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Stability Indicating UPLC Method for The Degradation Study of Ketorolac Tromethamine

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Abstract

UPLC stability indicating method was developed for determining ketorolac tromethamine in its degradation study at different conditions. An isocratic mobile phase composition of 60:10:30 (v/v) containing CH_3OH, CH_3CN and 5mM NaH_2PO4 and C18 column were used at a flow rate of 0.20 mL min^{-1}. Satisfactory retention time was found 2.13±0.05 min at 320 nm when monitored by DAD detector. Forced degradation studies of ketorolac tromethamine was also performed at the following conditions: acid and basic hydrolysis, heat (50-70°C for 1 hr), photolytic (UV and sunlight for up to 3 hr), oxidation (3% hydrogen peroxide for 1 hr). Forced degradation study revealed that ketorolac degraded significantly under thermal conditions. In 1N acid and base hydrolysis, degradation was moderately significant and comparable. It was degraded marginally in 0.1N acid-base hydrolysis which was comparable to oxidative conditions. But in photolytic condition ketorolac shows insignificant degradation. Method was also applied to pharmaceutical formulation.

Keywords: Forced degradation, ketorolac tromethamine, pharmaceutical formulation, UPLC

Stability Indicating untuk Studi Degradasi Ketorolak Trometamin dengan Metode UPLC

Abstrak

Stability indicating dengan metode UPLC telah dikembangkan untuk studi degradasi ketorolak trometamin pada berbagai kondisi. Metode isokratik dengan komposisi fase gerak CH_3OH, CH_3CN and 5mM NaH_2PO4 60:10:30 (v/v) dan kolom C18 digunakan dengan laju alir 0.20 mL min^{-1}. Waktu retensi yang diperoleh adalah 2.13±0.05 min pada 320 nm yang dimonitor oleh detektor DAD. Studi degradasi paksa dari ketorolak trometamin dilakukan pada kondisi berikut: hidrolisis asam dan basa, pemanasan (50-70°C selama 1 jam), fotolisis (UV dan cahaya matahari selama 3 jam), oksidasi (3% hidrogen peroksida selama 1 jam). Dari hasil studi degradasi paksa, diketahui bahwa ketorolak terdegradasi secara signifikan pada kondisi panas. Dalam hidrolisis 1N asam dan basa, degradasi cukup signifikan dan sebanding. Ketorolak terdegradasi sedikit dalam hidrolisis 0,1 N asam-basa jika dibandingkan dengan kondisi oksidatif. Pada kondisi fotolisis, ketorolak menunjukan degradasi yang tidak signifikan. Metode telah diaplikasikan pada sediaan farmasi.

Kata Kunci: Degradasi paksa, ketorolak trometamin, sediaan farmasi, UPLC

1. Introduction

Ketorolac tromethamine (KTR-Figure 1), 2-Amino-2-(hydroxymethyl) propane-1, 3-diol(1RS)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylate, is a non-steroidal anti-inflammatory drug (NSAID). It is a member of the heterocyclic acetic acid derivative family. It is used as an analgesic with an efficacy close to that of the opioid family.[1] It is also a potent antipyretic and anti-inflammatory. It is mainly used for the short term treatment of post-operative pain as it is highly selective for the COX-1 enzyme.[2]

Ketorolac (KTR), like other NSAIDs, competitively inhibits both cyclooxygenase (COX) isoenzymes, COX-1 and COX-2[3] by blocking arachidonate binding. Anti-inflammatory and analgesic effects have been attributed to the inhibition of prostaglandin synthesis.[4] Prostaglandins have been shown to be mediators of certain kinds of intraocular inflammation and also produce disruption of the blood-aqueous humour barrier, vasodilation, increased vascular permeability, leukocytosis, and increased intraocular pressure.[5] It is available in oral, intramuscular and topical dosage forms. Other adverse effects are similar to the ones associated with other NSAIDs. The most serious risks associated with ketorolac are those associated with other NSAIDs, i.e. gastrointestinal ulcers, bleeding and perforation; renal (kidney) events ranging from interstitial nephritis to complete kidney failure; hemorrhage, and hypersensitivity.[6] In these concern a fast, precise and accurate analytical method is paramount important.

The methods described in the literature for the analysis of ketorolac included flow injection analysis,[9] spectrophotometric,[10] spectrofluorometric,[11] micellar electrokinetic chromatography[12] and differential pulse polarography.[13] The chromatographic methods available for ketorolac tromethamine determination are HPLC,[2,14-16] LC/MS/MS,[17] high-performance thin layer chromatography (HPTLC),[18] capillary electrochromatography[19] and gas chromatography-mass spectrometry (GC-MS).[20]

Furthermore, forced degradation studies are used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient and drug product stability, and to provide information about degradation pathways and degradation products. Forced degradation studies of the bulk drug sample were also performed using the following conditions: acid hydrolysis (0.1N HCl, 1N HCl), basic hydrolysis (0.1 N NaOH, 1N NaOH), heat (60-80°C for 1 hr), photolytic (UV and sunlight for up to 3 hr), oxidation (3% hydrogen peroxide for 1 hr).[21-24] Still the degradation study is needed to get a clear scenario of its degradation ways.

The present study attempts to develop a stability indicating ultra-performance liquid chromatography (UPLC) method to study forced degradation to be performed at ambient temperature under different degradation conditions. Method was validated in terms of ICH[25] guidelines and applied to pharmaceutical formulation to check its validity.

2. Methods

2.1. Instruments

A Shimadzu (Tokyo, Japan) binary low-pressure gradient system was used for the chromatographic determination of the examined analyte (KTR). The solvent lines were mixed in an FCV-20AH2 mixer. Two Nexera LC-30AD pumps equipped with a Prominence CBM-20A communication bus module, permitting fully automated operation,

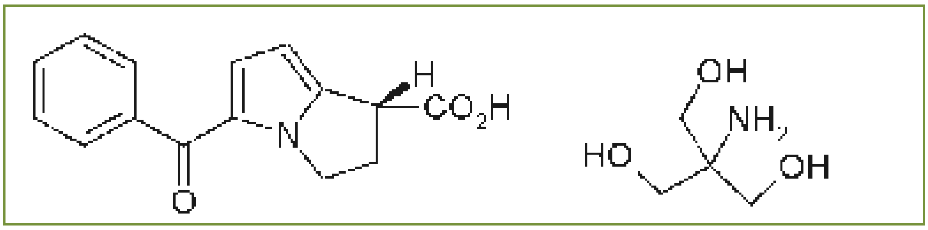

Figure 1. Structural formula for ketorolac tromethamine (MW=376.4)

was used to deliver the mobile phase to the analytical column. Sample injection was performed via a Nexera SIL-30AC auto-sampler. Detection was achieved by an SPD-M20A Photodiode Array Detector, complied with Data acquisition software Lab Solutions-Nexera PDA by Shimadzu.

