AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Stability Indicating HPLC Method for Simultaneous Determination of Diclofenac Potassium, Paracetamol and Methocarbamol

Maulikkumar R. Amin[1*], Paresh U. Patel[2], B.N. Suhagia[3], Madhabhai M. Patel[4]
1. Kalol Institute of Pharmacy, Kalol, Gujarat, India- -382721
2.Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva, Mehsana, Gujarat-382711
3. Dean, Faculty of Pharmacy, D.D. University College Road, Nadiad-387001
4. Shankersinh Vaghela Bapu Institute of Pharmacy, Gandhinagar, Gujarat-382650

ABSTRACT

A simple, specific, selective and accurate stability-indicating reversed phase high performance liquid chromatographic method was developed for simultaneous determination of Diclofenac potassium (DIC), Paracetamol (PCM) and Methocarbamol (MET). An isocratic RP-HPLC was achieved on younglin HPLC system using Varian C18 (250 X 4.6 mm i.d, 5 µm particle size) column with the mobile phase containing mixture of Methanol:water (80:20,v/v). The flow rate was 0.8 ml/min and the eluent was monitored at 225nm. The retention times of DIC, PCM and MET were found to be 3.51, 6.42 and 9.90 min respectively. The linearity was established for DIC, PCM and MET in the range of 10-60 µg/ml, 65-390µg/ml, 100-600µg/ml respectively. The percentage recoveries of DIC, PCM and MET were found to be in the range of 99.73%±0.109, 99.59%±0.085 and 99.50%±0.16 respectively. The LOD for DIC, PCM and MET were found to be 0.15, 2.40 and 1.82µg/ml respectively, while LOQ were 0.48, 7.29 and 5.53µg/ml respectively. All three drugs were subjected to acid, alkali, oxidation, and dry heat degradation. The degradation studies indicated DIC, PCM and MET showed degradation in acid, alkaline, H_2O_2, and in dry heat condition. The degradation products of DIC, PCM and MET were resolved well from the pure drug with significant differences in their retention time values. This method was also successfully employed for simultaneous quantitative analysis of DIC, PCM and MET in bulk drugs and formulations. The developed method is stability indicating and separate degradants and can be used to determine the stability of samples.

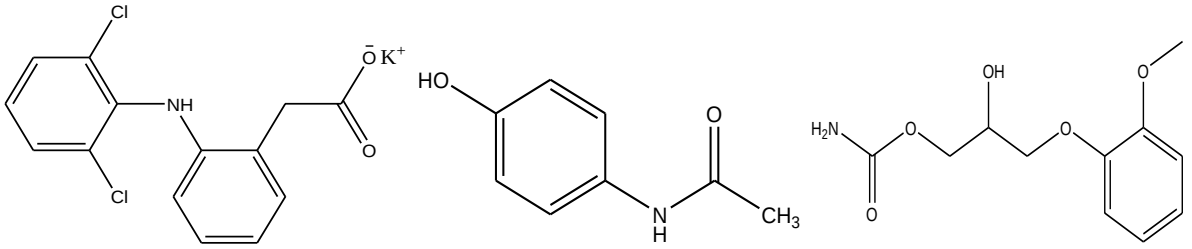
Keywords: DIC, PCM, MET, HPLC, LOD, LOQ

*Corresponding Author Email: amin_maulik2003@yahoo.co.in
Received 28 September 2012, Accepted 31 October 2012

Please cite this article in press as Amin MR *et al.*, Stability Indicating HPLC Method for Simultaneous Determination of Diclofenac Potassium, Paracetamol and Methocarbamol American Journal of PharmTech Research 2012.

INTRODUCTION

Diclofenac potassium(DIC) [2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, monopotassium salt], is analgesic which acts by non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models. The mechanism of action of diclofenac potassium tablets, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthetase inhibition[1-4]. It is official in Indian Pharmacopoeia and United States Pharmacopoeia. Paracetamol(PCM)[N-(4-Hydroxyphenyl)acetamide], an analgesic and antipyretic action with weak anti inflammatory activity. These effects are related to inhibition of prostaglandin synthesis[5-7]. It is official in Indian Pharmacopoeia and United States Pharmacopoeia. Methocarbamol(MET)[3-(2-methoxyphenoxy)-l,2-propanediol1-carbamate] is muscle relaxant which has no direct action on the contractile mechanism of striated muscle, the motor end plate or the nerve fiber[8-10]. It is official in United States Pharmacopoeia (Figure 1). Several UV and HPLC methods either single and in combination have been reported for estimation of diclofenac potassium(DIC), paracetamol(PCM) and methocarbamol(MET). Few HPTLC methods are also reported for estimation of DIC and PCM.



