Research Article

DEVELOPMENT OF A VALIDATED STABILITY-INDICATING HPLC ASSAY METHOD FOR DEXKETOPROFEN TROMETAMOL

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ABSTRACT

In the present study, comprehensive stress testing of Dexketoprofen trometamol was carried out according to ICH guideline Q1A (R2). Dexketoprofen trometamol is subjected to stress conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Additionally, the solid drug is subjected to 50 °C for 60 days in dry-bath, and to the combined effect of temperature and humidity, with and without light, at 40°C/75% RH. The drug was found to degrade significantly in oxidative and photo condition whereas it is found to be stable in acidic, alkaline and neutral condition. The drug is relatively stable in the solid-state, except formation of minor products under accelerated conditions. Successful separation of drug from degradation products formed under stress conditions is achieved on a Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing methanol: acetate buffer pH adjusted to 4 with glacial acetic acid (65: 35) at flow rate of 1 mL/min using UV detection at 255 nm. The method is validated according to ICH guidelines. The developed method is found to be precise, accurate, specific and selective.

Keywords: Dexketoprofen trometamol, HPLC, Stress studies, Stability indicating method, Validation.

INTRODUCTION

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH)[1] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and should be fully validated.
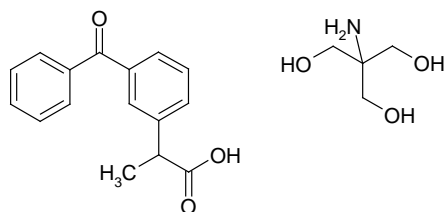

Fig. 1: Structure of Dexketoprofen trometamol

Dexketoprofen trometamol, (2S)-2-[3-(benzoyl)phenyl]propanoic acid (**Fig 1**), is a non-steroidal anti-inflammatory drug. It works by blocking the action of a substance in the body called cyclo-oxygenase. Cyclo-oxygenase is involved in the production of chemicals in the body called prostaglandins. Prostaglandins are produced in response to injury or certain diseases and would otherwise go on to cause swelling, inflammation and pain. Dexketoprofen blocks cyclo-oxygenase and prevents the production of prostaglandins and therefore reduces inflammation and pain. It possesses central analgesic action along with peripheral analgesic action[2].

Dexketoprofen trometamol is a new, quick acting analgesic for the treatment of painful musculoskeletal conditions such as osteo-arthritis and low back pain. It is also used as a treatment for post-operative pain, toothache and dysmenorrhoea. It is the active optical isomer (eutomer) of ketoprofen, a propionic acid non-steroidal anti-inflammatory drug (NSAID). The eutomer has been separated to halve the dosage required and halve the metabolic load. The inactive isomer (distomer) has been discarded in the hope of eliminating or reducing potential unnecessary side effects. It is presented as keral.

The tromethamine salt results in the drug being quickly absorbed and thus promotes rapidity of action[3].

Literature review reveals that determination of Dexketoprofen by HPLC have been reported[4], stability study for analysis of Dexketoprofen, Tramadol and Haloperidol by LC-DAD have been reported[5], in biological fluids determination of the plasma concentration of Dexketoprofen with HPLC in rabbits have been reported[6].

In the present study we have proposed new stability indicating simple and reliable RP-LC for determination of Dexketoprofen trometamol. The aim of the present work is to develop an accurate, specific, repeatable and stability-indicating HPLC method for the determination of Dexketoprofen trometamol in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its bulk dosage forms. The proposed method was validated as per ICH guidelines[7-9] and its updated international convention[10].

MATERIALS AND METHODS

Materials

Emcure Pharmaceuticals Ltd. Pune, India, kindly supplied pure drug sample of Dexketoprofen trometamol as a gift sample of Batch No.: 510KETF007. It was used without further purification and certified to contain 99.49 % (w/w) on dry weight basis. Fixed dose combination tablets (Infen 25) containing 25 mg Dexketoprofen trometamol was procured from local market**.** All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

Instrumentation

The LC system consisted of a Pump (model Jasco PU 2080), Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075) model operated at a wavelength of 255 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany.

Forced Degradation Studies

Dexketoprofen trometamol (100 mg) was weighed accurately and transferred into a 100 mL volumetric flask. Methanol (50 mL) was added and the flask was sonicated for 20 min, and then diluted up to the mark with methanol. An aliquot (1 mL) was further diluted to 100 mL with the same solvent. The final solution contained 10 μg of Dexketoprofen trometamol per mL of solution. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of Dexketoprofen after application (10 μg/mL) of seven replicates was obtained.