2.2. Chemicals

HPLC-grade methanol was supplied by SIGMA-ALDRICH (Germany), ACN was supplied by Scharlau (Scharlab S.L, Spain) and sodium dihydrogen phosphate was supplied by Applichem GmbH (Germany). Water used throughout the study was purified by the reverse osmosis method to gain high-purity water with a Milli-Q water purification system from Millipore (Millipore, Bedford, MA, USA). Purity of reference compounds was not less than 98%.

2.3. Preparation of calibration curve

Calibration curves were prepared for seven concentration levels ranged from 0.50-10.0 ng μL^{-1} of the analyte. The calibration curve was constructed by plotting peak area against theoretical concentrations which were fitted by a least squares linear regression to the equation: response ratio (y) = slope (m) × concentration (x) + intercept (c). Unknown concentration of the analyte was determined with reference to the calibration equation.

2.4. Chromatographic conditions

A standard solution at concentration level 5 $\mu g\ mL^{-1}$ was used for the optimization procedure. Reversed-phase Kinetex C18 (100 × 2.10 mm, 2.6 μm) column and 5 mM NaH_2PO_4 as buffer solution were used through the experiment. The injection volume was 10 μL. Prior to the analysis the column was equilibrated with mobile phase. The dihydrogen sodium phosphate solution was filtered in vacuum using 0.2 μm membrane, and the mobile phase was degassed prior to use by a stream of helium.

An UV scan of standard solution in the used mobile phase was done for the spectra of studied drugs in the range of 250 to 400 nm to detect the absorption maxima. To evaluate an efficient UPLC method special attention was given on optimization of the mobile phase composition to obtain satisfactory results with good resolution at reduced elution time and tailing problems. Several solvent gradients were performed to determine the optimum one to detectable peaks with high intensity in the chromatogram at considerable analysis time. The best flow rate was investigated with respect to sharpness and symmetry of the peaks. Different composition of mobile phase consisting CH_3OH, CH_3CN and NaH_2PO_4 under isocratic program was checked as the optimized conditions at a flow rate of 0.20 mL/min at ambient temperature.

2.5. Forced degradation studies

2.5.1. Preparation of standard solution

Forced degradation studies of the standard drug sample were performed using the following conditions: acid hydrolysis (0.1N HCl, 1N HCl), basic hydrolysis (0.1 N NaOH, 1N NaOH), heat (60-80°C for 1 hr), photolytic (UV radiation), oxidation (3% hydrogen peroxide). Standard Solution of 100 $\mu g\ mL^{-1}$ of ketorolac was prepared for forced degradation study and experimental solutions were prepared according to following procedure.

2.5.2. Acid hydrolysis

2.5 mL solution of standard ketorolac tromethamine in 0.1N HCl or 1N HCl was transferred to 25 mL volumetric flask to gain a concentration of 10 $\mu g\ mL^{-1}$ and was shaken in ultrasonic water bath for the period of 3 hours at ambient temperature. During the ultrasonic shaking after one hour of interval up to 3 hours 10 μL of acid degradation sample was subjected to UPLC and was analyzed under the optimized chromatographic conditions. In case of 1N HCl degradation additional 10 μL sample after 24 hours was subjected to UPLC.

2.5.3. Basic hydrolysis

2.5 mL solution of standard ketorolac tromethamine in 0.1N NaOH or 1N NaOH was transferred to 25 mL volumetric flask to gain a concentration of 10 $\mu g\ mL^{-1}$ and was shaken in ultrasonic water bath for the period of 3 hours at ambient temperature. During the ultrasonic shaking after one hour of interval up to 3 hours 10 µL of degradation sample was subjected to UPLC and was analyzed under the optimized chromatographic conditions.

2.5.4. Photo degradation

2.5 mL solution of standard ketorolac tromethamine in water was transferred to 25 mL volumetric flask to gain a concentration of 10 µg mL^{-1} and was placed in photo-stability chamber (UV-light) for the period of 3 hours at ambient temperature. After one hour of interval up to 3 hours 10 µL of degradation sample was subjected to UPLC and was analyzed under the optimized chromatographic conditions.

2.5.5. Oxidative degradation

2.5 mL solution of standard ketorolac tromethamine in 3% H2O2 was transferred to 25 mL volumetric flask to gain a concentration of 10 µg mL^{-1} and was stored at room temperature for the period of 3 hours. After one hour of interval up to 3 hours 10 µL of degradation sample was subjected to UPLC and was analyzed under the optimized chromatographic conditions.

2.5.6. Thermal Degradation

2.5 mL solution of standard ketorolac tromethamine in water was transferred to three different 25 mL volumetric flasks to gain a concentration of 10 µg mL-1 were exposed to heat at 50, 60 and 80 °C for one hour. 10 µL of degradation sample was subjected to UPLC and was analyzed under the optimized chromatographic conditions.

Calculation:

$$\textit{Relative Decrease}, C\ (\%) = \frac{(x_i - x_e)}{x_i} * 100$$

xi = initial conc. of KTR

xe = conc. after degradation

2.6. Validation Parameters

UPLC method was validated in terms of ICH[25] analytical performance parameters; precision, accuray, specificity, limit of detection, limit of quantitation, linearity and range, suitability and robustness.

2.6.1. Linearity

Linearity was determined by constructing the calibration curves for standard samples at certain concentration levels. Each concentration was analyzed in triplicate and curves were constructed using peak area versus nominal concentrations of the analytes. Least square linear regression analysis of the data gave slope, intercept, and correlation coefficient or coefficient of determination.

2.6.2. Recovery/Accuracy

Aliquots of 10 µL of the selected assay solutions at three concentration levels were injected into the UPLC system, and triplicate measurements were recorded for each concentration. The nominal contents of the drug in each solution were calculated from the linear regression equations. The results were expressed as percent recoveries of the particular components in the samples as [mean found concentration/theoretical concentration] × 100.