Diclofenac Potassium Paracetamol Methocarbamol

Figure 1: Structure of diclofenac potassium, paracetamol and methocarbamol

The International Conference on Harmonization (ICH) guideline entitled ‘stability testing of new drug substances and products’ requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances. An ideal stability indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine DIC, PCM and MET simultaneously in presence of its degradation products is rather a challenge for pharmaceutical analyst. Thus, thought necessary to study the stability of DIC, PCM and MET under acidic, alkaline, neutral hydrolysis, oxidative, and dry heat conditions. This paper describes validated stability-indicating HPLC method for simultaneous estimation of DIC, PCM and MET. Reversed-phase chromatographic method with UV detection has shown to be sensitive, accurate and suitable for analyzing a large support bioavailability and stability studies.

MATERIALS AND METHOD

Reagents and chemicals

Pure diclofenac potassium, paracetamol and methocarbamol were gifted by Zydus Cadila Ltd. (Ahmedabad, India). Tablet Robinaxol-D was purchased from the local market. Methanol and acetonitrile of HPLC grade were procured by Merck Ltd., India. Disodium hydrogen phosphate was procured from Merck Ltd., India. Sodium hydroxide, hydrochloric acid, disodium hydrogen phosphate and hydrogen peroxide of analytical reagent were procured from Merck Ltd., India.

Instrumentation and chromatographic conditions

The HPLC system used was Younglin equipped with binary solvent manager, manual, dual wavelength UV detector operated at 225nm. HPLC column incorporated solvent delivery model LC-10AT$_{VP}$. Moreover, HPLC column is Varian C-18 (250x4.6) mm i.d. 5 µm particle size with Microliter syringe (Rheodyne) injector. The HPLC was connected to personal computer with Class- Autochro-3000 software. The isocratic mobile phase consisted of methanol:water (80:20 v/v) and was delivered at a flow rate of 0.8 ml min^{-1}. Detection was carried out using a UV detector at 225nm. The column was maintained at ambient temperature and injection volume of 20µl was used.

Preparation of stock solutions

Accurately weighed DIC (50mg) was transferred in 100ml volumetric flask. The drug was dissolved in methanol with sonication and final volume was adjusted with methanol upto mark to prepare a 500µg/ml stock solution. Similarly, accurately weighed PCM (32.5mg) was transferred in 100ml volumetric flask. The drug was dissolved in methanol with sonication and final volume was adjusted with methanol upto mark to prepare a 325µg/ml stock solution. Lastly, accurately weighed MET (50mg) was transferred in 50ml volumetric flask. The drug was dissolved in methanol with sonication and final volume was adjusted with methanol upto mark to prepare a 500µg/ml stock solution.

PREPARATION OF SAMPLE SOLUTIONS

Preparation of standard working solution of DIC

From the stock solution (500µg/ml), an accurately measured 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml was transferred into separate 10ml volumetric flask and final volume was adjusted with methanol upto mark to prepare 10-60µg/ml solutions.

Preparation of standard working solution of PCM

From the stock solution (325µg/ml), an accurately measured 2, 4, 6, 8, 10 and 12ml was transferred into separate 10ml volumetric flask and final volume was adjusted with methanol upto mark to prepare 65-390µg/ml solutions.

Preparation of standard working solution of MET

From the stock solution (1000µg/ml), an accurately measured 1, 2, 3, 4, 5 and 6ml was transfer into separate 10ml volumetric flask and final volume was adjusted with methanol upto mark to prepare 100-600µg/ml solutions.

Preparation of sample solution

Twenty tablets were weighed accurately and finely powdered. Powder exactly equivalent to 50mg of DIC, 32.5mg of PCM and 500mg of MET was transferred to a 100ml volumetric flask. The powder was dissolved in 60ml of methanol with sonication for 15 minutes and volume was made up with methanol. 1ml of the solution was transferred into 10ml volumetric flask and diluted upto mark with methanol.

