAN			**268127.17**
STDEV			**10218.00**
% CV			**3.81**

% Stability: 98.46

Table- 6b: Room Temperature Stability of Ketoprofen (n=6)

FRESH SAMPLE Ketoprofen

SR NO	SAMPLE ID	DRUG	
		RETENTION TIME	PEAK AREA
1	FRESH SAMPLE	6.28	541442
2	FRESH SAMPLE	6.28	544228
3	FRESH SAMPLE	6.28	539300
4	FRESH SAMPLE	6.31	542516
5	FRESH SAMPLE	6.31	548217
6	FRESH SAMPLE	6.32	549720
MEAN			**544237.17**
STDEV			**4026.20**
% CV			**0.74**

STABILITY SAMPLE Ketoprofen

SR NO	SAMPLE ID	DRUG	
		RETENTION TIME	PEAK AREA
1	STABILITY SAMPLE	6.25	556734
2	STABILITY SAMPLE	6.27	540595
3	STABILITY SAMPLE	6.23	544243
4	STABILITY SAMPLE	6.27	522057
5	STABILITY SAMPLE	6.27	532256
6	STABILITY SAMPLE	6.25	540260
MEAN			**539357.50**
STDEV			**11643.84**
% CV			**2.16**

% Stability: 99.10

Coden : IJABPT, Copyrights@2014, ISSN : 0976-4550

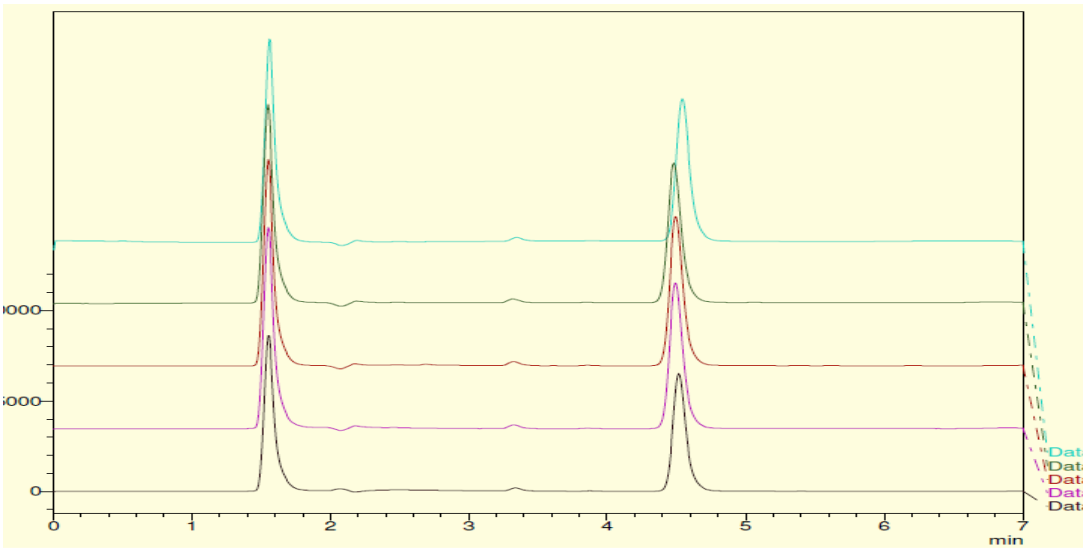


Figure-5a: Overlap Chromatogram showing the influence of various stress conditions on Paracetamol;

Data 1-Fresh sample;Data 2- Oxidative stress;Data 3- Photolytic stress;Data 4- Acid stress;Data 5-Alkaline stress

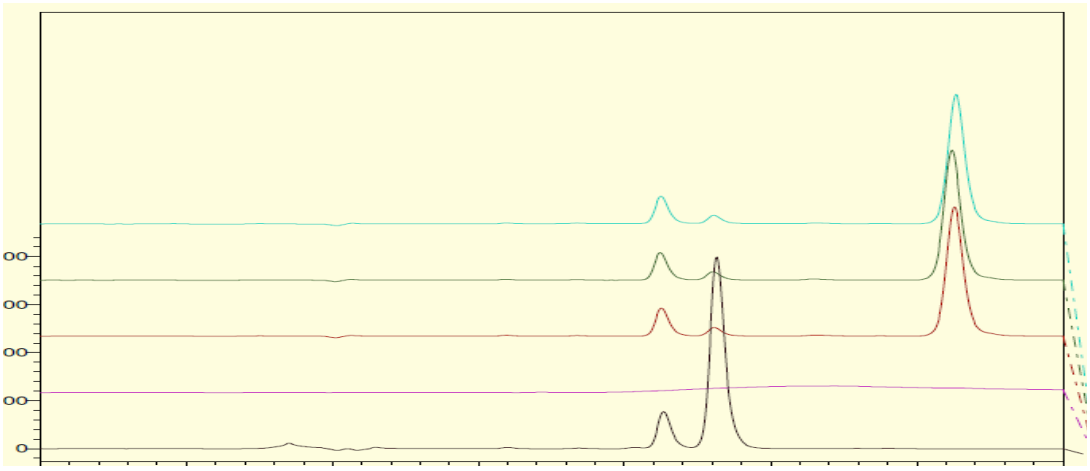


Figure-5b: Overlap Chromatogram showing the influence of various stress conditions on Ketoprofen;

Data 1-Fresh sample; Data 2-Oxidatve stress; Data 3- Photolytic stress; Data 4- Acid stress; Data 5-Alkaline stress