2.6.3. Precision

The precision, degree of repeatability of the method, was determined by calculating the standard deviation (SD) and the relative standard deviation (RSD) for the repeated measurements between nominal and measured concentrations by analyzing different concentrations. Within-day and between-day precision and accuracy of the assay were assessed by performing replicate analyses of drugs in standard solutions against a calibration curve. Within-day repeatability was determined in six replicates at three concentrations levels of drugs of standard in the same day. The procedure was repeated on six consecutive days, in standard samples at same concentration levels, to determine between-day repeatability.

2.6.4. Specificity/Selectivity

The specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrices. It was demonstrated showing that analytes were free of interference from degradation products or excipients in pharmaceutical formulations ensuring that peak response in the same retention times is due to examined components only.

2.6.5. Sensitivity

Sensitivity of the method has been tested by examining limit of detection (LOD), the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified, and the limit of quantitation (LOQ), the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The calculation method is based on the standard deviation of the response (S_{xy}) and the slope of the calibration curve (a). The limit of detection were calculated from calibration graph by the formula; $LOD=3\cdot S_{xy}/a$, and the limit of quantification; $LOQ=10\cdot S_{xy}/a$.

2.6.6. Stability

The stability of sample solutions was tested by the proposed method over a period of 90 days for long-term assay. The freshly prepared solutions at room temperature and the 90 days stored solutions in a refrigerator at both 4^oC were analyzed. Each sample was analyzed for intact compounds once daily for investigation of stability. Recovery and RSD of the stored samples were calculated and compared to that of freshly prepared samples.

2.6.7. Robustness

Robustness is the capacity of the method to remain unaffected by small deliberate variations in method parameters. It has been evaluated by varying method parameters set for optimum conditions such as flow rate, gradient program, concentration of buffer, and determining the effect (if any) on the results of the method.

2.6.8. Column Efficiency

The column efficiency parameters have been calculated for a representative chromatogram by the relations: retention factor, $k = t_R-t_0/t_0$, separation factor, $\alpha = t_{R2}-t_0/t_{R1}-t_0$, resolution factor, $Rs = 2\ (t_{R2}-t_{R1})/(w_2+w_2)$, theoretical plate number, $N = 16\ (t_R/w)^2$ and tailing or asymmetry factor, $T_f = (a+b)/2a$. Where, t_0, t_{R1} and t_{R2} are the retention times and w_1 and w_2 the baseline peak width of successive peaks.

3. **Results**

3.1. Chromatography

Initial experiments with the LC system using methanol or acetonitrile as organic modifier in the buffered mobile phase were performed for better separation of analytes. The combination of methanol and acetonitrile with 0.02M dihydrogen sodium phosphate served our intentions best. Reversed-phase Kinetex C18 (100 × 2.10 mm, 2.6 µm) column and 5 mM NaH_2PO_4 as buffer solution were used for the determination of Ketorolac. In particular, peak tailing observed was considerable. The absorption spectra of ketorolac showed maximum absorption wavelengths at 320 nm (Figure 2). To determine the optimum mobile phase flow-rate under optimized composition the effect on Rt, peak height and peak width was studied. As expected when the mobile-phase flow-rate was increased Rt decreased. A flow-rate of 0.20 mL min^{-1} was chosen as a compromise analysis time, because this value also maintains good peak shape. The mobile phase mixture of CH_3OH, CH_3CN and 5mM NaH_2PO_4 by the composition of 60:10:30 (v/v) was optimized at isocratic program (Table 1). The method was carried out for the detection and quantitation of the drug representing total elution time 2.13±0.05 min. A representative chromatogram as shown in Figure 3 was obtained from a standard solution under optimized conditions. No interfering of peaks was observed in the samples studied. Precision of retention times was examined to evaluate system suitability from within-day repeatability (mean value of six measurements, n = 18) and between-day precision (mean value of three measurements during six days, n = 54) at 0.5, 1, 2, 3, 4, 5 and 10 µg mL-1 level of drugs, which revealed RSD values of 2.57%.

The validated method developed herein was applied to various concentrations

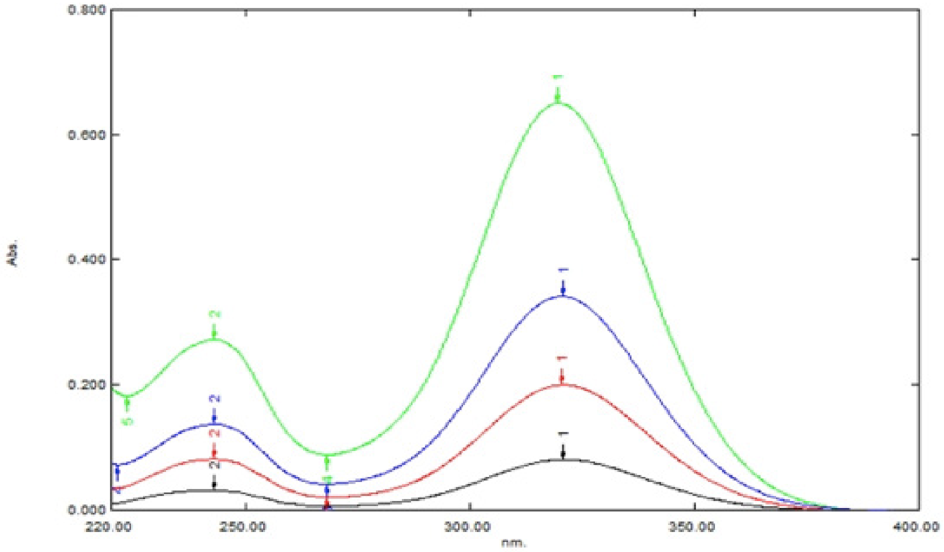

Figure 2. Electronic spectra of ketorolac standard solution in the used mobile phase

Tabel 1. Optimum gradient program for the proposed method

Program	Time (min)	Solvent composition			Flow rate (mL/min)	R. time (min)
		CH3CN	CH3OH	0.02 M NaH2PO4		
A*	0.01-3.0	10	60	30	0.20	2.13±0.05
B	0.01-3.0	10	60	30	0.22	2.00±0.05

*Program has been followed throughout the experiment

taken from the pharmaceutical products for determining the content of investigated drugs. Table 2 summarizes *within-day* and *between-day* precision and accuracy data, indicating that these values are acceptable and the method is accurate and precise. Table 3 shows the validation performance of the proposed UPLC method. Analytical data of system suitability, robustness and column efficiency data are placed in Table 4. Table 5 presents the analytical values on pharmaceutical formulations.