Selection of Detection Wavelength

Solutions of DIC, PCM and MET were scanned between 200 and 400nm. UV spectra of all three drugs show maximum absorbance at 225nm. (Figure 2)

Selection of mobile phase

Different mobile phases were tried and 20µL of mixed standard solution was injected. The mobile phase methanol: water (80:20) was selected as it gave well resolved sharp peaks with reasonable retention time for all the drugs. The analysis was carried out using Younglin series LC-10AT$_{VP}$ binary gradient system, Varian 5µm C-18 column (250x4.6 mm) with flow rate 1.5 mL/min.

METHOD VALIDATION

System Suitability Parameters

System suitability parameter was established to ensure that the validity of the analytical method was maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyzer. In case of liquid chromatography typical variations are composition of mobile phase, different lots or supplier of columns, temperature and flow rate.

Linearity and calibration graph

The linearity was evaluated by linear regression analysis. The series of dilution ranging from 10-60µg/ml for DIC, 65-390µg/ml PCM and 100-600µg/ml for MET was prepared and performed for linearity. All the solutions were filtered through 0.2 µm membrane filter and injected, chromatograms were recorded and it was repeated for six times.

Recovery studies

The recovery studies were performed to validate the accuracy of developed method. To prepared analysed sample solution, a definite concentration of standard drug was added and recovery was studied.

Precision

The precision of the procedure was determined by repeatability (intraday). Intraday precision was evaluated by assaying same concentration and during the same day. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of sample. Another replicate determination on three different days to estimate interday precision.

Limit of detection and limit of quantification

For HPLC method, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope by using calibration curves.

Robustness

For the HPLC method, robustness was determined by analysis of the samples under a variety of conditions making small changes in the percentage of mobile phase compounds (phosphate buffer: methanol in the ratios 68:32 and 42:58), in the flow rate (0.6 and 1.2 ml/min), in the temperature conditions (35 and 45°C), and changing the wavelength (268 and 276 nm).

FORCED DEGRADATION STUDY[11-16].

Forced degradation for all three drugs was carried out under conditions of acid/base/neutral hydrolysis, oxidation, and dry heat. For each study, six samples were prepared and injected then study was extended up to the formulation.

Acidic degradation

Accurately weighed 10mg of DIC, 32.5mg of PCM and 50mg of MET were transferred into 100ml volumetric flask and dissolved in 50ml methanol. 50ml of 0.1N HCl was added and kept at room temperature for 10 hours. 6ml of the solution was diluted upto 10ml with methanol and analysed by HPLC.

Alkaline degradation

Accurately weighed 10mg of DIC, 32.5mg of PCM and 50mg of MET were transferred into 100ml volumetric flask and dissolved in 50ml methanol. 50ml of 0.1 N NaOH was added and refluxed at 85°C for 24 hours (325µg/ml) and the solution was analysed by HPLC.

Oxidative degradation

Accurately weighed 10mg of DIC, 32.5mg of PCM and 50mg of MET were transferred into 100ml volumetric flask and dissolved in 50ml methanol. 50ml of 3% H_2O_2 was added and kept at room temperature for 72 hours. 6.0ml of the solution was diluted upto 10ml with methanol and analyzed by HPLC.

Thermal degradation

Accurately weighed 10mg of DIC, 32.5mg of PCM and 50mg of MET were transferred into

100ml volumetric flask and dissolved in 50ml methanol. The powder was dissolved in 50ml of methanol and diluted upto mark with methanol and the solution was analysed by HPLC.

Neutral degradation

Accurately weighed 10mg of DIC, 32.5mg of PCM and 50mg of MET were transferred into 100ml volumetric flask and dissolved in 50ml methanol. 50ml of water was added and refluxed at 40°C for 4 hours and analysed by HPLC.

