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Q Absorbance ration and area under curve method spectrophotometric method for the simultaneous estimation of Ketoprofen, Methyl Paraben and Propyl Paraben in their formulated gel form

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ABSTRACT

UV Derivative Spectrophotometric methods for Method I -Q absorbance ratioof Ketoprofen (KETO), Methyl Paraben (MP) and Propyl Paraben, (PP) in Gel were formulated in the present work. UV spectrophotometric method was performed at 288nm, 308nm and 309 nm for KETO, MP and PP respectively and Isoabsorptive point - 298nm The linearity lies between 10-50ug/ml for Ketoprofen and 2-10 ug/ml for Methyl Paraben (MP) ,0.2-1.0 ug/ml for Propyl Paraben, (PP) for all the three methods. Method II In the Area under curve method preparation of standard stock solution was same as mentioned in method I from the overlain spectra 290.5 to 297.5 (KETO) and 297.5 to 303 (MP) , 306.5 to 312.5 (PP) were selected for analysis. The calibration curve for Keto ,MP,PP were prepared in the Conc. range as mentioned in method I at the selected wavelength range in Methanol and distilled water (50:50). The linearity lies between 10-50ug/ml for Ketoprofen and 2-10 ug/ml for Methyl Paraben (MP), 0.2-1.0 ug/ml for Propyl Paraben, (PP) for all the three methods. The proposed method percentage recoveries for of KETO, MP and PP were found to be 100.02±1.5409,99.94% ±1.7891,99.97% and , 99.97%±0.7662 for this meth od I and 100.02 % ±1.659 ,99.99% ±1.112,100.00 % ±1.0 08Method II respectively in formulated Gel. The proposed methods are highly sensitive, precise and accurate and therefore can be used for its intended purpose. All the methods showed good reproducibility and recovery with % RSD less than 1. All method were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of in Ketoprofen (KETO), Methyl Paraben (MP) and Propyl Paraben, (PP) bulk and combined dosage form The various parameters, such as linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to (ICH) International Conference on Harmonization guidelines

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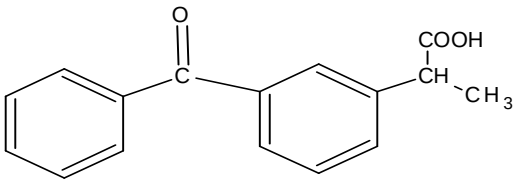
Keywords: Ketoprofen (KETO), Methyl (MP) and Propyl Paraben (PP), Q absorbance ratio, Area Under curve, Validation.

INTRODUCTION

Ketoprofen, (RS) 2-(3-benzoylphenyl)-propionic acid (*chemical formula* C16H14O3) belongs to the non-steroidal anti-inflammatory drugs group (NSAID), which has an important role in today's therapy due to its anti-inflammatory, analgesic and antipyretic action, recommended for the treatment of inflammatory rheumatismal affections, cardiovascular, genital, urological, stomatological diseases and many more [1, 2]. Recently, there have been a number of reports dealing with various analytical methods for the determination of ketoprofen, such as capillary electrophoresis [3,4],Preservatives Methyl paraben methyl 4-hydroxybenzoate. CAS Number: 99-76-3. *Chemical Formula*: C8H8O3 and Propyl paraben Propyl 4-Hydroxybenzoate. The molecular *formula* is C10H12O3are substances added to formulation in order to avoid the multiplication or development of microorganisms in the preparation. Formulators must be fully aware of the procedure for preservative systems in a product need to be analysed to establish their effectiveness throughout shelf life of the product [5]. Many existing analytical procedures are available in literature for the determination of present preservatives studied, either alone or

in combination with other drugs by HPLC and other techniques [6–21]. Steroids, alkaloids, antibiotics, preservatives and vitmnins ,which are often difficult to separate and analyse by other methods, have been determined successfully HPLC The spectrophotometric method was used to determine ketoprofen in capsules or vials with hydroxylamine hydrochloride, in a Na acetate medium and the product of reaction with o-chlorine was measured at 530 nm[22].