3.2. Method Validation

The described UPLC method was developed using a simple mobile phase to provide a rapid quality control determination of both drugs in standard and pharmaceutical formulations. The parameters linearity, selectivity, extraction recovery, precision, accuracy, stability and robustness were studied for the validation of the method.

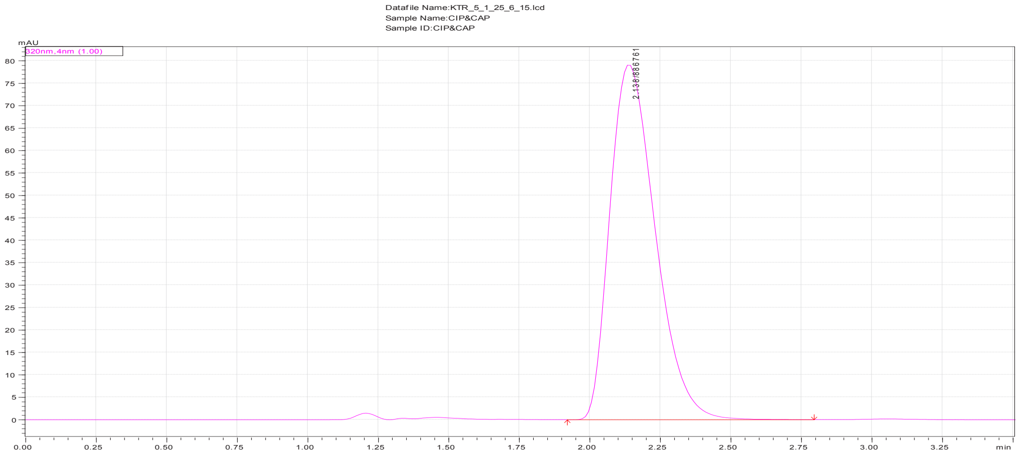

Figure 3. Typical UPLC chromatogram of the examined drug (5 μg mL^{-1}) in standard. Chromatographic conditions are described in text. Peaks: 2.13 min (KTR).

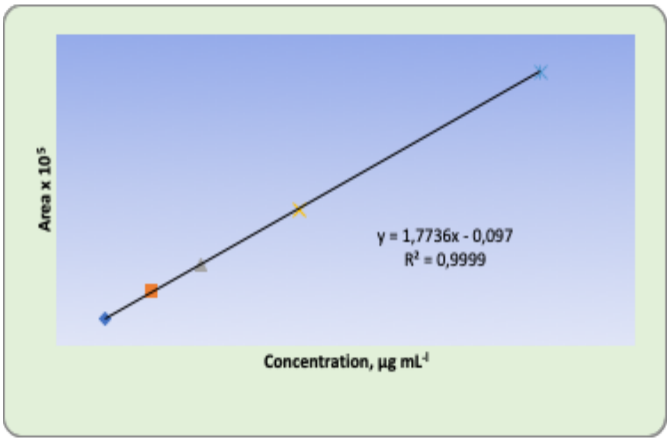

Figure 4. Calibration curve for the determination of ketorolac

3.2.1. Liniërity

The calibration curves constructed for standard using working concentration at levels 0.50, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0, 10.0 µg mL^{-1} of ketorolac. Calibration curves were constructed using peak area of drug versus nominal concentrations of the analyte. Calibration equations are y=1.7736x-0.097 for KTR determination. The calibration curves were linear in the range of 0.5-10 µg mL^{-1} for KTR. The coefficients of determination (r2) were 0.9999 for the drug. Figure 4 shows the calibration curve for the determination of ketorolac.

3.2.2. Sensitivity

The limit of detection were calculated from calibration graph by the formula; $LOD=3\cdot S_{xy}/a$, and the limit of quantification; $LOQ=10\cdot S_{xy}/a$. The LOD and LOQ were found to be 0.016 and 0.051 µg mL^{-1}, respectively. These results indicate that method is sensitive enough for therapeutic assay.

3.2.3. Accuracy

The results of recovery studies obtained from the within-day assay at six concentrations (n=6) by the proposed method was 98.0222-103.5116% and Between-day assay at six different days was 98.5027-103.0967% indicated high Accuracy of the drug. Within-day and between-day recovery data for proposed method are presented in Table 2.

3.2.4. Precision

The relative standard deviations (RSD) obtained for the within-day assay at six concentrations (n=6) in the range 0.14-3.08% for KTR and for Between-day assay the corresponding values in the range 2.12-4.02% for KTR indicating the high precision of the method. Within-day and between-

Tabel 2. Summarizes within-day and between-day precision and accuracy data

Analysis	Added value µg mL^{-1}	Found value µg mL^{-1}	Recovery (%)	RSD (%)
Intra day	1	1.03±0.005	103.0967	0.30
	2	1.97±0.004	98.5027	0.1253996
	3	2.96±0.032	98.9596	0.6335716
	4	5.03±0.02	100.6343	0.2387658
	5	9.96±0.04	99.9636	0.2490341
Inter day	1	1.73±0.01	103.5116	0.724698
	2	1.97±0.01	98.5272	0.577928
	3	2.94±0.03	98.0222	0.627065
	4	5.02±0.07	100.444	0.904664
	5	9.92±0.07	99.2073	0.447181

Tabel 3. Validation performance of the proposed UPLC method

Validation Parameters	(KTR)
Measurement wavelength (nm)	320
Linear range (μg mL-1)	0.01-10
Linearity equation	y=1.7736x-0.097
Standard deviation of the slope	0.004
Correlation coefficient (r)	0.9999
Relative standard deviation (% RSD)	
Intraday	0.12-0.63
Inter day	0.44-0.90
Relative standard deviation (% R)	
Intraday	98.50-103.09
Inter day	98.02-103.51
Limit of detection, LOD (μg mL-1)	0.016
Limit of quantification, LOQ (μg mL-1)	0.051

day precision data for proposed method are presented in Table 2. Validation performances of the proposed UPLC method are presented in Table 3.