Acid and base induced degradation

Acid decomposition studies were performed by exposing the solution of drug in 5 M hydrochloric acid at 80 °C for 8 h. The studies in alkaline conditions were carried out in 5 M sodium hydroxide at 80 °C for 8 h. The resultant solutions were diluted to obtain 10 μg/mL solutions and 20 μL were injected into the system.

Hydrogen peroxide induced degradation

To study hydrogen peroxide induced degradation, the sample was exposed to 3 % hydrogen peroxide at room temperature for a period of 5 min, and then heated in boiling water bath for 10 min to completely remove the excess of hydrogen peroxide. The resultant solutions were diluted to obtain 10 μg/mL solutions and 20 μL were injected into the system.

Photochemical degradation

The photochemical stability of the drug was studied by exposing the stock solution (100 μg/mL) to direct sunlight for 15 min on a wooden plank and kept on terrace. The solution was diluted with methanol to obtain a solution of 10 μg/mL and then 20 μL of the solution was injected into system.

The photochemical stability of the drug was also performed by keeping the stock solution (100 μg/mL) in the stability chamber for 28 h. The solution was diluted with methanol to obtain a solution of 10 μg/mL and then 20 μL of the solution was injected into system.

Neutral Hydrolysis

The degradation behavior of drug in neutral condition was studied by dissolving drug solution in water and solution was refluxed at 80 °C for 8 h.

Optimization of stability indicating HPLC method

The HPLC procedure was optimized with a view to develop stability indicating assay method. Pure drug along with its degraded products were injected and run in different solvent systems. Initially methanol and water in the ratio of (70: 30) was tried. It was found that the peak with hump shape was obtained. To improve the peak shape acetate buffer was used whose pH was adjusted to 4 with glacial acetic acid. It was found that methanol: acetate buffer with pH adjusted to 4.0 with glacial acetic acid (65: 35) as a mobile phase at a flow rate 1 mL/min gave acceptable retention time (R_t), theoretical plates and good resolution of drug and degradation products (Fig 2).
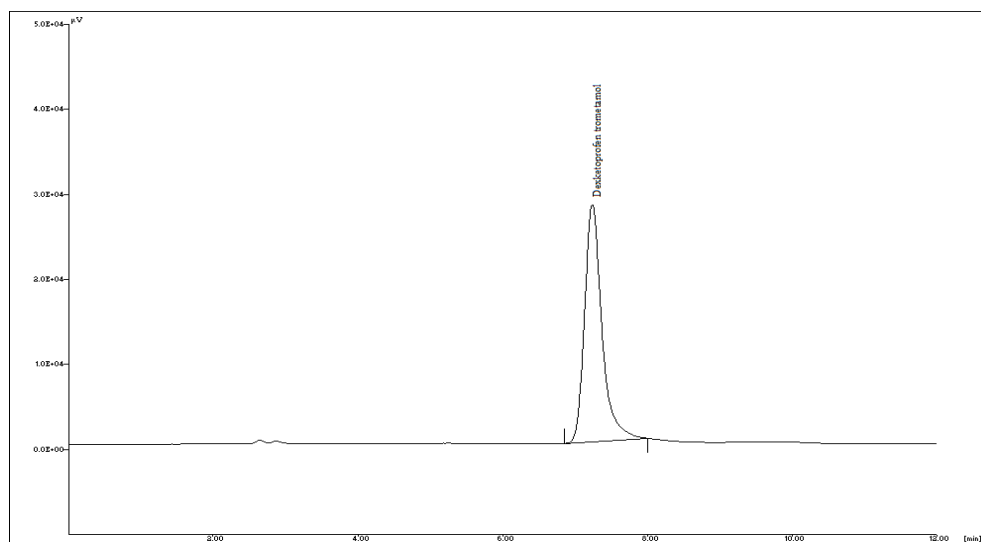

Fig. 2: Chromatogram of standard Dexketoprofen trometamol 10 μg/mL

Validation of the Method

Validation of optimized LC method was done with respect to following parameters.

Linearity and range

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range of 5-10 μg/mL in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (5, 7, 9 μg/ mL) of the drug in hexaplicate on the same day. Intermediate

precision of the method was checked by repeating studies on three different days. Additionally, the developed HPLC method was checked through separation studies on the mixture of reaction solutions on a different chromatographic system on a different day.