Literature survey reveals that Ketoprofen can be estimated by spectrophotometry[23], HPLC[24.25] methods individually or in combination with other drugs. Ketoprofen is reported to be estimated by spectrophotometry[26, 27] and HPLC[28] individually or in combination with other drugs. However, there is no analytical method reported for the estimation of MP and PP and KETO in a combined dosage formulation. Present work describes two methods for simultaneous estimation of MP and PP and KETO in Gel formulation



FIGNo.1 Ketoprofen

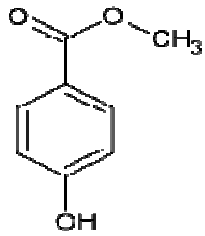


FIG No. 2 Methyl Paraben

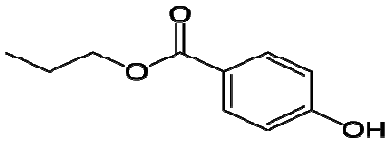


FIG 3 No. Propyl Paraben

MATERIALS AND METHODS

Apparatus:
A double beam UV/Visible spectrophotometer, Shimadzu UV- 1700 Pharma spec, was employed with a pair of 1 cm quartz cells for all analytical work.

Reagents and chemicals:
Ketoprofen was obtained from Zim Lab. Nagpur, Methyl Paraben and Propyl Paraben was obtained from Gen Pharmaceuticals Ltd. Pune, Maharashtra, India as gift sample and was used as working standards. Sodium hydroxide of analytical grade and double distilled water were used throughout the analysis.

Formulation of Ketoprofen Gel:
Ketoprofen gel formulation were prepared using 1% carbopol 940 and as a Gelling agent. Gelling agent was dispersed in a small quantity of distilled water 75ml and then stored overnight to ensure complete hydration. Ketoprofen in a suitable solvent (water) as added to the dispersion and make up weight with distilled water.

Other excipient (methyl paraben 1% and Propyl paraben 0.1%) were also added slowly with continuous stirring. In carbopol gels, pH. Of the vehicle was brought to neutral by using TEA (Triethanol amine).

The final weight of the gel was adjusted to to 100gm with distilled water. Entrapped air bubbles were removed by keeping the gels in vacuum desiccators and shown in the Table 1

Preparation of standard solution:
An accurately weighed quantity of 100 mg KETO was transferred to 100 mL volumetric flasks added 10mg MP and 10mg PP (in solution form) dissolved and diluted using Methanol: Water (50:50)as solvent up to 50 ml and volume make up with solvent and sonic ate up to 20 min .From this solution, 5.0 mL was transferred to 10.0 mL volumetric flask and diluted to the mark with mobile phase (Concentration 25 µg/mL PP and 25 µg/mL MP, and 1000 µg/mL KETO). Working standard solutions were scanned in the entire UV range to determine the λmax. The λmax of KETO, MP and PP were found to be 288nm, 308nm and 309nm respectively

Calibration curve:
Standard dilutions of each drug were prepared separately having concentrations of 10-50 µg/mL for KETO and 2-10 µg/mL MP and concentration of 0.2 -.1.0µg/mL for PP .The absorbance of these standard solutions were measured at 288nm, 308nm and 309nm and calibration curve was plotted. The absorptivity coefficients of the three drugs were determined using calibration curve graph shown below;

Preparation of sample solutions:
An accurately weighed quantity of Gel was weighed equivalent to about 1000mg of Ketoprofen and 400mg of Methyl Paraben and 40mg Propyl Paraben into a 1000-mL volumetric flask. And appropriate amount 500 ml of Methanol: Water (50:50) was then added. The 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 decant-ted and the extraction procedure was repeated. Working standard solution of 10 µg/mL concentration was prepared by appropriate dilution seven standard dilutions of concentrations of 5, 10, 15,20,25,30,35,40,45 and 50µg/mL was prepared from working standard solution. The absorbance of this sample solution was measured at 288nm, 308nm and 309nm and their concentrations were determined using proposed analytical methods.