3.2.5. Specificity/Selectivity

The specificity was demonstrated showing that drug was determined free of interference from potential impurities and degradation products by the absence of any peak in the same retention times. The selectivity of the method was checked by injecting Ketorolac tromethamine standard solution, background control sample. There was no interference at retention time of Ketorolac due to back ground control sample. Peak purity of ketorolac peak was passed in standard. From the chromatogram shown in Figure 5 it is evident that under the chosen chromatographic conditions KTR (Tr=2.13 min) the UPLC method did not suffer interference by the formulation excipients, since there was not another peaks on the retention times of KTR. Results indicate the high specificity of the method and can be used in a stability assay and routine analysis of the investigated drug.

3.2.6. Robustness

It was found that the percent recoveries were excellent under most conditions, and remained unaffected by small deliberate changes of experimental parameters including the flow rate and isocratic program (Table 1) though retention time and resolution was shortened as expected. There was no noticeable difference between the chromatograms when the wavelength was varied by ±3 nm.

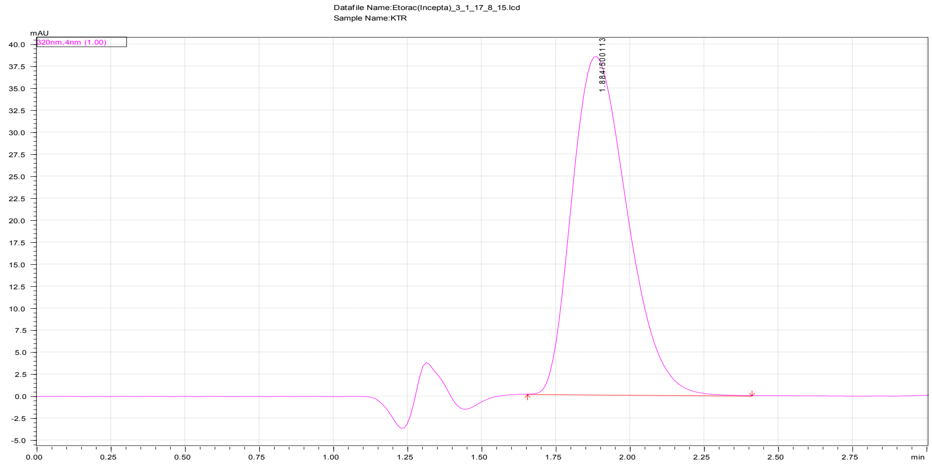

Figure 5. UPLC chromatogram of the examined drug (3 μg mL-1) in pharmaceutical formulation. Chromatographic conditions are described in text. Peaks: 2.15 min (KTR)

Tabel 4. Validation parameters in terms of suitability, robustness and column efficiency

Conc. (5 µg mL^{-1})	Suitability		Robustness	
			Recovery (%)	
	R.T (n=6)	Area (n=6)	Program A Flow rate (0.2 mL/min)	Program B Flow rate (0.22 mL/min)
Average	1.87±0.015	516845±0.03	99.75	98.95
RSD (%)	0.85	0.63	1.25	1.63
Column efficiency	NTP	HETP	T. F	C.F, k´
Average	763±5	203±1.4	1.36±0.01	0.733±0.03
RSD	0.69	0.72	0.37	0.37

Variation in the experimental parameters (flow rate, isocratic program) provided an indication of its reliability during normal use and concluded that the method was robust as shown in Table 4.

3.2.7. System Suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of KTR to be performed. The system suitability was assessed by replicate injections (n=6) of the sample at 5 µg mL-1 concentration level including within- and between-day assessments for standard. Precision of retention time and peak area was examined to evaluate the system suitability. RSD of the peak area 0.63% and that of retention time 0.85% indicates excellent suitability of the system as shown in Table 4.

3.2.8. Column Efficiency

The column efficiency parameters have been calculated for a representative chromatogram. This test is essential for the assurance of the quality performance of a chromatographic system. The calculated values; retention factor, k = 0.733±0.03, theoretical plate number, 763±5, and tailing factor, Tf = 1.36±0.01 shown in Table 4 revealed the excellent performance of analytical column.

3.2.9. Stability

The stability of KTR in methanol, stored in clear glassware in the fridge (4ºC) was tested at five intervals by the 90 days. The responses from the aged solutions were compared with those from freshly prepared standard solution. The results showed that the retention time and peak area of

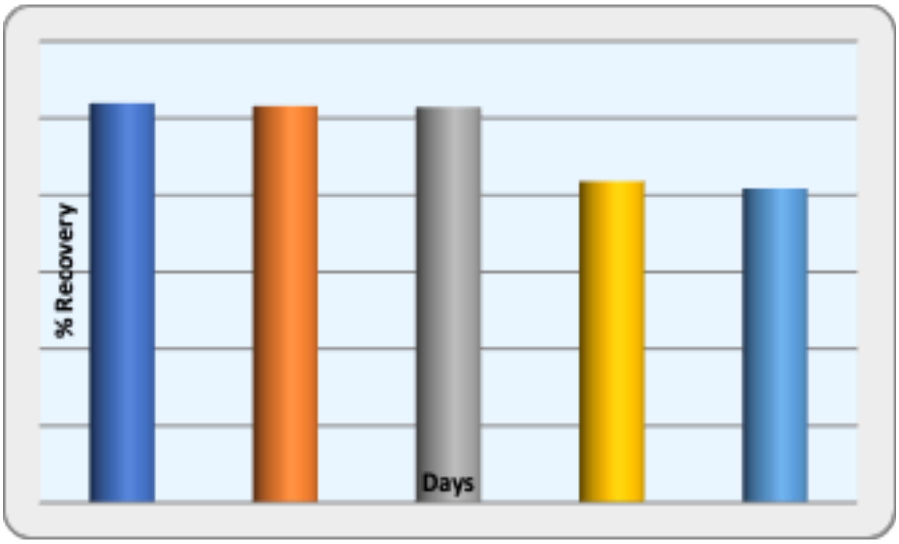

Figure 6. Long term stability graph for ketorolac

Tabel 5. Determination of Ketorolac in pharmaceutical degradation by the proposed method

