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A VALIDATED STABILITY–INDICATING HPLC METHOD ESTIMATION OF KETOPROFEN IN THE PRESENCE OF PRESERVATIVE IN THE BULK DRUG AND FORMULATED GEL

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ABSTRACT

A new stability-indicating high-performance liquid chromatographic method for simultaneous analysis of Ketoprofen, Methyl paraben and Propyl paraben in the bulk drug and Formulated Gel form was developed and validated. The chromatographic separation was performed on C18 Column (250mm × 4.6mm, 5µm particle size) using a mobile phase Methanol: Water (50:50v/v)at a flow rate of 1.0 ml/min and 30°C column temperature with the detection wavelength at 233nm using photodiode diode array detector. The retention times of Ketoprofen (RT 4.95 min) and Methyl paraben (RT 6.25 min) propyl parben (RT 9.70 min). The linearity was performed in the concentration range of 5 to 25 µg/mL 4 to 20 µg/mL and 2.5 to 12.5 µg/mL for Ketoprofen, Methyl paraben and Propyl paraben respectively with a squared correlation coefficient of 0.999, 0.999 and 0.9983 for MP, KETO and PP respectively. The percentage purity of Ketoprofen, Methyl paraben and Propyl paraben were found to be 99-101%. Precision (Inert-day) of the system was found to be 0.36% and 0.18%, 0.11% for Ketoprofen, Methyl paraben and Propyl paraben respectively and all the method was found to be specific and found to be within the limits of the acceptance criteria The limit of detection was 0.087 µg/mL and 0.037 µg/mL, 0.012 µg/mL for Ketoprofen, Methyl paraben and Propyl paraben respectively and limit of quantification were 0.77µg/mL and 0.34µg/mL,

0.13µg/mL for Ketoprofen, Methyl paraben and Propyl paraben respectively and the method was found to be specific. Forced degradation conditions of hydrolysis (neutral, acidic and alkaline), oxidation, photolysis and thermal stress, as suggested in the ICH guideline Q1A (R2). The drug showed instability in acid and oxide, while it remained stable in alkaline conditions. The method has proven specificity for stability indicating assay method.

Keyword: Ketoprofen, Methyl paraben. Propyl paraben, Validation, Force degradation studies

INTRODUCTION

Currently over 50 pharmaceuticals are known that, though different in their chemical structure, are classified as non-steroidal anti-inflammatory drugs (NSAIDs).[1] They are widely used in medical practice and are important components in the treatment of rheumatoid arthritis[2] and osteoarthritis.[3] The incidence of rheumatoid arthritis is high; yearly diagnostics add some 9.000 new patients, of which 67 -68% are of able-bodied age.[4] Ketoprofen, (Fig 1) [2-(3-benzoylphenyl) propionic acid] is a non-steroidal anti-inflammatory and analgesic agent.

A new pharmaceutical preparation (gel) containing Ketoprofen (KETO) as an active compound with anti-inflammatory and analgesic activity was developed for treatment of diseases of the muscolo-skeletal apparatus, in which a local action is preferred. In order to prevent bacterial growth during the storage of the formulation[5, 6], two commonly used preservatives—a mixture of the methyl ester and propyl ester of p-hydroxybenzoic acid (methyl paraben (MP) (Fig 2) and propyl paraben (PP) (Fig 3)-have been used gas chromatography–mass spectrometry (GC–MS) [7], capillary electro chromatography [8], high-performance liquid chromatography (HPLC) [9–11], HPLC–MS [12,13] or micellar chromatography [14] as well. Only one HPLC method has been found in literature [15] for simultaneous determination of KETO and its degradation products, but not in the presence of preservatives. Recently, preservatives in pharmaceuticals have to be quantified. HPLC analysis of MP and PP is frequently described in the literature [16–18]; another publication deals with simultaneous quantification of Ketoprofen and Parabens in a commercial gel formulation by RP–HPLC with UV detection [19], but there is no any HPLC method describing simultaneous determination of all three components—Ketoprofen, MP and PP—in pharmaceutical preparations with no any HPLC method describing simultaneous determination in this mobile phase with beneficial system suitability parameter.

HPLC is distinguished from traditional low pressure liquid chromatography because small sample amount separated in analytical HPLC. This gives HPLC superior resolving power when separating mixtures, which is a popular chromatographic technique. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase which is economic.

For such a formulation, a novel method capable to analyse simultaneously the active component Ketoprofen, its and two preservatives Methyl paraben and Propyl paraben were developed. Thereafter, this HPLC method [20] was successfully applied for the separation, quantification and stability study of all these compounds in formulated Ketoprofen gel 2.5%. It is, therefore, necessary to develop a new stability-indicating method for the determination and quantitative estimation of KETO, MP and PP from bulk and formulated gel. The advantages of HPLC are a technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. In the present work we are focused on to achieve the optimum chromatographic conditions for the simultaneous determination KETO, MP and PP in the bulk and formulated gel. The developed method can be applied successfully to quality control purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines.