Limit of detection and limit of quantitaiton

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was injected six times following the same method as explained above. The signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. The LOD and LOQ were experimentally verified by diluting known concentrations of standard solution of Dexketoprofen trometamol until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Robustness of the method

To evaluate robustness of HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of methanol in the mobile phase and solvents from different lot were taken. The resolution of drug in a mixture of stressed samples was studied by performing the analyses on a different chromatographic system. Robustness of the method was done at three different concentration levels 5, 7, 9 μg/mL for Dexketoprofen trometamol. Also robustness was verified by studying the resolution of drug in a mixture of degraded samples on different chromatographic system on a different day.

Specificity

The specificity of the HPLC method was determined by the complete separation of Dexketoprofen trometamol in presence of its degradation products along with other parameters like retention time, capacity factor, tailing or asymmetrical factor etc.

Accuracy

Accuracy of the developed method was tested by fortifying a mixture of decomposed reaction solutions with three concentrations of drug corresponding to 80, 100 and 120% and determining the recovery of added drug. At each level of the amount six determinations were performed.

Analysis of marketed formulation

To determine the content of Dexketoprofen trometamol (25 mg) (Brand name: Infen 25) tablets which was procured from local market, twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 25 mg of Dexketoprofen was transferred into a 50 mL volumetric flask containing 30-35 mL methanol, sonicated for 30 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (500 μg/mL). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 10 μg/mL. A 20 μL volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas of the spots were measured at 255 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

Stability indicating property

HPLC studies of samples obtained on stress testing of Dexketoprofen trometamol under different conditions using methanol: acetate buffer pH adjusted to 4 with glacial acetic acid (65: 35) as a mobile phase suggested the following degradation behavior.

Hydrolysis

Initially 1 M hydrochloric acid was refluxed at 80 °C but no degradation was found until 12 h, hence the strength of acid was increased. Subsequently, studies were performed in 5 M hydrochloric acid which was refluxed at 80 °C, still no degradation

was found until 12 h, hence Dexketoprofen trometamol was found to be stable to acid degradation.

The reaction in 1 M sodium hydroxide was used at 80 °C for 12 h but since no degradation was observed, the strength of base was increased. Subsequently, studies were performed in 5 M sodium hydroxide. The drug did not show any degradation until 12 h at 80 °C. Hence Dexketoprofen trometamol was found to be stable to base degradation.

Oxidation

The drug was found to be highly labile to oxidative degradation. The reaction in 3 % H_2O_2 was carried out at room temperature for 30 min but the degradation was so fast that around 100 % of the drug was degraded, hence the duration of exposure was decreased. Subsequently, studies were performed in 3 % H_2O_2 at room temperature for 5 min. The drug showed a degradation of around 45 % forming major degradation product at 2.56 min (Fig 3).

Photochemical degradation

Dexketoprofen trometamol was found to be unstable to photochemical degradation as around 25-30 % of the degradation was seen after exposing drug solution in methanol to sunlight for 15 min forming major degradation products at 8.66 min (Fig 4a).

Around 20-25 % of the degradation was observed after exposing the drug solution in photo stability chamber for 28 h forming major degradation product at 4.80 min and 6.68 min (Fig 4b).

Neutral degradation

Upon heating the drug solution in water at 80 °C for 12 h, no fall in the drug peak area was observed. Hence, the drug was found to be stable to neutral degradation.

Validation of the stability indicating method

The results of validation studies on the stability indicating method developed for Dexketoprofen trometamol in the current study involving methanol: acetate buffer pH adjusted to 4 with glacial acetic acid (65: 35) as a mobile phase are given below.