Name	Company name	Conc. μg mL^{-1}		%R	RSD (%)	Average R	Amount (mg)
		Added	Expt.± SD				
Minolac	ACI	1	1.45±0.09	145.14	0.67	142.32	14.2
		2	2.84±0.02	142.11	0.10		
		4	5.55±0.1	138.77	0.33		
Winop	ACME	1	1.01±0.01	101.03	0.07	104.73	10.47
		2	2.07±0.02	103.96	0.11		
		4	4.36±0.2	109.19	0.63		
Zidolac	BEXIMCO	1	1.03±0.09	103.11	0.91	102.61	10.26
		2	2.02±0.1	101.11	0.62		
		4	4.14±0.1	103.61	0.41		
Perilac	BIO-PHARMA	1	0.99±0.1	99.48	1.04	101.10	10.11
		2	2.02±0.2	101.11	1.03		
		4	4.10±0.1	102.71	0.37		
Zeropain	HEALTH-CARE	1	0.89±0.02	89.10	0.25	86.05	8.605
		2	1.70±0.06	85.02	0.35		
		4	3.36±0.1	84.02	0.36		
Etorac	INCEPTA	1	0.84±0.4	84.43	0.53	84.23	8.42
		2	1.66±0.03	83.47	0.20		
		4	3.39±0.2	84.80	0.64		
Ofpain	KEMIKO	1	0.81±0.4	81.31	5.81	83.67	8.37
		2	1.70±0.3	85.28	2.02		
		4	3.37±0.3	84.41	0.93		
Todol	OPSONIN	1	1.51±0.1	151.37	0.84	154.38	15.4
		2	3.07±0.1	153.52	0.47		
		4	6.32±0.5	158.23	0.84		
Rolac	RENATA	1	1.24±0.05	124.39	0.45	126.70	12.6
		2	2.52±0.1	126.02	0.58		
		4	5.18±0.4	129.69	0.84		
Torax	SQUARE	1	1.11±0.07	111.41	0.70	109.75	10.9
		2	2.17±0.05	108.89	0.27		
		4	4.35±0.2	108.93	0.56		

KTR remained almost unchanged and no significant degradation within the indicated period occurred. Recovery of the compound was ≥ 99 % up to three months at 4°C stored sample as shown in Figure 6 though it showed gradual decrease.

3.3. Application to Pharmaceutical formulations

The method developed here was applied to various concentrations i.e. 1.0, 2.0, 4.0, ng μL^{-1}) of solutions prepared from pharmaceutical products for determining the content of KTR. Typical UPLC chromatogram of the examined drug (5 μg mL^{-1}) in formulation is given in Figure 5. The values of the overall drug percentage recoveries and the RSD values of measurements are as presented in Table 5. Results indicate that measurements are acceptable with good precision. Recovery was almost same as that of levelled values for four tested samples. Some contain excessive large amount and some contain lower than labelled values. It is may be due to lack of proper quality management.

3.4. Degradation of ketorolac tromethamine

Forced degradation studies of the

Tabel 6. Relative decrease (%) and degradation kinetics of ketorolac tromethamine under various stress conditions

Hour	Decrease						Temp °C	% Decrease
	0,1 N HCl	1 N HCl	0,1 N NaOH	1 N NaOH	UV Radiation	3% H_2O_2		Thermal
1	5.1	14.1	5.9	12.8	1	6.28	50	13.8
2	11	15.3	11.4	13.2	1.45	8.85	60	19.45
3	11.3	17.1	14	13.9	2	10.26	80	25.4
24		38.5						
			Degradation kinetics					
k		0.018	4.05	0.017	0.50	1.98		0.373
$T_{1/2}$		37.94	0.17	40.76	1.39	0.35		1.86

bulk drug sample were performed using the following conditions: acid hydrolysis (0.1N HCl, 1.0 N HCl), basic hydrolysis (0.1 N NaOH, 1.0 N NaOH), heat (50-80°C for 1 hr), photolytic (UV radiation for 3 hr) and oxidation (3% hydrogen peroxide). Peak purity test was carried out for ketorolac by using UV detector. The stressed samples so prepared were injected in HPLC column and chromatograms were obtained using preliminarily optimized mobile phase. The chromatograms of degradation products are depicted in Figure 7.1-7.5. Percentage degradation of ketorolac tromethamine under various stress conditions was calculated and listed in Table 6.

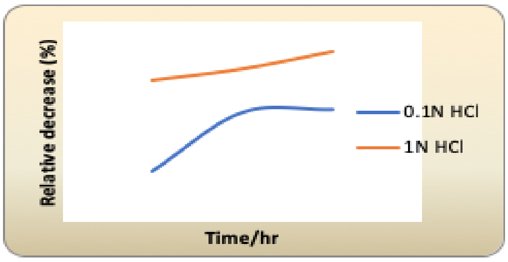

Figure 7.1. Relative decrease (%) of ketorolac tromethamine in acid hydrolysis

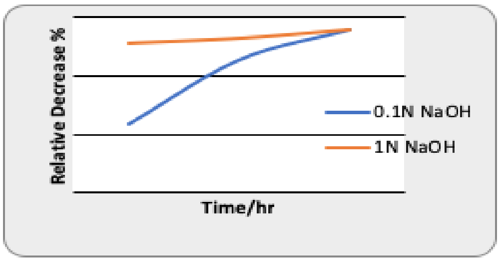

Figure 7.2. Relative decrease (%) of ketorolac tromethamine in alkaline hydrolysis.

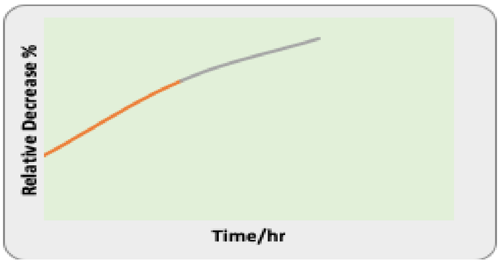

Figure 7.3. Relative decrease (%) of ketorolac tromethamine in oxidative degradation

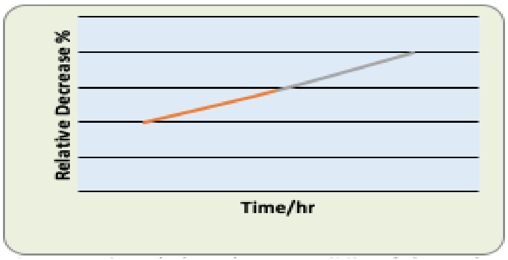

Figure 7.4. Relative decrease (%) of ketorolac tromethamine in photolytic degradation