MATERIALS AND METHODS

All the reagents as Methanol (HPLC grade), O-phosphoric LC system (Agilent LC Model-1200 with Ezchromelite Software) containing C8 (Qualisil BDS, 250×4.6 mm, 5 µ) column with PDA detection, Labindia -3000+ UV/Vis double beam spectrophotometer with a fixed slit width 1nm and 1cm matched quartz cells were employed in the present study. Reference standard ketoprofen and methyl paraben and propyl paraben were procured from ZIM laboratories Nagpur, India as gift samples. Formulated gel formulation (Ketoprofen 2.5% w/w)

Preparation of Standard Concentrations

Stock Solution KETO, MP and PP

Accurately weighed quantity (100 mg) of KETO, MP and PP respectively were transferred to 100.0 mL volumetric flask, dissolved and diluted up to the mark with mobile phase. From this solution, 5.0 mL was transferred to 50.0 mL volumetric flask and diluted to the mark with mobile phase (Concentration 100 µg/mL) KETO, MP and PP respectively. The solution was mixed and filtered through 0.2 µ membrane filter.

Sample solution of KETO, MP and PP

An accurately weighed quantity of 1000 mg KETO and 400 mg MP, 40mg PP were transferred to 100.0 ml volumetric flasks, 50 ml of mobile phase was added; the content was dissolved and diluted up to the mark with mobile phase. The resulting solution was mixed and filtered through Whatman filter paper 42 and filtrate was appropriately diluted to obtain final concentration (1000 µg/ml KETO and 400 µg/ml MP, 40 µg/ml PP respectively).The solution was mixed and filtered through 0.2 µ membrane filter. The solution was mixed and filtered through 0.2 µ membrane filter. Equal volume of standard and sample solution (10 µl) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions

Chromatographic Conditions

Different kinds of equipment like Analytical weighing balance, HPLC system (SHIMADZU-SPD 20A), Injector (Rheodyne,20 µl), Sonicator, pH meter, Vacuum filter pump, Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's was used throughout the experiment. Grace C18 ACME 9000, C18 reverse phase column of 250x 4.0mm i.e., 5µm dimensions Detector: UV, D2 lamp, 233 nm Column Temperature: Controlled room temperature (25°C) Injection: 10 µl sample loop was used for the separation Ketoprofen, Methyl Paraben, Propyl Paraben with the help of mobile phase consisted of a mixture.

Assay of Formulated Gel

Preparation of Standard Solution: Standard solution of KETO, MP and PP containing concentration of 1000 µg/ml KETO and 400 µg/ml MP, 40 µg/ml PP respectively was prepared in methanol.

Preparation of Formulated gel

Six Gel sample solutions were prepared and analyzed in following manner:

An accurately weighed quantity of gel was weighed equivalent to about 1000 mg of Ketoprofen and 400mg of Methyl paraben and 40 mg Propyl paraben into a 100.0ml volumetric flask, mobile phase was then added, mixture was ultra sonicated for 30 min with heating and allowed to cool at room temperature before adjusting to volume with mobile phase. The organic layer was decan-ted and the extraction procedure was repeated.

The resulting mixture was centrifuged at 3500 rpm for 20 min and 10 uL of the clear supernatant was injected directly onto the column. After centrifugation 10 µl of this mixture was injected into the chromatograph. The resulting solution was mixed and filtered through Whatman filter paper 42 and filtrate was appropriately diluted to obtain final concentration of 1000 µg/ml of KETO and 400 µg/ml of MP, 40 µg/ml of PP. The diluted solution was filtered through 0.2u filter.

Equal volume of standard and sample solution (10 µl) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peak for KETO, MP and PP were measured at 233 nm. Amount of KETO, MP and PP in sample (mg) were calculated by comparing the mean peak area of standard and sample solution.

The content KETO, MP and PP in sample were calculated using following equation.

$$\text{Amount of drug estimated in sample (mg)} = \frac{PA_{Sample}}{PA_{Standard}} \times C_S \times d_f$$

Where, PA_{Sample} = Peak area of sample,

$PA_{Standard}$ = Peak area of standard,

C_S = Concentration of standard (g/lit.),

d_f = Dilution factor.

Percent label claim was calculated using below equation.

$$\text{Percent label claim} = \frac{\text{Amount of drug estimated in sample (mg)} \times 100}{\text{Label claim (mg)}}$$

METHOD VALIDATION PROCEDURE

Specificity

Specificity was demonstrated showing that neither formulation excipients nor degradation products interfered with quantification of KETO and MP, PP indicating that the proposed method can also be used in a stability assay are depicted in Fig .4

Linearity of response

Ketoprofen: From standard stock solution A, aliquot portion was appropriately diluted to obtain concentration of 100 µg/ml from above solution take 0.5ml, 1ml, 1.5ml, 2.0ml and.2.5 were transferred individually to 10.0 ml volumetric flask and diluted to the mark with mobile phase (Concentration 5,10,15,20 and 25 µg/ml, respectively). The diluted solutions were filtered through 0.2µ membrane filter.