Quantitative equations method:
Method was based on Quantitative equation method. Primary stock solution was prepared by using Methanol: water (50:50). From this different dilutions were prepared to determine λmax and beer's law range. Calibration curve was prepared by using different concentrations of standard solution. KETO, MP and PP in dosage form were estimated by calibration curve[29, 30]. Developed method was validated as per ICH[31, 33] guidelines with the help of several parameters like accuracy, precision, LOD, LOQ, and stability.[34]

Estimation in the formulated Gel:
An accurately weighed quantity of pre-analysed gel equivalent to about 1000 mg KETO and 400mg MP and 40 mg PP was transferred individually in nine different 1000 mL volumetric flasks. Then added 500ml of Methanol was added to each flask and contents of the flask were ultrasonicated for 30mins with heating and allowed to cool at room temperature before adjusting to volume with Methanol: water (50:50).The solution was then filtered through what man filter paper no. 41. The solution was further diluted to get different concentrations in the range of 100ug/ml and 40ug/ml, 4ug/ml KETO, MP and PP respectively in the gel. The analysis procedure was repeated three times with the formulation. The result of analysis of the formulation is shown in Table 1.

METHOD I
Q-Absorbance ratio method (Method I): Q-Absorbance method uses the ratio of absorbances at three selected wavelengths, one at iso absorptive point and other being the λmax of one of the three compounds. From the stock solutions, working standard solutions of ketoprofen (100 ug/mL) and methyl paraben (25ug/mL) and propyl paraben (25 ug/mL were prepared by appropriate dilution and were scanned in the entire UV range to determine the maximum absorbance λmax and isoabsorptive point of ketoprofen and methyl paraben, propyl paraben have λmax at 288 nm and at 308.5 nm, 309nm respectively. Both the drugs were found to have same absorbance at 298 nm (iso absorptive point). The wavelengths selected for analysis were 278.5 nm and 312.5nm respectively (Fig.1). A series of standard solutions ranging from 10-50 _g/mL for ketoprofen and 2-10 ug/mL for methyl paraben and 2-10 ug/mL for propyl paraben were prepared and the absorbance of solutions was recorded at 278.5 nm and 312.5nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in the concentration range under study. Absorptivity values of ketoprofen and methyl paraben, propyl paraben were determined at selected wavelengths and are presented in Table-1. The concentration of two drugs in mixture was calculated by using following equations:

$$\text{Conc: of KETO} = \frac{Qm-Qy}{Qx-Qy} \times \frac{A}{ax1}$$

$$\text{Conc: of MP} = \frac{Qm-Qx}{Qy-Qx} \times \frac{A}{ay1}$$

$$\text{Conc: of PP} = \frac{Qm-Qy}{Qx-Qy} \times \frac{A}{az1}$$

Where, A1 and A2 are the absorbances of mixture at 278.5 nm and 312.5 nm and ax1 (64.4), ax2 (1.8) and ay1 (57.4), ay2 (82.1) are absorptivities E (1%, 1 cm) of ketoprofen and methyl paraben, propyl paraben at 278.5nm and 312.5 nm and Qm= A2/A1,

Qy = ay2/ay1 and Qx = ax2/ax1.