RESULTS AND DISCUSSION

The mobile phase consisting of methanol: water (80:20, v/v) at 0.8ml/min flow rate which gave sharp, well-resolved peak with minimum tailing factor for DIC, PCM and MET (Figure 6). The parameters like retention time, asymmetric factor, number of theoretical plates and tailing factors were evaluated for DIC, PCM and MET. The results of system suitability parameters are shown in table 1. The calibration curve for DIC, PCM and MET was found to be linear over the range of 10-60µg/ml for DIC, 65-390µg/ml for PCM and 100-600µg/ml for MET and results of linearity **(**Figure 3, Figure 4, and Figure 5) and calibration curve are shown in Table 2. The results of recovery studies are shown in Table 3. The proposed method was successfully applied to the determination of DIC, PCM and MET in market formulation. The %RSD of intraday and interday precision study for DIC, PCM and MET were found to be <2 as shown in Table 4. The LOD for DIC, PCM & MET were found to be 0.15µg/ml, 2.40µg/ml and 1.82µg/ml respectively, while LOQ were 0.48µg/ml, 7.29µg/ml and 5.53µg/ml respectively as shown in Table 5. The summary of all validated parameters is shown in Table 6. The degradation study indicated that DIC was susceptible to base and H_2O_2 under experimental conditions. Moreover, MET was not stable towards acidic and oxidative degradation conditions. It was noticed that except oxidation PCM found to stable for all the experimental conditions. The study revealed that DIC and PCM showed no degradation in 0.1 N HCl when reflux at 80°c for 10hr condition (Figure 7) and chromatogram showed no additional peak. In alkaline hydrolysis DIC and MET degraded as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the non-degraded drug, with giving additional degradation peak (Figure 8). In oxidative degradation all the three drugs were observed to be labile and more than 8% of degradation for MET was found (Figure 9). There was no degradation found under thermal and neutral conditions for DIC, PCM and MET. (Figure 10 & Figure 11). Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drug under non degradation condition. The summary of degradation studies is given in Table 7.

Table 1 Results of system suitability parameters

Parameter	RT*	AUC*	No. of theoretical plates*	Tailing factor*
DIC	3.51±0.03	50152.6±503.35	3590.4±90.52	1.04±0.02
PCM	6.42±0.04	312120.2±112.54	3437.4±139.80	1.08±0.01
MET	9.90±0.05	400120.0 ±3923.11	1263.12±7.005	0.96±0.008

*=Average result of six replicate samples

Table 2 Linearity and calibration curve

Drug	Linearity range	Y=mx+c		r^{2*}
		Slope*	Intercept*	
DIC	10-60µg/ml	5085	1345.1	0.9998
PCM	65-390µg/ml	4606.9	14242	0.9993
MET	100-600 µg/ml	4030.6	1086	0.9992

*=Average result of six replicate samples

Table 3 Results of recovery studies

Drug	Conc. Of Form. (µg/ml)	Conc. Of Std. added (µg/ml)	Conc. Recover (µg/ml)	% recovery±SD*
DIC	20	20	40.03	100.07%±0.046
	20	25	44.84	99.64%±0.109
	20	30	49.89	99.78%±0.046
PCM	165	135	299.89	99.96%±0.085
	165	165	326.94	99.07%±0.16
	165	195	361.83	100.50%±0.11
MET	200	250	450.05	100.02%±0.12
	200	200	400.87	100.21%±0.12
	200	250	448.13	99.58%±0.10

*= Average result of six replicate samples

Table 4 Results of Intraday & Inter day precision

Drug	Intra day		Inter day	
	Mean %assay*	%RSD*	Mean %assay*	%RSD*
DIC	98.84%±0.30	1.22	98.76%±0.25	1.26
PCM	98.29%±1.18	0.26	99.06%±0.36	0.48
MET	98.94%±0.12	0.14	99.86%±0.19	0.52

*= Average result of six replicate samples

Table 5 Limit of detection and limit of quantification

Drugs	Standard	Slope of calibration curve	LOD	LOQ
DIC	862.1	5085	0.56	1.69
PCM	3684.49	5051.4	2.4	7.29
MET	2759.5	4987.5	1.82	5.53

Table 6 Summary of validation parameters

Parameter	DIC	PCM	MET
Wavelength	225 nm	225 nm	225 nm
Range	10-60 µg/ml	65-390 µg/ml	100-600 µg/ml
Linearity	0.9998	0.9993	0.9992
Intercept	1345.1	14242	1086

Slope	5085	4606.9	4030.6
Intraday precision	1.22	0.26	0.14
Interday precision	1.26	0.48	0.52
LOD	0.56	2.40	1.82
LOQ	1.69	7.29	5.53