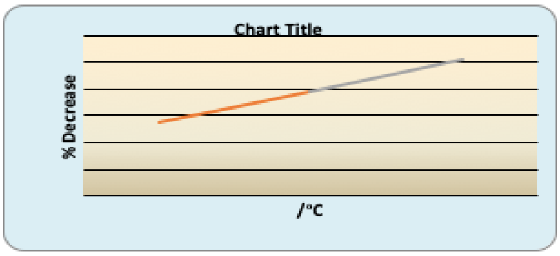

Figure 7.5. Relative decrease (%) of ketorolac tromethamine in thermal degradation

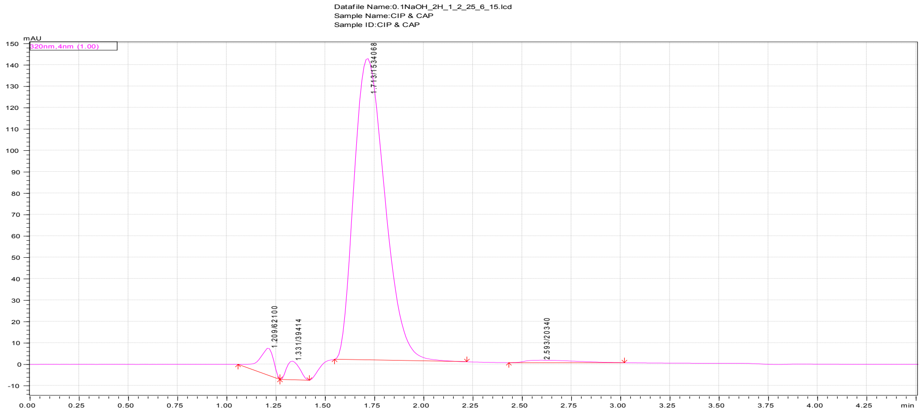

Figure 8.1. Typical UPLC chromatogram of the examined drug showing peaks 1.714 min for KTR and 2.593 min for degradation product in acidic condition 0.1N HCl

3.4.1. Acid hydrolysis

In the acid degradation study of ketorolac tromethamine showed no additional peak. In 0.1N HCl, relative decrease (%) of initial concentration is maximum 11.3 % after 3 hrs. Significant decrease (%) has been found in acid hydrolysis at 1.0N HCl up to 38.5 % after 24 hrs of shaking at room temperature. Relative decrease (%) in both acid concentrations has been plotted against the time as shown in Figure7.1. In the higher conc. of acid degradation was higher. In 1N HCl (upper curve) relative decrease happened proportionally with time whereas in 0.1N HCl maximum decrease was happened after 2 hrs.

3.4.2. Alkali hydrolysis

In the base degradation study of ketorolac tromethamine showed no additional peak. In both 0.1N NaOH and 1N NaOH, relative decrease (%) of initial concentration reaches to the maximum 14 % after 3 hrs of shaking at room temperature. Relative decrease (%) in both basic concentrations has been plotted against the time as shown in Figure 7.2. In the higher conc. of acid degradation was higher but after 3 hrs both reaches to the same degradation. In both 0.1N NaOH and 1N NaOH relative decrease happened almost proportionally with time but both reaches to the same point after 3 hrs hydrolysis.

3.4.3. Oxidative degradation

In the oxidative degradation study of ketorolac tromethamine showed no additional peak. In 3% H2O2 degradation, relative decrease (%) of initial concentration reaches to the maximum 10.26 % after 3 hrs of shaking at room temperature. Relative

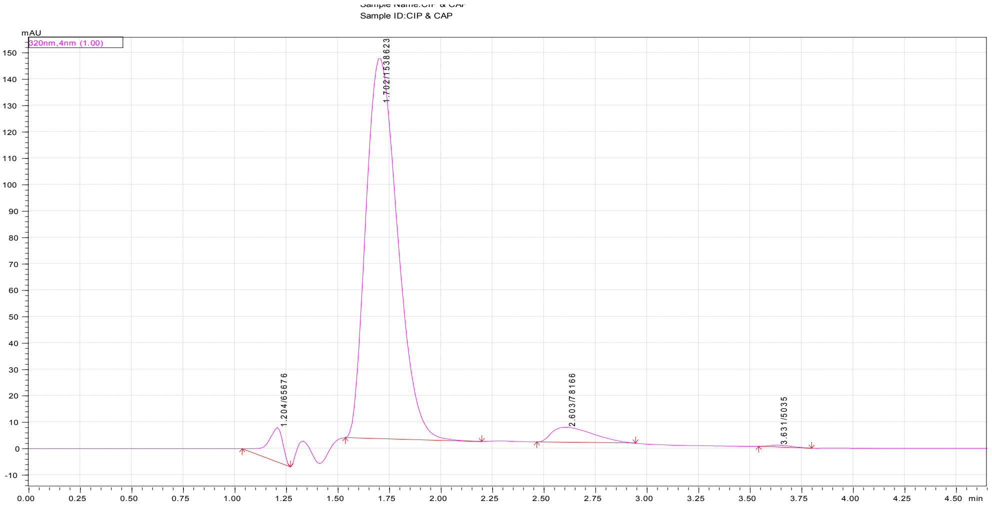

Figure 8.2. Typical UPLC chromatogram of the examined drug showing peaks 1.709 min for KTR, 2.603 min and 3.631 min for degradation product in basic condition 0.1N NaOH

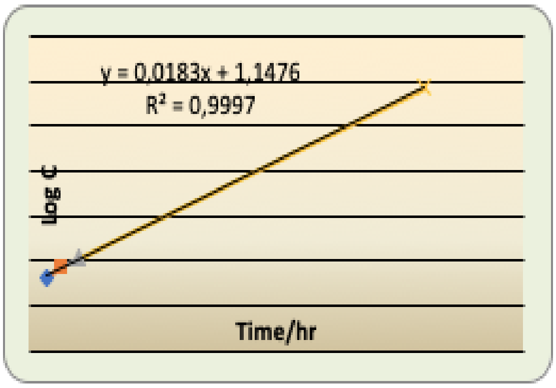

Figure 9. Degradation profile of ketorolac tromethamine in acid (1N HCl) hydrolytic degradation

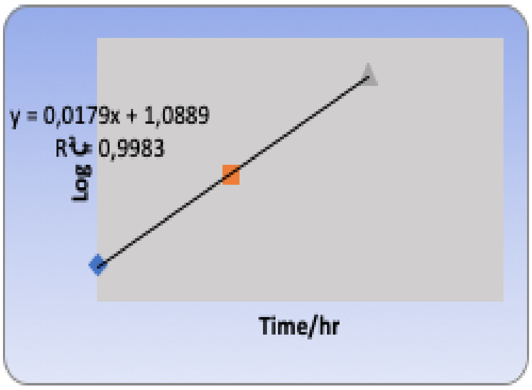

Figure 10. Degradation profile of ketorolac tromethamine in base (1N NaOH) hydrolytic degradation

decrease (%) in ketorolac concentration has been plotted against the time as shown in Figure 7.3. Relative decrease happened almost proportionally with time.