Q m- ratio of absorbtivity of a sample at 288 nm to 278.5
Qx = ratio of absorbtivity of KETO at 288 nm to 278.5

Qy = ratio of absorbtivity of a sample of MP308 nm to 278.5
Qz = ratio of absorbtivity of a sample of PP 308 nm to 278.5

Ax1= absorbtivity of KETO
Ay1- absorptivity of MP
A z1 - absorptivity of PP

METHOD II

Area under curve method: Area under curve method is used when broad spectrum of the drug is obtained. From the spectrum of mixture of Ketoprofen gel, In the Area under curve method preparation of standard stock solution was same as mentioned in method I from the overlain spectra 290.5 to 297.5 (KETO) and 297.5 to 303 (MP), 306.5 to 312.5 (PP) were selected for analysis. The calibration curve for Keto, MP, and PP was prepared in the Conc. Range as mentioned in method II at the selected wavelength range. Absorptivity values are determined for KETO, MP, PP were found to be 389.56/323.16/714.13,289.32/458.42/511.32and531.04/479.92/511.42 at 290.5 to 297.5 (KETO) 297.5 to 303 (MP) and 306.5 to 312.5 nm (PP) respectively. Using these absorptivity values following equation were developed for determining conc. of KETO, MP, PP in the Gel sample solution. Precision (interday and intraday) 100.11 ±0.111, 100.09±0.121,100.09±0.103100.01±0.119,100.01±0.102,100.02±0.110, 101.05±0.101,100.04±0.112 KETO ,MP,PP respectively

A1= 3895.61Ck+ 289.32Cm+ 531.04Cp----------1
A2= 3235.16Ck+ 4589.42Cm+ 4799.04Cp----------2
A3= 741.13Ck+ 511.32Cm+ 511.42Cp----------3

Where A1 and A2 and A3 are Area under curve of the sample KETO at 290.5 to 297.5nm MP- 297 to 303 and PP 306.5 to 312.5 nm Respective C keto is the conc. of ketoprofen and preparation of sample solution for analysis of Gel formulation was same as described under method I .the conc. Of keto, MP and PP were determination by using the equation 4, 5 and 6

Results of analysis of formulated gel are mention in table 1

The proposed Q-Absorbance ratio method I method allowed a rapid and accessible quantitation of KETO, MP and PP in Gel without any time consuming sample preparation. Moreover,the spectrophotometric method involved simple instrumentation compared with other instrumental techniques. The absorption spectra of KETO, MP and PP showed λmax was at 288 and 308 nm, 309 nm respectively. The first derivative method was based on derivative spectrophotometric method and absoebance nm, which was the wavelength used (Figure 1). The calibration curves were constructed in the range of expected concentrations (10-50ug/ml for Ketoprofen and 2-10 ug/ml for Methyl Paraben (MP), 0.2-1.0 ug/ml for Propyl Paraben, (PP)). The representative equation analysis was *Y= 0.0073x+ 0.0151, Y= 0.0453x+ 0.0068, Y= 0.0116x+ 0.0086* with a correlation coefficient of 0.9999 (Table 2). LOD and LOQ were found to be 0.022, 0.014, 0.010µg/mL and 0.011, 0.031, 0.025µg/mL and 0.031, 0.025, 0.013µg/mL and 0.022, 0.021, 0.011µg/mL respectively respectively, showing that the experimental values obtained for the determination of KETO, MP and PP in the samples indicated a satisfactory intra-day variability and inter-day variability (R.S.D. of 101.11 ±0.124, 101.09 ±0.121, 100.09 ±0.100, 100.01 ±0.098 100.01 ±0.102 100.02±0.130 KETO, MP and PP respectivly

0.047, 0.023 and 0.029%). A good accuracy of the method was verified with a mean recovery of 99.78, 99.84 and 99.70% (Table 3). Finally, the method showed to be specific for the determination of of KETO, MP and PP in the Gel

Method validation:

The method validation parameters like linearity, precision, accuracy, repeatability, limit of detection and limit of quantitation were checked as per ICH guidelines.

Linearity and range:

The linearity for KETO, MP and PP were determined at some concentration levels for KETO, MP and PP from 10-50 ug/ml and 2-10 µ/mL 0.2-1.0ug/ml and for PP ranging from 5-50µ/mL using working standards.