Methyl paraben: Standard stock solution B was appropriately diluted to obtain concentration of 100 µg/ml. From above solution, 0.45ml, 0.85ml.1.25ml, 1.65ml and 2.05ml were transferred individually to 10.0 ml volumetric flask and diluted to the mark with mobile phase (Concentration 4.5, 8.5, 12.5, 16.5 and 20.5 µg/ml, respectively). The diluted solutions were filtered through 0.2µ membrane filter.

Propyl paraben: Standard stock solution C was appropriately diluted to obtain concentration of 100µg/ml. From above solution, 0.25ml, 0.5ml, 0.75ml, 1.0ml and 1.25ml were transferred individually to 10.0 ml volumetric flask and diluted to the mark with mobile phase (Concentration 2.5, 5.0, 7.5, 10.0 and12.5µg/ml, respectively). The diluted solution was filtered through 0.2µ membrane filter.

Precision of the assay

Intra-day Precision

Intraday precision was determined by analyzing gel sample solutions at different time intervals on the same day. Gel sample solution was prepared and analysed in the similar manner as described under analysis of the gel formulation.

Inter-day Precision

Inter-day precision was determined by analyzing gel sample solutions on three different days. Gel sample solution was prepared and analysed in the similar manner as described in analysis of the gel formulation. (Table10). The RSD for both intra-day and inter-day precision study was found to be less than 2 indicating the repeatability and reproducibility of method

Limit of detection (LOD) and quantification (LOQ)

The limits of detection and quantification of the developed method were calculated using 3 σ /S and 10 σ /S phenomena, respectively. Where, σ is the standard deviation of the y-intercepts and S is the slope of the calibration curve. Limits of detection were 0.087µg/mL and 0.037

µg/mL, 0.012 µg/mL for KETO, MP and PP respectively and limit of quantification were 0.77µg/mL and 0.34µg/mL, 0.13µg/mL for KETO, MP and PP respectively shown in Table 11

Accuracy

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

Preparation of Standard Solutions: Standard solution D of KETO, MP and PP containing concentration of 1000 µg/ml KETO and 400 µg/ml MP, 40 µg/ml PP respectively was prepared in methanol.

Preparation of Sample Solution

An accurately weighed quantity of pre-analysed gel equivalent to about 1000 mg KETO and 400mg MP and 40mg PP were transferred individually in nine different 100.0 ml volumetric flasks. To each of the flask following quantities of KETO, MP and PP were added:

Flask No.1: 800 mg KETO + 320 mg MP +32mg PP

Flask No.2: 800 mg KETO + 320mg MP +32mg PP

Flask No.3: 800 mg KETO + 320mg MP +32mg PP

Flask No.4: 1000mg KETO + 400mg MP +40mg PP

Flask No.5: 1000 mg KETO + 400mg MP +40mg PP

Flask No.6: 1000 mg KETO + 400mg MP +40mg PP

Flask No.7: 1200 mg KETO + 480 mg MP +48mg PP

Flask No.8: 1200 mg KETO + 480mg MP +48mg PP

Flask No.9: 1200 mg KETO + 480mg MP +48mg PP

Then 50 ml mobile phase was added to each flask and content of the flask were ultrasonicated for 20 minutes, volume was then made up to the mark with mobile phase. The resulting solution was mixed and filtered through Whatman filter paper. 42 and filtrate was appropriately diluted to obtain final concentration 1000 µg/ml of KETO and 400 µg/ml of MP, 40 µg/ml of PP. The diluted solution was filtered through 0.2 µ filter.

Equal volume of standard and sample solution (10 µl) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions. The

corresponding chromatograms were recorded and area of each peak for KETO, MP and PP were measured at 233 nm. Results are shown in Table 8, 9

Amount of KETO, MP and PP in sample were calculated by comparing the mean peak area for standard and sample solution by above equation. (1)

Percent Recovery was calculated by using below equation.

$$\text{Percent Recovery} = \frac{A - B}{C} \times 100$$

Where, A – Total amount of drug estimated (mg)

B -- Amount of drug contributed by tablet powder (mg)

C-- Amount of Pure drug added (mg)

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on retention time and tailing factor were studied. The solution containing 1000 µg/mL of KETO and 400µg/mL of MP and 40 µg/mL of PP was injected (in triplicate) into sample injector of HPLC three times under the varied conditions. Robustness data is given in Table No. 12

Ruggedness

The gel formulation was analyzed by proposed method by different analyst and in different Laboratory. The results are shown in Table 15 for different analyst and Table 16 for different laboratory.

Force degradation studies

In order to evaluate the stability indicating property of the developed HPLC method stress studies were carried out under ICH recommended conditions. Intentional degradation was tried by exposing the tablet sample to following stress conditions: acid (0.1 N HCl at 60^0C for time 5hrs), base (0.1 N NaOH at 60^0C for time 5hrs), oxidation (3 % H2O2 at 60^0C for time 5hrs), and heat (60 ^{0}C for time 5hrs), and UV light (254 nm for time 5hrs). Ability of the proposed method to measure the analyte response in presence of its degradation products was studied. The results for forced degradation studies are included in Table 13. Typical chromatography obtained for KETO, MP and PP under different stress conditions are shown

in Fig. 8-12. The developed HPLC method could effectively resolve the drugs from their degradation products which confirm the stability indicating power of the developed method.