Linearity

The response for the drug was linear (r^2= 0.9991) in the concentration range between 5-10 μg/mL. The mean (±RSD) values of slope, intercept and correlation coefficient were 50061 (± 1011), 9262.1 (± 1451) and 0.9991 (± 0.121), respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in **Table 1**. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guideline. Separation of the drug and different degradation products in stressed samples was found to be similar when analysis was performed on different chromatographic system on different days.
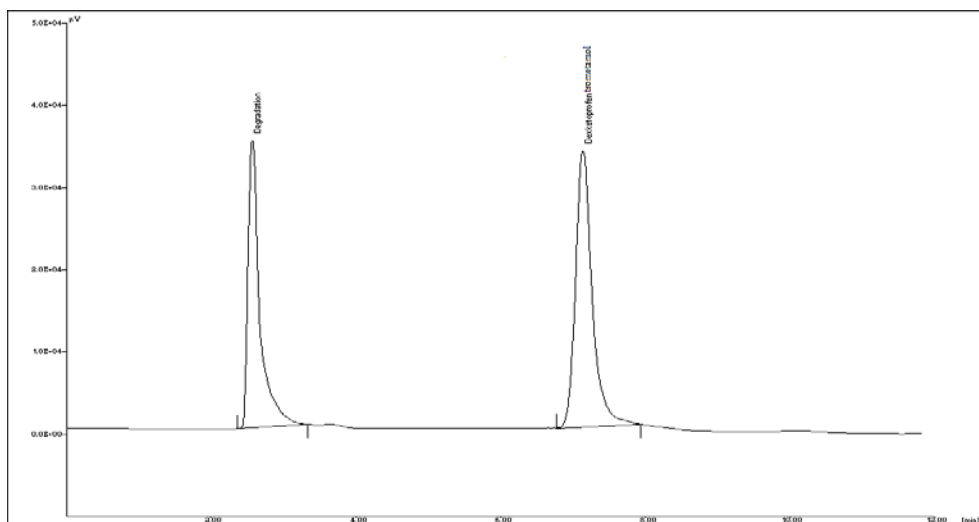
Fig. 3: Chromatogram of H_2O_2 (3 % H_2O_2 at room temperature for 5 min) treated Dexketoprofen trometamol
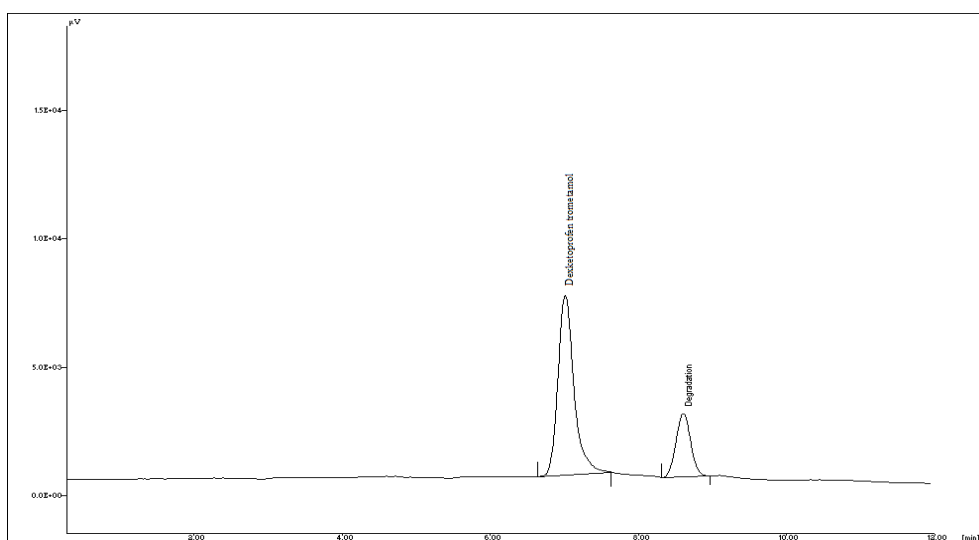

Fig. 4a: Chromatogram of photo (sunlight for 15 min) treated Dexketoprofen trometamol
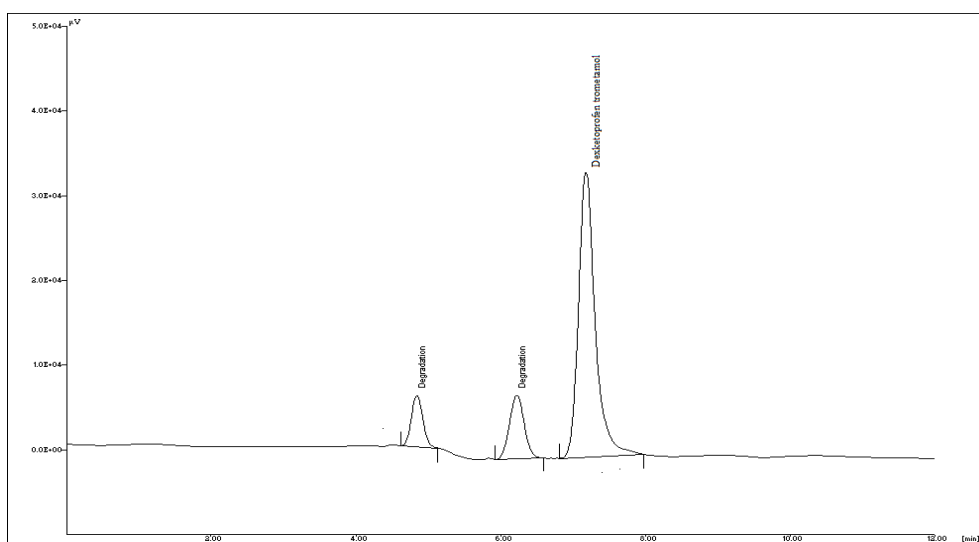