Precision and Accuracy:
Journal of Applied Pharmaceutical Science 01 (01); 2011: 46-49 the precision of the method was evaluated by interday and intraday variation studies. In intraday studies, working solutions of standard and sample were analyses thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the interday variation studies, working solution of standard and sample were analysed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is shown in table 3-5.

The accuracy of the method was determined by recovery studies. The recovery studies were performed by the standard addition method at 80%, 100% and 120% level and the percentage recoveries were calculated and are shown in Table 3-5.

Limit of detection and limit of quantitation:
The Limit of Detection (LOD) is the smallest concentration of the analyte that give the measurable response. LOD was calculated using the following formula and shown in Table 4-5.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 4-5.

$$LOQ = 10 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

RESULTS AND DISCUSSION

In the present work, new method, namely, Q-equation method (Vierordt's method) was used for the simultaneous spectroscopic estimation of KETO, MP and PP in in the formulated Gel dosage form. The concentrations in the range of 10-50 µg/mL of mixed working standard and three sampling wavelengths of 288nm (λ max of KETO), 308.5nm (λ max of MP) and 309.5nm (λ max of PP) gave optimum accuracy, precision, time, economy and sensitivity for this method. The proposed procedure was successfully applied to the determination of KETO, MP and PP in the formulated Gel dosage form. The recovery studies were carried out at different concentrations by spiking a known concentration of standard drug to the reanalyzed sample and contents were reanalyzed by proposed methods. The results of marketed formulation analysis and Recovery studies are depicted in Table2. The method was validated statistically for range, linearity, precision, accuracy, repeatability, LOD, and LOQ Table 4-5. Accuracy was ascertained on the basis of Recovery studies. Precision was calculated as inter and intraday Variation for both the drugs table 3-5. The percentage recoveries for of KETO, MP and PP were found to be 100.02±1.5409,99.94% ±1.7891,99.97% and , 99.97%±0.7662 for this method I and 100.02 % ±1.659 ,99.99% ±1.112,100.00 % ±1.008Method II respectively.

The relative standard deviation was found to be within the limit, indicating good accuracy, precision, and repeatability of the proposed method.

Table 1: 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)

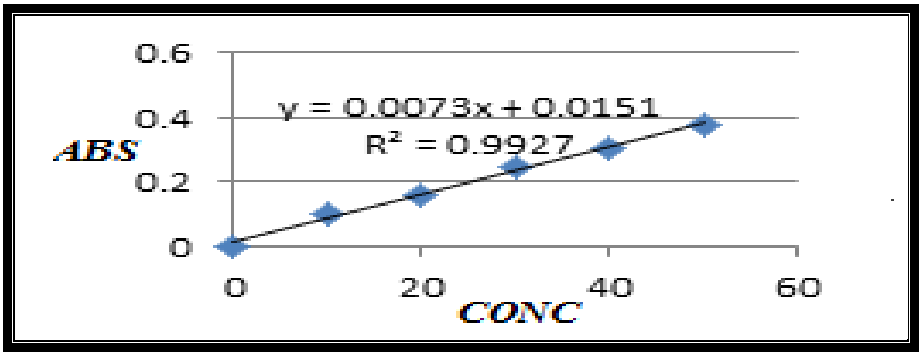


Fig No.4 KETOPROFEN Methanol: Water (50:50)