Table 7 Results of forced degradation study

Degradation condition	Drug	Conc. Of drug(µg/ml)	RT of observed peak*	AUC*	%drug*	% degradation*
Acidic	DIC	50	3.56	241732	99.14%±0.00	0.00%±0.00
	PCM	325	6.42	1420526	100%±1.01	0.00%±0.00
	MET	500	9.90	1807432	94.86%±1.04	0.00%±0.00
			5.49(I)	36123	0.00%±0.00	1.99%±0.98
			11.10(II)	84231	0.00%±0.00	4.36%±1.62
Alkaline	DIC	50	4.11	231415	95.34%±0.98	0.00%±0.00
			3.52(I)	9329	0.00%±0.00	4.03%±0.99
	PCM	325	6.39	1419311	99.21%±1.48	0.00%±0.00
	MET	500	9.90	1806415	94.12%±1.35	0.00%±0.00
			8.92(II)	95120	0.00%±0.00	5.5%±0.25
Oxidative	DIC	50	4.11	231811	95.39%±1.21	0.00%±0.00
			3.49(I)	9653	0.00%±0.00	4.16%±0.50
	PCM	325	6.39	1419769	91.76%±1.34	0.00%±0.00
			5.62(II)	11981	0.00%±0.00	8.64%±1.21
	MET	500	9.90	1806926	99.54%±1.21	0.00%±0.00
			10.32(III)	91824	0.00%±0.00	5.06%±0.50
Thermal	DIC	50	3.51	251830	98.99%±1.09	0.00%±0.00
	PCM	325	6.42	1500874	99.42%±1.09	0.00%±0.00
	MET	500	9.90	2007891	99.29%±1.21	0.00%±0.00
Neutral	DIC	50	4.11	24981	98.44%±12	0.00%±0.00
	PCM	325	6.39	1465896	98.97%±1.21	0.00%±0.00
	MET	500	9.90	1805341	94.76%±1.09	0.00%±0.00
			10.29(I)	98691	0.00%±0.00	5.46%±0.78