Stability

The stability evaluation of KETO and MP, PP in sample solutions (constituted with methanol) was performed up to 24 h. The sample solutions placed in the autosampler at room temperature were analysed periodically at 1, 2, 4, 12 and 24 h. The results are shown in Table 14. The peak areas of each drug were not considerably different from each other. Moreover, RSD value of each sample was <1%. The slight increase in peak area with the passage of time suggested that small evaporation of the solvent took place resulting in increased concentration of the sample. According to our results the drugs dissolved in methanol were stable at room temperature during analysis over a period of 24 h.

The corresponding chromatograms were recorded and area of each peak for KETO and MP, PP was measured at 233.0 nm. Amount of KETO and MP, PP in sample (mg) was calculated by comparing the mean peak area of standard and sample solution.

RESULTS AND DISCUSSION

In this study, separation of KETO, MP and PP were done on C18 column Optimization of mobile phase composition was performed based on resolutions among drugs and preservatives, asymmetric factor and theoretical plates. Methanol: water in the ratio (50:50 v/v) [pH 4.5adjusted with Ortho phosphoric acid (dil)] was selected as an appropriate mobile phase was acceptable system suitability parameters for KETO, MP and PP was delivered at a flow rate of 1 ml/min with detection wavelength 309 nm for KETO, 302 nm for MP and 287nm for PP. The injection volume was 20 µl. Analysis was performed at a temperature of 30^0C, which shown in Fig 4. For system precision six replicate injections of standard solution were given, tailing factor (T), resolution (R) and column efficiency (number of theoretical plates, N) were studied of KETO and MP, PP was recorded for each injection. The % RSD for peak area was calculated and is shown in Table 1. A linear relationship was observed at selected wavelength are shown in Table 2 and the standard calibration curves of Mean Peak Area vs. Concentration are depicted in Fig.5 and 6, 7 for KETO, MP and PP respectively. Results of analysis of sample laboratory mixture are shown in Table No. 3, 4.

The resulting mixture of formulated gel was recorded and area of each peak for KETO.MP and PP were measured at 233 nm. Amount of KETO, MP and PP in sample (mg) was

calculated by comparing the mean peak area of standard and sample solution. Results of analysis of Gel formulation are shown in Table 6, 7.

The results of precision studies were expressed in terms of relative standard deviation (RSD) of the percent label claim determined by developed method are shown in Table10. The RSD for both intra-day and inter-day precision study was found to be less than 2 indicating the repeatability and reproducibility of method. The limits of detection of the developed method were calculated 0.087µg/mL and 0.037 µg/mL, 0.012 µg/mL for KETO, MP and PP respectively and limit of quantification were 0.77µg/mL and 0.34µg/mL, 0.13µg/mL for KETO, MP and PP respectively shown in Table 11. It is apparent from this table that changing the column temperature had a noticeable effect on KETO, MP and PP chromatographic peak shape. While, changing the flow rate had an obvious effect on the content of KETO, MP and PP but almost no effect on KETO, MP and PP chromatographic peak shape.

The percent recovery at three levels (80 %, 100 % and 120 %) was found to be in the range of 98-102 %. Amount of KETO, MP and PP in sample was calculated by comparing the mean peak area for standard and sample solution Results of recovery studies are shown in Table 8, 9. The solution containing 25 µg/mL of KETO and 12.5 µg/mL of MP and .5 µg/mL of PP was injected (in triplicate) into sample injector of HPLC three times under the varied conditions. Robustness data is given in Table No. 12.

The stability evaluation of KETO and MP, PP in sample solutions (constituted with methanol) was performed up to 24 h. The sample solutions placed in the autosampler at room temperature were analysed periodically at 1, 2, 4, 8 and 24 h. The results are shown in Table 14. In the forced degradation studies KETO, MP and PP were found to degrade under acidic and oxidative stress conditions employed. However it was found to be stable to the alkaline and unstable in photo degradation, thermal and neutral conditions employed. The results for forced degradation studies are included in Table 13.

We determined the effect of flow rate and mobile phase ratio on retention time and tailing factor were studied, using sample solution for the durability study. The chromatographic conditions investigated are listed in Table 15.

Typical chromatography obtained for KETO, MP and PP under different stress conditions are shown in Fig. 8-12. The present HPLC method for simultaneous determination of KETO.MP and PP proved to be simple, rapid, precise, accurate and robust in their formulated Gel dosage and validated as per ICH guidelines. Moreover the method is economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories.

The Force degradation studies method was found to be simple, sensitive, selective, and suitable for determination of KETO.MP and PP in presence of its degradation products in acidic, alkaline and oxidation, thermal, dry heat condition. Statistical analysis proved that the method is repeatable, reproducible, accurate and specific for the analysis of KETO.MP and PP. The developed HPLC method which confirm the stability indicating power of the developed method.