Fig. 4b: Chromatogram of photo (photostability chamber for 28 h) treated Dexketoprofen trometamol

Table 1: Precision studies

Concentration (µg/mL)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured concentration ±SD	(%) RSD	Recovery (%)	Measured concentration ±SD	(%) RSD	Recovery (%)
5	4.91 ± 2.8	0.52	98.20	4.96 ± 3.1	0.77	99.20
7	7.12 ± 3.1	0.81	101.71	6.99 ± 2.9	1.01	99.85
9	9.10 ± 4.3	0.43	101.11	9.04 ± 5.5	0.96	100.44

LOD and LOQ

The signal to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 2 µg/mL and 4 µg/mL respectively.

Robustness of the method

Each factor selected was changed at three levels (-1, 0 and 1). One factor at a time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed (Table 2). The resolution of drug in the mixture of stressed sample was found to be similar when studies were performed on different chromatographic system on different days indicating that the method has sufficient ruggedness.

Specificity

The specificity of the HPLC method is illustrated in Figure 2, 3, 4a, 4b where complete separation of Dexketoprofen trometamol in presence of its degradation products was noticed. The peaks obtained were sharp and have clear baseline separation. The resolution factor for drug from nearest resolving was > 3. The photodiode array scanned all the components present in the mixture in whole wavelength range from 200 to 400 nm and it indicated that there is no degradation peak (hiding) under or unresolved from the analyte peak (pure drug), which also reflected the specificity of the method.

Recovery studies

As shown from the data in Table 3 good recoveries of the drug in the range from 98.60 to 100.06 % were made at various added concentrations, despite the fact that the drug was fortified to a mixture that contained drug as well as degradation product formed at various reaction conditions.

Analysis of marketed formulation

The drug content was found to be 99.26 % ± 0.93. Two different lots of commercially available Dexketoprofen trometamol tablets were analyzed using the proposed procedures and the results are summarized in Table 4.

Table 2: Robustness testing[a] (n = 3)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
A: Flow rate (mL/min)				
0.9	-1	7.29	1.91	1.34
1.0	0	7.23	1.89	1.30
1.1	+1	7.17	1.86	1.27
Mean ± SD (n = 3)		7.23 ± 0.06	1.88 ± 0.02	1.30 ± 0.03
B: % of methanol in the mobile phase (v/v)				
64	-1	7.30	1.92	1.33
65	0	7.23	1.89	1.30
66	+1	7.19	1.87	1.28
Mean ± SD (n = 3)		7.24 ± 0.05	1.89 ± 0.02	1.30 ± 0.02
C: Solvents of different lots				
First lot		7.23	1.89	1.30
Second lot		7.24	1.90	1.31
Mean ± SD (n = 3)		7.23 ± 0.01	1.89 ± 0.01	1.30 ± 0.01

[a] Three factors were slightly changed at three levels (-1, 0, 1)

Table 3: Recovery studies (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
25	20 (80%)	45	44.78 ± 0.41	99.51
25	25 (100%)	50	50.03 ± 0.98	100.06
25	30 (120%)	55	54.23 ± 1.05	98.60

Table 4: Analysis of commercial formulation

Dexketoprofen trometamol (25 mg) Brand name: Infen 25	Dexketoprofen trometamol found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	24.79 ± 1.27	99.16
2nd Lot	24.84 ± 1.63	99.36

CONCLUSION

HPLC method was developed and validated as per ICH guidelines for stability-indicating studies of Dexketoprofen trometamol. UV

detection allowed an accurate quantitation of chromophoric compounds.

In this study, intrinsic stability of Dexketoprofen trometamol was established using various ICH recommended stress conditions. The drug as such was very stable in solid form but was found to be unstable in methanolic solution. In the latter case, unknown decomposition products were formed under stress conditions. The drug was found to degrade significantly in oxidative and photo condition whereas it is found to be stable in acidic, alkaline and neutral condition.

The drug was analyzed in the presence of different degradation products by HPLC method using Thermo Hypersil BDS–C$_{18}$ (250 mm × 4.6 mm, 5.0 µ) column from Germany with isocratic conditions and simple mobile phase using methanol: acetate buffer pH adjusted to 4 with glacial acetic acid (65: 35) at flow rate of 1 mL/min using UV detection at 255 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 5-10 µg/mL. LOD was found to be 2 µg/mL and LOQ was found to be 4 µg/mL.

ACKNOWLEDGEMENT

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