Robustness study

Robustness is the measure of method capacity to remain unaffected by deliberate small changes in the chromatographic conditions. The experimental conditions were deliberately altered to evaluate the robustness of the method. The impact of flow-rate (0.9 ± 0.1 mL/min), and effect of mobile-phase composition (± 5%) on chromatographic parameters such as retention time, theoretical plates, and tailing factor, were studied**.** At normal flow rate, the retention time of Paracetamol was 1.5 ± 0.00 minutes (n=6) while that of Ketoprofen was 6.27 ± 0.01 minutes. At normal flow rate, the tailing factor for Paracetamol is 1.26 ± 0.00 while that of Ketoprofen was 1.13 ± 0.00. At higher flow rate, tailing factor for Ketoprofen remained unchanged as compared to normal flow while it is 1.307±0.01 for paracetamol. At a lower flow rate of 0.8 mL/min, Paracetamol and Ketoprofen eluted at 1.73 ± 0.01 and 7.42± 0.07 minutes respectively.

The tailing factor of Paracetamol and Ketoprofen were 1.167 ± 0.01 and 1.09 ± 0.0 respectively (n=6). At mobile phase composition of 75: 25 % v/v of Methanol and 0.1% v/v orthophosphoric acid the retention times of Paracetamol and Ketoprofen were 1.52 ± 0.01 and 5.25 ± 0.01 minutes (n=6). At mobile phase composition of 65:35 % v/v of Methanol and 0.1% v/v orthophosphoric acid the retention times of Paracetamol and Ketoprofen were 1.57 ± 0.00 and 8.69± 0.28minutes (n=6) respectively.

Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of Paracetamol and Ketoprofen. Also the method is validated for different parameters; hence it has been applied for the simultaneous estimation in pharmaceutical dosage forms. The amount of Paracetamol and Ketoprofen in the commercial tablet dosage form is within the pharmacopoeial specifications. None of the tablets ingredients interfered with the analyte peak.The spectrum of Paracetamol and Ketoprofen in the extracted tablet was matching with that of standard compounds indicating the purity of the compounds in the tablets.

CONCLUSIONS

The method gave accurate and precise results in the concentration range of 50.00 to 500µg/mL for Paracetamol and 20.05 to 200.54 µg/mL for Ketoprofen.The mobile phase composition consists of 70:30 % v/v of Methanol and 0.1% Orthophosphoric acid at the flow rate of 0.9 mL/min.The retention time of Paracetamol is 1.50 ± 0.00 minutes and that of Ketoprofen is 6.27 ± 0.01 minutes.The column is YMC, C4 (150 x 4.6 mm) with the particle size of 3µm.A rapid sensitive and specific method for the simultaneous estimation of Paracetamol and Ketoprofen in the pharmaceutical tablet formulations has been developed and validated.

REFERENCES

Ashraful SM; Abuzar SM; Paul PK (2011). Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of Paracetamol and Aceclofenac in marketed tablets, International journal of pharmacy and Life Science, volume 2, issue 12, pages 1267-1275.

Blanko M., Coello J., Iturriaga H., Maspoch S. and Perez-Maseda, C (1998). Chiral and nonchiral determination of ketoprofen in pharmaceuticals by capillary zone electrophoresis. J Chromatography A, volume 799, pages 301-307.

Dimal AS; Neel JP; Sunil, LB; Usman KC; Kashyap KB (2011). Stability Indicating LC-Method for Estimation of Paracetamol and Lornoxicam in Combined Dosage Form, J Sci Pharm, volume 79, pages 113-122 .

Dvorák J, (2004). Simultaneous HPLC determination of ketoprofen and its degradation products in the presence of preservatives in pharmaceuticals. J Pharm Biomed Anal. Volume 36, issue 3,pages 625-629.

International conference on harmonization. (2003). Q1A(R-2) stability testing of new drug substances and products International conference on harmonization. IFPMA, Geneva .

Kumble RM, Singh SG (2012). Stability-indicating RP-HPLC method for analysis of paracetamol and tramadol in a pharmaceutical dosage form. E Journal of Chemistry. Volume 9, issue 3, pages 1347-1356.

Kormosh Z., Hunka, I and Basel Y (2009). Spectrophotometric determination of ketoprofen and its application in pharmaceutical analysis. Acta Pol. Pharm.-Drug research, volume 66, pages 3-9.

Labbozzetta S., Valvo L., Bertocchi, P, Alimouti S, Gandiano M. and Manna L (2009). Focused microwave-assisted extraction and LC determination of ketoprofen in the presence of preservatives in a pharmaceutical cream formulation. Chromatographia, volume 69, pages 365-368.

Mannucci C., Bertini J., Cocchini A. and Perico F (1992). High performance liquid chromatography simultaneous quantitation of ketoprofen and parabiens in a commercial gel formulation. J Liquid Chromatography, volume 15, pages 327-335.

Patil, V. Raheja, A. Damre (2010). Simultaneous analysis of intestinal permeability markers, caffeine, paracetamol and sulfasalazine by reverse phase liquid chromatography: a tool for the standardization of rat everted gut sac model, Asian J Pharm Clin Res, volume 3, pages 204-207.

Pattan SR; Jamdar SG; Godge RK; Dighe NS; Dathankar AV; Nirmal SA; Pai MG (2009). RP-HPLC Method for simultaneous estimation of Paracetamol and Etoricoxib from Bulk and Tablets, volume 1, issue1, pages 329-335.

Coden : IJABPT, Copyrights@2014, ISSN : 0976-4550

Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonization. (1996) Washington, DC.

Wong, C., Yeh, M. and Wang, D, (1992). High-performance liquid chromatographic determination of ketoprofen in pharmaceutical dosage forms and plasma. J Liquid Chromatography, volume 15, pages 1215-1225.