As the method in cost effective and less time consuming, it may be more advantageous for regulatory quality control laboratories especially to process the post- marketing surveillance program. Thus, it can represent another good alternative for the already existing HPLC methods.

Table 1: System Suitability Parameters

Parameter	KETO	MP	PP
Resolution	2.456		4.456
Tailing factor (T)	1.18	1.61	1.01
No. of theoretical plates (N)	7540	5302	9451
System precision (% RSD for peak area)	0.788	0.414	0.156
Method precision (% RSD for peak area)	0.578	0.314	0.114

Table 2: Standard Calibration Table for Ketoprofen, Methyl paraben and Propyl paraben

KETO		MP		PP	
Concentration (µg/ml)	**Mean *Peak Area**	**Concentration (µg/ml)**	**Mean* Peak Area**	**Concentration (µg/ml)**	**Mean* Peak Area**
5	36882	4	35382	2.5	2213
10	75150	8	68065	5	4870
15	110648	12	928147	7.5	6641
20	147531	16	127530	10	9155
25	184413	20	156913	12.5	12068

*denotes average of three determination.

TABLE 3: Results of Analysis of Sample Mixture

Amount of drug/preservative taken (mg))			Mean Peak Area*			Amount of pure Drug/preservative estimated (mg)			% Estimation		
KETO	MP	PP	KETO	MP	PP	KETO	MP	PP	KETO	MP	PP
1000	400	40	74150	68728	9150	1000.3	399.8	40.21	100.02	99.87	100.9
1000	400	40	74134	69180	9145	999.91	400.3	40.32	99.98	100.23	100.9
1000	400	40	74150	69020	9115	1000.3	399.8	39.11	100.04	99.98	99.38
1000	400	40	74109	68784	9129	999.61	389.6	40.43	100.03	97.43	101.6
1000	400	40	74149	69182	9113	1000.0	400.3	39.16	100.0	100.23	99.63
1000	400	40	74180	69290	9130	1000.46	400.37	40.18	101.2	100.43	101.7

*denotes average of three determinations.

Table 4: Results of Statistical Validation for Sample Laboratory Mixture

Mean	100.26	101.20	100.09
S.D.	±0.5944	±0.4472	±0.4242
C.V	±0.5289	±0.4124	±0.4456

Table 5: Composition of the Carbapol and Pure Drug Ketoprofen as below

Ingredient	Quantity taken
Ketoprofen	2.5g
Methyl Paraben	1.0g
Propyl Parben	0.1g
Carbopol (1 %) as gel base	Q S
Double Distilled water	make up to 100ml
Triethanol amine	Q. S to neutralise gel

Q. S (Quality sufficient)

Table 6: Results of Analysis of Gel Formulation

Amount of Gel equivalent to KETO,MP and PP taken (g)	Mean Peak Area*			Amount of drug/ preservative estimated (mg)			% Label Claim		
	KETO	MP	PP	KETO	MP	PP	KETO	MP	PP
40.00	79285	70181	10112	1000	400	40	100.0	100.00	100.00
40.00	79192	70175	10101	999.8	399.1	39.10	99.52	99.80	99.16
40.00	79152	70145	10104	998.9	398.4	39.79	99.67	98.88	99.80
40.00	79100	70174	10112	989.9	399.4	40.00	98.67	99.88	100.00
40.00	79192	70175	10112	999.1	399.1	40.19	99.23	99.49	100.00
40.00	79150	70175	10112	998.9	399.7	40.00	99.81	99.49	100.00

*denotes average of three determinations.

Table 7: Results of Statistical Validation for of Gel Formulation

Mean	99.72	99.45	100.28
S.D.	±0.4143	±0.4189	±0.4147
C.V.	±0.4278	±0.4146	±0.4929

Table 8: Results of Accuracy Studies

Level of recover	**Weight of Gel taken (g)**	**Amount of drug added (mg)**			**Amount of drug recovered (mg)**			**% Recovery***		
		KETO	MP	PP	KETO	MP	PP	KETO	MP	PP
80 %	40.00	800.01	320.5	32.8	800.17	320.18	32.00	100.11	100.08	100.08
	40.00	800.21	321.5	32.0	800.30	320.18	32.23	100.29	100.09	101.08
	40.00	799.23	319.6	32.1	800.10	319.90	32.00	99.98	99.64	100.00
100 %	40.00	1000.1	400.9	40.8	999.97	400.11	40.11	99.90	100.04	100.69
	40.00	999.98	399.6	40.9	999.98	400.01	41.10	99.94	100.08	102.28
	40.00	998.52	400.13	39.8	998.99	400.00	40.00	99.98	100.00	100.00
120 %	40.00	1199.89	478.4	48.7	1200.10	478.91	47.91	100.03	99.90	99.90
	40.00	1200.00	480.2	47.9	1200.41	480.01	47.09	100.11	100.00	100.19
	40.00	1200.16	479.9	47.8	1199.98	481.01	47.95	99.85	99.98	99.83