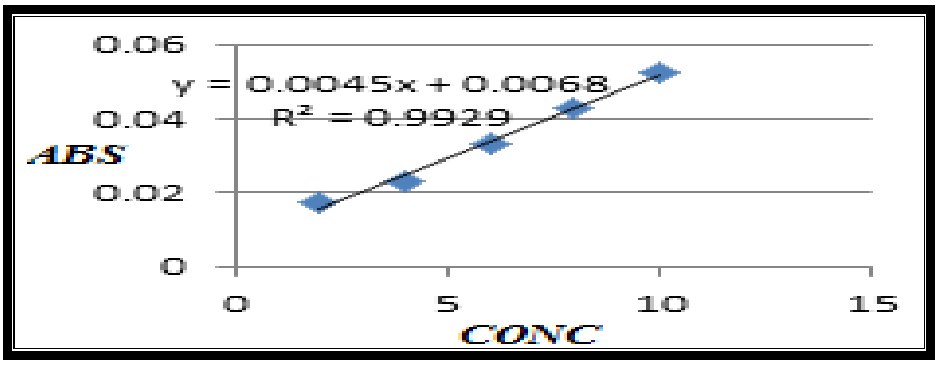


Fig No.5 METHYL PARABEN Methanol: Water (50:50)

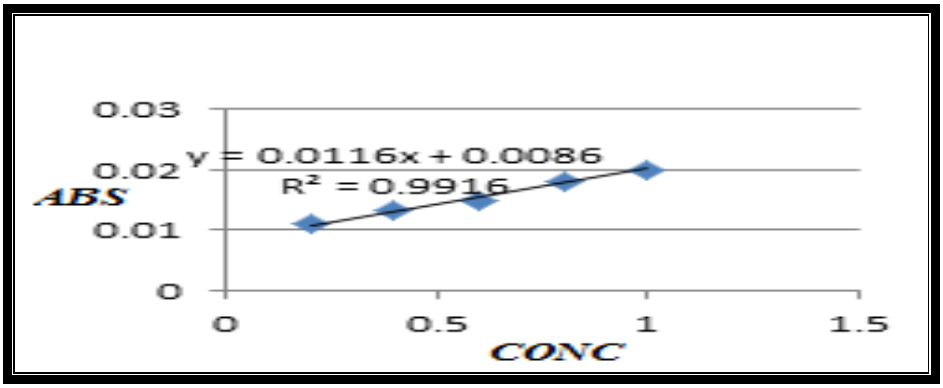


Fig No.6 PROPYL PARABEN: Methanol: Water (50:50

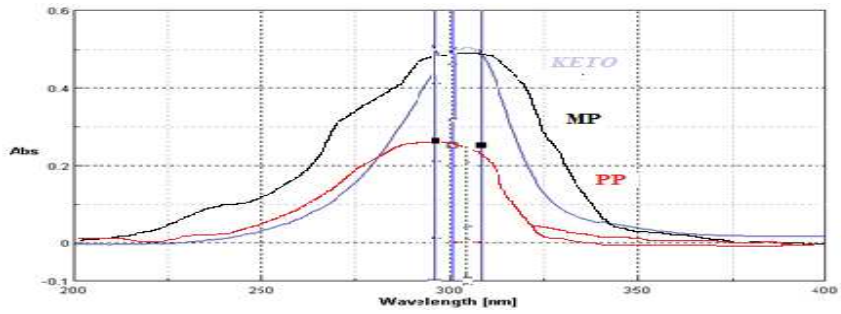


Fig: 7 Overlain spectra of KETO, MP, PP (Ketoprofen, Methyl Paraben and Propyl paraben

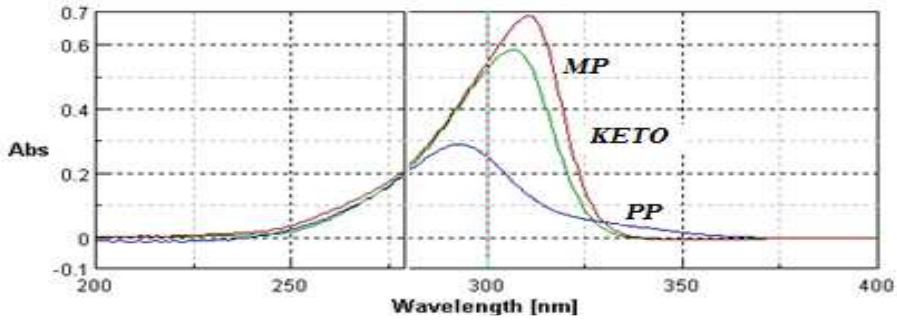


Fig: 8 Area under cuvre of KETO, MP, PP (Ketoprofen, Methyl Paraben and Propyl paraben