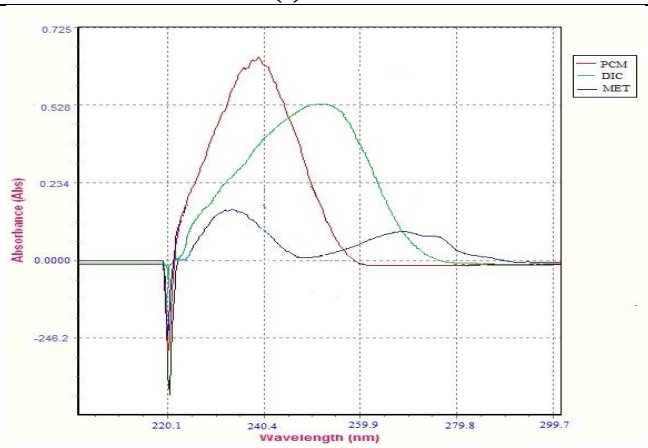


Figure 2: Overlay UV spectra of DIC, PCM and MET

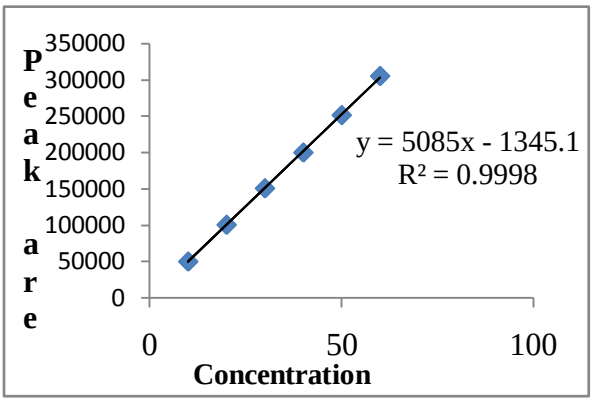


Figure 3: Calibration curve of DIC

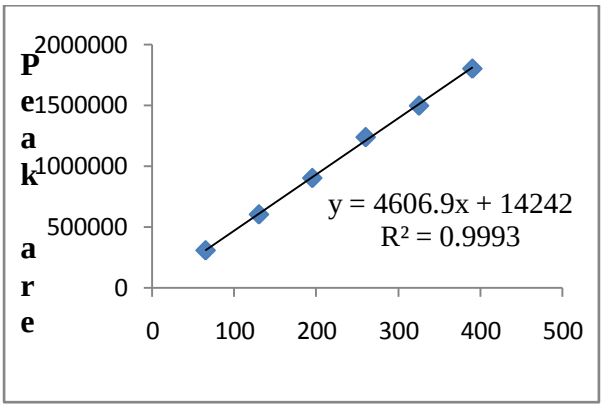


Figure 4: Calibration curve of PCM

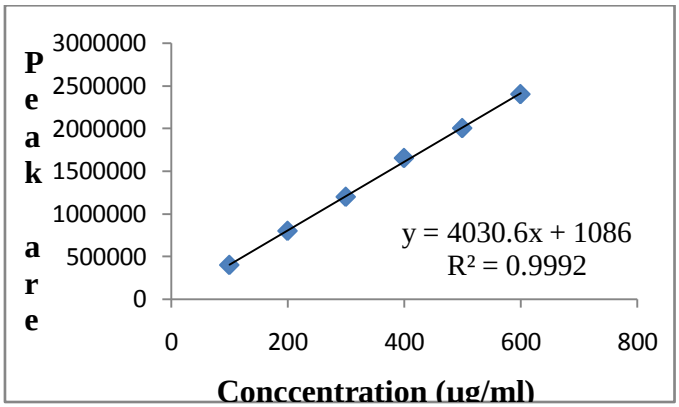


Figure 5: Calibration curve of MET

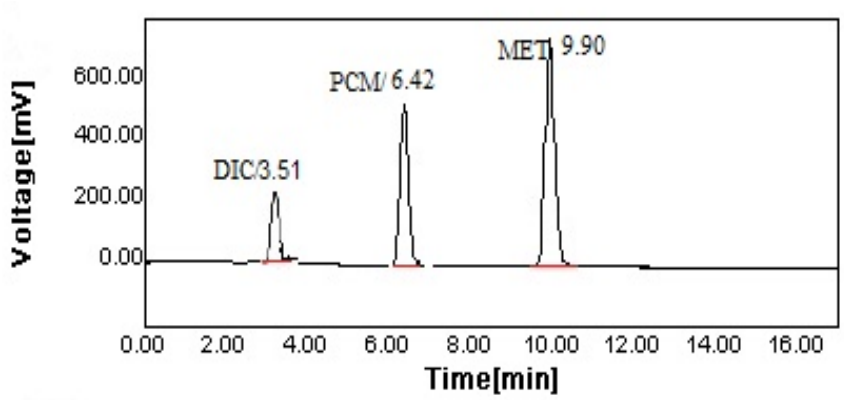


Figure 6: A typical Chromatogram for DIC, PCM and MET

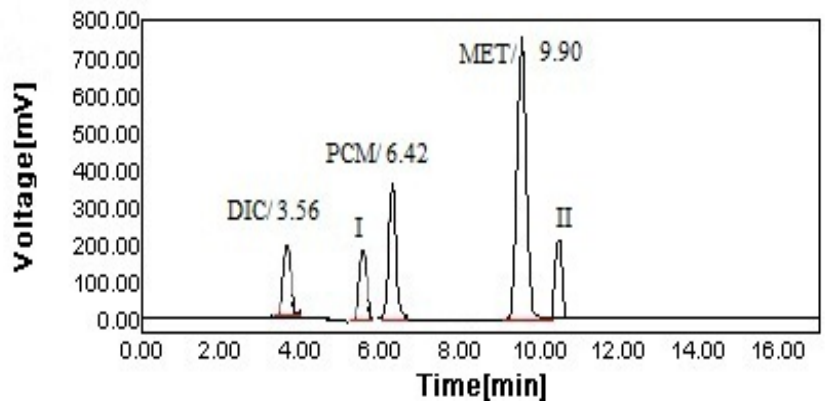


Figure 7: Forced degradation under acidic condition

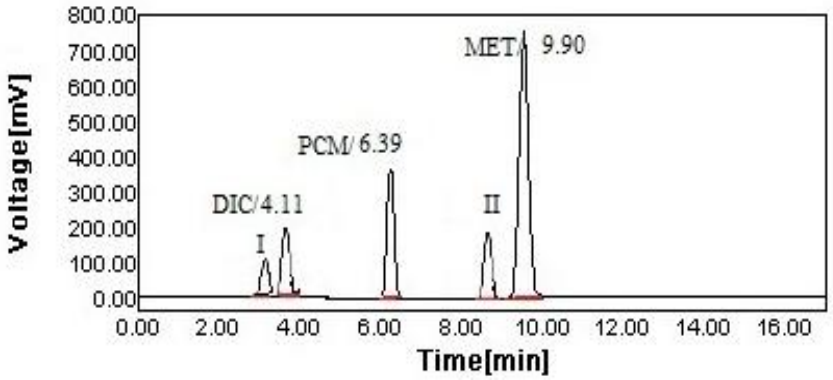


Figure 8: Forced degradation under alkaline condition

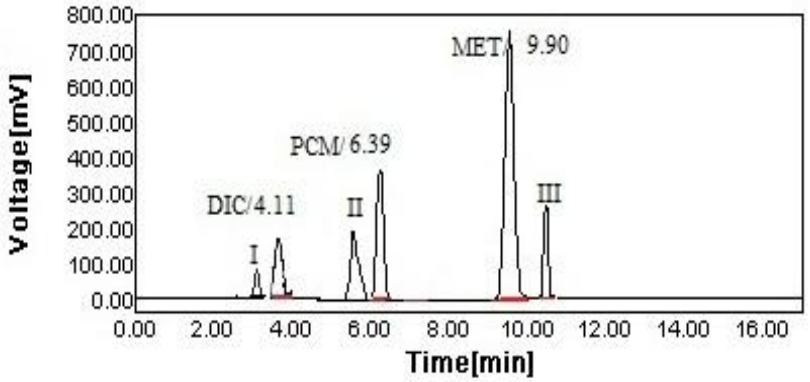


Figure 9: Forced degradation under oxidative condition

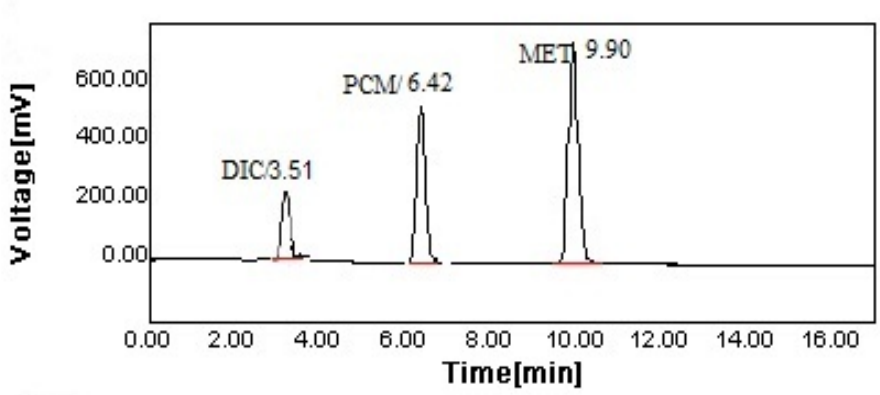


Figure 10: Forced degradation under thermal condition

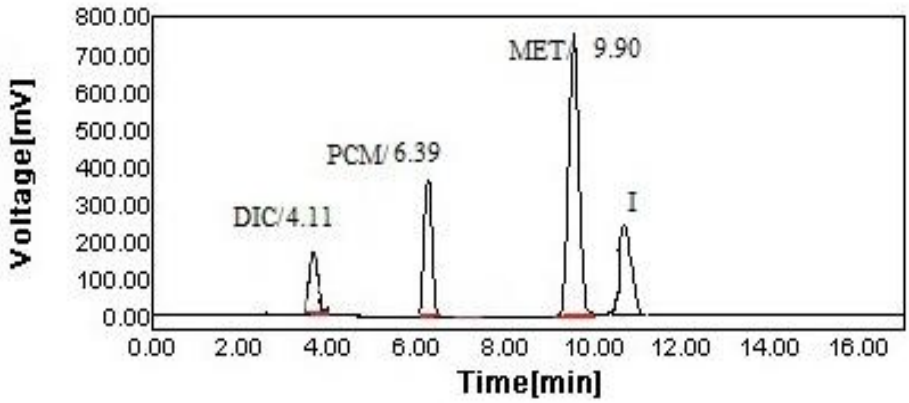


Figure 11: Forced degradation under neutral condition

CONCLUSION

The developed method was found to be simple, sensitive, selective, accurate, precise, and repeatable for analysis of diclofenac potassium, paracetamol and methocarbamol in market formulation without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. It was possible in this study to develop a stability indicating assay method for the drugs by subjecting ICH recommended stress conditions. The drugs and degradation products got well separated from each other in isocratic mode using a reversed phase C18 column and mobile phase composed of methanol: water (80:20). The results indicated suitability of this method to study stability of three drugs under various forced degradation conditions like acid, base, dry heat and oxidative degradation. There was no interference observed due to excipients or other components present in tablet dosage form. The developed method is stability indicating and separate degradants and can be used to determine the stability of samples.

ACKNOWLEDGEMENT

The authors are greaful to Zydus Cadila Ltd for providing gift samples of diclofenac potassium, paracetamol and methocarbamol respectively.

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