3.4.4. Photo degradation

Photolytic studies were carried out by exposure of ketorolac tromethamine in water to UV light (photo-stability chamber) for a period of 3 hrs. This photo degradation sample was analyzed under the optimized chromatographic conditions. In the photo degradation study, ketorolac tromethamine showed no additional peaks. Relative decrease (%) of ketorolac has been plotted against the time as shown in Figure 7.4. Relative decrease increases almost proportionally with time. Differences between means were less than 2%, and these small differences are generally considered clinically unimportant.

3.4.5. Thermal degradation

Thermal degradation was performed by keeping ketorolac in water in oven at three different temperature from 50 to 80ºC for a period of 1 hour. Over the study period, sample concentrations stored at either temperature retained not more than 14% of the initial ketorolac concentration. Relative decrease (%) in both acid concentrations has been plotted against the time as shown in Figure 7.5. In the increase in temperature, degradation increases proportionally with time. It is revealed that amount of degradation was significant.

3.4.6. Time dependent degradation kinetics

For the measurement of degradation profile of ketorolac tromethamine, acid (1N HCl) and base (1N NaOH) hydrolytic degradation was performed for the period of 3 hours. In 1N HCl it was done for up to 24 hours. Log C (C= % decrease) vs time (hour) curve was constructed for all degradations. These data were fit significantly better by a first-order rate. Different kinetics parameters like apparent first order rate constant (k) and half-life (t1/2, time for 50% potency left) were calculated and tabulated in table 7.

4. Discussion

UPLC method developed here was well validated in terms of parameters linearity, selectivity, extraction recovery, precision, accuracy, stability and robustness (Table 2, 3). Determination was free of interference from degradation products and no interference from the sample excipients could be observed at this detection wavelength, indicating the high specificity of the method. Method was successfully applied to formulations and KTR was estimated with good agreement to levelled values. Results of stability study conclude that there was no degradation product and the drug is stable at 4°C for at least 90 days, indicating the possibility of using the samples over a period of 90 days at refrigerator without degradation. This indicates good stability.

In table 7 and figures 7.1-7.5 the decrease in concentration of ketorolac at different time (hours) are represented.

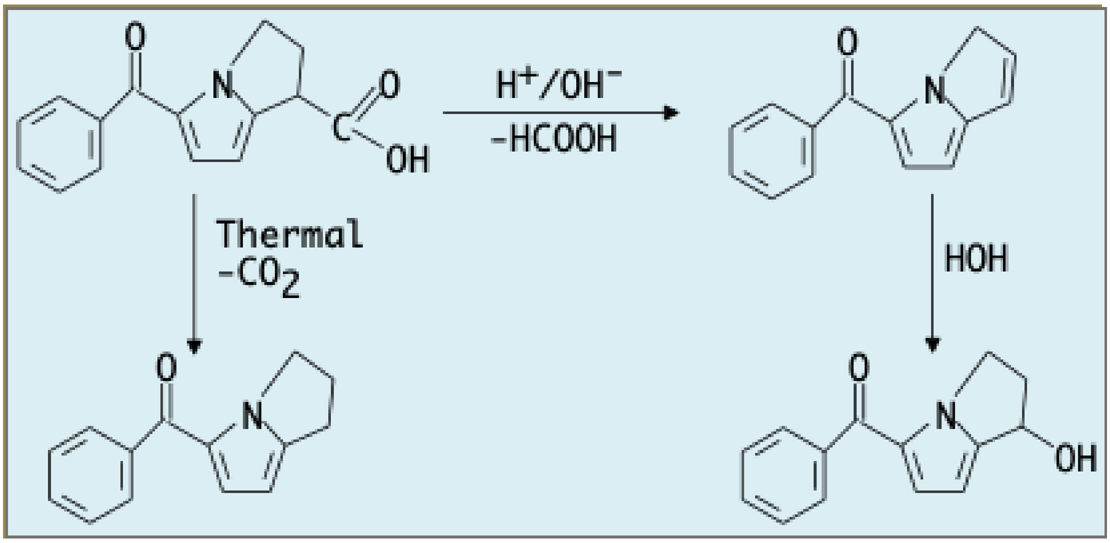

Figure 11. Degradation scheme of ketorolac tromethamine at different conditions

Degradation results indicated that ketorolac tromethamine degraded significantly under hydrolysis, thermal and oxidative conditions significantly. Degradation was comparable in 0.1N acid and 0.1N base hydrolysis which was comparable to oxidative conditions. But in photolytic condition ketorolac shows insignificant degradation. In the stress conditions of degradation study additional peak of degraded product was indicated at the monitoring weave length of 320 nm as shown in Figure 8.1-8.2. Scheme 1 shows the degradation products of ketorolac tromethamine at different conditions.[26] From the degradation kinetics data (Table 7), it can be concluded that ketorolac tromethamine is susceptible to acidic and alkaline degradation (Figure 9 and Figure 10).

rikut rendemen dari fraksi metanol-air, n-heksan dan fraksi etil asetat berturut-turut adalah 56,2%; 40,2% dan 0,88%. Fraksi etil asetat memiliki rendemen yang sangat kecil dibandingkan fraksi yang lain artinya senyawa semipolar yang tertarik oleh pelarut etil asetat lebih sedikit dibandingkan senyawa yang bersifat polar ataupun non polar.

5. Conclusion

A simple, sensitive, accurate and selective validated stability indicating UPLC method has been developed to study the degradation behavior of KTR under hydrolysis (acid, base, and neutral), oxidation, photolysis, and thermal stress conditions and determined the inherent stability of the drug. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation and specificity. The drug was found to degrade in all the stress conditions except it was exposed to photolytic conditions. The method was able to separate the peaks of active ingredients (KTR) from the degradation products. Estimation of KTR from different pharmaceutical formulations was free of interference from the sample excipients could be observed at this detection wavelength, indicating the high specificity of the method. Therefore, method might be applicable to routine analysis of in pharmaceutical laboratories.

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