Table no.2 Analysis Data of formulated Gel

Parameter	Method I			Method I		
	KETO	MP	PP	KETO	MP	PP
Drug Content	100.11	99.40	99.07	100.17	100.75	99.17
+-SD	0.300	0.166	0.101	0.386	0232	0.149
% RSD	0.311	0.181	0.035	0.032	0.212	0.107
S.E	0.101	0.032	0.021	0.256	0.123	0.067

Table 3 Result of recovery Study for Gel formulation

Drug	Amount taken mg	Amount added		% Recovery	
		%	mg	METHODI	METHODII
KETO	800	80%	800.1	100.01	100.05
MP	320	80%	320.13	100.12	100.09
PP	32	80%	32.14	100.04	99.64
KETO	1000	100%	1000	100.02	100.02
MP	400	100%	399.11	99.97	99.99
PP	40	100%	39.98	99.94	100.00
KETO	1200	120%	1198	99.97	99.88
MP	480	120%	482	100.06	100.03
PP	48	120%	48.69.	100.77	102.17

Table 4: Validation parameters for Q Absorbance method(isoabsorptive point (298nm)

SrNO	Parameter	Result method I		
		KETO	MP	PP
1	Absorption (nm)	288nm	308nm	309nm
2	Linearity range (µg/ml)	10-50ug/ml	2-10ug/ml	0.2-1.0ug/ml
3	Standard regression equation	Y= 0.073+0.0151	Y= 0.0045+0.0068	Y= 0.0116+0.0086
4	Correlation coefficient (r^2)	r^2-= 0.9927	r^2-= 0.9929	r^2-= 0.9916
5	A (1%, 1cm)	100.29± 0.148	99.97± 0.1315	99.94 ± 0.048
6	Accuracy (% recovery± SD)	100.02 ±0.117	99.97±0.117	99.94±0.117
7	Precision (% CV)	101.11 ±0.124 101.09 ±0.121	100.09 ±0.100 100.01 ±0.098	100.01 ±0.102 100.02±0.130
8	LOD	0.022	0.014	0.010
9	LOQ	0.031	0.025	0.013

Table no 5 .Validation parameters for Area under Curve

SrNO	Parameter	Result method II			
		KETO	MP	PP	Mixture
1	Absorption (nm)	290.5-297.5nm	297.5-303.0nm	306.5nm -312.5nm	294-304
2	Linearity range (µg/ml)	10-50ug/ml	2-10ug/ml	0.2-1.0ug/ml	10-50ug/ml
3	Standard regression equation	Y= 0.073+0.0151	Y= 0.0045+0.0068	Y= 0.073+0.0151	Y= 0.070+0.0145
4	Correlation coefficient (r^2)	r^2-= 0.9927	r^2-= 0.9929	r^2-= 0.9927	r^2-= 0.9911
5	A (1%, 1cm)	100.09± 0.111	99.09± 0.121	99.89± 0.011	99.12±0.121
6	Accuracy (% recovery± SD)	100.02±0.123	99.99±0.143	100.00±0.103	99.13±0.113
7	Precision (% CV)	100.11 ±0.111 100.09 ±0.121	100.09 ±0.103 100.01 ±0.119	100.01 ±0.102 100.02±0.110	101.05±0.101 100.04±0.112
8	LOD	0.011	0.010	0.009	0.013
9	LOQ	0.020	0.021	0.011	0.023

CONCLUSION

The proposed method based on the UV is suitable for determination of KETO, MP and PP in the commercial tablets. The methods are simple, reliable, fast and reproducible. The spectrophotometric method requires only wavelength scan and automatic calculation of the Q method and area under curve method. Furthermore, the proposed methods are inexpensive and low polluting, because small volumes are required for preparation of samples.

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