Table 9: Results of Statistical Validation for Accuracy Studies

Mean	100.18	101.48	101.22
S.D.	±0.4882	±0.3189	±0.218
C.V.	±0.4828	±0.3127	±0.2289

Table 10: Results of Precision

Intra-day Precision			
Drug	% Mean*	S. D.	C. V.
KETO	99.90	± 0.5889	0.5881
MP	99.87	± 0.1924	0.1948
PP	99.91	±0.1101	±0.1156
Inter-day Precision			
KETO	99.90	± 0.3773	0.3698
MP	99.79	± 0.1876	0.1875
PP	99.91	± 0.1191	0.1176

*denotes average of three determinations

Table 11: LOD and LOQ

Parameter	**KETO**	**MP**	**PP**
Limit of Detection (µg/mL)	0.0379	0.0179	0.0124
Limit of Quantification (µg/mL)	0.0516	0.0441	0.0341

Table 12: Results of Robustness Studies

Chromatographic Changes				
Factor	**Level**	**RT Value**		
Mobile phase composition (± 0.1 ml)		KETO	MP	PP
4.9: 5.1	– 0.1	4.91	6.29	9.45
5.0:5.0	0	4.93	6.40	9.71
5.1:4.9	+ 0.1	4.71	6.58	9.69
Amount of Mobile Phase(v/v)(± 1ml)		KETO	MP	PP
9	– 1.0	4.99	6.35	9.47
10	0	4.95	6.21	9.71
11	+1.0	4.77	6.42	9.61

Table 13: Results of Degradation Study

Sr. No.	Stress Condition	Percent assay of active substance (Ketoprofen)	RT Value of degraded product
1.	Acid (0.1 M HCl)	97.66	3.48, 4.05, 7.50
2.	Alkali(0.1M NaOH)	99.53	-
3.	Oxide (3 % H_2O_2)	97.76	3.85,4.80,5.58
4.	Heat (60^0C)	98.82	4.10,4.58
5.	UV (240nm)	98.19	4.53

Table 14: Results of Solution stability

Time	Amount of drug estimated KETO	% Label Claim*	S.D. (±)	% C.O.V.
20 min	99.98	99.88	0.376	0.452
1hr	99.78	99.67	0.311	0.389
2hr	99.04	99.89	0.378	0.392
4hr	100.00	10.00	0.408	0.455
12hr	100.10	10.07	0.455	0.478
24hr	99.98	99.91	0.377	0.394

Table 15 Results of Ruggedness Studies for Different Analyst

Sample	Percent estimation		
	KETO	MP	PP
Analyst-1	99.27	99.70	98.72
Analyst-2	98.42	98.45	98.99
Analyst-3	97.81	97.92	98.10
Mean	98.62	98.90	98.98
SD	± 0.64	±0.67	±0.98
CV	0.72	0.78	1.07

Table 16 Results of Ruggedness Studies for Different Laboratory

Sample	Percent estimation		
	KETO	MP	PP
Laboratory -1	99.78	99.87	98.74
Laboratory -2	99.82	99.42	98.56
Laboratory -3	98.50	98.18	98.86
Mean	98.36	99.15	98.72
SD	±0.75	±0.87	±0.15
CV	0.75	0.87	0.15

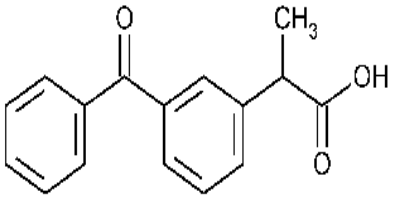

Fig.1: Ketoprofen

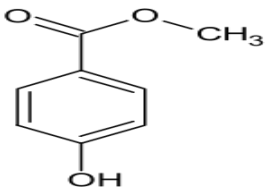

Fig. 2: Methyl Paraben

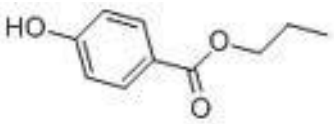

Fig.3: Propyl Paraben

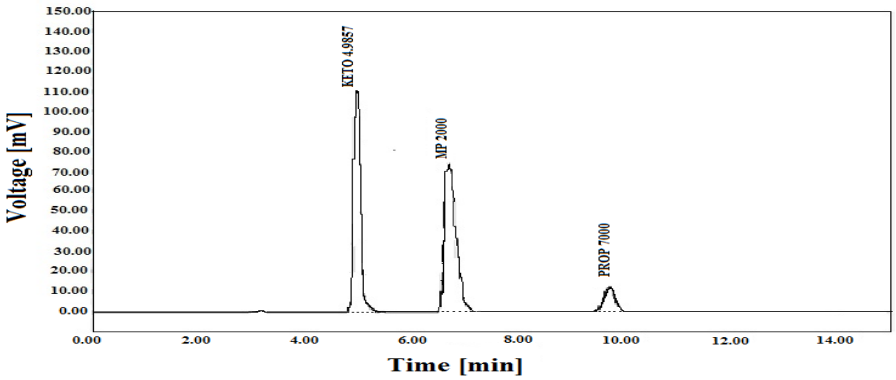

Fig. 4: Typical chromatogram of Ketoprofen (RT 4.95 min) And Methyl paraben (RT 6.25 min) Propyl paraben (RT 9.70 min).

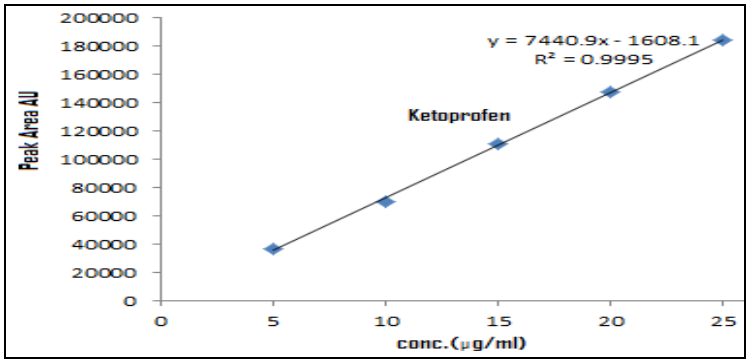

Fig. 5: Standard Calibration Curve for Ketoprofen

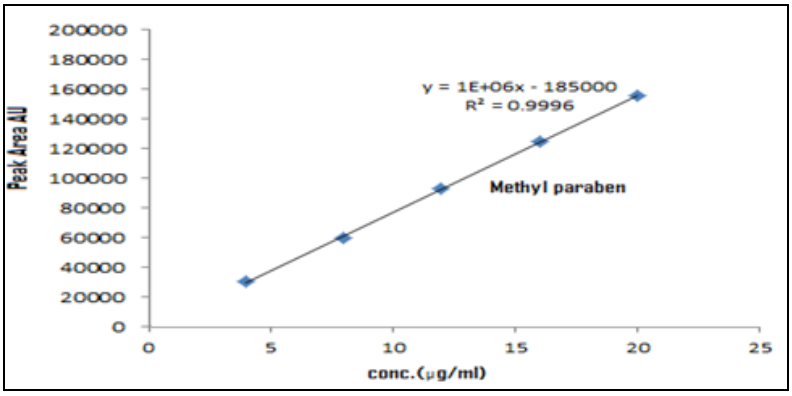

Fig. 6: Standard Calibration Curve for Methyl Paraben

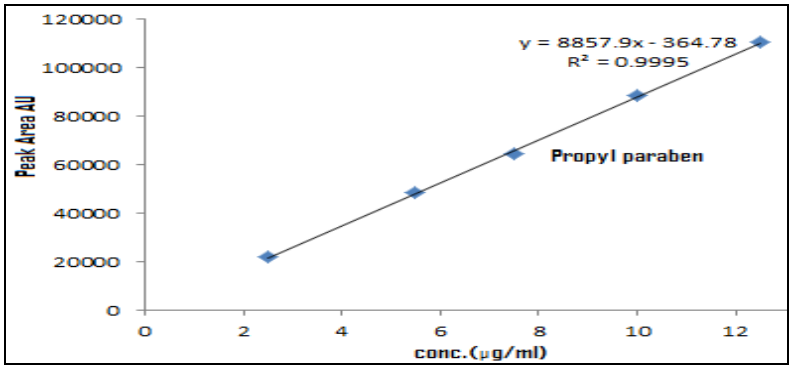

Fig. 7: Standard Calibration Curve for Propyl Paraben

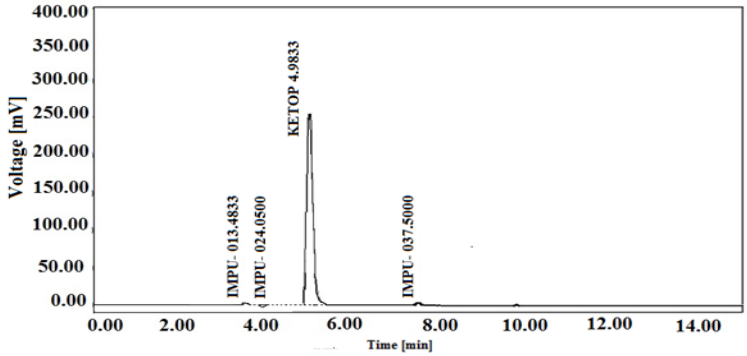

Fig. 8: Chromatogram of 0.1 M HCl Treated GEL Sample

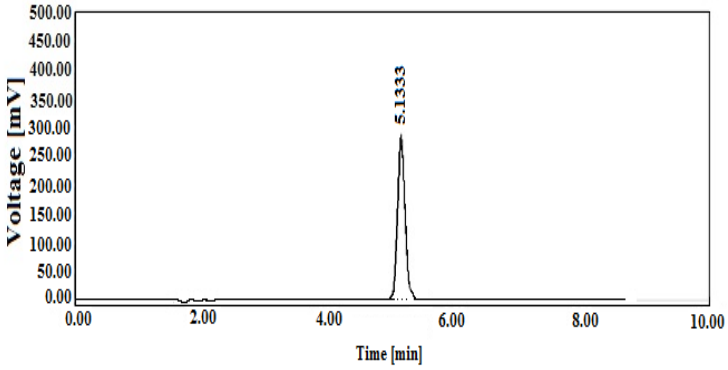

Fig. 9: Chromatogram of 0.1 M NaOH Treated GEL Sample

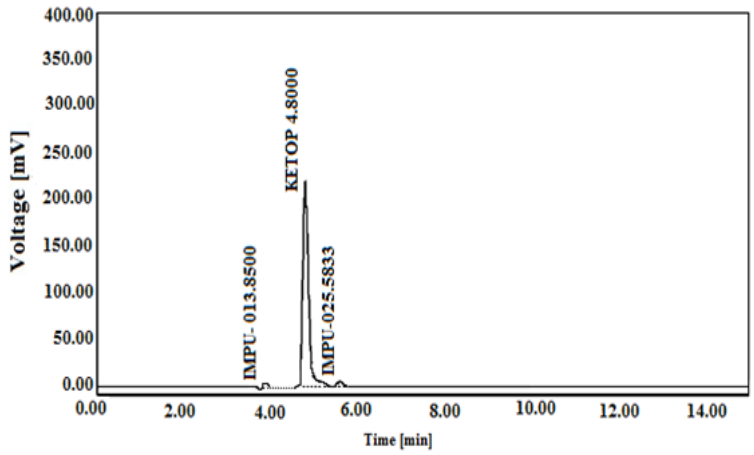

Fig.10: Chromatogram of H_2O_2(3 %) Treated GEL Sample

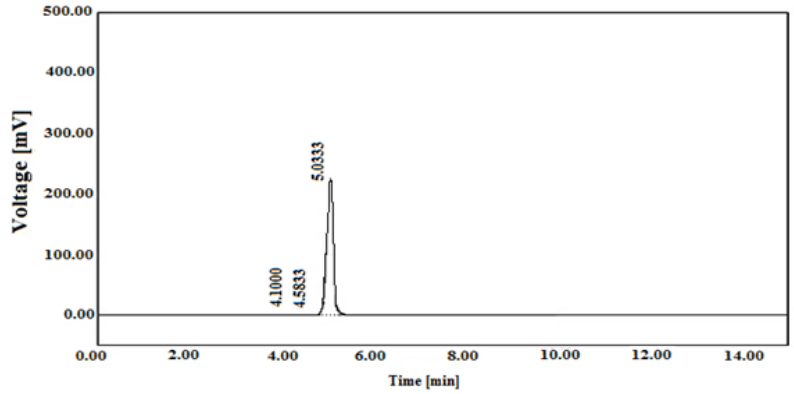

Fig. 11: Chromatogram of Dry Heat Treated GEL Sample

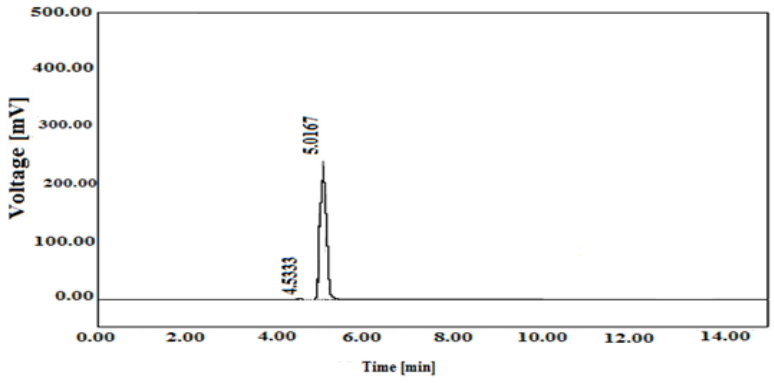

Fig. 12: Chromatogram of UV Radiation Treated GEL Sample

CONCLUSION

The present HPLC method for simultaneous determination of KETO.MP and PP proved to be simple, rapid, precise, accurate and robust in their formulated Gel dosage and validated as per ICH guidelines. Moreover, KETO.MP and PP were found stable in the sample solutions placed at room temperature up to 24 h. accordingly the proposed analytical procedure with

detection time of 10 min can be used for reliable simultaneous determination of KETO, MP and PP in bulk and formulated Gel. Moreover the method is economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories The Force degradation studies method was found to be simple, sensitive, selective, and suitable for determination of KETO.MP and PP in presence of its degradation products in acidic, alkaline and oxidation, thermal, dry heat condition. Statistical analysis proved that the method is repeatable, reproducible, accurate and specific for the analysis of KETO.MP and PP. The developed HPLC method which confirm the stability indicating power of the developed method. As the method in cost effective and less time consuming, it may be more advantageous for regulatory quality control laboratories especially to process the post-marketing surveillance program. Thus, it can represent another good alternative for the already existing HPLC methods.

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